

# First Report of *Myrothecium roridum* Causing Leaf and Stem Rot Disease on *Peperomia quadrangularis* in Korea

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**Abstract** In 2010, symptoms of leaf and stem rot were observed on potted plants (*Peperomia quadrangularis*) in a greenhouse in Yongin, Korea. The causative pathogen was identified as *Myrothecium roridum* based on morphological data, internal transcribed spacer sequence analysis, and pathogenicity test. To our knowledge, this is the first report of *M. roridum* causing leaf and stem rot disease on *P. quadrangularis* in Korea and elsewhere worldwide.

**Keywords** Leaf and stem rot, *Myrothecium roridum*, *Peperomia quadrangularis*

*Peperomia quadrangularis* (J. V. Thomps.) A. Dietr. (family Piperaceae) is native to South America, and it is cultivated for commercial purposes in Korea. The plant has ornamental value because of its striped leaves, and it grows well in household conditions. In January 2010, necrotic lesions were observed on the leaves and stems of *P. quadrangularis* in a farm located in Namsa-myeon, Yongin-si, Korea. The incidence of disease was up to 5% on potted plants propagated from stem cuttings. The disease was especially prevalent when cuttings were rooted in pot soils that remained excessively wet. The initial symptom of the disease was a brown discoloration on the stem base (Fig. 1A). Affected leaves turned completely dark brown as the disease spread upwards (Fig. 1B). As the disease progressed, black sporodochia were produced on older lesions (Fig. 1A, 1B, 1E, and 1F). The diseased leaves withered or became rotten and then detached.

A fungal isolate was obtained from sporodochia on lesions and deposited in the Korean Agricultural Culture Collection (KACC No. 93161P). The isolate was subcultured on potato dextrose agar (PDA) plates at 25°C for further studies. Morphological characteristics of the fungus were examined using a Zeiss AXIO Imager A1 microscope equipped with an AxioCam ICc3 camera (Carl Zeiss, Jena, Germany). Sporodochia were black and were surrounded by white tufts of mycelium (Fig. 1F). Conidiophores branched two to three times, and each branch bore two to five phialides in whorls (Fig. 1H). Phialides were hyaline, cylindrical, and 10~20 × 1.2~2 μm in size (Fig. 1H). Conidia were hyaline, single-celled, cylindrical to ellipsoid, rounded at both ends, and 5~7.5 × 1.5~2.5 μm in size (Fig. 1I). Colonies on PDA were 75~80 mm in diameter after 3 wk of incubation at 25°C under continuous fluorescent light (Fig. 1G). Colonies were pinkish white and floccose and formed sporodochia with conidial masses in black concentric rings. Based on these morphological characteristics, the fungus isolated from *P. quadrangularis* was identified as *Myrothecium roridum* Tode ex Fr. [1].

Genomic DNA was extracted from mycelial and conidial masses harvested from PDA plates according to a previously described method [2]. The internal transcribed spacer (ITS) rDNA region was amplified using the primers ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') [3]. The amplification was conducted with 5 min of initial denaturation at 95°C; 35 cycles of 1 min of denaturation at 95°C, 1 min of annealing at 58°C, and 2 min of extension at 72°C; and 10 min of final extension at 72°C. The PCR amplicons were purified and directly sequenced. The raw sequence data for

Mycobiology 2014 June, 42(2): 203-205  
<http://dx.doi.org/10.5941/MYCO.2014.42.2.203>  
pISSN 1229-8093 • eISSN 2092-9323  
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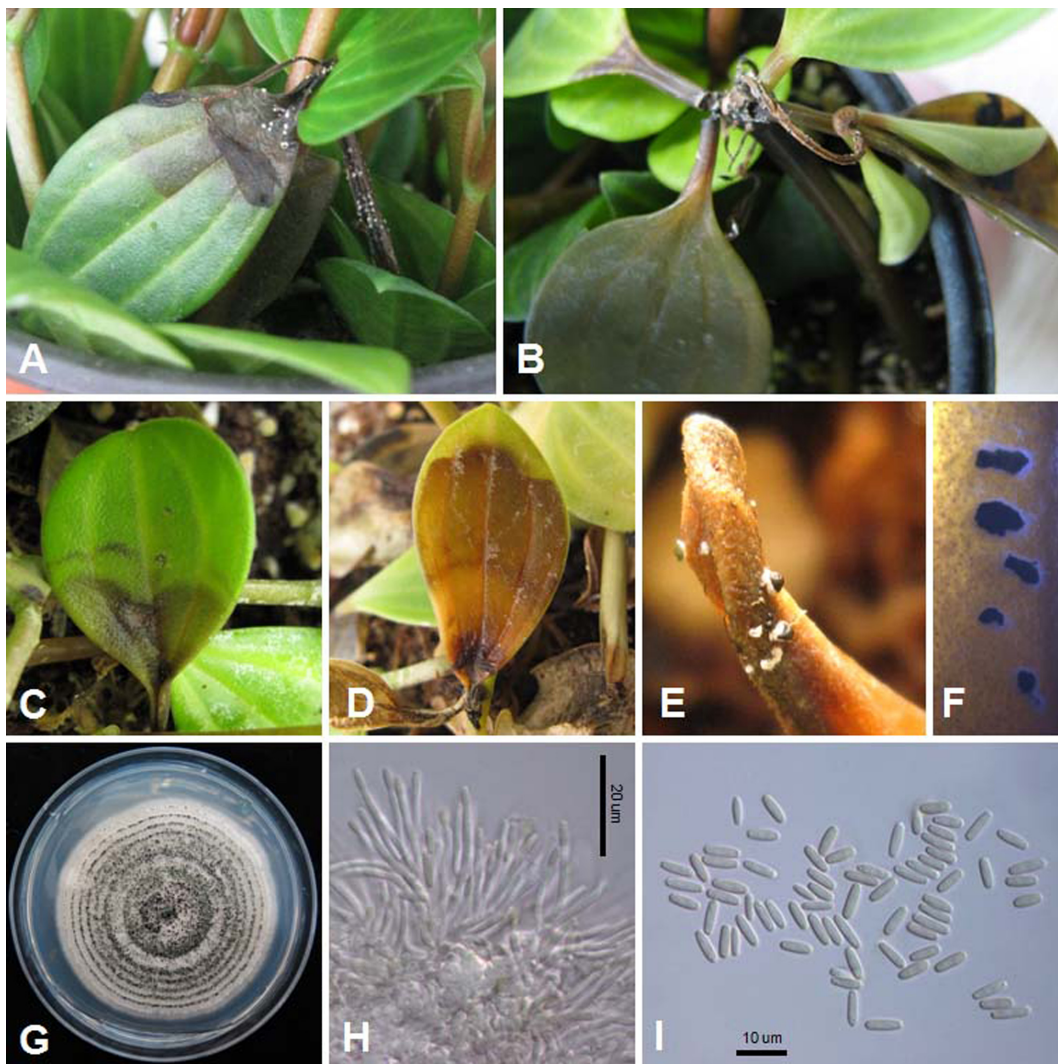
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**Received** February 23, 2014

**Revised** April 3, 2014

**Accepted** April 23, 2014

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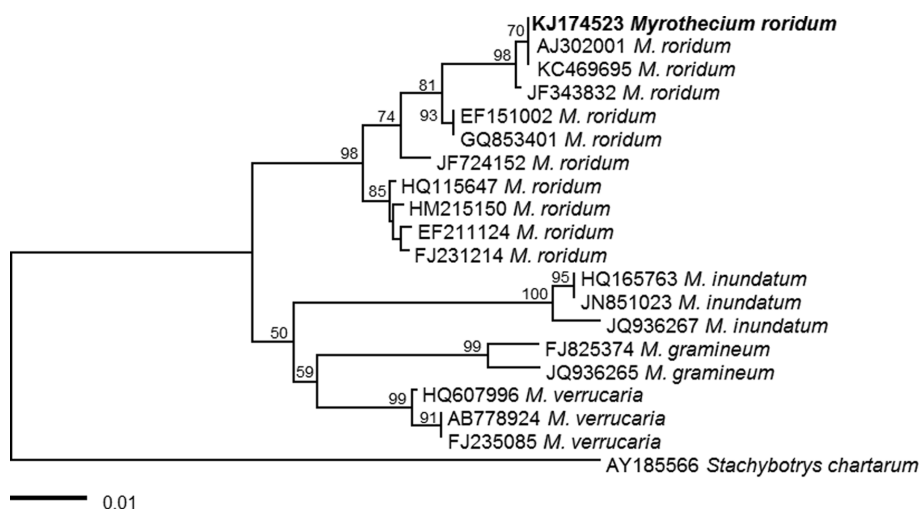
**Fig. 1.** Symptoms of leaf and stem rot on *Peperomia quadrangularis* and morphological characteristics of *Myrothecium roridum*. A, B, Symptoms from natural infection; C, D, Symptoms from artificial inoculation; E, F, Sporodochia produced on the petiole (E) and the leaf surface (F); G, Colony on potato dextrose agar after 3 wk of incubation; H, Conidiophores with phialides producing conidia; I, Conidia.

*M. roridum* were trimmed using SeqMan (DNASTAR Lasergene, Madison, WI, USA). The ITS sequences of the isolate have been submitted to GenBank (accession No. KJ174523). For phylogenetic analysis, 18 reference sequences of *Myrothecium* spp. were downloaded from GenBank. *Stachybotrys chartarum* (AY185566) was used as an outgroup. Sequence alignment and neighbor-joining analysis with the Tajima-Nei model were carried out in MEGA5 [4]. In the phylogenetic tree (Fig. 2), the isolate was placed in a distinct group with *M. roridum* sequences. Therefore, the molecular data confirmed the morphological identification of the causative fungus.

To fulfill Koch's postulates, the pathogenicity of the isolate was tested using 3-month-old plants in a greenhouse. An inoculum was prepared by harvesting conidial masses from 30-day-old cultures. An aqueous conidial suspension (approx.  $1 \times 10^5$  conidia/mL) was sprayed on leaves of three

young plants wounded with needles. Three plants sprayed with sterile water served as controls. Each plant was covered with a polyethylene bag to maintain high humidity and incubated in a growth chamber at 28°C for the first 48 hr. Necrotic symptoms appeared on the inoculated leaves within 7 days, and the infected leaves became withered or rotten a few days afterwards. The fungus was successfully re-isolated from the inoculated plants. The leaves of control plants did not show any symptoms.

*M. roridum* is a cosmopolitan plant pathogen with a broad host range. To date, leaf spot diseases caused by *M. roridum* have been recorded on two plants, watermelon and soybean, in Korea [5, 6]. In the genus *Peperomia*, *P. sandersii* has been reported to be a plant host of *M. roridum*, causing leaf spot disease in the USA [7, 8]. In addition, unidentified *Myrothecium* species have been known to cause leaf and stem rot on *Peperomia* spp. (i.e., *P.*



**Fig. 2.** Neighbor-joining tree of *Myrothecium* spp. based on internal transcribed spacer rDNA sequences. Bootstrap values  $\geq 50\%$  obtained from 10,000 replicates are indicated above the branches. The scale bar represents 0.01 nucleotide substitutions per site.

*bicolor*, *P. caperata*, and *P. obtusifolia*) in the USA [8, 9]. To our knowledge, this is the first report of leaf and stem rot disease caused by *M. roridum* on *P. quadrangularis* in Korea as well as in other countries worldwide. Because wet soil conditions favor the development of this disease, careful watering practices and soil sanitation are required to reduce economic losses in commercial cultivation of *P. quadrangularis*.

### ACKNOWLEDGEMENTS

We would like to thank Dr. Seung-Beom Hong (KACC, NAAS, RDA) for providing access to the microscopy facility.

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