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Research Papers

Phylogenetic diversity and pathogenicity of *Colletotrichum* species associated with avocado anthracnose in Chile

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Summary. Anthracnose, caused by *Colletotrichum* species, is a major disease of avocado (*Persea americana*) that significantly reduces fruit quality and export potential in Chile. *Colletotrichum* species associated with this disease were identified and their pathogenicity to avocado was assessed. In the summer of 2018, healthy fruits (*n* = 1,335) were sampled from three commercial groves located in the Metropolitan, O'Higgins and Valparaíso regions of Chile, and from a non-commercial grove and local markets. Fruits were stored until symptoms developed, indicating anthracnose incidence in commercial groves ranging from 10 to 50%. A total of 146 fungal isolates were obtained from symptomatic fruit and were initially identified as *Colletotrichum* spp. Fifty representative isolates were further identified through multilocus phylogenetic analyses. Ten species belonging to four species complexes were identified. *Colletotrichum* cf. *cigarro* was the most frequent taxon (*n* = 17), followed by *C. pyricola* (*n* = 9), *C. gloeosporioides* (*n* = 6), *C. jiangxiense* (*n* = 5), *C. karsti* (*n* = 4), *C. anthrisci* (*n* = 3), *C. brassicicola* (*n* = 3), *C. laurosilvaticum* (*n* = 1), *C. fructicola* (*n* = 1), and *C. perseae* (*n* = 1). Pathogenicity tests reproduced anthracnose symptoms in inoculated fruits, whereas control fruits remained symptomless, and revealed differences in virulence among isolates and species. This study provides the first report of *C. brassicicola, C. laurosilvaticum* and *C. pyricola* as causal agents of avocado anthracnose, highlights the diversity of *Colletotrichum* species in Chilean avocado groves, and provides insights for improved management strategies for avocado anthracnose.

Keywords. Etiology, *Persea americana*, postharvest disease.

INTRODUCTION

Avocado (*Persea americana* Mill.) is an economically important fruit crop in Chile, with approx. 33,000 ha cultivated mainly between the Coquimbo and O'Higgins regions (latitudes 29°30' to 35°45' S) (ODEPA-CIREN, 2024). The cultivar Hass dominates production and fruit of which are the only ones exported. During the 2024-2025 season, Chile produced approximately 240,000 tons of avocados, exporting 139,091 tons primarily to Europe (76.9%), Latin America (17.1%), Asia (3.5%), and North America (2.4%) (Comité de Paltas de Chile, 2025; FAOSTAT 2025). Avocado fruit is susceptible to anthracnose, a major postharvest disease caused by species of *Colletotrichum* (Dann *et al.,* 2013). Infections occur in the field, where these pathogens penetrate young fruit and remain latent until ripening after harvest (Prusky and Plumbley, 1992). Because of long distances to export markets, fruit is stored at low temperatures (4–5°C) for 17 to 45 d (Ferreyra and Defilippi, 2012). This period allows for fruit ripening and activation of latent pathogen infections, often resulting in anthracnose development and significant postharvest losses (Ramírez-Gil *et al.,* 2020).

During the last two decades, incidence of anthracnose in avocados has increased in Chile, particularly in high-density groves located in humid areas. The disease is most severe in coastal areas, where high relative humidity and prolonged dew periods favour infections. Symptoms first appear as small circular lesions on fruit skins that enlarge rapidly, becoming dark and sunken. Soon after, the pathogens produce acervuli that rupture fruit epidermis and sporulate, forming orange to pink, waxy masses of conidia (Nelson, 2008). As the disease progresses, the fruit pulp beneath the lesions begins to rot and separates easily from the skin, leaving characteristic cavities when skin is removed. In the field, damaged infected fruit may ripen prematurely, leading to pre-harvest fruit drop. Infected avocado leaves and twigs often remain attached to the trees, serving as inoculum sources for subsequent infection cycles (Fitzell, 1987).

Avocado anthracnose is caused by fungi of the genus *Colletotrichum*, with approx. 26 species reported. Most species associated with this disease belong to the *Colletotrichum gloeosporioides* species complex (CGSC), followed by the *C. acutatum* species complex (CASC), and the *C. boninense* species complex (CBSC). Comprehensive studies investigating species associated with avocado anthracnose have been conducted in several major avocado producing countries, including Mexico (Fuentes-Aragón *et al*., 2020), Colombia (Gañán *et al*., 2015), Kenya (Kimaru *et al*., 2018), Brazil (Soares *et al*., 2020), Chile (Bustamante *et al*., 2022), Israel (Sharma *et al*., 2017), and Vietnam (Thanh *et al*., 2025). In other important avocado-producing countries such as Indonesia, the Dominican Republic, and Peru, only *C. gloeosporioides sensu lato* has been documented (Zakaria, 2021). Additional reports of this disease from regions with low avocado production volumes include China (Li *et al*., 2022), the United States of America (Nelson, 2008, Faber *et al*., 2016), Australia (Shivas and Tan, 2009; Giblin *et al*., 2018), South Africa (Weir *et al*., 2012), New Zealand (Weir *et al*., 2012; Hofer *et al*., 2021), Türkiye (Akgül *et al*., 2016), Greece (Malandrakis *et al*., 2023), Sri Lanka (Dissanayake *et al*., 2021), and Ghana (Honger *et al*., 2016). Studies from countries with emerging avocado industries include Taiwan (Wu *et al*., 2023), Thailand (Armand and Jayawardena, 2024), and Italy (Guarnaccia *et al*., 2016). Species belonging to the *C. gigasporum* species complex have been reported in Sri Lanka, *C. magnum* species complex in Mexico, and *C. dematium* species complex (CDSC) in Chile. These represent infrequent detections compared with dominant species complexes associated with avocado anthracnose (Hunupolagama *et al*., 2015; Fuentes-Aragón *et al*., 2020; Bustamante *et al*., 2022).

In Chile, only *C. gloeosporioides sensu lato* and *C. anthrisci* have been formally documented as causal agents of avocado anthracnose (Morales *et al*., 1979; Bustamante *et al*., 2022). Research in other avocado-growing regions has shown that diversity and prevalence of *Colletotrichum* species vary considerably, depending on geographical location and environmental conditions. Accurate identification of *Colletotrichum* species is a critical prerequisite for the development of effective disease management strategies (Downling *et al*., 2020; Camiletti *et al*., 2022). Therefore, a clear understanding of the species composition and pathogenic potential of *Colletotrichum* associated with avocado anthracnose in Chile is required to support the implementation of integrated disease management programs. To address this gap, the present study aimed to isolate, identify, and assess the pathogenicity of *Colletotrichum* species associated with anthracnose symptoms on avocado fruits in Chile.

MATERIALS AND METHODS

Fruit sampling and incubation

During the 2018 growing season, a total of 1,335 apparently healthy avocado fruits (cv. Hass) were collected from three commercial groves, a non-commercial grove, and from local markets. The commercial groves were located in the Metropolitan (33°43'60"S), O'Higgins

(34°23'46"S), and Valparaíso (33°38'00"S) regions of Chile. The non-commercial grove was located approx. 10 km southeast of the commercial grove, near Naltahua in the Metropolitan region. Fruits were randomly collected from harvest bins, trees, and grove floors. Locally grown fruit from domestic markets within the Metropolitan region were also randomly sampled. In the laboratory, collected fruits were incubated in cardboard packaging at room temperature (20–25°C) under a 12 h photoperiod with fluorescent light and maintained at 40 to 50% humidity for 14 d until anthracnose symptoms developed.

Fungal isolations

Symptomatic fruits were inspected for anthracnose by observing acervuli formation and fruit rot underneath sunken lesions. Fruits were surface-disinfected by spraying with 70% ethanol, and isolations were carried out individually from one sporulating lesion per fruit, following methods of Hu *et al*. (2015). Symptomatic tissue pieces (each approx. 2 × 2 mm) beneath lesions were excised and plated onto potato dextrose agar (PDA) amended with streptomycin (100 mg L^{-1}). Pure cultures were established using single-conidium and hyphal tip isolation methods (Senanayake *et al*., 2020), and each isolate was obtained from a different symptomatic fruit. Preliminary identifications of isolated fungi were based on observation of acervuli, setae, and conidia using a microscope (Axiostar Plus, Carl Zeiss) at 100× and 400× magnifications. For isolates that did not sporulate on PDA, casitone-yeast extract agar (CYA: 1.7 g L^{-1} of casitone, 0.35 g L^{-1} of yeast extract, 2.0 g L^{-1} of glucose, 5.0 g L^{-1} of agar) was used to induce acervulus formation. Subsequently, isolates were cultured on PDA for colony observations, and representative isolates from each location were selected for phylogenetic analyses.

DNA extraction, amplification, and sequencing of representative isolates

Selected isolates (*n* = 50) were each cultured on PDA for 7 d, and DNA was extracted using the Fungi/Yeast Genomic DNA isolation kit (Norgen Biotek Corp.). The rDNA internal transcribed spacer (ITS), along with fragments of the glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) and beta-tubulin (*tub2*) genes, were amplified by PCR for all the selected isolates using the primer pairs ITS1/ITS4 for ITS (White *et al*., 1990), GDF1/GDR1 for *gapdh* (Guerber *et al*., 2003), and T1/Bt2b for *tub2* (Glass and Donaldson, 1995; O'Donnell and Cigelnik, 1997). Additionally, for isolates within the *C. gloeosporioides* species complex (CGSC), the glutamine synthetase (*gs*) gene was amplified using GSF1/GSR1 (Guerber *et al*., 2003), and the intergenic region between the DNA lyase (*Apn2*) and the mating type *MAT1-2* genes (referred to as ApMat) was amplified using AMF1/AMR1 (Silva *et al*., 2012b). For isolates within the *C. dematium* species complex (CDSC), the actin (*act*) gene was amplified using ACT-512F/ACT-783R, and the chitin synthase (*chs1*) gene was amplified using CHS-79F/CHS-354R (Carbone and Kohn, 1999). PCRs were each carried out in a final volume of 25 µL, containing 1× GoTaq® Green Master Mix (Promega), 200 nM of each primer, 50 to 100 ng of genomic DNA, and Nanopure water to complete the volume. The thermocycling conditions were: an initial denaturation at 94°C for 5 min, followed by 30 cycles each of 30 s at 94°C, 45 s at 48°C for ITS, or 52°C for *gapdh, tub2*, *gs* and ApMat, or 61°C for *act*, or 58°C for *chs1*, and 40 s at 72°C, then with final extension of 7 min at 72°C. PCR products were sequenced by Psomagen USA (Rockville, MD, USA), and consensus sequences were obtained by assembling forward and reverse sequences using CAP3 (Huang and Madan, 1999).

Phylogenetic analyses

Four data sets, consisting of sequences of different DNA markers (ITS, *gapdh*, *tub2*, *gs*, ApMat, *act*, and *chs1*) of *Colletotrichum* species, were constructed by combining reference sequences from the NCBI database with the sequences obtained from the Chilean isolates (Supplementary Tables S1 to S4). Sequences were aligned by locus using MAFFT 7 selecting the L-INS-i refinement method (Katoh *et al*., 2019), and were trimmed manually on BioEdit 7 (Hall, 1999). Each locus of the concatenated data sets was partitioned by coding and non-coding regions (but not by codon position), resulting in 20 partitioned subsets. Maximum likelihood (ML) reconstructions were carried out in IQ-TREE 2 (Minh *et al*., 2020), with the following options: linked branch lengths for partitioned analysis, 'greedy' algorithm for merging partitions, 'merge-model all' and 'merge-rate all', to employ the widest range of evolutionary models, corrected Akaike information criterion for model testing, 'allnni' for a more thorough tree search, and the ultrafast approximation of 1,000 bootstrap replicates for support values. Each species complex was analyzed separately using concatenated data sets of ITS-*gapdh*-*tub2*. When resolution was insufficient, the markers ApMat, *gs*, *act* and *chs1* were incorporated individually or in combination, to improve phylogenetic informativeness of the dataset. Bayesian inference (BI) was assessed using MrBayes 3.2.7 (Ronquist *et al*., 2012), following the methodology of Bourret

et al., (2018). Resulting ML and BI trees were examined, and support values were combined in TreeGraph2 (Stöver and Müller, 2010). Visual edits were carried out using Inkscape 0.92 (http://inkscape.org). Final sequences were submitted to GenBank following analyses.

Conidium characterization

Representative isolates of each *Colletotrichum* species were cultured on CYA plates at 22°C for 7 to 14 d until acervuli formed. For each isolate, 30 conidia were measured for length and width using a light microscope (Axiostar Plus, Carl Zeiss) at 400× magnification. Conidium dimensions were recorded as minimum, mean ± standard deviation, and maximum for lengths and widths, and length-to-width ratios were calculated from mean values. Conidium dimensions were compared to published data of reference strains of each species (Damm *et al.*, 2009; 2012a; 2012b; Liu *et al.*, 2015; Prihastuti *et al.*, 2009; Ramos *et al.*, 2016; Sharma *et al.*, 2017).

Pathogenicity tests

One or two isolates per species were selected ($n = 17$) to conduct assessments of Koch's postulates on healthy 'Hass' avocado fruits. Three fruits per isolate were inoculated, and each fruit received two inoculations. The isolates were cultured on CYA plates for 14 d at 22°C to induce acervulus formation. Conidia were washed from sporulating acervuli using sterile distilled water, and conidium suspensions were diluted up to 10^6 conidia mL^{-1} by counting with hemocytometer. Fruits were each disinfected with 0.5% sodium hypochlorite for 3 min and then rinsed with sterile distilled water. The fruits were each then wounded at two points using a sterile needle (0.5 mm diameter) to 1 mm of depth. Inoculations each consisted of pipetting 20 μL of the conidium suspension onto each wound, while inoculation control fruits received sterile water. Fruits were then incubated at 22°C for 7 d in humid chambers (>80% RH), which were polystyrene containers containing moistened paper towels. Evaluations were carried out by removing the skin from each fruit and measuring diameters of necrotic lesions, with each lesion measured in two perpendicularly opposite directions using a caliper. Re-isolations were carried out by culturing pulp pieces (each approx. 16 mm^2) from lesions margins on PDA for 7 d at 22°C. Resulting fungal colonies were identified morphologically. Data of lesion diameters were subjected to analysis of variance (ANOVA) using generalized linear models with the corresponding R packages in InfoStat v2008 (Grupo InfoStat, FCA), and means were separated using Fisher's least significant difference (LSD) test ($\alpha = 0.05$). The pathogenicity assessment was carried out twice, and data were combined for treatments that were not significantly different ($P > 0.05$) for the two experiments.

RESULTS

Symptoms on sampled fruit

Black, circular lesions developed on the fruit skins after ripening, with one to several lesions per fruit. Lesions appeared within 7 to 14 d after harvest, as fruits ripened under incubation at room temperature (20 to 25°C, 40–50% relative humidity). Over time, lesions expanded progressively, and waxy conidium masses developed in the lesion centres. Conidium masses varied in colour, from orange to pink (Figure 1 A), and less frequently, from white to grey (Figure 1 B). As the disease progressed, adjacent lesions coalesced and became sunken (Figure 1 C). Internally, the pulp beneath each lesions was dark brown, forming a rounded pattern toward the fruit centre. When symptomatic fruits were bisected, the necrotic pulp detached easily from the surrounding healthy tissues, leaving characteristic cavities (Figure 1 D).

Fungal isolations

Isolations from individual fruits yielded 146 fungal isolates: 48 were from a commercial grove in the Metropolitan region, 40 were from Valparaíso, 18 were from O'Higgins, 27 were from a non-commercial grove in the Metropolitan region, and 13 were from locally grown fruit purchased in markets. All isolates were morphologically consistent with *Colletotrichum* spp., showing diagnostic structures including acervuli, setae, and conidia on PDA and CYA cultures (Barnett and Hunter 1998). Colony morphology on PDA at 22°C had overlap among isolates, so was not used for species identifications. Representative isolates from each location were selected for multilocus phylogenetic analyses for species identification.

Phylogenetic analyses

The consensus sequence lengths ranged from 535 to 577 bp for ITS, 254 to 278 bp for *gapdh*, 724 to 751 bp for *tub2*, 922 to 997 bp for *gs*, 906 to 961 bp for ApMat, 252 to 254 bp for *act*, and 288 to 301 bp for *chs1*. BLAST searches confirmed that the 50 analyzed isolates belonged to *Colletotrichum*. Phylogenetic reconstruc-

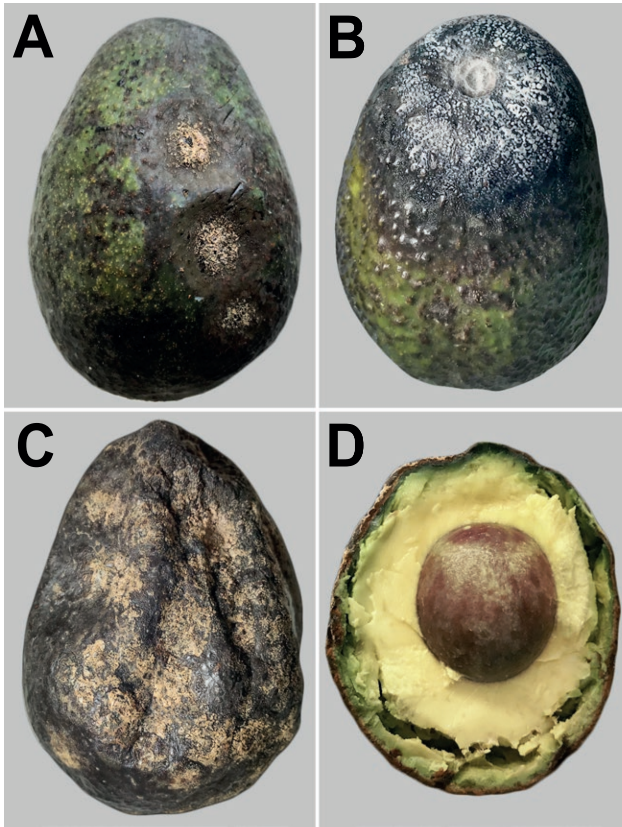

Figure 1. Symptoms of anthracnose on 'Hass' avocado fruits during postharvest. A, Lesions on fruit skin with orange to pink sporulation. B, Lesion on the stem end skin of a fruit showing the least frequent white to grey sporulation. C, Advanced symptoms showing coalescing, sunken lesions. D, Characteristic cavity formed between healthy and colonized pulp after cutting open an anthracnose symptomatic fruit.

tions using maximum likelihood (ML) and Bayesian posterior probability (PP) methods showed that the isolates clustered with reference strains of ten species across four species complexes (Figures 2 to 5). A predominance of members of the *C. gloeosporioides* species complex (CGSC; *n* = 30 isolates) was detected, followed by the species complexes *C. acutatum* (CASC; *n* = 9), *C. boninense* (CBSC; *n* = 8), and *C. dematium* (CDSC; *n* = 3).

For the CGSC, the combined dataset ITS-*gapdh-tub2*-ApMat-*gs* provided high resolution (Figure 2). Isolates formed strongly supported clades with reference strains of *C. fructicola*, *C. gloeosporioides, C. jiangxiense*, and *C. perseae*, all with ML/PP support values of 100%/1.0. The remaining isolates formed a well-supported cluster (100%/1.0) with the strains ICMP 12952, ICMP 12953, and PR432, previously reported as *C. cigarro*. However, the ex-type strain of *C. cigarro* (ICMP 18539) formed a separate clade as a sister taxon of *C. heleniense*. Consequently, these isolates were designated as *C.* cf. *cigarro*. For CASC and CBSC, the concatenated dataset ITS-*gapdh-tub2* was phylogenetically informative (Figures 3 and 4). Chilean isolates formed well-supported clades with reference strains of *C. pyricola* (100%/1.0), *C. karsti* (99%/1.0), *C. laurosilvaticum* (99%/1.0), and *C. brassicicola* (100%/1.0). The CDSC was resolved with the combined dataset ITS-*gapdh-tub2-act-chs1*, which revealed a well-supported clade between Chilean isolates and strains of *C. anthrisci* (100%/1.0) (Figure 5). Species identifications and their respective frequencies are summarized in Table 1. GenBank accession numbers of analyzed isolates are listed in Tables 2, 3 and 4.

Conidium characterization

Selected isolates of each *Colletotrichum* species produced acervuli on CYA plates after 7 to 14 days at 22°C. Isolates from the CGSC and CBSC formed cylindrical to oval, aseptate conidia with straight or slightly rounded ends. Isolates of *C. pyricola* (CASC) formed cylindrical to fusiform, aseptate conidia, each with one end slightly pointed and the other end rounded. In contrast, *C. anthrisci* (CDSC) produced curved fusiform conidia, which were almost straight in the central regions but bending abruptly toward both ends. Conidium dimensions of all analyzed isolates, together with reference strains, are presented in Table 5. Due to the high morphological variability within this group, 12 isolates of *C.* cf. *cigarro* were examined. Conidium lengths ranged from 12.0 to 21.0 µm and widths from 4.0 to 7.0 µm, exhibiting overlaps across isolates and significant differences in length, width, and length-to-width ratios (Figure S1).

Pathogenicity assessments

All assessed isolates induced anthracnose symptoms on inoculated avocado fruits within 7 days at 22 °C, whereas non-inoculated control fruits remained symptomless. Mean lesions diameters ranged from 20.9 to 34.1 mm, with significant differences detected among isolates ($P < 0.0001$) (Table 6). The most virulent isolates were *C. fructicola* (MER-06; mean lesion diameter = 34.1 mm) and *C.* cf. *cigarro* (MER-11; mean = 33.0 mm). Intermediate virulence was observed for *C.* cf. *cigarro* (SDO-03), *C. anthrisci* (NAL-54) and *C. pyricola* (FAM-20 and FAM-23), with mean lesion diameters between 28.3 and 30.9 mm. The remaining isolates of *C. gloeosporioides* (MER-07 and FAM-01), *C. jiangxiense* (SDO-38 and NAL-03), *C. perseae* (NAL-19), *C. karsti*

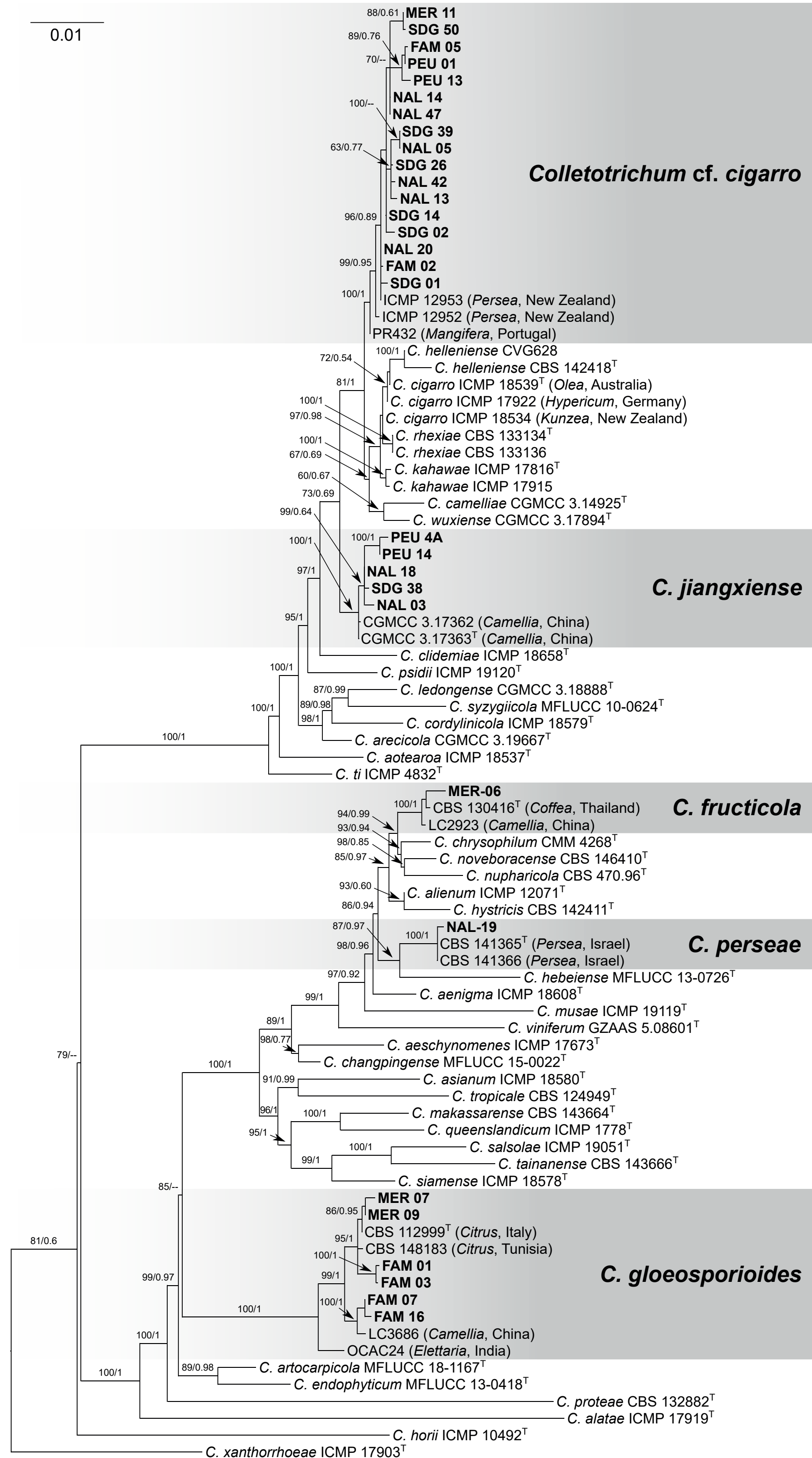

Figure 2. Maximum likelihood phylogenetic analysis of the *Colletotrichum gloeosporioides* species complex (CGSC). Chilean isolates are shown in bold font, and ex-type strains are each accompanied with a superscript T. Maximum likelihood bootstrap values and Bayesian posterior probabilities are indicated above the tree branches. The tree was inferred from a data set consisting of sequences of five DNA markers (ITS, *gapdh*, *tub2*, ApMat, and *gs*), and was rooted with *C. xanthorrhoeae*. Scale bar = substitutions per site.

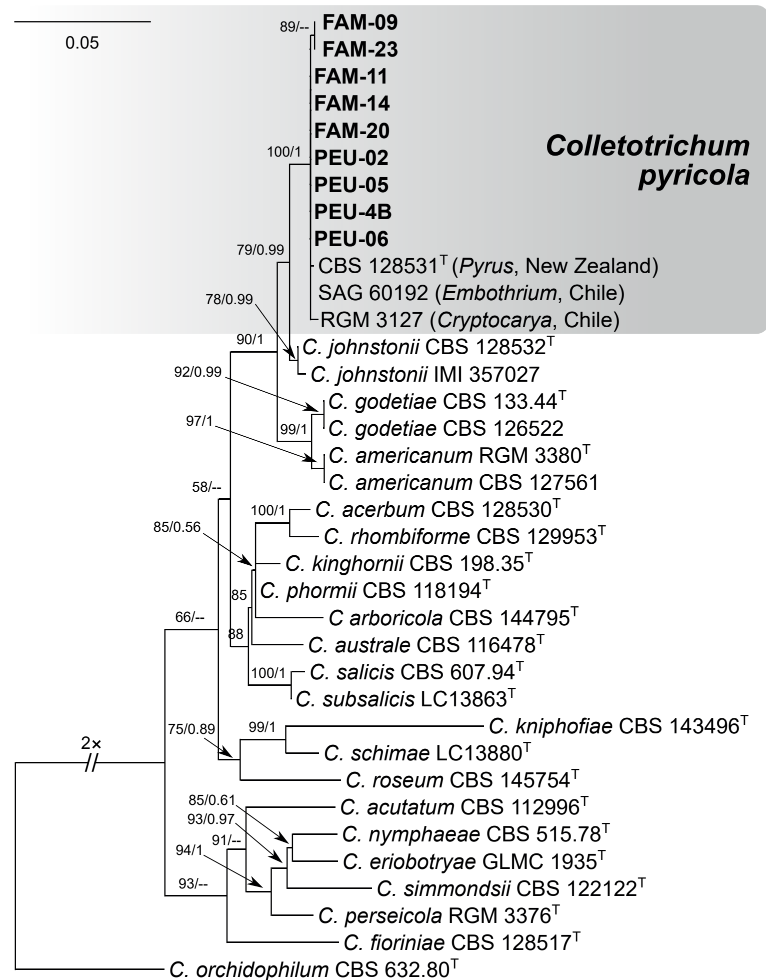

Figure 3. Maximum likelihood phylogenetic analysis of the *Colletotrichum acutatum* species complex (CASC). Chilean isolates are shown in bold font, and ex-type strains are each accompanied with a superscript T. Maximum likelihood bootstrap values and Bayesian posterior probabilities are indicated above the tree branches. The tree was inferred from a data set consisting of sequences of three DNA markers (ITS, *gapdh*, and *tub2*) and was rooted with *C. orchidophilum*. Scale bar = substitutions per site.

(NAL-33 and NAL-35), *C. anthrisci* (NAL-53), *C. brassicicola* (NAL-32 and FAM-06), and *C. laurosilvaticum* (NAL-17), were less virulent, with mean lesion diameters from 20.9 to 27.4 mm. Figure 6 illustrates representative species showing contrasting levels of virulence. The same isolates as those inoculated were consistently re-isolated on PDA plates from the necrotic pulp of symptomatic fruit, with identities confirmed by morphology, and no *Colletotrichum* colonies were recovered from the non-inoculated control fruits.

DISCUSSION

This study provides a comprehensive assessment of the etiology of avocado anthracnose in Chile. Ten *Colletotrichum* species were identified through combined morphological and multilocus phylogenetic analyses, representing four species complexes: CGSC (30 isolates), CASC (eight isolates), CBSC (eight isolates), and CDSC (three isolates). From other countries, approx. 26 *Colletotrichum* species have been previously identified causing avocado anthracnose, with members of the CGSC most frequently observed (Shivas and Tan, 2009; Cannon *et al*., 2012; Hunupolagama *et al*., 2015; Sharma *et al*., 2017; Giblin *et al*., 2018; Fuentes-Aragón *et al*., 2020; Soares *et al*., 2020; Hofer *et al*., 2021; Wu *et al*., 2023). The present study detected greater diversity of species in Chile than previously documented, including three species reported on avocado for the first time.

The most frequent species found in the present study was *C.* cf. *cigarro* (CGSC), detected across all sam-

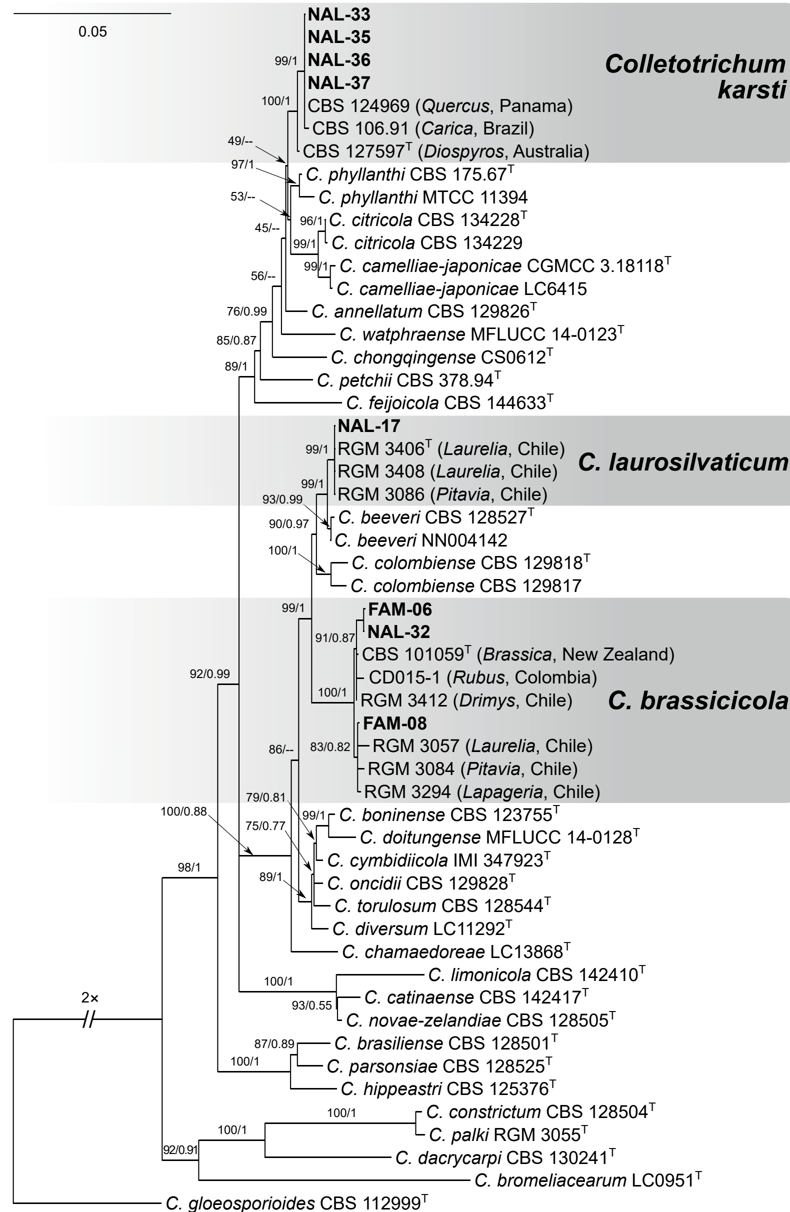

Figure 4. Maximum likelihood phylogenetic analysis of the *Colletotrichum boninense* species complex (CBSC). Chilean isolates are shown in bold font, and ex-type strains are each accompanied with a superscript T. Maximum likelihood bootstrap values and Bayesian posterior probabilities are indicated above the tree branches. The tree was inferred from a data set consisting of sequences of three DNA markers (ITS, *gapdh*, and *tub2*) and was rooted with *C. gloeosporioides*. Scale bar = substitutions per site.

pled sites. These isolates were morphologically diverse and indistinguishable from *C. jiangxiense* or *C. fructicola*, but clustered phylogenetically with strains reported as *C. cigarro* from avocado and mango. Their genetic divergence from the type strain of *C. cigarro* suggests they may represent a distinct, undescribed species, a hypothesis that requires further taxonomic investigation. The second most common species was *C. pyricola* (CASC), previously reported on various hosts in Australia, New Zealand, and Chile (Damm *et al*., 2012a; Zapata and Opazo, 2017). Its detection on avocado represents a new host record, and indicates a wider distribution in Chile than previously recognized, supporting the hypothesis by Zapata *et al*., (2024) of its endemic origin in southern South America.

Other notable species included *C. gloeosporioides* and *C. jiangxiense*, both members of the CGSC. The present study provides the first molecular confirmation of *C. gloe-*

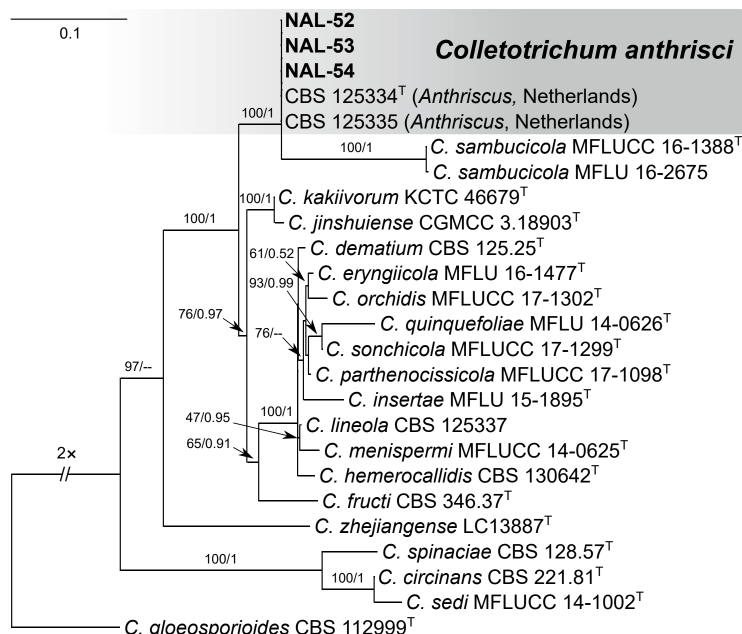

Figure 5. Maximum likelihood phylogenetic analysis of the *Colletotrichum dematium* species complex (CDSC). Chilean isolates are shown in bold font, and ex-type strains are each accompanied with a superscript T. Maximum likelihood bootstrap values and Bayesian posterior probabilities are indicated above the tree branches. The tree was inferred from a data set consisting of sequences of five DNA markers (ITS, *gapdh*, *tub2*, *act*, and *chs1*) and was rooted with *C. gloeosporioides*. Scale bar = substitutions per site.

Table 1. Isolate frequencies and geographical origins for *Colletotrichum* species associated with avocado anthracnose in Chile.

Species complex and species	Frequency of isolates (*n*)				
	Metropolitan Region	Valparaiso Region	O'Higgins Region	Total	Relative frequency (%)
C. gloeosporioides species complex (CGSC)					
C. cf. *cigarro*	9	6	2	17	34.0
C. gloeosporioides	6	0	0	6	12.0
C. jiangxiense	2	1	2	5	10.0
C. fructicola	1	0	0	1	2.0
C. perseae	1	0	0	1	2.0
C. acutatum species complex (CASC)					
C. pyricola	5	0	4	9	18.0
C. boninense species complex (CBSC)					
C. karsti	4	0	0	4	8.0
C. brassicicola	3	0	0	3	6.0
C. laurosilvaticum	1	0	0	1	2.0
C. dematium species complex (CDSC)					
C. anthrisci	3	0	0	3	6.0
Total	35	7	8	50	100.0

osporioides on avocado in Chile, clarifying previous morphology-based reports (Morales *et al*., 1979; Montealegre *et al*., 2002). Conversely, *C. jiangxiense*, previously reported only in Mexico on avocado (Ayvar-Serna *et al*., 2021), is here documented for the first time in Chile, extending the known geographical range of this pathogen.

Within the CBSC, *C. karsti*, *C. brassicicola*, and *C. laurosilvaticum* were identified at low frequencies.

Table 2. GenBank accession numbers and sampling sites of *Colletotrichum gloeosporioides* species complex (CGSC) isolates obtained from avocado anthracnose in Chile and included in phylogenetic analyses.

Species	Isolate	Sampling site	Location, region	GenBank accession number				
				ITS	*gapdh*	*tub2*	ApMat	*gs*
C. cf. *cigarro*	FAM-02	Non-commercial grove	Isla de Maipo, Metropolitan	PQ167786	PQ178841	PQ195572	PQ217744	PQ217774
C. cf. *cigarro*	FAM-05	Non-commercial grove	Isla de Maipo, Metropolitan	PQ167787	PQ178842	PQ195573	PQ217745	PQ217775
C. cf. *cigarro*	MER-11	Local market	Santiago, Metropolitan	PQ167788	PQ178843	PQ195574	PQ217746	PQ217776
C. cf. *cigarro*	NAL-05	Commercial grove	Isla de Maipo, Metropolitan	PQ167789	PQ178844	PQ195575	PQ217747	PQ217777
C. cf. *cigarro*	NAL-13	Commercial grove	Isla de Maipo, Metropolitan	PQ167790	PQ178845	PQ195576	PQ217748	PQ217778
C. cf. *cigarro*	NAL-14	Commercial grove	Isla de Maipo, Metropolitan	PQ167791	PQ178846	PQ195577	PQ217749	PQ217779
C. cf. *cigarro*	NAL-20	Commercial grove	Isla de Maipo, Metropolitan	PQ167792	PQ178847	PQ195578	PQ217750	PQ217780
C. cf. *cigarro*	NAL-42	Commercial grove	Isla de Maipo, Metropolitan	PQ167793	PQ178848	PQ195579	PQ217751	PQ217781
C. cf. *cigarro*	NAL-47	Commercial grove	Isla de Maipo, Metropolitan	PQ167794	PQ178849	PQ195580	PQ217752	PQ217782
C. cf. *cigarro*	PEU-01	Commercial grove	Peumo, O'Higgins	PQ167795	PQ178850	PQ195581	PQ217753	PQ217783
C. cf. *cigarro*	PEU-13	Commercial grove	Peumo, O'Higgins	PQ167796	PQ178851	PQ195582	PQ217754	PQ217784
C. cf. *cigarro*	SDO-01	Commercial grove	Santo Domingo, Valparaíso	PQ167797	PQ178852	PQ195583	PQ217755	PQ217785
C. cf. *cigarro*	SDO-02	Commercial grove	Santo Domingo, Valparaíso	PQ167798	PQ178853	PQ195584	PQ217756	PQ217786
C. cf. *cigarro*	SDO-14	Commercial grove	Santo Domingo, Valparaíso	PQ167799	PQ178854	PQ195585	PQ217757	PQ217787
C. cf. *cigarro*	SDO-26	Commercial grove	Santo Domingo, Valparaíso	PQ167800	PQ178855	PQ195586	PQ217758	PQ217788
C. cf. *cigarro*	SDO-39	Commercial grove	Santo Domingo, Valparaíso	PQ167801	PQ178856	PQ195587	PQ217759	PQ217789
C. cf. *cigarro*	SDO-50	Commercial grove	Santo Domingo, Valparaíso	PQ167802	PQ178857	PQ195588	PQ217760	PQ217790
C. fructicola	MER-06	Local market	Santiago, Metropolitan	PQ167773	PQ178828	PQ195559	PQ217731	PQ217761
C. gloeosporioides	FAM-01	Non-commercial grove	Isla de Maipo, Metropolitan	PQ167774	PQ178829	PQ195560	PQ217732	PQ217762
C. gloeosporioides	FAM-03	Non-commercial grove	Isla de Maipo, Metropolitan	PQ167775	PQ178830	PQ195561	PQ217733	PQ217763
C. gloeosporioides	FAM-07	Non-commercial grove	Isla de Maipo, Metropolitan	PQ167776	PQ178831	PQ195562	PQ217734	PQ217764
C. gloeosporioides	FAM-16	Non-commercial grove	Isla de Maipo, Metropolitan	PQ167777	PQ178832	PQ195563	PQ217735	PQ217765
C. gloeosporioides	MER-07	Local market	Santiago, Metropolitan	PQ167778	PQ178833	PQ195564	PQ217736	PQ217766
C. gloeosporioides	MER-09	Local market	Santiago, Metropolitan	PQ167779	PQ178834	PQ195565	PQ217737	PQ217767
C. jiangxiense	NAL-03	Commercial grove	Naltahua, Metropolitan	PQ167780	PQ178835	PQ195566	PQ217738	PQ217768
C. jiangxiense	NAL-18	Commercial grove	Naltahua, Metropolitan	PQ167781	PQ178836	PQ195567	PQ217739	PQ217769
C. jiangxiense	PEU-4A	Commercial grove	Peumo, O'Higgins	PQ167782	PQ178837	PQ195568	PQ217740	PQ217770
C. jiangxiense	PEU-14	Commercial grove	Peumo, O'Higgins	PQ167783	PQ178838	PQ195569	PQ217741	PQ217771
C. jiangxiense	SDO-38	Commercial grove	Santo Domingo, Valparaíso	PQ167784	PQ178839	PQ195570	PQ217742	PQ217772
C. perseae	NAL-19	Commercial grove	Isla de Maipo, Metropolitan	PQ167785	PQ178840	PQ195571	PQ217743	PQ217773

Table 3. GenBank accession numbers and sampling sites of isolates of the *Colletotrichum acutatum* (CASC) and *C. boninense* (CBSC) species complexes obtained from avocado anthracnose in Chile and included in phylogenetic analyses.

Species	Species complex	Isolate	Sampling site	Location, region	GenBank accession number		
					ITS	*gapdh*	*tub2*
C. brassicicola	CBSC	FAM-06	Non-commercial grove	Isla de Maipo, Metropolitan	PQ167805	PQ195525	PQ195542
C. brassicicola	CBSC	FAM-08	Non-commercial grove	Isla de Maipo, Metropolitan	PQ167806	PQ195526	PQ195543
C. brassicicola	CBSC	NAL-32	Commercial grove	Isla de Maipo, Metropolitan	PQ167807	PQ195527	PQ195544
C. karsti	CBSC	NAL-33	Commercial grove	Isla de Maipo, Metropolitan	PQ167808	PQ195528	PQ195545
C. karsti	CBSC	NAL-35	Commercial grove	Isla de Maipo, Metropolitan	PQ167809	PQ195529	PQ195546
C. karsti	CBSC	NAL-36	Commercial grove	Isla de Maipo, Metropolitan	PQ167810	PQ195530	PQ195547
C. karsti	CBSC	NAL-37	Commercial grove	Isla de Maipo, Metropolitan	PQ167811	PQ195531	PQ195548
C. laurosilvaticum	CBSC	NAL-17	Commercial grove	Isla de Maipo, Metropolitan	PQ167812	PQ195532	PQ195549
C. pyricola	CASC	FAM-09	Non-commercial grove	Isla de Maipo, Metropolitan	PQ167813	PQ195533	PQ195550
C. pyricola	CASC	FAM-11	Non-commercial grove	Isla de Maipo, Metropolitan	PQ167814	PQ195534	PQ195551
C. pyricola	CASC	FAM-14	Non-commercial grove	Isla de Maipo, Metropolitan	PQ167815	PQ195535	PQ195552
C. pyricola	CASC	FAM-20	Non-commercial grove	Isla de Maipo, Metropolitan	PQ167816	PQ195536	PQ195553
C. pyricola	CASC	FAM-23	Non-commercial grove	Isla de Maipo, Metropolitan	PQ167817	PQ195537	PQ195554
C. pyricola	CASC	PEU-02	Commercial grove	Peumo, O'Higgins	PQ167818	PQ195538	PQ195555
C. pyricola	CASC	PEU-4B	Commercial grove	Peumo, O'Higgins	PQ167819	PQ195539	PQ195556
C. pyricola	CASC	PEU-05	Commercial grove	Peumo, O'Higgins	PQ167820	PQ195540	PQ195557
C. pyricola	CASC	PEU-06	Commercial grove	Peumo, O'Higgins	PQ167821	PQ195541	PQ195558

Table 4. GenBank accession numbers and sampling sites of *Colletotrichum anthrisci* isolates obtained from avocado anthracnose in Chile included in phylogenetic analyses.

Species	Isolate	Sampling site	Location, region	GenBank accession number				
				ITS	*gapdh*	*tub2*	*act*	*chs1*
C. anthrisci	NAL-52	Commercial grove	Isla de Maipo, Metropolitan	MN203633	MN207466	MN207160	OM055666	OM037442
C. anthrisci	NAL-53	Commercial grove	Isla de Maipo, Metropolitan	MN203634	MN207467	MN207161	OM055667	OM037443
C. anthrisci	NAL-54	Commercial grove	Isla de Maipo, Metropolitan	MN203635	MN207468	MN207162	OM055668	OM037444

Table 5. Conidial dimensions of isolates of *Colletotrichum* spp. associated with avocado anthracnose in Chile compared to reference strains.

Species	Isolate/strain[a]	Conidial size (µm) (L × W)[b]	Mean (µm) (L × W)	Mean L/W ratio	Reference
C. anthrisci	CBS 125334^{T}	(22.0–)23.9–26.9(–28.5) × (3.0–)3.3–3.7(–4.0)	26.3 × 3.4	7.8	Damm *et al.* (2009)
C. anthrisci	NAL-53	(20.0–)22.0–25.3(–27.5) × (2.5–)2.3–3.1(–3.8)	23.6 × 2.7	8.7	This study
C. brassicicola	CBS 101059^{T}	(9.0–)11.4–13.4(–14.5) × (5.0–)5.3–5.9(–6.0)	12.2 × 5.6	2.2	Damm *et al.* (2012b)
C. brassicicola	FAM-06	(11.0–)11.8–12.3(–12.2) × (4.5–)4.9–5.2(–5.4)	12.0 × 5.0	2.4	This study
C. cigarro	ICMP 18534	(11.0–)12.4–14.5(–16.0) × (3.0–)3.5–4.5(–6.0)*	13.4 × 4.2	3.3	Cabral *et al.* (2020)
C. cigarro	ICMP 18539^{T}	(12.0–)16.0–19.5(–29.0) × (4.5–)5.0(–8.0)*	17.8 × 5.1	3.5	Weir *et al.* (2012)
C. cf. *cigarro*	ICMP 12953	(10.5–)13.0–14.4(–15.5) × (5.0–)5.5–6.6(–6.0)*	13.4 × 5.6	2.4	Cabral *et al.* (2020)
C. cf. *cigarro*	FAM-05	(14.0–)16.1–18.4(–20.0) × (4.0–)4.4–6.3(–7.0)	16.8 × 5.3	3.2	This study
C. cf. *cigarro*	MER-11	(12.0–)13.4–17.0(–20.0) × (5.0–)5.1–6.1(–6.0)	15.2 × 5.6	2.7	This study
C. cf. *cigarro*	NAL-05	(13.0–)14.3–16.7(–18.0) × (5.0–)4.8–6.0(–7.0)	15.4 × 5.2	3.0	This study
C. cf. *cigarro*	NAL-13	(15.0–)15.8–18.3(–20.0) × (5.0–)5.7–7.1(–7.0)	16.9 × 6.4	2.6	This study
C. cf. *cigarro*	NAL-42	(13.0–)14.2–16.6(–18.0) × (4.0–)4.9–6.0(–6.0)	15.4 × 5.4	2.9	This study
C. cf. *cigarro*	PEU-13	(14.0–)15.0–17.5(–19.0) × (5.0–)4.8–5.6(–6.0)	16.2 × 5.2	3.1	This study
C. cf. *cigarro*	PR432	(11.0–)12.5–14.0(–15.5) × (4.5–)5.5–6.3(–7.0)*	13.3 × 5.9	2.3	Cabral *et al.* (2020)
C. cf. *cigarro*	SDO-01	(15.0–)17.2–20.1(–21.0) × (5.0–)5.0–6.4(–7.0)	18.3 × 5.7	3.2	This study
C. cf. *cigarro*	SDO-02	(13.0–)14.2–16.4(–18.0) × (4.0–)4.9–6.0(–6.0)	15.3 × 5.5	2.8	This study
C. cf. *cigarro*	SDO-14	(14.0–)14.7–16.4(–18.0) × (4.0–)5.0–6.7(–7.0)	15.5 × 5.8	2.7	This study
C. cf. *cigarro*	SDO-26	(14.0–)14.8–17.0(–18.0) × (5.0–)4.8–5.7(–6.0)	15.9 × 5.2	3.1	This study
C. cf. *cigarro*	SDO-39	(14.0–)15.0–17.4(–19.0) × (5.0–)5.1–6.2(–7.0)	16.2 × 5.6	2.9	This study
C. cf. *cigarro*	SDO-50	(13.0–)15.0–19.3(–21.0) × (5.0–)5.0–6.5(–7.0)	17.2 × 5.7	3.0	This study
C. fructicola	ICMP 18581^{T}	(9.7–)10.5–12.6(–14.0) × (3.0–)3.2–3.9(–4.3)	11.4 × 3.5	3.3	Prihastuti *et al.* (2009)
C. fructicola	MER-06	(14.0–)14.1–14.8(–15.0) × (4.5–)4.6–5.0(–5.0)	14.4 × 4.8	3.0	This study
C. gloeosporioides	LC3312	(11.0–)12.3–14.7(–15.5) × (4.5–)5.2–5.8(–6.0)	13.5 × 5.5	2.5	Liu *et al.* (2015)
C. gloeosporioides	MER-07	(19.0–)19.4–20.5(–22.0) × (4.8–)4.9–5.0(–5.0)	19.9 × 5.0	4.0	This study
C. gloeosporioides	PR411	(17.8–)19.5(–21.6) × (4.8–)6.1(–6.9)	19.5 × 6.1	3.2	Ramos *et al.* (2016)
C. jiangxiense	CGMCC 3.17363^{T}	(13.0–)14.2–16.2(–19.0) × (4.0–)4.8–5.6(–6.0)	15.2 × 5.2	2.9	Liu *et al.* (2015)
C. jiangxiense	NAL-03	(12.0–)13.7–16.7(–18.0) × (6.0–)5.9–7.5(–8.0)	15.2 × 6.7	2.3	This study
C. karsti	CBS 127597^{T}	(12.0–)12.9–15.1(–16.5) × (5.5–)5.4–6.0(–6.5)	13.1 × 5.8	2.2	Damm *et al.* (2012b)
C. karsti	NAL-33	(14.5–)14.8–15.0(–15.0) × (5.0–)5.4–7.0(–7.0)	14.9 × 6.2	2.4	This study
C. laurosilvaticum	NAL-17	(12.3–)12.7–13.9(–14.0) × (6.0–)6.5–7.1(–7.0)	13.3 × 6.8	2.0	This study
C. laurosilvaticum	RGM 3406^{T}	(13.5–)12.9–14.3(–16.5) × (5.0–)6.1–6.7(–6.5)	13.6 × 6.4	2.1	Zapata *et al.* (2024)
C. perseae	CBS 141365^{T}	(13.0–)15.7(–19.0) × (4.0–)5.2(–6.5)	15.7 × 5.2	3.0	Sharma *et al.* (2017)
C. perseae	NAL-19	(15.0–)17.2–20.1(–21.0) × (5.0–)5.0–6.4(–7.0)	18.6 × 5.7	3.3	This study
C. pyricola	CBS 128531^{T}	(9.5–)13.8–17.0(–18.5) × (4.0–)4.4–5.2(–5.5)	15.4 × 4.8	3.2	Damm *et al.* (2012a)
C. pyricola	PEU-02	(14.2–)14.5–15.5(–16.2) × (4.5–)4.7–5.1(–5.2)	15.0 × 4.9	3.1	This study

[a] Type strains are highlighted in bold and a superscript T.
[b] L × W = length by width, data represents (minimum–) average–standard deviation [SD] – average+SD (–maximum).
Data marked with an asterisk (*) represents (minimum–) first quartile – third quartile (–maximum).

While *C. karsti* has been previously reported on avocado (Damm *et al.*, 2012b), this study provides the first records of *C. brassicicola* and *C. laurosilvaticum* on avocado, thereby expanding their known host ranges and distribution to central Chile (Zapata *et al.*, 2024). The species *C. anthrisci* (CDSC) was also recovered at low frequency, supporting its limited epidemiological relevance, in agreement with previous observations (Rose and Damm, 2024). Likewise, *C. fructicola* and *C. perseae* (CGSC) were detected only sporadically, extending their known geographical distributions to Chile yet suggesting that both species constitute minor and likely incidental components of the local pathogen composition.

The *Colletotrichum* identification results obtained in this study are consistent with previous reports of these pathogens on avocado and other hosts (Diao *et al.*, 2017; Sharma *et al.*, 2017; Armand and Jayawardena, 2024). Multilocus phylogenetic analyses were essential for accu-

Table 6. Mean lesion diameters (mm) caused by *Colletotrichum* species inoculated into healthy avocado fruits (cv. Hass) after 7 d at 20°C. Means accompanied by the same letter are not significantly different ($P > 0.05$) according to Fisher's LSD test.

Species	Isolate	Mean lesion diameter (mm)	Standard error	LSD test
C. fructicola	MER-06	34.05	0.55	a
C. cf. *cigarro*	MER-11	33.03	1.56	a b
C. cf. *cigarro*	SDO-03	30.93	1.17	b
C. anthrisci	NAL-54	30.30	1.59	b c
C. pyricola	FAM-23	30.08	1.36	b c
C. pyricola	FAM-20	28.32	1.81	b c d
C. gloeosporioides	MER-07	27.38	0.80	c d
C. jiangxiense	SDO-38	25.83	0.31	d
C. perseae	NAL-19	25.27	0.38	d e
C. jiangxiense	NAL-03	25.05	0.18	d e
C. gloeosporioides	FAM-01	25.05	1.03	d e
C. karsti	NAL-33	24.92	1.23	d e
C. anthrisci	NAL-53	24.35	1.03	d e
C. karsti	NAL-35	24.08	0.62	e
C. brassicicola	NAL-32	22.77	1.09	e f
C. brassicicola	FAM-06	22.70	1.41	e f
C. laurosilvaticum	NAL-17	20.91	0.25	f
Control	n/a	0.09	0.10	g

rate species delimitation. Concatenated ITS, *gapdh,* and *tub2* sequences resolved species within CASC and CBSC, as reported previously (Velho *et al*., 2015; Khodadadi *et al*., 2020). By contrast, in the CDSC, incorporation of *act* and *chs1* was required to resolve *C. anthrisci*, consistent with previous studies (Lee and Jung, 2018; Fu *et al*., 2019). Similarly, in the CGSC, inclusion of ApMat and *gs* was necessary to separate closely related taxa (Liu *et al*., 2015). Chilean isolates identified as *C.* cf. *cigarro* clustered with strains reported as *C. cigarro* from avocado in New Zealand and mango in Portugal, whereas the type strain grouped separately with isolates from other hosts in Germany and New Zealand. These results support previous observations that isolates identified as *C. cigarro* do not form a monophyletic clade, and may represent multiple species (Silva *et al*., 2012a; Doyle *et al*., 2013; Vieira *et al*., 2018; Cabral *et al*., 2020; Kreth *et al*., 2025). Additionally, Conidia of Chilean isolates of *C.* cf. *cigarro* were generally longer than those measured by Cabral *et al*., (2020), and although they overlapped with the type strain, statistically significant differences in conidium size were detected among isolates (Figure S1). Collectively, these results suggest that the Chilean isolates, along with strains ICMP 12952, ICMP 12953 and PR432, may constitute a new species that requires formal taxonomic evaluation. From a morphological perspective, conidium features of representative isolates from all identified species were consistent with type strain descriptions, although size variation was expected given the known overlap across species (Damm *et al*., 2009; 2012a; 2012b; Weir *et al*., 2012).

Pathogenicity tests confirmed all ten species as causal agents of avocado anthracnose. Isolates from the CGSC and CASC exhibited greater virulence than those from CBSC and CDSC, consistent with previous studies on avocado and other hosts (Munir *et al*., 2016; Sharma *et al*., 2017; Oo *et al*., 2018; Fuentes-Aragón *et al*., 2020; Wu *et al*., 2023). Variation in virulence among species complexes, species, and isolates highlights the importance of species-level identification for disease management (Guarnaccia *et al*., 2017; Chung *et al*., 2020; Riolo *et al*., 2021; Camiletti *et al*., 2022).

In conclusion, the present study documents an unprecedented diversity of *Colletotrichum* species associ-

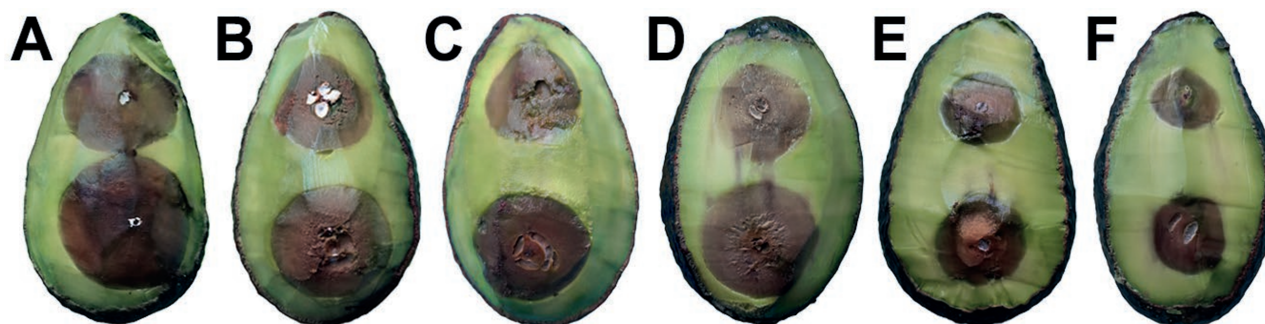

Figure 6. Pathogenicity tests showing internal lesions caused by the most frequently isolated *Colletotrichum* species with differential virulences on cv. 'Hass' avocado fruits at 7 d after inoculations. A and B, the most virulent species. C and D, moderately virulent species. E and F, least virulent species. A, *Colletotrichum* cf. *cigarro* isolate SDO-03. B, *C. pyricola* isolate FAM-23. C, *C. anthrisci* isolate NAL-54. D, *C. gloeosporioides* isolate MER-07. E, *C. karsti* isolate NAL-35. F, *C. brassicicola* isolate FAM-06.

ated with avocado anthracnose in Chile, including three species reported for the first time on avocado. These results provide information on the most frequent species, extend the known geographic distributions of several pathogens, reveal differences in virulence, and emphasize the need for further taxonomic clarification of *C.* cf. *cigarro*. Further research should address the epidemiology of the most prevalent *Colletotrichum* species to support the development of effective disease management strategies for Chilean avocado production.

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