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Neoscytalidium dimidiatum Associated With Canker Disease in *Spondias mombin* L. in Brazil

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ABSTRACT

In 2023, Cajá trees (*Spondias mombin*) exhibiting symptoms of canker and resinosis were collected from a commercial plantation in Teresina, Piauí, Brazil. Affected plants displayed small, irregular cankers on the trunks and branches, accompanied by abundant resin exudation, which coalesced into extensive necrotic lesions. A Scytalidium-like fungus was consistently isolated from symptomatic tissues. Based on morphology and phylogenetic analyses of concatenated multilocus sequences of the internal transcribed spacer (ITS), translation elongation factor 1-alpha (*tef1-α*), and β-tubulin (*tub2*), the isolates were identified as *Neoscytalidium dimidiatum*. Pathogenicity tests confirmed that all isolates caused typical canker symptoms on stems and fruit rot in *S. mombin*, *S. purpurea*, and *S. tuberosa*. The pathogen was re-isolated from inoculated tissues, and its morphology matched that of the original cultures, fulfilling Koch's postulates. Our results demonstrate the pathogenic potential of *N. dimidiatum* on economically important *Spondias* species. This is the first report of *N. dimidiatum* as the causal agent of canker in *S. mombin*.

Spondias mombin L. (common names: cajá or taperebá; *Anacardiaceae* family) is an evergreen tree native to tropical America and widely distributed across tropical regions worldwide (Mitchell and Daly 2015). In Brazil, the species is mainly exploited in humid and semi-arid areas of the North and Northeast regions, where its commercial use is largely based on extractivism with limited cultivation (Silva et al. 2024). In 2023, during phytosanitary inspections in a cultivated area of *S. mombin* in Teresina, Piauí, Brazil (5°05′13″ S, 42°48′41″ W), symptoms of canker and resinosis were observed in 17 mature trees. The symptoms consisted of sunken lesions on the outer bark, appearing as isolated spots on the trunk and main branches (Figure 1a,b). These lesions were associated with abundant exudation of dark resin and longitudinal cracking of the wood (Figure 1a–c). Internal vascular tissues showed pronounced darkening (Figure 1d), frequently accompanied by shoot dieback and premature leaf drop. In severe cases, affected trees exhibited progressive decline and eventual death. Three fungal isolates were obtained from symptomatic tissues of three cajá trees and deposited in the Coleção de Fitopatógenos da Universidade Federal de Alagoas (COUFAL) under the codes COUFAL0654, COUFAL0655, and COUFAL0656. The isolates were characterized by assessing colony colour (both the front and back of the plates), mycelial growth rate, and conidia size. After 14 days of growth at 27°C in the dark, we evaluated the colony colour and texture on Potato Dextrose Agar (PDA) and Oatmeal Agar (OMA). These isolates initially produced whitish-grey colonies with moderately dense aerial mycelium on PDA. After 14 days, colonies became dark grey to dark brown (Figure 2a). On OMA, the colonies showed an olive-grey coloration and dense

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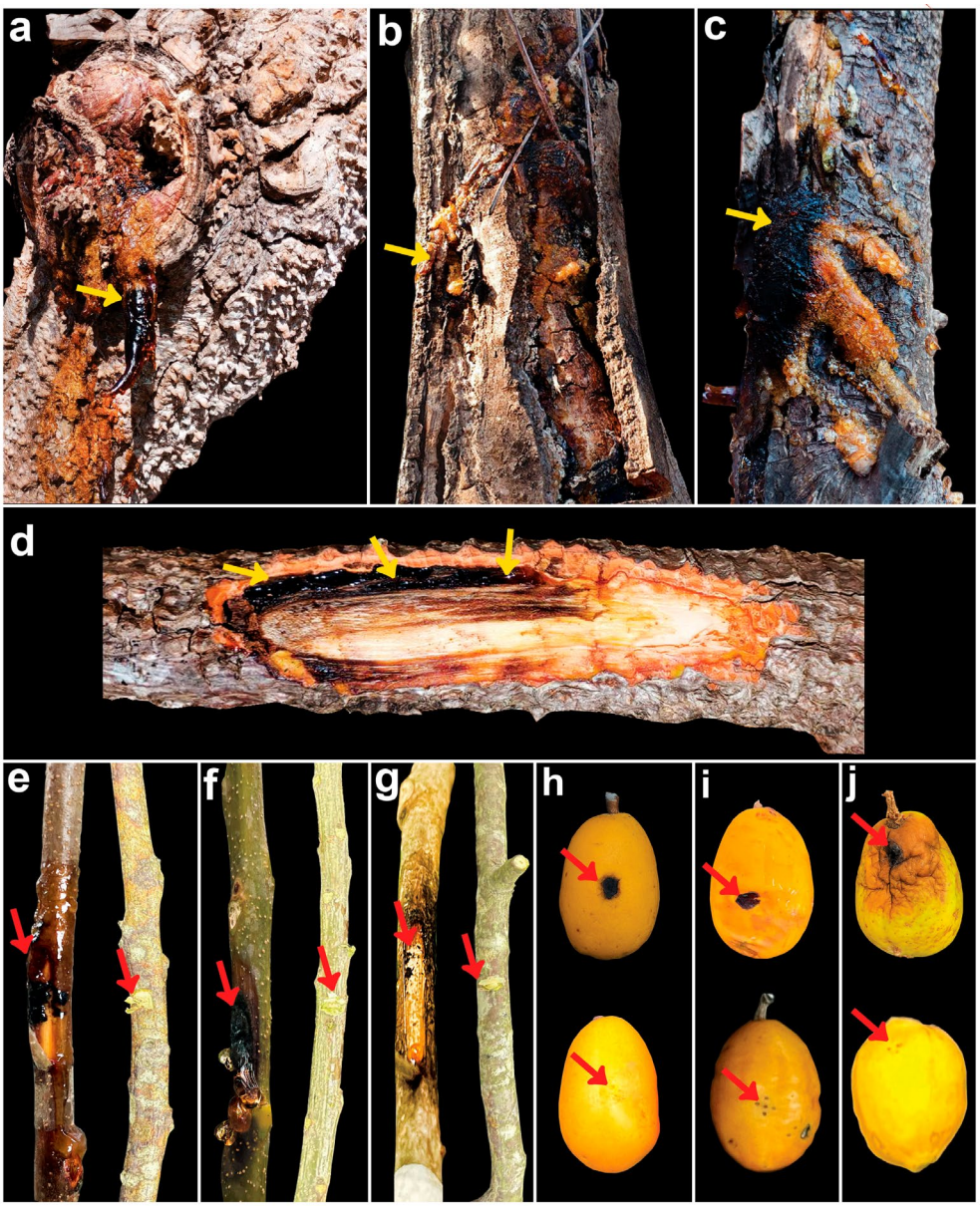


FIGURE 1 | Symptoms observed in the field and those induced in pathogenicity tests. (a–d) Canker and resinosis symptoms on *Spondias mombin* under field conditions. (d) Transverse section of bark tissue showing resin exudation, indicative of a host defence response. (e–j) Symptoms induced in pathogenicity assays on seedlings and fruits of *S. mombin* (cajá), (e, h) *S. purpurea* (siriguela) (f, i) and *S. tuberosa* (umbu). (g, j) Negative control treatments showed no symptoms. *Yellow arrows indicate resin production; red arrows indicate inoculation points.

mycelium (Figure 2b). Mycelial growth rates ranged from 19.2 to 20.7 mm day^{-1}, and colonies reached 9 cm in diameter within 2–4 days. Hyphae were greyish-brown, septate, and branched, with chain-like constrictions that fragmented into arthroconidia (Figure 2c). Arthroconidia were ellipsoidal to ovoid, hyaline to dark brown, thick-walled, and septate, measuring 7.62–11.59 × 1.91–3.82 µm ($n = 20$; mean = 8.82 × 2.53 µm), differing from previous descriptions that report wider and predominantly doliiform conidia (e.g., Yeken et al. 2025). However, these measurements fall within the known morphological variability of *Neoscytalidium dimidiatum*, which exhibits considerable variation in conidial size and shape. Dark, melanized, aggregated structures were observed on the colony surface; however, these structures remained infertile and did not develop into true pycnidia after 14 days of incubation (Figure 2d). Therefore, they are herein referred to as conidiomata primordia (or sclerotioid bodies), as previously suggested in the literature. Genomic DNA was extracted following Doyle and Doyle (1987). Partial sequences of ITS, *tef1-α*, and *tub2* were amplified with primers ITS1/ITS4 (White et al. 1990), EF1-688F/EF1-1251R (Alves et al. 2008), and Bt2a/Bt2b (Glass and Donaldson 1995), respectively. The PCR products were purified and sequenced. The newly obtained sequences were assembled and manually adjusted using CodonCode Aligner v. 4.1.1 (Codon Code Corporation, Dedham, MA, USA). Multiple sequence alignments for each marker were then generated using MEGA 11.0.13 (Tamura et al. 2021). Bayesian phylogenetic inference was conducted using a Markov chain Monte Carlo approach on the CIPRES web portal (Miller et al. 2010). Due to the observed morphological variability among the isolates, a

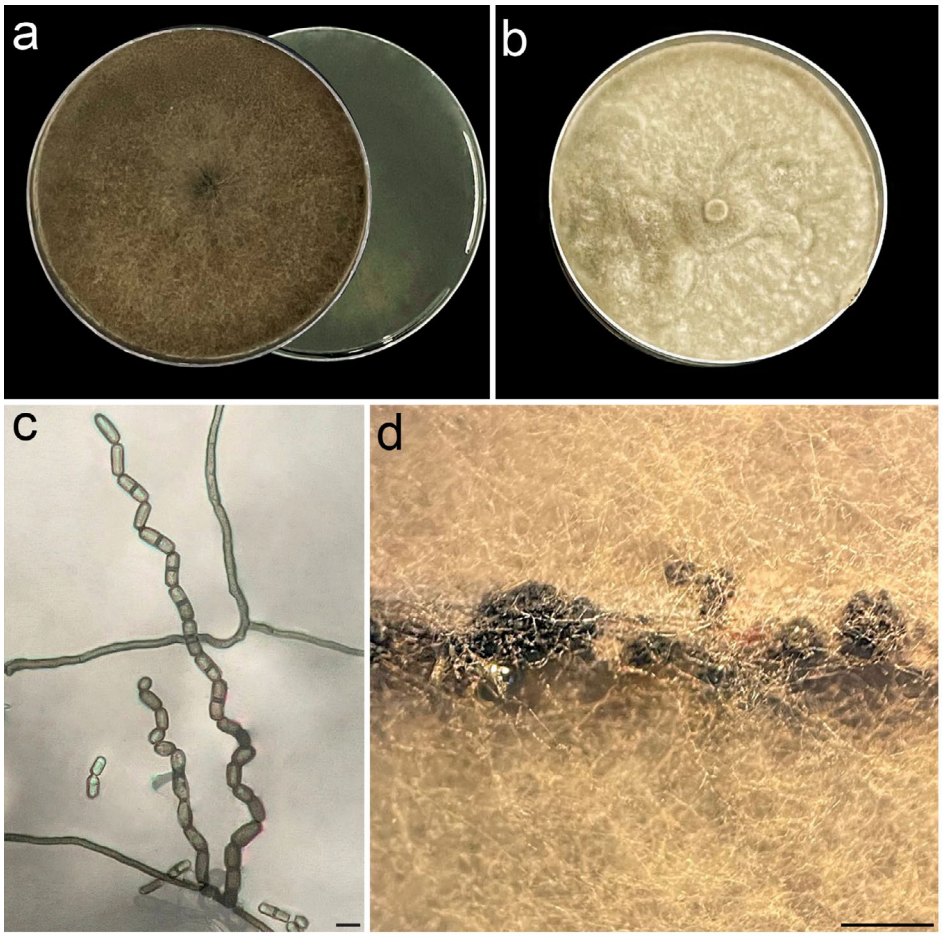


FIGURE 2 | Morphological features of the *Neoscytalidium dimidiatum*, isolate COUFAL0654: (a) Colony morphology on PDA. (b) Colony morphology on OMA. (c) Chains of dematiaceous arthroconidia. (d) Conidiomata primordia on pine needles after 14 days. Scale bars: (c) 10 μm; (d) 3 mm.

multigene phylogenetic analysis was performed to support species identification. The dataset was restricted to representative taxa closely related to *Neoscytalidium dimidiatum* to ensure a clear and interpretable phylogenetic topology, consistent with the concise format of this study (Table S1). In the Bayesian inference tree, isolates COUFAL0654, COUFAL0655, and COUFAL0656 clustered within a well-supported monophyletic clade of *Neoscytalidium dimidiatum*, together with reference sequences of the species (posterior probability = 0.96; Figure 3; Table S1). For the Bayesian Inference (BI) phylogenetic analyses, the HKY + G model was selected for both ITS and *tef1-α* based on the AIC, while F81 was chosen for *tub2*. Pathogenicity tests were conducted on fruits and seedlings of *Spondias mombin*, with additional cross-inoculations on *S. purpurea* and *S. tuberosa*. For fruit assays, wounds were made on the fruit surface using sterile needles, punctured centrally to a depth of 3 mm, and then inoculated with 5-mm mycelial plugs from 7-day-old PDA cultures. For seedlings, stem wounds were made approximately 30 cm above the substrate level using sterile scalpel blades. A single colonized rice grain prepared from fungal cultures was then inserted into each wound, which was subsequently wrapped with plastic film to maintain humidity and promote infection (Claudino and Soares 2014). Control treatments received sterile PDA plugs or non-colonized rice grains. Inoculated materials were maintained under humid conditions at 27°C for 48 h, and symptom development was assessed 7 days after inoculation. On fruits, irregular, sunken necrotic lesions developed within 2 days after inoculation, followed by tissue softening and pulp darkening by Day 7 (Figure 1h–j). In seedlings, resin exudation was observed from 4 days after inoculation, and necrotic canker-like lesions developed by Day 7 (Figure 1e–g). The pathogens were consistently re-isolated from symptomatic tissues, and the recovered isolates showed morphological characteristics identical to those of the original cultures, fulfilling Koch's postulates. Canker disease caused by species of the genus *Neoscytalidium* is considered one of the most destructive diseases affecting tropical and subtropical fruit trees worldwide (Dy et al. 2022). The pathogen has been reported in several crops, including pitaya (Xu et al. 2018), pistachio (Derviş et al. 2019), fig (Al-Bedak et al. 2018), grapevine (Rolshausen et al. 2013), olive (Güney et al. 2022), and apple (Ören et al. 2022). To the best of our knowledge, this is the first report of *Neoscytalidium dimidiatum* causing canker on *Spondias mombin* worldwide.

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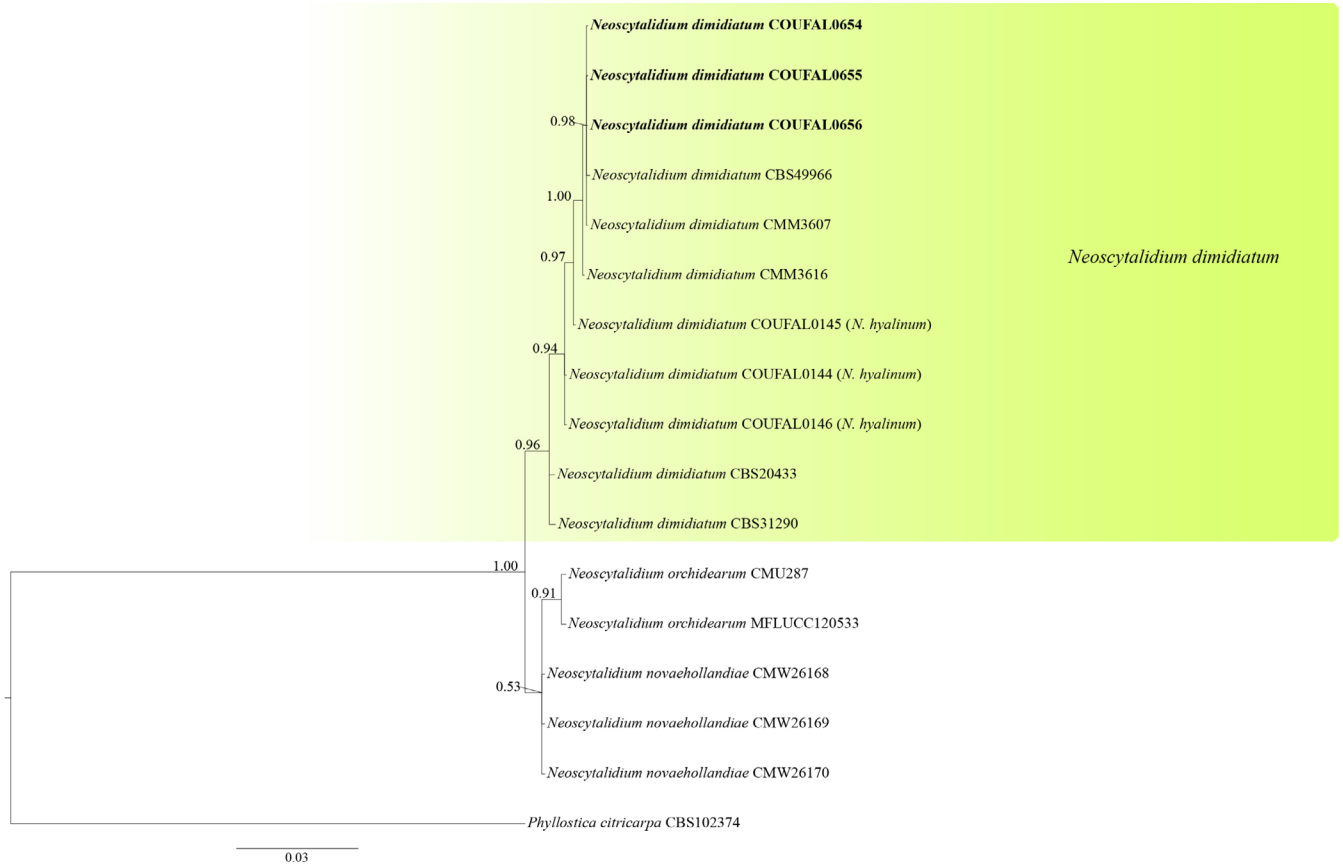


FIGURE 3 | Bayesian inference phylogenetic tree based on the combined sequences of the ITS, *tef-1α*, and *tub2* genomic regions. Bayesian posterior probability values are shown above the nodes. *Neoscytalidium* isolates obtained in this study are highlighted in bold. *Phyllostica citricarpa* (CBS 102374) was used as an outgroup.

Ethics Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Taxa used in the phylogenetic analysis, along with their respective GenBank accession numbers.