DOI: 10.22034/IJPP.2024.2021790.445

بیماری‌های گیاهی / جلد ۵۹ / شماره ۱/ سال ۱۴۰۲: ۶۳-۵۶

مقاله کوتاه پژوهشی

جنس *Leveillula* روی *Helichrysum* در ایران: کشف هاپلوتیپ جدید در جنس *Leveillula**

سیداکبر خداپرست[1]، حمیده دارسرائی[1] و محمدجواد پورمقدم[1]**

(تاریخ دریافت : ۱۴۰۲/۱۲/۲۰ ، تاریخ پذیرش: ۱۴۰۳/۰۲/۱۵)

چکیده

در چارچوب برنامه آزمایشگاه تحقیقات قارچ‌شناسی دانشگاه گیلان برای شناسایی و بارکدگذاری DNA قارچ‌های عامل سفیدک‌های پودری (*Erysiphaceae, Ascomycota*) از ایران، یک نمونه سفیدک پودری روی *Helichrysum* sp. از جاده زنجان - طارم جمع‌آوری و مورد بررسی ریخت‌شناختی و شناسایی مولکولی قرار گرفت. اگرچه این قارچ از نظر ریخت‌شناختی شبیه به *Leveillula helichrysi* و برخی از گونه‌های نزدیک دیگر مانند کمپلکس *L. taurica* است، اما توالی ITS تفاوت قابل اعتمادی را در برابر توالی *L. helichrysi* و *L. taurica* موجود در بانک ژن نشان داد. پیشنهاد ما این است که این نمونه ممکن است نشان دهنده یک گونه جدید و یا هاپلوتیپ دیگری از *L. helichrysi* باشد. بنابراین، تا به دست آوردن نمونه‌های بیشتر و توالی نمونه تیپ، این قارچ را به عنوان *Leveillula cf. helichrysi* گزارش می‌کنیم.

واژه‌های کلیدی: تنوع زیستی، سفیدکهای پودری، قارچهای ایران، *Erysiphaceae*، *Helotiales*

* بخشی از نتایج یک طرح تحقیقاتی ارایه شده به گروه گیاه‌پزشکی، دانشکده علوم کشاورزی، دانشگاه گیلان

** مسئول مکاتبات، پست الکترونیکی: khodaparast@guilan.ac.ir; blumeria2015@gmail.com

۱ گروه گیاه‌پزشکی، دانشکده علوم کشاورزی، دانشگاه گیلان، رشت، ایران

DOI: 10.22034/IJPP.2024.2021790.445

Short Research Article
Leveillula on *Helichrysum* in Iran: Unravelling a new *Leveillula* haplotype*

Seyed Akbar Khodaparast[1], Hamideh Darsaraei[1] and Mohammad Javad Pourmoghaddam[1]**

(Received: 10.03.2024; Accepted: 04.05.2024)

Abstract
Within the framework of the Mycology research laboratory's program at the University of Guilan focusing on the identification and DNA barcoding of powdery mildew fungi (*Erysiphaceae*, *Ascomycota*) from Iran, a powdery mildew specimen on *Helichrysum* was collected from Zanjan-Tarom road and subjected for morphological and molecular identification. Although this species morphologically resembles *Leveillula helichrysi*, and some other closely related species such as *L. taurica* complex, the ITS sequence differentiates this fungus from the sequences of *L. helichrysi* and *L. taurica* available in GenBank. We suggest this collection may represent a new species or new haplotype within *L. helichrysi*. Therefore, until we obtain more samples and sequence the type material, we report this fungus as *Leveillula cf. helichrysi* from Iran.

Keywords: Biodiversity, *Erysiphaceae, Helotiales,* Iran mycobiota, Powdery mildew

* A part of research project submitted to Department of Plant Protection, Faculty of Agricultural Sciences University of Guilan, Rasht, Iran

**Corresponding author, e-mail address: khodaparast@guilan.ac.ir; blumeria2015@gmail.com
1. Department of Plant Protection, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran

Introduction

The genus *Leveillula* G. Arnaud (*Erysiphaceae*, *Helotiales*) is a group of important powdery mildew fungi that infects several plant families including cultivated and non-cultivated species. The Mediterranean region and Central Asia are the main distribution zones for the genus, although now it is globally distributed (Braun & Cook 2012, Cerkauskas *et al.* 2011). The genus *Leveillula* includes about 48 species, including seven *Oidiopsis*-based names, (Braun & Cook 2012, www.indexfungorum.org, accessed December 2023). *Asteraceae* and *Fabaceae* plant families comprise the greatest number of host species for *Leveillula*, however, *Solanaceae* is considered one of the important economic plant families that host *Leveillula* spp. (Khodaparast *et al.* 2012, Cerkauskas *et al.* 2011). Reports of *Leveillula* powdery mildew on field and greenhouse crops from most parts of the world have been sporadic in the last century, however, new occurrences in some regions such as North America, Japan, North Europe, Australia, and other parts of the world are significantly increasing (Uchida 1995, Swart & Terblanche 2001, du Toit *et al*. 2004, Liberato *et al*. 2005, Liberato 2006, He *et al.* 2012, García-Gaytán *et al*. 2016, Cho *et al*. 2017, Meeboon *et al*. 2018, Damicone & Sutherland 1999, McGrath *et al*., 2001, Glawe *et al*., 2004, Garrido-Benavent *et al.* 2020, Kiss *et al*. 2020, Aydogdu *et al*. 2021). Greenhouse pepper and tomato are two main host species of *L. taurica* and the occurrence of *Leveillula* powdery mildew on them is significantly expanding around the world (Cerkauskas *et al*. 2011). Moreover, there are a large number of weeds and non-cultivated plants that harbor *Leveillula*, especially *L. taurica.* These plants may play important roles in the dispersal and epidemiology of the disease. In the present study, we have collected *Leveillula* on *Helichrysum* sp. and identified the fungus based on morphological and molecular characteristics.

Material and Methods

Anamorphic and teleomorphic structures were transferred into a drop of 1:1 lactic acid and glycerin drop using clear adhesive tape or a sterile needle. At least 20 fungal structures were measured and extreme values are shown in parentheses. All photos were taken using an Olympus BH2 microscope equipped with a Sony camera.

The whole DNA was extracted using a Thermolysis buffer (Zhang *et al.* 2010, Khodaparast *et al*. 2021). The ITS sequences were obtained using AITS (Bradshaw & Tobin 2020) /ITS4 (White *et al*. 1990). The amplicon was then sent to Codon Genetic Group, (Tehran, Iran) to be sequenced in two directions. The reaction components were 2–3 µl of total genomic DNA, 12.5 µl Taq DNA Polymerase Master Mix RED (Ampliqon, Denmark), 0.5 µl each primer (10 µM), and sterile reverse osmosis (RO) water up to a final volume of 25 µl. PCR conditions were as follows: activation for 3 min (95 °C), followed by 35 cycles of denaturation (95 °C) for 30 s, annealing (56 °C) for 30 sec, and elongation (72 °C) for 2 min (ramped up slowly at 1 °C per second), and a final elongation (72 °C) for 10 min.

The evolutionary history was inferred using the Minimum Evolution method (Rzhetsky & Nei 1992) in MEGA11 (Tamura *et al*. 2021). The evolutionary distances were computed using the Kimura 2-parameter method (Kimura 1980) and are in the units of the number of base substitutions per site. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). The ME tree was searched using the Close-Neighbor-Interchange (CNI) algorithm at a search level of 1. The Neighbor-joining algorithm (Saitou & Nei 1987) was used to generate the initial tree. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 580 positions in the final dataset.

Result and Discussion

Comparison of the obtained sequence with the sequences available in the GenBank showed at least 1.8% difference (identity 559/569, with 3 gaps) with all sequences of the genus *Leveillula* (about

265 sequences deposited in the GenBank). The difference between *Leveillula* species in this gene region is often very small. When this sequence was compared with the ITS sequence from *L. taurica* on *Zygophyllum fabago* L. (host plant for the holotype of *L. taurica*) and *Leveillula helichrysi* from Ukraine (on *Helichrysum* arenarium (L.) Moench, holotype host plant and locality; accession number LC565497) 12 substitutions (and 1-2 Gaps) were found. The interesting finding of these results is that the sequence of *Leveillula helichrysi* from Ukraine is 100% similar to *L. taurica* on *Zygophyllum* spp. In phylogenetic analysis, our sequence was clustered separately and far from *L. helichrysi*. *Leveillula helichrysi* is morphologically close to species in the *L. taurica* complex and is indistinguishable or difficult to distinguish. According to these results, several assumptions can be made to explain this situation. The first possibility is that the sample sequenced from Ukraine is representative of *L. helichrysi* species and our sequence belongs to a different species. In this case, we need to describe a new species to circumscribe our specimen. The second is that our sequence belongs to *L. helichrysi* and the sequence from *Leveillula* on *H. arenarium* (from Ukraine) belongs to a different species; although this possibility is unlikely because the sequence from Ukraine is from the holotype host plant and the same holotype locality. The third possibility is that the existence of different ITS sequences due to intragenomic variation, however intragenomic variation was found to be minimal in the powdery mildews. It should be noted that species in the genus *Leveillula* have not been heavily evaluated for intragenomic variation (Bradshaw *et al.* 2023). Although this assumption is not impossible, we consider it weaker than other possibilities. We suggest these sequences belong to different haplotypes and more likely a different species. The occurrence of more than one genotype of *Leveillula* on the same host plant species is not uncommon and has been reported for *Leveillula* on *Helianthus annuus* L., and *Eryngium* spp. (Khodaparast *et al*. 2012).

To find a clear answer to these questions, sequencing of more samples, and in particular the holotype material of *L. helichrysi*, is necessary. Morphological characteristics are insufficient for strong and accurate identification of *Helichrysum* powdery mildew from closely related species within the *L. taurica* complex. Hence, at this stage, we report this fungus as *Leveillula cf. helichrysi* from Iran.

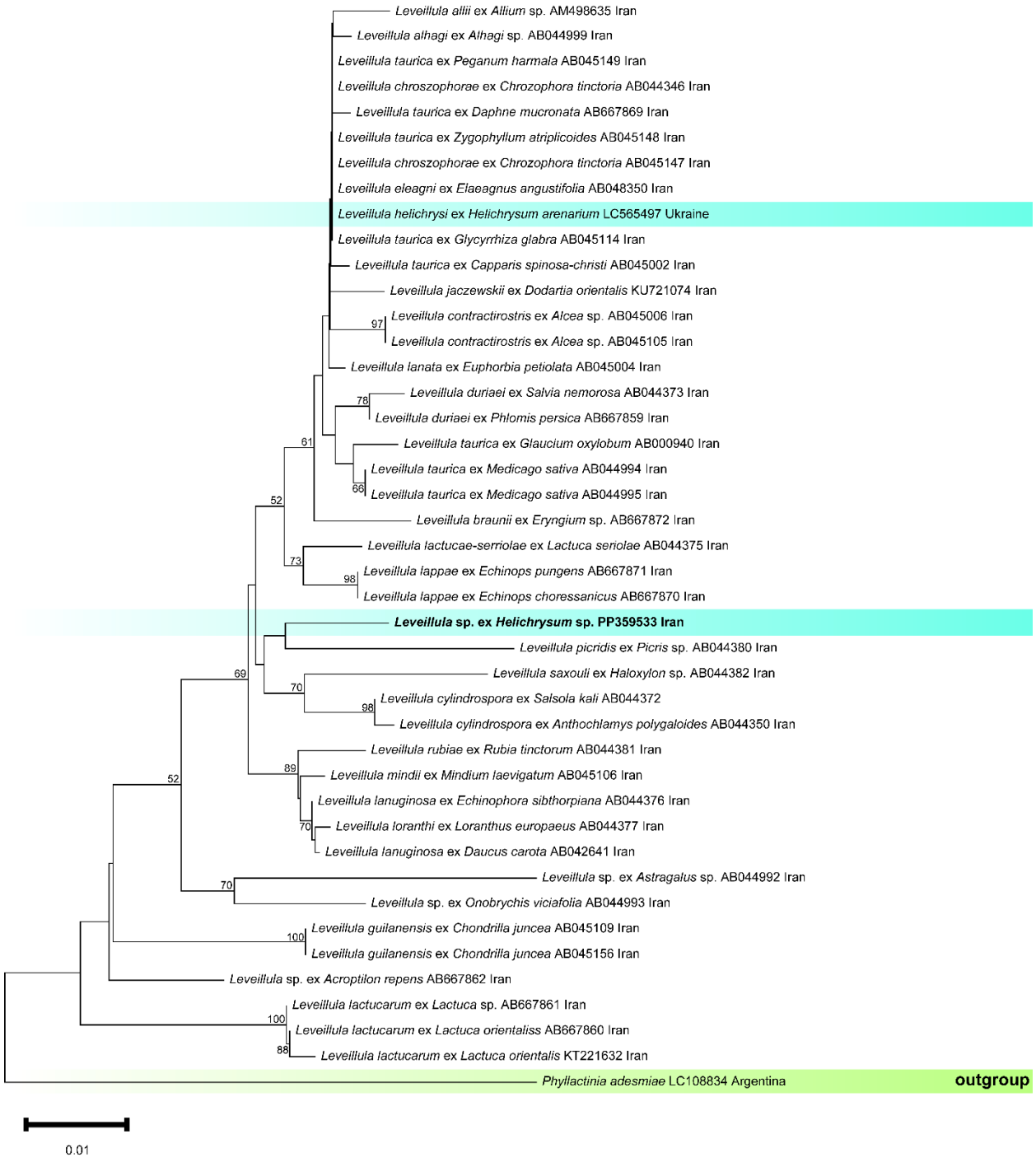

Fig. 1. The evolutionary history was inferred using the Minimum Evolution method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown above the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.

***Leveillula cf. helichrysi* V.P. Heluta & Simonyan, Biol. Zh. Armenii 41(10): 819 (1988)**

Mycelium on living leaves and stems, amphigenous, interwoven with tomentum of leaves, whitish, persistent, covering most parts or entire leaves. Hyphae 2–5 µm wide, sparingly branched, septate, hyaline, thin-walled. Primary conidia sparsely found, lanceolate, ellipsoid-lanceolate, with a pointed tip, 45–65 × 15–22.5 µm, length/width ratio 2.4–4.3; secondary conidia cylindric, subcylindric to cylindric-ellipsoid, rounded at the apex, subtruncate at the base, 42–57 × 16–20 µm; chasmothecia 175–225 (–250) µm diameter, appendage numerous, short, shorter than the chasmothecial diameter, hyaline at first, becoming fully pigmented at maturing, brawn, simple to irregularly branched, branching coral-like, sometimes 1–4 (–5) dichotomously branched, usually aseptate, 5–9 µm wide at base; asci numerous, ellipsoid, obovoid, 70–105 × 32–43 µm (including stalk), stalk 4–11 µm, sometimes longer, 2–spored; ascospores usually ellipsoid, ovoid, 37–47 × 20–25 µm.

Hosts and Distribution in Iran: On *Helichrysum* sp., Zanjan, Zanjan-Tarum road, 29 August 2023, S.A. Khodaparast and M.J. Pourmoghaddam (GUM 1973).

Molecular Data: Accession number for ITS1–5.8S–ITS2 rDNA sequences PP359533

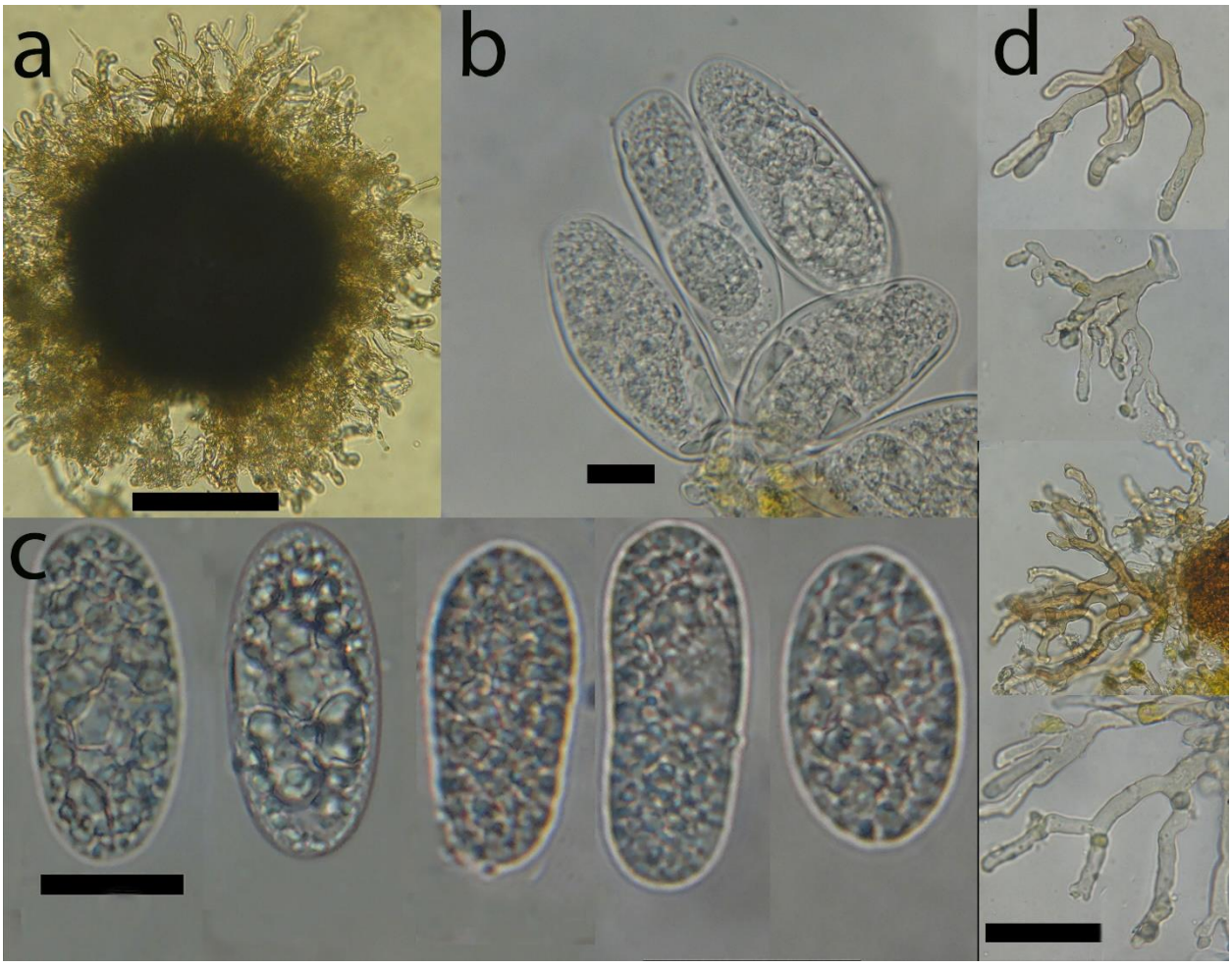

Fig. 2 *Leveillula cf. helichrysi* (a) Ascoma (b) Ascus (c) Ascospores (d) appendages, scale bar for a, d=100 µm, b, c=20 µm

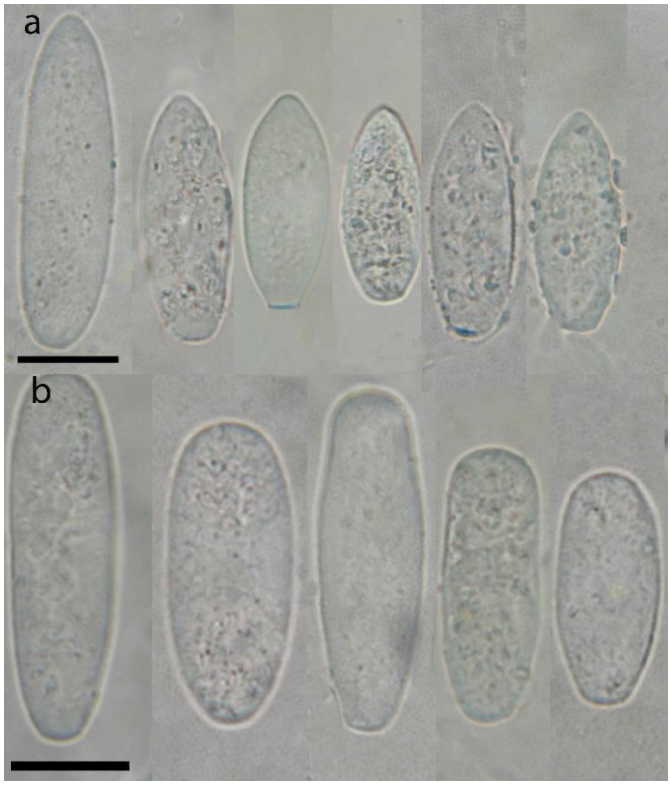

Fig. 3 *Leveillula cf. helichrysi*: (a) Primary conidia, (b) Secondary conidia, scale bar =20 µm

Acknowledgments This work was supported in part by Grants-in-Aid for Scientific Research (No. 5998) from the Deputy of Research and Technology of University of Guilan, Iran.

References

منابع

Aydogdu M., Sülü G., and Kurbetli I. 2021. Characterization of powdery mildew (*Leveillula taurica*) in globe artichoke (*Cynara scolymus*). Mediterranean Agricultural Sciences, 34(1), 17–24.

Bradshaw M. and Tobin P. C. 2020. Sequencing Herbarium Specimens of a Common Detrimental Plant Disease (Powdery Mildew). Phytopathology 110:1248–1254. https://doi.org/10.1094/PHYTO-04-20-0139-PER.

Bradshaw M. J., Aime M. C., Rokas A., Maust A., Moparthi S., Jellings K., Jellings K., Pane A. M., Hendricks D., Pandey B., Li Y. and Pfister D. H. 2023. Extensive intragenomic variation in the internal transcribed spacer region of fungi. Iscience, 26(8).107317.https://doi.org/10.1016/j.isci. 2023.107317

Braun U. and Cook R. 2012. Taxonomic manual of the *Erysiphales*. Utrecht: CBS–KNAW Fungal Biodiversity Centre

Cerkauskas R. F., Ferguson G. and Banik M. 2011. Powdery mildew (*Leveillula taurica*) on greenhouse and field peppers in Ontario–host range, cultivar response and disease management strategies. Canadian journal of plant pathology 33(4): 485–498.

Cho S. E., Choi I. Y. and Shin H. D. 2017. The Identity of Eggplant Powdery Mildews Collected in Korea. The Korean Journal of Mycology 45(2): 91–101.

Damicone J. P. and Sutherland A. J. 1999. First report of pepper powdery mildew caused by *Leveillula taurica* in Oklahoma. Plant disease 83(11): 1072–1072.

du Toit L. J., Glawe D. A. and Pelter G. Q. 2004. First report of powdery mildew of onion (*Allium cepa*) caused by *Leveillula taurica* in the Pacific Northwest. Plant Health Progress 5(1): 17. Doi: 10.1094/PHP-

2004-1129-01-HN

García-Gaytán V., García-Morales S., Silva-Rojas H. V., Trejo-Téllez L. I. and Gómez-Merino F. C. 2016. First report of powdery mildew in chilhuacle chili (*Capsicum annuum*) caused by *Leveillula taurica* in Southern Mexico. Plant disease 100(11): 2325–2325.

Garrido-Benavent I., Gomez-Bolea A. and Benitez G. 2020. *Leveillula guilanensis* (*Erysiphales*, *Ascomycota*): a new record from Europe with notes on the biogeography of the genus. Phytotaxa 451(3): 179–194.

Glawe D. A., du Toit L. J. and Pelter G. Q. 2004. First report of powdery mildew on potato caused by *Leveillula taurica* in North America. Plant Health Progress 5(1): 15. Doi: 10.1094/PHP-2004-1214-01-HN

He G., Xu B., Song J. G., Zhang L. L., Zhao Z. Y. and Wang G. 2012. First report of powdery mildew caused by *Leveillula taurica* on *Cynanchum kashgaricum* in China. Plant Disease 96(9): 1373–1373.

Khodaparast S. A., Takamatsu S., Harada M., Abbasi M. and Samadi S. 2012. Additional rDNA ITS sequences and its phylogenetic consequences for the genus *Leveillula* with emphasis on conidium morphology. Mycological Progress 11: 741–752.

Khodaparast S.A., Darsaraei H. and Abbasi M. 2021. The genus *Arthrocladiella*: a new report of powdery mildew fungi from Iran. Mycologia Iranica 8: 135–140

Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111–120.

Kiss L., Vaghefi N., Bransgrove K., Dearnaley J. D., Takamatsu S., Tan Y. P., Marston C., Liu S. Y., Jin D. N., Adorada, D. L. and Bailey J. 2020. Australia: A continent without native powdery mildews? The first comprehensive catalog indicates recent introductions and multiple host range expansion events, and leads to the re-discovery of *Salmonomyces* as a new lineage of the *Erysiphales*. Frontiers in Microbiology 11: 1571.

Liberato J. R. 2006. Powdery mildew on Passiflora in Australia. Australasian Plant Pathology 35: 73–75.

Liberato J. R., Wright J. G., Seth M. and Shivas R. G. 2005. *Oidiopsis* (*Erysiphaceae*) on *Euphorbia* spp. in Australia and Vanuatu. Australasian Plant Pathology 34(3): 409–411.

McGrath M. T., Shishkoff N., Bornt C. and Moyer D. D. 2001. First occurrence of powdery mildew caused by *Leveillula taurica* on pepper in New York. Plant disease 85(10): 1122–1122.

Meeboon J., Kokaew J. and Takamatsu S. 2018. Notes on powdery mildews (*Erysiphales*) in Thailand VI. *Phyllactinia* and *Leveillula*. Mycological Progress 17: 701–718.

Rzhetsky A. and Nei M. 1992. A simple method for estimating and testing minimum evolution trees. Molecular Biology and Evolution 9: 945–967.

Saitou N. and Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406–425.

Swart W. J. and Terblanche J. 2001. First report of powdery mildew of kenaf caused by *Leveillula taurica* in South Africa. Plant Disease 85(8): 923–923.

Tamura K. Stecher G. and Kumar S. 2021. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution. https://doi.org/10.1093/molbev/msab120.

Uchida J. Y., Aragaki M. and Ogata D. Y. 1995. First report of *Leveillula taurica* in Hawaii. Plant Disease, 79(9). DOI: 10.1094/PD-79-0966D.

White T. J., Bruns T., Lee S. and Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315–332 in: PCR Protocols: A Guide to Methods and Applications. M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, eds. Academic Press, San Diego.

Zhang Y. J., Zhang S., Liu X. Z., Wen H. A. and Wang M. 2010. A simple method of genomic DNA extraction suitable for analysis of bulk fungal strains. Letters in applied microbiology 51 (1): 114-118. https://doi.org/10.1111/j.1472-765X.2010.02867.x