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ORIGINAL PAPER

Botryosphaeriaceae associated with the die-back of ornamental trees in the Western Balkans

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Abstract Extensive die-back and mortality of various ornamental trees and shrubs has been observed in parts of the Western Balkans region during the past decade. The disease symptoms have been typical of those caused by pathogens residing in the Botryosphaeriaceae. The aims of this study were to isolate and characterize Botryosphaeriaceae species associated with diseased ornamental trees in Serbia, Montenegro, Bosnia and Herzegovina. Isolates were initially characterized based on the DNA sequence data for the internal transcribed spacer rDNA and six major clades were identified. Representative isolates from each clade were further characterized using DNA sequence data for the translation elongation factor 1-alpha, β-tubulin-2 and large subunit rRNA gene regions, as well as the morphology of the asexual morphs. Ten species of the Botryosphaeriaceae were identified of which eight, i.e., *Dothiorella sarmentorum*, *Neofusicoccum parvum*, *Botryosphaeria dothidea*, *Phaeobotryon cupressi*, *Sphaeropsis visci*, *Diplodia seriata*, *D. sapinea* and *D. mutila* were known taxa. The remaining two species could be identified only as *Dothiorella* spp. *Dichomera* syn-asexual morphs of *D. sapinea*, *Dothiorella* sp. 2 and *B. dothidea*, as well as unique morphological characters for a number of the known species are described. Based on host plants and geographic distribution, the majority of Botryosphaeriaceae species found represent new records. The results of this study contribute to our knowledge of the distribution, host associations and impacts of these fungi on trees in urban environments.

Keywords Botryosphaeriales · Morphology · Phylogeny · Tree pathogens · Urban tree pathology

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Introduction

During the course of last decade, extensive die-back and mortality of various ornamental trees and shrubs has been observed in Serbia, Montenegro, Bosnia and Herzegovina. Common external symptoms have included necrotic lesions, cankers and resin bleeding, which were often associated with top die-back and death of whole trees. Internal wood symptoms ranged from circular or irregular browning or reddening of the

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Fig. 1 Disease symptoms observed on ornamental trees in the Western Balkans. **a** Top die-back of Lawson cypress (*Chamaecyparis lawsoniana*), **b** stem canker of white fir (*Abies concolor*), **c** abundant resin flow from branch of Atlas cedar (*Cedrus atlantica*), and **d** discoloration of sapwood in the branch of giant sequoia (*Sequoiadendron giganteum*)

vascular tissue to brown vascular streaking visible as spots in cross sections (Fig. 1).

The cause of the sudden and severe die-back was unknown, but appeared to be associated with extreme recent weather conditions and Botryosphaeriaceae infections. During the last 15 years, the European continent has experienced a global rise in temperatures followed by a number of prolonged extreme high temperature events; "Heat waves", "Cold waves", severe droughts, storms and large scale flooding (Allen et al. 2010; IPCC 2014). According to the Hydrometerological Service of Serbia (accessed July 2015), the summer of 2012 was the hottest since records were first taken in 1887 and the highest temperature ever observed in Serbia (44.9 °C) was recorded in summer of 2007. Other extreme weather conditions during this period included extreme cold, heavy snowfall and large scale flooding (www.mod.gov.rs). Apart from human lives that were lost and extensive economic damage, the extreme weather conditions also placed significant stress on plants across the region.

Symptoms observed on ornamental trees in the Western Balkans were similar to those caused by the wound-infecting opportunistic canker pathogens belonging to the family of Botryosphaeriaceae. "*Botryosphaeria*-like" symptoms include leaf and shoot blights, gummosis, blue stain of the sapwood, tip die-back, cankers and in severe cases tree death (Slippers and Wingfield 2007; Urbez-Torres 2011). Diseases caused by the Botryosphaeriaceae typically emerge after hosts have been subjected to stress caused by environmental factors such as drought, flooding or high temperatures (Slippers and Wingfield 2007; Mehl et al. 2013). During the course of a last decade, the incidence of diseases caused by Botryosphaeriaceae species appear to have increased in Europe and climate change was suspected to be a driver of this increase (Desprez-Loustau et al. 2006; Fabre et al. 2011; Piškur et al. 2011).

Species of Botryosphaeriaceae are important pathogens of fruit and forest trees (e.g., Sánchez et al. 2003; Slippers et al. 2009; Chen et al. 2014b), but the diversity and role of these fungi on ornamental trees has not been extensively explored. Trees planted as ornamentals in cities tend to have low economic value, but they provide a wide range of ecosystem services. These include the reduction of the so called "heat island effects" and air pollutants, flood control, increasing people's feelings of well-being, carbon storage and carbon sequestration (Tubby and Webber 2010). Thus, when tree diseases emerge, they become a significant threat to the "public good" (Perrings et al. 2002) and this is particularly true in towns and cities where ornamental trees can be subjected to stress and Botryosphaeriacae diseases (Begoude et al. 2010; Heath et al. 2011).

Several species of the Botryosphaeriaceae, including *Botryosphaeria dothidea*, *Neofusicoccum parvum*, *Neofusicoccum luteum*, *Neofusicoccum batangarum*, *Neofusicoccum magniferae*, *Neofusicoccum mediterraneum*, *Neodeightonia palmicola*, *Lasiodiplodia theobromae*, *Lasiodiplodia pseudotheobromae*, *Lasiodiplodia mahajangana* and *Diplodia cupressi* have been found causing disease on ornamental trees worldwide (e.g., Begoude et al. 2010; Heath et al. 2011; Varela et al. 2011; Mayorquin et al. 2012; Ligoxigakis et al. 2013; Deng et al. 2015; Stanosz et al. 2015). With the exception of

Greece, not much is known regarding species of the Botryosphaeriaceae that occur in the Balkans. In Greece, seven species of the Botryosphaeriaceae have been reported from various trees, including *B. dothidea*, *Diplodia corticola*, *D. cupressi*, *N. palmicola*, *N. parvum*, *N. mediterraneum*, and *Neofusicoccum hellenicum* (Alves et al. 2006; Crous et al. 2007; Inderbitzin et al. 2010; Tsopelas et al. 2010; Ligoxigakis et al. 2013; Chen et al. 2015). *B. dothidea*, *Dothiorella iberica* and *Dothiorella parva* have been found associated with dying *Ostrya carpinifolia*, *Cotinus coggygria* and *Juniperus communis* in Slovenia (Piškur et al. 2011; Pavlic-Zupanc et al. 2015). Botryosphaeriaceae members have also been reported from fruit trees in the Western Balkans region, including *Diplodia bulgarica* on apple trees in Bulgaria (Phillips et al. 2012), *B. dothidea* on apple fruit in Serbia and olives in Montenegro (Latinović et al. 2013; Vasić et al. 2013), *N. parvum* on grapevine in Croatia and *Diplodia seriata* on shrubs of *Cotoneaster salicifolius* in Bulgaria and on olives in Croatia (Bobev et al. 2008; Kaliterna et al. 2012, 2013). On forest and ornamental trees in the Western Balkans, *B. dothidea*, *Diplodia sapinea* and *Sphaeropsis visci* have been identified, but the identification of species was based solely on morphological characteristics and is uncertain (Karadžić et al. 2000, 2004; Karadžić and Milijašević 2008).

The Botryosphaeriaceae are difficult to identify and the taxonomy of these species has been confused for many years (Phillips et al. 2013). These fungi have recently been taxonomically redefined using a combination of morphology and multiple gene sequence data. The family currently comprises 17 well defined genera (Phillips et al. 2013; Slippers et al. 2013). The Botryosphaeriaceae is known to include a large number of phylogentically closely related and morphologically similar cryptic species, which makes morphological identification of species tenuous or at best unreliable (Pavlic et al. 2009; Phillips et al. 2013). While traditional morphological descriptions are still widely used in the characterization and description of new species, differentiation most commonly now relies on molecular phylogenetic analyses for multiple gene regions (Phillips et al. 2013; Slippers et al. 2014).

This study sought to clarify the identity of the Botryosphaeriaceae associated with symptoms of dieback, cankers and abundant resin production on ornamental trees in the Western Balkans. Symptomatic material was collected from a wide variety of hosts from across the region and fungi were isolated from symptoms and fruiting structures. Botryosphaeriaceae-like isolates were characterized and identified using comparisons of DNA sequence data for the internal transcribed spacer (ITS) rDNA, translation elongation factor 1-alpha (TEF-1-α), β-tubulin-2 (BT2) and large subunit (LSU) rRNA gene regions.

Materials and methods

Disease surveys, sample collection and Botryosphaeriaceae isolation

Disease surveys were conducted from February 2009 to December 2014 in Serbia, Montenegro, Bosnia and Herzegovina. These included urban forests, public greens, parks and private gardens in 15 cities and three villages distributed across the study region. In total, 145 trees/shrubs were sampled. These included 118 ornamental trees (representing 35 species), 9 ornamental shrubs (representing 4 species) and other trees/shrubs were sampled in forests, forest plantations and ornamental nurseries (Tables 1; S1). All trees and shrubs displayed disease symptoms at the time of collection.

Samples of symptomatic tissues were transferred to the laboratory. Longitudinal and transverse sections were made from symptomatic stems and branches to observe possible internal symptoms. Isolations were made from the edges of necrotic lesions, resin impregnated chips of xylem tissues, discoloured wood, resinous twigs, needles or cone petioles. In some cases, isolations were also made from asymptomatic pieces taken from buds, needles and cones surrounding diseased parts. Small (3–4 mm) pieces of tissue were washed in tap water, surface disinfested (1 min in 70 % ethanol), rinsed in sterile distilled water, dried on sterile paper towels, flamed lightly and placed onto 2 % malt extract agar (MEA) plates (Merck KGaA, Darmstadt, Germany) complemented with lactic acid (2.5 ml/l, NRK Belgrade, Serbia). When fruiting bodies were present in the affected tissue, a single structure was removed using a sterile needle and transferred to a Petri dish containing AMEA. Petri dishes were sealed with parafilm and incubated at room temperature in the dark for 2 weeks. Botryosphaeriaceae-like isolates (mycelium greenish-grey, fast growing) were transferred aseptically to new Petri dishes containing MEA. Isolates were purified by hyphal tip transfers and

Table 1 Isolates used in the phylogenetic analyses

Isolate[a, b]	Identity	Host	Location[c–e]	Collector	GenBank Accession No.			
					ITS	EF1-α	β-Tubulin	LSU
CBS 112555	*Diplodia seriata*	*Vitis vinifera*	Portugal	A. J. L. Phillips	AY259094	AY573220	DQ458856	AY928050
CMW 39385	***D. seriata***	*Rubus fruticosus*	Bosnia and Herzegovina[c]	D. Karadžić	KF574991	KF575023	KF575087	KF575055
CMW 39384	***D. seriata***	*Thuja occidentalis*	Belgrade, Serbia	M. Zlatković	KF574992	KF575024	KF575088	KF575056
CMW 39377	***D. seriata***	*T. occidentalis*	Crvenareka, Serbia	M. Zlatković	KF574993	KF575025	KF575089	KF575057
CMW 39378	***D. seriata***	*Cedrus atlantica*	Belgrade, Serbia	M. Zlatković	KF574994	KF575031	KF575090	KF575061
CMW 39374	***D. seriata***	*Fraxinus excelsior*	Bosnia and Herzegovina	D. Karadžić	KF574995	KF575026	KF575091	KF575058
CMW 39376	***D. seriata***	*Chamaecyparis pisifera*	Belgrade, Serbia[d]	M. Zlatković	KF574996	KF575027	KF575092	KF575059
CMW 39379	***D. seriata***	*Ligustrum vulgare*	Belgrade, Serbia	M. Zlatković	KF574997	KF575032	KF575093	KF575060
CBS 119049	*D. seriata*	*Vitis* sp.	Italy	L. Mugnai	DQ458889	DQ458874	DQ458857	EU673266
CBS 393.84	*Diplodia sapinea*	*Pinus nigra*	Netherlands	H. A. van der Aa	DQ458895	DQ458880	DQ458863	EU754157
CMW 39341	***D. sapinea***	*Cedrus deodara*	Podgorica, Montenegro	M. Zlatković	KF574998	KF575028	KF575094	KF575062
CMW 39338	***D. sapinea***	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF574999	KF575029	KF575095	KF575063
CMW 39346	***D. sapinea***	*Picea omorika*	Belgrade, Serbia	M. Zlatković	KF575000	KF575030	KF575096	KF575064
CMW 44981	***D. sapinea***	*P. omorika*	Mt. Tara[c], Serbia	D. Karadžić	KF729198	KF729432	KT253569	KT253573
CBS 109725	*D. sapinea*	*Pinus patula*	South Africa	M. J. Wingfield	DQ458896	DQ458881	DQ458864	EU673270
CBS 112556	*Diplodia intermedia*	*Malus domestica*	Aveiro, Portugal	A. Alves	GQ923857	GQ923850	–	–
CBS 124462	*D. intermedia*	*Malus sylvestris*	Portugal	A. J. L. Phillips	GQ923858	GQ923826	–	–
CBS 118110	*Diplodia scrobiculata*	*Pinus banksiana*	USA	M. A. Palmer	KF766160	AY624258	AY624258	KF766326
CBS 113423	*D. scrobiculata*	*Pinus greggii*	Mexico	M. J. Wingfield	DQ458900	DQ458885	DQ458868	EU673267
CBS 136014	*Diplodia mutila*	*Populus alba*	Portugal	A. Alves	KJ361837	KJ361829	–	–
CMW 39356	***D. mutila***	*Aesculus hippocastanum*	Obrenovac, Serbia	D. Karadžić	KF575001	KF575033	KF575097	KF575066
CMW 39354	***D. mutila***	*P. halepensis*	Herceg Novi, Montenegro	M. Zlatković	KF575002	KF575034	KF575098	KF575067
CMW 39353	***D. mutila***	*Cupressus arizonica*	Herceg Novi, Montenegro	M. Zlatković	KF575003	KF575035	KF575099	KF575068
CBS 112553	*D. mutila*	*V. vinifera*	Portugal	A. J. L. Phillips	AY259093	AY573219	DQ458850	AY928049
CBS 124133	*Diplodia subglobosa*	*Lonicera nigra*	Spain	J. Luque	GQ923856	GQ923824	–	–

Table 1 continued

Isolate[a, b]	Identity	Host	Location[c–e]	Collector	GenBank Accession No.			
					ITS	EF1-α	β-Tubulin	LSU
CBS 124132	*D. subglobosa*	*F. excelsior*	Spain	J. Luque	DQ458887	DQ458871	–	–
CBS 116472	*Diplodia rosulata*	*Prunus africana*	Ethiopia, Gambo	A. Gure	EU430266	EU430268	EU673131	DQ377897
CBS 116470	*D. rosulata*	*P. africana*	Ethiopia, Gambo	A. Gure	EU430265	EU430267	EU673132	DQ377896
CBS 122527	*Sphaeropsis visci*	*Viscum album*	Ukraine	Á. Akulov	EU673327	–	–	–
CMW 39386	***S. visci***	*V. album*	Mt. Goč[c], Serbia	N. Keča	KF575004	KF575036	KF575100	KF575065
CBS 100163	*S. visci*	*V. album*	Luxemburg	H. A. van der Aa	EU673324	EU673292	EU673127	DQ377870
CBS 110496	*Sphaeropsis porosa*	*V. vinifera*	South Africa	J. M. van Niekerk	AY343378	AY343339	EU673130	DQ377894
CBS 110574	*S. porosa*	*V. vinifera*	South Africa	J. M. van Niekerk	AY343378	AY343339	–	–
IRAN 1458c	*Phaeobotryon cupressi*	*Cupressus sempervirens*	Gorgan, Iran	M. A. Aghajani	FJ919671	FJ919660	–	–
CMW 39387	***P. cupressi***	*C. sempervirens*	Podgorica, Montenegro	M. Zlatković/J. Lazarević	KF575005	KF575037	KF575101	KF575086
CBS 124700	*P. cupressi*	*C. sempervirens*	Gorgan, Iran	M. A. Aghajani	FJ919672	FJ919661	–	–
CBS 122980	*Phaeobotryon mamane*	*Sophora chrysophylla*	Hawaii	W. Gams	EU673332	EU673298	EU673121	EU673248
CPC 12445	*P. mamane*	*S. chrysophylla*	Hawaii	W. Gams	EU673336	EU673302	EU673122	EU673250
CMW 8000	*Botryosphaeria dothidea*	*Prunus* sp.	Switzerland	B. Slippers	AY236949	AY236898	AY236927	AY928047
CMW 39302	***B. dothidea***	*Pseudotsuga menziesii*	Belgrade, Serbia	M. Zlatković	KF575006	KF575038	KF575102	KF575077
CMW 39304	***B. dothidea***	*Sequoia sempervirens*	Belgrade, Serbia	M. Zlatković	KF575007	KF575039	KF575103	KF575078
CMW 39308	***B. dothidea***	*Sequoiadendron giganteum*	Valjevo, Serbia	N. Keča	KF575008	KF575040	KF575104	KF575079
CMW 44982	***B. dothidea***	*S. giganteum*	Valjevo, Serbia	N. Keča	KF729110	KT253575	KT253571	KT253572
MUCC 501	*B. dothidea*	*Eucalyptus marginata*	Yalgorup, Australia	K. M. Taylor	EF591916	EF591969	EF591952	EF591935
CBS 135219	*Botryosphaeria kuwatsukai*	*M. domestica*	Shaanxi, China[e]	C. S. Wang	KJ433388	KJ433410	–	–
PG 55	*B. kuwatsukai*	*M. domestica*	Shaanxi, China[e]	C. S. Wang	KJ433389	KJ433411	–	–
CBS 121769	*Botryosphaeria auasmontanum*	*Acacia mellifera*	Namibia	F. J. J van der Walt/J. Roux	EU101303	EU101348	–	–
ATCC 22927	*Botryosphaeria corticis*	*Vaccinium* sp.	USA	R. D. Millholland	DQ299247	EU673291	EU673108	EU673245
CBS 119047	*B. corticis*	*Vaccinium corymbosum*	New Jersey, USA	P. V. Oudemans	DQ299245	EU017539	EU673107	EU673244
IMI 63581b	*Dothiorella sarmentorum*	*Ulmus* sp.	England	E. A. Ellis	AY573212	AY573235	EU673102	AY928052
CMW 39366	***Do. sarmentorum***	*A. hippocastanum*	Belgrade, Serbia	I. Milenković	KF575009	KF575047	KF575105	KF575069

Table 1 continued

Isolate[a, b]	Identity	Host	Location[c–e]	Collector	GenBank Accession No.			
					ITS	EF1-α	β-Tubulin	LSU
CMW 39364	***Do. sarmentorum***	*Chamaecyparis lawsoniana*	Belgrade, Serbia	M. Zlatković	KF575010	KF575048	KF575106	KF575070
CMW 39370	***Do. sarmentorum***	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF575011	KF575049	KF575107	KF575071
CBS 115038	*Do. sarmentorum*	*Malus pumila*	The Netherlands	A. J. L. Phillips	AY573206	AY573223	EU673101	DQ377860
CBS 128309	*Dothiorella americana*	*V. vinifera*	Missouri, USA	K. Striegler/G. M. Leavitt	HQ288218	HQ288262	HQ288297	–
CBS 128310	*Do. americana*	*V. vinifera*	Missouri, USA	K. Striegler/G. M. Leavitt	HQ288219	HQ288263	HQ288298	–
CBS 124716	*Dothiorella* sp.	*Juglans regia*	Iran	J. Abdollahzadeh/A. Javadi	KC898232	KC898215	–	–
CBS 124717	*Dothiorella* sp.	*J. regia*	Iran	J. Abdollahzadeh/A. Javadi	KC898233	KC898216	–	–
CMW 39360	***Dothiorella* sp. 2**	*Fraxinus excelsior*	Bosnia and Herzegovina	D. Karadžić	KF575012	KF575052	KF575108	KF575072
CMW 39361	***Dothiorella* sp. 2**	*C. sempervirens*	Podgorica, Montenegro	M. Zlatković	KF729083	KT253576	KT253570	KT253574
CMW 39362	***Dothiorella* sp. 2**	*Thuja occidentalis*	Belgrade, Serbia	M. Zlatković	KF575013	KF575053	KF575109	KF575073
CMW 39363	***Dothiorella* sp. 2**	*C. lawsoniana*	Belgrade, Serbia	M. Zlatković	KF575014	KF575054	KF575110	KF575074
CMW 39123	***Dothiorella* sp. 1**	*Thuja plicata*	Belgrade, Serbia	M. Zlatković	KF040058	KF261728	KF261730	KF261726
CBS 135623								
CMW 39372	***Dothiorella* sp. 1**	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF575015	KF575050	KF575111	KF575075
CMW 39371	***Dothiorella* sp. 1**	*C. atlantica*	Belgrade, Serbia	M. Zlatković/I. Milenković	KF575016	KF575051	KF575112	KF575076
CMW 39122	***Dothiorella* sp. 1**	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF261725	KF261729	KF261731	KF261727
CBS 135622								
CMW 39373	***Dothiorella* sp. 1**	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729085	KF767530	KF767531	KF767532
MFLUCC 13-0497	*Do. symphoricarposicola*	*Symphoricarpos* sp.	Italy	Erio Camporesi	KJ742378	KJ742381	–	–
MFLUCC 13-0498	*Do. symphoricarposicola*	*Symphoricarpos* sp.	Italy	Erio Camporesi	KJ742379	KJ742382	–	–
BL 167	*Do. symphoricarposicola*	*Corylus avellana*	Italy	B. L. Linaldeddu	KP205496	KP205469	–	–
BL 174	*Do. symphoricarposicola*	*Corylus avellana*	Italy	B. L. Linaldeddu	KP205495	KP205468	–	–
BL 158	*Do. symphoricarposicola*	*Corylus avellana*	Italy	B. L. Linaldeddu	KP205493	KP205466	–	–

Table 1 continued

Isolate[a, b]	Identity	Host	Location[c–e]	Collector	GenBank Accession No.			
					ITS	EF1-α	β-Tubulin	LSU
BL 53	*Do. symphoricarposicola*	*Corylus avellana*	Italy	B. L. Linaldeddu	KP205492	KP205465	–	–
DAR 78992	*Dothiorella vidmadera*	*V. vinifera*	Eden Valley, Australia	W. M. Pitt/A. Loschiavo	EU768874	EU768881	HM800522	–
DAR 78993	*Do. vidmadera*	*V. vinifera*	Loxton, Australia	W. M. Pitt/A. Loschiavo	EU768876	EU768882	HM800523	–
CBS 115041	*Dothiorella iberica*	*Quercus ilex*	Aragón, Spain	J. Luque	AY573202	AY573222	EU673096	DQ377853
CBS 113188	*Do. iberica*	*Quercus suber*	Andalucia, Spain	M. E. Sánchez	AY573198	EU673278	EU673097	EU673230
JL 599	*Dothiorella parva*	*Corylus avellana*	Spain	J. Luque	EU673314	EU673281	EU673099	EU673233
CBS 124720	*Do. parva*	*C. avellana*	Iran, Ardabil	J. Abdollahzadeh/A. Javadi	KC898234	KC898217	–	–
CBS 124723	*Dothiorella prunicola*	*Prunus dulcis*	Portugal	A. J. L. Phillips	EU673313	EU673280	EU673100	EU673232
CBS 124722	*Dothiorella iranica*	*Olea europaea*	Iran, Golestan	A. Javadi	KC898231	KC898214	–	–
CBS 124719	*Dothiorella sempervirentis*	*C. sempervirens*	Iran, Golestan	M. A. Aghajani	KC898237	KC898220	–	–
CBS 124718	*Do. sempervirentis*	*C. sempervirens*	Iran, Golestan	M. A. Aghajani	KC898236	KC898219	–	–
MUCC 507	*Dothiorella moneti*	*Acacia rostellifera*	Yalgorup, Australia	K. M. Taylor	EF591922	EF591973	EF591956	EF591939
MUCC 505	*Do. moneti*	*A. rostellifera*	Yalgorup, Australia	K. M. Taylor	EF591920	EF591971	EF591954	EF591937
MUCC 509	*Dothiorella santali*	*Santalum acuminatum*	Yalgorup, Australia	K. M. Taylor	EF591924	EF591975	EF591958	EF591941
MUCC 508	*Do. santali*	*S. acuminatum*	Yalgorup, Australia	K. M. Taylor	EF591923	EF591974	EF591957	EF591940
CMW 36463	*Dothiorella brevicollis*	*Acacia karoo*	South Africa	F. Jami/M. Gryzenhout	JQ239403	JQ239390	JQ239371	JQ239416
CMW 36464	*Do. brevicollis*	*A. karoo*	South Africa	F. Jami/M. Gryzenhout	JQ239404	JQ239391	JQ239372	JQ239417
CBS 122068	*Dothiorella longicolis*	*Lysiphyllum cunninghamii*	Western Australia, Australia	T. I. Burgess	EU144054	EU144069	–	–
CBS 122067	*Do. longicolis*	*L. cunninghamii*	Western Australia	T. I. Burgess	EU144053	EU144068	–	–
CMW 9081	*Neofusicoccum parvum*	*Populus nigra*	New Zealand	G. J. Samuels	AY236943	AY236888	AY236917	AY928045
CMW 39328	***N. parvum***	*Pittosporum tobira*	Herceg Novi, Montenegro	M. Zlatković	KF575017	KF575041	KF575113	KF575080
CMW 39321	***N. parvum***	*Prunus laurocerasus*	Budva, Montenegro	M. Zlatković	KF575018	KF575042	KF575114	KF575081
CMW 39326	***N. parvum***	*Eucalyptus globulus*	Herceg Novi, Montenegro	M. Zlatković	KF575019	KF575043	KF575115	KF575082
CMW 39317	***N. parvum***	*E. globulus*	Bar, Montenegro	M. Zlatković	KF575020	KF575044	KF575116	KF575083
CMW 39325	***N. parvum***	*A. hippocastanum*	Belgrade, Serbia	I. Milenković	KF575021	KF575045	KF575117	KF575084
CMW 39318	***N. parvum***	*C. lawsoniana*	Belgrade, Serbia	N. Keča/M. Zlatković	KF575022	KF575046	KF575118	KF575085

Table 1 continued

Isolate[a, b]	Identity	Host	Location[c–e]	Collector	GenBank Accession No.			
					ITS	EF1-α	β-Tubulin	LSU
CMW 9071	*N. parvum*	*Ribes* sp.	Australia	M. J. Wingfield	EU339552	AY236880	AY236909	–
CMW 7772	*Neofusicoccum ribis*	*Ribes* sp.	New York, USA	B. Slippers/G. Hudler	AY236935	AY236877	AY236906	AY928044
CMW 7773	*N. ribis*	*Ribes* sp.	New York, USA	B. Slippers/G. Hudler	AY236936	DQ235142	AY236907	DQ246263
CMW 14058	*Neofusicoccum umdonicola*	*Syzygium cordatum*	Kosi Bay, South Africa	D. Pavlic	EU821904	EU821874	EU821844	–
CMW 14127	*N. umdonicola*	*S. cordatum*	South Africa	D. Pavlic	EU821926	EU821896	EU821866	–
CMW 28315	*Neofusicoccum batangarum*	*Terminalia catappa*	Cameroon	D. Begoude/J. Roux	FJ900606	FJ900652	FJ900633	–
CMW 28363	*N. batangarum*	*T. catappa*	Cameroon	D. Begoude/J. Roux	FJ900607	FJ900653	FJ900634	–
CBS 123639	*Neofusicoccum kwambonambiense*	*S. cordatum*	South Africa	D. Pavlic	EU821900	EU821870	EU821840	–
CBS 123641	*N. kwambonambiense*	*S. cordatum*	South Africa	D. Pavlic	EU821919	EU821889	EU821859	–
CMW 14054	*Neofusicoccum cordaticola*	*S. cordatum*	South Africa	D. Pavlic	EU821906	EU821876	EU821846	–
CMW 13992	*N. cordaticola*	*S. cordatum*	South Africa	D. Pavlic	EU821898	EU821868	EU821838	–
CBS 117448	*Pseudofusicoccum stromaticum*	*Eucalyptus* hybrid	Venezuela	S. Mohali	AY693974	AY693975	EU673094	DQ377931
CBS 117449	*P. stromaticum*	*Eucalyptus* hybrid	Venezuela	S. Mohali	DQ436935	DQ436936	EU673093	DQ377932

Culture collections: CMW: FABI, University of Pretoria, South Africa; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Collection of Pedro Crous housed at CBS; IRAN: Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Iran; ATCC, American Type Culture Collection; MUCC: Murdoch University Culture Collection; DAR: Plant Pathology Herbarium, Orange Agricultural Institute, DPI, Orange, NSW, Australia; IMI: CABI Genetic Resource Collection Bioscience, Egham, Surrey, UK; JL: J. Luque, IRTA, Barcelona, Spain; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand

[a] Isolates sequenced in this study are given in bold. Others were retrieved from the GenBank

[b] Isolate accession numbers in italics signify cultures linked morphologically to the type material

[c] Forest stand

[d] Nursery

[e] Xu et al. (2015)

stored on half-strength MEA slants at 4 °C. Representative isolates from each host were deposited in the Culture Collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI) with duplicates of two cultures representing *Dothiorella* sp. 1 deposited in the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands (CBS) and in the National Collection of Fungi, Pretoria, South Africa (PREM).

DNA extraction, PCR and sequencing

DNA was extracted using PrepMan Ultra reagent (Applied Biosystems, Foster City, California) following the manufacturer's protocol. DNA concentrations were quantified with a NanoDropH ND-1000 and accompanying software (NanoDrop Technologies, DuPont Agricultural Genomics Laboratories, Delaware). The original DNA extracts were dissolved in 50 µl of distilled Sabax water and stored at −20 °C prior to their utilization in amplification reactions. The ITS region of rDNA operon was amplified using primers ITS-1 and ITS-4 (White et al. 1990), part of the TEF-1-α gene using primers EF1-728F and EF1-986R (Carbone and Kohn 1999), part of the BT gene using primers Bt2a and Bt2b (Glass and Donaldson 1995) and part of the LSU gene using primers LR0 and LR5 (Vilgalys and Hester 1990). The 25 µl PCR reaction mixtures contained 2.5 µl of 10 mM PCR buffer (PCR buffer with $MgCl_2$), 1 µl of 100 mM of each dNTPs, 0.5 µl of 10 mM of each primer, 2–2.5 µl of diluted genomic DNA, 0.5 U of Taq polymerase (Roche Molecular Biochemicals, Indianapolis) and 18 µl of sterile distilled Sabax water (Adcock Ingram Ltd., Johannesburg, South Africa). All reactions were carried out alongside a non-template control containing sterile water. The amplification was performed as described by Jami et al. (2012).

Where the above-mentioned primer pairs failed to amplify in some isolates, modifications were made as follows: the mycelium was extracted using a CTAB-based protocol of Möller et al. (1992) and 1 µl of undiluted DNA extracts was used for PCR reactions. The PCR reaction mixture contained either an additional 1 µl of 25 mM $MgCl_2$ or additional 0.5 µl of Taq polymerase. ITS region was amplified using primers ITS1F (Gardes and Bruns 1993) and ITS-4 (White et al. 1990) and TEF-1-α gene using primers EF1-F and EF2-R (Jacobs et al. 2004) and annealing temperature of 60 or 62 °C.

Three µl of each PCR product were separated by electrophoresis on 1.5 % (w/v) agarose gels in 0.5× TAE buffer, stained with GelRed (Biotium, Hayward, California, USA) and visualized under UV illumination. The size of the products was estimated using DNA molecular weight marker (Gene Ruler™ 100 bp DNA ladder, Fermentas). The amplified PCR fragments were purified with Sephadex G-50 columns (Sigma, Steinheim, Germany) following the manufacturer's instructions. The sequencing PCR mixture and sequencing conditions were similar to those described in Begoude et al. (2010). The products were separated with an ABI PRISM 3500 genetic analyzer (Applied Biosystems). Sequencing was done in both directions, with the same primers used for the PCR reactions.

Sequence alignment and phylogenetic analyses

The nucleotide sequences of both strands were examined and assembled with the CLC Main Workbench 6.6.1 (CLC Bio, Aarhus, Denmark). Sequences were compared to those of the other Botryosphaeriaceae in GenBank using BLAST. Related sequences were extracted and included in the analyses. Alignments were done online using MAFFT version 7 (http://mafft.cbrc.jp/alignment/server/), checked manually for alignment errors in MEGA v. 6 (Tamura et al. 2013) and adjusted where necessary.

For maximum likelihood (ML) analyses the best nucleotide substitution model was determined with JModeltest v.0.1 (Posada 2008). ML tree construction was performed using an online version of PhyML 3.0 (Guindon et al. 2010). The reliability of each node was assessed using 1000 bootstrap replications (Felsenstein 1985). Maximum parsimony (MP) analyses were run using PAUP version 4.0b10 (Swofford 2003). The heuristic search function with 1000 random addition replicates was selected as well as tree bisection and reconstruction as branch swapping algorithm. Gaps were treated as fifth characters and all characters were unordered and of equal weight. Branches of zero length were collapsed and all resulting equally parsimonious trees were saved. Measures such as consistency index (CI) and retention index (RI) were recorded. Congruence between the different data sets was tested using the partition homogeneity test (PHT;

Farris et al. 1995) in PAUP v. 4 with the uninformative characters removed before analysis. Phylogenetic trees were visualized in MEGA v. 6. All sequences obtained in this study were deposited in GeneBank and the four gene alignment and MP tree in TreeBASE (accession no. S18025). Accession numbers of representative isolates included in the multigene analysis are shown in Table 1.

Morphological characterization and growth studies

Isolates of each species included in the multigene phylogenetic analyses were used for morphological studies. To induce sporulation, cultures were grown on water agar (WA; 2 % agar; Biolab, Johannesburg, South Africa) overlaid with triple sterilized pine needles or Lawson cypress twigs and incubated under near UV light at 25 °C for 3–7 weeks. Morphological observations were made with Carl Zeiss dissection and compound microscopes using HRc Axiocam digital camera and Axiovision 3.1 imaging software (Carl Zeiss Ltd., Munich, Germany). Fruiting structures were sectioned using Jung tissue freezing medium and a Leica CM1100 Cryotome or broken by hand and mounted in distilled water. At least 50 measurements were made of the lengths and widths of conidia and where present the spermatia. Dimensions of other fungal structures were given as the range of least 20 measurements. Shape, colour, presence and absence of septa and a mode of conidiogenesis were recorded.

Isolates were further used to study colony morphology and optimal temperature for mycelial growth. For each isolate, 6 mm diam. mycelial plugs were taken from the edges of 1-week-old cultures and transferred to the centres of 90 mm diam. Petri dishes containing 2 % MEA. Plates were incubated at nine temperatures (5, 10, 15, 20, 25, 30, 35, 37 and 40 °C) for 2 weeks in the dark. For each temperature, five replicate plates for each isolate were used. Two measurements of colony diameter at right angles to each other were taken daily at a fixed time until the mycelium of the fastest growing isolate had reached the edges of the plates. The averages of colony diameter were computed and the optimal temperature for growth was defined as the temperature at which maximum mycelial growth occurred. The entire experiment was repeated once. The colours of the resulting cultures were compared to those in the colour charts of Rayner (1970).

Statistical analyses of growth rates

Analyses were conducted using Statistica 8.0 (StatSoft, Inc., Tulsa, OK, USA). The datasets were first checked for normality using Kolmogorov–Smirnov test and for homogeneity of variances using Leven's test. The analyses of mycelial growth rates were further assessed using one-way ANOVA followed by post-hock LSD test and values of $p < 0.05$ were considered significant. Since the assumptions of parametric tests for the analyses of optimal temperature for growth were not met, and data transformations did not improve normality of variables, the analyses were assessed using non-parametric Kruskal–Wallis one-way ANOVA followed by multiple Mann–Whitney U tests for means comparisons.

Results

Botryosphaeriaceae isolation and molecular phylogenetic identification

In total, 306 Botryosphaeriaceae-like isolates were obtained in this study. Of these, 285 isolates were from ornamental trees, 14 isolates were from trees in forest stands, 3 isolates originated from forest plantations and 4 isolates originated from seedlings in ornamental nurseries (Tables 1; S1). Based on a preliminary screening using ITS sequences, thirty eight representative isolates residing in six genera (*Dothiorella*, *Neofusicoccum*, *Diplodia*, *Phaeobotryon*, *Sphaeropsis* and *Botryosphaeria*) were subjected to subsequent analyses using multiple gene regions and morphological characterization (Table 1).

MP analyses of the ITS, TEF-1-α, BT and LSU gene regions were conducted for each gene region separately and for the combined data set of four loci, whereas ML analyses were conducted for only the combined data set. The combined dataset contained 113 sequences including 37 sequences obtained in this study and 76 sequences retrieved from GenBank with *Pseudofusicoccum stromaticum* as an outgroup. The sequence datasets contained 527 characters for ITS (167 parsimony informative, 360 parsimony uninformative, CI = 0.6, RI = 0.9 and TL = 400), 386 for

TEF-1-α (302 parsimony informative, 84 parsimony uninformative, CI = 0.6, RI = 0.9 and TL = 871), 440 for BT (148 parsimony informative, 292 parsimony uninformative, CI = 0.7, RI = 0.9 and TL = 312), 559 for LSU (56 parsimony informative, 503 parsimony uninformative, CI = 0.7, RI = 0.9 and TL = 78) and 1912 for the combined data set (673 parsimony informative, 1239 parsimony uninformative, CI = 0.6, RI = 0.9, TL = 1714). The results of the PHT test were not significant and showed that four loci could be combined (P = 0.7). The model TrN+G was chosen for the ML analyses of the combined dataset (G = 0.2080). The final MP and ML analyses yielded trees with the similar topology and only minor differences with regards to the position of subclades, and therefore, only the MP tree is shown (Fig. 2). However, phylogenetic groups representing individual species and genera (*Dothiorella* and *Diplodia* in LSU analyses) occasionally collapsed in the analyses of individual gene regions.

Phylogenetic analyses revealed six major and strongly supported clades, each representing a separate genus, including *Dothiorella*, *Neofusicoccum*, *Botryosphaeria*, *Phaeobotryon*, *Sphaeropsis* and *Diplodia*. Ten species of the Botryosphaeriaceae were distinguished amongst these clades. *Dothiorella* and *Diplodia* each included three species, whereas one species was recognized in each of *Neofusicoccum*, *Botryosphaeria*, *Phaeobotryon* and *Sphaeropsis*. Eight known species namely *Dothiorella sarmentorum*, *N. parvum*, *B. dothidea*, *Phaeobotryon cupressi*, *S. visci*, *D. seriata*, *D. sapinea* and *D. mutila*, were identified and two additional species were recognised in *Dothiorella*.

Morphological characterization

Isolates were induced to sporulate and produced mature pycnidia on WA overlaid with sterilized pine needles or Lawson cypress twigs. No sexual structures were observed and therefore, morphological characterization of species was based on the morphology of the asexual morphs. All isolates produced spermatia and the majority of isolates formed chlamydospores, chlamydospore-like hyphae and pycnidial hyphae with surface ornamentations (Fig. 3; Table S2). *Dichomera* syn-asexual morphs were observed in some isolates of *B. dothidea*, *D. sapinea* and *Dothiorella* sp. 2 and their morphology is described below.

Taxonomy

The following section includes the description of *Dothiorella* sp. 1 and morphological characteristics for described species where these have not previously been observed.

Dothiorella sp. 1, *Figs. 1, 2, 3; Table 1*

Conidiomata pycnidial, immersed in agar or superficial, single or in groups, globose, subglobose, or long-necked, 200–1000 µm in diameter, 250–3000 µm high, smooth or covered with hyphal hairs, composed of two–three outer layers of dark brown thick-walled cells *textura angularis* and five–six layers of hyaline cells in the inner region. Pycnidia on the host *Cedrus atlantica* separate or aggregated, globose, black, immersed in the host tissue, becoming superficial, up to 2 mm wide. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* formed from the cells lining the inner walls of the pycnidial cavity, holoblastic, cylindrical to subcylindrical, hyaline, predominantly proliferating percurrently to form one–three annellations, occasionally proliferating at the same level giving rise to periclinal thickenings, 8–15 × 3–6 µm (av. 11.5 × 4.5, l/w 2.6) µm. *Conidia* on MEA oval to ovoid, (17.1–) 19–21 (–23.1) × (7–) 8–10 (–12) µm (av. 20.3 × 9, l/w 2.3) µm, on WA imbedded with sterilized Lawson cypress twigs or *P. nigra* needles slightly longer and wider, (18.2–) 20–22 (–23.9) × (8.6–) 9–11 (–13.6) µm (av. 21 × 10.1, l/w 2.1) µm, apex rounded and truncate base, constricted at the septum, initially hyaline and granulose, becoming dark brown with rough outer wall and one-septate while still attached to conidiogenous cells. *Spermatophores* hyaline, smooth, cylindrical, occasionally branched. *Spermatogenous cells* hyaline, smooth, cylindrical, phyalidic. *Spermatia* hyaline, smooth, aseptate, rod-shaped with rounded ends, occasionally slightly curved, 2–4 × 1.5–2 (av. 3 × 1.8, l/w 1.7) µm and similar in shape, but smaller, 1.5–2.1 × 0.4–0.8 (av. 1.8 × 0.6, l/w 3) µm. *Chlamydospores* globose to subglobose, ellipsoid, oval or subcylindrical, hyaline to golden-brown, thick-walled, smooth with granular content or rough, terminal on lateral hyphae, or arising laterally or intercalary, remaining attached in chains of 2–30, rarely single, (12–) 15– 21 (– 27) × (11–) 13–17 (–22) µm.

Cultural characteristics colonies initially white, edges remaining white, centre becoming olivaceous-

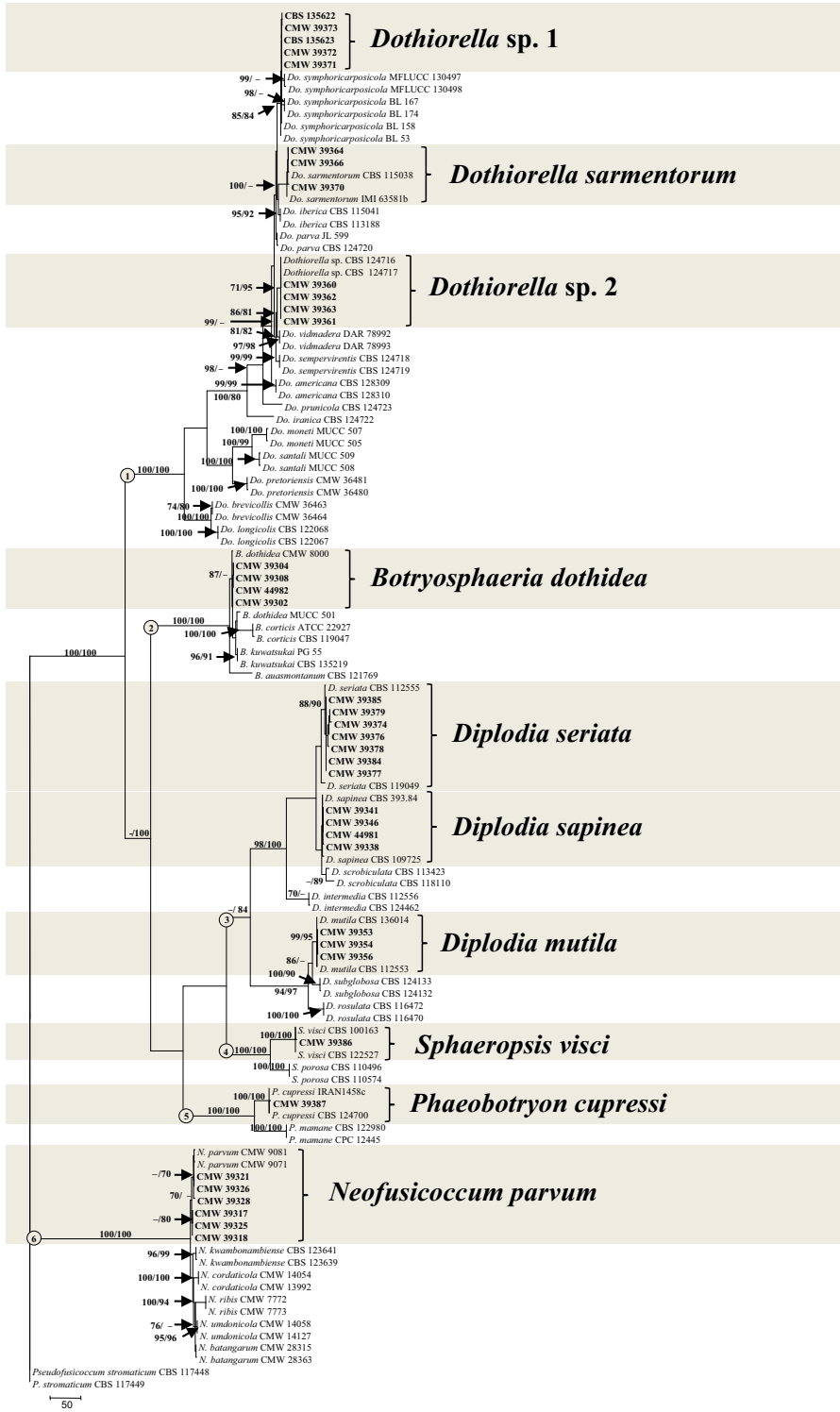

Fig. 2 The first of 1000 equally most parsimonious trees resulting from parsimony analyses of the combined ITS, TEF-1-α, β-tub and LSU alignment. The bootstrap support values (MP/ML $\geq$70 %) are indicated at the *nodes* and the *scale bar* represents the number of changes. The *tree* was rooted to *P. stromaticum* CBS 117448 and CBS 117449. Clades representing genera identified in this study are indicated by *circles* at the *nodes* and clades corresponding to species are *highlighted*

grey (21‴′b) to smoke grey (21‴′f) at the surface in 1 week, turning to smoke grey at the surface and pale olivaceous-grey (21‴′d) at the reverse in 2 weeks. Mycelium with lobed margins, giving a rosette appearance. Globose pycnidia immersed in the substrate (seen as round black structures on the reverse

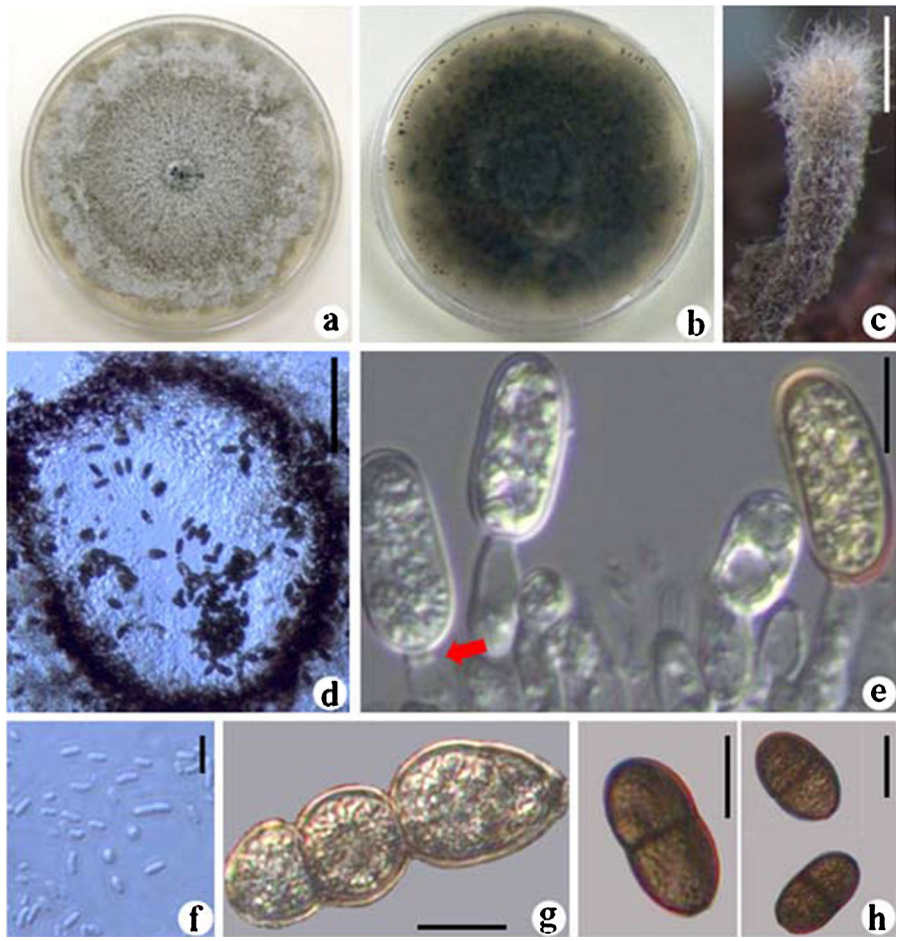

Fig. 3 Morphological features of *Dothiorella* sp. 1 (CMW 39123 = CBS 135623). **a** 14 Day-old culture growing on MEA at 25 °C (front), **b** 14 day-old culture growing on MEA at 25 °C (reverse) with globose pycnidia immersed in culture, **c** superficial, solitary, long-necked pycnidium formed on Lawson cypress twigs after 6 weeks under near UV light, **d** vertical section through pycnidium showing multiple layers of hyaline cells lining pycnidial cavity, **e** developing conidia and conidiogenous cell showing annellations (*arrow*), **f** spermatia, **g** chlamidospores, and **h** one-septate dark conidia with distinctly rough outer wall. *Scale bars* **c** = 1 cm, **e**, **g**, **h** = 10 μm, **f** = 3 μm

side of Petri dishes) readily forming from the middle of the colonies within 7–14 days at 5–25 °C in the dark. Superficial, solitary pycnidia forming 3–4 weeks after incubation, covering the entire surface of the colony. Cultures transferred onto WA with sterile Lawson cypress twigs or *P. nigra* needles formed numerous pycnidia on the surface of agar, immersed in the medium and on the surface of twigs/needles.

Cardinal temperatures for growth min. 5 °C and max. 35 °C. Optimum growth at 25 °C. Growth rate 10.7 mm per day at the optimal temperature, colonies covering 90 mm diam. Petri dishes after 6 days in the dark.

Sexual morph not seen.

Known hosts Thuja plicata, *C. atlantica*.

Known distribution Serbia (Belgrade).

Isolates examined Serbia, Belgrade, on resin-soaked branch lesion of *T. plicata*, June 2011, M. Zlatković (PREM 60884, living cultures CMW 39123 = CBS 135623). Serbia, Belgrade, on resin-soaked dead needles of *C. atlantica*, June 2011, M. Zlatković (living cultures CMW 39122 = CBS 135622). Serbia, Belgrade, on necrotic resinous stem lesion of *C. atlantica*, June 2011, M. Zlatković/I. Milenković (living culture CMW 39371). Serbia, Belgrade, on dead resin-soaked needles of *C. atlantica*, June 2011, M. Zlatković (living culture CMW 39372). Serbia, Belgrade, fruiting bodies on dead branch of *C. atlantica*, June 2011, M. Zlatković (living culture CMW 39373).

Notes isolates of *Dothiorella* sp. 1 and *Do. symphoricarposicola* formed a sub-clade (bootstrap support BS = 97 % MP) within *Dothiorella* species in the phylogenetic analyses. The separation of *Do. symphoricarposicola* from its sister taxon was strongly supported in the combined analyses of four gene regions (BS = 99 % MP), as well as in individual MP analyses of TEF-1-α (BS = 98 %). *Dothiorella symphoricarposicola* also differs morphologically from *Dothiorella* sp. 1 in its much smaller conidiomata (200–250 μm diam., 250–300 μm high) compared to

Dothiorella sp. 1 (200–1000 µm diam., 250–3000 µm high), smaller conidiogenous cells (4–12 × 1.5–6) µm compared to *Dothiorella* sp. 1 (8–15 × 3–6) µm, type of conidium development (phialidic) compared to *Dothiorella* sp. 1 (annelidic and phialidic) and smaller and smooth walled conidia (av. 17 × 8) µm compared to the larger and rough-walled conidia of *Dothiorella* sp. 1 (20.3 × 9 and 21 × 10.1) µm. Morphologically, conidia of *Dothiorella* sp. 1 resemble those of *Do. sempervirentis and Do. longicollis* and the pycnidia resemble those of *Do. longicollis* and *Do. moneti*. However, *Dothiorella* sp. 1 can be distinguished from these species based on its rough outer conidial walls and wider pycnidia with longer necks.

Dothiorella sarmentorum (Fr.) A.J.L. Phillips, J. Luque & A. Alves, Mycologia 97: 522. 2005. Figures 2, 4; S1; Tables 1; S2 =*Botryosphaeria sarmentorum* A.J.L. Phillips, J. Luque & A. Alves, Mycologia 97: 522. 2005.

Isolates examined Serbia, Belgrade, necrotic lesion on the lower stem of *Aesculus hippocastanum*, I. Milenković (living culture CMW 39366). Serbia, Belgrade, resin soaked branch lesion of *Chamaecyparis lawsoniana*, M. Zlatković (living culture CMW 39364). Serbia, Belgrade, resin soaked stem canker of *C. atlantica*, M. Zlatković (living culture CMW 39370).

Notes Phillips et al. (2005) showed that *Dothiorella* species differ from *Diplodia* in having conidia that are dark and one-septate within the pycnidial cavity, often while still attached to conidiogenous cells, whereas in *Diplodia* conidial darkening and septation takes place only after discharge. In this study, a large number of conidia remained hyaline long after discharge and became one–three septate and often rough walled after ageing.

Dothiorella sp. 2 *Syn-asexual morph Dichomera*-like, Figs. 2, 4; S1; Tables 1, S2

Conidia of the *Dichomera syn-asexual morph* oval, ovoid or ellipsoid, doliiform, muriform 11–14 × 6–8 (av. 13 × 6.6, l/w 2) µm, golden brown with 2–4 transversal septa, 0–2 longitudinal septa and 0–1 oblique septa. Conidia intermediate between *Dothiorella* and *Dichomera* golden brown–dark brown in colour, with 1–3 transverse septa, strongly constricted at the middle septum, 20–32 × 7–12 (av. 21.5 × 8.2, l/w 2.6) µm.

Isolates examined Montenegro, Podgorica, fruiting bodies on dead branch of *Cupressus sempervirens*, February 2010, M. Zlatković/J. Lazarević (living culture CMW 39361, producing muriform conidia and conidia intermediate between *Dothiorella* spp. and *Dichomera* spp.). Serbia, Belgrade, margin between healthy and dead branch wood of *Thuja occidentalis*, November 2010, M. Zlatković (living culture CMW 39362). Serbia, Belgrade, blue stained wood of a dead branch of *C. lawsoniana*, November 2010, M. Zlatković (living culture CMW 39363). Bosnia and Herzegovina, necrotic branch lesion of *Fraxinus excelsior*, April 2012, D. Karadžić (living culture CMW 39360).

Notes this species was included in *Dothiorella* by Phillips et al. (2008) and later found by Piškur et al. (2011), but remained unnamed because isolates obtained in those studies failed to sporulate and were thus considered not to be authentic. *Dothiorella* sp. 2 has recently been studied by other authors (Abdollahzadeh et al. 2014) and the species has not been described here. In the present study, conidia of the asexual morph, *Dichomera*-like conidia of the syn-asexual morph and conidia intermediate between the *Dichomera* and *Dothiorella* were observed in the same pycnidia. The conidia of intermediate morphology reported here displayed some similarities with spore forms intermediate between *Dichomera* and *Fusicoccum* described by Butin (1993). The mode of conidiogenesis giving rise to muriform conidia could not be observed.

Botryosphaeria dothidea (Moug. ex Fr.) Ces. & De Not., Comm. Soc. Crittog. Ital. 1: 212 (1863). Figures 2, 4; S1; Tables 1; S2 =*Fusicoccum aesculi* Corda, in Sturm Deutschl. Fl. 3: 111 (1829).

Syn-asexual morph Dichomera-like

Pycnidia of the *Dichomera syn-asexual morph* long-necked, 200–250 × 350–400 µm, covered with iron grey mycelium. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* hyaline, smooth, cylindrical, proliferating percurrently to form 1–2 annellations, 16–19 × 3–4 µm. *Conidia* round to oval, pyriform to limoniform, truncate at base, apex rounded or tapered, thick-walled, becoming dark and rough-walled with age, 8.4–15 × 5–7.8 (av. 12.5 × 6.4, l/w 1.9) µm or conidia ovoid to ellipsoid, doliiform, muriform, 8.6–12.7 × 4.7–6 (av. 10.6 × 5.4, l/w 2) µm, golden brown with 1–4 transversal septa and 0–1 oblique septa.

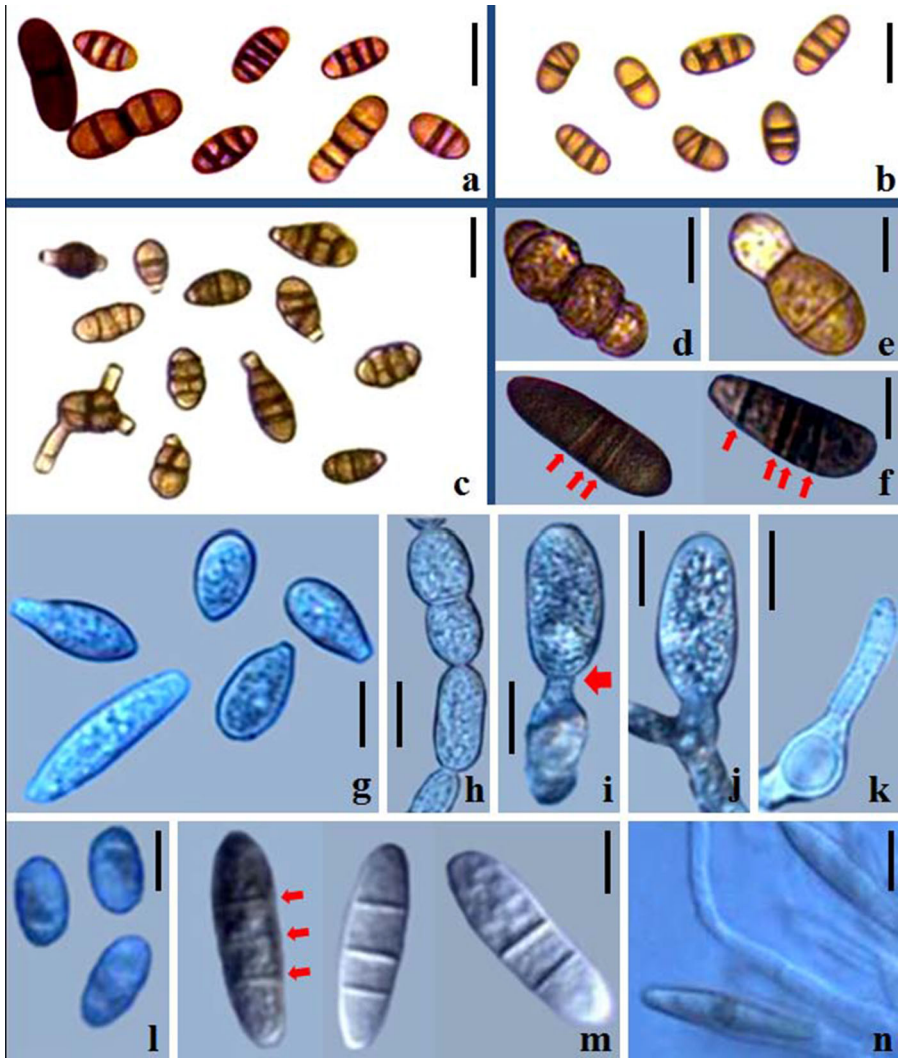

Fig. 4 Unique morphological characteristics of Botryosphaeriaceae identified in this study. **a** *Dothiorella* conidia, *Dichomera* conidia and conidia intermediate between *Dothiorella* and *Dichomera* of the *Dothiorella* sp. 2 CMW 39361, **b** *Dichomera* conidia of *Botryosphaeria dothidea* CMW 44982, **c** *Dichomera* conidia of *Diplodia sapinea* CMW 44981, **d** conidial chlamydospores of *D. sapinea* CMW 39338, **e** cylindrical conidium of *D. mutila* CMW 39354, **f** three-septate and four-septate conidia (*arrows*) of *D. sapinea* CMW 39338, **g** dark-walled *Dichomera*-like pyriform conidia of *B. dothidea* CMW 39304, **h** chlamidospores of *Phaeobotryon cupressi* CMW 39387, **i** conidiogenous cell showing annellation (*arrow*) and developing conidium of *P. cupressi* CMW 39387, **j** branched conidiophore with developing conidium of *P. cupressi*, **k** broad ampuliform conidiogenous cell of *P. cupressi* narrowing to a long neck, **l** dark-walled ellipsoid conidia of *Neofusicoccum parvum* CMW 39325, **m** two and three septate hyaline and dark conidia (*arrows*) of *B. dothidea* CMW 39302, and **n** fusiform dark-walled developing conidia and one-septate paraphyses of *B. dothidea* CMW 39302. *Scale bars* **a**–**f**, **h**–**k** = 10 μm, **g**, **l**–**n** = 5 μm

Isolates examined Serbia, Valjevo, resin-soaked bark of *Sequoiadendron giganteum*, April 2011, M. Zlatković (isolate CMW 44982, producing muriform conidia). Serbia, Belgrade, margin between healthy and dead branch tissue of *Pseudotsuga menziesii*, June 2011, M. Zlatković (living culture CMW 39302). Serbia, Belgrade, margin between healthy and dead shoot tissue of *Sequoia sempervirens*, September 2011, M. Zlatković/A. Knezević (living culture CMW 39304, producing dark pyriform conidia). Serbia, Valjevo, dead resin-soaked needles still attached to the tree of *S. giganteum*, April 2011, N. Keča (living culture CMW 39308).

Notes hyaline fusiform conidia typical of *Botryosphaeria* in the present study readily formed 1–3 septa

prior to germination or after ageing compared to conidia described in Phillips et al. (2013) that were hyaline, aseptate and rarely formed up to one septa. According to Phillips et al. (2013), species of *Botryosphaeria* have hyaline spores that may occasionally become darker with age. We occasionally observed dark-walled spores even at an early stage of development and while still attached to the conidiogenous cells.

Dichomera-like syn-asexual morphs from this study were morphologically different to those previously described (Barber et al. 2005; Inderbitzin et al. 2010; Slippers et al. 2014). Isolate CMW 39304 produced hyaline fusiform conidia, dark fusiform conidia and dark *Dichomera*-like pyriform to limoniform conidia in the same pycnidia. Pyriform conidia were rough-walled and aseptate compared to conidia illustrated by Slippers et al. (2014). This isolate also formed unusual long-necked pycnidia. Pycnidia in isolate CMW 44982 contained either hyaline fusiform conidia typical of *Botryosphaeria* or ovoid to ellipsoid muriform conidia typical of *Dichomera* spp., but unique in size and shape compared to those previously reported.

Neofusicoccum parvum (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips, Stud. Mycol. 55: 248. 2006. Figures 2, 4; S1; Tables 1; S2 =*Botryosphaeria parva* Pennycook & Samuels, Mycotaxon 24: 455. 1985.

Isolates examined Montenegro, bar, stem canker of *Eucalyptus globulus*, June 2011, M. Zlatković (living culture CMW 39317). Serbia, Belgrade, margin between healthy and dead branch wood of *C. lawsoniana*, April 2010, N. Keča/M. Zlatković (living culture CMW 39318). Montenegro, Budva, discoloured resin-soaked branch wood of *Prunus laurocerasus*, July 2011, M. Zlatković (living culture CMW 39321). Montenegro, Herceg Novi, margin between healthy and dead shoot of *Pittosporum tobira*, June 2011, M. Zlatković (living culture CMW 39328). Serbia, Belgrade, necrotic stem lesion of *A. hippocastanum*, March 2011, I. Milenković (living culture CMW 39325). Montenegro, Herceg Novi, bleeding stem canker of *E. globulus*, June 2011, M. Zlatković (living culture CMW 39326).

Notes conidia of *N. parvum* from this study became 1–3 septate after ageing, rather than only two-septate as described before (Phillips et al. 2013). Moreover, isolate CMW 39318 formed hyaline, fusiform conidia typical of the *Neofusicoccum* and smaller, dark brown, ellipsoid conidia in the same pycnidia, measuring 6.8–12.4 × 3.7–13.4 (av. 10.3 × 8.4, l/w 1.2) µm. Dark ellipsoid, *Dichomera*-like conidia observed in this study differed in size and shape from muriform subglobose to obpyriform conidia of the asexual morph described previously (Barber et al. 2005; Crous et al. 2006; Phillips et al. 2013).

Diplodia sapinea (Fr.) Fuckel, Jb. Nassau. Ver. Naturk. 23–24: 393. 1870. Figures 2, 4; S1; Tables 1; S2 Syn-asexual morph Dichomera-like

Conidia of the *Dichomera syn-asexual morph* variable and irregular in shape, mostly obpyriform, clavate, ellipsoidal, muriform with 0–4 transversal, 0–2 longitudinal and 0–2 oblique septa, 10.1–19.7 × 5.6–10.1 (av. 15.1 × 7.8, l/w 1.9) µm, rarely bearing 1–3 appendages, 3–17 × 3–5 µm.

Isolates examined Serbia, Tara Mt., resin-soaked cone petal of *Picea omorika*, April 2012, D. Karadžić (isolate CMW 44981, producing muriform conidia). Serbia, Belgrade, resin-soaked branch canker of *P. omorika*, September 2011, M. Zlatković/A. Knežević (living culture CMW 39346). Serbia, Belgrade, resin-soaked sapwood of *C. atlantica*, May 2011, M. Zlatković (living culture CMW 39338). Montenegro, Podgorica, resin-soaked sapwood of *Cedrus deodara*, September 2011, M. Zlatković (living culture CMW 39341).

Notes in the present study, conidia were rarely aseptate, mostly 1–4 septate and sometimes also with three horizontal and one oblique septum, compared to predominantly aseptate and only rarely up to one-septate conidia described in Phillips et al. (2013). Isolate CMW 44981 formed a *Dichomera*-like syn-asexual morph and dark, oblong, 0–2 septate *Diplodia*-like conidia in the same pycnidia. *D. sapinea* colonies are typically characterized by having grey fluffy mycelium (Palmer et al. 1987). However, isolate CMW 39338 formed colonies with flat, dense mycelial mat.

Diplodia mutila (Fr.) Mont., Ann. Sci. nat., sér. 2, 1: 302. 1834. Figures 2, 4; S1; Tables 1; S2 =*Botryosphaeria stevensii* Shoemaker, Canad. J. Bot. 42: 1299. 1964.

Isolates examined Serbia, Obrenovac, necrotic lesion on stem of *A. hippocastanum*, June 2011, D. Karadžić (living culture CMW 39356). Montenegro, Herceg Novi, fruit bodies on cone of *Cupressus arizonica*, September 2011, M. Zlatković (living culture CMW 39353). Montenegro, Herceg Novi, branch canker of *Pinus halepensis*, July 2011, M. Zlatković (living culture CMW 39354).

Notes isolates examined here occasionally produced both the typical hyaline oblong to ovoid conidia, as well as cylindrical conidia strongly constricted at the septum with one white cell and 1–2 dark brown cells, measuring 25–29 × 8.5–9.4 µm. Hyaline conidia from this study were frequently 1–2 septate becoming dark 0–2 septate with age.

Diplodia seriata De Not., Micr. Ital. Dec. 4: 6. 1942. Figures 2, 4; S1; Tables 1; S2 =*Botryosphaeria obtusa* (Schwein.) Shoemaker, Canad. J. Bot. 42: 1298. 1964.

Isolates examined Serbia, Belgrade, resin-soaked branch lesion of *T. occidentalis*, May 2011, M. Zlatković (living culture CMW 39384). Bosnia and Herzegovina, margin between healthy and dead stem wood of *Rubus fruticosus*, May 2010, D. Karadžić (living culture CMW 39385). Serbia, Crvena reka, resin-soaked wood of dead branch of *T. occidentalis*, May 2011, M. Zlatković (living culture CMW 39377). Serbia, Belgrade, dead resin-soaked needles still attached to *C. atlantica* trees, June 2011, M. Zlatković (living culture CMW 39378. Belgrade, Serbia, necrotic needles of seedling of *Chamaecyparis pisifera*, April 2011, M. Zlatković (living culture CMW 39376). Bosnia and Herzegovina, necrotic stem lesion of *F. excelsior*, June 2012, D. Karadžić (living culture CMW 39374). Serbia, Belgrade, branch canker on *Ligustrum vulgare*, October 2011, M. Zlatković (living culture CMW 39379).

Notes conidia from the present study often became dark 1–3 septate and rough-walled with age. Moreover, some of the conidia became septate without becoming dark.

Sphaeropsis visci (Alb. & Schwein.) Sacc., Michelia 2: 105. 1880. Figures 2, 4; S1; Tables 1; S2 =*Phaeobotryosphaeria visci* (Kalchbr.) A.J.L. Phillips & Crous, Persoonia 21: 47. 2008.

Isolate examined Serbia, Mt. Goč, fruit bodies on leaves of *Viscum album*, M. Zlatković (living culture CMW 39386).

Notes pycnidia from this study were immersed to partially erumpent and never superficial as has been described previously (Phillips et al. 2013). Moreover, conidia were larger than previously described, often spherical to ellipsoidal [(27–56 × 17–36 (av. 42.6 × 25)] µm, becoming golden brown and later dark brown and 1–2 septate with age.

Phaeobotryon cupressi Abdollahz., Zare & A.J.L. Phillips, Persoonia 23: 6. 2009. Figures 2, 4; S1; Tables 1; S2 Isolate examined Montenegro, Podgorica, resinous branch lesion of *C. sempervirens*. M. Zlatković/J. Lazarević (living culture CMW 39387).

Notes isolate from the present study occasionally formed conidiophores that have not been observed in previous collections. Conidiophores were hyaline, cylindrical, occasionally branched, measuring 14–18 × 4–5 µm. The conidiogenous cells observed here were occasionally broad ampuliform, often narrowing to form up to 20 µm long necks, proliferating internally, but also proliferating percurrently giving rise to annellations. Furthermore, conidia in the present study formed up to two septa prior to germination.

Growth studies

Botryosphaeriaceae species grew over a wide range of temperatures from 5 to 40 °C. Only *B. dothidea* exhibited growth at 40 °C. *D. sapinea*, *D. seriata*, *B. dothidea* and *S. visci* did not grow at 5 °C, whereas *Do. sarmentorum*, *Dothiorella.* sp. 2, *D. seriata* and *S. visci* showed no mycelial growth at 35 °C. There were statistically significant differences in the optimum temperature for mycelial growth and mycelial growth rates ($p < 0.05$) at an optimum temperature among species. The optimum temperature varied from 20 to 25 °C (*D. mutila*, *P. cupressi*) to 30 °C (*D. sapinea* CMW 39388, *B. dothidea*) and the optimum temperature for the majority of species was 25 °C. At their optimum temperatures, *N. parvum* had the most rapid growth rate of 21 mm/day, while *S. visci* had the lowest growth rate of 2.3 mm/day (Figs. 5, S1).

Discussion

This study represents the first attempt to determine the presence and identity of Botryosphaeriaceae species associated with diseased ornamental trees in the Western Balkans. It is also the first study to consider the Botryosphaeriaceae on a large number of tree species growing in this area. Ten Botryosphaeriaceae species

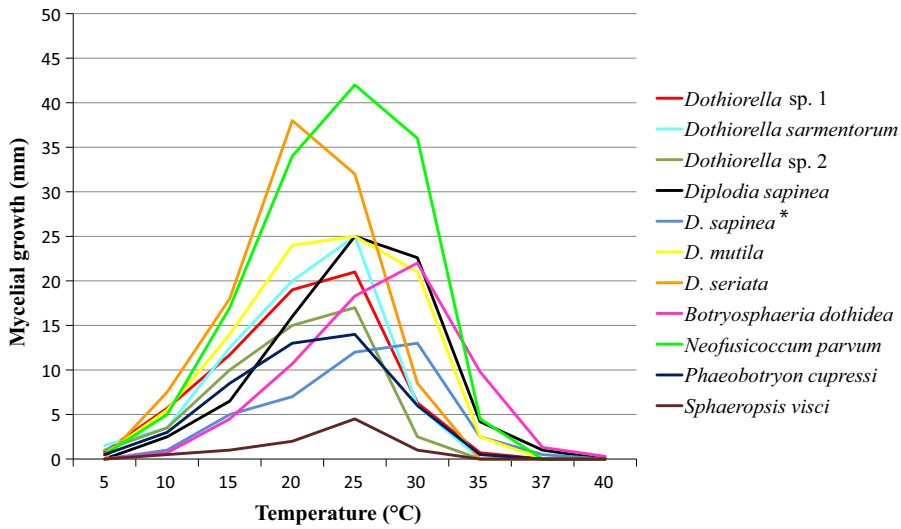

Fig. 5 Growth of Botryosphaeriaceae colonies in relation to temperature after 3 days in the dark. *Indicate *D. sapinea* CMW 39338. Growth values are averaged except in the case of *D. sapinea**

belonging to six genera were identified from Serbia, Montenegro, Bosnia and Herzegovina. These included eight known species (*B. dothidea*, *N. parvum*, *D. sapinea*, *D. mutila*, *D. seriata*, *Do. sarmentorum*, *P. cupressi* and *S. visci*) and two previously unnamed species residing in *Dothiorella*. With the exception of *P. cupressi* and *S. visci*, the host ranges for the previously known Botryosphaeriaceae were substantially expanded. The majority of records were of *B. dothidea*, *D. sapinea* and *N. parvum* and other species were isolated only occasionally.

Botryosphaeria dothidea was one of the most commonly isolated species, which is not surprising given that it has been reported from numerous tree species worldwide (Phillips et al. 2013). In the Balkans, *B. dothidea* has previously been reported causing disease on *O. carpinifolia* in Slovenia (Piškur et al. 2011), white rot of apple fruit in Serbia (Vasić et al. 2013) and rot of olives in Montenegro (Latinović et al. 2013). This species has also been isolated from English walnut, pistachio and kiwi fruit from Greece (Inderbitzin et al. 2010; Thomidis and Exadaktylou 2010; Chen et al. 2014b) and (based on morphological identification) from *S. giganteum*, *Quercus petraea*, *Q. cerris*, *Fagus sylvatica*, *Populus* × *euramericana* cl. I-214, *C. sempervirens* and *P. halepensis* from Serbia and Balkan (Karadžić et al. 2000). Although *B. dothidea* is a generalist species, it has been isolated only occasionally from gymnosperms. It was thus interesting that in the present study, the fungus was found for the first time associated with the two coniferous gymnosperms, *P. menziesii* and *S. sempervirens*.

The well-known pathogen of *Pinus* spp., *D. sapinea* (Swart and Wingfield 1991; Phillips et al. 2013) was found associated with three coniferous species in this study. These included *C. atlantica*, *C. deodara*, *P. omorika* and *P. omorika* is a new host record for the fungus. Previous *D. sapinea* records from the Balkans were based solely on morphology of conidia and cultures (Karadžić and Milijašević 2008). *D. sapinea* has only occasionally been isolated from conifers other than *Pinus* spp. (Gibson 1979), while the sibling species *D. scrobiculata* is known to occur more commonly on a broad range of conifers (Phillips et al. 2013). This association is of special concern for Serbian spruce (*P. omorika*) that is endemic to a small region of limestone mountains in Serbia, Bosnia and Herzegovina. This tree species is in danger of extinction due to its limited population distribution, climate change and loss of genetic diversity (Alberto et al. 2013), and is thus listed in the IUCN red list of threatened plants (http://www.iucnredlist.org/, accessed July 2015). Nothing is known regarding the possible pathogenicity of *D. sapinea* on *P. omorika* and this is a subject deserving of further study.

The plurivorous species *N. parvum* was frequently collected in this study. This species has been found associated with many forest, plantation and ornamental trees, including *Quercus suber*, *Eucalyptus* spp., *Populus* spp., *Salix* spp., *T. plicata*, *Rhododendron*

spp. and *S. giganteum* (Phillips et al. 2013). Its common occurrence on many ornamentals sampled in this study was thus not surprising. This is the first time the species has been reported on *C. lawsoniana* and three angiosperm ornamental trees and shrubs, namely *A. hippocastanum*, *P. laurocerasus* and *P. tobira*. *N. parvum* is a widely distributed species found on six continents (Phillips et al. 2013), but in the Balkans it has been reported only from pistachio trees and kiwi fruit in Greece (Rumbos and Phillips 2005) and from grapevine in Croatia (Kaliterna et al. 2013). This study provides the first record of *N. parvum* from Serbia and Montenegro.

Diplodia seriata was isolated from forest and ornamental trees in this study, which is consistent with previous reports (Alves et al. 2013; Luchi et al. 2014). It was also the only Botryosphaeriaceae species isolated from *R. fruticosus* consistent with previous findings that it is common on fruit trees (Damm et al. 2007; Slippers et al. 2007; Cloete et al. 2011). The host range of *D. seriata* includes many angiosperms and only a small number of gymnosperm species (Phillips et al. 2013). In the present study, the fungus was found for the first time in association with three angiosperm and three coniferous gymnosperms (Table 1). Although *D. seriata* has a worldwide distribution, in the Balkans the species has been known only from olives in Croatia (Kaliterna et al. 2012) and this study represents its first record from Serbia, Bosnia and Herzegovina.

Diplodia mutila, *Do. sarmentorum* and *Dothiorella* sp. 2 were only occasionally isolated in this study. *D. mutila* has been found associated with various hosts, including ornamental trees (Phillips et al. 2013), but is reported here for the first time from *A. hippocastanum*, *P. halepensis* and *C. arizonica*. Although the species has a world-wide distribution (Phillips et al. 2013), this study represents a first record for it from Serbia and Montenegro. This is also the first report of the species from the Balkans. *Do. sarmentorum* is plurivorous (Phillips et al. 2013), but there is only a single report of the species from gymnosperms (*Cupressus lusitanica*) by Alves et al. (2013). In the present study, *Do. sarmentorum* was found for the first time associated with the conifers *C. lawsoniana* and *C. atlantica* and the angiosperm *A. hippocastanum*. *Do. sarmentorum* has a cosmopolitan distribution (Phillips et al. 2013), but is reported here for the first time from Serbia and the Balkans. The unnamed *Dothiorella* sp. 2 (CMW 39363, CMW 39362, CMW 39360) has been isolated from four angiosperm tree species in previous studies in France, Italy and Iran (Phillips et al. 2008; Piškur et al. 2011; Abdollahzadeh et al. 2014; Pitt et al. 2015). In this study, it was recorded for the first time from Serbia, Bosnia and Herzegovina, Montenegro and the Balkans, and associated for the first time with three ornamental conifers (*C. sempervirens*, *T. occidentalis* and *C. lawsoniana*) and the angiosperm *F. excelsior*.

Dothiorella sp. 1 was occasionally isolated in this study. Interestingly the host range and geographic distribution of this taxon apparently does not overlap with that of the closely related *Dothiorella symphoricarposicola*. *Dothiorella* sp. 1 has been isolated from both *Pinaceae* and *Cupressaceae* including *C. atlantica* and *T. plicata* in Serbia. *Do. symphoricarposicola* has been isolated only from *Symphoricarpos* sp., an angiosperm shrub in Italy (Li et al. 2014).

One isolate each of *S. visci* and *P. cupressi* obtained in this study were included in the phylogenetic analyses. *S. visci* is known to occur only on *V. album* in Europe and is commonly found in the crowns of a wide range of woody species (Varga et al. 2012; Phillips et al. 2013). This host-association was confirmed in the present study. The species has been reported from Hungary (Varga et al. 2012), Luxemburg, Germany (Phillips et al. 2008) and (based on morphological identification) Serbia (Karadžić et al. 2004). *P. cupressi* was previously known to occur only on *C. sempervirens* and *Juniperus scopulorum* in Iran and the USA, respectively (Phillips et al. 2013) and it was not surprising to isolate it from *C. sempervirens* in Montenegro.

The results of this study confirm the fact that morphological characteristics of the Botryosphaeriaceae are shared between different genera and species and are thus of limited taxonomic value (Pavlic et al. 2009; Slippers et al. 2013, 2014; Phillips et al. 2013). There were many similarities between muriform spores of *B. dothidea*, *Dothiorella* sp. 2 and *D. sapinea* and these were thus uninformative for species identification. Slippers et al. (2013) showed that morphological characters of the Botryosphaeriaceae species are not expressed under all conditions in different genera. This would explain the presence of unusual small, dark-walled ellipsoid conidia in *N. parvum*, conidia that become dark-walled while attached to the conidiogenous cell in *B. dothidea* or *Dichomera*-like spores in some of the isolates of *B. dothidea*, *Dothiorella* sp. 2 and *D. sapinea*.

The Botryosphaeriaceae-associated diseases encountered in this study could be linked to climate extremes before and during the study period. Trees planted in the cities are likely to be especially vulnerable to high summer temperatures because of the "heat island effect" and additional stresses from air pollution, limited area for root expansion and heavy construction equipment and vehicles that move over the root zone and compacts the soil, reducing available oxygen to the roots (Tubby and Webber 2010; Alameda et al. 2012). These conditions would have contributed to stress on the trees, predisposing them to infections. This is consistent with the fact that these fungi are known to be opportunistic endophytes that contribute to tree-disease under conditions of stress (Slippers and Wingfield 2007; Mehl et al. 2013). Similarly, Piškur et al. (2011) reported unusual Botryosphaeriaceae (especially *B. dothidea*) related die-back of *O. carpinifolia* trees in Slovenia and Italy following extreme drought conditions.

Overall, the results of this study have shown that a great diversity of Botryosphaeriaceae species occurs on ornamental trees in the countries of the Western Balkans, in a geographically small area. The distribution of these fungi across the study area and their common association with cankers and other disease symptoms suggests that they could be contributing to the die-back and death of the sampled trees. The fact that the Botryosphaeriaceae are known to contribute to tree death under conditions of stress (Slippers and Wingfield 2007; Mehl et al. 2013) and the forecasted extremes in temperature and drought conditions (Tebaldi et al. 2006; IPCC 2014) suggests that these fungi deserve further study.

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References

Abdollahzadeh J, Goltapeh EM, Javadi A, Shams-Bakhsh M, Zare R, Phillips AJL (2009) *Barriopsis iraniana* and *Phaeobotryon cupressi*: two new species of the Botryosphaeriaceae from trees in Iran. Persoonia 23:1–8

Abdollahzadeh J, Javadi A, Zare R, Phillips AJL (2014) A phylogenetic study of *Dothiorella* and *Spencermatinsia* species associated with woody plants in Iran, New Zealand, Portugal and Spain. Persoonia 32:1–12

Alameda D, Anten NPR, Villar R (2012) Soil compaction effects on growth and root traits of tobacco depend on light, water regime and mechanical stress. Soil Tillage Res 120:121–129

Alberto FJ, Aitken SN, Alía R et al (2013) Potential for evolutionary responses to climate change-evidence from tree populations. Glob Change Biol 19:1645–1661

Allen CD, Macalady AK, Chenchouni H et al (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For Ecol Manag 259:660–684

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 Divers 23:1–15

Alves A, Barradas C, Phillips AJL, Correia A (2013) Diversity of Botryosphaeriaceae species associated with conifers in Portugal. Eur J Plant Pathol 135:791–804

Alves A, Linaldeddu BT, Deidda A, Scanu B, Phillips AJL (2014) The complex of *Diplodia* species associated with *Fraxinus* and some other woody hosts in Italy and Portugal. Fungal Divers 67:143–156

Barber PA, Burgess TJ, Hardy GESJ, Slippers B, Keane PJ, Wingfield MJ (2005) *Botryosphaeria* species from *Eucalyptus* in Australia are pleoanamorphic, producing *Dichomera* synanamorphs in culture. Mycol Res 109:1347–1363

Begoude BAD, Slippers B, Wingfield MJ, Roux J (2010) Botryosphaeriaceae associated with *Terminalia catappa* in Cameroon, South Africa and Madagascar. Mycol Prog 9: 101–123

Bobev SG, Lopes J, Phillips AJL (2008) First report of *Diplodia seriata* causing shoot blight and cankers of *Cotoneaster salicifolius* in Bulgaria. Plant Dis 92:976

Butin H (1993) Morphological adaptation and spore pleomorphism in the form-complex *Dichomera–Camarosporium* and *Fusicoccum–Dothiorella*. Sydowia 45:161–166

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91:553–556

Chen SF, Morgan DP, Hasey JK, Anderson K, Michailides TJ (2014) Phylogeny, morphology, distribution, and pathogenicity of Botryosphaeriaceae and Diaporthaceae from English walnut in California. Plant Dis 98:636–652

Chen SF, Li G, Liu F, Michailides TJ (2015) Novel species of Botryosphaeriaceae associated with shoot blight of

pistachio. Mycologia. http://www.mycologia.org/content/early/2015/05/05/14-242.abstract. Accessed 1 Oct 2015

Cloete M, Fourie PH, Damm U, Crous PW, Mostert L (2011) Fungi associated with dieback symptoms of apple and pear trees with a special reference to grapevine trunk disease pathogens. Phytopathol Mediterr 50(Supplement): 176–190

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. Stud Mycol 55:235–253

Crous PW, Groenewald JZ, Wingfield MJ, Phillips AJL (2007) *Neofusicoccum mediterraneum*. Fungal Planet 19. CBS-KNAW Fungal Biodiversity Centre, Utrecht

Damm U, Crous PW, Fourie PH (2007) Botryosphaeriaceae as potential pathogens of *Prunus* species in South Africa, with descriptions of *Diplodia africana* and *Lasiodiplodia plurivora* sp. nov. Mycologia 99:664–680

Deng TJ, Li QL, Chen XL et al (2015) First report of *Lasiodiplodia theobromae* associated with stem canker of *Cassia fistula* in Guangxi, South China. Plant Dis 99:288

Desprez-Loustau ML, Marçais B, Nageleisen LM, Piou D, Vannini A (2006) Interactive effects of drought and pathogens in forest trees. Ann For Sci 63:597–612

Fabre B, Piou D, Desprez-Loustau M, Marcais B (2011) Can the emergence of pine *Diplodia* shoot blight in France be explained by changes in pathogen pressure linked to climate change? Glob Change Biol 17:3218–3227

Farris J, Kallersjo M, Kluge A, Bult C (1995) Testing significance of incongruence. Cladistics 10:315–319

Felsenstein J (1985) Confidence intervals on phylogenetics: an approach using bootstrap. Evolution 39:783–791

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118

Gibson IAS (1979) Diseases of forest trees widely planted as exotics in the tropics and Southern Hemisphere. Part II. The genus *Pinus*. Commonw. Mycol. Inst., Surrey

Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microbiol 61:1323–1330

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol 59:307–321

Heath RN, Roux J, Slippers B, Drenth A, Pennycook SR, Wingfield BD, Wingfield MJ (2011) Occurrence and pathogenicity of *Neofusicoccum parvum* and *N. mangiferae* on ornamental *Tibouchina* species. For Pathol 41:48–51

Inderbitzin P, Bostock RM, Trouillas FP, Michailides TJ (2010) A six locus phylogeny reveals high species diversity in Botryosphaeriaceae from California almond. Mycologia 102:1350–1368

IPCC (2014) Climate change 2014: impacts, adaptation, and vulnerability. Part A: global and sectoral aspects. Contribution of the Working Group II to the Fifth Assessment Report of Intergovernmental Panel on Climate Change (IPCC). Cambridge University Press, Cambridge

Jacobs K, Bergdahl DR, Wingfield MJ, Halik S, Seifert KA, Bright DE, Wingfield BD (2004) *Leptographium wingfieldii* introduced into North America and found associated with exotic *Tomicus piniperda* and native bark beetles. Mycol Res 108:411–418

Jami F, Slippers B, Wingfield MJ, Gryzenhout M (2012) Five new species of the Botryosphaeriaceae from *Acacia karroo* in South Africa. Cryptogam Mycol 33:245–266

Kaliterna J, Milicevic T, Ivic D, Bencic D, Mesic A (2012) First report of *Diplodia seriata* as causal agent of olive dieback in Croatia. Plant Dis 96:290

Kaliterna J, Milicevic T, Bencic D, Duralija B (2013) First report of *Neofusicoccum parvum* associated with grapevine trunk diseases in Croatia. Plant Dis 97:1656

Karadžić D, Milijašević T (2008) The most important parasitic and saprophytic fungi in Austrian pine and Scots pine plantations in Serbia. Bull Fac For 97:147–170 **(in Serbian with English summary)**

Karadžić D, Milijašević T, Keča N (2000) Contribution to the study of the fungus *Botryosphaeria dothidea* agent of canker and tree dying. Bull Fac For 83:87–97 **(in Serbian with English summary)**

Karadžić D, Lazarev V, Milenković M (2004) The most significant parasitic and saprophytic fungi on common mistletoe (*Viscum album* L.) and their potential application in biocontrol. Bull Fac For 89:115–126 **(in Serbian with English summary)**

Latinović J, Mazzaglia A, Latinović N, Ivanović M, Gleason ML (2013) Resistance of olive cultivars to *Botryosphaeria dothidea*, causal agent of olive fruit rot in Montenegro. Crop Prot 48:35–40

Li W, Liu J, Bhat DJ, Camporesi E, Xu J, Hyde KD (2014) Introducing the novel species, *Dothiorella symphoricarposicola*, from snowberry in Italy. Cryptogam Mycol 35:257–270

Ligoxigakis EK, Markakis EA, Papaioannou IA, Typas MA (2013) First report of palm rot of *Phoenix* spp. caused by *Neodeightonia phoenicum* in Greece. Plant Dis 97:286

Luchi N, Oliveira Longa CM, Danti R, Capretti P, Maresi G (2014) *Diplodia sapinea*: the main fungal species involved in the colonization of pine shoots in Italy. For Pathol 44:372–381

Mayorquin JS, Eskalen A, Downer AJ, Hodel DR, Liu A (2012) First report of multiple species of the Botryosphaeriaceae causing bot canker disease of Indian laurel-leaf fig in California. Plant Dis 96:459

Mehl JWM, Slippers B, Roux J, Wingfield MJ (2013) Cankers and other diseases caused by Botryosphaeriaceae. In: Gonthier P, Nicolotti G (eds) Infectious forest diseases. CAB International, Boston, pp 298–317

Möller EM, Bahnweg G, Sandermann H, Geiger HH (1992) A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. Nucleic Acids Res 20:6115–6116

Palmer MA, Stewart EL, Wingfield MJ (1987) Variation among isolates of *Sphaeropsis sapinea* in the north central United States. Phytopathology 77:944–948

Pavlic D, Slippers B, Coutinho TA, Wingfield MJ (2009) Molecular and phenotypic characterization of three phylogenetic species discovered within the *Neofusicoccum parvum/N. ribis* complex. Mycologia 101:636–647

Pavlic-Zupanc D, Piškur B, Slippers B, Wingfield M, Jurc D (2015) Molecular and morphological characterization of

Dothiorella species associated with dieback of *Ostrya carpinifolia* in Slovenia and Italy. Phytopathol Mediterr 54:222–231

Perrings C, Williamson M, Barbier EB et al (2002) Biological invasion risks and the public good: an economic perspective. Conserv Ecol 6. http://www.consecol.org/vol6/iss1/art1. Accessed 1 Oct 2015

Phillips A, Alves A, Correia A, Luque J (2005) Two new species of *Botryosphaeria* with brown, 1-septate ascospores and *Dothiorella* anamorphs. Mycologia 97:513–529

Phillips AJL, Alves A, Pennycook SR, Johnston PR, Ramaley A, Akulov A, Crous PW (2008) Resolving the phylogenetic and taxonomic status of dark-spored teleomorph genera in the Botryosphaeriaceae. Persoonia 21:29–55

Phillips AJL, Lopes J, Abdollahzadeh J, Bobev S, Alves A (2012) Resolving the *Diplodia* complex on apple and other Rosaceae hosts. Persoonia 29:29–38

Phillips AJL, Alves A, Abdollahzadeh J, Slippers B, Wingfield MJ, Groenewald JZ, Crous PW (2013) The Botryosphaeriaceae: genera and species known from culture. Stud Mycol 76:51–167

Piškur B, Pavlic D, Slippers B, Ogris N, Maresi G, Wingfield MJ, Jurc D (2011) Diversity and pathogenicity of Botryosphaeriaceae on declining *Ostrya carpinifolia* in Slovenia and Italy following extreme weather conditions. Eur J For Res 130:235–249

Pitt WM, Úrbez-Torres JR, Trouillas FP (2015) *Dothiorella* and *Spencermartinsia*, new species and records from grapevines in Australia. Australas Plant Pathol 44:43–56

Posada D (2008) jModelTest: phylogenetic model averaging. Mol Biol Evol 25:1253–1256

Rayner RW (1970) A mycological color chart. CMI and British Mycological Society, Surrey

Ronquist F, Huelsenbeck JP (2003) MrBayes: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574

Rumbos IC, Phillips AJL (2005) A new disease of kiwifruit caused by *Botryosphaeria parva*. S Afr J Sci 101: XXII

Sánchez ME, Venegas J, Romero MA, Phillips AJL, Trapero A (2003) *Botryosphaeria* and related taxa causing oak canker in southwestern Spain. Plant Dis 87:1515–1521

Slippers B, Wingfield MJ (2007) The Botryosphaeriaceae as endophytes and latent pathogens of woody plants diversity, ecology and impact. Fungal Biol Rev 21:90–106

Slippers B, Smit WA, Crous PW, Coutinho TA, Wingfield BD, Wingfield MJ (2007) Taxonomy, phylogeny and identification of Botryosphaeriaceae associated with pome and stone fruit trees in South Africa and other regions of the world. Plant Pathol 56:128–139

Slippers B, Burgess T, Pavlic D, Ahumada R, Maleme H, Mohali S, Wingfield MJ (2009) A diverse assemblage of Botryosphaeriaceae infect *Eucalyptus* in native and non-native environments. South For 71:101–110

Slippers B, Boissin E, Phillips AJL, Groenewald JZ, Lombard L, Wingfield MJ, Postma A, Burgess T, Crous PW (2013) Phylogenetic lineages in the Botryosphaeriales: a systematic and evolutionary framework. Stud Mycol 76:31–49

Slippers B, Roux J, Wingfield MJ, Van der Walt FJJ, Jami F, Mehl JWM, Marais GJ (2014) Confronting the constraints of morphological taxonomy in the Botryosphaeriales. Persoonia 33:155–168

Stanosz G, Smith D, LaPhilliph P, Daughtrey ML (2015) Leyland cypress shoot blight and canker caused by *Diplodia cupressi* on Long Island, New York. Plant Dis 99:726

Swart WJ, Wingfield MJ (1991) Biology and control of *Sphaeropsis sapinea* on *Pinus* species in South Africa. Plant Dis 75:761–766

Swofford DL (2003) PAUP*: phylogenetic analyses using parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729

Tebaldi C, Hayhoe K, Arblaster JM, Meehl GA (2006) Going to the extremes. Clim Change 79:185–211

Thomidis T, Exadaktylou E (2010) First report of *Botryosphaeria dothidea* causing shoot blight of kiwifruit (*Actinidia deliciosa*) in Greece. Plant Dis 94:1503

Tsopelas P, Slippers B, Gonou-Zagou Z, Wingfield MJ (2010) First report of *Diplodia corticola* in Greece on kermes oak (*Quercus coccifera*). Plant Pathol 59:805

Tubby KV, Webber JF (2010) Pests and diseases threatening urban trees under a changing climate. Forestry 83:451–459

Urbez-Torres JR (2011) The status of Botryosphaeriaceae species infecting grapevines. Phytopathol Mediterr 50(Supplement): 5–45

Varela PC, Redondo Fernández V, Mansilla Vázquez JP, Aguín Casal O (2011) First report of dieback on hybrid Rhododendrons caused by *Neofusicoccum luteum* and *N. parvum* in Spain. Plant Dis 95:221

Varga I, Taller J, Baltazár T, Hyvönen J, Poczai P (2012) Leaf-spot disease on European mistletoe (*Viscum album*) caused by *Phaeobotryosphaeria visci*: a potential candidate for biological control. Biotechnol Lett 34:1059–1065

Vasić M, Duduk N, Vico I, Ivanović MS (2013) First report of *Botryosphaeria dothidea* causing white rot of apple fruit in Serbia. Plant Dis 97:1659

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* spp. J Bacteriol 172:4238–4246

White TJ, Bruns T, Lee S, Taylor J, Innis MA, Gelfand DH, Sninsky JJ, White TJ (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. Academic, New York, pp 315–322

Xu C, Wang C, Ju L (2015) Multiple locus genealogies and phenotypic characters reappraise the causal agents of apple ring rot in China. Fungal Divers 71:215–231