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Saprobic *Botryosphaeriaceae*, including *Dothiorella italica* sp. nov., associated with urban and forest trees in Italy

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Abstract

A collection of saprobic botryosphaeriaceous isolates (from dead aerial branches, stems, and dead leaves) was obtained from urban and forest tree species in Italy. A total of 52 isolates were characterized by multi-locus sequence analysis of the internal transcribed spacer region (ITS) and partial sequences of the translation elongation factor 1-alpha (TEF). According to morphological characteristics and analysis of multi-gene sequence data, 14 species in *Botryosphaeriaceae* belonging to five genera, viz. *Botryosphaeria*, *Diplodia*, *Dothiorella*, *Eutiarospora* and *Neofusicoccum* were identified, including *Dothiorella italica* sp. nov. The other known species isolated were *Botryosphaeria dothidea*, *B. auasmontanum*, *Diplodia alatafructa*, *Di. crataegicola*, *Di. mutila*, *Di. sapinea*, *Di. seriata*, *Dothiorella omnivora*, *Do. rhamnii*, *Do. sarmentorum*, *Do. sempervirentis*, *Eutiarospora dactylidis* and *Neofusicoccum parvum*. Of these, *Botryosphaeria dothidea* and *Diplodia seriata* were the most prevalent species among the isolates. Several new host-fungus relationships were established. This study indicates the fungal family *Botryosphaeriaceae* seems to be common and widespread on a broad range of hosts in Italy.

Key words – Botryosphaeriales – *Botryosphaeria* – *Diplodia* – *Eutiarospora* – multigene phylogeny – *Neofusicoccum*

Introduction

The family *Botryosphaeriaceae* Theiss. & Syd. comprise 23 genera found on a wide range of plant hosts (Dissanayake et al. 2016b). Many botryosphaeriaceous taxa can exist as endophytes (Slippers & Wingfield 2007), or are primary or opportunistic pathogens or saprobes (Schoch et al. 2006, Mehl et al. 2014) and are widespread in tropical and temperate regions (Phillips et al. 2013, Dissanayake et al. 2016b). Almost all *Botryosphaeriaceae* species are plant-associated and most of them cause disease symptoms on ecologically and economically significant plants in forestry and agriculture (Hyde et al. 2013, 2014, Phillips et al. 2013, Slippers et al. 2013). *Botryosphaeriaceae* consists of many well-known plant pathogens such as *Botryosphaeria dothidea* (Moug.) Ces. & De Not., *Diplodia seriata* De Not., *Dothiorella sarmentorum* (Fr.) A.J.L. Phillips, A. Alves & J. Luque, *Lasiodyplodia theobromae* (Pat.) Griffon & Maubl. *Neofusicoccum parvum* (Pennycook &

Samuels) Crous, Slippers & A.J.L. Phillips and many others causing severe infections (Slippers et al. 2013, Yan et al. 2013, Hyde et al. 2014). *Botryosphaeria* Ces. & De Not. species are frequently associated with diseases such as dieback, cankers, shoot blights, leaf spots, fruit and seed rots. *Diplodia* Fr. species are associated with diverse symptoms such as cankers, dieback, gummosis, fruit rots and twig blight (Lazzizzera et al. 2008, Giambra et al. 2016, Linaldeddu et al. 2016c). Though several *Diplodia* species such as *Di. mutila* (Fr.) Mont., *Di. pseudoseriata* C.A. Pérez, Blanchette, Slippers & M.J. Wingf., *Di. scrobiculata* J. de Wet, Slippers & M.J. Wingf. and *Di. seriata* have an extensive host range, other *Diplodia* species have been described from very limited plant species and may be host-specific (Phillips et al. 2013, Dissanayake et al. 2016b). The genus *Dothiorella* Sacc. is widely spread in woody hosts world-wide (Dissanayake et al. 2016a) and morphologically similar to *Spencermartinsia* A.J.L. Phillips, A. Alves & Crous in asexual morph, but treated as a distinct genus based on the presence of apiculi on ascospores of *Spencermartinsia* (Phillips et al. 2008, 2013). However, this separation was recently perceived as uncertain (Slippers et al. 2014). Except *Do. iberica* A.J.L. Phillips, J. Luque & A. Alves, *Do. omnivora* B.T. Linaldeddu, A. Deidda & B. Scanu and *Do. sarmentorum*; all other *Dothiorella* species were reported from very few plant species (Phillips et al. 2013, Dissanayake et al. 2016b). Compared to other genera of *Botryosphaeriaceae*, species of *Eutiarospora* Crous have different culture characteristics and conidial morphology (Jami et al. 2012, Thambugala et al. 2014, Crous et al. 2015). Although recent studies have reported *Eutiarospora* species from woody hosts (Jami et al. 2012, 2014), traditionally they have been associated with family Poaceae (Sutton & Marasas 1976, Nag Raj 1993, Li et al. 2016). The genus *Neofusicoccum* Crous, Slippers & A.J.L. Phillips comprises species with widespread geographical and plant host distribution, some of them of economic importance (Lopes et al. 2017). Although *Neofusicoccum* species are typically endophytes, under stress environments certain species can cause deterioration and dieback symptoms (Crous et al. 2006, Slippers & Wingfield 2007).

Before 2008, with exception of a few studies, all *Botryosphaeriaceae* species reported from Italy were identified based on morphological characters (Alves et al. 2004, Phillips et al. 2005, Linaldeddu et al. 2006, 2007), thus some of these reports are doubtful. Since 2008, numerous *Botryosphaeriaceae* species have been reported from Italy based on molecular data, either from symptomatic or asymptomatic native trees and/or from non-native trees (Lazzizzera et al. 2008, Spagnolo et al. 2011, Ismail et al. 2013, Li et al. 2014, Linaldeddu et al. 2014, 2015, 2016a, b, c, Carlucci et al. 2015, Pavlic-Zupanc et al. 2015, Giambra et al. 2016). However, less is known regarding *Botryosphaeriaceae* species in the provinces of Forlì-Cesena, Arezzo and Perugia, in Italy. Thus, the aim of the present study was to explore the species composition, host range and distribution of saprobic *Botryosphaeriaceae* fungi in multiple plant species in Italy.

Materials & Methods

Sample collection, fungal isolation and morphological characterization

Since 2012 to 2016, 52 *Botryosphaeriaceae* specimens were collected (Table 1) from dead aerial branches, stems and leaves of 34 host plants belonging to 23 host families (*Adoxaceae*, *Asteraceae*, *Betulaceae*, *Celastraceae*, *Cornaceae*, *Cupressaceae*, *Fabaceae*, *Fagaceae*, *Magnoliaceae*, *Malvaceae*, *Meliaceae*, *Oleaceae*, *Pinaceae*, *Poaceae*, *Ranunculaceae*, *Rhamnaceae*, *Rosaceae*, *Rubiaceae*, *Salicaceae*, *Sapindaceae*, *Tamaricaceae*, *Ulmaceae* and *Vitaceae*). These specimens were collected from plants in natural forest ecosystems and in urban environments in three provinces of Italy: Arezzo, Forlì-Cesena and Perugia. Specimens were observed and examined with a Motic SMZ 168 stereomicroscope. Fruiting bodies (ascomata and/or conidiomata) were detected in all collected samples and single spore isolations were made as previously described by Chomnunti et al. (2014). Conidial micro-morphological characteristics were observed with a Nikon ECLIPSE 80i compound microscope and images were captured with a Canon EOS 550D digital camera.

Table 1 *Botryosphaeriaceae* species studied in this study (Fig. 1 to 5). Ex-type of the species introduced in this study is in bold.

Species	Strain (MFLUCC)	Host	Habit	Locality	Collector	Collection date	GenBank accession numbers	
							ITS	EF
<i>Botryosphaeria dothidea</i>	13-0292	<i>Sambucus nigra</i>	Dead aerial branch	Forlì-Cesena	N. Camporesi	01.01.2013	MF398850	MF398902
<i>B. dothidea</i>	17-0940	<i>Sambucus ebulus</i>	Dead aerial stem	Forlì-Cesena	E. Camporesi	15.03.2013	MF398851	MF398903
<i>B. dothidea</i>	17-0943	<i>Galium sp.</i>	Dead aerial stem	Forlì-Cesena	E. Camporesi	10.02.2014	MF398852	MF398904
<i>B. dothidea</i>	17-0944	<i>Carpinus betulus</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	19.02.2014	MF398853	MF398905
<i>B. dothidea</i>	15-0913	<i>L. anagyroides</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	01.10.2014	MF398855	MF398907
<i>B. dothidea</i>	17-1069	<i>Vitis vinifera</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	21.02.2015	MF398856	MF398908
<i>B. dothidea</i>	17-0960	<i>Vitis vinifera</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	21.02.2015	MF398857	MF398909
<i>B. dothidea</i>	15-0927	<i>P. menziesii</i>	Dead land cone	Forlì-Cesena	E. Camporesi	01.06.2015	MF398859	MF398911
<i>B. dothidea</i>	17-0973	<i>Colutea arborescens</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	02.11.2015	MF398860	MF398912
<i>B. dothidea</i>	17-0986	<i>Cornus sanguinea</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	24.11.2015	MF398861	MF398913
<i>B. dothidea</i>	17-0994	<i>Salix sp.</i>	Dead aerial stem	Forlì-Cesena	E. Camporesi	26.01.2016	MF398862	MF398914
<i>B. dothidea</i>	17-0945	<i>Euonymus europaeus</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	20.02.2014	MF398854	MF398906
<i>B. auasmontanum</i>	15-0923	<i>Rosa canina</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	07.04.2015	MF398858	MF398910
<i>B. auasmontanum</i>	17-1071	<i>Alnus cordata</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	07.03.2016	MF398863	MF398915
<i>Diplodia alatafructa</i>	15-0924	<i>Picea excelsa</i>	Dead land cone	Forlì-Cesena	E. Camporesi	12.04.2015	MF398880	MF398932
<i>Di. crataegicola</i>	13-0192	<i>Crataegus sp.</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	03.11.2012	MF398867	MF398919
<i>Di. crataegicola</i>	13-0289	<i>Prunus sp.</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	23.12.2012	MF398870	MF398922
<i>Di. crataegicola</i>	15-0905	<i>Tilia sp.</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	22.05.2013	MF398871	MF398923
<i>Di. mutila</i>	15-0917	<i>Acer negundo</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	22.01.2015	MF398877	MF398929
<i>Di. mutila</i>	15-0918	<i>Colutea arborescens</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	26.10.2015	MF398882	MF398934
<i>Di. sapinea</i>	17-0938	<i>Picea excelsa</i>	Dead land cone	Forlì-Cesena	E. Camporesi	12.04.2012	MF398866	MF398918
<i>Di. sapinea</i>	13-0194	<i>Pinus pinaster</i>	Dead aerial needles	Arezzo	E. Camporesi	07.11.2012	MF398868	MF398920
<i>Di. seriata</i>	17-0936	<i>Euonymus europaeus</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	08.04.2012	MF398865	MF398917
<i>Di. seriata</i>	15-0903	<i>Quercus pubescens</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	02.12.2012	MF398869	MF398921

<i>Di. seriata</i>	17-0952	<i>Rosa canina</i>	Dead aerial spines	Forlì-Cesena	E. Camporesi	06.10.2014	MF398872	MF398924
<i>Di. seriata</i>	17-0950	<i>Rosa canina</i>	Dead aerial stem	Forlì-Cesena	E. Camporesi	17.10.2014	MF398876	MF398928
<i>Di. seriata</i>	17-0958	<i>Sambucus nigra</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	07.02.2015	MF398878	MF398930
<i>Di. seriata</i>	15-0965	<i>Rosa canina</i>	Dead aerial spines	Forlì-Cesena	E. Camporesi	25.03.2015	MF398879	MF398931
<i>Di. seriata</i>	17-0975	<i>Magnolia grandiflora</i>	Dead land leaf	Forlì-Cesena	E. Camporesi	15.09.2015	MF398881	MF398933
<i>Di. seriata</i>	17-0989	<i>Populus nigra</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	01.12.2015	MF398883	MF398935
<i>Di. seriata</i>	15-0760	<i>Cornus sanguinea</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	02.03.2012	MF398864	MF398916
<i>Di. seriata</i>	15-0914	<i>Rosa canina</i>	Dead aerial spines	Arezzo	E. Camporesi	13.10.2014	MF398875	MF398927
<i>Dothiorella omnivora</i>	17-0990	<i>Cornus sanguinea</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	21.12.2015	MF398895	MF398947
<i>Do. rhamnii</i>	17-1067	<i>Tamarix gallica</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	01.12.2012	MF398888	MF398940
<i>Do. rhamnii</i>	15-0922	<i>Rhamnus alaternus</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	04.03.2015	MF398893	MF398945
<i>Do. sarmentorum</i>	15-0901	<i>Clematis vitalba</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	26.03.2012	MF398884	MF398936
<i>Do. sarmentorum</i>	15-0912	<i>Ulmus minor</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	25.09.2014	MF398890	MF398942
<i>Do. sarmentorum</i>	15-0921	<i>Vitis vinifera</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	21.02.2015	MF398892	MF398944
<i>Do. sarmentorum</i>	17-1000	<i>Robinia pseudacacia</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	08.03.2016	MF398896	MF398948
<i>Do. sempervirentis</i>	17-1066	<i>Cytisus sp.</i>	Dead aerial branch	Perugia	E. Camporesi	06.11.2012	MF398886	MF398938
<i>Do. italica</i>	17-1065	<i>Cupressus sp.</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	13.04.2012	MF398885	MF398937
<i>Do. italica</i>	17-0954	<i>Prunus sp.</i>	Dead aerial branch	Perugia	E. Camporesi	06.11.2012	MF398887	MF398939
<i>Do. italica</i>	15-0908	<i>Rubus sp.</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	16.11.2013	MF398889	MF398941
<i>Do. italica</i>	17-0951	<i>Rosa canina</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	17.10.2014	MF398891	MF398943
<i>Do. italica</i>	17-0979	<i>Melia azedarach</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	29.09.2015	MF398894	MF398946
<i>Do. italica</i>	17-1002	<i>Ligustrum sp.</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	09.03.2016	MF398897	MF398949
<i>Eutiarosporella dactylidis</i>	MFLU 15-3473	<i>A. elatius</i>	Dead aerial stem	Forlì-Cesena	E. Camporesi	03.11.2013	MF398898	MF398950
<i>Neofusicoccum parvum</i>	17-0961	<i>Vitis vinifera</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	21.02.2015	MF398899	MF398951
<i>N. parvum</i>	17-0991	<i>Salix sp.</i>	Dead aerial branch	Forlì-Cesena	E. Camporesi	18.12.2015	MF398900	MF398952
<i>N. parvum</i>	17-1024	<i>E. cannabinum</i>	Dead aerial stem	Forlì-Cesena	E. Camporesi	14.08.2016	MF398901	MF398953

Observations and photographs were made from materials mounted in water. Measurements were made with the Tarosoft (R) Image Frame Work and images used for figures were processed with Adobe Photoshop CS3 Extended version 10.0. Cultures were incubated at 18°C in the dark and colony color was examined according to Rayner (1970) after 15 d of growth on PDA. Herbarium specimens are deposited in Mae Fah Luang University Herbarium (MFLU) while, ex-type living cultures are deposited at the Mae Fah Luang University Culture Collection (MFLUCC) in Thailand (Table 1).

DNA extraction, amplification and phylogeny

Mycelium from actively growing cultures was scraped from the surface of the plates. Total genomic DNA was extracted by the modified protocol of Dissanayake et al. (2016a). ITS and TEF gene regions were amplified using primer pairs ITS1/ITS4 (White et al. 1990) and EF-728F/EF-986R (Carbone & Kohn 1999) respectively.

PCR was performed in a BIORAD 1000™ Thermal Cycle in a total volume of 25 µl. The PCR mixtures contained TaKaRa Ex-Taq DNA polymerase 0.3 µl, 12.5 µl of 2 × PCR buffer with 2.5 µl of dNTPs, 1 µl of each primer, 9.2 µl of double-distilled water and 100–500 ng of DNA template. The thermal cycling protocol followed Dissanayake et al. (2016a). The PCR products were verified by staining with ethidium bromide on 1.2 % agarose electrophoresis gels and purified according to the manufacturer's instructions of Qiagen purification kit (Qiagen, Hilden, Germany). DNA sequencing of the genes were conducted by Sunbiotech Company, Beijing, China.

The systemic placement of taxa in this paper was based on the phylogeny of combined analysis of ITS and TEF (Phillips et al. 2013, Dissanayake et al. 2016b). DNASTAR V.5.1 and SEQMAN V.5.00 were used to obtain consensus sequences from sequences generated from forward and reverse primers. Sequences obtained from this study and other sequences originating from GenBank (Table 2) were aligned using default settings of MAFFT v.7 (Katoh & Toh 2008, <http://mafft.cbrc.jp/alignment/server/>) and manually adjusted using BIOEDIT V.7.0.9.0 (Hall 1999), where necessary. Maximum-parsimony (MP) analysis was performed using PAUP v. 4.0b10 (Swofford 2003) to obtain the most parsimonious trees. Gaps were treated as missing data. Trees were inferred using the heuristic search option with Tree Bisection Reconnection (TBR) branch swapping and 1000 random sequence additions. Maxtrees were set up to 1000 and branches of zero length were collapsed and all multiple parsimonious trees were saved. Descriptive tree statistics for parsimony such as Tree Length (TL), Consistency Index (CI), Retention Index (RI), Relative Consistency Index (RC) and Homoplasy Index (HI) were calculated. Clade stability was evaluated by 1000 bootstrap replications resulting from maximum parsimony analysis (Hillis & Bull 1993). The best model of evolution for each gene region was determined by using MrModeltest 2.2 (Nylander 2004). The general time-reversible model of evolution (Rodriguez et al. 1990), including estimation of invariable sites and assuming a discrete gamma distribution with six rate categories (GTR+I+G) was used for BI analyses for ITS and EF1- α sequence datasets. Posterior probabilities (PP) were determined by Markov Chain Monte Carlo sampling (BMCMC) in MrBayes v. 3.0b4 (Ronquist & Huelsenbeck 2003). Four simultaneous Markov chains were run for 1000000 generations and trees were sampled every 100th generation and 10000 trees were obtained. The first 2000 trees, representing the burn-in phase of the analyses, were discarded while remaining 8000 trees were used for calculating posterior probabilities in the majority rule consensus tree (critical value for the topological convergence diagnostic set to 0.01) (Crous et al. 2006). Phylogenetic trees were drawn using Treeview v. 1.6.6 (Page 1996). The DNA sequences generated in this study are deposited in GenBank (Table 1) and the alignments in TreeBase (<http://purl.org/phylo/treebase/phylovs/study/>). Taxonomic novelties were submitted to the Faces of Fungi database (Jayasiri et al. 2015) and Index Fungorum (Index Fungorum 2017).

Table 2 Isolates from GenBank used in phylogenetic analyses. Ex-type species are in bold.

Species	Isolate number	ITS	TEF
<i>Botryosphaeria agaves</i>	MFLUCC 11-0125	JX646791	JX646856

Species	Isolate number	ITS	TEF
<i>Botryosphaeria agaves</i>	MFLUCC 10-0051	JX646790	JX646855
<i>Botryosphaeria auasmontanum</i>	CMW 25413	KF766167	EU101348
<i>Botryosphaeria corticis</i>	CBS 119047	DQ299245	EU017539
<i>Botryosphaeria corticis</i>	ATCC 22927	DQ299247	EU673291
<i>Botryosphaeria dothidea</i>	CBS 115476	AY236949	AY236898
<i>Botryosphaeria dothidea</i>	CBS 110302	AY259092	AY573218
<i>Botryosphaeria fabicerciana</i>	CBS 127193	HQ332197	HQ332213
<i>Botryosphaeria fabicerciana</i>	CMW 27108	HQ332200	HQ332216
<i>Botryosphaeria fusispora</i>	MFLUCC 10-0098	JX646789	JX646854
<i>Botryosphaeria fusispora</i>	MFLUCC 11-0507	JX646788	JX646853
<i>Botryosphaeria minutispermata</i>	GZCC 16-0013	KX447675	KX447678
<i>Botryosphaeria minutispermata</i>	GZCC 16-0014	KX447676	KX447679
<i>Botryosphaeria ramosa</i>	CBS 122069	EU144055	EU144070
<i>Botryosphaeria scharifii</i>	CBS 124703	JQ772020	JQ772057
<i>Botryosphaeria scharifii</i>	CBS 124702	JQ772019	JQ772056
<i>Botryosphaeria sinensia</i>	CGMCC 3.17722	KT343254	KU221233
<i>Botryosphaeria sinensia</i>	CGMCC 3.17724	KT343256	KU221234
<i>Diplodia africana</i>	CBS 120835	EF445343	EF445382
<i>Diplodia africana</i>	CBS 121104	EF445344	EF445383
<i>Diplodia agrifolia</i>	CBS 132777	JN693507	JQ517317
<i>Diplodia agrifolia</i>	UCROK1429	JQ411412	JQ512121
<i>Diplodia alatafructa</i>	CBS 124931	FJ888460	FJ888444
<i>Diplodia alatafructa</i>	CBS 124933	FJ888478	FJ888446
<i>Diplodia allocellula</i>	CBS 130408	JQ239397	JQ239384
<i>Diplodia allocellula</i>	CBS 130410	JQ239399	JQ239386
<i>Diplodia bulgarica</i>	CBS 124254	GQ923853	GQ923821
<i>Diplodia bulgarica</i>	CBS 124135	GQ923852	GQ923820
<i>Diplodia corticola</i>	CBS 112549	AY259100	AY573227
<i>Diplodia corticola</i>	CBS 112546	AY259110	DQ458872
<i>Diplodia crataegicola</i>	MFLU 15-1311	KT290244	KT290248
<i>Diplodia cupressi</i>	CBS 168.87	DQ458893	DQ458878
<i>Diplodia cupressi</i>	CBS 261.85	DQ458894	DQ458879
<i>Diplodia estuarina</i>	CMW41231	KP860831	KP860676
<i>Diplodia estuarina</i>	CMW41230	KP860830	KP860675
<i>Diplodia fraxinii</i>	CBS 136010	KF307700	KF318747
<i>Diplodia galiicola</i>	MFLU 15-1310	KT290245	KT290249
<i>Diplodia insularis</i>	CBS 140350	KX833072	KX833073
<i>Diplodia intermedia</i>	CBS 124462	GQ923858	GQ923826
<i>Diplodia intermedia</i>	CBS 124134	HM036528	GQ923851
<i>Diplodia malorum</i>	CBS 124130	GQ923865	GQ923833
<i>Diplodia malorum</i>	CBS 112554	AY259095	DQ458870
<i>Diplodia mutila</i>	CBS 112553	AY259093	AY573219
<i>Diplodia mutila</i>	CBS 230.30	DQ458886	DQ458869
<i>Diplodia neojuniperi</i>	CPC 22753	KM006431	KM006462
<i>Diplodia olivarum</i>	CBS 121887	EU392302	EU392279
<i>Diplodia olivarum</i>	CBS 121886	EU392297	EU392274
<i>Diplodia pseudoseriata</i>	CBS 124906	EU080927	EU863181
<i>Diplodia pseudoseriata</i>	CBS 124907	EU080922	EU863179
<i>Diplodia quercivora</i>	CBS 133852	JX894205	JX894229
<i>Diplodia rosacearum</i>	CBS 141915	KT956270	KU378605
<i>Diplodia rosulata</i>	CBS 116470	EU430265	EU430267
<i>Diplodia rosulata</i>	CBS 116472	EU430266	EU430268
<i>Diplodia sapinea</i>	CBS 393.84	DQ458895	DQ458880
<i>Diplodia sapinea</i>	CBS 109725	DQ458896	DQ458881

Species	Isolate number	ITS	TEF
<i>Diplodia scrobiculata</i>	CBS 118110	AY253292	AY624253
<i>Diplodia scrobiculata</i>	CBS 109944	DQ458899	DQ458884
<i>Diplodia scrobiculata</i>	CBS 113423	DQ458900	DQ458885
<i>Diplodia seriata</i>	CBS 112555	AY259094	AY573220
<i>Diplodia seriata</i>	CBS 119049	DQ458889	DQ458874
<i>Diplodia subglobosa</i>	CBS 124133	GQ923856	GQ923824
<i>Diplodia tsugae</i>	CBS 418.64	DQ458888	DQ458873
<i>Dothiorella acacicola</i>	CBS 141295	KX228269	KX228376
<i>Dothiorella americana</i>	CBS 128309	HQ288218	HQ288262
<i>Dothiorella americana</i>	CBS 128310	HQ288219	HQ288263
<i>Dothiorella brevicollis</i>	CBS 130411	JQ239403	JQ239390
<i>Dothiorella californica</i>	CBS 141587	KX357188	KX357211
<i>Dothiorella capri-amissi</i>	CMW 25403	EU101323	EU101368
<i>Dothiorella capri-amissi</i>	CMW 25404	EU101324	EU101369
<i>Dothiorella casuarini</i>	CBS 120688	DQ846773	DQ875331
<i>Dothiorella casuarini</i>	CBS 120690	DQ846774	DQ875333
<i>Dothiorella dulcispinae</i>	CBS 130413	JQ239400	JQ239387
<i>Dothiorella iberica</i>	CBS 115041	AY573202	AY573222
<i>Dothiorella iberica</i>	CBS 113188	AY573198	EU673278
<i>Dothiorella iranica</i>	IRAN1587C	KC898231	KC898214
<i>Dothiorella longicollis</i>	CBS 122068	EU144054	EU144069
<i>Dothiorella longicollis</i>	CBS 122066	EU144052	EU144067
<i>Dothiorella moneti</i>	MUCC505	EF591920	EF591971
<i>Dothiorella moneti</i>	MUCC 507	EF591922	EF591973
<i>Dothiorella neclivorem</i>	DAR80992	KJ573643	KJ573640
<i>Dothiorella oblonga</i>	CMW 25407	EU101300	EU101345
<i>Dothiorella omnivora</i>	CBS 140349	KP205497	KP205470
<i>Dothiorella omnivora</i>	CBS 188.87	EU673316	EU673283
<i>Dothiorella parva</i>	IRAN1579C	KC898234	KC898217
<i>Dothiorella parva</i>	IRAN1585C	KC898235	KC898218
<i>Dothiorella pretoriensis</i>	CBS 130404	JQ239405	JQ239392
<i>Dothiorella pretoriensis</i>	CBS 130403	JQ239406	JQ239393
<i>Dothiorella prunicola</i>	CBS 124723	EU673313	EU673280
<i>Dothiorella rhamnii</i>	MFLUCC 14-0902	KU246381	N/A
<i>Dothiorella santali</i>	MUCC 509	EF591924	EF591975
<i>Dothiorella santali</i>	MUCC 508	EF591923	EF591974
<i>Dothiorella sarmentorum</i>	IMI 63581b	AY573212	AY573235
<i>Dothiorella sarmentorum</i>	CBS 115038	AY573206	AY573223
<i>Dothiorella sempervirentis</i>	IRAN1583C	KC898236	KC898219
<i>Dothiorella sempervirentis</i>	IRAN1581C	KC898237	KC898220
<i>Dothiorella striata</i>	ICMP16824	EU673320	EU673287
<i>Dothiorella striata</i>	ICMP16819	EU673320	EU673287
<i>Dothiorella symphoricarposicola</i>	MFULCC 13-0497	KJ742378	KJ742381
<i>Dothiorella symphoricarposicola</i>	MFLUCC 13-0196	KU234782	KU234796
<i>Dothiorella tectonae</i>	MFLUCC12-0382	KM396899	KM409637
<i>Dothiorella thailandica</i>	CBS 133991	JX646796	JX646861
<i>Dothiorella thripsita</i>	BRIP 51876	FJ824738	KJ573639
<i>Dothiorella ulmacea</i>	CBS 138855	KR611881	KR611910
<i>Dothiorella uruguayensis</i>	CBS 124908	EU080923	EU863180
<i>Dothiorella vidmadera</i>	DAR78992	EU768874	EU768881
<i>Dothiorella vidmadera</i>	DAR78993	EU768876	EU768882
<i>Dothiorella vinea-gemmae</i>	DAR81012	KJ573644	KJ573641
<i>Eutiarospora africana</i>	CBS 133854	KC769956	KC769852

Species	Isolate number	ITS	TEF
<i>Eutiarosporella africana</i>	CBS 135850	KC769957	KC769853
<i>Eutiarosporella dactylidis</i>	MFLUCC 13-0276	KM978944	KP031694
<i>Eutiarosporella dactylidis</i>	MFLUCC 15-0915	KU246378	N/A
<i>Eutiarosporella darliae</i>	DAR 82491	KP309793	KP309805
<i>Eutiarosporella darliae</i>	DAR 82493	KP309786	KP309807
<i>Eutiarosporella pseudodarliae</i>	DAR 82489	KP309796	KP309797
<i>Eutiarosporella pseudodarliae</i>	DAR 82490	KP309794	KP309800
<i>Eutiarosporella tritici</i>	CBS 118719	KC769961	KF531809
<i>Eutiarosporella tritici-australis</i>	DAR 82485	KP309788	KP309799
<i>Eutiarosporella tritici-australis</i>	DAR 82486	KP309787	KP309804
<i>Eutiarosporella urbis-rosarum</i>	CBS 130405	JQ239407	JQ239394
<i>Eutiarosporella urbis-rosarum</i>	CBS 130406	JQ239408	JQ239395
<i>Neofusicoccum algeriense</i>	CBS 137504	KJ657702	KJ657715
<i>Neofusicoccum algeriense</i>	ALG9	KJ657704	KJ657721
<i>Neofusicoccum andinum</i>	CBS 117453	AY693976	AY693977
<i>Neofusicoccum andinum</i>	CBS 117452	DQ306263	DQ306264
<i>Neofusicoccum arbuti</i>	CBS 116131	AY819720	N/A
<i>Neofusicoccum arbuti</i>	CBS 117090	AY819724	KF531791
<i>Neofusicoccum australe</i>	CMW 6837	AY339262	AY339270
<i>Neofusicoccum australe</i>	CMW 6853	AY339263	AY339271
<i>Neofusicoccum batangarum</i>	CBS 124924	FJ900607	FJ900653
<i>Neofusicoccum batangarum</i>	CBS 124923	FJ900608	FJ900654
<i>Neofusicoccum braziliense</i>	CMM1338	JX513630	JX513610
<i>Neofusicoccum cordaticola</i>	CBS 123634	EU821898	EU821868
<i>Neofusicoccum cordaticola</i>	CBS 123635	EU821903	EU821873
<i>Neofusicoccum cryptoaustrale</i>	CMW23785	FJ752742	FJ752713
<i>Neofusicoccum eucalypticola</i>	CBS 115679	AY615141	AY615133
<i>Neofusicoccum eucalypticola</i>	CBS 115766	AY615143	AY615135
<i>Neofusicoccum eucalyptorum</i>	CBS 115791	AF283686	AY236891
<i>Neofusicoccum eucalyptorum</i>	CMW 10126	AF283687	AY236892
<i>Neofusicoccum grevilleae</i>	CBS 129518	JF951137	N/A
<i>Neofusicoccum hellenicum</i>	CERC1947	KP217053	KP217061
<i>Neofusicoccum kwambonambiense</i>	CBS 123639	EU821900	EU821870
<i>Neofusicoccum kwambonambiense</i>	CBS 123641	EU821919	EU821889
<i>Neofusicoccum lumnitzeriae</i>	CMW41469	KP860881	KP860724
<i>Neofusicoccum lumnitzeriae</i>	CMW41228	KP860882	KP860725
<i>Neofusicoccum luteum</i>	CBS 110299	AY259091	AY573217
<i>Neofusicoccum luteum</i>	CBS 110497	EU673311	EU673277
<i>Neofusicoccum macroclavatum</i>	CBS 118223	DQ093196	DQ093217
<i>Neofusicoccum macroclavatum</i>	WAC 12446	DQ093197	DQ093218
<i>Neofusicoccum mangiferae</i>	CBS 118531	AY615185	DQ093221
<i>Neofusicoccum mangiferae</i>	CBS 118532	AY615186	DQ093220
<i>Neofusicoccum mangroviorum</i>	CMW41365	KP860859	KP860702
<i>Neofusicoccum mangroviorum</i>	CMW42481	KP860848	KP860692
<i>Neofusicoccum mediterraneum</i>	CBS 121718	GU251176	GU251308
<i>Neofusicoccum mediterraneum</i>	CBS 121558	GU799463	GU799462
<i>Neofusicoccum nonquaesitum</i>	CBS 126655	GU251163	GU251295
<i>Neofusicoccum nonquaesitum</i>	PD 301	GU251164	GU251296
<i>Neofusicoccum oculatum</i>	CBS 128008	EU301030	EU339509
<i>Neofusicoccum oculatum</i>	MUCC 286	EU736947	EU339511
<i>Neofusicoccum parvum</i>	CMW 9081	AY236943	AY236888
<i>Neofusicoccum parvum</i>	CBS 110301	AY259098	AY573221
<i>Neofusicoccum pennatisporum</i>	MUCC 510	EF591925	EF591976

Species	Isolate number	ITS	TEF
<i>Neofusicoccum protearum</i>	STE-U 4361	AF196295	N/A
<i>Neofusicoccum protearum</i>	STE-U 1775	AF452539	N/A
<i>Neofusicoccum ribis</i>	CBS 115475	AY236935	AY236877
<i>Neofusicoccum ribis</i>	CBS 121.26	AF241177	AY236879
<i>Neofusicoccum umdonicola</i>	CBS 123645	EU821904	EU821874
<i>Neofusicoccum umdonicola</i>	CBS 123646	EU821905	EU821875
<i>Neofusicoccum ursorum</i>	CMW 24480	FJ752746	FJ752709
<i>Neofusicoccum viticlavatum</i>	CBS 112878	AY343381	AY343342
<i>Neofusicoccum viticlavatum</i>	CBS 112977	AY343380	AY343341
<i>Neofusicoccum vitifusiforme</i>	CBS 110887	AY343383	AY343343
<i>Neofusicoccum vitifusiforme</i>	CBS 110880	AY343382	AY343344

Results

Sample collection

In this study, a collection of 52 saprobic specimens from different hosts in urban and forest tree species were collected and this resulted in identification of 14 *Botryosphaeriaceae* species. Of these, 12 isolates, each from *B. dothidea* (23 %) and *Di. seriata* (23%) were detected as the prominent species. Six isolates of the newly described *Do. italica* (12 %) were obtained, while all other species were in low numbers of isolates. Host ranges of the species were determined from available literature and as well as from the SMML Fungus-Host Distribution Database. Several new fungus-host associations were established (Table 1).

Phylogenetic analyses

In total, five multi-gene phylogenies were generated (Figs 1–5). With the support values of Bayesian analyses, maximum parsimony trees from each alignment are presented here. The analyses treated *Botryosphaeria* (Fig. 1), *Diplodia* (Fig. 2), *Dothiorella* (including *Spencermartinsia*, Fig. 3), *Eutiarosporella* (Fig. 4) and *Neofusicoccum* (Fig. 5). PCR amplifications of the ITS and TEF regions gave products of 0.5 and 0.4 kb, respectively.

The combined ITS and TEF data set of *Botryosphaeria* consists of 33 strains with *Macrophomina phaseolina* (CBS 227.33) as the outgroup taxon. The best scoring parsimony tree is shown in Fig. 1 and revealed two known species: *B. dothidea* and *B. auasmontanum* F.J.J. Van der Walt, Slippers & G.J. Marais. The combined ITS and EF1- α dataset consisted of 824 characters (including alignment gaps). Of those, 667 were constant and 79 were variable and parsimony uninformative. Maximum parsimony analysis of the remaining 78 parsimony informative characters resulted in 199 equally parsimonious trees (CI= 0.894, RI= 0.864, RC= 0.772, HI= 0.106). Alignment and trees were deposited in TreeBASE (S21254). The phylogenetic analysis of *Diplodia* revealed five known species *Di. alatafructa* J.W.M. Mehl & B. Slippers, *Di. crataegicola* Dissanayake, Camporesi & K.D. Hyde, *Di. mutila*, *Di. sapinea* (Fr.) Fuckel and *Di. seriata*. The combined ITS and EF1- α dataset consisted of 839 characters (including alignment gaps) for 64 ingroup and 1 outgroup taxa. Of the 839 characters 611 were constant and 87 were variable and parsimony uninformative. Maximum parsimony analysis of the remaining 141 parsimony informative characters resulted in 398 equally parsimonious trees (CI= 0.719, RI= 0.885, RC=0.49 0.6375, HI=0. 0.281) and the best scoring first MP tree is shown in Fig. 2. Alignment and trees were deposited in TreeBASE (S21255). The *Dothiorella* phylogeny revealed four known species *Do. omnivora*, *Do. rhamnii* Wanasinghe, Bulgakov, E.B.G. Jones & K.D. Hyde, *Do. sarmentorum*, *Do. sempervirentis* Abdollahz., Zare & A.J.L. Phillips and one taxonomic novelty which is treated below. The combined ITS and EF1- α dataset consisted of 737 characters (including alignment gaps) for 74 ingroup and 1 outgroup taxa. Of these, 484 were constant and 80 were variable and parsimony uninformative. Maximum parsimony analysis of the remaining 173 parsimony informative characters resulted in 644 equally parsimonious trees (CI= 0.564, RI= 0.832, RC= 0.469, HI= 0.436) and the best scoring first MP tree is shown in Fig. 3. Alignment and trees were

deposited in TreeBASE (S21256). The *Eutiarosporella* phylogeny discovered only one known species *E. dactylidis* (Thambug., Camporesi & K.D. Hyde) Dissanayake, Camporesi & K.D. Hyde. The combined ITS and EF1- α dataset consisted of 735 characters (including alignment gaps) for 17 ingroup and 1 outgroup taxa. Of the 735 characters 601 were constant and 601 were variable and parsimony uninformative. Maximum parsimony analysis of the remaining 72 parsimony informative characters resulted in six equally parsimonious trees (CI= 0.853, RI= 0.861, RC= 0.735, HI= 0.147) and the best scoring first MP tree is shown in Fig. 4. Alignment and trees were deposited in TreeBASE (S21257). Phylogenetic analysis of *Neofusicoccum* revealed only one known species *N. parvum*. The combined ITS and EF1- α dataset consisted of 817 characters (including alignment gaps) for 55 ingroup and 1 outgroup taxa. Of these characters 600 were constant and 89 were variable and parsimony uninformative. Maximum parsimony analysis of the remaining 128 parsimony informative characters resulted in 44 equally parsimonious trees (CI= 0.717, RI= 0.878, RC= 0.629, HI= 0.283) and the best scoring first MP tree is shown in Fig. 5. Alignment and trees were deposited in TreeBASE (S21258).

Taxonomy

Dothiorella italica Dissanayake, Camporesi & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF553566; *Facesoffungi number*: FoF03426; Fig. 6

Etymology – Referring to the locality where the specimen was collected.

Holotype – MFLU 17-0290.

Saprobic on dead aerial branch of *Rosa canina* (*Rosaceae*). **Sexual morph**: Undetermined.

Asexual morph: *Conidiomata* up to 680 μm wide, globose, pycnidial, stromatic, solitary, composed of dark brown thick-walled *textura angularis*. Ostiolate. *Ostirole* up to 65 μm in height. *Conidiogenous cells* 8–15 \times 3–6 μm , lining the pycnidial cavity, holoblastic, hyaline, subcylindrical. *Conidia* 28.7–43.2 \times 13.2–17.5 μm (35.7 \times 15.6 μm , n=20) initially hyaline and aseptate becoming pigmented brown and 1-septate often while still attached to conidiogenous cell, slightly constricted at the septum, ovoid with a broadly rounded apex and truncate base.

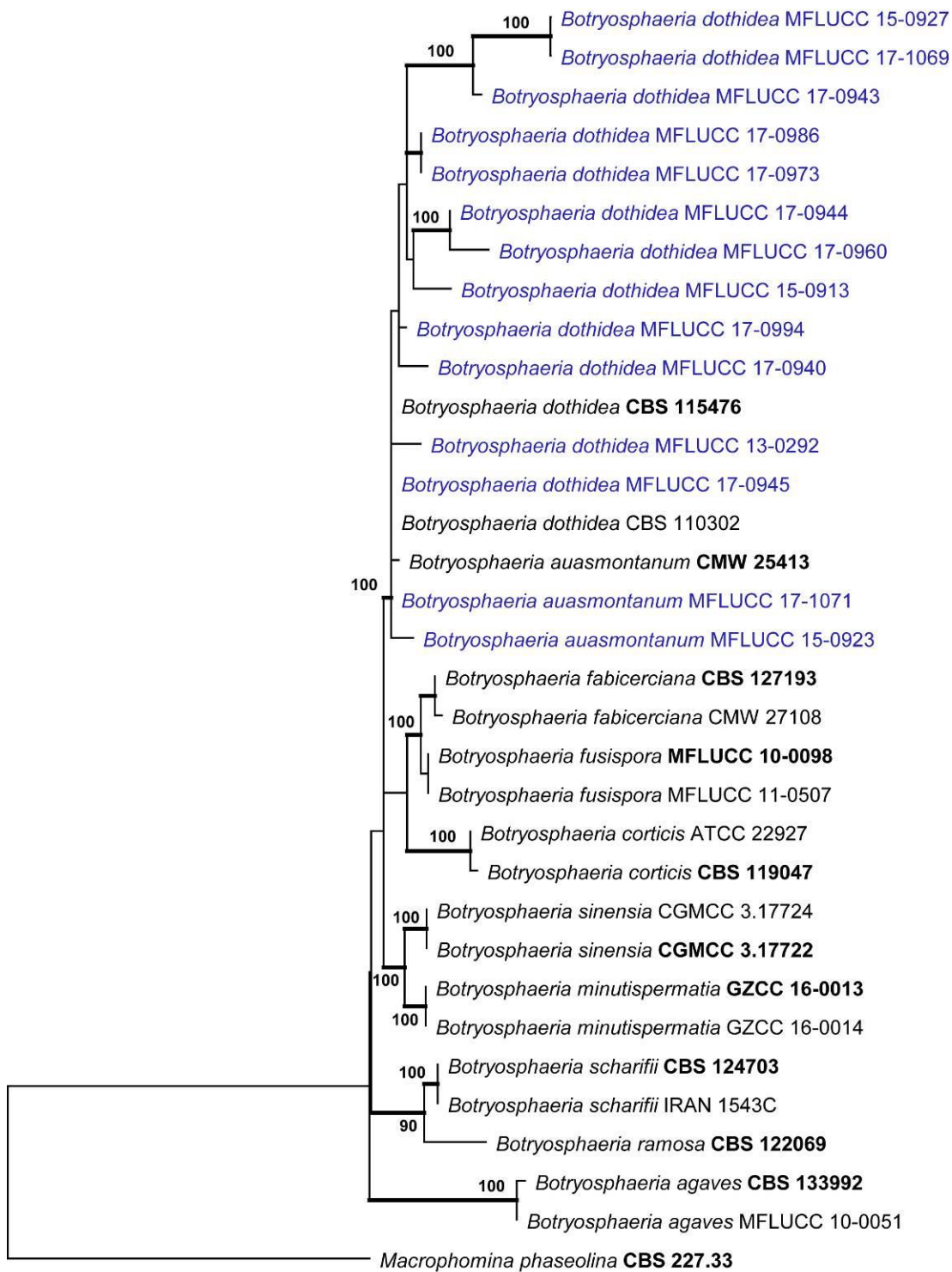
Culture characteristics – Colonies on PDA, covering entire petri dishes after 12 d in the dark at 25°C; circular, initially white, after 7 d becoming greyish brown to black; reverse grey to dark grayish green.

Material examined – ITALY. Province of Forlì-Cesena [FC], near Strada San Zeno - Galeata, on dead aerial branch of *Rosa canina* (*Rosaceae*), 17 October 2014, Erio Camporesi IT 2179 (MFLU 17-0290, holotype), ex-type living culture MFLUCC 17-0951. Details of studied additional materials are given in Table 1.

Notes – Based on multigene phylogenetic analyses, six isolates of *Do. italica* formed a clade close to *Do. prunicola* A.J.L. Phillips & Abdollahz. The novel species differs from *Do. prunicola* by 16 nucleotides in the concatenated alignment, in which five were distinct in the ITS region and eleven in the TEF region. Morphologically, the length of conidia of *Do. prunicola* are smaller (19–30.5 μm) compared to those of *Do. italica* (28.7–43.2 μm).

Discussion

Studies on *Botryosphaeriaceae*, dealing with the phylogenetic traits and morphology of isolates associated with various hosts, have increased in recent years, enabling the worldwide identification of taxa at the species level (Liu et al. 2012, Phillips et al. 2013, Dissanayake et al. 2016b). The current study provides details from sampling over the past five years which has led to characterization of 14 species in *Botryosphaeriaceae*, especially in the provinces of Forlì-Cesena, Arezzo and Perugia in Italy. Of these, *Dothiorella italica* is illustrated and described as a novel species. *Botryosphaeria auasmontanum* (Slippers et al. 2014), *Di. alatafructa* (Mehl et al. 2011) and *Do. sempervirentis* (Abdollahzadeh et al. 2014) were reported as new records for Italy. However, when considering the phylogenetic analysis of *Botryosphaeria* species (Fig. 1), *B. dothidea*/*B. auasmontanum* appear to be synonyms.



0.01

Figure 1 – Phylogram generated from maximum parsimony analysis of *Botryosphaeria* species based on combined ITS and TEF sequence data. Parsimony bootstrap support values for $MP \geq 90\%$ are indicated above the nodes and the branches are in bold indicate Bayesian posterior probabilities ≥ 0.9 . The tree is rooted with *Macrophomina phaseolina* (CBS 227.33). Isolate numbers of ex-types and reference strains are in bold. Species isolated in this study are in blue.

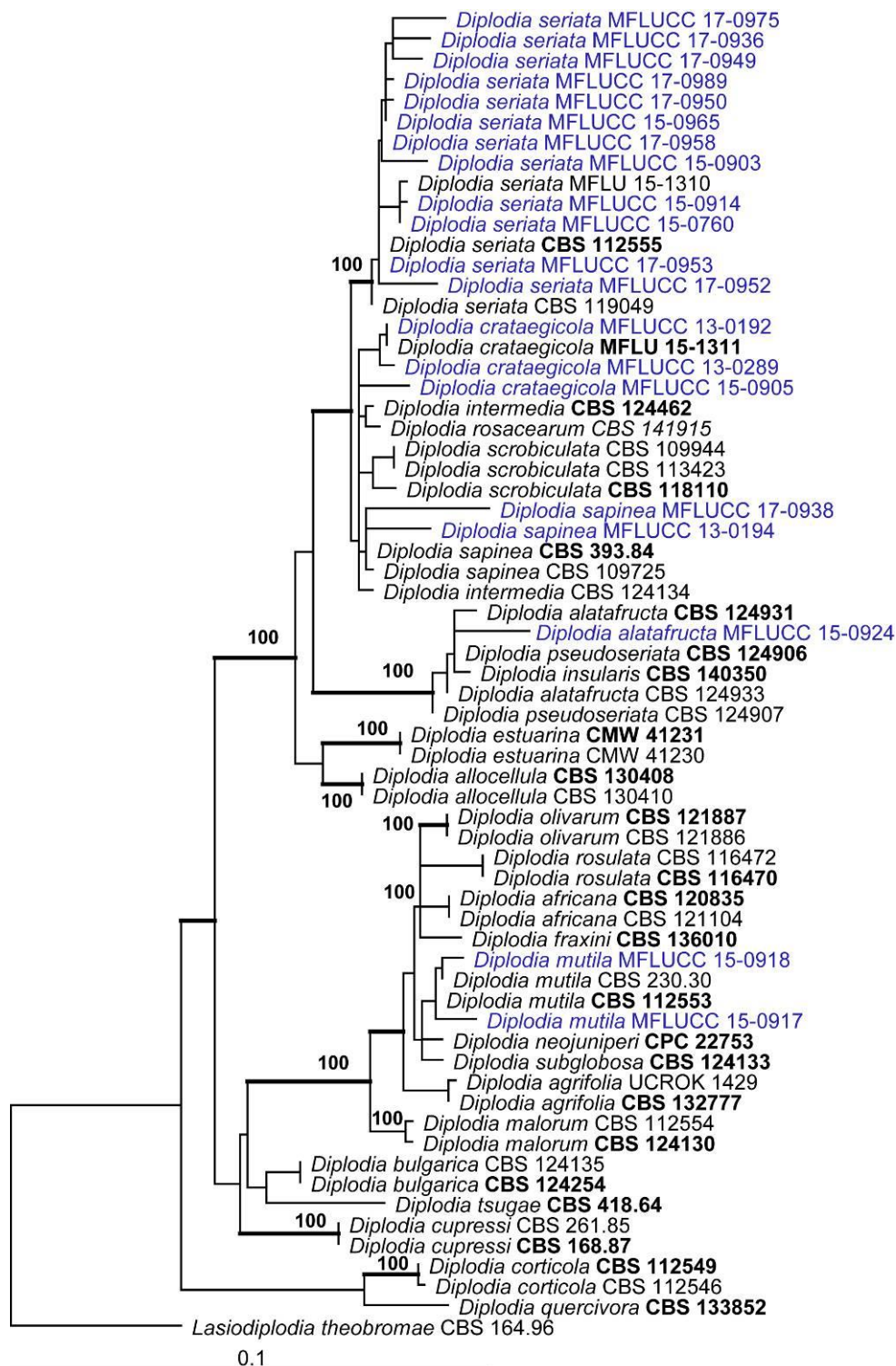


Figure 2 – Phylogram generated from maximum parsimony analysis of *Diplodia* species based on combined ITS and TEF sequence data. Parsimony bootstrap support values for MP \geq 90 % are indicated above the nodes and the branches are in bold indicate Bayesian posterior probabilities \geq 0.9. The tree is rooted with *Lasiodiplodia theobromae* (CBS 164.96). Isolate numbers of ex-types and reference strains are in bold. Species isolated in this study are in blue.

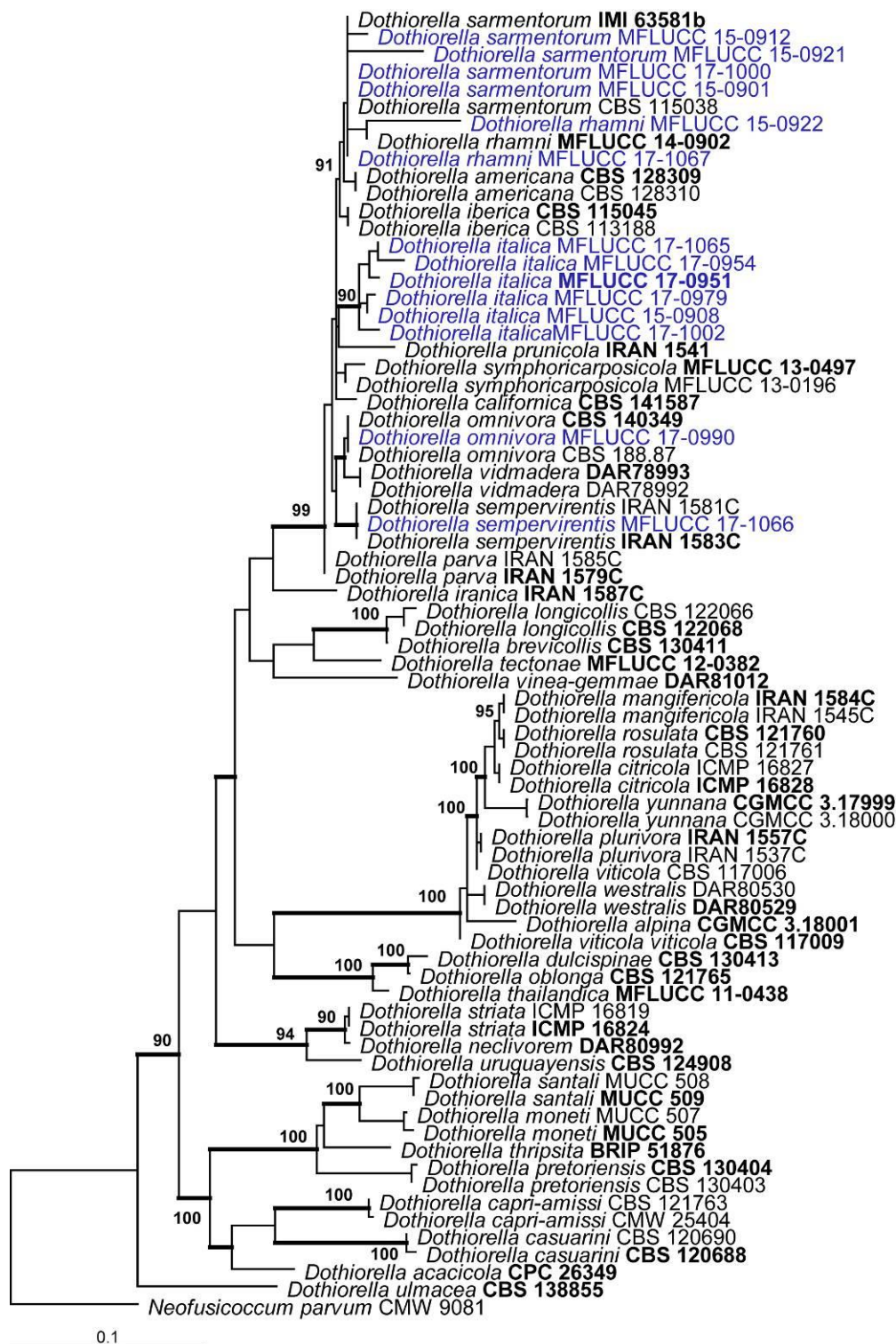


Figure 3 – Phylogram generated from maximum parsimony analysis of *Dothiorella* species based on combined ITS and TEF sequence data. Parsimony bootstrap support values for MP \geq 90 % are indicated above the nodes and the branches are in bold indicate Bayesian posterior probabilities \geq 0.9. The tree is rooted with *Neofusicoccum parvum* (CMW 9081). Isolate numbers of ex-types and reference strains are in bold. Species isolated in this study are in blue.

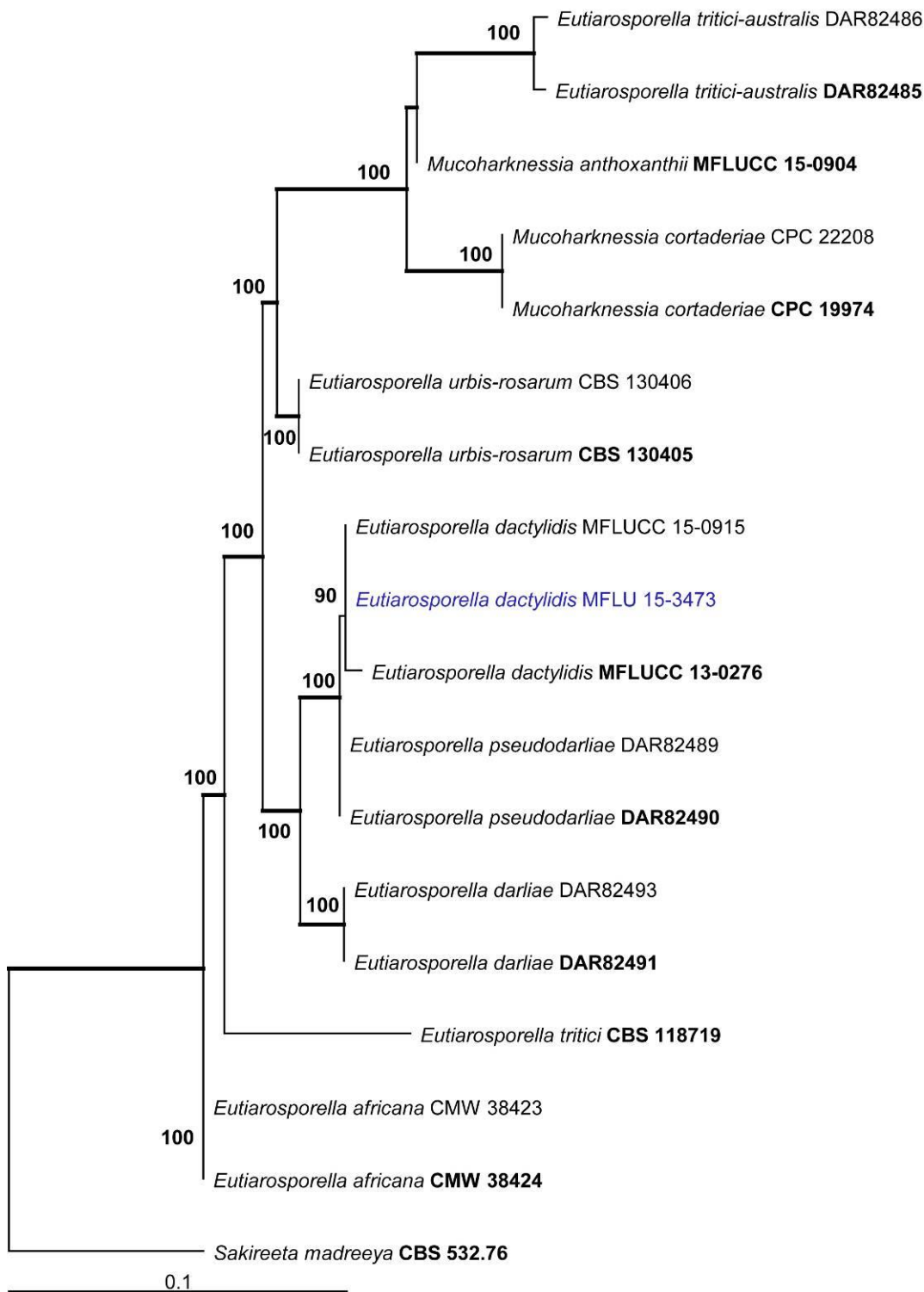


Figure 4 – Phylogram generated from maximum parsimony analysis of *Eutiarosporella* species based on combined ITS and TEF sequence data. Parsimony bootstrap support values for MP \geq 90 % are indicated above the nodes and the branches are in bold indicate Bayesian posterior probabilities \geq 0.9. The tree is rooted with *Sakireeta madreeya* (CBS 532.76). Isolate numbers of ex-types and reference strains are in bold. Species isolated in this study are in blue.

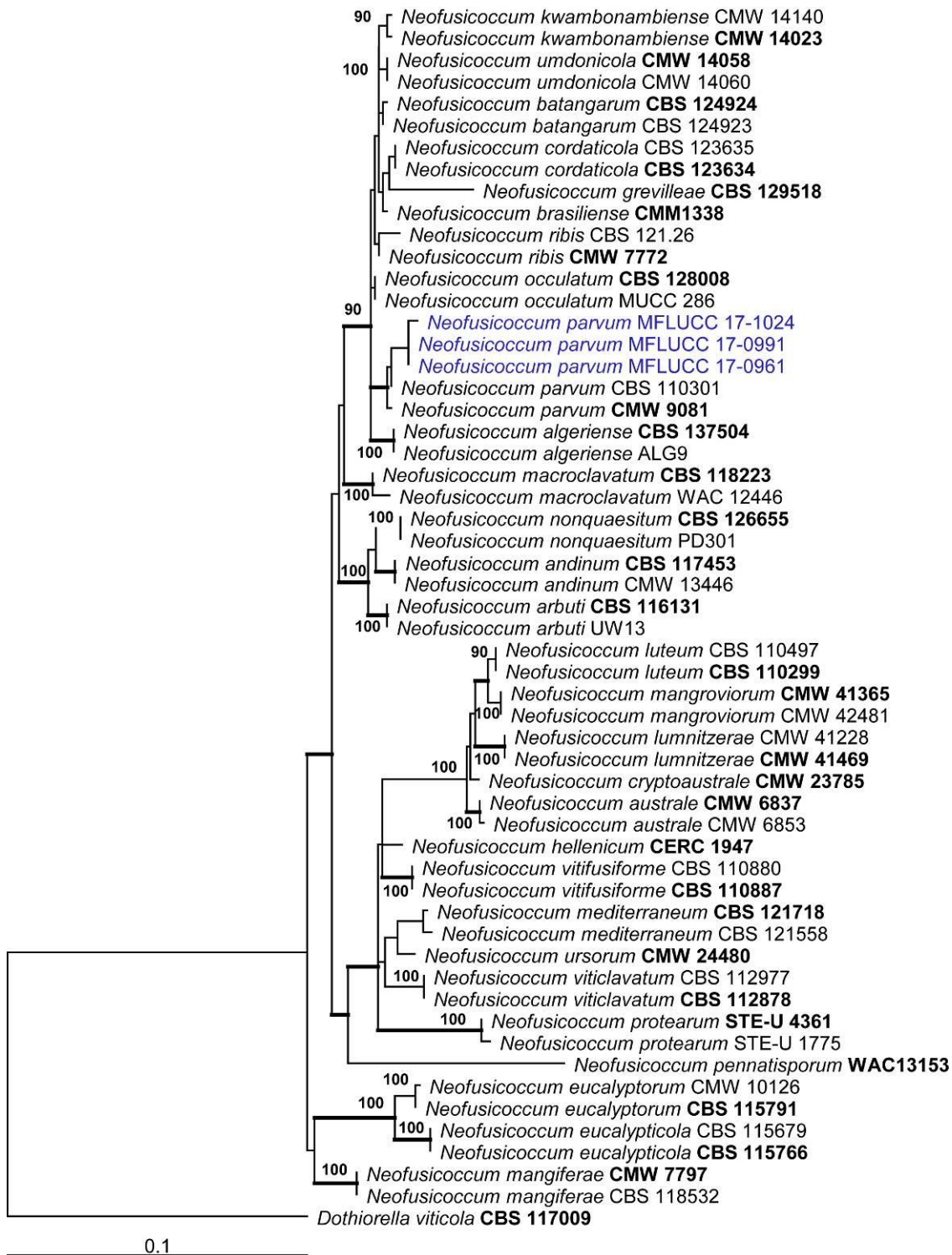


Figure 5 – Phylogram generated from maximum parsimony analysis of *Neofusicoccum* species based on combined ITS and TEF sequence data. Parsimony bootstrap support values for MP \geq 90 % are indicated above the nodes and the branches are in bold indicate Bayesian posterior probabilities \geq 0.9. The tree is rooted with *Dothiorella viticola* (CBS 117009). Isolate numbers of ex-types and reference strains are in bold. Species isolated in this study are in blue.

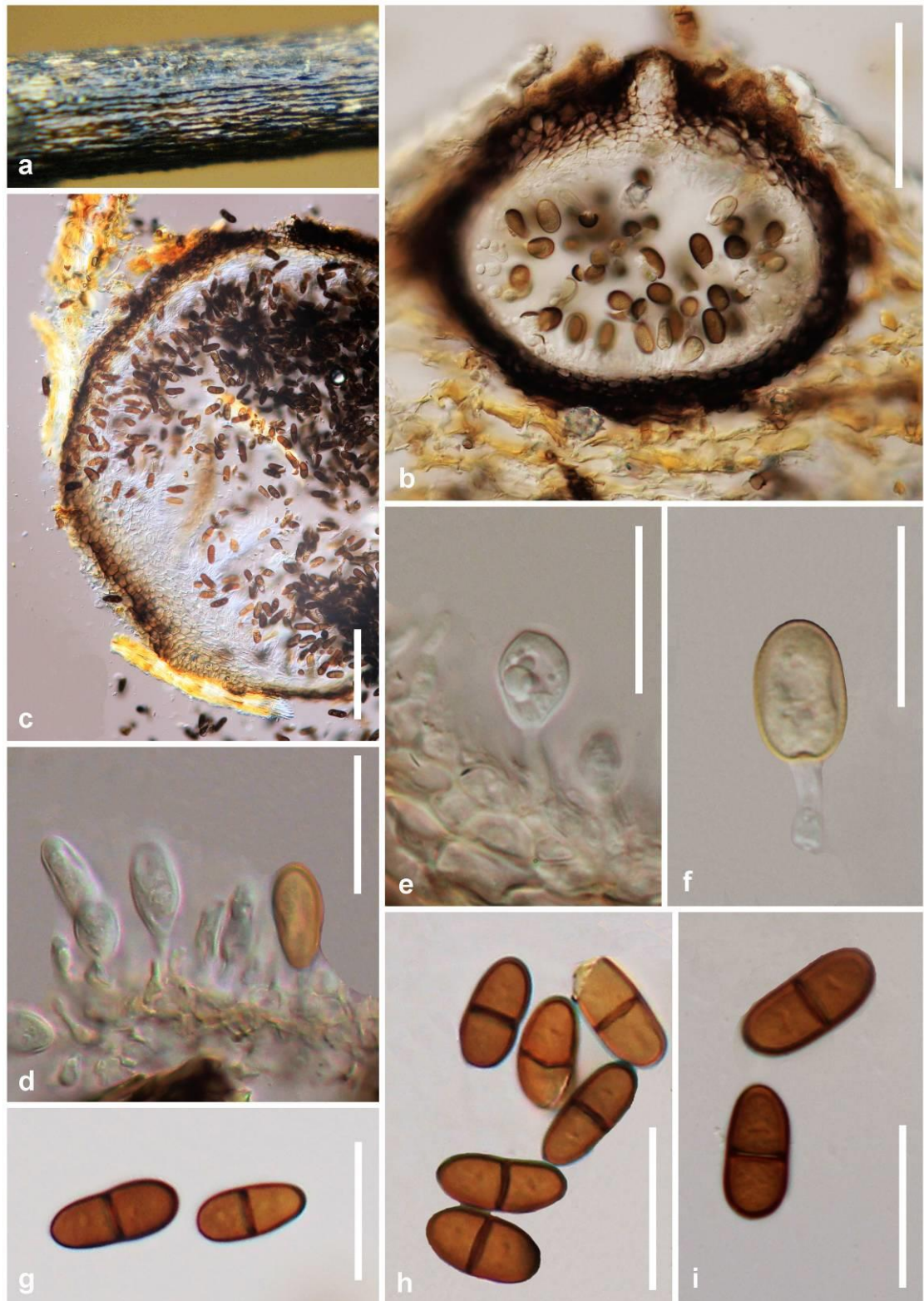


Fig. 6 *Dothiorella italica* (MFLU 17-0290). **a** – Conidiomata on host surface, **b** – Cross section of conidioma, **c** – Peridium, **d** – **e** Immature and mature conidia attached to conidiogenous cells. **g** – **i** Mature 1-septate brown conidia. Scale bars: b, c = 200 µm, d–i = 40 µm.

Hence we suggest a careful comparison of these two species morphologically and at the molecular level to clarify the possible synonymy of *B. auasmontanum* under *B. dothidea*. Ten species, *Botryosphaeria dothidea*, *Diplodia crataegicola*, *Di. mutila*, *Di. sapinea*, *Di. seriata*, *Dothiorella omnivora*, *Do. rhamnii*, *Do. sarmentorum*, *Eutiarospora dactylidis* and

Neofusicoccum parvum have already been reported from Italy (Dissanayake et al. 2016a, b). In this study, *B. dothidea* from *Galium* sp. (Rubiaceae), *Carpinus betulus* (Betulaceae), *Laburnum anagyroides* (Fabaceae), *Colutea arborescens* (Fabaceae) and *Euonymus europaeus* (Celastraceae); *Di. crataegicola* from *Prunus* sp. (Rosaceae) and *Tilia* sp. (Malvaceae); *Di. mutila* from *Acer negundo* (Sapindaceae) and *Colutea arborescens* (Fabaceae); *Do. omnivora* from *Cornus sanguinea* (Cornaceae); *Do. rhamni* from *Tamarix gallica* (Tamaricaceae); *Do. sarmentorum* from *Clematis vitalba* (Ranunculaceae) and *Robinia pseudacacia* (Fabaceae); *N. parvum* from *Eupatorium cannabinum* (Asteraceae) are described as new host records based on SMMML Fungus-Host Distribution Database. The gene regions commonly used to infer phylogenetic relationships within species in the *Botryosphaeriaceae* (ITS and TEF) made it potential to identify species with confidence.

Botryosphaeriaceae species in host plants in Italy vary in their abundance, distribution and host association (Giambra et al. 2016, Linaldeddu et al. 2016a). *Botryosphaeria* and *Diplodia* were the most frequently isolated genera in this study and this inference is consistent with several previous reports (Lazzizera et al. 2008, Linaldeddu et al. 2014, 2015, Giambra et al. 2016, Marsberg et al. 2017), which show the capability of species in these genera to occupy a wide range of plant species and geographic areas worldwide. Some *Diplodia* species have wide host ranges, for example *Di. seriata* which has been recorded on more than 250 hosts (Phillips et al. 2013, Dissanayake et al. 2016b). *Botryosphaeria dothidea* is one of the most common species on a large number of hosts and has been reported from hundreds of plant species with a broad global distribution (Phillips et al. 2013, Dissanayake et al. 2016b). Our study has confirmed this, by isolating *B. dothidea* from eleven host plant species (Table 1). *Dothiorella* species are endophytes, pathogens and saprobes of a wide range of woody hosts (Crous et al. 2006, Liu et al. 2012, Phillips et al. 2013, Hyde et al. 2013, 2014, Dissanayake et al. 2016a,). Although there are 390 species records for *Dothiorella* in Index fungorum (accessed in June 2017, <http://www.indexfungorum.org>), Dissanayake et al. (2016b) revealed that cultures are available for only 30 species. Among the species in this genus, *Do. sarmentorum* has a worldwide distribution associated with a variety of hosts (Gonzalez-Dominguez et al. 2016, Zlatkovic´ et al. 2016). Our study revealed four *Do. sarmentorum* isolates from four different hosts. The newly described *Do. italica* species was isolated from Forlì-Cesena and Perugia provinces in Italy and all isolates were recovered from dead aerial branches of *Cupressus* sp. (Cupressaceae), *Ligustrum* sp. (Oleaceae), *Melia azedarach* (Meliaceae), *Prunus* sp. (Rosaceae), *Rosa canina* (Rosaceae) and *Rubus* sp. (Rosaceae). The isolation of this novel species from different host plants implies its wide distribution and indistinct host association. *Diplodia sapinea* is a well-known pathogen of *Pinus* spp. worldwide (Phillips et al. 2013). Two isolates of *Di. sapinea* obtained in this study from *Picea excelsa* (Pinaceae) and *Pinus pinaster* (Pinaceae) confirmed that, this species is more specific in Pinaceae hosts. *Neofusicoccum parvum* is a common and cosmopolitan species on diverse host plants and recognized as an aggressive pathogen of *Actinidia* sp., *Juglans regia*, *Prunus laurocerasus*, *Vaccinium* sp. and *Vitis vinifera* (Yan et al. 2013, Boyzo-Marin et al. 2016, Yu et al. 2016, Zlatkovic et al. 2016, Diaz et al. 2017). Our study revealed three saprobic *N. parvum* isolates from *Eupatorium cannabinum* (Asteraceae), *Salix* sp. (Salicaceae) and *Vitis vinifera* (Vitaceae).

Members of Botryosphaeriaceae family represent a growing threat to agricultural crops, urban and natural forest ecosystems in Italy (Linaldeddu et al. 2014, 2015, 2016a). This finding raises questions about the origin, introduction and pathway of these fungi as well as underlining the need to develop suitable actions to limit their further spread.

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