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ORIGINAL PAPER

Diversity and pathogenicity of *Botryosphaeriaceae* species on forest trees in the north of Iran

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Abstract
From 2012 to 2015, several field surveys were conducted throughout forest areas in the north of Iran in order to determine the occurrence of species of *Botryosphaeriaceae* on forest trees. Fungal isolations were made from symptomatic branches of 20 tree genera including *Acer, Alnus, Carpinus, Crataegus, Cupressus, Cydonia, Diospyros, Fagus, Fraxinus, Gleditsia, Mespilus*, *Parrotia, Pinus, Populus, Prunus, Pterocarya, Punica, Quercus, Ulmus* and *Zelkova*, as well as fruiting bodies formed on the surface of woody debris. Morphological identification along with molecular analysis of the internal transcribed spacer region (ITS1-5.8S-ITS2) of the nuclear ribosomal DNA (rDNA) and a partial sequence of translation elongation factor 1-α (*tef*-1α) gene showed that at least nine species of *Botryosphaeriaceae* occur on forest trees in the north of Iran. These include *Dothiorella sarmentorum*, *Dothiorella plurivora*, *Neofusicoccum parvum*, *Botryosphaeria dothidea*, *Neoscytalidium novaehollandiae*, *Diplodia seriata*, *Diplodia sapinea*, *Lasiodiplodia mahajangana* and *Diplodia intermedia.* Pathogenicity tests were conducted for selected isolates from each species on six tree species (*Mespilus germanica, Punica granatum*, *Parrotia persica*, *Alnus glutinosa*, *Quercus castaneifolia* and *Pterocarya fraxinifolia*) under field conditions. Results of the pathogenicity tests showed a variation in lesion lengths (virulence) and re-isolation frequencies of inoculated species on branches of trees, *Neoscytalidium novaehollandiae, B. dothidea* and *D. intermedia* being the most virulence species. Based on host plant species, the majority of *Botryosphaeriaceae* species are new records. This is the first comprehensive study on the species identification, distribution and pathogenicity of *Botryosphaeriaceae* on forest trees in Iran. This is also the first report of *L. mahajangana* in this country.

Keywords Fungal pathogens · ITS · *tef*-1α · Trunk diseases · Woody debris

Introduction

The majority of Iran's forests are found in the north around the Caspian coastal plain. Adequate rainfall and favorable climates have created a beautiful green landscape and dense forest trees in this area of Iran. Unfortunately, significant proportions of the northern forests have been destroyed due to human activities, climatic conditions, fire and plant diseases. In forest trees, various fungal taxa are known to cause trunk diseases worldwide. These include species of *Botryosphaeriaceae* Theiss. & Syd. (Swart and Wingfield 1991; Stanosz and Cummings Carlson 1996), *Togniniaceae* Réblová, L. Mostert, W. Gams and Crous. (Halliwell 1966; Hausner et al. 1992; Mostert et al. 2006; Réblová and Mostert 2007; Réblová 2011; Kazemzadeh Chakusary et al. 2017), *Diatrypaceae* Nitschke, Verh. Naturhist. (Davidson and Lorenz 1938; Hinds 1981, Jurc et al. 2006; Senanayake et al. 2015; Černý et al. 2016), *Valsaceae* Tul. & C. Tul. (Wang et al. 2013; Dar and Rai 2014; Zhu et al. 2018), *Xylariaceae* Tul. & C. Tul. (Jurc and Ogris 2006; Henriques et al. 2014; Luchi et al. 2015) and a diverse group of basidiomycetes (Ryvarden and Gilbertson 1993; Sinclair and Lyon 2005; Crockatt et al. 2008; Prieto-Recio et al. 2012). In recent years, several fungal trunk pathogens have been reported as the main destructive and threatening factors of forest trees in northern Iran. *Biscogniauxia*

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mediterranea De Not. has been shown to be a damaging pathogen of *Quercus castaneifolia* C. A. Mey., *Q. brantii* Lindl. and *Zelkova carpinifolia* (Pall.) K. Koch trees in Iran (Mirabolfathy et al. 2011; Mirabolfathy 2013). The fungus has also been found causing charcoal disease on forest trees in other countries (Jurc and Ogris 2006; Nugent et al. 2005). According to Mohammadi et al. (2014), four species of *Togniniaceae*, namely *Phaeoacremonium parasiticum* (Ajello, Georg & C.J.K. Wang) W. Gams, Crous & M.J. Wingf., *P. minimum* Tul. and C. Tul., *P. iranianum* L. Mostert, Gräf., W. Gams & Crous and *P. rubrigenum* W. Gams, Crous & M.J. Wingf., and two species of *Botryosphaeriaceae*, *Botryosphaeria dothidea* (Moug. ex Fr) Ces. & De Not, and *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & Phillips, were associated with *Cupressus sempervirens* L. trunk diseases in Iran. During an investigation by Hashemi and Mohammadi (2016), some members of *Togniniaceae*, *Botryosphaeriaceae* and *Diatrypaceae* were isolated from willow and poplar trees in Iran. In a study considering the fungal trunk pathogens on forest trees in northern Iran, seven species of *Togniniaceae*, namely *P. parasiticum*, *P. alvesii* L. Mostert, Summerb., *P. minimum*, *P. iranianum*, *P. scolyti* L. Mostert, Summerb. & Crous, *P. fraxinopennsylvanicum* T.E. Hinds, and *P. croatiense* Essakhi, Mugnai, Surico & Crous, have been isolated and identified from various forest trees (Kazemzadeh Chakusary et al. 2017). In recent years, an extensive research effort has been concentrated on the role of *Botryosphaeriaceae* species in trunk diseases of native, ornamental and forest trees worldwide (Encinas 2001; Abdollahzadeh et al. 2009, 2013a; Alves et al. 2013; Jami et al. 2013; Pavlic-Zupanc et al. 2015).Various members of *Botryosphaeriaceae* are pathogenic fungi mostly of woody plants, including forest trees and shrubs worldwide (Slippers and Wingfield 2007; Slippers et al. 2009). Species of this family are predominantly associated with branch and trunk cankers, dieback as well as leaf yellowing and decline (Phillips 2000; Van Niekerk et al. 2004). This family consists of many well-known plant pathogens such as *Lasiodiplodia*, *Neoscytalidium*, *Neofusicoccum*, *Diplodia*, *Dothiorella* and *Botryosphaeria* species which have been reported from a wide range of plant species around the world (Phillips et al. 2013). Various species of *Botryosphaeriaceae* represent a growing threat to agricultural plants (Slippers et al. 2007; Van Niekerk et al. 2004), ornamental trees in urban areas (Begoude et al. 2010; Mayorquin et al. 2012; Hashemi and Mohammadi 2016; Linaldeddu et al. 2016; Hashemi et al. 2017) and forest ecosystem (Munck et al. 2017; Smahi et al. 2017; Zlatković et al. 2018). Some species of this family predominantly infect forest and ornamental trees worldwide. *Diplodia sapinea* shows a host preference mainly on *Pinus* species (Jankovský and Palovćiková 2003; Luchi et al. 2014; Adamson et al. 2015). Research shows that *Diplodia corticola* A.J.L. Phillips, A. Alves and J. Luque, is predominantly associated with oak trees. This taxon has been reported from trunk and branches of *Quercus suber* L. and cankered branch of *Q. ilex* L. (Alves et al. 2004). It was found to be associated with branch cankers and tip dieback on landscape live oak (*Q. virginiana*) in Florida (Dreaden et al. 2011) and the same disease symptoms on *Q. suber* in Algeria (Smahi et al. 2017). This fungus documented as the cause of stem and branch cankers on white oak (*Quercus alba* L.) in USA (Reed et al. 2018), trunk cankers and dieback of canyon live oak (*Quercus chrysolepis* Liebm.) in California (Úrbez-Torres et al. 2010), holm oak decline in Italy (Linaldeddu et al. 2014), bleeding cankers on black oak (*Quercus velutina* Lam.) (Munck et al. 2017), canker and dieback of *Quercus ilex* L., *Q. petraea* (Matt.) Liebl and *Q. suber* in France (Linaldeddu et al. 2017) and mortality of northern red oak (*Quercus rubra* L.) in Maine (Aćimović et al. 2016). *Sardiniella urbana* Linaldeddu, A. Alves and A.J.L. Phillips, as a new species in *Botryosphaeriaceae* has recently been isolated from diseased European hackberry (*Celtis australis* L.) trees showing sunken cankers, dieback and collar rot and stem exudates in an urban environment on the island of Sardinia in Italy (Linaldeddu et al. 2016). There are only a few studies regarding the impact of *Botryosphaeriaceae* species on trunk diseases of ornamental and forest trees in Iran (Mohammadi et al. 2014; Hashemi et al. 2017). Therefore, the aims of this study were to identify the *Botryosphaeriaceae* species occurring on forest trees in the north of Iran (Guilan province), using morphological and molecular characteristics. Furthermore, the pathogenicity of identified species was tested on some forest trees in field trials.

Materials and methods

Sample collection, fungal isolation and morphological characterization

From October 2012 to May 2015, various genera of forest trees (*Acer, Alnus, Carpinus, Crataegus, Cupressus, Cydonia, Diospyros, Fagus, Fraxinus, Gleditsia, Mespilus*, *Parrotia, Pinus, Populus, Prunus, Pterocarya, Punica, Quercus, Ulmus* and *Zelkova*) in Guilan province (in the north of Iran) were surveyed with the aim of isolating and identifying fungal trunk pathogens on forest trees showing disease symptoms. Wood samples were collected from trunks and branches of various trees showing yellowing, branch or trunk canker, branch dieback, sparse foliage, gummosis or death as well as woody debris. Fungal isolations were made from the discolored tissues of affected trees, as well as from fruiting bodies formed on the woody debris. From each symptomatic sample, 10–15 pieces (3 × 3 mm) of wood segments were cut from the margin between necrotic and apparently healthy tissue and placed onto malt extract agar (MEA: 2% malt extract, Merck, Darmstadt, Germany) supplemented with 100 mg/L streptomycin sulfate (MEAS). Isolations of fungi from wood debris were made directly from fruiting bodies (pycnidia) on MEA. In both cases, plates were incubated at 25 °C for 5–15 days. Pure cultures of *Botryosphaeriaceae*-like isolates were obtained by transferring single hyphal tips from the edge of colonies to fresh MEA plates.

Isolates were initially identified and grouped by comparing the main morphological and cultural characteristics (Phillips et al. 2013; Slippers et al. 2013). Pure isolates of each group were stimulated to produce fruiting body (pycnidia) by growing each isolate on water agar (Agar, 20 g l–1; Merck, Germany) containing 5–7 autoclaved segments of wood of trees (1.5 to 2 cm) or pine needles (Slippers et al. 2004). Plates were exposed to light with a 12-h photoperiod at 22–25 °C and examined for the formation of pycnidia, during an incubation period of 15–40 days. After sporulation, the main morphological characteristics of conidia (length and width, color, shape and presence or absence of septations) were observed and recorded (Slippers et al. 2004; Phillips 2006; Phillips et al. 2013). For each isolate, 40 to 50 conidia were measured and evaluated using a light microscope (BH2, Olympus Optical, Tokyo, Japan, at × 1000 magnification).

DNA isolation and amplification

Single hyphal tip of *Botryosphaeriaceae* isolates from each group was grown on 2% MEA at 25 °C for 8–10 days. Mycelial mat produced by each isolate was scraped directly from the medium and transferred to a cool mortar and mechanically disrupted by grinding to a fine powder using liquid nitrogen with a pestle. Total DNA was extracted using an AccuPrep Genomic DNA Extraction Kit (Bioneer, Daejeon, South Korea) following the manufacturer's instruction manual. Extracted DNA samples were visualized on 0.1% agarose gels (UltraPureTM Agarose; Invitrogen, Carlsbad, California, USA) stained with ethidium bromide, and were kept at − 20 °C for the next process.

Identification of the isolates was confirmed by sequence analysis of the internal transcribed spacer (ITS) of the rDNA and the translation elongation factor 1-alpha (*tef*-1α) regions using primers ITS1/ITS4 (White et al. 1990) and EF1-728F/EF1-986R (Carbone and Kohn 1999), respectively. The polymerase chain reaction (PCR) was performed in a Techne TC-312 Thermal Cycler (Techne, Cambridge, UK) as previously described by Hashemi and Mohammadi (2016). PCR products were visualized under UV light on a 1% agarose gel (UltraPureTM Agarose, Invitrogen) containing ethidium bromide. A 100-bp ladder (Gene Ruler, TMDNA Ladder Mix, Fermentas) was used as a molecular weight marker. PCR products were purified and sequenced by Bioneer Corporation (Daejeon, South Korea). All sequences were read and edited with BioEdit Sequence Alignment Editor v. 7.0.9.0 (Hall 2006). Edited sequences were run through the BLAST (Basic Local Alignment Search Tool, http://blast.ncbi.nlm.nih.gov/Blast.cgi) to determine basic identity of the isolates.

Phylogenetic analysis

ITS and *tef*-1α sequences of 62 isolates of *Botryosphaeriaceae* species obtained in this study were combined with the sequences of various *Botryosphaeriaceae* species from GenBank. Sequences of two isolates of *Pseudofusicoccum stromaticum* Mohali, Slippers. and M.J. Wingf. (CBS117448 and CBS117449) were added as out-groups. Sequences were aligned using MAFFT v. 7 (Katoh and Standley 2013). Alignments were checked, and manual adjustments were made where necessary. Phylogenetic information contained in indels (gaps) was incorporated into the phylogenetic analyses using simple indel coding as implemented by GapCoder (Young and Healy 2003). Phylogenetic analyses were carried out with PAUP for maximum parsimony (MP) analysis as described by Abdollahzadeh, et al. (2010). CI (tree length, consistency index), RI (retention index) and RC (rescaled consistency index) were calculated. Bootstrap support was estimated using 1000 replicates to assess the robustness of each clade. New sequences were deposited in GenBank (Table 1).

Pathogenicity trials and fungal re-isolations

The pathogenicity of the two isolates from each identified species was tested on branches of selected forest trees (12–15 years old) in the north of Iran. Six tree species, *Punica granatum* L., *Alnus glutinosa* (L.) Gaertn, *Parrotia persica* (DC.) C. A. Mey., *Pterocarya fraxinifolia* (Lam.) Spach., *Mespilus germanica* L., and *Q. castaneifolia,* were used for fungal inoculations. The region of each branch to be inoculated was surface-disinfested with 75% ethyl alcohol. A wound was made between two nodes of each branch using a cork borer of 4 mm diameter. Each wound was inoculated with a mycelial plug (4 mm in diameter) taken from the margin of an actively growing colony on MEA (10 days old) and then protected by moist cotton and wrapped immediately with a strip of Parafilm® (Pechiney Plastic Packaging, Menasha, USA). Five branches of six tree species per isolate were inoculated, and an equal number was also inoculated with sterile PDA plugs, which served as controls. The length of wood lesion, produced by inoculated isolates, was measured after two months, and re-isolations were done from the edges of discolored tissues on the treatment and control branches and placed on PDA. Petri dishes were incubated at 25 °C, and re-isolated fungi were identified based on colony

Table 1 *Botryosphaeriaceae* isolates used in the phylogenetic analyses (GenBank isolates are shown in bold face)

Isolates		Host	Location	GenBank accession numbers		References
Species	Code			ITS	EF	
Dothiorella sarmentorum	IRNHM-KZS1	*Quercus castaneifolia*	Iran	MG192331	MG192352	This study
	IRNHM-KZS2	*Quercus castaneifolia*	Iran	–	–	This study
	IRNHM-KZS3	*Alnus glutinosa*	Iran	MG192332	MG192353	This study
	IRNHM-KZS4	*Alnus glutinosa*	Iran	–	–	This study
	IRNHM-KZS5	*Quercus castaneifolia*	Iran	–	–	This study
	IMI 63581b	*Ulmus* sp.	UK	AY573212	AY573235	Phillips et al. (2005)
	CBS 115038	*Malus pumila*	The Netherlands	AY573206	AY573223	Phillips et al. (2005)
Dothiorella plurivora	IRNHM-KZm1	*Diospyros lotus*	Iran	MG192341	MG192362	This study
	IRNHM-KZm2	*Diospyros lotus*	Iran	–	–	This study
	IRNHM-KZm3	*Populus caspica*	Iran	MG192342	MG192363	This study
	IRNHM-KZm4	*Populus caspica*	Iran	–	–	This study
	IRNHM-KZm5	*Populus caspica*	Iran	–	–	This study
	IRNHM-KZM7	*Diospyros lotus*	Iran	MG198184	MG198171	This study
	IRNHM-KZm8	*Quercus castaneifolia*	Iran	MG198185	MG198172	This study
	IRNHM-KZm10	*Alnus glutinosa*	Iran	MG198186	MG198173	This study
	CBS 117006	*Vitis vinifera*	Spain	AY905555	AY905562	Luque et al. (2005)
Dothiorella americana	**CBS 128309**	*Vitis vinifera*	USA	HQ288218	HQ288262	Urbez-Torres et al. (2012)
	CBS 128310	*Vitis vinifera*	USA	HQ288219	HQ288263	Urbez-Torres et al. (2012)
Neofusicoccum parvum	IRNHM-KZP1	*Alnus glutinosa*	Iran	MG192322	MG192343	This study
	IRNHM-KZP2	*Alnus glutinosa*	Iran	–	–	This study
	IRNHM-KZP3	*Parrotia persica*	Iran	MG192323	MG192344	This study
	IRNHM-KZP4	*Parrotia persica*	Iran	–	–	This study
	IRNHM-KZP5	*Parrotia persica*	Iran	–	–	This study
	IRNHM-KZP6	*Carpinus betulus*	Iran	MG192324	MG192345	This study
	CBS:719.85	*Populus nigra*	New Zealand	AY236943	AY236888	Slippers et al. (2004)
Botryosphaeria dothidea	IRNHM-KZ5	*Populus caspica*	Iran	MG456721	MG456733	This study
	IRNHM-KZ11	*Prunus divaricata*	Iran	MG198187	MG198174	This study
	IRNHM-KZ27	*Punica granatum*	Iran	MG198188	MG198175	This study
	IRNHM-KZ30	*Crataegus pentagyna*	Iran	MG198189	MG198176	This study
	IRNHM-KZ39	*Populus caspica*	Iran	MG198190	MG198177	This study
	IRNHM-KZ49	*Populus caspica*	Iran	MG198191	MG198178	This study
	IRNHM-KZ52	*Punica granatum*	Iran	MG198192	MG198179	This study
Botryosphaeria dothidea	IRNHM-KZ77	*Parrotia persica*	Iran	MG198193	MG198180	This study
	IRNHM-KZ78	*Prunus divaricata*	Iran	MG198194	MG198181	This study
	IRNHM-KB168	*Parrotia persica*	Iran	MG198195	MG198182	This study
	IRNHM-KZ172	*Ulmus carpinifolia*	Iran	MG198196	MG198183	This study
	IRNHM-KB93	*Populus caspica*	Iran	MG456722	MG456734	This study
	CBS 110302	*Vitis vinifera*	Portugal	AY259092	AY573218	Alves et al. (2004)
	CBS 115476	*Prunus* sp.	Switzerland	KF766151	AY236898	Slippers et al. (2013)

Table 1 (continued)

Isolates		Host	Location	GenBank accession numbers		References
Species	Code			ITS	EF	
Neoscytalidium novae-hollandiae	IRNHM-KZN1	*Pinus* sp.	Iran	MG220373	MG220380	This study
	IRNHM-KZN2	*Crataegus pentagyna*	Iran	MG220374	MG220381	This study
	IRNHM-KZN3	*Carpinus betulus*	Iran	MG220375	MG220382	This study
	IRNHM-KZN4	*Fagus orientalis*	Iran	MG220376	MG220383	This study
	IRNHM-KZN5	*Alnus glutinosa*	Iran	–	–	This study
	IRNHM-KZN6	*Pinus eldarica*	Iran	–	–	This study
	IRNHM-KZN7	*Alnus glutinosa*	Iran	–	–	This study
	IRNHM-KZN8	*Crataegus pentagyna*	Iran	–	–	This study
	CBS122610	*Acacia synchronicia*	Australia	EF585536	EF585578	Pavlic et al. (2008)
	CBS122071	*Crotalaria medicag-inea*	Australia	EF585540	EF585580	Pavlic et al. (2008)
Diplodia seriata	KZD1	*Parrotia persica*	Iran	MG192325	MG192346	This study
	KZD2	*Acer cappadocicum*	Iran	MG192326	MG192347	This study
	KZD3	*Parrotia persica*	Iran	MG192327	MG192348	This study
	KZD4	*Zelkova carpinifolia*	Iran	MG192328	MG192349	This study
	KZD5	*Fraxinus excelsior*	Iran	MG192329	MG192350	This study
	KZD6	*Fagus orientalis*	Iran	MG192330	MG192351	This study
	KZD7	*Acer cappadocicum*	Iran	–	–	This study
	KZD59	*Fagus orientalis*	Iran	–	–	This study
	CBS 112555	*Vitis vinifera*	Portugal	KF766161	AY573220	Alves et al. (2004)
	CBS 119049	*Vitis* sp.	Italy	DQ458889	DQ458874	Alves et al. (2006)
	CBS 134700	*Prunus laurocerasus*	Italy	KC869996	KC869998	Quaglia et al. (2014)
Diplodia intermedia	IRNHM-KZI1	*Gleditsia caspica*	Iran	MG220378	MG220385	This study
	IRNHM-KZI2	*Fagus orientalis*	Iran	MG220379	MG220386	This study
Diplodia intermedia	IRNHM-KZI3	–	Iran	–	–	This study
	CBS 124462	*Malus sylvestris*	Portugal	GQ923858	GQ923826	Phillips et al. (2012)
Diplodia sapinea	IRNHM-KZ400	*Pinus eldarica*	Iran	MG456720	MG456732	This study
	IRNHM-KZ4001	*Pinus eldarica*	Iran	–	–	This study
	CBS 393.84	*Pinus nigra*	The Netherlands	DQ458895	DQ458880	Alves et al. (2006)
	CBS 109725	*Pinus patula*	Indonesia	DQ458896	DQ458881	Phillips et al. (2008)
Lasiodiplodia mahajangana	IRNHM-KZL1	*Pterocarya fraxinifolia*	Iran	MG192333	MG192354	This study
	IRNHM-KZL2	*Quercus castaneifolia*	Iran	MG192334	MG192355	This study
	IRNHM-KZL3	*Ulmus carpinifolia*	Iran	MG192335	MG192356	This study
	IRNHM-KZL4	*Zelkova carpinifolia*	Iran	MG192336	MG192357	This study
	IRNHM-KZL5	*Pterocarya fraxinifolia*	Iran	MG192337	MG192358	This study
	IRNHM-KZL6	*Quercus castaneifolia*	Iran	MG192338	MG192359	This study
	IRNHM-KZL7	*Quercus castaneifolia*	Iran	–	–	This study
	IRNHM-KZL8	*Pterocarya fraxinifolia*	Iran	–	–	This study
	IRNHM-KZL9	*Zelkova carpinifolia*	Iran	MG192339	MG192360	This study
	IRNHM-KZL10	*Ulmus carpinifolia*	Iran	MG192340	MG192361	This study
	CBS 124927	*Terminalia catappa*	Madagascar	FJ900597	FJ900643	Begoude et al. (2010)
Dothiorella omnivora	**CBS 188.87**	*Juglans regia*	France	EU673316	EU673283	Phillips et al. (2008)
	CBS 242.51	–	Italy	EU673317	EU673284	Phillips et al. (2008)
	IRAN 1570C	–	Iran	KC898233	KC898216	Abdollahzadeh et al. (2014)
	IRAN 1573C	–	Iran	KC898232	KC898215	Abdollahzadeh et al. (2014)

Table 1 (continued)

Isolates		Host	Location	GenBank accession numbers		References
Species	Code			ITS	EF	
Dothiorella semper-virentis	**IRAN1581C**	–	Iran	KC898237	KC898220	Abdollahzadeh et al. (2014)
	IRAN1583C	–	Iran	KC898236	KC898219	Abdollahzadeh et al. (2014)
Dothiorella parva	**IRAN1579C**	–	Iran	KC898234	KC898217	Abdollahzadeh et al. (2014)
	IRAN1585C	–	Iran	KC898235	KC898218	Abdollahzadeh et al. (2014)
Dothiorella iberica	**CBS 115041**	*Quercus ilex*	Spain	AY573202	AY573222	Phillips et al. (2005)
	CBS 113188	*Medicago sativa*	South Africa	EU673318	EU673285	Phillips et al. (2008)
Dothiorella mangiferae	**CBS 500.72**	*Medicago sativa*	South Africa	EU673318	EU673285	Phillips et al. (2008)
Dothiorella mangif-ericola	**CBS:124727**	*Mangifera indica*	Iran	KC898221	KC898204	Yang et al. (2017)
Dothiorella citricola	**ICMP16828**	*Citrus sinensis*	New Zealand	EU673323	EU673290	Phillips et al. (2008)
Dothiorella westralis	**CBS117007**	*Vitis vinifera*	Spain	AY905556	KX464623	Yang et al. (2017)
Dothorella viticola	**CBS 117009**	*Vitis vinifera*	Spain	KF766228	AY905559	Slippers et al. (2013)
Neofusicoccum brasil-iense	**CMM 1285**	*Mangifera indica*	Brazil	JX513628	JX513608	Marques et al. (2013)
Neofusicoccum corda-ticola	**CBS 123634**	*Syzygium cordatum*	South Africa	EU821898	EU821868	Pavlic et al. (2009)
Neofusicoccum umdonicola	**CBS 123645**	*Syzygium cordatum*	South Africa	KF766206	KF766427	Slippers et al. (2013)
Neofusicoccum kwam-bonambiense	**CBS 123639**	*Syzygium cordatum*	South Africa	EU821904	EU821874	Pavlic et al. (2009), Yang et al. (2017)
Neofusicoccum ribis	**CBS 115475**	*Ribes* sp.	USA	AY236935	AY236877	–
Neofusicoccum occu-latum	**CBS 128008**	*Eucalyptus grandis*	Australia	EU301030	EU339509	–
Botryosphaeria fabicerciana	**CBS 127193**	*Eucalyptus* sp.	China	HQ332197	HQ332213	Chen et al. (2011)
Botryosphaeria fusis-pora	**MFLUCC 10-0098**	*Entada* sp.	Thailand	JX646789	JX646854	Liu et al. (2012)
Botryosphaeria cortices	**CBS 119047**	*Vaccinium corymbo-sum*	USA	DQ299245	EU017539	Phillips et al. (2006)
Botryosphaeria ramosa	**CBS 122069**	*Eucalyptus camaldu-lensis*	Australia	KF766168	EU144070	Pavlic et al. (2008)
Botryosphaeria scharifii	**CBS 124703**	–	Iran	JQ772020	JQ772057	Abdollahzadeh et al. (2013b)
Botryosphaeria agaves	**CBS 133992**	*Agave* sp.	Thailand	JX646790	JX646855	Liu et al. (2012)
Neoscytalidium hya-linum	**CBS 145.78**	*Homo sapiens*	UK	KF531816	KF531795	Phillips et al. (2013)
	CBS 251.49	*Juglans regia*	USA	KF531819	KF531797	Phillips et al. (2013)
	CBS 499.66	*Mangifera indica*	Mali	KF531820	KF531798	Phillips et al. (2013)
Diplodia pseudoseriata	**CBS 124906**	*Blepharocalyx salici-folius*	Uruguay	EU080927	EU863181	Pérez et al. (2010)
Diplodia pseudoseriata	**CBS124907**	*Pterocarpus ango-lensis*	South Africa	FJ888478	FJ888446	–
Diplodia alatafructa	**CBS 124931**	*Pterocarpus ango-lensis*	South Africa	FJ888461	J888445	Mehl et al. (2011)
	CBS 124933	*Pterocarpus ango-lensis*	South Africa	FJ888478	FJ888446	Mehl et al. (2011)
Diplodia scrobiculata	**CBS 109944**	*Pinus greggii*	Mexico	DQ458899	DQ458884	Alves et al. (2006)

Table 1 (continued)

Isolates		Host	Location	GenBank accession numbers		References
Species	Code			ITS	EF	
	CBS 113423	*Pinus greggii*	Mexico	DQ458900	DQ458885	Alves et al. (2006)
Diplodia scrobiculata	**CBS 118110**	*Pinus banksiana*	USA	KF766160	KF766399	Slippers et al. (2013)
Diplodia allocellula	**CBS 130408**	*Acacia karroo*	South Africa	JQ239397	JQ239384	Jami et al. (2013)
	CBS 130410	*Acacia karroo*	South Africa	JQ239399	JQ239386	Jami et al. (2013)
Diplodia rosulata	**CBS 116470**	*Prunus africana*	Ethiopia	EU430265	EU430267	Phillips et al. (2008)
	CBS 116472	*Prunus africana*	Ethiopia	EU430266	EU430268	Phillips et al. (2008)
Diplodia rosacearum	**CB S141915**	*Eriobotrya japonica*	Italy	KT956270	KU378605	Giambra et al. (2016)
Lasiodiplodia exigua	**CBS 137785**	*Retama raetam*	Tunisia	KJ638317	KJ638336	Linaldeddu et al. (2015)
Lasiodiplodia caatinguensis	**CMM 1325**	*Citrus sinensis*	Brazil	KT154760	KT008006	–
Lasiodiplodia theobromae	**CBS 164.96**	fruit along coral reef coast	Papua New Guinea	AY640255	AY640258	Phillips et al. (2005)
	CBS 111530	–	–	EF622074	EF622054	Alves et al. (2008)
Lasiodiplodia brasiliensis	**CMM 4015**	*Mangifera indica*	Brazil	JX464063	JX464049	Marques et al. (2013)
Lasiodiplodia vitis	**CBS 124060**	*Vitis vinifera*	Italy	KX464148	KX464642	Yang et al. (2017)
Lasiodiplodia viticola	**UCD 2604MO**	*Vitis* sp.	USA	HQ288228	HQ288270	Urbez-Torres et al. (2012)
Lasiodiplodia iranensis	**CBS 124710**	*Salvadora persica*	Iran	GU945348	GU945336	Abdollahzadeh et al. (2010)
	CBS 124711	–	Iran	GU945347	GU945335	Abdollahzadeh et al. (2010)
Lasiodiplodia jatrophicola	**CMM 3610**	*Jatropha curcas*	Brazil	KF234544	KF226690	Machado et al. (2014)
Pseudofusicoccum stromaticum	**CBS 117448**	*Eucalyptus hybrid*	Venezuela	AY693974	AY693975	Slippers et al. (2013)
	CBS 117449	*Eucalyptus hybrid*	Venezuela	DQ436935	DQ436936	Mohali et al. (2006)

and conidial morphology. Recorded data were checked for normal distribution using the Shapiro–Wilk and Kolmogorov–Smirnov tests. Results were subjected to analysis of variance (ANOVA) using SAS v 9.1 (SAS Institute, Cary, NC, USA). The LSD test was used for comparison of treatment means at $p < 0.05$.

Results

Sample collection, fungal isolation and morphological characterization

In our study, 221 forest trees belonging to 20 species were surveyed and 765 wood samples were collected from trees showing yellowing, sparse foliage, branch and trunk canker, branch dieback, gummosis and death and various wood lesion types in cross sections. In total, 118 *Botryosphaeriaceae*-like isolates were obtained in this study. All isolates produced fruiting structures (pycnidia) and sporulated efficiently on pine needles or autoclaved segments of wood of trees on WA media within 15 to 35 days. Isolates were separated into five main groups based on cultural characteristics and conidial morphology.

Twenty-two isolates (8 from infected wood tissues and 14 from wood debris) were classified in group one: Conidia in this group were sub-ovoid to ellipsoid ovoid, initially hyaline and aseptate, dark brown 1-septate after discharge from the conidiomata and showing longitudinal striations. All these isolates were identified as *Lasiodiplodia* spp.

Fourteen isolates (8 from infected wood tissues and 6 from wood debris) were placed in the second group. Isolates of this group produced colonies with gray to dark gray aerial mycelia. These isolates produced chains of arthroconidia with brown walls in the aerial mycelia. These characteristics were consistent with the description of *Neoscytalidium* spp.

Nineteen isolates (12 from trees and 7 from wood debris) were put in group three. Isolates of this group produced diplodia-like conidia. Dark brown conidia were observed within the pycnidia, while they were attached to the conidiogenous cells. All these isolates were identified as *Diplodia* spp.

Forty-five isolates of *Botryosphaeriaceae* were classified in group four. These isolates were obtained from wood discolored tissues (29 isolates) and wood debris (16 isolates). Conidia narrowly fusiform or irregularly fusiform. According to morphological characteristics, these isolates were identified as *Neofusicoccum* sp. or *Botryosphaeria* sp.

The last fungal group included 18 isolates (11 from infected trees and 7 from wood debris). Conidia in these isolates were initially hyaline and became dark brown and 1-septate within the pycnidia often while still attached to the conidiogenous cells. Based on these morphological features, these isolates were identified as *Dothiorella* spp.

Phylogenetic analysis

The combined ITS and *tef*-1α sequences alignment for in-group and two out-group isolates contained 1683 characters including alignment gaps, of which 734 characters were excluded, 580 were constant, 32 were variable and parsimony-uninformative and 337 were parsimony-informative. Gaps were treated as "missing." A heuristic search of these 321 parsimony-informative characters resulted in 24 most parsimonious trees of 278 steps (CI = 0.74, HI = 0.16, RI = 0.96) with the same overall topology. One of the MP trees is shown in Fig. 1 with MP bootstrap support values at the nodes. According to the phylogenetic analyses and combination of ITS and *tef*-1α sequences, *Botryosphaeriaceae* isolates obtained in the current study were placed in nine clades representing *Dothiorella sarmentorum* (5 isolates), *Dothiorella plurivora* (8 isolates), *Neofusicoccum parvum* (6 isolates), *Botryosphaeria dothidea* (12 isolates), *Neoscytalidium novaehollandiae* (8 isolates), *Diplodia seriata* (8 isolates), *Diplodia sapinea* (2 isolates), *Lasiodiplodia mahajangana* (10 isolates) and *Diplodia intermedia* (3 isolates).

Host, frequency and distribution of *Botryosphaeriaceae* species

From affected wood tissues

During this study, 20 species of trees were studied in the forest areas of Guilan Province in the north of Iran. In total, 118 *Botryosphaeriaceae*-like isolates were obtained from symptomatic trees and wood debris from various forest areas (Fig. 2). Of these, 68 isolates (57.6% of total isolates) were recovered from affected wood tissues of tree species in nine forest areas. *Botryosphaeriaceae* isolates were obtained from 15 tree species belonging to 13 families. Five species of woody hosts, *M. germanica, Cydonia oblonga* Mill., *Acer cappadocicum* Gled., *Ulmus carpinifolia* Gled. and *C. sempervirens*, did not yield any *Botryosphaeriaceae* isolate. According to Table 2, one to five different species of *Botryosphaeriaceae* per individual tree species were isolated. Nine of 15 tree species yielded only one species, *Crataegus pentagyna* Waldst. & Kit. ex Willd., two species, *Fagus orientalis* Lipsky, *Carpinus betulus* L.*, P. persica* and *Q. castaneifolia* three species and *A. glutinosa* five species.

Nine *Botryosphaeriaceae* species, *L. mahajangana*, *N. novaehollandiae*, *D. seriata*, *B. dothidea*, *N. parvum*, *D. intermedia*, *Do. sarmentorum*, *Do. plurivora* and *D. sapinea*, were isolated and identified from affected trees based on morphology, culture characteristics and DNA sequence analysis. Of these, *B. dothidea* was the most common *Botryosphaeriaceae* species (33.8% of total isolates) followed by *L. mahajangana*, *N. novaehollandiae* and *D. seriata* (11.8% for each species), *Do. sarmentorum* (10.3%), *N. parvum* (8.8%), *Do. plurivora* (5.9%), *D. intermedia* (4.4%) and *D. sapinea* (1.4%).

Botryosphaeria dothidea was recovered from infected tissues of seven species of trees in four main regions including Manjil, Shaft, Masal and Rasht. Isolates of this species were obtained from *P. granatum* (21.8%), *A. glutinosa* (13.0%), *C. betulus* (17.4%), *P. persica* (17.4%), *C. pentagyna* (8.7%), *Populus caspica* Bornm. (13.0%) and *Prunus divaricata* Ledeb. subsp. *divaricata* (8.7%). *Lasiodiplodia mahajangana* was isolated from *P. fraxinifolia* (62.5%) in Asalem and *Q. castaneifolia* (37.5%) in Astara. *Noescytalidium novaehollandiae* was associated with *C. betulus* (50.0%) in Rasht and Masal, *F. orientalis* (12.5%) in Masal and *A. glutinosa* (37.5%) in Rasht and Shaft. *Diplodia seriata* was recovered at the frequency of 25% from each species of *F. orientalis* and *Z. carpinifolia* in Masal and *Fraxinus excelsior* L. and *P. persica* in Astara. Of the nine *Botryosphaeriaceae* species, seven isolates were identified as *Do. sarmentorum.* This species was obtained from *Q. castaneifolia* (71.4%) in Talesh and Rasht and *A. glutinosa* (28.6%) in Anzali. *Neofusicoccum parvum* occurred on three tree species, *A. glutinosa* (in Talesh), *P. persica* (in Saravan) and *C. betulus* (in Masal) with a frequency of 33.3, 50.0 and 16.7%, respectively. We obtained four isolates of *Do. plurivora* from *Diospyros lotus* L. (in Talesh), *Q. castaneifolia* (in Rasht) and *A. glutinosa* (in Anzali) with 25.0, 50.0 and 25.0%, respectively. Three isolates of *D. intermedia* were also detected from affected samples of *Gleditsia caspica* Desf. (66.7%) in Talesh and *F. orientalis* (33.3%) in Masal. In this work, only one isolate of *D. sapinea* was isolated from discolored wood tissues of *Pinus eldarica* Medw. in Rasht.

Botryosphaeriaceae on wood debris

Many pycnidia of *Botryosphaeriaceae* isolates were detected on the surface of the dead branches and debris collected in the seven forest regions visited. During this study, 50 isolates of *Botryosphaeriaceae* (42.4% total isolates) were obtained from wood debris of forest trees. Fruiting bodies of six *Botryosphaeriaceae* species

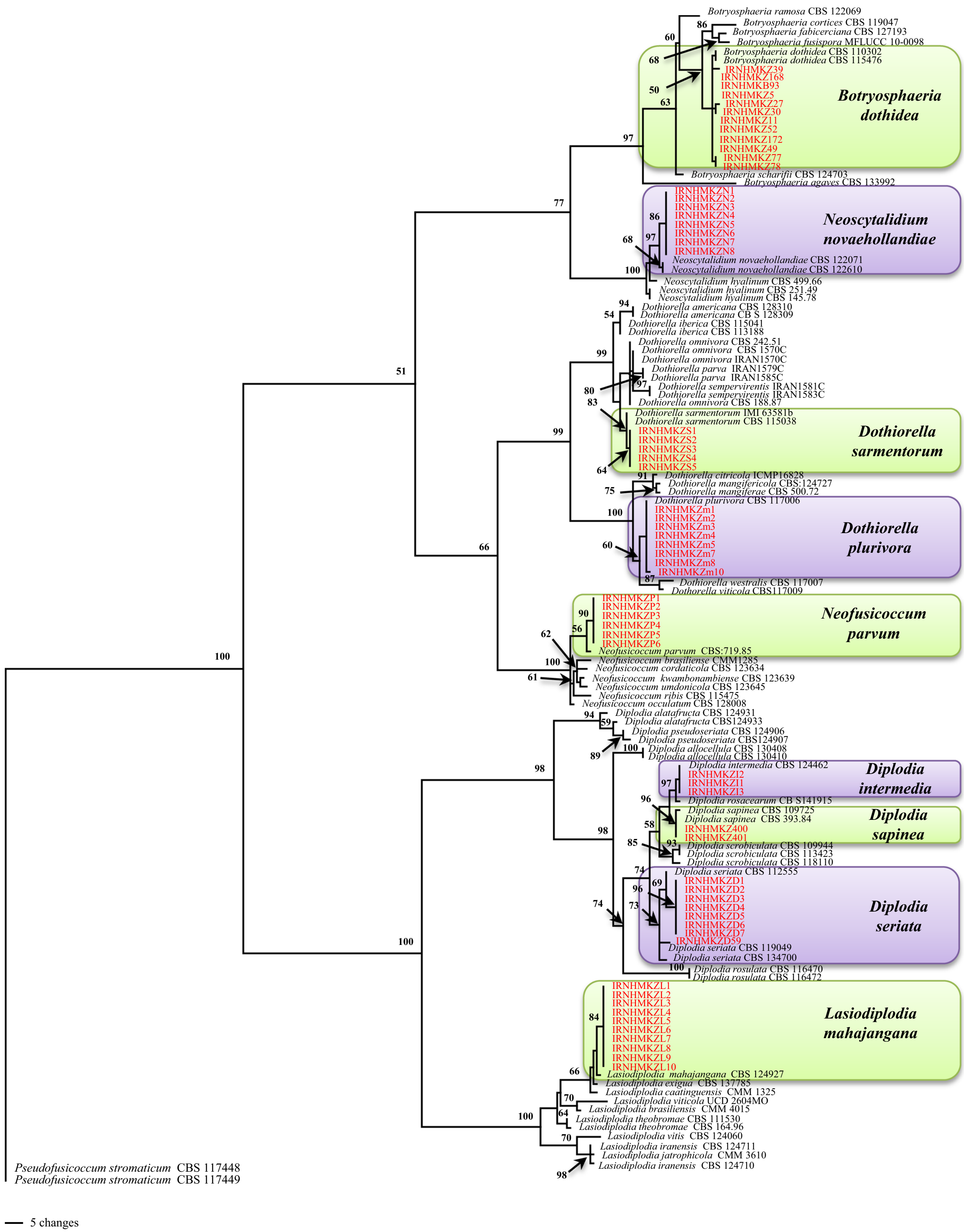

Fig. 1 One of the most parsimonious trees for *Botryosphaeriaceae* obtained from combined ITS and *tef-1α* sequence data. MP bootstrap values based on 1000 pseudoreplicates are given at the nodes. *Pseudofusicoccum stromaticum* (CBS 117448, CBS 117449) was used as out-group and Iranian isolates obtained in this study shown in red color. Bar represents five changes

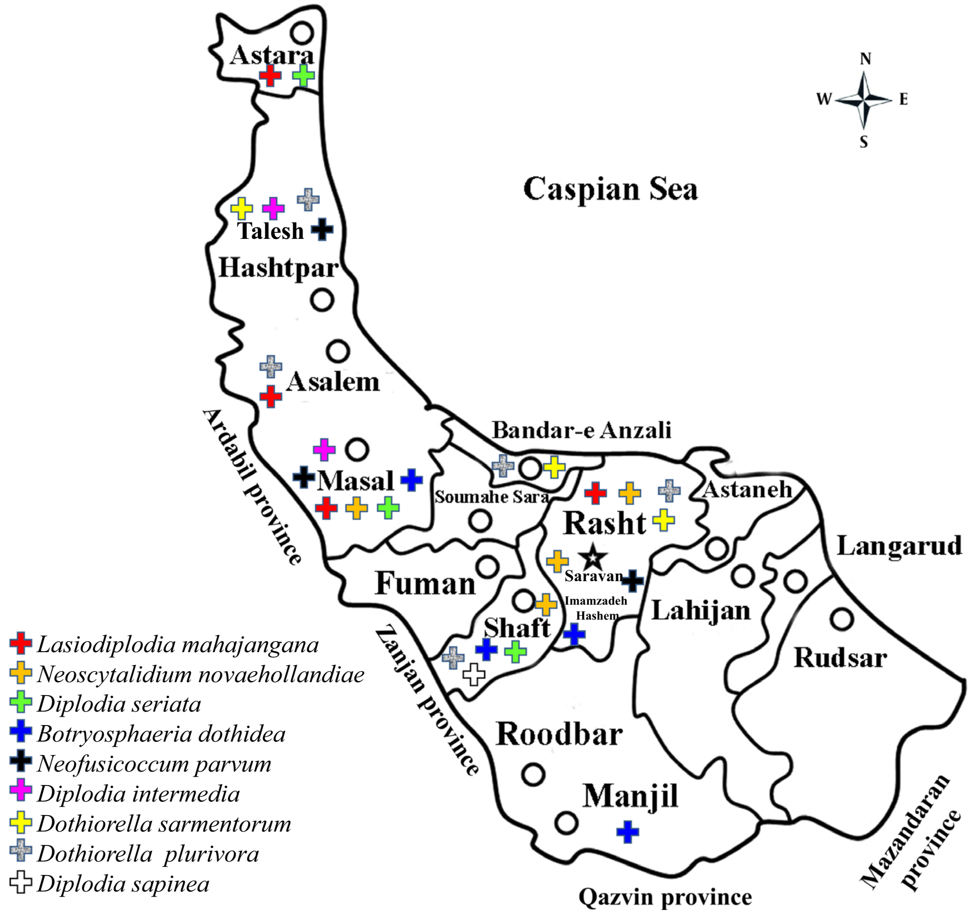

Fig. 2 Guilan Province map (northern Iran) indicating regions where forest trees were sampled and the species of *Botryosphaeriaceae* obtained from each site. The nine species of *Botryosphaeriaceae* are indicated by plus symbols with various colors

(*B. dothidea*, *L. mahajangana*, *N. novaehollandiae*, *D. seriata*, *Do. plurivora* and *D. sapinea*) were detected from wood debris of 12 tree species (Table 2). The highest percentages of *Botryosphaeriaceae* isolates (26.0%) were obtained from *P. caspica* debris, whereas the lowest recoveries (2.0%) were recorded for *P. eldarica* debris. *Botryosphaeria dothidea* with 16 (32.0%) and *L. mahajangana* with 14 (28.0%) isolates were the most frequent species. In this regard, *D. sapinea* with one isolate (2.0%) had the lowest isolation. *Botryosphaeria dothidea* and *L. mahajangana* were associated with wood debris of four tree species. *Botryosphaeria dothidea* was detected from wood debris of *P. granatum*, *C. betulus*, *C. pentagyna* and *P. caspica*, while the latter was observed on wood surface of *Z. carpinifolia*, *U. carpinifolia*, *Q. castaneifolia* and *P. fraxinifolia.* Three *Botryosphaeriaceae* species, i.e., *N. novaehollandiae*, *D. seriata* and *Do. plurivora*, were isolated from wood debris of two tree species. *Neoscytalidium novaehollandiae* was found on wood of *P. eldarica* and *C. pentagyna*. *Diplodia seriata* was isolated from *P. persica* and *A. cappadocicum*, and finally, *Do. plurivora* was associated with debris of *D. lotus* and *P. caspica.* *Diplodia sapinea* was only observed on the wood surface of *P. eldarica*. Based on our results, three species, *Do. sarmentorum*, *N. parvum* and *D. intermedia*, were only isolated from infected wood tissues.

Pathogenicity tests

According to Table 3, all inoculations with selected isolates of *Botryosphaeriaceae* obtained in this study resulted in visible brown to black wood lesions upward and downward from the point of inoculation on branches after 2 months. No wood lesions were observed on control shoots inoculated with sterile PDA plugs. Our results showed a variation in wood lesion lengths and re-isolation frequencies of inoculated isolates. Length of wood lesions and re-isolation frequencies of inoculated isolates varied within and between species. Additionally, a small irregular or wedge-shaped necrosis was frequently observed in cross sections of all inoculated branches. Three species of *B. dothidea*, *N. novaehollandiae* and *D. intermadia* produced the longest lesions on inoculated branches, whereas two species of *Diplodia*, *D. seriata* and *D. sapinea*, produced the smallest lesions. Re-isolation percentages were between 12.5% (*N. parvum* and *L. mahajangana*) and 100% (*B. dothidea* and *N. novaehollandiae*) on inoculated tree species, and no *Botryosphaeriaceae* were obtained from control shoots.

Table 2 Geographical origin and host plant species of *Botryosphaeriaceae* species in Guilan Province (northern Iran)

Species	Host		Number of the fungal isolates		Locality
	Scientific name	Family	Trees*	Wood debris*	
Botryosphaeria dothidea			**23**	**16**	
	Punica granatum L. 1753	*Punicaceae*	5	2	Manjil
	Alnus glutinosa (L.) Gaertn, 1790	*Betulaceae*	3	0	Shaft
	Carpinus betulus L. 1753	*Corylaceae*	4	4	Masal
	Parrotia persica (D.C.) C. A. Meyer. 1831	*Hammamelidaceae*	4	0	Shaft (Jirdeh)
	Crataegus pentagyna Waldst. & Kit. 1799	*Rosaceae*	2	2	Shaft
	Populus caspica Bornm. 1939	*Salicaceae*	3	8	Rasht (Imamzadeh Hashem)
	Prunus divaricata Ledeb. subsp. *divaricata*	*Rosaceae*	2	0	Shaft
Neofusicoccum parvum			**6**	**0**	
	Alnus glutinosa	*Betulaceae*	2	0	Talesh
	Parrotia persica	*Hammamelidaceae*	3	0	Saravan
	Carpinus betulus	*Corylaceae*	1	0	Masal
Lasiodiplodia mahajangana			**8**	**14**	
	Pterocarya fraxinifolia (Lam.) Spach. 1834	*Juglandaceae*	5	0	Asalem
	Quercus castaneifolia C. A. Mey. 1971	*Fagaceae*	3	0	Astara
	Zelkova carpinifolia (pall.) Dipp. 1892	*Ulmaceae*	0	3	Masal
	Ulmus carpinifolia Borkh. 1793	*Ulmaceae*	0	2	Astara
	Quercus castaneifolia	*Fagaceae*	0	5	Rasht
	Pterocarya fraxinifolia	*Juglandaceae*	0	4	Asalem
Neoscytalidium novaehollandiae			**8**	**6**	
	Carpinus betulus	*Corylaceae*	4	0	Rasht, Masal
	Fagus orientalis Lipsky. 1898	*Fagaceae*	1	0	Masal
	Alnus glutinosa	*Betulaceae*	3	0	Rasht, Shaft
	Pinus eldarica Medw. 1902	*Pinaceae*	0	2	Saravan
	Crataegus pentagyna	*Rosaceae*	0	4	Shaft (Jirfeh)
Diplodia seriata			**8**	**6**	
	Fagus orientalis	*Fagaceae*	2	0	Masal
	Fraxinus excelsior L. 1753	*Oleaceae*	2	0	Astara
	Zelkova carpinifolia	*Ulmaceae*	2	0	Masal
	Parrotia persica	*Hammamelidaceae*	2	0	Astara
	Parrotia persica	*Hammamelidaceae*	0	2	Shaft
	Acer cappadocicum Gled. 1784	*Aceraceae*	0	4	Shaft
Diplodia intermedia			**3**	**0**	
	Gleditsia caspica Desf. 1809	*Leguminosae/Caesalpinaceae*	2	0	Talesh
	Fagus orientalis	*Fagaceae*	1	0	Masal
Dothiorella sarmentorum			**7**	**0**	
	Quercus castaneifolia	*Fagaceae*	2	0	Talesh
	Quercus castaneifolia	*Fagaceae*	3	0	Rasht
	Alnus glutinosa	*Betulaceae*	2	0	Anzali

Table 2 (continued)

Species	Host		Number of the fungal isolates		Locality
	Scientific name	Family	Trees*	Wood debris*	
Dothiorella plurivora			**4**	**7**	
	Diospyros lotus L. 1753	*Ebenaceae*	1	0	Talesh
	Quercus castaneifolia	*Fagaceae*	2	0	Rasht
	Alnus glutinosa	*Betulaceae*	1	0	Anzali
	Diospyros lotus	*Ebenaceae*	0	2	Asalem
	Populus caspica	*Salicaceae*	0	5	Shaft
Diplodia sapinea			**1**	**1**	
	Pinus eldarica	*Pinaceae*	0	1	Shaft
	Pinus eldarica	*Pinaceae*	1	0	Rasht
Nine Botryosphaeriaceae species	17 tree species	13 families	68 isolates	50 isolates	9 main areas

* Bold numbers in columns represent the total isolates of each species obtained from trees or wood debris

On *P. granatum, B. dothidea* (IRNHM-KZ39) produced the longest lesions (135.00 mm) and was the most virulent species on this host. On this host, *D. seriata* (IRNHM-KZD5) caused the smallest lesions (13.00 mm) that were not significantly different from the control (5.00 mm) and could therefore be regarded as nonpathogenic on this tree. Re-isolation percentages were between 12.5% (*N. parvum*, isolate IRNHM-KZP1) and 100% (*N. novaehollandiae*, isolate IRNHM-KZN1) on *P. granatum.* On *A. glutinosa*, the average length of wood discoloration caused by all *Botryosphaeriaceae* isolates was significantly different from that occurring in the control treatment. On inoculated shoots of *A. glutinosa, N. novaehollandiae* (88.33 mm) caused the longest lesions and was the most virulent isolate of *Botryosphaeriaceae* on shoots of this tree. *Diplodia sapinea* (IRNHM-KZ400) was the less aggressive *Botryosphaeriaceae* species on this woody plant; however, produced lesions by this isolate (17.33 mm) were significantly different from the controls (4.33 mm). The lowest and highest percentage recoveries were between 25% (*L. mahajangana* isolate IRNHM-KZL10) and 83.3% (*D. seriata* isolate IRNHM-KZD5) on *A. glutinosa*, respectively. *Botryosphaeria dothidea* (isolate IRNHM-KZ39, 120.0 mm) and *N. novaehollandiae* (isolate IRNHM-KZN1, 116.33 mm) were the most aggressive isolates on *P. fraxinifolia* and produced lesions that are significantly different from the other isolates and controls (5.33 mm), while *D. seriata* (IRNHM-KZD5) gave the shortest lesions (16.33 mm) on inoculated shoot of this woody plant and there was no significant difference between the length of lesions produced by this isolate and controls. The highest percentage recovery (83.3%) was recorded for *N. novaehollandiae* (IRNHM-KZN1), *D. intermedia* (IRNHM-KZI1) and *D. sapinea* (IRNHM-KZ400), while the lowest (33.3%) was noted for *Do. plurivora* (IRNHM-KZm1), *L. mahajangana* (IRNHM-KZL10) and *D. seriata* (IRNHM-KZD5). On *P. persica,* two isolates of *N. novaehollandiae*, that is, IRNHM-KZN3 (mean lesion length = 76.00 mm) and IRNHM-KZN1 (mean lesion length = 72.00 mm), gave the longest wood lesions and there was no significant difference between lesion lengths caused by these two species. Lesions caused by two isolates of *D. seriata* (IRNHM-KZD2 and IRNHM-KZD5) were the smallest (10.00 mm and 17.33 mm) among the *Botryosphaeriaceae* species tested, and there was no significant difference between length of lesions produced by these isolates and control treatments (4.00 mm). The frequency of inoculated isolates varied from 25.0% (*D. sarmentorum* isolate, IRNHM-KZS3) to 100% (*B. dothidea,* isolate IRNHM-KZ5) on this tree. One isolate of *N. novaehollandiae* (IRNHM-KZN1) again caused the longest lesions and was the most virulent species of *Botryosphaeriaceae* on shoots of *M. germanica. Diplodia seriata* (IRNHM-KZD2) produced lesions of 9.67 mm, shorter than the other inoculated isolates on *M. germanica* shoots. Isolates were re-isolated from inoculated shoots of *M. germanica* in the range of 25.0% (*D. sapinea*, isolate KZ4001 and *D. seriata*, isolate IRNHM-KZD2) to 75.0% (*N. novaehollandiae*, isolate IRNHM-KZN3). On *Q. castaneifolia* shoots, one isolate of *D. intermedia* (IRNHM-KZI1) produced the longest lesions (mean lesion length = 125.00 mm) and displayed the highest level of pathogenicity. One isolate of *D. seriata* (IRNHM-KZD5) included in the pathogenicity tests produced the shortest lesions (mean lesion length = 19.67 mm) that were not statistically different from those of the uninoculated control (7.33 mm). Re-isolation of the inoculated isolates succeeded in the range of 12.5% (*L. mahajangana* isolate IRNHM-KZL10) to 83.3% (*N. novaehollandiae,* isolate IRNHM-KZN3 and *D. intermedia*, isolate IRNHM-KZI2) on shoots of this host.

Table 3 Mean lesion length and re-isolation frequencies of *Botryosphaeriaceae* species inoculated onto forest tree shoots in a pathogenicity trial

Isolates		Original host	Mean lesion length (mm) and re-isolation frequency % in parentheses					
Species	Cod (IRNHM-)		*Punica granatum*	*Alnus glutinosa*	*Pterocarya fraxinifolia*	*Parrotia persica*	*Mespilus germanica*	*Quercus castaneifolia*
Do. plurivora	KZm1	*Diospyros lotus*	24.67 **IJK** (83.3)	33.67 **GHI** (58.3)	41.33 **GH** (33.3)	30.67 **EFG** (66.6)	31.00 **FGHI** (50.0)	42.67 **GHI** (25.0)
	KZm3	*Populus caspica*	27.33 **HIJK** (37.5)	33.67 **GHI** (50.0)	32.00 **GHI** (58.3)	36.00 **DEF** (66.6)	32.67 **FGH** (50.0)	31.33 **HIJ** (33.3)
N. novaehollandiae	KZN1	*Pinus* sp.	75.33 **BC** (100.0)	88.33 **A** (75.0)	116.33 **A** (83.3)	72.00 **A** (50.0)	88.33 **A** (66.6)	118.00 **ABC** (33.3)
	KZN3	*Carpinus betulus*	68.00 **CDE** (50.0)	80.0 **AB** (58.3)	107 **ABC** (66.6)	76.00 **A** (50.0)	81.67 **AB** (75.0)	120.67 **AB** (83.3)
N. parvum	KZP1	*Alnus glutinosa*	48.67 **EFGH** (12.5)	58.33 **DE** (66.6)	49.67 **FG** (75.0)	54.00 **BC** (66.6)	62.67 **BC** (37.5)	77.67 **DEF** (25.0)
	KZP3	*Parrotia persica*	46.00 **EFGHI** (37.5)	50.67 **EF** (75.0)	34.67 **GHI** (66.6)	54.67 **BC** (58.3)	56.67 **CD** (50.0)	65.33 **EFG** (66.6)
B. dothidea	KZ5	*Populus caspica***	107.00 **B** (66.6)	45.67 **FG** (58.3)	98.33 **ABCD** (66.6)	55.00 **AB** (100.0)	37.00 **EFG** (66.6)	97.67 **BCD** (50.0)
	KZ39	*Populus caspica*	135.00 **A** (66.6)	55.00 **DEF** (58.3)	120.00 **A** (75.0)	40.37 **CDE** (75.0)	13.67 **HIJ** (66.6)	116.33 **ABC** (66.6)
D. intermedia	KZI1	*Gleditsia caspica*	60.00 **DEF** (66.6)	21.33 **J** (50.0)	110.00 **AB** (83.3)	65.67 **AB** (58.3)	76.67 **AB** (58.3)	125.00 **A** (66.6)
	KZI2	*Fagus orientalis*	43.33 **FGHI** (50.0)	35.67 **GH** (58.3)	87.33 **CDE** (75.0)	48.00 **CD** (50.0)	64.33 **BC** (37.5)	78.00 **DEF** (83.3)
L. mahajangana	KZL2	*Quercus castaneifolia*	106.67 **B** (50.0)	61.67 **CDE** (50.0)	113.33 **A** (58.3)	47.00 **CD** (66.6)	53.00 **CDE** (58.3)	93.67 **CD** (33.3)
	KZL10	*Ulmus carpinifolia*	93.33 **C** (37.5)	67.00 **CD** (25.0)	90.33 **BCDE** (33.3)	42.33 **CDE** (33.3)	50.00 **CDEF** (66.6)	87.00 **DE** (12.5)
D. sapinea	KZ400	*Pinus eldarica***	33.33 **GHIJ** (75.0)	17.33 **J** (75.0)	113.33 **A** (83.3)	33.33 **DEF** (58.3)	11.67 **IJ** (66.6)	61.67 **EFG** (75.0)
	KZ4001	*Pinus eldarica*	28.00 **HIJK** (66.6)	19.67 **J** (50.0)	83.00 **DE** (66.6)	28.33 **EFG** (58.3)	20.67 **GHIJ** (25.0)	34.33 **HI** (33.3)
Do. sarmentorum	KZS1	*Quercus castaneifolia*	46.33 **EFGHI** (75.0)	52.67 **EF** (66.6)	47.67 **FG** (50.0)	24.67 FGH (37.5)	20.67 **GHIJ** (58.3)	56.00 **FGH** (25.0)
	KZS3	*Alnus glutinosa*	55.33 **DEFG** (50.0)	71.00 **B** (37.5)	69.67 **EF** (50.0)	23.33 FGH (25.0)	38.67 **DEFG** (37.5)	71.33 **DEF** (25.0)
D. seriata	KZD2	*Acer cappadocicum*	23.00 **IJK** (37.5)	28.67 **HIJ** (37.5)	23.33 **HIJ** (50.0)	10.00 HI (58.3)	9.67 **J** (25.0)	39.33 **GHI** (25.0)
	KZD5	*Fraxinus excelsior*	13.00 **JK** (33.3)	22.33 **IJ** (83.3)	16.33 **IJ** (33.3)	17.33 GHI (37.5)	12.00 **IJ** (66.6)	19.67 **IJ** (33.3)
PDA plug (control)			5.00 **K** (0.0)	4.33 **K** (0.0)	5.33 **J** (0.0)	4.00 I (0.0)	5.33 **J** (0.0)	7.33 **J** (0.0)
LSD ($p < 0.05$)			23.655	12.063	22.11	14.873	19.531	26.882

Different letters in bold face indicate significant differences only within a column at $p = 0.05$

** Isolates obtained from wood debris

Discussion

This work is part of a larger effort to study the diversity of fungal trunk pathogens associated with various forest trees in the north of Iran. Nine *Botryosphaeriaceae* species were isolated and identified from various plant tissue types and wood debris of forest trees. These include *B. dothidea, L. mahajangana, N. novaehollandiae, N. parvum*, *D. seriata, D. sapinea*, *D. intermedia*, *Do. sarmentorum* and *Do. plurivora*. Of these, six species (*L. mahajangana*, *N. novaehollandiae*, *D. seriata*, *B. dothidea*, *Do. plurivora* and *D. sapinea*) were recovered from both infected wood tissues of various forest trees and wood debris, while three species (*Do. sarmentorum*, *N. parvum* and *D. intermedia*) were only associated with affected wood tissues of trees. The most abundant species of the *Botryosphaeriaceae* in the trees sampled was *B. dothidea*, which represented 33.8% of the total isolates associated with affected wood tissues. This fungus as one of the most common species of *Botryosphaeriaceae* has been reported from hundreds of plant species worldwide (Phillips et al. 2013). The current study has confirmed this, by isolating *B. dothidea* from seven tree species in six families, *P. granatum*, *A. glutinosa*, *C. betulus*, *P. persica*, *C. pentagyna*, *P. caspica* and *P. divaricata* subsp. *divaricata.* Sixteen isolates of this species were also isolated from surface of wood debris of *P. granatum*, *C. betulus*, *C. pentagyna* and *P. caspica*. This pathogen has previously been found on some forest and ornamental tree species such as *Juniperus communis* L.*, Cotinus coggygria* Scop. (Piškur et al. 2011; Pavlic-Zupanc et al. 2015), *Pseudotsuga menziesii* (Mirb.) Franco, *Sequoia sempervirens* (D. Don) Endl. (Zlatkovic´ et al. 2016), *Chamaecyparis* sp., *Pinus* spp. and *Sequoiadendron giganteum* (Lindl.) J. Buchholz. (Smith and Stanosz 2001; Flowers et al. 2003). *Botryosphaeria dothidea* has also been isolated from various forest trees including *Juniperus communis* L., *Populus nigra* L., *Fagus* sp., *Picea* sp., *Quercus* sp., *Rubus* sp., *Acer* sp. and *Salix* sp. in Iran (Abdollahzadeh et al. 2013b). It was thus interesting that in the present study, the fungus was found for the first time associated with *P. granatum*, *A. glutinosa*, *C. betulus*, *P. divaricata* subsp. *divaricata*, *P. caspica*, *P. persica* and *C. pentagyna*.

Three species of *Diplodia*, *D. seriata*, *D. intermedia* and *D. sapinea* were obtained from various forest trees in this study. *Diplodia* spp. as an important group of plant pathogens can cause various disease symptoms such as dieback, blight, canker and rot diseases on numerous plants (Lazzizera et al. 2008, Linaldeddu et al. 2011). A number of species of this genus have been previously recorded from forest and ornamental trees worldwide (Alves et al. 2004; Pérez et al. 2010; Linaldeddu et al. 2013; Phillips et al. 2013). *Diplodia seriata* was found associated with five tree species in the current study. These include *F. orientalis*, *F. excelsior*, *Z. carpinifolia*, *P. persica* and *A. cappadocicum*. This fungus is also regarded as a generalist species of *Botryosphaeriaceae* and has been recorded on more than 250 plant hosts (Phillips et al. 2013, Dissanayake et al. 2016). This species has been found on some forest and ornamental trees, *Pinus nigra* J.F. Arnold (Flowers et al. 2003), *P. glauca*, *Abies grandis* (Douglas ex D.Don) Lindl., *P. menziesii*, *Picea abies* (L.) Karst., *Cedrus* sp. (De Wet et al. 2008), *Chamaecyparis lawsoniana* (Murr.) Parl., *Thuja plicata* Donn. ex D. Don. (Alves et al. 2013), *Cedrus atlantica* (Endl.) Manetti ex Carrière., *F. excelsior*, *Chamaecyparis pisifera* (Sieb. & Zucc.) Endl. and *Ligustrum vulgare* L. (Zlatkovic´ et al. 2016). *Diplodia seriata* previously was isolated and reported from *Z. carpinifolia* with canker symptoms (Mirabolfathy 2013) and *Salix alba* L. (Hashemi and Mohammadi 2016) in Iran. Therefore, in this study we report this species from two novel woody hosts, *F. orientalis* and *P. persica.*

Only one isolate of *D. sapinea* was obtained from affected wood tissues of pine tree (*P. eldarica*) in this work, confirming that this species is more specific in pine trees. More than 150 host plants (mostly conifers) are reported for *D. sapinea* (Farr and Rossman 2013). This fungus is a well-known latent pathogen of pine trees in various countries of the world (Phillips et al. 2013). This pathogen has been found to cause needle and shoot blight, blue stain of the sapwood, branch and trunk cankers, dieback and tree death (Phillips et al. 2013). *Diplodia sapinea* has been isolated from 33 *Pinus* species (Punithalingam and Waterston 1970; Gibson 1979; Palmer et al. 1987; Phillips et al. 2013). In addition to the *Pinus* spp., other forest trees are also referred to as the host of this pathogen. These include *Abies concolor* (Gord. & Glend.) Lindl. ex Hildebr. (white fir), *C. atlantica*, *C. deodara, C. libani, Picea omorika* (Pančić) Purk.*, P. abies, P. pungens, Thuja occidentalis* L.*, C. sempervirens, Juniperus virginiana* L.*, P. nigra, P. sylvestris*, *P. pinea, P. pinaster, P. halepensis, P. radiata, P. menziesii, Juniperus horizontalis* Moench., *Ch. lawsoniana*, *Fagus sylvatica* L. (Karadžić and Stojadinović, 1988; Milijašević, 2003, 2009; Zlatković et al. 2016). In this work, only one isolate of *D. sapinea* was isolated from wood debris of *P. eldarica* (in Shaft).

Our study revealed three *D. intermedia* isolates from two different forest tree species. *Diplodia intermedia* was originally described from dead twigs of *Malus sylvestris* L. and *Cydonia* sp. in Portugal and an unknown woody plant in Iran (Phillips et al. 2012). This fungus has also been documented on grapevines in France (Comont et al. 2016) and apple in Uruguay (Delgado-Cerrone et al. 2016). Jabbari Firoozjah et al. (2015) also reported *D. intermedia* in medlar (*Mespilus germanica* L.) trees in Iran. Based on our knowledge, this is the first time that *D. intermedia* has been found on *G. caspica* and *F. orientalis*.

In the present work, *Do. sarmentorum* was obtained for the first time from *Q. castaneifolia* and *A. glutinosa*. Previous studies have shown that *Do. sarmentorum* is a plurivorous species in the family *Botryosphaeriaceae* and has been found associated with 34 different forest and fruit tree species worldwide (Phillips et al. 2013). Recently, *Do. sarmentorum* has also been isolated and reported from some conifers (*Ch. lawsoniana* and *C. atlantica*) and angiosperm (*Aesculus hippocastanum* L.) plants in the Western Balkans (Zlatkovic´ et al. 2016). In a study conducted on *Dothiorella* species in Italy, this fungus as the dominant species of *Botryosphaeriaceae* has been reported from *Ulmus minor* Mill., *Salix* sp., *Ulmus* sp., *Cornus sanguinea* L., *Euonymus europaeus* L., *Paliurus spina-christi* Mill. and *Coronilla emerus* L. (Dissanayake et al. 2016). This fungus previously been reported from fruit and ornamental trees such as *Ulmus carpinifolia* Gled. (Hashemi et al. 2017), *P. nigra* (Hashemi and Mohammadi 2016), *C. oblonga* and adult beetles of *Osphranteria coerulescens* Redt. (Mohammadi and Sharifi 2016) in Iran, and results of this study showed that *Do. sarmentorum* can also infect *Q. castaneifolia* and *A. glutinosa* in this country.

Neoscytalidium novaehollandiae was obtained from three woody hosts, *A. glutinosa*, *C. betulus* and *F. orientalis*. *Neoscytalidium novaehollandiae* was first isolated from asymptomatic branches of some endemic trees, including *Acacia synchronica* Maslin, *Adansonia gibbosa* (A. Cunn.) Guymer ex D.A. Baum, *Crotalaria medicaginea* Lam. and *Grevillea agrifolia* A. Cunn. ex R.Br., in Western Australia, and described by Pavlic et al. (2008). This fungus has been reported as a pathogen of *Mangifera indica* L. in Australia (Ray et al. 2010). This species has been isolated from the beetle galleries of *Ulmus densa* Litv. stems in China (Zhu and Liu 2012). Recently, *N. novaehollandiae* has also been isolated and reported from Orchid in Thailand (Huang et al. 2016). This work represents the first report of this species on *A. glutinosa*, *C. betulus* and *F. orientalis* worldwide.

Eleven isolates of *Do. plurivora* were obtained in this study. Of these, four isolates (36.4%) were isolated from branches of *D. lotus*, *Q. castaneifolia* and *A. glutinosa*. This taxon was recently reported from *Citrus* sp., *Casuarina equisetifolia* L., *Malus domestica* Borkh., *Prunus armeniaca* L., *Eucalyptus* sp. and *C. sempervirens* in Southern Iran and *Juglans regia* L., *M. domestica*, *P. armeniaca* and *Vitis vinifera* L. in Spain (Abdollahzadeh et al. 2014). This fungus is also reported here for the first time from branches of three forest trees, *D. lotus*, *Q. castaneifolia* and *A. glutinosa*, in the world.

Neofusicoccum parvum was another species of *Botryosphaeriaceae* which was recovered from three forest tree species in this work. This species is a cosmopolitan species on diverse plant species and reported as an aggressive pathogen on different trees (Úrbez-Torres and Gubler 2009; Inderbitzin et al. 2010; Zlatkovic´ et al. 2016). According to Phillips et al. (2013), this species has been found associated with many ornamental, forest and plantation trees worldwide. This species is one of the most virulent *Botryosphaeriaceae* species on grapevine (Van Niekerk et al. 2004; Úrbez-Torres and Gubler 2009) and a common trunk pathogen of pome and stone fruit trees (Slippers et al. 2007). In Iran, *N. parvum* has been isolated and reported from *V. vinifera* (Mohammadi et al. 2013), stone and pome fruit trees (Abdollahzadeh et al. 2013b; Soltaninejad et al. 2017), *J. regia* (Abdollahzadeh et al. 2013b), pistachio (Mohammadi et al. 2015) and some forest and ornamental trees such as *C. sempervirens* (Mohammadi et al. 2014) and *Salix* sp. (Abdollahzadeh et al. 2013b). This is the first time the species has been reported on *A. glutinosa, P. persica* and *C. betulus.*

Lasiodiplodia mahajangana was described for the first time from healthy branches of *Terminalia catappa* L. in Mahajanga, Madagascar (Begoude et al. 2010). However, since then this species has been isolated and reported from other plants such as *Euphorbia ingens* E. Mey. showing dieback symptoms in South Africa and *A. gibbosa* in Australia (van der Linde et al. 2011). Our results in this study indicated that this species can infect *P. fraxinifolia*, *Q. castaneifolia*, *Z. carpinifolia* and *U. carpinifolia* in forest areas in the north of Iran. Therefore, these woody trees are reported here as new hosts for this pathogen. In addition, *L. mahajangana* has not previously been reported from Iran and this study is the first report of this species in this country. In Iran, field studies and pathogenicity tests have shown clear evidence that *Botryosphaeriaceae* species are the main fungal trunk pathogens on ecologically and economically significant agricultural and urban trees (Mohammadi et al. 2013; Mohammadi 2014; Mohammadi et al. 2015; Hashemi and Mohammadi 2016; Mohammadi and Sharifi 2016; Hashemi et al. 2017; Soltaninejad et al. 2017).

Aside from internal wood infections, our study showed that some species of *Botryosphaeriaceae* were also present on surfaces of plant debris. Fruiting structures of six *Botryosphaeriaceae* species were detected from wood debris of 12 tree species in the seven forest regions. Except for *N. parvum*, *D. intermedia* and *Do. sarmentorum*, the other six species were isolated from wood debris of forest trees in the north of Iran. Similar results were reported in previous studies conducted on forest, fruit and grapevine debris. *Botryosphaeriaceae* species are common in nature and survive well on woody debris (Santini et al. 2008). Several studies have confirmed that *Botryosphaeriaceae* species associated with trunk diseases of grapevine were occurred saprophytically in pruning debris left in the vineyards (Hewitt and Pearson 1988; van Niekerk et al. 2003, 2004, 2010). Several species in the family *Botryosphaeriaceae* have been isolated and reported from pruning and woody debris. For example, *Diplodia sapinea* (Fr.) Fuckel has been isolated and identified from pycnidia on the bark of pruning debris of *Prunus* spp. in South Africa by Damm et al. (2007). Pérez et al. (2008) showed that *N. parvum* can survive and sporulate on *Eucalyptus grandis* W. Hill ex Maiden. debris. This species associated with *Sphaeropsis porosa* (Van Niekerk & Crous) A.J.L. Phillips & A. Alves were also isolated and reported from grapevine pruning debris in South Africa (van Niekerk et al. 2003, 2004). Therefore, plant debris as a major source of inoculum plays a role in the development and overwintering of these pathogens in gardens and forest areas. In northern Iran, due to high humidity and forest tree species diversity, the presence of fruiting bodies of *Botryosphaeriaceae* species on the plant debris and dead branches can be a very important primary inoculum source to infect trees.

The pathogenic ability of nine species of *Botryosphaeriaceae* on six forest tree species in the north of Iran showed that most of the identified species were pathogenic.

The results of our study showed significant differences in the degree of virulence within and between inoculated species, which is consistent with results in previous pathogenicity studies of various *Botryosphaeriaceae* species on grapevine (Úrbez-Torres and Gubler 2009; Amponsah et al. 2011; Ammad et al. 2014). Pathogenicity tests in this study showed that *N. novaehollandiae, B. dothidea* and *D. intermedia* are more aggressive than the other species. On branches of *A. glutinosa*, *P. fraxinifolia*, *P. persica* and *M. germanica* species of *N. novaehollandiae* gave the longest lesions and were the most virulent species on these hosts. Pavlic et al. (2008) recently described *N. novaehollandiae* as an endophyte of *G. agrifolia*, *A. gibbosa*, *A. synchronica* and *C. medicaginea* in north-western Australia. This fungus has been recognized as the main cause of mango dieback in the north, northwest of Australia (Ray et al. 2010). The high degree of aggressiveness observed in this study for *N. novaehollandiae* is consistent with the findings reported by Sakalidis et al. (2011) and Ray et al. (2010) on mango stems in Australia.

On *P. granatum* and *Q. castaneifolia*, two species of *B. dothidea* and *D. intermedia* were the most virulent species, respectively. *Diplodia intermedia* has been described in 2012, and currently, little studies have been done on the pathogenicity of this species. It has been reported as an aggressive pathogen on fruit and branches of mango in the Kimberley Region of Western Australia (Sakalidis et al. 2011) and excised mango stems in Australia (Ray et al. 2010). On the contrary, Comont et al. (2016) found it to be weakly pathogenic on grapevine in France. Among the nine *Botryosphaeriaceae* species tested on trees, the least aggressive was *D. seriata*. In this study, the pathogenicity of this species on inoculated forest trees was unclear. On branches of *P. granatum*, *P. fraxinifolia*, *P. persica* and *M. germanica*, both isolates of *D. seriata* produced lesions not significantly different from the controls. On branches of *A. glutinosa, D. seriata* isolates could be considered pathogenic, as their lesion lengths were significantly longer than the control treatments. On branches of *Q. castaneifolia*, one isolate of *D. seriata* was pathogenic, while the other isolate failed to produce significantly longer lesions than the negative controls. Overall, our results are in accordance with findings reported by other researchers who have been working on the pathogenicity of this species on different plants. Previous studies indicated that this species ranged from weakly to strongly virulent pathogen on woody plants. This taxon is regarded as an important pathogen on apple in the USA (Brown and Britton 1986, Brown-Rytlewski and McManus 2000), grapevine in California (Úrbez-Torres and Gubler 2009), Iran (Mohammadi et al. 2013), Chile (Auger et al. 2004), South Africa (van Niekerk et al. 2004) and Australia (Savocchia et al. 2007), as a weak pathogen on apple in England and New Zealand (Laundon 1973), quince trees in Iran (Mohammadi and Sharifi 2016), almond (Inderbitzin et al. 2010), olive (Moral et al. 2010), English walnut (Chen et al. 2014a) and pistachio (Chen et al. 2014b) in California, poplar in Iran (Hashemi and Mohammadi 2016) and nonpathogenic to grapevines in Western Australia (Taylor et al. 2005) and willow trees in Iran (Hashemi and Mohammadi 2016). Soltaninejad et al. (2017) also showed *D. seriata* to be pathogenic to apricot and cherry and nonpathogenic to peach, sour cherry and green gage. The interaction between fungal pathogens and their plant hosts is extremely complex. Therefore, these differences may be due to variations in virulence between species, genetic variability of the isolates, and differences in susceptibility and age of tree species. During this research, two isolates of *B. dothidea* and *D. sapinea* obtained from wood debris of trees also were pathogenic on inoculated trees. Similar outcomes were also reported for *N. parvum*, *B. dothidea*, *D. seriata* (Arabnezhad and Mohammadi 2013) and *S. porosa* (van Niekerk et al. 2004) on grapevine. Therefore, our results suggest that wood debris could act as a long-lasting inoculum source for these pathogens under forest conditions. Our work indicates that *Botryosphaeriaceae* species are common and widespread on various forest trees in the north of Iran. Most species of *Botryosphaeriaceae* reported in this study had previously been reported to be pathogenic or potentially pathogenic to agricultural crops (Mohammadi et al. 2013; Mohammadi 2014; Mohammadi et al. 2015; Mohammadi and Sharifi 2016; Soltaninejad et al. 2017) and ornamental trees (Mohammadi et al. 2014; Hashemi and Mohammadi 2016; Hashemi et al. 2017) in Iran, as well as in other countries, which show the capability of them to occupy a wide range of plant species and geographic areas. Although it is not clear whether *D. seriata* is a pathogen on some forest trees, there are reports of this species associated with serious trunk diseases on fruit (Mohammadi and Sharifi 2016; Soltaninejad et al. 2017) and ornamental (Hashemi and Mohammadi 2016) trees in Iran and other countries in the world (van Niekerk et al. 2004; Damm et al. 2007). Therefore, due to the diversity of trees in the northern forests of Iran, this fungus like the other *Botryosphaericeae* species may be a serious threat to other forest trees in this country. The distribution of *Botryosphaeriaceae* species in Guilan Province and their common association with trunk disease symptoms suggest that they could be contributing to decline of forest trees in other areas in the north of Iran. Further research is required to completely investigate the role of *Botryosphaeriaceae* species in the decline of forest trees in Iran.

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