Received: 2 October 2024 | Accepted: 3 November 2024

DOI: 10.1002/ndr2.70002

New Disease Reports Open Access BSPP WILEY

NEW DISEASE REPORT

First report of cassava witches' broom disease and *Ceratobasidium theobromae* in the Americas

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Funding information
United States Agency for International Development; Consortium of International Agricultural Research Centers

KEYWORDS
Cassava, emergent disease, witches' broom, Ceratobasidium

Cassava (*Manihot esculenta*) is a primary source of carbohydrates for millions of people in the tropics. In the Americas, cassava also holds deep cultural and economic significance for indigenous peoples (Parmar et al., 2017). However, the increased commercialisation and connectivity between cassava-producing regions has heightened the risks of the introduction of diseases that threaten this food security crop.

Symptoms of dwarfism, weak sprouts with short petioles and vascular necrosis were observed since 2023 in cassava-growing communes in French Guiana (Table 1). These symptoms resembled those of cassava witches' broom disease (CWBD) which has previously been reported only in Southeast Asia (Pardo et al., 2023) (Fig. 1). Recently, CWBD was associated with *Ceratobasidium theobromae* (Leiva et al., 2023; Landicho et al., 2024), the fungus causing vascular streak dieback of cacao (Keane et al., 1972).

To characterise the disease, on 13 May, 2024, we visited Saint-Georges-de-l'Oyapock (N3.731375, -W51.788637), the commune that had the largest number of affected fields in our previous survey (Table 1; Fig. 1). Petioles of the top part of cassava plants with and without symptoms were collected following a diagonal transect along the field (code name GUF-114). Petioles were wrapped in paper towels, placed in plastic bags, and kept at 4°C for 24 hours before processing.

For fungus isolation, petioles were disinfected using 1% hypochlorite followed by 75% ethanol following the protocol described by Gil-Ordóñez et al. (2024). For DNA extraction dry petioles were transferred into 2 mL tubes and ground in liquid nitrogen before using a DNeasy Plant Mini Kit (Qiagen, Germany). The rDNA internal transcribed spacer region was amplified using ITS4 and ITS5 primers (Landicho et al., 2024). A phylogenetic tree was generated using the Maximum Likelihood method (1000 replicates) with related sequences available in GenBank. PCR specific to *C. theobromae* targeting the CAMK gene, was conducted as described by Leiva et al. (2023).

TABLE 1 Number of fields visited, fields showing symptoms and proportion of fields showing disease symptoms over the period extending from 5 June to 29 February 2024.626

Commune	Number of fields visited	Number of affected fields	Proportion of affected fields
Apatou	21	11	0.52
Camopi	8	6	0.75
Iracoubo	6	4	0.67
Macouria	1	0	0.00
Mana	7	1	0.14
Maripasoula	2	2	1.00
Montsinéry-Tonnégrande	2	0	0.00
Papaïchton	2	2	1.00
Régina	1	0	0.00
Roura	1	1	1.00
Saint-Georges de l'Oyapock	16	13	0.81
Total	**67**	**40**	**0.60**

FIGURE 1 Symptoms of cassava witches' broom disease as observed in French Guiana. Broom-like growths were observed along the main stem of cassava (a), or at the top (b). Notice in A, dead leaves remain attached to the stem below the broom-like structure. In B, the white mycelium can be observed growing on the affected stem. In C, a cross-section of the diseased stem shows the vascular necrosis (top stem) associated with the disease in contrast with the stem of a healthy plant (bottom stem).

Characteristic flat and yellowish mycelia containing septa at every 90° branch were observed after incubating the petioles for three days at 25°C (Fig. 2). However, no basidiospores were produced which could be used to perform pathogenicity tests and attempts to infect plants using mycelia have so far failed, as observed in cacao (Keane et al., 1972). The ITS sequence (ITS GUF-114, GenBank Accession No. PQ475929) had 98.6% identity with *C. theobromae* isolated from cacao in India (KU310940) and 98.4% identity with *C. theobromae* isolate LAO1 isolated from cassava in Laos (PQ412515) (Fig. 3). The CAMK sequence (CAMK GUF-114: PQ384454) amplified in all diseased plants in the current study was >99% identical to *C. theobromae* isolates from Southeast Asia (OQ863061-OQ863086). This is the first report of CWBD and *C. theobromae* in the Americas and a phytosanitary alert that requires urgent regional action.

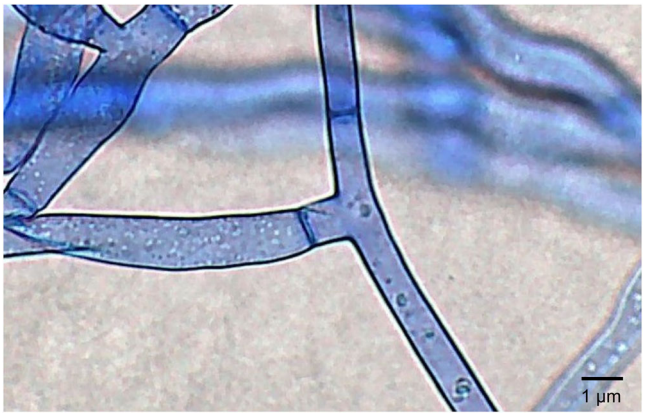

FIGURE 2 Isolation of *Ceratobasidium theobromae* from affected petioles. Characteristic structure of *Ceratobasidium* hyphae observed under the microscope (×400) after staining with 1% methylene blue. The fungal colony was grown in Corticium culture medium and stained as described in Gil-Ordóñez et al. (2024).

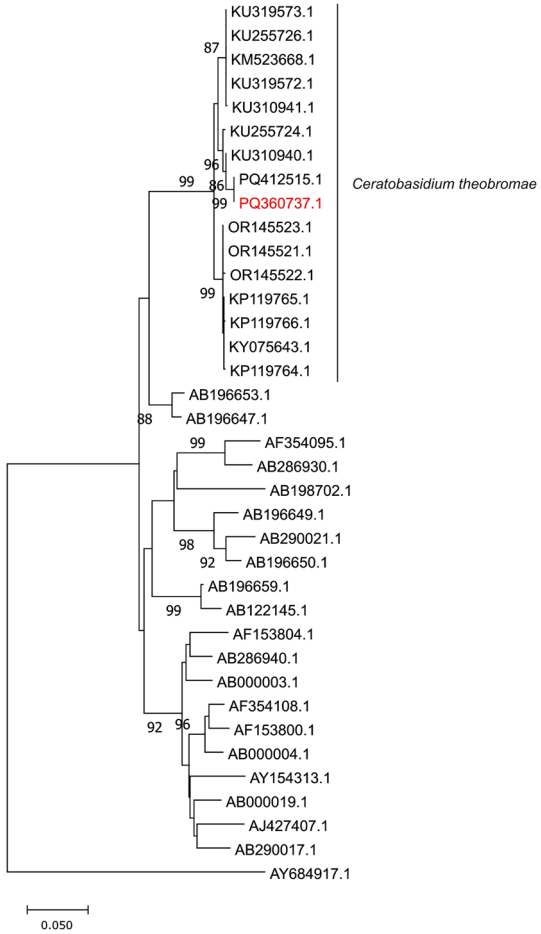

FIGURE 3 Phylogenetic tree showing the grouping of GUF-114 ITS (in red) with other ITS sequences of *Ceratobasidium theobromae* from GenBank. The tree was constructed with MEGA version 11.0.13 (1000 bootstrap replicates). The evolutionary distances were computed using the Maximum Composite Likelihood method (number of substitutions per site).

ACKNOWLEDGEMENTS

Thanks to the Alliance of Bioversity and CIAT for financial support through its institutional fund for strategic research activities and to the U.S. Agency for International Development.

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How to cite this article: Pardo, J.M., Gil-Ordóñez, A., Leiva, A.M., Enjelvin, L., Chourrot, A., Kime, S.C.K. et al. (2024) First report of cassava witches' broom disease and *Ceratobasidium theobromae* in the Americas. *New Disease Reports*, 50, e70002. https://doi.org/10.1002/ndr2.70002