Mycologia Iranica 8(2):119-133, 2021 Original Article DOI: 10.22092/MI.2022.359850.1229

New records of fungal species of the family *Didymellaceae* from Iran

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Abstract: The *Didymellaceae* is one of the most species-rich families in the *Pleosporales*, and contains numerous plant pathogenic, saprobic and endophytic specie, inhabiting a wide range of ecosystems. To identify the fungal species of the *Didymellaceae* in Iran, we investigated nine unidentified phoma-like strains obtained from the Iranian Fungal Culture Collection (IRAN) and six additional strains obtained from various diseased plants in Khuzestan Province, during 2019–2021. The strains under study were recognized by combining the results obtained from multi-gene phylogenetic analyses (ITS, LSU, *rpb2* and *tub2*), and morphological comparisons. Accordingly, six species belonging to four genera of the *Didymellaceae* were identified and described as follow: *Didymella aquatica*, *D. segeticola*, *Ectophoma multirostrata*, *Epicoccum dendrobii*, *E. tobaicum* and *Neomicrosphaeropsis Juglandis*. All these species are new records for the fungi of Iran. Furthermore, several new hosts are reported for these taxa.

Keywords: Morphology, Phoma-like, Phylogeny, Taxonomy

INTRODUCTION

The *Didymellaceae* is one of the core families within *Pleosporales* (Pleosporomycetidae, 19 *Dothideomycetes*, *Pezizomycotina*, *Ascomycota*) (Hyde et al. 2013, Hongsanan et al. 2020, Wijayawardene et al. 2020). This family comprises numerous plant pathogenic, saprobic, and endophytic species associated with a broad range of hosts (de Gruyter et al. 2009, Hyde et al. 2013, Chen et al. 2015a, Liu et al. 2015). Some of them are opportunistic pathogens in animals and human (Aveskamp et al. 2008). To date, more than 5,400 species from 31 genera in *Didymellaceae* have been recorded, including recently established genera such as *Dimorphoma* L.W. Hou, L. Cai & Crous and *Macroascochyta* L.W. Hou, L. Cai & Crous (Hou et al. 2020a).

The family *Didymellaceae* was proposed by de Gruyter et al. (2009) to accommodate three main genera, including *Ascochyta* Lib., *Didymella* Sacc. and *Phoma* Sacc., as well as several related phoma-like taxa. The type genus of this family is *Didymella*, with the type species *D. exigua* (Niessl) Sacc. (CBS 183.55). Chen et al. (2015a) performed a comprehensive molecular study on *Phoma* and phoma-like taxa, in which they proposed nine new genera in the *Didymellaceae* comprising *Allophoma* Q. Chen & L. Cai, *Calophoma* Q. Chen & L. Cai, *Heterophoma* Q. Chen & L. Cai, *Neoascochyta* Q. Chen & L. Cai, *Neodidymelliopsis* Q. Chen & L. Cai, *Nothophoma* Q. Chen & L. Cai, *Paraboeremia* Q. Chen & L. Cai, *Phomatodes* Q. Chen & L. Cai, and *Xenodidymella* Q. Chen & L. Cai. In this study, the genus *Microsphaeropsis* Höhn. was transferred to the family *Microsphaeropsidaceae*. However, *Microsphaeropsis* species were re-assessed using morphological and molecular data in the next studies, and accordingly they were again placed in *Didymellaceae* (Hyde et al. 2020, Hou et al. 2020a, b). Recently, several other new genera have been allocated to the *Didymellaceae* based on morphological and phylogenetic data, i.e. *Briansuttonomyces* Crous, *Chaetasbolisia* Speg.,

Submitted 16Aug 2021 accepted for publication 24 Nov 2021
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Coniothyrium Corda, *Cumuliphoma* Valenz.-Lopez et al., *Didymellocamarosporium* Wijayaw. & K.D. Hyde, *Didysimulans* Tibpromma, Camporesi & K.D. Hyde, *Dimorphoma*, *Ectodidymella* L.W. Hou, L. Cai, *Ectophoma* Valenz.-Lopez et al., *Endophoma* A. Tsuneda & M.L. Davey, *Heracleicola* Tibpromma, Camporesi & K.D. Hyde, *Juxtiphoma* Valenz.-Lopez et al., *Longididymella* L.W. Hou, L. Cai & Crous, *Macroascochyta*, *Neodidymella* Phookamsak, R.H. Perera & K.D. Hyde, *Neomicrosphaeropsis* Thambug. et al., *Nothomicrosphaeropsis* Crous, *Paramicrosphaeropsis* L.W. Hou, L. Cai & Crous, *Phaeomycocentrospora* Crous, H.D. Shin & U. Braun, *Pseudoascochyta* Valenz.-Lopez et al., *Remotididymella* Valenz.-Lopez et al., *Sclerotiophoma* L.W. Hou, L. Cai & Crous, *Similiphoma* Valenz.-Lopez et al., *Vacuiphoma* Valenz.-Lopez et al., *Vandijckomycella* Hern.-Restr. et al., and *Verrucoconiothyrium* Crous (Ariyawansa et al. 2015, Crous & Groenewald 2016, Crous et al. 2016, Thambugala et al. 2016, Wijayawardene et al. 2016, Valenzuela-Lopez et al. 2018, Hou et al. 2020a, b, Crous et al. 2021). However, some genera were not accepted in *Didymellaceae* due to inadequacy of genetic and morphological divergence, i.e., *Didymellocamarosporium*, *Didysimulans*, *Endocoryneum* Petr., *Endophoma* Petr., *Neodidymella*, *Platychora* Petr. and *Pseudohendersonia* Crous & M.E. Palm (Hou et al. 2020a).

The species of the family *Didymellaceae* have already been investigated in many studies in Iran (Ahmadpour et al. 2017a, b, Amirdehi et al. 2017, Babaahmadi et al. 2018, Khodaei et al. 2018, Larki et al. 2019, Bakhshi et al. 2019, Ahmadpour et al. 2022a, b). In these studies, new species of the genera *Allophoma*, *Ascochyta*, *Didymella*, *Ectophoma*, *Neodidymelliopsis*, *Paramicrosphaeropsis*, *Septoria* Sacc., and *Xenodidymella* were described and illustrated.

The current study aimed to elucidate unidentified phoma-like taxa deposited in IRAN collection and additional isolates associated with symptomatic various plants in Khuzestan Province. Here, 15 isolates of phoma-like taxa were evaluated using a polyphasic approach, combining morphological characteristics and phylogenetic analyses.

MATERIALS AND METHODS

Sample collection and fungal isolation

Nine unidentified phoma-like strains were obtained from the Iranian Fungal Culture Collection (IRAN). Six additional strains were isolated from symptomatic plants including *Kalanchoe blossfeldiana* Poelln., *Phragmites australis* Cav., *Mentha piperita* L., *Lactuca serriola* L. and *Trifolium alexandrium* L., collected from different areas in Khuzestan Province during 2019– 2020. For fungal isolation, small sections (0.5–0.8 cm) composed of both the asymptomatic and symptomatic tissues were excised and surface-decontaminated in 1% sodium hypochlorite (2–4 minutes), followed by rinsing in sterile distilled water and drying on sterile paper. Dried sections were placed in petri plates containing potato dextrose agar (PDA, potato extract 200–400 g L^{-1}, sucrose 10 g L^{-1}, agar 12 g L^{-1}) supplemented with streptomycin sulfate (30 mg L^{-1}). After 7–12 days of incubation at 25± 0.5 °C, individual colonies were subcultured to fresh PDA. Pure cultures were made using the single spore method (Babaahmadi et al. 2018, Larki et al. 2019). Subcultures of surveyed strains in this study are d deposited in the Iranian Fungal Culture Collection (Iranian Research Institute of Plant Protection, Tehran, Iran) and SCUA (Collection of Fungal Cultures, Department of Plant Protection, Shahid Chamran University of Ahvaz, Iran).

Morphological characterization

To evaluate microscopic features, colony characteristics, and growth speed, pure isolates were grown on oatmeal agar (OA; oatmeal 30–60 g L^{-1}, agar 12 g L^{-1}) at 25°C under 12 h photoperiod for 7–20 days.

Micro-morphological features were examined after mounting mature conidiomata and conidia in a drop of lactophenol. Pycnidial wall was studied using the microtome sections of 3-µm thickness, prepared with a Leica RM 2235 microtome, and stained with hematoxylin and eosin. Photomicrographs were taken with an OLYMPUS BX51 microscope equipped with an OLYMPUS DP12 digital camera, and at least, 50 measurements of each fungal structure were made with a Leitz Wetzlar (SM-LUX) Basic Biological Light Microscope at 400× and 1000× magnification.

DNA extraction, PCR and sequencing

The isolates were grown on PDA at 25 °C in darkness for 1–3 weeks. Mycelial biomass was scraped off from the surface of each culture using a sterile glass slide. DNA was extracted using protocol described by Raeder and Broda (1985), with some optimization (Ahmadpour et al. 2017a). Both the internal transcribed spacer regions 1 and 2 including the intervening 5.8S nuclear ribosomal DNA (ITS) and partial regions of nuclear 28S ribosomal DNA (LSU) were amplified using the primer pair of ITS1/ NL4 (White et al. 1990, O'Donnell 1993), and part of the RNA polymerase II second largest subunit (*rpb2*) with RPB2-5F2/ fRPB2-7cR (Liu et al. 1999, Sung et al. 2007) and partial β-tubulin (*tub2*) with Btub2Fd/ T2 (O'Donnell & Cigelnik 1997, Woudenberg et al. 2009). Polymerase chain reaction mixture was prepared according to Ahmadpour et al. (2022b).

The amplification was performed in a MJ Mini™ Gradient Thermal Cycler using the following parameters: 94 °C for 5 min; followed by 35 cycles at 94 °C for 30 s, the annealing temperature dependent on the amplified loci (56 °C for ITS-LSU, 58 °C for *tub* and *rpb2*) for 30 s and 72 °C for 90 s; and a final elongation step at 72 °C for 5 min. PCR products were analyzed and sequenced as described by Safi et al. (2020).

Phylogenetic analysis

The DNA sequences were analyzed using BioEdit v. 7.0.9.0 (Hall 1999) and DNA Baser Sequence Assembler v4 (2013, Heracle BioSoft, www.DnaBaser.com) and obtained consensus sequences were deposited in GenBank (Table. 1). BLASTn search algorithm was done for each gene region to identify closely related species in the NCBI's GenBank. The ITS, LSU, *tub2* and *rpb2* sequences of ex-type or authentic strains of the genera under study were retrieved from GenBank. All sequences of each region were aligned using Clustal W in BioEdit v. 7.0.9.0 (Hall 1999) and manually repaired. A primary single-locus phylogeny was performed to identify the phylogenetic position of the isolates under study (not shown) and then for the four loci combined (ITS + LSU + *tub2* + *rpb2*). The Multi-gene dataset was created by concatenation of all individual alignments. Maximum likelihood (ML) analysis was done using raxmlGUI 2.0 beta (Edler et al. 2019), and started with the following options: the general time-reversible model with a Gamma distributed and Invariant sites (GTR + G + I) rate variation and thorough bootstrapping analysis with 1000 replicates (MLBS). Maximum parsimony (MP) analyses were run in MEGA7 (Tamura et al. 2013) with the heuristic search option and 1000 pseudo-sampling in bootstrapping analysis. The Bayesian analysis (BI) was run using MrBayes v.3.2.6 (Ronquist et al. 2012), with the fittest evolutional models for each region estimated by jModelTest 2 (Darriba et al. 2012). Accordingly, GTR + G + I was used for ITS and *tub2* and GTR+I for LSU and *rpb2*. In BI analysis, the Markov chain Monte Carlo (MCMC) analysis was performed with the following options: four MCMC chains were sampled over 10,000,000 generations, sampling every 1000 generations, the standard deviation below 0.01 and posterior probability values (BPP) were determined after removing the first 25% of trees. New sequences were deposited in GenBank (Table 1).

Table 1. Strains used in phylogenetic analyses. The new sequences are designated in bold.

Taxon	Strain[a]	Source	GenBank accession numbers			
			ITS	*tub2*	*rpb2*	LSU
Didymella aquatica	LC 5556 [T]	Water	KY742055	KY742297	KY742140	KY742209
	IRAN 4377C	*Acer* sp., leaf spot	**OP163106**	**OM897462**	**OP562388**	—
D. bellidis	CBS 714.85	*Bellis perennis*	GU237904	GU237586	KP330417	GU238046
D. macrophylla	LC 8131 [T]	*Hydrangea acrophylla*	KY742070	KY742312	KY742154	KY742224
D. segeticola	CGMCC 3.17489 [T]	*Cirsium segetum*	KP330443	KP330399	KP330414	KP330455
	IRAN 4745C	*Quercus* sp., leaf spot	—	**OM897458**	—	—
	IRAN 4746C	unknown plant, leaf spot	—	**OM897459**	—	—
	IRAN 4747C	*Pterocarya fraxinifolia*, branch	—	**OM897460**	—	—
	IRAN 4748C	*Pterocarya fraxinifolia*, branch	—	**OM897461**	—	—
	IRAN 133C	*Camellia sinensis*, leaf spot	—	**OM897456**	—	—
	IRAN 4744C	*Diospyros lotus*, branch	—	**OM897457**	—	—
D. senecionicola	CBS 160.78	*Senecio jacobaea*	GU237787	GU237657	MT018177	GU238143
D. suiyangensis	LC 7439 [T]	Air	KY742089	KY742331	KY742168	KY742243
Ectophoma insulana	CBS 252.92 [T]	*Olea europaea*	MN973481	MT005581	MT018070	MN943685
E.iranica	CBS 144681[T]	*Catharanthus roseus*	MK519382	MK519562	—	MK519389
	IRAN 3355C	*Dracaena compacta*	MK519561	MK519388	—	MK519381
E.multirostrata	CBS27460 [T]	Soil from poultry farm	FJ427031	FJ427141	LT623265	GU238111
	IRAN 4150C; SCUA-Ah-D49	*Kalanchoe blossfeldiana*, stem rot	—	**OM897475**	—	—

Taxon	Strain[a]	Source	GenBank accession numbers			
			ITS	*tub2*	*rpb2*	LSU
Neomicrosphaeropsis alhagi pseudalhagi	TASM 6134 T	*Alhagi maurorum*	MH069664	MH069689	MH069682	MH069670
N. cytisicola	MFLUCC 18-0355	*Cytisus* sp.	MH069665	MH069690	—	MH069671
N. juglandis	MFLU 17-0517 T	*Juglans regia*	MN244223	MN871954	—	MN244206
	IRAN 159C	*Cupressus sempervirens* var. *horizontalis*	**OP163122**	**OM897440**	—	—
Neodidymelliopsis cannabis	CBS 12175 T	*Urtica dioica*	GU237761	GU237535	MT018288	GU237972
E. dendrobii	IRAN 4165C; SCUA-Ah-A12	*Lactuca serriola*, leaf spot	**OP163111**	**OM897443**	—	—
	IRAN 4167C; SCUA-Ah-A9-2	*Trifolium alexandrium*, leaf spot	**OP163112**	**OM897444**	—	—
	IRAN 4751C; SCUA-Ah-H268	*Phragmites australis*, stem necrotic lesions	**OP163121**	**OM897455**	—	—
	IRAN 4750C; SCUA-Ah-H262	*Mentha piperita*, leaf spot	**OP163115**	**OM897448**	—	—
Epicoccum dendrobii	LC 8145 T	*Dendrobium fimbriatum*	KY742093	KY742335	MT018084	KY742247
E. mezzettii	CBS 173.38 T	*Populus* pulp	MN973496	MT005596	MT018095	MN943701
E.nigrum	CBS 173.73 T	*Dactylis glomerata*	FJ426996	FJ427107	KT389632	GU237975
E.poae	LC 8160 T	*Poa annua*	KY742113	KY742355	KY742182	KY742267
E.pruni	MFLU 16-1794 T	*Prunus armeniaca*	KY711170	KY711168	—	KY711172
E. tobaicum	CBS 384.36 T	Heath soil	MN973493	MT005593	MT018092	MN943698
	IRAN 4749C; SCUA-Ah-H19	*Allium schoenoprasum*	—	**OM897451**	—	—
	IRAN 3307C	*Actinidia chinensis*	—	**OM897445**	—	—
E. pomi	CBS 26792 T	*Coffea arabica*	GU237814	GU237643	LT623263	GU238128
E.cedri	MFLU 16-1358 T	*Cedrus deodara*	KY711170	KY711168	—	KY711172
E. purpurascens	CBS 166.32	—	MN973487	MT005587	MT018082	MN943692

[a] Abbreviation of culture collections: CBS, Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; IRAN, Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Tehran, Iran; MFLUCC, Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; Others are not registered abbreviations. Newly generated sequences are in bold. T extype strains.

RESULTS

A multi-locus phylogeny was performed based on ITS, LSU, *tub2*, and *rpb2* loci consisted of 118 sequences from 37 ingroup taxa (Table 1), with *Neodidymelliopsis cannabis* CBS 12175 as outgroup. The concatenated alignment comprised 2149 bp, including gaps (ITS: 433 bp, LSU: 790 bp, *tub2*: 331 bp, *rpb2*: 595 bp). Among *those sites*, 1820 bp were constant and 326 bp were variable (287 bp were parsimony informative and 39 bp were parsimony-uninformative). Single-locus phylogeny did not show any conflicts in the tree topologies for the 70% reciprocal trees, which allowed to combine the four loci for a multi-locus phylogeny. The phylogenetic tree obtained from ML analysis showed a similar topology to the BI and MP trees in major clades (Fig. 1). In the phylogenetic tree (Fig. 1), the surveyed strains were clustered in four distinct clades representing *Didymella* (MLBS 100%, MPBS 100%, BPP 1.00), *Ectophoma* (MLBS 97%, MPBS 100%, BPP 1.00), *Epicoccum* (MLBS 100%, MPBS 100%, BPP 1.00), and *Neomicrosphaeropsis* (MLBS 100%, MPBS 99%, BPP 1.00). A multi-locus phylogeny, in combination with morphology, supported the identification of six species including *D. aquatica*, *D. segeticola*, *E. multirostrata*, *E. dendrobii*, *E. tobaicum* and *N. juglandis*. All these species are new for the funga of Iran. Moreover, this is the first report of *D. aquatica* on *Acer* sp.; *D. segeticola* on *Diospyros lotus*,

Pterocarya fraxinifolia, and *Quercus* sp.; *E. multirostrata* on *Kalanchoe blossfeldiana*; *E. dendrobii* on *Phragmites australis*, *Mentha piperita*, *Lactuca serriola*, and *Trifolium alexandrium*; *E. tobaicum* on *Allium schoenoprasum*; and *Actinidia chinensis* and *N. juglandis* on *Cupressus sempervirens* var. *horizontalis.*

TAXONOMY

***Didymella aquatica* Q. Chen, Crous & L. Cai, Studies in Mycology 87: 105 (2017) Fig.2**

Conidiomata pycnidial, solitary, sometimes aggregated, globose to subglobose, brown, glabrous or covered with some hyphal outgrowths, superficial, ostiolate or poroid, 152.4–331.5 (359.8) × (132.3–)150–288.1 µm, 95% confidence limits = 231.7–252.3 × 206.6–224.3 µm, ($\bar{x}$ ± SD = 242 ± 42.3 × 215.5 ± 37.2 µm). Ostioles 2–13, sometimes elongated as a short neck, papillate. Pycnidial wall pseudoparenchymatous, composed of oblong to isodiametric cells, 2–5 layers, 14–35 µm thick, outer wall 2–3-layers pigmented. Conidia ellipsoidal to oblong, globose, smooth and thin-walled, hyaline, aseptate, 3.4–5.5 × 1.8–2.6 µm, 95% confidence limits = 4.05–4.3 ×2.2–2.3 µm, ($\bar{x}$ ± SD = 4.2 ± 0.5 × 2.3 ± 0.2 µm). Conidial matrix cream. Culture characteristic: Colonies on OA 25-26 mm diam after 8 days of incubation at 25 ± 0.5 °C, margin regular and colorless, initially pale grey, becoming brown-grey with age, floccose, covered by pale grey aerial mycelium; reverse dark grey.

Specimen examined. IRAN, Mazandaran Province, Nur, leaf spot of *Acer* sp., Jul. 2018, A. Javadi Estahbanati, IRAN 4377C.

Note: *Didymella aquatica* was first introduced by Chen et al. (2017), with the characterization of a strain from water. In this study, the strain IRAN 4377C was associated with leaf spot symptoms in *Acer* sp. *Didymella aquatica* is phylogenetically closely related to *D. macrophylla* (MLBS 100%, MPBS 99%, BPP 1.00, Fig. 1). Morphologically, *D. aquatica* is clearly differentiated from *D. macrophylla* in the number of conidiomatal ostioles (2–13 vs. 1) (Chen et al. 2017). Accordingly, the new strain is similar to the type strain of *D. aquatica* (CGMCC 3.18349).

***Didymella segeticola* (Q. Chen) Q. Chen, Crous & L. Cai, Studies in Mycology 87: 138 (2017) Fig. 3**

Conidiomata pycnidial, solitary to confluent, on the agar surface, subglobose, glabrous, later developing to pyriform to irregular shaped with many hyphal outgrows and with a clear neck around the ostioles, 123.9–392.7 × 95.4–323.6 µm, 95% confidence limits = 209.9–281.1 × 180.2–237.5 µm, ($\bar{x}$ ± SD = 245.5 ± 80.4 × 208.9 ± 64.6 µm). Ostioles 1–2, on an elongated neck. Pycnidial wall pseudoparenchymatous. Conidia ellipsoidal to ovoid or cylindrical, thin-walled, smooth, hyaline, aseptate, 3.5–6.9 × 1.9–3 µm, 95% confidence limits = 3.9–4.1 ×2.2–2.4 µm, ($\bar{x}$ ± SD = 4 ± 0.4 × 2.3 ± 0.3 µm). Conidial matrix creme-white. Culture characteristics: Colonies on OA, 58–59 mm diam after 8 days of incubation at 25 ± 0.5 °C, margin regular, aerial mycelium woolly, white to grey; reverse gray olivaceous.

Specimens examined. IRAN, Guilan Province, Guilan, leaf spot of *Camellia sinensis* (L.) Kuntze., 18 Mar. 1992, Hosseini Moghadam, IRAN 133C; Fuman, branch of *Diospyrus lotus* L., 2 Aug. 2018, A. Javadi Estahbanati, IRAN 4744C; Siahkal-Deylaman, leaf spot of an unknown plant, 1 Aug. 2018, A. Javadi Estahbanati, IRAN 4746C; Siahkal-Deylaman, branch of *Pterocarya fraxinifolia* (Poir.) Spach, 1 Aug. 2018, A. Javadi Estahbanati, IRAN 4747C, IRAN 4748C; Mazandaran Province, Nur, leaf spot of *Quercus* sp., 1 Aug. 2018, A. Javadi Estahbanati, IRAN 4745C.

Note: Chen et al. (2015b) introduced *Phoma segeticola* with the characterization of several strains from diseased leaves of *Cirsium segetum*. Subsequently, Chen et al. (2017) transferred this species to the genus *Didymella* after the comprehensive revision of *Didymellaceae*. This species was also reported to cause leaf spots on *Camellia sasanqua*, *Camellia sinensis*, and *Nicotiana tabacum* (Ren et al. 2019, Guo et al. 2020). In phylogenetic tree, our six isolates clustered with the type strain of *D. segeticola* (CGMCC 3.17489; MLBS 95%, MPBS 91%, Fig. 1). Morphologically, the new strains differ from the type by producing larger pycnidia (123.9–392.7 × 95.4–323.6 µm vs. 90-105 × 75-95 µm). (Chen et al. 2015b).

***Ectophoma multirostrata* (P.N. Mathur, S.K. Menon and Thirum.) Valenz. -Lopez, J.F. Cano, Crous, Guarro & Stchigel, Studies in Mycology 90: 34 (2018) Fig. 4**

Conidiomata pycnidial, globose to subglobose or irregular, glabrous, solitary or confluent, frequently with some variously shaped necks, 145.9–382.7 × 95.4–323.6 µm, 95% confidence limits = 209.9–281.1 × 180.2–237.5 µm, ($\bar{x}$ ± SD = 245.5 ± 79.4 × 208.9 ± 64.6 µm, ($\bar{x}$ ± SD = 348.4 ± 57.8 × 247.9 ± 53.7 µm). Ostioles, papillate or non-papillate. Conidia oblong to ellipsoidal, sometimes eguttulate but usually with 2–3 small or large polar guttules, variable in dimensions, 2.2–5.8 × 1.7–2.9 µm, 95% confidence limits = 3.8–4.2 ×1.8–2.4 µm, ($\bar{x}$ ± SD = 4 ± 0.6 × 1.7 ± 0.2 µm). Conidial matrix creme-white. Culture characteristics: Colonies on OA, 56–57 mm diam.

After 8 days of incubation at 25 ± 0.5 °C, regular, colourless to weak olivaceous, with poorly developed, felted, white to grey olivaceous aerial mycelium or without any; reverse olivaceous.

Specimen examined. IRAN, Khuzestan Province, Ahvaz, greenhouse of Shahid Chamran University, stem rot of *Kalanchoe blossfeldiana* Poelln., 6 Nov. 2019, S.A. Ahmadpour, IRAN 4150C = SCUA-Ah-D49.

Note: *Sphaeronaema multirostratum* was originally described by Mathur & Thirumalachar (1959) from the soil in India (Boerema et al. 2004), and later transferred to the genus *Phoma* (Aveskamp et al. (2009a). Subsequently, Valenzuela-Lopez et al. (2018)

recombined this species into *Ectophoma* based on a multigene phylogenetic analysis. *Ectophoma multirostrata* was reported as thermo-tolerant species from leaves, stems, and roots of different plant species grown in tropical climates or under greenhouse conditions (Boerema et al. 2004). The new strain is phylogenetically closely related to *E. multirostrata* and *E. iranica* (MLBS 98%, MPBS 99%, BPP 0.99, Fig. 1). Morphologically, our strain is similar to the type of *E. multirostrata* (CBS 274.60) and slightly differentiated from *E. iranica* in producing longer conidia (2.2–5.8 µm vs. 1.9–4.4 µm) and smaller Pycnidia (145.9–382.7 vs. 232–438) (Aveskamp et al. 2009a, Larki et al. 2019).

***Epicoccum dendrobii* Q. Chen, Crous & L. Cai, Studies in Mycology 87: 140 (2017) Fig. 5**

Conidiomata sporodochial, aggregated, semi-immersed or superficial, clavate, pale brown. Conidiophores macronematous or semi-macronematous, unbranched, yellow to pale brown. Conidia globose, aseptate and smooth when young, later becoming multicellular-phragmosporous, verrucose, subglobose-pyriform, brown, with a basal cell, 11–14.4 × 10.4–13.5 µm, 95% confidence limits = 3.9–4.1 ×2.2–2.4 µm, ($\bar{x}$ ± SD = 12.4 ± 1 × 11.7 ± 1 µm). Culture characteristics: Colonies on OA, 45–50 mm diam after 8 days of incubation at 25 ± 0.5 °C, margin regular, covered by floccose aerial mycelia, dense, dark grey; reverse dark grey.

Specimens examined. IRAN, Khuzestan Province, Hamidiyeh, from stem necrosis of *Phragmites australis* Cav., 7 Dec. 2019, S.A. Ahmadpour, IRAN 4751C = SCUA-Ah-H268; Hamidiyeh, leaf spot of *Mentha piperita* L., 7 Dec. 2019, S.A. Ahmadpour, IRAN 4750C = SCUA-Ah-H262; Dezful, leaf spot of *Lactuca serriola* L., 4 Dec. 2018, S.A. Ahmadpour, IRAN 4165C = SCUA-Ah-A12; Dezful, leaf spot of *Trifolium alexandrium* L., 4 Dec. 2018, S.A. Ahmadpour, IRAN 4167C = SCUA-Ah-A9-2.

Note: *Epicoccum dendrobii* was introduced by Chen et al. (2017) with the characterization of the strains isolated from leaf spots of *Dendrobium fimbriatum* in China. Epicoccoid conidia of this species are similar in shape to those of *E. nigrum*, *E. poae* and *E. tobaicum*, but different in size. Our isolates are morphologically and phylogenetically similar to the type of *E. dendrobii* (LC 8145; MLBS 95%, MPBS 93%, Fig. 1).

***Epicoccum tobaicum* (Szilv) L.W. Hou, L. Cai & Crous, Studies in Mycology 96: 348 (2020) Fig. 6**

Conidiomata sporodochial, aggregated, superficial, clavate, brown. Conidiophores macronematous or semi-macronematous, unbranched, sometimes elongated and covered in mycelial hairs, yellow to pale brown. Conidia multicellular-phragmosporous, verrucose, subglobose-pyriform, with a basal cell, dark brown, 14.2–20 × 15–19.6 µm, 95% confidence limits = 18.1–19.4 ×17.4–18.3 µm, ($\bar{x}$ ± SD = 18.7 ± 3.8 × 19.6 ± 3.3 µm). Culture characteristics: Colonies on OA, 81–82 mm diam after 7 days of incubation at 25 ± 0.5 °C, margin regular, covered by floccose aerial mycelia, yellow to green-yellow, olivaceous; reverse yellow to saffron, with dark brown areas.

Specimens examined. IRAN, Khuzestan Province, Hamidiyeh, from leaf spot of *Allium schoenoprasum* L., 5 Feb. 2020, S.A. Ahmadpour, IRAN 4749C = SCUA-Ah-H19; Gilan Province, Shalman, endophyte of *Actinidia chinensis* Planch., Dec. 2016, S. Leyla Akbari, IRAN 3307C.

Note: *Toruloidea tobaica* was originally described from heath soil in Sumatra (Von Szilvinyi 1936). *Epicoccum mezzetti*, *E. oryzae*, *E. purpurascens* and *Toruloidea tobaica* were formerly regarded as synonyms of *E. nigrum* by Schol-Schwarz (1959). Recently, Hou et al. (2020a) resurrected *T. tobaica* from the synonymy of *E. nigrum* based on morphological and phylogenetic evidence, and recombined it into *Epicoccum*. *Epicoccum tobaicum* produces smaller epicoccoid conidia than *E. nigrum* (13–19.5 µm vs. 15–35 µm; Punithalingam et al. 1972) and larger than *E. mezzetti* (9–11 µm; Hou et al. 2020a). Our isolates are morphologically and phylogenetically similar to the type of *E. tobaicum* (CBS 38436; MLBS 95%, MPBS 95%, Fig. 1).

***Neomicrosphaeropsis juglandis* D. Pem, Selcuk, Jeewon & K.D. Hyde, Frontiers in Microbiology 11:1 (2020) Fig. 7**

Conidiomata pycnidial, scattered, solitary or aggregated, immersed, slightly erumpent, black, globose to subglobose, non-ostiolate, 127.6–368 × 112.6–292.6 µm, 95% confidence limits = 201.4–244.2 ×165–195.2 µm, ($\bar{x}$ ± SD = 222.8 ± 57.2 × 180.1 ± 40.3 µm, n= 60). Pycnidial wall comprising light to dark brown, thick-walled cells. Conidia yellow- or green-brown, aseptate, obovoid to ellipsoidal, smooth-walled, sometimes guttulate, 5–10.3 × 3.3–6.2 µm, 95% confidence limits = 6.6–7.3 ×3.9–4.2 µm, ($\bar{x}$ ± SD = 6.9 ± 1.1 × 4 ± 0.5 µm, n= 60). Culture characteristics: Colonies on OA, 7–8 mm diam after 8 days of incubation at 25 ± 0.5 °C, circular to irregular, flat to slightly raised, mycelium medium sparse, initially pale pink, with age becoming pink; reverse brown with the colorless edge.

Specimen examined. IRAN, Tehran Province, Chitgar Forest Park, from *Cupressus sempervirens* var. *horizontalis* (Mill.) Gord, 21 Jul. 1992, M. Mirabolfathy, IRAN 159C.

Note: *Neomicrosphaeropsis juglandis* was first introduced by Pem et al. (2020) from dead aerial stems of *Juglans regia* L. in Turkey. This species is characterized by large, aseptate conidia with a unique yellow- or green-brown color. In phylogenetic tree (Fig. 1), the new strain clustered with the ex-type strain *N. juglandis* MFLU:17-0517 and formed a well-supported monophyletic clade

(MLBS 100%, MPBS 100%, BPP 1.00). Morphologically, our strain differs from the type in the size of the conidia (5-10.3 × 3.3-6.2 µm vs. 8-11 × 6-7 µm) (Pem et al. 2020).

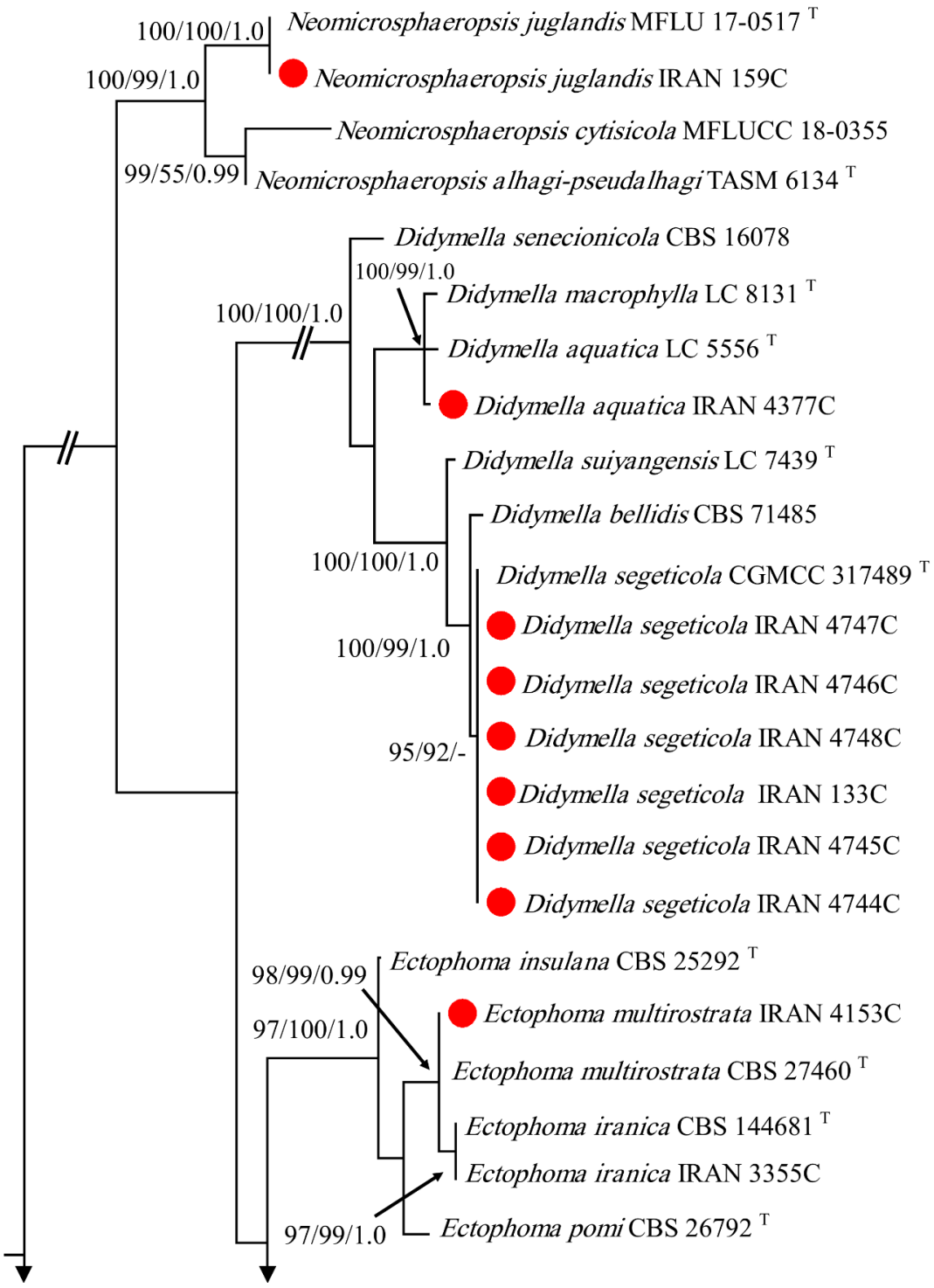

Fig. 1. Phylogenetic tree constructed from a maximum likelihood analysis based on the combined ITS, LSU, *tub2* and *rpb2* sequences. Bootstrap values obtained in maximum likelihood (MLBS) and maximum parsimony (MPBS) analyses ≥ 50% and Bayesian posterior probability values (BYPP) ≥ 0.95% are shown at the nodes, respectively. The scale bar shows the expected number of changes per site. The tree is rooted in *Neodidymelliopsis cannabis* strain CBS 121.75. Letter T indicates the ex-type strains. Taxa under study are shown with red-color filled circles.

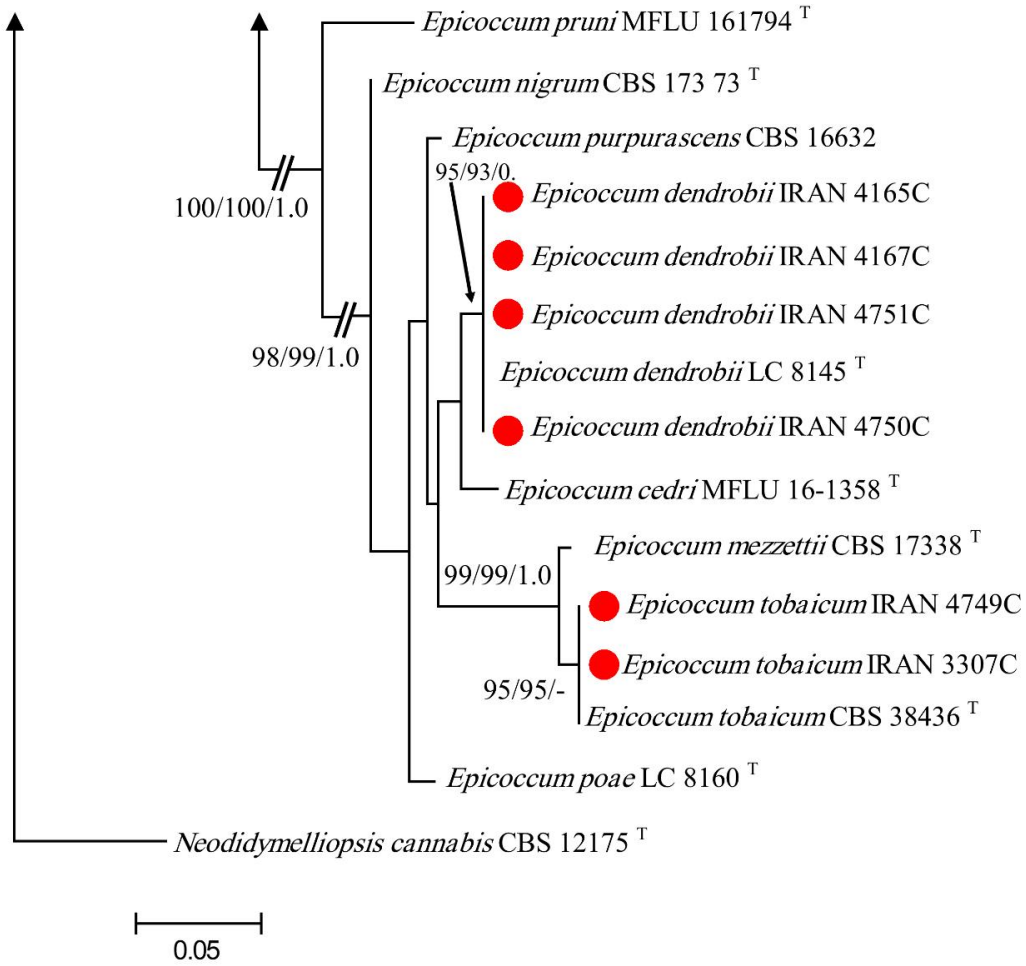

Fig. 1. (Continued).

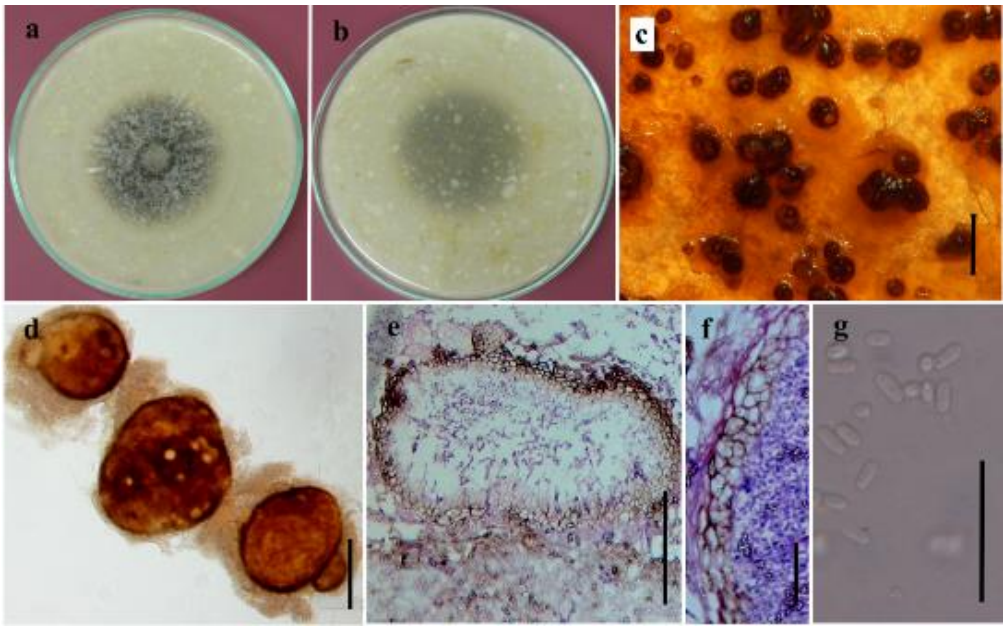

Fig. 2. *Didymella aquatica* (IRAN 4377C). a–b. colony on OA after 8 d at 28 °C (top and reverse); c–d. pycnidia; e–f. section of pycnidia; g. conidia. — Scale bars: c = 500 µm; d = 200 µm; e = 105 µm f, g = 20 µm.

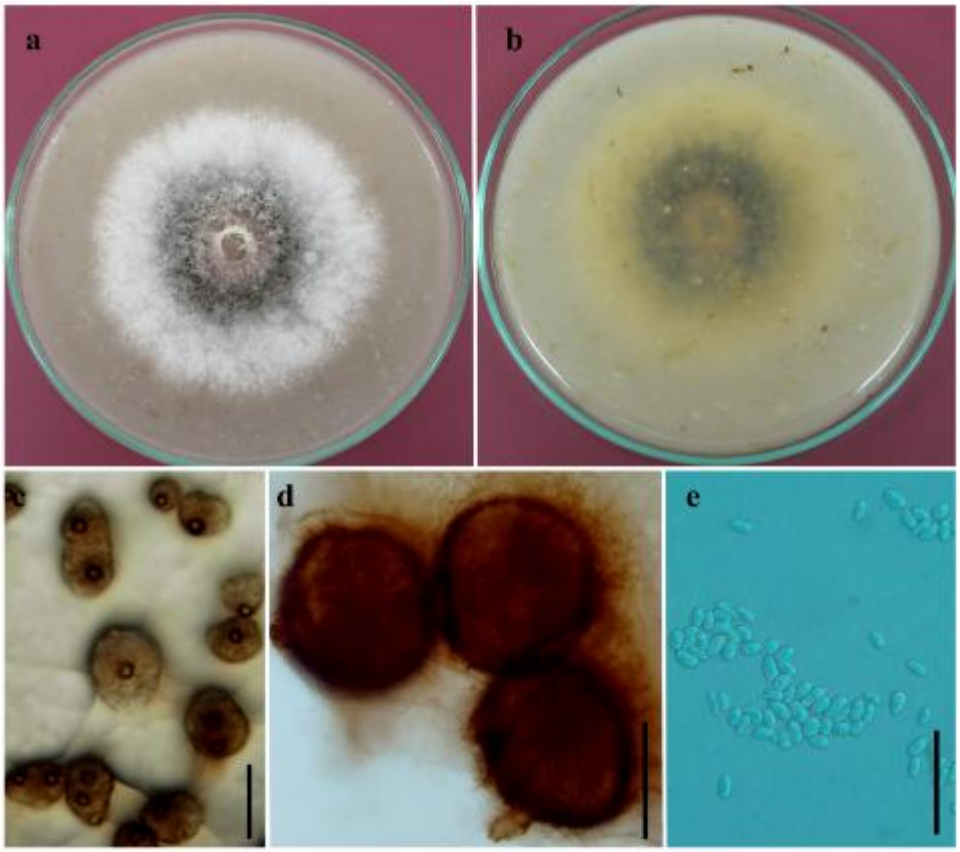

Fig. 3. *Didymella segeticola* (IRAN 4745C). a–b. colony on OA after 8 d at 28 °C (top and reverse); c–d. pycnidia; e. conidia. — Scale bars: c, d = 200 µm; e = 20 µm.

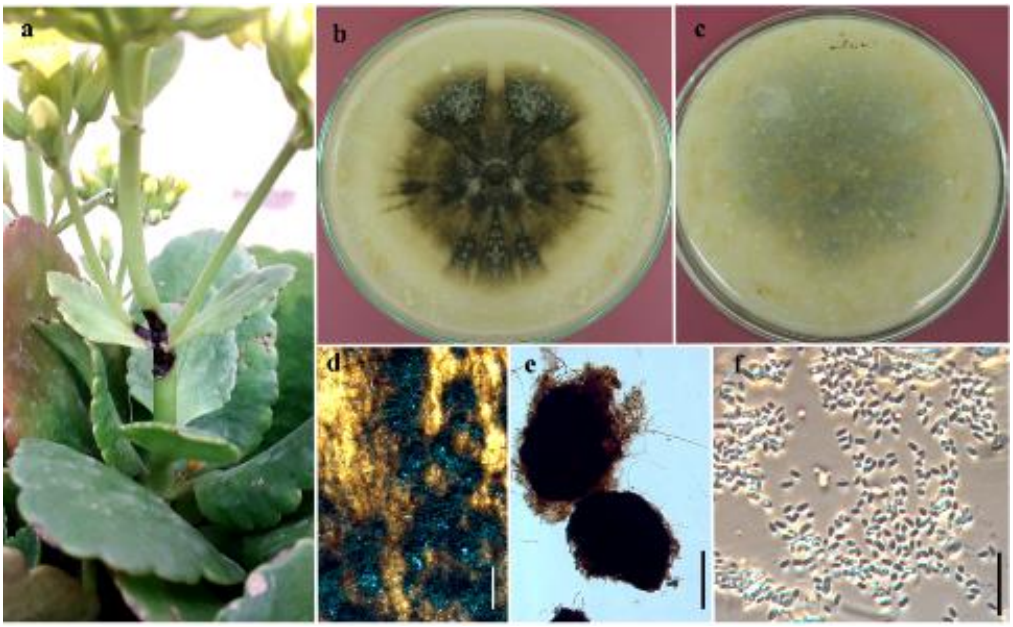

Fig. 4. *Ectophoma multirostrata* (IRAN 4150C). a. stem rot on *Kalanchoe blossfeldiana*; b–c. colony on OA after 8 d at 28 °C (top and reverse); d–e. pycnidia; f. conidia. — Scale bars: d = 500 µm; e = 200 µm; f = 20 µm.

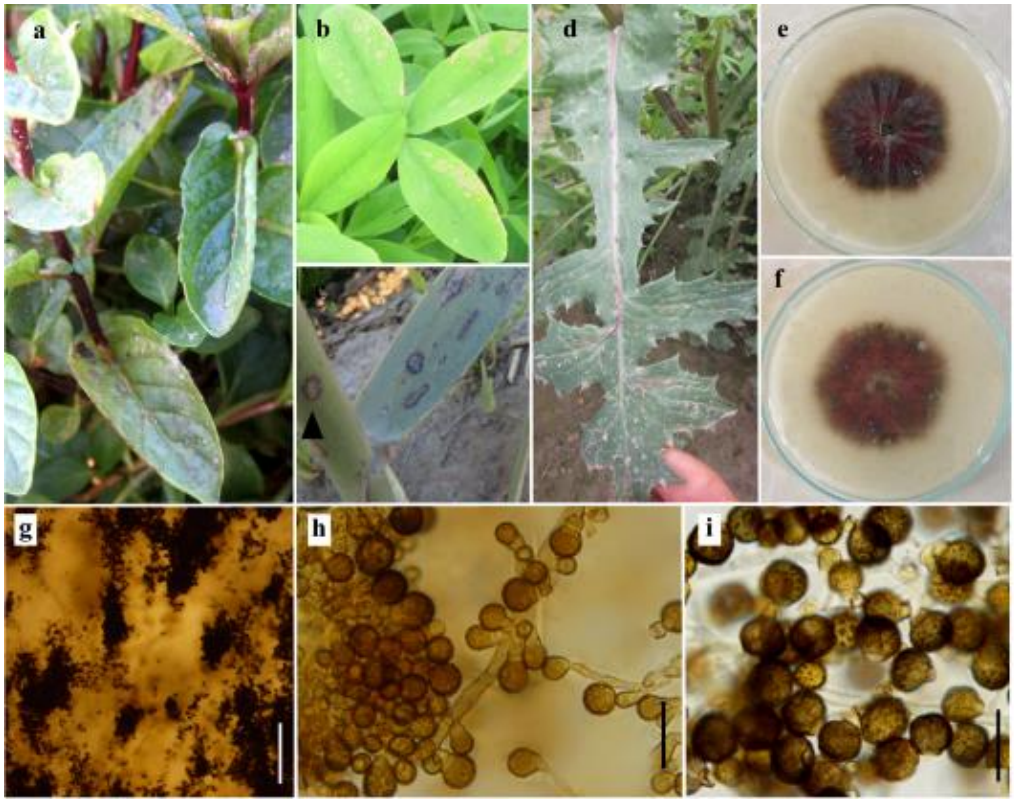

Fig. 5. *Epicoccum dendrobii* (IRAN 4751C). a–b. leaf spot on *Mentha piperita* and *Trifolium alexandrium*; c. stem necrotic lesions on *Phragmites australis*; d. leaf spot of *Lactuca serriola*; e–f. colony on OA after 8 d at 28 °C (top and reverse); g. sporodochia; h. Conidiogenous cells and conidia; i. conidia. — Scale bars: g = 200 µm; h–i = 20 µm.

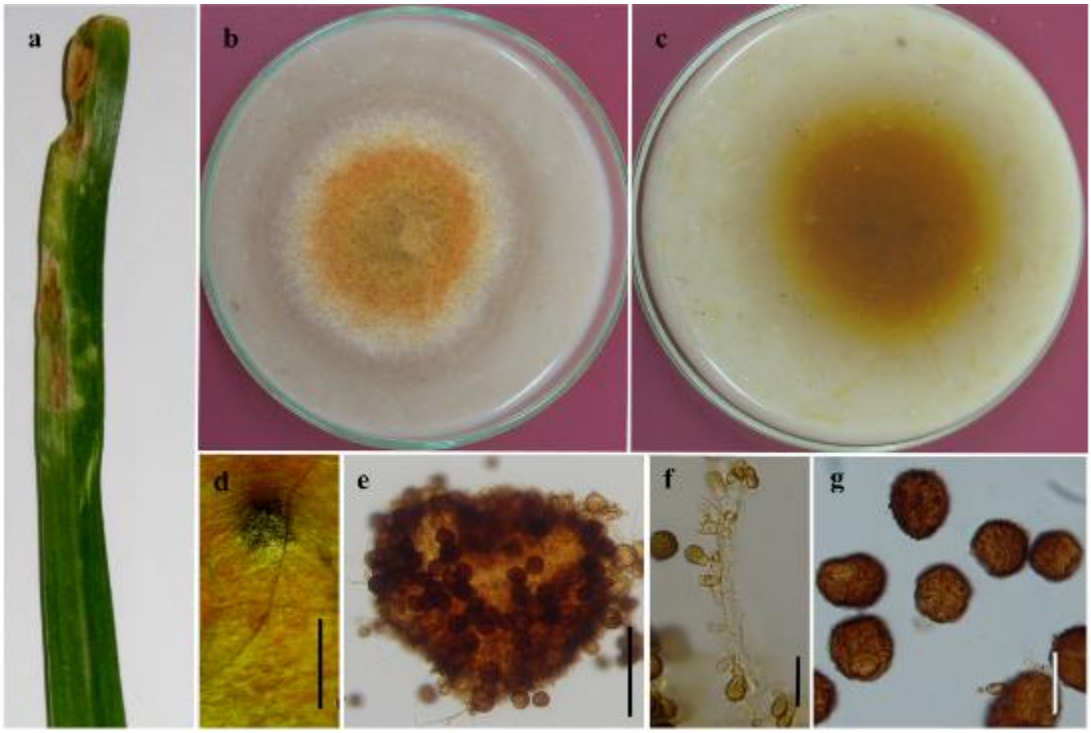

Fig. 6. *Epicoccum tobaicum* (IRAN 3307C). a. leaf spot on *Allium schoenoprasum*; b–c. colony on OA after 8 d at 28 °C (top and reverse); d–e. sporodochia; f. conidia; g. Conidiogenous cells — Scale bars: d = 500 µm; e = 50 µm; f–g = 20 µm.

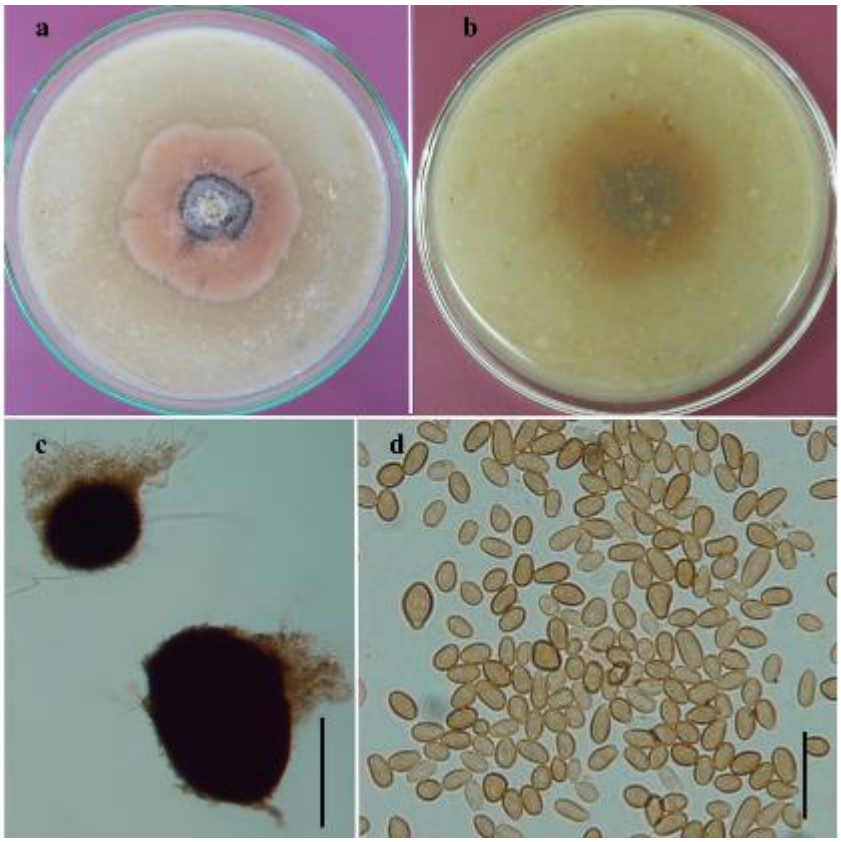

Fig. 7. *Neomicrosphaeropsis juglandis* (IRAN 2950C). a–b. colony on OA after 8 d at 28 °C (top and reverse); c. pycnidia; d. conidia. — Scale bars: c = 200 μm; d = 20 μm.

DISCUSSION

Phoma sensu lato is a large polyphyletic genus with different lineages of phoma-like species (de Gruyter et al. 2012). Most of these taxa were reclassified into *Didymellaceae* using molecular data, in combination with morphology (Aveskamp et al. 2009a, b, 2010, de Gruyter et al. 2009). The members of *Didymellaceae* and allied taxa subjected to a comprehensive taxonomic revision, based on their phylogenetic relationships (Chen et al. 2015a, 2017, Ariyawansa et al. 2015, Hyde et al. 2016, Valenzuela-Lopez et al. 2018, Hou et al. 2020a, b). In these studies, the appropriate delimitation of the genera and species within the *Didymellaceae* has been made possible by molecular phylogeny and morphological characterization

In this study, six new species belonging to four genera are reported for the fungi of Iran. Based on the literature, we combined the multi-locus data of ITS, LSU, *tub2* and *rpb2* for phylogenetic analysis. In four-locus-based phylogenetic trees (Fig. 1), the combined sequence was adequate to delimit closely related species in the genera under study.

In this study, *D. aquatica* is reported from a plant (*Acer* sp.) for the first time. Up to now, several other *Didymella* species were reported from *Acer* spp. including *D. macrostoma* and *D. sphaerellula* on *A. pseudoplatanus* and *D. nigricans* and *D. pinodella* on *A. palmatum* (Farr and Rossman 2022). *D. segeticola* has been reported from *Camellia sasanqu*a (Japan), *C. sinensis* (China), *Cirsium segetum* (China), and *Nicotiana tabacum* (China) (Ren et al. 2019, Guo et al. 2020). This is the first report of *D. segeticola* on *Diospyros lotus*, *Pterocarya fraxinifolia*, and *Quercus* sp.

Ectophoma multirostrata has been reported from *Cucumis sativus* and *Philodendron* sp. in the Netherlands and *Lilium* sp. and *Cicer arietinum* (causing root rot) in India (Aveskamp et al. 2009a, Valenzuela-Lopez et al. 2018, Chobe et al. 2020). In the current study, *E. multirostrata* is firstly reported to be associated with stem rot of *Kalanchoe blossfeldiana*. The genus *Epicoccum* is known as an air, seed and, soil-borne saprophyte. This species was reported to be a common contaminant of grass seeds but is also recorded as endophytic or weakly pathogenic from different parts of various other plants (Favaro et al. 2011). The species *E. dendrobii* has been reported from *Cunninghamia lanceolata*, *Dendrobium fimbriatum*, *Nicotiana tabacum* and *Prunus avium* (Chen et al. 2017, Bian et al. 2021, Hou et al. 2020a, Han et al. 2022). Another species, *E. tobaicum* has been reported from *Avena sativa*, *Camellia sinensis*, *Perilla* sp., *Prunus yedoensis*, *Weigela florida*, soil, and air (Chen et al. 2017, 2020, Hou et al. 2020a, Tian et al. 2021). This is the first report of *E. dendrobii* on

Phragmites australis, *Mentha piperita*, *Lactuca serriola*, and *Trifolium alexandrium*, and *E. tobaicum* on *Allium schoenoprasum* and *Actinidia chinensis*. *Neomicrosphaeropsis juglandis* has been reported from dead aerial stems of *Juglans regia* in Turkey (Pem et al. 2020). In the current study, *N. juglandis* is reported for the first time associated with *Cupressus sempervirens* var. *horizontalis*.

ACKNOWLEDGEMENTS

The authors would like to thank Hosseini Moghadam and Seyedeh Leyla Akbari for their foresight in depositing some examined strains in this study in the Iranian Fungal Culture Collection (IRAN).

Authors' contribution statement

Seyedeh Akram Ahmadpour carried out sample preparation, fungal isolation and purification, morphometric and morphological determination, DNA isolation and amplification, DNA and phylogenetic analysis and the writing of the manuscript. Mehdi Mehrabi-Koushki carried out the design and implementation of the research and revising of the manuscript. Reza Farokhinejad and Bita asgari, contributed to the implementation of the research and revising of the manuscript. Other authors, Alireza Javadi Estahbanati, Mansoureh Mirabolfathy and Kamran Rahnama, provided some fungal isolates and reviewed the manuscript.

FUNDING

This work was financially supported by grant (SCU.AP99.294) from Research Council of Shahid Chamran University of Ahvaz.

DATA AVAILABILITY

New sequences generated in the current study are deposited in NCBI GenBank (https://www.ncbi.nlm.nih.gov).

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گزارش جدید از گونه‌های قارچی خانواده *Didymellaceae* از ایران

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چکیده: تیره *Didymellaceae* یکی از غنی‌ترین تیره‌ها از نظر تنوع گونه‌ای در راسته *Pleosporales* محسوب می‌شود و شامل گونه‌های بیمارگر، پوده‌رست و درون‌رست‌های متعددی است که در طیف وسیعی از زیست‌بوم‌ها زندگی می‌کنند. در طی سال‌های ۱۴۰۰-۱۳۹۸، به‌منظور شناسایی گونه‌های متعلق به تیره *Didymellaceae* در ایران، نُه جدایه شناسایی نشده phoma-like از کلکسیون ملی قارچ‌های زنده ایران (IRAN) و شش جدایه بدست آمده از گیاهان بیمار نمونه‌برداری شده از استان خوزستان مورد بررسی قرار گرفت. جدایه‌های مورد بررسی در این مطالعه بر اساس ترکیب نتایج حاصل از تجزیه و تحلیل تبارزایی چند ژنی (ITS، LSU، *rpb2* و *tub2*) و داده‌های ریخت‌شناختی شناسایی شدند. بر این اساس، شش گونه متعلق به چهار جنس از تیره *Didymellaceae* شامل *Didymella aquatica* ، *D. segeticola*، *Ectophoma multirostrata*، *Epicoccum dendrobii*، *E. tobaicum* و *Neomicrosphaeropsis Juglandis* شناسایی شد که در اینجا توصیف می‌شوند. تمامی گونه‌های شناسایی شده برای قارچ‌های ایران جدید می‌باشند. علاوه بر این، چندین میزبان جدید برای این آرایه‌ها در اینجا گزارش می‌شود.

کلمات کلیدی: ریخت‌شناسی، تاکسونومی، تبارزایی، Phoma-like

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تاریخ دریافت: ۱۴۰۰/۵/۲۵ تاریخ پذیرش: ۱۴۰۰/۹/۳