First Report of *Phaeoacremonium minimum* associated with grapevine trunk diseases in China

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Grapevine trunk diseases (GTDs) are a complex of different diseases that strongly affect grape productivity, wine quality and vineyard longevity worldwide. The GTDs occur in diverse regions and in some areas the disease agents are present in up 100% of the vines (Pintos et al. 2018). During the last few years, species belonging to the genera *Botryosphaeria* and *Diaporthe* were identified as fungal pathogens associated with grapevine trunk diseases in China (Yan et al. 2013; Dissanayake et al. 2015). During 2017 to 2018, interveinal leaf necrosis, wood white decay where surrounded by brown necrosis in longitudinal section and black spots in cross section were observed in several grapevines (Cabernet) in Huailai county of Hebei Province, China. The disease incidence was up to 2-3% of the vineyard. The diseased samples (whole trunk and root of 19 years old grapevines) were collected and taken into the laboratory. The woody samples were cut into small chips to isolate the pathogens, and they were surface sterilized in 1.5% sodium hypochlorite for 3 min, followed by 70% ethanol for 30 sec., and rinsed three times with sterile distilled water. Once the samples were dried, they were placed onto potato dextrose agar (PDA) plates amended with ampicillin (0.1 g L^{-1}). The plates were incubated at 25°C under dark conditions. After 14 days of incubation, hyphal

tips of fungi growing from wood pieces were transferred onto new PDA plates and incubated until they produced conidia. One type of colony was consistently isolated from the discolored tissue, with honey brown colored mycelium, and producing a yellow pigment on PDA. Conidia were ellipsoid to allantoid, 3.8 to 6.2 μm long, and 1.6 to 3.2 μm wide (n=50). Morphologically these isolates resembled species belonging to genus *Phaeoacremonium* (Mostert et al. 2006). For species confirmation, genomic DNA of three representative isolates (JZB3190001, JZB3190003 and JZB3190005) was extracted. The PCR amplification was performed using two phylogenetic markers (actin and β-tubulin) amplified with primers ACT-513F/ ACT-783R (Carbone and Kohn 1999) and T1/Bt2b (Glass and Donaldson 1995). The sequences obtained in this study were deposited in GenBank under the accession numbers of MK994188, MK994189, MK994190, MK994191, MK994192 and MK994193. Phylogenetic analysis was conducted using maximum likelihood (ML) in RAXML which was accomplished using RAxML-HPC2 on XSEDE in the CIPRES Science Gateway platform (http://www.phylo.org/). In the phylogenetic tree, the isolates from the present study clustered together with *Phaeoacremonium minimum* (CBS 246.91), with 100 bootstrap values. Based on both morphological characters and phylogenetic results, the species isolated in this study were identified as *P. minimum* (Tul. & C. Tul.) D. Gramaje, L. Mostert & Crous (Gramaje et al.， 2015). The pathogenicity test was conducted on healthy, young, two months old, rooted 'Furcal' grapevines which were grown in inoculation chamber. The roots of plants were washed using flow water, and rinsed with sterile distilled water. The end of the sterile water treated roots (roughly 1cm) were cut using a sterilized scissor. Then, dipping the roots and base of trunk of 'Furcal' grapevines in a 10^6 per mL *P. minimum s*pore suspension for 30 minutes. Inoculated plants were immediately planted in the individual potted pot and 10 mL of spore suspension was added in the soil per pot. Sterile distilled water was used as control. Two *P. minimum* isolates (JZB3190001, JZB3190003) were used to do the pathogenicity test and ten plants were inoculated with each isolate and the control water. Temperature the of inoculation chamber was controlled between 24 and 25°C, and humidity was maintained at 60%. After 114 days of inoculation, the inoculated plants developed black necrosis at base of wood, but did not show leaves necrosis, while the control plants showed no symptoms both in wood and leaves. Koch's postulates were confirmed by re-isolating and identification based on cultural and morphological characters of the inoculated isolates.

To our knowledge, this is the first report of *P. minimum* associated with grapevine trunk diseases in

China. The results of this study will enhance the capability of controlling GTDs in China by correct identification of the causal organism.

Acknowledgements：

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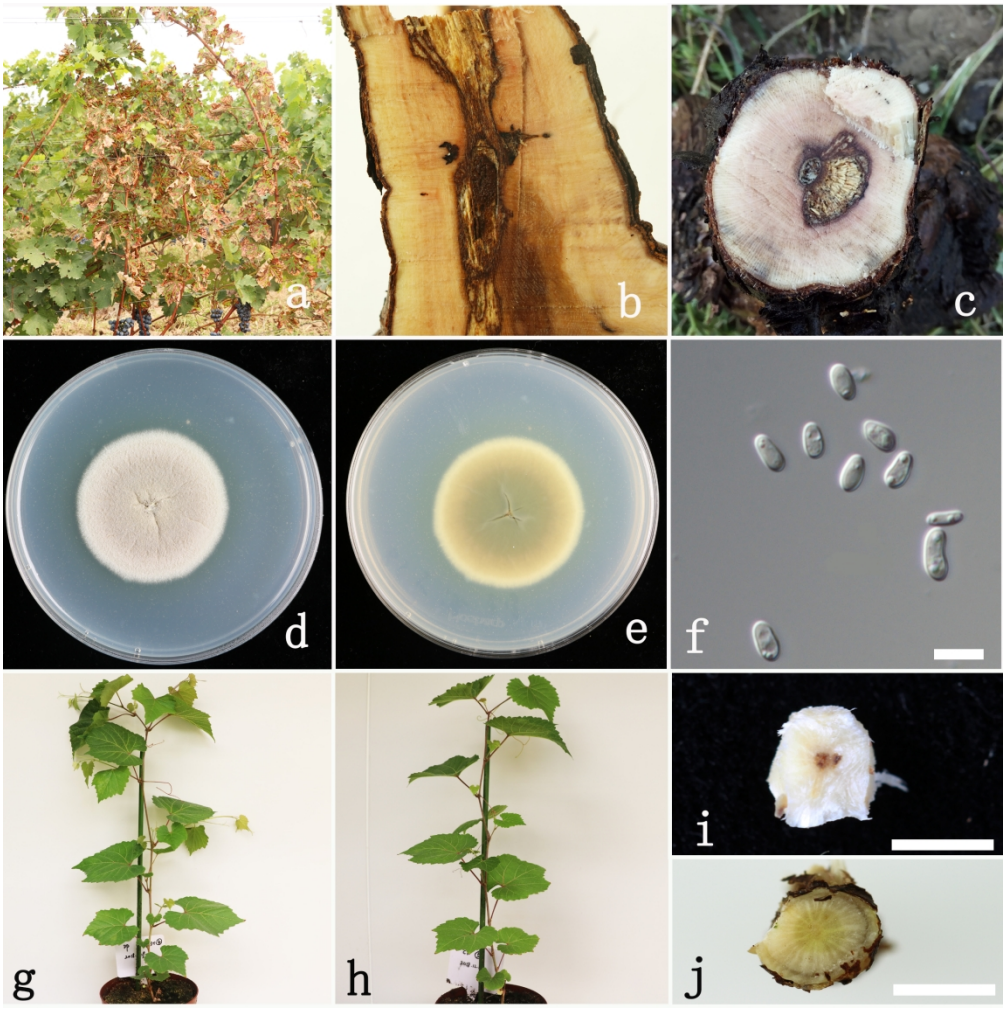

Figure 1. (a) Field symptoms of diseased grapevine from Hebei province. (b) longitudinal section of the infected wood. (c) Cross section of infected wood. (d-e) 30-day old P. minimum culture on PDA (d-up, e-down). (f) Conidia of P. minimum. (g, i) Plant inoculated with P. minimum and cause black necrosis in shoot after 114 days. (h, j) control. Scale Bar: f=10 μm, i-j=5 mm

209x210mm (300 x 300 DPI)

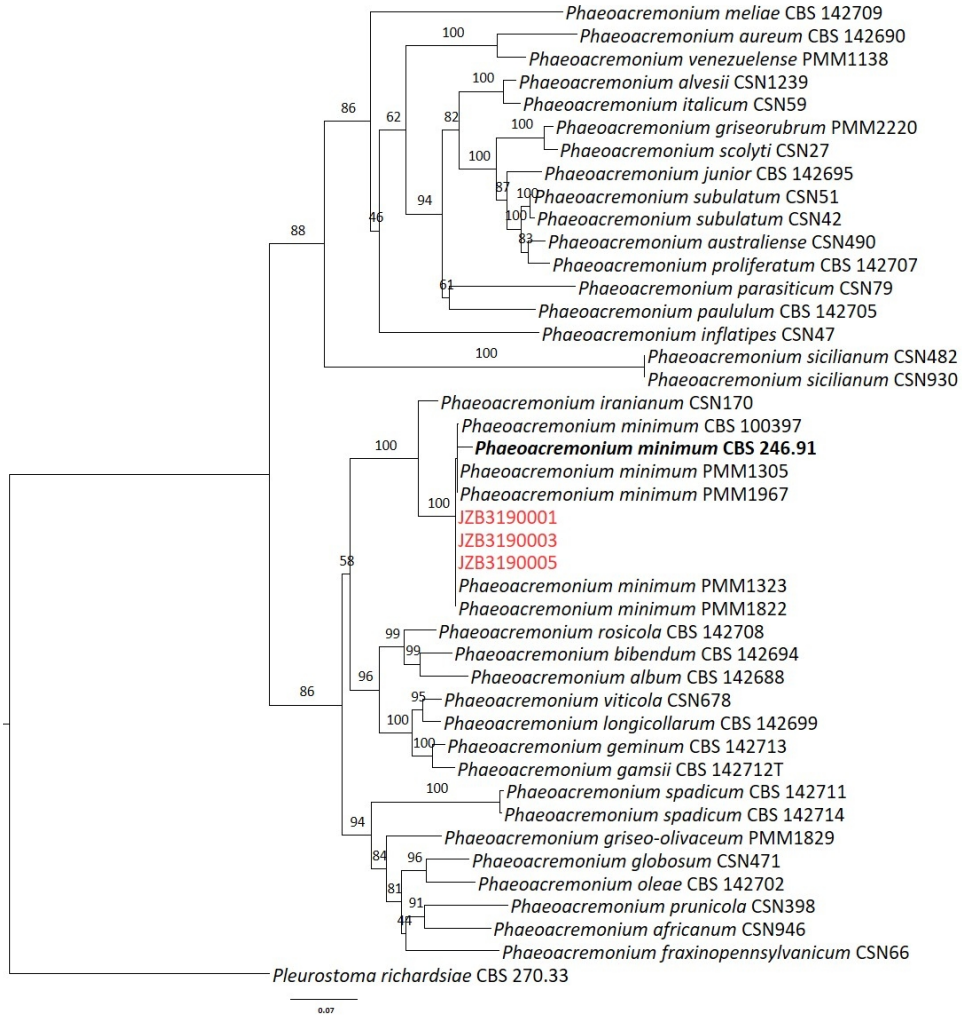

Figure 2. Phylogram generated from maximum likelihood analysis based on combined actin and beta-tubulin sequence data for species of Phaeoacremonium. The tree is rooted by Pleurostoma richardsiae (CBS 270.33). Bootstrap support values for ML which are equal to or greater than 50% are shown in nodes. The scale bar shows 0.07 changes. The ex-type strain of P. minimum is in bold. The isolates obtained in this study are in red.

191x196mm (150 x 150 DPI)