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First report of *Gilbertella persicaria* as the cause of soft rot of fruit of *Syzygium cumini*

Danilo B. Pinho · Olinto L. Pereira · Dartanha J. Soares

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Abstract A zygomycetous fungus causing fruit soft rot was found on *Sygyzium cumini* in Northeast Brazil. Based on morphological and phylogenetic analyses, the fungus was identified as *Gilbertella persicaria*. This is the first report of this fungus causing the decay of *S. cumini* fruit worldwide.

Keywords Choanephoraceae · Fruit decay · Mucorales · Myrtaceae · Zygomycetes

Fruits of *Syzygium cumini* (L.) Skeels (Myrtaceae) that showed soft rot symptoms caused by Mucorales (Zygomycetes) (Fig. 1a) were collected in the wet season of 2010 from Paraíba State (northeast Brazil). *Syzygium cumini*, known as jambolan or black plum, was naturalized in Brazil and has no commercial interest, despite its use in folk medicine and evidence of its pharmacological properties (Ayyanar and Subash-Babu 2012).

Soft roted fruits were examined under a stereo-microscope, which confirmed the presence of a *Mucor*-like fungus. The fungus was then isolated on potato dextrose-agar (PDA), by direct transfer of a spore mass using a sterile needle and the culture derived was deposited in the Culture Collection of the Universidade Federal de Viçosa (accession number COAD 1728) and also in the Culture Collection of Phytopathogenic Microorganisms of the Embrapa Algodão (accession number CCMF-CNPA 0627).

D. B. Pinho · O. L. Pereira
Departamento de Fitopatologia, Universidade Federal de Viçosa, 36570-900 Viçosa, MG, Brazil

D. J. Soares (✉)
Embrapa Algodão, Rua Oswaldo Cruz, 1143, Centenário, 58428-095 Campina Grande, PB, Brazil
e-mail: dartjs@yahoo.com.br

Identification of the fungus was based on its morphological characteristics, and the identity was later confirmed using molecular methods. Fungal structures obtained from the culture plates were mounted in lactic acid or water on microscope slides and were examined under a Leica DM2500 microscope equipped with DIC lenses. The genomic DNA was extracted from pure cultures grown on PDA using a Wizard® Genomic DNA Purification Kit (Promega Corporation, WI, U.S.A.), as described in Pinho et al. (2012). The target regions of the internal transcribed spacer (ITS) and the 28S rDNA large subunit (LSU) were amplified using ITS1/ITS4 and LR0R/LR5 primers, respectively (Vilgalys and Hester 1990; White et al. 1990). The sequences were edited using BioEdit software (Hall 2012). A BLAST search was performed to check for similarity with other sequences. The sequences used in the study were selected from a phylogenetic tree provided in Walther et al. (2013) and were aligned using the multiple sequence alignment program MUSCLE® (Edgar 2004) in MEGA v. 5 software (Tamura et al. 2011). Bayesian inference concatenated (BI) analyses were performed using the ITS dataset and the resulting alignment and tree were deposited in TreeBASE (http://www.treebase.org/) as accession number S15860. The best nucleotide substitution model selected according to the Akaike information criterion (AIC) was HKY+G for ITS. The Bayesian analysis was performed in the CIPRES Science Gateway (Miller et al. 2010) using MrBayes on XSEDE, and the tree was rooted to *Hyphomucor assamensis* CBS415.77.

The pathogenicity of the fungus was tested on *S. cumini* fruits that were surface sterilized and inoculated by touching the fungal spore mass, growth on PDA, with a sterile needle, which was then used to puncture the fruits. After inoculation, the fruits were maintained in a dew chamber in daylight on the bench (at~25 °C).

When grown on PDA at 25 °C, the fungus initially produced a white colony, which covered the entire 90 mm diam. Petri dish within 3 days, and became darker with age. A darker zone formed between colonies on the Petri dish used for culture isolation revealed the presence of the sexual structures of the fungus. Zygospores globose to subglobose, compressed

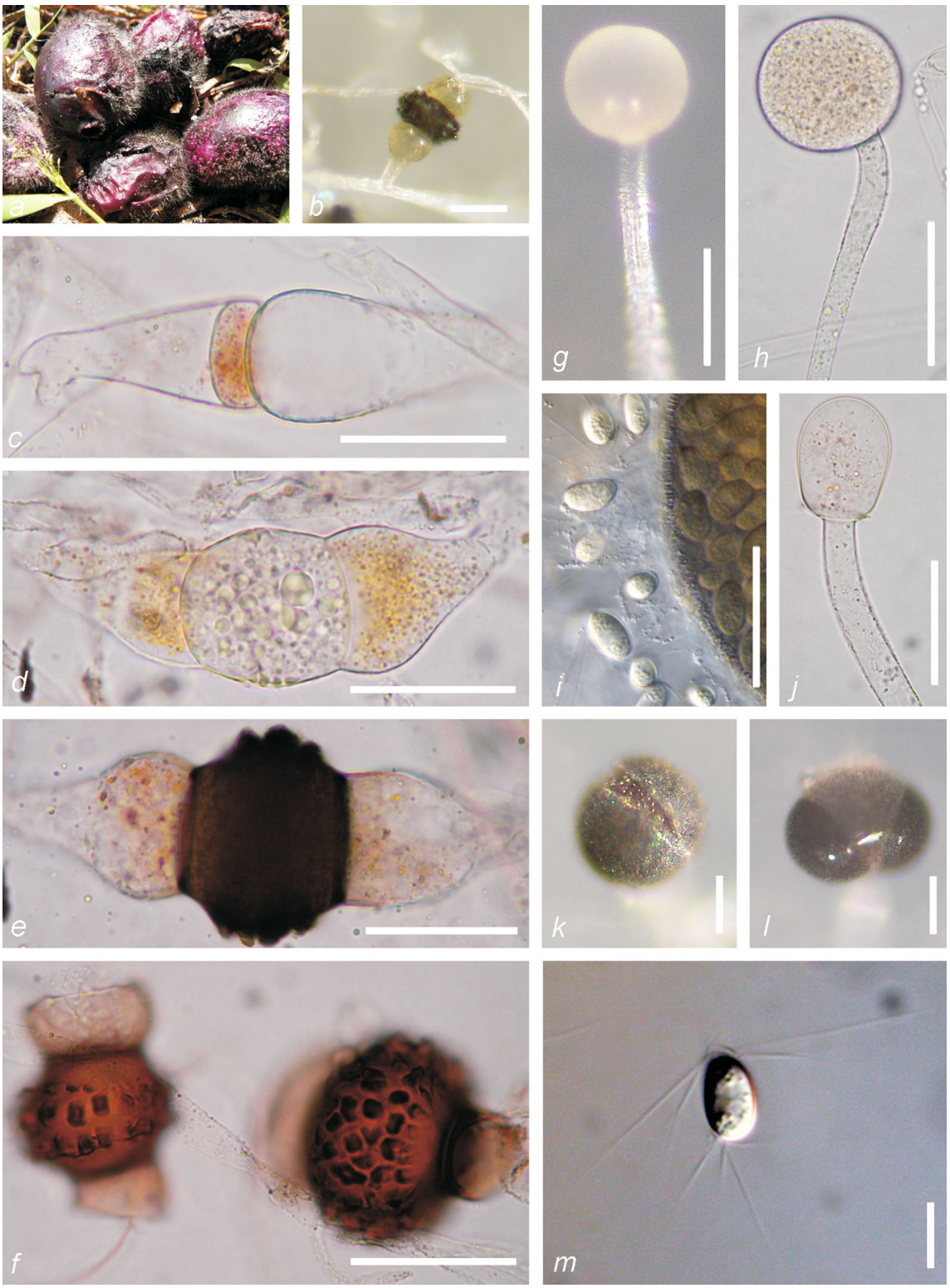

Fig. 1 *Gilbertella persicaria*. Typical image of the affected fruit of *Syzygium cumini* (**a**). Zygospore formation sequence (**b**–**f**). Immature sporophores (**g**–**h**). Higher magnification view of the sporangial wall showing spines (**i**). Columellae (**j**). Wall suturing in two equal halves (**k**–**l**). Sporangiospore with appendages (**m**). *Scale bars* = 50 μm, except in *m* in which the *bar* = 10 μm

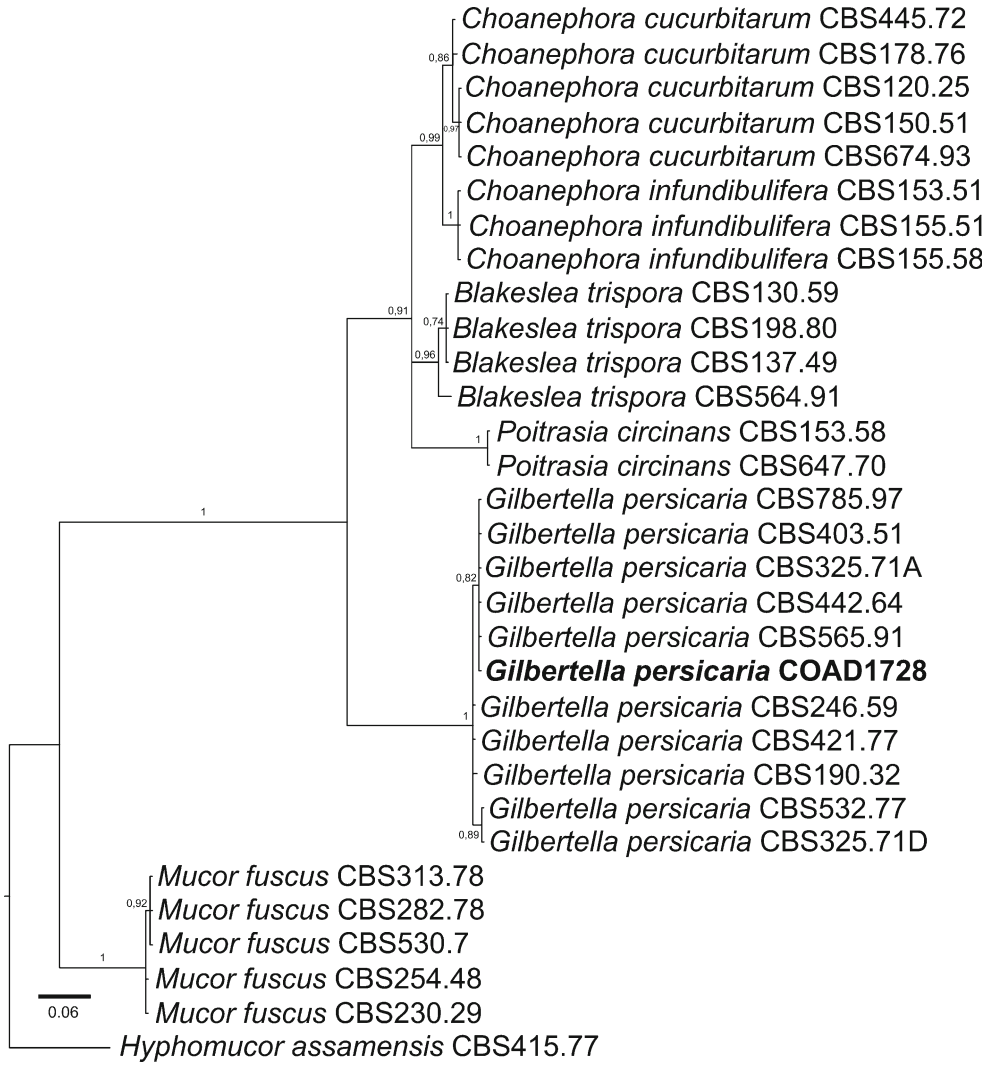

Fig. 2 Phylogenetic tree inferred from the Bayesian analysis based on the ITS sequences of the *Gilbertella persicaria* and related taxa. The Bayesian posterior probability values are indicated at the nodes. The tree was rooted to *Hyphomucor assamensis*. The specimen used in this study is highlighted in bold

between suspensors, with coarse projections (Fig. 1b–f). Sporophores hyaline, becoming pale brown with age, simple, sometimes branched, erect, curved below the sporangium. Sporangia spherical, initially white-yellowish, turning light to dark brown or black when mature (Fig. 1g–h); wall persistent, covered with spines (Fig. 1i), separating at maturity into two equal halves (Fig. 1k–l); columellae obvoid to globose, with a distinct basal collar (Fig. 1j). Sporangiospores variable in shape, mainly ellipsoid, hyaline, smooth, one-celled, with hyaline filiform appendages at both ends (Fig. 1m).

Based on the above-mentioned characteristics, the fungus was identified as a member of the genus *Gilbertella* Hesseltine. At least three names are recognized within *Gilbertella*, viz. *G. persicaria* (E.D. Eddy) Hesselt., *G. persicaria* var. *indica* B.S. Mehrotra & M.D. Mehrotra and *G. hainanensis* J.Y. Cheng & F.M. Hu, with *G. persicaria* being the type species (Hesseltine 1960); however, in a recent treatise concerning Mucorales, Walther et al. (2013) regarded those names as synonyms of *G. persicaria*, and the genus is now considered monotypic.

The Bayesian analysis confirmed that the fungus found on *S. cumini* belongs to the genus *Gilbertella* and can be grouped in the same clade as other taxa of the family Choanephoraceae (data not shown), according to the new classification adopted by Hoffmann et al. (2013). The BLAST search using the sequences ITS (KJ815093) and LSU (KJ815094) regions revealed 99 % identity with the ITS (Accession No. NR_111692) and LSU (Accession No. JN939197) sequences from the type material (strain CBS 190.32) of *G. persicaria*. Morphological examination and Bayesian analysis (Fig. 2) revealed that the fungus found on *S. cumini* is *G. persicaria*.

The first symptoms appeared between 16 and 24 h after inoculation in the form of a small depression of the fruit pulp at the point of inoculation. The fungus sporulated on fruits in less than 36 h, whereas the controls (non-inoculated fruits) remained intact (Fig. 3).

Gilbertella persicaria is known mainly as a postharvest rot fungus, but is frequently isolated from soil and dung

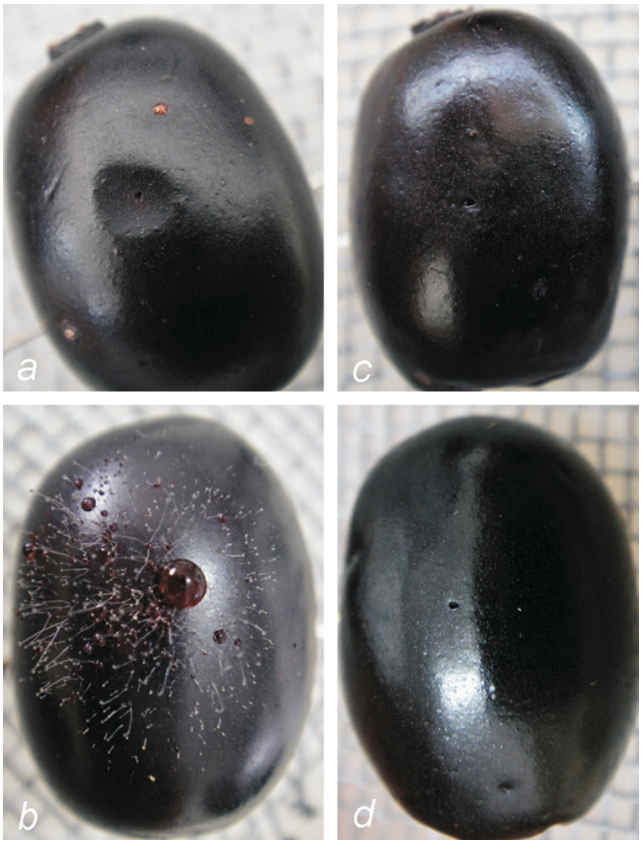

Fig. 3 Pathogenicity test of *Gilbertella persicaria* on *Syzygium cumini* fruits. Inoculated fruit 18 h after inoculation, showing the slight depression in the pulp (**a**) and at 36 h, showing the sporulating fungus (**b**). Non-inoculated fruit at 18 h (**c**) and 36 h (**d**), showing its intact pulp

(Hesseltine 1960; Mehrotra 1964; Benny 1991; Ginting et al. 1996; Santiago and Cavalcanti 2007; Guo et al. 2012). In Brazil, this fungus was first reported from soil samples in Rio de Janerio State, by Hesseltine (1960). It was subsequently reported from tapir, donkey and elk dung, in Pernambuco State, by Santiago and Cavalcanti (2007) who mistakenly regarded it as the first report in Brazil.

To our knowledge, this is the first report of *G. persicaria* associated with the fruit soft rot of *S. cumini* worldwide.

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