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Morphological and molecular characterization of *Spegazzinia tessarthra* (Ascomycota, Didymosphaeriaceae) from Iran

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Abstract: During the study of fungal taxa of gramineous plants, a hyphomycetous fungus with typical characteristics of the genus *Spegazzinia* was isolated from leaves of *Brachypodium* sp. (Poaceae), collected in Mazandaran province, Iran. The fungal species was determined as *Spegazzinia tessarthra* based on the combination of morphological characteristics and phylogenetic analysis of the internal transcribed spacer (ITS-rDNA) region and a partial region of the translation elongation factor 1-alpha gene (*tef1-α*) sequences. In this study, we introduce *S. tessarthra*, saprobic on *Brachypodium* sp. leaves, as a new record for the Funga of Iran and *Brachypodium* sp. as a new substrate for the species. The description and illustrations of *Spegazzinia tessarthra* from Iran have been provided, and its morphology and phylogenetic relationships with other species of *Spegazzinia* have been discussed. To our knowledge, this is the first report of a species from the genus *Spegazzinia* in Iran, and further research is needed to determine the diversity of *Spegazzinia* species in the country.

Keywords: Taxonomy, morphology, phylogeny, *Didymosphaeriaceae*, *Brachypodium*

INTRODUCTION

The genus *Spegazzinia* Sacc. was described by Saccardo (1880) with the type species *S. ornata*, which is now considered a synonym of *S. tessarthra* (Berk. & M.A. Curtis) Sacc. (Damon 1953, Hughes 1953). *Spegazzinia* is a hyphomycetous genus with a unique morphology of conidiophores and conidia, which sets it apart from other dematiaceous hyphomycetes. Most *Spegazzinia* species produce two types of conidia: α conidia (stellate-shaped) and β conidia (clover leaf-shaped). Conidia are brown to dark brown and may or may not have spine-like appendages (Ellis 1971, 1976, Cole 1974, Suwannarach et al. 2021). Conidia are produced blastically at the apex of a flask-shaped cell, and then a stalk extends from below the conidium, originating from the same flask-shaped cell. In most literature, this type of conidiogenesis in the genus *Spegazzinia* is referred to as basauxic. The stalk attached to the conidium is called the conidiophore, while the flask-shaped cell that produces both the conidium and the stalk is called the conidiophore mother cell (Hugehs 1953, Ellis 1971, Cole 1974, Tanaka et al. 2015). Kirschner et al. (2017) offered a different perspective on the conidiogenesis process in the *Spegazzinia* genus. They disputed the term "basauxic" and proposed that the stalk attached to the conidium is part of the conidium and should not be considered a conidiophore. Additionally, they suggested that the conidiophore mother cell should be regarded as the conidiogenous cell of a one-celled conidiophore. However, the precise nature of conidium production in the *Spegazzinia* genus remains unclear, and further research is needed to understand it better.

Hyde et al. (1998) considered *Spegazzinia* to be an Apiosporaceae (Sordariomycetes) member based on the morphology of basauxic conidiogenesis. Later on, Wijayawardene et al. (2012) regarded this genus as *incertae sedis* in Ascomycota. However, the phylogenetic analysis of SSU, LSU, and *tef1-α* sequences by Tanaka et al. (2015) showed that the genus belongs to the family Didymosphaeriaceae. Several recent studies have also accepted the placement of *Spegazzinia* in Didymosphaeriaceae (Wanasinghe et al. 2016, Thambugala et al. 2017, Jayasiri et al. 2019, Samarakoon et al. 2020a, b, Hongsanan et al. 2020, Jayawardena et al. 2022). Currently, 17 species are listed in *Spegazzinia* (https://www.speciesfungorum.org, accessed on 5th November 2023). However, sequence data are available for only 10 species in the GenBank nucleotide database (https://www.ncbi.nlm.nih.gov/). Therefore, it is necessary to recollect previously identified *Spegazzinia* species to redefine their

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species boundaries using phylogenetic and morphological approaches.

Spegazzinia species have been found in tropical, subtropical and temperate regions around the world and have been isolated as saprobes from dead plant materials (Ellis 1971, 1976, Sivasithamparam 1974, Leão-Ferreira & Gusmão 2010, Manoharachary & Kunwar 2010, Whitton et al. 2012, Mena-Portales et al. 2017, Thambugala et al. 2017, Jayasiri et al. 2019, Samarakoon et al. 2020b, Ren et al. 2022, Tennakoon et al. 2022, Farr & Rossman 2023), as endophytes from lichens and plants leaves (Manish et al. 2014, Crous et al. 2019, Suwannarach et al. 2021), as well as reported from soil (Ellis 1971). This study introduces *S. tessarthra*, saprobic on *Brachypodium* sp. leaves, as a new record for the Funga of Iran. The description and illustrations of *Spegazzinia tessarthra* from Iran have been provided, and its morphology and phylogenetic relationships with other species of *Spegazzinia* have been discussed.

MATERIALS AND METHODS

Fungus isolate and morphological examination

Leaf samples of *Brachypodium* sp. were collected from the Shahrpasht area of Nowshahr County, Mazandaran province, Iran. The leaves were incubated in a moist chamber at 25 °C without surface disinfection. The incubated leaves were examined using a stereomicroscope, and a part of the sporodochium formed on leaves was transferred onto a 2% Water Agar (WA) using a fine, sterile needle. The isolates were purified using the hyphal tip method, and purified isolates were stored on sterile filter paper at –20 °C. The fungus was grown on Potato Dextrose Agar (PDA), Potato Carrot Agar (PCA, containing 20 g potato, 20 g carrot, and 20 g agar in 1 L distilled water), and Malt Extract Agar (MEA, Merck, 48 g/L-1) and incubated at 25 °C in darkness for 14 days to investigate its morphological characteristics. Rayner's color charts were used to determine colony color (Rayner 1970). Measurements were made from slides prepared from PCA in lactic acid (90%) solution. Photomicrographs were taken using an Olympus AX70 compound microscope. The identified strain was deposited in the culture collection of the Iranian Research Institute of Plant Protection (IRAN).

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from mycelial mass harvested from 10-day-old fungal cultures on PDA, according to Ahmadpour et al. (2021). The internal transcribed spacer (ITS-rDNA) region and a partial region of the translation elongation factor 1-alpha gene (*tef1-α*) were amplified using the primer pairs ITS1/ITS4 (White et al. 1990) and TEF1-983F/TEF1-2218R (Rehner & Buckley 2005), respectively. The PCR reaction mixtures consisted of 10 μL of a Taq DNA polymerase 2X Master Mix (Ampliqon Company, Denmark), 0.4 μM of each primer, and about 10 ng of template DNA in a final volume of 30 μL. Both regions were amplified with a touchdown PCR method (Korbie & Mattick 2008) with modifications consisting of an initial denaturation step of 5 min at 95°C, followed by 35 cycles of 45 s at 95 °C, 45 s at 62–57 °C (annealing temperature decreased 0.5 °C per cycle in the first 10 cycles) and 45 s at 72 °C, and a final extension step at 72 °C for 5 min. The presence of expected amplicons was examined on a 1% agarose gel stained with FluoroStain™ DNA Fluorescent Staining Dye (SMOBIO, Taiwan) and viewed under ultra-violet light. The amplified PCR products were cleaned up and sequenced by the Beijing Genomics Institute (China).

Phylogenetic analyses

Newly generated sequences were seen and trimmed in Chromas 2.6.6 (http://technelysium.com.au/wp/chromas/) and deposited in the GenBank nucleotide database (Table 1). Preliminary identification was conducted by running a BLAST search (BLASTn, NCBI; https://blast.ncbi.nlm.nih.gov/Blast.cgi) of ITS-rDNA and *tef1-α* sequences against GenBank sequences. Published and authenticated DNA sequences of ITS-rDNA and *tef1-α* for the ex-type and reference *Spegazzinia* strains were obtained from the GenBank database (https://www.ncbi.nlm.nih.gov/) and included in the phylogenetic analyses (Table 1). The Multiple sequence alignments for each locus were conducted in the MAFFT v. 7 online program (Katoh et al. 2019) and manually trimmed and adjusted in MEGA 6.06 (Tamura et al. 2013). A combined sequence dataset comprising ITS-rDNA and tef1-α was generated using Mesquite v. 3.70 (Maddison & Maddison 2021), and used for Maximum Likelihood (ML), Maximum Parsimony (MP) and Bayesian inference (BI) phylogenetic analyses. ML analysis was performed in RAxML-HPC BlackBox v. 8.2.12 through the CIPRES Science Gateway portal (https://www.phylo.org/) (Miller et al. 2012) with 1000 bootstrap replicates, GTRGAMMA+I as substitution model and with the option to search for the best-scoring tree after bootstrapping. MP analyses were performed using heuristic searches, comprising 1,000 stepwise random addition replicates utilizing the tree-bisection-reconnection algorithm, alongside 1,000 bootstrap replicates in PAUP (Phylogenetic Analysis Using Parsimony) 4.0b10 (Swofford 2003). BI was conducted using the Markov Chain Monte Carlo (MCMC) method as four chains with 1,000,000 generations, sampling in every 1,000 generations and discarding the first 25% of trees in the burn-in phase in MrBayes 3.2.7 (Ronquist & Huelsenbeck 2003). The temperature value for the heated chain was set to 0.1, and the search was completed when the average standard deviation of split frequencies fell below 0.01.

The best-fit nucleotide evolutionary models for each partition were estimated in MrModeltest v. 2.3 (Nylander 2004) based on the Akaike Information Criterion (AIC). Sequences of *Laburnicola* *muriformis* Wanas., Camporesi, E.B.G. Jones & K.D. Hyde MFLUCC 16-0290 (Wanasinghe et al. 2016) served as the outgroup taxon. Phylogenetic trees were visualized using FigTree v. 1.4.4 (Rambaut, 2019) and edited using Adobe Illustrator® CC 2020.

Table 1. *Spegazzinia* strains used in the phylogenetic analyses in this study. Newly generated sequences are in bold.

Species	Culture collection number	Host/substrate	Location	GenBank accession numbers	
				ITS	*tef1-α*
Laburnicola muriformis	MFLUCC 16-0290[T]	*Laburnum anagyroides*	Italy	KU743197	KU743213
Spegazzinia bromeliacearum	URM 8084[T]	*Tillandsia catimbauensis*	Brazil	MK804501	-
S. camelliae	SDBR-CMU328[T]	*Camellia sinensis*	Thailand	MH734522	MH734524
S. deightonii	MFLUCC 20-0002	*Musa* sp.	Thailand	MN956768	MN927133
S.deightonii	MFLUCC 18-1625	*Hedychium coronarium*	Thailand	ON117291	ON158097
S. deightonii	MFLUCC 22-0180	Palm	Thailand	ON873998	ON885741
S. deightonii	Yone 66	*Arundo donax*	Japan	-	AB808557
S. deightonii	Yone 212	Herbaceous plant	Japan	-	AB808558
S.intermedia	CBS 249.89	Soil	Sudan	MH862171	-
S. jinghaensis	KUMCC 21-0495[T]	*Myristica yunnanensis*	China	OP058973	OP135946
S. jinghaensis	KUMCC 21-0496	*Myristica yunnanensis*	China	OP058974	OP135947
S. lobulata	CBS 361.58 [T]	*Hibbertia fasciculata*	Australia	MH857812	-
S. musae	MFLUCC 20-0001[T]	*Musa* sp.	Thailand	MN930512	MN927132
S. neosundara	MFLUCC 15-0456[T]	*Cortaderia* sp.	Thailand	KX965728	-
S. radermacherae	MFLUCC 17-2285[T]	*Radermachera sinica*	Thailand	MK347740	MK360089
S. tessarthra	SH 287	Balsa wood	Japan	-	AB808560
S. tessarthra	MFLUCC 18-1624	*Acacia auriculiformis*	Thailand	ON117290	ON158096
S. tessarthra	**IRAN 4932C**	***Brachypodium* sp.**	**Iran**	**OR782545**	**OR802133**

[T] indicates ex-type strains.

RESULTS

Phylogenetic analyses

PCR amplification of ITS-rDNA and *tef1-α* yielded DNA fragments of 501 and 864 bp, respectively. The combined dataset (ITS-rDNA + *tef1-α*) consisted of 1345 characters, of which 1080 were constant, 108 were variable and parsimony-uninformative, and 157 were parsimony-informative. A summary of phylogenetic information for the individual analyses and substitution models determined for each dataset is provided in Table 2. The phylogenetic trees resulted from maximum likelihood (Final ML Optimization Likelihood: –3957.000281), maximum parsimony (TL = 426, CI = 0.770, RI = 0.764, HI 0.230) and Bayesian Inference (BI) analyses on the combined dataset were congruent in terms of major topologies and results, of which the phylogenetic tree resulted from ML was used for phylogeny demonstration (Fig. 1). The molecular phylogenetic analyses revealed that our strain (IRAN 4932C) clustered with *S. tessarthra* strains (SH 287 and MFLUCC 18-1624) in a well-supported clade (90% MLBS, 99% MPBS and 1.0 BIPP) (Fig. 1). Additionally, pairwise sequence comparisons indicated that the ITS-rDNA sequence from IRAN 4932C was identical to *S. tessarthra* MFLUCC 18-1624. In addition, no noticeable difference was observed between our strain and other strains in pairwise sequence comparisons of *tef1-α* [from *S. tessarthra* MFLUCC 18-1624 by 2 bp (0.26%) and from SH 287 by 4 bp (0.46%)]. The phylogenetic position of the new strain is supported morphologically, and the congruence between molecular and morphological data confirms the strain IRAN 4932C as *S. tessarthra*.

Taxonomy

Spegazzinia tessarthra (Berk. & M.A. Curtis) Sacc., Syll. fung. (Abellini) 4: 758 (1886) **Fig. 2**

Basionym: *Sporidesmium tessarthrum* Berk. & M.A. Curtis, in Berkeley 1868

Saprobic on leaves of *Brachypodium* sp. Sexual morph: undetermined. Asexual morph: Hyphomycetous. On PCA: Sporodochia dry, powdery, velvety, dense, dark, 90–350 µm diam. Mycelium mostly superficial or immersed in agar medium, hyphae branched, septate, smooth, hyaline to pale brown. Conidiophore mother cell densely aggregated, hyaline when young, turning pale brown to medium brown, cylindrical to ampulliform, smooth, 3–5 (–6) × 5–8 µm ($\bar{x}$ = 4.36 × 6.1 µm, n = 50). Conidiophores basauxic, macronematous, arising singly from mother cells, aseptate, producing two types of conidia: α and β conidia. Conidiophores of α conidia erect, straight or flexuous, pale brown to mid brown, hyaline at the bottom near the conidiophore mother cell, unbranched, smooth or rough-walled, 2–3 µm wide up to 4 µm in apex, 28–140 ($\bar{x}$ = 58.14 µm) long (n = 50). Conidiophores of β conidia unbranched, hyaline, smooth, short, 1.5–2 µm wide, up to 2–3 µm at apex, 5–16 (–17) ($\bar{x}$= 1.87 × 9.88 µm) long (n = 50).

Conidia solitary, dry, acrogenous, two types: α conidia 14–25 × 15–25 µm ($\bar{x}$ = 18.16×19.62 µm, n = 50), mace shaped (stellate), 4 (–5) subglobose cells, brown to dark brown, with brown to dark brown spines scattered across the surface of the conidium,measuring up 2–10 (–12) × 1–2/5 (–3) ($\bar{x}$ = 5.4 × 1.88 µm, n = 50); β conidia 11–15 × 12–16 (–17) ($\bar{x}$ = 12.98 × 13.92 µm, n = 50), 8–9 µm wide in lateral view wide, clover leaf-shaped (rarely irregular shape), 4-celled (rarely 3 or 5-celled),

Table 2. Phylogenetic information of individual and combined sequence data sets used in phylogenetic analyses.

Parameter	ITS-rDNA	*tef1-α*	Combined
Number of taxa	15	14	18
Total characters	511	834	1345
Constant sites	343	737	1080
Variable sites	168	97	265
Parsimony informative sites	97	60	157
Parsimony uninformative sites	71	37	108
AIC substitution model	GTR+I+G	GTR+I	GTR+I+G
Lset nst, Rates	6, invgamma	6, propinv	6, invgamma
−lnL	-2000.726670	-1863.762344	-3957.000281

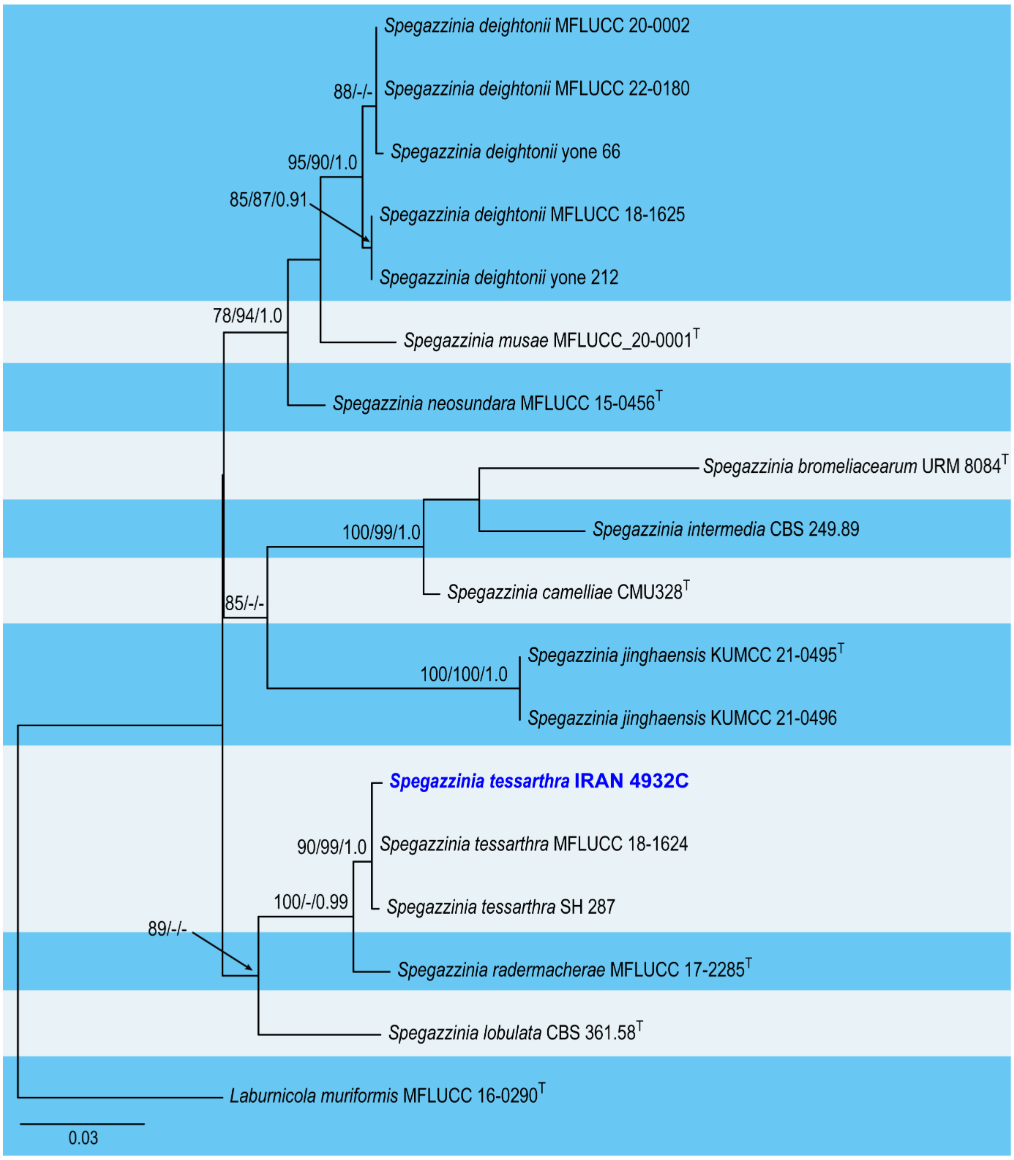

Fig 1. Phylogenetic tree generated from maximum likelihood analysis of the combined dataset of ITS-rDNA and *tef1-α* sequences of *Spegazzinia* species. The maximum likelihood (ML) and maximum parsimony (MP) bootstrap values (>70%) and Bayesian posterior probabilities (>0.90) are given at the nodes (MLBS/MPBS/BIPP). The tree was rooted to *Laburnicola muriformis* MFLUCC 16-0290, and the newly identified strain is bold and blue. [T] indicates ex-type strains.

hyaline when young, turning light brown to dark brown at maturity, smooth-walled, cruciately septate, constricted at the septa, dark brown in constricted areas, usually with conidiophore mother cell remnants after detachment from the conidiophore mother cell, more abundant than α conidia.

Colony on PDA 35 mm diam. after 1 week, effuse, margin regular, felty, with abundant aerial mycelium near the center, white when young, turning cream-white, isabelline near the margin after 2 weeks; reverse brown to vinaceous-cinnamon. Sporodochia formed as black dots on aerial and surface mycelium from the seventh day. Colony on MEA 40 mm diam. after 1 week, margin regular, effuse, felty, with abundant aerial mycelium, white when young, turning grayish to buff after 2 weeks; reverse same as front. Sporodochia formed as black dots on aerial and surface mycelium of the center and near the margin after 12 days. Colony on PCA 35 mm diam. after 1 week, transparent, margin regular, with white sparse aerial mycelium near the center; reverse same as front. Sporodochia abundantly formed as black dots scattered over the colony from the seventh day and became confluent by age.

Specimen examined: Iran, Mazandaran province, Nowshahr County, Shahrpasht area, 36°37'55.9"N, 51°30'08.7"E, saprobic on *Brachypodium* sp. leaf, 23 May 2021, E. Hashemlou, EH0501 (IRAN 4932C).

DISCUSSION

The present study introduces *S. tessarthra* as a new record for the Funga of Iran. A combination of morphological characteristics and phylogenetic analysis (ITS-rDNA + *tef1-α*) was used to identify the species. The Iranian strain (IRAN 4932C) with basauxic conidiophores, spined stellate α conidia, and cross-septate β conidia completely fits the morphological characteristics of the genus *Spegazzinia* and the characteristics mentioned in the literature for *S. tessarthra* (Ellis 1971; Kirschner et al. 2017; Tennakoon et al. 2022). Furthermore, based on the phylogenetic analysis (ITS-rDNA + *tef1-α*), our strain (IRAN 4932C) is clustered with other strains of *S. tessarthra* (SH 287 and MFLUCC 18-1624) in a well-supported clade (90% MLBS, 99% MPBS, and 1.0 BIPP).

Spegazzinia tessarthra is distinguished from other *Spegazzinia* species in terms of conidium morphology. *Spegazzinia tessarthra* produces α and β conidia and differs from *S. bromeliacearum* S.S. Nascimento & J.D.P. Bezerra (Crous et al. 2019), *S. intermedia* M.B. Ellis (Ellis 1976), *S. parkeri* Sivasith. (Sivasithamparam 1974), S. *subbramanianii* Bhat (Bhat 1994), and *S. xanthorrhoeae* Subram. (Subramanian 1994), which produces only one type of conidia. *Spegazzinia deightonii* (S. Hughes) Subram. produces 4–8-celled α conidia and 8-celled β conidia with blunt spines (Ellis 1971, Whitton et al. 2012, Samarakoon et al. 2020b) and *S. cruciata* Whitton, K.D. Hyde & McKenzie produces 8-celled α conidia and 4–celled β conidia (Whitton et al. 2012) compared to *S. tessarthra*, which produces 4-celled α and β conidia (Ellis 1971, Tennakoon et al. 2022, this study).

The production of the lobed β conidia in S. *flabellata* S.M. Leão & Gusmão (15–18 × 13–18 µm), *S. lobulata* Thrower (17–22.5 × 12 µm) and *S. sundara* Subram. (17–25 × 8–10 µm) distinguishes them from *S. tessarthra* (13–17 × 8–9 µm), which produces β conidia without lobes (McLennan et al. 1954, Ellis 1971, 1976, Leão-Ferreira & Gusmão 2010). The echinulate and larger β conidia in *S. affinis* J. Mena & Cantillo and smaller α and β conidia and shorter α conidium spines in *S. camelliae* N. Suwannarach, J. Kumla & S. Lumyong compared to *S. tessarthra* can be used to distinguish these species (Mena-Portales et al. 2017, Suwannarach et al. 2021). *Spegazzinia tessarthra*, *S. jinghaensis* G.C. Ren & K.D. Hyde, *S. musae* Samarak., Phookamsak, Wanas., Chomnunti & K.D. Hyde, and *S. neosundara* Thambug. & K.D. Hyde, exhibit similar morphological features of conidia, and it is not easy to distinguish these species based solely on the characteristics of conidia (Ren et al. 2012, Thambugala et al. 2017, Samarakoon et al. 2020b). The conidiophore length differs among these species, but it is not a helpful character to separate these species. Because the length of the conidiophore may vary in different experimental conditions of the isolates (Cole 1974), and on the other hand, various sizes have been reported in the literature for the conidiophore length of *S. tessarthra* strains (Ellis 1971, Tennakoon et al. 2022). Therefore, for a more accurate assessment of taxonomy in this genus, it is necessary to use both morphological and DNA sequence data.

Jayasiri et al. (2019) introduced a new species named *S. radermacherae* in the genus *Spegazzinia*, which is closely related to *S. tessarthra* in terms of phylogenetic and morphological aspects. *Spegazzinia tessarthra* and *S. radermacherae* have similar morphological characteristics of conidia.

The only marked difference is the length of the α conidium spines, which are shorter in *S. radermacherae* than in *S. tessarthra* (Jayasiri et al. 2019). ITS-rDNA and *tef1-α* sequences of *S. radermacherae* MFLUCC 17-2285 have been submitted with accession numbers MK347740 and MK360088, respectively. Jayasiri et al. reported 10 (3.1%) and 18 (2%) nucleotide differences between ITS-rDNA and *tef1-α* of these two species, respectively. Pairwise sequence comparison in this study indicates that the correct nucleotide differences in ITS-rDNA sequence between *S. tessarthra* strains and *S. radermacherae* MFLUCC 17-2285 are three nucleotides, including two gaps.

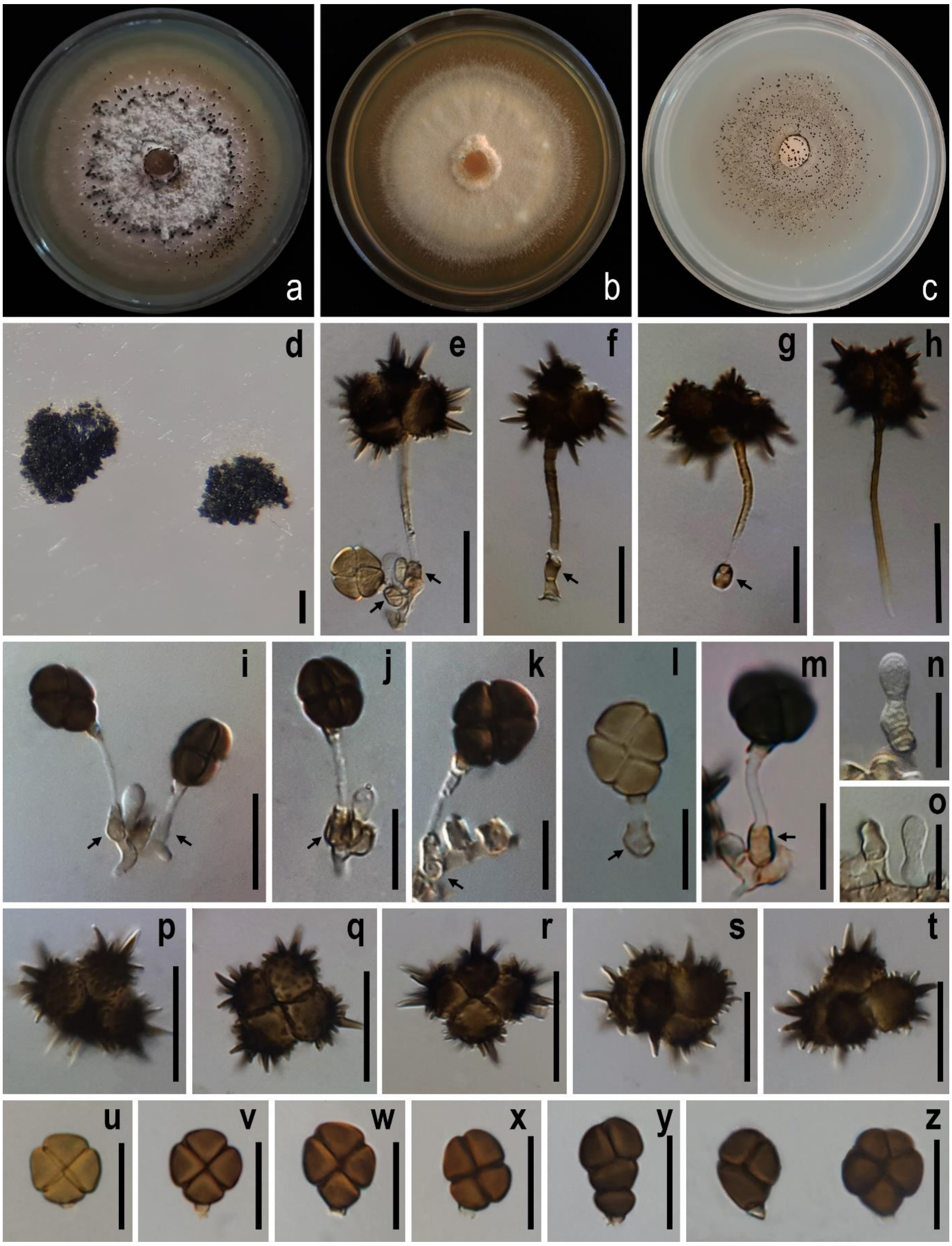

Fig. 2 *Spegazzinia tessarthra* (IRAN 4932C). Colony on PDA (a), MEA (b), and PCA (c) after 14 days. D. Sporodochia on PCA e. Conidiophores of α and β conidia with conidiophores mother cells (arrows). f–g. Conidiophores of α conidia with conidiophore mother cells (arrows). h. Conidiophores of α conidium detached from the conidiophore mother cell. I–M. Conidiophores of β conidia with conidiophore mother cells (arrows). N–O Conidiophore mother cell with blastic conidiation. P–T. α conidia. U–X. 4-celled β conidia. Y. Irregularly shaped β conidium. z. Rarely 3-celled (left) and 5-celled (right) β conidia. Scale bars: D= 50 µm, E–H and P–T = 20 µm, I–M and U–Z = 15 µm and N–O = 10 µm.

Therefore, the difference mentioned in the research of Jayasiri et al. (2019) does not seem correct, and they overestimated the differences. On the other hand, the *tef1-α* sequence (accession number: MK360088) seems to be mistakenly submitted to GenBank under the label *S. radermacherae* but belongs to *Rhytidhysteron rufulum* (Hysteriales, Hysteriaceae). A BLAST search showed 100% similarity between MK360088 and KU510399 (*Rhytidhysteron rufulum* MFLUCC 14-0577). If we consider MK360088 as the *tef1-α* sequence of *S. radermacherae*, the nucleotide differences between the two species (*S. tessarthra* and *S. radermacherae*) in *tef1-α* sequence would be 76 nucleotides (10%), which is not consistent with the 18 (2%) nucleotide differences reported by Jayasiri et al. (2019). Another *tef1-α* sequence was submitted for *Ramusculicola thailandica* MFLUCC 17-0909 with the GenBank accession number MK360089 (Jayasiri et al. 2019). According to the BLAST search results, this sequence is most similar to *tef1-α* sequences of *Spegazzinia* species and most likely belongs to *S. radermacherae*, but it was mistakenly submitted under the name of *Ra. thailandica*. If we consider this sequence as the correct *tef1-α* sequence for the *S. radermacherae* species, the nucleotide differences between this species and the *S. tessarthra* species will be 15–17 nucleotides (depending on the *S. tessarthra* strains), which is almost consistent with the 18 nucleotides reported in the Jayasiri et al. (2019). According to the given explanations, the *tef1-α* sequence of *S. radermacherae* should be corrected in GenBank, so that the correct sequences can be used for the subsequent phylogenetic analyses. In this study, the sequence with the accession number MK360089 was considered the *tef1-α* sequence of *S. radermacherae* and included in the phylogenetic analyses.

Spegazzinia tessarthra has been reported as an endophyte from lichens (Manish et al. 2014) and as a saprophyte of many plant species, including gramineous plants, in different countries (Ellis 1971, Tanaka et al. 2015, Kirschner et al. 2017; Calabon et al. 2021, Tennakoon et al. 2022, Farr & Rossman 2023). In the present study, *S. tessarthra* was isolated as a saprophyte from *Brachypodium* sp. in Iran. This is the first record of *S. tessarthra* for the Funga of Iran and the first record of *Brachypodium* sp. as a substrate for *S. tessarthra*. To our knowledge, this is the first report of a species from the genus *Spegazzinia* in Iran (Bakhshi et al. 2022, Ershad 2022), and further research is needed to determine the diversity of *Spegazzinia* species in the country.

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شناسایی ریخت‌شناختی و مولکولی گونه *Spegazzinia tessarthra* (Ascomycota ،Didymosphaeriaceae) از ایران

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چکیده: در طی بررسی گونه‌های قارچی گیاهان گرامینه، قارچ هیفومیستی با ویژگی‌های جنس *Spegazzinia* از برگ‌های گیاه *Brachypodium* sp. (Poaceae) جمع‌آوری شده از استان مازندران جداسازی شد. جدایه به دست آمده بر اساس تلفیق ویژگی‌های ریخت‌شناختی و واکاوی فیلوژنتیکی ITS-rDNA و *tef1-α* به عنوان *Spegazzinia tessarthra* شناسایی شد. در مطالعه حاضر گونه *S. tessarthra* به عنوان گزارش جدید برای فونگای ایران و گیاه *Brachypodium* sp. به عنوان بستره جدید برای این گونه معرفی می‌شود. توصیف دقیق ریخت‌شناختی، عکس‌ها و مقایسه آن با سایر گونه‌های جنس *Spegazzinia* از نظر ریخت‌شناسی و فیلوژنتیکی ارائه شده‌اند. بر اساس اطلاعات نگارندگان، این اولین گزارش از گونه‌های جنس *Spegazzinia* در ایران است و لازم است تا مطالعات بیشتری برای بررسی تنوع گونه‌های جنس *Spegazzinia* در کشور انجام شود.

کلمات کلیدی: رده‌بندی، ریخت‌شناسی، فیلوژنی، *Didymosphaeriaceae*، *Brachypodium*

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