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First Report of Collar Rot in Purple Passion Fruit (Passiflora edulis) Caused 1 2 by Neocosmospora solani in Yunnan province, China C.-H. Hao[†], X. Chai[†], F.-C. Wu^{*}, Z.-F. Xu^{*} 3 4 5 C.-H. Hao, Department of Gardening and Horticulture, Xishuangbanna Tropical Botanical Garden, 6 Chinese Academy of Sciences, Menglun, Mengla, Yunnan 666303, China; and College of Life Sciences, 7 University of Chinese Academy of Sciences, Beijing 100049, China; X. Chai, Key Laboratory of 8 Tropical Plant Resources and Sustainable Use, Xishuangbanna Tropical Botanical Garden, Innovation 9 Academy for Seed Design, Chinese Academy of Sciences, Menglun, Mengla, Yunnan 666303, China; 10 and College of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049, China; F.-C. Wu, Department of Gardening and Horticulture, Xishuangbanna Tropical Botanical Garden, Chinese 11 12 Academy of Sciences, Menglun, Mengla, Yunnan 666303, China; Z.-F. Xu, Key Laboratory of Tropical 13 Plant Resources and Sustainable Use, Xishuangbanna Tropical Botanical Garden, Innovation Academy 14 for Seed Design, Chinese Academy of Sciences, Menglun, Mengla, Yunnan 666303, China; and State 15 Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, College of Forestry, 16 Guangxi University, Nanning 530004, Guangxi, China 17 18 †These authors contributed equally to this work. 19 *Corresponding authors: F.-C. Wu, wfc@xtbg.org.cn; Z.-F. Xu, zfxu@gxu.edu.cn. 20 21 Purple passion fruit (*Passiflora edulis* Sims) is a perennial climbing vine native to South America that is grown worldwide as an edible tropical fruit with excellent nutritional value and high economic value 22 23 (Zibadi et al. 2007). With the increasing expansion of the plantation area in China, considerable economic 24 loss caused by collar rot has attracted wide attention. From 2018-2020, collar rot resulted in the death of 25 many plants of P. edulis 'Mantianxing', a commercial cultivar in China, in southwest China's Yunnan 26 province. The disease spread quickly, and field incidence reached more than 50%. Stem rot symptoms were observed at the base of the stem, about 5-10 cm from the ground, resulting in wilting, defoliation, 27 28 and death of plants. Representative symptomatic samples were collected from the base of five plants, 29 surface disinfested for 30 seconds with 75% ethanol and 15 min with 10% hypochlorite, washed three 30 times with sterile distilled water, then transferred to potato dextrose agar (PDA) dishes. After 2 days in 31 the dark at 28°C, emerging fungal colonies were purified on new PDA dishes cultured at 28°C for 7 days. 32 The mycelia were flocculent. The color of the surface and the reverse colony was white and cream,

respectively. On synthetic nutrient agar (SNA) medium, microconidia were oval, ellipsoidal or reniform,

34 0- or 1-septate, and 6.7-23.1 μm in length (n>30); macroconidia were straight to slightly curved, 3- or 5-35 septate, and 30.8-53.9 μm in length (n>30). Genomic DNA, extracted from six isolates, was amplified 36 with three pairs of primers, ITS1 and ITS4 (White et al. 1990), EF1-728F and EF1-986R (Carbone and Kohn 1999), and fRPB2-5F and fRPB2-7cR (Liu et al. 1999). The amplicons from all six isolates were 37 38 sequenced and identical sequences obtained. The sequence of one representative isolate was uploaded to 39 NCBI (National Center for Biotechnology Information) and analyzed with BLASTn in the Fusarium 40 MLST database (https://fusarium.mycobank.org). The sequence of the internal transcribed spacer 1 (ITS1) region (GenBank MN944550) showed 99.1% (449/453 bp) identity to Fusarium solani strain 41 NRRL 53667 (syn: Neocosmospora solani, GenBank MH582405). The sequence of the translation 42 43 elongation factor-1 (EF-1) gene (GenBank MN938933) showed 97.8% identity (263/269 bp) to F. 44 solani strain NRRL 32828 (GenBank DQ247135). The sequence of the second largest subunit of RNA 45 polymerase II (RPB2) gene (GenBank MW002686) showed 98.7% identity (810/821 bp) to F. solani strain NRRL 43441 (GenBank MH582407). Based on a multilocus phylogenetic analysis of the 46 47 ITS1, EF-1 and RPB2 sequences, coupled with the morphological characteristics, the isolate (designated as NsPed1) was considered to be Neocosmospora solani (syn: Fusarium solani) (Crespo et al. 2019). 48 49 Subsequently, three-month-old healthy seedlings and 45-day-old cuttings of P. edulis 'Mantianxing' 50 plants were inoculated with the isolate NsPed1 to test its pathogenicity. Stems were wounded, 51 approximately 1-2 mm deep, in the collar region of plants at 2 cm above the soil. A disk (9 mm in 52 diameter) of NsPed1-colonized PDA was placed on the wound. Sterile PDA served as controls. All plants 53 were kept in a growth chamber with 28-30°C, 60% relative humidity, and 16/8-h light/dark photoperiod. 54 Fifteen plants were used for each treatment and replicated three times. Two weeks after inoculation, the stems of the inoculated plants turned brown with a lesion, 2-5 cm in length, and the leaves wilted. These 55 56 symptoms were similar to those of the diseased plants in the field. The control plants were asymptomatic. 57 N. solani NsPed1 was re-isolated from the infected plants, satisfying Koch's postulates. Taken together, N. solani NsPed1 was identified as the causal pathogen of collar rot in P. edulis 'Mantianxing'. 58 59 Knowledge of the causal organism of collar rot in purple passion fruit will lead to improved measures to 60 prevent and control the disease in China and other countries.

61 Acknowledgements

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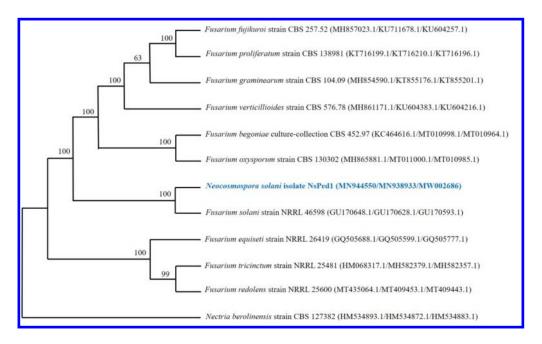
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Supplementary Figure 1 The symptoms of collar rot in purple passion fruit (P. edulis 'Mantianxing') and morphological characters of Neocosmospora solani isolate NsPed1. A, The symptoms of collar rot in purple passion fruit in the field. B, Transections (up) and longitudinal section (down) of stems of healthy (left) and infected (right) plants in the field. C, Colony morphology of N. solani NsPed1 observed from the top of a PDA Petri dish. D, Colony morphology of N. solani NsPed1 observed from the bottom of a PDA Petri dish. E, Macroconidia and microconidia of N. solani NsPed1 on synthetic nutrient agar (SNA) medium. Red arrows indicate macroconidia and green arrows indicate microconidia. F to H, 90-day-old seedlings of purple passion fruit were inoculated with sterile PDA (the two plants or stems on the left) and N. solani NsPed1 (the two plants or stems on the right) after two weeks. I to K, 45-day-old cuttings of purple passion fruit were inoculated with sterile PDA (the two plants or stems on the left) and N. solani NsPed1 (the two plants or stems on the right) after two weeks. All scale bars are 10 mm, except that in panel E is 50 µm.

255x191mm (350 x 350 DPI)



Supplementary Figure 2 The phylogenetic tree for Neocosmospora solani isolate NsPed1 and related Fusarium genus based on the combined sequence data sets for ITS1, EF-1 and RPB2 genes, constructed by neighbor-joining method using MEGA-X software (https://megasoftware.net/). Nectria berolinensis was used as the outgroup. Numbers at the branch nodes indicate bootstrap values based on 1000 replications.

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