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New records of powdery mildew fungi on landscape and ornamental plants from Iran

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Abstract: The Erysiphaceae are obligatory and biotrophic fungal parasites that infect many landscapes and ornamental plants, which results in a reduction of beauty and marketability of these plants. This study was performed to identify powdery mildew fungi on landscape and ornamental plants in four provinces (Isfahan, Chaharmahal & Bakhtiari, Markazi and Lorestan) of Iran. Consequently, 24 powdery mildew taxa on 28 host species were collected from 2017 to 2019. According to our findings, *Golovinomyces asperifolii* (on *Nonea* sp.) and *Podosphaera euphorbiae–helioscopiae* (on *Pedilianthus* sp.) are new records to Iran mycobiota. Three plant species, including *Coreopsis* sp., *Catharanthus roseus* and *Fragaria vesca* are new hosts for powdery mildew fungi of Iran. Moreover, *Podosphaera xanthii* on *Dahlia* sp. is reported for the first time from Iran.

Keywords: phytopathogen, host, phylogeny, morphology, taxonomy

INTRODUCTION

Powdery mildew fungi (Erysiphales, Ascomycota) have emerged as a serious threat to landscape and ornamental plants. These disease agents infect green leaves, stems, sprouts and flowers through which premature wither and falling of leaves can occur. Flowers infected by powdery mildews lose their ornamental value. The genus and species–level taxonomy of powdery mildews have been significantly changed based on new molecular and morphological approaches (Cook et al. 1997, Braun & Takamatsu 2000, Braun et al. 2002, Braun & Cook 2012). Many studies have been conducted by mycologists and plant pathologists, although research is still ongoing on taxonomy and several aspects of pathogenicity of this interesting group of fungi. Until now, no comprehensive study has been done on landscape and ornamental plants in Iran. However, sporadic publications show that these fungi are common on ornamental plants in the country (Sharifi et al. 2013, Sharifi et al. 2014, Khodaparast 2016).

Based on the status of the international center of ornamental plants (AIPH), Iran ranks 12th worldwide in terms of ornamental plants cultivation (http://aiph.org/statistical–yearbook/). In spite of vast areas under cultivation of ornamental plants, unfortunately, exports of these plants have not been satisfactory. Lack of enough information about pathogens of ornamental plants such as powdery mildew fungi is one of the challenges of producing and exporting these plants.

In this study, we have described and illustrated some powdery mildew species on ornamental plants from four provinces of Iran to identify the species by means of combined analyses of morphological and molecular data.

MATERIALS AND METHODS

Sample collection

Samples infected with powdery mildew fungi (disease agents covering green leaves, stems, sprouts with mycelium) were collected from four provinces of Iran during 2016–2018 (Isfahan, Chaharmahal & Bakhtiari, Markazi and Lorestan) and transferred to the laboratory of Plant Pathology, University of Lorestan.

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All the specimens were deposited in the University of Guilan Mycological Herbarium (GUM).

Morphological characterization

For microscopic analysis, different organs of the fungus were examined under an optical microscope, Olympus BH2. To examine asexual morphs, mycelia were strip off from the leaf surface using clear adhesive tape and mounted in a 50% lactic acid solution. For observation of sexual structures, chasmothecia were placed in 3 % NaOH solution. Along with morphological characteristics of sexual and asexual stages, host species names and other information related to the specimens were recorded. Measurements were conducted based on the data from 30 samples for each structure. All the images were provided by a digital camera (Sony, DSH–HX) attached to the microscope. The images were put together and were edited using Photoshop (Adobe Photoshop CS). Exact identification and confirmation of taxa were made using Braun (1987, 1995), Braun & Takamatsu (2000), Cook & Braun (2009) and Braun & Cook (2012).

DNA extraction, PCR amplification, sequencing

Total DNA was extracted from conidia, mycelia, and chasmothecia of powdery mildew fungi by the HotShot method (Montero–Pau et al. 2008). A universal primer set of ITS1/ITS4 (White et al. 1990) was used to amplify ITS region. The Polymerase chain reaction (PCR) was performed in a Thermal Cycler in a total volume of 25 ml. The PCR mixtures contained 12.5 µl of master mix (RNA Biotech company, Iran), 1 µL of each primer (0.4 pmol/µL), 3 µL of DNA template and 7.5 µL of double–distilled water. The PCR amplicons were electrophoresed on 1.5 % agarose gels in TAE buffer. The PCR products were sent to a commercial sequencing provider (Tao Yang, Beijing, China) for direct sequencing.

Phylogenetic analysis

All the obtained sequences were analyzed and edited using MEGA7.0 (Kumar et al. 2016) and subsequently compared with the available sequences in the NCBI GenBank nucleotide database using the BLASTN search. These sequences were aligned with other sequences retrieved from DNA databases using MUSCLE in MEGA 7 (Edgar 2004, Kumar et al. 2016). Phylogenetic trees were obtained using the minimum–evolution method in MEGA 7.0 (Kumar et al. 2016). In the ME method, the evolutionary distances were computed using the Kimura 2–parameter method (Kimura 1980). All the ambiguous positions were removed for each sequence pair. The strength of the internal branches from the resulting trees was statistically tested by bootstrap analysis with 1,000 replicates (Felsenstein 1985). The ITS sequences determined in this study were deposited in GenBank.

RESULTS AND DISCUSSION

Powdery mildew fungi collected in this study belong to the genera including *Erysiphe*, *Golovinomyces*, *Leveillula*, *Oidium*, *Podosphaera* and *Sawadaea* (Table 1). Comparison of the sequences showed that our sequences mostly were identical with some reliable sequences in GenBank (Fig. 1). Due to morphological complexity, molecular approaches, including ITS sequence and phylogenetic analysis, were useful for the identification of the species. Further information is given for each taxon under species description in the taxonomic part.

Golovinomyces asperifolii **(Erikss.) U. Braun & H.D. Shin, Mycobiology 46 (3): 198 (2018) Fig. 2**

Mycelium amphigenous, on stems and sepals, dense, persistent, forming regular or irregular white patches, effuse; hyphae hyaline, thin–walled, smooth, 2.5–7.5 µm wide; conidiophores erect, arising from the upper surface of hyphal mother cells, 100–200 µm long; foot–cells straight, cylindrical, 40–100 × 8–12.5 µm, foot–cells followed by 1–4 shorter cells, forming catenescent conidia; conidia ovoid to doliiform, 22–38 × 12–20 µm, length/width ratio 1.4–2.4, germ tubes Euoidium type. Teleomorph not found.

According to Braun & Cook (2012), members of *Boraginaceae* are infected with a single species *Golovinomyces cynoglossi* s. lat. Recently, this species has been divided into three species based on the phylogenetic analysis of rDNA ITS sequences and morphological re–examination (Braun et al. 2019). Blast search showed that our sequences is 100 % similar to *G. asperifolii* on *Myosotis sylvatica* (GLM–F079322, epitype). This is the first report of *G. asperifolii* on *Nonea* sp. in Iran.

Specimen examined. IRAN, Isfahan province, Isfahan, on *Nonea* sp., 18 May 2018, K. Sharifi, (GUM 1696), GenBank accession number: MT621669, ITS.

Leveillula cleomes **Simonyan & V.P. Heluta, Biol. Zh. Armenii 42(5): 481 (1989) Fig. 3**

Chasmothecia gregarious to scattered, often immersed in dense mycelia, 130–200 µm, appendages usually well developed, mycelioid, simple, mostly interlaced with each other and with the mycelium, hyaline to light brown, asci obovoid–clavate to subcylindrical, 65–100 × 20–45 µm, ascospores ellipsoid–ovoid, colourless, 20–32 × 12–18 µm. Primary conidia subcylindrical, usually with the parallel side which apically narrowed, wider in the upper half, 50–70 × 11.5– 17.5 µm, secondary conidia cylindrical, subcylindrical, sometimes slightly narrowed towards the base and slightly clavate, as large as the primary conidia, usually 55–75 × 11– 16.5 µm.

Based on the rDNA ITS sequences, this species is closely related to *L. taurica* (accession AB045000,

from *L. taurica* on *Zygophyllum fabago*, type host of this species). However, the sequence of this specimen showed variation in three positions in ITS sequence from AB045000. Several ITS sequences have been recorded for *Leveillula* species in GenBank; however, this is the first record of ITS sequence for *L. cleomes*. There is one ambiguous record of *Leveillula taurica* on *Cleome* (Amano, 1986), but this is the first reliable record of *L. cleomes* on this plant which is identified by morphological and molecular characteristics.

Specimen examined. IRAN, Isfahan province, Isfahan, on *Cleome* sp., 21 June 2018, K. Sharifi, (GUM 729), GenBank accession number: MT621677, ITS.

***Podosphaera euphorbiae–helioscopiae* (Tanda & Y. Nomura) U. Braun & S. Takam., Schlechtendalia 4: 28, (2000) Fig. 4**.

Mycelium amphigenous, white on stem and leaves, frequently infection on the terminal bud of the stem that causes the death of the bud, hyphae hyaline, persistent hyphae later turning brown with thin–walled, 5– 7.5 µm wide, appressoria nipple–shaped, conidiophores erect, straight and flexuous, up to 280 µm, foot cell cylindrical, up to 45–100 × 7.5–10 µm, followed by 1–4 shorter cells, forming catenescent conidia, mostly 3–8 conidia per chain, conidia cylindrical, ellipsoidoid, 20–57 × 9–14 µm, teleomorph not seen.

Sharifi et al. (2013) have already reported this powdery mildew as *P. euphorbiae–hirtae* (U. Braun & Somani) U. Braun & Takam on this plant from Guilan province. However, this identification was only based on conidial state and without molecular examination. The ITS sequence of both specimens from Guilan and Markazi provinces showed 100% similarity. Blast search showed that the similarity of our sequences to ITS–rDNA sequence of some taxa deposited in GeneBank is more than 99% (Accession numbers: KY661086 Ellingham Unpublished; MN216223 Pei & Zhu unpublished). We found one reliable ITS sequence of *P. euphorbiae–hirtae* (AB040306, Hirata et al. 2000), another *Podosphaera* species on *Euphorbiaceae*, which is easily distinguishable from *P. euphorbiae–helioscopiae* on the basis of molecular data.

Table 1: Fungi included in this study, their host plants and corresponding GenBank accession numbers.

Taxa	Host plant	Vochers	Genebak accession no.
Erysiphe Australiana	*Lagerstroemia indica*	GUM 1716	—
Erysiphe euonymicola	*Euonymus japonicus*	GUM 1719	—
Erysiphe howeana	*Oenothera biennis*	GUM 1714	—
Erysiphe multappendicis	*Berberis thunbergii*	GUM 1715	—
Erysiphe platani	*Platanus orientalis*	GUM 1718	—
Erysiphe rayssiae	*Spartium junceum*	GUM 1711	—
Erysiphe robiniae var. *roboniae*	*Robinia pseudoacacia*	GUM 1713	—
Erysiphe syringae– japonicae	*Syringa vulgaris*	GUM 1712	—
Erysiphe trifoliorum	*Trifolium* sp.	GUM 1717	—
Golovinomyces latisporus	*Zinnia elegans*	GUM 1707	—
Golovinomyces asperifolii	*Nonea* sp.	GUM 1696	MT621669
Golovinomyces biocellatus	*Mentha piperita*	GUM 1708	—
Golovinomyces orontii	*Antirrhinum majus*	GUM 1697	MT621670
Golovinomyces bolayi	*Abelmoschus esculentus*	GUM 1703	—
Golovinomyces orontii	*Viola pansy*	GUM 1704	—
Leveillula cleomes	*Cleome* sp.	GUM 729	MT621677
Leveillula taurica	*Catharanthus roseus*	GUM 731	—
Leveillula taurica	*Silybum marianum*	GUM 730	MT621676
Erysiphe sp.	*Cleome* sp.	GUM 1720	—
Podosphaera aphanis	*Fragaria vesca*	GUM 1721	—
Podosphaera erigerontis– canadensis	*Taraxacum* sp.	GUM 1710	—
Podosphaera euphorbiae–helioscopiae	*Pedilanthus* sp.	GUM 1698	MT621672
Podosphaera leucotricha	*Photinia* sp.	GUM 1699	MT621673
Podosphaera pannosa	*Rose* sp.	GUM 1705	—
Podosphaera xanthii	*Gerbera* sp.	GUM 1706	—
Podosphaera xanthii	*Dahlia pinnata*	GUM 1700	MT621674
Podosphaera xanthii	*Calendula officinalis*	GUM 1709	—
Podosphaera xanthii	*Coreopsis grandiflora*	GUM 1701	MT621675
Sawadaea negundinis	*Acer negundo*	GUM 1702	MT621671

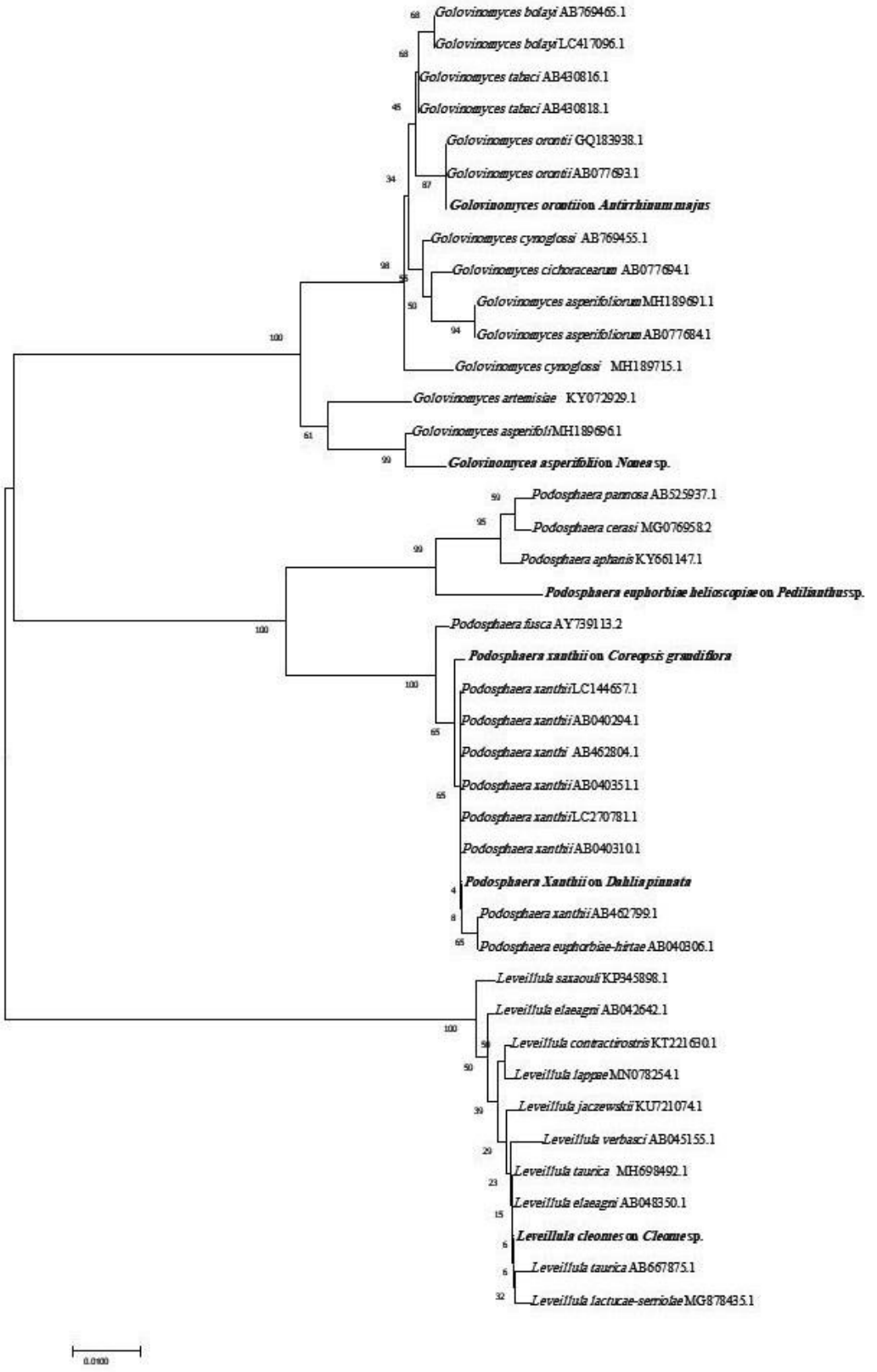

Fig. 1. Phylogenetic analysis of the rDNA ITS regions for 41 sequences of *Golovinomyces*, *Podosphaera* and *Leveillula* by the minimum–evolution method. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Numbers next to the branches shows the bootstraps values ≥ 50 %. Evolutionary analyses were conducted in MEGA 7.0

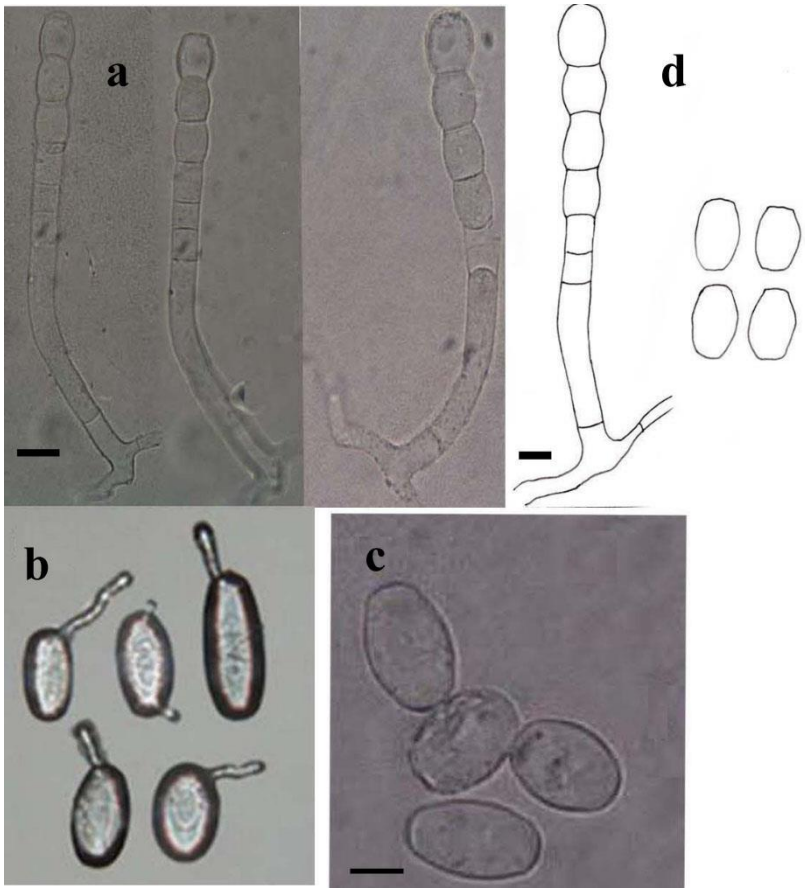

Fig. **2**. *Golovinomyces asperifolii*. a. Conidiophores; b. Germinated conidium; c. Conidia; d. Drawing conidia & conidiophore. — Scale bars: a–c = 10 µm, d = 20 µm.

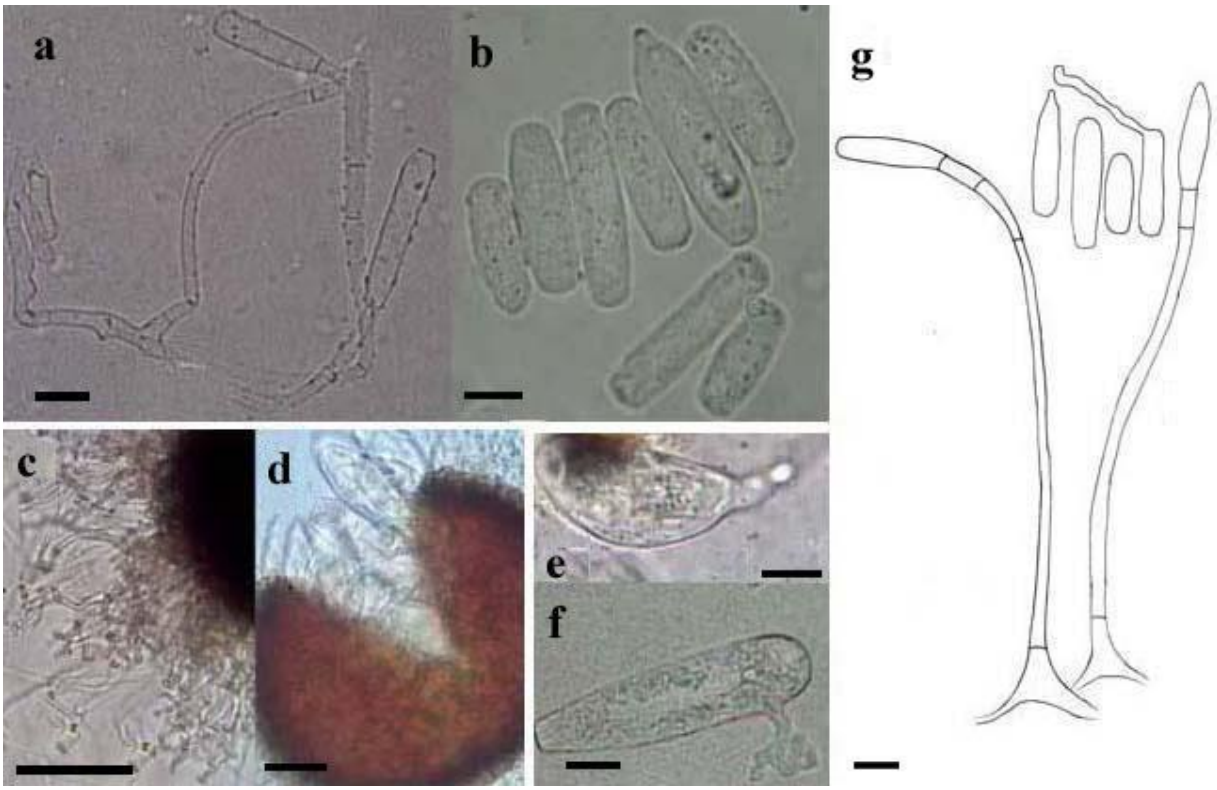

Fig. **3.** *Leveillula cleome.* a. Conidiophore; b. Conidia; c. Appendages; d. Chasmothecia with ascus; e. Ascus, f. germinated conidia; g. Drawing conidia & conidiophore. — Scale bars: a, f= 10 µm, b, c, d, e, g= 20µm.

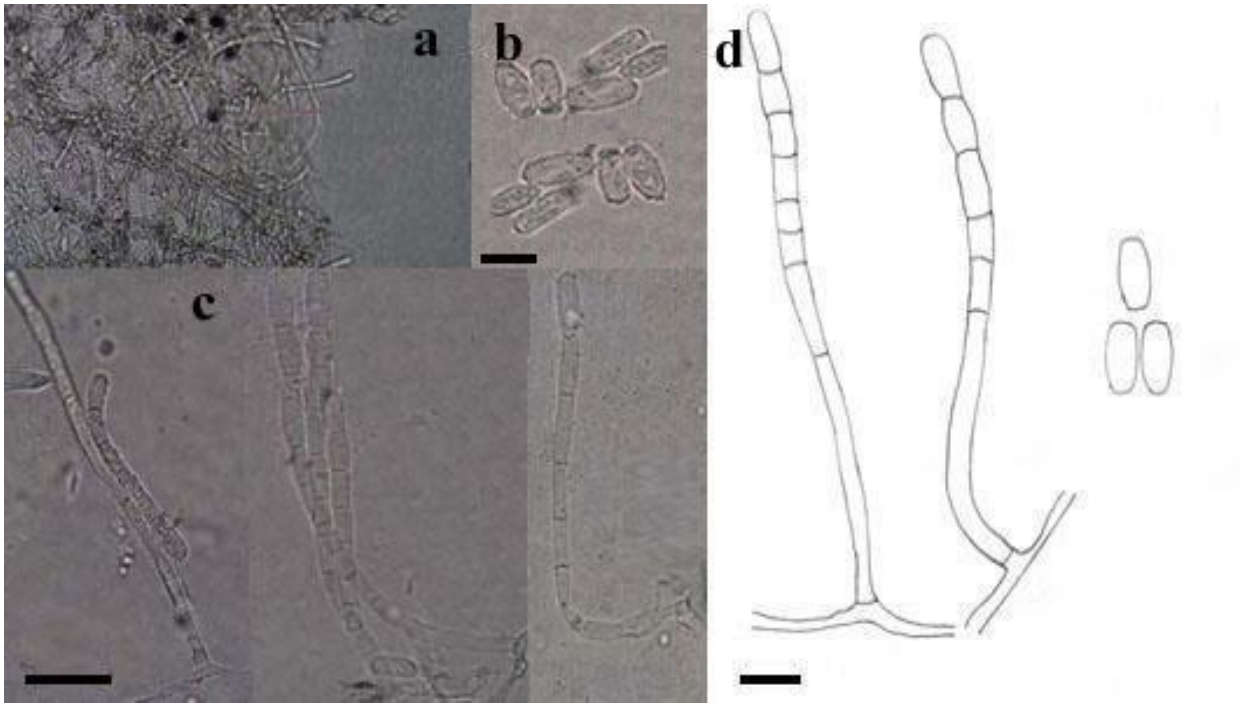

Fig. 4. *Podosphaera euphorbiae–helioscopiae.* a. Mycelium & conidiophores; b. Conidia; c. Conidiophores; d. Drawing from conidia & conidiophores. — Scale bars = 20μm

Specimen examined. IRAN, Markazi province, Mahalat, on *Pedilianthus* sp., 10 Aug. 2017, K. Sharifi, (GUM 1698), GenBank accession number: MT621672, ITS.

The following species have already been described, but their host plants are new to Iran. Furthermore, ITS sequences for some taxa are provided.

***Leveillula taurica* (Lév.) G. Arnaud, Annls Épiphyt. 7: 92 (1921) Fig. 5**

Specimen examined. IRAN, Isfahan province, Isfahan, on *Catharanthus roseus*, 10 Aug. 2017, K. Sharifi, (GUM 731).

This is the first report of *L. taurica* on *Catharanthus roseus* in Iran.

***Podosphaera aphanis* (Wallr.) U. Braun & S. Takam., Schlechtendalia 4: 26 (2000) Fig. 6**

Specimen examined. IRAN, Isfahan province, Isfahan, on *Fragaria vesca*, 10 Aug. 2017, K. Sharifi, (GUM 1721).

This is the first report of *P. aphanis* on *Fragaria vesca* in Iran.

***Podosphaera xanthii* (Castagne) U. Braun & Shishkoff, Schlechtendalia 4: 31, (2000) Fig. 7**

Already, *Golovinomyces ambrosiae* (Schwein.) U. Braun & R.T.A. Cook has been recorded on *Dahlia* from Iran (Khodaparast 2016). This is the first report of *P. xanthii* on *Dahlia pinnata* in Iran.

Specimen examined. IRAN, Isfahan province, Isfahan, on *Dahlia pinnata*, 12 June 2018, K. Sharifi, (GUM 1700), GenBank accession number: MT621674, ITS.

***Podosphaera xanthii* (Castagne) U. Braun & Shishkoff, Schlechtendalia 4: 31, (2000). Fig. 8**

Cho et al. (2016) have already reported *Podosphaera xanthii* on *Coreopsis verticillata* from Korea.

The blast search showed 100% similarity to several accessions such as AB525914.1 (from *Calendula officinalis*, Takamatsu et al. 2010); AB040349.1 (from *Syneilesis palmata*, Hirata et al. 2000) LC270781.1 (from *Cosmos sulphureus*, submitted by Meeboon & Takamatsu, unpublished) . All of these sequences assigned to *P. xanthii*.

Specimen examined. IRAN, Isfahan province, Isfahan, on *Coreopsis grandiflora*, 6 May 2018, K. Sharifi, (GUM 1701), GenBank accession number: MT621675, ITS.

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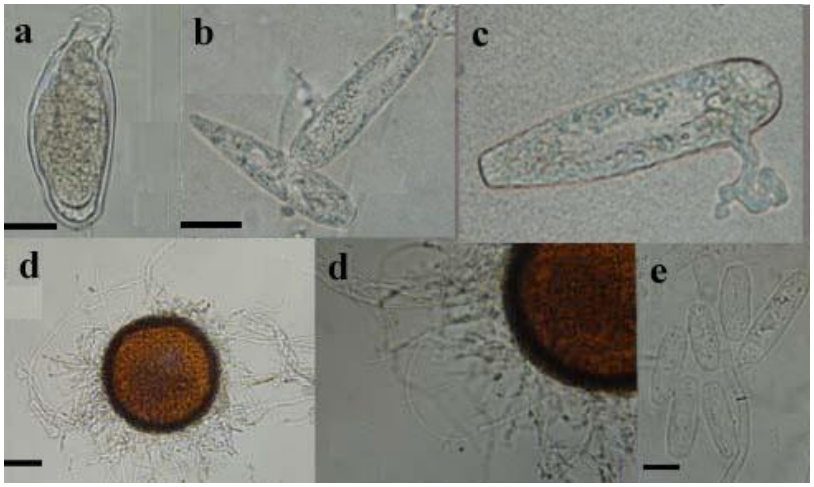

Fig. 5. *Leveillula taurica.* a. Asci & Appendages; b. Primary conidia; c. Conidia germination Appresorium; d. Chasmothecia; e. Secondary conidia & conidiophore; f. *Catharanthus roseus*. — Scale bars = 20µm.

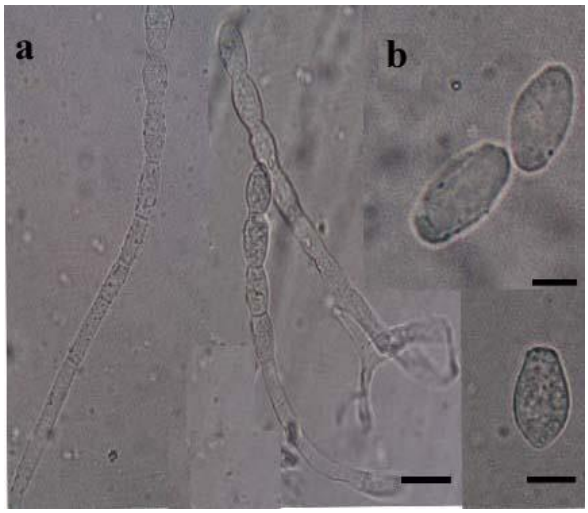

Fig. 6. *Podosphaera aphanis*. a. Conidiophores; b. Conidia. — Scale bars: A= 20 µm, B= 10µm.

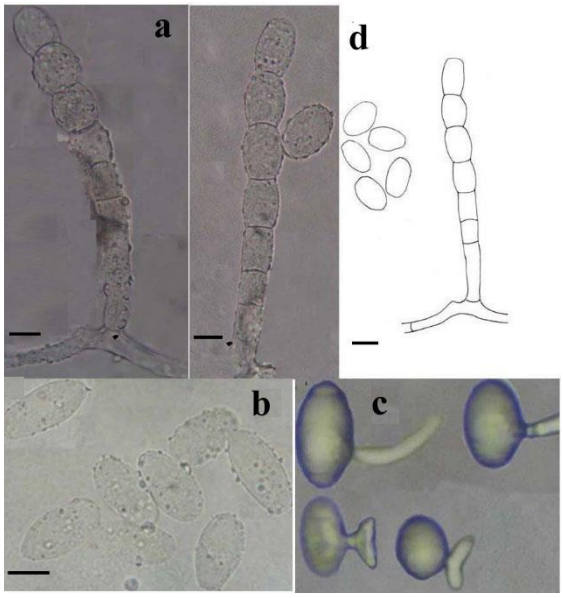

Fig. 7. *Podosphaera xanthii*. a. Conidiophores; b. Conidia; c. Germinated conidium; d. Drawing conidia & conidiophore. — Scale bars = 20µm.

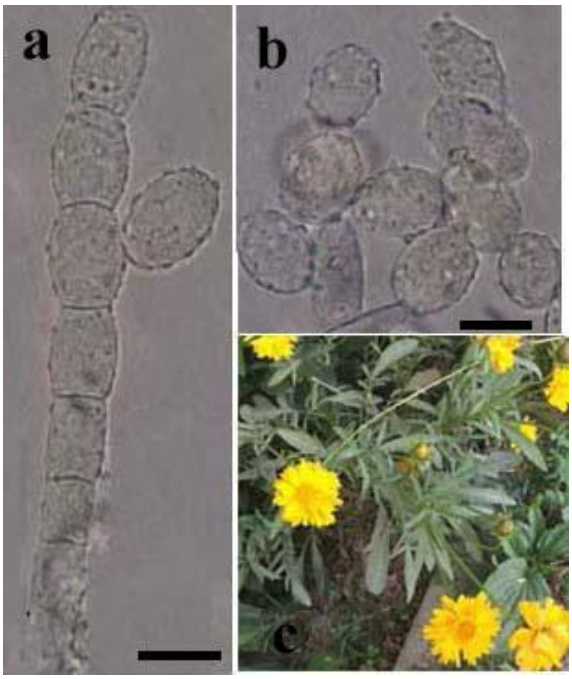

Fig. 8. *Podosphaera xanthii*. a. Conidiophores; b. Conidia; c. *Coreopsis grandiflora*. — Scale bars = 20µm.

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گزارش‌های جدید از سفیدک‌های پودری گیاهان زینتی و فضای سبز ایران

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چکیده: قارچ‌های تیره *Erysiphaceae* پارازیت اجباری میزبان‌های متنوعی از جمله گیاهان زینتی و فضای سبز هستند که موجب کاهش زیبایی و بازار پسندی این گیاهان می‌شوند. این مطالعه با هدف شناسایی قارچ‌های عامل سفیدک پودری روی گیاهان زینتی و فضای سبز چهار استان ایران (اصفهان، چهارمحال و بختیاری، مرکزی و لرستان) انجام شد. در کل، طی سال‌های ۱۳۹۸–۱۳۹۶، ۲۴ گونه سفیدک پودری از روی ۲۸ گونه میزبان جمع آوری و شناسایی شد. براساس یافته‌های این پژوهش، گونه *Golovinomyces asperifolii* روی *None* sp. و گونه *Podosphaera euphorbiae–helioscopiae* روی *Pedilianthus* sp.، آرایه های جدیدی برای میکوبیوتای ایران هستند. سه گونه گیاهی *Catharanthus roseus*، *Coreopsis* sp. و *Fragaria vesca* میزبان-های جدیدی برای سفیدک‌های پودری ایران هستند. علاوه بر این، *Podosphaera xanthii* روی *Dahlia* sp. برای اولین بار از ایران گزارش می شود.

کلمات کلیدی: بیمارگر گیاهی، میزبان، فیلوژنی، ریخت شناسی، تاکسونومی

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