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First Report of Rust Disease on *Erigeron strigosus* in Korea Caused by *Coleosporium asterum*

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In 2016, the typical symptoms of rust disease were observed on rough fleabane (*Erigeron strigosus* Muhl., Asteraceae) in Yongin–si, Korea. The diseased leaves were covered with yellow spots and chlorosis, and massive urediniospores covered the entire plant. Subsequently, a severe infection resulted in leaf blight and defoliation. To investigate the rust disease on rough fleabane, observations were conducted by using stereo microscopy, light microscopy, and scanning electron microscopy. The observations showed that the urediniospores were subglobose to polygonal and 20.4–32.3 × 14.7–23.6 μm in size and the teliospores were septate, obovoid to ellipsoid, and 72.1–85.1 × 21.0–24.0 μm in size. The phylogenetic analysis based on partial 28S rDNA sequences indicated that it was closely related to *Coleosporium asterum* (AF426241) isolated from *Aster* sp. Based on the results of the morphological and phylogenetic analyses, this is thought to be the first report of *C. asterum* as a causative agent of rust disease on rough fleabane in Korea.

Key words: Coleosporium asterum, rough fleabane, rust disease, Erigeron strigosus

INTRODUCTION

Erigeron spp. (*Asteraceae*) include approximately 300 species, originally from North America and Europe, but now distributed worldwide (Willis, 1985). *Erigeron strigosus*, known as rough fleabane, is a naturalized plant that was introduced to Korea; in particular, rough fleabane dominates orchards, fields, and fallow ground and is found nationwide (Kim *et al*., 2008). Furthermore, they may spread further than at present; they commonly occur in field and fallow ground and have a strong ability to adapt to natural conditions (Kim *et al*., 2008). In Korea, only a few diseases have been reported in the genus *Erigeron*: leaf spot caused by *Septoria erigerontis* was reported in rough fleabane (*E. strigosus*), and three diseases (leaf spot caused by *Phoma exigua* and *Septoria erigerontis*, powdery mildew caused by *Sphaerotheca fusca*, and hypophyllous white mold caused by *Cercosporella virgaureae*) were reported in daisy fleabane (*E. annuus*); however, there have not yet been any reports of rust disease on both *E. strigosus* and *E. annuus* (The Korean Society of Plant Pathology, 2009). In this study, we reported the first observation of rust disease on rough fleabane *(E. strigosus*) in Yongin–si, Korea, in 2016, studied the morphological characteristics, and performed phylogenetic analysis based on a partial sequence of the 28S rDNA gene to identify the rust disease.

MATERIALS AND METHODS

Sample collection and microscopic observation

In November 2016, diseased *E. strigosus* was found to be severely infected by rust fungi in Yongin, Korea. To clarify the symptoms, the diseased leaves were observed under a digital microscope (DIMIS–M; Siwon Optical Technology, Co., Ltd, Anyang, Korea). The morphological characteristics of the urediniospores and teliospores were observed using a light microscope (BX–50; Olympus, Tokyo, Japan) and the structures of the uredinia and urediniospores were investigated using a scanning electron microscope (SEM). The samples were treated for SEM examination as follows (Lee *et al*., 2013): the samples were prefixed with 2% paraformaldehyde and 2.5% glutaraldehyde in 0.05 M sodium cacodylate buffer, pH 7.2, for 24 h at 4°C. The samples were then washed in 0.05 M sodium cacodylate buffer solution for three periods of 15 min each. The samples were subsequently dehydrated in a graded ethanol series (30%, 50%, 70%, 90%, and absolute ethanol) for 20min at each concentration, and the dehydrated samples were dried with hexamethyldisilazane for 15 min. Finally, the dried samples were observed under SEM (S–3500N, Hitachi, Tokyo, Japan), with a particular focus on the structures of the uredinia and urediniospores.

Genomic DNA preparation and PCR amplification

Total genomic DNA was prepared by using the HiGene Genomic DNA Prep Kit (Biofact, Daejeon, Korea) in accordance with the manufacturer's protocol, and partial region of 28S rDNA was amplified and sequenced by using the NL1 (5'–GCA TAT CAA TAA GCG GAG GAA AAG–3')/NL4 (5'–GGT CCG TGT TTC AAG ACG G–3') primer pair (O'Donnell, 1993). PCR amplification was examined in 1 × *Taq* reaction buffer, which contains 0.4 μL of 10 mM dNTP, 1 μL of 10 pM of each of the NL 1/NL 4 primer pair, and 0.2 μL of *Taq* DNA polymerase (Solgent, Daejeon, Korea). The PCR was conducted in a Veriti 96–well thermal cycler (Applied Biosystems,

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Carlsbad, CA, USA) under the following conditions: 95°C for 5 min; 35 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 1 min; and 72°C for 5 min for the final extension. The PCR products were purified by using ExoSAP–IT (GE Healthcare, Buckinghamshire, UK) and sequenced directly by Solgent Co. Ltd (Daejeon, Korea).

Phylogenetic analysis

All the obtained sequences of the partial 28S rDNA gene were compared with the available sequence data by using a BLAST search against the NCBI GenBank database. The sequence alignment was performed by using CLUSTALW (Thompson *et al.*, 1994) with the 28S rDNA sequences of allied species of Coleosporium spp. retrieved from the GenBank database. The phylogenetic tree was constructed by the maximum likelihood method, with 1,000 bootstrap replications, using MEGA 7 software.

RESULTS AND DISCUSSION

The collected samples were covered with pale green–to–yellow spots on the surface and numerous reddish or orange–yellow spots were found under the infected

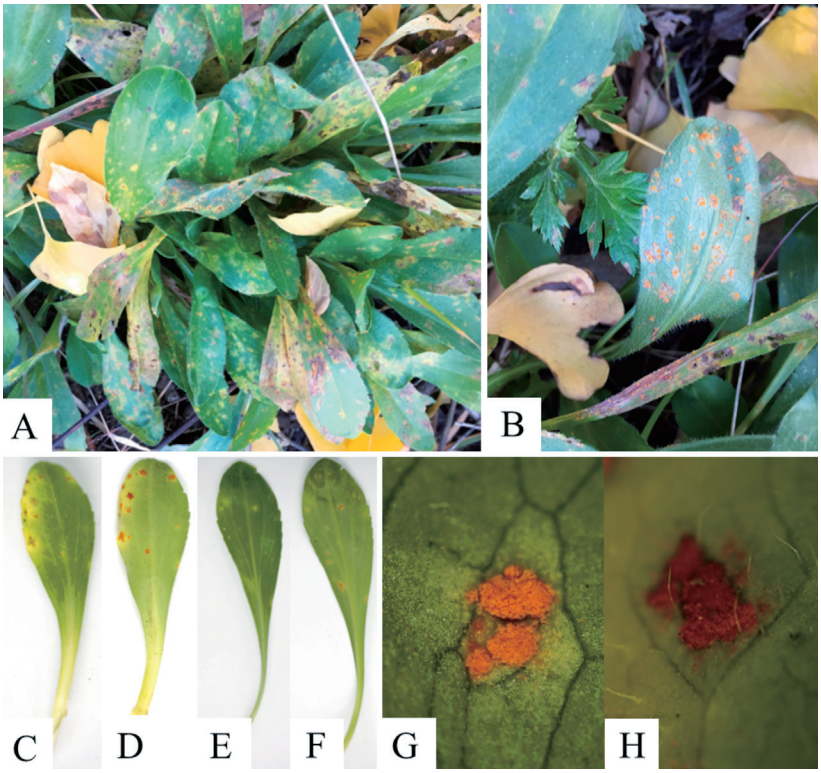

Fig. 1. Rust disease symptoms on rough fleabane leaves and photographs of the uredinium and telium. A: Yellow spots on the surface of severely infected leaves, B: Underside of diseased leaves, C and E: Surface of the infected leaf, D and E: Underside of C and E, G: Uredinium, H: Telium.

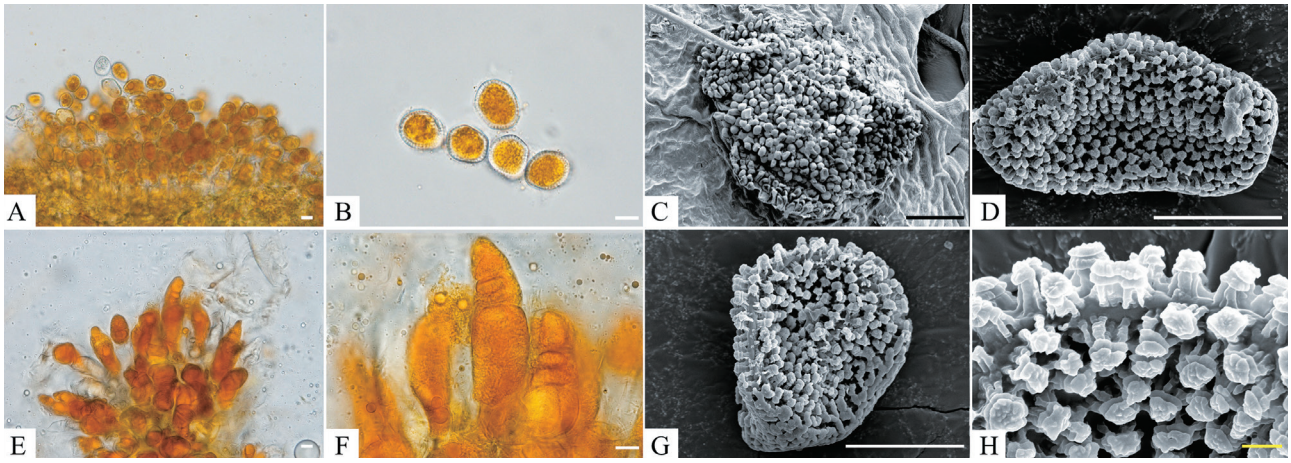

Fig. 2. Morphological characteristics of urediniospores and teliospores. A: Cross section of uredinium, B: Urediniospores observed under a light microscope, C: Uredinia observed under a scanning electron microscope, E: Cross section of telium, F: Teliospores observed under a light microscope, D and G: Feature of urediniospore, H: Ornamentation of urediniospore. White scale bar = 10 μm; black scale bar = 100 μm; yellow scale bar = 1 μm.

Table 1. Comparison of morphological characteristics of the urediniospores and teliospores of *Coleosporium* spp.

Characteristics		Present isolate	*Coleosporium plectranthi***	*Coleosporium asterum**	*Coleosporium asterum***
Urediniospore	Shape	ellipsoid to subglobose or polygonal	Subglobose, globose or ellipsoid	Subglobose to ellipsoid, but somewhat irregular and variable shape	Broadly ellipsoid to ellipsoid or polygonal
	Size	20.4–32.3 × 14.7–23.6	16.0–28.0 × 12.0–20.0	31.0–36.5 × 26.5–29.0	20.0–32.0 × 14.0–24.0
	Color	Orange–yellow	Yellow	Orange–yellow	Orange–yellow
	Size of verruca	0.8–1.1 broad 1.2–1.3 high	0.5–1.5 broad 0.8–2 high	–	0.8–2 broad 0.5–3 high
Teliospore	Shape	Septate, obovoid to ellipsoid	Oblong–ellipsoid to cylindrical	Single–celled and obovoid	Single–celled, obovoid or ellipsoid
	Size	72.1–85.1 × 21.0–24.0	42.0–85.0 × 16.0–23.0	73.0–86.5 × 22.0–37.0	45.0–90.0 × 19.0–30.0
	Color	Orange–red	Orange–red to orange	Orange–red	Orange–red

* Described by Back *et al*., 2014, ** Described by Hiratsuka *et al*., 1992.

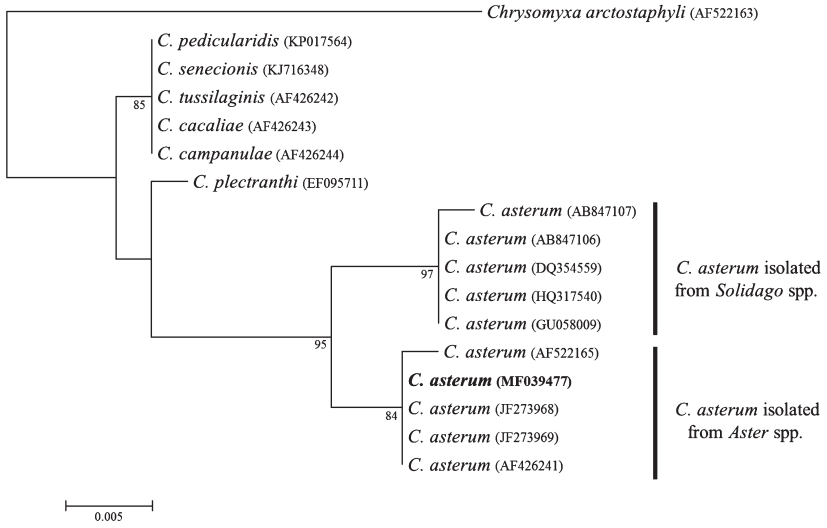

Fig. 3. Phylogenetic tree constructed by the maximum likelihood method, based on 28S rDNA gene sequences of isolated *C. asterum* sequences (MF039477) and allied species of *C. asterum* retrieved from NCBI GenBank. *Chrysomyxa arctostaphyli* (AF522163) was used as the outgroup. The numbers above the branches represent the bootstrap values obtained for 1,000 replicates (values over 80% were shown). The bar represents a phylogenetic distance of 0.005%.

leaves (Fig. 1A and 1B). The samples showed typical rust symptoms, with browning and premature senescence detected in severely infected leaves (Fig. 1A). To observe the detailed symptoms, each of the diseased leaves was observed separately. Yellow and green halo were found on the surface of diseased leaves (Fig. 1C–F), whereas orange–yellow uredinia and reddish telia were observed on the underside of the diseased leaves (Fig. 1G and 1H). Orange urediniospores were found scattered on severely infected leaves owing to the exposure of the urediniospores from the uredinia. The morphological characteristics of the urediniospores and teliospores were observed on diseased leaves of *E. strigosus* under the light microscope and SEM. The urediniospores were produced in a chain, yellow–orange, ellipsoid to subglobose or polygonal, verrucose with bluntly capitate, annulate tubercles, and 20.4–32.3 × 14.7–23.6 μm in size (Fig. 2A–D). The teliospores were septate, orange–red, obovoid to ellipsoid, and 72.1–85.1 × 21.0–24.0 μm in size (Fig. 2E–H). The morphological characteristics of the urediniospores and teliospores were similar to the description of *Coleosporium asterum*

(Hiratsuka, 1992). The comparison of the morphological characteristics among isolated rust fungi such as *C. plectranthus* and *C. asterum*, indicated that the isolated rust fungus was close to *C. asterum* (Table 1), although the isolated rust fungus was smaller than *C. asterum* found on Solidago sp. (Back *et al*., 2014).

The results of the sequencing analysis were obtained from rust fungal isolate and the partial 28S rDNA gene sequence (532 bp) was deposited in the NCBI GenBank database as MF039477. An NCBI search revealed that the obtained sequences showed 100% similarity to those of *Coleosporium asterum* (AF426241) found on *Aster ciliolatus*, of the family *Asteraceae*. The phylogenetic tree showed that the rust fungus isolated from rough fleabane was included in *C. asterum* group, but they were different from *C. asterum* that was isolated from *Solidago* spp. clade. Based on the phylogenetic and morphological analysis, the rust fungus observed on rough fleabane was confirmed as *C. asterum*.

The causal agent of pine needle rust, *C. asterum*, has been reported on *Erigeron* spp. in the USA and Canada (Farr and Rossman, 2013). However, there have been no previous reports on rough fleabane (*E. strigosus*). It has been reported that *C. asterum* is a host–alternating rust fungus between an herbaceous plant and *Pinus* spp. (Hedgecock, 1928). In Korea, *C. asterum* has been reported in nine hosts; tatarian aster, chrysanthemum, *Aster ciliolatus*, *A. ageratoides*, *A. scaber*, *A. pilosus*, and Korea goldenrod, red pine, and Korean pine (The Korean Society of Plant Pathology, 2009; Back *et al*., 2014; Park *et al*., 2012). Although *C. asterum* can infect numerous herbaceous plants, this was the first observation that *C. asterum* could infect *E. strigosus*. Thus, this is the first report of the morphological characteristics and phylogenetic analysis of *C. asterum* on rough fleabane and the occurrence of rust disease caused by *C. asterum* on rough fleabane in Korea. Furthermore, the additional investigation of rust disease on rough fleabane should be conducted to control and reduce the occurrence of pine needle rust disease in Korea.

AUTHOR CONTRIBUTIONS

S–Y. LEE designed the study, performed the microscopic observation, DNA analysis and wrote the paper. C–H. AHN performed the PCR experiment. H–Y. Jung and S. OHGA designed the study, supervised the work. All authors assisted in editing the manuscript and approved the final version.

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