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Exserohilum persianum sp. nov., a new species from Iran based on morphological and molecular characters

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Abstract: *Exserohilum* species are plant or human pathogens, saprobes or endophytes mostly associated with grasses including cultivated cereals. At present, 11 phylogenetic species have been accepted in this genus worldwide. In this study, we introduce a novel species, *Exserohilum persianum* on *Festuca* sp. (*Poaceae*, *Poales*) showing necrotic leaf lesions based on morphological characteristics and sequence data obtained from the internal transcribed spacer (ITS–rDNA) region, parts of glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and translation elongation factor-1 alpha (*TEF1*) genes. A detailed morphological description, illustration and comparison with closely allied species are provided.
Keywords: Taxonomy, Morphology, Molecular phylogeny, *Pleosporaceae*, Novel taxon.

INTRODUCTION

The genus *Exserohilum* K.J. Leonard & Suggs belongs to the family*Pleosporaceae* of the *Pleosporales* in *Dothideomycetes* (Ascomycota) (Zhang et al. 2012, Ariyawansa et al. 2015, Hernández-Restrepo et al. 2018, Marin-Felix et al. 2019), and was introduced by Leonard & Suggs (1974), with *E. turcicum* (Pass.) K.J. Leonard & Suggs as the type species. The genus is characterized by having brown, mono- or polytretic conidiogenous cells, and fusiform, cylindrical or obclavate, straight to curved, multi-distoseptate conidia with a distinctly protuberant hilum (Sivanesan 1987, Alcorn 1988, Hernández-Restrepo et al. 2018). *Exserohilum* is differentiated from the closely related *Bipolaris* Shoemaker, *Curvularia* Boedjin and *Pyrenophora* Fr. by producing conidia with a protruding hilum (Leonard & Suggs 1974, Sivanesan 1987, Alcorn 1988, Manamgoda et al. 2014, Hernández-Restrepo et al. 2018). Some species of *Exserohilum* have been linked to *Setosphaeria* K.J. Leonard & Suggs sexual morph (Leonard & Suggs 1974, 1976, Alcorn 1978, 1986, Sivanesan 1987). Rossman et al. (2015) recommend using of the name *Exserohilum* over *Setosphaeria* according to Article 57.2 of the International Code of Nomenclature for algae, fungi and plants (McNeill et al. 2012).

Exserohilum species are pathogens of plants, animals and humans, saprobes or endophytes mostly associated with grasses including cultivated cereals. Some species are important plant pathogens, including *E. turcicum* (causing northern leaf blight of corn), *E. pedicellatum* (A.W. Henry) K.J. Leonard & Suggs (causing root rot on maize and brown lesions on wheat roots) (Sivanesan 1987), and *E. rostratum* (Drechsler) K.J. Leonard & Suggs (causing leaf spot on banana, maize and wheat, foot rot of wheat, damping off of sugarcane seedlings, blackening and seed germination failure in cereals) (Sivanesan 1987, Lin et al. 2011, Farr & Rossman 2023). Three species, *E. longirostratum* (Subram.) Sivan., *E. macginnisii* A.A. Padhye & Ajello and *E. rostratum* were recognized as opportunistic human pathogens as the etiologic agent of sinusitis, which may extend to the central nervous system, keratitis and cutaneous and subcutaneous mycosis (de Hoog et al. 2000, Da Cunha et al. 2012, Farr & Rossman 2023).

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Exserohilum species are morphologically similar and hard to differentiate from one another. Therefore, molecular data are essential for the accurate identification of species within this genus. Sequences of the large subunit ribosomal RNA gene (*LSU*), the internal transcribed spacer (ITS–rDNA) region, actin (*act*), β-tubulin (*tub2*), calmodulin (*cam*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), histone H3 (*his*), translation elongation factor-1 alpha (*TEF1*) and RNA polymerase II second largest subunit (*RPB2*) have been used for delimitation of *Exserohilum* species (Berbee et al. 1999, Olivier et al. 2000, Zhang & Berbee 2001, Rossman et al. 2002, Kodsueb et al. 2006, Zhang et al. 2008, 2012, Amaradasa et al. 2014, Ariyawansa et al. 2015, Hernández-Restrepo et al. 2018, Marin-Felix et al. 2019). Recently the taxonomy and phylogeny of *Exserohilum* have been revised and based on molecular data 11 phylogenetic species have been accepted in this genus (Hernández-Restrepo et al. 2018, Marin-Felix et al. 2019). Up to now, five *Exserohilum* species have been reported from Iran (Ahmadpour et al. 2013, Heidarian et al. 2016, 2020, Nemati & Mostowfizadeh-Ghalamfarsa 2016, Ershad 2022) and most of the presently known *Exserohilum* species have been identified based on morphology (Nemati & Mostowfizadeh-Ghalamfarsa 2016, Ershad 2022). In this study, we introduce a new species isolated from necrotic leaf lesions of *Festuca* sp. in Miyandoab, West Azarbaijan province, Iran. Based on morphological characteristics combined with multi-gene phylogenetic analyses, it is recognized as a new species, *Exserohilum persianum*. A detailed description and illustration are provided and compared with the closely related allies.

MATERIALS AND METHODS

Fungal isolates

Leaf samples from *Festuca* sp. (*Poaceae*, *Poales*) showing necrotic lesions were collected from Miyandoab city, West Azarbaijan province, Iran, in 2020. The lesions were confined mostly to the midrib, at first oval to fusiform, brown, then expanding and coalescence into long fusiform lesions over time, dark brown at the margins and light brown to gray at the center with a yellowish halo (Fig. 2). The samples were surface disinfected using 1% sodium hypochlorite solution for 3 min., followed by rinsing in sterile distilled water and incubated in a moist chamber at 25 °C. The incubated leaves were inspected under the stereo microscope (SZ51, Olympus) and single spores were taken using a fine sterile needle and transferred to potato dextrose agar (PDA) medium. The isolates were purified using the single spore method and purified isolates were stored on sterile filter paper at –20 °C. To observe morphological characteristics, the isolates were grown on tap water agar with autoclaved wheat straw (TWA–wheat straw) medium and Petri dishes were incubated at 23–25 °C under the near ultraviolet light (NUV)/dark period of 12/12 h for 14 days (Sivanesan 1987, Hernández-Restrepo et al. 2018). Colony morphology was characterized by cultures grown on PDA medium after seven days at 25 °C in darkness, and colony color was determined using the color charts of Rayner (1970). Measurements and microphotographs were prepared from slide mounts in lactophenol and lactophenol cotton blue using an Olympus AX70 compound microscope with differential interference contrast (DIC) illumination. Adobe Photoshop 2020 v. 2.10.8 software (Adobe Inc., San Jose, California) was used for manual editing. Pure cultures of all identified isolates were deposited in the fungal culture collections of the Iranian Research Institute of Plant Protection (IRAN) and Urmia University (FCCUU). The taxonomic novelty was deposited in MycoBank (Crous et al. 2004).

DNA extraction, PCR amplification and sequencing

For total DNA extraction, the mycelial mass of each isolate harvested from 10-days-old PDA Petri dishes was homogenized in a standard sodium dodecyl sulfate (SDS) detergent lysis buffer and DNA was isolated using chloroform extraction and isopropanol precipitation method (Ahmadpour et al. 2021). The ITS–rDNA, parts of *GAPDH* and *TEF1* genes were amplified using the primer pairs ITS1/ITS4 (White et al. 1990), gpd1/gpd2 (Berbee et al. 1999) and TEF1-983F/TEF1-2218R (Rehner & Buckley 2005), respectively. Each polymerase chain reaction (PCR) mixture contained 0.4 µM of each primer, 10 µL of a ready master mix (Taq DNA polymerase 2X Master Mix Red, 2 mM $MgCl_2$, Ampliqon Company, Denmark) and about 10 ng of template DNA in a final volume of 30 µL. A touchdown PCR consisted of 35 cycles of 45 s at 95 °C, 45 s at 62–57 °C (annealing temperature decreased 0.5 °C per cycle in the first 10 cycles) and 45 s at 72 °C, and a final extension step at 72 °C for 5 min (Korbie & Mattick 2008). Amplicons were visualized on a 1 % agarose gel stained with GelRed™ (Biotium, Hayward, CA, USA) and viewed under ultra-violet light and sizes of amplicons were determined using a HyperLadder™ I molecular marker (Bioline). The amplified products were cleaned and sequenced using the same primer sets used for PCR amplification by Macrogen Corporation (Seoul, South Korea).

Phylogenetic analyses

DNA sequences generated for the new strains were seen and trimmed in MEGA 6.0 (Tamura et al. 2013), and were exported as FASTA files for further analyses. Preliminary identifications of the isolates were carried out using newly generated ITS, *GAPDH* and *TEF1* sequences using NCBI Basic Local Alignment Search Tool (BLAST). Pairwise sequence comparisons were performed for novel species with their respective closely related taxa using the BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) with default settings. Published and authenticated DNA sequences of different loci for the ex-type and reference *Exserohilum* strains were obtained from the

GenBank database (https://www.ncbi.nlm.nih.gov/) and included in the phylogenetic analyses (Hernández-Restrepo et al. 2018, Marin-Felix et al. 2019) (Table 1). For each locus, multiple sequence alignments were produced using MAFFT version 7 online program (Katoh et al. 2019) and were adjusted and trimmed manually in MEGA 6.0 where necessary. A final three-gene concatenated dataset (ITS + *GAPDH* + *TEF1*) was produced in Mesquite v. 3.61 (Maddison & Maddison 2019). Multi-locus phylogenetic analyses were done by using Maximum Likelihood (ML), Maximum Parsimony (MP) and Bayesian inference (BI) methods. Maximum likelihood (ML) was analyzed with gamma model of rate heterogeneity using the RAxML–HPC BlackBox v. 8.2.8 (Stamatakis 2014) online server of the CIPRES Science gateway portal (https://www.phylo.org/) (Miller et al. 2012). The maximum likelihood option was used to search for the best-scoring tree after bootstrapping. By default, the RAxML HPC BlackBox calculates statistical support for branches by rapid bootstrap analyses of 1000 replicates (Stamatakis 2014). Bayesian inference (BI) was performed in MrBayes v. 3.2.7 (Ronquist et al. 2012) by using the Markov Chain Monte Carlo (MCMC) method with four chains, 1M generations and temperature value of the heated chain of 0.1. Trees were saved every 1000 generations, Burn-in was set to 25% and posterior probabilities (PP) were determined from the remaining trees. For determining the best-fit evolutionary models required for BI, all individual alignments were evaluated in MrModeltest 2.3 (Nylander 2004) using Akaike Information Criterion (AIC). Maximum Parsimony (MP) analyses were performed in PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2002). Trees were inferred using the heuristic search option with 1000 random sequence additions and branch swapping with the tree-bisection-reconnection (TBR) algorithm and gaps were treated as missing data. The bootstrap values with 1000 replicates were performed to determine branch support. Descriptive tree statistics [Tree Length (TL), Consistency Index (CI), Retention Index (RI) and Homoplasy Index (HI)] were calculated for trees generated in the parsimony analysis. Sequences of *Bipolaris maydis* (CBS 137271) and *B. oryzae* (MFLUCC 10-0715) served as the outgroup taxa. The resultant phylogenetic trees were visualized in FigTree v. 1.4.4 (Rambaut 2019) and edited in graphic design software, Adobe Illustrator® CC 2020. The newly generated sequences were submitted in GenBank (Table 1).

RESULTS

Molecular phylogenetic analyses

The average size of individual DNA sequence alignments for ITS, *GAPDH* and *TEF1* was 559 bp, 517 bp and 963 bp, respectively. Since the topologies of single-locus trees were concordant, individual alignments were combined into a concatenated sequence alignment. The results of MrModeltest recommended a GTR+I, GTR+G and GTR+I+G models for ITS, *GAPDH* and *TEF1*, respectively. A total of 1841 characters (1539 constant, 302 variable, 53 parsimony-uninformative and 249 parsimony-informative sites) including gaps (454 for ITS, 491 for *GAPDH* and 896 for *TEF1*) were included in the phylogenetic analysis. BI, ML and MP (TL= 529; CI = 0.698; RI = 0.888; HI = 0.302) trees were similar in terms of major clades and topology. The resulting phylogram (Fig. 1) from the multi-locus phylogenetic analyses revealed that our isolates belong to a different *Exserohilum* species, which is described as a novel species below.

Taxonomy and morphology

Exserohilum persianum A. Ahmadpour, Z. Heidarian, Z. Alavi, F. Alavi & Y. Ghosta, *sp. nov.* Fig. 2

MycoBank MB 846935

Etymology – The name refers to the old name of Iran, Persia, from which it was collected.

Description – Hyphae branched, septate, light brown to brown, smooth, 2–4 µm wide. Conidiophores macronematous, mononematous, single or in small groups, straight to flexuous, septate, light brown to brown, geniculate towards the apex, unbranched, smooth-walled, but sometimes becoming finely verruculose near the conidiogenous loci, mostly with 1–3 scars or more, 250–560 × 6–8 µm, sometimes with a bulbous base up to 18 µm wide. Conidiogenous cells integrated, terminal and intercalary, mostly subcylindrical, mono- to polytretic, proliferating sympodially, with scars up to 5.5 µm wide. Conidia straight or mostly curved and rarely sigmoid, fusiform to obclavate-fusoid, occasionally ellipsoidal to subcylindrical, pale olivaceous brown to dark brown, smooth to verruculose, with the basal cell (and sometimes also the apical and middle cells) delimited by a dark septum, (5–)8–10(11)-distoseptate, (97–)112–125(–132) × (13–)15–17(–19) µm (n = 50), with a strongly protruding hilum 2–3 µm wide. Conidia germinated mono- or bipolar. Chlamydospores and sexual morph were not observed.

Culture characteristics – Colonies on PDA reaching 60–65 mm diam. after seven days at 25 ºC, entire, dark green to olivaceous brown with sparse, felty, some white to grey aerial mycelium.

Typification – IRAN, West Azarbaijan province, Miyandoab, isolated from infected leaves of *Festuca* sp. (*Poaceae*, *Poales*) with long light brown to brown lesions, 20 Sept. 2020, *A. Ahmadpour*, **holotype** IRAN 18245F, living Ex-type culture IRAN 3503C. GenBank: ITS = OQ119789; *GAPDH* = OQ123392; *TEF1* = OQ123394; West Azarbaijan province, Miyandoab, on infected leaves of *Festuca* sp., 20 Sept. 2020, *A. Ahmadpour*, FCCUU 1201 (Table 1).

Table 1. Fungal strains were used for phylogenetic analyses in this study. Newly generated sequences are shown in bold.

Species	Isolates[1]	Geographical origin (country, province, locality)	Substrate	GenBank accession numbers			References
				ITS	*GAPDH*	*TEF1*	
Bipolaris maydis	CBS 137271^{T}	USA	*Zea mays*	AF071325	KM034846	KM093794	Berbee et al. (1999), Manamgoda et al. (2014)
B. oryzae	MFLUCC 10-0715^{T}	Thailand	*Oryza sativa*	JX256416	JX276430	JX266585	Manamgoda et al. (2012)
Exserohilum corniculatum	BRIP 11426^{T}	Australia	*Oryza sativa*	LT837453	LT883533	LT883558	Hernández-Restrepo et al. (2018)
E. holmii	CBS 318.64	Unknown	*Dactyloctenium aegyptium*	LT837457	LT883537	LT883565	Hernández-Restrepo et al. (2018)
E. holmii	CBS 413.65^{IsoT}	USA	*Dactyloctenium aegyptium*	LT837459	LT715890	LT883567	Hernández-Restrepo et al. (2018)
E. holmii	CBS 505.90	Venezuela	*Sorghum vulgare*	KT265252	LT715889	LT883560	Hernández-Restrepo et al. (2018)
E. khartoumensis	IMI 249194^{IsoT}	Sudan	*Sorghum bicolor var. mayo*	LT837461	LT715888	LT883569	Hernández-Restrepo et al. (2018)
E. minor	BRIP 14612	Australia	Ascocarps formed by BRIP 13597	LT837467	LT715884	LT883577	Hernández-Restrepo et al. (2018)
E. minor	BRIP 14614	Australia	*Dactyloctenium aegyptium*	LT837468	LT715885	LT883578	Hernández-Restrepo et al. (2018)
E. minor	BRIP 14616^{T}	Australia	*Dactyloctenium aegyptium*	LT837470	LT883545	LT883580	Hernández-Restrepo et al. (2018)
E. monoceras	BRIP 11542	Australia	*Setaria italica*	LT837473	LT883546	LT896604	Hernández-Restrepo et al. (2018)
E. monoceras	BRIP 12236	Australia	*Echinochloa colona*	LT837472	LT715876	LT896603	Hernández-Restrepo et al. (2018)
E. monoceras	BRIP 12271^{A}	Australia	*Echinochloa colona*	LT837475	LT883548	LT896606	Hernández-Restrepo et al. (2018)
E. neoregeliae	CBS 132832^{T}	Japan	*Neoregelia carolinae*	LT837476	LT715886	LT896607	Hernández-Restrepo et al. (2018)
E. neoregeliae	CBS 132833^{IsoT}	Japan	*Neoregelia carolinae*	LT837477	LT715887	LT896608	Hernández-Restrepo et al. (2018)
E. oryzicola	CBS 502.90^{IsoT}	Colombia	*Oryza sativa*	HF934949	LT715878	LT896629	Amaradasa et al. (2014), Hernández- Restrepo et al. (2018)
E. oryzicola	CBS 376.76	Turkey	*Oryza sativa*	LT837456	LT883535	LT883562	Hernández-Restrepo et al. (2018)
E. pedicellatum	CBS 322.64^{ET}	USA	*Triticum aestivum*	KT265258	LT715902	LT896630	Amaradasa et al. (2014), Chowdhary et al. (2015), Hernández-Restrepo et al. (2018)
E. pedicellatum	BRIP 12040	Australia	*Oryza sativa*	LT837452	LT883532	LT883557	Hernández-Restrepo et al. (2018)
E. persianum	**IRAN 3503C**	**Iran**	*Festuca* sp.	**OQ119789**	**OQ123392**	**OQ123394**	**This study**
E. persianum	**FCCUU 1201**	**Iran**	*Festuca* sp.	**OQ119790**	**OQ123393**	**OQ123395**	**This study**
E. protrudens	BRIP 14816	Australia	*Dactyloctenium aegyptium*	LT631309	LT715881	LT896650	Hernández-Restrepo et al. (2018)
E. rostratum	BRIP 10995	Australia	*Zea mays*	LT837823	LT882566	LT896637	Hernández-Restrepo et al. (2018)
E. rostratum	BRIP 14916	Australia	*Zea mays*	LT837835	LT882552	LT896655	Hernández-Restrepo et al. (2018)
E. rostratum	BRIP 16078	Australia	*Spinifex hirsutus*	LT837826	LT882563	LT896640	Hernández-Restrepo et al. (2018)
E. rostratum	CBS 196.29	Japan	*Leptochloa chinensis*	LT837462	LT715896	LT883570	Hernández-Restrepo et al. (2018)
E. rostratum	CBS 297.80	Sudan	*Sorghum bicolor*	KT265244	LT715895	LT883563	Hernández-Restrepo et al. (2018)
E. rostratum	CBS 325.87	USA	*Homo sapiens*	KT265237	LT715898	HE664082	Chowdhary et al. (2015), Hernández- Restrepo et al. (2018)
E. rostratum	CBS 412.93	Cuba	Plant debris from forest soil	KT265246	LT715894	LT883556	Hernández-Restrepo et al. (2018)
E. rostratum	CBS 571.73	USA	*Zea mays*	LT837831	LT715892	LT896646	Hernández-Restrepo et al. (2018)
E. turcicum	BRIP 12267	Australia	*Sorghum bicolor*	LT837482	LT883553	LT896613	Hernández-Restrepo et al. (2018)
E. turcicum	BRIP 13326	Australia	*Sorghum sudanense*	LT837480	LT883551	LT896611	Hernández-Restrepo et al. (2018)
E. turcicum	CBS 690.71^{ET}	Germany	*Zea mays*	LT837487	LT882581	LT896618	Hernández-Restrepo et al. (2018)

[1] BRIP: Queensland Plant Pathology Herbarium, Brisbane, Australia; CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, UK; IRAN: the fungal culture collections of the Iranian Research Institute of Plant Protection, Iran; FCCUU: the fungal culture collections of Urmia University, Iran. ^{T}, ^{ET}, ^{IsoT} and ^{A} indicate ex-type, ex-epitype, ex-isotype and authentic strains.

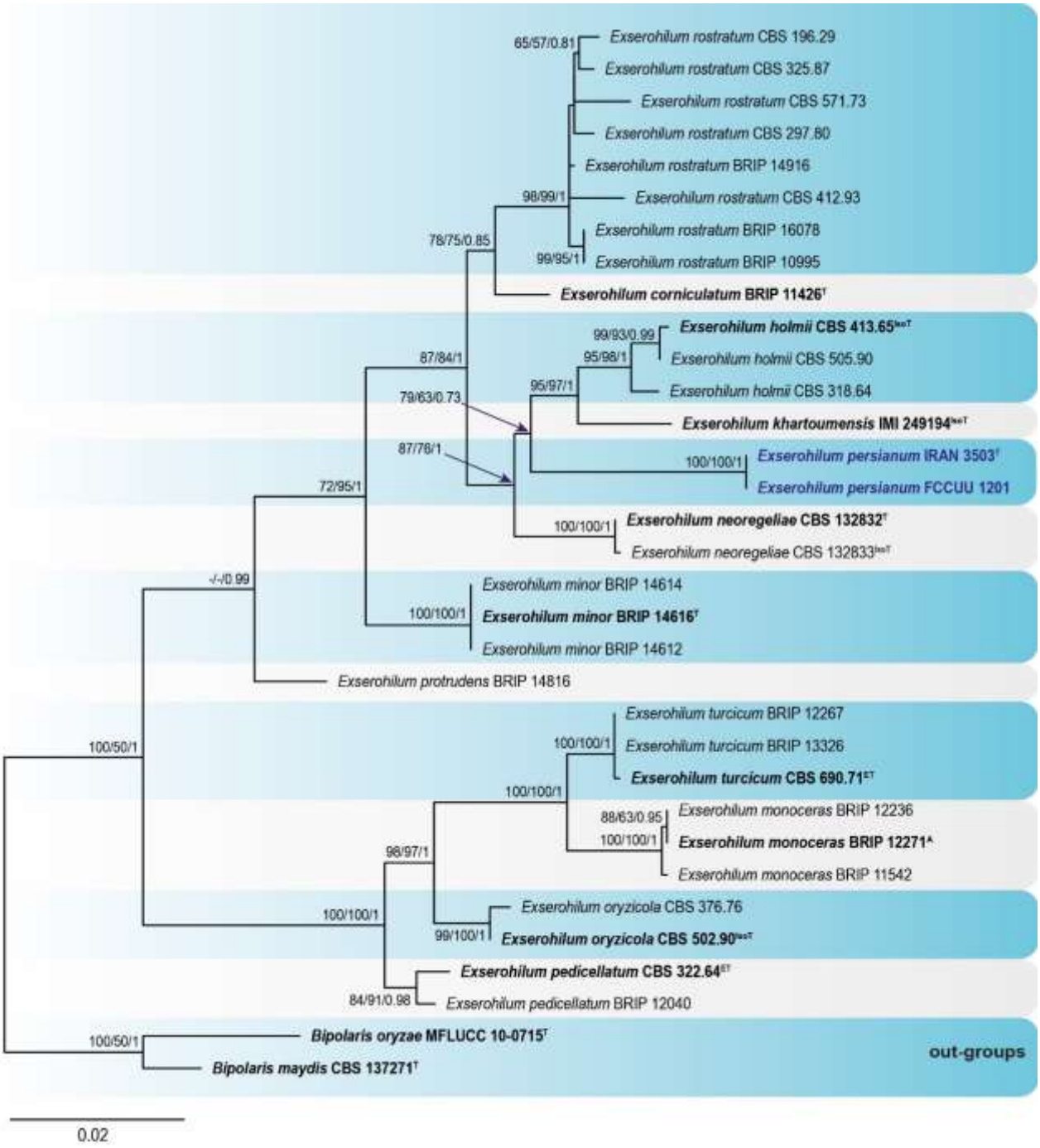

Fig. 1 Phylogenetic tree inferred from Maximum Likelihood (ML) of the combined dataset of ITS, *GAPDH* and *TEF1* of *Exserohilum* species. The Maximum Likelihood, Maximum Parsimony (MP) bootstrap supports (BS) and Bayesian posterior probabilities (PP) values >50% are given at the nodes (BS/PP). The tree was rooted to *Bipolaris maydis* (CBS 137271) and *B. oryzae* (MFLUCC 10-0715) and newly identified strains are in blue bold face. The scale bar indicates the number of nucleotide substitutions. $^{T, ET, IsoT}$ and ^{A} indicate ex-type, ex-epitype, ex-isotype and authentic strains.

Notes – Based on phylogenetic analyses (Fig. 1), *Exserohilum persianum* clusters in a distinct lineage with 100% ML/MP bootstraps and 1.0 BI posterior probabilities values, representing a new taxon (Fig. 1). This species is morphologically similar to *E. neoregeliae*, but it can be differentiated by the smaller and narrower conidia ((97–) 112–125 (–132) × (13–) 15–17 (–19) µm vs. 80.5–285 × 12.5–27 µm in *E. neoregeliae*) and fewer distosepta (5–11 vs. 6–20 in *E. neoregeliae*) (Sakoda & Tsukiboshi 2011, Hernández-Restrepo et al. 2018).

DISCUSSION

The traditional morphological attributes of conidia that have been used as taxonomical criteria at the generic rank for *Bipolaris*, *Curvularia*, *Exserohilum* and *Pyrenophora* are mainly the germination patterns of the conidia, septum ontogeny and hilum morphology (Leonard & Suggs 1974, Alcorn 1978, 1986, 1988, 1990, 1991, Sivanesan 1987). Among those features, the hilum morphology is the most valuable criterion to delineate *Exserohilum* species. Morphological variability in conidial shape, size and pigmentation has been already noticed in the natural substrate (Drechsler 1923) and external factors (carbon source, glucose concentration, type of culture media, light exposure and pH) might affect morphology in culture media (Leonard & Suggs 1976, Sivanesan 1987, Alcorn 1988). Therefore, species of *Exserohilum* are morphologically very variable and molecular analysis is required for correct species identification (Hernández-Restrepo et al. 2018, Marin-Felix et al. 2019). Currently, 40 names have been listed in index fungorum (http://www.indexfungorum.org) and MycoBank (http://www.mycobank.org) (accessed on 20th January 2023) under the genus *Exserohilum*. Based on a taxonomic re-evaluation of *Exserohilum* species, several species have been transferred to other graminicolous helminthosporoid genera including *Bipolaris*, *Curvularia* and *Sporidesmiella* (Amaradasa et al. 2014, Madrid et al. 2014, Manamgoda et al. 2014, Hernández-Restrepo et al. 2018). Hernández-Restrepo et al. (2018) recognized 11 phylogenetic species in *Exserohilum* genus and listed other 15 species as 'doubtful species' (i.e., *E. curvisporum*, *E. echinochloae*, *E. elongatum*, *E. frumentacei*, *E. glycinea*, *E. heteromorphum*, *E. israeli*, *E. lagenarioides*, *E. longisporum*, *E. oryzae*, *E. oryzinum*, *E. parlierense*, *E. phragmitis*, *E. psidii* and *E. sodomii*), need to be confirmed as members of *Exserohilum* by molecular data. Moreover, the results showed that *E. rostratum* is as conspecific to *E. antillanum*, *E. gedarefense*, *E. longirostratum*, *E. macginnisii*, *E. prolatum* and *Helminthosporium leptochloae* and proposed them as synonyms of *E. rostratum* (Hernández-Restrepo et al. 2018).

The combination of ITS, *GAPDH* and *TEF1* sequences data as well as morphological characteristics establishes that our isolates belong to *Exserohilum*, and are distinct from all other species previously included and described in this genus (Fig. 1). Species of *Exserohilum* formed a well-supported clade (100% BS/1.0 PP) and separated from *Bipolaris* species (Fig. 1). Also, our isolates formed a distinct lineage in a clade with close affinity to *E. khartoumensis*, *E. holmii* and *E. neoregeliae*. So, we introduced the new species, *Exserohilum persianum* based on morphological and molecular characteristics. A comparison of nucleotide differences in ITS, *GAPDH* and *TEF1* indicates that our isolate (IRAN 3503C) differs from *E. khartoumensis* (IMI 249194) by 35 bp (6.22%) in ITS, 28 bp (5.46%) in *GAPDH* and 25 bp (2.86%) in *TEF1*, from *E. holmii* (CBS 413.65) by 41 bp (7%) in ITS, 26 bp (5%) in *GAPDH* and 51 bp (6%) in *TEF1* and from *E. neoregeliae* (CBS 132832) by 24 bp (5.01%) in ITS, 25 bp (4.88%) in *GAPDH* and 24 bp (3%) in *TEF1*. Morphologically, *E. persianum* can be distinguished from *E. neoregeliae* and *E. khartoumensis* by the shape and size of conidia and the number of conidium distosepta (Sakoda & Tsukiboshi 2011, Hernández-Restrepo et al. 2018).

Species of the genus *Exserohilum* are frequently found as asexual morphs in nature, although the sexual morph was often obtained by combining compatible strains, except for *E. minor* and *E. khartoumensis* (Leonard & Suggs 1974, 1976, Alcorn 1978, 1986, Sivanesan 1987, Hernández-Restrepo et al. 2018). However, our efforts to induce the formation of sexual morph were unsuccessful. Considering the rich diversity of plant species in the mainland of Iran as a hotspot for the biodiversity of microfungi, more *Exserohilum* species are expected to be discovered in Iran. More studies are needed to elucidate the diversity of *Exserohilum* species associated with diverse *Poaceae* family plants in different geographical locations, determine the host range of the identified species in the studied area, and assess its pathogenicity as well.

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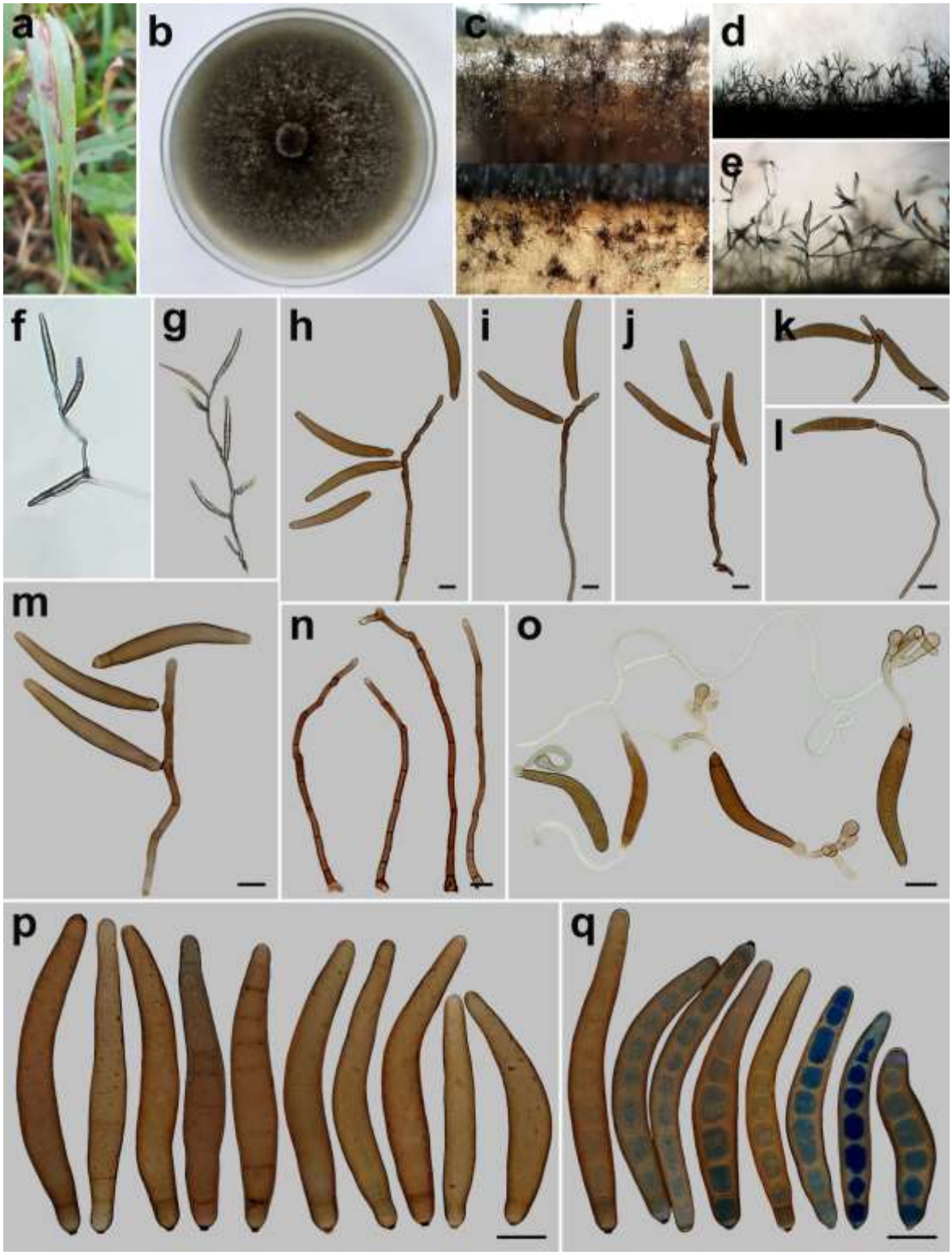

Fig. 2 *Exserohilum persianum* (IRAN 3503C). **a.** Symptoms on the leaf of *Festuca* sp. (*Poaceae*, *Poales*). **b.** Colony on PDA after 7 days. **c**–**e.** Sporulation pattern on TWA. **f**–**n.** Conidiophores. **o.** Germinated conidia. **p**–**q.** Conidia. Scale Bars = 20 µm

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Exserohilum persianum گونه جدیدی از ایران بر اساس ویژگی‌های ریخت‌شناختی و مولکولی

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چکیده: گونه‌های جنس *Exserohilum* بیمارگرهای گیاهی، انسانی، پوده‌رست یا اندوفیت، اغلب در ارتباط با گیاهان گرامینه از جمله غلات زراعی می‌باشند. در حال حاضر، ۱۱ گونه تبارشناختی در دنیا از این جنس گزارش شده است. در مطالعه حاضر، ما گونه جدیدی به نام *Exserohilum persianum* را از گیاه *Festuca* sp. (*Poaceae*, *Poales*) دارای نشانه‌های لکه‌برگی بر اساس تلفیق صفات ریخت‌شناختی و داده‌های توالی حاصل از ناحیه ITS–rDNA و بخش‌هایی از ژن‌های *GAPDH* و *TEF1* معرفی می‌کنیم. توصیف دقیق ریخت‌شناختی، عکس‌ها و مقایسه آن با گونه‌های نزدیک از نظر ویژگی‌های ریخت‌شناختی و تبارشناختی ارئه شده‌اند.

کلمات کلیدی: آرایه‌بندی، ریخت‌شناسی، تبارشناسی مولکولی، *Pleosporaceae*، آرایه جدید

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