Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Blight on *Paphiopedilum* Caused by *Curvcladiella* sp. (GZCC19-0342) in China

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Plants in the genus *Paphiopedilum* (Orchidaceae), known as “slipper orchids”, have unique and exotic flowers (Luan et al. 2019). Due to habitat destruction and overexploitation, *Paphiopedilum* species have become highly endangered. All wild species are listed in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (Luo et al. 2003). Large-scale production can meet the demand and protect wild resources effectively. In China, the *Paphiopedilum* industry was negatively influenced by diseases, including stem rot (*Fusarium oxysporum*) (Xu et al. 2014) and anthracnose (*Colletotrichum gloeosporioides*) (Xu et al. 2018). In August 2019, leaf blight was observed on about 10% of leaves of *Paphiopedilum* cultivated in the Guizhou Botanical Garden (26°37'N, 106°43'E), Guiyang, Guizhou province, China. Typical symptoms on leaves were irregular, brown, dead spots. The causal agent was isolated using a slightly modified tissue isolation: pieces of leaves (5 × 5 mm), each with half diseased and half healthy, were sterilized by 75% ethanol for 5 to 10 s, rinsed three times with sterilized distilled water (SDW), placed on PDA, and incubated at 25°C for 2 days; mycelia were transferred to PDA and incubated for 10 days at 25°C to get pure cultures (Fang 2001). The colony color became light beige to light yellow on the surface, brown on the reverse, and the edge was irregular. The artificial infection test showed that the colonies of the leaf blight were white, scattered, and hairy. Conidiophores were straight to flexuous, consisting of a stipe bearing a penicillate arrangement of fertile branches, stipe septate, hyaline, smooth; stipe extensions septate, straight or curved, dull and tapering toward the apex, 288.1 (128.5 to 549.9) µm long. The conidiogenous apparatus had

many branches, each terminal branch producing two to four phialides, phialides doliform to reniform, hyaline, aseptate, apex with minute periclinal thickening and inconspicuous collarette. Conidia cylindrical, rounded at both ends, straight, one-septate, hyaline, 34.2 (30.5 to 42.0) × 3.2 (2.6 to 3.9) µm. In the culture, chlamydospores were found, thick-walled, ellipsoidal or sphaeropedunculate, brown to hyaline, 16.3 (9.0 to 23.1) × 10.8 (8.1 to 15.4) µm. Morphological characteristics were similar to the description of *Curvcladiella* (Decock and Crous 1998), but there were some differences from *Curvcladiella cigna*, the only species of the genus. To confirm identity, the 28S large subunit (LSU), calmodulin (*cmdA*), histone H3 (*his3*), the internal transcribed spacer region and intervening 5.8S nrRNA (ITS), translation elongation factor 1- α (*tef1*), and β -tubulin (*tub2*) genes were amplified using primers LR0R/LR5, CAL-228F/CAL-2Rd, CYLH3F/CYLH3R, ITS5/ITS4, EF1-728F/EF2, and T1/CYLUB1R (Lombard et al. 2015), respectively. The sequences of the PCR products were deposited in GenBank as MT279199 (LSU), MT294104 (*cmdA*), MT294105 (*his3*), MT279198 (ITS), MT294103 (*tef1*), and MT294102 (*tub2*). Based on the phylogenetic tree and morphology, the fungus was identified as a likely new species of *Curvcladiella*. To complete Koch's postulates, 10 healthy plants of *Paphiopedilum* (2 years old) were washed with 75% ethanol, rinsed three times with SDW, punctured with a sterilized needle, and inoculated with 10-day-old mycelia (at 25°C on PDA) discs (3 mm in diameter) on the leaves. Five corresponding healthy plants were inoculated with PDA as controls. They were incubated at 25°C. Small brown spots were visible after 3 days. After 21 days, the symptoms were similar to those observed in the field. Early on, the brown lesions spread into a large area; later, the lesions became dark brown. Finally, whole leaves withered. Symptoms developed on all inoculated rhizomes but not on the controls. The same pathogenic fungus was reisolated from the infected rhizomes. This is the first report of leaf blight on *Paphiopedilum* caused by *Curvcladiella* sp. (GZCC19-0342) in the world.

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