Mycologia Iranica 6(1): 1 – 7, 2019 Original Article DOI: 10.22043/mi.2019.120420

Notes on two powdery mildew fungi (*Erysiphe magnifica* and *E. corylacearum*) from Iran

S. A. Khodaparast ✉
A. Ghobakhloo
Department of Plant Protection, Faculty of Agricultural Science, University of Guilan, Rasht, Iran

B. Asgari
F. Aliabadi
S. Sajedi
Department of Botany, Iranian Research Institute of Plant Protection, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran

Abstract: Two powdery mildew species were collected from Iran. For the first time, *Erysiphe magnifica* on *Magnolia* sp. was identified from Iran based on morphological and sequence data of ITS rDNA. *Erysiphe corylacearum* was collected for the second time in Iran and reported for the first time from Guilan province. There are some polymorphisms in ITS-rDNA sequences of *E. corylacearum* from Iran and those from GenBank. This polymorphism is worth of attention for better understanding of the taxonomy of the species and role of ITS sequences for species delimitation in Erysiphales. The ITS sequence of the Iranian specimen of *E. magnifica* were 100% identical to four ITS sequences of *E. magnifica* in GenBank. Morphologically, *E. magnifica* is characterized by curved, flexuous and relatively long foot cells, whereas, foot-cells of conidiophores have been reported in the literature as relatively short. According to these observations, it is recommended to check as many characters as possible for identification of morphologically and molecularly closely related species.

Key words: Biosystematics, morphology, DNA polymorphism, Erysiphales, *Magnolia*

INTRODUCTION

Powdery mildew fungi are widespread plant pathogenic fungi that are easily recognizable by their appearance on the plant surface as white powdery covering the leaves, stems, flowers and fruits. These fungi are well characterized by their asexual and sexual morphs, which produce a unique kind of meristem-arthric aseptate conidia and a kind of unique ascomata named chasmothecia. More than 1000 species have been described based on solely anamorphic or teleomorphic, or both states. Powdery mildew fungi are different in host range and distribution. Some species are strictly confined to a few host plant species so that they may occur just on a few species of one genus. On the other hand, some species have been reported on hosts belonging to a large number of plant families that are distantly related to each other (Braun 1987, Palti 1988, Braun & Cook 2012). Closely related species of a single genus on a common host sometimes are hardly distinguishable. For example, *Magnolia* s. l. species may be infected by four powdery mildews from the same genus (Braun & Cook 2012, Cho et al. 2014). According to the literature more than 100 species of powdery mildew have been reported from Iran; probably some further species might have not been observed and are waiting to be explored for the country (Khodaparast et al. 2001, Khodaparast 2007, Khodaparast & Abbasi 2009, Abbasi et al. 2013, Sharifi et al. 2013, Khodaparast et al. 2016, Arzanlou et al. 2017).

MATERIALS AND METHODS

Morphological examination

For microscopy of hyphae, conidiophores and conidia, these structures were stripped off from the leaf surface using clear adhesive tapes and subsequently mounted in glycerol/lactic acid solution (Heidari et al. 2015, Khodaparast et al. 2016a). An Olympus light microscope (BH-2, Japan) equipped with a Sony digital camera was used for microscopic

Submitted 9 Jan. 2019, accepted for publication 21 May 2019
✉ Corresponding Author E-mail: khodaparast@guilan.ac.ir

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observations and photomicrography. All measurements were done based on at least 20 to 30 observations. Morphological features of the species were compared to the description and illustration of related taxa available in the literature (Shin 2000, Braun & Cook 2012, Cho et al. 2014).

DNA sequencing and data analysis

Total DNA was extracted from fungal specimens by the Chelex method (Walsh et al. 1991; Hirata & Takamatsu 1996; Khodaparast et al. 2016 a, b). Primers ITS1 (5'–TCCGTAGGTGAACCTGCGG–3') and ITS4 (5'–TCCTCCGCTTATTGATATGC–3') were used for amplification and sequencing of the rDNA internal transcribed spacers (White et al. 1990).

The nucleotide sequences of the polymerase chain reaction (PCR) products were obtained using direct sequencing in an ABI 3730 xl sequencer (Applied Biosystems, USA). Obtained sequences were analyzed and edited using MEGA7.0 (Kumar et al. 2016) and subsequently, were compared to the sequences available in the NCBI GenBank nucleotide database using the BLASTN search method. Several sequences from GenBank were selected for phylogenetic analyses. Sequence alignment was performed using MUSCLE plug-in of MEGA 7.0 with the default settings (Edgar 2004). Phylogenetic trees were obtained using the minimum-evolution (Rzhetsky & Nei 1992) method in MEGA 7.0 (Kumar et al. 2016). In the minimum-evolution (ME) method, the evolutionary distances were computed using the Kimura 2-parameter method (Kimura 1980). The ME tree was searched using the close-neighbour-interchange (CNI) algorithm at a search level of 1 (Nei & Kumar 2000). The neighbor-joining algorithm was used to generate the initial tree. All 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 1000 replicates (Felsenstein 1985). The ITS sequences determined in this study were deposited in GenBank under the accession numbers MF668614 and MF668615.

RESULTS

In this study, several powdery mildew samples were examined from which two were new for Iran or Guilan province that are described here. Furthermore, some notes are provided for their morphology and ITS sequences.

Erysiphe magnifica (U. Braun) U. Braun & S. Takam., Schlechtendalia 4: 10 (2000) Fig. 1

Magnolia trees heavily infected with a powdery mildew fungus, forming patches or effuse mycelial growth on both sides of leaves. In small leaves, whole leaf was covered with mildew and leaf malformation was also observed. Hyphal cells 2–7 µm wide, hyphal appressoria single or in pairs, nipple-shaped or multilobed. Conidiophores arising from upper surface of mother cell, 45–200 µm long, foot cells cylindrical, straight, but mostly curved, flexuous or distinctly sinuous, 25–75 × 5–8.5 µm, followed by 1–2 shorter cells being clearly wider than foot cell. Conidia produced singly, ellipsoid, ovoid, doliform to ellipsoid-cylindrical, (27–)33–45(–50) × 14–18 µm, germ tube clearly terminal, usually short, sometimes very long, conidial appressoria simple or mostly multilobed.

Specimen examined. IRAN, Mazandaran province, Ramsar, on *Magnolia* sp., 6 July 2017, S. A. Khodaparast, GUM 775. GenBank accession number: MF668614, ITS.

Erysiphe corylacearum U. Braun & S. Takam., in Braun, Schlechtendalia 8: 33 (2002) Fig. 2

Mycelium on leaves, amphigenous, white, effuse, hyphal appressoria single or in pairs, lobed. Conidiophores arising from upper surface of mother cell, 54–160 µm long, foot cells cylindrical, straight, sometimes slightly sinuous, 40–70 × 7–9.5 µm, followed by 1–2 shorter cells. Conidia produced singly, variable in shape and size, ellipsoid-ovoid, doliiform, (27–)32–42 × 16–22 µm, germ tube terminal, usually short, to long, conidial appressoria simple or lobed.

Specimen examined. IRAN, Guilan province, Lahijan, on *Corylus avellana*, 11 May 2017, S. A. Khodaparast, Herbarium accession number: GUM 786, GenBank accession number: MF668615, ITS.

DISCUSSION

Magnolia powdery mildew in Iran, was found only in the asexual state in this study. This is not uncommon for powdery mildew species in Iran. Hence, some of powdery mildew fungi have only been identified based on the asexual stage and ITS sequences (Khodaparast et al. 2016). Identification of some powdery mildews is usually difficult or impossible without sexual stage.

Four species of *Erysiphe* sect. *Microsphaera viz*. *E. magnifica* (U. Braun) U. Braun & S. Takam., *E. magnoliae* (Sawada) U. Braun & S. Takam., *E. bulbosa* (U. Braun) U. Braun & S. Takam. (Braun and Cook 2012) and *E. magnoliicola* S.E. Cho, S. Takam. & H.D. Shin (Cho et al. 2014) have been recorded on *Magnolia* s.l. species. These species are well differentiated based on combined anamorphic and teleomorphic characters as well as ITS sequences (Cho et al. 2014). However, identification of *E. magnifica* and *E. magnoliicola* based on anamorph characteristic is difficult in practice. Cho et al. (2014) differentiated these two species based on foot cell length. According to their observations, the foot cell of conidiophores was relatively short in *E. magnifica* [(15–40 µm, up to 50 µm in Korean sample, according to Braun and Cook 2012)] versus long foot

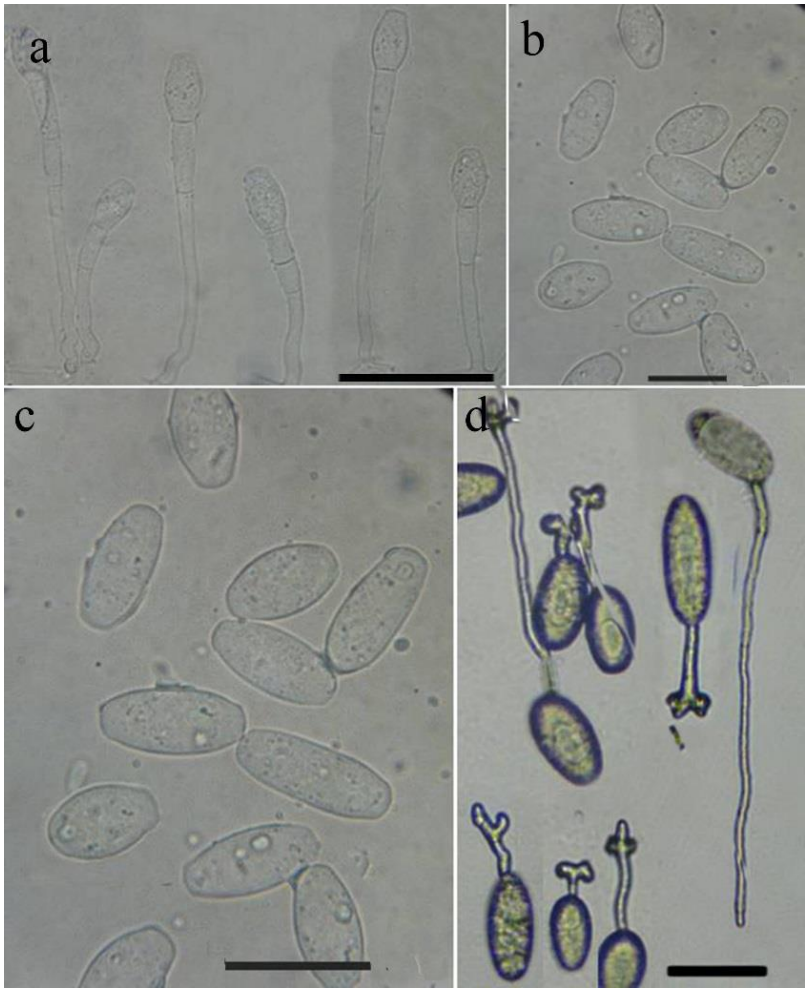

Fig. 1. *Erysiphe magnifica*. a. Conidiophores; b–c. Conidia; d. Germinated conidia. — Scale bars: a=50 µm, b–d=30 µm.

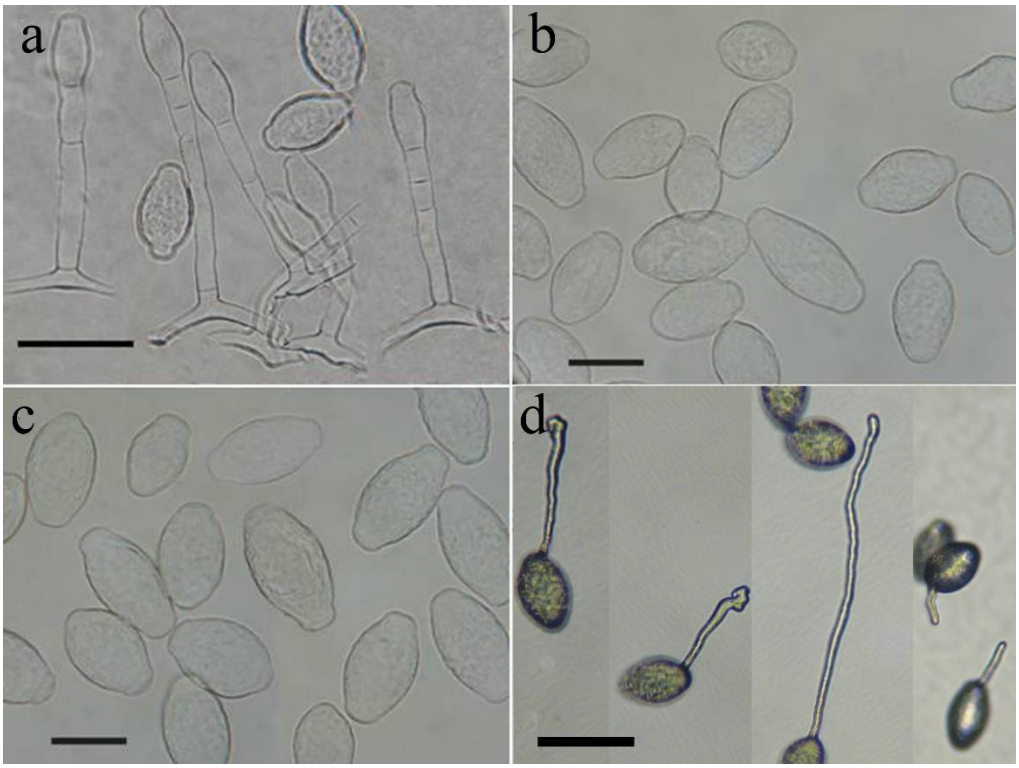

Fig. 2. *Erysiphe corylacearum*. a. Conidiophores; b–c. Conidia; d. Germinated conidia. — Scale bars: a =50 µm, b–c = 20 µm, d = 30 µm.

cell in *E. magnoliicola* (42.5–70 µm). However, the Iranian specimen showed foot cells as long as in *E. magnoliicola*. Nevertheless, the ITS sequence of the Iranian specimen was 100% identical to four ITS sequences of *E. magnifica* available in GenBank (from Argentina and Germany).

According to Braun & Cook (2012), two types of foot cell have been described for *E. magnifica*. In European and North American samples, foot cells are straight or slightly flexuous, while in the Korean sample, foot cell are distinctly sinuous. They stated that it was not clear whether these collections belonged to varieties of the same species or different taxa. Our specimen is well characterized with curved, flexuous or sinuous and relatively long foot cells. Dimorphism in conidiophores has been speculated to be dependent on physiological and ecological effects (Yarwood & Gardner 1970) or specific morphological varieties (Schmidt & Scholler 2006). Therefore, the length of the foot cells seems not to be a robust morphological marker for differentiation between *E. magnifica* and *E. magnoliicola*. According to these observations, for such difficult species, it is recommended to check as many morphological characters as possible and analyze ITS sequences.

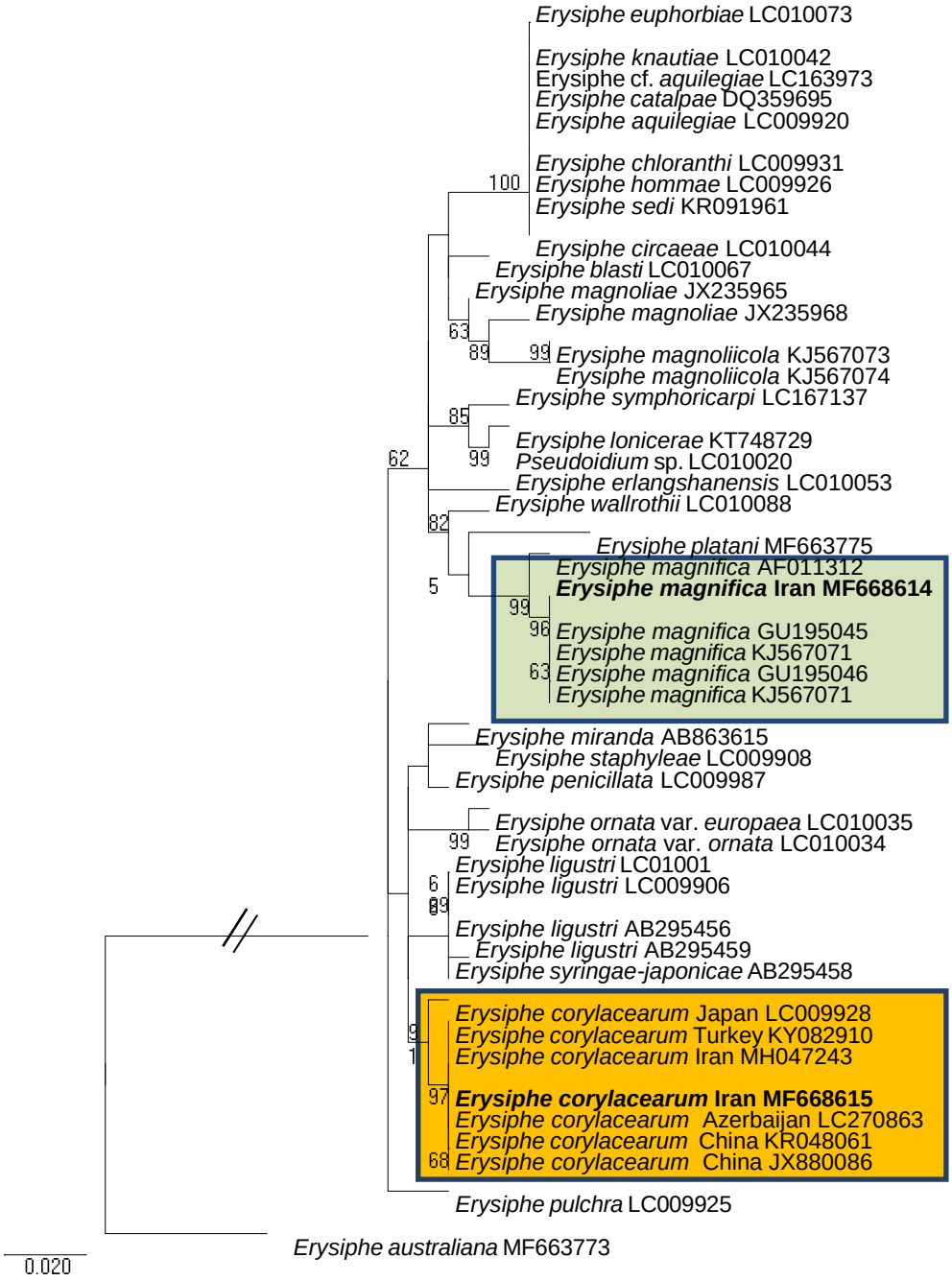

Fig. 3. A minimum-evolution (ME) tree (length = 0.49836697) based on ITS sequences of *Erysiphe* species. All ambiguous positions were removed for each sequence pair. Bootstrap support values > 50% (1000 replicates) are shown next to the nodes. The sequences generated in this study are shown in bold. *Erysiphe australiana* is outgroup.

There are more than 25 base substitutions on ITS sequences between *E. magnifica* (from this study) and *E. magnoliicola* (KJ567073, KJ567074) which are sufficient for species delimitation. This is the first report of *E. magnifica* from Iran.

Seven sequences are available for *E. corylacearum* in GenBank. Iranian specimens differed in 5 sites against a Japanese collection (LC009928) but were 100% identical to collections from Turkey and Azerbaijan (KY082910 and LC270863). However, one substitution and one deletion were found compared to two other sequences available in GenBank (JX880086 and KR048061), both from China. As shown in our phylogenetic analysis (Fig. 3), collections from Iran, Azerbaijan, Turkey and China were closely related to the Japanese specimen but clustered in a distinct clade.

The level of genetic diversity in the ITS sequences of the powdery mildew fungi requires precaution for taxonomic interpretation especially for species delimitation. Kovács et al. (2011) investigated this issue and showed that ITS sequences of some powdery mildew species may show 100% similarity. Moreover, small variation on ITS sequences from 1–5 nucleotide substitutions may occur within particular species such as *Golovinomyces orontii* species complex (Kovács et al. 2011). Braun et al. (2019) used one to few nucleotide differences in the ITS sequences for delimiting closely allied species in the *Golovinomyces orontii* species complex. Polymorphism in the ITS sequences of *E. corylacearum* is worth of attention for better understanding of the taxonomy of the species and role of ITS sequences for species delimitation.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Iran National Science Foundation.

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ملاحظاتی در مورد دو گونه قارچ عامل سفیدک پودری (*Erysiphe magnifica* و *E. corylacearum*) در ایران

سید اکبر خداپرست[1] ✉، علی قباخلو[1]، بیتا عسگری[2]، فائزه علی‌آبادی[2]، سپیده ساجدی[2]

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

۲- بخش تحقیقات رستنی‌ها، مؤسسه تحقیقات گیاه‌پزشکی کشور، سازمان تحقیقات، آموزش و ترویج کشاورزی، تهران، ایران

چکیده: دو گونه قارچ عامل سفیدک پودری از ایران جمع آوری شده است. برای اولین بار گونه *Erysiphe magnifica* از روی گیاه *Magnolia* sp. بر اساس ویژگی های ریخت‌شناختی و مولکولی تشخیص داده شد. گونه *Erysiphe corylacearum* برای دومین بار از کشور و اولین بار از استان گیلان جمع‌آوری شد. مقداری چند شکلی در توالی ITS-rDNA گونه *E. corylacearum* بین نمونه ایرانی و توالی‌های موجود در بانک ژن وجود دارد. این چندشکلی برای درک بهتر تاکسونومی این گونه و نقش توالی ITS-rDNA در تشخیص گونه ارزشمند است. توالی نمونه ایرانی *E. magnifica* ، ۱۰۰ درصد مشابه چهار توالی این گونه در بانک ژن بود. از نظر ریخت‌شناختی نمونه ایرانی *E. magnifica* با ویژگی هایی نظیر سلول پایه کنیدیوفور خمیده، انعطاف پذیر و نسبتا بلند توصیف شد، در حالی که سلول پایه کنیدیوفور در این گونه بر اساس منابع علمی نسبتا کوتاه است. براساس این مشاهدات، توصیه می شود گونه هایی که از نظر ریخت‌شناسی و مولکولی به هم نزدیک هستند، ویژگی های مختلف تا آنجا که ممکن است، مورد بررسی قرار گیرند.

واژه های کلیدی: بیوسیستماتیک، ریخت شناسی، چند شکلی DNA، راسته اریزیفالس، ماگنولیا

مکاتبه کننده: خداپرست Email: khodaparast@guilan.ac.ir

تاریخ دریافت: ۱۳۹۷/۱۰/۱۹ تاریخ پذیرش: ۱۳۹۸/۰۲/۳۱