RESEARCH NOTE

Grovesinia moricola occurring on *Parthenocissus tricuspidata*

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ABSTRACT

Between August and September 2017, zonate leaf spots with severe defoliation were observed on *Parthenocissus tricuspidata* in Gimcheon, Korea. The initial symptoms included water-soaked, gray to grayish brown, circular or irregular-shaped leaf spots. As the disease progressed, the spots enlarged, coalesced, and became cream-colored with characteristic target-shaped rings, leading to leaf blight and premature defoliation. Based on cultural and morphological characteristics, the fungus associated with the symptoms was identified as *Grovesinia moricola*. The identity of this fungus was confirmed by analyzing sequences of the internal transcribed spacer region of ribosomal DNA. The pathogenicity of the isolate was demonstrated by artificial inoculation. To our knowledge, this is the first report of *G. moricola* occurring on *P. tricuspidata* globally as well as in Korea.

Keywords: Boston ivy, *Cristulariella moricola*, *Grovesinia moricola*, Internal transcribed spacer rDNA

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Parthenocissus tricuspidata (Siebold & Zucc.) Planch., commonly known as Boston ivy, is a climbing vine in the Vitaceae family. It is native to East Asia including northern and eastern China, Japan, and Korea. The vine is planted worldwide as an ornamental plant of buildings, walls, and fences for its esthetic beauty and also to filter out a range of metals that pollute urban air [1]. During our routine foray of phytopathogenic fungi in Korea between August and September of 2017, zonate leaf spots with severe defoliation were observed on dozens of *P. tricuspidata* with 100% disease incidence in Gimcheon, Korea (36°07'05"N, 128°08'05"E). The initial symptoms included water-soaked, gray to grayish brown, circular or irregular-shaped leaf spots with or without a chlorotic halo. As the spots enlarged and coalesced, they became cream colored with characteristic target-shaped rings, leading to leaf blight and premature defoliation (Fig. 1A). A representative specimen was deposited in the Korea University herbarium (KUS-F29884).

For morphological observations, fresh propagules were detached from infected leaves using a sterile razor blade and mounted in a few drops of distilled water on glass slides. The morphological characteristics of the fungal structures were examined using brightfield light microscopy and differential interference contrast light microscopy. An Olympus BX51 microscope (Olympus, Tokyo, Japan) was used to take measurements, and a Zeiss AX10 microscope equipped with an AxioCam MRc5 (Carl Zeiss, Göttingen, Germany) was

used to photograph the samples. At least 20 measurements were taken for each morphological structure.

Conical white sporophores on the leaf lesions were dominantly hypophyllous, single, hyaline, erect, and easily detachable (Fig. 1B, 1C). The upper portion of the sporophores consisted of a broadly oval to ellipsoidal head measuring 450–600 × 80–110 μm (n = 20). The central stipe was hyaline and tapered to an acute or somewhat rounded apex (Fig. 1C, 1D). Globose cells were 7–28 μm in diameter and di- or trichotomously branched (Fig. 1E). The fungus from a lesion was isolated by culturing on potato dextrose agar at 15°C; whitish, cottony, and mycelial colonies were observed (Fig. 1F). Sclerotia were produced on

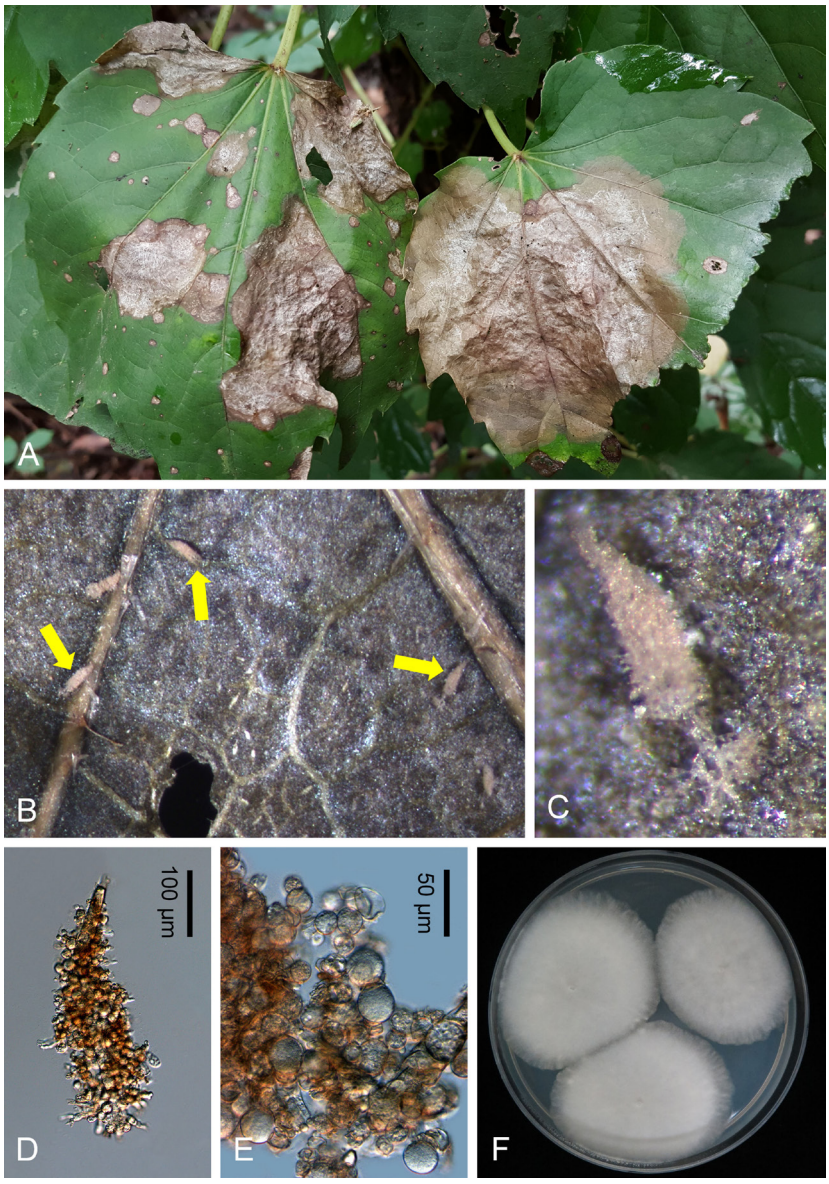

Fig. 1. *Grovesinia moricola* occurring on *Parthenocissus tricuspidata* causing zonate leaf spots. (A) Symptoms on leaves of *P. tricuspidata*. (B) White sporophores (arrow) on the abaxial surface of the lesion. Yellow arrows indicate sporophores. (C, D) Sporophores. (E) Globose cells. (F) One-week-old colonies of *G. moricola* on potato dextrose agar at 15°C.

the surfaces of the colonies after 2 weeks. These sclerotia appeared as black, irregularly shaped structures of approximately 2–4 mm in diameter. These characteristics were consistent with those of *Grovesinia moricola* (I. Hino) Redhead (= *Cristulariella moricola* [I. Hino] Redhead) [2, 3]. An isolate was deposited in the Korean Agricultural Culture Collection (KACC48409).

For molecular identification of the causal pathogen, genomic DNA was isolated from the fungal mycelia formed on an artificial medium, using MagListo 5M plant Genomic DNA Extraction Kit (Bioneer, Daejeon, Korea). Polymerase Chain Reaction (PCR) amplification was performed for the internal transcribed spacer (ITS) rDNA region using primers ITS1 and ITS4 [4]. The resulting amplicons were purified and sequenced by a DNA sequencing service (Macrogen, Seoul, Korea). The ITS rDNA sequence was edited using the DNASTAR software package version 5.05 (DNASTAR, Madison, WI, USA). The obtained 454 bp sequence was deposited in GenBank (Accession No. MG564341). Searches for homologous DNA sequences were carried out using the Basic Local Alignment Search Tool (BLAST) algorithm available from the National Center for Biotechnology Information. The BLAST search in GenBank revealed that the ITS sequence of the Korean isolate is identical with the reference sequences of *G. moricola* ex *Acer negundo* (JQ036181), *Humulus japonicus* (KC460209), and *Vigna angularis* (KX906373) [5-7]. A phylogenetic tree was constructed using the minimum evolution (ME) method in MEGA 7.0 [8]. In the phylogenetic tree (Fig. 2), the ITS sequence was grouped with other reference sequences of *G. moricola*, with a high supporting value of 94%, confirming the morphology-based identification.

To determine the pathogenicity of the fungus, sporophores were carefully detached from a lesion on the naturally infected leaf using fine needles. Each sporophores was transferred individually onto 4 areas of 3 detached healthy leaves. The leaves were placed in humid chambers at an RH 100% and incubated at 15°C.

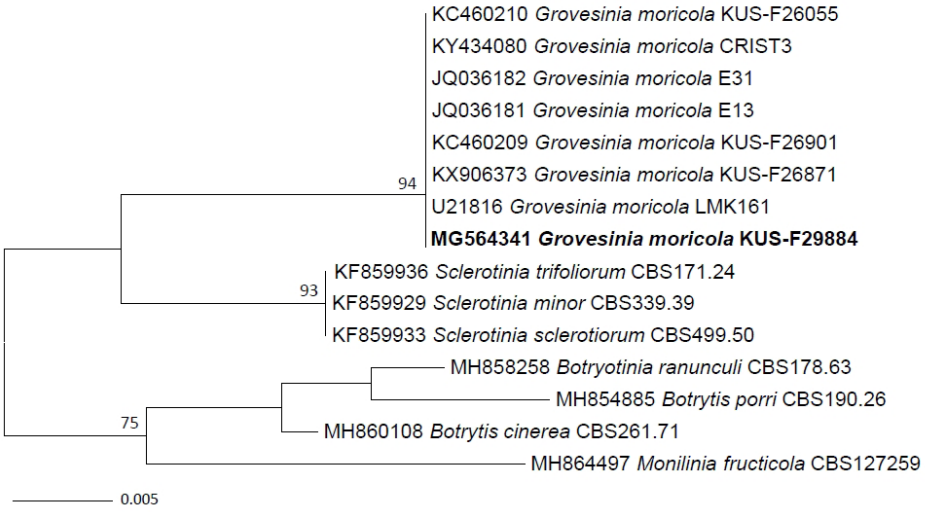

Fig. 2. Phylogenetic tree of *Grovesinia* and allied genera, including *G. moricola* parasitic to *Parthenocissus tricuspidata*, using the internal transcribed spacer (ITS) rDNA sequences. The numbers above the nodes are the bootstrap values obtained from 1000 replications. The Korean isolate presented in this study is indicated in bold. The scale bar indicates the number of nucleotide substitutions per site.

Disease symptoms developed after 3 or 4 days on all inoculated leaves. The lesions became discolored to light brown and enlarged rapidly, reaching 50–70 mm in diameter 2 weeks after inoculation. Sporophores and sclerotia were formed on the abaxial leaf surfaces 4 weeks after the inoculation. Re-isolation from the lesion confirmed the pathogenicity of the tested isolate. No symptoms were observed on the control leaves during the 2 weeks of observation.

Parthenocissus quinquefolia has been known to be associated with *G. moricola* in the southeastern regions of the United States [9]. To our knowledge, this is the first report of *G. moricola* occurring on *P. tricuspidata* globally as well as in Korea. Because the disease can be severe, it could become a significant concern for phytosanitary of *P. tricuspidata*.

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