Aspergillus olivicola, a new record for the funga of Iran

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Abstract

During a mycological survey of declining olive trees (*Olea europaea*) in the Kermanshah Province (Iran) in Dec. 2022, an *Aspergillus* strain belonging to sect. *Nidulantes* of subgenus *Nidulantes* was recovered from symptomatic bark and xylem tissues exhibiting dieback and discoloration. Based on comprehensive morphological characterization, our strain (IRAN 5531C) was identified as *A. olivicola* which is mainly characterized by having greenish-brown globose cleistothecial ascomata embedded in masses of brown, thin-walled Hülle cells, and reddish-brown, stellate ascospores. The morphological identification was further confirmed through multi-locus phylogenetic analysis using sequences of the internal transcribed spacer (ITS) region of rDNA and β-tubulin (*BenA*) gene, which resulted in clustering of Iranian strain with the ex-type strain of *A. olivicola* (CBS 119.37) with 100% bootstrap support. BLASTn analysis of the ITS and *BenA* sequences, revealed 98–100% sequence identity of the strain with the CBS 119.37. The present study represents the first record of *A. olivicola* for the funga of Iran.

Keywords: *Aspergillus* sect. *Nidulantes*, multi-locus phylogeny, *Olea europaea*, stellate ascospores, taxonomy

Aspergillus olivicola، گزارش جدید برای قارچ‌های ایران

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خلاصه

در یک بررسی قارچ‌شناسی روی درختان زیتون (*Olea europaea* L.) رو به زوال در استان کرمانشاه که در آذرماه ۱۴۰۱ انجام شد، یک جدایه آسپرژیلوس از بخش *Nidulantes* زیرجنس *Nidulantes* از بافت‌های چوب و پوست دارای علایم سرخشکیدگی به همراه تغییر رنگ بافت آوندی جداسازی شد. این جدایه براساس ویژگی‌های ریخت‌شناختی از جمله تولید آسکوکارپ‌های کروی کلیستوتسیومی به رنگ قهوه‌ای مایل به سبز، سلول‌های هول (Hülle cells) با دیواره نازک، قهوه‌ای و آسکوسپورهای ستاره‌ای به رنگ قرمز مایل به قهوه‌ای، به عنوان *Aspergillus olivicola* شناسایی شد. شناسایی ریخت‌شناختی از طریق واکاوی تبارشناسی چندژنی، با استفاده از توالی‌های ناحیه‌های ژنی ITS از rDNA و ژن بتا-توبولین (*BenA*) تایید شد. تحلیل BLASTn توالی‌های ITS و *BenA*، شباهت حدود ۱۰۰-۹۸ درصد هویت توالی را با جدایه تیپ گونه *A. olivicola* (CBS 119.37) نشان داد. در واکاوی تبارشناسی، جدایه این مطالعه با ضریب حمایت ۱۰۰ درصد در کنار جدایه تیپ قرار گرفت. مطالعه حاضر نخستین گزارش از *A. olivicola* برای قارچ‌های ایران است.

واژه‌های کلیدی: آسپرژیلوس بخش *Nidulantes*، آسکوسپورهای ستاره‌ای، تبارشناسی چندژنی، تاکسونومی، زیتون

Introduction

The genus *Aspergillus* is one of the most diverse and economically important fungal genera, comprising approximately 520 accepted species organized into six subgenera, 27 sections, and 75 series (Houbraken *et al*. 2020, Visagie *et al*. 2025). Subgenus *Nidulantes* (formerly *Emericella*), with nine sections (*Aenei*, *Bispori*, *Cavernicolarum*, *Nidulantes*, *Ochraceorosei*, *Raperorum*, *Silvatici*, *Sparsi*, and *Usti*), includes species with key ecological roles as saprobes in organic matter decomposition, endophytes/pathogens on woody hosts, industrial producers (e.g., echinocandins from *A. nidulans*), and mycotoxin producers (Raper & Fennell 1965, Frisvad & Samson 2004, Samson *et al*. 2014, Chen *et al*. 2016, Visagie *et al*. 2025).

Species in sect. *Nidulantes* are characterized by Hülle cells and cleistothecial ascomata (Chen *et al*. 2016). The *Stellati* series within this section is defined by unique stellate ascospores and includes species such as *A. olivicola*, *A. stellatus*, and *A. venezuelensis* (Zalar *et al*. 2008).

The olive tree (*Olea europaea* L.), a cornerstone of the Oleaceae, is a major global fruit crop spanning >10.5 million hectares, with Iran contributing 38,000 ha and 114,000 tons (FAOSTAT 2023). Despite its economic importance in Iran's expanding western olive regions and, fungal diversity associated with olive decline remains underexplored.

Materials and Methods

In Dec. 2022, samples were collected from symptomatic bark and xylem of declining olive trees in Kermanshah, Iran, focusing on branches with external bark cankers, and internal brown-to-dark vascular discoloration. Small wood fragments were excised from the margin between healthy and necrotic tissues, surface-sterilized, and plated onto potato dextrose agar (PDA) supplemented with antibiotics for fungal isolation. Pure cultures were obtained by single-spore isolation and maintained on PDA slants at 4 °C.

Microscopic observations of sexual (ascomata, Hülle cells, asci, ascospores) and asexual (conidiophores, phialides, conidia) stages, developed at 25 °C after 7 days on PDA and malt extract agar (MEA), used an Olympus BX51 microscope with DinoCapture camera. Dimensions of 50 structures each were measured using BioLoMICS; images processed in CorelDRAW 12. Mounts were in lactic acid–glycerin (1:1). Growth was tested by central inoculation on PDA and MEA at 10, 15, 20, 25, 30, and 38 °C (and 40 °C) for 7 days in darkness. Identification was based primarily on these combined macroscopic and microscopic characteristics, using established taxonomic keys and references (Hubka *et al*. 2016). Living culture of the strain examined in this study was preserved at the Iranian Fungal Culture Collection (IRAN 5531C) of Herbarium Ministerii Iranici Agriculturae "IRAN," Iranian Research Institute of Plant Protection (Tehran, Iran).

DNA was extracted using a Dena Zist Asia kit. The internal transcribed spacer (ITS) region of rDNA and the β-tubulin (*BenA*) gene were amplified using the ITS1/ITS4 and Bt2a/Bt2b primer pairs, respectively, with PCR conditions following established protocols (White *et al*. 1990, O'Donnell *et al*. 1998). The resulting sequences of ITS region and *BenA* gene were deposited in GenBank (www.ncbi.nlm.nih.gov/genbank) under accession numbers PX760330 and PX761504, respectively. For phylogenetic analyses, sequences were aligned using MAFFT Ver. 7.490 (Katoh *et al*. 2019) and refined in BioEdit Ver. 7.0.4.1, followed by removal of ambiguously aligned regions using Gblocks (under relaxed parameters). The final concatenated dataset comprised 992 positions for *Aspergillus* (ITS + *BenA*). Multi-locus phylogenetic analyses were inferred using both Maximum Parsimony (MP) and Maximum Likelihood (ML) methods in MEGA X (Kumar *et al*. 2018). For ML analysis, the best-fit K2P + G model was selected based on the Bayesian Information Criterion (genus-specific Gamma parameter: G = 0.1876). Initial heuristic search trees were constructed using Neighbor-Join and BioNJ algorithms applied to pairwise distances estimated by the Maximum Composite

Likelihood method. MP analysis employed the Subtree-Pruning-Regrafting (SPR) algorithm (search level 1; 1000 random replicates). A partial deletion option (allowing ≤5% gaps/missing data) was applied to all analyses, and clade robustness was assessed via 1000 bootstrap replicates (support values ≥90% considered high).

Results

Sequence analysis of the ITS region and *BenA* gene, complemented by BLASTn searches against the GenBank database, successfully identified our *Aspergillus* strain as *A. olivicola*. The strains showed 100% ITS identity with the reference strains of *A. oleicola*, i.e., CBS 597.65 and DTO 322-A9, and 97.98% *BenA* identity with the ex-type strain, CBS 119.37. It should be noted that, *A. oleicola*, as listed for strains CBS 597.65 and DTO 322-A9 in some databases, represents an orthographic variant of *A. olivicola*, and both names refer to the same taxon. The combined ITS and *BenA* sequence dataset used for the phylogenetic analysis of *Aspergillus* included 21 ingroup and one outgroup (*A. heterothallicus* belonging to sect. *Usti*) taxa, totaling 922 characters. Of these, 649 were constant, 297 variable but parsimony-uninformative, and 186 parsimony-informative. The subsequent MP analysis yielded a single most parsimonious tree with a tree length (TL) of 357 steps, consistency index (CI) of 0.76, retention index (RI) of 0.82, and rescaled consistency index (RCI) of 0.62. This analysis robustly confirmed the placement of our strain within the *A. olivicola* clade (Fig. 1). For comprehensive phylogenetic inference, ML trees were also reconstructed **(**SBL=0.62, log likelihood = -2853.36, model Kimura 2-parameter, Ts/Tv = 2.3, gamma = 0.32**)**. The ML trees were found to be topologically congruent with the MP trees, differing only in minor variations of bootstrap support values (Fig. 2).

Both MP and ML phylogenetic analyses placed the Iranian strain (IRAN 5531C) in a fully supported clade (100% bootstrap) including the ex-type strain of *A. olivicola* (CBS 119.37), and reference strains of this species (CBS 597.65, DTO 322-A9) (Figs 1–2). Morphological chracarteristics of our strain also fully aligned with the series *Stellati*.

The Iranian strain exhibited optimal growth at 30 °C, reaching colony diameters of 30 mm after 5 days on both PDA and MEA. No growth occurred at 10, 15, or 40 °C on either medium. Growth rates were 15 mm at 20 °C and 20 mm at 25 °C on PDA and MEA, respectively. The strain remained viable at 38 °C, with 10 mm growth after 5 days. On PDA and MEA, colonies were granular due to abundant dark-brown ascomata, lacking exudate droplets and aerial mycelium; the colony surface was initially yellowish to olive-brown, becoming dark brown with age; reverse yellowish to brown. On both media, our strain produced abundant greenish-brown, globose cleistothecial ascomata, measuring 320–720 µm ($\bar{x}$ = 540 µm, n = 50) diam., within 10 days. These structures were surrounded by characteristic brown, thin-walled Hülle cells, measuring 14.07–26.12 µm ($\bar{x}$ = 20.72 µm, n = 50) diam. Asci stellate in surface view, globose, 8-spored, measuring 10–15.9 µm ($\bar{x}$ = 12.29 µm, n = 50) diam. Ascospores orange to reddish-brown, measuring 6.8–9.5 µm ($\bar{x}$ = 7.5 µm, n = 50) diam., spore bodies smooth, in surface view stellate, in side view lenticular, and possessed two distinct equatorial crests with 2–3 µm long extensions (Fig. 1 a-f). Conidiophores arise as long, brownish, smooth-walled, and simple structures, measuring 150–350 × 3.5–5.5 µm (n = 50). Vesicles subglobose to subclavate, brownish, 9.2–17 µm ($\bar{x}$ = 12.96 µm, n = 50), upper half covered with metuale; metulae hyaline to brown, 6.5–8.5 × 2.0–3.5 µm. Phialides flask-shaped, hyaline to brown, 6–12 × 2–2.5 µm (n = 50). Conidia yellowish to greenish in mass, echinulate, globose to subglobose, 2.5–3.5 µm ($\bar{x}$ = 2.6 µm, n = 50), (Fig. 3).

Specimen examined: IRAN: Kermanshah Province, Rijab, from declining olive tree, 26.12.2022, S. Jamali (IRAN 5531C).

Discussion

In this study, we isolated an *Aspergillus* species from symptomatic branches of declining olive trees in Kermanshah Province, Iran. These branches exhibited dieback symptoms and brown-to-dark vascular discoloration in the bark and xylem tissues. The strain was identified as *A. olivicola*, belonging to sect. *Nidulantes* series *Stellati*, based on detailed morphological characterization and multi-locus phylogenetic analysis (ITS + *BenA*). Defining features of series *Stellati* include spreading colonies, green conidia en masse, biseriate conidiophores, hyaline to yellowish-brown, smooth stipes, globose Hülle cells, no growth at 40 °C, and an *Emericella*-type homothallic sexual morph (except in *A. caespitosus*) producing globose, stellate, or appendaged ascospores (Sklenář *et al.* 2020). The series *Stellati* encompasses *A. angustatus*, *A. astellatus*, *A. caespitosus*, *A. dromiae*, *A. filifer*, *A. miraensis*, *A. qinqixianii*, *A. stellamaris*, *A. stellatus*, *A. stelliformis*, *A. undulatus*, and *A. venezuelensis* (Houbraken *et al.* 2020). The smooth convex spore surface in *A. olivicola* distinguishes this species from the closely related *A. stellatus* which possesses roughened convex surfaces (Zalar *et al.* 2008, Hubka *et al.* 2016).

Members of *Aspergillus* sect. *Nidulantes* produce a wide array of secondary metabolites, such as aflatoxins and sterigmatocystins, echinocandins and mulundocandins, penicillins and terreins (Frisvad & Samson 2004, Chen *et al.* 2016). The ability of *A. olivicola* to produce aflatoxin B_1 and sterigmatocystin poses a significant threat to agricultural health and food security (Chen *et al*. 2016). This study provides the first record of *A. olivicola* from Iran, expanding its known geographic distribution, and reports the first isolation of this species from olive bark and xylem tissues worldwide. These findings highlight the need for further studies to elucidate its association with olive decline.

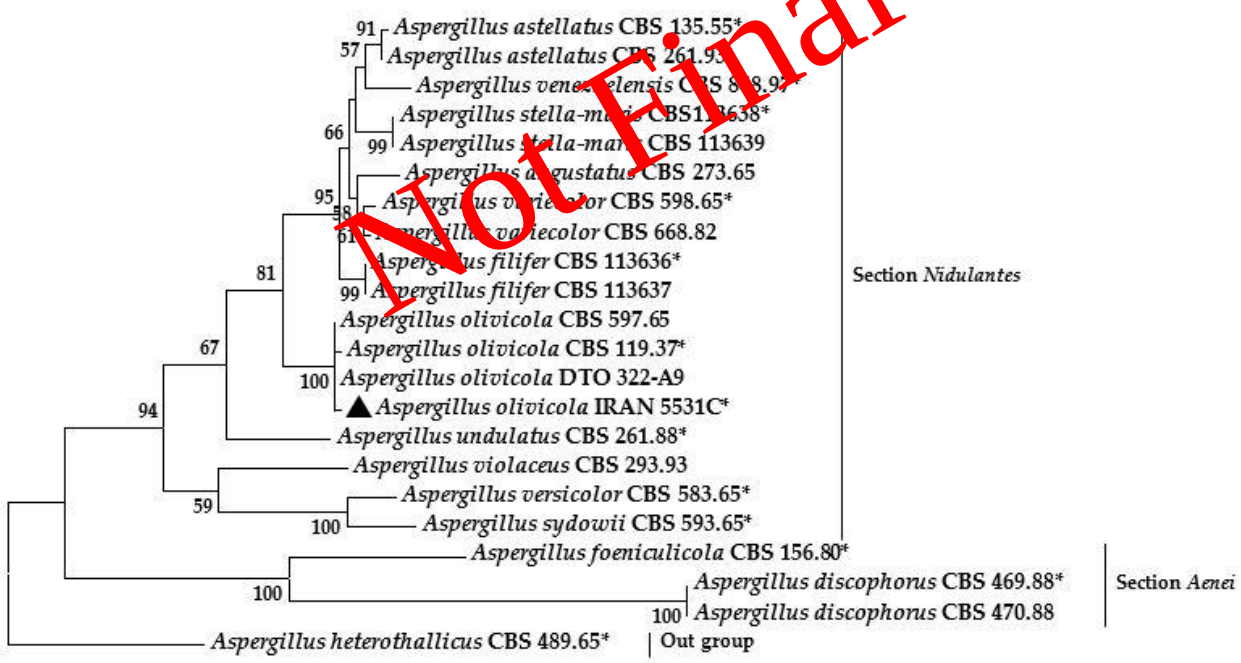

Fig. 1. Maximum parsimony consensus tree of *Aspergillus* species belonging to subgenus *Nidulantes* based on concatenated ITS and *BenA* sequences. Bootstrap support values (1,000 replicates) are displayed at the nodes. The strain characterized in this study is marked with a triangle (▲). Ex-type strains are indicated with a star (⋆).

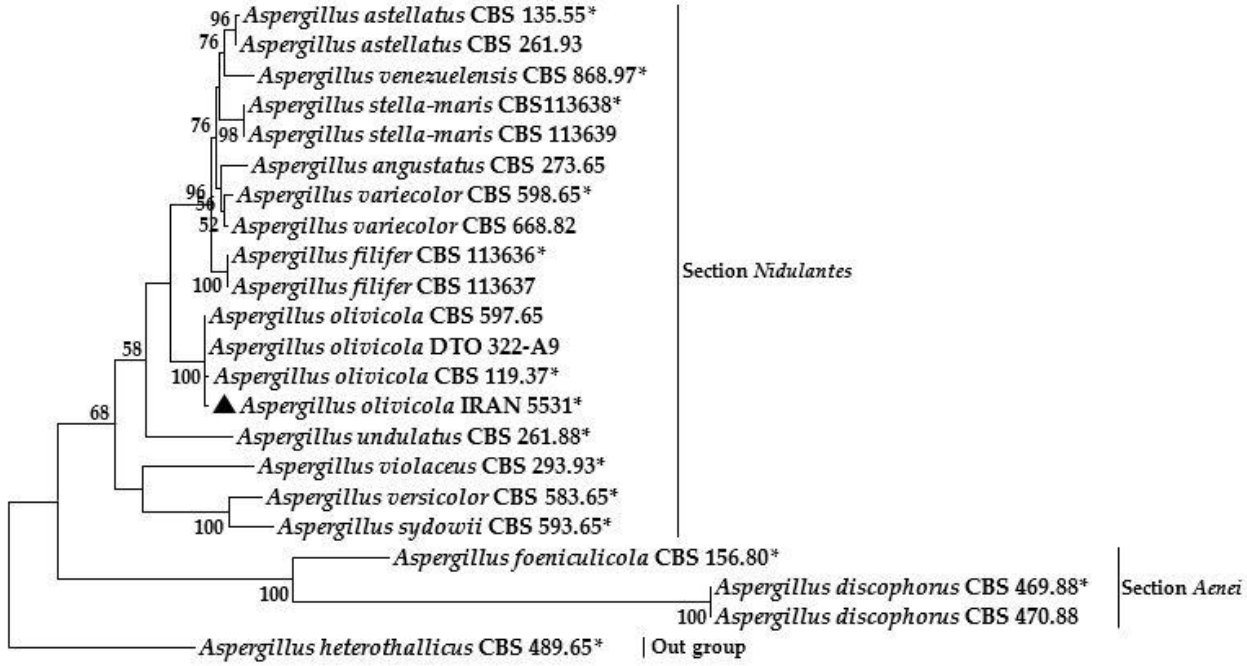

Fig. 2. Maximum likelihood consensus tree of *Aspergillus* species belonging to subgenus *Nidulantes* based on concatenated ITS and *BenA* sequence data. Bootstrap support values (1,000 replicates) are displayed at the nodes. The strain characterized in this study is marked with a triangle (▲). Ex-type strains are indicated with a star (⋆).

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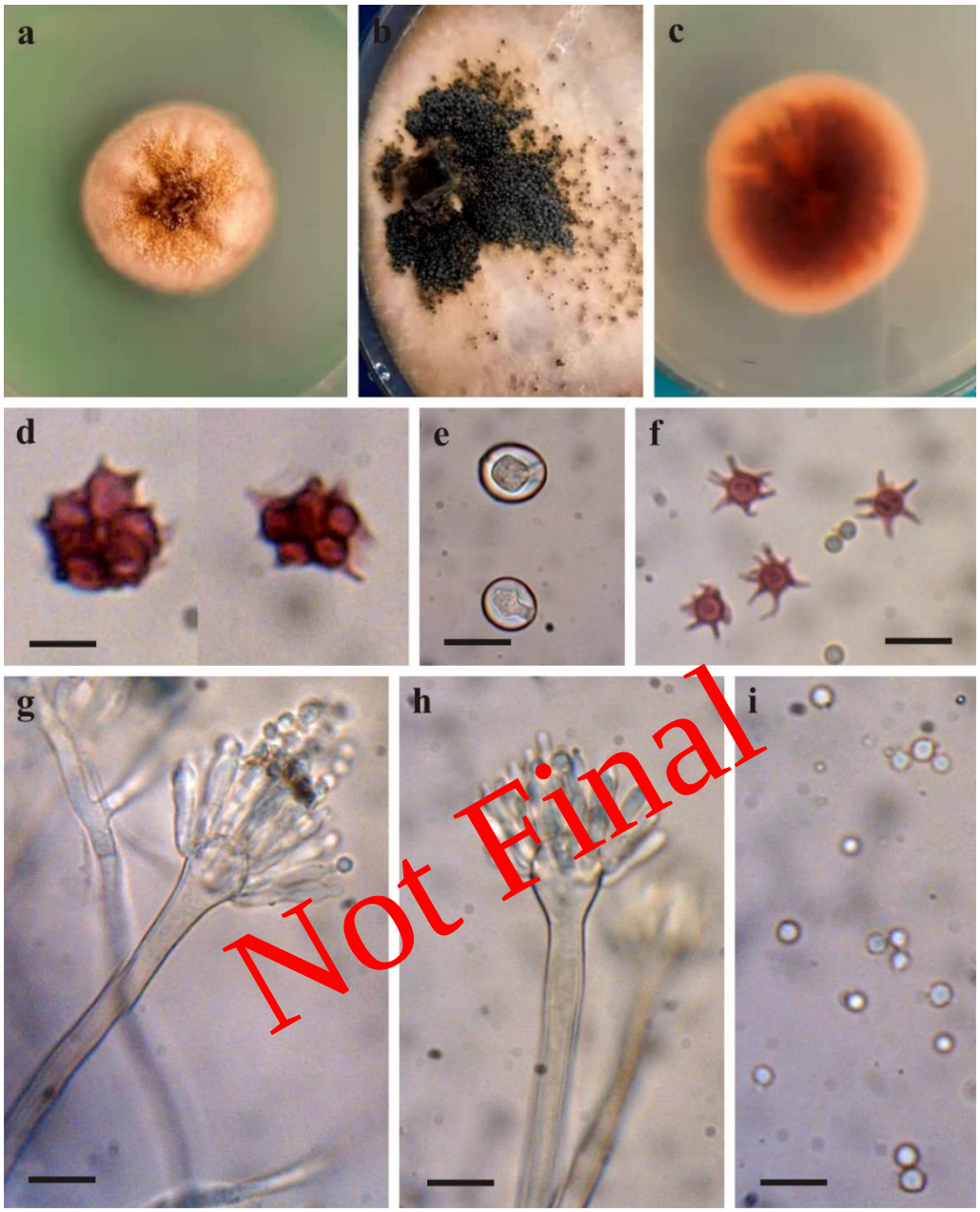

Fig. 3. *Aspergillus olivicola* (Strain IRAN 5531C): A. Colony on PDA after 5 days incubation at 25 °C, B. Ascocarps production after 21 days at 25 °C, C. Reverse side of the colony on PDA after 5 days incubation at 25 °C, D. Asci, E. Hülle cells, F. Ascospores, G, H. Conidiophores with metulae and phialides, I. Conidia (Bars: d, f, g, h, i = 10 µm; e = 20 µm).

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