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Curvularia curculiginis causes leaf spot and blight on *Curculigo capitulata* in China

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Abstract
Leaf spot and blight on *Curculigo capitulata* was found in Fair Lake Botanical Garden, Shenzhen, Guangdong, China in 2018. *Curvularia curculiginis*, identified by morphology, was associated with the symptoms. Molecular barcodes from the internal transcribed spacer region of rDNA (ITS), and partial fragments of glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) and translation elongation factor 1-α (*tef1*) genes were provided. Pathogenicity tests showed for the first time that *C. curculiginis* was pathogenic on leaves of its host.

Keywords Amaryllidaceae · *Curvularia* · Morphology · Phylogenetic analysis · Pathogenicity

Curculigo capitulata, belonging to the family Amaryllidaceae, has a long history of medical use in China and India (Kirtikar and Basu 1935; Nie et al. 2013). *Curvularia curculiginis*, *Pseudocercospora curculiginis* and *Puccinia curculiginis* were associated with *Curculigo capitulata* according to U.S. National Fungus Collections Fungus-Host Database (https://nt.ars-grin.gov/fungaldatabases/fungushost/fungushost.cfm) and previous publications (Arthur and Cummins 1936; Guo and Liu 1992; Zhang and Zhang 2003). Among them, *C. curculiginis* was described as a new species based on morphology by Zhang and Zhang (2003). The species was collected from living leaves of *Curculigo capitulata*, in Nanning city, Guangxi province, China. However, there is no mention about its pathogenicity (Zhang and Zhang 2003; Zhang and Sun 2010). In August 2018, leaf spot and blight of *Curculigo capitulata* was observed in Shenzhen Fair Lake Botanical Garden, Shenzhen city, Guangdong province, China. Many small to large, round or irregular brown necrotic spots surrounded with chlorotic halos occurred throughout the leaves. The apices were normally wilted. In serious cases, leaves were entirely withered (Fig. 1a-b).

Diseased leaf samples were cut into small pieces, and then placed into Petri-dishes with moist filter papers. After incubation at 25 °C, conidia similar to *Curvularia* spp. were encountered from tissues. To obtain pure cultures, conidia were singly picked using sterile glass needles and transferred onto potato dextrose agar (PDA) according to the method of Luo et al. (2018). Strains were deposited in the Culture Collection of Yangtze University (YZU) in Jingzhou, China. The colonies of those strains were identical to each other. Hence, one isolate YZU 181230 was selected for further study to identify the species. The colony characteristics were determined on PDA and conidial morphology was studied on water agar (WA) incubated at 25 °C in darkness for 7 days. Conidia were mounted in sterile distilled water and examined under a Nikon ECLIPSE Ni-U microscope system (Nikon, Japan). Colony on PDA was velutinous, margin fimbriate, pale smoke grey to greyish sepia, buffer to purplish grey, 77–78 mm diam. (Fig. 2a). On WA, conidiophores were straight or flexuous, normally 60–133 μm long, 4–6 μm wide. Conidia were ellipsoidal, obovoid or obclavate, 23–47 × 13–23 μm in size with 3 transverse septa among which the middle one was the thickest septum (Fig. 2b-d). Morphologically, the isolate was identical to *C. curculiginis* with the holotype of HSAUP 992140 (Zhang and Zhang 2003; Zhang and Sun 2010).

Genomic DNA of the strain YZU 181230 was extracted from fresh mycelia grown on PDA using the method of Cenis (1992). Three gene regions were amplified, namely the internal transcribed spacer region of rDNA (ITS), glyceraldehyde-

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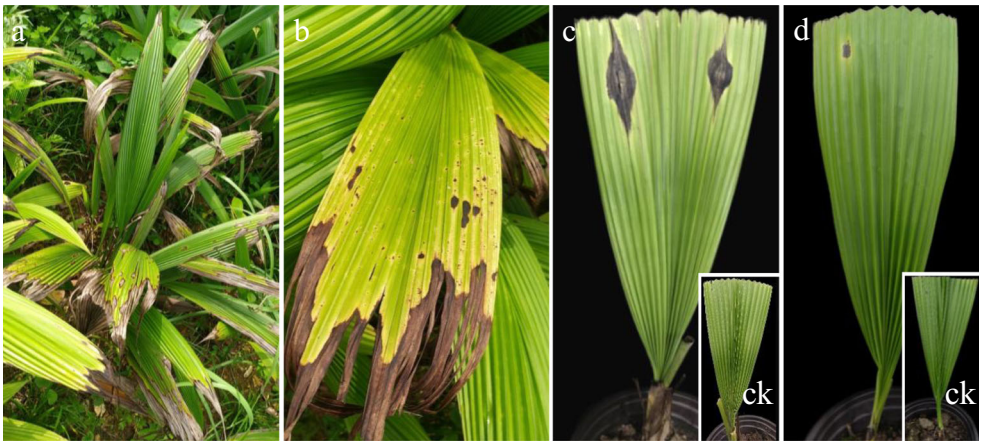

Fig. 1 Symptoms caused by *Curvularia curculiginis* on *Curculigo capitulata*: **a-b** leaf spot and blight in field, **c-d** necrotic spots induced by mycelia plugs and spore suspension, respectively, on wounded (left) and unwound (right) of living leaves after 10 days at 25 °C (ck = control)

3-phosphate dehydrogenase (*gapdh*) and translation elongation factor 1-α (*tef1*) using BIO-RAD T100™ Thermal Cycler (BioRad, CA, USA). The primer pairs were selected according to Tan et al. (2014): ITS5/ITS4 (White et al. 1990) for ITS, gpd1/gpd2 (Berbee et al. 1999) for *gapdh*, EF1983/EF12218R (Schoch et al. 2009) for *tef1*. The PCRs were amplified in 25 μL of reaction mixture including 12.5 μL 2 × Taq PCR Starmix (Genstar, Beijing, China), 1.25 μL of each primer, 2 μL template DNA, and 8 μL sterile distilled water. Successfully amplified products were sequenced by BGI (Beijing, China) with both primers. The obtained sequences were deposited in GenBank with accession numbers of MK507796 (ITS), MK507794 (*gapdh*) and MK507795 (*tef1*). A maximum likelihood tree was constructed using

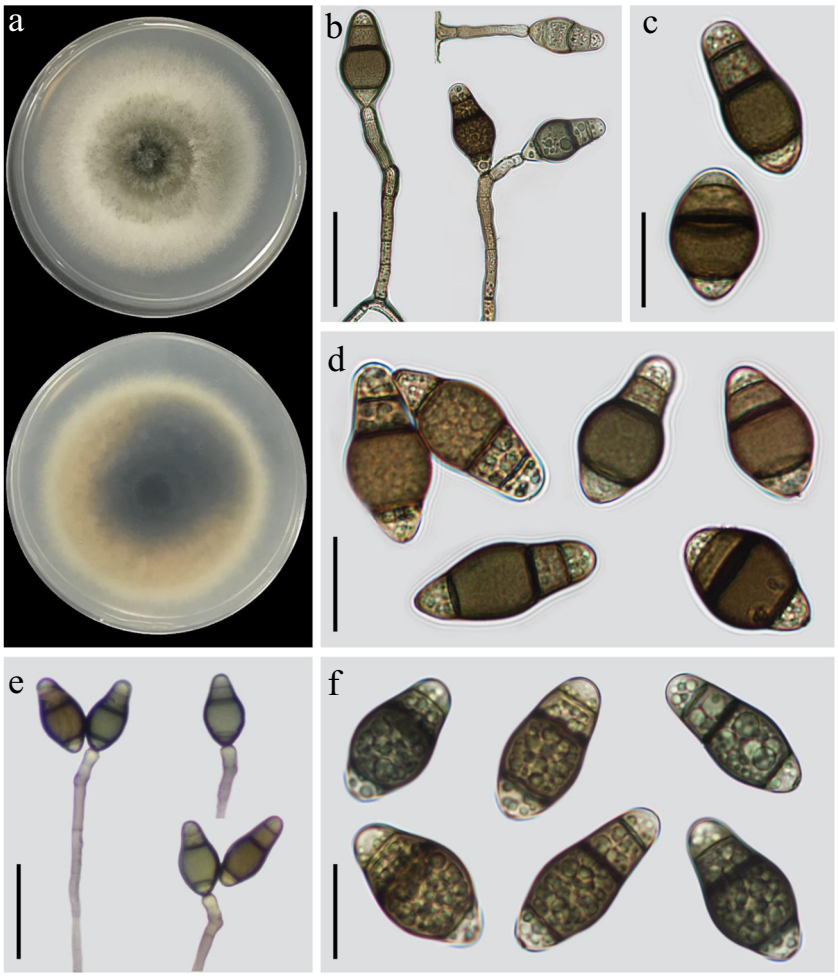

Fig. 2 Morphology of *Curvularia curculiginis* YZU 181230: **a** colony on PDA for 7 days at 25 °C, **b** sporulation patterns on WA at 25 °C, **c-d** conidia on media, **e** sporulation patterns from leaf symptoms at 25 °C, **f** conidia from host. Scale bars: b, e = 40 μm, c, d, f = 20μm

representative isolates of 24 relevant species (Tan et al. 2018) to determine the phylogenetic relationship (Fig. 3). Each gene sequence was aligned and concatenated for a combined analysis using MEGA v7.0 (Kumar et al. 2016). The maximum likelihood analysis with 1000 bootstrap replicates was performed in RAxML program (Stamatakis et al. 2008) using the GTRCAT model of nucleotide substitution. In the resulting phylogram, the present isolate was demonstrated as a separate species of *Curvularia* which supported the morphological results of *C. curculiginis* (Zhang and Zhang 2003). Its closest phylogenetic species is *C. reesii* collected from air in Australia (Tan et al. 2018).

Morphologically, conidia of *C. curculiginis* were larger than those of *C. reesii*, which were (28–) 31–35 (−39) × (10–) 12–13 (−14) μm in size (Tan et al. 2018). Conidia of *C. curculiginis* were similar to *C. oryzae* (24–40 × 12–22 μm) and *C. tuberculata* (23–52 × 13–20 μm) in size (Sivanesan 1987), but it was different from *C. oryzae* in having a thick middle septum of conidia and distinct from *C. tuberculata* without verruculose ornamented cell walls (Zhang and Sun 2010).

Pathogenicity of the isolate YZU 181230 was determined on leaves of potted *Curculigo capitulata*. The leaves (over 20 cm high) were sprayed with 70% ethanol prior to inoculation. Mycelia disks (3 mm in diam.) and 20 μL spore suspension (10^5 spores per mL) were placed onto needle-wounded and unwounded areas on leaves (Fig. 1). Controls were treated with pure PDA disk and distilled water. The inoculated plants were placed in a greenhouse with a 12 h fluorescent light period around 25 °C. Large necrotic spots (around 12 × 45 mm in size) surrounded with yellow halos were observed on mycelia disc inoculated leaves at 10 days, while the smaller similar symptoms (around 3 × 10 mm in size) were induced only on wounded leaves by spore suspension. Controls were symptomless after 10 days.

To examine the morphology of the fungus from host, the fungus was grown from the inoculated tissues using the method as previously described for isolation. Conidia were re-isolated and matched to *C. curculiginis*. At the same time, the sporulation patterns and conidial characteristics were observed (Fig. 2). The conidial morphology on the host was identical to that grown on WA. Only the conidia size was

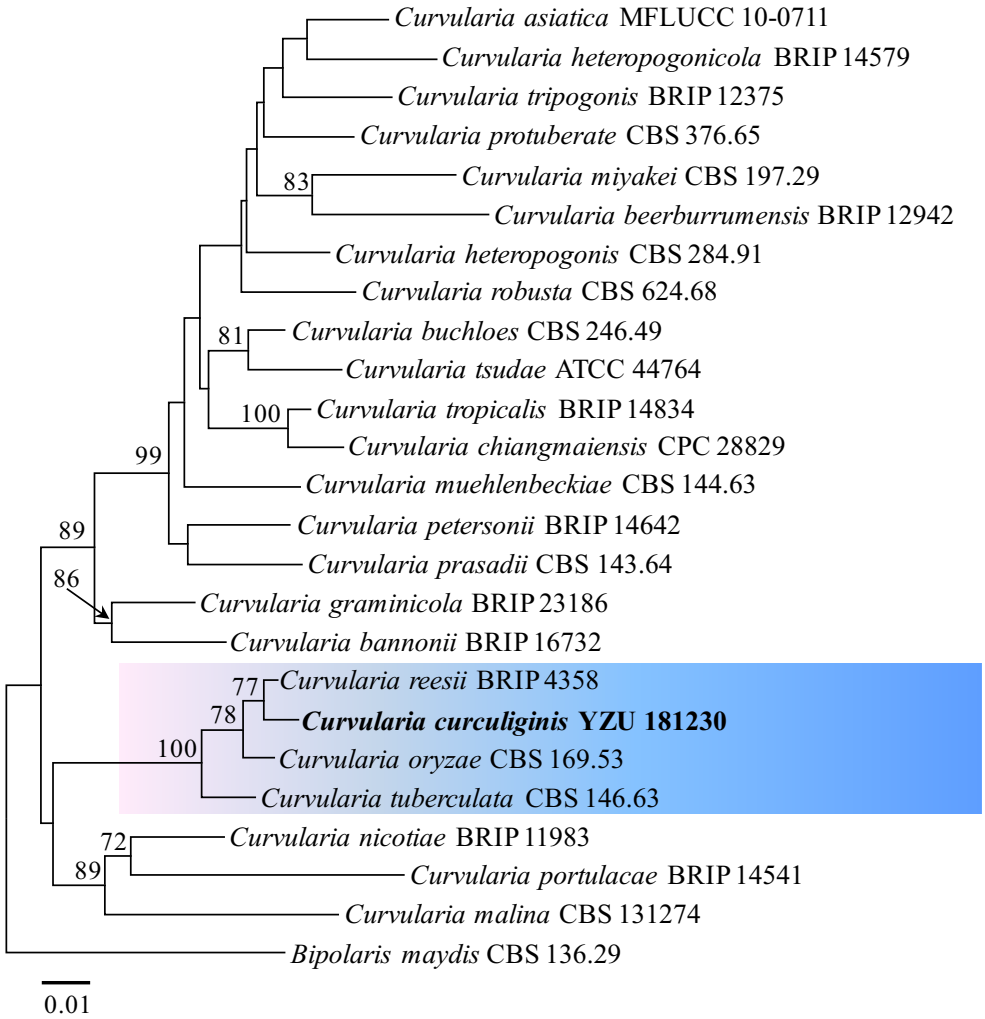

Fig. 3 Maximum likelihood phylogenetic tree of *Curvularia curculiginis* generated from the combined dataset of ITS, *gapdh* and *tef1* gene sequences. Bootstrap values (>70%) from 1000 replicates are shown at nodes. The bar indicates the number of substitutions per position

smaller at 28–41 × 13–20 μm (Fig. 2e-f). The results indicated that *Curvularia curculiginis* is a causal agent to induce leaf spot and blight on *Curculigo capitulata*.

We showed the species placement of *C. curculiginis* with a phylogenetic species concept based on three gene regions. This supported the morphological taxonomy described by Zhang and Zhang (2003). We further showed that *C. curculiginis* is a pathogen of *Curculigo capitulata*.

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