***Dothiorella* species associated with woody hosts in Italy**

Dissanayake AJ^{1,2}, Camporesi E³, Hyde KD^{2,4}, Phillips AJL⁵, Fu CY¹, Yan JY¹ and Li XH^{1,*}

¹ Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, People's Republic of China

² Center of Excellence in Fungal Research, School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand

³ A.M.B. Gruppo Micologico Forlivese "Antonio Cicognani", Via Roma 18, Forlì, Italy

⁴ Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People's Republic of China

⁵ UCIBIO-REQUIMTE, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal

* Corresponding author: Xinghong Li (lixinghong1962@163.com)

Dissanayake AJ, Camporesi E, Hyde KD, Phillips AJL, Fu CY, Yan JY, Li XH 2016 – *Dothiorella* species associated with woody hosts in Italy. Mycosphere 7(1), 51–63, Doi 10.5943/mycosphere/7/1/6

Abstract

Dothiorella species are pathogens of many woody hosts but their host distribution is poorly known. In this study, a collection of *Dothiorella* isolates obtained from different woody hosts, including *Acer*, *Cornus*, *Coronilla*, *Crataegus*, *Euonymus*, *Fraxinus*, *Laburnum*, *Laurus*, *Paliurus*, *Prunus*, *Salix*, *Sambucus* and *Ulmus* species in Italy were identified. Morphological and cultural characteristics, as well as ITS and EF1- α sequence data, were used to identify the isolates. The results show the diversity of *Dothiorella* species associated with twigs and branches of these woody hosts. Four species were identified. *Dothiorella sarmentorum* was the dominant species (65% of isolates), followed by *D. symphoricarposicola* (20%), *D. iberica* (8%) and *D. vidmadera* (8%). A host-fungus index for all *Dothiorella* species with available molecular data from the literature is provided.

Key words – *Botryosphaeriaceae* – EF1- α – Hosts – ITS – Multigene phylogeny

Introduction

Dothiorella belongs in the family *Botryosphaeriaceae*, order *Botryosphaeriales*, and class Dothideomycetes (Wijayawardene et al. 2014) and has been the subject of several recent publications (Adesemoye et al. 2014, Chen et al. 2014, Li et al. 2014, Liu et al. 2012, Lynch et al. 2014, Pitt et al. 2015). Species of *Dothiorella*, like other members of this family, are pathogens, endophytes and saprobes of a wide range of woody hosts (Crous et al. 2006, Hyde et al. 2013, 2014, Liu et al. 2012, Phillips et al. 2013); however, it is not clear whether species have narrow or wide host distributions. The genus encompassed a wide range of morphologies from thin-walled, hyaline, aseptate conidia to thick-walled, brown, 1-septate conidia (Phillips et al. 2005). Sivanesan (1984) confused the taxonomy of *Dothiorella* by treating *Dothiorella pyrenophora* Sacc. (1880) as a synonym of *Dothichiza sorbi* Lib., which has small, hyaline, aseptate conidia and is the asexual morph of *Dothiora pyrenophora* (Fr.) Fr. However, Sutton (1977) clarified the taxonomic history

Submitted 29 December 2015, Accepted 18 February 2016, Published online 23 February 2016

Corresponding author: Xinghong Li – e-mail– lixinghong1962@163.com

by placing *Dothiora pyrenophora* as a homonym of *Dothiorella pyrenophora*. Crous & Palm (1999) studied the holotype of *D. pyrenophora* and concluded that *Dothiorella* is a synonym of *Diplodia*. Based on morphology and molecular data, Phillips et al. (2005) revived *Dothiorella* for species with conidia that become brown and 1-septate, while they are still attached to the conidiogenous cells. Sexual morphs of *Dothiorella* have pigmented, 1-septate ascospores (Phillips et al. 2005, 2013). With the exception of *D. sarmentorum* (Fr.) Phillips, Alves & Luque and *D. iberica* Phillips, Alves & Luque, the sexual morphs of *Dothiorella* species are rarely encountered in nature and have never been produced in culture (Phillips et al. 2013).

Although there are 350 species records in *Dothiorella*, Phillips et al. (2013) revealed that cultures are available for only 17 species and of those four species have yet to be named. Abdollahzadeh et al. (2014) introduced three species names for these un-named taxa i.e. *D. parva* Abdollahz., Zare & A.J.L Phillips, *D. prunicola* A.J.L. Phillips & Abdollahz. and *D. striata* A.J.L. Phillips & Abdollahz. Presently, 23 species are accepted in the genus (Abdollahzadeh et al. 2014, Li et al. 2014, Phillips et al. 2013, Pitt et al. 2013, 2015, Slippers et al. 2014). All species, except *D. sarmentorum*, have been introduced since 2005.

A collection of *Dothiorella* isolates was obtained from branches of various woody hosts in Forlì-Cesena Province, in Italy. The aim of this study was to name these species and reveal the distribution of species on the hosts. Isolates were characterised in terms of morphology and their phylogenetic position in *Dothiorella*. A host index for *Dothiorella* species based on literature records with molecular identifications is provided.

Material and methods

Sample collection, specimen examination and isolations

Specimens were collected from woody branches of *Acer* L., *Cornus* L., *Coronilla* L., *Crataegus* L., *Euonymus* L., *Fraxinus* L., *Laburnum* Fabr., *Laurus* L., *Paliurus* Mill., *Prunus* L., *Salix* L., *Sambucus* L. and *Ulmus* L. species in Italy. Specimens were observed and examined with a Motic SMZ 168 stereomicroscope. Micro-morphological characters were determined with a Nikon ECLIPSE 80i compound microscope and images were captured with a Canon EOS 550D digital camera. 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. Single spore isolations were prepared following the method of Chomnunti et al. (2014). Spore germination on water agar (WA) was examined after 24 h and germinating spores were transferred to potato dextrose agar (PDA) media. Cultures were incubated at 18°C in the dark and cultural characteristics such as mycelium colour, shape, texture and growth rate were determined. Herbarium specimens are deposited in the Mae Fah Luang University Herbarium (MFLU) while, ex-type cultures are deposited at the Mae Fah Luang University Culture Collection (MFLUCC) with duplicates in the Kunming culture collection, China (KUMCC) and GuiZhou Culture Collection, China (GZCC).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from 10 day-old pure cultures grown on PDA, following a modified protocol of Armaleo & Clerc (1995). Amplification and sequencing of the internal transcribed spacer (ITS) region and part of the translation elongation factor (EF1- α) gene were performed as described by Dissanayake et al. (2015) using primer pairs ITS1 and ITS4 (White et al. 1990) and EF1-728F and EF1-986R (Carbone & Kohn 1999), respectively.

Phylogenetic analyses

All new sequences generated in this study were checked manually and nucleotides at ambiguous positions clarified with sequences from both strands. All reference sequences were obtained from GenBank based on previously published study by (Hyde et al. 2014) as listed in Table 1. Combined datasets of ITS and EF genes were aligned using MAFFT v.7 (Kato & Toh

2008, <http://mafft.cbrc.jp/alignment/server/>). The alignment was checked and improved manually where necessary using BioEdit (Hall 2006). Maximum-parsimony (MP) analysis was performed using PAUP v. 4.0b10 (Swofford 2003) to obtain the most parsimonious tree. Ambiguously aligned regions were excluded and 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).

Results

DNA phylogeny

The combined ITS and EF1- α dataset consisted of 727 characters (including alignment gaps) for 37 ingroup and 1 outgroup taxa. Of the 727 characters 480 were constant and 56 were variable and parsimony uninformative. Maximum parsimony analysis of the remaining 91 parsimony-informative characters resulted in 309 equally parsimonious trees (CI=0.673, RI=0.735, RC=0.495, HI=0.327) and the best scoring first MP tree is shown in Fig. 1. The *Dothiorella* isolates studied were distributed into four sub-clades in the phylogenetic analysis (Fig. 1). The three isolates from *Laburnum alpinum*, *Laurus nobili* and *Sambucus nigra* were identified as *Dothiorella symphoricarposicola* (Fig. 3); one isolate from *Acer opalus* was identified as *Dothiorella iberica* and a set of isolates from *Cornus sanguinea*, *Coronilla emerus*, *Crataegus* sp., *Euonymus europaeus*, *Paliurus spina-christi*, *Prunus dulcis*, *Salix* sp. and *Ulmus minor* clustered in a larger group identified as *D. sarmentorum* (Fig. 2). The remaining clade containing isolates from *Fraxinus ornus* represents *Dothiorella vidmadera*.

Discussion

In this study *Dothiorella* isolates obtained from different woody hosts, including *Acer*, *Cornus*, *Coronilla*, *Crataegus*, *Euonymus*, *Fraxinus*, *Laburnum*, *Laurus*, *Paliurus*, *Prunus*, *Salix*, *Sambucus* and *Ulmus* species in Italy were identified and provide additional information on the distribution of *Dothiorella* species on various hosts. The 23 *Dothiorella* species along with their known hosts, host families, orders and their distribution are presented in Table 2. *Dothiorella iberica* has been isolated from 12 woody hosts world-wide (Phillips et al. 2005, 2008, Lynch et al. 2013, 2014, Luque et al. 2005, Burgess et al. 2006, Zhou et al. 2010, Wet et al. 2009); and this study provides the first report of *D. iberica* isolated from *Acer opalus* (Table 2). Phillips et al. (2013) reported that *Dothiorella sarmentorum* is a cosmopolitan species and has been isolated from 34 different host species including *Malus*, *Menispermum*, *Prunus*, *Pyrus* and *Ulmus*. However, molecular studies have proven *Dothiorella sarmentorum* is described in only 17 woody hosts. Our study reveals the isolation of *D. sarmentorum* from five woody hosts in Italy and this is the first report of *D. sarmentorum* from these woody hosts (Table 2). An additional three isolates

Fig. 1 – One of 309 most parsimonious trees obtained from combined ITS and EF-1 α sequence data, for all ex-types from species in *Dothiorella*. Maximum parsimony bootstrap values (>70%) and Bayesian inference values (>0.9) are given on the nodes. The tree is rooted to *Spencermartinsia viticola* (CBS 117009).

Fig. 2 – *Dothiorella sarmentorum*. a, b - Conidiomata on host substrate, c – Cross section of conidioma, d – Peridium, e, f – Immature conidia attached to conidiogenous cells, g – Mature conidia attached to conidiogenous cells, h, i – Immature hyaline conidia, j, k – Mature conidia, l – Germinating conidia. Scale bars: c=100, d=50, e-l=20 µm.

Fig. 3 – *Dothiorella symphoricarposicola*. **a, b** - Conidiomata on host substrate, **c** – Cross section of conidioma, **d** – Ostiole, **e** – Immature/mature conidia attached to conidiogenous cells, **f** – Mature conidia, **g, h** – Immature hyaline conidia attached to conidiogenous cells, **i** – mature conidia attached to conidiogenous cell, **j, k** – Immature hyaline conidia, **l, m** – Mature conidia, **n** – Germinating conidia. Scale bars: c=100, d=50, e–f=40, g–n=20 μ m.

Table 1 *Dothiorella* isolates included in the phylogenetic analysis. Newly deposited sequences in GenBank are italicized

Species	Strain	Host	Locality	Collector	GenBank Accession numbers	
					ITS	EF 1- α
<i>Dothiorella americana</i>	CBS 128309	<i>Vitis vinifera</i>	Missouri, United States	K. Striegler & G.M. Leavitt	HQ288218	HQ288262
<i>D. brevicollis</i>	CBS 130411	<i>Acacia karroo</i>	Pretoria, South Africa	F. Jami & M. Gryzenhout	JQ239403	JQ239390
<i>D. casuarinae</i>	CBS 120688	<i>Casuarina</i> sp.	Canberra, Australia	M.J. Wingfield	DQ846773	DQ875331
<i>D. dulcispinae</i>	CBS 130413	<i>Acacia karroo</i>	Pretoria, South Africa	F. Jami & M. Gryzenhout	JQ239400	JQ239387
<i>D. iberica</i>	CBS 115041	<i>Quercus ilex</i>	Aragón, Spain	J. Luque	AY573202	AY573222
<i>D. iberica</i>	MFLUCC 15-0761	<i>Acer opalus</i>	Forlì-Cesena, Italy	E. Camporesi	<i>KU234781</i>	<i>KU234795</i>
<i>D. iranica</i>	IRAN1587C	<i>Olea europaea</i>	Golestan, Iran	A. Javadi	KC898231	KC898214
<i>D. longicollis</i>	CBS 122068	<i>Lysiphyllum cunninghamii</i>	Tunnel Creek, Australia	T.I. Burgess	EU144054	EU144069
<i>D. moneti</i>	MUCC 505	<i>Acacia rostellifera</i>	Yalgorup, Australia	K.M. Taylor	EF591920	EF591971
<i>D. neclivorem</i>	DAR80992	<i>Vitis vinifera</i>	Pokolbin, Australia	N. Wunderlich	KJ573643	KJ573640
<i>D. parva</i>	IRAN1579C	<i>Corylus avellana</i>	Ardabil, Iran	J. Abdollahzadeh & A. Javadi	KC898234	KC898217
<i>D. pretoriensis</i>	CBS 130404	<i>Acacia karroo</i>	Pretoria, South Africa	F. Jami & M. Gryzenhout	JQ239405	JQ239392
<i>D. prunicola</i>	IRAN1541	<i>Prunus dulcis</i>	Algarve, Portugal	E. Diogo	EU673313	EU673280
<i>D. santali</i>	MUCC 509	<i>Santalum acuminatum</i>	Yalgorup, Australia	K.M. Taylor	EF591924	EF591975
<i>D. sarmentorum</i>	IMI 63581b	<i>Ulmus</i> sp.	Warwickshire, England	E.A. Ellis	AY573212	AY573235
<i>D. sarmentorum</i>	MFLUCC 15-0760	<i>Cornus sanguinea</i>	Forlì-Cesena, Italy	E. Camporesi	<i>KU234779</i>	<i>KU234793</i>
<i>D. sarmentorum</i>	MFLUCC 13-0241	<i>Salix</i> sp.	Forlì-Cesena, Italy	E. Camporesi	<i>KU234780</i>	<i>KU234794</i>
<i>D. sarmentorum</i>	MFLUCC 13-0249	<i>Ulmus minor</i>	Forlì-Cesena, Italy	E. Camporesi	<i>KU234783</i>	<i>KU234797</i>
<i>D. sarmentorum</i>	MFLUCC 13-0250	<i>Crataegus</i> sp.	Forlì-Cesena, Italy	E. Camporesi	<i>KU234784</i>	<i>KU234798</i>
<i>D. sarmentorum</i>	MFLUCC 13-0251	<i>Euonymus europaeus</i>	Forlì-Cesena, Italy	E. Camporesi	<i>KU234785</i>	<i>KU234799</i>
<i>D. sarmentorum</i>	MFLUCC 13-0252	<i>Crataegus</i> sp.	Forlì-Cesena, Italy	E. Camporesi	<i>KU234786</i>	<i>KU234800</i>
<i>D. sarmentorum</i>	MFLUCC 13-0287	<i>Paliurus spina-christi</i>	Forlì-Cesena, Italy	E. Camporesi	<i>KU234787</i>	<i>KU234801</i>
<i>D. sarmentorum</i>	MFLUCC 13-0288	<i>Prunus dulcis</i>	Forlì-Cesena, Italy	E. Camporesi	<i>KU234788</i>	<i>KU234802</i>
<i>D. sarmentorum</i>	MFLUCC 13-0290	<i>Coronilla emerus</i>	Forlì-Cesena, Italy	E. Camporesi	<i>KU234789</i>	<i>KU234803</i>
<i>D. sempervirentis</i>	IRAN1583C	<i>Cupressus sempervirens</i>	Golestan, Iran	M.A. Aghajani	KC898236	KC898219
<i>D. striata</i>	ICMP16824	<i>Citrus sinensis</i>	Kerikeri, New Zealand	P.R. Johnston	EU673320	EU673287
<i>D. symphoricarposicola</i>	MFULCC 13-0497	<i>Symphoricarpos</i> sp.	Forlì-Cesena, Italy	E. Camporesi	KJ742378	KJ742381
<i>D. symphoricarposicola</i>	MFLUCC 13-0196	<i>Sambucus nigra</i>	Forlì-Cesena, Italy	E. Camporesi	<i>KU234782</i>	<i>KU234796</i>
<i>D. symphoricarposicola</i>	MFLUCC 15-0762	<i>Laurus nobilis</i>	Forlì-Cesena, Italy	E. Camporesi	<i>KU234790</i>	<i>KU234804</i>
<i>D. symphoricarposicola</i>	MFLUCC 15-0758	<i>Laburnum alpinum</i>	Forlì-Cesena, Italy	E. Camporesi	<i>KU234791</i>	<i>KU234805</i>
<i>D. thailandica</i>	CBS 133991	<i>Bambusa</i> sp.	Chang Rai, Thailand	D.Q. Dai	JX646796	JX646796
<i>D. thripsita</i>	BRIP 51876	<i>Acacia harpophylla</i>	Tallegalla, Australia	D.J. Tree & C.E.C. Tree	FJ824738	KJ573639
<i>D. ulmacea</i>	CBS 138855	<i>Ulmus leavis</i>	Germany	R.K. Schumacher	KR611881	KR611910
<i>D. uruguayensis</i>	CBS 124908	<i>Hexachlamis edulis</i>	Paysandu, Uruguay	C.A. Pérez	EU080923	EU863180
<i>D. vidmadera</i>	DAR78992	<i>Vitis vinifera</i>	Eden Valley, Australia	W.M. Pitt & A. Loschiavo	EU768874	EU768881
<i>D. vidmadera</i>	MFLUCC15-0759	<i>Fraxinus ornus</i>	Forlì-Cesena, Italy	E. Camporesi	<i>KU234792</i>	<i>KU234806</i>
<i>D. vinea-gemmae</i>	DAR81012	<i>Vitis vinifera</i>	Pokolbin, Australia	N. Wunderlich	KJ573644	KJ573641
<i>Spencermartinsia viticola</i>	CBS 117009	<i>Vitis vinifera</i>	Spain	-	KF766228	AY905559

Table 2 Distribution of *Dothiorella* species on host plants. Details of *Dothiorella* species studied in this study are in bold. Species distribution data is used only if identifications are supported by molecular data and are post-2005.

<i>Dothiorella</i> species	Host	Family	Order	Distribution	References
<i>Dothiorella americana</i>	<i>Vitis vinifera</i>	Vitaceae	Vitales	USA	Úrbez-Torres et al. 2012
<i>D. brevicollis</i>	<i>Acacia karroo</i>	Fabaceae	Fabales	South Africa	Jami et al. 2012
<i>D. casuarinae</i>	<i>Casuarina</i> sp.	Casuarinaceae	Fagales	Australia	Wet et al. 2009
<i>D. dulcispinae</i>	<i>Acacia karroo</i>	Fabaceae	Fabales	South Africa	Jami et al. 2012
<i>D. iberica</i>	<i>Acer opalus</i>	Sapindaceae	Sapindales	Italy	This study
	<i>Citrus sinensis</i>	Rutaceae	Sapindales	California	Adesemoye et al. 2014
	<i>Cupressus macrocarpa</i>	Cupressaceae	Pinales	Algeria	Azouaoui-Idjer et al. 2012
	<i>Juglans regia</i>	Juglandaceae	Fagales	California	Chen et al. 2014
	<i>Juniperus</i> sp.	Cupressaceae	Pinales	Portugal	Alves et al. 2013
	<i>Malus pumila</i>	Rosaceae	Rosales	Spain	Phillips et al. 2005
	<i>Olea europaea</i>	Oleaceae	Lamiales	California	Úrbez-Torres et al. 2013
	<i>Ostrya</i> sp.	Betulaceae	Fagales	Italy	Pavlic-Zupanc et al. 2015
	<i>Persea americana</i>	Lauraceae	Laurales	California	Mcdonald & Eskalen 2011, Twizeyimana et al. 2013
	<i>Pistacia</i> sp.	Anacardiaceae	Sapindales	California	Chen et al. 2014
	<i>Prunus dulcis</i>	Rosaceae	Rosales	California	Doll et al. 2015
	<i>Quercus agrifolia</i>	Fagaceae	Fagales	California	Lynch et al. 2014
	<i>Quercus ilex</i>	Fagaceae	Fagales	Spain	Phillips et al. 2005, 2008, Lynch et al. 2013, 2015, Luque et al. 2005, Burgess et al. 2006, Liu et al. 2012, Zhou et al. 2010, Wet et al. 2009
	<i>Vitis vinifera</i>	Vitaceae	Vitales	Australia, California, New Zealand, California	Úrbez-Torres et al. 2007, Qiu et al. 2011, Baskaratevan et al. 2012, Pitt et al. 2013, 2015, Lynch et al. 2013
<i>D. iranica</i>	<i>Olea europaea</i>	Oleaceae	Lamiales	Iran	Abdollahzadeh et al. 2014
<i>D. longicollis</i>	<i>Lysiphyllum cunninghamii</i>	Fabaceae	Fabales	Australia	Pavlic et al. 2008
<i>D. moneti</i>	<i>Acacia rostellifera</i>	Fabaceae	Fabales	Australia	Taylor et al. 2009
<i>D. neclivorem</i>	<i>Vitis vinifera</i>	Vitaceae	Vitales	Australia	Pitt et al. 2015
<i>D. parva</i>	<i>Corylus avellana</i> ,	Betulaceae	Fagales	Iran, Slovenia and Italy	Abdollahzadeh et al. 2014,
	<i>Ostrya carpinifolia</i>	Betulaceae	Fagales	Italy	Pavlic-Zupanc et al. 2015
<i>D. pretoriensis</i>	<i>Acacia karroo</i>	Fabaceae	Fabales	South Africa	Jami et al. 2012
<i>D. prunicola</i>	<i>Prunus dulcis</i>	Rosaceae	Rosales	Portugal	Abdollahzadeh et al. 2014
<i>D. santali</i>	<i>Santalum acuminatum</i>	Santalaceae	Santalales	Australia	Taylor et al. 2009
<i>D. sarmentorum</i>	<i>Acer platanoides</i>	Sapindaceae	Sapindales	Poland	Mulenko et al. 2008
	<i>Armeniaca vulgaris</i>	Rosaceae	Rosales	Ukraine	Dudka et al. 2004
	<i>Cornus sanguinea</i>	Cornaceae	Cornales	Italy	Li et al. 2014
	<i>Cornus sanguinea</i>	Cornaceae	Cornales	Italy	This study
	<i>Coronilla emerus</i>	Fabaceae	Fabales	Italy	This study
	<i>Crataegus</i> sp	Rosaceae	Rosales	Italy	This study

<i>Dothiorella</i> species	Host	Family	Order	Distribution	References
	<i>Euonymus europaeus</i>	<i>Celastraceae</i>	<i>Celastrales</i>	Italy	This study
	<i>Malus pumila</i>	<i>Rosaceae</i>	<i>Rosales</i>	Netherlands	Phillips et al. 2008, Wet et al. 2009, Chen et al. 2014
	<i>Menispermum canadense</i>	<i>Menispermaceae</i>	<i>Ranunculales</i>	Sweden	-
	<i>Paliurus spina-christi</i>	<i>Rhamnaceae</i>	<i>Rosales</i>	Italy	This study
	<i>Persica vulgaris</i>	<i>Rosaceae</i>	<i>Rosales</i>	Ukraine	Dudka et al. 2004
	<i>Pistacia</i>	<i>Anacardiaceae</i>	<i>Sapindales</i>	California	Chen et al. 2014
	<i>Prunus americana</i>	<i>Rosaceae</i>	<i>Rosales</i>	Florida	Perez et al. 2010
	<i>Prunus domestica</i>	<i>Rosaceae</i>	<i>Rosales</i>	Poland	Mulenko et al. 2008
	<i>Prunus dulcis</i>	<i>Rosaceae</i>	<i>Rosales</i>	California	Inderbitzin 2010, Lynch et al. 2013, Gramaje et al. 2012
	<i>Prunus dulcis</i>	<i>Rosaceae</i>	<i>Rosales</i>	Italy	This study
	<i>Prunus</i> sp.	<i>Rosaceae</i>	<i>Rosales</i>	North America	Gramaje et al. 2012
	<i>Prunus spinosa</i>	<i>Rosaceae</i>	<i>Rosales</i>	Poland, Ukraine	Mulenko et al. 2008, Dudka et al. 2004
	<i>Pyrus communis</i>	<i>Rosaceae</i>	<i>Rosales</i>	Oregon, Washington	Luque et al. 2005
	<i>Quercus ilex</i>	<i>Fagaceae</i>	<i>Fagales</i>	ballota Spain	Sanchez et al. 2003
	<i>Quercus suber</i>	<i>Fagaceae</i>	<i>Fagales</i>	Spain	Sanchez et al. 2003
	<i>Salix</i> sp.	<i>Salicaceae</i>	<i>Malpighiales</i>	Italy	This study
	<i>Ulmus</i> sp.	<i>Ulmaceae</i>	<i>Rosales</i>	England	Luque et al. 2005, Phillips et al. 2005
	<i>Ulmus minor</i>	<i>Ulmaceae</i>	<i>Rosales</i>	Italy	This study
	<i>Vitis vinifera</i>	<i>Vitaceae</i>	<i>Vitales</i>	New Zealand	Baskarathevan et al. 2012
<i>D. sempervirentis</i>	<i>Cupressus sempervirens</i>	<i>Cupressaceae</i>	<i>Pinales</i>	Golestan, Iran	Abdollahzadeh et al. 2014
<i>D. striata</i>	<i>Citrus sinensis</i>	<i>Rutaceae</i>	<i>Sapindales</i>	New Zealand	Abdollahzadeh et al. 2014
<i>D. symphoricarposicola</i>	<i>Symphoricarpos</i> sp.	<i>Caprifoliaceae</i>	<i>Dipsacales</i>	Italy	Li et al. 2014
	<i>Sambucus nigra</i>	<i>Adoxacea</i>	<i>Dipsacales</i>	Italy	This study
	<i>Laurus nobilis</i>	<i>Lauraceae</i>	<i>Laurales</i>	Italy	This study
	<i>Laburnum alpinum</i>	<i>Fabaceae</i>	<i>Fabales</i>	Italy	This study
<i>D. thailandica</i>	Bamboo	<i>Poaceae</i>	<i>Poales</i>	Thailand	Liu et al. 2012
<i>D. thripsita</i>	<i>Acacia harpophylla</i>	<i>Fabaceae</i>	<i>Fabales</i>	Australia	Shivas et al. 2009
<i>D. ulmacea</i>	<i>Ulmus leavis</i>	<i>Ulmaceae</i>	<i>Rosales</i>	Germany	Crous et al. 2015
<i>D. uruguayensis</i>	<i>Hexachlamys edulis</i>	<i>Myrtaceae</i>	<i>Myrtales</i>	Uruguay	Pérez et al. 2010
<i>D. vidmadera</i>	<i>Vitis vinifera</i>	<i>Vitaceae</i>	<i>Vitales</i>	Australia	Pitt et al. 2013
	<i>Fraxinus ornus</i>	<i>Oleaceae</i>	<i>Lamiales</i>	Italy	This study
<i>D. vinea-gemmae</i>	<i>Vitis vinifera</i>	<i>Vitaceae</i>	<i>Vitales</i>	Australia	Pitt et al. 2015

representing *D. sarmentorum* from *Prunus dulcis*, *Salix* sp. and *Ulmus minor* were collected. The other known reports of *D. sarmentorum* in above mentioned genera are *Prunus dulcis* (California), *Salix* sp. (Cyprus) and *Ulmus* sp. (UK). *Dothiorella symphoricarposicola*; a novel species which was recently introduced by Li et al. (2014) was isolated from *Symphoricarpos* sp. in Italy. Another three of our isolates from *Sambucus nigra*, *Laurus nobilis* and *Laburnum alpinum* clustered with *D. symphoricarposicola* (Fig. 1, table 2). The sexual morph of *D. vidmadera* was isolated from *Fraxinus ornus*, and the asexual morph of this species was isolated from *Vitis vinifera* in Australia (Pitt et al. 2013).

Eighteen *Dothiorella* species are known from only one host genus. Based on the information available; *Dothiorella americana*, *D. brevicollis*, *D. casuarinae*, *D. dulcispinae*, *D. iranica*, *D. longicollis*, *D. moneti*, *D. neclivorem*, *D. pretoriensis*, *D. prunicola*, *D. santali*, *D. sempervirentis*, *D. striata*, *D. thailandica*, *D. thripsita*, *D. ulmacea*, *D. uruguayensis* and *D. vinea-gemmae* species are limited to single host genera (Table 2). Although many *Dothiorella* species are host specific, many more collections are needed to confirm this. *Dothiorella parva* has been isolated from two host genera in a single host family *Betulaceae*, in the order *Fagales*, whereas *D. vidmadera* have been isolated from two host families; *Vitaceae* and *Oleaceae* (Table 2). Three *Dothiorella* species are known from a wide range of host genera or families. *Dothiorella iberica*, *D. sarmentorum* and *D. symphoricarposicola* occur across many host families and orders. *Dothiorella iberica* has been isolated from 12 host genera (from 6 host families), whereas *D. sarmentorum* has been isolated from 17 host genera (from 12 host families), while *D. symphoricarposicola* has been isolated from four host genera in four different host families. The information up to date suggests that most *Dothiorella* species have narrow host ranges, while a few species have very wide host ranges. As more hosts from diverse continents are studied, we should expect many more species to be discovered in this genus.

Our study illustrates the diversity of *Dothiorella* species associated with twig and branches of woody hosts. Four species were identified. *Dothiorella sarmentorum* was the dominant species (65% of isolates), followed by *D. symphoricarposicola* (20%), *D. iberica* (8%) and *D. vidmadera* (8%). All studied *Dothiorella* species occurred on more than one host, indicating that they are not host-specific, but host-generalists.

Acknowledgement

The research was supported by Innovation Funds of IPEP, Beijing Academy of Agriculture and Forestry Sciences and CARS-30 China. We gratefully acknowledge The Mushroom Research Foundation, Chiang Rai, Thailand; K.D. Hyde thanks the Chinese Academy of Sciences, project number 2013T2S0030, for the award of Visiting Professorship for Senior International Scientists at Kunming Institute of Botany.

References

- Abdollahzadeh J, Javadi A, Zare R, Phillips AJL. 2014 – A phylogenetic study of *Dothiorella* and *Spencermartinsia* species associated with woody plants in Iran, New Zealand, Portugal and Spain. *Persoonia* 32, 1–12.
- Adesemoye AO, Mayorquin JS, Wang DH, Twizeyimana M, Lynch SC, Eskalen A. 2014 – Identification of Species of *Botryosphaeriaceae* Causing Bot Gummosis in *Citrus* in California. *Plant Disease* 98, 55–61.
- Alves A, Barradas C, Phillips AJL, Correia A. 2013 – Diversity of *Botryosphaeriaceae* species associated with conifers in Portugal. *European Journal of Plant Pathology* 135, 791–804.
- Armaleo D, Clerc P. 1995 – A rapid and inexpensive method for the purification of DNA from lichens and their symbionts. *Lichenologists* 27, 207–213.
- Azouaoui-Idjer G, Rocca GD, Pecchioli A, Bouznad Z, Danti R. 2012 – First report of *Botryosphaeria iberica* associated with dieback and tree mortality of Monterey Cypress (*Cupressus macrocarpa*) in Algeria. *Plant Disease* 96, 1073.

- Baskarathevan J, Jaspers MV, Ridgway HJ, Jones E. 2012 – Incidence and distribution of botryosphaeriaceous species in New Zealand vineyards. *European Journal of Plant Pathology* 132, 549–560.
- Burgess TI, Barber PA, Mohali S, Pegg G, de Beer W, Wingfield MJ. 2006 – Three new *Lasiodiplodia* spp. from the tropics, recognized based on DNA sequence comparisons and morphology. *Mycologia* 98, 423–435.
- Carbone I, Kohn LM. 1999 – A method for designing primer sets for speciation studies in filamentous Ascomycetes. *Mycologia* 91, 553–556.
- Chen SF, Morgan DP, Michailides TJ. 2014 – *Botryosphaeriaceae* and *Diaporthaceae* associated with panicle and shoot blight of pistachio in California, USA. *Fungal Diversity* 67, 157–179.
- Chomnunti P, Hongsanan S, Aguirre-Hudson B, Tian Q, Peršoh D, Dhami MK, Alias AS, Xu J, Liu Z, Stadler M, Hyde KD. 2014 – The sooty moulds. *Fungal Diversity* 66, 1–36.
- Crous PW, Palm ME. 1999 – Reassessment of the anamorph genera *Botryodiplodia*, *Dothiorella* and *Fusicoccum*. *Sydowia* 51, 167–175.
- Crous PW, Schumacher RK, Wingfield MJ, Lombard L, Giraldo A, Christensen M, Gardiennet A, Nakashima C, Pereira OL, Smith AJ, Groenewald JZ. 2015 – Fungal Systematics and Evolution: FUSE 1. *Sydowia* 67, 81–118.
- Crous PW, Slippers B, Wingfield MJ, Rheeder J, Marasas WFO, et al. 2006 – Phylogenetic lineages in the *Botryosphaeriaceae*. *Studies in Mycology* 55, 235–253.
- Dissanayake AJ, Zhang W, Li XH, Zhou Y, Chethana KWT, Chukeatirote E, Hyde KD, Yan JY, Zhang G, Zhao S. 2015 – First report of *Neofusicoccum mangiferae* associated with grapevine dieback in China. *Phytopathologia Mediterranea* 54, 414–419.
- Doll DA, Rolshausen PE, Pouzoulet J, Michailides TJ. 2015 – First report of *Dothiorella iberica* causing trunk and scaffold cankers of almond in California Plant Disease. 99, 1185.
- Dudka IO, Heluta VP, Tykhonenko YY, Andrianova TV, Hayova VP, Prydiuk MP, Dzhagan VV, Isikov VP. 2004 – Fungi of the Crimean Peninsula. M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, 452 pages.
- Gramaje D, Agusti-Brisach C, Perez-Sierra A, Moralejo E, Olmo D, Mostert L, Damm U, Armengol J. 2012 – Fungal trunk pathogens associated with wood decay of almond trees on Mallorca (Spain). *Persoonia* 28, 1–13.
- Hall T. 2006 – Bioedit 7.5.0.3. Department of Microbiology, North Carolina State University. <http://www.mbio.ncsu.edu/BioEdit/Bioedit.html>.
- Hillis DM, Bull JJ. 1993 – An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* 42, 182–192.
- Hyde KD, Jones EGB, Liu JK, Ariyawansa H, Boehm E, Boonmee S, Braun U, Chomnunti P, Crous PW, Dai DQ, Diederich P, Dissanayake A, Doilom M, Doveri F, Hongsanan S, Jayawardena R, Lawrey JD, Li YM, Liu YX, Lücking R, Monkai J, Muggia L, Nelsen MP, Pang KL, Phookamsak R, Senanayake IC, Shearer CA, Suetrong S, Tanaka K, Thambugala KM, Wijayawardene NN, Wikee S, Wu HX, Zhang Y, Aguirre HB, Alias SA, Aptroot A, Bahkali AH, Bezerra JL, Bhat DJ, Camporesi E, Chukeatirote E, Gueidan C, Hawksworth DL, Hirayama K, Hoog S. de, Kang JC, Knudsen K, Li WJ, Li XH, Liu ZY, Mapook A, McKenzie EHC, Miller AN, Mortimer PE, Phillips AJL, Raja HA, Scheuer C, Schumm F, Taylor JE, Tian Q, Tibpromma S, Wanasinghe DN, Wang Y, Xu JC, Yacharoen S, Yan JY, Zhang M. 2013 – Families of Dothideomycetes. *Fungal Diversity* 63, 1–313.
- Hyde KD, Nilsson RH, Alias SA, Ariyawansa HA, Blair JE, Cai L, de Cock AWAM, Dissanayake AJ, Glockling SL, Goonasekara ID, Gorczak M, Hahn M, Jayawardena RS, van Kan JAL, Laurence MH, Lévesque CA, Li XH, Liu JK, Maharachchikumbura SSN, Manamgoda DS, Martin FN, McKenzie EHC, McTaggart AR, Mortimer PE, Nair PVR, Pawłowska J, Rintoul TL, Shivas RG, Spies CFJ, Summerell BA, Taylor PWJ, Terhem RB, Udayanga D, Vaghefi N, Walther G, Wilk M, Wrzosek M, Xu JC, Yan JY, Zhou N. 2014 – One stop shop: backbone trees for important phytopathogenic genera: I. *Fungal Diversity* 67, 21–125.

- 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.
- Jami F, Slippers B, Wingfield M J. 2012 – Five new species of the *Botryosphaeriaceae* from *Acacia karroo* in South Africa. *Cryptogamie Mycologie* 33, 245–266.
- Katoh K, Toh H. 2008 – Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics* 9, 276–285.
- Li W, Liu JK, Bhat DJ, Camporesi E, Xu J, Hyde KD. 2014 – Introducing the novel species, *Dothiorella symphoricarposicola*, from snowberry in Italy. *Cryptogamie Mycologie* 35, 257–270.
- Liu JK, Phookamsak R, Mingkhuan M, Wikee S, Li YM, Ariyawansa H, Boonmee S, Chomnunti P, Dai DQ, Bhat JD, Romero AI, Zhuang WY, Monkai J, Gareth Jones EB, Chukeatirote E, Ko Ko TW, Zhao YC, Wang Y, Hyde KD. 2012 – Towards a natural classification of *Botryosphaeriales*. *Fungal Divers* 57: 149–210.
- Luque J, Martos S, Phillips AJL. 2005 – *Botryosphaeria viticola* sp. nov. on grapevines: a new species with a *Dothiorella* anamorph. *Mycologia* 97, 1111–1121.
- Lynch SC, Eskalen A, Zambino PJ, Mayorquin JS, Wang DH. 2013 – Identification and pathogenicity of *Botryosphaeriaceae* species associated with coast live oak (*Quercus agrifolia*) decline in southern California. *Mycologia* 105, 125–140.
- Lynch SC, Zambino PJ, Scott TA, Eskalen A. 2014 – Occurrence, incidence and associations among fungal pathogens and *Agrilus auroguttatus*, and their roles in *Quercus agrifolia* decline in California. *Forest Pathology* 44, 62–74.
- McDonald V, Eskalen A. 2011 – *Botryosphaeriaceae* species associated with avocado branch cankers in California. *Pl. Dis.* 95, 1465–1473.
- Mulenko W, Majewski T, Ruskiewicz-Michalska M. 2008 – A Preliminary Checklist of Micromycetes in Poland. *W. Szafer Institute of Botany, Polish Academy of Sciences* 9, 752.
- Nylander JAA. 2004 – MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Sweden.
- Page RD. 1996 – TreeView: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* 12, 357–358.
- Pavlic D, Wingfield MJ, Barber B, Slippers B, Hardy GEJ, Burgess TI. 2008 – Seven new species of the *Botryosphaeriaceae* from baobab and other native trees in Western Australia. *Mycologia* 100, 851–866.
- Pavlic-Zupanc D, Piskur B, Slippers B. 2015 – Molecular and morphological characterization of *Dothiorella* species associated with dieback of *Ostrya carpinifolia* in Slovenia and Italy. *Phytopathologia Mediterranea* 54, 222–231.
- Pérez CA, Wingfield MJ, Slippers B, Altier NA, Blanchette RA. 2010 – Endophytic and canker-associated *Botryosphaeriaceae* occurring on non-native *Eucalyptus* and native *Myrtaceae* trees in Uruguay. *Fungal Diversity* 41, 53–69.
- 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, Abdollahzadeh J, Slippers B, Wingfield MJ, Groenewald JZ, Crous PW. 2013 – The *Botryosphaeriaceae*: genera and species known from culture. *Studies in Mycology* 76, 51–167.
- 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.
- Pitt WM, Úrbez-Torres JR, Trouillas FP. 2013 – *Dothiorella vidmadera*, a novel species from grapevines in Australia and notes on *Spencermartinsia*. *Fungal Diversity* 61, 209–219.
- Pitt WM, Úrbez-Torres JR, Trouillas FP. 2015 – *Dothiorella* and *Spencermartinsia*, new species and records from grapevines in Australia. *Australasian Plant Pathology* 44, 43–56.

- Qiu Y, Steel CC, Ash GJ, Savocchia S. 2011 – Survey of *Botryosphaeriaceae* associated with grapevine decline in the Hunter Valley and Mudgee grape growing regions of New South Wales. *Australasian Plant Pathology* 40, 1–11.
- Rodríguez F, Oliver JL, Marín A, Medina JR. 1990 – The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology* 142, 485–501.
- Ronquist FR, Huelsenbeck JP. 2003 – MrBayes3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572–1574.
- Sanchez ME, Venegas J, Romero MA, Phillips AJL, Trapero A. 2003 – *Botryosphaeria* and related taxa causing oak canker in south western Spain. *Plant Disease* 87, 1515–1521.
- Shivas RG, Tree DJ, Tan YP, Ballard EL. 2009 – *Dothiorella thripsita*. *Fungal Planet* 32, Persoonia 22, 168–169.
- Sivanesan A. 1984 – The bitunicate Ascomycetes and their anamorphs. Vaduz, Liechtenstein: J Cramer.
- 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, 55–168.
- Sutton BC. 1977 – Coelomycetes. VI. Nomenclature of generic names proposed for Coelomycetes. *Mycological Papers* 141, 1–253.
- Swofford DL. 2003 – PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4.0. Sunderland, Massachusetts, Sinauer Associates.
- Taylor K, Barber PA, St J Hardy GE, Burgess TI. 2009 – *Botryosphaeriaceae* from tuart (*Eucalyptus gomphocephala*) woodland, including descriptions of four new species. *Mycological Research* 113, 337–353.
- Twizeyimana M, McDonald V, Mayorquin JS. 2013 – Effect of Fungicide Application on the Management of Avocado Branch Canker (Formerly *Dothiorella* Canker) in California Plant Disease 97, 897–902.
- Úrbez-Torres JR, Gubler WD, Luque J. 2007 – First Report of *Botryosphaeria iberica* and *B. viticola* associated with grapevine decline in California. *Plant Disease* 91, 772.
- Úrbez-Torres JR, Peduto F, Striegler RK. 2012 – Characterization of fungal pathogens associated with grapevine trunk diseases in Arkansas and Missouri *Fungal Diversity* 52, 169–189.
- Úrbez-Torres JR, Peduto F, Vossen PM, Krueger WH, Gubler WD. 2013 – Olive Twig and Branch Dieback: Etiology, incidence, and distribution in California. *Plant Disease* 97, 231–244.
- Wet J de, Slippers B, Preisig O, Wingfield BD, Tsopelas P, Wingfield MJ. 2009 – Molecular and morphological characterization of *Dothiorella casuarini* sp. nov. and other *Botryosphaeriaceae* with diplodia-like conidia. *Mycologia* 101, 503–511.
- White TJ, Bruns T, Lee S, Taylor J. 1990 – Amplified and direct sequencing of fungal ribosomal RNA genes for phylogenies. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) *PCR protocols: a guide to methods and applications*. Academic, San Diego, pp 315–322.
- Wijayawardene NN, Crous PW, Kirk PM, Hawksworth DL, Boonmee S, Braun U, Dai DQ, D'souza MJ, Diederich P, Dissanayake AJ, Raja HA, Shivas RG, Slippers B, Taylor JE, Tian Q, Wang Y, Woudenberg JHC, Cai L, Jaklitsch WM, Hyde KD. 2014 – Naming and outline of Dothideomycetes–2014 including proposals for the protection or suppression of generic names. *Fungal Diversity* 69, 1–55.
- Zhou JP, Lu Q, Liang J, Decock C, Zhang XY. 2010 – *Lasiodiplodia pseudotheobromae*, a new record of pathogenic fungus from some subtropical and tropical trees in southern China. *Cryptogamie Mycologie* 31, 431–439.