



Colletotrichum asianum causes anthracnose in Philippine mango cv. Carabao

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Abstract

Anthracnose symptoms were observed on fruits and leaves of mango cv. Carabao at several locations in the Philippines. Single spore isolates were generated from affected leaves and fruits and identified as *Colletotrichum asianum* based on morphology and molecular analyses using several genes. Inoculation of *C. asianum* isolates onto healthy fruit of mango cv. Carabao resulted in the same symptoms as those observed initially. Koch's postulates were satisfied after isolates made from symptomatic inoculated fruits were morphologically identical as the original isolates. This is the first proof of *C. asianum* as causal fungus of anthracnose in mango cv. Carabao in the Philippines.

Keywords Anthracnose · *Mangifera indica* · *Colletotrichum asianum* · *Colletotrichum gloeosporioides*

The mango (*Mangifera indica*: Anacardiaceae) is one of the most popular fruits grown in the tropical and subtropical zones of the world (Alemu et al. 2014). Mango cv. Carabao is considered as premium agricultural export in the Philippines (Simeon 2017) being the third most important fruit crop based on export volume and value (Department of Agriculture-Bureau of Plant Industry 2016). Production of mango cv. Carabao dropped by 3.3% from 546,000 tons in the 3rd quarter of 2017 to 528,000 tons over the same period in 2018 (PSA 2018). Philippine Statistics Authority attributed the low production to several factors such as occurrence of rainfall, adverse effect of insect pests, particularly cecid fly, and fungal diseases.

There are many diseases of mango worldwide (Alemu 2014; Prakash and Misra 2000). Among them, anthracnose caused by *C. gloeosporioides*, is reported as one of the most important fungal disease and is the major pre- and post-harvest disease of mango in the Philippines and throughout

the tropics (Dodd et al. 1991; Jeffries et al. 1990). Throughout the years, *C. gloeosporioides* was reported to be the causative agent of anthracnose in mango cv. Carabao in the Philippines where most of the studies conducted focused more on the disease control (Alvindia and Acda 2015; Esguerra et al. 2006; Lizada et al. 1986). Weir et al. (2012) verified genetically by multi-gene phylogenies that *C. gloeosporioides* is a species complex which consisted of 22 taxa plus one subspecies. It is therefore possible that the anthracnose-causing fungi of mango cv. Carabao could be any of the 22 taxa or subspecies under the *C. gloeosporioides* species complex. The present study investigated the identity of the fungus causing anthracnose disease of Philippine mango cv. Carabao at several localities in the Philippines, using morphology, molecular analyses and pathogenicity studies.

In 2017, anthracnose-symptomatic fruit and leaf samples of mango cv. Carabao were collected through opportunistic sampling from a mango orchard in the province of Iba, Zambales in Luzon region and two orchards in Jordan, Guimaras in Visayas region. A total of 28 samples were collected in Zambales and 46 samples in Guimaras. The symptoms in leaves were irregularly shaped, brown to black spots and on fruit sunken, prominent, black necrotic spots. Yellow-orange acervuli were often present within the spots and no setae were present in the acervuli (Fig. 1). A single conidium isolates of the fungus were generated using the methods described in Nakashima et al. (2016). A mass of conidia from a single acervulus was suspended in a drop of sterile distilled water and the suspension was streaked across the surface of water

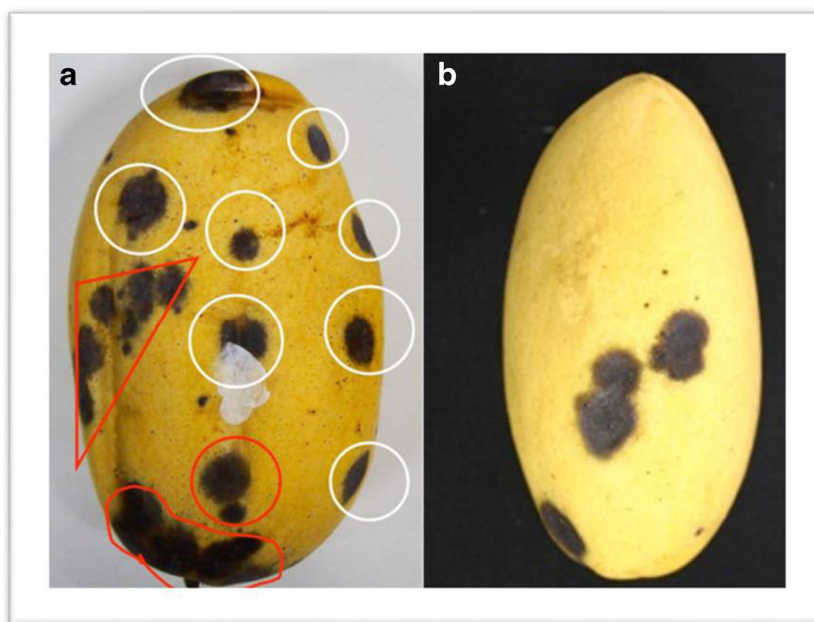
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Fig. 1 Anthracnose symptoms on mango cv. Carabao: Necrotic spots (A) and coalesced lesions (B)



agar in 9-cm-diameter petri dishes which were incubated for 24 h at room temperature (25°–30° C) under ambient light. Single germinating conidium was selected with the aid of a compound microscope and a circle of agar surrounding the conidium was excised using a modified loop and transferred to potato dextrose agar (PDA; Nissui) in similar petri dishes. The cultures were incubated at room temperature (25°–30° C) under ambient light. Single conidium isolates were also grown on synthetic nutrient-poor agar (SNA) under the same conditions to determine the morphology of appressoria and conidiogenous cells. Three isolates were subjected for further analyses PHP2017_Z23, PHP2017_Z25 and PHP2017_Z26. The isolates were deposited at the University of the Philippines Culture Collection (UPCC) housed at the Natural Science Research Institute (NSRI) with the Accession Numbers: UPCC 4139, UPCC 4138 and UPCC 4137, respectively.

Colonies of the three *Colletotrichum* isolates on PDA were white, later grayish green to black, white at the edge, with a mean colony diameter of 71.33 ± 2.1 mm ($n = 3$) from a single conidium after 10 days at 25 °C under ambient light; aerial mycelium was cottony, white, often formed concentric rings; acervuli were blackish with orange conidial masses scattered or formed concentric rings on the surface of the colony; reverse initially light orange and later black starting from the middle, (Fig. 2). Phialides were hyaline, $11\text{--}28 \times 2\text{--}4.5$ μm and conidia were aseptate, hyaline, cylindrical to fusiform, $14.5\text{--}18 \times 4\text{--}5.5$ μm . Appressoria in PDA slide cultures were brown to dark brown, subcircular to subfusoid, $4.5\text{--}10 \times 4.5\text{--}8$ μm (Fig. 3). Teleomorph was not produced on media. These morphological characters were identical to *Colletotrichum asianum* (Prihastuti et al. 2009).

To confirm the morphological identification, the *C. asianum* isolates, PHP2017_Z23, PHP2017_Z25 and PHP2017_Z26,

Fig. 2 *C. asianum* PHP2017_Z23 growing on PDA plate after 10 days at 25° C. (A) Upper plate showing acervuli around concentric rings (B) reverse side of the plate after 5 days at 25° C

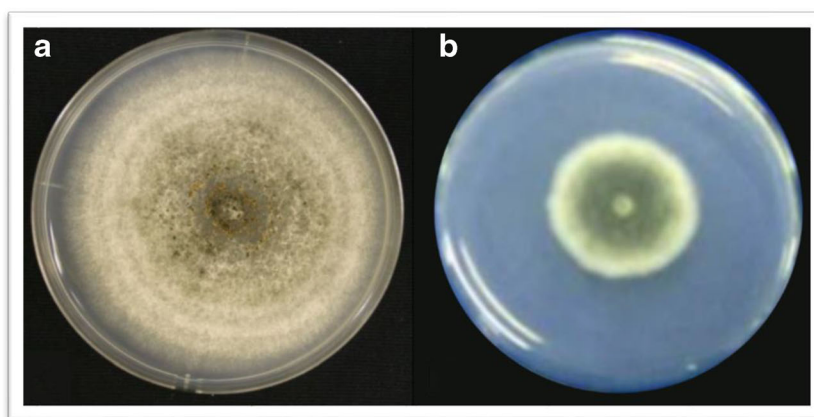
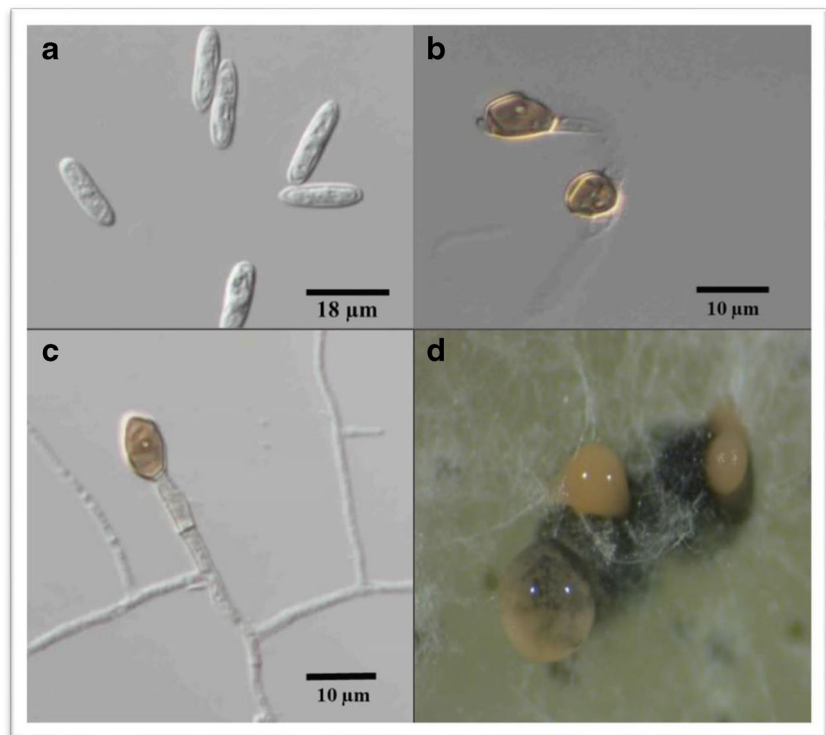


Fig. 3 Morphology and cultural characteristic of *Colletotrichum asianum*. (A) Conidia; (B and C) Appressoria; (D) conidial mass on plate culture

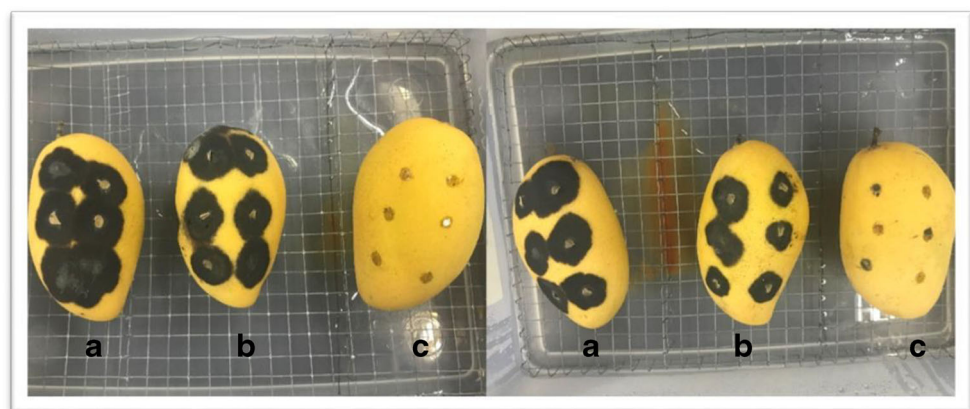


were subjected to amplification and sequencing of the internal transcribed spacer (ITS), actin (ACT), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Calmodulin (CAL), and chitin synthase (CHS-1) gene regions utilizing primers V9G/ITS4 (de Hoog and Gerrits van den Ende 1998; White et al. 1990), ACT512F/ ACT783R (Carbone and Kohn 1999), GDF/ GDR (Templeton et al. 1992), CL1-C/ CL2-C (Weir et al. 2012), and CHS-79F/ CHS-345R (Carbone and Kohn 1999), respectively. The NCBI GenBank Accession number for each gene (ITS, ACT, GAPDH, CAL and CHS) for each strain is LC458779, LC458783, LC458787 LC458791, LC458795 for PHP2017_23; LC458780, LC458784, LC458788, LC458792, LC458796 for PHP2017_25; and LC458781, LC458785, LC458789, LC458793 and LC458797 for PHP2017_26 respectively. The sequences were combined

and aligned with sequences recollected from NCBI GenBank and were analyzed by Maximum parsimony (MP) using PAUP* v. 4.0 b 10 (Swofford 2002). Phylogenetic analyses showed that the isolates obtained from mango cv. Carabao were grouped in a same clade with an ex-type culture of *Colletotrichum asianum* (Fig. 5). This clade was strongly supported with 100% in Bootstrap value. From these results, isolates associated with anthracnose symptoms were identified as *Colletotrichum asianum*.

The isolates, PHP2017_Z23, PHP2017_Z25 and PHP2017_Z26, identified as *Colletotrichum asianum* were inoculated onto fruit of mango cv. Carabao to confirm their pathogenicity. Three closed containers each containing three healthy mango fruits, labeled A (unwounded), B (wounded by piercing the skin with disinfected needle) and C (control) were prepared

Fig. 4 Symptoms of anthracnose on fruit of mango cv. Carabao, seven days after inoculation with *C. asianum* PHP_Z23; unwounded (A) and wounded (B) mango fruit (C) control



for each isolate. Six colonized agar blocks (10 mm in diameter) excised from seven-day old colonies of each isolate growing on PDA were placed on the surfaces of mango A and B (wounded area) while six blank agar blocks were placed on the surface of mango C. The fruits were incubated for seven days under ambient light and temperatures (25°-30° C). Symptoms and signs of inoculated fruits were very similar to that of originally observed on the affected mangoes in the orchards (Fig.1; that is

irregularly shaped, sunken lesions that later coalesced, with acervuli present in some of the infected fruits). The control fruits remained symptomless. The fungus was re-isolated from reproduced symptoms and the conidia exhibited the same morphological characters as the cultures used in the pathogenicity study (Fig. 4), thereby fulfilling the Koch's postulates.

Several reports on *C. asianum* associated with mango were reported among countries around the world, including

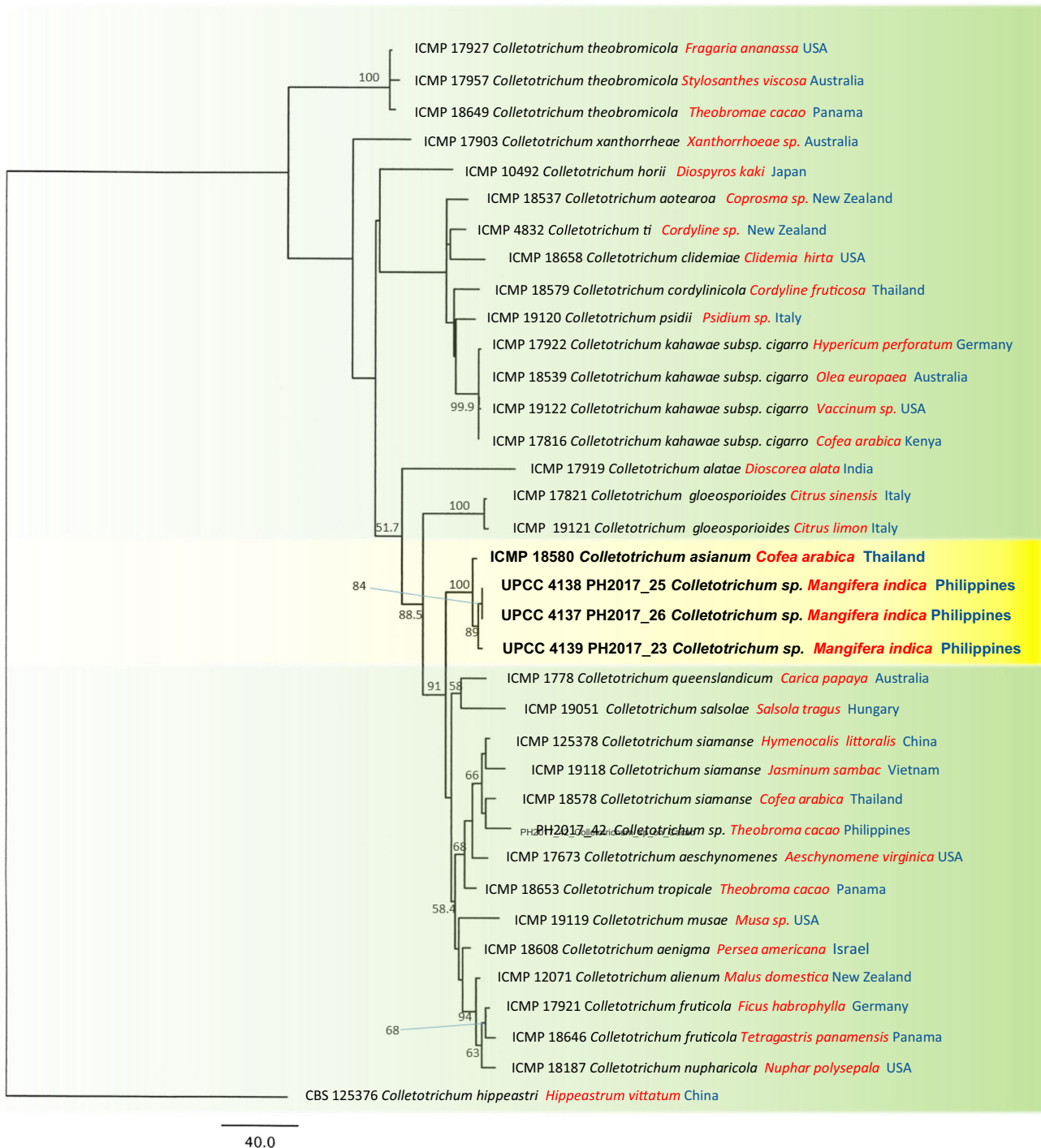


Fig. 5 Phylogenetic tree of *Colletotrichum* isolates grouped with ex-type *Colletotrichum asianum* strain

Australia, Colombia, Japan and Panama (Weir et al. 2012). This paper reports *Colletotrichum asianum* as the causal fungus of anthracnose disease of mango cv. Carabao in Luzon and Guimaras Islands, Philippines using morphology, molecular analyses and pathogenicity testing. While a study published by Weir et al. (2012) mentioned *C. asianum* (MAFF306627) obtained from Philippine mango isolated from fruit imported into Japan, the isolate was used only to understand the species complex of *C. gloeosporioides* and did not formally introduced *C. asianum* as the causal fungi of anthracnose in Philippine mango cv. Carabao. Additionally, the pathogenicity of the Japanese isolate (MAFF306627) has not been confirmed (T. Sato. personal comm.).

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