The Korean Journal of Mycology

www.kjmycology.or.kr

RESEARCH NOTE

Morphological and Molecular Characterization of *Podosphaera* Powdery Mildew on *Fatoua villosa* in Korea

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ABSTRACT

In Japan and China, *Podosphaera pseudofusca* causes powdery mildew in *Fatoua villosa*. During a taxonomic revision of Korean powdery mildew fungi, isolates newly collected from *F. villosa* were studied. Through morphological examination and molecular phylogenetic analyses of the internal transcribed spacer region and large subunit gene of rDNA, the powdery mildew fungus was identified as *Podosphaera xanthii*. In this study, we propose the merging of *P. pseudofusca* sensu Braun into the *P. xanthii* complex. To the best of our knowledge, this is the first report of *Podosphaera* powdery mildew on *F. villosa* in Korea.

Keywords: Anamorph, Erysiphaceae, *Podosphaera pseudofusca*, *Podosphaera xanthii*

OPEN ACCESS

pISSN : 0253-651X
eISSN : 2383-5249

Kor. J. Mycol. 2022 September, 50(3): 243-248
https://doi.org/10.4489/KJM.20220025

Received: May 04, 2022
Revised: September 02, 2022
Accepted: September 07, 2022

The current concept of the genus *Podosphaera* (Helotiales, Erysiphaceae) was established by Braun & Takamatsu in 2000 by combining two genera-*Podosphaera* sensu Kunze (1823) and *Sphaerotheca* sensu Léveille (1851) [1,2]. These two older genera were reduced to *Podosphaera* sect. *Podosphaera* and *Podosphaera* sect. *Sphaerotheca*, respectively [2]. The taxonomy and phylogeny of *Podosphaera* species have been studied mainly by splitting and partly lumping them into several distinct species based on the results of molecular phylogenetic analyses [3-7]. Nevertheless, the *Podosphaera xanthii* (Castagne) U. Braun & Shishkoff species complex still accommodates many isolates from diverse host families, such as Cucurbitaceae, Asteraceae, Fabaceae, and Malvaceae [2].

Only two host plant species belonging to the family Moraceae have been associated with *Podosphaera* powdery mildews. Of them, *Sphaerotheca fuliginea* (Schltdl.) Pollacci (currently included in the *P. xanthii* complex) was recorded on *Dorstenia contrajerva* L. in Japan [8]. However, the lack of additional records and herbarium material in Japan [9] and absence of powdery mildew on this plant species in its native range in Central and South America [10] limit the reliability of this data. In contrast, *Podosphaera pseudofusca* (U. Braun) U. Braun & S. Takam. has been recorded on *Fatoua villosa* (Thunb.) Nakai in Japan and China [9-11], thus representing the only confirmed host record from the family Moraceae.

The powdery mildew fungus infesting *F. villosa* was first named *Sphaerotheca pseudofusca* U. Braun in 1985 based on material from Japan [11]. Although the name was later changed to *P. pseudofusca* [1], the description was made based on teleomorph features without characterizing the anamorph. Nomura [9] later described anamorphic characteristics, and Ito & Takamatsu [3] provided the first sequence data of this species retrieved from Japanese material. Moreover, *P. pseudofusca* has also been recorded in China [12]. Nevertheless, information about this species is still insufficient. Therefore, this study aims to provide a detailed morphological description of the fungus through supporting photomicrographs of diagnostic features and elucidating its phylogeny based on the sequence data of the internal transcribed spacer (ITS) region and large subunit (LSU) of rDNA.

During our extensive field foray to study the diversity of Erysiphaceae in Korea, we collected seven samples and preserved them in the herbarium of Korea University (KUS; Seoul, Korea). The specimens are: KUS-F26422 (16 Nov 2011, Seoul), F27779 (31 Oct 2013, Busan), F27785 (4 Nov 2013, Seoul), F27797 (25 Nov 2013, Seoul), F29070 (12 Nov 2015, Mokpo), F29594 (20 Oct 2016, Jeju), and F32692 (29 Nov 2021, Wando).

For morphological examination, fungal structures were removed from the powdery mildew colonies on fresh leaves and mounted on a glass slide with distilled water. Identification of the pathogen was conducted through detailed examination under optical microscopes using bright-field and differential interference contrast. A minimum of 20 measurements were taken for each structure using an Olympus BX51 microscope (Olympus, Tokyo, Japan) at 100× and 1,000× magnification. Additionally, a Zeiss AX10 microscope equipped with an AxioCam MRc5 (Carl Zeiss, Göttingen, Germany) was used to capture photographs. The morphological description was mainly based on a representative sample of KUS-F27785 accommodating both anamorph and teleomorph states.

Mycelia on both sides of the leaves, on stems, and on inflorescences were sub-persistent to sub-evanescent, forming circular to irregular white patches or covering the entire surface with a thin layer of mycelium (Figs. 1A and B). Chasmothecia appeared on the leaves and stems in autumn (Figs. 1B-D). Hyphal appressoria were poorly developed, nipple-shaped, and single. Conidiophores were single on a hyphal cell, arising from the upper part of the mother cells, producing 2-4 (-6) swollen cells in chains with a crenate outline, 140-240 μm long. Foot-cells were straight, 32-76×10-12 μm, simple, with a basal septum at the branching point (Figs. 1E and F). Conidia were oval to ellipsoid, 29-42×16-20 μm (length/width ratio of 1.5-2.3), containing distinct fibrosin bodies, and producing germ tubes in the lateral position (Figs. 1G-K).

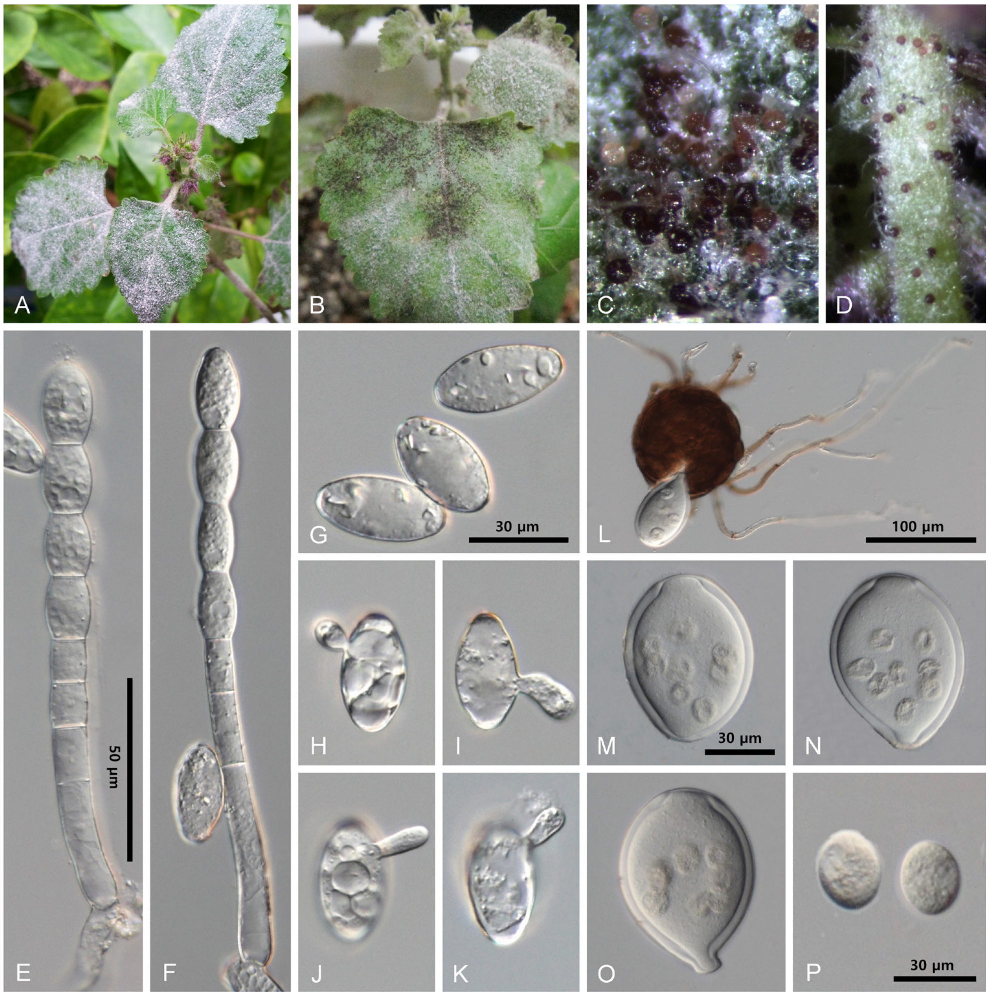

Fig. 1. *Podosphaera* powdery mildew on *Fatoua villosa*. A: The powdery mildew covers the leaves and stems of *F. villosa*. B: Numerous chasmothecia formed on the upper leaf surface. C, D: Close-up of chasmothecia formed on the leaf (C) and stem (D). E, F: Conidiophores. G: Conidia. Note conspicuous fibrosin bodies inside the cell. H-K: Conidia in germination. L: Chasmothecium with an ascus. M-O: Asci. Note sessile (M), sub-sessile (N) and short-stalked (O) base of the asci. P: Ascospores.

Chasmothecia were gregarious to somewhat scattered, 90-110 μm diam., and globose to subglobose (Figs. 1B-D, and L). Peridium cells were irregularly polygonal, conspicuous, and 20-35 μm diam. Appendages were few, positioned in the lower half, mycelioid, 0.5-3 times of the chasmothecial diam., approximately 4-7 μm wide, brown throughout or paler upwards, walls thin, and smooth to faintly rough. Ascus were subglobose to broadly ellipsoid, 60-82×50-75 μm, sessile or short-stalked, wall 2-3 μm thick, terminal oculus small, 12-15 (-18) μm diam., and 8-spored. Ascospores were mostly subglobose, 13-18×12-16 μm, and colorless. These morphological feature of the fungus was compatible with those of *Podosphaera xanthii* (Castagne) U. Braun & Shishkoff described previously [2].

The nucleotide sequence of the LSU and ITS region (ITS1 and ITS2) of rDNA were determined from specimens KUS-F27785, F27797, F29070, and F32692 using primer pairs PM3/NLP2 and ITS1F/PM6. Newly obtained sequences were assembled and deposited to GenBank under accession numbers ON365838-40 and ON387619 for ITS, and ON365841-43 and ON387618 for LSU. These sequences were later combined and aligned with closely related sequences of the genus *Podosphaera* using MUSCLE implemented in MEGA 11 [13]. *Cystotheca wrightii* Berk. & M.A. Curtis was selected as an outgroup. Furthermore, the phylogenetic analysis was conducted based on a combined dataset of ITS and LSU through PAUP* 4.0 using the maximum parsimony (MP) method [14]. The robustness of the MP tree was evaluated through bootstrap analysis from 1,000 replications. Tree scores, including tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency index (RC), were calculated.

A BLAST search revealed that our sequences were 100% identical to those of *P. xanthii* (KP219188, MT131256, LC270780 for ITS, MT919354, MT919340, LC270778, LC270782 for LSU) in GenBank for both loci of rDNA. A total of 20 sequences and 1,481 characters were used in MP analysis. Of them, 1,302 characters were constant, 95 were parsimony-informative, and 84 were variable and parsimony-uninformative. Sequences of Korean isolates in the resulting MP tree were clustered in a separate clade with the reference sequences of *P. xanthii* and a Japanese specimen of *P. pseudofusca*

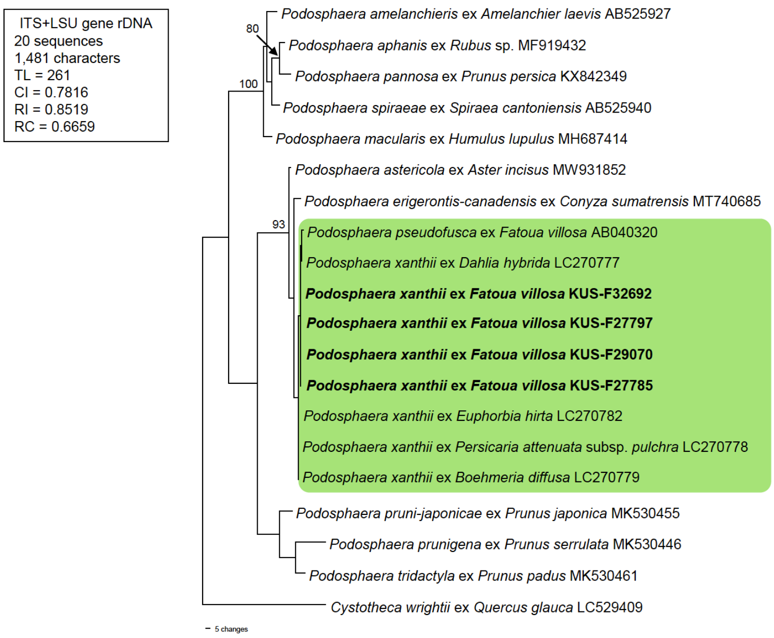

Fig. 2. Maximum parsimony tree of the *Podosphaera xanthii* complex generated from a combined dataset of internal transcribed spacer (ITS) and large subunit (LSU) of rDNA. The isolates obtained in this study are shown in bold. Bootstrap values (>70%) were indicated on related branches. TL = tree length; CI = consistency index; RI = retention index; RC = rescaled consistency index.

(AB040320) (Fig. 2), which supports our suggestion. Therefore, *P. pseudofusca* sensu Braun (1995) can be classified as *P. xanthii* rather than be maintained as a distinct species in the current species delimitation.

In this study, we report the detailed morphological characteristics of the isolate on *F. villosa*. To the best of our knowledge, this is the first report of *F. villosa* as a host plant for powdery mildew in Korea. After comparing with closely related powdery mildew species, we concluded that *P*. *pseudofusca* should be considered synonymous with *P. xanthii*.

CONFLICT OF INTERESTS

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

ACKNOWLEDGEMENTS

This work was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) through the Agriculture, Food and Rural Affairs Convergence Technologies Program for Educating Creative Global Leaders Program, funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA) (Project no.321001-03). H.D. Shin was supported by a grant (K2123391) from Korea University, Korea.

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