

***Curvularia austriaca*, a new record for the fungi of Iran**Zeinab Heidarian[✉] , Abdollah Ahmadpour 

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ABSTRACT

Curvularia species are associated with a wide range of ecological roles, functioning as pathogens of plants, animals, and humans, as well as occurring as epiphytes, saprophytes, or endophytes, predominantly on cultivated cereals. In this study, two putative isolates of the genus *Curvularia* were collected from leaves of *Setaria* sp. and *Sorghum* sp. in Rasht County, Guilan Province, Iran. Multi-locus phylogenetic analyses based on ITS, *GAPDH*, and *TEF1* sequences clustered the studied isolates in a well-supported lineage together with ex-type and representative strains of *Curvularia austriaca*. This species, previously known only as a human pathogen, is recorded for the first time from Iran. This study expands the known geographic distribution of *C. austriaca*, and reports *Setaria* sp. and *Sorghum* sp. as novel hosts worldwide, highlighting the importance of integrative morphological and molecular approaches for accurate species identification in *Curvularia*.

KEYWORDSMorphology, Multi-locus phylogeny, *Pleosporaceae*, Taxonomy.**INTRODUCTION**

The genus *Curvularia*, established by Boedijn (1933) with *C. lunata* as the type species, is a monophyletic member of the family *Pleosporaceae* (*Pleosporales*) and is characterized by asymmetrically inflated intermediate conidia, intercalary and terminal conidiogenous cells, and sympodial conidiophores (Sivanesan 1987, Manamgoda et al. 2015, Marin-Felix et al. 2020). Species of *Curvularia* are cosmopolitan, occurring on plants and in terrestrial and aquatic substrates as well as in the air. Several species are recognized as opportunistic human pathogens, causing diverse infections, particularly in immunocompromised individuals (Sivanesan 1987, Manamgoda et al. 2015, Heidarian et al. 2020, Marin-Felix et al. 2020, Ahmadpour et al. 2025a). *Curvularia* is morphologically similar to *Bipolaris*, rendering species delimitation based solely on morphological characteristics unreliable. Therefore, integrative taxonomic approaches that combine morphological observations with multi-locus molecular data —

including the internal transcribed spacer (ITS) region, parts of glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), and translation elongation factor-1 alpha (*TEF1*) genes—are essential for accurate species identification and recognition of cryptic diversity (Ahmadpour et al. 2025a, b). To date, approximately 189 *Curvularia* species have been accepted based on DNA barcode data (Ahmadpour et al. 2025a, Guo et al. 2025, Jaffey et al. 2025, Liang et al. 2025, Zhao et al. 2025), of which about 34 species have been recorded from Iran (Heidarian et al. 2020, Ershad 2022, Ahmadpour et al. 2025a). In the present study, *Curvularia austriaca* is delineated and illustrated based on detailed morphological characteristics, supported by phylogenetic analyses of ITS, *GAPDH*, and *TEF1* sequences.

MATERIALS AND METHODS

Leaf samples of *Setaria* sp. and *Sorghum* sp. were collected in Rasht County, Guilan Province, Iran, in 2012. Fungal isolation, purification, and preservation

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were performed following the protocols described by Ahmadpour et al. (2025a). All isolates were deposited as pure cultures in the Iranian Fungal Culture Collection (IRAN...C) of the “IRAN” Herbarium and at the Fungal Culture Collection Urmia University (FCCUU). Morphological observations were conducted on 10–14-day-old TWA–wheat straw cultures incubated under near-ultraviolet light (12 h photoperiod, 23–25 °C) (Ahmadpour et al. 2025a), as well as on colonies grown for seven days in the dark on potato dextrose agar (PDA; 39 g/L, Merck, Darmstadt, Germany), corn meal agar (CMA; 17 g/L, Quelab, Montreal, Canada), and malt extract agar

(MEA; 50 g/L, Quelab, Montreal, Canada) to assess colony characteristics.

Genomic DNA was extracted using the isopropanol precipitation and chloroform extraction method (Ahmadpour et al. 2021). Polymerase chain reaction (PCR) conditions and reaction mixtures followed Ahmadpour et al. (2025a). The ITS, *GAPDH*, and *TEF1* loci were amplified and sequenced. Multi-locus phylogenetic analyses were conducted using Maximum Likelihood (ML) and Bayesian inference (BI) methods via the CIPRES Science Gateway portal version 3.3 (<https://www.phylo.org/>) (Miller et al. 2010).

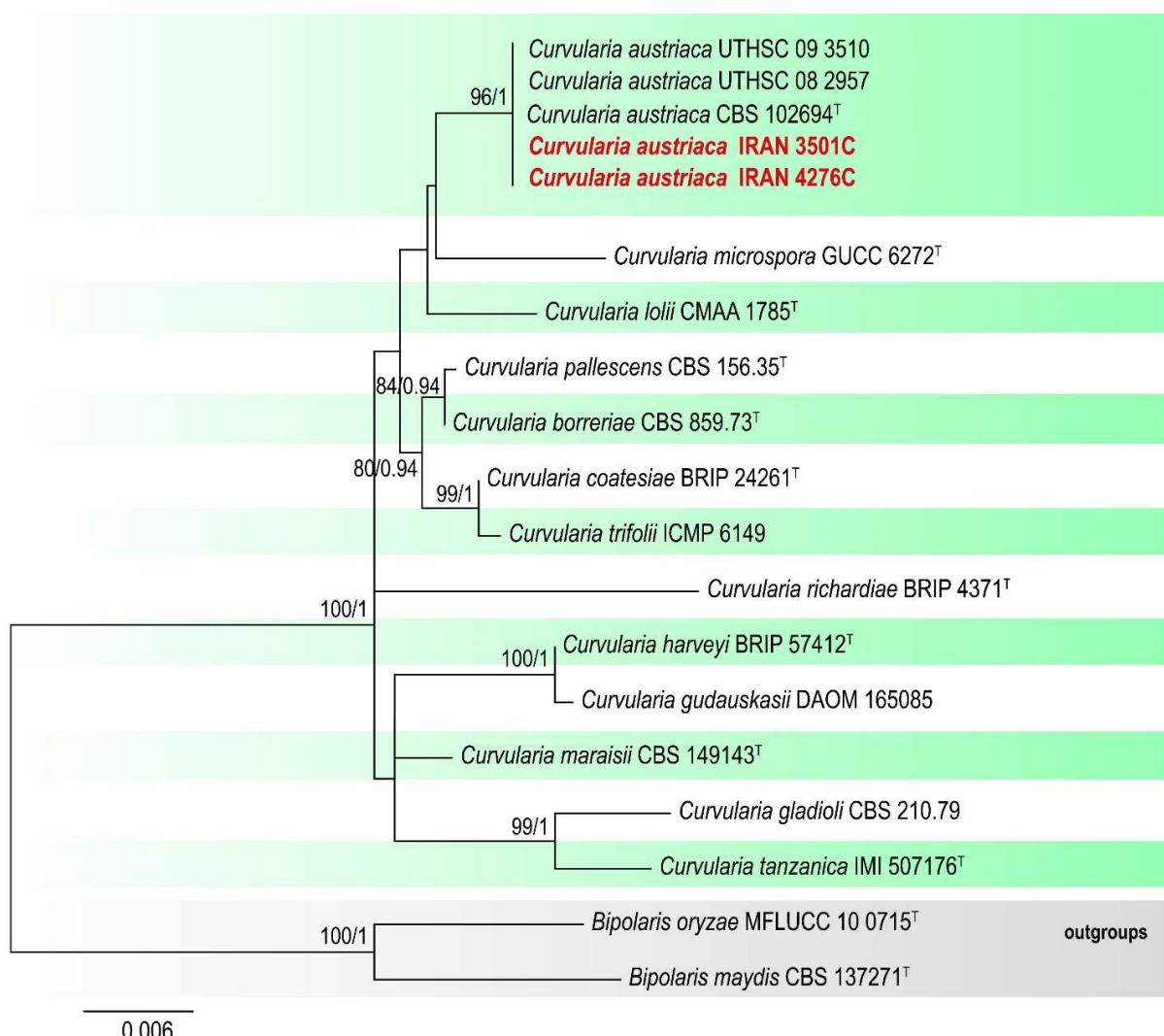


Fig. 1. Maximum Likelihood (ML) phylogenetic tree based on the combined ITS, *GAPDH*, and *TEF1* sequence dataset representing *Curvularia* species. Maximum Likelihood bootstrap support (MLBS) values of $\geq 70\%$, and the Bayesian posterior probabilities (BIPP) of ≥ 0.90 are indicated at the nodes. Newly studied strains are shown in red boldface. The tree is rooted with *Bipolaris maydis* (CBS 137271) and *B. oryzae* (MFLUCC 10-0715). The scale bar indicates the number of nucleotide substitutions per site, and ex-type strains are denoted by ^T.

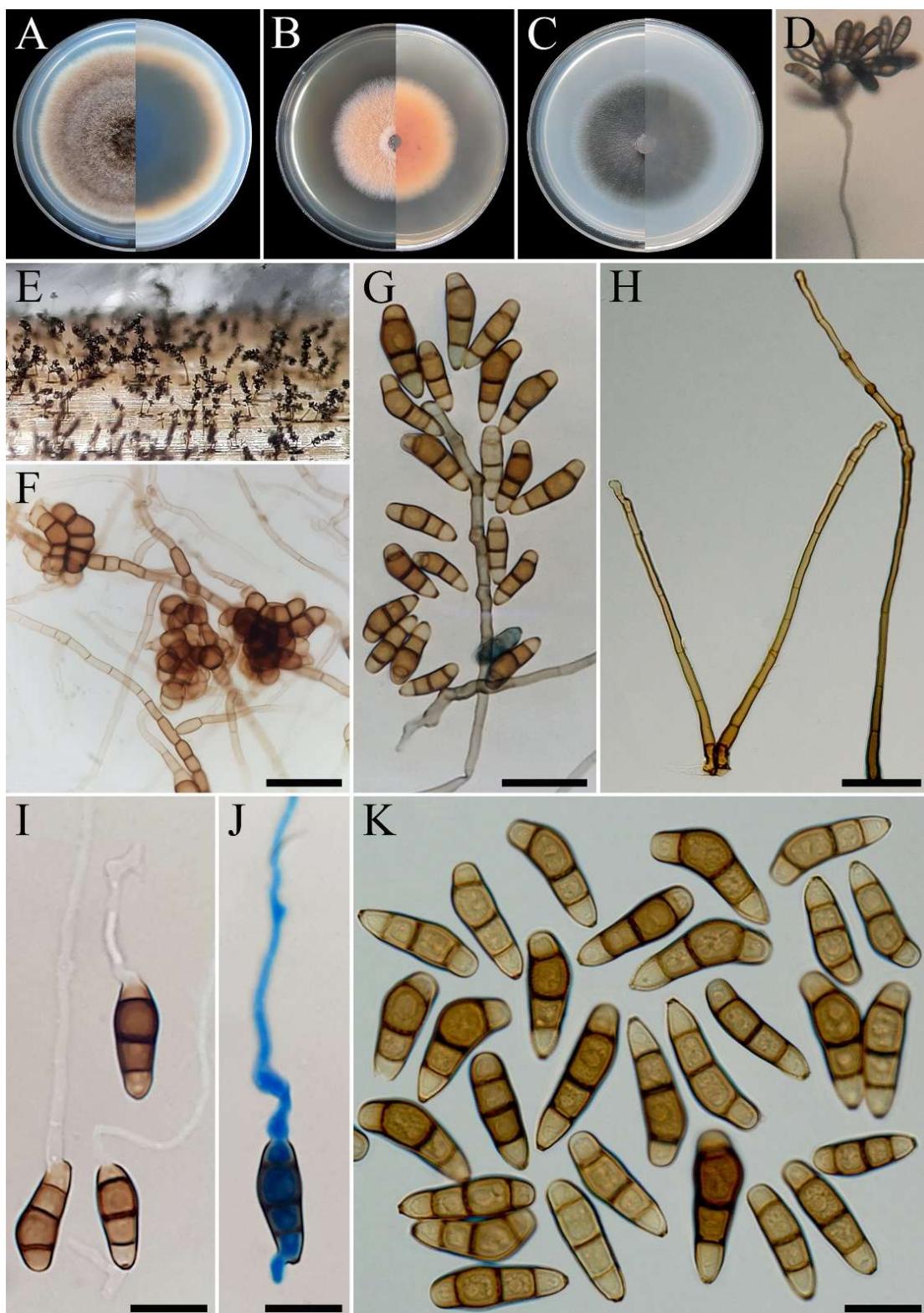


Fig. 2. *Curvularia austriaca* (IRAN 3501C). (A–C) Colonies (front and reverse) on PDA (A), MEA (B), and CMA (C) media after seven days, (D–E) Sporulation pattern on TWA medium, (H) Chlamydospores, (F–G) Conidiophores, (I–J) Germinated conidia, (K) Conidia. Scale bars: (F–H) 40 μ m, (I–K) 20 μ m.

RESULTS AND DISCUSSION

In this study, two isolates representing the genus *Curvularia* were obtained from leaves of *Setaria* sp. and *Sorghum* sp. Based on phylogenetic analyses, the two studied isolates formed a well-supported lineage together with the type and representative strains of *C. austriaca* (96% ML bootstrap support and 1.0 Bayesian posterior probability; Fig. 1). *Curvularia austriaca* was placed within the “trifoli” clade, which comprises 13 *Curvularia* species, namely *C. akaii*, *C. boreriae*, *C. coatesiae*, *C. gladioli*, *C. gudauskasii*, *C. harveyi*, *C. heteropogonis*, *C. lolii*, *C. microspora*, *C. pallescens*, *C. richardiae*, *C. tanzanica*, and *C. trifolii* (Madrid et al. 2014). Members of this clade predominantly produce 4-celled conidia with a protruding hilum (Fig. 2).

Curvularia austriaca Y. Marín & Crous, Mycol. Progr. 19(6): 564 (2020). Fig. 2.

Colonies on PDA reached 64 mm in diameter after seven days at 25 °C in the dark, circular, with entire margin, olivaceous green at the center with white to grey aerial mycelium, and white at the margin; reverse olivaceous-grey to olivaceous-black (Fig. 2A). Colonies on MEA reached 45 mm in diameter, circular, with entire margin, cottony with white aerial mycelium; reverse brown to pale brown from the center to the margin (Fig. 2B). Colonies on CMA reached 52 mm in diameter, circular, with entire margin, hairy, grey with sparse white to grey aerial mycelium; reverse olivaceous brown at the center and white at the margin (Fig. 2C). Asexual morph in TWA medium: Hyphae 2–5 µm wide, pale brown to brown, smooth, septate, and branched. Conidiophores (150–)300–400(–450) × 4–7 µm ($\bar{x} \pm SD = 350 \pm 50 \times 5.5 \pm 1.5$ µm, n = 50), mononematous, semi- to macronematous, simple, straight to flexuous, septate, arising singly or in groups, geniculate, pale brown to brown, paler toward the apex, simple to swollen at the base. Conidiogenous cells (5–)7–12(–13) × 5–8 µm ($\bar{x} \pm SD = 9.5 \pm 2.5 \times 6.5 \pm 1.5$ µm, n = 50), integrated, mono- to polytretic, proliferating sympodially, terminal or intercalary, subcylindrical to slightly swollen, pale brown to brown, smooth-walled to slightly verruculose, with thickened and darkened scars (Fig. 2G–H). Conidia 26–35 × 11–15 µm ($\bar{x} \pm SD = 30.5 \pm 4.5 \times 13 \pm 2$ µm, n = 50), brown, smooth-walled to finely verruculose, straight to curved, clavate to obovoid, occasionally ellipsoidal, tapering toward the rounded ends, broadest at the third cell from the base, apical and basal cells paler than the central cells, 3-distoseptate, germinated mono- or bipolar; hila 2–3 µm wide, protuberant, thickened and darkened (Fig. 2I–K). Chlamydospores 10–12 µm in diameter, brown to dark brown, globose to subglobose, thick-walled, formed terminally and intercalarily, in chains and clumps (Fig. 2F). Sexual morph, stroma, and microconidiation were not observed.

Specimens examined: IRAN, Guilan Province, Rasht County, on leaves of *Setaria* sp. (*Poaceae*, *Poales*), 20 September 2012, Z. Heidarian & A. Ahmadpour, isolate IRAN 3501C; *ibid*: on leaves of *Sorghum* sp. (*Poaceae*, *Poales*), 20 September 2012, Z. Heidarian & A. Ahmadpour, isolate IRAN 4276C. GenBank accession numbers: ITS: PP593894–PP593895, GAPDH: PP661364–PP661365, TEF1: PP661349–PP661350.

Notes: In the phylogenetic analyses, *C. austriaca* showed a close relationship with *C. microspora* and *C. lolii*. However, it could be differentiated from *C. lolii* by having longer conidiophores (up to 450 µm vs. up to 225 µm in *C. lolii*) and smaller conidia (26–35 × 11–15 µm vs. (26–)29–45(–48) × (11–)13–21(–23) µm in *C. lolii*) (Victoria Arellano et al. 2021). *Curvularia microspora* differs from *C. austriaca* in producing shorter conidiophores (up to 77.5 µm) and smaller conidia (4.5–11.5 × 2–6 µm) (Liang et al. 2018). *Curvularia austriaca* was previously known only as a human pathogen, isolated from the nasal cavity of a patient with sinusitis in Austria (Marin-Felix et al. 2020). To the best of our knowledge, *C. austriaca* represents a new record for the mycobiota of Iran, and *Setaria* sp. and *Sorghum* sp. are reported here for the first time worldwide as hosts of this species.

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AUTHOR CONTRIBUTION

ZH: Isolation of the strains, morphological identification, original draft preparation; **AA:** Identification, phylogenetic analysis, review and editing.

DATA AVAILABILITY

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

DECLARATION

The authors declare that there is no conflict of interest.

FUNDING

Not applicable.

ETHICS APPROVAL

Not applicable.

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گونه جدیدی برای قارچهای ایران *Curvularia austriaca*

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چکیده

گونه‌های *Curvularia* با نقش‌های متفاوت اکولوژیکی از جمله بیمارگرهای گیاهان، حیوانات و انسان‌ها و همچنین به صورت ابی‌فیت، ساپروفیت یا اندوفیت یافت می‌شوند و عمدهاً با غلات مرتبط هستند. در این مطالعه، دو جدایه متعلق به جنس *Curvularia* از برگ‌های گیاهان *Sorghum* sp. و *Setaria* sp. در شهرستان رشت، استان گیلان، ایران جمع‌آوری شدند. براساس تحلیل‌های تبارشناختی چندزنگاهی *ITS* و *GAPDH* و *TEFI*، نتایج نشان داد که جدایه‌های مورد مطالعه با جدایه‌های تیپ و شاخص گونه *C. austriaca* در یک دودمان و با پشتیبانی قوی قرار گرفتند. گونه *C. austriaca* که پیش از این تنها به عنوان پاتوژن انسانی شناخته می‌شد، برای اولین بار از ایران گزارش می‌شود. این مطالعه پراکنش شناخته شده *C. austriaca* را گسترش می‌دهد و گیاهان *Sorghum* sp. و *Setaria* sp. را به عنوان میزبان‌های جدید در سطح جهانی معرفی می‌گردد و اهمیت استفاده از روش‌های ترکیبی ریخت‌شناختی و مولکولی برای شناسایی دقیق گونه‌ها در جنس *Curvularia* را برجسته می‌سازد.

کلمات کلیدی: آرایه‌بندی، تبارشناختی چندزنگاهی، ریخت‌شناسی، *Pleosporaceae*