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Original Article

Didymellaceae species associated with Zagrosian forest trees in Khuzestan Province

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ABSTRACT

The family *Didymellaceae* comprises a large number of genera and species, as well as numerous host types and geographical spreads. In the present research, 30 *Didymellaceae* isolates were obtained from Zagros forests in Khuzestan Province. Using phylogenetic analysis and morphological features, these isolates were identified as follow: *Didymella glomerata*, *D. microchlamydospora*, *D. pinodella*, *D. pomorum*, *Neomicrosphaeropsis juglandis*, *Paramicrosphaeropsis iranica*, and *P. salandica*. New hosts are reported for all of these species including *Crataegus* and *Astragalus* plant genera for *D. glomerata*, *Crataegus* for *D. microchlamydospora* and *N. juglandis*, *Cerasus microcarpa* for *D. pinodella*, *Quercus brantii* for *D. pomorum*, *Celtis caucasica* and *Cerasus microcarpa* for *P. iranica*, and *Rhamnus persica* for *P. salandica*.

KEYWORDS

Fungi, Host, Morphology, Phylogeny, Taxonomy.

INTRODUCTION

The family *Didymellaceae* was established by de Gruyter et al. (2009) to accommodate several phoma-like lineages, including *Ascochyta* Lib., *Didymella* Sacc. and *Phoma* Sacc. Afterwards, multilocus phylogenetic studies have shown that *Didymellaceae* represents one of the most diverse families in *Pleosporales*. Members of this family are widespread, occurring in diverse habitats such as soil, water, plant debris, air, and on living plants. Many species appear as saprobes on plant debris; others are known as endophytes or pathogens of a wide range of host plants (Sutton 1980, Crous et al. 2004, Liu et al. 2015, Chen et al. 2017, Wanasinghe et al. 2018).

The genus *Didymella* is known to have the most species of the *Didymellaceae*. Saccardo (1880) introduced this genus, with *Didymella exigua* (Niessl) Sacc. as the type species (Thambugala et al. 2017, Wang et al. 2021). *Didymella* was first placed within the *Mycosphaerellaceae* and then transferred into the *Pleosporaceae*, *Venturiaceae*, and *Phaeosphaeriaceae* families (de Gruyter et al. 2009). *Didymella* was previously regarded as a taxon with uncertain placement in *Pleosporales*; however, recent multilocus phylogenetic studies verified its classification in *Didymellaceae* (de Gruyter et al. 2009, Chen et al. 2015, Hyde et al. 2016). Some studies regarded the genus as polyphyletic (Aveskamp et al. 2010), but molecular data corroborate its monophyly (Chen et al. 2015). Moreover, multigene phylogenetic analyses have demonstrated superior resolution compared to morphology in defining species boundaries and clarifying taxonomic relationships within *Didymellaceae* (Wang et al. 2024).

The genus *Neomicrosphaeropsis* was proposed by Thambugala et al., with *N. italica* Thambug., Camporesi & K.D. Hyde as the type species. Moreover, additional species were incorporated into the genus, including *N. novorossica* Thambug., Bulgakov & K.D. Hyde, *N. rossica* Thambug., Bulgakov & K.D. Hyde, and *N. tamaricicola* (Wanas. Camporesi, E.B.G. Jones & K.D. Hyde) Thambug., Wanas. & K.D. Hyde (Thambugala et al. 2016). Morphologically, members of *Neomicrosphaeropsis* resemble species of *Microsphaeropsis* Syd. & P. Syd.; however, molecular phylogenetic analyses distinguish these two genera (Thambugala et al. 2016). Among the investigated molecular markers,

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β-tubulin (*TUB2)* consistently gives the highest phylogenetic resolution for species delimitation within *Didymellaceae*, proving notably helpful in resolving *Neomicrosphaeropsis* lineages (Pem et al. 2020).

The *Paramicrosphaeropsis* L.W. Hou, L. Cai & Crous was introduced by Hou et al. (2020a) with *P. ellipsoidea* L.W. Hou, L. Cai & Crous, as the type. According to phylogenetic reconstructions, *Paramicrosphaeropsis* represents a separate and well-supported lineage within *Didymellaceae* and forms a sister group with *Neomicrosphaeropsis* and *Microsphaeropsis*. Morphologically, *Paramicrosphaeropsis* is different because its pycnidia have thin walls and stay pale after the conidia are released. *Paramicrosphaeropsis* and *Neomicrosphaeropsis* can also be distinguished by the shape of conidiogenous cells (subglobose, ampulliform vs typically subcylindrical; Hou et al. 2020). This evidence justified the establishment of *Paramicrosphaeropsis* as a separate genus.

In the present study, 30 *Didymellaceae* isolates obtained from symptomatic tree species in Khuzestan Province (Iran) were identified using morphological characteristics and phylogenetic analyses.

MATERIALS AND METHODS

Sample collection and fungal isolation

The plant specimens, showing disease symptom, were collected from various areas in the Zagros forests in Khuzestan Province, during 2021–2022. Plant species included *Astragalus* sp. (stem cankers), *Celtis caucasica* (dead branches), *Crataegus* sp. (stem cankers), *Cerasus microcarpa* (stem cankers), *Quercus brantii* (fruit lesions), and *Rhamnus persica* (stem cankers). Samples were examined under a stereomicroscope for fungal structures. Fungi were isolated from the margins of healthy and diseased tissues. Small pieces were surface-disinfected in 1–2% sodium hypochlorite for 1–3 min, rinsed in sterile distilled water, and dried on sterile paper. The pieces were plated in Petri dishes containing semi-selective potato dextrose agar medium (PSA, potato extract 200–400 gL^{-1}, sucrose 10 gL^{-1}, agar 12 gL^{-1}, streptomycin sulphate 50 mgL^{-1}) and maintained at 25°C for 7–15 days. Then, emerging colonies were sub-cultured on fresh medium using the hyphal- isolation methods to obtain pure cultures. All isolates in our research are stored at the Fungal Culture Collection of the Department of Plant Protection, Shahid Chamran University of Ahvaz (SCUA).

Morphological examination

Morphological observations were performed on PDA and oatmeal agar (OA; 30–60 g L^{-1} oatmeal, 12 g L^{-1} agar). Colony characteristics, such as color, rate of growth, and structure, were checked for a period of up to 14 days at 25°C and 30°C in both light and dark conditions. Two mounting media, including lactophenol and lactophenol cotton blue, were used to mount fungal structures. An Olympus BX-50 light microscope equipped with a TUCSEN GT-12 digital camera was used to observe and measure the characteristics of fungi and take the photomicrographs. At least 30 observations for each fungal structure were measured. For each structure, minimum–maximum ranges, mean ± standard deviation, and 95% confidence intervals were calculated.

DNA isolation and amplification

Fresh mycelia were collected from 1-week-old PDA cultures at 25°C in darkness and powdered in liquid nitrogen. Genomic DNA of the mycelia was extracted using a phenol-chloroform based organic protocol as described in previous research (Mehrabi-Koushki et al. 2018). The purity of the DNA was checked on agarose gels, and the samples were kept at −20°C. Polymerase chain reaction (PCR) was used to investigate a partial region of the β-tubulin gene (*TUB2)*. The primer combinations utilized were Btub2Fd and T2 (O'Donnell and Cigelnik 1997, Woudenberg et al. 2009). The PCR mixture in a volume of 30 µL consists of 3 µl DNA template, 0.4 mM dNTPs, 3 µL of 10× PCR buffer, 1.5 mM $MgCl_2$, 0.4 µM of each forward and reverse primers, 3 U of Prime Taq DNA polymerase, and ddH2O up to the final volume. The PCR conditions included 95 °C for 3 min, followed by 35 cycles of 95 °C for 30 s, 56 °C for 30 s, and 72 °C for 30 s, with the final extension at 72 °C for 5 min. The PCR products were checked using 1% agarose gel electrophoresis to confirm the presence of the expected *TUB2* fragment (approx. 400 bp) and sequenced at the Codon Genetics Institute in Tehran, Iran.

Phylogenetic analyses

Phylogenetic analyses were conducted using the *TUB2* sequence dataset. Consensus sequences were queried against NCBI using BLASTn search to reveal closely matched ex-type or known strains. The sequences of type strains belonging to related species were obtained from GenBank and imported into alignments. The *TUB2* gene matrix was aligned by BioEdit v. 7.2.5, considering gaps as missing data. To examine the evolutionary relationships, maximum likelihood (ML), maximum parsimony (MP), and Bayesian inference (BI) algorithms were used. The trees were rooted with *Vacuiphoma ferulae* strain (CBS 353.71). ML analysis was carried out using RAxML-HPC2 as implemented in raxmlGUI 2.0 beta (Edler et al. 2021) with 1,000 bootstrap repetitions (MLBS). MP analysis was carried out by MEGA v.7 (Tamura et al. 2013) with 1,000 bootstrap replicates (MPBS). The MrBayes v.3.2.6 implemented Markov Chain Monte Carlo sampling (MCMC) for calculating Bayesian posterior probabilities (BPP) values (Ronquist et al. 2012). The jModelTest 2 (Darriba et al. 2012) was used to determine the best-fit model of evolution for the alignment. Accordingly, GTR+I+G was selected for the *TUB2* dataset. Two independent runs, each comprising four concurrent Markov chains, were conducted with a burn-in of 0.25. Each run lasted for 3,000,000 generations, with sampling occurring every 300 generations. The DNA sequences generated during the current study were deposited in GenBank (Table 1).

Table 1. Strains used for phylogenetic analyses in this study. Newly generated sequences are shown in bold. T: ex-type strain.

Taxon	Strain[a]	Source	Origin	*TUB2*
Didymella arachidicola	CBS 333.75^T	*Arachis hypogaea*	South Africa	GU237554
D. aurea	CBS 269.93^T	*Medicago polymorpha*	New Zealand	GU237557
D. calidophila	CBS 448.83^T	Soil	Egypt	FJ427168
D. coffeae-arabicae	CBS 123380^T	*Coffea arabica*	Ethiopia	FJ427104
D. curtisii	CBS:288.29	*Nerine* sp.	The Nether-lands	FJ427148
D. exigua	CBS 183.55^T	*Rumex arifolius*	France	GU237525
D. glomerata	CBS 528.66	*Chrysanthemum* sp.	The Nether-lands	FJ427124
D. glomerata	**SCUA-IS-A4**	***Crataegus* sp.**	**Iran**	**PZ013121**
D. glomerata	**SCUA-IS-B8**	***Astragalus* sp.**	**Iran**	**PZ013122**
D. keratinophila	UTHSC DI16-200^T	Human	USA	LT592970
D. lethalis	CBS 504.85	*Olea europaea*	Italy	MN983864
D. microchlamydospora	CBS 105.95^T	*Eucalyptus* sp.	UK	FJ427138
D. microchlamydospora	**SCUA-IS-A12**	***Crataegus* sp.**	**Iran**	**PZ013123**
D. mitis	CBS:443.72^T	Soil	South Africa	MT005624
D. nigricans	CBS 444.81^T	*Actinidia chinensis*	New Zealand	GU237558
D. pinodella	CBS 318.90	*Pisum sativum*	The Nether-lands	FJ427161
D. pinodella	**SCUA-IS-E7**	***Cerasus microcarpa***	**Iran**	**PZ013124**
D. pinodes	CBS 525.77^T	*Pisum sativum*	Belgium	GU237572
D. pomorum	CBS 286.76	*Allium nutans*	Russia	FJ427164
D. pomorum	**SCUA-IS-A6**	***Astragalus* sp.**	**Iran**	**PZ013125**
D. pomorum	**SCUA-IS-D7**	***Quercus brantii***	**Iran**	**PZ013126**
D. rumicicola	CBS 683.79^T	*Rumex obtusifolius*	New Zealand	KT389800
D. sancta	CBS 281.83^T	*Ailanthus altissima*	South Africa	FJ427170
D. sinensis	CGMCC 3.18348^T	*Cerasus pseudocerasus*	China	KY742327
Neomicrosphaeropsis alhagi-pseudalhagi	MFLUCC17-0825^T	*Alhagi pseudoalhagi*	Uzbekistan	MH069689
N. elaeagni	MFLU 16-2389	*Elaeagnus angustifolia*	Russia	MH069691
N. juglandis	MFLU 17-0517^T	*Juglans regia*	Turkey	MN871954
N. juglandis	**SCUA-Is-B6**	***Crataegus* sp.**	**Iran**	**PZ013127**
Paramicrosphaeropsis amygdalus	IRAN 4452C^T	*Amygdalus scoparia*	Iran	MZ747174
P. iranica	IRAN 2929C^T	*Quercus brantii*	Iran	OK247743
P. iranica	**SCUA-Is-E10**	***Cerasus microcarpa***	**Iran**	**PZ013128**
P. iranica	**SCUA-Is-Z1**	***Celtis caucasica***	**Iran**	**PZ013129**
P. pistacicola	IRAN 4449C^T	*Pistacia khinjuk*	Iran	MZ747183
P. pistacicola	IRAN 4450C	*Pistacia atlantica*	Iran	MZ747184
P. salandica	IRAN 4376C^T	*Crataegus* sp.	Iran	MZ747180
P. salandica	IRAN4455C	*Nerium oleander*	Iran	MZ747178
P. salandica	**SCUA-Is-D8**	***Rhamnus persica***	**Iran**	**PZ013130**
P. vitis	IRAN 5248C^T	*Vitis vinifera*	Iran	PQ240635
P. zagrosensis	IRAN 4448C^T	*Quercus brantii*	Iran	MZ747172
P. zagrosensis	SCUA-Ar-Z1B	*Crataegus* sp.	Iran	MZ747173
Vacuiphoma ferulae	CBS 353.71	*Ferula communis*	Italy	MT005655

[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.

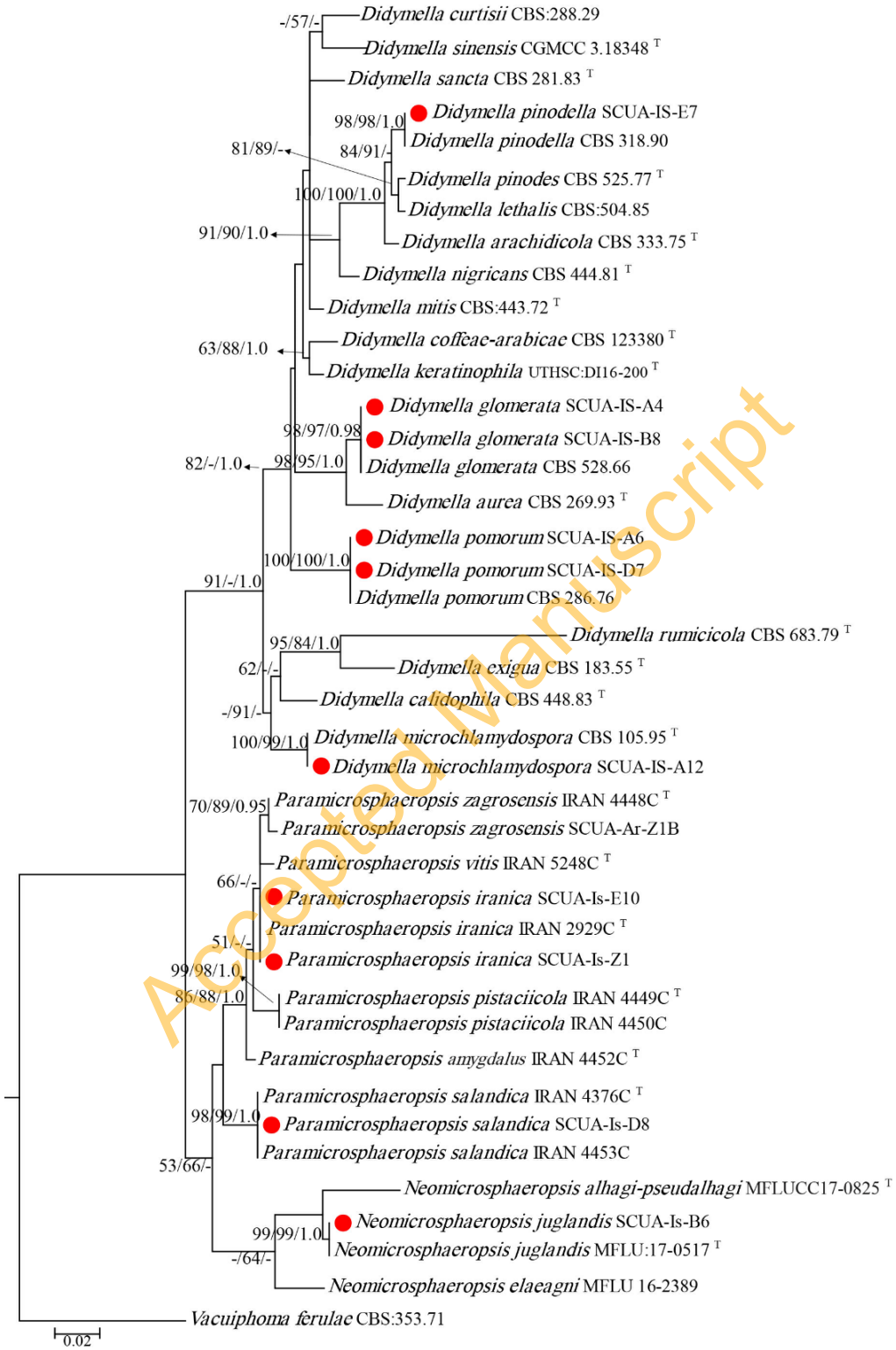

Fig. 1. Phylogenetic tree constructed from a maximum likelihood analysis based on *TUB2* sequences. Bootstrap values obtained in maximum likelihood (MLBS) and maximum parsimony (MPBS) analyses ≥ 50% and Bayesian posterior probability values (BYPP) ≥ 0.95% are shown at the nodes, respectively. The scale bar shows the expected number of changes per site. The tree is rooted in *Vacuiphoma ferulae* strain (CBS 353.71). Letter T indicates the ex-type strains. Taxa under study are shown with red-color filled circles.

RESULTS AND DISCUSSION

This research collected 30 *Didymellaceae* isolates from forest plants. Following morphological analysis, these isolates were divided into seven morphologically identical groups. These strains were obtained from diseased tissues of *Astragalus* sp. (seven isolates), *Celtis caucasica* (six isolates), *Crataegus* sp. (eight isolates), *Cerasus microcarpa* (four isolates), *Quercus brantii* (two isolates), and *Rhamnus persica* (three isolates). Ten isolates representing the above groups were selected for further morphological and phylogenetic analyses. Phylogenetic analyses were carried out using the *TUB2* gene alignment, which contained 40 sequences, with *Vacuiphoma ferulae* strain (CBS 353.71) as the outgroup (Table 1). The final single-gene dataset comprising 332 characters from the *TUB2* alignment (including gaps). With the aid of morphological and molecular data, we identified seven species of *Didymellaceae*, namely, *Didymella glomerata*, *D. microchlamydospora*, *D. pinodella*, *D. pomorum*, *Neomicrosphaeropsis juglandis*, *Paramicrosphaeropsis iranica*, and *P. salandica*.

Taxonomy

Didymella glomerata (Corda) Q. Chen & L. Cai, Studies in Mycology 82: 176 (2015). Fig. 2.

Pycnidia globose, subglobose or ellipsoidal, formed after a week, solitary, brown to dark brown, superficial or semi-immersed, ostiolate, 111–179 × 102–168 µm, 95% confidence limits = 137–149 × 127–139 µm, ($\bar{x}$ ± SD = 143 ± 16.4 × 133 ± 16 µm, n = 30). Conidia ellipsoidal to cylindrical, guttulate, aseptate, hyaline, smooth, 3.9–7 × 2–3.2 µm, 95% confidence limits = 5.5–5.9 × 2.5–2.7 µm, ($\bar{x}$ ± SD = 5.7 ± 0.6 × 2.6 ± 0.3 µm, n = 50). Conidial matrix hyaline to grey. Chlamydospores abundant, multicellular, dictyosporous, Alternarioid, dark brown, solitary or in short chains, intercalary or terminal, 15–38 × 9–20 µm.

Culture characteristic: Colonies on PDA attaining 78 mm diam after 1-week at 25 °C, and 70 mm diam at 30 °C, entire margin, brown to green with white margin and dark green in the middle, floccose; reverse similar. Colonies on OA attaining 65 mm diam after 1-week of incubation at 25 °C, and 30 mm diam at 30 °C, entire margin, dark brown with white margin, with sporulation in the center, floccose; reverse is similar.

Materials examined: Iran, Khuzestan Province, Dezpart, from the stem canker of *Astragalus* sp. and *Crataegus* sp. 8 Feb-2022, P. Eisvand, SCA-Is-A4 and SCUA-Is-B8.

Note: In BLASTn search, the closest match for the *TUB2* sequence of our strains was *Didymella glomerata* strain CBS 528.66 (*TUB2*: GenBank MW815128, identities = 100%). *Didymella glomerata* (Syn. *Phoma glomerata* (Corda) Wollenw. & Hochapfel) was introduced by Boerema (1993). This species was reassigned to the genus *Didymella*, following a revision of the family *Didymellaceae* (Chen et al. 2015). The phylogenetic tree (Fig. 1) shows two strains in a well-supported clade with *D. glomerata* (CBS 528.66; MLBS 98%, MPBS 97%, BPP 0.98). The morphology of our strains resembles that of the *D. glomerata* strain CBS 528.66 (Boerema 1993). In this study, *D. glomerata* is reported from *Crataegus* and *Astragalus* plant genera for the first time. In Iran, *D. glomerata* is reported from *Q. brantii* and *Q. libani* G.Olivier (Bashiri and Abdollahzadeh, 2024), *Cordia myxa* L. (Keshavarzi et al. 2023), *Vitis vinifera* L. (Mirabolfathy et al. 2020), *Citrus limon* (L.) Osbeck (Soleimani et al. 2018), *Q. brantii* (Alidadi et al. 2019), *Alyssum* sp., *Convolvulus* sp., *Chrysanthemum* sp., *Cupressus arizonica* Greene, *Inula helenium* L., Lolium sp., *Peganum harmala* L., Secale montanum Guss. and V. vinifera (Khodaei et al. 2018). In addition, *D. glomerata* has been isolated from a wide range of monocot and dicot hosts, including *Actinidia chinensis* Planch., *Bupleurum falcatum* L., *Cornus officinalis* Siebold & Zucc., *Leymus chinensis* (Trin.) Tzvelev, *Lonicera caerulea* L., *Prunus persica* (L.) Batsch, *Sophora tonkinensis* Gagnep. and *Zea mays* L. in China (Chen et al. 2017, Huang et al. 2018, Pan et al. 2018, Cui et al. 2021, Ma et al. 2022, Song et al. 2021, Tang et al. 2023, Yan et al. 2023), *Chrysanthemum* sp. in the Netherlands (Chen et al. 2017, Valenzuela-Lopez et al. 2018), *Pistacia vera* L. in Arizona (Moral et al. 2018, Nouri et al. 2019), *Vitis* sp. in Spain and Switzerland (Jayawardena et al. 2018), and *Rubus fruticosus* L. in Turkey (Derviş et al. 2024).

Didymella microchlamydospora (Aveskamp & Verkley) Q. Chen & L. Cai, Studies in Mycology 82: 178 (2015). Fig. 3.

Pycnidia glabrous or with hyphal outgrowths, normally solitary, rarely aggregated, mostly pyriform, subglobose or ellipsoidal, brown, superficial, semi-immersed or immersed, ostiolate, 118–181 × 111–170 µm, 95% confidence limits = 138–150 × 136–147 µm, ($\bar{x}$ ± SD = 143 ± 13 × 142 ± 12 µm, n = 50). Pycnidia produce 1(–2) necks. Conidia ellipsoidal, smooth, hyaline, aseptate, straight or slightly curved, 3.5–6.5 × 2–3.5 µm, 95% confidence limits = 4.5–5 × 2.5–2.8 µm, ($\bar{x}$ ± SD = 4.75 ± 0.6 × 2.6 ± 0.3 µm, n = 50). Chlamydospores intercalary or terminal, unicellular, dark brown, solitary or in short chains, 4.5–8(11.5) × 3.5–7.5(11) µm. Multicellular chlamydospores variable in size and shape, dictyosporous, and dark brown.

Culture characteristic: Colonies growing on PDA attaining 50 mm diam after 1-week at 25 °C, and 45 mm diam at 30 °C, regular margin, greyish with paler margin, floccose; reverse brownish with white margin. Colonies growing on OA attaining 43 mm diam after 1-week at 25 °C, and 17 mm diam at 30 °C, circular with regular margin, white to pink with a brown margin, floccose; reverse light brown with brown in the center.

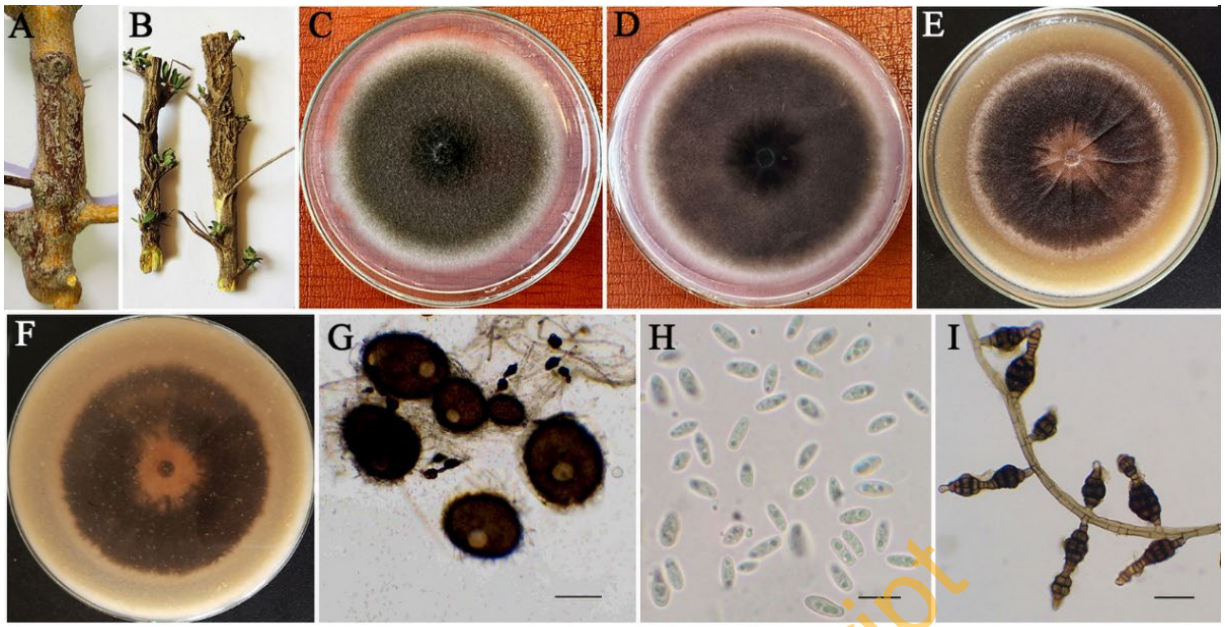

Fig. 2. *Didymella glomerata* (SCUA-Is-A4). (A, B) Stem canker on *Crataegus* sp. and *Astragalus* sp., Colonies on PDA (C, D), and OA (E, F) after 7 d at 25 °C (top and reverse), (G) Pycnidia, (H) Conidia, (I) Chlamydospores. Scale bars: (G) 70 µm, (H) 7 µm, (I) 20 µm.

Materials examined: Iran, Khuzestan Province, Dezpart, from stem canker of *Crataegus* sp., 8 Feb-2022, P. Eisvand, SCUA-Is-A12.

Note: The closest result in the BLASTn search for the *TUB2* sequence of the new strain (SCUA-Is-A12) was *Didymella microchlamydospora* strain CBS 105.95 (*TUB2:* GenBank FJ427138, identities = 100%). *Didymella microchlamydospora* (Syn. *Phoma microchlamydospora*) was introduced from *Eucalyptus* sp. in the United Kingdom (Aveskamp et al. 2009). After that, this species was transferred to the genus *Didymella* (Chen et al. 2015). The new strain is morphologically and phylogenetically similar to the type of *Didymella microchlamydospora* (CBS 105.95; MLBS 100%, MPBS 99%, BPP 1.00) (Fig. 1).

Didymella microchlamydospora has been documented on several host plants in Iran, such as *Morus nigra* L., *Nerium oleander* L., *Callistemon viminalis* (Sol. ex Gaertn.) G. Don, and *Olea europaea* L. (Ahmadpour et al. 2017). This is the first report of *D. microchlamydospora* on *Crataegus* sp.

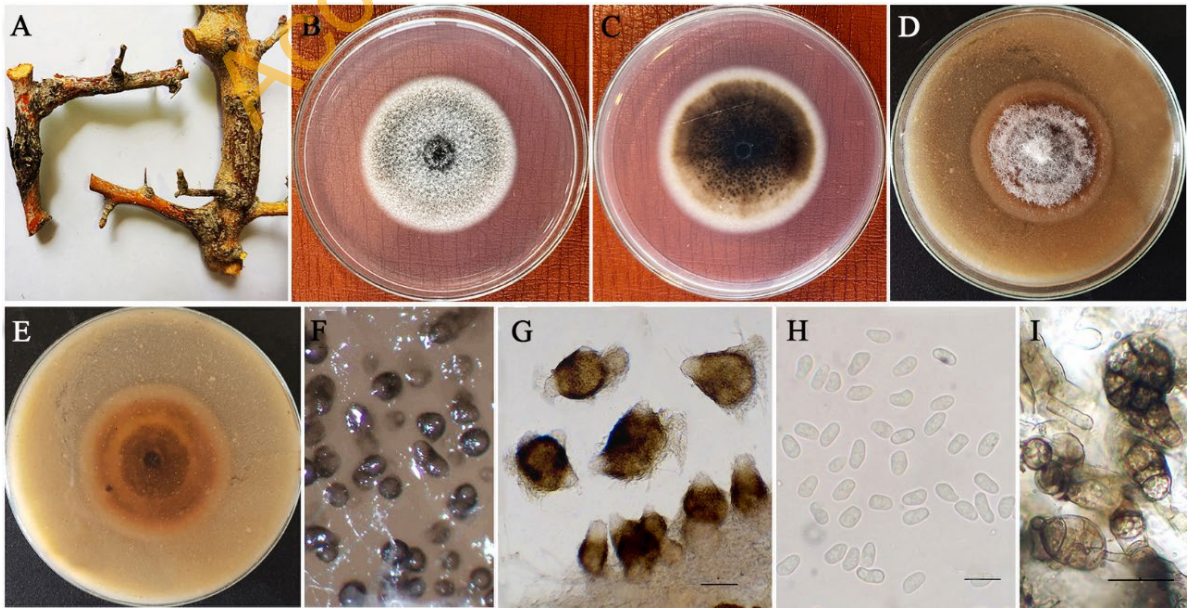

Fig. 3. *Didymella microchlamydospora* (SCUA-Is-A12). (A) Stem canker on *Crataegus* sp., Colonies on PDA (B, C), and OA (D, E) after 7 d at 25 °C (top and reverse), (F, G) Pycnidia, (H) Conidia, (I) Chlamydospores. Scale bars: (G) 70 µm, (H) 7 µm, (I) 20 µm.

Didymella pinodella (L.K. Jones) Qian Chen & L. Cai, in Chen, Jiang, Zhang, Cai & Crous, Stud. Mycol. 82: 178 (2015). Fig. 4.

Pycnidia globose or subglobose, glabrous or with hyphal outgrowths, mostly solitary, brown to dark brown, superficial, ostiolate, 70–191 × 68–186 µm, 95% confidence limits = 111–138 × 95–119 µm, ($\bar{x}$ ± SD = 125 ± 35 × 107 ± 33 µm, n = 50). Conidia ellipsoidal or cylindrical, smooth, hyaline, aseptate or septate, guttulate, 6–9 × 2.4–4.3 µm, 95% confidence limits = 7.1–8 × 3–3.6 µm, ($\bar{x}$ ± SD = 7.5 ± 0.8 × 3.3 ± 0.45 µm, n = 50). Conidial matrix cream to greyish. Chlamydospores intercalary or terminal, unicellular and multicellular, solitary or in chains. 4–10 × 5–12 µm.

Culture characteristic: Colonies growing on PDA attaining 73 mm diam after 1-week at 25 °C, and 61 mm diam at 30 °C, entire margin, dark green to brown with white margin, floccose; reverse brownish with white margin. Colonies growing on OA attaining 53 mm diam after 1-week at 25 °C, and 45 mm diam at 30 °C, regular margin, brown to dark brown with hyaline border, floccose; reverse similar.

Materials examined: Iran, Khuzestan Province, Izeh, from stem canker of *Cerasus microcarpa* K. Koch, 9 May-2022, P. Eisvand, SCUA-Is-E7.

Note: According on a Blastn search, the nearest relevant hits with the *TUB2* region of our strain were *Didymella pinodella* strain CBS 318.90 (*TUB2*: GenBank MZ073909, identities = 100%). *Didymella pinodella* was described by L.K. Jones (1927) under the name *Ascochyta pinodella* and then was transferred to *Phoma* (de Gruyter et al. 2002). In 2015, *Phoma pinodella* was reclassified, and recombined into *Didymella* (Chen et al. 2015). Phylogenetically, the new strain (SCUA-Is-E7) formed a monophyletic clade with the *D. pinodella* strain CBS 318.90 (MLBS 98%, MPBS 98%, BPP 1.00) (Fig. 1). Morphologically, our strain differs from *D. pinodella* strain CBS 531.66 in the size of the Conidia (6–9 µm vs. 4-7.5 µm; de Gruyter et al. 2002).

Didymella pinodella is firstly reported on *Pisum sativum* and is widely recognized as a causal agent of root rot in pea crops, particularly in Canada (Esmaeili Taheri et al. 2016, Bainard et al. 2017), Australia (Tran et al. 2014) and several European regions, including Germany, Denmark, France and Sweden (Persson et al. 1997, Pflughöft et al. 2012, Baćanović-Šišić et al. 2018, Šišić et al. 2019, Šišić et al. 2024). Reports consistently describe this species as a highly aggressive pathogen on pea (Tran et al. 2016, Schmidt et al. 2022). This species has been associated with root infections in other legumes, including lentils, chickpeas, and faba beans (Šišić et al. 2022, Farr and Rossman 2026). *Didymella pinodella* has been reported from *Trigonella foenum-graecum* L. in Iran (Amirdehi et al. 2017). In the current study, this is the first report of *D. pinodella* on *Cerasus microcarpa*.

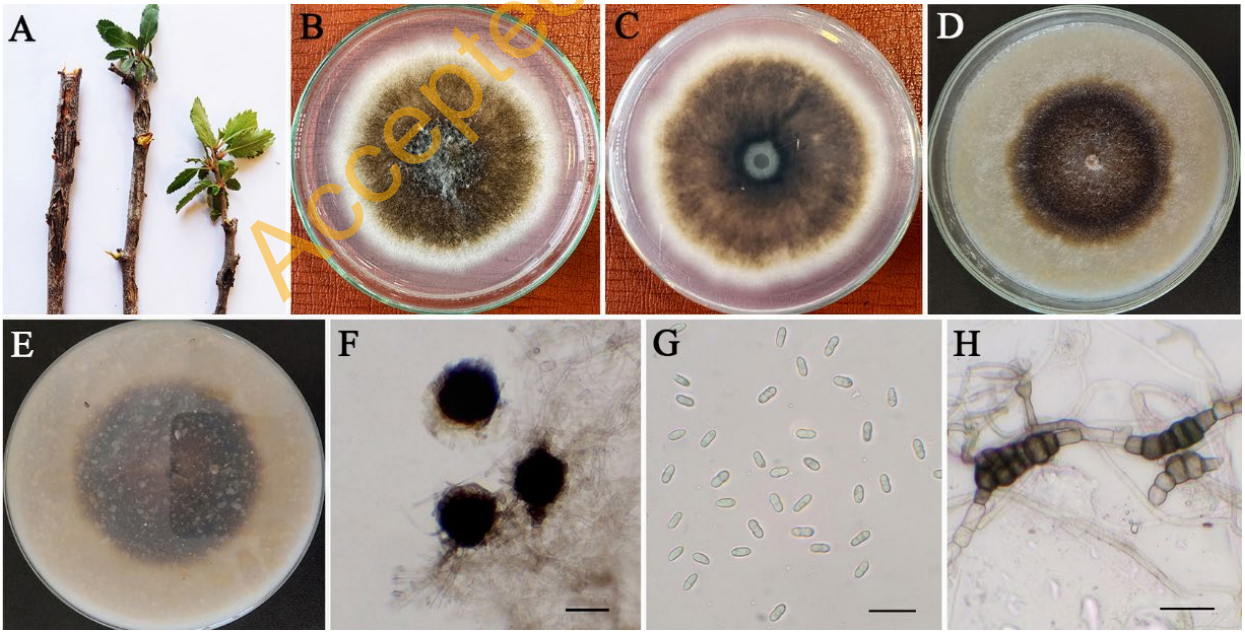

Fig. 4. *Didymella pinodella* (SCUA-Is-E7). (A) Stem canker on *Cerasus microcarpa*, Colonies on PDA (B, C), and OA (D, E) after 7 d at 25 °C (top and reverse), (F) Pycnidia, (G) Conidia, (H) Chlamydospores. Scale bars: (F) 70 µm, (G) 20 µm, (H) 20 µm.

Didymella pomorum (Thum) Chen & L. Cai, Studies in Mycology 82: 179 (2015). Fig. 5.

Pycnidia globose, subglobose or ellipsoidal, brown to dark brown, immersed or semi-immersed, solitary or grouped in clusters, glabrous or with hyphal outgrowths, ostiolate, 95–190 × 80–188 µm, 95% confidence limits = 133–149 × 122–137 µm, ($\bar{x}$ ± SD = 141 ± 25 × 129 ± 23 µm, n = 50). Conidia ellipsoidal or cylindrical, ovoid, smooth, aseptate, guttulate, 4–8.5 × 2.4–4 µm, 95% confidence limits = 6.2–6.5 × 3–3.3 µm, ($\bar{x}$ ± SD = 6.2 ± 0.4 × 3 ± 0.1 µm, n = 50). Chlamydospores unicellular or multicellular, intercalary or terminal, single or in short chains, 6.5–13.5 × 6–13 µm.

Culture characteristic: Colonies on PDA attaining 74 mm diam after 1-week at 25 °C, and 60 mm diam at 30 °C, entire margin, paler green with white margin, floccose; reverse dark brown with white margin. Colonies on OA attaining 58 mm diam after 1-week at 25 °C, and 50 mm diam at 30 °C, entire margin, grey to paler green with hyaline margin, floccose to cottony; reverse dark green with pale border.

Materials examined: Iran, Khuzestan Province, Dezpart, from stem canker *Astragalus* sp. and fruit lesions of *Quercus brantii*, 9 Mar-2022, 8 Feb-2022, P. Eisvand, SCUA-Is-A6 and SCUA-Is-D7.

Note: in BLASTn search, the closest match for the *TUB2* sequence of our strains was *Didymella pomorum* strain CBS 286.76 (*TUB2*: GenBank KT389799, identities = 100%). This species was originally described as *Phoma pomorum* Thüm (Boerema et al. 2004). This species was reassigned to *Didymella*, following phylogenetic and molecular investigations (Chen et al. 2015). The new strain clustered with *D. pomorum* strain CBS 286.76 and created a strongly supported monophyletic clade (MLBS 100%, MPBS 100%, BPP 1.00) (Fig. 1). Morphologically, our strain is similar to *D. pomorum* (CBS 539.66, Boerema 1993).

Didymella pomorum has been found in China on *Angelica sinensis* (Oliv.) Diels, *Dendrobium fimbriatum* Hook., *Gentiana straminea* Maxim., *Vitis* sp., and *Vitis vinifera* L. (Chen et al. 2017, Jayawardena et al. 2018, Wang et al. 2024), in Russia on *Heracleum dissectum* Ledeb. (Chen et al. 2015, Chen et al. 2017, Valenzuela-Lopez et al. 2018), in South Africa on *Malus domestica* (Suckow) Borkh. and *Triticum* sp. (Chen et al. 2017, Havenga et al. 2019), in the Netherlands on *Polygonum tataricum* L. (Hou et al. 2020b), and in Canada on *Rosa* sp. (Ilyukhin, 2022). According to these reports, *D. pomorum* can cause leaf spots on many plants. In the current study, *D. pomorum* is firstly reported to be associated with *Quercus brantii.*

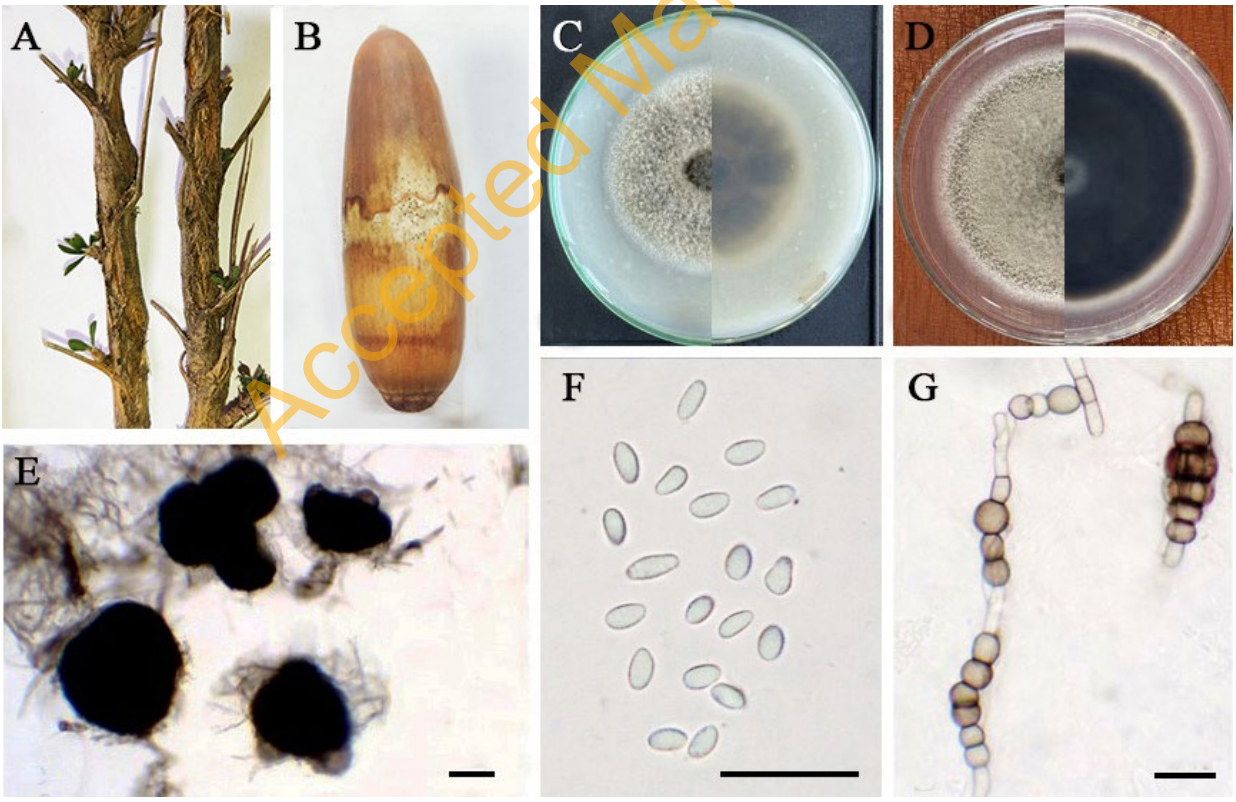

Fig. 5. *Didymella pomorum* (SCUA-Is-A6). (A) Stem canker on *Astragalus* sp., (B) fruit lesions of *Quercus brantii*, Colonies on PDA (C), and OA (D) after 7 d at 25 °C (top and reverse), (E) Pycnidia, (F) Conidia, (G) Chlamydospores. Scale bars: (E) 70 µm, (F) 20 µm, (G) 20 µm.

Neomicrosphaeropsis juglandis D. Pem, Selcuk, Jeewon & K.D. Hyde, Frontiers in Microbiology 11:1 (2020). Fig. 6.

Pycnidial globose, subglobose, dark brown, solitary or clustered, immersed or semi-immersed, ostiolate, 150–310 × 135–280 µm, 95% confidence limits = 175–210 × 153–182 µm, ($\bar{x}$ ± SD = 194 ± 36 × 167 ± 31 µm, n = 50). Conidia smooth, aseptate, mostly obovoid to ellipsoidal, pale brown to brown, 5.5–10.2 × 4.5–7 µm, 95% confidence limits = 7.5–8 × 5.2–5.6 µm, ($\bar{x}$ ± SD = 7.8 ± 1 × 5.3 ± 0.5 µm, n = 50). Chlamydospores unicellular, intercalary or terminal, single or in chains, 9–16 × 6–10.5 µm.

Culture characteristic: Colonies growing on PDA attaining 38 mm diam after nine days at 25 °C, and 13 mm diam at 30 °C, regular margin, cream with hyaline margin, floccose; reverse pinkish. Growing on OA attaining 44 mm diam after nine days at 25 °C, and 17 mm diam at 30 °C, regular margin, pinkish with a paler margin, floccose; reverse similar.

Materials examined: Iran, Khuzestan Province, Dezpart, from stem canker of *Crataegus* sp., 8 Feb-2022, P. Eisvand, SCUA-Is-B6.

Note: According to a BLASTn search, the closest hit to our strain was *Neomicrosphaeropsis juglandis* strain MFLU:17-0517 (*TUB2:* GenBank MN871954, identities = 100%). Originally, *Neomicrosphaeropsis juglandis* was described by Pem et al. (2020). It was isolated from dead branches of *Juglans regia* (walnut) in Turkey. Our strain clustered with *N. juglandis* strain MFLU:17-0517 (Fig. 1) and was morphologically similar to the type strain (MLBS 99%, MPBS 99%, BPP 1.00).

Neomicrosphaeropsis juglandis was isolated from *Cupressus sempervirens* var. *horizontalis* (Mill.) G. Don in Iran (Ahmadpour et al. 2021). Several taxa previously classified as *Neomicrosphaeropsis*, including *N. minimum*, *N. cytisi*, *N. cytisicola*, and *N. cytisina*, were re-evaluated using multilocus phylogenetic studies and reclassified as *Microsphaeropsis* (Artand et al. 2022). This study reports for the first time the association of *N. juglandis* with *Crataegus* sp.

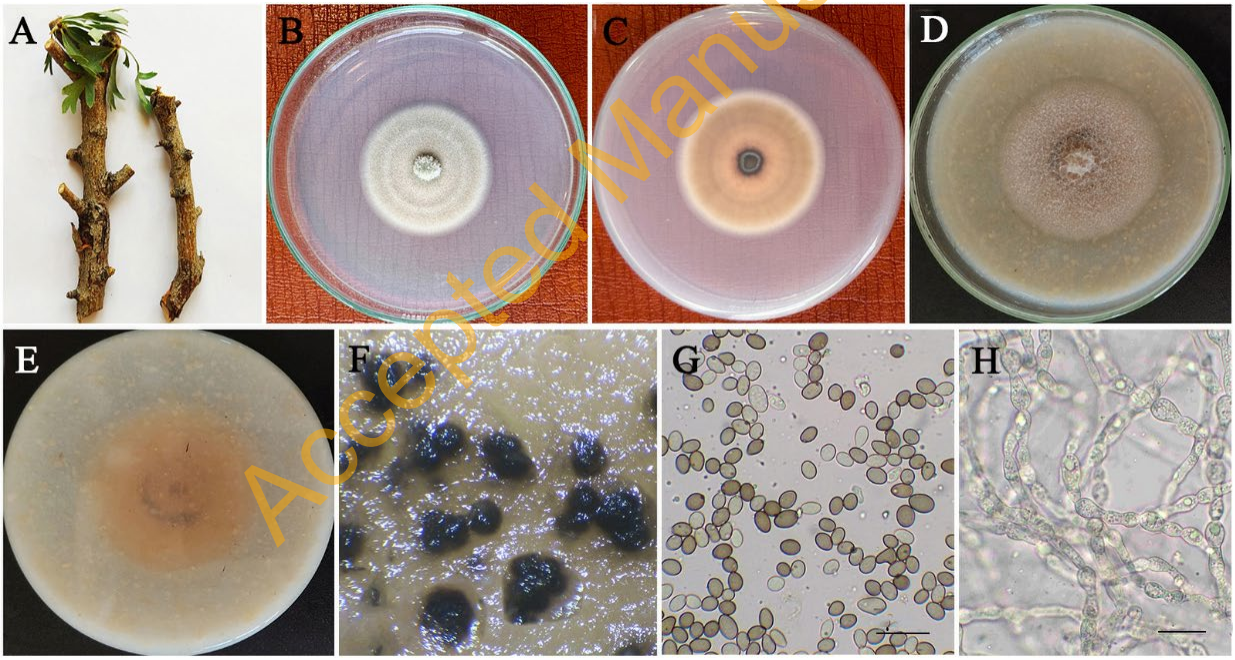

Fig. 6. *Neomicrosphaeropsis juglandis* (SCUA-Is-B6). (A) Stem canker on *Crataegus* sp., Colonies on PDA (B, C), and OA (D, E) after 9 d at 25 °C (top and reverse), (F) Pycnidia, (G) Conidia, (H) Chlamydospores. Scale bars: (G) 7 µm, (H) 20 µm.

Paramicrosphaeropsis iranica S.A. Ahmadp., M. Mehrabi, Farokh. & Asgari, Mycol. Progr. 21 (2, no. 28): 9 (2022). Fig. 7.

Pycnidia globose, subglobose or ellipsoidal, immersed or semi-immersed, formed after a week, solitary or clustered, brown to dark brown, ostiolate, 84–213 × 67–183 µm, 95% confidence limits = 146–176 × 112–139 µm, ($\bar{x}$ ± SD = 160 ± 40 × 130 ± 34 µm, n = 50). Conidia aseptate, occasionally varying from subglobose to ellipsoidal, ovoid, obpyriform, 4.5–8.5 × 3.8–6.8 µm, 95% confidence limits = 6.4–6.9 × 4.8–5.2 µm, ($\bar{x}$ ± SD = 6.6 ± 0.9 × 5 ± 0.6 µm, n = 50).

Culture characteristic: The diameter of two-week cultures on OA was 44 mm at 25 °C and 33 mm at 30 °C. The Colonies on OA showed initially cream, with age becoming dark brown with a paler margin and with white aerial mycelium, circular with an entire margin, floccose; reverse similar.

Materials examined: Iran, Khuzestan Province, Dezpart, from stem canker of *Cerasus microcarpa* K.Koch and dead branches of *Celtis caucasica* Willd., 9 Mar-2022, P. Eisvand, SCUA-Is-E10 and SCUA-Is-Z1.

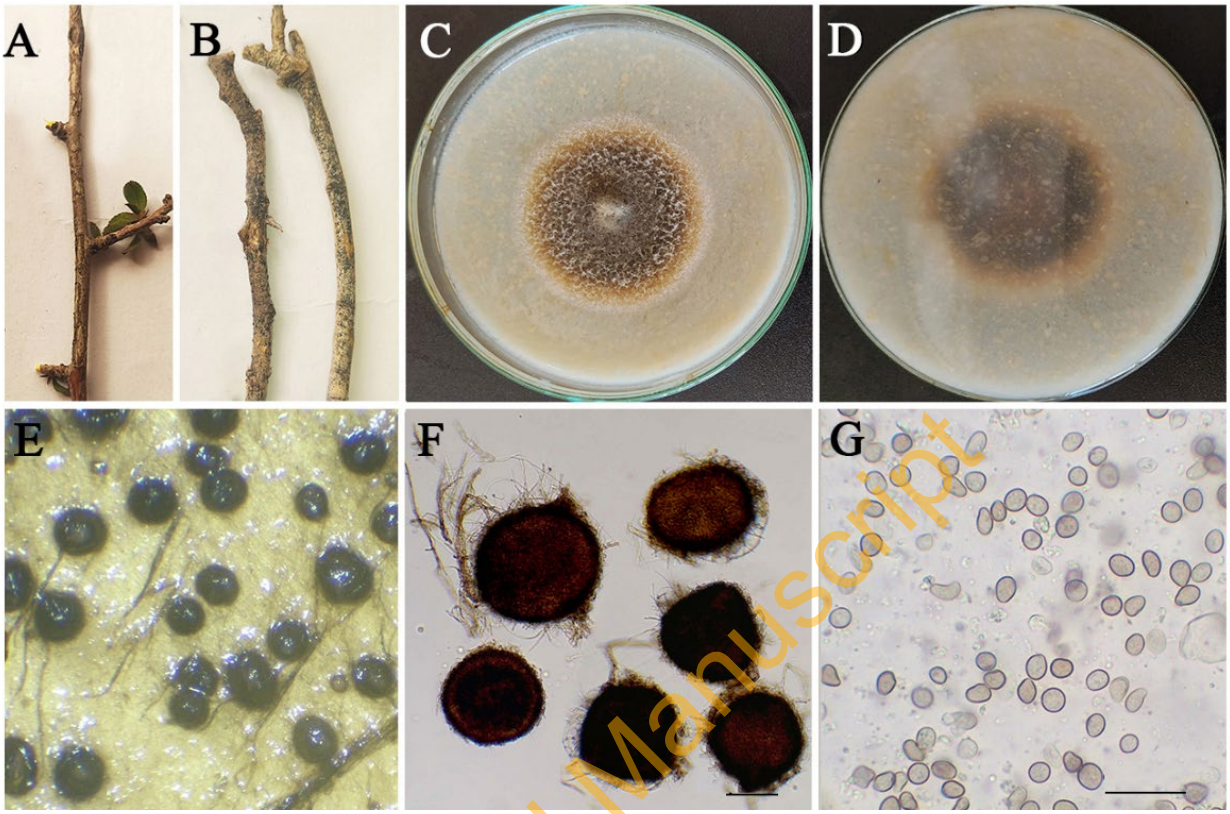

Fig. 7. *Paramicrosphaeropsis iranica* (SCUA-Is-E10). (A) stem canker on *Cerasus microcarpa*, (B) dead branches of *Celtis caucasica*, (C, D) Colony on OA after 14 d at 25 °C (top and reverse), (E, F) Pycnidia, (G) Conidia. Scale bars: (F) 70 µm, (G) 20 µm.

Note: In BLASTn search, the closest match for the *TUB2* sequence of our strains was *Paramicrosphaeropsis iranica* strain IRAN 2929C (*TUB2*: GenBank OK247743, identities = 99.8-100%). *Paramicrosphaeropsis iranica* was described by Ahmadpour et al. (2022) and isolated from stem canker on *Quercus brantii* in Iran. The new strain clustered with the type of *P. iranica* strain IRAN 2929C (Fig. 1) and was morphologically similar to it; however, slightly longer in size of conidia (4.5–8.5 µm vs. 4–7.7). In the *TUB2*-based phylogenetic tree, the clade comprising our isolate and its closest relative showed limited bootstrap and Bayesian support. This reduced statistical confidence is likely a consequence of the shorter sequence length of our isolate compared to the reference sequences, which limits the phylogenetic information available at this node and results in an unstable or weakly supported topology in a single-locus analysis. Nevertheless, the taxonomic placement of this isolate is robustly corroborated by both morphological features and BLAST sequence similarity, ensuring the accuracy of its phylogenetic position.

Paramicrosphaeropsis iranica was initially described from *Quercus brantii* in Iran (Ahmadpour et al. 2022). This species has been identified on other hosts, such as *Pistacia atlantica* Desf., *Quercus brantii*, and *Amygdalus scoparia* (Spach) C.K. Schneid (Artand et al. 2023). The current investigation is the initial report of *P. iranica* on *Celtis caucasica* and *Cerasus microcarpa*, thereby expanding its known host range.

Paramicrosphaeropsis salandica M. Mehrabi, Artand, K.D. Hyde & Jayaward., Cryptog. Mycol. 43 (7): 168 (2022). Fig. 8.

Pycnidia globose to sub-globose, immersed or in aerial mycelium, scattered, mostly solitary, brown with a paler wall, with age becoming black, with hyphal outgrowths, ostiolate, 115–224 × 88–207 µm, 95 % confidence limits = 156–182 × 136–157 µm, ($\bar{x}$ ± SD = 169 ± 34 × 146 ± 28 µm, n = 50). Conidia smooth, Conidia obpyriform, ellipsoidal, ovoid or irregular in shape, 0-septate, 3.8–7 × 3.5–4.2 µm, 95 % confidence limits = 5.2–5.7 × 3.4–3.6 µm, ($\bar{x}$ ± SD = 5.4 ± 0.7 × 3.5 ± 0.4 µm, n = 50).

Culture characteristic: Diameter of nine-day cultures on OA was 41 mm at 25 °C and 26 mm at 30 °C, regular margin, pinkish, with age becoming dark brown to grayish, floccose; reverse cream to pink with paler margin.

Materials examined: Iran, Khuzestan Province, Izeh, from stem canker of *Rhamnus persica*, 1 June 2022, P. Eisvand, SCUA-Is-D8.

Note: In the BLASTn search, the closest match for the *TUB2* sequence of the new strain (SCUA-Is-D8) was *Paramicrosphaeropsis salandica* strain IRAN 4376C (*TUB2*: GenBank MZ747180, identities = 100%). *Paramicrosphaeropsis salandica* was described by Artand et al. (2022) from symptomatic plants in Iran. Our strain clustered with the type strain *P. salandica* IRAN 4376C, forming a well-supported monophyletic clade (MLBS 98%, MPBS 99%, BPP 1.00) (Fig. 1). Morphologically, our strain is similar to the type strain but slightly differs in size of conidia [3.8–7 × 3.6–4.2 µm vs. (4.7–)5.9–6.5(–9.5) × (3.2–)4.6–5.2(–6.8) µm].

Paramicrosphaeropsis salandica was originally described from different species of plants, including fruit rot on *Crataegus* spp. and leaf spots on *Nerium oleander* L., *Quercus brantii,* and *Ziziphus* spp. (Artand et al. 2022). This study reports *P. salandica* for the first time from *Rhamnus persica*.

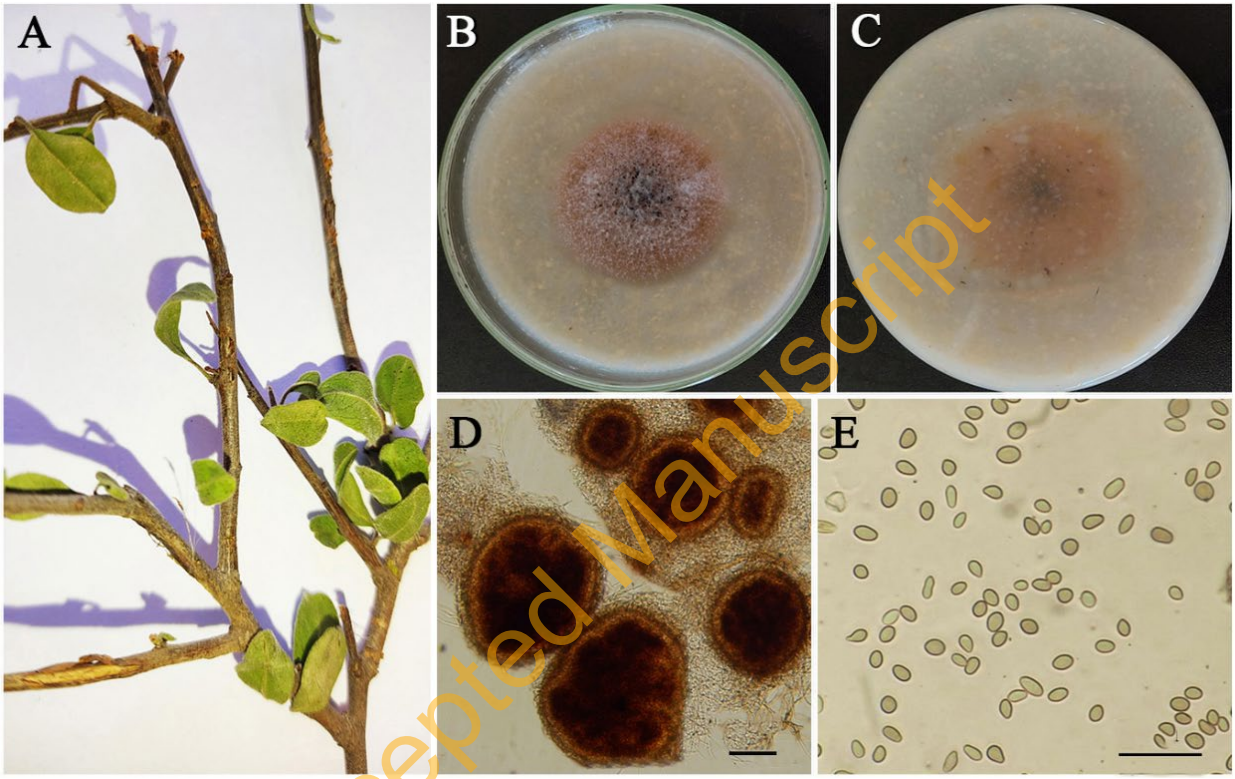

Fig. 8. *Paramicrosphaeropsis salandica* (SCUA-Is-D8). (A) Stem canker on *Rhamnus persica*, (B, C) Colony on OA after 9 d at 25 °C (top and reverse), (D) Pycnidia, (E) Conidia. Scale bars: (D) 70 µm, (E) 20 µm.

Until 2020, the only species known from *Paramicrosphaeropsis* was *P. ellipsoidea* L.W. Hou, L. Cai & Crous, which was isolated from decaying branches of *Quercus ilex* L. (Hou et al. 2020a). To date, several species have been introduced into the genus, including *P. iranica* S.A. Ahmadp., M. Mehrabi, Farokhinejad & Asgari, *P. amygdalus* M. Mehrabi, Artand, K.D. Hyde & Jayaward, *P. eriobotryae* Tavakolian & Mostowf, *P. pistacicola* M. Mehrabi, Artand & K.D. Hyde, *P. salandica* M. Mehrabi, Artand, K.D. Hyde & Jayaward, and *P. zagrosensis* M. Mehrabi, Artand, K.D. Hyde & Jayaward, *Paramicrosphaeropsis vitis* Abdollahz., Amirashayeri, Piri & Bashiri, and *Paramicrosphaeropsis sexualis* D. Pem, Gafforov & K.D. Hyde (Ahmadpour et al. 2022, Artand et al. 2022, Dong et al. 2023, Tavakolian and Mostowfizadeh-Ghalamfarsa 2023, Dong et al. 2025).

Didymella species act as plant pathogens and saprobes on a broad range of hosts. (Aveskamp et al. 2010, Chen et al. 2015, Luo et al. 2024). Some members of *Didymella* have been reported to cause infections on several host plants, such as *Angelica dahurica* (Hoffm.) Benth. & Hook.f. ex Franch. & Sav (Xu et al. 2016), *Bellis perennis* L. (Chen et al. 2015), *Chrysanthemum morifolium* (Ramat.) Hemsl. (Liu et al. 2019), *C. sinense* Sabine (Ren et al. 2019; Wang et al. 2021), *Eleocharis dulcis* (Burm.f.) Trin. ex Hensch. (Lv et al. 2011), *Lolium multiflorum* Lam. (Liu et al. 2023), and *Zanthoxylum bungeanum* Maxim. (Yang et al. 2023).

Fungal isolates in this study were identified based on their morphological characteristics and phylogenetic analyses. New forest hosts are reported for ten *Didymellaceae* species described above. Future studies are to focus on the pathogenicity or virulence of identified fungi on reported hosts.

AUTHOR CONTRIBUTION

Payam Eisvand 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 the revision of the manuscript.

DATA AVAILABILITY

New sequences generated in the current study are deposited in NCBI GenBank.

DECLARATION

The authors declare that they have no conflict of interest.

FUNDING

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ETHICS APPROVAL

Not applicable.

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مقاله کامل

گونه‌های تیره *Didymellaceae* مرتبط با درختان جنگل‌های زاگرس در استان خوزستان

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چکیده

تیره *Didymellaceae* شامل تعداد زیادی جنس و گونه بوده و از نظر دامنه میزبانی و پراکنش جغرافیایی، تنوع قابل‌توجهی دارد. در پژوهش حاضر، تعداد ۳۰ جدایه از این تیره از جنگل‌های زاگرس در استان خوزستان جمع‌آوری گردید. شناسایی جدایه‌ها بر اساس ویژگی‌های ریخت‌شناختی و تجزیه‌وتحلیل تبارزایی انجام شد. نتایج نشان داد که جدایه‌های مورد بررسی متعلق به گونه‌های *Didymella glomerata*، *D. microchlamydospora*، *D. pinodella*، *D. pomorum*، *Neomicrosphaeropsis juglandis*، *Paramicrosphaeropsis iranica* و *P. salandica* هستند. برای تمامی گونه‌های شناسایی‌شده، میزبان‌های جدیدی گزارش گردید؛ به‌طوری‌که جنس‌های گیاهی *Crataegus* و *Astragalus* به‌عنوان میزبان‌های جدید برای *D. glomerata*، جنس *Crataegus* برای *D. microchlamydospora* و *N. juglandis*، گونه *Cerasus microcarpa* برای *D. pinodella*، گونه *Quercus brantii* برای *D. pomorum*، گونه‌های *Celtis caucasica* و *Cerasus microcarpa* برای *P. iranica* و گونه *Rhamnus persica* برای *P. salandica* معرفی می‌شوند.

واژگان کلیدی: تاکسونومی، تبارزایی، ریخت‌شناسی، قارچ‌ها، میزبان.

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