



Dothiorella magnoliae, a new species associated with dieback of *Magnolia grandiflora* from China

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Abstract

Dothiorella magnoliae sp. nov. was identified and isolated from *Magnolia grandiflora* from Sichuan Province in China. The new taxon is described and illustrated based on unique morphological characteristics and phylogenetic analysis of the internal transcribed spacer (ITS) rDNA, and partial sequences of the elongation factor 1- α (*tef1- α*) gene. Morphologically, *D. magnoliae* produces conidia with conspicuous constriction at septum and it also differs from other described *Dothiorella* species in the dimensions of conidia. Molecular data reveal that *D. magnoliae* forms a sister clade to other species of *Dothiorella*, thus a new species is introduced here.

Key words – *Dothiorella* – Magnolia dieback – multigene phylogeny – taxonomy

Introduction

Dothiorella species, like other members of the family Botryosphaeriaceae, are known to be pathogens, endophytes, and saprobes on a wide range of mainly woody hosts (Phillips et al. 2008, 2013, Abdollahzadeh et al. 2014, Li et al. 2014, Pitt et al. 2015, Dissanayake et al. 2016a,b, Doilom et al. 2017). *Dothiorella* was resurrected by Phillips et al. (2005) based on the type species *D. pyrenophora*, which has brown, 1-septate conidia that turn brown while attached to their conidiogenous cells (Liu et al. 2012, Phillips et al. 2013, Pitt et al. 2013, 2015, Abdollahzadeh et al. 2014, Dissanayake et al. 2016a,b, Wijayawardene et al. 2016, Doilom et al. 2017).

Spencermartinsia, typified by *Spencermartinsia viticola* (A.J.L. Phillips & J. Luque) A.J.L. Phillips, A. Alves & Crous (Phillips et al. 2008), was delineated from *Dothiorella* based on the presence of an apiculus at either end of the 1-septate ascospores. The affinity of the two genera was supported by molecular phylogeny using ITS and *tef1- α* (Phillips et al. 2013), although the asexual morphs between *Spencermartinsia* and *Dothiorella* have little differences; the conidia of these two genera are brown and 1-septate while they are still attached to the conidiogenous cells. However, *Spencermartinsia* could not be resolved from *Dothiorella*, based on the phylogenetic analysis of six-gene regions (Slippers et al. 2013). Yang et al. (2017) demonstrated that species of *Spencermartinsia* clustered within *Dothiorella*, and the two genera were considered to be synonymous. Six described *Spencermartinsia* species were also transferred to *Dothiorella* by Yang et al. (2017).

During a 2015–2016 survey of forest pathogens causing canker or dieback disease in

southwestern China, one new species of *Dothiorella* were collected from *Magnolia grandiflora* Linn. in Daofu, Sichuan. This species is characterized by 1-septate conidia that become brown prior to the release from the conidiogenous cells, with obvious constriction at septum and it differs from all the described species. The aim of this study was to describe the novel taxon using both morphological and molecular data. Also, its phylogenetic relationship to other known species of *Dothiorella* is inferred using combined ITS and *tef1-α* sequence data.

Materials & Methods

Fungal Isolates

Two specimens were collected from branches or twigs of *M. grandiflora* with canker from southwestern China in June 2015. Single conidium isolate was prepared and transferred onto 1.8 % potato dextrose agar (PDA) in a Petri-dish, and incubated at 25 °C. The representative strains are maintained in the China Forestry Culture Collection Center (CFCC) and Beijing Forestry University (BJFU). Specimens are deposited in the Museum of the Beijing Forestry University (BJFC).

Morphology and culture characteristics

Fruiting bodies produced on infected plant tissues and cultural characteristics after inoculation were observed. Morphological characteristics of the fruiting bodies were recorded using a Nikon stereomicroscope (NI-U, Japan), including the size and shape of conidiophores and conidia. More than 20 pycnidia were sectioned and 50 conidia were selected randomly for measurement. The mean, standard deviation of the mean and 95% confidence interval for the lengths and widths of conidia per specimen were recorded, with extremes of length and width (minimum and maximum) given in parenthesis. Cultural characteristics of strains incubated on PDA in the dark at 25 °C were studied. The taxonomic information is deposited in MycoBank (MB 821470).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from 7 day-old colonies grown on PDA using the protocol of Cubero et al. (1999). The ITS region was amplified using primers ITS1 and ITS4 (White et al. 1990). The partial translation elongation factor 1- α (*tef1-α*) gene region was amplified using primers EF1-728F and EF1-986R (Carbone & Kohn 1999). DNA sequencing was performed using an ABI PRISM® 3730XL DNA Analyzer with BigDye® Terminator Kit v.3.1 (Invitrogen) at the Shanghai Invitrogen Biological Technology Company Limited (Beijing, China). DNA sequences were deposited in GenBank (Table 1).

Phylogenetic analysis

The new sequences generated in this study, and the reference sequences of all *Dothiorella ex-type* isolates selected from recent studies were included in the phylogenetic analyses (Table 1). Two species *Neofusicoccum luteum* (CBS 110299, CBS 110497) were used as outgroup. These sequences were checked manually and aligned in MAFFT v.7 (Katoh & Standley 2013). Phylogenetic analyses were performed on a combined dataset of ITS and TEF sequences by PAUP ver. 4.0b10 (Swofford et al. 2001) for maximum parsimony (MP), MrBayes v. 3.1.2 for Bayesian inference (BI) and PhyML v.7.2.8 for maximum likelihood (ML) (Ronquist & Huelsenbeck 2003). MP analysis was performed by a heuristic search algorithm (1,000 random-addition sequences) with tree bisection and reconnection (TBR) branch swapping. Maxtrees were set to 5,000, branches of zero length were collapsed and all equally parsimonious trees were saved. Descriptive tree statistics (Tree Length [TL], Consistency Index [CI], Retention Index [RI] and Rescaled Consistency [RC] were calculated. ML analysis was also performed with a GTR site substitution model. A bootstrapping (BS) analysis (1,000 replicates) was calculated to assess the branch support (Hillis & Bull 1993). Bayesian inference (BI) analysis was done by a Markov Chain Monte Carlo (MCMC) algorithm (Rannala & Yang 1996). A nucleotide substitution model was estimated by MrModeltest v.2.3.2.3 (Nylander 2004). For the BI analysis, two MCMC chains were run from

Table 1 Isolates of *Dothiorella* used for phylogenetic and taxonomic studies

Species	Culture no. ^{1,*}	Hosts	Locality	Collectors	GenBank Accession No.	
					ITS	TEF1- α
<i>Dothiorella americana</i>	CBS128309	<i>Vitis vinifera</i>	Missouri,United States	K.Striegler & G.M.Leavitt	HQ288218	HQ288262
<i>Dothiorella americana</i>	CBS128310	<i>V. vinifera</i>	Missouri,United States	K.Striegler & G.M.Leavitt	HQ288219	HQ288263
<i>D.brevicollis</i>	CMW36463	<i>Acacia karroo</i>	Pretoria,South Africa	F.Jami & M.Gryzenhout	JQ239403	JQ239390
<i>D.brevicollis</i>	CMW36464	<i>A. karroo</i>	Pretoria,South Africa	F.Jami & M.Gryzenhout	JQ239404	JQ239391
<i>D.capri-amissi</i>	CMW 25403	<i>A.erioloba</i>	Northern Cape, South Africa	F.J.J.van der Walt & G. J. Marais	EU101323	EU101368
<i>D.capri-amissi</i>	CMW25404	<i>A .erioloba</i>	Norther Cape, South Africa	F.J.J van der Walt & G.J.Marais	EU101324	EU101369
<i>D.casuarini</i>	CMW4855	<i>Casuarina</i> sp.	Canberra,Australia	M.J.Wingfield	DQ846773	DQ875331
<i>D.casuarini</i>	CMW4857	<i>Casuarina</i> sp.	Canberra,Australia	M.J.Wingfield	DQ846774	DQ875333
<i>D.dulcispinae</i>	CMW36460	<i>A. karroo</i>	Pretoria,South Africa	F.Jami & M.Gryzenhout	JQ239400	JQ239387
<i>D.dulcispinae</i>	CMW36461	<i>A. karroo</i>	Pretoria,South Africa	F.Jami & M.Gryzenhout	JQ239401	JQ239388
<i>D.dulcispinae</i>	CMW36462	<i>A. karroo</i>	Pretoria,South Africa	F.Jami & M.Gryzenhout	JQ239402	JQ239389
<i>D.dulcispinae</i>	CBS121764	<i>Acacia mellifera</i>	Rundu,Namibia	J.Roux	EU101299	EU101344
<i>D.dulcispinae</i>	CBS121765	<i>A. mellifera</i>	Pretoria,South Africa	R.N.Heath	EU101300	EU101345
<i>D.iberica</i>	CBS115041	<i>Quercus ilex</i>	Aragon,Spain	J.Luque	AY573202	AY573222
<i>D.iberica</i>	CBS113188	<i>Quercus suber</i>	Andalucia,Spain	M.E.Sanchez	AY573198	EU673278
<i>D.iranica</i>	CBS124722	<i>Olea europaea</i>	Golestan,Iran	A.Javadi	KC898231	KC898214
<i>D.longicollis</i>	CMW26165	<i>Lysiphyllum cunninghamii</i>	Tunnel Creek,Australia	T.I.Burgess	EU144053	EU144068
<i>D.longicollis</i>	CMW26166	<i>L. cunninghamii</i>	Tunnel Creek,Australia	T.I.Burgess	EU144054	EU144069
<i>D.moneti</i>	MUCC505	<i>Acacia rostelifera</i>	Yalgorup,Australia	K.M.Taylor	EF591920	EF591971
<i>D.moneti</i>	MUCC506	<i>A. rostelifera</i>	Yalgorup,Australia	K.M.Taylor	EF591921	EF591972
<i>D.neclivorem</i>	DAR80992	<i>Vitis vinifera</i>	Pokolbin,Australia	N.Wunderlich	KJ573643	KJ573640
<i>D.oblonga</i>	CMW25407	<i>A.mellifera</i>	Pretoria, South Africa	F.J.J. van der Walt & R.N. Heath	EU101300	EU101345
<i>D.oblonga</i>	CMW25408	<i>A.mellifera</i>	Pretoria, South Africa	F.J.J. van der Walt & R.N. Heath	EU101301	EU101346
<i>D.parva</i>	CBS124720	<i>Corylus avellana</i>	Ardabil,Iran	J.Abdollahzadeh & A.Javadi	KC898234	KC898217
<i>D.parva</i>	CBS124721	<i>C. avellana</i>	Ardabil,Iran	J.Abdollahzadeh & A.Javadi	KC898235	KC898218
<i>D.pretoriensis</i>	CMW36480	<i>Acacia karroo</i>	Pretoria,South Africa	F.Jami & M.Gryzenhout	JQ239405	JQ239392

Table 1 continued Isolates of *Dothiorella* used for phylogenetic and taxonomic studies

Species	Culture no. ^{1,*}	Hosts	Locality	Collectors	GenBank Accession No.	
					ITS	TEF1- α
<i>D.pretoriensis</i>	CMW36481	<i>A. karroo</i>	Pretoria,South Africa	F.Jami & M.Gryzenhout	JQ239406	JQ239393
<i>D.prunicola</i>	CBS124723	<i>Prunus dulcis</i> <i>Santalum</i>	Algarve,Portugal	E.Diogo	EU673313	EU673280
<i>D.santali</i>	MUCC508	<i>acuminatum</i>	Yalgorup,Australia	K.M. Taylor	EF591923	EF591974
<i>D.santali</i>	MUCC509	<i>S. acuminatum</i>	Yalgorup,Australia	K.M. Taylor	EF591924	EF591975
<i>D.sarmentorum</i>	IMI63581b	<i>Ulmus</i> sp.	Warwickshire,England	E.A. Ellis	AY573212	AY573235
<i>D.sarmentorum</i>	CBS115038	<i>Malus pumila</i> <i>Cupressus</i>	Delf,Netherlands	A.J.L. Phillips	AY573206	AY573223
<i>D.sempervirentis</i>	CBS124718	<i>sempervirens</i>	Golestan,Iran	M.A .Aghajani	KC898236	KC898220
<i>D.sempervirentis</i>	CBS124719	<i>C. sempervirens</i>	Golestan,Iran	M.A .Aghajani	KC898237	KC898219
<i>D.striata</i>	CBS124730	<i>Citrus sinensis</i>	Kerikeri,New Zealand	P.R.Johnston	EU673320	EU673287
<i>D.striata</i>	CBS124731	<i>C. sinensis</i>	Kerikeri,New Zealand	P.R.Johnston	EU673320	EU673287
<i>D.symphoricarposicola</i>	MFULCC130497	<i>Symphoricarpos</i> sp.	Forli-Cesena, Italy	Erio Camporesi	KJ742378	KJ742381
<i>D.symphoricarposicola</i>	MFULCC130498	<i>Symphoricarpos</i> sp.	Forli-Cesena, Italy	Erio Camporesi	KJ742379	KJ742382
<i>D.thailandica</i>	CBS133991	<i>Bambusa</i> sp. <i>Acacia</i>	Chang Rai,Thailand	D.Q.Dai	JX646796	JX646861
<i>D.thripsita</i>	BRIP51876	<i>harpophylla</i> <i>Hexachlamis</i>	Tallegalla,Australia	D.J.Tree & C.E.C.Tree	KJ573642	KJ573639
<i>D.uruguayensis</i>	CBS124908	<i>edulis</i>	Paysandu,Uruguay	C.A.Perez	EU080923	EU863180
<i>D.vidmaterata</i>	DAR78992	<i>Vitis vinifera</i>	Eden Valley,Australia	W.M. Pitt & A. Loschiavo	EU768874	EU768881
<i>D.vidmaterata</i>	DAR78993	<i>V. vinifera</i>	Loxton,Australia	W.M. Pitt & A. Loschiavo	EU768876	EU768882
<i>D.vinea-gemmae</i>	DAR81012	<i>V. vinifera</i>	Pokolbin,Australia	N. Wunderlich	KJ573644	KJ573641
<i>Dothiorella</i> sp.1	CBS242.51	Unknow	Italy	R. Ciferri	EU673317	EU673284
<i>Dothiorella</i> sp.1	CBS188.87	<i>Juglans regia</i>	France	Meylan	CBS188.87	EU673283
<i>Dothiorella</i> sp.1	CBS124716	<i>J. regia</i>	Jolfa,Iran	J. Abdollahzadeh & A.Javadi	KC898232	KC898215
<i>Dothiorella</i> sp.1	CBS124717	<i>J. regia</i>	Kermanshah, Iran	J.Abdollahzadeh & A.Javadi	KC898233	KC898216
<i>Dothiorella</i> sp.1	CMW25743	<i>Ostrya carpinifolia</i>	Lochere,Italy	G.Maresi	FM955386	FM955418
<i>Dothiorella</i> sp.1	DAR78991	<i>Vitis vinifera</i>	Eden Valley, Australia	W.M. Pitt & A. Loschiavo	EU768875	EU768880
<i>D.citricola</i>	CBS124728	<i>Citrus sinensis</i>	Kerikeri, New Zealand	P.R. Johnston	EU673322	EU673289
<i>D. citricola</i>	CBS124729	<i>C.sinensis</i>	Kerikeri, New Zealand	P.R. Johnston	EU673323	EU673290

Table 1 continued Isolates of *Dothiorella* used for phylogenetic and taxonomic studies

Species	Culture no. ^{1,*}	Hosts	Locality	Collectors	GenBank Accession No.	
					ITS	TEF1- α
<i>D.mangifericola</i>	CBS124726	<i>Mangifera indica</i>	Hormozgan,Iran	J.Abdollahzadeh & A.Javadi	KC898222	KC898205
<i>D.mangifericola</i>	CBS124727	<i>M. indica</i>	Hormozgan,Iran	J.Abdollahzadeh & A.Javadi	KC898221	KC898204
<i>D.plurivora</i>	CBS124724	<i>Citrus</i> sp.	Hormozgan,Iran	J.Abdollahzadeh & A.Javadi	KC898225	KC898208
<i>D.plurivora</i>	CBS124725	<i>Prunus armeniaca</i>	Hormozgan,Iran	J.Abdollahzadeh & A.Javadi	KC898230	KC898213
<i>D.plurivora</i>	DAR78869	<i>Vitis vinifera</i>	Eden Valley,Australia	W.M.Pitt & A.Loschiavo	EU603287	HM800507
<i>D.plurivora</i>	DAR78872	<i>V. vinifera</i>	Hills,Australia	W.M.Pitt & A.Loschiavo	EU603292	HM800510
<i>D.viticola</i>	CBS117009	<i>V. vinifera</i>	Vimbodi,Spain	J.Luque & S.Martos	AY905554	AY905559
<i>D.viticola</i>	CBS117010	<i>V. vinifera</i>	Sant Esteve Sesrovires,Spain	J.Luque & S.Martos	AY905558	AY905561
<i>D.westralis</i>	DAR80529	<i>V. vinifera</i>	Upper Swan,Australia	F.P.Trouillas	HM009376	HM800511
<i>D.westralis</i>	DAR80530	<i>V. vinifera</i>	Upper Swan,Australia	F.P.Trouillas	HM009377	HM800512
<i>D.westralis</i>	DAR80531	<i>V. vinifera</i>	Upper Swan,Australia	F.P.Trouillas	HM009378	HM800513
<i>D. rosulata</i>	CBS121760	<i>Acacia karroo</i>	Windhoek,Namibia	F.J.J.van der Walt & J. Roux	EU101290	EU101335
<i>D. rosulata</i>	CBS 121761	<i>A. mellifera</i>	Pretoria, South Africa	F.J.J.van der Walt & R.N. Heath	EU101293	EU101338
<i>D. yunnana</i>	CGMCC3.17999	<i>Camellia</i> sp.	Yunnan, China	Y. Zhang	KX499643	KX499649
<i>D. yunnana</i>	CGMCC3.17998	<i>Acer buergerianum</i>	Yunnan, China	Y. Zhang	KX499646	KX499652
<i>D. alpina</i>	CGMCC3.18001	<i>Platycladus orientalis</i>	Yunnan, China	Y. Zhang	KX499645	KX499651
<i>D. magnoliae</i>	CFCC 51563*	<i>Magnolia grandiflora</i>	Sichuan, China	C.J. You	KY111247	KY213686
<i>D. magnoliae</i>	CFCC 51564*	<i>Magnolia grandiflora</i>	Sichuan, China	C.J. You	KY111248	KY213687
<i>Neofusicoccum luteum</i>	CBS110299	<i>Vitis vinifera</i>	Oerias, Portugal	A.J.L. Phillips	AY259091	AY573217
<i>N. luteum</i>	CBS110497	<i>V. vinifera</i>	Portugal	A.J.L. Phillips	EU673311	EU673277

¹BRIP, Queensland Plant Pathology Herbarium, Queensland Department of Agriculture, Fisheries and Forestry, Dutton Park, Australia; CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CMW: CMW-FABI, University of Pretoria, South Africa; CFCC, China Forestry Culture Collection Center, Beijing, China; CGMCC, China General Microbiological Culture Collection Center, Beijing, China; DAR, Plant Pathology Herbarium, Orange Agricultural Institute, Department of Primary Industries, Orange, New South Wales, Australia; IMI, CABI Genetic Resource Collection Bioscience, Egham, Surrey, United Kingdom; MFLUCC, Mae Fah Luang University Culture Collection, Chiang Rai, Northern Thailand. *The ex-type strains are indicated in bold, and the newly generated sequences are marked by an asterisk

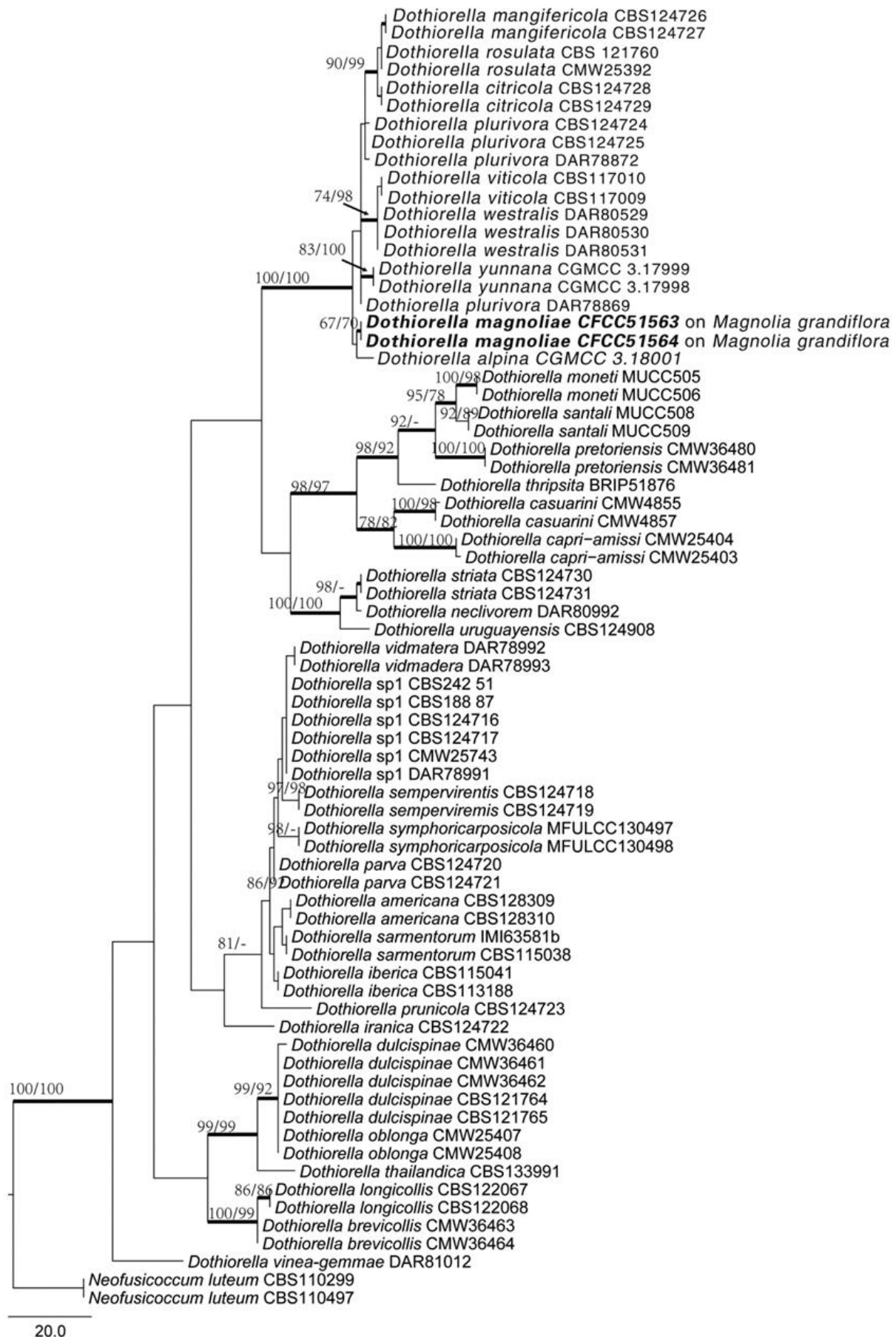


Figure 1 – Phylogram of *Dothiorella* based on combined ITS and TEF1- α genes. MP and ML bootstrap support values are shown at the first and second position (The posterior probabilities from BI are not shown). Scale bar = 30 nucleotide substitutions. New species in current study are in bold.

random trees for 1,000,000 generations, and trees were sampled every 100th generation, resulting in 10,000 total trees. The first 25 % of trees were discarded as the burn-in phase of each analysis. Branches with significant Bayesian posterior probabilities (BPP) were estimated in the remaining 7,500 trees. Trees are shown using FigTree v.1.4.0 (<http://tree.bio.ed.ac.uk/software/figtree>). Sequence alignments were deposited at TreeBase under the accession number S20262.

Results

Phylogenetic analysis

The combined ITS and *tef1-α* sequences comprised 70 ingroup strains corresponding to 35 species of *Dothiorella*, including two strains from this study are recognized as new species. Sequence alignment contained 677 characters, 460 for the ITS region, 217 for *tef1-α* gene. 677 characters were included in the final dataset, of which 497 characters were constant, 20 variable characters were parsimony-uninformative, and 160 were parsimony informative. The heuristic search generated one parsimonious tree (TL= 400, CI = 0.623, RI = 0.915, RC = 0.570) as shown in Fig. 1. Strains CFCC 51563 and CFCC 51564 formed a well-supported clade (MP/ML/BI = 67/70/0.91) representing a new phylogenetic species.

Taxonomy

***Dothiorella magnoliae* C.M. Tian, C.J. You sp. nov.**

Fig. 2

MycoBank 821470; Facesoffungi number: FoF 03374

Etymology – *magnoliae*, referring to *Magnolia grandiflora*, the only host known for this species.

Conidiogenesis – Conidiomata pycnidial, abundant, superficial, or semi-immersed, separate or aggregated into botryose cluster. Conidiogenesis holoblastic, with hyaline and cylindrical conidiogenous cells giving rise to periclinal thickenings. Conidia, initially hyaline, thin-walled, non-septate, becoming thick-walled, brown, 1-septate prior to release from the conidiogenous cells, always deeply constricted at septum, externally smooth, internally finely verruculose, rounded at both ends, (16.00–) 20.63–22.50 (–31.35) × (8.14–) 10.87–12.03 (–15.55) (av. of 50 conidia = 21.56 × 11.45 μm), and an average length to width ratio of 1.88.

Cultural characteristics – on PDA colonies flat and filamentous, initially leaden grey in the centre, becoming smoke grey at the margin, with white appressed mycelia radiating outwards, forming smoke-grey to olivaceous-dark at the surface and brown-dark at the reverse after 7–10 days. Colonies extending to ca. 66 mm diam after two weeks in the dark at room temperature.

Known distribution – Southwestern China.

Material examined – CHINA, Sichuan Province: Daofu, 29°59'36"N, 101°53'46"E, 3183 m asl, on twigs and branches of *Magnolia grandiflora*, coll. C.J. You, 10 June 2015 (BJFC-S2323, holotype), ex-type culture, CFCC 51563; Daofu, 29°59'36"N, 101°53'46"E, 3183 m asl, on twigs and branches of *Magnolia grandiflora*, coll. C.J. You, 10 June 2015 (BJFC-S2324, paratype), ex-paratype culture, CFCC 51564.

Notes – *Dothiorella magnoliae* differs from other species of *Dothiorella* in having 1-septate conidia deeply constricted at the septum (Fig. 1) (Table 2). Morphologically, *D. magnoliae* resembles *D. westralis* (W. M. Pitt et al.) Tao Yang & Crous (mean L/W: 1.9), but differs from this species in its larger conidia with rounded apex and base. The conidial size of *D. magnoliae* is comparable with those of *D. plurivora* (Abdollahz. et al.) Tao Yang & Crous (Table 2), but they differ in conidial shapes in that most conidia of *D. plurivora* have a truncate base, while those of *D. magnoliae* are unique with rounded ends (Abdollahzadeh et al. 2014, Yang et al. 2017).

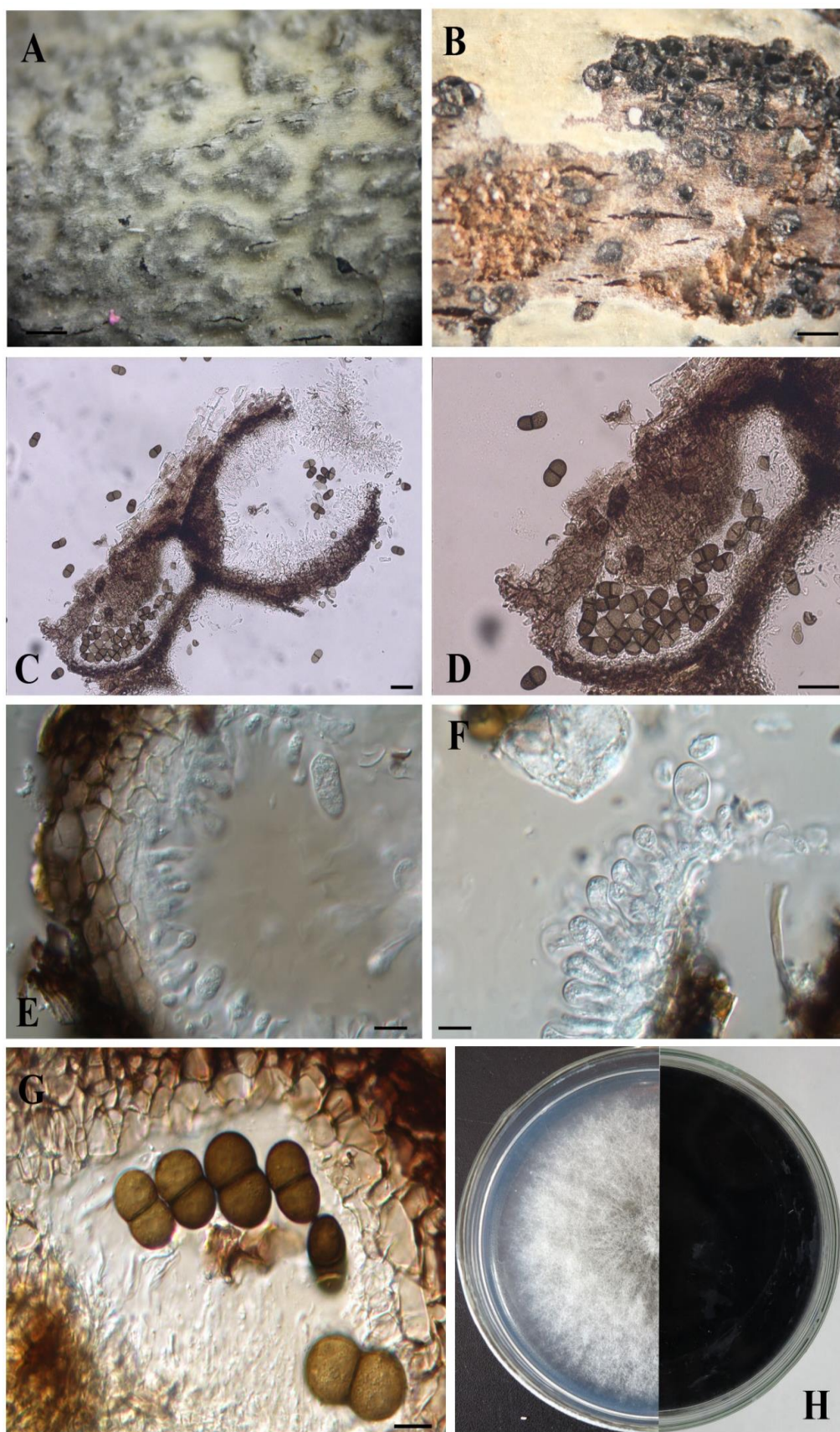


Figure 2 – *Dothiorella magnoliae* (Holotype CFCC 51563). A–B Black conidiomata on the host surface. C–D Vertical section of conidioma. E Section of peridium. F Conidiogenous cells and developing conidia. G Mature brown 1-septate conidia. H Culture on PDA (seven and 28 day-old colonies). Scale bars: A = 2 mm, B = 2000 μ m, C, D, = 20 μ m, E, F, G = 10 μ m.

Table 2 Conidial dimensions and L/W ratios of selected *Dothiorella* species

Species	Dimensions (μm)	Conidia		References
		Mean (μm)	L/W ratio	
<i>D. citricola</i>	24–27 × 10–12	25.8 × 12.2	2.1	Abdollahzadeh et al. (2014)
<i>D. mangifericola</i>	17–22 × 8–10	19 × 9	2.1	Abdollahzadeh et al. (2014)
<i>D. plurivora</i>	20–25 × 10–13	22.5 × 11	2.1	Abdollahzadeh et al. (2014)
<i>D. viticola</i>	20.2–20 × 9.2–9.4	20.4 × 9.3	2.2	Luque et al. (2005)
<i>D. westralis</i>	18.8–19.7 × 9.8–10.3	19.3 × 10.1	1.9	Pitt et al. (2015)
<i>D. rosulata</i>	21–24.2 × 10–10.5	22.6 × 10.4	2.2	Slippers et al. (2014)
<i>D. alpina</i>	19–20.4 × 8.4–9	19.7 × 8.7	2.2	Zhang et al. (2016)
<i>D. yunnana</i>	19.6–21 × 8.6–9.2	20.3 × 8.9	2.3	Zhang et al. (2016)
<i>D. magnoliae</i>	20.6–22.5 × 10.9–12.0	21.56 × 11.45	1.9	This study

Discussion

In the present study, the subclade of the new species *D. magnoliae* nested in the *Dothiorella* clade and formed a sister group to other species of *Dothiorella*. Morphologically, the deeply constricted conidia of *D. magnoliae* can be readily distinguished from all other reported species of *Dothiorella*, and may serve as a diagnostic character. The molecular data also indicated its uniqueness among all known species.

The taxonomic status of *Dothiorella* and its closely related genus *Spencermartinsia*, have been discussed by various mycologists (Phillips et al. 2008, 2013, Liu et al. 2012, Pitt et al. 2013, Abdollahzadeh et al. 2014, Li et al. 2014, Pitt et al. 2015, Yang et al. 2017). Yang et al. (2017) recommended that the two genera should be reduced to a single genus *Dothiorella*. Six described species, *S. mangiferae*, *S. rosulata*, *S. citricola*, *S. plurivora*, *S. viticola*, and *S. westrale* have been transferred to *Dothiorella*. Two new species to *Spencermartinsia*, i.e. *S. alpina* and *S. yunnana* from Yunnan province in China, newly reported by Zhang et al. (2016) should be transferred to *Dothiorella*. Our study including representatives of all *Dothiorella* and 8 *Spencermartinsia* species, also revealed that *Spencermartinsia* is paraphyletic and the distinction between the two genera was not completely resolved (Phillips et al. 2013, Abdollahzadeh et al. 2014, Slippers et al. 2013, Pitt et al. 2015). The apiculate ascospores is not a reliable feature to differentiate these two genera, and only one *Spencermartinsia* species has been demonstrated to produce the sexual stage.

The new species differed from all other most related eight species of *Dothiorella*, which were treated as *Spencermartinsia*, on the basis of its unique conidial morphology in having 1-septate conidia with deeply constricted septum (Table 2). Phylogenetically, *D. magnoliae* was closely related to *D. alpina*, but conidia of *D. magnoliae* differed from it, being significantly longer and broader (mean L/W: 1.9), and rounder at both ends than *D. alpina*. Furthermore, conidia of *D. magnoliae* were of similar size to *D. plurivora*, but *D. magnoliae* was unique phylogenetically.

In China, only one pathogen, *Pleomassaria magnoliae* Shear was reported to cause canker or dieback on *Magnolia denudata* (Tai 1979), *D. magnoliae* is described for the first time, which is associated with dieback of *Magnolia grandiflora* from China on *Magnolia*. Although pathogenicity of the species described in this study has not been determined, the pathogenicity, host specificity and geographic distribution of the characterized species remain unknown and should be considered in future studies.

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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.
- Carbone I, Kohn LM. 1999 – A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* 91, 553–556.
- Dissanayake AJ, Camporesi E, Hyde KD, Phillips AJL et al. 2016a – *Dothiorella* species associated with woody hosts in Italy. *Mycosphere* 7, 51–63.
- Dissanayake AJ, Phillips AJL, Li XH, Hyde KD. 2016b – Botryosphaeriaceae: Current status of genera and species. *Mycosphere* 7, 1001–1073.
- Doilom M, Dissanayake AJ, Wanasinghe AN, Boonmee S et al. 2017 – Microfungi on *Tectona grandis* (teak) in northern Thailand. *Fungal Divers* 82, 107–182.
- Hillis DM, Bull JJ. 1993 – An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* 42, 182–192.
- Katoh K, Standley DM. 2013 – MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology & Evolution* 30, 772–780.
- Li W, Liu J, 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, Doilom M, Wikee S et al. 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.
- Nylander JAA. 2004 – MrModeltest v. 2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Phillips A, Alves A, Correia A, Luque J. 2005 – Two new species of *Botryosphaeria* with brown, 1-septate ascospores and *Dothiorella* anamorphs. *Mycologia* 97, 513–529.
- Phillips AJL, Alves A, Pennycook SR, Johnston PR et al. 2008 – Resolving the phylogenetic and taxonomic status of dark-spored teleomorph genera in the Botryosphaeriaceae. *Persoonia* 21, 29–55.
- Phillips AJL, Alves A, Abdollahzadeh J, Slippers B et al. 2013 – The Botryosphaeriaceae: genera and species known from culture. *Studies in Mycology* 76, 51–167.
- Pitt WM, Huang R, Steel CC, Savocchia S. 2010 – Identification, distribution and current taxonomy of Botryosphaeriaceae species associated with grapevine decline in New South Wales and South Australia. *Australian Journal of Grape Wine Research* 16, 258–271.
- 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.
- Rannala B, Yang Z. 1996 – Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of Molecular Evolution* 43, 304–311.
- Ronquist F, Huelsenbeck JP, van der Mark P. 2005 – *MrBayes 3.1 Manual*.
- Slippers BJ, Roux MJ, Wingfield MJ, van der Walt FJJ et al. 2014 – Confronting the constraints of morphological taxonomy in the Botryosphaeriales. *Persoonia* 33, 155–168.
- Swofford DL, Waddell PJ, Huelsenbeck JP, Foster PG et al. 2001 – Bias in phylogenetic estimation and its relevance to the choice between parsimony and likelihood methods. *Systematic Biology* 50, 525–539.
- Tai FL. 1979 – *Sylloge Fungorum Sinicorum*. Beijing, China.
- White TJ, Bruns T, Lee S, Taylor J. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds), *PCR Protocols: a guide to methods and applications*. Academic Press, San Diego, U.S.A, pp 315–322.

- Wijayawardene NN, Hyde KD, Wanasinghe DN, Papizadeh M et al. 2016 – Taxonomy and phylogeny of dematiaceous coelomycetes. *Fungal Divers* 77, 1–316.
- Yang T, Groenewald JZ, Cheewangkoon R, Jami F et al. 2017 – Families, genera and species of Botryosphaeriales. *Fungal Biology* 121, 322–346.
- Zhang M, He W, Wu JR, Zhang Y. 2016 – Two new species of *Spencermartinsia* (Botryosphaeriaceae, Botryosphaeriales) from China. *Mycosphere* 7, 942–949.