Phytopathologia Mediterranea (2015) 54, 2, 222–231
DOI: 10.14601/Phytopathol_Mediterr-15011

RESEARCH PAPERS

Molecular and morphological characterization of *Dothiorella* species associated with dieback of *Ostrya carpinifolia* in Slovenia and Italy

Draginja PAVLIC-ZUPANC[1], Barbara PIŠKUR[2], Bernard SLIPPERS[3], Michael J. WINGFIELD[3,4] and Dušan JURC[2]

[1] Biosystematics Programme-Mycology Unit, Plant Protection Research Institute, Agricultural Research Council (ARC-PPRI), Pretoria, 0001, South Africa
[2] Department of Forest Protection, Slovenian Forestry Institute, Večna pot 2, SI-1000 Ljubljana, Slovenia
[3] Department of Genetics, and [4]Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), Centre of Excellence in Tree Health Biotechnology, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria, 0002, South Africa

Summary. Isolates that resemble *Dothiorella* (Botryosphaeriaceae, Ascomycota) species were isolated from dead twigs, asymptomatic and necrotized bark of European hop hornbeam (*Ostrya carpinifolia* Scop.), Eurasian smoke tree (*Cotinus coggygria* Scop.) and common juniper (*Juniperus communis* L.) growing in western Slovenia and northern Italy. They were identified based on anamorph morphology and phylogenetic analyses of the ITS rDNA and EF-1α sequences, and previously designated as *Dothiorella* sp. A, B and C. This study has clarified the identity of these species by comparing them with other *Dothiorella* species known from culture based on gene sequence data, as well as morphological characters of the anamorphs. The phylogenetic results revealed three species, *Dothiorella iberica*, *Dothiorella parva*, and a *Dothiorella* sp. Isolates identified in the phylogenetic analyses as *D. parva* differed from the original description of this species and are thus described here based on the anamorph morphology. Isolates of *D. parva* were identified from *O. carpinifolia* in western Slovenia and northern Italy, and *C. coggygria* in western Slovenia, and coexist with *Dothiorella* sp. on *O. carpinifolia* in northern Italy. *Dothiorella iberica* was identified on *J. communis* in western Slovenia, thus expanding the geographic range of this species. This is the first record of *D. parva* from these hosts and countries. Our results indicate that these *Dothiorella* species occur widely across the Mediterranean region, and on a variety of hosts.

Key words: Botryosphaeriaceae, European hop hornbeam, ITS, molecular phylogenetics, translation elongation factor EF-1α

Introduction

The Botryosphaeriaceae (Ascomycota) is a large monophyletic family of fungi that includes many common endophytes and opportunistic pathogens mainly of woody plants (Slippers and Wingfield, 2007; Slippers *et al.*, 2013). In a study considering the Botryosphaeriaceae on a variety of forest trees in western Slovenia and northern Italy, isolates resembling *Dothiorella* Sacc. species represented the second most prevalent group after *Botryosphaeria dothidea* (Moug.:Fr.) Ces. & De Not. (Piškur *et al.*, 2011). These were isolated from asymptomatic and necrotized tissues, but in pathogenicity trials they produced lesions not significantly longer than uninoculated experimental controls. Accordingly, although they were able to infect the wood, they were not considered major pathogens.

Recent taxonomic revision and phylogenetic reconstruction of Botryosphaeriaceae has revealed new phylogenetic relationships among species and genera (Phillips *et al.*, 2013; Slippers *et al.*, 2013). Following the re-introduction of *Dothiorella* (Phillips *et al.*, 2005), and the revision of the phylogenetic and taxonomic status of dark-spored sexual genera in the

Corresponding author: D. Pavlic-Zupanc
E-mail: pavlicdr@gmail.com

ISSN (print): 0031-9465
ISSN (online): 1593-2095
www.fupress.com/pm

Botryosphaeriaceae (Phillips *et al*., 2008), numerous new species were described in *Dothiorella* (Pavlic *et al*., 2008; Phillips *et al*., 2008; de Wet *et al*., 2009; Taylor *et al*., 2009; Pérez *et al*., 2010; Jami *et al*., 2012; Úrbez-Torres *et al*., 2012; Pitt *et al*., 2013, 2014; Abdollahzadeh *et al*., 2014; Li *et al*., 2014, Slippers *et al*., 2014). However, due to lack of herbarium specimens linked to type species and uncertainties of identification based only on morphology, this remains a difficult genus to deal with taxonomically.

In the study of Piškur *et al*. (2011), isolates resembling *Dothiorella* grouped in three clades based on phylogenetic analyses of ITS rDNA and EF-1α sequences, and these were designated as *Dothiorella* sp. A, B and C. The aim of the study reported here was to clarify the identity of these species by comparing them with other *Dothiorella* species known from culture, based on ITS rDNA and EF-1α sequence data, as well as morphological characters of the asexual states.

Materials and methods

Isolates

The isolates used in this study were collected during the survey of the Botryosphaeriaceae on various woody hosts showing bark necrosis and dieback, including *Ostrya carpinifolia* Scop. (Figure 1), *Juniperus communis* L. and *Cotinus coggygria* Scop. in the western part of Slovenia (Kras) during 2005 and 2006, and in the Italian provinces Trento and Bologna, in 2006 (Table 1). Isolations were made from necrotic bark, dead branches and asymptomatic, visually healthy bark of trees, as described in Piškur *et al*. (2011). All cultures used in this study are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa, and representative isolates have been deposited in the collection of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands.

DNA sequence comparisons

Sequence data for the isolates resembling *Dothiorella* spp. from woody hosts in Slovenia and Italy were produced in a previous study (Piškur *et al*., 2011). These data included those for two nuclear loci, the internal transcribed spacer rDNA (ITS1, 5.8S, and ITS2) and a part of the translation elongation factor 1-α (EF-1α). The sequences of all other *Dothiorella* spp. used in phylogenetic analyses in the present study were obtained from GenBank (Table 1).

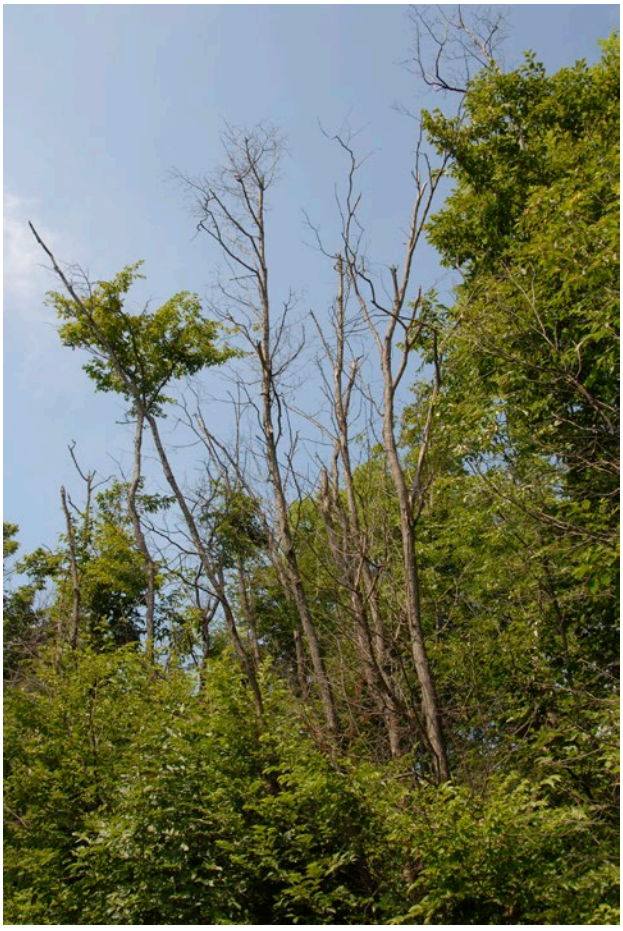

Figure 1. Extensive dieback symptoms on twigs and branches of *Ostrya carpinifolia* trees.

Phylogenetic analyses

The ITS and EF-1α sequences data matrices were aligned using MAFFT (http://align.bmr.kyushuu.ac.jp/mafft/online/server/) version 6 (Katoh *et al*., 2005), and manual adjustments were made where necessary. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura, 1980). The tree with the highest log likelihood (-2991.0867) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and

Table 1. Isolates included in the phylogenetic study.

Accession No. [1, 2]	Species	Host	Locality [3]	Reference	GenBank No. [4]	
					ITS	EF-1α
CMW25753	*Dothiorella parva*	*Ostrya carpinifolia*	Slovenia, Podgorje	Piškur *et al.* (2011)	FM955391	FM955423
CMW25751	*D. parva*	*Cotinus coggygria*	Slovenia, Gorjansko	Piškur *et al.* (2011)	FM955384	FM955416
CMW25754	*D. parva*	*O. carpinifolia*	Slovenia, Ravnje	Piškur *et al.* (2011)	FM955392	FM955424
CMW26361	*D. parva*	*O. carpinifolia*	Slovenia, Križ	Piškur *et al.* (2011)	FM955389	FM955421
CMW26362	*D. parva*	*O. carpinifolia*	Slovenia, Podgorje	Piškur *et al.* (2011)	FM955390	FM955422
CMW25750	*D. parva*	*O. carpinifolia*	Italy, S. Michele	Piškur *et al.* (2011)	FM955385	FM955417
CBS124720	*D. parva*	*Corylus avellana*	Iran, Ardabil	Abdollahzadeh *et al.* (2014)	KC898234	KC898217
CBS124721	*D. parva*	*C. avellana*	Iran, Ardabil	Abdollahzadeh *et al.* (2014)	KC898235	KC898218
JL599	*D. parva*	*C. avellana*	Spain	Phillips *et al.* (2008)	EU673314	EU673281
CMW25743	*Dothiorella* sp.	*O. carpinifolia*	Italy, Lochere	Piškur *et al.* (2011)	FM955386	FM955418
CMW25752	*Dothiorella iberica*	*Juniperus comumunis*	Slovenia, Križ	Piškur *et al.* (2011)	FM95583	FM955415
CBS115041	*D. iberica*	*Quercus ilex*	Spain, Aragon	Phillips *et al.* (2005)	AY573202	AY573222
CBS113188	*D. iberica*	*Q. suber*	Spain, Catalonia	Phillips *et al.* (2005, 2008)	AY573198	EU673278
CAA005	*D. iberica*	*Pistacia vera*	USA	Phillips *et al.* (2008)	EU673312	EU673279
UCD2252MO	*D. americana*	*Vitis vinifera*	USA, Missouri	Úrbez-Torres *et al.* (2012)	HQ288218	HQ288262
UCD2272MO	*D. americana*	*V. vinifera*	USA, Missouri	Úrbez-Torres *et al.* (2012)	HQ288219	HQ288263
CMW36463	*D. brevicollis*	*A. karroo*	South Africa, Pretoria	Jami *et al.* (2012)	JQ239403	JQ239390
CBS121763	*D. capri-amissi*	*Acacia erioloba*	South Africa, Northern Cape Province	Slippers *et al.* (2014)	EU101323	EU101368
CMW25404	*D. capri-amissi*	*A. erioloba*	South Africa, Northern Cape Province	Slippers *et al.* (2014)	EU101324	EU101369
CMW4855	*D. casuarini*	*Casuarina* sp.	Australia	de Wet *et al.* (2009)	DQ846773	DQ875331

(Continued).

Table 1. (Continued).

Accession No. [1, 2]	Species	Host	Locality [3]	Reference	GenBank No. [4]	
					ITS	EF-1α
CMW36460	*D. dulcispinae*	*Acacia karroo*	South Africa, Pretoria	Jami *et al.* (2012)	JQ239400	JQ239387
CBS124722	*D. iranica*	*Olea europaea*	Iran, Golestan	Abdollahzadeh *et al.* (2014)	KC898231	KC898214
CBS122068	*D. longicollis*	*Lysiphyllum cunninghamii*	Western Australia, Tunnel Creek NP	Pavlic *et al.* (2008)	EU144054	EU144069
MUCC506	*D. moneti*	*Allocasuarina rostellifera*	Western Australia	Taylor *et al.* (2009)	EF591921	EF591972
DAR80992	*D. neclivorem*	*V. vinifera*	Australia, Pokolbin	Pitt *et al.* (2014)	KJ573643	KJ573640
CBS121765	*D. oblonga*	*Acacia mellifera*	South Africa, Pretoria	Slippers *et al.* (2014)	EU101300	EU101345
CBS121766	*D. oblonga*	*A. mellifera*	South Africa, Pretoria	Slippers *et al.* (2014)	EU101301	EU101346
CMW36480	*D. pretoriensis*	*Acacia karroo*	South Africa, Pretoria	Jami *et al.* (2012)	JQ239405	JQ239392
CMW36481	*D. pretoriensis*	*A. karroo*	South Africa, Pretoria	Jami *et al.* (2012	JQ239406	JQ239393
CBS124723	*D. prunicola*	*Prunus dulcis*	Portugal	Abdollahzadeh *et al.* (2014)	EU673313	EU673280
MUCC509	*D. santali*	*A. rostellifera*	Western Australia	Taylor *et al.* (2009)	EF591924	EF591975
CBS115038	*D. sarmentorum*	*Malus pumila*	Netherlands, Delft	Phillips *et al.* (2005)	AY573206	AY573223
IMI63581b	*D. sarmentorum*	*Ulmus* sp.	England, Warwickshire	Phillips *et al.* (2005)	AY573212	AY573235
CBS124718	*D. sempervirentis*	*Cupressus sempervirens*	Iran, Golestan	Abdollahzadeh *et al.* (2014)	KC898236	KC898219
CBS124719	*D. sempervirentis*	*C. sempervirens*	Iran, Golestan	Abdollahzadeh *et al.* (2014)	KC898237	KC898220
ICMP16819	*D. striata*	*Citrus sinensis*	New Zealand	Abdollahzadeh *et al.* (2014)	EU673320	EU673287
ICMP16824	*D. striata*	*C. sinensis*	New Zealand	Abdollahzadeh *et al.* (2014)	EU673321	EU673288
MFLUCC130497	*D. symphoricarposicola*	*Symphoricarpos* sp.	Italy, Forli-Cesena	Li *et al.* (2014)	KJ742378	KJ742381
MFLUCC130498	*D. symphoricarposicola*	*Symphoricarpos* sp.	Italy, Forli-Cesena	Li *et al.* (2014)	KJ742379	KJ742382
MFLUCC110438	*D. thailandica*	*Bambusa* sp.	Thailand, Doi Pui	Liu *et al.* (2012)	JX646796	JX646861

(Continued).

Table 1. (Continued).

Accession No. [1, 2]	Species	Host	Locality [3]	Reference	GenBank No. [4]	
					ITS	EF-1α
BRIP51876	*D. thripsita*	*Acacia harpophylla*	Australia, Tallegalla	Pitt *et al.* (2014)	KJ573642	KJ573639
CBS124908	*D. uruguayensis*	*Hexachlamis edulis*	Uruguay, Paysandu	Pérez *et al.* (2010)	EU080923	EU863180
DAR78992	*D. vidmadera*	*V. vinifera*	Australia, Eden Valley	Pitt *et al.* (2013)	EU768874	EU768881
DAR78993	*D. vidmadera*	*V. vinifera*	Australia, Loxton	Pitt *et al.* (2013)	EU768876	EU768882
DAR81012	*D. vinea-gemmae*	*V. vinifera*	Australia, Pokolbin	Pitt *et al.* (2014)	KJ573644	KJ573641
CBS910.73	*Diplodia acerina*	*Acer pseudoplatanus*	München, Germany	Phillips *et al.* (2008)	EU673315	EU673282
CBS242.51	*D. coryli*	*Unknown*	Italy	Phillips *et al.* (2008)	EU673317	EU673284
CBS188.87	*D. juglandis*	*Juglans regia*	France	Phillips *et al.* (2008)	EU673316	EU673283
CBS117010	*Spencermartinsia viticola*	*V. vinfera*	Spain, Sant Esteve Sesrovires	Luque *et al.* (2005)	AY905558	AY905561
CBS117009	*S. viticola*	*V. vinfera*	Spain, Vimbodí	Luque *et al.* (2005)	AY905554	AY905559

[1] Abbreviations of isolates and culture collections: BRIP = Queensland Plant Pathology Herbarium, Queensland Department of Agriculture, Fisheries and Forestry, Dutton Park, Australia; CAA = Personal culture collection A. Alves, University of Averio, Portugal; CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CMW = Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; DAR = Plant Pahology Herbarium, Orange Agricultural Institute, Department of Primary Industries, Orange, New South Wales, Australia; ICMP = International Collection of Mictoorganisms from Plants, Landcare Research, Aukland, New Zealand; IMI = CABI Bioscience, Egham, U.K.; IRAN = Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Iran; JL = Personal culture collection, J. Luque, IRTA, Barcelona, Spain; MFLUCC = Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; MUCC = Murdoch University Culture Collection, Perth, Australia; UCD = University of California, Devis, California, USA.
[2] Accessions in bold indicate holotype cultures linked to the type material
[3] NP = National Park
[4] Sequences were obtained from the GenBank public database

BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (five categories (+*G*, parameter = 0.2966)). The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. Codon positions included were 1st + 2nd + 3rd + Noncoding. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 645 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura *et al*., 2013). A maximum likelihood (ML) tree was constructed and the robustness of the tree(s) obtained was evaluated by 500 bootstrap replicates. Bootstrap support values of greater than 70 % are indicated next to the nodes. The sequence alignment and phylogenetic tree have been deposited in TreeBASE (S16721).

Morphological characteristics

Isolates (Table 1) were induced to sporulate in cultures grown on 2% malt extract agar (MEA) or on 1.5%

water agar (WA) plates supplemented with pine needles (Pavlic *et al*., 2007). Conidia were mounted in lactophenol on microscope slides and studied using a light microscope. Fifty measurements of conidial lengths and widths were taken for each isolate and the ranges and averages, as well as length and width (L/W) ratios were calculated. Measurements were made and digital photographs captured with a HRc Axiocam digital camera and accompanying Axiovision 3.1 software (Carl Zeiss Ltd). Single conidium cultures grown on 2% MEA at 25ºC under continuous near fluorescent light were used to characterize culture morphology. Growth rates were determined for cultures grown on 2% MEA plates incubated in the dark at six different temperatures from 5 to 30ºC, at 5ºC intervals. Colony colours (upper surface and reverse) were compared with those in the colour charts of Rayner (1970).

Results

Phylogenetic analyses

Sequence alignment of the combined ITS and EF-1α sequences included 50 isolates, of which 48 represented *Dothiorella* spp., and two were of *Spencermartinsia viticola* (A.J.L. Phillips & J. Luque) A.J.L. Phillips, A. Alves & Crous (CBS 117010 and CBS 117009), to which the maximum likelihood (ML) tree was rooted (Figure 2, TreeBASE (S16721)).

Isolates of *Dothiorella* sp. A, B and C from Piškur *et al*. (2011) grouped in three distinct clades. One isolate, identified previously as *Dothiorella* sp. B, grouped with a *Dothiorella* sp. (=*Diplodia coryli* Fuckel) and a *Dothiorella* sp. (=*Diplodia juglandis* (Fr.) Fr.). This clade was most closely related to *Dothiorella vidmadera* W.M. Pitt, J.R. Úrbez-Torres & Trouillas. A single isolate representing *Dothiorella* sp. C clustered with ex-type isolate of *D. iberica* A.J.L. Phillips, J. Luque & A. Alves (CBS115041). A number of isolates identified by Piškur *et al*. (2011) as *Dothiorella* sp. A clustered within a *D. parva* Abdollahz., Zare & A.J.L. Phillips clade. One isolate recovered from GenBank as *Dothiorela* sp. (JL599) isolated from *Corylus avellana* L. in Spain, also clustered in this clade. Thus, all isolates previously identified as *Dothiorella* sp. A were considered to represent *D. parva*.

Morphological characteristics

The 1–3 septate conidia of *Dothiorella* sp. A distinguished this species from other *Dothiorella* spp., except from *D. iberica*. Although the original description of *D. iberica* suggests only 1-septate conidia, the isolate of *Dothiorella* sp. C identified here as *D. iberica,* formed 1–3 septate conidia (Piškur *et al*., 2011). *Dothiorella* sp. B could not be induced to sporulate in the study of Piškur *et al*. (2011), but it did sporulate in the present study. The conidia clearly resembled species of *Dothiorella*. Conidia were oval to ovoid, (15–) 19–21 (–26) × (9–) (10–11) (–11.5) μm (av. = 21 × 9 μm, L/W = 2.3), apices rounded and bases truncate, thick-walled, initially hyaline, unicellular, becoming cinnamon (13'') to sepia (13''k) and 1-septate while still attached to conidiogenous cells; detached conidia hyaline, cinnamon (13'') or sepia (13''k), unicellular or 1-septate. Given that only one isolate was available, it is not described here.

Taxonomy

Multiple gene sequence data revealed that isolates of *Dothiorella* sp. A represent recently described *D. parva* (Abdollahzadeh *et al*., 2014). However, isolates identified in phylogenetic analyses as *D. parva* differed from the original anamorph morphology description of this species and cannot be classified as *D. parva* using the key to *Dothiorella* species provided with its description (Abdollahzadeh *et al*., 2014). They are therefore fully described here as follows:

D. parva = *Dothiorella* sp. A *sensu* Piškur *et al*. Eur J Forest Res 130: 235–249 (2011) (Figure 3A-L).

Conidiomata semi-immersed, mostly solitary, with globose base (up to 500 μm diam) and short neck, up to 1 mm long, arising from the substrate, thick-walled, composed of dark brown thick-walled *textura angularis*, becoming thin-walled, hyaline towards inner region. *Conidiogenous cells* holoblastic, cylindrical to subcylindrical, hyaline, the first conidium produced holoblastically and subsequent conidia enteroblastically, (6–) 8–9 (–13) × (2–) 3–3.5 (–4) μm (av. = 8.9×3.3 μm). *Conidia* oval to ovoid, (15–) 19–21 (–27) × (7–) 9–10 (–12) μm (av. = 20.9 × 9.8 μm, L/W = 2.1), apices rounded and truncate base, thick-walled, initially hyaline, unicellular, becoming cinnamon (13'') to sepia (13''k) and 1–2 septate while still attached to conidiogenous cells; detached conidia, hyaline, cinnamon (13'') or sepia (13''k), unicellular or 1–3 septate. *Microconidiogenous* cells hyaline, smooth, cylindrical, holoblastic, 8–12 × 2–3 μm. *Microconidia* hyaline, smooth, aseptate, rod-shaped with rounded ends, 3.5–5.5 × 1–2 μm. *Cultural characteristics*.

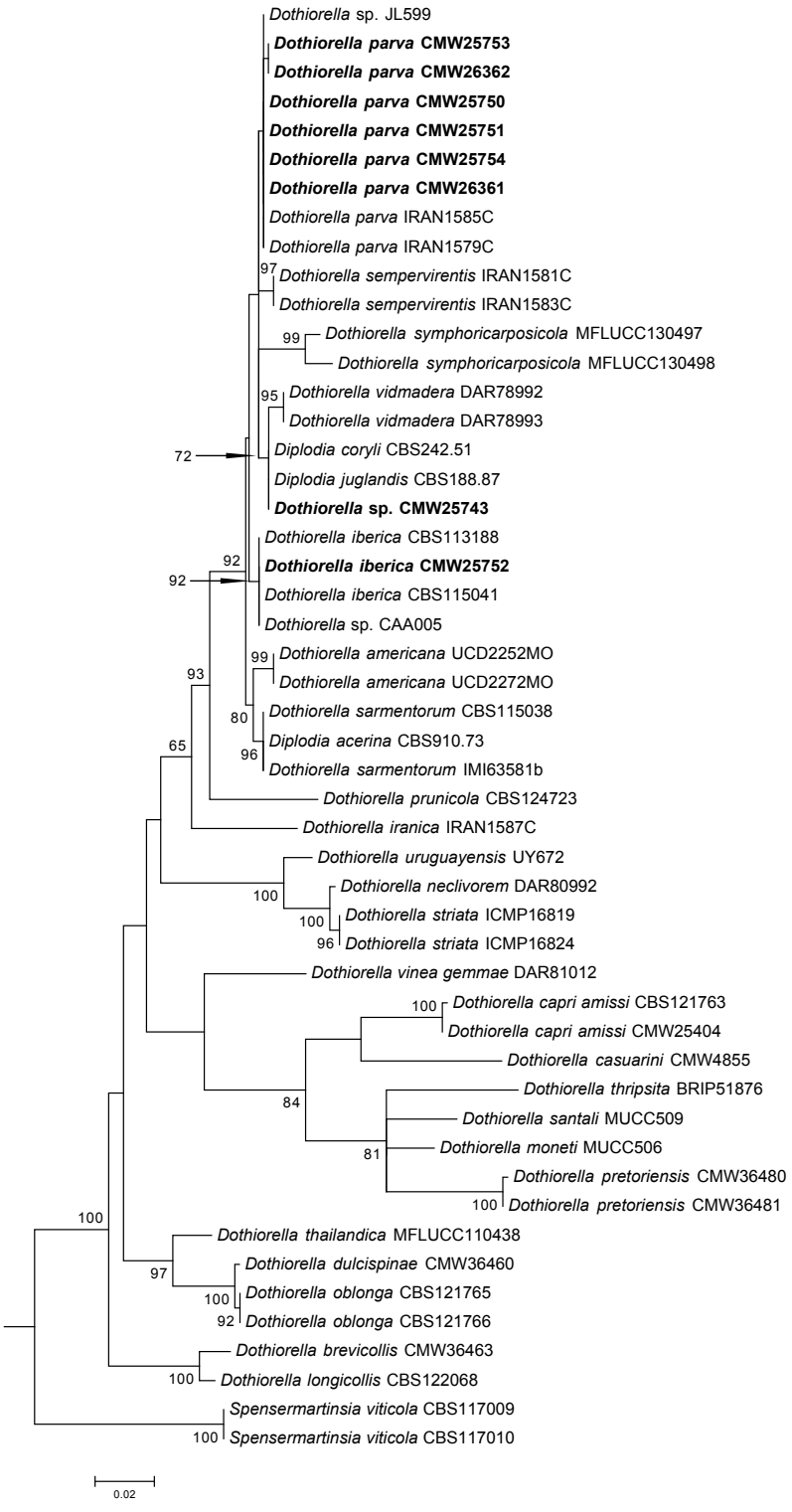

Figure 2. Maximum likelihood (ML) tree obtained from the combined ITS and EF-1α sequences of the *Dothiorella* species (Botryosphaeriaceae). Bootstrap support values greater than 70% are indicated next to the nodes. The tree was rooted to *Spencermartinsia viticola* (CBS 117010 and CBS 117009).

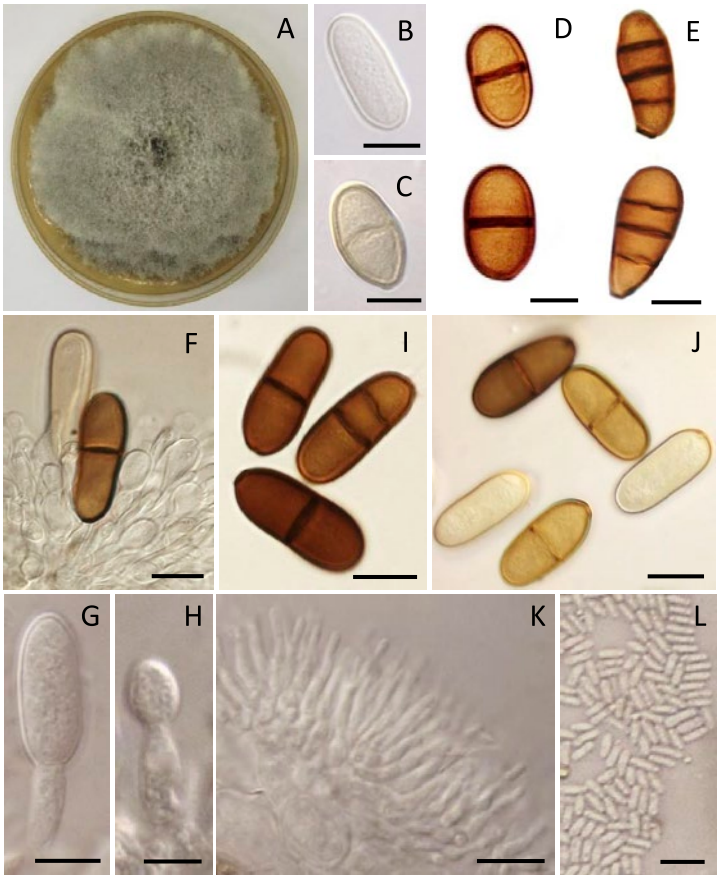

Figure 3. *Dothiorella parva*. A. Seven-day-old culture on 2% MEA (CMW25746). B. Hyaline, aseptate conidium (CMW25753). C. Hyaline, one-septate conidium (CMW25753). D. Dark, 1-septate conidia (CMW25746). E. Dark, 3-septate conidia (CMW26361). F. Conidiogeneous cells arising from the pycnidial wall, with conidia turning dark and 1-septate while still attached to conidiogeneous cells (CMW25753). G, H. Conidium attached to conidiogeneous cell (CMW25753). I. Dark, 1- and 2-septate conidia (CMW25753). J. Hyaline, aseptate and light and dark brown 1-septate conidia (CMW25753). K, L. Microconidiogenous cells and microconidia (CMW25751). Scale bars: B–K = 10 μm, L = 5 μm.

Colonies initially white to olivaceous-buff (21‴d), becoming greenish-olivaceous (23‴) to citrine (21k) from the middle of colonies within 7 d, iron grey (23‴″) (surface) and black (beneath) with age, with thick, cottony mycelium mats, edges irregular. Conidiomata readily formed from the middle of colonies within 7–10 d, covering the entire surface of the colony and immersed in the medium (seen as round black structures on the reverse side of Petri dishes) 14 d after incubation. Optimum growth at 20–25 ºC. *Teleomorph*: Not known. *Habitat*: Asymptomatic bark, necrotic bark and dead branches of *Ostrya carpinifolia* and *Cotinus coggygria* in western Slovenia and northern Italy (Table 1).

Discussion

Isolates from variety of woody hosts in western Slovenia and northern Italy that were designated as *Dothiorella* sp. A, B and C in a previous study (Piškur *et al*., 2011) were identified here as, respectively, *D. parva*, a *Dothiorella* sp., and *D. iberica*. The identity of these species was confirmed in comparisons with other *Dothiorella* species known from culture and based on the ITS rDNA and EF-1α sequences, as well as morphological characters of the asexual states. *Dothiorella parva* was recorded on *C. coggygria* in Slovenia, *O. carpinifolia* in Slovenia and Italy, and coexist with *Dothiorella* sp. on *O. carpinifolia* in Italy. *Dothiorella iberica* was identified on *J. communis* in

Slovenia, thus expanding the geographic range of this species. This is the first record of *D. parva* from these hosts and countries. *Dothiorella parva*, together with *D. iberica* and *D. sarmentorum* described from the Mediterranean region, are likely to be common in this region (Phillips *et al*., 2013). These two species, and others in the genus have been described from various, mostly woody hosts, including forest and fruit trees, grapevine and ornamentals, and recorded in many countries worldwide.

Dothiorella parva isolates identified in this study are morphologically similar to the other species with *Dothiorella* anamorphs, but differ from the original description of *D. parva* (Abdollahzadeh *et al*., 2014). The *D. parva* isolates have larger conidiomata with distinct necks, longer and narrower conidia with greater L/W ratios, and different cultural characteristics. *Dothiorella parva* isolates from *O. carpinifolia* differs from *Dothiorella* species other than *D. iberica* by its 1–3 septate conidia. Due to variation within the species and their overlap among *Dothiorella* species, morphological characteristics cannot be used with confidence to separate them. Their distinction is, however, well supported in the ITS and EF-1α sequence-based phylogenies (Abdollahzadeh *et al*., 2014; Li *et al*., 2014; Slippers *et al*., 2014).

One of the isolates (CMW25752) from hop hornbeam considered in this study was confirmed to represent *D. iberica* based on phylogenetic analyses of the ITS and EF-1α sequence data. This identification also shows that *D. iberica* conidia can form more than one septum, which is in contrast to the original description of the species by Phillips *et al*. (2005). *Dothiorella iberica* was described by Phillips *et al*. (2005) from *Quercus ilex* in Spain, and has been recorded on *J. communis* in Portugal (Alves *et al*., 2013). The isolate identified here as *D. iberica* was from dead twigs of *J. communis*, collected in Križ, Slovenia. This is the first record of *D. iberica* from Slovenia. The species is known from a variety of woody hosts worldwide (Phillips *et al*., 2013), and is likely to be widespread across the Mediterranean region.

One *Dothiorella* isolate (CMW25743), designated as *Dothiorella* sp. B by Piškur *et al*. (2011), grouped with a *Dothiorella* clade accommodating isolates identified as *Diplodia coryli* and *Diplodia juglandis*. Although the latter two species belong in the *Dothiorella* clade, their generic names have not been formally changed. As noted by Phillips *et al*. (2008), neither of these isolates is related to their respective type material and neither could be induced to sporulate, as was also the case with the isolate of *Dothiorella* sp. B in the study of Piškur *et al*. (2011). When recovered from the culture collection for the present study, the isolate produced fruiting structures in culture. Its asexual morphology conforms well to the morphological concept of the genus proposed by Phillips *et al*. (2005), having dark, septate conidia, which form septa and turn dark while still attached to conidiogenous cells. Description of this species requires a comprehensive taxonomic examination which is currently underway (Alan Phillips, personal communication).

Dothiorella parva was isolated from asymptomatic branch tissue, as well as from necrotised bark of two unrelated hosts, *O. carpinifolia* and *C. coggygria*. One isolate (JL599), originating from *Corylus avellana* in Spain and previously identified by Phillips *et al*. (2008), grouped also in the *D. parva* clade. This record extends the host range and geographic distribution for recently described *D. parva*. This indicates that *D. parva* is potentially also widespread across the Mediterranean region on a variety of hosts.

Dothiorella species treated here were described from necrotised host bark, dead material or as endophytes (Piškur *et al*., 2011). Most of *D. parva* isolates were obtained from necrotic bark and their ability to infect bark and to cause lesions was confirmed in inoculation experiments on hop hornbeam (Piškur *et al*., 2011). Although lesions produced by *D. parva* were not significantly larger than those of the uninoculated experimental controls in pathogenicity tests, their role in disease development still needs to be clarified.

Acknowledgments

This study was financed by the Slovenian Research Agency (research program P4-0107) and the Ministry of Agriculture and the Environment of Republic of Slovenia (Public Forestry Service). Molecular and morphological studies were carried out in laboratories of the Forestry and Agricultural Biotechnology Institute (FABI), the University of Pretoria, South Africa and funded by the DST/NRF Centre of Excellence in Tree Health Biotechnology. The authors thank N. Ogris (Department of Forest Protection, Slovenian Forestry Institute, Ljubljana, Slovenia) and G. Maresi (IASMA Research Center, Natural Resources Department, San Michele all'Adige, TN, Italy) for

providing isolates used in this study. We also thank the two anonymous reviewers for their helpful comments and suggestions to improve the manuscript.

Literature cited

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

Alves A., C. Barradas, A.J.L. Phillips and A. Correia, 2013. Diversity of Botryosphaeriaceae species associated with conifers in Portugal. *European Journal of Plant Pathology* 135, 791–804.

de Wet J., B. Slippers, O. Preisig, B.D. Wingfield, P. Tsopelas and M.J. Wingfield, 2009. Molecular and morphological characterization of *Dothiorella casuarini* sp. nov. and other Botryosphaeriaceae with diplodia-like conidia. *Mycologia* 101, 503–511.

Jami F., B. Slippers, M.J. Wingfield and M. Gryzenhout, 2012. Five new species of the Botryosphaeriaceae from *Acacia karroo* in South Africa. *Cryptogamie Mycologie* 33, 245–266.

Katoh K., K. Kuma, H. Toh and T. Miyata, 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Research* 33, 511.

Kimura M., 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16, 111–120.

Li W., J. Liu, D.J. Bhat, E. Camporesi, J. Xu and K.D. Hyde, 2014. Introducing the novel species, *Dothiorella symphoricarposicola*, from snowberry in Italy. *Cryptogamie Mycologie* 35, 257–270.

Liu J.-K., R. Phookamsak, M. Doilom, S. Wikee, Y.M. Li, H. Ariyawansha, *et al*., 2012. Towards a natural classification of Botryosphaeriales. *Fungal Diversity* 57, 149–210.

Luque J., S. Martos and A.J.L. Phillips, 2005. *Botryosphaeria viticola* sp. nov. on grapevines: a new species with a *Dothiorella* anamorph. *Mycologia* 97, 1111–1121.

Pavlic D., B. Slippers, T.A. Coutinho and M.J. Wingfield, 2007. Botryosphaeriaceae occurring on native *Syzygium cordatum* in South Africa and their potential threat to *Eucalyptus*. *Plant Pathology* 56, 624–636.

Pavlic D., M.J. Wingfield, P. Barber, B. Slippers, G.E.St.J. Hardy and T.I. Burgess, 2008. Seven new species of the Botryosphaeriaceae from baobab and other native trees in Western Australia. *Mycologia* 100, 851–866.

Pérez C.A., M.J. Wingfield, B. Slippers, N.A. Altier and R.A. Blanchette, 2010. Endophytic and canker-associated Botryosphaeriaceae occurring on non-native Eucalyptus and native Myrtaceae trees in Uruguay. *Fungal Diversity* 41, 53–69.

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

Phillips A.J.L., A. Alves, S.R. Pennycook, P.R. Johnston, A. Ramaey, A. Akulov and P.W. Crous, 2008. Resolving the phylogenetic and taxonomic status of dark-spored teleomorph genera in the Botryosphaeriaceae. *Persoonia* 21, 29–55.

Phillips A.J.L., A. Alves, J. Abdollahzadeh, B. Slippers, M.J. Wingfield, J.Z. Groenewald and P.W. Crous, 2013. The *Botryosphaeriaceae*: genera and species known from culture. *Studies in Mycology* 76, 51–167.

Piškur B., D. Pavlic, B. Slippers, N. Ogris, G. Maresi, M.J. Wingfield and D. Jurc, 2011. Diversity and pathogenicity of *Botryosphaeriaceae* on declining *Ostrya carpinifolia* in Slovenia and Italy following extreme weather conditions. *European Journal of Forest Research* 130, 235–249.

Pitt W.M., J.R. Úrbez-Torres and F.P. Trouillas, 2013. *Dothiorella vidmadera*, a novel species from grapevines in Australia and notes on *Spencermartinsia*. *Fungal Diversity*, DOI 10.1007/s13225-013-0244-7

Pitt W.M., J.R. Úrbez-Torres and F.P. Trouillas, 2014. *Dothiorella* and *Spencermartinsia*, new species and records from grapevines in Australia. *Australasian Plant Pathology*, DOI 10.1007/s13313-014-0332-5

Rayner R.W., 1970. *A Mycological Colour Chart*. Kew, UK: CMI and British Mycological Society.

Slippers B. and M.J. Wingfield, 2007. Botryosphaeriaceae as endophytes and latent pathogens of woody plants: diversity, ecology and impact. *Fungal Biology Reviews* 21, 90–106.

Slippers B., E. Boissin, A.J.L. Phillips, J.Z. Groenewald, L. Lombard, M.J. Wingfield, A. Postma, T.I. Burgess and P.W. Crous, 2013. Phylogenetic lineages in the *Botryosphaeriaceae*: a systematic and evolutionary framework. *Studies in Mycology* 76, 31–49.

Slippers B., J. Roux, M.J. Wingfield, F.J.J. van der Walt, F. Jami, J.W.M. Mehl and G.J. Marais, 2014. Confronting the constraints of morphological taxonomy in the Botryosphaeriales. *Persoonia* 33, 155–168.

Tamura K., G. Stecher, D. Peterson, A. Filipski and S. Kumar, 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30, 2725–2729.

Taylor K, P.A. Barber, G.E. St. J. Hardy and T.I. Burgess, 2009. Botryosphaeriaceae from tuart (*Eucalyptus gomphocephala*) woodland, including descriptions of four new species. Mycological Research 113, 337–353.

Úrbez-Torres J.R., F. Peduto, R.K. Striegler, K.E. Urrea-Romero, J.C. Rupe, R.D. Cartwright and W.D. Gubler, 2012. Characterization of fungal pathogens associated with grapevine trunk diseases in Arkansas and Missouri. *Fungal Diversity* 52, 169–189.

Accepted for publication: January 13, 2015
Published online: July 10, 2015