RESEARCH NOTE

First Report of Smut Caused by *Urocystis eranthidis* on *Anemone flaccida* in Korea

Diane Avalos-Ruiz[1], Gi-Seok Seol[1], Seong-Keun Lim[1], In-Kyu Kang[2], Seung-Yeol Lee[1,3,4,*], and Hee-Young Jung[1,3,4]

[1]College of Agriculture and Life Sciences, Kyungpook National University, Daegu 41566, Korea
[2]Department of Horticulture, Kyungpook National University, Daegu 41566, Korea
[3]Institute of Plant Medicine, Kyungpook National University, Daegu 41566, Korea
[4]Department of Plant Protection and Quarantine, Kyungpook National University, Daegu 41566, Korea

[*]Corresponding author: leesy1123@knu.ac.kr

ABSTRACT

Abnormal symptoms were observed on *Anemone flaccida* in Korea, indicating an infection caused by smut fungi. Morphological and molecular analyses of the strain KNUF-UB were consistent with previous reports of *Urocystis eranthidis*. Therefore, this is the first report of smut caused by *U. eranthidis* on *A. flaccida* in Korea.

Keywords: *Anemone flaccida,* Smut, *Urocystis eranthidis*

The genus *Anemone* (family Ranunculaceae) has more than 150 species of flowering plants distributed globally, but is native to temperate zones of both northern and southern hemispheres [1,2]. *Anemone flaccida* (Flaccid anemone), a medicinal perennial grass distributed globally, has been used to traditionally treat rheumatism and neuralgia and has reported anticonvulsant, anti-inflammatory, anticancer, immunosuppressive, and antiviral activities [1,3,4]. In Korea, *A. flaccida* is located in southern regions such as Jeolla, Gyeongnam provinces, and Jeju Island, and has a high academic and horticultural value [5,6]. Previous studies have reported *A. flaccida* as a plant host for pathogens such as *Puccinia japonica*, *Ceraceopsora elaeagni*, and *Urocystis pseudoanemones* [7-9], the last one reported in Japan. However, no study exists about the disease occurrence on *A. flaccida* in Korea so far.

In April 2021, abnormal spots were observed on leaves and stems of *A. flaccida* at the historical landmark of Ban-Gu Jeong, the native habitat of *A. flaccida* in Haman, Gyeongnam province, Korea. The symptomatic areas presented gray-colored swellings or blisters on leaves and stems, containing a black powdery mass of spores (Figs. 1A-E). The morphological characteristics of the designated KNUF-UB strain were observed through light microscopy using a BX50 microscope (Olympus, Tokyo, Japan), and the spores were measured using Olympus cellSens imaging software. Sori in leaves presented irregular, globose spore balls of 21.96-39.36×15.75-22.71 μm consisting of 1-3 spores, surrounded by a layer of pale yellow, sometimes collapsed sterile cells (Figs. 1F-I). The observed symptoms matched previous *Urocystis eranthidis* reports [10,11], and the aforementioned morphological characteristics of KNUF-UB isolate agreed well with those previously recorded for *U. eranthidis* [12,13].

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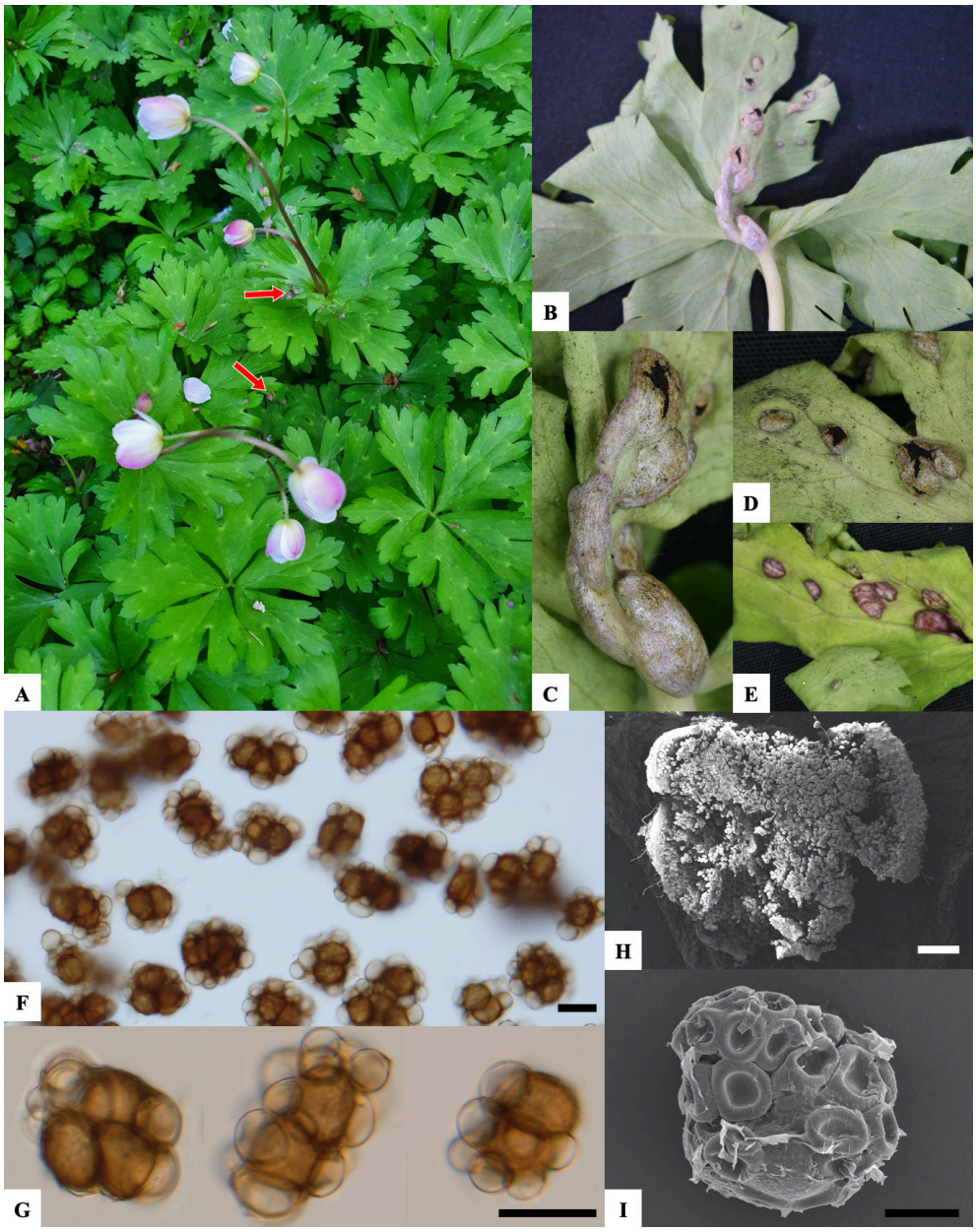

Fig. 1. Symptoms observed on *Anemone flaccida* and morphological characteristics of the KNUF-UB isolate. (A) Abnormal spots on leaves and stems. (B-E) Blisters containing a black powdery mass of spores. (F and G) Light microscopy of spore balls (H) SEM of spore balls (I) SEM of spore ball with collapsed sterile cells around. Red arrow: Natural symptoms on the host leaf; Black scale bar: 10 μm; White scale bar: 200 μm.

To identify the KNUF-UB strain, genomic DNA was extracted using a HiGene Genomic DNA Prep Kit (BIOFACT, Daejeon, Korea) following the manufacturer's protocol. Identification was conducted using the nuclear ribosomal internal transcribed spacer (ITS) region and the large subunit (LSU) ribosomal RNA gene [14]. For the ITS region, ITS1F/ITS4 [15,16] primer pair was used, and the LR0R/LR6 [17] primer pair was used for the partial LSU gene. Amplified PCR products were purified using ExoSAP-IT

PCR Product Cleaning Reagent (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced (Bioneer Co., Daejon, Korea). Sequences of 664 and 1,031bp were obtained from the ITS region and partial LSU gene, respectively. All obtained gene sequences were registered in the National Center for Biotechnology Information (NCBI) with the following GenBank accession numbers (OM250114, OM250116). A BLAST search in NCBI's database revealed that KNUF-UB had a 98.2% and 97.9% similarity with *U. eranthidis* MR95 (KY552912) and *U. eranthidis* hmk292 (JN367299) for the ITS region, respectively. For the LSU gene, KNUF-UB showed 99.8% similarity with *U. eranthidis* hmk292 (JN367324) and *U. eranthidis* M0065996 (EF517940). To identify the phylogenetic relationships, *Urocystis* spp. sequences were retrieved from NCBI's GenBank (Table 1), and a neighbor-joining phylogenetic analysis for each genetic marker was conducted using MEGA version X [18]. Consequently, the KNUF-UB strain was clustered together with *U. eranthidis* hmk292 in a clade with a high bootstrap value for both the ITS region and *LSU* gene (Figs. 2 and 3), strongly supporting the affiliation to the same species.

Urocystis eranthidis is a typical spring smut fungus first described in 1950 [19]. Smut fungi are pathogens that occur worldwide and mainly infect grasses by producing sori in various host plant organs [20,21]. The genus *Urocystis* (order Urocystidales, family Urocystidaceae) contains more than 170 species of smut fungi pathogenic to plants [22]. For the host plant family of Ranunculaceae, *Urocystis* can be observed causing sori on leaves, with spore balls of one to many fertile spores [11]. In *Anemone* species, *U. anemones*, *U. japonica*, *U. antipolitana*, and *U. pseudoanemones*, have been previously reported [9]. *U. eranthidis* has been reported as a pathogen for *Ceratocephalus falcatus* and *Eranthis hymenalis* [23-25]. However, it has not been previously reported on *Anemone* spp. In Korea, only three species of the genus *Urocystis* have been reported; *U. colchici, U. syncocca,* and *U. tritici* [26]. To the best of our knowledge, this is the first report of smut caused by *U. eranthidis* on *A. flaccida* in Korea, highlighting the importance of disease incidence research to understand and control this and other potential pathogens.

Table 1. GenBank accession numbers used in this study for phylogenetic analyses.

Species	Voucher	Country	ITS	LSU
Urocystis agropyri	WSP 72768	USA	KX057788	-
U. agropyri	WSP 72765	USA	KX057790	-
U. eranthidis	MR95	Iran	KY552912	-
U. eranthidis	hmk292	United Kingdom	JN367299	JN367324
U. eranthidis	WT1	China	HG934424	-
U. eranthidis	KNUF-UB	Korea	OM250114	OM250116
U. magica	Lluta 1	Chile	MH380193	MH380194
U. magica	Lluta 2	Chile	MK468896	MK474615
U. magica	Lluta 3	Chile	MK468897	MK474616
U. occulta	WSP 69497	USA	KX057774	-
U. occulta	WSP 69121	USA	KX057773	-
U. piptatheri	PUP Bot.202	Pakistan	MT073413	MT073414
U. tritici	WSP 72773	USA	KX057782	-
U. tritici	VPRI 19511	USA	KX057777	-
U. tritici	VPRI 18612	USA	KX057778	-
Ustilago sparsa	KVU892	India	JN367308	JN367335

ITS: Internal transcribed spacer region; LSU: Large subunit ribosomal RNA gene; -: Not available.

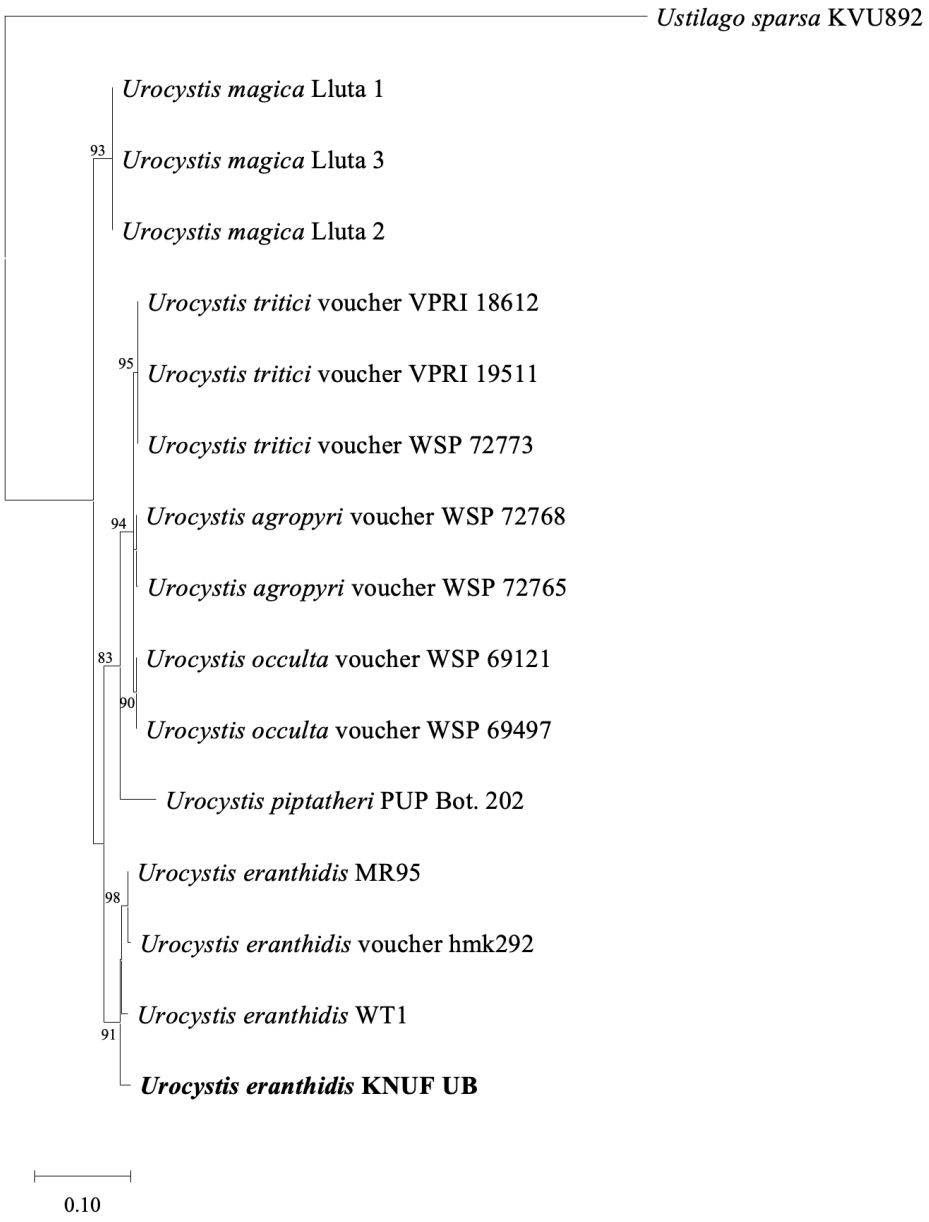

Fig. 2. Molecular phylogenetic analysis of internal transcribed spacer (ITS) region sequences of genus *Urocystis* inferred using the neighbor-joining method. The percentage in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Strain is shown in bold, and values lower than 70 are not shown.

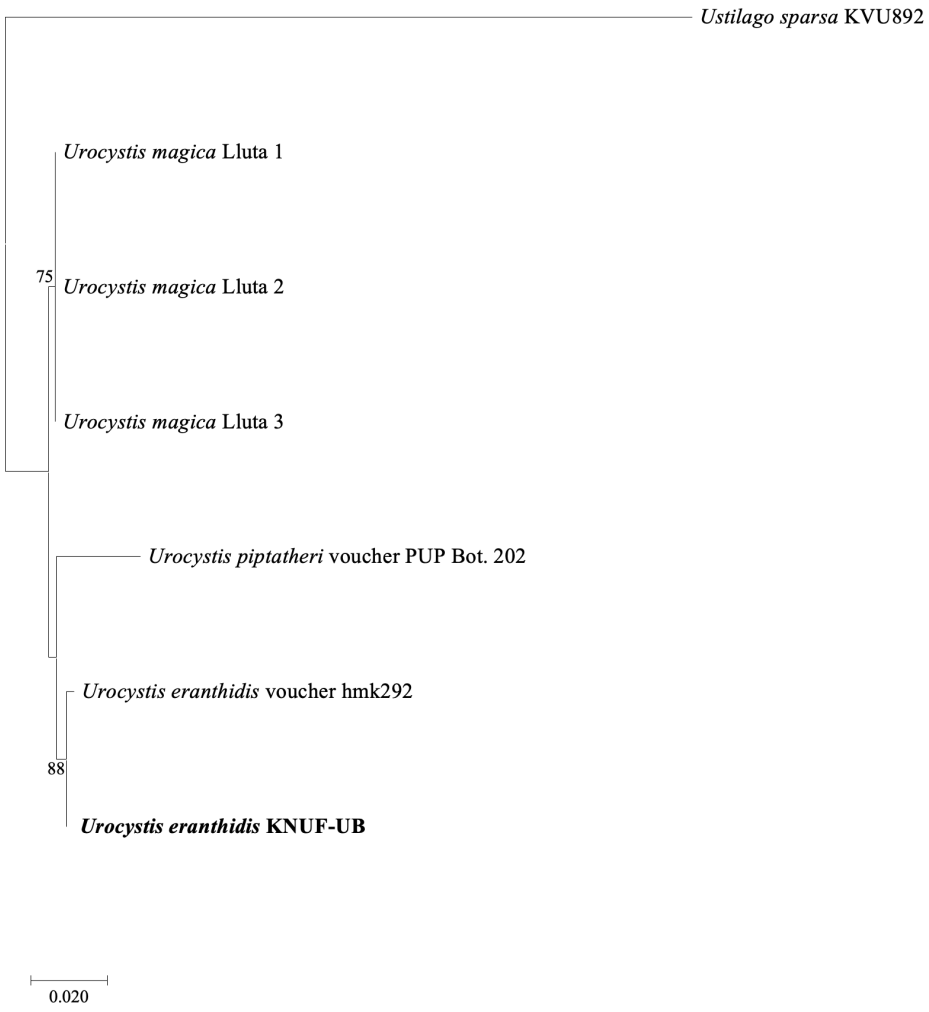

Fig. 3. Molecular phylogenetic analysis of large subunit (LSU) rRNA gene sequences of genus *Urocystis* inferred using the neighbor-joining method. The percentage in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Strain is shown in bold, and values lower than 70 are not shown.

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