

1 First Report of Leaf Spot in *Farfugium japonicum* Caused by
2 *Alternaria alternata* in China

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10 *Farfugium japonicum* (L.) Kitam (with the common name leopard plant) is
11 known as a garden and medicinal herb, and belongs to the family Asteraceae.
12 In May 2019, a leaf spot disease was observed on the upper leaf surface of *F.*
13 *japonicum* in Changsha city, Hunan province, China. More than 98% of the *F.*
14 *japonicum* plants were infected in a garden of Donghu district (28° 13' N; 112°
15 56' E). Leaf symptoms included small (1 to 10 mm in diameter), brown spots
16 that were circular, tan to gray in the center and distinct brownish-yellow
17 margins. Severely affected leaves were blighted and plants were dying. For
18 isolation, symptomatic leaf tissue was surface sterilized in 75% ethanol, rinsed
19 in sterile distilled water, and plated on potato dextrose agar (PDA) amended
20 with a 50 µg/ml streptomycin sulfate followed by incubation at 25°C in
21 darkness. By a single-spore isolation technique, pure fungal cultures were
22 obtained and displayed gray-brown and gray-white aerial mycelia after five
23 days of incubation. One representative isolate (HnAa-1) was selected for
24 further studies. Conidia of HnAa-1 were olive-brown, obpyriform, either
25 branched or unbranched with a short beak, 1 to 5 transverse septa, and 0 to 3
26 longitudinal or oblique septa. The conidia were 10 to 35 µm long and 2 to 12
27 µm wide. HnAa-1 was identified as an *Alternaria* sp. based on the
28 morphological characterization by Simmons (1). Further identification to

1 species level was made by molecular analyses. DNA of HnAa-1 was extracted
 2 from the regions internal transcribed spacer (ITS), glyceraldehyde-3-phosphate
 3 dehydrogenase (GAPDH) and partial Alt a 1 major allergen (ALT) gene.
 4 Amplification and sequencing were carried out with the method described
 5 by Woudenberg et al. (2). BLASTn searches showed that the ITS, GAPDH and
 6 ALT sequences had the highest similarity with *A. alternata* strains, with 100%
 7 (548/548) identities for ITS (GQ169728), 100% (567/567) identities for GAPDH
 8 (MK903028) and 99.36% (466/469) identities for ALT (MN184998). Moreover,
 9 the ITS, GAPDH and ALT sequences had more than 99% identities with the
 10 epitype CBS 916.96 of *A. alternata* (ITS: AF347031; GAPDH: AY278808; ALT:
 11 AY563301). The ITS, GAPDH and ALT sequences of HnAa-1 were submitted
 12 to GenBank (Accession No. MT767170, No. MW115639 and No. MW316727).
 13 Pathogenicity tests were conducted by spraying a 10 ml conidial suspension
 14 (1.0×10^5 conidia /mL) on surfaces of leaves of three healthy plants (8-week-old).
 15 Leaves of three healthy plants were sprayed with sterile distilled water as a
 16 control treatment. All inoculated plants were maintained in a growth chamber
 17 at 25°C with a 12-h photoperiod. The pathogenicity test was repeated twice.
 18 After five days of inoculation, typical brown spots and necrotic lesions similar
 19 to those observed in the field, had developed on all inoculated plants but not
 20 on water-treated control plants. *Alternaria alternata* was re-isolated from the
 21 symptomatic tissue of inoculated plants but not from the control plants, and re-
 22 identified with morphological and molecular methods, which fulfilled Koch's
 23 postulates. This host-pathogen association has been reported in Korea (3), but
 24 it is the first report of *A. alternata* causing leaf spots on *F. japonicum* in China.
 25 Since *A. alternata* is a ubiquitous and very important plant pathogen causing
 26 leaf spot diseases in over 100 species of plant, the occurrence of this disease is
 27 a serious threat to *F. japonicum* and might lead to economic losses. Therefore,
 28 appropriate prevention strategies for *F. japonicum* should be adopted.

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2 Fund of Hunan Agricultural University (YB2019005) and
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4 **References:**

- 5 (1) Simmons, E. G. 2007. *Alternaria*: An Identification Manual. CBS Fungal
6 Biodiversity Centre, Utrecht, the Netherlands.
7 (2) Woudenberg, J. H. C., et al. 2015. Stud. Mycol. 82:1-21.
8 (3) Lee, J. H., et al. 2013. Plant Dis. 97(10):1382.

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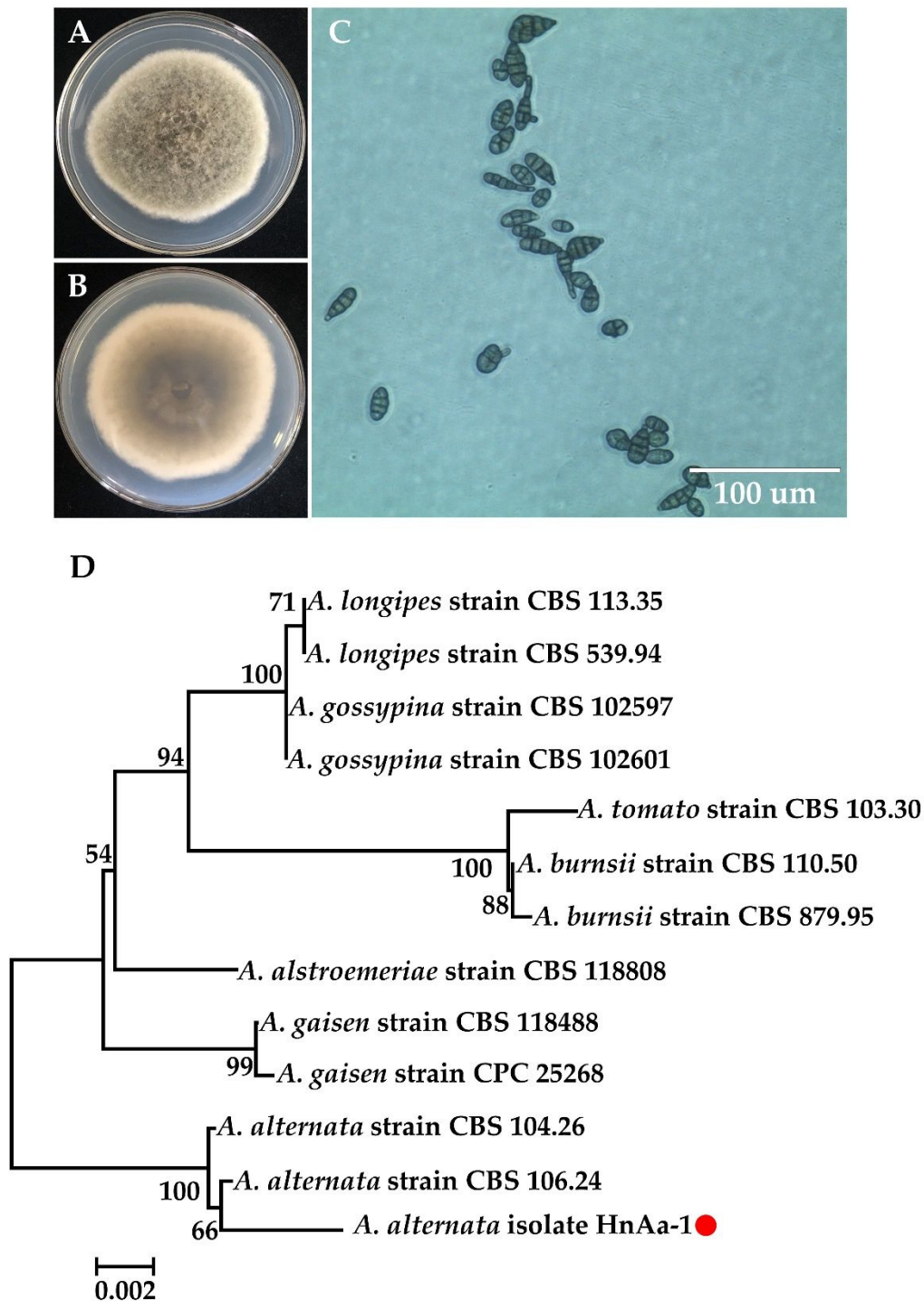


Figure S1. Morphology of a 7-day old culture of *Alternaria alternata* (HnAa-1) isolated from *Farfugium japonicum* and grown on PDA at 25°C in darkness; (A) colony surface, (B) colony reverse, and (C) conidia. Phylogenetic tree was constructed based on the ITS, GAPDH and ALT sequences of HnAa-1 and other *Alternaria* strains. HnAa-1 was indicated by a red dot.

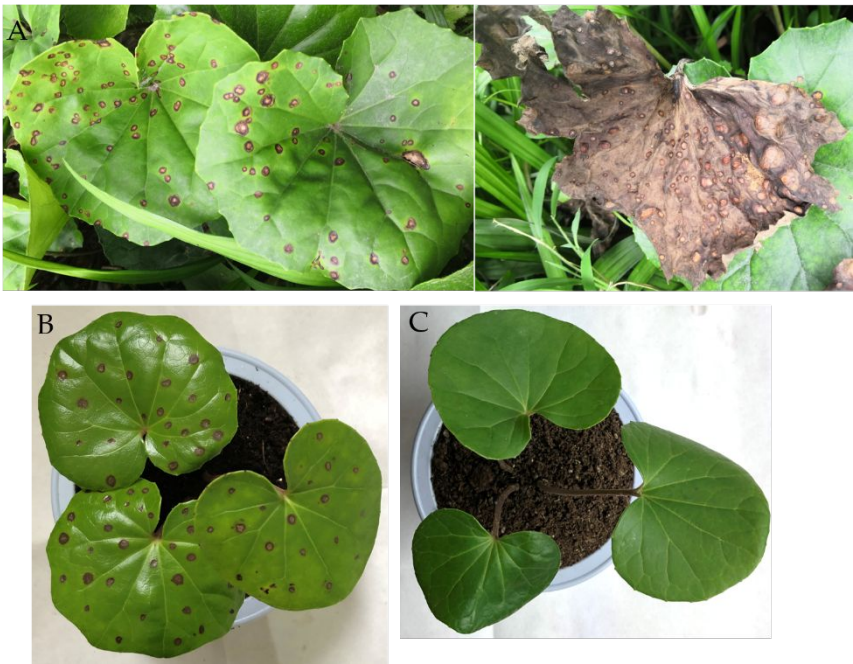


Figure S2. Symptoms caused by *Alternaria alternata* on *Farfugium japonicum*; **(A)** under field conditions, **(B)** 5-days after inoculation with spore suspension, and **(C)** noninoculated control plant.