cryptogamie
Mycologie
2022 • 43 • 7

Revision of the *Microsphaeropsis* complex with addition of four new *Paramicrosphaeropsis* L.W.Hou, L.Cai & Crous species from Zagrosian forest trees in Iran

Saeid ARTAND, Mehdi MEHRABI-KOUSHKI,
Saeid TABEIN, Kevin D. HYDE & Ruvishika S. JAYAWARDENA

art. 43 (7) — Published on 20 December 2022
www.cryptogamie.com/mycologie

PUBLICATIONS SCIENTIFIQUES
MUSÉUM NAL HIST NATURELLE

Couverture / *Cover*:
Extraits d'éléments de la Figure 4 / Extracts of the Figure 4

Cryptogamie, Mycologie est indexé dans / Cryptogamie, Mycologie *is indexed in:*
- Biological Abstracts
- Current Contents
- Science Citation Index
- Publications bibliographiques du CNRS (Pascal).

Cryptogamie, Mycologie est distribué en version électronique par / Cryptogamie, Mycologie *is distributed electronically by:*
- BioOne® (http://www.bioone.org/loi/crym)

Cryptogamie, Mycologie est une revue en flux continu publiée par les Publications scientifiques du Muséum, Paris
Cryptogamie, Mycologie *is a fast track journal published by the Museum Science Press, Paris*

Les Publications scientifiques du Muséum publient aussi / *The Museum Science Press also publishes:* ***Adansonia, Geodiversitas, Zoosystema, Anthropozoologica, European Journal of Taxonomy, Naturae,*** **Cryptogamie sous-sections** ***Algologie, Bryologie***, ***Comptes Rendus Palevol***.

Diffusion – Publications scientifiques Muséum national d'Histoire naturelle
CP 41 – 57 rue Cuvier F-75231 Paris cedex 05 (France)
Tél.: 33 (0)1 40 79 48 05 / Fax: 33 (0)1 40 79 38 40
diff.pub@mnhn.fr / http://sciencepress.mnhn.fr

ISSN (imprimé / *print*): 0181-1584/ ISSN (électronique / *electronic*): 1776-100

Revision of the *Microsphaeropsis* complex with addition of four new *Paramicrosphaeropsis* L.W.Hou, L.Cai & Crous species from Zagrosian forest trees in Iran

Saeid ARTAND
Department of Plant Protection, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Khuzestan Province (Iran)

Mehdi MEHRABI-KOUSHKI
Department of Plant Protection, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Khuzestan Province (Iran)
and Biotechnology and Bioscience Research Center, Shahid Chamran University of Ahvaz, Ahvaz, Khuzestan Province (Iran)
mhdmhrb@scu.ac.ir; mhdmhrb@gmail.com (corresponding author)

Saeid TABEIN
Department of Plant Protection, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Khuzestan Province (Iran)

Kevin D. HYDE
Ruvishika S. JAYAWARDENA
Center of Excellence in Fungal Research, Mae Fah Luang University, 57100 (Thailand)

Submitted on 1 November 2021 | Accepted on 23 September 2022 | Published on 20 December 2022

Artand S., Mehrabi-Koushki M., Tabein S., Hyde K. D. & Jayawardena R. S. 2022. — Revision of the *Microsphaeropsis* complex with addition of four new *Paramicrosphaeropsis* L.W.Hou, L.Cai & Crous species from Zagrosian forest trees in Iran. *Cryptogamie, Mycologie* 43 (7): 159-175. https://doi.org/10.5252/cryptogamie-mycologie2022v43a7. http://cryptogamie.com/mycologie/43/7

ABSTRACT

Paramicrosphaeropsis L.W.Hou, L.Cai & Crous is a monophyletic taxon in Didymellaceae and includes two species to date. In a survey on microsphaeropsis-like fungi associated with Zagrosian forest trees in Iran, four new *Paramicrosphaeropsis* species were identified and described including *P. amygdalus* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. from *Amygdalus scoparia* Spach., *P. pistacicola* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. from *Pistacia* spp., *P. salandica* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. from *Crataegus* sp., *Nerium oleander* L., *Quercus brantii* Lindl. and *Ziziphus* sp., and *P. zagrosensis* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. from *Crataegus* sp. and *Quercus brantii*. Phylogenetic analyses based on a combination of four genomic regions, including the partial nuclear 28S ribosomal DNA (LSU), internal transcribed spacer regions 1 and 2 including the intervening 5.8S nuclear ribosomal DNA (ITS), partial β-tubulin (*tub*2) and RNA polymerase II second largest subunit (*rpb*2), are provided to clarify the phylogenetic affinities of the new species and other taxa within the *Microsphaeropsis* complex. In addition, a new genus *Heteromicrosphaeropsis* M.Mehrabi-Koushki, K.D.Hyde & Jayaward., gen. nov. and five new combinations are introduced. The new combinations are *Heteromicrosphaeropsis ononidicola* (Thambug., Camporesi & K.D.Hyde) M.Mehrabi-Koushki, K.D.Hyde & Jayaward., comb. nov., *Microsphaeropsis cytisi* (W.J.Li & K.D.Hyde) M.Mehrabi-Koushki, K.D.Hyde & Jayaward., comb. nov., *M. cytisicola* (Wanas., Camporesi, E.B.G.Jones & K.D.Hyde) M.Mehrabi-Koushki, K.D.Hyde & Jayaward., comb. nov., *M. cytisinus* (Tennakoon, Camporesi & K.D.Hyde) M.Mehrabi-Koushki, K.D.Hyde & Jayaward., comb. nov. and *M. minima* (W.J.Li & K.D.Hyde) M.Mehrabi-Koushki, K.D.Hyde & Jayaward., comb. nov.

KEY WORDS
Iran,
Didymellaceae,
morphology,
multilocus phylogeny,
new combinations,
new species,
new genus.

RÉSUMÉ

Révision du complexe Microsphaeropsis *avec l'ajout de quatre nouvelles espèces de* Paramicrosphaeropsis *L.W.Hou, L.Cai & Crous provenant d'arbres de la forêt zagrosienne en Iran.*

Paramicrosphaeropsis L.W.Hou, L.Cai & Crous est un taxon monophylétique de la famille des Didymellaceae et comprend, à ce jour, deux espèces. Lors d'une étude sur les champignons de type *Microsphaeropsis* associés aux arbres de la forêt zagrosienne en Iran, quatre nouvelles espèces de *Paramicrosphaeropsis* ont été identifiées et décrites, dont *P. amygdalus* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. d'*Amygdalus scoparia* Spach., *P. pistacicola* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. de *Pistacia* spp., *P. salandica* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. de *Crataegus* sp., *Nerium oleander* L., *Quercus brantii* Lindl. et *Ziziphus* sp., et *P. zagrosensis* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. de *Crataegus* sp. et *Quercus brantii*. Des analyses phylogénétiques basées sur une combinaison de quatre régions génomiques, incluant l'ADN ribosomal 28S nucléaire partiel (LSU), les régions intercalaires transcrites internes 1 et 2 comprenant l'ADN ribosomal nucléaire 5.8S intermédiaire (ITS), la β-tubuline partielle (*tub2*) et la deuxième plus grande sous-unité de l'ARN polymérase II (*rpb2*), sont fournies pour clarifier les affinités entre nouvelles espèces et autres taxons du complexe *Microsphaeropsis*. En outre, un nouveau genre *Heteromicrosphaeropsis* M.Mehrabi-Koushki, K.D.Hyde & Jayaward., gen. nov. et cinq nouvelles combinaisons sont introduits. Les nouvelles combinaisons sont *Heteromicrosphaeropsis ononidicola* (Thambug., Camporesi & K.D.Hyde) M.Mehrabi-Koushki, K.D.Hyde & Jayaward., comb. nov., *Microsphaeropsis cytisi* (W.J.Li & K.D.Hyde) M.Mehrabi-Koushki, K.D.Hyde & Jayaward., comb. nov., *M. cytisicola* (Wanas., Camporesi, E.B.G.Jones & K.D.Hyde) M.Mehrabi-Koushki, K.D.Hyde & Jayaward., comb. nov., *M. cytisinus* (Tennakoon, Camporesi & K.D.Hyde) M.Mehrabi-Koushki, K.D.Hyde & Jayaward., comb. nov. et *M. minima* (W.J.Li & K.D.Hyde) M.Mehrabi-Koushki, K.D.Hyde & Jayaward., comb. nov.

MOTS CLÉS
Iran,
Didymellaceae,
morphologie,
phylogénie,
combinaisons nouvelles,
espèces nouvelles,
genre nouveau.

INTRODUCTION

The Zagros mountain forest steppe, with an area of about six million hectares (3.5% of Iran) is a broadleaf and mixed forests ecoregion in western Asia and extends along the Zagros mountains, stretching primarily in Iran, ranging northwest to southeast and roughly paralleling the Iran's western border (eastern Turkey and northern Iraq). These regions constitute 40% of the Iran's forested areas and are stretching over 12 provinces. The mountains of Segaryoo and Saland-Kooh in Dezful (Khuzestan Province), and Zaz and Mahroo in Aligudarz (Lorestan Province) are a very small part of these forests in Iran. These forested areas are home to many tree species including, hawthorn (*Crataegus* sp.), khinjuk (*Pistacia khinjuk* Stocks), Persian oak (*Quercus brantii* Lindl.), oleander (*Nerium oleander* L.), turpentine tree (*Pistacia atlantica* Desf.), and wild almond (*Amygdalus scoparia* Spach.). Due to the predominance of oak trees in the mentioned areas, these forests have also been called oak forests. According to the literature, these forest trees were reported to be hosts for a large number of pathogenic and non-pathogenic fungi (Ershad 2009; Farr & Rossman 2021). In recent years, different symptoms including the leaf spot, charcoal rot, stem necrosis, canker, and dead twigs and branches of trees have frequently been observed, especially in those which are under drought stress (Ershad 2002, 2009; Fotouhifar *et al*. 2010; Pirnia *et al*. 2012; Rostamian *et al*. 2016; Shamsi *et al*. 2019). The identification of fungi growing within these trees is a first step toward understanding the ecology of plant-fungus associations in forest trees.

Microsphaeropsis Höhn. was introduced by Höhnel (1917) and is known as a polyphyletic genus with 60 species epithets in GenBank (https://www.ncbi.nlm.nih.gov). This genus was originally placed in Montagnulaceae (Höhnel 1917), and then reclassified in the Phaeosphaeriacea (Barr 1987) and Didymellaceae (Barr 1987; de Gruyter *et al*. 2009, 2013; Aveskamp *et al*. 2010; Hyde *et al*. 2013). In a comprehensive molecular study on phoma-like taxa, two representative species of *Microsphaeropsis*, including *M. olivacea* (Bonord.) Höhn and *M. proteae* (Crous & Denman) Crous & Denman, grouped phylogenetically in a different lineage basal to the Didymellaceae, for which a new family Microsphaeropsidaceae was introduced (Chen *et al*. 2015). Subsequently, Thambugala *et al*. (2017) identified the microsphaeropsis-like fungi isolated from *Tamarix* species based on morphological characters and analyses of gene sequence data. In this study, microsphaeropsis-like strains clustered in a single lineage within Didymellaceae, distinct from the family Microsphaeropsidaceae. Accordingly, the genus *Neomicrosphaeropsis* Thambugala, Camporesi & K.D.Hyde was established by Thambugala *et al*. (2017) to accommodate the type species, *N. italica* Thambugala, Camporesi & K.D. Hyde, and three other species including *N. novorossica* Thambugala, Bulgakov & K.D.Hyde, *N. rossica* Thambugala, Bulgakov & K.D.Hyde, and *N. tamaricicola* (Wanas., Camporesi, E.B.G.Jones & K.D.Hyde) Thambugala, Wanasinghe & K.D.Hyde (synonym: *Phoma tamaricicola*). Lately, further species of *Neomicrosphaeropsis* were newly introduced and described including *N. alhagi-pseudalhagi* Wanas., Gafforov & K.D.Hyde (Wanasinghe *et al*. 2018), *N. cytisicola* Wanas., Camporesi, E.B.G.Jones & K.D.Hyde (Wanasinghe *et al*. 2018), *N. elaeagni* Wanas., Bulgakov, E.B.G.Jones &

K.D.Hyde (Wanasinghe *et al*. 2018), *N. juglandis* D.Pem, Selcuk, Jeewon & K.D.Hyde (Pem *et al*. 2020), and *N. minima* W.J.Li, Camporesi & K.D.Hyde (Hyde *et al*. 2016).

Moreover, the generic circumscription of *Microsphaeropsis* and *Neomicrosphaeropsis* were further emended to reveal more natural evolutionary relationships (Hou *et al*. 2020; Crous *et al*. 2021). As other taxa in Didymellaceae, molecular phylogenetic analysis based on DNA sequences of the same non-linked loci, including the internal transcribed spacers 1 and 2 with the intervening 5.8S nuclear ribosomal DNA (ITS), partial nuclear 28S ribosomal DNA (LSU), part of the β-tubulin gene (*tub*2), and partial RNA polymerase II second largest subunit (*rpb*2), led to an elucidation of the in-group and out-group taxa of *Microsphaeropsis* and *Neomicrosphaeropsis* (Hou *et al*. 2020; Crous *et al*. 2021). Subsequent to these revisions, two additional genera were introduced in the Microsphaeropsis complex within the Didymellaceae, namely *Paramicrosphaeropsis* L.W. Hou, L.Cai & Crous (Hou *et al*. 2020) and *Nothomicrosphaeropsis* Crous (Crous *et al*. 2021).

Hou *et al*. (2020) reclassified the members of *Microsphaeropsis* complex by including more taxa in the phylogenetic analyses. In this study, *Microsphaeropsis* and *Neomicrosphaeropsis* species accommodated inside the Didymellaceae, closely related to new genus *Paramicrosphaeropsis*. Therefore, the family Microsphaeropsidaceae was not accepted and reduced to synonym of Didymellaceae (Hou *et al*. 2020). Recently, a novel genus *Nothomicrosphaeropsis* was introduced with the description of *N. welwitschiae* Crous as the type species (Crous *et al*. 2021).

Paramicrosphaeropsis was first introduced, with the description of the species *P. ellipsoidea* L.W.Hou, L.Cai & Crous as the type (Hou *et al*. 2020). Very recently another species, *P. iranica* S.A.Ahmadp., M.Mehrabi-Koushki, Farokhinejad & Asgari, has been assigned to this genus (Ahmadpour *et al*. 2022). *Paramicrosphaeropsis* is characterized by globose to subglobose pycnidia with hyaline and thin pseudoparenchymatous walls, and a thin-walled, smooth, aseptate young conidia (Hou *et al*. 2020). *Paramicrosphaeropsis* and two previously related genera, including *Microsphaeropsis* and *Neomicrosphaeropsis* formed a well-supported lineage, distinct from other known genera of Didymellaceae (Hou *et al*. 2020). The members of this lineage show some consistent morphological characteristics including globose to subglobose pycnidia, hyaline conidiogenous cells, and hyaline conidia that become brownish with age (Chen *et al*. 2015; Hou *et al*. 2020).

Up to date, the *Microsphaeropsis* complex included only four genera mentioned above, across a wide range host around the world. Most species of this complex are reported as endophytes, saprobes, and pathogens of terrestrial plants (Thambugala *et al*. 2017; Espargham *et al*. 2020; Hou *et al*. 2020; Pem *et al*. 2020; Farr & Rossman 2021; Ahmadpour *et al*. 2022). Some species are known as opportunistic human pathogens (Guarro *et al*. 1999; Reppas *et al*. 2015).

This study aimed to identify the microsphaeropsis-like fungi associated with various trees growing in a small area of Zagros forests located in Khuzestan and Lorestan Province in Iran, through phylogenetic analyses combined with morphology. A new genus, four new species and five combinations are herein introduced.

MATERIAL AND METHODS

Sampling area and fungal isolation

The study region was a very small area of Zagros forests located in Segaryoo and Saland-Kooh (Dezful, Khuzestan Province), and Zaz and Mahroo (Aligudarz, Lorestan Province) in Iran. During 2020-2021, 30 symptomatic samples from leaves, stems, fruits and/or branches of trees and shrubs were collected in paper bags and processed for fungal isolation. The plants sampled were hawthorn (showing stem canker and fruit rot), khinjuk (showing leaf spot), Persian oak (showing leaf spot and stem canker and necrosis), oleander (showing leaf spot), turpentine tree (showing leaf spot and stem canker), and wild almond (showing stem canker and necrosis).

Fungal isolation was performed as described by Safi *et al*. (2020). Small pieces (0.2-0.6 cm) from samples excised from healthy and symptomatic margins were surface-sterilized with 1% sodium hypochlorite for two-four minutes and were then rinsed three times with sterile distilled water. Surface-sterilized pieces were dried on sterile filter paper and plated onto potato dextrose agar medium (PDA; potato extract 200-400 gL^{-1}, sucrose 10 gL^{-1}, agar 12 gL^{-1}, streptomycin 30 mgL^{-1}). The petri plates were incubated at 25°C for two-five days. Pycnidia-producing colonies of *Paramicrosphaeropsis* were subcultured on separate plates. The pure cultures of *Paramicrosphaeropsis* isolates were prepared by single spore isolation (Larki *et al*. 2019).

Holotypes of new species were transferred for permanent storage to the Herbarium Ministerii Iranici Agriculturae, Iranian Research Institute of Plant Protection, Tehran, Iran. The living cultures of ex-type strains are deposited in the Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Tehran, Iran and Collection of Fungal Cultures, Department of Plant Protection, Shahid Chamran University of Ahvaz, Iran (Table 1).

Growth and morphologic studies

Morphology of fungal structures was determined from cultures grown on PDA and oatmeal agar (OA, oatmeal 30-60 gL^{-1}, agar 12 gL^{-1}) at 25°C with 12 hours alteration of dark and light for 8-20 days. Circular agar plugs of 0.5 cm from the active-growing edge of the colony of each isolate was separately transferred into the center of Petri plates containing PDA and OA (three replicates for each medium). The diameter of each colony on PDA and OA was measured in two perpendicular directions after eight days of incubation at 25°C and 30°C in darkness. The data obtained from three replicates were used to estimate the average colony growth rate. Pycnidia were removed from the culture of each isolate using a dissecting forcep and mounted on a slide in in a drop of lactophenol or lactophenol cotton blue, followed by observing through 40× and 100× objective lens of a Leitz Wetzlar (SM-LUX) Basic Biological Light Microscope. At least, fifteen measurements were made from each fungal structure (pycnidia, conidiogenous cells, and conidia) and expressed as a maximum and minimum range, mean, standard deviation (S.D.) and 95% confidence intervals. Photomicrographs were obtained with an OLYMPUS DP12 digital camera coupled to an OLYMPUS BX51 microscope.

TABLE 1. — Strains used in phylogenetic analysis and their GenBank accession numbers. Generated sequences are indicated in **bold**. [T] **letter** indicates ex-type cultures. Abbreviations: **CBS**, Central Bureau of Fungal Cultures, Utrecht, The Netherlands; **CPC**, Culture collection of Pedro Crous, housed at CBS; **IRAN**, Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Iran; **MFLUCC**, Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **SCUA**, the Collection of Fungal Cultures, Department of Plant Protection, Shahid Chamran University of Ahvaz, Iran.

				GenBank accession numbers			
Species name	**Strains name**	**Isolation source**	**Origin**	**LSU**	**ITS**	***tub*2**	***rpb*2**
Heteromicrosphaeropsis ononidicola comb. nov.	MFLUCC 15-0459[T]	*Ononis spinosa*	Italy	MG967668	MG967670	MG973087	–
Microsphaeropsis cytisicola comb. nov.	MFLUCC 18-0355[T]	*Cytisus* sp.	Italy	MH069671	MH069665	MH069690	MH069683
M. cytisinus comb. nov.	MFLUCC 16-0790[T]	*Cytisus* sp.	Italy	KX611242	KX611243	–	–
M. cytisi comb. nov.	MFLUCC 13-0396[T]	*Verbascum* sp.	Italy	KX572342	KX572337	–	KX572355
M. fusca	CBS 116669	*Sarothamnus scoparius*	The Netherlands	EU754170	MN973572	MT005675	MT018219
M. fusca	CBS 116670	*Sarothamnus scoparius*	The Netherlands	MN943779	MN973573	MT005676	MT018220
M. hellebori	CBS 569.82	–	Belgium	MH873273	MH861529	–	–
M. minima comb. nov.	MFLUCC 13-0394[T]	*Cytisus* sp.	Italy	KX572341	KX572336	–	–
M. olivacea	CBS 233.77	*Pinus nigra subsp. laricio*	France	GU237988	GU237803	GU237549	KT389643
M. olivacea	CBS 320.76	–	France	MH872752	MH860982	GU237549	MT018214
M. proteae	CBS 111303	*Protea nitida*	South Africa	JN712561	JN712495	MT005677	MT018221
M. proteae	CBS 111319[T]	*Protea nitida*	South Africa	JN712563	JN712497	MT005679	MT018223
M. spartii-juncei	MFLU 16-0097	*Spartium junceum*	Italy	MH069669	MH069663	MH069688	MH069681
M. spartii-juncei	MFLU 16-0100[T]	*Spartium junceum*	Italy	MH069668	NR160346	MH069687	MH069680
M. taxicola	CBS 442.83[T]	*Taxus baccata*	The Netherlands	EU754171	GU237865	GU237547	MT018211
M. viridis	CBS 432.71[T]	*Sarothamnus* sp.	The Netherlands	JX681102	MH871969	GU237548	MT018209
M. viridis	CBS 639.80	*Abies alba*	Germany	MH873066	MH861301	MT005667	MT018208
Neomicrosphaeropsis alhagi-pseudalhagi	MFLUCC 17-0825[T]	*Alhagi pseudoalhagi*	Uzbekistan	MH069670	MH069664	MH069689	MH069682
N. elaeagni	MFLUCC 16-2389	*Elaeagnus angustifolia*	Russia	MH069672	MH069666	MH069691	MH069684
N. italica	MFLUCC 16-0284	*Tamarix* sp.	Italy	KU900296	KU900321	KX453299	KU714604
N. juglandis	MFLU17-0517	*Juglans regia*	Turkey	MN244206	MN244223	MN871954	MN593307
N. novorossica	MFLUCC 14-0578[T]	*Tamarix ramosissima*	Russia	KX198710	KX198709	–	–
N. rossica	MFLUCC 14-0586[T]	*Tamarix ramosissima*	Russia	KU729855	KU752192	–	–
N. tamaricicola	MFLUCC 14-0602[T]	*Tamarix gallica*	Italy	KM408754	KM408753	MH069692	MH069685
Nothomicrosphaeropsis welwitschiae	CPC 38879[T]	*Welwitschia mirabilis*	Namibia	MW883826	MW883434	MW890138	MW890067
Paramicrosphaeropsis amygdalus	SCUA-Ar-KS3-3[T]	*Amygdalus scoparia*	Iran	**MZ746125**	**MZ746101**	**MZ747174**	**MZ747195**
P. amygdalus sp. nov.	SCUA-Ar-S6A	Unknown plant	Iran	**MZ746127**	**MZ746103**	**MZ747176**	**MZ747197**
P. amygdalus sp. nov.	SCUA-Ar-SB2A	*Amygdalus scoparia*	Iran	**MZ746126**	**MZ746102**	**MZ747175**	**MZ747196**
P. ellipsoidea	CBS 194.97[T]	*Quercus ilex*	Spain	MN943781	NR170791	MT005681	MT018225
P. ellipsoidea	CBS 197.97	*Quercus ilex*	Spain	MN943780	MN973574	MT005680	MT018224
P. iranica	IRAN 2929C	*Quercus brantii*	Iran	OK257024	OK257016	OK247743	OK247737
P. iranica	SCUA-Ah-B	*Quercus brantii*	Iran	OK257025	OK257017	OK247744	OK247738
P. pistacicola sp. nov.	SCUA-Ar-SK11A[T]	*Pistacia khinjuk*	Iran	**MZ746135**	**MZ746111**	**MZ747183**	**MZ747205**
P. pistacicola sp. nov.	SCUA-Ar-SK1D	*Pistacia atlantica*	Iran	**MZ746136**	**MZ746112**	**MZ747184**	**MZ747206**
P. salandica sp. nov.	SCUA-Ar-S4D1	*Ziziphus* sp.	Iran	**MZ746131**	**MZ746107**	**MZ747179**	**MZ747201**
P. salandica sp. nov.	SCUA-Ar-S4D2	*Ziziphus* sp.	Iran	**MZ746134**	**MZ746110**	**MZ747182**	**MZ747204**
P. salandica sp. nov.	SCUA-Ar-S9C2	Unknown plant	Iran	**MZ746129**	**MZ746105**	**MZ747177**	**MZ747199**
P. salandica sp. nov.	SCUA-Ar-SB5A	*Quercus brantii*	Iran	**MZ746133**	**MZ746109**	**MZ747181**	**MZ747203**
P. salandica sp. nov.	SCUA-Ar-SKH13B	*Nerium oleander*	Iran	**MZ746130**	**MZ746106**	**MZ747178**	**MZ747200**
P. salandica sp. nov.	SCUA-Ar-SZ8C2[T]	*Crataegus* sp.	Iran	**MZ746132**	**MZ746108**	**MZ747180**	**MZ747202**
P. zagrosensis sp. nov.	SCUA-Ar-B10A[T]	*Quercus brantii*	Iran	**MZ746123**	**MZ746099**	**MZ747172**	**MZ747193**
P. zagrosensis sp. nov.	SCUA-Ar-Z1B	*Crataegus* sp.	Iran	**MZ746124**	**MZ746100**	**MZ747173**	**MZ747194**
Pseudoascochyta novae-zelandiae	CBS 141689	–	New Zealand	**LT592893**	**LT592892**	**LT592894**	**LT592895**
P. pratensis	CBS 141688	–	–	**NG069490**	**NR158273**	**LT223132**	**LT223133**
Vacuiphoma ferulae	CBS 353.71	*Ferula communis*	Italy	**MN943759**	**MN973552**	**MT005655**	**MT018196**

DNA EXTRACTION AND AMPLIFICATION

The fungal biomass of each strain grown on PDA at 25°C in the dark for 10-12 days was collected using a sterile glass slide and powdered in a mortar containing liquid nitrogen. Total genomic DNA was extracted from *Paramicrosphaeropsis* isolates as described by Raeder & Broda (1985), with some optimization (Ahmadpour *et al.* 2017).

Samples of DNA were qualified and quantified by loading on agarose gel together with known amounts of 100 bp Plus ladder (SinaClon, Iran). The primers ITS1 and NL4 (White *et al.* 1990; O'Donnell 1993) were used for the amplification and sequencing of the ITS and LSU (D1 + D2) regions; primers B*tub*2Fd and T2 (O'Donnell & Cigelnik 1997; Woudenberg *et al.* 2009) were used to amplify and sequence a fragment of partial *tub*2; and primers RPB2-5F2 and fRPB2-7cR (Liu *et al.* 1999; Sung *et al.* 2007) were used to amplify and sequence a fragment of the *rpb*2. The PCR mixture contained 1× PCR Buffer (GenetBio, South Korea), 3 µl of genomic DNA (20 ng), 1.2 µl of each primer (10 µM), 1.2 µl of dNTP mix (2.5 mM of each dNTP), 2.4 µl of $MgCl_2$ (25 mM), three units of Prime Taq DNA polymerase (GenetBio, South Korea) and DNase free milii-Q water up to final volume (30 µl).

The thermal cycle of PCR was performed in a MJ Mini™ Gradient Thermal Cycler and consisted of an initial denaturation step at 94°C for three minutes; 35 cycles of 30 seconds at 94°C for denaturation, 30 seconds at 54°C (ITS-LSU) or 56°C (*tub*2 and *rpb*2) for annealing, and 70 seconds at 72°C for extension; and five minutes at 72°C for the final extension.

SEQUENCING AND PHYLOGENETIC ANALYSES

The ITS-LSU and *tub*2 products were directly sequenced in in forward and reverse directions. The *rpb*2 products were separated by electrophoresis in 1% agarose gels in 1.0× Tris-acetic acid-EDTA (TAE) buffer, stained with a commercial DNA

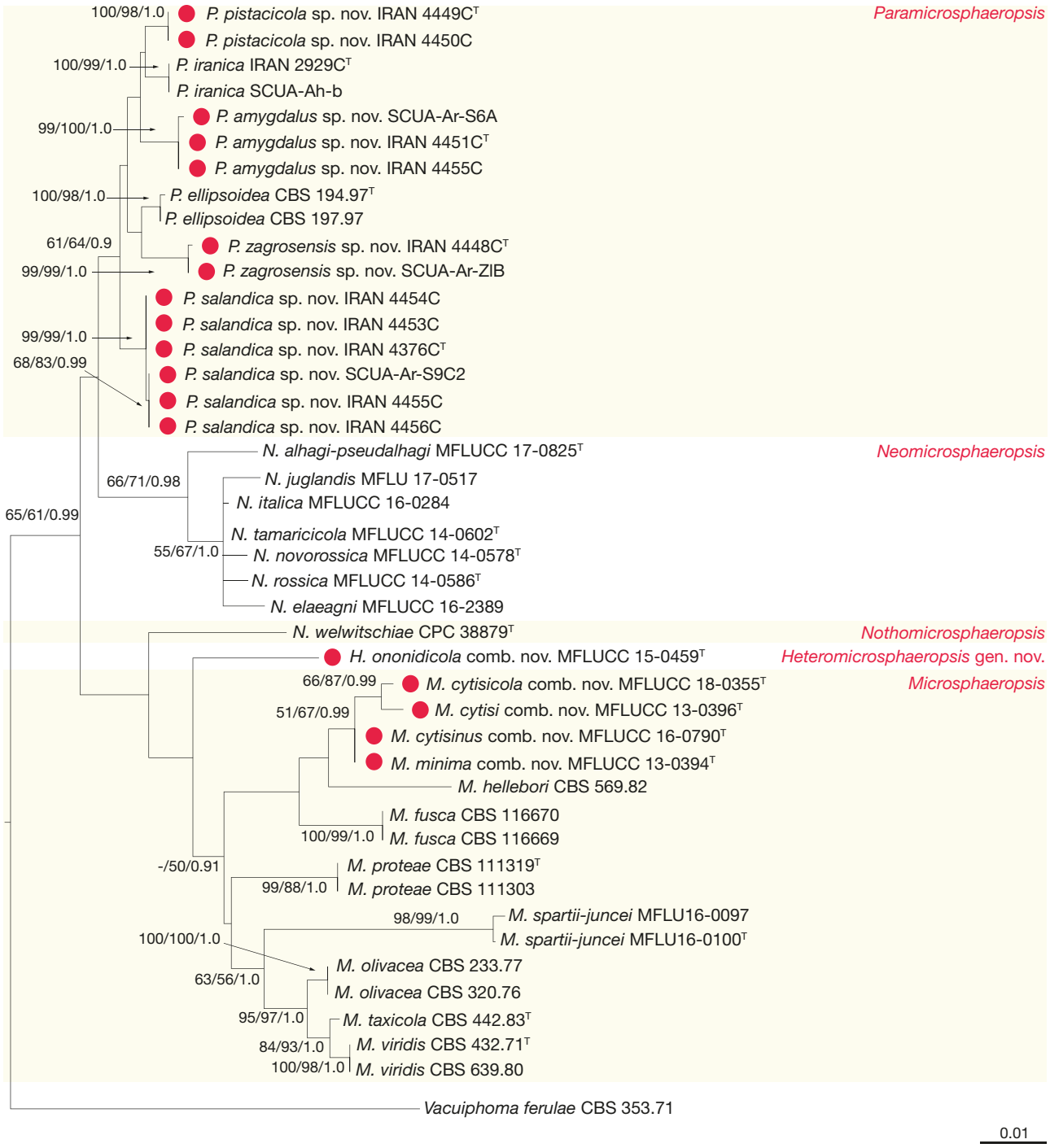

FIG. 1. — The ML phylogenetic tree obtained from the combined LSU-ITS, *tub2* and *rpb2* sequence data of strains belonging to the *Microsphaeropsis* complex. The tree was rooted to *Vacuiphoma ferulae* L.W.Hou, L.Cai & Crous. Taxa retrieved or reclassified newly are shown with **red dots**. Bootstrap values obtained in maximum likelihood (ML) and maximum parsimony (MP) analyses ≥ 0.50% and Bayesian posterior probability values (BYPP) ≥ 0.90% are shown at the nodes, respectively. The scale bar shows the expected number of changes per site. **T letter** indicates type strains.

safe stain (SinaClon, Iran) and visualized under a UV transilluminator. The PCR products of the expected size were excised and purified with the GF-1 AmbiClean Kit (Vivantis, Malaysia), followed by sequencing in both directions. The final sequences of each region were analyzed with BioEdit v.7.0.9.0 (Hall 1999) and DNA Baser Sequence Assembler v4 (2013, Heracle BioSoft, www.DnaBaser.com). New sequences were deposited in GenBank (Table 1). Sequences of the type strains used in the phylogenetic analyses were retrieved from GenBank (Table 1). The tree was rooted with *Vacuiphoma ferulae* (Pat.) L.W.Hou, L.Cai & Crous. Single-locus alignments were made using ClustalW in BioEdit v.7.0.9.0 and refined manually where necessary. Initial phylogenetic relationships within *Paramicrosphaeropsis* and allied genera were obtained using

phylogenetic analyses based on sequence data of each loci. Maximum likelihood (ML) phylogenetic analyses of DNA sequence data were performed with raxmlGUI 2.0 beta (Edler *et al*. 2020) and maximum parsimony analysis with MEGA 7 (Kumar *et al*. 2016). In ML analyses, a general time reversible model of evolution was used with a gamma-distributed rate variation and invariant sites (GTR + G + I) and thorough bootstrapping with 1000 replicates (MLBS). Bayesian inference analyses (BI) of concatenated alignment were done using the Markov chain Monte Carlo method with MrBayes v.3.2.6 (Ronquist *et al*. 2012). The best nucleotide substitution model for each region was estimated by jModelTest 2 (Darriba *et al*. 2012), and models were selected according to the lowest Akaike Information Criterion (AIC). The GTR + I + G model of evolution was used for ITS and *tub*2 regions, GTR for LSU, and SYM + G for *rpb*2. The BI analysis was performed with two simultaneous runs of ten millions generation, sampling every 1000 generations, discarding 25% of the first trees for calculating posterior probability values (BPP), and the standard deviation below 0.01. Single-locus and combined alignments were deposited in TreeBASE (http://www.treebase.org/) under accession number TB2: S28882.

RESULTS

We studied the species of the *Microsphaeropsis* complex in literature review of Didymellaceae and found that our strains represent four new species within the genus *Paramicrosphaeropsis*. The new species are identified and described based on four-locus phylogenetic analyses and morphology herein. In addition, a new genus and five combinations are introduced.

DNA analyses and phylogeny

Concatenated alignment used in the phylogenetic analyses contained 156 sequences from 42 ingroup taxa (strains of *Heteromicrosphaeropsis* M.Mehrabi-Koushki, K.D.Hyde & Jayaward., gen. nov., *Microsphaeropsis*, *Neomicrosphaeropsis*, *Nothomicrosphaeropsis* and *Paramicrosphaeropsis*) and four sequences from the outgroup (Table 1). This combined dataset was composed of 1655 nucleotide sites including gaps (LSU: 521 bp; ITS: 431 bp; *tub*2: 265 bp; *rpb*2: 438 bp). Of those 1428 sites are constant (LSU: 514 bp; ITS: 405 bp; *tub*2: 192 bp; *rpb*2: 317 bp), 47 variable sites are parsimony-uninformative (LSU: 2 bp; ITS: 11 bp; *tub*2: 20 bp; *rpb*2: 14 bp) and 175 variable sites are parsimony-informative (LSU: 5 bp; ITS: 11 bp; *tub*2: 52 bp; *rpb*2: 107 bp). The ML phylogenetic tree showed an overall similar topology with those obtained in the BI and MP analysis (Fig. 1). In the phylogenetic tree (Fig. 1), all microsphaeropsis-like strains were grouped in five moderately-supported lineages including *Microsphaeropsis* (MPBS 50%, BPP 0.91), *Neomicrosphaeropsis* (MLBS 66%, MPBS 71%, BPP 0.98), *Paramicrosphaeropsis* (MLBS 61%, MPBS 64%, BPP 0.90), and two monotypic lineages including *Nothomicrosphaeropsis* and new genus *Heteromicrosphaeropsis* M.Mehrabi-Koushki, K.D.Hyde & Jayaward., gen. nov. In our phylogenetic analyses based on combined LSU, ITS, *tub*2 and *rpb*2 sequence (Fig. 1), all strains isolated in this study clustered with *Paramicrosphaeropsis* lineage and delimited in four monophyletic, well-supported clades distinct from two previously known species of *Paramicrosphaeropsis*, including *P. amygdalus* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. (MLBS 99%, MPBS 100%, BPP 1.0), *P. pistacicola* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. (MLBS 100%, MPBS 98%, BPP 1.0), *P. salandica* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. (MLBS 99%, MPBS 99%, BPP 1.0), and *P. zagrosensis* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. (MLBS 99%, MPBS 99%, BPP 1.0). In the multilocus phylogenetic tree (Fig. 1), *Neomicrosphaeropsis cytisi* W.J.Li & K.D.Hyde, *Neomicrosphaeropsis cytisicola* Wanas., Camporesi, E.B.G.Jones & K.D.Hyde, *Neomicrosphaeropsis cytisinus* Tennakoon, Camporesi s& K.D.Hyde, and *Neomicrosphaeropsis minima* W.J.Li & K.D.Hyde grouped with *Microsphaeropsis* lineage. Hence, we transferred them to *Microsphaeropsis*.

Family Didymellaceae Gruyter, Aveskamp & Verkley

Genus *Heteromicrosphaeropsis* M.Mehrabi-Koushki, K.D.Hyde & Jayaward., gen. nov.

Type species. — *Heteromicrosphaeropsis ononidicola* (Thambug., Camporesi & K.D.Hyde) M.Mehrabi-Koushki, K.D.Hyde & Jayaward.

Etymology. — The name refers to *Microsphaeropsis* which is morphologically similar but phylogenetically different.

MycoBank. — MB 841486.

Description

Conidiomata pycnidial, immersed or erumpent, globose to subglobose, solitary or confluent, uni- to bi-loculate, ostiolate. Pycnidial wall of light to dark brown *textura angularis*. Conidiogenous cells phialidic, hyaline, cylindrical, discrete or integrated. Conidia thin- and smooth-walled, hyaline to yellowish brown, aseptate, obovoid to ellipsoidal, straight, and sometimes guttulate.

Heteromicrosphaeropsis ononidicola (Thambug., Camporesi & K.D.Hyde) M.Mehrabi-Koushki, K.D.Hyde & Jayaward., comb. nov.

Microsphaeropsis ononidicola Thambug., Camporesi & K.D.Hyde, *Current Research in Environmental & Applied Mycology* 8: 220 (Thambugala *et al*. 2018).

MycoBank. — MB 841491.

Description

See Thambugala *et al*. (2018).

Notes
Microsphaeropsis ononidicola was introduced with the description of a saprobic strain (MFLUCC 15-0459) isolated from dead aerial stems of *Ononis spinosa* L. (Fabaceae) in Italy (Thambugala *et al*. 2018). In the phylogenetic analyses of the present study (Fig. 1), this species is distant from the *Microsphaeropsis* lineage and other genera in the *Microsphaeropsis* complex. Therefore, a new genus *Heteromicrosphaeropsis* M.Mehrabi-Koushki, K.D.Hyde & Jayaward., gen. nov. is introduced to accommodate this species. Nucleotide comparison of *Heteromicrosphaeropsis* M.Mehrabi-Koushki, K.D.Hyde & Jayaward., gen. nov. with *Microsphaeropsis* species revealed a difference of 0.6% (3/521 bp) in the LSU region, 2.6% (11/431 bp) in the ITS and 11% (22/198 bp) in the *tub*2. However, this new genus is morphologically similar to *Microsphaeropsis* but phylogenetically different.

Genus *Microsphaeropsis* Höhn.

Hedwigia 59: 267 (Höhnel 1917).

Notes
Microsphaeropsis was introduced by Höhnel (1917), with *M. olivacea* (Bonord.) Höhn. as the type species. This genus is characterised by immersed or erumpent, subglobose, solitary or confluent, ostiolate pycnidia with a wall of *textura angularis*; phialidic, hyaline, ampulliform to doliiform or subcylindrical conidiogenous cells and a thin-walled, smooth or finely roughened, 0-1-septate conidia (Chen *et al*. 2015).

Microsphaeropsis cytisi
(W.J.Li & K.D.Hyde)
M.Mehrabi-Koushki, K.D.Hyde & Jayaward., comb. nov.

Neomicrosphaeropsis cytisi W.J.Li & K.D.Hyde, *Fungal Diversity* 80: 38 (Hyde *et al*. 2016).

MycoBank. — MB 841487.

Description
See Hyde *et al*. (2016).

Notes
Hyde *et al*. (2016) introduced *Neomicrosphaeropsis cytisi* for a saprobic fungus isolated from dead stem of *Cytisus* sp. (Fabaceae) in Italy. The analyses of combined LSU, ITS, *tub*2 and *rpb*2 sequence data for the *Microsphaeropsis* complex revealed that the type strain of *Neomicrosphaeropsis cytisi* (MFLUCC 13-0396) clustered within *Microsphaeropsis* lineage (Fig. 1). Therefore, we transfer *Neomicrosphaeropsis cytisi* to *Microsphaeropsis*. Nucleotide comparison of *Microsphaeropsis cytisi* (W.J.Li & K.D.Hyde) M.Mehrabi-Koushki, K.D.Hyde & Jayaward., comb. nov. with *Neomicrosphaeropsis* species showed a difference of 0.6% (3/521 bp) in the LSU region, 3% (13/431 bp) in the ITS and 10.5% (46/438 bp) in the *rpb*2.

Microsphaeropsis cytisicola
(Wanas., Camporesi, E.B.G.Jones & K.D.Hyde)
M.Mehrabi-Koushki, K.D.Hyde & Jayaward., comb. nov.

Neomicrosphaeropsis cytisicola Wanas., Camporesi, E.B.G.Jones & K.D.Hyde, *Studies in Fungi* 3 (1): 169 (Wanasinghe *et al*. 2018).

MycoBank. — MB 841488.

Description
See Wanasinghe e*t al*. (2018).

Notes
Neomicrosphaeropsis cytisicola was firstly isolated from dead aerial branches of *Cytisus* sp. (Fabaceae) in Italy and described by Wanasinghe *et al*. (2018) as a saprobic fungus. According to its position on the phylogenetic tree (Fig. 1) and SNP analysis of four genomic regions, we transfer *Neomicrosphaeropsis cytisicola* to *Microsphaeropsis*. Nucleotide comparison of *Microsphaeropsis cytisicola* (Wanas., Camporesi, E.B.G.Jones & K.D.Hyde) M.Mehrabi-Koushki, K.D.Hyde & Jayaward., comb. nov. with *Neomicrosphaeropsis* species showed a difference of 0.6% (3/521 bp) in the LSU region, 3% (13/431 bp) in the ITS, 13.6% (36/265 bp) in *tub*2 and 10% (44/438 bp) in the *rpb*2.

Microsphaeropsis cytisinus
(Tennakoon, Camporesi & K.D.Hyde)
M.Mehrabi-Koushki, K.D.Hyde & Jayaward., comb. nov.

Neomicrosphaeropsis cytisinus Tennakoon, Camporesi & K.D.Hyde, *Fungal Diversity* 80: 39 (Hyde *et al*. 2016).

MycoBank. — MB 841489.

Description
See Hyde *et al*. (2016).

Notes
Neomicrosphaeropsis cytisinus was originally described from a dead stem of *Cytisus scoparius* L. (Fabaceae) a saprobic fungus in Italy (Hyde *et al*. 2016). According to the phylogenetic analyses in the present study (Fig. 1) and SNP analysis, this species is transferred to *Microsphaeropsis*. Nucleotide comparison of *M. cytisinus* (Tennakoon, Camporesi & K.D.Hyde) M.Mehrabi-Koushki, K.D.Hyde & Jayaward., comb. nov. with *Neomicrosphaeropsis* species showed a difference of 0.6% (3/521 bp) in the LSU region and 3% (13/431 bp) in the ITS.

Microsphaeropsis minima
(W.J.Li & K.D.Hyde)
M.Mehrabi-Koushki, K.D.Hyde & Jayaward., comb. nov.

Neomicrosphaeropsis minima W.J.Li & K.D.Hyde, *Fungal Diversity* 80: 39 (Hyde *et al*. 2016).

MycoBank. — MB 841490.

Description
See Hyde *et al.* (2016).

Notes
Hyde *et al.* (2016) introduced *Neomicrosphaeropsis minima* as a saprobic fungus isolated from dead stems of *Verbascum* sp. (Scrophulariaceae) in Italy. In our analyses (Fig. 1), this species is related to *Microsphaeropsis* and transferred to this genus. SNP analysis of *M. minima* (W.J.Li & K.D.Hyde) M.Mehrabi-Koushki, K.D.Hyde & Jayaward., comb. nov. with *Neomicrosphaeropsis* species showed a difference of 0.6% (3/521 bp) in the LSU region and 3% (13/431 bp) in the ITS.

Genus *Paramicrosphaeropsis* L.W.Hou, L.Cai & Crous

Paramicrosphaeropsis amygdalus
M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov.
(Fig. 2)

Holotype. — **Iran**. Khuzestan Province, Dezful, Shahiyoon (forest mountains of Segeryoo), from stem canker of *Amygdalus scoparia*, I.2019, S. Artand (holo-, IRAN[18146F]; ex-type living culture, IRAN[4451C] = SCUA-Ar-KS3-3).

Additional specimens examined. — **Iran**. Khuzestan Province, Dezful, Sardasht (forest mountains of Saland-Kooh), from stem canker of *Amygdalus scoparia*, III.2021, S. Artand (IRAN[4452C] = SCUA-Ar-SB2A); from leaf spot of an unknown tree, III.2021, S. Artand (SCUA-Ar-S6A).

Etymology. — The name refers to the host genus *Amygdalus* L. from which it was isolated.

MycoBank. — MB 841492.

Description
Asexual morphology
Pycnidia scattered and irregular, solitary or aggregated, superficial on the medium or in aerial mycelium, globose to subglobose, sometime with a short neck, covered with hyphal outgrowths, usually inconspicuous ostiole, sometime with a conspicuous ostiole, slightly papillate or non-papillate, brown to dark brown with a paler wall, (107.2-)159.6-186.6(-268) × (102.2-)144.1-166.4(-241.2) µm, (x ± SD = 173.7 ± 6.7 × 155.6 ± 5.7 µm, n = 50). Pycnidial wall pseudoparenchymatous, composed of isodiametric to elongated cells, 3-6 layers, pale brown to brown, outer layers darker. Conidiogenous cells phialidic, hyaline, smooth-walled, discrete, ampulliform or doliiform. Conidia mostly ovoid to ellipsoidal but also allantoid, or irregular in shape, pale brown to brown, straight or curved, smooth- and thin-walled, guttulate, aseptate, (3.7-)5.9-6.9(-10.5) × (3.2-)4.0-4.3(-5.8) µm, (x ± SD = 6.3 ± 0.3 × 4.2 ± 0.1 µm, n = 50). Chlamydospores and swelling cells not observed.

Sexual morphology
Not observed.

Culture characteristics
Colonies on OA attaining 28-29 mm diam after eight days of incubation at 25 ± 0.5°C and 3-3.5 mm diam at 30 ± 0.5°C, circular with regular margin, floccose, initially white to pinkish white, with age becoming brownish grey with pale brown centre and hyaline border, pycnidia scattered but most abundant in the centre, reverse, dark brown with brown centre and hyaline border. Colonies on PDA attaining 29-30 mm diam after eight days of incubation at 25 ± 0.5°C and 3-3.5 mm diam at 30 ± 0.5°C, circular with regular margin, floccose, initially pale brown with dark centre, with age becoming brown to dark brown with a cottony white centre; reverse dark brown to black with paler centre.

Notes
In the phylogenetic tree (Fig. 1), *Paramicrosphaeropsis amygdalus* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. strains grouped together in a strongly supported monophyletic clade (MLBS 99%, MPBS 100%, BPP 1.0), closely related to the species *P. iranica* and *P. pistacicola* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. Single nucleotide polymorphisms (SNP) analysis of three regions showed that *P. amygdalus* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. and *P. iranica* (*P. pistacicola* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov.) had 1 (2) base pair difference across 431 nucleotides of the ITS region (0.25% and 0.5%), 4 (6) different base pairs across 265 nucleotides of the *tub*2 region (1.5% and 2.3%), and a difference of 12 (8) base pairs across 439 nucleotides of the *rpb*2 region (2.7% and 1.8%), respectively. *Paramicrosphaeropsis amygdalus* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. is closely related to *P. pistacicola* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. phylogenetically, but it can be distinguished morphologically. *Paramicrosphaeropsis amygdalus* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. differs from *P. pistacicola* in having larger pycnidia ($\bar{x}$ = 173.7 × 155.6 µm versus $\bar{x}$ = 120.5 × 97.6 µm) with a short neck in some of them, while *P. pistacicola* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. has smaller pycnida lacking neck. Pycnidial wall in *P. amygdalus* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. is composed of pseudoparenchymatous tissues which completely enclosed conidiomata, while pycnidial wall of *P. pistacicola* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. is mostly prosenchymatous.

Paramicrosphaeropsis pistacicola
M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov.
(Fig. 3)

Holotype. — **Iran**. Khuzestan Province, Dezful, Sardasht (forest mountains of Saland-Kooh), from leaf spot of *Pistacia khinjuk*, III.2021, S. Artand (holo-, IRAN[18145F]; ex-type cultures, IRAN[4449C] = SCUA-Ar-SK11A).

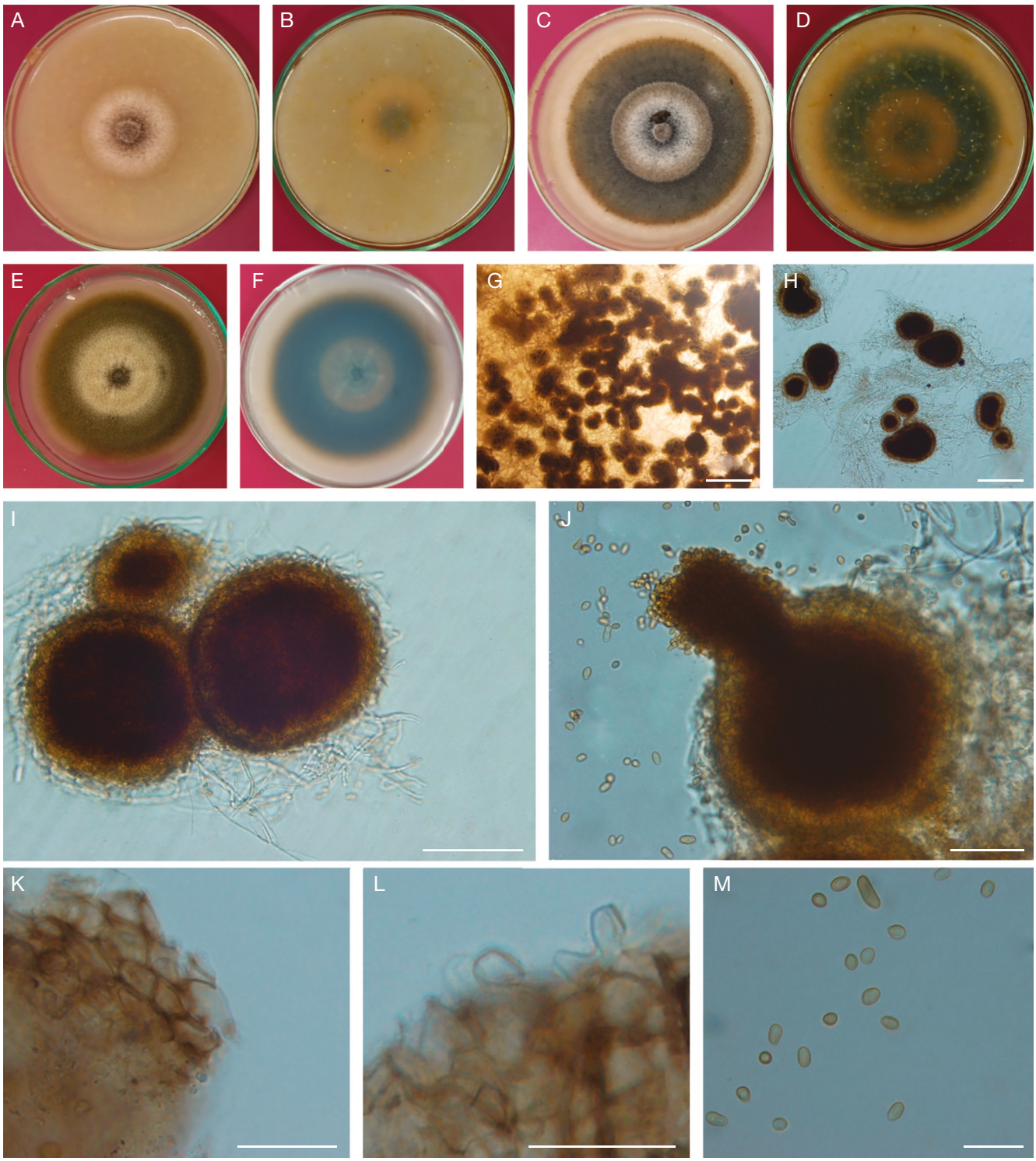

FIG. 2. — *Paramicrosphaeropsis amygdalus* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. (holo-, IRAN[17595F]): **A**, **B**, eight-days colony on OA (top and reverse); **C**, **D**, old colony on OA (top and reverse); **E**, **F**, old colony on PDA (top and reverse); **G-I**, pycnidia; **J**, pycnidium extruding conidia; **K**, pycnidal wall; **L**, conidiogenous cells; **M**, conidia. Scale bars: G, 500 µm; H, 200 µm; I, 105 µm; J, 50 µm; K-M, 20 µm.

ADDITIONAL SPECIMEN EXAMINED. — **Iran**. Khuzestan Province, Dezful, Sardasht (forest mountains of Saland-Kooh), from leaf spot of *Pistacia atlantica*, III.2021, S. Artand (IRAN[4450C] = SCUA-Ar-SK1D).

ETYMOLOGY. — The name refers to the host genus *Pistacia* L. from which it was isolated.

MYCOBANK. — MB 841493.

Description

Asexual morphology

Pycnidia scattered and irregular, mostly solitary, sometimes confluent, superficial on the medium or in aerial mycelium, globose to subglobose, occasionally flask-shaped, glabrous or covered with some hyphal outgrowths, with inconspicuous ostiole, rarely with a conspicuous ostiole, brown to dark brown with a paler wall, (78-)113.2-127(-208) × (53.6-)91.3-103.9 (-187.6) µm, (x ± SD = 120.5 ± 3.5 × 97.6 ± 3.1 µm, n = 50). Pycnidial wall prosenchymatous and pseudoparenchymatous, composed of branched hyphae and elongated cells, 3-12 layers, sometimes wall layers do not completely enclose conidiomata and the conidia can be seen through hyphal network, pale brown to brown. Conidiogenous cells phialidic, hyaline, smooth-walled, discrete, globose to subglobose. Conidia mostly subglobose to ellipsoidal but also ovoid, obpyriform or irregular in shape, pale brown to brown, straight or slightly curved, smooth- and thin-walled, guttulate, aseptate, (4.2-)5.5-6.2 (-8.4) × (3.2-)3.9-4.2(-5.3) µm, (x ± SD = 5.8 ± 0.2 × 4.1 ± 0.1 µm, n = 50). Chlamydospores and swelling cells not observed.

Sexual morphology

Not observed.

Culture characteristics

Colonies on OA attaining 30-33 mm diam after eight days of incubation at 25 ± 0.5°C and 4-4.4 mm diam at 30 ± 0.5°C, circular with regular margin, floccose, pinkish white with hyaline margin, abundant pycnidia scattered on the agar and in aerial mycelium; reverse similar. Colonies on PDA attaining 17-19 mm diam after eight days of incubation at 25 ± 0.5°C and 2.5-3 mm diam at 30 ± 0.5°C, circular with regular margin, floccose, pinkish grey, pycnidia scattered on the agar and in aerial mycelium; reverse similar.

Notes

In the phylogenetic tree (Fig. 1), both strains of *Paramicrosphaeropsis pistacicola* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. formed a well-separated monophyletic clade (MLBS 100%, MPBS 98%, BPP 1.0), closely related to the species *P. iranica*.

Paramicrosphaeropsis salandica M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov.

(Fig. 4)

Holotype. — **Iran**. Khuzestan Province, Dezful, Sardasht (forest mountains of Saland-Kooh), from fruit rot of *Crataegus* sp., III.2021, S. Artand (holo-, IRAN[18137F]; ex-type cultures, IRAN[4376C] = SCUA-Ar-SZ8C2).

Additional specimens examined. — **Iran**. Khuzestan Province, Dezful, Sardasht (forest mountains of Saland-kooh), from leaf spot of *Quercus brantii*, III.2021, S. Artand (IRAN[4453C] = SCUA-Ar-SB5A); from leaf spot of *Ziziphus* sp., III.2021, S. Artand (IRAN[4456C] = SCUA-Ar-S4D1 and IRAN[4454C] = SCUA-Ar-S4D2); from leaf spot of *Nerium oleander*, III.2021, S. Artand (IRAN[4455C] = SCUA-Ar-SKH13B); from leaf spot of an unknown shrub., III.2021, S. Artand (SCUA-Ar-S9C2).

Etymology. — The name refers to the Saland-Kooh Mountains, where this fungus was collected.

MycoBank. — MB 841494.

Description

Asexual morphology

Pycnidia scattered and irregular, solitary or confluent, superficial on the medium or in aerial mycelium, globose to subglobose, covered with hyphal outgrowths, with inconspicuous ostiole, brown to dark brown with a paler wall, (107.2-)167.9-198.5 (-321.6) × (93.8-)153-174.9(-268) µm, (x ± SD = 184.4 ± 7.5 × 163.9 ± 5.6 µm, n = 50). Pycnidial wall pseudoparenchymatous, composed of isodiametric to elongated cells, 3-6 layers, pale brown to brown, outer layers darker. Conidiogenous cells phialidic, hyaline, smooth-walled, discrete, ampulliform or doliiform. Conidia mostly ovoid to obpyriform but also irregular in shape, pale brown to brown, straight or sometime very slightly curved, smooth- and thin-walled, guttulate, aseptate, (4.7-)5.9-6.5(-9.5) × (3.2-)4.6-5.2(-6.8) µm, (x ± SD = 6.2 ± 0.1 × 4.9 ± 0.1 µm, n = 50). Chlamydospores and swelling cells not observed.

Sexual morphology

Not observed.

Culture characteristics

Colonies on OA attaining 33-34 mm diam after eight days of incubation at 25 ± 0.5°C and 7-7.5 mm diam at 30 ± 0.5°C, circular with regular margin, floccose, initially white with pale brown centre, with age becoming dark olive-green with grayish aerial mycelium, pycnidia scattered on the agar and in aerial mycelium; reverse greenish black with hyaline border. Colonies on PDA attaining 32-33 mm diam after eight days of incubation at 25 ± 0.5°C and 5-5.5 mm diam at 30 ± 0.5°C, circular with regular margin, floccose, initially dark olive-green with grayish aerial mycelium, with age becoming brown with light green aerial mycelium and a dark brown pigmentation in the centre; reverse greenish black with brown border.

Notes

In the phylogenetic tree (Fig. 1), *Paramicrosphaeropsis salandica* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. strains are grouped together in a well-supported monophyletic clade (MLBS 99%, MPBS 99%, BPP 1.0), distinct from other *Paramicrosphaeropsis* species. A comparison of nucleotides of *P. salandica* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. with *P. amygdalus* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov., *P. ellipsoidea P. iranica*, *P. pistacicola* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. and *P. zagrosensis* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. revealed 2, 2, 1, 0 and 0 base pair dif-

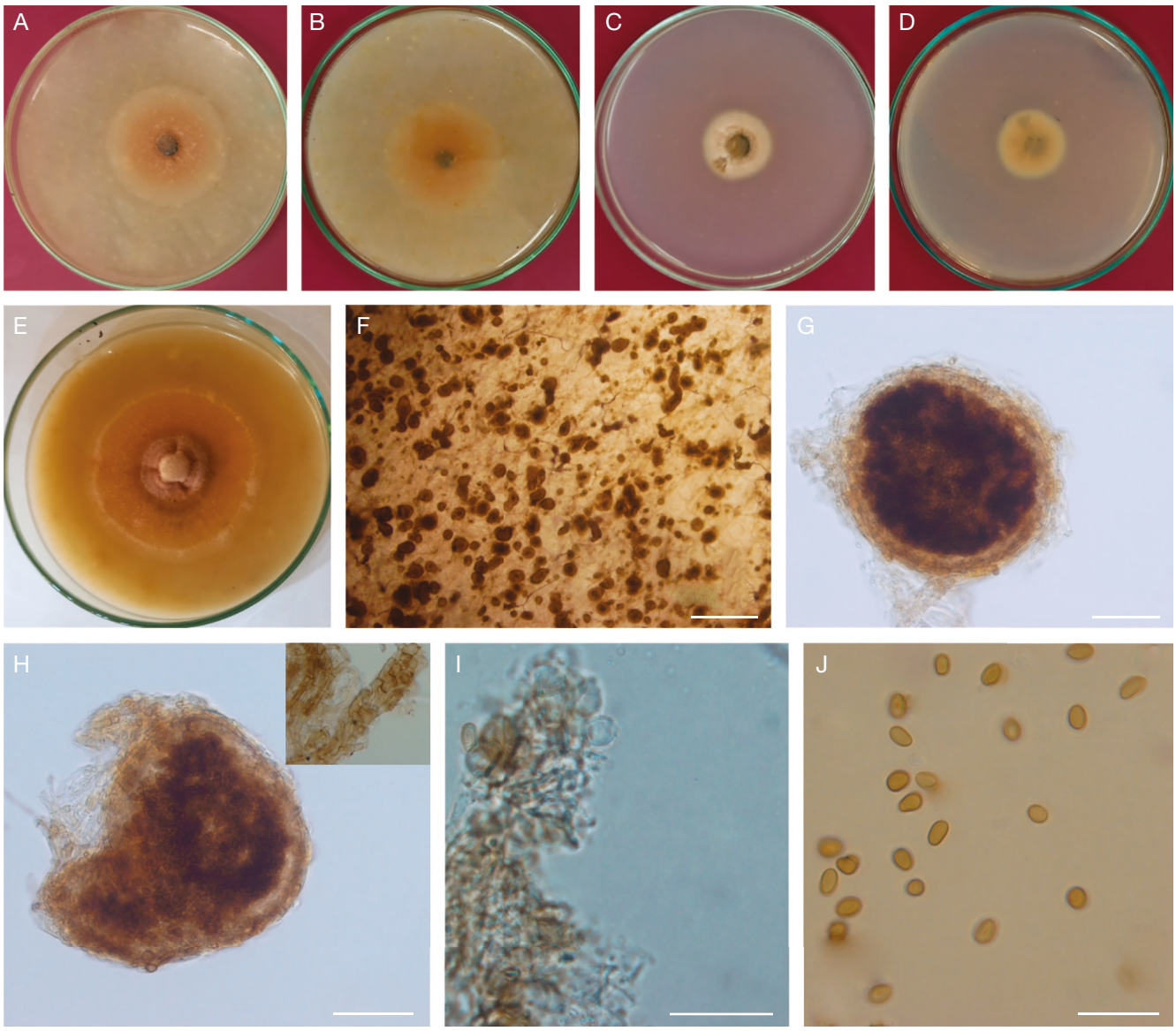

Fig. 3. — *Paramicrosphaeropsis pistacicola* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. (holo-, IRAN[17598F]): **A**, **B**, eight-days colony on OA (top and reverse); **C**, **D**, eight-days colony on PDA (top and reverse); **E**, old colony on OA (top); **F**, **G**, pycnidia; **H**, pycnidal wall; **I**, conidiogenous cells; **J**, conidia. Scale bars: F, 500 µm; G, H, 50 µm; I, J, 20 µm.

ference across 431 nucleotides of the ITS region (0.5, 0.5, 0.25, 0 and 0%), 10, 7, 10, 12 and 12 base pair difference across 265 nucleotides of the *tub*2 region (3.8, 2.6, 3.8, 4.5 and 4.5%) and 8, 7, 8, 6 and 12 base pair difference across 439 nucleotides of the *rpb*2 region (1.8, 1.6, 1.8, 1.4 and 2.7%), respectively. *Paramicrosphaeropsis salandica* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. differs from *P. zagrosensis* in having somewhat larger pycnidia (167.9-198.5 × 153-174.9 µm versus 128.3-147.5 × 119.3-135.7 µm) and conidia (5.9-6.5 × 4.6-5.2 µm versus 5.4-6.0 × 3.8-4.1 µm), and from *P. ellipsoidea* in having smaller pycnidia (167.9-198.5 × 153-174.9 µm versus 150-490 × 110-440 µm) and conidia (5.9-6.5 × 4.6-5.2 µm versus 5-11 × 3.5-6.5 µm).

Paramicrosphaeropsis zagrosensis M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. (Fig. 5)

Holotype. — **Iran**. Lorestan Province, Aligudarz, Zaz and Mahroo (forest mountains of Moolish), from leaf spot of *Quercus brantii*, IX.2020, S. Artand (holo-, IRAN[18144F]; ex-type cultures, IRAN[4448C] = SCUA-Ar-B10A).

Additional specimen examined. — **Iran**. Lorestan Province, Aligudarz, Zaz and Mahroo (forest mountains of Moolish), from stem canker of *Crataegus* sp., IX.2020, S. Artand (SCUA-Ar-Z1B).

Etymology. — The name refers to the Zagros forest, where this fungus was collected.

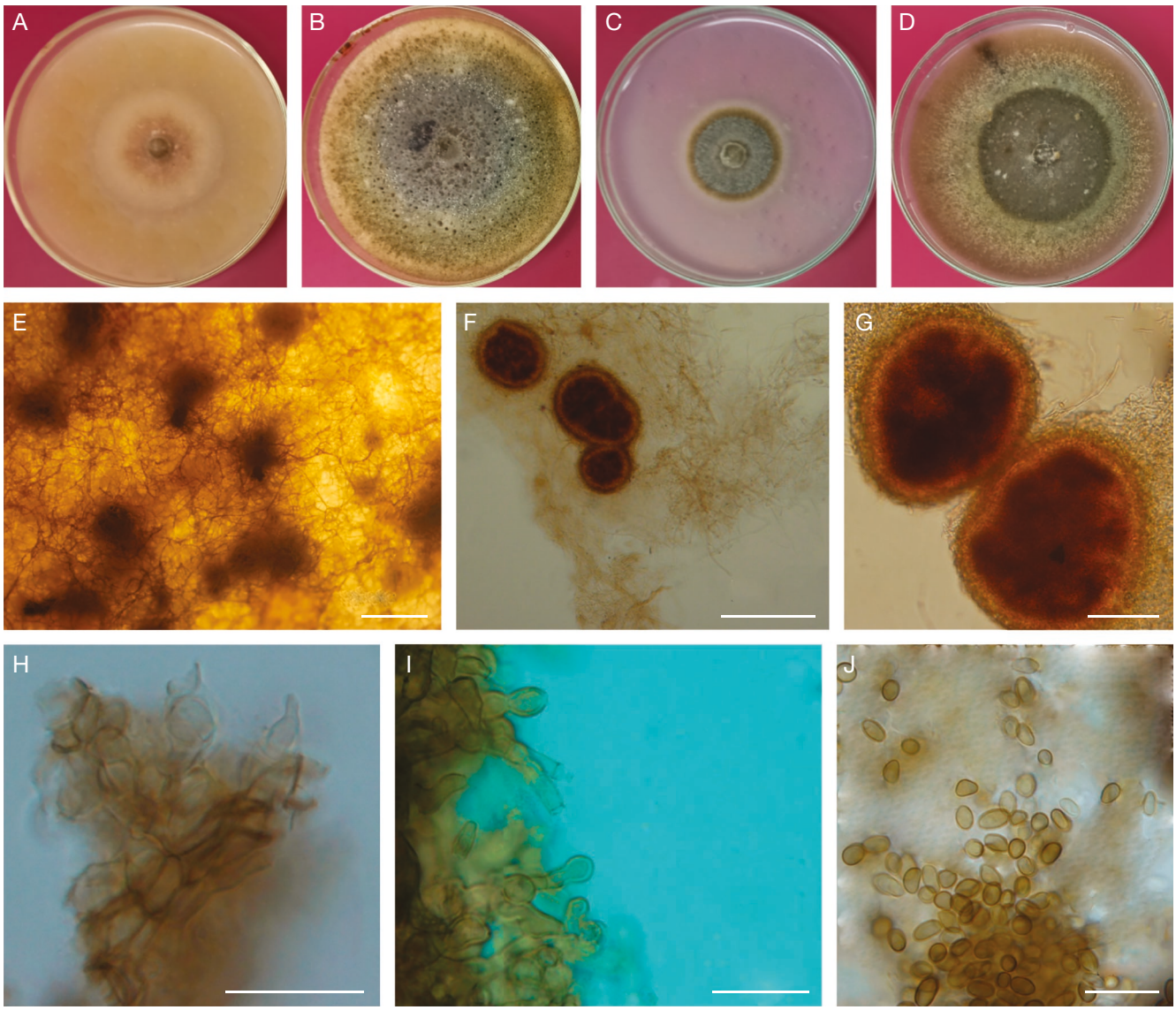

Fig. 4. — *Paramicrosphaeropsis salandica* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. (holo-, IRAN[18137F]): **A**, **B**, colony on OA (eight-days and old); **C**, **D**, colony on PDA (eight-days and old); **E-G** pycnidia; **H**, pycnidal wall; **I**, conidiogenous cells; **J**, conidia. Scale bars: E, 500 µm; F, 200 µm; G, 105 µm; H-J, 20 µm.

MycoBank. — MB 841495.

Description

Asexual morphology

Pycnidia scattered and irregular, solitary or confluent, superficial on the medium or in aerial mycelium, sometime semi-immersed in the agar, globose to subglobose, covered with hyphal outgrowths, with inconspicuous ostiole, brown to dark brown with a paler wall, (80.4-)128.3-147.5(-214.4) × (78-)119.3-135.7(-205) µm, (x ± SD = 138.4 ± 4.8 × 128 ± 4.2 µm, n = 50). Pycnidial wall pseudoparenchymatous, composed of isodiametric to elongated cells, 3-5 layers, pale brown to brown, outer layers darker and pigmented. Conidiogenous cells phialidic, hyaline, smooth-walled, discrete, subglobose or bottle-shaped. Conidia mostly globose to subglobose but also broadly ellipsoidal, ovate-ellipsoidal or irregular in shape, pale brown to brown, straight or sometimes very slightly curved, smooth- and thin-walled, guttulate, aseptate, (3.2-)5.4-6.0(-7.4) × (2.6-)3.8-4.1(-5.3) µm, (x ± SD = 5.7 ± 0.2 × 4.0 ± 0.1 µm, n = 50). Chlamydospores and swelling cells not observed.

Sexual morphology

Not observed.

Culture characteristics

Colonies on OA attaining 23-25 mm diam after eight days of incubation at 25 ± 0.5°C and 2-2.5 mm diam at 30 ± 0.5°C, circular with regular margin, floccose, hyaline to pinkish white, with age forming abundant pycnidia, pycnidia scattered but most numerous in the centre; reverse pinkish white to pinkish brown. Colonies on PDA

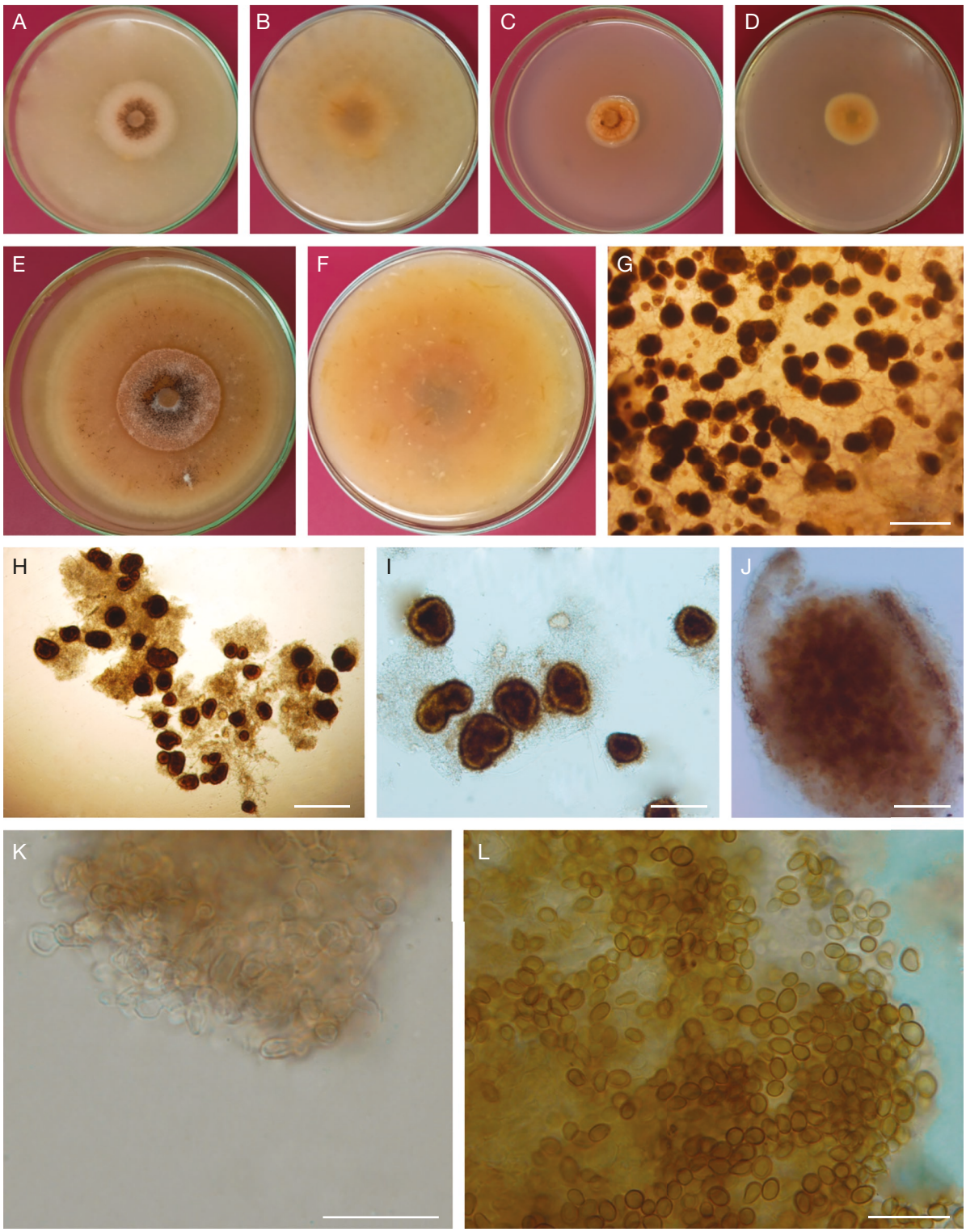

Fig. 5. — *Paramicrosphaeropsis zagrosensis* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. (holo-, IRAN[17600F]): **A**, **B**, eight-days colony on OA (top and reverse); **C**, **D**, eight-days colony on PDA (top and reverse); **E**, **F**, old colony on OA (top and reverse); **G-I**, pycnidia; **J**, pycnidal wall; **K**, conidiogenous cells; **L**, conidia. Scale bars: G, H, 500 µm; I, 200 µm; J, 50 µm; K, L, 20 µm.

attaining 15-17 mm diam after eight days of incubation at 25 ± 0.5°C and 1-1.2 mm diam at 30 ± 0.5°C, circular with entire margin, floccose, pale brown with hyaline border; reverse similar.

Notes

In the phylogenetic tree (Fig. 1), both strains of *Paramicrosphaeropsis zagrosensis* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. formed a highly supported

clade (MLBS 99%, MPBS 99%, BPP 1.0), closely related to the previously known species *P. ellipsoidea*. SNP analysis of three regions showed *P. zagrosensis* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. and the type strain of *P. ellipsoidea* (CBS 194.97) had 2 base pair differences (0.5%) across 431 nucleotides of the ITS region, 9 different base pairs (3.4%) across 265 nucleotides of the *tub*2 region, and a difference of 7 base pairs (1.6%) across 439 nucleotides of the *rpb*2 region. Phylogenetically, *Paramicrosphaeropsis zagrosensis* M.Mehrabi-Koushki, S.Artand, K.D.Hyde & Jayaward., sp. nov. is closely related to *P. ellipsoidea* (Hou *et al*. 2020), but it can easily be distinguished by smaller pycnidia (128.3-147.5 × 119.3-135.7 µm versus 150-490 × 110-440 µm) and conidia (5.4-6.0 × 3.8-4.1 µm versus 5-11 × 3.5-6.5 µm).

DISCUSSION

In this study, multilocus phylogenetic analyses combined with morphology revealed four new species of *Paramicrosphaeropsis* in the *Microsphaeropsis* complex within the Didymellaceae. The phylogeny based on combined LSU, ITS, *tub*2 and *rpb*2 sequence data provided sufficient resolution to delimit most species in this complex, as observed in previous studies for the members of the family Didymellaceae (Chen *et al*. 2015, 2017; Valenzuela-Lopez *et al*. 2018; Jayasiri *et al*. 2019; Hou *et al*. 2020). Based on single-locus trees (not shown), the *tub*2 and *rpb*2 were the best loci for delimitation and identification of the species in the *Microsphaeropsis* complex. Although the ITS region is the main fungal barcode it did not provide sufficient resolution to distinguish the species. In a SNP analysis of four genomic regions, the members of this complex shared 98.7% sequence identity in the LSU region (D1/D2, 521 bp) attributed to 7 bp SNPs, 94.9% sequence identity in the ITS (431 bp) attributed to 19 SNPs and 3 bp insertion/deletion, 74.4% sequence identity in the *tub*2 gene (265 bp) attributed to 67 SNPs and 1 bp insertion/ deletion, and 74.3% sequence identity in the *rpb*2 gene (439 bp). However, the combined use of *tub*2 and *rpb*2 regions proved to work well in distinguishing most species in this generic complex.

Up to date, four defined genera have been phylogenetically classified in the *Microsphaeropsis* complex, including *Microsphaeropsis*, *Neomicrosphaeropsis*, *Nothomicrosphaeropsis* and *Paramicrosphaeropsis* (Chen *et al*. 2015; Hyde *et al*. 2016; Thambugala *et al*. 2017, 2018; Wanasinghe *et al*. 2018; Hou *et al*. 2020; Pem *et al*. 2020; Crous *et al*. 2021). For them, there are 64 recorded epithets (*Microsphaeropsis*: 51; *Neomicrosphaeropsis*: 10; *Nothomicrosphaeropsis*: 1; *Paramicrosphaeropsis*: 2) in Index Fungorum (http://http://www.speciesfungorum.org). Some of these species have not yet been phylogenetically confirmed in these genera and key loci of their representative strains need to be sequenced and analyzed to determine their taxonomic placement within the Microsphaeropsis complex.

Paramicrosphaeropsis species were reported from the decayed twig of *Quercus ilex* (Fagaceae) in Spain and stem canker of *Q. brantii* in Iran (Hou *et al*. 2020; Ahmadpour *et al*. 2022). Up to now, no other species of *Microsphaeropsis* complex have been reported from these hosts worldwide, with the exception of *Microsphaeropsis olivacea* on *Quercus brantii* (Alidadi *et al*. 2019) in Iran. *Microsphaeropsis* species are found on a wide range of hosts with different geographic distributions, including some plant genera in Amaranthaceae, Apiaceae, Araliaceae, Arecaceae, Asparagaceae, Asteraceae, Bignoniaceae, Bromeliaceae, Caprifoliaceae, Celastraceae, Fabaceae, Melastomataceae, Myrtaceae, Oleaceae, Poaceae, Proteaceae, Ranunculaceae, Rosaceae, Rubiaceae, Verbenaceae, and Zosteraceae (Farr & Rossman 2021). Species of *Microsphaeropsis* are mostly known as endophytes, saprobes, and pathogens of terrestrial plants (Alfieri *et al*. 1984; Sun *et al*. 2007; Pethybridge *et al*. 2008; Espargham *et al*. 2020), while some species are also reported to be opportunistic human pathogens (Guarro *et al*. 1999; Reppas *et al*. 2015).

Species of *Neomicrosphaeropsis* have been so far isolated from several plant hosts including *Alhagi pseudalhagi* (Bieb.) Desv. (Fabaceae), *Cytisus* spp. (Fabaceae), *Elaeagnus angustifolia* L. (Elaeagnaceae), *Juglans regia* L. (Juglandaceae), *Tamarix* spp. (Tamaricaceae), and *Verbascum* sp. (Scrophulariaceae) (Hyde *et al*. 2016; Chen *et al*. 2017; Jiang *et al*. 2017; Wijayawardene *et al*. 2017; Thambugala *et al*. 2017; Valenzuela-Lopez *et al*. 2018; Wanasinghe *et al*. 2018; Pem *et al*. 2020). They are known as endophytes or pathogens (Wijayawardene *et al*. 2017). However, host-fungus records for *Microsphaeropsis* and *Neomicrosphaeropsis* species are of uncertain validity because of these two genera are known to be polyphyletic and a DNA sequence-based phylogenetic analyses of both genera including all strains deposited in international fungal culture collections should be performed to gain a more realistic understanding of the geographical distribution and host range of the species.

Several species of the *Microsphaeropsis* complex have been previously reported on herbaceous and woody plants from different areas of Iran, including *Microsphaeropsis arundinis* (S. Ahmad) B. Sutton on *Hypericum perforatum* L.; *Microsphaeropsis olivacea* on *Alhagi maurorum* Medik., *Amygdalus scoparia*, *Citrus* spp., *Pinus* spp., *Prunus cerasus* L., *P. avium* L., *Quercus brantii*, *Vitis vinifera* L., and *Robinia* sp.; *Microsphaeropsis proteae* on *Pinus* sp.; and *Neomicrosphaeropsis* sp. on *Prunus armeniaca* L. and *Robinia pseudoacacia* L. (Abdollahi Aghdam & Fotouhifar 2016; Razaghi & Zafari 2016; Ghobad-Nejhad *et al*. 2018; Alidadi *et al*. 2019; Shamsi *et al*. 2019; Espargham *et al*. 2020; Bahmani *et al*. 2021; Goodarzian *et al*. 2021).

In this study, five new taxa and five new combinations are proposed in *Microsphaeropsis* complex. Present and previous studies showed that this generic complex is polyphyletic and may represent more cryptic genera, which are morphologically similar, but phylogenetically different (Hyde *et al*. 2013, 2016; Ariyawansa *et al*. 2014; Phookamsak *et al*. 2014; Wijayawardene *et al*. 2014, 2016, 2018; Chen *et al*. 2015; Wijayawardene *et al*. 2016; Thambugala *et al*. 2017). Many *Microsphaeropsis* species recorded in Index Fungorum do not have DNA sequence data in GenBank and therefore they have not been phylogenetically confirmed in *Microsphaeropsis* and related to Didymellaceae. Molecular data and more

representative cultures of *Microsphaeropsis* and allied taxa are needed to resolve the systematics of these genera and their species within Didymellaceae.

Acknowledgements

This work was financially supported by grant (SCU.AP99.294) from Research Council of Shahid Chamran University of Ahvaz. The authors are thankful to anonymous reviewers for the beneficial comments and suggestions on the manuscript.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

Abdollahi Aghdam S. & Fotouhifar K. B. 2016. — New reports of endophytic fungi associated with cherry (*Prunus avium*) and sour cherry (*Prunus cerasus*) trees in Iran. *Mycologica Iranica* 3 (2): 75-85. https://doi.org/10.22043/mi.2017.112557

Ahmadpour S. A., Mehrabi-Koushki M. & Farokhinejad R. 2017. — *Neodidymelliopsis farokhinejadii*, a new fungal species from dead branches of trees in Iran. *Sydowia* 69: 171-182. https://doi.org/10.12905/0380.sydowia69-2017-0171

Ahmadpour S. A., Mehrabi-Koushki M., Farokhinejad R. & Asgari B. 2022. — New species of the family Didymellaceae in Iran. *Mycological Progress* 21: 28. https://doi.org/10.1007/s11557-022-01800-5

Alfieri Jr. S. A., Wehlburg C., Langdon K. R. & Kimbrough J. W. 1984. — *Index of Plant Diseases in Florida*. Bulletin 11, Division of Plant Industry, Gainsville, FL, USA: Florida Department of Agriculture and Consumer Services.

Alidadi A., Kowsari M., Javan-Nikkhah M., Salehi Jouzani G. R. & Ebrahimi Rastaghi M. 2019. — New pathogenic and endophytic fungal species associated with Persian oak in Iran. *Europian Journal of Plant Pathology* 155: 1017-1032.

Ariyawansa H. A., Tanaka K., Thambugala K. M., Phookamsak R., Phookamsak R., Tian Q., Camporesi E., Hongsanan S., Monkai J., Wanasinghe D. N., Mapook A. & Chukeatirote E. 2014. — A molecular phylogenetic reappraisal of the Didymosphaeriaceae (= Montagnulaceae). *Fungal Diversity* 68: 69-104.

Aveskamp M. M., de Gruyter J., Woudenberg J. H. C., Verkley G. J. M. & Crous P. W. 2010. — Highlights of the Didymellaceae: a polyphasic approach to characterise *Phoma* and related pleosporalean genera. *Studies in Mycology* 65 (1): 1-60. https://doi.org/10.3114/sim.2010.65.01

Bahmani Z., Abdollahzadeh J., Amini J. & Evidente A. 2021. — *Biscogniauxia rosacearum* the charcoal canker agent as a pathogen associated with grapevine trunk diseases in Zagros region of Iran. *Scientific Reports* 11: 14098. https://doi.org/10.1038/s41598-021-93630-w

Barr M. E. 1987. — *Prodromus* to class Loculoascomycetes. University of Massachusetts, Massachusetts, 168 p.

Chen Q., Jiang J. R., Zhang G. Z., Cai L. & Crous P. W. 2015. — Resolving the *Phoma* Enigma. *Studies in Mycology* 82: 137-217.

Chen Q., Hou L. W., Duan W. J., Crous P. W. & Cai L. 2017. — Didymellaceae revisited. *Studies in Mycology* 87: 105-159.

Crous P. W., Hernández-Restrepo M., Schumacher R. K., Cowan D. A., Maggs-Kölling G., Marais E., Wingfield M. J., Yilmaz N., Adan O. C. G., Akulov A., Álvarez Duarte E., Berraf-Tebbal A., Bulgakov T. S., Carnegie A. J., de Beer Z. W., Decock C., Dijksterhuis J., Duong T. A., Eichmeier A., Hien L. T., Houbraken J. A. M. P., Khanh T. N., Liem N. V., Lombard L., Lutzoni F. M., Miadlikowska J. M., Nel W. J., Pascoe I. G., Roets F., Roux J., Samson R. A., Shen M., Spetik M., Thangavel R., Thanh H. M., Thao L. D., van Nieuwenhuijzen E. J., Zhang J. Q., Zhang Y., Zhao L. L. & Groenewald J. Z. 2021. — New and Interesting Fungi. 4. *Fungal Systematics and Evolution* 7: 255-343.

Darriba D., Taboada G. L., Doallo R. & Posada D. 2012. — jModelTest 2: more models, new heuristics and parallel computing. *Natural Methods* 9: 772. https://doi.org/10.1038/nmeth.2109

de Gruyter J., Aveskamp M. M., Woudenberg J. H. C., Verkley G. J. M., Groenewald J. Z. & Crous P. W. 2009. — Molecular phylogeny of *Phoma* and allied anamorph genera: towards a reclassification of the *Phoma* complex. *Mycological Research* 113: 508-519.

de Gruyter J., Woudenberg J. H. C., Aveskamp M. M., Verkley G. J. M., Groenewald J. Z. & Crous P. W. 2013. — Redisposition of phoma-like anamorphs in Pleosporales. *Studies in Mycology* 75: 1-36.

Edler D., Klein J., Antonelli A. & Silvestro D. 2020. — raxmlGUI 2.0 beta: a graphical interface and toolkit for phylogenetic analyses using RAxML. *Methods in Ecology and Evolution* 12 (2): 373-377. https://doi.org/10.1111/2041-210X.13512

Ershad D. 2002. — A new *Cercospora* species from Iran. *Rostaniha* 3 (1): 15-17.

Ershad D. 2009. — *Fungi of Iran*. Third edition. Iranian Research Institute of Plant Protection, Tehran, 528 p.

Espargham N., Mohammadi H. & Gramaje D. 2020. — A Survey of trunk disease pathogens within *Citrus* Trees in Iran. *Plants* 9 (6): 754. https://doi.org/10.3390/plants9060754

Farr D. F. & Rossman A. Y. 2021. — Fungal Databases, U.S. National Fungus Collections, ARS, USDA. Available from https://nt.ars-grin.gov/fungaldatabases (accessed on 17 June 2021).

Fotouhifar K. B., Hedjaroude G. A. & Leuchtmann A. 2010. — ITS rDNA phylogeny of Iranian strains of *Cytospora* and associated teleomorphs. *Mycologia* 102 (6): 1369-1382. https://doi.org/10.3852/10-034

Ghobad-Nejhad M., Meyn R. & Langer E. 2018. — Endophytic fungi isolated from healthy and declining Persian oak (*Quercus brantii*) in western Iran. *Nova Hedwigia* 107 (3-4): 273-290. https://doi.org/10.1127/nova_hedwigia/2018/0470

Goodarzian K., Tajick Ghanbary M. A., Babaeizad V. & Mojerlou S. 2021. — Identification of root endophytic fungi from rangeland plants in Mazandaran province. *Iranian Journal of Forest and Range Protection Research* 18 (2): 216-232. https://doi.org/10.22092/ijfrpr.2020.351371.1434

Guarro J., Mayayo E., Tapiol J., Aguilar C. & Cano J. 1999. — *Microsphaeropsis olivacea* as an etiological agent of human skin infection. *Medical Mycology* 37 (2): 133-137. https://doi.org/10.1080/02681219980000211

Hall T. A. 1999. — BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95-98.

Höhnel F. von 1917. — Fungi imperfecti. Beiträge zur Kenntnis derselben. *Hedwigia* 59: 236-284.

Hou L. W., Groenewald J. Z., Pfenning L. H., Yarden O., Crous P. W. & Cai L. 2020. — The phoma-like dilemma. *Studies in Mycology* 96: 309-396.

Hyde K. D., Jones E. B. G., Liu J. K., Ariyawansa H. A., Boehm E., Boonmee S., Braun U., Chomnunti P., Crous P. W., Dai D. Q., Diederich P., Dissanayake A., Doilom M., Doveri F., Hongsanan S., Jayawardena R., Lawrey J. D., Li Y. M., Liu Y. X., Lücking R., Monkai J., Muggia L., Nelsen M. P., Pang K. L., Phookamsak R., Senanayake I. C., Shearer C. A., Suetrong S., Tanaka K., Thambugala K. M., Wijayawardene N. N., Wikee S., Wu H. X., Zhang Y.,

Aguirre-Hudson B., Alias S. A., Aptroot A., Bahkali A. H., Bezerra J. L., Bhat D. J., Camporesi E., Chukeatirote E., Gueidan C., Hawksworth D. L., Hirayama K., De Hoog S., Kan J. C., Knudsen K., Li W. J., Li X. H., Liu Z. Y., Mapook A., McKenzie E. H. C., Miller A. N., Mortimer P. E., Phillips A. J. L., Raja H. A., Scheuer C., Schumm F., Taylor J. E., Tian Q., Tibpromma S., Wanasinghe D. N., Wang Y., Xu J. C., Yacharoen S., Yan J. Y. & Zhang M. 2013. — Families of Dothideomycetes. *Fungal Diversity* 63: 1-313.

Hyde K. D., Hongsanan S., Jeewon R., Bhat D. J., McKenzie E. H. C., Jones E. B. G., Phookamsak R., Ariyawansa H. A., Boonmee S., Zhao Q., Richter C., Thongbai B., Stadler M. & Zhu L. 2016. — Fungal diversity notes 367-490: taxonomic and phylogenetic contributions to fungal taxa. *Fungal Diversity* 80: 1-270.

Jayasiri S. C., Hyde K. D., Jones E. B. G., McKenzie E. H. C., Jeewon R., Phillips A. J. L., Bhat D. J., Wanasinghe D. N., Liu J. K., Lu Y. Z., Kang J. C., Xu J. & Karunarathna S. C. 2019. — Diversity, morphology and molecular phylogeny of Dothideomycetes on decaying wild seed pods and fruits. *Mycosphere* 10: 1-186.

Jiang J. R., Chen Q. & Cai L. 2017 — Polyphasic characterisation of three novel species of *Paraboeremia*. *Mycological Progress* 16: 285-295.

Kumar S., Stecher G. & Tamura K. 2016. — MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33 (7): 1870-1874. https://doi.org/10.1093/molbev/msw054

Larki R., Mehrabi-Koushki M. & Farokhinejad R. 2019. — *Ectophoma iranica* sp. nov. and new hosts and records of *Allophoma* spp. in Iran. *Journal of Phytopathology* 167 (9): 538-545. https://doi.org/10.1111/jph.12840

Liu Y. J., Whelen S. & Hall B. D. 1999. — Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. *Molecular Biology and Evolution* 16 (12): 1799-1808. https://doi.org/10.1093/oxfordjournals.molbev.a026092

O'Donnell K. 1993. — *Fusarium* and its near relatives, *in* Reynolds D. R. & Taylor J. W. (eds), *The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics*. CAB International, Wallingford: 225-233.

O'Donnell K. & Cigelnik E. 1997. — Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics and Evolution* 7 (1): 103-116. https://doi.org/10.1006/mpev.1996.0376

Pem D., Jeewon R., Selcuk F., Ulukapi M., Bhat J., Doilom M., Lumyong S. & Hyde K. D. 2020. — Ribosomal and protein gene phylogeny reveals novel saprobic fungal species from *Juglans regia* and *Urtica dioica*. *Frontiers in Microbiology* 11: 1303.

Pethybridge S. J., Jones S. J., Shivas R. G., Hay F. S. & Wilson C. R. 2008. — Tan spot: a new disease of pyrethrum caused by *Microsphaeropsis tanaceti* sp. nov. *Plant Pathology* 57: 1058-1065.

Phookamsak R., Liu J. K., McKenzie E. H. C., Manamgoda D. S., Ariyawansa H. A., Thambugala K. M., Dai D. Q., Camporesi E., Chukeatirot E., Wijayawardene N. N., Bahkali A. H., Mortimer P. E., Hyde K. D. & Xu J. C. 2014. — Revision of Phaeosphaeriaceae. *Fungal Diversity* 68: 159-238.

Pirnia M., Zare R., Zamanizadeh H. R. & Khodaparast A. 2012. — New records of cercosporoid hyphomycetes from Iran. *Mycotaxon* 120: 157-169. https://doi.org/10.5248/120.157

Raeder U. & Broda P. 1985. — Rapid preparation of DNA from filamentous fungi. *Letters in Applied Microbiology* 1 (1): 17-20. https://doi.org/10.1111/j.1472-765X.1985.tb01479.x

Razaghi P. & Zafari D. 2016. — First report of *Microsphaeropsis olivacea* causing brown spine rot on *Alhagi maurorum* in Iran. *Journal of Plant Pathology* 98 (2): 685. http://doi.org/10.4454/JPP.V98I2.044

Reppas G., Gottlieb T., Krockenberger M., Halliday C. & Malik R. 2015. — *Microsphaeropsis arundinis*: an emerging cause of phaeohyphomycosis in cats and people. *Microbiology Australia* 36: 74-78.

Ronquist F., Teslenko M., Van Der Mark P., Ayres D. L., Darling A., Hohna S., Larget B., Liu L., Suchard M. A. & Huelsenbeck J. P. 2012. — MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539-542.

Rostamian M., Kavosi M. R., Bazgir E. & Babanezhad M. 2016. — First report of *Biscogniauxia mediterranea* causing canker on wild almond (*Amygdalus scoparia*). *Australasian Plant Disease Notes* 11: 30. https://doi.org/10.1007/s13314-016-0219-0

Safi A., Mehrabi-Koushki M. & Farokhinejad R. 2020. — *Amesia khuzestanica* and *Curvularia iranica* spp. nov. from Iran. *Mycological Progress* 19: 935-945. https://doi.org/10.1007/s11557-020-01612-5

Shamsi M. M., Akbarinia M., Mirabolfathy M., Manzari S. & Ahmadikhah A. 2019. — Dieback and decline of wild almond (*Amygdalus scoparia* Spach) in the Harat protected forest of Yazd Province, Iran. *Forest Pathology* 49: e12538.

Sun J.-Q., Guo L.-D. & Zang W. 2007. — Endophytic fungi VIII. Two new records from medicinal plants in China. *Mycosystema* 26: 591-593.

Sung G. H., Sung J. M., Hywel-Jones N. L. & Spatafora J. W. 2007. — A multi-gene phylogeny of Clavicipitaceae (Ascomycota, Fungi): identification of localized incongruence using a combinational bootstrap approach. *Molecular Phylogenetics and Evolution* 44 (3): 1204-1223. https://doi.org/10.1016/j.ympev.2007.03.011

Thambugala K. M., Daranagama D. A., Phillips A. J. & Bulgakov T. S. 2017. — Microfungi on *Tamarix*. *Fungal Diversity* 82: 239-306. https://doi.org/10.1007/s13225-016-0371-z

Thambugala K. M., Hyde K.D., Camporesi E. & Liu Z. Y. 2018. — *Microsphaeropsis ononidicola* sp. nov. (Microsphaeropsidaceae, Pleosporales) from *Ononis spinosa* L. *Current Research in Environmental & Applied Mycology* 8: 217-223.

Valenzuela-Lopez N., Cano-Lira J. F., Guarro J., Sutton D. A., Wiederhold N., Crous P. W. & Stchigel A. M. 2018. — Coelomycetous Dothideomycetes with emphasis on the families Cucurbitariaceae and Didymellaceae. *Studies in Mycology* 90: 1-69.

Wanasinghe D. N., Jeewon R., Peršoh D., Jones E. B. G., Camporesi E., Bulgakov T. S., Gafforov Y. S. & Hyde K. D. 2018. — Taxonomic circumscription and phylogenetics of novel didymellaceous taxa with brown muriform spores. *Studies in Fungi* 3: 152-175.

White T. J., Bruns T., Lee S. & Taylor J. 1990. — Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, *in* Innis M. A., Gelfand D. H., Sninsky J. J. & White T. J. (eds), *PCR protocols: a guide to methods and applications*. Academic Press, New York: 315-322.

Wijayawardene N. N., Crous P. W., Kirk P. M., Hawksworth D. L., Boonmee S., Braun U., Dai D., D'souza M. J., Diederich P., Dissanayake A., Doilom M., Hongsanan S., Jones E. B., Groenewald J. Z., Jayawardena R., Lawrey J. D., Liu J., Lücking R., Madrid H., Manamgoda D. S., Muggia L., Nelsen M. P., Phookamsak R., Suetrong S., Tanaka K., Thambugala K. M., Wanasinghe D. N., Wikee S., Zhang Y., Aptroot A., Ariyawansa H. A., Bahkali A. H., Bhat D. J., Gueidan C., Chomnunti P., De Hoog G. S., Knudsen K., Li W., McKenzie E. H., Miller A. N., Phillips A. J., Piątek M., Raja H. A., Shivas R. S., Slippers B., Taylor J. E., Tian Q., Wang Y., Woudenberg J. H., Cai L., Jaklitsch W. M. & Hyde K. D. 2014. — Naming and outline of Dothideomycetes. 2014 including proposals for the protection or suppression of generic names. *Fungal Diversity* 69: 1-55.

Wijayawardene N. N., Hyde K. D., Wanasinghe D. N., Papizadeh M., Goonasekara I. D., Camporesi E., Bhat D. J., McKenzie E. H. C., Phillips A. J. L., Diederich P., Tanaka K., Li W. J., Tangthirasunun N., Phookamsak R., Dai D. Q., Dissanay-

AKE A. J., WEERAKOON G., MAHARACHCHIKUMBURA S. S. N., HASHIMOTO A., MATSUMURA M., BAHKALI A. H. & WANG Y. 2016. — Taxonomy and phylogeny of dematiaceous coelomycetes. *Fungal Diversity* 77: 1-316.

WIJAYAWARDENE N. N., HYDE K. D., RAJESHKUMAR K. C., HAWKSWORTH D. L., SUETRONG S., TIAN Q., CASTAÑEDA-RUIZ R. F., BAHKALI A. H., PANG K. L., TANAKA K., DAI D. Q., SAKAYAROJ J., HUJSLOVÁ M., LOMBARD L., SHENOY B. D., SUIJA A., MAHARACHCHIKUMBURA S. S. N., THAMBUGALA K. M., WANASINGHE D. N., SHARMA B. O., GAIKWAD S., PANDIT G., ZUCCONI L., ONOFRI S., EGIDI E., RAJA H. A., KODSUEB R., CÁCERES M. E. S., PÉREZ-ORTEGA S., FIUZA P. O., MONTEIRO J. S., VASILYEVA L. N., SHIVAS R. G., PRIETO M., WEDIN M., OLARIAGA I., LATEEF A. A., AGRAWAL Y., SHAHZADEH FAZELI S. A., AMOOZEGAR M. A., ZHAO G. Z., PFLIEGLER W. P., SHARMA G., OSET M., ABDEL-WAHAB M. A., TAKAMATSU S., BENSCH K., DE SILVA N. I., DE KESEL A., KARUNARATHNA A., BOONMEE S., PFISTER D. H., LU Y. Z., LUO Z. L., BOONYUEN N., DARANAGAMA D. A., SENANAYAKE I. C., JAYASIRI S. C., SAMARAKOON M. C., ZENG X. Y., DOILOM M., QUIJADA L., RAMPADARATH S., HEREDIA G., DISSANAYAKE A. J., JAYAWARDANA R. S., PERERA R. H., TANG L. Z., PHUKHAMSAKDA C., HERNÁNDEZ-RESTREPO M., MA X., TIBPROMMA S., GUSMAO L. F. P., WEERAHEWA D. & KARUNARATHNA S. C. 2017. — Notes for genera: *Ascomycota*. *Fungal Diversity* 86: 1-594.

WIJAYAWARDENE N. N., HYDE K. D., LUMBSCH H. T., LIU J. K., MAHARACHCHIKUMBURA S. S. N., EKANAYAKA A. H., TIAN Q. & PHOOKAMSAK R. 2018. — Outline of *Ascomycota*: 2017. *Fungal Diversity* 88: 167-263.

WOUDENBERG J. H. C., AVESKAMP M. M., DE GRUYTER J., SPIERS A. G. & CROUS P. W. 2009. — Multiple *Didymella* teleomorphs are linked to the *Phoma clematidina* morphotype. *Persoonia* 22: 56-62. https://doi.org/10.3767/003158509X427808

Submitted on 1 November 2021;
accepted on 23 September 2022;
published on 20 December 2022.