Revisiting *Erysiphe magnoliae* with morphological and molecular data

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Erysiphe magnoliae known on *Magnolia obovata* is a powdery mildew hitherto considered to be native and endemic to Japan. The morphological characteristics of the species have been insufficiently known since its first description in 1951. Fresh samples of this species were collected on *M. obovata* for the first time in Korea where the species is introduced. Based on the Korean samples, both anamorph and teleomorph of this species are described and illustrated in detail. The inflated base of foot-cells in conidiophores is a unique character not found in the two related species, *E. magnifica* and *E. bulbosa*, known on *Magnolia* spp. The sequences of internal transcribed spacer (ITS) and 28S of ribosomal DNA obtained from Korean and Japanese samples confirmed the phylogenetic position of *E. magnoliae* in the section *Microsphaera* of the genus *Erysiphe*. A comparison of and a synoptic key to the three species of *Erysiphe* known on *Magnolia* spp. are provided.

Keywords: anamorph, *Magnolia*, *Microsphaera*, powdery mildew, taxonomy.

Three powdery mildew species belonging to *Erysiphe* sect. *Microsphaera* have been described on *Magnolia* spp. (Braun 1987, 1988; Braun & Cook 2012). *Erysiphe magnifica* (U. Braun) U. Braun & S. Takam. (syn. *Microsphaera magnifica* U. Braun) is characterized by having straight or flexuous foot-cells in conidiophores and regularly branched appendages with distinctly recurved tips (Braun 1987, Shin 2000). This species is known on several species of *Magnolia* from North America, South America and Asia, and recently from Europe (Braun *et al.* 2009, Braun & Cook 2012, Farr & Rossman 2012). Interestingly this species was found to be associated with a powdery mildew disease on *Nelumbo nucifera* Gaertn. in Germany (Kirschner 2010). Another species, *Erysiphe bulbosa* (U. Braun) U. Braun & S. Takam. (syn. *Microsphaera bulbosa* U. Braun), is morphologically close to *E. magnifica*, but differentiated from the latter species by having chasmothecial appendages with distinctly swollen base (Braun 1988). This is known to be endemic in China and recorded only on *Magnolia liliiflora* Desr. The third species, *Erysiphe*

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magnoliae (Sawada) U. Braun & S. Takam. (syn. *Microsphaera magnoliae* Sawada), is morphologically incompletely described and only known from *Magnolia obovata* Thunb. in Japan (Nomura 1997, Kobayashi 2007). The authors (SEC & HDS) collected both anamorph and teleomorph of *E. magnoliae* on *M. obovata* in 2010 and 2011 in Korea. Based on these Korean specimens, it is possible to supplement the knowledge about *E. magnoliae* with new morphological and molecular data.

Materials and methods

Collections used

In October 2010, a tree of *Magnolia obovata* in Suwon campus of Seoul National University (37°16'9"N, 126°59'23.08"E) was found to be heavily infected with a powdery mildew. In November 2010, some chasmothecia were formed on the powdery mildew colonies. The tree, fallen down by strong wind in 2009 summer, was cut down at the base of the trunk, but in summer 2010 many new sprouts were heavily infected with a powdery mildew. Interestingly, a dozen of trees of the same species nearby this tree were free from powdery mildew infections. In September 2011, about 20 trees of *M. obovata* planted in a public park in Osan city of Korea were found to be infected with an anamorphic powdery mildew, but later, during the first week of November, the leaves were covered with numerous chasmothecia. Representative specimens are deposited at the Korea University Herbarium (KUS), Seoul, Korea; and the Mie University Mycological Herbarium (MUMH), Tsu, Japan.

Morphological observations

The fungus was detached from the infected leaves and mounted in a few drops of distilled water on a glass slide for light microscopy. Morphological characteristics of the fungal structures in fresh samples were examined in bright field- and differential interference contrast (DIC)-light microscopy, using an Olympus BX51 microscope (Olympus, Tokyo, Japan) for measurements and a Zeiss AX10 microscope equipped with AxioCam MRc5 (Carl Zeiss, Göttingen, Germany) for photographs. Thirty measurements were performed at 100×, 200×, 400×, and 1000× magnifications.

Phylogenetic analyses

Genomic DNA was extracted from chasmothecia on the lower surface of the leaves using the chelex method (Walsh *et al.* 1991, Hirata & Takamatsu 1996). The 28S rDNA, including the domains D1 and D2, and internal transcribed spacers (ITS) region of the rDNA, including ITS1, 5.8S, and ITS2, were amplified by polymerase chain reaction (PCR) using protocols as described in Takamatsu & Kano (2001). The PCR products were purified using a Qiaquick Gel Extraction Kit (Qiagen, Hilden, Germany) and then,

sequenced on an ABI Prism TM 377 automatic DNA sequencer (Applied Biosystems, Foster City, CA, USA), using BigDye™ cycle sequencing kit version 3.1 (Applied Biosystems), with the same primers used as for PCR. The sequences were edited using the DNASTAR computer package version 5.05 (Lasergene, Madison, WI, USA) and resulting sequences were deposited in GenBank under the accession numbers of JX235964–JX235969. In order to construct an ITS phylogenetic tree, the data set included 24 taxa belonging to *Erysiphe* sect. *Erysiphe* (8 species) and *Erysiphe* sect. *Microsphaera* (12 species) retrieved from GenBank (http://www.ncbi.nlm.nih.gov/genbank/). *Erysiphe juglandis* (Golovin) U. Braun & S. Takam. was used as outgroup taxon based on Takamatsu *et al*. (1999). Phylogenetic analysis was carried out using Molecular Evolutionary Genetics Analysis Software (MEGA 5) (Tamura *et al*. 2011). A phylogenetic tree was constructed by using Maximum Likelihood method (ML) and the Tajima-Nei distance (Tajima & Nei 1984). The robustness of ML tree was evaluated by 1000 bootstrap replicates. The phylogenetic tree generated from ITS analysis was deposited in TreeBASE (http://www.treebase.org/) under the accession number of S13198.

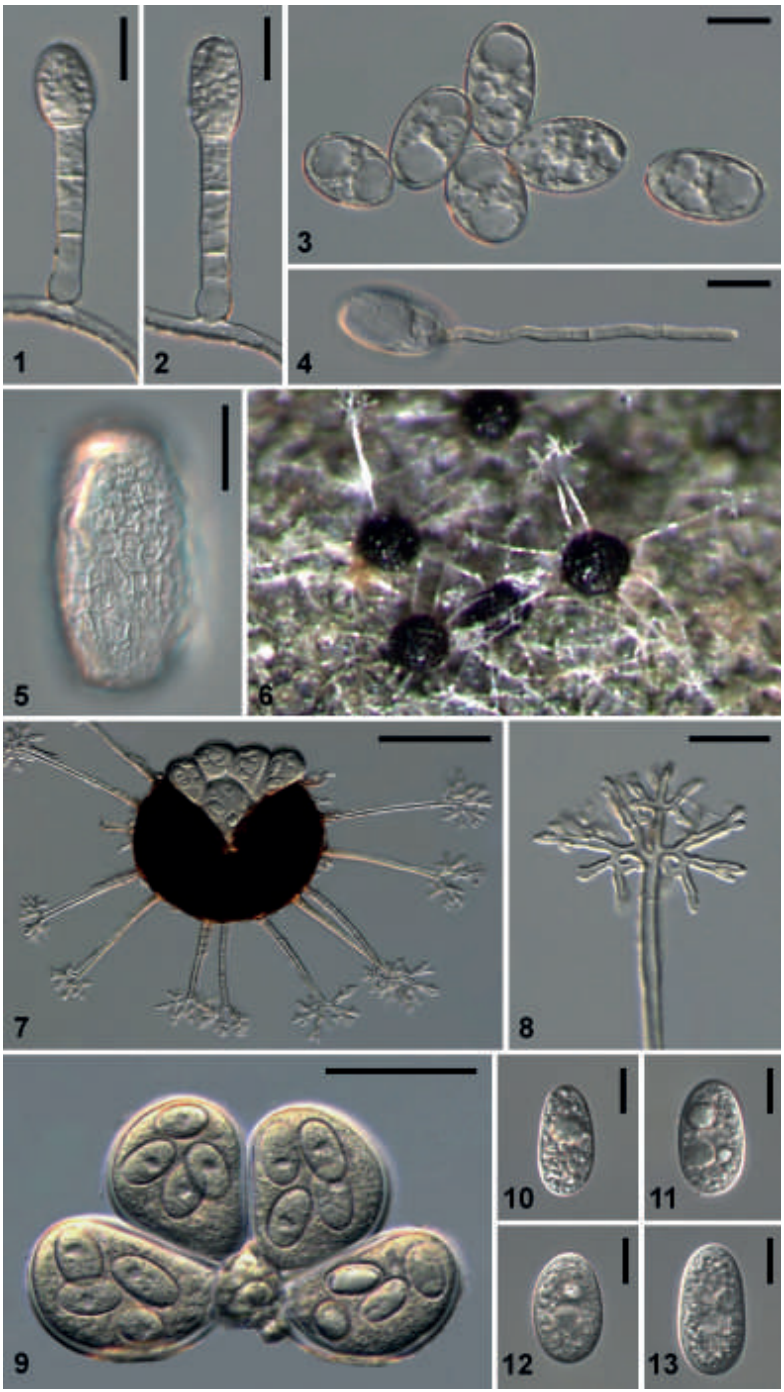
Results

Morphological characteristics

Mycelial colonies conspicuous, amphigenous, forming circular or irregular patches. Appressoria on the mycelia lobed, solitary or in opposite pairs. Conidiophores composed of 3–4 cells arising from the upper part of hyphae, 75–125 × 7.5–10 µm, producing conidia singly; foot-cells straight, short, somewhat inflated at the base, 15–25 µm (Figs. 1, 2). Primary conidia obovoid to ellipsoid, apically rounded, basally rounded to sub-truncate. Secondary conidia oblong to ellipsoid, 27–42 × 17.5–25 µm with a length/width ratio of 1.3–2.1, without fibrosin bodies (Fig. 3), and producing germ tubes in subterminal position (Fig. 4). The surface of the conidia with an angular/rectangular wrinkling pattern (Fig. 5). Chasmothecia amphigenous, mostly hypophyllous, scattered or partly clustered, dark brown, spherical, 90–120 µm in diameter, with 4–8 asci (Figs. 6, 7). Peridium cells irregularly polygonal to rounded, 8–20 µm wide. Appendages 4–6 times dichotomously branched, aseptate, 6–12 per chasmothecium, 120–180 × 5–9 µm, 1.2–1.8 times as long as the chasmothecial diam. and brown at the base, becoming paler towards the tip (Fig. 8). Asci ellipsoid to ovoid, saccate to short-stalked, 50–70 × 35–45 µm, with 3–4 ascospores (Fig. 9). Ascospores ellipsoid-ovoid to oblong-ellipsoid, 25–32 × 12–15.5 µm with a large length/width ratio of 1.7–2.6 and filled with numerous oil drops (Figs. 10–13).

Phylogenetic analyses

Based on the molecular phylogeny generated from the ML analyses of ITS and 28S rDNA regions, the three sequences of *E. magnoliae* from Korean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and Japanese isolates form an independent clade with strong bootstrap supports (86 % and 96 % in ITS and 28S trees; Figs. 14, 15). The status of *E. magnoliae* as a separate species is confirmed by the present phylogenetic analyses.

Discussion

Magnolia obovata is native to Japan and the adjacent Kurile Islands of Russia, and was introduced into Korea in 1920s (Choi 2009). The tree is one of the most common ornamental trees in gardens and parks. Nevertheless, no powdery mildew was recorded on this tree species in the monographic study published by Shin (2000), which was a result of 16 years' extensive foray in Korea. This suggests that *E. magnoliae* seems to be introduced recently from Japan.

Salmon (1908) described a first collection on *M. obovata* and referred it to *Microsphaera diffusa* Cooke & Peck, and Homma (1937) followed Salmon's wide species concept. *Microsphaera magnoliae* was introduced as a new species for "*Microsphaera diffusa*" on *Magnolia obovata* by Sawada (1951), but the anamorph of this fungus was not described in detail. Nomura (1997) first described and illustrated the holomorph of the fungus, but its conidiophores and ascospores were poorly characterized. Now, this species belongs to section *Microsphaera* of the genus *Erysiphe* as *E. magnoliae* (Sawada) U. Braun & S. Takam. (Braun & Takamatsu 2000). In this study, we found morphological characteristics which have not been described previously. This species is clearly distinguished from *M. diffusa* by having conidiophores with short, basally inflated foot-cells (cf. Nomura 1997). The ascospores have a large length/width ratio (1.7–2.6) and are filled with numerous oil drops. These are novel morphological characters of taxonomic value found in *E. magnoliae*. Phylogenetic analyses showed that *E. magnoliae* on *M. ovobata* forms an independent lineage separated from *E. magnifica* on *M. liliiflora* (AF011312, GU195046) and on *Nelumbo nucifera* (GU195045). Therefore, the status of *E. magnoliae* as a separate species was confirmed by phylogenetic analyses. *Erysiphe magnifica* was previously known to be associated with *Magnolia* spp. Interestingly Kirschner (2010) found *E. magnifica* on *Nelumbo nucifera* as powdery mildew and confirmed it on the basis of morphological characters and molecular data. It was the first record of *E. magnifica* on a host outside the *Magnoliales*. The anamorph of *E. bulbosa* is still unknown. Molecular analysis of this species has never been done. Therefore morphological and molecular data regarding *E. bulbosa* should be supplemented for a bet-

Fig. 1. Powdery mildew disease of *Magnolia obovata* associated with *Erysiphe magnoliae*: **1–2.** Conidiophores (bars: 20 µm). **3.** Conidia. **4.** A conidium in germination. **5.** Surface view of a conidium showing angular/rectangular wrinkling pattern (bars 3–5: 10 µm). **6.** Formation of chasmothecia on the abaxial leaf surface. **7.** Chasmothecium accommodating several asci (bar: 100 µm). **8.** Appendage (bar: 20 µm). **9.** Asci containing 4 ascospores each (bar: 50 µm). **10–13.** Ascospores containing numerous oil drops (bars: 10 µm).

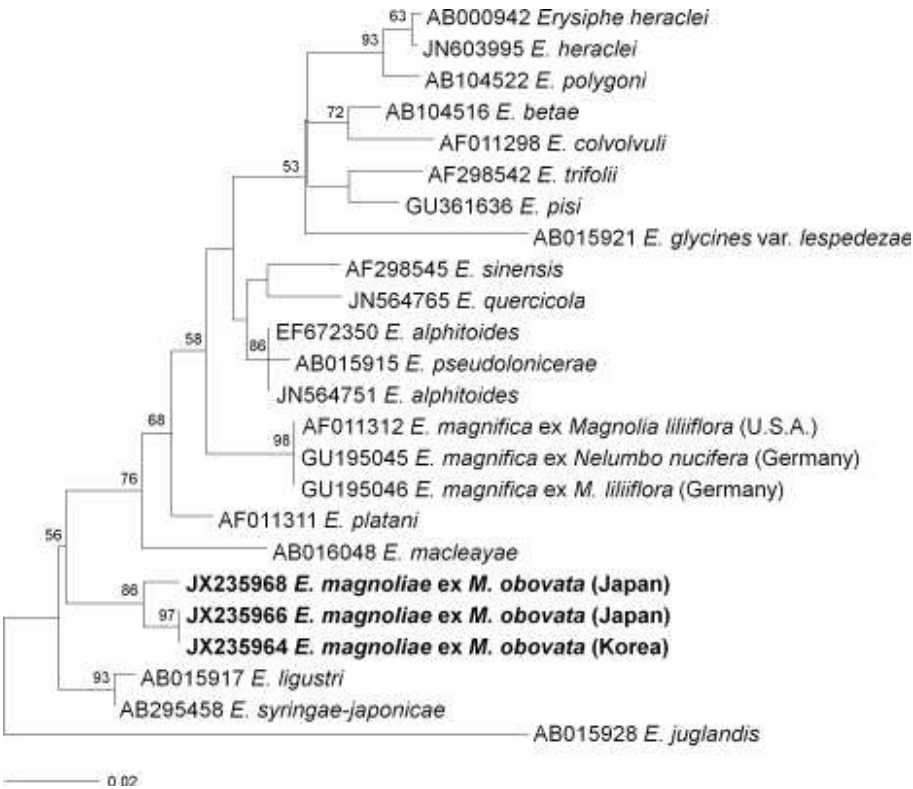

Fig. 14. Phylogenetic relationship between *Erysiphe magnoliae* isolates and some reference isolates retrieved from GenBank, inferred by Maximum likelihood method using the ITS rDNA region. Numbers above the branches represent the bootstrap values of over 50 % obtained from 1000 bootstrap replicates. Bar = Number of nucleotide substitutions per site.

ter understanding of *Magnolia-Erysiphe* associations. Until now, powdery mildew caused by *E. magnoliae* was recorded only on *M. obovata* in Japan (Braun & Cook 2012). In this study, both the anamorphic and teleomorphic state of *E. magnoliae* on *M. obovata* are described in detail and DNA sequences of the 28S and ITS regions of rDNA from the fungus are determined for the first time.

Based on the information in literatures and by the present study, we can provide a synoptic key for the three species of *Erysiphe* on *Magnolia* as follows;

Key to powdery mildew fungi belonging to *Erysiphe* sect. *Microsphaera* on *Magnolia* spp.

1. Chasmothecial appendages distinctly swollen at the base..... *E. bulbosa*
1*. Chasmothecial appendages not swollen at the base 2

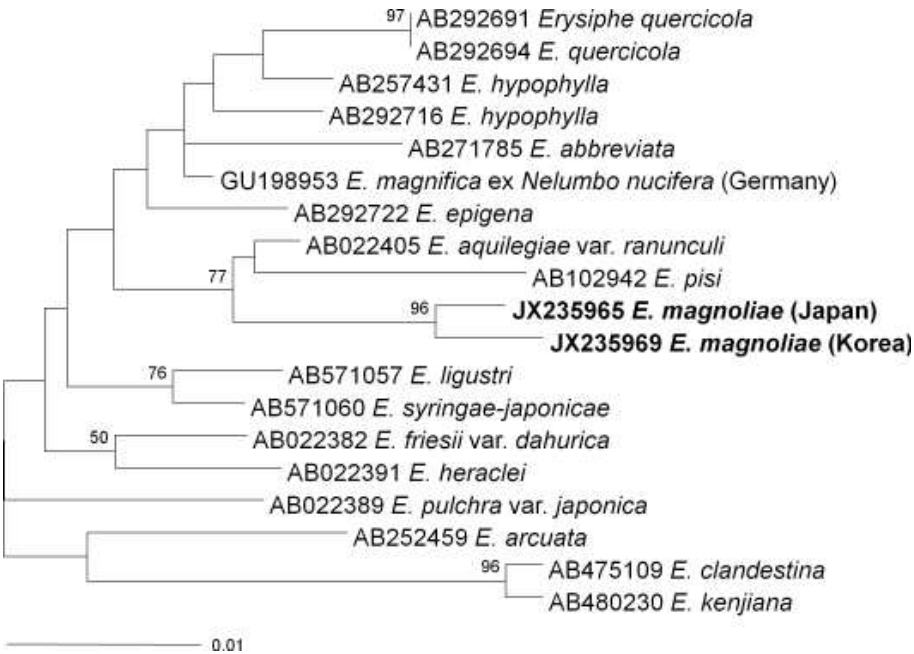

Fig. 15. Phylogenetic relationship between *Erysiphe magnoliae* isolates and some reference isolates retrieved from GenBank, inferred by Maximum likelihood method using the 28S rDNA region. Numbers above the branches represent the bootstrap values of over 50 % obtained from 1000 bootstrap replicates. Bar = Number of nucleotide substitutions per site.

2. Foot-cells of conidiophores relatively long (15–40 µm), straight to slightly sinuous at the base; chasmothecial appendages 0.5–1.5 times as long as the chasmothecial diameter ..... *E. magnifica*

2*. Foot-cells of conidiophores relatively short (15–25 µm), inflated at the base; chasmothecial appendages 1.2–1.8 times as long as the chasmothecial diameter ..... *E. magnoliae*

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