

Fruit internal brown rot caused by *Neoscytalidium dimidiatum* on pitahaya in Guangdong province, China

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Abstract In July 2013, an unknown fruit internal brown rot disease was observed on white-fleshed pitahaya (*Hylocereus undatus*) in Zhanjiang, Guangdong province, China. Based on morphological characteristics and phylogenetic analysis of ITS sequences, the pathogen was determined as *Neoscytalidium dimidiatum*.

Keywords *Hylocereus* · Fungal diseases · *Neoscytalidium dimidiatum*

Pitahaya (*Hylocereus undatus*), also known as strawberry pear or dragon fruit, is a cactaceous fruit crop cultivated widely in some tropical and subtropical countries because of its high nutrient content and numerous selling points such as the attractive shape, colour and the good internal properties for market (Le Bellec et al. 2006). In China, pitahaya is a great contributor to the regional farming economic development particularly in the Hainan, Guangdong, Fujian, Guangxi and Yunnan provinces; the cultivation area up to 13,000 ha on the mainland.

In July 2013, an unknown fruit internal brown rot disease was observed on white-fleshed pitahaya in several orchards of Zhanjiang, Guangdong province. The disease caused the fruit to develop slower than uninfected fruit in the initial stage, induced fruit internal brown rot and made the peel colour change from green to red 10–15 days in advance of the normal fruit peel which turned red 24–25 days after anthesis (Nerd et al. 1999). The brown rot first occurred below the persistent

style (Fig. 1a) which gradually spread and developed into a rotten area. The conspicuous grey-white mycelia developed after the diseased fruit was cut in half lengthways and kept in a sealed plastic box for 3 days (Fig. 1b). The incidence of the disease was approximately 15 % in the field.

Fruit with internal brown rot were collected from an orchard (21°8'52"N 110°18'28"E). After surface sterilisation with 75 % ethanol solution, a symptomatic piece of fruit tissue from the location of the brown rot was carefully extracted with sterile forceps and placed on potato dextrose agar (PDA) plates under aseptic conditions and then incubated at 25 °C in the dark for 36 h. Hyphae growing from the tissue pieces were transferred and sub-cultured to obtain pure cultures. To obtain single spored isolates, conidia from pure cultures were suspended in sterile water and streaked onto PDA plates. The single-conidia forming colonies were obtained after incubation at 25 °C in the dark for 24 h.

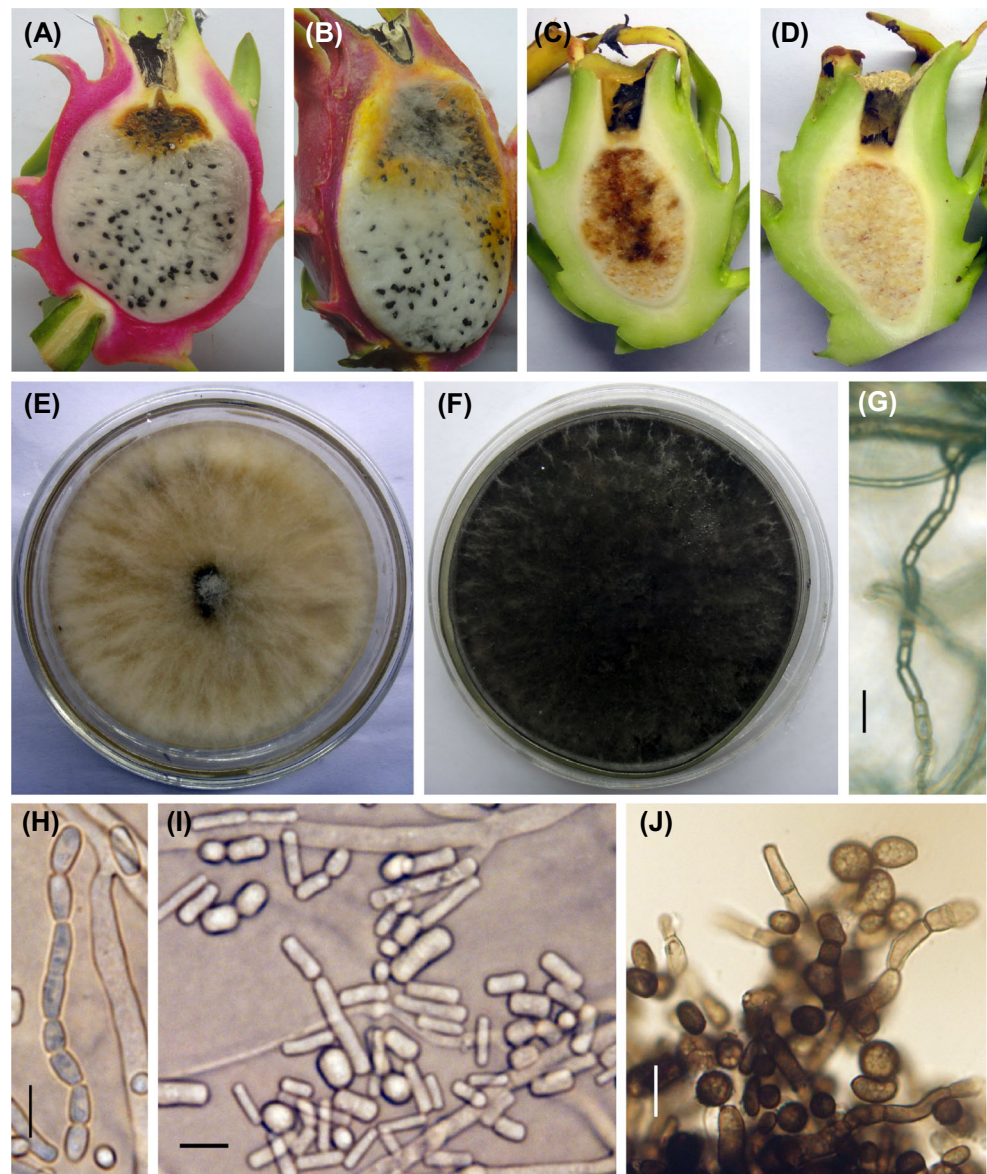
Healthy fruit were used for pathogenicity tests in the laboratory and field. A suspension of approximately 10⁴ spores/mL was prepared from 4-day-old cultures grown at 25 °C in the dark. Two hundred µl of the suspension was injected into the sarcocarp of healthy fruit with a sterile 32G hypodermic needle. Injection of isovolumetric sterile water served as a control. In the laboratory, all fruit were maintained in a sealed plastic box at 25 °C in the dark. The symptoms of brown rot were observed on inoculated fruit 7 days postinoculation (Fig. 1c). Fruit in the laboratory rotted more quickly than in the field, whereas control fruit did not develop any symptoms (Fig. 1d). The same fungi were subsequently re-isolated from the rotten tissues which confirmed the disease aetiology.

Fungal isolates grew quickly on PDA and the colony diameter reached up to 7.2±0.5 cm at 25 °C in the dark after 3 days. The colony was covered with dense, grey to black, cottony aerial mycelia. Colony colour originally was grey-white, and gradually became black on reverse and obverse

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Fig. 1 Symptoms of fruit flesh brown rot disease and the causative agent of *Neoscytalidium dimidiatum*. **a** Vertical section of diseased fruit, the flesh brown rot on the top. **b** Grey mycelia growing from the surface of diseased flesh after 3 days incubation in a sealed plastic box. **c** Artificial inoculation with spore suspension. **d** Artificial inoculation with sterile water. **e** and **f** Colony of *N. dimidiatum* on PDA, **e** Incubation for 5 days and **f** for 15 days at 25 °C in the dark. **g** and **h** Chains of arthroconidia. **i** and **j** The size-and-shape-variable arthroconidia, **(i)** Incubation for 5 days and **(j)** for 15 days at 25 °C in the dark. Scale bars: **(g)**, **(h)**, **(i)**=10 µm, **(j)**=20 µm



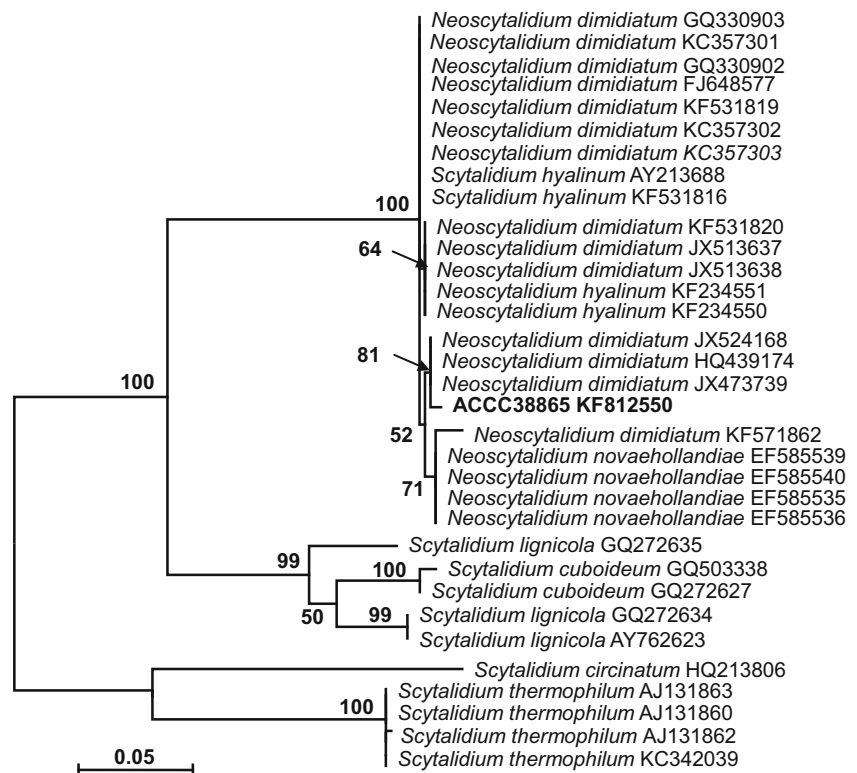
sides of Petri dishes (Fig. 1e and f). Mycelia were branched, septate, hyaline to brown hyphae which constricted into spore chains (Fig. 1g and h) and disarticulated into arthroconidia. The arthroconidia were orbicular to dolioform, hyaline to dark brown, thick-walled, and 0–1-septate, $2.64\text{--}6.75$ (av. 4.39 ± 0.88) \times $3.61\text{--}22.87$ (av. 8.72 ± 2.62) μm ($n > 50$) (Fig. 1i and j).

A representative isolate, ACCC38865, was selected for sequencing and phylogenetic analysis and deposited with Agricultural Culture Collection of China (ACCC). The internal transcribed spacer (ITS1-5.8S-ITS2) sequence was amplified using ITS1 (TCC GTA GGT GAA CCT GCG G) and ITS4 (TCC TCC GCT TAT TGA TAT GC) (White et al. 1990) according to the method of Lu et al. (2012). The sample was

denatured at 98 °C for 5 min, followed by 40 amplification cycles (98 °C for 10 s, 56 °C for 15 s, 68 °C for 1 min) and a final extension (68 °C for 10 min). The PCR amplification product was sequenced at the Sangon Biotech (Shanghai, China) Co., Ltd. A new sequence of 574 bp was obtained and deposited in GenBank (KF812550). The sequence was blasted with the sequences deposited in GenBank nucleotide database and the results showed greater than 99 % similarity to sequences of *Neoscytalidium dimidiatum* (KF000372, JX524168, JX473739 and HQ439174).

For the phylogenetic analysis, a total of 32 sequences of *Neoscytalidium* and *Scytalidium* species obtained from GenBank and ACCC38865 were used to construct the

Fig. 2 Molecular phylogenetic analysis of isolate ACCC38865 with *Neoscytalidium* spp. and *Scytalidium* spp. obtained from GenBank by Maximum Likelihood method based on the ITS sequences. Bootstrap values after 1000 replicates were expressed as percentages



phylogenetic tree. Nucleotide sequences were analysed using MEGA version 6 (Tamura et al. 2013) for Maximum Likelihood analyses, using the Kimura-2-parameter nucleotide substitution model. Bootstrap values were obtained from 1000 bootstrap replicates. The isolate ACCC38865 clustered in a clade with known isolates of *N. dimidiatum*, *N. novaehollandiae* and *Scytalidium hyalinum* with high bootstrap support (100 %), and in a subclade with three isolates of *N. dimidiatum* with 81 % bootstrap support (Fig. 2). *S. hyalinum* was considered to be a variety or a melanin-deficient cultural mutant of *N. dimidiatum* (Polizzi et al. 2011), and has never been isolated from plants or the environment (Machouart et al. 2004). *Neoscytalidium novaehollandiae* produced muriform, *Dichomera*-like conidia (Pavlic et al. 2008), which distinguished it from isolate ACCC38865. Therefore, the pathogen of fruit internal brown rot disease was identified as *N. dimidiatum* (Crous et al. 2006) based on the morphological characters as well as the ITS1-5.8S-ITS2 sequence.

Neoscytalidium dimidiatum is an opportunistic fungus of plants (Padin et al. 2005). This pathogen can cause pitahaya canker and spot on the surface of the stem or fruit (Lan et al. 2012; Chuang et al. 2012; Yi et al. 2013; Mohd et al. 2013) and internal black rot in fruit (Ezra et al. 2013). However, this is the first reported of internal browning in pitahaya in Guangdong Province, China.

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