

Two new species of *Ophioceras* and *Schizothecium* for funga of Iran

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Abstract: During a study on the biodiversity of fungal taxa associated with symptomatic plants in the forests and gardens of Guilan and Khorasan Razavi provinces (northern and northeastern Iran) two diseased plant samples were collected: one from large-leaved linden (*Tilia platyphyllos* Scop.) in Guilan, showing branch canker symptoms, and the other from a fig tree (*Ficus carica* L.) in Khorasan Razavi, exhibiting necrotic leaf spot symptoms. Two fungal isolates were recovered, identified, and characterized through a combination of morphological characteristics and phylogenetic analysis of the ITS rDNA genomic regions. These isolates were identified as *Ophioceras leptosporum* on large-leaved linden and *Schizothecium inaequale* on the fig tree. According to the literature, *O. leptosporum* and *S. inaequale* are new ascomycetous taxa for the fungal flora of Iran. Furthermore, large-leaved linden and fig tree are reported here for the first time as new hosts (matrix nova) for *O. leptosporum* and *S. inaequale*, respectively, worldwide.

Keywords: Ascomycota, Morphology, Phylogeny, ITS rDNA, Perithecia.

INTRODUCTION

The class *Sordariomycetes* O.E. Erikss. & Winka (*Ascomycota* Caval.-Sm) was first introduced by Eriksson & Winka in 1997. It is the second-largest class in the subphylum *Pezizomycotina* O.E. Erikss. & Winka, after *Dothideomycetes* O.E. Erikss. & Winka. *Sordariomycetes* are a taxonomically complex group of asexual and sexual ascomycetes that are common throughout the world, occupying various ecological niches. Many species are significant plant pathogens, while others are commonly isolated as endophytes from various plants. Some taxa are fungicolous, whereas others persist as saprobes, contributing to decomposition and nutrient recycling (Maharachchikumbura et al. 2022; Eriksson & Winka, 1997).

The family *Ophiocerales* Klaubauf, E.G. LeBrun & Crous, was first introduced by Klaubauf et al. (2014). The family is recognized with immersed to superficial, scattered to separate, globose to subglobose perithecial ascomata, periphysate necks, unitunicate 8-spored asci, and filiform, hyaline to olivaceous, septate or aseptate, ascospores without sheaths. The genus *Ophioceras* Sacc. as type genus of the *Ophiocerales*, was first introduced by Saccardo in 1883 to accommodate fungi with immersed, sub-carbonaceous, globose perithecia, having conical-cylindrical, filiform ostioles, elongate asci, and filiform, septate hyaline ascospores. Initially, the genus included seven species with *O. dolichostomum* (Berk. & M.A. Curtis) Sacc. as the type species. These species are commonly found as saprobes on wood and herbaceous plants in both aquatic and terrestrial habitats (Klaubauf et al. 2014; Jiang et al. 2021). Forty-nine *Ophioceras* species are listed in the Index Fungorum until December 2024. In the past year, Yang et al. (2023) described *O. thailandense* J. Yang, E.B.G. Jones & K.D. Hyde as a new species from decaying wood submerged in freshwater in Thailand. Similarly, Zhang et al. (2023) reported *O. guizhouen* J.F. Zhang & Jian K. Liu, as a new species from dead bamboo culms in China.

The family *Schizotheciaceae* Y. Marin & Stchigel was introduced by Marin-Felix et al. (2020), with the genus *Schizothecium* Corda and its type species, *S. fimicola* Corda, established as the type genus of *Schizotheciaceae*. This genus was created to accommodate lasiosphaeriaceous taxa located in a well-supported clade (clade VIII), that is distinct from *Lasio-sphaeriaceae* s. str. (clade V). The taxonomy of the genus *Schizothecium* has been subjected to some major revisions. Huang et al. (2021) proposed a new genus *Neoschizothecium* S.K. Huang & K.D. Hyde with the type species of *Neoschizothecium curvisporum* (Cain) S.K. Huang & K.D. Hyde, in the new family *Neoschizotheciaceae* S.K. Huang & K.D. Hyde. This new family was created to accommodate some species of *Podospora* and *Schizothecium* in the *Neoschizotheciaceae* clade such as *Podospora minicauda* Faurel & Locq.-Lin., *Schizothecium aloides* (Fuckel) N. Lundq., *Schizothecium carpnicola* (Mouch.) L. Cai, *Schizothecium conicum* (Fuckel) N. Lundq., *Schizothecium fimbriatum* (A. Bayer) Barrasa & Soláns, *Schizothecium glutinans* (Cain) N. Lundq., *Schizothecium inaequale* (Cain) N. Lundq., *Schizothecium selenosporum* (Stchigel, Guarro & M.

Calduch) Y. Marín & Stchigel, and *Schizothecium tetrasporum* (G. Winter) N. Lundq. However, Marin-Felix and Miller (2022) later revised the recent taxonomic changes within the *Sordariales* Chadef. ex D. Hawksw. & O.E. Erikss. They stated that the proposed genus *Neoschizothecium* in the family *Neoschizotheciaceae* is superfluous and should not be used. Until December 2024, thirty *Schizothecium* species

were listed in Index Fungorum (www.indexfungorum.org).

To date, species of *Ophioceras* and *Schizothecium* have not been studied in Iran, and no scientific reports exist on their presence in the country. Therefore, the main objective of this study was to identify two new fungal species belonging to these genera in the northern and northeastern regions of Iran.

Table 1. Fungal strains used for phylogenetic analyses. Newly generated sequences are in boldface.

Species	Collection number/Strain	Host/Substrate	Origin	GenBank
				accession no.
				ITS
<i>Ophioceras leptosporum</i>	ABRIICC 10339	<i>Tilia platyphyllos</i>	Iran	MZ226450
<i>O. leptosporum</i>	CPC:39147	<i>Syzygium cordatum</i>	South Africa	MW883435
<i>O. leptosporum</i>	N150, 7.4.4.7	Unknown	New Zealand	KP689126
<i>O. freycinetiae</i>	CBS 146781	<i>Freycinetia banksii</i>	New Zealand	NR173031
<i>O. freycinetiae</i>	CBS:146781	<i>Freycinetia banksii</i>	New Zealand	MZ064408
<i>O. junci</i>	CPC 42234	<i>Juncus effusus</i>	Netherlands	OK664750
<i>O. junci</i>	CBS 148450	<i>Juncus effusus</i>	Netherlands	NR175243
<i>O. aquaticum</i>	IFRDCC 3091	Submerged wood	China	NR165842
<i>O. aquaticum</i>	IFRDCC 3091	Submerged wood	China	JQ797440
<i>O. commune</i>	HKAS 92587	Submerged wood	China	MH795814
<i>O. commune</i>	HKAS 92640	Submerged wood	China	MH795813
<i>Schizothecium inaequale</i>	ABRIICC 10361	<i>Ficus carica</i>	Iran	OP622280
<i>S. inaequale</i>	CBS:109403	Unknown	Chile	MH862824
<i>S. fimbriatum</i>	CBS 144.54	Dung of horse	China	AY999115
<i>S. carpinicola</i>	CBS 228.87	<i>Carpinus betulus</i>	China	NR103589
<i>S. carpinicola</i>	CBS 228.87	<i>Carpinus betulus</i>	China	AY999118
<i>S. glutinans</i>	A620	Unknown	China	MK247326
<i>S. glutinans</i>	CBS 134.83	<i>Arctostaphylos uva-ursi</i>	Australia	AY999116
<i>S. selenosporum</i>	CBS 109403	Unknown	China	NR175136
<i>S. selenosporum</i>	CBS 356.49	<i>Daucus carota</i>	USA	NR175134
<i>S. selenosporum</i>	CBS 226.87	<i>Carpinus betulus</i>	China	AY999117
<i>Pleospora herbarum</i>	EGS 36-138.2	<i>Medicago sativa</i>	India	AF442785

MATERIALS AND METHODS

Sampling and fungal isolates

Branch and leaf samples showing canker and necrotic spot symptoms were collected from large-leaved linden (*Tilia platyphyllos* Scop.) and common fig (*Ficus carica* L.) trees in the Guilan and Khorasan Razavi provinces of Iran during the autumn and spring of 2021–2022, respectively. Fungal strains were isolated from the diseased plant samples using a method described by Refaei et al. (2011), with some minor modifications. At first, diseased plant tissues were cut into small pieces (1 cm²), and washed under running tap water for 10 min to remove surface contaminants. The plant pieces were then surface disinfected by immersion in 70% ethanol for 2 min and subsequently in 2% sodium hypochlorite (NaOCl) solution for 2 min (for the leaf sample, the immersion time in each solution was reduced to 30 seconds). After disinfection, all plant pieces were rinsed twice with sterile distilled water for 2 min, dried between sterile paper towels, and placed onto

2% water agar (WA). The inoculated Petri dishes were kept at 25±2 °C under a 12 h photoperiod for seven days. Fungal isolates were purified on potato dextrose agar (PDA) culture medium using the hyphal tip method and then incubated at 25±2 °C in continuous darkness until pure fungal colonies appeared. For long-term storage, fungal isolates were grown on sterile filter papers placed on PDA for seven to 10 days. The colonized filter papers were subsequently removed from the culture medium, and then dried at room temperature for four to five days, and stored at –20 °C for future use.

Morphology

The morphological characterization of the fungal isolates was performed based on the morphology of the colony, as well as features of fruiting bodies, including ascomata (perithecia), the texture of peridium, asci, and ascospores. Fungal features were assessed after 10 to 25 days of incubation of pure fungal colonies using an Olympus BH2 light microscope (Olympus, Japan). Microscopic slide

mounts were prepared with lactophenol or lactophenol cotton blue solutions. Morphological studies of the two obtained genera were performed on PDA, with cultures incubated under near-ultraviolet (nUV) light (12 h light/12 h darkness) at 25±2 °C. Colony diameter of the fungal strains was measured after seven days. Macro- and micromorphological features of the different recovered isolates were measured. Micro-morphological features and measurements were performed following the methods described by Walker (1980) and Mirza & Cain (1969). Photographs were taken using the Sony digital camera mounted on Olympus BH2 light microscope.

Phylogeny

After morphological identifications, the fungal isolates were prepared for molecular investigations. DNA was extracted from seven-day-old fungal mycelium, using the method provided by Zhong and Steffenson (2001). The complete internal transcribed spacer (ITS1-5.8S-ITS2) region of the 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-MgCl₂, 11 µL deionized water, 1 µL of each primer (10 pmol) and 2 µL of template DNA. The PCR amplification was performed in an Eppendorf Thermal Cycler with the following conditions: an initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation step at 94°C for 30 s, annealing at 52°C for 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 on a 1.5% agarose gel via electrophoresis with 1x Tris-Boric acid-EDTA buffer (TBE) and PCR products were sent to the Cardiogenetic Research Center (IRAN) for sequencing.

After sequencing, the 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 (456-495 bp) were subjected to BLAST search (Altschul et al. 1990) in the National Centre of Biological Information, GenBank (NCBI) (www.ncbi.nlm.nih.gov/genbank/) to find the most relevant sequences. For phylogenetic analyses, twenty-one ITS sequences of *Ophioceras* and *Schizothecium* species along with *Pleospora herbarum* (AF442785) as out-group taxon, were selected (Table 1). The sequences were aligned with Clustal W (Thompson et al. 1994). Maximum likelihood (ML) analysis (Felsenstein 1973) was conducted through a heuristic search using Mega X (Kumar et al. 2018). Bootstrap analysis (Felsenstein 1985) of the ML tree was performed with 1000

replicates, and the bootstrap values above 50 were indicated on the nodes.

RESULTS

In the present study, two fungal isolates were recovered from symptomatic large-leaved linden and fig trees. The morphological investigation, along with a phylogenetic study based on ITS rDNA sequences, revealed that these two isolates correspond to *Ophioceras leptosporum* and *Schizothecium inaequale*, which were recovered from *Tilia platyphyllos* and *Ficus carica*, respectively. In the reconstructed ML tree (Fig. 1), the recovered *O. leptosporum* (MZ226450) and *S. inaequale* (OP622280) isolates were grouped with the relevant taxa with 100% bootstrap support, confirming the accuracy of their morphological identification. The newly obtained nucleotide sequences of the ITS rDNA region in this study were deposited in the GenBank using the BankIt submission tools (NCBI, USA), with the accession no. of MZ226450 for *Ophioceras leptosporum* isolate UTOS-60, and OP622280 for *Schizothecium inaequale* isolate UTR12. In the ML tree, *Schizothecium* species were grouped in Clade II with 100% bootstrap support with other species of this genus from GenBank (NCBI). Our obtained isolate of *S. inaequale* (OP622280) was 100% similar to other isolates of this species from GenBank (MK926846 and NR175134), with 100% query coverage in BLAST search.

Additionally, the living cultures of *Ophioceras leptosporum* (UTOS-60), and *Schizothecium inaequale* (UTR12) were deposited in the Agricultural Biotechnology Research Institute of Iran Culture Collection, Karaj, Iran, with the accession numbers of ABRIICC 10339 and ABRIICC 10361, respectively.

Taxonomy

Ophioceras leptosporum (S.H. Iqbal) J. Walker, *Mycotaxon* 11 (1): 62 (1980)

Specimen examined: IRAN, Guilan Province, Asalem, N 37°43'52.5" E 48°57'38.0", recovered from branch with canker symptom of *Tilia platyphyllos* Scop., November 2021, Isolate code: UTOS-60, ABRIICC 10339, Collector; A. Atashi Khalilabad.

Colony on PDA, slow-growing, reaching 42 mm in diameter after seven days at 23 – 25°C under the near-ultraviolet (nUV) light (12 h light/12 h dark). Colony white, cottony, low convex, with crenate edges; Reverse: white at the margin, yellow to yellow-orange in the center.

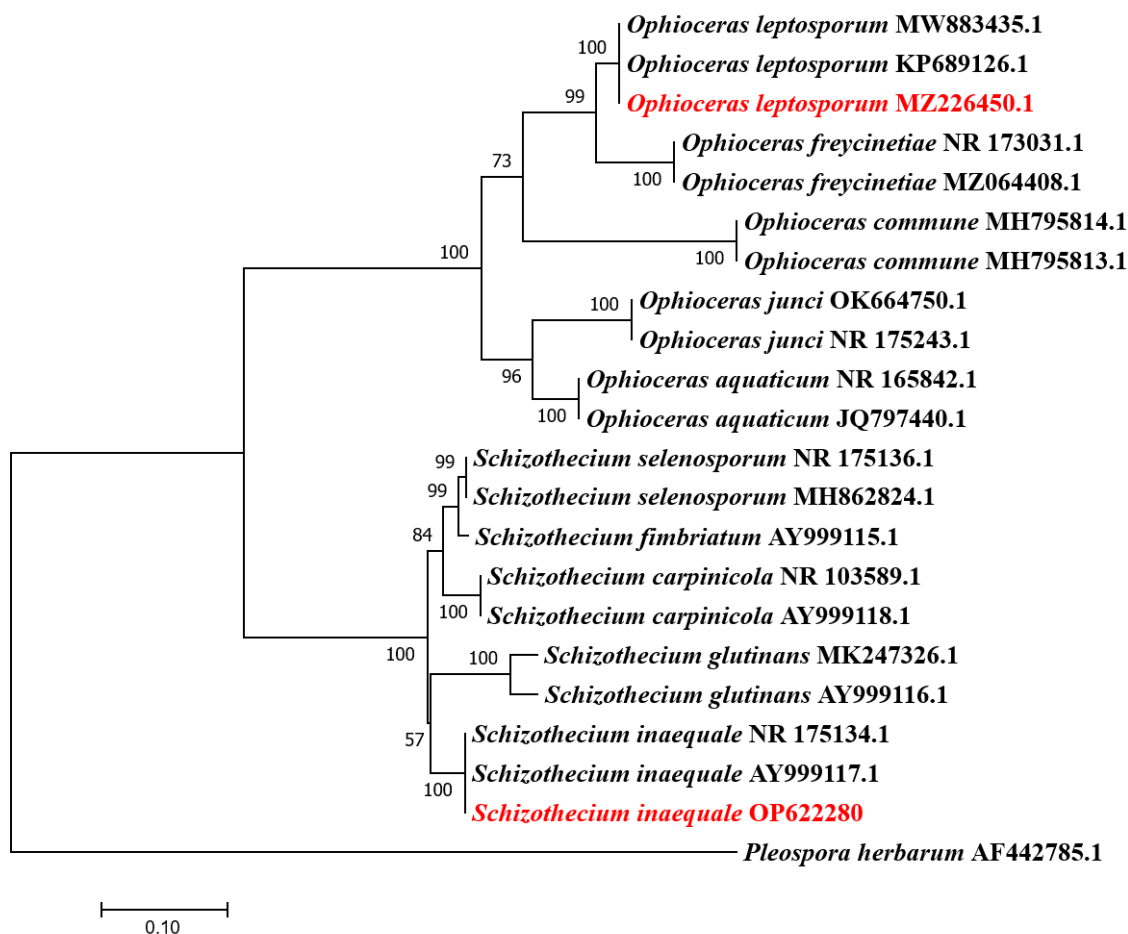


Fig. 1. Maximum likelihood (ML) tree generated in MEGA X, based on aligned sequences of the ITS rDNA regions of 21 isolates of *Ophioceras* and *Schizothecium* species and *Pleospora herbarum* as an out-group. Bootstrap values (1000 replicates) are indicated at the nodes. The strains indicated in red color were obtained in the present study. The scale bar indicates 0.05 expected nucleotide changes per site.

Sexual morph: Ascomata black to dark brown, perithecial, globose with long filiform neck, $450 - 700 \times 250 - 310 \mu\text{m}$ ($\bar{x} = 575 \times 275 \mu\text{m}$, $n = 20$), immersed or superficial, scattered or gregarious. Textura angularis of peridium with brown to pale brown cells, $10 - 15 \times 10 - 14 \mu\text{m}$ ($\bar{x} = 12.5 \times 12.4 \mu\text{m}$, $n = 30$). Neck dark brown (near the tip) to black, placed centrally on the perithecia. Ostiolar canal $20 - 40 \mu\text{m}$ wide. Periphyses and paraphyses absent. Asci cylindrical, hyaline, unitunicate, eight-spored, straight or slightly curved, $65 - 100 \times 5 - 7 \mu\text{m}$ ($\bar{x} = 80 \times 6.2 \mu\text{m}$, $n = 30$). Ascospores hyaline, pale yellowish in mass, parallel in the ascus, filiform, straight or slightly

curved or sigmoid, apex rounded, multiseptate ($3 - 7$ indistinct septa), $65 - 85 \times 1 - 1.5 \mu\text{m}$ ($\bar{x} = 75.5 \times 1/2 \mu\text{m}$, $n = 50$) in size (Fig. 2).

Asexual morph: Not observed.

The morphological features of the investigated isolate were similar to the species description provided by Walker (1980). To date, the asexual morph of *Ophioceras* has not been reported, and only 49 epithets are listed for this genus in the Index Fungorum database (2024). Additionally, LSU and ITS sequence data are mostly available for these species.

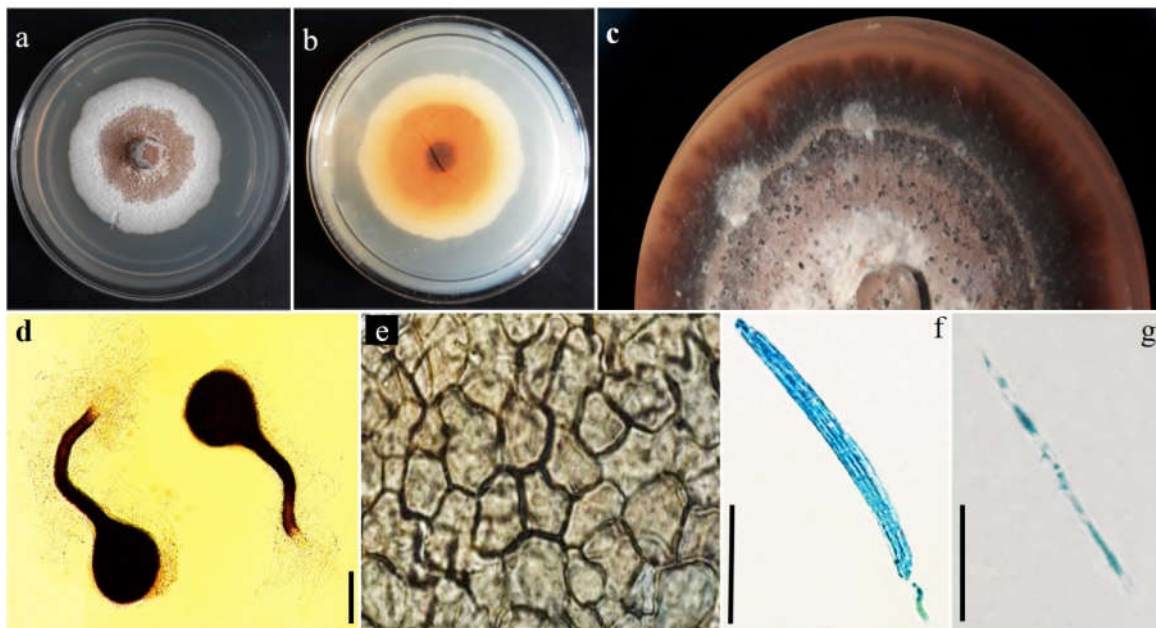


Fig. 2. *Ophioceras leptosporum* isolate UTOS-60: a-b. Upper (top) and reverse (bottom) sides of the colony on PDA, after seven days, c. colony on PDA, after twenty days of cultivation at 25 °C in 12/12 dark/nUV condition, d. Perithecia, e. Peridium, f. Ascus and ascospores, and g. Ascospore. Scale bars: d = 200 μ m, f = 40 μ m, g = 30 μ m.

***Schizothecium inaequale* (Cain) N. Lundq., Symbolae Botanicae Upsalenses 20 (1): 334 (1972)**
Specimen examined; IRAN, Khorasan Razavi Province, Bardaskan, N 35°14'34.7" E 57°57'15.3", recovered from necrotic leaf spot symptoms of *Ficus carica* L., May 2022, Isolate code: UTR12, ABRIICC 10361, Collector; A. Atashi Khalilabad.

Colony on PDA was velvety, grey-black, with an irregular margin, and reached 45 mm in diameter after seven days at 23 – 25°C under the near-ultraviolet (nUV) light (12 h light/12 h dark).

Sexual morph: Ascomata dark brown, perithecia superficial, globose to subglobose, or pyriform with short neck, 250 – 460 \times 120 – 220 μ m (\bar{x} = 300 \times 170 μ m, n = 20), superficial to immersed, scattered or gregarious. Textura angularis of peridium with brown to olivaceous brown cells, 9 – 15 \times 8 – 14 μ m (\bar{x} = 12.5 \times 12.4 μ m, n = 30). The neck is placed centrally on the perithecia. Asci hyaline, unitunicate, 4-spored, cylindrical, apical ring not distinct, 90 – 118 \times 10 – 15 μ m (\bar{x} = 105 \times 13 μ m, n = 20). Ascospores hyaline when immature, then dark brown to black with age, smooth, inequilateral in side view with one side almost straight, ellipsoid in face view, 16 – 24 \times 10 – 13 μ m (\bar{x} = 20 \times 12/5 μ m, n = 50). Pedicel cylindrical or clavate, hyaline, 5 – 8 \times 1 – 2.5 μ m (\bar{x} = 7.2 \times 1.8 μ m, n = 30). usually long persistent, germ pore eccentric (Fig. 3).

Asexual morph: Not observed.

The morphological features of the investigated isolate were similar to the species description provided by Mirza & Cain (1969). The known anamorphic stages of *Schizothecium* species are all phialidic (e.g. *S. aloides*, *S. conicum*, *S. fimbriatum*, *S. tetrasporum* and *S. vesticola*). The phialides are

flask-shaped or elongated clavates, some with distinct collarettes. Spores are generally small, hyaline, and globose or ovoid (Cai et al. 2005). Based on the literature, *S. inaequalis* has been reported as a relatively infrequent species with small perithecia without gelatinous appendages.

DISCUSSION

The present study was conducted in 2021–2022 at two locations in northern (Asalem district) and northeastern (Bardaskan county) Iran. In the present study, two species from different genera in the class *Sordariomycetes*, namely *Ophioceras leptosporum* and *Schizothecium inaequale*, were identified and described based on both morphological characteristics and molecular data from ITS rDNA sequences. Both species are new taxa for the funga of Iran. Furthermore, the plant hosts are reported as new hosts (matrix nova) for the respective identified fungal taxa worldwide. *Ophioceras leptosporum* was first described by Iqbal (1972) as *Gaeumannomyces leptosporus* (S.H. Iqbal) J. Walker from decaying submerged branches of an Umbelliferus plant in a river in England. Later, Walker (1980) transferred *G. leptosporus* to the genus *Ophioceras*, renaming it as *O. leptosporum*. This species is generally known as a saprophytic fungus on dead and decaying plants in aquatic environments. Morphologically, *O. leptosporum* closely resembles *O. sichuanense* H.B. Jiang, Phookamsak & K.D. Hyde and *O. tenuisporum* Shearer, J.L. Crane & W. Chen due to the size of the asci and ascospores, as well as, the extremely long ostiolar necks. However, *O. leptosporum* differs in its ascospore septation (3–7 indistinct septa) in

comparison to *O. sichuanense*, which has aseptate ascospores, and *O. tenuisporum* which has 3-septate ascospores. *Ophioceras leptosporum* belongs to the *Ophiocerales* family in the order *Magnaporthales*.

In the reconstructed ML tree based on the ITS rDNA sequence data, phylogenetic analyses revealed that obtained isolates from Iran are closely related to the sequences from GenBank (NCBI), forming two distinct clades. *Ophioceras* species were grouped in the Clade I with 100% bootstrap support with other species of this genus from GenBank. Our obtained isolate of *Ophioceras leptosporum* (MZ226450) was 100% similar to another isolate of this species from GenBank (MW883435), with 100% query coverage in the BLAST search.

Schizothecium inaequale was first introduced by Cain and Groves (1948) as *Sordaria inaequalis* Cain. Lundqvist (1972) transferred *S. inaequalis* to the genus *Schizothecium* as *S. inaequale*.

Schizothecium inaequale has been reported from *Carpinus betulus* (Cai et al. 2005), vegetables, soil, and horse dung (Doveri, 2008). *Schizothecium inaequale* belongs to family *Schizotheciaceae* family of the *Sordariales* order. In the genus *Schizothecium*, the morphology of the ascospore is important and is one of the most useful characters for separating species (Lundqvist, 1972). For example, *S. fimbriatum*, *S. inaequale*, and *S. curvisporum* are characterized by inequilateral ascospores, and the presence or absence of gelatinous appendages, which can help differentiate species in this genus. For example, *S. carpinicola*, *S. curvisporum*, and *S. inaequale* lack gelatinous appendages in their ascospores (Cai et al. 2005). In most *Schizothecium* species the gelatinous appendages are absent in their ascospores, likely due to growth in non-coprophilous habitats (Mirza and Cain, 1969).

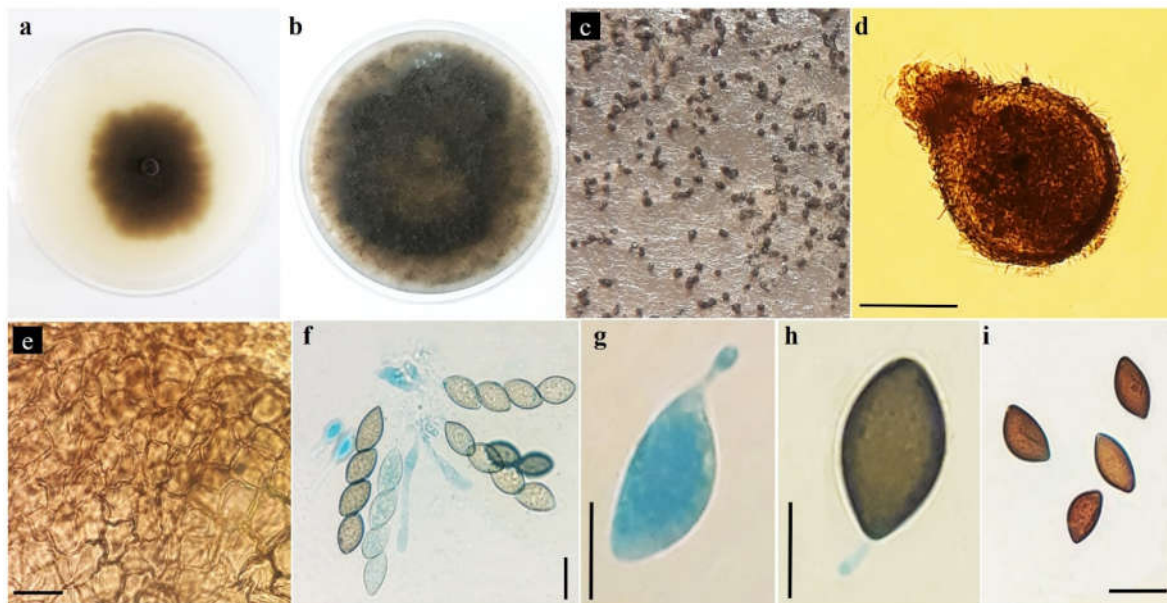


Fig. 3. *Schizothecium inaequale* isolate UTR12: a-b. Colony on PDA after seven- and fourteen-days growth at 25 °C in 12/12 dark/nUV condition, respectively. c. Perithecia formed on the surface of the PDA culture medium, d. Perithecium, e. Peridium, f. Asci and ascospores, and g-i. Ascospores. Scale bars: d = 250 µm, f = 20 µm, g-h = 10 µm, i = 20 µm.

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دو گونه جدید از *Schizothecium* و *Ophioceras* برای قارچهای ایران

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چکیده: در طی بررسی تنوع زیستی برخی آرایه‌های قارچی مرتبط با گیاهان بیمار و دارای علائم بیماری در جنگل‌ها و باغات استان‌های گیلان و خراسان رضوی (شمال و شمال شرق ایران) در پاییز و بهار سال‌های ۱۴۰۰ و ۱۴۰۱، دو نمونه گیاهی بیمار شامل درخت نمدار برگ بزرگ (*Tilia platyphyllos* Scop.)، گیلان) دارای علائم شاکر شاخه و انجیر (*Ficus carica* L.)، خراسان رضوی) با علائم لکه برگ‌گی نکروتیک جمع‌آوری شدند. دو جدایه قارچی به دست آمده با استفاده از ویژگی‌های ریخت‌شناختی روی محیط کشت سیب زمینی دکستروز آگار و همچنین بر اساس تجزیه و تحلیل فیلوژنتیکی به روش حداکثر احتمال و مبتنی بر داده‌های توالی نوکلئوتیدی ناحیه ITS rDNA مورد شناسایی قرار گرفتند. این دو جدایه به جنس‌های *Ophioceras* و *Schizothecium* تعلق داشتند و به عنوان گونه *Ophioceras leptosporum* از درخت نمدار برگ بزرگ و گونه *Schizothecium inaequale* از درخت انجیر شناسایی شدند. بر اساس بررسی‌های انجام گرفته تا کنون، گونه‌های *Ophioceras leptosporum* و *Schizothecium inaequale* آرایه‌های جدیدی برای فونگای ایران هستند. علاوه بر آن، درختان نمدار برگ بزرگ و انجیر، به عنوان میزبان‌های گیاهی جدیدی به ترتیب برای گونه‌های *Ophioceras leptosporum* و *Schizothecium inaequale* در دنیا گزارش می‌شوند.

کلمات کلیدی: آسکومیکوتا، ریخت‌شناسی، فیلوژنی، ITS rDNA، پریسیوم‌ها.