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New records of endophytic fungi on members of the subtribe *Triticinae* in Iran

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Abstract

During an investigation on endophytic fungi of grasses of the tribe *Triticeae* (subtribe *Triticinae*) in the west and northwestern provinces of Iran, two species, viz. *Sarocladium terricola* and *Selenophoma linicola*, and one genus, viz. *Septoriella* (*S. allojunci*), were newly identified for the funga of Iran using morphological traits and sequences of the internal transcribed spacer regions 1 and 2 including the intervening 5.8S nuclear ribosomal DNA (ITS). *Selenophoma linicola* and *Septoriella allojunci*, members of the class *Dothideomycetes*, were isolated from stem of *Aegilops cylindrica* and leaf of *A. cylindrica* in W. Azerbaijan and Lorestan Provinces, respectively. *Sarocladium terricola*, belonging to the class *Sordariomycetes*, was isolated from leaf of *Triticum aestivum* in Kermanshah Province. All identified species are described and illustrated herewith, and compared with closely related species. *Aegilops cylindrica* and *T. aestivum* are reported as new hosts (matrix nova) for these fungi.

Keywords: Biodiversity, phylogeny, *Poaceae*, symbiosis, taxonomy

گزارش‌های جدید از قارچ‌های اندوفیت اعضای زیرقبیله *Triticinae* در ایران

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خلاصه

طی بررسی قارچ‌های اندوفیت گیاهان علفی قبیله *Triticeae* (زیرقبیله *Triticinae*) در استان‌های غرب و شمال‌غرب ایران، دو گونه جدید *Sarocladium terricola* و *Selenophoma linicola* و یک جنس جدید *Septoriella* (*S. allojunci*)، براساس ویژگی‌های ریخت‌شناختی و توالی‌یابی ناحیه ITS از DNA ریبوزومی، برای قارچ‌های ایران شناسایی شدند. گونه‌های *Selenophoma linicola* و *Septoriella allojunci*، از اعضای رده *Dothideomycetes*، از ساقه *Aegilops cylindrica* و برگ *A. cylindrica* به ترتیب در استان‌های آذربایجان‌غربی و لرستان جداسازی شدند. همچنین، گونه *Sarocladium terricola* متعلق به رده *Sordariomycetes*، از برگ *Triticum aestivum* در استان کرمانشاه جداسازی شد. در این مقاله، تصاویر و توصیف‌های تمامی گونه‌های شناسایی شده ارایه و با گونه‌های نزدیک مقایسه شده است. گونه‌های گیاهی *A. cylindrica* و *T. aestivum* نیز به عنوان میزبان‌های جدید برای این قارچ‌ها گزارش می‌شوند.

واژه‌های کلیدی: تاکسونومی، تبارزایی، تنوع زیستی، گندمیان، هم‌زیستی

Introduction

The term 'endophyte' was first introduced in a broad definition by de Bary (1886), as "Any organism found within tissues of living plants including everything from virulent foliar pathogens to mycorrhizal root symbionts". Several researchers then proposed various definitions of endophytes; one of the most widely-accepted has been that of Petrini (1991), as "All organisms inhabiting plant organs at some time in their life that can colonize internal plant tissues without causing apparent harm to the host". Different groups of organisms such as fungi and bacteria (actinomycetes & mycoplasmas) have been reported as endophytes of plants (Pimentel *et al*. 2011, Singh & Dubey 2015). Endophytic fungi are known to promote plant growth (Mucciarelli *et al*. 2003), improve their ability to tolerate abiotic and biotic stresses (Rodriguez *et al*. 2008), and produce bioactive antimicrobial compounds, used in agriculture, commercial industry, and medicine (Leuchtmann *et al.* 2000, Tan & Ziu 2001, Strobel & Daisy 2003, Joseph & Priya 2011). However, to achieve these benefits, it is essential to explore the diversity of endophytic fungi in different ecosystems.

The *Triticeae*, consisting of 503 species in 27 genera, is an important tribe in *Poaceae* that includes genera with many domesticated species (Soreng *et al*. 2015). This tribe splits into two subtribes, viz. *Hordeinae* and *Triticinae* (Soreng *et al.* 2015). In *Hordeinae*, endophytic fungi have been found in many genera including *Agropyron* spp. (Yanagida *et al*. 2004, 2005), *Elymus* spp. (White 1987, Leuchtmann & Clay 1993, Saikkonen *et al*. 2000, Nan & Li 2001, Moon *et al*. 2004, Spooner & Kemp 2005, Sánchez Márquez *et al.* 2008, Charlton *et al*. 2012, Card *et al.* 2014), *Elytrigia* sp. (Nan & Li 2001), *Hysterix* sp. (Leuchtmann & Clay 1993), *Hordelymus* sp. (Moon *et al*. 2004, Oberhofer & Leuchtmann 2012, Leuchtmann & Oberhofer 2013), *Hordeum* spp. (Youssef & Dugan 2000, Nan & Li 2001, Moon *et al*. 2004), *Leymus* sp. (Nan & Li 2001), *Roegneria* sp. (Nan & Li 2001, Kang *et al*. 2011), and *Sitanion* sp. (White 1987).

In the subtribe *Triticinae* (treated in the present survey), endophytic fungi have been reported mainly in two genera including *Triticum* L. and *Aegilops* L. (Crous *et al*. 1995, Bishop *et al.* 1997, Marshall *et al.* 1999, Larran *et al.* 2007, Ofek-Lalzar *et al*. 2016, Comby *et al*. 2017). Crous *et al.* (1995) investigated the occurrence of endophytic fungi from leaves, roots and stems in cultivars of *Triticum aestivum* L. in South Africa. In their research, *Phoma glomerata* (Corda) Wollenw. & Hochapfel was not restricted to only one tissue type, whereas *Alternaria alternata* (Fr.) Keissl., basidiomycete sp. 1, *Epicoccum nigrum* Link and *Pleospora herbarum* (Pers.) Rabenh. occurred primarily in the leaves, and *Fusarium avenaceum* (Fr.) Sacc. was frequently found growing in roots.

Bishop *et al.* (1997) isolated *Neotyphodium* sp. from *Triticum aestivum* cv. Super Dwarf. However, Kwon & Anderson (2001) concluded that, the fungus described by Bishop *et al*. (*l.c.*) was misidentified and later described it as a species closely related to *Fusarium proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg. Marshal *et al.* (1999) isolated seedborne endophytic fungi from wild *Triticum* species in Turkey. They found two different endophytic fungi in *Triticum* species, i.e., members of the genus *Neotyphodium* in *T. dichasians* (Zhuk.) Bowden [now *Aegilops markgrafii* (Greuter) K. Hammer] and *T. tripsacoides* (Jaub. & Spach) Bowden [now *Amblyopyrum muticum* (Boiss.) Eig], and members of the genus *Acremonium* Link in *T. columnare* (Zhuk.) Morris & Sears [now *Aegilops columnaris* Zhuk.], *T. cylindricum* Ces. [now *Aegilops cylindrica* Host], *T. monococcum* L., *T. neglecta* Morris & Sears [now *Aegilops neglecta* Req. ex Bertol.], *T. recta* Morris & Sears [now *Aegilops neglecta* Req. ex Bertol.], *T. triunciale* (L.) Raspail [now *Aegilops triuncialis* L.], *T. turgidum* L., and *T. umbellulatum* (Zhuk.) Bowden [now *Aegilops umbellulata* Zhuk.].

Larran *et al.* (2007) studied the composition species of endophytes in healthy wheat plants in Argentina and determined their infection frequencies from leaves, stems, glumes, and grains. The frequency of microorganisms was higher in the grains than in the other organs and *Alternaria alternata*, *Cladosporium herbarum* (Pers.) Link, *Cryptococcus* sp., *Epicoccum nigrum*, *Fusarium graminearum* Schwabe, *Penicillium* sp., and *Rhodotorula rubra* (Schimon) F.C. Harrison were isolated in the highest frequency. In order to find the biological control agents for the management of fusarium head blight of wheat in France, Comby *et al.* (2017) isolated 27 endophytic fungi, mainly *Aureobasidium proteae* (Joanne E. Taylor & Crous) Joanne E. Taylor & Crous, *Chaetomium globosum* Kunze, *Cladosporium halotolerans* Zalar, de Hoog & Gunde-Cim., *Microdochium bolleyi* (R. Sprague) de Hoog & Herm.-Nijh., *Rhodotorula lysinophila* Nagah., Hamam., Nakase & Horikoshi, *Sarocladium kiliense* (Grütz) Summerb., and *Sporobolomyces roseus* Kluyver & C.B. Niel, from Caphorn and Apache cultivars of *Triticum aestivum*.

In an investigation on biodiversity of endophytic fungi of grasses of the tribe *Triticeae* (subtribe *Triticinae*) in the west and northwestern provinces of Iran (2018–19), three taxa were identified as new for Iranian funga that are characterized here using morphological and molecular data.

Materials and Methods

- Plant materials

During spring and summer of 2018–19, fresh asymptomatic leaves and stems of *Aegilops cylindrica* and *Triticum aestivum* were collected from the west and northwestern provinces of Iran. Samples were stored in zip-lock bags, transported on ice to the laboratory and kept refrigerated until processing within 48 h.

- Isolation of endophytic fungi

Fungal isolates were recovered from the healthy plant tissues using a surface-sterilization technique described in Florea *et al.* (2015) with slight modifications. Leaves and stems of each plant were cut into 5–10 mm long segments and were surface-sterilized (70% ethanol for 2 min, sterile distilled water three times, 2.5% sodium hypochlorite for 2 min, and sterile water three times), followed by draining on sterile filter paper. Plant tissues were placed on the potato-dextrose-agar (PDA; Merck, Germany) containing 150 mg/l each of penicillin G (Jiangxi Dongfeng Pharmaceutical Co., Ltd., China) and streptomycin sulfate (Sigma-Aldrich, Inc., USA). The plates were sealed, incubated for 2 months at 25 °C, and examined weekly for endophyte growth.

- Morphological identification

Potato-carrot-agar (PCA; 20 g potato, 20 g carrot, and 20 g agar per 1 L of distilled water; Kirk *et al.* 2008) and pine-needle-agar (PNA; 2% tap water agar, with sterile pine needles) (Crous *et al.* 2006) was used to induce fungal sporulation. Single-spore cultures were obtained by serial dilutions and transferring a single germinating conidium to a new Petri dish containing PDA. Colony morphology, color, and growth rate were determined on PDA and PCA at 25 °C in the dark (Rayner 1970). All microscopic observations, measurements and photographs were taken with samples mounted in 85% lactic acid, under a Dino Capture 2.0 image software installed on an Olympus BH-2 Microscope (Tokyo, Japan). Macroscopic observations were carried out using an Olympus SZH Stereo Microscope. Subcultures of all strains are preserved at the Fungal Culture Collection (IRAN) of the Iranian Research Institute of Plant Protection (Tehran, Iran).

- Molecular identification

Fresh fungal mycelium (25 mg) was scraped from surface of a PDA plate incubated at 20 ºC for 14 days and transferred into a 1.5 mL centrifuge tube. DNA extraction was performed according to Liu *et al.* (2000) with an initial step of grinding the mycelia in liquid nitrogen. The ITS-rDNA region (ITS1-5.8S-ITS2) was amplified using primers ITS1 [5′-tcc gta ggt gaa cct gcg g]

and ITS4 [5′-tcc tcc gct tat tga tat gc] (White *et al.* 1990). The PCR reaction (25 µL) contained 1 µL (10 pmol/µL) of each primer (Takapouzist Inc., Tehran), 1.0 µL genomic DNA (30 ng/µL), 2.5 µL 10× high yield PCR buffer (Jena Bioscience, Germany), 0.3 µL *Taq* DNA polymerase (5 units/µL, Jena Bioscience, Germany), 1 µL MgCl2 (25 mM), 0.5 µL dNTPs (10 mM), and 17.7 µL sterile distilled water. PCR amplification of ITS-rDNA region was carried out using a MyCycler Thermal Cycler (Bio-Rad, USA) following conditions described by Mehrabi *et al*. (2016). The PCR products were purified by Microsynth Company (Switzerland) and submitted for sequencing to a capillary sequencing machine (ABI 3730XL, Applied Biosystem, Foster City, CA) of the same company.

- Phylogenetic analyses

The sequences obtained in this study were compared with those of the NCBI's GenBank nucleotide database using the MegaBlast (Zhang *et al*. 2000). The alignments were obtained using MAFFT Ver. 7 (http://mafft.cbrc.jp/alignment/server/index.html) (Katoh *et al.* 2019), and manually optimized with MEGA Ver. 6 (Tamura *et al.* 2013).

Phylogenetic analyses of the aligned dataset were conducted using Maximum Likelihood (ML) and Bayesian Inference (BI). Maximum likelihood analysis was performed with RAxML-HPC2 on XSEDE Ver. 8.2.10 (Stamatakis 2014) on the CIPRES Science Gateway portal (Miller *et al*. 2010) using GTRGAMMA substitution model. Nonparametric bootstrap iterations were run with 1000 replicates. BI analysis was carried out using MrBayes Ver. 3.1.2 (Huelsenbeck & Ronquist 2001) with a Markov Chain Monte Carlo (MCMC) algorithm with Bayesian posterior probabilities (Rannala & Yang 1996). Using the AIC implemented in JModeltest 2 (Guindon & Gascuel 2003, Darriba *et al*. 2012), the Bayesian analysis employed the GTR+I+G model for the ITS matrix. Four MCMC chains were run from random trees for 10^6 generations, and the trees were sampled every 100 generation, which resulted in 10^4 total trees. The first 25% of the trees were discarded as the burn-in phase of each analysis and the posterior probabilities were calculated using the remaining 7500 trees. Trees were drawn with FigTree Ver. 1.4.0 (Rambaut 2012). The sequences generated in this study were deposited in GenBank (Table 1).

Table 1. Sequences used in the phylogenetic analyses

Taxon	Strain	Origin	GenBank accession number (ITS)	Reference
Saccharomyces cerevisiae	CBS 1171^T	Brewers top yeast, The Netherlands	NR_111007	Schoch *et al.* (2014)
Sarocladium bacillisporum	CBS 425.67^T	Soil, Ontario, Canada	HE608639	Giraldo *et al*. (2015)
S. bactrocephalum	CBS 749.69^T	*Ustilago* sp., Canada	HG965006	Giraldo *et al*. (2015)
S. bifurcatum	UTHSC 05-3311^T	Bronchoalveolar lavage fluid, USA	HG965009	Giraldo *et al*. (2015)
S. brachiariae	CGMCC 2192^T	Leaves of *Brachiaria brizantha*, China	EU880834	Liu *et al.* (2017)
S. gamsii	CBS 707.73^T	Dead stem of *Pandanus lerum*, Sri Lanka	HG965015	Giraldo *et al*. (2015)
S. glaucum	CBS 796.69^T	Woolen overcoat, Solomon Islands	FN691454	Giraldo *et al.* (2015)

Table 1 (contd)

S. hominis	UTHSC 04-1034^{T}	Leg, USA	HG965011	Giraldo *et al*. (2015)
S. implicatum	CBS 959.72^{NT}	Desert soil, Egypt	HG965023	Giraldo *et al*. (2015)
S. kiliense	CBS 122.29^{T}	Skin, Germany	FN691446	Perdomo *et al*. (2011)
S. ochraceum	CBS 428.67^{T}	*Zea mays*, Kenya	HG965025	Giraldo *et al*. (2015)
S. oryzae	CBS 180.74^{ET}	*Oryza sativa*, India	HG965026	Giraldo *et al*. (2015)
S. pseudostrictum	UTHSC 02-1892 ^{T}	Sputum, USA	HG965029	Giraldo *et al*. (2015)
S. spinificis	BCRC 34941^{T}	Root of *Spinifex littoreus*, Taiwan	KF269096	Yeh & Kirschner (2014)
S. strictum	CBS 346.70^{T}	*Triticum aestivum*, Germany	FN691453	Giraldo *et al*. (2015)
S. subulatum	MUCL 9939^{T}	Soil, Egypt	HG965031	Giraldo *et al*. (2015)
S. summerbellii	CBS 430.70^{T}	Soil from greenhouse, The Netherlands	HG965034	Giraldo *et al*. (2015)
S. terricola	CBS 243.59^{T}	Forest soil, USA	FN706553	Giraldo *et al*. (2015)
S. terricola	MUCL 12011	Leaf of *Milleta laurentii*, Democratic Republic of Congo	HG965039	Giraldo *et al*. (2015)
S. terricola	UTHSC 03-2933	Bronchial wash fluid, USA	HG965041	Giraldo *et al*. (2015)
S. terricola	**IRAN 3462C**	***Triticum aestivum*, Iran**	**OR053727**	**This study**
S. zeae	CBS 800.69^{T}	Stalk of *Zea mays*, USA	FN691451	Perdomo *et al*. (2011)
Selenophoma australiensis	CBS 124776^{T}	*Eucalyptus mineata*, Australia	GQ303293	Cheewangkoon *et al*. (2009)
S. linicola	CBS 468.48^{T}	*Linum usitatissimum*, Canada	NR_160075	Vu *et al*. (2019)
S. linicola	**IRAN 3461C**	***Aegilops cylindrica*, Iran**	**OR053726**	**This study**
S. mahoniae	CBS 388.92	Leaf of *Mahonia repens*, Colorado	KT693746	van Nieuwenhuijzen *et al*. (2016)
Septoriella agrostina	MFLU 18-0113^{HT}	*Agrostis stolonifera*, Italy	MG828945	Wanasinghe *et al*. (2018)
S. allojunci	MFLUCC 15-0701^{T}	*Juncus* sp., Italy	KU058718	Li *et al*. (2015)
S. allojunci	**IRAN 3468C**	***Aegilops cylindrica*, Iran**	**OR053728**	**This study**
S. germanica	CBS 145372^{T}	Culm of *Phragmites australis*, Germany	MK539965	Marin-Felix *et al*. (2019)
S. artemisiae	MFLUCC 17-0693^{T}	*Artemisia austriaca*, Russia	MG828929	Wanasinghe *et al*. (2018)
S. arundinicola	MFLU 16-0225^{HT}	*Arundo plinii*, Italy	MG828946	Wanasinghe *et al*. (2018)

Table 1 (contd)

S. arundinis	MFLUCC 15-0702^{T}	Reed (*Juncaceae*), Italy	KU058716	Li *et al*. (2015)
S. bromi	MFLUCC 13-0739^{T}	*Bromus sterilis*, Italy	KU058717	Li *et al*. (2015)
S. chlamydospora	MFLUCC 15-0177^{T}	*Dactylis* sp., Italy	KU163658	Jayasiri *et al*. (2015)
S. elongata	MFLUCC 12-4444^{T}	Unknown, Italy	KM491546	Li *et al*. (2015)
S. dactylidis	MFLU 15-2720^{HT}	*Dactylis* sp., Italy	KU163657	Jayasiri *et al*. (2015)
S. forlicesenica	MFLUCC 15-0470^{T}	*Dactylis glomerata*, Italy	KX926422	Thambugala *et al*. (2017)
S. garethjonesii	MFLUCC 15-0469^{T}	*Dactylis glomerata*, Italy	KX926425	Thambugala *et al*. (2017)
S. hirta	CBS 536.77^{ET}	*Agropyron repens*, Germany	KR873249	Crous *et al*. (2015)
S. hibernica	CBS 145371^{T}	On unidentified grass species, Ireland	MK539966	Marin-Felix *et al*. (2019)
S. hollandica	CBS 145374^{T}	*Phragmites australis*, The Netherlands	MK539967	Marin-Felix *et al*. (2019)
S. hubertusii	CBS 338.86^{T}	*Phragmites australis*, France	KF251230	Quaedvlieg *et al*. (2013)
S. italica	MFLUCC 13-0267^{T}	*Arundo plinii*, Italy	KX926421	Thambugala *et al*. (2017)
S. leuchtmannii	CBS 459.84^{IT}	*Phragmites australis*, Switzerland	KF251188	Quaedvlieg *et al*. (2013)
S. muriformis	MFLUCC 13-0277^{T}	*Lolium* sp., Italy	KX926415	Thambugala *et al*. (2017)
S. neoarundinis	MFLUCC 15-0027^{T}	*Arundo plinii*, Italy	KY706139	Thambugala *et al*. (2017)
S. neodactylidis	MFLUCC 13-0618^{T}	Dead upright stems of *Dactylis glomerata*, Italy	KP744432	Liu *et al*. (2015)
S. oudemansii	CBS 138012^{T}	*Phragmites australis*, The Netherlands	KR873250	Crous *et al*. (2015)
S. phragmitis	CBS 140065^{ET}	*Phragmites* sp., The Netherlands	KR873251	Crous *et al*. (2015)
S. poae	CBS 136766^{T}	*Poa* sp., The Netherlands	KJ869111	Crous *et al*. (2014)
S. pseudophragmitis	CBS 145417^{T}	Leaves of *Phragmites* sp., The Netherlands	MK560161	Marin-Felix *et al*. (2019)
S. rosae	MFLU 18-0114^{HT}	*Rosa canina*, Italy	MG828948	Wanasinghe *et al*. (2018)
S. subcylindrospora	MFLUCC 13-0380^{T}	Dead stem of *Dactylis glomerata*, Italy	KT314184	Ariyawansa *et al*. (2015)
S. tridentina	MFLUCC 15-0475^{T}	*Dactylis glomerata*, Italy	KX926424	Thambugala *et al*. (2017)
S. vagans	CBS 604.86	*Calamagrostis arundinacea*, Sweden	KF251193	Quaedvlieg *et al*. (2013)

T: ex-type, ET: ex-epitype, IT: ex-isotype, HT: holotype, and NT: ex-neotype strains. CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; IRAN: Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Tehran, Iran; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; MUCL: Mycothèque de l'Université Catholique de Louvain, Louvain-la-Neuve, Belgium; and UTHSC: Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio (Sequences generated in this study are marked in bold).

Results and Discussion

- Phylogeny

The sequences generated in this study were aligned against closely related sequences of *Sarocladium*, *Selenophoma*, and *Septoriella* species, mostly from Quaedvlieg *et al*. (2013), Giraldo *et al*. (2015), Thambugala *et al.* (2017), and Marin-Felix *et al*. (2019) (Table 1). The ITS sequence dataset comprised 23 isolates with a total alignment length of 838 bp, with *Saccharomyces cerevisiae* (Desm.) Meyen (CBS 1171) selected as outgroup. Analyses using ML and BI resulted in phylogenies with congruent topologies. Therefore, only ML topology is presented with RAxML-BS and BI-PP support values superimposed (Fig. 1). The ML analysis of the dataset yielded a best scoring tree with a final ML optimization likelihood value of − 4995.765086. The matrix had 460 distinct alignment patterns, with 33.66% of undetermined characters or gaps.

Parameters for the GTRGAMMA model of the ITS sequence dataset were as follows: estimated base frequencies A = 0.228921, C = 0.274226, G = 0.249810, and T = 0.247043; substitution rates AC = 1.180023, AG = 2.153025, AT = 2.021030, CG = 1.059852, CT = 3.545571, and GT = 1.000000; gamma distribution shape parameter α = 0.576370.

In this study, three fungal strains isolated from grasses of the tribe *Triticeae* in Iran as endophyte, i.e., IRAN 3461C, IRAN 3462C, and IRAN 3468C, were placed in three separate clades (Fig. 1), close to the type strains of species *Selenophoma linicola* (ML = 100%, BI = 1), *Sarocladium terricola* (ML = 98%, BI = 1), and *Septoriella allojunci* (ML = 93%, BI = 0.99), respectively.

- Taxonomy

1. *Sarocladium terricola* (J.H. Mill., Giddens & A.A. Foster) A. Giraldo, Gené & Guarro, Persoonia 34: 22 (2014)

Sexual morph undetermined. Asexual morph hyphomycetous. On PCA vegetative hyphae 1–2 µm wide, hyaline, thin-walled, septate, branched, smooth-walled. Conidiophores erect, simple, hyaline, smooth-walled. Phialides arising from vegetative hyphae, hyaline, slender, thin- and smooth-walled, 20–25 × 1–2 µm (av. = 23 × 1.2 µm, n = 10). Conidia arranged in long chains, aseptate, hyaline, thin- and smooth-walled, fusiform with sharply pointed ends, 4–5 × 1–1.5 µm (av. = 4.4 × 1.3 µm, n = 20). Chlamydospores not observed (Fig. 2).

Culture characteristics (25 °C, 7 d): Colonies on PDA attaining 20 mm diam., flat, floccose, white; margins regular; reverse orange (7). Colonies on PCA attaining 30 mm diam., flat, floccose, white; margins regular; reverse of the same obverse colony color.

Specimen examined: IRAN: Kermanshah Province, Gilan-e-gharb, 34˚04′19.41″ N, 45˚57′36.31″ E, leaf endophyte of *Triticum aestivum*, 13.5.2018, M. Mehrabi (IRAN 3462C).

Sarocladium W. Gams & D. Hawksw. (*Sordariomycetes*, *Hypocreales*) was introduced by Gams & Hawksworth (1976) with *S. oryzae* (Sawada) W. Gams & D. Hawksw. as the type species. Some of *Sarocladium* species were formerly placed in *Acremonium* Link (Summerbell *et al*. 2011), but it can be morphologically differentiated from *Acremonium* by its elongated phialides arising solitarily on vegetative hyphae or on conidiophores that are sparsely or repeatedly branched, the production of abundant adelophialides and elongated conidia (Giraldo *et al.* 2015). *Sarocladium* currently encompasses 29 species (Index Fungorum 2023).

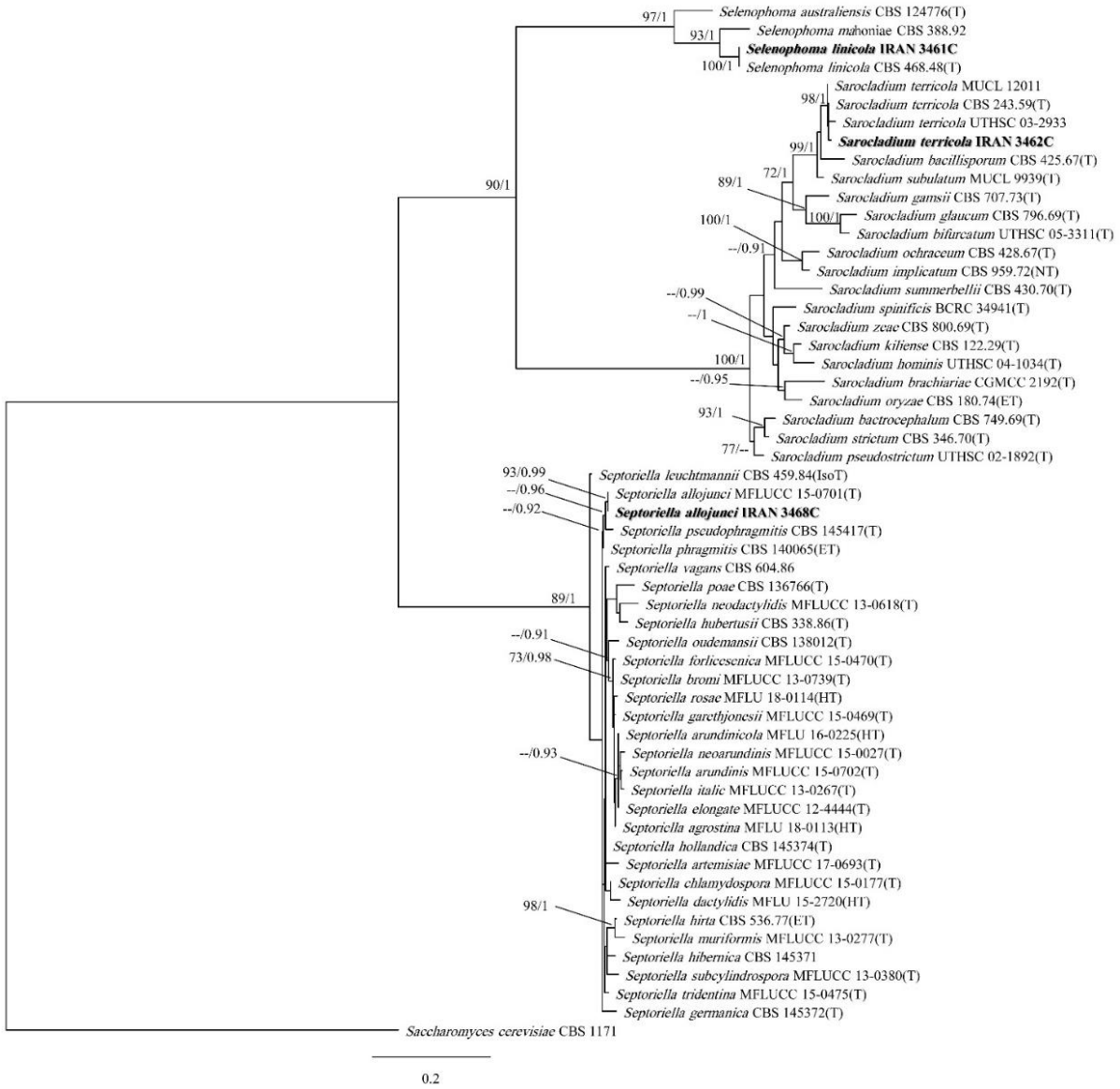

Fig. 1. Phylogeny obtained by RAxML analysis of the ITS rDNA of sequence alignment of *Sarocladium*, *Selenophoma*, and *Septoriella* species. Maximum likelihood bootstrap supports (≥70%) and Bayesian posterior probabilities support (≥0.9%) are given at the branches, respectively (Dashes replace non-significant values). Sequences generated in this study are marked with bold font. The tree was rooted with *Saccharomyces cerevisiae* CBS 1171.

The genus *Sarocladium* includes ecologically diverse species ranging from common saprotrophs on plant seeds and debris, bone, skin, water, and air or in soil (Giraldo *et al.* 2015) to endophytic taxa (Liu *et al.* 2017, Yeh & Kirschner 2014), human pathogens (Fincher *et al.* 1991, Perdomo *et al.* 2011), and plant pathogens (Gams & Hawksworth 1976, Ayyadurai *et al*. 2005). Previous studies identified nine *Sarocladium* species from various plants as endophytes, i.e., *S. zeae* (W. Gams & D.R. Sumner) Summerb. from leaf of *Zea mays* L. (Wicklow *et al.* 2005, Poling *et al.* 2008), *S. ochraceum* (Onions & G.L. Barron) Summerb. from seed of *Festuca callieri* Markgr. and *Aegilops triuncialis* L. (Tunali *et al*. 2000), *S. bacillisporum* (Onions & G.L. Barron) Summerb. and *S. strictum* (W. Gams) Summerb. from stem of *Prumnopitys andina* (Poepp. ex Endl.) de Laub. (Hormazabal & Piontelli 2009), *S. bactrocephalum* (W. Gams) Summerb. from seed of *Aegilops mutica* Boiss. and *Aegilops cylindrica* Host. (Tunali *et al.* 2000), *S. kiliense* (Grütz) Summerb. from leaf of *Triticum aestivum* (Comby *et al.* 2017), *S. brachiariae* X.B. Liu, G.X. Huang & Z.K. Guo from leaf of *Brachiaria*

brizantha (Liu *et al*. 2017), *S. spinificis* Y.H. Ye & R. Kirschner from root of *Spinifex littoreus* (Burm. f.) Merr. (Yeh & Kirschner 2014), and *S. terricola* from leaf of *Myracrodruon urundeuva* Allemão (Pádua *et al*. 2019).

In Iran, *Sarocladium oryzae* was reported from *Oryza sativa* L. (Naeemi *et al*. 2002). Ebrahimi & Fotouhifar (2016) also reported two species of *Sarocladium*, *S. kiliense*, and *S. strictum*, from scab or scab-like symptoms on leaves of various host plants in Iran. *Sarocladium subulatum* A. Giraldo, Gené & Guarro and *S. pseudokiliense* Rezakhani, Khodap., Masigol & Grossart, isolated from merged rotten leaves in Anzali lagoon, Iran (Rezakhani *et al*. 2019, Masigol *et al*. 2022).

Based on a MegaBlast search of NCBIs GenBank nucleotide database, the closest hit using the ITS sequence of our isolate (IRAN 3462C) was *S. terricola* (CBS 243.59, GenBank MH857853) with a similarity of 99% (528/530). Phylogenetic analyses based on the ITS sequence data also showed that, the isolate group of the present study with *S. terricola* (CBS 243.59) are with high statistical support value (ML = 98%, BI = 1.0, Fig. 1).

Morphological features of the investigated isolate in the present study are similar to description of *S. terricola* by Giraldo *et al.* (2015). Phylogenetically, *S. terricola* is close to *S. subulatum* and *S. bacillisporum* (Fig. 1), but it can be differentiated by its faster growth rate on PDA at 25 °C after 14 d (34 mm diam. in the strain of the present study vs. 20–24 mm diam. in *S. bacillisporum* and 17–20 mm diam. in *S. subulatum*) and conidial size. *Sarocladium bacillisporum* produces small rod-shaped conidia (4–6 × 1 µm) and *S. subulatum* has large conidia (5–8 × 1–2 µm), and its phialides are wider at the base (2–2.5 µm) than the other two species (Giraldo *et al.* 2015). *Sarocladium terricola* has been reported from different sources such as plants, soil, sputum, bronchial wash fluid, bronchoalveolar lavage fluid, sinus and bone (Giraldo *et al.* 2015), and also as endophyte from leaves of the medicinal plant *Myracrodruon urundeuva* in Brazil. This is the first report of this species from Iran and from *Triticum aestivum* as its host.

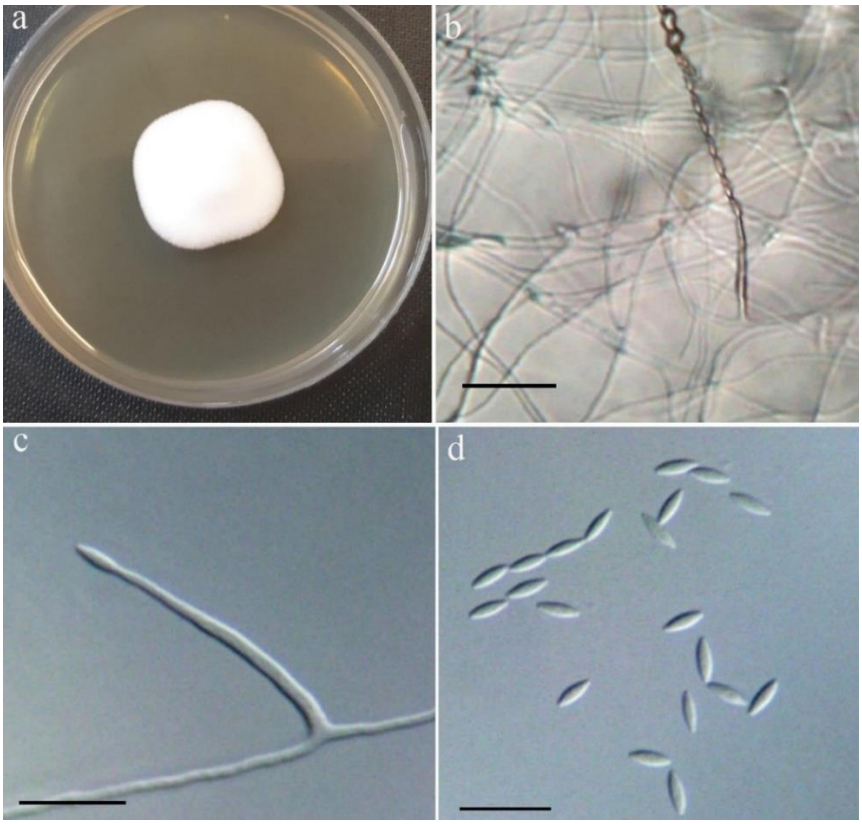

Fig. 2. *Sarocladium terricola* (IRAN 3462C): a. Colony morphology on PDA incubated for 7 d at 25 °C, b. Conidial chain formed on PCA, c. Phialide, D. Conidia (Bars: b = 20 µm, c, d = 10 µm).

2. *Selenophoma linicola* Vanterp., Mycologia 39(3): 346 (1947)

Sexual morph undetermined. Asexual morph hyphomycetous. On PCA vegetative hyphae 1–6 µm wide, subhyaline, thin- and smooth-walled, septate, soon becoming brown, thick-walled and chlamydospore-like. Conidiophores reduced to conidiogenous cells. Conidiogenous cells phialidic, discrete, subglobose, obovoid or slender, hyaline, smooth-walled, without collarettes, 1–8 × 1–6 µm (av. = 4.5 × 4 µm, n = 20). Conidia aseptate, hyaline, thin- and smooth-walled, lunate to falcate with sharply pointed ends, 13–25 × 2.5–4 µm (av. = 18 × 3 µm, n = 20).; germination is usually preceded by septation (Fig. 3).

Culture characteristics (25 °C, 7 d): Colonies on PDA attaining 20 mm diam. first flat, primrose (66), then becoming raised, smoke gray (105) to black, velvety, dense, lacking aerial mycelium; margins regular; reverse first primrose (66), then olivaceous black (108) to black. Colonies on PCA similar to PDA.

Specimen examined: IRAN: W. Azerbaijan Province, Khoy, 38˚41′21.86″ N, 44˚40′59.04″ E, stem endophyte of *Aegilops cylindrica*, 27.6.2018, M. Mehrabi (IRAN 3461C).

The genus *Selenophoma* Maire (*Dothideomycetes*, *Dothideales*) was established by Maire (1906) based on *S. catananches* as its type species, and currently includes 109 species (Index Fungorum 2023). This genus is characterized by septate, pale brown hyphae; pycnidial conidiomata with wall composed of 2–3 layers of brown, thick-walled cells of *textura angularis*; absence of conidiophores; enteroblastic, phialidic, discrete, determinate conidiogenous cells, and aseptate, falcate to fusiform and hyaline conidia (Sutton 1980). *Selenophoma* species are common parasites of the grass family, *Poaceae*, in the temperate and Arctic parts of the world (Park & Sprague 1953). Information on the morphology of the genus *Selenophoma* on the *Poaceae* was given by Sprague (1949), Sprague & Johnson (1940, 1950) and Park & Sprague (1953). However, Vanterpool (1947) described *S. linicola* from Saskatchewan on *Linum usitatissimum* L. In this species, the conidia are produced both freely on the mycelium and inside the pycnidium. In case of isolate of the present study (IRAN 3461C), pycnidia were not observed, but the morphological characteristics of conidia formed on the mycelium were similar to those of Vanterpool (1947).

There are several asexual fungi with phylogenetic affinity to *Selenophoma*, namely, *Kabatiella* Bubák, *Hormonema* Lagerb. & Melin and *Aureobasidium* Viala & G. Boyer (Yurlova *et al.* 1996, 1999, Zalar *et al.* 2008). These genera can be differentiated based on mechanisms of conidiogenesis and type of asexual fruiting bodies, but morphological distinction of species in culture is difficult as discussed by Cheewangkoon *et al.* (2009).

Based on a MegaBlast search of NCBIs GenBank nucleotide database, the closest hits using the ITS sequence of isolate of the present study were *S. linicola* [CBS 468.48, GenBank NR_160075, Identity = 480/488 (98%)], *Pseudoseptoria obscura* Quaedvl., Verkley & Crous [CBS 135103, GenBank KF251219, Identity = 483/496 (97%)], *Arxiella lunata* Ruscoe [CBS 476.71, GenBank MH860223, Identity = 491/510 (96%)], *Pseudoseptoria donacis* (Pass.) B. Sutton [CBS 313.68, GenBank MH859141, Identity = 489/512 (96%)], and *Hormonema schizolunatum* Middelhoven & de Hoog [CBS 707.95, GenBank MH862552, Identity = 455/471 (97%)].

Phylogenetically, the isolate (IRAN 3461C) of this study, clusters with ex-type culture of *S. linicola* (CBS 468.48) with high value (ML = 100%, BI = 1.0, Fig. 1). Besides, it possesses a similar anamorph which was observed in *S. linicola* by Vanterpool (1947). The development of both pycnidial conidiomata and spore formation on the mycelium was observed by Vanterpool (1947). Pycnidial conidiomata were not observed in the strain of the present study, but sporulation on hyphae matched that reported by Vanterpool (1947). Therefore, authors of this paper prefer to identify this isolate as

S. linicola. However, a comprehensive taxonomic revision of *Selenophoma* and related genera, especially *Pseudoseptoria* and *Hormonema*, is necessary. Until now, seven species of *Selenophoma*, i.e., *S. boissierae* Vienn.-Bourg., *S. bupleuri* Petr., *S. donacis* (Pass.) Sprag. & Johns. (now *Pseudoseptoria donacis*), *S. drabae* (Fuckel) Petr., *S. lunula* (V. Höhn.) Petr., *S. oxyospora* (Penz. & Sacc.) Syd., and *S. staussiana* (Sacc.) Petr. have been reported from Iran (Ershad 2022). This is the first report of *S. linicola* from *Aegilops cylindrica* in Iran.

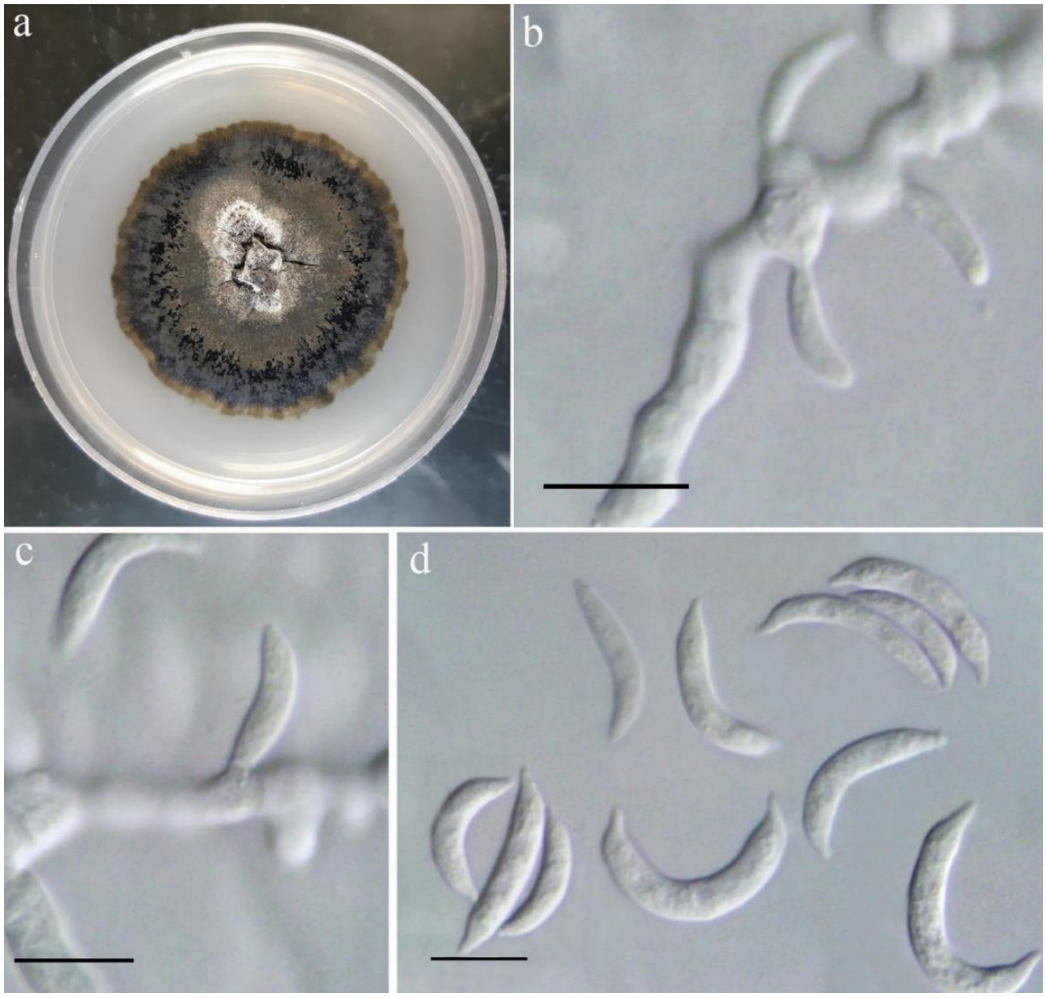

Fig. 3. *Selenophoma linicola* (IRAN 3461C): a. Colony morphology on PDA incubated for 7 d at 25 °C, b–d. Conidiogenous cells and conidia (Bars = 10 µm).

3. *Septoriella allojunci* W.J. Li, Camporesi, Bhat & K.D. Hyde, Mycosphere 6(6): 701 (2015)

Sexual morph undetermined. Asexual morph coelomycetous. On PCA vegetative hyphae 1–4 µm wide, subhyaline, thin- and smooth-walled, septate, branched. Conidiomata pycnidial, dark brown, solitary or aggregated, unilocular, globose, immersed, 100–290 µm high, 100–250 µm diam., ostiole centrally located, exuding a pale brown conidial mass; wall of conidiomata composed of brown, thick-walled cells of *textura angularis*. Conidiophores reduced to conidiogenous cells. Conidiogenous cells formed from the inner cells of the conidiomata, phialidic, ampulliform, hyaline, smooth-walled, 3–10 × 3–6 µm (av. = 6.5 × 4 µm, n = 10). Conidia, hyaline to subhyaline, subcylindrical, flexuous, with obtuse ends, 3–5-euseptate, smooth-walled, 40–67 × 3–5 µm (av. = 50 × 3.8 µm, n = 20) (Fig. 4).

Culture characteristics (25 °C, 7 d): Colonies on PDA attaining 60 mm diam., flat, floccose; with moderate aerial mycelium, sienna (8); margins irregular; reverse rust (39). Colonies on PCA attaining 60 mm diam., flat, floccose, ochreous (44); margins irregular; reverse of the same obverse colony color.

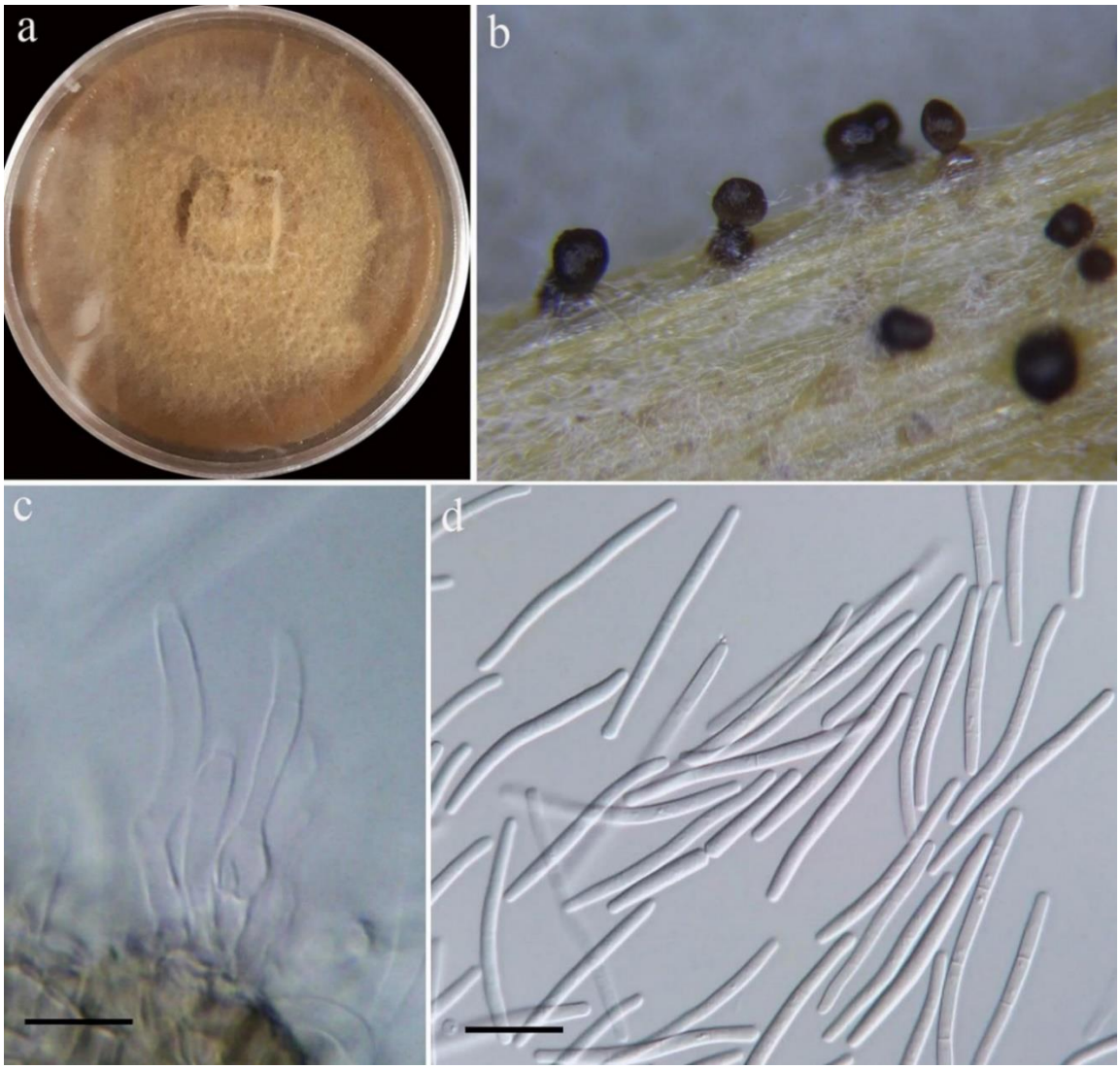

Fig. 4. *Septoriella allojunci* (IRAN 3468C): a. Colony morphology on PDA incubated for 7 d at 25 °C, b. Conidiomata on PNA, c. Conidiogenous cells. d. Conidia (Bars = 10 µm).

Specimen examined: IRAN: Lorestan Province, Khorramabad, 33˚17′53.5″ N, 48˚26′50.13″ E, leaf endophyte of *Aegilops cylindrica*, 18.5.2018, M. Mehrabi (IRAN 3468C).

Crous *et al*. (2015) fixed the application of the type species of *Septoriella*, *S. phragmitis* Oudem., and confirmed the placement of this genus in the *Phaeosphaeriaceae* (*Dothideomycetes*, *Pleosporales*). *Septoriella* is characterized by pycnidial, unilocular conidiomata, and cylindrical to fusoid, euseptate conidia bearing mucoid appendages at both ends (Crous *et al.* 2015). The genus *Septoriella* currently contains 53 species (Index Fungorum 2023). Most species of this genus are saprophytes, except for *S. hirta* (Sacc.) Hern.-Restr. & Crous, which is an important secondary pathogen of grasses (Sprague 1950). Li *et al.* (2015) described *S. allojunci* W.J. Li, Camporesi, Bhat & K.D. Hyde from dead stems of *Juncus* sp. in Italy.

Morphological characteristics of isolate of the present study (IRAN 3468C), were similar to description

of *S. allojunci* (Li *et al*. 2015), but no mucoid cap was observed at the apex of its conidia. *Septoriella allojunci* is close to *S. junci* (Desm.) B. Sutton and *S. canadensis* Nag Raj in form of conidiomata and conidiogenous cells, but it can be differentiated by its conidial size and septation (3–4-septate, 36–56 × 2.5–3.5 µm in *S. canadenesis* and 6–7-septate, 49–90 × 2–3 µm in *S. junci*) (Li *et al*. 2015). Phylogenetically, *S. allojunci* is related to *S. pseudophragmitis* Crous, Quaedvl. & Y. Marín and *S. phragmitis* Oudem. However, *S. allojunci* produces larger conidia than *S. pseudophragmitis* (24–28 × 3.5 µm) and *S. phragmitis* (32–40 × 3 µm).

Based on a MegaBlast search of NCBIs GenBank nucleotide database, the closest hit using the ITS sequence of isolate of the present investigation was ex-type of *S. allojunci* (MFLUCC 15-0701, GenBank KU058718) with a similarity of 100% (510/510). Based on phylogenetic analyses of the ITS sequence data, isolate of the present study, clustered with *S. allojunci* with high value (ML = 93%, BI = 0.99, Fig. 1). To the best of author's knowledge, this species has not been reported from the genus *Aegilops*, and this is a first report of this genus *Septoriella* from Iran.

Acknowledgments

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