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Plant pathogenic and endophytic *Botryosphaeriales* known from culture

Alan J.L. Phillips, Bernard Slippers, Johannes Z. Groenewald and Pedro W. Crous, editors

CBS-KNAW Fungal Biodiversity Centre,
Utrecht, The Netherlands
An institute of the Royal Netherlands Academy of Arts and Sciences

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Cover: Left column, Eucalyptus canker caused by *Neofusicoccum* sp. Right column, leaf spots caused by *Phyllosticta cussonia*. Central top row, asci of *Botryosphaeria corticis*, and conidia of *Phyllosticta cussonia*. Middle row, conidia of *Neofusicoccum arbuti*, and *Barriopsis iraniana*. Bottom row, vertical section through ascostromata of *Diplodia corticola*, and conidia of *Sphaeropsis sapinea*.

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edited by

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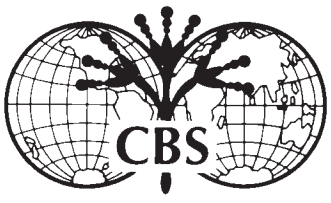

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INTRODUCTION

The present issue of *Studies in Mycology* revises the *Botryosphaeriales*, which represents a well-known order containing numerous plant pathogenic fungi associated with fruit rot, leaf spots, die-back, gummosis and cankers of Angiosperms (e.g. *Botryosphaeria*, *Diplodia*, *Phyllosticta*, *Sphaeropsis,* etc.), though some members are also associated with root rot (e.g. *Macrophomina*). Although the order has only recently been introduced (Schoch *et al*. 2006), and presently contains two families, *Botryosphaeriaceae* and *Planistromellaceae* (Minnis *et al*. 2012), it is clear that several taxa could not be well accommodated in this familial structure (Liu *et al.* 2012). The present issue focuses firstly on resolving the families that occur in the order, and secondly focuses on the species that are known from culture and DNA data, providing morphological keys to their identification, and associated DNA barcodes.

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Schoch CL, Shoemaker RA, Seifert KA, Hambleton S, Spatafora JW, Crous PW (2006). A multigene phylogeny of the *Dothideomycetes* using four nuclear loci. *Mycologia* **98**: 1041–1052.

DEDICATION: Robert (Bob) A. Shoemaker

Dr Shoemaker is well known to plant pathologists around the world for his taxonomic studies on *Dothideomycetes*, having monographed several genera within the class. His very thorough descriptions of ascomycete species in the class have set a high standard for the field of ascomycete taxonomy, and many systematists have adopted his format of description. He has published over 100 papers in his career (frequently being more than 100 pages in length), which provide a valuable resource to mycologists and plant pathologists alike. Several of these papers dealt with the *Botryosphaeria* complex, and therefore it is also fitting that the present issue of *Studies in Mycology* is dedicated to him. He has excelled in working out the life cycles of species in many genera, and has been a constant source of information for the community. He has also served in an editorial capacity on the *Canadian Plant Disease Survey* and *Fungi Canadensis*. Last but not least, Bob has helped build a world-class mycological fungarium at Biodiversity (Mycology and Microbiology), Agriculture and Agri-Food Canada, which is one of the most important fungaria in North America. Dr Robert Shoemaker has been recognised for having made an outstanding contribution to global mycology, and also has been honoured as Distinguished Mycologist of the Mycological Society of America. Throughout his 46-year-long career, Bob has collaborated with many plant pathologists on taxonomic issues pertaining to fungal plant diseases, and continues to provide valued advice to his colleagues, and to the clients of the Canadian National Identification Service.

The Editors

September 2013

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A phylogenetic re-evaluation of *Phyllosticta* (*Botryosphaeriales*)

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Abstract: *Phyllosticta* is a geographically widespread genus of plant pathogenic fungi with a diverse host range. This study redefines *Phyllosticta*, and shows that it clusters sister to the *Botryosphaeriaceae* (*Botryosphaeriales*, *Dothideomycetes*), for which the older family name *Phyllostictaceae* is resurrected. In moving to a unit nomenclature for fungi, the generic name *Phyllosticta* was chosen over *Guignardia* in previous studies, an approach that we support here. We use a multigene DNA dataset of the ITS, LSU, ACT, TEF and GPDH gene regions to investigate 129 isolates of *Phyllosticta*, representing about 170 species names, many of which are shown to be synonyms of the ubiquitous endophyte *P. capitalensis*. Based on the data generated here, 12 new species are introduced, while epitype and neotype specimens are designated for a further seven species. One species of interest is *P. citrimaxima* associated with tan spot of *Citrus maxima* fruit in Thailand, which adds a fifth species to the citrus black spot complex. Previous morphological studies lumped many taxa under single names that represent complexes. In spite of this *Phyllosticta* is a species-rich genus, and many of these taxa need to be recollected in order to resolve their phylogeny and taxonomy.

Key words: *Botryosphaeriales*, foliar pathogens, *Guignardia*, *Phyllosticta*, *Phyllostictaceae,* Multi-Locus Sequence Typing (MLST), systematics.
Taxonomic novelties: New species – *Phyllosticta abieticola* Wikee & Crous, *P. aloeicola* Wikee & Crous, *P. citrimaxima* Wikee, Crous, K.D. Hyde & McKenzie, *P. leucothoicola* Wikee, Motohashi & Crous, *P. mangifera-indica* Wikee, Crous, K.D. Hyde & McKenzie, *P. neopyrolae* Wikee, Motohashi, Crous, K.D. Hyde & McKenzie, *P. pachysandricola* Wikee, Motohashi & Crous, *P. paxistimae* Wikee & Crous, *P. podocarpicola* Wikee, Crous, K.D. Hyde & McKenzie, *P. rhaphiolepidis* Wikee, C. Nakash. & Crous, *P. rubra* Wikee & Crous, *P. vacciniicola* Wikee, Crous, K.D. Hyde & McKenzie; **New combinations** – *P. foliorum* (Sacc.) Wikee & Crous, *P. philoprina* (Berk. & M.A. Curtis) Wikee & Crous; **Epitypifications (basionyms)** – *P. concentrica* Sacc., *P. cussoniae* Cejp, *P. owaniana* G. Winter; **Neotypifications (basionyms)** – *Phyllosticta cordylinophila* P.A. Young, *Physalospora gregaria* var. *foliorum* Sacc., *Sphaeropsis hypoglossi* Mont., *Sphaeropsis minima* Berk. & M.A. Curtis.

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INTRODUCTION

The genus *Phyllosticta* was introduced by Persoon (1818) with *P. convallariae* (nom. inval., lacking description) designated as the type species (Donk 1968), which is a synonym of *P. cruenta* (van der Aa 1973), which van der Aa & Vanev (2002) cited as type of the genus. Species of *Phyllosticta* are mostly plant pathogens of a broad range of hosts, and responsible for numerous diseases, including leaf and fruit spots. Some of these pathogens cause diseases of significant economic importance, e.g., *P. citricarpa,* the cause of citrus black spot, which is regarded as a quarantine pest in Europe and the USA (Baayen *et al.* 2002, Glienke *et al.* 2011). Other economically important plant pathogenic species include the *P. ampelicida* species complex that causes black rot disease on grapevines (Wicht *et al*. 2012), and the *P. musarum* species complex that causes banana freckle disease (Pu *et al*. 2008, Wong *et al.* 2012). Some species of *Phyllosticta* have also been isolated as endophytes from a wide range of hosts, e.g., *P. capitalensis*. Other species are regarded as saprobes, e.g., *P. carpogena* and *P. ericae* (van der Aa 1973, Baayen *et al*. 2002, van der Aa & Vanev 2002, Glienke *et al*. 2011, Wikee *et al*. 2011). Presently there are approximately 3 340 epithets known for *Phyllosticta* (www.MycoBank.org; accessed August 2013), but many of these reflect old concepts of the genus, and have since been accommodated elsewhere (van der Aa & Vanev 2002). Many species also produce spermatial or sexual states, which in some cases have been named in *Leptodothiorella* and *Guignardia*, respectively (van der Aa 1973).

For many years researchers have confused the generic circumscription of *Phoma* and *Phyllosticta*. Both genera were recognised as pycnidial fungi forming unicellular, hyaline conidia. Allescher (1898) separated the two genera based on the infected part of the plant part, with *Phyllosticta* as foliar pathogens, and *Phoma* on other plant parts. This concept was further refined by Grove (1935) who regarded *Phyllosticta* as a parasite and *Phoma* as saprobe or wound parasite. Seaver (1922) and Grove (1935) separated "*Phyllosticta*" species based on host preference, as was common taxonomic practice in the 20th century. Seaver (1922) described 300 species, and Grove (1935) approximately 150. In both cases the host plant was the main criterion on which species were separated. Indeed, Seaver's classification was largely characterised on spore size on host plants, while Grove arranged species under the alphabetically arranged host genera. Many *Phyllosticta* species were given specific epithets based on the host family, genus or species. For example, *P. iridis* on *Iris versicolor*

(*Iridaceae*), *P. eugeniae* on *Eugenia buxifolia* (*Myrtaceae*), *P. minor* on *Vinca minor* (*Apocynaceae*), etc. (Seaver 1922). For the plant pathogenic *Phyllosticta* species, separation based on host species (or sometimes genus) has proven to be a good method to distinguish species, but this does not hold true for the endophytic or saprobic species.

Viala & Ravaz (1892) introduced *Guignardia* as a replacement name for *Laestadia* Auersw. (1869), which was a later homonym of *Laestadia* Kunth ex Lessing (1832). Viala & Ravaz applied the name only to *Sphaeria bidwellii* (≡ *G. bidwellii*), a species that is different from *L. alnea*, the type species of *Laestadia* Auersw. (Bissett 1986). Petrak (1957) concluded that *G. bidwellii* and related species could be accommodated in *Botryosphaeria,* and Barr (1970, 1972) agreed with Petrak and placed *Guignardia* and *Phyllosticta* in *Botryosphaeria*, and other related species in *Discosphaerina*.

Punithalingam (1974) suggested that the genus *Guignardia* must be confined to only those taxa with *Phyllosticta* morphs as typified by *G. bidwellii* (= *P. ampelicida*, see Zhang *et al*. 2013). He stated that *Botryosphaeria* usually has larger ascomata and ascospores, and also a multilocular stroma, features that distinguish it from *Guignardia*. Van der Aa (1973) also pointed out that these two genera had different growth characteristics in culture. Following molecular studies, Schoch *et al.* (2006) placed *Phyllosticta* in the *Botryosphaeriales.* Since *Botryosphaeria* has been shown to be poly- and paraphyletic, numerous genera have been distinguished in the *Botryosphaeriaceae* (Crous *et al*. 2006, Phillips *et al*. 2008, Liu *et al.* 2012). With the increasing use of molecular data to link asexual and sexual morphs, and the end of dual nomenclature for fungi (Hawksworth *et al.* 2011, Wingfield *et al.* 2012), the oldest, more important and commonly used name, *Phyllosticta*, was chosen over that of *Guignardia* (Glienke *et al*. 2011, Sultan *et al*. 2011, Wikee *et al.* 2011, 2013, Wong *et al*. 2012).

The principal character by which a fungus is recognised as a species of *Phyllosticta* is by the production of pycnidia containing aseptate, hyaline conidia that are usually covered by a mucoid layer and bearing a single apical appendage (van der Aa 1973). However, the mucoid layer and appendage is not necessarily a universal feature, and some species such as *P. colocasiicola*, *P. minima*, and *P. sphaeropsoidea* lack these features. Furthermore, mucoid appendages formed on agar media may disappear with age, or vary in size and shape when the same isolate is compared on different media, e.g., pine needle agar, oatmeal agar, or potato dextrose agar. Presently *Phyllosticta* is circumscribed by pycnidia that are usually globose to subglobose, flattened above, and closely connected with the subepidermal pseudostroma. They are mostly unilocular but may be multilocular. The conidia are commonly hyaline, aseptate, ovoid, obovoid to ellipsoid, or short cylindrical, seldomly pyriform, globose or subglobose, and usually covered by a mucoid layer and bearing a single apical appendage (van der Aa 1973). The sexual morph is characterised by erumpent ascomata that are globose to pyriform in section, often irregularly shaped, unilocular, and with a central ostiole. The peridium is thin, comprising a few layers of angular cells. Asci are 8-spored, bitunicate, clavate to broadly ellipsoid, with a wide, obtusely rounded or slightly square apex, tapering gradually to a small pedicel, and with a well-developed ocular chamber. Ascospores are ellipsoid to limoniform, sometimes slightly elongated, aseptate, hyaline, often multiguttulate or with a large central guttule, and may have mucilaginous polar appendages or a sheath. A spermatial state may form in culture. Spermatia are hyaline, aseptate, cylindrical to dumbbell-shaped with guttules at each end (van der Aa 1973).

Phyllosticta s. str. was first monographed by van der Aa (1973), who described and illustrated 46 species, and listed the sexual morphs for 12 species, and the spermatial morphs for 17 based mostly on material collected in Europe and North America. More recently van der Aa & Vanev (2002) revised all species names described in *Phyllosticta,* and provided a list of 190 accepted epithets, as well as a second list of excluded names that indicated their current disposition if known.

In recent years DNA sequencing of conserved loci has vastly improved our knowledge of fungal phylogeny. Several studies have shown that phylogenetic analysis can resolve the taxonomy and identification of *Phyllosticta* spp. (Baayen *et al.* 2002, Wulandari *et al*. 2009, Glienke *et al*. 2011, Wikee *et al.* 2011). Indeed, new species of *Phyllosticta* are increasingly described based on molecular results (Crous *et al*. 2012, Wang *et al.* 2012, Su & Cai 2012, Wong *et al*. 2012, Zhang *et al*. 2012).

Phyllosticta was placed in the order *Botryosphaeriales* by Schoch *et al*. (2006), who proposed that the *Botryosphaeriaceae* contained both *Botryosphaeria* and *Phyllosticta*, although no support was obtained for this relationship. Crous *et al*. (2006) and Liu *et al*. (2012) also classified *Phyllosticta* in the *Botryosphaeriaceae*. In both studies it was noted that *Phyllosticta* was distinct from other genera in the *Botryosphaeriaceae*, and that these authors eventually expected it to be placed elsewhere. Seaver (1922) used the order *Phyllostictales* and family *Phyllostictaceae* for the genus *Phyllosticta*. The family name *Phyllostictaceae* (as Phyllostictei) was first proposed by Fries (1849) and accepted by Hawksworth & David (1989). This family name is still available for use, and we suggest that *Phyllosticta* again be placed in this family, which is sister to the *Botryosphaeriaceae* (*Botryosphaeriales*).

Although phylogenetic analysis has become a standard approach in fungal identification, phylogenetic studies should combine both molecular and morphological data to help discriminate taxa (Crous & Groenewald 2005, Hyde *et al*. 2010). Suitable type material that can be sequenced is not available for many species of fungi, and thus neo- or epitypification is required in order to create a stable and workable taxonomy. The objectives of this study are: (1) to clarify relationships among species of *Phyllosticta* using multi-gene sequence data [internal transcribed spacer region (ITS), translation elongation factor 1-α gene (TEF1), actin gene (ACT), 28S rRNA gene (LSU) and glyceraldehyde-3-phosphate dehydrogenase gene (GPDH)] combined with morphological characteristics; (2) to provide a phylogenetic backbone for the genus *Phyllosticta*, and (3) to designate neo- or epitype specimens for fungal isolates that correlate well with original type material, thereby fixing the genetic application of these names.

MATERIAL AND METHODS

Isolates

A global collection of 160 strains of *Phyllosticta* associated with both leaf spot diseases and healthy leaves of various host plants were studied (Table 1). All isolates were sequenced and analysed together with sequences downloaded from GenBank. If fruit bodies were present on diseased tissue then a single spore isolation procedure as described by Chomnunti *et al*. (2011) was used to obtain cultures. To obtain isolates of *Phyllosticta* from diseased leaves of host plants when fruit bodies were not present, the leaf surface was cleaned by wiping with 70 % ethanol. Small pieces of

leaf were then cut from the interface between healthy and diseased tissue. These were surface sterilised in 70 % ethanol, washed and plated onto ½ strength potato dextrose agar (½PDA). For isolation of endophytic species, healthy leaves were washed in tap water and wiped with 70 % ethanol. They were then cut into small pieces (about 1 × 1 cm), suspended in 70 % ethanol for 15 min (three times) and washed in distilled water (three times) before placing on ½PDA. All plates were incubated at 27 ºC for 1 wk and observed daily. The growing tips of hyphae of *Phyllosticta* colonies that developed were cut out and transferred to fresh PDA plates. Isolates are deposited in Mae Fah Luang University Culture Collection (MFLUCC) and in the working collection of Pedro Crous housed at the CBS-KNAW Fungal Biodiversity Centre (CBS), Utrecht, The Netherlands (CPC). Other fungal isolates of representative *Phyllosticta* spp. were obtained from the CBS and added to this study (Table 1).

Morphology

Growth rates, culture characteristics, and morphology of the isolates were determined at 27 °C. Sporulation was induced by growth on pine needle agar (PNA) (Smith *et al*. 1996) and synthetic nutrient-poor agar (SNA) under near UV-light. Colony colour and growth rate were established on PDA, malt extract agar (MEA) and oatmeal agar (OA) according to Crous *et al.* (2009). Culture characteristics were assessed, and the colour of upper and lower sides of cultures was determined after 14 d in the dark at 27 °C. Colony colour on MEA, OA and PDA was determined with the colour charts of Rayner (1970). Nomenclatural novelties and descriptions were deposited in MycoBank (www.mycobank.org; Crous *et al*. 2004).

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from cultures grown on MEA for 2–3 d using the UltraClean™ Microbial DNA isolation kits (Mo Bio Laboratories, Inc., California, USA) according to the manufacturer's protocol. Partial regions of five loci were amplified including actin (ACT) using primers ACT-512F and ACT-783R (Carbone & Kohn 1999); the internal transcribed spacer region (ITS) of the nuclear rDNA using primers V9G (de Hoog & Gerrits van den Ende 1998) and ITS4 (White *et al.* 1990), the 28S large subunit nrDNA (LSU) using primers LROR (Moncalvo *et al.* 1995) and LR5 (Vilgalys & Hester 1990); translation elongation factor 1-α using primers EF1-728F (Carbone & Kohn 1999) and EF2 (O'Donnell *et al.* 1998); and glyceraldehyde-3-phosphate dehydrogenase (GPDH) using primers Gpd1-LM and Gpd2-LM (Myllys *et al*. 2002). For *Phyllosticta citricarpa* isolates, GPDH was amplified using primers Gpd1 (Guerber *et al.* 2003) and GPDHR2 (Glienke *et al.* 2011). The PCR reaction mixtures and cycling conditions were followed as described by Glienke *et al.* (2011).

Amplified fragments were sequenced in both directions using the same primers pairs used for amplification. For this purpose, the BigDye Terminator Cycle Sequencing Kit v. 3.1 (Applied Biosystems™, Foster City, CA, USA) containing AmpliTaq DNA Polymerase was used. The amplified products were analysed on an automated 3730xl DNA analyzer (Life Technologies Europe BV, Applied Biosystems™, Bleiswijk, The Netherlands). Sequences generated were assembled and aligned using MEGA v. 5.05 (Tamura *et al*. 2011) and MAFFT v. 6 (http://mafft.cbrc.jp/alignment/server/), respectively. The sequences were manually aligned as necessary.

Molecular phylogeny

Phylogenetic analyses were based on both Maximum Parsimony (MP) and Bayesian inference (BI). The MP analyses were done using PAUP (Phylogenetic Analysis Using Parsimony, v. 4.0b10; Swofford 2003). Phylogenetic relationships were estimated by heuristic searches with 1 000 random addition sequences. Tree bisection-reconnection was used, with the branch swapping option set on "best trees" only with all characters weighted equally and alignment gaps treated as fifth state. Tree length (TL), consistency index (CI), retention index (RI) and rescaled consistence index (RC) were calculated for parsimony and bootstrap analysis (Hillis & Bull 1993) was based on 1 000 replications.

For BI, the best evolutionary models for each partition were determined using MrModeltest (Nylander 2004) and incorporated into the analyses. MrBayes v. 3.2.1. (Ronquist & Huelsenbeck 2003) was used to generate phylogenetic trees under optimal criteria per partition. A Markov Chain Monte Carlo (MCMC) algorithm of four chains was started in parallel from a random tree topology with the heating parameter set at 0.3. The MCMC analysis lasted until the average standard deviation of split frequencies came below 0.01 with trees saved every 1 000 generations. The first 25 % of saved trees were discarded as the "burn-in" phase and posterior probabilities (PP) determined from the remaining trees.

RESULTS

Phylogenetic relationships were determined for the ITS and ACT gene sequences of 160 *Phyllosticta* strains (including one outgroup). The combined partial dataset of *Phyllosticta* comprised 883 characters (including gaps), of which 341 characters are constant, and 150 characters are variable and parsimony-uninformative. Parsimony analysis generated 1 000 trees, one of which is presented as shown in Fig. 1 (TL = 2099, CI = 0.481, RI = 0.898, RC = 0.432). The phylogenetic tree of the ITS and ACT region resolved 46 clades (see Table 1 for details). The Bayesian consensus tree confirmed the tree topology and bootstrap support of the strict consensus tree obtained with MP.

A second analysis including all isolates for which a complete dataset were available (129 strains including the outgroup) was run based on ITS, LSU, ACT, TEF1 and GPDH (Table 1). The combined partial dataset of *Phyllosticta* comprised 2 577 characters (including gaps), of which 1 547 characters are constant, 296 characters are variable and parsimony-uninformative. Parsimony analysis generated 1 000 trees, of which one is shown in Fig. 2 (TL = 3173, CI = 0.517, RI = 0.906, RC = 0.468). The phylogenetic tree using combined multi-gene data resolved 33 clades (see Table 1 for details). The Bayesian consensus tree confirmed the tree topology and bootstrap support of the strict consensus tree obtained with MP.

Taxonomy

Phyllosticta is distinct from members of the *Botryosphaeriaceae* in cultural characteristics (slow growing, black erumpent colonies vs. grey, fluffy, fast-growing colonies). Morphologically it is also distinct, having conidia encased in a mucoid sheath and often with an apical appendage. The sexual morph has ascomata unilocular, ascospores frequently with mucoid caps, and hamathecial tissue disintegrating at maturity, which collectively differs from those in the *Botryosphaeriaceae*. *Phyllosticta* is also phylogenetically

Table 1. *Phyllosticta* isolates investigated in this study.

Species	Culture no.[1]	Host	Country	GenBank no.[2]				
				ITS	LSU	TEF1	ACT	GPDH
Botryosphaeria obtusa	CMW 8232	Conifers	South Africa	AY972105	-	DQ280419	AY972111	-
Guignardia mangiferae	CPC 17469	*Cymbidium* sp.	India	KF206189	-	-	KF289285	-
	IMI 260576	*Mangifera indica*	India	JF261459	KF206222	JF261501	JF343641	JF343748
	CPC 20260	*Arecaceae*	Thailand	KF206193	KF206243	KF289187	KF289294	KF289114
G. rhodorae	CBS 901.69	*Rhododendron* sp.	Netherlands	KF206174	KF206292	KF289230	KF289256	KF289166
Phyllosticta abieticola	**CBS 112067**	*Abies concolor*	Canada	KF170306	EU754193	-	KF289238	-
P. aloeicola	CPC 21020	*Aloe ferox*	South Africa	KF154280	KF206214	KF289193	KF289311	KF289124
	CPC 21021	*Aloe ferox*	South Africa	KF154281	KF206213	KF289194	KF289312	KF289125
	CPC 21022	*Aloe ferox*	South Africa	KF154282	KF206212	KF289195	KF289313	KF289126
	CPC 21023	*Aloe ferox*	South Africa	KF154283	KF206211	KF289196	KF289314	KF289127
	CPC 21024	*Aloe ferox*	South Africa	KF154284	KF206210	KF289197	KF289315	KF289128
P. beaumarisii	**CBS 535.87** = IMI 298910	*Muehlenbekia adpressa*	Australia	AY042927	KF306229	KF289170	KF306232	KF289074
P. bifrenariae	**CBS 128855** = VIC30556	*Bifrenaria harrisoniae*	Brazil	JF343565	KF206209	JF343586	JF343649	JF343744
	CPC 17467	*Bifrenaria harrisoniae*	Brazil	KF170299	KF206260	KF289207	KF289283	KF289138
P. brazillianiae	**CBS 126270** = LGMF330	*Mangifera indica*	Brazil	JF343572	KF206217	JF343593	JF343656	JF343758
	LGMF 333	*Mangifera indica*	Brazil	JF343574	KF206216	JF343595	JF343658	JF343760
	LGMF 334	*Mangifera indica*	Brazil	JF343566	KF206215	JF343587	JF343650	JF343752
P. capitalensis	CBS 173.77	*Citrus aurantiifolia*	New Zealand	KF206179	KF306231	FJ538393	KF289244	KF289100
	CBS 226.77	*Paphiopedilum callosum*	Germany	FJ538336	KF206289	FJ538394	FJ538452	JF343718
	CBS 356.52	*Ilex* sp.	Unknown	FJ538342	KF206300	FJ538400	FJ538458	KF289087
	CBS 100175	*Citrus* sp.	Brazil	FJ538320	KF206327	FJ538378	FJ538436	JF343699
	CBS 101228	*Nephelium lappaceum*	Hawaii	FJ538319	KF206325	FJ538377	FJ538435	KF289086
	CBS 114751	*Vaccinium* sp.	New Zealand	EU167584	EU167584	FJ538407	FJ538465	KF289088
	CBS 115046	*Myracrodruon urundeuva*	Brazil	FJ538322	KF206319	FJ538380	FJ538438	KF289082
	CBS 115047	*Aspidosperma polyneuron*	Brazil	FJ538323	KF206318	FJ538381	FJ538439	KF289077
	CBS 115049	*Bowdichia nitida*	Brazil	FJ538324	KF206317	FJ538382	FJ538440	KF289084
	CBS 117118	*Musa acuminata*	Indonesia	FJ538339	JQ743603	FJ538397	FJ538455	KF289090
	CBS 119720	*Musa acuminata*	Hawaii	KF206178	KF206316	FJ538398	KF289240	KF289098
	CBS 120428	*Sansevieria* sp.	Netherlands	JN692544	KF206315	JN692532	JN692520	JN692509
	CBS 123373	*Musa paradisiaca*	Thailand	FJ538341	JQ743604	FJ538399	FJ538457	JF343703
	CBS 123404	*Musa paradisiaca*	Thailand	FJ538333	JQ743601	FJ538391	FJ538449	KF289095
	CBS 128856	*Stanhopea* sp.	Brazil	JF261465	KF206304	JF261507	JF343647	JF343776
	CPC 11337	*Eucalyptus grandis*	Brazil	KF206180	-	-	KF289259	-
	CPC 11867	*Acacia crassicarpa*	Thailand	KF206181	KF206283	KF289184	KF289260	KF289108
	CPC 12157	*Acacia crassicarpa*	Thailand	KF206182	-	-	KF289261	-
	CPC 13987	*Protea repens*	Portugal	KF206183	KF206281	KF289176	KF289263	KF289083
	CPC 14609	*Zyzygium* sp.	Madagascar	KF206184	KF206280	KF289175	KF289264	KF289081
	CPC 16590	*Citrus limon*	Argentina	KF206185	KF206272	KF289177	KF289271	KF289091
	CPC 16591	*Citrus limon*	Argentina	KF206186	KF206271	KF289179	KF289272	KF289093
	CPC 16592	*Citrus limon*	Argentina	KF206187	KF206270	KF289178	KF289273	KF289092
	CPC 17468	*Cymbidium* sp.	Brazil	KF206188	KF206259	KF289189	KF289284	KF289120
	CPC 17748	*Heliconia* sp.	Thailand	KF206190	KF206258	KF289180	KF289286	KF289096
	CPC 18848	*Stanhopea graveolens*	Brazil	JF261465	KF206255	JF261507	KF289289	JF343776
	CPC 20251	Wild plant	Thailand	KC291333	KF206252	KC342553	KC342530	KF289101
	CPC 20252	*Punica granatum*	Thailand	KC291334	KF206251	KC342554	KC342531	KF289097
	CPC 20253	*Scheffera venulosa*	Thailand	KF206192	KF206250	KF289181	KF289293	KF289102
	CPC 20254	*Saccharum officinarum*	Thailand	KC291335	KF206249	KC342555	KC342532	KF289103
	CPC 20255	*Arecaceae*	Thailand	KC291336	KF206248	KC342556	KC342533	KF289115
	CPC 20256	*Ophiopogon japonicus*	Thailand	KC291337	KF206247	KC342557	KC342534	KF289089
	CPC 20257	*Ficus benjamina*	Thailand	KC291338	KF206246	KC342558	KC342535	KF289099

Table 1. (Continued).

Species	Culture no.[1]	Host	Country	GenBank no.[2]				
				ITS	LSU	TEF1	ACT	GPDH
	CPC 20258	*Ophiopogon japonicus*	Thailand	KC291339	KF206245	KC342559	KC342536	KF289094
	CPC 20259	*Orchidaceae*	Thailand	KC291340	KF206244	KC342560	KC342537	KF289104
	CPC 20263	*Magnoliaceae*	Thailand	KC291341	KF206241	KC342561	KC342538	KF289085
	CPC 20265	*Euphobiaceae*	Thailand	KF206194	KF206239	KF289182	KF289297	KF289105
	CPC 20266	*Polyscias* sp.	Thailand	KC291342	KF206238	KC342562	KC342539	KF289109
	CPC 20267	*Baccaurea ramiflora*	Thailand	KF206195	KF206237	KF289173	KF306233	KF289078
	CPC 20268	*Hibiscus syriacus*	Thailand	KC291343	KF206236	KC342563	KC342540	KF289117
	CPC 20269	*Ophiopogon japonicus*	Thailand	KC291344	KF206235	KC342564	KC342541	KF289118
	CPC 20270	*Tectona grandis*	Thailand	KC291345	KF206234	KC342565	KC342542	KF289110
	CPC 20271	*Crinum asiaticum*	Thailand	KF206196	KF206233	KF289183	KF289298	KF289106
	CPC 20272	*Orchidaceae*	Thailand	KC291346	KF206232	KC342566	KC342543	KF289079
	CPC 20274	*Mangifera indica*	Thailand	KF206197	KF206231	KF289188	KF289299	KF289119
	CPC 20275	*Polyalthia longifolia*	Thailand	KC291347	KF206230	KC342567	KC342544	KF289107
	CPC 20278	*Euphorbia milii*	Thailand	KC291348	KF206227	KC342568	KC342545	KF289113
	CPC 20423	*Philodendron* sp.	Thailand	KC291349	KF206226	KC342569	KC342546	KF289116
	CPC 20508	*Ixora chinensis*	Thailand	KF206198	KF206225	KF289185	KF289302	KF289111
	CPC 20509	*Cordyline fruticosa*	Thailand	KF206199	KF206224	KF289186	KF289303	KF289112
	CPC 20510	*Pyrrosia adnascens*	Thailand	KF206200	KF206223	KF289174	KF289304	KF289080
	CPC 21035	*Citrus* sp.		KF206201	-	-	KF289305	-
	LGMF 219	*Citrus sinensis*	Brazil	KF206202	KF206220	JF261490	KF289306	JF343737
	LGMF 220	*Citrus sinensis*	Brazil	KF206203	KF206219	JF261488	KF289307	JF343735
	LGMF 222	*Citrus sinensis*	Brazil	KF206204	KF206218	JF261492	KF289308	JF343739
P. citriasiana	**CBS 120486**	*Citrus maxima*	Thailand	FJ538360	KF206314	FJ538418	FJ538476	JF343686
	CBS 120487	*Citrus maxima*	China	FJ538361	KF206313	FJ538419	FJ538477	JF343687
	CBS 120488	*Citrus maxima*	Thailand	JN692545	KF206312	JN692533	JN692521	KF289144
	CBS 123370	*Citrus maxima*	Vietnam	FJ538355	KF206310	FJ538413	FJ538471	JF343689
	CBS 123371	*Citrus maxima*	Vietnam	FJ538356	KF206309	FJ538414	FJ538472	JF343690
	CBS 123372	*Citrus maxima*	Vietnam	FJ538357	KF206308	FJ538415	FJ538473	KF289145
P. citribraziliensis	**CBS 100098**	*Citrus limon*	Brazil	FJ538352	KF206221	FJ538410	FJ538468	JF343691
	CPC 17464	*Citrus* sp.	Brazil	KF170300	KF206263	KF289224	KF289280	KF289159
	CPC 17465	*Citrus* sp.	Brazil	KF170301	KF206262	KF289225	KF289281	KF289160
	CPC 17466	*Citrus* sp.	Brazil	KF170302	KF206261	KF289226	KF289282	KF289161
P. citricarpa	CBS 102374	*Citrus aurantium*	Brazil	FJ538313	KF206324	GU349053	FJ538429	JF343679
	CBS 120489	*Citrus sinensis*	Brazil	FJ538315	KF206311	FJ538373	FJ538431	KF289150
	CBS 127454	*Citrus limon*	Australia	JF343583	KF206306	JF343604	JF343667	JF343771
	CBS 127452	*Citrus reticulata*	Australia	JF343581	KF206307	JF343602	KF289241	JF343769
	CBS 127455	*Citrus sinensis*	Australia	JF343584	KF206305	JF343605	JF343668	JF343772
	CBS 122482	*Citrus sinensis*	Zimbabwe	FJ538317	KF306230	FJ538375	KF289265	KF289146
	CPC 16586	*Citrus limon*	Argentina	KF170293	KF206274	KF289220	KF289269	KF289155
	CPC 16587	*Citrus limon*	Argentina	KF170294	KF206273	KF289219	KF289270	KF289154
	CPC 16603	*Citrus limon*	Uruguay	KF170295	KF206269	KF289213	KF289274	KF289147
	CPC 16604	*Citrus limon*	Uruguay	KF206191	-	-	KF289292	-
	CPC 16605	*Citrus limon*	Uruguay	KF170296	KF206268	KF289214	KF289275	KF289148
	CPC 16606	*Citrus limon*	Uruguay	KF170297	KF206267	KF289215	KF289276	KF289149
	CPC 16609	*Citrus* sp.	Argentina	KF170298	KF206266	KF289217	KF289277	KF289152
	CPC 16149	*Citrus* sp.	Argentina	KF170290	KF206277	KF289216	KF289266	KF289151
	CPC 16151	*Citrus* sp.	South Africa	KF170291	KF206276	KF289221	KF289267	KF289156
	CPC 16152	*Citrus* sp.	South Africa	KF170292	KF206275	KF289218	KF289268	KF289153
P. citrichinaensis	**ZJUCC 200956**	*Citrus reticulata*	China	JN791620	-	JN791459	JN791533	-
	ZJUCC 200964	*Citrus maxima*	China	JN791611	-	JN791461	JN791535	-

Table 1. (Continued).

Species	Culture no.[1]	Host	Country	GenBank no.[2]				
				ITS	LSU	TEF1	ACT	GPDH
	ZJUCC 2010150	*Citrus maxima*	China	JN791662	-	JN791514	JN791582	-
	ZJUCC 2010152	*Citrus sinensis*	China	JN791664	-	JN791515	JN791589	-
P. citrimaxima	**CPC 20276** = MFLUCC10-0137 = CBS 136059	*Citrus maxima*	Thailand	KF170304	KF206229	KF289222	KF289300	KF289157
P. concentrica	**CBS 937.70**	*Hedera helix*	Italy	FJ538350	KF206291	FJ538408	KF289257	JF411745
	CBS 134749 = CPC 18842	*Hedera* sp.	Spain	KF170310	KF206256	KF289228	KF289288	KF289163
P. cordylinophila	CPC 21880 = MUCCJ 521	*Cordyline fruticosa*	Japan	AB454357	AB454357	-	AB704244	-
	CPC 20261 = MFLUCC10-0166 = WK024	*Cordyline fruticosa*	Thailand	KF170287	KF206242	KF289172	KF289295	KF289076
	CPC 20277 = MFLUCC12-0014 = WK048	*Cordyline fruticosa*	Thailand	KF170288	KF206228	KF289171	KF289301	KF289075
P. cornicola	CBS 111639	*Cornus florida*	USA	KF170307	-	-	KF289234	-
P. cussonia	CPC 13812	*Cussonia* sp.	South Africa	KF170311	KF206282	KF289223	KF289262	KF289158
	CPC 14873	*Cussonia* sp.	South Africa	JF343578	KF206279	JF343599	JF343662	JF343764
	CPC 14875	*Cussonia* sp.	South Africa	JF343579	KF206278	JF343600	JF343663	JF343765
P. elongata	**CBS 126.22**	*Oxycoccus macrocarpos*	USA	FJ538353	AB095508	FJ538411	FJ538469	KF289164
P. ericarum	**CBS 132534** = CPC 19744	*Erica gracilis*	South Africa	KF206170	KF206253	KF289227	KF28291	KF289162
P. eugeniae	CBS 445.82	*Eugenia aromatica*	Indonesia	AY042926	KF206288	KF289208	KF289246	KF289139
P. foliorum	CBS 174.77	*Cryptomeria japonica*	USA	KF170308	KF206290	KF289200	KF289245	KF289131
	CBS 447.68	*Taxus baccata*	Netherlands	KF170309	KF206287	KF289201	KF289247	KF289132
P. gaultheriae	**CBS 447.70**	*Gaultheria humifusa*	USA	JN692543	KF206298	JN692531	KF289248	JN692508
P. hamamelidis	MUCC 149	*Hamamelis japonica*	Japan	KF170289	-	-	KF289309	-
P. hostae	**CGMCC 3.14355**	*Hosta plantaginea*	China	JN692535	-	JN692523	JN692511	JN692503
	CGMCC 3.14356	*Hosta plantaginea*	China	JN692536	-	JN692524	JN692512	JN692504
	CGMCC 3.14357	*Hosta plantaginea*	China	JN692537	-	JN692525	JN692513	JN692505
P. hubeiensis	**CGMCC 3.14986**	*Viburnum odoratissimim*	China	JX025037	-	JX025042	JX025032	JX025027
	CGMCC 3.14987	*Viburnum odoratissimim*	China	JX025038	-	JX025043	JX025033	JX025028
	CGMCC 3.14988	*Viburnum odoratissimim*	China	JX025039	-	JX025044	JX025034	JX025029
P. hymenocallidicola	**CBS 131309**	*Hymenocallis littoralis*	Australia	JQ044423	JQ044443	KF289211	KF289242	KF289142
	CPC 19331	*Hymenocallis littoralis*	Australia	KF170303	KF206254	KF289212	KF289290	KF289143
P. hypoglossi	CBS 101.72	*Ruscus aculeatus*	Italy	FJ538365	KF206326	FJ538423	FJ538481	JF343694
	CBS 167.85	*Ruscus hypoglossum*	Italy	FJ538366	KF206302	FJ538424	FJ538482	JF343696
	CBS 434.92	*Ruscus aculeatus*	Italy	FJ538367	KF206299	FJ538425	FJ538483	JF343695
P. ilicis-aquifolii	**CGMCC 3.14358**	*Ilex aquifolium*	China	JN692538	-	JN692526	JN692514	-
	CGMCC 3.14359	*Ilex aquifolium*	China	JN692539	-	JN692527	JN692515	-
	CGMCC 3.14360	*Ilex aquifolium*	China	JN692540	-	JN692528	JN692516	-
P. leucothoicola	**MUCC 553 = CBS 136073**	*Leucothoe catesbaei*	Japan	AB454370	AB454370	-	KF289310	-
P. mangifera-indica	**CPC 20274** = MFLUCC10-0029	*Mangifera indica*	Thailand	KF170305	KF206240	KF289190	KF289296	KF289121
P. minima	**CBS 585.84** = IFO 32917	*Acer rubrum*	USA	KF206176	KF206286	KF289204	KF289249	KF289135
P. neopyrolae	**CPC 21879** = MUCC 125	*Pyrola asarifolia*	Japan	AB454318	AB454318	-	AB704233	-
P. owaniana	**CBS 776.97** = CPC 1009	*Brabejum stellatifolium*	South Africa	FJ538368	KF206293	FJ538426	KF289254	JF343767
	CPC 14901	*Brabejum stellatifolium*	South Africa	JF261462	KF206303	JF261504	KF289243	JF343766
P. pachysandricola	**MUCC 124** = NBRC 102276	*Pachysandra terminalis*	Japan	AB454317	AB454317	-	AB704232	-
P. paxistimae	**CBS 112527**	*Paxistima mysinites*	USA	KF206172	KF206320	KF289209	KF289239	KF289140
P. philoprina	CBS 587.69	*Ilex aquifolium*	Spain	KF154278	KF206297	KF289206	KF289250	KF289137
	CBS 616.72	*Ilex aquifolium*	Germany	KF154279	KF206296	KF289205	KF289251	KF289136
P. podocarpicola	**CBS 728.79**	*Podocarpus maki*	USA	KF206173	KF206295	KF289203	KF289252	KF289134

Table 1. (Continued).

Species	Culture no.[1]	Host	Country	GenBank no.[2]				
				ITS	LSU	TEF1	ACT	GPDH
P. podocarpi	CBS 111646	*Podocarpus falcatus*	South Africa	AF312013	KF206323	KC357671	KC357670	KF289169
	CBS 111647	*Podocarpus lanceolata*	South Africa	KF154276	KF206322	KF289232	KF289235	KF268168
P. pseudotsugae	CBS 111649	*Pseudotsuga menziesii*	USA	KF154277	KF206321	KF289231	KF289236	KF289167
P. rhaphiolepidis	**MUCC 432**	*Rhaphiolepis indica*	Japan	DQ632660	-	DQ632724	AB704242	-
P. rubra	**CBS 111635**	*Acer rubrum*	USA	KF206171	EU754194	KF289198	KF289233	KF289129
P. sphaeropsoidea	CBS 756.70 = IFO 32905	*Aesculus hippocastanum*	Germany	AY042934	KF206294	KF289202	KF289253	KF289133
P. spinarum	CBS 292.90	*Chamaecyparis pisifera*	France	JF343585	KF206301	JF343606	JF343669	JF343773
P. styracicola	**CGMCC 3.14985**	*Styrax gradiflorus*	China	JX052040	-	JX025045	JX025035	JX025030
	CGMCC 3.14989	*Styrax gradiflorus*	China	JX052041	-	JX025046	JX025036	JX025031
P. telopeae	**CBS 777.97**	*Telopea speciosissima*	Tasmania	KF206205	KF206285	KF289210	KF289255	KF289141
P. vacciniicola	**CPC 18590**	*Vaccinium macrocarpum*	USA	KF170312	KF206257	KF289229	KF289287	KF289165
P. yuccae	CBS 112065	*Yucca elephantipes*	USA	KF206175	-	-	KF289237	-
Phyllosticta sp.	CPC 11336	*Eucalyptus globulus*	Spain	KF206177	KF206284	KF289199	KF289258	KF289130
	MUCC 147	*Rhododendron keiskei*	Japan	AB454319	AB454319	-	AB704234	-
	CPC 17454	*Mangifera indica*	Brazil	KF206206	KF206265	KF289192	KF289278	KF289123
	CPC 17455	*Mangifera indica*	Brazil	KF206207	KF206264	KF289191	KF289279	KF289122

[1]CPC: Culture collection of P.W. Crous, housed at CBS; IFO: Institute For Fermentation, Osaka, Japan; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.; LGMF: Culture collection of Laboratory of Genetics of Microorganisms, Federal University of Parana, Curitiba, Brazil; CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands; ZJUCC: Zhejiang University Culture Collection, China; MFLUCC: Mae Fah Luang University Culture Collection; CGMCC: China, General Microbiological Culture Collection, Beijing, China; MUCC: Culture Collection, Laboratory of Plant Pathology, Mie University, Tsu, Mie prefecture, Japan. Type and ex-type cultures are in bold.
[2]ITS: Internal transcribed spacers 1 and 2 together with 5.8S nrDNA; LSU: large subunit 28S nrDNA; TEF1: partial translation elongation factor 1-α gene; ACT: partial actin gene; GPDH: partial glyceraldehyde-3-phosphate dehydrogenase gene.

supported as distinct from members of the *Botryosphaeriaceae* (see Slippers *et al*. 2013, this volume), we choose to place it in the *Phyllostictaceae* that was originally erected to accommodate this genus.

Phyllostictaceae Fr. (as "Phyllostictei"), Summa veg. Scand., Section Post. (Stockholm): 420. 1849.

Foliicolous, plant pathogenic, endophytic or saprobic. *Ascomata* pseudothecial, separate to gregarious, globose, brown to black, with a central ostiole. *Asci* bitunicate, fissitunicate, clavate to subcylindrical, 8-spored, fasciculate, stipitate, with an ocular chamber. *Pseudoparaphyses* mostly absent at maturity (see Sultan *et al*. 2013), filamentous, branched, septate when present. *Ascospores* bi- to triseriate, hyaline, aseptate, ellipsoid-fusoid to limoniform, smooth-walled, usually with mucilaginous caps at ends, or surrounded by a mucilaginous sheath. *Asexual morph*: *Conidiomata* pycnidial globose, dark brown, separate to aggregated, with a central ostiole; wall of 3–6 layers of brown *textura angularis*. *Conidiogenous cells* lining the inner wall, hyaline, smooth, subcylindrical to ampulliform or doliiform, proliferating percurrently near apex, frequently covered in mucilaginous sheath. *Conidia* hyaline, smooth, ellipsoid-fusoid to obovoid or ovoid, aseptate, smooth-walled, guttulate or granular, frequently surrounded by a mucilaginous sheath, and bearing a single mucilaginous apical appendage.

Type genus: *Phyllosticta* Pers.

Phyllosticta Pers., Traité sur les Champignons Comestibles (Paris): 55. 147. 1818.

Conidiomata and *spermatogonia* pycnidial, immersed, subepidermal to erumpent, unilocular, rarely multilocular, glabrous, ostiolate, dark brown to black; ostiole circular to oval; pycnidial wall of thick-walled, dark brown *textura angularis*, with inner layers of hyaline to pale brown, thin-walled *textura prismatica* to *angularis*. *Conidiophores* lining the cavity of the conidioma, reduced to conidiogenous cells, invested in mucus. *Conidiogenous cells* discrete, producing macroconidia and spermatia (also produced in separate spermatogonia), ampulliform, lageniform, doliiform to subcylindrical, hyaline, smooth, proliferating several times percurrently near the apex, invested in a mucoid layer. *Spermatogenous cells* ampulliform to lageniform or subcylindrical, hyaline smooth, phialidic. *Conidia* ellipsoid-fusoid to obovoid or ovoid, rarely subcylindrical, aseptate, broadly rounded at the apex, often tapering strongly toward the base, unicellular, hyaline, smooth-walled, guttulate to granular, often enclosed in a persistent mucilaginous sheath, and bearing an unbranched, tapering, straight to curved, mucoid apical appendage. *Spermatia* hyaline, smooth, granular, subcylindrical or dumbbell-shaped, with rounded or blunt ends. *Ascomata* pseudothecial, separate to gregarious, globose to subglobose, brown to black, unilocular with a central ostiole. *Asci* bitunicate, fissitunicate, clavate to subcylindrical, 8-spored, fasciculate, stipitate, with an ocular chamber. *Pseudoparaphyses* mostly absent at maturity, filamentous, branched, septate when present. *Ascospores* bi- to triseriate, hyaline, guttulate to granular, aseptate, ellipsoid, ellipsoid-fusoid to limoniform, smooth-walled, usually with mucilaginous caps at ends, or surrounded by a mucilaginous sheath.

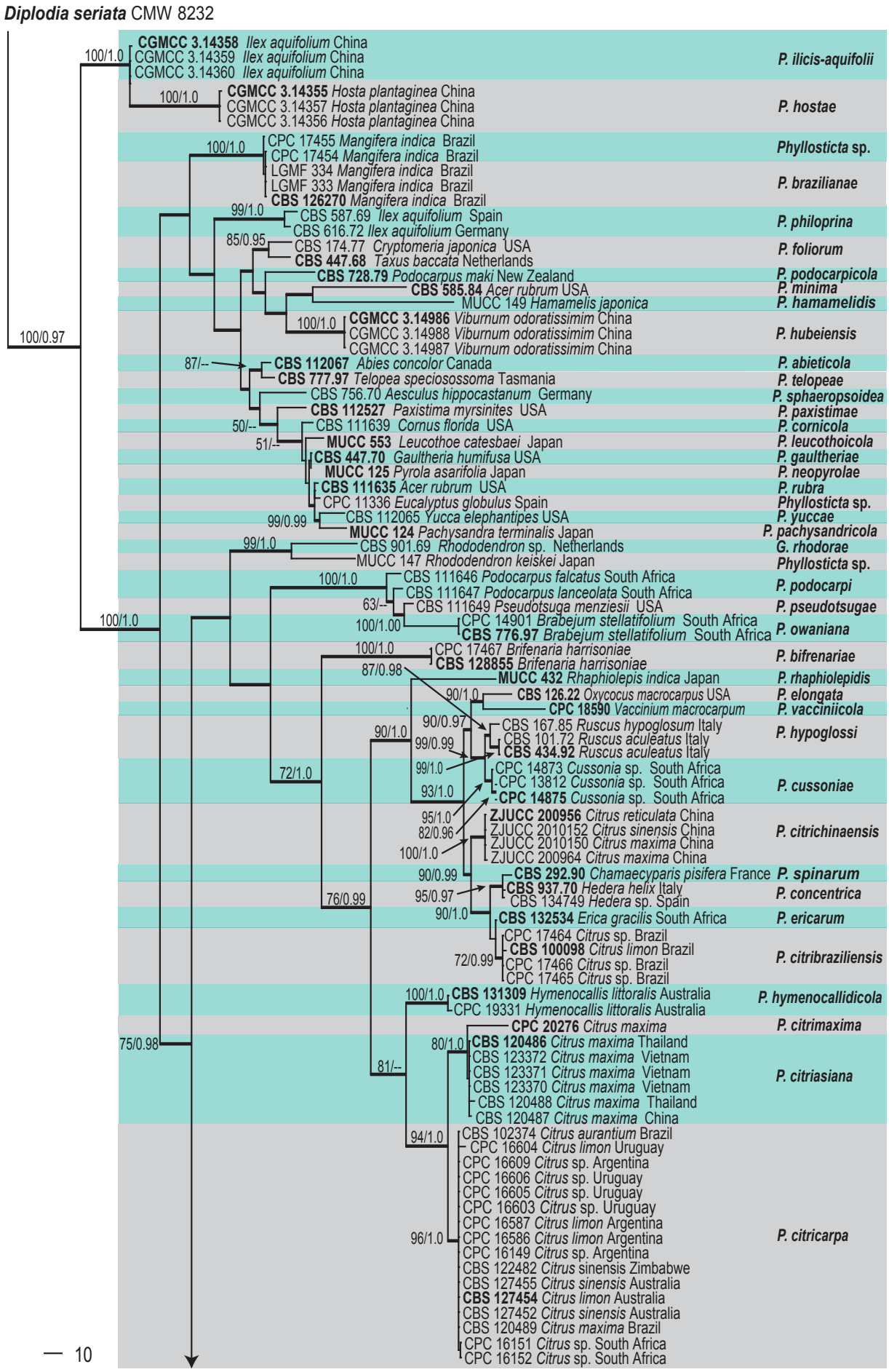

Fig. 1. One of 1 000 equally most parsimonious trees obtained from a heuristic search with 1 000 random taxon additions of the combined ACT and ITS sequence alignments. Bootstrap support values and Bayesian posterior probability values are indicated at the nodes. Branches present in both the consensus trees of the MP and BI are thickened. Substrate and country of origin, where known, are indicated next to the strain numbers. The tree was rooted to *Diplodia seriata* (CMW 8232)

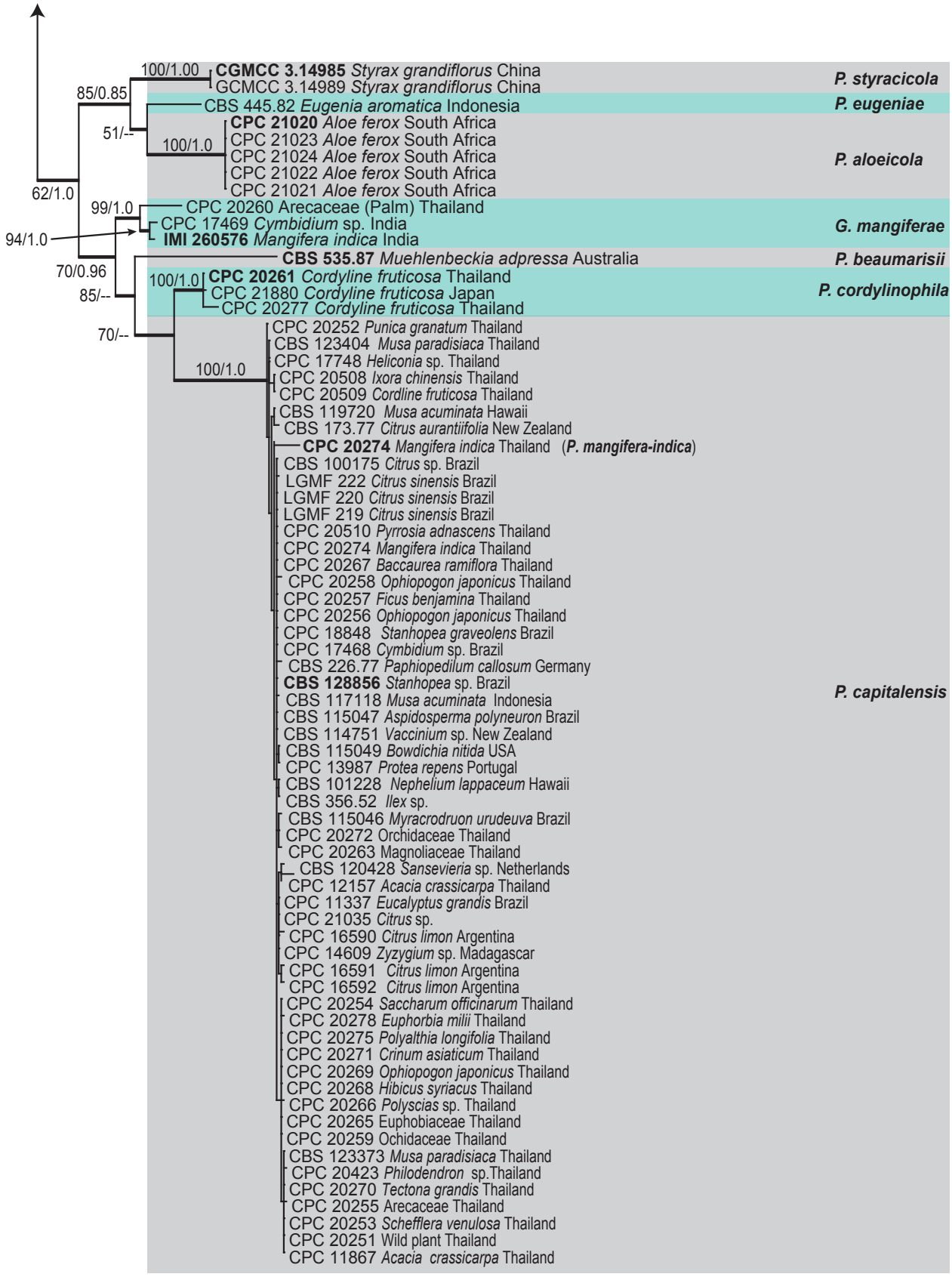

Fig. 1. (Continued).

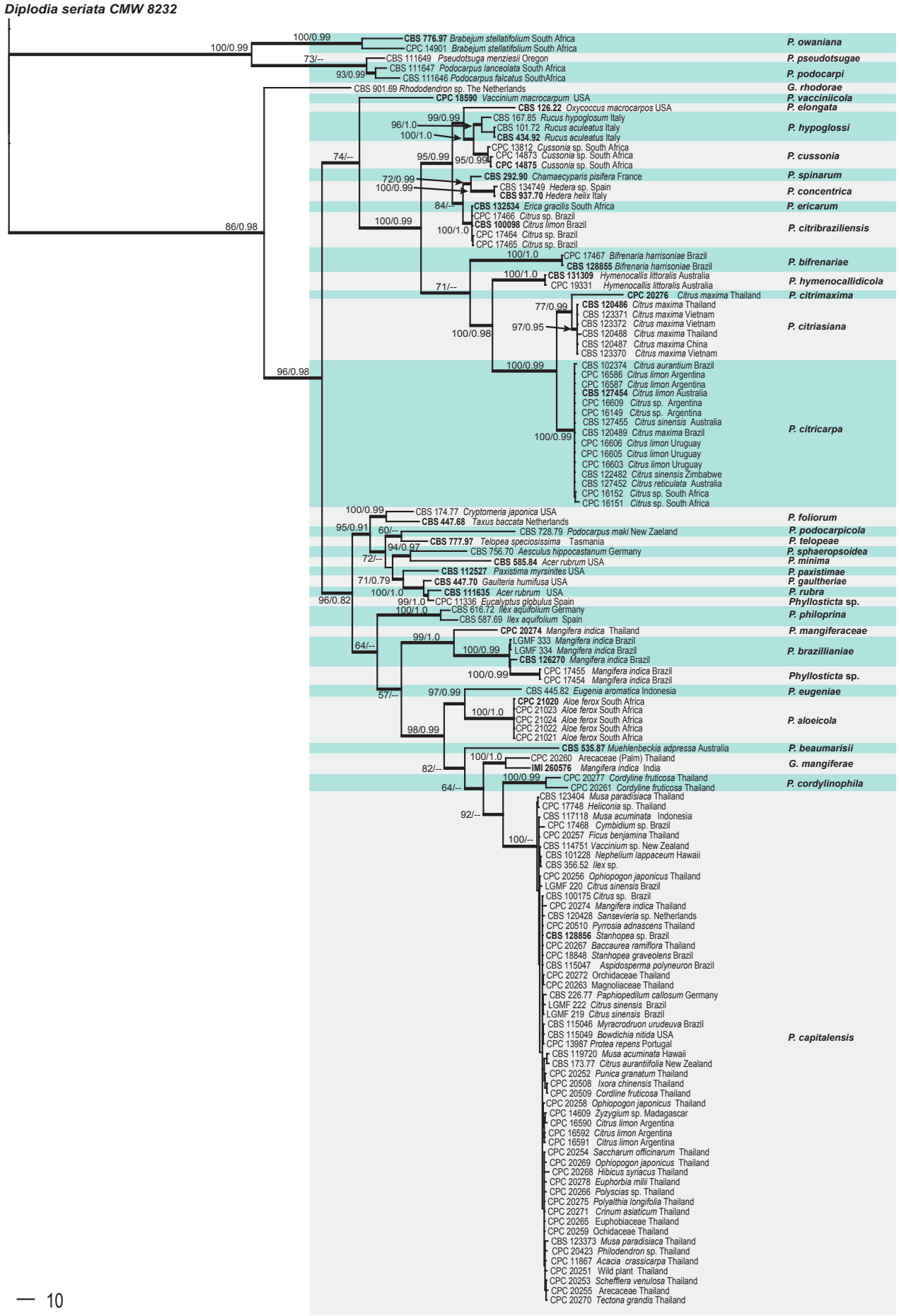

Fig. 2. One of 1 000 equally most parsimonious trees obtained from a heuristic search with 1 000 random taxon additions of the combined ACT, GPDH, ITS, LSU and TEF1 sequence alignments. Bootstrap support values and Bayesian posterior probability values are indicated at the nodes. Branches present in both the consensus trees of the MP and BI are thickened. Substrate and country of origin, where known, are indicated next to the strain numbers. The tree was rooted to *Diplodia seriata* (CMW 8232).

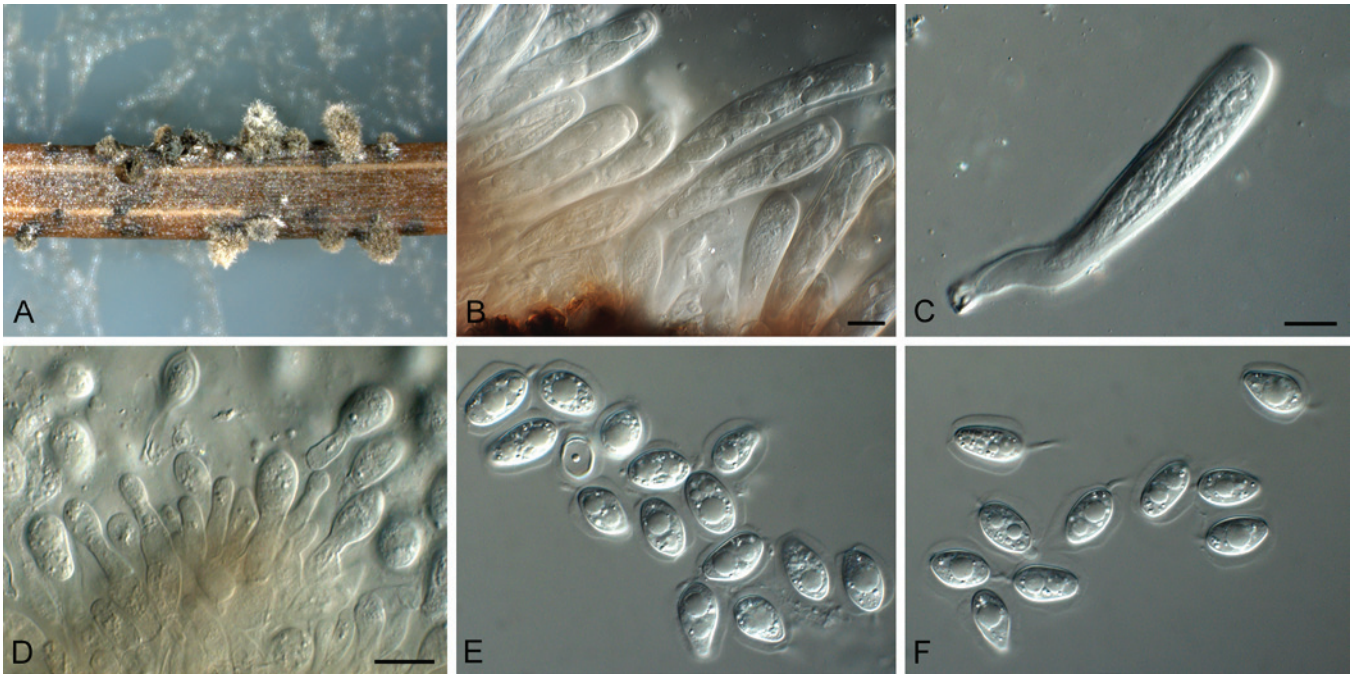

Fig. 3. *Phyllosticta abieticola* (CBS 112067). A. Conidiomata and ascomata forming on PNA. B, C. Asci with ascospores. D. Conidiogenous cells giving rise to conidia. E, F. Conidia with mucoid sheaths and apical appendages. Scale bars = 10 µm.

Type species: *P. convallariae* Pers., nom. inval. (= *P. cruenta* (Fr.) J. Kickx f.)

Phyllosticta abieticola Wikee & Crous, **sp. nov.** MycoBank MB805654. Fig. 3.

Etymology: Named after the host genus from which it was collected, *Abies.*

Conidiomata (on PNA) pycnidial, solitary, black, erumpent, globose, exuding colourless conidial masses; pycnidia up to 250 µm diam, elongated in culture on PNA; pycnidial wall of several layers of *textura angularis*, up to 30 µm thick; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 15 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1 supporting cell, that can be branched at the base, 10–25 × 4–6 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 7–15 × 3–5 µm; proliferating several times percurrently near apex. *Conidia* (11–)13–16(–18) × (7–)8 µm, solitary, hyaline, aseptate, thin and smooth-walled, granular, or with a single large central guttule, fusoid-ellipsoid, tapering towards a narrow truncate base, 2–3 µm diam, enclosed in a thin persistent mucoid sheath, 3–4 µm thick, and bearing a hyaline, apical mucoid appendage, (15–)20–25(–30) × 1.5(–2) µm, flexible, unbranched, tapering towards an acutely rounded tip. *Ascomata* similar to conidiomata in general anatomy. *Asci* bitunicate, hyaline, clavate to broadly fusoid-ellipsoid, with visible apical chamber, 2 µm diam, 65–120 × 12–15 µm. *Ascospores* bi- to multiseriate, hyaline, smooth, granular to guttulate, aseptate, straight, rarely curved, widest in the middle, limoniform with obtuse ends, (15–)16–18(–20) × (6–)7 µm.

Culture characteristics: Colonies erumpent, spreading with moderate aerial mycelium, covering dish after 1 mo at 25 ºC. On OA surface iron-grey. On PDA and MEA surface grey-olivaceous, reverse iron-grey.

Specimen examined. **Canada**, on living leaf of *Abies concolor*, Jan. 2001, M. Forve (**holotype** CBS H-21389, ex-type culture CBS 112067).

Notes: The present isolate of *P. abieticola* was originally identified as *P. abietis*, which is distinguished by having smaller conidia (7–12 × 6.5–9 µm), and a sheath up to 1.5 µm wide, with apical appendages up to 2.5 µm long when present (Bissett & Palm 1989).

Phyllosticta aloeicola Wikee & Crous, **sp. nov.** MycoBank MB805655. Fig. 4.

Etymology: Named after the host genus from which it was collected, *Aloe*.

Associated with leaf tip blight. *Conidiomata* (on PNA) pycnidial, solitary, black, erumpent, globose, exuding colourless conidial masses; pycnidia up to 250 µm diam; pycnidial wall of several layers of *textura angularis*, up to 40 µm thick; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 20 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 5–13 × 3–4 µm; proliferating several times percurrently near apex. *Conidia* (8–)14–18(–27) × (6.5–) 7–8(–9) µm, solitary, hyaline, aseptate, thin and smooth walled, granular, or with a single large central guttule, ellipsoid to obovoid or subcylindrical, tapering towards a narrow truncate base, 3–5 µm diam, enclosed in a thin, persistent mucoid sheath, 1–2 µm thick, and bearing a hyaline, apical mucoid appendage, (7–)15–20(–23) × 2–3(–3.5) µm, flexible, unbranched, tapering towards an acutely rounded tip.

Culture characteristics: Colonies erumpent, spreading, with sparse aerial mycelium and feathery margins, covering the dish in 1 mo. On MEA surface olivaceous-grey, reverse iron-grey; on OA and PDA iron-grey on surface and reverse.

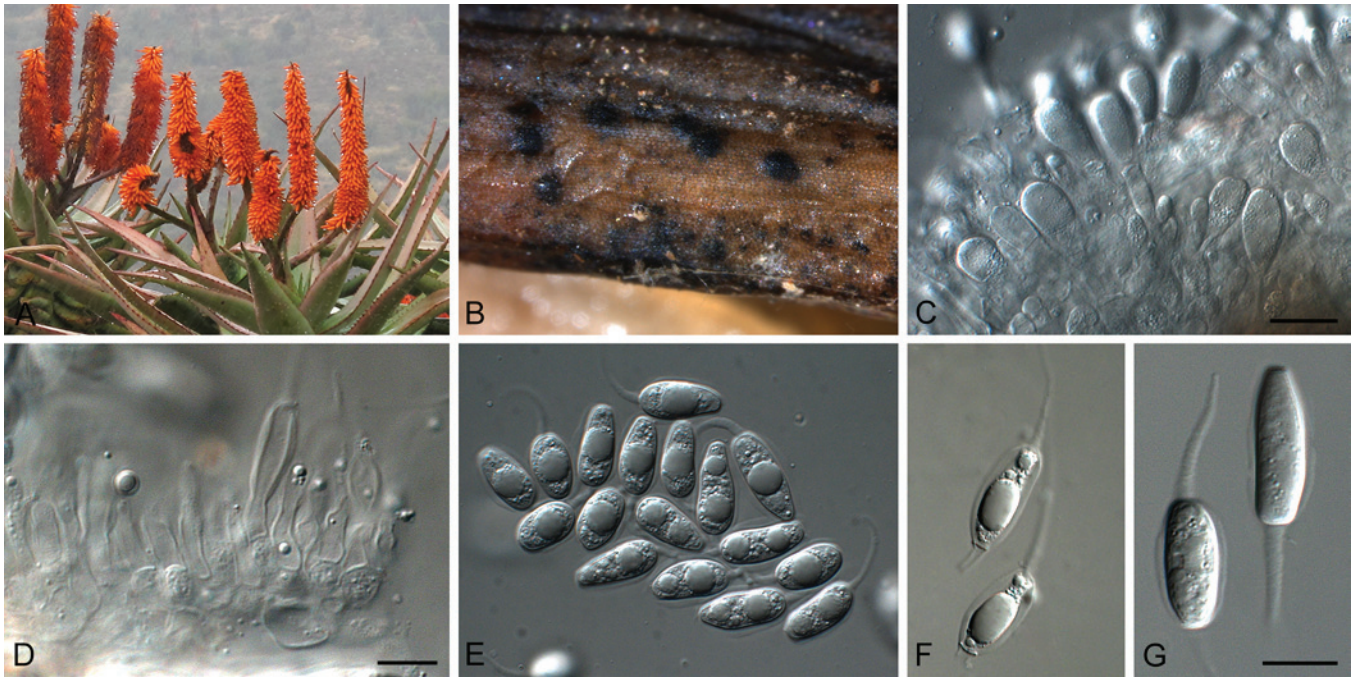

Fig. 4. *Phyllosticta aloeicola* (CPC 20677). A. *Aloe* with dead leaf tips that harbour *P. aloeicola*. B. Immersed conidiomata on leaf tissue. C, D. Conidiogenous cells giving rise to conidia. E–G. Conidia. Scale bars = 10 µm.

Specimen examined: **South Africa**, Free State Province, Bloemfontein Botanical Garden, Bloemfontein, on living leaf of *Aloe ferox*, 7 May 2012, P.W. Crous & W.J. Swart (**holotype** CBS H-21390, culture ex-type CPC 21020 = CBS 136058).

Notes: *Phyllosticta aloeicola* and *P. aloës* were both isolated from *Aloe latifolia* in South Africa. Van der Aa & Vanev (2002) examined the type specimen of *P. aloës* (deposited in B), and concluded that it was either a *Phoma* or *Asteromella* sp.

Phyllosticta citrimaxima Wikee, Crous, K.D. Hyde & McKenzie, **sp. nov.** MycoBank MB803675. Fig. 5.

Etymology: Named after this host on which it occurs, *Citrus maxima*.

Conidiomata pycnidial (on PNA), forming after 4 d of incubation, black, superficial, globose, 150–160 × 120–130 µm; wall 1–3 layers, 20–30 µm thick. *Conidiogenous cells* developing after 5 d, lining wall of pycnidium, phialidic, cylindrical, hyaline, 3–5 × 1–2 µm. *Conidia* ellipsoidal, hyaline, 1-celled, smooth, 5(–8) × (3–)4(–7) µm, surrounded by mucilaginous sheath, 1 µm thick, bearing a single, apical appendage, 2–16 µm long.

Culture characteristics: On OA, colonies flat, with irregular margin, initially hyaline with abundant mycelium, gradually becoming greenish after 2–3 d. On MEA colonies woolly, irregular, initially white with abundant mycelium, gradually becoming greenish to dark green after 2–3 d with white hyphae on the undulate margin, eventually turning black; reverse dark green to black. After 25 d in the dark at 27 ºC the colony covered the whole plate. On PDA, colonies were flat, rather fast growing, initially white with abundant mycelium, gradually becoming greenish to dark green after 2–3 d, with white hyphae at the margin, eventually turning black; reverse black and after 14 d in the dark at 27 ºC colony covered the whole plate.

Specimen examined: **Thailand**, Chiangrai, Weing Khaen, on fruit peel of *Citrus maxima*, Jun. 2011, S. Wikee (**holotype** MFLU 13-0001, ex-type culture CPC 20276 = MFLUCC10-0137 = CBS 136059).

Notes: *Phyllosticta citrimaxima* was isolated from tan spots on the fruit surface of *Citrus maxima*, which is grown as an economically important crop in Thailand and Asia. Recently, *P. citriasiana*, and *P. citrichinaensis* were described from *Citrus maxima* in Vietnam and China (Wulandari *et al.* 2009, Wang *et al*. 2012), and *P. citribraziliensis* from Brazil (Glienke *et al*. 2011). *Phyllosticta citrimaxima* is well supported phylogenetically (Fig. 1). Wang *et al.* (2012) provided a table in which they compared the morphology of five *Phyllosticta* species associated with citrus: *P. citricarpa*, *P. citriasiana*, *P. capitalensis*, *P. citribraziliensis*, and *P. citrichinaensis*. *Phyllosticta citrimaxima* produces smaller conidia (5–8 × 3–7 µm) than *P. citricarpa* (11–12 × 6–8 µm), *P. citriasiana* (12–14 × 6–7 µm), *P. capitalensis* (11–12 × 6–7 µm), *P. citribraziliensis* (10–12 × 6–7 µm) and *P. citrichinaensis* (8–12 × 6–9 µm), and has longer apical appendages (2–16 µm) than any of these four species, except *P. citrichinaensis* (14–26 µm).

Phyllosticta concentrica Sacc., Nuovo Giorn. Bot. Ital. 8: 203. 1876. Fig. 6.

Conidiomata (on PNA) pycnidial, solitary, black, erumpent, globose, exuding colourless conidial masses; pycnidia up to 400 µm diam, elongated in culture on PNA; pycnidial wall of several layers of *textura angularis*, up to 30 µm thick; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 25 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1 supporting cell, that gives rise to 1–2 conidiogenous cells, 12–20 × 4–6 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 7–10 × 3–6 µm; proliferating several times percurrently near apex. *Conidia* (10–)11–13(–14) × (6–)8(–9) µm, solitary, hyaline, aseptate, thin and smooth-walled,

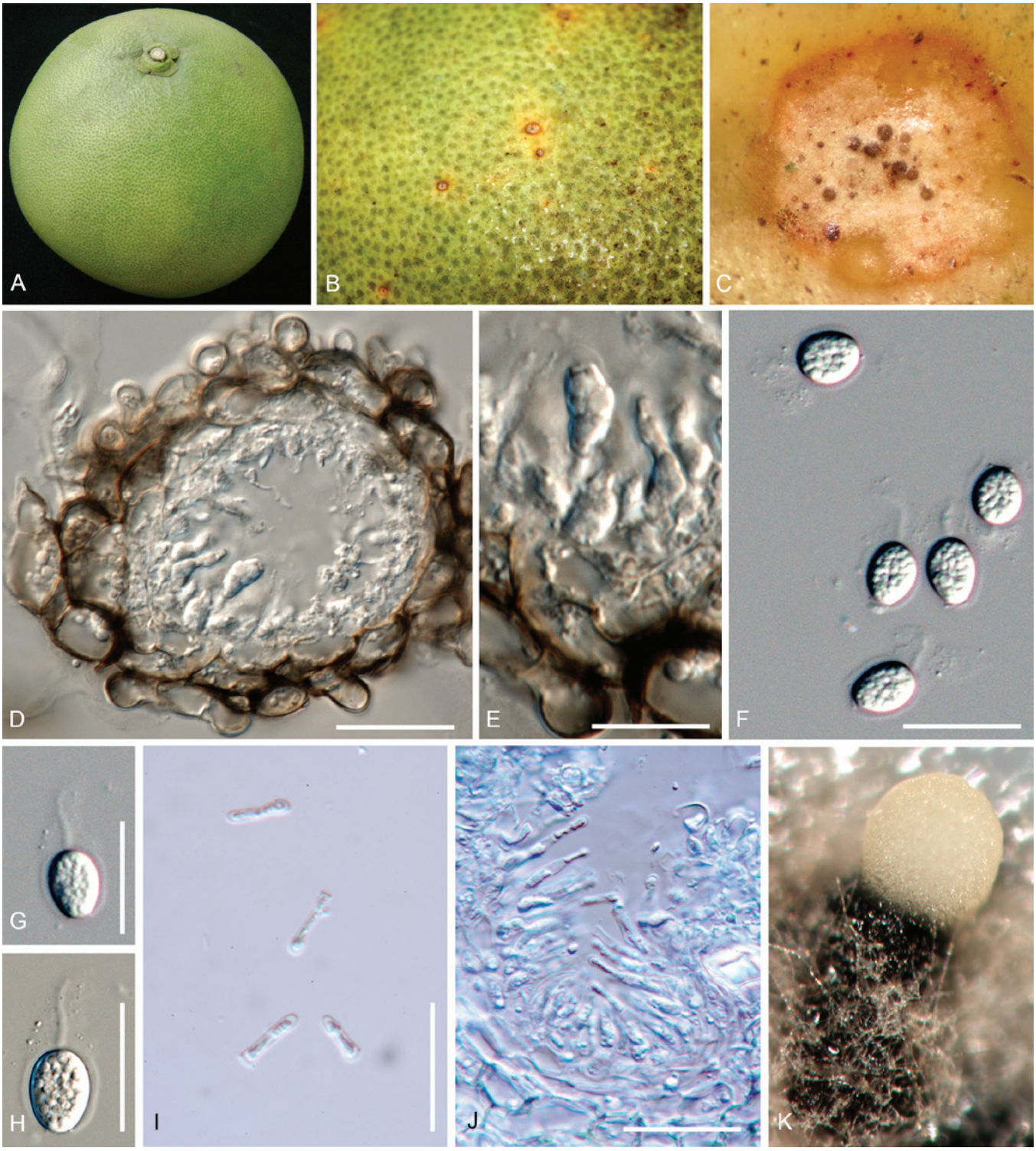

Fig. 5. *Phyllosticta citrimaxima* (CPC 20276). A–C. Symtoms on host. D, E. Vertical section through conidioma showing developing conidia. F–H.Conidia. I, J. Spermatial state, spermogonium. K. Conidia produced on OA. Scale bars: D = 30 µm; E–J = 10 µm.

granular, or with a single large central guttule, ellipsoid, tapering towards a narrow truncate base, 2–3 µm diam, enclosed in a thin persistent mucoid sheath, 1–2 µm thick, and bearing a hyaline, apical mucoid appendage, (5–)8–12(–15) × (1–)1.5 µm, flexible, unbranched, tapering towards an acutely rounded tip.

Culture characteristics: Colonies flat, spreading with sparse aerial mycelium, and feathery, lobate margins, reaching 30 mm after 2 wk at 25 ºC. On PDA surface greenish black, reverse iron-grey; on OA surface iron-grey; on MEA surface olivaceous-grey in centre, pale olivaceous-grey in outer region, olivaceous-grey underneath.

Specimens examined. **Italy**, Padua, on withering leaves of *Hedera helix*, Jul. 1875, **syntype** (L); Sardegna, Cologne near Oleina, leaf litter of *Hedera helix*, 31 Aug. 1970, W. Gams (**epitype designated here** CBS H-16992, culture ex-epitype CBS 937.70; MBT176244). **Spain**, on living leaf of *Hedera* sp., 10 Jul. 2010, U. Damm, culture CPC 18842 = CBS 134749.

Notes: *Phyllosticta concentrica*, and its purported sexual state, *Guignardia philoprina*, represent different taxa, with each name representing a species complex for which numerous old names are available. *Phyllosticta concentrica* was originally introduced by Saccardo for a species occurring on *Hedera helix* in Italy, but which appears to be common in Europe on this host. The present

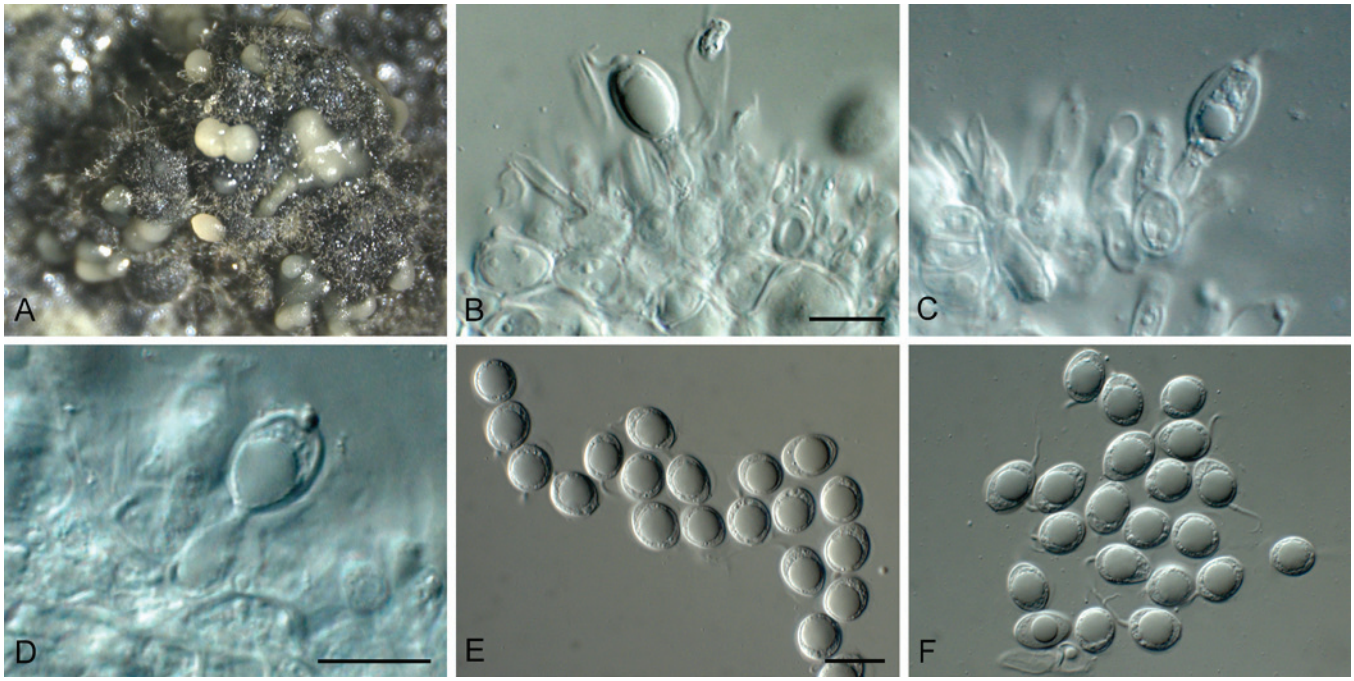

Fig. 6. *Phyllosticta concentrica* (CBS 937.70). A. Conidiomata sporulating on OA. B–D. Conidiogenous cells giving rise to conidia. E, F. Conidia. Scale bars = 10 µm.

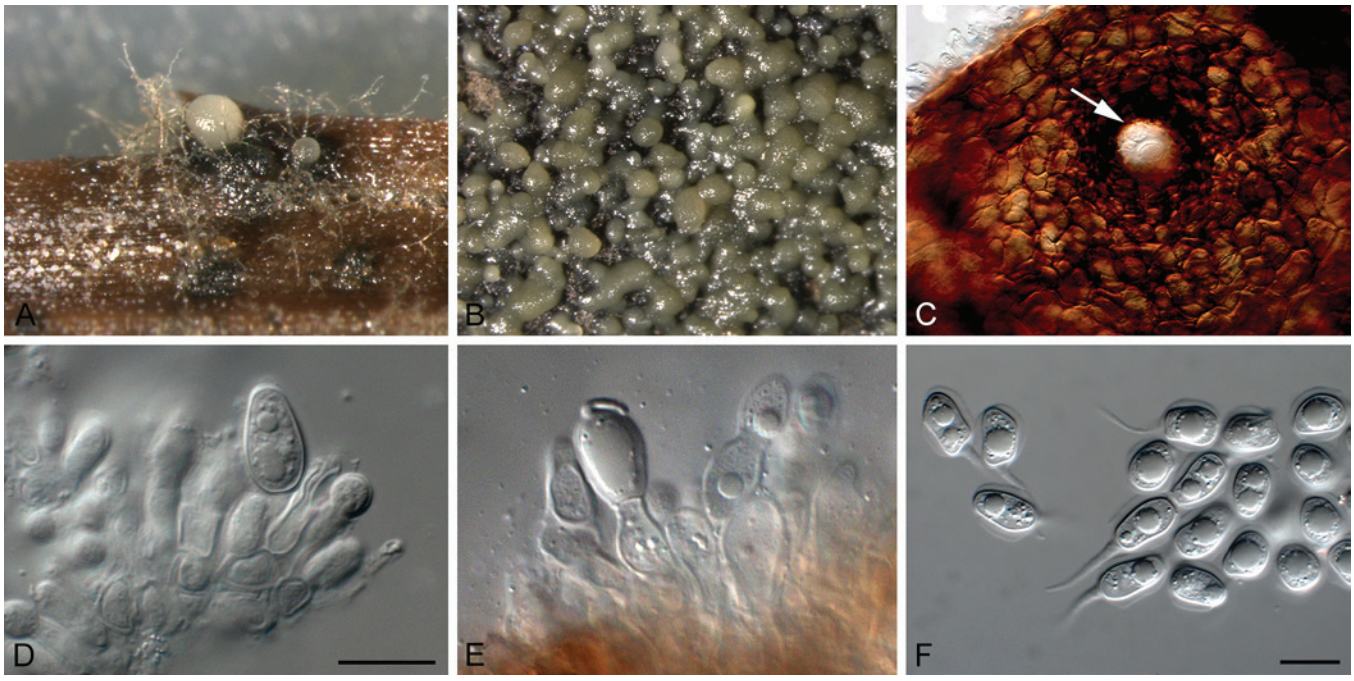

Fig. 7. *Phyllosticta cordylinophila* (CPC 20261). A. Conidiomata sporulating on PNA. B. Conidiomata sporulating on OA. C. Conidioma with ostiole (arrowed). D, E. Conidiogenous cells giving rise to conidia. F. Conidia. Scale bars = 10 µm.

collection closely matches the original description of *P. concentrica* in morphology, for which an epitype is designated.

Phyllosticta cordylinophila P.A. Young, Bulletin of the Bernice P. Bishop Museum, Honolulu, Hawaii 19: 133. 1925. Fig. 7.

Conidiomata (on PNA) pycnidial, solitary, black, erumpent, globose, exuding colourless to opaque conidial masses; pycnidia up to 200 µm diam; pycnidial wall of 3–6 layers of *textura angularis*, up to 40 µm thick; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 20 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1 supporting cell, at times branched at base, 10–20 × 4–6 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 10–17 × 3–6 µm; proliferating several times percurrently near apex. *Conidia* (10–)11–13(–15) × 7–8(–11) µm, solitary, hyaline, aseptate, thin and smooth walled, coarsely guttulate, or with a single large central guttule, ellipsoid to obovoid, tapering towards a narrow truncate base, 2–3 µm diam, enclosed in a thin, persistent mucoid sheath, 1–2 µm thick, and bearing a hyaline, apical mucoid appendage, (10–)20–35(–40) × 2(–3) µm, flexible, unbranched, tapering towards an acutely rounded tip.

Culture characteristics: Colonies spreading, erumpent, with sparse aerial mycelium and even, smooth margins. On MEA surface pale olivaceous-grey in centre, dirty white in outer region, reverse iron-

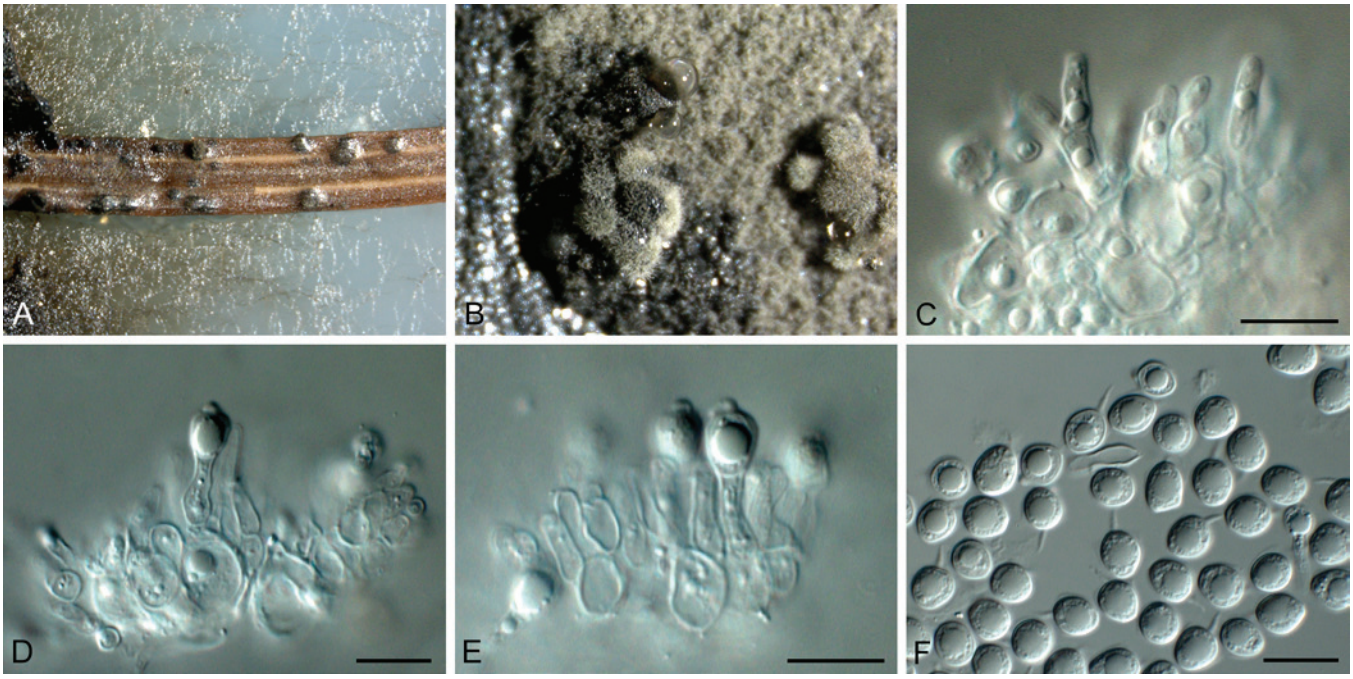

Fig. 8. *Phyllosticta cornicola* (CBS 111639). A. Conidiomata sporulating on PNA. B. Conidiomata forming on OA. C–E. Conidiophores giving rise to conidia. F. Conidia. Scale bars = 10 µm.

grey; on OA olivaceous-grey; on PDA olivaceous-grey on surface and reverse.

Specimens examined: **Thailand**, Chiangrai, Nang lae, Pasang, on leaf spot of *Cordyline fruticosa*, Nov. 2011, S. Wikee (**neotype designated here** CBS H-21391, ex-neotype culture CPC 20261 = WK024 = CBS 136244; MBT176245). **Japan**, Kagoshima, Amami-Ohshima, Amagi, on *C. fruticosa*, 22 Oct. 2003, Y. Ono & T. Kobayashi, culture ex-type MUCCJ 521 = CPC 21880 = CBS 136072.

Notes: Van der Aa (1973) did not locate type material, and the material studied by Petrak & Sydow (1927) was depauperate. As the present collections match the morphology of the original species description [conidia ellipsoid to ovoid, 7–12(–15) × 5–7.5(–8) µm], we herewith designate one specimen as neotype.

Phyllosticta cornicola (DC.) Rabenh., Klotzschii Herb. Viv. Mycol., Edn 2: no. 454. 1857. Fig. 8.
Basionym: *Sphaeria lichenoides* var. *cornicola* DC., in de Candolle & Lamarck, *Fl. franç.*, Edn 3 (Paris) 6: 148. 1815.

Conidiomata (on PNA) pycnidial, solitary, black, erumpent, globose, exuding colourless conidial masses; pycnidia up to 200 µm diam; pycnidial wall of several layers of *textura angularis*, up to 30 µm thick; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 10 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1–2 supporting cells, that can be branched at the base, 10–20 × 4–5 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 7–12 × 2.5–4 µm; proliferating several times percurrently near apex. *Conidia* (6–)7–8 × (5.5–)6(–7) µm, solitary, hyaline, aseptate, thin and smooth walled, granular, or with a single large central guttule, ellipsoid to obovoid, tapering towards a narrow truncate base, 2–3 µm diam, enclosed in a thin persistent mucoid sheath, 1 µm thick, and bearing a hyaline, apical mucoid appendage, (3–)4–5(–7) × 1(–1.5) µm, flexible, unbranched, tapering towards an acutely rounded tip.

Culture characteristics: Colonies erumpent, spreading with moderate aerial mycelium and feathery, lobate margins, covering dish after 1 mo at 25 ºC. On OA, MEA and PDA surface olivaceous-grey, reverse iron-grey.

Specimen examined. **USA**, on living leaf of *Cornus florida*, Jul. 1999, G. Carroll, CBS H-21392, culture CBS 111639.

Notes: The name *P. cornicola* is based on European collections (*Cornus sanguinea*, Czech Republic), and until fresh European material has been collected, we cannot be sure that the name is authentic for this taxon.

Phyllosticta cussoniae Cejp, Bothalia 10: 341. 1971. Fig. 9.

Leaf spots amphigenous, subcircular, pale to medium brown, 0.5–1 cm diam, frequently surrounded by a red-purple margin. *Conidiomata* (on PNA) pycnidial, solitary, black, erumpent, globose, exuding colourless to opaque conidial masses; pycnidia up to 200 µm diam; pycnidial wall of several layers of *textura angularis*; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 20 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1–2 supporting cells, branched at base, 10–25 × 3–5 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 5–10 × 3–4 µm; proliferating several times percurrently near apex. *Conidia* (10–)12–15(–17) × (6–)7(–8) µm, solitary, hyaline, aseptate, thin and smooth walled, coarsely guttulate, or with a single large central guttule, ellipsoid to obovoid, tapering towards a narrow truncate base, 3–4 µm diam, enclosed in a thin, persistent mucoid sheath, 2–4 µm thick, and bearing a hyaline, apical mucoid appendage, (8–)10–12(–13) × 2(–3) µm, flexible, unbranched, tapering towards an acutely rounded tip. *Spermatia* occurring in same conidioma with conidia, hyaline, smooth, guttulate to granular, bacilliform, 7–10 × 2–3 µm.

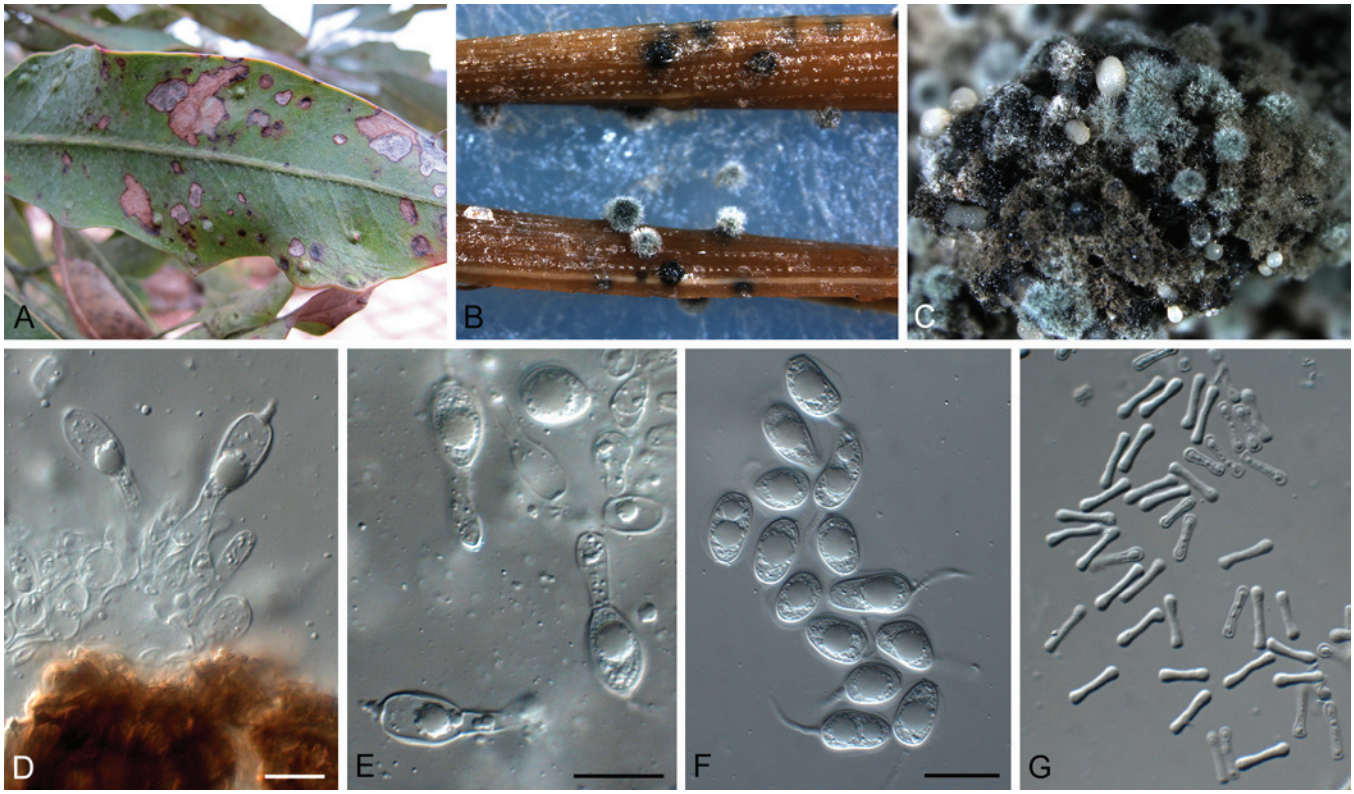

Fig. 9. *Phyllosticta cussoniae* (CPC 14873). A. Symptomatic leaf of *Cussonia* sp. B. Conidiomata forming on PNA. C. Conidiomata sporulating on OA. D, E. Conidiogenous cells giving rise to conidia. F. Conidia. G. Spermatia. Scale bars = 10 µm.

Culture characteristics: Colonies erumpent, spreading, with sparse aerial mycelium and feathery margins, covering the dish in 1 mo. On MEA surface olivaceous-grey, reverse iron-grey; on OA iron-grey; on PDA iron-grey on surface and reverse.

Specimens examined. **South Africa**, Mpumalanga, Schagen, Nelspruit, on *Cussonia umbellifera*, 25 Dec. 1933, L.C.C. Liebenberg, **holotype** PREM 32871; Eastern Cape, Graaff Reinet, Valley of Desolation, on leaf spot of *Cussonia* sp., 9 Jan. 2008, P.W. Crous (**epitype designated here** CBS H-21393, cultures ex-epitype CPC 14874, 14873 = CBS 136060; MBT176246); Gauteng, Walter Sisulu National Botanical Garden, on leaves of *Cussonia* sp., 2 Mar. 2007, P.W. Crous, cultures CPC 13812–13813.

Notes: *Phyllosticta cussoniae* occurs commonly on various *Cussonia* spp. throughout South Africa, where it causes a prominent leaf spot disease. All isolates collected from the various provinces where this host occurs, appear to have the same species (based on DNA sequence data) associated with the disease.

Phyllosticta foliorum (Sacc.) Wikee & Crous, **comb. nov.** MycoBank MB805656. Fig. 10.

Basionym: *Physalospora gregaria* var. *foliorum* Sacc., Syll. fung. (Abellini) 1: 435. 1882.

≡ *Pyreniella foliorum* (Sacc.) Theiss., Annls mycol. 14(6): 411. 1917 (1916).

≡ *Melanops foliorum* (Sacc.) Petr. (as "foliicola"), Kryptogamenflora Forsch. Bayer. Bot. Ges. Erforsch Leim. Flora 2(2): 165. 1931.

≡ *Botryosphaeria foliorum* (Sacc.) Arx & E. Müll., Beitr. Kryptfl. Schweiz 11(no. 1): 42. 1954.

Conidiomata (on PNA) pycnidial, solitary, black, erumpent, globose or with elongated body, exuding colourless to opaque conidial masses; pycnidia up to 400 µm diam; pycnidial wall of several layers of *textura angularis*; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 40 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1–2 supporting cells, branched at base or not, 10–25 × 4–5 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 8–20 × 3–4 µm; proliferating several times percurrently near apex. *Conidia* (12–)13–14(–15) × (9–)10(–11) µm, solitary, hyaline, aseptate, thin and smooth walled, coarsely guttulate, or with a single large central guttule, broadly ellipsoid, tapering towards a narrow truncate base, 2–3 µm diam, enclosed in a thin, persistent mucoid sheath, 2–3 µm thick, and bearing a hyaline, apical mucoid appendage, (10–)12–15(–20) × 1.5(–2) µm, flexible, unbranched, tapering towards an acutely rounded tip.

Culture characteristics: Colonies erumpent, spreading, with sparse aerial mycelium and feathery margins, covering the dish in 1 mo. On MEA surface olivaceous-grey, reverse iron-grey; on OA iron-grey; on PDA iron-grey on surface and reverse.

Specimens examined. **Italy**, on fallen leaves of *Taxus baccata*, **holotype** of *Physalospora gregaria* var. *foliorum*, Herb. P.A. Saccardo, PAD. **Netherlands**, Baarn, Maarschalksbos, on dead twigs and needles of *Taxus baccata*, Sep. 1969, H.A. van der Aa (**neotype designated here** CBS H-9495, culture ex-neotype CBS 447.68). **USA**, from bonsai tree of *Cryptomeria japonica*, 25 Feb. 1977, G.H. Boerema, specimens CBS H-13111, CBS H-619, culture CBS 174.77.

Notes: *Guignardia philoprina* (from *Ilex*) is a species complex with numerous old names. The oldest name linked to European specimens from *Taxus* appears to be *Physalospora gregaria* var. *foliorum*, which we recombine in *Phyllosticta*. As the holotype specimen in PAD only contains immature ascomata and spermatia, a neotype is herewith designated.

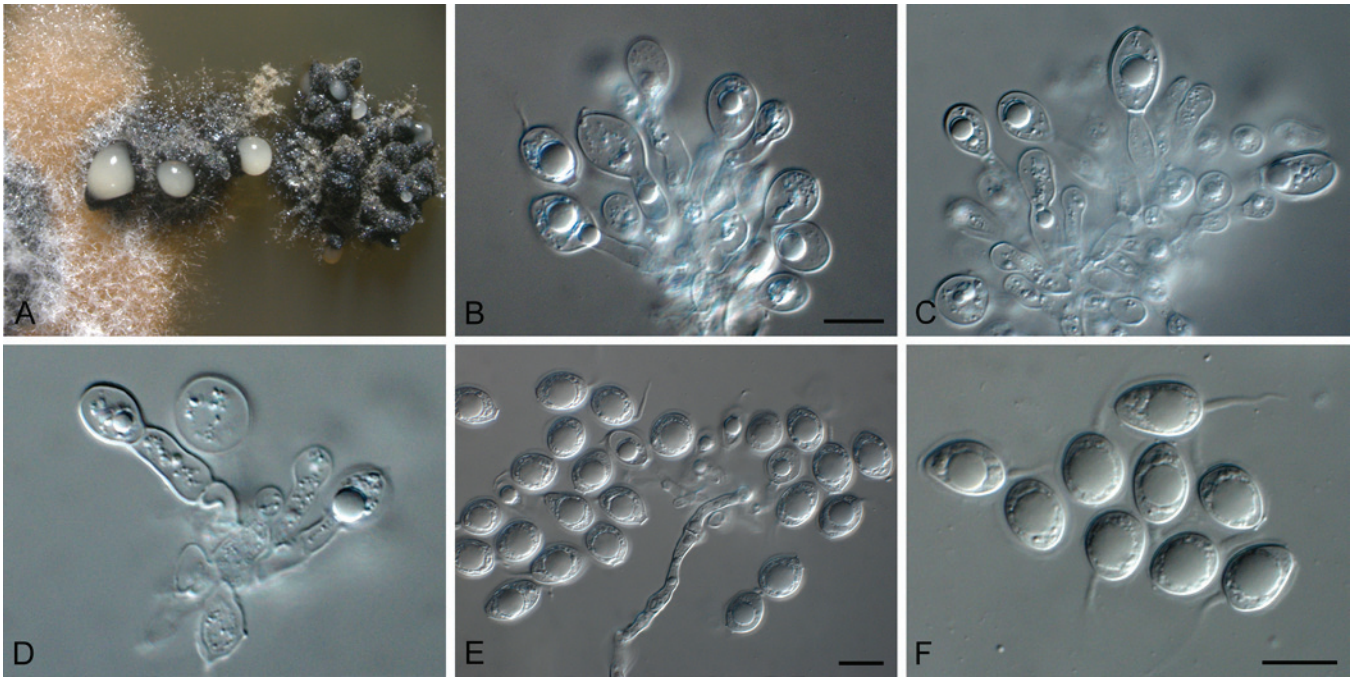

Fig. 10. *Phyllosticta foliorum* (CBS 447.68). A. Colony sporulating on MEA. B–D. Conidiogenous cells giving rise to conidia. E, F. Conidia. Scale bars = 10 µm.

Phyllosticta hypoglossi (Mont.) Allesch., Rabenh. Krypt.-Fl., Edn 2 (Leipzig) 1(6): 163. 1898. Fig. 11.
Basionym: *Sphaeropsis hypoglossi* Mont., Annls Sci. Nat., Bot., sér. 3 12: 307. 1849.

Conidiomata (on PNA) pycnidial, solitary, black, erumpent, globose, exuding colourless conidial masses; pycnidia up to 200 µm diam; pycnidial wall of several layers of *textura angularis*, up to 30 µm thick; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 15 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1–2 supporting cells, that can be branched at the base, 15–25 × 4–5 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 10–15 × 3–5 µm; proliferating several times percurrently near apex. *Conidia* (10–)11–12(–14) × (9–)10(–11) µm, solitary, hyaline, aseptate, thin and smooth walled, granular, or with a single large central guttule, broadly ellipsoid to obovoid or globose, tapering towards a narrow truncate base, 3–4 µm diam, enclosed in a thin, mucoid sheath, 1–3 µm thick, mostly not persistent, and bearing a hyaline, apical mucoid appendage, (8–)10–12(–15) × 1.5(–2) µm, flexible, unbranched, tapering towards an acute tip.

Culture characteristics: Colonies flat, spreading with sparse aerial mycelium and feathery, lobate margins, reaching 25 mm diam on MEA, 30 mm diam on PDA and 35 mm diam on OA after 2 wk at 25 ºC. On OA centre olivaceous-grey, outer zone with diffuse pale yellow pigment in agar. On PDA surface olivaceous-grey, reverse iron-grey. On MEA surface iron-grey in centre, pale grey-olivaceous in outer region, iron-grey in reverse.

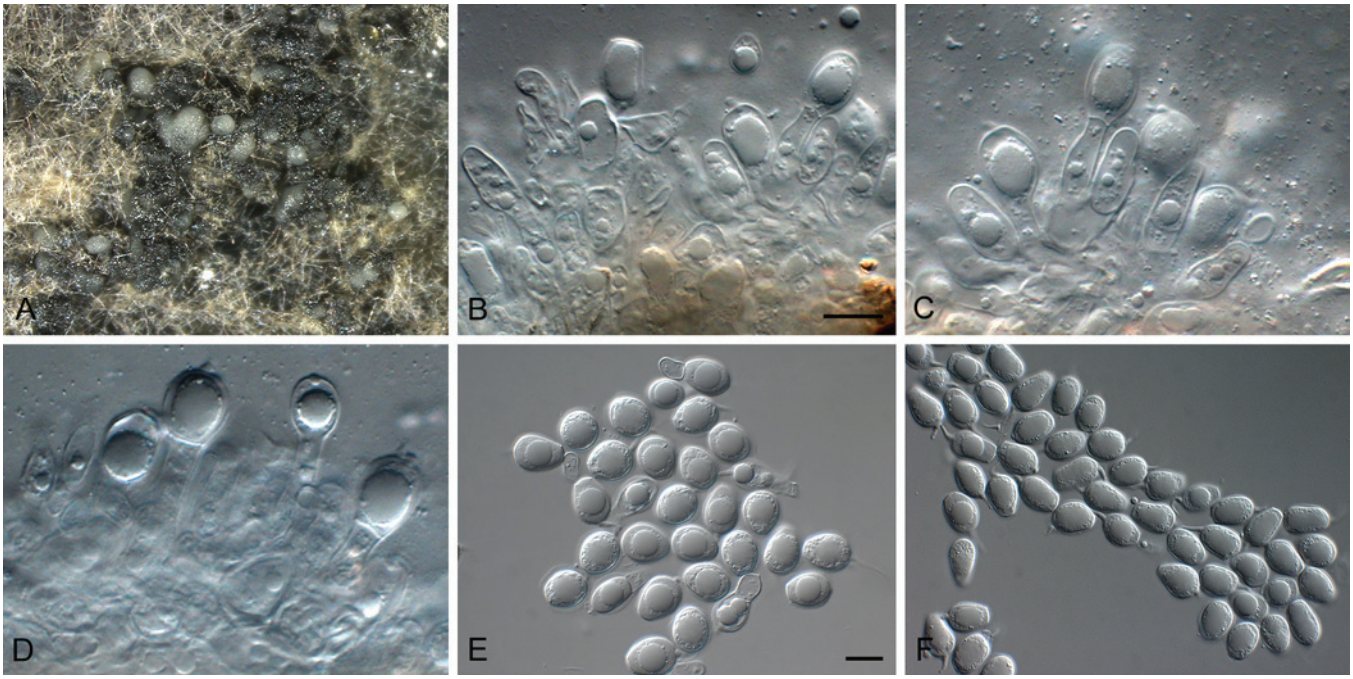

Fig. 11. *Phyllosticta hypoglossi* (CBS 434.92). A. Colony sporulating on OA. B–D. Conidiogenous cells giving rise to conidia. E, F. Conidia. Scale bars = 10 µm.

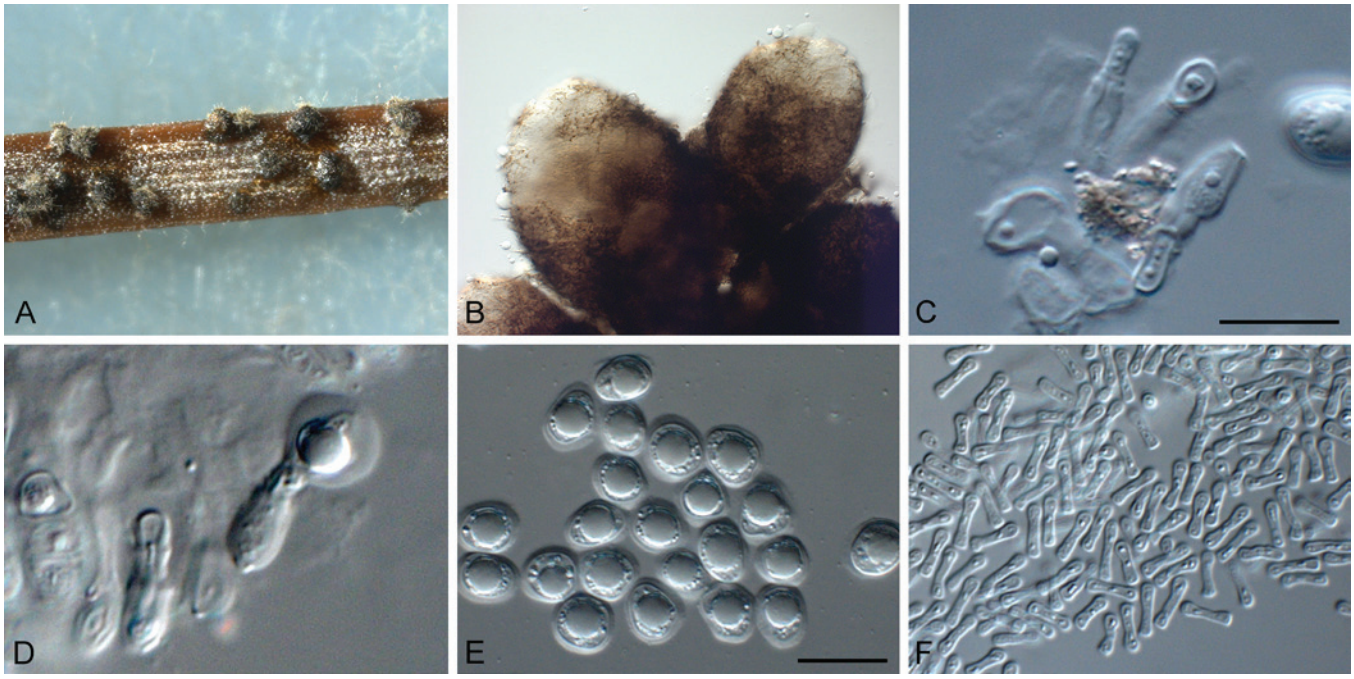

Fig. 12. *Phyllosticta leucothoicola* (MUCC 553). A. Conidiomata forming on PNA. B. Conidiomata. C, D. Conidiogenous cells giving rise to conidia. E. Conidia. F. Spermatia. Scale bars = 10 µm.

Specimens examined: **France**, near Marseille, on cladodes of *Ruscus hypoglossum*, 1845, J.L.M. Castagne, (type not found, presumably missing). **Italy**, Prov. Napoli, Cratere degli Astroni, on dead cladodes of *Ruscus aculeatus*, May 1992, W. Gams (**neotype designated here** CBS H-5331; ex-neotype culture CBS 434.92; MBT176248).

Notes: Judging from the number of specimens and cultures in the CBS collection, *P. hypoglossi* is a common European species on cladodes of *Ruscus hypoglossum.* The morphology of the neotype closely matches that described in the original description.

Phyllosticta leucothoicola Wikee, Motohashi & Crous, **sp. nov.** MycoBank MB805657. Fig. 12.

Etymology: Named after the host genus from which it was collected, *Leucothoe.*

Leaf spots purple-brown, scattered, enlarged and becoming confluent, subcircular to oblong, with brown to dark brown border (Takeuchi & Horie 1998). *Conidiomata* (on PNA) pycnidial, mostly aggregated in clusters, black, erumpent, globose to clavate or elongated with necks up to 500 µm long, exuding colourless to opaque conidial masses; pycnidia up to 300 µm diam; pycnidial wall of several layers of *textura angularis*, up to 40 µm thick; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 15 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1 supporting cell, 6–20 × 3–4 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 6–15 × 3–4 µm; proliferating several times percurrently near apex. *Conidia* (6–)7–8(–9) × 6(–7) µm, solitary, hyaline, aseptate, thin and smooth walled, coarsely guttulate, or with a single large central guttule, ovoid to irregularly ellipsoid, at times enclosed in a thin mucoid sheath, up to 1.5 µm thick; apical mucoid appendage not seen. *Spermatia* developing in same conidioma as conidia, bacilliform, smooth, hyaline, guttulate, 5–7 × 2–3 µm.

Culture characteristics: Colonies erumpent, spreading, with sparse aerial mycelium and feathery margins, covering the dish in 1 mo. On MEA surface olivaceous-grey, reverse iron-grey; on OA iron-grey; on PDA iron-grey on surface and reverse.

Specimen examined: **Japan**, Tokyo, on living leaf of *Leucothoe catesbaei*, May 1996, J. Takeuchi (holotype CBS H-21394, ex-type culture MUCC 553 = CPC 21881 = CBS 136073).

Notes: *Phyllosticta leucothoës* has been described from *Leucothoe acuminata*, although van der Aa & Vanev (2002) transferred this to *Fusicoccum* based on an examination of type material. *Phyllosticta leucothoicola* represents a distinct taxon on *L. catesbaei*, corroborating the morphological differences noted by Motohashi *et al.* (2009).

Phyllosticta mangifera-indica Wikee, Crous, K.D. Hyde & McKenzie, **sp. nov.** MycoBank MB805657. Fig. 13.

Etymology: Named after the host genus on which it occurs, *Mangifera indica*.

Conidiomata pycnidial (on PNA), initially forming after 4 d of incubation, black, superficial, subglobose or ellipsoidal, 220–300 × 160–180 µm; wall of 1–3 layers of brown *textura angularis*, 20–30 µm thick. *Conidiogenous cells* lining the inner wall, phialidic, cylindrical, hyaline, 3–5 × 3–4 µm. *Conidia* ellipsoidal, hyaline, aseptate, smooth, (6–)9(–13) × (4–)5(–6) µm, surrounded by mucilaginous sheath, 0.5–2 µm thick, bearing single apical appendage, 3–14 µm long.

Culture characteristics: On OA colonies appeared flat, with irregular margins, initially hyaline with abundant mycelium, gradually becoming greenish after 2–3 d. On MEA, colonies woolly, irregular, initially white with abundant mycelium, gradually becoming greenish to dark green after 2–3 d, with white hyphae at the undulate margin, eventually turning black; reverse dark green to black. After 25 d in

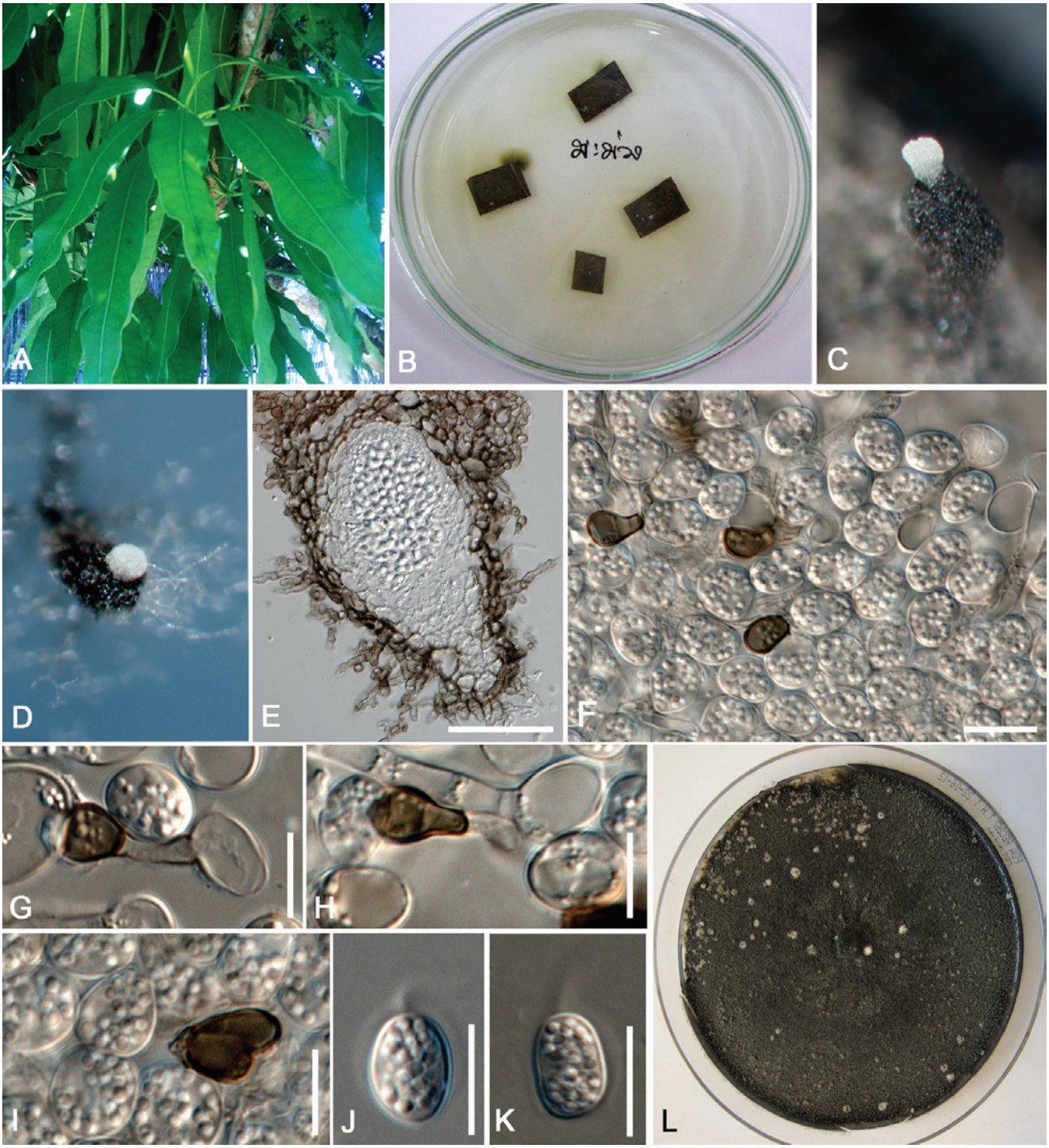

Fig. 13. *Phyllosticta mangifera-indica* (CPC 20274). A. Healthy leaf of *Mangifera indica*. B. Isolation on WA. C. Culture sporulating on OA. D. Culture sporulating on SNA. E. Vertical section through a conidioma showing developing conidia. F–I. Appressoria. J, K. Conidia. L. Culture on MEA. Scale bars: E = 100 µm, F–K = 10 µm.

the dark at 27 ºC colony covering the whole plate. On PDA colonies flat, rather fast growing, initially white with abundant mycelium, gradually becoming greenish to dark green after 2–3 d with white hyphae at the margin, eventually turning black; reverse black and after 14 d in the dark at 27 ºC colony covering the whole plate.

Specimen examined: **Thailand**, Chiangrai, Nanglae, on healthy leaf of *Mangifera indica*, July 2011, S. Wikee (**holotype** MFU13-0108; ex-type culture CPC 20274 = MFLUCC10-0029 = CBS 136061).

Notes: *Phyllosticta mangifera-indica* was isolated as an endophyte from a healthy leaf of *Mangifera indica*. Several species have been reported as pathogens on *M. indica* including *G. mangiferae* and *P. brazilianiae* (Glienke *et al.* 2011). *Phyllosticta mangifera-indica* produced abundant conidia on OA and formed appressoria within 2 d. Morphologically, it is distinct from *P. capitalensis* (conidia 8–11 × 5–6 µm) in having longer conidia (conidia 6–13 × 4–6), and represents a distinct lineage with 99 % bootstrap support with the inclusion of TEF1 and GPDH sequence data. It is phylogenetically distinct from *P. mangiferae*, and most closely related to *P. brazilianiae*, which occurs on the same host.

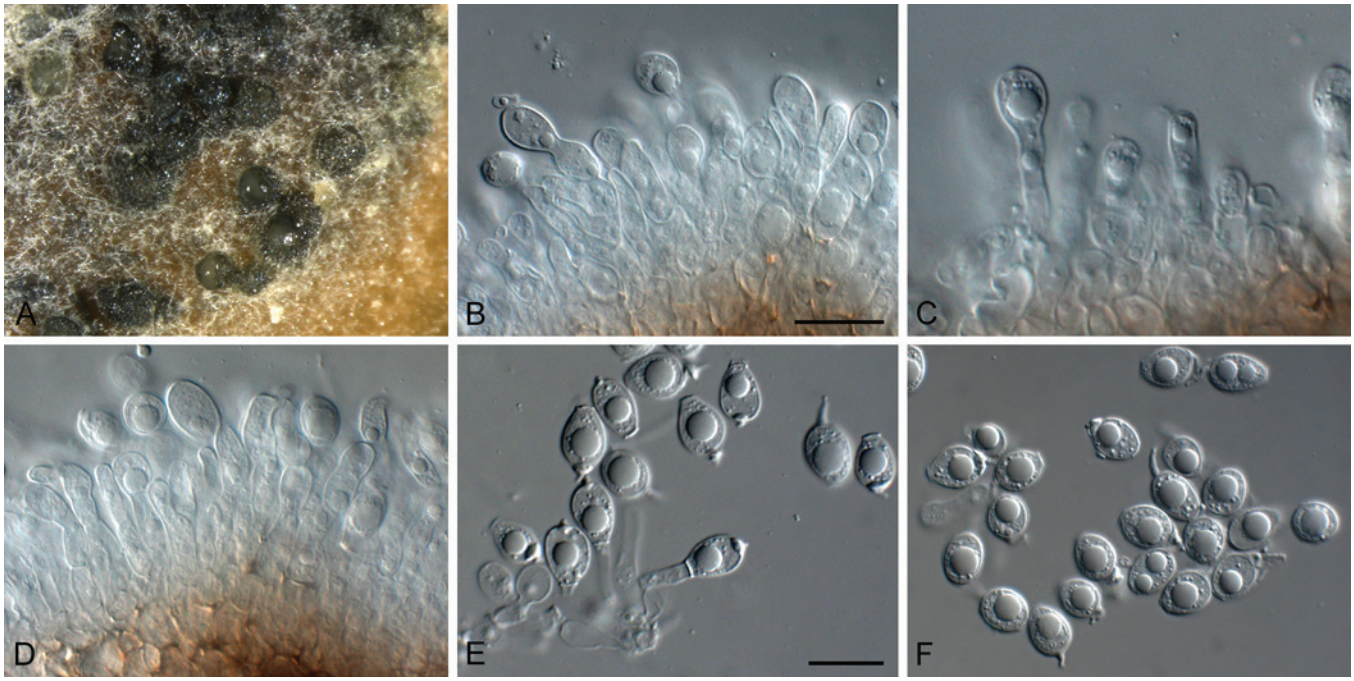

Fig. 14. *Phyllosticta minima* (CBS 585.84). A. Colony sporulating on MEA. B–E. Conidiogenous cells giving rise to conidia. F. Conidia. Scale bars = 10 µm.

Phyllosticta minima (Berk. & M.A. Curtis) Underw. & Earle, Bull. Alabama Agric. Exp. Stn. 80: 168. 1897. Fig. 14.
Basionym: *Sphaeropsis minima* Berk. & M.A. Curtis, N. Amer. Fungi: no. 418. 1874.

Conidiomata (on PNA) pycnidial, solitary, black, erumpent, globose, exuding colourless conidial masses; pycnidia up to 180 µm diam; pycnidial wall of several layers of *textura angularis*, up to 30 µm thick; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 15 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1–2 supporting cells, that can be branched at the base, 15–50 × 5–6 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 8–20 × 3–4 µm; proliferating several times percurrently near apex. *Conidia* (9–)10–11(–12) × (6–)7(–8) µm, solitary, hyaline, aseptate, thin and smooth walled, granular, or with a single large central guttule, broadly ellipsoid to obovoid or globose, tapering towards a narrow truncate base, 2–3 µm diam, enclosed in a thin mucoid sheath, absent at maturity or 1 µm thick, and bearing a hyaline, apical mucoid appendage, 6–7(–10) × 1.5(–2) µm, flexible, unbranched, tapering towards an acute tip.

Culture characteristics: Colonies flat, spreading with sparse aerial mycelium and feathery, lobate margins, reaching 15 mm diam on MEA, 40 mm diam on PDA and 8 mm diam on OA after 2 wk at 25 ºC. On OA surface olivaceous-grey. On PDA surface and reverse iron-grey. On MEA surface olivaceous-grey with patches of pale luteus.

Specimens examined: **USA**, North Dakota, New England, on *Acer rubrum*, R. Sprague 5314 (holotype not found); Tennessee, Gatlinburg, Great Smoky Mountains National Park, on leaf spot of *Acer rubrum*, June 1984, D.H. Defoe (**neotype designated here** CBS H-17023; ex-neotype culture CBS 585.84 = IFO 32917; MBT176250).

Note: This taxon appears to be common on *Acer* spp. in the USA, where it is associated with leaf spots (Bissett & Darbyshire 1984). The holotype could not be located in NY, LCR, IMI, S, K or BPI, and thus a neotype (from the original host in the USA) is designated.

Phyllosticta neopyrolae Wikee, Motohashi, Crous, K.D. Hyde & McKenzie, **sp. nov.** MycoBank MB803676. Fig. 15.

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

Leaf spots orbicular to ellipsoid, black. *Conidiomata* (on PNA) pycnidial, epiphyllous, sparse, solitary or aggregated, immersed at first, then erumpent breaking through the epidermis, brown to dark brown, subglobose, 60–100 × 60–113 µm; pycnidial wall composed of the depressed or irregular cells in 1–4 layers, brown to dark brown, hyaline or paler toward the inside, with a central ostiole, up to 10 µm diam. *Conidiophores* subcylindrical, reduced to conidiogenous cells, or with 1–2 supporting cells, branched at the base, 15–20 × 2–3 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 8–15 × 2–3 µm; proliferating several times percurrently near apex. *Conidia* (6–)7(–8) × (5–)6(–7) µm, solitary, hyaline, aseptate, thin and smooth walled, granular, or with a single large central guttule, broadly ellipsoid to globose, mucoid sheath and appendage lacking.

Culture characteristics: Colonies erumpent, spreading, with sparse aerial mycelium and feathery margins, covering the dish in 1 mo. On MEA surface olivaceous-grey, reverse iron-grey; on OA iron-grey; on PDA iron-grey on surface and reverse.

Specimen examined. **Japan**, Nagano, Sugadaira, on living leaf of *Pyrola asarifolia* subsp. *incarnata*, 17 June 2006, T. Hosoya (**holotype** TFM : FPH 7887, **isotype** CBS H-21395, ex-type culture MUCC 125 = CPC 21879 = CBS 134750).

Notes: Two species of *Phyllosticta* are known from *Pyrola* spp., namely *P. pyrolae* Ellis & Everh. and *P. pyrolae* (Ehrenb. : Fr) Allesch. Of these, the latter species is an illegitimate homonym, with morphological characteristics (conidia 3–4 µm long) that indicate that it should be excluded from *Phyllosticta* s. str. (van der Aa & Vanev 2002). The other species, *P. pyrolae* Ellis & Everh. (conidia ovoid to globose, 4.5–7.5 × 4–9 µm, with mucoid layer and an apical appendage) resembles *P. neopyrolae*. *Phyllosticta*

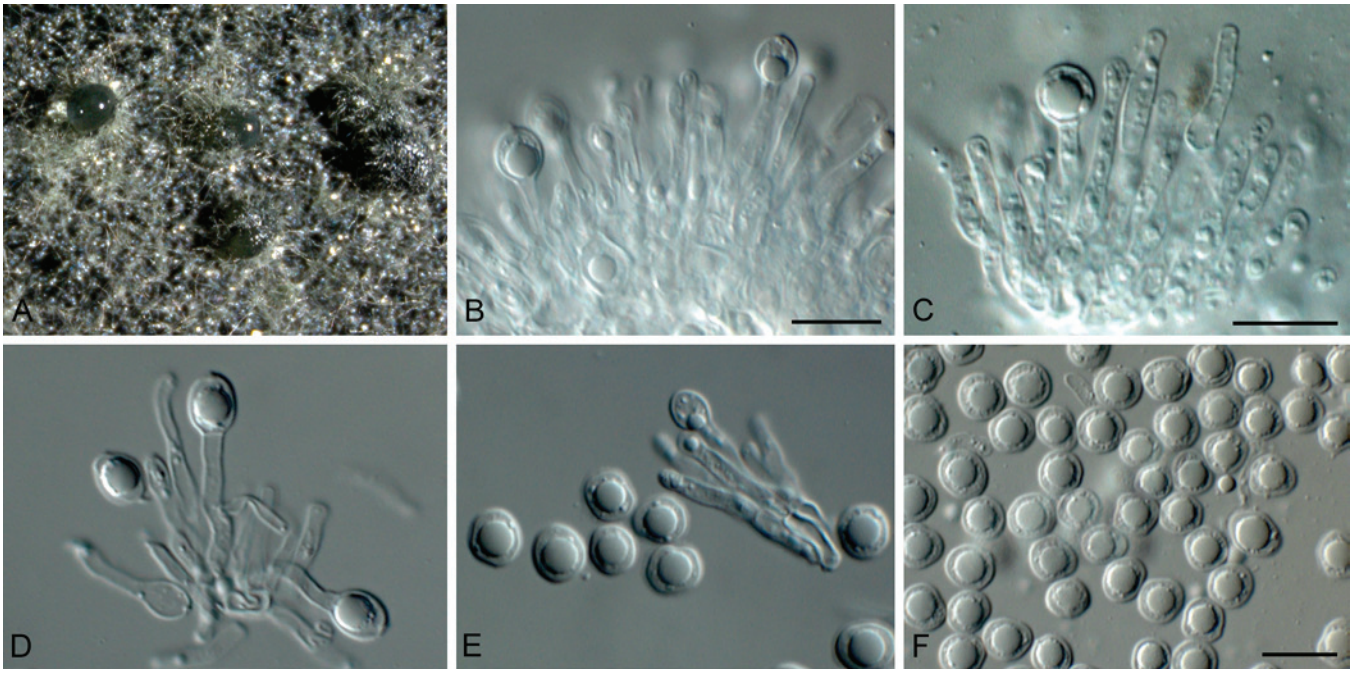

Fig. 15. *Phyllosticta neopyrolae* (CBS 134750). A. Colony sporulating on OA. B–E. Conidiogenous cells giving rise to conidia. F. Conidia. Scale bars = 10 µm.

neopyrolae differs from these two species by having conidia that lack a mucoid sheath and apical appendage.

Phyllosticta owaniana G. Winter, Hedwigia 24: 31. 1885. Fig. 16.

Leaf spots amphigenous, irregular to subcircular, pale to medium brown, turning greyish with age, surrounded by a broad purplish border, and chlorotic margin. *Conidiomata* (on PNA) pycnidial, solitary, black, erumpent, globose, exuding colourless conidial masses; pycnidia up to 300 µm diam, frequently with elongated neck on OA and MEA; pycnidial wall of several layers of *textura angularis*, up to 30 µm thick; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 10 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1–2 supporting cells, that can be branched at the base, 10–30 × 4–5 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 10–25 × 3–4.5 µm; proliferating several times percurrently near apex. *Conidia* (10–)11–12(–13) × (7–)8(–9) µm, solitary, hyaline, aseptate, thin and smooth walled, granular, or with a single large central guttule, ellipsoid to obovoid, tapering towards a bluntly obtuse or narrow truncate base, 2–3 µm diam, enclosed in a thin persistent mucoid sheath, 1–2 µm thick, and bearing a hyaline, apical mucoid appendage, (5–)8–12(–15) × (1–)1.5 µm, flexible, unbranched, tapering towards an acutely rounded tip.

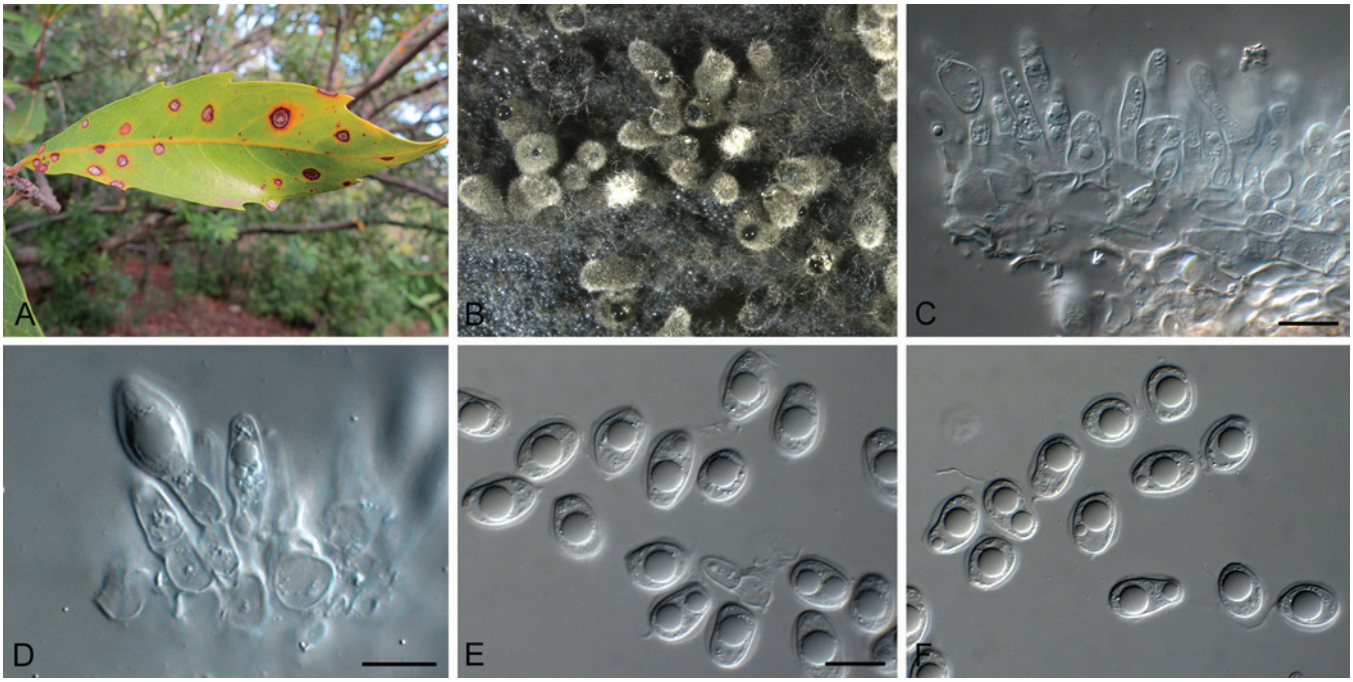

Fig. 16. *Phyllosticta owaniana* (CBS 776.97). A. Symptomatic leaf of *Brabejum stellatifolium*. B. Colony sporulating on OA. C, D. Conidiogenous cells giving rise to conidia. E, F. Conidia. Scale bars = 10 µm.

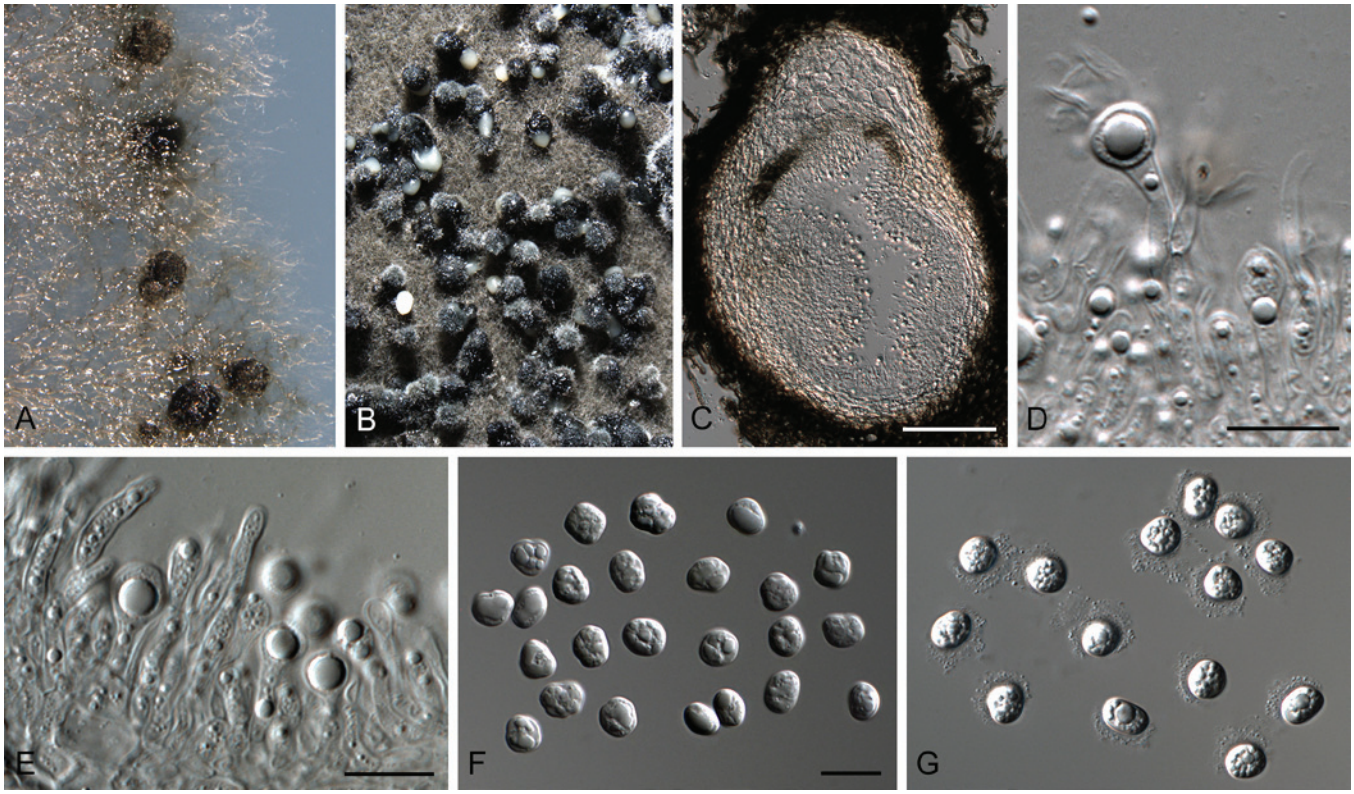

Fig. 17. *Phyllosticta pachysandricola* (NBRC 102276). A. Colony sporulating on SNA. B. Colony sporulating on PDA. C. Vertical section through conidioma. D, E. Conidiogenous cells. F. Conidia mounted in lactic acid. G. Conidia mounted in water. Scale bars: C = 35 µm, all others = 10 µm.

Culture characteristics: Colonies flat, spreading with sparse aerial mycelium and feathery, lobate margins, reaching 30 mm diam on MEA, 40 mm diam on PDA and 25 mm diam on OA after 2 wk at 25 ºC. On OA surface iron-grey. On PDA surface and reverse iron-grey. On MEA surface and reverse iron-grey.

Specimens examined: **South Africa**, Western Cape Province, Cape Town, Table Mountain, on leaves of *Brabejum stellatifolium*, 1884, P. McOwan, **holotype** in B; Western Cape Province, Jonkershoek Nature Reserve, on leaf spot of *Brabejum stellatifolium*, 3 Jan. 1995, A. den Breeÿen, (**epitype designated here** CBS H-21396, ex-epitype culture CPC 1009 = CBS 776.97; MBT176251).

Notes: *Phyllosticta owaniana* causes a serious leaf spot disease on *Brabejum stellatifolium*, and is generally found wherever this host occurs in South Africa. All isolates collected thus far (Crous, unpubl. data) are similar based on DNA sequence data, suggesting that it's a common species on this host.

Phyllosticta pachysandricola Wikee, Motohashi & Crous, **sp. nov.** MycoBank MB805658. Fig. 17.

Etymology: Named after the host genus from which it was collected, *Pachysandra.*

Leaf spots circular to ellipsoid, pale brown to brown, often extend with concentric rings, 6–16 mm diam, surrounded by a dark brown border. *Conidiomata* (on PNA) pycnidial, amphiphyllous, sparse, solitary or aggregated, immersed at first, then erumpent breaking through the epidermis, brown to dark brown, subglobular, 90–140 × 25–80 µm diam, with central ostiole; pycnidial wall composed of depressed or irregular cells with 1–4 layers, brown to dark brown, hyaline or paler toward the inside. *Conidiogenous cells* integrated, lining the innermost layer of the pycnidial wall, cylindrical, straight or slightly curved, hyaline, proliferating percurrently at least once, with minute periclinal thickenings, 5–12 × 2–2.5 µm. *Conidia* sporulating holoblastically, solitary, unicellular, spherical, ellipsoid to obovoid, 5.5–8.5 × 4.5–7.5 µm, truncate at the base or rounded at both ends, containing numerous greenish guttulae, surrounded by a mucous sheath, rarely with a short apical appendage.

Specimen examined: **Japan**, Hokkaido, Asahikawa, on *Pachysandra terminalis*, K. Motohashi, C. Nakashima & T. Akashi, 7 June 2006 (**holotype** TFM : FPH7877, **isotype** MUMH 10488, ex-type culture MUCC 124 = NBRC 102276).

Notes: One other species has been recorded from *Pachysandra*, *P. pachysandrae*, which van der Aa & Vanev (2002) excluded from *Phyllosticta s. str*. based on its conidia (unicellular, oblong, 4.5–6 × 1 µm) that indicate placement in *Asteromella*. The Japanese collection on *Pachysandra* is thus described as a new species, *P. pachysandricola*, in accordance to the morphological differences noted by Motohashi *et al*. (2009).

Phyllosticta paxistimae Wikee & Crous, **sp. nov.** MycoBank MB805659. Fig. 18.

Etymology: Named after the host genus from which it was collected, *Paxistima.*

Conidiomata (on PNA) pycnidial, solitary, black, erumpent, globose, exuding colourless conidial masses; pycnidia up to 200 µm diam; pycnidial wall of several layers of *textura angularis*, up to 30 µm thick; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 10 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1 supporting cell, 15–30 × 4–6 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline,

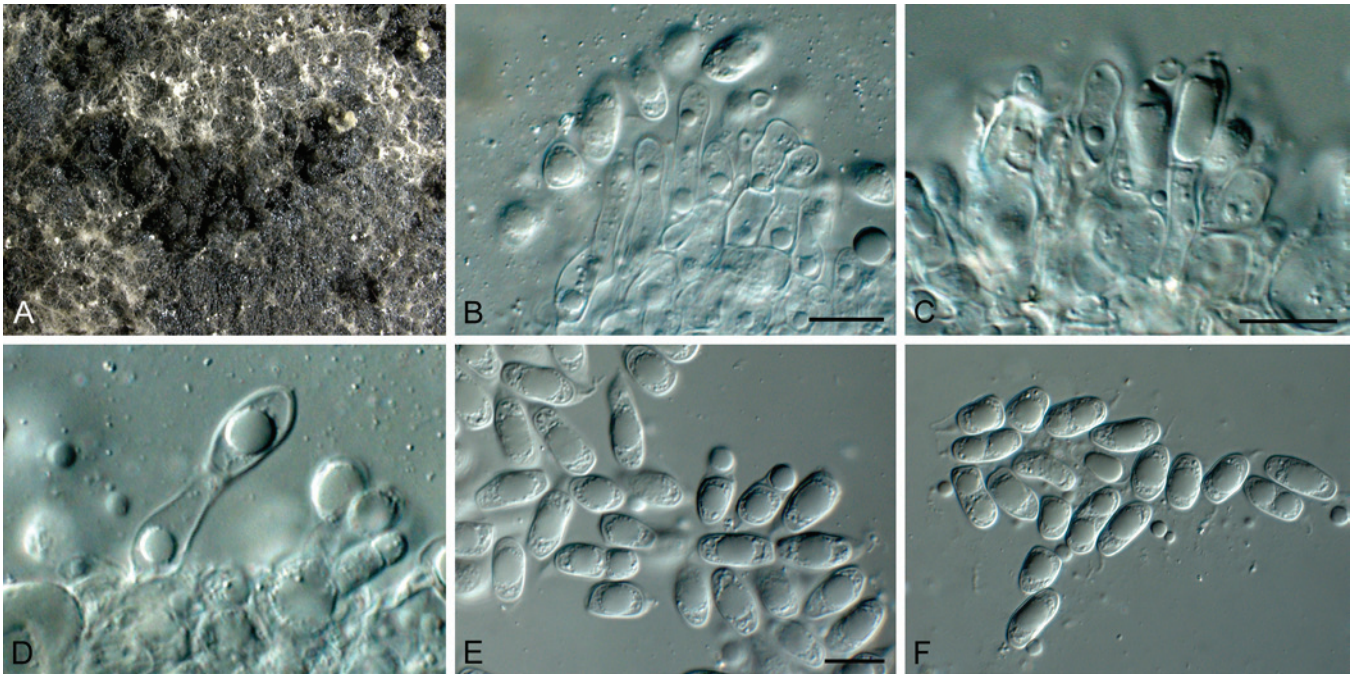

Fig. 18. *Phyllosticta paxistimae* (CBS 112527). A. Colony sporulating on OA. B–D. Conidiogenous cells giving rise to conidia. E, F. Conidia. Scale bars = 10 µm.

smooth, coated in a mucoid layer, 10–20 × 4–5 µm; proliferating several times percurrently near apex. *Conidia* (10–)12–14(–16) × 6–7(–8) µm, solitary, hyaline, aseptate, thin and smooth walled, granular, or with a single large central guttule, broadly ellipsoid, tapering towards a narrow truncate base, 2–3 µm diam, enclosed in a thin persistent mucoid sheath, 1 µm thick, and bearing a hyaline, apical mucoid appendage, (5–)9–11(–13) × 1.5(–2) µm, flexible, unbranched, tapering towards an acutely rounded tip.

Culture characteristics: Colonies erumpent, spreading with moderate aerial mycelium and feathery, lobate margins, reaching 60 mm diam after 1 mo at 25 ºC. On OA surface iron-grey with patches of olivaceous-grey. On PDA surface and reverse iron-grey. On MEA surface dirty white with patches of iron-grey, reverse iron-grey.

Specimen examined. **USA**, Oregon, on living leaf of *Paxistima myrsinites*, 1994, G. Carroll (**holotype** CBS H-21397, ex-type culture CBS 112527).

Notes: We have been unable to trace the holotype of *P. pachystimae* (**USA**, Wyoming, Hoback Canyon, near Granite Creek, on *Paxistima myrsinites*, 1 Aug. 1940, L.E. Wehmeyer No 1198). The conidia of *P. pachystimae* (9–14 × 4–5 µm; Wehmeyer 1946) are much narrower than those of *P. paxistimae* (10–16 × 6–8 µm).

Phyllosticta philoprina (Berk. & M.A. Curtis) Wikee & Crous, **comb. nov.** MycoBank MB805660.

Basionym: *Sphaeria philoprina* Berk. & M.A. Curtis, Grevillea 4 (32): 154. 1876.

≡ *Guignardia philoprina* (Berk. & M.A. Curtis) Aa, Stud. Mycol. 5: 44. 1973.

For additional synonyms see van der Aa (1973).

Specimens examined: **Spain**, on living leaf of *Ilex aquifolium*, July 1970, H.A. van der Aa, specimen CBS H-13113, culture CBS 587.69. **Germany**, on *Ilex aquifolium*, Aug. 1972, R. Schneider, CBS 616.72.

Notes: The oldest name for a *Phyllosticta* sp. occurring on *Ilex* is *Sphaeria philoprina*. However, this name was based on material collected in the USA, and the present isolates were derived from European collections.

Phyllosticta podocarpicola Wikee, Crous, K.D. Hyde & McKenzie, **sp. nov.** MycoBank MB805661. Fig. 19.

Etymology: Named after the host genus from which it was collected, *Podocarpus*.

Conidiomata (on PNA) pycnidial, solitary, black, erumpent, globose, exuding colourless to opaque conidial masses; pycnidia up to 200 µm diam; pycnidial wall of 3–6 layers of brown *textura angularis*; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 20 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1–2 supporting cells, at times branched at base, 10–25 × 4–6 µm. *Conidiogenous cells* terminal, subcylindrical to doliiform, hyaline, smooth, coated in a mucoid layer, 10–17 × 4–6 µm; proliferating several times percurrently near apex. *Conidia* 12–13(–16) × 8–9(–9.5) µm, solitary, hyaline, aseptate, thin and smooth walled, coarsely guttulate, or with a single large central guttule, broadly ellipsoid, tapering towards a narrow truncate base, 2–5 µm diam, enclosed in a thin, persistent mucoid sheath, 3–4 µm thick, and bearing a hyaline, apical mucoid appendage, (25–)30–45(–55) × 3–4(–5) µm, flexible, unbranched, tapering towards an acutely rounded tip.

Culture characteristics: Colonies after 3 wk reaching 30 mm diam on MEA, 60 mm on PDA and OA. Colonies flattened, spreading, with sparse aerial mycelium and feathery margins. On MEA surface and reverse olivaceous-grey; on OA olivaceous-grey; on PDA iron-grey on surface and reverse.

Specimen examined: **USA,** Florida, on seed of *Podocarpus maki* (intercepted in New Zealand), Sep. 1979, G. Laundon (**holotype** CBS H-13109; ex-type culture CBS 728.79).

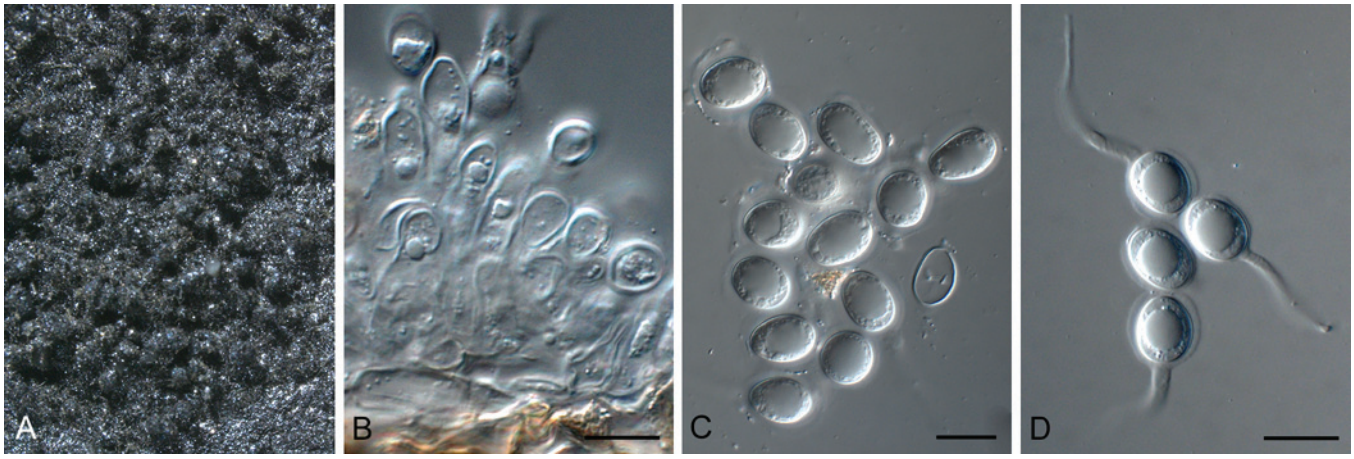

Fig. 19. *Phyllosticta podocarpicola* (CBS 728.79). A. Colony sporulating on OA. B. Conidiogenous cells giving rise to conidia. C, D. Conidia. Scale bars = 10 µm.

Notes: The isolate described here as *Phyllosticta podocarpicola* (CBS 728.79) was originally treated as part of the *G. philoprina* species complex, from which it is phylogenetically distinct (Figs 1, 2). It is also distinct from *Phyllosticta podocarpi*, which was originally described from *Podocarpus elongatus* leaf litter collected in South Africa [conidia (10–)14(–17) × (8–)9(–10) µm, appendages 10–40 × 1.5–2 µm; Crous *et al.* 1996].

Phyllosticta rhaphiolepidis Wikee, C. Nakash. & Crous, **sp. nov.** MycoBank MB805662. Fig. 20.

Etymology: Named after the host genus from which it was collected, *Rhaphiolepis.*

Leaf spots irregular, pale brown. *Conidiomata* (on PDA) pycnidial, amphiphyllous, immersed, subglobose to globose, composed of depressed or irregular cells in 2–3 layers, dark brown to black, hyaline or paler toward the inside, 85–175 × 100–110 µm diam, with central ostiole, 10–13 µm diam. *Conidiogenous cells* integrated, lining the inner layer of pycnidia, hyaline, lageniform, cylindrical or conical, 3–10 × 3–4 µm, proliferating percurrently near apex. *Conidia* unicellular, spherical, ellipsoid to obovoid, truncate at base, later rounded at both ends, surrounded by a mucoid layer, containing numerous minute guttules, 7.5–10 × 4.6–6 µm, with a slender and short apical appendage 4–6 × 1–2 µm.

Specimen examined: **Japan**, Kagoshima, Tokunoshima Is., on living leaf of *Rhaphiolepis indica* var. *umbellata*, T. Kobayashi & Y. Ono, 22 Oct. 2003 (**holotype** CBS H-21408, culture ex-type MUCC 432).

Notes: *Phyllosticta rhaphiolepicola*, which occurs on *Rhaphiolepis japonica* in Germany, has somewhat wider conidia (7–9 × 6–8 µm; van der Aa & Vanev 2002) than the Japanese collection. *Phyllosticta*

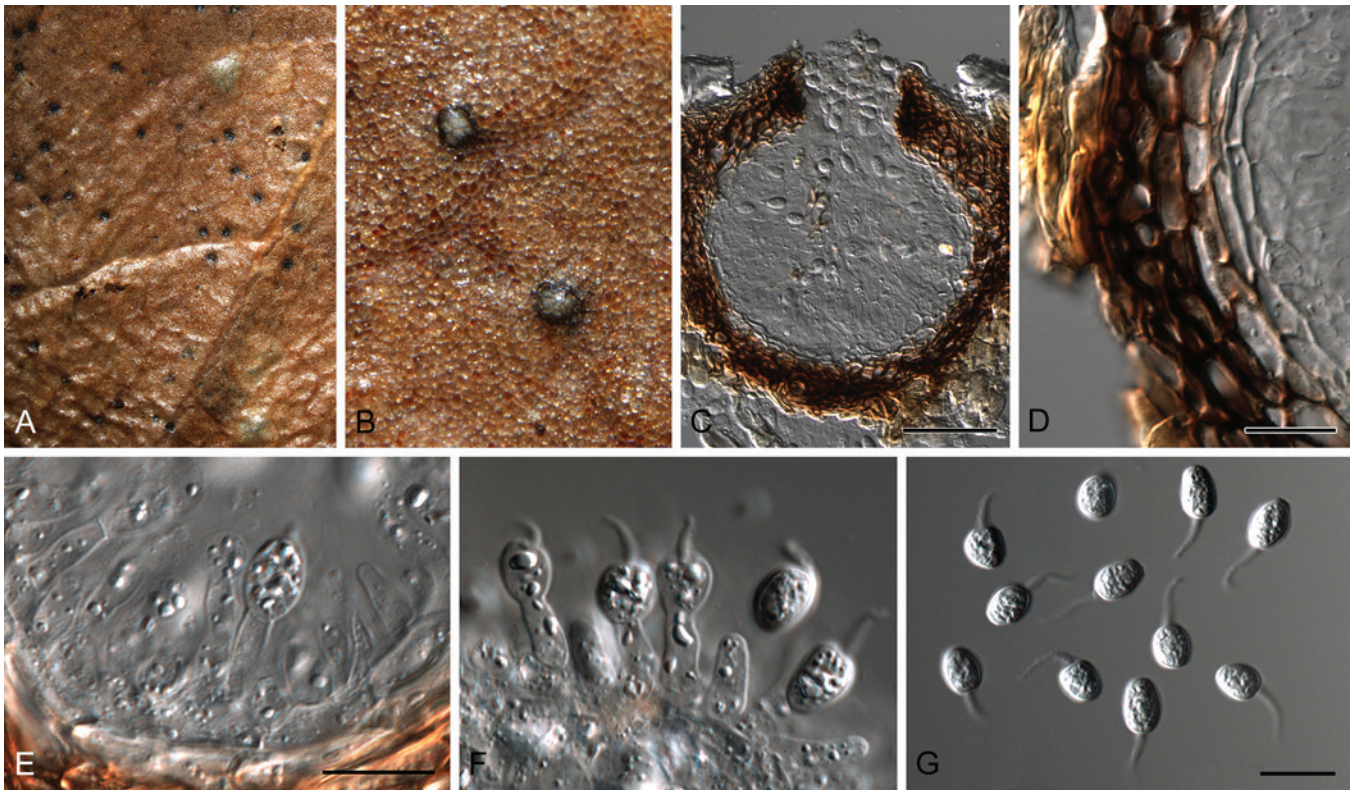

Fig. 20. *Phyllosticta rhaphiolepidis* (MUCC 432). A, B. Close-up of immersed conidiomata on leaf tissue. C. Vertical section through conidioma. D. Conidiomatal wall of *textura angularis*. E, F. Conidiogenous cells. G. Conidia. Scale bars: C = 25 µm, all others = 10 µm.

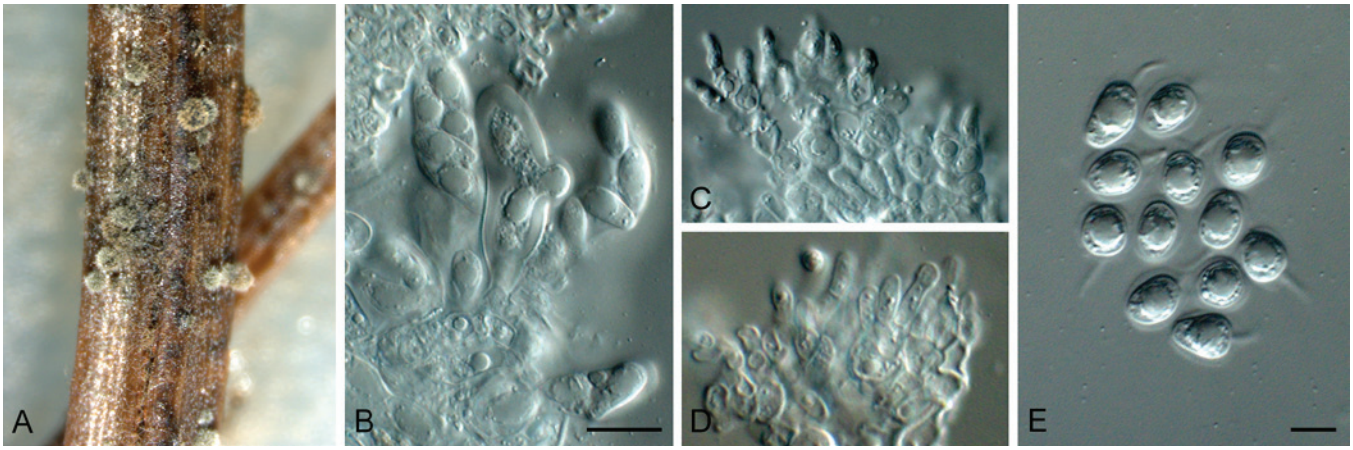

Fig. 21. *Phyllosticta rubra* (CBS 111635). A. Conidiomata forming on PNA. B. Asci with ascospores. C, D. Conidiogenous cells. E. Conidia. Scale bars = 10 µm.

rhaphiolepidis is also phylogenetically distinct from other species of *Phyllosticta* that have been deposited in GenBank (Figs 1, 2).

Phyllosticta rubra Wikee & Crous, **sp. nov.** MycoBank MB805663. Fig. 21.

Etymology. Named after the host species from which it was collected, *Acer rubrum.*

Conidiomata (on PNA) pycnidial, solitary, black, erumpent, globose, exuding colourless conidial masses; *pycnidia* up to 200 µm diam; pycnidial wall of several layers of *textura angularis*, up to 30 µm thick; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 15 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1 supporting cell, that can be branched at the base, 7–10 × 2–3 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 3–8 × 2–3 µm; proliferating several times percurrently near apex. *Conidia* (6–)6.5–7(–8) × (4–)5(–5.5) µm, solitary, hyaline, aseptate, thin and smooth walled, granular, or with a single large central guttule, ellipsoid to obovoid, tapering towards a narrow truncate base, 1.5–2 µm diam, enclosed in a thin persistent mucoid sheath, 1–1.5 µm thick, and bearing a hyaline, apical mucoid appendage, (5–)6–7(–9) × (1–)1.5 µm, flexible, unbranched, tapering towards an acutely rounded tip. *Ascomata* similar to conidiomata in general anatomy. *Asci* bitunicate, hyaline, clavate to broadly fusoid-ellipsoid, with visible apical chamber, 1 µm diam, 30–50 × 10–12 µm. *Ascospores* bi- to triseriate, hyaline, smooth, granular to guttulate, aseptate, straight, rarely curved, widest in the upper third, limoniform, (8–)9–10(–12) × (4–)5 µm.

Culture characteristics: Colonies erumpent, spreading with moderate aerial mycelium, covering dish after 1 mo at 25 ºC. On OA surface iron-grey. On PDA and MEA surface olivaceous-grey, to iron-grey, reverse iron-grey.

Specimen examined: **USA**, Missouri, on *Acer rubrum*, July 1999, G. Carroll, (**holotype** CBS H-21398, culture ex-type CBS 111635).

Notes: *Phyllosticta rubra* is part of the *P. minima* species complex. *Phyllosticta rubra* has larger conidia (10 µm long), than two proposed synonyms, namely *P. arida* (on *Acer negundo*, conidia 8–10 × 6–7 µm), and *P. acericola* (on *Acer rubrum*, conidia 5–8 × 3–3.5 µm) (see van der Aa 1973).

Phyllosticta spinarum (Died.) Nag Raj & M. Morelet, Bull. Soc. Sci. nat. Arch. Toulon et du Var 34 (219): 12. 1978. Fig. 22.

Basionym: *Phoma spinarum* Died., Krypt.-Fl. Brandenburg (Leipzig) 9: 148. 1912.

Conidiomata (on PNA) pycnidial, solitary, black, erumpent, globose, exuding colourless conidial masses; *pycnidia* up to 200 µm diam; pycnidial wall of several layers of *textura angularis*, up to 30 µm thick; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 15 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1 supporting cell, that can be branched at the base, 10–15 × 4–5 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 5–12 × 3–5 µm; proliferating several times percurrently near apex. *Conidia* (10–)12–14(–15) × (7–)7.5(–8) µm, solitary, hyaline, aseptate, thin and smooth walled, granular, or with a single large central guttule, ellipsoid to obovoid, tapering towards a narrow truncate base, 3–4 µm diam, enclosed in a thin persistent mucoid sheath, 1–2 µm thick, and bearing a hyaline, apical mucoid appendage, (7–)8–12(–20) × (2–)2.5(–3) µm, flexible, unbranched, tapering towards an acutely rounded tip.

Culture characteristics: Colonies flat, spreading with sparse aerial mycelium and feathery, lobate margins, reaching 70 mm diam after 1 mo at 25 ºC. On OA surface olivaceous-grey. On PDA surface olivaceous-grey, reverse iron-grey. On MEA surface pale olivaceous-grey in outer region, olivaceous-grey in centre; in reverse iron-grey in centre, smoke-grey in outer region.

Specimens examined: **France**, St. Denis en Val, on living leaf of *Chamaecyparis pisifera*, 1970, M. Morelet (CBS H-17034, culture CBS 292.90). **Germany**, Nieder Lauslitz: Colbus, on *Juniperus* sp., 4 Jul. 1910, Diedicke, **holotype** in B.

Notes: Nag Raj & Morelet (1979) provide a detailed description of the type specimen, which closely corresponds with isolate CBS 292.90 studied here.

Phyllosticta vacciniicola Wikee, Crous, K.D. Hyde & McKenzie, **sp. nov.** MycoBank MB805664. Fig. 23.

Conidiomata pycnidial, solitary, black, erumpent, globose, exuding colourless conidial masses; *pycnidia* up to 200 µm diam;

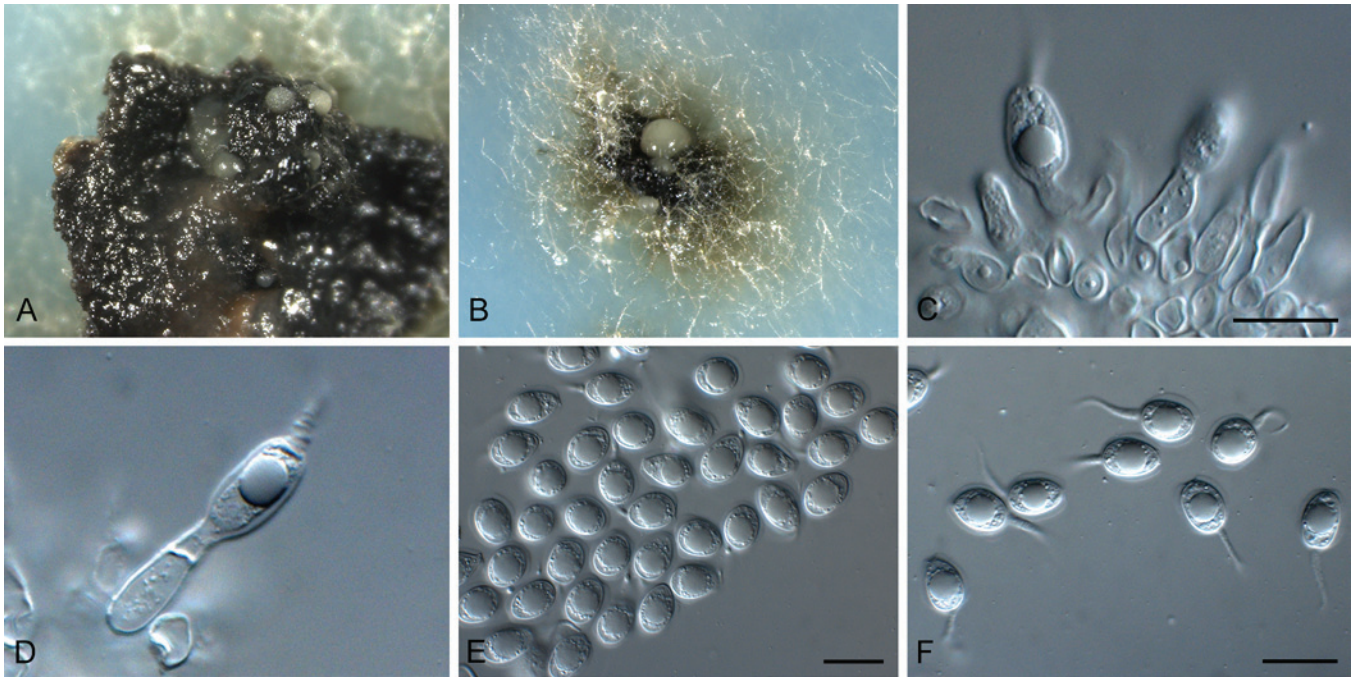

Fig. 22. *Phyllosticta spinarum* (CBS 292.90). A, B. Colony sporulating on SNA. C, D. Conidiogenous cells giving rise to conidia. E, F. Conidia. Scale bars = 10 µm.

pycnidial wall of several layers of *textura angularis*, up to 25 µm thick; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 20 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1 supporting cell, that can be branched at the base, 10–20 × 3–5 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 8–15 × 3–4 µm; proliferating several times percurrently near apex. *Conidia* (9–)10–12(–13) × (6–)7(–8) µm, solitary, hyaline, aseptate, thin and smooth walled, granular, with a single large central guttule, ellipsoid to obovoid, tapering towards a narrow truncate base, 3–4 µm diam, enclosed in a thin persistent mucoid sheath, 0.5–1 µm thick, and bearing a hyaline, apical mucoid appendage, 7–25 × (1.5–)2 µm, flexible, unbranched, tapering towards an acutely rounded tip.

Culture characteristics: Colonies flat, spreading with sparse aerial mycelium and feathery, lobate margins, reaching 15 mm diam after 2 wk at 25 ºC. On OA surface iron-grey. On PDA surface and reverse iron-grey. On MEA surface pale olivaceous-grey to olivaceous-grey, reverse olivaceous-grey.

Specimen examined: **USA**, on living leaf of *Vaccinium macrocarpum*, Mariusz Tadych, (**holotype** CBS H-21399, ex-type culture CPC 18590 = CBS 136062).

Notes: A recent study published by Zhang *et al*. (2013) revealed *P. vaccinii* (ex-epitype ATCC 46255) to be distinct from *Guignardia vaccinii* (ex-holotype CBS 126.22), and also revealed that several undescribed *Phyllosticta* spp. are associated with *Vaccinium*, one of which is described here as *P. vacciniicola*. The correct name for

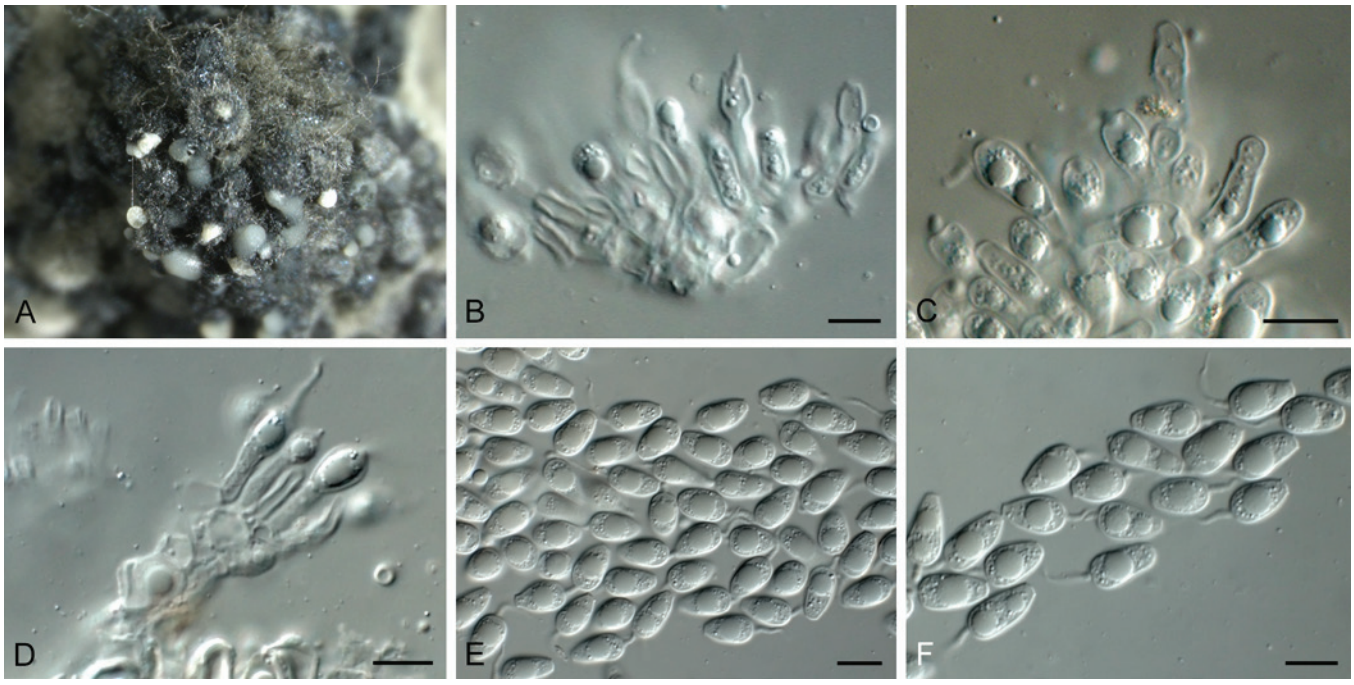

Fig. 23. *Phyllosticta vacciniicola* (CPC 18590). A. Colony sporulating on OA. B–D. Conidiogenous cells giving rise to conidia. E, F. Conidia. Scale bars = 10 µm.

G. vaccinii should thus be that of its asexual morph *P. elongata*, in accordance with Weidemann *et al*. (1982).

Phyllosticta sp.

Specimen examined: **Spain**, on living leaf of *Eucalyptus globulus*, 4 Jan. 2004, M.J. Wingfield, culture CPC 11336.

Notes: Two species of *Phyllosticta* are known from *Eucalyptus*. Van der Aa & Vanev (2002) treated *P. eucalyptorum* (on *E. grandis* from Brazil, conidia (7.5–)11–20 × (5–6(–6.5) µm; Crous *et al*. 1993) as synonymous with *P. eucalyptina* (on *E. globulus*, Tunisia, conidia 18–20 × 5–6 µm). No cultures of *P. eucalyptina* are available, and *P. eucalyptorum* was considered a synonym of *P. capitalensis* (Fig. 1). Although the present collection appears to represent a novel species, it is not treated further as the cultures proved to be sterile.

Phyllosticta sp.

Specimens examined: **Brazil**, São Paulo, Pompeia, on living leaf of *Mangifera indica*, 14 May 2007, C. Glienke & D. Stringari CPC 17454; ibid., CPC 17455.

Notes: Although phylogenetically distinct (Fig. 2), both cultures of this species proved to be sterile, and thus are not treated further.

DISCUSSION

The resurrection of the *Phyllostictaceae*, and its separation from the *Botryosphaeriaceae* is justified based on morphology and DNA phylogeny (Crous *et al.* 2006, Liu *et al.* 2012, Slippers *et al*. 2013, this volume). *Phyllosticta* is a well-established genus, distinct from genera in the *Phoma* complex (Aveskamp *et al*. 2010, de Gruyter *et al*. 2009, 2012, 2013), while the *Botryosphaeria* complex has also been shown to represent numerous genera (Crous *et al*. 2006, Phillips *et al*. 2008, Liu *et al*. 2012), and even families (Slippers *et al*. 2013, this volume).

Traditionally species of *Phyllosticta* have been chiefly identified by their host association. Several recent papers have shown that many traditional morphological species represent complexes of species, e.g. *P. citricarpa* on citrus, *P. musarum* on banana (Glienke *et al.* 2011, Wang *et al*. 2012), and *P. elongata* on *Vaccinium* (Zhang *et al*. 2013).

Freckle disease of banana was usually referred to in literature under its sexual name, *Guignardia musae*, or that of its purported asexual morph, *Phyllosticta musarum*. By employing multigene DNA analysis combined with a morphological comparison, Wong *et al.* (2012) demonstrated that these two names were not conspecific, and that the the common species occurring on banana cultivar Cavendish was in fact a novel taxon, which they described as *P. cavendishii*. The commonly occurring species in Southeast Asia and Oceania on non-Cavendish bananas was in fact another taxon, *P. maculata*. A third species on bananas, *P. musarum* was confirmed from India and Thailand. The most recent studies focusing on the taxonomy of *Phyllosticta* species associated with citrus black spot is that of Glienke *et al*. (2011) and Wang *et al*. (2012). Surprisingly, several species of *Phyllosticta* were shown to cause these symptoms on *Citrus*, although there was a difference in their host range and preference. The citrus black spot pathogen which is presently subjected to phytosanitary legislation in the EU and United States, *P. citricarpa*, was isolated from lemons, mandarins and oranges in China, although Wang *et al*. (2012) did define two subclades, one from mandarins, and another from oranges and lemons. *Phyllosticta citriasiana* was newly described on *Citrus maxima* in Asia by Wulandari *et al.* (2009), while Glienke *et al*. (2011) described *P. citribraziliensis* on *Citrus limon* from Brazil. Wang *et al.* (2012) also described *P. citrichinaensis* on *C. maxima* and *C. reticulata* from China. The present study adds yet a fifth species to this complex, namely *P. citrimaxima*, which is associated with tan spots on the fruit rind of *Citrus maxima* in Thailand. When considering that *P. capitalensis* can still co-occur as an endophyte in fruit or leaf lesions caused by these five species (Wikee *et al*. 2013), it is clear that these taxa are best distinguished by DNA sequence data. This has important biosecurity implications for the *Citrus* industries in many countries.

Guignardia philoprina (asexual morph *P. concentrica*) has been known as the taxon occurring on hosts such as *Rhododendron*, *Hedera*, *Ilex, Magnolia,* and *Taxus* (von Arx & Müller 1954). Not surprisingly, this turned out to represent a species complex, with numerous names available for consideration under the sexual and asexual morph. Although some of these names have been resurrected and applied in the present study, e.g. *P. concentrica* on *Hedera helix*, *P. foliorum* on *Taxus*, and *P. philoprina* on *Ilex*, many taxa still need to be recollected to resolve their phylogeny and correct taxonomy.

One aim of the present paper was to employ multigene DNA sequence analysis to discriminate among all species of *Phyllosticta* that were available to us from the CBS culture collection, supplemented by our own working collections, which resulted in a total of 160 strains. In addition to dealing with old synonymies that represented names that had to be resurrected, a further challenge has been to also merge *Phyllosticta* and *Guignardia* epithets, to obtain the best possible unit nomenclature for these species (Wingfield *et al.* 2012). In the present study we described 12 novel species, and designated a further eight epitype or neotype specimens. From the results obtained here, it is clear that in the case of epitypification, epitypes need to be designated based on the same host, recollected in the same geographic region (see Cannon *et al.* 2012). This is extremely difficult, as American names are commonly used for European of Asian taxa, and also vice versa (see the same situation in *Cercospora* and *Pseudocercospora*; Crous *et al*. 2013, Groenewald *et al*. 2013). In these cases the application of names to collections from other countries that appear morphologically similar, can at best be regarded as tentative, pending further collections.

Results obtained here clearly show that a multi-gene approach works well for distinguishing these taxa. In this study the intron dominated genes (ITS, ACT, TEF), and highly conserved gene coding regions (LSU, GPDH) were used. The result from the five gene analysis compared with the two gene analysis were similar (Figs 1, 2), indicating that the phylogeny of *Phyllosticta* derived from the ITS and ACT gene loci is sufficiently robust to distinguish most taxa, except those closely related to *P. capitalensis*. The biggest challenge ahead is to recollect specimens representative of the more than 3 000 names that exist in this complex.

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Phylogenetic lineages in the *Botryosphaeriales*: a systematic and evolutionary framework

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Abstract: The order *Botryosphaeriales* represents several ecologically diverse fungal families that are commonly isolated as endophytes or pathogens from various woody hosts. The taxonomy of members of this order has been strongly influenced by sequence-based phylogenetics, and the abandonment of dual nomenclature. In this study, the phylogenetic relationships of the genera known from culture are evaluated based on DNA sequence data for six loci (SSU, LSU, ITS, EF1, BT, mtSSU). The results make it possible to recognise a total of six families. Other than the *Botryosphaeriaceae* (17 genera), *Phyllostictaceae* (*Phyllosticta*) and *Planistromellaceae* (*Kellermania*), newly introduced families include *Aplosporellaceae* (*Aplosporella* and *Bagnisiella*), *Melanopsaceae* (*Melanops*), and *Saccharataceae* (*Saccharata*). Furthermore, the evolution of morphological characters in the *Botryosphaeriaceae* were investigated via analysis of phylogeny-trait association. None of the traits presented a significant phylogenetic signal, suggesting that conidial and ascospore pigmentation, septation and appendages evolved more than once in the family. Molecular clock dating on radiations within the *Botryosphaeriales* based on estimated mutation rates of the rDNA SSU locus, suggests that the order originated in the Cretaceous period around 103 (45–188) mya, with most of the diversification in the Tertiary period. This coincides with important periods of radiation and spread of the main group of plants that these fungi infect, namely woody Angiosperms. The resulting host-associations and distribution could have influenced the diversification of these fungi.

Key words: *Aplosporellaceae*, *Melanopsaceae*, molecular dating, *Phyllostictaceae*, *Planistromellaceae*, *Saccharataceae*, systematics.
Taxonomic novelties: New families – *Aplosporellaceae* Slippers, Boissin & Crous, *Melanopsaceae* Phillips, Slippers, Boissin & Crous, *Saccharataceae* Slippers, Boissin & Crous.

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INTRODUCTION

DNA sequence-based phylogenetics has dramatically influenced both the taxonomy and systematics of the *Botryosphaeriaceae* during the course of the past decade (Crous *et al*. 2006), as it has done in most other groups of *Fungi* (James *et al*. 2006, Hibbett *et al.* 2007). At a higher taxonomic level, DNA sequence data have led to the recognition that the *Botryosphaeriaceae* represents a distinct order within the *Dothideomycetes*, leading Schoch *et al*. (2006) to introduce the *Botryosphaeriales*. The circumscription of the *Botryosphaeriales* has suffered from insufficient sampling and it was only recently that Minnis *et al*. (2012) provided molecular evidence to show that the *Planistromellaceae* resides in this order. In a subsequent study, Liu *et al*. (2012) provided a comprehensive phylogenetic analysis of genera in the *Botryosphaeriales* and they also concluded that, other than the *Botryosphaeriaceae* and *Planistromellaceae*, a number of clearly defined evolutionary lineages exist.

Apart from the *Planistromellaceae*, the genera traditionally associated with *Botryosphaeria* and *Phyllosticta* have sexual morphs that are clearly distinct phylogenetically, morphologically and ecologically. However, both are still grouped within the *Botryosphaeriaceae*. Members of the *Botryosphaeria* group are common endophytes of leaf and woody tissue of many woody plant species, have hyaline to dark ascospores, multilocular ascomata, and a wide range of asexual morphs that typically lack a mucoid sheath and apical appendage. Species in the *Guignardia* group (= *Phyllosticta*) typically infect leaves and fruit, less commonly wood, have unilocular ascomata with smaller ascospores that typically have mucoid appendages, and *Phyllosticta* asexual morphs. The *Phyllostictaceae* has been resurrected to accommodate this group of taxa (see Wikee *et al*. 2013b, this volume).

Substantial changes to the definition of sexual and asexual genera linked to the *Botryosphaeriaceae* have been made during the past decade (e.g. Crous *et al*. 2006, Phillips *et al*. 2008, Liu *et al.* 2012). Only a selection of the most common examples is discussed here. The first DNA sequence data for the *Botryosphaeriaceae* appeared to reveal a distinction between asexual morphs with hyaline fusicoccum-like conidia and those with pigmented diplodia-like conidia, termed sections *Hyala* and *Brunnea* (Jacobs & Rehner 1998, Denman *et al*. 2000, Zhou & Stanosz 2001). This distinction became increasingly less obvious as sampling increased and it was evident that conidial pigmentation is a feature that evolved more than once. It was, for example, shown that dark, septate and even muriformly septate dichomera-like conidia could be synasexual morphs of well-known genera such as *Fusicoccum*

and *Neofusicoccum* (Barber *et al*. 2005, Phillips *et al.* 2005). Furthermore, dark, septate ascospores were shown to be a polyphyletic character of several genera and more common than previously believed (Phillips *et al.* 2008). As the true phylogenetic diversity within the group emerged, a number of new genera were described (e.g. *Botryobambusa*, *Cophinforma*, *Neofusicoccum*, *Neoscytalydium*, *Pseudofusicoccum*, etc.) or older genera re-defined (e.g. *Auerswaldia*, *Barriopsis*, *Dothiorella*, etc.) (e.g. Crous *et al*. 2006, Phillips *et al*. 2008, Liu *et al.* 2012). The most recent work by Liu *et al*. (2012) reviewed these genera, and this study reflects a growing consensus regarding the circumscription of the majority of the genera (29 in total, of which sequence data are available for 20).

DNA-based sequence analyses have also resulted in significant changes to the nomenclature, identification and circumscription of species in the *Botryosphaeriaceae*. These changes have resulted in the implementation of a single nomenclature for all morphs of a species (Crous *et al.* 2006, Hawksworth *et al*. 2011, Wingfield *et al*. 2012). For the *Botryosphaeriaceae*, this has included the description of cryptic species based on DNA sequence data, where morphological characters were not variable enough for this purpose (Pavlic *et al*. 2009a, Sakalidis *et al.* 2011).

Insights gained from contemporary studies on the *Botryosphaeriaceae* have led to uncertainty regarding the application of names published in the older literature. The analyses show for example that morphological characters typically used for species identification (chiefly conidia or ascospore dimensions, shape, septation and pigmentation) are frequently unreliable. Even ecological and geographical data are difficult to interpret, with some species occurring on numerous hosts, and single locations or hosts often yielding numerous co-occurring species (Slippers & Wingfield 2007, Slippers *et al*. 2009). For this reason (together with the significant changes in generic descriptions mentioned above) many, if not most, of the taxa dealt with before the introduction of DNA sequence-based phylogenetic inference will need to be redefined (possibly neo- or epitypified), to allow meaningful comparisons with currently applied names (also see the discussion in Phillips *et al*. 2013, this volume). Where it is not possible to follow this approach, older names may have to be ignored and new species introduced that are supported by DNA data (see Slippers *et al.* 2014).

The *Botryosphaeriales* is an important group of fungi due to the ecological and economic significance of many of its species. All species are plant-associated, and many are classified as pathogens, known to cause disease on a wide range of ecologically and economically important plants (Mehl *et al.* 2012). Some species are also known to cause opportunistic infections in humans (de Hoog *et al.* 2000). Most species exist as endophytes living in healthy plant tissues for extended periods of time (Slippers & Wingfield 2007). Their roles as endophytes or pathogens often overlap, as is for example found in the case of *Diplodia sapinea*. This well-known pathogen of *Pinus* (Swart *et al*. 1991) is also a common endophyte in branches, the trunks and seed cones of these trees. In an extreme example, *D. sapinea* has been isolated from the wood of *Pinus* in South Africa, where it must have existed without causing disease subsequent to the tree being infected as long as a decade previously (Bihon *et al.* 2011).

Unlike the case for *D. sapinea*, the ecological roles for the majority of species of *Botryosphaeriaceae* are unknown. The changes to the taxonomy of the group are already strongly promoting an ability to characterise the diversity in this group. In turn, this is providing an evolutionary framework making it possible to study the ecological role that remains obscure for the majority of these fungi.

In this paper, the phylogenetic relationships of all the genera known from culture and considered to reside in the *Botryosphaeriales* and *Botryosphaeriaceae* are determined based on DNA sequence data for six loci. The *Planistromellaceae* is well defined within the *Botryosphaeriales*. As expected, *Phyllosticta* (= *Guignardia*) also forms a strongly supported monophyletic lineage, recognised as the *Phyllostictaceae* (see Wikee *et al*. 2013b, this volume). *Saccharata*, however, groups separately with respect to all other genera in the *Botryosphaeriales*, as does *Aplosporella, Bagnisiella* and *Melanops*. The nomenclatural changes necessary to reflect these distinctions are considered in this study. With the well-supported phylogeny provided by these analyses, we also test hypotheses regarding the evolution of major morphological features typically used in taxonomy of the *Botryosphaeriaceae*. Finally, we use the nuclear ribosomal subunit data to date the divergence in the major groups of the *Botryosphaeriales*.

MATERIALS AND METHODS

Isolates and DNA extractions

A total of 96 strains corresponding to 85 species were grown on 2 % potato dextrose agar (PDA) plates incubated at 25 ºC. Genomic DNA was extracted from mycelium using the PrepMan™ Ultra protocol (Applied Biosystems). Sequences from additional species were retrieved from GenBank. A total of 140 taxa were included in the ingroup and six taxa in the outgroup (see Table 1 for details).

PCR and sequencing

A total of six partial gene portions were used in this study: the nuclear ribosomal small subunit (SSU), the nuclear ribosomal large subunit (LSU), the intergenic spacer (ITS), the translation elongation factor 1-alpha (EF1), the β-tubulin gene (BT) and the mitochondrial ribosomal small subunit (mtSSU).

The primers used were NS1 and NS4 (White *et al*. 1990) for SSU, LROR and LR5 (Vilgalys Laboratory, Duke university, www.biology.duke.edu/fungi/mycolab/primers.htm) for LSU, ITS-1 and ITS-4 (White *et al*. 1990) for ITS, EF-AF and EF-BR (Sakalidis *et al*. 2011) for EF1, BT2A and BT2B (Glass & Donaldson 1995) for BT and mrSSU1 and mrSSU3R (Zoller *et al.* 1999) for mtSSU. All PCR reactions were conducted in 15 µL containing 1.5 mM of $MgCl_2$, 0.5 mM of dNTP, 1 × final concentration of buffer, 1 µM of each primer, 0.25 U of FastStart *Taq* Polymerase (Roche), 1.5 µL of DNA template and Sabax sterilised water (Adcock Ingram) to complete up to 15 µL. The cycling parameters were as follows: a first step of denaturation at 95 ºC for 5 min followed by 35 cycles of (i) denaturation at 95 ºC for 60 s, (ii) annealing at optimal temperature (55 ºC for ITS, EF1, LSU and 45 ºC for SSU, mtSSU, BT) for 80 s, (iii) elongation at 72 ºC for 90 s, and a final elongation step of 5 min was applied.

Sephadex columns (Sigma-Aldrich) were used to clean the samples both before and after the sequencing reactions. The sequencing PCRs were performed in 10 µL containing 1 µL of PCR product, 0.7 µL of Big Dye Terminator v. 3.1 (Applied Biosystems), 2.5 µL of sequencing buffer (provided with Big Dye), 1 µL of primer (10 µM) and 4.8 µL of Sabax sterilised water. Cycling parameters consisted of 25 cycles with three steps each: 15 s at 95 ºC, 15 s at 55 ºC (for ITS, EF1, LSU) or 45 °C (for SSU, mtSSU, BT) and 4 min at 60 ºC. The sequencing PCR products were sent to a

partner laboratory for sequencing of both strands (Sequencing Facility, University of Pretoria).

Data analyses

Sequences were aligned using MAFFT v. 6 online (Katoh & Toh 2008) and refined visually. In order to retrieve the genetic information from indels, GapCoder (Young & Healy 2003) was used to code the gaps contained in the EF1 and the mSSU alignments. Analyses were run both with gaps coded and not coded.

The datasets were combined because this is believed to increase phylogenetic accuracy (Bull *et al*. 1993, Cunningham 1997). The six phylogenies resulting from the six data sets were first inspected visually to check whether there were conflicts between the histories of the genes that would preclude the combination of data. Additionally, the Incongruence Length Difference (ILD) test (Farris *et al.* 1995a, b) also known as the partition homogeneity test was run using PAUP v. 4.0b10 (Swofford 2003).

MrAIC (Nylander 2004) was used to determine the best fit model of nucleotide substitutions for each gene. Phylogenetic relationships of the samples were investigated using both Maximum Likelihood (ML) and Bayesian Inference (BI). The ML analysis was conducted using PhyML online (Guindon *et al.* 2005). The reliability of each node was assessed using the bootstrap (Felsenstein 1985) resampling procedure (100 replicates).

The BI was conducted using the software BEAST v. 1.7.4 (Drummond *et al*. 2012). The phylogenetic relationships were estimated by running 10 000 000 generations and sampling every 100th generation. Bayes Factors were computed to choose between the different options available in BEAST (four clock models: strict clock, exponential or lognormal uncorrelated relaxed clocks, and random local clock and two tree priors: Yule process or Birth-Death speciation model). The Birth-Death speciation model and a relaxed uncorrelated exponential clock were selected as best fitting our data. Six independent runs were performed and outputs were combined using LogCombiner (in the BEAST package). The programme TRACER v. 1.5 (available on the BEAST website) was used to check that the effective sampling sizes (ESS) were above 200 (as advised by the programmers to ensure an accurate estimation of phylogeny and parameters of interest). The programme Tree Annotator (available in the BEAST package) was used to summarize the resulting trees using the maximum clade credibility option. The final tree was visualised in FigTree v. 1.4.

Molecular clock dating

Using a mean molecular rate of 1–1.25 % per lineage per 100 million years, commonly accepted in fungi for the SSU gene (Berbee & Taylor 2010), a rough estimation of the times to the most recent common ancestors of groups of interest was assessed using BEAST. A normal distribution was used as prior and this was centred on a 95 % interval spanning 1.0–1.25 % (mean = 0.000113; standard deviation = 0.000006). The dating of the distinct groups of interest and their 95 % highest posterior density (HPD) were retrieved using TRACER v. 1.5.

Analysis of phylogeny-trait association

In order to investigate the evolution of morphological characters in the *Botryosphaeriaceae*, ancestral trait reconstructions and tests for phylogenetic signal were conducted in Mesquite v. 2.74 (Maddison & Maddison 2010). The characters considered were 1) ascospore colour; 2) presence or absence of ascospore septa; 3) conidial colour; 4) presence or absence of conidial septa; and 5) presence or absence of a mucus sheath. Both parsimony and ML reconstructions were used in Mesquite to test for phylogenetic signal. The observed distribution of character states at the tips of the phylogeny was compared to null distributions obtained when reshuffling the tip characters on the tree topology (10 000 times). Where the number of steps (or likelihood value) in the observed trait reconstruction fell outside the 95 % range of the null distribution, this was seen to indicate that character states are not distributed randomly on the phylogeny (i.e. there is a phylogenetic component).

RESULTS

Phylogenetic relationships

The alignment included a total of 4498 bp from six gene portions. The results from the ILD test were not significant and supported a decision to combine the 6 gene datasets. The number of polymorphic and parsimony informative sites ranged from 108 for the SSU, 165 for the mtSSU, 170 for the LSU, 222 for BT, 335 for the EF1 to 361 for the ITS.

The Maximum Likelihood (ML) and Bayesian Inference (BI) phylogenetic reconstructions were similar, and the BI tree is shown on Fig. 1. Species residing in the genera *Aplosporella* and *Bagnisiella* (in one clade), *Melanops, Saccharata* and *Kellermania* had a basal position on the tree with respect to other genera in the *Botryosphaeriales*. The remaining genera in the *Botryosphaeriales* clustered together with a bootstrap support value of 94 % and Posterior Probability (PP) value of 1. The first cluster to split from the rest of the main group was formed by species of *Phyllosticta*, hereafter treated as *Phyllostictaceae* (see Wikee *et al*. 2013b, this volume). The main clade below *Phyllostictaceae* was defined as *Botryosphaeriaceae* s. str. (0.99 PP and 89 % bootstrap).

Pseudofusiccocum was basal within the *Botryosphaeriaceae*, followed by *Endomelanconiopsis*. The remaining species formed a clade having strong bootstrap support v (99 %) and could be further subdivided into four sub-clades. Sub-clade 1 encompassed species of the genera *Diplodia*, *Neodeightonia*, *Lasiodiplodia*, *Macrovalsaria*, *Phaeobotryosphaeria*, *Phaeobotryon*, *Barriopsis*, *Botryobambusa* and *Tiarosporella*. The recently described *Auerswaldia lignicola* clustered in sub-clade 1, together with *Lasiodiplodia*. Sub-clade 2 encompassed species of *Neoscytalidium*, *Cophinforma*, *Botryosphaeria* (= *Fusicoccum*) and *Macrophomina* together with *Dichomera saubinetti*. Sub-clade 3 accommodated species in the genera *Dothiorella*, *Spencermartinsia* and the recently described *Auerswaldia dothiorella*. Sub-clade 4 included species of *Neofusicoccum* and *Dichomera.*

Molecular dating

Based on the models used, families split between 57 (28–100) – 103 (45–188) mya. Divergence within some families was also very ancient, such as the split between *Pseudofusicoccum* [65 (28–112) mya] and *Endomelanconiopsis* [52 (27–78) mya] and the rest of the *Botryosphaeriaceae*. The split between sub-clades 1 to 4 within the *Botryosphaeriaceae* was estimated to be between 33 (15–55) and 44 (25–64) mya.

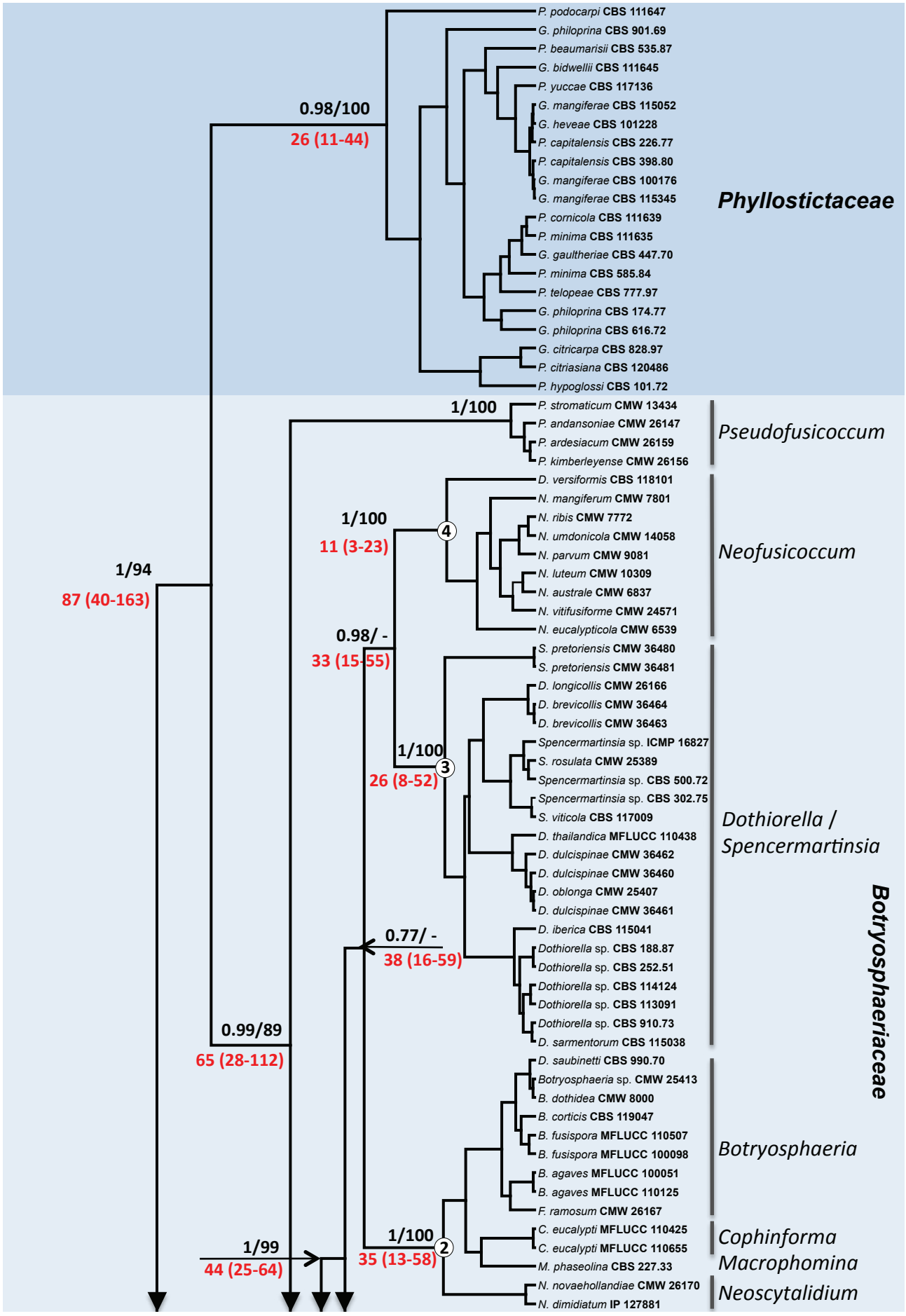

Fig. 1. Phylogenetic relationships of the *Botryosphaeriales* using Bayesian reconstruction and six gene portions (LSU, SSU, ITS, EF1, BT and mtSSU). Numbers above branches indicate bootstrap values/posterior probabilities. Numbers highlighted in red below branches indicate estimated dates in million years with the 95 % Highest Posterior Density interval given in brackets. Clades 1–4 in the *Botryosphaeriaceae* are indicated by a circled number on the corresponding node.

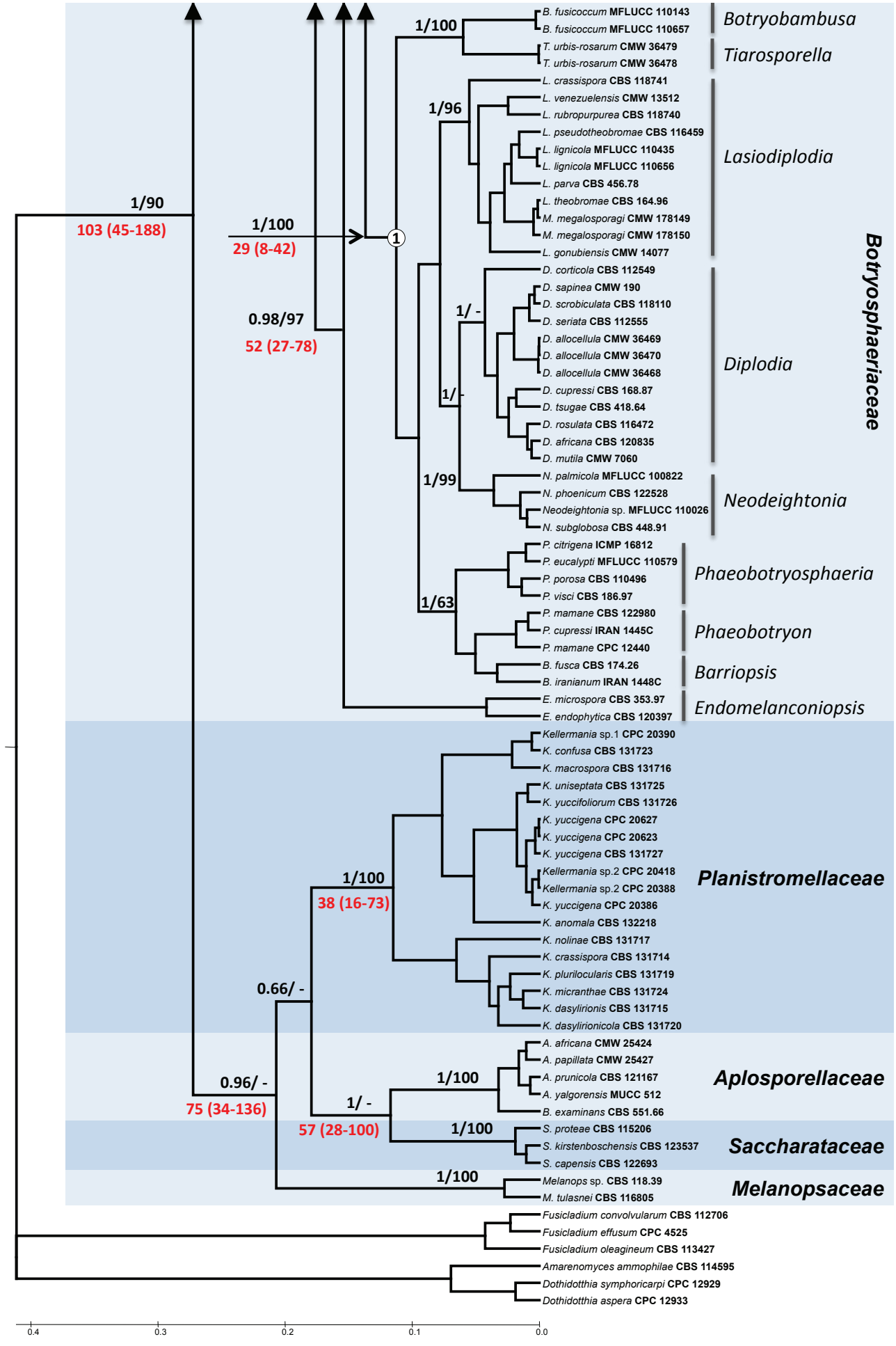

Fig. 1. (Continued).

Table 1. Isolates subjected to DNA analysis in this study.

Species	Isolate No.[1]	GenBank Accession No.					
		SSU	LSU	ITS	EF1	BT	mtSSU
Amarenomyces ammophilae	CBS 114595	N/A	KF766314	KF766146	KF766394	N/A	N/A
Aplosporella africana	CMW 25424, CBS 121777	KF766283	KF766366	KF766196	N/A	N/A	KF766475
Aplosporella papilata	CMW 25427, CBS 121780	KF766284	N/A	KF766197	N/A	N/A	KF766476
Aplosporella prunicola	CBS 121167	KF766229	KF766315	KF766147	N/A	N/A	KF766440
Aplosporella yalgorensis	MUCC512	N/A	EF591944	EF591927	EF591978	EF591961	
							N/A
Bagnisiella examinans	CBS 551.66	EU167562	KF766316	KF766148	GU349056	KF766126	KF766441
Barriopsis fusca	CBS 174.26	KF766230	KF766317	KF766149	KF766395	EU673109	N/A
Barriopsis iranianum	IRAN 1448C	KF766231	KF766318	KF766150	FJ919652	KF766127	N/A
Botryobambusa fusicoccum	MFLUCC110143	JX646826	JX646809	JX646792	JX646857	N/A	N/A
	MFLUCC110657	JX646827	JX646810	JX646793	JX646858	N/A	N/A
Botryosphaeria agaves	MFLUCC100051	JX646824	JX646807	JX646790	JX646855	JX646840	N/A
	MFLUCC110125	JX646825	JX646808	JX646791	JX646856	JX646841	N/A
Botryosphaeria corticis	CBS 119047	KF766232	EU673244	DQ299245	EU017539	EU673107	N/A
Botryosphaeria dothidea	CMW 8000, CBS 115476	KF766233	KF766319	KF766151	AY236898	AY236927	FJ190612
Botryosphaeria fusispora	MFLUCC100098	JX646823	JX646806	JX646789	JX646854	JX646839	N/A
	MFLUCC110507	JX646822	JX646805	JX646788	JX646853	JX646838	N/A
Botryosphaeria ramosa	CMW 26167	KF766253	KF766333	KF766168	EU144070	N/A	N/A
Botryosphaeria sp.	CMW 25413	KF766252	KF766332	KF766167	N/A	N/A	N/A
Cophinforma eucalypti	MFLUCC110425	JX646833	JX646817	JX646800	JX646865	JX646848	N/A
Dichomera saubinetii	CBS 990.70	KF766236	DQ377888	KF766153	KF766396	N/A	N/A
	MFLUCC110655	JX646834	JX646818	JX646801	JX646866	JX646849	N/A
Dichomera versiformis	CMW 15210, CBS 118101	KF766237	KF766321	KF766154	N/A	KF766128	N/A
Diplodia africana	CBS 120835	KF766238	KF766322	KF766155	KF766397	KF766129	KF766442
Diplodia allocellula	CBS 36468	N/A	JQ239410	JQ239397	JQ239384	JQ239378	N/A
	CBS 36469	N/A	JQ239411	JQ239398	JQ239385	JQ239379	N/A
	CBS 36470	N/A	JQ239412	JQ239399	JQ239386	JQ239380	N/A
Diplodia corticola	CBS 112549	KF766239	KF766323	KF766156	AY573227	DQ458853	KF766443
					KF766398		
Diplodia cupressi	CBS 168.87	KF766240	EU673263	KF766157	DQ458878	DQ458861	KF766444
Diplodia mutila	CMW 7060	KF766241	KF766324	KF766158	AY236904	AY236933	N/A
Diplodia rosulata	CBS 116472	EU673212	DQ377897	EU430266	EU430268	EU673131	N/A
Diplodia sapinea	CMW 109	KF766242	KF766325	KF766159	AY624251	AY624256	N/A
Diplodia scrobiculata	CMW 189, CBS 118110	KF766243	KF766326	KF766160	N/A	N/A	KF766445
Diplodia seriata	CBS 112555	KF766244	KF766327	KF766161	AY573220	DQ458856	N/A
Diplodia tsugae	CMW 100325, CBS 418.64	KF766234	DQ377867	DQ458888	DQ458873	DQ458855	N/A
Dothidotthia aspera	CPC 12933	EU673228	EU673276	N/A	N/A	N/A	N/A
Dothidotthia symphoricarpi	CPC 12929	EU673224	EU673273	N/A	N/A	N/A	N/A
Dothiorella brevicollis	CMW 36463	N/A	JQ239416	JQ239403	JQ239390	JQ239371	N/A
	CMW 36464	N/A	JQ239417	JQ239404	JQ239391	JQ239372	N/A
Dothiorella dulcispinae	CMW 36460	N/A	JQ239413	JQ239400	JQ239387	JQ239373	N/A
	CMW 36461	N/A	JQ239414	JQ239401	JQ239388	JQ239374	N/A
	CMW 36462	N/A	JQ239415	JQ239402	JQ239389	JQ239375	N/A
Dothiorella iberica	CBS 115041	KF766245	AY928053	AY573202	AY573222	EU673096	N/A
Dothiorella longicollis	CMW 26166, CBS 122068	KF766246	KF766328	KF766162	EU144069	KF766130	KF766447
Dothiorella oblonga	CMW 25407, CBS 121765	KF766247	KF766329	KF766163	N/A	N/A	KF766448
Dothiorella sarmentorum	CBS 115038	KF766248	DQ377860	AY573206	AY573223	EU673101	KF766446
Dothiorella sp.	CBS 114124	EF204515'	EF204498'	N/A	N/A	N/A	N/A
	CBS 113091	EF204516'	EF204499'	N/A	N/A	N/A	N/A
Dothiorella sp. (=*Diplodia acerina*)	CBS 910.73	EU673160	EU673234	EU673315	EU673282	N/A	N/A

Table 1. (Continued).

Species	Isolate No.[1]	GenBank Accession No.					
		SSU	LSU	ITS	EF1	BT	mtSSU
Dothiorella sp. (=*Diplodia coryli*)	CBS 252.51	EU673162	EU673235	EU673317	EU673284	EU673105	N/A
Dothiorella sp. (=*Diplodia juglandis*)	CBS 188.87	EU673161	DQ377891	EU673316	EU673283	EU673119	N/A
Dothiorella thailandica	MFLUCC110438	JX646829	JX646813	JX646796	JX646861	JX646844	N/A
Endomelanconiopsis endophytica	CBS 120397	KF766249	EU683629	KF766164	EU683637	KF766131	KF766449
Endomelanconiopsis microspora	CBS 353.97	KF766250	KF766330	KF766165	EU683636	N/A	KF766450
Fusicladium convolvularum	CBS 112706	AY251124	N/A	AY251082	N/A	N/A	N/A
Fusicladium effusum	CPC 4525	N/A	EU035430'	AY251085	KF766428	N/A	N/A
Fusicladium oleagineum	CBS 113427	KF766251	KF766331	KF766166	N/A	N/A	N/A
Guignardia bidwellii (= *Phyllosticta parthenocissi*)	CBS 111645	EU673223	DQ377876	EU683672	EU683653	FJ824777	N/A
Guignardia citricarpa (= *Phyllosticta citricarpa*)	CBS 828.97	KF766254	KF766334	FJ538318	FJ538376	N/A	N/A
Guignardia gaultheriae	CBS 447.70	N/A	KF766335	KF766169	KF766400	N/A	FJ190646
Guignardia heveae (= *Phyllosticta capitalensis*)	CBS 101228	KF766255	KF766336	FJ538319	FJ538377	N/A	KF766452
Guignardia mangiferae (= *Phyllosticta capitalensis*)	CBS 100176	N/A	KF766337	FJ538321	FJ538379	N/A	N/A
	CBS 115052	N/A	KF766338	FJ538321	FJ538379	N/A	N/A
	CBS 115345	N/A	KF766339	FJ538331	FJ538389	N/A	N/A
Guignardia philoprina (= *Guignardia rhodorae*)	CBS 901.69	KF766258	KF766342	KF766172	KF766403	N/A	N/A
Guignardia philoprina (= *Phyllosticta foliorum*)	CBS 174.77	KF766256	KF766340	KF766170	KF766401	N/A	KF766453
Guignardia philoprina (= *Phyllosticta philoprina*)	CBS 616.72	KF766257	KF766341	KF766171	KF766402	N/A	N/A
Kellermania anomala	CBS 132218	KF766259	KF766343	KF766173	KF766404	KF766133	KF766454
Kellermania confusa	CBS 131723	KF766260	KF766344	KF766174	KF766405	KF766134	KF766455
Kellermania crassispora	CBS 131714	KF766261	KF766345	KF766175	KF766406	KF766135	KF766456
Kellermania dasylirionicola	CBS 131720	KF766262	KF766346	KF766176	KF766407	KF766136	KF766457
Kellermania dasylirionis	CBS 131715	KF766263	KF766347	KF766177	KF766408	KF766137	KF766458
Kellermania macrospora	CBS 131716	KF766264	KF766348	KF766178	KF766409	KF766138	KF766459
Kellermania micranthae	CBS 131724	KF766265	KF766349	KF766179	KF766410	KF766139	KF766460
Kellermania nolinae	CBS 131717	KF766266	KF766350	KF766180	KF766411	KF766140	KF766461
Kellermania plurilocularis	CBS 131719	KF766267	KF766351	KF766181	KF766412	KF766141	KF766462
Kellermania sp. 1	CPC 20390	KF766268	KF766352	KF766182	KF766413	KF766142	KF766463
Kellermania sp. 2	CPC 20418	KF766269	KF766353	KF766183	KF766414	N/A	KF766464
	CPC 20386	KF766273	KF766357	KF766187	KF766418	N/A	KF766467
	CPC 20388	KF766274	KF766358	KF766188	KF766419	N/A	KF766468
Kellermania uniseptata	CBS 131725	KF766270	KF766354	KF766184	KF766415	KF766143	KF766465
Kellermania yuccifoliorum	CBS 131726	KF766271	KF766355	KF766185	KF766416	KF766144	N/A
Kellermania yuccigena	CBS 131727	KF766272	KF766356	KF766186	KF766417	KF766144	KF766466
	CPC 20623	KF766275	KF766359	KF766189	KF766420	N/A	KF766469
	CPC 20627	KF766276	KF766360	KF766190	KF766421	N/A	KF766470
Lasiodiplodia crassispora	CBS 118741	EU673190	DQ377901	DQ103550	EU673303	EU673133	N/A
Lasiodiplodia gonubiensis	CMW 14077, CBS 115812	KF766277	KF766361	KF766191	DQ458877	DQ458860	N/A
Lasiodiplodia lignicola	MFLUCC110435	JX646830	JX646814	JX646797	JX646862	JX646845	N/A
	MFLUCC110656	JX646831	JX646815	JX646798	JX646863	JX646846	N/A
Lasiodiplodia parva	CBS 456.78	KF766278	KF766362	KF766192	EF622063	N/A	KF766471
Lasiodiplodia pseudotheobromae	CBS 116459	KF766279	EU673256	KF766193	EF622057	EU673111	KF766481
Lasiodiplodia rubropurpurea	CBS 118740	EU673191	DQ377903	DQ103553	EU673304	EU673136	N/A
Lasiodiplodia theobromae (*Botryosphaeria rhodina* in CBS)	CBS 164.96	EU673196	EU673253	AY640255	AY640258	EU673110	N/A

Table 1. (Continued).

Species	Isolate No.[1]	GenBank Accession No.					
		SSU	LSU	ITS	EF1	BT	mtSSU
Lasiodiplodia venezuelensis	CMW 13512	KF766280	KF766363	KF766194	EU673305	N/A	N/A
Macrophomina phaseolina	CBS 227.33	KF766281	KF766364	KF766195	KF766422	N/A	KF766473
Macrovalsaria megalosporagi	CMW 178150	FJ215707	FJ215701	N/A	KF766399	N/A	N/A
	CMW 178149	FJ215706	FJ215700	N/A	N/A	N/A	N/A
Melanops sp. (*Botryosphaeria quercuum* in CBS)	CBS 118.39	FJ824763	DQ377856	FJ824771	FJ824776	FJ824782	N/A
Melanops tulasnei	CBS 116805	KF766282	KF766365	FJ824769	KF766423	FJ824780	KF766474
Neodeightonia palmicola	MFLUCC100822	HQ199223	HQ199222	HQ199221	N/A	N/A	N/A
Neodeightonia phoenicum	CBS 122528	KF766285	EU673261	KF766198	EU673309	EU673116	N/A
Neodeightonia sp.	MFLUCC110026	JX646837	JX646821	JX646804	JX646869	JX646852	N/A
Neodeightonia subglobosa	CBS 448.91	KF766286	DQ377866	KF766199	EU673306	EU673137	N/A
Neofusicoccum australe	CMW 6837	KF766287	KF766367	KF766200	AY339270	AY339254	KF766477
Neofusicoccum eucalypticola	CMW 6539, CBS 115679	KF766288	KF766368	KF766201	AY615133	AY615125	N/A
Neofusicoccum lutea	CMW 10309	KF766289	KF766369	KF766202	KF766424	DQ458848	N/A
Neofusicoccum mangiferum	CMW 7801	KF766290	KF766370	KF766203	KF766425	AY615174	KF766479
Neofusicoccum parvum	CMW 9081	KF766291	KF766371	KF766204	KF766426	AY236917	KF766480
Neofusicoccum ribis	CMW 7772, CBS 115475	KF766292	KF766372	KF766205	DQ677893	AY236906	KF766481
Neofusicoccum umdonicola	CMW 14058, CBS 123645	KF766293	KF766373	KF766206	KF766427	KF766145	KF766482
Neofusicoccum vitifusiforme	CMW 24571	KF766235	KF766320	KF766152	FJ752707	N/A	N/A
Neoscytalidium dimidiatum	IP127881	AF258603	DQ377925'	AY819727	EU144063	FM211167	N/A
Neoscytalidium novaehollandiae	CMW 26170, CBS 122071	KF766294	KF766374	KF766207	EF585580	N/A	N/A
Phaeobotryon cupressi	IRAN 1445C	KF766295	N/A	KF766208	N/A	N/A	N/A
Phaeobotryon mamane	CPC 12440	EU673184	EU673248	EU673332	EU673298	EU673121	KF766483
				KF766209			
	CBS 398.80	KF766301	KF766378	KF766213	KF766430	N/A	KF766486
Phaeobotryosphaeria citrigena (*Botryosphaeria fusca* in CBS)	ICMP 16812	EU673180	EU673246	EU673328	EU673294	EU673140	N/A
Phaeobotryosphaeria eucalypti	MFLUCC110579	JX646835	JX646819	JX646802	JX646867	JX646850	N/A
Phaeobotryosphaeria porosa	CBS 110496	KF766297	KF766375	KF766210	N/A	EU673130	N/A
Phaeobotryosphaeria visci	CBS 186.97	KF766298	KF766393	KF766211	EU673293	EU673128	N/A
Phyllosticta beaumarisii	CBS 535.87	KF766299	KF766376	KF766212	KF766429	N/A	KF766484
Phyllosticta capitalensis	CBS 226.77	KF766300	KF766377	FJ538336	FJ538394	N/A	KF766485
	CBS 398.80	N/A	N/A	N/A	N/A	N/A	N/A
Phyllosticta citriasiana	CBS 120486	KF766302	KF766379	FJ538360	FJ538418	N/A	N/A
Phyllosticta cornicola	CBS 111639	N/A	KF766380	KF766214	KF766431	N/A	KF766487
Phyllosticta hypoglossi	CBS 101.72	KF766303	KF766381	FJ538365	FJ538423	N/A	N/A
Phyllosticta minima	CBS 585.84	N/A	KF766382	KF766216	KF766433	N/A	N/A
Phyllosticta minima (= *Phyllosticta rubrum*)	CBS 111635	N/A	EU754194	KF766215	KF766432	N/A	N/A
Phyllosticta podocarpi	CBS 111647	KF766304	KF766383	KF766217	KF766434	N/A	N/A
Phyllosticta telopeae	CBS 777.97	N/A	KF766384	KF766218	KF766435	N/A	N/A
Phyllosticta yuccae	CBS 117136	KF766305	KF766385	KF766219	KF766436	N/A	N/A
Pseudofusicoccum adansoniae	CMW 26147, CBS 122055	KF766306	KF766386	KF766220	EF585571	N/A	KF766488
Pseudofusicoccum ardesiacum	CMW 26159, CBS 122062	KF766307	KF766387	KF766221	EU144075	N/A	KF766489
Pseudofusicoccum kimberleyense	CMW 26156, CBS 122058	KF766308	KF766388	KF766222	EU144072	N/A	KF766490
Pseudofusicoccum stromaticum	CMW 13434, CBS117448	KF766309	KF766389	KF766223	KF766437	EU673094	N/A
Saccharata capensis	CMW 22200, CBS 122693	N/A	KF766390	KF766224	EU552095	N/A	KF766491
Saccharata kirstenboschensis	CBS 123537	KF766310	FJ372409	KF766225	N/A	N/A	KF766492
Saccharata proteae	CBS 115206	KF766311	DQ377882	KF766226	KF766438	N/A	KF766493
Spencermartinsia pretoriensis	CMW 36480	N/A	JQ239418	JQ239405	JQ239392	JQ239376	N/A
	CMW 36481	N/A	JQ239419	JQ239406	JQ239393	JQ239377	N/A

Table 1. (Continued).

Species	Isolate No.[1]	GenBank Accession No.					
		SSU	LSU	ITS	EF1	BT	mtSSU
Spencermartinsia rosulata	CMW 25389, CBS 121760	KF766312	KF766391	KF766227	KF766439	N/A	KF766494
Spencermartinsia sp. (*Botryosphaeria* sp. in ICMP)	ICMP 16827	EU673171	EU673241	EU673322	EU673289	EU673144	N/A
Spencermartinsia sp. (*Diplodia medicaginis* in CBS)	CBS 500.72	EU673167	EU673237	EU673318	EU673285	EU673118	N/A
Spencermartinsia sp. (*Diplodia spegazziniana* in CBS)	CBS 302.75	N/A	EU673238	EU673319	EU673286	EU673135	N/A
Spencermartinsia vitícola	CBS 117009	KF766313	KF766392	KF766228	AY905559	EU673104	N/A
Tiarosporella urbis-rosarum	CMW 36478	N/A	JQ239421	JQ239408	JQ239395	JQ239382	N/A
	CMW 36479	N/A	JQ239422	JQ239409	JQ239396	JQ239383	N/A

[1]CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CMW: Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; IRAN: Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Iran; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Mai, Thailand; MUCC: Culture Collection, Laboratory of Plant Pathology, Mie University, Tsu, Mie prefecture, Japan.

Table 2. Test of phylogenetic signal for each of the 5 traits investigated using Parsimony and Maximum Likelihood reconstructions.

Phylogenetic Signal	Parsimony (number of steps)			Maximum Likelihood (-log likelihood values)		
	Observed	Expected Mean (Range)	P-value	Observed	Expected Mean (Range)	P-value
Ascospore colour	3	5,89 (3-7)	ns	11,35	12,87 (10,6-13,7)	ns
Ascospore septation	4	5,29 (3-6)	ns	11,7	12,79 (8,9-13,7)	ns
Conidial colour	6	8,99 (5-13)	ns	15,51	17,76 (15,1-18,4)	ns
Conidial septation	8	8,90 (5-12)	ns	18,02	17,77 (15,8-18,6)	ns
Mucus	4	3,98 (2-4)	ns	13,59	14,09 (8,0-18,1)	ns

Analysis of phylogeny-trait association

None of the five morphological traits that were investigated had a significant phylogenetic signal (Table 2; Fig. 2A–E).

Taxonomy

Based on the phylogenetic distinctions found in this study, as well as the morphological and in some cases ecological distinction between the major groups in the *Botryosphaeriales*, six families are recognised. Of these, the *Planistromellaceae* (accommodating *Kellermania*) and *Phyllostictaceae* (accommodating *Phyllosticta*) are accepted as previously described (Minnis *et al.* 2012, Wikee *et al.* 2013b, this volume). The *Botryosphaeriaceae* is redefined, while the *Aplosporellaceae*, *Saccharataceae* and *Melanopsaceae* are newly described.

Botryosphaeriaceae Theis. & P. Syd., Ann. Mycol. 16: 16. 1918.

Type genus: *Botryosphaeria* Ces. & De Not., Comment. Soc. Crittog. Ital. 1: 211. 1863.

Type species: *B. dothidea* (Moug. : Fr.) Ces. & De Not., Comment. Soc. Crittog. Ital. 1: 212. 1863.

Genera included based on support by DNA sequence data: *Barriopsis, Botryobambusa, Botryosphaeria* (= *Fusicocccum*, incl. *Dichomera pro parte*), *Cophinforma, Dothiorella, Diplodia, Endomelanconiopsis, Lasiodiplodia* (incl. *Auerswaldia, Macrovalsaria*), *Macrophomina, Neodeightonia, Neofusicoccum* (incl. *Dichomera pro parte*), *Neoscytalidium, Phaeobotryon, Phaeobotryosphaeria, Pseudofusicoccum, Spencermartinsia, Tiarosporella.*

Genera lacking DNA sequence data: *Auerswaldiella, Leptoguignardia, Microdiplodia, Phyllachorella, Pyrenostigma, Septorioides, Sivanesania, Thyrostroma, Vestergrenia* (Liu *et al*. 2012).

Ascostromata uni- to multilocular, solitary or in clusters, fully or partially erumpent at maturity, with multi-layered, dark brown walls, infrequently embedded in stromatic tissue. *Asci* bitunicate, fissitunicate, chiefly 8-spored, with a thick endotunica and well-developed apical chamber, short stipitate, clavate. *Pseudoparaphyses* intermixed with asci, hyaline, septate, frequently constricted at septa, hyphae-like, branched or not, frequently deliquescing at maturity. *Ascospores* 2–3 seriate, hyaline to pigmented, smooth to verruculose, septate or not, fusoid to ellipsoid or ovoid, with or without a mucoid sheath or rarely with appendages. *Asexual morphs* mostly have uni-, rarely multilocular pycnidial *conidiomata*, infrequently embedded in stromatic tissue. *Conidiophores* mostly reduced to conidiogenous cells. *Conidiogenous cells* hyaline, phialidic, proliferating percurrently or via periclinal thickening, with or without collarettes. *Conidia* hyaline to pigmented, aseptate, one or multi-septate, sometimes muriform, smooth or striate, thin to thick-walled, and sometimes with mucoid sheaths or appendages. *Synasexual morphs* coelomycetous or hyphomycetous (see Crous *et al*. 2006). *Spermatogonia* similar to conidiomata in anatomy. *Spermatogenous cells* ampulliform to lageniform or subcylindrical, hyaline smooth, phialidic. *Spermatia* developing in conidiomata or spermatogonia, hyaline, smooth, granular, subcylindrical or dumbbell-shaped, with rounded ends.

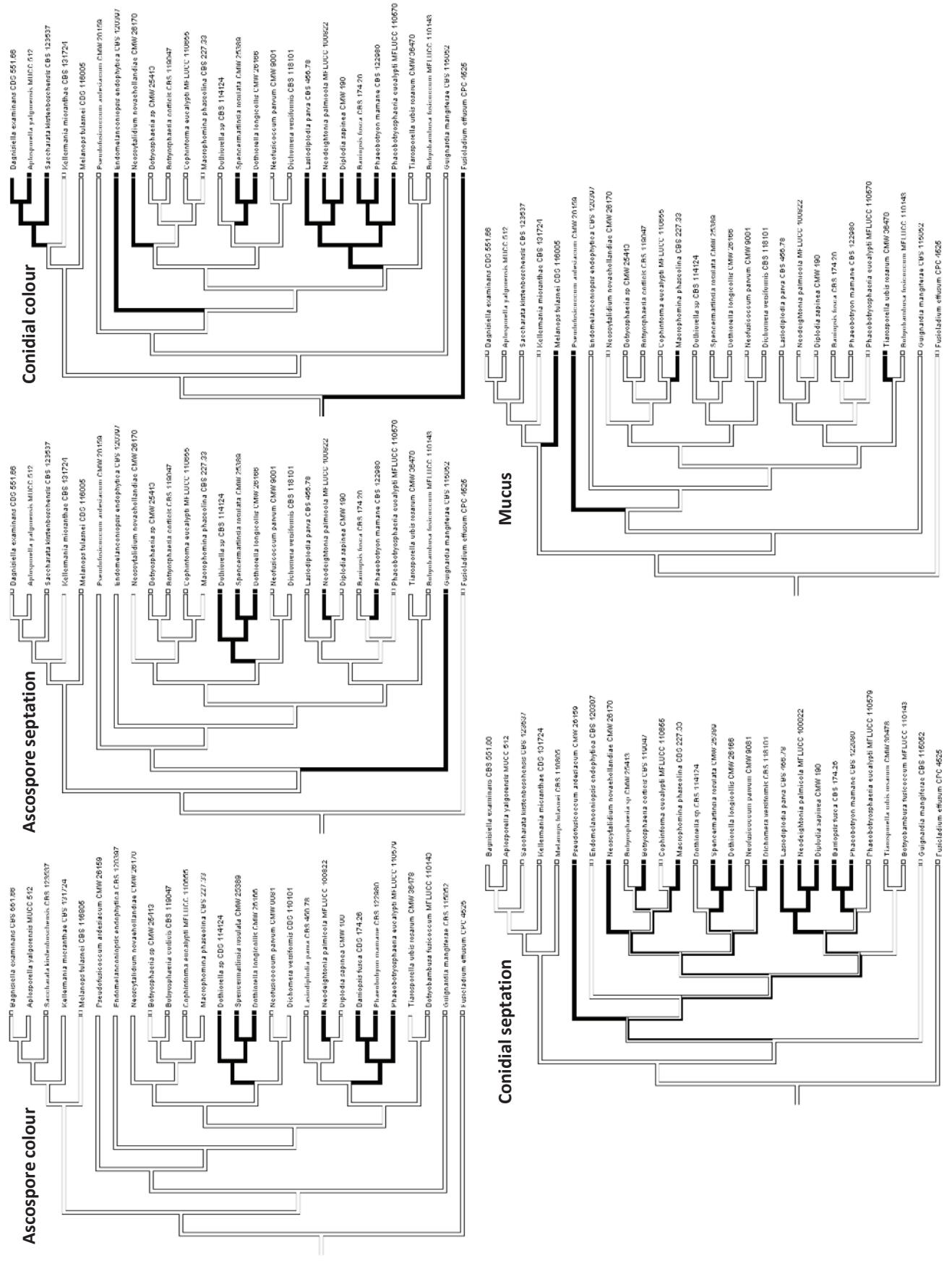

Fig. 2. Ancestral state reconstruction using parsimony and mapping the five traits onto the phylogenetic tree. Traits are coded as presence (in black) or absence (in white).

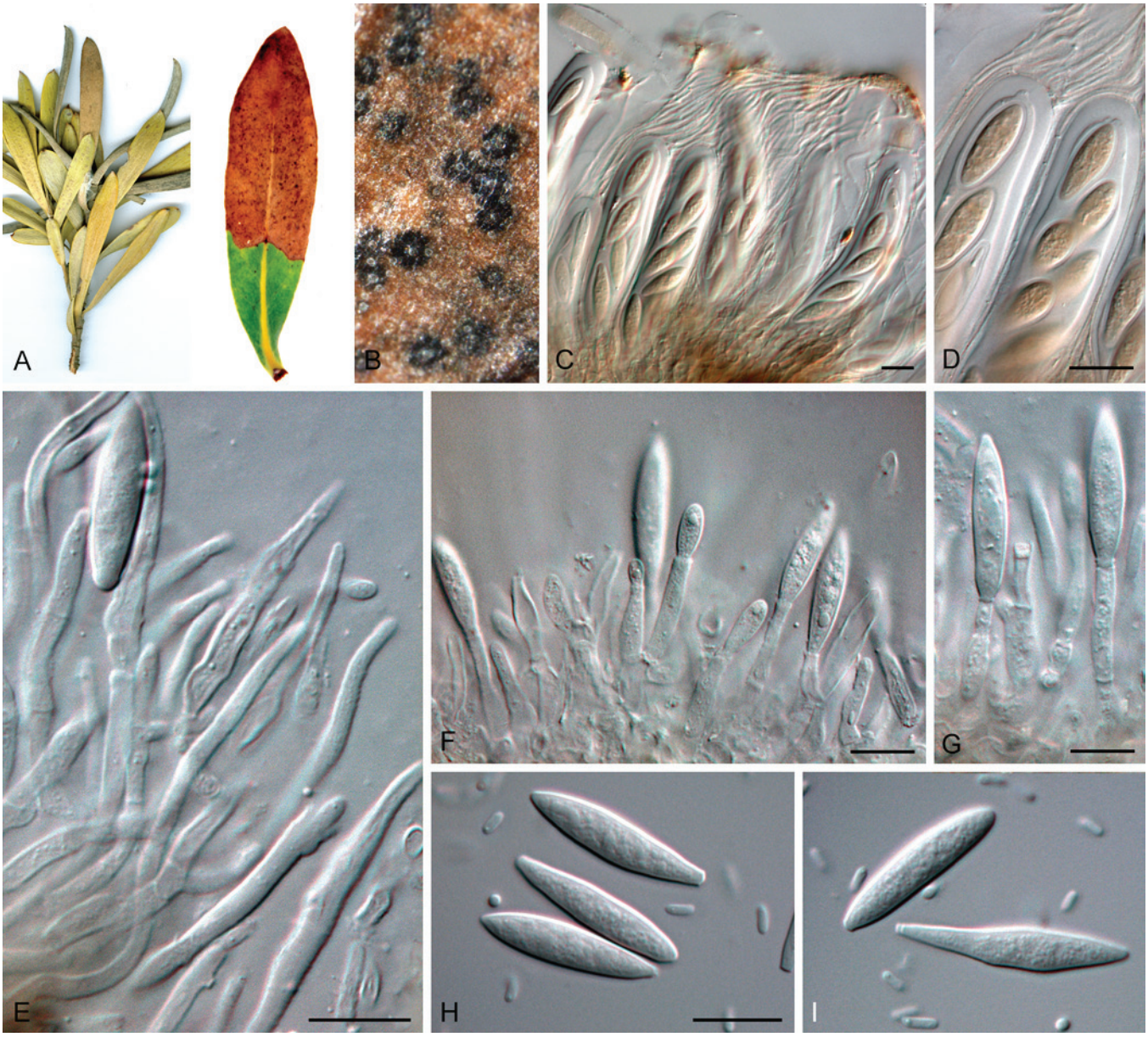

Fig. 3. *Saccharataceae* (*Saccharata proteae,* CBS 121406). A. Symptomatic leaves with tip die-back. B. Superficial view of immersed ascomata, showing clypeus-like structure. C, D. Asci and ascospores. E–G. Conidiogenous cells and paraphyses. H, I. Conidia and spermatia. Scale bars = 10 µm.

Saccharataceae Slippers, Boissin & Crous, **fam. nov.** MycoBank MB805794. Fig. 3.

Type genus: *Saccharata* Denman & Crous, In: Crous *et al.*, CBS Biodiversity Ser. (Utrecht) 2: 104. 2004.

Type species: *S. proteae* (Wakef.) Denman & Crous, In: Crous *et al.*, CBS Biodiversity Ser. (Utrecht) 2: 104. 2004.

Genus supported by DNA sequence data: *Saccharata.*

Ascomata pseudothecial, unilocular, solitary or in clusters, with multilayered dark brown walls, infrequently embedded in stromatic tissue, with upper ascomatal layer darkened and thickened. *Asci* bitunicate, fissitunicate, 8-spored, with a thick endotunica, stalked or sessile, clavate, with a well-developed apical chamber. *Pseudoparaphyses* intermixed with asci, hyaline, septate, hyphae-like, branched or not. *Ascospores* hyaline to pigmented, granular, septate or not, ellipsoid to ovoid, without mucoid appendages or sheath. *Asexual morph* has unilocular pycnidial conidiomata, infrequently embedded in stromatic tissue with thickened, darkened upper layer. *Conidiophores* sparingly branched, hyaline, subcylindrical, or reduced to conidiogenous cells. *Conidiogenous cells* hyaline, smooth, phialidic, proliferating via periclinal thickening or percurrent proliferation, with or without collarettes. *Conidia* hyaline, thin-walled, granular, fusoid, aseptate. *Synasexual morph* formed in separate conidiomata, or in same conidiomata with asexual morph. *Synasexual conidia* pigmented, thick-walled, finely verruculose, ellipsoid or oval, aseptate. *Spermatogonia* similar to conidiomata in anatomy. *Spermatogenous cells* ampulliform to lageniform or subcylindrical, hyaline smooth, phialidic. *Spermatia* developing in conidiomata or spermatogonia, hyaline, smooth, granular, subcylindrical or dumbbell-shaped, with rounded ends.

Aplosporellaceae Slippers, Boissin & Crous, **fam. nov.** MycoBank MB805795. Fig. 4.

Type genus: *Aplosporella* Speg., Anal. Soc. cient. argent. 10(5–6): 158. 1880.

Type species: *A. chlorostroma* Speg., Anal. Soc. cient. argent. 10(5–6): 158. 1880.

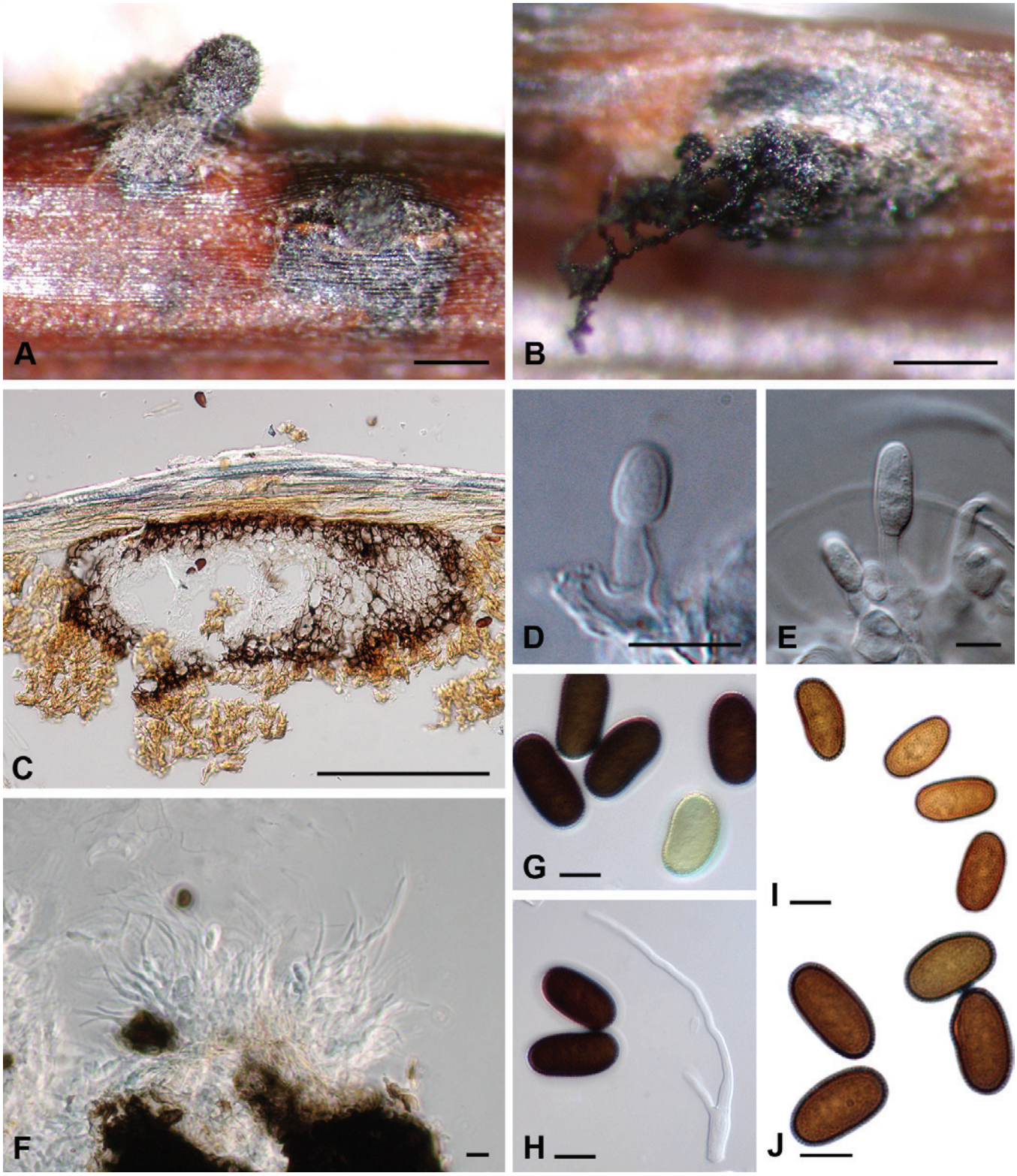

Fig. 4. *Aplosporellaceae* (*Aplosporella prunicola,* CBS 121167). A, B. Oozing spore masses from submerged conidiomata. C. Transverse section through multilocular conidioma. D, E. Conidiogenous cells. F. Paraphyses. H. Conidia and branched paraphyses. G–J. Conidia. Scale bars: A–C = 250 µm, F = 20 µm, D, E, G–J = 10 µm (adapted from Damm *et al.* 2007).

Genera supported by DNA sequence data: *Aplosporella*, *Bagnisiella.*

Ascomata pseudothecial, mostly multilocular with multilayered dark brown walls, embedded in stromatic tissue. *Asci* bitunicate, with a thick endotunica, stalked or sessile, clavate, with a well-developed apical chamber, intermixed with hyaline, septate, hyphal-like pseudoparaphyses, branched or not. *Ascospores* hyaline to pigmented, septate or not, ellipsoid to ovoid, without mucoid appendages or sheath. *Asexual morphs* with uni- to multilocular pycnidial conidiomata, embedded in stromatic tissue. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* hyaline, phialidic, proliferating percurrently or with periclinal thickening at apex. *Paraphyses* present or absent, hyaline, smooth-walled, septate, branched or not, hyphae-like. *Conidia* ellipsoid to subcylindrical, initially hyaline becoming pigmented, aseptate, thin-walled and smooth, becoming thick-walled and spinulose.

Melanopsaceae Phillips, Slippers, Boissin & Crous, **fam. nov.** MycoBank MB805796. Figs 5, 6.
Type genus: *Melanops* Nitschke ex Fuckel, In: Fuckel, Jahrb. Nassau. Ver. Naturk. 23–24: 225. 1870.
Type species: *Melanops tulasnei* Nitschke, In: Fuckel, Jahrb. Nassau. Ver. Naturk. 23–24: 225. 1870.

Genus supported by DNA sequence data: *Melanops* (see Phillips & Alves 2009).

Ascomata pseudothecial, multiloculate, immersed, partially erumpent at maturity, black, subglobose, thick-walled; wall composed of thick-walled *textura angularis*. *Asci* 8-spored, bitunicate, fissitunicate, stipitate, clavate. *Pseudoparaphyses* hyaline, thin-walled, hyphal-like, septate. *Ascospores* hyaline, aseptate, thin-walled, ellipsoid to rhomboid, with a persistent mucus sheath. *Conidiomata* indistinguishable from ascomata and often formed in the same stroma. *Paraphyses* hyaline, septate, branched or not, filiform, arising from between the conidiogenous cells. *Conidiophores* hyaline, smooth, 1–2-septate, branched or not, or reduced to conidiogenous cells. *Conidiogenous cells* subcylindrical, hyaline, branched or unbranched, discrete, formed from the inner wall of the conidioma, proliferating percurrently at apex, or with periclinal thickening. *Conidia* hyaline, aseptate, fusoid, with a persistent mucus sheath, rarely with minute marginal frill.

Phyllostictaceae Fr. (as "Phyllostictei"), Summa veg. Scand., Section Post. (Stockholm): 420. 1849.
Type genus: *Phyllosticta* Pers., Traité sur les Champignons Comestibles (Paris): 55. 147. 1818.
Type species: *P. convallariae* Pers., Traité sur les Champignons Comestibles (Paris): 148. 1818.

Genus supported by DNA sequence data: *Phyllosticta* (see Wikee *et al*. 2013b, this volume).

Planistromellaceae M.E. Barr, Mycotaxon 60: 433. 1996. Fig. 7.
Type genus: *Planistromella* A.W. Ramaley, Mycotaxon 47: 260. 1993.
Type species: *P. yuccifoliorum* A.W. Ramaley, Mycotaxon 47: 261. 1993 (= *Kellermania yuccifoliorum*)

Genus supported by DNA sequence data: *Kellermania* (= *Alpakesa, Piptarthron, Planistroma, Planistromella, Septoplaca* (possibly), see Minnis *et al*. 2012).

Ascomata pseudothecial, multi- or uniloculate, immersed to erumpent, solitary to gregarious, with papillate, periphysate ostiole; walls of several layers of dark brown *textura angularis*. Hamathecium mostly lacking pseudoparaphyses at maturity. *Asci* 8-spored, bitunicate, fissitunicate, thick-walled, oblong to clavate or subcylindrical, stipitate, with well-developed ocular chamber. *Ascospores* 1–3-seriate, hyaline or pale brown, guttulate, ellipsoid to broadly obovoid, aseptate or with 1–2 transverse septa, thin-walled, with or without a gelatinous sheath. *Conidiomata* pycnidial to acervular, subepidermal, dark brown, immersed to semi-erumpent, solitary to gregarious; wall comprising several layers with cells of dark brown *textura angularis*, becoming hyaline towards the inner region. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* ampulliform to sub-cylindrical, hyaline, smooth, phialidic, proliferating via percurrent proliferation or periclinal thickening. *Conidia* obclavate to ellipsoid-cylindrical, aseptate or transversely multiseptate, hyaline to brown, smooth to verruculose, with or without one or more apical appendages, a persistent mucoid sheath, and a basal marginal frill. *Spermatogonia* similar to conidiomata in anatomy. *Spermatogenous cells* ampulliform to lageniform or subcylindrical, hyaline smooth, phialidic. *Spermatia* developing in conidiomata or spermatogonia, hyaline, smooth, granular, sub-cylindrical or dumbbell-shaped, with rounded ends.

DISCUSSION

Using the DNA sequence data for the six loci analysed in this study, together with unique morphological and ecological characteristics (as discussed below), we have distinguished six families in the *Botryosphaeriales*. The *Planistromellaceae* that has recently been defined based on DNA sequence data (Minnis *et al*. 2012) has been retained. Furthermore, the *Aplosporellaceae*, *Melanopsaceae*, and *Saccharataceae* are distinguished from the *Botryosphaeriaceae* and introduced as novel families. These families are also phylogenetically distinct from the *Phyllostictaceae*, which has been defined in a separate study (Wikee *et al*. 2013b, this volume).

Botryosphaeriaceae

The *Botryosphaeriaceae* as it has been defined in this study includes the type genus *Botryosphaeria* (asexual morph *Fusicoccum*), as well as 16 other genera. This group corresponds to a group traditionally referred to as botryosphaeria-like. This term is, however, now understood to be much more restricted, including *B. dothidea* and a few closely related species (as defined in Slippers *et al*. 2004, Crous *et al*. 2006, Phillips *et al*. 2013, this volume). Using *Botryosphaeria* to refer to the assemblage of genera including *Diplodia*, *Lasiodiplodia*, *Neofusicoccum* and others, of which the sexual morphs were formerly described in *Botryosphaeria*, is thus taxonomically incorrect. These groups are now referred to using a single genus name, which is typically the asexual morph, irrespective of whether a sexual morph is known or not. This convention has been applied subsequent to the taxonomic changes introduced by Crous *et al*. (2006), and is also consistent with the recent decisions to abolish the dual nomenclatural system for fungal taxonomy (Hawksworth *et al*. 2011, Wingfield *et al*. 2012).

The data presented in this study, together with those emerging from more focused earlier studies, as well as the recent changes resulting in the abandonment of a dual nomenclature, necessitates reducing a number of genera in the *Botryosphaeriaceae* to synonymy with others. Most importantly, there is no longer just cause to maintain *Botryosphaeria* and *Fusicoccum* as distinct genera. In the interests of maintaining taxonomic stability, and the fact that *B. dothidea* is the type species of the order and family, it is recommended that *Botryosphaeria* be retained (Slippers *et al*. 2004, Schoch *et al*. 2006, Phillips *et al*. 2013, this volume). *Botryosphaeria* must thus be redefined to include species where only the asexual (*Fusicoccum* and dichomera-like) morphs are known. Species such as *F. ramosum* and *Dichomera saubinetti* must then be redefined in *Botryosphaeria*. For *F. ramosum,* an ex-type isolate was available and it has thus been redescribed in *Botryosphaeria* in a companion paper (Phillips *et al*. 2013, this volume).

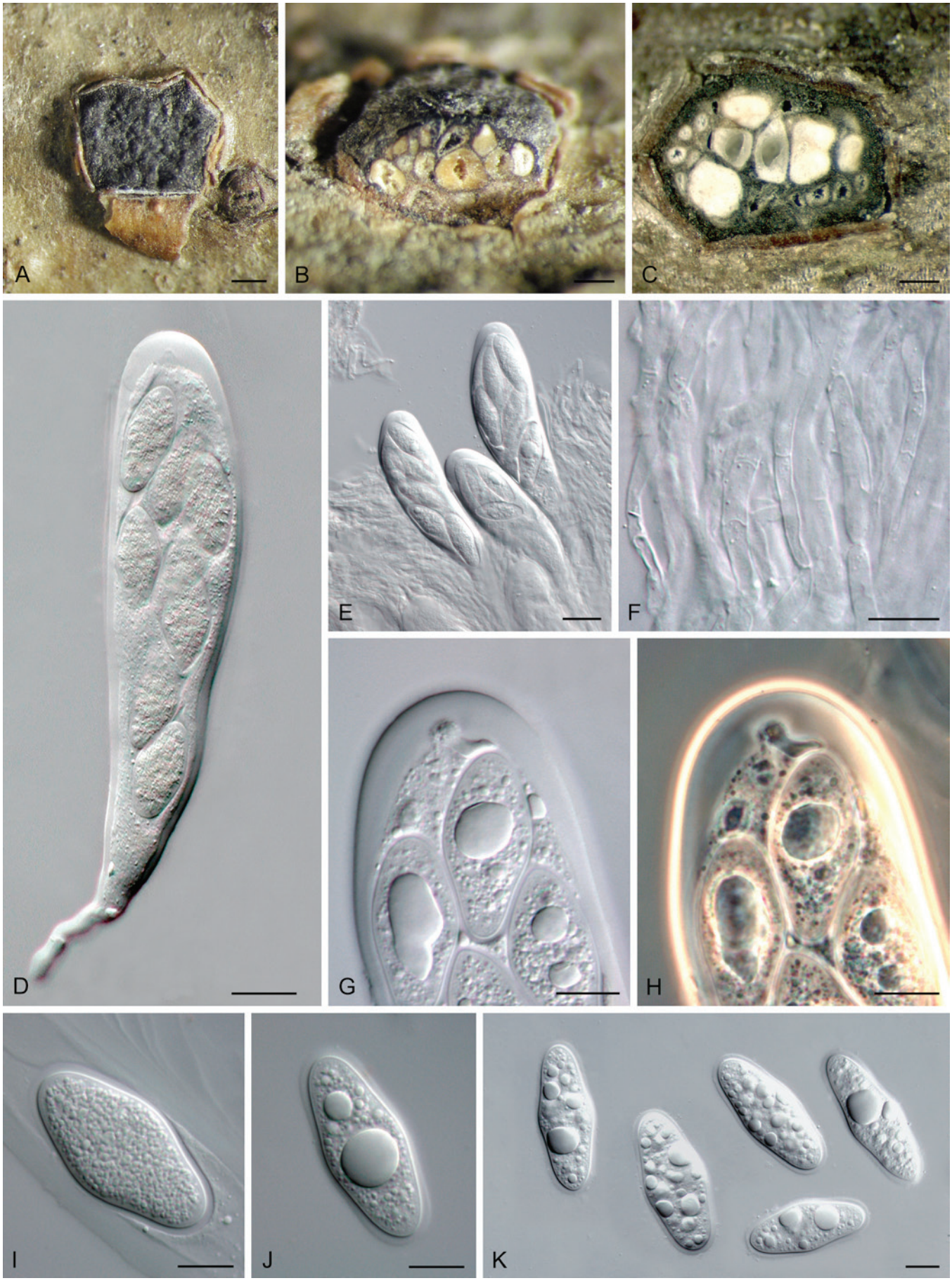

Fig. 5. *Melanopsaceae* (*Melanops tulasnei*, LISE 95179). A. Stroma erumpent through bark. B, C. Sections through stromata revealing ascomata and conidiomata. D. Ascus. E. Asci and pseudoparaphyses. F. Pseudoparaphyses. G, H. Ascus tips viewed under differential interference contrast (G) and phase contrast (H). I–K. Ascospores. Scale bars: A–C = 250 µm, D, E = 20 µm, F–K = 10 µm (adapted from Phillips & Alves 2009).

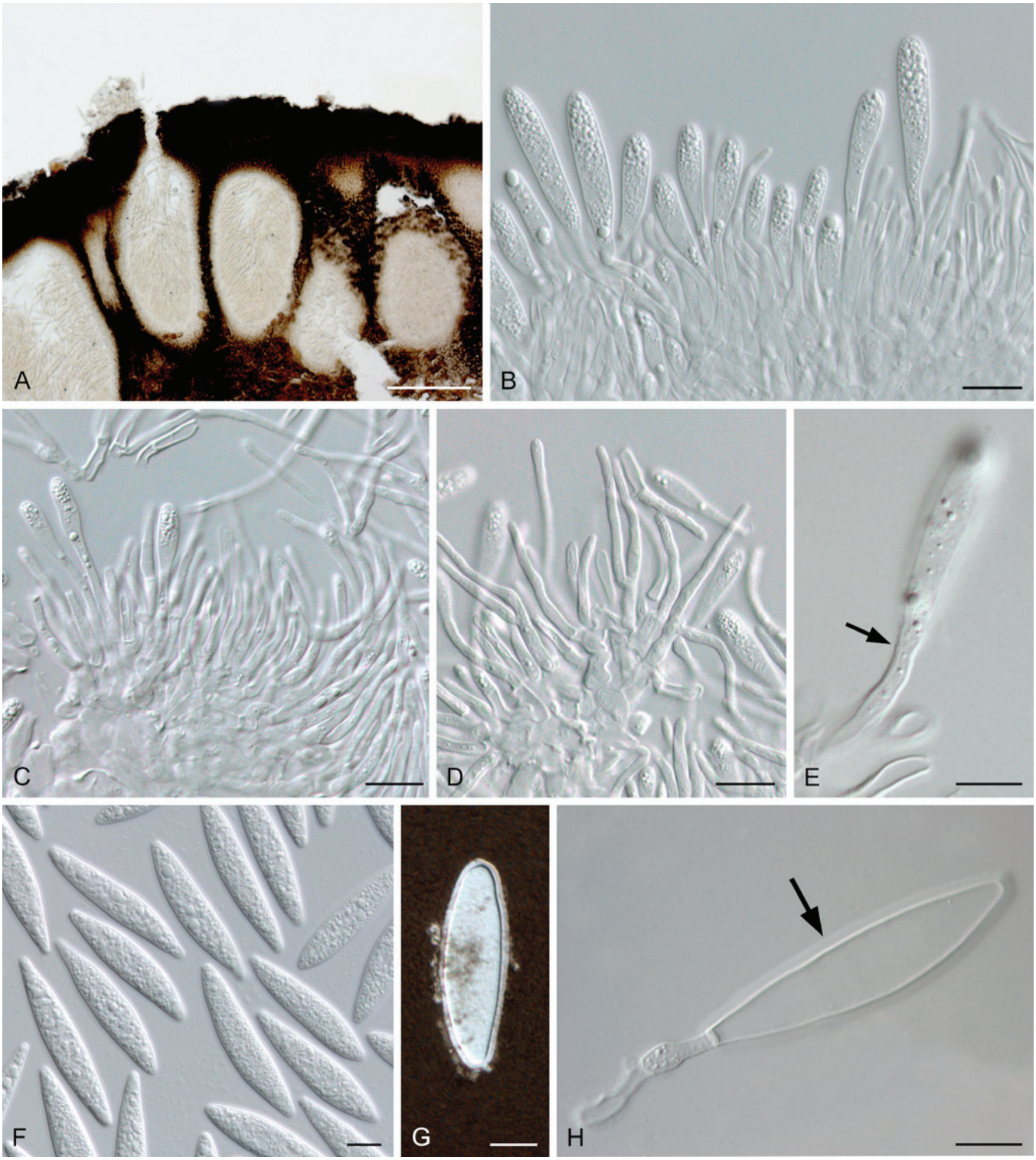

Fig. 6. *Melanopsaceae* (*Melanops tulasnei*, LISE 95179). A. Section through conidiomata. B. Conidiogenous layers with developing conidia among paraphyses. C. Immature conidiogenous cells. D. Paraphyses. E. Conidiogenous cell with percurrent proliferations (arrowed). F. Conidia. G. Conidium in indian ink, revealing sheath. H. Conidium attached to conidiogenous cell with mucus sheath (arrowed). Scale bars: A = 200 µm, B–D, F, G = 10 µm, E, H = 5 µm (adapted from Phillips & Alves 2009).

Dichomera is polyphyletic and most likely also includes synasexual morphs of other genera. Two of the species included in this analysis, *D. eucalypti* and *D. versiformis*, clearly group in *Neofusicoccum* and we consider them as synonyms of species in this genus. *Dichomera saubinetti* grouped with *Botryosphaeria* and should be redescribed in this genus. Unfortunately no ex-type isolates of these species are presently available.

A number of genera grouped in *Lasiodiplodia* s. lat. in our analyses and are possibly synonyms of this genus. *Macrovalsaria* (see Sivanesan 1975) clearly grouped amongst species of *Lasiodiplodia*. This was also pointed out by Liu *et al.* (2012), but they did not find the available LSU and SSU data sufficiently convincing to make taxonomic changes. Isolates of *Macrovalsaria* however, grouped extremely closely with *L. theobromae* and we view them as representing a synonym of *Lasiodiplodia*, rather than *Lasiodiplodia* being polyphyletic. *Lasiodiplodia* and *Macrovalsaria* are both tropical fungi. Our analyses differ from those of Liu *et al.* (2012), indicating that *Auerswaldia* is a synonym of *Lasiodiplodia*. This was also confirmed in Phillips *et al*. (2013, this volume), who redescribed *A. lignicola* as *L. lignicola*. These findings suggest that

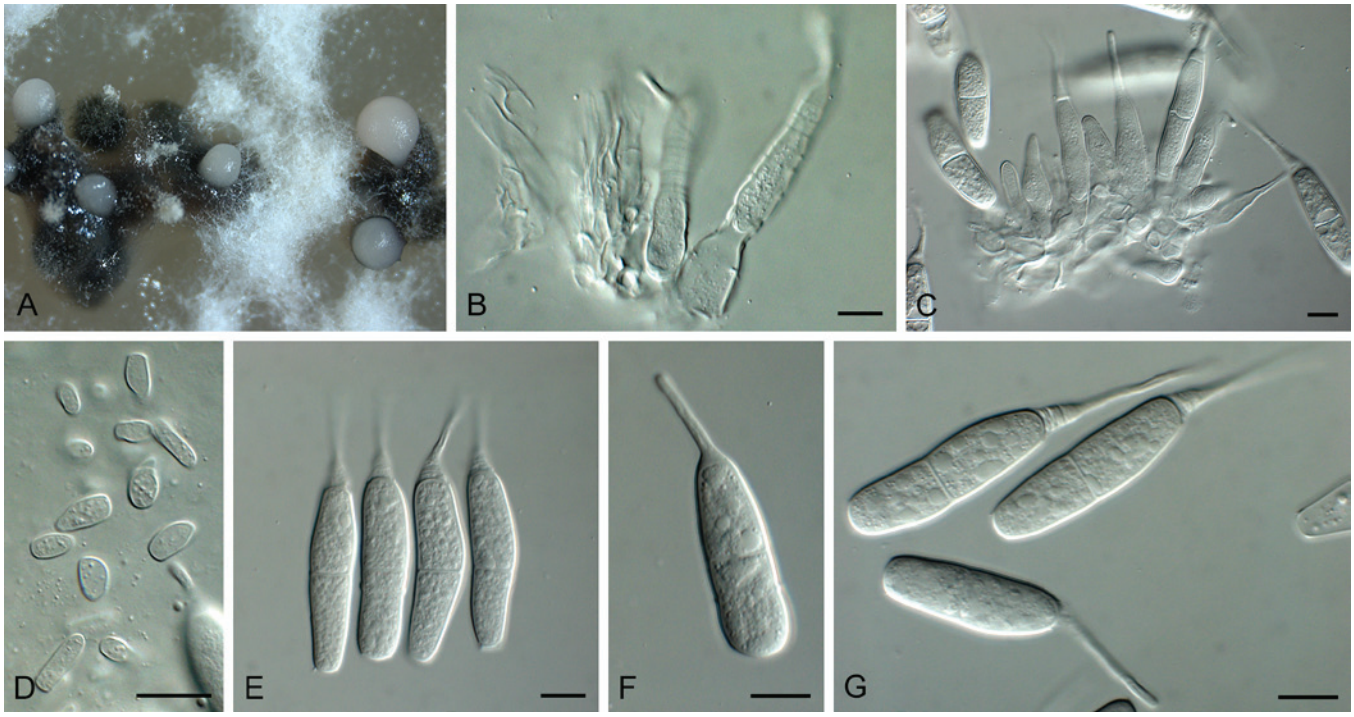

Fig. 7. *Planistromellaceae* (*Kellermania yuccigena*, CPC 20627). A. Conidiomata sporulating on OA. B, C. Conidiogenous cells showing percurrent proliferation. D. Spermatia. E–G. One-septate macroconidia with apical appendages. Scale bars = 10 µm.

the taxonomy of *Lasiodiplodia* needs to be re-evaluated, but for the present we do not recognize *Auerswaldia* as a genus in the Botryosphaeriaceae.

Diplodia juglandis and *D. corylii* both grouped in *Dothiorella*, which is consistent with the results of a previous study (Phillips *et al*. 2008). Type specimens were not available for these species and they were, therefore, not re-described. These species require epitypification. It is likely that a number of other *Diplodia* species will similarly reside in *Dothiorella* or *Spencermartinsia*, or *vice versa*, given the confusion of these names in the past (Phillips *et al*. 2005, 2008). The conidia of these genera remain difficult to distinguish, which can create problems when interpreting older descriptions or poorly preserved herbarium specimens.

There was no statistically significant pattern that could be discerned in the *Botryosphaeriaceae* with respect to the evolution of hyaline or pigmented conidia or ascospores. These characters appear to be more or less randomly spread amongst the clades of this section of the phylogenetic tree for the family. This would suggest that these characters predate the divergence of the genera in this family, and that they have been independently lost or suppressed (character not expressed under all conditions) in different groups. This would also explain the "appearance" of darker or even dark muriform conidia in genera such as *Botryosphaeria* and *Neofusicoccum* that were traditionally considered not to have such synasexual morphs (Barber *et al.* 2005, Phillips *et al*. 2005b, Crous *et al*. 2006). Clearly these characters, which have traditionally been commonly used for phylogenetic and taxonomic purposes, have very little phylogenetic and taxonomic value above the genus level.

The distinction and more narrow definition of the *Botryosphaeriaceae* is important when considering the economic and ecological importance of this group. Many of the *Botryosphaeriaceae* species share a common ecology in being endophytic and latent pathogens in virtually all parts of woody plants (Slippers & Wingfield 2007). While not all species have been isolated as endophytes, or from all plant parts, most of those that have been carefully studied have conformed to this pattern, and it is thus expected for the group as a whole. Many of the genera in the family are also very widespread, with wide host ranges and broad levels of environmental tolerance (e.g. *N. parvum*, *N. australe*, *B. dothidea*, *L. theobromae*, *L. pseudotheobromae*). Sakalidis *et al*. (2013) for example reported *N. parvum* from 90 hosts in 29 countries on six continents. This broad ecological range, together with their cryptic nature as endophytes, makes these fungi important to consider as a group prone to being spread with living plant material. Ample evidence exists that these fungi can infect both native and non-native trees, once they have been introduced into a region. The observed (Dakin *et al*. 2010, Piškur *et al*. 2011) and expected (Desprez-Loustau *et al*. 2006) increase of the importance of this group due to pressure on plant communities as a result of climate change provides another reason to focus future efforts on characterising the diversity, distribution and pathogenicity of this group of fungi.

Aplosporellaceae

An unexpected outcome of this study was the consistent connection between *Aplosporella* and *Bagnisiella* and their distinction from other members of the *Botryosphaeriales*. While it has been suggested that some *Aplosporella* spp. might be asexual morphs of *Bagnisiella*, this connection has never been proven. Neither of these genera were treated in molecular phylogenetic re-evaluations of the *Botryosphaeriaceae* until very recently. The first analyses to include DNA sequence data for *Aplosporella* (Damm *et al*. 2007, Liu *et al*. 2012) and *Bagnisiella* (Schoch *et al*. 2009) hinted at a distant relationship with other *Botryosphaeriales*. However, none of these studies included both genera. The phylogenetic relationship between these genera revealed in this study is further supported by their remarkably similar multiloculate sporocarps, expressed in both the asexual morphs and sexual morphs, which is thus

not the product of parallel evolution in two distinct groups. There are, however, undoubtedly many species of *Aplosporella* and *Bagnisiella* that would not be connected to the phylogenetic clade identified here, because both genera are heterogeneous and likely contain unrelated species.

The data presented in this study suggest that *Aplosporella* and *Bagnisiella* are not only related, but that they should be synonymized. Both genera were described in 1880, and historical precedence can thus not be used to choose an appropriate genus. *Aplosporella* includes many more species (352) than *Bagnisiella* (65) (www.MycoBank.org, accessed August 2013). More species have also recently been described in the former genus, possibly because the asexual structures are more common than the sexual structures (as is found in other *Botryosphaeriales*). An argument based on taxonomic stability, relevance and frequency of occurrence is thus favoured and has led us to decide that *Bagnisiella* should be reduced to synonymy with *Aplosporella*.

Examination of the distribution of species of *Aplosporella* and *Bagnisiella* is complicated by the fact that the literature is old (which means the taxonomic accuracy is difficult to judge) and commonly lacking relevant information. However, most of the well-known and recently characterised species, and all species included here, are from the Southern Hemisphere (Damn *et al*. 2007, Taylor *et al*. 2009). This result suggests the possibility of a Southern Hemisphere and Gondwanan origin and divergence pattern, which should be considered in future studies based on more robust sampling.

Melanopsaceae

Melanops tulasnei and an undescribed *Melanops* sp. grouped most basal in the *Botryosphaeriales*, together with the *Aplosporellaceae*, *Planistromellaceae* and *Saccharataceae*. This group is unique amongst these families in having persistent mucous sheath around its ascospores and conidia (Phillips & Alves 2009). Conidia in *M. tulasnei* are typically hyaline and fusoid and it resembles the *Aplosporellaceae* in having multiloculate ascomata and conidiomata, often with locules at different levels. Little is known regarding the ecology and distribution of this group, given the paucity of recent reports that could be verified using DNA sequence data. It appears similar, however, to other *Botryosphaeriales* that infect woody tissue of plants, and sporulates on the dead tissue. Whether it is pathogenic or endophytic is not known.

Saccharataceae

Saccharata (the only genus in the *Saccharataceae*) grouped separately from all other families that were basal in the phylogenetic tree, suggesting a long, separate evolutionary history. The genus was first described by Crous *et al.* (2004) from *Proteaceae* in the South Western Cape region of South Africa. Subsequently, three additional species were added to the genus, two also from *Proteaceae* and one from *Encephalartos* (Marincowitz *et al*. 2008, Crous *et al*. 2008, 2009). All known species are thus from the same region on indigenous flora. These species are typically associated with leaf spots and stem cankers and they appear to be pathogens. Separate studies have also shown that they are endophytes (Swart *et al*. 2000, Taylor *et al.* 2001), similar to members of the *Botryosphaeriaceae*.

Apart from its restricted distribution and host range, *Saccharata* is also unique in its asexual morphology, which includes a hyaline, fusicoccum-like and a pigmented diplodia-like asexual morph. These characters are shared with its related families; fusicoccum-like conidia in *Melanopsaceae* and pigmented diplodia-like conidia in *Aplosporellaceae*. It is clear that these variations in conidial morphology are very old (tens of millions of years) ancestral characters that must have existed prior to the divergence of this group from other *Botryosphaeriales*. It is thus remarkable how similar, especially in the fusicoccum-like conidia and ascospore morphology, the spores of these fungi have remained over time. The diplodia-like state is somewhat different from other *Botryosphaeriales* in that the conidia are typically almost half the size of other *Diplodia* conidia. We do not currently have enough data to address the selective pressure that could have played a role in the development these interesting morphological changes.

Phyllostictaceae and Planistromellaceae

Phyllosticta clearly warrants a separate family to accommodate this morphologically and ecologically unique, widespread and economically important genus in the *Phyllostictaceae*. These fungi typically infect leaves and fruit, rather than woody tissue, and they can cause serious damage (Glienke *et al.* 2011, Wong *et al*. 2012). Species of *Phyllosticta* are also known to have an endophytic phase (Wikee *et al.* 2013a), as is true for most other *Botryosphaeriales*. The *Phyllostictaceae* is also morphologically unique in terms of the ascospores and conidia (Van der Aa & Vanev 2002). The species included in this study are only representative of a small extent of the diversity in this group and a more complete analysis of the *Phyllostictaceae* is presented in Wikee *et al*. (2013b, this volume).

The *Planistromellaceae* has previously been recognised as distinct within the *Botryosphaeriales* (Minnis *et al*. 2012) and this is supported by the analyses in the present study. The family is currently considered to include only species of *Kellermania*. Species in the *Planistromellaceae* have unique conidia with fairly long appendages that are quite distinct from other genera in the *Botryosphaeriales*. *Kellermania* spp. are mostly leaf infecting, and one species has been associated with Yucca Leaf Blight in California and Florida in the USA (Horst 2008). Species are commonly collected sporulating on dead leaves of *Agavaceae*, and appear to be endophytic, as they have been isolated from healthy leaves in many countries where *Yucca* spp. are grown as ornamentals (P.W. Crous, unpubl data). Minnis *et al*. (2012) have also shown apparent patterns of host specificity and co-evolution with major plant lineages. As is true for many other families in the *Botryosphaeriales*, additional sampling is needed for a better understanding of species diversity, host range and geographic distribution.

Molecular dating

The molecular dating on the radiations within the *Botryosphaeriales* in this study, based on general estimated mutation rates of the rDNA SSU locus by Taylor & Berbee (2010), must be viewed as preliminary. From the dating that was conducted, the group appears to have originated in the Cretaceous period around 103 (45–188) mya, with most of the subsequent diversification within the families occurring in the Tertiary period. This date is well within the estimated date of the emergence of the *Dothideomycetes* (Berbee & Taylor 2010, Gueidan *et al*. 2011). This coincides with important periods of Angiosperm radiation and spread, which is the main group of plants on which these fungi are found and that might be expected to have influenced the diversification of these fungi. Of

particular relevance is the rapid diversification of the Eurosid and other dominant woody Angiosperm groups and their prominence in Angiosperm dominated forests from around 110 mya onwards (Soltis *et al.* 2008, Fawcett *et al.* 2009, Wang *et al*. 2009, Bell *et al*. 2010). De Wet *et al.* (2008) pointed out that the members of the *Botryosphaeriaceae* are most diverse on Angiosperms, and showed that ancestral state reconstruction suggests that this is the main group of plants on which the *Botryosphaeriaceae* co-evolved. A much smaller number of species, especially those in *Diplodia*, occur commonly on coniferous hosts and appear to have emerged and diversified more recently. They also tend to be more host-specific than some of the other genera discussed above that are more common on Angiosperms (De Wet *et al*. 2008, Sakalidis *et al*. 2013), suggesting a specific acquired trait that allowed them to infect coniferous hosts.

The major changes in dominant plant hosts in forests globally during the Cretaceous period would be expected to have influenced fungal evolution beyond the Botryosphaeriales, and this indeed appears to be the case. For example, studies on another *Dothideomycetes* order, the sooty molds in the *Capnodiales*, also appear to have been influenced by the rise of Angiosperm forests in the Cretaceous period (Schmidt *et al*. 2013). Furthermore, the divergence of a prominent fungal complex, *Fusarium*, was estimated to be later at around 93 mya, and is also thought to have been influence by this Angiosperm divergence (O'Donnell *et al*. 2013).

Molecular dating, together with the geographic distribution (and restriction) of different groups in the *Botryosphaeriales* should provide rich information to explore in future to understand the patterns that have shaped the diversity of this important group of plant-associated fungi. For example, the *Saccharataceae* has previously been known only from southern Africa, and is most diverse on the *Proteaceae*. Recent research has shown, however, that it has also been introduced as endophyte into other countries where South African *Proteaceae* are now being cultivated (Marincowitz *et al*. 2008). It is known that this plant family, which has a high endemic richness in southern Africa, has evolved in the region for more than 100 million years (Barker *et al.* 2007). This date allows for the estimated 57 (28–100) mya (based on rDNA SSU) of separation of the *Saccharataceae* to have evolved with these endemic plant hosts in the region.

Diversification within the most diverse family, the *Botryosphaeriaceae*, occurred between 52–65 (27–112) mya for the two earliest diverging (and least diverse) genera, *Pseudofusicoccum* and *Endomelanconiopsis*. These dates correspond to the diversification between some other families in the order [e.g. the *Aplosporellaceae*, *Melanopsaceae*, *Planistromellaceae* and *Saccharataceae* that split between 57–75 (28–136) mya]. At present, however, there does not appear sufficiently robust morphological or ecological validation for a further split of the *Botryosphaeriaceae* to accommodate *Pseudofusicoccum* and *Endomelanconiopsis* in distinct families. If these genera were to be considered as residing in distinct families, the question would arise as to whether further family level distinction in the *Botryosphaeriaceae* is necessary. The other major lineages within the *Botryosphaeriaceae* (clades 1–4) appeared around 11–35 (3–58) mya. The most recent diversification for which there was support was within *Neofusicoccum* clade, dated at around 11 (3–23) mya.

This study has provided a systematic framework for future taxonomic and ecological studies of the *Botryosphaeriales*. It has also highlighted a number of interesting host association and geographic patterns amongst the genera that are worthy of further investigation. It is hoped that this framework, in conjunction with the growing body of DNA–based sequence data reflecting the species diversity and their distribution will ultimately lead to a model supporting an improved understanding of the co-evolution of woody plants and their fungal endophytes/latent pathogens.

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The *Botryosphaeriaceae*: genera and species known from culture

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Abstract: In this paper we give an account of the genera and species in the *Botryosphaeriaceae*. We consider morphological characters alone as inadequate to define genera or identify species, given the confusion it has repeatedly introduced in the past, their variation during development, and inevitable overlap as representation grows. Thus it seems likely that all of the older taxa linked to the *Botryosphaeriaceae*, and for which cultures or DNA sequence data are not available, cannot be linked to the species in this family that are known from culture. Such older taxa will have to be disregarded for future use unless they are epitypified. We therefore focus this paper on the 17 genera that can now be recognised phylogenetically, which concentrates on the species that are presently known from culture. Included is a historical overview of the family, the morphological features that define the genera and species and detailed descriptions of the 17 genera and 110 species. Keys to the genera and species are also provided. Phylogenetic relationships of the genera are given in a multi-locus tree based on combined SSU, ITS, LSU, EF1-α and β-tubulin sequences. The morphological descriptions are supplemented by phylogenetic trees (ITS alone or ITS + EF1-α) for the species in each genus.

Key words: *Botryosphaeriales*, canker pathogens, *Diplodia*, *Fusicoccum*, *Lasiodiplodia,* Multi-Locus Sequence Analysis, *Sphaeropsis,* systematics.
Taxonomic novelties: New species – ***Neofusicoccum batangarum*** Begoude, Jol. Roux & Slippers. **New combinations** – *Botryosphaeria fabicerciana* (S.F. Chen, D. Pavlic, M.J. Wingf. & X.D. Zhou) A.J.L. Phillips & A. Alves, *Botryosphaeria ramosa* (Pavlic, T.I. Burgess, M.J. Wingf.) A.J.L. Phillips & A. Alves, *Cophinforma atrovirens* (Mehl & Slippers) A. Alves & A.J.L. Phillips, *Cophinforma mamane* (D.E. Gardner) A.J.L. Phillips & A. Alves, *Dothiorella pretoriensis* (Jami, Gryzenh., Slippers & M.J. Wingf.) Abdollahz. & A.J.L. Phillips, *Dothiorella thailandica* (D.Q. Dai., J.K. Liu & K.D. Hyde) Abdollahz., A.J.L. Phillips & A. Alves, *Dothiorella uruguayensis* (C.A. Pérez, Blanchette, Slippers & M.J. Wingf.) Abdollahz. & A.J.L. Phillips, *Lasiodiplodia lignicola* (Ariyawansa, J.K. Liu & K.D. Hyde) A.J.L. Phillips, A. Alves & Abdollahz., *Neoscytalidium hyalinum* (C.K. Campb. & J.L. Mulder) A.J.L. Phillips, Groenewald & Crous, *Sphaeropsis citrigena* (A.J.L. Phillips, P.R. Johnst. & Pennycook) A.J.L. Phillips & A. Alves, *Sphaeropsis eucalypticola* (Doilom, J.K. Liu, & K.D. Hyde) A.J.L. Phillips, *Sphaeropsis porosa* (Van Niekerk & Crous) A.J.L. Phillips & A. Alves. **Epitypification (basionym)** – *Sphaeria sapinea* Fries. **Neotypifications (basionyms)** – *Botryodiplodia theobromae* Pat., *Physalospora agaves* Henn, *Sphaeria atrovirens* var. *visci* Alb. & Schwein.

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INTRODUCTION

The *Botryosphaeriaceae* encompasses a range of morphologically diverse fungi that are either pathogens, endophytes or saprobes, mainly on woody hosts. They are found in all geographical and climatic areas of the world, with the exception of the polar regions. Their frequent association with plant diseases has stimulated substantial interest in these fungi, and much of this interest has been focussed on the systematics of species and genera.

Historical overview

The *Botryosphaeriaceae* was introduced by Theissen & Sydow (1918) as a sub-family in the *Pseudosphaeriaceae*. Although Theissen (1916) had earlier allocated the *Pseudosphaeriaceae* to the *Myriangiales*, Theissen & Sydow (1917) believed that the *Pseudosphaeriaceae* should be united with the *Dothideaceae* (Luttrell 1951). Theissen & Sydow (1918) established the sub-class the *Dothideineae* to accommodate the order *Pseudosphaeriales*, family *Botryosphaeriaceae*, and the genus *Botryosphaeria*. Petrak (1923) rejected Theissen & Sydow's (1918) classification and placed *Botryosphaeria* in the sub-family *Pseudosphaerieae*, which he placed in the *Pleosporaceae* (*Sphaeriales*).

Miller (1928) showed that there was a fundamental difference between the tissues forming the ascoma and those forming the boundary of the locules. He also showed how these different tissue types were correlated with features of the ascocarp centrum. Taxa allocated to the *Sphaeriales* had true perithecial ascomata and paraphyses, while those assigned to the *Dothideales* had ascostromatic ascomata lacking paraphyses. Thus, *Botryosphaeria* species (*Pseudosphaeriaceae*) were allocated to the *Dothideales* because they lacked true perithecial walls (Miller 1928).

Nannfeldt (1932) re-grouped the *Euascomycetes* into three orders. The ascostromatic forms, where asci form in cavities in pre-formed stromata, were accommodated in the *Ascoloculares*. The true *Sphaeriales*, i.e., species in which the asci developed in a hymenium, were accommodated in the *Ascohymeniales*. Although these groups were not widely accepted at the time, they were consistent with the bitunicate and unitunicate groups later proposed by Luttrell (1955).

Concepts based on morphological features resulting from the ontogeny of the perithecial wall and the development of centrum

tissues were further developed by Miller (1938) and three orders were recognised. The *Sphaeriales* had perithecia and paraphyses, and the *Dothideales* encompassed ascostromatic forms with interthecial threads that appeared in the ascomatal cavity before the asci developed. Miller (1938) retained *Botryosphaeria* in the family *Pseudosphaeriaceae*. Thus, *Botryosphaeria* was accommodated in the *Pseudosphaeriales* and not in the *Dothideales*.

Luttrell (1951) recognised two major morphological groups in the pyrenomycetous fungi. He also emphasised the significance of ontogenetic characters of the ascomata in classification. The two major groups were those with single-walled asci or the unitunicate ascomycetes, and the loculoascomycetes, commonly referred to as the bitunicate ascomycetes (Luttrell 1955). Luttrell also identified eight forms of centrum development and highlighted the taxonomic value of sterile interthecial tissues. Since the type of the family *Pseudosphaeriaceae*, and the type of the genus *Pseudosphaeria* had been transferred to the *Dothideales*, the order *Pseudosphaeriales* was no longer tenable. Therefore, Luttrell (1955) replaced the name *Pseudosphaeriales* with *Pleosporales*, based on the most important genus in the group with that type of centrum development, and assigned *Botryosphaeria* to the *Pleosporales*.

In Barr's earlier work (1972, 1976), she had not studied specimens of *B. dothidea* in which the interthecial tissues were clearly visible, and despite the clear demonstration by Parguey-Leduc (1966) that *B. dothidea* exhibited a *Pleospora* centrum type, she classified *Botryosphaeria* in the *Dothideales*. Later, however, Barr (1979) acknowledged that *Botryosphaeria* species had a centrum typical of the *Pleosporales* and she concluded that the genus should reside in this order. This view was retained in subsequent publications (Barr 1983, 1987).

According to von Arx & Müller (1975) and von Arx (1981, 1987) the orders proposed by Lutrell (1955, 1973) and Barr (1972, 1987) comprised a collection of unrelated genera and the taxonomic characters used to separate the orders overlapped. Moreover, von Arx & Müller (1975) did not support the placement of closely related genera such as *Guignardia* and *Botryosphaeria* in different orders, i.e. the *Dothideales* and *Pleosporales* respectively (Luttrel 1973). For this reason von Arx & Müller (1975) placed all bitunicate ascomycetes in the single order *Dothideales*, comprising two suborders and 34 families, including the *Botryosphaeriaceae*. To complicate matters further, Sivanesan (1984) placed both *Botryosphaeria* and *Guignardia* in the *Dothideales* whereas Hawksworth *et al.* (1995) listed *Botryosphaeria* under the *Botryosphaeriaceae* and *Guignardia* under the *Mycosphaerellaceae*, both in the *Dothideales*. Hence the two major systems of classification were those of Barr (1987) in which *Botryosphaeria* is placed in the *Pleosporales*, and von Arx & Müller (1975) who placed the genus in the *Dothideales*. Eriksson (1981), however, emphasised that *Botryosphaeria* species have a centrum typical of the *Pleosporales* with pseudoparaphyses and pseudothecia.

The advent of DNA sequencing methods provided taxonomists with powerful tools to determine phylogenetic relationships in fungi at various taxonomic levels from species to orders. Berbee (1996) used gene sequences of the 18S rRNA gene (SSU) to study phylogenetic relationships amongst genera and orders of loculoascomycetes. However, the positions of the two *Botryosphaeria* species included in that study changed depending on the analysis used. Thus, in the neighbour-joining trees of Berbee (1966) these species usually clustered with species of *Dothidea* in the *Dothideales*, but in a single maximum likelihood tree they clustered in the *Pleosporales*. In a subsequent study of 18S rRNA sequence data, Silva-Hanlin & Hanlin (1999) could not determine whether the *Botryosphaeria-Guignardia* clade corresponded to the *Dothideales* or the *Pleosporales*.

Schoch *et al.* (2006) constructed a multigene phylogeny based on SSU, 28S rRNA gene (LSU), translation elongation factor 1-alpha (EF1-α) and RNA polymerase second largest subunit (RPB2) sequence data for 96 taxa in the *Dothideomycetes*. Species of *Botryosphaeria* and *Guignardia* formed a clade that could not be associated with any other order. For this reason they proposed a new order *Botryosphaeriales* accommodating the single family, the *Botryosphaeriaceae*.

Characteristics of the *Botryosphaeriaceae*

Detailed descriptions of the family *Botryosphaeriaceae* have been presented by several authors (von Arx & Müller 1954, 1975, Hawksworth *et al.* 1995, Eriksson 1981, Sivanesan 1984, Barr 1987). Members of the family are pathogenic, necrotrophic or saprobic, especially on woody plants.

The *Botryosphaericeae* were characterised primarily on the basis of their large, ovoid to oblong, usually hyaline, aseptate ascospores. Although this could appear to be an inadequate basis for recognition of a family, ascospores with this morphology have been considered as an unusual spore type among loculoascomycetes (Luttrel 1973, Eriksson 1981, Sivanesan 1984, Barr 1987). More recently, however, at least six lineages in the family have been recognised as having pigmented ascospores, and in three of these genera the ascospores are septate (Phillips *et al.* 2008). Therefore, this simple circumscription can no longer be considered suitable for the *Botryosphaeriaceae*. Liu *et al.* (2012) recently provided a comprehensive definition of the family in which they considered ascospores to be hyaline and aseptate, but that could become pigmented and septate with age. This is an equally unsuitable definition because ascospores in some genera become pigmented and 1-septate at an early stage of their development, long before they can be considered aged. Furthermore, a circumscription based solely on the sexual state is not suitable especially since some species are known only from their asexual state, while in others the sexual state is extremely uncommon. Given these conditions a modified circumscription of the family is provided by Slippers *et al.* (2013, this volume).

Genera in the *Botryosphaeriaceae*

When Theissen & Sydow (1918) introduced the *Botryosphaeriaceae* they included three genera, namely *Botryosphaeria*, *Phaeobotryon* and *Dibotryon*. Further genera were included over the years and the addition of separate generic names for asexual and sexual morphs resulted in the inclusion of at least 78 genera in the family (MycoBank, http://www.mycobank.org, accessed May 2013). Many of these genera have been determined to be synonyms, some new genera have been introduced, some of the older genera have been resurrected, yet others have been removed to other families. Liu *et al.* (2012) recognised 29 genera of which 17 are known in culture.

The application of DNA sequence analysis and phylogenetic inference has had a major impact on the systematics of the *Botryosphaeriaceae*. Crous *et al.* (2006) used DNA sequence data of the 28S rRNA gene to resolve 10 lineages within the family. The phylogenetic clades correlated with distinct morphological features and corresponded to separate genera. However, the LSU dataset that Crous *et al.* (2006) used could not resolve a large clade

that comprised *Diplodia*, *Lasiodiplodia* and related genera with pigmented conidia.

Phillips *et al.* (2008) attempted to resolve the phylogenetic and taxonomic status of species of *Botryosphaeriaceae* with pigmented ascospores. In a phylogeny based on SSU, the internal transcribed spacers and intervening 5.8S rRNA gene (ITS) and LSU together with EF1-α and β-tubulin sequence data they resolved six clades in the *Diplodia/Lasiodiplodia* complex and an additional four clades in the *Botryosphaeriaceae*. Damm *et al.* (2007) showed that *Aplosporella* represents yet another genus in the *Botryosphaeriaceae* while Rojas *et al.* (2008) determined that *Endomelanconiopsis* also resides in this family. Phillips & Alves (2009) considered *Melanops* to be a genus in the *Botryosphaeriaeae*. In a phylogeny based on SSU, ITS, LSU and RNA polymerase largest subunit (RPB1) sequences, Minnis *et al.* (2012) included *Kellermania* in the *Planistromellaceae*, sister to the *Botryosphaeriaceae*. Furthermore, Wikee *et al.* (2013, this volume) reinstated the *Phyllostictaceae* to accommodate *Phyllosticta* (= *Guignardia*), which they recognised as distinct from the *Botryosphaeriaceae*. Finally, Slippers *et al.* (2013, this volume) introduced new families to accommodate *Saccharata* (*Saccharataceae*), *Melanops* (*Melanopsaceae*), *Aplosporella* and *Bagnisiella* (*Aplosporellaceae*). Thus, 17 genera can now be recognised phylogenetically in the *Botryosphaeriaceae*. We consider morphological characters alone as inadequate to define genera or identify species, given the confusion it has caused in the past. Slippers *et al*. (2013, this volume) also illustrates how misleading some of the prominent conidial and ascospore characters can be to reflect evolutionary origin, given independent origins or losses of these characters over time. We therefore focus this paper on the 17 genera that can now be recognised phylogenetically, which concentrates on the species that are presently known from culture.

Circumscription of genera

Characters that are used to differentiate genera in the *Botryosphaeriaceae* have largely relied on the morphological features of the ascospores (Barr 1987, 1989, Hsieh & Chen 1994, Phillips *et al.* 2008) and especially the conidial states (Crous *et al.* 2006, Phillips *et al.* 2008). The most informative characters are conidial features such as pigmentation, wall thickness, and septation, but other characters such as presence or absence of paraphyses in the conidiomata can be useful. The phylogenetic value of these characters can only be meaningfully interpreted, however, in combination with additional data (e.g. sequence based molecular data), as illustrated by their misinterpretation in the past, and the multiple independent origins and losses of shared characters throughout the evolutionary history of the family (see Slippers *et al*. 2013, this volume).

Sexual morph morphology

Ascomata

Ascomata range from uniloculate, discrete structures (Fig. 1A, B) through to relatively large multiloculate structures (Fig. 1C, D). The uniloculate forms occur either individually and scattered over the host (Fig. 1E), or they can be aggregated in botryose clusters (Fig. 1F) of several hundred ascomata that are often united on a submerged basal stroma. In the species with multiloculate ascomata, conidiomata can occur within the same stroma. Sometimes the ascomata develop at the periphery of a central conidioma (Fig. 1G) and are united with the conidioma in a single stroma. When cut through horizontally the contents are typically brilliant white (Fig. 1H). Irrespective of the form they take, ascomata in *Botryosphaeria* species are typical of the loculoascomycetes in which the asci are formed within locules that develop in a pre-formed stroma. The tissues of the stromata are of *textura angularis* and made up of brown, thick-walled cells that turn blue-black in KOH and red-brown in lactic acid. The thickness of the stromata varies considerably not only between species but also with any given species. The walls can be as thin as just 5 or 6 cells layers, or it can be up to 30 or even more. The locules are lined with thin-walled, hyaline, flattened cells.

The centrum is of the *Pleospora* type in which the asci are interspersed with pseudoparaphyses that grow downwards and fuse at the base of the locule. The form of the ascomata is of little taxonomic value since even within a species ascomata can vary from uniloculate with relatively thin walls to complex multiloculate with thick walls and extensive stromatic tissue. This variation is probably in response to the substrate or the conditions under which the ascomata are formed. For example, ascomata in *B. dothidea* can be either simple, uniloculate structures scattered individually over the surface of the host tissue, or they can be aggregated in large botryose clusters. They can also be formed in large multiloculate stromata united with conidiomata. Furthermore, there does not appear to be any correlation between the form of the ascomata and the asexual genus associated with a particular species.

Asci

Asci are bitunicate of the fissitunicate type with a relatively thin ectotunica and a thick endotunica (Fig. 2A–C). The apex of the endotunica (Fig. 2D) is modified to form a well-defined apical chamber, which results from a displacement of the endotunica by the cytoplasm within the body of the ascus. No other structures can be detected in the ascus apex. Asci are clavate to elongate-clavate approaching cylindrical, but they are never truly cylindrical. They often have a short, indistinct stipe that terminates in a hoof-shaped cell attached to the inner wall of the base of the ascoma. Asci arise from a basal hymenium and grow up through the pseudoparaphyses (Fig. 2E). Ascospores are discharged forcibly by what has become known as the "Jack-in-the-box" process whereby the ectotunica splits transversely near the middle of the ascus and the endotunica elongates expelling the spores.

Ascospores

Ascospores are arranged within the asci in an irregular, overlapping biseriate manner (Fig. 2A–C). Typically they are hyaline and aseptate (Fig. 2H–J), but they can be pale or dark brown (Fig. 2F, G), sometimes 1-septate, and may have an apiculus at one or both ends (Fig. 2G). The walls are smooth and in most species they are usually thin, but in some, notably those species with *Diplodia* asexual morphs, it can be moderately thick. Ascospores can be hyaline or coloured, aseptate or 1–2-septate. In species with hyaline, aseptate ascospores the spores can become translucent brown and 1–2-septate with age (Fig. 2K), and the walls may appear roughened (Fig. 2L) due to the deposition of melanin granules on the inner surface, giving the spores a somewhat verruculose appearance. Shapes range from fusiform to ovoid. They are usually widest in the middle part and the ends are subobtusely rounded.

Pseudoparaphyses

Pseudoparaphyses are hyphal-like, hyaline with thin walls and frequent septa (Fig. 2E), branched, frequently anatomosing. Often they are constricted at the septa. As the asci develop and mature,

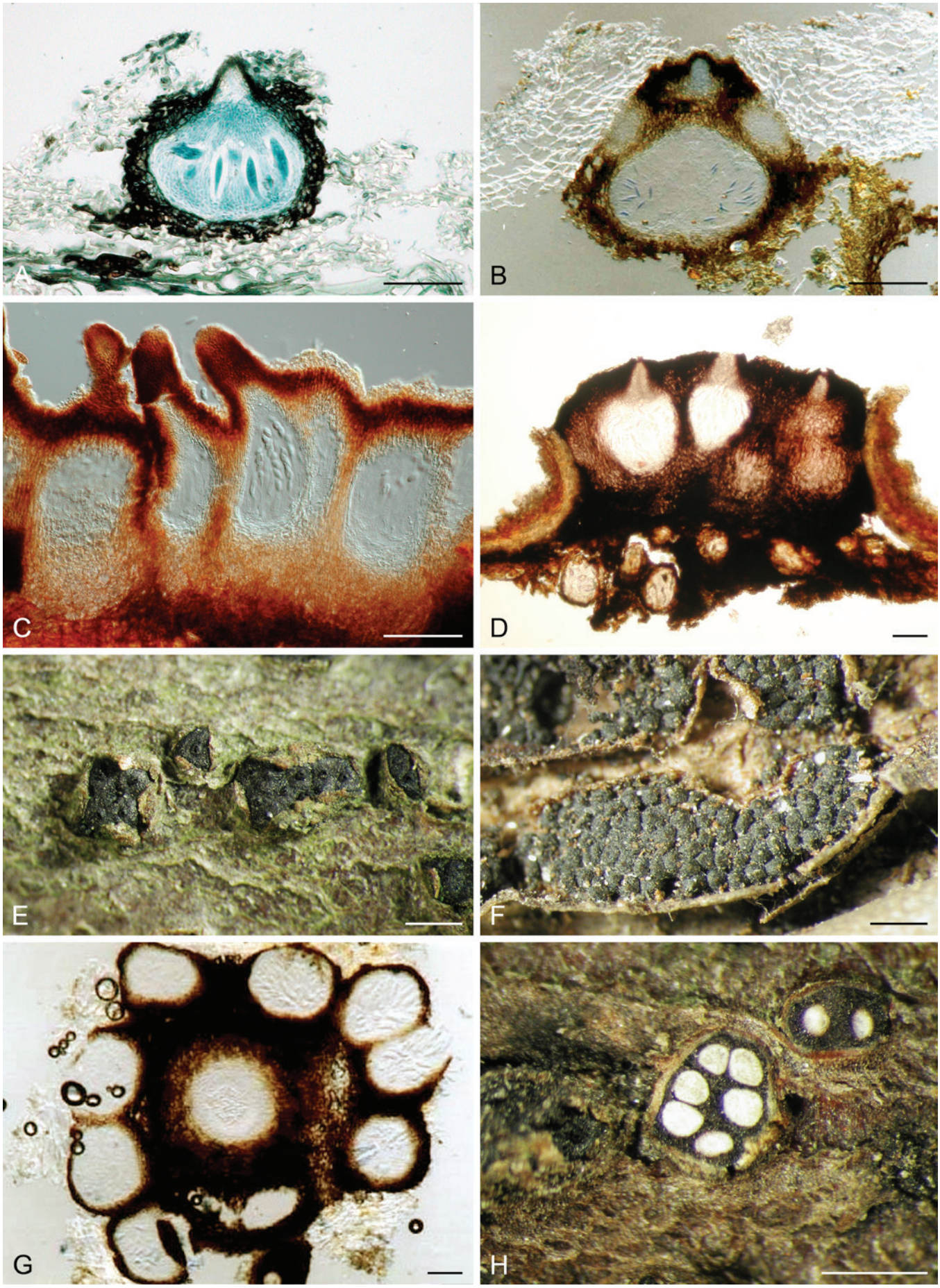

Fig. 1. Ascomata characters. A–D. Vertical sections through ascomata. E. Ascomata erumpent through host bark. F. Botryose clusters of ascomata. G. Transverse section through a central conidioma surrounded by ascomata. H. Ascomata cut through horizontally revealing the brilliant white contents. Scale bars: A–D, G = 100 µm, E = 1 mm, F, H = 500 µm.

Fig. 2. Asci and ascospores. A–C. Asci. D. Ascus tip showing the apical chamber. E. Pseudoparaphyses. F. Brown, 1-septate ascospores. G. Brown, aseptate ascospore with an apiculus at either end. H–J. Hyaline, aseptate ascospores. K, L. Pale brown, 2-septate, aged ascospores in two different focal planes to reveal the verruculose inner surface of the wall. Scale bars: A–C = 20 um, D–L = 10 µm.

the pseudoparaphyses gradually dissolve and only traces can be found in older ascomata.

Asexual morph morphology

Conidiomata

As with ascomata, conidiomata take on a variety of forms ranging from thin-walled uniloculate pycnidial to large, complex multiloculate forms. Irrespective of the form, the conidiomata are stromatic, that is, the pycnidial cavity develops within a preformed stroma (Fig. 3). The tissues that make up the stromatal and conidiomata walls are identical to those found in the ascostroma.

Conidiophores

Conidiophores are not always present in all species. Even within a species, conidiophores may be present or absent. When present,

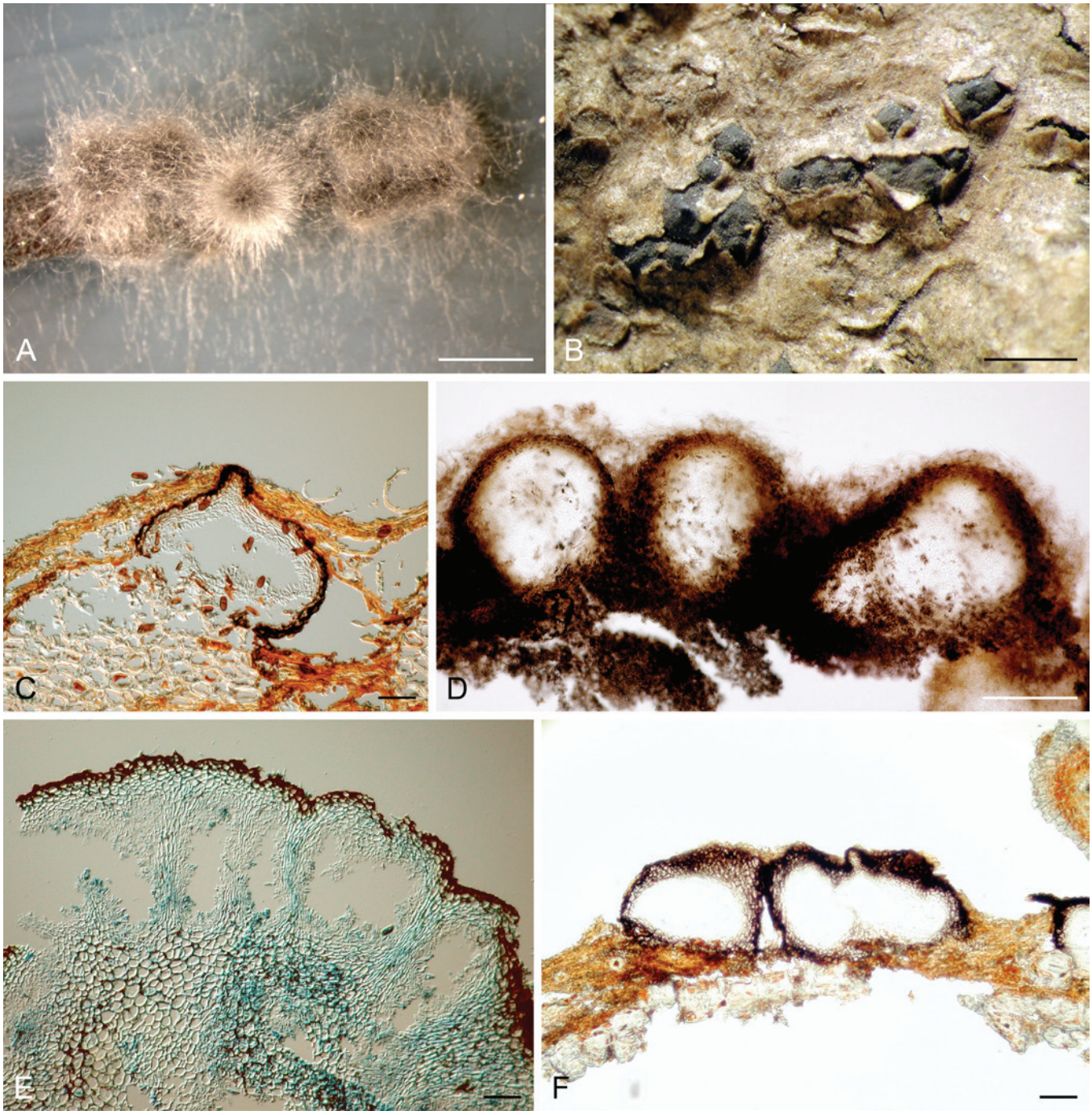

Fig. 3. Conidiomata. A. Conidiomata covered with mycelium, formed on pine needle in culture. B. Conidiomata erumpent through the host bark. C. Vertical section through a thin-walled conidioma. D. Section through conidiomata formed in culture. E. Transverse section through a stroma with several pycnidial locules. F. Vertical section through conidiomata. Scale bars: A = 1 mm, B, D = 500 µm, C, E, F = 50 µm.

they are hyaline, thin-walled and more or less cylindrical. Mostly they are not branched, but branched, septate conidiophores do occur.

Conidiogenesis

The first conidia are formed holoblastically at the tips of conidiogenous cells. Subsequent conidia are formed either by internal proliferation of the conidiogenous cells resulting in periclinal thickenings, or they may proliferate percurrently giving rise to two or three close or widely spaced annellations. Both types of proliferation can sometimes be seen on a single conidiogenous cell.

Conidiogenous cells are hyaline with a smooth, thin wall. Shapes vary from long cylindrical to short lageniform or ampuliform. In species with fusicoccum-like asexual morphs, the conidiogenous cells are generally smaller and more slender than the more robust types found in species with diplodia-like asexual morphs (Fig. 4).

Conidia

Conidia of the *Botryosphaeriaceae* display the greatest variation between genera and species. Although variation between species is wide, variability within a species can also be quite considerable. Two basic types of conidia can be distinguished, namely those that are thin-walled, narrow and fusicoccum-like, and the thick-walled, wider, diplodia-like conidia. In addition to these two basic types of conidia, coloured, muriform conidia are found in the *Dichomera* synasexual morph of some *Botryosphaeria* and *Neofusicoccum*

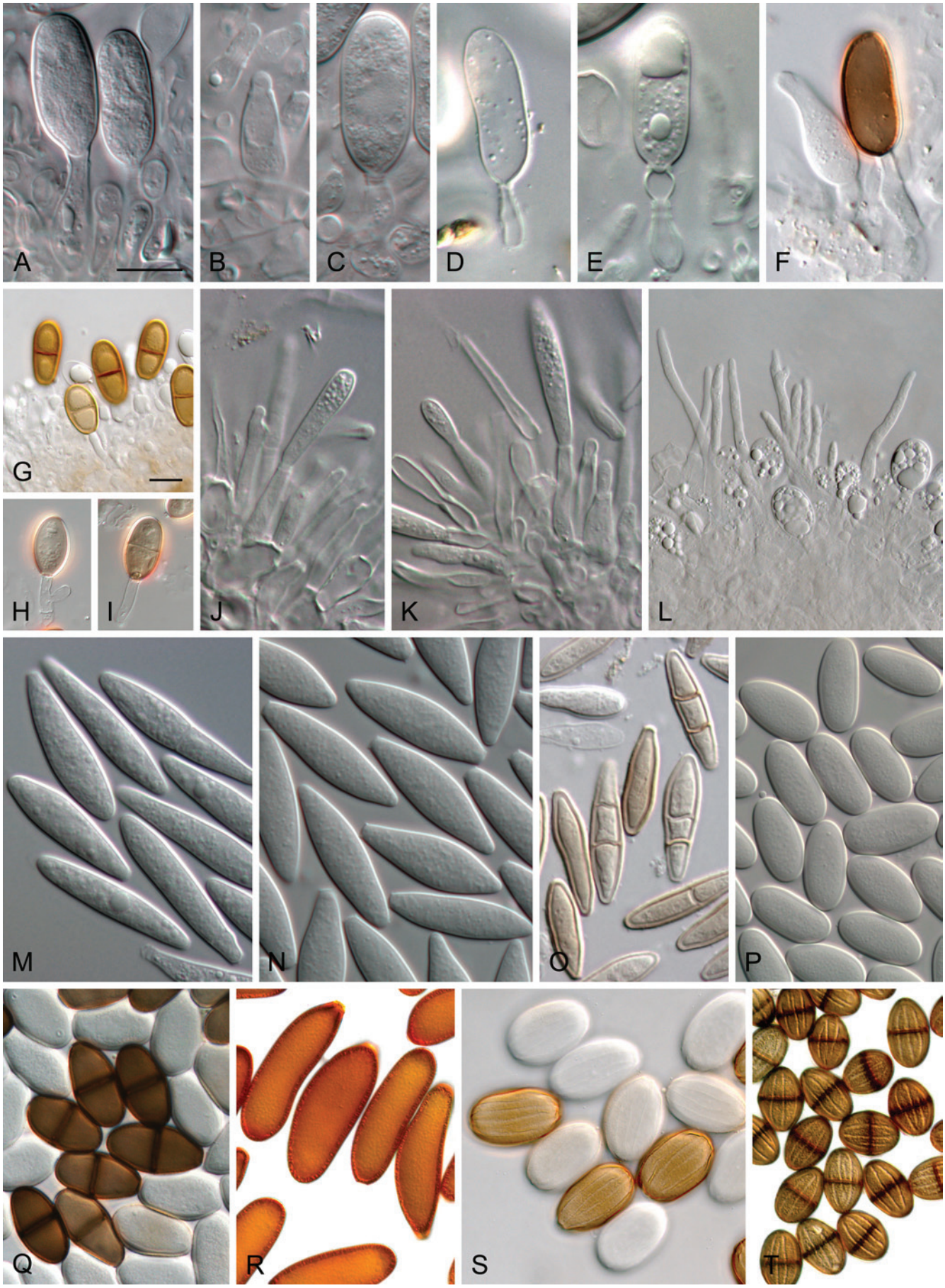

Fig. 4. Conidiogenous cells and conidia. A–K. Conidiogenous cells with periclinal thickenings (B, K) and annellations (A, C, D), the annellate cell in E has formed a secondary conidiogenous cell. F. Coloured, aseptate conidium of *Diplodia intermedia* attached to a conidiogenous cell. G. H, I. Coloured, 1-septate conidia of *Dothiorella* sp. attached to conidiogenous cells. L. Paraphyses arising between developing conidia in a *Lasiodiplodia* species. M. Hyaline, aseptate, thin-walled conida of *Botryosphaeria dothidea*. N. Hyaline, aseptate, thin-walled conida of *Neofusicoccum arbuti*. O. Coloured, septate conidia of *B. dothidea*. P. Hyaline, aseptate, thick-walled conidia of *Diplodia mutila*. Q. Hyaline, aseptate, coloured, 1-septate conidia of *Diplodia malorum*. R. Coloured, aseptate conidia of *Diplodia sapinea*. S. Striate, mature and immature conidia of *Barriopsis iraniana*. T. Striate, coloured, 1-septate conidia of a *Lasiodiplodia* species. Scale bars A, G = 10 µm. Scale bar in A applies to B–F, J, K, M–O. Scale bar in G applies to H–I, L, P–T.

species. Furthermore, arthric chains of dry, powdery conidia are a prominent feature of *Neoscytalidium* species.

The thin-walled, fusicoccum-like conidia range from fusiform to ovoid or elliptical, and typically they are hyaline and aseptate. However, the wall can become thicker and pale brown, and this may be related to aging. Other changes can take place just before germination when the normally hyaline, aseptate conidia can develop one or two septa and in some species they may become pale brown. In others, only the central cell becomes pigmented.

The diplodia-like conidia are relatively thick-walled and they can be hyaline or brown. Furthermore, they may be aseptate or 1-septate, sometimes two or even more septa can form. They are mostly ovoid with both ends broadly rounded. Externally the walls are smooth, but melanin deposits on the inner surface of the walls often give the conidia a verruculose appearance. In some species, especially those that have been assigned to the genus *Lasiodiplodia*, these deposits are arranged in longitudinal rows giving the conidia a striate appearance.

The timing of the onset of pigmentation varies considerably. In most *Diplodia* species, the conidia remain hyaline for a long time, and indeed they may never become brown. However, if they do become brown and septate, this occurs only after they have been discharged from the conidiomata, and in this case large numbers of brown, 1-septate conidia can be found on the surface of the host, surrounding the pycnidia. Nevertheless, in the group of species characterised by their brown, aseptate conidia (such as *D. seriata* and *D. sapinea*) pigmented conidia can be seen within the pycnidia, and often while the conidia are attached to the conidiogenous cells. In *Lasiodiplodia*, the conidia usually remain hyaline for a long time after they are formed, but they can become brown and 1-septate whilst enclosed within the pycnidia. Normally, however, pigmentation and septation happen after they have been discharged. Furthermore, in *Lasiodiplodia* the conidia invariably take on a striate appearance.

Conidia of some diplodia-like species become brown at an early stage of their development. For example, conidia of *D. seriata* become brown before they are discharged from the pycnidia. This pattern of development is also seen in *D. sapinea* and its close relative *D. scrobiculata*. In these three species (*D. sapinea, D. scrobiculata* and *D. seriata*) the conidia do not form septa, although one or more can develop at the time of germination. In one group of diplodia-like species the conidia become brown and septate at a very early stage, even before they are released from the conidiogenous cells. The genus *Dothiorella* was resurrected to accommodate these species (Phillips *et al.* 2005) and later *Spencermartinsia* was introduced to accommodate species with apiculate ascospores (Phillips *et al.* 2008).

Conidia of some species, both those in the diplodia-like group and the fusicoccum-like group, undergo morphological changes just before they germinate, and these changes can have diagnostic value. Thus, normally hyaline, aseptate conidia can develop one or two septa and become pale translucent brown just before germination. This pigmentation can be either uniform over the entire conidium, or one or more cells may be differentially pigmented. However, this aspect of morphological and colour changes at the time of germination has not been standardised, nor has it been studied for all species. Similarly, as the conidia age they may become darker and some develop septa. The effect of aging on morphological features of these fungi is even less well standardised and can be difficult to interpret.

Paraphyses

The presence or absence of paraphyses can be a useful character for differentiating genera in the *Botryosphaeriaceae*. However, in practice this can be difficult to apply because paraphyses are not well-defined. In this work we refer to paraphyses as sterile hyphal elements that form between and intermingled with conidiogenous cells. We further regard paraphyses as only those elements that extend beyond the height of conidiogenous cells and this helps to distinguish paraphyses from immature, or developing conidiogenous cells. In working through published descriptions of species, any mention of paraphyses was critically re-examined and only those that comply with the above definition were accepted.

An example where paraphyses are useful taxonomic characters is in the differentiation of *Lasiodiplodia* from *Neodeightonia*. Both *Lasiodiplodia* and *Neodeightonia* have striate conidia, but only *Lasiodiplodia* species have paraphyses. Likewise, presence of paraphyses in *Sphaeropsis* differentiates this genus from *Diplodia*, which does not have pycnidial paraphyses. Length of the paraphyses and their morphology, especially the presence or absence of a swelling at the tip can also aid in the differentiation of species.

Spermatogonia

Spermatial states are common in the *Dothideomycetes*, and also known in several species in the *Botryosphaeriaceae*. However, where seen they are not consistently formed by all isolates of a particular species, that is, they can be present or absent. Thus, their importance in the taxonomy and discrimination of species and genera is of questionable value.

The aim of the current paper was to consider all the genera and species in the *Botryosphaeriaceae* known from culture, based on their morphological characters and DNA sequence based phylogenetic relationships. The intention is to provide a comprehensive and up to date document that can serve as a foundation on which future descriptions of species and other genera can build. Of the older taxa linked to the *Botryosphaeriaceae*, and for which cultures or DNA sequence data are not available, very few, if any, can be linked to the current species that are known from culture. Such older taxa will have to be disregarded for future use unless they are epitypified. The current document will serve as a starting point for that process.

MATERIALS AND METHODS

Morphology

Fresh collections and type specimens were examined for most of the species included in this study. However, where the type (or other suitable specimens) could not be obtained, and no fresh collections were available, the descriptions were adapted from the orginal published descriptions. Isolations were made directly from ascomata or conidiomata on the host whenever possible. The sporocarps were cut through vertically with a sterile scalpel, one half was crushed in a drop of sterile water and then spread over the surface of a plate of 1/2 strength Difco potato-dextrose agar (PDA; Becton, Dickinson & Co, Sparks, USA). After incubation for up to 24 h, single germinating spores were transferred to fresh plates of PDA. The other half of the fruit body was placed in a drop of water on a microscope slide and the fertile tissues (asci or conidiogenous layer) were dissected and mounted in 100 %

lactic acid for microscopy. This method, when used for ascomata, allowed unambiguous connection to be established between the sexual and asexual morph.

Cultures were induced to sporulate by culturing on 2 % water agar bearing double-autoclaved poplar twigs, or pine needles. After a suitable period of incubation, ranging from 1–4 wk, conidiomata were cut through vertically, the conidiogenous layer dissected and mounted in 100 % lactic acid. Observations on micromorphological features were made with Leica MZ95 and Leica DMR microscopes and digital images were recorded with Leica DC300 and Leica DFC320 cameras, respectively. Measurements were made with the measurement module of the Leica IM500 image management system (Leica Micro-systems GmbH, Wetzlar, Germany). Mean, standard deviation (S.D.) and 95 % confidence intervals were calculated for asci, ascospores, and conidia. Minimum and maximum dimensions are given in parenthesis. Cultures were deposited in the CBS culture collection, taxonomic descriptions and nomenclature were deposited in MycoBank (www.MycoBank.org; Crous *et al.* 2004).

DNA isolation, sequencing and phylogenetic analyses

Most of the sequences used in this work were obtained from GenBank. Methods for DNA isolation, purification and sequencing of new sequences are detailed below. New sequences were deposited in GenBank, and the alignment in TreeBASE. Isolates and GenBank Accession numbers are listed in Table 1.

DNA isolation

Isolates were grown on PDA plates in darkness at 25 °C until they completely covered the medium surface. The mycelium was then scraped off and collected in a 2 mL Eppendorf tube with 50 µL of autoclaved glass micro spheres (230–320 µm diam). The tubes were then placed in liquid nitrogen for 5 min and transferred to ice. To separate organic and aqueous phases, 250 µL of phenol and 250 µL of chloroform were added, together with 500 µL of lysis buffer (100 mM NaCl, 10 mM Tris-HCl pH 8.0, 1 mM EDTA, 2 % Triton X-100, 1 % SDS). Tubes were vortexed for 20 min and then centrifuged (19000 × g, 4 °C, 25 min). The aqueous phase was transferred to a new 1.5 mL tube and the nucleic acids precipitated with an equal volume of cold absolute isopropanol. The tubes were centrifuged again (19 000 × g, 4 °C, 10 min), the supernatants discarded and the pellets washed with 1 mL of cold 70 % ethanol. After a further centrifugation (19 000 × g, 4 °C, 5 min), the supernatants were discarded and the pellets dried at RT with the tubes open in an inverted position. RNA was digested by incubating the pellets with 50 µL of TE (10 mM Tris, 1 mM EDTA) + RNAse A (Sigma®) (50 µg/mL) at 55 °C for 15 min.

DNA sequencing

A portion of the nuclear ribosomal 18S RNA gene (SSU) was amplified with primers NS1 and NS4 (White *et al.* 1990). The nucleotide sequence was determined using the above primers along with the internal sequencing primers NS2 and NS3 (White *et al*. 1990). The amplification and sequencing were done as described by Phillips *et al*. (2008).

Part of the nuclear rRNA cluster comprising the ITS region plus the D1/D2 variable domains of the ribosomal 28S RNA gene (LSU) was amplified using the primers ITS1 (White *et al.* 1990) and NL4 (O'Donnell 1993) as described by Alves *et al.* (2005). Nucleotide sequences of the ITS and D1/D2 regions were determined as described previously (Alves *et al*. 2004, 2005) using the primers ITS4 (White *et al*. 1990) and NL1 (O'Donnell 1993) as internal sequencing primers.

The primers EF1-688F (Alves *et al*. 2008) and EF1-986R (Carbone & Kohn 1999) and Bt2a and Bt2b (Glass & Donaldson 1995) were used to amplify and sequence part of the translation elongation factor 1-alpha (EF1-α) gene and part of the β-tubulin gene, respectively. Amplification and nucleotide sequencing of the EF1-α and β-tubulin genes were performed as described previously (Alves *et al*. 2006, 2008).

The amplified PCR fragments were purified with the JETQUICK PCR Purification Spin Kit (GENOMED, Löhne, Germany). Both strands of the PCR products were sequenced at STAB Vida Lda (Portugal) or GATC Biotech (Germany). The nucleotide sequences were read and edited with FinchTV v. 1.4.0 (Geospiza Inc.). All sequences were checked manually and nucleotide arrangements at ambiguous positions were clarified using both primer direction sequences

DNA sequencing and phylogenetic analysis

A phylogenetic analysis based on sequence data from five loci, namely SSU, ITS, LSU, EF1-α and β-tubulin, was done to define the phylogenetic position of genera in the *Botryosphaeriaceae*. Phylogenetic analyses based on ITS or ITS+EF1-α sequences were done for the species in each of the genera, except where there are few species in the genus.

Sequences were aligned with ClustalX v. 1.83 (Thompson *et al*. 1997), using the following parameters: pairwise alignment parameters (gap opening = 10, gap extension = 0.1) and multiple alignment parameters (gap opening = 10, gap extension = 0.2, transition weight = 0.5, delay divergent sequences = 25 %). Alignments were checked and manual adjustments were made where necessary.

Phylogenetic analyses of sequence data were done using PAUP v. 4.0b10 (Swofford 2003) for Maximum-Parsimony (MP) analyses and MEGA v. 5 (Tamura *et al.* 2011) for Maximum-Likelihood (ML) analyses. The general time reversible model of evolution (Rodriguez *et al.* 1990), including estimation of invariable sites and assuming a discrete gamma distribution with six rate categories (GTR+Γ+G) was used for the ML analysis. Trees were rooted using an outgroup and visualised with TreeView (Page 1996).

MP analyses were performed using the heuristic search option with 1 000 random taxa addition and tree bisection and reconnection (TBR) as the branch-swapping algorithm. All characters were unordered and of equal weight and gaps were treated as missing data. Maxtrees were set to 500, branches of zero length were collapsed, and all multiple equally most parsimonious trees were saved. The robustness of the most parsimonious trees was evaluated from 1 000 bootstrap replications. Other measures used were consistency index (CI), retention index (RI) and homoplasy index (HI).

ML analyses were performed on a MP starting tree automatically generated by the software. Nearest-Neigbour-Interchange (NNI) was used as the heuristic method for tree inference and 1 000 bootstrap replicates were performed.

Table 1. GenBank and culture collection accession numbers of species treated in the phylogenies.

		GenBank accession numbers				
Species[1]	**Cultures**[2]	**SSU**	**ITS**	**LSU**	**EF1-α**	**β-tubulin**
Barriopsis fusca	CBS 174.26 ex-type	EU673182	EU673330	DQ377857	EU673296	EU673109
Barriopsis iraniana	CBS 124698 ex-type	N/A	FJ919663	N/A	FJ919652	N/A
	IRAN 1449C	N/A	FJ919665	N/A	FJ919654	N/A
Botryobambusa fusicoccum	CBS 134113 ex-type	JX646826	JX646792	JX646809	JX646857	N/A
	MFLUCC 11-0657	JX646827	JX646793	JX646810	JX646858	N/A
Botryosphaeria agaves	CBS 133992 ex-neotype	JX646825	JX646791	JX646808	JX646856	JX646841
	MFLUCC 10-0051	JX646824	JX646790	JX646807	JX646855	JX646840
Botryosphaeria corticis	CBS 119047 ex-epitype	EU673175	DQ299245	EU673244	EU017539	EU673107
	ATCC 22927	EU673176	DQ299247	EU673245	EU673291	EU673108
Botryosphaeria dothidea	CBS 115476 ex-epitype	EU673173	AY236949	AY928047	AY236898	AY236927
	CBS 110302	EU673174	AY259092	EU673243	AY573218	EU673106
Botryosphaeria fabicerciana	CBS 127193 ex-type	N/A	HQ332197	N/A	HQ332213	N/A
	CMW 27108	N/A	HQ332200	N/A	HQ332216	N/A
Botryosphaeria fusispora	MFLUCC 10-0098 ex-type	JX646823	JX646789	JX646806	JX646854	JX646839
	MFLUCC 11-0507	JX646822	JX646788	JX646805	JX646853	JX646838
Botryosphaeria ramosa	CBS 122069 ex-type	N/A	EU144055	N/A	EU144070	N/A
Botryosphaeria scharifii	CBS 124703 ex-type	N/A	JQ772020	N/A	JQ772057	N/A
	CBS 124702	N/A	JQ772019	N/A	JQ772056	N/A
Cophinforma atrovirens	CBS 124934 ex-type	N/A	FJ888473	N/A	FJ888456	N/A
	CBS 124935	N/A	FJ888476	N/A	FJ888457	N/A
Cophinforma atrovirens	MFLUCC 11-0425 ex-type	JX646833	JX646800	JX646817	JX646865	JX646848
	MFLUCC 11-0655	JX646834	JX646801	JX646818	JX646866	JX646849
Cophinforma atrovirens	CBS 117444	KF531821	KF531822	DQ377855	KF531801	KF531802
	CBS 117450	N/A	EF118051	N/A	GU134937	N/A
Diplodia africana	CBS 120835 ex-type	N/A	EF445343	N/A	EF445382	N/A
	CBS 121104	N/A	EF445344	N/A	EF445383	N/A
Diplodia alatafructa	CBS 124931 ex-type	N/A	FJ888460	N/A	FJ888444	N/A
	CBS 124933 ex-paratype	N/A	FJ888478	N/A	FJ888446	N/A
Diplodia allocellula	CBS 130408 ex-type	N/A	JQ239397	JQ239410	JQ239384	JQ239378
	CBS 130410 ex-paratype	N/A	JQ239399	JQ239412	JQ239386	JQ239380
Diplodia agrifolia	CBS 132777 ex-type	N/A	JN693507	N/A	JQ517317	JQ411459
	UCROK 1429	N/A	JQ411412	N/A	JQ512121	JQ411443
Diplodia bulgarica	CBS 124254 ex-type	N/A	GQ923853	N/A	GQ923821	N/A
	CBS 124135	N/A	GQ923852	N/A	GQ923820	N/A
Diplodia corticola	CBS 112549 ex-type	EU673206	AY259100	AY928051	AY573227	DQ458853
	CBS 112546	EU673207	AY259110	EU673262	DQ458872	EU673117
Diplodia cupressi	CBS 168.87 ex-type	EU673209	DQ458893	EU673263	DQ458878	DQ458861
	CBS 261.85	EU673210	DQ458894	EU673264	DQ458879	DQ458862
Diplodia intermedia	CBS 124462 ex-type	N/A	GQ923858	N/A	GQ923826	N/A
	CBS 124134	N/A	HM036528	N/A	GQ923851	N/A
Diplodia malorum	CBS 124130 ex-epitype	N/A	GQ923865	N/A	GQ923833	N/A
	CBS 112554	N/A	AY259095	N/A	DQ458870	N/A
Diplodia mutila	CBS 112553	EU673213	AY259093	AY928049	AY573219	DQ458850
	CBS 230.30	EU673214	DQ458886	EU673265	DQ458869	DQ458849
Diplodia olivarum	CBS 121887 ex-type	N/A	EU392302	N/A	EU392279	HQ660079
	CBS 121886	N/A	EU392297	N/A	EU392274	N/A
Diplodia pseudoseriata	CBS 124906 ex-type	N/A	EU080927	N/A	EU863181	N/A
	CBS 124907 ex-paratype	N/A	EU080922	N/A	EU863179	N/A
Diplodia quercivora	CBS 133852 ex-type	N/A	JX894205	N/A	JX894229	N/A
	CBS 133853	N/A	JX894206	N/A	JX894230	N/A

Table 1. (Continued).

		GenBank accession numbers				
Species[1]	**Cultures[2]**	**SSU**	**ITS**	**LSU**	**EF1-α**	**β-tubulin**
Diplodia rosulata	CBS 116470 ex-type	EU673211	EU430265	DQ377896	EU430267	EU673132
	CBS 116472	EU673212	EU430266	DQ377897	EU430268	EU673131
Diplodia sapinea	CBS 393.84 (A) ex-epitype	EU673219	DQ458895	DQ377893	DQ458880	DQ458863
	CBS 109725 (C)	EU673222	DQ458896	EU673270	DQ458881	DQ458864
Diplodia scrobiculata	CBS 118110 ex-type	N/A	AY253292	N/A	AY624253	AY624258
	CBS 109944	EU673218	DQ458899	EU673268	DQ458884	DQ458867
	CBS 113423	EU673217	DQ458900	EU673267	DQ458885	DQ458868
Diplodia seriata	CBS 112555 ex-epitype	EU673215	AY259094	AY928050	AY573220	DQ458856
	CBS 119049	EU673216	DQ458889	EU673266	DQ458874	DQ458857
Diplodia tsugae	CBS 418.64 ex-isotype	EU673208	DQ458888	DQ377867	DQ458873	DQ458855
Dothiorella americana	CBS 128309 ex-type	N/A	HQ288218	N/A	HQ288262	HQ288297
	CBS 128310	N/A	HQ288219	N/A	HQ288263	HQ288298
Dothiorella brevicollis	CBS 130411 ex-type	N/A	JQ239403	JQ239416	JQ239390	JQ239371
	CBS 130412 ex-paratype	N/A	JQ239404	JQ239417	JQ239391	JQ239372
Dothiorella casuarinae	CBS 120688 ex-type	N/A	DQ846773	N/A	DQ875331	N/A
	CBS 120690	N/A	DQ846774	N/A	DQ875333	N/A
Dothiorella dulcispinae	CBS 130413 ex-type	N/A	JQ239400	JQ239413	JQ239387	JQ239373
	CBS 130414 ex-paratype	N/A	JQ239401	JQ239414	JQ239388	JQ239374
	CBS 130415 ex-paratype	N/A	JQ239402	JQ239415	JQ239389	JQ239375
	CBS 121764	N/A	EU101299	N/A	EU101344	N/A
	CBS 121765	N/A	EU101300	N/A	EU101345	N/A
Dothiorella iberica	CBS 115041 ex-type	EU673155	AY573202	AY928053	AY573222	EU673096
	CBS 113188	EU673156	AY573198	EU673230	EU673278	EU673097
	CAA 005	EU673157	EU673312	EU673231	EU673279	EU673098
Dothiorella longicollis	CBS 122068 ex-type	N/A	EU144054	N/A	EU144069	N/A
	CBS 122067	N/A	EU144052	N/A	EU144067	N/A
Dothiorella moneti	MUCC 505 ex-type	N/A	EF591920	EF591937	EF591971	EF591954
	MUCC 507	N/A	EF591922	EF591939	EF591973	EF591956
Dothiorella pretoriensis	CBS 130404 ex-type	N/A	JQ239405	JQ239418	JQ239392	JQ239376
	CBS 130403 ex-paratype	N/A	JQ239406	JQ239419	JQ239393	JQ239377
Dothiorella santali	MUCC 509 ex-type	N/A	EF591924	EF591941	EF591975	EF591958
	MUCC 508	N/A	EF591923	EF591940	EF591974	EF591957
Dothiorella sarmentorum	IMI 63581b ex-type	EU673158	AY573212	AY928052	AY573235	EU673102
	CBS 115038	EU673159	AY573206	DQ377860	AY573223	EU673101
Dothiorella thailandica	CBS 133991 ex-type	JX646829	JX646796	JX646813	JX646861	JX646844
Dothiorella thripsita	BRIP 51876 ex-type	N/A	FJ824738	N/A	N/A	N/A
Dothiorella uruguayensis	CBS 124908 ex-type	N/A	EU080923	N/A	EU863180	N/A
Dothiorella sp.1	CBS 188.87	EU673161	EU673316	DQ377891	EU673283	EU673119
	CBS 242.51	EU673162	EU673317	EU673235	EU673284	EU673105
Dothiorella sp.2	JL 599	EU673164	EU673314	EU673233	EU673281	EU673099
Dothiorella sp.3	CBS 124723	EU673163	EU673313	EU673232	EU673280	EU673100
Dothiorella sp.4	CBS 124731	EU673170	EU673321	EU673240	EU673288	EU673143
	CBS 124730	EU673169	EU673320	EU673239	EU673287	EU673142
Endomelanconiopsis endophytica	CBS 120397 ex-type	N/A	EU683656	EU683629	EU683637	N/A
	CBS 122550	N/A	EU683664	EU683634	EU683645	N/A
Endomelanconiopsis microspora	CBS 353.97 ex-type	N/A	EU683655	EU683628	EU683636	N/A
Lasiodiplodia citricola	CBS 124707 ex-type	N/A	GU945354	N/A	GU945340	N/A
	CBS 124706	N/A	GU945353	N/A	GU945339	N/A
Lasiodiplodia crassispora	CBS 118741 ex-type	N/A	DQ103550	N/A	EU673303	N/A
	WAC 12534	N/A	DQ103551	N/A	DQ103558	N/A

Table 1. (Continued).

Species[1]	Cultures[2]	GenBank accession numbers				
		SSU	ITS	LSU	EF1-α	β-tubulin
	CBS 110492	EU673189	EF622086	EU673251	EF622066	EU673134
Lasiodiplodia egyptiacae	CBS 130992 ex-type	N/A	JN814397	N/A	JN814424	N/A
	BOT-29	N/A	JN814401	N/A	JN814428	N/A
Lasiodiplodia gilanensis	CBS 124704 ex-type	N/A	GU945351	N/A	GU945342	N/A
	CBS 124705	N/A	GU945352	N/A	GU945341	N/A
Lasiodiplodia gonubiensis	CBS 115812 ex-type	EU673193	AY639595	DQ377902	DQ103566	DQ458860
	CBS 116355	EU673194	AY639594	EU673252	DQ103567	EU673126
Lasiodiplodia hormozganensis	CBS 124709 ex-type	N/A	GU945355	N/A	GU945343	N/A
	CBS 124708	N/A	GU945356	N/A	GU945344	N/A
Lasiodiplodia iraniensis	CBS 124710 ex-type	N/A	GU945346	N/A	GU945334	N/A
	CBS 124711	N/A	GU945347	N/A	GU945335	N/A
Lasiodiplodia lignicola	CBS 134112 ex-type	JX646830	JX646797	JX646814	JX646862	JX646845
	MFLUCC 11-0656	JX646831	JX646798	JX646815	JX646863	JX646846
Lasiodiplodia margaritacea	CBS 122519 ex-type	N/A	EU144050	N/A	EU144065	N/A
	CBS 122065	N/A	EU144051	N/A	EU144066	N/A
Lasiodiplodia mahajangana	CBS 124927 ex-type	N/A	FJ900597	N/A	FJ900643	N/A
	CBS 124925 ex-type	N/A	FJ900595	N/A	FJ900641	N/A
Lasiodiplodia missouriana	CBS 128311 ex-type	N/A	HQ288225	N/A	HQ288267	N/A
	CBS 128312	N/A	HQ288226	N/A	HQ288268	N/A
Lasiodiplodia parva	CBS 456.78 ex-type	N/A	EF622083	N/A	EF622063	N/A
	CBS 494.78	EU673201	EF622084	EU673258	EF622064	EU673114
	CBS 356.59	EU673200	EF622082	EU673257	EF622062	EU673113
Lasiodiplodia plurivora	CBS 120832 ex-type	N/A	EF445362	N/A	EF445395	N/A
	CBS 121103	N/A	AY343482	N/A	EF445396	N/A
Lasiodiplodia pseudotheobromae	CBS 116459 ex-type	EU673199	EF622077	EU673256	EF622057	EU673111
	CBS 447.62	EU673198	EF622081	EU673255	EF622060	EU673112
Lasiodiplodia rubropurpurea	CBS 118740 ex-type	EU673191	DQ103553	DQ377903	EU673304	EU673136
	WAC 12536	N/A	DQ103554	N/A	DQ103572	N/A
Lasiodiplodia theobromae	CBS 164.96 ex-neotype	EU673196	AY640255	EU673253	AY640258	EU673110
	CBS 124.13	EU673195	DQ458890	AY928054	DQ458875	DQ458858
	CBS 111530	N/A	EF622074	N/A	EF622054	N/A
	CAA 006	EU673197	DQ458891	EU673254	DQ458876	DQ458859
Lasiodiplodia venezuelensis	CBS 118739 ex-type	EU673192	DQ103547	DQ377904	EU673305	EU673129
	WAC 12540	N/A	DQ103548	N/A	DQ103569	N/A
Lasiodiplodia viticola	CBS 128313 ex-type	N/A	HQ288227	N/A	HQ288269	HQ288306
	CBS 128315	N/A	HQ288228	N/A	HQ288270	HQ288307
Macrophomina phaseolina	CBS 227.33	KF531823	KF531825	DQ377906	KF531804	KF531806
	CBS 162.25	KF531824	KF531826	DQ377905	KF531803	KF531805
Neodeightonia palmicola	MFLUCC 10-0822 ex-type	HQ199223	HQ199221	HQ199222	N/A	N/A
	MFLUCC 10-0823	HQ199226	HQ199224	HQ199225	N/A	N/A
Neodeightonia phoenicum	CBS 122528 ex-type	EU673205	EU673340	EU673261	EU673309	EU673116
	CBS 169.34	EU673203	EU673338	EU673259	EU673307	EU673138
Neodeightonia subglobosa	CBS 448.91 ex-type	EU673202	EU673337	DQ377866	EU673306	EU673137
	MFLUCC 11-0163	N/A	JX646794	JX646811	JX646859	JX646842
Neofusicoccum andinum	CBS 117453 ex-type	N/A	AY693976	N/A	AY693977	N/A
	CBS 117452	N/A	DQ306263	N/A	DQ306264	N/A
Neofusicoccum arbuti	CBS 116131 ex-type	KF531814	AY819720	DQ377915	KF531792	KF531793
	CBS 117090	KF531813	AY819724	DQ377919	KF531791	KF531794
Neofusicoccum australe	CMW 6837 ex-type	N/A	AY339262	N/A	AY339270	AY339254
	CMW 6853	N/A	AY339263	N/A	AY339271	AY339255

Table 1. (Continued).

		GenBank accession numbers				
Species[1]	**Cultures[2]**	**SSU**	**ITS**	**LSU**	**EF1-α**	**β-tubulin**
Neofusicoccum batangarum	CBS 124924 ex-type	N/A	FJ900607	N/A	FJ900653	FJ900634
	CBS 124923	N/A	FJ900608	N/A	FJ900654	FJ900635
Neofusicoccum cordaticola	CBS 123634 ex-type	N/A	EU821898	N/A	EU821868	EU821838
	CBS 123635	N/A	EU821903	N/A	EU821873	EU821843
Neofusicoccum corticosae	CBS 120081 ex-type	N/A	DQ923533	N/A	N/A	N/A
Neofusicoccum eucalypticola	CBS 115679 ex-type	N/A	AY615141	N/A	AY615133	AY615125
	CBS 115766	N/A	AY615143	N/A	AY615135	AY615127
	CBS 115791 ex-type	N/A	AF283686	N/A	AY236891	AY236920
	CMW 10126	N/A	AF283687	N/A	AY236892	AY236921
Neofusicoccum grevilleae	CBS 129518 ex-type	N/A	JF951137	JF951157	N/A	N/A
Neofusicoccum kwambonambiense	CBS 123639 ex-type	N/A	EU821900	N/A	EU821870	EU821840
	CBS 123641	N/A	EU821919	N/A	EU821889	EU821859
Neofusicoccum luteum	CBS 110299 ex-type sexual morph	EU673148	AY259091	AY928043	AY573217	DQ458848
	CBS 562.92 ex-type asexual morph	N/A	N/A	N/A	N/A	N/A
	CBS 110497	EU673149	EU673311	EU673229	EU673277	EU673092
Neofusicoccum macroclavatum	CBS 118223 ex-type	N/A	DQ093196	N/A	DQ093217	DQ093206
	WAC 12445	N/A	DQ093197	N/A	DQ093218	DQ093208
Neofusicoccum mangiferae	CBS 118531	EU673153	AY615185	DQ377920	DQ093221	AY615172
	CBS 118532	EU673154	AY615186	DQ377921	DQ093220	AY615173
Neofusicoccum mediterraneum	CBS 121718 ex-type	N/A	GU251176	N/A	GU251308	GU251836
	CBS 121558	N/A	GU799463	N/A	GU799462	GU799461
Neofusicoccum nonquaesitum	CBS 126655 ex-type	N/A	GU251163	N/A	GU251295	GU251823
	PD 301	N/A	GU251164	N/A	GU251296	GU251824
Neofusicoccum occulatum	CBS 128008 ex-type	N/A	EU301030	N/A	EU339509	EU339472
	MUCC 286	N/A	EU736947	N/A	EU339511	EU339474
Neofusicoccum parvum	ATCC 58191 ex-type	EU673151	AY236943	AY928045	AY236888	AY236917
	CBS 110301	EU673150	AY259098	AY928046	AY573221	EU673095
Neofusicoccum pennatisporum	WAC 13153 ex-type	N/A	EF591925	EF591942	EF591976	EF591959
Neofusicoccum protearum	STE-U 4361 ex-type asexual morph	N/A	AF196295	N/A	N/A	N/A
	CBS 114176 ex-type sexual morph	N/A	AF452539	N/A	N/A	N/A
Neofusicoccum ribis	CBS 115475 ex-type	N/A	AY236935	N/A	AY236877	AY236906
	CBS 121.26	N/A	AF241177	N/A	AY236879	AY236908
Neofusicoccum umdonicola	CBS 123645 ex-type	N/A	EU821904	N/A	EU821874	EU821844
	CBS 123646	N/A	EU821905	N/A	EU821875	EU821845
Neofusicoccum viticlavatum	CBS 112878 ex-type	N/A	AY343381	N/A	AY343342	N/A
	CBS 112977	N/A	AY343380	N/A	AY343341	N/A
Neofusicoccum vitifusiforme	CBS 110887 ex-type	N/A	AY343383	N/A	AY343343	N/A
	CBS 110880	N/A	AY343382	N/A	AY343344	N/A
Neoscytalidium hyalinum	CBS 499.66	KF531818	KF531820	DQ377925	KF531798	KF531800
	CBS 251.49	KF531817	KF531819	DQ377923	KF531797	KF531799
	CBS 145.78 ex-isotype	KF531815	KF531816	DQ377922	KF531795	KF531796
Neoscytalidium novaehollandiae	CBS 122071 ex-type	N/A	EF585540	N/A	EF585580	N/A
	CBS 122610	N/A	EF585536	N/A	EF585578	N/A
Phaeobotryon cercidis		N/A	N/A	N/A	N/A	N/A
Phaeobotryon cupressi	CBS 124700 ex-type	N/A	FJ919672	N/A	FJ919661	N/A
	IRAN 1458C	N/A	FJ919671	N/A	FJ919660	N/A
Phaeobotryon mamane	CBS 122980 ex-type	EU673184	EU673332	EU673248	EU673298	EU673121
	CPC 12442	EU673185	EU673333	DQ377899	EU673299	EU673124
Pseudofusicoccum adansoniae	CBS 122055 ex-type	N/A	EF585523	N/A	EF585571	N/A
	WAC 12689	N/A	EF585534	EF585554	EF585567	N/A

Table 1. (Continued).

Species[1]	Cultures[2]	GenBank accession numbers				
		SSU	ITS	LSU	EF1-α	β-tubulin
Pseudofusicoccum ardesiacum	CBS 122062 ex-type	N/A	EU144060	N/A	EU144075	N/A
	WAC 13294	N/A	GU172405	N/A	GU172437	N/A
Pseudofusicoccum kimberleyense	CBS 122058 ex-type	N/A	EU144057	N/A	EU144072	N/A
	CBS 122059	N/A	EU144056	N/A	EU144071	N/A
Pseudofusicoccum olivaceum	CBS 124939 ex-type	N/A	FJ888459	N/A	FJ888437	N/A
	CBS 124940	N/A	FJ888462	N/A	FJ888438	N/A
Pseudofusicoccum stromaticum	CBS 117448 ex-type	EU673146	AY693974	DQ377931	AY693975	EU673094
	CBS 117449	EU673147	DQ436935	DQ377932	DQ436936	EU673093
Pseudofusicoccum violaceum	CBS 124936 ex-type	N/A	FJ888474	N/A	FJ888442	N/A
	CBS 124937	N/A	FJ888458	N/A	FJ888440	N/A
Spencermartinsia viticola	CBS 117009 ex-type	EU673165	AY905554	DQ377873	AY905559	EU673104
	CBS 302.75	EU673168	EU673319	EU673238	EU673286	EU673135
Spencermartinsia sp.1	ICMP 16827	EU673171	EU673322	EU673241	EU673289	EU673144
	ICMP 16828	EU673172	EU673323	EU673242	EU673290	EU673145
Spencermartinsia sp.2	CBS 500.72	EU673167	EU673318	EU673237	EU673285	EU673118
Spencermartinsia sp.3	CBS 117006	EU673166	AY905555	EU673236	AY905562	EU673103
Sphaeropsis citrigena	ICMP 16812 ex-type	EU673180	EU673328	EU673246	EU673294	EU673140
	ICMP 16818	EU673181	EU673329	EU673247	EU673295	EU673141
Sphaeropsis eucalypticola	CBS 133993 ex-type	JX646835	JX646802	JX646819	JX646867	JX646850
	MFLUCC 11-0654	JX646836	JX646803	JX646820	JX646868	JX646851
Sphaeropsis porosa	CBS 110496 ex-type	EU673179	AY343379	DQ377894	AY343340	EU673130
	CBS 110574	N/A	AY343378	N/A	AY343339	N/A
Sphaeropsis visci	CBS 122526 ex-neotype	N/A	EU673324	N/A	EU673292	N/A
	CBS 186.97	EU673178	EU673325	DQ377868	EU673293	EU673128
	CBS 100163	EU673177	EU673324	DQ377870	EU673292	EU673127
Tiarosporella graminis var. *karoo*	CBS 118718	KF531827	KF531828	DQ377939	KF531807	KF531808
Tiarosporella madreeya	CBS 532.76	N/A	KC769960	DQ377940	N/A	N/A
Tiarosporella tritici	CBS 118719 ex-type	KF531829	KF531830	DQ377941	KF531809	KF531810
Tiarosporella urbis-rosarum	CBS 130405 ex-type	N/A	JQ239407	JQ239420	JQ239394	JQ239381
	CBS 130406 ex-paratype	N/A	JQ239408	JQ239421	JQ239395	JQ239382

[1]Type species of each genus are given in bold typeface.

[2]Acronyms of culture collections: ATCC: American Type Culture Collection, Virginia, USA; BRIP: Culture collection, Queensland Department of Agriculture and Fisheries, Queensland, Australia; CAA: Personal culture collection A Alves, University of Aveiro, Portugal; CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CMW: Tree Patholgy Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; ICMP: International Collection of Microorganisms from Plants, Landcare Research, Aukland, New Zealand; IMI: International Mycological Institute, CBI-Bioscience, Egham, Bakeham Lane, UK; IRAN: Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Iran; JL: Personal culture collection, J Luque, IRTA, Barcelona, Spain; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; PD: Culture collection, University of California, Davis, USA; STE-U: Culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa; UCROK: Culture collection, University of Riverside, California, USA; WAC: Department of Agriculture, Western Australia Plant Pathogen Collection, South Perth, Western Australia.

RESULTS

DNA phylogeny

After alignment the combined five-locus dataset consisted of 3 362 characters (including alignment gaps) for 94 ingroup taxa and one outgroup taxon. Of the 3 362 characters, 2 418 were constant and 159 were variable and parsimony-uninformative. Maximum parsimony analysis of the remaining 785 parsimony-informative characters resulted in 16 equally most parsimonious trees of 3 010 steps (CI = 0.499, RI = 0.846, HI = 0.501), one of which is shown in Fig. 5. The phylogenetic tree resulting from ML analyses using the general time reversible model of DNA evolution (Rodriguez *et al.* 1990), including estimation of invariable sites and assuming a discrete gamma distribution with six rate categories (GTR+Γ+G), had a topology identical to the MP tree presented.

In both analyses (MP and ML) a clade corresponding to the family *Botryosphaeriaceae* received a bootstrap support of 100 %. The genera *Saccharata* (used as outgroup) and *Melanops* are clearly excluded from the family. Within the *Botryosphaeriaceae* 17 clades corresponding to an equal number of genera could be readily recognised. All clades received moderate to high bootstrap support (> 70 %). The only exception was the *Dothiorella* clade, which had very low bootstrap support in both MP and ML analyses.

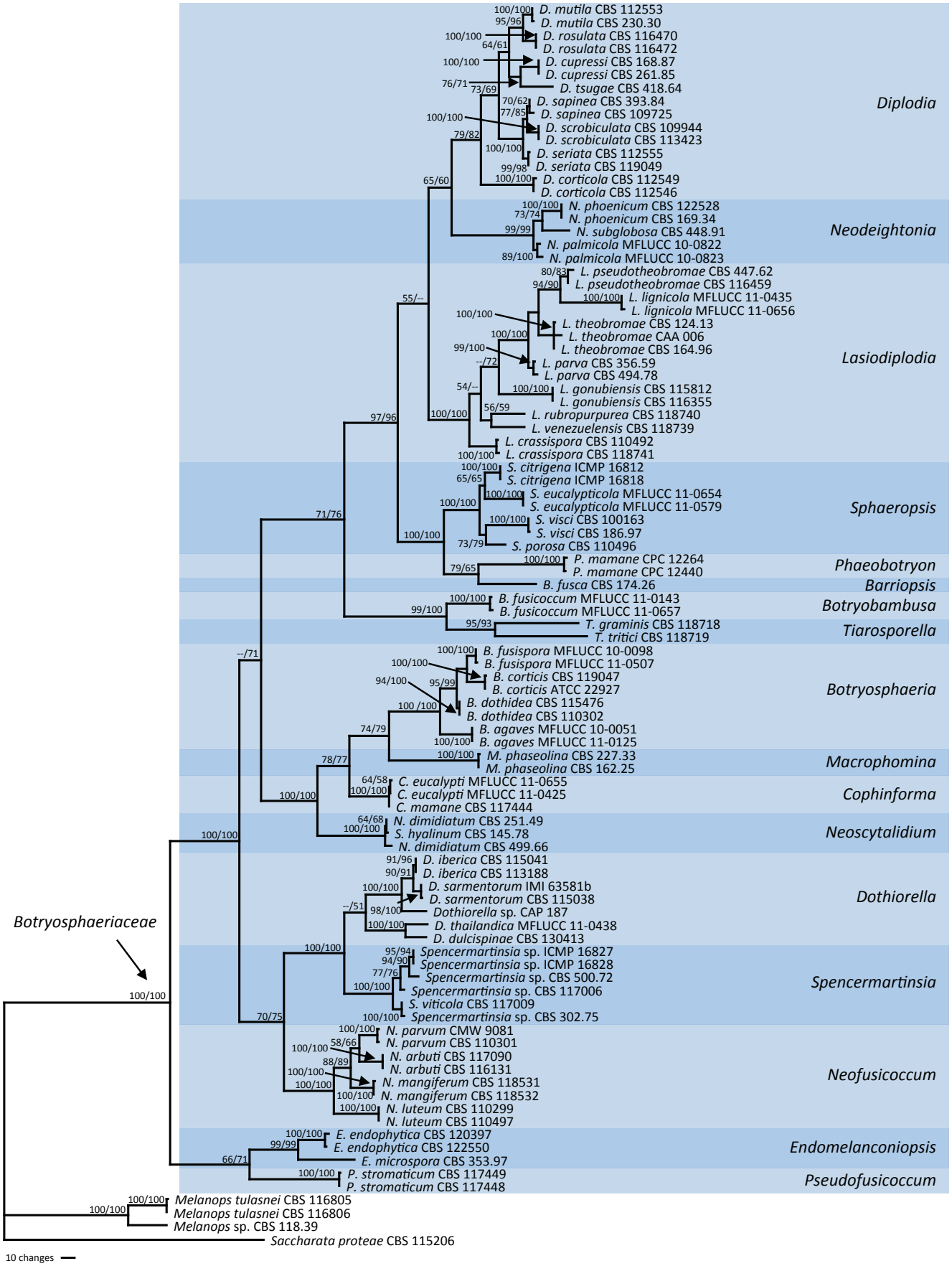

Fig. 5. One of 16 equally most parsimonious trees obtained from the combined analysis of 5 loci (SSU, LSU, ITS, EF1-α and β-tubulin), for all genera in the *Botryosphaeriaceae* that are known from culture. Gaps were treated as missing data. MP/ML values (> 50 %) resulting from 1000 bootstrap replicates are given at the nodes. The tree was rooted to *Saccharata proteae* CBS 115206. Clades corresponding to genera within the family *Botryosphaeriaceae* are highlighted.

Although Liu *et al.* (2012) included *Auerswaldia* in the *Botryosphaeriaceae*, our analysis of the sequences of their isolates revealed that *A. dothiorella* is in fact a species of *Dothiorella* while *A. lignicola* apears to be best placed in *Lasiodiplodia*. Thus, at this stage there is no evidence to indicate that *Auerswaldia* should be considered as a genus in the *Botryosphaeriaceae*.

Therefore, we accept 17 genera in the *Botryosphaeriaceae*. These genera, defined as clades in the five-locus phylogeny, are also supported by morphological characteristics. These morphological characters provide the basis for the following key to the genera.

Key to the genera[1]

1. Conidia formed within a pycnidium 2
1. Conida formed as dry powdery arthric chains ..... *Neoscytalydium*

2. Conidia hyaline (only rarely turn brown with age) 3
2. Conidia brown (can remain hyaline for some time before becoming brown) 8

3. Conidia hyaline, with persistent mucous sheath 4
3. Conidia hyaline, mucous sheath absent 5

4. Conidia fusiform with apical mucoid appendages ..... *Tiarosporella*
4. Conidia cylindrical, not reaching 50 µm long ..... *Pseudofusicoccum*

5. Conidia thin-walled 6
5. Conidia thick-walled 9

6. Conidia mostly fusoid to ellipsoidal 7
6. Conidia cylindrical to cylindro-clavate ..... *Botryobambusa*

7. Most conidia longer than 30 µm ..... *Cophinforma*
7. Conidia mostly less that 30 µm long ..... *Botryosphaeria/Neofusicoccum*[2]

8. Conidia with a single germ slit ..... *Endomelanconiopsis*
8. Germ slit absent 9

9. Conidia with longitudinal striations 10
9. Conidia not striate 12

10. Immature, hyaline conidia striate ..... *Barriopsis*
10. Immature conidia not striate 11

11. Pycnidial paraphyses present ..... *Lasiodiplodia*
11. Pycnidial paraphyses absent ..... *Neodeightonia*

12. Conidia aseptate 13
12. Conidia with 1 or more septa 15

13. Pycnidial paraphyses present ..... *Sphaeropsis*
13. Pycnidial paraphyses absent 14

14. Conidiogenous cells and conidia enclosed in mucoid sheath ..... *Macrophomina*
14. Mucoid sheath absent ..... *Diplodia*

15. Conidia become brown and septate only after dehiscence 16
15. Conidia become brown and 1-septate while attached to the conidiogenous cells before dehiscence ..... *Dothiorella/Spencermartinsia*[3]

16. Conidia frequently 2-septate ..... *Phaeobotryon*
16. Conidia 1-septate only rarely becoming 2-septate ..... *Diplodia*

[1]This key is based on morphology of the asexual morph because the sexual morph is not known for some genera, is very uncommon for others and has not been induced in culture for many of the genera.
[2]It is difficult to separate these two genera morphologically but phylogenetically they are distinct.
[3]These two genera cannot be separated on the morphology of the conidial states but the presence of apiculi on the ascospores of *Spencermartinsia* distinguishes it from *Dothiorella*.

Generic and species descriptions

Barriopsis A.J.L. Phillips, A. Alves & Crous, Persoonia 21: 39. 2008. MycoBank MB511712.
Type species: *Barriopsis fusca* (N.E. Stevens) A.J.L. Phillips, A. Alves & Crous, Persoonia 21: 39. 2008.

Ascomata pseudothecial, scattered or clustered, brown to black, wall composed of several layers of *textura angularis*, ostiole central. *Pseudoparaphyses* hyaline, smooth, multiseptate, constricted at septa. *Asci* bitunicate, clavate, stipitate, thick-walled with thick endotunica and well-developed apical chamber. *Ascospores* aseptate, ellipsoid to ovoid, brown when mature, without terminal apiculi. *Conidiomata* stromatic, pycnidial, superficial, dark brown to black, un- or multilocular. *Ostiole* central, circular, non-papillate. *Paraphyses* arising from the conidiogenous layer, extending above the level of developing conidia, thin-walled, hyaline, mostly aseptate. *Conidiophores* absent. *Conidiogenous cells* hyaline, thin-walled, smooth, cylindrical, holoblastic, proliferating at the same level forming periclinal thickenings. *Conidia* thick-walled, initially hyaline, aseptate with longitudinal striations, striations visible on immature hyaline conidia even while attached to conidiogenous cells, oval, both ends broadly rounded, becoming brown, aseptate or 1–3-septate, with prominent longitudinal striations, wall smooth. *Chlamydospores* catenate, intercalary, brown, smooth, thick-walled, formed within the agar medium.

Notes: The absence of apiculi on the ascospores differentiate this genus from *Sphaeropsis* and *Phaeobotryosphaeria*. The aseptate, brown ascospores without apiculi are unique in the *Botryosphaeriaceae*, as are the striate immature conidia. The genus is currently represented by two species that can be distinguished based on their conidial dimensions. Thus, conidia of *B. fusca* (20–28 × 11–16 µm) are smaller than those of *B. iraniana* (23–30 × 13–21.5 µm).

Species descriptions

Barriopsis fusca (N.E. Stevens) A.J.L. Phillips, A. Alves & Crous, Persoonia 21: 39. 2008. MycoBank MB511713. See Phillips *et al*. (2008) for illustrations.
Basionym: *Physalospora fusca* N.E. Stevens, Mycologia 18: 210. 1926.

≡ *Phaeobotryosphaeria fusca* (N.E. Stevens) Petr., Sydowia 6: 317. 1952.
= *Sphaeria disrupta* Berk. & M.A. Curtis, Grevillea 4 (no. 32): 149. 1876.
≡ *Physalospora disrupta* (Berk. & M.A. Curtis) Sacc., Syll. fung. (Abellini) 1: 438. 1882.
≡ *Phaeobotryon disruptum* (Berk. & M.A. Curtis) Petr. & Syd., Annls mycol. 23(3/6): 255. 1925.
≡ *Botryosphaeria disrupta* (Berk. & Curtis) Arx & Müller, Beitr. Kryptfl. Schweiz 11(1): 37. 1954.

Ascomata scattered, immersed, brown to black, separate or aggregated, wall composed of *textura angularis*, uniloculate, ostiole single, central. *Pseudoparaphyses* hyaline, smooth, 3–4.5 µm wide, multiseptate with septa 14–18 µm apart. *Asci* bitunicate, clavate, 8-spored, stipitate, thick-walled with thick endotunica and well-developed apical chamber, 125–180 × 30–36 µm. *Ascospores* biseriate, aseptate, ellipsoid to oval, straight or slightly curved, apex and base obtuse, without terminal apiculi, wall externally smooth, internally finely verruculose, brown, widest in the middle, (30–)31–36.5(–38.5) × (15.5–)16–18.5(–21) µm, 95 % confidence limits = 32.6–33.4 × 17.0–17.5 µm (av. ± S.D. = 33.0 ± 1.5 × 17.2 ± 1.0 µm), L/W ratio = 1.9 ± 0.15.

Type: **Cuba**, Herradura, on twigs of *Citrus* sp., 15 Jan. 1925, N.E. Stevens, **holotype** BPI 599052.

Culture: CBS 174.26 (ex-type).

Host: *Citrus* sp. (Stevens 1926, pathogenicity not known).

Known distribution: USA; Cuba (Stevens 1926), Florida (BPI 500054 collected by Shear 1923, determined by N.E. Stevens).

Notes: Von Arx & Müller (1954) placed *P. fusca* as a synonym of *Botryosphaeria disrupta*, along with various species in *Phaeobotryon* and *Phaeobotryosphaeria*. However, the broad concept of *Botryosphaeria* followed by von Arx & Müller (1954) encompassed such genera as *Phaeobotryon* and *Phaeobotryosphaeria* that Phillips *et al.* (2008) showed to be phylogenetically distinct from *Botryosphaeria*.

Phillips *et al.* (2008) could not induce the ex-type culture to sporulate, no doubt because it had been in culture for more than 80 years. According to Stevens (1926) the asexual morph is lasiodiplodia-like and he described it as, "Conidia initially hyaline, aseptate and thick-walled becoming dark brown and septate with irregular longitudinal striations, (20–)23–25(–28) × (11–)12–13(–16) µm". Stevens (1926) placed this species in *Physalospora*, but he was obviously hesitant to do so, judging from his statement, "To place in the genus *Physalospora* a fungus with colored ascospores is of course to do violence to the ideas of that genus". On account of the bitunicate asci and brown ascospores of this species, *Physalospora* is clearly unsuitable for it. Petrak & Deighton (1952) transferred this species to *Phaeobotryosphaeria* Speg. as *Phaeobotryosphaeria fusca* (N.E. Stevens) Petr., presumably because it has dark ascospores. Phillips *et al.* (2008) examined the type species of *Phaeobotryosphaeria* (*P. yerbae*) and found that it had terminal apiculi on the ascospores. Therefore, *Phaeobotryosphaeria* was also unsuitable and for that reason they proposed the new genus *Barriopsis* for this fungus.

Barriopsis iraniana Abdollahz., Zare & A.J.L. Phillips, Persoonia 23: 4. 2009. MycoBank MB513235. Fig. 6.

Ascomata not reported. *Conidiomata* stromatic, pycnidial, superficial, dark brown to black, covered with dense mycelium, on pine needles mainly unilocular and up to 600 µm diam, on *Populus* twigs mostly multilocular, individual or aggregated, thick-walled, ostiolate. *Ostiole* central, circular, non-papillate. *Paraphyses* arising from the conidiogenous layer, extending above the level of developing conidia, up to 70 µm long, 3.5 µm wide, thin-walled, hyaline, usually aseptate, sometimes becoming up to 2–3-septate, not constricted at the septa, tip rounded, occasionally branched. *Conidiophores* absent. *Conidiogenous cells* 7–12 × 3–5 µm, hyaline, thin-walled, smooth, cylindrical, holoblastic, proliferating at the same level, with visible periclinal thickening. *Conidia* thick-walled, initially hyaline, aseptate with longitudinal striations, striations visible on hyaline conidia even while attached to conidiogenous cells, oval, both ends broadly rounded, becoming brown, aseptate or 1–3-septate, with prominent longitudinal striations, wall smooth, (22.5–)24–30 × (12.8–)14–18(–21.5) µm, 95 % confidence limits

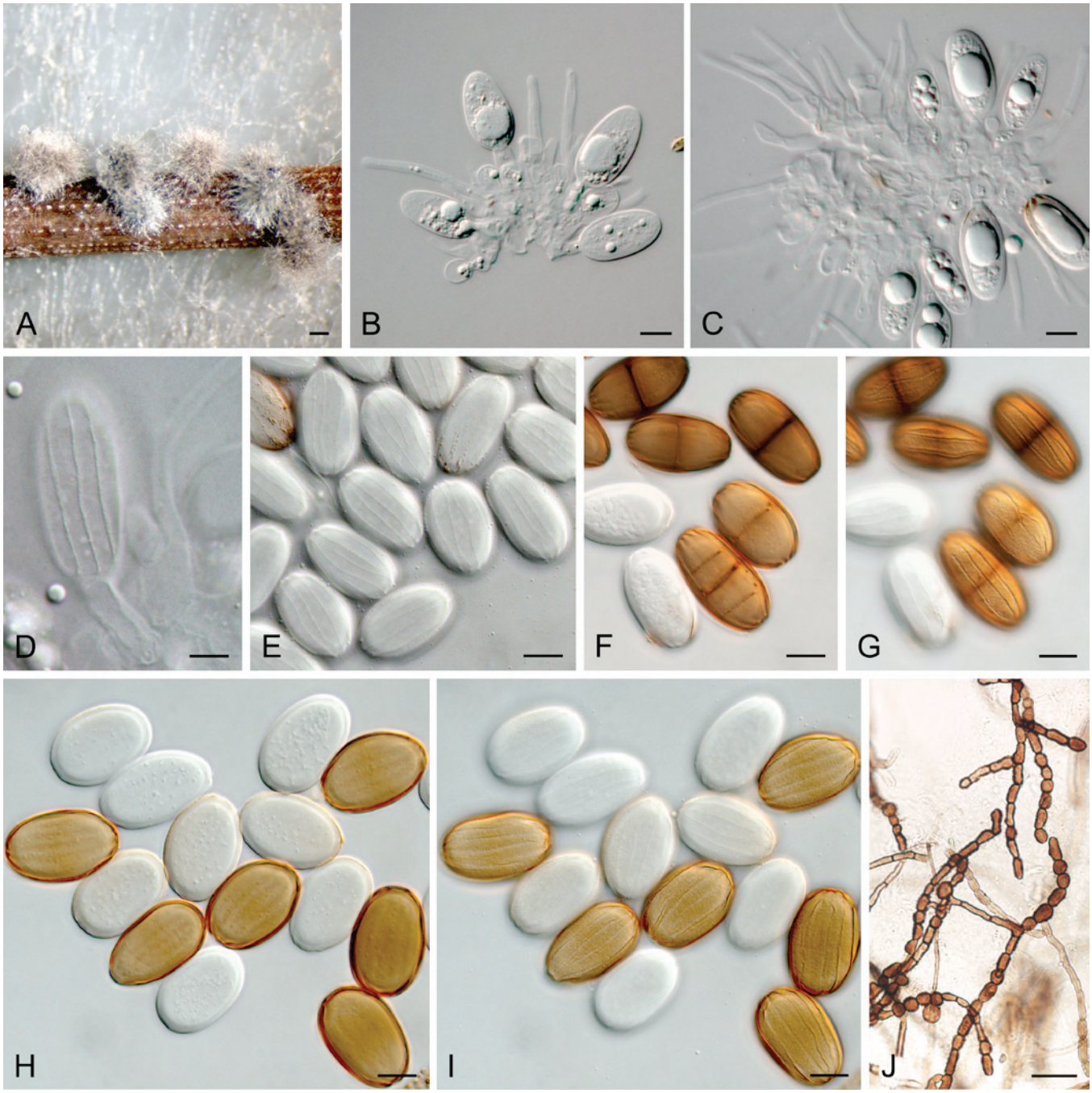

Fig. 6. *Barriopsis iraniana*. A. Conidiomata on pine needles in culture. B, C. Conidia developing on conidiogenous cells between paraphyses. D. Young, immature conidium attached to a conidiogenous cell, longitudinal striations are visible on the conidium. E. Hyaline, immature, striate conidia. F–I. Hyaline and brown, striate conidia, 1- and 3-septate conidia can be seen in F and G. J. Catenulate chlamydospores formed within the agar medium. Scale bars: A = 250 µm, B, C, E–I = 10 µm, D = 5 µm, J = 40 µm.

= 27–27.4 × 16.2–16.6 µm (av. ± S.D. = 27.2 ± 1.8 × 16.4 ± 1.3 µm), L/W ratio = 1.7 ± 0.16. *Chlamydospores* catenate, intercalary, brown, smooth, thick-walled, formed within the agar medium.

Culture characteristics: *Colonies* with appressed mycelial mat and fluffy aerial mycelium in the middle, becoming dull green to olivaceous-black at the surface, and dull green to grey-olivaceous at the reverse after 2 wk in the dark at 25 °C. Colonies reaching 45–50 mm diam on MEA after 4 d in the dark at 25 °C. Cardinal temperatures for growth: min 5 °C, max > 35 °C, opt 25–30 °C.

Type: **Iran**, Hormozgan Province, Minab, Hajikhademi, on twigs of *Mangifera indica*, 27 Feb. 2007, J. Abdollahzadeh & A. Javadi, **holotype** IRAN 13939F.

Cultures: IRAN 1448C = CBS 124698 (ex-type).

Hosts: Endophytic in stems of *Citrus* sp., *Mangifera indica* and *Olea* sp. (Abdollahzadeh *et al.* 2009).

Known distribution: Iran (Hormozgan Province) (Abdollahzadeh *et al.* 2009).

Notes: Conidia of *Barriopsis iraniana* are significantly larger than those reported by Stevens (1926) for *B. fusca*, the only other species known in this genus. The only available culture of *B. fusca* (CBS 174.26, ex-type) has lost its ability to sporulate. According to Stevens (1926) the asexual morph is lasiodiplodia-like with hyaline conidia that become dark-brown and septate with irregular longitudinal striations. However, in contrast to *Lasiodiplodia*, the conidia of *Barriopsis* are

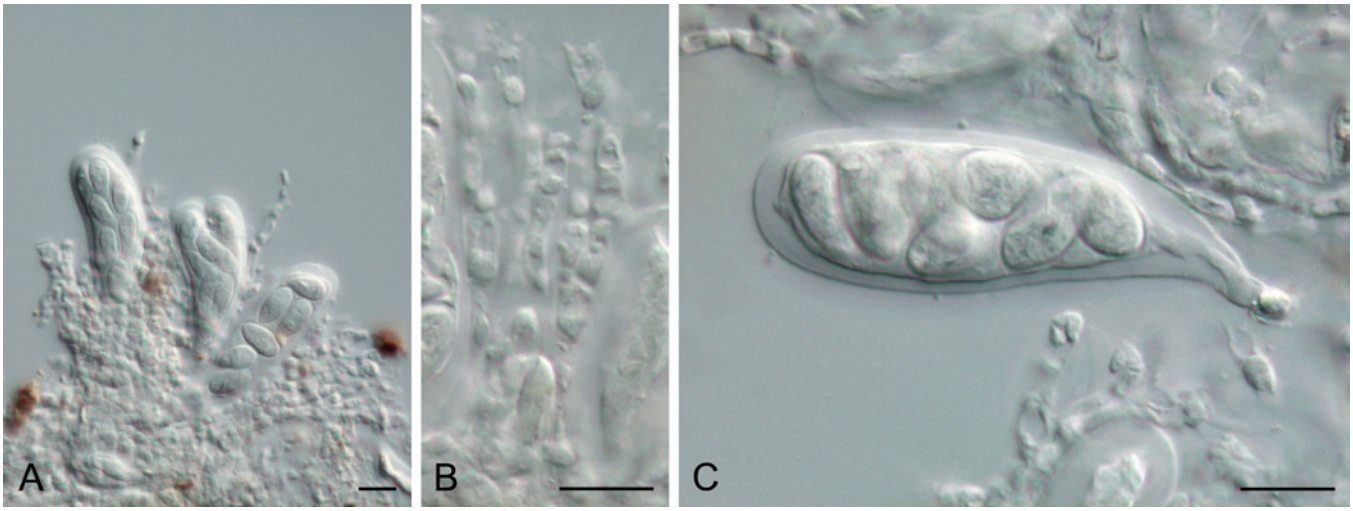

Fig. 7. *Botryobambusa fusicoccum*. A. Asci with ascospores. B. Pseudoparaphyses. C. Ascus with ascospores. Scale bars = 10 µm.

striate at a very early stage of development and the striations are clearly visible in young, hyaline conidia (Fig. 6). This is an unusual character not found in any other genus of the *Botryosphaeriaceae*. The sexual morph of *B. iraniana* has not been seen.

Botryobambusa R. Phookamsak, J.K. Liu & K.D. Hyde, Fungal Divers. 57: 166. 2012. MycoBank MB801313.
Type species: *Botryobambusa fusicoccum* R. Phookamsak, J.K. Liu & K.D. Hyde, Fungal Divers. 57: 166. 2012.

Ascomata dark brown to black, immersed under host epidermis to erumpent, gregarious, multiloculate, locules individual globose to subglobose or fused, vertical to the host surface, with a central, papillate, periphysate ostiole. *Asci* 8-spored, bitunicate, fissitunicate, clavate to cylindro-clavate, pedicellate, with well-developed ocular chamber. *Ascospores* hyaline, aseptate, smooth-walled, ellipsoidal to obovoid, thick-walled, surrounded by mucilagenous sheath. *Conidiomata* developing in stromatic clusters, fused, multiloculate, individually globose to subglobose, wall composed of several layers of *textura angularis*, broader at the base, outer layers dark-brown and thick-walled, inner layers hyaline and thin-walled. *Conidiogenous cells* holoblastic, hyaline, cylindrical to ellipsoidal, smooth. *Conidia* hyaline, aseptate, cylindrical to cylindro-clavate, thin-walled.

Notes: *Botryobambusa* was introduced by Liu *et al.* (2012) as a monotypic genus for *B. fusicoccum*. The genus is distinguished from the morphologically similar *Botryosphaeria* by its smaller asci and ascospores that are surrounded by a mucilagenous sheath. Phylogenetically the two genera are clearly distinct.

Species description

Botryobambusa fusicoccum R. Phookamsak, J.K. Liu & K.D. Hyde, Fungal Divers. 57: 166. 2012. MycoBank MB801314. Fig. 7.

Ascomata 90–152 µm diam, 104–152 µm high, dark brown to black, immersed under epidermis to erumpent, gregarious, visible as black dots or paillae on host surface, multiloculate, individual locules globose to subglobose or fused, vertical to the host surface, wall 12–20 µm thick, composed of several layers of cells with thick brown wall. *Ostiole* central, papillate, persiphystae necks 40–60 µm diam, 30–55 µm long. *Pseudoparaphyses* frequently septate, constricted at septum. *Asci* (45–)55–66(–82) × 14–17(–18) µm, 8-spored, bitunicate, fissitunicate, clavate to cylindro-clavate, pedicellate, apically rounded with well-developed ocular chamber. *Ascospores* (8–)11–13(–14) × 5–7 µm, irregularly biseriate, hyaline, aseptate, ellipsoidal to obovoid, usually wider in the upper third, thick-walled, surrounded by an irregular mucilagenous sheath. *Conidiomata* superficial, clustered in a stroma, multiloculate, globose to subglobose, wall composed of several layers of *textura angularis*, outer layers dark and thick-walled, inner layers hyaline and thin-walled. *Conidiogenous cells* (8–)10–14(–16) × 3–5 µm, holoblastic, cylindrical to ellipsoidal, smooth-walled, hyaline. *Conidia* (21–)22–25(–26) × 5–7 µm, hyaline, asepatte, cylindrical to cylindro-clavate, thin-walled, with rough walls.

Type: **Thailand**, Lampang Province, Jae Hom District, Mae Yuag Forestry Plantation, on dead culms of *Bambusa* sp., 19 Aug. 2010, R. Phookamsak, **holotype** MFLU 11-0179.

Cultures: CBS 134113 = MFLUCC 11-0143 (ex-type), MFLUCC 11-0657.

Host: *Bambusa* sp. (Liu *et al.* 2012).

Known distribution: Thailand (Liu *et al.* 2012).

Notes: The genus *Botryobambusa* is presently monotypic, and only known from *Bambusa* sp. in Thailand. The sexual morph is characterised by having ascospores surrounded by an irregular sheath, while the asexual morph is fusicoccum-like in morphology (Liu *et al*. 2012).

Botryosphaeria Ces. & De Not., Comm. Soc. crittog. Ital. 1: 211. 1863; emend. Sacc., Michelia 1: 42. 1877. MycoBank MB635.

= *Fusicoccum* Corda, in Sturm, Deutschl. Flora, III (Pilze) 2: 111. 1829.
= *Thuemenia* Rehm, Flora 62: 123. 1878.
= *Coutinia* J.V. Almeida & Sousa da Câmara, Rev. Agron. Lisboa 1: 392. 1903.
= *Cryptosporina* Höhn. Öst. bot. Z. 55: 54. 1905.
= *Amerodothis* Theiss. & Syd., Ann. mycol. 13: 295. 1915.

= *Epiphyma* Theiss., Verh. zool.-bot. Ges. Wien 66: 306. 1916.
= *Pyreniella* Theiss., Verh. zool.-bot. Ges. Wien 66: 371. 1916.
= *Desmotascus* F. Stevens, Bot. Gaz. 68: 476. 1919.
= *Creomelanops* Höhn. Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 129: 146. 1920.
= *Macrophomopsis* Petr., Ann. mycol. 22: 108. 1924.
= *Rostrosphaeria* Tehon & E.Y. Daniels, Mycologia 19: 112. 1927.
= *Apomella* Syd. Annls mycol. 35: 47. 1937.
= *Caumadothis* Petr., Sydowia 24: 276. 1971.

Type species: *Botryosphaeria dothidea* (Moug. : Fr.) Ces. & De Not., Comment. Soc. Crittog. Ital. 1: 212. 1863.

Mycelium immersed, consisting of branched, septate, smooth, hyaline hyphae. *Ascomata* eustromatic, scattered, solitary, aggregated or forming botryose clusters, externally black, uniloculate, with a thick pseudoparenchymatic wall composed of *textura angularis* or *textura globosa* with the outer layers blackened and their cells more thickened, ostiolate, embedded in the substrate and partially erumpent at maturity. *Pseudoparaphyses* thin-walled, hyaline, frequently septate, constricted at the septa, deliquescing from the basal parts when the asci mature. *Asci* clavate or cylindric-clavate, stipitate, bitunicate, ectotunica thin, endotunica rather thick, 3-layered (*sensu* Eriksson 1981), with a prominent apical chamber, 8-spored, developing on a broad basal hymenial layer. *Ascospores* irregularly biseriate in the ascus, hyaline, sometimes becoming pale brown with age, thin-walled, ovoid, fusoid, fusoid-ellipsoid, usually widest in the middle, straight or inequilateral, smooth, one-celled sometimes becoming 1–2 septate with age, contents smooth or granular, may be guttulate. *Conidiomata* stromatic, pycnidial, solitary or aggregated, often occurring within the same stroma as the ascomata, walls composed of dark brown, thick-walled *textura angularis*, becoming thin-walled and hyaline towards the inner layer. *Ostioles* indistinct to well-defined, round or irregular. *Paraphyses* hyaline, cylindrical, tapering to rounded ends, septate, arising between the conidiophores and conidiogenous cells. *Conidiophores* when present hyaline, cylindrical, branched at the base, smooth, 0–1 septate. *Conidiogenous cells* enteroblastic, integrated, hyaline, smooth, cylindrical, first-formed conidium holoblastic, determinate or proliferating percurrently with 1–2 indistinct annellations, or proliferating at the same level resulting in typical phialides (*sensu* Sutton 1980) with periclinal thickenings. *Conidia* hyaline, sometimes becoming olivaceous or darker with age, thin-walled, smooth, aseptate, occasionally forming one or two septa with age or before germination, with shapes varying from elliptical to fusiform or clavate, finely guttulate, apex subobtuse to obtuse, base conspicuously truncate with a minute marginal basal frill.

Notes: When Cesati & De Notaris (1863) introduced *Botryosphaeria* Ces. & De Not. they listed nine species (plus another six that they did not recombine in the genus) but they did not designate a type. Subsequently, Saccardo (1877) emended the genus to exclude hypocreaceous species. Von Höhnel (1909) designated *B. berengeriana* De Not. as the type, but this species was not included in the original description of the genus, although it was published soon after (De Notaris 1864). Theissen & Sydow (1915) suggested *B. quercuum* (Schwein.) Sacc. as the type since it was typical of Saccardo's (1877) emendation of *Botryosphaeria*, and this was accepted by von Arx & Müller (1954). However, *B. quercuum* also was not one of the original species of the genus and therefore is unsuitable as the type. Barr (1972) proposed *B. dothidea* (Moug. : Fr.) Ces. & De Not. as lectotype because it was one of the original species described, it conforms with Saccardo's (1877) emendation and it is an earlier synonym of *B. berengeriana*, von Höhnel's (1909) designated type. The proposal of Barr (1972) has been accepted generally, and Slippers *et al.* (2004a) provided a revised description of this species based on the type specimen and fresh collections, and they designated a neotype and epitype.

Species in *Botryosphaeria* were described largely on the basis of the morphology of their ascomata and host associations, and this led to a proliferation of names. Von Arx and Müller (1954) examined 183 taxa and reduced them to 11 species, with extensive synonymies under *B. dothidea* and *B. quercuum,* together with nine new combinations. However, because von Arx and Müller (1954) did not take into account the characters of the asexual morphs and because species of *Botryosphaeria* are difficult to separate on the basis of sexual morph characters, these synonymies have not always been accepted (Shoemaker 1964, Sivanesan 1984, Slippers *et al.* 2004a).

The genus *Botryosphaeria*, based on the type species *B. dothidea*, typically has ascospores that are hyaline and aseptate, although they can become pale brown and septate with age (Shoemaker 1964, Sivanesan 1984, Denman *et al.* 2000, Alves *et al.* 2004, Phillips *et al*. 2005). Because some species of *Botryosphaeria* have ascospores that become brown with age, von Arx & Müller (1954) placed *Dothidea visci* with brown ascospores in *Botryosphaeria* as *B. visci*, and later they (von Arx & Müller 1975) also placed the dark-spored *Neodeightonia subglobosa* in *Botryosphaeria*. Since it is the type species of *Neodeightonia*, this genus was reduced to synonymy with *Botryosphaeria*. In recognising these synonymies, von Arx & Müller (1954, 1975) broadened the concept of *Botryosphaeria* to include species with brown ascospores.

Phillips *et al.* (2005) resurrected the genus *Dothiorella* for species with 1-septate conidia that darken at an early stage of development, and have sexual morphs with brown, 1-septate ascospores. Phylogenetically (ITS+EF1-α) the two species studied by Phillips *et al.* (2005) fell within *Botryospheria* as defined by the broad morphological concept recognised by von Arx & Müller (1954, 1975). For these reasons, Phillips *et al.* (2005) described the sexual morphs of *Dothiorella* as two new species of *Botryosphaeria* with brown, 1-septate ascospores. Subsequently, Luque *et al.* (2005) described another dark-spored *Botryosphaeria*, namely *B. viticola*, with a *Dothiorella* asexual morph.

At least 18 asexual genera have been associated with *Botryosphaeria s. lat*. (Denman *et al.* 2000) including *Diplodia*, *Dothiorella*, *Fusicoccum*, and *Lasiodiplodia*. The morphological diversity of the asexual morphs linked to species of *Botryosphaeria,* together with the broad concept of the sexual genus was clear evidence that *Botryosphaeria* encompassed several distinct genera. Thus, through a study of partial sequences of the LSU gene, Crous *et al.* (2006) showed that *Botryosphaeria s. lat.* is composed of 10 phylogenetic lineages, each of which corresponds to different asexual genera. To avoid the unnecessary introduction of new generic names, these authors chose to use existing asexual generic names for most of the lineages, and restricted the use of *Botryosphaeria* to *B. dothidea* and *B. corticis*. Seven species are currently recognised in *Botryospheria*.

DNA phylogeny

In an ITS phylogeny the ex-type isolate of *B. mamane* and isolates previously regarded as *B. mamane* clustered in *Cophinforma* together with *C. atrovirens* (Fig. 8). Based on combined ITS and EF1-α sequence data seven species are currently recognised in *Botryosphaeria* (Fig. 8). Apart from *B. fabicerciana* all species clades are supported by high bootstrap values.

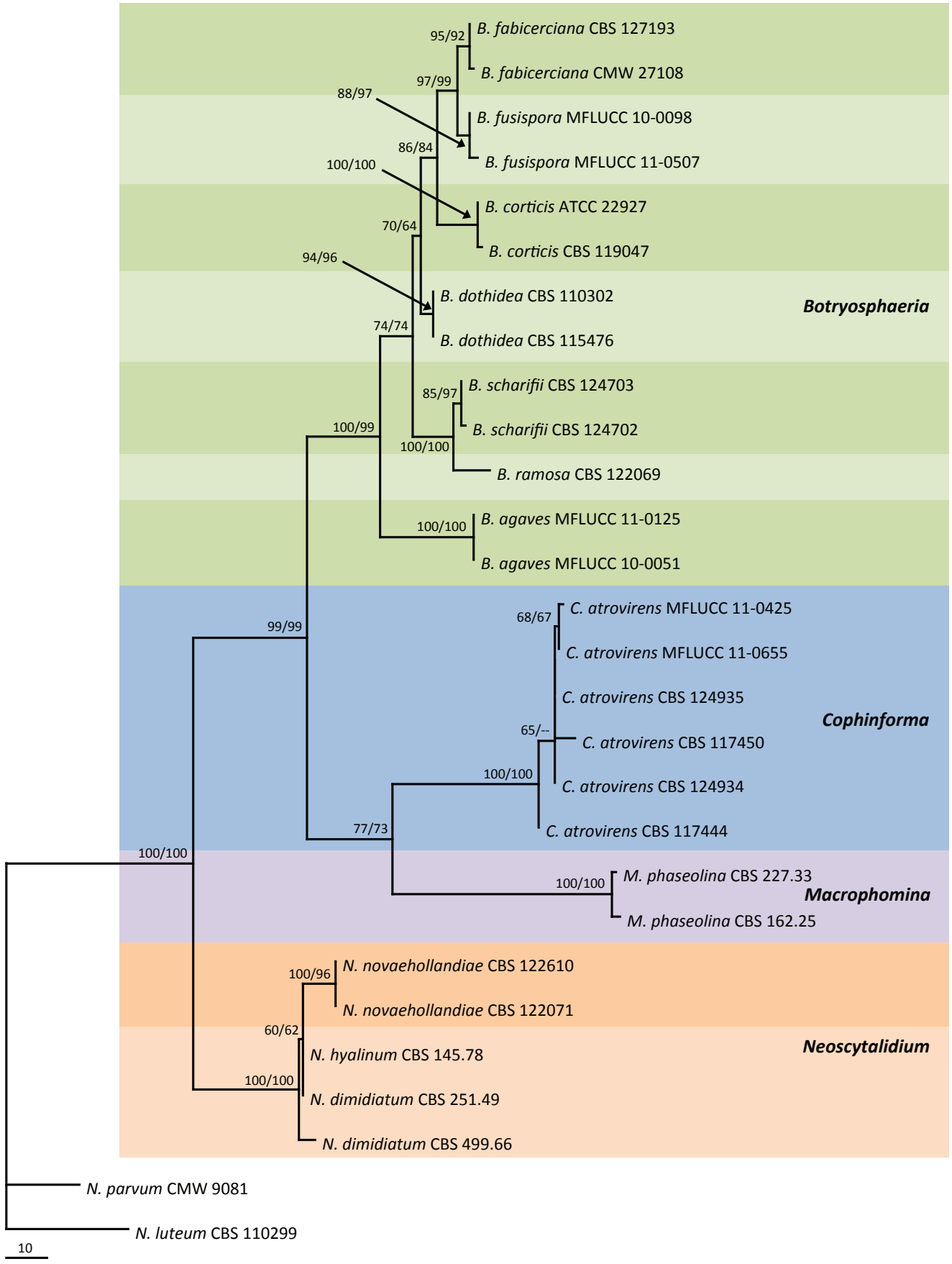

Fig. 8. One of 18 equally most parsimonious trees obtained from the combined analysis of ITS and EF1-α sequences from species of the genera *Botryosphaeria*, *Cophinforma*, *Macrophomina* and *Neoscytalidium*. The phylogenetic tree resulting from ML analysis using the general time reversible model of DNA evolution and assuming a discrete gamma distribution (GTR+G) had a topology identical to the MP tree presented. MP/ML values (> 50 %) resulting from 1000 bootstrap replicates are given at the nodes. The tree was rooted to *N. parvum* and *N. luteum*. Clades corresponding to genera and species are highlighted.

Key to *Botryosphaeria* spp.

1. Conidia 12–17 µm long 2
1. Most conidia longer than 18 µm 3

2. Average conidial length greater than 15 µm ..... *B. scharifii*
2. Average conidial length less than 15 µm ..... *B. ramosa*

3. On *Vaccinium* species, conidia 23.5–32.5 µm long ..... *B. corticis*
3. On hosts other than *Vaccinium* 4

4. Conidia 16–22 µm long ..... *B. fusispora*
4. Conidia mostly longer than 20 µm 5

5. Conidial L/W ratio greater than 4.5 ..... *B. dothidea*
5. Conidial L/W ratio less than 4.0 ..... *B. fabicerciana*

Notes: This key is based only on characters of the asexual morphs, because the sexual morphs are generally uncommon or have not been induced to form in culture. *Botryosphaeria agaves* was not included in the key because the asexual morph has never been reported.

Species descriptions

Botryosphaeria agaves (Henn.) E.J. Butler, Ann. Mycol. 9: 415. 1911. MycoBank MB119799. See Liu *et al.* (2012) for illustrations.
Basionym: *Physalospora agaves* Henn., Bot. Jb. 34: 51. 1905.

Ascomata 140–260 µm high × 600–800 µm diam, circular blackened areas on host tissue, immersed to erumpent on host, uni to multiloculate, aggregated, individually globose to subglobose, wall composed of several layers of dark brown walled cells of *textura angularis*. *Ostiole* circular, central, papillate. *Pseudoparaphyses* 3–5 µm wide, aseptate. *Asci* 91–122 × 27–38 µm, 8-spored, bitunicate with a thick endotunica, fissitunicate, clavate to cylindro-clavate, short pedicellate, with well-developed apical chamber. *Ascospores* 21–43 × 8–12 µm, biseriate in the ascus, hyaline, aseptate, ellipsoidal, fusiform, or inequilateral, usually wider at the middle, wall rough, surrounded by a mucilaginous sheath. *Conidiomata* not reported.

Type: **Tanzania**, Zanzibar, on leaves of *Agave sisalana*, Zimmerman, holotype presumably lost (not in B). **Thailand**, Chiang Rai Province, Mae Fah Luang District, Doi Tung, on living and dead leaves of *Agave* sp., 16 Jun. 2010, *R. Phookamsak*, **neotype designated here** MFLU 11–0161; MBT176241.

Cultures: MFLUCC 11–0125 = CBS 133992 (ex-neotype), MFLUCC 10-0051.

Host: *Agaves* sp. (Liu *et al.* 2012).

Known distribution: Thailand (Liu *et al.* 2012).

Notes: Liu *et al.* (2012) proposed a specimen from *Agave* sp. collected in Thailand (MFLU 11-0161) to serve as epitype for *B. agaves*. However, as they did not cite nor examine the holotype, their epitypification is invalid. We have also been unable to trace the holotype, thus designate the Thailand specimen as neotype to rectify this situation.

Botryosphaeria corticis (Demaree & Wilcox) Arx & E. Müll., Beitr. Kryptfl. Schweiz 11(1): 43. 1954. MycoBank MB293807. Figs 9, 10.
Basionym: *Physalospora corticis* Demaree & Wilcox, Phytopathology 32: 1074. 1942.

Ascomata abundant, embedded in the host becoming partially erumpent at maturity, up to 250 µm diam, conical with a dark brown to black wall composed of up to six cell layers of thick-walled *textura angularis* giving way to hyaline, thinner-walled cells lining the ascomata. *Asci* hyaline, clavate and stipitate, bitunicate with a thick endotunica and well-developed apical chamber, eight-spored, 145–165 × 25–28 µm, irregularly biseriate, formed amongst hyaline, thin-walled, septate *pseudoparaphyses*. *Ascospores* ellipsoid to fusoid, (24–)25.5–33(–34.5) × (9.5–)10–12.5(–13.5) µm, 95 % confidence limits = 28.5–30.1 × 11.2–11.9 µm (av. ± S.D. of 32 conidia = 29.3 ± 2.4 × 11.6 ± 1.0 µm), L/W = 2.5 ± 0.23. Ascospores germinate within 24 h at 25 °C and form unbranched germ tubes. *Conidiomata* developing in culture on pieces of poplar twigs after 14 d and producing conidia after 28 d, solitary to aggregated, dark brown to black, globose, up to 450 µm diam. *Conidiophores* cylindrical, hyaline, smooth, thin-walled, septate, branched in the upper parts, 7.5–14 × 3.5–4.5 µm, lining the entire inner surface of the conidiomata. *Conidiogenous cells* lageniform, hyaline, thin-walled, smooth, 12.5–17.5 × 2.5–4.5 µm, holoblastic producing a single conidium at the tip, rarely proliferating at the same level giving rise to periclinal thickenings. *Conidia* fusiform, widest in the middle to upper third, hyaline, thin-walled, smooth, apex acute, base truncate with a minute marginal frill and persistent mucous sheath, (20.5–) 23.5–32.5(–34.5) × (5.0–)5.5–7(–7.5) µm, 95 % confidence limits = 27.7–30.2 × 6.2–6.7 µm (av. ± S.D. of 26 conidia = 28.9 ± 3.4 × 6.4 ± 0.7 µm), L/W = 4.5 ± 0.46. *Spermatogonia* globose, dark brown to black. *Spermatophores* cylindrical, hyaline, branched, 11–14 × 2–3 µm. *Spermatogenous cells* hyaline, thin-walled, smooth, 14.5–20.5 × 1.5–2.3 µm, producing conidia at their tips, proliferating internally to form periclinal thickenings. *Spermatia* rod-shaped with obtuse ends, hyaline, thin-walled, smooth, 4–6 × 1.5–2 µm.

Culture characteristics: *Colonies* on CMA reaching 28–40 mm diam after 7 d at 25 °C, initially white becoming olive-green with clumps of loosely aggregated hyphae.

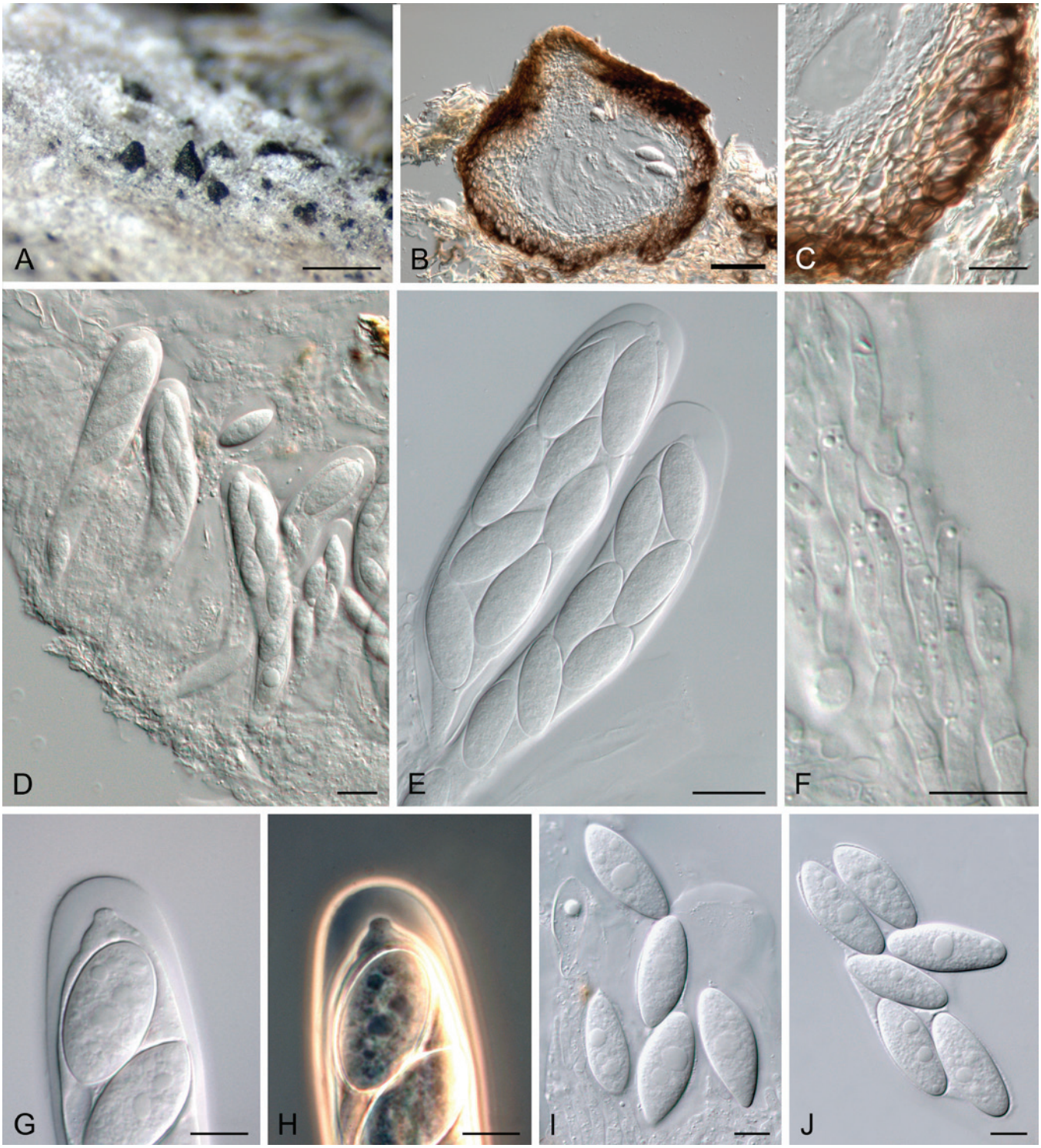

Fig. 9. *Botryosphaeria corticis*. A. Ascomatal necks emerging through the bark of *Vaccinium*. B. Section through an ascoma. C. Section through the ascomal wall. D, E. Asci with ascospores. F. Septate pseudoparaphyses. G, H. Apical chamber at tip of an ascus as seen in interference contrast (G) or phase contrast (H). I, J. Ascospores. Scale bars: A = 0.5 mm, B = 50 µm, C, E = 20 µm, D, F–J = 10 µm.

Type: **USA**, North Carolina, Atkinson, *Vaccinium corymbosum*, 14 Feb. 1940, J.B. Demaree, **holotype** BPI 598729; New Jersey, Hammonton, on cankered stems of *V. corymbosum*, May 2005, P.V. Oudemans, CBS H-19706 **epitype** (designated by Phillips *et al.* 2006a).

Cultures: CBS 119047, CBS 119048 (ex-epitype).

Hosts: *Vaccinium* species including *V. corymbosum*, *V. ashei*, *V. tenellum* and *V. virgatum* (Phillips *et al.* 2006, Wright & Harmon 2010).

Known distribution: USA (Florida, Georgia, Maryland, Mississippi, New Jersey, North Carolina) (Phillips *et al.* 2006, Wright & Harmon 2010).

Notes: This species appears to be restricted to *Vaccinium* spp. and has not been reported outside of the continental USA. The mucilaginous sheath surrounding the conidia is unusual in *Botryosphaeria*.

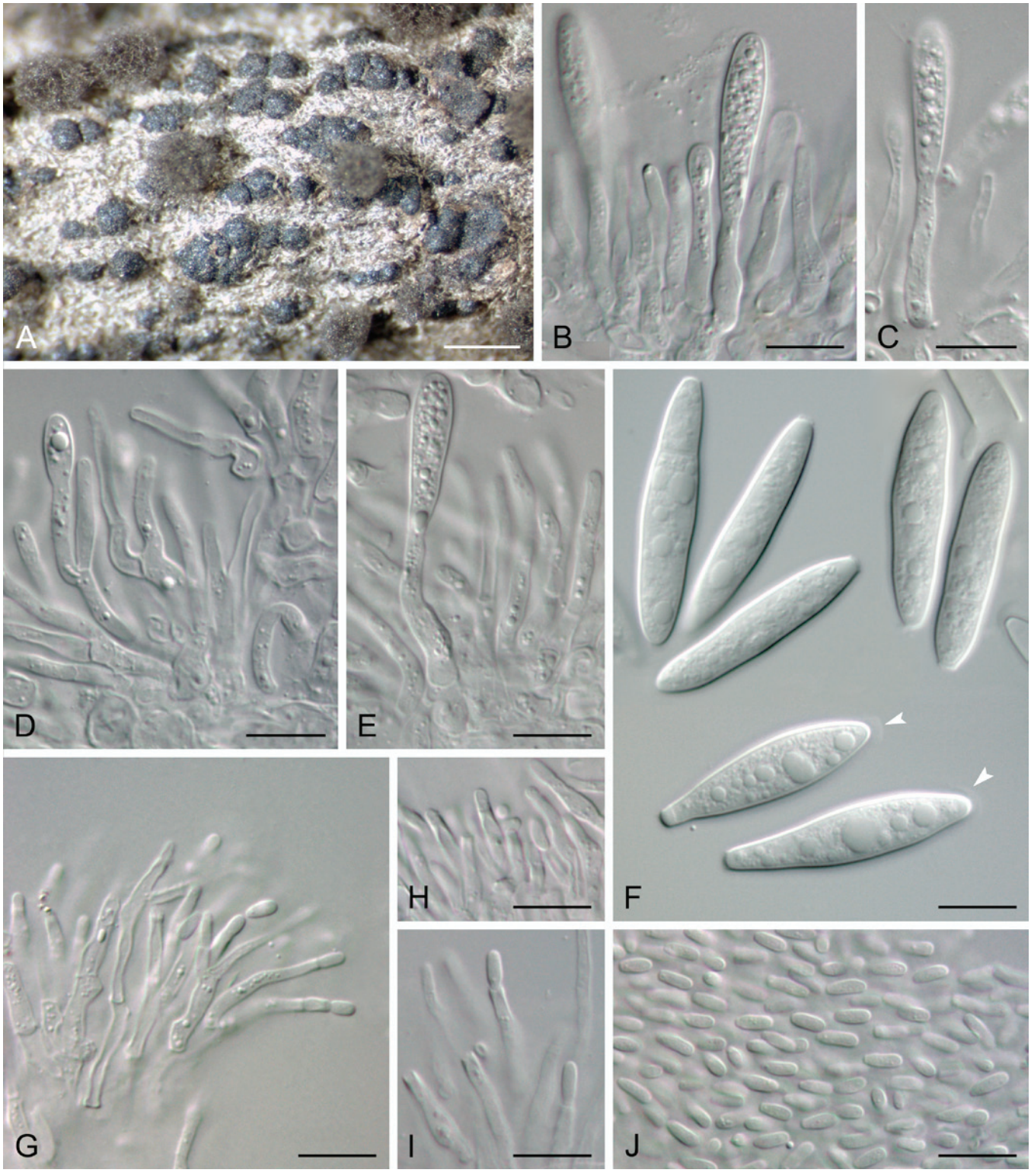

Fig. 10. *Botryosphaeria corticis*. A. Conidiomata formed on poplar twigs in culture. B–E. Conidiogenous cells and paraphyses. F. Conidia with mucous sheath (arrowheads). G–I. Spermatogenous cells. J. Spermatia. Scale bars: A = 0.5 mm, B–J = 10 µm.

Botryosphaeria dothidea (Moug.: Fr.) Ces. & De Not., Comm. Soc. Crittog. Ital. 1: 212. 1863. MycoBank MB183247. Figs 11, 12.

Basionym: *Sphaeria dothidea* Moug., *In*: Fries, *Syst. Mycol.* (Lundae) 2(2): 423. 1823.

= *Botryosphaeria berengeriana* De Not., Sfer. Ital. 82. 1863 [1864].
= *Fusicoccum aesculi* Corda, *In*: Sturm, Deutschl. Fl., Abth. 3, 2: 111. 1829.
= *Sphaeria coronillae* Desm., Annls Sci. Nat., Bot., sér. 2 13: 188. 1840.
≡ *Macrophoma coronillae* (Desm.) Höhn., Ber. Deutsch. Bot. Ges. 28: 479. 1910.
≡ *Macrophomopsis coronillae* (Desm.) Petr., Annls mycol. 22(1/2): 108. 1924.
≡ *Dothiorella coronillae* (Desm.) Petr., Sydowia 16(1–6): 188. 1963.
≡ *Fusicoccum coronillae* (Desm.) Vanev. & Aa, *In*: van der Aa & Vanev, *A Revision of the Species Described in Phyllosticta* (Utrecht): 192. 2002.
= *Phyllosticta divergens* Sacc., Malpighia 5: 274. 1891.

Ascostroma erumpent, 200−500 µm diam. *Ascomata* pseudothecial, forming a botryose aggregate of up to 100, sometimes solitary, globose with a central ostiole, ¼ to ½ emergent, rarely embedded, papillate or not, brown to black, pseudothecial wall comprising 5–15

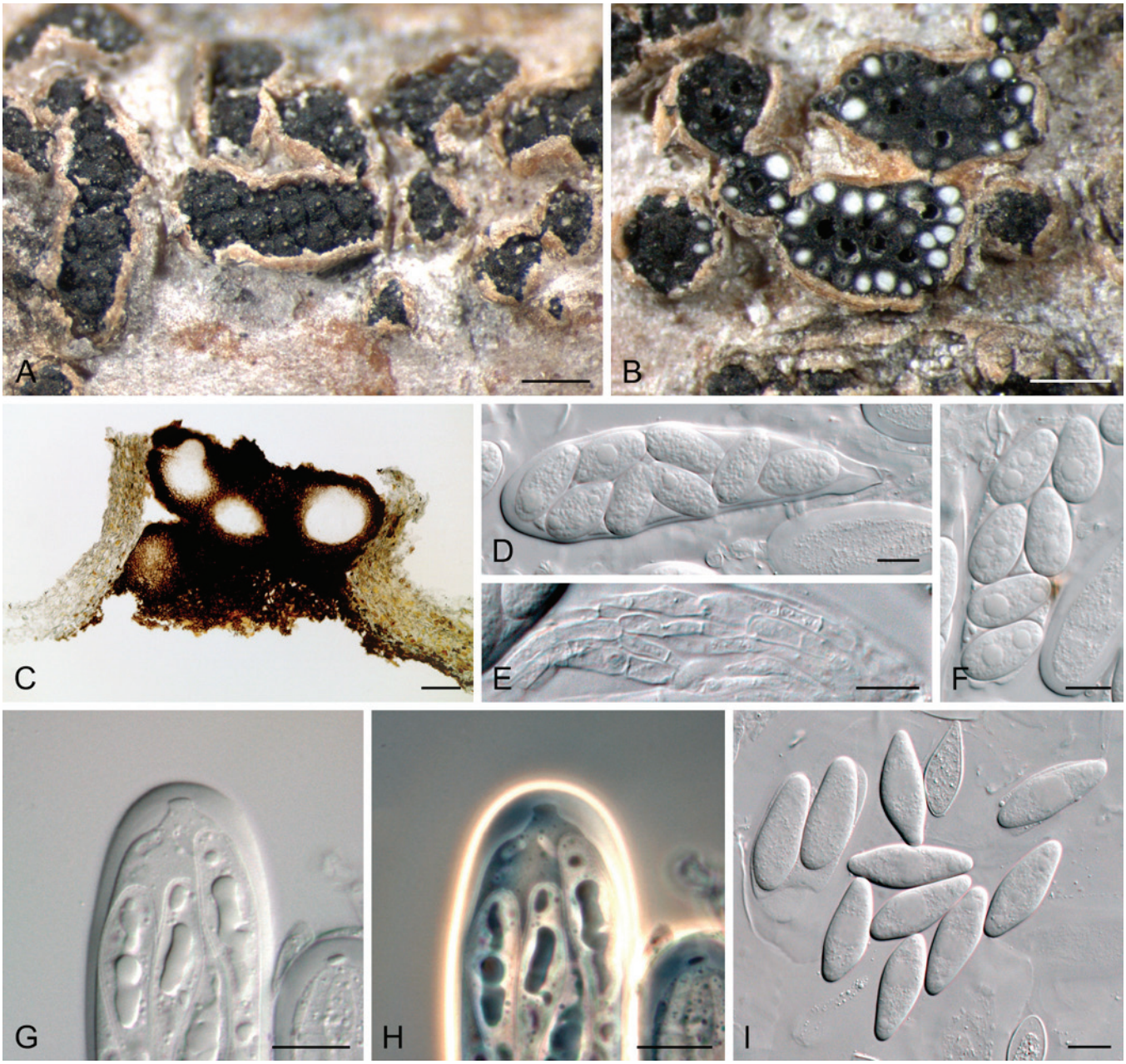

Fig. 11. *Botryosphaeria dothidea*. A. Botryose clusters of ascomata erumpent through the bark of a poplar twig. B. Transverse section through ascomata revealing brilliant white contents. C. Section through ascomata. D. Ascus with ascospores. E. Septate pseudoparaphyses. F. Ascospores. G, H. Ascus tip showing apical chamber as seen by interference contrast (G) or phase contrast (H). I. Ascospores. Scale bars: A, B = 0.5 mm, C = 100 µm, D–I = 10 µm.

layers of *textura angularis*, outer region of dark brown cells, inner region of 2–4 layers of hyaline cells lining the locule. *Asci* bitunicate, clavate, 63−125 × 16–20 µm, forming between pseudoparaphyses. *Pseudoparaphyses* filiform, septate, constricted at the septa, rarely branched, 2–4 µm wide. *Ascospores* fusoid to ovoid, sometimes with tapered ends and appearing spindle-shaped, biseriate in the ascus, (17–)19–24(–32) × (6–)7–8(–10) µm (av. of 102 ascospores = 22.7 × 7.8 µm), L/W = 2.9. *Conidiomata* stromatic, morphologically indistinguishable from the ascomata. *Paraphyses*, when present hyaline, septate, up to 110 µm long, 2.5–6 µm wide at the base tapering to acutely rounded apices, 2–2.5 µm wide at the tip. *Conidiophores* hyaline, smooth, thin-walled, rarely branched at the base, cylindrical, formed from the cells lining the locule wall, 23–35 × 4–5 µm, or reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, hyaline, sub-cylindrical, 6–20 × 2–5 µm, proliferating percurrently to produce 1–2 annellations or proliferating internally resulting in periclinal thickenings and typical "phialides" (*sensu* Sutton 1980). *Conidia* narrowly fusiform, or irregularly fusiform, base subtruncate to bluntly rounded, (17–) 19.5–30(–34) × 4–6(–7.5) µm, 95 % confidence limits of 350 conidia = 25.8–26.5 × 5.3–5.4 µm (av. ± S.D. of 350 conidia = 26.2 ± 3.1 × 5.4 ± 0.7 µm), L/W = 4.9 ± 0.96 with 95 % confidence limits of 4.8–5.0, rarely forming a septum before germination, smooth with granular contents, in some isolates becoming dark-walled and septate with age, (9.5–) 10.5–20(–23) × (3–)4–6(–6.5) µm (av. ± S.D. of 150 conidia = 15.5 ± 2.7 × 5.1 ± 0.6 µm). *Spermatophores* hyaline, smooth, occasionally branched, cylindrical to subcylindrical septate, 4−15 × 1−3.5 µm. *Spermatogenous cells* discrete or integrated, hyaline, smooth, cylindrical, holoblastic or proliferating via phialides with periclinal thickenings, 7–10 × 2–3 µm. *Spermatia* unicellular, hyaline, allantoid to rod-shaped, 3−6 × 1.5−2 µm.

Culture characteristics: *Colonies* oliveaceous becoming grey with reverse black. Mycelial mat moderately dense, margin smooth. Optimum temperature for growth 25(–30) °C, colony on ½ PDA reaching 50 mm radius after 4 d at 25 °C in the dark.

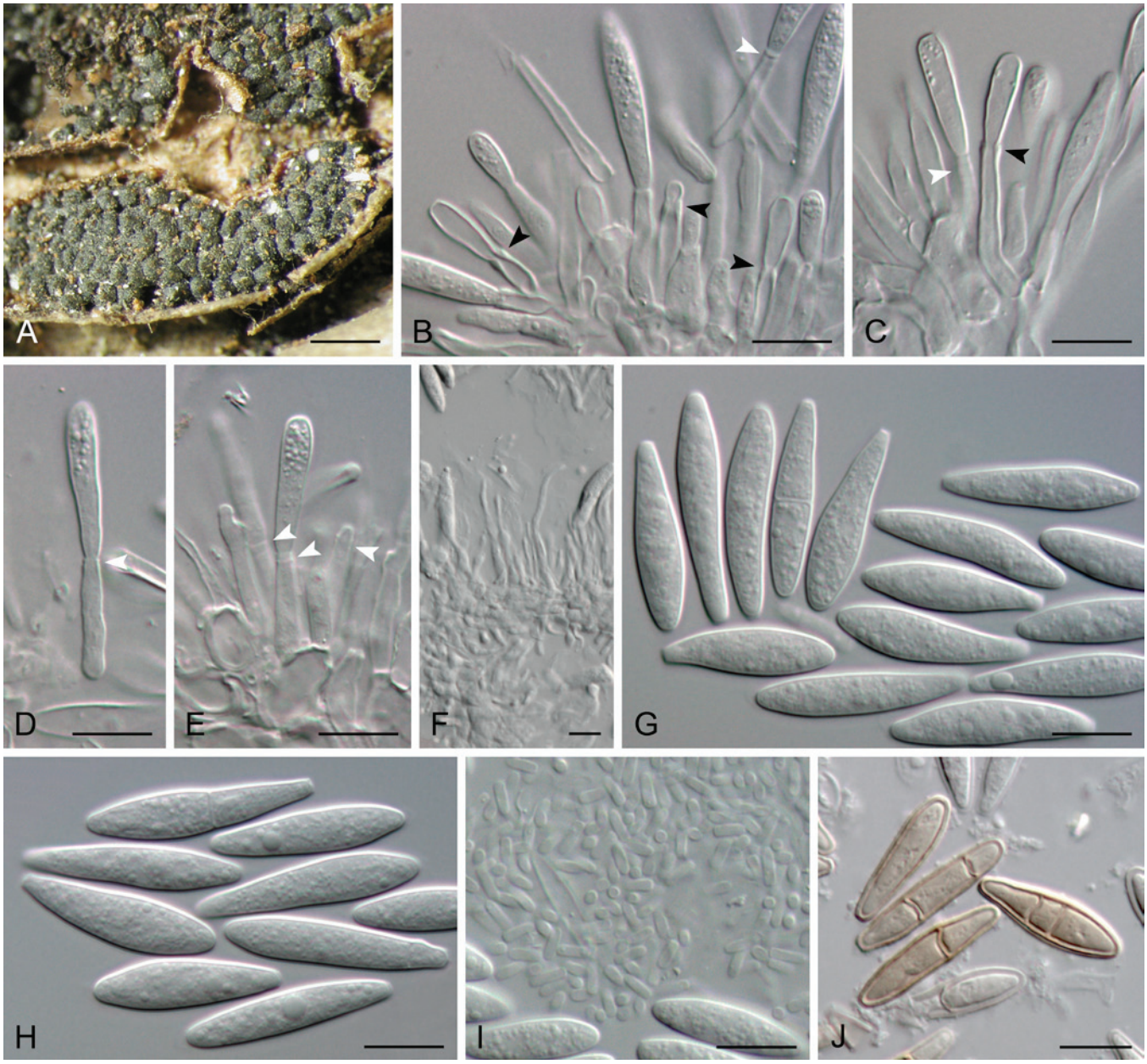

Fig. 12. *Botryosphaeria dothidea*. A. Botryose cluster of conidiomata erumpent through the bark of a poplar twig. B–E. Conidiogenous cells with periclinal thickenings (black arrowheads) or annellations (white arrowheads). F. Paraphyses. G, H. Conidia. I. Spermatia. J. Pigmented, thick-walled, septate conidia. Scale bars: A = 0.5 mm, B–J = 10 µm.

Type: **France**, *Rosa* sp., 1823, Fries ex Mougeot. Herbarium S (**neotype** of *Sphaeria dothidea* designated by Slippers *et al.* 2004a). **Switzerland**, Ticino, Crocifisso, *Prunus* sp., Oct. 2000, B. Slippers, PREM 57372 (**epitype** designated by Slippers *et al.* 2004a). **Italy**, on branches of *Aesculus* sp., P.A. Saccardo, PAD, (neotype of asexual morph designated by Crous & Palm 1999).

Cultures: CBS 115476 = CMW 8000 (ex-epitype).

Hosts: Woody plants in numerous families. Reports of hosts prior to 2000 are unreliable because the concept of this species was not clear until Slippers *et al.* (2004a) redefined it and proposed neotype, epitype and ex-epitype cultures. It is highly probable that before that time some of the host associations may have been of species of *Neofusicoccum*. However, some recent reports can confirm the following hosts: *Cistus ladanifer* (Sánchez *et al.* 2002), *Fraxinus*, *Ostrya*, *Prunus*, *Populus*, (Slippers *et al.* 2004a), *Acacia rostellifera*, *Eucalyptus marginata* (Taylor *et al.* 2009) *Vitis vinifera*, *Olea europaea* (Phillips *et al.* 2005, Lazzizera *et al.* 2008b), *Quercus suber*, *Q. ilex* (Sánchez *et al.* 2003), *Cistus ladanifer* (Sánchez *et al.*, 2002), *Juniperus communis*, *Acer* sp., *Actinidia deliciosa*, *C. limon*, *Fagus* sp., *Juglans regia*, *Mangifera indica*, *Olea europaea*, *Picea* sp., *Populus nigra*, *Prunus persica*, *Quercus* sp., *Rubus* sp., *Salix* sp., *V. vinifera* (Abdollahzadeh *et al.* 2013).

Known distribution: Probably worldwide and cosmopolitan.

Notes: The description of *S. dothidea* (Fries 1823) refers to a fungus on twigs of *Fraxinus* sp. According to Slippers *et al.* (2004a) the specimen of *S. dothidea* in the Fries collection that has been cited as the holotype (von Arx & Müller 1954) is on what appears to be a *Rosa* sp. and thus cannot be the holotype. Phillips & Lucas (1997) and Slippers *et al.* (2004a) examined the only other specimen of *S. dothidea* in the Fries herbarium and found it to be immature with no spores. Slippers *et al.* (2004a) designated that specimen as the neotype and also designated an epitype (PREM 57372) on *Prunus* sp. collected from Crocifisso, Switzerland, with an ex-epitype culture (CBS 115476 = CMW 8000).

Pennycook & Samuels (1985) described two new species of *Botryosphaeria* (*B. parva* and *F. luteum*) on kiwifruit, and suggested that *B. dothidea* may be a complex of species. This suggestion led to doubts about the earlier identifications of *B. dothidea*. However, the name *B. dothidea* continued to be used in a broad sense. Only after gene sequence data were used to clarify species concepts in the genus (e.g. Phillips *et al.* 2002, Slippers *et al.* 2004a) it became apparent that some of the earlier reports of *B. dothidea* in association with plant diseases may have been misidentifications. Thus, the earlier reports of *B. dothidea* prior to 2004 should be interpreted with circumspection.

Botryosphaeria fabicerciana (S.F. Chen, D. Pavlic, M.J. Wingf. & X.D. Zhou) A.J.L. Phillips & A. Alves, **comb. nov.** MycoBank MB805457. See Chen *et al.* (2011) for illustrations.
Basionym: *Fusicoccum fabicercianum* S.F. Chen, D. Pavlic, M.J. Wingf. & X.D. Zhou, Plant Pathol. 60: 746. 2011.

Ascomata not reported. *Conidiomata* developing in culture on pine needles after 10 d and producing conidia after 14 d, superficial, solitary to aggregated, dark brown, globose, covered with hyphae, (245–)346–470(–525) µm, wall composed of three layers: an outer of thick-walled dark to light brown *textura angularis*, a middle layer of thin-walled light brown cells, and an inner layer of thin-walled hyaline cells. *Conidiophores* absent. *Conidiogenous cells* cylindrical to lageniform, hyaline, smooth, thin-walled, holoblastic producing a single conidium at the tip, rarely proliferating at the same level giving rise to periclinal thickenings, (6.5–)10.5–13.5(–16) × (2–)2.5–3.5(–4.5) µm (av. of 50 conidiogenous cells = 12 × 3 µm). *Paraphyses* not seen. *Conidia* hyaline, thin-walled, smooth with granular contents, unicellular, aseptate, fusiform, widest in the middle to upper third, apex acute, base truncate with a minute marginal frill, forming one or two septa before germination, (16.5–)19.5–24.5(–26) × (4.5–)5–6.5(–7.5) µm (av. of 100 conidia = 22.0 × 5.8 µm), L/W = 3.8.

Culture characteristics: *Colonies* with fluffy mycelium, initially white turning smoke-grey from the middle of colonies within 4–6 d, with an appressed mycelial mat, sparse to moderately dense. Cottony aerial mycelium toward the edge of colony, becoming pale olivaceous-grey, and greenish black (reverse) within 12–16 d. Optimal temperature for growth 25(–30) ºC, colony covering the 90 mm diam Petri dish after 5 d in the dark at 25 °C.

Type: **China**, FuJian Province, from senescing twigs of an unknown *Eucalyptus* sp., Aug. 2007, M.J. Wingfield, **holotype** PREM 60449.

Cultures: CBS 127193 = CMW 27094 (ex-type).

Hosts: *Eucalyptus* sp., *E. urophylla* × *E. tereticornis*, *Eucalyptus grandis* hybrid (Chen *et al.* 2011).

Known distribution: China (FuJian, HaiNan and GuangXi Provinces) (Chen *et al.* 2011).

Notes: *Botryosphaeria fabicerciana* is morphologically similar to *B. dothidea* (size of conidia = 24.5 × 5 µm in culture, 19.5 × 5 µm on a natural *Prunus* sp. (Slippers *et al.* 2004a), but differs from other species in the genus. Conidia of *B. fabicerciana* are larger than those of *B. ramosa* (av. size of conidia = 13.4 × 5.7 µm in culture; Pavlic *et al.* 2008) and *B. scharifii* (av. size of conidia = 15.4 × 5.2 µm; Abdollahzadeh *et al.* 2013), but smaller than those of *Botryosphaeria corticis* (av. size of conidia = 28.9 × 6.4 µm; Phillips *et al.* 2006).

Botryosphaeria fusispora Boonmee, J.K. Liu & K.D. Hyde, Fungal Divers. 57: 171. 2012. MycoBank MB801319. See Liu *et al.* (2012) for illustrations.

Ascomata dark brown to black, immersed in the host, becoming erumpent, clustered, gragarious or scattered, subglobose with indistinct ostiole, 137–210 µm high × 160–230 µm diam, wall composed of 3–4 layers of dark brown cells of *textura angularis*. *Pseudoparaphyses* 2.5–5 µm wide, aseptate. *Asci* 8-spored, bitunicate, broadly cylindrical, short pedicellate with a well-developed apical chamber, 77.5–112.5 × 20–25 µm. *Ascospores* biseriate, partially overlapping, hyaline, aseptate, ellipsoidal to fusiform, smooth-walled, thin-walled, 20–27.5 × 10–12.5 µm. *Conidiomata* stromatic, solitary, semi-immersed, dark brown to black, 140–180 × 160–210 µm, walls composed of thick-walled dark brown cells of *textura angularis*, beconming thinner-walled and hyaline towards the inner region. *Conidiophores* hyaline, septate, cylindrical, smooth, 2–4.5 µm wide. *Conidiogenous cells* holoblastic, hyaline, cylindrical, integrated, producing a single apical conidium. *Conidia* hyaline, thin-walled, aseptate, fusiform to ellipsoidal, sometimes irregular ellipsoidal, smooth, apex obtuse, base truncate or bluntly rounded, 16–22 × 4–5.5 µm.

Culture characteristics: *Colonies* on MEA growing rapidly, reaching 9 cm diam within 7 d at room temperature, aerial mycelium at first white becoming dark grey to black.

Type: **Thailand**, Chiang Rai, Doi Tung, on dried bark of *Entada* sp., 10 Jun. 2009, S. Boonmee, **holotype** MFLU 10-0028.

Culture: MFLUCC 10-0098 (ex-type).

Hosts: *Caryota* sp., *Entada* sp. (Liu *et al.* 2012).

Known distribution: Thailand (Liu *et al.* 2012).

Notes: The shorter conidia separate this species from *B. corticis*, *B. dothidea* and *B. fabicerciana*. Conidia of *B. fusispora* are larger than those of *B. ramosa* and *B. scharifii*.

Botryosphaeria ramosa (Pavlic, T.I. Burgess, M.J. Wingf.) A.J.L. Phillips & A. Alves, **comb. nov.** MycoBank MB805458. Fig. 13.
Basionym: *Fusicoccum ramosum* Pavlic, T.I. Burgess & M.J. Wingf., Mycologia 100: 861. 2008.

Ascomata not reported. *Conidiomata* semi-immersed, solitary, globose, papillate, chestnut, covered by hyphal hairs, up to 510 µm diam, sometimes with a neck to 1.7 mm long, arising from the substrate. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* smooth, cylindrical to subcylindrical, hyaline, the first conidium produced holoblastically and subsequent conidia enteroblastically, (6–)7.5–10(–11) × (2–)2–3(–3.5) µm. *Conidia* fusiform to ellipsoid to oval, rounded at apex, base truncate,

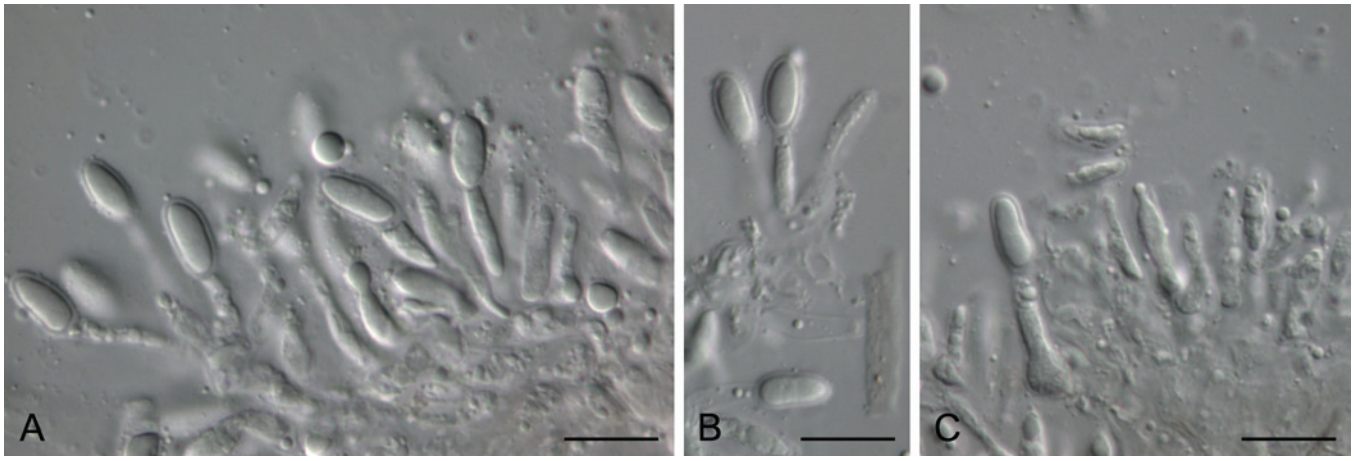

Fig. 13. *Botryosphaeria ramosa*. A–C. Conidia developing on conidiogenous cells. Scale bars = 10 µm.

smooth with fine granular contents, hyaline, thin-walled, aseptate, (11–)12–15(–16) × (4.7–)5–6(–7) µm, L/W ratio = 2.3.

Culture characteristics: *Colonies* initially white turning grey-olivaceous from the middle of colonies within 5–7 d, with appressed mycelial mat and white moderately dense, cottony aerial mycelium toward the edge of colony, becoming smoke grey to olivaceous-grey (surface) and iron grey (beneath) within 10–14 d. Optimum growth at 25 °C, covering the 9 cm diam Petri dish after 4 d in the dark.

Type: **Australia**, Western Australia, Bell Gorge, on *Eucalyptus camaldulensis*, Jul. 2006, T.I. Burgess, **holotype** PREM 59846.

Cultures: CBS 122069 = CMW 26167 (ex-type).

Host: Asymptomatic branches of *Eucalyptus camaldulensis* (Pavlic *et al.* 2008).

Known distribution: Western Australia (Pavlic *et al.* 2008).

Notes: No sexual morph has been reported, but phylogenetically this is clearly a species of *Botryosphaeria*. Only one culture of *B. ramosa* is known. Although Pavlic *et al.* (2008) reported long, branched conidiophores, we could not find such structures in the holotype. No *Dichomera* synasexual morph was reported by Pavlic *et al.* (2008). The conidia of *B. ramosa* are significantly shorter than those of any other species in this genus, although they are of a similar length to *B. scharifii*.

Botryosphaeria scharifii Abdollahz., Zare, A.J.L. Phillips, Mycologia 105: 213. 2013. MycoBank MB564800. Fig. 14.

Ascomata not reported. *Conidiomata* stromatic, pycnidial, produced on pine needles on WA within 2–4 wk, solitary or aggregated, dark brown to black, globose, up to 760 µm diam, superficial, mostly

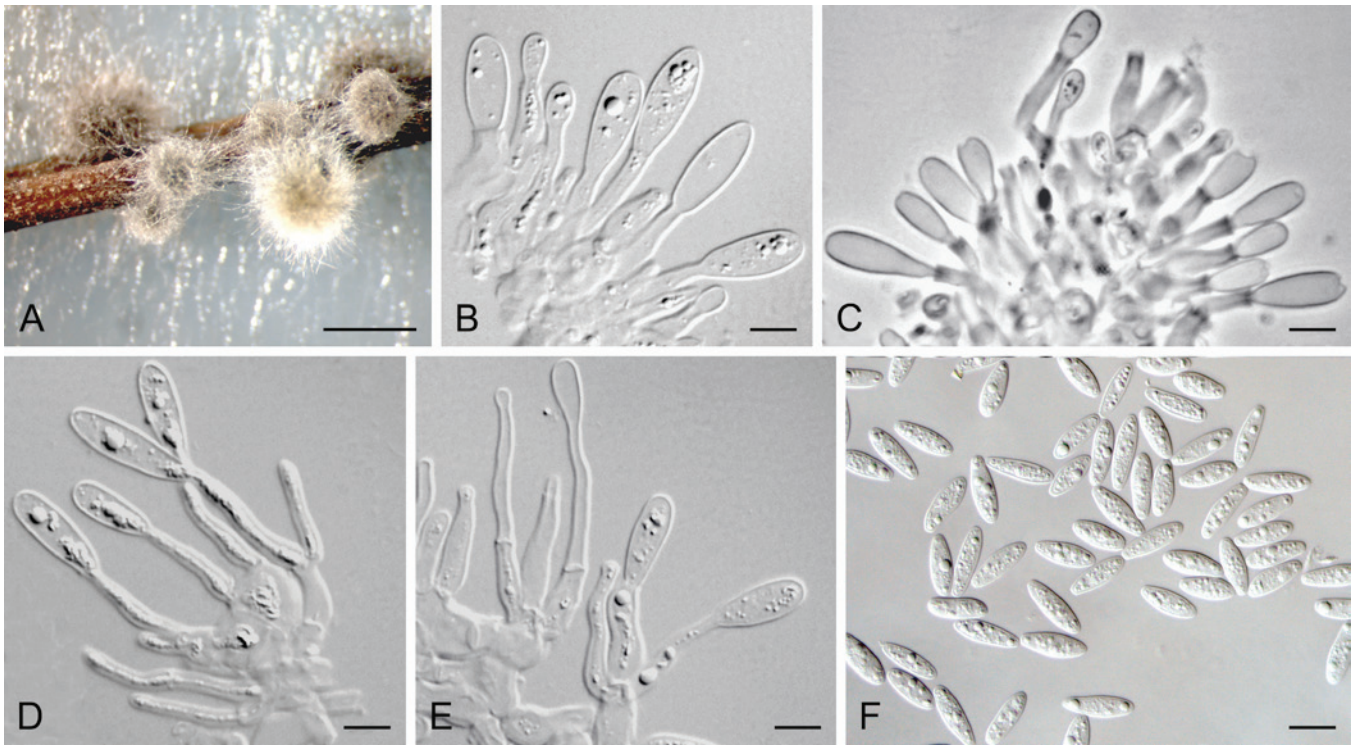

Fig. 14. *Botryosphaeria scharifii*. A. Conidiomata on pine needles in culture. B. Conidia developing on conidiogenous cells. C. Conidiogenous cells with periclinal thickenings. D, E. Conidiogenous cells and conidiophores. F. Conidia. Scale bars: A = 1 mm, B–E = 5 µm, F = 10 µm.

uniloculate, thick-walled, non-papillate with a central ostiole. *Conidiophores* cylindrical, hyaline, smooth, thin-walled, septate, branched at apex, 7.5–33.5 × 2–4.5 µm, lining the entire inner surface of the conidiomata. *Conidiogenous cells* cylindrical to lageniform, hyaline, thin-walled, smooth, 7–15 × 1.5–3.5 µm, holoblastic, phialidic with periclinal thickening. *Conidia* fusiform, unicellular, hyaline, thin-walled, smooth, apex obtuse, base subtruncate to bluntly rounded, (11.5–)13–17(–19) × 4–6.5 µm, 95 % confidence limits = 15.2–15.6 × 5.2–5.4 µm (av. ± S.D. = 15.4 ± 1.4 × 5.2 ± 0.5 µm), L/W ratio = 2.7.

Culture characteristics: *Colonies* with abundant aerial mycelium reaching to the lid of Petri dishes, aerial mycelium becoming smoke-grey to olivaceous-grey at the surface and greenish olivaceous to dull green at the reverse after 2 wk in the dark at 25 °C. Colonies reaching 84 mm on MEA after 3 d in the dark at 25 °C. Cardinal temperatures for growth: min ≤ 5 °C, max ≥ 35 °C, opt 25 °C.

Type: **Iran**, Tehran, on fruits of *Mangifera indica* imported from Pakistan, Aug. 2006, J. Abdollahzadeh, **holotype** IRAN 14275F.

Cultures: CBS 124703 = IRAN 1529C (ex-type).

Host: On twigs and fruits of *Mangifera indica* (Abdollahzadeh *et al.* 2013).

Known distribution: Iran (Hormozgan and Kurdistan Provinces and Tehran) (Abdollahzadeh *et al.* 2013).

Notes: *Botryosphaeria scharifii* is phylogenetically closely related to *B. ramosa*. Conidia of *B. scharifii* and *B. ramosa* are considerably shorter than all other species in the *Botryosphaeria* clade. However, the slightly longer conidia of *B. scharifii* distinguish it from *B. ramosa*. This speces was found on twigs of mango trees in Hormozgan Province (Minab) and from mango fruits, imported from Pakistan, in Kurdistan Province (Sanandaj) and Tehran.

Cophinforma Doilom, J.K. Liu & K.D. Hyde, Fungal Divers. 57: 174. 2012. MycoBank MB801315.
Type species: *Cophinforma atrovirens* (Mehl & Slippers) A. Alves & A.J.L. Phillips, **comb. nov.**

Ascomata initially immersed under host epidermis, becoming semi-immersed to erumpent, gregarious and fused, uniloculate, globose to subglobose ostiolate. *Ostiole* central, papillate, periphysate. *Asci* 8-spored, bitunicate, fissitunicate, clavate to cylindro-clavate, pedicellate, apex rounded with well-developed ocular chamber. *Ascospore* overlapping uniseriate to biseriate, hyaline, aseptate, smooth-walled, ellipsoidal to obovoid, slightly wider above the centre. *Conidiomata* indistinguishable from ascomata. *Paraphyses* absent. *Conidiogenous cells* enteroblastic, integrated, hyaline, smooth, cylindrical, first-formed conidium holoblastic, proliferating at the same level resulting in typical phialides (*sensu* Sutton 1980) with periclinal thickenings. *Conidia* hyaline, thin-walled, smooth, aseptate, fusiform. *Spermatophores* reduced to conidiogenous cells, occurring intermingled among conidiogenous cells in same conidioma, subcylindrical, hyaline, smooth. *Spermatia* hyaline, smooth, granular, subcylindrical.

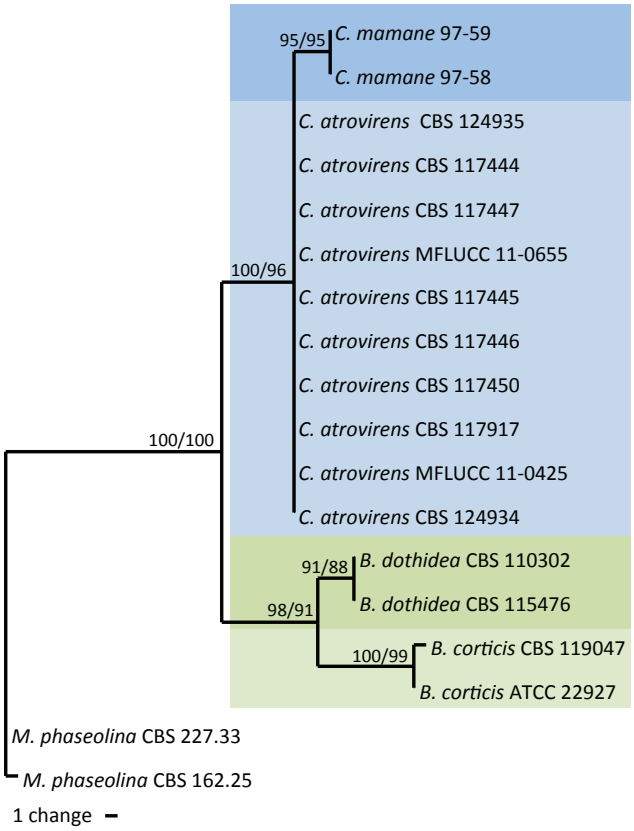

Fig. 15. Single most parsimonious tree obtained from the analysis of ITS sequences from species of the genera *Botryosphaeria* and *Cophinforma*. The phylogenetic tree resulting from ML analysis using the general time reversible model of DNA evolution and assuming a discrete gamma distribution with invariant sites (GTR+G+I) had a topology identical to the MP tree presented. MP/ML values (> 50 %) resulting from 1000 bootstrap replicates are given at the nodes. The tree was rooted to *M. phaseolina*.

Notes: *Cophinforma* was introduced by Liu *et al.* (2012) as a monotypic genus for *C. eucalypti*. Here we show that two species previously included in *Botryosphaeria* are better accommodated in *Cophinforma*. Conidia of the two known species of *Cophinforma* are longer than any known species in *Botryosphaeria*. In all other aspects the two genera are morphologically similar but are phylogenetically distinct. Two species are currently recognised in *Cophinforma*.

DNA phylogeny

The first 87 bases of the ITS sequences of the two *C. atrovirens* isolates appear to have many sequencing errors and were excluded from the analyses. In the ITS + EF1-α phylogeny (Fig. 8) the two isolates of *C. atrovirens* clustered with *C. eucalypti* and since the sequences were identical we consider this to represent a single species. The oldest epithet is *atrovirens*, thus *C. eucalypti* becomes a synonym. Unfortunately, no EF-α sequences are available for *C. mamane* and no cultures are extant, and thus we could not include *C. mamane* in the combined ITS + EF-α phylogeny. Nevertheless, in the ITS phylogeny (Fig. 15), 3 bp differences separate *C. mamane* from *C. atrovirens* and for this reason we consider these to represent two distinct species.

Key to *Cophinforma* spp.

The two species are morphologically very similar, with significant overlap in conidial dimensions, suggesting that they can only clearly be distinguished based on DNA data.

1. Conidia 30–40 × 8–9 µm ..... *C. mamane*
1. Conidia 31–36 × 7–10 µm ..... *C. atrovirens*

Species descriptions

Cophinforma atrovirens (Mehl & Slippers) A. Alves & A.J.L. Phillips, **comb. nov.** MycoBank MB805459. Fig. 16.
Basionym: *Fusicoccum atrovirens* Mehl & Slippers, Mycologia 103: 543. 2011.
= *Cophinforma eucalypti* Doilom, J.K. Liu & K.D. Hyde, Fungal Divers. 57: 174. 2012.

Ascomata not reported. *Conidiomata* on pine needles and host material pycnidial, superficial, multilocular, dark brown to black, eustromatic, complex, effuse, globose, covered with hyphae; wall composed of three layers, an outer of thick-walled dark to light brown *textura angularis*, a middle layer of thin-walled light brown cells, and an inner layer of thin-walled hyaline cells, (180–)215–275(–285) µm diam. *Conidiomata* indistinguishable from ascomata. *Conidiophores* absent. *Conidiogenous cells* hyaline, holoblastic, smooth, discrete, cylindrical, proliferating percurrently to form one or two distinct annellations, or proliferating at the same level giving rise to periclinal thickenings (10.5–)13.5–19(–22) × (2–)3.5–4.5(–5.5) µm (av. of 50 conidiogenous cells = 16.3 × 3.8 µm). *Paraphyses* absent. *Conidia* hyaline, thin-walled, unicellular, aseptate, rarely becoming septate on germination, granular, ellipsoid to obovoid, (27–)31–36(–40) × (6–)7–10(–12) µm (av. of 50 conidia = 33.5 × 8.5 µm). *Spermatophores* reduced to *Spermatogenous cells*, occurring intermingled among conidiogenous cells in same conidioma, subcylindrical, hyaline, smooth, 5–20 × 3–5 µm. *Spermatia* hyaline, smooth, granular, subcylindrical, straight or slightly curved, apex obtuse, base truncate, 5–8 × 3–4 µm.

Culture characteristics: *Colonies* fluffy, initially white to olivaceous in the center, edges becoming olivaceous to greenish black with age. Submerged mycelia (reverse) initially white to dark amber on the edges to olivaceous in the center, becoming olivaceous to greenish black with age. Optimum temperature for growth 30 °C.

Type: **South Africa**, Mpumalanga Province, Mawewe Nature Reserve, from an asymptomatic branch of *Pterocarpus angolensis*, Dec. 2005, J.W.M. Mehl & J. Roux, **holotype** PREM 60341; **paratype** PREM 60342.

Cultures: CBS 124934 = CMW 22674 (ex-holotype), CBS 124935 = CMW 22682 (ex-paratype).

Hosts: Asymptomatic branches and twigs of *Pterocarpus angolensis* (Mehl *et al.* 2011), on dead branch of *Eucalyptus* sp. (Liu *et al*. 2012) as *C. eucalypti*.

Known distribution: South Africa (Mehl *et al.* 2011), Thailand (Liu *et al*. 2012).

Notes: Morphologically this species is closely related to *C. mamane* but the highly divergent ITS phylogeny and several morphological characters separate the two species. Conidia can be 1- or 2-septate in *C. mamane* (Mohali *et al.* 2007) but remain aseptate until germination in *C. atrovirens*.

Cophinforma mamane (D.E. Gardner) A.J.L. Phillips & A. Alves, **comb. nov.** MycoBank MB805460. See Gardner (1997) for illustrations.
Basionym: *Botryosphaeria mamane* D.E. Gardner, Mycologia 89: 299. 1997.

Stromata erumpent through host tissue, black, 0.5−1.25 mm diam, multiloculate, locules spherical to ovoid, ostiolate, 100−200 µm diam. *Ascomata*, *conidiomata* and *Spermatogonia* distinct but often formed in the same stroma. *Ascomata* with a short neck, opening through a nonperiphysate ostiolar canal. *Asci* bitunicate, clavate, 8-spored, 100−180 × 25−35 µm, associated with filamentous *pseudoparaphyses*. *Ascospores* aseptate, hyaline, with granular or reticulately textured contents, oval to broadly fusiform, 25−39 × 15−20 µm. *Conidiogenous cells* simple, uniformly lining the locule wall. *Conidia* at first produced holoblastically, later enteroblastically, hyaline, 1-celled, fusiform, with truncate base when newly formed, (19−)30−44(−55) × (7−)8−9(−10) µm. *Spermatia* hyaline, rod-like to allantoid, 3−9 × 2−4 µm.

Type: **USA**, Hawaii, Hawaii Island, Hawaii Volcanoes National Park, Kipuka Ki, on bark of a swollen branch of *Sophora chrysophylla*, 1 May 1996, D.E. Gardner, **holotype** BISH 644614; **isotype** BISH 737731; **paratypes** BPI 737732, BPI 737733.

Cultures: No ex-type cultures are known to be extant. CBS 117444 and CBS 117450 are reportedly *B. mamane* but they were isolated from *Eucalyptus* and *Acacia* in Venezuela and their ITS sequences differ by 3 bp from the ex-type isolate of *B. mamane* collected by Gardner in 1996 and thus represent a different species.

Hosts: *Sophora chrysophylla* (Gardner 1997).

Diseases: Witch's brooms (Gardner 1997).

Known distribution: USA (Hawaii) (Gardner 1997).

Notes: Originally reported from Hawaii, this species is thought to be restricted to *Sophora chrysophylla*. Mohali *et al.* (2007) reported what they considered to be *B. mamane* on *Acacia mangium* (CBS 117445/CBS 117450) and *Eucalyptus urophylla* (CBS 117444/CBS 117917) in Venezuela. They based this conclusion on an ITS phylogeny and similarity in conidial characters and dimensions of their isolates with those of the ex-type strains of *B. mamane*. Unfortunately, the ex-type isolates of *B. mamane* no longer exist. Apparently D.E. Gardner sent sub-cultures to G. Stanosz at the

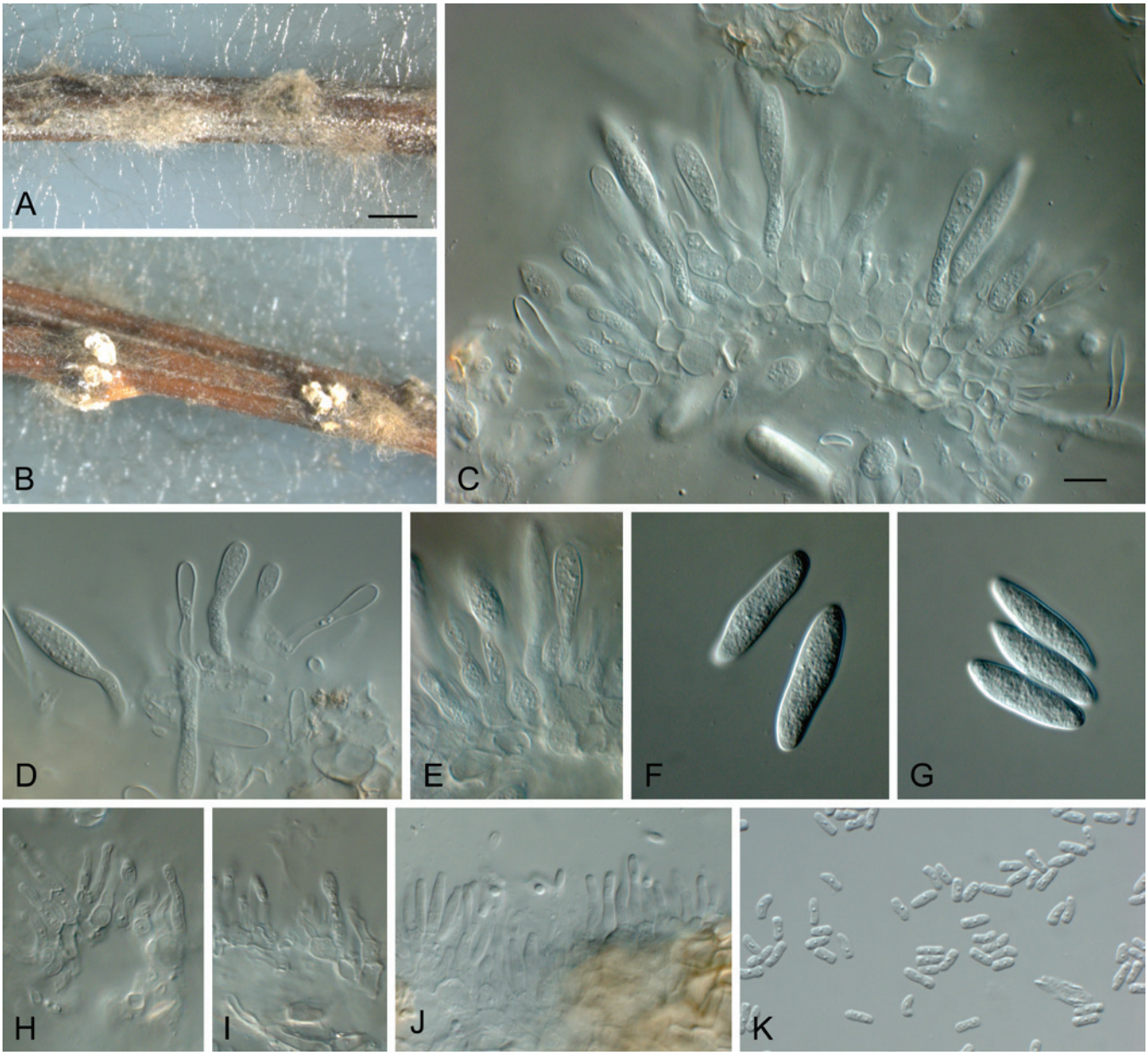

Fig. 16. *Cophinforma atrovirens*. A, B. Conidiomata formed on pine needles in culture. Conidia are oozing from the conidiomata in B. C–E. Conidiogenous cells. F, G. Conidia. H–J. Spermatogenous cells. K. Spermatia. Scale bars: A = 0.5 mm, C = 10 µm. Scale bar in A applies to B. Scale bar in C applies to D–K.

University of Wisconsin and these were given the codes 97-58 and 97-59. Zhou & Stanosz (2001) sequenced ITS of these two strains and the sequences are available in GenBank as AF246929 and AF246930. Unfortunately no other sequences were generated and these two isolates have since been lost.

In the ITS phylogeny generated by Mohali *et al.* (2007) isolates from *E. urophylla* and *A. mangium* clustered with the two ex-type isolates of *B. mamane*. However, three base pairs in ITS separate the ex-type isolates of *C. mamane* from the Venezuelan isolates. Furthermore, ITS sequences of the Venezuelan isolates of *C. mamane* are exactly the same as the ITS sequence of *C. atrovirens*. Therefore we consider the Venezuelan isolates to represent *C. atrovirens*.

Diplodia Fr., *In*: Mont., Ann. Sci. Nat. Bot., sér. 2, 1: 302. 1834. MycoBank MB8047.

Type species: *Diplodia mutila* Fr., *In*: Mont., Ann. Sci. Nat. Bot., sér. 2, 1: 302. 1834.

Ascomata unilocular, solitary or clustered, immersed, partially erumpent when mature, dark brown to black, thick-walled, wall composed of outer layers of thick-walled, dark brown *textura angularis*, inner layers of thin-walled, hyaline *textura angularis*. *Ostiole* central, circular, papillate, periphysate. *Pseudoparaphyses* hyaline, branched, septate. *Asci* clavate, stipitate, bitunicate, containing eight, biseriate ascospores. *Ascospores* fusiform, hyaline, thin-walled, smooth, aseptate, rarely becoming light brown and 1–2-septate with age. *Mycelium* immersed or superficial, branched, septate, melanised, dark brown. *Conidiomata* pycnidial, ostiolate, formed in uni- or multiloculate stromata, immersed, becoming erumpent at maturity. *Ostiole* central, circular, papillate. *Paraphyses* lacking. *Conidiophores* (when present) hyaline, simple, occasionally septate, rarely branched, cylindrical, arising from the cells lining the pycnidial cavity. *Conidiogenous cells* holoblastic, hyaline, cylindrical, determinate or proliferating at the same level giving rise to periclinal thickenings, or proliferating percurrently and forming two or three annellations. *Conidia* initially hyaline, aseptate, thick-walled, becoming 1–2-septate and pale

transluscent brown after discharge from the pycnidia, but the colouration is often delayed or never occurs, in some species the conidia become pigmented while still enclosed in the conidioma and in these species the conidia rarely become septate.

Notes: Two distinct conidial morphologies are seen in *Diplodia* species. In one type the conidia are initially hyaline and aseptate and later become pale to dark brown and 1-septate. Pigmentation is often delayed and in some species dark conidia are never seen. In the other type, the conidia become pigmented at an early stage of development, even while they are still enclosed within the pycnidia. These conidia only rarely become septate. These two morphological groups are supported by two distinct phylogenetic lineages.

Key to *Diplodia* spp.

1. Conidia hyaline and aseptate, becoming brown and 1-septate only with age 2
1. Conidia dark brown and aseptate before discharge from pycnidia 11

2. Av. conidial length greater than 29 µm 3
2. Av. conidial length less than 29 µm 5

3. Conidia 18–22 µm wide ..... *D. tsugae*
3. Conidia not more than 16 µm wide 4

4. On *Quercus*, av. conidial length 29.9 × 13.5 µm ..... *D. corticola*
4. On hosts other than *Quercus*, colonies rosulate ..... *D. rosulata*

5. On *Malus*, conidia pale brown ..... *D. bulgarica*
5. Conidia hyaline, becoming pigmented and 1-septate with age 6

6. On *Cupressus* or *Juniperus* spp. ..... *D. cupressi*
6. On other hosts 7

7. Av. conidial length 28 µm or longer 8
7. Av. conidial length less than 28 µm 9

8. Conidia up to 17 or more µm wide ..... *D. malorum*
8. Conidia never reach 17 µm wide 10

9. On *Quercus* ..... *D. quercivora*
9. On other hosts ..... *D. africana*

10. Av. conidial length greater than 27 µm (27.7 µm) ..... *D. agrifolia*
10. Av. conidial length less than 27 µm 11

11. Av. conidial size 24.5 × 12.5 µm, on *Olea* ..... *D. olivarum*
11. Av. conidial size 25.5 × 13.5 µm, on other hosts ..... *D. mutila*

12. Av. conidial length greater than 35 µm 13
12. Av. conidial length less than 35 µm 14

13. Conidial length exceeding 50 µm (up to 54 µm) ..... *D. sapinea*
13. Conidial length never exceeding 50 µm (up to 41.5 µm) ..... *D. scrobiculata*

14. Av. conidial length greater than 28 µm ..... *D. intermedia*
14. Av. conidial length less than 28 µm 15

15. Av. conidial length greater than or equal to 25 µm 16
15. Av. conidial length less than 25 µm ..... *D. allocellula*

16. Conidial length never exceeding 30 µm ..... *D. seriata*
16. Conidial length exceeding 30 µm ..... *D. alatafructa*/*D. pseudoseriata*[1]

[1]These two species cannot be distinguished based on their morphology.

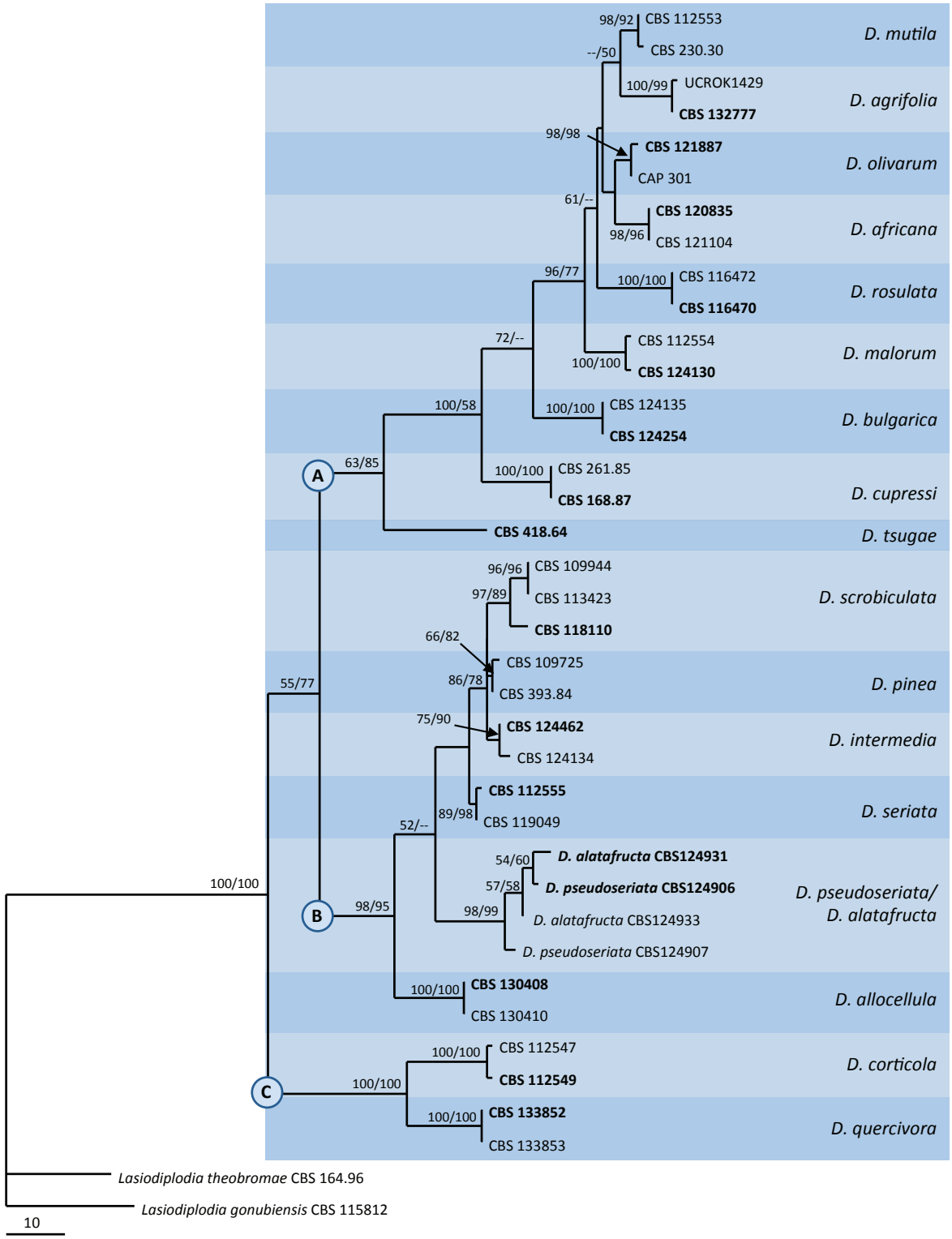

Fig. 17. One of 75 equally most parsimonious trees (tree length = 371, CI = 0.752, RI = 0.673, HI = 0.248) obtained from the combined analysis of ITS and EF1-α sequences from *Diplodia* species. Phylogenetic information contained in alignment gaps was incorporated into the phylogenetic analyses using simple indel coding as implemented by GapCoder (Young & Healy 2003). The phylogenetic tree resulting from ML analysis using the general time reversible model of DNA evolution and assuming a discrete gamma distribution (GTR+G) had a topology identical to the MP tree presented. MP/ML values (> 50 %) resulting from 1000 bootstrap replicates are given at the nodes. The tree was rooted to *Lasiodiplodia theobromae* CBS 164.96 and *Lasiodiplodia gonubiensis* CBS 115812. Clades corresponding to the 17 recognised species within the genus *Diplodia* are highlighted.

DNA phylogeny

Phylogenetic analysis based on combined ITS and EF1-α sequences revealed three major clades, A, B and C (Fig. 17). Most of the species in clade A have hyaline conidia that become pigmented and 1-septate only some time after discharge from the pycnidia. The exception is *D. bulgarica*, which has pale brown conidia, but these become more darkly pigmented and 1-septate with time. Eleven species can be distinguished in this clade and all are supported by moderate to high bootstrap values. In clade B the species all have conidia that become pigmented soon after they have formed, sometimes while still attached to the conidiogenous cell and usually before discharge from the pycnidia. Only rarely do they become septate. Bootstrap support for some of the species,

such as *D. pinea* and *D. intermedia*, is quite low. *Diplodia alatafructa* and *D. pseudoseriata* could not be separated clearly because none of the polymorphisms between isolates in this clade are fixed or consistent within a species. Clade C contains only two species, *D. corticola* and *D. quercivora*, and the conidia of these species have a morphology similar to that found in clade A, but they tend to be larger.

Species descriptions

Diplodia africana Damm & Crous, Mycologia 99: 671. 2008. MycoBank MB501323. See Damm *et al.* (2007) for illustrations.

Ascomata not reported. *Conidiomata* pycnidial, stromatic, produced on pine needles on SNA in 2–4 wk, solitary, globose to ovoid, dark brown, up to 500 µm wide, semi-immersed to erumpent, unilocular, sometimes multilocular in vitro, with a short neck and a central ostiole, wall 6–8 cell layers thick, outer layers composed of dark-brown *textura angularis*, becoming thin-walled and hyaline toward the inner region. *Conidiophores* 1–2 celled, hyaline, 10–25 × 3.5–6 µm. *Conidiogenous cells* holoblastic, hyaline, cylindrical, sometimes ampulliform, proliferating percurrently near the apex, sometimes with periclinical thickening, 3–15 × 3–6 µm. *Conidia* aseptate, hyaline, thick-walled, smooth, subcylindrical to oblong-elliptical, sometimes slightly curved, with rounded ends, hyaline after discharge from pycnidia, a few of them becoming brown, septate and finely verruculose with age, (17–)25.5–33(–34) × (10–)12–14(–15) µm (av. ± SD = 29.2 ± 3.6 × 13 ± 1.1 µm), L/W ratio = 2.2.

Culture characteristics: *Colonies* on PDA in the dark: mycelium pale olivaceous-grey, surface pale olivaceous-grey to dark grey-olivaceous, reverse olivaceous-black, umbonate with irregular zonation and lobate edges. Colonies under near ultraviolet: mycelium and surface greenish olivaceous to dark grey-olivaceous; reverse greenish olivaceous to olivaceous-black. Colonies reaching 26.8 mm diam after 2 d, reaching the edge the Petri dish within 5 d. Cardinal temperatures for growth: min 5 °C, max 35 °C, opt 20 °C.

Type: **South Africa**, Western Cape Province, Paarl, from wood section close to pruning wound of *Prunus persica*, 10 Jun. 2004, *U. Damm*, **holotype** CBS H-19843.

Cultures: CBS 120835 = STE-U 5908 (ex-type), STE-U 6289.

Host: *Prunus persica* (Damm *et al.* 2007).

Known distribution: South Africa (Western Cape Province) (Damm *et al.* 2007).

Notes: Conidia of *D. africana* are hyaline and thick-walled even after discharge from conidiomata and only a few conidia become brown and septate with age. It shares these features with *D. mutila*, *D. corticola*, *D. cupressi*, *D. rosulata*, *D. quercivora* and *D. tsugae*.

Diplodia agrifolia S.C. Lynch, A. Eskalen, Mycologia 105: 135. 2013. MycoBank MB800443. See Lynch *et al.* (2013) for illustrations.

Ascomata not reported. *Conidiomata* single or in groups, immersed to erumpent when mature, black and globose, 189 × 171–836 × 721 µm, wall composed of three layers; an outer layer of dark, thick-walled cells, middle layer with dark brown, thin-walled cells, and an inner layer of thin-walled hyaline cells. *Ostiole* central, circular, apapillate to papillate. *Conidiophores* absent. *Conidiogenous cells* holoblastic, discrete, cylindrical, hyaline, smooth, indeterminate, proliferating at the same level giving rise to periclinal thickenings or proliferating percurrently to form one to two indistinct annellations, 18.0 ± 7.4 × 8.1 ± 2.4 µm. *Conidia* in equal proportions hyaline, aseptate and pale to dark brown and 1-septate before and after discharge, smooth, thick-walled, oblong to ovoid, straight, both ends broadly rounded, (21.5–)27–36.5 × (12–)14.5–18 µm (av. ± S.D. = 27.7 ± 2.2 × 14.7 ± 1.2 µm), L/W = 1.9.

Type: **USA**, California, San Diego County, Mataguay Scout Camp, on cankered branch of *Quercus agrifolia*, 23 Feb. 2010, S.C. Lynch & A. Eskalen, **holotype** BPI 884095 (dried culture of *D. agrifolia*).

Cultures: CBS 132777 = ATCC MYA-4895 = UCROK 732 (ex-type).

Hosts: *Quercus agrifolia* and *Q. kelloggii* (Lynch *et al.* 2013).

Known distribution: **USA**, Coast range of north-central California southward to northern Baja California, with *Q. kelloggii* extending as far north as Eugene, Oregon (Lynch *et al.* 2013).

Notes: Phylogenetically, *D. agrifolia* is distinct from but closely related to *D. mutila*. *Diplodia agrifolia* differs from *D. mutila* in the conidia that are longer and wider than *D. mutila*. Conidia of *D. agrifolia* are hyaline and aseptate, but most become dark brown and 1-septate before discharge from pycnidia, whereas conidia of *D. mutila* are hyaline, aseptate, rarely becoming pale brown and 1-septate with age.

Diplodia alatafructa Mehl & Slippers, Mycologia 103: 542. 2011. MycoBank MB513498. See Mehl *et al.* (2011) for illustrations.

Ascomata not reported. *Conidiomata* on both pine needles and host material stromatic, superficial, unilocular, dark brown to black, mostly solitary, more or less globose/circular, covered with mycelium/hyphae, wall composed of three layers; an outer thick-walled dark brown *textura angularis*, a middle layer of light brown to reddish brown thin-walled cells, and an inner layer of hyaline thin-walled cells, (114–)130–155(–160) µm diam (av. of 50 conidioma = 141.4 µm). *Ostiole* central, circular. *Conidiophores* absent. *Conidiogenous cells* holoblastic, hyaline, discrete, spherical to cylindrical, proliferating percurrently to form two or three distinct annellations, or proliferating at same level giving rise to periclinal thickenings, (10–)12.5–18(–23) × (8–)11–14(–15.5) µm (av. of 40 conidiogenous cells = 15.4 × 12.5 µm). *Conidia* initially hyaline becoming pigmented and dark brown with age, unicellular, rarely septate or biseptate, rarely striate, ellipsoid to obovoid, thick-walled, granular, rounded at apices, eguttulate, smooth, (22.5–)24.5–29(–33) × (9.5–)11–14(–16) µm (av. of 50 conidia = 26.9 × 12.4 µm).

Culture characteristics: *Colonies* with fluffy mycelium, initially white to amber in the centre turning dark amber within 7 d and becoming white to dark amber, almost olivaceous with age; submerged

mycelium (reverse) same except becoming white to dark amber, almost olivaceous, at the periphery, and olivaceous in the centre with age. Optimum temperature for growth 25 °C.

Type: **South Africa**, Mpumalanga Province, Sudwala Caves area, from a stem wound on *P. angolensis*, Dec. 2005, J.W.M. Mehl & J. Roux, **holotype** PREM 60337.

Culture: CBS 124931 (ex-type).

Host: *Pterocarpus angolensis* (Mehl *et al.* 2011).

Known distribution: South Africa (Mehl *et al.* 2011).

Note: See notes to *D. pseudoseriata*.

Diplodia allocellula Jami, Gryzenh., Slippers & M.J. Wingf., Cryptogamie Mycol. 33: 257. 2012. MycoBank MB564140. See Jami *et al.* (2012) for illustrations.

Ascomata not reported. *Conidiomata* immersed on MEA, solitary, globose, brown, up to 100 µm diam. *Conidiogenous cells* holoblastic, smooth, unicellular, cylindrical to sub-cylindrical, hyaline (4–)4.5–5(–5.5) × (10.5–)13.5–23.5(–27.5) µm. *Conidia* ovoid to ellipsoid, smooth with fine granular contents, apex rounded, base truncate, thick-walled, aseptate, initially hyaline, becoming dark brown, aseptate (20–)21.5–25(–30) × (9–)10–12.5(–14.5) µm.

Type: **South Africa**, Gauteng Province, Pretoria, from branch of *Acacia karroo* with dieback, Nov. 2009, M. Gryzenhout & F. Jami, **holotype** PREM 60701.

Cultures: CBS 130408 (ex-type) CBS 130409, CBS 130410 (paratype).

Host: *Acacia karroo* (Jami *et al.* 2012).

Known distribution: South Africa (Gauteng Province) (Jami *et al.* 2012).

Notes: Phylogenetically *D. allocellula* falls within the group of species with conidia that become brown and aseptate at an early stage of their development. Morphologically it is most similar to *D. seriata* and *D. alatafructa/D. pseudoseriata* but can be distinguished from these species on account of its generally smaller conidia.

Diplodia bulgarica A.J.L. Phillips, J. Lopes & S.G. Bobev, Persoonia 29: 33. 2012. MycoBank MB19632. Fig 18.

Ascomata not reported. *Conidiomata* pycnidial, stromatic, produced on pine needles on WA after 7–21 d, solitary, immersed, partially erumpent when mature, dark brown to black, globose to ovoid, up to 600 µm diam and 700 µm high, wall composed of an outer layer of dark brown, thick-walled *textura angularis*, a middle layer of dark brown thin-walled cells, an inner layer of thin-walled hyaline cells. *Ostiole* central, circular, papillate. *Conidiophores* absent. *Conidiogenous cells* hyaline, cylindrical, holoblastic, forming a single conidium at the tip, discrete, smooth, indeterminate, proliferating internally giving rise to periclinal thickenings, or proliferating percurrently to form 1–5 annellations, 9–18 × 2–5 µm. *Conidia* aseptate, smooth, thick-walled, oblong to ovoid, straight, both ends broadly rounded, (22.5–)24–27(–28) × (14.5–)15.5–18(–18.5) µm, 95 % confidence limits = 25.0–25.7 × 16.6–17.0 µm (av. ± S.D. of 50 conidia = 25.4 ± 1.2 × 16.8 ± 0.7 µm), L/W ratio = 1.5.

Type: **Bulgaria**, Plovdiv, on dead twigs of *Malus sylvestris*, 2005, S.G. Bobev, **holotype** CBS H-20189 (a dried culture of CBS 124254 grown on pine needles).

Culture: CBS 124254 (ex-type).

Hosts: *Malus* spp. (Phillips *et al.* 2012).

Known distribution: Bulgaria, Iran (Phillips *et al.* 2012).

Notes: This species is morphologically distinct from all other *Diplodia* species reported from apples. Conidia are shorter and wider than both *D. malorum* and *D. intermedia*. Furthermore, the conidia are distinctive in that they become pale brown soon after they are formed. Phylogenetically this species is closely related to *D. cupressi* and *D. tsugae*.

Diplodia corticola A.J.L. Phillips, A. Alves & J. Luque, Mycologia 96: 603. 2004. MycoBank MB488568. Figs 19, 20.

= *Botryosphaeria corticola* A.J.L. Phillips, A. Alves & J. Luque, Mycologia 96: 603. 2004.

Pseudothecia stromatic, immersed, partially erumpent when mature, dark brown to black, more or less circular, up 1 mm diam, multiloculate, individual locules 200–300 µm diam, thick-walled, wall composed of outer layers of thick-walled, dark brown *textura angularis*, inner layers of thin-walled, hyaline *textura angularis*. *Ostiole* circular, central, papillate, periphysate. *Pseudoparaphyses* hyaline, branched, septate, 2–3 µm wide. *Asci* clavate, stipitate, bitunicate, containing eight, biseriate ascospores, 160–250 × 30–35 µm (including stipe). *Ascospores* broadly fusiform to rhomboid, widest in the middle, both ends obtuse, hyaline, moderately thick-walled (ca. 1 µm), smooth-walled, aseptate, rarely becoming light brown and 1–2-septate with age, (28.5–)30–38(–40.5) × (13–)14–18.5(–19) µm, 95 % confidence limits = 33.6–35 × 15.3–16.2 µm (av. ± S.D. of 90 ascospores = 34.3 ± 2.4 × 15.8 ± 1.5 µm), L/W ratio = 2.2. *Conidiomata* eustromatic, immersed, partially erumpent when mature, dark brown to black, more or less circular, up to 1 mm diam, multiloculate, individual locules 200–300 µm diam, wall composed of three layers, an outer of dark brown, thick-walled *textura angularis*, a middle layer of dark brown thin-walled cells, and an inner layer of thin-walled hyaline cells. *Ostiole* central, circular, papillate. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, discrete, cylindrical, hyaline, smooth, indeterminate, proliferating at the same level giving rise to periclinal thickenings, or proliferating percurrently to form one or two indistinct annellations, 12–19(–24) × 4–6 µm. *Conidia* hyaline, aseptate, eguttulate or sometimes with a large central guttule, contents granular, smooth, thick-walled, oblong to cylindrical, straight, both ends broadly rounded, rarely becoming brown and septate when aged, (23.5–)26–34.5(–46) × (9–)12–16(–18.5) µm, 95 % confidence limits = 29.6–30.3 × 13.4–13.8 µm (av. ± S.D. of 250 conidia = 29.9 ± 2.5 × 13.6 ± 1.4 µm), L/W ratio = 2.2.

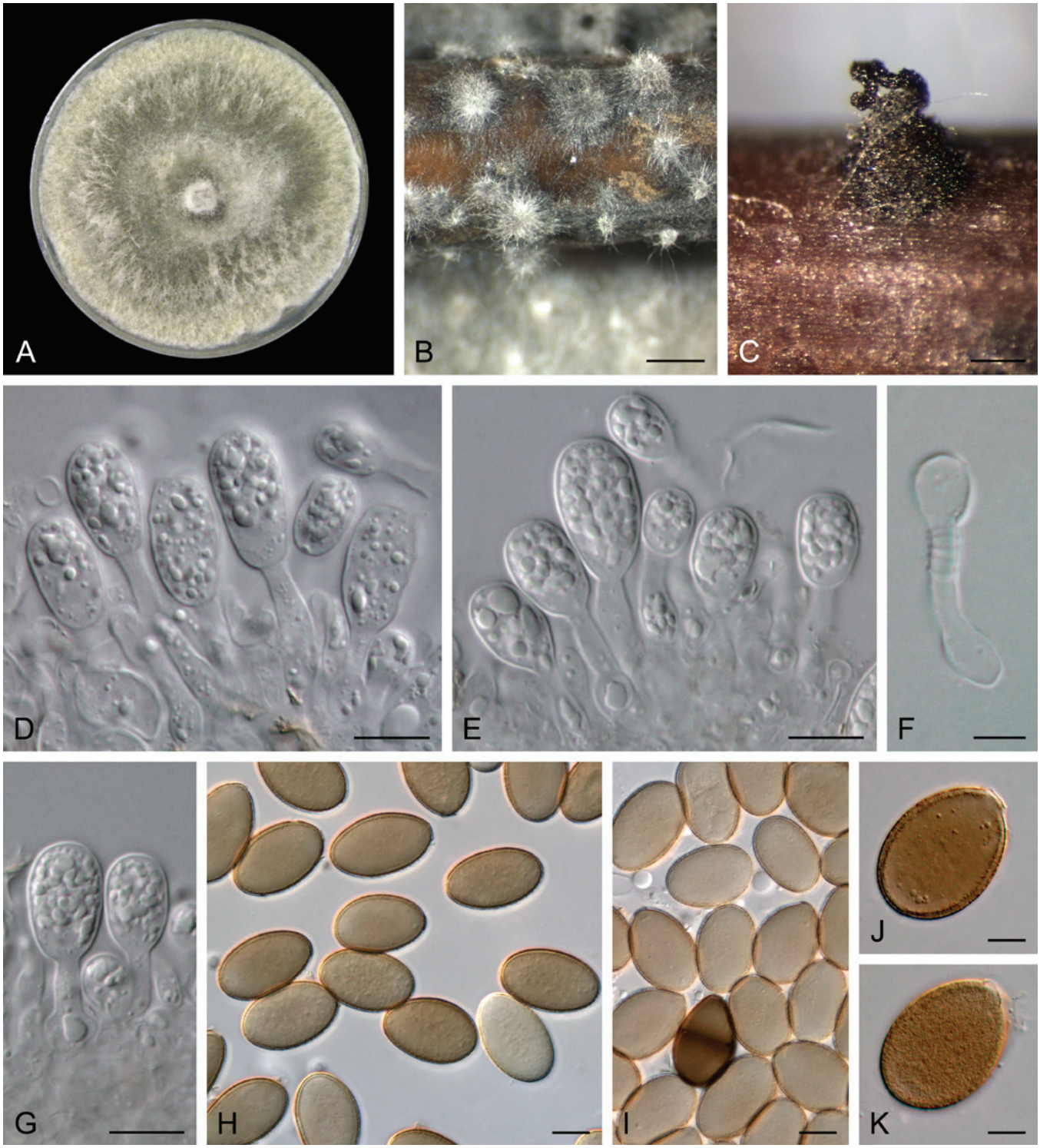

Fig. 18. *Diplodia bulgarica*. A. Culture grown on PDA. B. Conidiomata developing on pine needles in culture. C. Conidioma on pine needles oozing conidia. D–G. Conidiogenous cells with developing conidia. H. Pale brown, aseptate conidia. I. Pale brown, aseptate conidia and one 2-celled conidium. J, K. Brown conidium in two focal planes showing the finely verruculose inner surface of the wall. Scale bars: B = 0.5 mm, C = 200 µm, D–I = 10 µm, J, K, = 5 µm.

Culture characteristics: *Colonies* reaching 36−44 mm diam on PDA after 4 d in the dark at 25 °C. Cardinal temperatures for growth: min 5 °C, max < 35 °C, opt 20−25 °C.

Type: **Portugal**, Beira Littoral, Requeixo near Aveiro, on dead branches of *Quercus suber*, Feb. 2002, A. Alves, **holotype** LISE 94839.

Culture: CBS 112549 (ex-type).

Hosts: *Quercus* spp. (Alves *et al.* 2004).

Known distribution: Iberian Peninsula, Italy, N. America (Alves *et al.* 2004).

Notes: Conidia of this species are larger than in any other species of *Diplodia*. Phylogenetically *D. corticola* groups with *D. quercivora* (also an oak pathogen) in a distinct clade. It is responsible for dieback and cankers on *Q. suber* and *Q. ilex* and has been implicated as contributing to the general decline of cork oaks in the Iberian Peninsula and other regions of the Mediterranean.

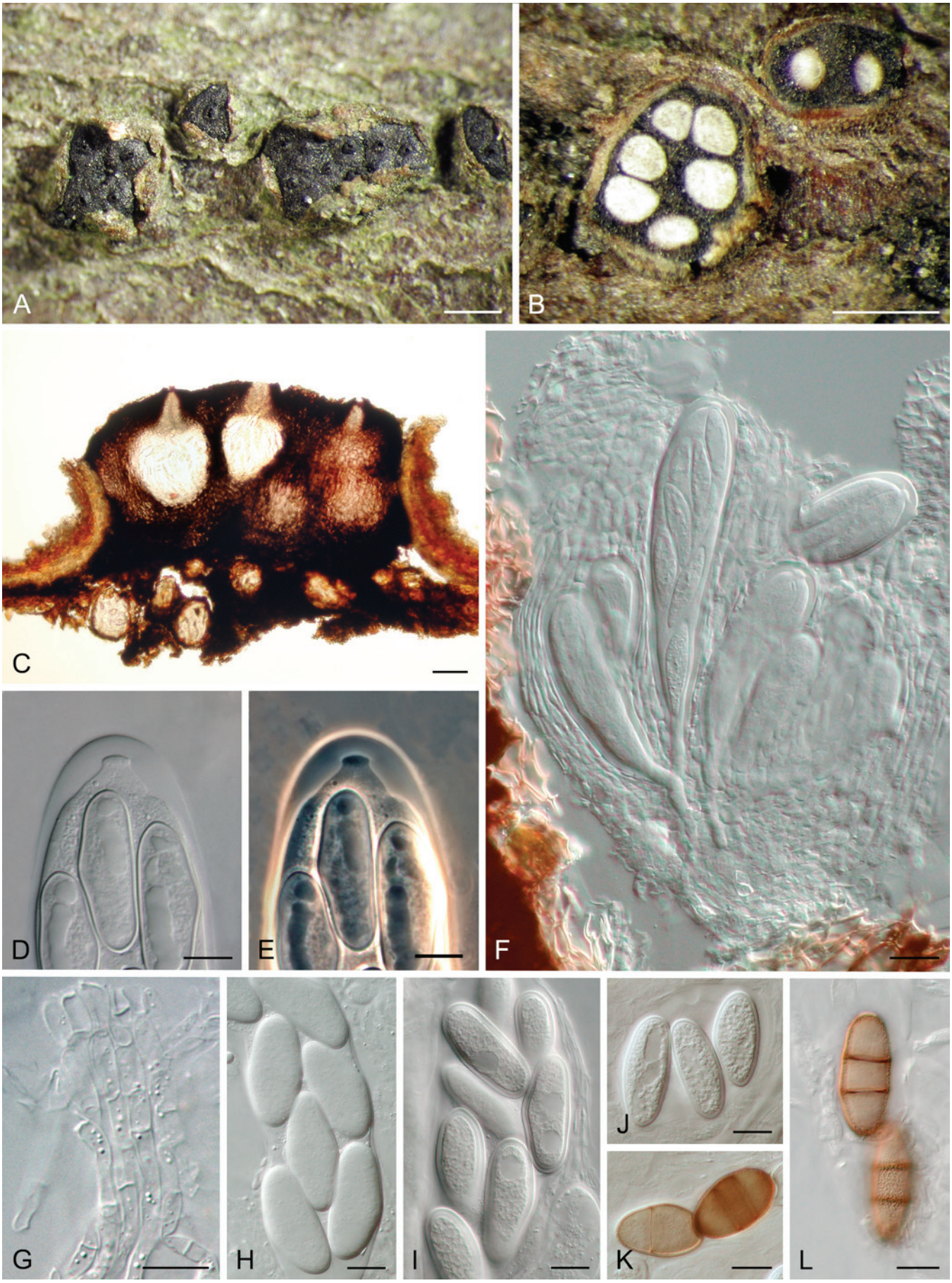

Fig. 19. *Diplodia corticola*. A. Ascomata partially erumpent through the host bark. B. Multilocular ascoma cut through horizontally revealing the brilliant white contents. C. Vertical section through an ascoma showing the thick wall and three locules opening through periphysate ostioles. D, E. Ascus tip as seen by interference contrast (D) and phase contrast (E) showing the well-developed apical chamber. F. Mature ascus containing ascospores, several immature asci and pseudoparaphyses. G. Pseudoparaphyses. H–J. Ascospores. K, L. Brown, 2-septate ascospores. Scale bars: A = 1 mm, B = 500 µm, C = 100 µm, D, E, G = 10 µm, F = 20 µm, H–L = 5 µm.

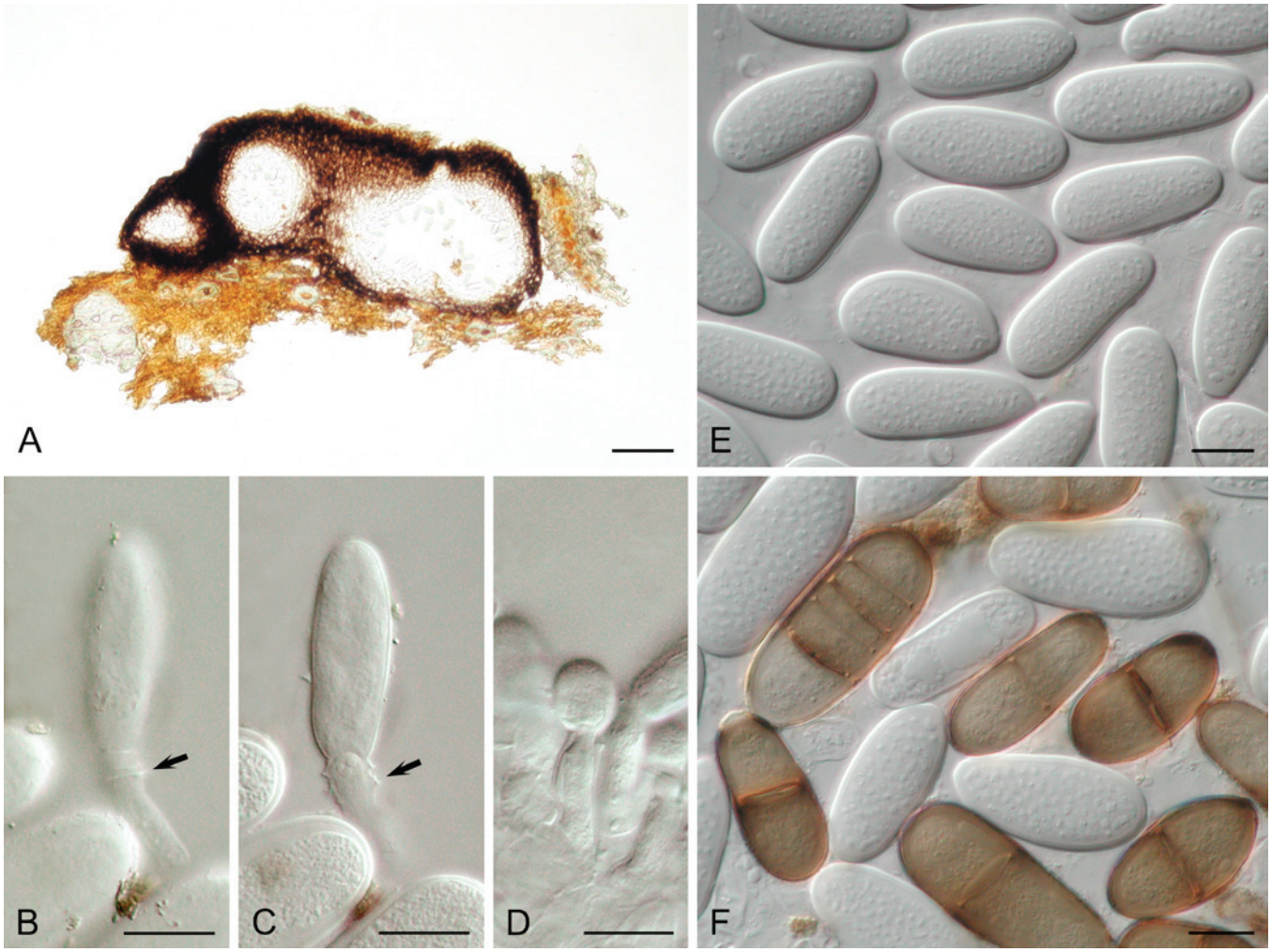

Fig. 20. *Diplodia corticola*. A. Sectioned conidiomata showing thick wall and three locules. B, C. Percurrently proliferating conidiogenous cells in surface view (B) and optical section (C) with annellations arrowed. D. Phialide with periclinal thickenings. E. Conidia. F. Brown and septate conidia. Scale bars: A = 100 µm, B–F = 10 µm.

Diplodia cupressi A.J.L. Phillips & A. Alves, Fungal Divers. 23: 9. 2006. MycoBank MB510136. Fig. 21.

Ascomata not reported. *Conidiomata* up to 300 µm diam, solitary, separate, uniloculate, dark brown to black, globose, ostiolate, wall composed of thick-walled *textura angularis*, becoming thin-walled and hyaline toward the inner region. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* hyaline, smooth, holoblastic forming conidia at their tips, proliferating internally giving rise to periclinal thickenings or proliferating percurrently with 1–4 close or widely spaced annellations, formed from the inner wall of the pycnidium, 12.5–20 × 4–4.5 µm. *Conidia* thick-walled, wall up to 2 µm wide, ovoid with both ends rounded, aseptate, hyaline and remaining so for a long time, becoming brown and 1-septate after discharge from the pycnidia, (21.5–)23.5–28.5(–30.5) × (12–) 13.5–15(–16) µm, 95 % confidence limits = 24.4–25.4 × 13.9–14.5 µm, (av. ± S.D. of 50 conidia = 24.9 ± 1.9 × 14.2 ± 0.9 µm), L/W = 1.76. *Spermatophores* hyaline, smooth, cylindrical, up to 10 µm long, 2.5–3 µm wide. *Spermatogenous cells* discrete or integrated, hyaline, smooth, cylindrical, holoblastic or proliferating via determinate phialides with periclinal thickening, 10–14 × 2–2.5 µm. *Spermatia* hyaline, smooth, aseptate, rod-shaped with rounded ends, 4–5 × 1.5 µm.

Type: **Israel**, Bet Dagan, dried culture from cankered stems of *Cupressus sempervirens*, 1986, Z. Solel, **holotype** IMI 303475.

Culture: CBS 168.87 (ex-type).

Hosts: *Cupressus* and *Juniperus* spp. (Alves *et al.* 2006, Solel *et al.* 1987).

Known distribution: Cyprus, Greece, Israel, Italy, Morocco, South Africa, Tunisia, USA (De Wet *et al*. 2009, Alves *et al.* 2006, Solel *et al.* 1987).

Notes: Solel *et al.* (1987) considered this fungus to be a sub-population of *Diplodia pinea* and named it *Diplodia pinea* f. sp. *cupressi*. Swart *et al.* (1993) challenged this assumption and showed that *D. pinea* f. sp. *cupressi* differed morphologically from *D. pinea* in terms of conidial dimensions, shape, colouration and this was supported by isozyme profiles. The observations of Swart *et al.* (1993) were supported by ITS sequence data by Zhou & Stanosz (2001). Finally, Alves *et al.* (2006) introduced the name *D. cupressi* for the Cypress pathogen. This species is morphologically similar to *D. mutila* but the conidia of *D. cupressi* are wider than are typical for *D. mutila* (Alves *et al.* 2004).

Diplodia intermedia A.J.L. Phillips, J. Lopes & A. Alves, Persoonia 29: 33. 2012. MycoBank MB19633. Fig. 22.

Ascomata unilocular, solitary or clustered, immersed, partially erumpent when mature, globose, up to 400 µm diam, dark brown to

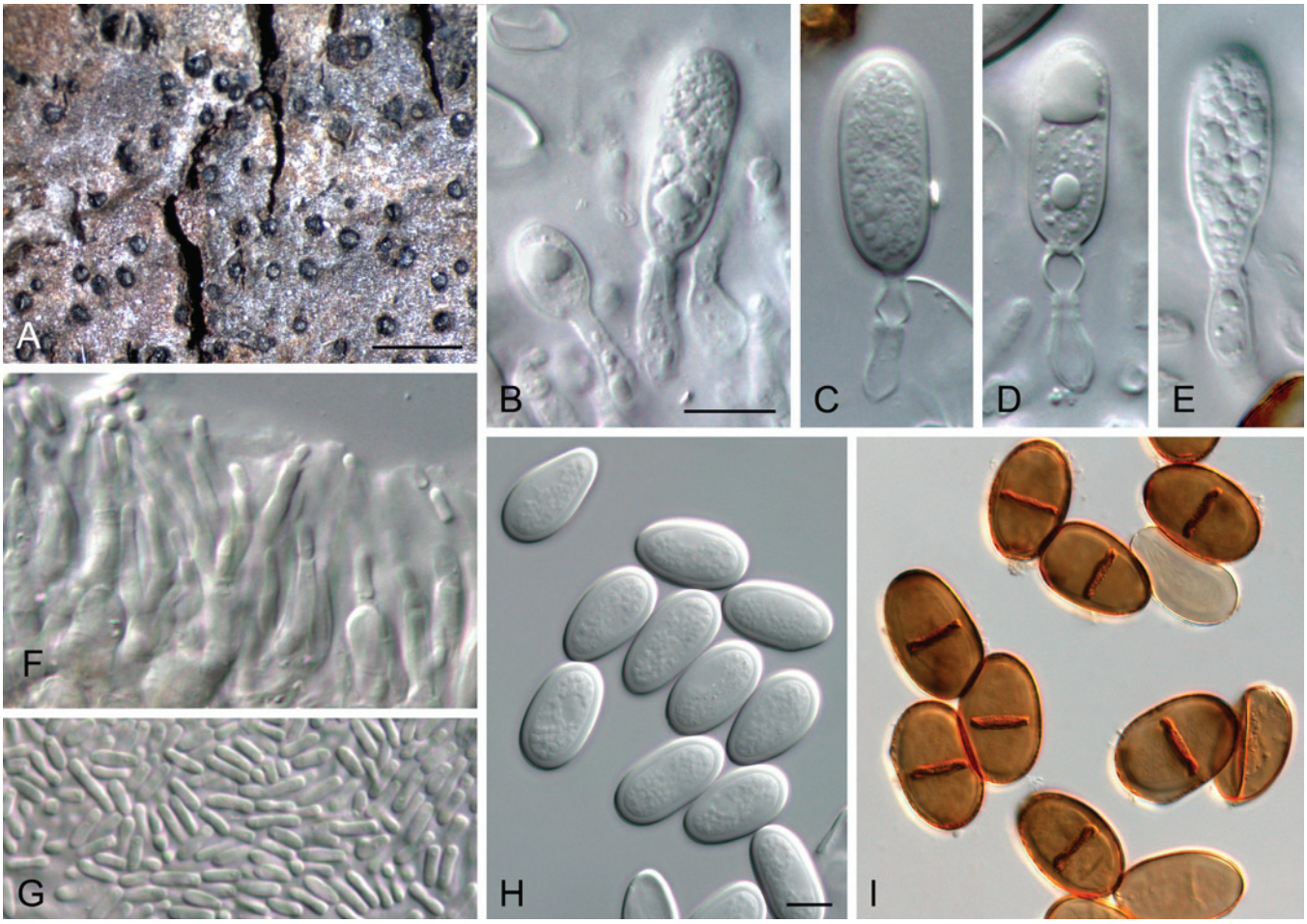

Fig. 21. *Diplodia cupressi*. A. Conidiomata on host bark. B–E. Conidiogenous cells. F. Spermatogenous cells. G. Spermatia. H. Hyaline, aseptate conidia. I. Mature dark-walled, 1-septate conidia. Scale bars: A = 1 mm, B, H = 10 µm. Scale bar of B applies to C–G. Scale bar of H applies to I.

black, thick-walled, wall composed of outer layers of thick-walled, dark brown *textura angularis*, inner layers of thin-walled, hyaline *textura angularis*. *Ostiole* central, circular, nonpapillate, periphysate. *Pseudoparaphyses* hyaline, branched, septate, constricted at the septum, 2–3 µm wide. *Asci* clavate, stipitate, bitunicate, containing eight ascospores biseriate in the ascus, 85–160 × 22–28 µm. *Ascospores* fusiform, widest in the upper third, hyaline, thin-walled, smooth, aseptate, 32–37(–40) × 6–8 µm. *Conidiomata* pycnidial, stromatic, solitary or clustered, immersed in the host, partially erumpent at maturity, dark brown to black, ostiolate, nonpapillate, thick-walled, outer and inner layers composed of dark brown and thin-walled hyaline *textura angularis*, respectively. *Conidiogenous cells* hyaline, thin-walled, smooth, cylindrical, swollen at the base, discrete, producing a single conidium at the tip, indeterminate, proliferating internally giving rise to periclinal thickenings or proliferating percurrently forming 2–3 annellations. *Conidia* aseptate, ovoid, widest in the middle, with obtuse apex and truncate or rounded base, initially hyaline, becoming dark brown before release from the pycnidia, wall moderately thick, externally smooth, internally roughened, (24.5–)29–33.5(–37) × (10–)11–16(–17.5) µm, with 95 % confidence limits = 30.2–31.1 × 13–13.6 µm (av. ± S.D. of 150 conidia = 30.6 ± 1.9 × 13.3 ± 1.8 µm), L/W = 2.3. *Spermatia* hyaline, aseptate, smooth, oblong, ends rounded, 5.5–9.5 × 4–6.5 µm. *Spermatogenous cells* not seen.

Type: **Portugal**, Setúbal, Monte da Caparica, dead twigs of *Malus sylvestris*, Mar. 2006, A.J.L. Phillips, **holotype** CBS H-20190.

Culture: CBS 124462 (ex-type).

Hosts: *Cydonia*, *Malus* (Phillips *et al.* 2012).

Known distribution: Portugal (Phillips *et al.* 2012).

Notes: Phylogenetically this species is very closely related to *D. sapinea*. However, on account of its smaller conidia, apparent preference for *Rosaceae* hosts, and the distinct clade it forms in the ITS + EF1-α phylogenies, Phillips *et al.* (2012) considered it to represent a distinct and separate species.

Diplodia malorum Fuckel, Jb. Nassau. Ver. Naturk. 23–24: 395. 1870. MycoBank MB 246351. Fig. 23.

Ascomata not reported. *Conidiomata* pycnidial, stromatic, immersed, erumpent, dark brown to black, aggregated, internally white, ostiolate, ostiole circular, central, short papilla. *Conidiophores* absent. *Conidiogenous cells* cylindrical, thin-walled, hyaline, holoblastic, indeterminate, proliferating at the same level to produce periclinal thickenings, or proliferating percurrently giving rise to 2–3 indistinct annellations. *Conidia* oblong with broadly rounded ends, smooth-walled, thick walled, hyaline, eguttulate, aseptate, becoming dark brown and 1-septate soon after release from the pycnidium, (24–)26–32(–36) × (12–)13–17.5(–18.5) µm, 95 % confidence limits = 28.0–28.3 × 14.3–14.5 µm (av. ± S.D. = 28.1 ± 2.4 ×14.4± 1.4 µm), L/W = 1.9.

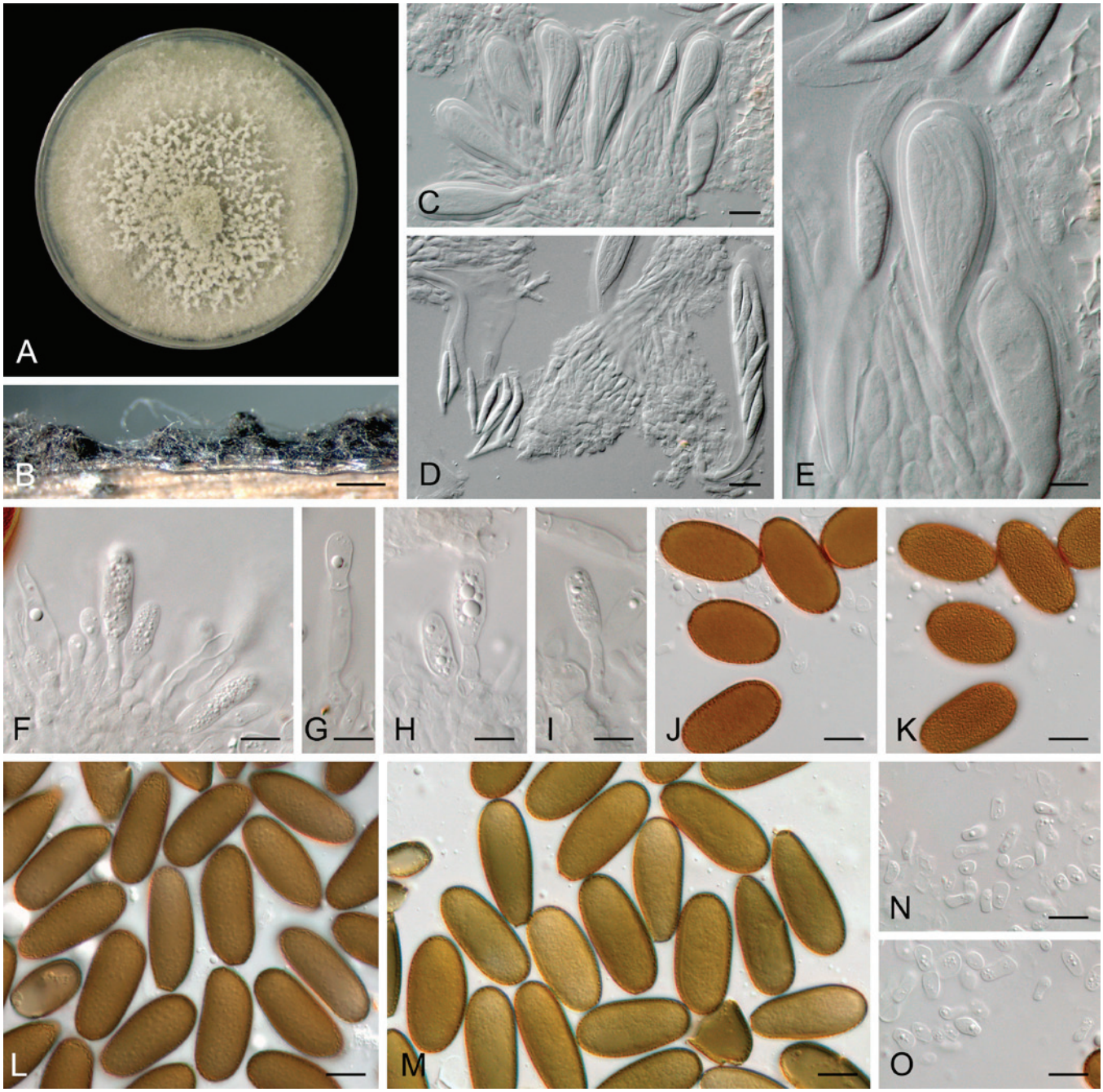

Fig. 22. *Diplodia intermedia*. A. Culture grown on PDA. B. Conidiomata developing on pine needles in culture. C. Asci. D, E. Ascus, ascospores and pseudoparaphyses. F–I. Conidiogenous cells. J, K. Conidia in two focal planes to show finely verruculose inner surface of the wall. L, M. Conidia. N, O. Spermatia. Scale bars: B = 0.5 mm, C, D = 20 µm, E–M = 10 µm, N, O = 5 µm.

Type: **Germany**, Rhineland, on *Malus* sp., 1870, J. Fuckel, Fuckel, Fungi rhenani Nº 1706, **holotype** in G, **isotypes** K and M. **Portugal**, Setúbal, Monte da Caparica, *Malus sylvestris*, Feb. 2006, A.J.L. Phillips, **epitype** CBS H-201888.

Culture: CBS 124130 (ex-epitype).

Hosts: *Malus* spp. (Phillips *et al.* 2012).

Known distribution: Germany, Portugal (Phillips *et al.* 2012).

Notes: Since the time that it was introduced by Fuckel (1870), the name *D. malorum* has been used infrequently, while the name *D. mutila* was applied to the apple pathogen. However, *D. malorum* is morphologically and phylogenetically distinct from *D. mutila*. The conidia are larger than those of *D. mutila* and they frequently become brown and 1-septate soon after discharge from the conidioma.

Diplodia mutila (Fr.) Mont., Ann. Sci. nat., sér. 2, 1: 302. 1834. MycoBank MB201741. Fig. 24.

Basionym: *Sphaeria mutila* Fr., Syst. Mycol. (Lundae) 2: 424. 1823.

≡ *Physalospora mutila* (Fr.) N.E. Stevens, Mycologia 28: 333. 1936.

= *Botryosphaeria stevensii* Shoemaker, Canad. J. Bot. 42: 1299. 1964.

Further synonyms are given by Stevens (1933).

Ascomata unilocular, solitary or clustered, immersed, partially erumpent when mature, globose, up to 300 µm diam, dark brown to black, thick-walled, wall composed of outer layers of thick-walled,

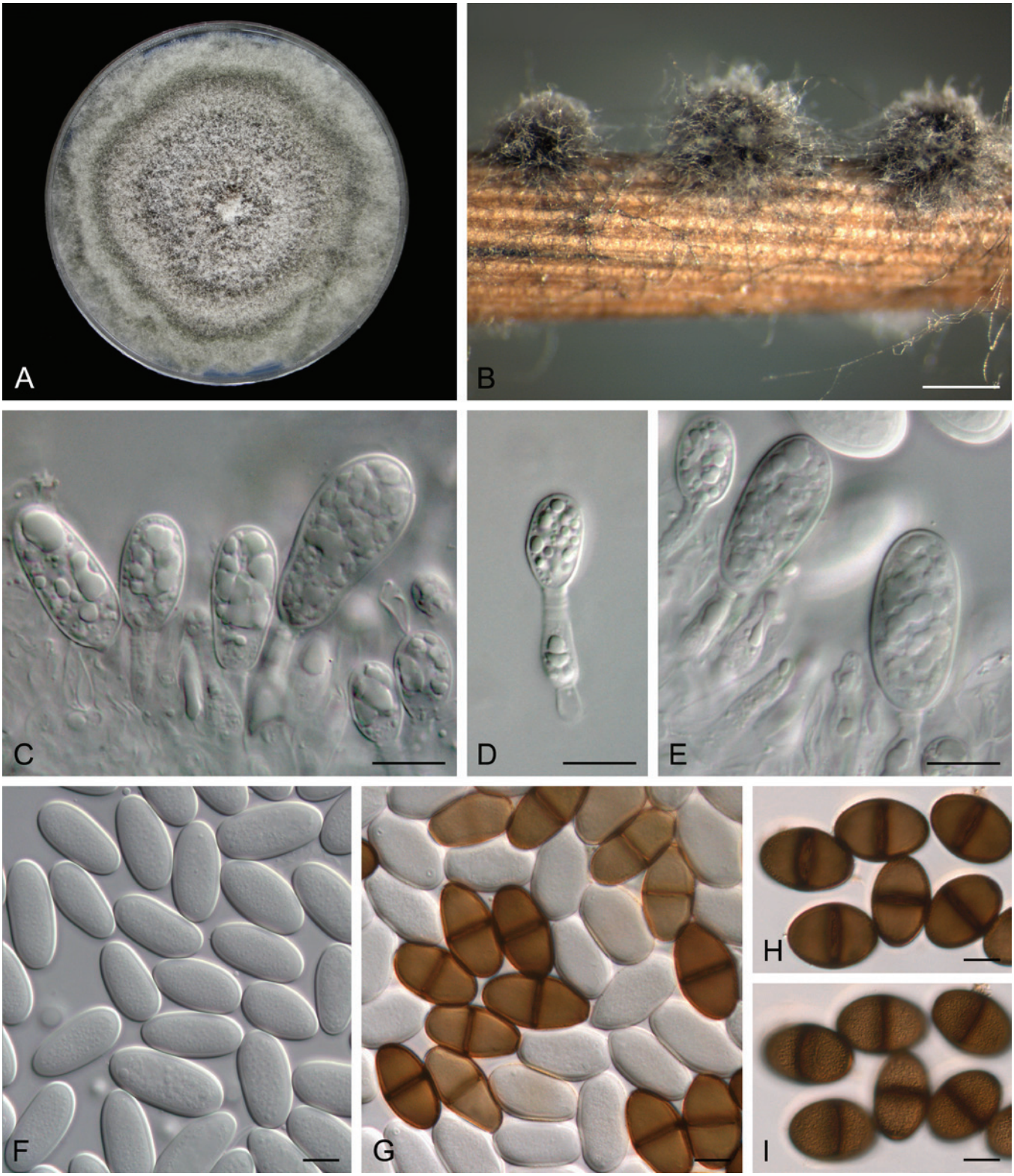

Fig. 23. *Diplodia malorum*. A. Culture growing on PDA. B. Pycnidia formed on pine needles. C–E. Conidiogenous cells. F. Hyaline aseptate conidia. G. Hyaline and 1-septate brown conidia. H, I. Brown conidia at two different planes of focus to show the finely verruculose inner surface of the wall. Scale bars: B = 500 µm, C–I = 10 µm.

dark brown *textura angularis*, inner layers of thin-walled, hyaline *textura angularis*. *Ostiole* central, circular, papillate, periphysate. *Pseudoparaphyses* hyaline, branched, septate, 2−3 µm wide. *Asci* clavate, stipitate, bitunicate, containing eight, biseriate ascospore, 100−160 × 14−22 µm (including stipe). *Ascospores* fusiform, widest in the middle, both ends obtuse, hyaline, thin-walled, smooth, aseptate, rarely becoming light brown and 1–2-septate with age, (24.5−)28–35(−36) × (9.5−)10−13(−13.5) µm, 95 % confidence limits = 30.8–32.1 × 11.2–11.7 µm (av. ± S.D. of 50 ascospores = 31.5 ± 2.3 × 11.4 ± 0.9 µm), L/W = 2.8. *Conidiomata* solitary or aggregated in clusters of up to five or more, immersed, partially erumpent when mature, dark brown to black, more or less globose, up to 600 µm diam, wall composed of three layers, an outer of dark brown, thick-walled *textura angularis*, a middle layer of dark brown thin-walled cells, an inner layer of thin-walled hyaline cells. *Ostiole* central, circular, papillate. *Conidiophores* absent.

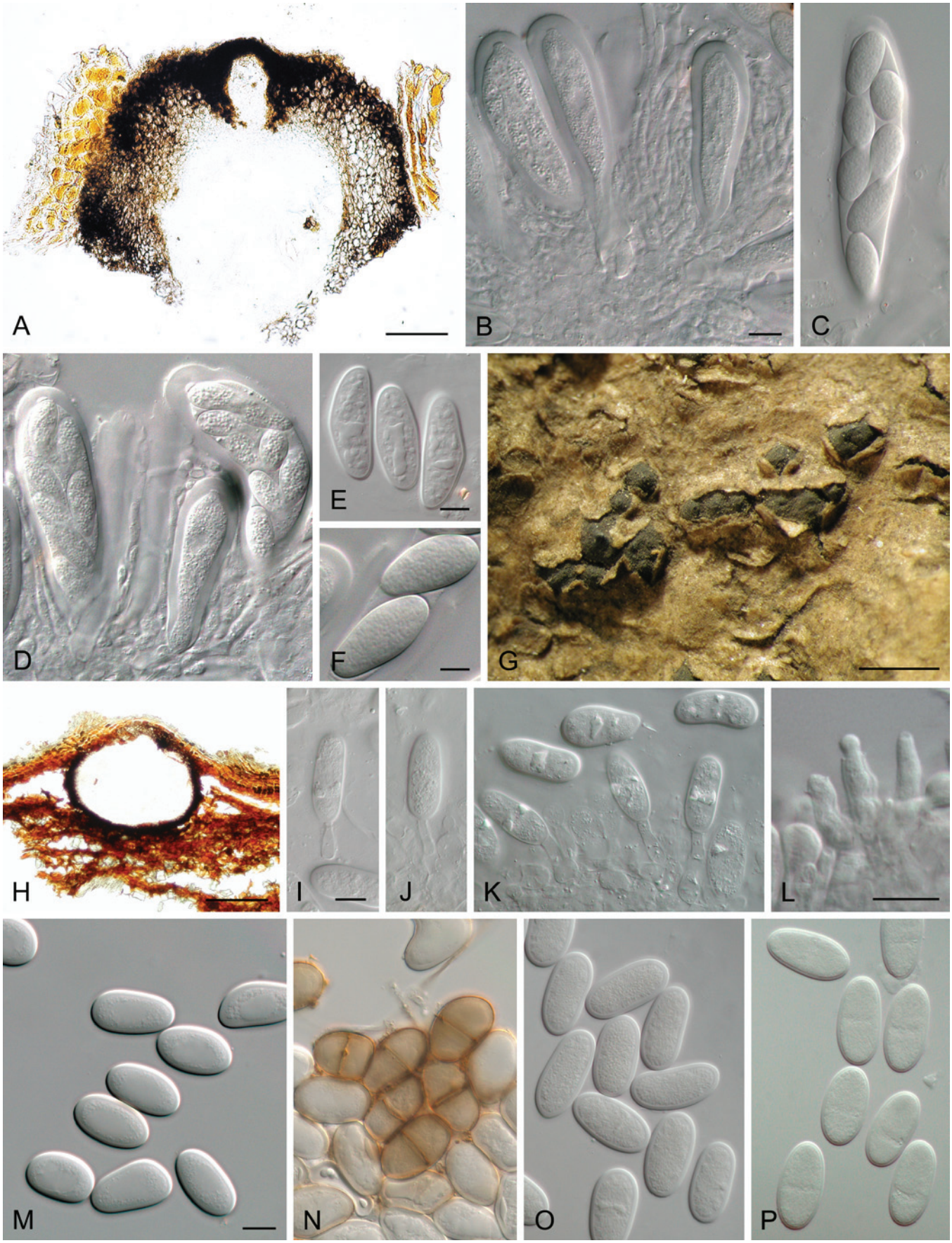

Fig. 24. *Diplodia mutila*. A. Sectioned ascoma. B. Immature asci and pseudoparaphyses. C, D. Asci with ascospores. E, F. Ascospores. G. Conidiomata partially erumpent through host. H. Sectioned conidioma. I–L. Conidiogenous cells. M–P. Conidia. M. Hyaline, aseptate conidia of CBS 112553. N. Pale brown, 1-septate conidia of CBS 112553. O. Hyaline, aseptate conidia of BPI 599153. P. Hyaline, aseptate conidia of K(M) 99664. Scale bars: A = 100 µm, B = 10 µm, E, F = 10 µm, G = 500 µm, H = 100 µm, I, L = 10 µm, M = 10 µm. Scale bar in B applies to C, D. Scale bar in I applies to J, K. Scale bar in M applies to N–P.

Conidiogenous cells holoblastic, discrete, cylindrical, hyaline, smooth, indeterminate, proliferating at the same level giving rise to periclinal thickenings, or proliferating percurrently to form one or two indistinct annellations, 11−15 × 4−5 µm. *Conidia* hyaline, aseptate, smooth, thick-walled, oblong to ovoid, straight, both ends broadly rounded, rarely becoming pale brown and septate when aged, (23.5−)24.5−27(−27.5) × (12.5−)13−14(−14.5) µm, 95 % confidence limits = 25.1–25.7 × 13.2–13.5 µm (av. ± S.D. of 50 conidia = 25.4 ± 1.0 × 13.4 ± 0.5 µm), L/W ratio = 1.9.

Type: of *Physalospora mutila* (designated by Alves *et al.* 2004): **UK**, England, Cornwall, Saltash, on bark of *Malus* sp., 22 Aug. 1935, N.E. Stevens, *lectotype* BPI 599153. Of *Diplodia mutila*: **France**, Ardenne, Sedan, on bark of *Populus nigra*, date unknown, *Montagne* sp., **isotype** K(M)99664.

Cultures: No ex-type, or authentic cultures of either state are known. CBS 112553 has been regarded, unofficially, as a standard isolate of *D. mutila* (Alves *et al.* 2004, Damm *et al.* 2008).

Hosts: While Farr *et al.* (2013) list 55 hosts for *D. mutila* it is now clear that many of the earlier reports of this fungus could be misidentifications (Alves *et al.* 2004, Alves *et al.* 2006, Lazzizera *et al.* 2008, Phillips *et al.* 2012). The following are confirmed hosts: *Chamaecyparis lawsoniana*, *Fraxinus*, *Malus*, *Populus*, *Taxus baccata*, *Vitis vinifera*.

Known distribution: England, France, Italy, Portugal, South Africa, USA (California).

Notes: The taxonomic history of *D. mutila* and the controversy surrounding the characters that define this fungus have been explained by Sutton (1980) and Alves *et al.* (2004). However, in the interests of presenting a comprehensive analysis, these explanations are repeated here.

Fries (1823) described *Sphaeria mutila* and distributed two exsiccati under that name as *Scler. Suec.* 164 and 385. Alves *et al.* (2004) examined material of these two exsiccati in STR and found both to be devoid of spores. Stevens (1933) and Sutton (1980) also reported that these two exsiccati in BPI and K had no spores. Sutton (1980) reported that 164 was an ascomycete of the *Botryosphaeria* type and pointed out that *Sphaeria mutila* should be adopted for the ascomycetous element it represents. Montagne sent Fries a fungus that was identified as *S. mutila*. The record was listed under *S. mutila* Fr. by Montagne (1834) with the note that this species would become the type of a new genus, *Diplodia,* later characterised by Fries (1849). Therefore, the name of the pycnidial fungus dates from Montagne (1834); it is typified by his material and the correct citation is *Diplodia mutila* Fr. in Montagne (1834).

Montagne distributed this fungus in his exsiccatus No. 498. According to Françoise Deluzarche of the Institut de Botanique, Strasbourg, France, no material of this could be found in STR (Alves *et al.* 2004). However, according to Alves *et al.* (2004), Montagne's specimen of *D. mutila* in Kew, K(M)99664 (isotype), agrees in all aspects with Stevens' (1933) account of Montagne's exs. 498 but differs from the description given by Sutton (1980). While Sutton (1980) referred to the conidia as initially hyaline with a large central guttule, later becoming dark brown and medianly one eusepate, Alves *et al.* (2004) reported that the vast majority of conidia in K(M)99664 are hyaline and aseptate, although pale brown and one- or two-septate conidia are seen rarely. The conidia usually have a large central guttule. Furthermore, the dimensions that Sutton (1980) reported (27–31 × 12–13.5 µm) are somewhat larger than Alves *et al.* (2004) found (23.5–27.5 × 12–14 µm). Stevens (1933) reported the conidia as (20–)25–27 × 10–12(–16) µm.

In the original description, Montagne (1834) described the conidia as "Asci [conidia] elliptico-oblongi, didymi, sporidiis binis referti." Stevens (1933) studied slides of Montagne's exsiccatus in STR and described the conidia as hyaline and aseptate with a thick smooth, glassy wall, although pale brown, 1-septate conidia sometimes were present. Both Shoemaker (1964) and Laundon (1973) agreed with Stevens' concept. Sutton (1980), however, described the conidia as hyaline at first but becoming dark brown and 1-septate when mature. In his illustration of this species he depicts a predominance of dark conidia. Alves *et al.* (2004) re-examined the isotype in K and concluded that the conidia are predominantly hyaline, although some are dark and 1-septate. The consensus was that conidia of *D. mutila* are (20–)25–27.5 × 10–12 µm (Stevens 1933, Shoemaker 1964, Laundon 1973, Sivanesan 1984), but Sutton (1980) considered they can be up to 31 µm long.

Stevens (1936) reported on the sexual morph of *D. mutila* that he found on apple and ash in England. The connection between this fungus and *D. mutila* was established through single ascospore isolations and Stevens applied the name *Physalospora mutila* (Fr.) N.E. Stevens. Shoemaker (1964) considered this to be a species of *Botryosphaeria* and applied the new name *B. stevensii* Shoemaker because the name *Botryosphaeria mutila* was already taken. Stevens (1936) referred to a specimen on cut sticks of *Fraxinus excelsior* as the type.

When Alves *et al.* (2004) examined the type specimen of *P. mutila* in BPI 599151 they could find no ascomycete. There was, however, ample material of the sexual morph on BPI 599153, which is a specimen of *P. mutila* on apple collected by Stevens from the same locality and at the same time that he collected BPI 599151. Since this specimen conformed in all ways with the protologue, Alves *et al.* (2004) designated this specimen as lectotype. Unfortunately, no ex-type cultures exist. The type host of *P. mutila* is *Fraxinus excelsior* whereas the type host of *Diplodia mutila* is a *Populus* sp.

Diplodia olivarum A.J.L. Phillips, Frisullo & Lazzizera, Fungal Divers. 31: 67. 2008. MycoBank MB511402. Fig. 25.

Ascomata not reported. *Conidiomata* pycnidial, stromatic, produced on pine needles on WA after 7–14 d, solitary, globose to ovoid, dark brown to black, up to 150 µm wide, wall composed of dark brown, thick-walled *textura angularis*, becoming thin-walled and hyaline towards the inner region, semi-immersed to erumpent, unilocular, with a short neck. *Ostiole* circular, central. *Conidiophores* hyaline, cylindrical, 10–15 × 3.5–5 µm. *Conidiogenous cells* hyaline, cylindrical, holoblastic forming a single conidium at the tip, proliferating internally to form periclinal thickenings or proliferating percurrently giving rise to 2–3 annellations, 8–12 × 3–6 µm. *Conidia* hyaline, aseptate, smooth, thick-walled, oblong to oval, widest in the middle, apex broadly rounded, base rounded or truncate, rarely becoming pale brown, internally verruculose, 1-septate after discharge from the pycnidia, (21.5–)22–27.5(–28.5) × (10–)11–13.5(–14.5) µm, 95 % confidence limits = 23.9–24.8 × 12.2–12.7 µm, av. ± S.D. = 24.4 ± 1.6 × 12.4 ± 1 µm), L/W = 2.0.

Type: **Italy**, Puglia, Lecce, Scorrano, Basco Belvedere, on rotting drupes of *Olea europaea*, Dec. 2004, S. Frisullo, **holotype** CBS H-19914.

Fig. 25. *Diplodia olivarum*. A–C. Conidia developing on conidiogenous cells. D. Hyaline, aseptate conidia. E. Dark pigmented, one-septate conidia. Scale bars = 10 µm.

Culture: CBS 121887 (ex-type).

Host: *Olea europaea* (Lazzizera *et al.* 2008).

Known distribution: Italy (Lazzizera *et al.* 2008), Spain (Gramaje *et al*. 2012).

Notes: This species is similar to *D. mutila* but the two can be distinguished based on minor differences in the dimensions of their conidia. Although the ranges of dimensions overlap considerably, mean dimensions of conidia of *D. olivarum* are smaller than *D. mutila*.

Diplodia pseudoseriata C.A. Pérez, Blanchette, Slippers & M.J. Wingf., Fungal Divers. 41: 63. 2010. MycoBank MB513545. Fig. 26.

Ascomata not reported. *Conidiomata* (formed in culture on sterilised pine needles) semi-immersed or superficial, solitary, globose, black, covered by mycelium, up to 430 µm diam. *Conidiogenous cells* cylindrical, discrete, producing a single conidium at the tip, with no evident annellations. *Conidia* initially hyaline becoming dark brown, wall externally smooth, roughened on the inner surface, sometimes 1-septate, ovoid, apex obtuse, base truncate, (23–)25.5–26.5(–30.5) × (10–)11.5–12(–14) µm.

Type: **Uruguay**, Paysandu, Guaviyu, isolated from asymptomatic twig of *Blepharocalyx salicifolius*, Aug. 2006, C. Pérez, **holotype** PREM 60264.

Culture: CBS 124906 (ex-type).

Hosts: *Acca sellowiana*, *Blepharocalyx salicifolius*, *Eugenia uniflora*, *Eugenia involucrata*, *Hexachlamis edulis*, *Myrceugenia euosma*, *Myrciaria tenella*, *Myrcianthes cisplatensis* (Pérez *et al.* 2010).

Known distribution: Uruguay (Pérez *et al.* 2010).

Notes: *Diplodia pseudoseriata* was described from native *Myrtaceae* trees in Uruguay (Pérez *et al*. 2010) while *D. alatafructa* was described from *Pterocarpus angolensis* in South Africa (Mehl *et al*. 2011). In the phylogeny constructed by Phillips *et al.* (2012) and in the present work, isolates of both of these species formed a cluster suggesting that they represent several phylogenetic species. Nevertheless, sequences of the ex-type isolates are divergent and indicate two separate species. Thus, it seems likely that either cultures or sequences of the other isolates of these two species have been mislabelled. Furthermore, the isolates in this cluster should be studied in detail to determine if they represent a complex of species.

Diplodia quercivora Linaldeddu & A.J.L. Phillips, Mycologia 105: 1269. 2013. MycoBank MB801757. Fig 27.

Ascomata not reported. *Conidiomata* pycnidial, stromatic, produced on poplar twigs on PDA within 14 d, superficial, dark brown to black, mostly uniloculate, solitary, globose, thick-

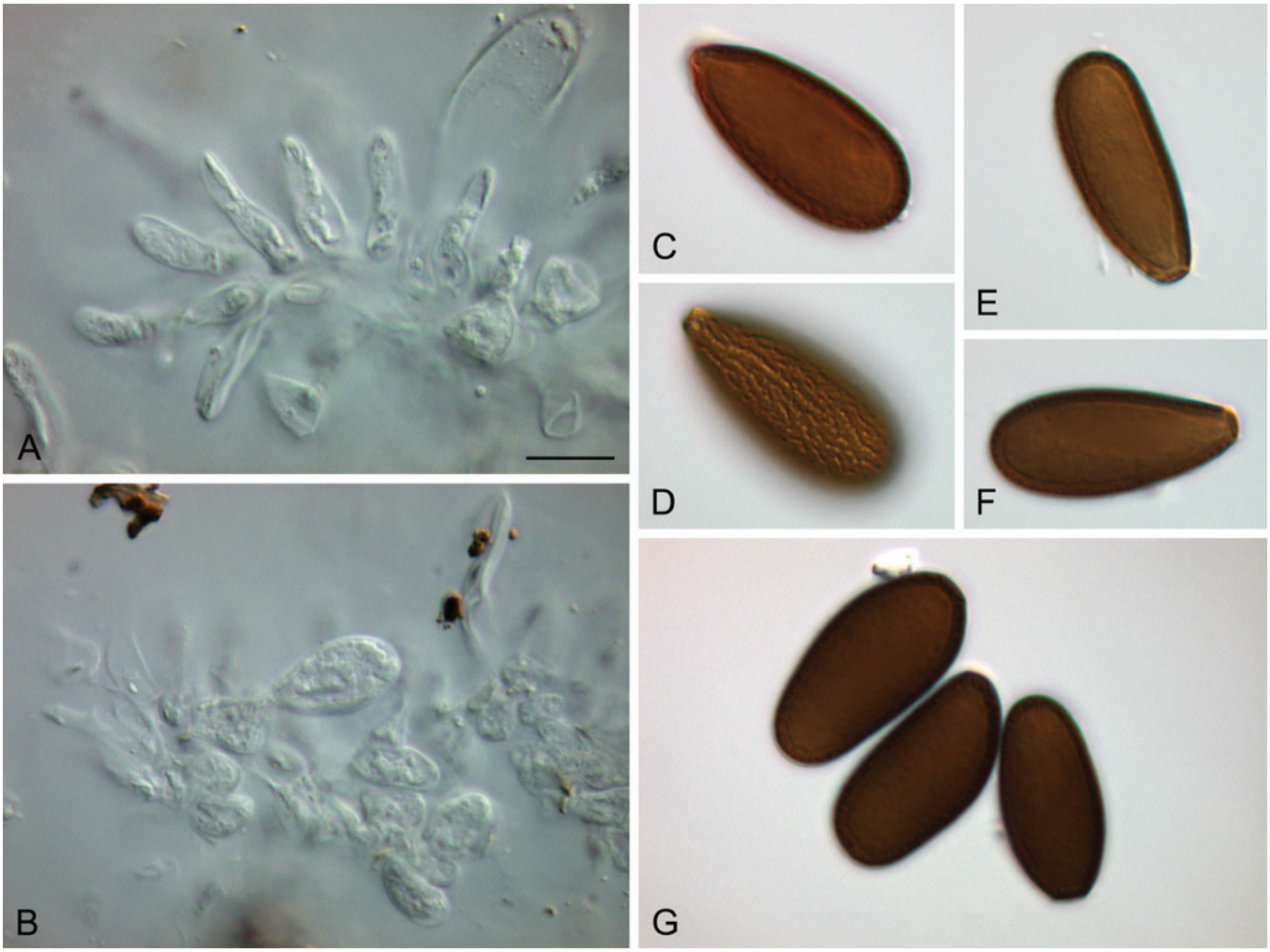

Fig. 26. *Diplodia pseudoseriata*. A, B. Conidiogenous cells. C–G. Conidia. The conidium in C, D is shown at two different focal planes revealing the ornamentation on the inner surface of the conidium wall. Scale bar A = 10 µm. Scale bar in A applies to B–G.

walled, non-papillate with a central ostiole. *Paraphyses* not seen. *Conidiogenous cells* hyaline, smooth, cylindrical, sometimes slightly swollen at the base, holoblastic forming conidia at their tips, proliferating internally giving rise to periclinal thickenings, 9.1–13.5 × 3.5–6 µm. *Conidia* hyaline, aseptate, smooth, thick-walled, subcylindrical to oblong-elliptical, widest at the middle, both ends broadly rounded, rarely becoming brown and 1-septate with age, (23–)28(–30.5) × (11.5–)14(–14.5) µm, 95 % confidence limits = 27.7–28.5 × 12.9–13.2 µm (av. ± S.D. of 50 conidia = 28.1 ± 1.4 × 13.8 ± 0.6 µm), L/W = 2.16.

Culture characteristics: Cardinal temperatures for growth: min < 5 ºC, max > 35 ºC and opt 20–25 ºC. All isolates failed to grow at 40 ºC, but mycelium resumed growth when plates were moved to 25 ºC.

Type: **Tunisia**, Tabarka, isolated from branch cankers of *Quercus canariensis*, 20 Sep. 2006, B.T. Linaldeddu, **holotype** LISE 96110 (a dried culture sporulating on holm oak twigs).

Culture: CBS 133852 (ex-type).

Host: *Quercus canariensis* (Linaldeddu *et al.* 2013).

Known distribution: North-west Tunisia (Linaldeddu *et al.* 2013).

Note: *Diplodia quercivora* is similar to *D. corticola* but the two species are readily distinguishable by conidial shape and size.

Diplodia rosulata Gure, Slippers & Stenlid, Mycol. Res. 109: 1010. 2005. MycoBank MB344348. Fig. 28.

Ascomata not reported. *Conidiomata* (formed on WA on sterilised pine needles and seeds after 45 d), pycnidial, stromatic, erumpent, solitary, globose with a central ostiole, papillate, wall composed of outer layers of thick-walled, dark brown *textura angularis*, becoming thin-walled and hyaline towards the inner layers. *Conidiogenous cells* holoblastic, hyaline, cylindrical, proliferating percurrently with indistinct annellations, 8–12 × 2–4 µm. *Conidia* oval to ellipsoid or ovoid, ends obtuse, initially hyaline, aseptate, granular contents, wall 1.5–2 µm thick and smooth, often turning light brown and 1-septate after discharge, (21–)25–32(–36) × (10–)11–17.5(–19.5) µm (av. size of 106 conidia = 28 × 14.5 µm), L/W ratio = 1.93.

Culture characteristics: *Colonies* initially beige to whitish (upper surface), becoming greenish grey from above, bluish-grey with whitish centre from below, cultures partially translucent after 2 wk, becoming opaque after 3 wk. Colony margin forming a concentric ring after 3–4 d with smooth margins, followed by additional rings forming as small sectors along the circumference of the colony, creating a lobed rosette appearance after 4–5 d. Mycelium dense,

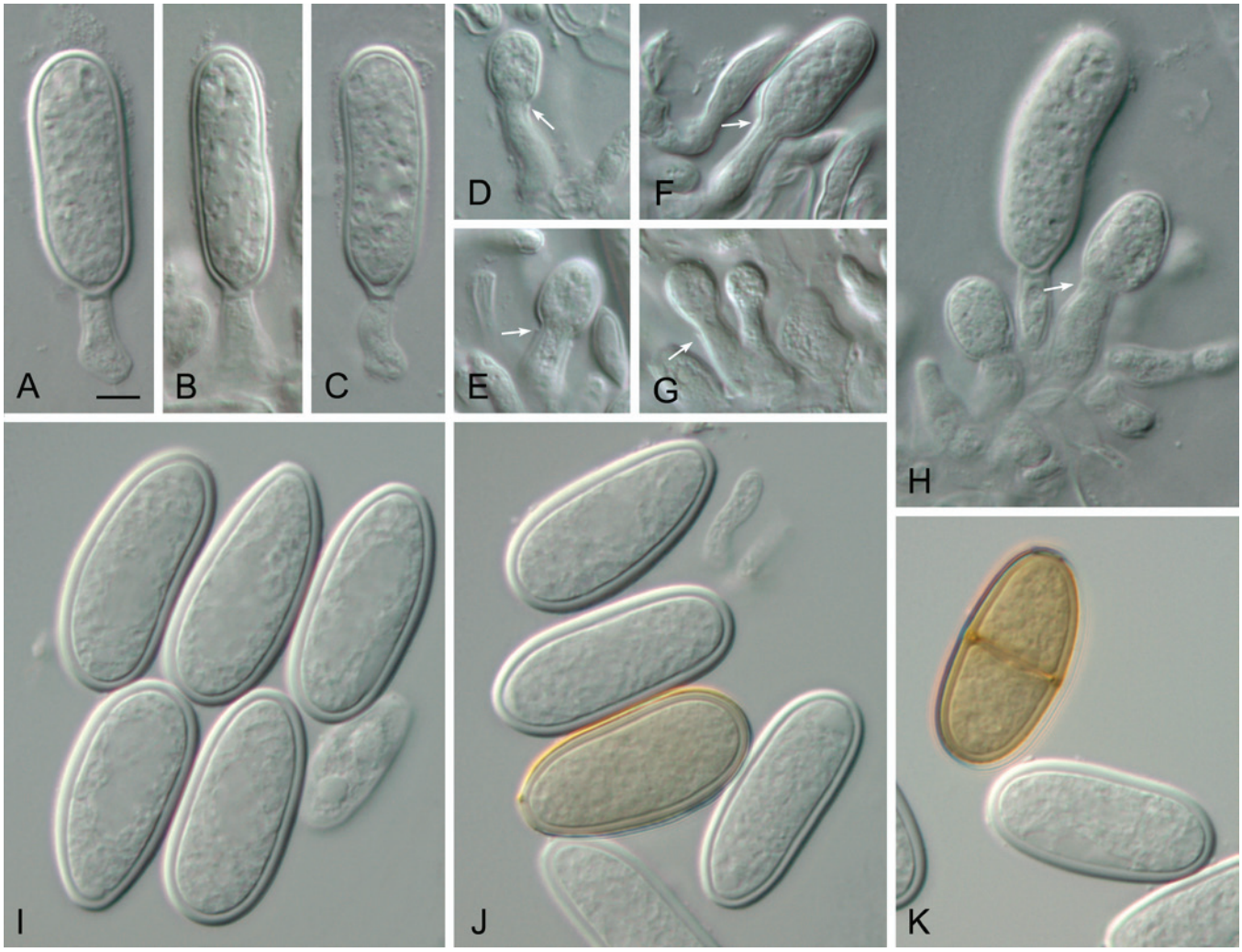

Fig. 27. *Diplodia quercivora*. A–H. Conidiogenous cells with developing conidia, arrows in D–H indicate periclinal thickenings. I. Hyaline, aseptate conidia. J. Hyaline, aseptate conidia and one pale brown conidium. K. Hyaline, aseptate conidium and one pale brown, one-septate conidium. Scale bar A = 5 µm. Scale bar in A applies to B–K.

forming an appressed mat, average growth rate approximately 7 and 8.5 mm filling the 9 cm Petri dishes within 12 and 10 d at 20 °C and 25 °C, respectively.

Type: **Ethiopia**, Southeastern Oromia, Gambo, Munessa-Shashamane Forest Enterprise, from seeds of *Prunus africana*, 20 Jul. 2001, A. Gure, **holotype** CBS H-12357.

Culture: CBS 116470 (ex-type).

Host: *Prunus africana* (Gure *et al.* 2005).

Known distribution: Ethiopia (Gure *et al.* 2005).

Notes: *Diplodia rosulata* has a distinct rosulate colony morphology, which separates it from all other *Diplodia* spp. including the closely related *D. africana* and *D. olivarum*. Iranian isolates of *D. bulgarica* have also rosulate colonies, but the conidia of *D. rosulata* (28 × 14.5 µm, L/W = 1.93) are longer and narrower than those of *D. bulgarica* (25.4 × 16.8 µm, L/W = 1.5).

Diplodia sapinea (Fr.) Fuckel, J. nassau. Ver. Naturk. 23–24: 393. 1870. MycoBank MB146913. Fig. 29.
Basionym: *Sphaeria sapinea* Fr., Syst. Mycol. 2: 491. 1823.

Synonyms see Sutton & Dyko (1989).

Ascomata not reported. *Conidiomata* pycnidial, stromatic, globose, immersed, sometimes appearing superficial, separate or aggregated, dark brown to black, unilocular, 0.3–0.5 mm diam, wall 6–8 layers, 30–60 µm thick, outer wall of dark brown thick-walled *textura angularis*, cells darker around the the ostiole. *Ostiole* central, circular single. *Conidiophores* absent. *Conidiogenous cells* lageniform to cylindrical, occasionally proliferating percurrently, discrete, indeterminate, hyaline, smooth, arising from the inner wall of the locule, 8.5–15 × 4–7.5 µm. *Conidia* oblong to clavate, straight to slightly curved, at first aseptate, sometimes much later becoming 1-euseptate, walls 0.5–1 µm thick, outer surface of wall smooth, or appearing pitted, apex obtuse, base truncate, (25.5–)30.5–52.5(–54) × (10–)12.5–20(–21) µm (av. ± S.D. of 200 conidia = 40.8 ± 4.9 × 15.5 ± 2.1 µm).

Type: **Sweden**, Suecia Smaland, Femsjo, on *Pinus* sp., E. Fries, *Scleromyceti Sueciae Exsiccati* No 126, *Sphaeria sapinea* Fries, **lectotype**: B, **isotypes**: G, K, E, UPS, C, BR, FH. **The Netherlands**, Gelderland, Schovenhorst, Putten, Pinetum, on cones of *Pinus nigra*, June 1984, H.A. van der Aa. **epitype designated here** CBS H-18340; MBT176178, culture ex-epitype CBS 393.84.

Cultures: CBS 393.84 (ex-epitype), CBS 109725.

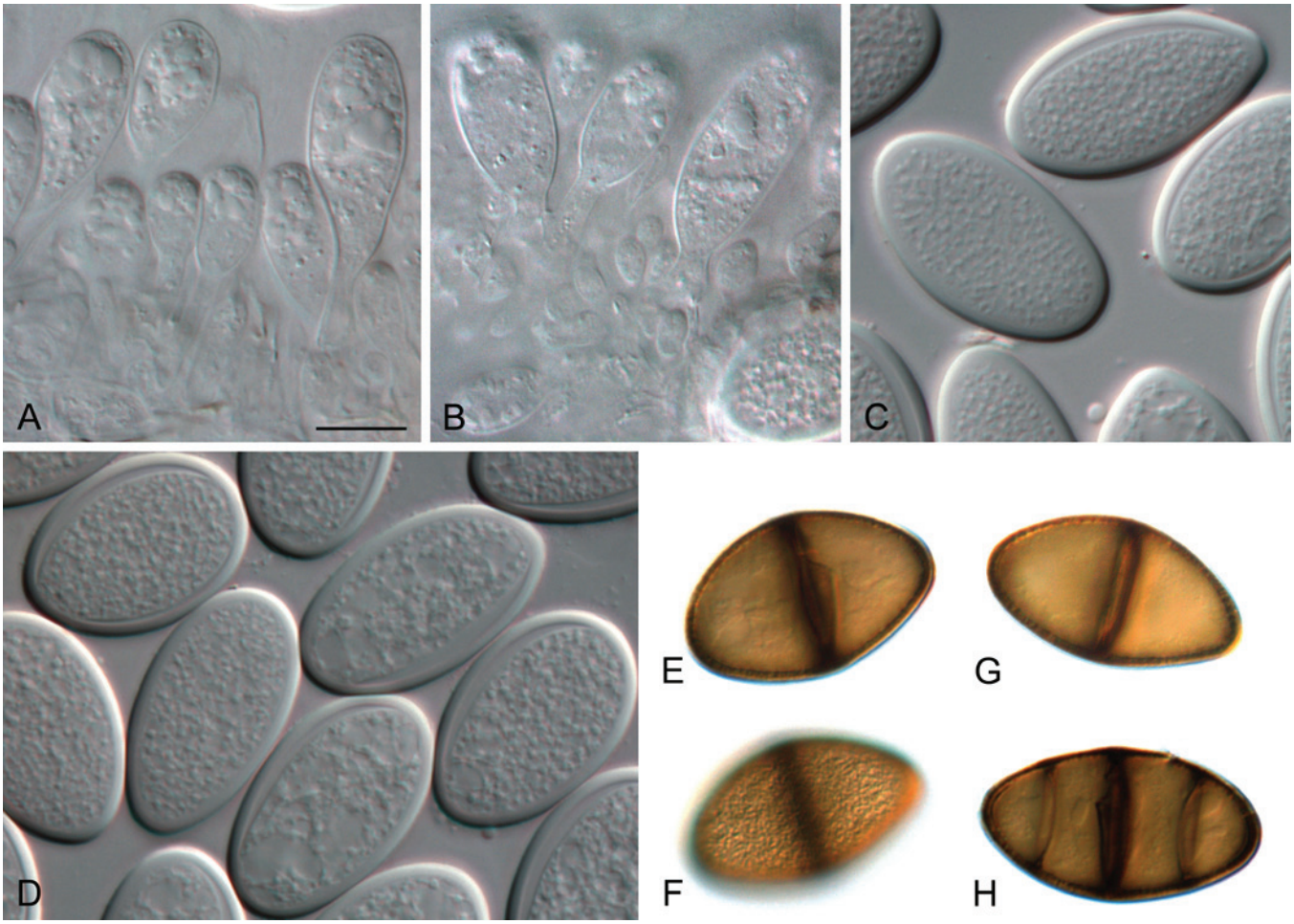

Fig. 28. *Diplodia rosulata*. A, B. Conidiogenous cells. C, D. Hyaline, aseptate conidia. E–H. Brown, one-septate conidia. Scale bar A = 10 µm. Scale bar in A applies to B–H.

Hosts: Host range includes *Abies*, *Larix*, *Picea*, *Thuja*, *Pseudotsuga*, and 33 species of *Pinus* (Palmer *et al.* 1987).

Known distribution: Worldwide wherever pines are grown (Palmer *et al.* 1987).

Notes: The history of this species has been explained by Sutton & Dyko (1989). Briefly, the pine pathogen was known for many years as *Diplodia pinea* (Desm.) Kickx. and later as *Sphaeropsis sapinea* (Fr.) Dyko & Sutton. According to Sutton & Dyko (1989) *S. sapinea* is based on *Sphaeria sapinea* Fr. and they proposed the specimen of Fries exsiccata in B as lectotype. Sutton & Dyko (1989) give extensive synonymies for *S. sapinea* including *Diplodia pinea*. We examined *Sphaeria pinea* Desm. (Desmaziéres No 1277 in PC) and found that the conidia are smaller (25–32 × 12–15 µm) than those reported by Sutton & Dyko (1989) for the type of *S. sapinea* and thus they represent two distinct species. Furthermore, average conidial dimensions of the common pine pathogen fall within the range of 33–39 × 11.5–13 µm (Palmer *et al.* 1987, Swart *et al.* 1991), thus corresponding to *S. sapinea*. Therefore we consider that the correct name to apply to the common pine pathogen is *Diplodia sapinea* based on *Sphaeria sapinea*.

Differences in colony appearance and growth rate were reported for isolates of *D. sapinea* from the north central United States and these two colony types were referred to as morphotypes A and B (Palmer *et al.* 1987). Isolates of the A morphotype were described as producing fluffy, white to grey-green mycelium and faster growth on PDA than isolates of the B morphotypes which produced white to black mycelium closely appressed to the agar (Palmer *et al.* 1987). Other differences between the two morphotypes have been suggested including differences in radial growth, conidial dimensions and conidial septation (Palmer *et al.* 1987), and texture of the conidium wall (Wang *et al.*1985). However, each of these differences has been shown to vary substantially within each group, or to be similar for each group. Nevertheless, according to Palmer *et al.* (1987) conidia of type A isolates are larger than conidia of type B isolates. They also considered that although conidia of both morphotypes were mostly aseptate, when septa were present the type B isolates had up to three septa while the type A isolates only ever formed a single septum.

De Wet *et al*. (2002) used RAPD markers and morphological characters to distinguish a third morphotype, which they referred to as the C morphotype. The C morphotypes had considerably larger conidia than the A morphotypes (De Wet *et al*. 2002) and were significantly more virulent than the A and B morphotypes (De Wet *et al*. 2002). Also see the notes for *D. scrobiculata* below.

Diplodia scrobiculata J. de Wet, Slippers & M.J. Wingf., Mycol. Res. 107: 562. 2003. MycoBank MB372427. Fig. 30.

Ascomata not reported. *Conidiomata* pycnidial, stromatic, covered in mycelium, dark, immersed in pine needles or in the agar, single, papillate ostiole, (100–)150(–250) µm diam. *Conidiogenous cells*

Fig. 29. *Diplodia sapinea*. A. Annellate conidiogenous cells. B–E. Conidia developing on conidiogenous cells. F–I. Conidia, the ones in H have up to 2 septa. J, K. Conidium in two different focal planes to show verruculose inner side of the wall. Scale bars = 10 µm. Scale bar in A applies to B. Scale bar in C applies to D–K.

Fig. 30. *Diplodia scrobiculata*. A, B. Conidiogenous layer with developing conidia. C. Conidia. Scale bar = 10 µm. Scale bar in A applies to B and C.

discrete, dark, smooth, 10 mm in diameter, holoblastic with limited percurrent proliferation forming a small number of annellations. *Conidia* clavate to truncate, dark mouse grey, (37.5–)39.5(–41.5) × (13–)14(–15.5) µm, 1–3 septa, thick, pitted walls (Wang *et al.* 1985).

Culture characteristics: *Colonies* pale mouse-grey to mouse-grey viewed from the top of the Petri dish, dark mouse-grey to fuscous black viewed from the bottom of the Petri dish, colonies with sinuate edges. Optimal growth at 25 °C, covering the medium surface (9 cm Petri dishes) in 8 d. Mycelium dark, septate, appressed to the agar surface.

Type: **USA**, Wisconsin, Jackson County, *Pinus banksiana*, 1987, M.A. Palmer, **holotype** PREM 57461.

Cultures: CMW 189 = CBS 118110 (ex-type). Other authentic culture CBS 117836.

Hosts: *Pinus banksiana*, *P. resinosa*, and *P. greggii* (De Wet *et al*. 2003).

Known distribution: Europe (France, Italy), Mexico and, USA (California, Minnesota, Wisconsin) (De Wet *et al*. 2003).

Notes: The differences in morphology and behaviour of the various morphotypes of *D. sapinea* were considered insufficient to justify separation into distinct species. However, De Wet *et al.* (2003) showed that differences in partial sequences of six protein coding genes and six microsatellite markers were consistent between the A and B morphotypes and they considered this to be sufficient evidence to consider them as two distinct species. On this basis they described the B morphotypes as *Diplodia scrobiculata*, while the A and C morphotypes were regarded as *Diplodia pinea*, now treated as *D. sapinea*.

Diplodia seriata De Not., Micr. Ital. Dec. 4: 6. 1942. MycoBank MB180468. Fig. 31.

= *Sphaeria obtusa* Schwein., Trans. Amer. Phil. Soc. II, 4: 220. 1832.
≡ *Physalospora obtusa* (Schwein.) Cooke, Grevillea 20: 86. 1892.
≡ *Botryosphaeria obtusa* (Schwein.) Shoemaker, Canad. J. Bot. 42: 1298. 1964.
= *Diplodia pseudodiplodia* Fuckel, Jb. Nassau. Ver. Naturk. 23–24: 393. 1870.
= *Physalospora cydoniae* G. Arnaud, Annals d'École National d'Agric. de Montpellier, Série 2, 12(1): 9. 1911.
= *Physalospora malorum* Shear, N.E. Stevens & Wilcox, J. Agric. Res. 28: 596. 1924.
= *Diplodia profusa* De Not., Micr. Ital. Dec. 4: No 8. 1842.

Ascomata stromatic, immersed, solitary to botryose up to 3 mm wide. *Asci* bitunicate, fissitunicate, clavate, 90–120 × 17µm. *Pseudoparaphyses* hyaline, branched, septate, 2−3 µm wide. *Asci* clavate, stipitate, bitunicate, containing eight, biseriate ascospores, 95−100 × 15−20 µm (including stipe). *Ascospores* irregularly biseriate in the ascus, broadly fusoid, widest in the middle, smooth, hyaline, aseptate, 25–33 × 7–12 µm. *Conidiomata* stromatic, separate or aggregated and confluent, immersed in the host, partially emergent at maturity, dark brown to black, ostiolate, non-papillate, thick-walled, outer layers composed of dark brown *textura angularis*, inner layers of thin-walled hyaline *textura angularis*. *Conidiogenous cells* hyaline, thin-walled, smooth, cylindrical, swollen at the base, discrete, producing a single conidium at the tip, indeterminate, proliferating internally giving rise to periclinal thickenings or proliferating percurrently forming 2–3 annellations, 3–5.5 × 7–10(–15) µm. *Conidia* initially hyaline, becoming dark brown, moderately thick-walled (ca. 0.5 µm thick), wall externally smooth, roughened on the inner surface, aseptate, ovoid, widest in the middle, apex obtuse, base truncate or rounded, (21.5–)22–27(–28) × (11–)11.5–14.5(–15.5) µm, 95 % confidence limits = 24.3–25.4 × 12-6–13.2 µm (av. ± S.D. of 50 conidia = 24.9 ± 1.9 × 12.9 ± 1.1 µm), L/W = 1.9.

Type: **Italy**, on dead stems of *Jasminium* sp., 18 Aug. 1837, De Notaris, **holotype** HERB RO. **Portugal**, Montemor-o-Novo, on dead stems of *Vitis vinifera*, 31 Jul. 1997, A.J.L. Phillips, **epitype** CBS H-19809.

Culture: CBS 112555 (ex-epitype).

Hosts: Apparently plurivorous.

Known distribution: Apparently worldwide.

Notes: The connection between the sexual and asexual morph was established by Hesler (1916) and confirmed by Shear, Stevens and Wilcox (1925) and Stevens (1936). When Shoemaker transferred this name to *Botryosphaeria* he decided not to apply a name to the asexual morph and for many years it was referred to as *B. obtusa*. After Crous *et al.* (2005) transferred this species to *Diplodia*, no valid name was available.

A great deal of controversy has surrounded the correct name for this fungus. Peck (1881) found what he considered to be the conidial state of this species in New York, and reported it as *Sphaeropsis malorum* (Berk.) Berk. According to Stevens (1933), *S. malorum* (Berk.) Berk. is a synonym of *Diplodia mutila* Fr., which has hyaline conidia. Stevens (1933) studied Peck's collection and confirmed that the conidia are dark and aseptate.

This fungus has also been referred to as *S. malorum* Peck. This name came about when Saccardo (1884) transferred *S. malorum* (Berk.) Berk. to the genus *Phoma* on account of its hyaline conidia. Because Peck's collection had brown conidia, Saccardo considered it not the same as Berkley's collection, and used the name *S. malorum* Peck. Thus, Peck did not name a new species and even if he had proposed the name *S. malorum* in 1880, it would be an illegitimate later homonym of *S. malorum* (Berk.) Berk. (1860). Since *S. malorum* Peck is illegitimate and *S. malorum* (Berk.) Berk. is a synonym of *D. mutila,* neither of these names can be used for this species.

Slippers *et al.* (2007) initially regarded *Diplodia malorum* Fuckel to be a more appropriate name for this fungus. However, after studying the type specimen in G (Fungi rhenani 1706) they rejected this possibility. Therefore, *D. malorum* is not the asexual morph of "*Botryosphaeria*" *obtusa*. Finally, through a study of type specimens Phillips *et al.* (2007) determined that *D. seriata* was the oldest name available for the asexual morph of what had been referred to as"*B.*" *obtusa*.

Diplodia tsugae (A. Funk) A.J.L. Phillips & A. Alves, Persoonia 29: 35. 2012. MycoBank MB801409. See Funk (1964) for illustrations.

Basionym: *Botryosphaeria tsugae* A. Funk, Canad. J. Bot. 42: 770. 1964.

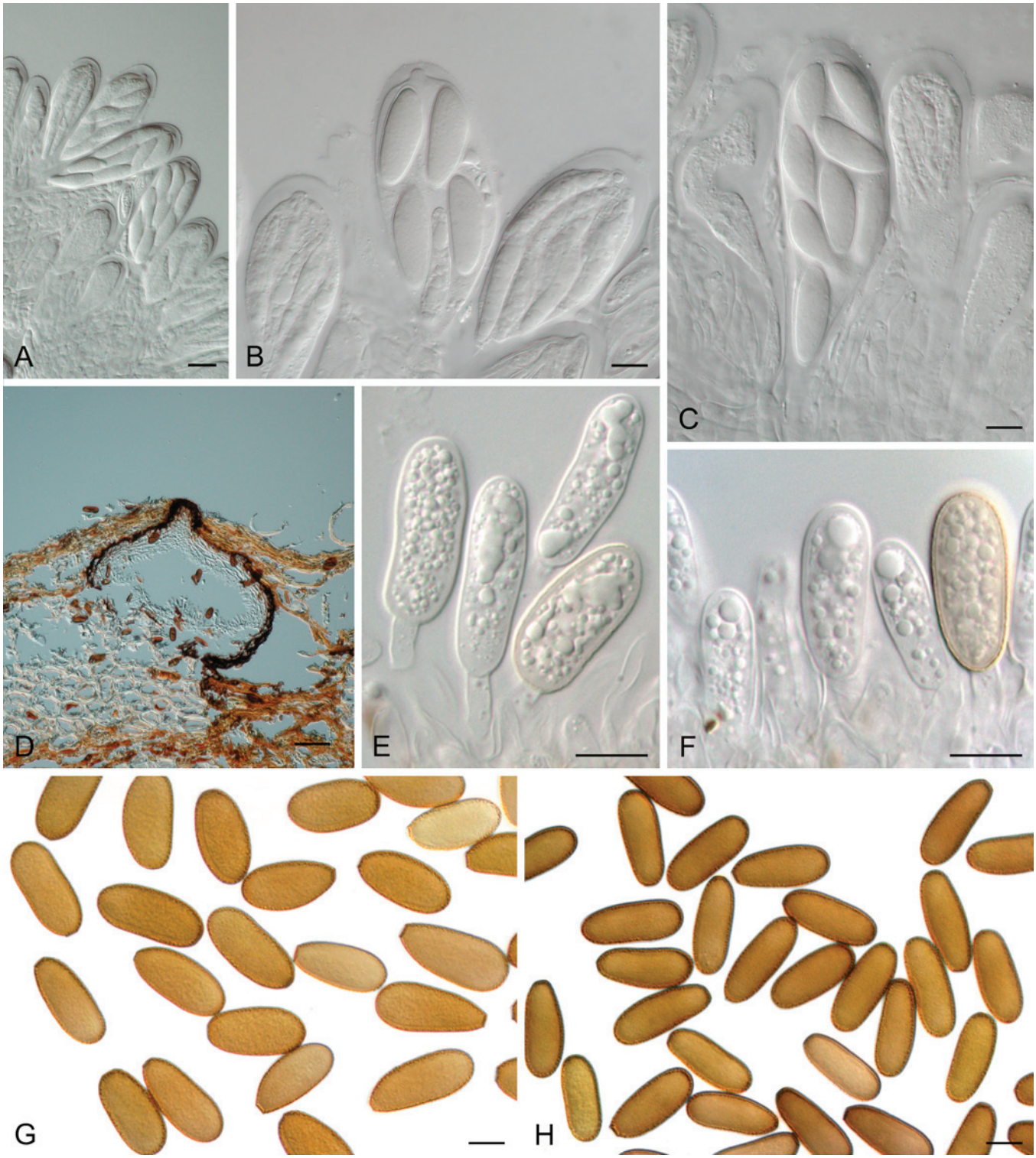

Fig. 31. *Diplodia seriata*. A–C. Asci with ascospores. D. Sectioned conidioma. E, F. Conidia developing on conidiogenous cells, one conidium in F is starting to become coloured. G, H. Brown, aseptate conidia. Scale bars: A = 20 µm, B, C = 10 µm, D = 50 µm, E–H = 10 µm.

Ascomata pseudothecial, black, globose or subglobose, immersed, uniloculate, with a short apical beak which becomes ostiolate and breaks through the periderm, 360−540 µm diam, wall pseudoparenchymatous, large-celled, 60−70 µm thick. *Asci* clavate, short-stalked, bitunicate, formed between pseudoparaphyses, 140−180 × 30−36 µm. *Ascospores* ellipsoid to fusoid-ellipsoid, sometimes inequilateral, one-celled, hyaline, 42−47 × 13−18 µm. *Conidiomata* pycnidial, stromatic, black, immersed, globose or subglobose, uniloculate, with a short papilla which breaks through the periderm, 400−540 µm diam, wall pseudoparenchymatous, 35−45 µm thick. *Conidiophores* simple, bearing a single conidium at the tip. *Conidia* oblong to ovoid, one-celled, hyaline, 36−41 × 18−22 µm.

Type: **Canada**, British Columbia, near Coola (Snootli Creek), on branches of *Tsuga heterophylla*, 11 Sep. 1963, A. Funk, **holotype** DAVFP 15485. Lake Cowichan, 1 Nov. 1962, A. Funk, **isotype** CBS H-6790.

Culture: CBS 418.64 = IMI 197143 (ex-isotype).

Host: *Tsuga heterophylla* (Funk 1964).

Known distribution: Canada (British Columbia) (Funk 1964).

Notes: When Funk (1964) introduced *B. tsugae* he did not name the asexual morph, but referred to it as a species of *Macrophoma*. However, morphologically and phylogenetically it is undoubtedly a species in *Diplodia* and for this reason Phillips *et al.* (2012) transferred it to *Diplodia* as *D. tsugae*.

Dothiorella Sacc., Michelia 2: 5. 1880. MycoBank MB8098.
Type species: *Dothiorella pyrenophora* Sacc., Michelia 2: 5. 1880.

Ascomata immersed becoming erumpent, finally appearing superficial, usually aggregated, often in rows or in small rounded groups, at times connected at sides, globose, sphaeroid or ovoid, medium sized, rarely small; apex rounded, with short or well developed papilla, often opening widely by rounded ostiole, lined with hyaline cells; surface smooth or roughened with protruding cells or bearing short to elongate hyphal appendages; peridium wide, composed externally of rows of large, brown-walled, pseudoparenchymatous cells, often blackened over surface, internally of more compressed rows of pallid cells, at times wedge-shaped groups of cells extending from lower sides, or basal portion of peridium thickened and hypostromatic, hyphae dark brown, coarse, forming slight or well-developed subiculum beneath and connecting ascomata. *Asci* bitunicate, basal, clavate or oblong, endotunica thickened. *Pseudoparaphyses* cellular, usually wide. *Ascospores* dull brown or dark reddish brown, ellipsoid, fusoid, obovoid, ends obtuse or somewhat acute, straight, inequilateral or slightly curved, one- to two-septate, infrequently one-celled, not or slightly constricted at septum; contents minutely granular; wall thick, smooth or verruculose at times; overlapping biseriate in the ascus. *Conidiomata* stromatic, ostiolate, individual or in loose clusters of up to 10 conidiomata, immersed, breaking through the bark when mature. *Ostiole* circular, central, non-papillate or papillate. *Paraphyses* absent. *Conidiophores* absent. *Conidiogenous cells* holoblastic, hyaline, smooth-walled, cylindrical and slightly swollen at the base, determinate or indeterminate and proliferating at the same level to form periclinal thickenings, rarely proliferating percurrently to produce two or three indistinct annellations, borne directly on the cells lining the pycnidial cavity. *Conidia* initially hyaline, becoming dark brown and one-euseptate within the pycnidial cavity often while still attached to the conidiogenous cell, ellipsoid to ovoid, thick-walled, externally smooth or striate, internally verruculose.

Notes: The genus *Dothiorella* has been the source of much confusion in the past and the name has been used in more than one sense. *Dothiorella* has been used for asexual morphs with hyaline, aseptate conidia similar to those normally associated with *Fusicoccum* and *Neofusicoccum*. Presumably this confusion started when Petrak (1922) transferred *F. aesculi* to *Dothiorella*, citing the species as the conidial state of *B. berengeriana* (Sutton 1980). In later years, *Dothiorella* was used for fusicoccum-like asexual morphs with multiloculate conidiomata (Grossenbacher & Duggar 1911, Barr 1987, Rayachhetry *et al.* 1996). Sivanesan (1984) confused matters further by reducing *Dothiorella pyrenophora* to synonymy with *Dothichiza sorbi*, which has small, hyaline, aseptate conidia and is the asexual morph of *Dothiora pyrenophora* (Fr.) Fr. However, he was referring to *Dothiorella pyrenophora* Sacc. (1884), which is a later homonym of *Dothiorella pyrenophora* Sacc. 1880 (Sutton 1977). The taxonomic history of *Dothiorella* has been explained by Sutton (1977) and Crous & Palm (1999), and is illustrated by Crous & Palm (1999).

Dothiorella was reduced to synonymy under *Diplodia* by Crous & Palm (1999), who used a broad morphological concept for *Diplodia*. Phillips *et al.* (2005) re-examined the type of *Dothiorella pyrenophora* Sacc. (K 54912) and found that it differed from *Diplodia* by having conidia that are brown and 1-septate early in their development, while they are still attached to the conidiogenous cells. In *Diplodia* conidial darkening and septation takes place after discharge from the conidiomata. Crous *et al.* (2006) re-examined the types of both *Diplodia* and *Dothiorella* and confirmed these morphological differences.

Sexual morphs of *Dothiorella* have pigmented, septate ascospores. Phillips *et al.* (2005) and Luque *et al.* (2005) broadened the concept of *Botryosphaeria* to include species with brown, 1-septate ascospores. Their reasons for doing this were based on the fact that ITS phylogenies placed *D. sarmentorum* and *D. iberica* within the boundaries of *Botryosphaeria* as it was circumscribed at that time. In a phylogeny based on partial sequences of the LSU gene Crous *et al.* (2006) revealed that *Botryosphaeria sensu lato* is composed of a number of distinct lineages that represent different genera. They suggested that the species with dark brown, 1-septate ascospores should be accommodated in *Dothidotthia*. Phillips *et al.* (2008) showed that *Dothidotthia symphoricarpa* (the type species of *Dothidotthia*) belongs in a distinct family within the *Pleosporales* while *D. sarmentorum*, *D. iberica* and *D. viticola* fall within two separate genera in the *Botryosphaeriaceae* and a new genus, *Spencermartinsia* was introduced to accommodate *D. viticola*.

More than 350 species names exist in *Dothiorella*, but presently cultures are available for only 17 species in fungal collections. Of these, ten species are known in *Dothiorella*, two species introduced in *Spencermatinsia* should be transferred to *Dothiorella*, *Auerswaldia dothiorella* is re-combined here as *D. thailandica* and the other four species remain unnamed. All of these, except *D. sarmentorum*, have been introduced since 2005. Considering the earlier problems surrounding the circumscription of this genus especially the confusion with *Diplodia*, it is likely that many more species will be found. The sexual stage of the species is rarely encountered in nature and under experimental conditions and no ascomata have been observed for any of the species, except for *D. sarmentorum* and *D. iberica*. Therefore, differentiation of species has mostly been done based on asexual morphs and cultural characteristics.

Key to *Dothiorella* species

1. Conidiomata papillate 2
1. Conidiomata non-papillate 6

2. Conidiomata with long necks (up to 1.5 mm) ..... *D. longicollis*
2. Conidiomata with short necks (less than 0.5 mm) 3

3. Conidia length not exceeding 22 µm (16–22 × 7–10 µm) ..... *D. dulcispinae*
3. Conidial length exceeding 22 µm (up to 33 µm) 4

4. Conidial width less than 12 µm (conidia fed by thrips) ..... *D. thripsita*
4. Conidial length greater than 12 µm (up to 14 µm) 5

5. Colony growth rate on MEA in the dark at 25 ºC > 20 mm/d ..... *D. pretoriensis*
5. Colony growth rate on MEA in the dark at 25 ºC < 20 mm/d ..... *D. brevicollis*

6. Conidial length less than 16 µm (av. length 15 µm) ..... *D. americana*
6. Conidial length 16 µm or more (av. length > 18 µm) 7

7. Average width of conidia greater than 10 µm 8
7. Average width of conidia less than 10 µm 9

8. Conidia 23–31 × 9–11 µm (av. 27.1 × 10.8 µm) ..... *D. casuarini*
8. Conidia 23–23.4 × 10.8–11 µm (av. 23.2 × 10.9 µm) ..... *D. iberica*

9. Average length of conidia greater than 20 µm 10
9. Average length of conidia less than 20 µm 11

10. Conidia 21.4–21.9 × 9.7–9.9 µm (L/W ratio 2.2) ..... *D. sarmentorum*[1]
10. Conidia 22–22.5 × 9–9.5 µm (L/W ratio 2.4) ..... *D. uruguayensis*[1]

11. Conidia with slight undulating striations on the surface ..... *D. thailandica*
11. Conidia smooth 12

12. Conidial L/W ratio 2 ..... *D. santali*
12. Conidial L/W ratio 2.4 ..... *D. moneti*

[1]It is difficult to distinguish these two species in terms of morphology but phylogenetically they are distinct.

DNA phylogeny

Phylogenetic analyses revealed two main clades representing the two distinct genera *Dothiorella* and *Spencermartinsia*. These two genera cannot be separated based on ITS sequence data and it is necessary to combine the ITS with EF1-α or other protein coding genes. The phylogeny based on ITS and EF1-α sequence data revealed 16 subclades representing 16 distinct species in *Dothiorella*. Most of these sub-clades received high bootstrap support (BS) in the MP analysis. But, these values are quite low for some internal nodes that can be improved with more sampling and gene loci (Fig. 32). It is important to note that all of the known species of *Dothiorella* in culture and studied here can be separated based solely on ITS, although bootstrap support values for some of the internal nodes are quite low (Fig. 33). Based on multi-gene phylogenies, *Auerswaldia dothiorella*, a species recently described by Liu *et al*. (2013) was found to reside in *Dothiorella* closely related to *D. dulcispinae* and a new name is introduced here. *Spencermartinsia pretoriensis* and *S. uruguayensis*, two recently described species were also found to reside in *Dothiorella*, and are treated below.

Species descriptions

Dothiorella americana Úrbez-Torres, Peduto & Gubler, Fungal Divers. 52: 184. 2011. MycoBank MB519956. See Úrbez-Torres *et al.* (2011) for illustrations.

Ascomata not reported. *Conidiomata* pycnidial, stromatic, produced on PDA within 2 wk, solitary, globose, black, covered with moderate mycelium, up to 650 µm wide, thick-walled, unilocular, with a central ostiole. *Conidiophores* absent. *Conidiogenous cells* holoblastic, hyaline, cylindrical to subcylindrical 7–16 × 4–6 µm. *Conidia* initially hyaline, unicellular, becoming light brown to dark brown and 1-septate while still attached to the conidiogenous cells, light to dark brown, thin-walled, oval to ovoid, round apex and truncate base, (13.5–)14–156(–17) × (5–)5.5–6.5(–8) µm (av. of 60 conidia = 15 × 6.1), L/W ratio = 2.4.

Culture characteristics: *Colonies* on PDA suppressed, initially olivaceous buff in the centre of the colony and white at the edge, becoming olivaceous within 7 d, turning dark green within 28 d on the surface, violaceous grey at the reverse after 28 d. Colonies reaching 90 mm diam on PDA after 5 d in the dark at 25 °C. Cardinal temperatures for growth: min 10 °C, max 35 °C, opt 20–25 °C.

Type: **USA**, Missouri, Purdy, on diseased interspecific grape cultivar Vignoles (Ravat51), R.K. Striegler & G.M. Leavitt, **holotype** UCD2252MO.

Cultures: CBS 128309 (ex-type), CBS 128310.

Hosts: *Vitis* spp. (Úrbez-Torres *et al*. 2011).

Known distribution: USA (Missouri) (Úrbez-Torres *et al*. 2011).

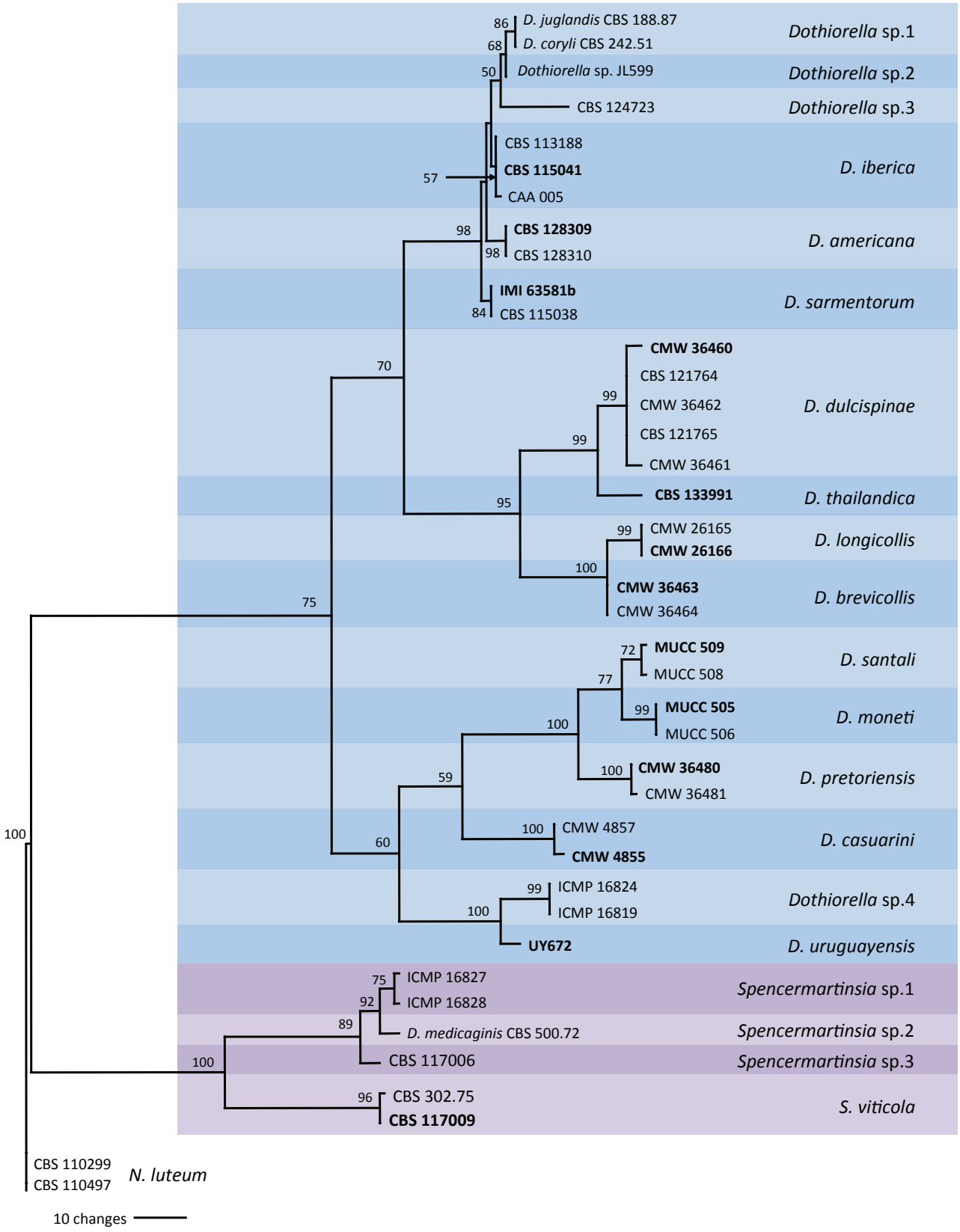

Fig. 32. Single most parsimonious tree obtained from combined ITS and EF-1α sequence data, for species in *Dothiorella* and *Spencermartinsia*. MP bootstrap values are given based on 1000 pseudoreplicates on the nodes. The tree is rooted to *Neofusicoccum luteum* (CBS 110299, CBS 110497).

Notes: Based on ITS and EF1-α sequence data, *D. americana* is closely related to *D. iberica* and *D. sarentorum*. But, morphologically conidia of this species are smaller than those in any other in *Dothiorella* sp. and obviously is a distinct species. Úrbez-Torres *et al*. (2011) considered this species to be a weak pathogen on grapevines.

Dothiorella brevicollis Jami, Gryzenh., Slippers & M.J. Wingf., Cryptog. Mycol. 33: 260. 2012. MycoBank MB564142. See Jami *et al.* (2012) for illustrations.

Ascomata not reported. *Conidiomata* pycnidial, stromatic, produced on *Acacia karroo* twigs on MEA within 2–4 wk, brown, solitary, up

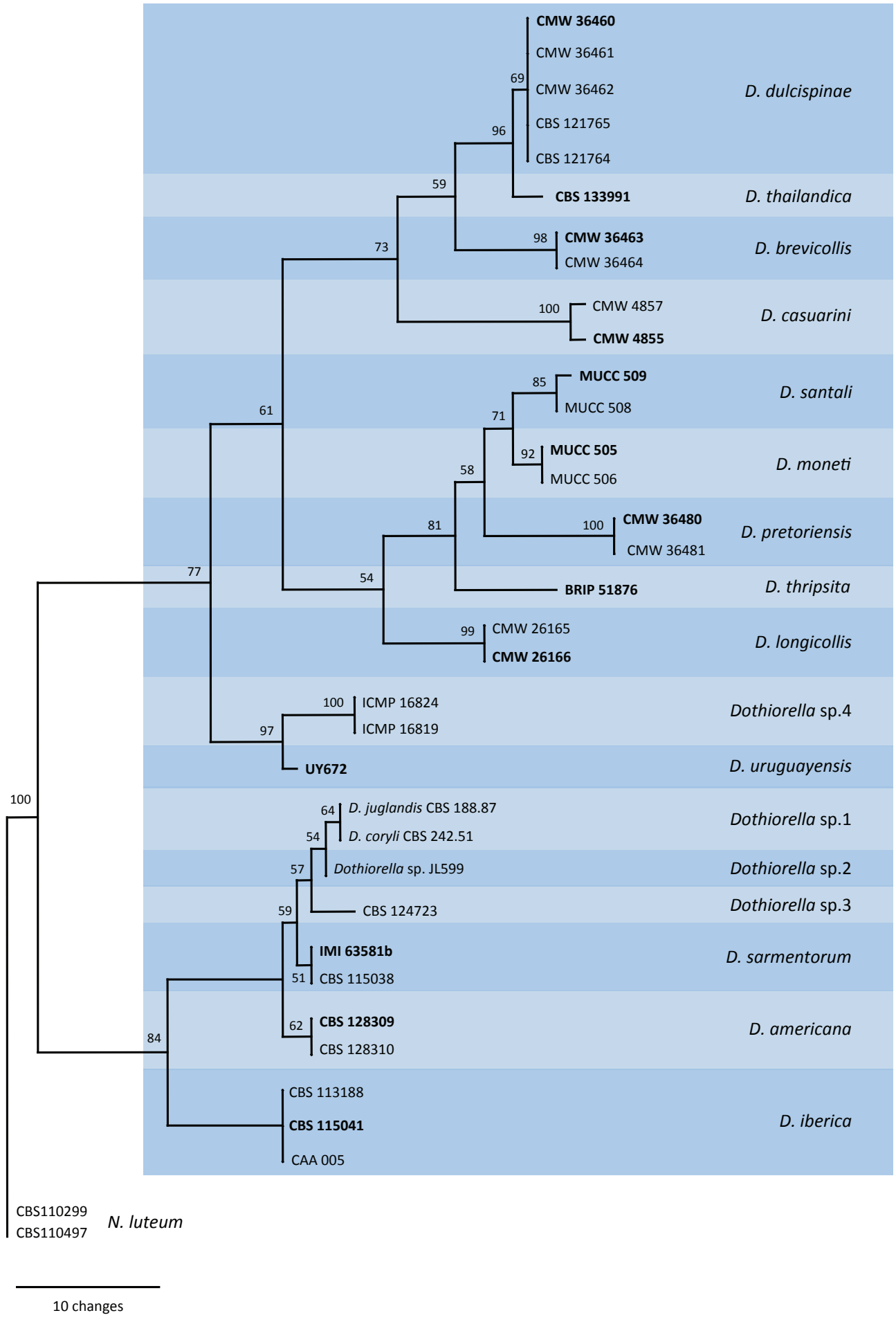

Fig. 33. Single most parsimonious tree obtained from ITS sequence data for species in *Dothiorella*. MP bootstrap values are given based on 1000 pseudoreplicates on the nodes. The tree is rooted to *Neofusicoccum luteum* (CBS 110299, CBS 110497).

to 200 µm wide, semi-immersed, unilocular, globose, papillate with a short neck, wall 5–7 cell layers, outer layers composed of dark-brown *textura angularis*, becoming thin-walled and hyaline toward the inner region. *Conidiophores* absent. *Conidiogenous cells* holoblastic, hyaline, cylindrical, (3–)3.5–7.5(–9) × (3–)3.5–4 µm. *Conidia* initially hyaline and aseptate, becoming dark brown and 1-septate, with 2 cells of equal length, thick-walled, ovoid, smooth with fine granular content, rounded apices, (20–)21.5–26(–27) × (8–)9–12(–13) µm.

Culture characteristics: *Colonies* on MEA appressed, conidiomata emerging after 9–10 d under near UV light, becoming pale olivaceous-grey to dark olivaceous-grey at the surface, and olivaceous-black to iron-grey at the reverse, with irregular edges. Colonies reaching 90 mm diam on PDA after 6 d (17.6 mm/d) in the dark at 25 °C. Cardinal temperatures for growth: min 5 °C, max 35 °C, opt 25 °C.

Type: **South Africa**, Gauteng Province, Pretoria, from healthy wood section of *Acacia karroo*, Nov. 2009, F. Jami, **holotype** PREM 60704.

Cultures: CBS 130411 = CMW 36463 (ex-type), CBS 130412 = CMW 36464.

Host: *Acacia karroo* (Jami *et al*. 2012).

Known distribution: South Africa (Gauteng Province) (Jami *et al*. 2012).

Notes: Phylogenetically this species is closely related to *D. longicollis* and *D. dulcispinae* and in terms of morphology it resembles *D. thripsita* and *D. dulcispinae*. All of these species produce papillate conidiomata. *Dohiorella longicollis* differs from the other three species by having very long necks (up to 1.5 mm). Moreover, conidia of *D. longicollis* (20.4 × 8.7 µm) are smaller than those of *D. brevicollis* (21.5–26 × 9–12 µm) and longer than those of *D. dulcispinae* (16–22 × 7–10 µm). Conidia of *D. brevicollis* are clearly larger (21.5–26 × 9–12 µm) than those of *D. dulcispinae* (16–22 × 7–10 µm). It is difficult to distinguish *D. brevicollis* from *D. thripsita* (av. size of conidia 20–25 × 8.5–11.5 µm) but phylogenetically, based on ITS sequence data, they are distinct (Fig. 33).

Dothiorella casuarini J. de Wet, Slippers & M.J. Wingf., Mycologia 101: 505. 2009. MycoBank MB510856. See De Wet *et al.* (2009) for illustrations.

Ascomata not reported. *Conidiomata* pycnidial, stromatic, few produced on pine needles, black, globose, solitary, scattered and immersed in water agar, ostiolate. *Conidiophores* absent. *Conidiogenous cells* emerging directly from cells lining the pycnidial cavity, holoblastic, hyaline, smooth-walled, subcylindrical, determinate or indeterminate and proliferating at the same level resulting in periclinal thickening, very rarely proliferating percurrently to produce two or three indistinct annellations. *Conidia* initially aseptate and hyaline, becoming brown to dark brown or sepia and 1-septate within the conidiomata, rarely 2–3-septate, ellipsoid to ovoid, rarely narrow ellipsoid, apex obtuse, base truncate, (22–)23–31(−38) × (8–)9–12 (−13.5) µm (av. of 60 conidia = 27.1 × 10.8 µm).

Culture characteristics: *Colonies* smooth to fluffy, pale greenish grey to greenish grey from above, becoming lighter or white around the edges, light bluish or sky grey from below, colony margins irregular, rosette-like. *Mycelium* thick-walled, branched, septate, melanised, pale to dark brown, with strings of dark brown chlamydospore-like hyphal swellings.

Type: **Australia**, Canberra, Cotter River, on *Casuarina* sp., 2000, M.J. Wingfield, **holotype** PREM 59650.

Cultures: CBS 120688 = CMW 4855 (ex-type), CBS 120690 = CMW 4857.

Host: *Casuarina* sp. (De Wet *et al*. 2009).

Known distribution: Australia (Canberra) (De Wet *et al*. 2009).

Note: Phylogenetically this species formed a distinct highly supported clade and morphologically conidia of *D. casuarini* are longer (27.1 × 10.8 µm) than those of any other *Dothiorella* species.

Dothiorella dulcispinae Jami, Gryzenh., Slippers & M.J. Wingf., Cryptogam. Mycol. 33: 258. 2012. MycoBank MB564141. See Jami *et al.* (2012) for illustrations.

Ascomata not reported. *Conidiomata* pycnidial, stromatic, produced on *Acacia karroo* twigs on MEA within 2–4 wk, solitary, dark brown, up to 200 µm wide, semi-immersed, unilocular, globose papillate with a short neck (100–300 µm), wall 6–8 cell layers, outer layers composed of dark-brown *textura angularis*, becoming thin-walled and hyaline toward the inner region. *Conidiophores* absent. *Conidiogenous cells* 1–2-celled, holoblastic, hyaline, cylindrical, proliferating percurrently. *Conidia* initially hyaline and aseptate, becoming dark brown or sepia and 1-septate, with 2 cells of unequal length, thick-walled, ovoid, smooth with fine granular content, rounded apices, (14–)16–22(–24) × (6–)7–10(–11) µm.

Culture characteristics: *Colonies* on MEA developing dense aerial mycelium with age, becoming pale olivaceous-grey to olivaceous-black at the surface, and olivaceous black at the reverse, umbonate with irregular zonation and lobate edges. Colonies reaching 90 mm diam on PDA after 5 d (17.9 mm/d) in the dark at 25 °C. Cardinal temperatures for growth: min 5 °C, max 35 °C, opt 25 °C.

Type: **South Africa**, Gauteng Province, Pretoria, from die-back wood section of *Acacia karroo*, Nov. 2009, F. Jami, **holotype** PREM 60706.

Cultures: CBS 130413 = CMW 36460 (ex-type), CBS 130414 = CMW 36461, CBS 130415 = CMW 36462, CBS 121764, CBS 121765.

Host: *Acacia karroo* (Jami *et al*. 2012).

Known distribution: South Africa (Gauteng Province) (Jami *et al*. 2012).

Notes: See notes for *D. brevicollis*.

Dothiorella iberica A.J.L. Phillips, J. Luque & A. Alves, Mycologia 97: 524. 2005. MycoBank MB344530. Fig. 34.

= *Botryosphaeria iberica* A.J.L. Phillips, J. Luque & A. Alves, Mycologia 97: 524. 2005.

Ascomata dark brown to black, globose pseudothecial, up to 350 µm diam, submerged in the substrate, partly erumpent at maturity, ostiole circular, central, papillate, wall up to 50 µm thick, composed of dark brown thick-walled *textura angularis*, cells 8–17 × 6–10 µm and lined with thinner-walled, hyaline, *textura angularis*. *Pseudoparaphyses* thin walled, hyaline, frequently septate, slightly constricted at the septum, 2.5–3.5(–4) µm wide. *Asci* 100–125 × 18–25 µm, stipitate, arising from the base of the ascoma, clavate, thick-walled, bitunicate with a well-developed apical chamber, stipitate, (4–)8-spored, irregularly biseriate. *Ascospores* oblong, ovate to sub-clavate, (0–)1-septate, slightly constricted at the septum, dark brown, moderately thick-walled, finely verruculose on the inner surface, straight or inequilateral, widest in the lower 1/3 to middle of the apical cell, basal cell tapering towards the rounded end, (17.5–)22.5–23.5(–29) × (8.5–)10–10.5(–12.5) µm (av. ± S.D. of 50 ascospores = 23.1 ± 2.1 × 10.2 ± 0.8 µm). *Conidiomata* pycnidial, stromatic, solitary, globose, up to 450 µm wide, thick walled, composed of dark brown thick-walled *textura angularis*, becoming thin-walled and hyaline towards the inner region. *Conidiophores* absent. *Conidiogenous cells* lining the pycnidial cavity, holoblastic, hyaline, subcylindrical, 8–15 × 3–5(–6.5) µm, proliferating at the same level giving rise to periclinal thickenings, or rarely proliferating percurrently forming one or two indistinct annellations. *Conidia* initially hyaline, becoming dark brown and one-euseptate often while still attached to the conidiogenous cell, ovoid with a broadly rounded apex and truncate base, brown walled, 1-septate, slightly constricted at the septum, (17–)23–23.5(–28.5) × (8–)10.5–11(–16) µm (av. ± S.D. of 400 conidia = 23.2 ± 1.9 × 10.9 ± 1.2 µm), L/W ratio = 2.2. *Spermatia* not seen. Cardinal temperatures for growth: min 5 °C, max < 35 °C, opt 20–25 °C.

Type: **Spain**, Zaragoza province, Aragon, Tarazona, on dead twigs of *Quercus ilex*, Dec. 2002, J. Luque, **holotype** LISE 94944.

Cultures: CBS 115041 (ex-type), CBS 113188.

Hosts: *Cupressus* (Azouaoui-Idjer *et al*. 2012), *Juniperus communis* (Alves *et al*. 2013), *Malus* (Phillips *et al.* 2005), *Persea* (Mcdonald & Eskalen 2011), *Pistacia* (Phillips *et al*. 2008), *Quercus* (Phillips *et al.* 2005, 2008, Lynch *et al*. 2013), *Vitis* (Úrbez-Torres *et al*. 2007, Qiu *et al*. 2011, Baskaratevan *et al*. 2012) and probably many more.

Known distribution: Algeria (Azouaoui-Idjer *et al*. 2012), Australia (Qiu *et al*. 2011), Italy (Phillips *et al*. 2005), New Zealand (Baskaratevan *et al*. 2012), Portugal (Alves *et al*. 2013), Spain (Phillips *et al*. 2005, 2008), USA (Phillips *et al*. 2008, Úrbez-Torres *et al*. 2007, Mcdonald & Eskalen 2011, Lynch *et al*. 2013).

Notes: This species is similar to *D. sarmentorum* but can be distinguished on characteristics of the asci, ascospores and conidia. Thus, in *D. iberica* the asci are shorter and more clavate, the ascospores characteristically taper towards the base, and on average the conidia are slightly longer. Also see notes for *D. americana*.

Dothiorella longicollis Pavlic, T.I. Burgess & M.J. Wingf., Mycologia 100: 859. 2008. MycoBank MB512053. See Pavlic *et al.* (2008) for illustrations.

Ascomata not reported. *Conidiomata* semi-immersed, mostly solitary, with globose base (up to 550 µm diam), papillate with long neck (sometimes branching) up to 1.5 mm, arising from the substrate. *Conidiophores* absent. *Conidiogenous cells* holoblastic, cylindrical to subcylindrical, hyaline, the first conidium produced holoblastically and subsequent conidia enteroblastically, (5–)6–8(–10) × (2.5–)3–4(–4.5) µm (av. of 30 conidiogenous cells = 7.3 × 3.4 µm). *Conidia* initially hyaline, unicellular, becoming cinnamon to sepia and 1-septate while still attached to conidiogenous cells, oval to ovoid, apices rounded and base truncate, (17–)19–22(–23) × (7–)8–9.5(–10.5) µm (av. of 50 conidia = 20.4 × 8.7 µm), L/W ratio = 2.3.

Culture characteristics: *Colonies* initially white to olivaceous buff, becoming greenish olivaceous to citrine from the middle of colonies within 7 d, iron-grey (surface) and black (reverse) with age, with suppressed, moderately fluffy mycelium, edges smooth appearing sinuate as the colony darkens with age. Conidiomata readily formed from the middle of colony within 7–10 d, covering the entire surface of the colony and immersed in the medium (seen as round black structures on the reverse side of Petri dishes) 14 d after incubation. Optimum growth at 25 °C, covering 90 mm diam Petri dishes after 4 d in the dark.

Type: **Australia**, Western Australia, Tunnel Creek National Park, on healthy branches of *Lysiphyllum cunninghamii*, Jul. 2006, T.I. Burgess, **holotype** PREM 59485.

Cultures: CMW 26166 = CBS 122068 (ex-type), CMW 26165 = CBS 122067.

Hosts: Asymptomatic branches of *L. cunninghamii* (*Caesalpiniaceae*) and *Terminalia* sp. (*Combretaceae*) (Pavlic *et al.* 2008).

Known distribution: Australia (Western Australia) (Pavlic *et al.* 2008).

Notes: This species differs from all othe *Dothiorella* species by having papillate pycnidia with very long necks (up to 1.5 mm). Also see notes for *D. brevicollis*.

Dothiorella moneti K. Taylor, Barber, G.E. Hardy & T.I. Burgess, Mycol. Res. 113: 342. 2009. MycoBank MB511825. See Taylor *et al.* (2009) for illustrations.

Ascomata not reported. *Conidiomata* pycnidial, stromatic, superficial, dark brown-grey, cylindrical, mostly solitary, covered in mycelium, 0.5–1.5 mm in length and 0.1–0.5 mm in diam. *Conidiophores* absent. *Conidiogenous cells* holoblastic, hyaline, cylindrical to flask shaped, (4–)6–12(–16) × 2–4(–5) (av. of 150 conidiogenous cells = 8.4 × 2.6 µm). *Conidia* initially hyaline and aseptate becoming dark brown and 1-septate sometimes while still attached to conidiogenous cell, ellipsoid, smooth-walled, apex obtuse, frequently base truncate, often strongly constricted at the septum, usually widest at the middle of apical cell, (13–)17–22(–32) × (6–)7–10(–11) µm (av. of 300 conidia = 19.8 × 8.4 µm), L/W ratio = 2.4.

Fig. 34. *Dothiorella iberica*. A. Vertical section through an ascoma. B. Ascus with brown, 1-septate ascospores. C. Immature asci and one ascus with four ascospores. D. Details of the ascoma wall. E. Pseudoparaphyses. F. Ascospores. G. Ascospore. H. Young conidiogenous cells. I. Conidiogenous cells with developing conidia. J, K. Conidia viewed at two different focal planes to show verruculose inner surface of the wall. L, M. Conidia. N. Germinating conidia. Scale bars: A = 50 µm, B–N = 10 µm.

Culture characteristics: *Colonies* composed of appressed mycelial mat with diffuse irregular edges, initially white, edge remaining white, centre turning olive-grey to dark greenish grey and entire culture becoming dark olive-grey by day 8 and very dark greenish grey with age. Conidiomata produced profusely in the centre of culture within 8 d. Cardinal temperatures for growth: min 5 °C, max < 35 °C, opt 25 °C.

Type: **Australia**, Western Australia, Yalgorup National Park, from healthy stem of *Acacia rostellifera*, Jun. 2005, K.M. Taylor, **holotype** PERTH 07692978.

Cultures: MUCC 505 = WAC 13154 (ex-type), MUCC 506.

Host: *Acacia rostellifera* (Taylor *et al*. 2009).

Known distribution: Australia (Western Australia) (Taylor *et al*. 2009).

Notes: In the original description, Taylor *et al*. (2009) mention that pycnidial paraphyses are very rare, but they did not provide an illustration of these structures although they do show young conidiogenous cells. Since pycnidial paraphyses have not been reported in any other *Dothiorella* species, other than *D. santali*, it is possible that Taylor *et al*. (2009) were referring to immature conidiogenous cells rather than paraphyses. Morphologically and phylogenetically, *D. moneti* is closely related to *D. santali*. This species is quite different in nucleotide sequences from *D. santali* (6–7 substitutions in ITS, 11 substitutions and 9 insertions/deletions in EF1-α) and thus are easily separated based on ITS sequence data (Fig. 33). Moreover, it can be distinguished by having longer conidia (19.8 × 8.4 µm, L/W ratio = 2.4) compare with *D. santali* (18.2 × 9 µm, L/W ratio = 2).

Dothiorella pretoriensis (Jami, Gryzenh., Slippers & M.J. Wingf.) Abdollahz. & A.J.L. Phillips, **comb. nov.** MycoBank MB803995. See Jami *et al.* (2012) for illustrations.
Basionym: *Spencermartinsia pretoriensis* Jami, Gryzenh., Slippers & M.J. Wingf., Cryptogam. Mycol. 33(3): 261. 2012.

Conidiomata (on sterile twigs of *Acacia karroo*) pycnidial, up to 200 µm diam, semi-immersed, unilocular, with short necks; wall of 5–7 layers of thick, dark-brown cells of *textura angularis*. *Conidiophores* reduced to conidiogenous cells, or a supporting cell. *Conidiogenous cells* 1–2-celled, hyaline, subcylindrical, proliferating percurrently near apex, (3–)3.5–7.5(–9) × (3–)3.5–4 µm. *Conidia* ovoid, smooth, granular, apices rounded, thick-walled, initially hyaline, aseptate, becoming dark brown and 1-septate, apex obtuse, base bluntly rounded, (18–)20–28(–33) × (6.5–)7–14(–11) µm (Jami *et al.* 2012).

Culture characteristics: Colonies on MEA appressed; surface pale olivaceous to dark greenish olivaceous; reverse olivaceous-black, with regular zonation and lobate margins. Colonies growing at 5–25 °C, reaching up to 22.5 mm / d at 25 °C.

Type: **South Africa**, Gauteng, Pretoria, from wood of *Acacia karroo* with die-back symptoms, Nov. 2009, F. Jami, **holotype** PREM 60709.

Cultures: CMW 36481 = CBS 130404 (ex-type).

Host: *Acacia karroo* (Jami *et al*. 2012).

Known distribution: South Africa (Gauteng Province) (Jami *et al*. 2012).

Note: *Dothiorella pretoriensis* induced dieback when inoculated into healthy branches of *A. karroo*, suggesting that it is a pathogen of this host (Jami *et al*. 2012).

Dothiorella santali K. Taylor, Barber & T.I. Burgess, Mycol. Res. 113: 345. 2009. MycoBank MB511828. See Taylor *et al.* (2009) for illustrations.

Ascomata not reported. *Conidiomata* pycnidial, stromatic, mostly superficial, dark brown to black, globose, solitary, occasionally covered in mycelium, 100–600 µm in length and 50–650 µm in diam. *Conidiophores* absent. *Conidiogenous cells* holoblastic, hyaline, cylindrical to flask-shaped, (4–)6–12(–17) × 2–3(–4) (av. of 50 conidiogenous cells = 8.6 × 2.4 µm). *Conidia* initially hyaline and aseptate becoming pigmented brown and 1-septate often while still attached to conidiogenous cell, ellipsoid, apex obtuse, sometimes base truncate, sometimes slightly constricted at the septum, usually widest at the middle of apical cell, (15–)16–20(–22) × 7–11(–13) µm (av. of 100 conidia = 18.2 × 9.0 µm), L/W ratio = 2.0.

Culture characteristics: *Colonies* initially white, appressed mycelial mat, within 8 d turning greenish to dark greenish grey and fluffy, becoming very dark greenish grey to black with age. Conidiomata produced on the agar. Cardinal temperatures for growth: min 5 ºC, max < 35 ºC, opt 25 ºC.

Type: **Australia**, Western Australia, Yalgorup National Park, from healthy stem of *Santalum acuminatum*, Jun. 2005, K.M. Taylor, **holotype** PERTH 07693028.

Cultures: MUCC 509 = WAC 13155 (ex-type), MUCC 508.

Host: *S. acuminatum* (Taylor *et al.* 2009).

Known distribution: Australia (Western Australia) (Taylor *et al.* 2009).

Note: See notes for *D. moneti*.

Dothiorella sarmentorum (Fr.) A.J.L. Phillips, J. Luque & A. Alves, Mycologia 97: 522. 2005. MycoBank MB501403. Fig. 35.
Basionym: *Sphaeria sarmentorum* Fr., K. svenska Vetensk-Acad. Handl. 39: 107. 1818.
≡ *Diplodia sarmentorum* (Fr.) Fr., Summ. veg. Scand. (Stockholm) 2: 417. 1849.
= *Diplodia pruni* Fuckel, Jahrb. Nassauischen Vereins Naturk., 23–24: 169. 1870 [1869].
= *Botryosphaeria sarmentorum* A.J.L. Phillips, J. Luque & A. Alves, Mycologia 97: 522. 2005.

Ascomata dark brown to black, globose pseudothecial, 350–400 µm diam, submerged in the substrate, partially erumpent at maturity, ostiolate; ostiole circular, central, papillate; wall 50–75 µm thick, composed of dark brown thick-walled *textura angularis*, cells 10–

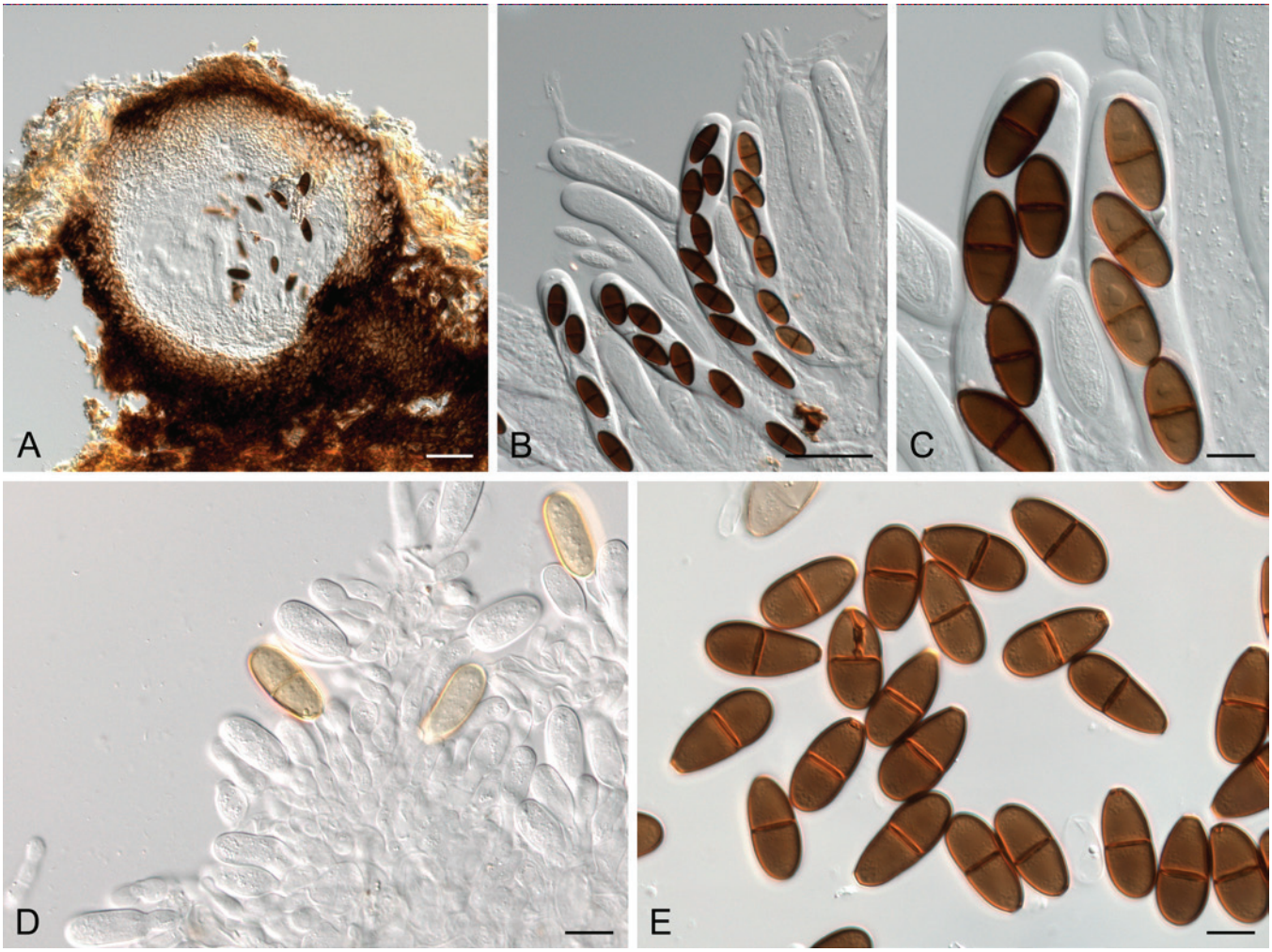

Fig. 35. *Dothiorella sarmentorum*. A. Vertical section through an ascoma. B. Cylindrical to clavate asci bearing eight brown ascospores. C. Details of ascus tip and ascospores. D. Conidiogenous layer with developing conidia. E. Dark brown, 1-septate conidia. Scale bars: A, B = 50 µm, C–E = 10 µm.

17 × 6–9 µm, lined with thinner-walled, hyaline, *textura angularis*. *Pseudoparaphyses* thin-walled, hyaline, frequently septate, often constricted at the septa, 3–4 µm wide. *Asci* 140–210 × 17–24 µm, stipitate, arising from the base of the ascoma, cylindric-clavate, bitunicate, endotunica thick-walled, with a well-developed apical chamber, 4–6(–8)-spored, obliquely uniseriate or irregularly biseriate. *Ascospores* oblong to ovate, widest in the middle part, straight, (0–)1-septate, slightly constricted at the septum, dark brown, moderately thick-walled, surface smooth, finely verruculose on the inner surface, (21–)24.5–25.5(–30.5) × (10–)11.5–12.5(–14) µm (av. ± S.D. = 25.0 ± 2.0 × 12.1 ± 0.9 µm). *Conidiomata* pycnidial, stromatic, solitary, globose, up to 450 µm wide, wall 5–8 cell layers thick, composed of dark brown thick-walled *textura angularis*, becoming thin-walled and hyaline towards the inner region. *Conidiophores* absent. *Conidiogenous cells* lining the pycnidial cavity, holoblastic, hyaline, subcylindrical, 7–15 × 3–7 µm, proliferating at the same level giving rise to periclinal thickenings, or rarely proliferating percurrently to form one or two close, indistinct annellations. *Conidia* initially hyaline and aseptate becoming pigmented brown and 1-septate often while still attached to conidiogenous cell, brown walled, slightly constricted at the septum, ovoid with a broadly rounded apex and truncate base, (17.5–)21.5–22(–25) × (8–)9.5–10(–11.5) µm (av. ± S.D. = 21.6 ± 1.5 × 9.8 ± 0.9 µm), L/W ratio = 2.2. *Spermatogenous cells* discrete or integrated, hyaline, smooth, cylindrical, holoblastic or proliferating via phialides with periclinal thickenings, 7–10 × 2–3 µm. *Spermatia* hyaline, smooth, aseptate, rod-shaped with rounded ends, 4–5.5 × 2 µm. Cardinal temperatures for growth: min 5 °C, max < 35 °C, opt 20–25 °C.

Type: Of the sexual morph; **UK**, England, Warwickshire, on *Ulmus* sp., Aug. 1956, E.A. Ellis, **holotype** IMI 63581b (as *Otthia spiraeae*); of the asexual morph; **Sweden**, Lund, Botanical Garden, on *Menispermum canadense*, 1818, E.M. Fries Scleromyc. Suec. 18, **holotype** UPS-FRIES (as *Sphaeria sarmentorum*); **isotype** of the asexual morph, K(M) 104852.

Cultures: IMI 63581b (ex-type), CBS 115038.

Hosts: *Dothiorella sarmentorum* is a plurivorous species and has been isolated from 34 different host species including *Malus*, *Menispermum*, *Prunus*, *Pyrus*, *Ulmus*, etc.

Known distribution: This species is cosmopolitan distributed worldwide and has been found across six continents.

Notes: In proposing 145 species as synonyms of *D. sarmentorum*, Wollenweber (1941) reported a wide range of dimensions for the conidia, namely, (15–)20–24(–35) × (7–)7.4–11.5(–15) µm. Some species in *Dothiorella* are separated by minor differences in conidium dimensions. It is therefore possible that some of Wollenweber's synonyms are in fact distinct species. Also see notes for *D. americana* and *D. iberica*.

Dothiorella thailandica (D.Q. Dai., J.K. Liu & K.D. Hyde) Abdollahz., A.J.L. Phillips & A. Alves, **comb. nov.** MycoBank MB805461. See Liu *et al.* (2012) for illustrations.
Basionym: *Auerswaldia dothiorella* D.Q. Dai., J.K. Liu & K.D. Hyde, Fungal Divers. 57: 162. 2012.

Saprobic on dead bamboo. *Ascomata* not reported. *Conidiomata* pycnidial, 400–800 µm wide, 200–250 µm high, 250–500 µm diam, immersed in the host tissue and becoming erumpent at maturity, globose, coriaceous, dark brown in the erumpent part. *Conidiomata wall* 15–50 µm wide, with brown to dark brown outer layers and hyaline to light brown inner layers, comprising several layers with cells of *textura angularis*, cells 3–9.5 × 2–6 µm. *Conidiophores* reduced to conidiogenous cells which are 2–5.5 × 1.5–4.5 µm, holoblastic, discrete, hyaline, cylindrical to ellipsoidal, smooth, straight or curved, formed from cells lining the innermost layer of the pycnidium. *Conidia* 15–20 × 6.5–8 µm (av. 20 conidia = 18.5 × 7 µm), initially hyaline and aseptate, becoming brown at maturity, 1–septate, slightly constricted at the septa, oblong to ellipsoidal, ends rounded, with slight undulating striations on the surface, occasionally curved, lower cell smaller, thick-walled.

Culture characteristics: *Colonies* on PDA, slow growing, 15 mm diam after 45 d at 23–25 °C, circular, with uneven margin, greyish brown after 7 d, becoming cottony and brown at the centre and dark brown towards the edge. *Chlamydospores* produced after 30 d.

Type: **Thailand**, Chiang Rai Province, Doi Pui, on dead bamboo culm, 1 Sep. 2011, D.Q. Dai, **holotype** MFLU 12–0751.

Culture: MFLUCC 11-0438 = CBS 133991 (ex-type).

Host: Bamboo (Liu *et al*. 2012).

Known distribution: Thailand (Liu *et al.* 2012).

Notes: This species is phylogenetically closely related to *D. dulcispinae*. Furthermore, in terms of morphology it resembles *D. santali* and *D. moneti*. But *D. thailandica* can easily be separated from those three species by its striate conidia.

Dothiorella thripsita R.G. Shivas & D.J. Tree, Fungal Planet No. 32. 2009. MycoBank MB513166. See Shivas *et al.* (2009) for illustrations.

Ascomata not reported. *Conidiomata* pycnidial, stromatic, solitary, immersed, partially erumpent when mature, dark brown, globose to ellipsoidal, papillate with a central ostiole, up to 300 × 200 µm diam, uniloculate, wall composed of an outer layer of dark brown, thick-walled *textura angularis*, and an inner layer of thin-walled hyaline cells. *Conidiophores* absent. *Conidiogenous* cells 10–15 × 3–6 µm, holoblastic, discrete, cylindrical, hyaline, smooth, indeterminate. *Conidia* initially hyaline, becoming dark brown and 1-euseptate often while still attached to the conidiogenous cell, aseptate and pale brown when young, becoming septate and brown when mature, often with a guttule in each cell, cylindrical to clavate, straight, both ends broadly rounded, 20–25 × 8.5–11.5 µm, conidial wall densely and minutely verruculose, profile smooth in LM, verruculose in SEM.

Culture characteristics: *Colonies* on 10 % potato-dextrose agar (Difco) reaching to 65 mm diam after 5 d in the dark at 23 °C, covered the entire plate after 3 wk in the dark followed by 5 d under black light, and were olivaceous-black to charcoal with sparse aerial mycelium, reverse greyish black to charcoal. Abundant conidia produced on Sachs' agar supporting sterilised pieces of maize leaf.

Type: **Australia**, Queensland, Tallegalla, on dead stems and phyllodes of *Acacia harpophylla*, Mar. 2008, D.J. Tree & C.E.C. Tree, **holotype** BRIP 51876.

Culture: BRIP 51876 (ex-type).

Host: *Acacia harpophylla* (Shivas *et al.* 2009).

Known distribution: Australia (Queensland) (Shivas *et al.* 2009).

Notes: Larvae and adults of the thrips *Mecynothrips hardyi* feed almost exclusively on conidia of *D. thripsita* (Shivas *et al.* 2009). Only ITS sequence data are available for the single isolate of this species. Based on ITS sequence data *D. thripsita* constitutes a completely distinct clade from all other species in *Dothiorella* (Fig. 33). In morphology it resembles *D. brevicollis*.

Dothiorella uruguayensis (C.A. Pérez, Blanchette, Slippers & M.J. Wingf.) Abdollahz. & A.J.L. Phillips, **comb. nov.** MycoBank MB803999. For illustrations see Pérez *et al*. (2010).
Basionym: *Spencermartinsia uruguayensis* C.A. Pérez, Blanchette, Slippers & M.J. Wingf., Fungal Divers. 41: 65. 2010.

Conidiomata (on PNA) pycnidial, superficial, solitary, globose, black, non-papillate, covered with mycelium, up to 350 µm diam. *Conidiogenous cells* hyaline, subcylindrical. *Conidia* (17–)22–22.5(–26.5) × (7–)9–9.5(–12) µm, dark brown, 1-septate, slightly constricted at septum, ovoid with broadly rounded apex and truncate base (from Pérez *et al.* 2010).

Type: **Uruguay**, Paysandu, Tres Bocas, endophytic on twigs of *Hexachlamis edulis*, Aug. 2006, C.A. Pérez, **holotype** PREM 60268.

Cultures: UY672 = CMW 26763 = CBS 124908 (ex-type).

Host: *Hexachlamis edulis* (Pérez *et al.* 2010).

Known distribution: Uruguay (Pérez *et al.* 2010).

Notes: Inoculation results suggest that *D. uruguayensis* is a weak pathogen on *Hexachlamis edulis*. It also proved to be uncommon in the area, and not pathogenic to *Eucalyptus* (Pérez *et al.* 2010).

Endomelanconiopsis Rojas & Samuels, Mycologia 100: 770. 2008. MycoBank MB511837.
Type species: *Endomelanconiopsis endophytica* Rojas & Samuels, Mycologia 100: 770. 2008.

Mycelium immersed, branched, septate, hyaline to pale brown. *Conidiomata* stromatic, immersed, peridermal to subepidermal,

separate, irregularly multilocular, walls composed of small-celled, pale brown, thin-walled *textura angularis*, becoming hyaline towards the conidiogenous region. Dehisence irregular. *Conidiophores* absent. *Conidiogenous cells* holoblastic, determinate, discrete, cylindrical, tapered markedly or gradually towards the apices, hyaline, smooth, thin-walled, formed from the walls of the locules. *Conidia* aseptate, pyriform to limoniform, dark brown, thick-walled, smooth, base often protruding and papillate, often with a central guttule and a single germ slit.

Notes: *Endomelanconiopsis* was introduced by Rojas *et al.* (2008) for *E. endophytica* and *E. microspora*. The genus is similar to *Endomelanconium* Petrak but belongs to the *Botryosphaeriaceae* and the conidia are non-papillate. Only two species are currently known in culture and the main difference between them is that chlamydospores are abundant in *E. microspora* but absent in *E. endophytica*.

Species descriptions

Endomelanconiopsis endophytica Rojas & Samuels, Mycologia 100: 770. 2008. MycoBank MB511838. See Rojas *et al.* (2008) for illustrations.

Conidiomata stromatic, scattered throughout colony, varying from globose to cylindrical, 1–3 cylindrical necks, superficial or immersed in the agar; often cylindrical papillae protruding from the agar in groups of a few; wall composed of pale brown and black angular cells, becoming hyaline and more hyphal toward the conidiogenous cells; locule convoluted, completely lined with conidiogenous cells. *Conidiogenous cells* formed from the inner cells all over the conidiomata wall, discrete, determinate, cylindrical, tapered toward the apex, hyaline, holoblastic, rarely with a single percurrent proliferation, 7.5–23.5 × 1–3.5 µm at apex, 1.5–4 µm at base (av. = 14.2 × 1.6 µm at apex, 14.2 × 2.2 µm at base). *Conidia* ellipsoidal to limoniform, apex rounded, base flat to rounded, aseptate, hyaline when immature, dark brown with a single longitudinal slit three-quarters of the length of the conidia when mature, (4.5–)5.5–7.5(–10) × (3–)3.5–4.5(–6) µm. *Spermatia* forming in the same locules as conidia from densely arranged, enteroblastic, phialidic conidiogenous cells, appearing to arise from the inner cells of the conidioma wall, ellipsoidal to allantoid, formed on PDA and SNA, 2–7(–10) × (1–2(–3) µm. *Chlamydospores* not observed.

Culture characteristics: *Colonies* at first colourless with hyaline immersed hyphae, after 4 d colonies olivaceous in center and concentric rings with irregular shape, after 10 d aerial mycelium dense dark olivaceous or grey or shiny black with little aerial mycelium. Optimum temperature at 30–37 °C; colony radius 43–55 mm after 5 d on PDA.

Type: **Panama**, Nombre de Dios, isolated from leaves of *Theobroma cacao*, 2000, E. Rojas, L. Mejía & Z. Maynard, **holotype** BPI 878370.

Culture: CBS 120379 (ex-type).

Hosts: *Heisteria concinna*, *Theobroma cacao* (Rojas *et al.* 2008).

Distribution: Panama (Rojas *et al.* 2008).

Notes: The germ slit in the conidia of *E. endophytica* and *E. microspora* is an unusual feature in the *Botryosphaeriaceae*. While *Neodeightonia subglobosa* was reported to have conidia with germ slits (Punithalingam 1969), and these were interpreted by Crous *et al.* (2006) as striations similar to those seen in *Lasiodiplodia*.

Endomelanconiopsis microspora (Verkley & Aa) E.I. Rojas & Samuels, Mycologia 100: 772. 2008. MycoBank MB511839.
Basionym: *Endomelanconium microsporum* Verkley & Aa, Mycologia, 89: 967. 1997.

Conidiomata stromatic, solitary and globose to subglobose, or convoluted with merging cavities, superficial or immersed in the agar, at first pale olivaceaous, later black, glabrous, often with an apical papilla but seldom a functional ostiole, mostly dehiscing by bursting or partial dissolution of upper wall tissue, 200–500 µm diam. *Conidiomatal wall* composed of two layers, an outer layer of brown to olivaceous *textura epidermoidea-angularis*, and an inner layer variable in thickness of hyaline *textura angularis-globulosa*. *Conidiogenous cells* formed from the inner cells all over the conidiomatal wall, discrete, determinate, cylindrical, but tapering towards the apex, hyaline, holoblastic, rarely with a single percurrent proliferation, mostly 6–10 × 5–7 µm. *Conidia* ellipsoidal to pyriform, apex rounded, base with an inconspicuous scar, aseptate, smooth, hyaline when liberated, soon becoming dark brown with a single longitudinal hyaline slit, containg one large and a few smaller oil droplets, (4.5–)5.5–6.5(–7) × (3.5–)4–4.5) µm. *Chlamydospores* abundant in immersed mycelium, intercalary and terminal, when interclary, subglobose to fusiform, single or catenate (2–5), when terminal, globose to clavate-pyriform, occasionally with a small basal, apophysis-like cell or an apical papilla, thick-walled, brown, often verruculose, filled with oil droplets, mostly 9–17 × 6–10 µm. In older cultures additional chlamydospores forming in basipetal succession behind the terminal ones.

Type: **Papua New Guinea**, Central Province, 22 km E of Port Moresby, Varirata National Park near Varirata Lookout, soil in dry secondary forest with *Casuarina* and *Eucalyptus*, and conglomerate rock outcrops, 23 Oct. 1995, A. Aptroot, H.A. van der Aa 12183 (a dried culture on oatmeal agar), **holotype** CBS H-12183.

Culture: CBS 353.97 (ex-type).

Substrate: Soil (Verkley & van der Aa 1997).

Known distribution: Papua New Guinea (Verkley & van der Aa 1997).

Note: *Endomelanconiopsis microspora* is characterised by having stromatic conidiomata that give rise to brown, aseptate conidia, and abundant terminal, and intercalary chlamydospore-like structures that are formed in culture (Verkley & van der Aa 1997).

Lasiodiplodia Ellis & Everh., Bot. Gaz. 21: 92. 1896. MycoBank MB8708.
Type species: *Lasiodiplodia theobromae* (Pat.) Griff. & Maubl., Bull. trimest. Soc. Mycol. Fr. 25: 57. 1909.

Mycelium immersed or superficial, branched, septate, dark brown. *Ascomata* eustromatic, dark brown to black, uniloculate with thick pseudoparenchymatic wall, ostiolate, embedded in the substrate and partially erumpent at maturity. *Pseudoparaphyses* hyaline, septate. *Asci* bitunicate with thick endotunica and well-developed apical chamber, clavate, stipitate, 8-spored. *Ascospores* irregularly biseriate, initially hyaline, becoming dark brown, aseptate. *Conidiomata* stromatic, immersed or superficial, separate or aggregated and confluent, globose, dark brown, uni- or multilocular; wall of dark brown, thick-walled *textura angularis*, paler and thinner-walled towards the conidiogenous region, often with dark brown superficial hyphae over the surface. *Ostiole* central, single, papillate. *Conidiophores* often reduced to conidiogenous cells, if present hyaline, simple, sometimes septate, rarely branched, cylindrical, arising from the inner layers of cells lining the locules. *Conidiogenous cells* hyaline, smooth, cylindrical to subobpyriform, holoblastic, discrete, determinate or indeterminate and proliferating percurrently with one or two distinct annellations, or proliferating at the same level giving rise to periclinal thickenings, formed from cells lining the inner wall of the conidiomata. *Conidia* hyaline when young, later becoming medianly 1-euseptate, dark brown with longitudinal striations, thick-walled, oblong to ellipsoid, straight, broadly rounded at the apex, base truncate. *Paraphyses* hyaline, cylindrical, septate.

Notes: *Lasiodiplodia* was introduced by Ellis in 1894 with *L. tubericola* as the type species. Although Ellis did not describe it, Clendenin (1896) provided a description of the genus and the species, attributing both to Ellis and Everhardt. Griffin & Maublanc (1909) considered that on account of the pycnidial paraphyses, the cocoa pathogen, *Botryodiplodia theobromae*, was more suitably accommodated in *Lasiodiplodia*. Since the epithet *theobromae* (1892) is older than *tubericola* (1896), *L. theobromae* should be regarded as the type species of *Lasiodiplodia*. Unfortunately, neither Patouillard (1892) nor Clendenin (1896) referred to any type or other specimens of the genus or species. Pavlic *et al.* (2004) could not locate the types, and they also could not find any specimens from the original hosts or origins.

It has been thought that *Lasiodiplodia* could represent a possible synonym of *Diplodia* (Denman *et al.* 2000). However, phylogenetic studies by Zhou & Stanosz (2001), Slippers *et al.* (2004) and Phillips *et al.* (2008) show that it clusters separately from *Diplodia*. On account of the phylogenetic and morphological differences there is no reason to consider the two as synonymous. Morphologically the two genera are also clearly distinct. Thus, striations on the conidia distinguish *Lasiodiplodia* from *Diplodia*, the conidiomatal paraphyses distinguish it from *Neodeightonia*, which also has striate conidia. Although *Barriopsis* has striate conidia, they are unique in the *Botryosphaeriaceae* because they are also present on immature, hyaline conidia. The sexual morph has been reported only for *L. theobromae*, but the connection with the asexual morph has not been confirmed (see notes under *L. theobromae*). While 27 species names are listed in MycoBank, only 18 species are currently known in culture and all, except *L. theobromae*, have been introduced since 2004. Species can be differentiated based on conidial morphology (especially dimensions) and morphology of the paraphyses.

Key to *Lasiodiplodia* spp.

1. Conidia sub-globose, L/W ratio less than 1.5 2
1. Conidia ellipsoidal to ovoid, L/W ratio greater than 1.5 3

2. Conidia 13.5–21.5 × 10–14 um (av. length 17.5 µm) ..... *L. mahajangana*
2. Conidia 12–19 × 10–12.5 µm (av. length 15.3 µm) ..... *L. margaritacea*

3. L/W ratio greater than 2.0 4
3. L/W ratio less than 2.0 5

4. Conidia 26–33 × 12–15 µm (av. length 28.4 µm) ..... *L. venezuelensis*
4. Conidia 17–23 × 8–11 µm (av. length 19.5 µm) ..... *L. viticola*

5. Longest paraphyses more than 100 µm long 6
5. Longest paraphyses less than 100 µm long 9

6. Average conidial length less than 25 µm 7
6. Average conidial length greater than 25 µm 8

7. Average conidial width = 13 µm ..... *L. iraniensis*
7. Conidia average width = 11.5 µm ..... *L. parva*

8. Conidia 22–35 µm long (av. 29.6 µm), L/W ratio = 1.9 ..... *L. plurivora*
8. Conidia 20–31 µm long (av. 24.5 µm), L/W ratio = 1.6 ..... *L. citricola*

9. Average width of conidia less than 16 µm 10
9. Average width of conidia 16 µm or more 15

10. Conidia small, mostly less than 25 µm long 11
10. Conidia large, mostly longer than 25 µm, up to 30 µm or more 14

11. Average width of conidia less than 10 µm 12
11. Average width of conidia greater than 10 µm 13

12. Length of paraphyses up to 15, conidia up to 17.5 µm ..... *L. lignicola*
12. Length of paraphyses up to 55, conidia up to 21 µm ..... *L. missouriana*

13. Paraphyses up to 55 µm long, conidial L/W ratio = 2 ..... *L. egyptiacae*
13. Paraphyses up to 85 µm long, conidial L/W ratio = 1.7 ..... *L. hormozganensis*

14. Conidiomata dark brown to black ..... *L. theobromae*
14. Conidiomata reddish-purple ..... *L. rubropurpurea*

15. Conidia not exceeding 35 µm long 16
15. Conidia up to 39 µm long 17

16. Paraphyses mostly septate ..... *L. crassispora*
16. Paraphyses mostly aseptate ..... *L. pseudotheobromae*

17. Paraphyses up to 95 µm long ..... *L. gilanensis*
17. Paraphyses not exceeding 65 µm, never reaching 95 µm ..... *L. gonubiensis*

DNA phylogeny

Combined analysis of ITS and EF1-α separates the 18 species currently recognised in this genus (Fig. 36). Some of the species, such as *L. citricola* / *L. parva* / *L. hormozganensis*, are distinguishable mainly from differences in their EF1-α sequences. Furthermore, bootstrap support for some of the inner branches is quite low. This would suggest that a reappraisal of the species in *Lasiodiplodia* based on more gene loci should be undertaken.

Lasiodiplodia citricola Abdollahz., Javadi & A.J.L. Phillips, Persoonia 25: 4. 2010. MycoBank MB16777. Fig. 37.

Ascomata not reported. *Conidiomata* stromatic, produced on pine needles on WA within 2–4 wk, superficial, dark brown to black, covered with dense mycelium, mostly uniloculate, up to 2 mm diam, solitary, globose, thick-walled, non-papillate with a central ostiole. *Paraphyses* hyaline, cylindrical, thin-walled, initially aseptate, becoming up to 1–5 septate when mature, occasionally branched, rounded at apex, occasionally basal, middle or apical cells swollen, up to 125 µm long, 3–4 µm wide. *Conidiophores* absent. *Conidiogenous cells* holoblastic, discrete, hyaline, smooth, thin-walled, cylindrical, proliferating percurrently with 1–2 annellations, 11–16 × 3–5 µm. *Conidia* initially hyaline, aseptate, ellipsoid to ovoid, with granular content, both ends broadly rounded, wall < 2 µm, becoming pigmented, verruculose, ovoid, 1-septate with longitudinal striations, (20–)22–27(–31) × (11–)12–17(–19) µm, 95 % confidence limits = 24.1–24.9 × 15–15.7 µm (av. ± S.D. = 24.5 ± 0.2 × 15.4 ± 1.8 µm, L/W ratio = 1.6).

Culture characteristics: *Colonies* with abundant aerial mycelia reaching to the lid of Petri plate, aerial mycelia becoming smoke grey to olivaceous-grey or iron-grey at the surface and greenish grey to dark slate blue at the reverse after 2 wk in the dark at 25 °C. Colonies reaching 85 mm on MEA after 2 d in the dark at 25 °C. Cardinal temperatures for growth: min ≤ 10 °C, max ≥ 35 °C, opt 25–30 °C.

Type: **Iran**, Gilan Province, Chaboksar, on twigs of *Citrus* sp., Jun. 2007, J. Abdollahzadeh & A. Javadi, **holotype** IRAN 14270F.

Cultures: IRAN 1522C = CBS 124707 (ex-type), IRAN 1521C = CBS 124706.

Hosts: *Citrus* sp. (Abdollahzadeh *et al.* 2010), *Juglans regia* (Chen *et al*. 2013).

Known distribution: Iran (Chaboksar, Gilan Province; Sari, Mazandaran Province; Northern Iran) (Abdollahzadeh *et al.* 2010), USA (California) (Chen *et al*. 2013).

Notes: Phylogenetically, *Lasiodiplodia citricola* is closely related to *L. parva*, but conidia of *L. citricola*, (20–)22–27(–31) × (11–)12–17(–19) µm, are longer and wider than those of *L. parva* (15.5–)16–23.5(–24.5) × (10–)10.5–13(–14.5) µm. In terms of morphology it resembles *L. plurivora* but on average the conidia of *L. citricola* (av. length = 24.5 µm) are shorter than those of *L. plurivora* (av. length = 29.6 µm). This species produces a pink pigment in PDA cultures at 35 °C.

Lasiodiplodia crassispora T.I. Burgess & Barber, Mycologia 98: 425. 2006. MycoBank MB500235. Fig. 38.

Ascomata not reported. *Conidiomata* stromatic, superficial, mostly solitary, conical, smooth, iron grey, 0.5–1 mm diam. *Paraphyses* cylindrical, septate, hyaline (21–)30–62(–66) × 2–3.5(–4) µm (av. of 50 paraphyses = 45.7 × 2.7 µm). *Conidiophores* absent. *Conidiogenous cells* holoblastic, hyaline, subcylindrical to cylindrical to ampulliform, proliferating percurrently, (6–)8–16(–19) × 3–7 µm (av. of 50 conidiogenous cells = 11.8 × 5 µm). *Conidia* produced in culture initially hyaline, unicellular, ellipsoid to obovoid, thick-walled (2–3 µm, av. of 50 conidia = 2.6 µm) with granular content, round at apex, occasionally truncate at base, becoming pigmented with one septum when mature or before germination, developing longitudinal striations when mature, 27–30(–33) × 14–17 µm (av. ± S.D. = 28.8 × 16.0 µm, L/W ratio = 1.8).

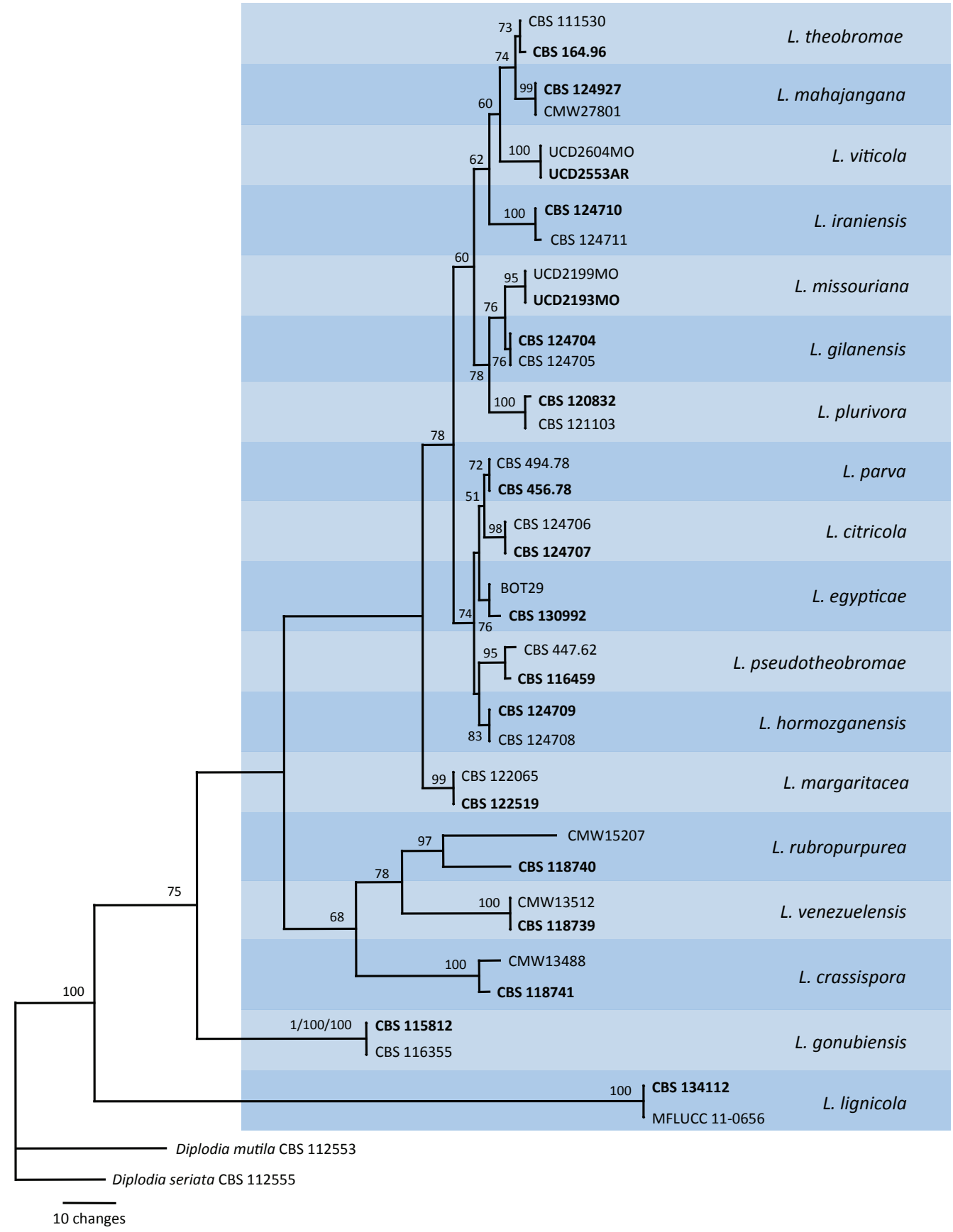

Fig. 36. One of the two equally most parsimonious trees obtained from combined ITS and EF-1α sequence data for species in *Lasiodiplodia*. MP bootstrap values are given based on 1000 pseudoreplicates on the nodes. The tree is rooted to *Diplodia mutila* (CBS 112553) and *D. seriata* (CBS 112555).

Culture characteristics: *Colonies* moderately dense, with appressed mycelial mat, initially white to buff turning pale olivaceous-grey within 7 d and darkening with age. After 7 d the submerged mycelia are olivaceous-grey, becoming black with age. Optimum temperature for growth 30 °C, reaching 74 mm on PDA after 3 d at 30 °C in the dark.

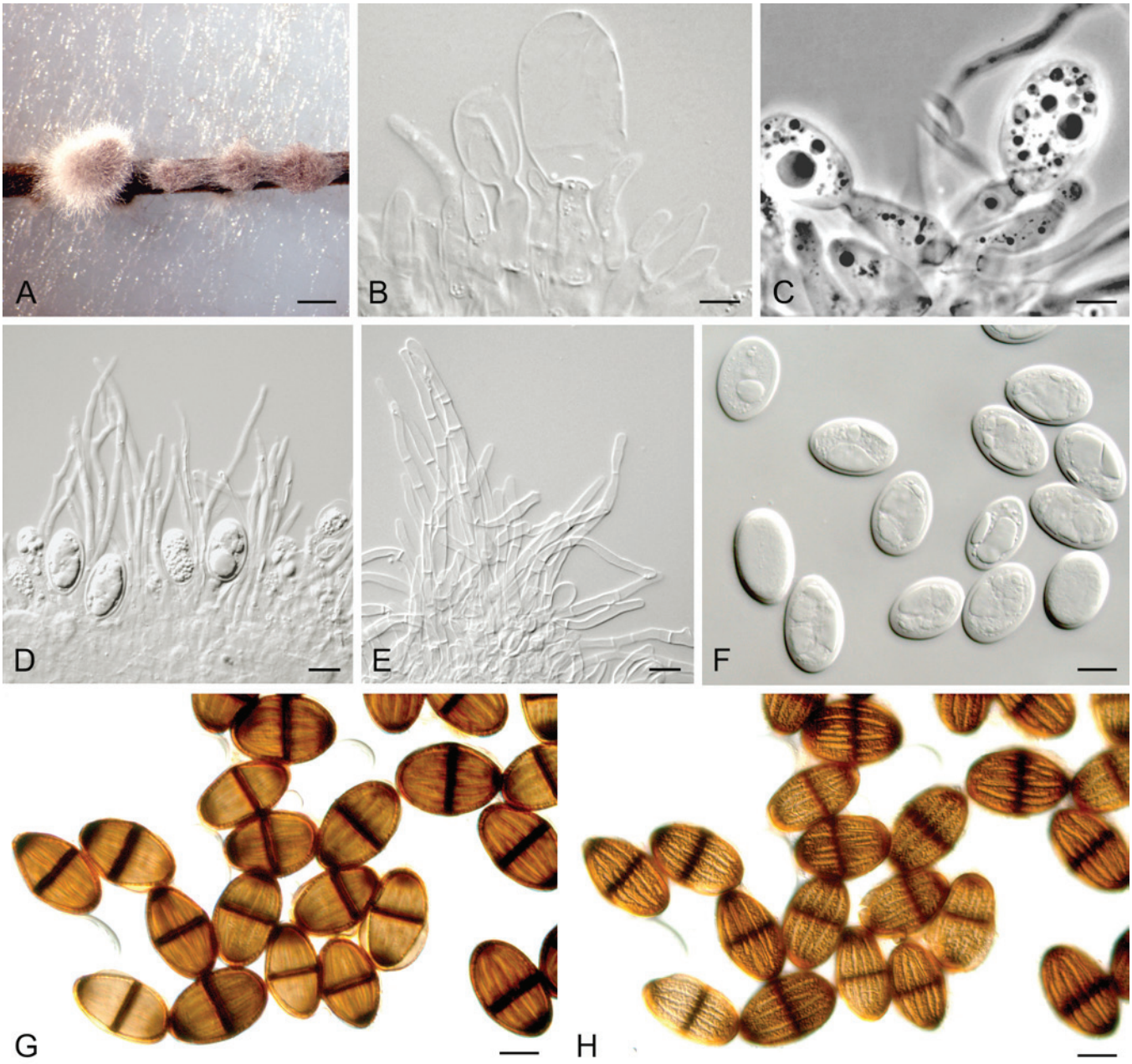

Fig. 37. *Lasiodiplodia citricola*. A. Conidiomata on pine needles in culture. B. Conidia developing on conidiogenous cells. C. Annellations on conidiogenous cell. D. Conidia developing on conidiogenous cells between paraphyses. E. Septate paraphyses. F. Hyaline, immature conidia. G, H. Mature conidia in two different focal planes to show the longitudinal striations. Scale bars: A = 1 mm, B, C = 5 µm, D–H = 10 µm.

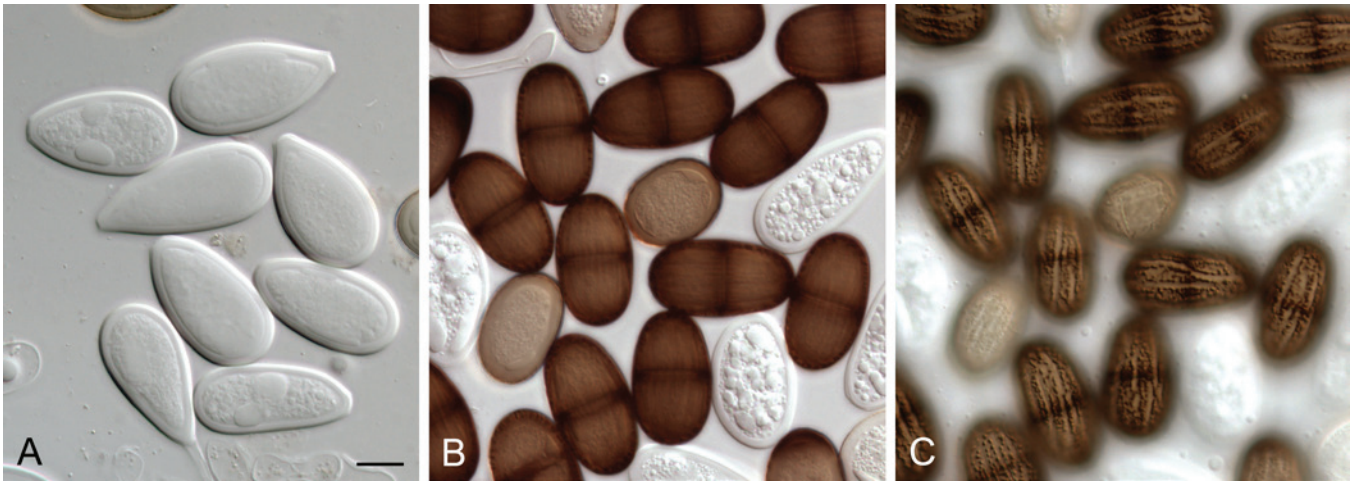

Fig. 38. *Lasiodiplodia crassispora*. A. Hyaline, aseptate conidia. B, C. Dark brown, 1-septate conidia in two focal planes to show the longitudinal striations. Scale bar A = 10 µm. Scale bar in A applies to B and C.

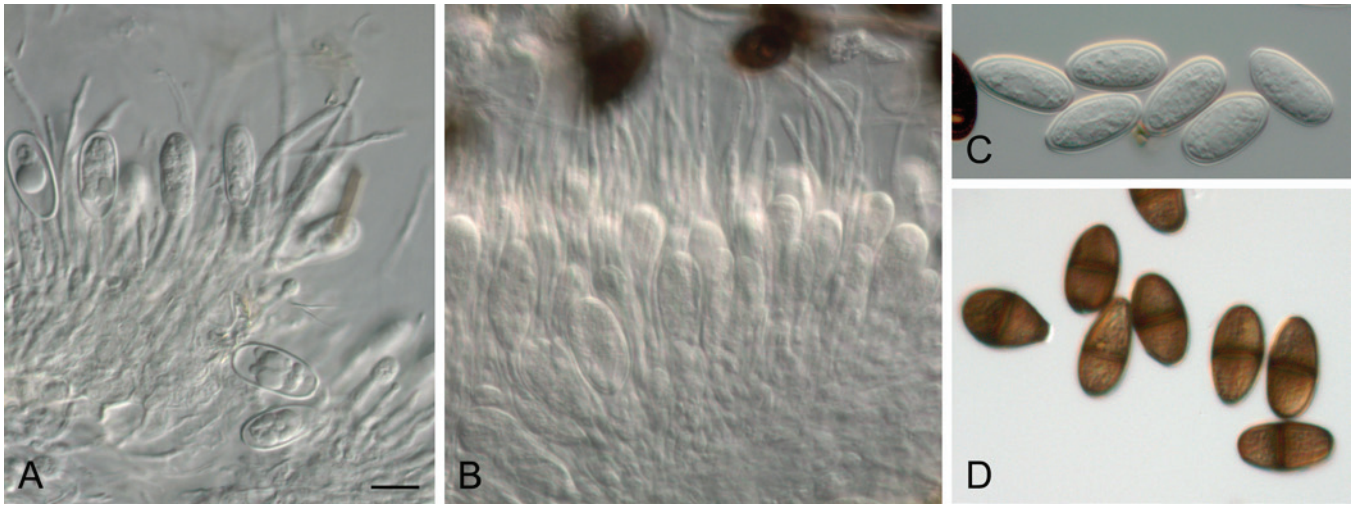

Fig. 39. *Lasiodiplodia egypticae*. A, B. Conidiogenous layer with conidia developing on conidiogenous cells between paraphyses. C. Hyaline, aseptate conidia. D. Dark-walled, 1-septate conidia. Scale bar A = 10 µm. Scale bar in A applies to B–D.

Type: **Australia**, Western Australia, Kununurra, from canker on *Santalum album*, Dec. 2003, T.I. Burgess, **holotype** MURU 407.

Cultures: WAC 12533 = CMW 14691 (ex-type), CMW 13448.

Hosts: *Santalum album* (Burgess *et al.* 2006), *Eucalyptus urophylla* (Perez *et al.* 2010), *Vitis vinifera* (Úrbez-Torres *et al.* 2010, van Niekerk *et al.* 2010).

Known distribution: Australia (Western Australia) (Burgess *et al.* 2006), South Africa (van Niekerk *et al.* 2010), Uruguay (Perez *et al.* 2010), USA (California) (Úrbez-Torres *et al.* 2010).

Notes: This species is phylogenetically closely related to *L. rubropurpurea* and *L. venezuelensis*, but can be distinguished from *L. rubropurpurea* by the absence of red-purple conidiomata. Furthermore, conidia of *L. crassispora* (av. = 28.8 × 16 µm) are wider than those of *L. venezuelensis* (av. = 28.4 × 13.5 µm). In terms of morphology *L. crassispora* resembles *L. pseudotheobromae* and the only feature that distinguishes the two species is that in *L. crassispora* the pseudoparaphyses are mostly septate, while in *L. pseudotheobromae* they are mostly aseptate.

Lasiodiplodia egyptiacae A.M. Ismail, L. Lombard & Crous, Australas. Plant Path. 41: 655. 2012. MycoBank MB564516. Fig. 39.

Ascomata not reported. *Conidiomata* stromatic, mostly solitary, dark-grey to black, globose to subglobose. *Paraphyses* hyaline, subcylindrical, aseptate, up to 57 µm long, 2–3 µm wide. *Conidiophores* absent. *Conidiogenous cells* holoblastic, hyaline, cylindrical, proliferating percurrently, 5–11 × 3–5 µm. *Conidia* initially hyaline, smooth, thick-walled, aseptate, obovoid to ellipsoid, granular, mostly somewhat tapered at apex, and rounded at base, becoming brown, 1-septate, with longitudinal striations when mature, (17–)20–24(−27) × 11–12(−13) µm (av. ± S.D. = 22 ± 2 × 12 ± 1 µm, L/W ratio = 2).

Culture characteristics: *Colonies* on PDA with moderately dense, raised mycelium mat, initially white to smoke-grey, turning greenish grey on the surface and greenish grey in reverse, becoming dark slate-blue with age. Cardinal temperatures for growth: min 15 °C, max 35 °C, opt 25 °C.

Type: **Egypt**, Sharkia Province, El Menayar, from *M. indica* leaf, Feb. 2010, A.M. Ismail, **holotype** CBS H-20736.

Cultures: BOT-10 = CBS 130992 (ex-type), BOT-29.

Host: *Mangifera indica* (Ismail *et al.* 2012).

Known distribution: Brazil (Marques *et al.* 2013), Egypt (Ismail *et al.* 2012).

Notes: This species is morphologically and phylogenetically closely related to *L. citricola*, *L. hormozganensis*, *L. parva* and *L. pseudotheobromae*, but can be distinguished based on the dimensions of conidia and paraphyses.

Lasiodiplodia gilanensis Abdollahz., Javadi & A.J.L. Phillips, Persoonia 25: 5. 2010. MycoBank MB16778. Fig. 40.

Ascomata not reported. *Conidiomata* stromatic, produced on pine needles on WA within 2–4 wk, superficial, dark brown to black, covered with dense mycelium, mostly uniloculate, up to 940 µm diam, solitary, globose, thick-walled, non-papillate with a central ostiole. *Paraphyses*, hyaline, cylindrical, thin-walled, initially aseptate, becoming up to 1–3 septate when mature, rarely branched, rounded at apex, up to 95 µm long, 2–4 µm wide. *Conidiophores* absent. *Conidiogenous cells* holoblastic, discrete, hyaline, smooth, thin-walled, cylindrical, 11–18 × 3–5 µm. *Conidia* initially hyaline, aseptate, ellipsoid to ovoid, with granular content, rounded at apex, base mostly truncate, wall < 2 µm, becoming pigmented, verruculose, ellipsoid to ovoid, 1-septate with longitudinal striations, (25–)28–35(–39) × (14.5–)15–18(–19) µm, 95 % confidence limits = 30.6–31.4 × 16.5–16.7 µm (av. ± S.D. = 31 ± 2.4 × 16.6 ± 1 µm, L/W ratio = 1.9).

Culture characteristics: *Colonies* with abundant aerial mycelia reaching to the lid of Petri plate, aerial mycelia becoming smoke-grey to olivaceous-grey at the surface and greenish grey to dark

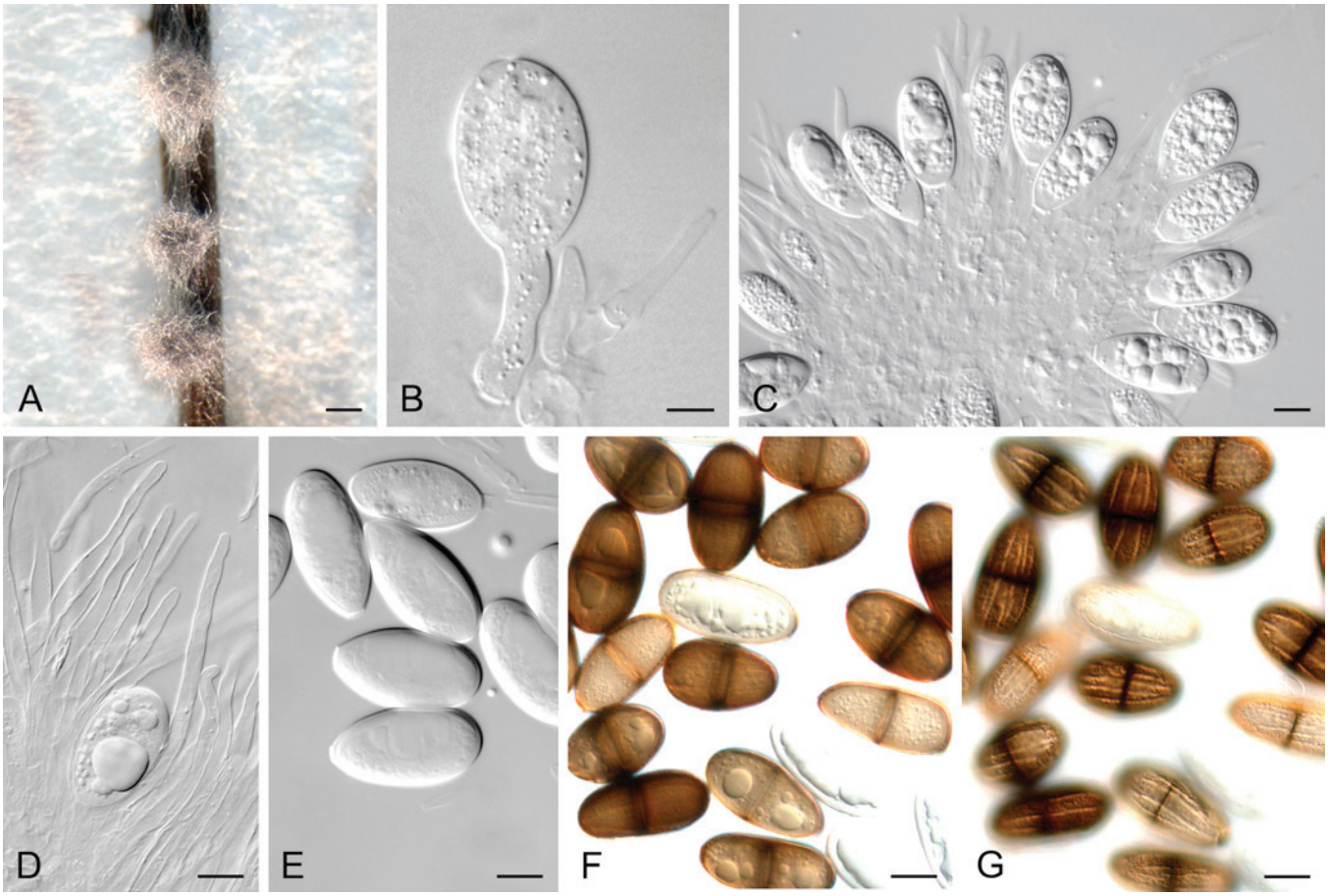

Fig. 40. *Lasiodiplodia gilanensis*. A. Conidiomata on pine needles in culture. B. Conidia developing on conidiogenous cells. C. Conidia developing on conidiogenous cells between paraphyses. D. Paraphyses. E. Hyaline, immature conidia. F, G. Mature conidia in two different focal planes to show the longitudinal striations. Scale bars: A = 1 mm, B = 5 µm, C–G = 10 µm.

slate blue at the reverse after 2 wk in the dark at 25 °C. Colonies reaching 80 mm on MEA after 2 d in the dark at 25 °C. Cardinal temperatures for growth: min ≤ 10 °C, max ≥ 35 °C, opt 25–30 °C.

Type: **Iran**, Gilan Province, Rahimabad-Garmabdost, on twigs of unknown woody plant, Jun. 2007, J. Abdollahzadeh & A. Javadi, **holotype** IRAN 14272F.

Cultures: IRAN 1523C = CBS 124704 (ex-type), IRAN 1501C = CBS 124705.

Hosts: Unknown (isolated from twigs of an unknown woody plant).

Known distribution: Rahimabad-Garmabdost, Gilan Province, Northern Iran (Abdollahzadeh *et al.* 2010).

Notes: Phylogenetically, *L. gilanensis* is closely related to *L. plurivora* and *L. missouriana*, but the three species can be distinguished on average conidial dimensions. Moreover, the paraphyses of *L. gilanensis* are up to 95 µm long and 4 µm wide, whereas paraphyses of *L. plurivora* are up to 130 µm long and 10 µm wide (Damm *et al.* 2007). Also, the 1–3 basal cells of *L. plurivora* paraphyses often are broader than the apical cells whereas, in *L. gilanensis* they are the same as the apical cells. In terms of morphology, *L. gilanensis* is similar to *L. gonubiensis*, but paraphyses of *L. gilanensis* (up to 95 µm) are longer than those of *L. gonubiensis* (up to 65 µm). Moreover, conidia of *L. gilanensis* (av. ± S.D. = 31 × 16.6 µm) are slightly shorter than in *L. gonubiensis* (av. ± S.D. = 33.8 × 17.3 µm). This species produces a pink pigment in PDA cultures at 35 °C.

Lasiodiplodia gonubiensis Pavlic, Slippers & M.J. Wingf., Stud. Mycol. 50: 318. 2004. MycoBank MB500079. See Pavlic *et al.* (2004) for illustrations.

Ascomata not reported. *Conidiomata* stromatic, formed on WA on sterilised pine needles within 7–21 d, semi-immersed, solitary, globose, papillate, leaden-black, covered by mycelium, up to 460 µm diam. *Paraphyses* cylindrical, aseptate, hyaline, (14–)26.5–47(–65) × (1.5–)2–2.5(–3) µm. *Conidiophores* absent. *Conidiogenous cells* holoblastic, cylindrical, hyaline, (6.5–)10–15(–18) × (1–)2–4(–4.5) µm. *Conidia* initially hyaline, unicellular, ellipsoid to obovoid, thick-walled with granular content, rounded at apex, occasionally truncate at base becoming cinnamon to sepia with longitudinal striations, forming one to three septa, (28–)32–36(–39) × (14–)16–18.5(–21) µm (av. of 100 conidia = 33.8 × 17.3 µm, L/W ratio = 1.9).

Culture characteristics: *Colonies* initially white to smoke-grey with fluffy, aerial mycelium, becoming olivaceous-grey on the surface after 3–4 d, with dense aerial mycelium, margins slightly irregular; reverse side of the colonies dark slate-blue. Optimum temperature for growth 25 °C, covering the medium surface (90 mm Petri dish) after 5 d in the dark. Isolates growing at 35 °C produced a coral red pigment within 4 d.

Type: **South Africa**, Eastern Cape Province, Gonubie, isolated from *Syzygium cordatum,* Jul. 2002, D. Pavlic, **holotype** PREM 58127 (conidiomata on needles of *Pinus* sp. on WA).

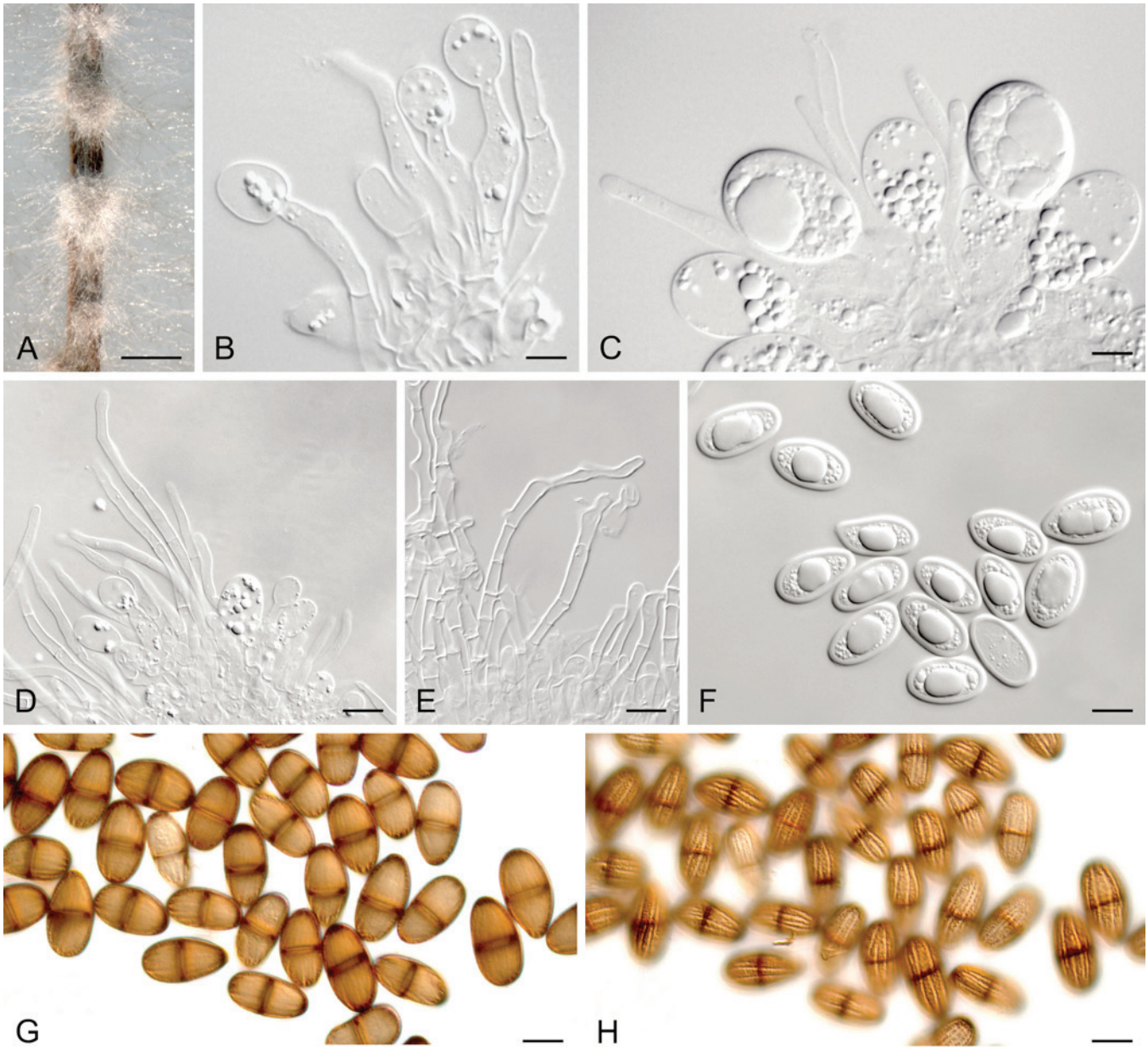

Fig. 41. *Lasiodiplodia hormozganensis*. A. Conidiomata on pine needles in culture. B, C. Conidia developing on conidiogenous cells between paraphyses. D, E. Septate and aseptate paraphyses. F. Hyaline immature conidia. G, H. Mature conidia in two different focal planes to show the longitudinal striations. Scale bars: A = 1 mm, B, C = 5 µm, D–H = 10 µm.

Cultures: CMW 14077 = CBS 115812 (ex-type), CMW 14078 = CBS 116355.

Hosts: *Syzygium cordatum* (Pavlic *et al.* 2004).

Known distribution: South Africa (Gonubie, Eastern Cape Province) (Pavlic *et al.* 2004).

Notes: Phylogenetically this species is clearly distinct from all other *Lasiodiplodia* species. In terms of morphology, conidia of *L gonubiensis* are larger than those of any other species presently known in the genus.

Lasiodiplodia hormozganensis Abdollahz., Zare & A.J.L. Phillips, Persoonia 25: 6. 2010. MycoBank MB16779. Fig 41.

Ascomata not reported. *Conidiomata* stromatic, produced on pine needles on WA within 2–4 wk, superficial, dark brown to black, covered with dense mycelium, mostly uniloculate, up to 950 µm diam, solitary, globose, thick-walled, non-papillate with a central ostiole. *Paraphyses*, hyaline, cylindrical, thin-walled, initially aseptate, becoming up to 1–7-septate when mature, rarely branched, occasionally basal, middle or apical cells swollen, rounded at apex, up to 83 µm long, 2–4 µm wide. *Conidiophores* absent. *Conidiogenous cells* holoblastic, discrete, hyaline, smooth, thin-walled, cylindrical, 9–15 × 3–5 µm. *Conidia* initially hyaline, aseptate, ellipsoid to cylindrical, with granular contents, rounded at apex, base round or truncate, wall < 2 µm, becoming pigmented, verruculose, ellipsoid to ovoid, 1-septate with longitudinal striations, (15.5–)18–24(–25) × 11–14 µm, 95 % confidence limits = 21.2–21.7 × 12.4–12.6 µm (av. ± S.D. = 21.5 ± 1.9 × 12.5 ± 0.8 µm, L/W ratio = 1.7).

Culture characteristics: *Colonies* with abundant aerial mycelia reaching to the lid of Petri dish, aerial mycelia becoming smoke grey to olivaceous-grey at the surface and greenish grey to dark slate blue at the reverse after 2 wk in the dark at 25 °C. Colonies reaching 83 mm on MEA after 2 d in the dark at 25 °C. Cardinal temperatures for growth: min ≤ 10 °C, max ≥ 35 °C, opt 25–30 °C.

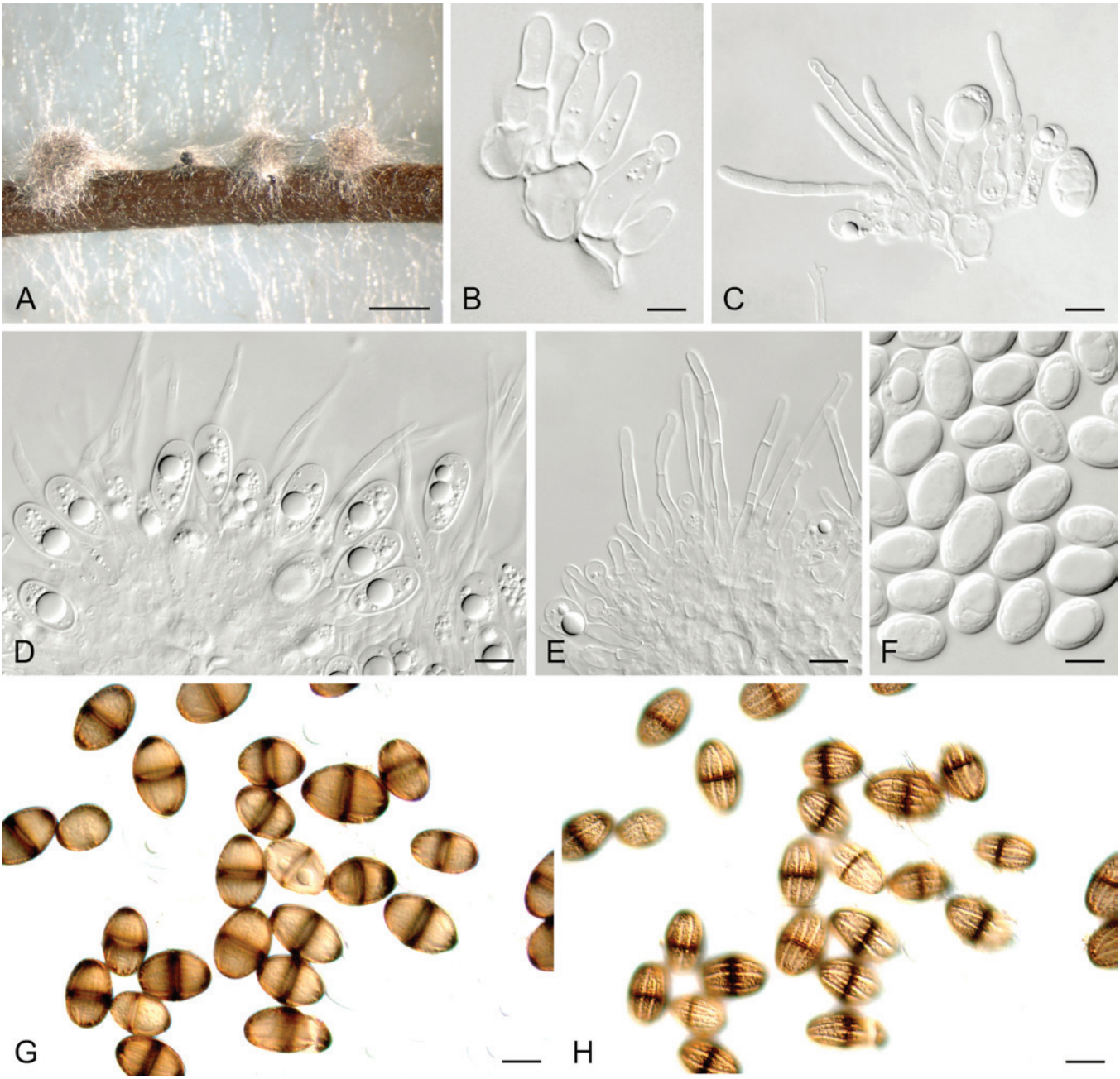

Fig. 42. *Lasiodiplodia iraniensis*. A. Conidiomata on pine needles in culture. B. Conidia developing on conidiogenous cells. C, D. Conidia developing on conidiogenous cells between paraphyses. E. Paraphyses. F. Hyaline, immature conidia. G, H. Mature conidia in two different focal planes to show the longitudinal striations. Scale bars: A = 1 mm, B, C = 5 µm, D–H = 10 µm.

Type: **Iran**, Hormozgan Province, Rodan, on twigs of *Olea* sp., Jun. 2007, J. Abdollahzadeh & A. Javadi, **holotype** IRAN 14271F.

Cultures: IRAN 1500C = CBS 124709 (ex-type), IRAN 1498C = CBS 124708.

Hosts: *Mangifera indica*, (Abdollahzadeh *et al.* 2010, Marques *et al.* 2013), *Olea* sp. (Abdollahzadeh *et al.* 2010).

Known distribution: Iran (Hormozgan Province) (Abdollahzadeh *et al.* 2010), Brazil (Marques *et al.* 2013).

Notes: Phylogenetically and morphologically, this species is closely related to *L. citricola*, *L. egyptiacae*, *L. parva* and *L. pseudotheobromae*, but can be distinguished based on average conidial dimensions and paraphyses length. This species does not produce a pink pigment in PDA cultures at 35 °C.

Lasiodiplodia iraniensis Abdollahz., Zare & A.J.L. Phillips, Persoonia 25: 8. 2010. MycoBank MB16780. Fig 42.

Ascomata not reported. *Conidiomata* stromatic, produced on pine needles on WA within 2–4 wk, superficial, dark brown to black, covered with dense mycelium, mostly uniloculate, up to 980 µm diam, solitary, globose, thick-walled, non-papillate with a central ostiole. *Paraphyses*, hyaline, cylindrical, thin-walled, initially aseptate, becoming up to 1–6 septate when mature, rarely branched, occasionally basal, middle or apical cells swollen, rounded at apex, up to 127 µm long, 2–4 µm wide. *Conidiophores* absent. *Conidiogenous cells* holoblastic, discrete, hyaline, smooth, thin-walled, cylindrical, 9–16 × 3–5 µm. *Conidia* initially hyaline, aseptate, subglobose to subcylindrical, with granular content, both ends rounded, wall < 2 µm, becoming pigmented, verruculose, ellipsoid to ovoid, 1-septate with longitudinal striations, (15.5–) 17–23(–29.5) × 11–14 µm, 95 % confidence

limits = 20.6–20.8 × 13–13.1 µm (av. ± S.D. = 20.7 ± 2 × 13 ± 0.9 µm, L/W ratio = 1.6).

Culture characteristics: *Colonies* with abundant aerial mycelia reaching to the lid of Petri dish, aerial mycelia becoming smoke grey to olivaceous-grey at the surface and greenish grey to dark slate blue at the reverse after 2 wk in the dark at 25 °C. Colonies reaching 80 mm on MEA after 2 d in the dark at 25 °C. Cardinal temperatures for growth: min ≤ 10 °C, max ≥ 35 °C, opt 25–30 °C.

Type: **Iran**, Hormozgan Province, Bandar Abbas, Geno mountain, on twigs of *Salvadora persica*, Mar. 2007, J. Abdollahzadeh & A. Javadi, **holotype** IRAN 14268F.

Cultures: IRAN 1520C = CBS 124710 (ex-type), IRAN 1519C.

Hosts: *Citrus* sp., *Eucalyptus* sp., *Juglans* sp., *Mangifera indica*, *Salvadora persica*, *Terminalia catapa* (Abdollahzadeh *et al.* 2010).

Known distribution: Brazil (Marques *et al.* 2013), Iran (Hormozgan & Golestan Provinces) (Abdollahzadeh *et al.* 2010).

Notes: Phylogenetically this species is closely related to *L. mahajangana*, *L. theobromae* and *L. viticola*. This species can be easily separated from the first two species based on conidial dimensions. Conidia of *L. iraniensis* (av. = 20.7 × 13 µm) are larger and smaller than those of *L. mahajangana* (av. = 17.5 × 11.5 µm) and smaller than *L. theobromae* (av. = 26.2 × 14.2 µm). Conidia of *L. viticola* (av. = 19.5 × 9.5 µm) are shorter and narrower than those of *L. iraniensis*. Furthermore, the paraphyses in *L. iraniensis* are longer than 100 µm, while they are less than 100 µm in *L. viticola*. Although, conidial dimensions of *L. iraniensis* are similar to those of *L. parva*, the average width of conidia of *L. iraniensis* (13 µm) is greater than in *L. parva* (av. width = 11.5 µm). This species produces a pink pigment in PDA cultures at 35 °C.

Lasiodiplodia lignicola (Ariyawansa, J.K. Liu & K.D. Hyde) A.J.L. Phillips, A. Alves & Abdollahz., **comb. nov.** MycoBank MB805462. Fig. 43.
Basionym: *Auerswaldia lignicola* Ariyawansa, J.K. Liu & K.D. Hyde, Fungal Divers. 57: 161. 2012.

Saprobic on dead wood. *Ascomata* 0.5–0.75 mm diam, 0.75–1 mm high, dark brown to black, developing on host tissue, semi-immersed, globose to subglobose, coriaceous, multiloculate, with 4–5 locules, with individual ostioles, cells of ascostromata brown-walled *textura angularis*. *Locules* 100–130 × 110–130 µm, with individual papillate ostioles. *Peridium* of locules 30–60 µm diam, thick-walled, wall composed of outer layers of thick-walled, dark brown cells of *textura angularis*, inner layers of thin-walled cells of *textura angularis*. *Pseudoparaphyses* not observed. *Asci* bitunicate, fissitunicate, clavate to broadly clavate, with short and narrow pedicel, rounded at the apex with an ocular chamber, 80–90 × 15–25 µm. *Ascospores* uniseriate or partially overlapping, reddish brown to dark brown, aseptate, fusiform to ellipsoid with narrowly rounded ends, smooth-walled, 15–20 × 8–10 µm (av. of 40 ascospores = 19 × 9 µm). *Conidiomata* indistinguishable from ascomata. *Paraphyses* aseptate, thin-walled, with slightly bulbous tip up to 15 µm long. *Conidiophores* hyaline, thin-walled, cylindrical, 6–12 × 2.5–3 µm. *Conidiogenous cells* hyaline, thin-walled, smooth, cylindrical, forming a single conidium at the tip, holoblastic, proliferating at the same level giving rise to periclinal thickenings, 10–15 × 2.5–3.5 µm. *Conidia* hyaline, smooth, thick-walled, globose to ovoid, becoming dark brown with longitudinal striations, (15–)16–17.5 × (8–)8.5–10.5(–11) µm, L/W ratio = 1.7.

Culture characteristics: *Colonies* growing slowly on MEA, reaching 3 mm after 5 d at 27 °C, effuse, velvety, with entire to slightly undulate edge, dark brown to black.

Type: **Thailand**, Chiang Rai Province, Muang District, Bandu, on dead wood, 30 Sep. 2011, A.D. Ariyawansa, **holotype** MFLU 12–0750.

Cultures: MFLUCC 11-0435 = CBS 134112 (ex-type), MFLUCC 11-0656.

Hosts: Dead wood of unknown host (Liu *et al*. 2012).

Known distribution: Thailand (Liu *et al.* 2012).

Notes: This species was introduced by Liu *et al.* (2012) under *Auerswaldia lignicola*. However, in the phylogenenies presented here, it is obviously a distinct species in *Lasiodiplodia* and formed a clade as a group basal to all other species. This is one of the few species in which the asexual morph and sexual have been definitively linked, and the dark brown ascospores (Liu *et al.* 2012) are assumed to be a typical feature of the genus.

Lasiodiplodia mahajangana Begoude, Jol. Roux & Slippers, Mycol. Prog. 9: 110. 2010. MycoBank MB514012. See Didier Begoude *et al.* (2010) for illustrations.

Ascomata not reported. *Conidiomata* stromatic, produced on pine needles on MEA within 2 wk, up to 300 µm diam, solitary and covered by mycelium, superficial, conical, unilocular, with long necks (up to 200 µm) and single ostioles at the tips, locule walls thick, consisting of two layers: an outer dark brown *textura angularis*, lined with inner thin-walled, hyaline cells. *Paraphyses* rare, cylindrical, hyaline, aseptate 1-celled, (27.5–)33.5–52.5(–66) × (2–)2.5–3.5(–5) µm, (av. of 50 paraphyses = 43 × 3 µm), rounded at the tips, unbranched. *Conidiophores* absent. *Conidiogenous cells* holoblastic, discrete, hyaline, cylindrical, (10–)10.5–18(–26) × (3–)3.5–5.5(–6) µm (av. of 50 conidiogenous cells = 14.5 × 4.5 µm, L/W ratio = 3.2). *Conidia* initially aseptate, hyaline, ellipsoid to ovoid, thick-walled (< 2.5 µm), granular content, becoming 1-septate and pigmented after release, vertical striations observed at maturity, (13.5–)15.5–19(–21.5) × (10–)11.5–13(–14) µm (av. of 50 conidia = 17.5 × 11.5 µm, L/W ratio = 1.4).

Culture characteristics: *Colonies* initially white, fluffy with abundant aerial mycelium, becoming pale olivaceous-grey after 4 d, with the reverse sides of the colonies olivaceous-grey. Optimum temperature for growth 25–30 °C, covering a 90 mm Petri dish after 3 d on MEA in the dark, no growth observed at 10 °C.

Type: **Madagascar**, Mahajanga, isolated from healthy branches of *Terminalia catappa*, Oct. 2007, J. Roux, PREM 60288 **holotype** (a dry culture of CMW 27801 = CBS 124925 on pine needles); isolated from healthy branches of *Terminalia catappa*, Oct. 2007, J. Roux, **paratype** PREM 60289.

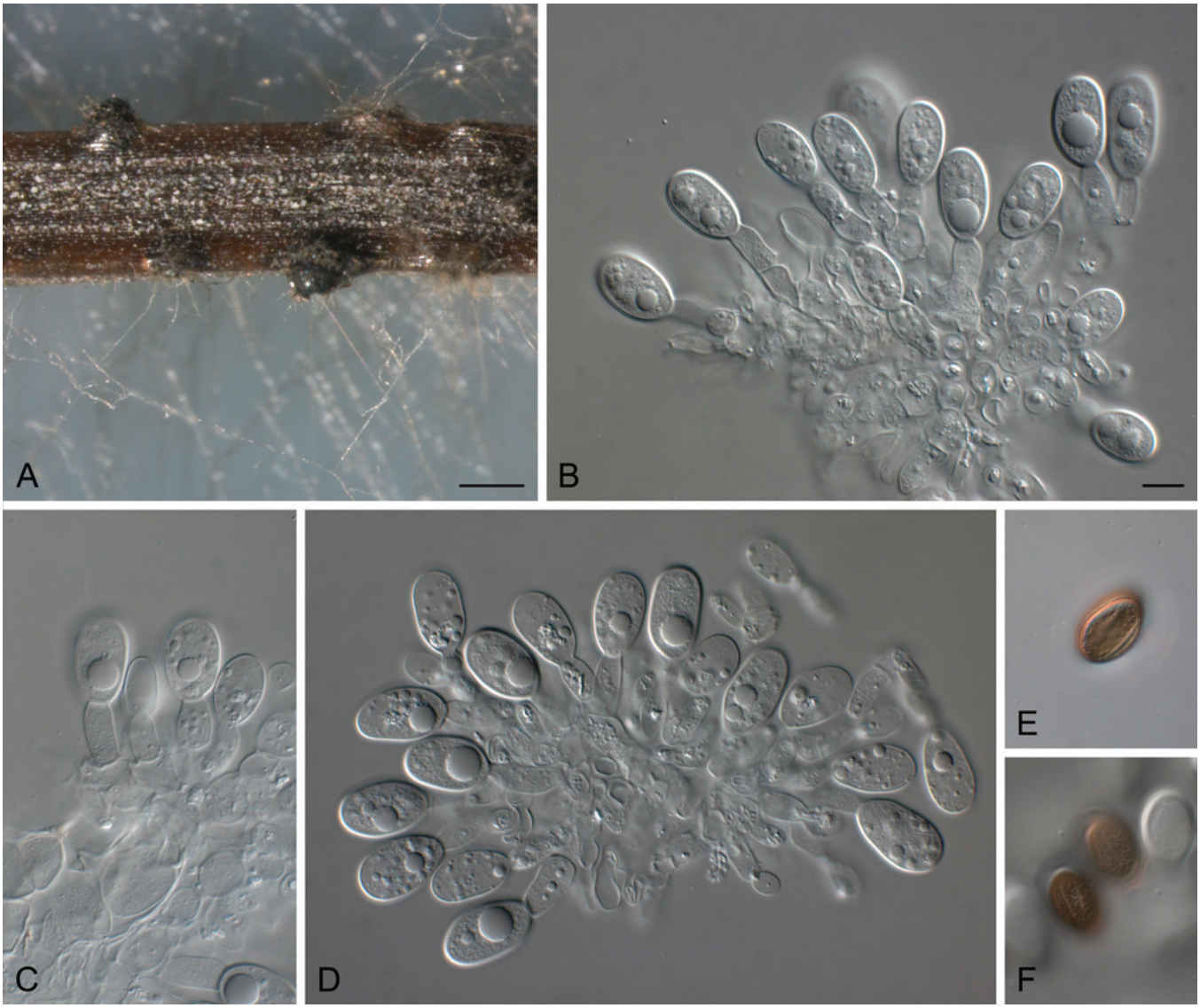

Fig. 43. *Lasiodiplodia lignicola*. A. Conidiomata developing on pine needles in culture. B–D. Conidiogenous cells. E, F. Brown, striate conidia. Scale bars: A = 500 µm, B = 10 µm. Scale bar in B applies to C–F.

Cultures: CMW 27820 = CBS 124927, CMW 27801 = CBS 124925 (ex-type).

Host: *Terminalia catappa* (Didier Begoude *et al.* 2010).

Known distribution: Madagascar (Mahajanga) (Didier Begoude *et al.* 2010).

Notes: Conidia of *L. mahajangana* are smaller than those of its closest relative, *L. theobromae*. Paraphyses of *L. mahajangana* are aseptate while those of *L. theobromae* are septate. In terms of morphology it is similar to *L. margaritacea* and the two can be distinguished only on the average lengths of their conidia (*L. mahajangana* = 17.5 µm, *L. margaritacea* = 15.3 µm).

Lasiodiplodia margaritacea Pavlic, T.I. Burgess & M.J. Wingf., Mycologia 100: 860. 2008. MycoBank MB512052. See Pavlic *et al.* (2008) for illustrations.

Ascomata not reported. *Conidiomata* stromatic, semi-immersed, solitary, globose, papillate, black, covered by hyphal hairs, up to 520 µm diam. *Paraphyses* cylindrical, 1–2-septate, hyaline, (19–)28–46(–54) × (1.5–)2–2.5(–3) µm (av. = 37.1 × 2.2 µm), formed among conidiogenous cells. *Conidiophores* absent. *Conidiogenous cells* holoblastic, cylindrical to subcylindrical, hyaline, the first conidium produced holoblastically and subsequent conidia enteroblastically, (6–)10–11(–19.5) × (2–)3–4(–4.5) µm (av. = 10.3 × 3.3 µm). *Conidia* globose to subglobose to obovoid, (12–)14–17(–19) × (10–)11–12(–12.5) µm (av. of 50 conidia = 15.3 × 11.4 µm, L/W ratio = 1.3), with granular content, thick-walled (1–2 µm), initially unicellular, hyaline, becoming cinnamon to sepia, forming one septum and longitudinal striations with maturation.

Culture characteristics: *Colonies* initially white to smoke grey with woolly aerial mycelium, becoming pale olivaceous-grey within 5–7 d, olivaceous-grey to iron-grey with age, margins regular. Submerged mycelium dense, reverse grey olivaceous to olivaceous-black after 7 d, becoming black with age. Optimum growth at 30 °C, covering the 90 mm Petri dish after 3 d in the dark.

Type: **Australia**, Western Australia, Tunnel Creek Gorge, on *Adansonia gibbosa*, Jul. 2006, T.I. Burgess, **holotype** PREM 59844 (a dry culture of CMW 26162 on pine needles).

Cultures: CMW 26162 = CBS 122519 (ex-type), CMW 26163 = CBS 122065.

Host: Asymptomatic branches of *Adansonia gibbosa* (Pavlic *et al*. 2008).

Known distribution: Australia (Western Australia) (Pavlic *et al*. 2008).

Notes: The small sub-globose conidia clearly distinguish this species from all species other than *L. mahajangana*, and these two can be separated morphologically only on average conidial lengths (*L. mahajangana* = 17.5 µm, *L. margaritacea* = 15.3 µm). Phylogenetically, however, they are clearly two distinct species.

Lasiodiplodia missouriana Úrbez-Torres, Peduto & Gubler, Fungal Divers. 52: 181. 2012. MycoBank MB519954. See Úrbez-Torres *et al.* (2012) for illustrations.

Ascomata not reported. *Conidiomata* stromatic, superficial, formed on PDA within 2–3 wk, black, covered with mycelium, up to 320 µm diam, globose to ovoid, thick-walled, unilocular, with a central ostiole, often oozing conidia. *Paraphyses* hyaline, cylindrical, aseptate, not branched, round at apex, up to 55 µm long, 2–3 µm wide. *Conidiophores* absent. *Conidiogenous cells* holoblastic, hyaline, smooth, cylindrical. *Conidia* produced in culture initially hyaline, unicellular, ellipsoid to ovoid, thick-walled (1–2 µm), contents granular, becoming dark brown, 1-septate, with longitudinal striations while still inside the conidiomata, (16–)17.5–19.5(–21) × (8–)9–10.5(–11.5) µm (av. of 60 conidia ± 18.5 × 9.8 µm, L/W ratio = 1.9).

Culture characteristics: *Colonies* on PDA with moderately dense aerial mycelium, initially white becoming pale olivaceous-grey within 7 d and turning iron grey to greenish black within 28 d; reverse dark slate blue after 28 d. Colonies covering the dish on PDA after 48 h in the dark at 25 °C. Cardinal temperatures for growth: min 10 °C, max 35 °C, opt 25–30 °C.

Type: **USA**, Saint James, on *Vitis vinifera* × *V. labrusca* hybrid cv. Catawba, Jun. 2006, R.K. Striegler & G.M. Leavitt, **holotype** UCD2193MO.

Cultures: UCD2193MO = CBS 128311 (ex-type), UCD2199MO = CBS128312.

Hosts: *Vitis* spp. (Úrbez-Torres *et al*. 2012).

Known distribution: USA (Missouri) (Úrbez-Torres *et al*. 2012)

Notes: The small conidia of this species distinguish it morphologically from all others except *L. hormozganensis* and these two can be distinguished only by small differences in conidial widths (*L. missouriana* = 8–12 µm, *L. hormozganensis* = 11–14 µm). Nevertheless, phylogenetically they are clearly two distinct species.

Lasiodiplodia parva A.J.L. Phillips, A. Alves & Crous, Fungal Divers. 28: 9. 2007. MycoBank MB510942. Fig. 44.

Ascomata not reported. *Conidiomata* stromatic, formed on poplar twigs in culture, uniloculate, dark brown to black, immersed in the host becoming erumpent when mature. *Paraphyses* hyaline, cylindrical, septate, ends rounded, up to 105 µm long, 3–4 µm wide arising amongst the conidiogenous cells. *Conidiophores* absent. *Conidiogenous cells* hyaline, smooth, cylindrical, slightly swollen at the base, holoblastic, proliferating percurrently to form one or two annellations, or proliferating at the same level giving rise to periclinal thickenings. *Conidia* ovoid, apex broadly rounded, base rounded or truncate, widest in the middle or upper third, thick-walled, initially hyaline and aseptate and remaining so for a long time, becoming 1-septate and dark-walled only some time after release from the conidiomata, with melanin deposits on the inner surface of the wall arranged longitudinally giving a striate appearance to the conidia, (15.5–)16–23.5(–24.5) × (10–)10.5–13(–14.5) µm, 95 % confidence limits = 19.8–20.5 × 11.4–11.7 µm (av. ± S.D. = 20.2 ± 1.9 × 11.5 ± 0.8 µm, L/W ratio = 1.8).

Type: **Colombia**, Dep. Meta, Villavicencio, cassava field soil, 1978, O. Rangel, **holotype** CBS H-19915.

Cultures: CBS 456.78 (ex-type), CBS 494.78.

Hosts: Cassava-field soil, *Theobroma cacao* (Alves *et al.* 2008).

Known distribution: Colombia, Sri Lanka (Alves *et al.* 2008).

Notes: This species can be separated from its closest relatives, *L. citricola*, *L. egypticae*, *L. hormozganensis* and *L. pseudotheobromae* based on conidial and paraphyses dimensions. In terms of morphology it is similar to *L. iraniensis* and the two species can be separated only on the average width of conidia, but phylogenetically they are clearly distinct.

Lasiodiplodia plurivora Damm & Crous, Mycologia 99: 674. 2007. MycoBank MB501322. See Damm *et al.* (2007) for illustrations.

Ascomata not reported. *Conidiomata* stromatic, produced on pine needles on SNA within 2–4 wk, solitary, globose to ovoid, dark brown, up to 400 µm diam, embedded in needle tissue, semi-immersed, unilocular, with a central ostiole; wall 4–7 cell layers thick, outer layers composed of dark brown *textura angularis*, becoming thin-walled and hyaline toward the inner region. *Conidiophores* absent. *Conidiogenous cells* holoblastic, discrete, hyaline, cylindrical, proliferating percurrently several times near the apex, 8–13 × 4–7 µm. *Paraphyses* hyaline, cylindrical, 2–7-celled, the 1–3 basal cells often broader than the apical cells, apical cell with rounded tip, sometimes branched, up to 130 µm long, 2–5 µm broad at the upper part and up to 10 µm broad at the lower part (basal cells). *Conidia* initially aseptate, thick-walled (< 3 µm), hyaline, ellipsoidal to obovate, sometimes somewhat irregular, with granular content, becoming 1-septate after release, brown, obovate, verruculose and with longitudinal striations, (22–)26.5–32.5(–35) × (13–)14.5–17(–18.5) µm (av. ± S.D. = 29.6 ± 2.9 × 15.6 ± 1.2 µm, L/W ratio = 1.9).

Culture characteristics: *Colonies* on PDA in the dark: mycelium and surface white to pale olivaceous-grey, reverse pale olivaceous-buff to pale grey-olivaceous, flat with undulate margins. Under near-ultraviolet light: mycelium and surface white to pale mouse-grey, reverse pale olivaceous-buff to smoke-grey. Colonies 76 mm after 2

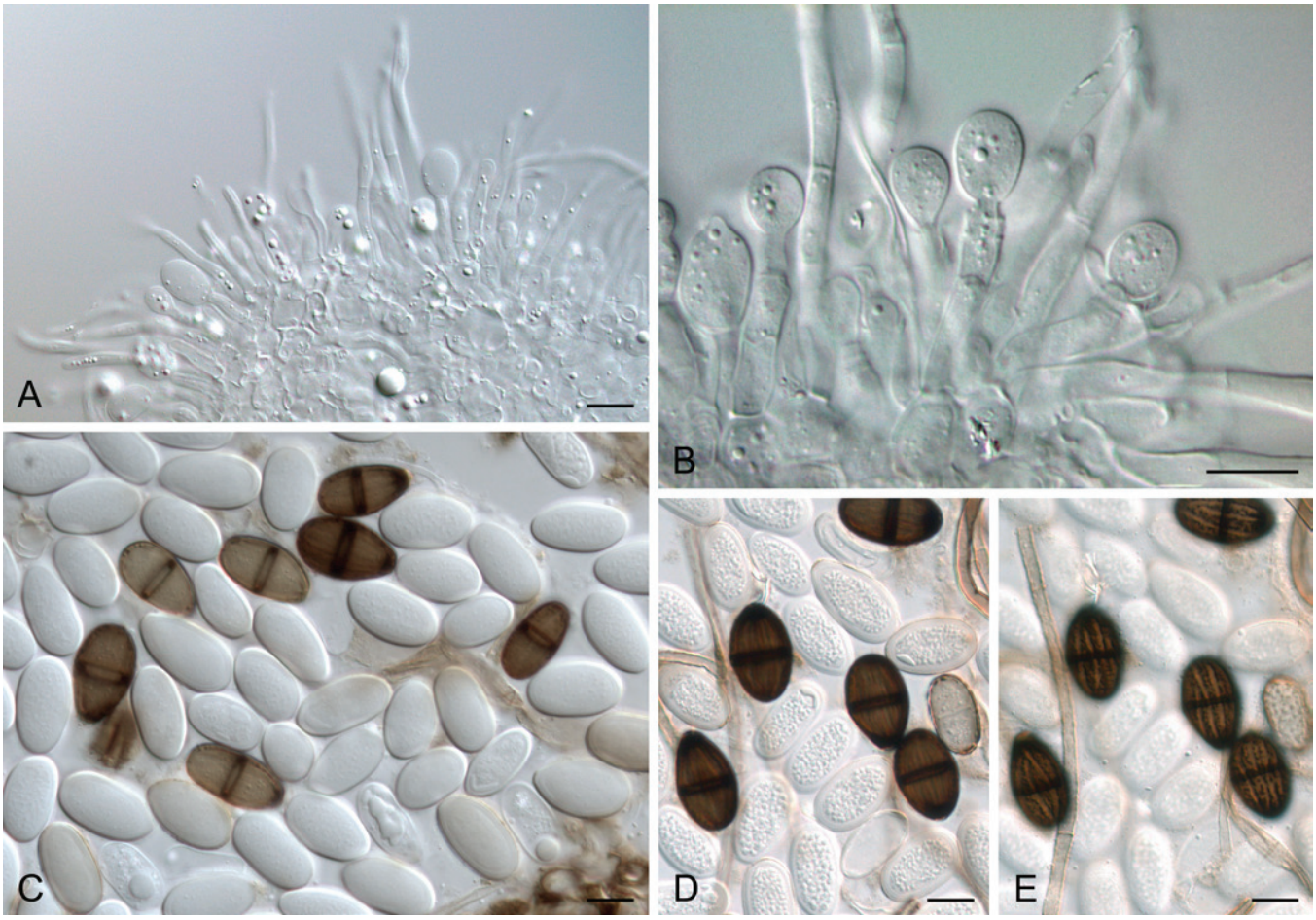

Fig. 44. *Lasiodiplodia parva*. A. Conidiogenous layer with paraphyses and developing conidia. B. Percurrently proliferating conidiogenous cells. C. Hyaline, aseptate conidia and dark-walled, septate conidia. D, E. Mature conidia at two different focal planes showing the striations on the inner side of the conidial wall. Scale bars = 10 µm.

d, reaching the edge the Petri dish after 3 d. Cardinal temperatures for growth: min 10 °C, max ≥ 35 °C, opt 30 °C.

Type: **South Africa**, Western Cape Province, Stellenbosch, from V-shaped necrotic lesion of *P. salicina*, May 2004, U. Damm, **holotype** CBS H-19844.

Cultures: CBS 120832 = STE-U 5803 (ex-type), CBS 121103 = STE-U 4583.

Hosts: *Prunus salicina*, *Vitis vinifera* (Damm *et al.* 2007).

Known distribution: South Africa (Western Cape Province) (Damm *et al.* 2007).

Notes: Phylogenetically this species is close to *L. gilanensis* and *L. missouriana*, but it can be separated from that species based on conidial dimensions and paraphyses length and shape. Conidia of *L. gilanensis* (av. = 29.6 × 15.6 µm) are larger than those of *L. missouriana* (av. = 18.5 × 9.8 µm), but compared to *L. gilanensis* they are slightly shorter. Moreover, paraphyses of *L. plurivora* (up to 130 µm) are longer than 100 µm, while in *L. gilanensis* and *L. missouriana* they are consistently less than 100 µm. In terms of morphology it is close to *L. citricola*, but conidia of *L. citricola* (av. = 24.5 × 15.4 µm) are quite small compared with *L. plurivora* (av. = 29.6 × 15.6 µm).

Lasiodiplodia pseudotheobromae A.J.L. Phillips, A. Alves & Crous, Fungal Divers. 28: 8. 2007. MycoBank MB510941. Fig. 45.

Ascomata not reported. *Conidiomata* stromatic, formed on poplar twigs in culture, uniloculate, dark brown to black, immersed in the host becoming erumpent when mature. *Paraphyses* hyaline, cylindrical, mostly aseptate, sometimes branched, ends rounded, up to 58 µm long, 3–4 µm wide arising amongst the conidiogenous cells. *Conidiophores* absent. *Conidiogenous cells* hyaline, smooth, cylindrical, slightly swollen at the base, holoblastic, proliferating percurrently to form one or two closely spaced annellations. *Conidia* ellipsoidal, apex and base rounded, widest at the middle, thick-walled, initially hyaline and aseptate and remaining so for a long time, becoming 1-septate and dark brown only some time after release from the conidiomata, with melanin deposits on the inner surface of the wall arranged longitudinally giving a striate appearance to the conidia, (22.5–)23.5–32(–33) × (13.5–)14–18(–20) µm, 95 % confidence limits = 27.5–28.5 × 15.5–16.5 µm (av. ± S.D. = 28.0 ± 2.5 × 16.0 ± 1.2 µm, L/W ratio = 1.7).

Type: **Costa Rica**, San Carlos, on *Gmelina arborea*, J. Carranza-Velazquez, **holotype** CBS H-19916.

Cultures: CBS 116459 (ex-type), CBS 447.62.

Hosts: *Acacia mangium, Citrus aurantium*, *Coffea* sp., *Gmelina arborea*, *Rosa* sp. (Alves *et al.* 2008).

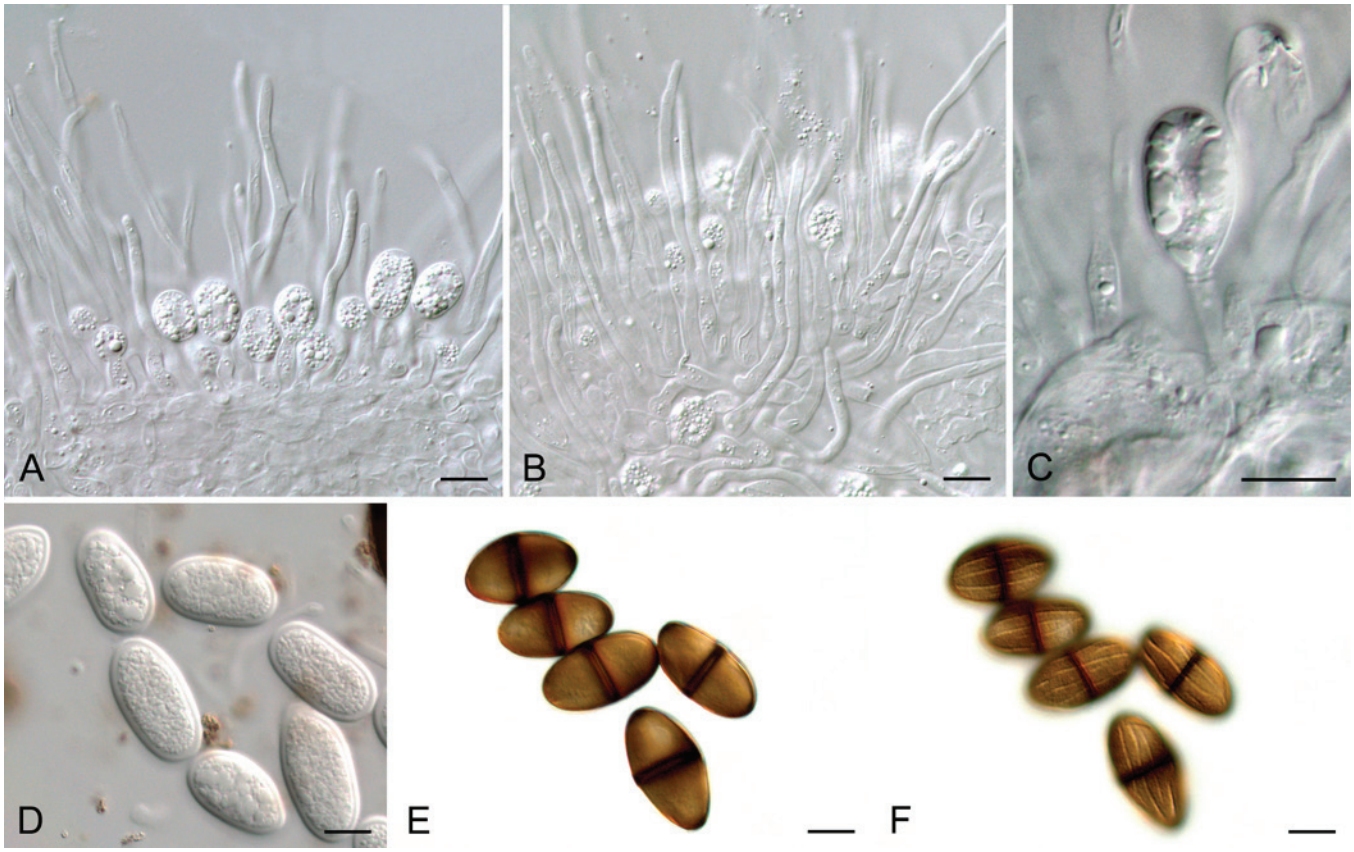

Fig. 45. *Lasiodiplodia pseudotheobromae*. A. Conidiogenous layer with developing conidia and paraphyses. B. Paraphyses. C. Conidium developing on an annellidic conidiogenous cell. D. Immature, hyaline conidia. E, F. Mature, dark-walled, one-septate, striate conidia in two different focal planes to show the striations on the inner side of the wall. Scale bars = 10 µm.

Known distribution: Costa Rica, Netherlands, Suriname, Zaire (Alves *et al.* 2008).

Notes: This species can be separated from its closest relatives, *L. citricola*, *L. egypticae*, *L. hormozganensis* and *L. parva* and as previously mentioned under *L. plurivora*. In terms of morphology it is close to *L. crassispora* but the two species differ in that the pseudparaphyses of *L. crassispora* are mostly septate, while in *L. pseudotheobromae* they are mostly aseptate.

Lasiodiplodia rubropurpurea Burgess, Barber & Pegg, Mycologia 98: 431. 2006. MycoBank MB500236. See Burgess *et al.* (2006) for illustrations.

Ascomata not reported. *Conidiomata* stromatic, superficial, globose, red to dark vinaceous, mostly solitary, 0.5–1.5 mm diam and covered with mycelium. *Paraphyses* cylindrical, aseptate, hyaline (30–)32–52(–58) × 1.5–3.5 µm (av. of 50 paraphyses = 42.4 × 2.6 µm). *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, hyaline, subcylindrical to ampulliform, 7–13(–15) × 3–5 µm (av. of 50 conidiogenous cells = 10.2 × 4 µm), proliferating percurrently with a single annellation. *Conidia* initially hyaline, unicellular, ellipsoid to obovoid, thick-walled (1 µm) with granular contents, rounded at apex, occasionally truncate at base, initially hyaline and unicellular, becoming pigmented with one septum when mature or before germination, longitudinal striations observed at maturation, 24–33 × 13–17 µm (av. of 100 conidia = 28.2 × 14.6 µm, L/W ratio = 1.9).

Culture characteristics: *Colonies* moderately dense, with appressed mycelial mat, colonies initially white to buff turning to pale olivaceous-grey within 7 d and becoming darker with age. After 7 d submerged mycelia olivaceous-grey, becoming black with age. Optimum temperature for growth 25–30 °C, reaching 76 mm on PDA after 3 d at both 25 °C and 30 °C in the dark.

Type: **Australia**, Queensland, Tully, from canker on *Eucalyptus grandis*, May 2003, T.I. Burgess, **holotype** MURU 409.

Cultures: WAC12535 = CMW 14700 = CBS 118740 (ex-type), WAC12536 = CMW 15207.

Host: *Eucalyptus grandis* (Burgess *et al.* 2006).

Known distribution: Australia (Queensland) (Burgess *et al.* 2006).

Note: The red-purple conidiomata of *L. rubropurpurea* are unique in this genus and distuinguish it from all other species (Burgess *et al.* 2006).

Lasiodiplodia theobromae (Pat.) Griff. & Maubl., Bull. Soc. Mycol. Fr. 25: 57. 1909. MycoBank MB188476. Fig. 46.

Basionym: *Botryodiplodia theobromae* Pat., Bull. Soc. Mycol. Fr. 8: 136. 1892.

≡ *Diplodia theobromae* (Pat.) W. Nowell, Diseases of Crop Plants in the Lesser Antiles: 158. 1923.

= *Sphaeria glandicola* Schwein., Trans. Am. phil. Soc., Ser. 2 4(2): 214. 1832.

≡ *Physalospora glandicola* (Schwein.) N.E. Stevens, Mycologia 25: 504. 1933.

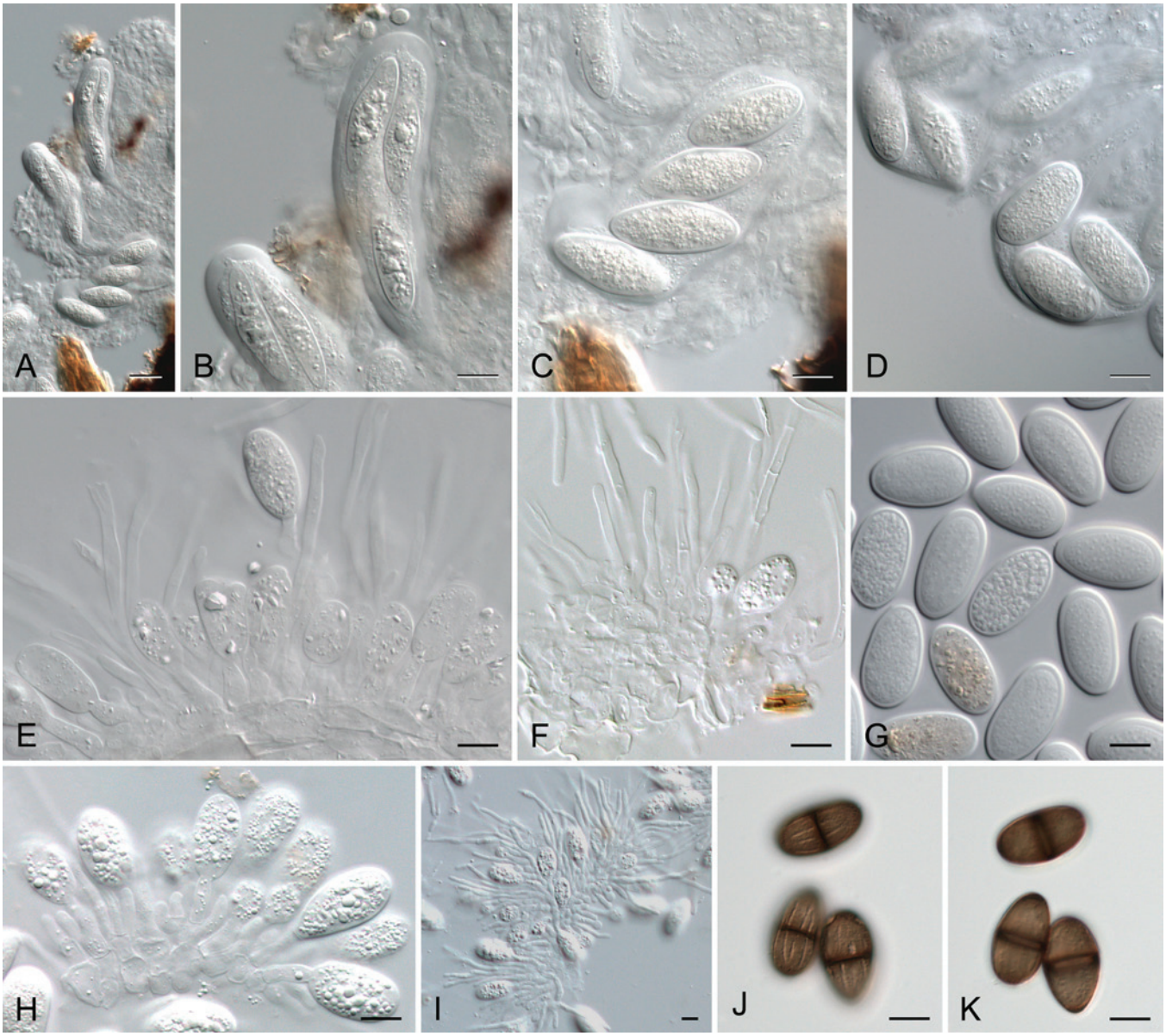

Fig. 46. *Lasiodiplodia theobromae* (A–D from holotype of *Sphaeria rhodina*). A, B. Asci. C, D. Ascospores. E, I. Conidiogenous layer with conidiogenous cells and paraphyses. F. Paraphyses. G. Immature hyaline conidia. H. Developing conidia. J, K. Mature, dark-walled, one-septate, striate conidia in two different focal planes. Scale bars = 10 µm.

= *Physalospora rhodina* Berk. & M.A. Curtis, Grevillea 17: 92. 1889.
≡ *Botryosphaeria rhodina* (Berk. & M.A. Curtis) Arx, Gen. Fungi Sporul. Cult. (Lehr): 143. 1970.
= *Diplodia gossypina* Cooke, Grevillea 7: 95. 1879.
= *Macrophoma vestita* Prill. & Delacr., Bull. Soc. Mycol. Fr. 10: 165. 1894.
= *Diplodia cacaoicola* Henn., Bot. Jb. 22: 80. 1895.
= *Lasiodiplodia tubericola* Ellis & Everh., Bot. Gaz. 21: 92. 1896.
≡ *Diplodia tubericola* (Ellis & Everh.) Taubenh., Am. J. Bot. 2: 328. 1915.
≡ *Botryodiplodia tubericola* (Ellis & Everh.) Petr., Ann. Mycol. 21: 332. 1923.
= *Botryodiplodia gossypii* Ellis & Barth., J. Mycol. 8: 175–176. 1902.
= *Botryodiplodia elasticae* Petch., Ann. R. Bot. Gdns Peradeniya 3: 7. 1906.
= *Diplodia arachidis* Petch., Ann. R. Bot. Gdns Peradeniya 3: 6. 1906.
= *Chaetodiplodia grisea* Petch., Ann. R. Bot. Gdns Peradeniya 3: 6. 1906.
= *Lasiodiplodia nigra* Appel & Laubert, Arbeiten Kaiserl. Biol. Anst. Ld.-u. Forstw. 5: 147. 1907.
= *Diplodia rapax* Massee, Bull. Misc. Inf., Kew: 3. 1910.
= *Diplodia natalensis* Pole-Evans Transvaal Dept. of Agricult. Sci. Bull. 4: 15. 1911 (1910).
= *Diplodia manihoti* Sacc. (as "*maniothi*"), Ann. Mycol. 12: 310. 1914.
≡ *Botryodiplodia manihoti* (Sacc.) Petr. (as "*maniothi*"), Ann. Mycol. 22: 83. 1924.
= *Botryodiplodia manihotis* Syd. & P. Syd., Ann. Mycol. 14: 202. 1916.
= *Diplodia corchori* Syd. & P. Syd., Ann. Mycol. 14: 196. 1916.
= *Diplodia musae* Died., Ann. Mycol. 14: 200. 1916.
= *Lasiodiplodia triflorae* B.B. Higgins, Bull. Georgia Exp. Stn 118: 16. 1916.
= *Diplodia ananassae* Sacc., Atti Acad. Sci. Ven.-Tren.-Istr. 10: 75. 1917.
≡ *Botryodiplodia ananassae* (Sacc.) Petr., Ann. Mycol. 27: 365. 1929.
= *Physalospora gossypina* N.E. Stevens, Mycologia 17: 198. 1925.
= *Botryodiplodia manihoticola* Petr., *In*: Petrak & Syd., Feddes Repert., Beih. 42: 143. 1926.

Ascomata dark brown to black, aggregated, thick-walled, wall composed of dark brown, thick-walled *textura angularis*, becoming thinner and hyaline towards the inner layers, 250–400 µm diam. *Asci* bitunicate, clavate, stipitate, 8-spored, 90–120 µm long. *Ascospores* irregularly biseriate, hyaline, aseptate (24–)30–35(–42) × (7–)11–14(–17) µm. *Conidiomata* stromatic, simple or aggregated, immersed in the host becoming erumpent when mature, dark brown, unilocular, thick- or thin-walled, wall formed of dark brown thick-walled *textura angularis*, frequently setose, up to 5 mm wide, ostiole central, single, papillate. *Paraphyses* hyaline, cylindrical, septate, occasionally branched, ends rounded, up to 55µm long, 3–4 µm wide. *Conidiophores* hyaline, simple, sometimes septate, rarely branched, cylindrical, arising from the inner layers of

cells lining the locules. *Conidiogenous cells* hyaline, thin-walled, smooth, cylindrical to sub-obpyriform, holoblastic, discrete, determinate or indeterminate and proliferating percurrently with one or two distinct annellations, or proliferating at the same level, giving rise to periclinal thickenings. *Conidia* subovoid to ellipsoid-ovoid, apex broadly rounded, tapering to truncate base, widest in middle to upper third, thick-walled, contents granular, initially hyaline and aseptate, remaining hyaline for a long time, becoming dark brown and 1-septate only a long time after discharge from the conidiomata, with melanin deposits on the inner surface of the wall arranged longitudinally giving a striate appearance to the conidia, (19–)21.5–31.5(–32.5) × (12–)13–17 (–18.5) µm, 95 % confidence limits = 26.2–27 × 14–14.4 µm (av. ± S.D. = 26.2 ± 2.6 × 14.2 ± 1.2 µm, L/W ratio = 1.9).

Type: **Ecuador**, on *Theobroma cacao*, Lagerheim, holotype not found, and presumably lost. **Papua New Guinea**, Madang, Jais Aben, from unidentified fruit along coral reef coast, No. 1995, A. Aptroot, CBS H-21411, **neotype designated here**; MBT176098, culture ex-neotype CBS 164.96.

Cultures: CBS 164.96 (ex-neotype), CBS 111530.

Hosts: Punithalingam (1976) refers to a wide host range. Considering that the original concept of *L. theobromae* now refers to a complex of species (Alves *et al.* 2008), many of the older records of this fungus are unreliable.

Known distribution: Widely distributed in tropical and subtropical regions (Punithalingham 1976).

Notes: *Botryodiplodia theobromae* was originally described from *Theobroma cacao* in Ecuador. In spite of searching through literature and many herbaria, we have been unable to locate the holotype specimen. In recent years numerous new species have been described, but in spite of this, the generic application of the name, *L. theobromae*, has not been resolved. To address this issue, we thus designate CBS 164.96 as ex-neotype culture, and have deposited a dried specimen as neotype. Although this isolate, from an unidentified fruit on a coral reef coast in Papua New Guinea, is from neither the type locality (Equador) nor the type substrate (cocoa plant), it has long been regarded as a reference strain for *L. theobromae*. For this reason we consider that it best serves to stabilise this species by continuing to use this isolate as a reference strain and to elevate its status to ex-neotype.

The connection between *L. theobromae* and its sexual morph has not been proven conclusively. Stevens (1925) made single ascospore cultures from a fungus that he referred to as *Physalospora gossypina* on cotton stems in Florida, and from *Hicoria*, *Ilex*, *Liquidambar*, *Quercus* and *Vitis*. In all cases the conidia formed in these cultures were morphologically identical to those of *L. theobromae*. Stevens (1926) then determined that the fungus he called *P. gossypina* was in fact *Physalospora rhodina* Cooke, which was later transferred by von Arx (1970) to *Botryosphaeria* as *B. rhodina* (Cooke) Arx. However, there have been no subsequent reports to confirm this connection, leaving some doubts about its authenticity. Thus the connection between the sexual morph and asexual morph has not been established beyond all doubt and the value of the above description of the sexual morph is questionable. Phylogenetically this species is close to *L. mahajangana*, but it is easily separated by its larger conidia (av. = 26.2 × 14.2 µm) compared with *L. mahajangana* (av. = 17.5 × 11.5 µm). In terms of morphology *L. theobromae* is similar to *L. rubropurpurea*, but it differs from *L. rubropurpurea* by the absence of red-purple conidiomata. Moreover, conidial length of this species (av. length = 26.2 µm) is slightly shorter than in *L. rubropurpurea* (av. length = 28.2 µm).

Lasiodiplodia venezuelensis T.I. Burgess, Barber & Mohali, Mycologia 98: 432. 2006. MycoBank MB500237. See Burgess *et al.* (2006) for illustrations.

Ascomata not reported. *Conidiomata* stromatic, superficial, smooth, cylindrical, mostly solitary, 0.5–1 mm diam, often oozing immature conidia. *Paraphyses* cylindrical, septate, hyaline (12–)16–41(–45) × (1.5–)2–5 µm (av. of 50 paraphyses = 28.3 × 3.5 µm). *Conidiophores* absent. *Conidiogenous cells* holoblastic, hyaline, subcylindrical to cylindrical to ampulliform, (5–)7–14(–15) × 3–4.5(–5), proliferating percurrently. *Conidia* initially hyaline, unicellular, ellipsoid to obovoid, thick-walled (1.5–)2.5(–3) µm, av. of 50 conidia = 1.96 µm) with granular contents, rounded at apex, occasionally truncate at base, becoming pigmented with one septum when mature or before germination, developing longitudinal striations when mature, 26–33 × 12–15 µm (av. of 75 conidia = 28.4 × 13.5 µm, L/W ratio = 2.1).

Culture characteristics: *Colonies* moderately dense, with appressed mycelial mat, initially white to buff turning pale olivaceous-grey within 7 d and becoming darker with age. After 7 d submerged mycelia olivaceous-grey, becoming black with age. Optimum temperature for growth 25 °C, reaching 75 mm on PDA after 3 d at 25 °C in the dark.

Type: **Venezuela**, Estado Portuguesa, Acarigua, from wood of living *Acacia mangium*, Oct. 2003, S. Mohali, **holotype** MURU 413.

Cultures: WAC12539 = CMW 13511 = CBS 118739 (ex-type), WAC12540 = CMW 13512.

Host: *Acacia mangium* (Burgess *et al.* 2006).

Known distribution: Venezuela (Burgess *et al.* 2006).

Notes: Phylogenetically, this species is closely related to *L. crassispora* and *L. rubropurpurea*, but can be distinguished from *L. rubropurpurea* by the absence of red-purple conidiomata. Furthermore, conidia of *L. venezuelensis* are narrower (av. = 28.4 × 13.5 µm) than those of *L. crassispora* (av. = 28.8 × 16 µm). In terms of morphology this species is similar to *L. viticola*, but conidia of *L. venezuelensis* (av. = 28.4 × 13.5 µm) are considerably larger than those of *L. viticola* (av. = 19.5 × 9.5 µm).

Lasiodiplodia viticola Úrbez-Torres, Peduto & Gubler, Fungal Divers. 52: 183. 2011. MycoBank MB519966. See Úrbez-Torres *et al.* (2010) for illustrations.

Ascomata not reported. *Conidiomata* stromatic, solitary, formed on PDA within 3–4 wk, black, covered with moderately dense mycelium, up to 900 µm wide, globose to ovoid, thick-walled, unilocular, with a central ostiole, often oozing conidia. *Paraphyses* hyaline, cylindrical, aseptate, not branched, round at apex, up to

60 µm long, 2–3 µm wide. *Conidiophores* absent. *Conidiogenous cells* holoblastic, hyaline, smooth, cylindrical. *Conidia* produced in culture initially hyaline, unicellular, ellipsoidal, base rounded or truncate, thick-walled (1–2 µm), granular content, becoming dark brown, 1-septate, with longitudinal striations while still inside the conidiomata, (16.5–)18–20.5(–23) × (8–)9–10.1(–10.5) µm (av. of 60 conidia = 19.5 × 9.5 µm, L/W ratio = 2.05). *Colonies* on PDA with dense aerial mycelium, mycelium initially white becoming pale olive-buff within 7 d and turning iron grey to greenish black within 28 d, reverse dark slate blue after 28 d, reaching 90 mm on PDA after 48 h in the dark at 25 °C. Cardinal temperatures for growth: min 10 °C, max 35 °C, opt 25–30 °C.

Type: **USA**, Arkansas, Altus, on interspecific hybrid grape Vignoles cv. Ravat 51R, D. Cartwright & W. D. Gubler, **holotype** UCD2553AR.

Cultures: UCD2553AR = CBS 128313 (ex-type), UCD2604MO = CBS 128314.

Hosts: *Vitis* hybrids (Úrbez-Torres *et al.* 2010).

Known distribution: USA (Arkansas and Missouri) (Úrbez-Torres *et al.* 2010).

Note: Phylogenetically this species is closely related to *L. mahajangana*, *L. theobromae* and *L. iraniensis*, but can be easily distinguished based on conidial and paraphyses dimensions (see notes for *L. iraniensis*).

Macrophomina Petr. Ann. Mycol. 21: 314. 1923. MycoBank MB8814.
Type species: *Macrophomina phaseolina* (Tassi) Goid., Annali Sper. agr. N.S. 1: 457. 1947.

Mycelium superficial or immersed, brown to hyaline, branched, septate, often dendroid in culture. *Ascomata* not reported. *Conidiomata* pycnidial, stromatic, separate, globose, dark brown, immersed, unilocular, thick-walled, wall consisting of an outer layer of dark brown thick-walled *textura angularis*, becoming hyaline towards the inside. *Ostiole* central, circular, papillate. *Conidiophores* absent. *Conidiogenous cells* enteroblastic, phialidic, determinate, discrete, lageniform to doliiform, hyaline, smooth, with wide aperture and minute collarette, formed from the inner cells of the pycnidial wall. *Conidia* hyaline, aseptate, obtuse at each end, straight, cylindrical to fusiform, thin-walled, smooth, guttulate. *Sclerotia* black, smooth, hard, formed of dark brown, thick-walled cells.

Note: Of the five species listed in MycoBank, only one (*M. phaseolina*) is known in culture.

Macrophomina phaseolina (Tassi) Goid., Annali Sper. agr. N.S. 1: 457. 1947. MycoBank MB300023. See Crous *et al.* (2006) for illustrations.
Basionym: *Macrophoma phaseolina* Tassi, Bull. Lab. Ort. bot. Siena 4: 9. 1901.
≡ *Tiarosporella phaseolina* (Tassi) Aa, *In*: von Arx, Gen. Fungi Sporul. Cult., Edn 3 (Vaduz): 208. 1981.
Additional synonyms listed by Holliday & Punithalingam (1988).

Sclerotia occurring in host tissue or in soil, black, smooth, hard, 100–1000 µm diam. *Ascomata* not reported. *Conidiomata* pycnidial, stromatic, dark brown to black, solitary or gregarious, up to 200 µm diam, opening by a central ostiole, wall multilayered, cells dark brown, thick-walled. *Conidiophores* reduced to conidiogenous cells that are arranged along the inner lining of the conidioma, hyaline, short obpyriform to subcylindrical, proliferating several times percurrently near the apex, 6–12 × 4–6 µm, young conidiogenous cells having a mucous layer that extends over the apex of the developing conidium. *Conidia* ellipsoid to obovoid, (16–)20–24(–32) × (6–)7–9(–11) µm; immature conidia hyaline, enclosed in a mucous sheath that upon dehiscence encloses the top half of the conidium, becoming two lateral tentaculiform, apical mucoid appendages (type C, Nag Raj 1993); mature conidia becoming medium to dark brown, with a granular outer layer that in some cases appears pitted, without any mucoid appendages; conidium hilum frequently with a marginal frill.

Cultures: **Niger**, *Vigna minima*, M. Ndiaye, CPC 11052, 11070. **Senegal**, soil, M. Ndiaye, CPC 11079, 11085, 11106, 11108. **Uganda**, *Eucalyptus* sp., Jan. 1925, CBS 162.25; **Unknown**, *Zea mays,* Jun. 1933, S.F. Ashby, CBS 227.33.

Hosts: Plurivorous.

Known distribution: Cosmopolitan.

Notes: Although *Macrophomina phaseolina* can have apical mucoid appendages as found in *Tiarosporella* (Sutton & Marasas 1976), it is distinguished by having percurrently proliferating conidiogenous cells, which are not seen in any species of *Tiarosporella sensu* Nag Raj (1993), nor in those investigated by Crous *et al.* (2006), and conidia that become dark brown at maturity, and the presence of microsclerotia. Based on these differences (and in the absence of authentic cultures of *T. paludosa*), Crous *et al.* (2006) chose to retain the genus *Macrophomina* and the name *M. phaseolina*.

Neodeightonia Booth, in Punithalingam, Mycol. Pap. 19: 17. 1970 [1969]. MycoBank MB3450.
Type species: *Neodeightonia subglobosa* Booth, in Punithalingam, Mycol. Pap. 119: 19. 1970 [1969].

Ascostromata immersed, dark brown to black, with a single aparaphysate locule, wall composed of pseudoparenchymatic cells many layers thick, asci developing amongst partially disintegrating sterile thin-walled tissue in locule. Neck of ascostromata narrow, opening by an apical ostiole, formed by the disintegration of the central thin-walled cells. *Pseudoparaphyses* hyphae-like, septate, constricted at the septa. *Asci* parallel, more or less separated from one another by stromatic tissue, clavate to cylindric-clavate, 8-spored, bitunicate with a thick endotunica. *Ascospores* biseriate, initially hyaline, brown when mature, oval to broadly ellipsoidal with a single transverse septum, surrounded or not by a mucilagenous sheath. *Conidiomata* brown to black, solitary or aggregated, sometimes intermixed with ascomata, globose, uni- to multilocular, stromatic, wall composed of dark-brown thick-walled *textura angularis*. *Paraphyses* absent. *Conidiogenous cells* holoblastic, hyaline, aseptate, cylindrical to sub-cylindrical. *Conidia* spherical to globose, initially hyaline, pale to dark brown when mature, thick-walled, smooth to finely rough-walled with fine striations.

Notes: *Neodeightonia* was introduced by Booth (Punithalingam 1969). Von Arx & Müller (1975) transferred *N. subglobosa* to *Botryosphaeria*, and because this is the type species of the genus, they reduced *Neodeightonia* to synonymy under *Botryosphaeria*. However, morphologically (based on the dark, 1-septate ascospores) and phylogenetically (Phillips *et al.* 2008), this genus is distinguishable from *Botryosphaeria,* and the genus was reinstated by Phillips *et al.* (2008). Punithalingam (1969) referred to germ slits in the conidia. Crous *et al.* (2006) suggested that these were in fact striations on the conidial wall, and that more than one could occur per conidium, a feature confirmed by Phillips *et al.* (2008). The striate walls suggest an affinity to *Lasiodiplodia*. Nevertheless, *Neodeightonia* can be distinguished from *Lasiodiplodia* by the absence of conidiomatal paraphyses. Thus, conidial striations distinguish *Neodeightonia* from *Diplodia*, and the absence of conidiomatal paraphyses distinguishes it from *Lasiodiplodia*.

DNA phylogeny

The three species fall in three clades with *N. palmicola* distantly related to the other two known species (Fig. 47).

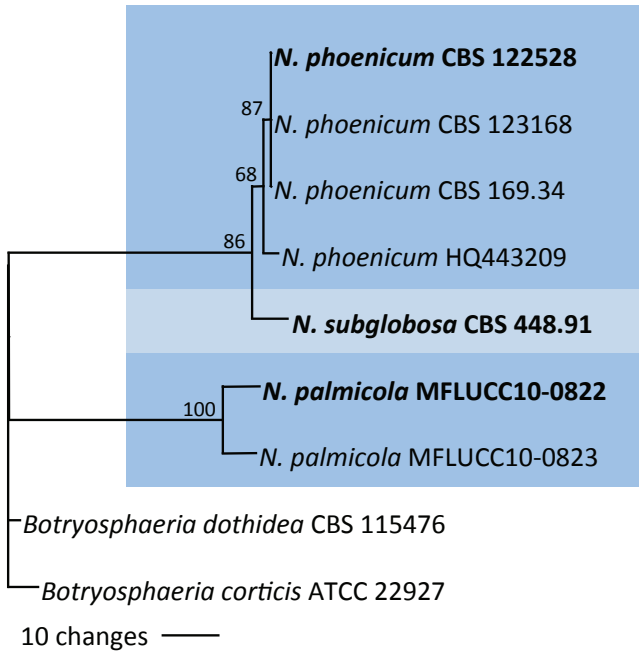

Fig. 47. The single most parsimonious tree obtained from ITS sequences of *Neodeightonia* species. Bootstrap values from 1000 replicates are given at the nodes.

Key to *Neodeightonia* spp.

The three species known in culture can be separated on conidial length:

1. Conidia less than 15 µm long, 9–12 µm long ..... *N. subglobosa*
1. Conidia longer than 15 µm 2

2. Conidia 15.5–21.5 µm long ..... *N. phoenicum*
2. Conidia never shorter than 17 µm, 17.5–24.5 µm long ..... *N. palmicola*

Species descriptions

Neodeightonia palmicola J.K. Liu, Phookamsak & K.D. Hyde, Sydowia 62: 268. 2010. MycoBank MB518804. Fig. 48.

Ascomata uniloculate, immersed to erumpent in host tissue, globose to subglobose, brown to dark brown, rounded at the base, 180–230 µm high (excluding the neck), 270–420 µm diam. *Ostiole* circular, central, papillate. *Peridium* 26–55 µm wide, comprising several layers of brown-walled cells, the outer stratum of 1–3 cells comprising thick, dark brown walls, the inner layer 3–5 cells, *textura angularis* comprising pale brown to hyaline, thin-walled cells. *Pseudoparaphyses* thin-walled, hyaline, frequently septate, often constricted at the septa, up to 3–5 µm diam. *Asci* 8-spored, bitunicate, fissitunicate, with thick endotunica, clavate to cylindrical-clavate, stipitate, apically rounded, with a well-developed ocular apical chamber, arising from the base of ascoma, (80–)110–210(–225) × 17–22.5(–24) µm (av. = 154.2 × 20.5 µm). *Ascospores* obliquely uniseriate or irregularly biseriate, ellipsoidal-fusiform or fusiform, widest in the middle, both ends obtuse, 1-celled, aseptate, hyaline, smooth, thin-walled, with bipolar germ pores, surrounded by a wing-like hyaline sheath, 23–31.5 × 8.5–12.5 µm (av. = 27 × 10 µm). *Conidioma* (formed on WA on sterilised pine needles within 21–28 d) uniloculate, semi-immersed, solitary, globose, covered by mycelium, up to 240 µm wide, wall 4–8 cell layers thick, composed of dark brown thick-walled *textura angularis*, becoming thin-walled and hyaline toward the inner region. *Conidiogenous cells* holoblastic, cylindrical to subcylindrical, hyaline, 9–20 × 3–6 µm. *Conidia* initially hyaline, unicellular, ellipsoid to obovoid, thick-walled with granular content, rounded at apex, occasionally truncate at base, aging conidia becoming cinnamon to sepia, forming a single septum, 17.5–24.5 × 9.5–12.5 µm (av. of 50 conidia = 21.5 × 11.0 µm).

Type: **Thailand**, Chiang Rai, Muang District, Khun Korn Waterfall, on dead leaves of *Arenga westerhoutii*, 18 Dec. 2009, Jian-Kui Liu, **holotype** MFLU10 0407.

Culture: MFLUCC10 0822 = CBS 136074 (ex-type).

Host: *Arenga westerhoutii* (Liu *et al*. 2010).

Known distribution: Thailand (Liu *et al*. 2010).

Notes: This species is unusual in having ascospores surrounded by a mucilagenous sheath and pycnidial paraphyses, features not seen in other species of *Neodeightonia*. Furthermore, there are no striations on the conidia and it is also phylogenetically somewhat divergent from other *Neodeightonia* species.

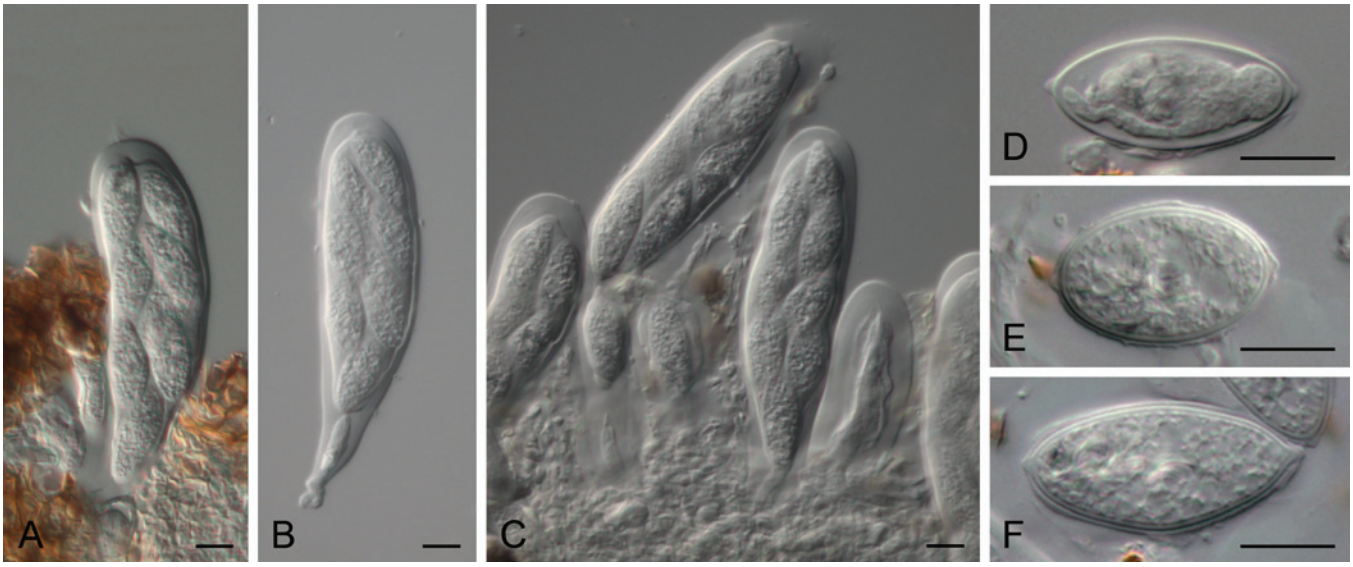

Fig. 48. *Neodeightonia palmicola*. A–C. Asci. D–F Ascospores with apiculi at either end. Scale bars = 10 µm.

Neodeightonia phoenicum A.J.L. Phillips & Crous, Persoonia 21: 43. 2008. MycoBank MB511708. Fig. 49.

= *Macrophoma phoenicum* Sacc., Annuar. R. Ist. Bot. Roma 4: 195. 1890.

≡ *Diplodia phoenicum* (Sacc.) H.S. Fawc. & Klotz, Bull. Calif. Agric. Exp. Sta. 52: 8. 1932.

≡ *Strionemadiplodia phoenicum* (Sacc.) Zambett., Bull. trimest. Soc. mycol. Fr. 70: 235. 1955 (1954).

Ascomata not reported. *Conidiomata* formed on pine needles in culture pycnidial, stromatic, multiloculate, dark brown to black, immersed in the host becoming erumpent when mature. *Paraphyses* absent. *Conidiogenous cells* hyaline, smooth, cylindrical, swollen at the base, holoblastic, proliferating percurrently to form one or two annellations, or proliferating at the same level giving rise to periclinal thickenings. *Conidia* ovoid to ellipsoid, apex and base broadly rounded, widest in the middle to upper third, thick-walled, initially hyaline and aseptate, becoming dark brown and 1-septate some time after discharge from the pycnidia, with melanin deposits on the inner surface of the wall arranged longitudinally giving a striate appearance to the conidia, (14.5–)15.5–21.5(–24) × (9–)10–12(–14) µm, 95 % confidence limits = 18.6–19.5 × 11.2–11.8 µm (av. ± S.D. = 19.1 ± 1.7 × 11.5 ± 1.1 µm), L/W ratio = 1.7.

Type: **Spain**, Catalonia, Tarragona, Salou, on *Phoenix* sp., F. Garcia, **holotype** CBS H-20108.

Cultures: CBS 122528 (ex-type), CBS 123168, CBS 169.34.

Hosts: *Phoenix* spp. (Phillips *et al.* 2008).

Known distribution: Spain, USA (California) (Phillips *et al.* 2008).

Neodeightonia subglobosa C. Booth, Mycol. Pap. 119: 19. 1970 (1969). MycoBank MB318601. Fig. 50.

≡ *Botryosphaeria subglobosa* (C. Booth) Arx & E. Müll., Stud. Mycol. 9: 15. 1975.

= *Sphaeropsis subglobosa* Cooke, Grevillea 7: 95. 1879.

≡ *Macrodiplodia subglobosa* (Cooke) Kuntze, Revis. gen. pl. 3: 492. 1898.

≡ *Coniothyrium subglobosum* (Cooke) Tassi, Bulletin Labor. Orto Bot. de R. Univ. Siena 5: 25. 1902.

Ascomata immersed, up to 300 µm wide, dark brown to black with a single locule, aparaphysate, locule filled with disintegrating sterile thin-walled tissue, amongst which the asci develop, neck narrow, cone-shaped, opening by an apical ostiole. *Asci* bitunicate, clavate, with well-developed apical chamber, 110–140 × 16–20 µm, 8-spored. *Ascospores* hyaline, aseptate, becoming brown and 1-septate, ovoid to broadly ellipsoidal, smooth or with a finely roughened surface, 20–26 × 7–10 µm. *Conidiomata* brown to black, solitary or aggregated, sometimes intermixed with ascomata, globose, uni- to multilocular, stromatic, up to 200 µm broad. *Paraphyses* absent. *Conidiogenous cells* holoblastic, simple, hyaline. *Conidia* spherical to globose, pale to dark brown when mature, smooth to finely rough-walled, 9–12 × 6–9 µm.

Type: **Sierra Leone**, Njala (Kori), on dead culms of *Bambusa arundinacea*, 17 Aug. 1954, F.C. Deighton, **holotype** IMI 57769(f).

Culture: CBS 448.91 (ex-type).

Host: *Bambusa arundinacea* (Punithalingam 1969).

Known distribution: Sierra Leone (Punithalingam 1969).

Notes: According to Phillips *et al.* (2008) the type specimen of *Neodeightonia subglobosa* contains only immature asci with hyaline ascospores. However, Punithalingam (1969) clearly described and illustrated the ascospores as brown and 1-septate. According to Punithalingam (1969) this species is homothallic and forms asci in culture.

Neofusicoccum Crous, Slippers & A.J.L. Phillips, Stud. Mycol. 55: 247. 2006. MycoBank MB500870.

Type species: *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips, Stud. Mycol. 55: 248. 2006.

Synasexual morph: Dichomera-like.

Notes: *Neofusicoccum* was introduced by Crous *et al.* (2006) for species that are morphologically similar to, but phylogenetically distinct from *Botryosphaeria* and thus could no longer be accommodated in that genus. Morphologically *Neofusicoccum*

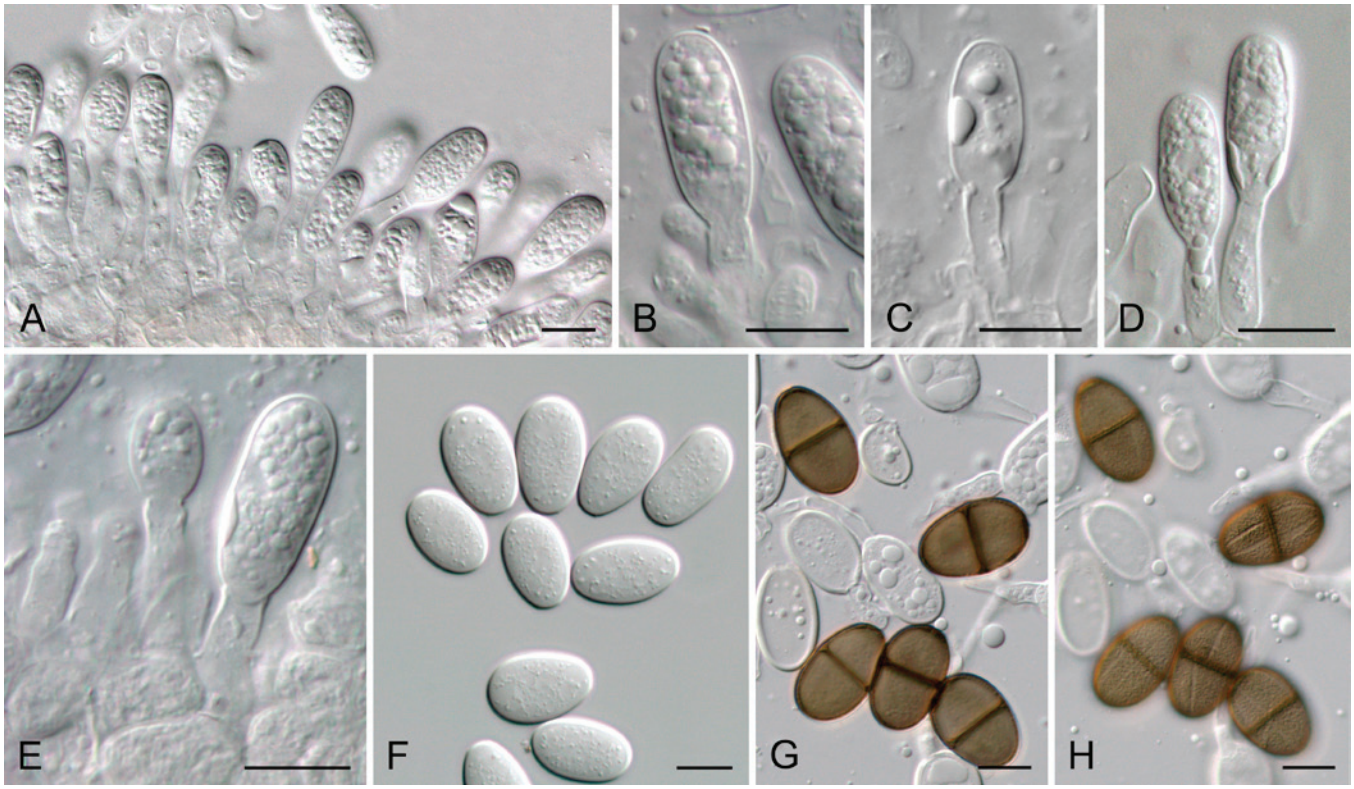

Fig. 49. *Neodeightonia phoenicum*. A. Conidiogenous layer. B–E. Conidiogenous cells. F. Hyaline, aseptate conidia. G, H. Brown, 1-septate conidia with longitudinal striations. Scale bars = 10 µm.

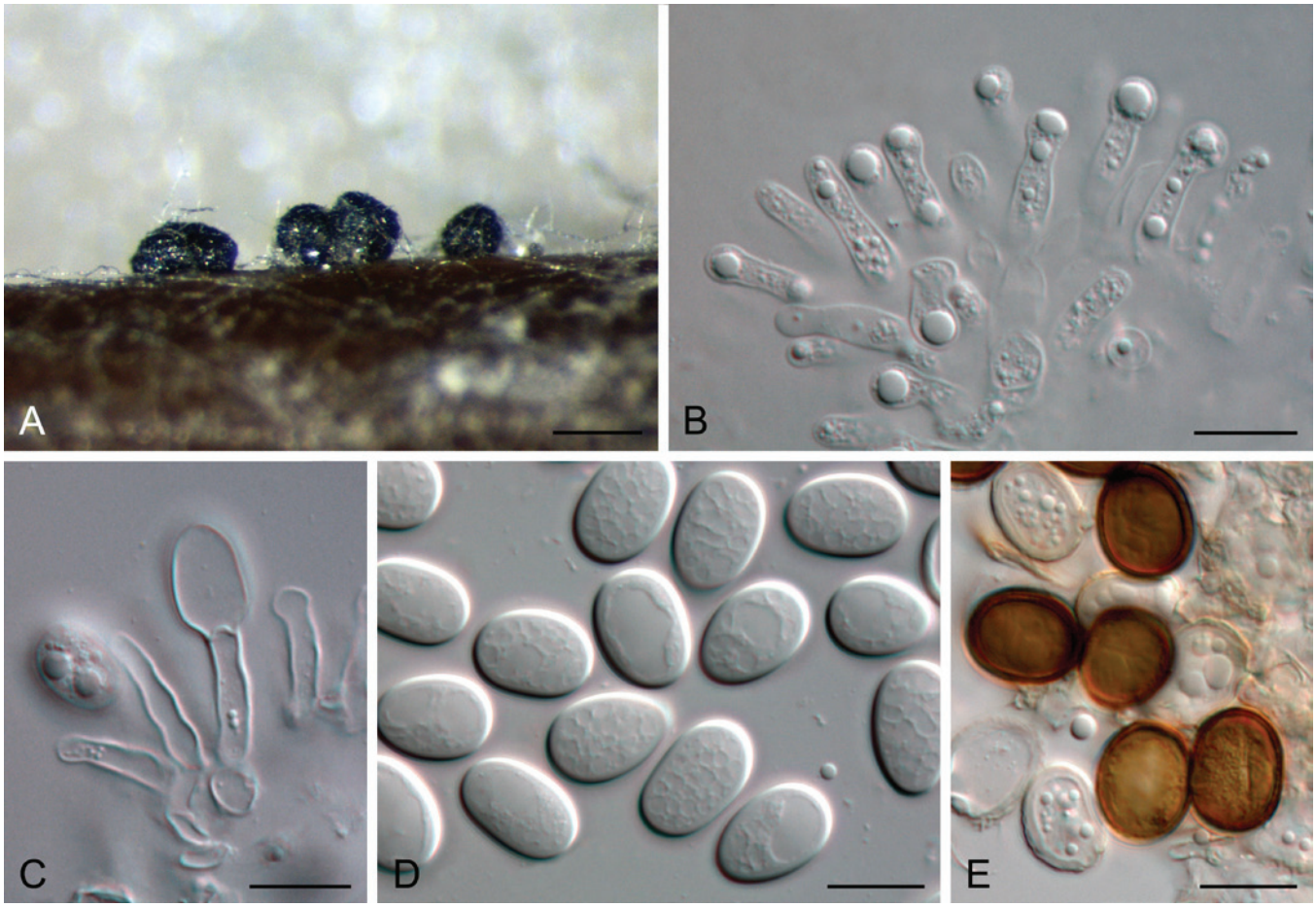

Fig. 50. *Neodeightonia subglobosa*. A. Globose conidiomata. B, C. Conidiogenous cells. D. Hyaline conidia. E. Mature, brown conidia with faint striations. Scale bars: A = 250 µm, B–E = 10 µm.

resembles *Botryosphaeria* and it can be difficult to separate the two genera. The presence of a *Dichomera* synasexual morph in *Neofusicoccum* has been used to differentiate it from *Botryosphaeria*. However, not all *Neofusicoccum* species, or even

all isolates of any given species form such a synasexual morph, and some isolates of *B. dothidea* have been reported to form dichomera-like conidia (Phillips *et al.* 2005, Barber *et al.* 2005). Paraphyses have not been reported in conidiomata of any *Neofusicoccum* species, but have been seen in most of the currently accepted *Botryosphaeria* species. However, the similarity of paraphyses to developing conidiogenous cells makes this feature difficult to apply as a general rule to separate the two genera. Conidial L/W ratios of the fusicoccum-like state are normally less than 4 and the condidia are more ellipsoidal than in the definitely fusiform ones of *Fusicoccum s. str*.

Currently 22 species are recognised in *Neofusicoccum* and they have been separated on the basis of conidial dimensions and pigmentation, pigment production in culture media and ITS sequence data, although taxonomic significance of some of these characters have recently been questioned (Abdollahzadeh *et al.* 2013). Species in some of the species complexes are morphologically indistinguishable and are defined almost exclusively on sequence of ITS often together with loci of other genes. In some cases, multi-gene sequence data are essentail to unambiguously identify the species.

Species in *Neofusicoccum* appear to be evolving quite rapidly and this is reflected in the appearance of distinct groups of isolates in various geographic regions with fixed nucleotide differences in ITS and EF1-α and other regions of the genome. Some have already been described as new species (Pavlic *et al.* 2009) while others are regarded as local variants (e.g. Lazzizera *et al.* 2008, Spagnolo *et al.* 2010). Many of the species in *Neofusicoccum* are morphologically similar and can be very difficult to distinguish from one another. *Neofusicoccum* species are notoriously variable and the full range of variability within species has not been determined for most of the species. Nevertheless, an attempt has been made to differentiate all species in the key presented here, but it must be stressed that the outcome should be checked carefully against the description of that species. Host association has been used in this key for some species that have thus far not been found on any other host. However, it must be borne in mind that this apparent host specialisation may not be absolute. For example, *N. vitifusiforme* was originally considered to be restricted to *Vitis* (van Niekerk *et al.* 2004), but was later isolated from rotting olive drupes in Southern Italy (Lazzizera *et al.* 2008) and shown to be pathogenic on that host. Some species may well be truly host specific, such as *N. arbuti* (Farr *et al.* 2005) and *N. protearum* (Denman *et al.* 2003), which have not yet been found on any other host since they were first described.

Some species can be determined relatively easily. For example, the conidia of *N. macroclavatum* and *N. pennatosporum* are far longer than any other species in the genus and these two species are easily differentiated on the shape and dimensions of their conidia.

Key to *Neofusicoccum* spp.

1. Average length of conidia 30 µm or more 2
1. Average length of conida less than 30 µm 3

2. Conidia fusiform, up to 50 µm long ..... *N. pennatisporum*
2. Conidia clavate-fusiform, length not exceeding 41 µm ..... *N. macroclavatum*

3. Average length of conidia 25 µm or more 4
3. Average length of conidia less than 25 µm 7

4. Average conidial width less than 6 µm ..... *N. andinum*
4. Average conidial width 7 µm or more 5

5. On *Eucalyptus* spp. ..... *N. eucalypticola*
5. On hosts other than *Eucalyptus* 6

6. On *Grevillea* spp., conidial length not exceeding 32 µm ..... *N. grevilleae*
6. On hosts other than *Grevillea*, conidial length up to 40 µm ..... *N. protearum*

7. Average length of conidia 20 µm or more 8
7. Average length of conidia less than 20 µm 15

8. Conidial L/W ratio 4 or more 9
8. Conidial L/W ratio less than 4 11

9. Average conidial width 6 µm, L/W ratio 4 ..... *N. mediterraneum*
9. Average conidial width less than 6, L/W ratio greater than 4 10

10. No yellow pigment, on *Syzygium cordatum* ..... *N. cordaticola*
10. Yellow pigment on PDA, on hosts other than *Syzygium* ..... *N. australe*

11. Average conidial width 7 µm or more 12
11. Average conidial width less than 7 µm 13

12. Conidial width less than 11 µm ..... *N. nonquaesitum*[1]
12. Conidial width up to 12 µm ..... *N. eucalyptorum*[1]

13. Broad host range, average conidial width less than 6 µm ..... *N. ribis*
13. Narrow host range, average conidial width greater than 6 µm 14

14. On *Syzygium cordatum*, from South Africa ..... *N. kwambonambiense*
14. On hosts other than *Syzygium*, from outside Africa ..... *N. arbuti*

15. Average conidial length less than 15 µm ..... *N. mangiferae*
15. Average conidial length greater than 15 µm 16

16. Conidial L/W ratio less than 3 17
16. Conidial L/W ratio greater than 3 18

17. Conidia fusoid to ovoid, L/W ratio 2.9 ..... *N. batangarum*
17. Conidia ellipsoid to clavate, L/W ratio 2.4 ..... *N. viticlavatum*

18. Average conidial length less than 18 µm ..... *N. parvum*
18. Average conidial length greater than 18 µm 19

19. Yellow pigment on PDA ..... *N. luteum*
19. No yellow pigment 20

20. Conidia L/W ratio 3.3 ..... *N. vitifusiforme*
20. Conidia L/W ratio 3.5 21

21. Conidia fusiform to oval, average length greater than 19 µm ..... *N. umdonicola*
21. Conidia fusiform to ellipsoid, average length less than 19 µm ..... *N. occulatum*

[1]Morphologically it is very difficult to separate these two species, but phylogenetically they are clearly distinct.

Notes: In this key we have used conidial morphology and dimensions, cultural characteristics, host association and geographic distribution to separate all the 22 described *Neofusicoccum* species. But, it is important to consider that there is overlap between species in some of those characters. Furthermore, some characters are not stable between populations or individuals of a given species. For example, not all isolates of *N. luteum* and *N. australe* produce a yellow pigment in culture media and recently we found this pigment production in some isolates of *N. parvum*. Thus, definitive identification of most of the species is dependent on the use sequence data for the ITS region alone, or more often in combination with EF1-α sequence data.

DNA phylogeny

Phylogenetic analyses were done using ITS alone and ITS combined with EF1-α. No EF1-α sequences are available for *N. protearum*, *N. corticosae* and *N. grevilleae*. Thus, the phylogenetic position of these species was deduced based on ITS phylogeny. Phylogenetic analysis using ITS sequence data revealed 21 *Neofusicoccum* species (Fig. 51). With the exception of *N. ribis* and *N. occulatum*, all of the species in the *N. ribis* / *N. parvum* species complex can be separated based on ITS alone (Fig. 51). However, the bootstrap support was quite low for most of them. In the ITS phylogeny, *D. eucalypti* and *N. corticosae* were grouped with *N. vitifusiforme* in a single clade but with only 63 % support. On the other hand, in the phylogenetic analysis based on ITS and EF1-α, *D. eucalypti* was grouped with *N. vitifusiforme* (Fig. 52), which suggests that *D. eucalypti* is a synasexual morph of *N. vitifusiforme*. Despite the absence of *N. corticosae* in the ITS/EF1-α phylogeny, in the ITS phylogenetic tree it is clear that *N. corticosae* is a synonym of *N. vitifusiforme*.

Species descriptions

Neofusicoccum andinum **(**Mohali, Slippers & M.J. Wingf.) Mohali, Slippers & M.J. Wingf., Stud. Mycol. 55: 247. 2006. MycoBank MB500871. See Mohali *et al.* (2006) for illustrations.

Basionym: *Fusicoccum andinum* Mohali, Slippers & M.J. Wingf., Mycol. Res. 110: 408. 2006.

Ascomata not reported. *Conidiomata* stromatic, superficial, produced abundantly on the surface of MEA at 25 °C, oozing conidia after 30 d at 25 °C on MEA, solitary or botryose, globose, (331–)374–597(–740) × (302–)339–557(–671) µm (av. of 50 conidiomata = 486 × 448 µm); conidiomata wall, composed of brown *textura angularis*, 6–8 cell layers thick. *Conidiogenous cells* holoblastic, hyaline, smooth, cylindrical, producing a single apical conidium, proliferating enteroblastically, (8–)11–17(–23) × (1.5–)2–2.5(–3) µm. *Conidia* hyaline, granular, clavate to slightly navicular, apex obtuse and base truncate, 0–1 septa, (19–)23–31(–40) × (4–)5–6(–8) µm (av. of 50 conidia = 27 × 5.5 mm), L/W ratio = 4.84. *Dichomera* synasexual morph not reported.

Culture characteristics: *Colonies* on MEA at 25 °C in darkness for 15 d fluffy and flat becoming pale olivaceous-grey (surface) and olivaceous buff (reverse), producing columns of mycelium reaching the Petri dish lid after 30 d at 25 °C, reaching 80 mm diam on MEA after 4 d in the dark at 25 °C. Cardinal temperatures for growth: min 15 °C (reaching an average 24 mm diam), max < 35 °C, opt 20–30 °C.

Type: **Venezuela**, Mérida State, Merida, Mucuchies (3140 m), Cordillera of Los Andes, on branches of *Eucalyptus* sp., Feb. 2003, S. Mohali, **holotype** PREM 58238.

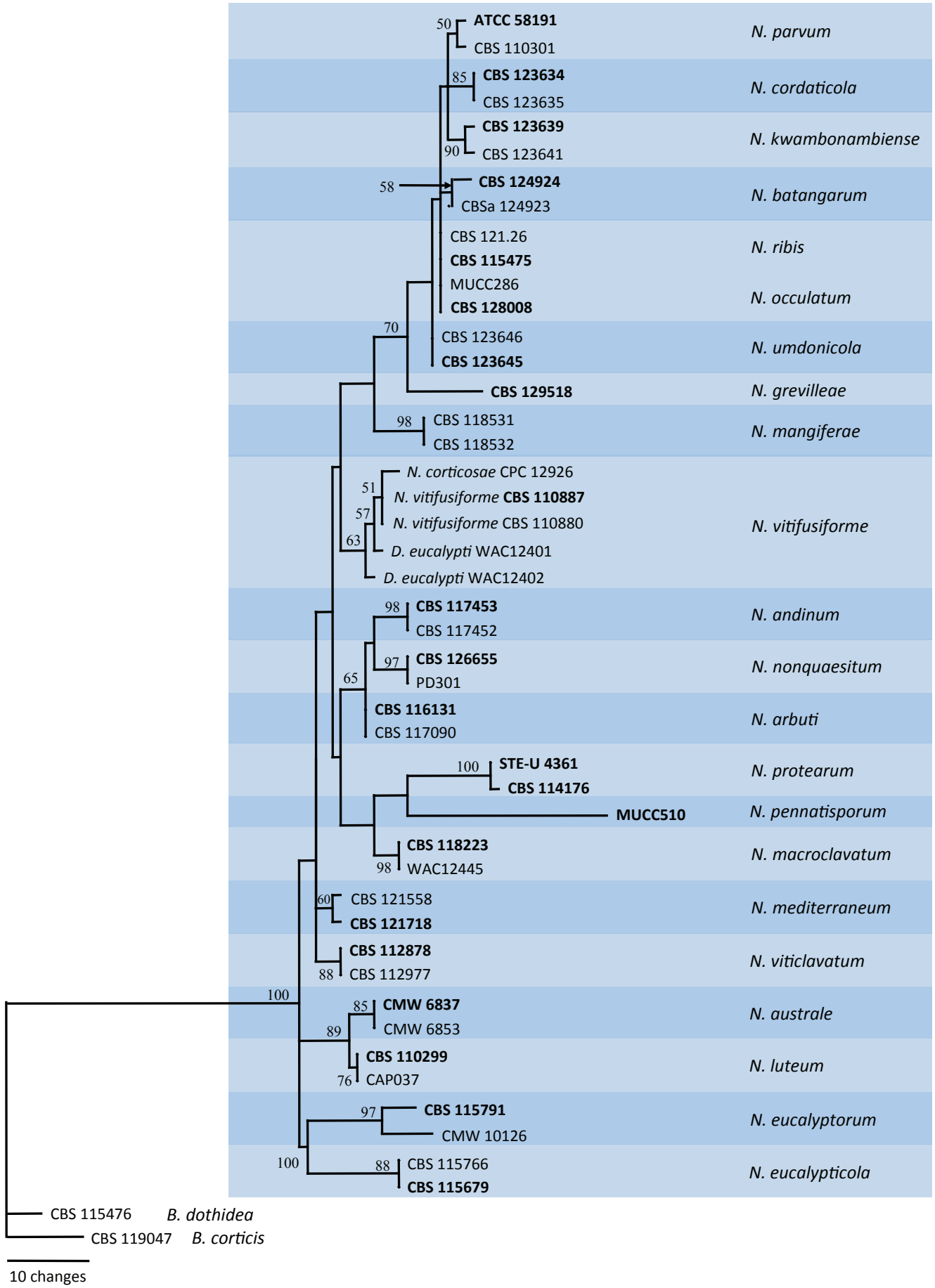

Fig. 51. Single most parsimonious tree obtained from ITS sequence data of *Neofusicoccum* species. MP bootstrap values from 1000 pseudoreplicates are given at the nodes. The tree is rooted to *Botryosphaeria dothidea* (CBS 115476) and *B. corticis* (CBS 119047).

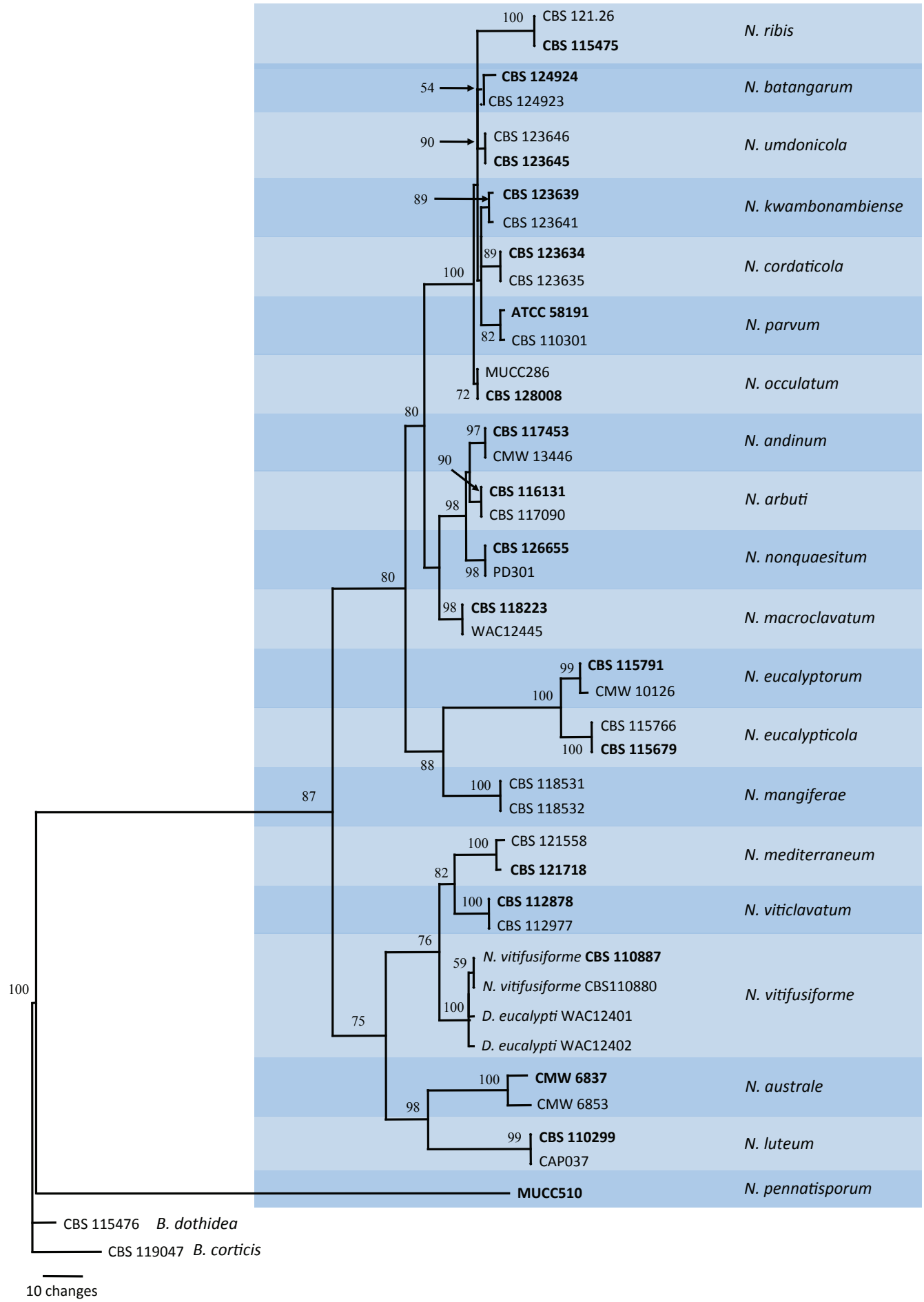

Fig. 52. Single most parsimonious tree obtained from combined ITS and EF-1α sequence data of *Neofusicoccum* species. MP bootstrap values from 1000 pseudoreplicates are given at the nodes. The tree is rooted to *Botryosphaeria dothidea* (CBS 115476) and *B. corticis* (CBS 119047).

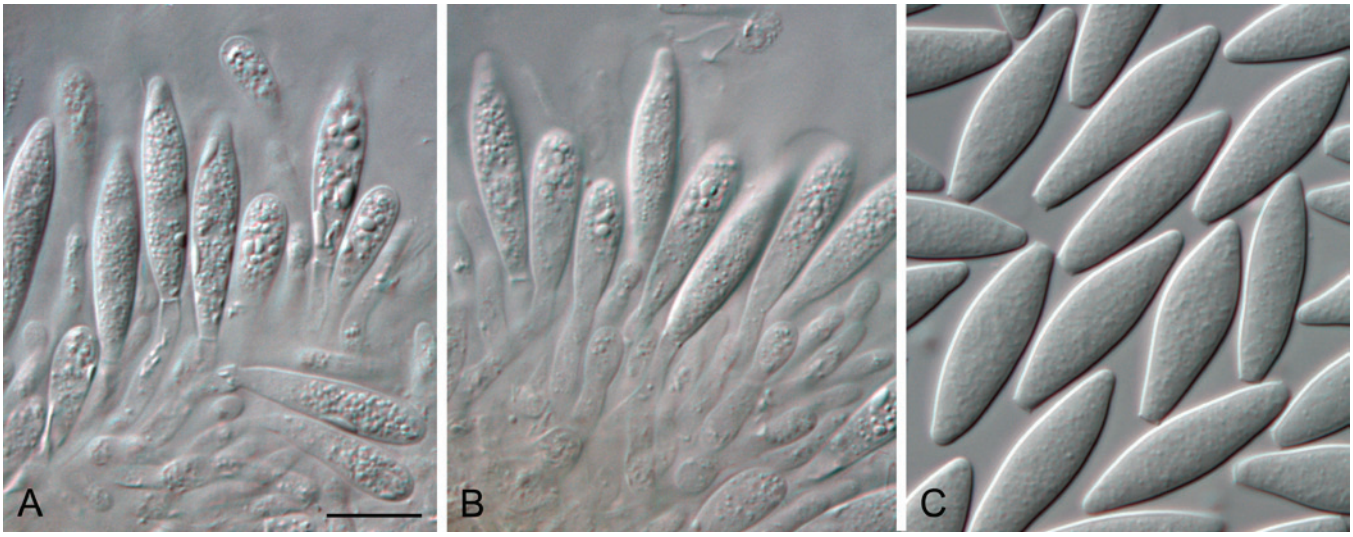

Fig. 53. *Neofusicoccum arbuti*. A, B. Conidiogenous cells. C. Conidia. Scale bar A = 10 µm. Scale bar in A applies to B and C.

Cultures: CBS 117453 = CMW 13445 (ex-type), CBS 117452 = CMW 13446.

Host: *Eucalyptus* sp. (Mohali *et al.* 2006).

Known distribution: Venezuela (Mohali *et al.* 2006).

Notes: *Neofusicoccum andinum* was introduced by Mohali *et al.* (2006) for isolates from *Eucalyptus* sp. in Venezuela. There have been no subsequent reports of this species. Based on phylogenetic inference, (ITS, EF1-α) it is most closely related to *N. arbuti* and *N. nonquaesitum*. The clavate to slightly navicular conidia of *N. andinum* separate it from *N. arbuti*, which has obovoid to fusiform conidia. Conidia of *N. andinum* are longer and narrower (27 × 5.5 µm) than those of *N. nonquaesitum* (23 × 7.5 µm).

Neofusicoccum arbuti (D.F. Farr & M. Elliott) Crous, Slippers & A.J.L. Phillips, Stud. Mycol. 55: 247. 2006. MycoBank MB500872. Fig. 53.

Basionym: *Fusicoccum arbuti* D.F. Farr & M. Elliott, Mycologia 97: 731. 2005.

Ascomata not reported. *Conidiomata* black, scattered, uniloculate to multiloculate, 0.5–1.5 × 1.5–3 mm, becoming clumped irregular in shape, papillate, stromata in longitudinal section of dark brown *textura intricata*, locule walls of several layers of thick-walled, dark-brown *textura angularis*, becoming hyaline towards conidiogenous region. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, cylindrical to subobpyriform, hyaline, discrete, determinate, occasionally indeterminate and proliferating percurrently resulting in periclinal thickenings or rarely indistinct annellations, lining inner wall of pycnidium, 9–16.5 × 2.5–3.5 µm. *Conidia* obovoid, fusiform, base truncate, apex obtuse to subobtuse, hyaline, guttulate, non-spetate, older conidia may become brownish and septate before germination, on sterile twig 18.5–27.5 × 5.5–7.5 µm (av. of 235 conidia = 22.8 × 6.4 µm), L/W ratio = 3.6. *Spermatia* cylindric to allantoid, flexuous or somewhat dumbbell-shaped, hyaline, smooth, aseptate, 3.4–6.3 × 1–1.5 µm, av. of 37 spermatia = 4.3 ± 0.6 × 1.2 ± 0.14 µm. *Dichomera* synasexual morph not reported.

Culture characteristics: *Mycelium* immersed, of branched, septate, smooth, hyaline hyphae, becoming brown, constricted with age, forming sparse, brown, thick-walled, intercalary, serial chlamydospores. *Colonies* on PDA at 25 ºC in darkness for 8 d, light yellow to olive-grey or olive-brown, darkest around plug, pigmentation extending about 2/3 of the colony width, outer area of colony white, reverse same, surface mycelium cottony except around plug where the mycelium is appressed, obscurely zonate, margin irregular, not producing yellow pigments diffusing into the agar. Cardinal temperatures for growth: opt. 25 °C, max. < 35 °C (25 mm at 15 °C, 63 mm at 20 °C, 70 mm at 25 °C, 37 mm at 30 °C, no growth at 35 °C).

Type: **USA**, Washington, King Co., Seattle, Magnolia Bluffs, isolated from cankers of *Arbutus menziesii*, Oct. 2003, collected by M. Elliott, isolated by A. Rossman, **holotype** BPI 843970.

Cultures: AR 4036 = CBS 116131 (ex-type), CBS 117090 = UW 13.

Hosts: *Arbutus menziesii* (Pacific madrone) (Farr *et al.* 2005), *Vaccinium* spp. (Espinoza *et al.* 2009).

Known distribution: Western USA and Canada from British Columbia to California (Farr *et al.* 2005), Chile (Espinoza *et al.* 2009).

Notes: This species is phylogenetically most closely related to *N. andinum* and *N. nonquasetinum*. The three species can be distinguished on the shapes and dimensions of their conidia. See notes for *N. andinum*.

Neofusicoccum australe (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips, Stud. Mycol. 55: 248. 2006. MycoBank MB500873. Fig. 54.

Basionym: *Fusicoccum australe* Slippers, Crous & M.J. Wingf., Mycologia 96: 1035. 2004.

= *Botryosphaeria australis* Slippers, Crous & M.J. Wingf., Mycologia 96: 1035. 2004.

Ascostromata erumpent through the host bark, 1.2 mm diam. *Ascomata* pseudothecial, forming botryose aggregates of 2−10,

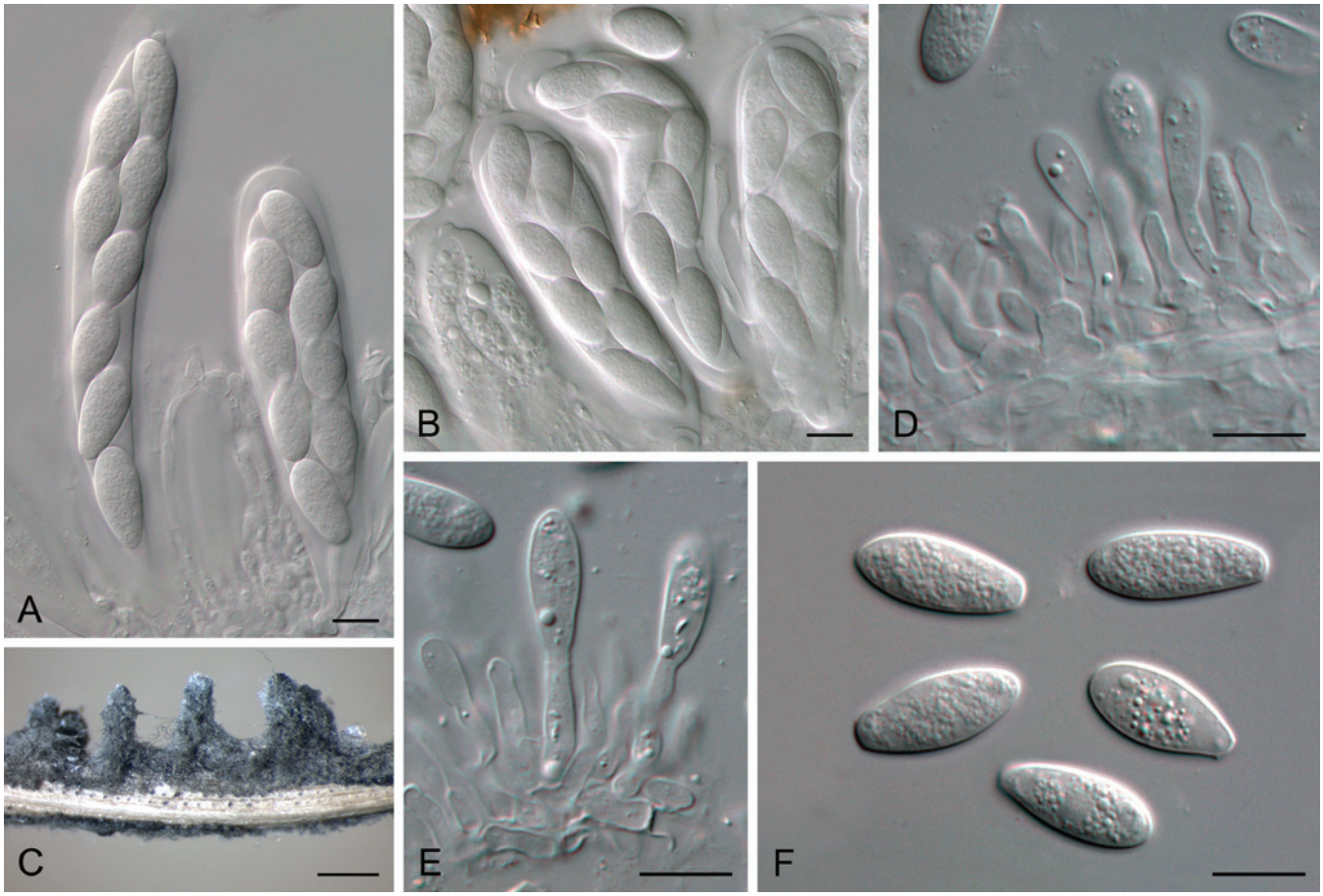

Fig. 54. *Neofusicoccum australe*. A, B. Asci with ascospores. C. Conidiomata on pine needles in culture. D, E. Conidiogenous cells. F. Conidia. Scale bars: A, B, D–F = 10 µm, C = 1 mm.

sometimes solitary, globose with a central ostiole, papillate or not, embedded with only the papilla emerging up to 2/3 emergent, black, 100−300 µm; pseudothecial wall comprising 5−8 layers of *textura angularis*, outer region of dark brown or brown cells, inner region 3−6 layers of hyaline cells lining the locules. *Asci* bitunicate, clavate, 8-spored, 60−125 × 16−26 µm. *Pseudoparaphyses* filiform, septate, rarely branched, 3−4 µm wide. *Ascospores* fusoid to ovoid, unicellular, hyaline, smooth with granular contents, biseriate in the ascus, 20−23(−25) × 7−8(−9) µm (av. of 50 ascospores = 21.9 × 7.6 µm), L/W = 2.9. *Conidiomata* stromatic, superficial, globose, mostly solitary. *Conidiogenous cells* holoblastic, hyaline, subcylindrical, phialidic with periclinal thickenings or proliferating percurrently with 1−4 annellations, 10−14 × 2−3 µm. *Conidia* hyaline, fusiform, base subtruncate to bluntly rounded, non-septate, rarely forming a septum before germination, smooth with granular contents, (18−)23−26(−30) × 5−6(−7.5) µm (av. of 240 conidia = 24.7 × 5.1 µm), L/W ratio = 4.8. *Spermatia* not seen. *Dichomera* synasexual morph: *Conidia* subglobose, obpyriform or obovoid, apex obtuse, base truncate to bluntly rounded, (9.5–)10.5–14.5(–17.5) × (7–)9–11 µm, pale brown when immature with 1–2 transverse septa, 0–1 longitudinal septa, and 0–2 oblique septa, becoming dark brown and muriform when mature with 1–3 transverse septa, 1–4 longitudinal septa, and 0–3 oblique septa.

Culture characteristics: *Colonies* buff to light primrose, light yellowish pigment diffusing into the medium, most noticeably at 15–20 °C in the dark, becoming olivaceous buff to olivaceous-grey after 5–6 d with sparse to moderately dense, appressed mycelial mat in centre with sparse tufts of aerial mycelium around the edges, margin smooth. Optimum temperature for growth 25 °C, colony reaching 48 mm diam on PDA after 4 d at 25 °C in the dark.

Type: **Australia**, Victoria, Batemans Bay, *Acacia* sp., M.J. Wingfield, **holotype** PREM 57589.

Cultures: CMW 6837 (ex-type), CMW 6853.

Hosts: *Acacia* sp. (Slippers *et al.* 2004c), *Acacia cochlearis*, *Acacia rostellifera*, *Agonis flexuosa* (Dakin *et al.* 2010), *Allocasuarina fraseriana*, *Banksia grandis*, *Callitris preissii*, *Citrus* sp. (Adesemoye & Eskalen 2011), *Chamaecyparis lawsoniana, Picea abies, Pinus pinaster, P. pinea, Sequoia sempervirens, Taxus baccata, Thuja plicata, Thujopsis dolabrata* (Alves *et al.* 2013) *Elaeocarpus holopetalus* (Cunnington *et al.* 2007), *Eucalyptus gomphocephala*, *Eucalyptus marginata*, *Santalum acuminatum* (Taylor *et al.* 2009), *Eucalyptus globulus* (Burgess *et al.* 2005, 2006), *Eucalyptus diversicolor* (Barber *et al.* 2005), *Malus domestica*, *Prunus domestica*, *Prunus dulcis*, *Prunus persica*, *Prunus salicina*, *Pyrus communis* (Damm *et al.* 2007, Slippers *et al.* 2007, Gramaje *et al.* 2012), *Olea europaea* (Lazzizera *et al.* 2008), *Persea americana* (McDonald *et al.* 2009, Auger *et al.* 2013), *Phoenix canariensis* (Cunnington *et al.* 2007), *Pistacia vera* (Armengol *et al.* 2008), *Protea cynaroides*, *Protea* sp. (Denman *et al.* 2003 (as *N. luteum*), Marincowitz *et al.* 2008), *Quercus robur* (Barradas *et al.* 2013), *Rubus* sp. (Phillips *et al.* 2006), *Salix* sp. (Cunnington *et al.* 2007), *Syzygium cordatum* (Pavlic *et al.* 2007), *Vaccinium corybosum* (Cunnington *et al.* 2007, Espinoza *et al.* 2009); *Vitis vinifera* (van Niekerk *et al.* 2004, Úrbez-Torres *et al.* 2006b, Úrbez-Torres &

Gubler 2009, Martin *et al*. 2011, White *et al.* 2011, Besoain *et al*. 2013), *Widdringtonia nodiflora* (Slippers *et al.* 2005b).

Known distribution: Australia (Slippers *et al.* 2004c, Barber *et al*. 2005, Burgess *et al.* 2005, Cunnington *et al.* 2007, Taylor *et al.* 2009), Chile (Espinosa *et al.* 2009, Auger *et al*. 2013, Besoain *et al*. 2013), Italy (Lazzizera *et al.* 2008), Portugal (van Niekerk *et al.* 2004, Phillips *et al*. 2006, Alves *et al.* 2013, Barradas *et al*. 2013), South Africa (Damm *et al*. 2007, Denman *et al*. 2003, Slippers *et al.* 2005b, Pavlic *et al.* 2007, Slippers *et al.* 2007, White *et al.* 2011), Spain (Armengol *et al.* 2008, Marincowitz *et al.* 2008, Martin *et al*. 2011, Gramaje *et al*. 2012), Spain (Tenerife) (Marincowitz *et al.* 2008), Uraguay (Perez *et al.* 2010), USA (California) (Úrbez-Torres *et al*. 2006b, McDonald *et al.* 2009, Úrbez-Torres & Gubler 2009, Adesemoye & Eskalen 2011).

Notes: This is a sister species to *N. luteum* and the two differ mainly in the intensity of the yellow pigment produced in culture, although conidia of *N. australe* are generally larger (24.7 × 5.1 µm, L/W ratio = 4.8) than those of *N. luteum* (19.7 × 5.6 µm, L/W ratio = 3.6). Slippers *et al.* (2004) first reported this species from Australia and South Africa, and mentioned a single isolate from pistachio in Italy. Nevertheless, they regarded this as a species restricted to the Southern Hemisphere. In their study of "*Botryosphaeria*" species on grapevines, van Niekerk *et al.* (2004) included an isolate of *N. australe* from *Robinia pseudoacacia* collected in Portugal. An isolate of a "*Botryosphaeria*" from *Rubus* sp., also in Portugal was also identified as *N. australe* (Phillips *et al*. 2006) and it has been isolated frequently from *Oleae europaea* in southern Italy (Lazzizera *et al.* 2008). These reports thus suggest that *N. australe* is a widespread and plurivorous species. Interestingly, *N. australe* is the dominant associate of natural woody vegetation in the south-west of Western Australia, while *N. parvum*, a species commonly isolated elsewhere in the world, is only found associated with dying trees in the peri-urban landscape. Isolates from olives in southern Italy consistently differ from typical isolates of *N. australe* by 1 bp in their ITS and 3 bp in their EF1-α sequences (Lazzizera *et al.* 2008).

Neofusicoccum batangarum Begoude, Jol. Roux & Slippers, **sp. nov.** MycoBank MB514013. See Didier Begoude *et al.* (2010) for illustrations.

≡ *Neofusicoccum batangarum* Begoude, Jol. Roux & Slippers, Mycol. Prog. 9: 113. 2010. Nom. inval., Art 37.7.

Ascomata not reported. *Conidiomata* stromatic produced on pine needles within 14 d, solitary and covered by mycelium, initially embedded, 3/4 erumpant through the pine needles at maturity, obpyriform to ampulliform with a central and circular ostiole at the neck, unilocular, locule wall thick consisting of two layers: an outer layer of dark brown *textura* cells, lined with an inner layer of of thin-walled, hyaline cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, hyaline, smooth, cylindrical, proliferating percurrently, sometimes forming a periclinal thickening, (11–)12.5–19(–27) × (2–)2.5–3 (–3.5) µm. *Conidia* non-septate, hyaline, smooth, fusoid to ovoid, thin-walled, (12–)14–17.5(–20) × (4–)4.5–6(–6.5) µm (av. of 50 conidia = 15.5 × 5.5 µm), L/W ratio = 2.9. *Spermatia* not reported. *Dichomera* synasexual morph not reported.

Culture characteristics: *Colonies* on MEA forming concentric rings, mycelium white and immersed at the leading edge, becoming smokey grey to grey-olivaceous from the old ring after 5 d on MEA. Cardinal temperatures for growth: opt 25 °C (covering the 90 mm diam Petri plate after 4 d on MEA in the dark), little growth observed at 10 and 35 °C.

Type: **Cameroon**, Kribi, Beach, isolated from healthy branches of *Terminalia catappa*, Dec. 2007, D. Begoude & J. Roux, a dry culture on pine needles, **holotype** PREM 60285.

Cultures: CMW 28363 = CBS 124924 (ex-type), CMW 28320 = CBS 124923.

Hosts: *Terminalia catappa* (Didier Begoude *et al.* 2010), *Schinus terebinthifolius* (Shetty *et al.* 2011).

Known distribution: Kribi, Cameroon (Didier Begoude *et al.* 2010), Florida, USA (Shetty *et al.* 2011).

Notes: The original description of *N. batangarum* is invalid, as no type specimen was designated, only an "ex-paratype specimen", which was in fact a typing error, as it should have read "holotype". This issue is now addressed, and the name validly published.

Based on ITS and EF1-α sequence data, *N. batangarum* is most closely related to *N. ribis* and can be distinguished from it based only on four fixed unique single nucleotide polymorphisms (SNPs) in four gene regions (ITS, EF1-α, β-tubulin and BOTF15). It can be discriminated from other species in the *N. ribis* / *N. parvum* complex by the formation of concentric rings on MEA, a characteristic that has not been observed in any other species of the complex. Furthermore, the small conidia (15.5 × 5.5 µm, L/W ratio = 2.9) clearly distinguish this species from all other species in the *N. ribis* / *N. parvum* complex. Shetty *et al.* (2011) isolated *N. batangarum* from seeds of *Schinus terebinthifolius* and showed that it is an aggressive pathogen and potential biocontrol agent of this invasive exotic tree.

Neofusicoccum cordaticola Pavlic, Slippers & M.J. Wingf., Mycologia 101: 643. 2009. MycoBank MB512498. See Pavlic *et al.* (2009) for illustrations.

Ascomata not reported. *Neofusicoccum cordaticola* is morphologically similar to other species in the *N. parvum* / *N. ribis* species complex. *Conidia* hyaline, unicellular, narrowly fusiform to oval, apices rounded, 18–28 × 4.5–7 µm (av. of 150 conidia = 23.3 × 5.3 µm), L/W = 4.3. It differs from other species in the *N. parvum* / *N. ribis* complex by uniquely fixed nucleotides in five nuclear loci: ITS (EU821898) position 141 (C), 372 (G) and 416 (C); EF1-α (EU821868) positions 58 (C) and 221 (C); β-tubulin (EU821838) position 32 (T), 96 (T) and 316 (G); locus BotF15 (EU821802) position 121 (T) and 122 (C); RNA polymerase II subunit (EU821928) positions 100 (A), 112 (T), 265 (A) and 409 (C).

Type: **South Africa**, Kwazulu-Natal Province, Sodwana Bay, on symptomless branches and leaves, dying branches and pulp of ripe fruits of *Syzygium cordatum*, Mar. 2002, D. Pavlic, a dry culture on pine needles **holotype** PREM 60066.

Cultures: CMW 13992 = CBS 123634 (ex-type), CMW 14056 = CBS 123635.

Host: *Syzygium cordatum* (Pavlic *et al.* 2009).

Known distribution: South Africa (Pavlic *et al.* 2009).

Notes: Although variation in conidial dimensions is evident in the *N. parvum* / *N. ribis* complex, it is difficult to separate all the species in this complex. Furthermore, precise identification of these species is dependent on DNA sequence comparisons.

Neofusicoccum eucalypticola (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips, Stud. Mycol. 55: 248. MycoBank MB500874. See Slippers *et al.* (2004) for illustrations.

Basionym: *Fusicoccum eucalypticola* Slippers, Crous & M.J. Wingf., Stud. Mycol. 50: 351. 2004.

= *Botryosphaeria eucalypticola* Slippers, Crous & M.J. Wingf., Stud. Mycol. 50: 351. 2004.

Ascomata pseudothecia, mostly solitary, sometimes forming a botryose aggregate of 2–3 structures, globose with a central ostiole, papillate, embedded with 1/3–2/3 emerging, black, 160–340 µm diam pseudothecial wall comprising 5–8 layers of *textura angularis*, outer region of dark or medium brown cells, inner region of hyaline cells lining the locule. *Asci* bitunicate, clavate, 8-spored, 70–110 × 20–25 µm. *Pseudoparaphyses* filiform, septate, rarely branched in the upper parts, 2–4 µm wide. *Ascospores* fusoid to ovoid, unicellular, hyaline, smooth with granular contents, biseriate in the ascus, 20–22(–23.5) × 7–8 µm (av. of 50 ascospores = 21.7 × 7.6 µm), L/W 2.8. *Conidiomata* formed on WA on sterilised pine needles within 7–21 d, stromatic, superficial, globose, mostly solitary, and covered by mycelium. *Conidia* produced in culture fusiform to rod-shaped, often bent or irregularly shaped, apex obtuse, bases subtruncate to bluntly rounded, hyaline, unicellular, sometimes forming 1–2 transverse septa before germination, smooth with finely granular contents, (20–)25–27(–35) × (5–)7–9(–10) µm (av. of 135 conidia = 26.3 × 7.2 µm), L/W = 3.6. *Spermatia* not reported. *Dichomera* synasexual morph not reported.

Culture characteristics: *Colonies* white to buff or olivaceous-grey, sometimes becoming olivaceous-black at the centre after 7 d, with a dense mat of aerial mycelium, edges smooth to crenulate, sometimes not reaching the edge of the plate. Optimum temperature for growth 25 °C, reaching 34–43 mm radius on PDA after 4 d at 25 °C in the dark.

Type: **Australia**, Victoria, Orbost, on *Eucalyptus grandis*, 2001, *M.J. Wingfield*, holotype PREM 57848.

Culture: CBS 115679 = CMW 6539 (ex-type), CBS 115766 = CMW 6217.

Hosts: *Eucalyptus* spp. (Slippers *et al.* 2004, Burgess *et al.* 2006).

Known distribution: Australia (Slippers *et al.* 2004).

Notes: *Neofusicoccum eucalypticola* is phylogenetically most closely related to *N. eucalyptorum*, and the two species can be separated on the shapes and dimensions of their conidia in culture. Thus, conidia of *N. eucalypticola* are fusiform and longer (25–27 µm) than the ovoid to clavate conidia of *N. eucalyptorum*, which are 20–23 µm long. Slippers *et al.* (2004) found that *N. eucalyptorum* was the dominant species collected from *Eucalyptus* species in eastern Australia.

Neofusicoccum eucalyptorum (Crous, H. Sm. ter. & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips, Stud. Mycol. 55: 248. 2006. MycoBank MB500875. See Smith *et al.* (2001) for illustrations.

Basionym: *Fusicoccum eucalyptorum* Crous, H. Sm. ter. & M.J. Wingf., Mycologia 93: 280. 2001.

= *Phoma australis* Cooke, Grevillea 15: 17. 1886.

≡ *Idiocercus australis* (Cooke) H.J. Swart, Trans. Brit. Mycol. Soc. 90: 283. 1988.

= *Botryosphaeria eucalyptorum* Crous, H. Sm. ter. & M.J. Wingf., Mycologia 93: 280. 2001.

Ascomata embedded in host tissue, up to 300 µm diam, becoming erumpent, solitary or botryose, stromatic, dark brown to black, with central, black ostioles. *Asci* clavate, 8-spored, bitunicate with a well-developed apical chamber, 70−140 × 15−21 µm. *Pseudoparaphyses* filiform. *Ascospores* irregularly biseriate, hyaline, aseptate, granular contents, becoming light brown with age, prominantly inequilateral when young, less so when mature, fusoid, widest in the middle, base obtuse, apex obtuse or subobtuse, (20−)23−26(−28) × (7−)8−9(−11) µm. *Conidiomata* embedded in host tissue, solitary or botryose, stromatic, globose, up to 450 µm diam, wall 6−8 layers thick, composed of brown *textura angularis*, becoming hyaline towards the inner region. *Conidiogenous cells* holoblastic, hyaline, subcylindrical, proliferating percurrently with 1−3 annellations, or proliferating at the same level with minute periclinal thickenings, 10−25 × 3.5−6 µm. *Conidia* hyaline, granular, ovoid to slightly clavate, apex obtuse, tapering towards a subtruncate or bluntly rounded base, sometimes with a minute marginal frill visible on younger conidia, (20−)22−25(−28) × (6−)7−8(−9) µm *in vivo*, (18−)20−23(−25) × 7−8(−12) µm *in vitro*. *Spermatia* not reported. *Dichomera* synasexual morph not reported.

Culture characteristics: *Colonies* on MEA iron-grey (reverse) and olivaceous-grey (surface) with extensive grey aerial mycelium, and smooth margins, attaining a radius of 21–24 mm after 4 d in darkness at 25 °C. Cardinal temperatures for growth: min > 5 °C, max < 35 °C, opt 25 °C.

Type: of sexual morph: **South Africa**, Mpumalanga, Sabie, *Eucalyptus grandis*, 1995, H. Smith, **holotype** PREM 56603; of asexual morph: **South Africa**, Mpumalanga, Sabie, *E. grandis*, 1995, H. Smith, **holotype** PREM 56604.

Cultures: The ex-type isolate was not designated in the original publication and could not be traced. Slippers *et al.* (2004b) regarded the following as representatives CBS 115791 = CMW10125, CMW 10126.

Hosts: *Eucalyptus* spp. (Burgess *et al.* 2006, Smith *et al.* 2001, Slippers *et al.* 2004b, Perez *et al.* 2010), *Myrceugenia glaucescens*, *Myrrhinium atropurpureum* var. *octandrum*, *Blepharocalyx salicifolius* (Perez *et al.* 2010).

Known distribution: Australia (Slippers *et al.* 2004b), South Africa (Smith *et al.* 2001), Uraguay (Perez *et al.* 2010).

Notes: *Neofusicoccum eucalyptorum* is a sister species to *N. eucalypticola* and the two can be separated on the shapes and dimensions of conidia formed in culture. See notes for *N. eucalypticola*.

Neofusicoccum grevilleae Crous & R.G. Shivas, Persoonia 26: 117. 2011. MycoBank MB560162. See Crous *et al.* (2011) for illustrations.

Leaf spots medium brown, situated along leaf margins, surrounded by a dark red-brown border, spots extending to the midrib, up to 7 mm diam, and up to 2 cm long. *Conidiomata* amphigenous, stromatic, up to 200 µm diam (on sterilised pine needles). Wall consisting of 3–5 layers of brown *textura angularis*. *Conidiophores* lining the inner layer of conidioma, hyaline, smooth, 0–1-septate, 15–30 × 3–5 µm. *Conidiogenous cells* holoblastic, integrated, doliiform to subcylindrical, phialidic, proliferating 2–3 times percurrently near apex, 15–25 × 3–4 µm. *Conidia* hyaline, smooth, thin-walled, with granular cytoplasm, fusoid-ellipsoidal, widest in middle or in upper third of conidium, apex subobtuse, base truncate, (20–)25–28(–32) × (6–)7–8(–10) µm (av. size of conidia = 25.7 × 7.5 µm), L/W = 3.4.

Culture characteristics: *Colonies* after 14 d at 25 °C in darkness flat, spreading, with abundant, grey aerial mycelium, covering the dish after 7 d, on PDA, OA and MEA iron-grey, sporulating poorly on water agar supplemented with sterile pine needles. *Spermatia* not reported. *Dichomera* synasexual morph not reported.

Type: **Australia**, Queensland, Brisbane, on leaves of *Grevillea aurea*, 14 Jul. 2009, P.W. Crous & R.G. Shivas, **holotype** CBS H-20578.

Cultures: CBS 129518 = CPC 16999 (ex-type).

Host: *Grevillea aurea* (Crous *et al.* 2011).

Known distribution: Australia, Western Australia (Crous & Shivas 2011).

Notes: Based on ITS sequence data, *N. grevilliae* is most closely related to the *N. ribis* / *N. parvum* complex, but conidia of *N. grevilliae* (25.7 × 7.5 µm) are larger than those of all seven species in that complex.

Neofusicoccum kwambonambiense Pavlic, Slippers & M.J. Wingf., Mycologia 101: 643. 2009. MycoBank MB512499. See Pavlic *et al.* (2009) for illustrations.

Ascomata not reported. *Neofusicoccum kwambonambiense* is morphologically similar to other related species in the *N. parvum* / *N. ribis* species complex. *Conidia* hyaline, unicellular, fusiform to ellipsoid, apices rounded 16–28 × 5–8 mm (av. 140 conidia 22.3 × 6.3 µm), L/W 3.6. It differs from other species in the *N. parvum* / *N. ribis* complex by uniquely fixed nucleotides in four nuclear loci: ITS (EU821900) position 163 (T) and 173 (G); β-tubulin (EU821840) position 175 (T), 235 (A) and 251 (A); locus BotF15 (EU821804) position 87, and 172; RNA polymerase II subunit (EU821930) positions 49 (G), 382 (A), 421 (A) and 526 (C). *Spermatia* not reported. *Dichomera* synasexual morph not reported.

Type: **South Africa**, Kwazulu-Natal Province, Kwambonambi, on symptomless branches and leaves, dying branches and pulp of ripe fruits of *Syzygium cordatum*, Mar 2002, D. Pavlic, a dry culture on pine needles, **holotype** PREM 60067.

Cultures: CMW 14023 = CBS 123639 (ex-type), CMW 14140 = CBS 123641.

Host: *Syzygium cordatum* (Pavlic *et al.* 2009).

Known distribution: South Africa (Pavlic *et al.* 2009).

Note: See notes for *N. cordaticola*.

Neofusicoccum luteum (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips, Stud. Mycol. 55: 248. 2006. MycoBank MB500876. Fig. 55.

Basionym: *Fusicoccum luteum* Pennycook & Samuels, Mycotaxon 24: 456. 1985.

= *Botryosphaeria lutea* A.J.L. Phillips, Sydowia 54: 70. 2002.

Ascomata initially immersed, later becoming erumpent through the host tissue, black, < 0.5 mm diam, uni- or multilocular, locules spherical to ovoid, 150–200 µm diam, ascomata and conidiomata often formed in the same stroma, opening through a nonperiphysate ostiole, with a short neck, wall consisting of 8–12 layers of dark brown to black, thick-walled cells, forming pseudoparenchymatic *textura angularis*, up to 60 µm thick, with 3–4 layers of thin-walled, hyaline cells lining the cavity. *Asci* bitunicate, cylindrical, to clavate, stipitate, 84–176 × 16–24 µm, 8-spored, associated with filamentous pseudoparaphyses. *Pseudoparaphyses* hyaline, septate, branched, 2–3.5 µm wide. *Ascospores* irregularly biseriate in the ascus, hyaline, guttulate, smooth, aseptate, oval to broadly fusiform, widest in the middle or upper third of the ascospore, tapering to the obtuse base and apex 18–22.5(–24) × 7.5–12 µm. *Conidiomata* frequently formed on the same stromata as the ascomata, stromatic, separate or confluent, dark brown to black, uni- or multilocular immersed in the host, sub-peridermal, locules up to 150 µm diam, walls consisting of a dark brown *textura angularis*, becoming smaller, thinner-walled and hyaline towards the conidiogenous region. *Ostioles* papillate, circular. *Conidiophores* hyaline, smooth, thin-walled, rarely branched at the base, cylindrical, formed from the cells of the inner locule wall, 8–19 × 3–4 µm. *Conidiogenous cells* holoblastic, discrete, integrated, hyaline, smooth, cylindrical, producing the first conidium holoblastically and subsequent conidia enteroblastically, proliferating percurrently with 2–3 indistinct percurrent proliferations, or proliferating internally forming typical phialides (*sensu* Sutton, 1980) and periclinal thickening, (6–)8–16(–18) × (2.5–)3–4(–4.5) µm. *Conidia* hyaline, thin-walled, aseptate, smooth, ellipsoidal, widest in the middle or upper third of the conidium, apex subobtuse, base truncate (15–)16.5–22.5(–24) × 4.5–6(–7.5) µm, 95 % confidence limits of 242 conidia = 19.4–19.9 µm (av. ± S.D. of 242 conidia = 19.7 ± 1.8 × 5.6 ± 0.6 µm), L/W ratio = 3.6 ± 0.5 with 95 % confidence limits = 3.5, often with a minute basal frill. *Spermatia* hyaline, rod-shaped to reniform with either truncate or rounded ends 3–5 × 1 µm. *Dichomera* synasexual morph not reported.

Type: of sexual morph: **Portugal**, Estremadura, Oeiras, Quinta do Marquês, on cane of *Vitis vinifera* cv. Galego Dourado, Mar.

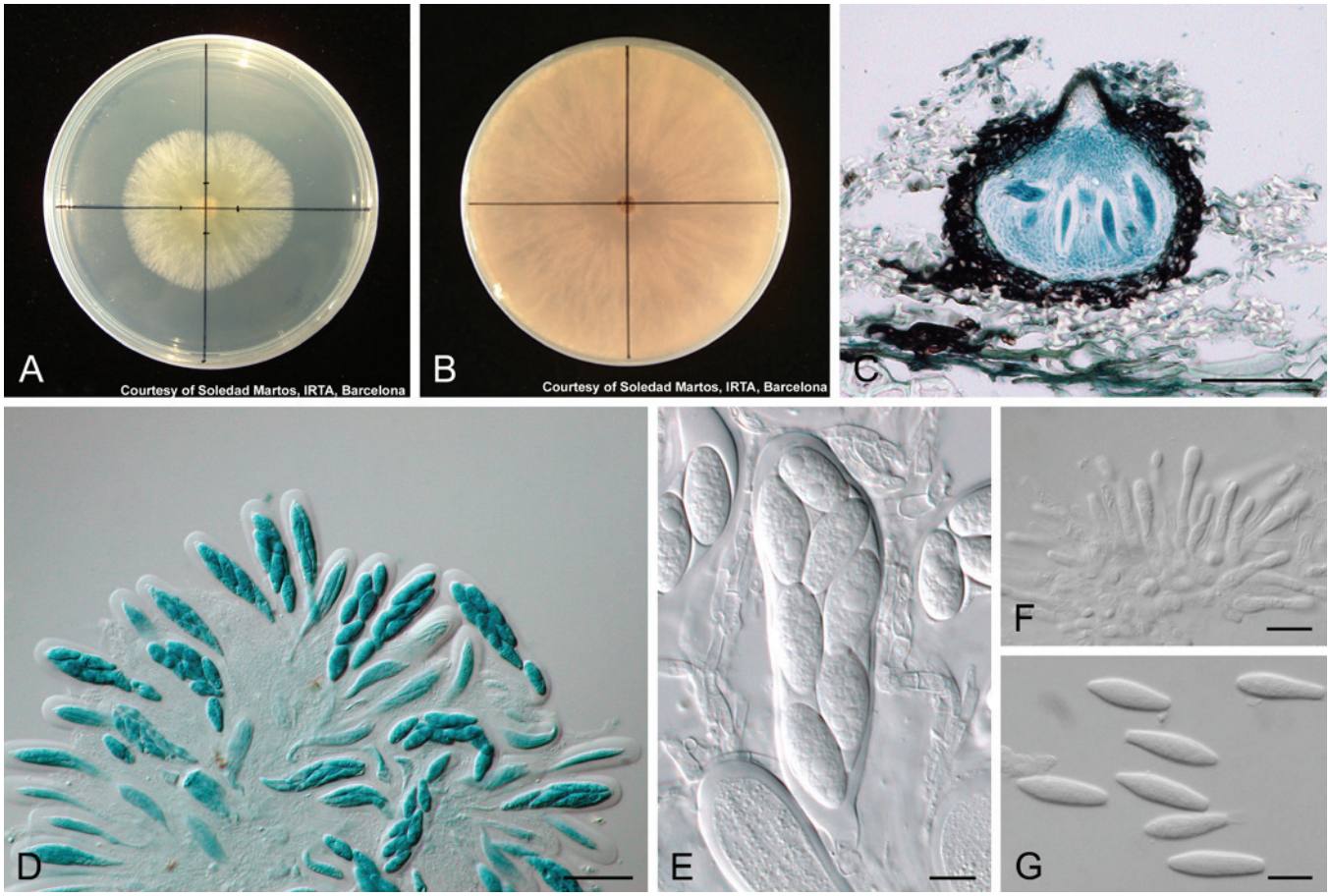

Fig. 55. *Neofusicoccum luteum*. A, B. Cultures of *N. luteum* on PDA after 2 days (A) and 4 days (B) of incubation at 25 ºC. A pale yellow pigment is produced at first (A) that later becomes violaceous (B). C. Vertical section through an ascoma. D. Asci stained with cotton blue. E. Ascus with eight ascospores. F. Conidiogenous cells. G. Conidia. Scale bars: C = 100 µm, D = 50 µm, E–G = 10 µm.

1996, A.J.L. Phillips, **holotype** LISE 94070; of asexual morph: **New Zealand**, Bay of Plenty, Te Puke, No 1 Road, DSIR Research Orchard, from lesions on ripe fruit of *Actinidia deliciosa*, 6 Oct. 1982, S.R. Pennycook, **holotype** PDD 45400.

Cultures: PDDCC 8004 = ATCC 58193 (ex-type of asexual morph) / CBS 110299 (ex-type of sexual morph), CAP037.

Hosts: Plurivorous including *Actinidia chinensis*, *Actinidia deliciosa* (Gadgil *et al.* 2005, Pennycook & Samuels 1985), *Banksia* sp., *Buckinghamia* sp. (Denman *et al.* 2003), *Chamaecyparis lawsoniana*, *Cupressus sempervirens*, *C. lusitanica, Juniperus communis, Pinus pinea, Sequoia sempervirens, Thujopsis dolabrata*, *Thuja plicata* (Alves *et al.* 2013), *Chrysanthemoides monilifera* (Cunnington *et al.* 2007), *Crataegus mexicana* (Adesmoye *et al.* 2013), *Diospyros kaki* (Gadgil *et al.* 2005), *Eucalyptus* sp. (Denman *et al.* 2003), *Ficus microcarpa* (Mayorquin *et al.* 2012), *Fraxinus angustifolia* (Phillips *et al.* 2002), *Malus domestica* (Gadgil *et al.* 2005), *Olea europaea* (Sergeeva *et al.* 2009), *Persea americana* (McDonald & Eskalen 2011), *Protea cynaroides* (Denman *et al.* 2003), *Pyrus communis* (Gadgil *et al.* 2005), *Pyrus pyrifolia* (Gadgil *et al.* 2005), *Quercus robur* (Barradas *et al.* 2013), *Rhododendron* sp. (Varela *et al.* 2011), *Salix fragilis* (Cunnington *et al.* 2007), *Salix magnifica* (Gadgil *et al.* 2005), *Sophora japonica* (Phillips *et al.* 2002), *Syzygium cordatum* (Pavlic *et al.* 2007), *Vitis vinifera* (van Niekerk *et al.* 2004, Úrbez-Torres *et al.* 2006b).

Known distribution: eastern Australia (Denman *et al.* 2003, Cunnington *et al.* 2007, Sergeeva *et al.* 2009), USA (California) (Úrbez-Torres *et al.* 2006b, McDonald & Eskelen 2011, Mayorquin *et al.* 2012), Italy (Lazzizera *et al.* 2008), New Zealand (Gadgil *et al.* 2005, Pennycook & Samuels 1985), Portugal (Phillips *et al.* 2002, Alves *et al.* 2013, Barradas *et al.* 2013), South Africa (Denman *et al.* 2003, van Niekerk *et al.* 2004, Pavlic *et al.* 2007), Spain (Varela *et al.* 2011), Uruguay (Peréz *et al.* 2010).

Notes: The morphology of the conidiomata varies depending on the substrate on which this species is found. Thus, Phillips *et al.* (2002) reported that on grapevine canes they were thick-walled and eustromatic while on leaves they were thin-walled and globose. Phylogenetically it groups with *N. australe*. See notes for *N. australe*.

Neofusicoccum macroclavatum (T.I. Burgess, Barber & Hardy) T.I. Burgess, Barber & Hardy, Stud. Mycol. 55: 248. 2006. MycoBank MB500877. See Burgess *et al.* (2005) for illustrations.

Basionym: *Fusicoccum macroclavatum* T.I. Burgess, Barber & Hardy, Austral. Pl. Pathol. 34: 562. 2005.

Ascomata not reported. *Conidiomata* stromatic, formed on water agar on sterilised pine needles within 21 d, superficial, globose, mostly solitary, 1–2 mm diam, covered with mycelium, single or multiloculate. *Conidiogenous cells* holoblastic, hyaline, subcylindrical to cylindrical to ampuliform, proliferating percurrently with up to 2 annellations, (4.5–)5.5–10.5(–13) × 2–3.5(–4.5) µm.

Conidia produced in culture on pine needles elongate-clavate to fusiform, base subtruncate to bluntly rounded, hyaline, unicellular, occasionally 1–4-septate when mature or before germination, smooth wall with fine granular contents, (19–)25–35(–41) × (5–)6–8(–10) µm (av. of 125 conidia = 30.3 × 7.1 µm), L/W = 4.2. *Spermatia* observed in culture hyaline, cylindrical, sub-cylindrical or clavate, base truncate with rounded apex, 4.5–9.5(–13) × 2–3.5(–4.5) µm (av. of 50 spermatia = 7.7 × 2.6 µm). *Dichomera* synasexual morph not reported.

Culture characteristics: *Colonies* on half strength PDA initially white to buff turning olivaceous-grey within 7 d and becoming black with age, moderately dense, appressed mycelial mat with irregular very dense aerial aggregations. Optimum temperature for growth 25 °C, reaching 53 mm in diameter on half strength PDA after 4 d at 25 °C in the dark.

Type: **Australia**, Western Australia, Denmark, from wood of living *Eucalyptus globulus*, Oct. 2002, T.I. Burgess, **holotype** MURU 400.

Cultures: WAC 12444 = CBS 118223 (ex-type), WAC 12445 = CMW 15948.

Hosts: *Eucalyptus globulus*, *E. saligna* (Burgess *et al.* 2005).

Known distribution: Western Australia (Burgess *et al.* 2005).

Notes: Phylogenetically *N. macroclavatum* is closely related to *N. andinum*, *N. nonquaesitum* and *N. arbuti*. It can be distinguished from all other species in *Neofusicoccum* on the characteristic shape of its conidia that are considerably larger than most other known species in this genus; only *N. pennatisporum* has longer conidia.

Neofusicoccum mangiferae (Syd. & P. Syd.) Crous, Slippers & A.J.L. Phillips, Stud. Mycol. 55: 248. 2006. MycoBank MB500878. See Slippers *et al.* (2005) for illustrations.

Basionym: *Dothiorella mangiferae* Syd. & P. Syd., Ann. Mycol., 14: 192. 1916.

≡ *Nattrassia mangiferae* (Syd. & P. Syd.) B. Sutton & Dyko, Mycol. Res. 93: 484. 1989.

≡ *Fusicoccum mangiferae* (Syd. & P. Syd.) Johnson, Slippers & M.J. Wingf., Mycologia 97 (1): 106. 2005.

≡ *Fusicoccum mangiferae* (Syd. & P. Syd.) G.I. Johnson, Slippers & M.J. Wingf. (as "*mangiferum*"), Mycologia 97 (1): 106. 2005.

= *Hendersonula cypria* Nattrass, *A first list of Cyprus fungi*: 43. 1937.

= *Exosporina fawcettii* E.E. Wilson, Hilgardia 17 (12): 427. 1947.

Ascomata not reported. *Conidiomata* stromatic, erumpent, dark brown to black, uni- to multi-loculate; walls composed of thick-walled, brown *textura angularis*, locules opening by means of separate ostioles; spherical, 150–400 µm diam. *Conidiophores* absent. *Conidiogenous cells* lageniform to ampulliform, hyaline, discrete, arising from the inner wall of the stroma, producing a succession of conidia at one level, collarette absent, periclinal thickening and cytoplasmic channel wide, 6.5–14 × 2.5–4 µm. *Conidia* holoblastic, ellipsoid to nearly fusiform, at first aseptate, then becoming 1–2 euseptate, central cell dark brown, end cells hyaline to pale brown, smooth (11–)12–15(–17.5) × 5–6.6 µm (av. of 54 conidia = 13.6 × 5.4 µm).

Type: **India**, Lucknow, on *Mangifera indica*, F. Bahadur (E.J. Butler 1724), 22 Oct. 1908, **holotype** HCIO.

Cultures: Cultures linked to the type could not be located and probably do not exist. Slippers *et al.* (2005) regarded the following as representatives: CBS 118531 = CMW7024, CBS 118532 = CMW7797.

Host: *Mangifera indica* (Slippers *et al.* 2005).

Known distribution: Australia, India (Slippers *et al.* 2005).

Notes: Phylogenetically this species is closely related to *N. eucalypticola* and *N. eucalyptorum*, but the conidia of *N. mangiferae* are distinct from all other *Neofusicoccum* spp. in their shorter average length (~13–14 mm) and smaller length/width ratio (2–2.5). The conidia often become 1- or 2-septate, light brown with distinctly darker middle cells. This feature is shared with *N. parvum* and *N. mediterraneum*, but is not seen in all isolates of these two latter species.

Neofusicoccum mediterraneum Crous, M.J. Wingf. & A.J.L. Phillips, Fungal Planet No. 19: 2. 2007. MycoBank MB504461. Fig. 56.

Ascomata not reported. *Conidiomata* amphigenous, stromatic, brown, up to 450 µm diam on pine needles, ostiolate, exuding conidia in a white mucoid mass, wall consisting of 3–5 layers of brown *textura angularis*. *Conidiophores* lining the inner layer of the conidioma, hyaline, smooth, 0–1-septate, 15–40 × 3–5 µm. *Conidiogenous cells* holoblastic, hyaline, integrated, phialidic, subcylindrical, rarely ampulliform, proliferating several times percurrently near apex, rarely with minute periclinal thickening, 15–30 × 3–5 µm. *Conidia* hyaline, smooth, thin-walled, fusoid-ellipsoidal, widest in the middle or in the upper third, apex subobtuse, base subtruncate, somewhat flattened with minute marginal frill, with granular cytoplasm, (19–)22–26(–27) × (5.5–)6(–6.5) µm *in vitro* (av. size of conidia = 24 × 6 µm), L/W = 4. *Spermatia* not reported. *Dichomera* synasexual morph not reported.

Culture characteristics: *Colonies* on 2 % MEA fluffy, iron-grey, with abundant grey aerial mycelium, fertile on water agar overlaid with autoclaved pine needles.

Type: **Greece**, Rhodes, Rhodos Palace Hotel parking lot, on branches and leaves of *Eucalyptus* sp., 12 Jun. 2006, collected by P.W. Crous, M.J. Wingfield & A.J.L. Phillips, **holotype** CBS H-19921.

Cultures: CBS 121718 (ex-type), CBS 121558.

Hosts: *Citrus* sp. (Inderbitzin *et al.* 2010, Abdollahzadeh *et al.* 2013), *Ficus microcarpa* (Mayorquin *et al.* 2012), *Fortunella* sp., *Fraxinus* sp., *Juniperus* sp., *Persea americana*, *Pistacia vera*, *Prunus dulcis*, *Rubus* sp., *Sequoiadendron giganteum* (Inderbitzin *et al.* 2010), *Eucalyptus* (Crous *et al.* 2007, Inderbitzin *et al.* 2010), *Juglans regia* (Inderbitzin *et al.* 2010, Trouillas *et al.* 2010), *Mangifera indica* (Abdollahzadeh *et al.* 2013), *Olea europaea* (Lazzizera *et al.* 2008), *Vitis vinifera* (Úrbez-Torres *et al.* 2010, Inderbitzin *et al.* 2010, Martin *et al.* 2011, Pintos Varela *et al.* 2011).

Known distribution: USA (California) (Úrbez-Torres *et al.* 2010, Trouillas *et al.* 2010, Inderbitzin *et al.* 2010, Mayorquin *et al.* 2012),

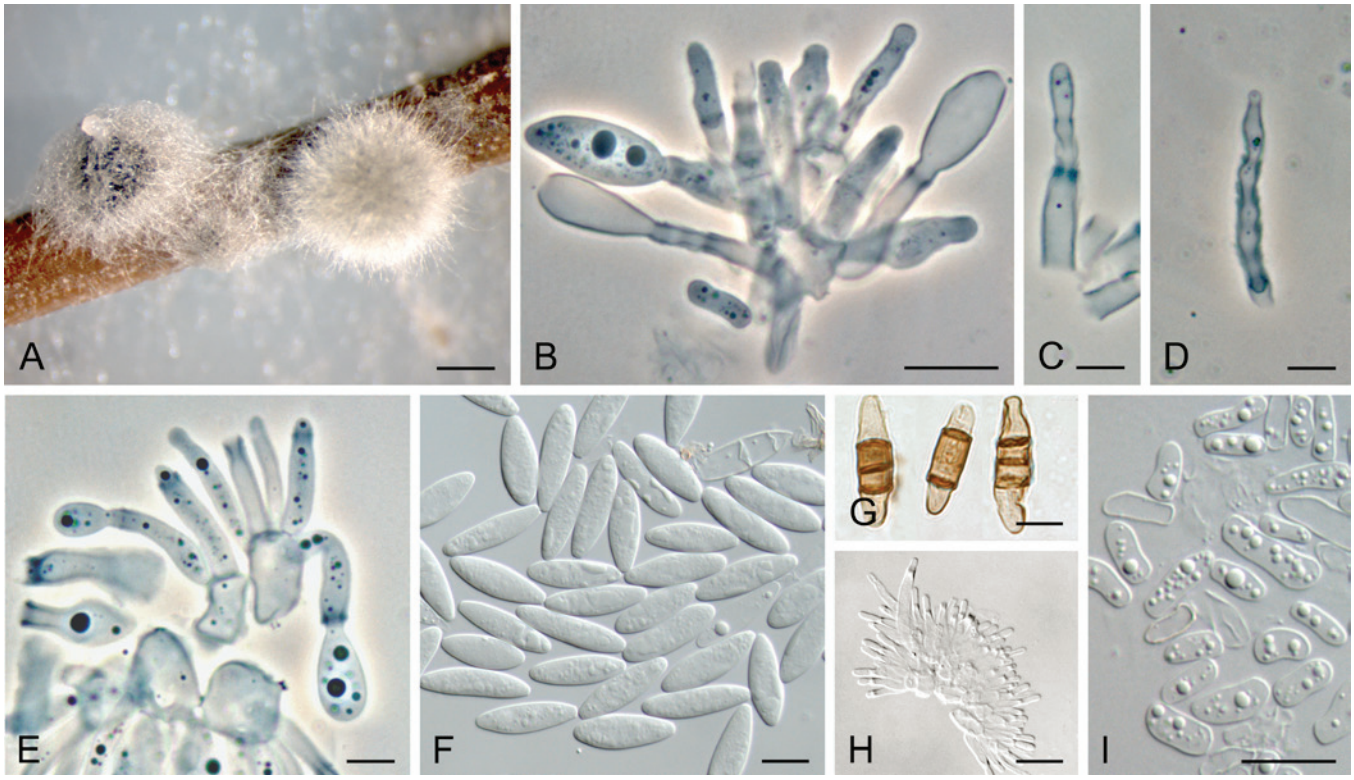

Fig. 56. *Neofusicoccum mediterraneum*. A. Conidiomata formed in culture on poplar twig. B–E. Conidiogenous cells. F. Hyaline, aseptate conidia. G. Coloured, septate conidia. H. Spermatogenous cells. I. Spermatia. Scale bars: A = 500 µm, B, F–H = 10 µm, C–E, I = 5 µm.

Greece (Crous *et al.* 2007), Iran (Abdollahzadeh *et al*. 2013), Italy (Lazzizera *et al.* 2008), Spain (Martin *et al.* 2011, Pintos Varela *et al.* 2011).

Notes: *Neofusicoccum mediterraneum* is phylogenetically most closely related to *N. viticlavatum* and *N. vitifusiforme*, but it can be separated by having larger conidia (24 × 6 µm) than those of *N. viticlavatum* (16–18 × 6.5–7.5 µm) and *N. vitifusiforme* (19–21 × 5.5–6.5 µm). Conidia in some isolates become septate, light brown with distinctly darker middle cells; a feature seen in *N. mangiferum* and *N. parvum*, but can be distinguished from these two species in having larger conidia.

A search of GenBank revealed a wide range of variation amongst the ITS sequences for isolates of *N. mediterraneum*. Furthermore, in the six-locus phylogeny of Inderbitzin *et al.* (2010), two distinct clades were resolved for this species. Therefore, as mentioned by Abdollahzadeh *et al*. (2013), it seems that *N. mediterraneum* is a complex of species that should be examined in more detail using greater numbers of isolates and additional gene loci.

Neofusicoccum nonquaesitum Inderb., Trouillas, Bostock & Michailides, Mycologia 102: 1360. 2010. MycoBank MB518135. See Inderbitzin *et al.* (2010) for illustrations.

Ascomata not reported. *Conidiomata* stromatic, single or in groups, immersed or immersed-erumpent, lenticular to subglobose, 200–500 × 150–400 µm, sometimes with a short neck, wall up to 50 µm wide, three-layered, outer layer composed of dark, thick-walled cells, intermediate layer lighter pigmented, cells smaller, inner layer hyaline, cells thin-walled. *Conidiophores* short, undifferentiated, originating from the inner pycnidial wall, branching at times, up to 30 µm long, 1.5–2 µm wide, bearing single, unbranched conidiogenous cells, of similar dimensions as conidiophores. *Conidiogenous cells* holoblastic proliferating percurrently with up to five proliferations. *Conidia* hyaline, fusiform to oval, base truncate, rarely 1–3-septate, sometimes becoming pigmented, 17–29 × 5.5–10.5 µm (av. size of conidia = 23.2 × 7.6 µm), L/W ratio = 3.1. *Spermatia* when present most abundant in upper part of pycnidium, cylindrical, with rounded or truncate apices, curved at times, 4–10 × 2–4 µm, rarely up to 15 × 5 µm. *Dichomera* synasexual morph not reported.

Culture characteristics: *Colonies* on half strength PDA plate with cork oak or pistachio leaf after 12 d under continuous light on a laboratory bench white to olive-brown or olivaceous-black, reverse white to olivaceous-black, conidioma forming mainly on leaf, black, some covered by mycelium, immersed-erumpent, up to 600 µm diam and of variable shape, conidia and spermatia present.

Type: **USA**, California, Napa County, St Helena, on cankered branch of *Umbellularia californica*, 12 Nov. 2004, F.P. Trouillas, **holotype** UC1946389 (dried branch of *U. californica* inoculated with PD484).

Cultures: CBS 126655 = PD484 (ex-type), PD301.

Hosts: *Umbellularia californica*, *Prunus dulcis* (Inderbitzin *et al.* 2010), *Vaccinium corymbosum* (Espinoza *et al.* 2009) *Sequoiadendron giganteum* (Rooney-Latham *et al*. 2012).

Known distribution: USA (California) (Inderbitzin *et al.* 2010), Chile (Espinoza *et al.* 2009), North America (Rooney-Latham *et al*. 2012).

Note: See notes for *N. andinum*.

Neofusicoccum occulatum Sakalidis & T.I. Burgess, Mol. Phylogenet. Evol. 60: 340. 2011. MycoBank MB518777. See Sakalidis *et al.* (2011) for illustrations.

Ascomata not reported. *Conidiomata* on *Populus* sp. twigs stromatic, solitary often or in groups, rapidly covered with mycelium, superficial, conical or spherical or obpyriform, unilocular. *Conidiogenous cells* holoblastic, hyaline, oval to fusiform, 4–14 × 0.5–2.5 µm (av. size = 8 × 1 µm). *Conidia* hyaline, unicellular, fusifom to ellipsoid to cymbiform, apices obtuse, base truncate, sometime both apices taper, aseptate, smooth-walled, 14–22 × 3.5–7.5 µm (av. size of conidia = 18.3 × 5.2 µm), L/W = 3.5. *Dichomera* synasexual morph: *Conidiogenous cells* holoblastic, hyaline, globose to turbinate 11.5 × 1.5 µm. *Conidia* two forms observed "irregular long" and "irregular round" both brown and muriform "irregular round" 1–3 transverse septa, 0–1 long septa and 0–3 oblique septa, 7.5–13.5 × 5.5–8.5 µm (av. size of conidia = 9.8 × 7 µm), L/W = 1.4, rarely found "irregular long" 1–5 transverse septa, 0–2 oblique septa, 11.5–20.5 × 4–7.5 µm (av. of 20 conidia = 15.5 × 5.8 µm), L/W = 2.7.

Culture characteristics: *Colonies* white, flattened with tufts of white mycelium, becoming very to dark greenish grey colour after 14 d with the reverse side of the colonies greenish black. Optimal temperature for growth 30 ºC, covering a 90 mm Petri dish on MEA in 3–4 d, limited growth occurred at 4 ºC and 10 ºC.

Type: **Australia**, Queensland, Karanda, symptomless branches of *Eucalyptus grandis* hybrid, Mar 2002, T.I. Burgess, dried culture sporulating on *Populus* sp. twigs, **holotype** MURU467.

Cultures: MUCC 227 = CBS 128008 (ex-type), MUCC 286 = WAC 12395.

Host: *Eucalyptus* (Sakalidis *et al.* 2011).

Known distribution: Australia (Sakalidis *et al.* 2011).

Notes: A pale yellowish pigment was observed once in the media of three isolates MUCC 270 and MUCC 296 and MUCC 232 (Sakalidis *et al*. 2011). *Neofusicoccum occulatum* is morphologically similar to other closely related species in the *N. parvum* / *N. ribis* species complex and differs from other species in the complex by one uniquely fixed nucleotide difference in partial EF1-α (EU339509) position 164 (A). See notes for *N. cordaticola*.

Neofusicoccum parvum (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips, Stud. Mycol. 55: 248. 2006. MycoBank MB500879. Fig. 57.

Basionym: *Fusicoccum parvum* Pennycook & Samuels, Mycotaxon 24: 455. 1985.

= *Botryosphaeria parva* Pennycook & Samuels, Mycotaxon 24: 455. 1985.

Ascomata forming botryose clusters 2–5 mm diam, each comprising up to 100 ascomata, erumpent through the bark, globose, with a short, conical papilla, dark brown to black, smooth, thick-walled, wall composed of dark brown thick-walled cells, lined with thin-walled, hyaline cells, locules 150–250 µm diam, contents conspicuously white when dry. *Asci* clavate, 8-spored, bitunicate, 75–143(–210) × 17–21 µm. *Ascospores* broadly ellipsoidal to fusoid, often with an apiculus at each end, hyaline, smooth, aseptate, occasionally becoming 1-septate, (14–)18–23(−26) × (7–)8−10(–11) µm (av. of 73 ascospores = 20.8 × 9.2 µm), L/W = 2.2. Conidiomatal aggregates morphologically indistinguishable from ascomatal aggregates. *Conidiomata* globose and non-papillate to pyriform with a short, acute papilla, entire locule lined with conidiogenous cells. *Conidiogenous cells* holoblastic, hyaline, subcylindrical, proliferating percurrently to form 1–2 annellations, or proliferating at the same level to form periclinal thickenings. *Conidia* ellipsoidal with apex round and base flat, unicellular, hyaline, old conidia becoming 1–2-septate hyaline, or light brown with the middle cell darker than the terminal cells, (12–)13.5–21(–24) × 4–6(–10) µm, 95 % confidence limits of 320 conidia = 16.9–17.3 × 5.4–5.6 µm (av. ± S.D. of 320 conidia = 17.1 ± 2.1 × 5.5 ± 0.8 µm), L/W ratio = 3.2 ± 0.6 with 95 % confidence limits of 3.1–3.2. *Dichomera* synasexual morph: *Conidia* subglobose to obpyriform, brown, apex obtuse, base truncate, 8–10.5(–12) × (6.5–)7–8(–9) µm, 1–3 transverse septa, 1–2 longitudinal septa, and 1–2 oblique septa.

Type: **New Zealand**, Bay of Plenty, Te Puke, No 3 Road, Baldwin Orchard, on small dead branch of *Populus nigra*, 17 Dec. 1981, S.R. Pennycook, **holotype** PDD 45438.

Cultures: PDDCC 8003 = ATCC 58191 (ex-type) = ICMP 8003 = CMW 9081.

Hosts: Plurivorous including *Actinidia deliciosa* (Pennycook & Samuels 1985, Abdollahzadeh *et al*. 2013), *Araucaria heterophylla* (Slippers *et al.* 2005b), *Citrus sinensis* (Cunnington *et al*. 2007), *Citrus* sp. (Adesemoye *et al.* 2011), *Cupressus funebris* (Li *et al.* 2010), *Diospyros kaki* (Gadgil *et al.* 2005), *Eriobotrya japonica* (Gadgil *et al.* 2005), *Eucalyptus citriodora*, *Eucalyptus globulus*, *Eucalyptus grandis*, *Eucalyptus saligna* (Gezahgne *et al.* 2004), *Eucalyptus pellita* (Barber *et al.* 2005), *Eucalyptus urophylla* (Mohali *et al.* 2007), *Ficus microcarpa* (Mayorquin *et al*. 2012), *Grevillea robusta* (Toljander *et al.* 2007), *Heteropyxis natalensis* (Slippers *et al.* 2004a), *Juglans regia* (Inderbitzin *et al.* 2010, Abdollahzadeh *et al*. 2013), *Juniperus communis, Pinus pinea, Thuja plicata, Thujopsis dolabrata* (Alves *et al.* 2013), *Kolkwitzia amabilis* (Cunnington *et al.* 2007), *Leucadendron* sp. (Marincowitz *et al.* 2008), *Leucospermum* sp. (Marincowitz *et al.* 2008), *Lilium lancifolium* (Woodward *et al.* 2006), *Malus domestica* (Pennycook & Samuels 1985), *Mangifera indica* (Javier-Alva *et al.* 2009), *Olea africana* (Cunnington *et al.* 2007), *Olea europaea* (Lazzizera *et al.* 2008), *Persea americana* (Hartill 1991, Cunnington *et al.* 2007, Zea-Bonilla *et al*. 2007, McDonald & Eskalen 2011, Molina-Gayosso *et al*. 2012), *Pistacia vera* (Cunnington *et al.* 2007, Inderbitzin *et al.* 2010), *Populus* sp. (Gadgil *et al.* 2005), *Protea cynaroides* (Marincowitz *et al.* 2008), *Prunus armeniaca* (Gramaje *et al*. 2012), *Prunus dulcis* (Inderbitzin *et al.* 2010), *Prunus persica* (Cunnington *et al.* 2007), *Prunus avium* (Abdollahzadeh *et al*. 2013), *Pseudopanax laetus* (Gadgil *et al.* 2005), *Psidium guajava* (Mohali *et al.* 2007), *Pyrus* sp. (Abdollahzadeh *et al*. 2013), *Pyrus communis* (Gadgil *et al.* 2005), *Pyrus pyrifolia* (Shen *et al.* 2010), *Pinus* sp. (Abdollahzadeh *et al*. 2013), *Quercus suber* (Linaldeddu *et al.* 2007), *Rhododendron* sp. (Varela *et al.* 2011), *Ribes* sp. (Slippers *et al.* 2004a), *Rubus fruticosus* (Abdollahzadeh *et al*. 2013), *Salix* sp. (Abdollahzadeh *et al*. 2013), *Sequoia gigantea* (Slippers *et al.* 2004a), *Syzygium cordatum* (Pavlic *et al.* 2007), *Syzygium paniculatum* (Ploetz *et al*. 2008), *Terminalia catappa* (Didier Begoude *et al.* 2010), *Trachycarpus fortunei* (Taylor & Hyde 2003), unknown, palm (Taylor & Hyde 2003), *Vaccinium*

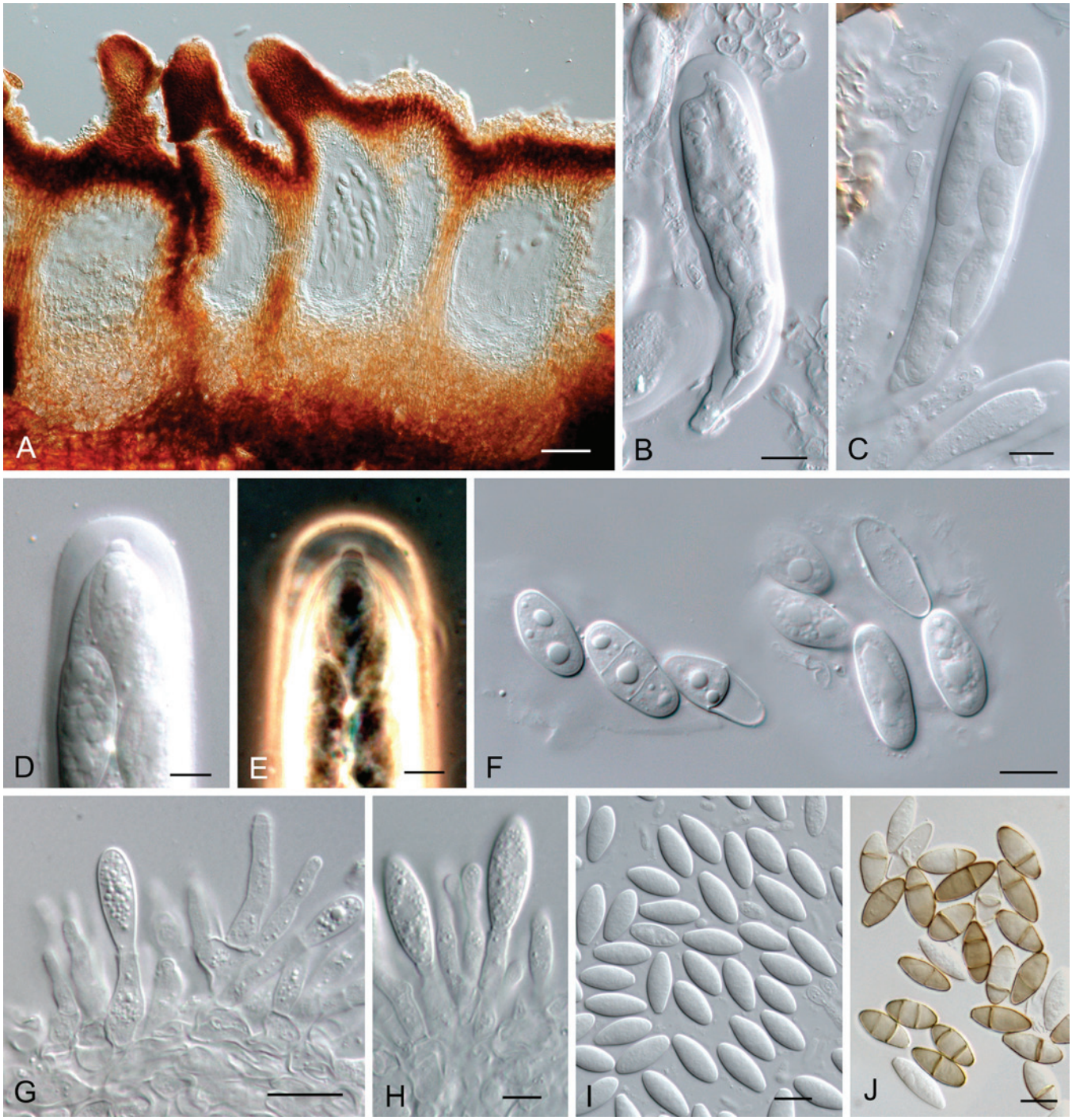

Fig. 57. *Neofusicoccum parvum*. A. Vertical section through an aggregate ascoma. B, C. Asci. D, E. Details of ascus apex as seen by interference contrast (D) or phase contrast (E). F. Ascospores. G, H. Conidiogenous cells. I. Hyaline, aseptate conidia. J. Coloured, 1- and 2-septate conidia. Scale bars: A = 50 µm, B, C, F, G = 10 µm, D, E, H–J = 5 µm.

corymbosum (Espinoza *et al.* 2009), *Vitis vinifera* (Cunnington *et al.* 2007, Mohammadi *et al*. 2008, Phillips, *et al.* 2006, Úrbez-Torres *et al.* 2006, Díaz *et al.* 2011, White *et al.* 2011).

Known distribution: Probably worldwide. Australia (Barber *et al.* 2005, Cunnington *et al.* 2007, Slippers *et al.* 2004a, Taylor & Hyde 2003), USA (California) (Úrbez-Torres *et al.* 2006b, Adesemoye & Eskalen 2011, Inderbitzin *et al.* 2010, McDonald & Eskalen 2011, Mayorquin *et al.* 2012), Chile (Díaz *et al.* 2011, Espinoza *et al.* 2009), China (Li *et al.* 2010, Taylor & Hyde 2003), Ethiopia (Gezahgne *et al.* 2004), USA (Florida) (Ploetz *et al.* 2008), USA (Georgia) (Woodward *et al.* 2006), Greece (Inderbitzin *et al.* 2010), USA (Hawaii) (Marincowitz *et al.* 2008), Iran (Mohammadi *et al.* 2008, Abdollahzadeh *et al.* 2013), Italy (Lazzizera *et al.* 2008, Linaldeddu *et al.* 2007), Mexico (Molina-Gayosso *et al.* 2012), New Zealand (Gadgil *et al.* 2005, Hartill 1991, Pennycook & Samuels 1985, Slippers *et al.* 2005b), Peru (Javier-Alva *et al.* 2009), Portugal (Phillips *et al.* 2006, Alves *et al.* 2013), South Africa (Didier Begoude *et al.* 2010, Pavlic *et al.* 2007, Slippers *et al.* 2004a, Slippers *et al.* 2004b, White *et al.* 2011), Spain (Úrbez-Torres *et al.* 2006a, Zea-Bonilla *et al.* 2007, Varela *et al.* 2011, Gramaje *et al.* 2012), Taiwan (Shen *et al.* 2010), Uganda (Toljander *et al.* 2007), Venezuela (Mohali *et al.* 2007).

Notes: Phylogenetically, this species lies within a cluster of morphologically highly similar species that can be distinguished

only on the basis of ITS and EF1-α sequence data. *Neofusicoccum parvum* has, however, been distinguished by different researchers from other species in this cluster based on the colour and septation of conidia at the time of germination. Thus, the conidia become 2-septate and the central cells become pale brown in *N. parvum*, while in the other species there is no colouration of the conidia at the time of germination. But recently, in a phylogenetic study on *Neofusicoccum* and *Botryosphaeria* species in Iran, Abdollahzadeh *et al*. (2013) studied 34 *N. parvum* isolates and found that in all of them the old conidia remained hyaline even after 10 wk. Furthermore, the production of a yellow pigment on PDA was reported in some isolates of an Iranian population of *N. parvum*, which is a feature never seen previously. *Neofusicoccum parvum* is emerging as a common and cosmopolitan species on a wide variety of hosts. It is now recognised as an aggressive pathogen of grapevines (e.g., Phillips 1998) as *B. dothidea*, van Niekerk *et al.* 2004), and possibly other woody hosts.

Neofusicoccum pennatisporum K. Taylor, Barber & T.I. Burgess, Mycol. Res. 113: 346. 2009. MycoBank MB511826. See Taylor *et al.* (2009) for illustrations.

Ascomata not reported. *Conidiomata* stromatic, superficial, dark-brown to black, cylindrical to triangular to irregular, mostly solitary, rough with some mycelium, 300–1000 µm long and 100–500 µm diam on pine needles but up to 2 mm long on agar. *Conidiogenous cells* holoblastic, hyaline, cylindrical to flask shaped, 4–10(–12) × (1–)2–3(–4) µm. *Conidia* hyaline, usually aseptate, often with 1 septum but can have up to 5 septa with age, typically fusiform, smooth-walled, apex obtuse, base frequently truncate but sometimes rounded, (31–)40–50(–64) × 6–10 (–12) µm (av. of 100 conidia = 45.4 × 9.7 µm), L/W ratio = 4.6. *Spermatia* hyaline, aseptate, fusiform, either rounded or truncate at both ends, (2–)3–6(–7) × 1–2 µm (av. of 100 spermatia = 4.4 × 1.5 µm). *Dichomera* synasexual morph not reported.

Culture characteristics: *Colonies* composed of appressed mycelial mat with diffuse irregular edges, white centre, darkening slightly with age, pycnidia produced profusely.

Type: **Australia**, Western Australia, Yalgorup National Park, from healthy stem of *Allocasuarina fraseriana*, Jun. 2005, K.M. Taylor, **holotype** PERTH 07693044.

Cultures: WAC 13153 = MUCC 510 (ex-type).

Host: *Allocasuarina fraseriana* (Taylor *et al.* 2009).

Known distribution: Western Australia (Taylor *et al.* 2009).

Notes: The conidia of *N. pennatisporum* are unusually long (40–50 × 6–10 µm), when compared with other *Neofusicoccum* spp., including *N. macroclavatum* (25–35 × 6–8 µm), which is also found in Western Australia, and *N. protearum* (25–30 × 7–8 µm), which is the most closely related species to *N. pennatisporum* based on ITS sequence data. In the phylogeny based on ITS and EF1-α sequences, this species resides in a distinct clade as a sister group to all other *Neofusicoccum* species. According to Taylor *et al.* (2009), an isolate of *N. pennatisporum* produced the sexual morph once on pine needles in culture. The ascospores have distinctive protrusions at either end unlike ascospores of other *Neofusicoccum* spp.

Neofusicoccum protearum (Denman & Crous) Crous, Slippers & A.J.L. Phillips, Stud. Mycol. 55: 249. 2006. MycoBank MB500880. See Denman *et al.* (2003) for illustrations.

Basionym: *Fusicoccum protearum* Denman & Crous, *Mycologia* 95: 301. 2003.

= *Botryosphaeria protearum* Denman & Crous, *Mycologia* 95: 301. 2003.

Ascomata pseudothecial, embedded in host tissue, up to 600 µm diam, becoming erumpent, solitary or botryose, stromatic, dark brown to black, with central, black ostioles; pseudothecial wall 6–15 layers thick, composed of brown *textura angularis*. *Asci* clavate to subcylindrical, 8-spored, bitunicate, with a well-developed apical chamber that becomes inconspicuous at maturity, 110–200 × 15–21 µm. *Pseudoparaphyses* filiform, branched, septate, 3–5 µm wide. *Ascospores* irregularly biseriate, hyaline, nonseptate, granular, becoming light brown with age, fusiform, widest in the middle with obtuse ends, sometimes inequilateral, (25–)26–33(–37) × (9–)10–12(–13) µm. *Conidiomata* stromatic, embedded in host tissue, solitary or botryose, stromatic, globose, up to 500 µm diam, wall 4–8 layers thick, composed of brown *textura angularis*, becoming hyaline towards the inner region. *Conidiophores* 0–1-septate, hyaline, subcylindrical, rarely branched, 7–20(–30) × 3–5 µm. *Conidiogenous cells* holoblastic, hyaline, subcylindrical, rarely proliferating percurrently with 1−2 anellations, proliferating predominantly at the same level with minute periclinal thickenings, which become more prominent in older conidiogenous cells, 7−12 × 3−5 µm. *Conidia* hyaline, granular, ovoid to clavate when young, becoming irregularly fusoid when mature, widest in the middle with an obtuse apex and bluntly rounded or slightly flattened base, (20–)25–30(–40) × 7–8(–10) µm *in vivo*. *Spermatia* produced in same conidiomata as conidia, or in separate conidiomata. *Spermatophores* hyaline, smooth, branched, cylindrical, 0−2-septate, straight, unbranched or branched above, 12–17 × 2–3 µm. *Spermatogenous cells* discrete or integrated, hyaline, smooth, cylindrical, proliferating via phialides with periclinal thickenings, 5–12 × 1.5–2.5 µm. *Spermatia* hyaline, smooth, aseptate, rod-shaped with rounded ends, 3–6 × 1–1.5 µm. *Dichomera* synasexual morph not reported.

Type: Of sexual morph: **South Africa**, Western Cape, Porterville, Baanbreek Farm, on stems of *Protea magnifica*, 27 Jul. 1997, S. Denman, **holotype** PREM 57329; of asexual morph: **South Africa**, Western Cape, Devon Valley, Protea Heights Farm, on stems of *Leucadendron salignum*, 31 Oct. 1997, S. Denman & J. Taylor, **holotype** PREM 57330.

Cultures: STE-U 4361 = CPC 4361 (ex-type culture of sexual morph), STE-U 1775 = CBS 114176 (ex-type culture of asexual morph).

Hosts: *Protea* and *Leucadendron* spp. (Denman *et al*. 2000, 2003, Marincowitz *et al.* 2008), *Santalum acuminatum* (Taylor *et al.* 2009).

Known distribution: Australia, Portugal (continental and Madeira), South Africa, Spain (Tenerife), USA (Hawaii) (Denman *et al.* 2000, 2003, Marincowitz *et al.* 2008, Taylor *et al.* 2009).

Notes: *Neofusicoccum protearum* was originally thought to be restricted to *Proteaceae*, but it was recently isolated from *Santalum acuminatum* (Taylor *et al.* 2009). See notes for *N. pennatisporum*.

Neofusicoccum ribis **(**Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips, Stud. Mycol. 55: 249. 2006. MycoBank MB500881. See Slippers *et al*. (2004) for illustrations.

Basionym: *Fusicoccum ribis* Slippers, Crous & M.J. Wingf., Mycologia 96: 96. 2004.

= *Botryosphaeria ribis* Grossenb. & Duggar, Tech. Bull. N.Y. Agric. Exp. St. 18: 128. 1911.

Ascostroma erumpent through the bark, pulvinate, 100–400 µm diam. *Ascomata* pseudothecial, forming botryose aggregates of up to 5–50, globose with central ostiole, papillate or not, brown to black, 175–250 µm, pseudothecial wall comprising 5–15 layers of *textura angularis*, outer region of dark brown or brown cells, inner region 2−4 layers of hyaline cells lining the locule. *Asci* bitunicate, clavate, 8-spored, 80–120 × 17–20 µm. *Pseudoparaphyses* filiform, septate, rarely branched, 2–4 µm wide. *Ascospores* fusoid to ellipsoid, often round at the ends then broadly ellipsoidal, hyaline, unicellular, smooth with granular contents, biseriate in the ascus, (14–)18–23(–27) × 6–8(–10) µm (av. of 80 ascospores = 20.5 × 7.1 µm), L/W = 2.9. *Conidiomata* in same stromata as ascomata and morphologically indistinguishable from them, or solitary and embedded in young host shoots. *Conidiogenous cells* holoblastic, hyaline, subcylindrical, proliferating percurrently with 1–2 annellations, or proliferating at the same level to form periclinal thickenings, 6–22 × 2–5 µm. *Conidia* fusiform, sometimes irregularly fusiform, base subtruncate to blunt, hyaline, unicellular, rarely septate with age, smooth with granular contents, (16–)19–23(–24) × 5–6(–7) µm (av. of 90 = conidia 20.8 × 5.5 µm), L/W = 3.8. *Spermatia* not reported. *Dichomera* synasexual morph: *Conidia* subglobose, obpyriform or rarely obovoid to broadly fusiform or fusiform, apex subobtuse to obtuse, base truncate to bluntly rounded. Subglobose, obpyriform conidia (7–)8–13.5(–17) × (6.5–)7–9.5(–10.5) µm, hyaline to pale brown when immature with one transverse septum and 0–2 longitudinal septa, becoming brown when mature with 1–4 transverse septa, 0–3 longitudinal septa, and 0–4 oblique septa. Broadly fusiform to fusiform conidia (12–)13.5–22.5(–24) × (5–)5.5–8 µm, brown with 2–7 transverse septa, and 0–2 oblique septa.

Type: Of asexual morph; **USA**, New York, Ithaca, *Ribes* sp., 2000, G. Hudler, holotype PREM 57368, **lectotype** of sexual morph; **USA**, New York, Geneva, on *Ribes vulgare*, 1911, J.G. Grossenbacher & B.M. Duggar, **holotype** CUP-A (F.Col. 3408).

Cultures: CBS 115475 = CMW 7772 (ex-type), CMW 7054.

Hosts: More than 250 hosts are listed for *N. ribis* (Farr *et al.* 2012). However, many of the reports were published before the concept of *N. ribis* (as *Botryosphaeria ribis*) was clarified by Slippers *et al*. (2004) and thus the identifications are not reliable.

Known distribution: Although this species has been considered to be distributed worldwide on numerous hosts this is based on reports published prior to the establishment of a stable concept for *N. ribis* (Slippers *et al.* 2004). Thus far it has been verified only on *Ribis* sp. in NY state, USA (Slippers *et al.* 2004).

Notes: For many years, *B. ribis* was was regarded as a synonym of *B. dothidea* (e.g., Witcher & Clayton 1963, Barr 1972, English *et al.* 1975, Maas & Uecker 1984, Pennycook & Samuels 1985, Brown & Britton 1986, Smith *et al.* 1994), while others regarded them as distinct species (e.g., Punithalingam & Holliday 1973, Morgan-Jones & White 1987, Rayachhetry *et al*. 1996, Smith & Stanosz 2001). The debate was finally settled when Slippers *et al.* (2004) demonstrated that the two were phylogenetically and morphologically distinct and Crous *et al.* (2006) showed that *B. dothidea* and *N. ribis* reside in two distinct phylogenetic lineages. Phylogenetically *N. ribis* resides in a cluster of cryptic species that are difficult to separate based on morphology.

Neofusicoccum umdonicola Pavlic, Slippers & M.J. Wingf., Mycologia 101: 644. 2009. MycoBank MB512500. See Pavlic *et al.* (2009) for illustrations.

Ascomata not reported. *Neofusicoccum umdonicola* is morphologically similar to other related species in the *N. parvum* / *N. ribis* species complex. *Conidia* hyaline, unicellular, fusiform to oval, apices tapered 15–23.5 × 4.5–6.5 µm (av. of 310 conidia = 19.4 × 5.5 µm), L/W = 3.5). *Neofusicoccum umdonicola* differs from other species in the *N. parvum* / *N. ribis* complex by uniquely fixed nucleotides in four nuclear loci: ITS (EU821904) position 168 (C); EF1-α (EU821874) positions 62 (T); β-tubulin (EU821844) position 40 (A); RNA polymerase II subunit (EU821934) position 280 (T).

Type: **South Africa**, Kwazulu-Natal Province, Kosi Bay from symptomless branches and leaves, dying branches and pulp of ripe fruits of *Syzygium cordatum*, Mar. 2002, D. Pavlic, a dry culture on pine needles, **holotype** PREM 60068.

Cultures: CMW 14058 = CBS 123645 (ex-type), CMW 14060 = CBS 123646.

Host: *Syzygium cordatum* (Pavlic *et al.* 2009).

Known distribution: South Africa (Pavlic *et al.* 2009).

Nots: See notes for *N. cordaticola*.

Neofusicoccum viticlavatum (Van Niekerk & Crous) Crous, Slippers & A.J.L. Phillips, Stud. Mycol. 55: 249. 2006. MycoBank MB500882. See van Niekerk *et al.* (2004) for illustrations.

Basionym: *Fusicoccum viticlavatum* Van Niekerk & Crous, Mycologia 96: 792. 2004.

Ascomata not reported. *Conidiomata* stromatic, embedded in host tissue, solitary, stromatic, globose, up to 450 µm wide, wall 4–8 cell layers thick, of brown *textura angularis*, becoming hyaline toward inner region. *Conidiophores* 0–1-septate, hyaline, subcylindrical, 10–20 × 2.5–3.5 µm. *Conidiogenous cells* holoblastic, hyaline, subcylindrical, proliferating percurrently with 1–3 proliferations, or proliferating at same level (phialidic) with minute periclinal thickening, 7–15 × 2.5–3.5 µm. *Conidia* hyaline, guttulate, ellipsoid to clavate, widest in upper third, with an obtuse apex and flattened, subtruncate base, aseptate, (15–)16–18(–20) × (6–)6.5–7.5(–8)

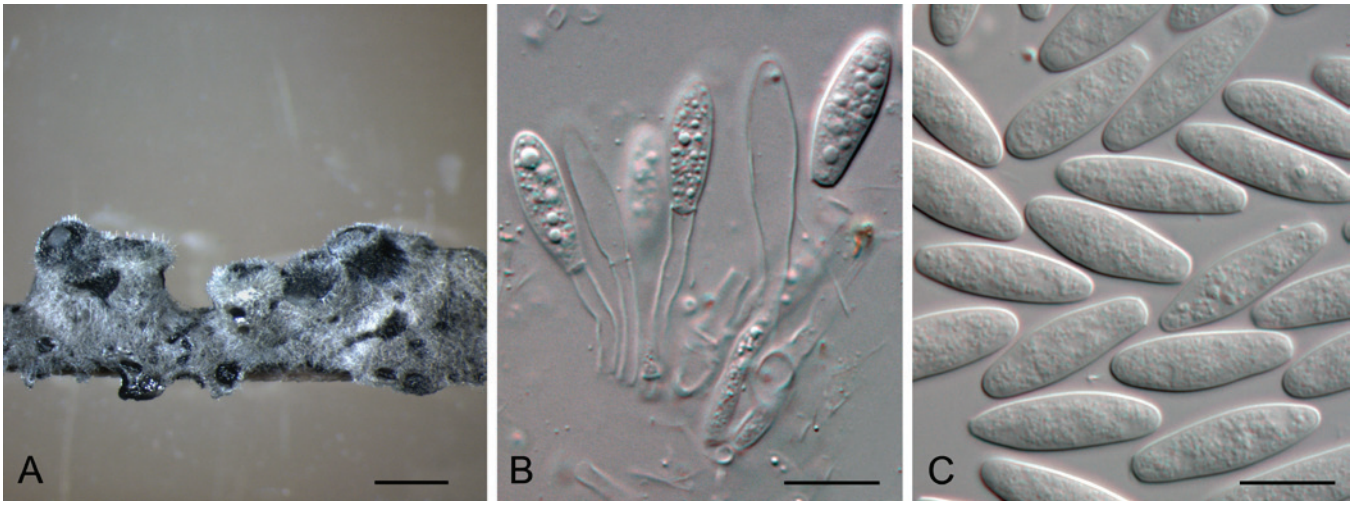

Fig. 58. *Neofusicoccum vitifusiforme*. A. Conidiomata on pine needles in culture. B. Conidiogenous cells. C. Conidia. Scale bars: A = 1 mm, B, C = 10 µm.

µm, L/W ratio = 2.4. *Spermatia* not reported. *Dichomera* synasexual morph not reported.

Culture characteristics: *Colonies* umbonate with undulating margins, olivaceous on the surface, and dull green reverse, reaching a radius of 26 mm after 3 d at 25 °C. Cardinal temperatures for growth: min 10 °C, max 35 °C, opt 30 °C.

Type: **South Africa**, Western Cape Province, Stellenbosch, on *V. vinifera,* 2002, F. Halleen, **holotype** CBS H-7755.

Cultures: STE-U 5044 = CBS 112878 (ex-type), STE-U 5041 = CBS 112977.

Host: *Vitis vinifera* (van Niekerk *et al.* 2004).

Known distribution: South Africa (Western Cape Province) (van Niekerk *et al.* 2004).

Notes: *Neofusicoccum viticlavatum* is closely related to *N. mediterraneum* and *N. vitifusiforme*. It can be differentiated from *N. vitifusiforme* based on the characteristic clavate conidia of *N. viticlavatum* and its smaller conidia. Conidia of this species are much smaller (16–18 × 6.5–7.5 µm) than those of *N. mediterraneum* (24 × 6 µm).

Neofusicoccum vitifusiforme (Van Niekerk & Crous) Crous, Slippers & A.J.L. Phillips, Stud. Mycol. 55: 249. 2006. MycoBank MB500883. Fig. 58.

Basionym: *Fusicoccum vitifusiforme* Van Niekerk & Crous, Mycologia 96: 793. 2004.

Synasexual morph: *Dichomera eucalypti* (G. Winter) B. Sutton, Mycol. Pap. 138: 182 1975.

Basionym: *Camarosporium eucalypti* G. Winter, Revue Mycol., Toulouse 8 (32): 212. 1886.

= *Neofusicoccum corticosae* Crous & Summerell, Fungal Divers. 23: 337. 2006.

Ascomata not reported. *Conidiomata* stromatic, solitary, globose to obpyriform, up to 450 µm diam, conidioma wall 6–15 cell layers thick, of brown *textura angularis*, becoming hyaline toward inner region. *Conidiophores* 0–1-septate, hyaline, subcylindrical, 10–45 × 2.5–5 µm. *Conidiogenous cells* holoblastic, hyaline, subcylindrical, proliferating percurrently with numerous proliferations, or proliferating at the same level (phialidic) with minute periclinal thickening, 10–30 × 2.5–3.5 µm. *Conidia* hyaline, granular, fusoid to ellipsoid, widest in the upper third with an obtuse apex and flattened, subtruncate base, (18–)19–21(–22) × (4.5–)5.5–6.5(–8) µm *in vitro*, L/W ratio = 3.3. *Spermatia* not reported. *Dichomera* synasexual morph: *Conidia* subglobose, obpyriform or obovoid, apex obtuse, base truncate to bluntly rounded, (9–)9.5–13(–14.5) × (6.5–)8–10.5(–11) µm, hyaline to pale brown when immature with 0–3 transverse septa, 0–2 longitudinal septa, and 0–2 oblique septa, becoming brown when mature with 1–3 transverse septa, 0–3 longitudinal septa, and 0–2 oblique septa.

Culture characteristics: *Colonies* effuse with even, smooth margins, white on the surface, and greenish olivaceous underneath, reaching a radius of 31 mm after 3 d at 25 °C. Cardinal temperatures for growth: min 10 °C, max 35 °C, opt 30 °C.

Type: **South Africa**, Western Cape Province, Stellenbosch, on *V. vinifera,* 2002, J.M. van Niekerk, **holotype** CBS H-7756.

Cultures: STE-U 5252 = CBS 110887 (ex-type), STE-U 5050 = CBS 110880.

Hosts: *Eucalyptus corticosa* (as *N. corticosae*) (Summerell *et al.* 2006), *Eucalyptus* sp., *Eucalyptus camaldulensis*, *Eucalyptus diversicolor*, *E. pauciflora*, *Eucalyptus marginata*, *Eucalyptus rubida*, *Eucalyptus viminalis* (as *D. eucalypti*) (Barber *et al.* 2005, Taylor *et al.* 2009, Sutton 1980), Olea *europaea* (Lazzizera *et al.* 2008, Úrbez-Torres *et al.* 2013), *Prunus armeniaca*, *Prunus persica*, *Prunus salicina* (Damm *et al.* 2007), *Vaccinium corymbosum* (Kong *et al.* 2010), *Vitis vinifera* (van Niekerk *et al.* 2004, Úrbez-Torres *et al.* 2012).

Known distribution: Australia (Sutton 1980, Barber *et al.* 2005, Summerell *et al.* 2006, Taylor *et al.* 2009), China (Kong *et al.* 2010), Italy (Lazzizera *et al.* 2008), South Africa (van Niekerk *et al.* 2004, Damm *et al.* 2007), USA (Úrbez-Torres *et al.* 2012, Úrbez-Torres *et al.* 2013).

Notes: The fusiform conidia of *N. vitifusiforme* separate this species from its closest relative *N. viticlavatum*, which has clavate conidia.

This species was originally thought to be restricted to *Vitis* species, but it was later isolated from *Olea europaea* in Italy (Lazzizera *et al.* 2008). The same authors showed that it is phylogenetically indistinguishable from *Dichomera eucalypti*, which was confirmed in the present study based on ITS and EF1-α. Thus, *D. eucalypti* becomes a synonym and *Eucalyptus* can be regarded as an additional host for the fungus. Furthermore, as mentioned earlier, in the ITS phylogeny, *N. corticosae* grouped with *N. vitifusiforme* and *D. eucalypti* and despite the lack of EF1-α sequence data for *N. corticosae* it would appear that these three species are synonyms, more information is needed to confirm this.

Neoscytalidium Crous & Slippers, Stud. Mycol. 55: 244. 2006. MycoBank MB500868.
Type species: *Neoscytalidium hyalinum* (C.K. Campb. & J.L. Mulder) A.J.L. Phillips, Groenewald & Crous.
Coelomycetous synasexual morph: *Hendersonula* Speg., Anal. Soc. Cient. Arg. 10: 160. 1880.

Ascomata not reported. *Conidia* occurring in arthric chains in aerial mycelium, powdery to the touch, disarticulating, cylindrical-truncate, oblong-obtuse to doliiform, dark brown, thick-walled, 0–2-septate. Coelomycetous synasexual morph: *Mycelium* immersed, branched, septate, hyaline. *Conidiomata* stromatic and irregularly multilocular, or pycnidial and unilocular, blackish brown. *Conidiophores* absent. *Conidiogenous cells* discrete, determinate or indeterminate, hyaline, smooth, ampulliform, doliiform or cylindrical, proliferating enteroblastically with conidia seceding at the same level or at successively higher levels, periclinal thickening distinct or not, with occasionally a single percurrent proliferation. *Conidia* holoblastic, pale brown, smooth or verruculose, thin-walled, 1–3 (mostly 3)-euseptate, septa thick and prominent, cylindrical to fusiform, apex obtuse, base truncate, eguttulate, occasionally with a mucilagenous apical appendage.

Species descriptions

Neoscytalidium hyalinum (C.K. Campb. & J.L. Mulder) A.J.L. Phillips, Groenewald & Crous, **comb. nov.** Fig. 59. MycoBank MB805648.
Basionym: *Scytalidium hyalinum* C.K. Campb. & J.L. Mulder, Sabouraudia, 15: 163, 1977.
= *Torula dimidiata* Penz., Michelia 2: 466. 1882.
≡ *Scytalidium dimidiatum* (Penz.) B. Sutton & Dyko, Mycol. Res. 93: 484. 1989.
≡ *Fusicoccum dimidiatum* (Penz.) D.F. Farr, Mycologia 97: 740. 2005.
≡ *Neoscytalidium dimidiatum* (Penz.) Crous & Slippers, Stud. Mycol. 55: 244. 2006.
= *Hendersonula toruloidea* Nattrass, Trans. Br. Mycol. Soc. 18: 197. 1933.

Ascomata not reported. *Conidia* occurring in arthric chains in aerial mycelium, powdery to the touch, disarticulating, cylindrical-truncate, oblong-obtuse to doliiform, dark brown, thick-walled, 0–2-septate, 4–16.5 × 8.5 µm. Coelomycetous synasexual morph: *Conidiomata* stromatic, immersed, eventually erumpent, dark brown to black, unilocular to multilocular, globose, up to 2 mm diam, wall of 7–12 cell layers, up to 20–43 µm thick, outer wall of irregular, thick-walled, dark brown *textura angularis*, inner wall of hyaline, thinner-walled *textura angularis*. *Ostiole* central to each locule, circular, papillate. *Conidiophores* absent. *Conidiogenous cells* lageniform to ampulliform, hyaline, discrete, collarette absent, periclinal thickenings and cytoplasmic channel wide, arising from the inner wall of the locules, 6.5–14 × 2.5–4 µm. *Conidia* holoblastic, ellipsoid to nearly fusiform, hyaline, at first aseptate, then becoming 1–2(–3)-euseptate, central cell dark brown, end cells hyaline to pale brown, 10–16(–21) × 3.5–6.5 µm.

Lectotype: **United Kingdom**, sole of human foot, 20 Nov. 1973, C.K. Campbell, CBS H-7745 (isotype of *Scytalidium hyalinum*).

Cultures: CBS 145.78 (ex-isotype).

Hosts: Human skin and nails (Campbell & Mulder 1977). According to Sutton & Dyko (1989) it is plurivorous causing gummosis, dieback, wilt and cankers on *Acacia auriculiformis, Agathis palmerstoni, Agave americana, Agave sisalana, Ananas comosus, Ananas sativa, Citrus sinensis, Eucalyptus, Eucalyptus globulus, Ficus carica, Fucraea sp., Ipomoea batatas, Juglans regia, Malus pumila, Mangifera indica, Manihot utilissima, Melia azaderach, Morus alba, Musa, Philidendron bipinnatifidum, Plumeria obtusa, Populus alba, Prunus armeniaca, Sanseveria guineensis.*

Known distribution: Tropical and sub-tropical regions of Europe, Africa, Asia, North and South America.

Notes: Nattrass (1933) first described this fungus under the name *Hendersonula toruloidea* . Gentles and Evans (1971) reported the same fungus from a dermatomycosis in patients from tropical areas and a few years later, Campbell and Mulder (1977) introduced the new species *S. hyalinum* as the cause of the same clinical lesions as *H. toruloidea*. Since these first descriptions, the production of both arthroconidial and pycnidial synanamorphs has been shown and led to several controversies in the nomenclature. Sutton and Dyko (1989) transferred *H. toruloidea* to *Nattrassia mangiferae* with the mycelial synanamorph named *Scytalidium dimidiatum* based on *Torula dimidiata*. Farr *et al.* (2005) concluded from a phylogenetic analysis that *Nattrassia mangiferae* and *Scytalidium dimidiatum* belong in *Fusicoccum* and introduced the name *Fusicoccum dimidiatum* to replace *Scytalidium dimidiatum*. Crous *et al.* (2006) in a taxonomic revision of the *Botryosphaeriaceae* concluded that *Scytalidium* is polyphyletic and proposed the genus *Neoscytalidium* to accommodate *S. dimidiatum* as *N. dimidiatum*. It has been suggested that *S. dimidiatum* and *S. hyalinum* might be conspecific and a new name (*N. dimidiatum* var. *hyalinum*) has been suggested (Madrida *et al.* 2009). Although Crous *et al.* (2006) included an isolate of *S. hyalinum* in their study, they were not aware at the time that the isolate is in fact linked to the isotype of *S. hyalinum*. Since *S. hyalinum* is phylogenetically indistinguishable from *N. dimidiatum* and is the older epithet we transfer *S. hyalinum* to *Neoscytalidium* and reduce *N. dimidatum* to synonymy. Diseases reported to be associated with this fungus tend to be more common in tropical countries. It has been associated with freeze-damaged limbs of *Citrus* spp. in California, and appears to be a wound pathogen of this host. In Italy, it causes a shoot blight, canker and gummosis disease of *Citrus* (Polizzi *et al*. 2009, 2011).

Neoscytalidium novaehollandiae Pavlic, T.I. Burgess, M.J. Wingf., Mycologia 100: 862. 2008. MycoBank MB512103. See Pavlic *et al.* (2008) for illustrations.

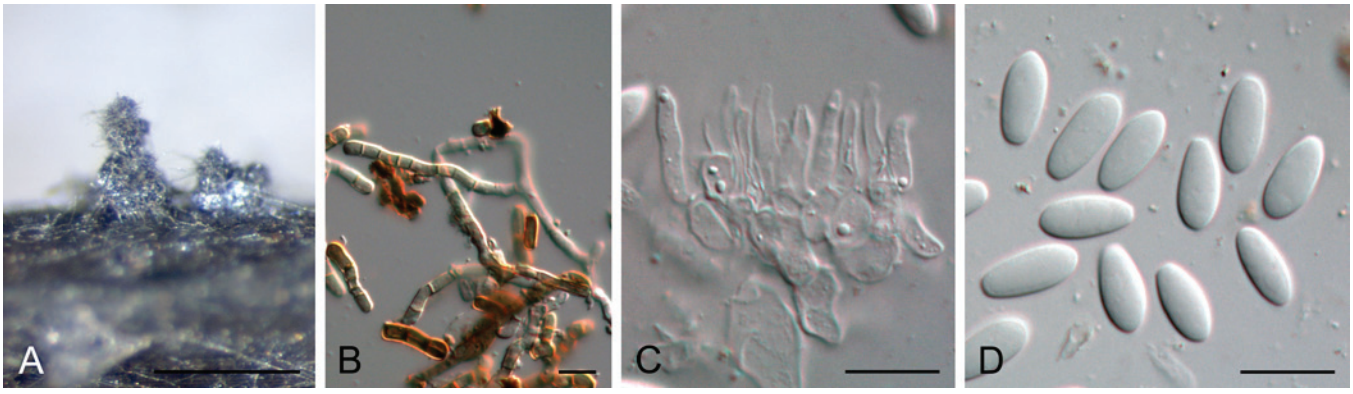

Fig. 59. *Neoscytalidium hyalinum*. A. Conidiomata formed on pine needles in culture. B. Arthric chains of conidia. C. Conidiogenous cells of coelomycetous state. D. Conidia of coelomycetous state. Scale bars: A = 500 µm, B–D = 10 µm.

Ascomata not reported. *Conidiomata* semi-immersed or superficial, solitary or in multilocular stromata, black, with globose base, up to 300 µm diam and long neck, up to 600 µm long. *Conidiogenous cells* holoblastic, cylindrical to subcylindrical, hyaline, the first conidium produced holoblastically and subsequent conidia enteroblastically, (6–)7–10(–11) × (2–)2–3(–4) µm (av. = 8.6 × 2.5 µm). *Conidia* of two types: (i) ellipsoidal to oval, apices rounded, initially hyaline, unicellular, becoming cinnamon to sepia, and 0–1-septate or 2-septate with darker central cell, (8–)10.5–12.5(–14) × (3–)4–5(–5) µm (av. = 11.5 × 4.4 µm, L/W = 2.6); (ii) variable in shape, globose, subglobose to obpyriform with muriform septa, initially hyaline becoming cinnamon to sepia, (8–)8.5–12.5(–15.5) × (5–)5.5–7.5(–8) µm (av. = 10.6 × 6.9 µm, L/W = 1.5). *Aerial mycelium* forms chains of arthroconidia, (5–)5.5–7.5(–9.5) × (3–)3.5–4.5(–5) µm (av. = 6.5 × 4 µm, L/W = 1.6), unicellular, powdery to the touch, disarticulating, cylindrical, oblong to obtuse to doliiform, thick-walled, initially hyaline becoming becoming cinnamon to sepia and 0–1-septate.

Culture characteristics: *Colonies* initially white to olivaceous-buff, becoming greenish olivaceous to citrine from the middle of colonies within 7 d, and black (surface and beneath) with age, with suppressed, moderately fluffy mycelium, edges smooth. Optimum growth at 35 ºC, covering the 90 mm diam Petri dish after 3 d in the dark.

Type: **Australia**, Western Australia, Bell Gorge, on *Crotalaria medicaginea*, Jul. 2006, T.I. Burgess, **holotype** PREM 60069.

Cultures: CMW 26170 = CBS 122071 (ex-type).

Hosts: Asymptomatic branches (sapwood) of *Acacia synchronica*, *Adansonia gibbosa*, *Crotalaria medicaginea* and *Grevillia agrifolia* (Pavlic *et al.* 2008). Pathogen of *Mangifera indica* and *Ficus carica* (Ray *et al.* 2010).

Known distribution: northern Western Australia.

Notes: Although *N. novaehollandiae* is morphologically and phylogenetically similar to *N. dimidiatum* (Punithalingam & Waterston 1970, Crous *et al.* 2006), Pavlic *et al.* (2008) reported muriform, dichomera-like conidia in the isolates that they studied and for this reason they regarded it as a distinct species.

Phaeobotryon Theiss. & Syd., Ann. Mycol. 13: 664. 1915. MycoBank MB3892.
Type species: *Phaeobotryon cercidis* (Cooke) Theiss. & Syd., Ann. Mycol. 13: 664. 1915.

Ascomata black, immersed to erumpent, subglobose to ovoid, multilocular, wall composed of layers of dark brown *textura angularis*. *Pseudoparaphyses* hyphae-like, septate, constricted at septa. *Asci* 8-spored, bitunicate, fissitunicate, clavate to cylindro-clavate, short-pedicellate, apically rounded with an ocular chamber. *Ascospores* hyaline to brown, 2-septate, ellipsoid to broad fusiform, with an apiculus at each end, immature asci surrounded by a mucilagenous sheath. *Conidiomata* pycnidial, stromatic, black, ostiolate, separate or aggregated, immersed to erumpent, unilocular or multilocular, ostiolate. *Ostiole* circular, central, papillate. *Paraphyses* hyaline, thin-walled, usually aseptate, sometimes becoming 1–2-septate. *Conidiogenous cells* holoblastic, hyaline, thin-walled, smooth, cylindrical to doliiform. *Conidia* ellipsoidal to oblong or obovoid, ends rounded, moderately thick-walled, initially hyaline, becoming brown, mostly 2-septate at maturity.

Notes: *Phaeobotryon* was introduced by Theissen & Sydow (1915) to accommodate *Dothidea cercidis*. This taxon was considered a distinct genus on account of its pale brown, 2-septate ascospores, which were reported as hyaline in the original description. In their broad concept of *Botryosphaeria,* von Arx & Müller (1954, 1975) considered *Phaeobotryon* as a synonym of *Botryosphaeria*. Phillips *et al.* (2008) reinstated *Phaeobotryon* after they showed that it is morphologically and phylogenetically distinct from all other genera in the *Botryosphaeriaceae*. The 2-septate, brown ascospores with an apiculus at each end are characteristic for the genus. Only two species (*P. mamane* and *P. cupressi*) are currently known in culture and they can be separated on the size of their conidia.

Species descriptions

Phaeobotryon mamane Crous & A.J.L. Phillips, Persoonia 21: 45. 2008. MycoBank MB506581. See Phillips *et al.* (2008) for illustrations.

Ascomata pseudothecial, dark brown to black, stromatic, globose, aggregated in botryose clusters or separate, immersed, becoming erumpent, ostiolate, up to 350 µm diam, wall consisting of 4–6 cell

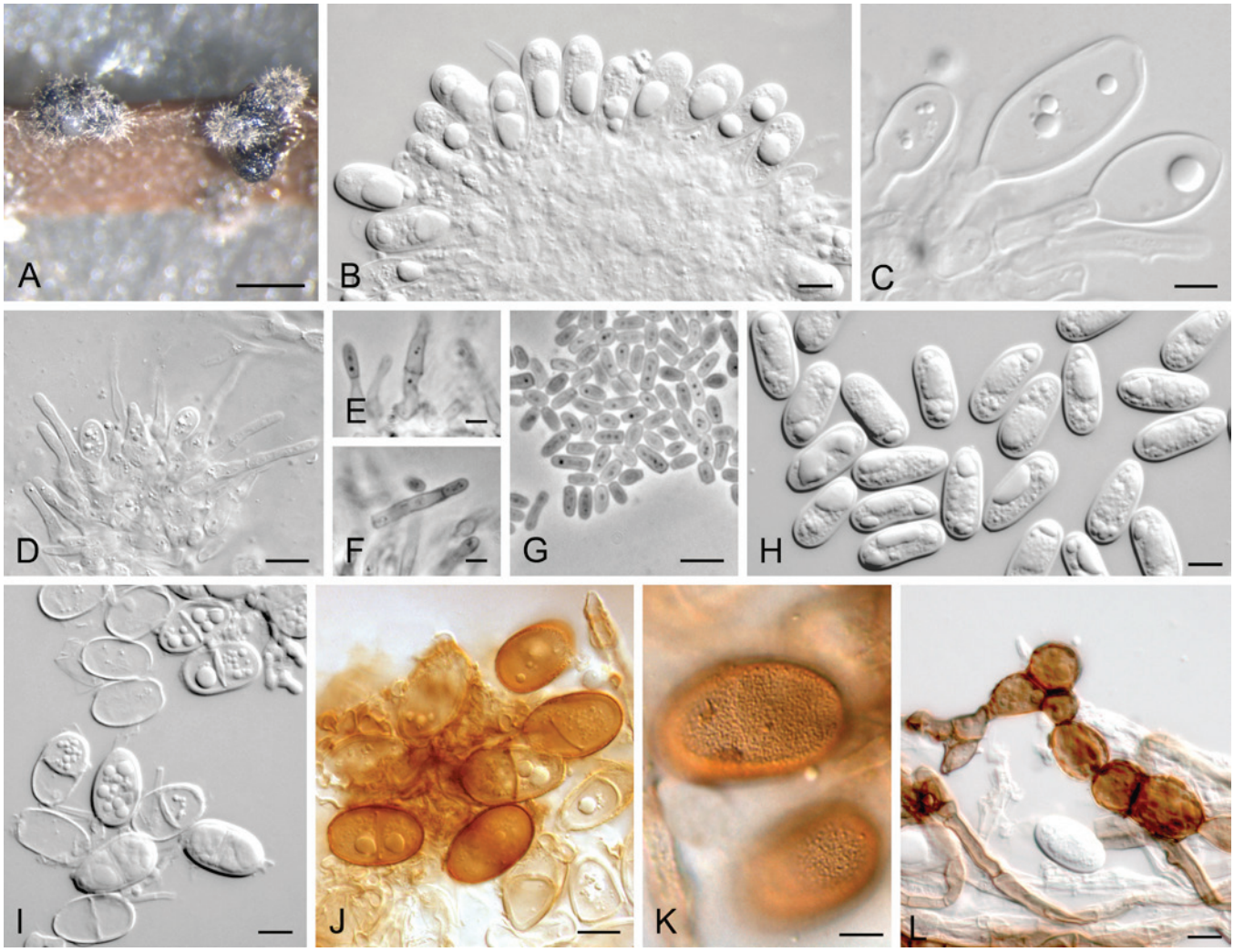

Fig. 60. *Phaeobotryon cupressi*. A. Conidiomata formed on pine needles in culture. B, C. Conidia on conidiogenous cells D. Paraphyses and developing conidia. E, F. Spermatogenous cells. G. Spermatia. H. Hyaline immature conidia. I. Mature and germinated, hyaline and septate or aseptate conidia. J, K. Mature, brown septate or aseptate conidia in two different focal planes to show verruculose inner surface of the wall. L. Brown chlamydospores. Scale bars: A = 500 µm, B, D, H–J, L = 10 µm, C, G, K = 5 µm, E, F = 2.5 µm.

layers of dark brown *textura angularis*. *Pseudoparaphyses* hyaline, smooth, multiseptate, with septa 10–23 µm apart, constricted at septa, 3–4 µm wide. *Asci* bitunicate, 8-spored, stipitate, thick-walled with thick endotunica and well-developed apical chamber, 120–150(–200) × 25–30 µm, with biseriate ascospores. *Ascospores* ellipsoid to ovate, (30–)37–40(–45) × (11–)13–15(–16) µm, 2-septate, with three cells of equal length, not constricted at septa, finely verruculose, widest in middle with conical apiculus at one or both ends. *Spermatogonia* morphologically similar to conidiomata, also formed in culture. *Spermatia* hyaline, rod-shaped with rounded ends, 3–5 × 2 µm. *Conidiomata* pycnidial, stromatic, ostiolate, separate or aggregated, globose, black, immersed to erumpent, unilocular, up to 350 µm diam, wall consisting of 4–6 layers of brown *textura angularis*. *Conidiogenous cells* cylindrical to doliiform, hyaline, smooth, proliferating percurrently near apex, 10–14 × 4–8 µm. *Conidia* ellipsoid to oblong or subcylindrical or obovoid, brown, smooth to finely verruculose, moderately thick-walled, granular, guttulate, ends rounded, 1(–2)-septate, base with inconspicuous scar, slightly flattened, (30–)35–38(–43) × (12–)14–15(–16) µm.

Type: **USA**, Hawaii, Manna Koa Park, Saddle Road, on stems of *Sophora chrysophylla*, Jul. 2005, W. Gams, **holotype** CBS H-20109.

Cultures: CPC 12440 = CBS 122980 (ex-type).

Host: *Sophora chrysophylla* (Phillips *et al.* 2008).

Known distribution: USA (Hawaii) (Phillips *et al.* 2008).

Note: Asexual morph dothiorella/spencermartinsia-like, but with up to two transverse septa and apiculi at either end of the ascospores.

Phaeobotryon cupressi Abdollahz., Zare & A.J.L. Phillips, Persoonia 23: 6. 2009. MycoBank MB513236. Fig. 60.

Ascomata not reported. *Conidiomata* pycnidial, stromatic, superficial, dark-brown to black, mostly unilocular on pine needles and up to 650 µm diam, mostly multilocular on *Populus* twigs, individual or aggregated, thick-walled, ostiolate. *Ostiole* central, circular, non-papillate. *Paraphyses* hyaline, thin-walled, arising from the conidiogenous layer, extending above the level of developing conidia, up to 42 µm long, 4.8 µm wide, usually aseptate, sometimes becoming up to 2-septate, tip rounded, occasionally branched. *Conidiophores* absent. *Conidiogenous cells* hyaline, smooth, thin-walled, cylindrical, holoblastic, phialidic, proliferating internally with visible periclinal thickening, 7–14 × 2–5

µm. *Conidia* thick-walled, initially hyaline, oval, both ends broadly rounded, aseptate, forming a single septum at germination, rarely becoming brown and 1-septate, internally verruculose when aged, (19.5–)21–28(–30) × (10–)11–15(–17) µm, 95 % confidence limits = 24–25 × 12–12.5 µm (av. ± S.D. = 24.8 ± 1.9 × 12.4 ± 1.3 µm), L/W ratio = 2. *Spermatogonia* globose, dark-brown to black, superficial, occasionally immersed in pine needle or *Populus* tissue. *Spermatophores* cylindrical, hyaline, aseptate becoming 1–2-septate, branched, 7–13 × 1.5–2.5 µm. *Spermatogenous cells* hyaline, thin-walled, phialidic, proliferating internally, giving rise to periclinal thickening, 6–10 × 1–2 µm. *Spermatia* oval, thin-walled, hyaline, aseptate 2–4 × 1–2 µm. *Chlamydospores* intercalary, brown, smooth, thick-walled, formed within the agar medium.

Culture characteristics: *Colonies* on PDA with abundant aerial mycelium towards periphery, appressed in the centre, becoming grey-olivaceous to olivaceous-grey at the surface, and grey-olivaceous in reverse after 2 wk in the dark at 25 °C, reaching 46–53 mm diam after 4 d in the dark at 25 °C. Cardinal temperatures for growth: min 5 °C, max > 35 °C, opt 25 °C.

Type: **Iran**, Golestan Province, Gorgan, City Park, on twigs of *Cupressus sempervirens*, 15 Aug. 2006, M.A. Aghajani, **holotype** IRAN 13940F.

Cultures: IRAN 1455C = CBS 124700 (ex-type).

Host: *Cupressus sempervirens* (Abdollahzadeh *et al*. 2009), *Juniperus scopulorum* (Alves *et al*. 2013).

Known distribution: Iran (Abdollahzadeh *et al*. 2009), USA (Alves *et al*. 2013).

Notes: This species differs from *P. quercicola* and *P. mamane* in its smaller conidia, and has been collected only from *Cupressus* species. The hyaline, aseptate conidia of *P. cupressi* are superficially similar to those of other *Diplodia* species with hyaline conidia. Furthermore, conidial dimensions of *P. cupressi* are similar to those of *Diplodia cupressi* (21.5–30.5 × 12–16 µm) as reported by Alves *et al.* (2006). It is thus possible that *P. cupressi* has been mistaken for *D. cupressi* in the past. Pycnidial paraphyses in *Phaeobotryon* clearly distinguish this genus from *Diplodia*.

Pseudofusicoccum Mohali, Slippers & M.J. Wingf., Stud. Mycol. 55: 249. 2006. MycoBank MB500884.
Type species: *Pseudofusicoccum stromaticum* (Mohali, Slippers & M.J. Wingf.) Mohali, Slippers & M.J. Wingf., Stud. Mycol. 55: 249. 2006.

Resembling species of *Fusicoccum*, but distinct in having conidia encased in a persistent mucous sheath. Conidia are also more cylindrical than in *Fusicoccum* species.

Notes: *Pseudofusicoccum* was introduced by Crous *et al.* (2006) for species that are morphologically similar to *Fusicoccum* and *Neofusicoccum* but phylogenetically distinct from both of these genera. While it was originally based on *Ps. stromaticum* a further five species have subsequently been added to the genus. Species are distinguished primarily on the dimensions of their conidia and on pigment production in culture. Thus far no sexual morphs have been found. The species appear to be restricted to tropical or sub-tropical regions and occur mainly as endophytes. There is no evidence of host-specificity.

Key to *Pseudofusicoccum* spp.

1. Forms a violet pigment in culture 2
1. No violet pigment in cultures 3

2. Conidia on average greater than 30 µm long ..... *Ps. violaceum*
2. Conidia on average smaller than 25 µm long ..... *Ps. adansoniae*

3. Conidia on average smaller than 30 µm long 4
3. Conidia on average = 30 µm or more long ..... *Ps. kimberleyense*

4. Conidia on average 7 µm or more wide 5
4. Conidia on average smaller than 7 µm wide ..... *Ps. stromaticum*

5. Conidia 20–26 × 6.5×7.5 µm ..... *Ps. olivaceum*
5. Conidia 21–29 × 7–8 µm ..... *Ps. ardesiacum*

DNA phylogeny

Six species can be distinguished in the ITS phylogeny (Fig. 61). Support for *Ps. ardesiacum* and *Ps. kimberleyensis* is very low and the branch lengths for these two species are very short. Morphologically they are also very similar, although conidia of *Ps. kimberleyensis* are, on average, longer than those of *Ps. ardesiacum*.

Species descriptions

Pseudofusicoccum adansoniae Pavlic, T.I. Burgess, M.J. Wingf., Mycologia 100: 855. 2008. MycoBank MB512048. See Pavlic *et al.* (2008) for illustrations.

Ascomata not seen. *Conidiomata* semi-immersed, solitary, globose, papillate, chestnut, covered by hyphal hairs, up to 500 µm diam. *Conidiogenous cells* holoblastic, smooth, cylindrical, hyaline, the first conidium produced holoblastically and subsequent conidia enteroblastically, (9–)10–15(–16) × (1.5–)2–

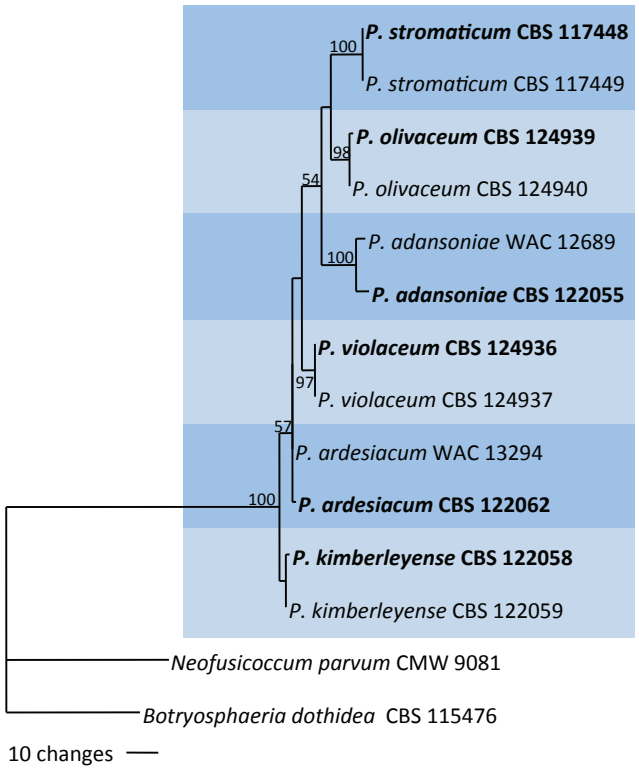

Fig. 61. One of six equally most parsimonious trees obtained from combined ITS and EF1-α sequence data for species in *Pseudofusicoccum*. Bootstrap values from 1000 replicates are given at the nodes.

3(–3.5) µm (av. 12.7 × 2.4 µm). *Conidia* ellipsoid, occasionally slightly bent or irregularly shaped, apices rounded, smooth with fine granular content, hyaline, thin-walled, covered with a persistent mucus layer, unicellular, forming 1 or 2 septa before germination, (19–)21–24(–26) × (3.5–) 4.5–6(–6.5) µm (av. size of conidia = 22.5 × 5.2 µm), L/W = 4.3.

Culture characteristics: *Colonies* initially white with moderately dense, appressed mycelial mat, submerged mycelium turning grey-olivaceous to olivaceous-black from the middle of colony after 3–5 d and becoming dark slate-blue with age, aerial mycelium slightly fluffy, becoming dense, cottony with age, sometimes remaining white to smoke-grey, usually turning pale olivaceous-grey within 7 d and becoming olivaceous-grey to iron grey with age; conidiomata readily formed from the middle of colony within 7–10 d, covering the entire surface of the colony and immersed in the medium. Optimum growth at 30 °C, covering the 90 mm diam Petri dish after 4 d in the dark.

Type: **Australia**, Western Australia, Derby, on *Adansonia gibbosa*, Jul. 2006, T.I. Burgess, **holotype** PREM 59841 (a dry culture on pine needles).

Cultures: CBS 122055 = CMW 26147 (ex-type).

Hosts: *Adansonia gibbosa*, *Acacia synchronica*, *Eucalyptus* sp., *Ficus opposita* (Pavlic *et al.* 2008), *Adansonia gregorii*, *Grevillea agrifolia* (Sakalidis *et al.* 2011).

Known distribution: Australia (Pavlic *et al.* 2008, Sakalidis *et al.* 2011).

Notes: This species appears to be a non-specialised endophyte since it has been found on asymptomatic hosts residing in five widely separate genera. It has been found only in Australia.

Pseudofusicoccum ardesiacum Pavlic, T.I. Burgess, M.J. Wingf., Mycologia 100: 858. 2008. MycoBank MB512051. See Pavlic *et al.* (2008) for illustrations.

Ascomata not seen. *Conidiomata* semi-immersed, solitary, globose, papillate, chestnut, covered by hyphal hairs, up to 510 µm diam. *Conidiogenous cells* holoblastic, smooth, cylindrical, hyaline, the first conidium produced holoblastically and subsequent conidia enteroblastically, (6–)7.5–10(–11) × (2.7–)3–4(–4.5) µm (av. = 8.6 × 3.5 µm). *Conidia* ellipsoid to rod-shape, straight or slightly bent, apices rounded, smooth with fine granular content hyaline, thin-walled, covered with a persistent mucus layer, unicellular, forming 1–3 septa before germination, (17.5–)21–29(–32) × (6.5–)7–8(–9) µm (av. = 25 × 7.5 µm), L/W = 3.3.

Culture characteristics: *Colonies* initially white with sparse to moderately dense appressed mycelial mat; submerged mycelium dark violet to dark blue (middle of the colony) and smoke grey to grey-olivaceous toward edges within 3–5 d, becoming violaceous grey to slate blue with age, aerial mycelium slightly fluffy, becoming dense, cottony with age, turning smoke grey to pale purplish grey in the middle of colony and smoke grey to grey-olivaceous toward edges after 5–7 d, becoming lavender grey with age; occasional columns of aerial mycelium in the middle of colony, reaching the lid, colonies slightly irregular with sinuate edges, conidiomata readily formed in culture and immersed in aerial mycelia on the entire colony surface within 7–10 d. Optimum growth at 30 ºC, covering the 90 mm diam Petri dish after 4 d in the dark.

Type: **Australia**, Western Australia, Mount Hardman, Great Northern Highway, on *Adansonia gibbosa*, Jul. 2006, T.I. Burgess, **holotype** PREM 59843 (a dry culture on pine needles).

Cultures: CMW 26159 = CBS 122062 (ex-type).

Hosts: *Adansonia gibbosa*, *Eucalyptus* sp. (Pavlic *et al.* 2008).

Known distribution: Western Australia (Pavlic *et al.* 2008).

Notes: This species is probably an endophyte not restricted to any host since it has been found on dying branches of *Adansonia* and in asymptomatic branches of *Eucalyptus* sp. (Pavlic *et al.* 2008). It is known only from Australia.

Pseudofusicoccum kimberleyense Pavlic, T.I. Burgess, M.J. Wingf., Mycologia 100: 857. 2008. MycoBank MB512049. See Pavlic *et al.* (2008) for illustrations.

Ascomata not seen. *Conidiomata* semi-immersed, solitary, globose, papillate, chestnut brown, covered by hyphal hairs, up to 500 µm diam. *Conidiogenous cells* holoblastic, smooth, cylindrical to subcylindrical, hyaline, the first conidium produced holoblastically and subsequent conidia enteroblastically, (7–) 8.5–11(–14) × (2.5–) 3–3.5(–4) µm (av. = 9.8 × 3.3 µm). *Conidia* ellipsoid, straight or slightly curved, apices rounded, smooth

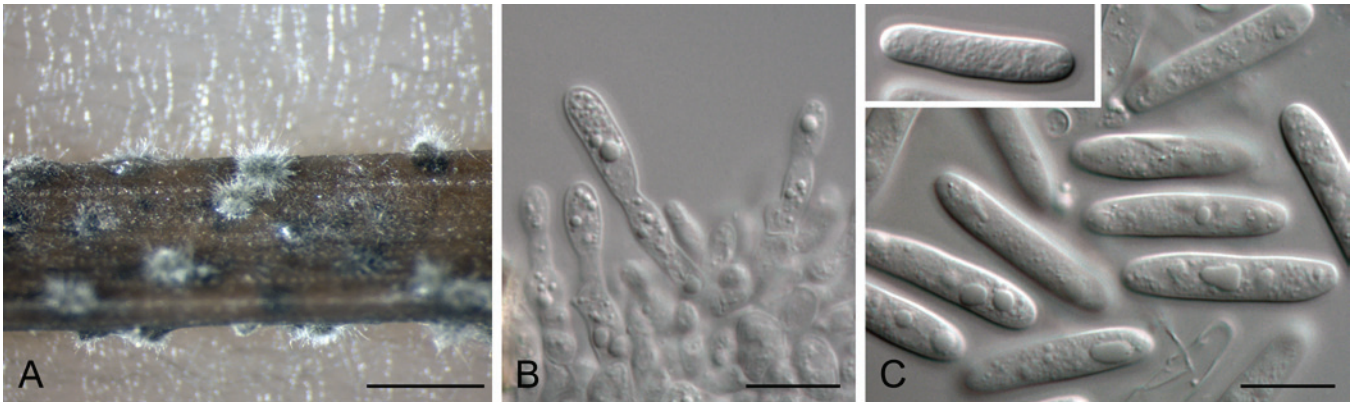

Fig. 62. *Pseudofusicoccum stromaticum*. A. Conidiomata developing on pine needle in culture. B. Conidiogenous cells. C. Conidia. The mucilagenous sheath is visible on the conidium in the insert. Scale bars: A = 1 mm, B, C = 10 µm.

with fine granular content, hyaline, thin-walled, covered with a persistent mucus layer, unicellular, forming 1–4 septa before germination, (24–)28–33(–34) × (6.5–)7–8(–8.5) µm (av. = 30.7 × 7.4 µm), L/W = 4.1.

Culture characteristics: *Colonies* slightly irregular with sinuate edges, initially white, forming a moderately dense, appressed mycelial mat, submerged mycelium citrine to grey-olivaceous from the middle of colony after 3–5 d, becoming olivaceous-black to black with age, aerial mycelium slightly fluffy, becoming dense, cottony with age, smoke-grey to pale olivaceous-grey. Optimum growth at 30 ºC, covering a 90 mm diam Petri dish after 4 d in the dark.

Type: **Australia**, Western Australia, Tunnel Creek National Park, on *Acacia synchronica*, Jul. 2006, T.I. Burgess, **holotype** PREM 59842 (a dry culture on pine needles).

Cultures: CMW 26156 = CBS 122058 (ex-type).

Hosts: *Adansonia gibbosa*, *Acacia synchronica*, *Eucalyptus* sp. and *Ficus opposita* (Pavlic *et al.* 2008).

Known distribution: Western Australia (Pavlic *et al.* 2008).

Note: The wide range of hosts and absence of symptoms on the hosts suggest that this species is a non-specialised endophyte known only in Australia.

Pseudofusicoccum olivaceum Mehl & Slippers, Mycologia 103: 537. 2011. MycoBank MB513501. See Mehl *et al.* (2011) for illustrations.

Ascomata not seen. *Conidiomata* on host and on pine needles on water agar pycnidial, stromatic, subcuticular, unilocular, dark brown, mostly solitary, applanate, covered with hyphae/mycelium, wall composed of three layers: an outer layer of thick-walled dark to light brown *textura angularis*; a middle layer of thin-walled light brown cells; an inner layer of thin-walled hyaline cells, (480–)530–650(–690) µm diam. *Ostiole* central, circular, papillate. *Conidiogenous cells* hyaline, holoblastic, smooth, cylindrical, guttulate, proliferating percurrently to form one or two indistinct annellations, or proliferating at the same level giving rise to periclinal thickenings. *Paraphyses* (3–)4.5–8.5(–12.5) × (1.5–)3–4.5(–6.5) µm (av. = 6.6 × 3.7 µm). *Conidia* hyaline, thin-walled, unicellular, aseptate, occasionally granular, guttulate, surrounded by a persistent mucoid sheath, apex and base blunt to broadly rounded, bacilliform, (18–)20–25.5(–30.5) × (6–)6.5–7.5(–9) µm (av. = 22.8 × 7.0 µm).

Culture characteristics: *Cultures* fluffy, initially white to amber at the centre, olivaceous at the edges, becoming white to olivaceous with age. Optimum temperature for growth 25 °C.

Type: **South Africa**, Mpumalanga Province, Kruger National Park, Pretoriuskop, on an asymptomatic branch of *Pterocarpus angolensis*, Sep. 2005, J. Roux, **holotype** PREM 60328.

Cultures: CMW 20881 = CBS 124939 (ex-type), CMW 22637 = CBS 124940, CMW 22643 = CBS 124941 (ex-paratype).

Host: *Pterocarpus angolensis* (Mehl *et al.* 2011).

Known distribution: South Africa (Mehl *et al.* 2011).

Notes: In addition to the host on which it was described, this species has also been found on *Terminalia sericea* (Mehl *et al.* (2011), suggesting it is a common endophyte on other tree species.

Pseudofusicoccum stromaticum (Mohali, Slippers & M.J. Wingf.) Mohali, Slippers & M.J. Wingf., Stud. Mycol. 55: 249. 2006. MycoBank MB500885. Fig. 62.
Basionym: *Fusicoccum stromaticum* Mohali, Slippers & M.J. Wingf., Mycol. Res. 110: 408. 2006.

Ascomata not seen. *Conidiomata* large, superficial, multilocular, locule totally embedded without ostioles when formed on on MEA, smaller, uniloculate, ostiolate on pine needles; eustromatic, covered with hyphae, locule walls consisting of a dark brown *textura angularis*, becoming thinner and hyaline towards the conidiogenous region. *Conidiogenous cells* hyaline, holoblastic, smooth, cylindrical, producing a single apical conidium, the first conidium produced holoblastically and subsequent conidia produced enteroblastically, proliferating at the same level forming periclinal thickenings, (10–)11–15(–17) × (1.5–)2–3 µm (av. = 13 × 2.5 µm, L/W = 5.3). *Conidia* hyaline, thin to slightly thick-walled, aseptate, granular, cylindrical, straight to slightly curved, apex and base blunt to bluntly rounded, surrounded by a persistent mucous sheath, (19–)20–23(–24) × (4–)5–6 µm (av. = 21.5 × 5.5 µm), L/W = 4.

Culture characteristics: *Colonies* fluffy, greenish olivaceous with reverse olivaceous after 15 d on MEA at 25 °C, reaching 70–75 mm diam on MEA after 4 d in the dark at 25 °C. Cardinal temperatures for growth: min 15 °C (little or no growth), max < 40 °C (no growth at 40 °C), opt 30–35 °C.

Type: **Venezuela**, Portuguesa State, Acarigua, Smurfit Company, on branches of *Eucalyptus urophylla*, Feb. 2003, S. Mohali, **holotype** PREM 58237.

Cultures: CMW 13366 (ex-holotype), CMW 13434 = CBS 117448, CMW 13435 = CBS 117449.

Hosts: *Eucalyptus* spp. (Mohali *et al.* 2006), *Acacia mangium* (Mohali *et al.* 2006), *Mangifera indica* (Marques *et al.* 2012).

Known distribution: Brazil (Marques *et al.* 2012), Venezuela (Mohali *et al.* 2006).

Notes: *Pseudofusicoccum stromaticum* was originally isolated from asymptomatic as well as dead and dying branches and stems of *Eucalyptus* and *Acacia mangium* trees in Venezuela. The presence of the fungus in asymptomatic branches of two different host genera suggests that it is a generalist endophyte. However, it has been reported to cause die-back of *Mangifera indica* in Brazil (Marques *et al.* 2013).

Pseudofusicoccum violaceum Mehl & Slippers, Mycologia 103: 542. 2011. MycoBank 513500. See Mehl *et al.* (2011) for illustrations.

Ascomata not seen. *Conidiomata* on the host and on pine needles on water agar pycnidial, stromatic, superficial, unilocular, dark brown, mostly solitary, more or less globose/circular, covered with hyphae/mycelium, wall composed of three layers: an outer layer of thick-walled, dark to light brown *textura angularis*, a middle layer of thin-walled light brown cells, and an inner layer of thin-walled hyaline cells, (470–)500–615(–660) µm diam. *Ostiole* central, circular, papillate. *Conidiogenous cells* hyaline, holoblastic, smooth, cylindrical, proliferating percurrently to form one or two distinct annellations, or proliferating at the same level giving rise to periclinal thickenings, (5.5–)6–11(–17) × (2.5–)3.5–5(–6.5) µm (av. = 8.6 × 4.3 µm). *Paraphyses* not seen. *Conidia* hyaline, thin-walled, unicellular, aseptate, granular, guttulate, surrounded by a persistent mucoid sheath, apex and base blunt to broadly rounded, cylindrical, (26.5–)29.5–36(–39.5) × (8–)8.5–10.5(–11.5) µm (av. = 33.0 × 9.5 µm).

Culture characteristics: Cultures with fluffy mycelium, initially white to amber in the center and violet on the edges, turning olivaceous to greenish black in the centre and becoming olivaceous to greenish black with age. Optimum temperature for growth 30 °C.

Type: **South Africa**, Mpumalanga Province, Mawewe Nature Reserve, on an asymptomatic branch of *Pterocarpus angolensis*, Dec. 2005, J.W.M. Mehl & J. Roux, **holotype** PREM 60333.

Cultures: CMW 22679 = CBS 124936 (ex-type), CMW 22671 = CBS 124938 (ex-paratype).

Host: *Pterocarpus angolensis* (Mehl *et al.* 2011).

Known distribution: South Africa (Mehl *et al.* 2011).

Notes: The violet pigment formed in cultures of this species was considered to be distinctive for *Ps. violaceum* (Mehl *et al.* 2011). However, a similar pigment is also found in *Ps. ardesiacum* (Pavlic *et al.* 2008). Nevertheless, the two species can be distinguished based on conidial dimensions and are clearly differentiated in ITS and EF1-α phylogenies. The wide host range suggests that this is a non-specialised endophyte.

Spencermartinsia A.J.L. Phillips, A. Alves & Crous, Persoonia 21: 51. 2008. MycoBank MB511762.
Type species: *Spencermartinsia viticola* (A.J.L. Phillips & J. Luque) A.J.L. Phillips, A. Alves & Crous, Persoonia 21: 51. 2008.

Ascomata pseudothecial, ostiolate. *Pseudoparaphyses* thin-walled, hyaline, septate, constricted at septa. *Asci* bitunicate, 8-spored, clavate, stipitate, developing amongst thin-walled, septate pseudoparaphyses, with biseriate ascospores. *Ascospores* hyaline when young, brown when mature, uniseptate with an apiculus at each end. *Conidiomata* pycnidial, stromatic. *Conidiophores* absent. *Conidiogenous cells* lining inner surface of conidiomata, holoblastic, proliferating internally producing periclinal thickenings, or proliferating percurrently to form annellations. *Conidia* initially hyaline, becoming dark brown and 1-euseptate within the pycnidial cavity often while still attached to the conidiogenous cell, thick-walled, externally smooth, internally verruculose, broadly rounded at the apex, base truncate.

Notes: *Spencermartinsia* was introduced by Phillips *et al.* (2008) for species similar to *Dothiorella* but that differ in having 2-celled ascospores with an apiculus at either end of the ascospores. This minor difference was considered to be taxonomically meaningful since the presence or lack of apiculi on ascospores also separates other genera in this family, such as *Barriopsis* (no apiculus) from *Sphaeropsis* (apiculus present), and this was supported phylogenetically. Nevertheless, this is a tenuous and difficult morphological character to apply, especially since a sexual morph has been reported only for *S. viticola* and it is not clear whether this is a consistent character for the genus. Furthermore, with the addition of further species in *Dothiorella,* the phylogenetic distinction between the two genera is becoming less obvious. However, we continue to recognise *Spencermartinsia* as a separate genus pending further phylogenetic and morphological studies including additional species. *Spencermartinsia* is presently monotypic based on *S. viticola*. Based on phylogenetic analyses, two recently described species, *S. uruguayensis* and *S. pretoriensis* have been re-combined in *Dothiorella* (see above).

DNA phylogeny

Based on ITS and EF1-α sequence data, *Spencermartinsia* is clearly separated from *Dothiorella*. In the phylogenetic analyses two main clades are recognised in *Spencermartinsia* (Figs 32, 33). The first clade constitutes *S. viticola* while the other includes three subclades including four isolates CBS 500.72 (*Diplodia medicaginis*), CBS 117006, ICMP 16827 and ICMP 16828, representatives of three distinct species. Isolate CBS 117006 identified by Luque

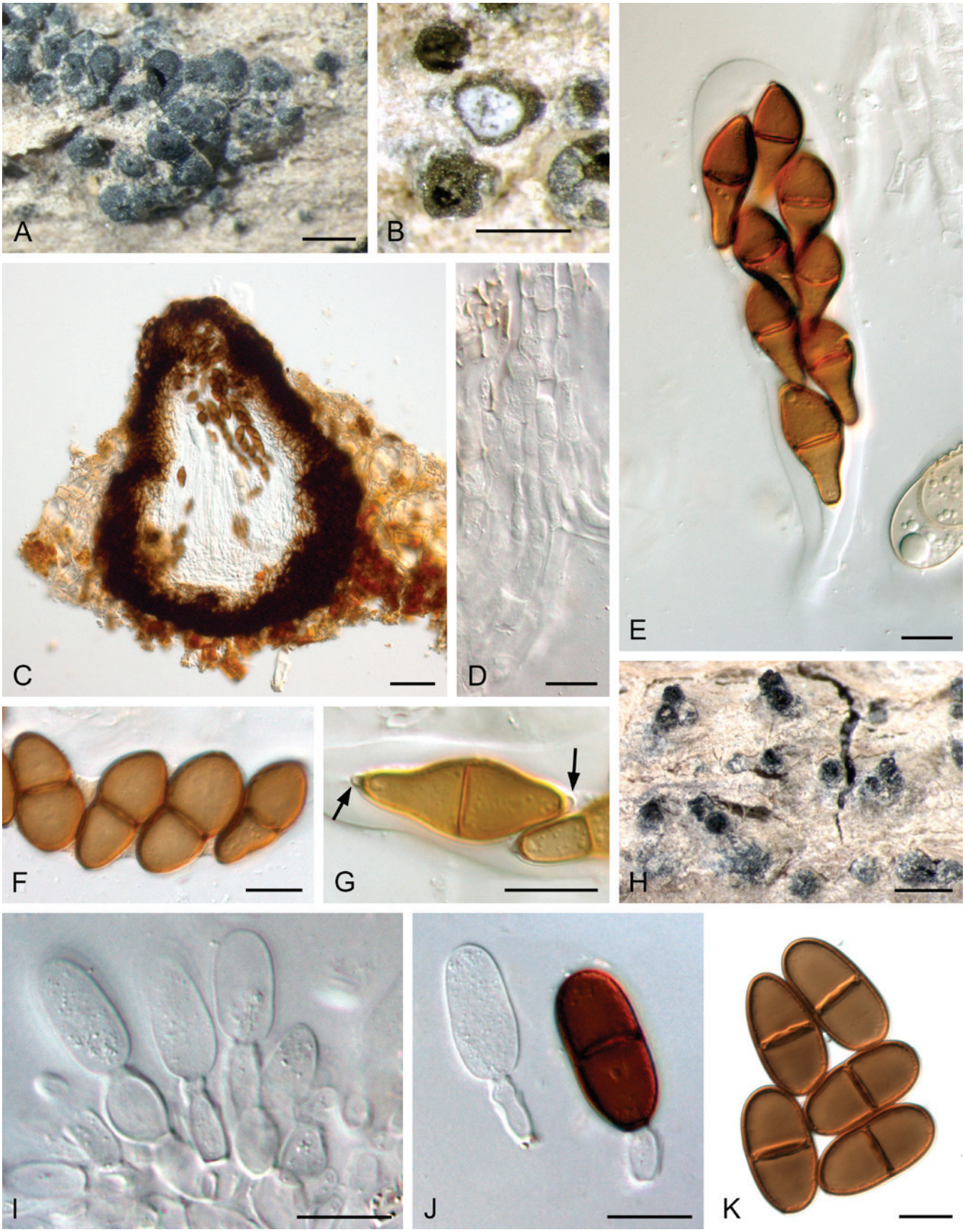

Fig. 63. *Spencermartinsia viticola*. A. Ascomata erumpent through the host bark. B. Ascoma cut through horizontally revealing the white contents with dark spots corresponding to asci and ascospores. C. Vertical section through an ascoma. D. Septate paraphyses. E. Clavate ascus containing eight biseriate, dark brown, 1-septate ascospores. F. Ascospores. G. Ascospores with small, rounded apiculi (arrows). H. Conidiomata partially erumpent through the host bark. I, J. Conidiogenous cells. K. Conidia. Scale bars: A, H = 500 µm, B = 250 µm, C = 50 µm, D–G, I–K = 10 µm.

et al. (2005) as *B. viticola*, exhibited some differences in culture morphology and sequence data from the ex-type strain and other strains as discussed by Phillips *et al*. (2008), reside in a distinct clade. The two isolates ICMP 16827 and ICMP 16828 on *Citrus sinensis* from New Zealand constitute a distinct clade as representatives of a new species. Furthermore, isolate CBS 500.72

previously characterised as *Diplodia medicaginis* formed another distinct clade and is clearly a missidentification. These species are not described here due to their uncertain taxonomic status.

Species descriptions

Spencermartinsia viticola (A.J.L. Phillips & J. Luque) A.J.L. Phillips, A. Alves & Crous, Persoonia 21: 51. 2008. MycoBank MB511763. Fig. 63.

Basionym: *Dothiorella viticola* A.J.L. Phillips & J. Luque, Mycologia 97: 1116. 2005.

= *Botryosphaeria viticola* A.J.L. Phillips & J. Luque, Mycologia 97: 1116. 2005.

Ascomata dark brown to black, stromatic, pyriform, pseudothecial, isolated or in botryose clusters up to 2 mm diam, initially immersed in host, partially erumpent at maturity, up to 240 µm diam, ostiole circular, central, papillate, wall up to 60 µm thick, of dark brown thick-walled *textura angularis*, and lined with thin-walled, hyaline cells. *Pseudoparaphyses* thin-walled, hyaline, frequently septate, slightly constricted at septum, 3.5–4.5(–5) µm wide. *Asci* arising from base of ascoma, stipitate, clavate, thick-walled, bitunicate with a thick endotunica and a well-developed apical chamber, 8-spored, irregularly biseriate, 100–110 × 25–30 µm. *Ascospores* oblong, ovate to sub-clavate, mostly 1-septate, slightly constricted at septum, dark brown, moderately thick-walled, finely verruculose on inner surface, often inequilateral, widest in lower 1/3 to middle of apical cell, often with a small rounded projection at tip and base of spore, basal cell tapering towards obtuse base, (19–) 22.5–23.5(–27) × (8.5–)10.5–11(–14.5) µm (av. ± S.D. = 23.1 ± 0.2 × 10.9 ± 0.1 µm). *Conidiomata* pycnidial, stromatic, separate or aggregated into botryose clusters up to 2 mm diam, individual conidiomata spherical to globose, black, immersed, partially erumpent when mature, unilocular, 200–360 µm diam, thick-walled, wall consisting of three layers: an outer layer of dark brown, thick-walled *textura angularis*, a median layer of dark brown thin-walled cells *textura angularis*, and an inner layer of thin-walled, hyaline cells. *Ostiole* single, central, circular, papillate. *Conidiophores* absent. *Conidiogenous cells* discrete or integrated, cylindrical to broad lageniform, (5–)8.5–10(–14) × (3–)4.5–5(–7) µm, hyaline, smooth, holoblastic, indeterminate, proliferating at same level to form periclinal thickenings or rarely proliferating percurrently giving rise to 1–2 annellations. *Conidia* brown, oblong to subcylindrical, septate, occasionally slightly constricted at septum, moderately thick-walled, externally smooth, internally finely verruculose, ends rounded, often with a truncate base, (16–)20–20.5(–26) × (7–)9–9.5(–12) µm (av. ± S.D. = 20.4 ± 0.1 × 9.3 ± 0.1 µm), L/W ratio = 2.2.

Culture characteristics: *Colonies* on PDA reaching 40 mm in radius after 3 d at 25 °C. Aerial mycelium present, colonies cottony, dark olive to greyish, darkening from the center of the colony after 3 d, colony fully darkened after 6–10 d. Conidiomata produced after 20–30 d in culture at 23 °C under near UV black light (12/12 h photoperiod). Cardinal temperatures for growth: min 5 °C, max < 35 °C, opt 20–25 °C.

Type: **Spain**, Catalonia, Vim-bodí, near the Monastery of Poblet, on pruned canes of *Vitis vinifera* cv. Garnatxa Negra, Aug. 2004, J. Luque & S. Martos, **holotype** LISE 95177.

Cultures: CBS 117009 (ex-type), CBS 302.75.

Hosts: *Citrus* sp. (Adesemoye & Eskalen 2011, Inderbitzin *et al.* 2010), *Populus cathayana* (Zhang *et al.* 2009), *Poniciana gilliesii* (Phillips *et al*. 2008), *Prunus persica* and *P. salicina* (Damm *et al.* 2007), *Vitis vinifera* (de Wet *et al.* 2009, Luque *et al.* 2005, Qiu *et al.* 2011, Úrbez-Torres *et al.* 2007).

Known distribution: Australia (Qiu *et al.* 2011), China (Zhang *et al.* 2009), France (Phillips *et al*. 2008), South Africa (Damm *et al.* 2007, de Wet *et al.* 2009), Spain (Luque *et al.* 2005) and USA (Adesemoye & Eskalen 2011, Inderbitzin *et al.* 2010, Úrbez-Torres *et al.* 2007).

Notes: The sexual morph is extremely rare compared to the abundant asexual morph. The ex-type isolate of *Spencermartinsia viticola* (CBS 117009) clustered with an isolate previously identified as *Diplodia spegazziniana* (CBS 302.75), which is clearly a misidentification.

Sphaeropsis Sacc., Michelia 2: 105. 1880. MycoBank MB9992.

= *Phaeobotryosphaeria* Speg., Ann. Inst. Rech. Agron. 17, 10: 120. 1908.

Type species: *Sphaeropsis visci* (Alb. & Schwein.) Sacc., Michelia 2: 105. 1880.

Ascomata pseudothecial, brown to black, unilocular, thick-walled. *Pseudoparaphyses* hyaline, septate. *Asci* bitunicate, 8-spored, thick-walled with thick endotunica and well-developed apical chamber. *Ascospores* brown, aseptate with small apiculus at either end. *Conidiomata* pycnidial, stromatic, immersed to erumpent, thick-walled, wall composed of several layers of dark-brown *textura angularis*. *Ostiole* single, central, papillate. *Paraphyses* hyaline, aseptate, thin-walled. *Conidiogenous cells* hyaline, discrete, proliferating internally to form periclinal thickenings. *Conidia* oval, oblong or clavate, straight, aseptate, moderately thick-walled.

Notes: *Sphaeropsis* was introduced by Saccardo (1880) for species of *Diplodia* with brown, aseptate conidia with *S. visci* as the type species. Since then more than 600 species have been described (MycoBank accessed 10 Jul. 2013) mostly on the basis of host association. However, few of these names are currently in use and cultures are not available for the species that define them. The well-known pine pathogen that has been known as *Sphaeropsis sapinea* is clearly not a species of *Sphaeropsis* and is retained in *Diplodia*.

Phillips *et al.* (2008) established the connection between the asexual and the sexual morph in *S. visci*. A bitunicate ascomycete, with characters corresponding to *Phaeobotryosphaeria*, occurring on *Viscum album* produced in culture a coelomycete with large, brown, aseptate conidia typical of *Sphaeropsis* and corresponding to the current concept of *S. visci*. Phillips *et al.* (2008) applied the one fungus-one name concept and chose *Phaeobotryosphaeria* in favour of *Sphaeropsis*. However, following the ammendments to the ICBN ratified at the 18th Botanical Congress in Melbourne, it is now clear that priority of names will no longer be based on the life stage of the fungus. Thus, the older name *Sphaeropsis* (1880) takes priority over *Phaeobotryosphaeria* (1908). To correct this, new combinations are introduced here together with the descriptions of the species considered by Phillips *et al.* (2008). Pycnidial paraphyses distinguish *Sphaeropsis* morphologically

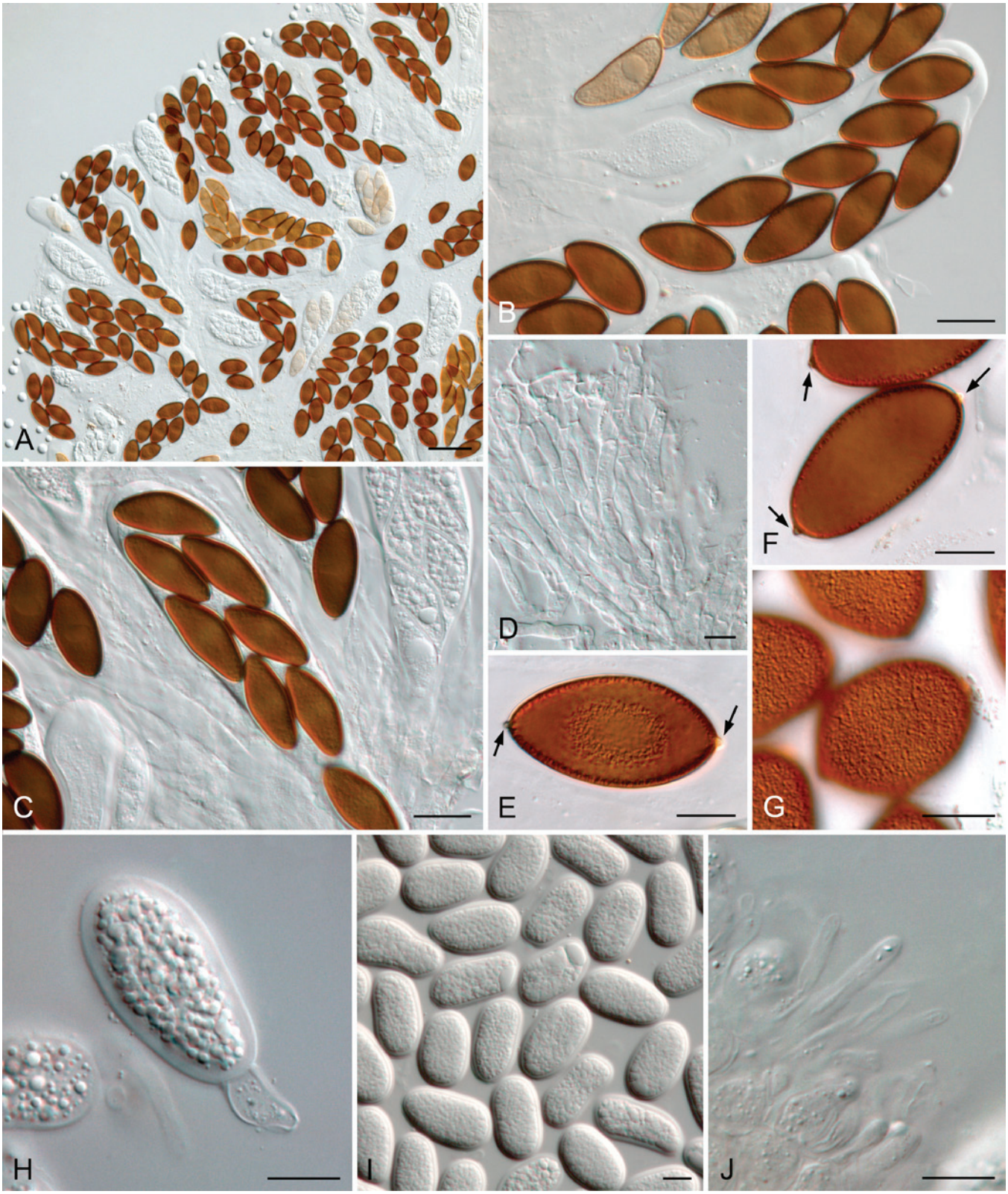

Fig. 64. *Sphaeropsis citrigena*. A–C. Asci with brown ascospores. D. Pseudoparaphyses. E–G. Brown, aseptate ascospores with apiculi (arrows). H. Conidium developing on a conidiogenous cell with periclinal thickenings. I. Hyaline, aseptate conidia. J. Conidiomatal paraphyses. Scale bars A = 50 µm, B–D = 20 µm, E–J = 10 µm.

from *Diplodia* while the striate conidia of *Lasiodiplodia* differentiate it from *Sphaeropsis*, which has smooth-walled conidia. Although more than 600 names exist in *Sphaeropsis,* only four species are currently known in culture. The distinctly pitted conidial walls of *S. porosum* distinguish it from the other two species. The paraphyses with swollen tips and conidia that soon become pigmented distinguish *S. visci* from *S. citrigena* in which conidia remain hyaline for long periods, rarely become pigmented and paraphyses tips are not swollen. The only known cultures of *S. eucalypti* have not sporulated and thus could not be included in the key, which relies on characters of the asexual morph.

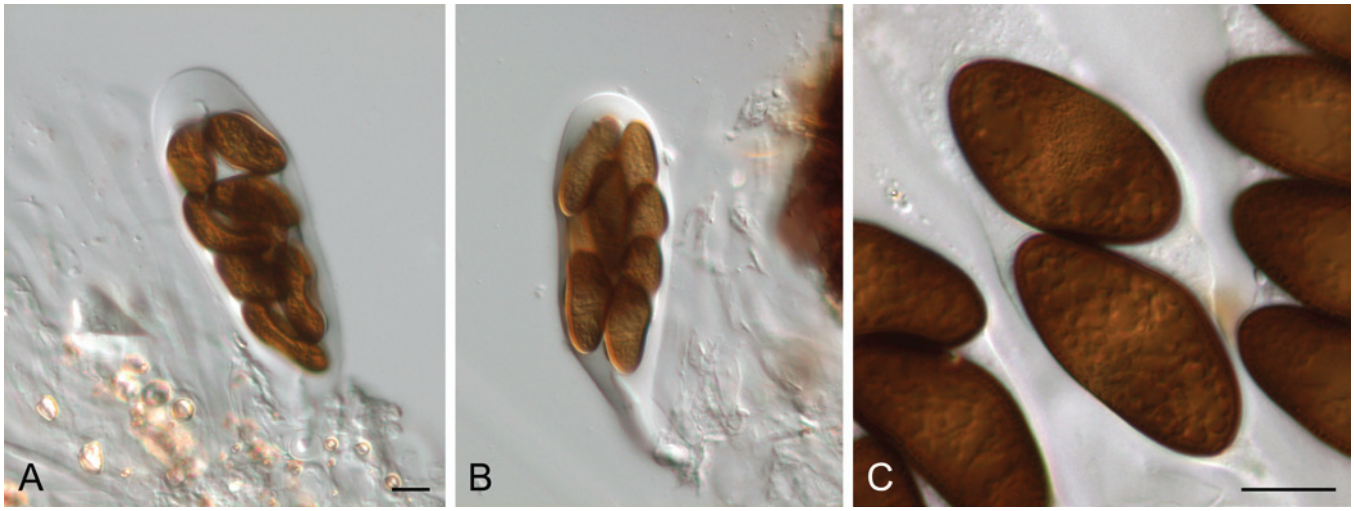

Fig. 65. *Sphaeropsis eucalypti*. A, B. Asci with ascospores. C. Ascospores. Scale bars = 10 µm. Scale bar in A applies to B.

Key to *Sphaeropsis* spp.

1. Conidial wall distinctly pitted ..... *S. porosa*
1. Conidial wall not pitted 2

2. Conidiomatal paraphyses with swollen tips ..... *S. visci*
2. Conidiomatal paraphyses not swollen at tip ..... *S. citrigena*

Sphaeropsis citrigena (A.J.L. Phillips, P.R. Johnst. & Pennycook) A.J.L. Phillips & A. Alves, **comb. nov.** MycoBank MB805463. Fig. 64.
Basionym: *Phaeobotryosphaeria citrigena* A.J.L. Phillips, P.R. Johnst. & Pennycook, *Persoonia* 21: 50. 2008.

Ascomata pseudothecial, brown to black, separate or aggregated, immersed, becoming erumpent, ostiolate, wall composed of several layers of dark brown *textura angularis*. *Pseudoparaphyses* hyaline, smooth, 4–6 µm wide, multiseptate, with septa 11–26 µm apart, constricted at septa. *Asci* bitunicate, 8-spored, stipitate, thick-walled with thick endotunica and well-developed apical chamber, 180–230 × 35–43(–50) µm, with biseriate ascospores. *Ascospores* reddish-brown when mature, ellipsoid to ovoid with both ends rounded, with an apiculus at either end, aseptate, externally smooth, internally finely verruculose, widest in middle to upper third, (27.5–)29–37.5(–38.5) × (14.5–)15.5–18(–19.5) µm. *Conidiomata* immersed to erumpent and superficial, unilocular, up to 500 µm wide, wall composed of several layers of dark brown *textura angularis. Paraphyses* hyaline, aseptate, up to 25 µm long and 3–3.5 µm wide, apex not swollen. *Conidiogenous cells* hyaline, discrete, proliferating internally to form periclinal thickenings, 8–11 × 4–6.5 µm. *Conidia* oval, apex obtuse, base obtuse or truncate, moderately thick-walled, initially hyaline, becoming brown, externally smooth, internally finely verruculose, aseptate, (27–)28–33(–34) × (14.5–)15–18.5(–19) µm.

Type: **New Zealand**, Northland, Kerikeri, Davies Orchard (#2), Inlet Road, on recently dead bark-covered twigs of *Citrus sinensis*, 6 Sep. 2006, S.R. Pennycook, P.R. Johnston & B.C. Paulus, **holotype** PDD 89238.

Culture: ICMP 16812 (ex-type).

Notes: Conidia of *P. citrigena* remain hyaline for long periods and dark conidia are rarely encountered. Conidial dimensions of this species are similar to those of *S. visci*, but its ascospores are reddish-brown in contrast to the pale brown ones of *S. visci*. Furthermore, *S. visci* appears to be specific to *Viscum* species while *S. citrigena* has been found only on *Citrus* species.

Sphaeropsis eucalypticola (Doilom, J.K. Liu, & K.D. Hyde) A.J.L. Phillips, **comb. nov.** MycoBank MB805464. Fig. 65.
Basionym: *Phaeobotryosphaeria eucalypti* Doilom, J.K. Liu & K.D. Hyde, Fungal Divers. 57: 190. 2012.

Ascomata black, dark brown, aggregated, initially immersed in tissue becoming erumpent through cracks in bark, solitary, or gregarious, multiloculate, globose to subglobose, wall composed of several layers of dark brown cells of *textura angularis*. *Pseudoparaphyses* 3–4 µm wide, septate, constricted at septa. *Asci* 8–spored, bitunicate, fissitunicate, cylindro-clavate or clavate, with a short pedicel, apically rounded with an ocular chamber, (90–)97−110(−125) × 28–30 µm (av. = 106 × 29 µm). *Ascospores* overlapping biseriate, hyaline when young, becoming dark brown when mature, aseptate, ellipsoid to ovoid, ends rounded, with a minute apiculus at each end, smooth, widest in the middle, 27–35 × 11–14 µm (av. = 30 × 12 µm). *Asexual* state not seen.

Type: **Thailand**, Chiang Rai Province, Muang District, on dead twig of *Eucalyptus* sp., 8 Aug. 2011, M. Doilom, **holotype** MFLU 12-0753.

Cultures: MFLUCC 11-0579 = CBS 133993.

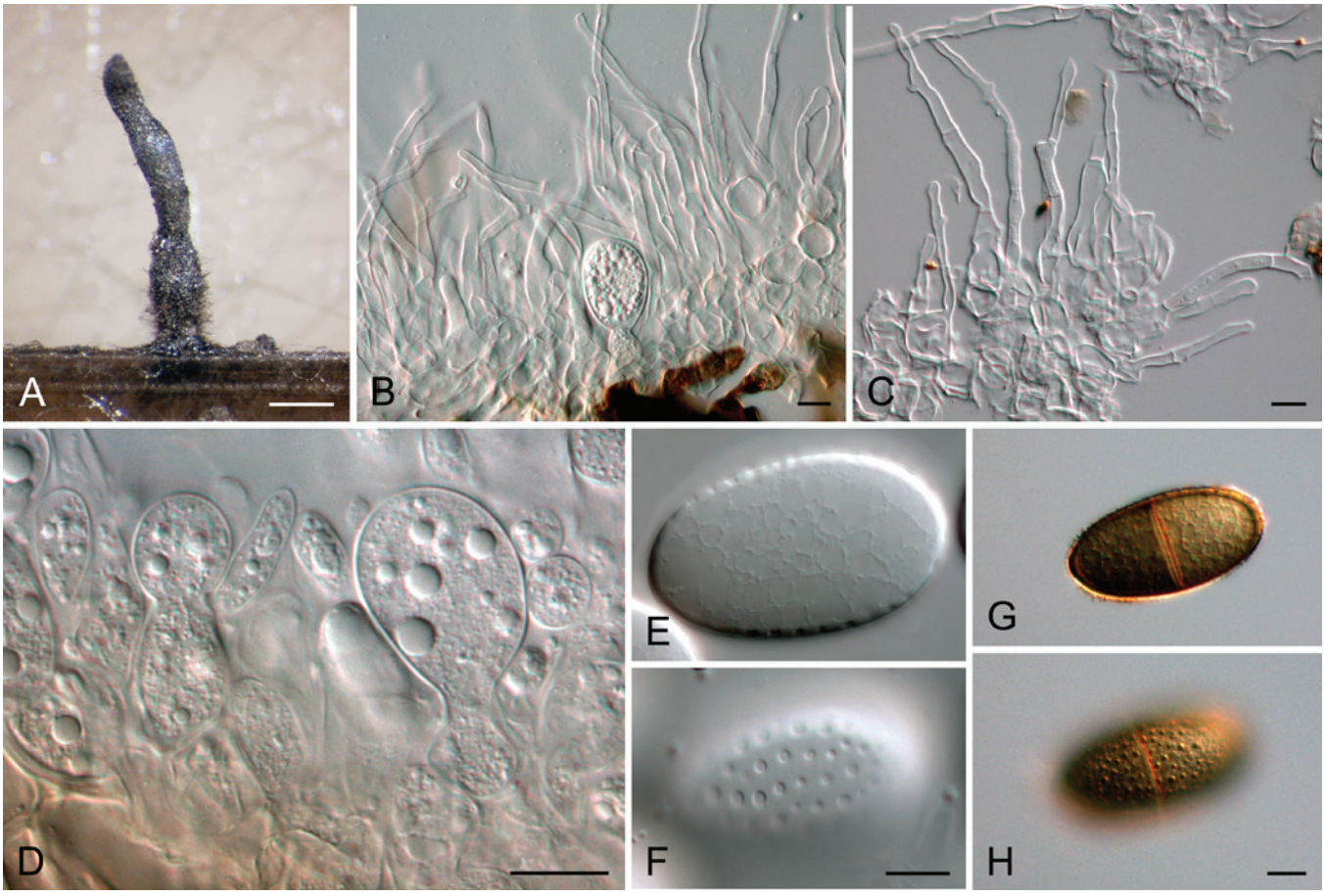

Fig. 66. *Sphaeropsis porosa*. A. Pycnidium with elongated neck. B. Conidium developing between paraphyses. C. Paraphyses. D. Conidia and conidiogenous cells. E, F. Immature conidium at two different levels of focus to show the pores in the conidium wall. G, H. Mature conidium at two different levels of focus to show verruculose inner surface of the wall. Scale bars: A = 500 µm, B–H = 10 µm.

Hosts: *Eucalyptus* sp. (Liu *et al.* 2012).

Known distribution: Thailand (Liu *et al.* 2012).

Notes: Liu *et al.* (2012) could not induce asexual sporulation of *S. eucalypti* in culture and our attempts with the ex-type culture were also unsuccessful.

Sphaeropsis porosa (Van Niekerk & Crous) A.J.L. Phillips & A. Alves, **comb. nov.** MycoBank MB805465. Fig. 66.
Basionym: Diplodia porosum Van Niekerk & Crous, Mycologia 96: 790. 2004.
= *Phaeobotryosphaeria porosa* (Van Niekerk & Crous) Crous & A.J.L. Phillips, *Persoonia* 21: 51. 2008.

Ascomata not reported. *Conidioma* solitary, unilocular, ostiolate, globose to obpyriform, up to 400 µm wide, conidioma wall 4–8 cell layers thick, of dark brown *textura angularis*, becoming hyaline toward inner region. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* lining cavity, holoblastic, hyaline, subcylindrical to ampulliform, 6–10 × 5–7 µm, rarely proliferating percurrently. *Conidia* hyaline, guttulate, ovoid to broadly ellipsoid with a bluntly rounded apex, and flattened base, wall 2 µm thick, with pores 1 µm wide, becoming medium brown with age, (38–)42–45(–47) × (20–)22–25(–30) µm in vitro, L/W = 1.9.

Culture characteristics: *Colonies* flat with undulating margins, dark green on the surface and dull green underneath, reaching a radius of 32 mm after 3 d at 25 °C. Cardinal temperatures for growth: min 10 °C, max 30 °C, opt 25 °C.

Type: **South Africa**, Western Cape Province, Stellenbosch, on *Vitis vinifera,* 2002, J.M. van Niekerk, **holotype** CBS H-12039.

Cultures: STE-U 5132 = CBS 110496 (ex-type).
Host: *Vitis vinifera* (van Niekerk *et al.* 2004).

Known distribution: South Africa (Western Cape Province) (van Niekerk *et al.* 2004).

Notes: Van Niekerk *et al.* (2004) did not mention pycnidial paraphyses in *Diplodia porosum*, but they were clearly seen when their isolates were re-examined (Fig. 3). This species is unique within the *Botryosphaeriaceae* because of its large, thick-walled conidia with large pores (1 µm wide) that are easily seen by light microscopy. However, the pitted walls, although unique and distinctive, should be regarded as informative at the species level in the same way that this character was regarded in the original description.

Sphaeropsis visci (Alb. & Schwein.) Sacc., Michelia 2: 105. 1880. MycoBank MB281898. Fig. 67.
Basionym: *Sphaeria atrovirens* var. *visci* Alb. & Schwein., Consp. fung. (Leipzig): 48. 1805.
≡ *Ceuthospora visci* (Alb. & Schwein.) Sollm., Hedwigia 2: 189. 1863.
≡ *Sphaeropsis visci* (Alb. & Schwein.) Sacc., Michelia 2(no. 6): 105. 1880.
≡ *Sphaeropsis visci* (Alb. & Schwein.) Sacc. f. *visci*, Michelia 2(no. 6):

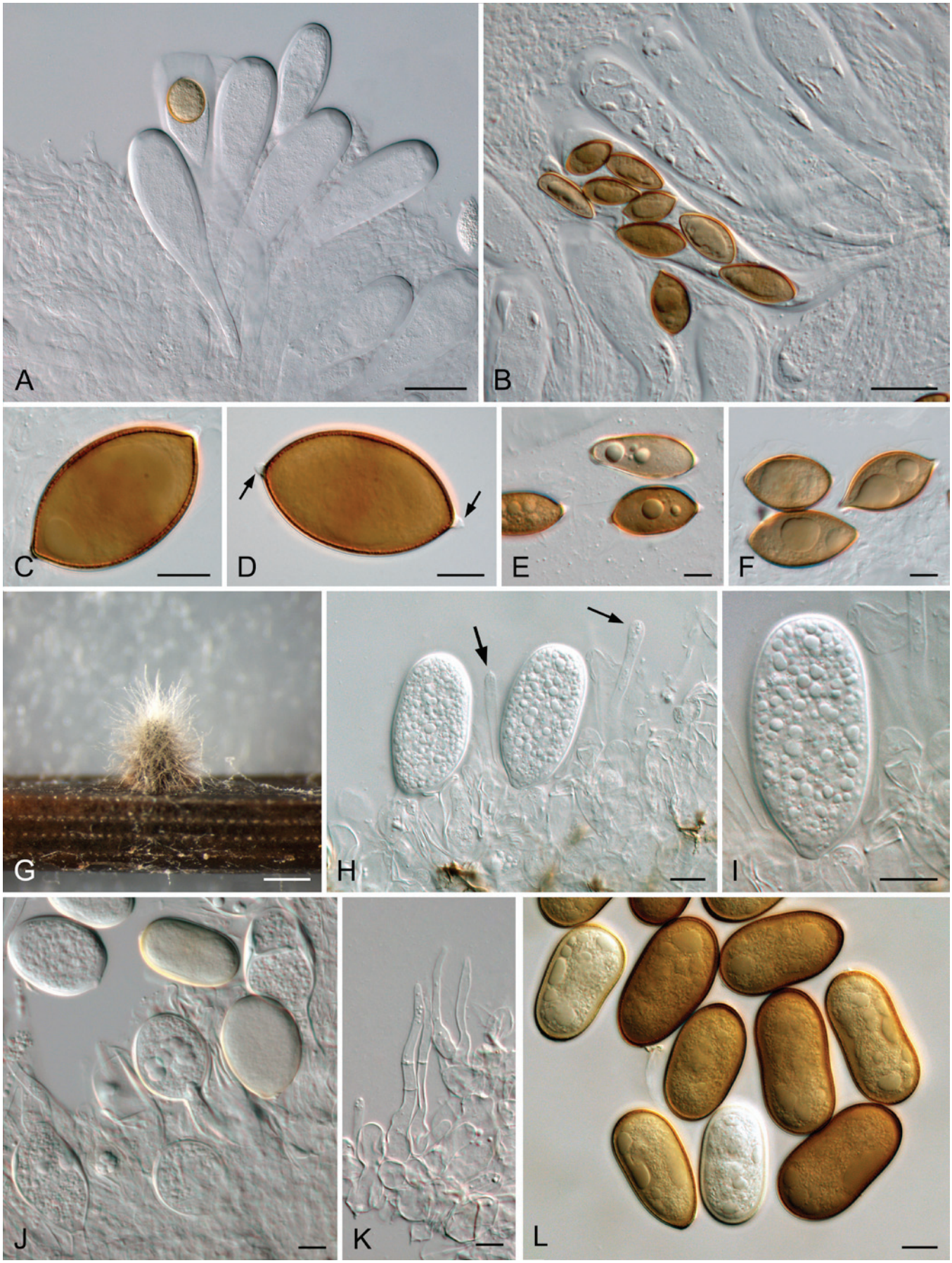

Fig. 67. *Sphaeropsis visci*. A. Immature asci. B. Mature ascus with brown, aseptate ascospores. C–F. Brown, aseptate ascospores with apiculi (arrows). G. Conidioma formed in culture on a pine needle. H, I. Conidia forming on conidiogenous cells between paraphyses (arrows). J. Developing conidia. K. Paraphyses. L. Brown, aseptate mature conidia. Scale bars: A, B = 20 µm, C–F, H–L = 10 µm, G = 50 µm.

105. 1880.
≡ *Botryosphaerostroma visci* (Alb. & Schwein.) Petr., Beih. Rep. spec. nov. regn. veg. 42: 127. 1926.
= *Sphaeria visci* DC., in de Candolle & Lamarck, Fl. franç., Edn 3 (Paris) 6: 146. 1815.
≡ *Diplodia visci* (DC.) Fr., Summa veg. Scand., Section Post. (Stockholm): 417. 1849.
≡ *Microdiplodia visci* (DC.) Potebnia, Ann. Mycol. 8(1): 63. 1910.
≡ *Ascochytella visci* (DC.) Petr., Ann. Mycol. 23(1/2): 111. 1925.
≡ *Botryosphaerostroma visci* (DC.) Petr., Ann. Mycol. 23(1/2): 111. 1925.
≡ *Pseudodiplodia visci* (DC.) Petr., Sydowia 7(5–6): 304. 1953.
≡ *Metadiplodia visci* (DC.) Zambett., Bull. trimest. Soc. mycol. Fr. 70(3): 295. 1955.
= *Dothidea visci* Kalchbr., Hedwigia 8: 117. 1869.
≡ *Anthostomella visci* (Kalchbr.) Sacc., Syll. fung. (Abellini) 1: 293. 1882.
≡ *Anthostoma visci* (Kalchbr.) Sacc., Nuovo G. bot. ital. 23(2): 224. 1916.
≡ *Phaeobotryon visci* (Kalchbr.) Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 128: 591. 1919.
≡ *Botryosphaeria visci* (Kalchbr.) Arx & E. Müll., Beitr. Kryptfl. Schweiz 11(no. 1): 41. 1954.
≡ *Phaeobotryosphaeria visci* (Kalchbr.) A.J.L. Phillips & Crous, Persoonia 21: 47. 2008.
= *Macrophoma visci* Aderh., Arb. biol. Anst. Land-u. Forstw. 4: 462. 1905.

Ascomata pseudothecial, brown to black, uni- or multiloculate, separate, immersed, ostiolate, up to 500 µm diam, wall composed of several layers of dark brown *textura angularis*. *Pseudoparaphyses* hyaline, smooth, 4–6 µm wide, multiseptate, with septa 11–19(–26) µm apart, constricted at septa. *Asci* bitunicate, 8-spored, ascospores biseriate in the ascus, stipitate, thick-walled with thick endotunica and well-developed apical chamber, 180–230 × 35–50 µm. *Ascospores* pale-brown when mature, ovoid, aseptate, externally smooth, internally finely verruculose, widest in middle with an apiculus at either end, (27.5–)31–37.5(–38.5) × (14.5–)15–19(–19.5) µm. *Conidiomata* immersed to erumpent and superficial, unilocular, up to 300 µm wide, wall composed of dark brown *textura angularis*. *Paraphyses* hyaline, aseptate, up to 40 µm long and 4 µm wide with a bulbous tip 5 µm diam. *Conidiogenous cells* hyaline, discrete proliferating internally to form periclinal thickenings, (4–)8.5–11 × 4–6.5 µm. *Conidia* oval, apex obtuse, base obtuse or truncate, moderately thick-walled, initially hyaline, becoming brown, externally smooth, internally finely verruculose, (27–)29–33(–50) × (14.5–)15.5–19(–22) µm.

Holotype: **Germany**, on *Viscum album*, Albertini & Schweinitz, holotype not found and presumably lost. **Ukraine**, National Nature Park 'Svjatie Gory', on branches of *Viscum album*, 10 Mar. 2007, Á. Akulov, **neotype here designated** CWU (MYC) AS 2271 (MBT176099).

Cultures: CBS 122526, CBS 122527 (ex-neotype).

Host: *Viscum album* (Sutton 1980, Phillips *et al*. 2008).

Known distribution: Austria, Czechoslovakia, Egypt, Romania (Sutton 1980), Ukraine (Phillips *et al.* 2008).

Notes: *Sphaeropsis* was introduced by Saccardo (1880) for *Diplodia* species with brown, aseptate conidia. He designated *S. visci*, based on *Sphaeria atrovirens* var. *visci*, as the type species. The connection between the asexual and sexual morphs was established by Phillips *et al.* (2008). Single ascospore isolations from a botryosphaeria-like ascomycete on CWU (MYC) AS 2271 resulted in cultures of a coelomycete indistinguishable from *S. visci*, thus proving the connection between the two states. This specimen is herein designated as neotype. Features that distinguish the sexual morph from others with brown ascospores in the *Botryosphaeriaceae* are the aseptate ascospores with an apiculus at either end.

Tiarosporella Höhn, Ber. Deutsch. Bot. Ges. 37: 159. 1919. MycoBank MB10233.

Type species: *Tiarosporella paludosa* (Sacc. & Fiori ex P. Syd.) Höhn., *In*: Weese, Mitt. bot. Inst. tech. Hochsch. Wien 1(3): 83. 1924.

Characterised by having conidia formed from smooth, hyaline conidiogenous cells that lack periclinal thickenings and percurrent proliferations. The hyaline, subcylindrical to fusiform conidia have irregular mucoid appendages.

Tiarosporella graminis* var. *karoo B. Sutton & Marasas, Trans. Brit. Mycol. Soc. 67 (1): 73. 1976. MycoBank MB353200. For illustrations see Sutton & Marasas (1976).

Aerial mycelium composed of hyaline to light brown, septate, branched, smooth, encrusted, thin-walled hyphae and strands of coarse, thick-walled, dark brown, smooth or verrucose hyphae, 6–12 µm wide and consisting of cylindrical cells, 12–45 µm long which sometimes round off to form chains of globose, 1-celled, thick-walled, dark brown, chlamydospore-like cells. Pycnidia begin to develop after 7 d, embedded in the surface of the agar, single or in small groups, dark brown to black, rostrate and the elongate necks are covered with grey-olivaceous to brown, simple, septate, smooth or verruculose, straight or flexuous pycnidial hairs with obtuse ends. *Conidiogenous cells* formed from the cells lining the inner walls of the pycnidia, holoblastic, determinate, simple, cylindrical and slightly tapered towards the apex, hyaline, 12–18 × 1.5–2.5 µm. *Conidia* acrogenous, solitary, hyaline, smooth, thin-walled, straight, fusiform with truncate base and obtuse apex, 21–28 × 5–8 µm. During development, conidia are enclosed in a gelatinous sheath that may remain as an apical, hyaline, cone-like appendage.

Type: **South Africa**, Cape Province, Colesberg, on *Eriocephali* sp., 16 Feb. 1971, W.F.O. Marasas, **holotype** IMI 186782.

Cultures: IMI 186783 = CBS 118718.

Hosts: *Eriocephalus* sp., *Nestlera* sp., *Tribulus terrestris* (Sutton & Marasas 1976)

Known distribution: South Africa (Sutton & Marasas 1976)

Notes: Conidia of *T. graminis* var. *graminis* resemble those of *T. graminis* var. *karroo* in shape, though they are somewhat larger (20–29.5 × 7–9 µm), than those of *T. graminis* var. *karroo* (21–28 × 5–8 µm) (Sutton & Marasas 1976).

Tiarosporella tritici B. Sutton & Marasas, Trans. Brit. Mycol. Soc., 67 (1): 74. 1976. MycoBank MB324614.

Aerial mycelium composed of hyaline to light brown, septate, branched, smooth or encrusted thin-walled hyphae and strands of

very coarse, thick-walled, dark brown to black, verrucose hyphae 7.5–16.5 µm wide and consisting of cylindrical cells, 12–40 µm long that sometimes round off to form chains of intercalary, globose, thick-walled, smooth or verruculose chlamydospore-like cells, 8–14 µm diam. Immersed mycelium dark brown to black. *Pycnidia* begin to develop after 7 d and numerous mature pycnidia are present throughout the Petri dish after 14 d, particularly on PDA, semi-immersed in the surface of the agar, single or 2–15 aggregated in large, pulvinate, botryose, stromatoid groups up to 3 mm diam, dark brown to black, globose, rostrate, unilocular or multilocular, up to 200 µm diam, walls thick, composed of large, thick-walled, dark brown pseudoparenchymatous cells that become paler and thin-walled towards the inner conidiogenous region, ostiole circular, up to 65 µm diam, formed at the apex of an apical beak that is up to 400 µm long and covered with hyaline to light brown, simple, septate, straight or flexuous, smooth or verruculose pycnidial hairs with obtuse ends. *Conidiogenous cells* formed from the cells lining the inner wall of the pycnidia, holoblastic, determinate, simple, hyaline, cylindrical, 9–14 × 4–5 µm. *Conidia* acrogenous, solitary, hyaline, smooth, thin-walled, eguttulate, straight, oval to fusiform, apex obtuse, base truncate, 29–38 × 12–17 µm. During development some conidia are enclosed in a gelatinous sheath that later becomes everted into an apical, irregularly infundibuliform appendage up to 23 µm long and 29 µm wide.

Type: **South Africa**, Orange Free State, Heilbron, dried culture isolated from dead stems of *Triticum aestivum*, 18 Jan. 1973, W.F.O. Marasas, **holotype** PREM 44966.

Cultures: IMI 186786 = CBS 118719 (ex-type).

Host: *Triticum aestivum* (Sutton & Marasas 1976)

Known distribution: Free State Province, South Africa (Sutton & Marasas 1976).

Notes: Conidia of *T. tritici* are much larger than those of all other known species of *Tiarosporella* and the shape of the appendage is also different. Of the 14 species of *Tiarosporella* that have been named to date, DNA sequence data are only available for *T. graminis, T. madreeya*, *T. tritici* and *T. urbis-rosarum* (Crous *et al*. 2006, Jami *et al*. 2012).

Tiarosporella urbis-rosarum Jami, Gryzenh., Slippers & M.J. Wingf., Cryptogam. Mycol. 33: 256. 2012. For illustrations see Jami *et al*. (2012).

Conidiomata (on sterile twigs of *Acacia karroo*) pycnidial, dark black, up to 200 µm diam, immersed, unilocular, with long necks (4–9 mm); wall of 5–7 layers of dark brown *textura angularis*, becoming thin-walled towards inner region. *Conidiogenous cells* holoblastic, hyaline, cylindrical, (5–)5.5–9.5(–11) × (3–)3.5–4(–5) µm. *Conidia* ovoid, smooth, granular, thin-walled, aseptate, apices rounded, (21–)23.5–29.5(–34) × (8–)9–10(–11) µm (from Jami *et al.* 2012).

Culture characteristics: *Colonies* on MEA appressed, centres dirty white, becoming dark grey at the edges; reverse dark grey to black. Growth at 5–35 °C, with optimal growth rate of 14.4 mm / d at 25 °C.

Type: **South Africa**, Free State Province, Bloemfontein, on healthy wood of *Acacia karroo*, Jun. 2008, M. Gryzenhout, **holotype** PREM 60698.

Cultures: CMW 36477 = CBS 130405 (ex-type).

Host: *Acacia karroo*.

Known distribution: Free State and Gauteng Provinces of South Africa.

Note: *Tiarosporella urbis-rosarum* is morphologically similar to *T. tritici* (conidia 29–38 × 12–17 µm), but has smaller conidia (23.5–29.5 × 9–10 µm).

DISCUSSION

In this paper we considered only those genera and species of the *Botryosphaeriaceae* that are known to exist in culture, and thus accept 17 genera in the family. These genera are characterised based on 17 lineages in a multi-locus phylogeny. In a recent phylogenetic study of the *Botryosphaeriales*, Liu *et al.* (2012) included *Auerswaldia* in the *Botryosphaeriaceae* based on fresh collections of *A. lignicola* and *A. dothiorella*. However, they did not include ITS sequence data in their analyses because they claimed that it was not suitable to segregate taxa at the generic and species level. In our analyses, *A. lignicola* clustered within *Lasiodiplodia* and *A. dothiorella* in *Dothiorella*. For this reason, we argue that there is no evidence to suggest that *Auerswaldia* should be regarded as a distinct genus in the *Botryosphaeriaceae*. Indeed, Liu *et al.* (2012) state that depending on the method used to generate the phylogeny, *A. lignicola* clustered in the *Diplodia* / *Lasiodiplodia* clade in the RAxML analysis, but in *Dothiorella* when Maximum Parsimony was used. Furthermore, in the combined EF1-α and β-tubulin analysis, this species always clustered in *Dothiorella* irrespective of the phylogenetic method used. In the present paper we found that a combination of SSU, ITS, LSU, EF1-α and β-tubulin gave a clear separation of the genera and this was consistent between the different phylogenetic methods (MP, ML). This is also consistent with a previous multi-locus phylogeny (Phillips *et al.* 2008) of a smaller sub-set of the family.

Most of the genera revealed by the multi-locus phylogeny in this study can be distinguished based on their morphology. This is especially true for characteristics of the conidia and to a lesser extent on the presence or absence of paraphyses in the conidiomata. However, some genera cannot be separated using morphological characters. For example, conidia of *Botryosphaeria* are indistinguishable from those of *Neofusicoccum* when the range of variation for each genus is taken into consideration. Although there is some evidence that pycnidial paraphyses are found only in *Botryosphaeria*, this has not been confirmed for all the species. Nevertheless, paraphyses have never been reported in any *Neofusicoccum* species.

Another difficult pair of genera to distinguish is *Spencermartinsia* and *Dothiorella*. The conidial characters of species in both of these genera are identical, being pigmented and 1-septate. In both genera, the conidia become pigmented and septate even while they are attached to conidiogenous cells, and this character distinguishes them from *Diplodia*. Phillips *et al.* (2008) introduced *Spencermartinsia* for species similar to *Dothiorella* but differed in the presence of an apiculus on the ascospores, which is absent

from *Spencermartinsia* species. Although this is a small difference, it is supported by phylogenetic data and is also a useful character to separate *Barriopsis* (no apiculus) from *Phaeobotryosphaeria* (apiculus present). However, the status of these two genera needs to be re-evaluated in the light of the multi-locus analysis presented here and by Slippers *et al.* (2013, this volume), in which the phylogenetic distinction is unclear.

Although ITS alone was usually sufficient to separate species within each genus of the *Botryosphaeriaceae*, inclusion of EF1-α resulted in a more robust separation, and was considered essential in some genera such as *Diplodia*, *Lasiodiplodia* and *Neofusicoccum*. We therefore recommend at least these two loci for species separation within the *Botryosphaeriaceae*. With the increase in the number of species recognised in phylogenetic studies, the use of morphological data for species identification is becoming less useful. Although we have provided keys for species identification in each genus, the resulting identification should be interpreted with caution. For example, in *Neofusicoccum* the range of variation within a species is becoming more apparent as additional isolates are studied and often the variation overlaps considerably with other species. Furthermore, phylogenetic inference is revealing cryptic species complexes that cannot be distinguished based on morphology alone (see for example Pavlic *et al.* 2009a, b, Sakalidis *et al*. 2011). In this regard, in addition to ITS and EF1-α sequence data, data from the β-tubulin, RPB2 and other loci have been useful, and were at times necessary to provide convincing evidence of multigene phylogenetic concordance to separate cryptic species (see also Sakalidis *et al.* 2012).

Recognising the isolate identified by Liu *et al.* (2012) as *A. lignicola* is in fact a species in *Lasiodiplodia* has helped to resolve a long-standing problem regarding the connection between the asexual and the sexual morphs in *Lasiodiplodia*. As explained in the notes for *L. theobromae*, the connection between the asexual and sexual morphs of *L. theobromae* has not been definitively confirmed, and thus the characteristics of the sexual morph are also not clear. Liu *et al.* (2012) clearly demonstrate the asexual / sexual morph connection for *L. lignicola* and confirmed that mature ascospores are dark brown. This has also recently been observed for other species of *Lasiodiplodia* (Crous, unpubl. data). For this reason, we have amended the description of *Lasiodiplodia* to include brown ascospores. In recent studies, several new species have been introduced in *Lasiodiplodia,* and frequently these species are recognised based on minor differences in ITS sequences with great emphasis placed on EF1-α sequence data (Abdollahzadeh *et al.* 2010). It would seem that this genus should be the subject of a more detailed analysis based on additional gene loci to provide a robust phylogenetic basis for species definitions.

In each genus of the *Botryosphaericaceae* the species share a common general morphology, which complies to a great extent with the definition of a genus (Singer 1975, Booth 1978, Crous *et al.* 2009). However, in *Diplodia,* several different morphologies are seen and these lie within separate phylogenetic lineages. The typical morphology, as seen in *D. mutila* and related species, consists of hyaline, aseptate, thick-walled conidia that become dark brown and 1-septate. Another major group, which includes *D. seriata*, *D. pinea* and their relatives, has conidia that turn brown at an early stage of development and remain aseptate. These two morphological groups cluster in two well-supported clades. This would give the impression that *Diplodia* consists of two separate genera. However, *D. corticola* and *D. quercivora* have the characteristics typical of the *D. mutila* group, but form a clade near the root of the *Diplodia* phylogenetic tree. Furthermore, *D. cupressi* and *D. tsugae* with conidia indistinguishable from *D. mutila*, cluster with *D. bulgarica* (pale brown, aseptate conidia) in another clade that lies between the *D. mutila* and *D. seriata* clades. Thus, for the present, we have chosen to consider *Diplodia* as a genus with two morphologies rather than to provide separate genera or sections for them.

Following the recent changes to the nomenclature of pleomorphic fungi, and in particular the abolition of dual nomenclature for a single fungus, we have introduced some new combinations. With regard to *Botryosphaeria* / *Fusicoccum,* the oldest genus is *Fusicoccum* Corda (1829), not *Botryosphaeria* Ces. & De Not. (1863). However, *Botryosphaeria* is the type genus of *Botryosphaericeae* and *Botryosphaeriales*, and is well entrenched in the user community. For these reasons we have retained *Botryosphaeria* and have made several recombinations of *Fusicoccum* species.

Phillips *et al.* (2008) reinstated *Phaeobotryosphaeria* for species with dark brown, aseptate ascospores that have a hyaline apiculus at either end, and asexual morphs in *Sphaeropsis*. In the present paper we decided to revert to using the generic name *Sphaeropsis* for these species. *Sphaeropsis* Sacc. (1880) is an older name than *Phaeobotryosphaeria* Speg. (1908), and is also better established with the plant pathological community. Although *Sphaeropsis* has been applied incorrectly in the past, we believe that the confusion has now been resolved and the genus is clearly circumscribed.

Ever since Crous *et al.* (2006) sub-divided *Botryosphaeria* the position of *B. mamane* has been uncertain, apparently residing outside of *Botryosphaeria*. Furthermore, conidia of *B. mamane* are considerably larger than those of any other species in *Botryosphaeria*. In our ITS phylogenies the ex-type cultures of *B. mamane* formed a clade within *Cophinforma* confirming that this is a suitable genus for it.

The present study provides the first phylogenetic overview and morphological synthesis of the species of *Botryosphaeriaceae* that are presently known from culture. We trust that this will provide a stable platform to accommodate the numerous undescribed species that still await description, or recollection and epitypification to ensure a stable genetic application of names in the family.

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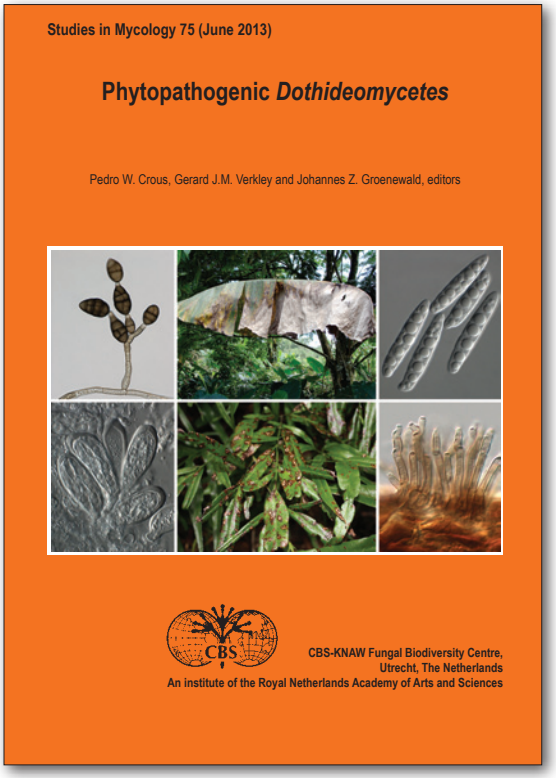


Studies in Mycology 75: Phytopathogenic *Dothideomycetes*

P.W. Crous, G.J.M. Verkley and J.Z. Groenewald (eds)

This volume of Studies in Mycology is dedicated to the plant health officers of the world, who are constantly confronted by a range of plant pathogenic fungi that cause devastating diseases of agricultural and forestry crops. Five main groups of fungi are dealt with, namely *Alternaria*, *Cercospora*, *Phoma*, *Pseudocercospora* and *Septoria*. In the first paper *Phoma* sections *Plenodomus*, *Heterospora* and *Pilosa* were reinvestigated, resulting in the introduction of several novel genera and species. The second paper deals with the paraphyletic genus *Pseudocercospora*; host specificity was considered for 146 species of *Pseudocercospora* occurring on 115 host genera from 33 countries. From these results we concluded that the application of European and American names to Asian taxa, and vice versa, was often not warranted. The third paper deals with the genus *Cercospora*, which contains more than 5 000 different species. Isolates used in the molecular phylogeny were obtained from 161 host species, 49 host families and 39 countries. Although some species were found to host-specific, others were isolated from a wide host range. The fourth paper deals with phylogenetic lineages within the genus *Alternaria*, which was revealed to represent a well-supported node containing 24 internal clades and six monotypic lineages. Several genera were placed in synonymy with *Alternaria*, for which 16 new sections were proposed. Two papers deal with the genus *Septoria*, which was shown to be poly- and paraphyletic, leading to the introduction of 15 new genera, and more than 40 new species. Although some species were shown to be highly specific, other taxa were revealed to occur on hosts in more than six different plant families. For all taxa investigated multi-gene DNA data were deposited in GenBank and other databases to expedite future identification of these plant pathogenic fungi. No single locus was found to be the ideal DNA barcode gene for these taxa, and species identification will have to be based on a combination of gene loci and morphological characters.

406 pp., fully illustrated with colour pictures (A4 format), paperback, 2013. € 70

Studies in Mycology 74: Development of *Aspergillus niger*

J. Dijksterhuis and H.A.B. Wösten (eds)

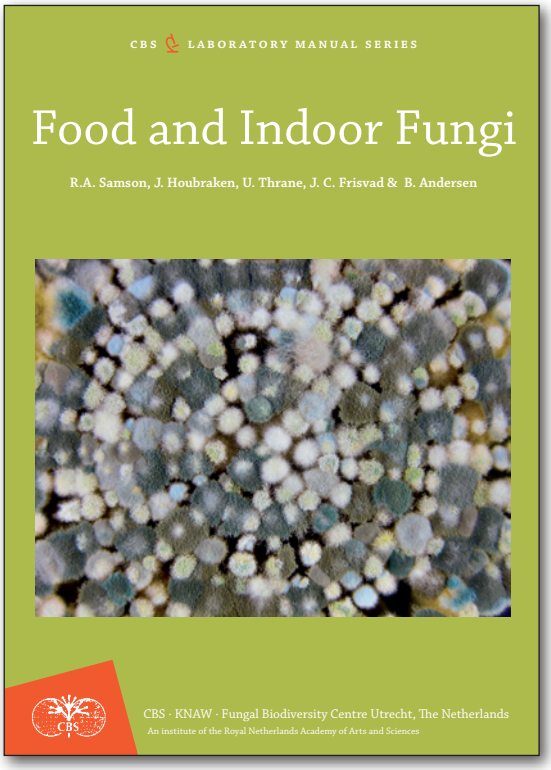
This issue of Studies in Mycology deals with vegetative growth and development of *Aspergillus* in general and *A. niger* in particular. *Aspergillus* niger is a member of the *Aspergillus* section *Nigri*, a group of 26 species that are dubbed “the black Aspergilli”. *Aspergillus niger* is a cosmopolitan fungus. It can be isolated from all continents and is not very selective with respect to environmental conditions. *Aspergillus niger* is used as a cell factory for the production of enzymes and metabolites such as organic acids.

The issue starts with a review on molecular mechanisms underlying differentiation processes in the vegetative mycelium and during asexual and sexual development of aspergilli.

The articles of van Leeuwen *et al*. show that the RNA composition of dormant conidia is highly different from that of germinating conidia (i.e. of conidia during isotropic and polarised growth). The transcriptome of conidia changes most dramatically during the first two hours of germination enabling initiation of protein synthesis and respiration. The antifungal natamycin does neither affect differential expression of genes nor germination of *A. niger* conidia during the first 2 h of the process. Notably, subsequent stages of germination were effectively blocked by the anti-fungal compound, and the transcriptome inside the cells had changed thoroughly. The article of van Veluw *et al*. focusses on stages following germination namely the formation of micro-colonies. It is shown that micro-colonies of a control strain are smaller and more heterogeneous in size when compared to strains in which pigmentation genes are inactivated. These results are of interest from a biotechnological point of view since productivity is related to the morphology of micro-colonies. The results of Van Veluw *et al*. also indicate the existence of transcriptionally and translationally highly active and lowly active hyphae in 1 mm wide micro-colonies of *A. niger* as was previously shown in macro-colonies with a diameter of about 5–7 cm. However, the existence of distinct populations of hyphae with high and low transcriptional and translational activity seems to be less robust when compared to macro-colonies. Why colonies have hyphae with different transcriptional and translational activity is still not clear but it may have a role in survival in an environment where conditions are dynamic. The article of Bleichrodt *et al*. focusses on sporulating colonies. Evidence is presented that GFP but not mRNA streams from the vegetative mycelium to conidiophores. Apparently, flow of molecules to the reproductive structure is selective. Absence of RNA streaming would explain why distinct RNA profiles were found in the aerial mycelium when compared to the vegetative mycelium. Future studies should reveal why GFP flows but mRNA does not.

85 pp., fully illustrated with colour pictures (A4 format), paperback, 2013. € 40

CBS Laboratory Manual Series



CBS Laboratory Manual Series 2: Food and Indoor Fungi

R.A. Samson, J. Houbraken, U. Thrane, J.C. Frisvad and B. Andersen

This book is the second in the new CBS Laboratory Manual Series and is based on the seventh edition of INTRODUCTION TO FOOD AND AIRBORNE FUNGI. This new version, FOOD AND INDOOR FUNGI, has been transformed into a practical user's manual to the most common micro-fungi found in our immediate environment – on our food and in our houses. The layout of the book starts at the beginning with the detection and isolation of food borne fungi and indoor fungi in chapters 1 and 2, describing the different sampling techniques required in the different habitats. Chapter 3 deals with the three different approaches to identification: morphology, genetics and chemistry. It lists cultivation media used for the different genera and describes step by step how to make microscope slides and tape preparations for morphological identification. The chapter also describes how to do molecular and chemical identification from scratch, how to evaluate the results and warns about pitfalls. Chapter 4 gives all the identification keys, first for the major phyla (*Ascomycetes*, *Basidiomycetes* and *Zygomycetes*) common on food and indoors, then to the different genera in the *Zygomycetes* and the *Ascomycetes*, with a large section on the anamorphic fungi and a section for yeasts. The section on anamorphic fungi contains two keys to the different genera: a dichotomous key and a synoptic key. For each genus a key to the species treated is provided, followed by entries on the different species. For each species colour plates are accompanied by macro- and a micro-morphological descriptions, information on molecular and chemical identification markers, production of mycotoxins, habitats and physiological and ecological characteristics. The book is concluded with an extensive reference list and appendices on the associated mycobiota on different food types and indoor environments, mycotoxins and other secondary metabolites, a glossary on the mycological terms used in the book and lastly a detailed appendix on the media used for detection and identification.

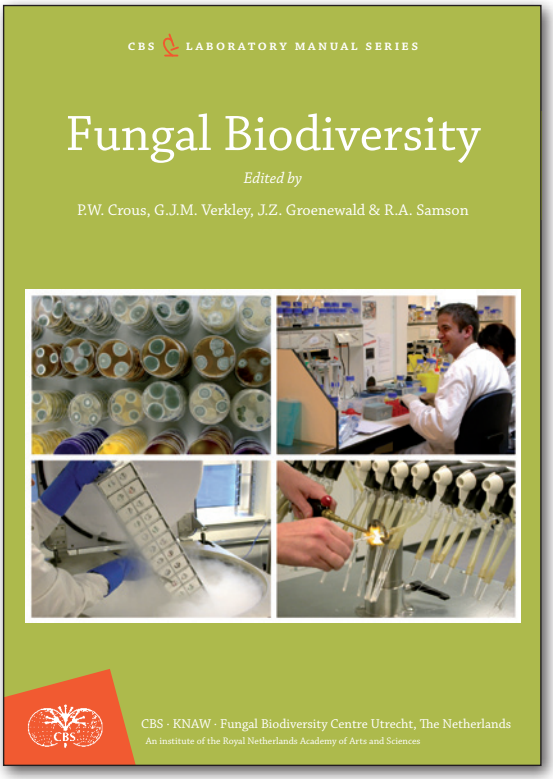
390 pp., fully illustrated with colour pictures (A4 format). Hardbound, 2010. € 70

CBS Laboratory Manual Series 1: Fungal Biodiversity

P.W. Crous, G.J.M. Verkley, J.Z. Groenewald and R.A. Samson (eds)

This book is the first in the new "CBS Laboratory Manual Series", and focuses on techniques for isolation, cultivation, molecular and morphological study of fungi and yeasts. It has been developed as a general text, which is based on the annual mycology course given at the CBS-KNAW Fungal Biodiversity Centre (Centraalbureau voor Schimmelcultures). It provides an introductory text to systematic mycology, starting with a concise treatise of *Hyphochytridiomycota* and *Oomycota*, which have long been subject of study by mycologists, but are now classified in the Kingdom *Chromista*. These are followed by sections on the groups of "true fungi": *Chytridiomycota*, *Zygomycota*, *Ascomycota* and *Basidiomycota*. This descriptive part is illustrated by figures of life-cycles and schematic line-drawings as well as photoplates depicting most of the structures essential for the study and identification of these fungi. Special attention is given to basic principles of working with axenic cultures, good morphological analysis, and complicated issues for beginners such as conidiogenesis and the understanding of life-cycles. Exemplar taxa for each of these fungal groups, in total 37 mostly common species in various economically important genera, are described and illustrated in detail. In a chapter on general methods a number of basic techniques such as the preparation and choice of media, microscopic examination, the use of stains and preparation of permanent slides, and herbarium techniques are explained. Further chapters deal with commonly used molecular and phylogenetic methods and related identification tools such as BLAST and DNA Barcoding, fungal nomenclature, ecological groups of fungi such as soil-borne and root-inhabiting fungi, water moulds, and fungi on plants and of quarantine importance. Some topics of applied mycology are also treated, including fungi in the air- and indoor environment and fungi of medical importance. Common mycological terminology is explained in a glossary, with reference to illustrations in the book. A chapter providing more than 60 mycological media for fungal cultivation, and a comprehensive list of cited references are also provided. The book is concluded with an index, and dendrograms reflecting our current understanding of the evolutionary relationships within the *Fungi*.

270 pp., fully illustrated with colour pictures (A4 format). Hardbound, 2009. € 50



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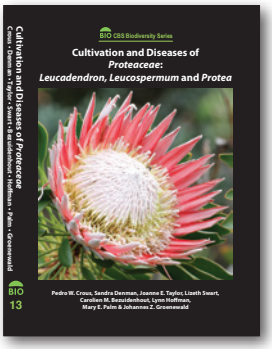


No. 13: Cultivation and Diseases of *Proteaceae*: *Leucadendron*, *Leucospermum* and *Protea*

Pedro W. Crous, Sandra Denman, Joanne E. Taylor, Lizeth Swart, Carolien M. Bezuidenhout, Lynn Hoffman, Mary E. Palm and Johannes Z. Groenewald

Proteaceae represent a prominent family of flowering plants in the Southern Hemisphere. Because of their beauty, unique appearance, and relatively long shelf life, *Proteaceae* cut-flowers have become a highly desirable crop for the export market. The cultivation of *Proteaceae* is a thriving industry that provides employment in countries where these flowers are grown, often in areas that are otherwise unproductive agriculturally. Diseases cause a loss in yield, and also limit the export of these flowers due to strict phytosanitary regulations. In this publication the fungi that cause leaf, stem and root diseases on *Leucadendron*, *Leucospermum* and *Protea* are treated. Data are provided pertaining to the taxonomy, identification, host range, distribution, pathogenicity, molecular characteristics and control of these pathogens. Taxonomic descriptions and illustrations are provided and keys are included to distinguish species in genera where a number of species affect *Proteaceae*. Disease symptoms are described and colour photographs are included. Where known, factors that affect disease epidemiology are discussed. Disease management strategies are also presented that will assist growers and advisors in making appropriate choices for reducing disease in specific areas. Information is also provided relating to crop improvement, cultivation techniques, harvesting and export considerations. Further development and expansion of this industry depends on producing and obtaining disease-free germplasm from countries where these plants are indigenous. For that reason it is important to document the fungi that occur on *Proteaceae*, and to establish the distribution of these fungi. These data are essential for plant quarantine services for use in risk assessments.

360 pp., fully illustrated (A4 format). Hardbound, 2013. € 75

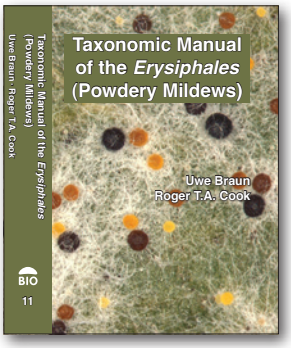
No. 12: Ophiostomatoid Fungi: Expanding Frontiers

Keith A. Seifert, Z. Wilhelm de Beer and Michael J. Wingfield (eds)

The 1992 Convention on Biological Diversity created a new awareness of the economic impact of living organisms. Regulators and quarantine specialists in governments all over the world now scrutinise dots on maps, as real-time online disease mapping and prediction models allow us to track (and try to prevent) the spread of diseases across borders. Woodlands are more managed, include less genetic diversity, and seem to be more susceptible to rapidly spreading disease. Different jurisdictions use different terminology, Biosecurity, Alien Invasive Species, Quarantine, but it is now commonplace to see large signs in airports, along highways, and on public hiking trails, warning citizens not to accidentally or deliberately facilitate the spread of unwanted pests or microbes. With the ophiostomatoid fungi, scientists have to cope with the overlapping behaviour of a triumvirate of kingdoms, the fungi, the animals (bark beetles, mites or nematodes), and how all of these impact trees in our forests and cities.

This book includes 21 papers divided among five themes, plus an appendix. It is a sequel to *Ceratocystis* and *Ophiostoma*: Taxonomy, Ecology, and Pathogenicity, published by the APS Press in 1993, and like that book is derived from an international symposium, this one held on North Stradbroke Island, Australia prior to the 9th International Mycological Congress. A year before this volume was completed, mycological taxonomy formally abandoned the historical two name system, known as dual nomenclature, and we are now adopting a single name binomial system. The appendix to this book provides a preliminary view of the nomenclature of the ophiostomatoid fungi using the new single name system. In an attempt at consistency, this naming system is used in all chapters.

337 pp., fully illustrated (A4 format). Hardbound, 2013. € 75

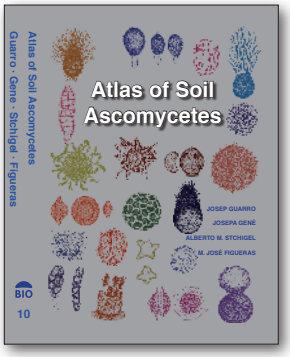


No. 11: Taxonomic Manual of the *Erysiphales* (Powdery Mildews)

Uwe Braun and Roger T.A. Cook

The "Taxonomic Manual of the *Erysiphales* (Powdery Mildews)" is a fully revised, expanded new version of U. Braun's former monograph from 1987, which is out of print. The present book covers the taxonomy of all powdery mildew fungi. New chapters have been prepared for phylogenetic relationships, conidial germination, conidia as viewed by Scanning Electron Microscopy, fossil powdery mildews, and holomorph classification. The treatment of the *Erysiphales*, its tribes and genera are based on recent molecular phylogenetic classifications. A key to the genera (and sections), based on teleomorph and anamorph characters is provided, supplemented by a key solely using anamorph features. Keys to the species are to be found under the particular genera. A special tabular key to species based on host families and genera completes the tools for identification of powdery mildew taxa. In total, 873 powdery mildew species are described and illustrated in 853 figures (plates). The following data are given for the particular species and subspecific taxa: bibliographic data, synonyms, references to descriptions and illustrations in literature, full descriptions, type details, host range, distribution and notes. A further 236 taxonomic novelties are introduced, comprising the new genus *Takamatsuella*, 55 new species, four new varieties, six new names and 170 new combinations. A list of excluded and doubtful taxa with notes and their current status is attached, followed by a list of references and a glossary. This manual deals with the taxonomy of the *Erysiphales* worldwide, and provides an up-todate basis for the identification of taxa, as well as comprehensive supplementary information on their biology, morphology, distribution and host range. This monograph is aimed at biologists, mycologists and phytopathologists that encounter or study powdery mildew diseases.

707 pp., fully illustrated with 853 pictures and line drawings (A4 format). Hardbound, 2012. € 80



No. 10: Atlas of Soil Ascomycetes

Josep Guarro, Josepa Gené, Alberto M. Stchigel and M. José Figueras

This compendium includes almost all presently known species of ascomycetes that have been reported in soil and which sporulate in culture. They constitute a very broad spectrum of genera belonging to very diverse orders, but mainly to the *Onygenales*, *Sordariales*, *Eurotiales*, *Thelebolales*, *Pezizales*, *Melanosporales*, *Pleosporales*, *Xylariales*, *Coniochaetales* and *Microascales*. The goal of this book is to provide sufficient data for users to recognise and identify these species. It includes the description of 146 genera and 698 species. For each genus a dichotomous key to facilitate species identification is provided and for each genus and species the salient morphological features are described. These descriptions are accompanied by line drawings illustrating the most representative structures. Light micrographs, supplemented by scanning electron micrographs and Nomarski interference contrast micrographs of most of the species treated in the book are also included. In addition, numerous species not found in soil but related to those included in this book are referenced or described. This book will be of value not only to soil microbiologists and plant pathologists concerned with the soilborne fungi and diseases, but also to anyone interested in identifying fungi in general, because many of the genera included here are not confined to soil. Since most of the fungi of biotechnological or clinical interest (dermatophytes, dimorphic fungi and opportunists) are soil-borne ascomycetes, the content of this book is of interest for a wide range of scientists.

486 pp., fully illustrated with 322 pictures and line drawings (A4 format). Hardbound, 2012. € 70

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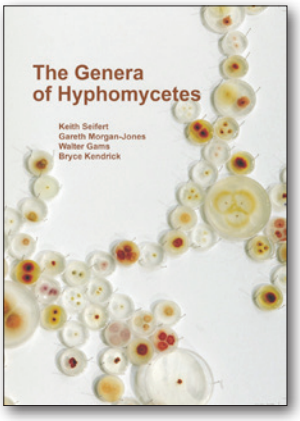
CBS Biodiversity Series

No. 9: The Genera of Hyphomycetes

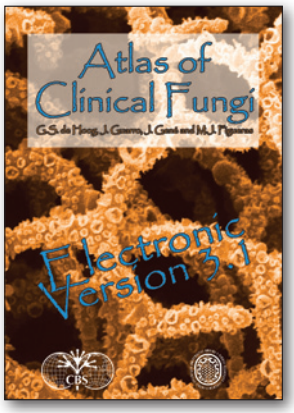


Keith Seifert, Gareth Morgan-Jones, Walter Gams and Bryce Kendrick

The Genera of Hyphomycetes is the essential reference for the identification of moulds to all those who work with these fungi, including plant pathologists, industrial microbiologists, mycologists and indoor environment specialists, whether they be professionals or students. The book compiles information on about 1480 accepted genera of hyphomycetes, and about 1420 genera that are synonyms or names of uncertain identity. Each accepted genus is described using a standardized set of key words, connections with sexual stages (teleomorphs) and synanamorphs are listed, along with known substrates or hosts, and continental distribution. When available, accession numbers for representative DNA barcodes are listed for each genus. A complete bibliography is provided for each genus, giving the reader access to the literature necessary to identify species. Most accepted genera are illustrated by newly prepared line drawings, including many genera that have never been comprehensively illustrated before, arranged as a visual synoptic key. More than 200 colour photographs supplement the line drawings. Diagnostic keys are provided for some taxonomic and ecological groups. Appendices include an integrated classification of hyphomycete genera in the phylogenetic fungal system, a list of teleomorph-anamorph connections, and a glossary of technical terms. With its combination of information on classical morphological taxonomy, molecular phylogeny and DNA diagnostics, this book is an effective modern resource for researchers working on microfungi.

997 pp., fully illustrated with colour pictures and line drawings (A4 format). Hardbound, 2011. € 80

Other CBS publications



Atlas of Clinical Fungi CD-ROM version 3.1

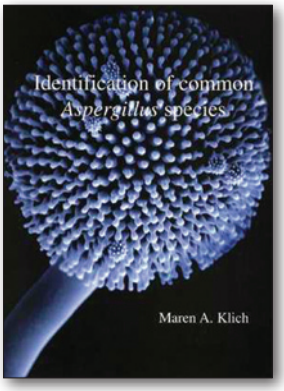
G.S. de Hoog, J. Guarro, J. Gené and M.J. Figueras (eds)

A new electronic version of the 3rd edition is available since November 2011. It will allows fast and very comfortable search through the entire Atlas text the engine is fully equiped for simple as well as advanced search. Items are strongly linked enabling direct use of the electronic version as a benchtool for identification and comparison. Text boxes with concise definitions appear explaining all terminology while reading. Illustrations are of highest quality and viewers are provided for detailed observation. The Atlas is interactive in allowing personal annotation which will be maintained when later versions will be downloaded.

The electronic version has been developed by T. Weniger. The third edition will contain about 530 clinically relevant species, following all major developments in fungal diagnostics. Regular electronic updates of the Atlas are planned, which should include numerous references to case reports, as well as full data on antifungals. Future features will include links to extended databases with verified molecular information. Note: The Atlas runs on Windows only! Not compatible with Mac

Atlas of Clinical Fungi version 3.1, interactive CD-ROM, 2011. € 105

Identification of Common *Aspergillus* Species



Maren A. Klich

Descriptions and identification keys to 45 common *Aspergillus* species with their teleomorphs (*Emericella*, *Eurotium*, *Neosartorya* and *Sclerocleista*). Each species is illustrated with a one page plate and three plates showing the most common colony colours.

116 pp., 45 black & white and 3 colour plates (Letter format), paperback, 2002. € 45

A revision of the species described in *Phyllosticta*

Huub A. van der Aa and Simon Vanev

2936 taxa are enumerated, based on the original literature and on examination of numerous herbarium (mostly type) specimens and isolates. 203 names belong to the genus *Phyllosticta s.str.*, and are classified in 143 accepted species. For seven of them new combinations are made and for six new names are proposed. The great majority, 2733 taxa, were redisposed to a number of other genera. A complete list of these novelties, as included in the book's abstract, can also be consulted on the web-site of CBS.

510 pp. (17 x 25 cm), paperback, 2002. € 55

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Studies in Mycology (ISSN 0166-0616)

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