# *Colletotrichum* spp. and other fungi associated with anthracnose on *Coffea arabica* L. in Mérida State, Venezuela

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**ABSTRACT**

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In tropical and subtropical regions worldwide, diseases can be major limiting factors to coffee (*Coffea arabica*) production, a highly valued crop internationally. Our aim was to identify *Colletotrichum* spp. and other fungal species associated with Coffee Berry Disease (CBD) and anthracnose on coffee twigs, which can greatly inhibit crop production. Concatenated phylogenetic analyses of ApMat and GS loci were used to identify two *Colletotrichum* species. *Colletotrichum siamense* was isolated from symptomatic mature and green berries that were both infested and uninfected with Coffee Berry Borer (CBB) and from twigs displaying anthracnose symptoms. *Colletrotrichum alienum* was isolated from twigs showing anthracnose symptoms. Along with these two *Colletotrichum* species, association of *Fusarium incarnatum* (= *Fusarium semitectum*) and *Fusarium solani* was found. Identification of *Fusarium* species was obtained through combined datasets of partial TEF1 and RPB2. *Fusarium* isolates came from ripe coffee fruits displaying symptoms of CBD, infested or not with CBB, and coffee twigs. In addition, concatenation of four gene regions (ITS, TEF1, CAL, TUB2) allowed the identification of another fungus, together with isolates from coffee twigs with anthracnose, *Diaporthe pseudomangiferae.* This is the first report of *Colletotrichum siamense* and *Colletotrichum alienum*, along with the fungi *Fusarium solani* and *Diaporthe pseudomangiferae*, associated with berry diseases on *Coffea arabica* in the state of Mérida, Venezuela.

**Keywords**: Coffee Berry Disease, *Diaporthe pseudomangiferae, Fusarium incarnatum, Fusarium solani*

**RESUMEN**

Mohali-Castillo, S.R.; Miller, S.; Stewart, J. *Colletotrichum* spp., y otros hongos asociados con la antracnosis en *Coffea arabica* L. en el estado Mérida, Venezuela. ***Summa Phytopathologica,*** v.48, n.3, p.99-111, 2022.

En las regiones tropicales y subtropicales de todo el mundo, las enfermedades pueden ser factores limitantes importantes para la producción de café (*Coffea arabica*), un cultivo muy valorado a nivel internacional. Nuestro objetivo era identificar *Colletotrichum* spp., y otras especies de hongos asociadas con la enfermedad de las Bayas o Cerezas del café (CBD) y de la antracnosis en las ramitas de café, que pueden inhibir en gran medida la producción de cultivos. Se utilizaron análisis filogenéticos, concatenando los locus ApMat y GS para identificar dos especies de *Colletotrichum*. Se aisló *Colletotrichum siamense* desde bayas verdes y maduras sintomáticas que estaban infestadas y no por Broca del fruto del cafeto (CBB) y desde ramitas que presentaban síntomas de antracnosis. *Colletrotrichum alienum* se aisló de ramitas que mostraban síntomas de antracnosis. Junto con estas dos especies de *Colletotrichum*, se encontraron asociados *Fusarium incarnatum* (= *Fusarium semitectum*) y *Fusarium solani*. La identificación de la especie *Fusarium* se realizaron utilizando conjuntos de datos combinados del parcial TEF1 y RPB2. Los aislamientos de *Fusarium* provinieron de frutos de café maduros que mostraban síntomas de CBD, infestados o no con CBB y ramitas de café. Asimismo, mediante la concatenación de cuatro regiones genéticas (ITS, TEF1, CAL, TUB2) se identificó otro hongo junto con aislamientos de ramitas de café con antracnosis, *Diaporthe pseudomangiferae*. Este es el primer reporte de *Colletotrichum siamense* y *Colletotrichum alienum*, junto con los hongos *Fusarium solani* y *Diaporthe pseudomangiferae*, asociados con enfermedades de las bayas en *Coffea arabica* en el estado de Mérida, Venezuela.

**Palabras Claves**: Enfermedad de las Bayas o Cerezas del Café, *Diaporthe pseudomangiferae, Fusarium incarnatum, Fusarium solani*.

**RESUMO**

Mohali-Castillo, S.R.; Miller, S.; Stewart, J. *Colletotrichum* spp. e outros fungos associados à antracnose em *Coffea arabica L.* no estado de Mérida, Venezuela. ***Summa Phytopathologica,*** v.48, n.3, p.99-111, 2022.

Nas regiões tropicais e subtropicais do mundo todo, as enfermidades podem ser fatores limitantes importantes para a produção do café (*Coffea arabica*), que possui grande valor no mercado internacional. O objetivo do presente trabalho foi identificar as espécies de *Colletotrichum* spp. e de outros fungos associados a queda dos dos frutos do café (QFC) e a antracnose dos ramos que podem reduzir a produtividade. Foram utilizados análises filogenéticas, de locos ApMat e GS. Foi identificado *Colletotrichum siamense* nos frutos verdes e maduros sintomáticos infestados ou não pela broca do café (BFC) como também nos ramos com sintomas de antracnose onde se isolou *Colletotrichum alienum. A*ssociados a outras espécies de *Colletotrichum*, foi isolado do fungo *Fusarium incarnatum* (= *Fusarium semitectum* e *Fusarium solani)*, identificados com a combinação TEF e RPB2. Mediante a amplificação de regiões genéticas (ITS, TEF1, CAL, TUB2), foi possível identificar outro fungo nos ramos com antracnose: *Diaporthe pseudomangiferae*. Este é o primeiro relato da ocorrência de *Colletotrichum siamense* e *Colletotrichum alienum*, junto com os fungos *Fusarium solani* e *Diaporthe pseudomangiferae*, associados com os frutos de *Coffea arabica* no estado de Mérida, Venezuela.

**Palavras Chaves**: Quadra dos frutos de café, *Diaporthe pseudomangiferae, Fusarium incarnatum, Fusarium solani*.

*Coffea arabica* L. is considered one of the most important crops worldwide, contributing significantly to the economy of different countries. The largest global producers of green *Coffea arabica* include Brazil, Colombia, Ethiopia and Honduras; meanwhile, in Venezuela, *Coffea arabica* production has faced a steady decline since 2007 (18). Biotic agents, particularly fungal agents, are known as major limiting factors to coffee production. Increased fungal pressure can greatly impact food security in certain countries, especially those where coffee is a major income source for small-scale growers concerning purchase of food and supplies for grain cultivation (1).

Pathogens of the genus *Colletotrichum* are responsible for anthracnose diseases on several economically important crops, and *Colletotrichum gloeosporioides* Penz. is the predominant pathogen in tropical and subtropical regions (5, 8, 35, 42, 53). *Colletotrichum* species have been reported on coffee as endophytes, epiphytes or pathogens (42) capable of causing leaf necrosis, coffee berry diseases and dieback (35).

*Colletotrichum kahawae* subsp. *kahawae* has been reported in multiple African countries, including Kenya, Angola, Cameroon and Malawi (54), causing Coffee Berry Disease (CBD), damaging green berries and inducing premature fruit drop and/or fruit mummification (37). This disease reduces yields by as much as 70-80% (14).

Even though *C. kahawae* is most virulent, sixteen *Colletotrichum* species have been identified on *Coffea arabica*, and six of them are presently reported in Vietnam and Mexico (5, 35). There are reports of *Colletotrichum asianum* Prihastuti, L. Cai & K.D. Hyde, *C. fructicola* Prihastuti, L. Cai & K.D. Hyde, *C. siamense* Prihastuti, L. Cai & K.D. Hyde, and *C. cordylinicola* Phoulivong, L. Cai & K.D. Hyde causing diseases on green and red berries in Laos and Thailand (41, 42); *Colletotrichum acutatum* Simmonds also causes minor disease on ripening berries (31). In Mexico, at least six *Colletotrichum* species were identified causing anthracnose on fruits and leaves of *C. arabica,* including *C. gloeosporioides*, *C. siamense*, *C. gigasporum* E.F. Rakotoniriana & F. Munauton, *C. theobromicola* Delacr., *C. karstii* Y.L. Yang, Zuo Y. Liu, K.D. Hyde & L. Cai and one *Colletotricum* sp. (5). In Costa Rica, *Colletotrichum costaricense* Damm, P.F. Cannon & Crous was isolated as pathogenic/endophytic on berries of *C. arabica* (6, 19). In Brazil, *Colletotrichum boninense* Moriwaki, Toy, Sato & Tsukib., and *C. gloeosporioides* have been reported on *C. arabica* and *Coffea canephora* L. trees, producing dieback and necrosis on leaves and berries (9). Furthermore, *Colletotrichum coffeanum* Noak & Pflanzenk. and *C. gloeosporioides* have also been reported as endophytic on healthy leaves of *C. arabica* in Brazil (36). In Colombia, *Colletotrichum gigasporum* was reported as an endophytic fungus on *C. arabica* (43).

For Venezuelan coffee production, there is little information about the involved diseases. In 1984 and 1985, *C. arabica* fields were surveyed in Lara and Portuguesa States, Venezuela, to determine the causal agent of leaf and branch anthracnose, fruit diseases and tree dieback. *Colletotrichum gloeosporioides* was identified, at altitudes between 700 and 1300 m, as the causal agent of anthracnose (29, 30). *Colletotrichum capsici* (Syd.) E. J. Butler & Bisby and a species similar to *C. gossypii* Southworth were reported as the causal agents of basal rot on coffee plants in Trujillo State (51). The aim of the present study was to characterize *Colletotrichum* species and fungal species associated with anthracnose and CBD in Venezuela using both morphological and genetic species identifications.

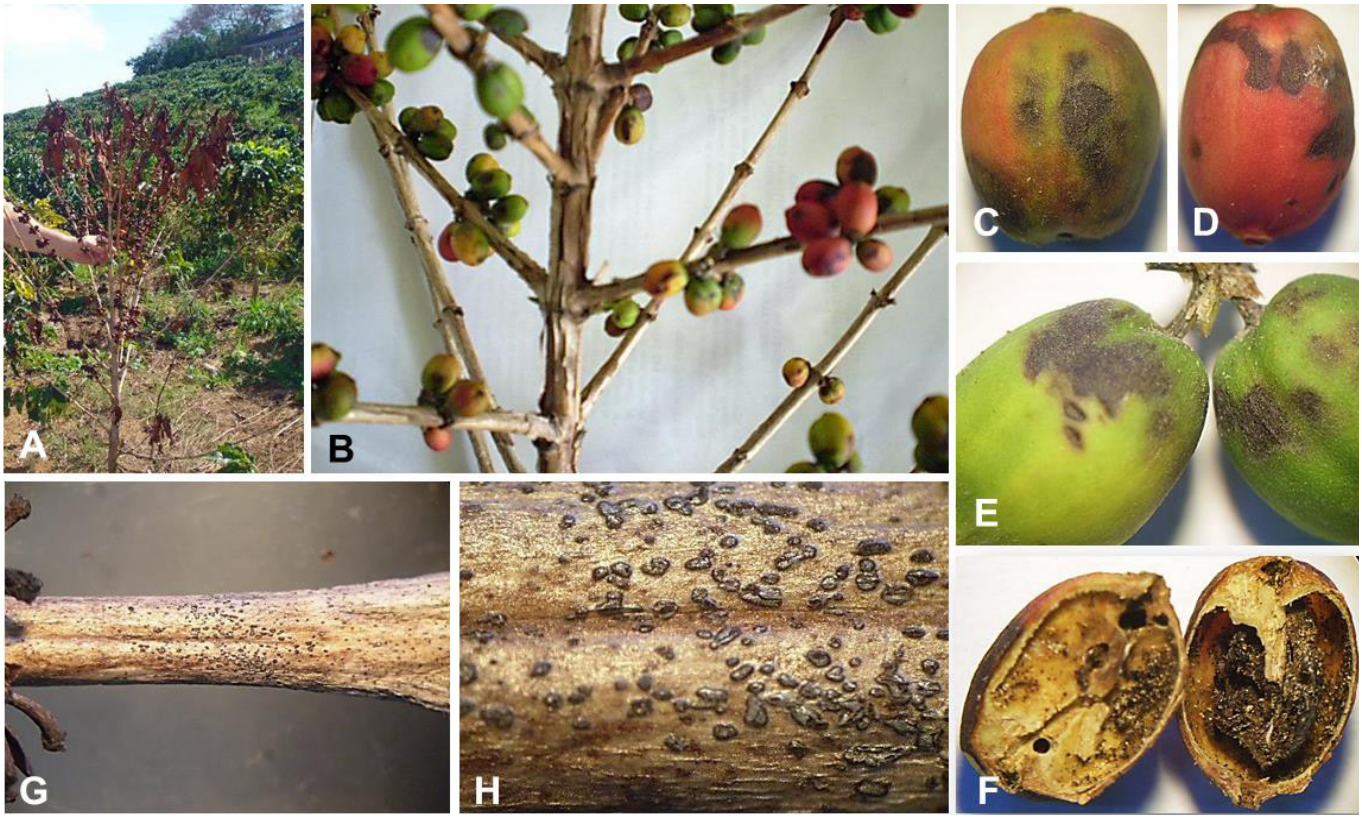


**Figure 1.** Coffee plant with anthracnose symptom. (**A**) Dead coffee plant due to anthracnose; (**B**) Berries with anthracnose symptoms or coffee berry disease (CBD); (**C**, **D**) CBD on ripe berries; (**E**) CBD on unripe or green berries; (**F**) Coffee berry borer (CBB); (**G**, **H**) Black acervuli on coffee twig with anthracnose.

## MATERIALS AND METHODS

**Isolation of fungal species from infected coffee berries and twigs**

Samples were collected in two parishes from Antonio Pinto Salina Municipality (8°24′33.12″ N, 71°39′5.4″ W), Mesa de Las Palmas parish and Agropecuaria Las Canales farm, Santa Cruz de Mora parish, both belonging to Mérida State, Venezuela. Fungi were isolated from green and mature fruits symptomatic of CBD, infested or not with CBB, and from twigs with anthracnose (Figure 1). Samples were transferred to the Phytopathology Laboratory of the School of Forestry Engineering, University of Los Andes (ULA), Mérida State, Venezuela. Fungi were isolated from anthracnose lesions on infected berries and twigs by excising 5×5mm margins of infected tissue. The tissues were then dipped in 1% sodium hypochlorite for 1 minute, immersed in 70% ethanol for 1 minute, rinsed three times with sterile water and finally dried in sterile tissue paper. Samples were placed on water-agar and incubated at room temperature, 25-28°C. The growing edges of any fungal hyphae, developing from the tissues, were then transferred aseptically to potato dextrose agar (PDA; 20 g potato dextrose agar in 1L distilled water; Difco Laboratories, Detroit, MI). Fungi were identified following sporulation. Single spore subcultures were obtained according to the procedure described by Choi et al. (4). After overnight room temperature incubation, single germinated spores were picked up with a sterile needle, transferred to PDA and incubated at 25°C for 7 days or until mycelia were observed growing from the samples. Fungal mycelium was sub-cultured onto PDA and hyphal tipping methods were performed to obtain pure cultures.

**Morphological analyses**

Mycelial plugs (5-mm diameter) were taken from the periphery of actively growing cultures and transferred to 9-cm Petri dishes containing PDA and malt extract agar (MEA, Difco Laboratories, Detroit, MI, USA). Following incubation at room temperature (25°C) for 7 days, colony characteristics and pigment production were noted; colony diameters were measured after 7-to-10-day growth. Colony coloration was rated according to Rayner (45). Cultures were examined periodically for ascoma development. Conidia produced on PDA and MEA were mounted in lactophenol to obtain dimensions and perform morphological analyses. If a fungus was not sporulating on PDA and MEA, morphological characteristics were described from synthetic nutrient-poor agar (SNA). Hyphal appressoria were observed on colonies grown on PDA. At least 50 measurements per structure were taken at x1000 magnification under a compound light microscope (Nikon Eclipse Ci).

**DNA extraction, amplification and sequencing**

DNA was extracted from 7-day-old axenic cultures, grown on half-strength potato dextrose agar medium (1/2 PDA: 10 g potato dextrose agar in 1L distilled water; Difco Laboratories, Detroit, MI) added of 0.1 g streptomycin sulphate (Sigma-Aldrich, USA). Cultures were grown on sterile MF-Millipore membrane filters (Millipore Sigma, Burlington, MA) and incubated at 25°C. DNA was extracted, using the ZR Fungal/Bacterial DNA MiniPrep (Zymo Research, Irvine, CA), following the manufacturer's protocol. Polymerase chain reactions (PCR) were performed with a reaction mixture that consisted of 30 ng fungal genomic DNA, 2.5 µl of 10x Standard Tag Reaction Buffer (New England BioLabs (NEB), Ipswich, MA), 0.5 µl of 10 mM dNTP (Roche Applied Science, Penzberg, Germany), 1 µl of each of 10 µM primer, 0.125 µl Taq DNA polymerase (NEB), and sterile deionized water for a total volume of 25 µl. For certain fungal genera, 1.5 µl $MgCl_2$ had to be included in the PCR reaction mixture for amplification (Roche Diagnostics, Mannheim, Germany). PCR products were cleaned with ExoSAP-IT PCR Product Cleanup (Thermo Fisher Scientific, Grand Island, NY) and sequenced at Eurofins Scientific (www.eurofinsus.com).

Several genes were amplified and sequenced for phylogenetic species recognition considering the isolates within each genus, including 5.8S rDNA and two flanking internal transcribed spacers (ITS), partial sequence of the translation elongation factor 1-α (TEF1), glutamine synthetase (GS), Apn2-Mat1-2 intergenic spacer and partial mating type (Mat1-2) gene (ApMat), calmodulin (CAL), β-tubulin (TUB2), and RNA polymerase II second largest subunit (RPB2). Primer pairs were used for the following genera: ***Colletotrichum*** sp.: **GS**: GSLF2 (TACACGAGSAAAAGG ATACGC) and GSLR1 (AGRCGCACATTGTCAGTATCG) (25); **ApMAT**: AMF1 (TCATTCTACGTATGTGCCCG) and AMR1 (CCAGAAATACACCGAACTTGC) (50); ***Fusarium*** sp.: **TEF1**: EF1 (ATGGGTAAGGA(A/G)GACAAGAC) and EF2 (GGA(G/A)GTA CCAGT(G/C)ATCATGTT) (38); **RPB**2: fRPB2-5f2 (GGGGWGAYCAGAAGAAGGC) and fRPB2-7cr (CCCATRGCTTGYTTRCCCAT) (27); ***Diaporthe*** sp.: **ITS**: ITS1F (CTTGGTCATTTAGAGGAAGTAA) (11) and ITS4 (TCCTCCGCTTATTGATATGC) (55); **TEF1**: EF1-728F (CATCGAGAAGTTCGAGAAGG) and EF1-986R (TACTTGAAGGAACCCTTACC) (2); **CAL**: CAL-228F (GAGTTCAAGGAGGCCTTCTCCC) and CAL-737R (CATCTTTCTGGCCATCATGG) (2); **TUB2**: Bt2a (GGTAACCAAATCGGTGCTGCTTTC) and Bt2b (ACCCTCAGTGTAGTGACCCTTGGC) (12), respectively.

PCR thermal cycle programs were performed as described by each previous author. The optimum annealing temperatures were: ApMat, GS, ITS, TEF1 (EF1-728F+ EF1-986R): 55°C; TUB2: 55 or 60°C; TEF1 (EF1+EF2) and CAL: 61°C; RPB2: 62°C. Some isolates required a change in the annealing temperatures due to either excessive or no bands amplified at the original annealing temperature.

**Phylogenetic analyses**

Phylogenetic analyses compared the sequenced isolates from the current study with holotypes and ex-types of closely related taxa. Preliminary sequence alignments for each locus and genus were completed using either ClustalX v. 2.1 (22) or BioEdit version 7.2.5 (15). Sequence data for closely related species considering genus were obtained from GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Manual adjustments and concatenation of loci were conducted using MAFFT for final alignments (20, 21) within Geneious Pro v. 9.0.5.

Maximum likelihood (ML) and Bayesian inference (BI) algorithms were used to reconstruct phylogenies for alignments using PhyML and MrBayes v. 3.2.1 (46) in Geneious v6.1.3 (Biomatters Inc.). A best-fit substitution model for each dataset was selected using the Bayesian Information Criterion (BIC) implemented in DT ModSel (33). Models HKY+G, SYM+I+G and TrN+I+G were chosen for the combined loci for *Colletotrichum*, *Fusarium* and *Diaporthe*, respectively. For ML analyses within PhyML, phylogenies were run with 1000 bootstraps. Bayesian Markov Chain Monte Carlo analyses were carried out using MrBayes (17). Six chains were run for 2000000 generations and trees were sampled every 100 generations. The first 25000 trees were discarded as burn-in, the remaining trees were used to estimate posterior probabilities (PP) for the majority rule consensus tree. Phylogenies

were estimated for each locus separately and multiple loci together for each genus.

Phylogenies for multiple loci datasets were estimated using Bayesian analyses, implemented in Bayesian Evolutionary Analysis Sampling Trees (BEAST v1.7.5) (7). BEAST does not use concatenation, but rather co-estimates the individual gene trees embedded inside the summary species tree. Bayesian Evolutionary Analysis Utility (BEAUti), version 1.7.5, was used to create XML-formatted input files for BEAST v1.7.5. In BEAST, a Markov Chain Monte Carlo algorithm was used to sample the posterior distribution of trees by conducting five independent runs of 100 million generations each using a constant size tree prior, strict molecular clock, and uniform priors. Trees were sampled every 1000 generations and the first 20% were discarded as burn-in. Post burn-in trees were combined with the program Log Combiner (BEAST v1.7.5), and chains were assumed to converge when the average standard deviation of split frequencies was < 0.01. The maximum clade credibility tree with posterior probability of each node was computed with the program Tree Annotator (BEAST v 1.7.5). Log files and tree files were visualized in Tracer v1.5 (http://tree.bio.ed.ac.uk/software/tracer/) and FigTree v1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/), respectively.

## RESULTS AND DISCUSSION

Isolates were divided into five groups according to mycelium growth, color on culture media, and conidial shape and size after 8 days. Two groups of *Colletotrichum* and *Fusarium* and one *Diaporthe* were separated.

### *Colletotrichum* Corda

Sixteen isolates of *Colletotrichum* spp. (CSM 143-1, CSM 143-2,

**Table 1**. GenBank and culture collection accession numbers of *Colletotrichum* spp. included in the current study.

| Species | Accession number | Host | Locality | Collector | GenBank accession number | |
|---|---|---|---|---|---|---|
| | | | | | GS | ApMat |
| *Colletotrichum aenigma* | ICMP 18608 | *Persea americana* | Israel | S. Freeman | JX010078 | KM360143 |
| *C. aeschynomenes* | ICMP 17673 | *Aeschynomene virginica* | USA | D. TeBeest | JX010081 | KM360145 |
| *C. alatae* | CBS 304.67 | *Dioscorea alata* | India | K.L. Kothari/J. Abramham | JX010065 | KC888932 |
| *C. alienum* | ICMP 12071 | *Malus domestica* | New Zealand | P.R. Johnston | JX010101 | KM360144 |
| *C. alienum* | LF322 | *Camellia sinensis* | China | F. Liu | KJ954982 | KJ954545 |
| *C. alienum* | **CSM 145** | ***Coffea arabica*-twigs** | **Venezuela** | **S. R. Mohali** | **MG584613** | **MG584592** |
| *C. alienum* | **CSM 146** | ***Coffea arabica*-twigs** | **Venezuela** | **S. R. Mohali** | **MG584612** | **MG584591** |
| *C. alienum* | **CSM 147** | ***Coffea arabica*-twigs** | **Venezuela** | **S. R. Mohali** | **MG584611** | **MG584590** |
| *C. alienum* | **CSM 156** | ***Coffea arabica*-twigs** | **Venezuela** | **S. R. Mohali** | **MG584610** | **MG584589** |
| *C. alienum* | **CSM 157** | ***Coffea arabica*-twigs** | **Venezuela** | **S. R. Mohali** | **MG584609** | **MG584588** |
| *C. aotearoa* | ICMP 18537 | *Coprosma* sp. | New Zealand | B. Weir | JX010113 | KC888930 |
| *C. asianum* | CBS 130418 | *Coffea Arabica* (berry) | Thailand | H. Prihastuti | JX010096 | FR718814 |
| *C. aeschynomenes* | ICMP 17673 | *Aeschynomene virginica* | USA | D. TeBeest | JX010081 | KM360145 |
| *C. camelliae* | LC1364 | *Camellia sinensis* | China | P. Tan | KJ954932 | KJ954497 |
| *C. clidemiae* | ICMP 18658 | *Clidemia hirta* | USA | S.A Ferreira/K. Pitz | JX010129 | KC888929 |
| *C. cordylinicola* | ICMP 18579 | *Cordyline fruticosa* | Thailand | S. Phoulivong | JX010122 | JQ899274 |
| *C. fructicola* | CBS 130416 | *Coffea Arabica* (berry) | Thailand | H. Prihastuti | JX010095 | JQ807838 |
| *C. gloeosporioides* | CBS 112999 | *Citrus sinensis* | Italy | G. Goidánich | JX010085 | JQ807843 |
| *C. henanense* | LF238 | *Camellia sinensis* | China | M. Zhang/R. Zang | KJ954960 | KJ954524 |
| *C. horii* | ICMP 10492 | *Diospyros kaki* | Japan | N. Nishihara | JX010137 | JQ807840 |
| *C. jiangxiense* | LF687 | *Camellia sinensis* | China | Y. Zhang | KJ955051 | KJ954607 |
| *C. jiangxiense* | LF684 | *Camellia sinensis* | China | Y. Zhang | KJ955048 | KJ954604 |
| *C. kahawae* | ICMP 17816 | *Coffea arabica* | Kenya | D.M. Masaba | JX010130 | JQ894579 |
| *Colletotrichum musae* | CBS 116870 | *Musa* sp. | USA | M. Arzanlou | JX010103 | KC888926 |
| *C. nupharicola* | CBS 470.96 | *Nuphar lutea subsp. polysepala* | USA | D.A. Johnson | JX010088 | JX145319 |
| *C. psidii* | CBS 145.29 | *Psidium* sp. | Italy | M. Curzi | JX010133 | KC888931 |
| *C. queenslandicum* | ICMP 1778 | *Carica papaya* | Australia | J.H. Simmonds | JX010104 | KC888928 |
| *C. salsolae* | ICMP 19051 | *Salsola tragus* | Hungary | D. Berner | JX010093 | KC888925 |
| *C. siamense* | LF139 | *Camellia* sp. | China | F. Liu | KJ954938 | KJ954503 |
| *C. siamense* | LF177 | *Camellia oleifera* | China | F. Liu | KJ954943 | KJ954508 |
| *C. siamense* | CBS130417,ICMP 18578 | *Coffea arabica* | Thailand | H. Prihastuti | JX010094 | JQ899289 |
| *C. siamense* (syn. *C. hymenocallidis*) | CBS125378,ICMP 18642 | *Hymenocallis americana* | China | Y. L. Yang | JX010100 | JQ899283 |

*continued...*

**Table 1**. Continuation

| Species | Accession number | Host | Locality | Collector | GenBank accession number | |
|---|---|---|---|---|---|---|
| | | | | | GS | ApMat |
| *C. siamense* | **CSM 143-1** | ***Coffea arabica*-twigs** | **Venezuela** | **S. R. Mohali** | **MG584608** | **MG584587** |
| *C. siamense* | **CSM 143-2** | ***Coffea arabica*-twigs** | **Venezuela** | **S. R. Mohali** | **MG584607** | **MG584586** |
| *C. siamense* | **CSM 144** | ***Coffea arabica*-twigs** | **Venezuela** | **S. R. Mohali** | **MG584606** | **MG584585** |
| *C. siamense* | **CSM 148** | ***Coffea arabica*-twigs** | **Venezuela** | **S. R. Mohali** | **MG584605** | **MG584584** |
| *C. siamense* | **CSM 149** | ***Coffea arabica*-twigs** | **Venezuela** | **S. R. Mohali** | **MG584604** | **MG584583** |
| *C. siamense* | **CSM 150** | ***Coffea arabica*-twigs** | **Venezuela** | **S. R. Mohali** | **MG584603** | **MG584582** |
| *C. siamense* | **CSM 151** | ***Coffea arabica*-twigs** | **Venezuela** | **S. R. Mohali** | **MG584602** | **MG584581** |
| *C. siamense* | **CSM 152** | ***Coffea arabica*-twigs** | **Venezuela** | **S. R. Mohali** | **MG584601** | **MG584580** |
| *C. siamense* | **CSM 153** | ***Coffea arabica*-twigs** | **Venezuela** | **S. R. Mohali** | **MG584600** | **MG584579** |
| *C. siamense* | **CSM 154** | ***Coffea arabica*-twigs** | **Venezuela** | **S. R. Mohali** | **MG584599** | **MG584578** |
| *C. siamense* | **CSM 155** | ***Coffea arabica*-twigs** | **Venezuela** | **S. R. Mohali** | **MG584598** | **MG584577** |
| *C. siamense* | **CSM 158** | ***Coffea arabica*-twigs** | **Venezuela** | **S. R. Mohali** | **MG584597** | **MG584576** |
| *C. siamense* | **CSM 159** | ***Coffea arabica*-twigs** | **Venezuela** | **S. R. Mohali** | **MG584596** | **MG584575** |
| *C. siamense* | **CSM 163** | ***Coffea arabica*-ripe fruit** | **Venezuela** | **S. R. Mohali** | **MG584595** | **MG584574** |
| *C. siamense* | **CSM 166** | ***Coffea arabica*-ripe fruit** | **Venezuela** | **S. R. Mohali** | **MG584594** | **MG584573** |
| *C. siamense* | **CSM 167** | ***Coffea arabica*-ripe fruit** | **Venezuela** | **S. R. Mohali** | **MG584593** | MG584572 |
| *Colletotrichum theobromicola* | CBS 124945, ICMP18649 | *Theobroma cacao* | Panama | E. J. Rojas | JX010139 | KC790726 |
| *C. ti* | ICMP 4832 | *Cordyline* sp. | New Zealand | J.M. Dingley | JX010123 | KM360146 |
| *C. tropicale* | CBS 124949 | *Theobroma cacao* | Panama | E.I. Rojas/L.C. Mejia/ Z. Maynard | JX010097 | KC790728 |
| *C. viniferum* | CBS 130644 | *Vitis vinifera*, cv. 'Hongti' | China | L.J. Peng | JN412784 | KJ623242 |
| *C. xanthorrhoeae* | CBS 127831 | *Xanthorrhoea preissii* | Australia | F.D. Podger | JX010138 | KC790689 |

ICMP: International Collection of Microorganisms from Plants, Auckland, New Zealand; LC: Working collection of Lei Cai, housed at CAS, China; LF: Working collection of Fang Liu, housed at CAS, China; CBS: Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; CSM: Personal culture collection deposited in the Department of Bioagricultural Sciences & Pest Management, Colorado State University, USA.

CSM 144, CSM 148, CSM 149, CSM 150, CSM 151, CSM 152, CSM 153, CSM 154, CSM 155, CSM 158, CSM 159, CSM 163, CSM 166, CSM 167) presented colonies white to pale brown, reverse pale yellow, aerial mycelium greyish white, dense, cottony; conidia one-celled, smooth-walled, guttulate, hyaline, fusiform with obtuse to slightly rounded ends, sometimes oblong, 8-18.5 x 4-8.5 µm (mean = 12.7 x 5.5 µm, n = 50). Other five isolates (CSM 145, CSM 146, CSM 147, CSM 156, CSM 157) had colonies cottony, grey aerial mycelium and orange conidial ooze, reverse dark grey to pale orange in center; conidia were one-celled, cylindric with broadly rounded ends, 13-21 x 3-6 µm (mean = 15.5 x 5.0 µm, n = 50) (54) (Table 1).

Bayesian and maximum likelihood phylogenetic trees were generated for a concatenated dataset of ApMat and GS loci with 1838 base pairs. The phylogenies included 21 isolates of *Colletotrichum* species from the present study and 26 additional closely-related taxa and were rooted with *C. xanthorrhoeae* CBS 127831 (Table 1). Sixteen *C. siamense* isolates and five *Colletotrichum alienum* B. Weir & P.R. Johnst. isolates formed two separate and well-supported clades with values of BS/PP = 78/0.98 for *Colletotrichum siamense* and BS/PP = 94/0.97 for *C. alienum* (Figure 2).

Coffee production has often been limited by CBD, which is caused by *Colletotrichum kahawae* in Kenya and other countries of Eastern Africa. At high altitudes, CBD is particularly devastating, damaging green berries and inducing premature fruit drop and fruit mummification, leading to production losses of 50 to 80% (16, 34). Interestingly, *Colletotrichum siamense* was isolated from symptomatic CBD in green and mature fruits, infested or not with CBB, and from twigs with anthracnose symptoms, whereas *C. alienum* was only isolated from twigs with anthracnose. *Colletotrichum siamense* was first identified and reported in isolates from lesions in healthy berry tissues, as well as from the surface of coffee berries in northern Thailand (42). In three municipalities in Puebla State, Mexico, *C. siamense* was isolated from leaves showing typical and atypical *Cercospora* lesions, as well as from symptomatic unripe fruits of coffee trees (5). In Chiapas, Mexico, 21 fungal genera were isolated from coffee berries infested with CBB by *Hypothenemus hampei*; however, *Colletotrichum* was not present in these isolates (40). In the current study, *C. siamense* was found associated with CBB in coffee grown in Venezuela.

*Colletotrichum alienum* was isolated and identified for the first time on ripe fruit rot from *Persea americana* in Australia (54), later in Israel (47), on *Malus domestica* fruit rot in New Zealand (54), associated with anthracnose diseases on *Proteaceae* in Australia, South Africa and Europe (24), and on *Nerium oleander* in Australia (48). This is the first report of such a species affecting coffee in Venezuela; however, as this species was recently named and separated from the *C. gloeosporioides* complex, it will likely be found in numerous regions where species within the *C. gloeosporioides* complex have been found (54). Recently, 80 *Colletotrichum* isolates from an Australian culture collection were analyzed on a molecular phylogenetic level, and *C. alienum* was distinguished from other species within the *C. gloeosporioides* species

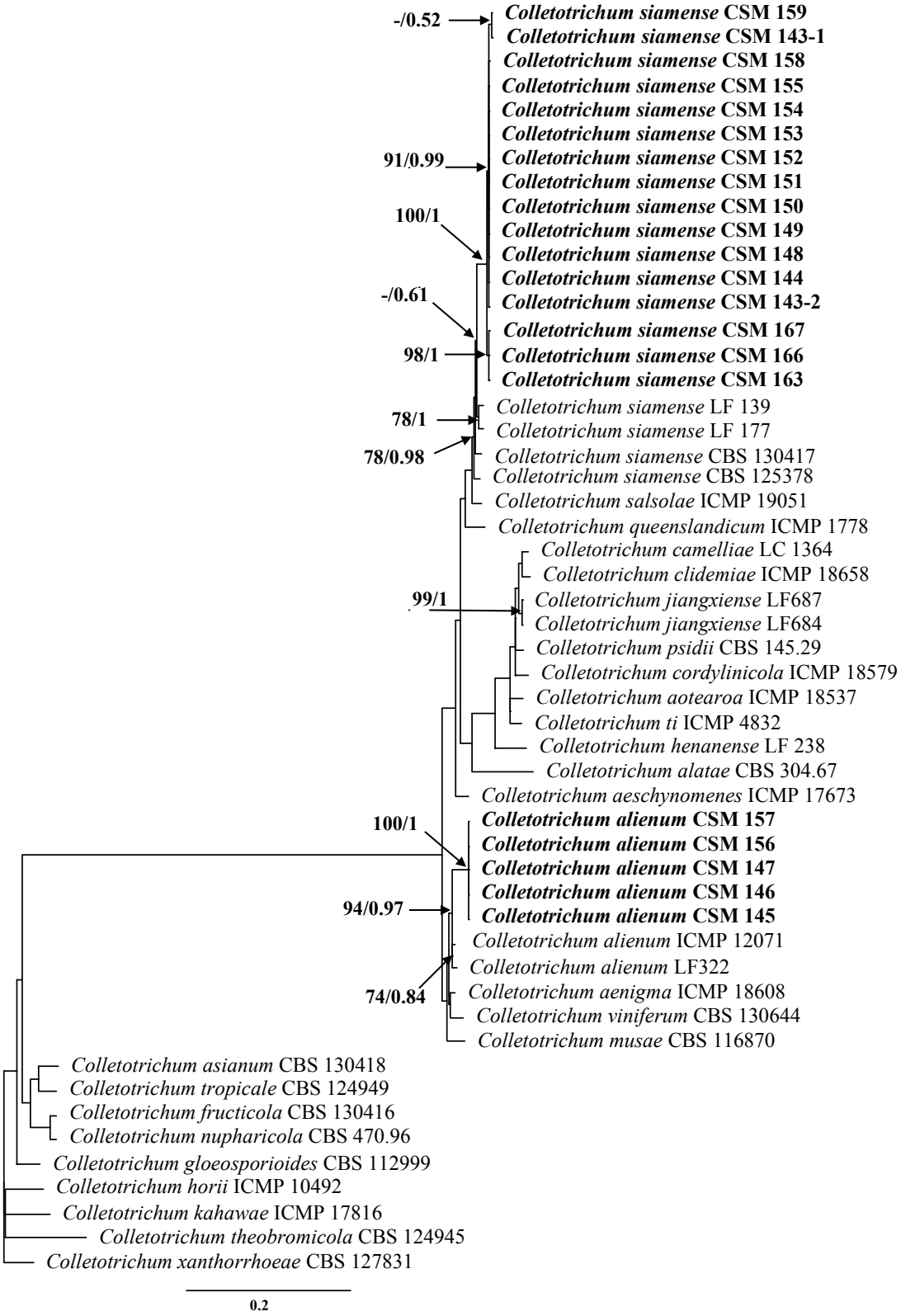


**Figure 2.** Phylogenies generated from Bayesian analysis based on combined ApMat and GS sequences of *Colletotrichum* species. The phylogenies were rooted with *C. xanthorrhoeae* CBS 127831. Bootstrap test ≥ 50%: 1000 replicates and Bayesian posterior probabilities ≥ 0.50 (BS/PP) are highlighted by arrows. The isolates included in the current is study are in oblique and coffee isolates are in bold and oblique. The phylogeny includes annotation of species boundaries for taxa that belong to the *C. gloeosporioides* complex. Bootstrap (BS) values below 50% are marked with a hyphen (-) on the phylogenetic trees.

complex using GS sequences (49, 54).

Sequence data of the concatenated ApMat and GS loci have served as a barcode for species identification of isolates belonging to the *C. gloeosporioides* species complex. Recently, Lui et al. (26) used these loci to re-construct a phylogenetic tree of *Colletotrichum* species from *Camellia* spp., highlighting that the topology of ApMat-GS phylogram was similar to that of a phylogram encompassing sequence data from 8 loci (25). Mating-related genes have been suggested to evolve at a faster rate and have a higher sequence variability, which dominates the topology of the multi-locus phylogram (25), allowing ApMat-GS to be informative for species identification.

***Fusarium* Link ex Grey**.

Four isolates of *Fusarium* spp. (CSM 134, CMS 135, CMS 137, CMS 138) had cultures showing dense aerial mycelia, initially white to beige or brown with age; Chlamydospores present; Macroconidia 3 to 5 septate, apical cell curved and tapering to a point, basal cell foot-shaped, 20-41.5 x 2.0-4.5 µm (mean = 19.3 x 3.1 µm, n = 50). Other cultures of five isolates (CSM 136, CMS 139, CSM 140, CSM 141, CSM 142) usually are white to cream with sparse mycelium; Chlamydospores abundant; Macroconidia 5 to 7 septate, wide and straight, apical cell blunt and rounded, basal cell straight to almost cylindrical, 13.5-44 x 3.0-6 µm (mean = 20.5 x 3.6 µm, n = 50); Microconidia oval to ellipsoid, 0 or 1 septum (23, 28) (Table 2).

The combined datasets of the partial TEF1 and RPB2 was comprised of 1659 base pairs from 27 taxa collected from this study and 43 reference species (Table 2). *Fusarium lyarnte* RBG5331 served as the outgroup. Four *F. incarnatum* (Roberge) Sacc (= *Fusarium semitectum* Berk. & Ravenel) isolates clustered together with reference species, forming a *Fusarium incarnatum-equiseti* species complex (FIESC). Five *Fusarium solani* (Mart.) Sacc)/ sexual morph *Neocosmospora solani* (Mart.) L. Lombard & Crous isolates clustered together. The BS and PP values for each *F. incarnatum* and *F. solani* well-support clades were BS/PP = 100/1 (Figure 3).

*Fusarium* species have long been known as latent pathogens of different tree species, existing as endophytes before causing disease (3). Numerous *Fusarium* species have previously been reported on coffee, as an endophyte or as a pathogen. The present study found *Fusarium incarnatum* and *F. solani* associated with anthracnose and as endophytes. In Colombia, Hawaii, Mexico and Puerto Rico, *Fusarium* sp. was the second most common endophyte out of 843 isolated fungi, while *Colletotrichum* sp. was the most commonly isolated species (52). A survey conducted from 1979 to 1981 in Puerto Rico, to evaluate anthracnose incidence and severity, found that *Fusarium stilboides* (Wollenw.), *F. oxysporum* (Schltdl.) and *F. semitectum* were associated with anthracnose symptoms (32), indicating that multiple *Fusarium* are capable of causing anthracnose on coffee. In Minas Gerais, Brazil, *Fusarium equiseti* (Corda) Sacc. and *F. semitectum* were found to be associated with the mycobiota, both internally and externally, on four coffee bean cultivars (*C. arabica*) without observable symptoms (39) and, similar to our study, *Fusarium incarnatum* was reported on coffee beans in Campo Elias Municipality, Trujillo State, Venezuela (51).

*Fusarium solani* and *Fusarium* sp. have been reported as part of the mycobiota associated with the cuticle, gut, feces and galleries of CBB, *Hypothenemus hampei*, in coffee plantations in Chiapas, Mexico (40). Interestingly, in the current study, *Fusarium incarnatum* and

**Table 2**._GenBank and culture collection accession numbers of *Fusarium* spp. included in the current study.

| Species | Accession number | Host | Locality | Collector | GenBank accession number | |
|---|---|---|---|---|---|---|
| | | | | | TEF1 | RPB2 |
| *Fusarium avenaceum* | FRC R-09495 | - | - | - | GQ915502 | GQ915486 |
| *F. aywerte* | RBG5743 | - | Australia | RBG | KP083250 | KP083278 |
| *F. beomiforme* | RBG4549 | - | Australia | RBG | HQ667157 | HQ646396 |
| *F. burgessii* | RBG5319 | Soil | Australia | B.Wang/A. Fraser | HQ667149 | HQ646392 |
| *F. coicis* | RBG5368 | *Coix gasteenii* | Australia | RBG | KP083251 | KP083274 |
| *F. commune* | NRRL28387 | *Dianthus caryophyllus* | The Netherlands | NRRL | AF246832 | JX171638 |
| *F. culmorum* | RBG3558 | - | Australia | RBG | HQ667167 | HQ646401 |
| *F. fujikuroi* | NRRL13566 | *Oryza sativa* | Taiwan | NRRL | AF160279 | EF470116 |
| *F. gaditjirri* | F15048 | *H. triticeus* | Australia | L.W. Burgess | AY639634 | HQ662690 |
| *F. goolgardi* | RBG5411 | *Xanthorrhoea glauca* | Australia | D.M Robinson/M.H. Laurence | KP101123 | KP083280 |
| *F. hostae* | RBG3994 | - | Australia | RBG | HQ667160 | HQ646390 |
| *F. incarnatum* | CBS 132194 | - | - | - | KF255470 | KF255542 |
| *F. incarnatum* | CBS 132894 | - | - | - | KF255480 | KF255545 |
| *F. incarnatum* | **CSM 134** | ***Coffea arabica*** | **Venezuela** | **S. R. Mohali** | **MG049934** | **MG049943** |
| *F. incarnatum* | **CSM 135** | ***Coffea arabica*** | **Venezuela** | **S. R. Mohali** | **MG049933** | **MG049942** |
| *F. incarnatum* | **CSM 137** | ***Coffea arabica*** | **Venezuela** | **S. R. Mohali** | **MG049932** | **MG049941** |
| *F. incarnatum* | **CSM 138** | ***Coffea arabica*** | **Venezuela** | **S. R. Mohali** | **MG049931** | **MG049940** |
| *F. lyarnte* | RBG5331 | - | Australia | RBG | EF107118 | HQ662691 |
| *F. miscanthi* | RBG4177 | - | Australia | RBG | HQ667166 | HQ662693 |
| *F. mundagurra* | RBG5717 | From soil Carnarvon Gorge National Park | Australia | S.M. Dunstan/M.H. Laurence | KP083256 | KP083276 |

*continued...*

**Table 2**. *Continuation*

| Species | Accession number | Host | Locality | Collector | GenBank accession number | |
|---|---|---|---|---|---|---|
| | | | | | TEF1 | RPB2 |
| *F. napiforme* | NRRL13604 | *Pennisetum typhoides* | Namibia | A.Lübben | AF160266 | EF470117 |
| *Fusarium nelsonii* | NRRL13338 | Soil | Australia | NRRL | GQ505402 | GQ505466 |
| *F. newnesense* | RBG5443 | - | Australia | RBG | KJ397074 | KP083277 |
| *F. nisikadoi* | RBG4043 | - | Australia | RBG | HQ667165 | HQ662692 |
| *Fusarium nelsonii* | NRRL13338 | Soil | Australia | NRRL | GQ505402 | GQ505466 |
| *F. newnesense* | RBG5443 | - | Australia | RBG | KJ397074 | KP083277 |
| *F. nisikadoi* | RBG4043 | - | Australia | RBG | HQ667165 | HQ662692 |
| *F. oxysporum* | RBG5413 | - | Australia | RBG | HQ667161 | HQ646385 |
| *F. oxysporum* | RBG5414 | - | Australia | RBG | HQ667163 | HQ646388 |
| *F. oxysporum* | CSM 105 | *Theobroma cacao* | Venezuela | S. R. Mohali | MF436042 | MF447156 |
| *F. oxysporum* | CSM 109 | *Theobroma cacao* | Venezuela | S. R. Mohali | MF436041 | MF447155 |
| *F. proliferatum* | NRRL43665 | Contact lens | USA | NRRL | EF452996 | EF470035 |
| *F. pseudograminearum* | RBG3580 | - | Australia | RBG | HQ667168 | HQ646400 |
| *F. redolens* | RBG4173 | - | Australia | RBG | HQ667159 | HQ646391 |
| *F. sibiricum* | NRRL53429 | *Avena sativa* | Russia | L. S. Malinovskaya/ E. A. Pirjazeva | HM744683 | HQ154471 |
| *F. solani* | CBS 138564 | Homo sapiens | Turkey | - | KT272100 | KT272102 |
| *F. solani* | CBS 131775 | wheat | Iran | M. Davari | JX118990 | JX237778 |
| *F. solani* | CSM 103 | *Theobroma cacao* | Venezuela | S. R. Mohali | MF436040 | MF436050 |
| *F. solani* | CSM 250 | *Theobroma cacao* | Venezuela | S. R. Mohali | MF436033 | MF436046 |
| *F. solani* | **CSM 136** | ***Coffea arabica*** | **Venezuela** | **S. R. Mohali** | **MG049930** | **MG049938** |
| *F. solani* | **CSM 139** | ***Coffea arabica*** | **Venezuela** | **S. R. Mohali** | **MG049929** | **MG049939** |
| *F. solani* | **CSM 140** | ***Coffea arabica*** | **Venezuela** | **S. R. Mohali** | **MG049928** | **MG049937** |
| *F. solani* | **CSM 141** | ***Coffea arabica*** | **Venezuela** | **S. R. Mohali** | **MG049927** | **MG049936** |
| *F. solani* | **CSM 142** | ***Coffea arabica*** | **Venezuela** | **S. R. Mohali** | **MG049926** | **MG049935** |
| *F. tjaetaba* | RBG5361 | *Sorghum interjectum* | Australia | J.L. Walsh. | KP083263 | KP083275 |
| *F. verticillioides* | NRRL43656 | Contact lens | USA | NRRL | EF452987 | EF470026 |

RBG: Royal Botanic Gardens Trust, Sydney, New South Wales, Australia; NRRL: Agricultural Research Service Culture Collection, Peoria, Illinois USA; F: University of Sydney, Sydney, New South Wales, Australia; CBS: Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands. CSM: Personal culture collection deposited in the Department of Bioagricultural Sciences & Pest Management, Colorado State University, USA; FRC: Fusarium Research Center, Penn State University, University Park, USA; -: not provided in literatures.

*F. solani* were isolated from ripe coffee fruits, with or without CBD infestations in Mérida, Venezuela, indicating that there may not be a close association of these *Fusarium* species with CBB.

***Diaporthe* Nitschke (asexual morph *Phomopsis* (Sacc.) Bubák)**.

Three isolates (CSM 133, CSM 222, CSM 224) with similar characteristics to genus *Phomopsis*/sexual morph *Diaporthe* produced cultures with a surface dirty white to ochreous, reverse umber, very large, coalescing stromata with lodged, elongated pycnidia necks forming dense, cream-colored droplets of conidia. Alpha conidia abundant, base truncate, smooth, aseptate, hyaline, guttulate, fusiform, tapering towards both ends, apex acutely rounded, 5.5-10 x 2-3 µm (mean = 8.0 x 2.5 µm, n = 50), beta conidia not seen (13) (Table 3).

A concatenated dataset of four gene regions was used for *Diaporthe* species identification. The ITS, TEF1, CAL and TUB2 data had 2095 base pairs and included 03 taxa isolated in the current study plus 31 reference species of *Diaporthe*. *Diaporthella corylina* was included as an outgroup CBS 121124 (Table 3). Three *Diaporthe pseudomangiferae* R.R. Gomes, C. Glienke & Crous isolates were clustered with BS/PP = 76/0.99 (Figure 4).

*Diaporthe* species (asexual morph = *Phomopsis*) have been previously isolated from coffee as both pathogens and endophytes around the globe; however, *Diaporthe* species have varied depending on location. In the present study, *Diaporthe pseudomangiferae* could be isolated from coffee twigs with anthracnose symptoms in Venezuela. *Phomopsis* sp. was previously isolated in Venezuela from a coffee leaf spot in Trujillo (51). In Puerto Rico, *Phomopsis* sp. was found on branches and dead nodes of coffee trees with *C. gloeosporioides* and mummified berries (32). Furthermore, *Diaporthe acutispora* Y.H. Gao & L. Cai and *Diaporthe yunnanensis* Y.H. Gao & L. Cai were also isolated from healthy leaves of *Coffea* sp. in Yunnan Province of China (10).

In Kenya, Rayner (44) isolated and reported *Phomopsis* sp. as a coffee endophyte after having sterilized the surfaces of healthy leaves, pedicels, stems, and green berries; an endophyte was also isolated from asymptomatic coffee tissues of berries and leaves in Colombia,

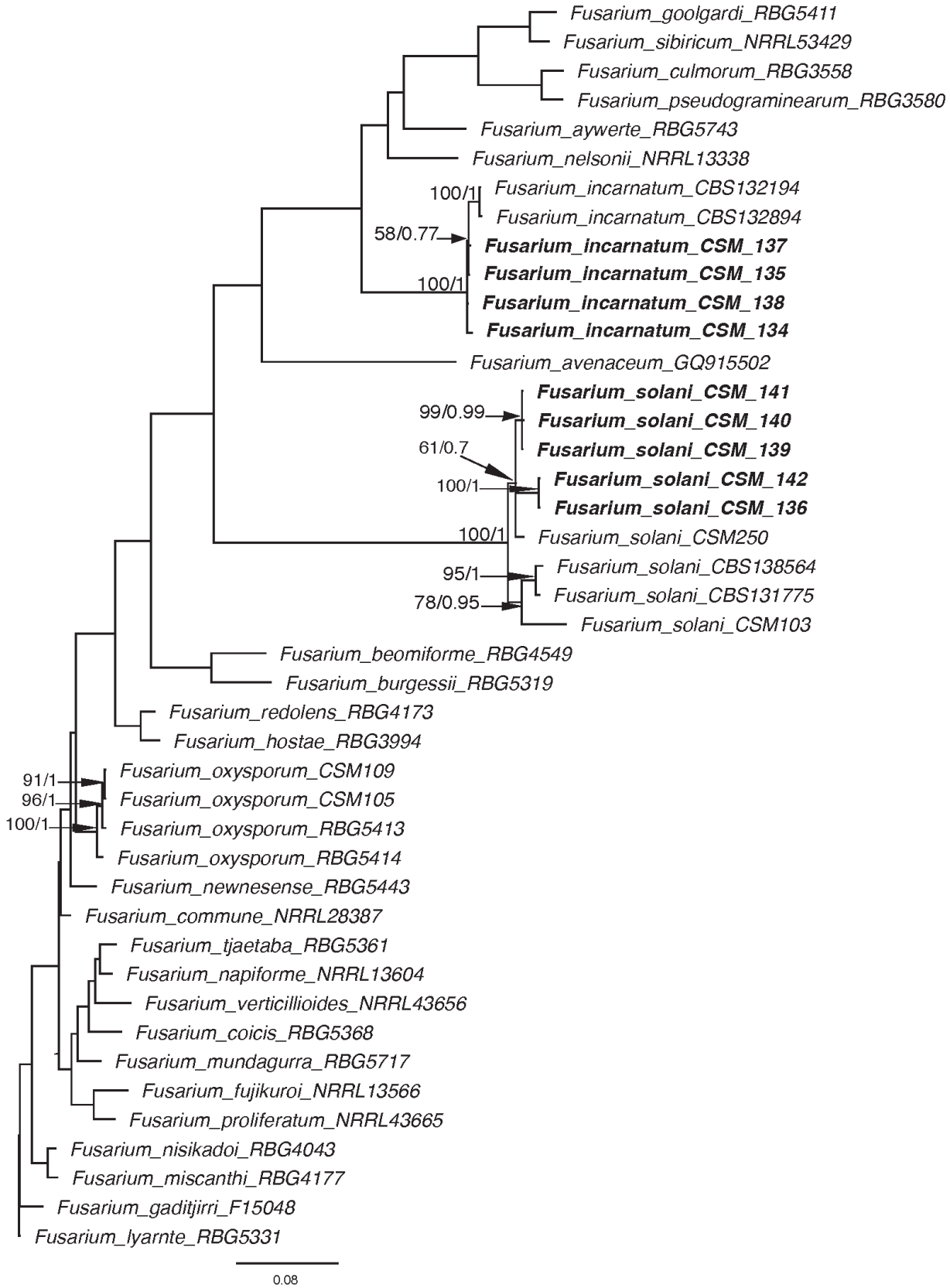


**Figure 3.** Phylogenies generated from Bayesian analysis based on combined EF1 and RPB2 sequences of *Fusarium* species. The phylogenies were rooted with *Fusarium lyarnte* (RBG 5331). Bootstrap test ≥ 50%:1000 replicates and Bayesian posterior probabilities ≥ 0.70 (BS/PP) are highlighted by arrows. The new coffee isolates included in the current study are in oblique and bold.

**Table 3.** GenBank and culture collection accession numbers of *Diaporthe* spp. included in the current study.

| Species | Accession number | Host | Locality | Collector | GenBank accession number | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | ITS | TEF1 | TUB2 | CAL |
| *Diaporthe acutispora* | LC6161 | *Coffea* sp. | China | W.J.Duan | KX986764 | KX999155 | KX999195 | KX999274 |
| *D. arecae* | CBS 161.64 | *Areca catechu* | India | H.C. Srivastava | KC343032 | KC343758 | KC344000 | KC343274 |
| *D. arengae* | CBS 114979 | *Arenga engleri* | Hong Kong | K.D. Hyde | KC343034 | KC343760 | KC344002 | KC343276 |
| *D. cf. heveae 2* | CBS 681.84 | *Hevea brasiliensis* | India | K. Jayarathnam | KC343117 | KC343843 | KC344085 | KC343359 |
| *D. eugeniae* | CBS 444.82 | *Eugenia aromatica* | West Sumatra | R. Kasim | KC343098 | KC343824 | KC344066 | KC343340 |
| *D. fraxini-angustifoliae* | BRIP 54781 | *Fraxinus-angustifolia* subsp. *oxycapa* | Australia | L. Smith | JX862528 | JX852534 | KF170920 | - |
| *D. hongkongensis* | CBS 115448 | *Dichora febrifuga* | Hong Kong | K.D. Hyde | KC343119 | KC343845 | KC344087 | KC343361 |
| *D. litchicola* | BRIP 54900 | *Litchi chinensis* | Australia | K.R.E. Grice | JX862533 | JX862539 | KF170925 | - |
| *D. lithocarpus* | LC0785 | *Lithocarpus glabra* | China | W. Sun | KF576274 | KF576249 | KF576298 | KF576226 |
| *D. lithocarpus* | CGMCC 3.17095 | *Lithocarpus glabra* | China | W. Sun | KF576285 | KF576260 | KF576309 | KF576234 |
| *D. musigena* | CBS 129519 | *Musa* sp. | Australia | P.W. Crous & R.G. Shivas | KC343143 | KC343869 | KC344111 | KC343385 |
| *D. pascoei* | BRIP 54847 | *Persea americana* | Australia | I.G. Pascoe | JX862532 | JX862538 | KF170924 | - |
| *D. perseae* | CBS 151.73 | *Perseae gratissima* | Netherlands Antilles | E. Laville | KC343173 | KC343899 | KC344141 | KC343415 |
| *D. pescicola* | MFLUCC 16-0105 | *Prunus persica* | China | X. H. Li | KU557555 | KU557623 | KU557579 | KU557603 |
| *D. podocarpi-macrophylli* | LC6194 | *Podocarpus macrophyllus* | China | W.J. Duan | KX986785 | KX999156 | KX999196 | KX999275 |
| *D. pseudomangiferae* | CBS 101339 | *Mangifera indica* | Dominican Republic | P. de Leeuw | KC343181 | KC343907 | KC344149 | KC343423 |
| *D. pseudomangiferae* | CBS 388.89 | *Mangifera indica* | Mexico | P. de Leeuw | KC343182 | KC343908 | KC344150 | KC343424 |
| *D. pseudomangiferae* | **CSM 133** | ***Coffea arabica*** | **Venezuela** | **S.R. Mohali** | **MG576129** | **MG584619** | **MG584622** | **MG584616** |
| *D. pseudomangiferae* | **CSM 222** | ***Coffea arabica*** | **Venezuela** | **S.R. Mohali** | **MG576128** | **MG584618** | **MG584621** | **MG584615** |
| *D. pseudomangiferae* | **CSM 224** | ***Coffea arabica*** | **Venezuela** | **S.R. Mohali** | **MG576127** | **MG584617** | **MG584620** | **MG584614** |
| *D. pseudophoenicicola* | CBS 462.69 | *Phoenix dactylifera* | Spain | H.A. van der Aa | KC343184 | KC343910 | KC344152 | KC343426 |
| *D. pseudophoenicicola* | CBS 176.77 | *Mangifera indica* | Iraq | M.S.A. A1-Momen | KC343183 | KC343909 | KC344151 | KC343425 |
| *D. pterocarpicola* | MFLUCC 10-0580a | *Piterocarpus indicus* | Thailand | D. Udayanga | JQ619887 | JX275403 | JX275441 | JX197433 |
| *D. pterocarpicola* | MFLUCC 10-0580b | *Piterocarpus indicus* | Thailand | D. Udayanga | JQ619888 | JX275404 | JX275442 | JX197434 |
| *D. sennae* | CFCC 51636 | *Senna bicapsularis* | China | Q. Yang | KY203724 | KY228885 | KY228891 | KY228875 |
| *D. taoicola* | MFLUCC 16-0117 | *Prunus persica* | China | X.H. Li | KU557567 | KU557635 | KU557591 | - |
| *D. tectonigena* | MFLUCC 12-0767 | *Tectona grandis* | Thailand | M. Doilom | KU712429 | KU749371 | KU743976 | KU749358 |
| *D. undulata* | LC8110 | Unknown host | China | F. Liu | KY491545 | KY491555 | KY491565 | - |
| *D. xishuangbanica* | LC6744 | *Camellia sinensis* | China | F. Liu | KX986784 | KX999176 | KX999217 | - |
| *D. yunnanensis* | LC8106 | *Coffea* sp. | China | W.J. Duan | KY491541 | KY491551 | KY491561 | KY491571 |
| *D. yunnanensis* | LC8107 | *Coffea* sp. | China | W.J. Duan | KY491542 | KY491552 | KY491562 | KY491572 |
| *Diaporthella corylina* | CBS 121124 | *Corylus* sp. | China | L.N. Vassiljeva | KC343004 | KC343730 | KC343972 | KC343246 |

CSM: Personal culture collection deposited in the Department of Bioagricultural Sciences & Pest Management, Colorado State University, USA; CBS: Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; MFLUCC: Mae Fah Luang University Culture Collection; BRIP: Australian plant pathogen culture collection, Queensland; CGMCC: China General Microbiological Culture Collection Center; CFCC: China Forestry Culture Collection Center; LC: Working collection of Lei Cai, housed at Institute of Microbiology, CAS, China. -: not provided in literatures.

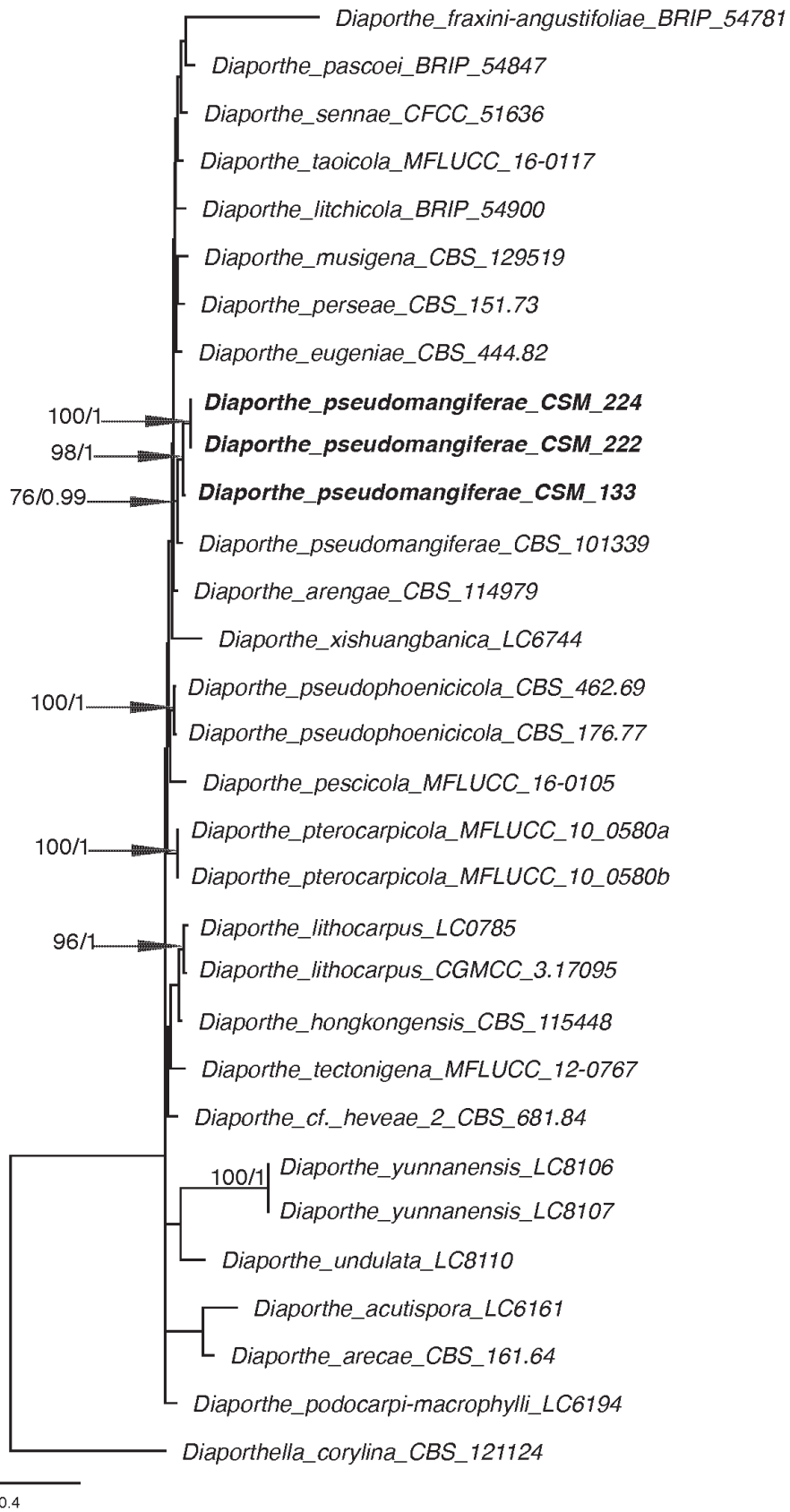


**Figure 4.** Phylogenies generated from Bayesian analysis based on combined ITS, TEF1, CAL and TUB2 sequences of *Diaporthe* species. The phylogenies were rooted with *Diaporthella corylina* CBS 121124. Bootstrap test ≥ 75%:1000 replicates and Bayesian posterior probabilities ≥ 0.99 (BS/PP) are highlighted by arrows. The new coffee isolates included in the current study are in oblique and bold.

Hawaii, Mexico and Puerto Rico (52). *Diaporthe liquidambaris* (C.Q. Chang, Z.D. Jiang & P.K. Chi) Udayanga & Castl., and *D. phaseolorum* (Cooke & Ellis) Sacc. are endophytic fungi which have been isolated from mature healthy leaves of *Coffea Arabica,* in both conventional and organic coffee production systems, in the municipality of Garanhuns, Pernambuco, Brazil (36). *Diaporthe acutispora* Y.H. Gao & L. Cai and *Diaporthe yunnanensis* Y.H. Gao & L. Cai were also isolated from healthy leaves of *Coffea* sp. in Yunnan Province of China (10). It is not entirely clear whether *Diaporthe* spp. play a role as latent pathogens in *Coffea* spp., but species in these genera are known as endophytes and pathogens (13).

Although CBD caused by *C. kahawae* is so far restricted to Africa, in 1986 and 1994 isolates similar to *C. gloeosporioides* that cause coffee berry diseases were reported in the states of Lara, Portuguesa and Trujillo States, Venezuela, based on morphological identification. This species has been reported as the cause of anthracnose on leaves and branches, fruit diseases and dieback in coffee crops (29, 30, 51). In the current study, both morphological and molecular species identification detected absence of *C. gloeosporioides* but presence of other *Colletotrichum* species, including *C. siamense* and *C. alienum*. These reported *Colletotrichum* species are capable of and known for causing berry diseases and anthracnose on *Coffea* spp. shoots. Thus, appropriate management measures for pathogen control must be adopted to prevent the introduction of this disease to other coffee growing areas, specially to neighboring regions in South America. The current study is the first report of *D. pseudomangiferae* associated with anthracnose in coffee plantations in Venezuela. The pathogen can be considered an endophyte or a latent pathogen together with *Fusarium* species in the global trade market.

## DECLARATION OF COMPETING INTEREST

The authors declare no potential conflict of interest for this study, and the present research has not been published previously.

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