Mycologia Iranica 9(1): 117 – 121, 2022 **Short Report** DOI: 10.22092/MI.2022.360591.1240

New host and record of *Didymella prosopidis* from Iran

Z. Baaji
R. Farokhinejad
M. Mehrabi-Koushki ✉

Department of Plant Protection, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Khuzestan Province, Iran

M. Mehrabi-Koushki ✉

Biotechnology and Bioscience Research Center, Shahid Chamran University of Ahvaz, Ahvaz, Iran

The *Narcissus* flower (*Narcissus tazetta* L., *Amaryllidaceae*) is one of the most important decorative flowers in Iran. This plant hosts a large number of endophytic and pathogenic fungi (Farr & Rossman 2022). During 2020-2021, *N. tazetta* plants growing in the natural resource areas of Behbahan in Khuzestan province (southwestern Iran) were visually inspected for disease symptoms. A typical brown spot on narcissus leaves was observed, which was collected for isolation of the potential fungal pathogen. The leaves were cut into approx. 5 mm pieces at the healthy and symptomatic margin. The pieces were then surface disinfected for 60–90 seconds in 1% sodium hypochlorite (NaOCl) and washed three times with sterilised distilled water, followed by drying on sterilised filter paper. Disinfected leaf pieces were plated on potato dextrose agar medium (PDA, potato extract 200–400 g L^{-1}, sucrose 10 g L^{-1}, agar 12 g L^{-1}) supplemented with 30 mgL^{-1} of streptomycin and incubated at 25°C until ten days. The fungal hyphae growing from the leaf pieces were subcultured on PDA and then purified on water agar (WA) using the hyphal tipping method. Five morphologically identical phoma-like strains were isolated, and two of them (SCUA-Ba-NB2 and SCUA-Ba-NB24 isolates) were used for further morphological and molecular analyses.

Morphological characteristics were determined from cultures grown on oatmeal agar (OA, oatmeal 30–60 g L^{-1}, agar 12 g L^{-1}) after ten days of incubation at 25 °C under a photoperiod of 12 h. Colonies on OA grew to a diameter of 60–72 mm (mean = 65 mm) after seven days of incubation at 25 °C ± 0.5.; circular with filiform margin, pale olivaceous-grey with darker margin, with aerial mycelium that was dense and cottony. Conidiomata were pycnidial, globose to subglobose, pale brown to brown, immersed in the agar or superficial, 1-3-ostiolate, 117.5-313.5 × 107-293 µm, 95% confidence limits = 166.5-211.5 × 152-193.5 µm, (x ± SD = 189 ± 50 × 172.5 ± 46.5 µm, n =50). The pycnidial wall was pseudoparenchymatous, composed of isodiametric angular cells, 3–5 layered, brown, with age becoming darker. Conidiogenous cells were hyaline, ampulliform, and phialidic. Conidia were hyaline, smooth- and thin-walled, ellipsoid, 0-septate, with rounded ends, 4.-6.5 × 2.5-3.5 µm, 95% confidence limits = 5.1-5.6 × 3.1-3.3 µm, (x ± SD = 5.4 ± 0.5 × 3.2 ± 0.2 µm, n =40). Chlamydospores were unicellular or multicellular, globose to subglobose, solitary or in the chain, intercalary or terminal, and brown to dark brown (Fig. 1).

For molecular identification, the mycelial biomass of each strain produced on PDA was harvested by a sterile glass slide and powdered in liquid nitrogen. DNA was isolated according to a chloroform- and phenol-based organic method described by Mehrabi-Koushki et al. (2018). The internal transcribed spacer regions 1 and 2 including the intervening 5.8S nuclear ribosomal DNA (ITS) and a partial sequence of the β-tubulin gene (*tub2*) were amplified and sequenced using the primer pairs ITS1/ITS4 (White et al. 1990) and Btub2Fd/ Btub4Rd (Woudenberg et al. 2009), respectively. PCR amplification and DNA analyses were performed by following methods described by Safi et al. (2021). Phylogenetic analyses were performed using reference sequences from related species of the strains under survey (Table 1). A combined ITS-*tub2* DNA matrix was made, and then a two-locus maximum likelihood (ML) tree was constructed in the raxmlGUI 2.0 beta program (Edler et al. 2020) using the following options: general time-reversible (GTR) model of evolution, a gamma-distributed rate variation (G) and thorough bootstrapping analysis with 1000 replicate (MLBS). Maximum parsimony (MP) analysis was performed using MEGA 7 software (Tamura et al. 2013) with 1000 pseudo-sampling in bootstrapping analysis. Bayesian analysis (BI) was performed by MrBayes v.3.2.6 program (Ronquist et al. 2012) and using the GTR + G + I model for both loci, estimated by jModelTest 2 (Darriba et al. 2012). The BI and MP analyses showed a similar tree topology to that obtained in the ML analysis.

Submitted 11 April 2022, accepted for publication 31 May 2022
✉ Corresponding Author: E-mail: mhdmhrb@scu.ac.ir

https://mij.areeo.ac.ir

In phylogenetic tree (Fig. 2), both isolates (SCUA-Ba-NB2 and SCUA-Ba-NB24) clustered with the type strain of *Didymella prosopidis* (Crous & A.R. Wood) L.W. Hou, L. Cai & Crous (CBS 136414) in a moderately-supported clade (MLBS 76%, MPBS 95%, BPP 0.79). The ITS (accession numbers; OP821092 and OP821093) and *tub2* (accession numbers; OP828921 and OP828922) sequences are deposited in GenBank.

According to both phylogenetic and morphological analyses, Iranian isolates were identified as *D. prosopidis*. This is the first record of *D. prosopidis* for mycobiota of Iran. This species was originally isolated from diseased stems of *Prosopis* sp. in South Africa and introduced as *Peyronellaea prosopidis* Crous & A.R. Wood (Crous et al. 2013). Later, Hou et al. (2020) recombined this species with *Didymella* (Hou et al. 2020). The genus *Didymella* is a fungus belonging to the *Didymellaceae* family and contains several pathogenic species mainly distributed in the field and ornamental crops as well as in wild plants (Chen et al. 2015, Ahmadpour et al. 2021, 2022). Many *Didymella* species are also saprobes that are commonly found in living or dead tissues of herbaceous and wooden plants (Chen et al. 2015); some species also act as mutualistic endophytes with some plant species (Rayner 1922). In this study, *D. prosopidis* was isolated from *Narcissus tazetta* showing leaf spot symptoms. So far, no other species from the family *Didymellaceae* has been reported from this genus, except *Didymella curtisii* (Berk.) Qian Chen & L. Cai in Armenia, Australia, and Poland (Boerema et al. 2004, Farr & Rossman 2022).

Key words: *Didymella prosopidis* , Iran, *Narcissus tazetta*

Table I. *Didymella* species used in phylogenetic analysis and their GenBank accession numbers of sequences.

Taxon	**Strain**[a]	**Source**	**Origin**	**GenBank accession numbers**	
				ITS	***tub2***
D. aeria	LC 7441	Air	China	KY742051	KY742293
D. anserina	CBS 285.29	*Calluna* sp.	UK	KT389499	KT389796
D. arachidicola	CBS 333.75	*Arachis hypogaea*	South Africa	GU237833	GU237554
D. aurea	CBS 269.93	*Medicago polymorpha*	New Zealand	GU237818	GU237557
D. coffeae-arabicae	CBS 123380	*Coffea arabica*	Ethiopia	FJ426993	FJ427104
D. combreti	CBS 137982	*Combretum mossambiciensis*	Zambia	MN973525	MT005626
D. eucalyptica	CBS 249.79	*Eucalyptus* sp.	Australia	MN972832	MN983849
D. glomerata	CBS 287.76	*Rubus idaeus*	Russia	FJ427006	FJ427117
D. keratinophila	UTHSC DI16-200	Human finger-hand lesion	USA	LT592901	LT592970
D. lethalis	CBS 504.85	*Olea europaea*	Italy	MN972849	MN983864
D. mitis	CBS 443.72	Soil	South Africa	MN973523	MT005624
D. musae	CBS 463.69	*Mangifera indica*	India	FJ427026	FJ427136
D. nigricans	CBS 444.81	*Actinidia chinensis*	New Zealand	GU237867	GU237558
D. pinodella	CBS 300.53	*Pinus nigra* var. *austriaca*	Italy	MN973526	MT005627
D. pinodes	CBS 525.77	*Pisum sativum*	Belgium	GU237883	GU237572
D. pomorum	CBS 838.84	*Hordeum vulgare*	Germany	MN972915	MN983930
D. prosopidis	CBS 136414	*Prosopis* sp.	South Africa	KF777180	MT005631
D. prosopidis	*SCUA-Ba-NB24*	*Narcissus tazetta*	Iran	OP821093	OP828922
D. prosopidis	SCUA-Ba-NB2	*Narcissus tazetta*	Iran	OP821092	OP828921
D. protuberans	CBS 381.96	*Lycium halifolium*	The Netherlands	GU237853	GU237574
D. sancta	CBS 281.83	*Ailanthus altissima*	South Africa	FJ427063	MT005619
D. sinensis	CGMCC 3.18348	*Cerasus pseudocerasus*	China	KY742085	KY742327
D. subglobispora	CBS 364.91	*Ananas sativus*	-	MN973531	MT005634

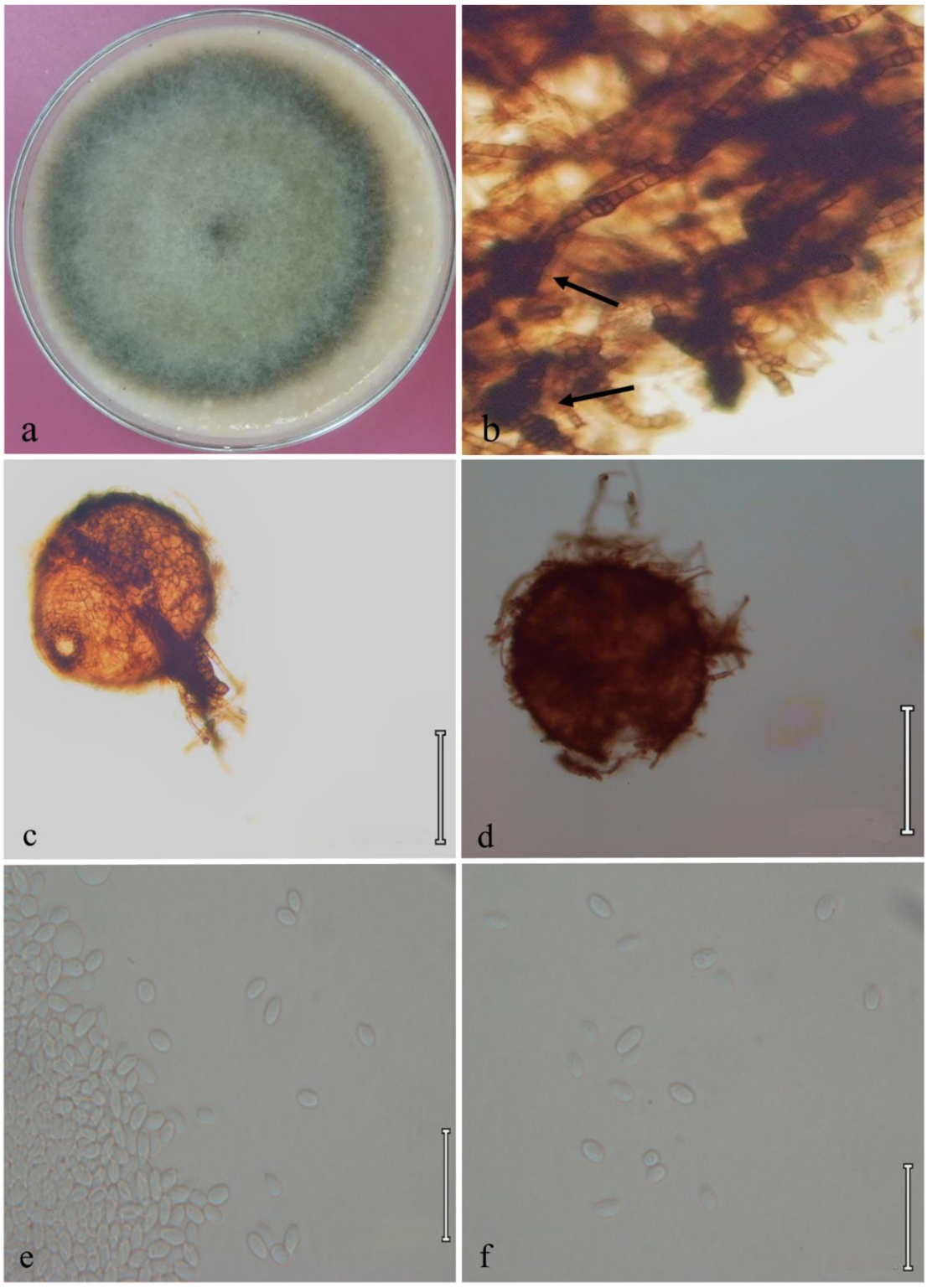

Fig. 1. *Didymella prosopidis*: a. 8-days colony on OA in the top side, b. Unicellular or multicellular (arrow) chlamydospores, c–d. Pycnidia, e–f. Conidia. Scale bars: c–d = 200 µm, e–f = 20 µm.

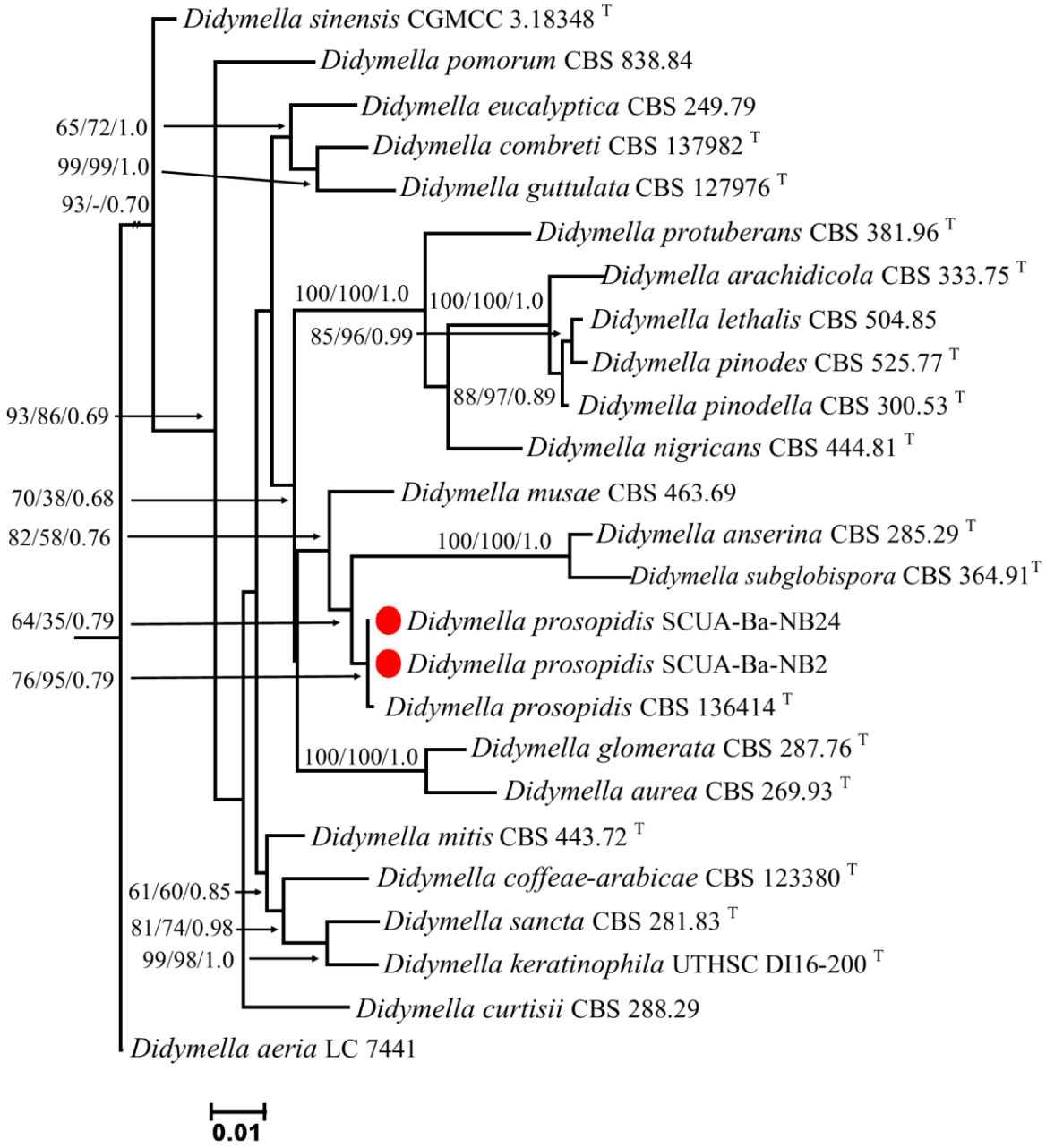

Fig. 2. Maximum likelihood phylogenetic tree constructed based on combined ITS and *tub2* regions. Iranian isolates were indicated with a red-color-filled circles. Bootstrap values obtained in maximum likelihood (MLBS) and maximum parsimony (MPBS) analyses ≥ 50% and Bayesian posterior probability values (BYPP) ≥ 0.5 are shown at the nodes, respectively. The tree is rooted to *Didymella aeria* (LC 7441). Letter T indicates the ex-type strains.

REFERENCES

Boerema GH, De Gruyter J, Noordeloos ME, Hamers MEC. 2004. Phoma identification manual: differentiation of specific and infra-specific taxa in culture. CABI Publishing, 470 pages.

Ahmadpour SA, Mehrabi-Koushki M, Farokhinejad R, Asgari B, Javadi-Estahbanati A, Mirabolfathy M, Rahnama K. 2021. New records of fungal species of the family Didymellaceae from Iran. Mycologia Iranica 8: 119-133.

Submitted 11 April 2022, accepted for publication 31 May 2022
✉ Corresponding Author: E-mail: *mhdmhrb@scu.ac.ir*

http://mij.areeo.ac.ir

Ahmadpour SA, Mehrabi-Koushki M, Farokhinejad R, Asgari B. 2022. New species of the family Didymellaceae in Iran. Mycological Progress 21. DOI.org/10.1007/s11557-022-01800-5

Chen Q, Jiang J, Zhang G, Cai L, Crous P. 2015. Resolving the Phoma enigma. Studies in Mycology 82: 137–217.

Crous PW, Wingfield MJ, Guarro J, Cheewankoon R, Van der bank M et al. 2013. Fungal Planet description sheets: 154–213. Persoonia 31: 188–296.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: 772.

Edler D, Klein J, Antonelli A. Silvestro. 2020. raxmlGUI 2.0 beta: a graphical interface and toolkit for phylogenetic analyses using RAxMl. bioRxiv DOI: 10.1101/800912

Farr DF, Rossman AY. 2022. Fungal Databases: U.S. National Fungus Collections, ARS, USDA. Internet Resource: https://nt.ars-grin.gov/fungaldatabases/ (Retrieved November 11 2022).

Hou LW, Groenewald JZ, Pfenning LH, Yarden O, Crous PW, Cai L. 2020a. The phoma-like dilemma. Studies in Mycology 96: 309–396.

Mehrabi-Koushki M, Khodadadi-Pourarpanahi S, Jounbozorgi S. 2018. Fungal endophytes associated with some thermotolerant plants in salt-stress ecosystem. Mikologiya i Fitopatologiya 52: 187–195.

Rayner MC. 1922. Nitrogen fixation in Ericaceae. Botanical Gazette 73: 226–235.

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542.

Safi A, Mehrabi-Koushki M. Farokhinejad R. 2021. Plenodomus dezfulensis sp. nov. causing leaf spot of Rapeseed in Iran. Phytotaxa 523: 141–154.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729.

White TJ, Bruns T, Lee S. Taylor J. 1990. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. Pp. 315–322. In: "PCR Protocols: A Guide to Methods and Applications" (Gelfand, M.A., Sninsky, D.H. & White, T.J., eds). Academic Press, San Diego, CA.

Woudenberg JHC, Aveskamp MM, de Gruyter J, Spiers AG, Crous PW. 2009. Multiple Didymella teleomorphs are linked to the Phoma clematidina morphotype. Persoonia 22: 56–62