

## Short Article

## First occurrence of *Golovinomyces bolayi*, the cause of powdery mildew disease on Prickly lettuce (*Lactuca serriola*) in Iran

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### ABSTRACT

During September–November 2022 and 2023, white, powdery fungal growth was observed on leaves of wild-grown prickly lettuce (*Lactuca serriola* L.) in the Firouragh county (Khoy, West Azerbaijan Province), Iran. Infected leaves were collected and subjected to morphotaxonomic and phylogenetic analyses. The integration of morphological and molecular characteristics revealed the identity of the species as *Golovinomyces orontii* species complex. Based on a phylogeny inferred using ITS-rDNA sequences obtained in the present study and sequence data from GenBank, the isolates from prickly lettuce clustered together with *G. bolayi* from the other *Lactuca* spp. such as *L. sativa* and *L. serriola*. On the members of the genus *Lactuca*, *G. bolayi* has been previously reported on *L. sativa* and *L. tuberosa* in Iran. This study provides the first report on the occurrence of *G. bolayi* on *L. serriola* in Iran. Hitherto, *Leveillula lactucae-serriolae* has been reported on *L. serriola* in Iran. With this study, we provide a comprehensive illustration of *G. bolayi* on *L. serriola* and further discuss the phylogeny, host range, and ecology of this species.

### KEYWORDS

*Golovinomyces orontii*, Iran, ITS-rDNA, *Lactuca*, *Leveillula lactucae-serriolae*.

### INTRODUCTION

The genus *Lactuca* L., commonly known as lettuce, resides in the family Asteraceae, comprising up to 100 species, with worldwide distribution (Chadha et al. 2021). *Lactuca sativa* L. (lettuce) is a well-known species in this genus, which is mostly cultivated as a leaf vegetable for use in green salads. Many species in this genus, such as *L. serriola* L., are known as economically important weeds in agroecosystems (Chadha et al. 2021); while some species, *viz.*, *L. sativa*, *L. indica* L. Mant. Pl., and *L. serriola*, possess medicinal properties and have been used in traditional medicine (Abdel Bar et al. 2023). The species diversity of the genus *Lactuca* is relatively high in Iran, with approximately 12 species known to grow in the mainland of Iran (Mozaffarian 2015).

Powdery mildews are biotrophic fungal pathogens residing in the family *Erysiphaceae* (the order *Helotiales*, Class *Leotiomycetes*), occurring on a wide range of plant species except gymnosperms (Braun and

Cook 2012). Powdery mildews are among the economically significant plant pathogens affecting many agricultural, landscape, and ornamental plants, including grapevines, wheat, cucurbits, roses, and others (Gan et al. 2025, Bradshaw et al. 2024). Several powdery mildew species are known to occur on *Lactuca* species worldwide, including *Golovinomyces bolayi* S. Takam., Lebeda & M. Götz, *Leveillula lactucae-serriolae* Khodap. & Hedjar., *L. lactucarum* Durrieu & Rostam and *L. taurica* (Lév.) G. Arnaud (Braun and Cook 2012). Until the present, three powdery mildew species have been reported on *Lactuca* spp. from Iran, including *G. bolayi* on *L. sativa* L. and *L. tuberosa* Jacq.; *Leveillula lactucae-serriolae* on *L. scarioloides* Boiss., *L. serriola* and *L. azerbaijanica* Rech.fil. and *Leveillula lactucarum* Durrieu & Rostam on *L. orientalis* (Boiss.) Boiss. (Khodaparast and Abbasi 2024). In this study, we report the occurrence of *G. bolayi* on *L. serriola* in Iran for the first time.

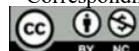
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## MATERIALS AND METHODS

During September–November 2022 and 2023, signs of powdery mildew were observed on wild-grown prickly lettuce (*Lactuca serriola*) in the Firouzagh County (Khoy, West Azerbaijan Province, Iran). Samples were collected from infected leaves and subjected to morphological and molecular studies at the Mycology laboratory of the Faculty of Agriculture, University of Tabriz, for species identification. Microscopic slide mounts were prepared from fresh fungal structures in distilled water and examined at  $\times 1000$  magnification using an Olympus BX41 light microscope. At least 30 measurements were made for each microscopic fungal structure, and 95% percentiles were derived for the measurements, and the extreme values were presented in parentheses. An Olympus digital camera system (DP 25) was used to capture high-resolution photographs of microscopic fungal structures. Subsequently, photoplates were edited using Adobe Photoshop CS6 (Adobe Systems Inc., USA). Dried specimens were maintained in the Fungal Herbarium of the Plant Protection Department, University of Tabriz, Iran (CCTU-H 125).

For molecular characterization, genomic fungal DNA was extracted from fresh fungal hyphae and conidial masses according to the protocol described by Arzanlou and Narmani (2017). In brief, a small mass of fungal mycelia and conidia was picked up from the surface of the leaf using a sterile inoculation needle and placed in PCR tubes containing 50  $\mu$ l of TE buffer and then gently crushed using a sterile pipette tip. The mixture was heated at 95 °C for five minutes in a thermocycler machine, and the resulting DNA was used for PCR amplification and stored at -20 °C for future use.

The universal primer set ITS1 (5'-TCCGTAGGTGAACCTGGG-3') and ITS4 (5'-TCCTCCGC TTATTGATATGC-3') was applied to amplify the internal transcribed spacer region (ITS1–5.8S–ITS2) of the rDNA operon via the polymerase chain reaction (White et al. 1990). The reaction reagents and PCR cycling program were the same as Arzanlou and Narmani (2017). The amplified PCR products were then analyzed using electrophoresis in a 1% agarose gel with 1X TAE buffer. A Gel documentation device was used to visualize the amplicon.

The same primer set (ITS1 and ITS4) was used to sequence the PCR product in both directions at Topazgene Company (Karaj, Iran). SeqMan (DNASTAR, LaserGene, Madison, USA) software was used to edit the raw sequence files, and a consensus file was subsequently extracted. For the similarity match, a BLAST search was conducted against GenBank sequence data at the NCBI (National Center for Biotechnology Information) database. The sequence data with high homology, including reference sequences, were downloaded from GenBank and subjected to phylogenetic analyses. The sequences

generated in this study were deposited in GenBank under the accession number PX588544.

## RESULTS AND DISCUSSION

The disease was prevalent, and almost 90 percent of prickly lettuce plants in sampling areas showed symptoms of powdery mildew disease. The disease severity was significantly high i. e. 15–90 percent of the leaves on plants were covered with fungal mass. The disease signs appeared as whitish mycelia, mainly on the upper surface of the leaves as evanescent to persistent, irregular, distinct, scattered patches; subsequently, thin and diffuse colonies formed on the underside of the leaves, leading to visible changes (Fig. 1). Sexual morph did not develop on the leaves and chasmothecia were not observed.

A Megablast search in NCBI's GenBank nucleotide database showed high similarity with the holotype sequence (LC417106) of *Golovinomyces* species from GenBank. Phylogenetic analyses were performed based on the ITS-rDNA sequence obtained in this study, together with sequence data from GenBank. The final sequence alignment, comprising 25 internal taxa, had 512 characters and 92 unique site patterns. *Golovinomyces eurybiarum* M. Bradshaw FH00941263 (ON367032) served as the outgroup taxon. Bayesian analyses were performed using the best-fitting substitution model (GTR+G) and resulted in 932 generations. After discarding the first 25% of generations as burn-in, the remaining 700 (75%) generations were used to calculate the consensus Bayesian tree and posterior probabilities. Results indicated that the sequence generated in this study clustered with *Golovinomyces bolayi* (Fig. 1).

*Golovinomyces bolayi* S. Takam., Lebeda & M. Götz, Mycol. Progr. 18(3): 341 (2019). Fig 2

The hyphae developed as patches or effused on the upper and lower sides of the leaves, branched, septate, hyaline, thin-walled and smooth with 4–8  $\mu$ m wide. Appressoria on the mycelium were poorly developed to nipple-shaped, solitary, 5–9  $\mu$ m diam. Conidiophores arising from superficial hyphae, on the upper surface of mother cells or lateral, central, or usually somewhat towards one septum of the mother cells, to slightly lateral, 80–195  $\mu$ m long. Foot-cells cylindrical or subcylindrical, straight or slightly curved to sinuous, (50–)84.08–103.66(–160)  $\times$  (10–)10.90–11.34(–12)  $\mu$ m, followed by 1–4(–5) shorter cells. Conidia ellipsoid to ovate, doliform, formed in chains with 1–7, hyaline, smooth, lake fibroin bodies, (25–)29.51–31.78(–36)  $\times$  (13–)16.41–17.68(–19)  $\mu$ m diam. Germ tubes developed on the perihilar position on conidia (*Euoidium* type) (Fig. 2).

Based on the morphological characters, the fungus was identified as the conidial stage of the genus *Golovinomyces*. *Golovinomyces orontii* encompasses a heterogenous assemblage with a wide host range (Takamatsu et al. 2013). Morphologically *G. orontii*

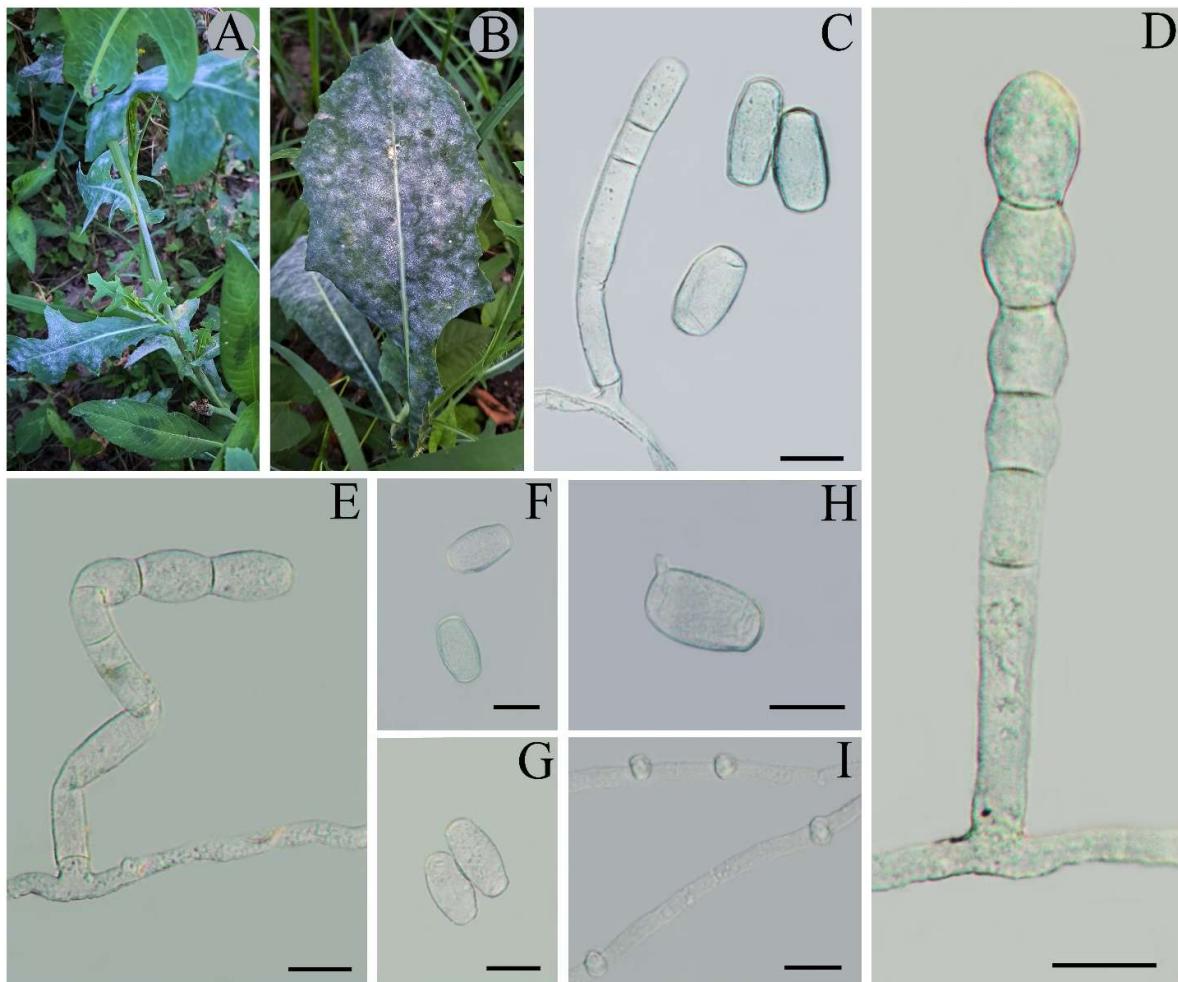
is closely related to *G. cichoracearum*-like powdery mildew occurring on the members of subfamily *Ciconiidae* in the family *Asteraceae* (Braun and Cook 2012, Matsuda and Takamatsu 2003). Recently, based on morpho-phylogenetic evidence, *G. orontii* was segregated into several well-supported and genetically distinct clades apart from *G. orontii* s. str. clades (Buran et al. 2019); hence, four species clades, namely *G. tabaci* (group 1), *G. orontii* s. str. (group 2), *G. bolayi* (group 3) and *G. vinacae* were recognized. Based on asexual and sexual morphs, *G. bolayi* has been recorded from *Asteraceae* (*Cichorieae*, *Cichorium* and *Lactuca* spp.); while

phylogenetic analyses have revealed a wider host range for this species, which is known only based on asexual morphs (Braun et al. 2019).

*Golovinomyces bolayi* has been previously reported from Iran on *Cichorium intybus* L., *C. endivia* L., *Lactuca sativa*, *L. tuberosa*, *Abelmoschus esculentus* Moench., *Taraxacum glaucum* Boiss., *T. montanum* DC., *T. syriacum* Boiss. and *Veronica persica* Poir. (Golmohammadi et al. 2019; Taheri Ardestani et al. 2020; Khodaparast et al. 2024). To the best of our knowledge, this study provides the first occurrence of *G. bolayi* on prickly lettuce (*L. serriola*) in Iran.



**Fig. 1.** Consensus phylogram resulting from a Bayesian analysis of ITS-rDNA sequence alignment using MrBayes v. 3.2.2 of *Golovinomyces* species. The scale bar indicates 0.08 expected changes per site. The tree was rooted to *Golovinomyces eurybuarum* FH00941263 (ON367032). HT= ex-holotype, ET= ex-epitype, LT= ex-lectotype, IT= ex-isotype, NT= ex-neotype, RS= reference sequence(s).



**Fig. 2.** *Golovinomyces bolayi* (CCTU-H 125). (A, B) Disease symptoms on Prickly lettuce (*Lactuca serriola*) leaves, (C–E) Conidiophores and conidial chains, (F–H) Conidia, (I) Nipple-shaped hyphal appressorium. Scale bars: (C–I) 20 µm.

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#### AUTHOR CONTRIBUTION

Mahdi Arzanlou: Conceptualization, Investigation, Writing – Original Draft, and Writing – Review & Editing. Abolfazl Narmani: Data Analysis, Data Curation, and Laboratory Work.

#### DATA AVAILABILITY

The datasets used during the current study are available from the corresponding author upon request.

#### DECLARATION

The authors declare no conflicts of interest.

#### FUNDING

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#### ETHICS APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

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## اولین گزارش از وقوع قارچ *Golovinomyces bolayi*, عامل بیماری سفیدک پودری کاهوی خاردار وحشی

در ایران (*Lactuca serriola*)

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### چکیده

در طول ماههای شهریور تا آبان ۱۴۰۱ و ۱۴۰۲، پوشش قارچی سفید رنگ و پودری شکل روی برگ‌های کاهوی خاردار وحشی (*Lactuca serriola*) در شهرستان فیروزق (خوی، استان آذربایجان غربی) ایران مشاهده شد. برگ‌های آلوده جمع‌آوری و نمونه‌های قارچی تحت بررسی‌های مورفوتاکسونومیکی و تحلیل‌های فیلوجنتیکی قرار گرفتند. ادغام ویژگی‌های مورفولوژیکی و مولکولی، هویت جدایه‌های قارچی را به عنوان عضوی از کمپلکس گونه‌ای *Golovinomyces orontii* تایید کرد. نتایج واکاوی فیلوجنتیکی مبتنی بر توالی‌های ITS-rDNA حاصل از این مطالعه و داده‌های توالی دریافت شده از ژن‌بانک (GenBank)، نشان داد که جدایه‌های کاهوی خاردار به همراه *G. bolayi* در یک خوشه قرار می‌گیرند و از سایر توالی‌های عامل سفیدک پودری روی گونه‌های دیگر *Lactuca sativa* و *L. serriola* متمایز می‌باشند. روی اعضای جنس *Lactuca* گونه *G. bolayi* قبلًاً روی *L. sativa* و *L. tuberosa* در ایران گزارش شده است. این مطالعه اولین گزارش از وقوع *G. bolayi* در ایران است. تاکنون، *G. bolayi* روی *Leveillula lactucae-serriolae* در *L. serriola* در ایران گزارش شده است. در این مطالعه شرح و توصیف کامل گونه *G. bolayi* روی *L. serriola* ارائه داده شده است و فیلوجنی، دامنه میزبانی و اکولوژی این گونه مورد بحث قرار گرفته است.

**کلمات کلیدی:** ایران، ITS-rDNA، *Leveillula lactucae-serriolae*، *Golovinomyces orontii*، *Lactuca*