Characterization of *Colletotrichum* Isolates from Tamarillo, Passiflora, and Mango in Colombia and Identification of a Unique Species from the Genus

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ABSTRACT

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This study was conducted to identify the species of *Colletotrichum* infecting tamarillo, mango, and passiflora in Colombia and to assess whether cross-infection between host species is occurring. Isolates of *Colletotrichum* spp. from tamarillo (n = 54), passiflora (n = 26), and mango (n = 15) were characterized by various molecular methods and by morphological criteria. Morphological characterization grouped the tamarillo isolates as *C. acutatum* and the passiflora and mango isolates as *C. gloeosporioides*. Species-specific primer analysis was reliable and confirmed grouping of the tamarillo isolates (besides Tom-6) as *C. acutatum* and the mango isolates (besides Man-76) as *C. gloeosporioides*. However, DNA of the passiflora isolates was not amplified by either *C. acutatum*- or *C. gloeosporioides*-specific primers, but reacted with a new primer, *Col*1, designed according to the internal transcribed spacer (ITS) 1 region of these isolates. Isolates Tom-6 and Man-76 also reacted positively with the *Col*1 primer. All the isolates reacting with the *C. acutatum*- and *C. gloeosporioides*-specific primers failed to react with primer *Col*1. Isolate Pass-35 from passiflora did not react with any of the taxon-specific primers. Arbitrarily primed polymerase chain reaction (ap-PCR), random amplified polymerase DNA (RAPD)-PCR, and A+T-rich DNA analyses delineated representative isolates into subgroups within the designated species. Molecular analyses indicated that the *C. acutatum* tamarillo isolates were uniform or clonal, whereas the *C. gloeosporioides* mango isolates and *Colletotrichum* passiflora isolates were heterogeneous. Likewise, sequence analysis of the complete ITS (ITS1–5.8S–ITS2) region identified certain isolates to their respective species: tamarillo isolates as *C. acutatum*; mango isolates as *C. gloeosporioides*; passiflora, Tom-6, and Man-76 isolates as a *Colletotrichum* sp. as yet undefined; and the Pass-35 isolate as an additional undefined *Colletotrichum* sp. Molecular analyses of the population of *Colletotrichum* isolates from passiflora, Tom-6 from tamarillo, and Man-76 from mango indicate that this population may not be host specific.

Additional keywords: *Glomerella cingulata,* phylogeny, rDNA.

Colombian tamarillo (*Solanum betacea* cav. Sendt) cultivation comprises more than 4,500 ha, with the largest plantation areas located in the Antioquia, Boyaca, Caldas, Cauca, and Cundinamarca states (4). Increased planting of tamarillo in Colombia has caused the spread and development of diseases such as anthracnose. Anthracnose has become a major threat to the tamarillo industry, not only causing severe damage to fruit but also affecting flowers and leaves. Symptoms include depressed black lesions on fruit accompanied by erupting pink spore masses in lesions, and round to irregular necrotic lesions on leaves with depressed lesions on main veins (4). Yield losses due to anthracnose of tamarillo can be greater than 50%.

The causal agent of anthracnose in tamarillo and other tropical fruit in Colombia, such as mango, custard apple, avocado, passiflora species, solanaceous species, citrus, and guava, has been referred to as *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz. (7). *C. gloeosporioides* strains obtained from these hosts have shown a wide range of variability in morphology and pathogenicity and, in several cases, have been referred to as a collective species (21). Characterization and taxonomic determination of *Colletotrichum* spp. has relied mainly on morphology and host range criteria. High variability of *Colletotrichum* spp. under culture conditions and the plasticity of this fungus for host preferences have made these two criteria unreliable for identifying isolates to species (2,5,11). *Colletotrichum* spp. have a wide host range, with cases of multiple pathogens on a single host as well as single species on diverse hosts (12). Cross-infection potential among different species of *Colletotrichum* has been well documented (3,5,17). The importance for species differentiation is critical for control purposes. For example, in mixed populations of *Colletotrichum* spp., such as *C. acutatum* and *C. gloeosporioides*, sensitivity of one species to a certain fungicide (e.g., benomyl) as opposed to the other may cause a shift in population structure (2,5,12,22).

Molecular tools have been widely implemented for more accurate differentiation between *Colletotrichum* spp. Species-specific polymerase chain reaction (PCR) primers, random amplified polymorphic DNA (RAPD), arbitrarily primed (ap)-PCR (16), A+T-rich analyses (15), and sequence analyses of the internal transcribed spacer (ITS) regions of ribosomal DNA (rDNA) (28) all have been used to reliably determine intra- and interspecific genetic diversity in *Colletotrichum* spp.

This study was undertaken to evaluate the composition of *Colletotrichum* spp. infecting tamarillo, mango, and passiflora in Colombia and to determine whether or not cross-infection between host species was occurring. We used a molecular approach for identifying the species infecting the various crops, because morphological criteria were not reliable enough for this purpose. Species-specific primer, ap-PCR, RAPD-PCR, A+T-rich DNA,

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and sequence analyses of the ITS regions of rDNA were performed to identify the *Colletotrichum* spp. responsible for anthracnose of tamarillo, mango, and passiflora and to assess the genetic diversity within the *Colletotrichum* populations from each host.

MATERIALS AND METHODS

Fungal cultures and growth conditions. *Colletotrichum* strains associated with anthracnose disease symptoms on tamarillo, mango, and passiflora were isolated over the period of 1998 to 2000 from several regions of Colombia (Table 1). All *Colletotrichum* isolates were grown on potato dextrose agar (Difco Laboratories, Detroit) at 24°C and single-spore isolates were prepared from each culture for morphological and molecular characterization. Isolates from tamarillo, passiflora, and mango, that were not clearly defined by molecular methods, were sent to P. F. Cannon of CABI Bioscience (Egham, Surrey, UK) for morphological identification and the cultures were deposited in the CABI culture collection. The tamarillo (Tom-6; IMI 386922), passiflora (Pass-35; IMI 386920), and mango (Man-76; IMI 386921) isolates were identified within the *C. gloeosporioides* species aggregate, with several unusual features (P. F. Cannon, *personal communication*), and are described in Table 2. In addition, isolates Tom-9 from tamarillo, Pass-62 from passiflora, and Man-75 from mango also were thoroughly characterized morphologically by us in this study. For determination of size, 40 conidia of each isolate (Tom-6, Tom-9, Pass-35, Pass-62, Man-75, and Man-76) were selected per microscopic field on PDA medium, and their average measurements were calculated (Table 2). Representative *Colletotrichum* cultures used in this study for molecular analyses included isolate AVO-37-4B of *C. gloeosporioides* from avocado (13) and isolate TUT-5954 of *C. acutatum* from strawberry (10).

Isolation and purification of fungal DNA. All *Colletotrichum* isolates (54 from tamarillo, 26 from passiflora, and 15 from mango) were grown in 100 ml of potato dextrose broth (PDB) at 24°C for 7 days. Thereafter, the mycelium was collected by vacuum filtration and lyophilized until dry. DNA was extracted and purified as previously described (15). The DNA was dissolved in 0.5 ml of Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA; pH 8.0) to an approximate concentration of 200 to 500 μg/ml and diluted to a final concentration of 10 to 100 ng/μl for PCR reactions.

PCR amplification. For ap-PCR, primers were derived from microsatellite or repeat sequences as follows: CAGCAGCAGC-

TABLE 1. Sources of *Colletotrichum* spp. isolates used in this study

Isolate[a]	Host species	Location[b]	Tissue	Collection date
Tom-2	Tamarillo	Entrerios, Antioquia	Fruit	1998
Tom-3	Tamarillo	Entrerios, Antioquia	Fruit	1998
Tom-4	Tamarillo	Santa Rosa, Antioquia	Fruit	1998
Tom-5	Tamarillo	Santa Rosa, Antioquia	Flower	1998
Tom-6	Tamarillo	San Pedro, Antioquia	Leaf	1998
Tom-7	Tamarillo	Entrerios, Antioquia	Flower	1998
Tom-8	Tamarillo	Rionegro, Antioquia	Leaf	1998
Tom-9	Tamarillo	Rionegro, Antioquia	Fruit	1998
Tom-10	Tamarillo	Rionegro, Antioquia	Fruit	1998
Tom-11	Tamarillo	Santa Rosa, Antioquia	Fruit	1998
Tom-12	Tamarillo	Santa Rosa, Antioquia	Flower	1998
Tom-13	Tamarillo	Don Matias, Antioquia	Fruit	1998
Tom-14	Tamarillo	Santa Rosa, Antioquia	Flower	1998
Tom-15	Tamarillo	Don Matias, Antioquia	Fruit	1998
Tom-16	Tamarillo	Guarne, Antioquia	Leaf	1998
Tom-17	Tamarillo	San Pedro, Antioquia	Fruit	1998
Tom-18	Tamarillo	Guarne, Antioquia	Flower	1998
Tom-21	Tamarillo	San Vicente, Antioquia	Flower	1998
Tom-22	Tamarillo	La Unión, Antioquia	Flower	1998
Tom-23	Tamarillo	Guarne, Antioquia	Flower	1998
Tom-24	Tamarillo	La Unión, Antioquia	Fruit	1998
Tom-25	Tamarillo	La Unión, Antioquia	Leaf	1998
Tom-26	Tamarillo	Guarne, Antioquia	Fruit	1998
Tom-27	Tamarillo	Guarne, Antioquia	Flower	1998
Tom-28	Tamarillo	Guarne, Antioquia	Flower	1998
Tom-30	Tamarillo	Don Matias, Antioquia	Leaf	1998
Tom-31	Tamarillo	Guarne, Antioquia	Fruit	1998
Tom-32	Tamarillo	Guarne, Antioquia	Fruit	1998
Tom-38	Tamarillo	Guarne, Antioquia	Flower	1998
Tom-39	Tamarillo	Santa Rosa, Antioquia	Fruit	1998
Tom-41	Tamarillo	San Vicente, Antioquia	Leaf	1998
Tom-42	Tamarillo	Don Matias, Antioquia	Fruit	1998
Tom-43	Tamarillo	Carmen de Viboral, Antioquia	Fruit	1998
Tom-44	Tamarillo	San Vicente, Antioquia	Fruit	1998
Tom-45	Tamarillo	Urrao, Antioquia	Fruit	1998
Tom-46	Tamarillo	Urrao, Antioquia	Fruit	1998
Tom-47	Tamarillo	Don Matias, Antioquia	Fruit	1998
Tom-48	Tamarillo	Don Matias, Antioquia	Flower	1998
Tom-49	Tamarillo	Rionegro, Antioquia	Fruit	1998
Tom-50	Tamarillo	Don Matias, Antioquia	Fruit	1998
Tom-54	Tamarillo	La Unión, Antioquia	Fruit	1998
Tom-55	Tamarillo	La Unión, Antioquia	Fruit	1998
Tom-57	Tamarillo	La Unión, Antioquia	Flower	1998
Tom-60	Tamarillo	La Unión, Antioquia	Fruit	1998
Tom-80	Tamarillo	Santa Rosa, Antioquia	Flower	1998

(*continued on next page*)

[a] Tom = tomato tree or tamarillo (*Solanum betacea*), Pass = *Passiflora*, different species, and Man = mango (*Mangifera indica* L.).
[b] Locations of commercial and backyard orchards in Colombia from which isolates were obtained.

AGCAG (26), AGGAGGAGGAGGAGG, GACACGACACGACAC (20), and GACAGACAGACAGACA (30). In the text, these primers have been designated $(CAG)_5$, $(AGG)_5$, $(GACAC)_3$, and $(GACA)_4$, respectively. For RAPD-PCR, a 10-base oligomer primer, OPF-08 (GGGATATCGG) also was used (Operon Technologies, Alameda, CA). Universal PCR primers were used (ITS1, TCCGTAGGTGAACCTGCGG and ITS4, TCCTCCGCTTATTGATATGC) for amplification of the ITS1 and ITS2 regions between the small and large nuclear rDNA, including the 5.8S rDNA, as described by White et al. (31). PCR primers for taxon-specific amplification included the ITS4 primer coupled with specific primers for *C. acutatum* (*Ca*Int2) (GGGGAAGCCTCTCGCGG) and *C. gloeosporioides* (*Cg*Int) (GGCCTCCCGCCTCCGGGCGG) (6). PCR primers for specific amplification of the passiflora population (including isolates TOM-6 and Man-76) of *Colletotrichum* spp. (not amplified with the *Ca*Int2 and *Cg*Int primers) included the ITS4 primer coupled with primer *Col*1 (GCCGTCCCCTGAAAAG), synthesized from the ITS1 region corresponding with the *Ca*Int2 and *Cg*Int primers. PCR reactions were performed in a total volume of 20 µl, containing 10 to 100 ng of genomic DNA, 50 mM KCl, 10 mM Tris-HCl, 0.2 mM each of dATP, dCTP, dGTP, and dTTP, 1.5 mM $MgCl_2$, 1 unit of *Taq* DNA polymerase (Promega, Corp., Madison, WI) and 1 µM primer. All the reactions were incubated in a PTC-100 thermocycler (MJ Research, Inc., Watertown, MA) starting with 5 min of denaturation at 95°C. For ap-PCR, this was followed by 30 cycles consisting of 30 s at 95°C, 30 s at either 60°C (for $(CAG)_5$ and $(AGG)_5$) or 48°C (for $(GACA)_4$ and $(GACAC)_3$) and 1.5 min at 72°C. For RAPD-PCR with OPF-08 primer, denaturation was followed by 44 cycles consisting of 1 min at 94°C, 1 min at 34°C, and 2 min at 72°C. For rDNA amplification, denaturation was followed by 40 cycles consisting of 30 s at 95°C, 30 s at 50°C, and 1.5 min at 72°C. For taxon-specific PCR, reactions were performed under conditions for primer $(CAG)_5$, with 0.5 µM ITS4 primer coupled with either 0.5 µM primer *Ca*Int2 for *C. acutatum*-specific detection or 0.5 µM primer *Cg*Int for *C. gloeosporioides*-specific detection. For *Col*1-specific PCR, the reaction was performed using 0.5 µM primer *Col*1 coupled with 0.5 µM primer ITS4, with denaturation of 5 min at 95°C, followed by 40 cycles consisting of 30 s at 95°C, 30 s at 65°C, and 1.5 min at 72°C. Amplification products were separated in agarose gels (1.5%, wt/vol; 15-cm width by 10-cm length) in Tris-acetate-EDTA buffer (27) electrophoresed at 80 V for 2 h. All PCR experiments were repeated at least four times with identical results

TABLE 1. (*continued from preceding page*)

Isolate[a]	Host species	Location[b]	Tissue	Collection date
Tom-90	Tamarillo	Don Matias, Antioquia	Leaf	1998
Tom-98	Tamarillo	Don Matias, Antioquia	Fruit	1998
Tom-99	Tamarillo	Don Matias, Antioquia	Branch	1998
Tom-104	Tamarillo	Urrao, Antioquia	Fruit	1998
Tom-106	Tamarillo	Guarne, Antioquia	Fruit	1998
Tom-109	Tamarillo	Urrao, Antioquia	Fruit	1998
Tom-110	Tamarillo	Don Matias, Antioquia	Fruit	1998
Tom-112	Tamarillo	San Vicente, Antioquia	Fruit	1998
Pass-19	*Passiflora mollissima*	Rionegro, Antioquia	Leaf	1999
Pass-20	Hybrid *P. mollissima*	Rionegro, Antioquia	Fruit	1999
Pass-33	Acc. 396097	Rionegro, Antioquia	Fruit	1999
Pass-34	*P. mollissima*	Rionegro, Antioquia	Fruit	1999
Pass-35	*Passiflora* spp.	Rionegro, Antioquia	Leaf	1999
Pass-36	*P. alnifolia*	Rionegro, Antioquia	Leaf	1999
Pass-40	*P. edulis*	Rionegro, Antioquia	Fruit	1999
Pass-52	*Passiflora* spp.	Rionegro, Antioquia	Leaf	1999
Pass-55	*P. edulis*	Rionegro, Antioquia	Leaf	1999
Pass-61	*P. edulis*	Rionegro, Antioquia	Leaf	1999
Pass-62	*Passiflora* spp.	Rionegro, Antioquia	Leaf	1999
Pass-63	Acc. 396047	Rionegro, Antioquia	Leaf	1999
Pass-64	*P. alnifolia*	Rionegro, Antioquia	Leaf	1999
Pass-65	*P. cuadragelis*	Rionegro, Antioquia	Fruit	1999
Pass-66	*Passiflora* spp.	Rionegro, Antioquia	Branch	1999
Pass-67	*P. maliformis*	Rionegro, Antioquia	Leaf	1999
Pass-68	*Passiflora* spp.	Rionegro, Antioquia	Leaf	1999
Pass-96	*P. cuadragelis*	Santa Fé de Antioquia, Antioquia	Fruit	1999
Pass-101	*P. ligularis*	Rionegro, Antioquia	Leaf	1999
Pass-102	*P. adenopoda*	Rionegro, Antioquia	Leaf	1999
Pass-103	*P. assiflora spp.*	Rionegro, Antioquia	Leaf	1999
Pass-107	*P. mollissima*	Rionegro, Antioquia	Leaf	1999
Pass-108	*P. ligularis*	Rionegro, Antioquia	Leaf	1999
Pass-113	*P. alnifolia*	Rionegro, Antioquia	Leaf	1999
Pass-114	*P. ligularis*	Rionegro, Antioquia	Leaf	1999
Pass-115	*P. moliformis*	Rionegro, Antioquia	Leaf	1999
Man-51	*Mangifera indica* cv. Heyden	Sevilla, Magdalena	Fruit	1998
Man-53	*M. indica* cv. Criollo	La Pintada, Antioquia	Leaf	2000
Man-59	*M. indica* cv. Corazón	Santa Fé de Antioquia, Antioquia	Leaf	1999
Man-69	*M. indica* cv. Heyden	Santa Fé de Antioquia, Antioquia	Flower	1999
Man-70	*M. indica* cv. Criollo	Barbosa, Antioquia	Fruit	1999
Man-72	*M. indica* cv. Papaya	Malagana, Bolivar	Fruit	1998
Man-73	*M. indica* cv. Azúcar	Sevilla, Magdalena	Fruit	1998
Man-75	*M. indica* cv. Azúcar	Cerete, Sucre	Fruit	1998
Man-76	*M. indica* cv. Criollo	Barbosa, Antioquia	Fruit	1999
Man-82	*M. indica* cv. Irwin	Turipana, Cordoba	Fruit	1998
Man-87	*M. indica* cv. Manzanita	Malagana, Bolivar	Fruit	1998
Man-91	Unknown	Santa Fé de Antioquia, Antioquia	Fruit	1998
Man-94	Unknown	Tierra Alta, Cordoba	Fruit	1998
Man-95	*M. indica* cv. Criollo	La Pintada, Antioquia	Flower	2000
Man-97	*M. indica* cv. Sufaida verde	Santa Fé de Antioquia, Antioquia	Leaf	1999

being achieved. Variation according to ap-PCR, RAPD-PCR, and A+T-rich DNA analyses (described below) was not quantified, but diversity was interpreted according to overall banding patterns.

A+T-rich DNA analyses. A+T-rich DNA was analyzed by *Hae*III digestion of total genomic DNA, which cleaves DNA at GGCC sites. *Hae*III digests nuclear DNA to fragments mainly less than 2 kb in size, whereas A+T-rich sequences are cleaved less frequently (15,18). A+T-rich DNA is partially associated with the mitochondrial genome, although contaminating nuclear A+T-rich DNA also may be present. Procedures used for *Hae*III restriction enzyme digestion and agarose gel electrophoresis were similar to those previously described (15).

Sequencing procedure. PCR-amplified rDNA products from representative isolates of *Colletotrichum* spp., using the primer pair ITS1 and ITS4 (31), resulted in a product of ≈560 bp, which was extracted from agarose gels using the Jetsorb kit (Genomed GmbH, Germany). The Big Dye Terminator DNA sequencing kit (Perkin-Elmer Inc., Branchburg, NJ) was used for determining sequence of the ITS1-2 regions (31). The sequence was determined using an ABI prism 377 DNA sequencer (Applied Biosystem Inc., Foster City, CA) and was performed by the Molecular Biology Center, Ness Ziona, Israel.

Phylogenetic analysis. Analyses of ITS sequences were carried out using the ARB program package (29). Alignment of sequences was performed with the ARB automated alignment tool and alignments were refined manually. Phylogenetic analyses were performed by applying ARB parsimony, distance matrix, and maximum-likelihood methods. To determine the robustness of phylogenetic trees, analyses were performed both on the original data set and on a data set from which highly variable positions were removed by use of a 50% conservation filter for the members of *Colletotrichum*, to reduce potential tree artifacts that may result from multiple base changes. Analysis was conducted on the ITS2 sequences of DNA (which is more informative as far as diversity is concerned than ITS1 alone) (14) from 15 representative isolates of *Colletotrichum* spp. from tamarillo (Tom-6, Tom-9, Tom-12, and Tom-21), passiflora (Pass-33, Pass-35, Pass-52, Pass-55, Pass-62, Pass-65, Pass-67, and Pass-103), and mango (Man-53, Man-69, and Man-76). Complete ITS1-2 sequences of the isolates were submitted to GenBank; accession numbers are shown in Table 3. Additional *Colletotrichum* sequences retrieved from the GenBank are included in this study for comparison (Table 3).

RESULTS

Morphological characterization of *Colletotrichum* isolates. Isolates from tamarillo (Tom-6; IMI 386922), passiflora (Pass-35; IMI 386920), and mango (Man-76; IMI 386921) that were not clearly defined by molecular methods were identified and characterized within the *C. gloeosporioides* species aggregate by morphological criteria by P. F. Cannon (CABI Bioscience, Egham, Surrey, UK); however, several features are unusual (Table 2). For example, Pass-35 conidia possess an unusually high length/breadth ratio and the appressoria appear to be formed from nodose brown swellings, and Man-76 appressoria are also complex and compound, distinguishing these two strains from typical *C. gloeosporioides*. In addition, isolates Tom-9 from tamarillo, Pass-62 from passiflora, and Man-75 from mango were characterized by us (Table 2). Conidia from Tom-9 were typical for *C. acutatum,* possessing tapering, acute ends, while those from Man-75 were typical for *C. gloeosporioides,* possessing cylindrical, rounded ends. Pass-62 was similar in morphology to isolate Man-76.

Species-specific primer analyses. DNA from 97 *Colletotrichum* spp. isolates collected from tamarillo (n = 54), passiflora (n = 26), mango (n = 15), and representatives of *C. acutatum* (isolate TUT-5954) and *C. gloeosporioides* (isolate AVO-37-4B), was amplified with the taxon-specific primers for *C. acutatum* (*Ca*Int2) and *C. gloeosporioides* (*Cg*Int) species as well as with *Col*1 (Table 4). PCR reactions with representative isolates of each population are presented in Figure 1. A 490-bp DNA fragment was amplified with the *C. acutatum*-specific primers *Ca*Int2 and ITS4 from all tamarillo isolates (except Tom-6) and the representative *C. acutatum* TUT-5954 isolate; however, no amplification product was evident with any of the passiflora and mango isolates (Fig. 1A; Table 4). A 450-bp DNA fragment was amplified with the *C. gloeosporioides*-specific primers *Cg*Int and ITS4 from 14 mango isolates and the representative *C. gloeosporioides* AVO-37-4B isolate, whereas no amplification product was observed from any of the passiflora isolates, isolate Tom-6 from tamarillo, or isolate Man-76 from mango (Fig. 1B; Table 4). A 520-bp DNA fragment was amplified from all passiflora isolates (except Pass-35), isolate Tom-6 from tamarillo, and isolate Man-76 from mango with *Col*1, the new primer designed for amplification of DNA from this unique population of *Colletotrichum*, but no amplification product was observed with the other tamarillo, mango, or representative *C. gloeosporioides* and *C. acutatum* isolates (Fig. 1C; Table 4). Specific amplification products indicate that 53 of the 54 tested

TABLE 2. Conidia type, colony morphology in culture, and species identification of representative isolates of *Colletotrichum* spp. from tamarillo, mango, and passiflora species in Colombia

Isolate[a]	Host	Conidia morphology and size	Colony morphology in culture	Species designation
Tom-6 (IMI 386922)	Tamarillo	Oblong, one end tapered and the other round, measuring 13.0 to 18.0 by 4.4 to 6.6 µm	White mycelium, turning dark salmon with age due to proliferation of spore masses; no sclerotia present; setae present but scarce	*Colletotrichum* sp.[b]
Tom-9	Tamarillo	Fusiform, tapered at both ends, measuring 10.3 to 18.7 by 3.7 to 5.6 µm	White mycelium turning gray and powdery with pink spore masses, salmon in color, produced outward in circles from the center of the culture	*C. acutatum*
Pass-35 (IMI 386920)	Passiflora	Fusiform with rounded ends, measuring 12.0 to 16.0 by 5.0 to 7.0 µm	White mycelium with pink spore masses, salmon in color, originating from large black sclerotia	*Colletotrichum* sp.[b]
Pass-62	Passiflora	Fusiform with rounded ends, measuring 12.0 to 16.0 by 5.0 to 7.0 µm	White cottony mycelium becoming gray with pink spore masses, salmon in color; large black sclerotia	*Colletotrichum* sp.[b]
Man-76 (IMI 386921)	Mango	Oblong with rounded ends, measuring 11.0 to 18.0 by 5.0 to 7.0 µm	White cottony mycelium with pink spore masses, salmon in color; large black sclerotia	*Colletotrichum* sp.[b]
Man-75	Mango	Cylindrical and lenticular, measuring 12.0 to 14.5 by 5.0 to 7.0 µm	White mycelium becoming gray and aerial with pink spore masses, salmon in color mainly at the center of the colony	*C. gloeosporioides*

[a] Isolates deposited in the culture collection of CABI Bioscience were designated with an additional code as indicated in parentheses.
[b] Identified as *C. gloeosporioides* by P. F. Cannon (CABI Bioscience).

tamarillo isolates are *C. acutatum* and 14 of the 15 mango isolates are *C. gloeosporioides*, whereas the passiflora isolates (together with Tom-6 from tamarillo and Man-76 from mango) belong to a population of *Colletotrichum* as yet unidentified. DNA of isolate Pass-35 from passiflora did not amplify with any of the primers.

Ap-PCR and RAPD analyses. Amplification products were obtained from all 95 (tamarillo, passiflora, and mango) isolates tested with the microsatellite derived primers $(CAG)_5$, $(AGG)_5$, $(GACA)_4$, and $(GACAC)_3$ and random primer OPF-08. Among the tested isolates from tamarillo, a low level of variability was observed, which would be consistent with a clonally propagating population. Mango isolates were more variable and the population from passiflora also was diverse when comparing isolates within each population. Isolates from passiflora were equally variable when comparing representative isolates between the tamarillo and mango populations. Gels showing diversity among and between representative isolates of each population from tamarillo, passiflora, and mango, using the respective primers $(AGG)_5$ and $(GACA)_4$, are presented (Fig. 2A and B). Similar degrees of diversity within and among populations were obtained using primers $(CAG)_5$, $(GACAC)_3$, and OPF-08 (data not shown).

A+T-rich DNA analyses. A+T-rich DNA analysis was carried out by *Hae*III digestion of total DNA from 16 representative isolates, used previously, of *Colletotrichum* from tamarillo, passiflora, and mango (Fig. 3). Results obtained using this method corroborated the findings observed with species-specific, microsatellite, and random primer analyses, whereby isolates from tamarillo belong to a homogeneous population but isolates from mango and passiflora showed a high level of variation. A+T-rich DNA analysis again allowed grouping of the tamarillo isolates within a single population, differentiating them from those identified as *C. gloeosporioides*. At the same time, clonality of the *C.*

TABLE 3. Internal transcribed spacer 1 and 2 sequences of *Colletotrichum* isolates used in this study

Species	Isolate	Host	EMBL accession
C. trichellum	89489[a]	*Hedera helix*	Z 33033
C. fructigenum	4885[a]	*Acmena smithii*	Z 32907
C. orbiculare	172.59[a]	*Cucumis sativus*	Z 33379
C. destructivum	319[a]	*Medicago sativa*	Z 32940
C. graminicola	DR1[b]	*Poa annua*	AF 059676
C. gloeosporioides	CG 231[c]	*Fragaria* × *ananassa*	AF 272780
C. gloeosporioides	AVO-37-4B[c]	*Persea americana*	AF 207792
C. gloeosporioides	APL 7[c]	*Malus domestica*	AF 272779
C. acutatum	TUT 5954[c]	*Fragaria* × *ananassa*	AF 207794
C. acutatum	PCN 5[c]	*Carya illinoensis*	AF 272786
C. acutatum	PCH 8[c]	*Prunus persica*	AF 272788
C. acutatum	ANE-HV83C[c]	*Anemone coronaria*	AF 272782
C. acutatum	STR 3[c]	*Fragaria* × *ananassa*	AF 272784
C. acutatum	ALM-US-4[c]	*Prunus dulcis*	AF 207793
C. acutatum	IMI 223120[c]	*Anemone coronaria*	AF 272783
C. acutatum	APL 2[c]	*Malus domestica*	AF 272787
C. acutatum	ANE-NL12A[c]	*Anemone coronaria*	AF 272781
C. acutatum	IMI 345026[c]	*Fragaria* × *ananassa*	AF 272789
Colletotrichum sp.	ALM-KSH-10[c]	*Prunus dulcis*	AF 207791
C. acutatum	Tom-9[d]	*Solanum betacea*	AF 521205
C. acutatum	Tom-12[d]	*Solanum betacea*	AF 521210
C. acutatum	Tom-21[d]	*Solanum betacea*	AF 521196
C. gloeosporioides	Man-53[d]	*Mangifera indica* sp.	AF 521198
C. gloeosporioides	Man-69[d]	*Mangifera indica* sp.	AF 521209
Colletotrichum sp.	Man-76[d]	*Mangifera indica* sp.	AF 521204
Colletotrichum sp.	Pass-33[d]	*Passiflora* sp.	AF 521202
Colletotrichum sp.	Pass-52[d]	*Passiflora* sp.	AF 521206
Colletotrichum sp.	Pass-55[d]	*Passiflora* sp.	AF 521199
Colletotrichum sp.	Pass-62[d]	*Passiflora* sp.	AF 521203
Colletotrichum sp.	Pass-65[d]	*Passiflora* sp.	AF 521200
Colletotrichum sp.	Pass-67[d]	*Passiflora* sp.	AF 521208
Colletotrichum sp.	Pass-103[d]	*Passiflora* sp.	AF 521201
Colletotrichum sp.	Tom-6[d]	*Solanum betacea*	AF 521207
Colletotrichum sp.	Pass-35[d]	*Passiflora* sp.	AF 521197
C. acutatum	BBA62126[e]	*Coffea arabica*	AJ 301924
Colletotrichum sp.	BBA70539[e]	*Coelogyne* sp.	AJ 301939
C. gloeosorioides	BBA71334[e]	*Citrus* sp.	AJ 301974
C. lupini	BBA71330[e]	*Urtica dioica*	AJ 301975
C. lupini	BBA71249[f]	*Lupinus albus*	AJ 301959
C. truncatum	BBA70523[f]	*Hosta* sp.	AJ 301937
Glomerella cingulata	BBA65797[f]	*Syringa vulgaris*	AJ 301925
Colletotrichum sp.	AHU9748[g]	Unknown	AB 076800
C. dematium	IMI-080025[h]	Unknown	AB 046608
C. linicola	CBS 172.51[h]	Unknown	AB 046608
C. truncatum	9969473[i]	*Lens culinaris*	AF 451902
C. truncatum	UQ349[i]	*Lens culinaris*	AF 451909

[a] Isolates sequenced by Sreenivasaprasad et al. (28).
[b] Isolate sequenced by Travanty et al. (EMBL direct submission).
[c] Isolates sequenced by Freeman et al. (13).
[d] Isolates sequenced by the authors.
[e] Isolate sequenced by Hagedorn et al. (EMBL direct submission).
[f] Isolate sequenced by Nirenberg et al. (24).
[g] Isolate sequenced by Tanaka et al. (EMBL direct submission).
[h] Isolate sequenced by J. Moriwaki and T. Tsukiboshi (EMBL direct submission).
[i] Isolate sequenced by R. Ford and P. W. J. Taylor (EMBL direct submission).

acutatum tamarillo population, represented in this case by isolates Tom-9, Tom-12, and Tom-21, was further verified (Fig. 3).

Sequence analyses. In order to verify the species identification performed using taxon-specific primers, a set of 15 representative isolates from tamarillo, mango, and passiflora was analyzed by comparing the ITS2 sequences of these *Colletotrichum* isolates with previously reported sequences of other *Colletotrichum* spp. Sequence analysis was conducted on isolates Tom-9, Tom-12, and Tom-21, identified as *C. acutatum*; isolates Man-53, Man-69, and Man-75, identified as *C. gloeosporioides*; and isolates Tom-6, Man-76, Pass-33, Pass- 35, Pass-52, Pass-55, Pass-65, Pass-62, Pass-67, and Pass-103, not corresponding to either *C. acutatum* or *C. gloeosporioides* according to the taxon-specific analysis, but classified as *Colletotrichum* spp. by morphological criteria. Sequence data of the isolates TUT 5954 of *C. acutatum* and AVO-37-4B of *C. gloeosporioides* were used as references together with the sequence data previously reported for additional representative isolates of the species: *C. trichellum*, *C. fructigenum*, *C. orbiculare*, *C. acutatum*, *C. lupini*, *C. destructivum*, *C. truncatum*, *C. gloeosporioides*, *C. graminicola*, *C. dematium*, *C. truncatum*, *C. linicola*, and *C. trifolii* (Table 3).

Phylogenetic analyses of the ITS2 sequence performed by applying ARB parsimony, distance matrix, and maximum-likelihood methods produced similar tree topologies. Removing highly variable positions from the sequence analysis did not affect tree topology. Sequence analysis confirmed species identification for the tamarillo population as *C. acutatum* and grouped them with *C. lupini* isolates within a new subgroup V of *C. acutatum* (Fig. 4). ITS2 sequence placed the mango population within the typical *C. gloeosporioides* cluster with isolates from avocado and apple. However, the passiflora isolates and Tom-6 and Man-76 from tamarillo and mango, respectively, were placed within a separate cluster containing three recently submitted sequences of *Colletotrichum* isolates (BBA 70539, BBA 71334, and AHU 9748) (Table

TABLE 4. Species-specific identification of *Colletotrichum* isolates from tamarillo (T), passiflora (P), mango (M), and representatives from avocado (A) and strawberry (S) using primers *Ca*Int2/ITS4, *Cg*Int/ITS4, and *Col*1/ITS4[a]

Host	Isolate	Primer reaction			Species	Host	Isolate	Primer reaction			Species
		*Ca*Int2	*Cg*Int	*Col*1				*Ca*Int2	*Cg*Int	*Col*1	
T	Tom-2	+	–	–	*C. acutatum*	T	Tom-98	+	–	–	*C. acutatum*
T	Tom-3	+	–	–	*C. acutatum*	T	Tom-99	+	–	–	*C. acutatum*
T	Tom-4	+	–	–	*C. acutatum*	T	Tom-104	+	–	–	*C. acutatum*
T	Tom-5	+	–	–	*C. acutatum*	T	Tom-106	+	–	–	*C. acutatum*
T	Tom-6	–	–	+	*C. acutatum*	T	Tom-109	+	–	–	*C. acutatum*
T	Tom-7	+	–	–	*C. acutatum*	T	Tom-110	+	–	–	*C. acutatum*
T	Tom-8	+	–	–	*C. acutatum*	P	Pass-19	–	–	+	*Colletotrichum* sp.
T	Tom-9	+	–	–	*C. acutatum*	P	Pass-20	–	–	+	*Colletotrichum* sp.
T	Tom-10	+	–	–	*C. acutatum*	P	Pass-33	–	–	+	*Colletotrichum* sp.
T	Tom-11	+	–	–	*C. acutatum*	P	Pass-34	–	–	+	*Colletotrichum* sp.
T	Tom-12	+	–	–	*C. acutatum*	P	Pass-35	–	–	–	*Colletotrichum* sp.
T	Tom-13	+	–	–	*C. acutatum*	P	Pass-36	–	–	+	*Colletotrichum* sp.
T	Tom-14	+	–	–	*C. acutatum*	P	Pass-40	–	–	+	*Colletotrichum* sp.
T	Tom-15	+	–	–	*C. acutatum*	P	Pass-52	–	–	+	*Colletotrichum* sp.
T	Tom-16	+	–	–	*C. acutatum*	P	Pass-55	–	–	+	*Colletotrichum* sp.
T	Tom-17	+	–	–	*C. acutatum*	P	Pass-61	–	–	+	*Colletotrichum* sp.
T	Tom-18	+	–	–	*C. acutatum*	P	Pass-62	–	–	+	*Colletotrichum* sp.
T	Tom-21	+	–	–	*C. acutatum*	P	Pass-63	–	–	+	*Colletotrichum* sp.
T	Tom-22	+	–	–	*C. acutatum*	P	Pass-64	–	–	+	*Colletotrichum* sp.
T	Tom-23	+	–	–	*C. acutatum*	P	Pass-65	–	–	+	*Colletotrichum* sp.
T	Tom-24	+	–	–	*C. acutatum*	P	Pass-66	–	–	+	*Colletotrichum* sp.
T	Tom-25	+	–	–	*C. acutatum*	P	Pass-67	–	–	+	*Colletotrichum* sp.
T	Tom-26	+	–	–	*C. acutatum*	P	Pass-68	–	–	+	*Colletotrichum* sp.
T	Tom-27	+	–	–	*C. acutatum*	P	Pass-96	–	–	+	*Colletotrichum* sp.
T	Tom-28	±	–	–	*C. acutatum*	P	Pass-101	–	–	+	*Colletotrichum* sp.
T	Tom-30	±	–	–	*C. acutatum*	P	Pass-102	–	–	+	*Colletotrichum* sp.
T	Tom-31	+	–	–	*C. acutatum*	P	Pass-103	–	–	+	*Colletotrichum* sp.
T	Tom-32	±	–	–	*C. acutatum*	P	Pass-107	–	–	+	*Colletotrichum* sp.
T	Tom-38	+	–	–	*C. acutatum*	P	Pass-108	–	–	+	*Colletotrichum* sp.
T	Tom-39	+	–	–	*C. acutatum*	P	Pass-113	–	–	+	*Colletotrichum* sp.
T	Tom-41	+	–	–	*C. acutatum*	P	Pass-114	–	–	+	*Colletotrichum* sp.
T	Tom-42	+	–	–	*C. acutatum*	P	Pass-115	–	–	+	*Colletotrichum* sp.
T	Tom-43	+	–	–	*C. acutatum*	M	Man-51	–	+	–	*C. gloeosporioides*
T	Tom-44	+	–	–	*C. acutatum*	M	Man-53	–	+	–	*C. gloeosporioides*
T	Tom-45	±	–	–	*C. acutatum*	M	Man-59	–	+	–	*C. gloeosporioides*
T	Tom-46	+	–	–	*C. acutatum*	M	Man-69	–	+	–	*C. gloeosporioides*
T	Tom-47	±	–	–	*C. acutatum*	M	Man-70	–	+	–	*C. gloeosporioides*
T	Tom-48	+	–	–	*C. acutatum*	M	Man-72	–	+	–	*C. gloeosporioides*
T	Tom-49	+	–	–	*C. acutatum*	M	Man-73	–	+	–	*C. gloeosporioides*
T	Tom-50	+	–	–	*C. acutatum*	M	Man-75	–	±	–	*C. gloeosporioides*
T	Tom-54	+	–	–	*C. acutatum*	M	Man-76	–	–	+	*Colletotrichum* sp
T	Tom-55	+	–	–	*C. acutatum*	M	Man-82	–	+	–	*C. gloeosporioides*
T	Tom-56	+	–	–	*C. acutatum*	M	Man-87	–	+	–	*C. gloeosporioides*
T	Tom-57	+	–	–	*C. acutatum*	M	Man-91	–	+	–	*C. gloeosporioides*
T	Tom-60	+	–	–	*C. acutatum*	M	Man-94	–	+	–	*C. gloeosporioides*
T	Tom-80	+	–	–	*C. acutatum*	M	Man-95	–	+	–	*C. gloeosporioides*
T	Tom-90	+	–	–	*C. acutatum*	M	Man-97	–	+	–	*C. gloeosporioides*
T	Tom-112	+	–	–	*C. acutatum*	A	AVO-37-4B	–	+	–	*C. gloeosporioides*
S	TUT-5954	+	–	–	*C. acutatum*						

[a] Taxon-specific primers *Ca*Int2 (*C. acutatum*), *Cg*Int (*C. gloeosporioides*), and *Col*1 (unidentified *Colletotrichum* sp.) were coupled with primer ITS4 for species identification; a positive (+) or negative (–) reaction with fungal DNA of each isolate is designated; (±) designates a weak reaction.

3; Fig. 4). The isolates within this cluster were more distant to the *C. gloeosporioides* complex but more closely related to *C. graminicola, C. dematium* (Fig. 4), and other *Colletotrichum* spp. (data not shown).

Homology values were calculated according to a data matrix calculated from the sequence divergence (data not shown). Sequence analysis based on the ITS2 region indicated respective homology levels of 99.5 and 97.8% among tamarillo isolates Tom-9, Tom-12, and Tom-21 and reference isolates of clusters I and II, belonging to the *C. acutatum* species (14). Respective homologies of the *C. acutatum* tamarillo isolates compared with isolate Tom-6 from tamarillo ranged between 89.0 to 91.2%; with passiflora isolates between 89.8 to 92.1%; and with the mango isolates between 91.0 to 92.1%. Homology between the typical *C. gloeosporioides* isolates (AVO-37-4B, APL-7, CG-231, Man-53, and Man-69) and those from passiflora (Man-76 and Tom-6) ranged from 94.5 to 95.5%, whereas similarity among the *C. gloeosporioides* isolates ranged between 98.9 and 100%. The Pass-35 isolate was associated with an outgroup, clustering with isolates of two *Colletotrichum* spp., *C. fructigenum* and *C. trichellum* (Fig. 4). Homology of Pass-35 with tested tamarillo, passiflora, and mango isolates of *C. gloeosporioides* and *C. acutatum* sequences was between 76.7 and 80.0%, further corroborating results observed with the taxon-specific analysis (Fig. 1).

DISCUSSION

This study was carried out to identify the species of *Colletotrichum* causing anthracnose of tamarillo, mango, and passiflora in Colombia, and to evaluate whether cross-infection between these host crops is occurring. Molecular methods were used for characterization of the species responsible for anthracnose of the various crops because morphological descriptors were not reliable for pathogen identification. Genetic diversity of the different populations also was assessed for each host-derived population.

The *C. acutatum*- and *C. gloeosporioides*-specific primers used here have been utilized in numerous studies for identification of *Colletotrichum* populations affecting various hosts. For example, using this approach, it was confirmed that *C. acutatum* is the causal species of almond anthracnose in California (9), and that two *Colletotrichum* spp., *C. acutatum* and *C. gloeosporioides*, are responsible for anthracnose of citrus in Florida (6). In this study, these primers were reliable for differentiating between the isolates from tamarillo and mango at the species level, but not for isolates from passiflora. Based on the species-specific primer analysis, all tamarillo isolates, except Tom-6, were identified as *C. acutatum,* whereas the mango isolates, other than Man-76, were identified as *C. gloeosporioides*. Although the passiflora, Man-76, and Tom-6 isolates were identified as *C. gloeosporioides* according to morphological criteria, they did not react with the *C. gloeosporioides*-specific primer (Fig. 1B). However, using the *Col*1 primer, a specific product was obtained, but only from this population (Fig. 1C). These results suggest that this population belongs to an as yet unidentified species of *Colletotrichum* based on taxon-specific amplification and sequence analysis. It should be noted, however, that two of the three additional isolates clustering with this population (BBA 70539 and AHU 9748) (Table 3; Fig. 4) were identified as *Colletotrichum* spp.; and, indeed, this cluster does not correspond to typical *C. gloeosporioides* based on ITS sequence. However, a third isolate from citrus (BBA 71334) was classified as *C. gloeosporioides*. This further demonstrates the incongruence between morphological criteria and molecular methodology for species identification in *Colletotrichum*.

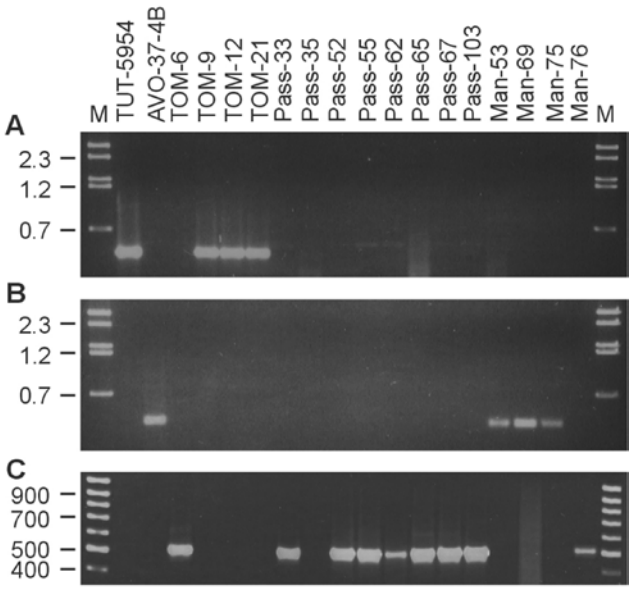

Fig. 1. Taxon-specific identification of **A,** *Colletotrichum acutatum* (primer *Ca*Int2 in combination with primer internal transcribed spacer [ITS]4), **B,** *C. gloeosporioides* (primer *Cg*Int in combination with primer ITS4) and **C,** an unidentified *Colletotrichum* population (primer *Col*1 in combination with primer ITS 4) according to amplification products of genomic DNA from *C. acutatum* isolates from strawberry (representative TUT-5954) and tamarillo (representatives Tom-9, Tom-12, and Tom-21); from *C. gloeosporioides* isolates from avocado (representative AVO 37-4B) and mango (representatives Man-53, Man-69, and Man-75); and from a population of *Colletotrichum* isolates from tamarillo (Tom-6), passiflora (representatives Pass-33, Pass-52, Pass-55, Pass-62, Pass-65, Pass-67, and Pass-103), and mango (Man-76). An additional unidentified isolate from passiflora (Pass-35) did not react with any of the polymerase chain reaction primers. Lane M: DNA markers with sizes in kilobases.

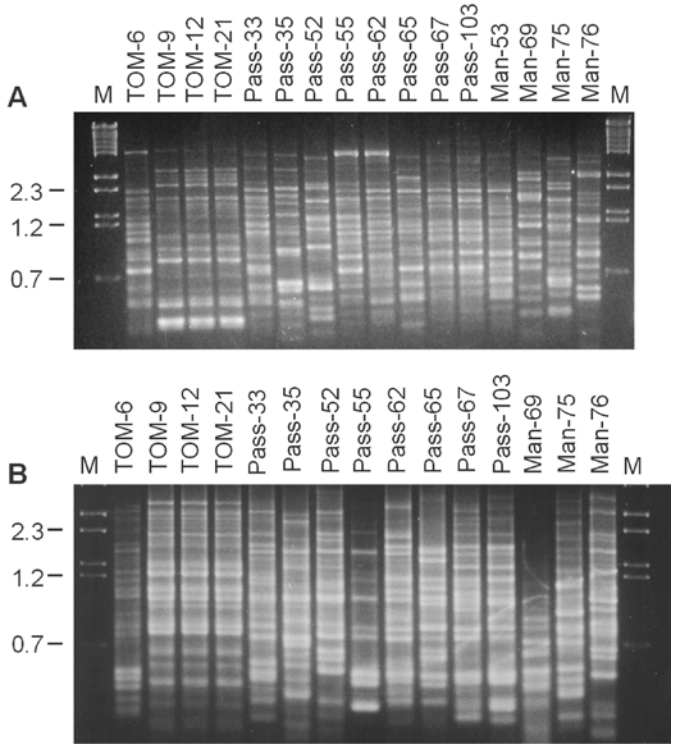

Fig. 2. Band patterns of arbitrarily primed polymerase chain reaction amplified genomic DNA of representative *Colletotrichum* isolates: *C. acutatum* isolates from strawberry (representative TUT-5954) and tamarillo (representatives Tom-9, Tom-12, and Tom-21); from *C. gloeosporioides* isolates from avocado (representative AVO 37-4B) and mango (representatives Man-53, Man-69, and Man-75); and from a population of *Colletotrichum* isolates from tamarillo (Tom-6), passiflora (representatives Pass-33, Pass-52, Pass-55, Pass-62, Pass-65, Pass-67, and Pass-103), and mango (Man-76). Primers used: **A,** $(AGG)_5$ and **B,** $(GACA)_4$. Lane M: DNA markers with sizes in kilobases.

ITS1-2 sequence analysis is reliable for phylogeny and systematics of *Colletotrichum* spp. (1,13,23,28) as opposed to other filamentous fungi such as *Fusarium* spp. (25). Therefore, this approach was applied to a representative set of isolates to verify identification of the *Colletotrichum* spp. causing anthracnose of tamarillo, mango, and passiflora in various regions of Colombia. In a previous study, based on ITS2 sequence data, four separate clusters or subgroups of *C. acutatum* isolates were identified (14). Subgroup I comprised mainly U.S. isolates from the various fruit; subgroup II comprised mainly anemone, olive, and strawberry isolates; subgroup III was represented by isolate ALM-KSH-10 and *C. acutatum* strawberry isolate IMI 345026 from Spain; and an anemone isolate NL-12A from the Netherlands represented subgroup number IV. In this study, sequence analysis of the ITS2 region was reliable and consistent, corroborating the taxon-specific analysis regarding the tamarillo isolates, by grouping this population within a new cluster of *C. acutatum* isolates, subgroup V, with *C. lupini* (Fig. 4). The mango isolates clustered within a group of previously defined isolates of *C. gloeosporioides* from various fruit according to specific amplification and sequence analysis (13). However, the *C. gloeosporioides*-specific primer did not react with DNA from the passiflora, Tom-6, and Man-76 isolates (Fig. 1) and ITS2 sequence analysis placed these isolates in a cluster distant from the *C. gloeosporioides* complex, but closer to representatives of *C. dematium* and *C. graminicola*. The Pass-35 isolate also was not identified to species according to the taxon-specific primers, including *Col*1 (Fig. 1). However, sequence analysis and morphological criteria placed this isolate within genus *Colletotrichum*, clustering with two isolates of *C. destructivum* and *C. orbiculare*, further indicating the importance of sequence data for identification of species within this genus. Therefore, it appears that a large database of ITS1-2 sequences of *Colletotrichum* isolates should be generated in order to compile a reliable phylogenetic tree for differentiating between species of this genus.

Genetic diversity of the populations was assessed using ap-PCR, RAPD-PCR, and A+T-rich DNA analyses. Within the tamarillo *C. acutatum* population, uniformity of banding patterns was observed, indicating clonality. Similar results were reported for *C. acutatum* infecting almond (9) and strawberry (12), indcating that minimal variability may be associated with host specificity. On the other hand, genetic diversity of *C. gloeosporioides* has been reported on many occasions (5,8,11,19). Likewise, in this study, the mango *C. gloeosporioides* isolates and those from passiflora were heterogeneous according to the molecular methods employed. Variability was observed within the population of *Colletotrichum* isolated from passiflora, which included the tamarillo (Tom-6) and mango (Man-76) isolates. If we assume that this population of isolates belongs to a single species, the genetic complexity and heterogeneity may be explained by the presence of a perfect stage (12). Furthermore, the diversity observed within this subpopulation may be associated with adaptation of these isolates to a nonspecific, broad host range. For example, the *Colletotrichum* isolates from passiflora originated from various species and cultivars of the host, relieving the pressure for selection within the population. In contrast, variation was not observed among isolates of the tamarillo population, originating from a region where a single cultivar of this host is cultivated, a factor that may promote selection for clonality and homogeneity.

In summary, the molecular methods used for studying the anthracnose pathogens of tamarillo, mango, and passiflora, mainly

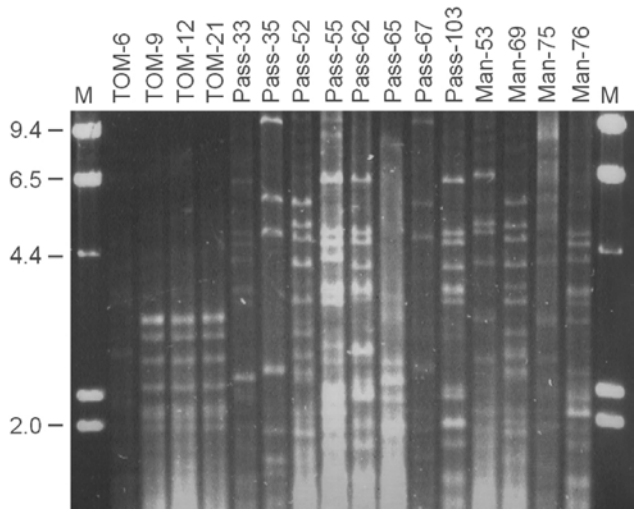

Fig. 3. Band patterns of mitochondrial DNA from *Colletotrichum* isolates: *C. acutatum* isolates from strawberry (representative TUT-5954) and tamarillo (representatives Tom-9, Tom-12, and Tom-21); from *C. gloeosporioides* isolates from avocado (representative AVO 37-4B) and mango (representatives Man-53, Man-69, and Man-75); and from a population of *Colletotrichum* isolates from tamarillo (Tom-6), passiflora (representatives Pass-33, Pass-52, Pass-55, Pass-62, Pass-65, Pass-67, and Pass-103), and mango (Man-76). Isolates were digested with *Hae*III for A+T-rich DNA analysis. DNA was electrophoresed until the major G+C-rich fragments were eluted from the gel. Lane M: DNA markers with sizes in kilobases.

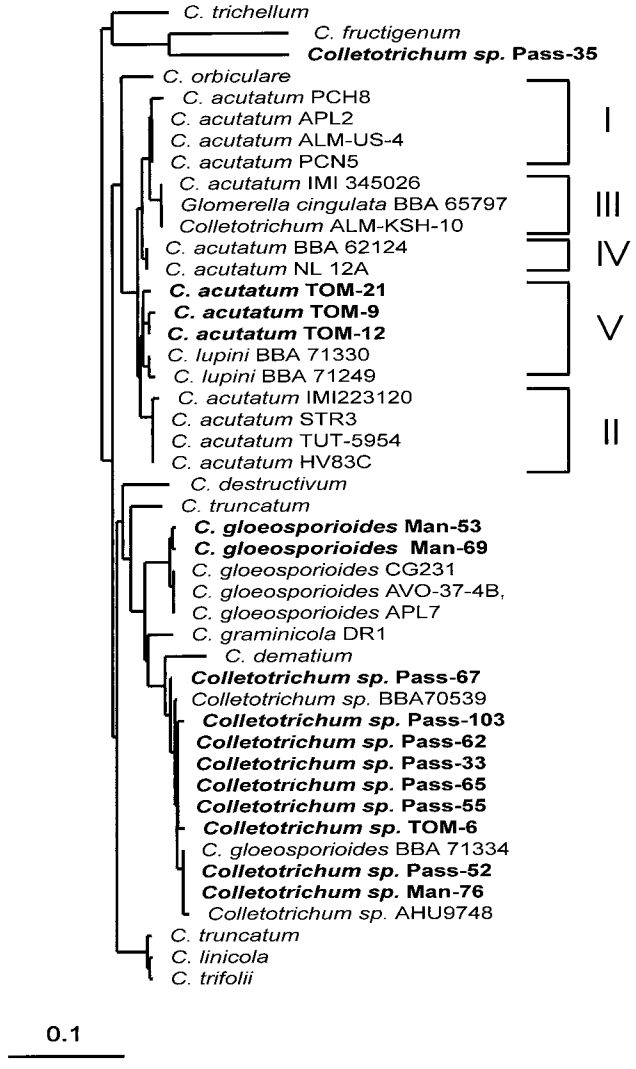

Fig. 4. Internal transcribed spacer 2-based phylogenetic tree of *Colletotrichum* isolates and published sequences. The tree was produced using the neighbor-joining algorithm. The order of branching was similar in all tree construction approaches used. Scale bar indicates estimated 10% sequence divergence.

from Antioquia, Colombia, identified the tamarillo population as limited to this host and clonal, belonging to *C. acutatum*, and the mango population as *C. gloeosporioides*. Another as yet undefined species of *Colletotrichum* also was identified, including isolates from passiflora, tamarillo (Tom-6), and mango (Man-76), according to sequence of the ITS region, indicating that this population may not be host specific.

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