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Towards a natural classification of *Botryosphaeriales*

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Abstract The type specimens of *Auerswaldia*, *Auerswaldiella*, *Barriopsis*, *Botryosphaeria*, *Leptoguignardia*, *Melanops*, *Neodeightonia*, *Phaeobotryon, Phaeobotryosphaeria, Phyllachorella, Pyrenostigme, Saccharata*, *Sivanesania*, *Spencermartinsia* and *Vestergrenia* were examined and fresh specimens of *Botryosphaeriales* were collected from Thailand. This material is used to provide a systematic treatment of *Botryosphaeriales* based on morphology and phylogeny. Two new genera, *Botryobambusa* and *Cophinforma* are introduced and compared with existing genera. Four species new to science, *Auerswaldia dothiorella*, *A. lignicola*, *Botryosphaeria fusispora* and *Phaeobotryosphaeria eucalypti,* are also described and justified. We accept 29 genera in *Botryosphaeriales*, with *Macrovalsaria* being newly placed. In the phylogenetic tree, the 114 strains of *Botyrosphaeriales* included in the analysis cluster into two major clades with 80 %, 96 % and 1.00 (MP, ML and BY) support, with Clade A containing the family type of *Botryosphaeriaceae*, and Clade B containing *Phyllosticta*, *Saccharata* and *Melanops* species. This group may represent *Phyllostictaceae*. In Clade A the taxa analyzed cluster in eight sub-clades (Clades A1–8). Clade A1 comprises three distinct subclusters corresponding to the genera *Diplodia* (*Diplodia* Clade), *Neodeightonia* (*Neodeightonia* Clade) and *Lasiodiplodia* (*Lasiodiplodia* Clade). Clade A2 clusters into

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three groups representing *Phaeobotryosphaeria* (100 %), *Phaeobotryon* (100 %) and *Barriopsis* (94 %). Clade A3 incorporates 17 strains that cluster into three well-supported genera (*Dothiorella* (86 %), *Spencermartinsia* (100 %) and *Auerswaldia* (63 %); the position of *Macrophomina* is not stable. Clade A4 is a single lineage (100 %) representing the new genus *Botryobambusa*. Clade A5 is a well-supported subclade incorporating *Neofussicoccum*. Clade A6 represents the type species of *Botryosphaeria*, three other *Botryosphaeria* species and two other genera, *Neoscytalidium* and *Cophinforma* gen. nov. Clade A7 comprises two *Pseudofusicoccum* species and Clade A8 has two *Aplosporella* species. These sub-clades may eventually require separate families but this requires analysis of a much larger dataset. Our data advances the understanding of *Botryosphaeriales,* there is, however, still much research to be carried out with resolution of families and genera, linkage of sexual and asexual morphs and differentiation of cryptic species.

Keywords Asexual morphs · *Botryosphaeria* · *Botryosphaeriaceae* · Classification · *Diplodia* · Generic types · *Lasiodiplodia* · Multi-gene phylogeny · New genera · *Phyllosticta*

Introduction

Botryosphaeria was introduced by Cesati and De Notaris (1863). Saccardo (1877) emended the initial generic description and transferred the hypocreaceous species amongst them to *Gibberella* and *Lisea*. Because Cesati and De Notaris (1863) did not designate a type species, von Höhnel (1909) suggested *Botryosphaeria berengeriana* De Not., while Theissen and Sydow (1915) suggested *B. quercuum* (Schwein.) Sacc., which could be regarded as generic lectotypes. Neither proposal was accepted because these species were not included in the original description of the genus (Cesati and De Notaris 1863). Therefore, Barr (1972) proposed *B. dothidea* (Moug. : Fr.) Ces. & De Not, one of the species originally included by Cesati and De Notaris (1863), as the lectotype of this genus. This proposal has generally been accepted and Slippers et al. (2004b) proposed a neotype and epitype to stabilize the type species *B. dothidea* and provided a modern description of this genus based on these new types.

Species of *Botryosphaeria* are cosmopolitan in distribution and occur on a wide range of monocotyledonous, dicotyledonous and gymnosperm hosts; on woody branches, herbaceous leaves, stems and culms of grasses; and on twigs and in the thalli of lichens (Barr 1987; Denman et al. 2000; Mohali et al. 2007; Lazzizera et al. 2008a; Marincowitz et al. 2008. Taxa range in habit from saprobic to parasitic or endophytic (Smith et al. 1996; Denman et al. 2000; Phillips et al. 2006; Slippers and Wingfield 2007; Huang et al. 2008; Pérez et al. 2010; Ghimire et al. 2011; González and Tello 2011), and cause die-back and canker diseases of numerous woody hosts (von Arx 1987; Damm et al. 2007a; Phillips et al. 2007; Slippers et al. 2007; Alves et al. 2008; Lazzizera et al. 2008b; Marincowitz et al. 2008; Zhou et al. 2008; Pérez et al. 2010; Adesemoye and Eskalen 2011; Urbez-Torres et al. 2012). Species of *Botryosphaeria* have also been isolated from marine environments in sea grasses (Sakayaroj et al. 2010).

The *Botryosphaeriales* was introduced by Schoch et al. (2006), following molecular analysis, and comprises a single family *Botryosphaeriaceae*. This family however, has a rather varied past as can be seen from inclusion of genera by various authors (Table 2). Von Arx and Müller (1954) included 15 genera, but later reduced it to 14 genera by von Arx and Müller (1975). Barr (1987) was much more conservative and included only nine genera, mostly different from those of von Arx and Müller (1954), while Hawksworth et al. (1995) listed five genera and numerous synonyms of *Botryosphaeria*. With the use of molecular data it has been possible to add more new genera to the family *sensu* Hawksworth et al. (1995). Lumbsch and Huhndorf (2010) included 11 genera, while Hyde et al. (2011) and Wijayawardene et al. (2012) listed 20 asexual genera. Phillips and Alves (2009) restudied the botryosphaeriaceous *Melanops*, epitypifying the generic type. In the present study, we accept 29 genera based on molecular data and examination of generic types.

Botryosphaeriaceae has been well circumscribed, and can be defined as forming uni- to multilocular ascostromata with multi-layered walls, occurring singly or sometimes in botryose clusters or pulvinate stromata (e.g. *Auerswaldiella*), often united with conidiomata on a common basal stroma and embedded in the host and becoming partially erumpent at maturity (von Arx and Müller 1954; Eriksson 1981; Sivanesan 1984) We follow the concept for “*Ascostromata*” given by Ulloa and Hanlin (2000) as follows: “ascostromata: A stromatic ascocarp resulting from ascolocular ontogeny, with the asci produced in locules or cavities, the walls of which consist only of stromal tissue. No separable wall is formed around them. If a single cavity is present it is a unilocular (uniloculate) ascostroma, and if several locules are formed it is a multilocular (multiloculate) ascostroma”. This is not always clear, but we have tried to be consistent in using ascostromata even when only single locules are present and ascomata might therefore be more appropriate. Asci are bitunicate, fissitunicate, with a thick endotunica, and clavate, with a short or long pedicel and with a well-developed ocular chamber. The asci form in a basal hymenial layer, intermixed among hyaline, septate, pseudoparaphyses, that are often constricted at the septum. Pseudoparaphyses are frequently present in the centrum of immature ascostromata, but they gradually disappear as the asci develop and mature. Ascospores are hyaline, thin-walled, aseptate and vary from fusoid to ellipsoid or ovoid, bi- to triseriate and are irregularly biseriate in the ascus, mostly without a mucilaginous sheath or appendages, some with apiculus at each end. Ascospores may turn brown and become septate and even slightly verruculose upon germination or with age and have projection or appendage at the ends (von Arx and Müller 1954; Shoemaker 1964; Eriksson 1981; Sivanesan 1984; Hsieh and Chen 1994; Denman et al. 2000; Alves et al. 2004; Slippers et al. 2004b; Phillips et al. 2005, 2008; Crous et al. 2006; Schoch et al. 2006; Phillips and Alves 2009).

The asexual morphs of *Botryosphaeriaceae* have been assigned to several coelomycete genera, including *Aplosporella*, *Diplodia*, *Dothiorella*, *Fusicoccum, Lasiodiplodia, Macrophomina, Microdiplodia, Neofusicoccum, Neoscytalidium, Pseudofusicoccum* and *Sphaeropsis* (Crous and Palm 1999; Denman et al. 2000; Crous et al. 2004, 2006; Pavlic et al. 2004, 2008, 2009a, b; Phillips and Pennycook 2004; Slippers et al. 2004a; Phillips et al. 2005; Alves et al. 2006, 2008; Damm et al. 2007b; Lazzizera et al. 2008b) Denman et al. (2000) recognized only two of these, namely *Diplodia* and *Fusicoccum*. Recent studies on the taxonomy of *Botryosphaeria* have employed molecular methods to reveal phylogenetic relationships among species (Jacobs and Rehner 1998) and to resolve species complexes (Smith et al. 2001; Phillips et al. 2002; Denman et al. 2003; Alves et al. 2004; Slippers et al. 2004c; Phillips et al. 2005). Two major clades corresponding to species with *Diplodia* and *Fusicoccum* asexual morphs were revealed based on the phylogenies resulting from ITS sequence analyses (Jacobs and Rehner 1998; Denman et al. 2000). Later studies including additional species and a larger suite of DNA-based markers supported this grouping (Zhou and Stanosz 2001; Alves et al. 2004; Slippers et al. 2004d). When Crous et al. (2004) described the species *Saccharata proteae* Denman & Crous (as *Botryosphaeria proteae* (Wakef.) Denman & Crous with *Fusicoccum* and *Diplodia* synanamorphs), this well supported grouping was questioned, as it is morphologically and phylogenetically distinct from representatives of the *Diplodia*-like and *Fusicoccum*-like groups. *Lasiodiplodia* Ellis & Everh. has been treated as a distinct genus from *Diplodia* Fr. by many authors due to its distinct phylogeny (usually ITS or EF-1α) and morphology (striated or smooth conidia and presence or absence of pseudoparaphyses). Pavlic et al. (2004) employed morphological and phylogenetic data to separate *Lasiodiplodia* from *Diplodia*. Later, Phillips et al. (2005) broadened the concept by including *Dothiorella* within *Botryosphaeria*. *Dichomera* Cooke has been linked to *Botryosphaeria* species with *Fusicoccum* anamorphs by Barber et al. (2005). In a phylogenetic study based on 28S rDNA sequence data, Crous et al. (2006) recognised ten lineages within *Botryosphaeriaceae* corresponding to different genera. Subsequently, Damm et al. (2007b) added a further genus, *Aplosporella*, while Phillips et al. (2008) recognised five additional genera. Asexual genera for *Botryosphaeriaceae* were listed in Hyde et al. (2011) as *Aplosporella, Bahusutrabeeja, Barriopsis, Dichomera, Diplodia, Dothichiza, Dothiorella, Fusicoccum, Lasiodiplodia, Macrophomina, Microdiplodia, Neofusicoccum, Neoscytalidium, Phaeobotryon, Phyllosticta, Pseudofusicoccum* and *Sphaeropsis*, with *Camarosporium, Dichomera* and *Hendersonula* listed under *Botryosphaeriales incertae sedis.*

In this paper, we present a systematic treatment of *Botryosphaeriaceae* and its related asexual morph genera based on type specimens sourced from various herbaria and a morphological study of 17 fresh specimens of botryosphaeriaceous taxa from northern Thailand as well as a molecular phylogenetic analysis of sequence data from four genes. Two monotypic genera and four new species are introduced, one in *Botryosphaeria*, one in *Phaeobotryosphaeria* and two in *Aeurswaldia*. These taxa are fully described and their taxonomy is discussed.

Materials and methods

Examination of herbarium material and fresh specimens

The type specimens of *Auerswaldia*, *Auerswaldiella*, *Barriopsis*, *Botryosphaeria*, *Leptoguignardia*, *Melanops*, *Neodeightonia*, *Phaeobotryon, Phaeobotryosphaeria, Phyllachorella, Pyrenostigme, Saccharata, Sivanesania*, *Spencermartinsia* and *Vestergrenia* were obtained from BPI, K, IMI, LISE, LPS, PREM and S. Fresh material was collected from Chiang Mai, Chiang Rai, Lampang and Phayao provinces in Thailand. Seventeen freshly collected samples were grown on malt extract agar (MEA) and/or potato dextrose agar (PDA). Methods for examining the type material and isolation from fresh material were as in Boonmee et al. (2011), Chomnunti et al. (2011) and Liu et al. (2011). To increase the chances of sporulation 3–5 single ascospore cultures were placed around the Petri-dish so that mixing of mycelia occurred. Observations and photomicrographs were made from material mounted in water using a Nikon ECLIPSE 80i microscope. India ink was added to water mounts to detect the presence of gelatinous sheaths or ascospore appendages. Measurements were made with Tarosoft (R) Image Frame Work (Liu et al. 2010).

DNA extraction, PCR amplification and sequencing

Fungal isolates were grown on PDA for 1 week at 28 °C in the dark. Genomic DNA was extracted from the fresh mycelium using the Biospin Fungus Genomic DNA Extraction Kit (BioFlux®) following the manufacturer's protocol (Hangzhou, P.R. China).

DNA amplification was performed by polymerase chain reaction (PCR). Primer pairs NS1 and NS4 (White et al. 1990) were used to amplify a region spanning of the nuclear ribosomal SSU gene. LROR and LR5 primer pairs (Vilgalys and Hester 1990) were used to amplify a segment of the large subunit rRNA gene. Primer pairs ITS4 and ITS5 (White et al. 1990) were used to amplify the internal transcribed spacers. Primers EF1–728 F and EF1–986R (Carbone and Kohn 1999) and Bt2a and Bt2b (Glass and 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 by Alves et al. (2006, 2008). The PCR products were checked on 1 % agarose electrophoresis gels stained with ethidium bromide.

PCR products were purified using minicolumns, purification resin and buffer according to the manufacturer's protocols (Amersham product code: 27–9602–01). The sequences were carried out by Shanghai Sangon Biological Engineering Technology & Services (Shanghai, P.R. China). For each fungal strain, sequences obtained for the respective primers (ITS5 and ITS4, LROR and LR5, NS1 and NS4, EF1-728 F and EF1-986R, Bt2a and Bt2b) were manually aligned to obtain an assembled sequence using Bioedit (Hall 1999). The reference nucleotide sequences of ITS, LSU, SSU, EF1-α, β-tubulin regions of various taxa were obtained from GenBank (Table 1)

Phylogenetic analysis

Sequences generated from different primers were analyzed with other sequences obtained from GenBank. A Blast search was performed to reveal the closest matches with taxa in *Botryosphaeriales*. In addition, fungal members from different genera of the *Botryosphaeriales* and close orders were also included in the analyses. Sequences were aligned using Bioedit (Hall 1999) and ClustalX v. 1.83 (Thompson et al. 1997). The alignments were checked visually and improved manually where necessary. Phylogenetic analyses were performed by using PAUP v. 4.0b10 (Swofford 2002) for Maximum-parsimony (MP) and MrBayes v. 3.0b4 (Ronquist and Huelsenbeck 2003) for Bayesian analyses.

Maximum-parsimony analyses were performed using the heuristic search option with 1000 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 unlimited, branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Clade stability was assessed using a bootstrap (BT) analysis with 1000 replicates, each with 10 replicates of random stepwise addition of taxa (Hillis and Bull 1993). The phylogram with bootstrap values above the branches is presented in Fig. 1 by using graphical options available in TreeDyn v. 198.3 (Chevenet et al. 2006).

A maximum likelihood analysis was performed at the CIPRES webportal (Miller et al. 2010) using RAxML v. 7.2.8 as part of the "RAxML-HPC2 on TG" tool (Stamatakis 2006; Stamatakis et al. 2008). A general time reversible model (GTR) was applied with a discrete gamma distribution and four rate classes. Fifty thorough maximum likelihood (ML) tree searches were done in RAxML v. 7.2.7 under the same model, with each one starting from a separate randomised tree and the best scoring tree selected with a final ln value of −13974.356237. One thousand non parametric bootstrap iterations were run with the GTR model and a discrete gamma distribution. The resulting replicates were plotted on to the best scoring tree obtained previously.

The model of evolution was estimated by using MrModeltest 2.2 (Nylander 2004). Posterior probabilities (PP) (Rannala and Yang 1996; Zhaxybayeva and Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (BMCMC) in MrBayes v. 3.0b4 (Huelsenbeck and Ronquist 2001). Six simultaneous Markov chains were run for 1000000 generations and trees were sampled every 100th generation (resulting in 10000 total trees). The first 2000 trees, representing the burn-in phase of the analyses, were discarded and the remaining 8000 trees used for calculating posterior probabilities (PP) in the majority rule consensus tree (Cai et al. 2006).

Phylogenetic trees were drawn using Treeview (Page 1996). Sequences derived in this study are deposited in GenBank.

Results

DNA sequencing—combined LSU, SSU, EF1-α and β-tubulin gene phylogenies

The combined 28S (LSU), 18S (SSU), elongation factor 1-α (EF1-α) and β-tubulin gene data set consists of 126 taxa, with *Dothidea insculpta* and *D. sambuci* as the outgroup taxa. The dataset consists of 2582 characters after alignment, of which 1861 sites are included in the ML and MP analysis. Of the included bases, 946 sites (36.64 %) are parsimony-informative. A heuristic search with random addition of taxa (1000 replicates) and treating gaps as missing characters generated six equally parsimonious trees. All trees were similar in topology and not significantly different (data not shown). The first of 1 000 equally most parsimonious trees is shown in Fig. 1. Bootstrap support (BS) values of MP and ML (equal to or above 50 % based on 1,000 replicates) are shown on the upper branches. Values of the Bayesian posterior probabilities

Table 1 Isolates used in this study. Newly deposited sequences are shown in bold

Taxon	Culture Accession No.[1]	GenBank Accession No.[2]				
		ITS	SSU	LSU	EF1-α	β-tubulin
Amniculicola lignicola	CBS 123094 ^{T}	–	EF493863	EF493861	–	–
Aplosporella prunicola	STE-U 6327	–	–	EF564378	–	–
Aplosporella prunicola	STE-U 6326 ^{T}	EF564376	–	EF564377	–	–
Aplosporella yalgorensis	MUCC 512	EF591927	–	EF591944	EF591978	EF591961
Aplosporella yalgorensis	MUCC 511 ^{T}	EF591926	–	EF591943	EF591977	EF591960
Auerswaldia dothiorella	MFLUCC 11–0438 ^{T}	**JX646796**	**JX646829**	**JX646813**	**JX646861**	**JX646844**
Auerswaldia lignicola	MFLUCC 11–0435 ^{T}	**JX646797**	**JX646830**	**JX646814**	**JX646862**	**JX646845**
Auerswaldia lignicola	MFLUCC 11-0656	**JX646798**	**JX646831**	**JX646815**	**JX646863**	**JX646846**
Barriopsis fusca	CBS 174.26 ^{T}	EU673330	EU673182	DQ377857	EU673296	EU673109
Botryobambusa fusicoccum	MFLUCC 11–0143 ^{T}	**JX646792**	**JX646826**	**JX646809**	**JX646857**	–
Botryobambusa fusicoccum	MFLUCC 11-0657	**JX646793**	**JX646827**	**JX646810**	**JX646858**	–
Botryosohaeria melanops	CBS 118.39	FJ824771	FJ824763	DQ377856	FJ824776	FJ824782
Botryosphaeria agaves	MFLUCC 10-0051	**JX646790**	**JX646824**	**JX646807**	**JX646855**	**JX646840**
Botryosphaeria agaves	MFLUCC 11–0125 ^{T}	**JX646791**	**JX646825**	**JX646808**	**JX646856**	**JX646841**
Botryosphaeria corticis	CBS 119047 ^{T}	DQ299245	EU673175	EU673244	EU017539	EU673107
Botryosphaeria corticis	ATCC 22927	DQ299247	EU673176	EU673245	EU673291	EU673108
Botryosphaeria dothidea	CMW 8000 ^{T}	AY236949	EU673173	AY928047	AY236898	AY236927
Botryosphaeria dothidea	CBS 110302	AY259092	EU673174	EU673243	AY573218	EU673106
Botryosphaeria fusispora	MFLUCC 10–0098 ^{T}	**JX646789**	**JX646823**	**JX646806**	**JX646854**	**JX646839**
Botryosphaeria fusispora	MFLUCC 11-0507	**JX646788**	**JX646822**	**JX646805**	**JX646853**	**JX646838**
Capnodium coffeae	CBS 147.52	–	–	DQ247800	–	–
Cochliobolus heterostrophus	CBS 134.39	–	AY544727	AY544645	–	–
Cophinforma eucalypti	MFLUCC 11–0425 ^{T}	**JX646800**	**JX646833**	**JX646817**	**JX646865**	**JX646848**
Cophinforma eucalypti	MFLUCC 11-0655	**JX646801**	**JX646834**	**JX646818**	**JX646866**	**JX646849**
Dichomera eucalypti	MUCC 498	EF591913	–	EF591932	EF591966	EF591949
Didymella exigua	CBS 183.55 ^{T}	–	EU754056	EU754155	–	–
Diplodia corticola	CBS 112549 ^{T}	AY259100	EU673206	AY928051	AY573227	DQ458853
Diplodia corticola	CBS 112546	AY259090	EU673207	EU673262	EU673310	EU673117
Diplodia cupressi	CBS 168.87 ^{T}	DQ458893	EU673209	EU673263	DQ458878	DQ458861
Diplodia cupressi	CBS 261.85	DQ458894	EU673210	EU673264	DQ458879	DQ458862
Diplodia mutila	CBS 112553 ^{T}	AY259093	EU673213	AY928049	AY573219	DQ458850
Diplodia mutila	CBS 230.30	DQ458886	EU673214	EU673265	DQ458869	DQ458849
Diplodia scrobiculata	CBS 113423	DQ458900	EU673217	EU673267	DQ458885	DQ458868
Diplodia scrobiculata	CBS 109944	DQ458899	EU673218	EU673268	DQ458884	DQ458867
Dothidea insculpta	CBS 189.58	AF027764	DQ247810	DQ247802	–	–
Dothidea sambuci	DAOM 231303	DQ491505	AY544722	AY544681	–	–
Dothidotthia symphoricarpi	CPC 12929 ^{T}	–	EU673224	EU673273	–	–
Dothiorella iberica	CBS 115041 ^{T}	AY573202	EU673155	AY928053	AY573222	EU673096
Dothiorella iberica	CBS 113188	AY573198	EU673156	EU673230	EU673278	EU673097
Dothiorella sarmentorum	IMI 63581b ^{T}	AY573212	EU673158	AY928052	AY573235	EU673102
Dothiorella sarmentorum	CBS 115038	AY573206	EU673159	DQ377860	AY573223	EU673101
Falciformispora lignatilis	BCC 21117	NG_016526	GU371834	GU371826	–	–
Falciformispora lignatilis	BCC 21118	–	GU371835	GU371827	–	–
Gloniopsis subrugosa	CBS 123346	–	FJ161170	FJ161210	–	–
Guignardia bidwellii	CBS 111645	FJ824766	EU673223	DQ377876	FJ824772	FJ824777
Guignardia citricarpa	CBS 102374	FJ824767	FJ824759	DQ377877	FJ538371	FJ824778
Guignardia philoprina	CBS 447.68	FJ824768	FJ824760	DQ377878	FJ824773	FJ824779

Table 1 (continued)

Taxon	Culture Accession No.[1]	GenBank Accession No.[2]				
		ITS	SSU	LSU	EF1-α	β-tubulin
Herpotrichia juniperi	AFTOL-ID 1608	–	DQ678029	DQ678080	–	–
Hysterium angustatum	CBS 123334	–	FJ161167	FJ161207	–	–
Lasiodiplodia crassispora	CBS 110492	EF622086	EU673189	EU673251	EF622066	EU673134
Lasiodiplodia crassispora	CBS 118741 T	DQ103550	EU673190	DQ377901	EU673303	EU673133
Lasiodiplodia gonubiensis	CBS 115812 T	DQ458892	EU673193	DQ377902	DQ458877	DQ458860
Lasiodiplodia gonubiensis	CBS 116355	AY639594	EU673194	EU673252	DQ103567	EU673126
Lasiodiplodia parva	CBS 356.59	EF622082	EU673200	EU673257	EF622062	EU673113
Lasiodiplodia parva	CBS 494.78 T	EF622084	EU673201	EU673258	EF622064	EU673114
Lasiodiplodia pseudotheobromae	CBS 447.62	EF622081	EU673198	EU673255	EF622060	EU673112
Lasiodiplodia pseudotheobromae	CBS 116459 T	EF622077	EU673199	EU673256	EF622057	EU673111
Lasiodiplodia theobromae	CBS 124.13	DQ458890	EU673195	AY928054	DQ458875	DQ458858
Lasiodiplodia theobromae	CBS 164.96 T	AY640255	EU673196	EU673253	AY640258	EU673110
Lasiodiplodia theobromae	CAA 006	DQ458891	EU673197	EU673254	DQ458876	DQ458859
Lasiodiplodia theobromae	MFLUCC 11-0508	**JX646799**	**JX646832**	**JX646816**	**JX646864**	**JX646847**
Leptosphaerulina australis	CBS 939.69	–	EU754068	EU754167	–	–
Macrophomina phaseolina	CBS 227.33	–	–	DQ377906	–	–
Macrophomina phaseolina	CBS 162.25	–	–	DQ377905	–	–
Macrophomina phaseolina	CPC 11108	–	–	DQ377912	–	–
Macrophomina phaseolina	CPC 11085	–	–	DQ377910	–	–
Macrophomina phaseolina	CPC 11106	–	–	DQ377911	–	–
Macrovalsaria megalospora	178150	–	FJ215707	FJ215701	–	–
Macrovalsaria megalospora	178149	–	FJ215706	FJ215700	–	–
Melanomma pulvis-pyrius	IFRD 002 T	–	FJ201985	FJ201984	–	–
Melanops tulasnei	CBS 116805 T	FJ824769	FJ824761	FJ824764	FJ824774	FJ824780
Melanops tulasnei	CBS 116806	FJ824770	FJ824762	FJ824765	FJ824775	FJ824781
Murispora rubicunda	IFRD 2017	–	GU456308	FJ795507	–	–
Mycosphaerella punctiformis	AFTOL-942	–	–	DQ470968	–	–
Neodeightonia palmicola	MFLUCC 10–0822 T	HQ199221	HQ199223	HQ199222	–	–
Neodeightonia palmicola	MFLUCC 10-0823	HQ199224	HQ199226	HQ199225	–	–
Neodeightonia phoenicum	CBS 169.34	EU673338	EU673203	EU673259	EU673307	EU673138
Neodeightonia phoenicum	CBS 122528 T	EU673340	EU673205	EU673261	EU673309	EU673116
Neodeightonia phoenicum	CBS 123168	EU673339	EU673204	EU673260	EU673308	EU673115
Neodeightonia sp	MFLUCC 11-0026	**JX646804**	**JX646837**	**JX646821**	**JX646869**	**JX646852**
Neodeightonia subglobosa	MFLUCC 11-0163	**JX646794**	–	**JX646811**	**JX646859**	**JX646842**
Neodeightonia subglobosa	CBS 448.91	EU673337	EU673202	DQ377866	EU673306	EU673137
Neofusicoccum luteum	CBS 110299 T	AY259091	EU673148	AY928043	AY573217	DQ458848
Neofusicoccum luteum	CBS 110497	EU673311	EU673149	EU673229	EU673277	EU673092
Neofusicoccum mangiferum	CBS 118531	AY615185	EU673153	DQ377920	–	AY615172
Neofusicoccum mangiferum	CBS 118532 T	AY615186	EU673154	DQ377921	DQ093220	AY615173
Neofusicoccum parvum	MFLUCC 11-0184	**JX646795**	**JX646828**	**JX646812**	**JX646860**	**JX646843**
Neofusicoccum parvum	CMW 9081 T	AY236943	EU673151	AY928045	AY236888	AY236917
Neofusicoccum parvum	CBS 110301	AY259098	EU673150	AY928046	AY573221	EU673095
Neoscytalidium dimidiatum	CBS 251.49	FM211430	–	DQ377923	–	FM211166
Neoscytalidium dimidiatum	CBS 499.66	FM211432	–	DQ377925	EU144063	FM211167
Neoscytalidium novaehollandiae	WAC 12691 T	EF585543	–	EF585548	EF585574	–
Neoscytalidium novaehollandiae	WAC 12688	EF585542	–	EF585549	EF585575	–
Otthia spiraeae 1	CBS 114124	–	EF204515	EF204498	–	–

Table 1 (continued)

Taxon	Culture Accession No.[1]	GenBank Accession No.[2]				
		ITS	SSU	LSU	EF1-α	β-tubulin
Otthia spiraeae 2	CBS 113091	–	EF204516	EF204499	–	–
Phaeobotryon mamane	CPC 12440 ^{T}	EU673332	EU673184	EU673248	EU673298	EU673121
Phaeobotryon mamane	CPC 12442	EU673333	EU673185	DQ377899	EU673299	EU673124
Phaeobotryon mamane	CPC 12443	EU673334	EU673186	EU673249	EU673300	EU673120
Phaeobotryon mamane	CPC 12444	EU673335	EU673187	DQ377900	EU673301	EU673123
Phaeobotryon mamane	CPC 12445	EU673336	EU673188	EU673250	EU673302	EU673122
Phaeobotryosphaeria citrigena	ICMP 16812 ^{T}	EU673328	EU673180	EU673246	EU673294	EU673140
Phaeobotryosphaeria citrigena	ICMP 16818	EU673329	EU673181	EU673247	EU673295	EU673141
Phaeobotryosphaeria eucalypti	MFLUCC 11–0579 ^{T}	**JX646802**	**JX646835**	**JX646819**	**JX646867**	**JX646850**
Phaeobotryosphaeria eucalypti	MFLUCC 11-0654	**JX646803**	**JX646836**	**JX646820**	**JX646868**	**JX646851**
Phaeobotryosphaeria porosa	CBS 110496 ^{T}	AY343379	EU673179	DQ377894	AY343340	EU673130
Phaeobotryosphaeria porosa	CBS 110574	AY343378	–	DQ377895	AY343339	–
Phaeobotryosphaeria visci	CBS 186.97	EU673325	EU673178	DQ377868	EU673293	EU673128
Phaeobotryosphaeria visci	CBS 100163	EU673324	EU673177	DQ377870	EU673292	EU673127
Phyllosticta brazilianiae	LGMF 330 ^{T}	JF343572	–	–	JF343593	–
Phyllosticta brazilianiae	LGMF 333	JF343574	–	–	JF343595	–
Phyllosticta brazilianiae	LGMF 334	JF343566	–	–	JF343587	–
Pleomassaria siparia	AFTOL-ID 1600	–	DQ678027	DQ678078	–	–
Pleospora herbarum	CBS 191.86 ^{T}	GU238232	DQ247812	DQ247804	–	–
Pseudofusicoccum adansoniae	WAC 12689 ^{T}	EF585534	–	EF585554	EF585567	–
Pseudofusicoccum adansoniae	WAC 12718	EF585533	–	EF585555	EF585568	–
Pseudofusicoccum stromaticum	CBS 117448	AY693974	EU673146	DQ377931	AY693975	EU673094
Pseudofusicoccum stromaticum	CBS 117449	DQ436935	EU673147	DQ377932	DQ436936	EU673093
Psiloglonium simulans	CBS 206.34	–	FJ161139	FJ161178	–	–
Pyrenophora phaeocomes	DAOM 222769	–	DQ499595	DQ499596	–	–
Saccharata capensis	CBS 122693 ^{T}	EU552130	–	EU552130	EU552095	–
Saccharata proteae	CBS 115206	AF452560	GU296194	DQ377882	GU349030	–
Spencermartinsia viticola	CBS 117006	AY905555	EU673166	EU673236	AY905562	EU673103
Spencermartinsia viticola	CBS 112870	AY343376	–	DQ377872	AY343337	–
Spencermartinsia viticola	CBS 117009 ^{T}	AY905554	EU673165	DQ377873	AY905559	EU673104
Trematosphaeria pertusa	CBS 122368 ^{T}	FJ201991	FJ201991	FJ201990	–	–
Trematosphaeria pertusa	CBS 122371	FJ201993	GU348999	FJ201992	–	–

AFTOL Assembling the Fungal Tree of Life; *ATCC* American Type Culture Collection, Virginia, USA; *BCC* BIOTEC Culture Collection, Bangkok, Thailand; *CAA* A. Alves, Universidade de Aveiro, Portugal; *CBS* Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; *CMW* Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; *CPC* Collection of Pedro Crous housed at CBS; *DAOM* Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada; *ICMP* International Collection of Micro-organisms from Plants, Landcare Research, New Zealand; *IFRDCC* Culture Collection, International Fungal Research & Development Centre, Chinese Academy of Forestry, Kunming, China; *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; *MFLUCC* Mae Fah Luang University Culture Collection, ChiangRai, Thailand; *MUCC* Murdoch University Algal Culture Collection, Murdoch, Western Australia; *STE-U* Culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa; *WAC* Department of Agriculture Western Australia Plant Pathogen Collection, South Perth, Western Australia. *T* ex-type/ex-epitype isolates

(PP) (equal to or above 90 % based on 1,000 replicates) from MCMC analyses are shown under the branches.

An effort was made to use ITS gene sequences, but it was found not suitable to segregate the taxa at generic/species level. Therefore, ITS gene data are not included in the multi-genes analyses of this study, but deposited in GenBank as it is preferred loci for use in fungal phylogenetics.

In the phylogenetic tree (Fig. 1), the 114 strains of *Botyrosphaeriales* included in the analysis cluster into two major clades with 80 %, 96 % and 1.00 (MP, ML and BY)

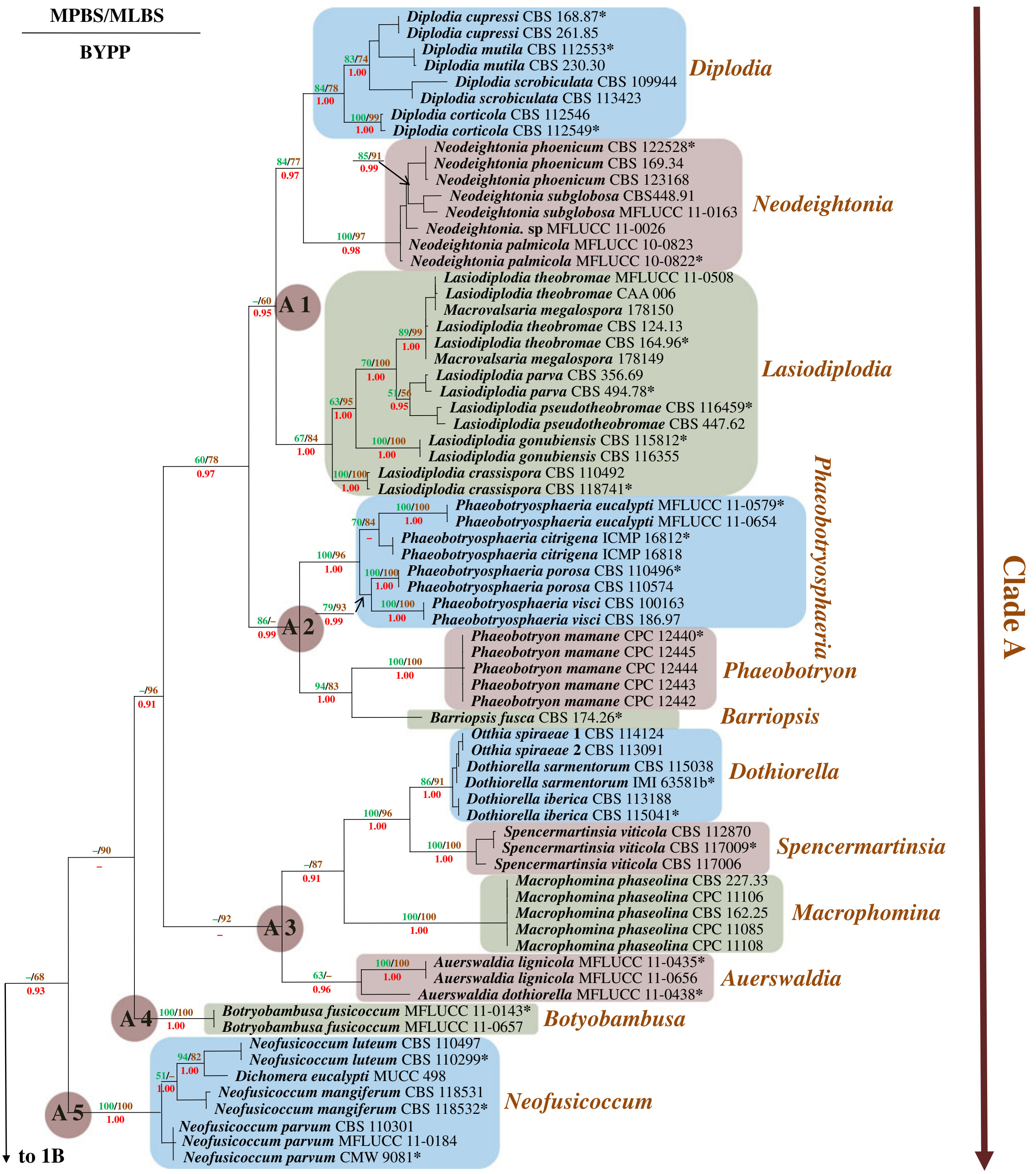

Fig. 1 **A–B** The first of 1 000 equally most parsimonious trees obtained from a heuristic search with 1000 random taxon additions of the combined dataset of SSU, LSU EF1-α and β-tubulin sequences alignment using PAUP v. 4.0b10. The scale bar shows 10 changes. Bootstrap support values for maximum parsimony (MP) and maximum likelihood (ML) greater than 50 % above the nodes. The values below the nodes are Bayesian posterior probabilities above 0.95. Hyphen ("–") indicates a value lower than 50 % (BS) or 0.90 (PP). The original isolate numbers are noted after the species names, ex-type/ex-epitype isolates are marked by an asterisk "*". The tree is rooted to *Dothidea insculpta* and *Dothidea sambuci*

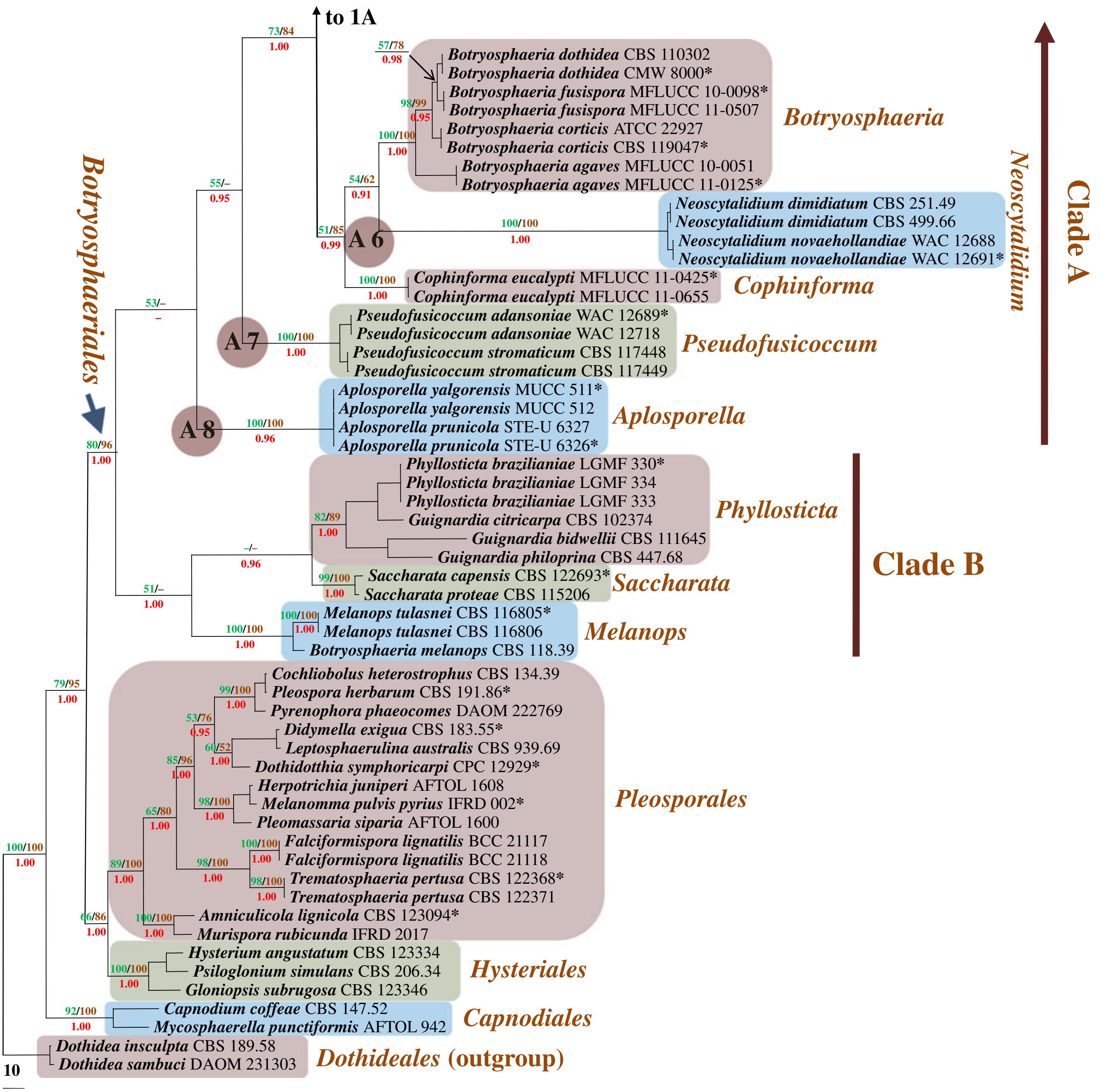

Fig. 1 (continued)

support, with Clade A containing the family type of *Botryosphaeriaceae*, and Clade B containing *Phyllosticta*, *Saccharata* and *Melanops* species. Clade B may represent one family and *Phyllostictaceae* Fr. (1849) could be used. In Clade A the taxa analyzed cluster in eight sub-clades named Clades A1–8. Clade A1 comprises three distinct subclusters corresponding to the genera *Diplodia* (*Diplodia* Clade), *Neodeightonia* (*Neodeightonia* Clade) and *Lasiodiplodia* (*Lasiodiplodia* Clade). All genera have asexual morphs with hyaline spores which become brown at maturity. The sexual morph is only known for *Neodeightonia.* Clade A2 clusters into three groups representing *Phaeobotryosphaeria* (100 %), *Phaeobotryon* (100 %) and *Barriopsis* (94 %). Clade A3 incorporates 17 strains that cluster into three well-supported genera *Dothiorella* (86 %), *Spencermartinsia* (100 %) and *Auerswaldia* (63 %), while the position of the fourth genus *Macrophomina* is not stable. Clade A4 is a single lineage (100 %) representing the new genus *Botryobambusa,* which is introduced below. Clade A5 is a well-supported subclade incorporating species of *Neofussicoccum* and one strain of *Dichomera* which may be a synonym. Clade A6 represents the type species of

Botryosphaeria and three other *Botryosphaeria* species and two other genera, *Neoscytalidium* and *Cophinforma* gen. nov. Clade A7 comprises two *Pseudofusicoccum* species and Clade A8 has two *Aplosporella* species.

Taxonomy

As a result of detailed morphological characterization and molecular analysis of taxa of *Botryosphaeriaceae*, two new genera, *Botryobambusa* and *Cophinforma* are introduced and compared with existing genera. Four species new to science are also described in existing genera *Auerswaldia lignicola*, *A. dothiorella, Botryosphaeria fusispora* and *Phaeobotryosphaeria eucalypti*. The new taxa are differentiated by molecular phylogeny and morphology and are described and compared with similar taxa. A list of possible synonyms are given for genera and species, however this synonymy needs to be confirmed with molecular data as the order is now arranged mostly on the basis of molecular data. We also provide a list of unstudied genera and provide brief notes for these.

Taxonomic treatment

Botryosphaeriales C.L. Schoch, Crous & Shoemaker

Ascostromata uni- to multiloculate, with dark brown to blackened walls, occurring singly or in clusters, often immersed, sometimes superficial or frequently embedded in stromatic tissues, or in ascostromata which form superficial cushion-like structures, exposed dry internal contents often white when cut. *Pseudoparaphyses* hyphae-like, frequently disappearing at maturity. Asci bitunicate, fissitunicate, pedicellate, clavate to cylindro-clavate. Ascospores hyaline to pigmented, septate or aseptate. *Asexual morphs* with uni to multilocular pycnidial conidiomata, frequently embedded in stromatic tissue, with hyaline, phialidic conidiogenous cells. *Conidia* hyaline to pigmented, mostly aseptate.

Botryosphaeriaceae Theiss. & P. Syd.

Ascostromata uni- to multilocular, with multi-layered walls, single or in clusters, with or without basal stroma, fully or partially erumpent at maturity, exposed dry internal contents often white when cut. *Pseudoparaphyses* hyphae-like, branched or unbranched, septate, constricted at the septum, frequently disappearing at maturity. *Asci* bitunicate, fissitunicate, with thick endotunica, short or long pedicellate, clavate to cylindro-clavate, apically rounded with an ocular chamber. *Ascospores* hyaline to brown, smooth to verrucose, thin-walled, aseptate to septate, fusoid to ellipsoid or ovoid, bi- to triseriate, with or without a mucoid sheath or rarely with appendages. *Asexual morphs* with uni to multilocular pycnidial conidiomata, frequently embedded in stromatic tissue, with hyaline, phialidic conidiogenous cells. *Conidia* hyaline to pigmented, thin to thick-walled conidia which sometimes have mucoid appendages or sheaths, striations, verrucose walls and germ slits.

Kirk et al. (2008) estimated that there are 26 genera and 1517 species in the family. Following this study we accept 29 genera (Table 2) and approximately 1485 species (based on estimates for species in genera in Kirk et al. 2008). From our study, however we suspect that there are numerous undescribed species and several species complexes. *Macrovalsaria* Petr. is newly placed in this family.

Auerswaldia Sacc., Syll. Fung. 2:626 (1883)
MycoBank: MB463

Saprobic on dead wood. *Ascostromata* black, superficial, gregarious, becoming erumpent at maturity, but still under host surface, flattened at the upper surface, globose to subglobose, with 4 to numerous locules, with individual ostioles, cells of ascostromata brown-walled *textura angularis*. *Peridium* of locules two-layered, outer layer composed of small heavily pigmented thick-walled cells of *textura angularis*, inner layer composed of hyaline thin-walled cells of *textura angularis. Pseudoparaphyses* not observed. *Asci* 6–8–spored, bitunicate, fissitiunicate, clavate to cylindro-clavate, with a short pedicel, apically rounded, with a small ocular chamber. *Ascospores* hyaline to brown, aseptate, oblong to ovate. *Conidiomata* pycnidial, immersed in the host tissue and becoming erumpent at maturity, globose, coriaceous, dark brown in the erumpent part. *Conidiomata wall* with brown to dark brown outer layers and hyaline to light brown inner layers, comprising several layers with cells of *textura angularis*. *Conidiophores* reduced to *conidiogenous cells*, holoblastic, discrete, hyaline, cylindrical to ellipsoidal, smooth, straight or curved, formed from cells lining the innermost later of the pycnidium. *Conidia* 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, lower cell smaller.

Notes: *Auerswaldia* was established by Saccardo in 1883 with *A. chamaeropis* (Cooke) Sacc*, A. pringlei* (Peck) Sacc and *A. scabies* (Kalchbr. and Cooke) Sacc. Von Arx and Müller (1954) suggested that *Auerswaldia* differs from the similar genus *Auerswaldiella* by the number of locules (40–50) within the ascostroma and its larger brown ascospores; in *Auerswaldiella* ascostroma have only 4–6 locules and small, hyaline to light brown ascospores. In addition, the types of these two genera were found on different substrates (wood and leaves). Combined sequence analysis of our fresh collections of *Auerswaldia* shows this to be a well-supported and distinct genus in *Botryosphaeriaceae* (Fig. 1). There is no sequence data for *Auerswaldia* or *Auerswaldiella* in GenBank*,* however

Table 2 Accepted genera according to various treatments of *Botryosphaeriaceae*

Von Arx and Müller 1954	Barr 1987	Hawksworth et al. 1995	Lumbsch and Huhndorf 2010	Present study[a]
Auerswaldia	*Auerswaldia*	*Amarenomyces*	*Auerswaldiella*	*Aplosporella*
Auerswaldiella	*Auerswaldiella*	*Auerswaldiella*	*Barriopsis*	*Auerswaldia*
Bagnisiella	*Botryosphaeria*	*Botryosphaeria*	*Botryosphaeria*	*Auerswaldiella*
Botryosphaeria	*Discochora* (= *Guignardia*)	*Dothidotthia*	*Guignardia*	*Barriopsis*
Cleistosphaeria	*Dothidotthia*?	*Sivanesania*	*Leptoguignardia*	*Botryobambusa*
Ellisiodothis	*Homostegia*		*Neodeightonia*	***Botryosphaeria***/*Fusiccocum*[b]
Guignardia	*Leptoguignardia*		*Phaeobotryon*	*Cophinforma*
Montagnellina	*Neodeightonia*		*Phaeobotryosphaeria*	*Diplodia*
Microdothiella	*Phyllachorella*		*Saccharata*	*Dothiorella*
Muyocopron			*Sivanesania*	*Endomelanconiopsis*
Parastigmatea			*Spencermartinsia*	*Lasiodiplodia*
Pilgeriella				*Leptoguignardia*
Pyrenostigme				*Macrophomina*
Trabutia				*Macrovalsaria*
Vestergrenia				*Melanops*
				Neodeightonia
				Neofusicoccum
				Neoscytalidium
				Phaeobotryon
				Phaeobotryosphaeria/*Sphaeropsis*[c]
				Phyllachorella
				Phyllosticta/*Guignardia*[d]
				Pseudofusicoccum
				Pyrenostigme
				Saccharata
				Sivanesania
				Spencermartinsia
				?*Tiarosporella*
				Vestergrenia

[a] If two names are known for the genus both names are listed. The name that should be used following the introduction of the rule requiring a genus to have a single name is listed first and in bold

[b] *Botryosphaeria* is preferred over *Fusicoccum*, even though the latter is the older name because this name has been used against *Fusicoccum* in recent publications, it is the type of the order and family, it is more commonly recorded in publications and as a pathogen (e.g. Slippers et al. 2004b; Crous et al. 2006)

[c] *Phaeobotryosphaeria* is preferred over *Sphaeropsis*; even through the latter is the older name because this name has been used against *Sphaeropsis* in recent publications (e.g. Phillips et al. 2008). *Sphaeropsis* is also likely to be polyphyletic

[d] A case has already been presented for using *Phyllosticta* in Wikee et al. (2011a)

we treat both as distinct genera in *Botryosphaeriaceae*, although fresh collections may show this to be incorrect. We have examined and illustrated the generic type of *Auerswaldia* although it is not in good condition. We also found two new species during collections in Thailand which are described below. One is the asexual morph which we link for the first time to *Auerswaldia*. Von Arx and Müller (1975) synonymised *Dothidea examinans* under *Bagnisiella*. We have examined the type material of *B. australis* Speg. (Fig. 3) which is immature, but does not appear to be *botryosphaeriaceous* based on the characters of the sunken ascostromata and cylindrical asci (Fig. 3). Schoch et al. (2009a) used a strain named *Bagnisiella examinans* (= *Auerswaldia examinans*) following the synonymy of von Arx and Müller (1975) in their phylogenetic tree, which placed this genus in *Botryosphaeriaceae*. However we believe that *Bagnisiella* is not the same as *Auerswaldia* and the former should be retained in *Dothideaceae* pending fresh collections.

Generic type: *Auerswaldia examinans* (Mont. & Berk.) Sacc.

Auerswaldia examinans (Mont. & Berk.) Sacc., Syll. Fung. 2:266 (1883)

MycoBank: MB165896 (Fig. 2)

≡ *Dothidea examinans* Mont. & Berk., London J. Bot. 4:335 (1844)

≡ *Melogramma examinans* (Mont. & Berk.) Cooke, Grevillea 13(no. 68): 108 (1885)

≡ *Bagnisiella examinans* (Mont. & Berk.) Arx & E. Müll., Stud. Mycol. 9: 17 (1975)

Saprobic on dead wood. *Ascostromata* 450–610 μm wide, black, gregarious, superficial, becoming erumpent, partially under the host surface, flattened at the upper surface, globose to subglobose, coriaceous, with numerous locules, with individual ostioles, cells of ascostromata brown-walled *textura angularis*. *Peridium* of locules 22–38 μm thick at the sides, two-layered, with outer layer composed of small heavily pigmented thick-walled cells *textura angularis*, with inner layer composed of hyaline thin-walled cells *textura angularis*. *Pseudoparaphyses* not observed. *Asci* 79–88×16–22 μm ($\overline{x} = 84 \times 19\,\mu m, n = 10$), (4-)8–spored, bitunicate, fissitunicate, clavate to cylindro-clavate, with a short pedicel, apically rounded ,with a small ocular chamber. *Ascospores* 16–21×9–12 μm ($\overline{x} = 20 \times 11\,\mu m, n = 15$), over-lapping 2–seriate, uniseriate near the base, brown, aseptate, oblong to ovate, smooth-walled. *Asexual state* not established.

Material examined: INDONESIA, Java, on decayed branches bursting through the bark, collector Zollinger, n 520. (K 76513, type).

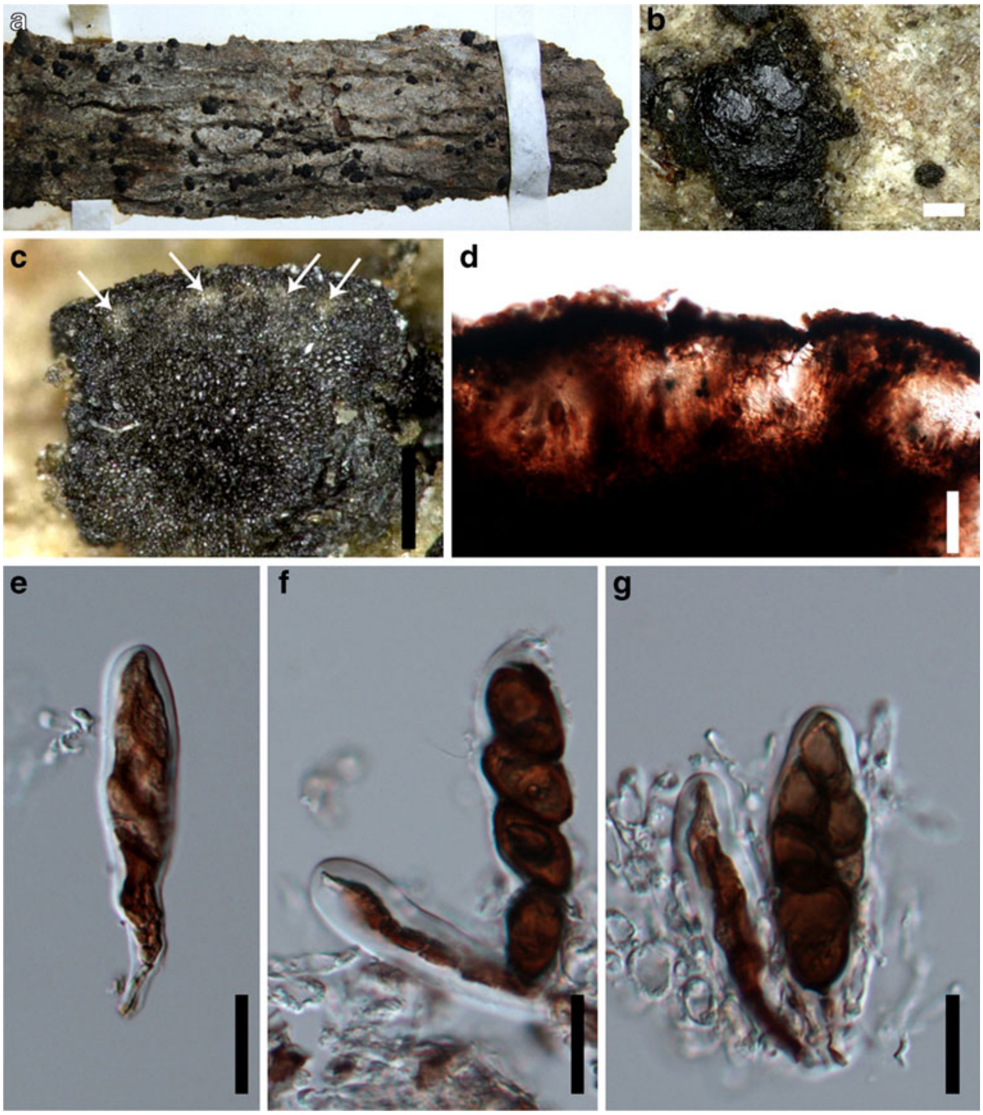

Fig. 2 *Auerswaldia examinans* (K 76513, holotype). **a–c** Appearance of ascostromata on the host substrate. Note the *arrowheads* indicate locules in **c**. **d** Vertical section through ascostroma. **e–g** Asci. Scale bars: **b–c** = 600 μm, **d** = 200 μm **e–g** = 20 μm

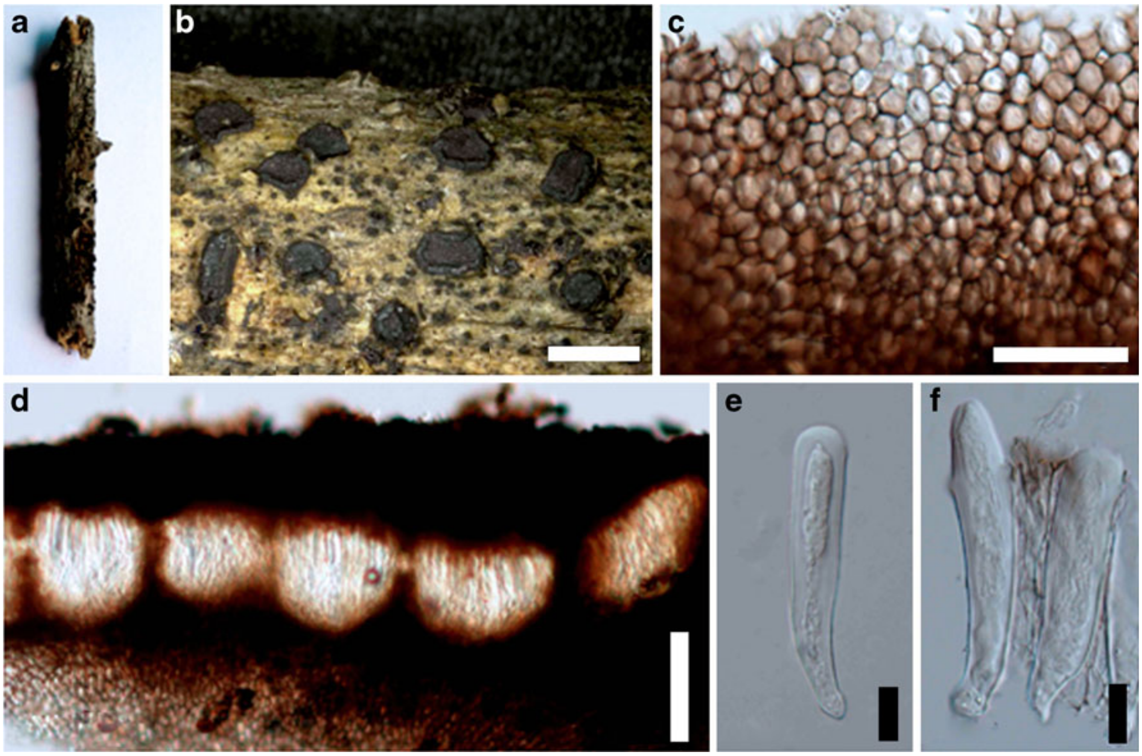

Fig. 3 *Bagnisiella australis* (LPS 322, holotype) **a** Herbarium specimen. **b** Appearance of ascostromata on the host substrate. **c** Cells of ascostromata **d** Vertical section through ascostroma showing locules. **e**–**f** Cylindrical asci. Scale bars: **b** = 800 µm, **c** = 50 µm, **d** = 100 µm, **e**–**f** = 20 µm

Material examined: ARGENTINA, Buenos Aires, San José de Flores, on the branch of *Acacia bonariensis*, June 1880, C.L. Spegazzini, (LPS 322, holotype) (Figs. 3 and 4).

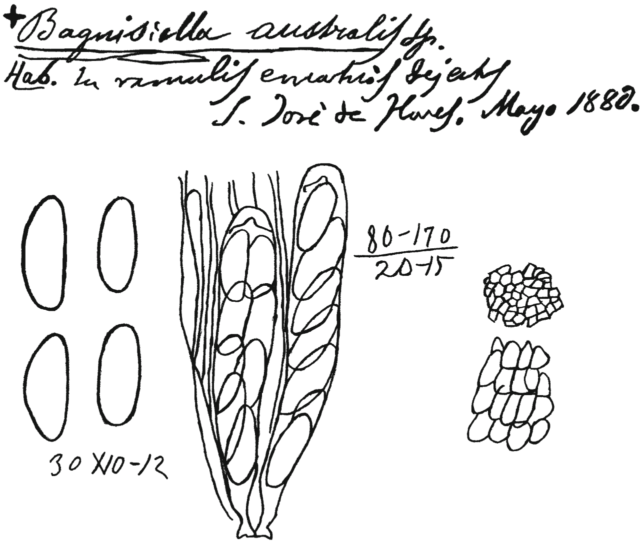

Fig. 4 Redrawing of *Bagnisiella australis* based on the original drawing (LPS 322, holotype)

Auerswaldia lignicola Ariyawansa, J.K. Liu & K.D. Hyde, sp. nov.

MycoBank: MB 801317 (Fig. 5)

Etymology: from *Lignin* and loving Latin = *icola*, in reference to habit on wood.

Saprobic on dead wood. *Ascostromata* 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 µm diam × 110–130 µm high ($\bar{x} = 115 \times 120\,\mu m, n = 10$), with individual papillate ostioles. *Peridium* of locules 30–60 µm diam ($\bar{x} = 50\,\mu m, n = 10$), 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* 80–90 × 15–25 µm ($\bar{x} = 85 \times 20\,\mu m, n = 20$), bitunicate, fissitunicate, clavate to broadly clavate, with short and narrow pedicel, rounded at the apex with an ocular chamber. *Ascospores* 15–20 × 8–10 µm ($\bar{x} = 19 \times 9\,\mu m, n = 40$), uniseriate or partially overlapping, reddish brown to dark brown, aseptate, fusiform to ellipsoid with narrowly rounded ends, smooth-walled. *Asexual state* not established.

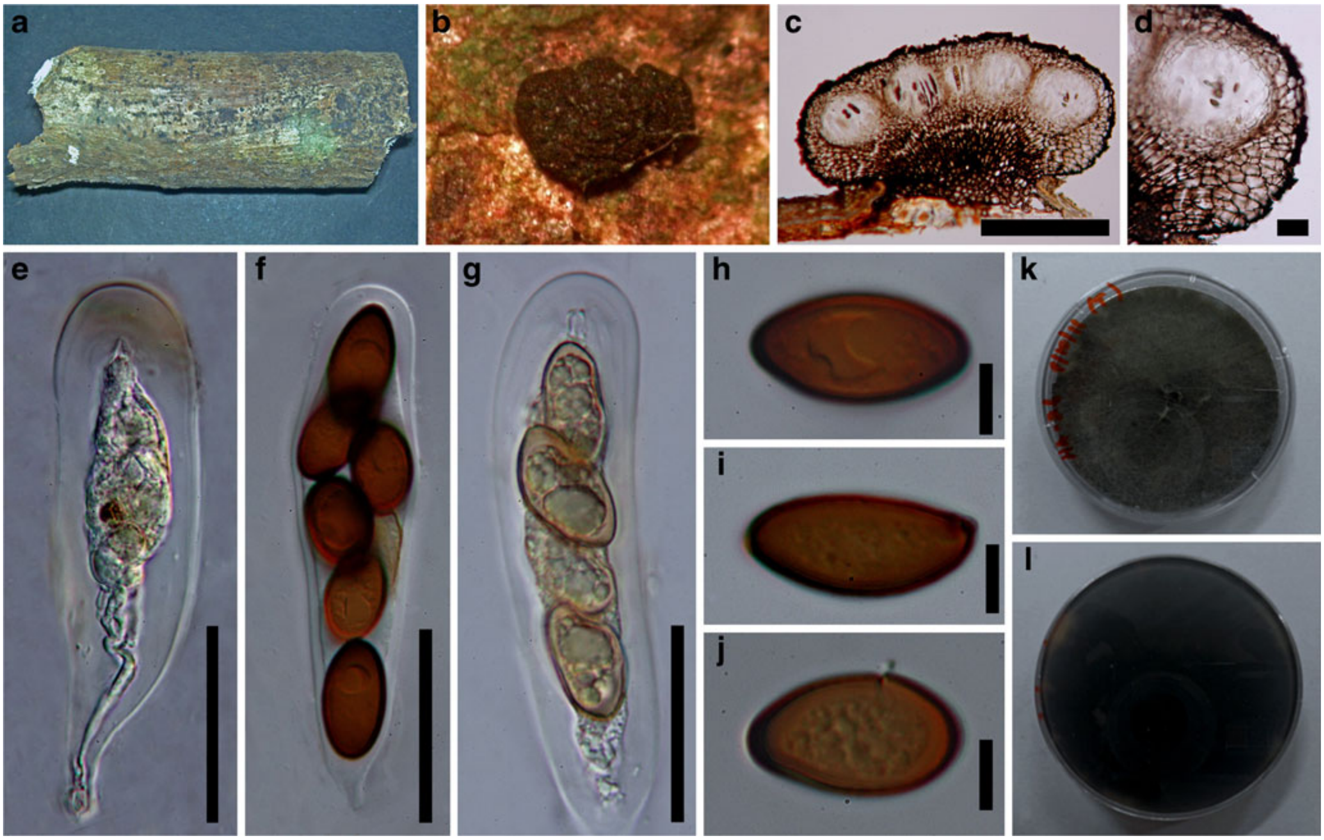

Fig. 5 *Auerswaldia lignicola* (MFLU 12–0750, holotype). **a**–**b** Ascostromata on host substrate. **c** Section of ascostromata showing 4–5 locules (TS). **d** Close up of peridium surrounding the locules comprising two cell layers and arrangement of cells in ascostromata. **e**–**g** Asci with 4–8 ascospores. **h**–**j** Immature and mature ascospores with smooth walls. **k**–**l** Colonies from above (k) and below (l). Scale bars: **c** = 350 μm, **d** = 50 μm, **e**–**g** = 30 μm, **h**–**j** = 5 μm

Cultural characteristics: Ascospores germinating on WA within 18 h and producing germ tubes from each septum. Colonies growing slowly on MEA, reaching a diam of 3 mm after 5 d at 27 °C, effuse, velvety, with entire to slightly undulate edge, dark brown to black. After 4 months, only superficial, branched, septate, smooth, brown mycelium produced, no asexual-morph produced on MEA and WA following incubation.

Material examined: THAILAND, Chiang Rai Province., Muang District, Bandu, on dead wood, 30 September 2011, A.D Ariyawansa, HA026 (MFLU 12–0750, holotype), ex-type living culture in MFLUCC11–0435; *Ibid*, living culture MFLUCC 11–0656.

Notes: The raised, pulvinate ascostromata of this taxon, isolated from wood, fit well with those of *Auerswaldia*. However, the species is distinct in producing short broad pedicellate asci with large brown ascospores. This fungus is phylogenetically most similar to *Auerswaldia dothiorella*, described below, (97 % bootstrap support) based on EF1-α gene sequence data. However, when multi-gene analyses were carried out, the species segregated into two distinct taxa. We therefore introduce *A. lignicola* as a new species.

Auerswaldia dothiorella D.Q. Dai., J.K. Liu & K.D. Hyde, sp. nov.

MycoBank: MB 801318 (Fig. 6)

Etymology: From the conidial shape which is similar to "*Dothiorella*" conidia

Saprobic on dead bamboo. *Conidiomata* pycnidial, 400–800 μm long, 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 $(\overline{x} = 4.2 \times 3\,\mu m, n = 10)$, holoblastic, discrete, hyaline, cylindrical to ellipsoidal, smooth, straight or curved, formed from cells lining the innermost later of the pycnidium. *Conidia* 15–20×6.5–8 μm $(\overline{x} = 18.5 \times 7\,\mu m, n = 20)$, 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. *Sexual state* not established.

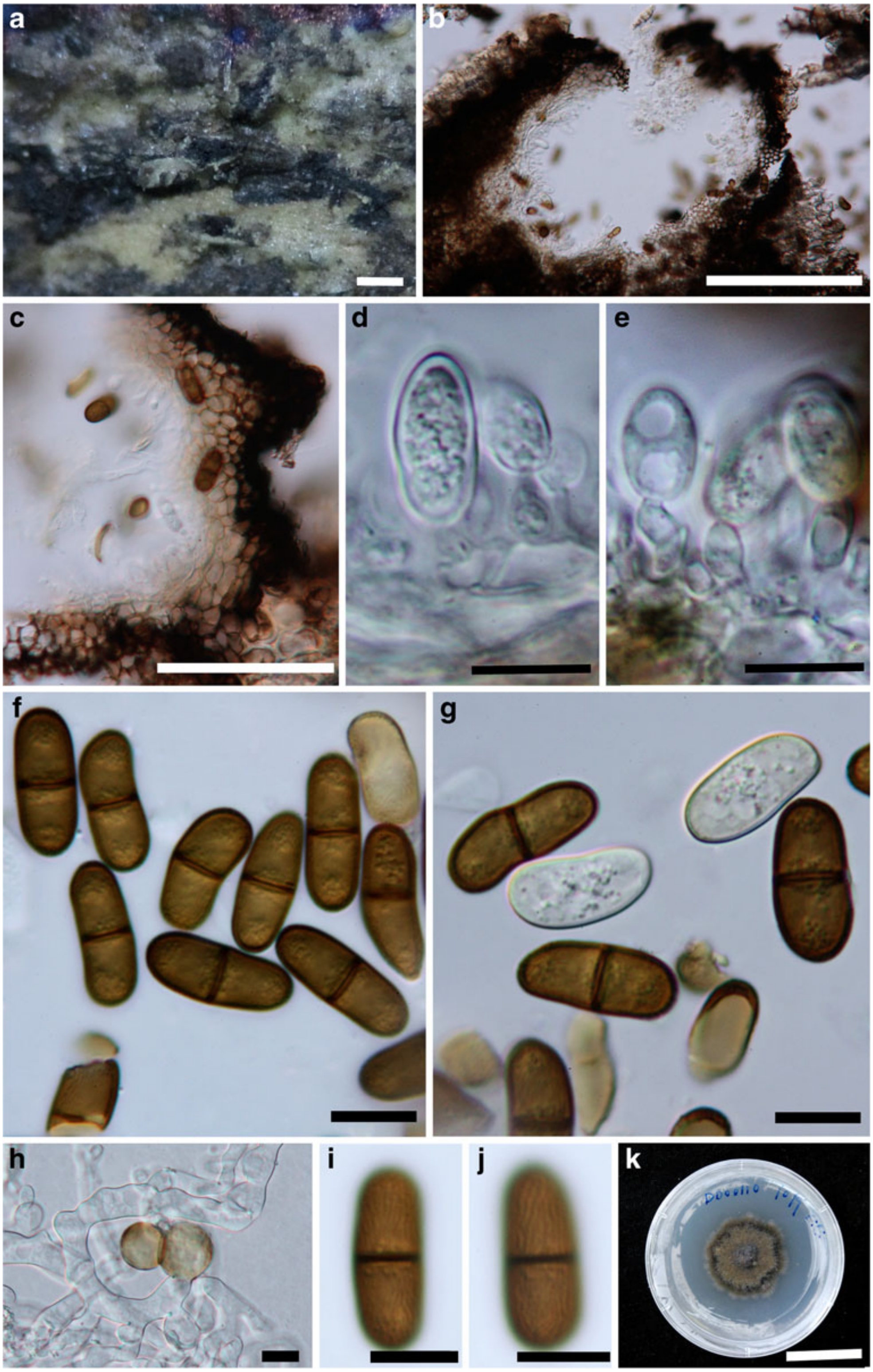

Fig. 6 *Auerswaldia dothiorella* (MFLU 12–0751, holotype). **a** Pycnidia on bamboo host. **b** Section of pycnidia. **c** Wall of pycnidium showing the cell characters. **d**–**e** *Conidiogenous cells* and developing conidia. **f**–**g** Brown conidia with 1–septa and hyaline young aseptate conidia. **h** Geminating conidia. **i**–**j** brown conidia with slight undulating striations. **k** Culture on PDA after 45 d. Scale Bars: **a** = 500 μm, **b** = 100 μm, **c** = 50 μm, **d**–**j** = 10 μm, **k** = 15 mm

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.

Material examined: THAILAND, Chiang Rai Province, Doi Pui, on dead bamboo culm, 1 September 2011, Dongqin Dai, DDQ00110 (MFLU 12–0751, holotype), ex-type living culture MFLUCC 11–0438.

Notes: *Auerswaldia dothiorella* is characterized by pycnidial conidiomata which are immersed in the host tissue, becoming erumpent at maturity. *Conidiophores* are reduced to *conidiogenous cells* which are holoblastic, discrete, hyaline, and cylindrical to ellipsoidal. Conidia are brown, 1–septate, oblong to ellipsoidal and with undulating striations on the surface. The new taxon is morphologically close to *Dothiorella*, but the hyaline conidia become brown with age and thus *A. dothiorella* differs from *Dothiorella* where conidia are brown, and septate while still attached to the conidiogenous cell (Crous et al. 2006). Phylogenetic data also confirms that this taxon can be distinguished from *Dothiorella* species. We did not encounter the sexual morph of *A. dothiorella* and it did not form in culture. The asexual stage did not sporulate in the ex-type culture.

Auerswaldiella Theiss. & Syd., Ann. Mycol. 12: 278 (1914)

MycoBank: MB454

Possible synonyms:

Dimeriellina Chardón, Bol. Soc. Venez. Cienc. Nat. 5(no. 40): 339 ('239') (1939)

Stichodothis Petr., Ann. Mycol. 25: 198 (1927)

Saprobic on leaves. *Ascostromata* black, solitary, scattered, superficial on lower side, globose, rough, papillate, pulvinate, multiloculate, cells of ascostromata brown-walled *textura angularis*. *Peridium* of locules two-layered, outer layer composed of small heavily pigmented thick-walled cells of *textura angularis*, inner layer composed of hyaline thin-walled cells of *textura angularis. Pseudoparaphyses* hyphae-like, numerous, septate. *Asci* 8–spored, bitunicate, fissitunicate, cylindro-clavate, with a pedicel and an ocular chamber. *Ascospores* biseriate, hyaline to light brown, obovoid to ellipsoidal with rounded ends, smooth–walled. *Asexual state* not established.

Notes: *Auerswaldiella* presently comprises nine epithets (*Index Fungorum*) with the latest species being introduced by Farr (1989). This unusual genus forms raised ascostromata on the surface of leaves comprising four to six locules with densely packed asci and unicellular hyaline to light brown ascospores. The asci are typical of *Botryosphaeriaceae*, however, the raised, pulvinate ascostromata on leaves and large numbers of pseudoparaphyses are atypical; the minute ascospores also differentiate this from other genera in *Botryosphaeriaceae*.

Generic type: *Auerswaldiella puccinioides* (Speg.) Theiss. & Syd.

Auerswaldiella puccinioides (Speg.) Theiss. & Syd., Ann. Mycol. 12: 278 (1914)

MycoBank: MB155192 (Figs. 7 and 8)

≡ *Auerswaldia puccinioides* Speg., Anales Soc. Ci. Argent. 19: 247 (1885)

= *Phyllachora viridispora* Cooke, Grevillea. 13(no. 67): 65 (1885)

= *Dothidea viridispora* (Cooke) Berl. & Voglino, in Sacc., Syll. Fung. Addit. I-IV: 243 (1886)

= *Bagnisiella pruni* Henn., Hedwigia. 48: 6 (1908)

Saprobic on lower surface of leaves. *Ascostromata* 0.8–0.9 mm diam, 0.4–0.5 mm high, black, raised on host tissue, solitary, scattered, superficial, pulvinate, globose, rough, multiloculate, containing 4–6 locules, with individual papillate ostioles, cells of ascostromata brown-walled *textura angularis*. *Locules* 320–370×450–500 μm. *Peridium* of locules two-layered, up to 30–40 μm wide, outer layer composed of small heavily pigmented thick-walled cells of *textura angularis*, inner layer composed of hyaline thin-walled cells of *textura angularis. Pseudoparaphyses* hyphae-like, septate, numerous. *Asci* 138–185×32–36 μm $(\bar{x} = 164 \times 35\,\mu m, n = 15)$, 8–spored, bitunicate, fissitunicate, cylindro–clavate, with a long pedicel and wide shallow ocular chamber. *Ascospores* 9–12×3–6 μm $(\bar{x} = 11 \times 5\,\mu m, n = 30)$, biseriate, hyaline to light brown, obovoid to ellipsoidal, flattened in one plane, with rounded ends, smooth–walled. *Asexual state* not established.

Material examined: PARAGUAY, Villa Rica; Mbocaiaté, on leaves of *Prunus sclerocarpa*, 15 January 1882, B. Balansa No 3443 (LPS 281, holotype)

Notes: The type specimen examined is relatively immature and it was very hard to find asci and ascospores. This is a very distinct fungus and should be recollected and epitypified. The smaller spores in Fig. 8 were not observed on the type specimen.

Barriopsis A.J.L. Phillips, A. Alves & Crous, Persoonia 21: 39 (2008)

MycoBank: MB511712

Saprobic on dead twigs. *Ascostromata* brown to black, immersed, aggregated or in clusters, scattered, erumpent at maturity, discoid to pulvinate or hemisphaerical, discrete, multiloculate. *Ostiole* central. *Pseudoparaphyses* hyphae-like, septate, embedded in gelatinous matrix. *Asci* 8–spored, bitunicate, clavate to sub-clavate, short stalked. *Ascospores* biseriate, brown to dark brown, aseptate, ellipsoid-oval, inequilateral, slightly curved, widest in the median to supra-median, smooth or verrucose, without sheath. *Asexual state* is *Lasiodiplodia*-like: *Conidiomata* stromatic, pycnidial, superficial, dark brown to black, multilocular, individual or aggregated, thick-walled, ostiolate. *Ostiole* central, circular, non-papillate. *Paraphyses* hyaline, thin-walled, usually aseptate, constricted at the septa, occasionally branched. *Conidiogenous cells* holoblastic, hyaline, thin-walled, cylindrical, with visible periclinal thickening. *Conidia* initially hyaline, oval, both ends broadly rounded, thick-walled, aseptate with longitudinal striations, striations visible on hyaline conidia even while attached to *conidiogenous cells*, becoming brown, aseptate or 1–3–septate, with prominent longitudinal striations (asexual morph description follows Stevens 1926; Abdollahzadeh et al. 2009).

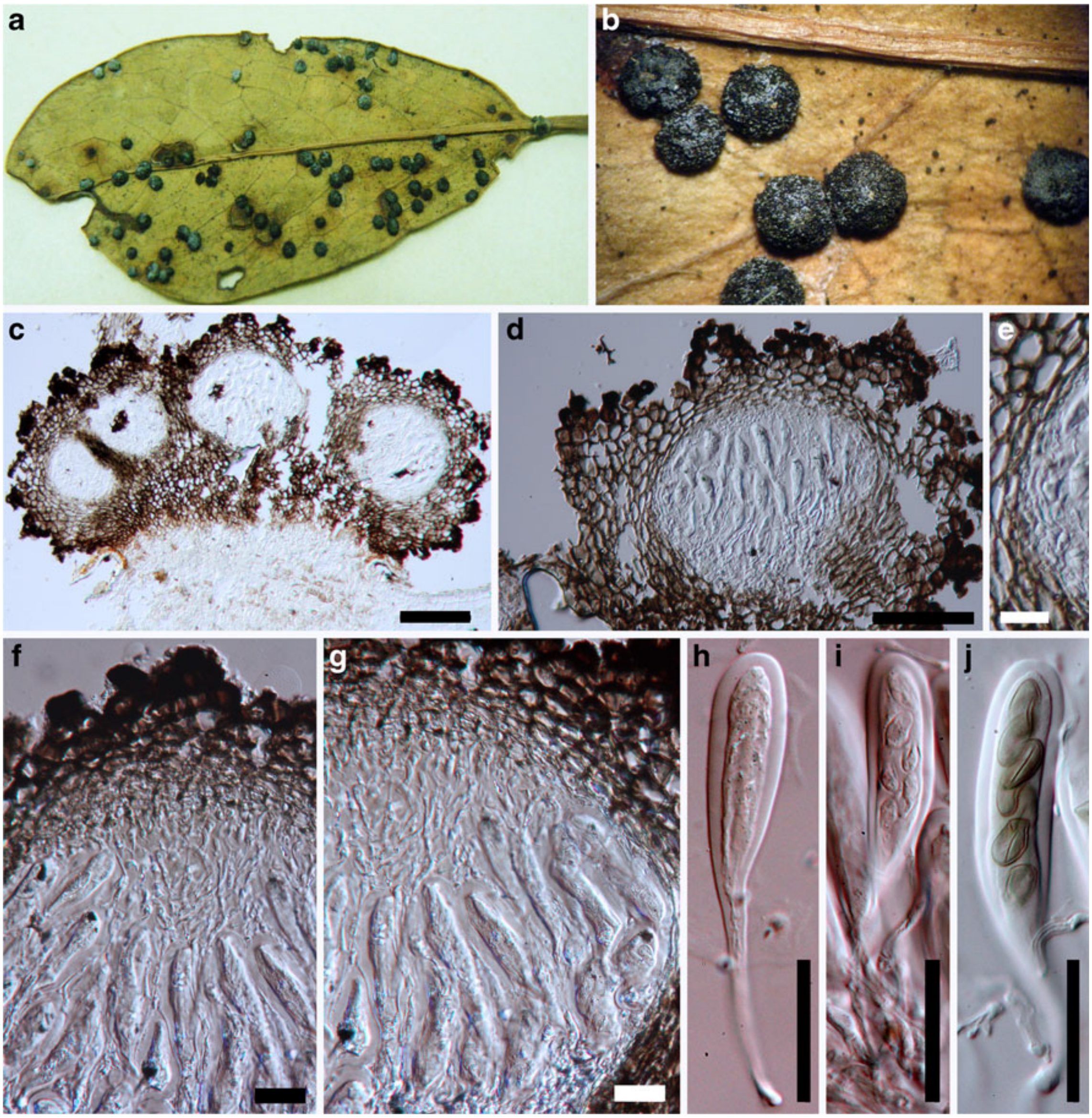

Fig. 7 *Auerswaldiella puccinioides* on *Prunus sclerocarpa* leaf (LPS 281, holotype). **a**–**b**: Ascostromata on the host. **c**–**d, f**–**g** Sections of ascostromata. **e** Peridium. **h**–**j** Ascus with hyaline and light brown ascospores. Scale bars: **c–d** = 100 μm, **e** = 10 μm, **f–g** = 20 μm, **h–j** = 30 μm

Notes: *Barriopsis* was introduced as a monotypic genus by Phillips et al. (2008) based on *Physalospora fusca,* and a second species, *Barriopsis iraniana* Abdoll., Zare & A.J.L. Phillips, was added by Abdollahzadeh et al. (2009). *Barriopsis* accommodates species having brown, aseptate ascospores, which are lighter in the centre, without apiculi and with a *Lasiodiplodia*-like asexual morph (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). It is listed as a member of *Dothidotthiaceae* in *Index Fungorum*, but Lumbsch and Huhndorf (2010) treated it as a member of *Botryosphaeriaceae*. Phillips et al. (2008) used phylogenetic data to confirm its identity as a member of the *Botryosphaeriaceae*. This is confirmed in the phylogenetic tree (Fig. 1).

Generic type: *Barriopsis fusca* (N.E. Stevens) A.J.L. Phillips, A. Alves & Crous.

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

MycoBank: MB511713 (Fig. 9)

≡ *Physalospora fusca* N.E. Stevens, Mycologia 18: 210 (1926)

= *Phaeobotryosphaeria fusca* (N.E. Stevens) Petr., Sydowia 6: 317 (1952)

Saprobic on dead twigs. *Ascostromata* (430-)546.5–520 μm diam×328–349 μm high $(\bar{x} = 520 \times 338\,\mu m)$,

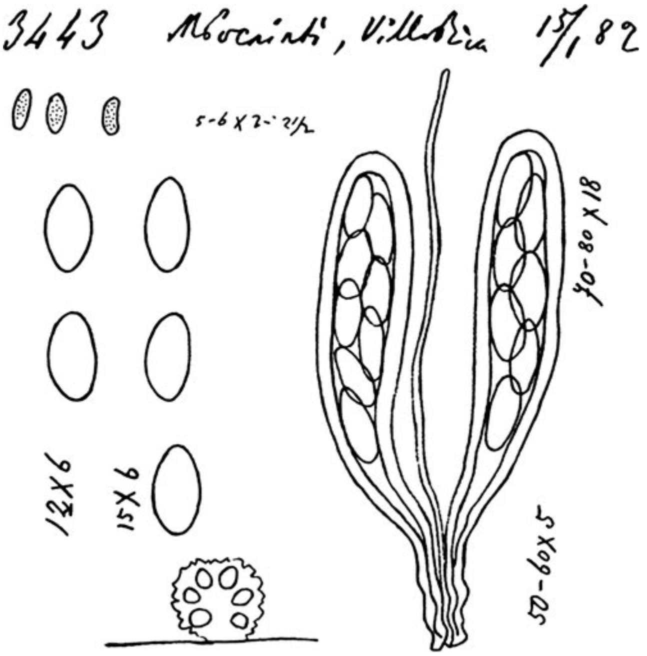

Fig. 8 *Auerswaldiella puccinioides* on *Prunus sclerocarpa* leaf. Redrawing from the original type species drawing (LPS 281, holotype)

black, immersed, aggregated or some clustered, scattered, composed of one or up to three ascomata in each ascostroma, developing in the substrate and erumpent through the bark at maturity, discoid to pulvinate or hemisphaerical, discrete or wide-spreading with surface slightly convex, with thickened peridium. *Pseudoparaphyses* (3-)4–4.5 µm wide, hyphae-like, septate, embedded in a gelatinous matrix. *Asci* (109-)124–154.5(−166)×32–39(−41) µm ($\bar{x} = 139 \times 36\,\mu m$), 8–spored, bitunicate, fissitunicate, clavate to sub-clavate, broad, with a short pedicel and apically rounded with an ocular chamber. *Ascospores* (29.5-)31–34× (13-)15–15.5 µm ($\bar{x} = 31.5 \times 15\,\mu m$), biseriate, brown to dark brown, aseptate, ellipsoid-oval, inequilateral, slightly curved, widest in the median to supramedian, ends rounded, light brown in the centre, smooth or verrucose, without a gelatinous sheath. *Conidiomata* stromatic, pycnidial, dark brown to black, superficial, mostly multilocular, individual or aggregated, thick-walled, ostiolate. *Ostiole* central, circular, non-papillate. *Paraphyses* hyaline, usually aseptate, sometimes becoming up to 2–3–septate, not constricted at the septa, thin-walled, tip rounded, occasionally branched. *Conidiogenous cells* 7–12×3–5 µm, holoblastic, hyaline, cylindrical, thin-walled, smooth, proliferating at the same level, with visible periclinal thickening. *Conidia* (20-)23–25 (−28)×(11-)12–13(−16) µm, initially hyaline, aseptate and thick-walled becoming dark brown and septate with irregular longitudinal striations (asexual morph description follows Stevens 1926; Abdollahzadeh et al. 2009).

Material examined: CUBA, Herradura, on twigs of *Citrus* sp., 15 January 1925, N. E. Stevens (BPI599052, holotype).

Notes: The asexual morph was not observed in the type and the ex-type culture which was isolated more than 80 years ago and has lost its ability to sporulate. The second species *Barriopsis iraniana* was introduced with only an asexual morph as no sexual stage was formed in culture. The morphological characters (the conidia are striate at an early stage of development and the striations are clearly visible in young, hyaline conidia) confirmed that the asexual morph of *Barriopsis* is linked to a *Lasiodiplodia*-like morph. *Barriopsis fusca* differs from *B. iraniana* by its distinctly smaller conidia (23–25×12–13 µm vs. 24–30×14–18 µm) (Abdollahzadeh et al. 2009; Stevens 1926).

Botryobambusa R. Phookamsak, J.K. Liu & K.D. Hyde, gen. nov.

MycoBank: MB 801313

Etymology: Referring to the host *Bambusa* and its placement in *Botryosphaeriaceae.*

Saprobic on dead bamboo. *Ascostromata* dark brown to black, immersed under epidermis to erumpent, gregarious, visible as minute black dots or papilla on the host tissue, multiloculate, locules individual globose to subglobose or fused, coriaceous, vertical to the host surface, with a central ostiole. *Neck* central, papillate, periphysate. *Asci* 8–spored, bitunicate, fissitunicate, clavate to cylindro-clavate, pedicellate, with well-developed ocular chamber. *Ascospores* hyaline, velvety, aseptate, ellipsoidal to obovoid, smooth and thick-walled, surrounded by a mucilaginous sheath. *Pycnidia* developing in stromatic clusters, fused, multiloculate, individually globose to subglobose. *Peridium* comprising several layers of *textura angularis*, broader at the base, outer layers dark to 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* is introduced as a monotypic genus for *B. fusicoccum* which is characterized by multiloculate *ascostromata*, clavate, short pedicellate, fissitunicate asci and velvety, thick-walled, hyaline, aseptate, sheathed ascospores. It is so far only known from bamboo. The ascomata are tightly clustered under the bamboo host surface and can be considered as ascostromatic in a broad sense. This is obvious in culture where the pycnidia are clearly stromatic. The genus can be distinguished from the closely similar *Botryosphaeria* by its smaller asci, aseptate, velvety, hyaline, sheathed ascospores and *Fusicoccum*-like asexual stage with large conidia. Phylogenetically, these two genera are markedly distinguished.

Generic type: *Botryobambusa fusicoccum* R. Phookamsak, J.K. Liu & K.D. Hyde

Botryobambusa fusicoccum R. Phookamsak, J.K. Liu & K.D. Hyde, sp. nov.

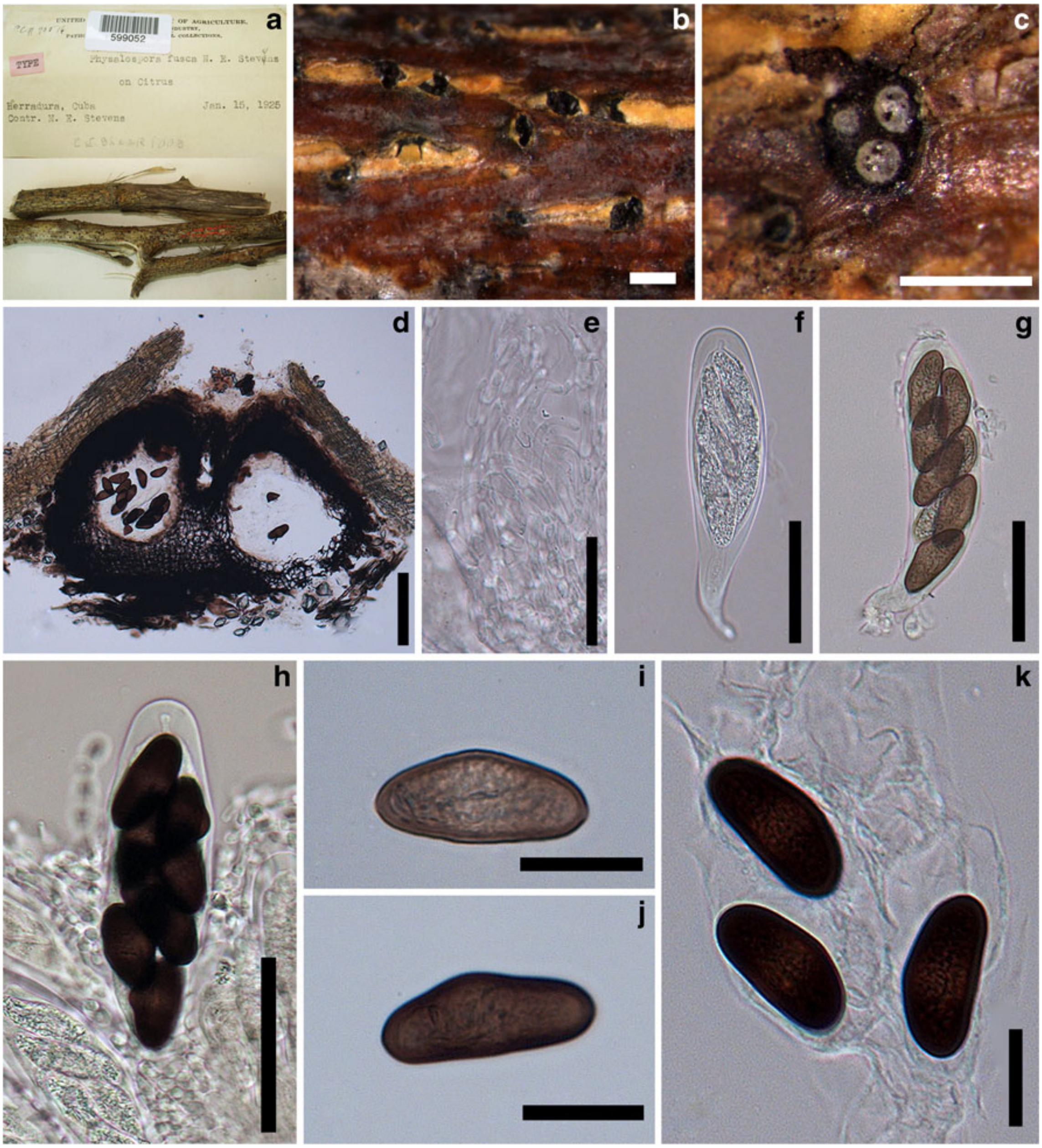

Fig. 9 *Barriopsis fusca* (BPI 599052, holotype) **a** Herbarium material. **b**–**c** Ascostromata forming beneath the bark of substrate, note the cross section in surface view in c. **d** Section through erumpent ascostromata and peridium. **e** Pseudoparaphyses. **f**–**h** Ascus with ocular chamber at apex and containing young and mature ascospores. **i**–**k** Immature and mature ascospores. Scale bars: **b**–**c** = 500 μm, **d** = 100 μm, **e** = 20 μm, **f**–**h** = 50 μm, **i**–**k** = 20 μm

MycoBank: MB 801314 (Figs. 10 and 11)

Etymology: Referring the asexual stage "*Fusicoccum*-like".

Saprobic on dead bamboo. *Ascostromata* 103.5–152 μm high (including neck), 95–152 μm diam, dark brown to black, immersed under epidermis to erumpent, gregarious, visible as minute black dots or papilla on host tissue, multiloculate, *locules* individual globose to subglobose or fused, coriaceous, vertical to the host surface, with a central ostiole. *Neck* 42–59 μm diam, 31–54 μm high, central, papillate, periphysate. *Peridium* 12–20 μm wide, comprising several layers of cells, with relatively thick brown to back-walls, arranged in *textura angularis*, broader at the base. *Pseudoparaphyses* not observed. *Asci* (48-)55–66(−82)× 14–17(−18) μm $(\bar{x} = 60 \times 15.5\,\mu m, n = 25)$, 8–spored, bitunicate, fissitunicate, clavate to cylindro-clavate, pedicellate, apically rounded with well-developed ocular chamber (2–3 μm wide, *n*=5). *Ascospores* (8-)11–13(−14)×5–7 μm $(\bar{x} = 11.5 \times 6\,\mu m, n = 30)$, uni–seriate at the base or irregularly biseriate, hyaline, velvety, aseptate, ellipsoidal to obovoid, usually wider in the apex, thick-walled,

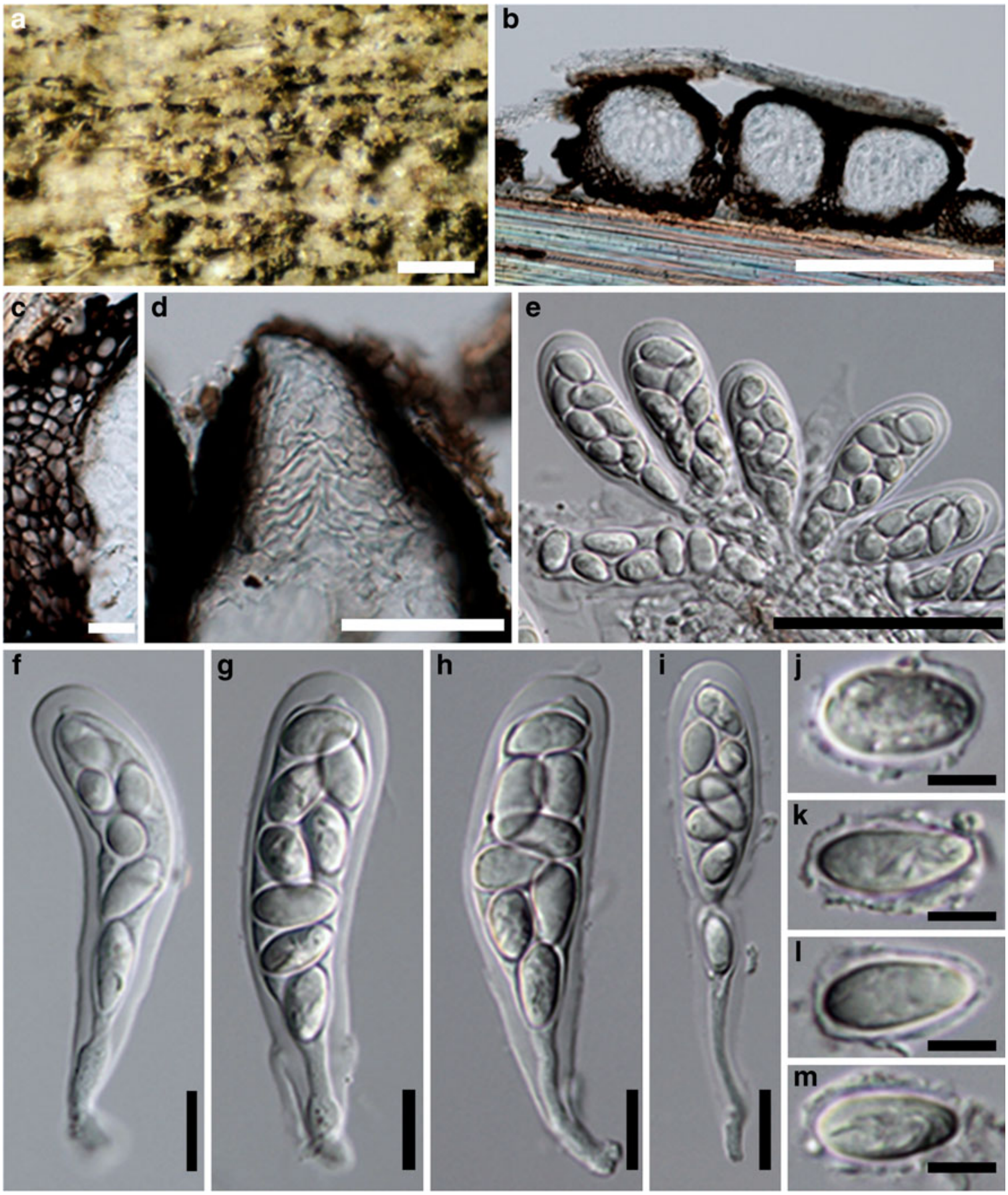

Fig 10 *Botryobambusa fusicoccum* (MFLU 11–0179, holotype) on dead culm of *Bambusa* sp. **a** Ascostromata on host substrate. **b** Section through multiloculate ascostromata. **c** Section through ascostromata showing arrangement of cells. **d** Neck with periphyses. **e**–**i** Asci. **j**–**m** Ascospores. Scale bars: **a** = 500 µm, **b** = 200 µm, **c** = 20 µm, **d**–**e** = 50 µm, **f**–**i** = 10 µm, **j**–**m** = 5 µm

surrounded by distinctive structured mucilaginous sheath. *Pycnidia* (formed on WA on sterilized pine needles within 10 days) superficial on host surface, clustered in a stroma, multiloculate, globose to subglobose. *Peridium* comprising several layers of cells *textura angularis*, broader at the base, outer layers dark to dark-brown and thick-walled, inner layers hyaline and thin-walled. *Conidiogenous cells* (8-)10−14(−16)×3–5 µm holoblastic, hyaline, cylindrical to ellipsoidal, smooth-walled. *Conidia* (21-)22–25(−26)×5–7 µm ($\bar{x} = 23.5 \times 6\,\mu m, n = 30$), hyaline, aseptate, cylindrical to cylindro-clavate, thin-walled, with rough wall.

Culture characteristics: Colonies on PDA reaching 50 mm diam after 4 d at 25–30 °C, fast growing; circular, whitened in a few days, after one week becoming grey to

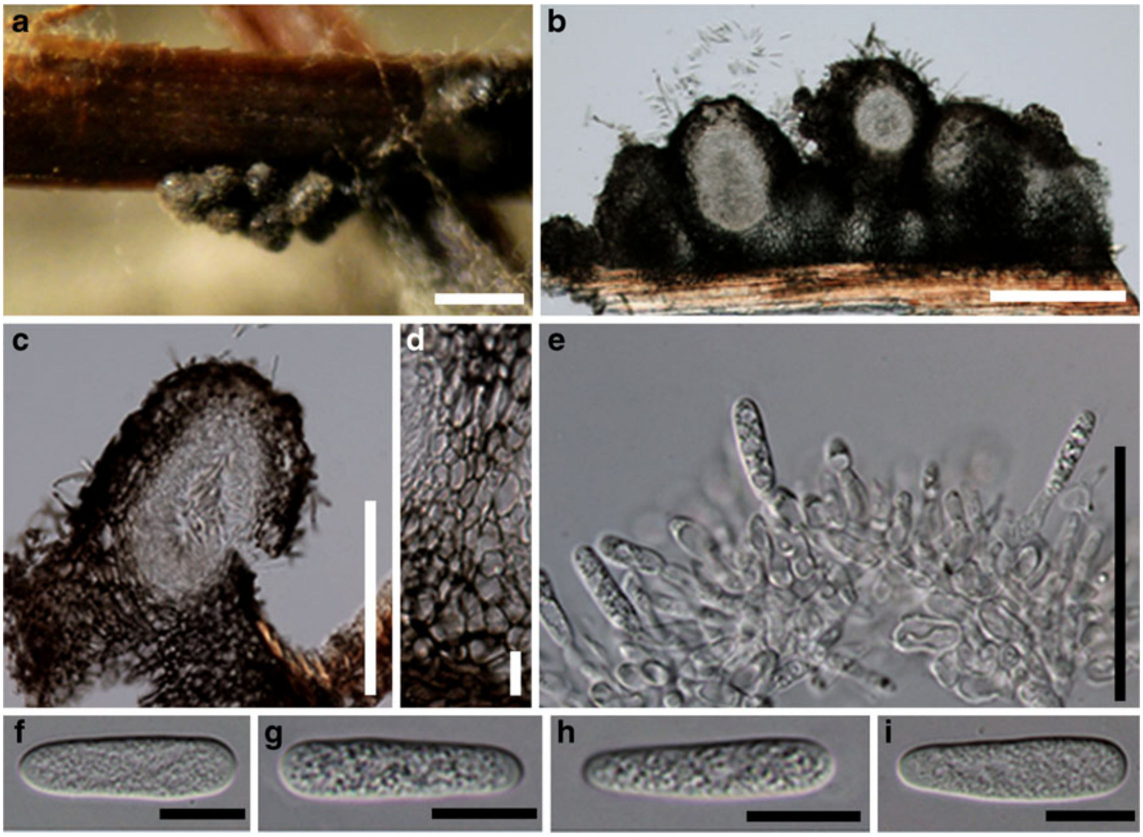

Fig. 11 Asexual morph of *Botryobambusa fusicoccum* on the sterilized pine needles after 10 days (MFLU 11–0179, holotype). **a** Conidiomata on host tissue. **b** Section through multiloculate conidiomata. **c** Section through pycnidia neck **d** Section through peridium. **e** Conidiogenous cells. **f–i** Conidia. Scale bars: **a** = 500 µm, **b–c** = 200 µm, **d** = 20 µm, **e** = 50 µm, **f–i** = 10 µm

green-black; flattened, fairly dense, surface smooth with crenate edge, filamentous; reverse grey to black, pigments not produced in media.

Material examined: THAILAND, Lampang Province, Jae Hom District, Mae Yuag Forestry Plantation, on dead culms of *Bambusa* sp., 19 August 2010, R. Phookamsak, RP0059 (MFLU11–0179, holotype), ex-type living culture MFLUCC11–0143; *Ibid.*, living culture MFLUCC 11–0657.

***Botryosphaeria* Ces. & De Not.,** Comm. Soc. Crittog. Ital. 1: 211 (1863)

Mycobank: MB635

Possible synonyms

Amerodothis Theiss. & Syd., Ann. Mycol. 13: 295 (1915)

Apomella Syd., Ann. Mycol. 35: 47 (1937)

Caumadothis Petr., Sydowia 24): 276 (1971) [1970]

Coutinia J.V. Almeida & Sousa da Câmara, Revta agron., Lisb. 1: 392 (1903)

Creomelanops Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 129: 146 (1920)

Cryptosphaeria Ces. & De Not., Comm. Soc. Crittog. Ital. 1(4): 231 (1863)

Cryptosporina Höhn., Öst. Bot. Z. 55: 54 (1905)

Desmotascus F. Stevens, Bot. Gaz. 68: 476 (1919)

Epiphyma Theiss., Verh. Zool.-bot. Ges. Wien 66: 306 (1916)

Fusicoccum Corda, in Sturm, Deutschl. Fl., 3 Abt. (Pilze Deutschl.) 2: 111 (1829)

Polythecium Bonord., Bot. Ztg. 19: 203 (1861)

Pyreniella Theiss., Verh. Zool.-bot. Ges. Wien 66: 371 (1916)

Rostrosphaeria Tehon & E.Y. Daniels, Mycologia 19: 112 (1927)

Thuemenia Rehm, in Thümen, Mycoth. Univ., cent.: no. 971 (in sched.) (1878)

Hemibiotrophic or *saprobic* on leaves and wood. *Ascostromata* 300–500 mm diam., often erumpent through the bark, comprising a botryose aggregate, sometimes solitary, globose, brown to black, individual locules, with a central ostiole, papillate or not, cells of ascostromata having dark brown walls and arranged in a *textura angularis*. *Peridium* of locules two-layered, outer layer composed of small heavily pigmented thick-walled cells of *textura angularis*, inner layer composed of hyaline thin-walled cells of *textura angularis*. *Pseudoparaphyses* hyphae-like, wide, septate. *Asci* 8–spored, bitunicate, fissitunicate, clavate, short pedicellate, apically rounded with a small ocular chamber. *Ascospores* biseriate, hyaline, aseptate, fusoid to ovoid, often with tapered ends, smooth-walled, with granular contents, with or without a mucilaginous sheath. *Conidiomata* pycnidial in nature. *Conidiogenous cells* holoblastic, hyaline, subcylindrical, proliferating percurrently with 1–2 proliferations and periclinical thickening. *Conidia* hyaline, aseptate, narrowly fusiform, or irregularly fusiform, base subtruncate to bluntly rounded, rarely forming a septum before germination, smooth with granular contents (asexual morph description follows Slippers et al. 2004b).

Notes: As the type of *Botryosphaeriaceae*, *Botryosphaeria* was introduced with type species *B. dothidea* by Cesati and De Notaris (1863). In the original description, Mougeot (in Fries 1823, as *Sphaeria dothidea*), did not designate any type specimen but the collection from fallen branches of *Fraxinus* sp was listed in the reference. However, the only material under this name available in the Fries herbarium was described from *Rosa* sp. As no type material existed, Slippers et al. (2004b) designated a neotype for the remaining *S. dothidea* sample from Fries collection. The material, however, was immature as noted by von Arx and Müller (1954), and thus does not bear characteristics that would make it possible to clearly define the name. In order to stabilize the name, Slippers et al. (2004b) epitypified the type species *Botryosphaeria dothidea* based on morphology and phylogeny (combined multi-gene, ITS, EF1-α and β-tubulin). Numerous species have been described in the genus *Botryosphaeria*, but later transferred to other genera (Crous et al. 2004, 2006; Phillips and Pennycook 2004; Phillips et al. 2005, 2008; Phillips and Alves 2009). Crous et al. (2006) restricted the use of *Botryosphaeria* to *B. dothidea* and *B. corticis*. In our phylogenetic trees, two additional species, namely *B. agaves* (which we have epitypified) and *B. fusispora* sp. nov. clustered in this clade. The asexual morphs of *Botryosphaeria* were reported as *Dichomera*, *Diplodia*, and *Fusicoccum* (Crous and Palm 1999; Slippers et al. 2004b; Crous et al. 2006).

Generic type: *Botryosphaeria dothidea* (Moug. : Fr.) Ces. & De Not.

Botryosphaeria dothidea (Moug. : Fr.) Ces. & De Not., Comment Soc. crittog. Ital. 1:212 (1863).

MycoBank: MB183247 (Fig. 12)

≡ *Sphaeria dothidea* Moug., in Fries, Syst. Mycol. 2: 423 (1823)

= *Botryosphaeria berengeriana* De Not., Sfer. Ital. 82 (1863) [1864]

= *Fusicoccum aesculi* Corda in Sturm, Deutschl. Fl., Abth. 3, 2:111 (1829)

Hemibiotrophic or *saprobic* on leaves and wood. *Ascostromata* erumpent through the bark, 300–500 mm diam., comprising a botryose aggregate, sometimes solitary, globose, brown to black, individual locules, with a central ostiole, papillate or not. *Peridium* of locules two-layered, outer layer composed of dark brown or brown thick-walled cells of *textura angularis*, inner layer composed of hyaline thin-walled cells of *textura angularis* lining the locule. *Pseudoparaphyses* 2–4 µm wide, hyphae-like, septate. *Asci* 63–125×16–20 µm, 8–spored, bitunicate, fissitunicate, clavate, short pedicellate, apically rounded with a small ocular chamber. *Ascospores* 20–25×7–9 µm, biseriate, hyaline, aseptate, fusoid to ovoid, sometimes with tapered ends giving a spindle shaped appearance, smooth with granular contents. *Conidiomata* pycnidial in nature. *Conidiogenous cells* 6–20×2–5 µm, holoblastic, hyaline, subcylindrical, proliferating percurrently with 1–2 proliferations and periclinical thickening. *Conidia* (17-)18–20(−22)×4–5 µm ($\bar{x} = 19.5 \times 4.8\,\mu m, n = 35$), hyaline, aseptate, narrowly fusiform, or irregularly fusiform, base subtruncate to bluntly rounded, rarely forming a septum before germination, smooth with granular contents (asexual morph description follows Slippers et al. 2004b).

Material examined: SWITZERLAND, Ticino, Crocifisso, *Prunus* sp., October 2000, B. Slippers (PREM57372, epitype)

Botryosphaeria agaves (Henn.) E.J. Butler, Ann. Mycol. 9: 415 (1911).

MycoBank: MB119799 (Fig. 13)

≡ *Physalospora agaves* Henn., Bot. Jb. 34: 51 (1905)

Hemibiotrophic or *saprobic* on leaves. *Ascostromata* 140–260 µm high (excluding the papilla), 600–880 µm diam, circular, blackened areas on host tissue, immersed to erumpent on host tissue, visible as minute black dots or papilla on host tissue, uni to multi loculate, gregarious, individually globose to subglobose. *Ostiole* circular, central, papillate. *Locules* 120–200 µm high, 140–250 µm diam. *Peridium* of locules up to 19–50 µm wide, comprising several layers of brown to dark brown walled cells of *textura angularis*, broader at the base. *Pseudoparaphyses* 3–5 µm wide, hyphae-like, aseptate, numerous. *Asci* 90.5–122×27–38 µm ($\bar{x} = 105.5 \times 31\,\mu m, n = 20$), 8–spored, bitunicate, fissitunicate, clavate to cylindro-clavate, short pedicellate, apically rounded with an ocular chamber (7–9 µm wide, n=10). *Ascospores* 21–43× 8–12 µm ($\bar{x} = 28 \times 10\,\mu m, n = 30$), 2(−3) –seriate at the ascus apex, 1–seriate at the base, hyaline, aseptate, ellipsoidal, fusiform, or inequilateral, usually wider in the middle, wall rough, surrounded by a mucilaginous sheath. *Asexual state* not established.

Culture characteristics: Colonies on MEA, 50–60 mm diam after 10 d at 25–30 °C, fast growing; circular, white to white-black in the centre, whitened in the margin; reverse grey to black in the centre, white in the margin; flattened, fluffy, fairly dense, aerial, smooth surface with crenate edge, filamentous, pigments and asexual morphs not formed in culture.

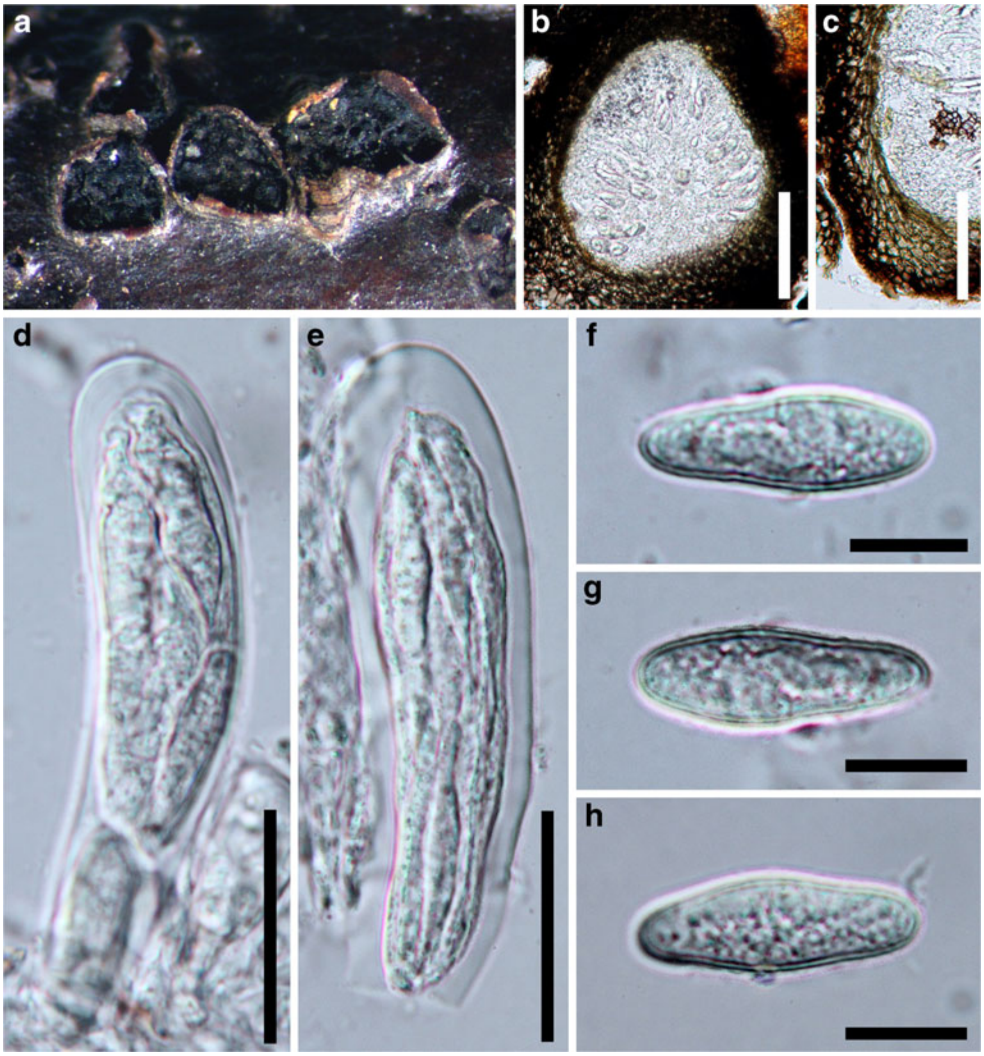

Fig. 12 *Botryosphaeria dothidea* (PREM57372, epitype) **a** Ascostromata on host substrate **b** Section through ascostromata. **c** Peridium. **d–e** Asci. **f–h** Ascospores. Scale Bars: **b–c** = 100 μm, **d–e** = 30 μm, **f–h** = 10 μm

Material examined: THAILAND, Chiang Rai Province, Mae Fah Luang District, Doi Tung, on living leaves and dead leaves of *Agave* sp., 16 June 2010, R. Phookamsak, RP0041, (MFLU 11–0161, epitype designated here), ex-epitype living culture MFLUCC 11–0125; Chiang Mai Province, Doi Nang Khaw., on living leaf of *Agave* sp., 16 June 2009, Putarak Chomnunti, DPC012 (MFLU 09–0648), living culture MFLUCC 10–0051.

Notes: This taxon was isolated from a living leaf of *Agaves* sp. and is identical to *Botryosphaeria agaves*. Therefore, we epitypify the species *B. agaves* with our collection which has living material and sequence data. In addition, this taxon has been shown to be a typical *Botryosphaeria* species (Crous et al. 2006) based on the phylogeny analyses in this study (Fig. 1).

Botryosphaeria fusispora Boonmee, J.K. Liu & K.D. Hyde, sp. nov.

MycoBank: MB 801319 (Figs. 14 and 15)

Etymology: Referring to the fusiform shape of ascospores.

Hemibiotrophic or *saprobic* on leaves and wood. *Ascostromata* 137.5–210 μm high × 160–230 μm diam, dark-brown to black, immersed under epidermis in host tissue, becoming erumpent, clustered, gregarious, or scattered, coriaceous, subglobose, with indistinct ostiole. *Peridium* up to 22.5–37.5 μm thick, comprising 3–4 (−5) layers of dark brown cells of *textura angularis*. *Pseudoparaphyses* 2.5–5 μm wide, hyphae-like, aseptate, dense, embedded in a gelatinous matrix. *Asci* 77.5–112.5 × 20–25 μm ($\bar{x} = 99.5 \times 22\,\mu m$), 8–spored, bitunicate, fissitunicate, broadly cylindrical, ellipsoidal, short-pedicellate, apically rounded with an ocular chamber, up to 1 μm wide at the thickened gelatinous apex. *Ascospores* 20–27.5 × 10–12.5 μm ($\bar{x} = 24.6 \times 11.5\,\mu m$), biseriate, partially overlapping, hyaline, aseptate, ellipsoidal to fusiform, smooth-walled. *Conidiomata* 140–180 × 160–210 μm. ($\bar{x} = 165 \times 180\,\mu m$), pycnidia, solitary, semi-immersed to immersed, dark brown to black walls, composed of thick–walled dark brown cells of *textura angularis*, becoming hyaline towards the inner region. *Conidiophores* 2–4.5 μm wide ($\bar{x} = 3\,\mu m$), hyaline, septate, cylindrical, smooth.

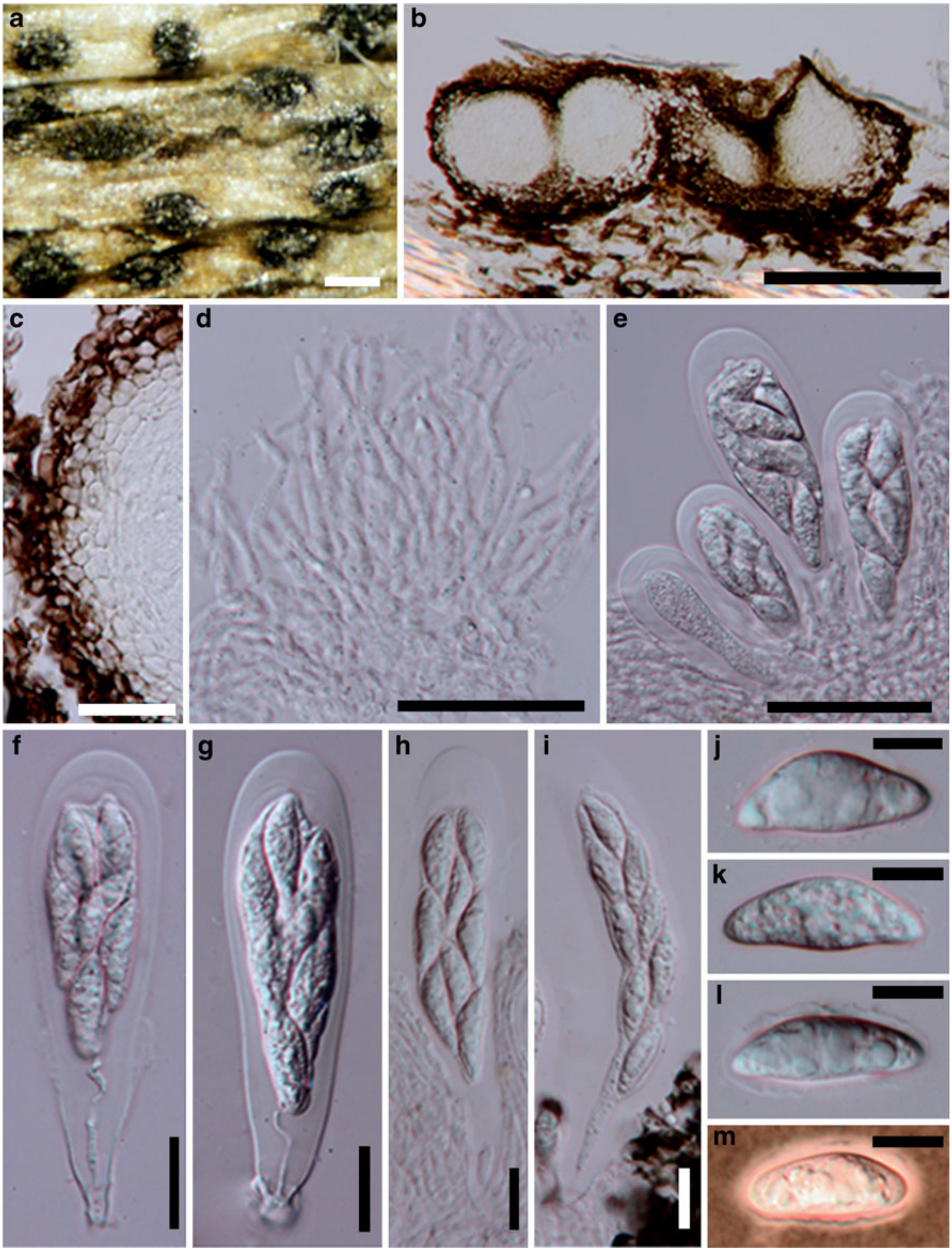

Fig. 13 *Botryosphaeria agaves* (MFLU 11–0161, epitype) on living and dead leaves of *Agave* sp. **a** Ascostromata on host substrate. **b** Section through multiloculate ascostroma. **c** Section through peridium. **d** Pseudoparaphyses **e** Asci with pseudoparaphyses. **f**–**i** Asci. **j**–**l** Ascospores. **m** Ascospore with India ink showing sheath. Scale bars: **a** = 500 μm, **b** = 200 μm, **c**–**e** = 50 μm, **f**–**i** = 20 μm, **j**–**m** = 10 μm

Conidiogenous cells holoblastic, hyaline, cylindrical, integrated, proliferating, producing a single apical conidium. *Conidia* 16–22×4–5.5 μm wide $\left(\overline{x} = 20 \times 5\,\mu m, n = 20\right)$, hyaline, aseptate, fusiform to ellipsoidal, sometimes irregular ellipsoidal, smooth, apex obtuse, base subtruncate or bluntly round, granular.

Culture characteristics: Ascospores germinating from one or both ends. Colonies on MEA growing rapidly,

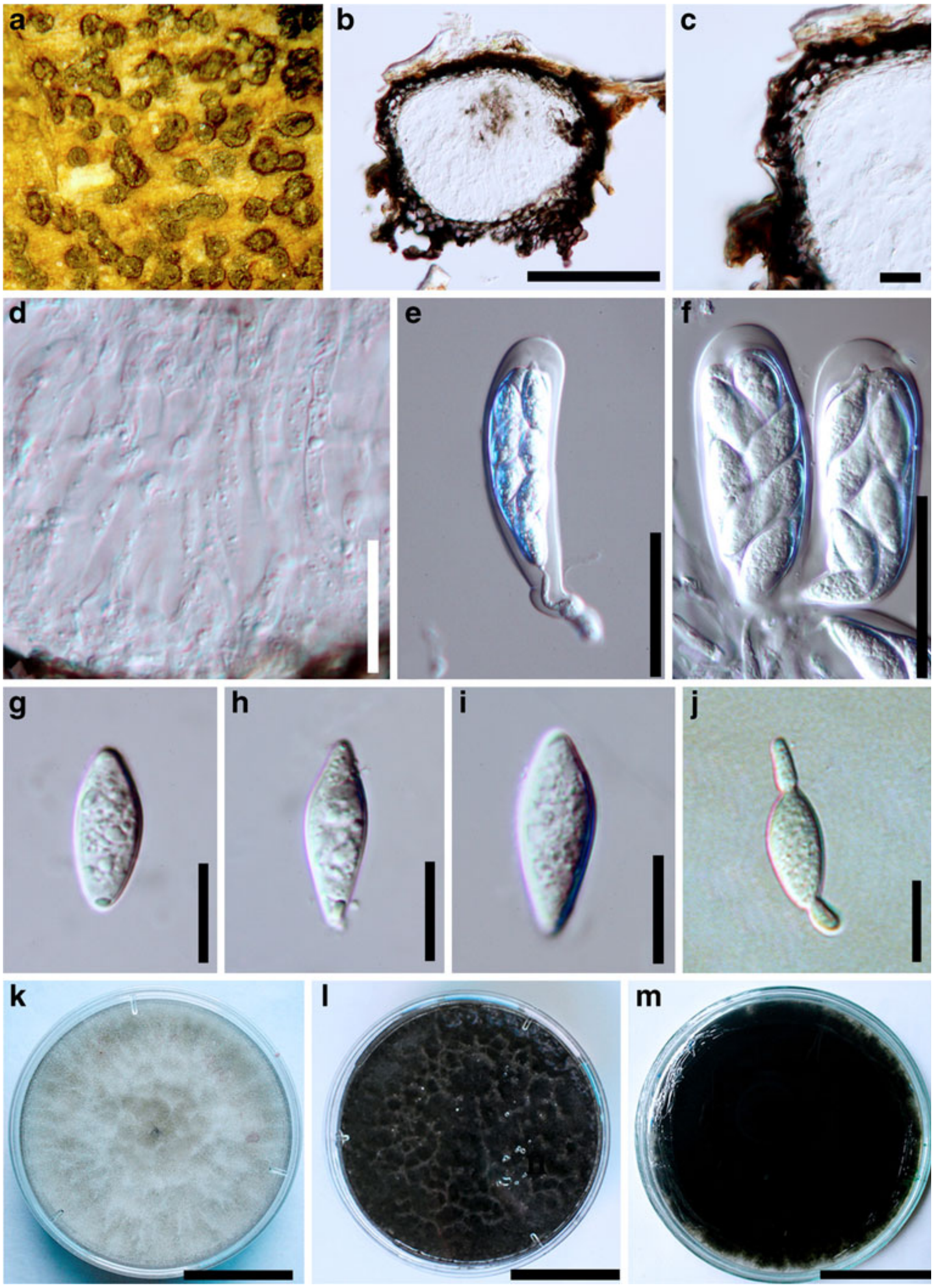

Fig. 14 *Botryosphaeria fusispora* (MFLU 10–0028, holotype). **a** Ascostromata on host substrate. **b** Section through ascostromata. **c** Peridium. **d** Pseudoparaphyses. **e**–**f** Asci with 8-spores and short stalk. **g**–**i** Ascospores. **j** Germinating ascospore. **k**–**m** Colonies on MEA. Scale bars: **b** = 100 μm, **c** = 20 μm, **d**–**f** = 40 μm, **g**–**j** = 10 μm, **k**–**m** = 2 cm

reaching 9 cm diam in a week, at room temperature. Aerial mycelium at first white and later becoming dark-grey to black, and no sporulating structures were produced in cultures within 3 months.

Material examined: THAILAND, Chiang Rai, Doi Tung, on dried bark of *Entada* sp., 10 June 2009, Saranyaphat Boonmee (MFLU 10–0028, holotype), ex-type culture MFLUCC 10–0098; Chiang Mai, Chiang Mai University, on dead leaves of *Caryota* sp., 15 April 2010, Ratchadawan Cheewangkoon, JKC009, living culture MFLUCC 11–0507.

Notes: *Botryosphaeria fusispora* was found on dried bark of *Entada* sp. It is characterised by clusters or gregarious ascostromata, scattered, dark-brown to black, immersed under epidermis and erumpent at maturity on the bark of the host substrate. The ascospores are aseptate, ellipsoid to fusiform, hyaline and smooth and lacking sheaths. The asexual stage was also founded on the palms and is "Fusicoccum"-like. This species phylogenetically belongs to *Botryosphaeria sensu stricto* (Crous et al. 2006). *Botryosphaeria fusispora* is introduced here based on morphology and phylogeny. The combined gene sets (LSU,

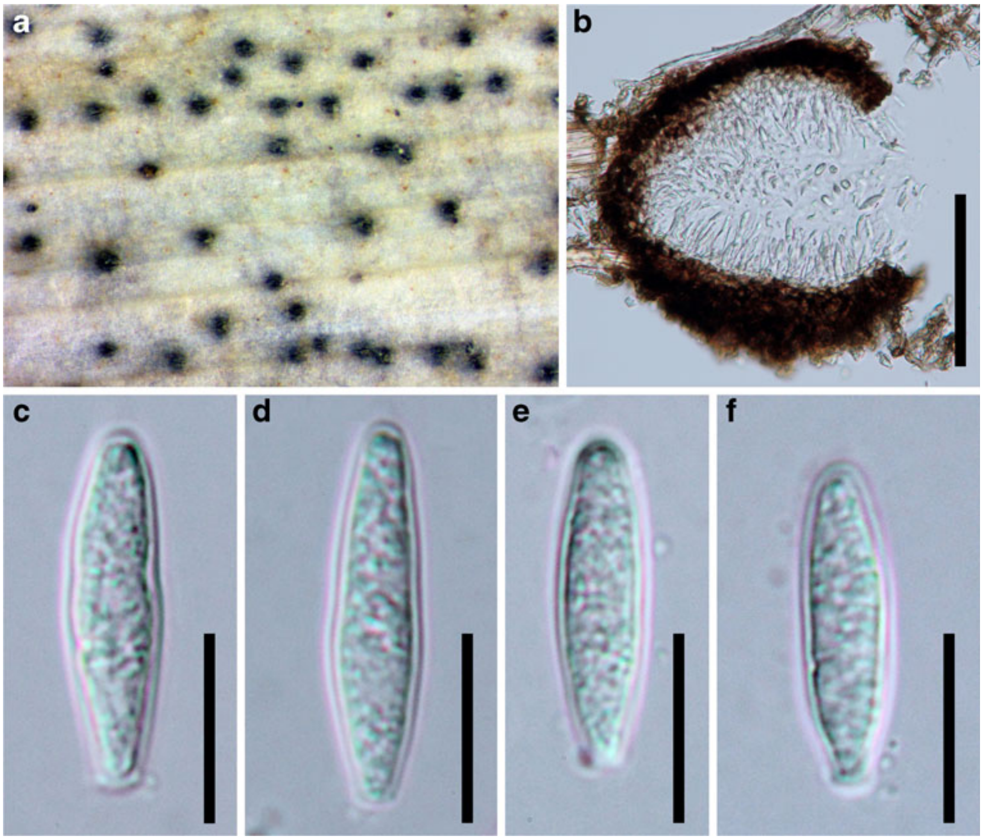

Fig. 15 Asexual morph of *Botryosphaeria fusispora*. **a** Conidiomata on dead leaves of *Caryota* sp. **b** Section through conidioma. **c**–**f** Conidia. Scale bars: **b** = 100 μm, **c**–**f** = 10 μm

SSU, EF1-α and β-tubulin and EF1-α and β-tubulin) indicate this species is a typical *Botryosphaeria* with strong bootstrap support values (Fig. 1).

Cophinforma Doilom, J.K. Liu & K.D. Hyde, gen. nov.
MycoBank: MB 801315

Etymology: From the Latin *cophinus,* referring to the ascospore coffin-like shape.

Saprobic on recently fallen wood. *Ascostromata* initially immersed under host epidermis, becoming semi-immersed to erumpent, breaking through cracks in bark, gregarious and fused, uniloculate, globose to subglobose, membraneous, visible white contents distinct when cut, ostiolate. *Ostiole* central, papillate, pale brown, relatively broad, periphysate. *Peridium* broader at the base, comprising several layers of relatively think-walled, dark brown to black-walled cells, arranged in a *textura angularis*. *Pseudoparaphyses* hyphae-like, numerous, embedded in a gelatinous matrix. *Asci* 8–spored, bitunicate, fissitunicate, clavate to cylindro-clavate, pedicellate, apex rounded with an ocular chamber. *Ascospores* overlapping, uniseriate to biseriate, hyaline, aseptate, ellipsoidal to obovoid, slightly wide above the centre, smooth-walled. *Asexual state* not established.

Notes: We introduce this new genus based mainly on the molecular phylogeny, which shows that this is a distinct genus. The most similar genus is *Botryosphaeria*. *Cophinforma* has morphologically unique ascospores which are hyaline and aseptate.

Generic type: *Cophinforma eucalypti* Doilom, J.K. Liu & K.D. Hyde.

Cophinforma eucalypti Doilom, J.K. Liu & K.D. Hyde., sp. nov.

MycoBank: MB 801316 (Fig. 16)

Etymology: Referring to the host "*Eucalyptus* sp.," on which the fungus was collected.

Saprobic on recently fallen wood. *Ascostromata* (88-)112–125(−130) μm high×(135-)172–185(−195) μm wide ($\bar{x} = 112 \times 165\,\mu m, n = 10$), initially immersed under host epidermis, becoming semi-immersed to erumpent, breaking through cracks in bark, gregarious and fused, uniloculate, globose to subglobose, membraneous, visible white contents distinct when cut, ostiolate. *Ostiole* (33-)43–52 μm high, (31-)40–48 μm wide, central, papillate, pale brown, relatively broad, periphysate. *Peridium* (13-) 28–34 μm wide, broader at the base, comprising several layers of relatively think-walled, dark brown to black-walled cells arranged in a *textura angularis*. *Pseudoparaphyses* hyphae-like, numerous, embedded in a gelatinous matrix. *Asci* 74–90(−123)×17–23 μm ($\bar{x} = 89 \times 20\,\mu m, n = 10$), 8-spored, bitunicate, fissitunicate, clavate to cylindro-clavate, sometimes short pedicellate, mostly long pedicellate, apex rounded with an ocular chamber. *Ascospores* 21–26×8–

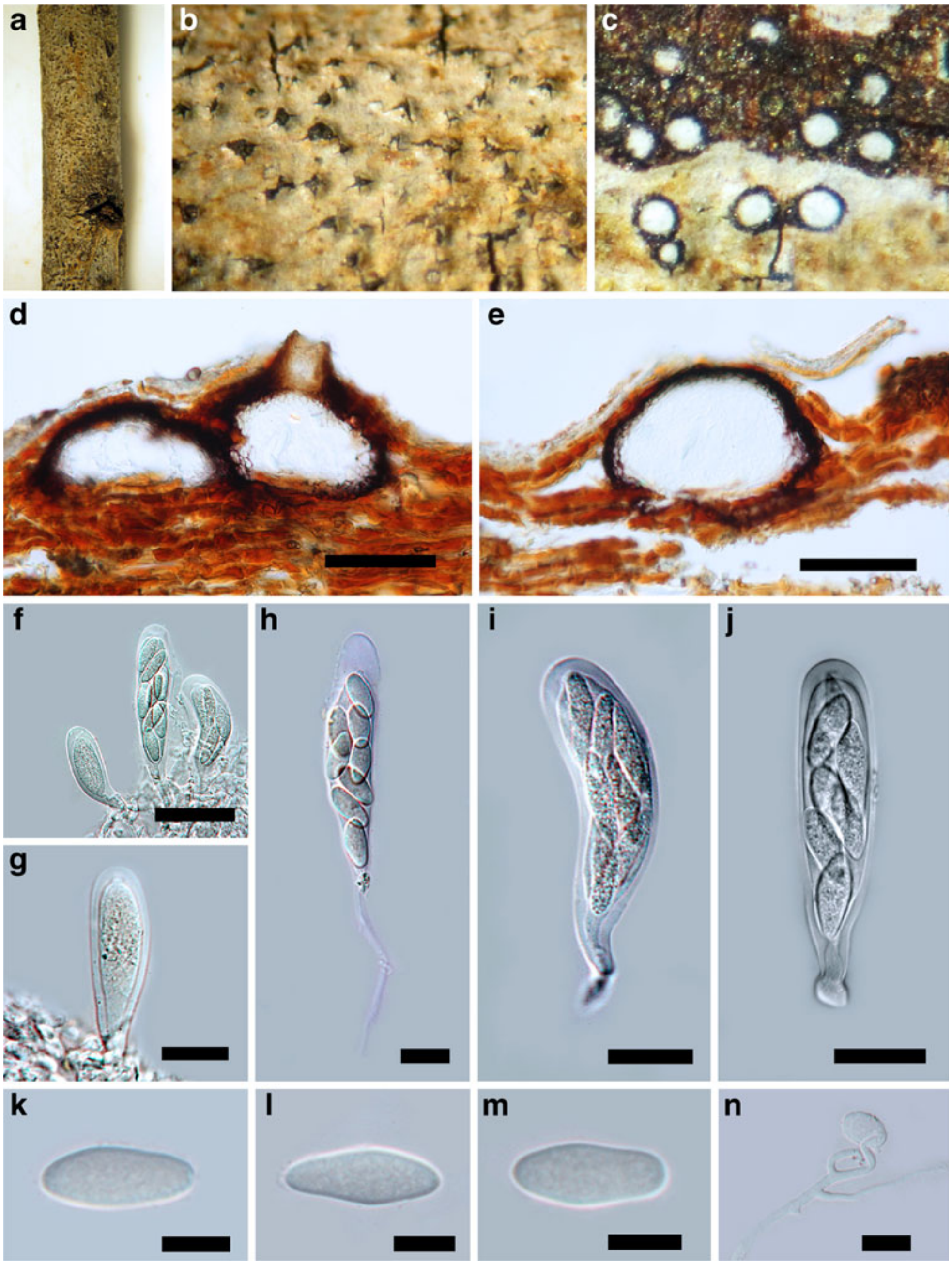

Fig. 16 *Cophinforma eucalypti (*MFLU 12–0752, holotype*)* **a-b.** Ascostromata on dead twigs of *Eucalyptus* sp. **c.** Ascostromata cut horizontally showing the white contents. **d–e.** Vertical section through ascostromata. **f.** Immature asci and mature asci. **g.** Immature ascus. **h–j.** Asci. **k–m.** Ascospores. **n**. Germinating ascospore. Scale bars: **d–e** = 100 μm, **f** = 50 μm, **g–j**, **n** = 20 μm, **k–m** = 10 μm

11 μm ($\bar{x} = 23.5 \times 9\,\mu m, n = 20$), overlapping uniseriate to biseriate, hyaline, aseptate, ellipsoidal to obovoid, slightly wide above the centre, minutely guttulate, smooth-walled. *Asexual state* not established.

Culture characteristics: Ascospores germinating on PDA within 8–15 h. Germ tubes produced from both ends of the ascospore. Colonies growing on PDA 80 mm diam after 3 d at 25–30 °C, fast growing; fimbriate, flat or effuse, dense, initially white after a few days becoming pale grey starting form the centre, finally dark grey to black, convex with papillate surface, reaching the edge the Petri dish after 4 d.

Material examined: THAILAND, Chiang Rai Province, Muang District, Thasud Sub District, on dead branch of *Eucalyptus* sp., 5 October 2011, M. Doilom, (MFLU 12–0752, holotype), ex-type living culture MFLUCC 11–0425; *Ibid.*, living culture MFLUCC 11–0655.

Lasiodiplodia Ellis & Everh., Bot. Gaz. 21: 92 (1896)
MycoBank: MB8708

Saprobic on recently dead wood, often attached to tree, and *endophytic. Sexual morph* link not equivicoally proven. *Conidiomata* dark brown to black, uniloculate, immersed in the host becoming erumpent when mature. *Paraphyses* hyaline, septate, cylindrical, ends rounded. *Conidiogenous cells* hyaline, cylindrical, holoblastic. *Conidia* initially hyaline and aseptate, subovoid to ellipsoid-ovoid, with granular content, apex broadly rounded, remaining hyaline for a long time, becoming dark brown, 1–septate, thick-walled, base truncate or round, with longitudinal striations from apex to base.

Notes: *Lasiodiplodia* was introduced by Clendenin (1896) with *L. tubericola* Ellis & Everh. as the type species; the current name is *L. theobromae*. Alves et al. (2008) provide a recent description of the species which is widespread on many hosts. With the large number of hosts and its known morphological variability (Punithalingam 1980), it is possible that *L. theobromae* comprises a number of cryptic species (Alves et al. 2008). The type strain has questionably been linked to *Botryodiplodia rhodina* (Cooke) Arx, but this link has not been equivicoally proven (Alves et al. 2008; Phillips et al. 2008). Further work is required to establish the characters of the sexual morphs of *Lasiodiplodia sensu stricto* species, especially with the large number of potential cryptic species. Therefore no characters are given in the generic description; however, these are likely to be "Botryosphaeria"-like. The asexual morph can be distinguished by its distinctive striate brown conidia and numerous long paraphyses (Alves et al. 2008). It is hard to distinguish between species in this genus solely by morphology and analysis of EF1-α, β-tubulin and RPB2 gene sequences (Alves et al. 2008; Abdollahzadeh et al. 2010) or Genealogical Sorting Index (GSI), which has been used to resolve the asexual morph of *Neofusicoccum* (Sakalidis et al. 2011), is needed to resolve species.

Generic type: *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl.

Lasiodiplodia theobromae (Pat.) Griffon & Maubl., Bull. Soc. Mycol. Fr. 25: 57 (1909)

MycoBank: MB188476 (Fig. 17)

≡ *Botryodiplodia theobromae* Pat., Bull. Soc.Mycol. Fr. 8: 136 (1892)

= *Diplodia gossypina* Cooke, Grevillea 7: 95(1879)

For other possible synonyms see *Index Fungorum*

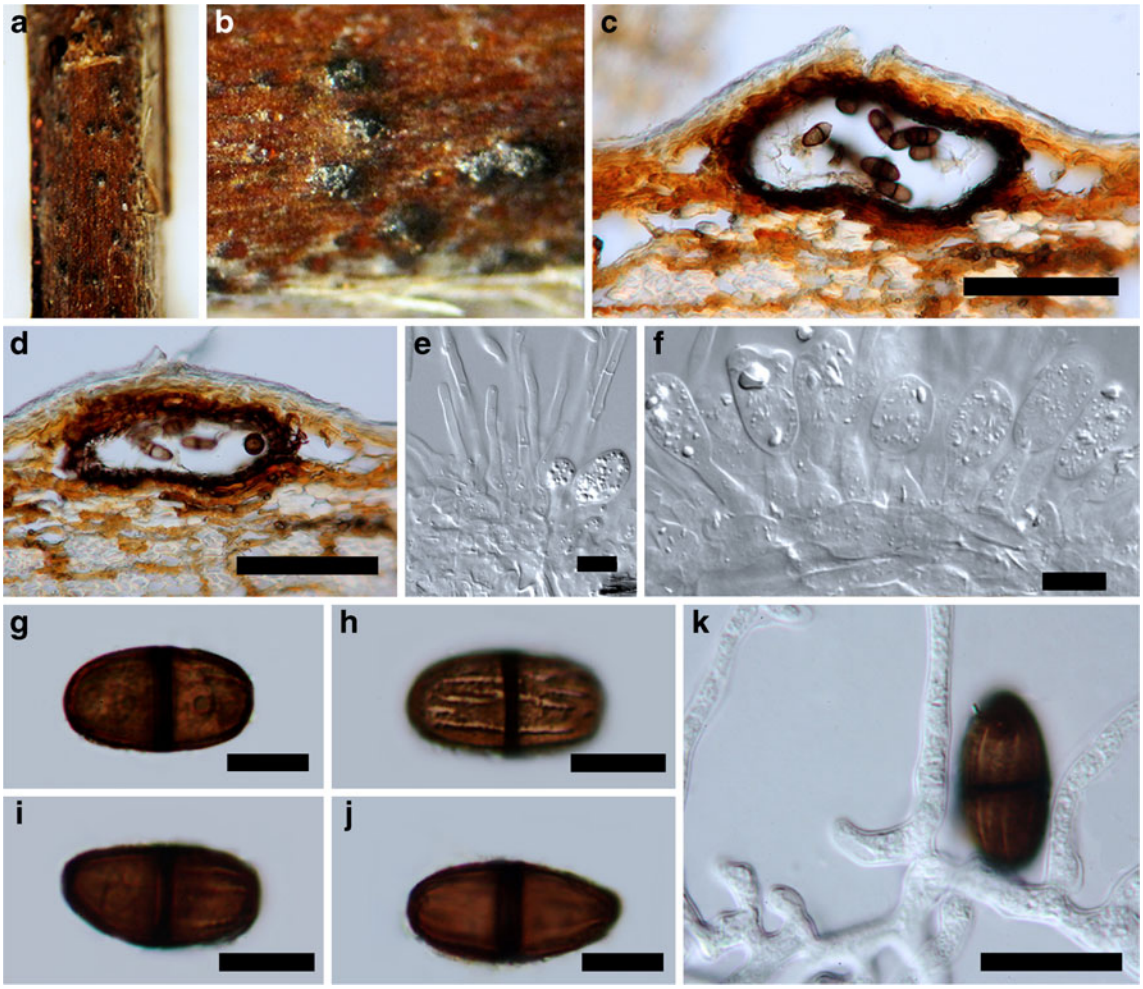

Fig. 17 *Lasiodiplodia theobromae* (MFLU 12–0760) **a-b.** Conidiomata on dead twigs. **c-d.** Section through conidioma. **e** Paraphyses. **f** Conidiogenous cells and young conidia. **g-j.** Mature conidia in two different focal planes to show the longitudinal striations. **k.** Spore germinated. **l-m.** Culture on PDA. Scale bars: **c-d** = 100 μm, **e-j** = 10 μm, **k** = 30 μm

Saprobic on recently dead wood, often attached to tree, and endophytic. *Sexual state* not established. *Conidiomata* dark brown to black, uniloculate, immersed in the host becoming erumpent when mature. *Paraphyses* hyaline, septate, cylindrical, ends rounded, up to 40 μm long, 3–4 μm wide. *Conidiogenous cells* hyaline, cylindrical, holoblastic. *Conidia* (17-)21–27(−31)×12.5–15.5 μm ($\bar{x} = 23 \times 13\,\mu m, n = 15$), initially hyaline and aseptate, subovoid to ellipsoid-ovoid, with granular content, apex broadly rounded, remaining hyaline for a long time, finally becoming dark brown, 1–septate, thick-walled, base truncate or round, with longitudinal striations from apex to base.

Material examined: THAILAND, Chiang Rai Province., Muang District, Thasud Sub District, on dead twig of *Eucalyptus* sp., 8 August 2011, M. Doilom (MFLU 12–0760), living culture MFLUCC 11–0508.

Leptoguignardia E. Müll., Sydowia 9: 216 (1955)

MycoBank: MB2777

Hemibiotrophic or *saprobic* on petioles. *Ascostromata* black, scattered, clustered or fusing in groups of 2–3, initially immersed, becoming erumpent but still under host tissue, ovoid to globose, coriaceous. *Papilla* central, ostiole with a pore. *Pseudoparaphyses* sparse, hyphae-like, not commonly observed in herbarium material. *Peridium* comprising small heavily pigmented thick-walled cells of *textura angularis, Asci* 8–spored, bitunicate, fissitunicate, with a short blunt pedicel, ocular chamber not clear. *Ascospores* hyaline, 2–septate, fusiform, asymmetrical, central cells widest, ends cells longer and tapering, smooth-walled. Asexual "Dothichiza"-like morph forming on same tissue. *Pycnidia* black, scattered, or fusing in groups or with locules, immersed, becoming erumpent, but still under host tissue, ovoid, coriaceous, scattered amongst locules. *Conidiogenous cells* hyaline, cylindrical, holoblastic. *Conidia* hyaline, 1–septate, septum nearer to apex, slightly constricted, ovoid with round ends.

Notes: *Leptoguignardia* was introduced by Müller (1955) and is monotypic represented by the generic type *Leptoguignardia onobrychidis* E. Müll. The taxon occurs on dead petioles of *Onobrychidis montanae* in France. There is no sequence data available for this species, but based on its ascomata and ascial characters, it fits well into *Botryosphaeriaceae*, although new collections are required to confirm this.

Generic type: *Leptoguignardia onobrychidis* E. Müll.

Leptoguignardia onobrychidis E. Müll., Sydowia 9: 217 (1955)

MycoBank: MB299536 (Figs. 18 and 19)

Hemibiotrophic or saprobic on petioles. *Ascostromata* 100–110 μm high×170–180 μm diam., black, scattered, clustered or fusing in groups of 2–3, initially immersed, becoming erumpent but still under host tissue, ovoid to globose, coriaceous. *Papilla* central, ostiole with a pore opening, 38–40 μm long. *Peridium* up to 23 μm wide, comprising composed of small heavily pigmented thick-walled cells of *textura angularis*. *Pseudoparaphyses* sparse, hyphae-like, not commonly observed in herbarium material or visible in drawing in protologue. *Asci* 50–70×5–8 μm, 8–spored, bitunicate, fissitunicate, with a short blunt pedicel, ocular chamber not clear. *Ascospores* 30–33×7–8 μm, overlapping 1–2–seriate in base and 2–3 seriate at apex, hyaline, fusiform, asymmetrical, two-septate, central cells widest, ends cells longer and tapering, one end longer than other, but not related to position in ascus, constricted at the septum, smooth-walled and lacking a sheath. Asexual "Dothichiza"-like morph forming on same tissue. *Pycnidia* 116–150(−200) μm diam., 145–150 μm high, scattered, or fusing in groups or with ascomata, immersed, becoming erumpent, but still under host tissue, ovoid, black, coriaceous, scattered amongst ascomata. *Conidiogenous cells* hyaline, cylindrical, holoblastic. *Conidia* 11–16×2.7–4 μm ($\bar{x} = 13 \times 3.5\,\mu m$), 1–sepate, septum nearer to apex, slightly constricted, hyaline, ovoid, and apical cells narrowing to the apex, basal cells widest, thin-walled.

Material examined: FRANCE, Queyras, Abriés, on dead petioles of *Onobrychidis montanae* 12 June 1954, E. Müller & K.H. Richle (ZT, ZT Myc 2232, holotype, Myc 2231, Myc 2225).

Macrovalsaria Petr., Sydowia 15: 298 (1962) [1961]

MycoBank: MB2971

Saprobic on dead twigs, leaf rachis, wood, bamboo and culms of a wide range of hosts. *Ascostromata* dark brown to black, immersed to erumpent, solitary to a few in a group, oblate, sphaeroid to subsphaerical, with a central ostiole. *Peridium* comprising brown and small-celled *textura angularis. Asci* 8–spored, bitunicate, fissitunicate, cylindro-clavate, with a short fine pedicel, apically rounded with a small ocular chamber. *Ascospores* uniseriate to irregularly uniseriate, 1–septate, brown, elliptical-fusoid, slightly constricted at septum, surface smooth to spinulose. *Asexual state* not established.

Notes: *Macrovalsaria* is a monotypic genus with a circumglobal distribution in the tropics. Sivanesan (1975) examined type material of *M. megalospora* (≡ *Sphaeria megalospora* Mont.) and several other species including *M. leonensis* (Deighton) Petr., the generic type, and synonymised them all under *Macrovalsaria megalospora* which is the oldest epithet. The brown, uniseptate ascospores that are constricted at the septum and the skull cap-like germ apparatus at the base are diagnostic features for the genus (Sivanesan 1975, Hyde et al. 2000). Cultures were obtained from material sampled from Hianan Province, China (Li and Zhuang 2009). Phylogenetic analysis based on sequence analyses of 18S rDNA showed the genus to be related to *Botryosphaeriales* (Li and Zhuang 2009). No asexual morph was observed in the collection. The two strains of *M.*

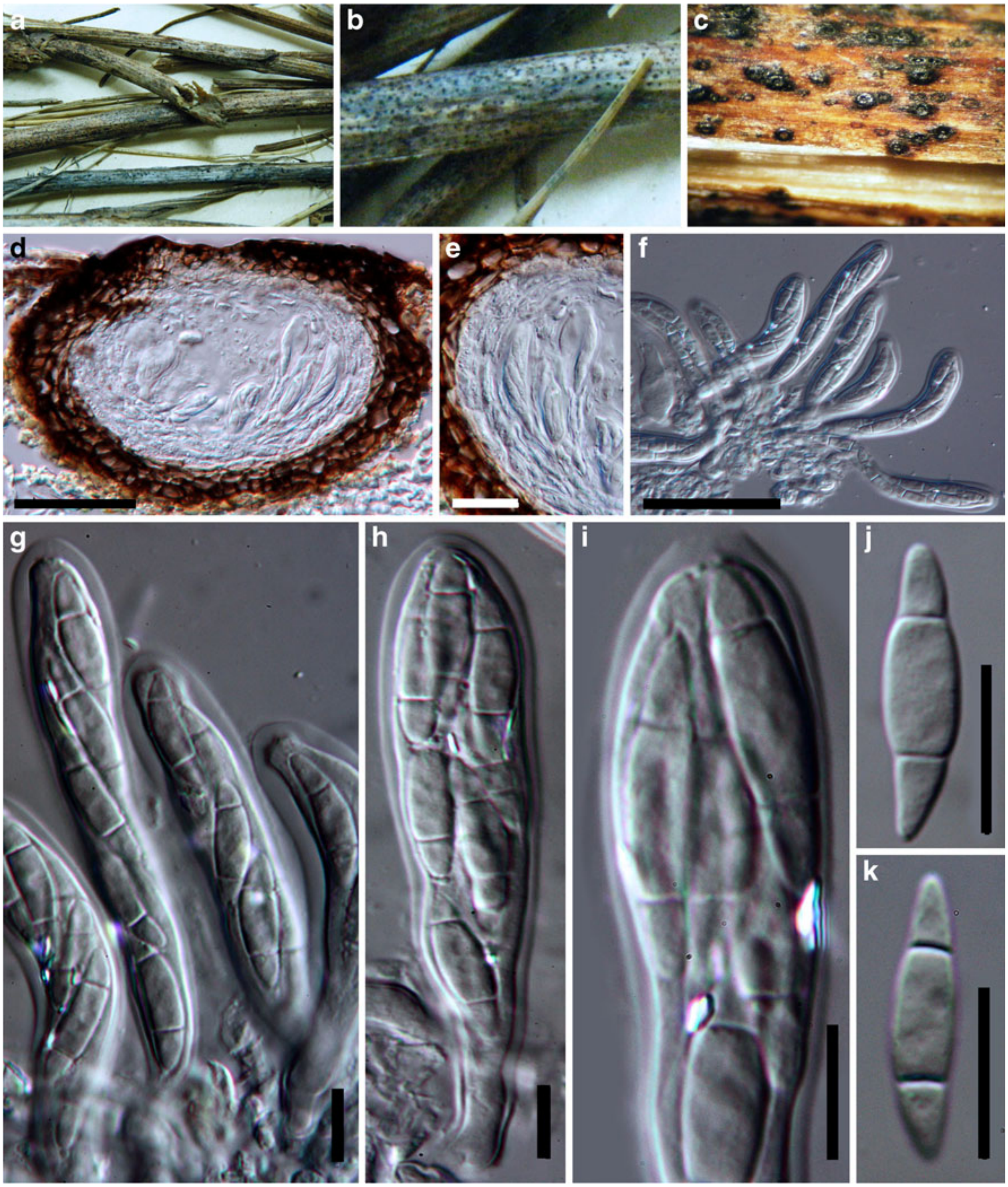

Fig. 18 *Leptoguignardia onobrychidis* (Myc 2232, holotype) **a–c** Habit and appearance of ascostromata on host substrate. **d–e** Section trough ascostromata showing developing of asci. **f–i** Asci. **j–k** Ascospores. Scale bars: **d–f** = 50 µm, **g–k** = 10 µm

megalospora clustered in the *Lasidodiplodia* clade (Fig. 1, Clade A1) and based on our data we might place *Macrovalsaria* in *Botryosphaeriaceae*. However, LSU and SSU sequence data is only available for the two strains of *Macrovalsaria* and thus it cannot be conclusively shown that *Macrovalsaria* is a synonym of *Lasiodiplodia*.

Generic type: *Macrovalsaria leonensis* (Deighton) Petr.

Macrovalsaria megalospora (Mont.) Sivan., Trans. Br. Mycol. Soc. 65: 400 (1975)

MycoBank: MB317110 (Fig. 20)

≡ *Sphaeria megalospora* Mont., Annls Sci. Nat., Bot., sér. 2, 14: 324 (1840)

≡ *Amphisphaeria megalospora* (Mont.) Sacc., Syll. Fung. 1: 724 (1882)

≡ *Melogramma megalospora* (Mont.) Cooke, Grevillea 13(no. 68): 109 (1885)

= *Amphisphaeria bambusina* Sydow, Philipp. Jour. Sci. 8: 247 (1913)

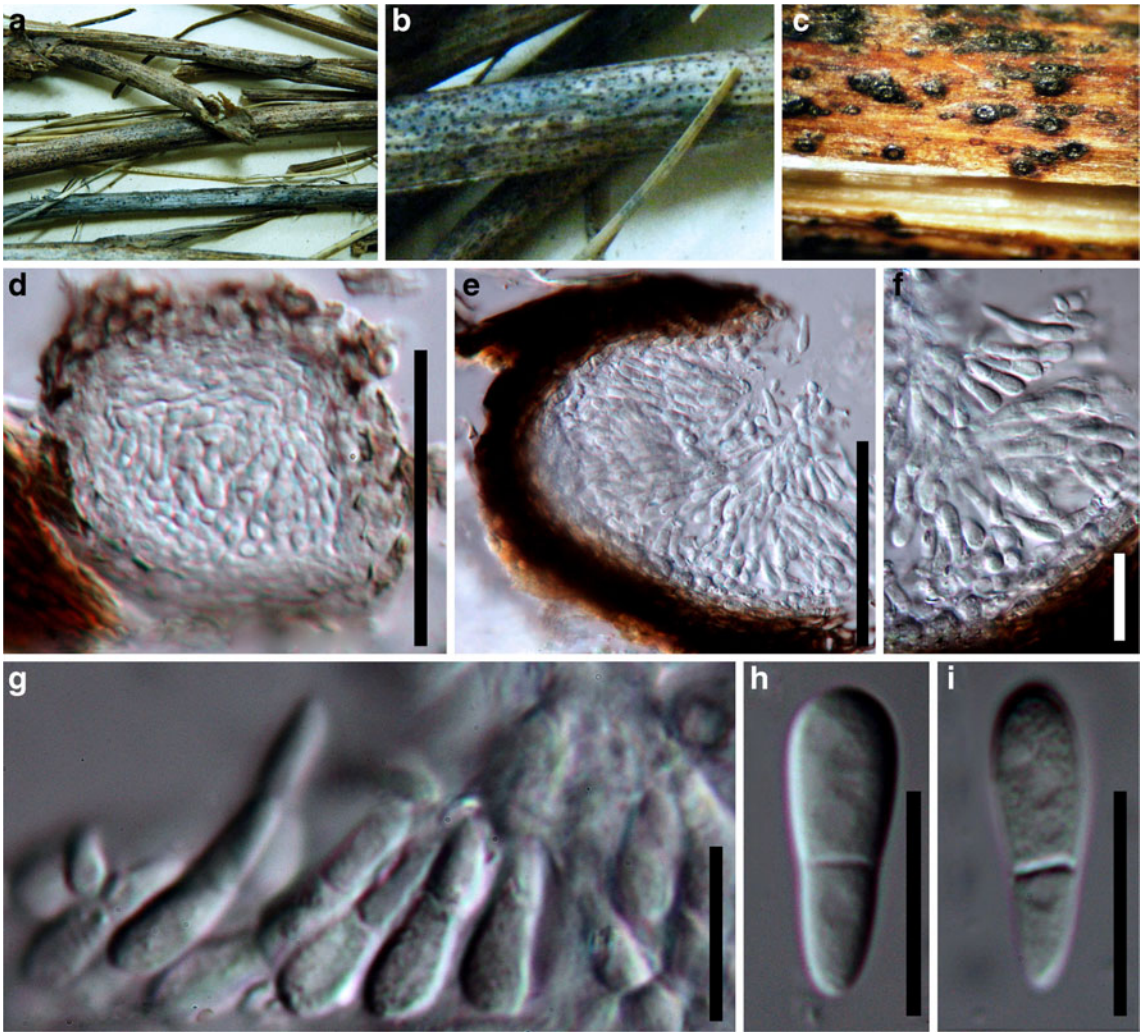

Fig. 19 Asexual morph of *Leptoguignardia onobrychidis* (Myc 2232, holotype) **a–c** Habit and appearance of conidiomata on host substrate. **d–f** Section through pycnidia. **g** Conidiogenous cells. **h–i** Conidia. Scale bars: **d–f** = 50 μm, **g-h** = 10 μm

= *Valsaria leonensis* Deighton, Sydowia 6: 321 (1952)

≡*Macrovalsaria leonensis* (Deighton) Petr., Sydowia 15: 300 (1961)

= *Amphisphaeria lantanae* K. Ramakr., Proc. Ind. Acad. Sci. 42: 249 (1955)

Saprobic on dead twigs, leaf rachis, wood, bamboo and culms of a wide range of hosts. *Ascomata* 706−1064× 538−728 μm $(\bar{x} = 887 \times 600\,\mu m, n = 10)$, on the dead twigs and branches of shrubs, immersed to erumpent, solitary to a few in a group, oblate spheroid to subsphaerical, dark brown to black, with a central ostiole. *Peridium* 41−75 μm thick, consisting of brown and small-celled *textura angularis*, ostiole periphysate. *Asci* 135−206×22−30 μm $(\bar{x} = 171 \times 26.3\,\mu m, n = 20)$, 8–spored, bitunicate, fissitunicate, cylindrical-clavate, with a short fine pedicel at base, apically rounded with a small ocular chamber. *Ascospores* 36.5−45.5×15.7−21 μm $(\bar{x} = 42.2 \times 18.2\,\mu m, n = 25)$, uniseriate, brown, 1–septate, broadly subfusoid, constricted at septum, with skull cap-like germ apparatus at the lower end, surface smooth, granular to verrucose. *Asexual state* not established.

Culture characteristics: On PDA, colonies appeared woolly, fast growing, colonies 90 mm diam. at 25 °C after 3 d, greyish brown to black, reverse becoming dark brown with age, aerial mycelium greyish brown, optimum growth temperature 25–28 °C. *Conidia* not observed.

Material examined: CHINA, Hainan, Sanya, alt. 300 m, on dead twigs, 21 September 2006, W.Y. Li 7441, 7443, 7447, 7511, HMAS 178153, 178152, 178149, 178150; Hainan, Ledong, alt. 1100 m, on dead twigs, 22 September 2006, W.Y. Li 7475, HMAS 178151.

Melanops Nitschke, in Fuckel., Jahrb. Nassauischen Vereins Naturk. 23–24: 225 (‘1869–70’)

MycoBank: MB3078

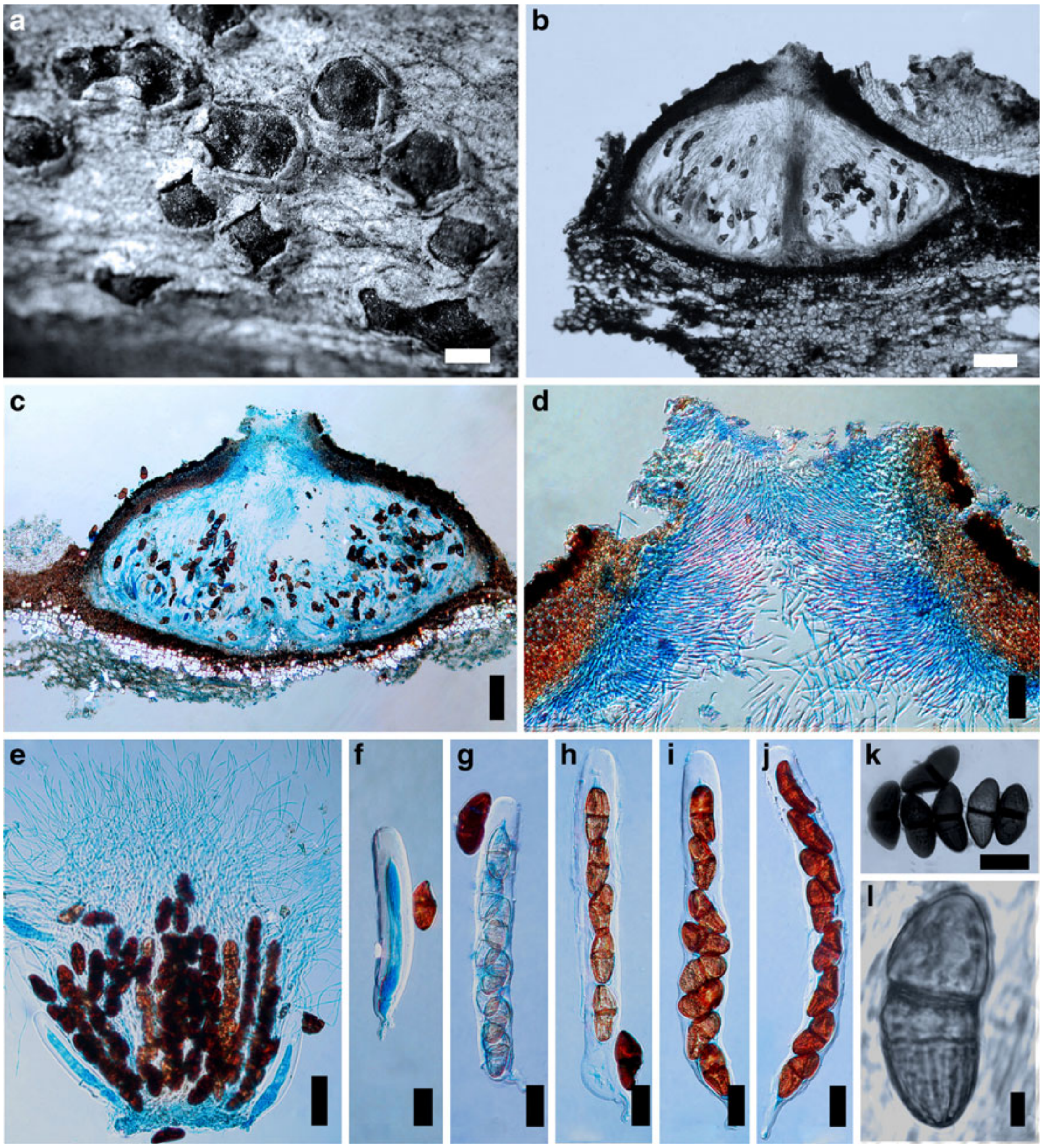

Fig. 20 *Macrovalsaria megalospora* (HMAS 178149): **a** Ascostromata on host substrate. **b, c** Section showing structure of ascostroma. **d** Ostiole with periphyses. **e** Asci associated with pseudoparaphyses. **f–j** Ascus at different stages of development. **k** Ascospores. **l** An ascospore at higher magnification. Note skull cap-like germ apparatus. Scale bars: **a** = 0.5 mm, **b–c** = 100 µm, **d** = 25 µm, **e** = 50 µm, **f–k** = 25 µm, **l** = 5 µm

Saprobic on dead wood. *Ascostromata* black, immersed, erumpent at maturity, multiloculate, thick-walled, wall composed of thick-walled *textura angularis*. *Peridium* of locules two-layered, outer layer composed of small heavily pigmented thick-walled cells of *textura angularis*, inner layer composed of hyaline thin-walled cells of *textura angularis. Pseudoparaphyses* hyphae-like, septate, constricted at the septa. *Asci* 8–spored, bitunicate, fissitunicate, clavate, pedicellate, apically rounded with an ocular chamber. *Ascospores* hyaline, ellipsoid to rhomboid, aseptate, with a persistent mucilaginous sheath. *Conidiomata* often found in the same ascostroma. *Paraphyses* hyphae-like, arising from between the conidiogenous cells. *Conidiogenous cells* cylindrical, hyaline, branched or unbranched, discrete. *Conidia* hyaline, aseptate, fusiform, with sheath.

Notes: *Melanops* Nitschke ex Fuckel was introduced by Fuckel (1870) to accommodate *Melanops tulasnei*, which was described as *Dothidia melanops* by Tulasne (1856) and *M. mirabilis* Fuckel. Later, a new combination *Botryosphaeria melanops* (Tul.) G. Winter was made to accommodate *D. melanops* by Winter (1887). Von Arx and Müller (1954) synonymised *B. melanops* under their broad concept of *B. quercuum*. Phillips and Pennycook (2004) detailed the taxonomy of *M. tulasnei*, the present type species of the genus and accepted this as a member of *Botryosphaeria*, but suggested that the correct name is *B. melanops* with designation of a neotype. Recently, Phillips and Alves (2009) epitypified the type species *Melanops tulasnei* and retained *Melanops* as a separate genus in the *Botryosphaeriaceae* based on morphology and phylogeny. They suggested that the large ascomata and conidiomata that occur within the same stroma and the mucus sheath surrounding the ascospores and conidia are unique in the *Botryosphaeriaceae*.

Generic type: *Melanops tulasnei* Nitschke ex Fuckel

Melanops tulasnei Nitschke ex Fuckel, Jahrb. Nassauischen Vereins Naturk. 23–24: 225 ('1869–70').

MycoBank: MB150956 (Fig. 21)

= *Dothidea melanops* Tul. & C. Tul., Annls Sci. Nat., Bot., sér. 4 5: 116 (1856)

≡ *Botryosphaeria melanops* (Tul. & C. Tul.) G. Winter, Rabenh. Krypt.-Fl. Ed. 2, 1: 800 (1886) [1887]

Saprobic on dead wood. *Ascostromata* black, immersed, erumpent at maturity, multilocular, thick-walled, composed of thick-walled, brown cells of *textura angularis*. *Locules* 150–300 µm diam, globose to subglobose. *Ostioles* central on each locule and circular. *Peridium* of locules two-layered, outer layer composed of small heavily pigmented thick-walled cells of *textura angularis*, inner layer composed of hyaline thin-walled cells of *textura angularis*. *Pseudoparaphyses* hyphae-like, up to 3–4 µm, septate, constricted at the septum. *Asci* 130–245×26–42 µm, 8–spored, bitunicate, fissitunicate, clavate, pedicellate, apically rounded with an ocular chamber. *Ascospores* 35–43×14–17 µm, irregularly biseriate in the ascus, hyaline, aseptate, ellipsoid to rhomboid, smooth, thin-walled, widest in the middle, with a mucilaginous sheath. *Conidiomata* often found in the same ascostroma. *Paraphyses* hyphae-like, branched, arising between the conidiogenous cells. *Conidiogenous cells* hyaline, cylindrical, sometimes branched at the base, discrete. *Conidia* 42–47(−55)×8.5–12.5 µm, hyaline, aseptate, fusiform, widest in the middle, apex acute, base truncate with a minute marginal frill, surrounded by a mucilaginous sheath.

Material examined: GERMANY, Bavaria, Munich, English Garden, on dead twigs of *Quercus robur*, 8 July 2004, A.J.L. Phillips (LISE 95179, epitype).

Neodeightonia C. Booth, in Punithalingam, Mycol. Pap. 119: 17 (1970) [1969]

Saprobic on dead wood and leaves of monocotyledons. *Ascostromata* brown to dark brown, uniloculate, immersed to erumpent, globose to subglobose. *Ostiole* circular, central. *Peridium* of dark brown-walled cells of *textura angularis*. *Pseudoparaphyses* hyphae-like, septate, constricted at the septa. *Asci* 8−spored, bitunicate, fissitunicate, clavate to cylindrical-clavate, apically rounded with an ocular chamber. *Ascospores* uniseriate or irregularly biseriate, hyaline, aseptate, ellipsoidal-fusiform or fusiform, surrounded or not surrounded by a complex sheath. *Pycnidia* uniloculate or multilocular, semi-immersed, solitary, globose, covered by mycelium, wall composed of dark brown thick-walled *textura angularis*, becoming thin-walled and hyaline toward the inner region. *Paraphyses* hyaline, cylindrical. *Conidiogenous cells* holoblastic, hyaline, aseptate, cylindrical to subcylindrical. *Conidia* initially hyaline, aseptate, ellipsoid to obovoid, thick-walled with granular content, rounded at apex, occasionally truncate at base. Aged conidia becoming cinnamon to sepia, and 1−septate, brown to dark brown.

Notes: *Neodeightonia* was introduced by Booth (Punithalingam 1969). However, von Arx and Müller (1975) transferred the type of the genus, *N. subglobosa,* to *Botryosphaeria*, reducing *Neodeightonia* to synonymy. Phillips et al. (2008) reinstated this genus which is distinguishable from *Botryosphaeria* morphologically (based on the dark, 1−septate ascospores) and phylogenetically (Phillips et al. 2008, Abdollahzadeh et al. 2009) and described a new species *N. phoenicum*. Liu et al. (2010) added the fourth species, *N. palmicola* based on studies on morphology of the sexual and asexual morphs and phylogenetic data.

Generic type: *Neodeightonia subglobosa* C. Booth

Neodeightonia subglobosa C. Booth, in Punithalingam, Mycol. Pap. 119: 19 (1970) [1969]

MycoBank: MB318601 (Figs. 22 and 23)

≡ *Botryosphaeria subglobosa* (C. Booth) Arx & E. Müll., Stud. Mycol. 9: 15 (1975)

≡ *Coniothyrium subglobosum* (Cooke) Tassi, Bulletin Labor. Orto Bot. de R. Univ. Siena 5: 25 (1902)

= *Macroplodia subglobosa* (Cooke) Kuntze, Revis. gen. pl. 3: 492 (1898)

≡ *Sphaeropsis subglobosa* Cooke, Grevillea 7(no. 43): 95 (1879)

Saprobic on dead bamboo. *Ascostromata* 140–200 µm high, 210–360 µm diam, dark brown, uniloculate, semi-immersed in host tissue, with protruding papilla or erumpent, developing under raised, dome-shaped regions. *Ostiole* 45–75×50–80 µm, central, papillate. *Peridium* 15–40 µm wide, comprising several layers of dark brown-walled cells of *textura angularis*. *Pseudoparaphyses* up to 3–5 µm wide, hyphae-like, cellular, numerous, embedded in a hyaline gelatinous matrix. *Asci* (70-)81.5–100(−117)×18–22.5(−23) µm ($\bar{x} = 89.2 \times 20.7\,\mu m, n = 20$), 8-spored, bitunicate, fissitunicate, clavate to cylindro-clavate, with a short rounded pedicle, apically rounded with an ocular chamber (2.5–4.5 µm wide,

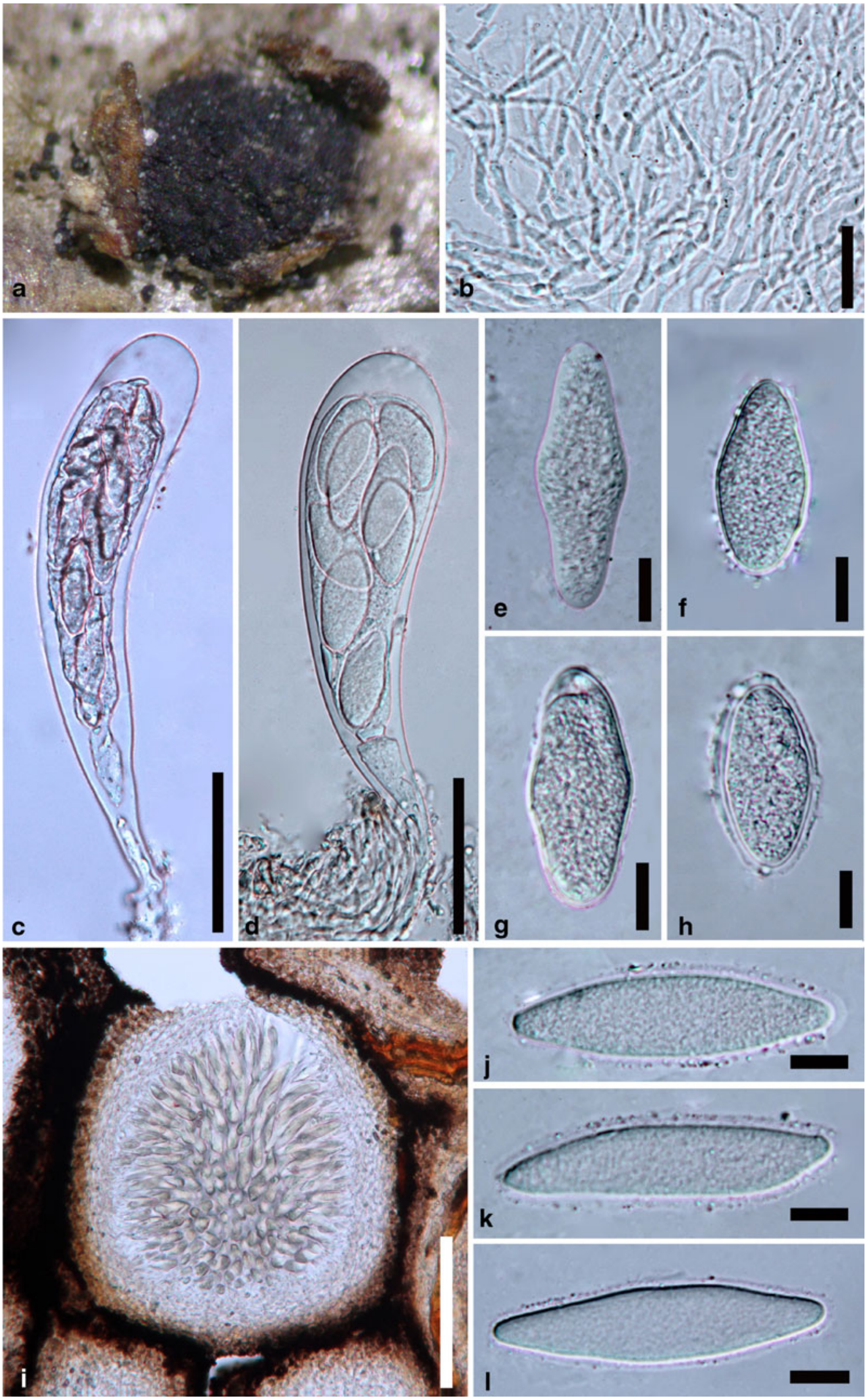

Fig. 21 Sexual (**a**–**h**) and asexual (**i**–**l**) morphs of *Melanops tulasnei* (LISE 95179, epitype) **a**–**c** Ascostromata on host substrate **b** Pseudoparaphyses. **c**–**d** Asci. **e**–**h** Ascospores. **i** Section through conidioma. **j**–**l** Conidia. Scale Bars: **b** = 30 μm, **c**–**d** = 50 μm, **e**–**f** = 10 μm, **i** = 100 μm, **j**–**l** = 10 μm

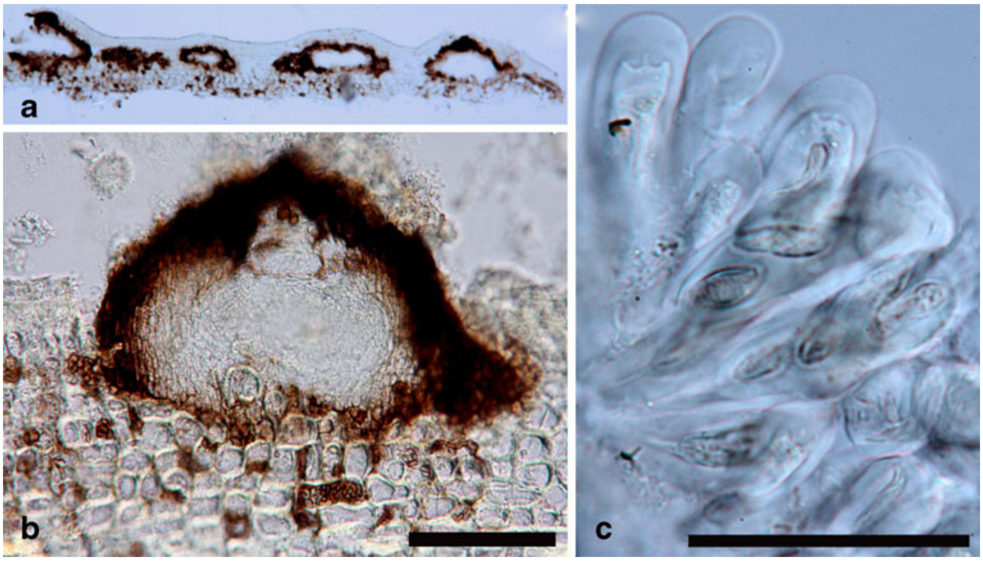

Fig. 22 *Neodeightonia subglobosa* (IMI 57769 c, holotype) **a–b** Section through ascostromata. **c** Developing asci. Scale bars: **b–c** = 50 μm

$n=5$). *Ascospores* (19.5-)21–26(−28)×(6.5-)7.5–9.5(−10) μm ($\bar{x} = 23.4 \times 8.5\,\mu m, n = 30$), uniseriate at the base, biseriate at the apex, hyaline, aseptate, ellipsoidal to fusiform, usually widest in the middle, rough-walled, with bipolar germ pores, surrounded by distinctive structured mucilaginous sheath. *Pycnidia* 150–200 μm diam., brown to black, solitary or aggregated sometimes intermixed amongst ascostromata, unilocular or multilocular, spherical to globose, wall stromatic, composed of several layers of laterally compressed brown cells. *Conidia* (*phialospores*) 9–12×6–9 μm, mature ones light brown to dark brown, spherical to subglobose (asexual morph description follows Punithalingam 1969).

Material examined: SIERRA LEONE, Njala (Kori), on dead culms of *Bambusa arundinacea*, 17 August 1954, F.C. Deighton (IMI 57769 c, holotype); THAILAND, Lampang Province., Jae Hom District, Mae Yuag Forestry Plantation, on dead culms of *Bambusa* sp., 19 August 2010, R. Phookamsak, RP0079 (MFLU 11–0199), living culture MFLUCC 11–0163.

Notes: MFLU 11–0199 is a fresh collection of *Neodeightonia subglobosa* from *Bambusa* sp., and is similar to *N. palmicola*, which also has hyaline, aseptate ascospores surrounded by a wing-like hyaline sheath. However, MFLU 11–0199 differs from *N. palmicola* in having smaller asci and ascospores lacking bipolar germ pores. The original description of *N. subglobosa* reported that the ascospores become 1–septate, and brown to dark brown when mature, and this was not observed in *N. palmicola* and no asexual morph was formed in culture. In Fig. 1 the new isolate clustered together with a strain of *N. subglobosa* (CBS 448.91). This new collection is tentatively named *N. subglobosa*.

Neodeightonia palmicola J.K. Liu, R. Phookamsak and K.D. Hyde. Sydowia. 62: 268 (2010)

MycoBank: MB518804 (Figs. 24 and 25)

Saprobic on dead leaves. *Ascostromata* 180–230 μm high, 270–420 μm diam., uniloculate, immersed to erumpent in host tissue, globose to subglobose, brown to dark brown, rounded at the base. *Ostiole* circular, central. *Peridium* 26–55 μm wide, comprising several layers of brown-walled cells, the outer stratum of 1–3 cells comprising thick, dark brown walls *textura angularis*, the inner layer comprising pale brown to hyaline, thin-walled cells *textura angularis*. *Pseudoparaphyses* up to 3–5 μm wide, hyphae-like, frequently septate, often constricted at the septa. *Asci* (80-)110−210 (−225)×17−22.5(−24) μm, 8−spored, bitunicate, fissitunicate, clavate to cylindro-clavate, pedicellate, apically rounded, with a well developed ocular chamber. *Ascospores* 23–31.5×8.5−12.5 μm ($\bar{x} = 27 \times 10\,\mu m$), obliquely uniseriate or irregularly biseriate, hyaline, aseptate, ellipsoidal or fusiform, widest in the middle, both ends obtuse, smooth and thin-walled, with bipolar germ pores, surrounded by a wing-like hyaline sheath. *Pycnidia* 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. *Paraphyses* hyaline, cylindrical. *Conidiogenous cells* 9–20×3–6 μm, holoblastic, hyaline, aseptate, cylindrical to subcylindrical. *Conidia* 17.5−24.5×9.5−12.5 μm ($\bar{x} = 21.5 \times 11\,\mu m$), initially hyaline, aseptate, ellipsoid to obovoid, thick-walled with granular content, rounded at apex, occasionally truncate at base. Aged conidia becoming cinnamon to sepia, and 1–septate.

Material examined: THAILAND, Chiang Rai Province., Muang District, Khun Korn Waterfall, on dead leaves of *Arenga westerhoutii*., 18 Dec 2009, J.K. Liu, JKA0022 (MFLU 10–0407, holotype); Chiang Rai Prov., Muang District, Khun Korn Waterfall, on living leaves of

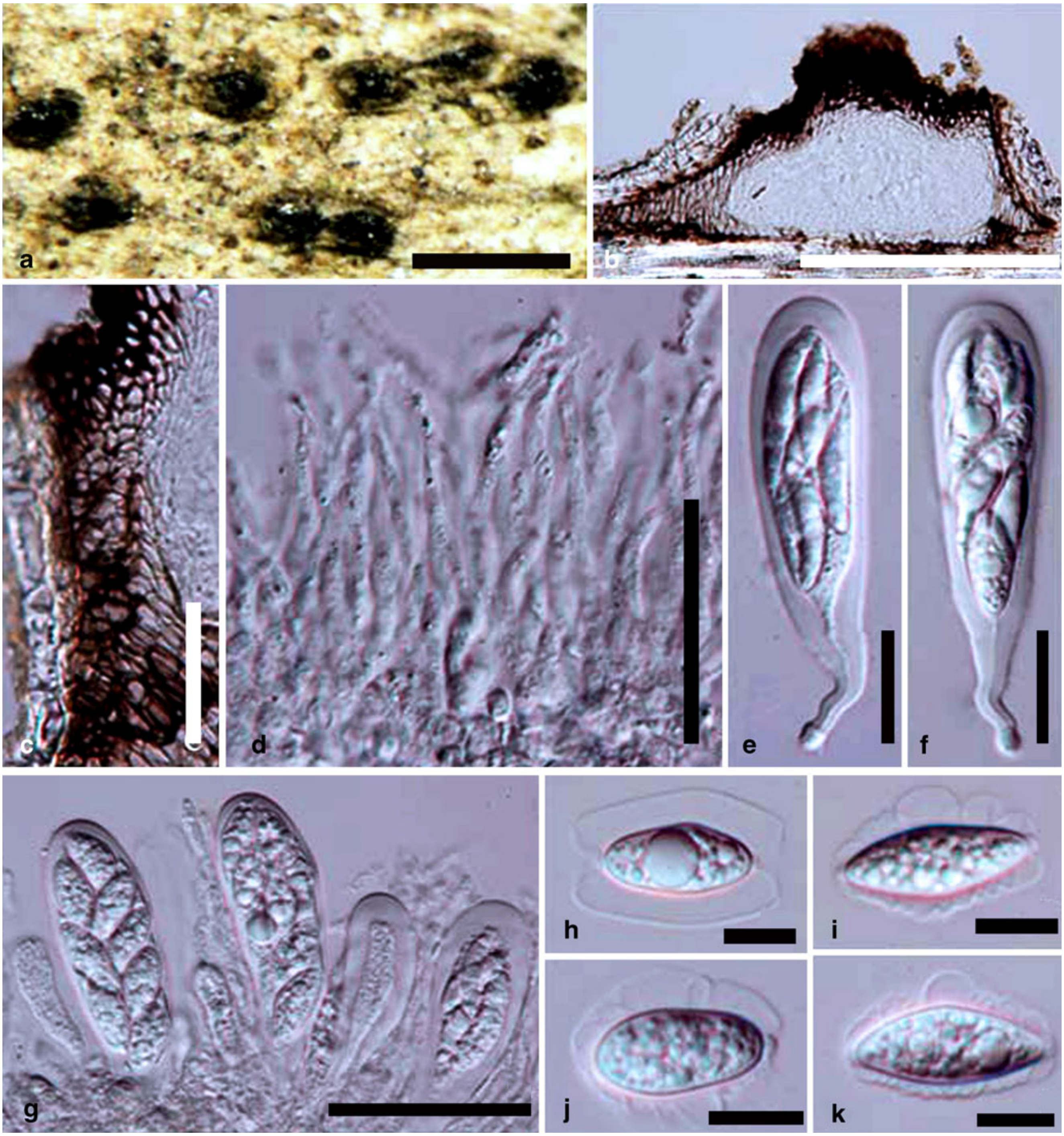

Fig. 23 *Neodeightonia subglobosa* (MFLU 11−0199). **a** Ascostromata on host substrate. **b** Section through ascostroma. **c** Section through peridium. **d** Pseudoparaphyses. **e−f** Asci. **g** Asci with pseudoparaphyses. **h−k** Ascospores. Scale bars: **a** = 500 µm, **b** = 200 µm, **c−d, g** = 50 µm, **e−f** = 20 µm, **h−k** = 10 µm

Caryota urens., 22 Jul 2009, R. Phookamsak, RP0004 (MFLU 10–0409).

Neofusicoccum Crous, Slippers & A.J.L. Phillips, Stud. Mycol. 55: 247 (2006)

Synonym

Nattrassia B. Sutton & Dyko, Mycol. Res. 93: 483 (1989)

MycoBank: MB500870

Saprobic on dead wood. *Ascostromata* visible as minute black dots or papilla on host tissue, semi-immersed to erumpent under epidermis, individually globose to subglobose,

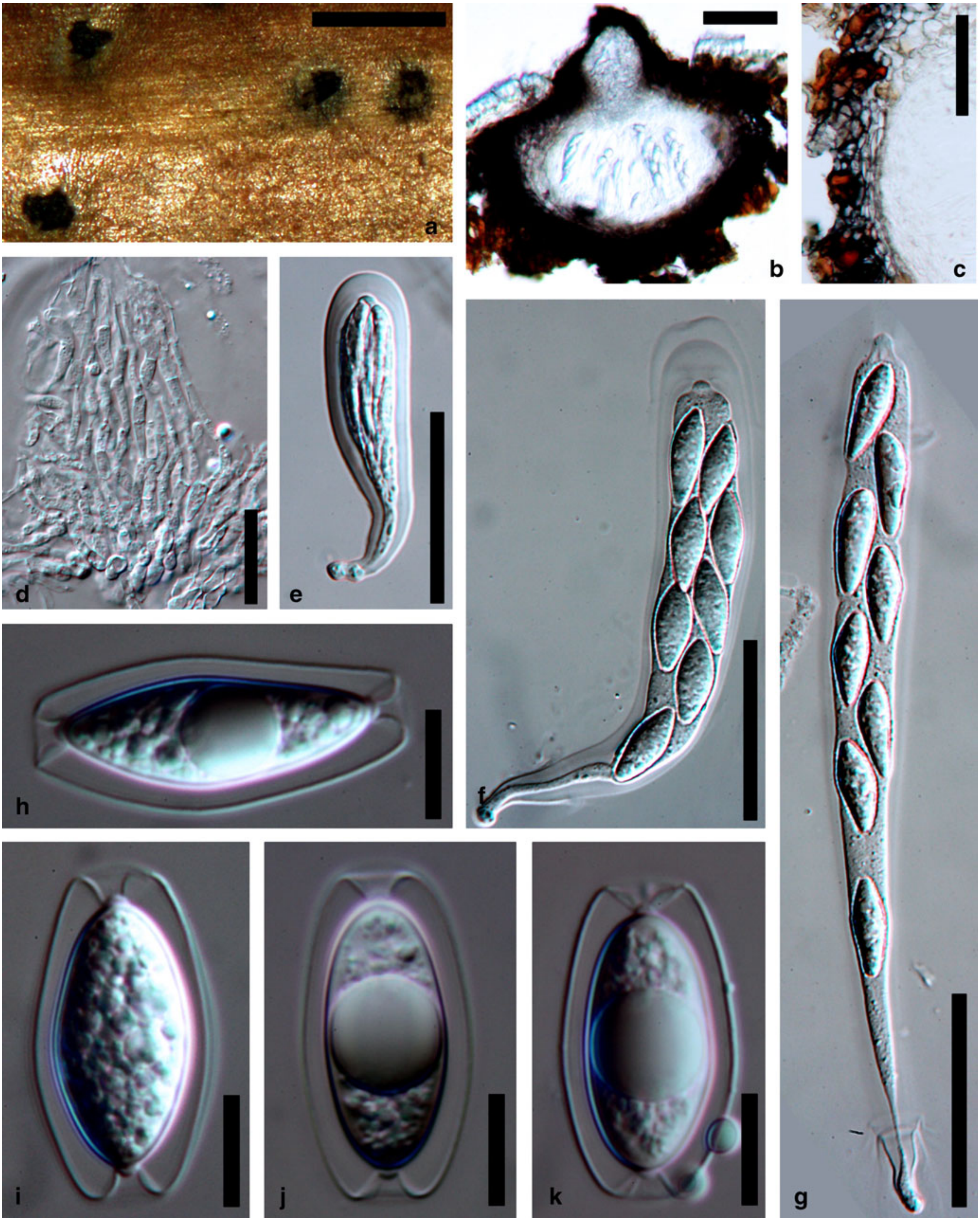

Fig. 24 *Neodeightonia palmicola* (MFLU 10–0407, holotype). **a** Appearance of ascostromata on host substrate. **b** Section of ascostroma. **c** Section of peridium comprising a few cells layers of *textura angularis*. **d** Pseudoparaphyses. **e–g** Asci. **h–k** Ascospores with a wing-like sheath. Scale bars: **a** = 1 mm, **b–c** = 100 μm, **d–g** = 30 μm, **h–k** = 10 μm

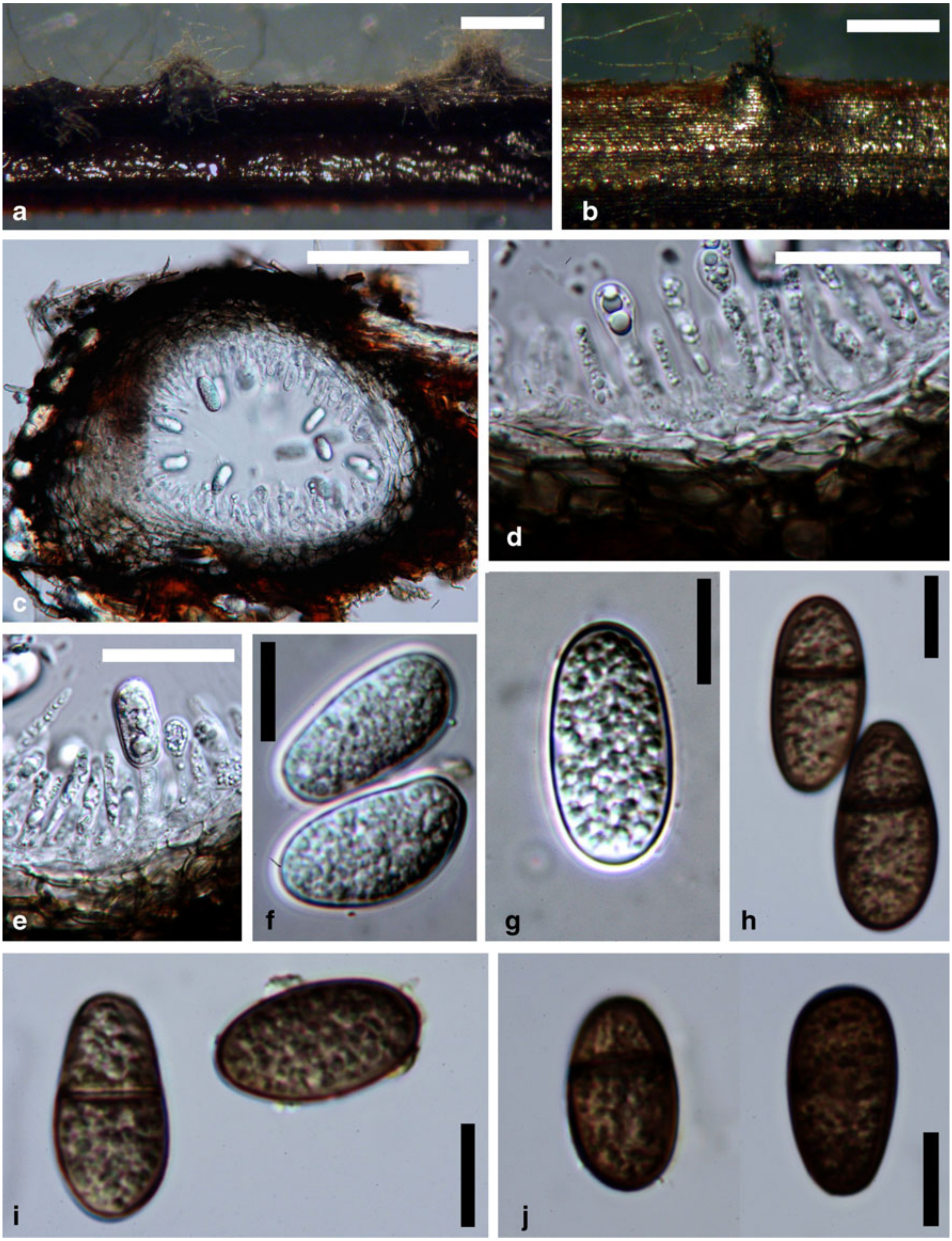

Fig. 25 Asexual morph of *Neodeightonia palmicola* (MFLU 10–0407). **a**–**b** Conidiomata on pine needles**. c** Section of conidioma. **d**–**e** Conidiogenous cells. **f**–**g** Young conidia. **h**–**i.** Mature conidia with septa. Scale bars: **a**–**b** = 500 μm, **c** = 100 μm, **d**–**e** = 30 μm, **g**–**j** = 10 μm

solitary or clustered, longitudinal axis vertical to the host surface. *Ostiole* central, circular, papillate. *Peridium* of locules two-layered, outer layer composed of brown to dark brown, thick-walled cells of *textura angularis*, inner layer composed of hyaline thin-walled cells of *textura angularis. Pseudoparaphyses* hyphae-like, numerous, septate, slightly constricted at septum. *Asci* 8–spored, bitunicate, fissitunicate, clavate to cylindro-clavate, short pedicellate, apically rounded with an ocular chamber. *Ascospores* hyaline, aseptate, ellipsoidal to fusiform, thick-walled. Pycnidial aggregates morphologically indistinguishable from ascomatal aggregates. *Pycnidia* globose and non-papillate to pyriform, with a short, acute papilla; pycnidium a locule created within stromal tissue; pycnidial wall not differentiated from surrounding tissue. *Conidiogenous cells* holoblastic, hyaline, subcylindrical, proliferating percurrently with 1–2 proliferations and periclinical thickening. *Conidia* ellipsoidal with apex round and base flat, hyaline, aseptate, becoming light brown and 1–2 septate with age (asexual morph description follows Pennycook and Samuels 1985).

Notes: *Neofusicoccum* was introduced for an asexual morph which occurs with a "Dichomera"-like synanamorph by Crous et al. (2006). They considered that the name is more informative of the morphological state. Most of the species of the genus had previously been treated as *Fusicoccum*, and Crous et al. (2006) proposed new combinations for 13 species based on the sequence data from cultures. Pennycook and Samuels (1985) listed *Fusicoccum parvum* as the asexual morph when they described *Botryosphaeria parvum* (= *Neofusicoccum parvum*). In the present study we found the sexual morph of *Neofusicoccum parvum*, the type species of the genus, on a branch of *Linum usitatissimum*. The isolate clustered with the type strain of *N. parvum* with 100 % bootstrap support (Fig. 1). Morphologically our collection is identical to the original description of *N. parvum.*

Generic type: *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips

Neofusicoccum parvum (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips, Stud. Mycol. 55: 248 (2006)

MycoBank: MB500879 (Fig. 26)

≡ *Fusicoccum parvum* Pennycook & Samuels, Mycotaxon 24: 455 (1985)

≡ *Botryosphaeria parva* Pennycook & Samuels, Mycotaxon 24: 455 (1985)

Saprobic on dead branch. *Ascostromata* 500–1,040 μm diam, 120–200 μm high (including the papilla), visible as minute black dots or papilla on host tissue, semi-immersed to erumpent under epidermis, individually globose to subglobose, solitary or clustered, longitudinal axis vertical to the host surface. *Locules* 170–260 μm diam, 117–193 μm high. *Ostiole* central, circular, papillate. *Peridium* of locules up to 20–50 μm wide, two-layered, outer layer composed of brown to dark brown, thick-walled cells of *textura angularis*, inner layer composed of hyaline thin-walled cells of *textura angularis. Pseudoparaphyses* 2–3.5 μm wide, hyphae-like, numerous, septate, slightly constricted at septum. *Asci* (64-)73−97.5(−104.5)×(15.5-)17−22.5(−24) μm ($\bar{x} = 82.4 \times 20.7\,\mu m, n = 25$), 8–spored, bitunicate fissitunicate, clavate to cylindro-clavate, short pedicellate, apically rounded with well developed ocular chamber (3–4 μm wide, n=5). *Ascospores* 18−22(−23)×7–9 μm ($\bar{x} = 20.1 \times 8\,\mu m$, $n = 30$), uni−seriate at the base, 2–3−seriate at the apex, hyaline, aseptate, ellipsoidal to fusiform, usually wider in the centre, thick and rough-walled. Pycnidial aggregates morphologically indistinguishable from ascomatal aggregates; several Pycnidia in each aggregate. *Pycnidia* globose and non-papillate to pyriform, with a short, acute papilla; pycnidium a locule (100–150 μm diam.) created within stromal tissue; pycnidial wall not differentiated from surrounding tissue. *Conidiogenous cells* holoblastic, hyaline, subcylindrical, proliferating percurrently with 1–2 proliferations and periclinical thickening. *Conidia* (11-)14−18 (−23)×5–7 μm, ellipsoidal with apex round and base flat, hyaline, aseptate, becoming light brown and 1–2 septate with age (asexual morph description follows Pennycook and Samuels 1985).

Culture characteristics: Colonies on PDA, 50 mm diam after 4 d at 25−30 °C, fast growing; circular, white at first, becoming gray to grey-black after two weeks; reverse white to pale white in first week, after one to two weeks becoming black; flattened, fluffy, fairly dense, aerial, surface smooth with raised edge, filamentous, pigments not produced.

Material examined: THAILAND, Chiang Mai Province., Jom Tong District, Doi Inthanon Royal Project, on dead branch of *Linum usitatissimum*, 16 November 2010, R. Phookamsak, RP0100 (MFLU 11–0220); living culture MFLUCC 11–0184.

Phaeobotryon Theiss. & Syd., Ann. Mycol. 13: 664 (1915)

MycoBank: MB3892

Saprobic on dead wood. *Ascostromata* black, immersed to erumpent, subglobose to ovoid, multilocular. *Ostiole* opening with a pore. *Peridium* consisting of layers of dark brown-walled cells of *textura angularis*. *Pseudoparaphyses* hyphae-like, septate, constricted at septa. *Asci* 8−spored, bitunicate, fissitunicate, clavate to cylindrical-clavate, short-pedicellate, apically rounded with an ocular chamber. *Ascospores* hyaline to brown, 2–septate, ellipsoid to broad fusiform, with or without an apiculus at each end, surrounded by a mucilaginous sheath. *Conidiomata* pycnidial, black, ostiolate, separate or aggregated, immersed to erumpent, unilocular or multilocular, ostiolate. *Ostiole* central, circular, non-papillate. *Paraphyses* hyaline, thin-walled, usually aseptate, sometimes becoming up to 2−septate. *Conidiogenous cells* holoblastic, hyaline, cylindrical to doliiform, smooth. *Conidia* brown, ellipsoid to oblong or obovoid, moderately thick-walled, ends rounded, 1 (−2)–septate, mostly 2–septate, not constricted at septa (asexual morph description follows Phillips et al. 2008; Abdollahzadeh et al. 2009). Asexual morph is "Dothiorella"-like, but having conidia with up to two transverse septa.

Notes: *Phaeobotryon* was introduced by Theissen and Sydow (1915) to accommodate *Dothidea cercidis.* This

Fig 26 *Neofusicoccum parvum* on dead branch of *Linum usitatissimum* (MFLU 11–0220). **a** Ascostromata on host tissue. **b** Section through ascostroma. **c** Section through peridium. **d** Pseudoparaphyses. **e** Asci with pseudoparaphyses. **f–i** Asci. **j–k** Ascospores. Scale bars: **a** = 500 μm, **b** = 200 μm, **c–d** = 20 μm, **e–i** = 30 μm, **j–m** = 10 μm

taxon was considered to belong to a distinct genus due to its pale brown to brown, 2–septate ascospores which were reported as hyaline in the original description. Using a broader concept for *Botryosphaeria*, von Arx and Müller (1954, 1975) treated *Phaeobotryon* as a synonym of *Botryosphaeria*. However, Phillips et al. (2008) reinstated *Phaeobotryon* as they found it to be morphologically and phylogenetically distinct from other genera in the *Botryosphaeriaceae*. Phillips et al. (2008) considered the 2–septate, brown ascospores with a conical apiculus at each end, were characteristic of the genus and further described two new species, *P. mamane* Crous & A.J.L. Phillips and *P. quercicola* (A.J.L. Phillips) Crous & A.J.L. Phillips. Subsequently, Abdollahzadeh et al. (2009) introduced an endophytic species, *P. cupressi* Abdollahzadeh, Zare & A.J.L. Phillips, isolated from stems of *Cupressus sempervirens*. Molecular sequence data is available for *P. mamane* and *P. cupressi*. Asexual morphological characters and conidial dimensions are used to distinguish the species. However, the remaining species *P. cercidis*, *P. disruptum* (Berk. & M.A. Curtis) Petr. & Syd and *P. euganeum* (Sacc.) Höhn., were described based on the morphology of the sexual stage only and no asexual characters have been reported. Presently there are seven species listed in the genus (*Index Fungorum*, MycoBank).

Generic type: *Phaeobotryon cercidis* (Cooke) Theiss. & Syd.

Phaeobotryon cercidis (Cooke) Theiss. & Syd., Ann. Mycol. 13: 664 (1915)

MycoBank: MB124247 (Fig. 27)

≡ *Dothidea cercidis* Cooke, Grevillea 13: 66. 1885, as 'Dothidea Bagnisiella'.

≡ *Bagnisiella cercidis* (Cooke) Berl. & Voglino, Add. Syll. Fung. 1–4: 223 (1886)

≡ *Auerswaldia cercidis* (Cooke) Theiss. & Syd., Ann. Mycol. 12: 270 (1914)

Saprobic on dead wood. *Ascostromata* 242–251 µm high × 218−253 µm diam, immersed, erumpent, but still under host tissue, subglobose to ovoid, rough, multilocular, with 3–4 *locules* in one ascostroma,. *Ostiole* opening with a pore, 98–110 µm long. *Peridium* 200–250 µm wide, one-layered, composed of brown-walled cells of *textura angularis*. *Pseudoparaphyses* hyphae-like, septate, constricted at septa. *Asci* 125–130×22–24 µm, 8−spored, bitunicate, fissitunicate, pedicellate, apically rounded with an ocular chamber. *Ascospores* 29–34×9–13 µm ($\bar{x} = 31 \times 12\,\mu m, n = 25$), 1–2–seriate, ellipsoid to broad fusiform with broadly to narrowly rounded ends, hyaline, surrounded by a mucilaginous sheath. *Asexual state* not established.

Material examined: USA, Carolina, on bark of *Cercis canadensis*, ex Herb. MC Cooke No 795 (K134204, holotype).

Notes: The type material that we examined had hyaline, aseptate ascospores, surrounded by a mucilaginous sheath, which cncurs with the original description. Theissen and Sydow (1915) reported that the ascospores became brown with age. It is possible that the material examined by us was not mature.

Phaeobotryosphaeria Speg., Ann. Inst. Rech. Agron. 17, 10: 120. 1908

Synonym

Sphaeropsis Sacc., Michelia 2(no. 6): 105 (1880)

Other possible synonyms

Botryosphaerostroma Petr. & Syd., Beih. Reprium nov. Spec. Regni veg. 42: 126 (1926) [1927]

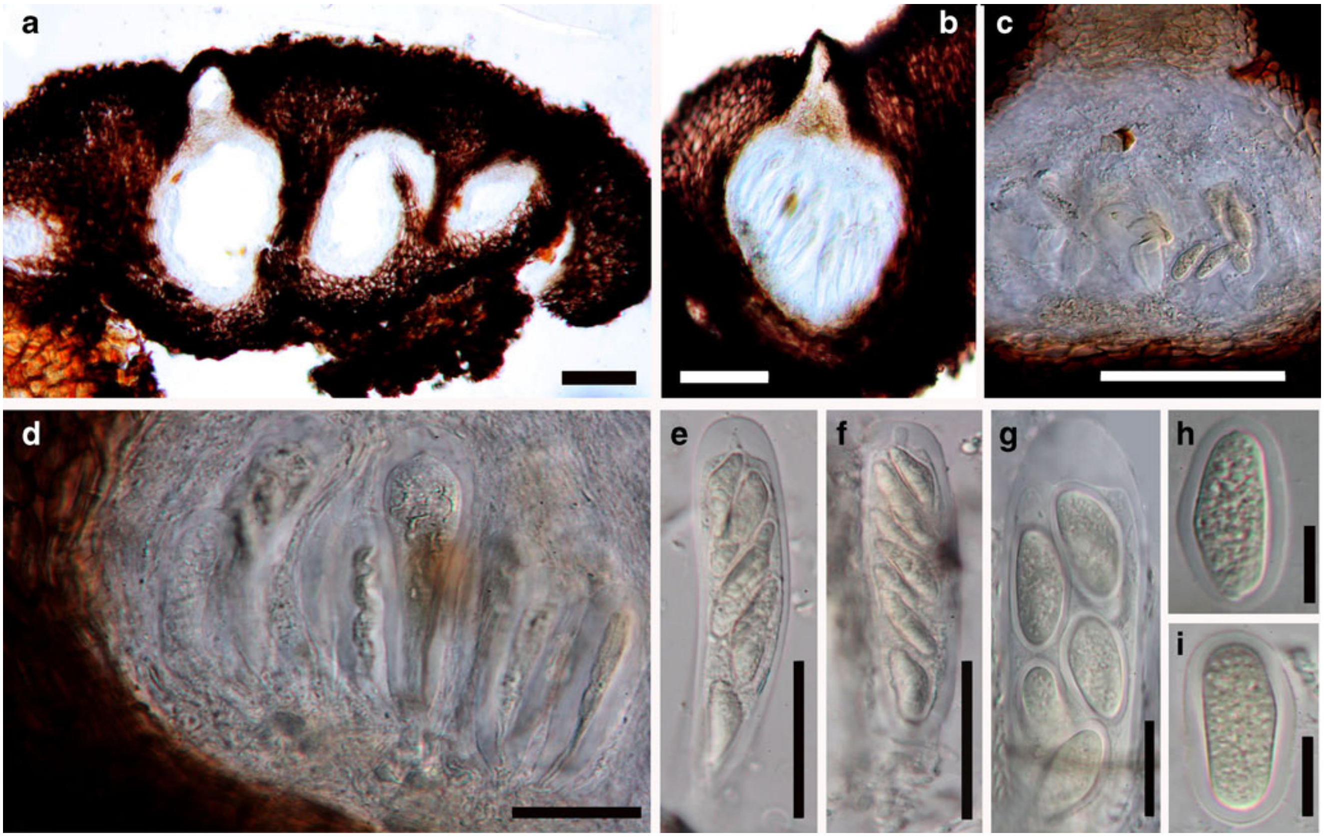

Fig. 27 *Phaeobotryon cercidis* (K134204, holotype) **a−b** Section of ascostromata showing locules. **c−d** Locule. **e−g** Asci. **h−i** Ascospores with mucilaginous sheath. Scale bars: **a−d** = 100 µm, **e−g** = 50 µm, **h−I** = 10 µm

Botrysphaeris Clem. & Shear, Gen. Fung., Edn 2: 361 (1931)

Catosphaeropsis Tehon, Mycologia 31: 542 (1939)

Granulodiplodia Zambett. ex M. Morelet, Bull. Soc. Sci. nat. Arch. Toulon et du Var 203: 12 (1973)

Gyratylium Preuss, Linnaea 26: 722 (1855)

Macrophoma (Sacc.) Berl. & Voglino, Atti Soc. Veneto-Trent. Sci. Nat. 10(1): 172 (1886)

Macroplodia Westend., Bull. Acad. R. Sci. Belg., Cl. Sci., sér. 2 2: 562 (1857)

Neosphaeropsis Petr., Ann. Mycol. 19: 67 (1921)

Phoma subgen. *Macrophoma* Sacc., Syll. Fung. 3: 66 (1884)

Phomatosphaeropsis Ribaldi, Annali Sper. Agr., n.s. 7 (3): 847 (1953)

Sphaeropsis Lév., in Demidov, Voyage dans la Russie Meridionale et la Crimeé, par la Hongrie, la Valachie et la Moldavie 2: 112 (1842)

MycoBank: MB3893

Saprobic on dead wood. *Ascostromata* erumpent, irregularly scattered or multiloculate in groups, fusiform. *Locules* in a single layer, flask-shaped, with short neck. composed of dark brown-walled cells of *textura angularis. Pseudoparaphyses* abundant, hyphae-like, septate. *Asci* 8–spored, bitunicate, fissitunicate, clavate, short or long pedicellate, apically rounded with an ocular chamber. *Ascospores* brown, aseptate, elliptical to ovoid, navicular, rhomboid when young, thick walled, with a hyaline apiculus at either end. *Conidiomata* pycnidial, immersed to erumpent, thick-walled, wall composed of several layers of dark brown *textura angularis*, eustromatic, unilocular. *Ostiole* central, papillate. *Paraphyses* hyaline, aseptate, thin-walled. *Conidiogenous cells* hyaline, discrete, proliferating internally to form periclinal thickenings. *Conidia* hyaline, becoming brown to dark brown, aseptate, oval, oblong or clavate, straight, thick-walled (asexual morph description follows Phillips et al. 2008).

Notes: *Phaeobotryosphaeria* was introduced as a monotypic genus by Spegazzini (1908) represented by *P. yerbae*, from *Ilex paraguayensis* collected from Argentina. Von Arx and Müller (1954) considered *Phaeobotryosphaeria* as a synonym of *Botryosphaeria* Ces. & De Not. However, Phillips et al. (2008) reinstated it showing that it is morphologically and phylogenetically distinct from *Botryosphaeria* in the *Botryosphaeriaceae*.

Generic type: *Phaeobotryosphaeria yerbae* Speg.

Phaeobotryosphaeria yerbae Speg., Anales del Museo Nacional de Historia Natural de Buenos Aires 17: 120 (1908)

MycoBank: MB182015 (Fig. 28)

Saprobic on dead branch. *Ascostromata* erumpent, irregularly scattered or multiloculate in groups (up to 6), fusiform. *Locules* in a single layer, flask-shaped, 200–290×300–350 μm, with a short neck 80–140 μm long. *Peridium* of locules single layer, composed of dark brown-walled cells of *textura angularis*. *Pseudoparaphyses* abundant, hyphae-like, septate, constricted at septa. *Asci* 180–200×30–35 μm, 8–spored, bitunicate, fissitunicate, clavate, with a 30–50 μm long pedicel, apically rounded with an ocular chamber. *Ascospores* 30−45(−50)×14–17 μm, brown to dark brown, aseptate, elliptical to ovoid, navicular, rhomboid when young, thick-walled, smooth, brown, with a hyaline apiculus at either end. *Asexual state* not established.

Material examined: ARGENTINA, Misiones, Campo de las Cuias, on branches of *Ilex paraguayensis*, February 1907, C. Spegazzini (LPS 2926 lectotype designated here); Departamento Iguazú, Parque Nac. Iguazú, on fallen unidentified branches, 17 March 1993, Carmarán 222 (BAFC33591 −identified as *Botryosphaeria ingiae* Kar & Maity).

Notes: The type material at LPS comprises four collections (LPS 2923, 2924, 2925, and 2926) under the name *Phaeobotryosphaeria yerbae,* all collected from the same place on the same date and are thus syntypes. Phillips et al. (2008) examined one collection (LPS 2926) and interpreted this as the holotype. We also studied LPS 2926 and designate this as the lectotype. Romero and Carmarán (1997) reported *Botryosphaeria ingae* A.K. Kar & Maity also from Argentina, but we have studied the material kept at BAFC Fungi Collection (BAFC33591) and it is identical to *Phaeobotryosphaeria yerbae*.

Phaeobotryosphaeria eucalypti Doilom, J.K. Liu & K.D. Hyde, sp. nov.

MycoBank: MB 801320 (Fig. 29)

Etymology: Referring to *Eucalyptus*, the host on which the fungus was collected.

Saprobic on dead wood. *Ascostromata* black, dark brown spot, aggregated, convex, on host tissue, initially immersed in tissue, becoming semi-immersed, appearing through cracks in bark, solitary, or gregarious, when cut horizontally, locules visible with white contents and, multiloculate, globose to subglobose. *Peridium* of locules composed of several layers of dark brown-walled cells of *textura angularis*, broader at the base. *Pseudoparaphyses* 3–4 μm wide, 5–10(−15) μm long, hyphae-like, numerous, septate, constricted at septa. *Asci* (90-)97−110(−126)×28−31 μm $(\overline{x} = 106 \times 29\,\mu m, n = 20)$, 8–spored, bitunicate, fissitunicate, cylindro-clavate or clavate, with a short pedicel, apically rounded with an ocular chamber. *Ascospores* 27–35×11–14 μm $(\overline{x} = 30 \times 12\,\mu m, n = 30)$, overlapping biseriate, hyaline when young, becoming pale brown or reddish brown when mature, aseptate, ellipsoid to ovoid, ends rounded, with an apiculus at each end, thick-walled, smooth, widest in the centre. *Asexual state* not established.

Culture characteristics: Ascospores germinating on PDA within 5–10 h. Germ tubes produced from germ pore

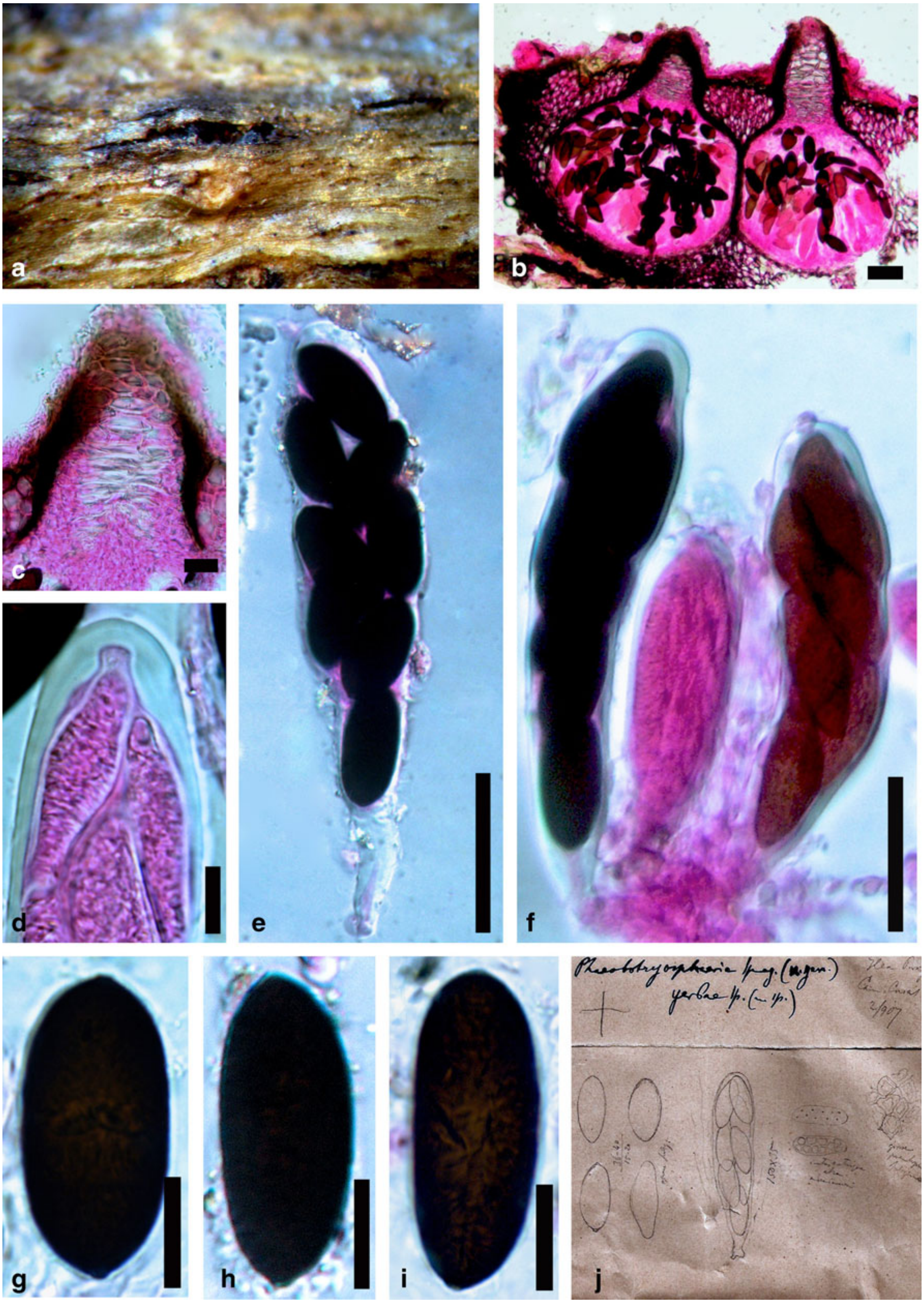

Fig. 28 *Phaeobotryosphaeria yerbae* (LPS 2926, lectotype). **a** Ascostromata immersed in the substrate. **b** Longitudinal section of ascostromata. **c** Longitudinal section through neck. **d** Young ascus apex with an ocular chamber. **e** Ascus. **f** Three asci in different stages of development. **g–h** Ascospores. **j** Original drawings by Spegazzini (LPS 2926) on the envelope. Scale Bars: **a** = 0.5 mm, **b** = 50 μm; **c** = 20 μm, **d, g –i** = 10 μm, **e–f** = 50 μm

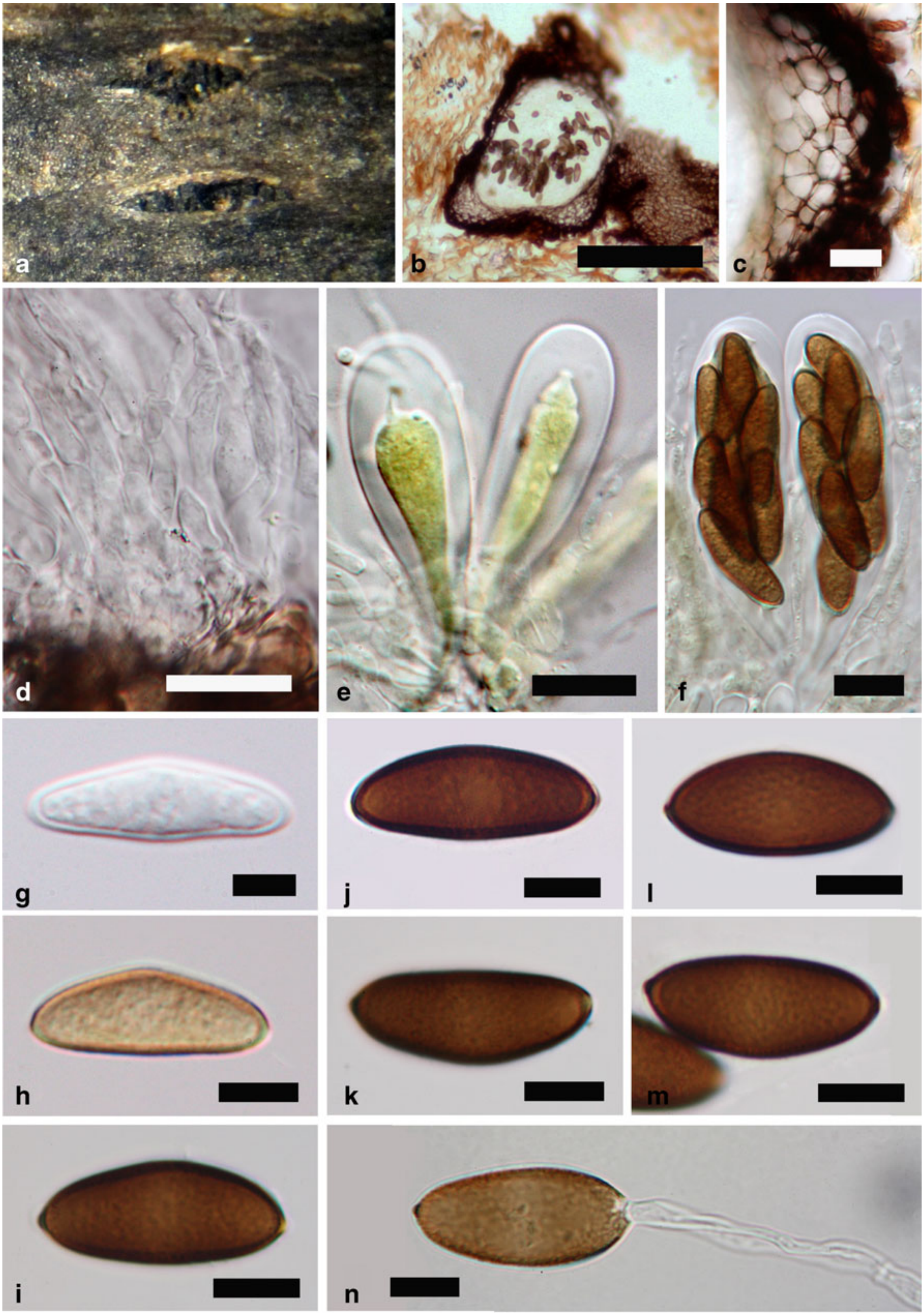

Fig. 29 *Phaeobotryosphaeria eucalypti (*MFLU12−0753, holotype*)* **a** Ascostromata on host substrate. **b** Section through ascostroma. **c** Peridium. **d** Pseudoparaphyses. **e** Immature asci in Melzers' reagent. **f** Mature asci. **g** Immature ascospore. **h−i** Pale brown or reddish brown ascospores with an apiculus. **n** Germinating ascospore. Scale bars: **b** = 200 μm, **c−f** = 20 μm, **g−n** = 10 μm

of ascospores. Colonies growing on PDA, fast growing, reaching 70 mm diam after 6 d at 25−30 °C, flat or effuse, fimbriate, initially white and cotton-like, bright white at edge after a few days becoming pale grey from the centre, reaching the edge of the Petri dish after 8 d. No asexual morphs were formed in culture even after 3 months.

Material examined: THAILAND, Chiang Rai Province, Muang District, Thasood Sub District, on dead twig of *Eucalyptus* sp., 8 August 2011, M. Doilom (MFLU 12–0753, holotype), ex-type living culture MFLUCC 11–0579; *Ibid*, living culture MFLUCC 11–0654.

Notes: This new taxon was collected from a dead twig of *Eucalyptus* spp.; its morphological characters, the brown aseptate ascospores with an apiculus at either end, fit well with *Phaeobotryosphaeria* and it is a characteristic species of this genus. Molecular sequence data is available for *P. citrigena*, *P. porosa* and *P. visci*. We have included these sequences in our analyses (Fig. 1). *Phaeobotryosphaeria eucalypti* clustered in the clade of *Phaeobotryosphaeria* in the *Botryosphaeriaceae* and formed a sister group with the other three species, although being distinguished from them with strong bootstrap support (83 %). The genus type of *Sphaeropsis, S. visci* DC. was shown to be the asexual morph of *Phaeobotryosphaeria* by Phillips et al. (2008), the culture did not form asexual morph in this study.

Phyllachorella Syd., Ann Mycol. 12: 489 (1914)

MycoBank: MB4050

Epiphytes on the host leaf surface, forming conspicuous ascostromata. *Ascostromata* gregarious, with numerous ascomata clustering together forming black, superficial, velvety patches. *Peridium* of locules laterally, thinner at the apex and the base, coriaceous, two-layered, outer layer composed of small heavily pigmented thick-walled cells *textura angularis*, inner layer composed of hyaline thin-walled cells *textura angularis*. *Pseudoparaphyses* not observed. *Asci* 8−spored, bitunicate, cylindrical to clavate, with a short narrow twisted pedicel, apically rounded; with a small ocular chamber. *Ascospores* irregularly arranged to uniseriate near the base, hyaline, septate, deeply constricted at the septum, oblong to ovate, with broadly to narrowly rounded ends, the upper cell often broader than the lower one, smooth, guttulate. *Asexual state* not established.

Notes: *Phyllachorella* was formally established by Sydow (1914) in "Phyllachoracearum" as a monotypic genus represented by *P. micheliae.* The genus is characterized by its "phyllachorae stroma" on the host surface. Kar and Maity (1971) recorded the type species of this genus in India and gave a full description of this genus based on its "hypophyllous, 2–3 sometimes coalescing stromata and cylindro-clavate, pedicellate asci".

We have re-examined the type specimen of this genus, which has hyaline ascospores as recorded in the protologue (Sydow 1914). According to Kar and Maity (1971) ascospore are brown inside the asci. It is not clear whether their collection was *Phyllachorella.*

There has been no phylogenetic study of this genus, however many of its characters (ascostromata, thick wall of relatively thick-walled brown-cells *textura angularis/globulosa*, characteristic asci and aseptate ascospores), suggest it should be included in *Botryosphaeriaceae*.

Generic type: *Phyllachorella micheliae* Syd.

Phyllachorella micheliae Syd., Ann. Mycol 12: 489 (1914)

≡ *Vestergrenia micheliae* (Syd.) Arx & E. Müll., Beitr. Kryptfl. Schweiz 11(no. 1): 75 (1954)

MycoBank: MB239498 (Fig. 30)

Epiphytes on the host leaf surface, forming conspicuous ascostromata. *Ascostromata* black, 170–220 μm high × 180–210 diam., gregarious, with numerous ascomata clustering together forming black, velvety patches, superficial. *Peridium* of locules up to 22–38 μm thick, laterally, thinner at the apex and the base, coriaceous, two-layered, outer layer composed of small heavily pigmented thick-walled cells *textura angularis*, inner layer composed of hyaline thin-walled cells *textura angularis*. *Pseudoparaphyses* not observed. *Asci* 70–94 × 18–24 μm $(\bar{x} = 88 \times 21\,\mu m, n = 10)$, 8–spored, bitunicate, cylindrical to clavate, with a short narrow twisted pedicel, apically rounded with a small ocular chamber. *Ascospores* 16–21 × 5–8 μm $(\bar{x} = 18 \times 7\,\mu m, n = 10)$, irregularly arranged to uniseriate near the base, hyaline, aseptate, deeply constricted at the centre, oblong to ovate, with broadly to narrowly rounded ends, the upper part often broader than the lower part, smooth-walled, guttulate. *Asexual state* not established.

Material examined: INDIA, Madras, Presidency, Ootacamund, Nilgris, on living leaves of *Michaelia niliginica*, 23 December 1912, W. Mac Rae, (SF5795, holotype).

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

MycoBank: MB9384

Possibly synonymy

Caudophoma B.V. Patil & Thirum., Sydowia 20: 36 (1968) [1966]

Guignardia Viala & Ravaz, Bull. Soc. Mycol. Fr. 8: 63 (1892)

Laestadiella Höhn., Ann. Mycol. 16: 50 (1918)

Leptasteromella Petr., Sydowia 20: 235 (1968) [1966]

Leptodothiorella Höhn., Hedwigia 60: 173, 175 (1918)

Leptodothiorella Aa, Stud. Mycol. 5: 13 (1973)

Leptophacidium Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 127: 331 [3 repr.] (1918)

Macrophyllosticta Sousa da Câmara, Anais Inst. sup. Agron. Univ. Téc. Lisboa 3: 36 (1929)

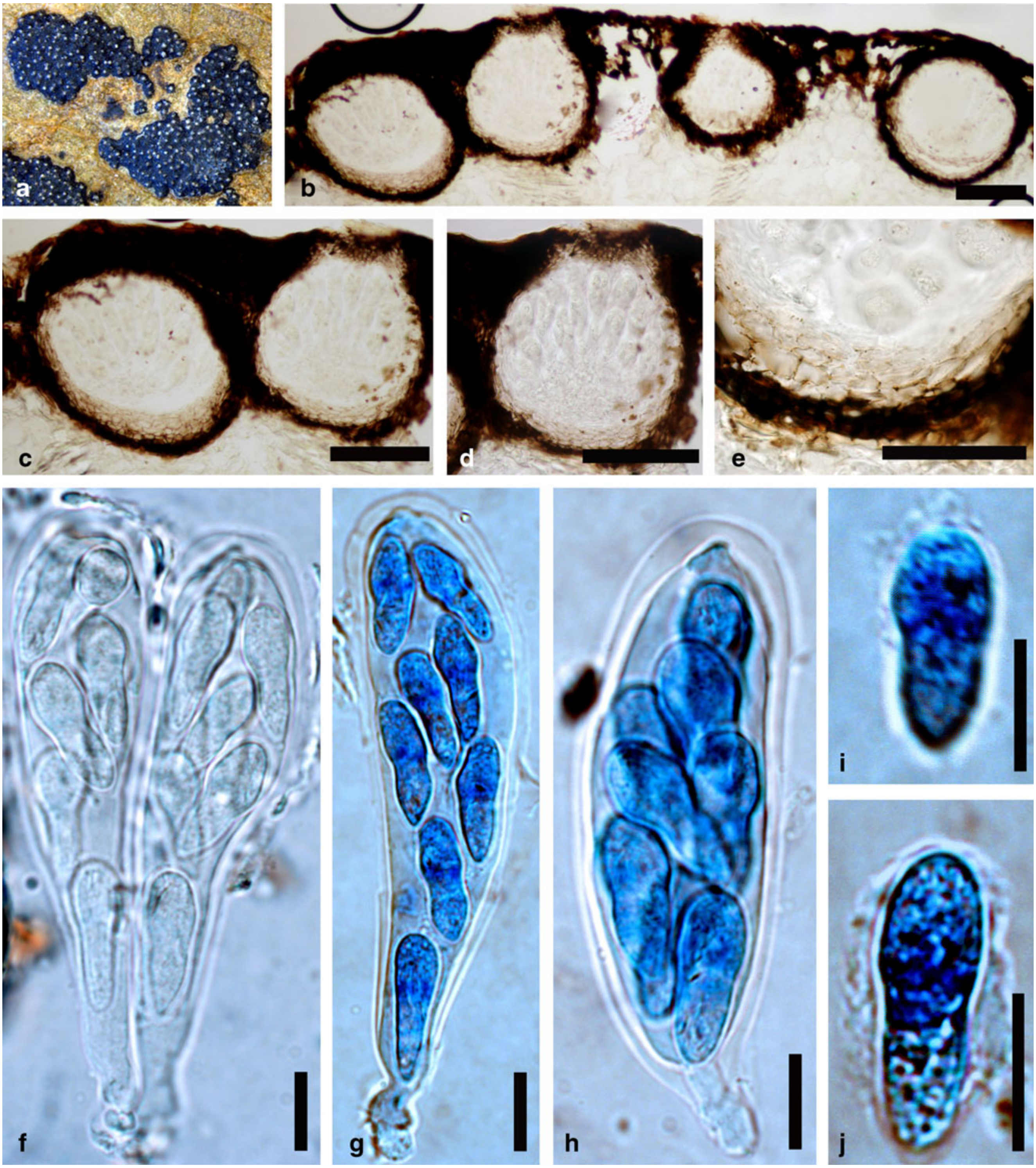

Fig. 30 *Phyllachorella micheliae* (SF5795, holotype) **a** Appearance of ascostromata on the host substrate. **b–d** Vertical section through ascostroma. **e** Vertical section illustrating the peridium. **f** Asci. **g–h** Asci in lactophenol cotton blue reagent. **i–j** Ascospores in the lactophenol cotton blue. Scale bars: **a** = 1 mm, **b–e** = 100 μm, **f–j** = 10 μm

Montagnellina Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 121: 387 [49 repr.] (1912)

Myriocarpa Fuckel, Jb. Nassau. Ver. Naturk. 23–24: 116 (1870) [1869–70]

Pampolysporium Magnus, Verh. Zool.-Bot. Ges. Wien 50: 444 (1900)

Phyllosphaera Dumort., Comment. Bot.: 86 (1822)

Phyllostictina Syd. & P. Syd., Ann. Mycol. 14: 185 (1916)

Polysporidium Syd. & P. Syd., Ann. Mycol. 6: 528 (1908)

Endophytic or *pathogenic* on leaves of a wide range of hosts. *Ascomata* gregarious, circular, brown to black, coriaceous, with a central ostiole. *Asci* (6-)8–spored, bitunicate, fissitunicate, clavate, with a gelatinous pedicel and ocular chamber. *Ascospores* irregularly biseriate, hyaline, aseptate, ellipsoid to broadly fusoid, but much wider in the middle, smooth walled, usually with mucilaginous pads at one or both ends or surrounded by a mucilaginous sheath. *Pycnidia* circular, brown to black, coriaceous, with a central ostiole. *Peridium* comprising brown cells of *textura angularis*. *Conidiogenous cells* lining wall of pycnidium, phialidic, cylindrical, hyaline. *Conidia* hyaline, ellipsoidal, aseptate, smooth-walled, surrounded by a mucilaginous sheath bearing a single apical appendage.

Notes: *Phyllosticta* has been reviewed by Wikee et al. (2011a) and there have also been several other modern treatments of the genus (Wulandari et al. 2009; Glienke et al. 2011; Wong et al. 2012). The generic type (*Phyllosticta convallariae* Pers.) lacks any recent collections or sequence data and this is certainly required. The sexual state *Guignardia* is clearly linked to *Phyllosticta* and Wikee et al. (2011a) proposed that *Phyllosticta* should be used for this genus with *Guignardia* listed as a synonym and this has already been used in *Index Fungorum* and by various authors (Glienke et al. 2011; Wikee et al. 2011b; Wong et al. 2012). As *Phyllosticta* is the older and more commonly used name there should be no difficulty in reaching a consensus on using *Phyllosticta* to represent all species in the biological genus with sexual and asexual morphs.

The sexual “Guignardia” state is represented by *Phyllosticta ampelicida* (Engelm.) Aa (= *Guignardia bidwellii* (Ellis) Viala & Ravaz) and causes leaf spots on grape vines in the USA. Other important species are *Phyllosticta citricarpa* (McAlpine) Aa which causes black spot of citrus and is of quarantine concern (Wulandari et al. 2009; Wong et al. 2012) and *P. citriasiana* Wulandari, Crous & Gruyter which causes tan spot of pomelo. Freckle disease of banana is caused by a complex of species of *Phyllosticta* (Wong et al. 2012). *Phyllosticta capitalensis* is a weak pathogen and appears to be a ubiquitous endophyte. Below we choose this species to illustrate the genus with both sexual and asexual morphs (Fig. 31).

Generic type: *Phyllosticta convallariae* Pers.

Phyllosticta capitalensis Henn., Hedwigia 48: 13 (1908)

Mycobank: MB168326 (Fig. 31)

Endophytic or *pathogenic* on leaves of a wide range of hosts. *Ascomata* 65−153 μm long, 64−130 diam ($\bar{x} = 112.5 \times 90.5\,\mu m, n = 15$), on the upper leaf surface, brown to black, gregarious, unilocular, circular, coriaceous, with a central ostiole, when mature, up to 230 μm. *Asci* 54−60×11−13 μm ($\bar{x} = 57.5 \times 12$ μm, $n = 10$), (6-)8–spored, bitunicate, fissitunicate, attached on the basal peridium, clavate, with a gelatinous pedicel and ocular chamber. *Ascospores* 10−15×4−6 μm ($\bar{x} = 13 \times 5$ μm, $n = 15$), irregularly biseriate, hyaline, aseptate, unicellular, ellipsoid to broadly fusoid, but much wider in the middle, smooth, thick-walled, with mucilaginous pads at each end. *Pycnidia* 65−153 μm long, 64−130 μm diam ($\bar{x} = 113 \times 90.5$ μm, $n = 15$), on the upper leaf surface, gregarious, circular, brown to black, coriaceous, with a central ostiole. *Peridium* 7−10 μm ($\bar{x} = 8\,\mu m, n = 10$) thick, comprising brown cells of *textura angularis*. *Conidiogenous cells* lining wall of pycnidium, phialidic, hyaline, cylindrical. *Conidia* 9−11.5×5.5−6.5 μm ($\bar{x} = 10 \times 6.5$ μm, $n = 15$), ellipsoidal, hyaline, aseptate, smooth-walled, surrounded by a mucilaginous sheath 0.7− 0.9 μm ($\bar{x} = 0.83$ μm, $n = 15$) thick, bearing a single apical appendage, usually 2–5 μm long ($\bar{x} = 4.5$ μm, $n = 15$).

Culture characteristics: On OA, Colonies appeared flat with an irregular margin, initially hyaline with abundant mycelium, gradually becoming greenish after 3–4 d. *Conidiophores* produced conidial masses on media. On MEA, colonies appeared woolly, puffy, flat, irregular, initially white with abundant mycelium, gradually becoming greenish to dark green after 2–3 d and white hyphae on the undulate margin, eventually turning black; reverse dark green to black. At 27 °C, in the dark, mycelium reached the edge of the Petri-dish in 20 d with a growth rate of 0.45 cm per day. On PDA, colonies appeared woolly, rather fast growing, initially white with abundant mycelium, gradually becoming greenish to dark green after 2–3 d and white hyphae on the undulate margin, eventually turning dark green to black; reverse black. After 15 days in the dark at 27 °C, mycelium reached the edge of the Petri-dish with a growth rate of 0.60 cm per day.

Material examined: THAILAND, Chiang Rai, Muang District, T. Nanglae, Pa Sang Wiwat, on necrotic leaf spot on leaf of *Crinum* sp. July 2011, S. Wikee CPC20271 (MFLUCC 10–0132).

Pyrenostigme Syd., Ann. Mycol. 24: 370 (1926)

MycoBank: MB4602

Parasitic on living leaves of *Siparunea patelliformis*. *Ascomata* black to dark brown, semi-immersed to superficial, scattered, globose to subglobose, thick walled. *Peridium* composed of brown to black, darkly pigmented, small, thick-walled cells of *textura angularis*. *Pseudoparaphyses* not observed. *Asci* 8–spored, bitunicate, fissitunicate, clavate to broadly-clavate, with a short, narrow, furcate pedicel, and with an ocular chamber. *Ascospores* biseriate, hyaline, aseptate, fusiform to ellipsoid. *Asexual state* not established.

Notes: This genus is clearly typical of *Botryosphaeriales* and appears to be distinct from other genera in the order. We accept it in this study but it should certainly be recollected and sequenced to confirm its uniqueness as a genus.

Generic type: *Pyrenostigme siparunae*

Pyrenostigme siparunae Syd., Ann. Mycol. 24: 370 (1926)

MycoBank: MB278247 (Fig. 32)

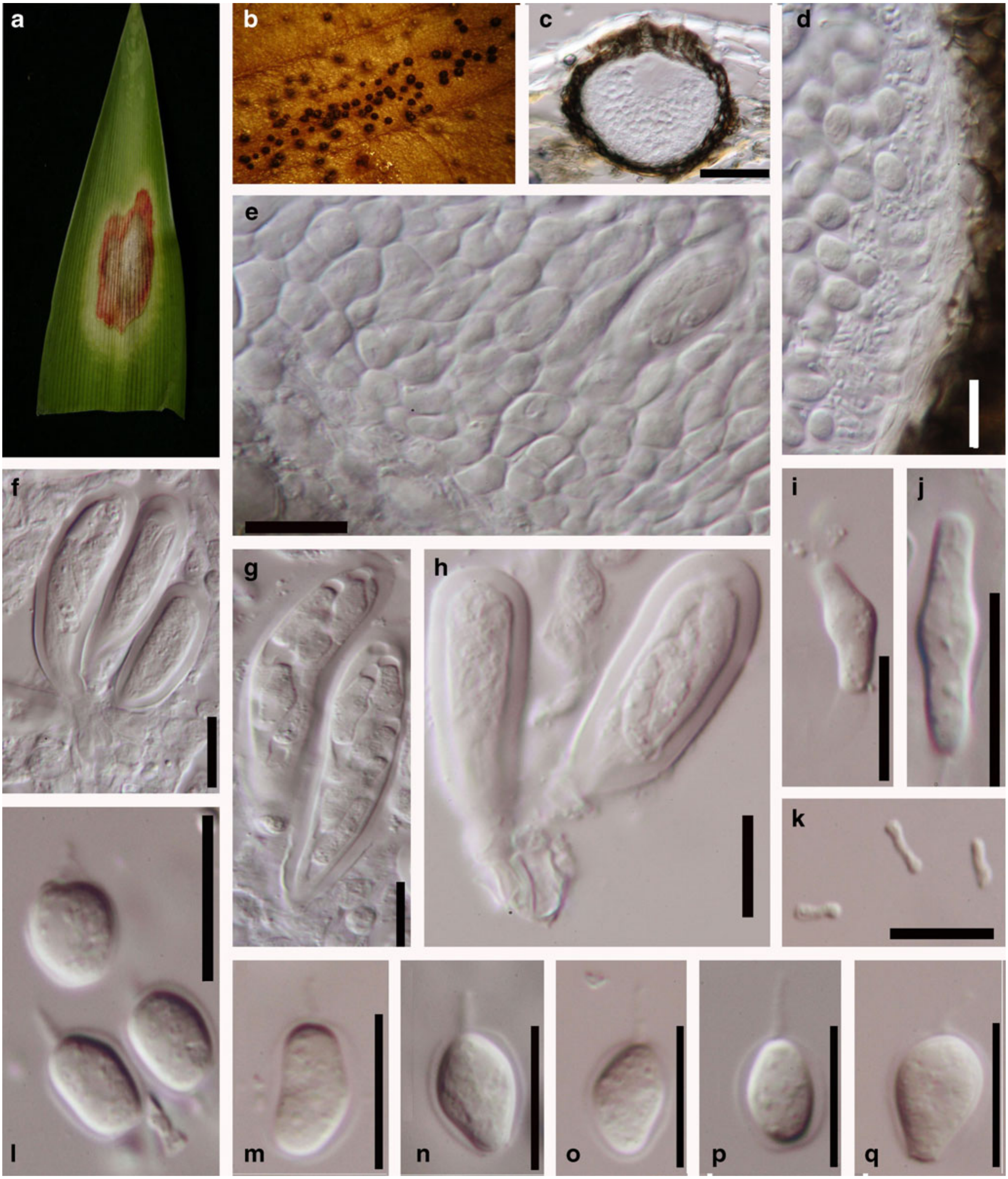

Fig. 31 *Phyllosticta capitalensis* on *Crinum* sp. (CPC20271) **a** Disease symptoms on living leaves of *Crinum* sp. **b** Pycnidia and ascostromata developing on host substrate. **c**–**e** Section through pycnidia showing conidiophores, conidia and spermatia. **f**–**h** Asci. **i**–**j** Ascospores. **k** Spermatia state **l**–**q** Conidia. Scale bars **c** = 50 µm, **e**–**d** = 10 µm, **f**–**h** = 20 µm, **i**–**q** = 10 µm

Parasitic on living leaves of *Siparunea patelliformis*. *Ascomata* 130–170 µm high, 150–180 µm wide ($\bar{x} = 156 \times 169\,\mu m, n = 10$), semi-immersed to superficial, scattered, globose to subglobose, black to dark brown, thick-

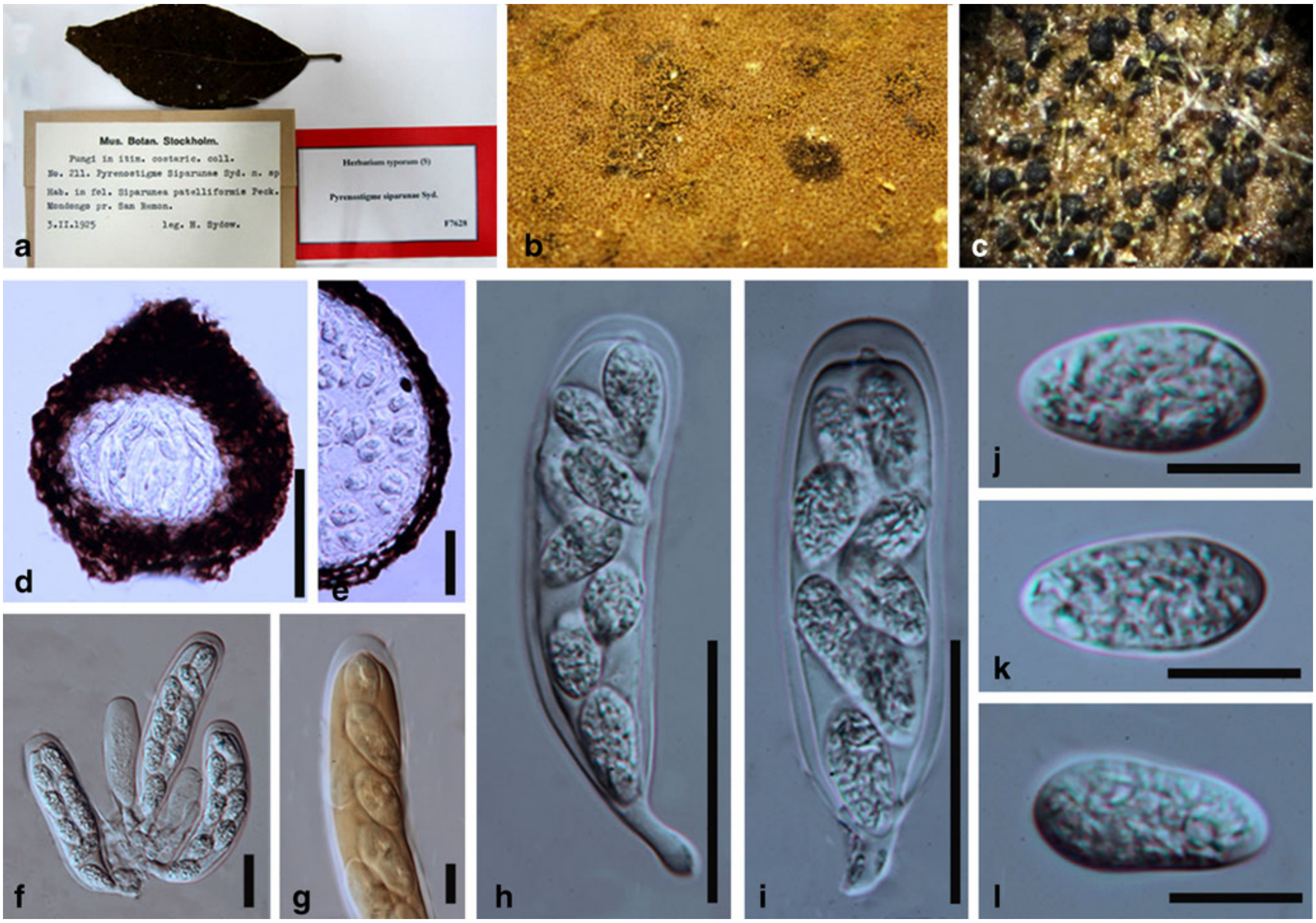

Fig. 32 *Pyrenostigme siparunae* (S−F7628, lectotype) **a** Herbarium packet **b−c** Ascostromata on host substrate. **d** Section of ascostroma (TS). **e**. Section of peridium comprising a few layers of cells. **f−i** Asci. **j−l** Ascospores. Scale bars: **d** = 80 μm, **e** = 50 μm, **f−g** = 20 μm, **h−I** = 50 μm, **j−l** = 10 μm

walled, apex usually widely porate, papillate. *Peridium* up to 15–40 μm wide $(\bar{x} = 21\,\mu m, n = 10)$, 3–10 layered, composed of brown to black, darkly pigmented, small, thick-walled cells *textura angularis*, with outer peridial wall fused with the host cells. *Pseudoparaphyses* not observed. *Asci* 60–90×13–20 μm $(\bar{x} = 75 \times 20\,\mu m, n = 20)$, 8−spored, bitunicate, fissitunicate, clavate to broadly-clavate, with a short, narrow, furcate pedicel, rounded at apex with a 3–5 μm high ocular chamber. *Ascospores* 15–20×7–10 μm $(\bar{x} = 17 \times 8\,\mu m, n = 40)$, biseriate or distichously arranged, partially overlapping, hyaline, aseptate, fusiform to ellipsoid, straight or somewhat curved, with verrucose spore wall. *Asexual state* not established.

Material examined: COSTA RICA, Alajuela, near Mondongo, on living leaves of *Siparunea patelliformis* Peck, 3 February 1925, San Ramon, H. Sydow 211, (S −F7628, lectotype designated here)

Saccharata Denman & Crous, CBS Diversity Ser. 2: 104 (2004)

MycoBank: MB28918

Saprobic on dead leaves. *Ascomata* black, erumpent, solitary, scattered, subglobose to ovoid, rough-walled, papillate. *Papilla* central, with a short neck. *Peridium* composed of brown pseudoparenchymatous cells of *textura globulosa*. *Pseudoparaphyses* hyphae-like, anastomosing mostly above the asci. *Asci* 8–spored, bitunicate, fissitunicate, cylindrical to fusiform, pedicellate, apically rounded with an ocular chamber. *Ascospores* uniseriate, hyaline, aseptate, guttulate, ellipsoidal, clavate, fusiform to broad fusiform, tapering to obtuse ends, smooth-walled. *Conidiomata* pycnidial, dark brown, eustromatic, immersed, subepidermal, separate, uni−to multilocular, walls consisting of dark brown *textura angularis*, ostiolate. *Fusicoccum* asexual morph: *Conidiophores* hyaline, smooth, branched, subcylindrical, 1–3 septate, formed from the inner layer of the locule, intermingled with hyaline, septate paraphyses. *Conidiogenous cells* enteroblastic, phialidic, hyaline, smooth, cylindrical, discrete or intergrated. *Conidia* hyaline, aseptate, smooth, clavate, thin-walled, apex subobtuse, base truncate. The *microconidial state* occurs in the same or in separate conidiomata to the *Fusicoccum* asexual morph.

Microconidiophores hyaline, cylindrical, 1–3 septate, smooth, branched. *Microconidiogenous cells* phialidic, hyaline, smooth, cylindrical, discrete or integrated. *Microconidia* brown, aseptate, subcylindrical to narrowly ellipsoid with rounded ends, thick-walled, finely verruculose, guttulate. The *spermatial state* occurs in conidiomata with the *Fusicoccum* asexual morph, or in separate *spermatogomia*. *Spermatiophores* hyaline, 1–3 septate, cylindrical, smooth, branched. *Spermatiogenous cells* hyaline, cylindrical, discrete or integrated, smooth. *Spermatia* hyaline, aseptate, rod−shape with rounded ends, smooth (asexual morph description follows Denman et al. 1999).

Notes: *Saccharata* was introduced as a monotypic genus based on *S. proteae* (≡ *Phyllachora proteae* Wakef) by Crous et al. (2004) to accommodate species having unilocular, immersed ascomata, as well as a "Fusicoccum"-like asexual morph, with a "Diplodia"-like synanamorph with brown, narrowly ellipsoidal, thick-walled, conidia. Doidge (1942) suggested that *Botryosphaeria* would possibly be a better genus to place *Phyllachora proteae* (Wakefield 1922) based on the ascomatal wall being continuous with, and smaller in structure to the clypeus. Denman et al. (1999) observed a "Fusicoccum"-like asexual morph which was formed in culture and proposed a new combination in *Botryosphaeria proteae* for *Phyllachora proteae* based on its bitunicate asci and ascospore morphology. By employing ITS DNA molecular sequence data, Denman et al. (2000) recognized two correlating clades of *Botryosphaeria*, namely *Diplodia* and *Fusicoccum*. However, *B. proteae* was not congeneric with these two clades. Recent phylogenetic studies using single and combined genes (Crous et al. 2006; Schoch et al. 2009a) showed *Saccharata* to be a distinct genus that is basal in the *Botryosphaeriales*. In this study, *Saccharata* clustered together with *Phyllosticta* and formed a clade with *Melanops* at the base of the *Botryosphaeriales*. This basal clade may be a distinct family in *Botryosphaeriales*.

Generic type: *Saccharata proteae* (Wakef.) Denman & Crous

Saccharata proteae (Wakef.) Denman & Crous., CBS Diversity Ser. 2: 104 (2004)

MycoBank: MB370531 (Fig. 33)

≡ *Phyllachora proteae* Wakef., Bull. Misc. Inf., Kew: 164 (1922)

Saprobic on dead leaves. *Ascostromata* black, 190–230 µm high×240–340 µm diam., immersed, becoming erumpent, but still under host tissue, solitary, scattered, or in small groups of 2–3, subglobose to ovoid, rough-walled, papillate. *Papilla* central, with a short neck, ostiole with a pore, up to 100 µm long. *Peridium* 30–40 µm wide, one-layered, up to 6–23 µm wide, composed of brown pseudoparenchymatous cells of *textura globulosa*, cell wall 2–3 µm thick, near the base composed of hyaline hyphae with numerous asci, up to 20 µm thick. *Pseudoparaphyses* 0.8−1.5 µm broad, hyphae-like, anastomosing mostly above the asci. *Asci* 90–110×7.5−10 µm ($\overline{x} = 97 \times 9\,\mu m, n = 10$), 8–spored, bitunicate, fissitunicate, cylindrical to fusiform, with a 17.5−27.5 µm long bifurcate pedicel, apically rounded with a large ocular chamber up to 2.5 µm wide×4 µm high. *Ascospores* 14−15.5×(5.5-)6−7.5 µm ($\overline{x} = 7 \times 14.5\,\mu m,\ n = 10$), uniseriate, hyaline, aseptate, ellipsoidal, clavate, fusiform to broad fusiform, tapering to obtuse ends, guttulate, smooth-walled. *Conidiomata* pycnidial, dark brown, eustromatic, to 450 µm diam, immersed, subepidermal, separate, uni- to multilocular, walls consisting of dark brown *textura angularis*, ostiolate. *Fusicoccum* asexual morph: *Conidiophores* 20–40×3–4.5 µm, hyaline, subcylindrical, 1–3 septate, smooth, branched, formed from the inner layer of the locule, intermingled with hyaline, septate paraphyses. *Conidiogenous cells* 20–30×2.5−3.5 µm enteroblastic, phialidic, hyaline, cylindrical, discrete or intergrated, smooth. *Conidia* (20-)22−25(−30)×(4.5-)5−6 µm, hyaline, aseptate, clavate, smooth, thin-walled, widest in the middle or upper third of the conidium, apex subobtuse, base truncate. The *microconidial state* occurs in the same or in separate conidiomata to the *Fusicoccum* asexual morph. *Microconidiophores* 15–25×2–3 µm, hyaline, cylindrical, 1–3 septate, smooth, branched. *Microconidiogenous cells* 6–10×2−3 µm, phialidic, hyaline, cylindrical, smooth, discrete or integrated. *Microconidia* (7-)8−11(−14)×2.5−3.5 µm brown, aseptate, subcylindrical to narrowly ellipsoid with rounded ends, thick-walled, finely verruculose, guttulate. The *spermatial state* occurs in conidiomata with the *Fusicoccum* asexual morph, or in separate spermatogomia. *Spermatiophores* 15–20×3–4 µm, hyaline, cylindrical, 1–3 septate, smooth, branched. *Spermatiogenous cells* 10–12×2–3 µm, hyaline, cylindrical, discrete or integrated. *Spermatia* 5–7×1.5−2 µm, hyaline, aseptate, rod-shape with rounded ends, smooth.

Material examined: SOUTH AFRICA, Western Cape Province, Klapmuts, on dead leaves of *Protea repens* (as *P. mellifera*), 5 June, 1997, P. Van Der Bijl. No. 357 (PREM 32915, holotype).

Sivanesania W.H. Hsieh & Chi Y. Chen, Mycol. Res. 100: 1106 (1996)

MycoBank: MB26498

Pathogenic on stems and petioles of *Rubi kawakamii*. *Ascostromata* immersed, erumpent, becoming superficial, scattered, multilocular, subcuticular to subepidermal, pulvinate, cells of ascostromata of brown-walled cell of *textura globulosa to angularis*. *Locules* numerous, globose to compressed, forming in a single layer. *Ostioles* inconspicuous. *Peridium* composed of dark brown cells. *Pseudoparaphyses* hyphae-like, septate, branched. *Asci* 8–spored, bitunicate,

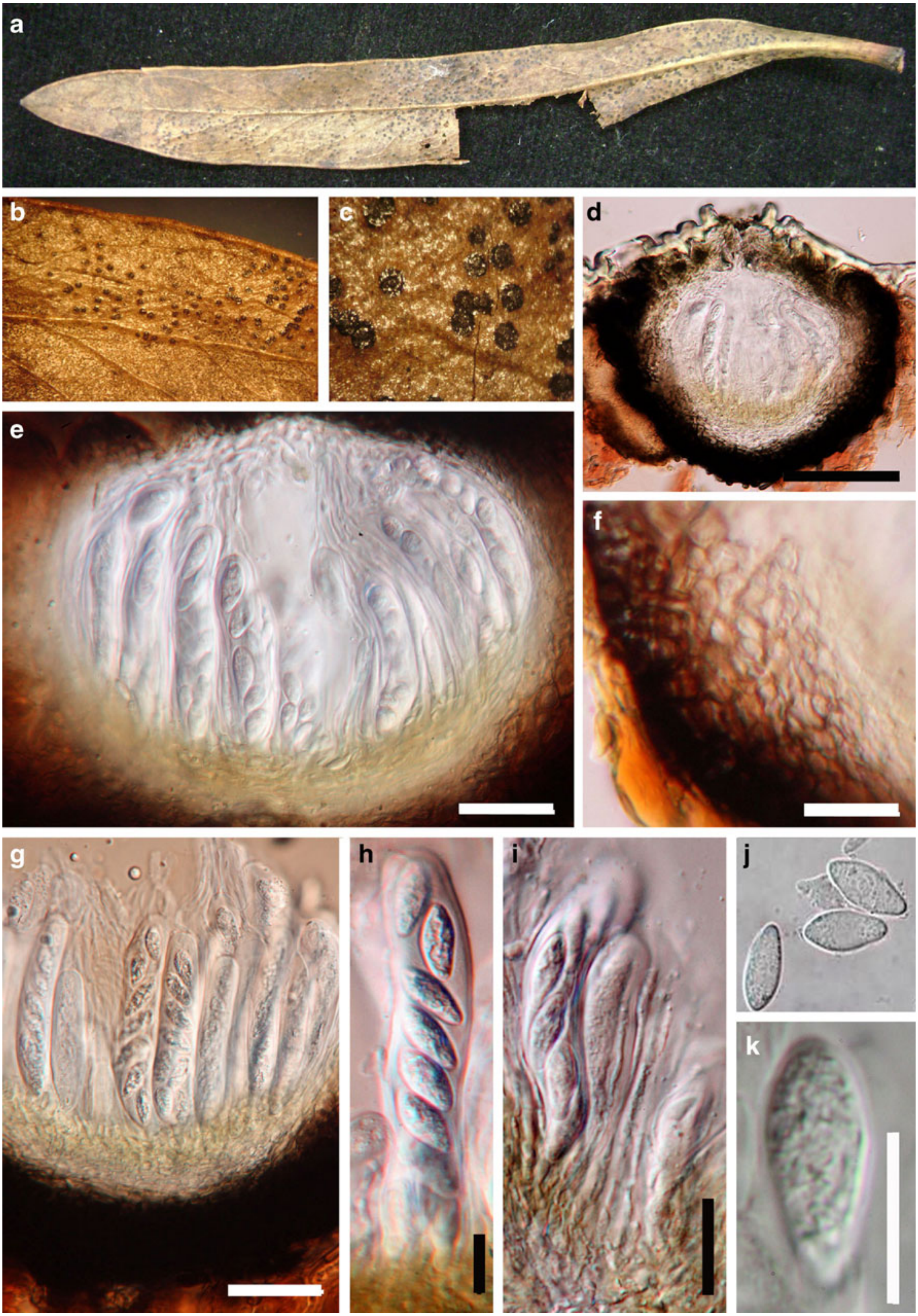

Fig. 33 *Saccharata proteae* (PREM 32915, holotype). **a–c** Habit, ascostromata on the host substrate. **d–e** Section of ascostroma. **e, g–i** Asci. **f** Peridium. **j–k** Ascospores. Scale bars **d** = 50 µm, **e, g** = 20 µm, **f** = 10 µm, **h–I, k** = 10 µm

fissitunicate, clavate, short pedicellate, apically rounded and thickened, with an inconspicuous ocular chamber. *Ascospores* hyaline to brown when old, ovoid, with a hyaline, filiform, simple appendage. *Asexual state* not established.

Notes: *Sivanesania* was introduced as a monotypic genus by Hsieh and Chen (1994) based on *Sivanesania rubi* W.H. Hsieh & Chi Y. Chen which is pathogenic on stems and petioles of *Rubi kawakamii.* The morphological characters of the fungus such as immersed, erumpent, multilocular ascostromata, hyaline, septate pseudoparaphyses and hyaline to brown, aseptate ascospores with an appendage fit well with *Botryosphaeriaceae*. It is most similar to *Botryosphaeria*, *Phyllosticta* and *Saccharata*, but differs by its multilocular ascostromata (*Phyllosticta* and *Saccharata* have uni-locular ascostromata) and aseptate ascospores with a basal filiform, hyaline, simple appendage. No asexual morph has been reported for this genus. No molecular sequence data is available, and therefore fresh collections are needed to confirm the phylogeny. In this study, we accept this genus in *Botryosphaeriaceae* based on morphology.

Generic type: *Sivanesania rubi* W.H. Hsieh & Chi Y. Chen

Sivanesania rubi W.H. Hsieh & Chi Y. Chen, Mycol. Res. 100: 1106 (1996)

MycoBank: MB415938 (Fig. 34)

Pathogenic on stems and petioles of *Rubi kawakamii*. *Ascostromata* immersed, erumpent, becoming superficial, scattered, multilocular, subcuticular to subepidemal, slightly convex, hyphae penetrating the underlying plant host tissue beneath the ascostromata, cells of ascostromata of brown-walled cell of *textura globulosa to angularis*. *Locules* numerous, formed in a single layer, globose to compressed globose, up to 190 μm wide. *Ostiole* central, inconspicuous. *Peridium* of locule a single thin layer, 100−120 μm wide. *Pseudoparaphyses* hyphae-like, septate, branched. *Asci* 85–110×17–22 μm, 8–spored, bitunicate, fissitunicate, clavate, with a short pedicel, apically rounded and thickened, with an inconspicuous ocular chamber. *Ascospores* 16–25×8–11 μm, irregularly biseriate in the ascus, hyaline to brown when old, ovoid to nongranulose, with a basal cellular, hyaline, simple, filiform appendage. *Asexual state* not established.

Material examined: TAIWAN, Hsianyang, Taitung Hsien, pathogenic on petiole of *Rubi kawakamii* (*Rosaceae*), 10 May 1991, C.Y. Chen, NCHUPP 2234 (IM1356634, holotype).

Spencermartinsia A.J.L. Phillips, A. Alves & Crous, Persoonia 21: 51 (2008)

MycoBank: MB511762

Saprobic or *endophytic* on plants. *Ascostromata* black, multilocular, solitary or in botryose clusters, immersed, erumpent, with four to numerous locules, with individual ostioles, cells of ascostromata of brown-walled *textura angularis*. *Peridium* of locules two-layered, outer layer composed of small heavily pigmented thick-walled cells of *textura angularis*, inner layer composed of hyaline thin-walled cells of *textura angularis. Pseudoparaphyses* hyphae-like, septate, constricted at septa. *Asci* 8–spored, bitunicate, fissitunicate, clavate, pedicellate, with an ocular chamber. *Ascospores* hyaline to brown, uniseptate with an apiculus at each end. *Conidiomata* stromatic.

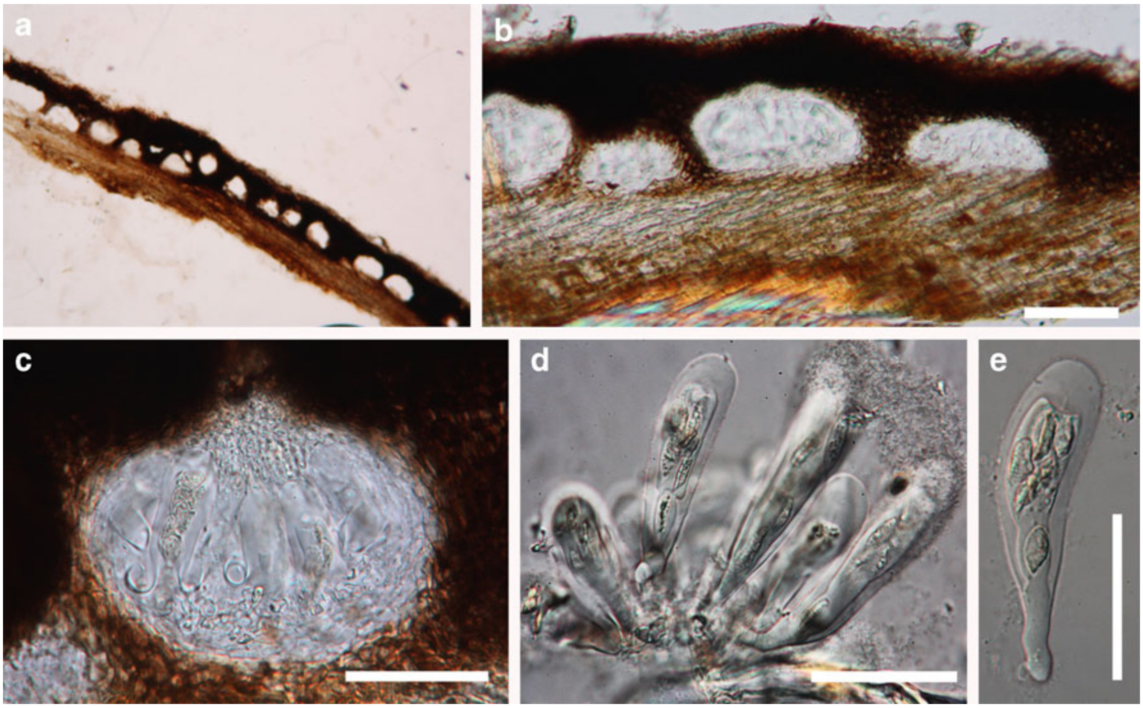

Fig. 34 *Sivanesania rubi* (IM1356634, holotype) **a−b** Sections of ascostromata. **b** Section through ascostroma. **d−e** Asci. Scale bars: **b−e** = 50 μm

Conidiogenous cells lining inner surface of conidiomata, cylindrical to broadly lageniform, holoblastic. *Conidia* hyaline to brown, oblong to subcylindrical, septate, constricted at the septum, thick-walled, often with a truncate base.

Notes: Phillips et al. (2008) introduced *Spencermartinsia* as a monotypic genus for *S. viticola* (A.J.L. Phillips & J. Luque) A.J.L. Phillips, A. Alves & Crous. It is close to *Botryosphaeria iberica* and *B. sarmentorum* due to the similar morphology of asexual morph "Dothiorella". However, they can be distinguished by their ascospores as in *S. viticola* ascospores have a rounded projection at the tip and base. When the new genus *Spencermartinsia* was introduced, *Dothiorella viticola* was reclassified as *S. viticola* (Phillips et al. 2008). Subsequently, Pérez et al. (2010) described the second species, an endophyte, *Spencermartinsia uruguayensis* C.A. Pérez, R.A. Blanchette, B. Slippers & M.J. Wingfield, based on the phylogeny and morphology of the asexual morph formed in culture. *Spencermartinsia* formed a complex group with *Dothiorella* and as it is difficult to distinguish them based on asexual-morphs, a combined gene phylogenetic analysis has thus been used to differentiate these genera. In this study, *Dothiorella* and *Spencermartinsia* have been shown to be distinct genera in *Botryosphaeriaceae* (Fig. 1).

Generic type: *Spencermartinsia viticola* (A.J.L. Phillips & J. Luque) A.J.L. Phillips, A. Alves & Crous

Spencermartinsia viticola (A.J.L. Phillips & J. Luque) A.J.L. Phillips, A. Alves & Crous, Persoonia 21: 51 (2008)

MycoBank: MB511763 (Fig. 35)

≡ *Botryosphaeria viticola* A.J.L. Phillips & J. Luque, Mycologia 97: 1118 (2006) [2005]

Saprobic on canes of *Vitis*. *Ascostromata* black, pseudothecial, solitary or in botryose clusters, initially immersed in host, erumpent at maturity, multilocular, with four to numerous locules, with individual ostioles, *Ostiole* circular, central, papillate; up to 40 μm thick, dark brown comprising cells of thick-walled *textura angularis*, cells of ascostromata brown-walled *textura angularis*. *Peridium* of locules two-layered, outer layer composed of small heavily pigmented thick-walled cells of *textura angularis*, inner layer composed of hyaline thin-walled cells of *textura angularis. Pseudoparaphyses* hyphae-like, septate, slightly constricted at septum, up to 3–4 μm wide. *Asci* 100–110×25–30 μm, 8–spored, bitunicate, fissitunicate, clavate, pedicellate, with a well-developed ocular chamber, arising from base of the ascoma. *Ascospores* irregularly biseriate, 21–24×9–11.5 μm, 1–septate, brown to dark brown, ovate to subclavate, slightly constricted at septum, thick-walled, often with a small rounded projection at the apex and base, with basal cell tapering into an obtuse base. *Conidiomata* pycnidial, black, separate or aggregated into botryose clusters, immersed, then erumpent, spherical to globose, unilocular, thick-walled, wall of three layers of dark brown cells *textura angularis*. *Ostiole* single, central, circular. *Conidiophores* hyaline, cylindrical. *Conidiogenous cells* discrete or integrated, holoblastic, hyaline, smooth, cylindrical to broad lageniform. *Conidia* 17–21×9–10 μm brown, oblong to sub-cylindrical, septate, slightly constricted at septum, thick-walled, often with a truncate base.

Material examined: SPAIN, Catalonia, Vimbodí, near the Monastery of Poblet, on pruned canes of *Vitis vinifera* cv. Garnatxa Negra, 12 Aug. 2004, J. Luque & S. Martos, (LISE 95177, holotype).

Vestergrenia Rehm, Hedwigia 40: 101 (1901)

MycoBank: MB5733

Saprobic on leaves. *Ascostromata* solitary, scattered, or in small groups, especially forming on leaf veins, superficial, subglobose or globose, black, coriaceous. *Peridium* composed of a single stratum, comprising 3–4 layers of brown pseudoparenchymatous cells of *textura angularis/globulosa*. *Pseudoparaphyses* not observed. *Asci* 8–spored, bitunicate, broadly clavate to ovoid, with a long pedicel, apically rounded with an ocular chamber. *Ascospores* irregularly 2–3–seriate, hyaline, aseptate, ellipsoidal-ovoid. *Asexual state* not established.

Notes: This appears to be a poorly studied genus with the last species, *Vestergrenia ixorae* C. Ramesh, being described in 1988 (Ramesh 1988). The genus has 23 epithets (*Index Fungorum*, MycoBank). *Vestergrenia* was introduced by Rehm (1901) in the "Sphaeriaceae" as a monotypic genus represented by *V. nervisequia*. Luttrell (1973) transferred this genus into *Dothideaceae* based on separate ascomata, broad-clavate to ovoid asci which lie in long, slender stalks of varying lengths and standing at differing heights in the locule and unicellular ascospores. There has been no phylogenetic study of this genus to confirm its taxonomic placement in *Dothideaceae*. However, the generic type is completely different to generic type of *Dothidea* where superficial pulvinate ascostromata contain numerous locules in an outer layer, and ascospores are 2-celled (Schoch et al. 2009a) The genus is more typical of *Botryosphaeriaceae* in having unicellular ascospores, widely clavate asci with distinct pedicels and ascomata with brown, relatively thick-walled cells of *textura angularis/globulosa*. We tentatively include *Vestergrenia* in *Botryosphaeriaceae* until fresh collections are made and this can be verified with phylogenetic analysis. The other species in the genus need examining to check their placement.

Generic type: *Vestergrenia nervisequia* Rehm.

Vestergrenia nervisequia Rehm, Hedwigia 40: 101 (1901)

MycoBank: MB221417 Fig. 36

= *Guignardiella nervisequia* (Rehm) Sacc. & P. Syd., Syll. Fung. 16: 465 (1902)

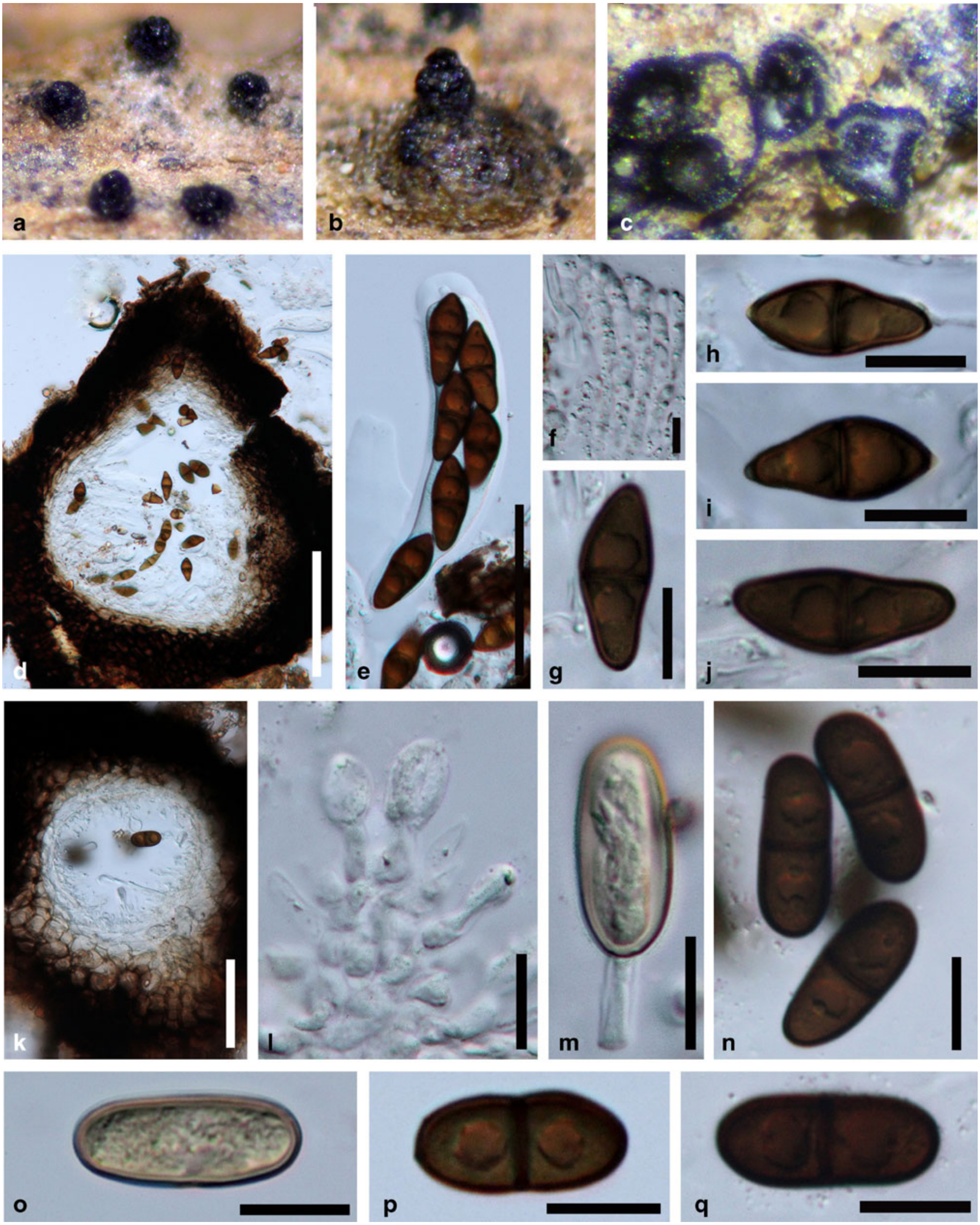

Fig. 35 Sexual (**a**–**j**) and asexual (**k**–**q**) morphs of *Spencermartinsia viticola* (LISE 95177, holotype) **a**–**c** Ascostromata on host substrate, note the cross section in surface view in c. **d** Section through ascostromata and peridium **e** Ascus. **f** Pseudoparaphyses. **g**–**j** Ascospores. **k** Section through conidioma. **l**–**m** Conidiogenous cells and developing conidia. **n**–**q** Dark brown conidia with 1–septa and light brown young aseptate conidia. Scale Bars: **d** = 100 μm, **e** = 50 μm, **f**–**j** = 10 μm, **k** = 50 μm, **l**–**q** = 10 μm

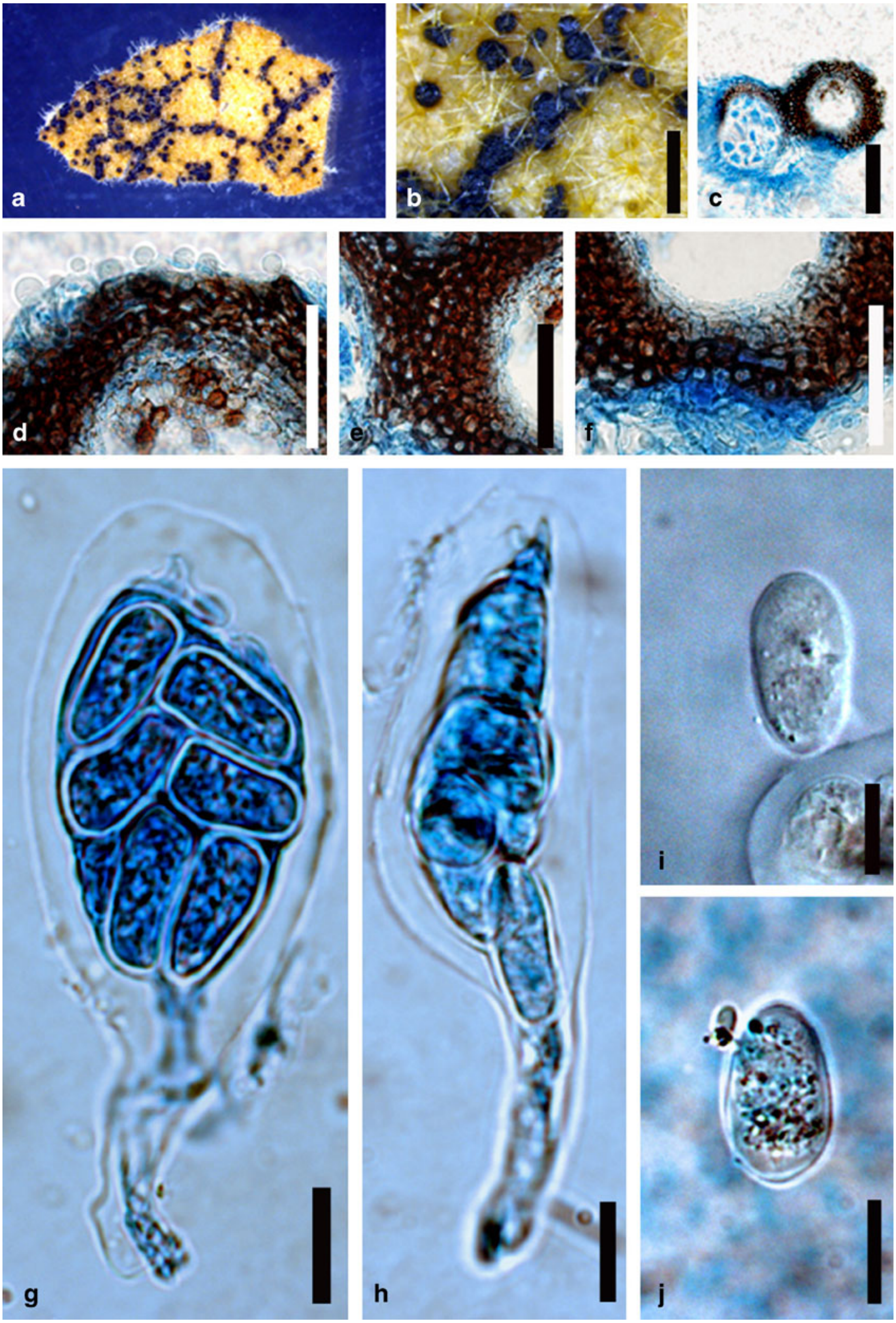

Fig. 36 *Vestergrenia nervisequia* (SF10703, holotype) **a** Appearance of ascostromata on host substrate, scattered mostly on leaf veins. **b** Appearance of ascostromata. **c−f** Vertical sections through ascostromata illustrating the peridium (in lactophenol in cotton blue). **g−h** Asci stained in lactophenol in cotton blue. **i−j** Ascospores. Note the guttules. Scale bars: **a** = 1 mm, **b** = 500 μm, **c** = 100 μm, **d−f** = 50 μm, **g−j** = 10 μm

Saprobic on the upper surface of leaves. *Ascostromata* 170–280 µm diam × 140–160 µm high, solitary, scattered, or in small groups of 2–6, especially forming on leaf veins, superficial, subglobose or globose, black, membranaceous, apapillate. *Ostioles* not distinct. *Peridium* 14–35 µm wide, composed of a single stratum, up to 16−31 µm thick, comprising 3–4 layers of brown pseudoparenchymatous cells of *textura angularis/globulosa*. *Pseudoparaphyses* not observed. *Asci* 62–68 × 25–29 µm ($\overline{x} = 65.5 \times 27.5\, \mu m, n = 15$), 8–spored, bitunicate, fissitunicate, broadly clavate to ovoid, with a 18–20 µm long pedicel, apically rounded with an ocular chamber. *Ascospores* 18–23 × 11–14 µm ($\overline{x} = 20.5 \times 12.5\, \mu m, n = 20$), irregularly 2–3–seriate, hyaline, aseptate, ellipsoidal-ovoid, guttulate, smooth-walled. *Asexual state* not established.

Material examined: BRAZIL, Rio de Janeiro, on leaves of *Solani*, 20 July 1887, Ule no. 734. H. Bresl. (SF10703, holotype).

Genera not studied

Aplosporella Speg., Anales Soc. Ci. Argent. 10: 157 (1880)

Possible synonyms

Epicyta Syd., Ann. Mycol. 24: 413 (1926)

Haplosporella subgen. *Pleosphaeropsis* (Died.) Petr. & Syd., Beih. Reprium nov. Spec. Regni veg. 42: 103 (1926)

Microhaplosporella Sousa da Câmara, Agron. lusit. 11: 63 (1949)

Pleosphaeropsis Died., Ann. Mycol. 14: 203 (1916)

Podosporium Bonord., Handb. Allgem. Mykol. 227 (1851)

Podosporium Sacc. & Schulzer, (1884)

Notes: A new species of *Aplosporella* was described by Damm et al. (2007b) and was shown to belong in *Botryosphaeriaceae.* Two species of *Aplosporella* cluster in *Botryosphaeriaceae* in Fig. 1 in this study. The genus appears to have no designated generic type and its 330 epithets are likely to be polyphyletic (Damm et al. 2007b) and thus the genus requires further study.

Dichomera Cooke, Nuovo G. Bot. Ital. 10: 24 (1878)

Notes: This genus has 48 epithets and has also been recorded as a synanamorph of some genera of *Botryosphaeriaceae* and requires a modern treatment.

Diplodia Fr., in Montagne, Annls Sci. Nat., Bot., sér. 2 1: 302 (1834)

Possible synonyms

Cryptosphaeria Grev., Scott. Crypt. Fl. 1: pl. 13 (1822)

Holcomyces Lindau, Verh. Bot. Ver. Prov. Brandenb. 45: 155 (1904)

Notes: This is a well-supported genus in *Botryosphaeriaceae* (Fig. 1). It has 1245 epithets and seriously needs a modern treatment. The type has been studied by Alves et al. (2004) and is characterized by erumpent conidiomata in which hyaline conidia develop which become pale brown (dark brown in some species) and 1–septate at maturity. The generic type *Diplodia mutila* Fr. has a "Botryosphaeria stevensii" sexual state.

Dothiorella Sacc., Michelia 2(no. 6): 5 (1880)

Possible synonym

Macrophomopsis Petr., Ann. Mycol. 22: 108 (1924)

Notes: This is a well-supported genus in *Botryosphaeriaceae* (Phillips et al. 2005 and Fig. 1 in this study). The generic type is *Dothiorella pyrenophora* Berk. ex Sacc., Michelia 2(no. 6): 5 (1880) and the genus has 362 epithets and seriously needs a modern treatment. Jami et al. (2012) described two new species in the genus. There may be some confusion over the generic type which is listed under *Diplodia* in *Index Fungorum* and does not appear to have been recently treated or have sequence data.

Endomelanconiopsis E.I. Rojas & Samuels, Mycologia 100: 770 (2008)

Notes: This new genus was described as a distinct lineage of *Botryosphaeriaceae* based on multigene analysis of LSU, ITS and EF1-α. The taxon was isolated as an endophyte from leaves of *Theobroma cacao* and a second species combined *Endomelanconium microsporum* Verkley & van der Aa (Rojas et al. 2008). The genus is distinct in having small brown ellipsoidal to limoniform conidia which are dark brown with a single longitudinal slit three-quarters of the length of the conidia when mature and hyaline microconidia.

Macrophomina Petr., Ann. Mycol. 21: 314 (1923)

Notes: Based on eight isolates of *Macrophomina phaseolina* (Tassi) Goid. This is a well-supported genus in *Botryosphaeriaceae* (Crous et al. 2006, Fig. 1 this study). The generic type is *Macrophomina philippinensis* Petr. and has not been subjected to phylogenetic study. The genus has seven epithets and needs a modern treatment.

Microdiplodia Allesch., Rabenh. Krypt.-Fl., Edn 2 1(7): 78 (1901) [1903]

Possible synonyms

Microbotryodiplodia Sousa da Câmara, Agron. Lusit. 13: 206 (1951)

Syndiplodia Peyronel, Mem. R. Accad. Sci. Torino, Ser. 2 66(10): 35 (1915)

Notes: This genus is likely to be polyphyletic; the generic type *Microdiplodia conigena* Allesch. is linked to *Botryosphaeriaceae* in *Index Fungorum*. With 382 epithets this genus needs a modern treatment.

Neoscytalidium Crous & Slippers, Stud. Mycol. 55: 244 (2006)

Notes: This is a well supported genus which has two species (Crous et al. 2006, Fig. 1 this paper) and a "Scytalidium"-like synanamorph (Pavlic et al. 2008; Madrid et al. 2009).

Pseudofusicoccum Mohali, Slippers & M.J. Wingf., Stud. Mycol. 55: 249 (2006)

Notes: This is a well-supported genus in *Botryosphaeriaceae* with six species (Crous et al. 2006, Pavlic et al. 2008, Fig. 1 this paper).

Tiarosporella Höhn., Mitt. Bot. Inst. Tech. Hochsch. Wien 1(3): 82 (1924)

Notes: Jami et al. (2012) described one new species of *Tiarosporella* which is resolved in *Botryosphaeriaceae.* The generic type *Tiarosporella paludosa* (Sacc. & Fiori ex P. Syd.) Höhn. is, however, listed as an asexual state of *Darkera* (Helotiales) in *Index Fungorum*; and thus the four *Tiarosporella* species (Jami et al. 2012) in *Botryosphaeriaceae* may need a new genus to accommodate them depending on the placement of *Tiarosporella paludosa*.

Thyrostroma Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 120: 472 [94 repr.] (1911)

Possible synonyms

Thyrostromella Syd., Ann. Mycol. 22: 406 (1924)

Wilsonomyces Adask., J.M. Ogawa & E.E. Butler, Mycotaxon 37: 283 (1990)

Notes: This genus comprises 22 epithets mostly linked to *Dothidotthia.* No generic type is presently listed and the genus needs a modern treatment.

Discussion

In this paper we use morphology and sequence data from fresh collections and sequence data (types) downloaded from GenBank to detail the *Botryosphaeriales,* treating 15 type genera and describing two new genera and six new species from Thailand.

Phylogenetic resolution of Botryosphaeriales

The 28S rRNA gene (LSU) has been shown to be suitable for distinguishing many ascomycetes at the generic level due to its relatively conserved nature (Crous et al. 2006; Schoch et al. 2006; Hibbett et al. 2007). By choosing comparisons of sequences of LSU, Crous et al. (2006) recognized ten lineages within the *Botryosphaeriaceae* and accepted several genera, including those genera with sexual and/or asexual morphs. Separate names were not introduced for morphs of the newly proposed genera when sexual and asexual morphs were known. With the addition of EF1-α and β-tubulin genes, and molecular data being available for more botryosphaeriaceous taxa, it is now possible to use combined multi-gene data to resolve complex groups such as *Diplodia/Lasiodiplodia*, *Phaeobotryon/Barriopsis* and *Dothiorella/Spencermartinsia* which have yet to be resolved. In addition, new asexual genera and cryptic species have been introduced (Alves et al. 2008; Sakalidis et al. 2011). By combining EF1-α and β-tubulin genes with ITS, Phillips et al. (2005, 2008) reinstated the genus *Neodeightonia* in the *Diplodia/Lasiodiplodia* complex and also showed that the latter asexual genera are morphologically and phylogenetically distinct.

ITS gene sequence data have been used to distinguish the species within the genera of *Botryosphaeriales* (Denman et al. 2000, 2003; Denman et al. 2003; Alves et al. 2004; Barber et al. 2005). However, it has not been possible to apply ITS alone in resolving species in this study, because *Botryosphaeriaceae* embodies species complexes. It is evident that at the generic level, the combined EF1-α and β-tubulin gene analysis is best for delimiting genera of *Botryosphaeriaceae*, as well as the species in several genera of *Botryosphaeriales*. It has also been recommended that the RPB2 gene should be considered in similar multi-combined genes analyses of genus and species levels of *Botryosphaeriales* (Pavlic et al. 2009a, b) and that some new approaches might be used for complex groups, such as Genealogical Sorting Index (GSI), which has been used to resolve the asexual morph of *Neofusicoccum* (Sakalidis et al. 2011).

Maximum Parsimonious (MP), Randomized Axelerated Maximum Likelihood (RAxML) and Mr. Bayes are models for generating phylogenetic trees and were used in this study. Most phylograms were similar when using different models, however the bootstrap values differed. RAxML and Mr. Bayes have been shown to be suitable models for phylogeny at higher taxonomic levels (class, order and family) and large data analysis (Hibbett et al. 2007; Schoch et al. 2009a, b; Suetrong et al. 2009; Liu et al. 2011; Zhang et al. 2012). Felsenstein (2004) suggested that the Bayesian methods are closely related to the likelihood methods, differing only in the use of a prior distribution of the quantity being inferred, which would typically be the tree. Maximum parsimony analysis has been shown to be a better method for establishing taxonomy at the family, genus and species levels. In our molecular data analysis, some of the new species taxonomic positions were not consistent when using the different methods. For example *Auerswaldia lignicola* clustered in the *Diplodia / Lasiodiplodia* clade in both Mr. Bayes and RAxML analysis, but with the *Dothiorella/Spencermartinsia* clade when using the Maximum Parsimony (MP) method. Furthermore, this only occurred in the combined multi-gene (LSU, SSU, EF1-α and β-tubulin) analysis, however when combined EF1-α and β-tubulin analysis was carried out they always clustered in the *Dothiorella / Spencermartinsia* clade. Maximum Parsimony may therefore be a better method for resolving the phylogeny and taxonomy in *Botryosphaeriales*. We also recommend that LSU, EF1-α, β-tubulin and RPB2 genes should be sequenced for differentiating genera, while the latter three genes can resolve cryptic species.

Genera accepted in Botryosphaeriales

Von Arx and Müller (1954) included 15 genera in *Botryosphaeriaceae* (Table 2). This study suggests that *Auerswaldia*, *Auerswaldiella*, *Botryosphaeria*, *Pyrenostigme* and *Vestergrenia* were correctly placed in the family, indicating that von Arx and Müller (1954) were remarkably astute in their understanding and observations. Many of the genera that von Arx and Müller (1954) included were subsequently removed from *Botryosphaeriaceae* by various researchers (Table 2) and in Lumbsch and Huhndorf (2010) only 11 genera were listed for the order. *Bagnisiella* is presently included in *Dothideaceae* (Lumbsch and Huhndorf 2010) as discussed above under *Auerswaldia*. *Cleistosphaeria* as represented by *C. macrostegia* Syd. & P. Syd. is presently included in *Parodiopsidaceae* (Lumbsch and Huhndorf 2010). The ascospores are unicellular and typical of *Botryosphaeriaceae,* whereas the asci are unusual in being widely clavate and ascomata have a peridium comprising a single cell layer (S. Boonmee, pers. obs.). *Montagnellina* is now considered a synonym of *Phyllosticta* (= *Guignardia*) (Wikee et al. 2011a; Wong et al. 2012). *Muyocopron* is typical of *Botryosphaeriaceae* but the almost thyriothecoid ascomata are atypical and molecular data of Wu et al. (2011) exclude this genus. *Ellisiodothis* is treated as a synonym of *Muyocopron* in *Index Fungorum*, while *Microdothella* as represented by *M. culmicola* Syd. & P. Syd. is also probably a synonym. *Trabutia* is a synonym of *Phyllachora* (Barr 1987), while we have not been able to examine *Pilgeriella.* In the present study, we include 29 genera in *Botryosphaeriales*; this includes several genera (i.e. *Dothiorella* and *Phyllosticta*) whose name were previously based on the sexual morphs and thus not treated by von Arx and Müller (1954). *Planistromella* A.W. Ramaley*, Planistroma* A.W. Ramaley, *Mycosphaerellopsis* Höhn., and *Comminutispora* A.W. Ramaley with their asexual states appear to belong in *Botryosphaeriaceae* J. Monkai et al. pers. comm.).

Otthia (Cooke 1871, 1890; Massee 1887; Stevens 1936; Bisby and Mason 1940) which was introduced from *Ulmus* sp., with six species, but without a generic type being named (Fuckel 1870), might be considered for inclusion in *Botryosphaeriaceae.* Booth (1958) selected a lectotype in *O. spiraeae* and considered *Diplodia sarmentorum* (Fr.) Fr. to be the asexual morph. Phillips et al. (2005) redescribed and illustrated *Otthia spiraeae* and placed *Diplodia sarmentorum* in a new species named *Botryosphaeria sarmentorum* A.J.L. Phillips, Alves & Luque. They considered the holotype of *Otthia spiraeae* and the specimen illustrated by Booth (1958) to be from different genera, with *O. spiraeae* having cylindrical asci with a thin endotunica, while Booth's specimen (Fig. 1 in Booth 1958) had clavate asci with a thick endotunica more typical of *Botryosphaeriaceae.* Schoch et al. (2009a) sequenced two strains named *Otthia spiraeae* from CBS (isolated from *Ulmus glabra* by K. & L. Holm in 1987, Sweden, Herbarium, UPS) and these clustered in *Botryosphaeriaceae* (see Fig. 1). However, it is not clear whether the strains used in Schoch et al. (2009a) were correctly identified and therefore the placement of *Otthia* (synonym = *Otthiella* (Sacc.) Sacc. & D. Sacc., Syll. Fung. (Abellini) 17: 662 1905) in *Botryosphaeriaceae* cannot be confirmed until fresh collections identical to the holotype are made and sequenced. It is evident however, that the *Dothiorella* Clade (Fig. 1, Clade A6) in our study, which includes the sequences from putative *Otthia* species, is a distinct genus.

The asexual morphs of *Botryosphaeriaceae* include species with brown, unicellular or bi-celled conidia (*Aplosporella*, *Diplodia*, *Dothiorella*, *Macrophomina*, *Neoscytalidium* and *Lasiodiplodia*) and species with hyaline conidia (*Fusicoccum*, *Neofusicoccum* and *Pseudofusicoccum*). In Table 2 we list the sexual morph against the asexual morph and provide an argument for which name should be used now that only a single name is available for each genus and taxon. Each plate was inoculated with more than three (generally five) single ascospores, derived cultures. We ensured this primarily to obtain secondary or dikaryotic mycelium, which enhanced the formation of sexual or asexual morphs.

It is evident that several groups of botryosphaeriaceous taxa are species complexes and these need to be resolved using multi-gene sequence analysis which should include protein genes. For example, the genus *Lasiodiplodia* is likely to comprise several species complexes (Burgess et al. 2006; Alves et al. 2008; Abdollahzadeh et al. 2010). Other genera which may also comprise species complexes are *Aplosporella, Botryosphaeria, Dothiorella, Neofusicoccum* and *Spencermartinsia* (Phillips et al. 2005; Crous et al. 2006; Damm et al. 2007b; Pavlic et al. 2009a, b; Sakalidis et al. 2011). Cryptic species have also been resolved in several other pathogenic genera using multigene analysis including *Colletotrichum, Fusarium* and *Phyllosticta* (Hyde et al. 2010; Summerell et al. 2010, 2011; Cai et al. 2011; Ko-Ko et al. 2011; Wikee et al. 2011a, b; Damm et al. 2012a, b).

Conclusion and future work

Our data analysis indicates that the order *Botryosphaeriales* may comprise more families than the presently accepted

Botryosphaeriaceae (Lumbsch and Huhndorf 2010). Clade B could be represented by *Phyllostictaceae,* while Clade A splits into three major clades, A1-A3. Clade A1 comprises *Diplodia*, *Neodeightonia* and *Lasiodiplodia* and is characterized by dark brown, septate, striate conidia. Clade A2 comprises *Barriopsis*, *Phaeobotryon* and *Phaeobotryosphaeria*, and characterized by dark to dark brown, aseptate or 2-septate ascospores, with or without an apiculus. Clade A3 includes *Auerswaldia*, *Dothiorella* and *Spencermartinsia*. In these genera the ascospores become brown inside the asci, while the conidia become brown when still attached to the conidiogenous cells. Clade A6 (*Botryosphaeriaceae*) which includes the family type (*Botryosphaeria dothidea*) is characterized by hyaline, aseptate ascospores. We refrain from introducing new families for these clades at this stage until a larger dataset can confirm this.

In this paper we have re-examined the type specimens of 15 genera of *Botryosphaeriales,* collected six new species from Thailand and used 124 *Botryosphaeriaceae* strains with sequence data to derive a modern treatment for the order. There is however still much research to be carried out with resolution of families and genera, linkage of sexual and asexual morphs and differentiation of cryptic species.

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