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First Confirmed Report of *Corynespora cassiicola* Causing Target Leaf Spot on *Handroanthus serratifolius* and *Tabebuia rosea* in Brazil

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ABSTRACT

Handroanthus serratifolius (yellow lapacho) and *Tabebuia rosea* (pink poui) are forest species of ecological and economic importance in Brazil. In March 2023, leaves exhibiting target spot symptoms were collected from a forest nursery in São Lourenço da Mata, Pernambuco, Brazil. Fungal isolates were obtained and purified from the symptomatic tissue. Based on morphological characterization, multilocus phylogenetic analysis (*ITS*, *TEF-1α*, *TUB2* and *LSU*), and pathogenicity tests, the causal agent was identified as *Corynespora cassiicola*. This represents the first confirmed global report of *C. cassiicola* infecting *H. serratifolius* and *T. rosea* supported by molecular phylogeny.

Handroanthus serratifolius (Vahl) S.D. Grose and *Tabebuia rosea* DC. are forest species found in urban landscaping and as native vegetation in Brazil (Lorenzi 2020). Despite their hardiness, these species are susceptible to various diseases observed in urban areas, natural ecosystems, and nursery environments (Auer 2001). In March 2023, dark brown necrotic spots with greyish centres, characteristic of target spot, were observed on seedlings of *H. serratifolius* (Figure 1A) and *T. rosea* (Figure 1B) in a forest nursery located in São Lourenço da Mata, Pernambuco, Brazil (8°00′07″ S, 35°01′04″ W). Approximately 500 seedlings of each species were present, with a target leaf spot incidence of 80%. To isolate the pathogen, three leaflets from each host species were collected. Fragments of symptomatic leaves were surface-sterilized in 70% ethanol for 1 min, followed by 1% sodium hypochlorite for 1 min, rinsed with sterile distilled water, dried under a laminar flow hood, and transferred to potato dextrose agar (PDA) amended with 0.5 g L^{-1} streptomycin sulphate. The plates were then incubated at 28°C with a 12-h photoperiod. After 21 days, six single-spore isolates were obtained, exhibiting dark brown colonies with a velvety texture, well-defined margins and abundant aerial mycelium (Figure 1C,D). The isolates produced septate, pale brown hyphae and macronematous, mononematous conidiophores, solitary, straight to slightly curved, smooth and tapering (Figure 1E). Conidiogenous cells were monophialidic. Conidia were solitary or catenate, straight to slightly curved, obclavate, pale brown, and 4–10 pseudoseptate, measuring 63.5–234.7 × 7.2–9.6 μm ($n = 50$) (Figure 1F). Based on these morphological characteristics, the isolates were identified as *Corynespora* spp. according to Ellis (1971). The observed morphological characteristics are consistent with those described for the type species *Corynespora cassiicola*, particularly regarding conidial morphology, septation, pigmentation and conidiophore structure (Index Fungorum 2026). Two isolates (CFC 1784 and CFC 1785, from *H. serratifolius* and *T. rosea*, respectively) were selected for further molecular analysis.

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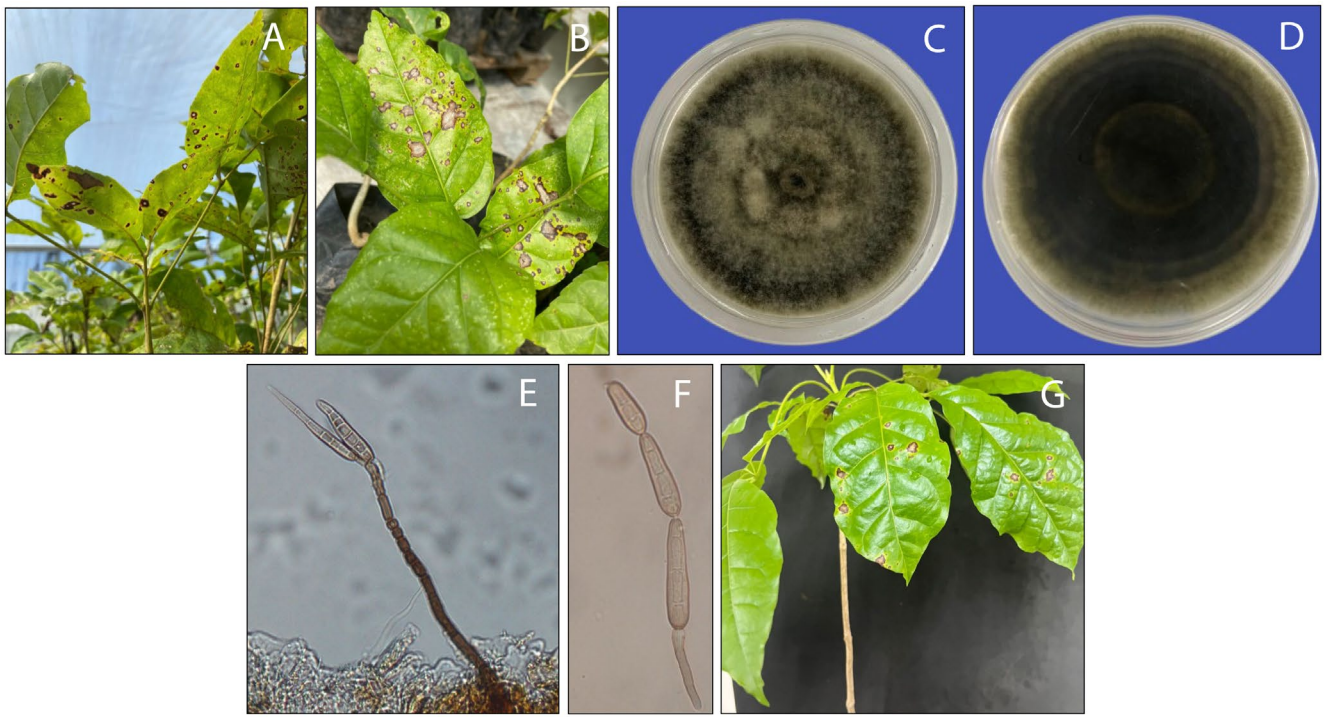


FIGURE 1 | Nursery symptoms of target leaf spot on *Handroanthus serratifolius* (A) and *Tabebuia rosea* (B), morphological characteristics of *Corynespora cassiicola* upper (C) and reverse sides (D) of the colony on potato dextrose agar after 21 days, conidiophores and conidiogenous cells (E), conidia of *C. cassiicola* (F), and symptoms in inoculated *T. rosea* with *C. cassiicola* CFC 1784 in the pathogenicity test two months after inoculation (G).

Total DNA was extracted and the internal transcribed spacer (*ITS*) region and partial gene sequences of the translation elongation factor 1-α (*TEF-1α*), β tubulin (*TUB2*), and large subunit ribosomal RNA (*LSU*) were amplified and sequenced using the primers ITS1/ITS4 (White et al. 1990), Ef1-688F/Ef1-1251R (Alves et al. 2008), Bt2A/Bt2B (Glass and Donaldson 1995) and 28S1-F/28S3-R (Xia et al. 2017), respectively. Sequences of the isolates were deposited in GenBank. A BLAST search showed that *ITS* sequences (PX353592 and PX353593) had 100% similarity to isolate FJ852578, the *TEF-1α* sequences (PX313664 and PX380366) had 100% similarity to isolate PP725563, the *TUB2* sequences (PX313665 and PX313666) had 99.77% similarity to isolate AB539206, and *LSU* sequences (PX433289 and PX433290) had 99.55% similarity to isolate MH869486, both identified as *C. cassiicola*. Maximum likelihood analysis of the combined gene sequences was performed using IQ-TREE, with simultaneous inference of the best substitution models for each gene (TIM2e + G4 for *ITS*, TNe for *TEF-1α*, HKY + F + I for *TUB2* and TNe + I for *LSU*) like based on the Bayesian information criterion (BIC), following recent multilocus phylogenetic approaches applied to *Corynespora* spp. (Liu et al. 2023; Huang et al. 2026). The phylogenetic tree showed that isolates CFC 1784 and CFC 1785 clustered with *C. cassiicola* isolate CBS100822, in a clade with 93.9% support (Figure 2). The isolates were deposited in the Culture Collection of Phytopathogenic Fungi from Cariri (CFC) at the Federal University of Cariri (Crato, Brazil). Pathogenicity tests with these isolates were performed twice on healthy, 1-year-old *H. serratifolius* and *T. rosea* plants cultivated in plastic pots. The plants were sprayed with 10 mL of conidial suspension (2×10^4 conidia mL^{-1}), while control plants received sterile water. Each isolate and the control treatment were tested on ten replicate plants of each species. All plants were covered with plastic bags to simulate a humid chamber (≈85% relative humidity) for 7 days and maintained in a greenhouse at 24°C–30°C. Two months after inoculation, inoculated leaves showed symptoms similar to those observed in the nursery (Figure 1F), whereas control plants remained asymptomatic. The pathogens were reisolated and exhibited the same morphology as the inoculated isolates, fulfilling Koch's postulates. *Corynespora cassiicola* was previously reported as the causal agent of target leaf spots on *H. serratifolius* and *T. rosea* in Brazil (Ferreira and Alfenas 1980), but isolates were identified based solely on morphological characteristics. There are no other records of *C. cassiicola* in these forest species worldwide (USDA 2026). To our knowledge, this is the first confirmed molecular phylogeny-based report of *C. cassiicola* causing target leaf spot on *H. serratifolius* and *T. rosea* in Brazil and worldwide.

Author Contributions

Project conceptualization: Humberson Rocha Silva, Ingryd Luzia de Farias Andrade and Marcos Paz Saraiva Câmara. Methodology: Marcos Paz Saraiva Câmara, Kamila Câmara Correia and Sami Jorge Michereff. Investigation: Ana Gabriele Gurgel Amaral, André Nunes de Oliveira and Ingryd Luzia de Farias Andrade. Resources: Humberson Rocha Silva and Marcos Paz Saraiva Câmara. Data analysis: Kamila Câmara Correia, Marcos Paz Saraiva, Rosana Blawid and Sami Jorge Michereff. Preparation of the draft manuscript: Ingryd Luzia de Farias Andrade. Review and editing manuscript: Kamila Câmara Correia, Marcos Paz Saraiva Câmara, Rosana Blawid and Sami Jorge Michereff. All authors read and approved the final manuscript.

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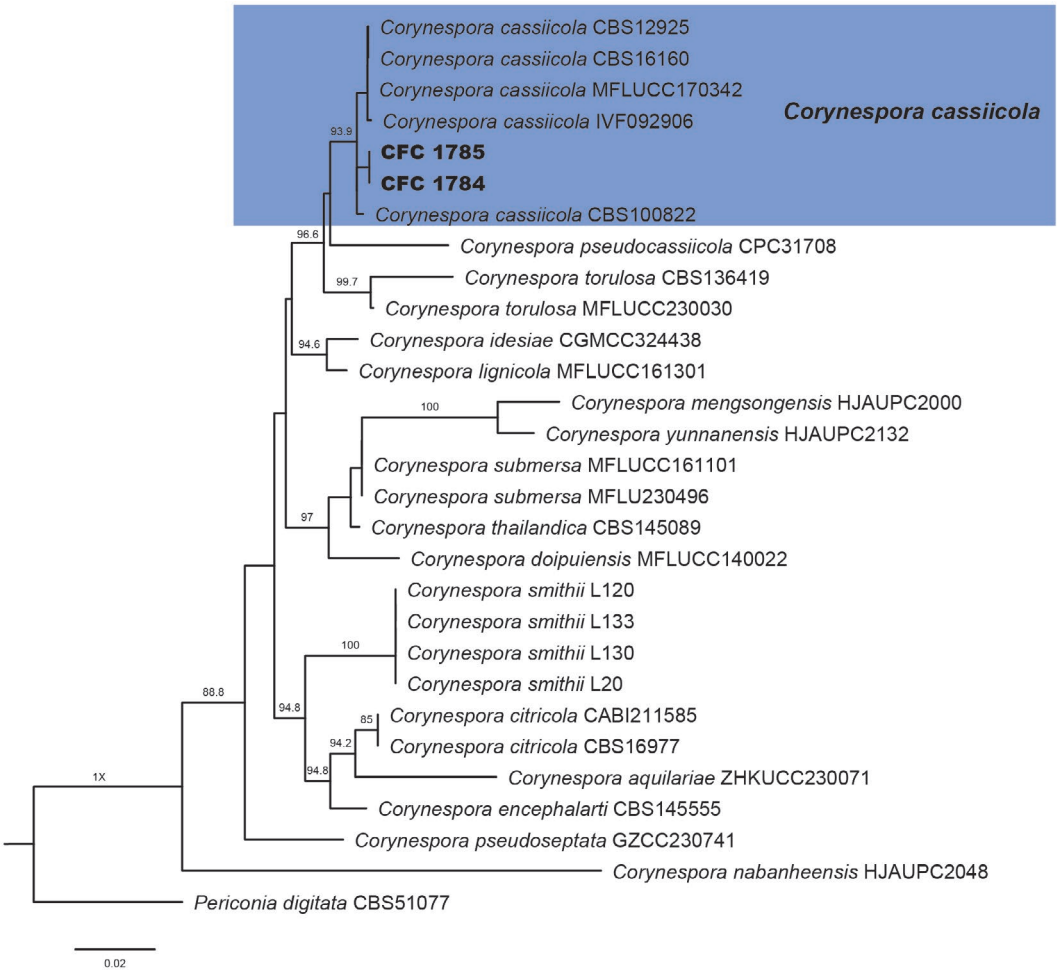


FIGURE 2 | Maximum likelihood phylogeny inferred from the combined internal transcribed spacer (*ITS*) region and partial gene sequences of the translation elongation factor 1-α (*TEF-1α*), β tubulin (*TUB2*), and large subunit ribosomal RNA (*LSU*) sequences used to infer the relative position of *Corynespora cassiicola*. Support values (Maximum Likelihood [ML] bootstrap) are given at the nodes. *Periconia digitata* (CBS51077) was used as an outgroup.

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Ethics Statement

All authors declare that this material has not been published in whole or in part elsewhere; the manuscript is not currently being considered for publication in another journal. We declare that our manuscript complies with all ethical rules applicable to this journal.

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.

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