Cryptogamie, Mycologie, 2014, 35 (3): 257-270

Introducing the novel species, *Dothiorella symphoricarposicola*, from snowberry in Italy

*Wenjing LI[a,b,c,d], Jiankui LIU[c,d], D. Jayarama BHAT[e], Erio CAMPORESI[f], Jianchu XU[a,b] & Kevin D. HYDE[*a,b,c,d]*

[a]World Agroforestry Centre, East and Central Asia, 132 Lanhei Road, Kunming 650201, China

[b]Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences, Lanhei Road No 132, Panlong District, Kunming, Yunnan Province, 650201, PR China

[c]Institute of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand, e-mail: winnie20070653026@gmail.com

[d]School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand

[e]Formerly, Department of Botany, Goa University, Goa 403206, India

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

Abstract – Species of *Dothiorella* are common plant pathogens or saprobes found mainly on a variety of woody hosts, with a cosmopolitan distribution. Strains of *Dothiorella* were isolated from the stems of *Symphoricarpos* sp. and *Cornus sanguinea* in Italy. Morphological characters, as well as phylogenetic analyses of the internal transcribed spacer region (ITS4, ITS5) and partial sequences of the translation elongation factor 1-α genes were used to characterize and distinguish the two isolates. One is conspecific to *D. sarmentorum* previously from *Menispermum canadense* collected in Sweden, and a description for this species is provided. The second species could not be assigned to any known species of *Dothiorella*. *Dothiorella symphoricarposicola* sp. nov. from *Symphoricarpos* is described and illustrated herein, and compared with similar *Dothiorella* taxa.

Asexual morph / *Botryosphaeriaceae* / *Dothiorella* / Phylogeny / Taxonomy

INTRODUCTION

The order Botryosphaeriales has been a taxon of great research interest in recent years and has undergone considerable revision (Liu *et al.*, 2012; Slippers *et al.*, 2013; Hyde *et al.,* 2013). The order now comprises six families, viz *Aplosporellaceae*, *Botryosphaeriaceae*, *Phyllostictaceae*, *Planistromellaceae*,

* Corresponding author: Kevin D. Hyde, email address: kdhyde3@gmail.com

doi/10.7872/crym.v35.iss3.2014.257

Melanopsaceae and *Saccharaceae* (Slippers *et al.*, 2013). Genera of the *Botryosphaeriaceae*, have also undergone considerable revision, based on molecular data and thus numerous new species have been introduced in the genera included in the family (Jami *et al.*, 2012; Liu *et al.*, 2012; Hyde *et al.*, 2013; Marques *et al.*, 2013; Abdollahzadeh *et al.*, 2014). The genus *Dothiorella* is no exception (Hyde *et al.*, 2014) with 18 species being introduced in eight years. Species of *Dothiorella* are cosmopolitan in distribution, as endophytes in living plants, saprobes on dead plant parts or phytopathogens on various hosts such as grapevine, *Prunus* and *Citrus* (Crous *et al.*, 2006; Liu *et al.*, 2012; Hyde *et al.*, 2013, 2014; Phillips *et al.*, 2013; Pitt *et al.*, 2013; Abdollahzadeh *et al.*, 2014). Many species of this genus are known associated with canker diseases of woody hosts, branch die-back and discoloured canes of grapevines (Urbez-Torres, 2011; Pitt *et al.*, 2013). During surveys of *Botryosphaeriaceae*-infected vineyards in south-eastern Australia, *Dothiorella* species were found to be second in abundance only to *Diplodia* (Pitt *et al.*, 2013). However, unlike their more virulent counterparts *Dothiorella* species were found throughout most grape growing regions and threatened the productivity and longevity of grapevines in Australia (Pitt *et al.*, 2010; Úrbez-Torres, 2011; Liu *et al.*, 2012, Pitt *et al.*, 2013).

The genus *Dothiorella* was introduced by Saccardo (1880) with *D. pyrenophora* (Berk.) ex Sacc. as the type species (Saccardo, 1880; Sutton, 1977). *Dothiorella* species have conidiomata varying from pycnidial to multilocular and eustromatic, and hyaline, branched conidiophores that produce brown, ellipsoidal, 1-septate conidia (Saccardo, 1880; Sutton, 1977; Crous & Palm, 1999; Liu *et al.*, 2012). In the past decade, a great deal confusion has surrounded the type specimen and generic concept of *Dothiorella*, and this has been attributed mainly to lack of cultures and DNA sequence data, resulting in a wide concept for this genus (Crous and Palm, 1999; Crous *et al.*, 2006; Liu *et al.*, 2012). Petrak (1922) had further confused matters by transferring *Fusicoccum aesculi* to *Dothiorella*, citing the species as the conidial state of *Botryosphaeria berengeriana* (Sutton, 1980). Similarly, the genus *Dothiorella* was used for fusicoccum-like asexual morphs with multiloculate conidiomata, by several authors (Grossenbacher & Duggar, 1911; Barr, 1987; Rayachhetry *et al.*, 1996). Crous and Palm (1999) regarded *Dothiorella* as a synonym of *Diplodia* based on the morphology of the type species.

Phylogenetic studies using ITS and EF1-α sequences by Phillips *et al.* (2005a) showed this genus clustered with *Neofusicoccum*. However, the conidial characters of the type, *Dothiorella pyrenophora*, do not conform to, and cannot be accommodated in *Neofusicoccum* (Crous and Palm, 1999; Crous *et al.*, 2006; Pitt *et al.*, 2013). Phillips *et al.* (2005a) re-examined the type of *D. pyrenophora* Berk. ex Sacc. (K 54921) and found that it differed from *Diplodia* in having conidia that are brown and 1-septate early in their development while they are still attached to the conidiogenous cells, whereas in *Diplodia* conidial darkening and septation takes place after discharge (Sutton, 1977, 1980; Crous and Palm, 1999; Alves *et al.*, 2004; Phillips *et al.*, 2005a, 2013; Crous *et al.*, 2006). Subsequently, Crous *et al.* (2006) re-examined the types of both *Diplodia* and *Dothiorella* and concurred with Phillips (2005a) that species of *Dothiorella* have distinct conidial characteristics from those in *Diplodia*. *Dothiorella* species are also phylogenetically distinct from *Diplodia* (Phillips *et al.*, 2005a, 2008; Crous *et al.*, 2006; Pitt *et al.*, 2013; Hyde *et al.*, 2014).

Sexual morphs of *Dothiorella* have pigmented, 1-septate ascospores (Crous and Palm, 1999; Crous *et al.*, 2006; Phillips *et al.*, 2005a, 2013; Hyde *et al.*, 2014). Barr (1989) had regarded *Dothidotthia* as the sexual morph of *Dothiorella*

based on these characteristics. However, studies of Dothideomycetes by Schoch *et al.* (2006) show that *Dothidotthia* is unrelated to the family *Botryosphaeriaceae*, and Phillips *et al.* (2008) introduced a new family *Dothidotthiaceae* to accommodate it. Since *Dothidotthia* was shown to fall in the order Pleosporales, this name is no longer available for the sexual morph of *Dothiorella*, and therefore Phillips *et al.* (2008) proposed that asexual name *Dothiorella*, be used for the asexual and sexual states of the genus (Phillips *et al.*, 2008; Pitt *et al.*, 2013).

A search of Index Fungorum (August 2014) lists 373 names in *Dothiorella*, while MycoBank (August 2014) lists 395 species names. Phillips *et al.* (2013), however, included only 17 species (four unnamed) with available cultures in *Dothiorella*. Subsequently, *D. vidmadera* and *D. iranica* were introduced by Pitt *et al.* (2013). Abdollahzadeh *et al.* (2014) introduced five new species in *Dothiorella*, namely *D. iranica*, *D. parva*, *D. prunicola*, *D. sempervirentis*, and *D. striata* based on morphological characters and phylogenetic analyses of DNA sequences for ITS and TEF1-α.

In the present study, morphology and molecular data are used to characterize the Italian isolates. A new species, *Dothiorella symphoricarposicola* sp. nov. from *Symphoricarpos* sp. is described and illustrated herein, while the second species is *Dothiorella sarmentorum*.

MATERIAL AND METHODS

Collection and examination of specimens

Fresh specimens were collected from Forlì-Cesena in Italy on *Symphoricarpos* sp. and *Cornus sanguinea*, dried and sent to Thailand for examination. Samples were processed and examined, following the method described in Chomnunti *et al.* (2014). Conidiomata were removed directly from the dried material. Hand-sectioning of pycnidial structures was carried out using a razor blade. Thin sections were mounted in water for microscopic study and photomicrography. Conidiomata, conidia and conidiogenous structures were examined under a Nikon ECLIPSE 80i compound microscope and photographed by a Canon 550D digital camera fitted to the microscope. Measurements were made with the Tarosoft (R) Image Frame Work program and images used for figures were processed with Adobe Photoshop CS3 Extended version 10.0 software (Adobe Systems, USA) (Chomnunti *et al.*, 2011; Liu *et al.*, 2011).

Cultures were made from single spores following the method of Chomnunti *et al.* (2014). The aqueous suspension of conidia was prepared in a glass container or on a glass slide, crushed with a few drops of sterile distilled water, the spore suspension was transferred to Potato-dextrose agar (PDA) using a sterile needle and incubated at 25C overnight. After 24 h, individual germinating conidia were transferred to fresh PDA plates. The holotypes are deposited at the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand. Isolates are deposited at Mae Fah Luang University Culture Collection (MFLUCC). Duplicate cultures are deposited in Mycothèque de l'Université catholique de Louvain (MUCL).

DNA extraction, PCR amplification and sequencing

Isolates were grown on PDA plates in the darkness at 25C until completely covering the agar surface. The mycelium (about 50 mg) was scraped off and collected in a 1.5 ml micro centrifuge tube. The Biospin Fungus Genomic DNA Extraction Kit (BioFlux®) following the manufacturer's protocol (Hangzhou, P.R. China) was used to extract Genomic DNA.

DNA amplification was performed by polymerase chain reaction (PCR). Primer pairs ITS4 and ITS5 as defined by White *et al.* (1990) were used to amplify the internal transcribed spacers. Primer EF1-728 F and EF1-986R (Carbone & Kohn, 1999) was used to amplify sequence part of the translation elongation factor 1-alpha (EF1-α) gene. The amplifications were performed in a 25 µl reaction volume as follows: 1 µg DNA template, 1 µl of each forward and reverse primers, 12.5 µl of 2 × Taq PCR SuperMix (Mixture of 0.1 U Taq Polymerase/µl, 500 µm Dntp each, 20 mM Tris-HCL PH8.3, 100 Mm KCl, 3 mM $MgCl_2$ and optimized buffer) (TIANGEN BIOTECH Co., Ltd., Chaoyang District, Beijing, PR China) and 9.5 µl sterilized distilled water (Cai *et al.*, 2009). The amplification conditions were as follows: initially 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30-50 seconds, annealing at 55°C for 1 minute, elongation at 72°C for 90 seconds, and final extension at 72°C for 5-10 minutes (Alves *et al.*, 2006, 2008; Liu *et al.*, 2011). The PCR products were checked on 1% agarose electrophoresis gels stained with ethidium bromide. The sequencing of PCR products were sent to Beijing Bai Mai Hui Kang Biological Engineering Technology Co. Ltd (Beijing, P. R. China) for sequencing.

DNA sequence data analysis

The sequence data from *Dothiorella* strains from *Cornus sanguinea* and *Symphoricarpos* sp., including ex-type specimens identified in previous studies of *Dothiorella* were included in phylogenetic analyses (Table 1).

Bioedit (Hall, 1999) and Clustal X v. 1.83 (Thompson *et al.*, 1997) were used to align the sequence data. The alignments were checked visually and improved manually where necessary. Phylogenetic analyses of sequence data were carried out using PAUP v.4.0b 10 (Swofford, 2002) for Maximum-Parsimony (MP) analyses and MrBAYES v. 3.0b4 (Ronquist & Huelsenbeck, 2003) for Bayesian analyses. Trees were rooted using *Spencermartinsia viticola* as the outgroup taxon and visualized with TreeView (Page, 1996).

Maximum-parsimony analyses were performed using the heuristic search option with 1000 random taxa addition and tree bisection and reconnection (TBR) as the branch-swapping algorithm. All characters were unordered and of equal weight and gaps were treated as missing data. Maxtrees were unlimited, branches of zero length were collapsed and all multiple equally parsimonious trees were saved. The robustness of the most parsimonious trees was evaluated from 1000 bootstrap replications (Hillis & Bull, 1993). Tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency index (RC) were calculated and the resulting trees were printed with TreeView v. 1.6.6 (Page, 1996).

Bayesian analysis was performed on a MP starting tree automatically generated by using software PAUP 4.0b 10 (Swofford, 2002), MrModeltest2.3 (Nylander, 2004). The performed procedures were followed as described previously (Liu *et al.*, 2012).

Table 1. GenBank and culture collection accession numbers of species treated in the phylogenies. The newly generated sequences are indicated in bold and ex-type strains are marked by an asterisk "*"

Taxon	*Culture Accession No.1*	*Host*	*Location*	*Collecter*	*GenBank Accession No.2*	
					ITS	*EF1-α*
Dothiorella americana	UCD2252MO/CBS 128309*	*Vitis vinifera*	Missouri, USA	K. Striegler & G.M. Leavitt	HQ288218	HQ288262
D. americana	UCD2272MO/CBS 128310	*V. vinifera*	Missouri, USA	K. Striegler & G.M. Leavitt	HQ288219	HQ288263
D. brevicollis	CMW 36463/CBS 130411*	*Acacia karroo*	Pretoria,South Africa	F. Jami & M. Gryzenhout F.	JQ239403	JQ239390
D. brevicollis	CMW 36464/CBS 130412	*A. karroo*	Pretoria,South Africa	F. Jami & M. Gryzenhout F.	JQ239404	JQ239391
D. casuarini	CMW 4855/CBS 120688*	*Casuarina* sp.	Canberra, Australia	M.J. Wingfield	DQ846773	DQ875331
D. casuarini	CMW 4857/CBS 120690	*Casuarina* sp.	Canberra, Australia	M.J. Wingfield	DQ846774	DQ875333
D. dulcispinae	CMW 36460/CBS 130413*	*A. karroo*	Pretoria, South Africa	F. Jami & M. Gryzenhout	JQ239400	JQ239387
D. dulcispinae	CMW 36462/CBS 130415	*A. karroo*	Pretoria, South Africa	F. Jami & M. Gryzenhout	JQ239402	JQ239389
D. dulcispinae	CMW 36461/CBS 130414	*A. karroo*	Pretoria, South Africa	F. Jami & M. Gryzenhout	JQ239401	JQ239388
D. iberica	CBS 115041*	*Quercus ilex*	Aragón, Spain	J. Luque	AY573202	AY573222
D. iberica	CBS 113188	*Q. suber*	Andalucia, Spain	M.E. Sánchez	AY573198	EU673278
D. iberica	CAA 005	*Pistacia vera*	USA	–	EU673312	EU673279
D. iranica	IRAN1587C/CBS 124722*	*Olea europaea*	Iran, Golestan	A. Javadi	KC898231	KC898214
D. longicollis	CMW 26166/CBS 122068*	*Lysiphyllum cunninghamii*	Western Australia, Australia	T.I. Burgess	EU144054	EU144069
D. longicollis	CMW 26165/CBS 122067	*L. cunninghamii*	Western Australia, Australia	T.I. Burgess	EU144052	EU144067
D. moneti	MUCC 505*/WAC 13154	*A. rostellifera*	Yalgorup, Australia	K.M. Taylor	EF591920	EF591971
D. moneti	MUCC 507	*A. rostellifera*	Yalgorup, Australia	K.M. Taylor	EF591922	EF591973
D. parva	IRAN1579C*/CBS 124720	*Corylus avellana*	Iran, Ardabil	J. Abdollahzadeh/A. Javadi	KC898234	KC898217
D. parva	IRAN1585C/CBS 124721	*C. avellana*	Iran, Ardabil	*J. Abdollahzadeh/A. Javadi*	*KC898235*	*KC898218*

Table 1. GenBank and culture collection accession numbers of species treated in the phylogenies. The newly generated sequences are indicated in bold and ex-type strains are marked by an asterisk "*" *(continued)*

Taxon	*Culture Accession No.1*	*Host*	*Location*	*Collecter*	*GenBank Accession No.2*	
					ITS	*EF1-α*
D. pretoriensis	CMW 36481/CBS 130404*	*A. karroo*	South Africa	F. Jami/M. Gryzenhout	JQ239406	JQ239393
D. pretoriensis	CMW 36480	*A. karroo*	South Africa	F. Jami/M. Gryzenhout	JQ239405	JQ239392
D. prunicola	IRAN1541/CBS 124723*	*Prunus dulcis*	Portugal, Algarve	E. Diogo	EU673313	EU673280
D. santali	MUCC 509*/WAC 15155	*Santalum acuminatum*	Yalgorup, Australia	K.M. Taylor	EF591924	EF591975
D. santali	MUCC 508	*S. acuminatum*	Yalgorup, Australia	K.M. Taylor	EF591923	EF591974
D. sarmentorum	IMI63581b*	*Ulmus* sp.	Warwickshire, England	E.A. Ellis	AY573212	AY573235
D. sarmentorum	CBS 115038	*Malus pumila*	Delft, Netherlands	A.J.L. Phillips	AY573206	AY573223
D. sarmentorum	MFLUCC 13-0489	*Cornus sanguinea*	Forlì-Cesena, Italy	Erio Camporesi	KJ742380	KJ742383
D. sempervirentis	IRAN1583C*/CBS 124718	*Cupressus sempervirens*	Iran, Golestan	M.A. Aghajani	KC898236	KC898219
D. sempervirentis	IRAN1581C/CBS 124719	*C. sempervirens*	Iran, Golestan	M.A. Aghajani	KC898237	KC898220
D. striata	ICMP16824/CBS 124731*	*Citrus sinensis*	New Zealand	S.R. Pennycook/P.R. Johnston	EU673321	EU673288
D. striata	ICMP16819/CBS 124730	*C. sinensis*	New Zealand	S.R. Pennycook/P.R. Johnston	EU673320	EU673287
D. symphoricarposicola	**MFULCC 13-0497***	***Symphoricarpos* sp.**	**Forlì-Cesena, Italy**	**Erio Camporesi**	**KJ742378**	**KJ742381**
D. symphoricarposicola	**MFULCC 13-0498**	***Symphoricarpos* sp.**	**Forlì-Cesena, Italy**	**Erio Camporesi**	**KJ742379**	**KJ742382**
D. thailandica	MFLCC 11 0438*/CBS 133991	Bamboo culm	Chiang Rai, Thailand	D.Q. Dai	JX646796	JX646861
D. thripsita	BRIP 51876*	*A. harpophylla*	Queensland, Australia	D.J. Tree & C.E.C. Tree	FJ824738	–
D. uruguayensis	UY672/CBS 124908*	*Hexalamis edulis*	Uruguay	C. Perez	EU080923	EU863180
D. vidmadera	DAR78992*	*V. vinifera*	Eden,Valley, Australia	W.M. Pitt & A. Loschiavo	EU768874	EU768881
D. vidmadera	DAR78993	*V. vinifera*	Loxton, Australia	W.M. Pitt & A. Loschiavo	EU768876	EU768882
D. vidmadera	DAR78994	*V. vinifera*	Barossa,Valley, Australia	W.M. Pitt & A. Loschiavo	EU768877	EU768883
Spencermartinsia viticola	CBS 117009*	*V. vinifera*	Vimbodi, Spain	J. Luque & S. Martos	AY905554	AY905559

RESULTS AND DISCUSSION

Phylogenetic analyses

The combined ITS-EF1-α gene datasets comprised 40 taxa including the outgroup *Spencermartinsia viticola* (Table 1) which is based on the data setup used by Hyde *et al.* (2014) for the genus *Dothiorella* in *Botryosphaeriaceae*. The data setup contains 761 characters, 480 from the ITS region and 281 from the TEF region. Thus 761 characters were included in the final dataset, of which 549 are conserved, 35 variable characters are parsimony-uninformative and the numbers of parsimony-informative characters is 177. The Maximum Parsimony (MP) and Bayesian Inference (BI) phylogenetic reconstructions were similar, and the MP tree is shown on Fig. 1 with Bayesian posterior probabilities and MP bootstrap support values at the nodes.

In this paper, we use morphology and sequence data of fresh collections from Italy and sequence data (types) downloaded from GenBank to describe *D. sarmentorum* and introduce one new species *D. symphoricarposicola*. ITS gene sequence data have been used to distinguish the species within *Botryosphaeriaceae* (Denman *et al.*, 2000, 2003; Liu *et al.*, 2012; Phillips *et al.*, 2013; Slippers *et al.*, 2013). However, it has not been possible to apply ITS alone in resolving species in species complexes. It is evident that at the species level, the combined ITS and EF1-α gene analyses are better for resolving the species complexes of *Botryosphaeriaceae* (Liu *et al.*, 2012; Phillips *et al.*, 2013, Abdollahzadeh *et al.*, 2014). Therefore, phylogenetic analyses were based on a combination of the ITS and the EF1-α gene.

The phylogeny based on ITS and EF1-α sequence data revealed 20 subclades corresponding to 20 species of *Dothiorella*. Most of these sub-clades have high bootstrap support (BS) in the MP and BY analyses. In phylogenetic analyses, stain MFLUCC 13-0489 (IT-154) clusters with *D. sarmentorum* and is well-supported (79% MP and 0.97 PP). The comparisons of ITS and EF1-α showed that strain MFLUCC 13-0489 (IT-154) differed from ex-type (CBS 115038) by only five nucleotides. Morphologically, MFLUCC 13-0489 (IT-154) and ex-type (CBS 115038) share similar characters by having brown and 1-septate conidia early in their development, while they are still attached to the conidiogenous cells, but differ in size of the conidiomata (250-300 µm diam. in MFLUCC 13-0489 strain versus up to 450 µm diam. in the ex-type CBS 115038) and conidiogenous cells (phialidic in MFLUCC 13-0489 strain versus holoblastic in ex-type CBS 115038). However, the differences noted here similarly reflect reasonable intraspecific variation (Luque *et al.*, 2005; Phillips *et al.*, 2008, 2013; Pitt *et al.*, 2013). Hence, we provide here a description of this species for further reference.

Strains MFLUCC 13-0497 and MFLUCC 13-0498 clustered with ex-type strains of specimens of *D. iberica* (CBS 115041), *D. americana* (UCD 2252MO), *D. sarmentorum* (IMI 63581b), *D. parva* (IRAN 1579C), *D. sempervirentis* (IRAN 1583C) and *D. vidmadera* (DAR 78992) with 87% MP and 0.98 PP bootstrap support. *Dothiorella symphoricarposicola* however is clearly distinct from *D. iberica*, *D. americana* and *D. sarmentorum* and occupies a separate clade with *D. vidmadera*, *D. sempervirentis* and *D. parva* (Fig. 1). The nine strains in this clade clustered in four well-supported species, three strains of *D. vidmadera* (BS = 100%), and two strains of *D. sempervirentis* (BS = 98%), *D. parva* (BS = 70%) and *Dothiorella symphoricarposicola* (BS = 100%). However,

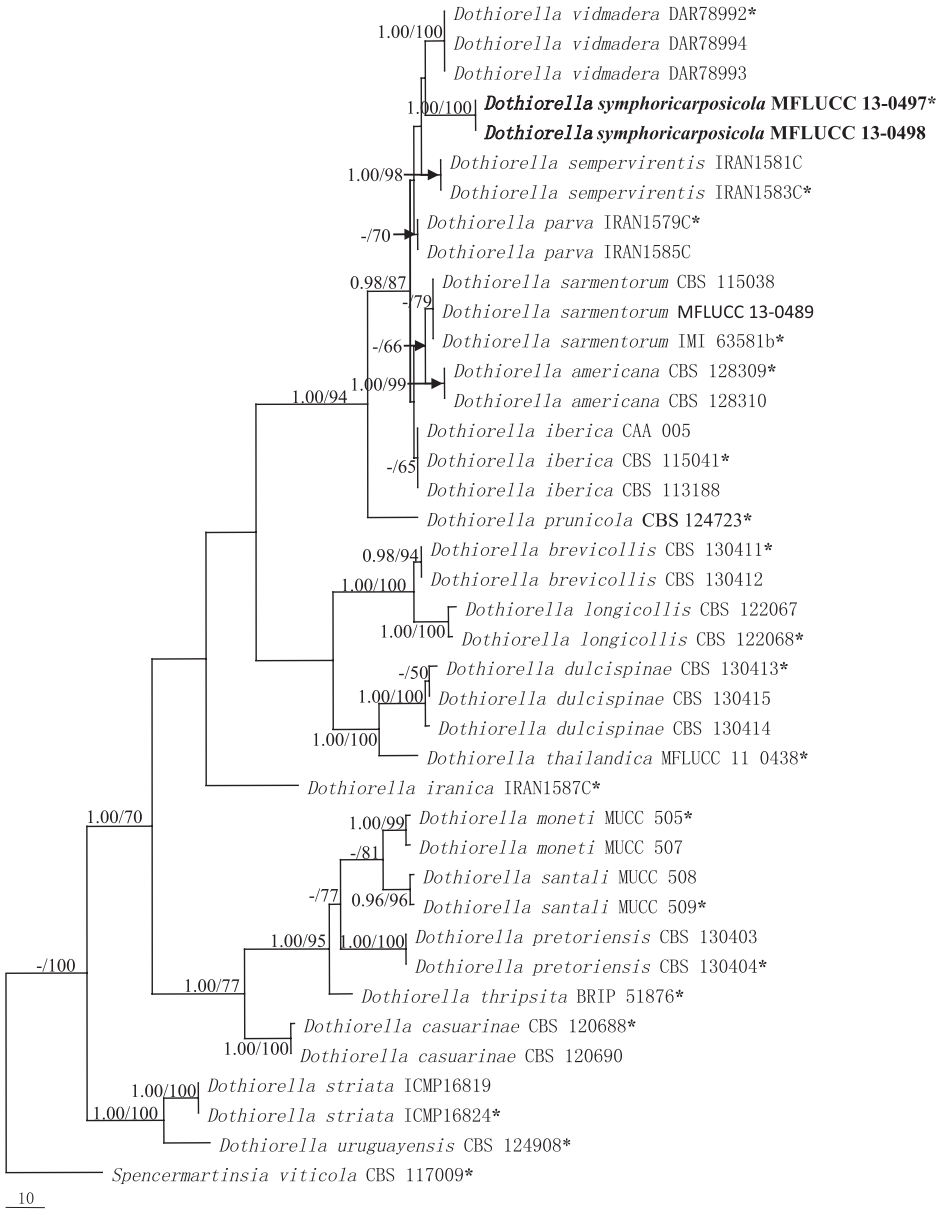

Fig. 1. One of 92 equally most parsimonious tree obtained from combined ITS and EF-1α sequence analyses, for species within *Dothiorella*. Bootstrap support (BS) values of MP (equal to or greater 50% based on 1,000 replicates) and values of the Bayesian posterior probabilities (PP) (equal to or greater 0.95) are shown nodes. Hyphen ("–") indicates a value lower than 50% (BS) or 0.95 (PP). New species strains are in bold and ex-type strains are marked by an asterisk "*". The tree is rooted to *Spencermartinsia viticola*.

sequences of *Dothiorella symphoricarposicola* strain appeared distinct from those species, and formed a basal sub-clade to *D. vidmadera.* Morphologically, conidia of *D. symphoricarposicola* differ from *D. vidmadera*, being slightly shorter and narrower than *D. vidmadera* (21.6 × 9.7 µm). In addition, the phialidic conidiogenous cells of *Dothiorella symphoricarposicola* were also distinct, being holoblastic in the holotype *D. vidmadera* (Pitt *et al.*, 2013). Combined with morphological data, the phylogenetic placement of the *Dothiorella* strains cited above provides sufficient evidence to justify the introduction of a new species, which we describe herein as *Dothiorella symphoricarposicola.*

Twenty species (including *Dothiorella symphoricarposicola*) are presently included in *Dothiorella* but many more species are likely to be described. Therefore, there is still much research to be carried out in future studies.

Taxonomy

Dothiorella symphoricarposicola W.J. Li, J.K. Liu & K.D. Hyde, **sp. nov.** **Fig. 2**

Index Fungorum number: IF550587
Face of Fungi number: FOF 000024
Etymology: Referring to the host on which the fungus was found.

Saprobic on dead bark of *Symphoricarpos* sp., forming conspicuous, rounded, black, papillate conidiomata. *Sexual state*: Unknown. *Asexual state*: *Conidiomata* 200-250 µm diam., 250-300 µm high, submerged in the substrate, solitary, immersed to semi-immersed or partially erumpent at maturity, pyriform, black, ostiolate (Fig. 2d). *Ostiole* 30-70 µm diam., single, central, with a well-developed neck, thick-walled, sometimes papillate (Fig. 2e). *Peridium* 30-50 µm wide, composed of 6-7-layers, with outer 3-4-layers brown and inner 1-2-layers hyaline, composed of thick-walled cells of *textura angularis* (Fig. 2c). *Conidiophores* reduced to conidiogenous cells, arising from all around the basal region of the conidioma. *Conidiogenous cells* 4-12 µm long × 1.5-6 µm wide, cylindrical, sometimes slightly curved, phialidic, hyaline, thick and smooth-walled (Fig. 2f-j). *Conidia* 14-20 × 6.5-9 µm ($\bar{x}$ = 17 × 8 µm; n = 20), ovoid, rounded at both ends, guttulate, initially hyaline and aseptate, becoming pigmented brown and 1-septate at maturity, slightly curved, smooth-walled (Fig. 2l-n).

Culture on PDA: *Colonies* fast growing, reaching 50 mm diam. after 4 d at 20-25C, circular, white in first few days, the center becoming grey to green-grey after one week, and finally black after two weeks, flattened, felt-like, sparse, aerial, surface smooth with crenate edge, filamentous.

Material examined: ITALY. Forlì-Cesena [FC], Berleta-Santa Sofia, on dead bark of *Symphoricarpos* sp., 18 May 2013, Erio Camporesi, IT-1075 (MFLU 14-0217, holotype); ex-type cultures = MFLUCC 13-0497 = MUCL and MFLUCC 13-0498 = MUCL. *ibid*. Forlì-Cesena [FC], Spinello-Santa Sofia, on dead bark of *Symphoricarpos* sp., 28 June 2013, Erio Camporesi, IT-1075A (MFLU 14-0297, paratype).

Dothiorella sarmentorum (Fr.) A.J.L. Phillips, J. Luque & A. Alves, Mycologia 97: 522. 2005 **Fig. 3**

Index Fungorum number: IF 501403
Face of Fungi number: FoF 00171
≡ *Sphaeria sarmentorum* Fr., K. svenska Vetensk-Acad. Handl. 39: 107. 1818.

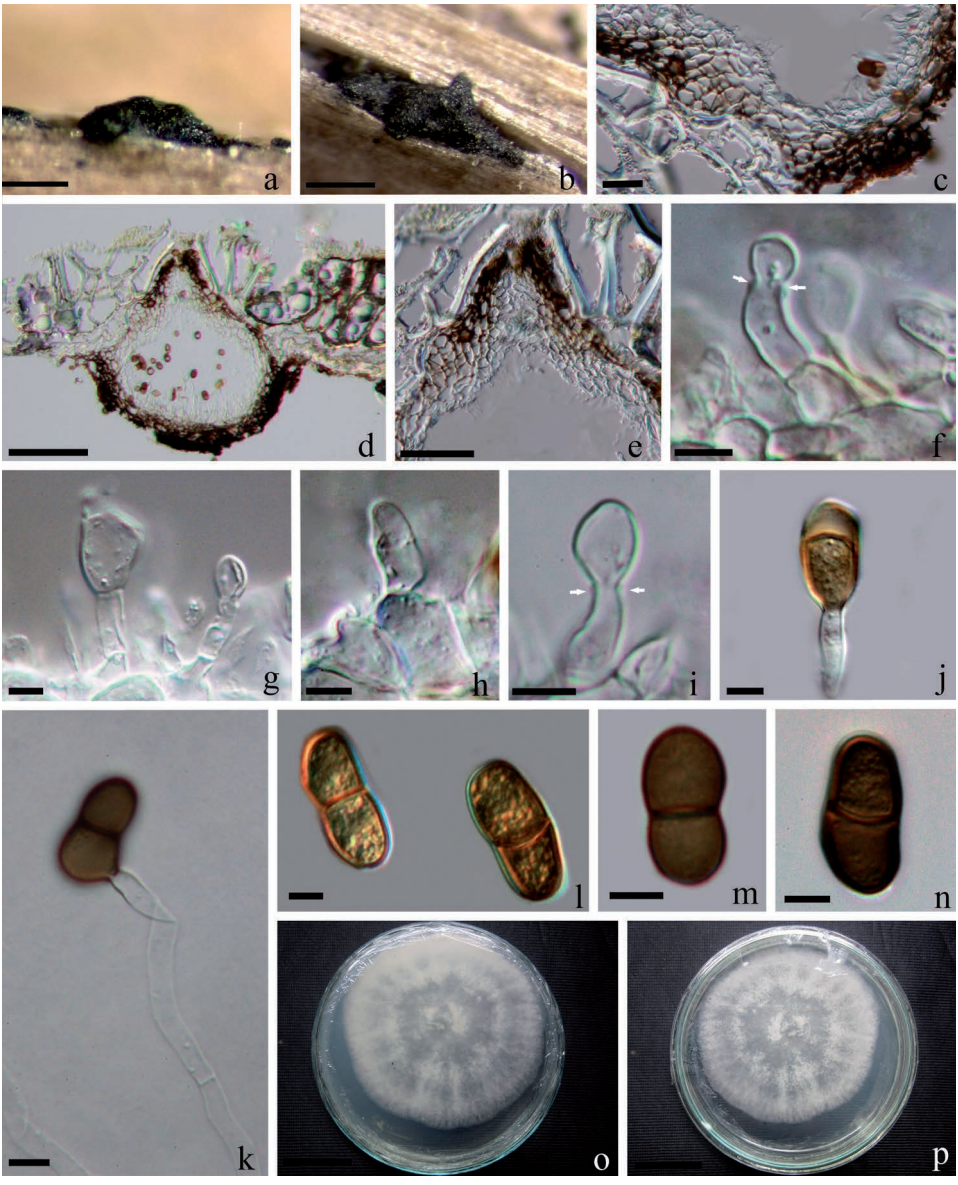

Fig. 2. ***Dothiorella symphoricarposicola*** (Holotype: MFLU 14-0217). **a-b.** Black conidiomata on the host surface. **c.** Section of peridium. **d.** Vertical section of conidioma. **e.** Ostiole. **f-j.** Conidiogenous cells and developing conidia. **k.** Germinating spore. **l-n.** Conidia. **o-p.** Culture on PDA (note o reverse). **Scale bars** a-b = 200 µm, c = 20 µm. d = 100 µm. e = 50 µm. f-j = 5 µm. k = 5 µm. l-n = 5 µm. o-p = 25 mm.

Fig. 3. ***Dothiorella sarmentorum*** (MFLU 14-0218). **a.** Material specimen. **b, c.** Black conidiomata on the host surface. **d.** Vertical section of conidioma. **e.** Ostiole. **f.** Section of peridium. **g-m.** Conidiogenous cells and developing conidia. **n.** Conidia. **o.** Germinating spore. **p.** Culture on PDA. **Scale bars** b = 500 µm. c = 200 µm. D = 100 µm. e = 50 µm. f = 20 µm. g-m = 5 µm. n-o = 5 µm. p = 25 mm.

Saprobic or *parasitic* on terrestrial plants, forming numerous, conspicuous, rounded, black, conidiomata. *Sexual state*: Unknown. *Asexual state*: *Conidiomata* 150-300 µm diam., 250-300 µm high, solitary, gregarious or confluent, globose to subglobose, composed of thick-walled cells of *textura angularis*, becoming thin-walled and hyaline towards the inner region (Fig. 3d). *Ostiole* 35-60 µm diam., centrally located, well-developed (Fig. 3e). *Peridium* composed of brown, thick-walled cells of *textura angularis* (Fig. 3f). *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 3-11 µm long × 3-8 µm wide, phialidic, integrated, subcylindrical, hyaline (Fig. 3g-m). *Conidia* 13-20 × 7-11 µm ($\bar{x}$ = 15 × 8.5 µm; n = 20), ovoid with a broadly rounded apex and truncate base, initially hyaline and aseptate, becoming pigmented, yellowish and 1-septate at maturity, often remain attached to conidiogenous cell, brown to dark brown, slightly constricted at the septum, smooth-walled (Fig. 3n).

Culture on PDA: Colonies fast growing, reaching 50 mm diam. after one week, circular, whitened in the first few days, the outside area becoming grey after one week, after two weeks becoming black, flattened, felt-like, sparse, aerial, surface smooth with crenate edge, filamentous.

Material examined: ITALY, Forlì-Cesena [FC], Collinaccia-Castrocaro Terme, on dead twig of *Cornus sanguinea*, 20 February 2013, Erio Camporesi, IT-154 (MFLU 14-0218), living culture = MFLUCC 13-0489 = MUCL; *ibid.* on dead twig of *Cornus sanguinea*, 12 June 2013, Erio Camporesi, IT-154A (MFLU 14-0219); on dead twig of *Cornus sanguinea*, 28 August 2013, Erio Camporesi, IT-154B (MFLU 14-0220).

Note: Dothiorella sarmentorum was introduced by Phillips *et al.* (2005a) based on distinctive morphological characters and phylogenetic analyses of DNA sequence of ITS and EF-1α. The sexual morph is linked to *Botryospheria sarmentorum* A.J.L. Phillips, A. Alves & J. Luque (Phillips *et al.*, 2008). This species has been re-described and illustrated from culture by Phillips *et al.* (2013). The species is cosmopolitan with a worldwide distribution and has been found across six continents where it occurs on various other hosts, such as *Malus*, *Menispermum*, *Prunus*, *Pyrus* and *Ulmus* (Phillips *et al.*, 2013).

Acknowledgments. We would like to thank the CGIAR Research Program 1.2 – *Humidtropics: Integrated systems for the humid tropics*, for partially funding this work. KD 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. The authors also thank MFLU Dothideomycetes grant for supporting this study.

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