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RESEARCH NOTE

Erysiphe magnoliicola Comprises the Powdery Mildew Found on *Magnolia kobus* in Korea

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ABSTRACT

The powdery mildew found on *Magnolia kobus* was recorded as *Microsphaera alni* for the first time in Korea in 1975. After splitting *M. alni* into several distinct species, this mildew was regarded as *Microsphaera magnifica*, now *Erysiphe magnifica*. Since *E. magnifica* is known to be a North American species, the powdery mildew found on *M. kobus* in Korea was studied to clarify its identity. Based on morphological characteristics and sequencing results of the internal transcribed spacer and large subunit rDNA gene, the powdery mildew found on *M. kobus* in Korea was identified as *Erysiphe magnoliicola*.

Keywords: *Erysiphe magnifica*, *Erysiphe magnoliae*, kobus magnolia, Magnoliaceae

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Magnolia L. species comprise well-known deciduous trees with high decorative value, naturally growing in Asia, America, and the West Indies. Among them, *M. kobus* DC., known as kobus magnolia or kobushi magnolia, is native to Japan and Korea. The forests of Jeju island of Korea consist of a natural habitat for this plant [1]. Hundreds of these trees are cultivated predominantly in arboretums and botanical gardens in temperate regions, mainly in the southern part of the Korean Peninsula. Considering the narrow distribution of *M. kobus*, it is understandable that concrete information on powdery mildew infection of this plant in Korea is lacking. Hence, this issue prompted further research on *Magnolia-Erysiphe* associations in Korea [2,3].

Historically, Lee [4] was the first in Korea to report *Microsphaera alni* (DC.) G. Winter sensu lato as the powdery mildew on *M. kobus*. Later, Shin [5] listed this record under *Microsphaera magnifica* U. Braun (now *Erysiphe magnifica* (U. Braun) U. Braun & S. Takam.), which was regarded as the only accepted species name for powdery mildew found on *Magnolia* spp. at that time. Nevertheless, Lee's record in 1975 provided neither morphological characteristics of taxonomic value nor information about preserved herbarium material, which makes it impossible to confirm this record.

Later, Cho et al. [3] introduced a new species, *Erysiphe magnoliicola* S.E. Cho, S. Takam. & H.D. Shin, and revealed that *E. magnoliae* (Sawada) U. Braun & S. Takam. and *E. magnoliicola* are causative agents of powdery mildew diseases on *Magnolia* spp. in Japan and Korea, suggesting that *E. magnifica* is a North American species that is absent in East Asia. Moreover, they noted that the presence of *E. magnifica* on

M. kobus in Korea is obscure. Therefore, the identity of powdery mildew on *M. kobus* should thus be examined and reconsidered with morphological and molecular data.

In this study, during field forays to the arboretums and botanical gardens in the southern part of Korea, three samples of *M. kobus* infected by powdery mildew were collected and preserved in the Korea University herbarium (KUS). They were named KUS-F27350 (12 Jul 2013, Busan, Daejeo-dong), F30020 (14 Sep 2017, Jeju, Halla Ecological Forest), and F32686 (29 Nov 2021, Wando, Wando Arboretum). Detailed morphological characteristics of the powdery mildew were observed using an Olympus BX50 microscope (Olympus, Tokyo, Japan). Photomicrographs were taken with a Zeiss AX10 microscope equipped with an AxioCam MRc5 camera (Carl Zeiss, Oberkochen, Germany).

Morphological characteristics were as follows. Hyphae were straight or wavy, hyaline or almost hyaline, septate, branched, and 4-7 μm wide. Hyphal appressoria were well-developed, moderately lobed to multi-lobed, and solitary or in opposite pairs. Conidiophores were 86-120×8-10 μm, producing conidia solitary, and followed by 2-3 cells. Foot-cells of conidiophores were mostly or almost straight, relatively long, and 40-62 μm (Fig. 1A-C). Conidia were hyaline, ellipsoidal to oval, and 32-42(-46)×15-20 μm, with a length/width ratio of 1.8-2.4 (Fig. 1D). They lacked fibrosin bodies and produced germ tubes at the perihilar position (Fig. 1E). Chasmothecia were amphigenous, cauligenous, scattered to gregarious, dark brown, spherical, and 96-132 μm in diameter, and contained 4-7 asci per chasmothecium (Fig. 1F-I). Chasmothecial appendages were equatorial, 6-18 in number, mostly aseptate or uniseptate, 4-5 times dichotomously branched, 0.8-1.2 times as long as the chasmothecial diameter, 76-110×7-9 μm, with distinctly recurved tips, and brown at the base or hyaline throughout (Fig. 1H and I). Asci were obovoid, saccate, short-stalked, and 56-72×40-48 μm and contained 4-6 spores per ascus (Fig. 1I). Ascospores were hyaline to subhyaline, oblong-ovoid, and 22-30×12-14 μm (Fig. 1I).

Among three species of *Erysiphe* known to inhabit on *Magnolia* spp., *E. magnoliae* has conidiophores with a bulbose base [2] and thus is not related to our fungus. The remaining two species, *E. magnifica* and *E. magnoliicola*, share some common characteristics in terms of the morphology of chasmothecia [6]. However, *E. magnifica* differs from *E. magnoliicola* by its shorter conidiophores (up to 100 μm vs. 85-137 μm) and shorter foot-cells (15-45 μm vs. 42-70 μm). Therefore, the description of our fungus is in well agreement with that of *E. magnoliicola*.

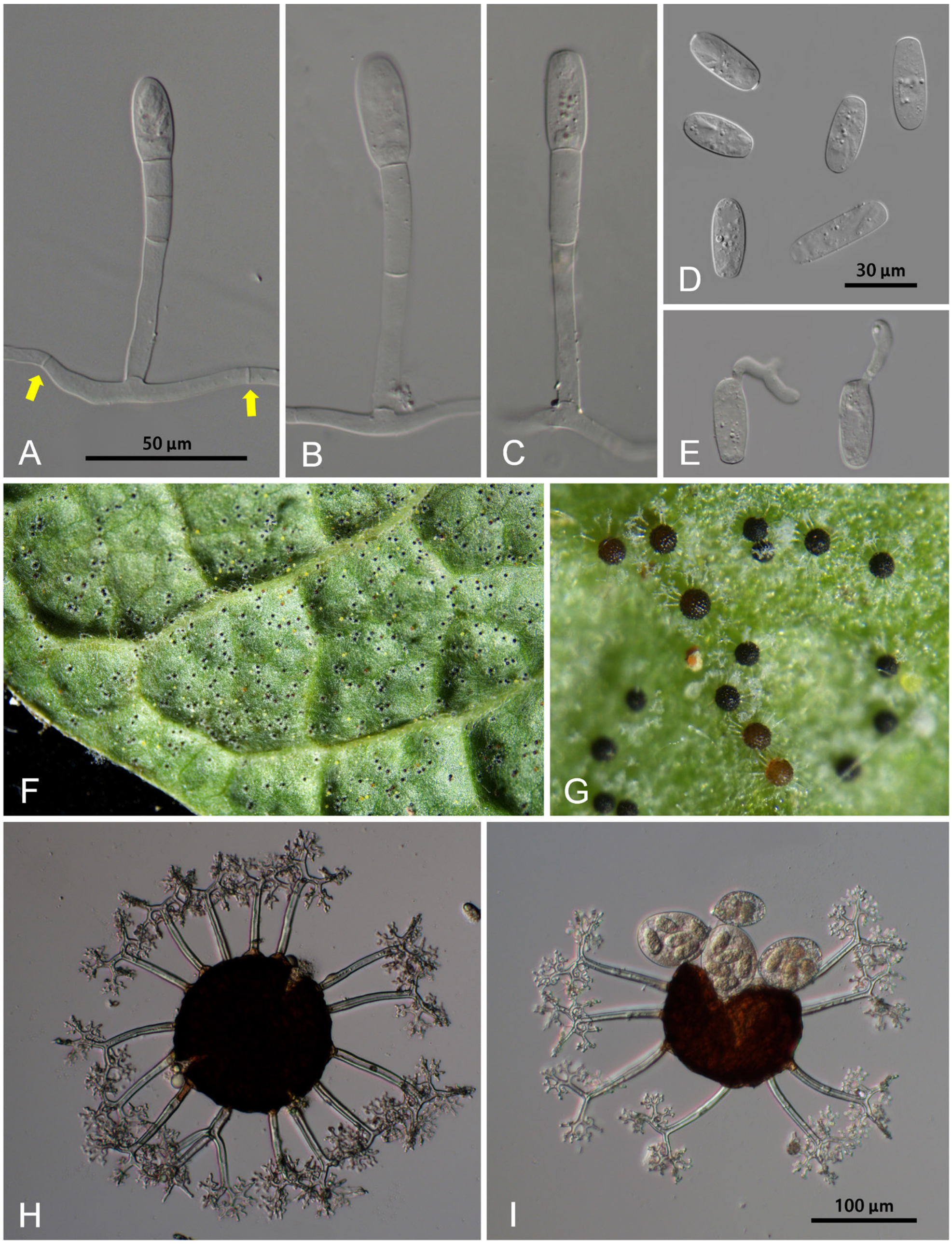

Fig. 1. *Erysiphe magnoliicola*, a powdery mildew found on *Magnolia kobus*. A-C: Conidiophores. Note the hyphal septa, indicated by yellow arrows. All images are presented at the same magnification. D: Conidia. E: Conidia in germination. D and E comprise the same magnification. F: Numerous chasmothecia formed on the lower leaf surface. G: Close-up view of chasmothecia. H: Chasmothecium with 15 appendages. I: Chasmothecium with asci and ascospores. H and I are presented at the same magnification.

To confirm the morphology-based identification, DNA extraction and polymerase chain reaction (PCR) was performed according to Choi et al. [6] using ITS1F/PM6 and PM3/NLP2 primer sets for the amplification of internal transcribed spacers (ITS) and large subunit (LSU) regions of rDNA, respectively.

PCR products were sent to Macrogen Inc. (Seoul, Korea) for sequencing. The resulting sequences were assembled in MEGA11 and deposited into GenBank under the following accession numbers: OM535821 and OM535822 for ITS; OM535823, OM535824, and OM535825 for LSU. A comparison of obtained sequences from the BLASTn search showed 99.8% similarity with sequences of *E. magnoliicola* (KJ567072) for ITS and 99.25% similarity with *E. magnoliicola* (KJ567067) and 98.2% with *E. magnoliae* (JX235969) for LSU. For the phylogenetic analysis, two datasets for ITS and LSU were prepared separately in MEGA11 and aligned using the MUSCLE command [7]. The first alignment included only the ITS regions of 21 closely related sequences, whereas the second analysis of the LSU dataset consisted of 18 sequences of the genus *Erysiphe*. *Erysiphe nothofagi* was selected as an outgroup according to Choi et al. [6]. Phylogenetic trees were generated in PAUP* 4.0.b using the maximum parsimony method [8]. The strength of the internal branches in the resulting trees was tested by bootstrap (BS) analysis using 1,000 replications. In both trees, sequences obtained in this study were placed in the distinct clade of *E. magnoliicola*, which was supported by the highest BS values (Fig. 2).

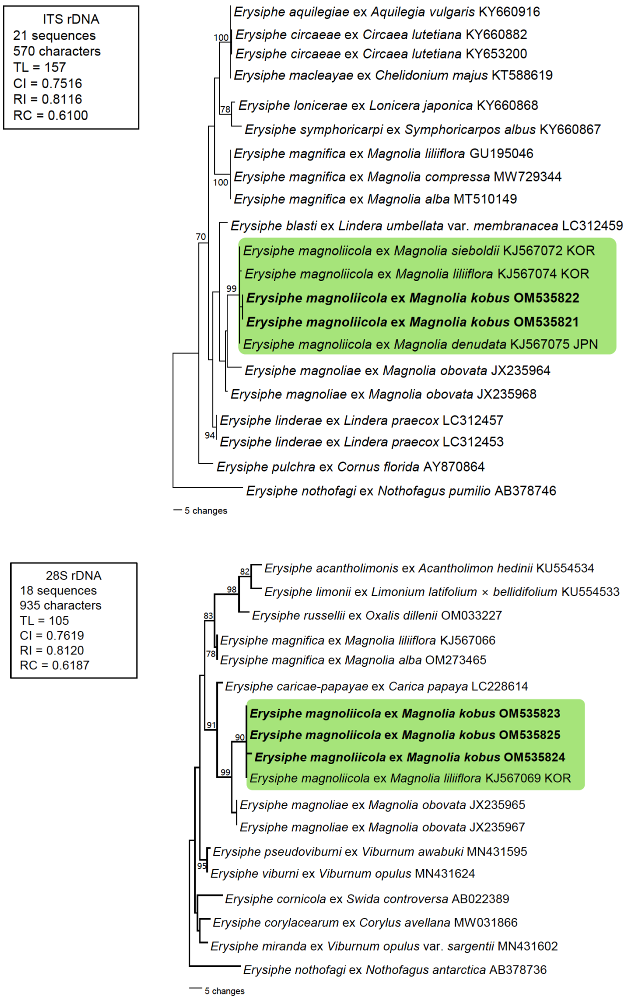

Fig. 2. Maximum parsimony trees of the *Erysiphe magnoliicola* based on the sequences of ITS (A) and LSU (B) regions of the rDNA. The isolates obtained in this study are shown in bold. Bootstrap values (>70%) are indicated on related branches.

Based on morphological data and molecular analysis of the three samples, the powdery mildew found on *M. kobus* in Korea was determined to be *E. magnoliicola*. This is the first study to document *E. magnoliicola* as a powdery mildew found on *M. kobus*. *E. magnifica* had been known as a North American species found on *Magnolia* spp. [9,10], but recently, this fungus started its geographical expansion to Europe (Germany and Switzerland, 2009; the Netherlands, 2009; Italy, 2011; the United Kingdom, 2011; Ukraine, 2012; and Slovakia, 2014) and Northeast Asia (Taiwan, 2020; Korea, 2021) [6,11,12]. In Korea, Choi et al. [6] recorded *E. magnifica* for the first time on *M. compressa*, which is an endangered indigenous species here.

Such recent studies on the diversity of powdery mildews on *Magnolia* species suggest global expansion of the three species of *Erysiphe*, possibly through the introduction of ornamental magnolia species. Therefore, the continuous monitoring of powdery mildew diversity on *Magnolia* plants should be considered. The diversity of powdery mildews on indigenous and introduced species of *Magnolia* would be helpful to *Magnolia* breeders and the associated breeding programs.

CONFLICT OF INTERESTS

No conflict of interest was reported by the author(s).

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