

SHORT COMMUNICATION OPEN ACCESS

Leaf Spot and Fruit Rot on Achachairu: Novel Diseases Caused by *Lasiodiplodia theobromae*

Kevison Romulo da Silva França  | Marcus Vinícius Santos da Silva | Mayra Machado de Medeiros Ferro | Denise de Santana Silva | Jaqueline Figueredo de Oliveira Costa | Gaus Silvestre de Andrade Lima | Iraildes Pereira Assunção

Universidade Federal de Alagoas, Campus de Engenharias e Ciências Agrárias, Rio Largo, AL, Brazil

Correspondence: Kevison Romulo da Silva França (kevison.franca@ceca.ufal.br)

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ABSTRACT

The achachairu (*Garcinia humilis*) is an exotic tropical fruit native to Bolivia that has recently gained commercial popularity in Brazil. In a producing region of Pernambuco, Brazil, spots and rot symptoms were observed on the leaves and fruit of *G. humilis*. Fungal isolates from the *Lasiodiplodia* genus were obtained from symptomatic tissue. Based on multigenic analyses (*TEF1-α*, ITS and *TUB2*) and morphological characteristics, the isolates were identified as *L. theobromae*, which were confirmed to be pathogenic on achachairu leaves and fruits. This report presents two new diseases in achachairu caused by *L. theobromae*. This study improves our understanding of the achachairu pathosystem and establishes a foundation for future disease management research.

1 | Introduction

Garcinia humilis (Vahl) C. D. Adam (*Clusiaceae* Family), commonly known as achachairu, is a fruit tree native to the Santa Cruz region of Bolivia and has recently been widely cultivated in Brazil. This lesser-known species is frequently mistaken for other members of the *Garcinia* genus outside its native range, including bacuripari (*G. brasiliensis*) and mangosteen (*G. mangostana*). However, achachairu exhibits strong potential for commercial fruit production and plays a significant role in forest conservation and the restoration of degraded areas (Silva et al. 2018; Nunes et al. 2019). It is a medium-sized perennial tree well adapted to the tropical and subtropical regions of South America. The tree produces a highly palatable fruit characterised by a soft, mucilaginous texture and a well-balanced combination of sweetness and acidity, which is harvested from both cultivated and wild populations (Sanchez

et al. 2024). Furthermore, achachairu fruit has an extended shelf life and demonstrates resilience to handling and transportation, conferring a competitive advantage over other commercially available fruits (Tome et al. 2019).

Despite its agricultural and ecological significance, research on diseases affecting achachairu remains limited, likely due to its relatively recent commercial expansion and the scarcity of scientific studies. The most recent reports include *Rhizopus stolonifer*, identified as a postharvest pathogen affecting the fruit (Souza et al. 2024), as well as *Colletotrichum siamense* and *C. theobromicola*, which have been documented as causative agents of diseases affecting both the fruit and leaves of achachairu in Brazil (da Silva Anjos et al. 2025).

In 2024, we observed small spots and necrotic lesions on leaf margins and symptoms of fruit rot in achachairu (Figure 1a,b).

Kevison Romulo da Silva França and Marcus Vinícius Santos da Silva contributed equally to this article.

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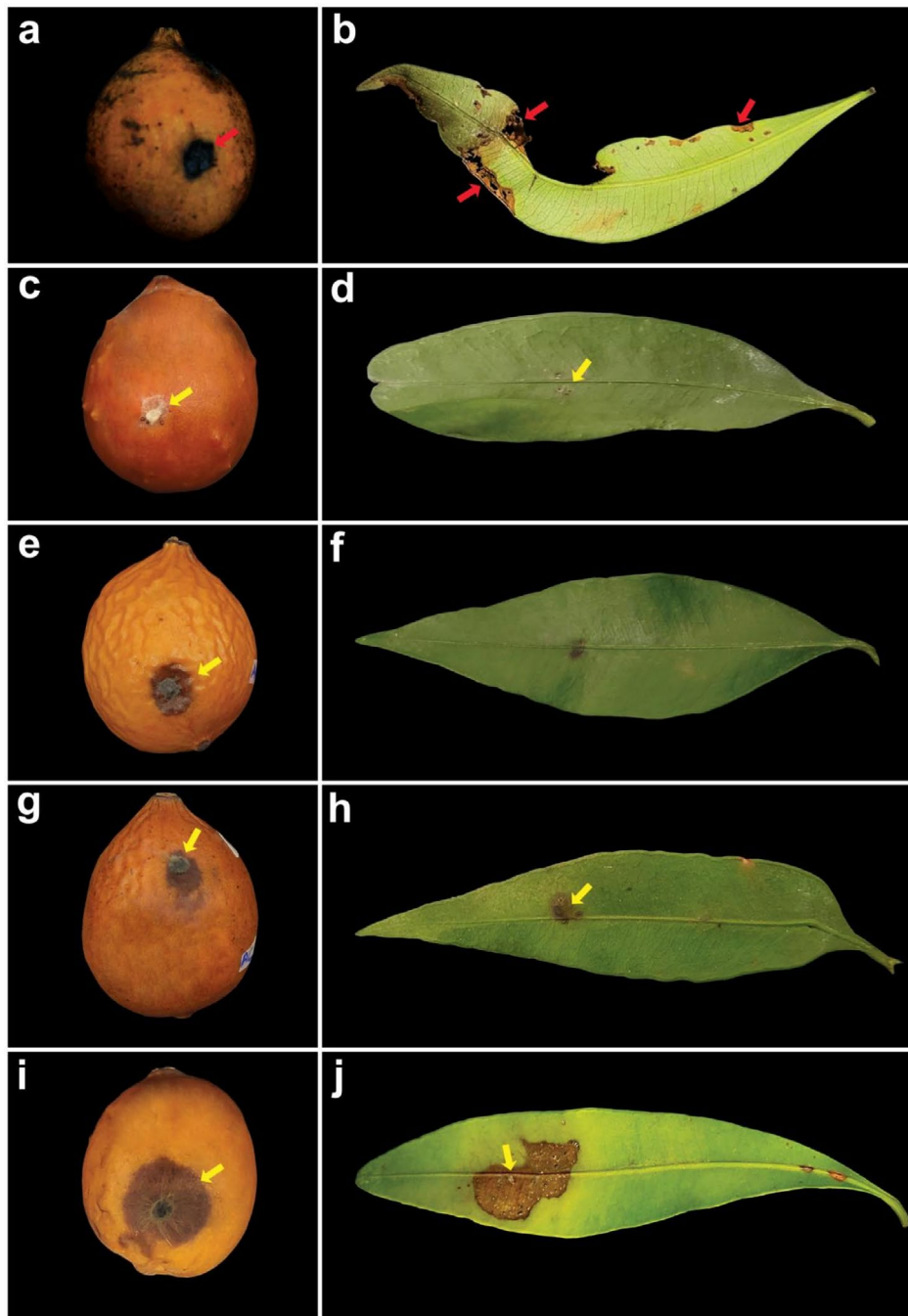


FIGURE 1 | Symptoms observed on the leaves (including spots and necrotic lesions) and fruit (showing rot) of *Garcinia humilis* in the field (a, b), along the pathogenicity test conducted on detached leaves and fruit (c–j). Asymptomatic fruit and leaf (negative control) (c, d). Symptoms induced by the isolates COUFAL0637 (e, f), COUFAL0638 (g, h) and COUFAL0639 (i, j), respectively.

Initially, older leaves exhibited small brown spots with irregular edges. As the disease progressed, leaf deformation and perforations developed in the affected areas. Additionally, small dark spots emerged on the fruit surface, eventually leading to black rot. Internally, the pulp became soft and dark in colour. Given the lack of comprehensive studies on achachairu diseases, we aimed to document and characterise new pathogenic associations affecting this species worldwide. The identification of causal agents was conducted based on morphological and cultural characteristics, multilocus phylogenetic analysis, and pathogenicity tests.

2 | Material and Methods

2.1 | Sampling and Isolation

Fungal isolates were obtained from symptomatic leaves exhibiting necrotic spots and from fruits displaying rot symptoms (Figure 1a,b) in a commercial production area in Vitória de Santo Antão, Pernambuco, Brazil. The plant material was washed under running water and dried using sterile paper towels. Fragments were excised from the transition zone between

diseased and healthy tissues using sterilised scalpel blades. The samples were then surface-disinfected by sequential immersion in 70% ethanol for 30s, 1% sodium hypochlorite for 1min, followed by two rinses in sterile distilled water for 30s each. The disinfected fragments were air-dried and transferred to potato dextrose agar (PDA) medium, where they were incubated at 25°C under a 12-h photoperiod.

After 3 days, mycelial disks were excised from the colony margins and transferred to fresh PDA plates to obtain pure cultures. Upon observing reproductive structures characteristic of the *Lasioidiplodia* genus (Phillips et al. 2013), isolates were further cultured on water agar to facilitate purification using the hyphal tip method. All purified isolates were deposited in the Coleção de Fitopatógenos at the Universidade Federal de Alagoas (COUFAL).

2.2 | Morphological and Cultural Characterisation

Colony morphology, including colour and aerial mycelium characteristics, was assessed after 7 and 15 days of growth on PDA at 25°C in darkness (Rayner 1970). To induce pycnidia production, isolates were grown on PDA supplemented with pine needles and incubated at 25°C in darkness for 30 days. Conidial morphology was examined by macerating pycnidia in a drop of lactoglycerol on glass slides. The fungal structures were observed and documented using a Zeiss microscope equipped with a Nikon Eclipse camera. The dimensions of 50 mature and immature conidia were measured to characterise their morphological features.

2.3 | DNA Extraction, PCR and Sequencing

Genomic DNA was extracted from the aerial mycelium of 7-day-old according to Doyle and Doyle (1987). The internal transcribed spacer (ITS) regions and partial sequences of the β -tubulin (*TUB2*) and translation elongation factor 1- α (*TEF1*- α) genes were amplified using polymerase chain reaction (PCR) for multilocus analysis. These regions are recommended for better species identification in the *Botryosphaeriaceae* family (Phillips et al. 2013).

The PCR reactions were performed in a total volume of 30 μ L. It comprised 3.0 μ L of 10 $\times$ buffer (containing 100mM KCl, 100mM Tris-HCl pH9.0, and 1% Triton-X), 2.4 μ L of 2.5mM dNTPs, 0.9 μ L of 50mM MgCl₂, 0.2 μ L of Taq DNA polymerase, 1.0 μ L of each oligonucleotide (at a concentration of 10 μ M), 1.0 μ L of total DNA (50ng) and 18.5 μ L of PCR-grade water. The amplification conditions were as follows: initial denaturation at 95°C for 2min, followed by 35 cycles of 94°C for 30s, annealing at 52°C–55°C (52°C for *TEF1*- α ; 55°C for *TUB2* and ITS) for 30s, 72°C for 1min and a final extension at 72°C for 10min. The target regions were amplified with primer pairs EF1-688F/EF1-1251R for *TEF1*- α (Alves et al. 2008) ITS1-F/ITS4 for ITS (White et al. 1990) and Bt2a/Bt2b for *TUB2* (Glass and Donaldson 1995). The PCR products were purified and sequenced by ACTGene Análises Moleculares in Alvorada, Rio Grande do Sul, Brazil. The sequences obtained were deposited in the GenBank nucleotide database.

2.4 | Phylogenetic Analysis

The *TEF1*- α , ITS and *TUB2* were assembled and edited using the CodonCode Aligner v. 4.1.1 software (www.codoncode.com) and analysed with the BLASTn algorithm and the GenBank nonredundant nucleotide database (<http://www.ncbi.nlm.nih.gov>) to identify closely related *Lasioidiplodia* species. Sequences were obtained from GenBank using BLAST results and relevant publications (Wu et al. 2021; Zhang et al. 2021). Each locus was aligned using MEGA software v. 11.0.13 and adjusted manually when necessary.

The phylogenetic Bayesian inference was carried out using the Markov chain Monte Carlo method on the CIPRES web portal (Miller et al. 2010). This analysis involved the sequences of each locus both separately and combined. The software used for this analysis was Mr. Bayes v. 3.2.3 (Ronquist et al. 2012). The best nucleotide substitution models for the data from each genomic region were determined using MrModeltest 2.3 (Posada and Buckley 2004) based on the Akaike Information Criterion (AIC), implemented in PAUP v.4.0b10. The analysis was conducted over 10 million generations with four chains, sampling every 1000 generations for a total of 10,000 trees. The first 2500 trees were discarded from each run during the burn-in phase, and subsequent probabilities (Rannala and Yang 1996) were determined from a majority rule consensus tree generated with the remaining 7500 trees. The trees were visualised and edited using FigTree v.1.4 (tree.bio.ed.ac.uk/software/figtree) and Adobe Illustrator v.25.4.1.

2.5 | Pathogenicity Tests

Pathogenicity tests were conducted on detached leaves and fruits of achachairu. The plant material was washed with detergent and running water, immersed in a 1% NaOCl solution for 3min and subsequently rinsed with sterilised distilled water. After drying, artificial wounds were made in the central region of each sample using sterilised needles. Mycelial disks (5mm in diameter) obtained from 7-day-old cultures were placed on the wound surface. A negative control was established using PDA disks without fungal growth.

Following inoculation, the plant material was placed in plastic trays lined with moist paper towels and enclosed in polyethylene bags to maintain high humidity for 48h. Disease progression was assessed every 24h for 7 days. To fulfil Koch's postulates, the fungal pathogens were re-isolated from the symptomatic tissues and compared to the original isolates to confirm their identity.

3 | Results

3.1 | Morphological and Cultural Characterisation

Two isolates were obtained from leaves exhibiting necrotic spots (COUFAL0637 and COUFAL0638), and one isolate was obtained from fruit displaying rot symptoms (COUFAL0639). On PDA, the colonies initially appeared greyish-white with dense aerial mycelium, gradually turning grey to olive green. The

reverse side of the colonies also transitioned from greyish-white to olive green or black (Figure 2a,d,g).

The conidia were ellipsoid to ovoid, with a rounded apex and a truncated base. Immature conidia were hyaline, unicellular and contained granular inclusions with smooth walls (Figure 2b,e,h). In contrast, mature conidia were dark brown, featuring a median septum and longitudinal striations (Figure 2c,f,i). The conidial dimensions ranged from 25.5 to 33.8×14.3 to 20.9 μm (length×width), with an average of 29.6±2.0×18.9±1.2 μm (n=50).

3.2 | Phylogenetic Analysis

BLASTn searches for isolates COUFAL0637, COUFAL0638, and COUFAL0639 revealed that *TEF1-α* sequences exhibited high similarity to *Lasiodiplodia theobromae* and *L. pseudotheobromae* isolates, with nucleotide identity ranging from 99.4% to 100%. Similarly, *TUB2* sequences showed high similarity to *L. theobromae* and *L. pseudotheobromae*, with nucleotide identity between 99.8% and 100%. The ITS sequences closely matched

those of *L. theobromae*, *L. brasiliense*, *L. pseudotheobromae*, *L. hormozganensis*, *L. laeliocattleyae* and *L. parva*, with nucleotide identity ranging from 99.8% to 100%.

Bayesian inference phylogenetic analysis, based on concatenated sequences of *TEF1-α*, *TUB2* and ITS, confirmed that the isolates obtained in this study belong to the same taxonomic group. The isolates COUFAL0637, COUFAL0638 and COUFAL0639 formed a well-supported clade closely associated with *L. theobromae* isolates, with a posterior probability value of 0.95 (Figure 3).

Sequence lengths were determined as follows: 570 bases for ITS, 563 for *TEF1-α*, and 459 for *TUB2*. The final alignment consisted of 1592 characters, including 94 parsimony-informative sites, 1407 conserved sites and 183 variable sites. The locus boundaries within the alignment were 1–570 for ITS, 571–1133 for *TEF1-α* and 1134–1592 for *TUB2*. For Bayesian inference phylogenetic analysis, the most suitable nucleotide substitution models were selected based on the Akaike Information Criterion (AIC): SYM+I for ITS, HKY+G for *TEF1-α* and GTR+I for *TUB2*.

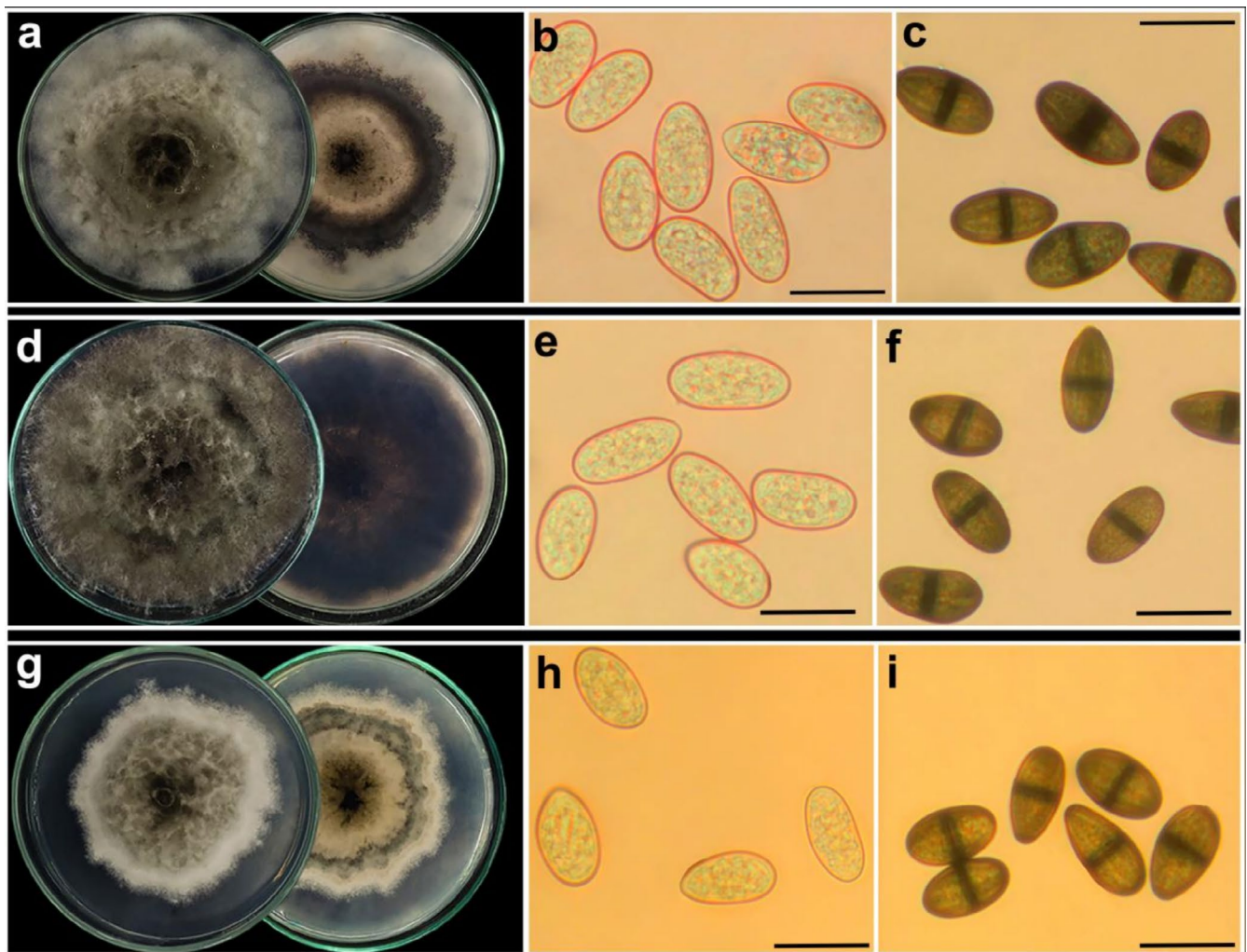


FIGURE 2 | Morphological markers from *Lasiodiplodia theobromae* strains isolated in this study. Isolates COUFAL0637 (a–c), COUFAL0638 (d–f) and COUFAL0639 (g–i). Colony characteristics (a, d, g), immature, hyaline conidia (b, e, h) and mature conidia with longitudinal striations (c, f, i). Scale bar: 20 μm.

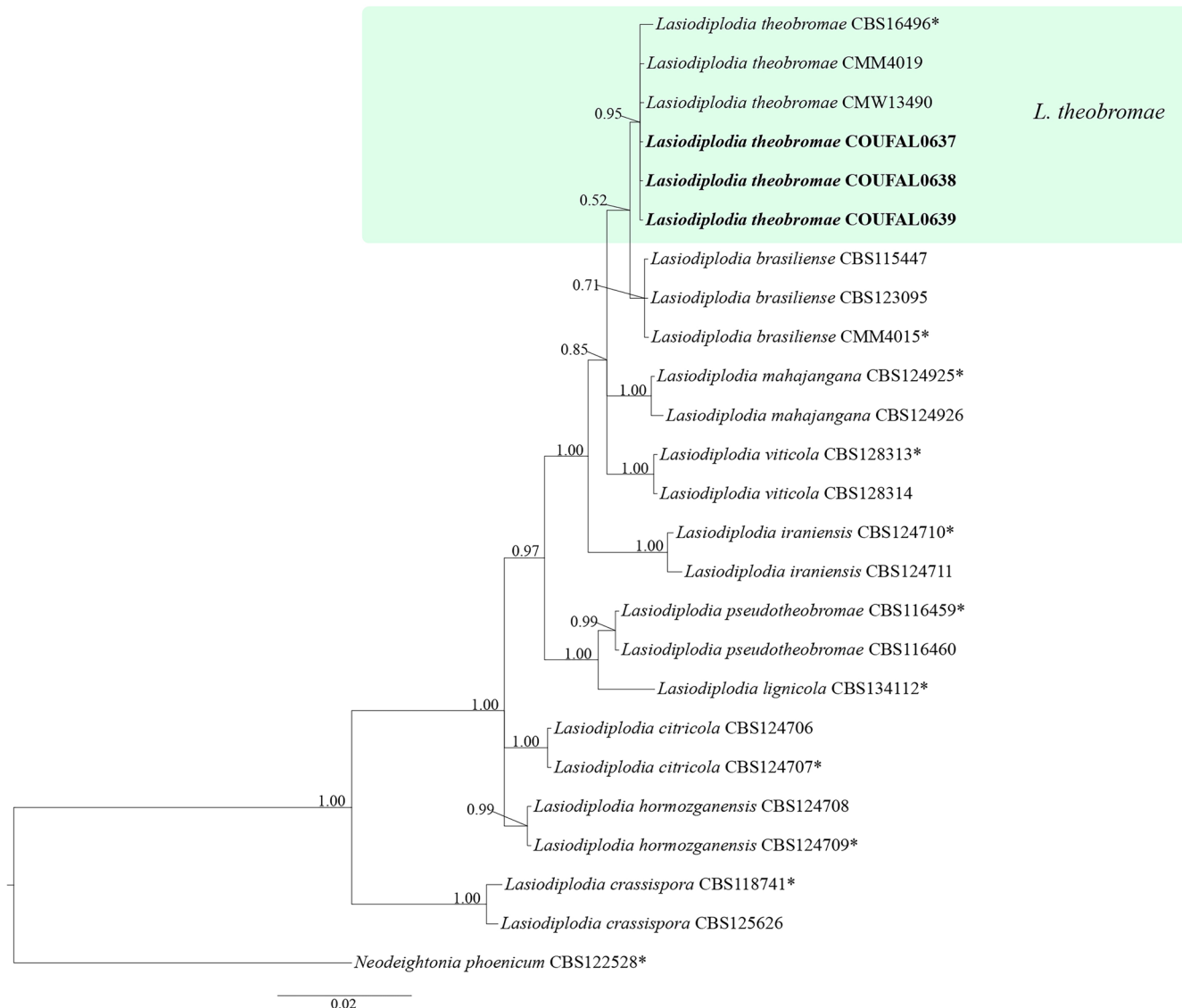


FIGURE 3 | Bayesian phylogenetic tree of *Lasiodiplodia* spp. isolates from *G. humilis*. The tree was inferred using concatenated sequences of the ITS region and *TEF-1α* and *TUB2* genes. The isolates obtained in this study are highlighted in bold. This tree is rooted with *Neodeightonia phoenicum*. Significant supports (PP ≥ 0.95) are shown above the branches.

3.3 | Pathogenicity Tests

All *Lasiodiplodia* isolates were pathogenic to both detached leaves and fruit of achachairu, exhibiting varying levels of aggressiveness (Figure 1e–j). The lesion diameters on leaves and fruit were 9.5 and 13.5 mm for isolate COUFAL0637, 12.0 and 19.5 mm for isolate COUFAL0638, and 25.4 and 27.5 mm for isolate COUFAL0639. Among them, COUFAL0639 caused the largest lesions and was therefore identified as the most aggressive isolate (Figure 1i,j).

Necrotic lesions began to develop on the surfaces of leaves and fruit within 2 days of inoculation, progressively expanding until the seventh day. No symptoms were observed in the negative control (Figure 1c,d). The fungi were successfully re-isolated from diseased tissues, and the recovered isolates displayed morphological characteristics identical to those of the original cultures, thus fulfilling Koch's postulates.

4 | Discussion

Achachairu (*Garcinia humilis*) is a fruit species native to Bolivia and cultivated in tropical and subtropical regions. Although not widely known, this fruit has been extensively studied for its pharmacological and nutritional properties, including antioxidant activity (Tome et al. 2019; Almeida et al. 2021), anti-inflammatory effects (Nunes et al. 2021), diuretic potential (Mariano et al. 2022) and applications in food supplementation (Barros et al. 2020; Gomes et al. 2025). Despite its growing market potential, the occurrence of pre- and postharvest diseases poses a significant risk to its commercial viability.

In this study, a combination of morphological and phylogenetic analyses identified *Lasiodiplodia theobromae* as the causal agent of leaf spots and fruit rot in achachairu. *L. theobromae* (syn. *Botryodiplodia theobromae*) is a cosmopolitan

phytopathogen predominantly found in tropical and subtropical regions (Punithalingam 1980). This species is a member of diverse endophytic communities and is recognised as a generalist opportunistic pathogen responsible for various plant diseases, including canker (Aguilera-Cogley et al. 2022; Sharma et al. 2023), dieback (Rusin et al. 2020; Huda-Shakirah et al. 2022), gummosis (Guajardo et al. 2018; Endes et al. 2016), and root, collar and fruit rot (Latha et al. 2009; Gnanesh et al. 2022; Adikaram et al. 2020). It is also associated with leaf spots (Khoo et al. 2022), as well as branch and leaf blights (Qiu et al. 2020; Sumaya et al. 2024). In Brazil, *L. theobromae* is one of the most widely distributed phytopathogens, affecting a broad range of hosts, particularly tropical fruit trees (Cardoso et al. 2002; Pereira et al. 2006; Marques et al. 2013; Coelho et al. 2022; Rusin et al. 2020; de Souza et al. 2024).

This study reports two new diseases affecting achachairu caused by *L. theobromae*. The isolates obtained demonstrated pathogenic potential, causing symptoms analogous to those observed in the field, including fruit darkening and softening, as well as necrotic leaf spots. The findings presented here contribute to a better understanding of the achachairu pathosystem and provide foundational knowledge for future research aimed at developing effective disease management strategies.

Ethics Statement

The authors have nothing to report.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Peer Review

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/jph.70094>.

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