

New Rust Disease of Korean Willow (*Salix koreensis*) Caused by *Melampsora yezoensis*, Unrecorded Pathogen in Korea

Yeo Hong Yun¹, Geum Ran Ahn¹, Seong Kwon Yoon¹, Hoo Hyun Kim¹, Seung Yeol Son¹ and Seong Hwan Kim^{1,2,*}

¹Department of Microbiology, Dankook University, Cheonan 31116, Korea

²Institute of Biodiversity, Dankook University, Cheonan 31116, Korea

Abstract During the growing season of 2015, leaf specimens with yellow rust spots were collected from *Salix koreensis* Andersson, known as Korean willow, in riverine areas in Cheonan, Korea. The fungus on *S. koreensis* was identified as the rust species, *Melampsora yezoensis*, based on the morphology of urediniospores observed by light and scanning electron microscopy, and the molecular properties of the internal transcribed spacer rDNA region. Pathogenicity tests confirmed that the urediniospores are the causal agent of the rust symptoms on the leaves and young stems of *S. koreensis*. Here, we report a new rust disease of *S. koreensis* caused by the rust fungus, *M. yezoensis*, a previously unrecorded rust pathogen in Korea.

Keywords ITS, *Melampsora yezoensis*, Rust, *Salix koreensis*

The genus *Melampsora* was established by Castagne in 1843 [1]. More than half of *Melampsora* spp. have been described on Salicaceae, which comprises the genus *Salix* (willows) and the genus *Populus* (poplars) [2, 3]. *Salix koreensis* Andersson, commonly known as Korean willow, is a deciduous, broad-leaved tree in the family of Salicaceae, and is distributed in East Asia, including Korea and China. The tree grows naturally along wetlands and rivers [4]. Since twig bark of *S. koreensis* is known to be effective for pain relief, the tree has been applied for medicinal use [5].

In June of 2015, leaf rust spots were found on several *S. koreensis* trees growing wild in areas surrounding Cheonhoji, an agricultural water reservoir in Cheonan (36°50'11.6" N, 127°10'22.0" E), Korea. Representative plants were infected

with rust on more than 10% of their leaves. Numerous yellow uredinia, fruiting bodies of rust fungi, were present on leaves and petioles of the willow (Fig. 1A~1C). In Korea, *Melampsora larici-capraearum* is listed as a causal agent of willow rust on *Salix hultenii* Floderus without molecular characterization [6], but there are no reports internationally of rust disease on *S. koreensis*. This study identified the causal fungus on *S. koreensis* and confirmed its pathogenicity. Fungal isolates, coded as DUCC508 and DUCC509, were used for detailed study. The morphological features of the isolates, were examined using a stereoscopic microscope (SZ61 stereo microscope; Olympus Optical Co., Tokyo, Japan), a light microscope (Axioskop 40; Karl Zeiss, Jena, Germany) and a scanning electron microscope (SEM, Hitachi 4300; Hitachi, Tokyo, Japan) [7]. The heads of the paraphyses had uniformly thickened walls. SEM examination revealed that the surfaces of urediniospores were evenly echinulate. Light microscopy showed that the urediniospores were mostly ovoid in shape with yellow contents and uniformly thick hyaline walls. The urediniospores measurements were 10~14 × 13~21 μm. Based on these morphological characters, the isolates were identified as *Melampsora* sp. To identify the specimens at the species level, genomic DNA was extracted from DUCC508 and DUCC509 with a drilling method [8]. The common DNA barcode of the internal transcribed spacer (ITS) region in ribosomal DNA (rDNA) was amplified with ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TTC CGC TTA TTG ATA TGC-3') primer set [9]. The PCR products were purified

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***Corresponding author**

E-mail: piceae@dankook.ac.kr

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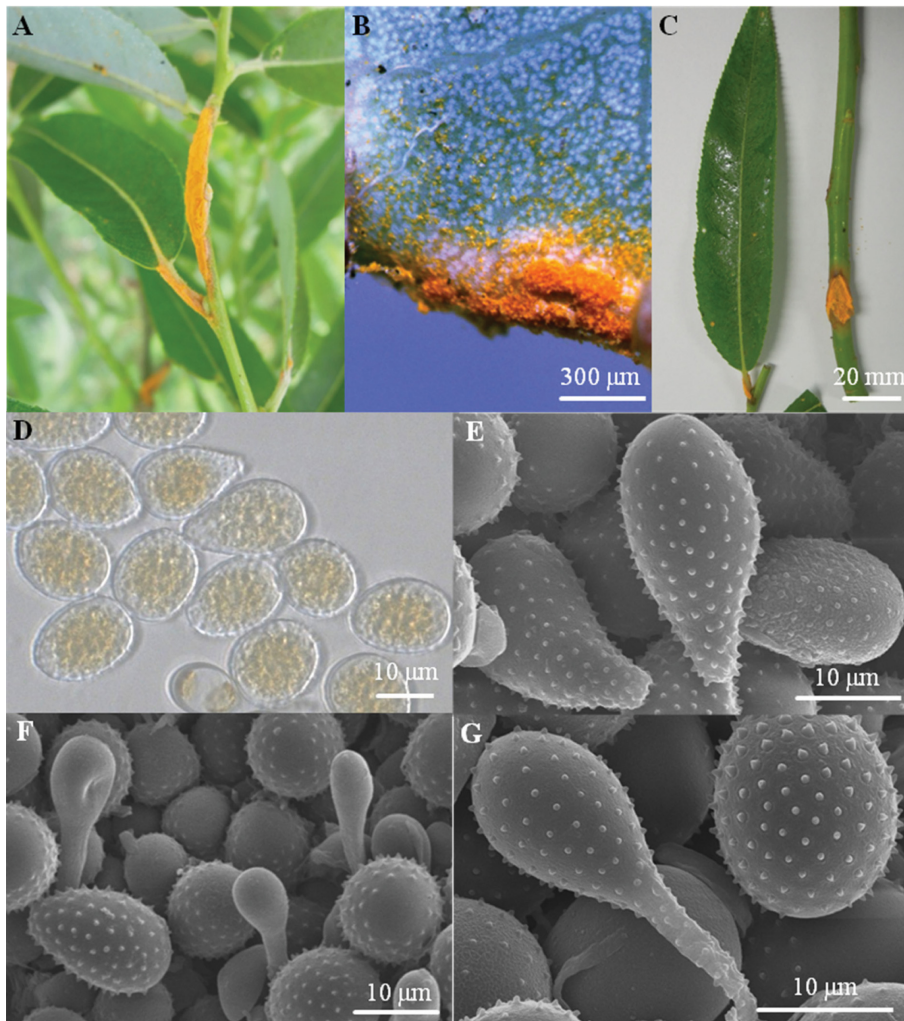


Fig. 1. Symptoms of rust caused by *Melampsora yezoensis* on *Salix koreensis*, and morphological features of the pathogen. A, Disease symptoms of plants in the field; B, Urediniospores on leaves; C, Artificial infection on leaves and young stem of *S. koreensis*; D, E, Urediniospores; F, G, Uredinial paraphyses (scale bars: B = 300 μm , C = 20 μm , D~G = 10 μm).

with a High Pure PCR Purification Kit (Roche, Basel, Switzerland), and sequencing was carried out by Macrogen Inc. (Seoul, Korea). The resulting nucleotide sequences were searched with BLASTN at the GenBank database (<http://www.ncbi.nlm.nih.gov/BLAST/>) and compared with sequences of other fungal ITS1 and ITS4 regions of ITS rDNA. A phylogenetic analysis based on the ITS rDNA sequences was constructed using a maximum likelihood method in the MEGA 5 program [10]. Bootstrap values were generated with 1,000 replicates. This analysis established that the ITS rDNA sequences were identical to each other and shared 99.8% identity (577 of 578 bp) with a *Melampsora yezoensis* isolated from *Chelidonium majus* var. *asiaticum* in Japan (GenBank accession No. AB646768). Based on these morphological and molecular results (Fig. 2) the isolates were identified as *M. yezoensis* [11]. One of the identified specimens DUCC509 was deposited in the National Institute of Biological Resource (NIBRFG0000144293), and the analyzed ITS rDNA sequences of DUCC508 and DUCC509

were deposited in the GenBank DNA database under accession numbers KT199020 and KT199021, respectively.

When a urediniospore suspension (1×10^6 spores/mL sterile water) prepared from infected lesions of the DUCC509 was sprayed onto young leaves and stems of a willow tree, rust spots with yellow uredinia formed on the inoculated leaves and stems (Fig. 1). Meanwhile, a control spray with sterile water produced no rust spots (data not shown). Koch's postulates for identifying *M. yezoensis* as a pathogen were satisfied by re-isolating *M. yezoensis* from rusted lesions on the artificially inoculated leaves and stems. *M. yezoensis* is known to infect diverse *Salix* species including *S. eriocarpa*, *S. pierotii*, *S. serissaefolia*, *S. iessoensis*, *S. serissaefolia*, *S. eriocarpa*, and *S. pierotii* [10]. However, there is no record available currently on *S. koreensis* as its host. Thus, to our knowledge, this is the first disease report of willow rust on *S. koreensis* caused by *M. yezoensis*. *S. koreensis* is added as a new uredinial host of *M. yezoensis*. In addition, we report *M. yezoensis* is an unrecorded rust

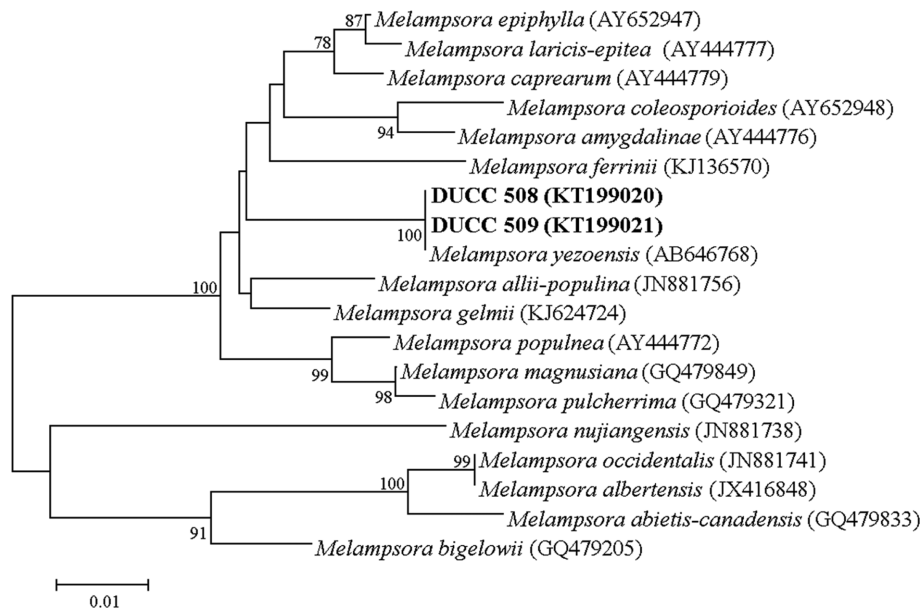


Fig. 2. Phylogenetic tree for *Melampsora yezoensis* and related species based on neighbor-joining analysis of internal transcribed spacer rDNA sequence using MEGA 5.0. The numbers at the nodes indicate the bootstrap support calculated for 1,000 repetitions. The scale bar indicates 0.01 substitutions per nucleotide position.

fungal pathogen in Korea. Since this host plant is widely distributed in Korea, our report should be valuable for better management of rust disease in *S. koreensis*. Further study is needed to find the aeciospore stage of *M. yezoensis* and define the life cycle of this species including its host range in Korea.

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