

First report of leaf spot on *Clematis brevicaudata* DC. caused by *Alternaria alternata* in China

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Clematis brevicaudata DC. is distributed in China, Korea, Mongolia, Russia and Japan. This plant is both ornamental and medical, used in the treatment of nervous disease, dyskinesia and other diseases. In September, 2019, a leaf spot on *C. brevicaudata* was first found in a 5 ha *C. brevicaudata* plantation in Harbin, Heilongjiang Province, China. The incidence was about 80%. The symptoms were elliptical, circular, or irregular brown to black necrotic lesions in leaf apex and leaf margin. Ten fresh sample leaves with typical symptoms were collected from ten *C. brevicaudata* plants. The tissues (5mm × 5mm) between symptomatic and healthy junction were cut and surface disinfected in 75% ethanol, and with 7% NaClO for 1 min, then rinsed three times with sterilized water, 30s each time. The sterilized tissues were inoculated on potato dextrose agar (PDA) plates for 7 days at 25°C. The colonies were obtained and transferred onto new PDA and potato carrot agar (PCA) plates by single spore method to further purify. After 7 days, the colonies on PDA were 50 to 63 mm in diameter, circular, grayish brown, with white aerial hyphae. A total of 150 conidia on PCA were single or in chains, ovoid, inverted pear, 2 to 7 transverse septa, 0 to 3 longitudinal or oblique septa, 17.5 to 57.5 × 7.5 to 17.5 µm. Beaks and supposititious beaks were mostly columnar, rarely conical, 2.5 to 6.0 × 2.0 to 3.0 µm. Conidiophores were solitary or clustered, pale brown, erect or bent, branched or unbranched, separated, 112.0 to 151.0 × 5.1 to 14.7 µm. Ten isolates purified on PDA were obtained. Morphological identification showed the ten isolates were similar and appeared to be *Alternaria alternata* (Simmons, 2007). Two strains from ten isolates were selected for molecular identification. Genomic DNA was extracted from mycelia of two isolates (LD2020520 and LD2020521) on PDA using a modified CTAB method. Internal transcribed spacer rDNA regions (ITS), RNA polymerase II second largest subunit gene (RPB2), *Alternaria* major allergen (Alt a 1), endopolygalacturonase (endoPG) and glyceraldehyde 3-phosphate dehydrogenase (gpd) were amplified and sequenced using two directional sequencing with the primers ITS1/ITS4, RPB2-F/RPB2-R, Alt-F/Alt-R, end-F/end-R and gpd-F/gpd-R (Woudenberg et al. 2015). The sequences obtained were deposited in GenBank (ITS: MT501762, OK571395; RPB2: MT506027, OK631891; Alt a 1: MT506026, OK631890; endoPG: ON054189, ON054188; gpd: ON054191, ON054190). The phylogenetic analysis of maximum-likelihood tree by MEGA 7 software showed that the two isolates had 99% identity with the *A. alternata* CBS 916.96. For pathogenicity testing, eighteen leaves of six 5-week-old plants were sprayed with spore suspensions (1×10⁶ spores /mL) of the 7 days-old isolates LD2020521 and LD2020520 (Each isolate infected three plants and each infected three leaves). Three plants were sprayed with sterile distilled water as a control group. The plants were incubated at 25°C. After 15 days, taupe irregular spots appeared on the leaves. The pathogenicity test was repeated three times. The same fungi were re-

isolated from the inoculated leaves and with the same morphological and molecular characteristics as LD2020520 and LD 2020521, fulfilling Koch's postulates. No fungi were isolated from the control group. This is the first report of leaf spot on *C. brevicaudata* caused by *A. alternata*. Leaf spot can reduce the yields of *C. brevicaudata*. This study provides a reference for the prevention and treatment to the leaf spot of *C. brevicaudata*.

References

Simmons E. G, (2007) *Alternaria*: an identification manual. CBS Fungal Biodiversity Centre, Utrecht, The Netherlands.

Woudenberg, J. H. C., et al. 2015. Stud. Mycol. 82:1.

Acknowledgments

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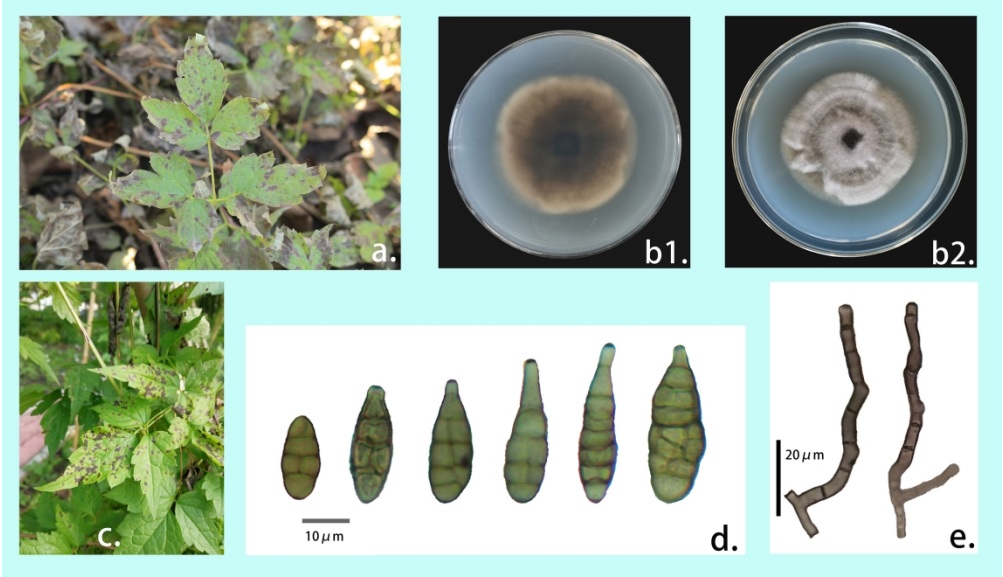


Fig. 1. Symptoms and morphology of *Alternaria alternata* on *Clematis brevicaudata* DC. (a) Symptoms of leaf spot on *C. brevicaudata* DC. (b1-b2) Colony on PDA. (c) The leaf spots of the inoculated plants (pathogenicity test). (d) Conidia. (e) Conidiophores.

299x173mm (300 x 300 DPI)

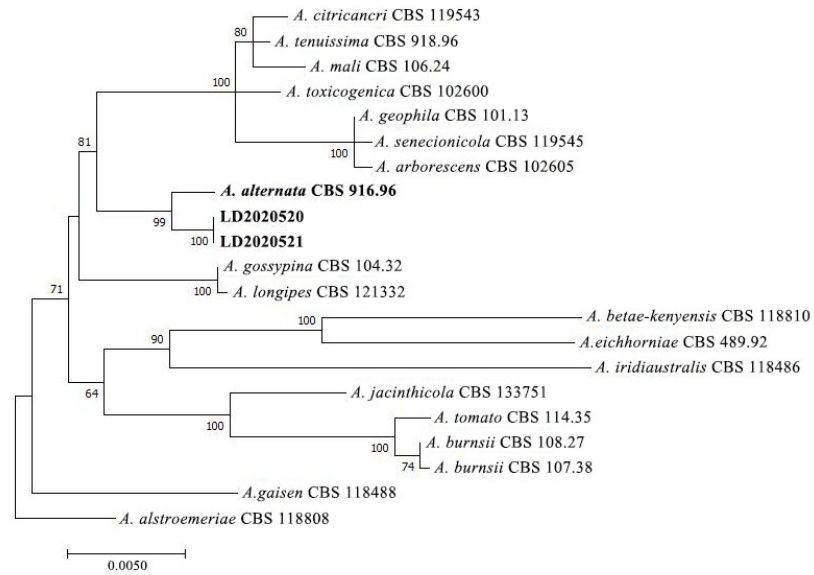


Fig. 2. The Maximum Likelihood tree (The evolutionary history was inferred by using the Maximum-Likelihood based on the Tamura-Nei mode) from combined ITS, RPB2, Alt a 1, gpd and endoPG gene sequences of the isolates LD2020520, LD2020521 and its related species. Bootstrap values were calculated from 1000 replicates. Phylogenetic analysis was performed in MEGA 7.

253x195mm (96 x 96 DPI)