Penicillium bialowiezense, new report for the funga of Iran

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Abstract

Penicillium is a globally distributed fungal genus with diverse ecological roles, habitat niches, and biotechnological applications over the past decades. During a targeted survey in 2024 of fungal entomopathogens associated with the Sunn pest in E. Azerbaijan Province (Iran), the authors of the paper isolated and identified 30 strains of *Penicillium* from *Chalcophorella* sp. cadavers collected beneath *Astragalus* spp. Morphological assessment, supported by *β-tubulin* (*BenA*) gene sequencing and phylogenetic analysis, confirmed the identity of all isolates as *Penicillium bialowiezense.* The present findings represent the first record of *P. bialowiezense* in Iran and, to the authors' knowledge, the first report of this species from a Coleoptera host worldwide. Iranian isolates showed some phenotypic variation regarding the protologue in the conidiophores' branching patterns. In the phylogenetic analysis the representative isolate (IRAN 5372C) clustered with the type and reference strains of *Penicillium bialowiezense*. Results derived from the present study, contribute to fungal biodiversity documentation of the mainland of Iran, and highlight the necessity for further study on the host range and geographical distribution of said fungus in natural environments.

Keywords: Aspergillaceae, *Brevicompacta*, mycobiota, new host, Varzeqan

Penicillium bialowiezense، گزارش جدید برای قارچ‌های ایران

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خلاصه

Penicillium یکی از جنس‌های قارچی با پراکنش جهانی است که دارای نقش‌های بوم‌شناختی متنوع، زیستگاه‌ها و کاربردهای زیست‌فناورانه گوناگون در دهه‌های اخیر بوده است. در یک بررسی هدفمند در سال ۱۴۰۳، برای شناسایی قارچ‌های بیماری‌زای حشرات مرتبط با آفت سن گندم در استان آذربایجان‌شرقی، نگارندگان مقاله، تعداد ۳۰ جدایه متعلق به جنس *Penicillium* از لاشه‌های *Chalcophorella* sp. جمع‌آوری‌شده در زیر بوته‌های گیاه گون جداسازی و شناسایی کردند. بررسی‌های ریخت‌شناختی همراه با توالی‌یابی ژن بتاتوبولین (*BenA*) و تجزیه و تحلیل‌های تبارشناختی نشان داد که تمامی جدایه‌ها متعلق به *Penicillium bialowiezense* هستند. یافته‌های تحقیق حاضر، نخستین گزارش از حضور این گونه در ایران و همچنین نخستین گزارش از وقوع آن در یک میزبان حشره‌ای (بالپوش‌داران) در سطح جهان می‌باشد. جدایه‌های ایرانی برخی تفاوت‌های فنوتیپی در الگوهای انشعاب کنیدیوم‌برها را نشان می‌دهند. در تجزیه و تحلیل‌های فیلوژنتیکی جدایه نماینده (IRAN 5372C) با جدایه تیپ و جدایه‌های مرجع گونه *P. bialowiezense* در یک خوشه قرار گرفت. این یافته‌ها به مستندسازی تنوع زیستی قارچ‌ها در کشورمان ایران کمک می‌کند و لزوم انجام پژوهش‌های بیشتر برای بررسی دامنه میزبانی و پراکنش جغرافیایی جدایه مذکور در محیط‌های طبیعی را برجسته می‌سازد.

واژه‌های کلیدی: میزبان جدید، میکوبیوتا، ورزقان، Aspergillaceae، *Brevicompacta*

Introduction

The revised classification divides *Penicillium* into two subgenera (*Aspergilloides* and *Penicillium*) and 32 sections and 89 series (there are 19 sections in the subgen. *Aspergilloides*, comprising over 331 species and 13 sections in the subgenus. *Penicillium*, containing over 145 species) (Houbraken *et al.* 2020). Based on the latest publications, *Penicillium* sect. *Brevicompacta*, in subgen. *Penicillium*, currently comprises 15 species (11 species in 2020), which are placed inside four series, namely, *Brevicompacta*, *Buchwaldiorum*, *Olsoniorum*, and *Tularensia*. (Houbraken *et al.* 2020, Liang *et al.* 2025). Series *Brevicompacta* was introduced by Houbraken & Frisvad in 2020 with *P. brevicompactum* Dierckx as the type species, whose colonies are restricted in growth and exhibit a velvety texture. Conidiophores are predominantly terverticillate, though biverticillate or multiramulate forms may also occur; they are smooth-walled and relatively broad.

To the best of author's knowledge, only one species from *Penicillium* sect. *Brevicompacta*, namely, *P. brevicompactum* has been recorded from Iran (Bakhshi *et al.* 2022, Ershad 2022). In the present study, penicillium-like fungal isolates were recovered from insect cadavers collected beneath *Astragalus* spp. plants in the E. Azerbaijan Province (Iran). Given this information, the study focused on the morphological characteristics and phylogenetic analysis of the *β-tubulin* gene in *Penicillium* species.

Materials and Methods

- Sampling, isolation, and storage of isolates

To identify fungal entomopathogens of the wheat pest *Eurygaster integriceps* Puton, during the spring of 2024, 45 cadavers of the Sunn pest, as well as remnants of other dead *Chalcophorella* sp. were collected from beneath *Astragalus* spp. plants at various overwintering sites across different regions of E. Azerbaijan Province (Iran). The fungal isolates were obtained using the method described by Gül *et al.* (2021). Pure fungal isolates were established by transferring the hyphal tips technique to 2% water agar (WA).

- Morphological characterization

Obtained isolates were cultured on Malt Extract Agar (MEA), Czapek Yeast Autolysate Agar (CYA), and CYA with 5% NaCl (CYAS) at 15, 25 and 30 °C in the dark for seven days. Species identification was based on growth rate, colony morphology, and microscopic features, including conidiophores, branching patterns, and conidia. These fungal structures were examined from slides prepared from 7–10-day-old MEA cultures with Shear's solution mounting liquid. Living culture from the Pen1 isolate, which was used as a representative for morphological and molecular identification, was deposited in the Iranian Fungal Culture Collection ("IRAN"), Iranian Research Institute of Plant Protection (Tehran, Iran).

- Molecular identification

For molecular identification, primers Bt2a and Bt2b were used to amplify the partial *β-tubulin* gene (*BenA*) as a DNA barcode (Glass & Donaldson 1995). The PCR product was sent to the services of Microsynth (Balgach, Switzerland) through Topaz Gene Research (Alborz, Iran) for sequencing, which was performed using the Bt2a primer.

- Phylogenetic analysis

The raw trace file was examined and manually edited using MEGA Ver. 7, and the consensus sequence was assembled (Kumar *et al.* 2016). The resulting sequence was compared against the NCBI GenBank database using the mega BLAST algorithm to determine taxonomic affiliation. Homologous sequences with the other species from the sect. *Brevicompacta* within the *Penicillium* genus retrieved from GenBank, along with the newly obtained sequence, were aligned using the online version of MAFFT with default settings (Katoh & Standley 2013). Final alignments were reviewed and regions of ambiguous alignment were observed in MEGA Ver. 7 and excluded from analysis. Phylogenetic analyses of the finally aligned dataset were conducted using Maximum Likelihood (ML) and Bayesian Inference (BI). Maximum likelihood analyses were performed in raxmlGUI software Ver. 2.0.15 (Edler *et al.* 2021) using the ML + rapid bootstrap setting and the GTRGAMMA substitution model with 1000 bootstrap replicates (MLBS). Bayesian inference for phylogenetic analyses was performed using MrBayes Ver. 3.2.6 (Ronquist *et al.* 2012). The most appropriate evolutionary model was selected through MrModelTest Ver. 2.3 (Nylander 2004).

Results

Through multiple surveys of overwintering habitats in E. Azerbaijan Province (Iran), 30 fungal isolates were recovered from insect cadavers of *Chalcophorella* sp. collected beneath *Astragalus* spp. plants. Preliminary examination revealed that, all *Penicillium* isolates belonged to the species *P. bialowiezense*. Following a detailed morphological assessment, one representative isolate (IRAN 5372C) was selected for further morphological and molecular characterization.

- Taxonomy

Penicillium bialowiezense K. Zaleski, Bull. Int. Acad. Polon. Sci., Cl. Sci. Math., Sér. B., Sci. Nat. 1927: 450 (1927) (Fig. 1). *In*: *Penicillium* subgen. *Penicillium*, sect. *Brevicompacta*, ser. *Brevicompacta.*

Colony characters: on MEA, CYA and CYAS at 25 °C after 7 d, colony diameters reached 18–20 mm, 20–25 mm, and 22–27 mm, respectively. On CYA and MEA at 15 °C after 7 d, 15–19 mm and 10–13 mm respectively. On CYA and MEA at 30 °C after 7 d, 0 mm in diameter. On CYA (at 25 °C after 7 d), the colonies were protuberant and velutinous, in meadow green to greyish green texture, conidium color dull green to grey green, and reverse color beige to cream. On MEA (at 25 °C after 7 d), the colonies were protuberant and radially sulcate, with dull green to grey green color, margins lobed; reverse pale or light brown. Sporulation strong on MEA and appeared as a dull green to grey green. No exudates or soluble pigments were observed in both media. Sclerotia and sexual morphs were absent.

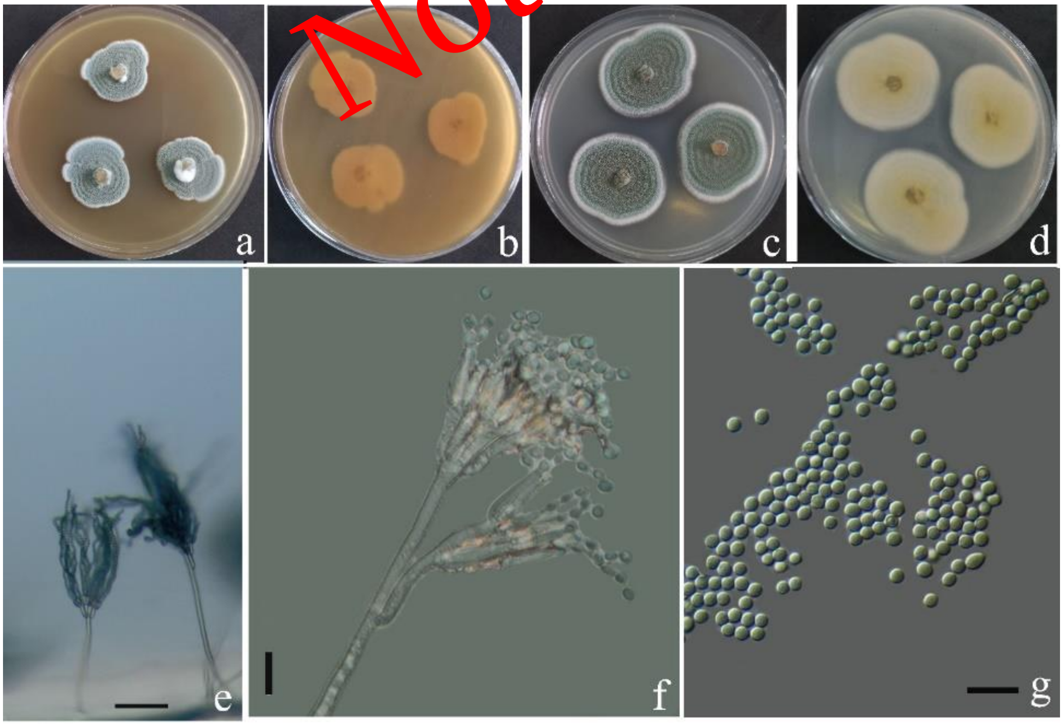

Fig. 1. *Penicillium bialowiezense* (isolate: IRAN 5372C): a-b. Colonies obverse and reverse on MEA, c-d. Colonies obverse and reverse on CYA, e. Conidial head, f. Terverticillate conidiophore, j. Conidia (Bars: e = 50 µm, f-j = 10 µm).

- Micromorphology

Conidiophores terverticillate, and appressed. Stipes were smooth-walled, 200.68–385.25 × 3.64–5.37 µm. Rami 14.28–26.10 × 3.68–5.23 µm. Metulae 9.45–13.17 × 3.73–5.09 µm. Phialides were cylindrical with gradually tapering collula, 9–14.65 × 2.95–3.23 µm. Conidia smooth-walled to roughened, ranging from globose to slightly ovoid, formed in chains, 2.50–3.59 × 2.10–3.20 µm in diameter.

Note: *Penicillium bialowiezense* is recognized as the sister species to *P. anthracinoglaciei* based on multi-gene molecular analyses, as well as only *BenA* gene phylogenetic analysis (Perini *et al.* 2023). According to the valid descriptions of *P. bialowiezense* (based on CBS 227.28), this species only produces terverticillate conidiophores (Frisvad & Samson 2004). However, the strains discussed here, in addition to the predominant terverticillate conidiophores, produce other, less common types of conidiophores, including monoverticillate, divaricate, and quaterverticillate conidiophores. Therefore, this study reports for the first time the presence of these types of conidiophores' branching patterns in this species.

Specimen examined: IRAN: E. Azerbaijan Province, Varzeqan, on *Chalcophorella* sp. cadaver, 38°29'56" N 46°44'07" E, 30.1.2024, H. Golmohammadi (CCTU Pen1; living culture IRAN 5372C).

- Molecular phylogeny

The obtained sequence of *β-tubulin* gene was submitted to GenBank under accession number: PV696547 (http://www.ncbi.nlm.nih.gov). BLAST analysis of the obtained sequence of the Iranian isolate (IRAN 5372C) revealed 100% identity with the type sequence and multiple *P. bialowiezense* isolates such as *P. bialowiezense* strain CBS 227.28 (Accession number: AY674439), *P. bialowiezense* strain CBS 116044 (Accession number: EU587342), *P.* bialowiezense strain 12a15 (Accession number: KX928874) and *P. bialowiezense* strain 5i16 (Accession number: KX928919) available in GenBank.

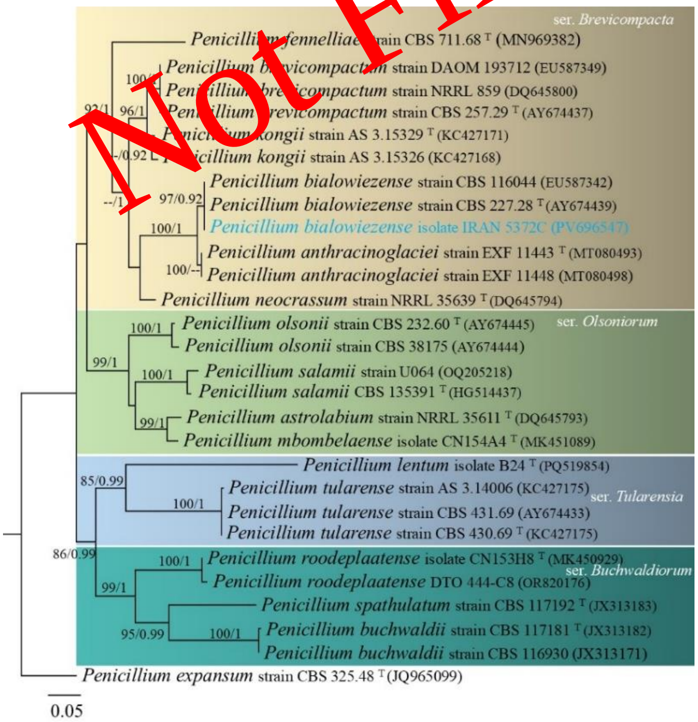

Fig. 2. The phylogenetic tree constructed using RAxML analysis of the *BenA* gene for various species belonging to the *Penicillium* sect. *Brevicompacta*. Maximum likelihood bootstrap supports (≥70%) and Bayesian posterior probabilities support (≥0.9) are given at the branches, respectively (Dashes replace non-significant values). The sequenced isolate in this study is highlighted in blue, and ex-type isolates are marked with the letter 'T'. The scale bar represents 0.05 expected substitutions per nucleotide site. The tree was rooted by *P. expansum* CBS 325.48. In parentheses, there are the NCBI GenBank accession number for the *BenA* gene.

Phylogenetic trees generated using Maximum Likelihood (ML) and Bayesian Inference (BI) on *β-tubulin* sequences from 28 taxa retrieved from GenBank showed similar branching patterns. Therefore, only ML topology is presented with raxmlGUI software (Ver. 2.0.15), and BI-PP support values superimposed (Fig. 2). The ML analyses of the dataset yielded a best scoring tree with a final ML optimization likelihood value of -3278.416787. The final alignment matrix comprised 498 characters and 269 distinct alignment patterns, with 11.47% of undetermined characters or gaps. In BI analyses, based on model selection using MrModelTest, was performed under the HKY+I+G substitution model, which incorporated invariant sites and gamma-distributed rate variation, with Dirichlet-distributed base frequencies. The analysis was run for 1,000,000 generations, and 248 trees were sampled. After discarding the first 25% of trees as burn-in, the consensus tree and posterior probabilities (PP) were calculated from the remaining 186 trees.

In the present study, the resulting phylogeny (Fig. 2) places the Iranian isolate, *P. bialowiezense* isolated from the coleopteran insect (*Chalcophorella* sp.) cadaver in Iran (IRAN 5372C), within a well-supported clade of *P. bialowiezense* type strain (ML = 97%, BI = 0.92).

Discussion

Penicillium bialowiezense, is characterized by a rather complicated taxonomic history. Previous studies have highlighted uncertainties regarding the authenticity of the former type strains, due to the mistake made in determining the type culture in previous years, until Scott *et al.* (2008) analyzed the ITS rDNA and *β-tubulin* sequences of DAOM 239766 (derived from CBS 227.28), and assigned it as an epitype according to the International Code of Botanical Nomenclature. Their molecular analyses confirmed the identity of this strain and clarified that, *P. bialowiezense* has nomenclatural priority over *P. biourgeianum* K. Zaleski (current name: *P. brevicompactum*), which is now accepted by mycologists.

Peniciilium bialowiezense has been isolated from a diverse array of substrates, including sugar beans, forest soils, household dust, fruiting bodies of basidiomycetes, ferns, fresh coconut, and various plant leaves such as *Berberis*, *Rhododendron*, and *Prunus cerasus* L. (Wang & Wang 2013). It has also been reported from wheat, seaweed, soil under *Salix* sp. in dried root segment, soil under *Nothofagus* sp., bread, barley, *Thymus vulgaris* L., and margarine. The species has been documented in several countries, including Denmark, Faroe Islands, Poland, Italy, Slovenia, Chile, Wyoming state (Western United States), Canada, China, Germany, and Saudi Arabia (Frisvad & Samson 2004, Wang & Wang 2013). A comprehensive review of Iran's mycological records revealed no previous report of this species within the country. In this study, *P. bialowiezense* is documented for the first time in Iran and obtained from the insect cadaver. To the authors' knowledge, this is the first report worldwide of *P. bialowiezense* with a coleopteran insect host (*Chalcophorella* sp.).

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