



NEW DISEASE REPORT

Colletotrichum gigasporum causing coffee berry anthracnose in the Benguet Highlands of the Philippines

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KEYWORDS*Coffea arabica*, fungal plant disease

In the Philippines, four coffee species are grown, *Coffea arabica* (Arabica coffee), *C. canephora*, *C. liberica* var. *dewevrei* and *C. liberica* var. *liberica*, with Arabica coffee being valued significantly higher. Between November 2022 and February 2023, green and ripe berries of Arabica coffee in Benguet, Philippines (16° 27.169'N; 120° 34.223'E) were observed with dark and sunken lesions that are typical of anthracnose disease (Fig. 1). Tissues obtained from the advancing necrotic lesions were surface sterilised with 10% NaOCl for two minutes and washed three times with sterile distilled water. Blot-dried tissues were plated on potato dextrose agar then incubated for seven days at room temperature (25 ± 2°C) with a 12 hour photoperiod. Pure cultures were obtained from single spore isolation. The fungal colony had a mean growth rate of 10.2 mm per day with grey to dark grey mycelia and black conidial masses (Fig. 2). Conidia (n = 90) were hyaline, cylindrical, aseptate and measured 24.6 ± 0.5 × 7.3 ± 0.2 µm (Fig. 3a). Appressoria were single or in small clusters, shape varying from elliptical to irregular outline, entire or deeply lobed, germ pore observed, with a mean size of 16.7 ± 4.9 × 8.8 ± 2.2 µm (Fig. 3b). These morphocultural characters are consistent with descriptions of *Colletotrichum gigasporum* (Rakotoniriana et al., 2013; Saini et al., 2022).

For molecular characterisation, the DNA of three isolate types (13D, 16B and 27B) was extracted from seven-day old cultures using



FIGURE 1 Naturally infected red (a) and green (b) Arabica coffee berries showing dark and sunken lesions

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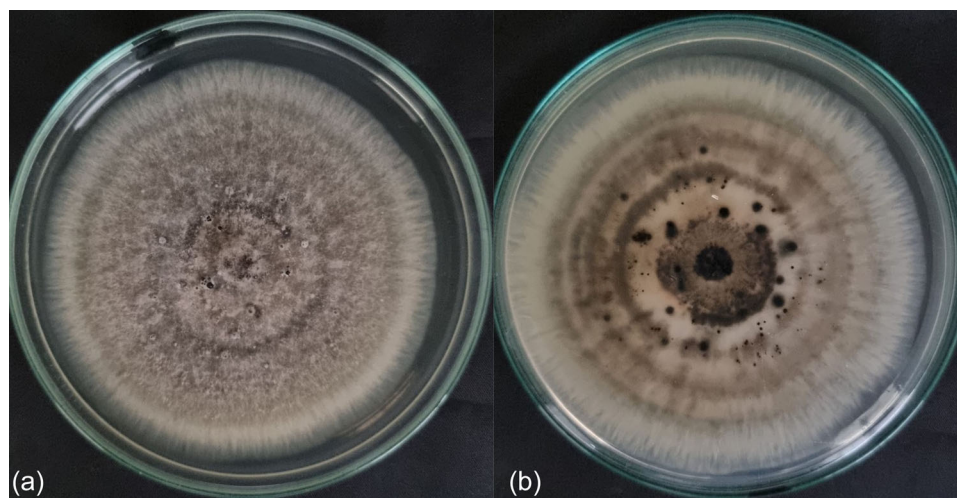


FIGURE 2 Obverse (a) and reverse (b) colony of *Colletotrichum gigasporum* grown on potato dextrose agar for seven days

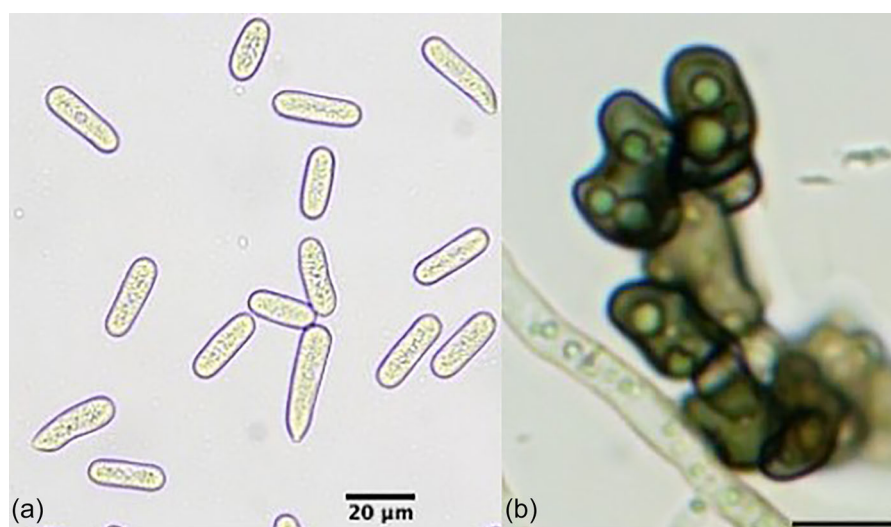


FIGURE 3 Large, hyaline, cylindrical and aseptate conidia (a) and clustered appressoria (b) of *Colletotrichum gigasporum*. Bar = 20 μ m

the CTAB method. Partial regions of the actin (ACT), chitin synthase (CHS-1), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and the β -tubulin (TUB2) genes were amplified and sequenced. BLAST analysis showed 100% identity to ACT, CHS-1, and GAPDH and 99.4% identity to TUB2 gene sequences of *C. gigasporum* (GenBank Accession Nos. KF687796, KF687758, KF687830 and KF687880, respectively). The sequence data have been submitted to GenBank (PQ824946-PQ824948 for ACT; PQ824949-PQ824951 for CHS-1; PQ824952-PQ824954 for GAPDH; and PQ824955-PQ824957 for TUB2). Phylogenetic analyses through Bayesian phylogeny and Maximum Likelihood identified the fungal isolates as *C. gigasporum* (Fig. 4).

For each isolate type, pathogenicity testing was conducted using four berries in a detached berry assay ($n = 12$). Detached healthy green berries of *C. arabica* cv. Red Bourbon were surface sterilised with 1% NaOCl for three minutes and washed three times with sterile

distilled water. An aliquot of 6 μ L of 2×10^6 conidia/ml or sterile distilled water (as control) was inoculated on unwounded and wounded (syringe-pricked) berries. Inoculated berries were placed on top of a sterile petri dish inside a humid container and incubated for 14 days at $23 \pm 2^\circ\text{C}$ with a 12 hour photoperiod. Typical symptoms of anthracnose developed 6 and 10 days post inoculation on wounded and unwounded berries, respectively, while no symptoms developed on control berries (Fig. 5). The experiment was performed twice with similar results. The fungus was re-isolated from inoculated berries with morphocultural characters similar to the original cultures, thus fulfilling the Koch's postulates.

Colletotrichum gigasporum (previously *C. thailandicum*) has been reported as the causal pathogen of *Hibiscus rosa-sinensis* leaf anthracnose in Thailand (Noireung et al., 2012). More recently *C. gigasporum* was reported as an endophyte associated with *Centella asiatica*

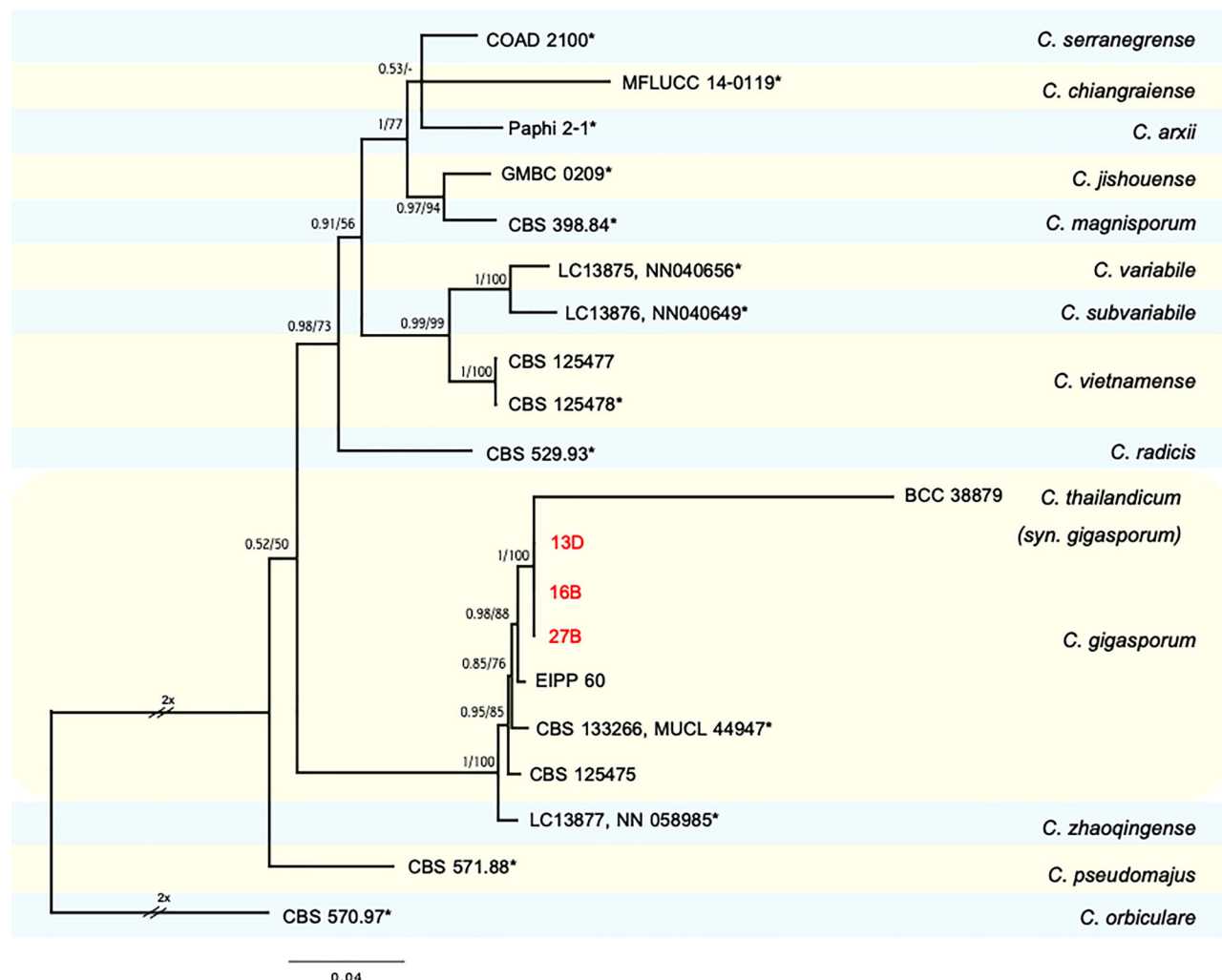


FIGURE 4 Bayesian phylogeny for the TII isolates (shown in red) together with other species within the *Colletotrichum gigasporum* complex based on a 50% majority rule consensus tree of the concatenated ACT, TUB2, CHS-1 and GAPDH sequence alignment. The posterior probabilities (PP) and maximum likelihood bootstrap support (BS) values (>50%) are shown in the nodes (PP/BS). The type/holotype strains are indicated with an asterisk. The lengths of the two branches with 2x were halved for the tree layout. *Colletotrichum orbiculare* was used as an outgroup

(Madagascar), *Stylosanthes guianensis* (Mexico) and *Coffea arabica* (Colombia) (Rakotoniriana et al., 2013). Recent reports have also implicated *C. gigasporum* as a pathogen of various diseases, such as anthracnose of ornamental plants in Iran (Alizadeh et al., 2015), anthracnose of sour soup tree and sugar apple trees in Brazil (Costa et al., 2019), cauliflower anthracnose in India (Saini et al., 2022), and leaf anthracnose of *Dalbergia odorifera* and papaya fruit rot in China (Wan et al., 2018; He et al., 2024). To the best of our knowledge, this study reports the first record of *C. gigasporum* in the Philippines causing Arabica coffee berry anthracnose. Assessment of the aggressiveness of this newly recorded pathogen to the four coffee species grown in the Philippines as well as sensitivity screening to chemicals and biocontrol agents will be crucial to disease management.

ACKNOWLEDGEMENTS

The authors thank the coffee farmers, staff of the Municipal Agriculturist Office of the 13 Local Government Units of Benguet Province

and PMGEN Oliver Enmodias for their valuable assistance during sample collection, the Department of Science and Technology-ASTHRDP and the Institute of Plant Breeding- University of the Philippines Los Baños for funding and laboratory support. In particular, O.D. Balanban wants to thank Jessica Enmodias for her ongoing support over the last 10 years.

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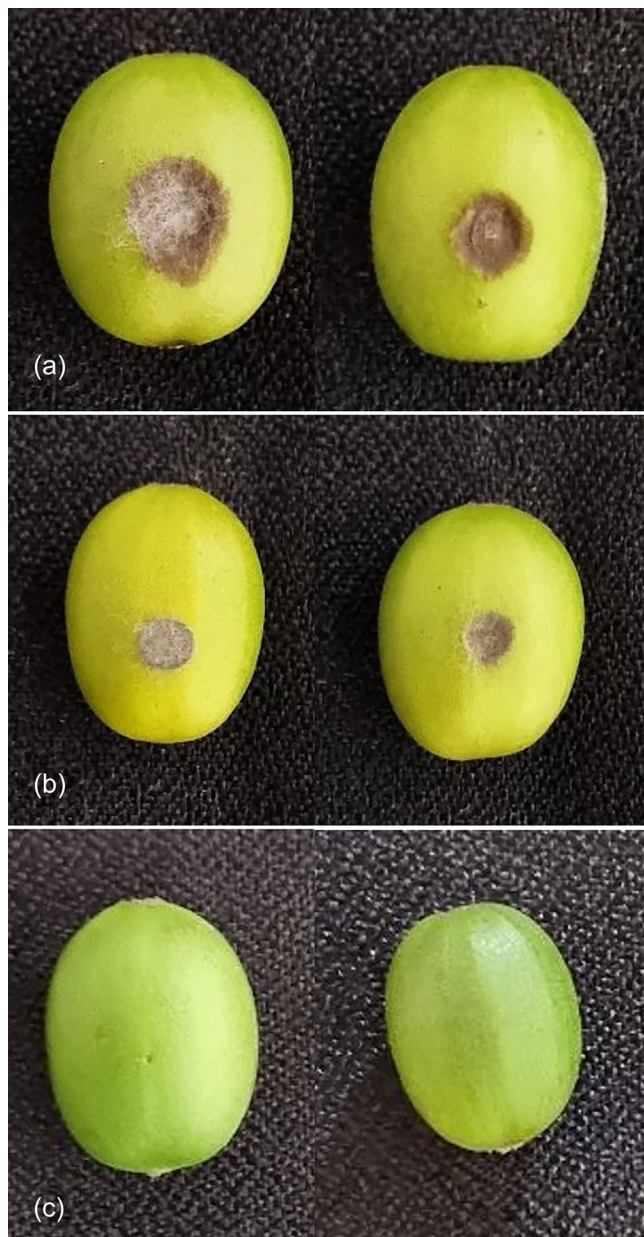


FIGURE 5 Symptoms developed on wounded (a) and unwounded (b) green berries at 6 and 10 days post inoculation, respectively. No symptoms were observed on berries inoculated with sterile distilled water (c).

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How to cite this article: Balanban, O.D., Pangga, I.B., Diaz, M.G.Q., Tiongco, R.L. & Cueva, F.M.D. (2025) *Colletotrichum gigasporum* causing coffee berry anthracnose in the Benguet Highlands of the Philippines. *New Disease Reports*, vol.51, e70034. <https://doi.org/10.1002/ndr2.70034>