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Three new Pleosporalean genera for the funga of Iran

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Abstract: *Pleosporalean* fungi are important plant pathogens, saprobes, and endophytes found in a wide range of economically important plants. To identify the fungi associated with branch and stem canker symptoms in plants, the gardens and forests of Guilan and Mazandaran provinces were surveyed, and infected plant samples were collected from common hawthorn (*Crataegus monogyna*), common rue (*Ruta graveolens*), and oriental persimmon (*Diospyros kaki*) plants during the autumn of 2021. Fungal strains were isolated and purified by common procedures and then were morphologically identified. Molecular identification of the fungal strains was performed using the sequence data of the ITS rDNA region. Based on the combined data, three fungal genera and their related species belonging to the order *Pleosporales* including *Acrocalymma walkeri* from oriental persimmon, *Setophaeosphaeria badalingensis* from common rue, and *Tremateia chromolaenae* from common hawthorn, were identified and characterized. All these three species are new to the funga of Iran. In addition, *Diospyros kaki, Crataegus monogyna*, and *Ruta graveolens* have been reported as new hosts (*matrix nova*) for the respective identified fungal taxa worldwide.

Keywords: *Ascomycota*, *Pleosporales*, Morphology, Phylogeny, ITS rDNA**.**

INTRODUCTION

In the *Dothideomycetes* (*Ascomycota*), the order *Pleosporales*, commonly known as pleosporalean fungi, is the most species-rich and the largest order in this class, including more than 10,000 species. The *Pleosporales* was first proposed by Luttrell (1955) and formally established by Barr (1987). This order is characterized by pseudothecioid ascomata, usually with a papillate apex, ostioles with or without periphyses, presence of cellular pseudoparaphyses, bitunicate asci, and ascospores of various shapes, pigmentations, and septations. Pleosporalean fungi are cosmopolitan and adapted to various habitats, sometimes with extreme environmental conditions. In recent years, molecular studies coupled with morphological evidence have revealed numerous novel families, genera, and species within the *Pleosporales* (Wanasinghe et al., 2020; Lu et al., 2022; Wanasinghe et al., 2022).

The genus *Ruta* L. belonging to the *Rutaceae* family with the common name of "Iranian Sodab or Sadab", has two species in Iran; *R. chalepensis* L. and *R. graveolens* L. (Soleimani et al., 2009). Some fungal species have been reported from *Ruta* plants. Liberato and Barreto (2006) have reported *Oidiopsis haplophylli* (Magnus) Rulamort from *Ruta graveolens* in Brazil. The common hawthorn (*Crataegus monogyna* Jacq.), a wild fruit tree, is one of the most interesting species of the *Rosaceae* family (Gundogdu et al., 2014). This plant is commonly found as a shrub or small tree, with small dark red fruit, commonly called haw (Sallabanks, 1992). The largest number of natural populations of this plant can be found in the different regions of Iran (Mozafarian, 1996). Several fungi have been reported from common hawthorn. Montecchio et al. (2002) have reported *Coniothyrium sporulosum* (W. Gams & Domsch) Aa, from *C. monogyna* in Italy. In an investigation, *Gymnosporangium globosum* (Farl.) Farl. was first reported from American hawthorn by Yun et al. (2008) in South Korea. According to the surveys in Mexico, species of *Alternaria* Nees, *Aureobasidium* Viala & G. Boyer, *Drechslera* S. Ito, *Fusarium* Link, *Paecilomyces* Bainier, and *Ulocladium* Preuss were reported as the fungal community associated with *Crataegus* sp. (Salazar-Cerezo et al., 2020). Also, Chen et al. (2022) investigated the associated fungus with hawthorn in China, and reported *Diplocarpon mespilicola* as a new species. Several fungal species such as *Gymnosporangium confusum* Plowr., *Phyllactinia mali* (Duby) U. Braun, *Podosphaera clandestine* (Wallr.) Lév. and *Rosellinia necatrix* Berl. ex Prill. have been reported from *Crataegus monogyna* in Iran (Ershad 2022).

The genus *Diospyros* L., (persimmon) of the Ebenaceae family, has many species, including Japanese or oriental persimmon (*Diospyros kaki* L.),

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which is a common edible cultivated species (Mansoory et al. 2022). Like other plants, many fungi have been reported from persimmon. In an investigation, *Cladosporium cladosporioides* (Fresen.) G.A. de Vries, was reported as sooty mold fungus from *D. kaki* by Kwon and Park (2003) in South Korea. Kwon et al. (2012), based on the morphological features, pathogenicity tests, and molecular data, have identified *Zygophiala wisconsinensis* Batzer & Crous (current name: *Schizothyrium wisconsinense* (Batzer & Crous) Crous & Batzer) on sweet persimmon in South Korea. Yamamoto et al. (2012) have isolated and characterized *Adisciso kaki* Kaz. Tanaka, J. Yamam. & Toy. Sato from *D. kaki* in Japan. Moyo et al. (2016) have studied several fungi associated with persimmon tree based on morphological features and molecular data. As a result, many fungal species such as *Diaporthe infecunda* R.R. Gomes, Glienke & Crous, *Diplodia mutila* (Fr.) Mont., *Eutypa lata* (Pers.) Tul. & C. Tul., *Eutypella citricola* Speg., *Phaeoacremonium parasiticum* (Ajello, Georg & C.J.K. Wang) W. Gams, Crous & M.J. Wingf., and *Neofusicoccum australe* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips were isolated and reported from South Africa. Furthermore, Asadi and Babaeizad (2016) have reported *Alternaria alternata* (Fr.) Keissl. from the persimmon tree in Iran. *Colletotrichum horii* B.S. Weir & P.R. Johnst., was also isolated and reported from persimmon by Jeon et al. (2017) in South Korea. *Pestalotiopsis kaki* K. Das, S.Y. Lee & H.Y. Jung, was introduced as a novel species from the persimmon tree by Das et al. (2021) in South Korea. In Iran, several fungal species such as *Ganoderma austral* (Fr.) Pat., *Pseudocercospora kaki* Goh & W.H. Hsieh and *Stereum hirsutum* (Willd.) Pers. have been reported from *D. kaki* (Ershad 2022).

The main aim of the present study was the morphological and phylogenetic identification and characterization of the new fungal taxa belonging to the pleosporalean fungi in Guilan and Mazandaran provinces, Iran.

MATERIALS AND METHODS

Fungal isolation

Branches and stems with canker symptoms were collected from common hawthorn (*C. monogyna*), common rue (*R. graveolens*), and oriental persimmon (*D. kaki*) plants from Guilan and Mazandaran provinces during the autumn of 2021. Fungal strains were isolated from diseased plant samples bearing canker symptoms using the method described by Refaei et al. (2011) with some minor modifications. At first, diseased tissues were cut in small pieces (1 cm^2), and then washed in running tap water for 10 min to eliminate the surface contaminants. After surface disinfection by immersion of plant pieces in 70% ethanol for 2 min and subsequently in 2% sodium hypochlorite (NaClO) solution for 2 min, all plant pieces were rinsed twice with sterile distilled water. Then, disinfested plant pieces were dried between sterile paper towels and placed onto 2% water agar (WA), and Petri dishes were kept at 25ºC under a 12 h photoperiod in the incubator for seven days. Fungal isolates were purified on potato dextrose agar (PDA) culture medium using the hyphal tip method, and then incubated at 25±1 °C until the pure fungal colonies appeared. For long–term storage, fungal isolates were grown on sterile filter papers placed on the PDA for 7–10 days. Subsequently, colonized filter papers were taken from the surface of the culture medium, dried at room temperature for four to five days, and then stored at -20 °C for future use.

Morphology

The morphological characterization of the fungal isolates was performed based on the morphology of colony, as well as features of the fruiting bodies such as conidiomata (pycnidia), conidiogenous cells, conidia, ascomata, asci, and ascospores. Morphological studies of the three obtained genera were performed on PDA, and the cultures were incubated under near–utraviolet (nUV) light (12 h light/12 h darkness) at 25 °C. After 10–14 days of incubation of the pure fungal colonies, the fungal features were assessed by light microscope using the microscopic slides mounts prepared in lacto–phenol or lacto–phenol cotton blue solutions. Colony diameter of the fungal strains was measured usually after 10 and 14 days. Macro– and micromorphological features of the different recovered isolates were measured. Micro–morphological features and measurements were performed according to Shoemaker et al. (1991), Crous et al. (2014) and Mapook et al. (2020). Photographs were taken using the BH2 light microscope (Olympus, Japan).

Phylogeny

After morphological identifications, DNA was extracted from the seven-day-old fungal mycelium of the recovered fungal strains using the method described by Zhong and Steffenson (2001). The complete internal transcribed spacer (ITS1-5.8S-ITS2) region of rDNA was amplified using the ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCGCTTATTGATATGC) primers (White et al. 1990). PCR amplification was carried out in a final volume of 25 µl containing 10 µL of *Taq* DNA polymerase Mix Red–$Mgcl_2$ (Sinaclon, Iran), 11 µL deionized water, 1 µL of each primer (10 pmol) and 2 µL of template DNA. The PCR amplification

was performed in Eppendorf Thermal Cycler (Mastercycler, Germany) with an initial denaturation at 94ºC for 3 min, followed by 35 cycles of denaturation step at 94ºC for 30 s, annealing at 60°C f or 30 s, and extension at 72°C for 30 s, and terminated with a final extension step at 72°C for 10 min The PCR product was analyzed in 1.5% agarose gel by gel electrophoresis technique with 1x Tris-Boric acid-EDTA buffer (TBE) and finally PCR products were sent to Cardiogenetic Research Center (Tehran, Iran) for sequencing.

Newly obtained DNA sequences were manually edited with Chromas 2.6.6 software (Technelysium, Australia), and the edited sequences were saved in FASTA format. The resulting sequences (450-620 bp) were subjected to BLAST search (Altschul et al. 1990) to find the most similar sequences in the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih. gov/genbank/). Thirty-eight reference ITS sequences of *Setophaeosphaeria, Acrocalymma*, *Tremateia* species as well as the ITS sequences of *Sarcinomyces crustaceus* (AJ244258) and *Daldinia concentrica* (JX658475) as outgroup were selected for phylogenetic analyses (Table 1). Then, the sequences were aligned with Clustal W (Thompson et al. 1994). Maximum likelihood (ML) analysis (Felsenstein 1973) of the aligned sequences was performed by heuristic search with Mega X (Kumar et al. 2018). Bootstrap analysis (Felsenstein 1985) of the ML tree was performed with 1000 replicates (Fig. 2). Sequences generated in the current study were deposited in the GenBank. Detailed information of the examined sequences in this study are provided in Table 1.

Table 1. ITS rDNA sequences used for phylogenetic analyses. Newly generated sequences are in boldface.

Species	Collection number/Strain	Host/Substrate	Origin	GenBank accession no.	Reference
				ITS	
Setophaeosphaeria hemerocallidis	CBS 138006	*Hemerocallis fulva*	China	NR171715	Crous et al. (2014)
	CBS 138006	*Hemerocallis fulva*	China	KJ869161	Crous et al. (2014)
	Strain BRPET38	*Broussonetia papyrifera*	China	MT658111	Xu (2020)
S. badalingensis	**ABRIICC 10360**	***Ruta graveolens***	**Iran**	**ON544079**	**This study**
	CBS 138007	*Hemerocallis fulva*	China	NR171716	Crous et al. (2014)
	CBS 138007	*Hemerocallis fulva*	China	KJ869162	Crous et al. (2014)
S. sidae	CBS 135108	*Sida* sp.	Brazil	NR156261	Quaedvlieg et al. (2013)
	CBS 135108	*Sida* sp.	Brazil	KF251149	Quaedvlieg et al. (2013)
S. citricola	KACC 49591	*Amaranthus patulus*	South Korea	MW412749	Choi (2020)
	CBS 143179	*Citrus australasica*	Australia	MH107916	Crous et al. (2018)
S. citri	Strain CPC 27148	*Citrus reticulata*	Italy	MG263524	Guarnaccia and Crous (2017)
S. microspora	-	Soil	China	MK329132	Zhang et al. (2021)
	CGMCC 3.19301	Soil	China	NR172843	Zhang and Cai (2018)
	-	Soil	China	MK329131	Zhang and Cai (2018)
Acrocalymma pterocarpi	MFLUCC 17-0926	*Pterocarpus indicus*	Thailand	NR163327	Jayasiri (2018)
	Voucher C233	*Pterocarpus indicus*	Thailand	MK347732	Jayasiri et al. (2019)
A. medicaginis	Yeh 0049	*Ipomoea pes-caprae*	Taiwan	MW376531	Yeh (2020)
	CPC 24340	*Medicago sativa*	Australia	KP170620	Trakunyingcharoen et al. (2014)
A. ampeli	NCYU 19-0008	*Ficus ampelas*	Taiwan	MW063151	Tennakoon et al. (2021)
	MFLU 19-2734	*Ficus ampelas*	Taiwan	MW063150	Tennakoon et al. (2021)
A. walkeri	**ABRIICC 10353**	***Diospyros kaki***	**Iran**	**OL376691**	**This study**
	CBS 257.93	-	Australia	MH862398	Vu et al. (2019)
A. vagum	Strain 1186	*Nervilia fordii*	China	MZ400559	Tan et al. (2021)
	Strain DSE1	*Glycyrrhiza uralensis*	China	MW042345	Li (2020)
A. fici	NTOU 4481	-	Taiwan	MZ422889	Cha et al. (2021)
	BR68	*Calamus castaneus*	Malaysia	MN637807	Azuddin and Zakaria (2019)
A. aquatica	MFLUCC 11-0208	-	China	NR121544	Schoch et al. (2014)
	MFLUCC11-0208	-	China	JX276951	Zhang et al. (2012)
Tremateia chiangraiensis	MFLUCC 17-1428	*Chromolaena odorata*	Thailand	NR168867	Mapook (2020)
	MFLUCC 17-1429	*Chromolaena odorata*	Thailand	MT214356	Mapook (2020)
T. chromolaenae	**ABRIICC 10342**	***Crataegus monogyna***	**Iran**	**MZ226451**	**This study**
	MFLUCC 17-1425	*Chromolaena odorata*	Thailand	NR168868	Mapook (2020)
T. thailandensis	MFLUCC 17-1430	*Chromolaena odorata*	Thailand	NR168869	Mapook (2020)
	MFLUCC 17-1430	*Chromolaena odorata*	Thailand	MT214361	Mapook et al. (2020)
T. murispora	HKAS 104642	Decaying wood	China	NR165916	Feng (2019)
	GZCC 18-2787	Decaying wood	China	MK962245	Feng et al. (2019)
T. camporesii	MFLU 19-2109	Dead branch	Thailand	NR169985	Samarakoon and Hyde (2019)
	MFLU 19-2109	Dead branch	Thailand	MN473061	Samarakoon and Hyde (2020)
Sarcinomyces crustaceus	CBS 156.89	*Endocronartium harknessii* gall	Netherlands	AJ244258	de Hoog et al. (1999)
Daldinia concentrica	CBS 117124	*Platanus* sp.	Greece	JX658475	Stadler et al. (2013)

RESULTS

In the present study, three species belonging to three different pleosporalean genera including *Acrocalymma walkeri, Setophaeosphaeria badalingensis*, and *Tremateia chromolaenae* were obtained and characterized based on both morphological criteria and molecular data. All three genera are new taxa for the funga of Iran. Furthermore, *Diospyros kaki, Ruta graveolens*, and *Crataegus monogyna* are reported as new hosts (*matrix nova*) for the respective recovered fungal species worldwide.

Molecular phylogeny

As shown in Fig. 1, phylogenetic analyses and obtained tree topology revealed that the obtained isolates from Iran are closely related to the relevant sequences which were deposited in the GenBank and placed in three distinct clades from top to bottom of the phylogenetic tree, corresponding to fungal families of *Phaeosphaeriaceae* (Clade I), *Acrocalymmaceae* (Clade II), and *Didymosphaeriaceae* (Clade III) of the *Pleosporales* (class *Dothideomycetes*) in the Ascomycota. species identified in this study can be distinguished from other reported species according to their substrate (host plant). *Setophaeosphaeria* species were placed in Clade I of the phylogenetic tree, with 80% bootstrap support. The *S. badalingensis* isolate (accession no. ON544079) was 99% identical to that of other isolates of this species from GenBank (NR171716 and KJ869162). *Acrocalymma* species were situated in Clade II of the phylogenetic tree, with 98% bootstrap support. The *A. walkeri* isolate (accession no. OL376691) was 100% identical to that of another isolate from GenBank (MH862398). Different *Tremateia* species were placed in Clade III, with 100% bootstrap support. The partial ITS rDNA sequence of the recovered *T. chromolaenae* isolate (accession no. MZ226451) was 100% identical to that of another isolate from GenBank (NR168868).

Taxonomy

Acrocalymma walkeri (Shoemaker, C.E. Babcock & J.A.G. Irwin) Crous & Trakun., IMA Fungus 5 (2): 407 (2014)

Colony on PDA attaining 46 mm in diam. after 14 days at 25 °C under near ultraviolet (nUV) light, 12 h light/12 h dark. Colony from above: medium dense, circular, with an entire smooth edge, slightly raised, fluffy to velvety, olivaceous to grayish at the margin, white in the center; reverse: white at the margin, dark brown to black in the center. Sexual morph: Ascomata 180–280 × 170–270 µm ($\bar{x}$ = 220 × 210 µm, n = 20), solitary or aggregated, immersed to semi-immersed, dark brown, globose, without ostiole, wall of textura angularis, with brown to pale brown cells. Asci 55–85 × 8–12 µm ($\bar{x}$ = 75 × 10 µm, n = 30), hyaline, bitunicate, fissitunicate, cylindrical, with a short stalk having eight overlapping biseriate ascospores. Ascospores 17–23 × 5–6 µm ($\bar{x}$ = 20 × 5 µm, n = 50), overlapping, obliquely biseriate overlapping, hyaline, fusiform, first with 1 -septate, becoming 3-septate when mature, with four guttules, smooth, narrowly rounded ends, constricted at the median septum. Asexual morph: not observed (Fig. 2).

Specimen examined. Iran, Mazandaran province, Babol, N 36°32'60.0" E 52°40'60.0, recovered from the branch of *Diospyros kaki* with canker symptom, November 2021, Abbas Atashi Khalilabad, isolate UZB-RB, GenBank Accession No. OL376691, ABRIICC 10353.

Note: Morphological features of the investigated isolate were similar to the description of *Acrocalymma walkeri* provided by Shoemaker et al. (1991). In the phylogenetic tree, our *Acrocalymma walkeri* isolate (OL376691) was grouped with other species of the genus in the clade II with 98% bootstrap support (Fig. 1). Among the 18 reported *Acrocalymma* species, only A. *arengae* Konta & K.D. Hyde, *A. pterocarpi* Jayasiri, E.B.G. Jones & K.D. Hyde, *A. chuxiongense* Y.W. Liu & X.Y. Zeng, *A. hongheense* Mortimer, and *A. walkeri* have known teleomorph, and the other 13 species have only anamorphic state. The morphological difference between *A. walkeri* with other related species is that *A. walkeri* has bigger ascospores compared to *A. pterocarpi* and smaller ascospores compared to *A. arengae*, *A. chuxiongense* and *A. hongheense* (Konta et al. 2023, Liu & Zeng. 2022).

Setophaeosphaeria badalingensis Crous & Y. Zhang ter, Persoonia 32: 271 (2014)

Colony on PDA slow growing, attaining 29 mm in diam. after 10 days at 25 °C under near ultraviolet (nUV) light, 12 h light/12 h dark. Colony from above: fluffy to velvety at the center and covered by floccose aerial mycelia, circular, with an entire smooth edge, initially white, becoming smoke grey on the surface; reverse: olivaceous grey to dark grey with distinct sectors. Conidiomata pycnidial, covered with hyphae, 220–310 × 210–300 µm ($\bar{x}$ = 250 × 260 µm, n = 20), aggregated or solitary, pale brown with dark central ostiole. Ostiole surrounded by brown, unbranched, thick-walled, septate, verruculose setae. *Textura angularis*, pale brown. Conidiophores absent. Conidiogenous cells, hyaline, smooth, ampulliform to doliiform, 5–7 × 3.5–4.5 µm ($\bar{x}$ = 6 × 3/5 µm, n = 20), phialidic with prominent periclinal thickening. Conidia solitary, hyaline, aseptate, smooth, two biguttulate to multiguttulate, subcylindrical with obtuse ends, straight or gently curved, 4.5–7 × 2.5–3.5 µm ($\bar{x}$ = 6 × 3 µm, n = 50). Sexual morph: not observed (Fig. 3).

Specimen examined. Iran, Gilan province, Asalem, N 37°43'52.5" E 48°57'38.0", recovered from stem of *Ruta graveolens* with canker symptom, November 2021, Abbas Atashi Khalilabad, isolate UT2021, GenBank Accession No. ON544079, ABRIICC 10360.

Note: Morphological features of the investigated isolate were similar to the description of *Setophaeosphaeria badalingensis* provided by Crous et al. (2014). Phylogenetic analyses indicated that *S. badalingensis* is a sister species to *S. hemerocallidis* Crous & Y. Zhang ter (CBS 138006 and BRPET38) with 76% bootstrap support (Fig. 1). However, morphologically, *S. hemerocallidis* differs from *S. badalingensis* in having bigger conidia (11–)13–16(–19) × (3–)3.5(–4) µm (Crous et al. 2014).

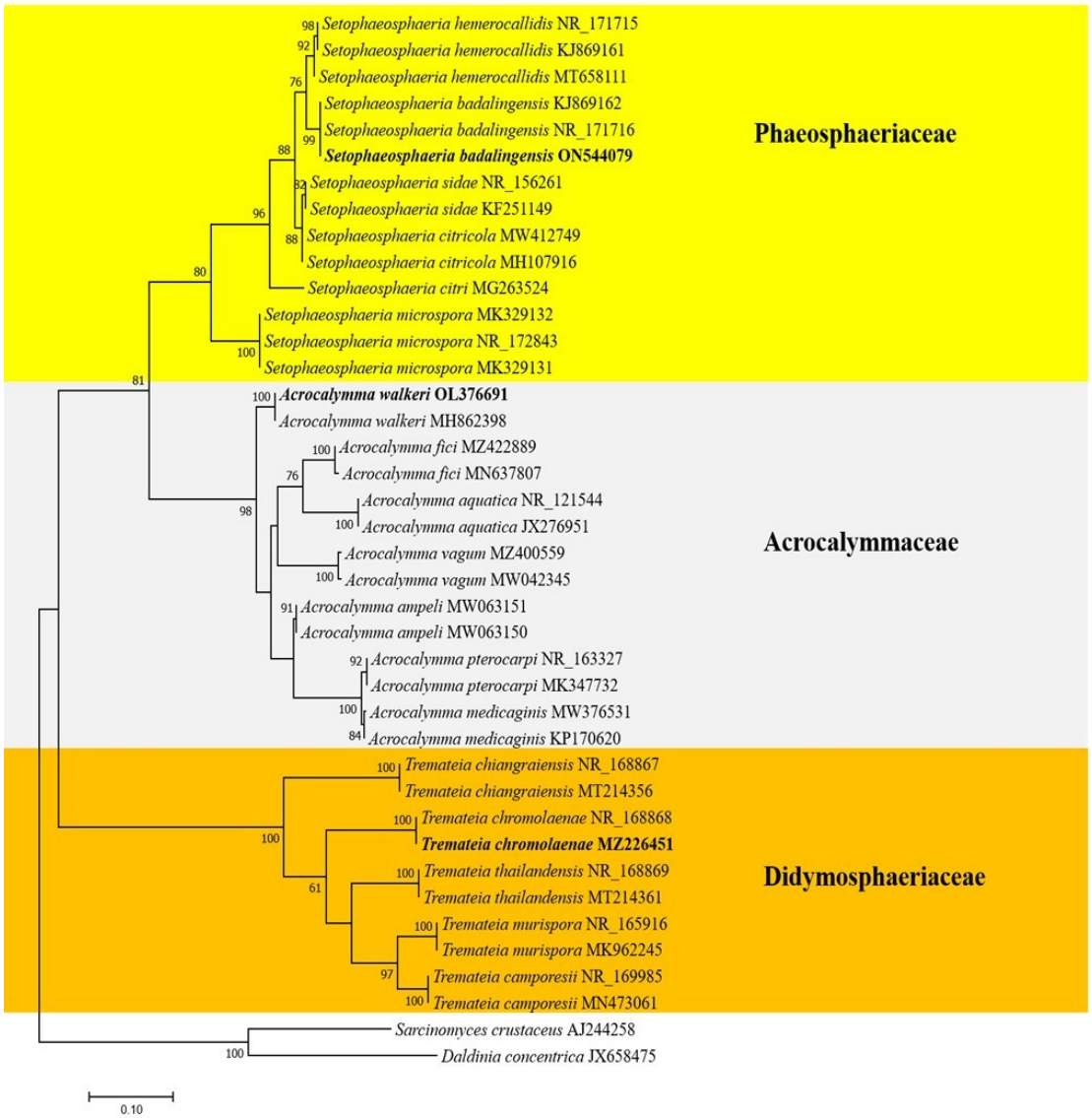

Fig. 1. Maximum likelihood (ML) tree generated in MEGA X, based on aligned sequences of the ITS rDNA regions of 38 isolates of *Setophaeosphaeria*, *Acrocalymma*, *Tremateia* species, and *Sarcinomyces crustaceus* (AJ244258) and *Daldinia concentrica* (JX658475) as the out-group sequences. Bootstrap values (1000 replicates) are indicated at the nodes. Sequences generated in the current study are in boldface. The scale bar indicates 0.10 expected nucleotide changes per site.

Tremateia chromolaenae Mapook & K.D. Hyde, Fungal Diversity 101: 40 (2020)

On PDA, the colony attained a diameter of 54 mm after 14 days at 25°C under near-ultraviolet light., 12 h light/12 h dark. Colony from above: circular, with smooth margin, mycelium slightly raised, initially white, becoming pale pinkish white on the surface; reverse: brown to pale brown, white to creamy white at margins. Sexual morph: Ascomata 150–225 × 160–220 µm diam ($\bar{x}$ = 195 × 188 µm, n = 10), immersed, solitary or aggregated, pale to dark brown to brown, globose, with ostiolar protruding neck, arranged in a

textura angularis with brown to pale brown cells, Asci 75–100 × 14–19 µm ($\bar{x}$ = 88 × 17.5 µm, n = 20), hyaline, bitunicate, fissitunicate, clavate to cylindric-clavate, with eight ascospores, straight or slightly curved, apically rounded, pedicellate. Ascospores 17–21 × 6–9 µm ($\bar{x}$ = 19 × 7.8 µm, n = 50), overlapping uni- two seriate, initially hyaline, becoming golden-brown at maturity, muriform, oval, ellipsoidal to subfusiform, straight or slightly curved, 3–6 transversely septate, with 1–2 vertical septa, constricted at the central septum, surrounded by a distinct hyaline gelatinous sheath. Asexual morph: not observed (Fig. 4).

Specimen examined. Iran, Gilan province, Asalem, N 37°44'03.8"E 48°57'07.9", recovered from branch of *Crataegus monogyna* with canker symptom, November 2021, Abbas Atashi Khalilabad, isolates UTFS-17, GenBank Accession No. MZ226451, ABRIICC 10342.

Note: The morphological features of the investigated isolate were similar to the description of *Trematеia chromolaenae* provided by Mapook et al. (2020). In the phylogenetic analyses, sequences of five *Trematеia* species were used. Our isolate (ABRIICC 10342) clustered with another isolate of *T. chromolaenae* (NR168868) with maximum bootstrap support of 100% (Figure. 1). *Trematеia chromolaenae* differs from *T. thailandensis* Mapook & K.D. Hyde, one of the nearest species, in having smaller ascomata, asci, and ascospores (Mapooket al. 2020).

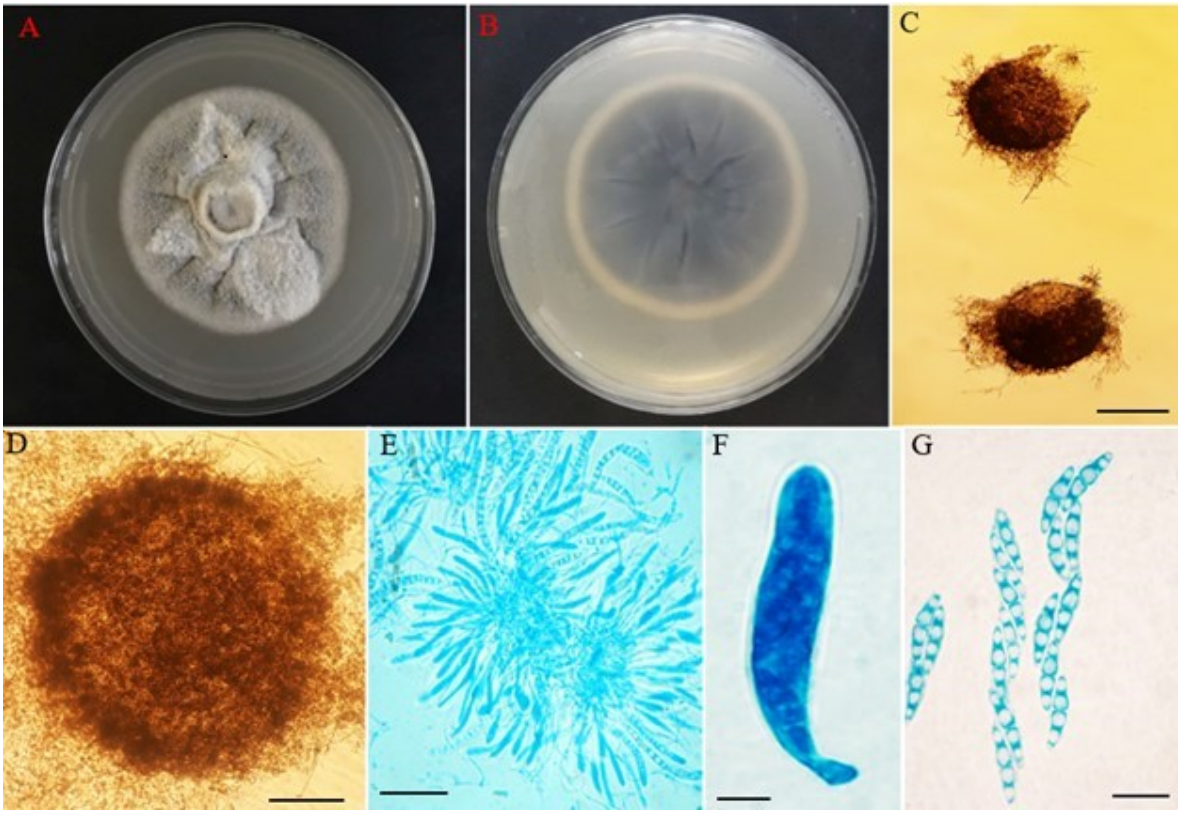

Fig. 2. *Acrocalymma walkeri* isolate UZB-RB. A.-B. Colony obverse and reverse on PDA incubated for 14 d at 25 °C in 12/12 h dark/nUV condition, C.-D. Ascomata, E.-F. Asci, and G. Ascospores. Scale bars: C = 200 µm. D= 50 µm. E–G = 10 µm.

DISCUSSION

In this study, three fungal isolates were obtained from the stems and branches of growing plants with canker symptoms in Guilan and Mazandaran, Iran. After examination of cultural and morphological features, the isolates were grouped into three categories. Based on morphological and molecular analyses, these three isolates were identified as *Acrocalymma walkeri, Setophaeosphaeria badalingensis*, and *Trematеia chromolaenae* in *Diospyros kaki* (oriental persimmon), *Ruta graveolens* (common rue), and *Crataegus monogyna* (common hawthorn), respectively. Isolate UT2021 was identified as *S. badalingensis* based on both the morphological description provided by Crous et al. (2014) and molecular data. Until 2022, only seven species of *Setophaeosphaeria* have been identified and described including *S. badalingensis S. citri* Guarn. & Crous, *S. Citricola* Crous & M.J. Wingf., *S. Hemerocallidis*, *S. microspora* Z.F. Zhang & L. Cai, *S. Setosa* (Leuchtm.) Crous and *S. Sidae* (Quaedvl., Verkley, R.W. Barreto & Crous) Crous (https://www.mycobank.org). *Setophaeosphaeria*

badalingensis can be distinguished from other species of the genus based on the nucleotide sequence of the ITS region.

This species was previously reported from *Hemerocallis fulva* (L.) L. (Crous et al. 2014). This is the first report of *S. badalingensis* as a new taxon for the funga of Iran. In addition, *S. badalingensis* is

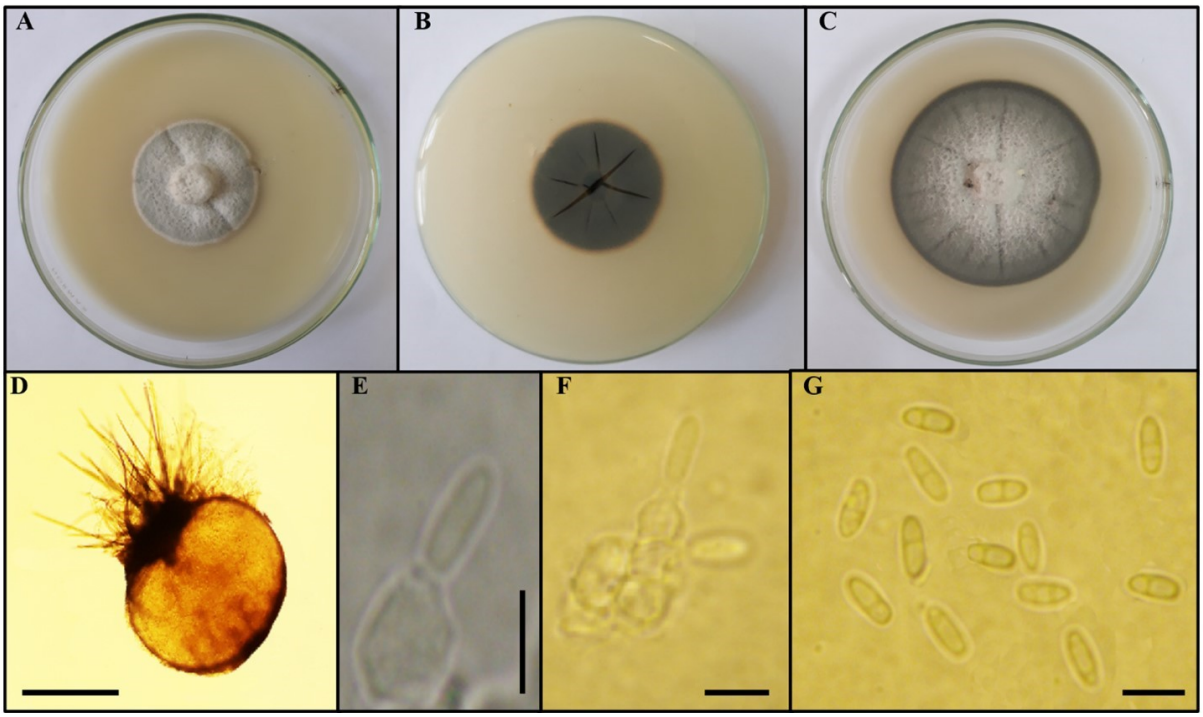

Fig. 3. *Setophaeosphaeria badalingensis*, isolate UT2021. A.-B. Colony obverse and reverse on PDA incubated for seven d at 25 °C in 12/12 h dark/nUV condition, C. Colony obverse on PDA after 14 d at 25 °C in 12/12 h dark/nUV condition, D. Pycnidium with setae, E.-F. Conidiogenous cells, and G. Conidia. Scale bars: D = 100 µm. E–G = 5 µm.

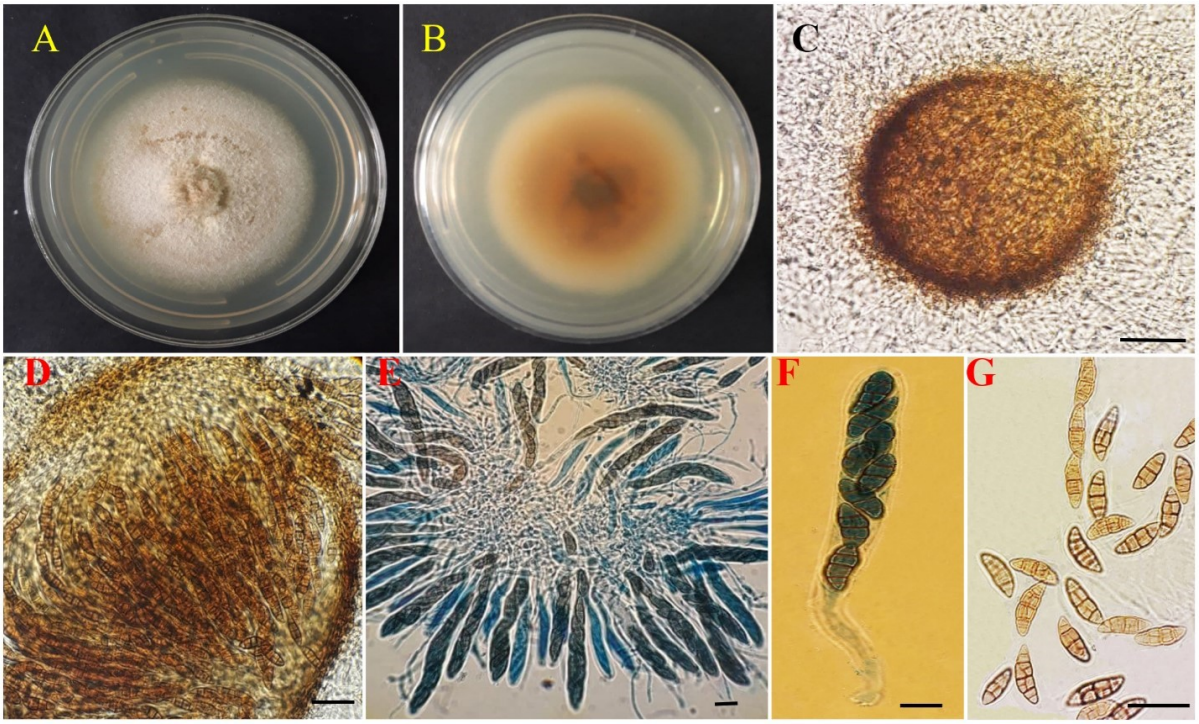

Fig. 4. *Tremateia chromolaenae* isolate UTFS-17. A.-B. Colony obverse and reverse on PDA incubated for 14 d at 25 °C in 12/12 h dark/nUV condition, C.-D. Ascomata, E.-F. Asci, and G. Ascospores. Scale bars: C = 60 µm. D= 30 µm. E–G = 20 µm.

reported here for the first time as a fungus associated with stem canker symptoms in *R. graveolens*. Isolate UZB-RB was identified as *Acrocalymma 26* based on the description provided by Shoemaker et al. (1991) as well as molecular data (Vu et al. 2019). *Acrocalymma walkeri* was originally described by Shoemaker et al. (1991) as *Massarina 26alker* Shoemaker, C.E. Babc. & J.A.G. Irwin with *Acrocalymma medicaginis* as its an asexual morph of this fungus. A recent phylogenetic analysis by Trakunyingcharoen et al. (2014) based on the sequence of the ITS rDNA region revealed that *M. walkeri* is nestled inside the broader *Acrocalymma* lineage. Therefore, Trakunyingcharoen et al. (2014) transferred *M. walkeri* to the genus *Acrocalymma* and treated *A. medicaginis* and *A. walkeri* as distinct species. This species has been previously reported in *Medicago sativa* (Trakunyingcharoen et al. 2014). This is the first report of *A. walkeri* as a new taxon for the funga of Iran. In addition, *A. walkeri* was reported for the first time as a fungus associated with branch canker symptoms in *D. kaki* trees worldwide. The isolate UTFS-17 was identified as *Trematеia chromolaenae* based on the description provided by Mapook et al. (2020) as well as molecular data. *Trematеia chromolaenae* differs from other species of this genus based on its smaller ascomata. This species was previously reported as a saprobic fungus from *Chromolaena odorata* (Mapook et al. 2020). This is the first report of *T. Chromolaenae* as a new taxon for the funga of Iran. In addition, *T. chromolaenae* is reported for the first time as a fungus associated with branch canker symptoms in *C. monogyna* trees worldwide.

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سه جنس جدید از راسته *Pleosporales* برای فونگای ایران

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چکیده: قارچ های متعلق به راسته (Pleosporalean) *Pleosporales* بیمارگرهای گیاهی، پوده‌زی و یا اندوفیت طیف وسیعی از گیاهان مهم از نظر اقتصادی هستند. به منظور شناسایی قارچ‌های همراه با علائم شانکر شاخه و ساقه در گیاهان، در پاییز ۱۴۰۰ باغات و جنگل‌های استان‌های گیلان و مازندران مورد بازدید قرار گرفت و نمونه‌های گیاهی دارای علائم از درخت زالزالک معمولی (*Crataegus monogyna* Jacq.)، بوته سداب معمولی (*Ruta graveolens* L.) و درخت خرمالوی شرقی (*Diospyros kaki* L.) جمع‌آوری شدند. استرین‌های قارچی با روش های معمول جداسازی و خالص سازی شدند و بر اساس خصوصیات ریخت شناختی شناسایی شدند. در نهایت، شناسایی مولکولی استرین های قارچی هم با استفاده از توالی یابی ناحیه ITS rDNA انجام شد. بر اساس تلفیق داده‌ها، در نهایت سه جنس و گونه قارچی متعلق به راسته *Pleosporales* شامل گونه *Acrocalymma walker* از خرمالو شرقی، گونه *Setophaeosphaeria badalingensis* از سداب معمولی و گونه *Tremateia chromolaenae* از زالزالک معمولی شناسایی و توصیف شدند. در تحقیق حاضر، هر سه گونه قارچی برای فونگای ایران جدید هستند. همچنین گیاهان خرمالوی شرقی، زالزالک معمولی و سداب معمولی به عنوان میزبان‌های جدید (matrix nova) برای گونه‌های قارچی مورد بررسی در جهان گزارش می شوند.

کلمات کلیدی: آسکومیکوتا، *Pleosporales*، ریخت شناسی، فیلوژنی، ITS rDNA

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