Plant Pathology (2017) **66**, 254–267 Doi: 10.1111/ppa.12572

Colletotrichum species associated with chili anthracnose in Australia

D. D. De Silva[a], P. K. Ades[b], P. W. Crous[ac] and P. W. J. Taylor[a]*

[a]*Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Vic., 3010, Australia;* [b]*Faculty of Science, The University of Melbourne, Vic., 3010, Australia; and* [c]*CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT, Utrecht, Netherlands*

Phylogenetic relationships were determined for 45 *Colletotrichum* isolates causing anthracnose disease of chili in Queensland, Australia. Initial screening based on morphology, ITS and *TUB2* genes resulted in a subset of 21 isolates being chosen for further taxonomic study. Isolates in the *C. acutatum* complex were analysed using partial sequences of six gene regions (ITS, *GAPDH*, *ACT*, *CHS-1*, *TUB2* and *HIS3*), and in the *C. gloeosporioides* complex were analysed using four gene regions (ITS, *TUB2*, *ApMat* and *GS*). Phylogenetic analysis delineated four *Colletotrichum* species including *C. siamense*, *C. simmondsii*, *C. queenslandicum*, *C. truncatum* and a new *Colletotrichum* species, described here as *C. cairnsense* sp. nov. This is the first reported association of *C. queenslandicum*, *C. simmondsii* and *C. siamense* with chili anthracnose in Australia; these species were previously associated with anthracnose on papaya and avocado. Furthermore, the dominant species causing anthracnose of chili in Southeast Asia, *C. scovillei*, was not detected in Australia. Inoculations on chili fruit confirmed the pathogenicity of *C. cairnsense* and the other four species in the development of chili anthracnose in Australia.

Keywords: anthracnose, chili, *Colletotrichum cairnsense*, phylogeny, taxonomy

Introduction

Chili (*Capsicum* spp.) is an important vegetable crop and has great popularity as an essential culinary ingredient in many food recipes throughout the world. There are five domesticated chili species including *Capsicum annuum*, *Capsicum chinense*, *Capsicum frutescens*, *Capsicum baccatum* and *Capsicum pubescens* of which *C. annuum* is the most commonly grown commercial species in Southeast Asia and Australia (Mongkolporn & Taylor, 2011). Chili originated from South America and has been mostly grown in tropical and subtropical regions of the world with India being the largest chili producer and exporter followed by China and Peru (FAOSTAT, 2015). Australia also produces chili and capsicum, mainly in the Bowen-Burdekin and Bundaberg regions in Queensland, with 38 913 tonnes cultivated across 1722 ha (ABS, 2014).

Chili anthracnose is a major disease of chili fruit worldwide and causes significant yield loss, as well as reducing the marketability of the fruit. Anthracnose disease of chili is caused by a complex of *Colletotrichum* species, reported as *C. fructicola* and *C. siamense* in India (Sharma & Shenoy, 2014); *C. gloeosporioides* in Korea (Kim *et al.*, 1999), Thailand (Than *et al.*, 2008) and Indonesia (Voorrips *et al.*, 2004); *C. truncatum* in Australia, China, India and Thailand (Sharma *et al.*, 2005; Ranathunge *et al.*, 2012; Diao *et al.*, 2015); *C. acutatum* from almost all chili-growing countries, including China, India, Korea, New Zealand, Sri Lanka, Thailand, the USA and Indonesia (Simmonds, 1968; Harp *et al.*, 2008; Than *et al.*, 2008; Damm *et al.*, 2012); and *C. coccodes* in New Zealand and India (Johnston & Jones, 1997; Sharma *et al.*, 2011; Cannon *et al.*, 2012). The identities of many of these species are uncertain as they have not been confirmed by modern molecular methods.

Recent advances in *Colletotrichum* taxonomy have revealed *C. acutatum* to be a species complex with isolates from infected chili fruit from Thailand being re-identified as *C. scovillei* and from Indonesia as *C. nymphaeae* (Damm *et al.*, 2012). The status of the *C. acutatum* complex from the other chili-producing countries in Southeast Asia remains uncertain. Similarly, the *C. gloeosporioides* complex was shown to contain the chili anthracnose pathogens *C. siamense*, *C. fructicola* and *C. asianum*, which have been reported in Thailand and India (Phoulivong *et al.*, 2012; Sharma & Shenoy, 2014). There have been few studies on chili anthracnose in Australia, with *C. truncatum* and *C. brisbanense* being identified as pathogens of *C. annuum* and *C. frutescens* (Ranathunge *et al.*, 2009; Shivas & Tan, 2009; Damm *et al.*, 2012).

Colletotrichum is one of the most important genera of plant pathogenic fungi, causing disease in many crop plants worldwide (Damm *et al.*, 2012; Phoulivong *et al.*, 2012; Udayanga *et al.*, 2013). Recent taxonomic studies of *Colletotrichum* spp. resulted in the recognition of many new species from different hosts, thus making it

*E-mail: paulwjt@unimelb.edu.au

Published online 25 July 2016

more important to confirm the status of *Colletotrichum* taxonomy in Australia (Weir *et al.*, 2012; Barimani *et al.*, 2013). Many tropical countries close to Australia produce, export and consume a range of fruits and vegetables, and hence the accurate identification of postharvest pathogens will have a significant impact on agriculture, biosecurity and quarantine (Than *et al.*, 2008; Phoulivong *et al.*, 2012).

A polyphasic approach, combining the application of molecular methods with morphological and pathogenicity data, was recognized as being the most reliable method for resolving species complexes of *Colletotrichum* (Cai *et al.*, 2009; Cannon *et al.*, 2012). The objectives of this study were to investigate the taxonomy and phylogenetic relationships of *Colletotrichum* isolates associated with chili anthracnose in Queensland, Australia based on morphology and multigene phylogenetic analysis. The pathogenicity of different *Colletotrichum* species on chili fruit was then assessed.

Materials and methods

Collection and isolates

A total of 45 isolates associated with anthracnose disease symptoms on chili fruit were collected from different geographical locations in Queensland, Australia. The collection comprised 11 isolates from Cairns, 6 from the Brisbane region, and 28 isolates from Bundaberg. Isolates were grown on potato dextrose agar (PDA), and synthetic nutrient-poor agar (SNA) and incubated for 5–7 days at 27 °C as described by Damm *et al.* (2012). Single-spore isolation was carried out for all the *Colletotrichum* cultures and they were subcultured onto fresh PDA plates (Choi *et al.*, 1999).

Type specimens of new species were deposited in the Plant Pathology Herbarium (BRIP), Queensland, Australia, and ex-type living cultures deposited in the University of Melbourne culture collection (UOMCT) and in CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands (CBS).

Analysis of morphology

Morphological characters included conidia and appressorial size and shape, presence or absence of setae, culture morphology and growth rate. Colony colours were rated after 10 days using the colour charts of Rayner (1970). Production of acervular conidiomata was observed on dried, sterilized chili peduncles inoculated with mycelia and incubated on water agar (WA) and SNA medium. Structures were mounted in lactic acid, and the size of conidiomata and conidia were examined using a Leica DM6000 LED compound microscope and Leica LAS v. 4.4 software. Length and width were measured for 30 randomly selected conidia for each isolate, with the range, mean and standard deviation calculated. Size and shape of appressoria was examined after 10 days' incubation on WA, using a slide culture technique (Johnston & Jones, 1997).

Multigene phylogenetic analysis

PCR amplification and sequencing

Genomic DNA was extracted from fresh mycelia of isolates grown on PDA using the DNeasy Plant Mini kit (QIAGEN), diluted to 2 ng μL^{-1} and stored at −20 °C. All the *Colletotrichum* colonies were primarily identified by cultural characteristics on PDA, morphology of the spores, and internal transcribed spacer and intervening 5.8S nrRNA gene (ITS) sequence data. A total of 24 isolates were identified as *C. truncatum* and four representative isolates (UOMCT 4, 7, 10, 13) were used for further gene analysis. Eleven isolates of the *C. acutatum* complex were analysed with partial gene sequences of six genomic loci: ITS, an intron sequence of the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), partial sequences of the chitin synthase 1 (*CHS-1*), actin (*ACT*), *β*-tubulin (*TUB2*) and histone 3 (*HIS3*) genes. Ten isolates of the *C. gloeosporioides* complex were analysed with four gene sequences: ITS, *TUB2*, the *Apn2–Mat1–2* intergenic spacer and partial mating type (*Mat1–2*) gene (*ApMat*) and glutamine synthetase (*GS*) genes. These were amplified and sequenced using the primer pairs ITS-1F (Gardes & Bruns, 1993) + ITS4 (White *et al.*, 1990), GDF1 + GDR1 (Guerber *et al.*, 2003), CHS-354R + CHS-79F (Carbone & Kohn, 1999), ACT-512F + ACT-783R (Carbone & Kohn, 1999) and BT2Fd + BT4R (Woudenberg *et al.*, 2009) and CYLH3F + CYLH3R (Crous *et al.*, 2004b), AMF1 + AMR1 (Silva *et al.*, 2012) and GSF1 + GSR1 (Stephenson *et al.*, 1997), respectively. The PCR was performed in a 2720 Thermal Cycler (Applied Biosystems) in a total volume of 25 μL. The 25 μL reaction mixture comprised 1× PCR buffer, 0.2 mM dNTP, 0.4 μM of each primer, 2 mM $MgCl_2$, 1 U *Taq* DNA polymerase (Mango*Taq* DNA polymerase; Bioline) and 6 ng template DNA and components were adjusted as required. PCR amplification protocols were performed as described by Damm *et al.* (2012) and Silva *et al.* (2012), but the annealing temperatures were adjusted to 55 °C for ITS, *GAPDH*, *TUB2*, 58 °C for *ACT*, 60 °C for *GS* and 62 °C for *ApMat*. All the PCR products were then purified with the QIAquick PCR Purification kit (QIAGEN), according to manufacturer's instructions. Sequencing of the PCR products using their respective primers was carried out at the Australian Genome Research Facility (AGRF, Melbourne). PCR products were sequenced in both forward and reverse directions, and the consensus sequences were obtained by alignment of these sequences using GeneiousPro v. 7.0.6 (Kearse *et al.*, 2012). The consensus sequences were deposited in GenBank (Table 1), and taxonomic novelties in MycoBank (Crous *et al.*, 2004a).

Sequence alignment and phylogenetic analysis

The sequences for each isolate were examined using GeneiousPro v. 7.0.6, aligned by clustalW2 (Larkin *et al.*, 2007), and edited manually where necessary. All ITS and *TUB2* sequences were analysed to determine which clade each isolate belonged to and a phylogenetic tree was produced with maximum likelihood analysis (ML). Concatenated datasets comprising ITS, *CHS-1*, *ACT*, *GAPDH*, *HIS3* and *TUB2* gene sequences for 11 isolates from the *C. acutatum* complex and concatenated gene sequences of ITS, *TUB2*, *ApMat*, *GS* for 10 isolates from the *C. gloeosporioides* complex with selected reference type strains (Table 1) were generated (Damm *et al.*, 2012; Weir *et al.*, 2012). Phylogenetic analyses were performed using MrBayes v. 3.2.2 (Ronquist *et al.*, 2012) for Bayesian inference analysis (BI), and RAxML v. 7.2.6 (Stamatakis *et al.*, 2008) for ML analysis.

For BI analysis, the best nucleotide substitution model for each locus was determined by MrModelTest v. 2.3 (Nylander, 2004) to be (SYM+I) for ITS and *TUB2*; and (GTR+G) for *ACT*, *CHS-1*, *GAPDH*, *GS* and *HIS3*; and (HKY+G) for *ApMat*. Posterior probabilities were determined by Markov

Table 1 Isolates of the *Colletotrichum* species with details of host, location and GenBank accession numbers of gene sequences

Species	Accession number	Host	Locality	GenBank accession number							
				ITS	*GAPDH*	*CHS-1*	*HIS3*	*ACT*	*TUB2*	*ApMat*	*GS*
C. acutatum	CBS 127539, CPC 11738	*Aspalathus linearis*, anthracnose on stems and leaves	South Africa	JQ948377	JQ948708	JQ949038	JQ949368	JQ949698	JQ950028	—	—
	CBS 112996, ATCC 56816, STE-U 5292[a]	*Carica papaya*	Australia	JQ005776	JQ948677	JQ005797	JQ005818	JQ005839	JQ005860	—	—
C. aenigma	ICMP 18608[a]	*Persea americana*	Israel	JX010244	—	—	—	—	JX010389	KM360143	JX010078
C. aeschynomenes	ICMP 17673, ATCC 201874[a]	*Aeschynomene virginica*	USA	JX010176	—	—	—	—	JX010392	KM360145	JX010081
C. alatae	CBS 304.67, ICMP 17919[a]	*Dioscorea alata*	India	JX010190	—	—	—	—	JX010383	KC888932	JX010065
C. alienum	ICMP 12071[a]	*Malus domestica*	New Zealand	JX010251	—	—	—	—	JX010411	KM360144	JX010101
C. aotearoa	ICMP 18537[a]	*Coprosma* sp.	New Zealand	JX010205	—	—	—	—	JX010420	KC888930	JX010113
C. asianum	ICMP 18580[a], CBS 130418	*Coffea arabica*	Thailand	FJ972612	—	—	—	—	JX010406	FR718814	JX010096
C. brisbanense	CBS 292.67, DPI 11711[a]	*Capsicum annuum*	Australia	JQ948291	JQ948621	JQ948952	JQ949282	JQ949612	JQ949942	—	—
C. cairnsense	BRIP 63641	*C. annuum*	Australia	KU923671	KU923703	KU923709	KU923721	KU923715	KU923687	—	—
	BRIP 63642[a], CBS 140847	*C. annuum*	Australia	KU923672	KU923704	KU923710	KU923722	KU923716	KU923688	—	—
	BRIP 63643	*C. annuum*	Australia	KU923673	KU923705	KU923711	KU923723	KU923717	KU923689	—	—
	BRIP 63644	*C.annuum*	Australia	KU923674	KU923706	KU923712	KU923724	KU923718	KU923690	—	—
	BRIP 63645	*C. annuum*	Australia	KU923675	KU923707	KU923713	KU923725	KU923719	KU923691	—	—
	BRIP 63646	*C. annuum*	Australia	KU923676	KU923708	KU923714	KU923726	KU923720	KU923692	—	—
C. camelliae	CGMCC 3.14925, LC1364[a]	*Camellia sinensis*	China	KJ955081	—	—	—	—	KJ955230	KJ954497	KJ954932
C. chrysanthemi	CBS 126518, PD 84/520	*Carthamus* sp., twisted stem	Netherlands	JQ948271	JQ948601	JQ948932	JQ949262	JQ949592	JQ949922	—	—
	IMI 364540, CPC 18930	*Chrysanthemum coronarium*, leaf spot	China	JQ948273	JQ948603	JQ948934	JQ949264	JQ949594	JQ949924	—	—
C. clidemiae	ICMP 18658[a]	*Clidemia hirta*	USA, Hawaii	JX010265	—	—	—	—	JX010438	KC888929	JX010129
C. cordylinicola	MFLUCC 090551[a], ICMP 18579	*Cordyline fruticosa*	Thailand	JX010226	—	—	—	—	JX010440	JQ899274	JX010122
C. cosmi	CBS 853.73, PD 73/856[a]	*Cosmos* sp., seed	Netherlands	JQ948274	JQ948604	JQ948935	JQ949265	JQ949595	JQ949925	—	—
C. dematium	CBS 125.25			GU227819	GU228211	GU228309	GU228015	GU227917	GU228113	—	—
C. fioriniae	CBS 293.67, DPI 13120	*P. americana*	Australia	JQ948310	JQ948640	JQ948971	JQ949301	JQ949631	JQ949961	—	—
C. fructicola	ICMP 18581[a], CBS 130416	*C. arabica*	Thailand	JX010165	—	—	—	—	JQ807838	JX010405	JX010095
C. gloeosporioides	IMI 356878[a], ICMP 17821, CBS 112999	*Citrus sinensis*	Italy	JX010152	—	—	—	—	JQ807843	JX010445	JX010085
C. godetiae	CBS 133.44[a]	*Clarkia hybrida*, cv. Kelvon Glory, seed	Denmark	JQ948402	JQ948733	JQ949063	JQ949393	JQ949723	JQ950053	—	—
C. grevilleae	CBS 132879, CPC 15481[a]	*Grevillea* sp.	Italy	KC297078	KC297010	—	—	—	KC297102	—	KC297033

(continued)

Table 1 (continued)

Species	Accession number	Host	Locality	GenBank accession number							
				ITS	*GAPDH*	*CHS-1*	*HIS3*	*ACT*	*TUB2*	*ApMat*	*GS*
C. guajavae	IMI 350839, CPC 18893[a]	*Psidium guajava*, fruit	India	JQ948270	JQ948600	JQ948931	JQ949261	JQ949591	JQ949921	—	—
C. henanense	LC3030, CGMCC 3.17354, LF238[a]	*C. sinensis*, pathogen	China	KJ955109	—	—	—	—	KJ955257	KJ954524	KJ954960
C. horii	NBRC 7478[a], ICMP 10492	*Diospyros kaki*	Japan	GQ329690	—	—	—	—	JX010450	JQ807840	JX010137
C. indonesiense	CBS 127551, CPC 14986[a]	*Eucalyptus* sp.	Indonesia	JQ948288	JQ948618	JQ948949	JQ949279	JQ949609	JQ949939	—	—
C. jiangxiense	LC3463, CGMCC 3.17363, LF687[a]	*C. sinensis*	China	KJ955201	—	—	—	—	KJ955348	KJ954607	KJ955051
C. kahawae subsp. *kahawae*	IMI 319418[a], ICMP 17816	*C. arabica*	Kenya	JX010231	—	—	—	—	JX010444	JQ894579	JX010130
C. laticiphilum	CBS 112989, IMI 383015, STE-U 5303[a]	*Hevea brasiliensis*	India	JQ948289	JQ948619	JQ948950	JQ949280	JQ949610	JQ949940	—	—
C. lupini	CBS 109225, BBA 70884[a]	*Lupinus albus*	Ukraine	JQ948155	JQ948485	JQ948816	JQ949146	JQ949476	JQ949806	—	—
C. musae	CBS 116870[a], ICMP 19119	*Musa* sp.	USA	JX010146	—	—	—	—	HQ596280	KC888926	JX010103
C. nupharicola	CBS 470.96[a], ICMP 18187	*Nuphar lutea* subsp. *polysepala*	USA	JX010187	—	—	—	—	JX010398	JX145319	JX010088
C. simmondsii	CBS 122122, BRIP 28519[a]	*C. papaya*, fruit	Australia	JQ948276	JQ948606	JQ948937	JQ949267	JQ949597	JQ949927	—	—
	CBS 295.67, DPI 16518	*Fragaria* sp., fruit	Australia	JQ948278	JQ948608	JQ948939	JQ949269	JQ949599	JQ949929	—	—
	BRIP 63647	*Capsicum* sp.	Australia	KT957917	KT957919	KT957921	KT957922	KT957920	KT957918	—	—
	BRIP 63648	*Capsicum* sp.	Australia	KU199246	KU199248	KU199250	KU199251	KU199249	KU199247	—	—
	BRIP 63649	*Capsicum* sp.	Australia	KU199252	KU199254	KU199256	KU199257	KU199255	KU199253	—	—
	BRIP 63650	*Capsicum* sp.	Australia	KU199258	KU199260	KU199262	KU199263	KU199261	KU199259	—	—
	BRIP 63651	*Capsicum* sp.	Australia	KU199264	KU199266	KU199268	KU199269	KU199267	KU199265	—	—
C. nymphaeae	CBS 515.78[a]	*Nymphaea alba*, leaf spot	Netherlands	JQ948197	JQ948527	JQ948858	JQ949188	JQ949518	JQ949848	—	—
	CBS 126528, PD 94/921-2, BBA 70348	*Capsicum* sp.	Indonesia	JQ948219	JQ948549	JQ948880	JQ949210	JQ949540	JQ949870	—	—
	CBS 482.82	*Protea* sp.	Australia	JQ948213	JQ948543	JQ948874	JQ949204	JQ949534	JQ949864	—	—
C. orbiculare	LARS 414, 104T, CBS 514.97			JQ005778	—	—	—	—	JQ005862	—	—
C. paxtonii	IMI 165753, CPC 18868[a]	*Musa* sp.	Saint Lucia	JQ948285	JQ948615	JQ948946	JQ949276	JQ949606	JQ949936	—	—
C. psidii	CBS 145.29[a], ICMP 19120	*Psidium* sp.	Italy	JX010219	—	—	—	—	JX010443	KC888931	JX010133
C. proteae	CBS 132882, CPC 14859[a]	*Protea* sp.	South Africa	KC297079	—	—	—	—	KC297101	—	KC297032
C. queenslandicum	ICMP 1778[a]	*C. papaya*	Australia	JX010276	—	—	—	—	JX010414	KC888928	JX010104
	BRIP 63695	*C. annuum*	Australia	KU923677	—	—	—	—	KU923693	KU923727	KU923737
	BRIP 63696	*C. annuum*	Australia	KU923678	—	—	—	—	KU923694	KU923728	KU923738
	BRIP 63697	*C. annuum*	Australia	KU923679	—	—	—	—	KU923695	KU923729	KU923739
	BRIP 63698	*C. annuum*	Australia	KU923680	—	—	—	—	KU923696	KU923730	KU923740
	BRIP 63699	*C. annuum*	Australia	KU923681	—	—	—	—	KU923697	KU923731	KU923741
	BRIP 63700	*C. annuum*	Australia	KU923682	—	—	—	—	KU923698	KU923732	KU923742

(continued)

Table 1 (continued)

Species	Accession number	Host	Locality	GenBank accession number							
				ITS	*GAPDH*	*CHS-1*	*HIS3*	*ACT*	*TUB2*	*ApMat*	*GS*
C. salicis	CBS 607.94[a]	*Salix* sp., leaf, spot	Netherlands	JQ948460	JQ948791	JQ949121	JQ949451	JQ949781	JQ950111	—	—
C. salsolae	ICMP 19051[a]	*Salsola tragus*	Hungary	JX010242	—	—	—	—	JX010403	KC888925	JX010093
C. scovillei	CBS 126529, PD 94/921-3, BBA 70349[a]	*Capsicum* sp.	Indonesia	JQ948267	JQ948597	JQ948928	JQ949258	JQ949588	JQ949918	—	—
C. siamense	ICMP 18578[a], CBS 130417	*C. arabica*	Thailand	JX010171	—	—	—	—	JX010404	JQ899289	JX010094
	BRIP 63701	*C. annuum*	Australia	KU923683	—	—	—	—	KU923699	KU923733	KU923743
	BRIP 63702	*C. annuum*	Australia	KU923684	—	—	—	—	KU923700	KU923734	KU923744
	BRIP 63703	*C. annuum*	Australia	KU923685	—	—	—	—	KU923701	KU923735	KU923745
	BRIP 63704	*C. annuum*	Australia	KU923686	—	—	—	—	KU923702	KU923736	KU923746
C. siamense (syn. *C. jasmini-sambac*)	CBS 130420, ICMP 19118	*Jasminum sambac*	Vietnam	HM131511	—	—	—	—	JX010415	JQ807841	JX010105
C. siamense (syn. *C. hymenocallidis*)	CBS 125378, ICMP 18642, LC0043	*Hymenocallis americana*	China	JX010278	—	—	—	—	JX010410	JQ899283	JX010100
C. sloanei	IMI 364297, CPC 18929[a]	*Theobroma cacao*, leaf	Malaysia	JQ948287	JQ948617	JQ948948	JQ949278	JQ949608	JQ949938	—	—
C. tamarilloi	CBS 129814, T.A.6[a]	*Solanum betaceum*, fruit, anthracnose	Colombia	JQ948184	JQ948514	JQ948845	JQ949175	JQ949505	JQ949835	—	—
C. ti	ICMP 4832[a]	*Cordyline* sp.	New Zealand	JX010269	—	—	—	—	JX010442	KM360146	JX010123
C. tropicale	CBS 124949, ICMP 18653, MTCC 11371[a]	*T. cacao*	Panama	JX010264	JX010007	—	—	—	JX010407	KC790728	JX010097
C. truncatum	CBS 151.35[a]	*Phaseolus lunatus*	USA	GU227862	—	—	—	—	GU228156	—	—
	UOMCT 4	*C. annuum*	Australia	KU985043	—	—	—	—	KU985047	—	—
	UOMCT 7	*C. annuum*	Australia	KU985044	—	—	—	—	KU985048	—	—
	UOMCT 10	*C. annuum*	Australia	KU985045	—	—	—	—	KU985049	—	—
	UOMCT 13	*C. annuum*	Australia	KU985046	—	—	—	—	KU985050	—	—
C. theobromicola	CBS 124945[a], ICMP 18649	*T. cacao*	Panama	JX010294	—	—	—	—	JX010447	KC790726	JX010139
C. walleri	CBS 125472, BMT(HL)19[a]	*Coffea* sp., leaf tissue	Vietnam	JQ948275	JQ948605	JQ948936	JQ949266	JQ949596	JQ949926	—	—
C. xanthorrhoeae	BRIP 45094[a], ICMP 17903, CBS 127831	*Xanthorrhoea preissii*	Australia	JX010261	JX009927	JX009823	—	JX009478	JX010448	KC790689	JX010138

ATCC: American Type Culture Collection; CBS: culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, Netherlands; CPC: working collection of Pedro W. Crous, housed at CBS, Netherlands; CGMCC: China General Microbiological Culture Collection; DPI: Department of Primary Industries; IMI: culture collection of CABI Europe UK Centre, Egham, UK; MFLUCC: Mae Fah Luang University Culture Collection, ChiangRai, Thailand; BBA: culture collection of the Biologische Bundesanstalt für Land- und Forstwirtschaft, Berlin, Germany; BRIP: Plant Pathology Herbarium, Department of Employment, Economic, Development and Innovation, Queensland, Australia; ICMP: International Collection of Microorganisms from Plants, Auckland, New Zealand; LC: working collection of Lei Cai, housed at CAS, China; LF: working collection of Fang Liu, housed at CAS, China; STE-U: culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa; HKUCC: the University of Hong Kong Culture Collection, Hong Kong, China; PD: Plantenziektenkundige Dienst Wageningen, Netherlands; NBRC: NITE Biological Resource Center, Chiba, Japan; UOMCT: University of Melbourne Culture Collection.

[a]Ex-holotype or ex-epitype cultures.

chain Monte Carlo sampling (MCMC) in MrBayes v. 3.2.2 (Huelsenbeck & Ronquist, 2001; Ronquist *et al.*, 2012). Eight simultaneous Markov chains were run for 10^6 generations and trees were sampled every 1000 generations for the *C. acutatum* complex and every 100 generations for the *C. gloeosporioides* complex. The heating parameter was set to 0.2 and analyses stopped once the average standard deviation of split frequencies was below 0.01. The first 25% trees, representing the burn-in phase of the analyses were discarded and the remaining trees in each analysis were used to calculate posterior probabilities. The generated 50% majority rule consensus tree was viewed in treeview v. 1.6.6 (Page, 1996). Branch support for ML analyses using the GTR-gamma model was applied to the individual alignments with 1000 pseudoreplicates using RAxML v. 7.2.6 (Stamatakis *et al.*, 2008).

Pathogenicity assay

Pathogenicity tests on chili fruit were conducted using two representative isolates from each species. Detached chili fruits (mature red *C. annuum*) from the Victoria market in Melbourne were used for the pathogenicity assay. Conidial suspensions were prepared from 7-day-old cultures by adding 5–10 mL of sterile distilled water into each culture, scraping the mycelia from plates, and filtering through two layers of muslin cloth. Red fruits were surface sterilized using 1% (a.i.) sodium hypochlorite for 5 min followed by washing three times with sterile distilled water. Fruits were placed in sterilized plastic containers then inoculated with conidial suspension (10^6 conidia mL^{-1}) of each isolate by both non-wounding and wounding methods. For the wounding method, the cuticle and epidermis of chili fruit were wounded *c.* 2 cm from the peduncle by pricking with a needle to about 1 mm depth and placing 6 μL of conidial suspension over the wound. For the non-wounding method, 6 μL of conidial suspension was placed on the fruit surface *c.* 2 cm from the base of the fruit. Control fruits were treated with 6 μL sterile distilled water. The containers were sealed and maintained at high humidity in an incubator at 27 °C. After 7–10 days lesion sizes were scored on a 1–9 scale according to Montri *et al.* (2009). Three replicate fruit were tested per isolate with both inoculation methods and the experiment was carried out three times.

Disease severity was calculated according to the equation: Disease severity = lesion length × 100/fruit length.

A disease severity scale of chili fruit infected by *Colletotrichum* spp. was used for scoring the different severity levels (Montri *et al.*, 2009).

Results

Phylogenetic analyses of the combined datasets

Colletotrichum isolates were preliminarily identified, based on the phylogenetic analysis of the combined ITS and *TUB2* alignment (Fig. 1), as belonging to the *C. acutatum* species complex (11 isolates), *C. gloeosporioides* species complex (10 isolates) and *C. truncatum* (4 isolates).

Colletotrichum acutatum *complex*

The six-gene (ITS, *TUB2*, *GAPDH*, *CHS-1*, *ACT* and *HIS3*) phylogenetic analysis of the *C. acutatum* species complex included sequences of 36 isolates with *C. xanthorrhoeae* (CBS 127831) as the out-group (Fig. 2). The dataset comprised 2653 characters including the alignment gaps. Bayesian analysis of the combined alignment, based on 629 unique site patterns (ITS: 78, *TUB2*: 130, *GAPDH*: 157, *CHS-1*: 92, *ACT*: 61 and *HIS3*: 111) lasted 1 060 000 generations, resulting in 2122 total trees of which 1592 trees were used to calculate the posterior probabilities. Bootstrap support values of ML analysis and BI posterior probabilities are plotted at the nodes, which are congruent between the ML tree and the Bayesian phylogeny.

Isolates from infected chili fruits in the *C. acutatum* complex clustered in two clades: five isolates clustered with the ex-type isolate of *C. simmondsii*, whereas six isolates formed a distinct clade, which was phylogenetically different from other species and hence was identified as a potential new species. The *C. simmondsii* isolates showed the typical colony morphologies described by Damm *et al.* (2012). However, they had different colony growth rates and slight differences in spore measurements. The morphological characters for the potential new species were compared with closely related type strains from culture collections.

Colletotrichum gloeosporioides *species complex*

The four-gene (ITS, *TUB2, ApMat* and *GS*) phylogenetic analysis of the *C. gloeosporioides* species complex included 39 isolates with *C. proteae* (CBS 132882) as the out-group (Fig. 3). The dataset comprised 2857 characters including the alignment gaps. Bayesian analysis of the combined alignment, based on 1046 unique site patterns (ITS: 59, *TUB2*: 140, *ApMat*: 486 and *GS*: 361) lasted 95 000 generations, resulting in 1902 total trees of which 1428 trees were used to calculate the posterior probabilities. Bootstrap support values of ML analysis and BI posterior probabilities are plotted at the nodes, which are congruent between the ML tree and the Bayesian phylogeny.

The phylogenetic analysis of the *C. gloeosporioides* species complex showed that all the chili isolates clearly separated into two groups representing different species (Fig. 3). Four isolates collected from Bundaberg formed a small subgroup with the reference species *C. siamense* and fall within the intraspecific variation for this species. Another six isolates from the Brisbane region showed a close relationship to the available sequences of the reference species *C. queenslandicum* and fall within the intraspecific variation of ITS and *TUB2* sequences available for additional *C. queenslandicum* strains on GenBank.

Morphological analysis

Morphological characters including both colony and conidial characters were variable for all the five *Colletotrichum* species identified as causing anthracnose of chili in Australia (Table 2). However, the growth rates of *C. queenslandicum* and *C. siamense* (*C. gloeosporioides* complex) were faster than *C. cairnsense* and *C. simmondsii* (*C. acutatum* complex).

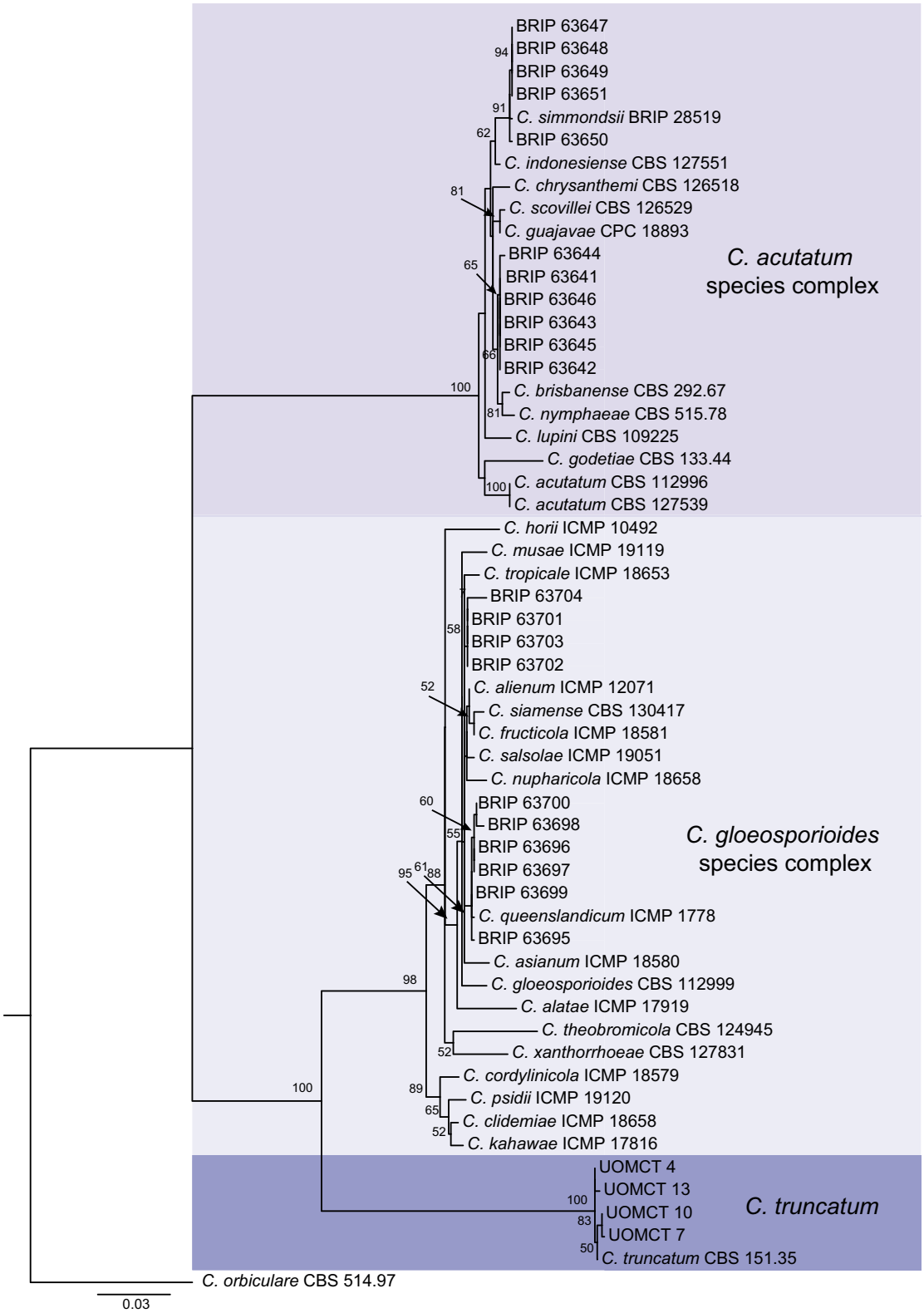

Figure 1 Maximum likelihood (ML) consensus tree of the combined gene analysis of ITS and *TUB2* sequence alignment showing separation of *Colletotrichum* isolates into the *C. acutatum* species complex, *C. gloeosporioides* species complex and *C. truncatum* (indicated by blocks of different colours). Bootstrap support values (ML > 50) are given at the nodes. The scale bar shows the number of substitutions per nucleotide position. *Colletotrichum orbiculare* CBS 514.97 is used as out-group. [Colour figure can be viewed at wileyonlinelibrary.com].

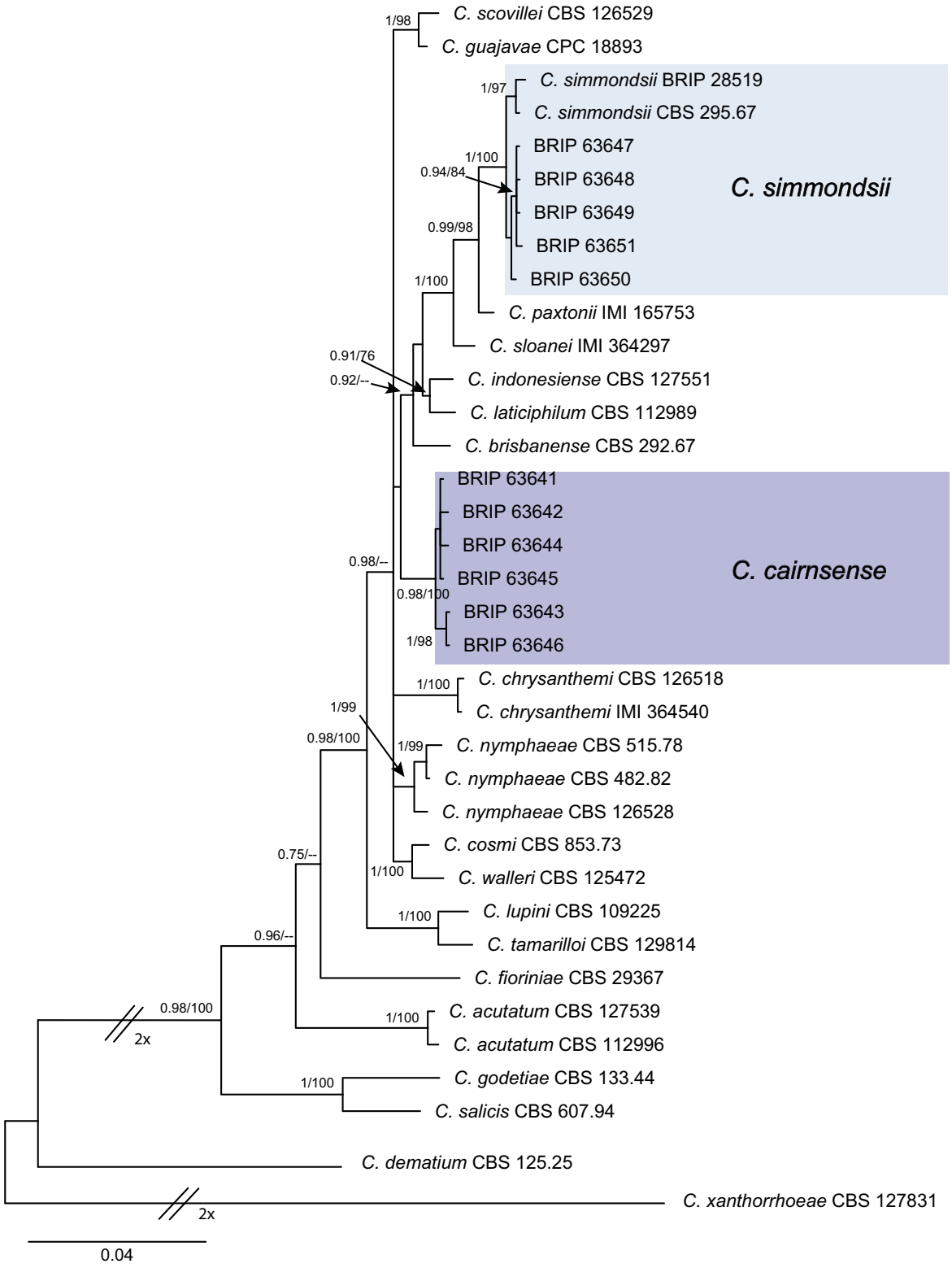

Figure 2 Bayesian phylogeny (BI) based on a 50% majority rule consensus tree of the combined ITS, *TUB2*, *GAPDH*, *ACT*, *CHS-1* and *HIS3* sequence alignment showing phylogenetic affinities of Australian chili isolates of the *Colletotrichum acutatum* species complex. The two clades containing chili isolates are indicated by blocks of different colours. The BI posterior probabilities and bootstrap support values (>75% based on 100 replicates) of maximum likelihood analysis are given at the nodes. The lengths of the two branches with 2× on them were halved for the layout of the tree. The scale bar shows the expected number of substitutions per site. *Colletotrichum xanthorrhoeae* CBS 127831 was used as the out-group. [Colour figure can be viewed at wileyonlinelibrary.com].

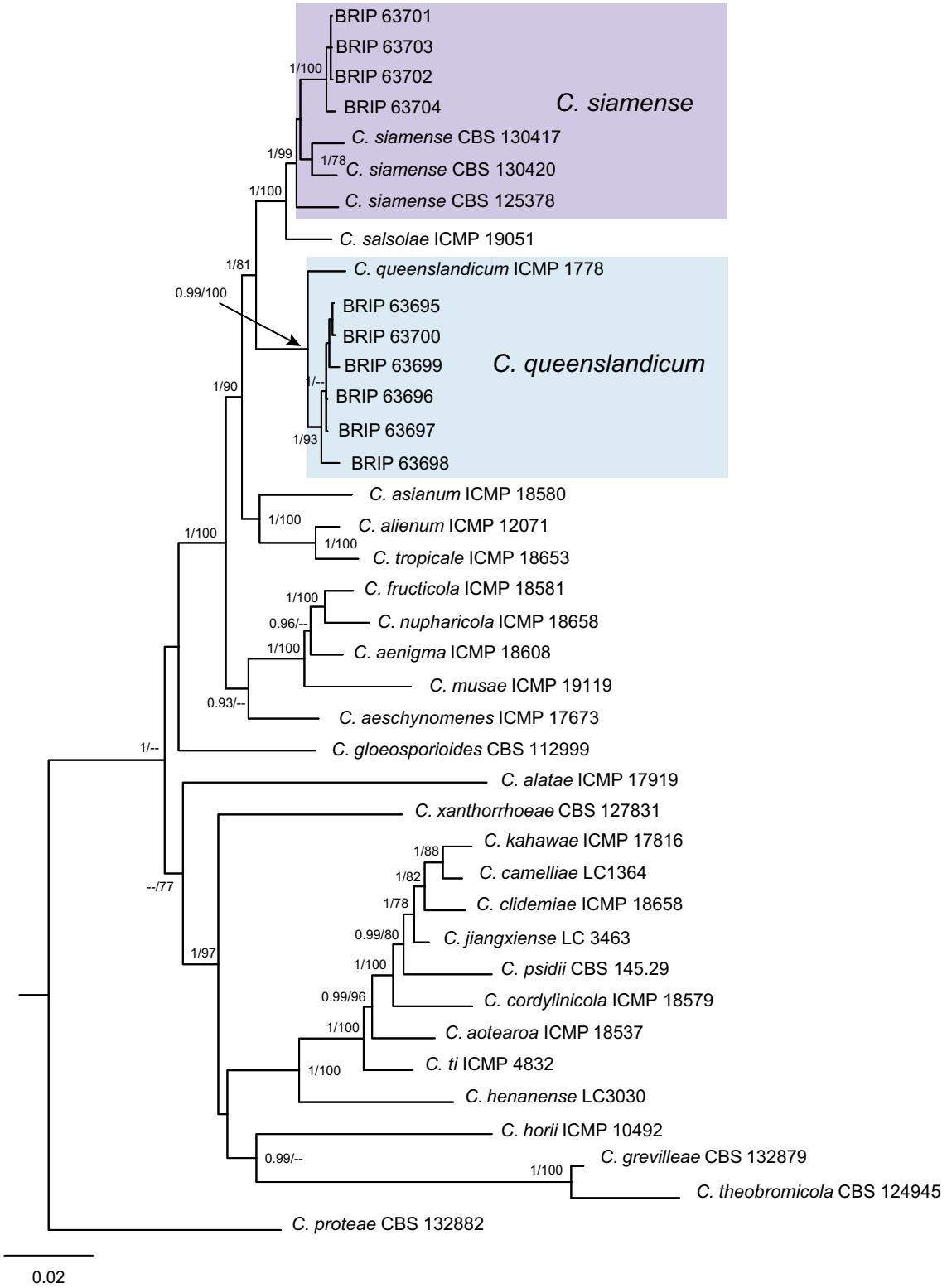

Figure 3 Bayesian phylogeny (BI) based on a 50% majority rule consensus tree of the combined ITS, *TUB2*, *ApMat* and *GS* sequence alignment showing phylogenetic affinities of the Australian chili isolates of the *Colletotrichum gloeosporioides* species complex. The two clades containing chili isolates are indicated by blocks of different colours. The BI posterior probabilities and bootstrap support values (>75% based on 100 replicates) of maximum likelihood analysis are given at the branch. The scale bar shows the number of substitutions per nucleotide position. *Colletotrichum proteae* CBS 132882 was used as the out-group. [Colour figure can be viewed at wileyonlinelibrary.com].

Table 2 Morphological characters and conidial measurements of *Colletotrichum* spp. causing anthracnose of chili in Australia

Taxon	Colony morphology	Conidial morphology					Growth rate (mm day^{-1})
		Length (μm)		Width (μm)		Shape	
		Range	Mean	Range	Mean		
C. queenslandicum	Pale, white grey to pale yellow aerial mycelium with abundance of many acervuli conidiomata that ooze orange conidial masses	10.5–17.0	13.8 ± 0.13	4.5–6.0	5.2 ± 0.03	Fusiform to cylindrical	6.0 ± 2.1
C. cairnsense sp. nov.	White grey to olivaceous-grey mycelia with whitish margin	12.5–16.5	14.3 ± 0.15	3.5–4.0	3.7 ± 0.04	Cylindrical with two ends acute or one end slightly obtuse	3.5 ± 1.4
C. simmondsii	Pale pink to orange mycelia with centre covered with short whitish to pale grey aerial mycelium	12.0–17.0	13.8 ± 0.19	3.0–4.5	3.6 ± 0.04	Fusiform	3.8 ± 0.4
C. siamense	Pale white, grey dense cottony mycelia with orange acervuli conidiomata at the centre	13.0–14.0	13.9 ± 0.80	3.0–4.0	3.8 ± 0.20	Fusiform to cylindrical	5.7 ± 1.5
C. truncatum	Grey-white mycelia with black acervuli condiomata arranged in concentric rings	17.0–23.5	23.0 ± 1.29	2.5–3.0	2.6 ± 0.15	Curved	8.5 ± 0.3

Taxonomy of *Colletotrichum cairnense* sp. nov

Morphological observations and phylogenetic analyses suggested that the *Colletotrichum* species isolated from anthracnose-like lesions on chili fruits collected from Cairns, Australia was a new species, for which the name *Colletotrichum cairnsense* is proposed below.

***Colletotrichum cairnsense* D. D. De Silva, R. Shivas & P. W. J. Taylor, sp. nov.**

Etymology: Named after the city where the diseased specimen was collected, Cairns, Queensland, Australia.

Conidiomata on SNA inconspicuous, erumpent, 50–200 μm diam., forming on a brown central stroma, but lacking setae. *Conidiophores* hyaline, smooth, subcylindrical, 1–4-septate, branched, 30–50 × 3–4 μm. *Conidiogenous cells* hyaline, smooth, subcylindrical, straight to gently curved, 12–25 × 3–4 μm, phialidic, with visible periclinal thickening at truncate apex, 1.5–2 μm diam. *Conidia* hyaline, smooth, aseptate, straight, cylindrical with acutely rounded ends, guttulate, granular (12–)14–15(–16.5) × (3.5–)4(–4.5) μm. *Appressoria* single, medium brown, smooth-walled, subglobose, ovoid to ellipsoidal, 8–13 × 4–5 μm, the outline entire, rarely undulate (Fig. 4).

Colonies on PDA grew 3.5 mm day^{-1} and were 24 mm diameter after 7 days, aerial mycelium pale white-grey to olivaceous grey with whitish margin; reverse pink to straw with pale olivaceous grey to pink-orange in the centre. *Chlamydospores* not observed. *Conidiomata* present (near the inoculation point), conidiophores formed directly on hyphae. Setae not observed. *Conidiophores* hyaline to pale brown, smooth-walled, septate and branched. *Conidia* hyaline, smooth-walled, aseptate, straight, cylindrical with two ends acute or one end slightly obtuse (12.5–) 13–15 (–16.5) × (3.5–) 4 (–4.5) μm, L:W ratio = 3.7. *Appressoria* single or in loose groups, medium to dark brown, smooth-walled, subglobose, ovoid to ellipsoidal, 7.5–10.5 × 5–6.5 μm the outline entire, rarely undulate (Fig. 4).

Mycelia on fruit peduncles were colourless to off-white. Masses of bright orange, smooth-walled conidia were produced. Conidia hyaline, smooth-walled, aseptate, straight, cylindrical with two ends acute or one end slightly obtuse; 12.4–16.3 × 3.7–6 (mean 14.5 ± 1.22 × 4.6 ± 0.7 μm).

Notes: The new species was isolated from infected chili fruits collected from a retail fruit market in Cairns, Australia. This species formed a distinct clade that could be clearly distinguished from other species in the *C. acutatum* complex (Fig. 2). The BLASTN search with the ITS sequence of this strain showed 100% similarity to GU183315.1 *C. simmondsii* isolate. The closest matches in a BLASTN search in GenBank with the *GAPDH* sequence were KT321123.1 *C. acutatum* strain (97% identity, 9 bp differences), and KM252207.1 *C. nymphaeae* isolate (97% identity, 9 bp differences), KJ947270.1 *C. guajavae* isolate (96% identity, 9 bp differences). The closest matches with the *TUB2* sequence (with 99% identity, 3–4 bp differences) were *C. simmondsii*, GU183290.1 and *C. sloanei*.

Holotype: Australia, Queensland, Cairns, on fruit of *Capsicum annuum*, 7 June 2015, R. G. Shivas (BRIP 63642) culture ex-type = CBS 140847 = UOMCT 12; MycoBank MB815396.

Pathogenicity assay

All the *Colletotrichum* isolates showed symptoms of anthracnose on detached chili fruit inoculated by the

Figure 4 Culture characteristics and microscopic features of the *Colletotrichum cairnsense* sp. nov. Samples of diseased chili fruits in which the pathogen was isolated (a), production of conidiomata on chili peduncle (b), 1-week-old culture on potato dextrose agar (c, d), conidiomata (e), appressoria on synthetic nutrient-poor agar (f–h), germinating conidia (i, j), conidiophores (k, l), and asexual conidia (m). Scale bars: i–m = 10 μm. f, g, h = 20 μm. [Colour figure can be viewed at wileyonlinelibrary.com].

wounding method while only *C. cairnsense* and *C. siamense* were able to infect non-wounded fruits. Both *C. cairnsense* and *C. siamense* isolates were virulent in non-wounded fruit and were able to produce symptoms. On wounded fruit, isolates of *C. cairnsense* and *C. queenslandicum* showed the highest disease severity producing large necrotic lesions with disease scores in the range 5–9, 5–7 respectively (Fig. 5; Table 3).

Discussion

Phylogenetic affinities of *Colletotrichum* species associated with diseased chili fruits in Queensland, Australia were determined together with evidence of their pathogenicity. *Colletotrichum cairnsense* belongs to the *C. acutatum* species complex, and was identified and described as a new species responsible for anthracnose disease of chili in northern Queensland, Australia. The only previously reported species from the *C. acutatum* complex causing anthracnose in chili in Australia was *C. brisbanense*, which was formerly identified as *C. simmondsii* in the *C. acutatum* group D or A2 (Shivas & Tan, 2009; Damm *et al.*, 2012).

Colletotrichum queenslandicum, *C. siamense* and *C. simmondsii* were reported for the first time in Australia causing anthracnose of chili. *Colletotrichum simmondsii* was previously identified as a pathogen of papaya in Australia and has been shown to cross infect between hosts (Shivas & Tan, 2009; Damm *et al.*, 2012; Phoulivong *et al.*, 2012). Phylogenetic analysis clearly differentiated the three species with morphological data including colony characters, conidial measurements and growth rates being similar or within the range of the type specimens (Shivas & Tan, 2009; Weir *et al.*, 2012). The most frequently reported species from other Southeast Asian countries, *C. scovillei*, has yet to be identified in Australia. The multilocus phylogeny clearly showed the new species, *C. cairnsense*, to cluster in a distinct phylogenetic clade

Figure 5 Pathogenicity assay conducted on detached chili fruits (*Capsicum annuum*) and the symptoms caused by *C. siamense* (a), *C. cairnsense* (b), *C. queenslandicum* (c), *C. simmondsii* (d), 10 days after inoculation by a wounding method. [Colour figure can be viewed at wileyonlinelibrary.com].

Table 3 Disease score on a 0–9 scale of chili fruits (*Capsicum annuum*) for different *Colletotrichum* species inoculated by wounding or non-wounding methods

	Average score range	
Species name	Wounding	Non-wounding
C. siamense BRIP 63701	3–7	1
C. siamense BRIP 63702	3–7	1–3
C. cairnsense BRIP 63641	5–9	1–3
C. cairnsense BRIP 63642	5–9	3
C. queenslandicum BRIP 63695	5–7	0
C. queenslandicum BRIP 63696	5–7	0
C. simmondsii BRIP 63647	3–7	0
C. simmondsii BRIP 63648	3–7	0

from the other species. *Colletotrichum cairnsense* showed different morphological characters compared to other species in the *C. acutatum* complex and was clearly distinguished by *GAPDH* and *TUB2* sequences, which are considered as the most informative gene markers to differentiate isolates in the *C. acutatum* complex. Pathogenicity tests confirmed *C. cairnsense* as a highly virulent species on chili fruits. Further analysis needs to be conducted to identify the effect of this pathogen on plant growth and leaf infection.

Species in the *C. gloeosporioides* complex were identified from a combined multilocus gene analysis, which included the *ApMat* gene. Phylogenetic trees built from *ApMat* and *GS* gene sequences provided similar information and support as the concatenated tree of four genes (*ApMat*, ITS, *TUB2*, *GS*) demonstrating that the *ApMat* marker was an informative marker that distinguished most species in the *C. gloeosporioides* species complex. In contrast, the combined gene analysis of ITS and *TUB2* data was not informative enough to separate the above species. The *ApMat* gene has been shown to improve the systematics of the *C. gloeosporioides* species complex (Silva *et al.*, 2012), and was applied in more recent molecular phylogenetic analyses of the species in this complex (Liu *et al.*, 2015). The study by Liu *et al.* (2015) showed that the *ApMat* gene provided superior phylogenetic information compared to other loci, and the concatenated *ApMat* and *GS* alignment could delimit all the species in the *C. gloeosporioides* species complex that infected *Camellia* in China.

The two species identified from the *C. gloeosporioides* complex, *C. queenslandicum* and *C. siamense*, showed nearly similar colony morphologies, with small variations in colony growth rates and spore sizes between the isolates. The combined gene analyses of *ApMat* and *GS* (trees not shown) data, supported the distinction of the above two taxa from the other isolates in the group. In recent studies, *C. siamense* was shown to be a separate species complex within the *C. gloeosporioides* complex, with many new divergent species identified (Weir *et al.*, 2012; Udayanga *et al.*, 2013). Due to a lack of sequence data of the *ApMat* gene for some reference strains, it was difficult to determine the placement of the tested isolates. These isolates represent potential new lineages inside the subcomplexes, and further work is needed to phylogenetically reassess the *C. gloeosporioides* species complex. Although *C. queenslandicum* and *C. siamense* were previously reported from Australia, they have never been reported as pathogens of chili in Australia (Weir *et al.*, 2012; James *et al.*, 2014). The main chili-pathogenic species within the *C. gloeosporioides* species complex, *C. asianum* and *C. fructicola* (Phoulivong *et al.*, 2012; Sharma & Shenoy, 2014) have been recorded in Thailand and India, but have yet to be identified in Australia.

Among all *Colletotrichum* species, *C. truncatum* (formerly *C. capsici*) (Damm *et al.*, 2009) was the most frequently isolated from chili in Australia and was detected in 47% of the collected samples. Morphological characters of these isolates were consistent and showed very

distinctive curved conidia typical of the species. *Colletotrichum truncatum* has been reported as a pathogen of chili in several countries and an important pathogenic species that infects other plant crops causing serious damage (Sharma *et al.*, 2005; Than *et al.*, 2008; Mongkolporn & Taylor, 2011; Diao *et al.*, 2014). Microsatellite-based marker analysis of *C. truncatum* populations from chili-growing regions in Southeast Asia revealed a high allele flow within and between populations, showing a high adaptive capacity of this pathogen to overcome host resistance (Ranathunge *et al.*, 2009, 2012).

Different *Colletotrichum* species showed varying degrees of virulence on infected chili fruits. All the *Colletotrichum* isolates were pathogenic on wounded fruit; however, only isolates of *C. cairnsense* and *C. siamense* were able to infect non-wounded fruit. The quantitative difference in infection severity between isolates reflects the aggressiveness of pathogens. Further pathogenicity analysis with more isolates is needed to confirm whether pathotype variations exist within the different species. In previous studies, pathotypes that showed qualitative difference in infection severity were identified by fruit bioassays conducted on fruit wounded prior to inoculation (Montri *et al.*, 2009; Mongkolporn *et al.*, 2010; Mongkolporn & Taylor, 2011). Mongkolporn *et al.* (2010) identified pathotype differences between isolates of *C. scovillei*, *C. truncatum* and *C. siamense* at different stages of physiological maturity on four *Capsicum* varieties. These studies were conducted on wounded chili fruits, which disregarded the importance of the cuticle and epidermis as resistance barriers to initial infection. Auyong *et al.* (2015) showed the importance of the *C. truncatum* enzyme cutinase to break down the structural defence of the host tissue in the initial infection of chili fruit.

The identification of a new *Colletotrichum* species causing chili anthracnose in Australia and the lack of identification of important species that occur in Southeast Asia reflects the importance for further research in *Colletotrichum* taxonomy to mitigate the risk to the Australian chili fruit industry.

Acknowledgements

The first author gratefully acknowledges the financial support from the Melbourne International Research Scholarship (MIRS) and Melbourne International Fee Remission Scholarship (MIFRS) awarded by the University of Melbourne; and also Dr Ewald (J. Z.) Groenewald for sequence data processing and valuable suggestions on the manuscript.

References

ABS (Australian Bureau of Statistics), 2014. 7121.0 Agricultural Commodities, Australia, 2011–12. [www.abs.gov.au/]. Accessed 20 August 2015.

Auyong ASM, Ford R, Taylor PWJ, 2015. The role of cutinase and its impact on pathogenicity of *Colletotrichum truncatum*. *Journal of Plant Pathology and Microbiology* **6**, 259.

Barimani M, Pethybridge SJ, Vaghefi N, Hay FS, Taylor PWJ, 2013. A new anthracnose disease of pyrethrum caused by *Colletotrichum tanaceti* sp. nov. *Plant Pathology* **62**, 1248–57.

Cai L, Hyde KD, Taylor PWJ *et al.*, 2009. A polyphasic approach for studying *Colletotrichum*. *Fungal Diversity* **39**, 183–204.

Cannon PF, Damm U, Johnston PR, Weir BS, 2012. *Colletotrichum* – current status and future directions. *Studies in Mycology* **73**, 181–213.

Carbone I, Kohn L, 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* **91**, 553–6.

Choi YW, Hyde KD, Ho W, 1999. Single spore isolation of fungi. *Fungal Diversity* **3**, 29–38.

Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G, 2004a. MycoBank: an online initiative to launch mycology into the 21st century. *Studies in Mycology* **50**, 19–22.

Crous PW, Groenewald JZ, Risede JM, Simoneau P, Hywel-Jones NL, 2004b. *Calonectria* species and their *Cylindrocladium* anamorphs: species with sphaeropedunculate vesicles. *Studies in Mycology* **50**, 415–30.

Damm U, Woudenberg JHC, Cannon PF, Crous PW, 2009. *Colletotrichum* species with curved conidia from herbaceous hosts. *Fungal Diversity* **39**, 45–87.

Damm U, Cannon PF, Woudenberg JHC, Crous PW, 2012. The *Colletotrichum acutatum* species complex. *Studies in Mycology* **73**, 37–113.

Diao Y, Zhang C, Lin D, Liu XL, 2014. First report of *Colletotrichum truncatum* causing anthracnose of tomato in China. *Plant Disease* **98**, 687–8.

Diao Y, Zhang C, Xu J *et al.*, 2015. Genetic differentiation and recombination among geographic populations of the fungal pathogen *Colletotrichum truncatum* from chili peppers in China. *Evolutionary Applications* **8**, 108–18.

FAOSTAT, 2015. Food and Agriculture Organization of the United Nations Statistics Division. [http://faostat.fao.org/]. Accessed 5 January 2016.

Gardes M, Bruns TD, 1993. ITS primers with enhanced specificity for basidiomycetes–application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**, 113–8.

Guerber JC, Liu B, Correll JC, Johnston PR, 2003. Characterization of diversity in *Colletotrichum acutatum sensu lato* by sequence analysis of two gene introns, mtDNA and intron RFLPs, and mating compatibility. *Mycologia* **95**, 872–95.

Harp TL, Pernezny K, Ivey MLL, Miller SA, Kuhn PJ, Datnoff L, 2008. The etiology of recent pepper anthracnose outbreaks in Florida. *Crop Protection* **27**, 1380–4.

Huelsenbeck JP, Ronquist F, 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**, 754–5.

James R, Ray J, Tan Y, Shivas R, 2014. *Colletotrichum siamense*, *C. theobromicola* and *C. queenslandicum* from several plant species and the identification of *C. asianum* in the Northern Territory, Australia. *Australasian Plant Disease Notes* **9**, 138.

Johnston PR, Jones D, 1997. Relationships among *Colletotrichum* isolates from fruit-rots assessed using rDNA sequences. *Mycologia* **89**, 420–30.

Kearse M, Moir R, Wilson A *et al.*, 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**, 1647–9.

Kim KD, Oh BJ, Yang J, 1999. Differential interactions of a *Colletotrichum gloeosporioides* isolate with green and red pepper fruits. *Phytoparasitica* **27**, 97–106.

Larkin MA, Blackshields G, Brown NP *et al.*, 2007. clustalW and clustalX version 2.0. *Bioinformatics* **23**, 2947–8.

Liu F, Weir BS, Damm U *et al.*, 2015. Unravelling *Colletotrichum* species associated with *Camellia*: employing ApMat and GS loci to resolve species in the *C. gloeosporioides* complex. *Persoonia* **35**, 63–86.

Mongkolporn O, Taylor PWJ, 2011. Capsicum. In: Cole C, ed. *Wild Crop Relatives: Genomic and Breeding Resources. Vegetables*. Berlin, Germany: Springer, 43–57.

Mongkolporn O, Montri P, Supakaew T, Taylor PWJ, 2010. Differential reactions on mature green and ripe chili fruit infected by three *Colletotrichum* spp. *Plant Disease* **94**, 306–10.

Montri P, Taylor PWJ, Mongkolporn O, 2009. Pathotypes of *Colletotrichum capsici*, the causal agent of chili anthracnose, in Thailand. *Plant Disease* **93**, 17–20.

Nylander JAA, 2004. *MrModelTest v2. Program Distributed by the Author*. Uppsala, Sweden: Evolutionary Biology Centre, Uppsala University.

Page RD, 1996. TreeView: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* **12**, 357–8.

Phoulivong S, McKenzie EHC, Hyde KD, 2012. Cross infection of *Colletotrichum* species; a case study with tropical fruits. *Current Research in Environmental and Applied Mycology* **2**, 99–111.

Ranathunge NP, Ford R, Taylor PWJ, 2009. Development and optimization of sequence-tagged microsatellite site markers to detect genetic diversity within *Colletotrichum capsici*, a causal agent of chilli pepper anthracnose disease. *Molecular Ecology Resources* **9**, 1175–9.

Ranathunge NP, Mongkolporn O, Ford R, Taylor PWJ, 2012. *Colletotrichum truncatum* pathosystem on *Capsicum* spp: infection, colonization and defence mechanisms. *Australasian Plant Pathology* **41**, 463–73.

Rayner RW, 1970. *A Mycological Colour Chart*. Kew, UK: Commonwealth Mycological Institute/British Mycological Society.

Ronquist F, Teslenko M, van der Mark P *et al*., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**, 539–42.

Sharma G, Shenoy BD, 2014. *Colletotrichum fructicola* and *C. siamense* are involved in chilli anthracnose in India. *Archives of Phytopathology and Plant Protection* **47**, 1179–94.

Sharma PN, Kaur M, Sharma OP, Sharma P, Pathania A, 2005. Morphological, pathological and molecular variability in *Colletotrichum capsici*, the cause of fruit rot of chilies in the subtropical region of North-Western India. *Journal of Phytopathology* **153**, 232–7.

Sharma PN, Katoch A, Sharma P, Sharma SK, Sharma OP, 2011. First report on association of *Colletotrichum coccodes* with chili anthracnose in India. *Plant Disease* **95**, 1584.

Shivas RG, Tan YP, 2009. A taxonomic re-assessment of *Colletotrichum acutatum*, introducing *C. fioriniae* comb. et stat. nov. and *C. simmondsii* sp. nov. *Fungal Diversity* **39**, 111–22.

Silva DN, Talhinhas P, Várzea V, Cai L, Paulo OS, Batista D, 2012. Application of the Apn2/MAT locus to improve the systematics of the *Colletotrichum gloeosporioides* complex: an example from coffee (*Coffea* spp.) hosts. *Mycologia* **104**, 396–409.

Simmonds JH, 1968. Type specimens of *Colletotrichum gloeosporioides* var. *minor* and *Colletotrichum acutatum*. *Queensland Journal of Agricultural and Animal Science* **25**, 178A.

Stamatakis A, Hoover P, Rougemont J, 2008. A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* **57**, 758–71.

Stephenson SA, Green JR, Manners JM, Maclean DJ, 1997. Cloning and characterisation of glutamine synthetase from *Colletotrichum gloeosporioides* and demonstration of elevated expression during pathogenesis on *Stylosanthes guianensis*. *Current Genetics* **31**, 447–54.

Than PP, Jeewon R, Hyde KD, Pongsupasamit S, Mongkolporn O, Taylor PWJ, 2008. Characterization and pathogenicity of *Colletotrichum* species associated with anthracnose on chilli (*Capsicum* spp.) in Thailand. *Plant Pathology* **57**, 562–72.

Udayanga D, Manamgoda DS, Liu X, Chukeatirote E, Hyde KD, 2013. What are the common anthracnose pathogens of tropical fruits? *Fungal Diversity* **61**, 165–79.

Voorrips RE, Finkers R, Sanjaya L, Groenwold R, 2004. QTL mapping of anthracnose (*Colletotrichum* spp.) resistance in a cross between *Capsicum annuum* and *C. chinense*. *Theoretical and Applied Genetics* **109**, 1275–82.

Weir BS, Johnston PR, Damm U, 2012. The *Colletotrichum gloeosporioides* species complex. *Studies in Mycology* **73**, 115–80.

White TJ, Bruns T, Lee S, Taylor J, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Gelfand DH, Innis MA, Sninsky JJ, White TJ, eds. *PCR Protocols: A Guide to Methods and Applications*. New York, NY, USA: Academic Press, 315–22.

Woudenberg JHC, Aveskamp MM, de Gruyter J, Spiers AG, Crous PW, 2009. Multiple *Didymella* teleomorphs are linked to the *Phoma clematidina* morphotype. *Persoonia* **22**, 56–62.