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Original Article

Stagonospora pseudoperfecta and *S. uniseptata*, two new records from wetland plants in Iran

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ABSTRACT

The genus *Stagonospora* (*Massarinaceae*, *Pleosporales*, *Dothideomycetes*) is both morphologically and phylogenetically diverse, with more than 500 species epithets reported; however, only a limited number of species have been critically evaluated using molecular data. As part of surveys of fungi associated with wetland plants in Iran, twelve isolates with morphological characteristics resembling *Stagonospora* were obtained. These isolates were subjected to detailed morphological and phylogenetic analyses based on multi-locus sequence data (ITS and LSU). Two species, *Stagonospora pseudoperfecta* Kaz. Tanaka & K. Hiray and *S. uniseptata* Quaedvl., Verkley & Crous, were identified and are reported here as new records for the Iranian funga. This study provides comprehensive morphological descriptions and illustrations of the identified species, and discusses their habitats, distribution, and phylogenetic placement. The findings expand the known diversity of *Stagonospora* in Iran, refine species delimitation within *Massarinaceae*, and underscore the ecological significance of wetlands as reservoirs of fungal diversity.

KEYWORDS

Cyperaceae, *Juncaceae*, *Massarinaceae*, Molecular phylogeny, Morphology, Pycnidial fungi, *Typhaceae*.

INTRODUCTION

The genus *Stagonospora* (Sacc.) Sacc., typified by *S. paludosa* (Sacc. & Speg.) Sacc. was described by Saccardo and Spegazzini (1884). It is currently placed in the family *Massarinaceae* (*Pleosporales*, *Dothideomycetes*, *Pezizomycotina*, *Ascomycota*) (Quaedvlieg et al. 2013, Tanaka et al. 2015). The genus is morphologically characterized in its asexual morph by globose, immersed, ostiolate pycnidial conidiomata; conidiophores reduced to holoblastic conidiogenous cells with percurrent proliferations; and doliiform, cylindrical to ellipsoid, hyaline, guttulate conidia (Quaedvlieg et al. 2013). In its sexual morph, *Stagonospora* produces globose, brown ascomata with a peridium composed of textura angularis, hyaline and septate pseudoparaphyses, and bitunicate, clavate asci containing eight ascospores. The ascospores are ellipsoidal, 1–3-septate depending on the species, and each cell contains guttules (Quaedvlieg et al. 2013, Tanaka et al. 2015).

Historically, *Stagonospora* was delimited under a broad morphological concept (Sutton 1980) and thought to be related to the *Phaeosphaeriaceae* (Zhang et al. 2012). However, multi-locus phylogenetic analyses (ITS, LSU, *RPB2*, *TEF1*, and *TUB*) have revealed its polyphyletic nature, with *Stagonospora sensu stricto* resolved within the *Massarinaceae* (Quaedvlieg et al. 2013). To accommodate unrelated lineages that were previously included in the genus *Stagonospora*, several new genera—including *Neostagonospora* and *Parastagonospora*—were established within the *Phaeosphaeriaceae* (Quaedvlieg et al. 2013). Tanaka et al. (2015) subsequently revised the *Massarinaceae*, formally recognizing 12 species of *Stagonospora* based on combined morphological and molecular evidence. Since then, additional species have been described, further expanding the genus (Crous et al. 2017, 2022, Thambugala et al. 2017, Brahmanage et al. 2020, Bhagya et al. 2024, Liu et al. 2025). Currently, twelve genera are

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recognized within the *Massarinaceae*: *Byssothecium*, *Haplohelminthosporium*, *Helminthosporiella*, *Helminthosporium*, *Massarina*, *Mirohelminthosporium*, *Pseudodidymosphaeria*, *Pseudosplanchnonema*, *Semifissispora*, *Stagonospora*, *Suttonomyces*, and *Synhelminthosporium* (Hyde et al. 2024). Although approximately 533 epithets are listed under *Stagonospora* in Index Fungorum (2025) (https:// www. Indexfungorum .org/Names/), relatively few species have been critically reassessed and confirmed through molecular phylogenetic analyses (Quaedvlieg et al. 2013, Tanaka et al. 2015, Brahmanage et al. 2020, Bhagya et al. 2024, Liu et al. 2025). In Iran, 11 species of *Stagonospora* were reported (Ershad 2022), but recent taxonomic revisions have reassigned most of them to other genera, such as *Depazea*, *Didymella*, *Hendersonia*, *Longiseptatispora*, *Parastagonospora*, and *Septoria* (Quaedvlieg et al. 2013, Tanaka et al. 2015, Bakhshi et al. 2022). These revisions underscore the critical role of molecular phylogenetic analyses and integrative taxonomy in clarifying species boundaries and generic affiliations within the *Massarinaceae* and related groups.

As part of our ongoing surveys of fungi associated with wetland plants in different regions of Iran, 12 fungal strains morphologically resembling the genus *Stagonospora* were isolated and purified. Two species, *Stagonospora pseudoperfecta* Kaz. Tanaka & K. Hiray and *S. uniseptata* Quaedvl., Verkley & Crous, were identified and are reported here as new records for the Iranian funga. The aims of this study were to: (i) provide detailed morphological descriptions and illustrations of the collected isolates; (ii) determine their phylogenetic affinities within *Massarinaceae* using multi-locus sequence data; and (iii) expand the current knowledge of the diversity, distribution, and taxonomy of *Stagonospora* in Iran.

MATERIALS AND METHODS

Sample Collection and Fungal Isolates

A total of 36 symptomatic leaf and culm samples exhibiting brown lesions and blight symptoms were collected between 2019 and 2021 from wetland plants belonging to the families *Cyperaceae*, *Juncaceae*, and *Typhaceae* in two provinces of Iran (Ardabil and West Azarbaijan). Samples were labeled, stored under low-temperature conditions, and transported to the laboratory. Fungal isolation, purification, and preservation followed the protocols of Ahmadpour et al. (2021, 2025b). All isolates were preserved as pure cultures in the fungal culture collections of the Iranian Research Institute of Plant Protection (IRAN) and Urmia University (FCCUU).

Morphological Study

Fungal strains were cultured on Potato Dextrose Agar (PDA; Merck, Germany), Malt Extract Agar (MEA; Quelab, Canada), and Oatmeal Agar (OA; 30 g oatmeal, 15 g agar, 1,000 mL distilled water), and incubated at 23–25 °C in darkness for 14 days (Quaedvlieg et al. 2013, Tanaka et al. 2015). Microscopic features were examined from 10–14-day-old cultures grown on Tap Water Agar (TWA) with autoclaved host tissues under near-UV light (12 h photoperiod, 23–25 °C) (Quaedvlieg et al. 2013). Colony characteristics (color, growth pattern, diameter) were recorded, and pigmentation was determined using Rayner's (1970) color charts. For each structure, 20–50 measurements were taken. Observations were made using an Olympus AX70 microscope equipped with differential interference contrast (DIC) optics from slide mounts in lactic acid or lactophenol cotton blue. Photomicrographs were processed using Adobe Photoshop 2020 v. 2.10.8 (Adobe Inc., San Jose, California, USA).

DNA extraction and PCR amplification

Genomic DNA was extracted from fresh mycelium obtained from 10-day-old PDA cultures using the method of Ahmadpour et al. (2021, 2025b). The internal transcribed spacer (ITS) region and large subunit ribosomal RNA gene (LSU) were amplified with primer pairs ITS1/ITS4 (White et al. 1990) and LR0R/LR5 (Vilgalys and Hester 1990), respectively. PCR was performed in a SimpliAmp™ Thermal Cycler (Applied Biosystems™, Thermo Fisher Scientific Inc., USA) in 30 µL reactions containing 0.4 µM of each primer, 10 µL of 2X Taq DNA Polymerase Master Mix Red with 2 mM $MgCl_2$ (Ampliqon, Odense, Denmark), and ~10 ng of template DNA. Cycling conditions were: initial denaturation at 95 °C for 5 min; 35 cycles of 95 °C for 45 s, annealing at 62–57 °C (decreasing 0.5 °C per cycle for the first 10 cycles) for 45 s, and extension at 72 °C for 45 s; and a final extension at 72 °C for 7 min. PCR products were visualized on 1% agarose gels stained with FluoroVue™ (SMOBIO Technology Inc., Hsinchu, Taiwan), and fragment sizes were estimated with a FluoroBand™ 100 bp + 3K DNA ladder (SMOBIO Technology Inc.). Amplicons were purified and sequenced by Macrogen Inc. (Seoul, South Korea) using the same primers. Newly generated sequences were deposited in GenBank (Table 1).

Phylogenetic analyses

Preliminary identification was based on BLAST searches of ITS and LSU sequences against the NCBI database (www.ncbi.nlm.nih.gov/blast/). Sequences from the type or representative isolates were retrieved from GenBank (Table 1) and used in a combined phylogenetic analysis of ITS and LSU datasets. Alignments were generated with the online MAFFT v.7 server (https://mafft.cbrc.jp/alignment/server/) (Katoh et al. 2019). The best-fit nucleotide substitution models were determined using the Akaike Information Criterion (AIC) in MrModeltest 2.3 (Nylander 2004). Maximum

Table 1. Strains used for phylogenetic analyses in this study. Newly generated sequences are shown in bold. ^{T}: ex-type strain.

Species	**Culture no.**	**Host/Substratum**	**Location**	**GenBank Accession Numbers**	
				LSU	**ITS**
Massarina cisti	CBS 266.62^{T}	*Cistus albidus*	France	AB807539	LC014568
Stagonospora bicolor	ATCC 42652^{T}	*Saccharum officinarum*	Kenya	–	NR_155862
Stagonospora cf. paludosa	CBS 130005	*Carex* sp.	Russia	KF251757	KF251254
Stagonospora chrysopyla	CBS 137792^{T}	*Scirpus microcarpus*	USA	–	NR_172529
Stagonospora cylindrica	CP2	*Cyperus involucratus*	Taiwan	MH423485	MH423482
Stagonospora cylindrica	BRIP 14187^{T}	*Cyperus brevifolius*	Australia	MZ734408	MZ734404
Stagonospora duoseptata	CBS 135093^{T}	*Carex acutiformis*	Netherlands	MH877612	NR_156563
Stagonospora endophytica	CMML 20-37	*Zoysia japonica*	Korea	PQ741509	PQ741484
Stagonospora endophytica	CMML 20-93	*Zoysia japonica*	Korea	PQ741510	PQ741485
Stagonospora forlicesenensis	MFLUCC 15-0054^{T}	*Phragmites australis*	Italy	KX655547	KX655557
Stagonospora imperaticola	MFLUCC 15–0026^{T}	*Imperata cylindrical*	Thailand	KY706133	KY706143
Stagonospora lomandrae	CBS 143447^{T}	*Lomandra longifolia*	Australia	NG_058524	NR_156671
Stagonospora multiseptata	MFLUCC 15–0449^{T}	dead grass leaves	Thailand	KX954404	KX965735
Stagonospora paludosa	CBS 135088^{T}	*Carex acutiformis*	Netherlands	KF251760	NR_155787
Stagonospora paspali	CBS 331.37	*Paspalum notatum*	USA	EU754172	KP170653
Stagonospora perfecta	KT 1726A	*Carex* sp.	Japan	AB807579	AB809642
Stagonospora perfecta	CBS 135099^{T}	*Carex acutiformis*	Netherlands	MH878233	KF251258
Stagonospora poaceicola	MFLU 17-0769^{T}	Dead grass leaves	China	MT199604	MT199603
Stagonospora pseudocaricis	CBS 135414	*Carex acutiformis*	France	KF302407	KF302401
Stagonospora pseudocaricis	S610^{T}	*Carex acutiformis*	France	KF251763	KF251260
Stagonospora pseudopaludosa	CPC 22654^{T}	*Phragmites australis*	South Africa	KF777239	NR_137840
Stagonospora pseudoperfecta	CBS 144607	*Typha* sp.	Germany	MK442561	MK442625
Stagonospora pseudoperfecta	KT 889^{T}	*Typha latifolia*	Japan	AB807577	AB809641
Stagonospora pseudoperfecta	**IRAN 4781C**	***Carex* sp.**	**Iran**	**PX234419**	**PX234413**
Stagonospora pseudoperfecta	**FCCUU 2000**	***Carex* sp.**	**Iran**	**PX234420**	**PX234414**
Stagonospora pseudoperfecta	**FCCUU 2001**	***Juncus acutus***	**Iran**	**PX234421**	**PX234415**
Stagonospora pseudoperfecta	**FCCUU 2002**	***Eleocharis* sp.**	**Iran**	**PX234422**	**PX234416**
Stagonospora pseudovitensis	S602	*Carex acutiformis*	Netherlands	KF251765	KF251262
Stagonospora samroiyotensis	MFLUCC24-0052^{T}	Dead *Typha* leaves	Thailand	PP463950	PP463951
Stagonospora sp.	CBS 135096; 652	*Carex acutiformis*	France	KF251766	KF251263
Stagonospora sp.	KT 903	–	–	AB807578	–
Stagonospora sp.	MFLUCC 13-0281	–	–	KY000646	KX965736
Stagonospora tainanensis	KT 1866	on dead leaves of herbaceous plant	Japan	AB807580	AB809643
Stagonospora tainanensis	ATCC 38204^{T}	*Saccharum officinarum*	Taiwan	–	NR_155769
Stagonospora tauntonensis	BRIP 70684	*Sporobolus natalensis*	Australia	OM220036	OM220030
Stagonospora tauntonensis	BRIP 70573^{T}	*Sporobolus natalensis*	Australia	NG_088192	NR_182369
Stagonospora trichophoricola	CBS 136764^{T}	*Trichophorum cespitosum*	Netherlands	NG_058081	NR_156586
Stagonospora uniseptata	CPC 22150	*Carex acutiformis*	Netherlands	KF251769	KF251266
Stagonospora uniseptata	CPC 22151	*Carex acutiformis*	Netherlands	KF251768	KF251265
Stagonospora uniseptata	CBS 135090^{T}	*Carex acutiformis*	Netherlands	KF251767	KF251264
Stagonospora uniseptata	**IRAN 4270C**	***Sparganium elactum***	**Iran**	**PX234423**	**PX234417**
Stagonospora uniseptata	**FCCUU 2003**	***Sparganium elactum***	**Iran**	**PX234424**	**PX234418**
Stagonospora victoriana	CBS 143403^{T}	*Poaceae*	Australia	NG_058518	NR_156666

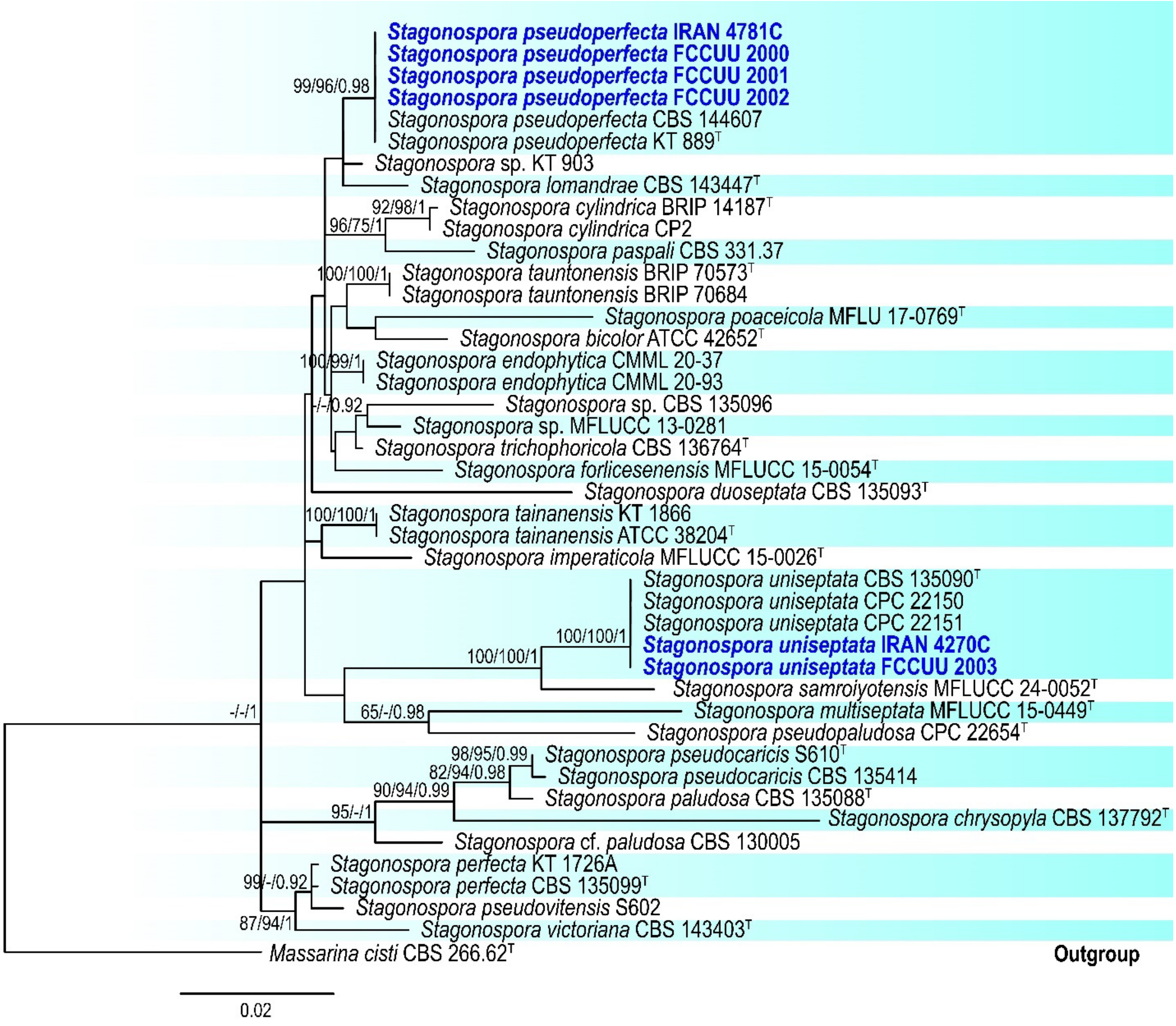

Fig. 1. Phylogenetic tree inferred using Maximum Likelihood (ML) analysis of the combined ITS and LSU dataset of *Stagonospora* species. Bootstrap support values from ML and MP analyses (MLBS/MPBS) ≥ 60% and Bayesian posterior probabilities (BIPP) ≥ 0.90 are indicated at the nodes. The tree is rooted with *Massarina cisti* strain CBS 266.62. Newly identified strains are shown in blue boldface, and ex-type strains are marked with "T". The scale bar represents the number of nucleotide substitutions per site.

Likelihood (ML), Bayesian inference (BI), and Maximum Parsimony (MP) phylogenetic analyses were conducted via the CIPRES Science Gateway portal version 3.3 (https://www.phylo.org/) (Miller et al. 2010), employing the following tools: RAxML-HPC BlackBox v. 8.2.12 with the GTR + GAMMA model and 1000 bootstrap iterations (Stamatakis 2014); MrBayes on ACCESS v. 3.2.7a, using the Markov Chain Monte Carlo (MCMC) method with four chains, 1,000,000 generations, a sampling frequency of 1000, and a 25% burn-in phase (Ronquist et al. 2012); and PAUP on ACCESS v. 4.a168, utilizing the heuristic search option with branch swapping via the tree-bisection-reconnection (TBR) algorithm and 1000 bootstrap replicates (Swofford 2002). Descriptive tree statistics [Tree Length (TL), Consistency Index (CI), Retention Index (RI), and Homoplasy Index (HI)] were calculated for MP analysis. *Massarina cisti* strain CBS 266.62 was used as the outgroup taxon (Quaedvlieg et al. 2013). Phylogenetic trees were visualized in FigTree v. 1.4.4 (Rambaut 2019) and finalized with Adobe Illustrator® CC 2021 (Adobe Inc., San Jose, California, USA).

RESULTS

Molecular phylogenetic analyses

A total of 12 isolates were obtained from host plants belonging to the families *Cyperaceae*, *Juncaceae,* and *Typhaceae*. All isolates were examined morphologically, and six representative isolates from different host plants were selected for phylogenetic analyses. Forty-two ITS and 40 LSU sequences were aligned (including nucleotides and gaps), resulting in datasets comprising 509 and 838 characters, respectively. The combined two-gene dataset for 43 strains comprised 1,347 characters, of which 1,094 were constant, 117 were variable but parsimony-uninformative, and 136 were parsimony-informative. The most parsimonious tree had the following values: TL = 548; CI = 0.588; RI = 0.695; HI = 0.412. Model selection using MrModeltest suggested the GTR+I+G model for ITS and the HKY+I+G model for LSU. Maximum Likelihood (ML), Maximum Parsimony (MP), and Bayesian Inference (BI) analyses produced trees with congruent topologies and revealed no significant conflicts. Analysis of the combined dataset in RAxML yielded the best-scoring tree (Fig. 1) with a final ML optimization likelihood value of –4749.154260. Estimated base frequencies were: A = 0.233932, C = 0.225776, G = 0.279755, T = 0.260537; substitution rates: AC = 2.079830, AG = 3.114978, AT = 2.019563, CG = 0.377054, CT = 10.419318, GT = 1.000000; gamma distribution shape parameter: α = 0.654682. All six isolates were assigned to the genus *Stagonospora* (Fig. 1). Based on morphological characteristics and multi-locus phylogeny (ITS and LSU), two species were identified: *Stagonospora pseudoperfecta* and *S. uniseptata*. Both species are reported here as new to the Iranian funga. Their morphology, habitat, distribution, and phylogenetic relationships with other *Stagonospora* species are fully described and illustrated.

Taxonomy

Stagonospora pseudoperfecta Kaz. Tanaka & K. Hiray., Stud. Mycol. 82: 106 (2015). Fig. 2

Description: On infected culms of *Carex* sp. (*Cyperaceae*), *Eleocharis* sp. (*Cyperaceae*), and *Juncus acutus* (*Juncaceae*), grey to brown spots were observed. Asexual morph on TWA medium containing host culms: Conidiomata pycnidial, immersed to semi-immersed, solitary or aggregated, scattered, brown to dark brown, globose to subglobose with central ostiole, papillate, 300–420 × 300–350 µm ($\bar{x}$ = 370 × 330 µm, n = 30). Pycnidial wall thick, consisting of 2–3 layers of pale brown to brown cells of textura angularis. Conidiophores reduced to conidiogenous cells. Conidiogenous cells phialidic, hyaline, smooth, aggregated, ampulliform to subcylindrical, lining the inner cavity, formed from inner cells of the conidiomata, with percurrent proliferation at the apex, 5–8(–10) × 3–5 µm ($\bar{x}$ = 7.5 × 4 µm, n = 30). Conidia 25–30 × 5–7 µm ($\bar{x}$ = 27.5 × 6.2 µm, n = 50), solitary, hyaline, straight, smooth, thin-walled, fusoid-ellipsoid, with obtuse apex and truncate to bluntly rounded base, (2–)3-septate, guttulate. Sexual morph and chlamydospores were not observed.

Culture characteristics: Colonies on PDA reaching 78 mm diameter after 14 days at 25 °C; white, floccose aerial mycelia, olivaceous grey at the center, white at the margin; reverse pale brown at the center, hyaline at the margin. Colonies on MEA reaching 79 mm diameter after 14 days at 25 °C; white aerial mycelia, surface white to grey; reverse cinnamon at the center, hyaline at the margin. Colonies on OA reaching 76 mm diameter after 14 days at 25 °C; surface white to pale olivaceous, floccose aerial mycelia; reverse buff, pale olivaceous near the center, hyaline at the margin.

Specimens examined*:* IRAN, West Azarbaijan Province, Khoy County, Salkadeh Village, on infected culms of *Carex* sp. (*Cyperaceae*), 5 Jul. 2020, A. Ahmadpour, isolate IRAN 4781C;—*ibid.* on infected culms of *Juncus acutus* (*Juncaceae*), 15 Sep. 2021, A. Ahmadpour, isolate FCCUU 2001; Urmia County, on infected culms of *Eleocharis* sp. (*Cyperaceae*), 20 Oct. 2020, A. Ahmadpour, isolate FCCUU 2002; Ardabil Province, Ardabil County, Neor Lake, on infected culms of *Carex* sp. (*Cyperaceae*), 18 Jun. 2021, A. Ahmadpour, isolate FCCUU 2000 (Table 1).

Habitat and distribution*:* On *Typha latifolia* and *Typha* sp. (*Typhaceae*) from Germany and Japan (Tanaka et al. 2015, Crous et al. 2019, Farr et al. 2025), and on *Carex* sp., *Juncus acutus*, and *Eleocharis* sp. from Iran (this study).

Notes*: Stagonospora pseudoperfecta* was first described from dead leaves of *Typha latifolia* (*Typhaceae*) and collected in Japan (Tanaka et al. 2015) and later reported from Germany on *Typha* sp. (Crous et al. 2019). This species is known to be homothallic (Tanaka et al. 2015). In the phylogenetic analyses, the four studied isolates clustered closely with *S. pseudoperfecta* type and representative strains in a distinct lineage, supported by 99/96% ML/MP bootstrap values and a Bayesian posterior probability of 0.98 (Fig. 1).

Stagonospora uniseptata Quaedvl., Verkley & Crous, Stud. Mycol. 75: 380 (2013). Fig. 3

Description: On infected leaves of *Sparganium erectum* (*Typhaceae*), grey to brown spots, 1–20 cm long. Asexual morph on TWA medium containing host culms: Conidiomata pycnidial, immersed to semi-immersed, solitary or aggregated, scattered, brown to black, globose to subglobose with central ostiole, papillate, exuding yellow conidial masses, 250–550 × 250–530 µm ($\bar{x}$ = 390 × 380 µm, n = 30). Pycnidial wall thick, comprising 3–5 layers of pale brown to brown cells of textura angularis. Conidiophores reduced to conidiogenous cells. Conidiogenous cells phialidic, hyaline, smooth, aggregated, ampulliform to subcylindrical, lining the inner cavity and formed from inner cells of the

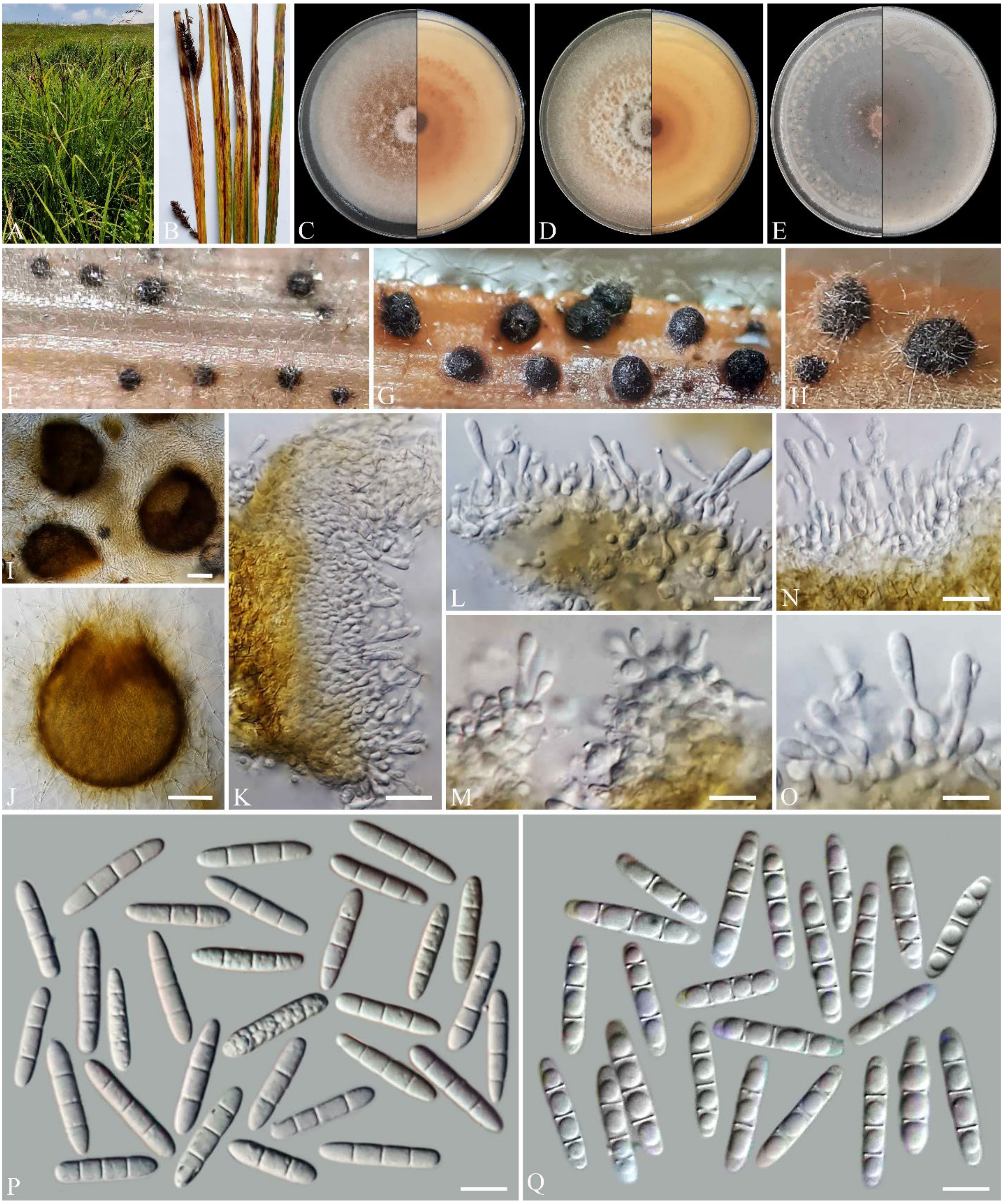

Fig. 2. *Stagonospora pseudoperfecta* (IRAN 4781C). (A, B) Symptoms on *Carex* sp., (C–E) Colonies on PDA (C), MEA (D), and OA (E) media after 14 days (front and reverse), respectively, (F–J) Conidiomata formed on TWA medium containing host culms, (K–O) Conidiogenous cells, (P–Q) Conidia. Scale bars: (I–J) 100 µm, (K) 20 µm, (L–Q) 10 µm.

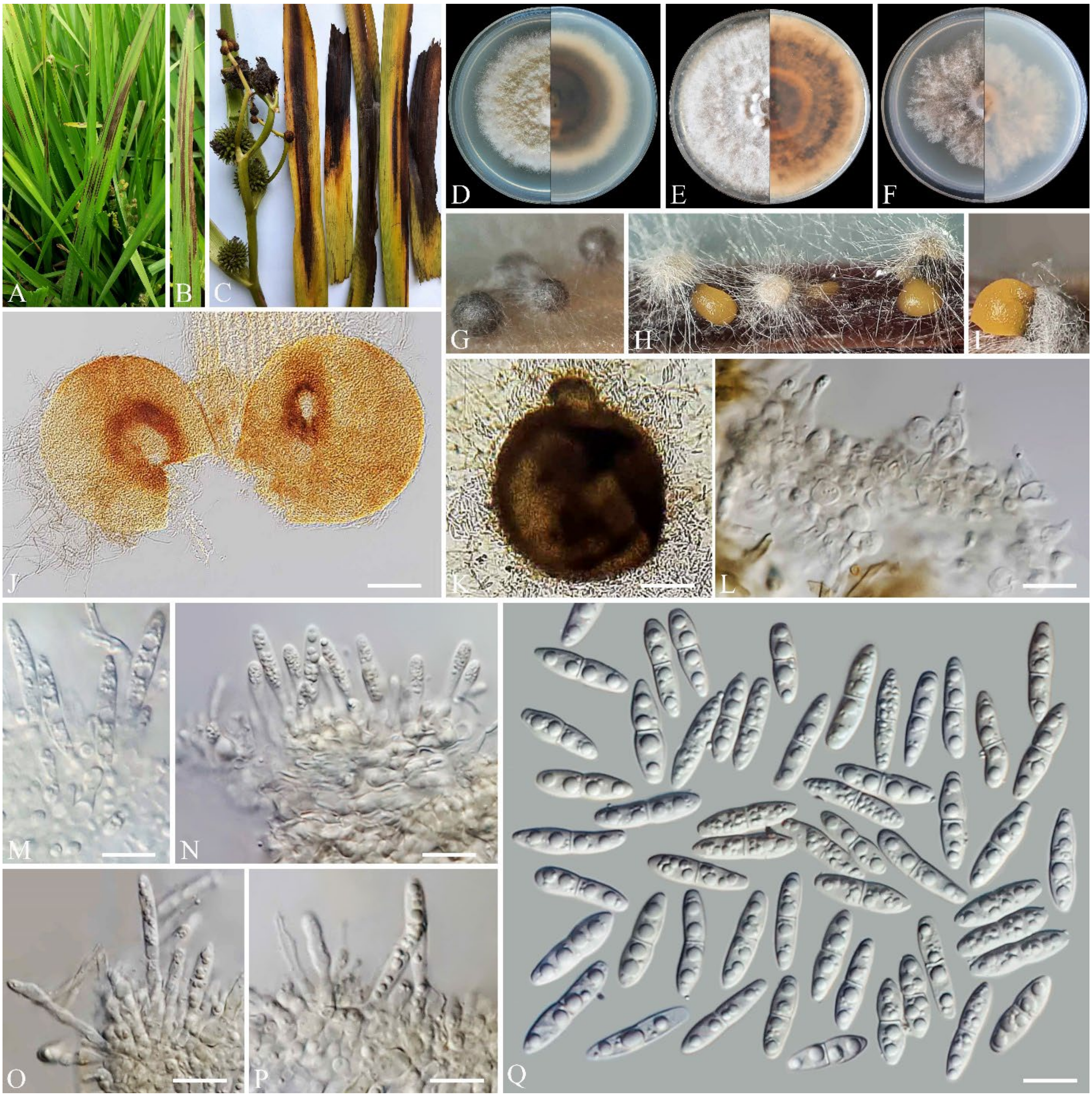

Fig. 3. *Stagonospora uniseptata* (IRAN 4270C). (A–C) Symptoms on *Sparganium erectum*, (D–F) Colonies on PDA (C), MEA (D), and OA (E) media after 14 days (front and reverse), respectively, (G–K) Conidiomata formed on TWA medium containing host culms, (L–P) Conidiogenous cells, (Q) Conidia. Scale bars: (J–K) 100 µm, (L–Q) 10 µm.

conidiomata, with percurrent proliferation at the apex, 4–6(–8) × 3–4 µm ($\bar{x}$ = 6.5 × 3.5 µm, n = 30). Conidia hyaline, smooth, thin-walled, fusoid to ellipsoidal, with obtuse apex and truncate to bluntly rounded base, 1-septate, slightly constricted at septum, straight to slightly curved, smooth-walled, guttulate, 19–30 × 5–6 µm ($\bar{x}$ = 25 × 5.5 µm, n = 50). Sexual morph and chlamydospores were not observed.

Culture characteristics: Colonies on PDA reaching 70 mm diameter after 14 days at 25 °C; white, floccose aerial mycelia, olivaceous grey at the center, white at the margin; reverse pale brown at the center, hyaline at the margin. Colonies on MEA reaching 78 mm diameter after 14 days at 25 °C; white aerial mycelia, surface white to grey; reverse cinnamon at the center, hyaline at the margin. Colonies on OA reaching 67 mm diameter after 14 days at 25 °C; surface white to pale olivaceous, floccose aerial mycelia; reverse buff, pale olivaceous near the center, hyaline at the margin.

Specimens examined*:* IRAN, West Azarbaijan Province, Khoy County, Salkadeh Village, on infected leaves of *Sparganium erectum* (*Typhaceae*, *Poales*), 20 Sep. 2019, A. Ahmadpour, isolates IRAN 4270C and FCCUU 2003 (Table 1).

Habitat and distribution*:* On leaves of *Carex acutiformis* (*Cyperaceae*) from the Netherlands (Quaedvlieg et al. 2013) and *Sparganium erectum* (*Typhaceae*) from Iran (this study).

Notes*:* *Stagonospora uniseptata* was originally described on leaves of a *Carex acutiformis* (*Cyperaceae*) in the Netherlands (Quaedvlieg et al. 2013). In the present phylogenetic analyses, the two studied isolates clustered with *S. uniseptata* type and representative strains in a distinct lineage, supported by 100% ML/MP bootstrap values and a Bayesian posterior probability of 1.0 (Fig. 1). *Stagonospora uniseptata* is phylogenetically related to *S. samroiyotensis* (Fig. 1) but can be distinguished morphologically by conidial shape: fusoid to ellipsoidal with an obtuse apex and truncate to bluntly rounded base in *S. uniseptata* versus oblong to cylindrical with a rounded apex and tapered base in *S. samroiyotensis* (Bhagya et al. 2024).

DISCUSSION

We re-examined the records of *Stagonospora* species reported from Iran. Among the 11 species previously recorded, only four species (*S. alliina, S. caricinella, S. citrorum,* and *S. iranica*) were confirmed as belonging to this genus. However, all of these species were reported decades ago and likely lack living cultures or available sequence data, leaving their phylogenetic positions within *Stagonospora* uncertain.

In this study, we report two new records of *Stagonospora* for the Iranian funga, namely *S. pseudoperfecta* and *S. uniseptata*, based on detailed morphological characterization and multi-locus phylogenetic analyses. The identification of both species was supported by morphological and molecular evidence, underscoring the necessity of integrative taxonomy in resolving the often complex and polyphyletic relationships within the *Massarinaceae*.

These findings expand the known diversity of *Stagonospora* in Iran, refine species boundaries within *Massarinaceae*, and emphasize wetlands as important reservoirs of fungal biodiversity.

Most *Stagonospora* species are associated with plants in the families *Cyperaceae*, *Poaceae*, and *Typhaceae* (Quaedvlieg et al. 2013, Tanaka et al. 2015, Thambugala et al. 2017). Members of the genus have been reported across a wide latitudinal range in both Hemispheres, from tropical regions such as Thailand to temperate areas including Italy, France, and the Netherlands (Quaedvlieg et al. 2013, Tanaka et al. 2015). *Stagonospora* species inhabit diverse ecosystems ranging from grasslands to wetlands, reflecting their ecological adaptability (Quaedvlieg et al. 2013, Tanaka et al. 2015, Thambugala et al. 2017, Brahmanage et al. 2020, Bhagya et al. 2024, Liu et al. 2025). Previously, *S. pseudoperfecta* was reported from *Typha latifolia* and other *Typha* species (*Typhaceae*) (Tanaka et al. 2015, Crous et al. 2019), whereas *S. uniseptata* was described from *Carex acutiformis* (*Cyperaceae*) (Quaedvlieg et al. 2013). In the present study, the host range of these fungi in Iran was found to be broader than previously known. *Stagonospora pseudoperfecta* was isolated from multiple hosts, including *Carex* sp., *Juncus acutus*, and *Eleocharis* sp., representing both *Cyperaceae* and *Juncaceae*, while *S. uniseptata* was recovered from *Sparganium erectum* (*Typhaceae*). These findings not only represent the first records of these species from Iran but also suggest that they may have a wider ecological amplitude than previously recognized, colonizing a range of wetland-associated monocotyledonous hosts.

As part of our broader investigations into fungal diversity in Iranian wetlands, numerous fungi have been isolated from *Cyperaceae* and *Juncaceae* hosts. Several taxa representing *Alternaria*, *Bipolaris*, *Curvularia*, *Macgarvieomyces*, and *Stemphylium* have been identified as morphologically and phylogenetically distinct (Ahmadpour et al. 2024, 2025a, b, c, d). These genera include fungi with diverse ecological roles, ranging from endophytes to plant pathogens, thereby reflecting the complexity of wetland mycobiota. The frequent occurrence of these fungi in aquatic and semi-aquatic environments further underscores wetlands as important reservoirs of fungal diversity. Although still relatively understudied, Iranian wetlands appear to harbor unique assemblages of *Stagonospora* species, potentially indicating endemism or ecological specialization. The discovery of *S. pseudoperfecta* and *S. uniseptata* in these habitats highlights the importance of continued mycological surveys to uncover cryptic diversity and host associations in wetland ecosystems.

Despite recent taxonomic advances, *Stagonospora* remains comparatively understudied in relation to its close relatives. Many historically described species lack molecular data, leaving their phylogenetic placement unresolved. Future research that incorporates multi-locus phylogenies with morphological traits, host associations, and ecological data will be essential for more precise species delimitation, clarification of evolutionary relationships within the *Pleosporales*, and a better understanding of the ecological role of *Stagonospora* in wetland ecosystems.

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AUTHOR CONTRIBUTION

A.A. and Y.G. designed and supervised the project, conducted sampling, fungal isolation, experiments, writing, and editing. F.A. provided photographic documentation. A.A. and F.A. performed the phylogenetic analyses. All authors read and approved the final version of the manuscript.

DATA AVAILABILITY

All data are available in online repositories. Requests for more data and materials should be addressed to A. Ahmadpour or Y. Ghosta.

DECLARATION

The authors declare that there is no conflict of interest.

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Not applicable.

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Stagonospora pseudoperfecta و *S. uniseptata* دو گزارش جدید از گیاهان تالابی در ایران

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چکیده

جنس *Stagonospora* (*Massarinaceae*، *Pleosporales*، *Dothideomycetes*) از نظر ریخت‌شناسی و تبارشناسی متنوع بوده و بیش از ۵۰۰ نام گونه‌ای برای آن گزارش شده است؛ با این حال تنها تعداد محدودی از گونه‌ها با استفاده از داده‌های مولکولی به‌طور دقیق ارزیابی شده‌اند. به عنوان بخشی از بررسی قارچ‌های مرتبط با گیاهان تالابی در ایران، دوازده جدایه با ویژگی‌های ریخت‌شناختی مشابه با جنس *Stagonospora* به دست آمد. جدایه‌ها بر اساس ویژگی‌های دقیق ریخت‌شناختی و تجزیه و تحلیل‌های تبارشناختی مبتنی بر توالی چند-ژنگاهی (ITS و LSU) مورد مطالعه قرار گرفتند. دو گونه، *Stagonospora pseudoperfecta* Kaz. Tanaka & K. Hiray و *S. uniseptata* Quaedvl., Verkley & Crous شناسایی شدند و در اینجا به‌عنوان گزارش‌های جدید برای قارچ‌های ایران گزارش می‌شوند. این مطالعه توصیفات و تصاویر ریخت‌شناختی جامعی از گونه‌های شناسایی شده را ارائه می‌دهد و زیستگاه‌ها، پراکنش و جایگاه تبارشناسی آنها را مورد بحث قرار می‌دهد. یافته‌های این پژوهش، تنوع شناخته شدۀ جنس *Stagonospora* در ایران را گسترش می‌دهد، مرزبندی گونه‌ها را در خانوادۀ *Massarinaceae* اصلاح می‌کند و بر اهمیت اکولوژیکی تالاب‌ها به‌عنوان مخازن تنوع قارچی تأکید می‌کند.

کلمات کلیدی: *Cyperaceae*، *Juncaceae*، *Massarinaceae*، تبارشناسی مولکولی، ریخت‌شناسی، قارچ‌های پیکنیدیوم‌دار، *Typhaceae*.

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