

First Report of Leaf Rust Caused by *Puccinia caricis* in *Farfugium japonicum* in Korea

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Abstract *Farfugium japonicum* is used in traditional medicine and as an edible herb in China and Korea. In July 2013, leaf spots were observed in *F. japonicum* seedlings at Ulleung Island, Gyeongsangbuk Province, Korea. Early symptoms on the leaf adaxial surface included roughly circular yellow spots that later developed brown, necrotic centers. The aecia were hypophyllous, cupulate, yellowish, 180~430 µm in diameter, clustered, and erumpent with a peridium with a recurved margin. The aeciospores were globose, 14~17 × 13~16 µm, light yellow or colorless, and densely verrucose. The 28S rDNA sequence of the isolate was identical to each other and shared 99% identity with *Puccinia caricis*. This is the first report of rust caused by *P. caricis* in *F. japonicum* in Korea or elsewhere in the world.

Keywords 28S rDNA, *Farfugium japonicum*, *Puccinia caricis*, Rust

Farfugium japonicum (L.) Kitam. is a species of flowering plants of the family Asteraceae. *F. japonicum* grows naturally along streams and coasts and in moist meadows in Korea, China, and Japan. In Korea, these plants have been used as an anti-pyretic as well as an ingredient in folk remedies for cough, wound healing, bronchitis, and diarrhea. Recently, the essential oil of *F. japonicum* was reported to exert anti-inflammatory effects [1].

Rust fungi (Pucciniales) are important phytopathogens, known to comprise more than 7,000 species over 100 genera [2]. The genus *Puccinia* is the largest in the Pucciniales order and considered the most economically destructive genus of biotrophic fungi [3]. Moreover, this genus includes serious pathogens causing diseases in major cereal crop species. In 2004, two types of severe leaf spot disease caused by *Phoma* spp. and black leaf spot disease caused by *Alternaria cinerariae* were reported in Japan [4, 5]. However, no reports are available for fungal diseases caused by *Puccinia*

in *F. japonicum*. The aim of this study was to identify the causal agent associated with leaf rust in *F. japonicum*, based on morphological characteristics and molecular analysis.

In July 2013, yellow and circular spots were observed on the leaves of *F. japonicum* seedlings at Ulleung Island, Gyeongsangbuk Province, Korea. The specimen was coded DUCC507 and studied in further detail. The morphological features of the fungus isolated from DUCC507 was examined by light microscopy (Axioskop 40; Carl Zeiss, Oberkochen, Germany) and scanning electron microscopy (Hitachi 4300; Hitachi, Tokyo, Japan) [6]. For species-level identification of this fungus, genomic DNA extraction was undertaken [7]. The D1 and D2 regions of 28S ribosomal DNA were amplified with the LROR (5'-ACC CGC TGA ACT TAA GC-3') and LR4 (5'-ACC AGA GTT TCC TCT GG-3') primer set [8]. The PCR products were purified with High Pure PCR Purification Kit (Roche, Basel, Switzerland) and sequencing was performed by Macrogen Inc. (Seoul, Korea). The nucleotide sequences obtained were searched using BLASTN available with the GenBank database (<http://www.ncbi.nlm.nih.gov/BLAST/>) and compared with sequences of other fungal 28S rDNA D1 and D2 regions. Phylogenetic analysis based on the 28S rDNA sequences was performed using a maximum likelihood method in MEGA 5 program [9]. Bootstrap values were generated with 1,000 replicates. The nucleotide sequences of the 28S rDNA sequence of *Puccinia* spp. and related species were obtained from the GenBank database and used for phylogenetic tree construction.

Representative *F. japonicum* plants in the population from where DUCC507 was sampled were infected with aecia on > 40% of their leaves. The early symptoms on the adaxial leaf surface consisted of roughly circular light yellow spots that later developed brown, necrotic centers

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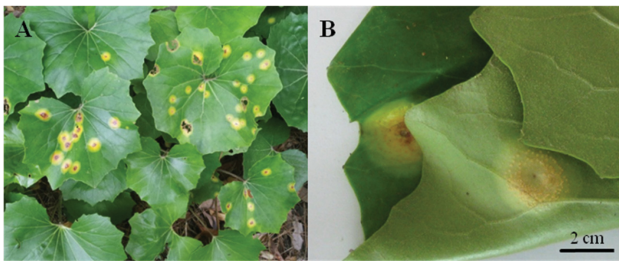


Fig. 1. Symptoms of leaf rust caused by *Puccinia caricis* in *Farfugium japonicum*. A, The infected leaves showed browning, followed by rotting; B, The adaxial and abaxial leaf surfaces of *F. japonicum*.

(spot size, 11~14 mm in diameter) (Fig. 1A). On the abaxial side, numerous yellow aecia were visible (Fig. 1B). The morphological characters of the fungus isolated from DUCC507 are described in Table 1 and shown in Fig. 2. Aecia were hypophyllous, cupulate, yellowish, 180~430 μm in diameter, clustered, and erumpent with a recurved margin. Peridial cells were whitish to yellowish, linear rhomboidal, inner walls with small papillae, 16~22 × 18~26 μm (average, 18.4 × 21.5 μm). The aeciospores were globoid, 14~17 × 13~16 μm (average, 15.2 × 14.8 μm), light yellow or colorless, and densely verrucose. On the basis of these morphological characters, the fungus in DUCC507 was identified as *Puccinia* sp.

To further identify the fungus isolated from DUCC507, molecular analysis of the D1 and D2 regions of 28S rDNA was performed. A PCR product of 945-bp-sized partial D1

Table 1. Comparison of the morphological characters of the present isolate and *Puccinia caricis*

	<i>P. caricis</i> [10]	Present isolate
Aecia		
Color	Honey yellow	Yellow
Shape	Cupulate	Cupulate
Size (μm)	200~350	180~430
Aeciospore		
Color	Colorless	Colorless or light yellow
Shape	Globoid or ellipsoid; verrucose	Globoid; verrucose
Size (μm)	16~25 × 15~23	14~17 × 13~16

and D2 regions of 28S rDNA sequence was obtained and sequenced. The determined nucleotide sequence was subjected to similarity search with the GenBank database using the BLASTN program. The 28S rDNA sequence of the fungus isolated from DUCC507 shared 99% similarity with that of *Puccinia caricis* (DQ354514). The sequence of the D1 and D2 regions of the fungal 28S rDNA sequence was deposited in GenBank DNA database under the accession number KM110789. A phylogenetic tree showed that the fungus isolated from DUCC507 positioned with *P. caricis* (Fig. 3). Based on these molecular results and the morphological data obtained, we identified the fungus isolated from DUCC507 as *P. caricis* [10]. To the best of our knowledge, this is the first published report of rust caused by *P. caricis* in *F. japonicum* in Korea or elsewhere in the world.

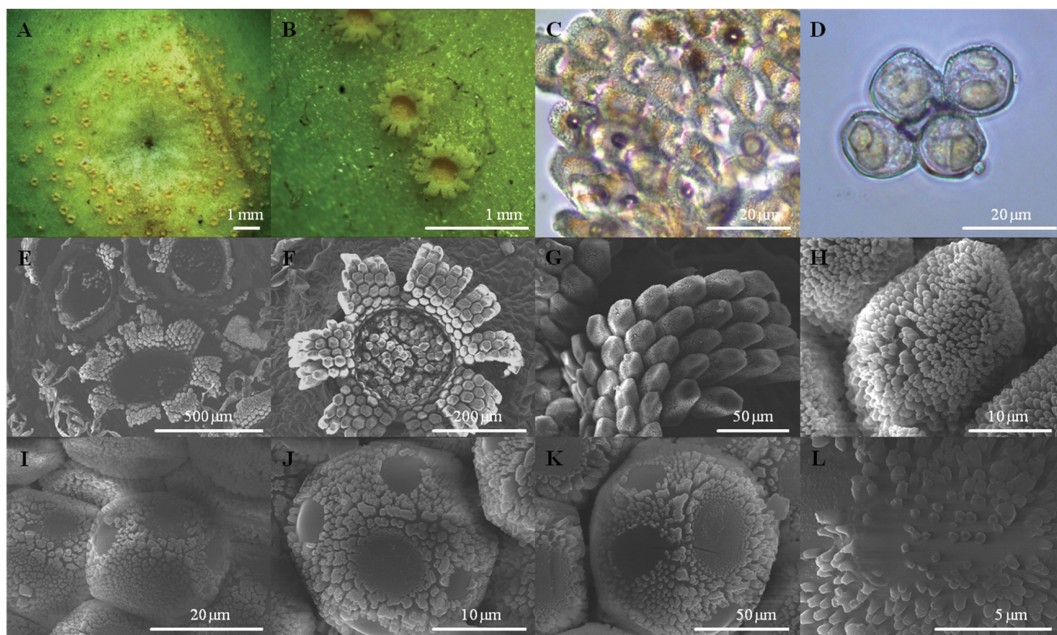


Fig. 2. Morphology of the aecial stage of *Puccinia caricis* in *Farfugium japonicum*. A, B, Stereoscopic microscopy images of the aecia on the adaxial and abaxial leaf surfaces; C, Peridial cells; D, Aeciospores as observed by light microscopy; E, F, Aecia on the abaxial leaf surface as observed by scanning electron microscopy (SEM); G, H, Peridial cells as observed by SEM; I~K, Aeciospores as observed by SEM; L, Verrucose shape of aeciospores as observed by SEM.

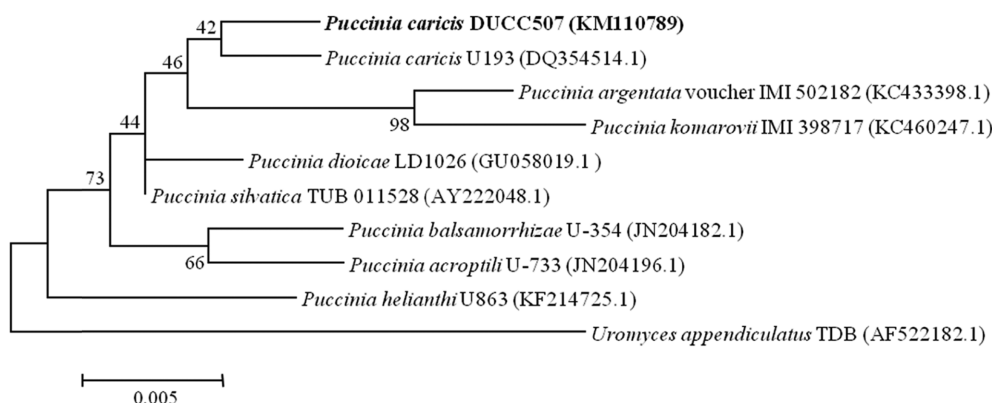


Fig. 3. Phylogenetic tree based on the 28S rDNA sequences of *Puccinia caricis* DUCC507 and other species. A phylogram was constructed by the maximum likelihood method using MEGA 5. The numbers above the nodes are the supporting percentages obtained from 1,000 bootstrap replicates. *Uromyces appendiculatus* was considered an outgroup. The NCBI GenBank accession numbers are indicated in parentheses.

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