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Peltaster fructicola, a newly recorded species from China associated with sooty blotch and flyspeck

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Abstract — *Peltaster fructicola* is first reported and described from China. This fungus was isolated from peels of crabapple and hawthorn collected from Liaoning and Shaanxi Provinces and exhibited the punctate mycelial type of sooty blotch and flyspeck on both hosts. Our isolates were identified based on morphological characteristics and DNA sequence analysis.

Key words — *Rosaceae,* taxonomy, morphology, phylogeny

Introduction

Sooty blotch and flyspeck (SBFS) is a commonly occurring disease complex in humid and temperate regions worldwide, causing blemishes on apple, pear, banana, and other cultivated crops that result in economic losses (Williamson & Sutton 2000, Batzer et al. 2005, Gleason et al. 2011). The SBFS fungi colonize the waxy cuticle of plants and display various mycelial types. The term "sooty blotch" refers to colonies on hosts that consist of a dark mycelial mat with or without sclerotium-like bodies, whereas "flyspeck" denotes a cluster of black dots without a visible mycelial mat. "Sooty blotch" was attributed to a single fungal species for more than 75 years (Colby 1920, Williamson & Sutton 2000) until the late 1990s, when Johnson et al. (1996, 1997) found three "sooty blotch" species based on morphological examination of sooty blotch isolates from apples in USA. One newly described species from that work was *Peltaster fructicola* (Johnson et al. 1996).

During a recent survey of SBFS complex on fruit of rosaceous hosts in China, crabapples and hawthorns were found to display colonies of SBFS. Based on analysis of morphology and DNA sequences of nuclear ribosomal ITS region, our isolates were identified as *P. fructicola.*

Materials & methods

Isolates and morphology

Crabapple *(Malus ×micromalus)* and hawthorn *(Crataegus pinnatifida)* fruit with SBFS signs were collected from Liaoning and Shaanxi Provinces. Two isolates were obtained from hawthorns in Liaoning and Shaanxi Provinces, and three from crabapples in Liaoning Province. Peels displaying SBFS were examined, photographed and preserved. Thalli were transferred directly from peels to potato-dextrose agar (PDA) and cultured at 25°C in the dark (Sun et al. 2003). After one month, pure isolates were transferred to PDA and water agar (WA, 2% agar) for morphological examination. Colony descriptions were based on cultures on PDA after 3 weeks in the dark at 25°C. Morphological descriptions were made on WA and synthetic nutrient-poor agar (SNA) after 3 weeks using the method of Zhang et al. (2009). Specimens and representative dried culture were deposited in the Fungal Herbarium of Northwest A&F University (HMUABO), Yangling, Shaanxi Province, China.

DNA extraction, PCR and sequencing

Genomic DNA was extracted from fungal mycelium according to the protocol of Barnes et al. (2001). The primers ITS1-F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) were used to amplify the internal transcribed spacer (ITS) region of nuclear ribosomal DNA. The PCR conditions followed the methods of Zhuang et al. (2010). PCR products were sequenced by Sangon Biotech (Shanghai), China.

Sequence alignment and phylogenetic analysis

Sequences generated in this study were added to other sequences of *Peltaster* spp., as well as the outgroup species *Mycosphaerella lateralis* and *Schizothyrium pomi* obtained from GenBank. Preliminary alignments were performed using CLUSTAL X (Thompson et al. 1997), and then manually adjusted using BioEdit v. 5.0.9.1 (Hall 1999). Phylogenetic analysis of aligned sequences was performed using PAUP v. 4.0b 10 (Swofford 2003). The heuristic search option was 1000 random taxa addition and tree bisection-reconnection (TBR) as the branch-swapping algorithm. The robustness of clades and internal branches was evaluated by 1000 bootstrap replications. Tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency index (RC) were also calculated. Sequences generated in this study were deposited in GenBank. Alignment and the representative tree were deposited in TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S13540).

Results

Phylogenetic analysis

The manually adjusted ITS alignment contained 27 sequences (including the outgroup sequences). Of the 544 characters used for the phylogenetic analysis, 153 were parsimony-informative, 95 were variable and parsimony-uninformative, and 296 were constant. The first 100 equally most parsimonious trees were obtained from the parsimonious analysis, and the first of which is shown in Fig. 1 (TL = 403 steps, CI = 0.8734, RI = 0.9373, RC = 0.8187). The *Peltaster* species formed a strongly supported clade with a bootstrap value of

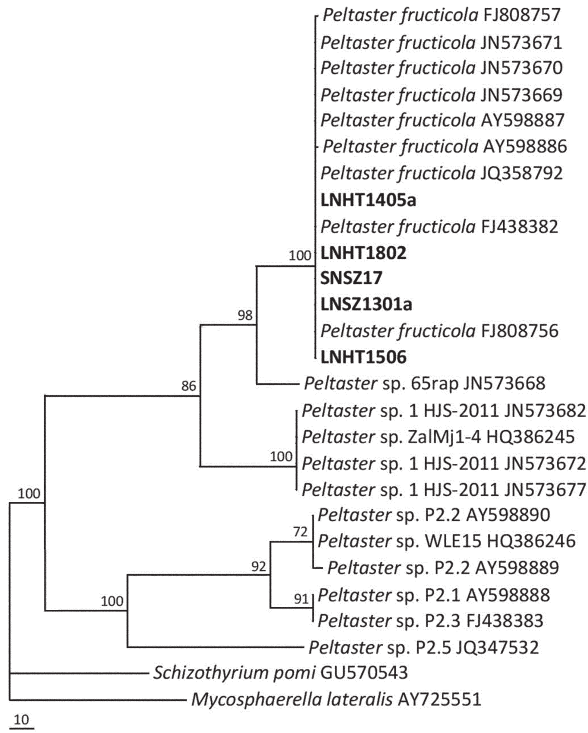

Fig. 1. One of 100 equally most parsimonious trees obtained from a heuristic search with 1000 random taxon additions of the ITS sequence alignment. The bootstrap support values (50%) based on 1000 replicates are shown at the nodes. The tree was rooted to *Mycosphaerella lateralis* and *Schizothyrium pomi*. The scale bar shows 10 changes. Strains investigated in this paper are presented in bold.

100%, which further divided into two subclades. Our five strains clustered together with *Peltaster fructicola* within one of the subclades with a high bootstrap value of 100%, indicating that they might represent the same species (Fig. 1).

Taxonomy

Peltaster fructicola Eric M. Johnson, T.B. Sutton & Hodges, Mycologia 88(1): 120, 1996 Fig. 2

Hyphae smooth, septate, branched, hyaline and turning to pale brown as culture aged, 1.5–2.0 μm wide. Conidiophores absent. Conidiogenous cells hyaline, monoblastic, intercalary, formed directly on undifferentiated hyphae. Conidiogenous loci inconspicuous, stub-shaped, concolorous with conidiogenous cells. Conidia solitary, unicellular, hyaline, elliptic to ovoidal, produced in basipetal order from the conidiogenous loci and aggregated in

masses near the conidiogenous cells, 4–6.5(–7) × 1.5–2.5 µm. Microcyclic conidiation observed in culture, with expansion and germination of conidia. Conidia on WA slightly smaller in size than those on SNA, 4–6(–7) × 1.5–2.0 µm.

Cultural characteristics — On PDA erumpent, compact, with smooth, crenate margin and sparse to no aerial mycelium, surface strongly folded, outer zone light gray, center greenish gray to olivaceous-gray, reverse black with yellow masses of conidia at the bottom of the colony; reaching (13–)14(–16) mm diam after 21 d at 25°C in the dark.

On peels of crabapple fruit, showing punctate mycelial type, visible mycelial mat with dull brown, flattened, circular or irregular sclerotium-like bodies, 68–145 µm diam. On peels of hawthorn fruit similar, sclerotium-like bodies 62–223 µm diam.

Specimens examined (all conserved in HMUABO): **CHINA, Liaoning Province**: Huludao City, Suizhong County, 40°19′32″N 120°17′28″E, on fruit surface of crabapple (*Malus* ×*micromalus* Makino), Sept. 2010, Zhuang JL LNHT1506 (GenBank JX961608), LNHT1802 (GenBank JX961609), LNHT1405a (GenBank JX961610); on fruit surface of hawthorn (*Crataegus pinnatifida* **Bunge**), Sept. 2010, Zhuang JL LNSZ1301a (GenBank JX961607). **Shaanxi Province**: Shangluo City, Shangnan County, on fruit surface of hawthorn (*Crataegus pinnatifida*), 21 Oct. 2011, Chen C & Dang JL SNSZ17 (GenBank JX961611).

Discussion

The genus *Peltaster* was established by Sydow & Sydow in 1917; the type species is *Peltaster hedyotidis* Syd. & P. Syd. Sutton and his colleagues described *P. fructicola* (Johnson et al. 1996) as one of the pathogens of sooty blotch and later associated it with punctate mycelial type on apple (Williamson et al. 2004). Since then, this fungus has been found widely in the eastern and midwestern United States (Johnson et al. 1997, Batzer et al. 2005, Díaz Arias et al. 2010). As the etiology of SBFS is studied worldwide, *P. fructicola* has been reported to cause sooty blotch in many other countries and regions, including Serbia, Montenegro, and Poland (Ivanović et al. 2010, Mirzwa-Mróz & Wińska-Krysiak 2011), indicating that *P. fructicola* is among the most prevalent SBFS species.

Based on phylogenetic analysis of the ITS region and morphological comparison, we identified our isolates as *Peltaster fructicola*, a newly recorded species for China. To our knowledge, this is also the first report of SBFS on crabapple and hawthorn caused by *P. fructicola*.

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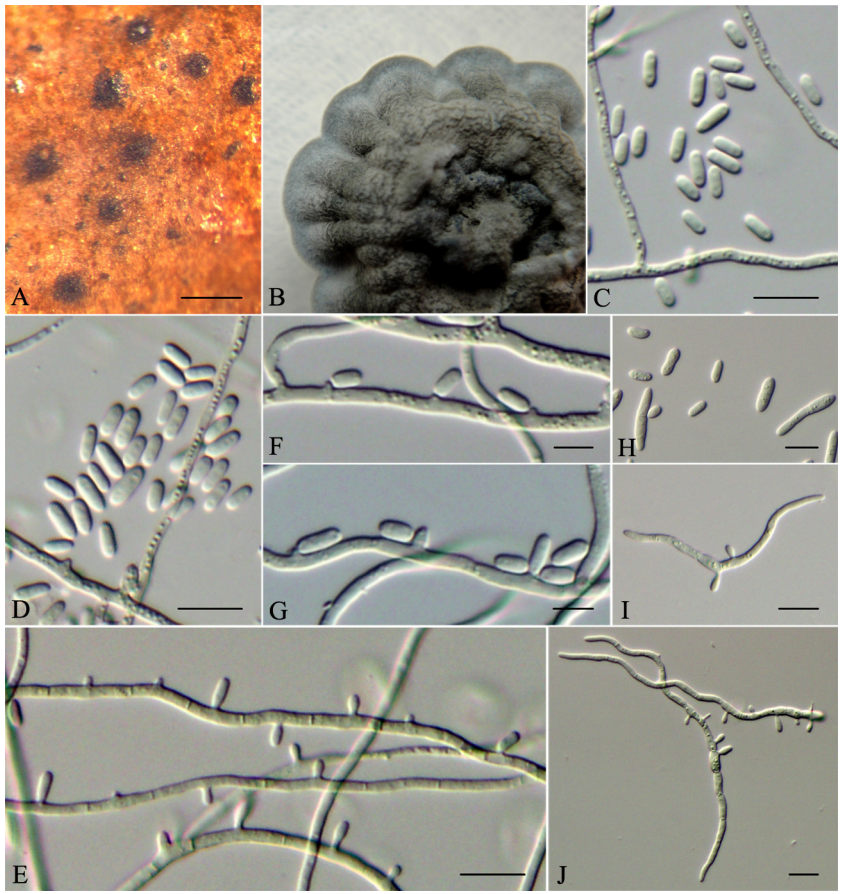

Fig. 2. *Peltaster fructicola* (LNHT1506). A. Signs on crabapple. B. Colony on PDA. C, D. Conidia and conidiogenous cells. E–G. Conidiogenous cells giving rise to conidia, with visible conidiogenous loci. H–J. Conidia expanding and germinating and microcyclic conidiation. Scale bars: A = 0.2 mm; C–E, H–J = 10 µm; F, G = 5 µm.

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