Author/s:
Mongkolporn, O;Taylor, PWJ

Title:
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Date:
2018-08-01

Citation:
Mongkolporn, O. & Taylor, P. W. J. (2018). Chili anthracnose: Colletotrichum taxonomy and pathogenicity. Plant Pathology, 67 (6), pp.1255-1263. https://doi.org/10.1111/ppa.12850.

DR. ORARAT MONGKOLPORN (Orcid ID : 0000-0002-3530-7913)
PROFESSOR PAUL WILLIAM JAMES TAYLOR (Orcid ID : 0000-0003-3076-2084)

Article type : Review Article

Running head recto: Chili anthracnose pathogen

Running head verso: O. Mongkolporn & P. W. J. Taylor

REVIEW

Chili anthracnose: *Colletotrichum* taxonomy and pathogenicity

O. Mongkolporn[a]* and P. W. J. Taylor[b]

[a]*Department of Horticulture, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Nakhon Pathom, Thailand 73140; and* [b]*Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, VIC, 3010, Australia*

*E-mail: orarat.m@ku.th

Chili anthracnose is caused by *Colletotrichum* species mostly associated with the acutatum, truncatum and gloeosporioides complexes. Since 2009 the *Colletotrichum* taxonomy has been extensively revised based on multigene phylogenetics, which has had a large impact on the number of species known to cause anthracnose disease of chili. This review discusses (i) the taxonomy of *Colletotrichum* spp. infecting chili, and (ii) the impact of *Colletotrichum* pathotypes on breeding for resistance to anthracnose. To date, 24 *Colletotrichum* species have been identified as pathogens of chili anthracnose, with the three main pathogens being *C. scovillei*, *C. truncatum* and *C. siamense*. Identification of several pathotypes within these three *Colletotrichum* species, particularly pathotypes that can overcome resistance in the related *Capsicum* species, *Ca. chinense* and *Ca. baccatum*, will be of major concern to plant

breeders as they develop resistant chili genotypes from the transfer of resistance genes from these *Capsicum* species into *Ca. annuum*. Accurate identification of the *Colletotrichum* species causing anthracnose and improved understanding of the biology of the *Colletotrichum* species and their interaction with the host will enable the application of improved integrated disease management techniques.

Keywords: *Colletotrichum* species, differential host reaction, pathotype, species identification

Introduction

Chili anthracnose is caused by a complex of *Colletotrichum* species. Molecular analyses based on multigene phylogenetics, and pathogenicity bioassays are now standard protocols to identify *Colletotrichum* species. This paper reviews the *Colletotrichum* species that have been reported to be pathogens of chili. Since the introduction of multigene phylogenetic analyses in 2009, the number of *Colletotrichum* species has dramatically increased from 66 to currently over 200 species, and as such the number of *Colletotrichum* species that cause chili anthracnose has also increased. However, this increase is not only due to advancements in methodologies to identify species but also due to more systematic surveys and collections having taken place, particularly in Australia, Southeast Asia and China. Nevertheless, species identification can still be hindered by problems with sequence integrity and compliance with international protocols on naming new species, which is also discussed in the review.

The assessment of pathogenicity of *Colletotrichum* species infecting chili are mostly performed on detached chili fruit, which are either pre- or nonwounded before inoculation. Wounding involves the breaking of the cuticle and epidermal cells of the fruit and hence disregards the importance of these tissues as the first barrier of defence to pathogen infection. The issue of a fruit wounding bioassay to determine pathogenicity and in turn host resistance is also discussed in this review. The chili fruit bioassay is a key step in screening chili genotypes for anthracnose resistance. *Capsicum annuum* is the most important *Capsicum* species worldwide but lacks resistance. However, immune resistance to anthracnose has been identified in *Ca. chinense* and *Ca. baccatum* by the World Vegetable Center (WorldVeg; formerly the Asian Vegetable Research and Development Center (AVRDC); Mongkolporn & Taylor, 2011). Pathogenicity studies of *Colletotrichum* species on differential host genotypes are important for understanding host–pathogen interactions, especially with resistant chili

genotypes. The review concludes with an overview of the importance of identifying pathotypes of the most widespread and pathogenic *Colletotrichum* species for resistance breeding.

Taxonomy of *Colletotrichum* species infecting chili

The genus *Colletotrichum* (Sordariomycetes, Ascomycota) infects over 3000 species of herbaceous and woody crops worldwide (O'Connell *et al*., 2012). *Colletotrichum* was first reported in 1790 as *Vermicularia*, and the name *Colletotrichum* was introduced in 1831 as reported in Hyde *et al*. (2009b). Recently, *Colletotrichum* was ranked the eighth most important pathogenic fungal genus in the world (Dean *et al*., 2012).

In the past, the identification of *Colletotrichum* species was problematic because of uncritical use of species names based on the assumptions of host specificity (Cannon *et al*., 2012). All species were incorrectly assumed to be host specific, which led to a huge number of described taxa. Species misidentification causes complications in (i) understanding host pathogen relationships, (ii) developing effective control strategies, (iii) establishing cost effective quarantine programmes (De Silva *et al*. 2017b), and (iv) breeding for resistance. Conventionally, species identification has relied on conidia and appressoria morphology, presence of setae, presence of sclerotia, teleomorphic state and mycelial culture characteristics. However, morphological characteristics are limited by large variations within species and in the rare teleomorphic stage.

The first molecular applications to distinguish *Colletotrichum* species based on comparative ITS rDNA gene sequences were reported in 1992 (Mills *et al*., 1992; Sreenivasaprasad *et al*., 1992). Since these early reports, sequences of fungal genes have been used to develop multigene phylogenetic analyses for the revision of the taxonomy of *Colletotrichum* species. The polyphasic approach (Cai *et al*., 2009) that includes multigene phylogenetic analyses and culture characteristics is now used as the basis to describe species of *Colletotrichum*. Genes currently used in *Colletotrichum* species identification are dependent on species complexes (Hyde *et al*., 2009a; Cannon *et al*., 2012). Genes summarized by Marin-Felix *et al*. (2017) included ITS rDNA (ribosomal internal transcribed spacer), *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase), *CHS1* (chitin synthase 1), *ACT* (actin-like protein), *HIS3* (histone 3), *TUB2* (β-tubulin), *GS* (glutamine synthetase), *CAL*

(calmodulin), *SOD2* (manganese superoxide dismutase), *APN2* (DNA lyase 2) and *ApMat*; (MAT1/APN2 mating type gene/DNA lyase 2). Recent molecular analyses of *Colletotrichum* have identified over 200 species (Marin-Felix *et al*., 2017).

The three key species of chili anthracnose that had been widely reported in Southeast Asia, originally identified as *C. capsici*, *C. gloeosporioides* and *C. acutatum* (Than *et al*., 2008; Montri *et al*., 2009; Mongkolporn *et al*., 2010), were reclassified as *C. truncatum* (Damm *et al*., 2009; Ranathunge *et al*., 2012), *C. siamense* and *C. scovillei* (Damm *et al*., 2012; De Silva *et al*., 2017a), respectively. Hence, *C. capsici* is no longer a valid name. *Colletotrichum acutatum sensu lato* has been identified as a pathogen of *Capsicum annuum* but only in Sri Lanka (Damm *et al*., 2012), while *C. gloeosporioides sensu lato* has been identified causing anthracnose of chili in China and India (Liu *et al*., 2016; Diao *et al*., 2017; Katoch *et al*., 2017). Morphological characteristics of these three main *Colletotrichum* species that cause chili anthracnose are described in Table 1.

A recent extensive study conducted in China (Diao *et al*., 2017) collected isolates from chili from 50 locations in 29 eastern provinces. Multigene phylogenetic analysis identified 14 *Colletotrichum* species in China with *C. fioriniae*, *C. fructicola*, *C. gloeosporioides*, *C. scovillei* and *C. truncatum* being the most prominent. *Colletotrichum conoides*, *C. grossum* and *C. liaoningense* were newly described.

The current status of *Colletotrichum* species causing chili anthracnose in various countries, based on pathogenicity and phylogenetic analyses of available ITS and *TUB2* gene sequences, are shown in Tables 2 and 3, and Figure 1. A total of 24 species have been identified, seven of which belong to the acutatum complex, and nine to the gloeosporioides complex.

Multigene phylogenetic analysis has had an impact on the revision of the nomenclature of the *Colletotrichum* species infecting chili. De Silva *et al*. (2017a) reported that the major *Colletotrichum* species causing chili anthracnose in Australia belonged to the species complexes of acutatum, gloeosporioides and truncatum. The study also identified a new species *C. cairnsense*, which was grouped within the acutatum complex that also contained the chili anthracnose pathogens *C. scovillei* and *C. simmondsii*. *Colletotrichum scovillei* has yet to be identified in Australia; thus, this species poses a biosecurity risk to *Capsicum* production. Conversely, *C. cairnsense* has yet to be identified outside of Australia. The chili anthracnose pathogens in the gloeosporioides complex comprised *C. siamense* and

C. queenslandicum. These two species were first reported in Australia as pathogens of advocado, *Pistacia* (*C. siamense*) and papaya (*C. queenslandicum*) (Weir *et al*., 2012).

Colletotrichum aenigma in Diao *et al*. (2017) was excluded from the phylogenetic analysis, because the gene sequences for isolate GAU26, i.e. KP145439 (ITS) and KP145467 (*TUB2*), were identified as *C. gloeosporioides* in Table 2a of Diao *et al*. (2017), and in GenBank. Although GAU26 was reported to be a pathogen of chili in China and belonged to a clade with the type species of *C. aenigma* in the phylogenetic tree, further verification of the gene sequences is required. *Colletotrichum sichuanensis* was also omitted from the analysis because a type specimen is missing for the isolate described in Liu *et al*. (2016), and therefore this species is invalid according to section 8.4 of the International Code for Nomenclature of Algae, Fungi, and Plants (Melbourne Code) (http://www.iapt-taxon.org/nomen/main.php). The ITS and *TUB2* sequences for *C. coccodes* used in the phylogenetic analysis were from a *Capsicum* species isolated from Serbia (Liu *et al.*, 2013), because the *TUB2* sequences for the Indian isolates (Katoch *et al.*, 2017) were inconsistent and could not be used in the concatenated tree. Both reports of *C. cliviae* from *Capsicum* sp. in China (Diao *et al*., 2017) and India (Saini *et al*., 2017) did not include an ITS sequence, hence this species was omitted from the two-gene phylogenetic tree.

The most widespread and commonly reported *Colletotrichum* species causing anthracnose in chili throughout Southeast Asia and South America are *C. truncatum*, *C. siamense* and *C. scovillei* (P. W. J. Taylor, unpublished observations).

Symptoms of chili anthracnose and *Colletotrichum* lifestyles

Chili anthracnose develops during the wet season in the tropics and subtropics around the world, especially in Asian and tropical American countries, reducing chili production. *Colletotrichum* spp. can infect chili plants at all stages of development, with the fruit being more severely infected. In field grown plants, typical anthracnose symptoms are usually found on ripe chili fruit as sunken necrotic lesions with concentric rings of black acervuli (Fig. 2). Anthracnose can also be considered as a seedborne disease because seed in chili fruit heavily infected by *C. truncatum* becomes infected, hence seed infection is an important part of the life cycle of the pathogen (Ranathunge *et al*., 2012). Seed infection causes pre- and post-emergence death of the seedlings after germination. In addition, leaves can be infected

but in young leaves the pathogens enter a quiescent stage. The infected leaves remain healthy due to a suppressed fungal growth until the leaves start to senesce, at which time the anthracnose symptoms develop. The infected senesced leaves subsequently serve as a primary source of inoculum for infection of the fruit and leaves. Chili anthracnose has also been reported as a significant post-harvest disease (Ranathunge *et al*., 2012; Ali *et al*., 2014; De Silva *et al*., 2017a), due to the pathogen's ability to develop a latent or quiescent lifestyle in infected fruit.

Colletotrichum conidia develop in acervuli in fruit lesions and are dispersed by water-splash or wind and then become attached to the leaf and fruit surfaces of a suitable host (De Silva *et al*., 2017b). Under favourable conditions of high leaf and fruit moisture, the conidia germinate and form appressoria normally within 24 h. *Colletotrichum* appressoria then produce infection hyphae (De Silva *et al*., 2017b). For many *Colletotrichum* species, penetration can be promoted by the aid of fungal cutinases, which hydrolyse the plant cuticle. Cutinases were shown to have a major role in the infection of chili fruit by *C. truncatum.* Auyong *et al*. (2015) showed that transgenic lines with reduced expression of cutinase due to mRNAi silencing were not able to infect and cause disease of chili fruit. The cutinase-silenced lines of *C. truncatum* were only able to infect fruit after the cuticle was wounded prior to inoculation.

Once penetration succeeds, the pathogen colonizes the host cells, which then become densely infected, and finally necrotic lesions and hyphae become visible (Gomes *et al*., 2012). Secondary hyphae grow extensively inter- and intracellular across cell walls. Host cuticle collapse eventually occurs after extensive infection. The pathogen then forms a mass of mycelium culminating with cuticle rupture and formation of an acervulus.

Colletotrichum species have complicated lifestyles and thus understanding the pathogens' lifestyle is essential for efficient control of the disease. Different lifestyles of the *Colletotrichum* spp., thoroughly reviewed by De Silva *et al*. (2017b), include necrotrophic, biotrophic, latent or quiescent and endophytic lifestyles. Hemibiotrophy is the most common lifestyle for the major *Colletotrichum* species causing anthracnose of chili (Kim *et al*., 2004; O'Connell *et al*., 2012; Ranathunge *et al*., 2012; De Silva *et al*., 2017b), in which the lifestyle is not truly biotrophic but consists of a short biotrophic phase followed by a necrotrophic stage.

Different *Colletotrichum* species have various degrees of hemibiotrophy due to their lifestyle patterns, which are highly regulated by specific gene families (Gan *et al*., 2016). Hemibiotrophy followed by a very short endophytic phase was identified as the main infection and colonization process for *C. truncatum* infection of chili fruit. Auyong *et al*. (2012) studied colonization of chili fruit with a genetically modified strain of *C. truncatum* that expressed green fluorescent protein (*gfp*) to enable visualization of infection *in situ*. The *C. truncatum*::*gfp* transformant's growth revealed that after initial infection, the hyphae colonized intramurally within the parenchyma tissue of healthy chili fruit without further development of the secondary biotrophic structures. Then after 2 h the infecting hyphae became necrotrophic.

Pathogenicity of *Colletotrichum* infecting chili

In the field situation, natural infection of chili fruit occurs through spore attachment to the cuticle, appressoria formation and direct infection. Wounding of fruit pericarp through abrasion or insect damage also provides a direct entry for infection. Most pathogenicity studies to identify the virulence of *Colletotrichum* species have been performed on chili fruit that had been wounded by puncturing the cuticle and periderm with a needle prior to placement of inoculum consisting of a spore suspension. This type of inoculation bioassay overlooks the role the cuticle plays as a first line of host defence against *Colletotrichum* infection (Ranathunge *et al*., 2012; Auyong *et al*., 2015; De Silva *et al*., 2017b). Very few studies have actually been performed using nonwounding methods. Inoculation of wounded fruit generally results in faster lesion development than in nonwounded fruit (De Silva *et al*., 2017a). However, nonwounding inoculation is important in determining the pathogenicity of new species of *Colletotrichum*. Therefore, a minimum protocol for pathogenicity testing should be a fruit bioassay that involves both wounded and nonwounded fruit before inoculation.

Pathogenic variability is a qualitative characteristic used to measure pathogenic severity. Pathogenicity can be measured qualitatively or quantitatively based on the degree of infection of differential genotypes. Isolates of a species are referred to as pathotypes when a subclass or group of isolates can be differentiated from others of the same species by the level of virulence on a specific host genotype (Taylor & Ford, 2007).

Montri *et al*. (2009) and Mongkolporn *et al*. (2010) identified the existence of pathotypes *of Colletotrichum truncatum, C. siamense* and *C. scovillei* based on the qualitative differences in infection of a set of chili species and genotypes. A set of differential chili genotypes with disease scores ranging from 0 to 9 were used to identify pathotypes within three *Colletotrichum* species. The differentiation of host reactions was considered based on qualitative differences of whether the host was infected (scores 1–9) or not infected (score 0). Differential host reactions can also be affected by specific host–pathogen relationships. Within the *Colletotrichum truncatum* population, differential host reactions were found in the *Capsicum chinense* genotypes; while within *Colletotrichum scovillei* pathotypes were differentiated in the *Capsicum baccatum* genotypes. In addition, fruit maturity has been shown to have an important role in differential host reactions (Temiyakul *et al*., 2012), with pathotypes being discriminated by the different fruit maturity (Table 4). Three pathotypes were identified based on the host reactions on ripe fruit and two on mature green fruit within *C. truncatum*. Likewise, one *C. scovillei* pathotype was differentiated from ripe fruit reactions and three from the mature green fruit reactions (Table 5).

Colletotrichum siamense appeared to be the least virulent pathogen compared to *C. truncatum* and *C. scovillei*. Not all *C. siamense* isolates could infect all *Ca. baccatum* genotypes and *Ca. chinense* 'PBC932'. Pathotypes of *C. siamense* were therefore derived from differential reactions of *Ca. chinense* 'C04714', and all genotypes of *Ca. annuum* and *Ca. frutescens* (Table 6). Five pathotypes were identified based on ripe fruit reactions, while six pathotypes were identified from mature green fruit reactions.

In contrast, quantitative differences or levels of aggressiveness based on degrees of infection ranging from low to high on a set of differential chili genotypes were used by Park *et al*. (2009) to record seven pathotypes, while Sharma *et al*. (2005) claimed that 15 pathotypes existed based on quantitative lesion sizes, which were then arbitrarily divided into two reactions, resistant and susceptible. Quantitative disease severity reflects a natural distribution of aggressiveness within a population, ranging from low to high, which according to Taylor & Ford (2007) is not a true measure of pathogenic differences between isolates. Both studies (Sharma *et al*., 2005; Park *et al*., 2009) used a set of differential chili genotypes that expressed different sizes of lesions.

Pathotype identification based on the qualitative differences on chili fruit following Montri *et al*. (2009) and Mongkolporn *et al.* (2010) was found to be in concordance with the

genetic studies of anthracnose resistances in *Ca. chinense* and *Ca. baccatum*. Genetic analyses of the resistances in chili populations derived from *Ca. chinense* and *Ca. baccatum* revealed similar results, in that the resistances were classified based on symptomless fruit, which was an outcome of hypersensitive reaction of the resistance mechanism reported in these two *Capsicum* species (Mahasuk *et al*., 2009a,b). The genetic analyses in all chili populations also supported the fact that the resistances expressed at different fruit maturity stages were controlled by different genes (Mahasuk *et al*., 2009a,b, 2013, 2016; Sun *et al*., 2015). Consequently, fruit bioassays performed at different fruit maturity stages become a basic requirement in chili anthracnose resistance genetic studies and breeding.

Conclusions and remarks

Chili anthracnose is caused by a complex of *Colletotrichum* species. Previously only *C. acutatum*, *C. capsici* and *C. gloeosporioides* were recognised as the causal species. Since 2009, *Colletotrichum* taxonomy has been extensively revised based on multigene phylogenetics, which has had a large impact on the nomenclature of species and also the identification of new species. To date, 24 *Colletotrichum* species infecting chili have been identified; however, the three main species *C. scovillei* (previously identified as *C. acutatum*), *C. truncatum* (syn. *C. capsici*) and *C. siamense* (previously identified as *C. gloeosporioides*) remain the most common pathogenic species. Correct taxonomy is important to help to understand their host–pathogen relationships, develop effective control strategies, and establish cost effective quarantine programmes.

The ultimate goal for sustainable control of chili anthracnose is to breed for durable anthracnose resistance, which should contain different resistance gene loci to a broad range of *Colletotrichum* species and pathotypes. *Colletotrichum* pathotypes should be differentiated based on qualitative differential host reactions (infected versus not infected). Pathotype identification based on qualitative difference of infection on differential chili cultivars with known resistance genes may lead to future *Colletotrichum* race identification as reported in *C. lindemuthianum* infecting bean (*Phaseolus vulgaris*; Mesquita *et al*., 1998; Halvorson *et al*., 2016). Races of *Colletotrichum* species that cause chili anthracnose have never been characterized. A major obstacle for characterization of races in chili anthracnose is the lack of standardization of the resistance assessments (bioassay, measure of and determination of

resistance) between research groups. Knowledge of pathogen races that can overcome specific resistance genes on resistant cultivars would necessitate a better management of the use of these cultivars. Anthracnose resistant genotypes have been identified in two *Capsicum* species including *Ca. chinense* and *Ca. baccatum*. These resistant genotypes are useful in identifying *Colletotrichum* pathotypes because they provide differential reactions to different *Colletotrichum* isolates. Breeding for resistance to races would broaden the resistance base of chili cultivars through gene pyramiding of multiple resistance genes into one cultivar. The establishment of a uniform procedure to identify pathotypes or races of *Colletotrichum* species for anthracnose assessment, covering a standard set of chili host genotypes, and consolidated inoculation and disease evaluation methods would be necessary. Finally, an extensive collaboration among chili breeders and pathologists is encouraged.

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Figure legends

Figure 1 Maximum likelihood consensus tree of the combined ITS and *TUB2* sequence data of the currently accepted species of *Colletotrichum* that cause anthracnose on *Capsicum* spp. Bootstrap support values >50% are indicated at the nodes and branches. The scale bar shows the number of substitutions per nucleotide position. The tree is rooted with *Monilochaetes infuscans* CBS 869.96. GenBank accession numbers are listed in Table 3.

Figure 2 Typical anthracnose symptom on a chili fruit, caused by *Colletotrichum truncatum*.

Table 1 Morphological characteristics and conidial measurements of three common Colletotrichum species causing chili anthracnose

Taxon	Colony characteristics	Growth rate (mm/day)	Conidia		
			Length (µm)	Width (µm)	Shape
C. truncatum[a]	Flat with entire margin, no aerial mycelium, surface buff, covered with olivaceous-grey to iron-grey acervuli, reverse buff to pale olivaceous-grey, conidia in mass whitish, buff to pale saffron	2.8	15.0–20.0	3.5–4.5	Falcate
C. siamense[b]	Cottony, dense greyish white aerial mycelium, pale yellowish to pinkish colony	6.9–8.0	13.6–15.2	4.8–5.0	Cylindrical
C. scovillei[c]	Flat with entire margin, surface covered with short floccose whitish to pale olivaceous grey aerial mycelium, margin rosy buff, reverse rosy buff, olivaceous grey in the centre, conidia in mass salmon	3.3–3.5	14.4–15.0	3.5–4.1	Cylindrical to clavate with one end round, one end ± acute

[a]Damm et al. (2009) & [c]Damm et al. (2012): conidia characteristics based on cultures on synthetic nutrient-poor agar, colony characteristics based on cultures on oatmeal agar.

[b]Weir et al. (2012): cultures on potato dextrose agar.

Table 2 Colletotrichum species that cause anthracnose of chili identified based on multigene phylogenetic analyses, reported countries and pathogenicity based on prewounding (PW) or nonwounding (NW) of fruit

			Pathogenicity[c]	
Major clade[a]	Species	Reported countries[b]	PW	NW
Acutatum	C. acutatum	Sri Lanka[1]	ND	ND
	C. brisbanense	Australia[1]	ND	ND
	C. cairnsense	Australia[2]	Yes	Yes
	C. fioriniae	China[3]	Yes	ND
	C. nymphaeae	India[5], Indonesia[1], Malaysia[4]	Yes	ND
	C. scovillei	Brazil[6,7,20], China[3,8,9], Indonesia[1], Japan[10], Korea[21], Taiwan[11], Thailand[1,12]	Yes	Yes
	C. simmondsii	Australia[2]	Yes	No
Boninense	C. karstii	China[3], India[5,13]	Yes	ND
Gloeosporioides	C. conoides	China[3]	Yes	ND
	C. fructicola	China[3,8], India[5,14]	Yes	ND
	C. gloeosporioides	China[3,8], India[5]	Yes	ND
	C. grossum	China[3]	Yes	ND
	C. kahawae	India[5]	ND	ND
	C. queenslandicum	Australia[2]	Yes	No
	C. siamense	Australia[2], Brazil[6,20], China[3,8], India[14], Thailand[12]	Yes	Yes
	C. tropicale	Brazil	Yes	ND
	C. viniferum	China[3]	Yes	ND

Spaethianum	C. incanum	China[3]	Yes	ND
Truncatum	C. truncatum	Brazil[6], China[3,8], India[5], Pakistan[15], Thailand[12,16,17]	Yes	Yes
—	C. brevisporum	Brazil[6,18,20], China[3,8]	Yes	ND
—	C. cliviae	China[3], India[19]	Yes	ND
—	C. coccodes	India[5], Serbia[22]	ND	ND
—	C. nigrum	Argentina[22]	ND	ND
—	C. liaoningense	China[3]	Yes	ND

[a]Major clades follow De Silva et al. (2017a) and Marin-Felix et al. (2017).

[b]References: [1]Damm et al. (2012); [2]De Silva et al. (2017a); [3]Diao et al. (2017); [4]Nasehi et al. (2016); [5]Katoch et al. (2017); [6]Silva et al. (2017); [7]Caires et al. (2014); [8]Liu et al. (2016); [9]Zhao et al. (2016); [10]Kanto et al. (2014); [11]Liao et al. (2012); [12]Mongkolporn et al. (2010); [13]Saini et al. (2016); [14]Sharma & Shenoy (2014); [15]Tariq et al. (2017); [16]Than et al. (2008); [17]Montri et al. (2009); [18]De Almeida et al. (2017); [19]Saini et al. (2017); [20]De Oliveira et al. (2017); [21]Oo et al. (2017); [22]Liu et al. (2013).

[c]Pathogenicity tests on fresh chili fruit: 'Yes', successful infection; 'No', unsuccessful infection; 'ND', pathogenicity test not determined.

Table 3 Colletotrichum species causing chili anthracnose with their GenBank accession numbers, ITS (ribosomal internal transcribed spacer) and TUB2 (β-tubulin) gene sequences used to construct the maximum likelihood tree in Figure 1

Major clade	Species	Locality	Host	Accession	ITS	TUB2
Acutatum	C. acutatum	Sri Lanka	Capsicum annuum	CBS 144.29	JQ948401	JQ950052
	C. acutatum	Australia	Carica papaya	CBS 112996^{T}	JQ005776	JQ005860
	C. brisbanense	Australia	Ca. annuum	CBS 292.67^{T}	JQ948291	JQ949942
	C. cairnsense	Australia	Ca. annuum	BRIP63642^{T}	KU923672	KU923688
	C. fioriniae	China	Ca. annuum	CAUA24	KP145017	KP145081
	C. fioriniae	USA	Fiorinia sp.	CBS 128517^{T}	JQ948292	JQ949943
	C. nymphaeae	Indonesia	Ca. annuum	CBS 126528	JQ948219	JQ949870
	C. nymphaeae	Netherlands	Nymphaea alba	CBS 515.78^{T}	JQ948197	JQ949848
	C. scovillei	Indonesia	Capsicum sp.	BBA 70349^{T}	JQ948267	JQ949918
	C. simmondsii	Australia	Capsicum sp.	BRIP 63647	KT957917	KT957918
	C. simmondsii	Australia	C. papaya	CBS 122122^{T}	JQ948276	JQ949927
Boninense	C. karstii	China	Ca. annuum	CAUOS1	KP890103	KP890110
	C. karstii	China	Vanda sp.	CBS 132134^{T}	HM585409	HM585428

Gloeosporioides	C. conoides	China	Ca. annuum	CGMCC3.17615^{T}	KP890168	KP890174
	C. fructicola	China	Ca. annuum	CAUG1	KP145416	KP145444
	C. fructicola	Thailand	Coffea arabica	ICMP 18581^{T}	JX010165	JX010405
	C. gloeosporioides	China	Ca. annuum	CAUG2	KP145417	KP145445
	C. gloeosporioides	Italy	Citrus sinensis	IMI 356878^{T}	JX010152	JX010445
	C. grossum	China	Ca. annuum	CGMCC3.17614^{T}	KP890165	KP890171
	C. kahawae	Kenya	C. arabica	ICMP 17816^{T}	JX010231	JX010444
	C. kahawae	India	Ca. annuum	CG217	HQ264180	HG764600
	C. queenslandicum	Australia	Ca. annuum	BRIP 63695	KU923677	KU923693
	C. queenslandicum	Australia	C. papaya	ICMP 1778^{T}	JX010276	JX010414
	C. siamense	Australia	Ca. annuum	BRIP 63701	KU923683	KU923699
	C. siamense	Thailand	C. arabica	ICMP 18578^{T}	JX010171	JX010404
	C. tropicale	Panama	Theobroma cacao	ICMP 18653^{T}	JX010264	JX010407
	C. tropicale	Brazil	Ca. annuum	COUFAL0052	KY319116	KY319107
	C. viniferum	China	Vitis vinifera	GZAAS5.08601^{T}	JN412804	JN412813

	C. viniferum	China	Ca. annuum	CAUG27	KP145440	KP145468
Spaethianum	C. incanum	Canada	Phaseolus vulgaris	ATCC 64682[T]	KC110789	KC110816
	C. incanum	China	Ca. annuum	CAUCT34	KP145641	KP145675
Truncatum	C. truncatum	USA	Phaseolus lunatus	CBS 151.35[T]	GU227862	GU228156
	C. truncatum	Australia	Ca. annuum	UOMCT 4	KU985043	KU985047
—	C. brevisporum	Thailand	Neoregalia sp.	BCC 38876[T]	JN050238	JN050244
	C. brevisporum	Brazil	Ca. annuum	COUFAL0053	KY319117	KY319108
—	C. cliviae	China	Clivia miniata	CBS 125375[T]	GQ485607	GQ849440
	C. cliviae	China	Ca. annuum	CAUOS6	—[a]	KP890115
—	C. coccodes	Netherlands	Solanum tuberosum	CBS 369.75[T]	HM171679	JX546873
	C. coccodes	Serbia	Capsicum sp.	CBS 125342	JX546832[b]	JX546879[b]
—	C. nigrum	Argentina	Capsicum sp.	CBS 169.49[T]	JX546838	JX546885
—	C. liaoningense	China	Ca. annuum	CGMCC3.17616[T]	KP890104	KP890111

[T]Type or epitype isolate for the specific species.

[a]Both reports of C. cliviae from Capsicum sp. in China and India did not include an ITS sequence.

[b]The ITS and TUB2 sequences for C. coccodes were from a Capsicum sp. isolated from Serbia (Liu et al., 2013), as the TUB2 sequences for Indian isolates (Katoch et al., 2017) were inconsistent and could not be used in the concatenated tree.

Table 4 Pathotype identification of Colletotrichum truncatum based on qualitative differential host reactions on mature green and ripe fruit using microinjection inoculation method (modified from Montri et al., 2009; Mongkolporn et al., 2010)

Pathotype		Capsicum annuum 3 genotypes		Capsicum baccatum 3 genotypes		Capsicum chinense PBC932		Capsicum chinense C04714		Capsicum frutescens 2 genotypes	
Ripe	Green	Ripe	Green	Ripe	Green	Ripe	Green	Ripe	Green	Ripe	Green
I	I	Y	Y	N	N	Y	N	Y	Y	Y	Y
II	II	Y	Y	N	N	Y	N	N	N	Y	Y
III	—	Y	—	N	—	N	—	N	—	Y	—

Y, infected; N, not infected; —, not available.

Table 5 Pathotype identification of Colletotrichum scovillei based on qualitative differential host reactions on mature green and ripe fruit using microinjection inoculation method (modified from Mongkolporn et al., 2010)

Pathotype		Capsicum annuum/chinense/ frutescens (all genotypes)		Capsicum baccatum					
				PBC80		PBC81		CA1422	
Ripe	Green	Ripe	Green	Ripe	Green	Ripe	Green	Ripe	Green
I	I	Y	Y	N	Y	Y	Y	Y	Y
—	II	—	Y	—	N	—	Y	—	Y
—	III	—	Y	—	N	—	N	—	Y

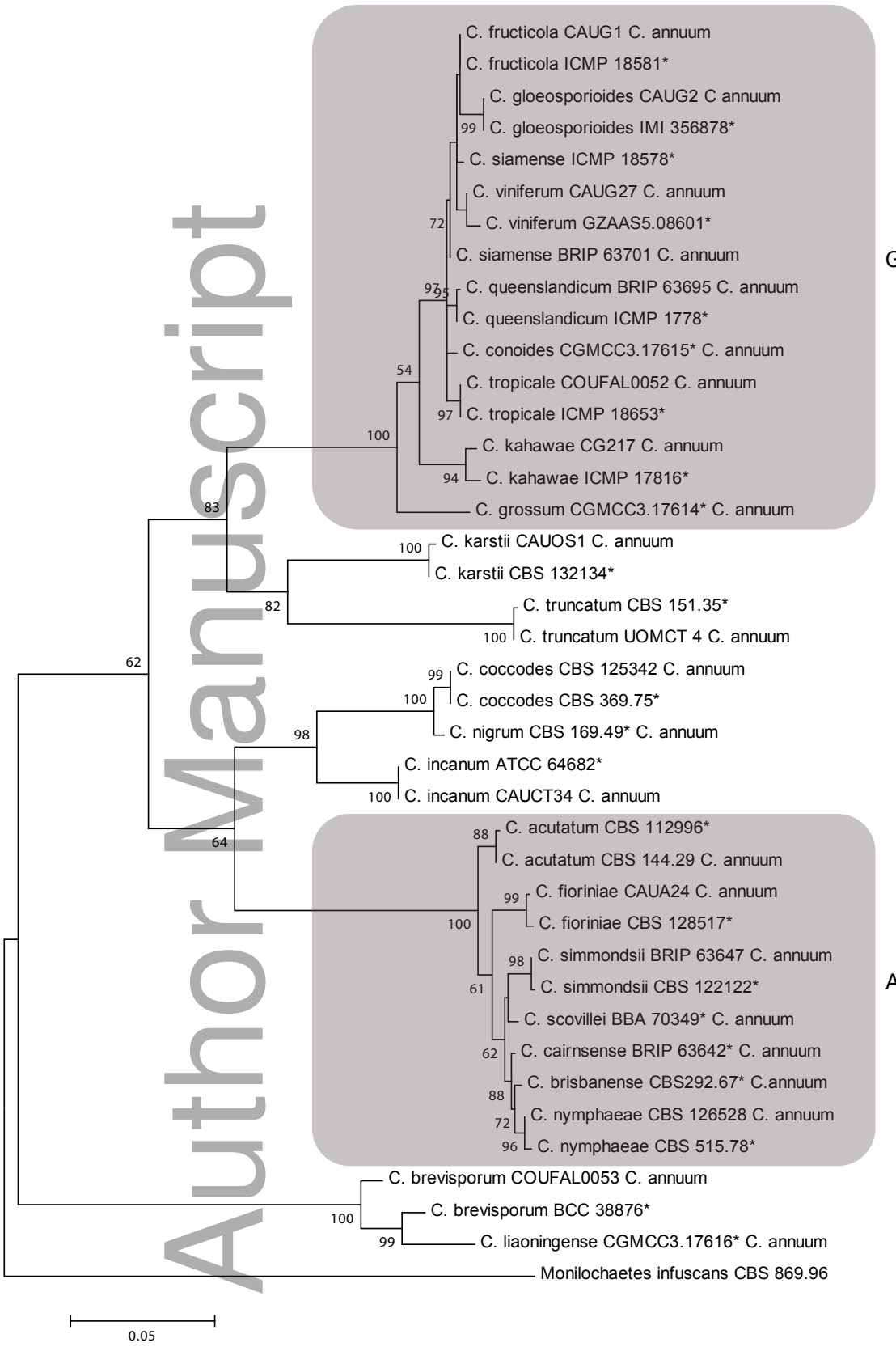
Y, infected; N, not infected; —, not available.

Table 6 Pathotype identification of Colletotrichum siamense based on qualitative differential host reactions on mature green and ripe fruit using microinjection inoculation method (modified from Mongkolporn et al., 2010)

Pathotype		Capsicum chinense		Capsicum annuum						Capsicum frutescens			
		C04714		Jinda		Bangchang		83-168		Khee Noo		Karen	
Ripe	Green	Ripe	Green	Ripe	Green	Ripe	Green	Ripe	Green	Ripe	Green	Ripe	Green
I	I	Y	Y	Y	N	Y	N	Y	Y	Y	Y	Y	Y
II	II	Y	N	N	Y	Y	Y	Y	Y	Y	Y	Y	N
III	III	Y	N	N	N	N	N	Y	Y	Y	Y	Y	Y
IV	IV	N	N	Y	N	N	N	Y	Y	Y	N	Y	Y
V	V	N	N	N	N	Y	N	Y	N	Y	Y	Y	N
—	VI	—	N	—	N	—	N	—	N	—	N	—	Y

Y, infected; N, not infected; —, not available.

Each row presents differential host reaction derived from a pathotype. Pathotypes I (top row) to V (bottom row) were identified on ripe fruit, and pathotypes I (top row) to VI (bottom row) in mature green fruit.



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