A New Species and Genus Distribution Record from China: *Neoscytalidium novaehollandiae*

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Abstract One isolate from bark beetle galleries was identified and characterized using morphological and molecular methodology, and described and illustrated based on these data. These results identified a new species distribution record in China for *Neoscytalidium novaehollandiae*. Species morphology and micrographs are provided in this paper. The specimens are stored in Northeast Forestry University.

Keywords *Neoscytalidium novaehollandiae* · *Ulmus densa* · New record · China

Introduction

The genus *Neoscytalidium* was first described by Crous and Slippers in 2006, and a new combination recognized as the type species *N. dimidiatum* (Penz.) Crous and Slippers. The species is characterized by the presence of powdery disarticulating aerial mycelium [1]. Pavlic et al. discovered

isolates representing *Neoscytalidium novaehollandiae*, which had not yet been described when investigating fungi associated with baobab, and surrounding endemic tree species in northwestern Australia. The cultures were isolated from asymptomatic or dying branches (sapwood) of *Acacia synchronica*, *Adansonia gibbosa*, *Crotalaria medicaginea* and *Grevillia agrifolia*. The species was subsequently described as *N. novaehollandiae* Pavlic et al. [2] from Western Australia, then the only known species distribution. Ray et al. [3] later reported first distribution record for *N. novaehollandiae* associated with mango dieback in Australia. However, *N. novaehollandiae* and *Neoscytalidium* have not previously been reported in China.

Materials and Methods

Isolates

Infested elm (*Ulmus densa* Litw.) stems were obtained from streets located in the Fukang District, Xinjiang, China in September 2010. Infested stems (approximately 10 cm in diameter) were cut, and transported to the laboratory.

Methods

Isolation of Fungi from Beetle Galleries

The bark beetle infested stems were cut into 5 cm long segments, and the surface of each segment was rinsed with 95 % ethanol. Each segment was split aseptically to expose brood galleries, and cut longitudinally into 3 × 5 cm sections. Five samples were placed on potato dextrose agar (PDA) (potato [peeled] 200 g; glucose 20 g; agar 15 g;

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Fig. 1 *Neoscytalidium novaehollandiae*. 1, 2 Pycnidia formed in culture on pine needles, 3 conidiogenous cells, 4, 5 Aseptate conidia, 6–9 Arthroconidia. Bars 2, 7 = 50 μm , others = 10 μm (zxm #0018)

distilled H_2O 1 L). The cultures were incubated for 3–7 days at 25 °C under dark conditions until colonies formed, and subsequently purified by further replating [4]. Fungal linings were taken directly from the purified

cultures using sterile forceps, and a spore suspension was prepared using sterile distilled water. The spore suspension was spread on a water agar (WA) (agar 18 g; distilled H_2O 1 L) surface using a sterilized streaking loop. Twenty

plates were incubated at 25 °C under dark conditions for approximately 12 h. Single germinating conidia were transferred onto malt extract agar (MEA) (malt extract powder 20 g; peptone 1.0 g; glucose 20 g; agar 15 g; distilled H₂O 1 L), and incubated at 25 °C and under dark conditions until colonies formed. The fungal cultures were maintained on PDA slants at 4 °C with periodic transfers. Cultures were inoculated onto sterilized pine needles placed on the surface of 2 % WA, and incubated at 25 °C under near-UV light to induce sporulation [1]. A single pycnidium was placed in a drop of lactoglycerol on a microscope slide, and cut in pieces with a sterile medical needle before adding the cover slip. Fifty released conidia, and 30 pycnidia, conidiogenous cells, and conidia were measured, and ranges and averages computed. Measurements and digital images were obtained with an OLYMPUS BX5.

Molecular Methods

DNA was extracted from fungal mycelium of 7 days old single-conidial cultures using the DNAsure Plant Kit (Tiangen Biotech, Co., Ltd., Beijing). The internal transcribed spacer (ITS) region of the ribosomal RNA operon was amplified from the isolate via PCR. The PCR reaction mixture (50 µl final volume) consisted of 6 µl DNA, 5 µl 10× PCR reaction buffer, 4 µl dNTPs, 2 µl ITS1 (TCCGTAGGTGAACCTGCGG), 2 µl ITS4 (TCCTCGCTTATTGATATGC), 0.4 µl *Taq* DNA polymerase, and 29.4 µl double distilled water. The PCR cycle parameters included initialization at 95 °C for 2 min; denaturation at 95 °C for 30 s; elongation at 55 °C for 1 min; and final elongation at 72 °C for 1 min; cycle to step 2, 35 cycles; elongation at 72 °C for 10 min. Samples were stored at 4 °C. PCR products were sent to Sangon Biotech (Beijing) Co., Ltd. for sequencing. NCBI Blast analysis was employed to assess phylogenetic relationships between *N. novaehollandiae*, and other species deposited in the GenBank database. Following sequence generation, *N. novaehollandiae* data were deposited in GenBank.

Results

Morphology

Colonies covered a 90 mm diameter MEA Petri dish after 3 days under dark conditions at 25 °C. Colonies were initially white, turning greenish and black (surface and beneath) with age, and exhibiting suppressed, fluffy mycelium, and smooth edges.

Pycnidia formed in pine needle cultures, semi-immersed or superficial, solitary or in multilocular stromata, black, with a globose base, up to 110 µm in diameter, and long necked. Conidiogenous cells holoblastic, cylindrical to subcylindrical, hyaline, 11–15 × 2–4 µm (av. 13 × 2.7 µm, l/w 4.8). Conidia ellipsoidal to oval, 11–13 × 3–5 µm (av. 11.7 × 4.5 µm, l/w 2.6), apices rounded, initially hyaline, unicellular, becoming sepia, and 0–1-septate or 2-septate with darker central cells. Aerial mycelium formed chains of arthroconidia, 5–8 × 3.5–5 mm (av. 6.6 × 4.2 µm, l/w 1.6), unicellular, powdery to the touch, disarticulating, cylindrical, oblong, obtuse, to doliiform, thick-walled, initially hyaline becoming sepia, and 0–1-septate.

Habitat Isolated from bark beetle galleries in *U. densa* Litw., Fukang, Xinjiang Province, China. Collected by Xiao-mei Zhu, September 2010 (zxm #0018).

Known distribution Western Australia, China (Fig. 1).

Sequence data were compared using Blast analysis of ITS1 and ITS4 regions deposited in NCBI; 100 % homology was found to *N. novaehollandiae*. Sequence data were submitted to GenBank (ACC #HQ845383).

Discussion

Neoscytalidium novaehollandiae isolates are morphologically similar to the type specimen *N. dimidiatum*. However *N. novaehollandiae* produce muriform, *Dichomera*-like conidia that distinguish this species from known *Neoscytalidium* species [1].

Although the fungus species was isolated from the beetle gallery system, few reports indicated *Neoscytalidium* or *N. novaehollandiae* associated with beetles. Consequently, further studies are required to determine if a symbiotic relationship exists between *N. novaehollandiae* and the beetle species. In addition, the role of *N. novaehollandiae* in *U. densa* ecology will be considered in future studies. Finally, the disjunct distribution pattern of the species is of biogeographic interest, and should be further explored.

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