


## Original Article

# *Diaporthe* species associated with Blackberry and Blueberry from Guilan Province, Iran

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## ABSTRACT

Species of *Diaporthe* (syn. *Phomopsis*) are important as endophytes, saprobes, and pathogens. They have a broad host range and are economically important in crop diseases. Several studies have reported *Diaporthe* species on *Rubus* and *Vaccinium* species worldwide. However, these plants have not yet been studied for the presence of *Diaporthe* species in Iran. From samples collected in various cities within Guilan Province, six isolates were obtained and identified based on morphological and molecular data as *Diaporthe* species. For molecular analyses, Internal transcribed spacers (ITS), translation elongation factor 1- $\alpha$  (*tef1*), and beta-tubulin (*tub2*) sequencing were employed. As a result, isolates were identified as *Diaporthe arecae* and *D. amygdali*. *Diaporthe arecae* is a new record for Iran mycobiota. Additionally, this is a new report of the association of *Diaporthe* species with *Rubus* and *Vaccinium* genera in Iran.

## KEYWORDS

*Diaporthaceae*, *Phomopsis*, Phylogeny, *Rubus*, *Vaccinium*.

## INTRODUCTION

The genus *Diaporthe* Nitschke 1870 (*Diaporthales*, *Ascomycota*) was established in 1870 based on *D. eres* Nitschke as the type species from *Ulmus* sp. in Germany. Rossman et al. (2015) proposed using the name *Diaporthe* instead of *Phomopsis*, its anamorph name, due to its priority by the publication date, following the International Code of Nomenclature for algae, fungi, and plants rules. Species of *Diaporthe* are cosmopolitan and mainly plant pathogens, endophytes, or saprobes with potential as producers of secondary metabolites (Chepkirui and Stadler 2017, Marin-Felix et al. 2019) and have been placed in the *Diaporthaceae* with 17 other genera, including *Apioporthella* Petr., *Apiosphaeria* Höhn., *Chaetoconis* Clem., *Chiangraiomycetes* Senan. & K.D. Hyde., *Hyaliappendispora* Senan., Camporesi & K.D. Hyde., *Leucodiaporthe* M.E. Barr & Lar. N. Vassiljeva., *Massariothea* Syd., *Mazzantia* Mont., *Ophiodiaporthe* Y.M. Ju, H.M. Hsieh, C.H. Fu, Chi Y. Chen & T.T.

Chang, *Paradiaporthe* Senan., Camporesi & K.D. Hyde, *Phaeocytostroma* Petr., *Phaeodiaporthe* Petr., *Pseudophaeocytostroma* Monkal & Phookamsak, *Pustulomyces* D.Q. Dai, Bhat & K.D. Hyde, *Pulvinaticonidioma* X. Tang, Jayaward., J.C. Kang & K.D. Hyde, *Stenocarpella* Syd. & P. Syd., *Subellipsoidispora* X. Tang, Jayaward., J.C. Kang & K.D. Hyde (Hyde et al. 2020, 2024).

Morphologically, *Diaporthe* is characterized by both morphs (sexual and asexual). The sexual morph is characterized by immersed ascomata and an erumpent pseudostroma with elongated perithecial necks. Asci are unitunicate, clavate to cylindrical. Ascospores are fusoid, ellipsoid to cylindrical, hyaline, biseriate to uniseriate in the ascus, and sometimes with appendages. The asexual morph is characterized by ostiolate conidiomata, with cylindrical phialides producing three types of hyaline, aseptate conidia (alpha, beta, and gamma) (Jiang et al. 2021).

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Historically, *Diaporthe* species were identified mainly based on host association as well as morphological characteristics such as the size and shape of sexual and asexual structures, including ascoma, asci, ascospores, conidiomata, and conidia (Rehner and Uecker 1994, Santos and Phillips 2009, Udayanga et al. 2014). Nonetheless, several taxonomic studies have proved that morphological criteria are inadequate for species identification in this genus (Udayanga et al. 2011, Pereira et al. 2023). Thus, multi-gene phylogenies have been used to resolve *Diaporthe* taxonomy during recent years which resulted in description of a large number of species (Udayanga et al. 2015, Huang et al. 2015, Gao et al. 2017, Guarnaccia and Crous 2017, Yang et al. 2018, Hilário et al. 2020, Jiang et al. 2021, Lambert et al. 2023, Aumentado and Balendres 2024, Jia et al. 2023, Zhu et al. 2024).

As plant pathogens, *Diaporthe* species are broadly distributed and have a wide range of hosts, including commercial agricultural and forest trees and ornamental plants, such as species of *Rosaceae*, *Pyrus*, *Rubus*, and *Vaccinium* (Vrandecic et al. 2011, Elfar et al. 2013, Lombard et al. 2014, Santos et al. 2017, Guo et al. 2020, Hilário et al. 2020, Guarnaccia et al. 2020, 2022; González and Ciordia 2023). Leaf spot and stem canker symptoms on blackberry, blueberry, and raspberry have occasionally been observed in the Guilan Province. In the present study, based on modern taxonomic approaches, we identified two species of *Diaporthe* from Iran. Detailed morphological descriptions, illustrations, and phylogenetic information are provided.

## MATERIALS AND METHODS

### Fungal isolation

From June to November 2022, symptomatic samples showing leaf spots and stem canker (5–10 samples from each tree) from infested *Rubus* spp. and *Vaccinium corymbosum* L. were collected from Guilan Province. Surface-sterilized pieces of samples (1–3 min in 0.5% sodium hypochlorite) were transferred to potato dextrose agar (PDA) and incubated at 25 °C. The fungal isolates were purified by the single-spore technique on 2% water agar (WA). Phenotypic features of the isolates were recorded on PDA, carnation leaf agar (CLA), and pine needles agar (PNA) in a 12 h near-ultraviolet light/12 h dark cycle at 20 °C. Living pure cultures were deposited in the culture collection of the Iranian Research Institute of Plant Protection, Tehran, Iran (IRAN). Dried pure cultures were deposited in the University of Guilan Mycological Fungarium (GUM), Rasht, Iran.

### Fungal observation and light microscopy

Fungal structures were mounted in sterilized water and examined using a Leica DM1000 light microscope equipped with a Canon digital camera (600D). Morphological characters of fungal structures, including

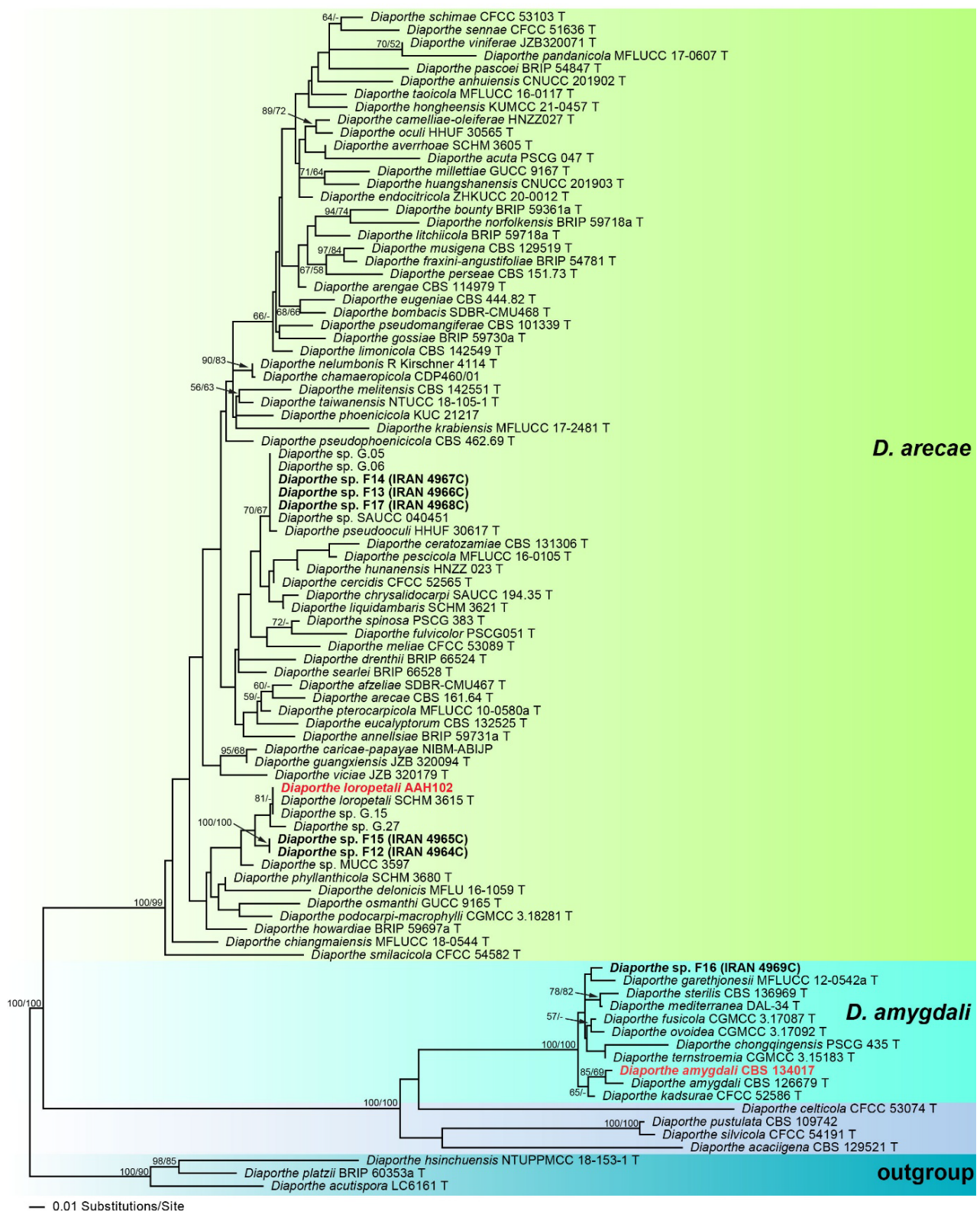
conidiomata, conidiophores, and conidia, were studied. Dimensions of fungal structures were calculated in sterilized water, based on at least 20 measurements for conidiomata and conidiophores and 100 measurements for conidia. The morphological characteristics of the isolates were compared with the descriptions of the related species available in Udayanga et al. (2011), Dissanayake et al. (2017), Santos et al. (2017), Hilário et al. (2021), and Hongsanan et al. (2023).

### DNA extraction, PCR, and sequencing

DNA extraction of fresh cultures was extracted using the Thermolysis method (Zhang et al. 2010). Three different loci were amplified, i.e. the internal transcribed spacer region (ITS), translation elongation factor 1- $\alpha$  (*tef1*), and beta-tubulin (*tub2*) genes. The ITS was amplified and sequenced using the primers ITS4 and ITS5 (White et al. 1990), *tef1* with EF1-688F and EF1-986R (Alves et al. 2008/Carbone and Kohn 1999), and *tub2* with T1D and T22D (Carbone and Kohn 1999). The amplification conditions consisted of an initial denaturation of 3 minutes at 95 °C, followed by 34 cycles of 30 seconds at 95 °C, 30 seconds at 52 °C, 30 seconds at 72 °C, and a final extension of 5 minutes at 72 °C for the ITS gene; for the *tef1* gene, an initial denaturation of 5 minutes at 94 °C, followed by 40 cycles of 45 seconds at 94 °C, 30 seconds at 52 °C, 90 seconds at 72 °C, and a final extension of 6 minutes at 72 °C and for the *tub2* gene, an initial denaturation of 5 minutes at 94 °C, followed by 37 cycles of 30 seconds at 94 °C, 60 seconds at 55 °C, 90 seconds at 72 °C, and a final extension of 10 minutes at 72 °C. The amplicons were then sent to Codon Genetic Group (Tehran, Iran) to be sequenced. The sequence data generated in this study are provided in Table 1.

### Phylogenetic analyses

For identifying closely related taxa, BLASTn searches were done for three loci. Also, type and reference sequences of related taxa were retrieved from the National Center for Biotechnology Information, based on recent publications on the genus *Diaporthe* (Hilário et al. 2021; Pereira et al. 2023; Dissanayake et al. 2024). All alignments were produced with the server versions of MAFFT v. 7.490 (<http://mafft.cbrc.jp/alignment/server/>; Katoh et al. 2019) and checked and refined manually using MEGA Ver. 7 (Kumar et al. 2016). For the phylogenetic analyses, 89 isolates of *Diaporthe* and three outgroup taxa (*Diaporthe acutispora* Y.H. Gao & L. Cai, *D. hsinchuensis* H.A. Ariyawansa & I. Tsai, and *D. platzii* Y.P. Tan & R.G. Shivas) were included (Supplementary Table 1). The alignments of individual gene loci were concatenated in Mesquite v. 3.10 (Maddison and Maddison 2015). Maximum Likelihood (ML) analyses were performed with RAxML (Stamatakis 2006) as implemented in raxmlGUI 1.3 (Silvestro and Michalak 2012) using the



**Fig. 1.** ML phylogram resulted from RAxML analysis of the combined ITS–*tef1*–*tub2* matrix of selected *Diaporthe* species. Isolates in black-bold were sequenced in this study. Isolates in red-bold have previously been collected from Iran. Bootstrap supports (ML/MP) >50% are given at the nodes. <sup>T</sup> = Ex-type strain.

**Table 1.** Isolates were sequenced in the present study.

Strain	Species	Host	GenBank accession numbers		
			ITS	<i>tefl</i>	<i>tub2</i>
<i>Diaporthe amygdali</i>	IRAN 4969C	<i>Vaccinium corymbosum</i>	PP830790	PP830784	PP810243
<i>Diaporthe arecae</i>	IRAN 4965C	<i>Rubus idaeus</i>	PP830785	PP830779	PP810238
<i>Diaporthe arecae</i>	IRAN 4964C	<i>Rubus fruticosus</i>	PP830786	PP830780	PP810239
<i>Diaporthe arecae</i>	IRAN 4966C	<i>Rubus fruticosus</i>	PP830787	PP830781	PP810240
<i>Diaporthe arecae</i>	IRAN 4968C	<i>Rubus idaeus</i>	PP830788	PP830782	PP810241
<i>Diaporthe arecae</i>	IRAN 4967C	<i>Rubus fruticosus</i>	PP830789	PP830783	PP810242

ML + rapid bootstrap setting and the GTRGAMMA substitution model with 1000 bootstrap replicates.

Maximum Parsimony (MP) analyses were performed with PAUP v. 4.0a169 (Swofford 2002). All characters were unordered with equal weight and gaps treated as missing data. The COLLAPSE command was set to MINBLEN. MP analysis of the combined multilocus matrix was done using 1000 replicates of heuristic search with random addition of sequences and subsequent TBR branch swapping (MULTREES option in effect, steepest descent option not in effect). Bootstrap analyses with 1000 replicates were performed in the same way, but using 10 rounds of random sequence addition and subsequent branch swapping during each bootstrap replicate. Consistency index (CI), homoplasy index (HI), retention index (RI) and tree length (TL) were also calculated.

## RESULTS

### Molecular phylogeny

The concatenated alignment of three loci (ITS: 464, *tefl*: 410, *tub2*: 468) contained 1342 characters. Of these, 405 were parsimony informative (ITS: 80, *tefl*: 191, *tub2*: 134). The best ML tree (lnL = -11655.7990) obtained by RAxML analyses is shown in Fig. 1. The MP analysis resulted in 12 most parsimonious trees (TL = 1917, CI = 0.439, RI = 0.754, and HI = 0.561) with similar topology.

In phylogenetic analyses, our isolates were placed in three distinct subclades within two *Diaporthe* species complexes. The first subclade (containing three Iranian isolates (IRAN 4966C/IRAN 4967C/IRAN 4968C) together with strains G.05, G.06, SAUCC 040451, and the type strain of *D. pseudooculi* Mochiz. & Kaz. Tanaka) was found within *Diaporthe arecae* complex. A comparison of the sequence data in this subclade revealed nine nucleotide differences (ITS: one substitution, *tefl*: three substitutions, *tub2*: five substitutions) between Iranian isolates and the type strain of *D. pseudooculi*.

The second subclade (within the first large clade of *Diaporthe arecae* complex), including Iranian isolates IRAN 4964C and IRAN 4965C, was found close to a

subclade containing the type strain of *Diaporthe loropetali* (C.Q. Chang, Z.D. Jiang & P.K. Chi) Y.H. Gao & L. Cai (SCHM 3615).

If we were to follow the narrow species concept, specimens from subclade one would be classified as *D. pseudooculi*. Similarly, specimens from subclade two would be identified as *D. loropetali*, or they would need to be assigned to a new species.

Recently, Pereira et al. (2023) synonymized several species in *D. arecae* complex. As we prefer to follow Pereira et al. (2023), here we characterised our isolates placed in these two subclades as *D. arecae*.

The third subclade, containing our isolate IRAN 4969C and the type strain of *D. garethjonesii* Dissan., Tangthir. & K.D. Hyde, was formed within the *Diaporthe amygdali* complex. Although significant differences are observed among the species within this clade—for example, there is a 32-nucleotide difference between our sample and the type sequence of *D. amygdali*, highlighting the distinct genetic variation within the clade—this has been accepted as intraspecific variation (Hilario et al. 2021). This approach has recently been followed by various researchers (Pereira et al. 2023, Dissanayake et al. 2024, Pereira and Phillips 2024) for both *D. amygdali* and *D. arecae*.

### Taxonomy

***Diaporthe arecae*** (H.C. Srivast., Zakia & Govindar.) R.R. Gomes, Glienke & Crous, Persoonia 31: 16 (2013)

**Description.** Asexual morph: Conidiomata pycnidial, scattered or aggregated, black, erumpent, superficial, subglobose, exuding white or yellowish creamy conidial droplets from central ostioles after 7 days on CLA or 28 days on PNA, 0.25 – 0.76 mm diam; pycnidial wall consists of black to dark brown, thin-walled cells. Conidiophores 8–35 × 1–3 µm, hyaline, phialidic, unbranched, tapering towards the apex, swelling at the base, subcylindrical, aseptate, smooth, straight or slightly curved. Alpha conidia aseptate, hyaline, ellipsoid to fusiform, acutely round at both ends, sometimes obtuse at both ends, multi- or bi-guttulate, 5.35–9 × 1.4–3.1 µm ( $\bar{X}$  = 7.2 × 2.25 µm, n = 100). Beta conidia less frequent than alpha conidia,

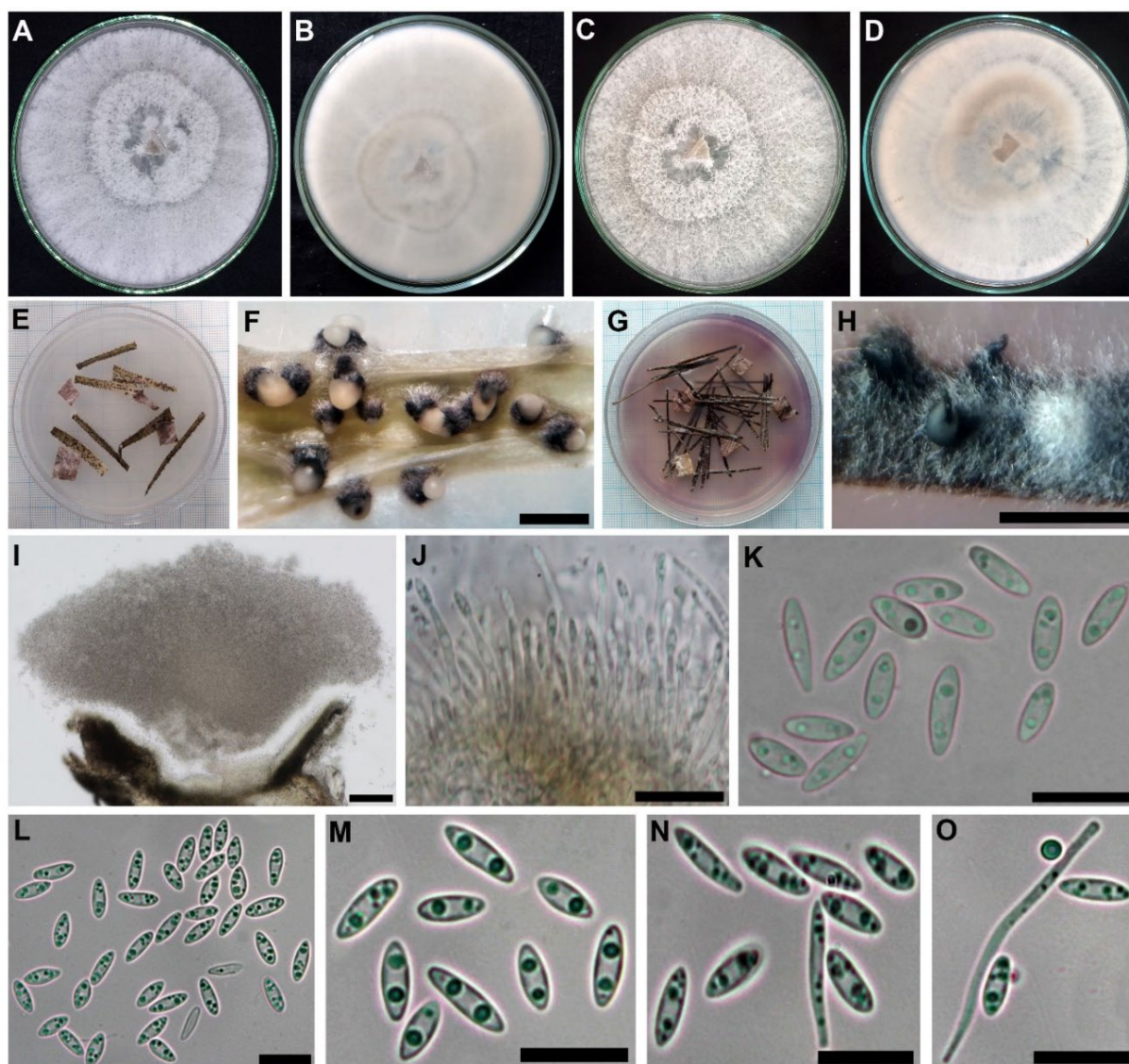


filiform, hyaline, straight or slightly curved, aseptate, base subtruncate, tapering towards the base,  $17.2\text{--}32.5 \times 0.8\text{--}1.6 \mu\text{m}$  ( $\bar{X} = 24.9 \times 1.2 \mu\text{m}$ ,  $n = 26$ ). Gamma conidia less frequent than alpha conidia, aseptate, smooth, hyaline, straight to slightly curved, multiguttulate,  $9\text{--}16.55 \times 0.7\text{--}2 \mu\text{m}$  ( $\bar{X} = 12.8 \times 1.35 \mu\text{m}$ ,  $n = 13$ ).

**Culture characteristics:** On PDA aerial mycelium abundant, white, reverse white to pale yellow, with a concentric zonation, irregular margin, pycnidia forming after 20 days, 15–30 mm diam/day at 20 °C; On CLA obverse and reverse white, pycnidia forming after 7

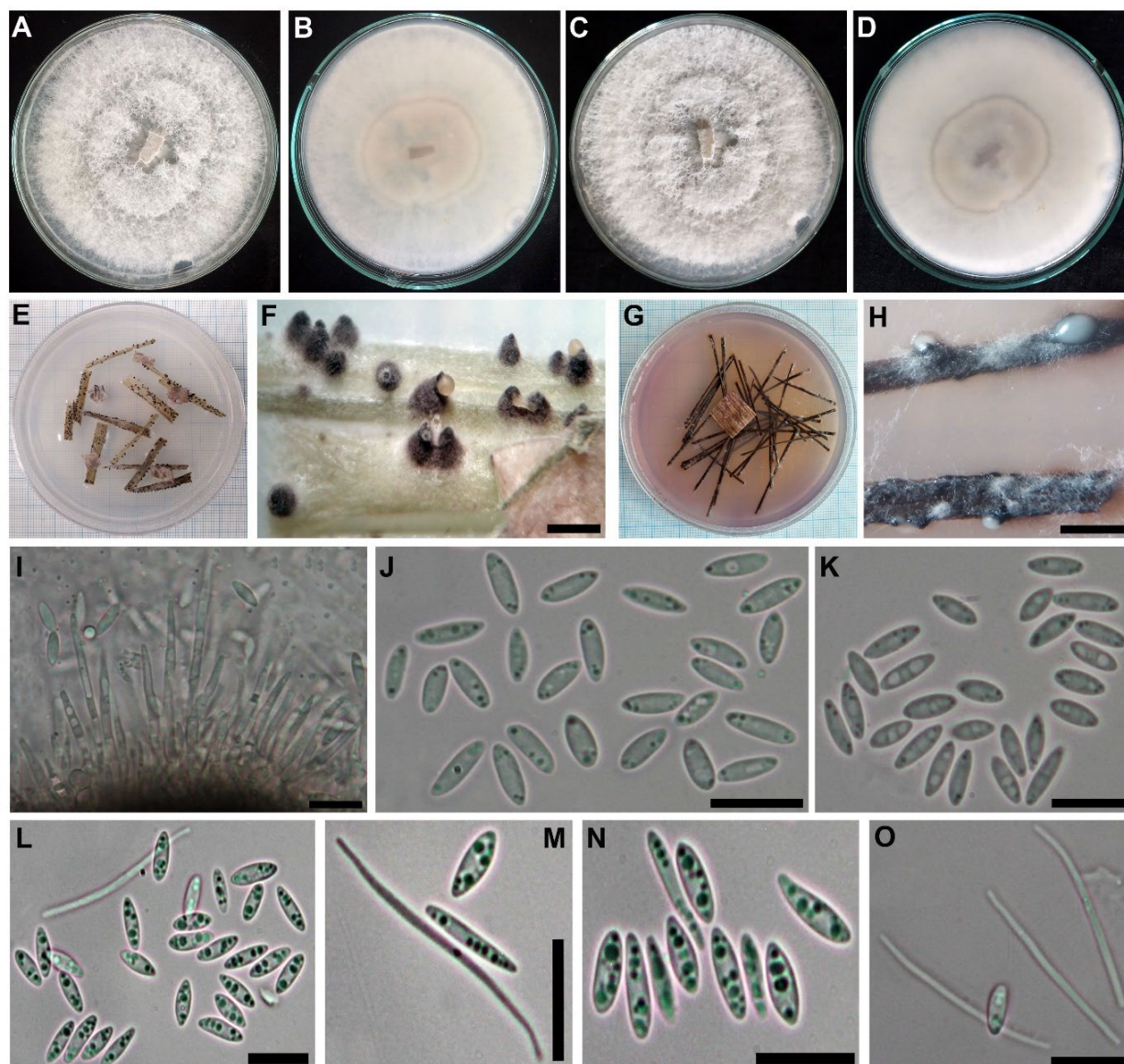
days; On PNA obverse and reverse with pink to purple pigmentation, pycnidia forming after 28 days (Figs 2, 3).

**Specimens examined:** Iran, Guilan Province, Sangar County, 37°13'04"N, 49°42'35.2"E, from the diseased stem of *Rubus fruticosus* (Blackberry), 4 August 2022, (IRAN 4966C); Guilan Province, Fuman County, 37°15'31.2"N, 49°16'45.6"E, from the diseased stem of *Rubus idaeus* (Raspberry), 13 November 2022, (IRAN 4968C); Guilan Province, Fuman County, 37°15'31.2"N, 49°16'45.6"E, from the diseased stem of *Rubus fruticosus* (Blackberry), 13 November 2022, (IRAN 4967C); Guilan Province, Fuman County, 37°15'31.2"N, 49°16'45.6"E, from the diseased stem of



**Fig. 2.** *Diaporthe arecae* (IRAN 4966C, from the first subclade and close to *Diaporthe pseudooculi*). (A–B) surface and reverse of the colony after 7 days on PDA, (C–D) surface and reverse of the colony after 14 days on PDA, (E) colonies on CLA after 7 days, (F) conidiomata with yellow sporulation on CLA, (G) colonies on PNA after 28 days with pink to purple pigmentation, (H) conidiomata with white to cream sporulation on PNA, (I) longitudinal section of conidioma in water, (J) conidiophores and conidia in water, (K) alpha conidia on CLA in water, (L–O) conidia on SNA, (L) alpha conidia in water, (M) alpha conidia in water, (N) alpha and gamma conidia in water, (O) alpha and beta conidia in water. Scale bars: (F, H) 1 mm, (I) 100  $\mu\text{m}$ , (J) 20  $\mu\text{m}$ , (K–M) 10  $\mu\text{m}$ .





**Fig. 3.** *Diaporthe arecae* (IRAN 4965C, from the second subclade and close to *Diaporthe loropetali*). (A–B) surface and reverse of colony after 7 days on PDA, (C–D) surface and reverse of colony after 14 days on PDA, E. colonies on CLA after 7 days, F. conidiomata with yellow sporulation on CLA, (G) colonies on PNA after 28 days with pink to purple pigmentation, (H) conidiomata with white to cream sporulation on PNA, (I) conidiophores and conidia in water, (J–K) alpha conidia on CLA in water, (L–O) conidia on SNA, (L) alpha and beta conidia in water, (M) alpha, beta and gamma conidia in water, (N) alpha and gamma conidia in water, (O) alpha and beta conidia in water. Scale bars: (F, H) 1 mm, (I–O) 10  $\mu$ m.

*Rubus idaeus* (Raspberry), 13 November 2022, (IRAN 4965C); Guilan Province, Sangar County, 37°13'04"N, 49°42'35.2"E, from the diseased stem of *Rubus fruticosus* (Blackberry), 4 August 2022, (IRAN 4964C). All isolates were collected by F. Ghahremani.

**Note:** *Diaporthe arecae* was first introduced by Srivastava et al. (1962) as *Subramanella arecae* from *Areca catechu* in India and then accommodated in *Diaporthe* by Gomes et al. (2013). Numerous studies have shown that the inability of single and multi-locus phylogenies of *Diaporthe* species to resolve the phylogenetic relationship of *D. arecae* and its allies resulted in a clade known as *D. arecae* species complex (Huang et al. 2015, Pereira et al. 2023). In recent years,

many species from various substrates have been identified in the *D. arecae* species complex around the world. Pereira et al. (2023), based on the Genealogical Concordance Species Concept (GCSC) and PTP coalescent models, re-examined species boundaries within the *D. arecae* species complex, containing 55 species, of which only three species *D. arecae*, *D. Chiangmaiensis* and *D. smilacicola* were accepted and 52 previously introduced species reduced to synonyms of *D. arecae*.

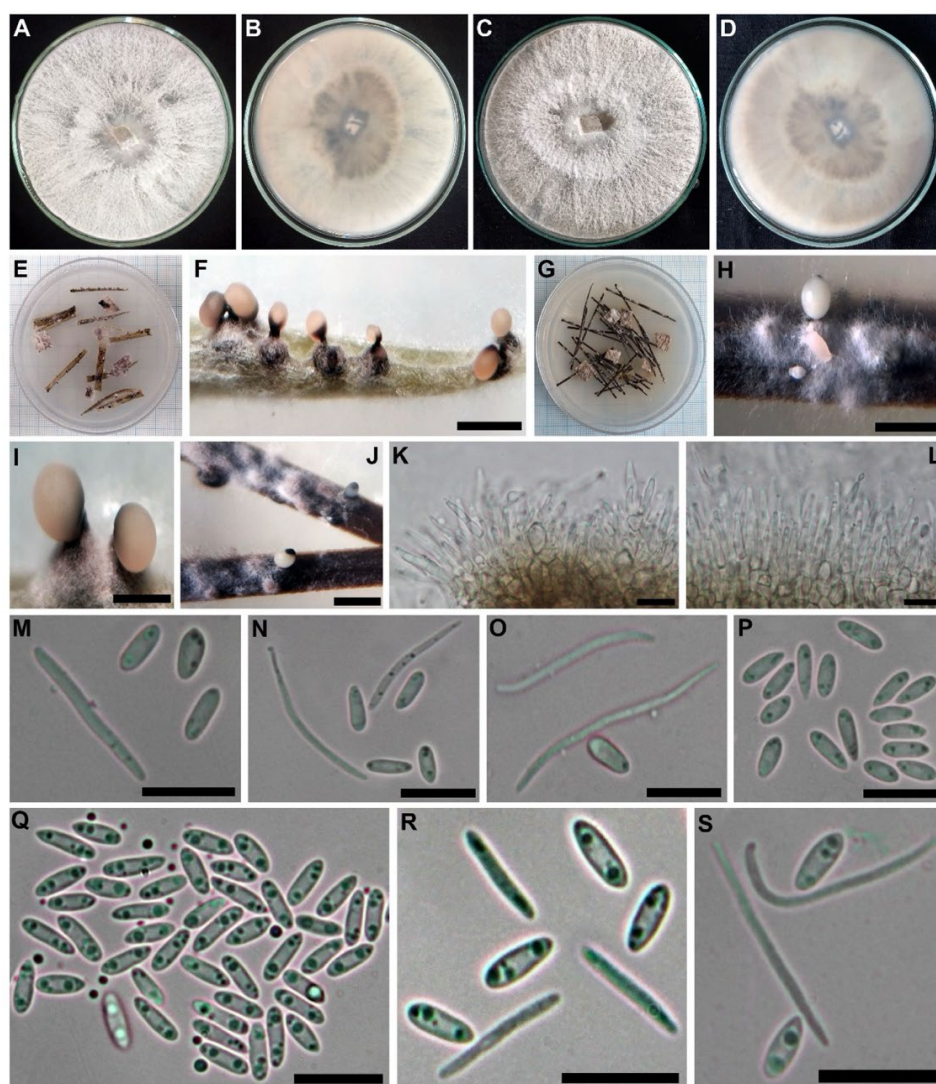
The morphological characters of the Iranian specimens are in accordance with all the descriptions in the *D. arecae* complex, which was compared by Pereira et al. (2023). However, our isolates produced gamma

conidia, which are usually absent in this complex and have only been observed in *D. limoncola*, *D. musigena* and *D. perseae*. The morphological variability among taxa belonging to the *D. arecae* complex, such as the absence or presence of paraphyses and beta- or gamma-conidia, is likely to be a result of character plasticity due to environmental conditions (Pereira et al. 2023).

***Diaporthe amygdali*** (Delacr.) Udayanga, Crous & K.D. Hyde, Fungal Diversity 56: 166 (2012)

**Description:** Conidiomata pycnidial, scattered or aggregated, black, crumpled, superficial, subglobose, exuding white or yellowish creamy conidial droplets from central ostioles after 7 days on CLA or 28 days on PNA, 0.5 × 1.2 mm diam; pycnidial wall consists of

black to dark brown, thin-walled cells. Conidiophores 14–20 × 1.5–2 µm, hyaline, phialidic, unbranched, tapering towards the apex, swelling at the base, subcylindrical, septate, smooth, straight or slightly curved. Alpha conidia aseptate, hyaline, ellipsoidal to fusiform, acutely round at both ends or obtuse at both ends, bi- or multi-guttulate, 5–8.6 × 1.1–2.5 µm ( $\bar{X}$  = 6.8 × 2.35 µm, n = 100). Beta conidia filiform, hyaline, straight or slightly curved, aseptate, base subtruncate, tapering towards the base, 16–33 × 0.7–1.4 µm ( $\bar{X}$  = 24.5 × 1 µm, n = 50). Gamma conidia aseptate, smooth, hyaline, straight to slightly curved, multiguttulate, 9–15.9 × 0.85–2 µm ( $\bar{X}$  = 12.45 × 1.4 µm, n = 50).



**Fig. 4.** *Diaporthe amygdali* (IRAN 4969C). (A–B) surface and reverse of colony after 7 days on PDA, (C–D) surface and reverse of colony after 14 days on PDA, (E) colonies on CLA after 7 days, (F, I) conidiomata with yellow sporulation on CLA, (G) colonies on PNA after 28 days, (H, J) conidiomata with white to cream sporulation on PNA, (K–L) conidiophores in water, (M–P) conidia on CLA, (M) alpha and gamma conidia in water, (N) alpha, beta and gamma conidia in water, (O) alpha and beta conidia, (P) alpha conidia in water, (Q–S) conidia on SNA, (Q) alpha conidia in water (R) alpha and gamma conidia in water, (S) alpha and beta conidia in water. Scale bars: (F, H–J) 1 mm, (K–S) 10 µm.



**Culture characteristics:** Cultures incubated on PDA, CLA, and PNA at 20 °C in a 12 h near-ultraviolet light/12 h dark cycle. The growth rate on PDA 20–22.5 mm diam/day, aerial mycelium abundant, white, becoming dark with age, greyish at the center, reverse white to greyish, with a concentric zonation, irregular margin, pycnidia forming after 20 days; On CLA obverse and reverse entirely white, pycnidia forming after 7 days; On PNA, obverse and reverse white, pycnidia forming after 28 days (Fig 4).

**Specimen examined:** Iran, Guilan Province, Fuman County, 37°15'31.2"N, 49°16'45.6"E, from the diseased petiole of *Vaccinium corymbosum* (Blueberry), 13 November 2022, collected by F. Ghahremani (IRAN 4969C).

**Notes:** Several species of *Diaporthe*, such as *D. amygdali*, *D. chongqingensis*, *D. fusicola*, *D. garethjonesii*, *D. kadsurae*, *D. ovoicicola*, *D. sterilis*, *D. ternstroemia*, and *D. mediterranea*, usually form a monophyletic group in phylogenetic analyses (Manawasinghe et al. 2019, Zhou and Hou 2019) and are referred as members of *D. amygdali* complex. Hilário et al. (2021) re-examined species boundaries within the *D. amygdali* complex based on the Genealogical Concordance Species Concept (GCSC) and coalescence-based models and reduced all the aforementioned species to the synonyms of *D. amygdali*.

Our isolate produced all three types of alpha, beta, and gamma conidia, while the presence of all three types of conidia together in a species within *D. amygdali* complex has not been reported. *Diaporthe chongqingensis*, *D. fusicola*, *D. kadsurae*, *D. mediterranea*, and *D. ternstroemia* produce only alpha conidia. *D. garethjonesii* and *D. ovoidea* produce alpha and beta conidia. *D. amygdali sensu stricto* produces alpha and gamma conidia (Hilário et al. 2021).

## DISCUSSION

Over several past decades, a huge number of *Diaporthe* species have been described based on morphology and host specificity. The classification and identification of *Diaporthe* species based on morphology host specificity have always been challenging for mycologists. During recent years, single-locus phylogenetic analyses have led to further challenges. Therefore, mycologists should be cautious to erect the new species based on just morphology or single-locus sequencing or just conduct a matrix with some loci (Hilário et al. 2021, Lambert et al. 2023). To overcome the challenges, mycologists offered a polyphasic approach, encompassing multi-locus phylogenies, together with morphology and ecology, which has been used for the investigation of species boundaries in the genus (Marin-Felix et al. 2019, Jiang et al. 2021, Xiao et al. 2023). During the last two decades, most researchers have followed the polyphasic approaches in taxonomic studies of the genus *Diaporthe*. These studies have resulted in some species

complexes (Gao et al. 2014, Lombard et al. 2014, Hyde et al. 2016, Zhou and Hou 2019, Yang et al. 2018, Guo et al. 2020). Therefore, a robust delimitation of species boundaries in this genus is still an ongoing challenge. Some mycologists have examined species boundaries using the Genealogical Concordance Species Concept (GCSC) (Hilário et al. 2021, Pereira et al. 2023, Dissanayake et al. 2024, Pereira and Phillips 2024). Based on these studies, they have found that a lot of nucleotide differences represent intraspecific variations. As a result, they synonymized many recently recognized species and tried to define new species boundaries in the genus *Diaporthe*. Although based on multi-gene sequencing and narrow species concept, we have identified two species; however, we follow recent works and identified our specimens as *D. amygdali* and *D. arecae*.

There are few studies on the identification and diversity of *Diaporthe* (syn. *Phomopsis*) species in Iran. To the best of our knowledge, eighteen species have so far been reported in the country. These species have solely been identified based on morphology or the combination of morphological and molecular characteristics. These include *Diaporthe* cf. *actinidiae* N.F. Sommer & Beraha from *Actinidia chinensis* Planch. (Mousakhah et al. 2014, morphological studies); *Diaporthe amygdali* (Delacr.) Udayanga, Crous & K.D. from *Corylus avellana* L. (Mirabolfathi et al. 2013, morphology and ITS-rDNA and *tefl* sequences); *Diaporthe cinerascens* Sacc. from *Ficus carica* L. (Banihashemi and Javadi 2009; Bolboli et al. 2023, morphology and ITS-rDNA, *tub2* and *his3* sequences); *Diaporthe citri* (H.S. Fawc.) F.A. Wolf from *Citrus aurantium* L., *C. limettoides* Tanaka and *C. sinensis* (L.) Ossbeck (Roohibakhsh and Ershad 1997, morphological studies); *Diaporthe eres* Nitschke from *Gleditsia caspia* Desf. and *Rosa* sp. (Ershad 2022; Mirabolfathy and Ershad 2004, morphological studies); *Diaporthe foeniculina* (Sacc.) Udayanga & Castl. from *Ficus benjamina* L. (Esmaeilzadeh et al. 2020, morphology and *tub2* sequences); *Diaporthe helianthi* Munt.-Cvetk., Mihaljč. & M. Petrov from *Helianthus annuus* L. (Ershad 2022, morphological studies); *Diaporthe longicolla* (Hobbs) J.M. Santos, Vrandečić & A.J.L. Phillips from *Abutilon* sp. (Mousavi et al. 2012, ?); *Diaporthe loropetali* (C.Q. Chang, Z.D. Jiang & P.K. Chi) Y.H. Gao & L. Cai from *Bauhinia* (*Phanera*) *purpurea* (Bavand Savadkuhi et al. 2019, morphology and rDNA-ITS sequences); *Diaporthe neoviticola* Udayanga, Crous & K.D. Hyde from *Vitis vinifera* L. (Ershad 2022, morphological studies); *Diaporthe novem* J.M. Santos, Vrandečić & A.J.L. Phillips from *Cichorium intybus* L. (Hatamzadeh et al. 2023, morphology and ITS, *tefl*, *tub2* sequences); *Diaporthe phaseolorum* (Cooke & Ellis) Sacc. from *Glycine max* (L.) Merr. (Ershad 2022, morphological studies); *Diaporthe quercina* Fuckel from unknown host (Ershad 2022, morphological studies); *Diaporthe subordinaria* (Desm.) R.R. Gomes, Glienke & Crous from *Plantago*



*major* L. (Ershad 2022, morphological studies); *Diaporthe vexans* (Sacc. & P. Syd.) Gratz from *Solanum melongena* L. (Ershad 2022, morphological studies); *Phomopsis malvacearum* (Westend.) Died. from *Alcea rosea* L. and *Abutilon theophrasti* Medic. (Babaeizad and Sayari 2012; Bavand Savadkuhi et al. 2019, morphology and rDNA-ITS and *tefl* sequences); *Phomopsis oryzae-sativae* Punith. from *Oryza sativa* L. (Ershad 2022, morphological studies); *Phomopsis theicola* Curzi from *Robinia* sp. (Bavand Savadkuhi et al. 2019, Morphology and ITS and *tefl* sequences); *Phomopsis* sp. from *Camellia sinensis* (L.) Kunze (Khodaparast et al. 1993). As we have shown, some species have been identified based on morphology or single-gene analyses. According to what has been presented earlier in this article, identifying *Diaporthe* species solely based on morphology or single-gene analysis, particularly the ITS region sequence, is nearly impossible. These species need to be identified with greater precision using multi-gene analysis. We hope the data presented in this paper will enhance our understanding of the taxonomy and biodiversity of *Diaporthe* in Iran.

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## AUTHOR CONTRIBUTION

Seyed Akbar Khodaparast planned and supervised the work; F. Ghahremani and M.J. Pourmoghaddam performed DNA sequencing; F. Ghahremani, M.J. Pourmoghaddam prepared the color photos; M.J. Pourmoghaddam performed the phylogenetic analysis; All authors reviewed the manuscript and commented on the manuscript.

## DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author upon request.

## DECLARATION

The authors declare no conflicts of interest.

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## ETHICS APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

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## گونه‌های *Diaporthe* مرتبط با گونه‌های تمشک و سیاه گیله از استان گیلان، ایران

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### چکیده

جنس *Diaporthe* (مترادف *Phomopsis*)، دارای گونه‌های اندوفیت، ساپروفیت و بیمارگر مهمی می باشد. این گونه‌ها دارای دامنه میزبانی وسیع بوده و از دیدگاه اقتصادی با بیماری‌های مهم گیاهی مرتبط هستند. گزارش‌های متعددی از گونه‌های این جنس روی دو جنس گیاهی تمشک و سیاه گیله در سراسر دنیا وجود دارد. با اینحال، گزارشی از این دو میزبان گیاهی از این جنس از ایران در دسترس نیست. بر همین اساس و به دنبال نمونه برداری از شهرهای مختلف استان گیلان، شش جدایه از این جنس به دست آمد. برای شناسایی این جدایه‌ها از ویژگی‌های ریختی و توالی ناحیه ریبوزومی فاصله‌انداز رونویسی شونده داخلی (ITS)، بخشی از ژن فاکتور طول‌سازی ترجمه-۱ آلفا (*tefl*) و بتاتوبولین (*tub2*) استفاده شد. نتایج نشان داد که نمونه‌ها متعلق به دو گونه *Diaporthe arecae* و *D. amygdali* می‌باشند. گونه *Diaporthe arecae* گونه جدید برای قارچهای ایران است و گیاهان تمشک و سیاه گیله به عنوان میزبان‌های جدید برای جنس *Diaporthe* معرفی می‌شوند.

### کلمات کلیدی

فیلوژنی، *Vaccinium*، *Rubus*، *Phomopsis*، *Diaporthaceae*.