

RESEARCH NOTE

Morphology and Phylogeny of *Erysiphe euonymicola* on *Euonymus* Species in Korea

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ABSTRACT

Five species of *Erysiphe* have been described globally as powdery mildew fungi on *Euonymus* species. In Korea, *Erysiphe euonymicola* was recorded as powdery mildew on *Euonymus japonicus* and *Eu. fortunei* var. *radicans*. To elucidate the identity of *Euonymus* powdery mildew in Korea and to provide detailed morphological and molecular data for the powdery mildew occurring on *Euonymus* species in Korea, 41 available specimens were examined morphologically, and seven representative specimens were analyzed using nucleotide sequences of the internal transcribed spacer regions (ITS1 and ITS2) and the large subunit gene (LSU) of the rDNA. The DNA was extracted from fungal colonies on the leaves of *Eu. japonicus* and *Eu. fortunei* var. *radicans*, and from fruits of *Eu. japonicus*. Morphological and phylogenetic analyses confirmed all specimens as *E. euonymicola*, including one collected from infected fruits of *Eu. japonicus*. This study provides information on the anamorphic characteristics of *E. euonymicola* and documents its occurrence on the fruit of *Eu. japonicus*.

Keywords: Conidial germination, Evergreen spindletree, Molecular phylogeny, *Oidium euonymi-japonici*, *Pseudoidium euonymi-japonici*

Powdery mildew fungi that occur on *Euonymus* species are currently classified into five species [1]. Among them, the two major species, *Erysiphe euonymi* and *E. euonymicola*, are closely related but distinct, differing mainly in host association and morphological characteristics [2,3]. *Erysiphe euonymi* occurs mainly on deciduous *Euonymus* species, whereas *E. euonymicola* is associated with evergreen *Euonymus* species, especially *Eu. japonicus* Thunb. and *Eu. fortunei* var. *radicans* Rehder [1,2]. The latter *Erysiphe* species usually occurs as an anamorph, and chasmothecia are rarely observed, which has contributed to its complicated taxonomic history.

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The powdery mildew of *Eu. japonicus*, in particular, has been treated under multiple taxonomic concepts [4]. It was originally described as *Oidium euonymi-japonici* and has subsequently been treated under several synonyms, including *Microsphaera euonymi-japonici* and *E. euonymi-japonici* [4]. However, Braun and Cook [2] noted that the common anamorph on *Eu. japonicus* had never been conclusively linked to the teleomorph described as *M. euonymi-japonici*. They, therefore, introduced *E. euonymicola* as a distinct species based on anamorphic and teleomorphic material collected from *Eu. japonicus* [2].

In Korea, powdery mildews on *Eu. japonicus* have been recorded under the names *O. euonymi-japonici* and *E. euonymi-japonici* [5–7], but both names are now regarded as taxonomic synonyms of *E. euonymicola* [2]. In addition, powdery mildews associated with *Eu. fortunei* var. *radicans* have been recorded as *E. euonymicola* based on morphological characteristics and internal transcribed spacer (ITS) sequence data [8]. However, these previous identifications were all based on the characteristics of the anamorph, and while *E. euonymicola* is distributed on evergreen *Euonymus* species worldwide, several aspects of its anamorphic morphology remain poorly documented. Specifically, information on primary conidia, conidial germination patterns, and the affected plant organs is limited. Therefore, the present study aimed to re-examine the identities of the powdery mildews on *Eu. japonicus* and *Eu. fortunei* var. *radicans* based on the current taxonomic framework and to provide additional information on the anamorph characteristics of *E. euonymicola*.

A total of 41 powdery mildew specimens, 35 from *Eu. japonicus* and 6 from *Eu. fortunei* var. *radicans*, were examined for morphological investigation. All of the specimens were collected throughout Korea between 1989 and 2025 and are preserved in the Korea University Herbarium (KUS). Among the 41 specimens, which were each examined morphologically, seven well-preserved specimens were selected for molecular analyses, with preference given to specimens collected from different localities. These specimens are listed in Table 1.

Table 1. Powdery mildew specimens from *Euonymus* species used for molecular analyses in this study

Specimen number	Host	Infected organ	Locality	Collection date	GenBank accession number	
					ITS	LSU
KUS-F31502	<i>Euonymus japonicus</i>	Leaves	Jeju	19-Nov-2019	PZ542729	PZ542737
KUS-F31636	<i>Euonymus japonicus</i>	Leaves	Seoul	02-May-2020	PZ542731	PZ542740
KUS-F32768	<i>Euonymus japonicus</i>	Leaves	Jeonju	28-Apr-2022	PZ542732	PZ542742
KUS-F33267	<i>Euonymus japonicus</i>	Fruits	Jeonju	24-Sep-2022	PZ542734	PZ542744
KUS-F27836	<i>Euonymus fortunei</i> var. <i>radicans</i>	Leaves	Seoul	07-Jun-2014	PZ545116	PZ542768
KUS-F28747	<i>Euonymus fortunei</i> var. <i>radicans</i>	Leaves	Seoul	28-Jul-2015	PZ545117	PZ542748
KUS-F29160	<i>Euonymus fortunei</i> var. <i>radicans</i>	Leaves	Seoul	20-May-2016	PZ542738	PZ542745

ITS: internal transcribed spacer; LSU: large subunit; KUS-F: Fungal specimens of Korea University Herbarium.

Powdery mildew symptoms were commonly observed on the leaves and young stems of *Eu. japonicus* and *Eu. fortunei* var. *radicans* (Fig. 1A). Colonies developed on infected leaves, with chlorotic spots often forming on the adaxial surface when colonies were abundant on the abaxial surface (Fig. 1B). When infections were severe, dense mycelial growth covered the entire leaf, resulting in distorted and malformed leaves (Fig. 1C and D). Notably, powdery mildew was also observed on the fruits of *Eu. japonicus*, and the fruits were frequently covered with mycelia (Fig. 1E and F).



Fig. 1. *Erysiphe euonymicola*, a powdery mildew fungus found on *Euonymus japonicus*. A: Powdery mildew symptoms on leaves. B: Chlorotic spots formed on the adaxial surface. C: Dense mycelial growth covering entire leaves. D: Leaf distortion and malformation caused by severe infection. E: Powdery mildew infections on fruits. F: Close-up view of infected fruits. G–I: Appressoria on the hypha. J–L: Conidiophores. Note the mostly flexuous foot-cells (arrows). M: Conidiophore bearing a primary conidium (arrow). N: Diversity of conidia. O, P: Primary conidia. Q: Conidia in germination.

For morphological characterization, mycelia and conidia were removed from the infected tissue using a sterile scalpel, mounted in distilled water, and examined using an Olympus BX53-32XDIC light microscope equipped with an Olympus DP74 digital camera (Olympus, Tokyo, Japan). For each morphological feature, measurements were based on at least 30 structures. Conidial germination patterns were examined following the method of Sivapalan [9], with conidia suspended in distilled water.

Hyphae were straight to flexuous, measuring 4–5 μm wide. Hyphal appressoria were lobed to multilobed, occurring singly or in opposite pairs, and measured 7–12 μm wide (Fig. 1G–I). Conidiophores were erect, cylindrical, and 62–83 μm long, arising from the upper surface of the mother cell. Foot-cells were cylindrical and mostly flexuous, measuring $17\text{--}27 \times 8\text{--}9 \mu\text{m}$, and they were followed by 1–2 shorter cells (Fig. 1J–M). The basal septum was consistently located at the branching point (Fig. 1J–L). Conidia were produced singly and lacked distinct fibrosin bodies (Fig. 1N). Primary conidia were ellipsoidal, measuring $32\text{--}35 \times 15\text{--}18 \mu\text{m}$, with rounded apices and subtruncate bases (Fig. 1O and P). Secondary conidia were cylindric-ellipsoidal to oblong-elliptical, measuring $34\text{--}44 \times 13\text{--}18 \mu\text{m}$. The germ tube arose from a subterminal position on the conidium and terminated in a lobed appressorium, hook-shaped apex, or simple and rounded end. Occasionally, two germ tubes arose from a single conidium (Fig. 1Q). Although specimens were collected over multiple years and across a broad seasonal range (May–December), chasmothecia were not observed in any of the materials examined. The observed morphological characteristics corresponded well with previous descriptions of *Erysiphe euonymicola* U. Braun [2].

To confirm the morphology-based identification and clarify the phylogenetic relationships of powdery mildew fungi infecting *Euonymus* species in Korea, genomic DNA was extracted from seven representative specimens: three collected from *Eu. japonicus* leaves (KUS-F31502, F31636, and F32768), one from *Eu. japonicus* fruits (KUS-F33267), and three from *Eu. fortunei* var. *radicans* leaves (KUS-F27836, F28747, and F29160). The nucleotide sequences of the ITS regions and the large subunit (LSU) gene of the rDNA were amplified using the primer pairs ITS1-F/PM6 [10] and PM3/TW14 [11], respectively. Sequencing was performed by Cosmogenetech (Seoul, Korea), and the resulting sequences were deposited in GenBank under the accession numbers listed in Table 1. Sequence alignment and editing were conducted using the MUSCLE algorithm implemented in MEGA11 [12]. Phylogenetic relationships were inferred using the maximum parsimony method in PAUP* 4.0a, with heuristic searches performed through the tree-bisection-reconnection algorithm [13]. All sites were treated as unordered and unweighted, and gaps were treated as missing data. A bootstrap analysis with 1,000 resamples was conducted to evaluate branch support. *Arthrocladiella mougeotii* HMJAU-PM91837 and *Golovinomyces latissporus* KUS-F30175 were selected as outgroup taxa [14]. BLASTn searches showed high sequence similarity to both *E. euonymicola* and *E. alphitoides*. Separate phylogenetic analyses of the ITS and LSU datasets showed no conflict in the placement of the Korean specimens, which grouped with reference sequences of *E. euonymicola* in both analyses. The final dataset consisted of 38 combined ITS+LSU sequences with 1,356 characters. In the resulting maximum parsimony tree, all Korean specimens formed a single clade with the reference *E. euonymicola* sequences from Japan and Taiwan, and the clade was clearly separated from *E. alphitoides*.

and the other *Erysiphe* species (Fig. 2). The *E. euonymicola* clade received 93% bootstrap support, whereas the subclade containing all Korean specimens was supported by a bootstrap value of 86%. These phylogenetic results support the placement of all powdery mildew occurring on *Eu. japonicus* and *Eu. fortunei* var. *radicans* in Korea into a single species, *E. euonymicola*.

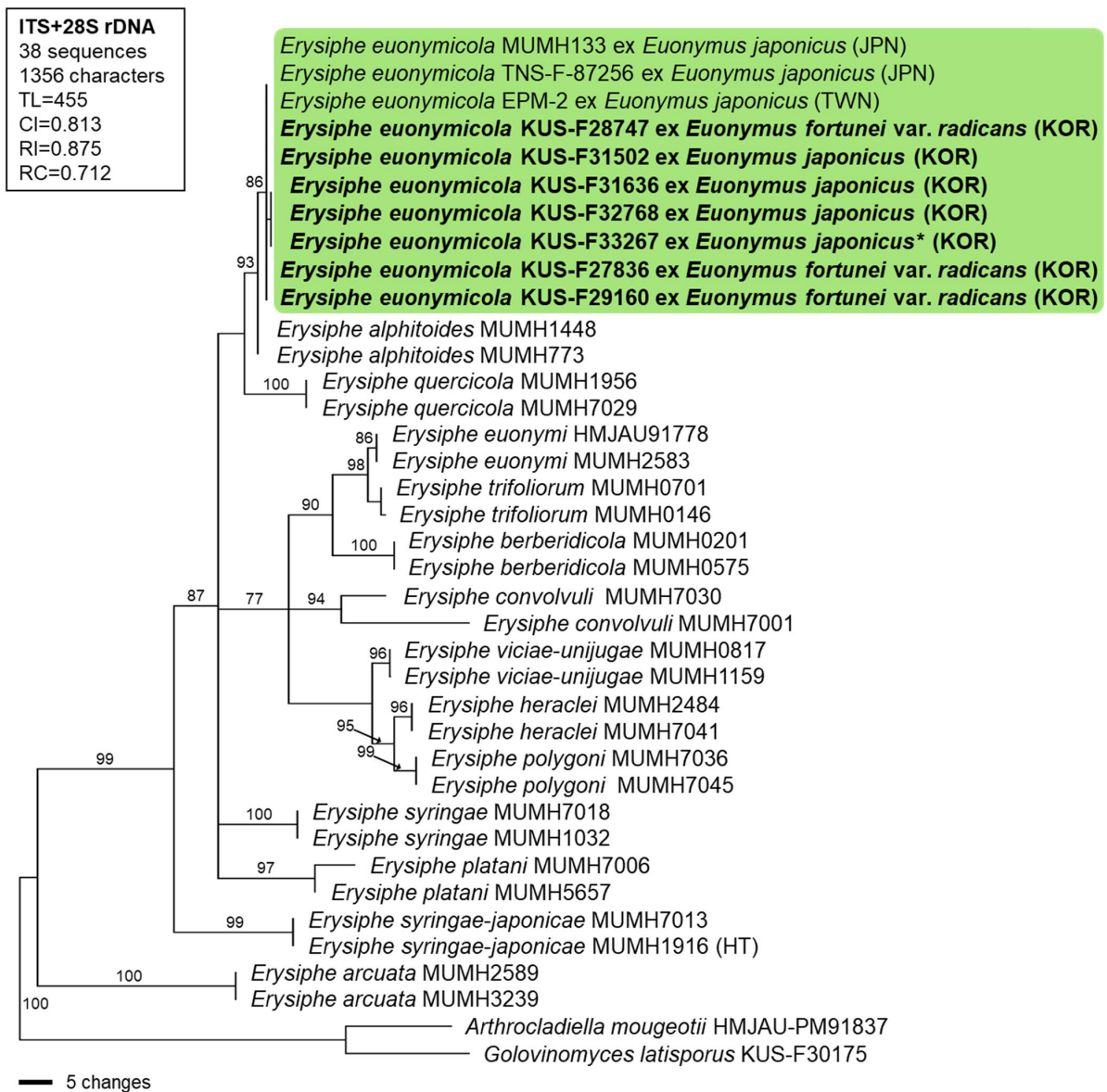


Fig. 2. Maximum parsimony phylogenetic tree inferred from combined ITS and LSU rDNA sequences showing the relationships of Korean powdery mildew specimens collected from *Euonymus japonicus* and *Eu. fortunei* var. *radicans* with related species in the *Erysiphe alphitoides* complex. Bootstrap values $\geq 70\%$ obtained from 1,000 replications are indicated above the branches. Korean specimens examined in this study are shown in bold. *Arthrocladiella mougeotii* (HMJAU-PM91837) and *Golovinomyces latisporus* (KUS-F30175) were used as outgroup taxa. An asterisk (*) indicates the specimen collected from fruits of *Eu. japonicus* (KUS-F33267). ITS: internal transcribed spacer; 28S: 28S ribosomal DNA (large subunit, LSU); TL: tree length; CI: consistency index; RI: retention index; RC: rescaled consistency index; HT: holotype; JPN: Japan; TWN: Taiwan; KOR: Korea; MUMH: Mie University Mycological Herbarium; TNS-F: National Museum of Nature and Science, Tsukuba, Japan; HMJAU: Herbarium of Mycology, Jilin Agricultural University; KUS-F: Fungal specimens of Korea University Herbarium; EPM: *Euonymus* Powdery Mildew (Isolate name designated by the original researchers).

Powdery mildew fungi recorded on *Euonymus* species are currently classified into five species: *E. euonymi*, *E. euonymicola*, *E. lianyungangensis*, *E. mayumi*, and *E. pseudopusilla* [1]. Among these, *E. euonymi* and *E. euonymicola* are of particular interest because they are morphologically similar and have historically been confused. *Erysiphe euonymi* commonly produces chasmothecia and is widely distributed in Europe, western and central Asia, and parts of North America. In contrast, *E. euonymicola* is associated mainly with evergreen *Euonymus* species and has been reported from East Asia as well as other regions where its hosts are cultivated [1,2,4]. Despite examining specimens collected from evergreen *Euonymus* hosts at different times of year over multiple decades, no chasmothecia were observed in any specimen, but the anamorphic characteristics were generally consistent with previous descriptions of *E. euonymicola* [2,8]. Furthermore, all sequenced specimens formed a single clade with reference *E. euonymicola* sequences. These ecological, morphological, and phylogenetic characteristics support the identification of the Korean specimens as *E. euonymicola*.

Minor differences were noted between the anamorphic characteristics of the Korean specimens and those in the previous descriptions of *E. euonymicola* [2,8]. Previous studies described the foot-cells of conidiophores as straight to moderately flexuous-sinuuous or usually straight [2,3,14,15]. In contrast, the Korean specimens examined here had foot-cells that were mostly flexuous and only occasionally straight. In addition, primary conidia were consistently distinguishable from secondary conidia in Korean specimens. Primary conidia were slightly shorter and characterized by rounded apices and subtruncate bases, whereas secondary conidia were cylindric-ellipsoidal to oblong-elliptical. Although primary conidia have previously been reported for *E. euonymicola* [8], detailed morphological observations and photographic documentation of these structures remain limited. Germ tube morphology also differed slightly from previous descriptions [2]. In a germination test of 105 conidia, all germ tubes originated from a subterminal position on the conidium, which corresponds with a report from Azerbaijan [15]. These observations provide additional morphological information for *E. euonymicola*, particularly regarding foot-cell morphology, primary conidia, and germination characteristics.

Powdery mildews on *Eu. japonicus* in Korea had previously been recorded as *O. euonymi-japonici* and *E. euonymi-japonici*, reflecting earlier nomenclatural treatments predating the recognition of *E. euonymicola*. A previous Korean record of *E. alphitoides* on *Eu. japonicus* requires confirmation because associations between *E. alphitoides* and *Euonymus* species have rarely been reported, and species delimitation within the *E. alphitoides* complex remains difficult [16,17]. In the present study, all specimens collected from *Eu. japonicus*, including a specimen obtained from infected fruit, and from *Eu. fortunei* var. *radicans* were identified as *E. euonymicola*. To our knowledge, fruit infection by *E. euonymicola* has not been previously reported for *Eu. japonicus*, so the specimen examined here provides evidence of a new infection type.

Among powdery mildew species known from the genus *Euonymus* worldwide, *E. lianyungangensis*, *E. mayumi*, *E. pseudopusilla*, and *E. euonymi* have not been found in Korea. Because *Eu. americanus* and *Eu. europaeus*, known hosts of *E. pseudopusilla* and *E. euonymi*, respectively, have been introduced into and are cultivated in Korea, continued monitoring is warranted to detect potential changes in the diversity of

powdery mildew fungi infecting *Euonymus*. Given that *Euonymus* shrubs are increasingly recognized as important functional carbon sinks in urban ecosystems [18], tracking potential fungal outbreaks is crucial to preventing the degradation of their health and subsequent carbon uptake efficiency.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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