



Resolving the phylogenetic and taxonomic status of dark-spored teleomorph genera in the Botryosphaeriaceae

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Key words

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SSU

Abstract Species in the Botryosphaeriaceae are common plant pathogens and saprobes found on a variety of mainly woody hosts. Teleomorphs typically have hyaline, aseptate ascospores. However, some have been reported with brown ascospores and their taxonomic status is uncertain. A multi-gene approach (SSU, ITS, LSU, EF1- α and β -tubulin) was used to resolve the correct phylogenetic position of the dark-spored '*Botryosphaeria*' teleomorphs and related asexual species. *Neodeightonia* and *Phaeobotryon* are reinstated for species with brown ascospores that are either 1-septate (*Neodeightonia*) or 2-septate (*Phaeobotryon*). *Phaeobotryosphaeria* is reinstated for species with brown, aseptate ascospores that bear an apiculus at either end. The status of *Sphaeropsis* is clarified and shown to be the anamorph of *Phaeobotryosphaeria*. Two new genera, namely *Barriopsis* for species having brown, aseptate ascospores without apiculi and *Spencermartinsia* for species having brown, 1-septate ascospores with an apiculus at either end are introduced. Species of *Dothiorella* have brown, 1-septate ascospores and differ from *Spencermartinsia* in the absence of apiculi. These six genera can also be distinguished from one another based on morphological characters of their anamorphs. Although previously placed in the Botryosphaeriaceae, *Dothidotthia*, was shown to belong in the Pleosporales, and the new family Dothidotthiaceae is introduced to accommodate it.

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INTRODUCTION

The genus *Botryosphaeria* based on the type species, *B. dothidea*, typically has ascospores that are hyaline and aseptate, although they can become brown and septate with age (Saccardo 1877, von Arx & Müller 1954, 1975, Denman et al. 2000). Because some species of *Botryosphaeria* have ascospores that become brown with age, von Arx & Müller (1954) placed *Dothidea visci* with brown ascospores in *Botryosphaeria* as *B. visci*. Later, von Arx & Müller (1975) also placed the dark-spored *Neodeightonia subglobosa* in *Botryosphaeria*. Since this is the type species of *Neodeightonia* (1970), this genus was reduced to synonymy with *Botryosphaeria* (1863). In recognising these synonymies, von Arx & Müller (1954, 1975) broadened the concept of *Botryosphaeria* to include species with brown ascospores.

At least 18 anamorph genera have been associated with *Botryosphaeria*. Denman et al. (2000) recognised only two of these, namely *Fusicoccum* and *Diplodia*. However, in view of the range of morphologies found in *Botryosphaeria* anamorphs, the

proposal by Denman et al. (2000) is probably too conservative. Although Denman et al. (2000) suggested that *Lasiodiplodia* could be a synonym of *Diplodia*, authors of recent papers accept these as distinct genera (Pavlic et al. 2004, Burgess et al. 2006, Damm et al. 2007, Alves et al. 2008).

Phillips et al. (2005) resurrected the genus *Dothiorella* for species with 1-septate conidia that darken at an early stage of development, and teleomorphs that have brown, 1-septate ascospores. Phylogenetically (ITS+EF1- α) these species fell within the broad morphological concept of *Botryosphaeria* (Phillips et al. 2005) as recognised by von Arx & Müller (1954, 1975). For these reasons, Phillips et al. (2005) described the teleomorphs of *Dothiorella* as two new species of *Botryosphaeria* with brown, 1-septate ascospores. Subsequently, Luque et al. (2005) described another dark-spored *Botryosphaeria*, namely *B. viticola*, with a *Dothiorella* anamorph. Crous et al. (2006) referred to the clade with *Dothiorella* anamorphs as *Dothidotthia* because of the strong resemblance of the teleomorphs to that genus. However, in a morphological study of *Dothidotthia aspera* from diverse hosts, Ramaley (2005) showed that the anamorph is a hyphomycete, *Thyrostroma negundinis*, and that this species and possibly *D. symphoricarpi*, type of *Dothidotthia*, are unrelated to the Botryosphaeriaceae (Schoch et al. 2006).

Although the teleomorphs of *Botryosphaeria* tend to be morphologically conserved, the anamorphs display a wide range of morphologies. Based on the morphological diversity of the anamorphs linked to species of *Botryosphaeria*, Crous et al. (2006) suggest that these taxa represent more than a single genus. By including teleomorphs with brown ascospores in

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Table 1 Isolates studied in this paper.

Species	Accession number ¹	Host	Locality	SSU	LSU	ITS	GenBank ²	EF1- α	β -tubulin
<i>Barriopsis fusca</i>	CBS 1174.26	Citrus sp.	Cuba	EU673182	DQ377857	EU673330	EU673330	EU673296	EU673109
<i>Bimuria novae-zelandiae</i>	CBS 107.79	soil	New Zealand	AY016338	AY016356	-	-	-	-
<i>Botryosphaeria corticis</i>	CBS119047	<i>Vaccinium corymbosum</i>	USA	EU673175	EU673244	DQ299245	DQ299245	EU017539	EU673107
	ATCC 22927	<i>Vaccinium</i> sp.	USA	EU673176	EU673245	DQ299247	DQ299247	EU673291	EU673108
<i>Botryosphaeria dothidea</i>	CBS 1154.76	<i>Prunus</i> sp.	Switzerland	EU673173	AY928047	AY236894	AY236894	AY236898	AY236927
<i>'Botryosphaeria' tsugae</i>	CBS110302	<i>Vitis vinifera</i>	Portugal	EU673174	DQ377867	AY529092	AY529092	AY573218	EU673106
<i>Bysothecium circinans</i>	CBS 418.64	<i>Tsuga heterophylla</i>	Canada	EU673208	DQ377867	DQ458888	DQ458888	DQ458873	DQ458855
<i>Capnodium coffeae</i>	CBS 675.92	<i>Medicago sativa</i>	USA	AY016339	AY016357	-	-	-	-
<i>Capnodium coffeae</i>	CBS 147.52	<i>Coffea robusta</i>	Zaire	DQ247808	DQ247800	-	-	-	-
<i>Cochliobolus heterostrophus</i>	AFTOL 54	<i>Zea mays</i>	Unknown	AY544727	AY544645	-	-	-	-
<i>Davidiella tassiana</i>	AFTOL 1591	man, skin, foot	Netherlands	DQ678022	DQ678074	-	-	-	-
<i>Delitschia winteri</i>	AFTOL 1599	dung of rabbit	Netherlands	DQ678026	DQ678077	-	-	-	-
<i>Diadymella cucurbitacearum</i>	IMI 373225	Unknown	USA	AY293779	AY293792	-	-	-	-
<i>Diplodia acerina</i>	CBS 910.73	<i>Acer pseudoplatanus</i>	Germany	EU673160	EU673234	EU673315	EU673315	EU673282	EU673139
<i>Diplodia corticola</i>	CBS 1125.49	<i>Quercus suber</i>	Portugal	EU673206	AY928051	AY259700	AY259700	AY573227	DQ458853
<i>Diplodia coryli</i>	CBS 1125.46	<i>Quercus ilex</i>	Spain	EU673207	EU673262	EU673310	EU673310	EU673219	EU673117
<i>Diplodia cupressi</i>	CBS 242.51	Unknown	Italy	EU673162	EU673235	EU673317	EU673317	EU673284	EU673105
<i>Diplodia juglandis</i>	CBS 168.87	<i>Cupressus sempervirens</i>	Israel	EU673209	EU673263	DQ458893	DQ458893	DQ458878	DQ458862
<i>Diplodia mutila</i>	CBS 261.85	<i>Cupressus sempervirens</i>	Israel	EU673210	EU673264	DQ458894	DQ458894	DQ458879	DQ458862
	CBS 188.87	<i>Juglans regia</i>	France	EU673161	DQ377891	EU673316	EU673316	EU673283	EU673119
	CBS 1125.53	<i>Vitis vinifera</i>	Portugal	EU673213	AY928049	AY259093	AY259093	AY573219	DQ458850
	CBS 230.30	<i>Phoenix dactylifera</i>	USA	EU673214	EU673265	DQ458886	DQ458886	DQ458869	DQ458849
<i>Diplodia pinea</i> A	CBS 393.84	<i>Pinus nigra</i>	Netherlands	EU673219	DQ377893	DQ458895	DQ458895	DQ458880	DQ458863
<i>Diplodia pinea</i> C	CBS 1097.27	<i>Pinus radiata</i>	South Africa	EU673220	EU673269	DQ458897	DQ458897	DQ458882	DQ458866
	CBS 1097.25	<i>Pinus patula</i>	South Africa	EU673222	EU673270	DQ458896	DQ458896	DQ458881	DQ458864
	CBS 1099.43	<i>Pinus patula</i>	Indonesia	EU673221	EU673271	DQ458898	DQ458898	DQ458883	DQ458866
<i>Diplodia rosulata</i>	CBS 1164.70	<i>Prunus africana</i>	Ethiopia	EU673211	DQ377896	EU430265	EU430265	EU430267	EU673132
<i>Diplodia scrobiculata</i>	CBS 1134.23	<i>Prunus greggii</i>	Ethiopia	EU673212	DQ377897	EU430266	EU430266	EU430268	EU673131
	CBS 1099.44	<i>Pinus greggii</i>	Mexico	EU673217	EU673267	DQ458900	DQ458900	DQ458885	DQ458868
	CBS 1125.55	<i>Vitis vinifera</i>	Mexico	EU673218	EU673268	DQ458899	DQ458899	DQ458884	DQ458867
<i>Diplodia seriata</i>	CBS 1190.49	<i>Vitis</i> sp.	Portugal	EU673215	AY928050	AY259094	AY259094	AY573220	DQ458856
	AFTOL 274	Unknown	Italy	EU673216	EU673266	DQ458889	DQ458889	DQ458874	DQ458857
<i>Dothidea sambuci</i>	CPC 12928	<i>Fendlera rupicola</i>	Unknown	EU673225	EU673272	-	-	-	-
<i>Dothiodothia</i> sp.	CPC 12930	<i>Euonymus alatus</i>	USA	EU673226	EU673274	-	-	-	-
<i>Dothiodothia</i> sp.	CPC 12932	<i>Acer negundis</i>	USA	EU673227	EU673275	-	-	-	-
<i>Dothiodothia</i> sp.	CPC 12933	<i>Acer negundis</i>	USA	EU673224	EU673273	-	-	-	-
<i>Dothiodothia symphoricarpi</i>	AFTOL 1359	<i>Symphoricarpos rotundifolia</i>	USA	DQ479933	DQ479984	-	-	-	-
<i>Dothiora carnabinae</i>	CBS 1150.41	<i>Daphne carnabina</i>	India	EU673155	AY928053	AY573202	AY573202	AY573222	EU673096
<i>Dothiorella iberica</i>	CBS 1131.88	<i>Quercus ilex</i>	Spain	EU673156	EU673230	AY573198	AY573198	EU673278	EU673097
<i>Dothiorella</i> sp.	IMI 63581b	<i>Quercus suber</i>	Spain	EU673158	AY928052	AY573212	AY573212	AY573235	EU673102
<i>Dothiorella</i> sp.	CBS 1150.38	<i>Ulmus</i> sp.	United Kingdom	EU673159	DQ377860	AY573206	AY573206	AY573223	EU673101
	CAA 005	<i>Melus pumila</i>	Netherlands	EU673157	EU673231	EU673312	EU673312	EU673279	EU673098
<i>Dothiorella</i> sp.	JL 599	<i>Pistacia vera</i>	Portugal	EU673163	EU673232	EU673313	EU673313	EU673280	EU673100
<i>Dothiorella</i> sp.	AFTOL 1360	<i>Corylus avellana</i>	Spain	EU673164	EU673233	EU673314	EU673314	EU673281	EU673099
<i>Eisinoë veneta</i>	CBS 1116.45	<i>Rubus</i> sp.	Unknown	DQ678007	DQ678060	-	-	-	-
<i>Hysteropateia clavispora</i>	AFTOL 1305	<i>Parthenocissus quinquefolia</i>	USA	DQ678007	DQ377876	-	-	-	-
<i>Lasiodiplodia crassispora</i>	CBS 1187.41	<i>Salix</i> sp.	USA	DQ678006	AY541493	-	-	-	-
	CBS 1158.12	Unknown	Unknown	EU673189	EU673251	EF622086	EF622086	EF622066	EU673134
	CBS 1163.55	<i>Santalum album</i>	Australia	EU673190	DQ377901	DQ103550	DQ103550	EU673303	EU673133
	CBS 356.59	<i>Syzygium cordatum</i>	South Africa	EU673193	DQ377902	DQ458892	DQ458892	DQ458877	DQ458860
	CBS 494.78	<i>Syzygium cordatum</i>	South Africa	EU673194	EU673252	AY639594	AY639594	DQ103567	EU673126
		<i>Theobroma cacao</i>	Sri Lanka	EU673200	EU673257	EF622082	EF622082	EF622062	EU673113
		Cassava-field soil	Colombia	EU673201	EU673258	EF622084	EF622084	EF622064	EU673114

<i>Lasiodiplodia pseudothobromae</i>	CBS 447.62	<i>Citrus aurantium</i>	Suriname	EU673198	EU673255	EF622081	EU673112
	CBS 116459	<i>Gmelina arborea</i>	Costa Rica	EU673199	EU673256	EF622060	EU673111
<i>Lasiodiplodia rubropurpurea</i>	CBS 118740	<i>Eucalyptus grandis</i>	Queensland	EU673191	DQ377903	EF622077	EU673136
<i>Lasiodiplodia theobromae</i>	CBS 124.13	Unknown	USA	EU673195	AY928054	DQ458890	DQ458858
	CBS 164.96	Fruit along coral reef coast	New Guinea	EU673196	EU673253	AY640255	EU673110
	CAA 006	<i>Vitis vinifera</i>	USA	EU673197	DQ458891	DQ458876	DQ458859
	CBS 118739	<i>Acacia mangium</i>	Venezuela	EU673192	DQ377904	EU673305	EU673129
	CBS 559.71	sandy desert soil	Algeria	DQ384068	DQ384106	—	—
	AFTOL 277	Unknown	Unknown	DQ470993	DQ470946	—	—
	AFTOL 267	<i>Medicago rugosa</i>	Australia	DQ677994	DQ678044	—	—
	AFTOL 1581	<i>Prunus spinosa</i>	Switzerland	DQ678017	DQ678069	—	—
	AFTOL 1574	<i>Platanus occidentalis</i>	USA	DQ678013	DQ678065	—	—
	CBS 619.86	<i>Phragmites australis</i>	Switzerland	DQ813573	DQ813509	—	—
	AFTOL 1734	<i>Opuntia</i> sp.	Unknown	AF164370	DQ678086	—	—
	AFTOL 942	<i>Quercus robur</i>	Netherlands	DQ471017	DQ470968	—	—
	CBS 260.36	<i>Chrysomphalus aonidium</i>	Argentina	AY016347	AY016365	—	—
	CBS 169.34	<i>Phoenix dactylifera</i>	USA	EU673203	EU673259	EU673307	EU673138
	CBS 123168	<i>Phoenix canariensis</i>	Spain	EU673204	EU673260	EU673308	EU673115
	CBS 122528	<i>Phoenix dactylifera</i>	Spain	EU673205	EU673261	EU673309	EU673116
	CBS 448.91	keratomycosis in eye	United Kingdom	EU673202	DQ377866	EU673306	EU673137
	CBS 110299	<i>Vitis vinifera</i>	Portugal	EU673148	AY928043	AY573217	DQ458848
	CBS 110497	<i>Vitis vinifera</i>	Portugal	EU673149	EU673229	EU673277	EU673092
	CBS 118531	<i>Mangifera indica</i>	Australia	EU673153	DQ377920	DQ093221	AY615172
	CBS 118532	<i>Mangifera indica</i>	Australia	EU673154	DQ377921	DQ093220	AY615173
	CMW 9081	<i>Pinus nigra</i>	New Zealand	EU673151	AY928045	AY236888	AY236917
	CBS 110301	<i>Vitis vinifera</i>	Portugal	EU673150	AY928046	AY573221	EU673095
	CBS 115475	<i>Ribes</i> sp.	USA	EU673152	AY928044	—	—
	CPC 12264	<i>Sophora chrysohylla</i>	Hawaii	EU673183	DQ377898	EU673297	EU673125
	CPC 12440	<i>Sophora chrysohylla</i>	Hawaii	EU673184	EU673248	EU673298	EU673121
	CPC 12442	<i>Sophora chrysohylla</i>	Hawaii	EU673185	DQ377899	EU673299	EU673124
	CPC 12443	<i>Sophora chrysohylla</i>	Hawaii	EU673186	EU673249	EU673300	EU673120
	CPC 12444	<i>Sophora chrysohylla</i>	Hawaii	EU673187	DQ377900	EU673301	EU673123
	CPC 12445	<i>Sophora chrysohylla</i>	Hawaii	EU673188	EU673250	EU673302	EU673122
	ICMP 16812	<i>Citrus sinensis</i>	New Zealand	EU673180	EU673246	EU673294	EU673140
	ICMP 16818	<i>Citrus sinensis</i>	New Zealand	EU673181	EU673247	EU673295	EU673141
	CBS 110496	<i>Vitis vinifera</i>	South Africa	EU673179	DQ377894	AY343379	EU673130
	CBS 100163	<i>Viscum album</i>	Luxembourg	EU673177	DQ377870	EU673292	EU673127
	CBS 186.97	<i>Viscum album</i>	Germany	EU673178	DQ377868	EU673325	EU673128
	CBS 122526	<i>Viscum album</i>	Ukraine	—	—	—	—
	CBS 122527	<i>Viscum album</i>	Ukraine	—	—	—	—
	AFTOL 280	Unknown	Unknown	AY544725	AY544684	—	—
	AFTOL 1575	Unknown	Unknown	DQ678014	DQ678066	—	—
	CBS 480.64	man, hair	Brazil	AY016349	AY016366	—	—
	AFTOL 1600	<i>Betula verrucosa</i>	Netherlands	DQ678027	DQ678078	—	—
	AFTOL 282	Unknown	Unknown	AY544726	AY544686	—	—
	CBS 117448	<i>Eucalyptus hybrid</i>	Venezuela	EU673146	DQ377931	AY693975	EU673094
	CBS 117449	<i>Eucalyptus hybrid</i>	Venezuela	EU673147	DQ377932	DQ436936	EU673093
	—	Unknown	Unknown	AY545724	AY545728	—	—
	CBS 117009	<i>Vitis vinifera</i>	Spain	DQ377873	DQ377873	AY905554	EU673104
	CBS 117006	<i>Vitis vinifera</i>	Spain	EU673166	EU673236	AY905555	EU673103
	CBS 500.72	<i>Medicago sativa</i>	South Africa	EU673237	EU673285	AY905562	EU673118
	CBS 302.75	<i>Panicum gilliesii</i>	France	EU673168	EU673238	EU673319	EU673135
	ICMP 16827	<i>Citrus sinensis</i>	New Zealand	EU673171	EU673241	EU673322	EU673144
	ICMP 16828	<i>Citrus sinensis</i>	New Zealand	EU673172	EU673242	EU673323	EU673145
	ICMP 16819	<i>Citrus sinensis</i>	New Zealand	EU673169	EU673239	EU673320	EU673142
	ICMP 16824	<i>Citrus sinensis</i>	New Zealand	EU673170	EU673240	EU673321	EU673143
	AFTOL 51	downed rotting wood	USA	AY544692	AY544648	—	—

¹ Acronyms of culture collections: AFTOL – Assembling the Fungal Tree of Life; CAA – A. Alves, Universidade de Aveiro, Portugal; CAP – Alan J.L. Phillips, Universidade Nova de Lisboa, Portugal; CBS – Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CMW – M.J. Wingfield, FABI, University of Pretoria, South Africa; IMI – CABI Bioscience, Egham, U.K.; JL – J. Luque, IRTA, Spain.

² Sequence numbers in *italics* were retrieved from GenBank. All others were obtained in the present study.

Botryosphaeria, Phillips et al. (2005) broadened the concept of the genus even further. Through a study of partial sequences of the LSU gene, Crous et al. (2006) showed that *Botryosphaeria* s.l. is composed of 10 phylogenetic lineages that correspond to different anamorph genera. To avoid the unnecessary introduction of new generic names, they opted to use existing anamorph generic names for most of the lineages, and restricted the use of *Botryosphaeria* to *B. dothidea* and *B. corticis*. In their phylogeny, a large clade consisting of *Diplodia* and *Lasiodiplodia* species was largely unresolved. Within this clade are species known to have hyaline ascospores, e.g. *B. corticola*, *B. stevensii*, and others reported to have dark ascospores, e.g. *B. subglobosa* and *B. visci*.

The aim of the present study was to use a multigene approach to determine the correct taxonomy and phylogeny of the dark-spored *Botryosphaeria*-like teleomorphs and their associated anamorphs and to resolve the phylogenetic position of the genus *Dothidotthia*.

MATERIALS AND METHODS

DNA isolation, PCR amplification and sequencing

Genomic DNA was extracted from mycelium following the method of Alves et al. (2004). PCR reactions were carried out with *Taq* polymerase, nucleotides and buffers supplied by MBI Fermentas (Vilnius, Lithuania) and PCR reaction mixtures were prepared according to Alves et al. (2004), with the addition of 5 % DMSO to improve the amplification of some difficult DNA templates. All primers used were synthesised by MWG Biotech AG (Ebersberg, Germany).

A portion of the nuclear ribosomal SSU gene was amplified with primers NS1 and NS4 (White et al. 1990). The amplification conditions were as follows: initial denaturation of 5 min at 95 °C, followed by 35 cycles of 45 s at 94 °C, 45 s at 48 °C and 90 s at 72 °C, and a final extension period of 10 min at 72 °C. The nucleotide sequence of the SSU region was determined using the above primers along with the internal sequencing primers NS2 and NS3 (White et al. 1990).

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

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

The amplified PCR fragments were purified with the JETQUICK PCR Purification Spin Kit (GENOMED, Löhne, Germany). Both strands of the PCR products were sequenced according to the procedures described previously (Alves et al. 2004), while

some were sequenced by STAB Vida Lda (Portugal). The nucleotide sequences were read and edited with FinchTV 1.4.0 (Geospiza Inc. <http://www.geospiza.com/finchtv>). All sequences were checked manually and nucleotide arrangements at ambiguous positions were clarified using both primer direction sequences.

Phylogenetic analyses

Sequences were aligned with ClustalX v. 1.83 (Thompson et al. 1997), using the following parameters: pairwise alignment parameters (gap opening = 10, gap extension = 0.1) and multiple alignment parameters (gap opening = 10, gap extension = 0.2, transition weight = 0.5, delay divergent sequences = 25 %). Alignments were checked and manual adjustments were made where necessary. Phylogenetic information contained in indels (gaps) was incorporated into the phylogenetic analyses using simple indel coding as implemented by GapCoder (Young & Healy 2003).

Phylogenetic analyses of sequence data were done using PAUP v. 4.0b10 (Swofford 2003) for Maximum-parsimony (MP) analyses and Mr Bayes v. 3.0b4 (Ronquist & Huelsenbeck 2003) for Bayesian analyses. Trees were visualised with Tree-View (Page 1996).

Maximum-parsimony analyses were performed using the heuristic search option with 1 000 random taxa addition and tree bisection and reconnection (TBR) as the branch-swapping algorithm. All characters were unordered and of equal weight and gaps were treated as missing data. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. The robustness of the most parsimonious trees was evaluated from 1 000 bootstrap replications (Hillis & Bull 1993). Other measures used were consistency index (CI), retention index (RI) and homoplasy index (HI).

Bayesian analyses employing a Markov Chain Monte Carlo method were performed. The general time-reversible model of evolution (Rodríguez et al. 1990), including estimation of invariable sites and assuming a discrete gamma distribution with six rate categories (GTR+I+G) was used. Four MCMC chains were run simultaneously, starting from random trees for 1 000 000 generations. Trees were sampled every 100th generation for a total of 10 000 trees. The first 1 000 trees were discarded as the burn-in phase of each analysis. Posterior probabilities (Rannala & Yang 1996) were determined from a majority-rule consensus tree generated with the remaining 9 000 trees. This analysis was repeated three times starting from different random trees to ensure trees from the same tree space were sampled during each analysis.

In this study we assessed the possibility of combining the individual datasets by comparing highly supported clades among trees generated from the different datasets to detect conflict. High support typically refers to bootstrap support values ≥ 70 % and Bayesian posterior probabilities ≥ 95 % (Alfaro et al. 2003). If no conflict exists between the highly supported clades in trees generated from these different datasets, it is likely that the genes share similar phylogenetic histories and phylogenetic resolution and support could ultimately be increased by combining the datasets (Miller & Huhndorf 2004).

RESULTS

Phylogenetic analyses

Partial nucleotide sequences of the SSU ribosomal DNA (1134 bp), the ITS region (500–600 bp), the D1/D2 variable domains of the LSU ribosomal DNA (614 bp), β -tubulin (approx. 400 bp) and EF1- α genes (approx. 300 bp) were determined for several isolates. The other sequences used in the analyses were retrieved from GenBank (Table 1). Sequences of the five genes were aligned and analysed separately by MP and Bayesian analyses, and the resulting trees were compared. No conflicts were detected between single gene phylogenies indicating that the datasets could be combined. New sequences were deposited in GenBank (Table 1) and the alignments in TreeBASE (SN 3881).

Combined SSU and LSU rDNA sequences of *Dothidotthia symphoricarpi* isolates were aligned with a set of sequences retrieved from GenBank (Table 1) representing several orders in the Dothideomycetes, as well as two Sordariomycetes sequences that were selected as outgroup taxa (*Sordaria fimicola* and *Xylaria hypoxylon*). The combined SSU+LSU alignment consisted of 38 taxa and contained 1742 characters including

coded alignment gaps. Indels were coded separately and added to the end of the alignment as characters 1682–1742. Of the 1742 characters, 1203 were constant, while 168 were variable and parsimony uninformative. Maximum parsimony analysis of the remaining 371 parsimony informative characters resulted in a single tree with TL = 1443, CI = 0.5038, RI = 0.7056 and HI = 0.4962. The overall topology of the 50 % majority-rule consensus tree of 10 000 trees sampled during the Bayesian analysis was similar to the MP tree. The MP tree is presented in Fig. 1 with bootstrap support above the branches. The Bayesian tree is available in TreeBASE (SN 3881).

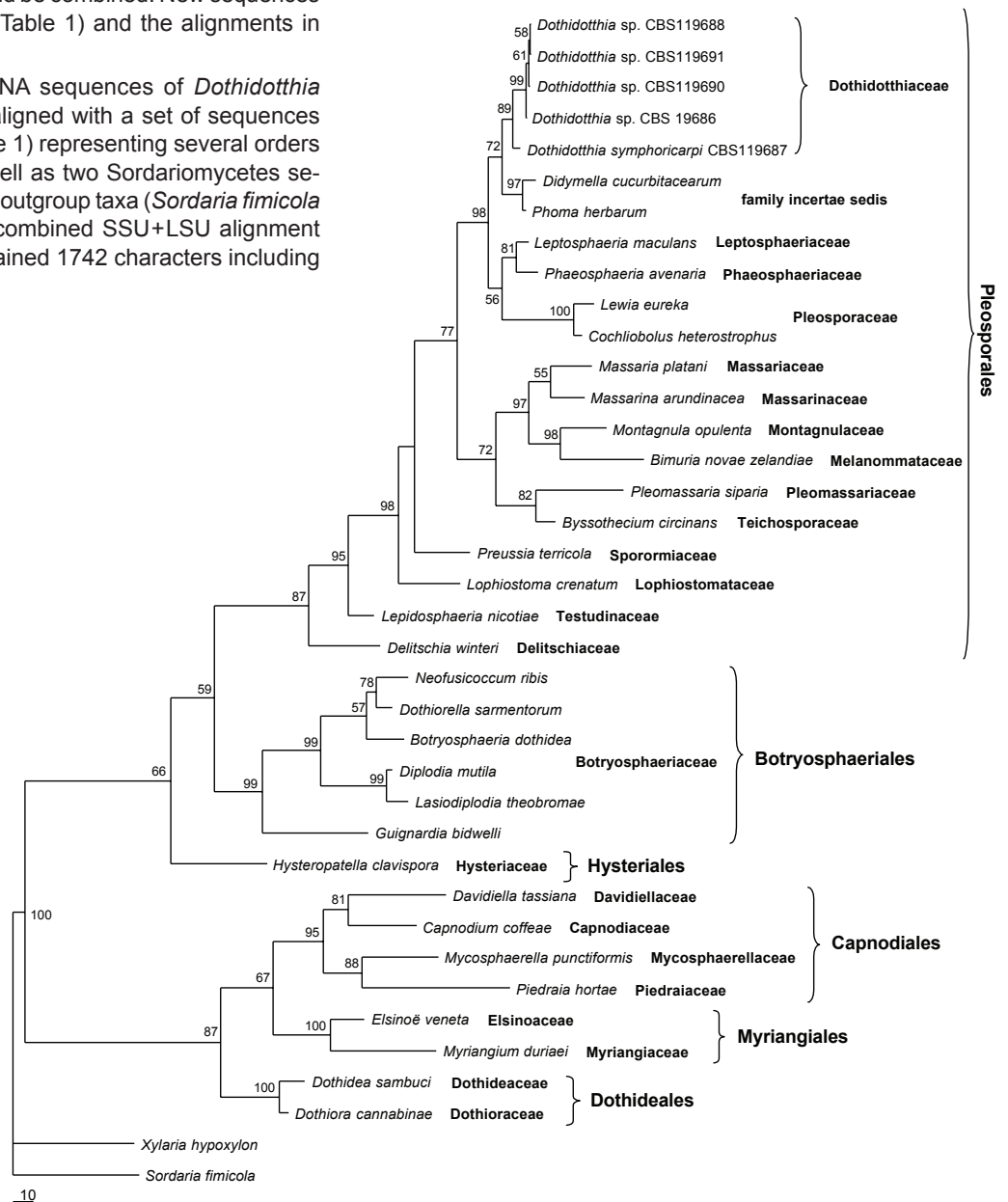
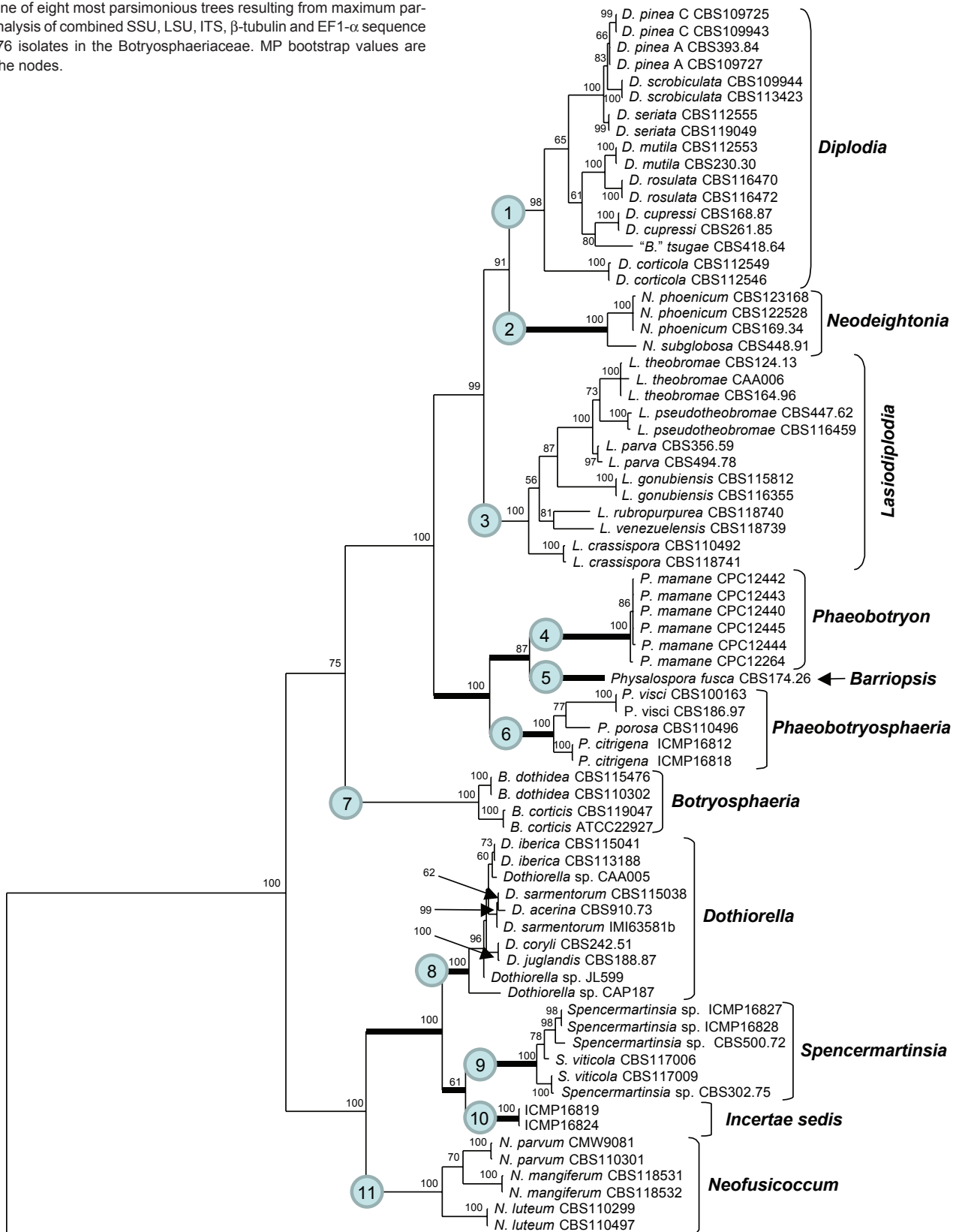


Fig. 1 Single most parsimonious tree resulting from maximum parsimony analysis of combined SSU and LSU sequence data for 38 taxa. MP bootstrap values are given at the nodes.

Fig. 2 One of eight most parsimonious trees resulting from maximum parsimony analysis of combined SSU, LSU, ITS, β -tubulin and EF1- α sequence data for 76 isolates in the Botryosphaeriaceae. MP bootstrap values are given at the nodes.



Pseudofusicoccum stromaticum CBS117449
Pseudofusicoccum stromaticum CBS117448

Within the ingroup taxa six well-supported clades could be identified, which correspond to known orders belonging to the Dothideomycetes, namely the Dothideales, Myriangiales, Capnodiales, Hysteriales, Botryosphaeriales and Pleosporales. The isolates identified as *D. symphoricarpi* formed a distinct and well-supported subclade (MP bootstrap = 89 %, posterior probability = 1.00) within the Pleosporales clade. The *D. symphoricarpi* clade was clearly separated from all families included in the analyses. In both MP and Bayesian analyses the isolates grouped close to *Didymella cucurbitacearum* and *Phoma herbarum* (family incertae sedis; de Gruyter et al. in prep.).

The object of the multigene dataset (SSU, LSU, ITS, β -tubulin and EF1- α) analyses was to determine the phylogenetic relationships of the species with brown ascospores. Therefore *Pseudofusicoccum stromaticum* was used as outgroup because it lies basal to the Botryosphaeriaceae (Crous et al. 2006). The alignment of 76 isolates consisted of 3470 characters including alignment gaps. Indels were coded separately and added to the end of the alignment as characters 3255–3470. In the analyses, alignment gaps were treated as missing data.

The combined dataset contained 3470 characters, of which 83 were variable and parsimony-uninformative and 2555 were constant. Maximum parsimony analysis of the remaining 832 parsimony-informative characters resulted in eight equal, most parsimonious trees (TL = 1966 steps, CI = 0.6175, RI = 0.9103, RC = 0.5621, HI = 0.3825). The 50 % majority-rule consensus tree of 10 000 trees sampled during the Bayesian analysis had an overall topology similar to the MP trees. One of the MP trees is shown in Fig. 2 with bootstrap support at the branches. The Bayesian tree is available in TreeBASE (SN 3881). In both analyses 11 clades were identified within the ingroup. For convenience these clades are numbered 1–11 in Fig. 2. All of the clades received high bootstrap (87–100 %) and posterior probabilities (0.99–1.00) support.

TAXONOMY

Position of *Dothidotthia*

Until now the genus *Dothidotthia* has been regarded as a member of the Botryosphaeriaceae (Barr 1987). One species of *Dothidotthia*, *D. aspera*, was recently shown to have a hyphomycetous anamorph in *Thyrostroma* (Ramaley 2005), quite unlike the pycnidial anamorphs of members of the Botryosphaeriaceae. The type species of *Dothidotthia*, *D. symphoricarpi*, was included in an analysis of the Dothideomycetes (Fig. 1). These data show that *Dothidotthia* belongs in the Pleosporales, outside any of the known families, and thus a new family in the Pleosporales is introduced.

Dothidotthiaceae Crous & A.J.L. Phillips, *fam. nov.* — MycoBank MB511706

Ascomata aggregata, erumpescentia, globosa, atrobrunnea. Pseudoparaphyses hyalinus, septatis. Asci octisporis, bitunicati, clavati. Ascospores brunneae, uniseptatae, ellipsoidae.

Typus. *Dothidotthia* Höhn.

Anamorph. *Thyrostroma*.

Ascomata in gregarious clusters, rarely solitary, erumpent, globose, dark brown; wall consisting of 3–6 layers of dark brown *textura angularis*; basal region giving rise to dark brown, thick-walled hyphae, that extend from the bottom of the ascomata into the substrate. *Pseudoparaphyses* hyaline, septate, branched in upper part above asci. *Asci* 8-spored, bitunicate, sessile, clavate, straight to curved. *Ascospores* brown, ellipsoid, transversely 1-septate. *Anamorph* hyphomycetous, *Thyrostroma*.

Dothidotthia Höhn., Ber. Deutsch. Bot. Ges. 36: 312. 1918

Type species. *Dothidotthia symphoricarpi* (Rehm) Höhn.

Dothidotthia symphoricarpi (Rehm) Höhn., Ber. Deutsch. Bot. Ges. 36: 312. 1918. — Fig. 3–5

Basionym. *Pseudotthia symphoricarpi* Rehm, Ann. Mycol. 11: 169. 1913.

≡ *Dibotryon symphoricarpi* (Rehm) Petr., Ann. Mycol. 25: 301. 1927.

≡ *Gibbera symphoricarpi* (Rehm) Arx, Acta Bot. Neerl. 3: 85. 1954.

Anamorph. *Thyrostroma negundinis* (Berk. & M.A. Curtis) A.W. Ramaley, Mycotaxon 94: 131. (2005) 2006.

Basionym. *Coryneum negundinis* Berk. & M.A. Curtis, Grevillea 2: 153. 1874.

For additional synonyms see Ramaley (2005).

Ascomata pseudothecial, in gregarious clusters, rarely solitary, erumpent, up to 550 μm diam and 500 μm high; apex somewhat papillate to depressed; wall consisting of 3–6 layers of dark brown *textura angularis*, 20–80 μm wide, giving rise to dark brown, thick-walled hyphae, 4–6 μm wide, that extend from the bottom of the ascomata into the substratum; reduced to short lateral projections (10–15 μm long) elsewhere on the outer ascomatal wall. *Pseudoparaphyses* hyaline, septate, 2–3 μm wide, generally not constricted at septa, and branched in upper part above asci. *Asci* 8-spored, bitunicate, sessile, clavate, 70–120 \times 15–22 μm , straight to curved. *Ascospores* uniformly pale brown, (20–)22–23(–26) \times (8–)9–10(–11) μm , ellipsoid, tapering towards subacutely rounded ends, medianly 1-septate, prominently constricted at septum, widest just above septum, smooth.

Specimens examined. *Dothidotthia symphoricarpi*. USA, North Dakota, on branches of *Symphoricarpos occidentalis*, holotype of *D. symphoricarpi* herb. NY; Colorado, San Juan Co, c. 0.5 mile up Engineer Mountain Trail from turnoff at mile 52.5, Hwy 550, dead twigs of *Symphoricarpos rotundifolius*, 24 June 2004, A.W. Ramaley 0410, epitype designated here as BPI 871823, culture ex-epitype CPC 12929 = CBS 119687.

Notes — Barr (1989) introduced the combination *Dothidotthia aspera*, but incorrectly listed *D. symphoricarpi* as synonym. *Dothidotthia aspera* (Fig. 6) has ascospores that are ellipsoidal with rounded ends, constricted at the medium septum, widest just above the septum, medium brown, smooth to finely verruculose, (20–)32–35(–37) \times (12–)13–14(–15) μm . Ascospores of *D. symphoricarpi* are much smaller, namely (20–)22–23(–26) \times (8–)9–10(–11) μm , ellipsoid with rounded ends, constricted at the median septum, widest above septum, finely verruculose, pale brown, and not medium brown as in *D. aspera*. Ramaley (2005) collected several specimens in this complex, one of which, BPI 871823, is selected to serve as epitype of *D. symphoricarpi*. Given the new circumscription of



Fig. 3 *Pseudotthia symphoricarpi* holotype NY. a. Ascomata; b. immature asci; c. asci and pseudoparaphyses; d. mature ascus with pale brown 1-septate ascospores; e. brown 1-septate ascospores; f. base of sessile ascus; g. brown 1-septate ascospores; h–j. conidia of *Thyrostroma* anamorph in association with ascomata. — Scale bars: a = 500 μ m; b–h = 10 μ m.

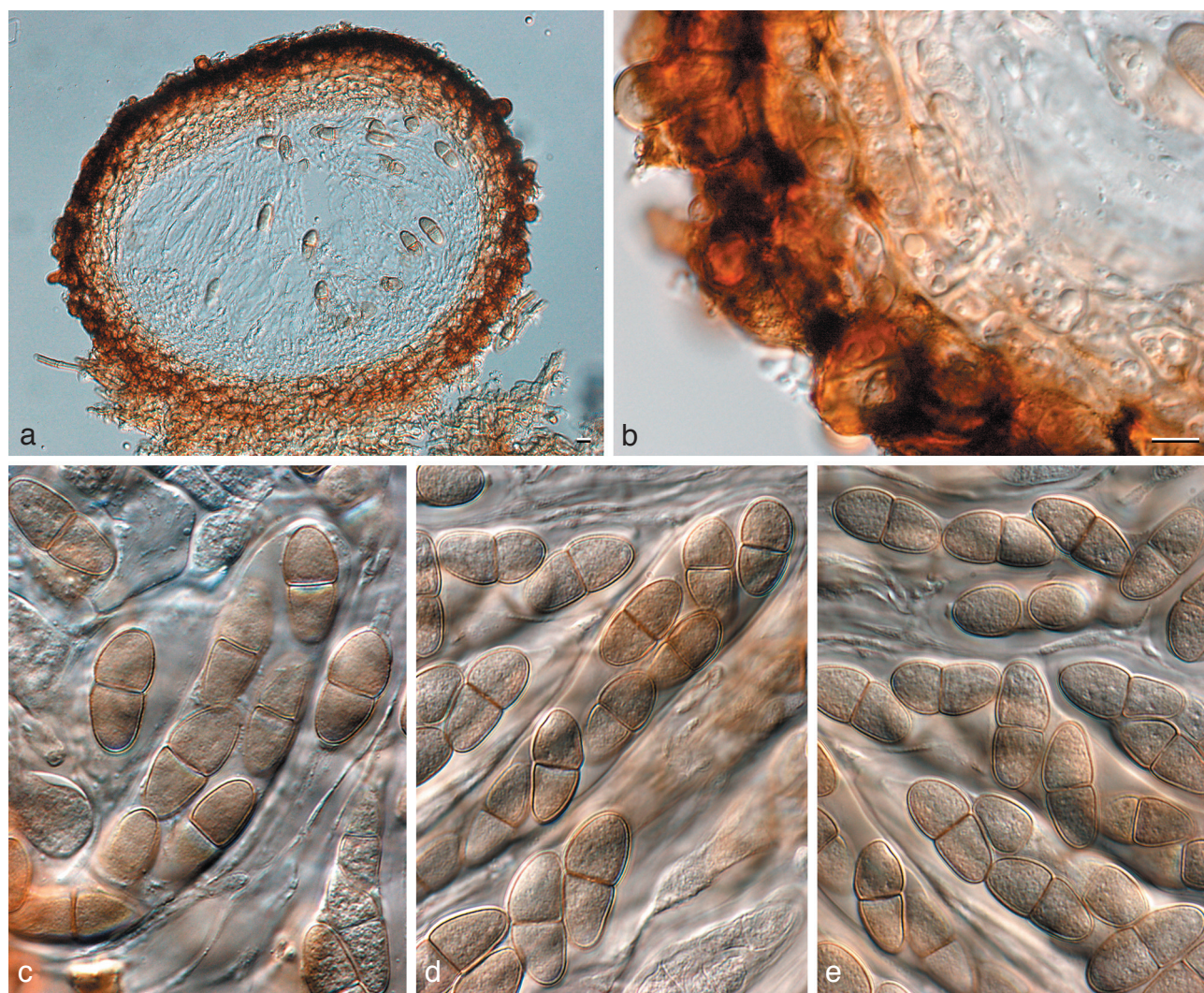


Fig. 4 *Dothidotthia symphoricarpi* epitype BPI 871823. a. Longitudinal section through an ascoma; b. detail of a section through the ascoma wall; c, d. asci with pale brown, 1-septate ascospores; e. pale brown, 1-septate ascospores. — Scale bars = 10 μ m.

this species, the other specimens treated by Ramaley (2005) appear to represent *D. aspera*, which is morphologically and phylogenetically distinct from both *D. symphoricarpi* based on the larger ascospores.

Dothidotthia aspera (Ellis & Everh.) M.E. Barr, Mycotaxon 34: 519. 1989 — Fig. 6

Basionym. *Amphisphaeria aspera* Ellis & Everh., Bull. Torrey Bot. Club 27: 52. 1900.

Ascomata pseudothecial, gregarious in groups, rarely solitary, erumpent, up to 600 μ m diam, 500 μ m high; apex rounded, short papillate to depressed; wall consisting of 3–6 layers of dark brown *textura angularis*, 20–80 μ m wide, giving rise to dark brown, thick-walled hyphae, 4–6 μ m wide, that extend from the bottom of the ascomata into the substratum; reduced to short lateral projections elsewhere on the outer ascomatal wall. *Pseudoparaphyses* hyaline, septate, 2–3 μ m wide, not constricted at the septa, branched in the upper parts. *Asci*

8-spored, bitunicate, sessile, clavate, 65–140 \times 10–23 μ m. *Ascospores* medium brown, ellipsoidal with rounded ends, 1-septate, constricted at the median septum, smooth to finely verruculose, (20–)32–35(–37) \times (12–)13–14(–15) μ m.

Specimens examined. *Dothidotthia aspera*. USA, Colorado, E. Bethel 517, holotype of *Amphisphaeria aspera* herb. NY. — *Dothidotthia* spp. USA, Colorado, Durango, 7 Animas Place, dead twigs of *Euonymus alatus*, 29 June 2004, A.W. Ramaley 0411, BPI 871820, culture CPC 12930 = CBS 119688; Colorado, Durango, between Animas Place and Animas River, dead twigs of *Acer negundo*, 8 July 2004, A.W. Ramaley 0414, BPI 871819, anamorph culture CPC 12933 = CBS 119691, teleomorph CPC 12932 = CBS 119690; Colorado, La Plata Co, c. 1.75 mile up Carbon Junction Trail, dead twigs of *Fendlera rupicola*, 11 May 2004, A.W. Ramaley 0403, BPI 871821, culture CPC 12928 = CBS 119686.

Taxonomy of species with brown ascospores in the Botryosphaeriaceae

The multigene phylogeny revealed 11 clades within the dataset of isolates studied (Fig. 2). Valid generic names are available and currently in use for clade 1 (*Diplodia*), clade 3 (*Lasiodiplodia*),

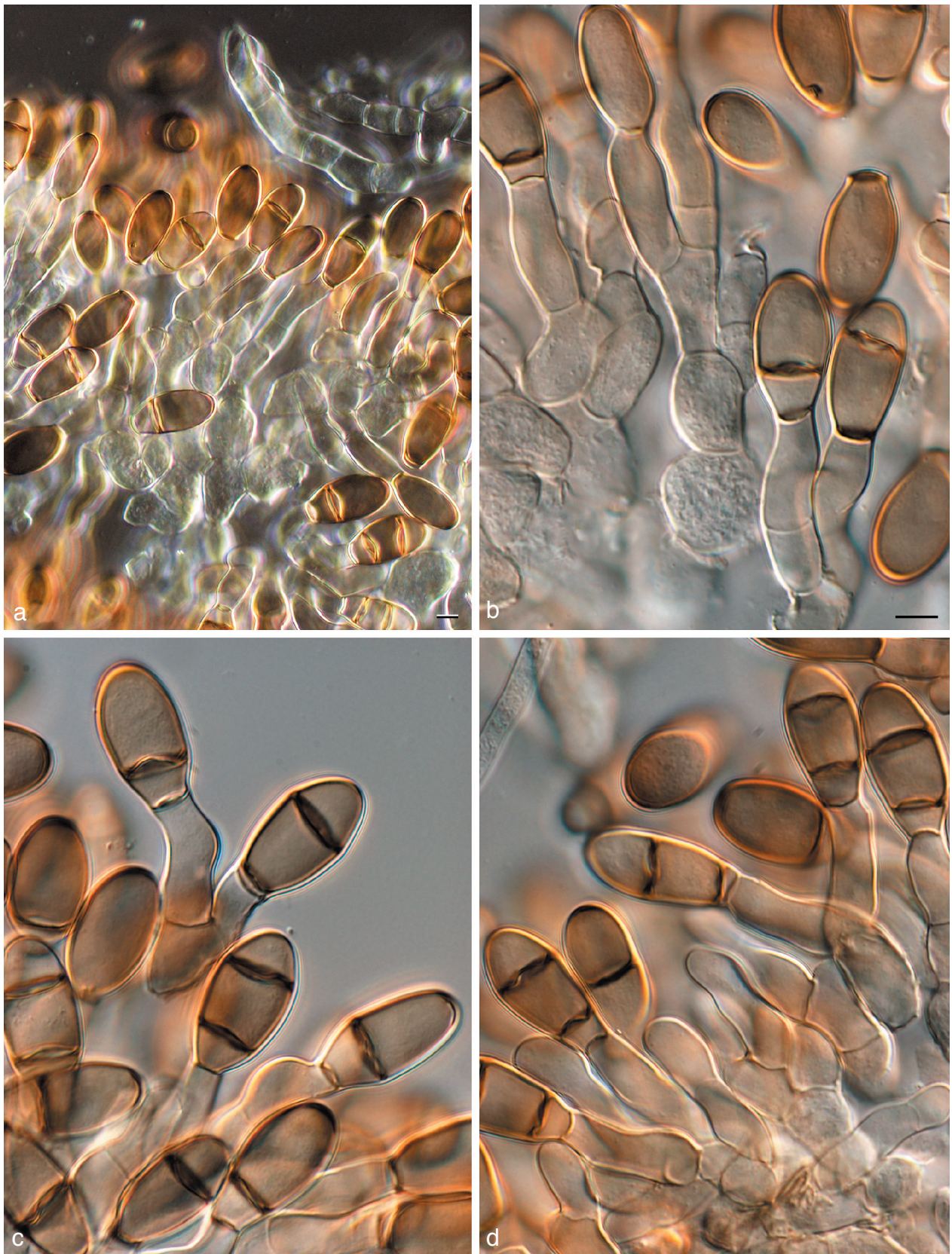


Fig. 5 *Thyrostroma* sp. CBS 119691, anamorph of *Dothidotthia aspera*. a–d. Conidia and conidiophores. — Scale bars = 10 μm.

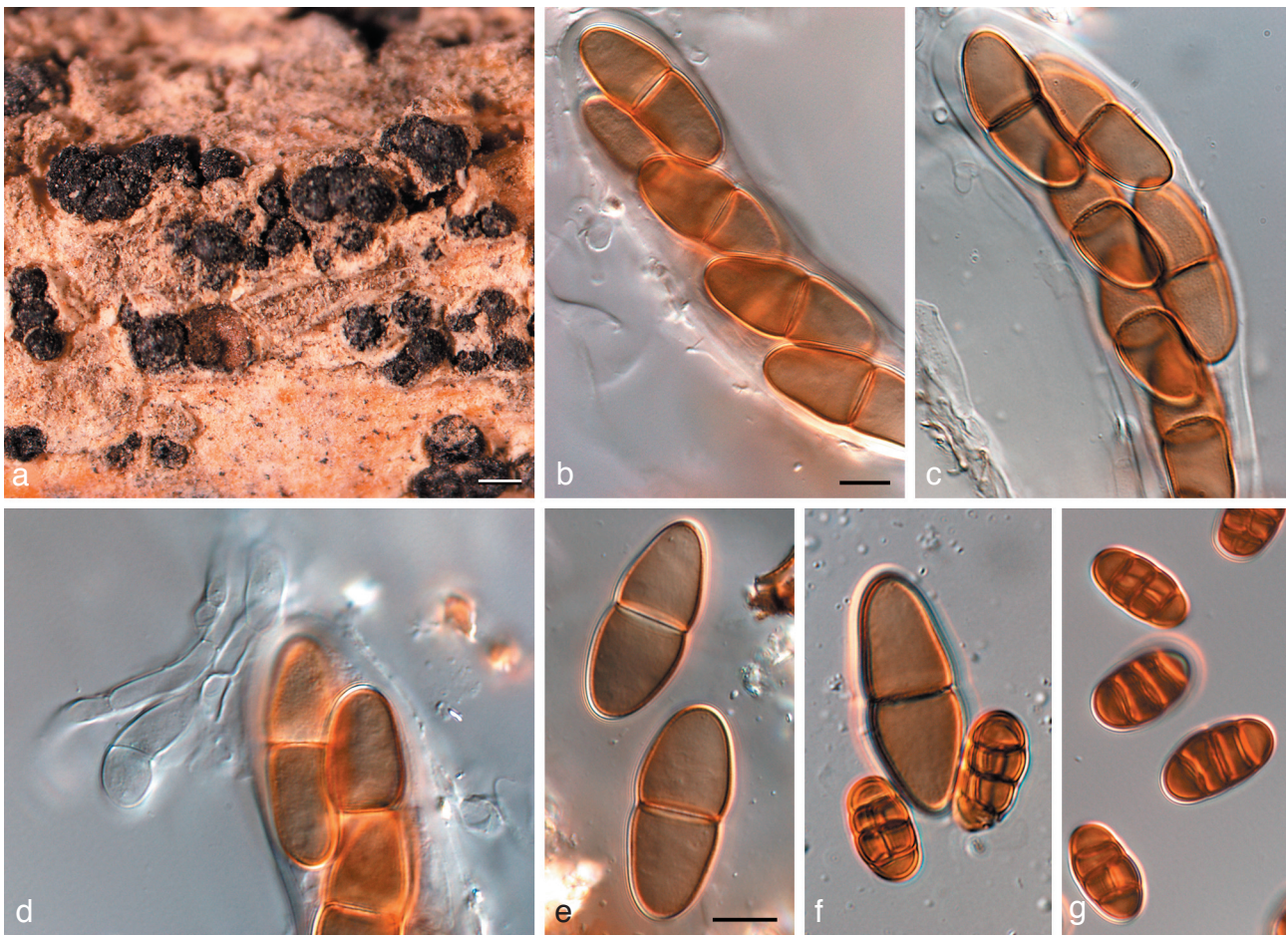


Fig. 6 *Amphisphaeria aspera* holotype NY. a. Ascomata; b, c. asci with ascospores; d. pseudoparaphyses and ascospores; e. ascospores; f. ascospore and two conidia; g. conidia. — Scale bars: a = 500 μ m ; b–g = 10 μ m.

clade 7 (*Botryosphaeria*), clade 8 (*Dothiorella*) and clade 11 (*Neofusicoccum*). *Neodeightonia* and *Phaeobotryon* are reinstated for clades 2 and 4, respectively. *Phaeobotryosphaeria* is reinstated for clade 6 and shown to be the teleomorph of *Sphaeropsis*, the status of which is clarified. No generic names are available for the remaining clades and for this reason *Barriopsis* and *Spencermartinsia* are introduced for clades 5 and 9, respectively. The status of clade 10 remains unresolved.

Barriopsis A.J.L. Phillips, A. Alves & Crous, *gen. nov.* — MycoBank MB511712

Ascomata pseudothecia, brunnea vel nigra. Pseudoparaphyses hyalinae, septatae. Asci clavati, stipitati, octospori, bitunicati cum loculo apicali bene evoluto. Ascosporae ellipsoides, unicellulares, ovoidea, brunnea.

Type species. *Barriopsis fusca* A.J.L. Phillips, A. Alves & Crous.

Etymology. Named in honour of Margaret E. Barr, who dedicated a large part of her career to resolving the taxonomy of the Dothideomycetes.

Ascomata pseudothecial, scattered or clustered, brown to black, wall composed of several layers of *textura angularis*, ostiole central. *Pseudoparaphyses* hyaline, smooth, multiseptate, constricted at septa. *Asci* bitunicate, clavate, stipitate, thick-walled

with thick endotunica and well-developed apical chamber. *Ascospores* aseptate, ellipsoid to ovoid, brown when mature, without terminal apiculi.

Note — The absence of apiculi differentiate this genus from *Sphaeropsis* and *Phaeobotryosphaeria*. The aseptate, brown ascospores without apiculi are unique in the Botryosphaeriaceae.

Barriopsis fusca (N.E. Stevens) A.J.L. Phillips, A. Alves & Crous, *comb. nov.* — MycoBank MB511713; Fig. 7

Basionym. *Physalospora fusca* N.E. Stevens, Mycologia 18: 210. 1926. = *Phaeobotryosphaeria fusca* Petr., Sydowia 6: 317. 1952. = *Botryosphaeria disrupta* (Berk. & Curtis) Arx & E. Müll., Beitr. Kryptogamenfl. Schweiz 2, 1: 37. 1954.

Ascomata scattered, immersed, brown to black, separate or aggregated, wall composed of *textura angularis*, uniloculate, ostiole single, central. *Pseudoparaphyses* hyaline, smooth, 3–4.5 μ m wide, multiseptate with septa 14–18 μ m apart. *Asci* bitunicate, clavate, 8-spored, stipitate, thick-walled with thick endotunica and well-developed apical chamber, 125–180 \times 30–36 μ m. *Ascospores* biseriata, aseptate, ellipsoid to oval,

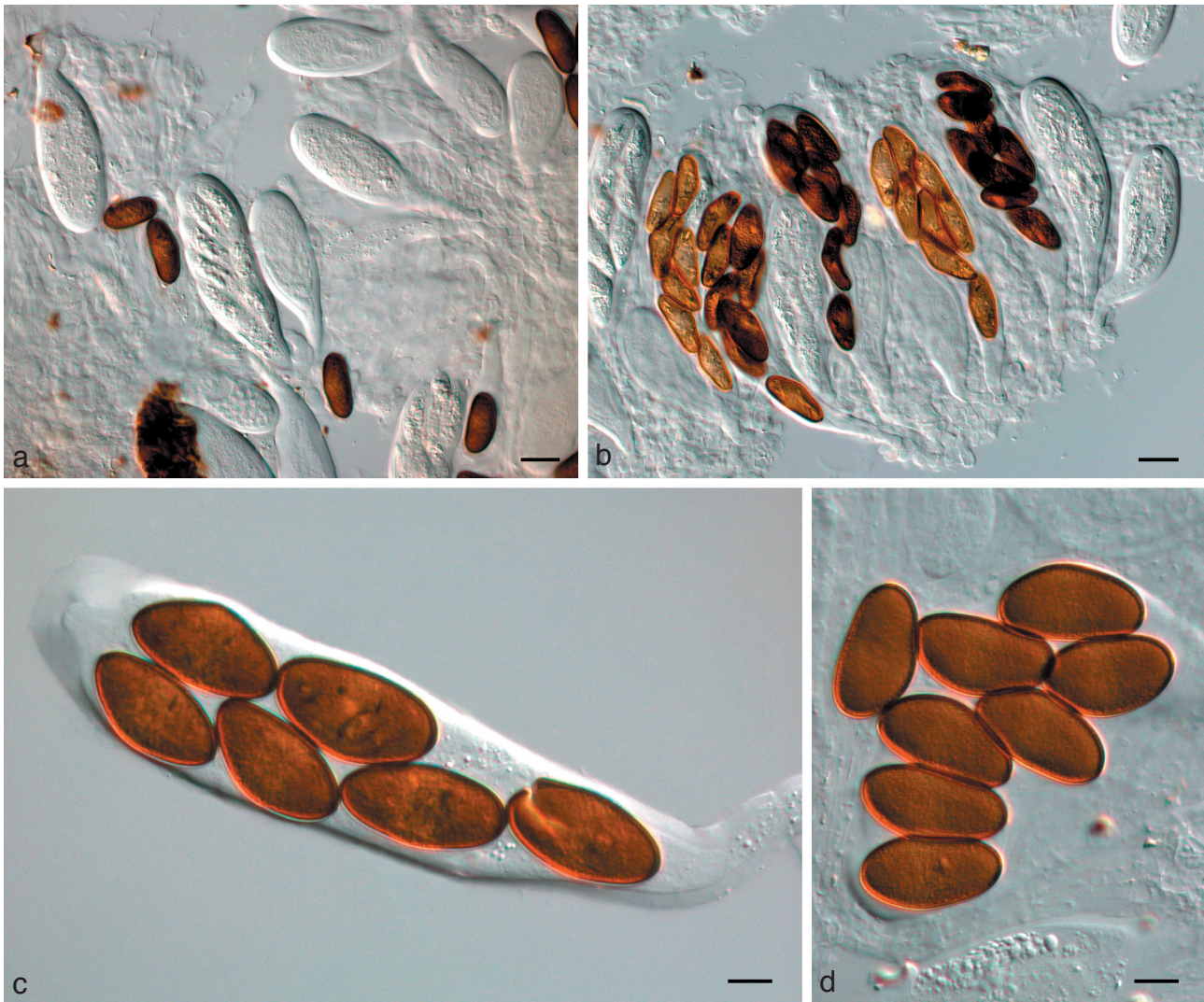


Fig. 7 *Barriopsis fusca* BPI 599052. a. Immature asci; b. mature asci with brown ascospores; c. ascus with ascospores; d. ascospores. — Scale bars: a, b = 20 μ m; c, d = 10 μ m.

straight or slightly curved, apex and base obtuse, without terminal apiculi, wall externally smooth, internally finely verruculose, brown, widest in the middle, (30–)31–36.5(–38.5) \times (15.5–)16–18.5(–21) μ m, 95 % confidence limits = 32.6–33.4 \times 17.0–17.5 μ m ($\bar{x} \pm$ S.D. = 33.0 \pm 1.5 \times 17.2 \pm 1.0 μ m, L/W ratio = 1.9 \pm 0.15).

Specimens examined. CUBA, Herradura, on twigs of *Citrus* sp., 15 Jan. 1925, N.E. Stevens, holotype BPI 599052, culture ex-type CBS 174.26. – USA, Florida, Orlando, on twigs of *Citrus* sp., 20 Feb. 1923, C.L. Shear, BPI 599054.

Notes — The ex-type culture could not be induced to sporulate, no doubt because it has been in culture for more than 80 years. According to Stevens (1926) the anamorph is lasiodiplodia-like and he described it as follows: “conidia initially hyaline, aseptate and thick-walled becoming dark brown and septate with irregular longitudinal striations, (20–)23–25(–28)

\times (11–)12–13(–16) μ m”. Stevens (1926) placed this species in *Physalospora*, but he was obviously hesitant to do so, judging from his statement “To place in the genus *Physalospora* a fungus with coloured ascospores is of course to do violence to the ideas of that genus”. On account of the bitunicate asci and brown ascospores of this species, *Physalospora* is clearly unsuitable. Petrak & Deighton (1952) transferred this species to *Phaeobotryosphaeria* as *Phaeobotryosphaeria fusca*, presumably on account of its dark ascospores. We examined the type species of *Phaeobotryosphaeria* (*P. yerbae*) and found it to have terminal apiculi on the ascospores. Therefore, *Phaeobotryosphaeria* would also appear to be unsuitable. For this reason we propose the new genus *Barriopsis* for this fungus. The brown, aseptate ascospores without terminal apiculi are characteristic of this new genus.



Fig. 8 *Dothiorella iberica*. a–g: LISE 94944; h–n: CBS 115041. a. Vertical section through an ascoma; b. ascus with brown, 1-septate ascospores; c. immature asci and one ascus with four ascospores; d. details of ascoma wall; e. pseudoparaphyses; f. ascospores; g. ascospore; h. young conidiogenous cells; i. conidiogenous cells with developing conidia; j, k. conidia viewed at two different levels of focus to show internally verruculose wall; l, m. conidia; n. germinating conidia. — Scale bars: a = 50 μ m; b–n: 10 μ m.

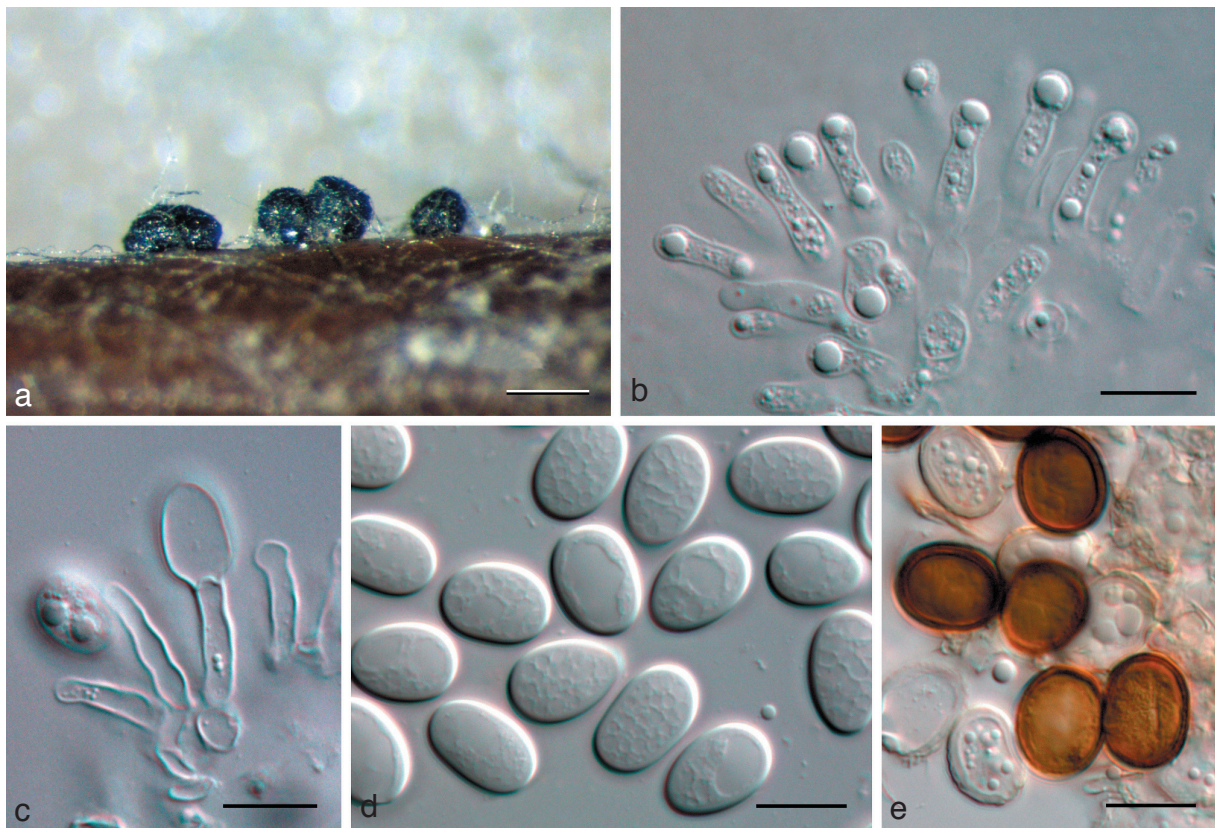


Fig. 9 *Neodeightonia subglobosa* CBS 448.91. a. Globose conidiomata; b, c. conidiogenous cells; d. hyaline conidia; e. mature brown conidia. — Scale bars: a = 250 μ m; b–e = 10 μ m.

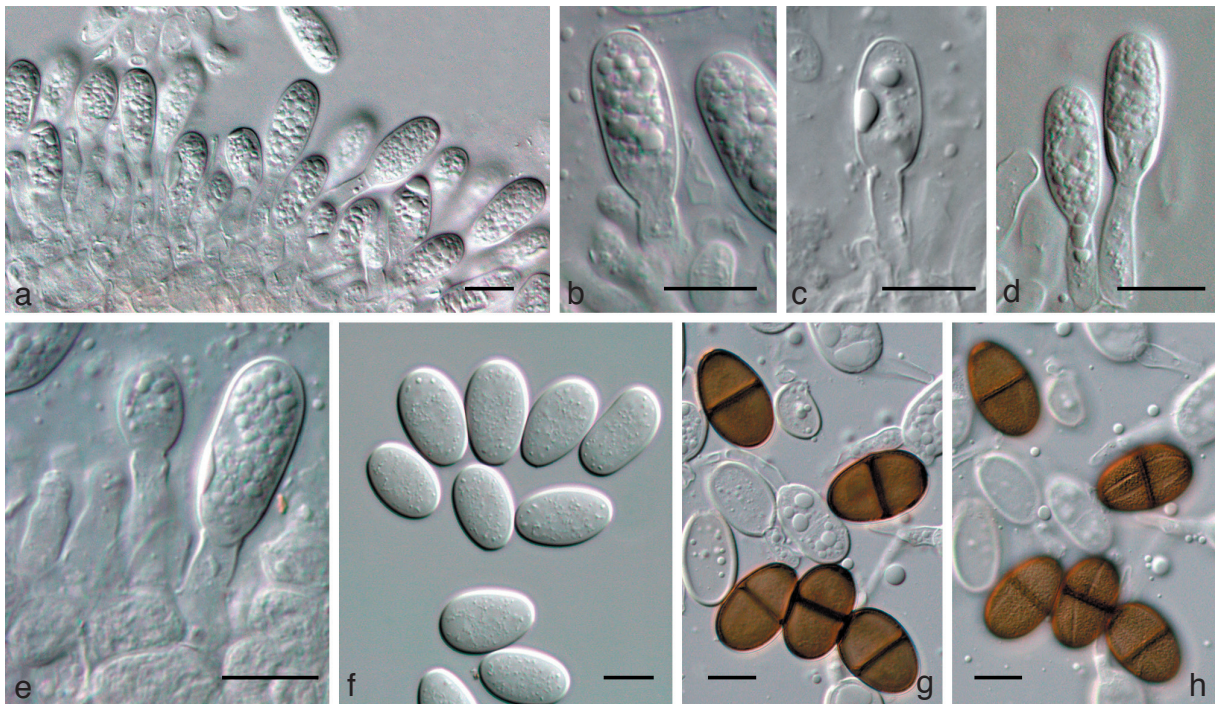


Fig. 10 *Neodeightonia phoenicum* CBS 122528. a. Conidiogenous layer; b–e. conidiogenous cells; f. hyaline, aseptate conidia; g, h. brown, 1-septate conidia with longitudinal striations. — Scale bars = 10 μ m.

***Dothiorella* Sacc., Michelia 2: 5. 1880**

Type species. *Dothiorella pyrenophora* Sacc.

***Dothiorella pyrenophora* Sacc., Michelia 2: 5. 1880**

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

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

***Dothiorella sarmentorum* (Fr.) A.J.L. Phillips, A. Alves & J. Luque, Mycologia 97: 522. 2005**

Basionym. *Sphaeria sarmentorum* Fr., Kongl. Vetensk. Acad. Handl., n.s. 39: 107. 1818.

= *Diplodia sarmentorum* (Fr.) Fr., Summa Veg. Scand. 2: 417. 1849.

= *Diplodia pruni* Fuckel, Jahrb. Nassauischen Vereins Naturk. 23–24: 169. (1869–1870) 1870.

Teleomorph. *Botryosphaeria sarmentorum* A.J.L. Phillips, A. Alves & J. Luque, Mycologia 97: 522. 2005.

Specimens examined. ENGLAND, Warwickshire, on *Ulmus* sp., Aug. 1956, E.A. Ellis, holotype of *Othia spiraeae*, IMI 63581b, culture ex-holotype IMI 63581b. — SWEDEN, Lund, Botanical Garden, on *Menispermum canadense*, 1818, E.M. Fries (holotype of *Sphaeria sarmentorum*) Scleromyc. Suec. 18, isotype K(M) 104852.

***Dothiorella iberica* A.J.L. Phillips, J. Luque & A. Alves, Mycologia 97: 524. 2005 — Fig. 8**

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

Specimens examined. SPAIN, Aragon, Tarazona, on dead twigs of *Quercus ilex*, 18 Dec. 2002, J. Luque, holotype of *B. iberica* LISE 94944, culture ex-type CBS 115041, LISE 94942 = CBS 115035.

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

Within the *Dothiorella* clade there is a subclade consisting of *Diplodia coryli* (CBS 242.51) and *Diplodia juglandis* (CBS 188.87). However, neither of these two isolates is authentic, and neither could be induced to sporulate. Thus, their identity and distinction from other species could not be determined. Two other isolates (CAP187 from *Prunus dulcis* in Portugal, and JL599 from *Corylus avellana* in Spain) identified as *Dothiorella* spp. formed two further clades. However, these clades are represented by single isolates and were not considered any further. Nevertheless, it is clear that *Dothiorella* is genetically diverse and further collections will undoubtedly yield more species.

***Neodeightonia* C. Booth, Mycol. Pap. 119: 17. (1969) 1970.**

Type species. *Neodeightonia subglobosa* C. Booth.

***Neodeightonia subglobosa* C. Booth, Mycol. Pap. 119: 19. (1969) 1970.**

Anamorph. *Sphaeropsis subglobosa* Cooke, Grevillea 7: 95. 1879, as '*subglobosum*'.

Specimens examined. SIERRA LEONE, Njala (Kori), on dead culms of *Bambusa arundinacea*, 17 Aug. 1954, F.C. Deighton, holotype IMI 57769 (c). — UNKNOWN LOCATION, human, keratomycosis of eye, Aug. 1991, Kirkness, CBS 448.91.

Note — We examined the type specimen of *Neodeightonia subglobosa* and found only immature asci with hyaline ascospores. However, Punithalingam (1969) clearly described and illustrated the ascospores as brown and 1-septate. Von Arx & Müller (1954) transferred *N. subglobosa* to *Botryosphaeria*, and because this is the type species of the genus, they reduced *Neodeightonia* to synonymy under *Botryosphaeria*. However, morphologically (based on the dark, 1-septate ascospores) and phylogenetically, this genus is distinguishable from *Botryosphaeria*, and the genus is therefore reinstated here. Punithalingam (1969) referred to a germ slit in the conidia. Crous et al. (2006) suggested that this is in fact a striation on the conidial wall, and that more than one could occur per conidium, a feature confirmed in the present study (Fig. 9). Such striate walls suggest an affinity to *Lasiodiplodia*. Nevertheless, *Neodeightonia* can be distinguished from *Lasiodiplodia* by the absence of pycnidial paraphyses. Thus, conidial striations distinguish *Neodeightonia* from *Diplodia*, and the absence of pycnidial paraphyses distinguishes it from *Lasiodiplodia*.

***Neodeightonia phoenicum* A.J.L. Phillips & Crous, sp. nov. — MycoBank MB511708; Fig. 10**

Conidiomata brunnea vel nigra, in contextu hospitis inclusa, multilocularia, globosa. Cellulae conidiogenae holoblasticae, hyalinae, cylindricae, percurrenter cum 1–2 proliferationibus prolificentes, vel in plano eodem peridinaliter incrassatae. Conidia (14.5–)17–21(–24) × (9–)10–12.5(–14) µm ovoidea vel ellipsoidea, apicibus rotundato, in fundo rotundato, parietibus crassis, primaria hyalinae, cum maturitate brunnea, longitudinaliter striata et unum septa formantia.

Anamorph. Macrophoma phoenicum Sacc., Annuario Reale Ist. Bot. Roma 4: 195. 1890.

≡ *Diplodia phoenicum* (Sacc.) H.S. Fawc. & Klotz, Bull. Calif. Agric. Exp. Station 522: 8. 1932.

≡ *Strionemadiplodia phoenicum* (Sacc.) Zambett., Bull. Trimestriell Soc. Mycol. France 70: 235. (1954) 1955.

Conidiomata formed on pine needles in culture pycnidial, multiloculate, dark brown to black, immersed in the host, becoming erumpent when mature. *Conidiogenous cells* hyaline, smooth, cylindrical, swollen at base, holoblastic, proliferating percurrently to form one or two annellations, or proliferating at same level giving rise to periclinal thickenings. *Conidia* ovoid to ellipsoid, apex and base broadly rounded, widest in middle to upper third, thick-walled, initially hyaline and aseptate, becoming dark brown and 1-septate some time after discharge from pycnidia, with melanin deposits on inner surface of wall arranged longitudinally giving a striate appearance to conidia, $(14.5\text{--}17\text{--}21\text{--}24) \times (9\text{--}10\text{--}12.5\text{--}14) \mu\text{m}$, 95 % confidence limits = $18.6\text{--}19.5 \times 11.2\text{--}11.8 \mu\text{m}$ ($\bar{x} \pm \text{S.D.} = 19.1 \pm 1.7 \times 11.5 \pm 1.1 \mu\text{m}$, l/w ratio = 1.7 ± 0.2).

Specimens examined. SPAIN, Catalonia, Tarragona, Salou, on *Phoenix* sp., *F. Garcia*, holotype CBS H-20108, culture ex-type CBS 122528; Catalonia, Barcelona, Vilanova i la Geltrú, on *Phoenix canariensis*, 17 May 2004, *M. Rojo*, CBS 123168. – USA, California, on *Phoenix dactylifera*, Mar. 1934, *H.S. Fawcett*, CBS 169.34.

Notes — Zambettakis (1955) placed this species in *Strionemadiplodia* (based on the striate conidia). However, the teleomorphic genus *Neodeightonia* is available for this species. The absence of pycnidial paraphyses distinguishes *Neodeightonia* from *Lasiodiplodia*, while the striate conidia distinguish it from *Diplodia*. Although Punithalingam (1969) reported that the teleomorph of *N. subglobosa* forms in culture, our isolates of *N. phoenicum* failed to do so, even after long periods of incubation (> 3 mo).

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

Type species. Phaeobotryon cercidis (Cooke) Theiss. & Syd.

Phaeobotryon cercidis (Cooke) Theiss. & Syd., Ann. Mycol. 13: 664. 1915. — Fig. 11

Basionym. 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.

Specimen examined. USA, Carolina, on bark of *Cercis canadensis*, ex Herb. MC Cooke No 795, K134204.



Fig. 11 *Bagnisiella cercidis* K 134204. a. Ascomata; b. immature ascus; c. asci with immature ascospores; d. hyaline ascospores; e–g. hyaline, aseptate ascospores with terminal apiculi (arrows); h. broken, brown, 2-septate ascospore. — Scale bars: a = 400 μm ; b–h = 10 μm .

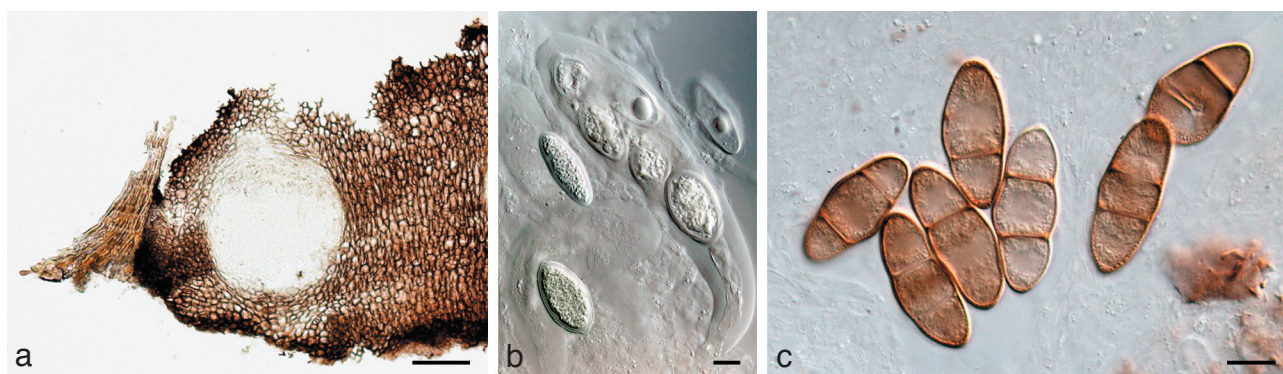


Fig. 12 *Phaeobotryon quercicola* Fungi Rehnani 534 in G. a. Vertical section through an ascoma; b. immature ascus; c. mature, 2-septate brown ascospores. — Scale bars: a = 100 μ m; b, c = 10 μ m.

Notes — In the original description of *Dothidea cercidis*, ascospores were reported as hyaline. However, Theissen & Sydow (1914) observed them to become brown with age, $32\text{--}38 \times 12\text{--}13 \mu\text{m}$. Subsequently Theissen & Sydow (1915) introduced the genus *Phaeobotryon* Theiss. & Syd. to accommodate this species. The asci are clavate, bitunicate, approx. $150\text{--}170 \times 30\text{--}35 \mu\text{m}$. The pseudoparaphyses are branched, septate, constricted at septa, anastomosing, $4\text{--}5 \mu\text{m}$ wide. The ascospores are ellipsoidal, initially hyaline, becoming pale brown, turning brown at maturity, 2-septate (cells equal in length), with a characteristic punctiform outgrowth (conical apiculus) at each end of the spore, $(27\text{--})30\text{--}35\text{--}(38) \times (8\text{--})12\text{--}14\text{--}(15) \mu\text{m}$. The latter features, namely 2-septate, brown ascospores with a conical apiculus at each end, are considered characteristic for the genus.

Phaeobotryon quercicola (A.J.L. Phillips) Crous & A.J.L. Phillips, *comb. nov.* — MycoBank MB511711; Fig. 12

Basionym. *Botryosphaeria quercicola* A.J.L. Phillips, *Mycologia* 97: 526. 2005 (based on *Othia quercus* Fuckel, *Jahrb. Nassauischen Vereins Naturk.*, 23–24: 170. (1869–1870) 1870.

Notes — As illustrated by Phillips et al. (2005), *Phaeobotryon quercicola* has brown, 2-septate ascospores with a conical apiculus at each end, thus suggesting that it would be better accommodated in *Phaeobotryon* than *Botryosphaeria*.

Phaeobotryon mamane Crous & A.J.L. Phillips, *sp. nov.* — MycoBank MB506581; Fig. 13

Phaeobotryon cercidis similis sed ascosporae majoribus, $(30\text{--})37\text{--}40\text{--}(45) \times (11\text{--})13\text{--}15\text{--}(16) \mu\text{m}$.

Anamorph. Dothiorella-like, but with up to two transverse septa.

Etymology. Named for its host, *Sophora chrysophylla*, which is known as 'mamane' in Hawaii.

Ascomata pseudothecial, dark brown to black, stromatic, globose, aggregated in botryose clusters or separate, immersed, becoming erumpent, ostiolate, up to 350 μm diam; wall consisting of 4–6 cell layers of dark brown *textura angularis*. *Pseudoparaphyses* hyaline, smooth, multiseptate, with septa

$10\text{--}23 \mu\text{m}$ apart, constricted at septa, $3\text{--}4 \mu\text{m}$ wide. *Asci* bitunicate, 8-spored, stipitate, thick-walled with thick endotunica and well-developed apical chamber, $120\text{--}150\text{--}(200) \times 25\text{--}30 \mu\text{m}$, with biseriolate ascospores. *Ascospores* ellipsoid to ovate, $(30\text{--})37\text{--}40\text{--}(45) \times (11\text{--})13\text{--}15\text{--}(16) \mu\text{m}$, 2-septate, with 3 cells of equal length, not constricted at septa, finely verruculose, widest in middle with conical apiculus at one or both ends. *Spermatogonia* morphologically similar to conidiomata, also formed in culture. *Spermatia* hyaline, rod-shaped with rounded ends, $3\text{--}5 \times 2 \mu\text{m}$. *Conidiomata* pycnidial, ostiolate, separate or aggregated, globose, black, immersed to erumpent, unilocular, up to 350 μm diam; wall consisting of 4–6 layers of brown *textura angularis*. *Conidiogenous cells* cylindrical to doliform, hyaline, smooth, proliferating percurrently near apex, $10\text{--}14 \times 4\text{--}8 \mu\text{m}$. *Conidia* ellipsoid to oblong or subcylindrical or obovoid, brown, smooth to finely verruculose, moderately thick-walled, granular, guttulate, ends rounded, 1(–2)-septate, base with inconspicuous scar, slightly flattened, $(30\text{--})35\text{--}38\text{--}(43) \times (12\text{--})14\text{--}15\text{--}(16) \mu\text{m}$.

Specimen examined. HAWAII, Manna Koa Park, Saddle Road, on stems of *Sophora chrysophylla*, July 2005, W. Gams, holotype CBS H-20109, culture ex-type-CPC 12440 = CBS 122980.

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

Type species. *Phaeobotryosphaeria yerbae* Speg.

Anamorph. *Sphaeropsis* Sacc., *Michelia* 2: 105. 1880, nom. cons.

Ascomata pseudothecial, brown to black, unilocular, thick-walled. *Pseudoparaphyses* hyaline, septate. *Asci* bitunicate, 8-spored, thick-walled with thick endotunica and well-developed apical chamber. *Ascospores* brown, aseptate with small apiculus at either end. *Conidiomata* pycnidial, eustromatic, immersed to erumpent, thick-walled, wall composed of several layers of dark brown *textura angularis*. *Ostiole* single, central, papillate. *Paraphyses* hyaline, aseptate, thin-walled. *Conidiogenous cells* hyaline, discrete, proliferating internally to form periclinal thickenings. *Conidia* oval, oblong or clavate, straight, aseptate, moderately thick-walled.

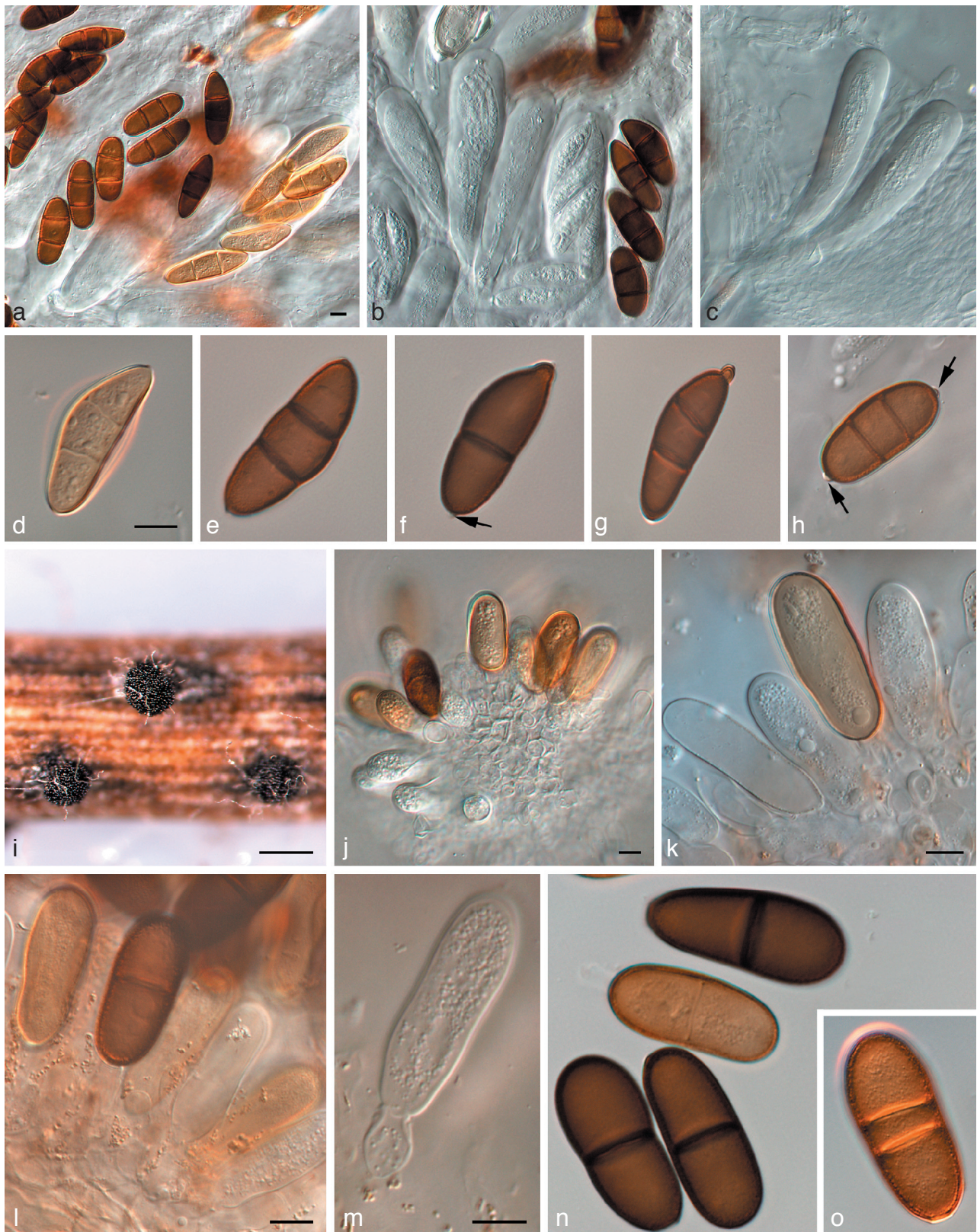


Fig. 13 *Phaeobotryon mamane*. a–h: CBS H-20109; i–o: CBS 122980. a–c. Asci and ascospores; d–h. ascospores with terminal apiculi (arrows); i. conidiomata forming on pine needle; j–m. conidiogenous cells with developing conidia; n. aseptate and 1-septate conidia; o. 2-septate conidium. — Scale bars: a–h, j–o = 10 μ m; i = 350 μ m.

Phaeobotryosphaeria yerbae Speg., Ann. Inst. Rech. Agron. 17, 10: 120. 1908 — Fig. 14

Anamorph. Not reported but presumably a *Sphaeropsis* species.

Ascomata pseudothecial, brown to black, multiloculate, immersed, becoming erumpent, ostiolate, papillate, up to 500 µm diam, wall composed of several layers of dark brown *textura angularis*. *Pseudoparaphyses* hyaline, smooth, 4–6 µm wide, multiseptate, with septa 10–18(–22) µm apart, constricted at septa. *Asci* bitunicate, clavate, 8-spored, ascospores biseriate in the ascus, stipitate, thick-walled with thick endotunica and well-developed apical chamber, 120–150 × 25–30 µm. *Ascospores* dark brown when mature, ovoid, (32–)34–42(–48) × (14–)16–18(–20) µm, aseptate, externally smooth, internally

finely verruculose, widest in middle with a hyaline apiculus at either end.

Specimen examined. ARGENTINA, Misiones, Campo das Cuias, y San Pedro, on branches of *Ilex paraguayensis*, Feb. 1907, C. Spegazzini, holotype LPS 2926.

Phaeobotryosphaeria visci (Kalchbr.) A.J.L. Phillips & Crous *comb. nov.* — MycoBank MB512100; Fig. 15

Basionym. *Dothidea visci* Kalchbr., Hedwigia 8: 117. 1869.
 ≡ *Phaeobotryon visci* (Kalchbr.) Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. kl., Abt I 128: 591. 1919.
 ≡ *Botryosphaeria visci* (Kalchbr.) Arx & E. Müll., Beitr. Kryptogamenfl. Schweiz, Band II, Heft I: 41. 1954.
Anamorph. *Sphaeropsis visci* (Fr.) Sacc., Michelia 2: 105. 1880.
 For synonyms see Sutton (1980).

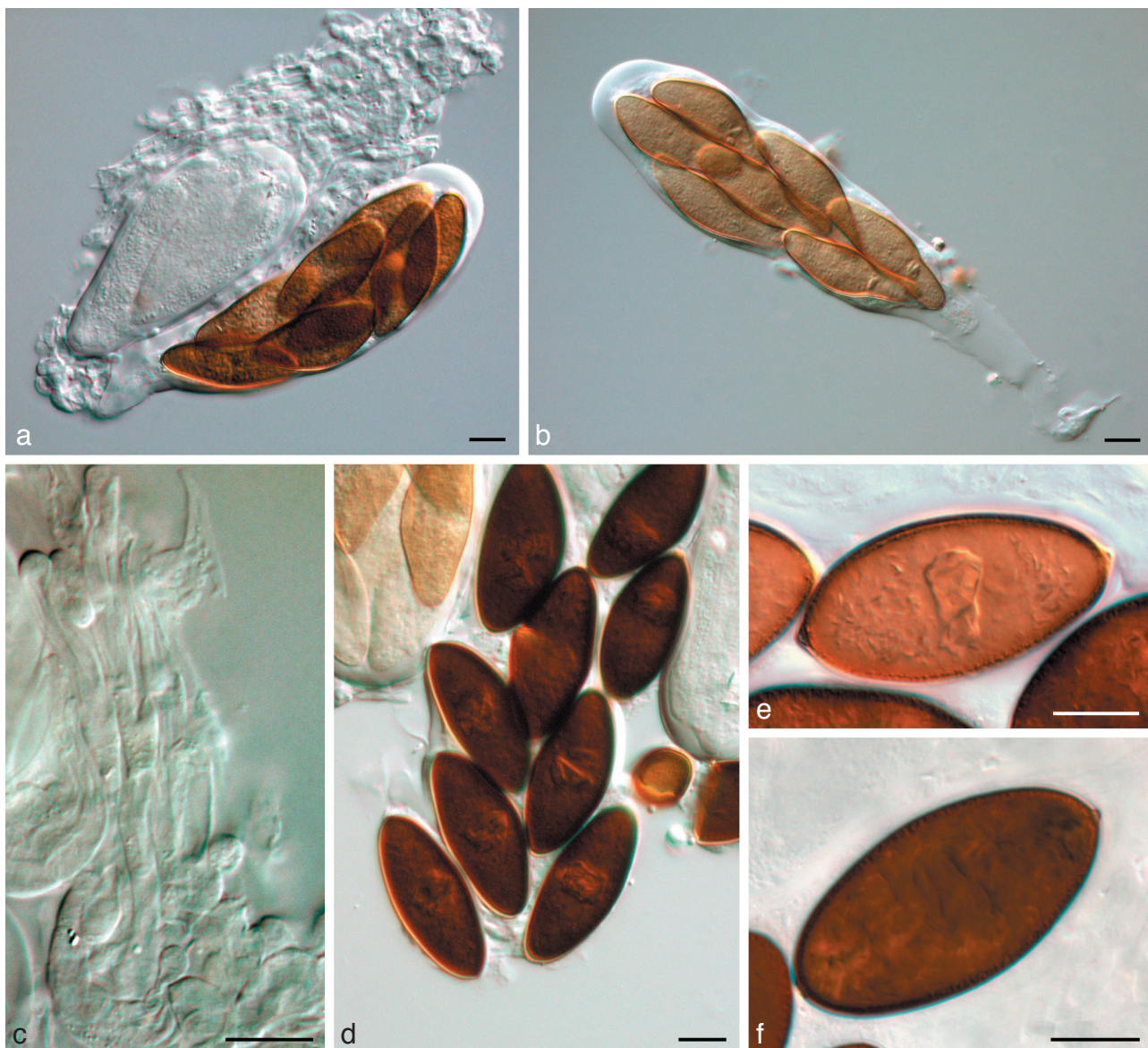


Fig. 14 *Phaeobotryosphaeria yerbae* LPS 2926. a. Immature (left) and mature (right) asci; b. mature ascus with brown, aseptate ascospores; c. septate pseudoparaphyses; d. dark brown, aseptate ascospores; e, f. dark brown, aseptate ascospores with apiculi. — Scale bars = 10 µm.

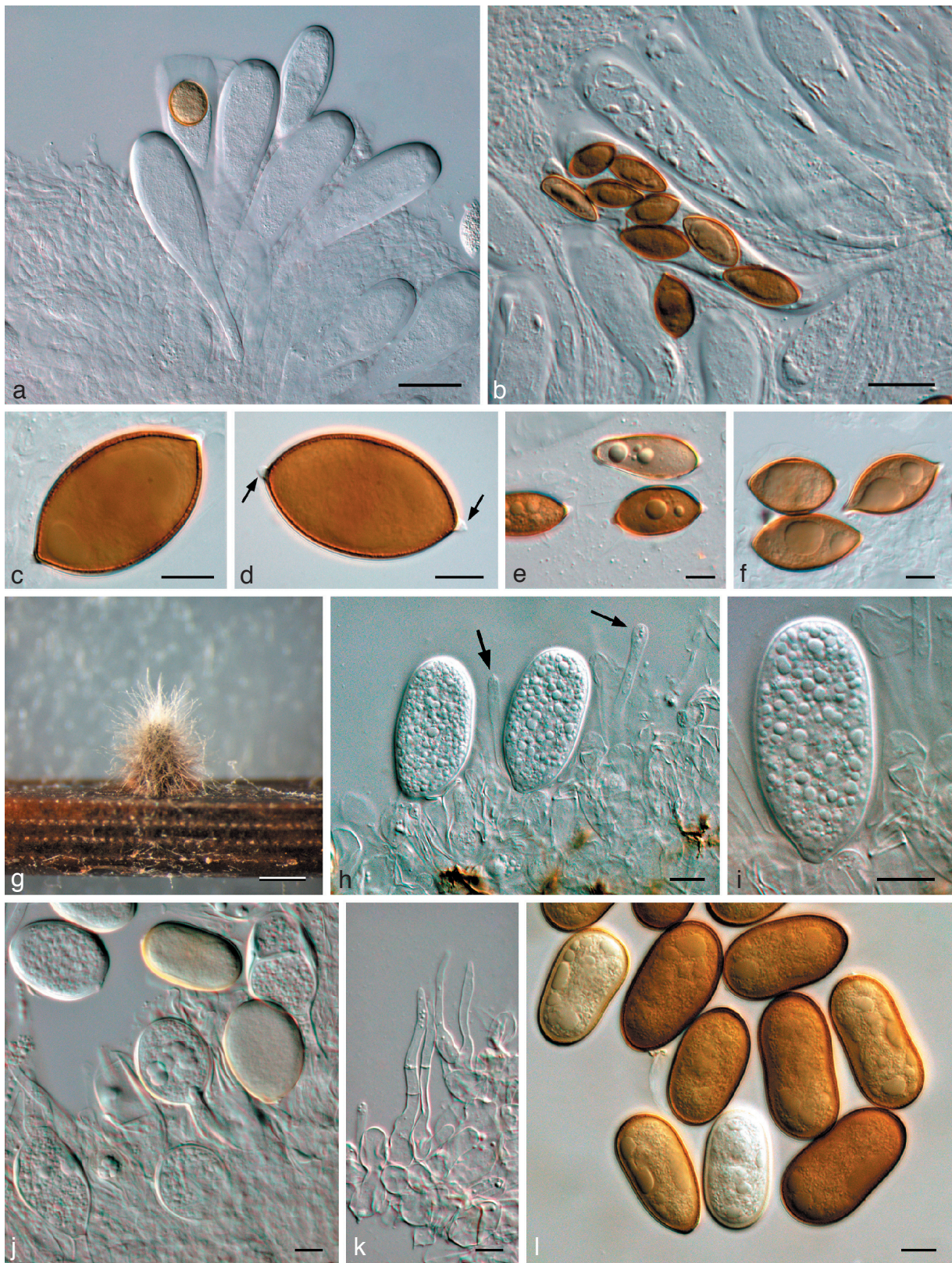


Fig. 15 *Phaeobotryosphaeria visci*. a–f: CWU (MYC) AS 2271; g–l: CBS 122527. a. Immature asci; b. mature ascus with brown, aseptate ascospores; c–f. brown, aseptate ascospores with apiculi (arrows); g. conidioma formed in culture on a pine needle; h, i. conidia forming on conidiogenous cell between paraphyses (arrows); j. developing conidia; k. paraphyses; l. conidia. — Scale bars: a, b = 20 μ m; c–f, h–l = 10 μ m; g = 50 μ m.

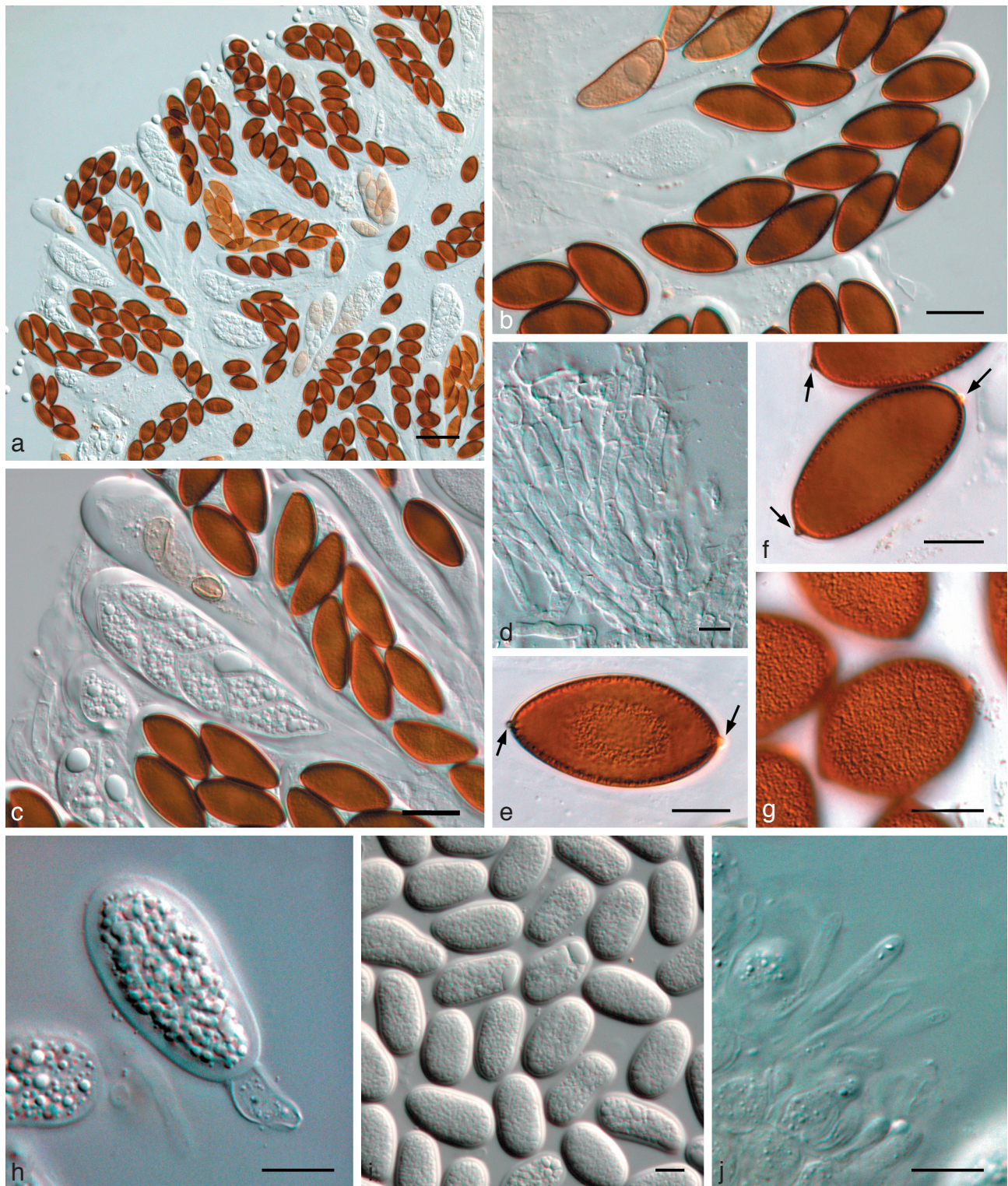


Fig. 16 *Phaeobotryosphaeria citrigena*. a–g: PDD 89238; h–j: ICMP 16812. a–c. Asci with brown ascospores; d. pseudoparaphyses; e–g. brown, aseptate ascospores with apiculi (arrows); h. conidium developing on a conidiogenous cell; i. hyaline, aseptate conidia; j. conidiomatal paraphyses. — Scale bars: a = 50 μ m; b–d = 20 μ m; e–j = 10 μ m.

Ascomata pseudothecial, brown to black, uni- or multiloculate, separate, immersed, ostiolate, up to 500 µm diam, wall composed of several layers of dark brown *textura angularis*. *Pseudoparaphyses* hyaline, smooth, 4–6 µm wide, multiseptate, with septa 11–19(–26) µm apart, constricted at septa. *Asci* bitunicate, 8-spored, ascospores biseriata in the ascus, stipitate, thick-walled with thick endotunica and well-developed apical chamber, 180–230 × 35–50 µm. *Ascospores* pale-brown when mature, ovoid, (27.5–)31–37.5(–38.5) × (14.5–)15–19(–19.5) µm, aseptate, externally smooth, internally finely verruculose, widest in middle with an apiculus at either end. *Conidiomata* immersed to erumpent and superficial, unilocular, up to 300 µm wide, wall composed of dark brown *textura angularis*. *Paraphyses* hyaline, aseptate, up to 40 µm long and 4 µm wide with a bulbous tip 5 µm diam. *Conidiogenous cells* hyaline, discrete proliferating internally to form periclinal thickenings, (4–)8.5–11 × 4–6.5 µm. *Conidia* (27–)29–33(–50) × (14.5–)15.5–19(–22) µm, oval, apex obtuse, base obtuse or truncate, moderately thick-walled, initially hyaline, becoming brown, externally smooth, internally finely verruculose.

Specimens examined. GERMANY, Klein Ziethen, near Angermünde, on fallen twigs of *Viscum album*, 22 July 1996, T. Graefenhan, CBS 186.97. – LUXEMBOURG, Weilenbach, near Echternach, on fallen twigs of *Viscum album*, 14 June 1997, H.A. van der Aa, CBS 100163. – UKRAINE, National Nature Park

'Svjatje Gory', Donetsk district, on branches of *Viscum album*, 10 Mar. 2007, Á. Akulov, CWU (MYC) AS 2271, cultures CBS 122526, CBS 122527.

Note — Until now the connection between *Phaeobotryosphaeria* and its anamorph has not been proven. On the specimen examined here there is a *Botryosphaeria*-like ascomycete with brown ascospores. Single ascospore isolations from this specimen resulted in cultures of *S. visci*, thus proving the connection between the two states. Features that distinguish this teleomorph from others with brown ascospores in the Botryosphaeriaceae are the aseptate ascospores with an apiculus at either end.

Phaeobotryosphaeria citrigena A. J. L. Phillips, P. R. Johnst. & Pennycook, *sp. nov.* — MycoBank MB511714; Fig. 16

Phaeobotryosphaeria visci similis sed ascosporae rufus-brunneae, et conidia minoribus, (27–)28–33(–34) × (14.5–)15–18.5(–19) µm.

Anamorph. *Sphaeropsis* sp.

Etymology. Named for its association with *Citrus*.

Ascomata pseudothecial, brown to black, separate or aggregated, immersed, becoming erumpent, ostiolate, wall composed of several layers of dark brown *textura angularis*. *Pseudopara-*

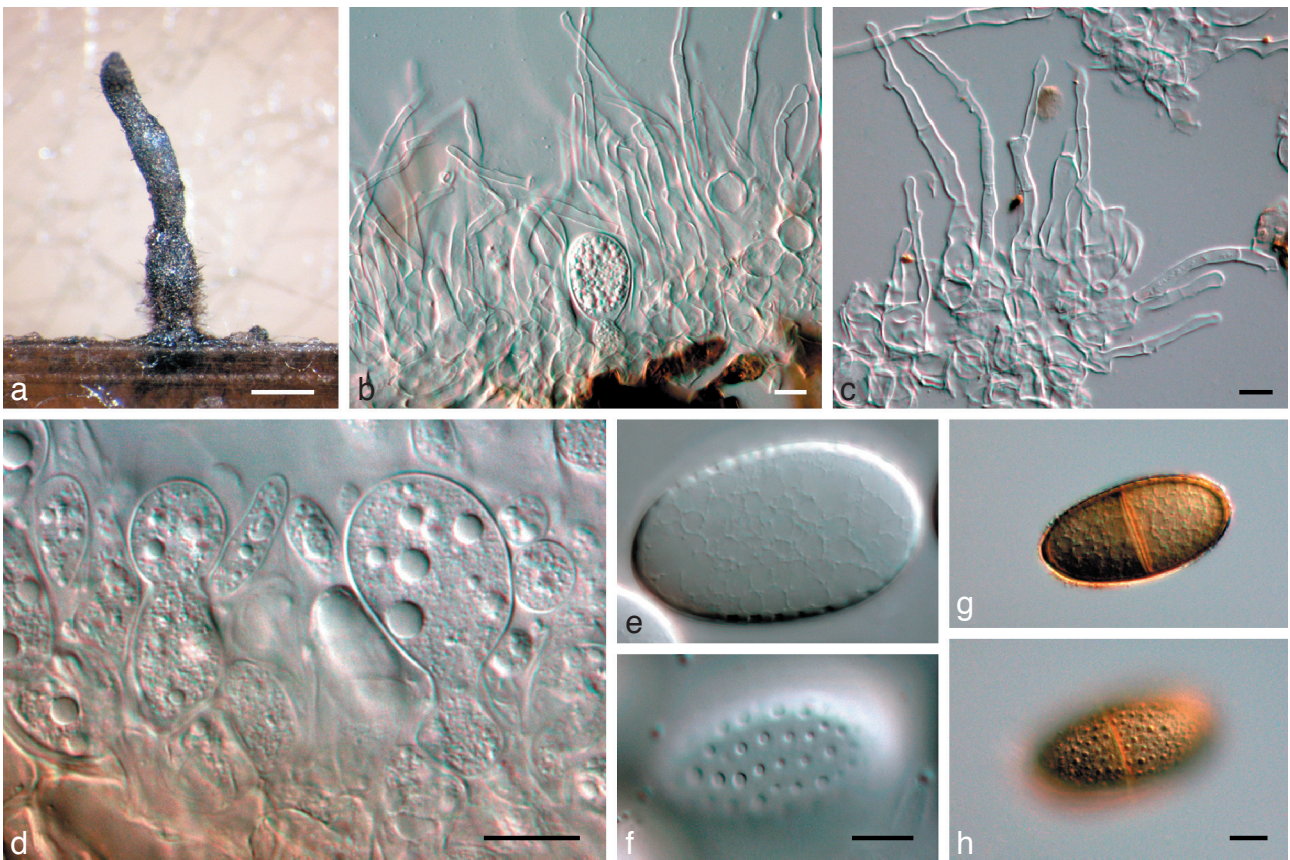


Fig. 17 *Phaeobotryosphaeria porosa* CBS 110496. a. Pycnidium with elongated neck; b. conidium developing between paraphyses; c. paraphyses; d. conidia and conidiogenous cells; e, f. immature conidium at two different levels of focus to show pores in the conidium wall; g, h. mature conidium at two different levels of focus to show verruculose inner surface of the wall. — Scale bars: a = 500 µm, b–h = 10 µm.

physes hyaline, smooth, 4–6 µm wide, multiseptate, with septa 11–26 µm apart; constricted at septa. *Asci* bitunicate, 8-spored, stipitate, thick-walled with thick endotunica and well-developed apical chamber, 180–230 × 35–43(–50) µm, with biseriate ascospores. *Ascospores* reddish brown when mature, ellipsoid to ovoid with both ends rounded, (27.5–)29–37.5(–38.5) × (14.5–)15.5–18(–19.5) µm, with an apiculus at either end, aseptate, externally smooth, internally finely verruculose, widest in middle to upper third. *Conidiomata* immersed to erumpent and superficial, unilocular, up to 500 µm wide, wall composed of several layers of dark brown *textura angularis*. *Paraphyses* hyaline, aseptate, up to 25 µm long and 3–3.5 µm wide. *Conidiogenous cells* hyaline, discrete, proliferating internally to form periclinal thickenings, 8–11 × 4–6.5 µm. *Conidia* (27–)28–33(–34) × (14.5–)15–18.5(–19) µm, oval, apex obtuse, base obtuse or truncate, moderately thick-walled, initially hyaline, becoming brown, externally smooth, internally finely verruculose, aseptate.

Specimens examined. NEW ZEALAND, Northland, Kerikeri, Davies Orchard (#2), Inlet Road, on recently dead bark-covered twigs of *Citrus sinensis*, 6 Sept. 2006, S.R. Pennycook, P.R. Johnston & B.C. Paulus, holotype PDD 89238, culture ex-type ICMP 16812; Northland, Kerikeri, Davies Orchard (#3), Inlet Road, on recently dead bark-covered twigs of *Citrus sinensis*, 6 Sept. 2006, S.R. Pennycook, P.R. Johnston & B.C. Paulus, PDD 89239, culture ICMP 16818.

Notes — *Conidia* of *P. citrigena* remained hyaline for long periods and only rarely did we observe dark conidia. Conidial dimensions of this species are similar to *S. visci*, but its ascospores are reddish brown in contrast to the pale brown ones of *S. visci*.

Phaeobotryosphaeria porosa (Van Niekerk & Crous) Crous & A.J.L. Phillips, *comb. nov.* — MycoBank MB511715; Fig. 17

Basionym. *Diplodia porosum* Van Niekerk & Crous, *Mycologia* 96: 790. 2004.

Specimen examined. SOUTH AFRICA, Western Cape Province, Stellenbosch, on shoots of *Vitis vinifera*, 2002, J.M. van Niekerk, holotype CBS H-12039, culture ex-type CBS 110496.

Notes — Van Niekerk et al. (2004) did not mention pycnidial paraphyses, but these were clearly seen when these isolates were re-examined (Fig. 17). This species is unique within the Botryosphaeriaceae because of its large, thick-walled conidia with large pores (1 µm wide) that are clearly visible by light microscopy. However, the pitted walls, although unique and distinctive, should be regarded as informative at the species level in the same way that this character was regarded in the original description.

Spencermartinsia A.J.L. Phillips, A. Alves & Crous, *gen. nov.* — MycoBank MB511762.

Ascomata pseudothecia, ostiolati. *Asci* bitunicati, octo-sporei, clavati, stipitati, pseudoparaphysibus multis filiformibus, septatis, latis interspersi. *Ascosporeae* biseriatati, uniseptati cum terminali apiculi. *Conidiomata* stromatiformia. *Cellulae conidiogena*e holoblasticase, proliferatione percurrenti, ut videtur annellationibus, vel in plano eodem periclinaliter incrassate. *Conidia* brunnea, uniseptata.

Type species. *Spencermartinsia viticola* (A.J.L. Phillips & J. Luque) A.J.L. Phillips, A. Alves & Crous.

Etymology. Named in honour of Prof. dr Isabel Spencer-Martins, founder of the Centro de Recursos Microbiológicos in Portugal.

Ascomata pseudothecial, ostiolate. *Pseudoparaphyses* thin-walled, hyaline, septate, constricted at septa. *Asci* bitunicate, 8-spored, clavate, stipitate, developing amongst thin-walled, septate pseudoparaphyses, with biseriate ascospores. *Ascospores* hyaline when young, brown when mature, uniseptate with an apiculus at each end. *Conidiomata* stromatic. *Conidiogenous cells* lining inner surface of conidiomata, holoblastic, proliferating internally producing periclinal thickenings, or proliferating percurrently to form annellations. *Conidia* brown, 1-septate.

Note — *Spencermartinsia* differs from *Dothiorella* in having 2-celled ascospores with an apiculus at either end of the ascospores.

Spencermartinsia viticola (A.J.L. Phillips & J. Luque) A.J.L. Phillips, A. Alves & Crous, *comb. nov.* — MycoBank MB511763; Fig. 18

Basionym. *Botryosphaeria viticola* A.J.L. Phillips & J. Luque, *Mycologia* 97: 1116. (2005) 2006.

Anamorph. *Dothiorella viticola* A.J.L. Phillips & J. Luque, *Mycologia* 97: 1116. (2005) 2006.

Specimens 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, holotype LISE 95177, culture ex-type CBS 117009; ditto, 28 May 2003, J. Luque & Mateu, LISE 95178, culture CBS 117006.

Notes — The ex-type isolate of *Spencermartinsia viticola* (CBS 117009) clustered with an isolate previously identified as *Diplodia spegazziniana* (CBS 302.75). The latter isolate is misidentified and is not representative of this species. An additional isolate originally identified by Luque et al. (2005) as *B. viticola* (CBS 117006), exhibited some differences in culture morphology from the ex-type strain and other strains (Luque et al. 2005). For example, the reverse side of cultures of CBS 117006 became red-brown after 3–5 d on PDA at 25 °C with a progressive darkening of the pigment after 6–10 d. Furthermore, there were some differences in ITS and EF1- α sequences between CBS 117006 and CBS 117009 (one substitution and one deletion in ITS and nine substitutions in EF1- α). Although these morphological and phylogenetic differences may reflect species differences, no name was applied to CBS 117006 because only one isolate was available for study.

Also contained within *Spencermartinsia* was a single isolate of '*Diplodia*' *medicaginis* (CBS 500.72), which formed a unique clade. Again, only a single isolate was available, the name of which is unresolved. Isolates ICMP 16827 and ICMP 16828 from *Citrus sinensis* in New Zealand formed another subclade, and thus would be regarded as a distinct phylogenetic species. However, neither of the isolates could be induced to sporulate, and no morphological data are available. Therefore, no names will be applied until their morphology can be determined.

Isolates ICMP 16819 and ICMP 16824, also from *Citrus sinensis* in New Zealand, formed a sister clade to *Spencermartinsia* that was supported by a high MP bootstrap value

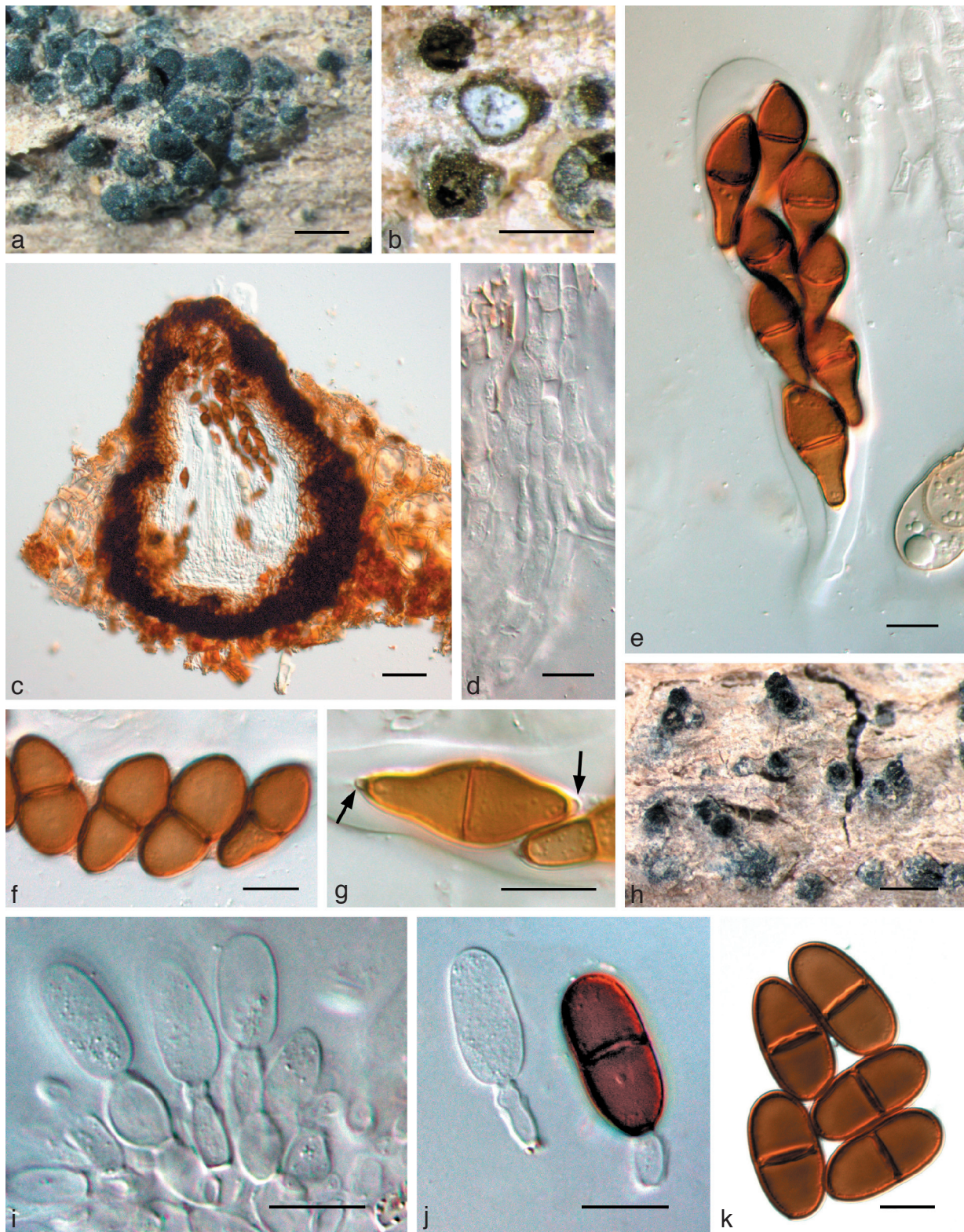


Fig. 18 *Spencermartinsia viticola*. a–h: LISE95177; i–k: CBS 117009. a. Ascomata erumpent through host bark; b. ascoma cut through horizontally showing the white contents with dark spots corresponding to asci with ascospores; c. vertical section through an ascoma; d. septate pseudoparaphyses; e. clavate ascus containing eight biserial, dark brown, 1-septate ascospores; f. ascospores; g. ascospores with small rounded apiculi (arrows); h. conidiomata partially erumpent through the host bark; i. conidiogenous cells; j. conidiogenous cells with annellations. The cell on the right has a dark brown, 1-septate conidium attached; k. conidia. — Scale bars: a, h = 500 µm; b = 250 µm; c = 50 µm; d–g, i–k = 10 µm.

(100 %). Neither of these two isolates has been induced to sporulate. In view of the lack of morphological data, the status of these two isolates remains uncertain.

DISCUSSION

In this paper the phylogenetic position and taxonomy of species of Botryosphaeriaceae with brown ascospores were studied. The taxonomic position of *Dothidotthia* was resolved, and within the Botryosphaeriaceae we recognise a number of genera with brown ascospores. For some of these genera we reinstate old names, while others are described as new. In keeping with the proposal to use a single name for pleomorphic fungi (Rossman & Samuels 2005) we propose a single generic name for each clade. For example, since *Dothidotthia* was shown to fall in the Pleosporales, this name is no longer available for the teleomorph of *Dothiorella*, and therefore we propose that the anamorph genus name, *Dothiorella*, be used for both the anamorph and the teleomorph of this clade.

Within the Botryosphaeriaceae, species with brown ascospores are found in three separate lineages, which lead to at least six genera. These lineages are dispersed randomly in different branches of the phylogenetic tree. Considering that brown ascospores are a common feature in other families in the Dothideomycetes (von Arx & Müller 1954, 1975), it is possible that this character has been retained in these lineages from the ancestors of the family, rather than being a character that has evolved at different times.

Other morphological features that were used to differentiate genera were the presence or absence of apiculi on ascospores, septation of ascospores, striations on conidia, and the presence or absence of paraphyses in conidiomata. It is interesting to note that striations are strongly developed in *Lasiodiplodia*, weaker in *Neodeightonia* and absent from *Diplodia*. Furthermore, conidiomatal paraphyses are found in *Lasiodiplodia* but are absent from *Neodeightonia* and *Diplodia*.

Two of the lineages with brown ascospores lie within a clade that was previously regarded to contain species with *Lasiodiplodia* and *Diplodia* anamorphs (Crous et al. 2006). In their phylogenetic study (Crous et al. 2006), based on LSU sequences, this clade could not be resolved. In the present study it could be resolved only by combining sequences of two protein coding genes with sequences of three ribosomal genes. In this way this clade was resolved into six genera, four of which have dark ascospores.

One lineage (clade 2, Fig. 2) clustered between *Diplodia* and *Lasiodiplodia*. The name *Neodeightonia* already exists for this genus and it is reinstated in this paper. This genus was introduced by Booth (in Punithalingam 1969) for a single species, namely *N. subglobosa*. Von Arx & Müller (1975) transferred this species to *Botryosphaeria* within their broad concept of the genus. When Crous et al. (2006) reassessed *Botryosphaeria*, reducing it to *B. corticis* and *B. dothidea*, their isolate of *B. subglobosa* resided in an unresolved clade consisting of *Diplodia*, *Lasiodiplodia* and *Tiarosporella*. From the data presented here it is clear that *Neodeightonia subglobosa*, type of *Neodeightonia*, is phylogenetically and morphologically

distinct from other genera in the Botryosphaeriaceae. *Diplodia phoenicum* was shown to be another species in this genus. Rather than introduce a new anamorph genus to accommodate *Diplodia phoenicum* we followed the procedures suggested by Rossman & Samuels (2005) and used the teleomorph genus name for this species. Conidia of both *N. subglobosa* and *N. phoenicum* have striations on the conidial wall similar to those seen in *Lasiodiplodia*, albeit somewhat less distinct. However, anamorphs of *Neodeightonia* do not have paraphyses, which are typical of *Lasiodiplodia*, and the striate conidial wall distinguishes *Neodeightonia* from *Diplodia*.

A second lineage, basal to *Diplodia*, *Lasiodiplodia* and *Neodeightonia*, was resolved into three clades (clades 4–6) that could be distinguished from one another on the morphology of the teleomorphs, especially septation of the ascospores and the presence or absence of ascospore apiculi. These genera can also be differentiated on morphology of the anamorphs. *Phaeobotryon* is available for one of these clades, *Phaeobotryosphaeria* for another, but as far as we could tell, no suitable names are available for the third one, and *Barriopsis* is introduced to accommodate *Physalospora fusca*, which has aseptate ascospores without apiculi. Von Arx & Müller (1954, 1975) placed *Phaeobotryon* in synonymy with *Botryosphaeria*. However, as determined here, *Phaeobotryon* is morphologically and phylogenetically distinct from all the other genera we studied. For this reason we have reinstated the generic name *Phaeobotryon* for isolate CBS 122980, and for other isolates in the same clade. The 1–2-septate ascospores of these fungi with an apiculus at either end correspond with *Bagnisiella cercidis* K134204, which is the basionym of *Phaeobotryon cercidis* and type species of the genus *Phaeobotryon*. Ascospores of the isolates from *Sophora chrysophylla* are larger than *P. cercidis* and for this reason these isolates were described as a new species. Although Von Arx & Müller (1954) considered *Phaeobotryosphaeria* a synonym of *Botryosphaeria*, in this study we show that it is morphologically and phylogenetically distinct from the other two genera in this clade and the name is reinstated for species with brown, aseptate ascospores with terminal apiculi.

The anamorph of *Phaeobotryosphaeria* was shown to correspond to *Sphaeropsis*. Although we have adopted to follow the system of one name for one genus, it is important to clarify some of the controversy surrounding the genus *Sphaeropsis*. This genus has been the subject of considerable debate, much of which has revolved around the question of a suitable genus name for the pine pathogen sometimes referred to as *Sphaeropsis sapinea*. The main point of debate has been whether this species should revert to its older name of *Diplodia pinea* or whether it should remain in *Sphaeropsis*. From the literature it seems that this species has been regarded as typical of the genus *Sphaeropsis*, both morphologically and phylogenetically. For example, the phylogenetic studies of Jacobs & Rehner (1998) and Denman et al. (2000) placed *Sphaeropsis sapinea* in the *Diplodia* clade, which prompted Denman et al. (2000) to suggest that *Sphaeropsis* is a synonym of *Diplodia*. This decision was also supported by subsequent studies (Zhou & Stanosz 2001, Alves et al. 2004). When Sutton (1980) stated that percurrently proliferating conidiogenous cells are a feature of *Sphaeropsis*

that are not found in *Diplodia* it is not clear if he was referring to *S. visci* or *S. pinea*. Nevertheless, Denman et al. (2000) referred to percurrent proliferations in *Diplodia*, further confirming their suggestion that *Sphaeropsis* is a synonym of *Diplodia*. Phillips (2002) and Alves et al. (2004) confirmed that this type of conidiogenesis occurs in *Diplodia mutila*. However, it is important to point out that when Saccardo (1880) established *Sphaeropsis* for species of *Diplodia* with dark conidia, he cited *S. visci* as the type species. We examined a number of strains isolated from *Viscum album* that correlate in all ways with the original description of *S. visci* and could find only internal proliferation of the conidiogenous cells, resulting in periclinal thickenings and typical phialides (sensu Sutton 1980). Moreover, this was the only type of conidiogenesis that we could detect in the other species that we consider to belong in *Sphaeropsis* (*D. porosum* and *S. citrigena*). As we illustrate here, the anamorphs of *Sphaeropsis* are morphologically (pycnidial paraphyses) and phylogenetically distinct from *Diplodia*. Thus, as revealed by the phylogeny presented here, *Sphaeropsis*, typified by *S. visci*, is a valid and distinct genus. Moreover, the pine pathogen often referred to as *S. sapinea* resides in *Diplodia*.

The other species in *Phaeobotryosphaeria* deserve some mention. *Phaeobotryosphaeria porosa* is distinct in the large pits in the conidial wall. When this species was described from grapevines in South Africa (van Niekerk et al. 2004) it was placed in *Diplodia*, although the authors suggested that its unique conidial morphology might necessitate a new genus. At that time *Sphaeropsis* was not clearly defined and indeed had been suggested as being a synonym of *Diplodia*. Despite the unique character of conidial pits, *D. porosum* has features that place it within the morphological concept of *Phaeobotryosphaeria*. These features include relatively large, thick-walled conidia, phialidic conidiogenous cells with periclinal thickenings, and pycnidial paraphyses. Phylogenetically (Fig. 2) it also falls within *Phaeobotryosphaeria*. Thus, it seems that conidial pits are of taxonomic significance at species level only, in the same way as they were regarded when this species was first described by van Niekerk et al. (2004). Finally, a third species is described in *Phaeobotryosphaeria*, namely *P. citrigena* from dead citrus twigs in New Zealand.

The third lineage (clades 8–10) is sister to *Neofusicoccum*, and the name *Dothiorella* has been used for the anamorphs of these species. This lineage was resolved into at least two, possibly three genera. Clades 8 and 9 could be distinguished from one another on the morphology of their ascospores. No teleomorph is yet known for clade 10. *Dothiorella* is already available for clade 8, and a new genus *Spencermartinsia* is introduced for clade 9. *Dothiorella* is based on *D. pyrenophora*, but no cultures are available for this species. When Phillips et al. (2005) reinstated *Dothiorella*, they determined that *D. sarmentorum* corresponded in all ways with the concept for this genus.

A clade sister to *Dothiorella* was composed of two subclades (clades 9 and 10). It is not entirely clear if these two clades represent two genera or a single genus. *Spencermartinsia viticola* was considered to be a species of *Dothiorella* by Luque et al. (2005), who pointed out that some morphological aspects of the anamorph (colony morphology) differentiated this species from others in *Dothiorella*. A more detailed examination of

this species revealed that the ascospores bear an apiculus at either end. This feature, together with the phylogenetic difference indicates that this clade represents another genus closely related to *Dothiorella*, and for which we introduce the name *Spencermartinsia*. The distinct apiculi differentiate this genus from *Dothiorella*, and for this reason we propose it as a new genus. This clade (9) is phylogenetically diverse and appears to be composed of several species. The type species (*S. viticola*) is represented in Fig. 2 by the ex-type culture of *Do. viticola* (CBS 1187009). Another isolate with this name (CBS 117006) resides in a separate clade, and thus probably represents another species. Since we have only a single example of this species we decline at this stage to apply a species name to it. Similarly, CBS 500.72 (*D. medicaginis*) is another distinct species represented by a single isolate, which we also decline to name. The two isolates from *Citrus* (ICMP 16827 and ICMP 16828) did not sporulate in culture during the course of this work and thus cannot be fully characterised. Nevertheless, they too represent a third species in *Spencermartinsia*. The conidia from which these isolates were grown match closely those illustrated by Gure et al. (2005) from an isolate from *Podocarpus falcatus* seeds, which these authors referred to *Dothiorella*. We are continuing to study these isolates with the aim of applying species epithets.

Isolates ICMP 16819 and ICMP 16824 form a further clade (clade 10). These isolates were grown from 1-septate, dark brown, striate conidia collected from twigs of *Citrus*. The conidia become pigmented and septate while still attached to the conidiogenous cell, a characteristic of *Dothiorella* and *Spencermartinsia*. This fungus failed to sporulate in culture, and has yet to be linked to a teleomorph. For this reason we were unable to determine if these two isolates form a distinct genus and such a decision will have to wait until more isolates in this clade have been studied.

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REFERENCES

- Alfaro ME, Zoller S, Lutzoni F. 2003. Bayes or Bootstrap? A simulation study comparing the performance of Bayesian Markov Chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. *Molecular Biology and Evolution* 20: 255–266.
- Alves A, Correia A, Luque J, Phillips AJL. 2004. *Botryosphaeria corticola*, sp. nov. on *Quercus* species, with notes and description of *Botryosphaeria stevensii* and its anamorph, *Diplodia mutila*. *Mycologia* 96: 598–613.
- Alves A, Correia A, Phillips AJL. 2006. Multi-gene genealogies and morphological data support *Diplodia cupressi* sp. nov., previously recognized as *D. pinea* f. sp. *cupressi*, as a distinct species. *Fungal Diversity* 23: 1–15.
- Alves A, Crous PW, Correia A, Phillips AJL. 2008. Morphological and molecular data reveal cryptic species in *Lasioidiplodia theobromae*. *Fungal Diversity* 28: 1–13.

- Alves A, Phillips AJL, Henriques I, Correia A. 2005. Evaluation of amplified ribosomal DNA restriction analysis (ARDRA) as a method for the identification of *Botryosphaeria* species. *FEMS Microbiology Letters* 245: 221–229.
- Arx JA von, Müller E. 1954. Die Gattungen der amersporen Pyrenomyceten. Beiträge zur Kryptogamenflora der Schweiz 11: 1–434.
- Arx JA von, Müller E. 1975. A re-evaluation of the bitunicate ascomycetes with keys to families and genera. *Studies in Mycology* 9: 1–159.
- Barr ME. 1987. *Prodromus to Class Loculoascomycetes*. Amherst, Massachusetts: Published by the author.
- Barr ME. 1989. The genus *Dothidotthia* (Botryosphaeriaceae) in North America. *Mycotaxon* 34: 517–526.
- Burgess TI, Barber PA, Mohali S, Pegg G, Beer W de, Wingfield MJ. 2006. Three new *Lasiodiplodia* spp. from the tropics, recognized based on DNA sequence comparisons and morphology. *Mycologia* 98: 423–435.
- Carbone I, Kohn LM. 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* 91: 553–556.
- Crous PW, Palm ME. 1999. Reassessment of the anamorph genera *Botryodiplodia*, *Dothiorella* and *Fusicoccum*. *Sydowia* 52: 167–175.
- Crous PW, Slippers B, Wingfield MJ, Rheeder J, Marasas WFO, Phillips AJL, Alves A, Burgess T, Barber P, Groenewald JZ. 2006. Phylogenetic lineages in the Botryosphaeriaceae. *Studies in Mycology* 55: 235–253.
- Damm U, Crous PW, Fourie PH. 2007. Botryosphaeriaceae as potential pathogens of *Prunus* in South Africa, with descriptions of *Diplodia africana* and *Lasiodiplodia plurivora* sp. nov. *Mycologia* 99: 664–680.
- Denman S, Crous PW, Taylor JE, Kang J-C, Pascoe I, Wingfield MJ. 2000. An overview of the taxonomic history of *Botryosphaeria*, and a re-evaluation of its anamorphs based on morphology and ITS rDNA phylogeny. *Studies in Mycology* 45: 129–140.
- Glass NL, Donaldson GC. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* 61: 1323–1330.
- Grossenbacher JG, Duggar BM. 1911. A contribution to the life-history, parasitism and biology of *Botryosphaeria ribis*. New York Agricultural Experimental Station, Technical Bulletin 18: 113–190.
- Gure A, Slippers B, Stenlid J. 2005. Seed-borne *Botryosphaeria* spp. from native *Prunus* and *Podocarpus* trees in Ethiopia, with a description of the anamorph *Diplodia rosulata* sp. nov. *Mycological Research* 109: 1005–1014.
- Hillis DM, Bull JJ. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* 42: 182–192.
- Jacobs KA, Rehner SA. 1998. Comparison of cultural and morphological characters and ITS sequences in anamorphs of *Botryosphaeria* and related taxa. *Mycologia* 90: 601–610.
- Luque J, Martos S, Phillips AJL. 2005. *Botryosphaeria viticola* sp. nov. on grapevines: a new species with a *Dothiorella* anamorph. *Mycologia* 97: 1111–1121.
- Miller AN, Huhndorf SM. 2004. Using phylogenetic species recognition to delimit species boundaries within *Leptosphaeria*. *Mycologia* 96: 1106–1127.
- Niekerk JM van, Crous PW, Groenewald JZ, Fourie PH, Halleen F. 2004. DNA phylogeny, morphology and pathogenicity of *Botryosphaeria* species on grapevines. *Mycologia* 96: 781–798.
- O'Donnell K. 1993. *Fusarium* and its near relatives. In: Reynolds DR, Taylor JW (eds), *The fungal holomorph: Mitotic, meiotic and pleomorphic speciation in fungal systematics*: 225–233. CAB International, Wallingford, UK.
- Page RD. 1996. *TreeView: an application to display phylogenetic trees on personal computers*. *Computer Applications in the Biosciences* 12: 357–358.
- Pavlic D, Slippers B, Coutinho TA, Gryzenhout M, Wingfield MJ. 2004. *Lasiodiplodia gonubiensis* sp. nov., a new *Botryosphaeria* anamorph from native *Syzygium cordatum* in South Africa. *Studies in Mycology* 50: 313–322.
- Petrak F. 1922. Beiträge zur kenntnis der Pilzflora der südlichen Alpenländer und Norditaliens. *Annales Mycologici editi in notitiam scientiae mycologicae universalis* 20: 126–159.
- Petrak F, Deighton FC. 1952. Beiträge zur Pilzflora von Sierra Leone. *Sydowia* 6: 309–322.
- Phillips AJL. 2002. *Botryosphaeria* species associated with diseases of grapevines in Portugal. *Phytopathologia Mediterranea* 41: 3–18.
- Phillips AJL, Alves A, Correia A, Luque J. 2005. Two new species of *Botryosphaeria* with brown, 1-septate ascospores and *Dothiorella* anamorphs. *Mycologia* 97: 513–529.
- Punithalingam E. 1969. *Studies on Sphaeropsidales in culture*. *Mycological Papers* 119: 1–24.
- Ramaley AW. 2005. The connection of *Dothidotthia aspera* (Botryosphaeriaceae) to a hyphomycetous anamorphic fungus, *Thyrostroma negundinis*. *Mycotaxon* 94: 127–132.
- Rannala B, Yang Z. 1996. Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of Molecular Evolution* 43: 304–311.
- Rayachhetry MB, Blakeslee GM, Webb RS, Kimbrough JW. 1996. Characteristics of the *Fusicoccum* anamorph of *Botryosphaeria ribis*, a potential biological control agent for *Melaleuca quinquernervia* in South Florida. *Mycologia* 88: 239–248.
- Rodriguez F, Oliver JF, Marin A, Medina JR. 1990. The general stochastic model of nucleotide substitutions. *Journal of Theoretical Biology* 142: 485–501.
- Ronquist F, Huelsenbeck JP. 2003. MrBayes3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
- Rossmann AY, Samuels GJ. 2005. Towards a single scientific name for species of fungi. *Inoculum* 56: 3–6.
- Saccardo PA. 1877. *Fungi Veneti novi vel critici vel Mycologiae Venetae addendi*. *Michelia* 1: 1–72.
- Saccardo PA. 1880. *Fungi gallici, ser. II*. *Michelia* 2: 38–135.
- Schoch CL, Shoemaker RA, Seifert KA, Hambleton S, Spatafora JW, Crous PW. 2006. A multigene phylogeny of the Dothideomycetes using four nuclear loci. *Mycologia* 98: 1041–1052.
- Sivanesan A. 1984. The bitunicate ascomycetes and their anamorphs. Cramer, Vaduz, Liechtenstein.
- Stevens NE. 1926. Two species of *Physalospora* on Citrus and other hosts. *Mycologia* 18: 206–217.
- Sutton BC. 1977. Coelomycetes. IV. Nomenclature of generic names proposed for Coelomycetes. *Mycological Papers* 141: 1–253.
- Sutton BC. 1980. The Coelomycetes, Fungi imperfecti with acervuli, pycnidia and stromata. Commonwealth Mycological Institute, Kew, UK.
- Swofford DL. 2003. PAUP*. *Phylogenetic Analysis Using Parsimony (*and other methods)* Version 4. Sunderland, Massachusetts: Sinauer Associates.
- Theissen F, Sydow H. 1914. Dothideazeen studien II. *Annales Mycologici* 12: 268–281.
- Theissen F, Sydow H. 1915. Die Dothideales. *Annales Mycologici* 13: 149–746.
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25: 4876–4882.
- White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds), *PCR Protocols: a guide to methods and applications*: 315–322. Academic Press, San Diego, California, USA.
- Young ND, Healey J. 2003. GapCoder automates the use of indel characters in phylogenetic analysis. *BMC Bioinformatics* 4: art. 6.
- Zambettakis CE. 1955. Recherches anatomiques et biologiques sur les Sphaeropsidales Phaeodidymae des Fungi imperfecti. *Archives du Museum National Histoire Naturelle (Paris)* 7: 7–145.
- Zhou S, Stanosz GR. 2001. Primers for amplification of mt SSU rDNA, and a phylogenetic study of *Botryosphaeria* and associated anamorphic fungi. *Mycological Research* 105: 1033–1044.

