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Molecular and phenotypic characterization revealed six *Colletotrichum* species responsible for anthracnose disease of small cardamom in South India

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Abstract Small cardamom (*Elettaria cardamomum* Maton) is extensively cultivated in the Western Ghats of South India either as a monocrop under the forest trees or as an intercrop along with arecanut and coffee plantations. *Colletotrichum* species responsible for severe outbreaks of anthracnose on small cardamom in South India are reported. Small cardamom anthracnose, popularly known as "Chenthal", manifests itself on the foliage as yellowish lesions, which later coalesce to form large blighted areas. In advanced stages, the affected leaves dry up giving a burnt appearance to the plant. Twenty-five isolates of *Colletotrichum* were isolated from leaves of small cardamom in Karnataka, Kerala and Tamil Nadu states of India. The isolates were characterized through morphological studies and multilocus phylogenetic analysis (*ITS, ACT, CHS-1, GAPDH, TUB2, CYLH3, GS* and *ApMat* gene regions) to test whether different species are present and identified: *C. karstii* (2 isolates), *C. gloeosporioides* (1), *C. siamense* (7), *C. syzygicola* (6), *Colletotrichum* sp (5), and *C. guajavae* (4), as the cause of anthracnose on small cardamom for the first time. Pathogenicity of the six species was confirmed. To our knowledge, this is the first detailed study of *Colletotrichum* species which cause anthracnose diseases on small cardamom.

Keywords Small cardamom · *Elettaria cardamomum* · Anthracnose · *Colletotrichum* · Multilocus phylogeny

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Introduction

Small cardamom (*Elettaria cardamomum* Maton), belonging to the family Zingiberaceae, is the world's third-most-expensive spice crop (Reyes et al. 2006). It is popularly known as the "Queen of spices" and mainly used in culinary and confectionery preparations apart from medicine and perfumes (Reyes et al. 2006). Guatemala, with a production of 23,000 tonnes, is the largest producer of cardamom followed by India and Tanzania (Sasikumar et al. 2012). Other producers include Sri Lanka, Papua New Guinea, El Salvador, Laos, and Vietnam. In India, small cardamom has been commercially cultivated in the hilly tracts of Karnataka, Kerala and Tamil Nadu states for 150 years and it was responsible for establishing the sea route from Europe to the Far East. It is grown in an area of 0.69 million ha either as a mono crop under the shade of forest trees or as an intercrop along with arecanut and coffee with production of 9,470 MT (www.indianspices.com).

Leaf blight, commonly known as "Chenthal", has emerged as the most destructive foliar disease affecting

small cardamom in India (Praveena et al. 2013). The symptoms initially manifest on the leaves as yellow lesions which later elongate to form necrotic streaks that run parallel to the veins. Several such lesions later coalesce to form yellowish-brown to reddish-brown patches, which subsequently wither off. In the advanced stages of disease development, lesions develop on both young as well as older leaves which dry up and give a burnt appearance to the affected plants. The disease, which appears during mid-monsoon, becomes severe during late monsoon periods and declines by March. Intermittent rains and prevalence of misty conditions in the plantations favour the incidence and spread of the disease. Earlier studies reported *Colletotrichum gloeosporioides* as the casual organism responsible for anthracnose on small cardamom based on morphology (Govindaraju et al. 1998; Praveena and Biju 2012; Saju et al. 2013).

Traditional methods of characterization and identification of *Colletotrichum* based on morphology, optimal temperature for mycelial growth, host affiliation and benomyl sensitivity (Cai et al. 2009; Freeman et al. 1998; Peres et al. 2005; Sutton 1992) are time- consuming, tedious and are not reliable (Damm et al. 2009; Freeman et al. 1998). Molecular methods involving sequence analysis of the *ITS* region of the rDNA region (Brown et al. 1996; Mills et al. 1992; Sreenivasaprasad et al. 1994), restriction fragment length polymorphism analysis of rDNA, analysis of A + T-rich mitochondrial DNA, comparison of arbitrarily primed (AP)-PCR or random amplified polymorphic DNA fingerprints (Freeman et al. 1993, 1998, 2000; Talhinhas et al. 2005) have been used to differentiate populations of *Colletotrichum* species. In view of the recent changes on the species concepts in *Colletotrichum* (Cai et al. 2009; Hyde et al. 2009; Prihastuti et al. 2009; Phoulivong et al. 2010; Wikee et al. 2011; Cannon et al. 2012; Damm et al. 2012a, b; Weir et al. 2012), the use of a polyphasic approach involving morphology and multiple-locus sequence comparisons of different genes (e.g., partial β-tubulin, actin, calmodulin, glutamine synthase, and glyceraldehyde-3-phosphate-dehydrogenase) have been advocated for resolving the closely-related *Colletotrichum* species. The objective of the present study was to characterize the species of *Colletotrichum* responsible for severe outbreaks of anthracnose on small cardamom in India based on the polyphasic taxonomic approach.

Materials and methods

Collection of anthracnose-affected samples

Small cardamom leaves showing typical symptoms of anthracnose (Fig. 1) were collected from small production fields located in Karnataka, Kerala and Tamil Nadu states of South India, where severe disease epidemics occurred, during the southwest monsoon period (June to September) of 2011 and 2012 (Fig. 2). When most of the small cardamom fields in these localities were infected, leaves with single lesions were collected. Each sample consisted of three infected leaves from each of five plants within a disease focus. The details of the isolates used in this study are shown in Table 1.

Isolation of *Colletotrichum* species

To obtain fungal isolates, leaves were washed in sterile distilled water and dried with sterilized tissue paper. Then, a small piece (5 by 5 mm) of foliage taken from the leading edge of the infected area was washed in sterile distilled water, surface disinfected in 70 % ethanol for 30s and 1 % sodium hypochlorite for 1 min, rinsed three times in sterile distilled water and placed on Potato dextrose agar (PDA) amended with streptomycin (100 μg/ml). Plates were incubated at 25 ± 1 °C with a 12–h photoperiod provided by fluorescent light for 5 days. The growing edges of fungal hyphae developing from the tissues were then transferred aseptically to PDA. Single-spore isolates were derived according to the method described by Goh (1999). Pure cultures were maintained on PDA slants at 5 °C by sub-culturing at 4-week intervals. For long-term storage, three agar plugs (3 mm diam) from actively growing cultures on PDA were suspended in 5 ml of 20 % glycerol: 17 % skimmed milk (1:1) solution and stored at -80 ° C (Chowdappa et al. 2009).

Morphological assessments

For morphological analyses, 5-mm-diameter mycelial plugs from the actively growing margin of 5 day old

Fig. 1 Symptoms of small cardamom anthracnose

cultures were placed in the centre of 90-mm Petri plates containing 15 ml of PDA (Himedia, Mumbai, India) and incubated at 25 ± 1 °C under continuous fluorescent light for 7 days. The colony colour and diameter of the isolates were recorded during 7 days. The colony colour was scored using the mycological colour chart (Rayner 1970). The colony diameter was measured daily and the mycelial growth rate (mm day^{-1}) was calculated. After 7 days, conidia were directly harvested from cultures and mounted in sterile distilled water. Appressoria were

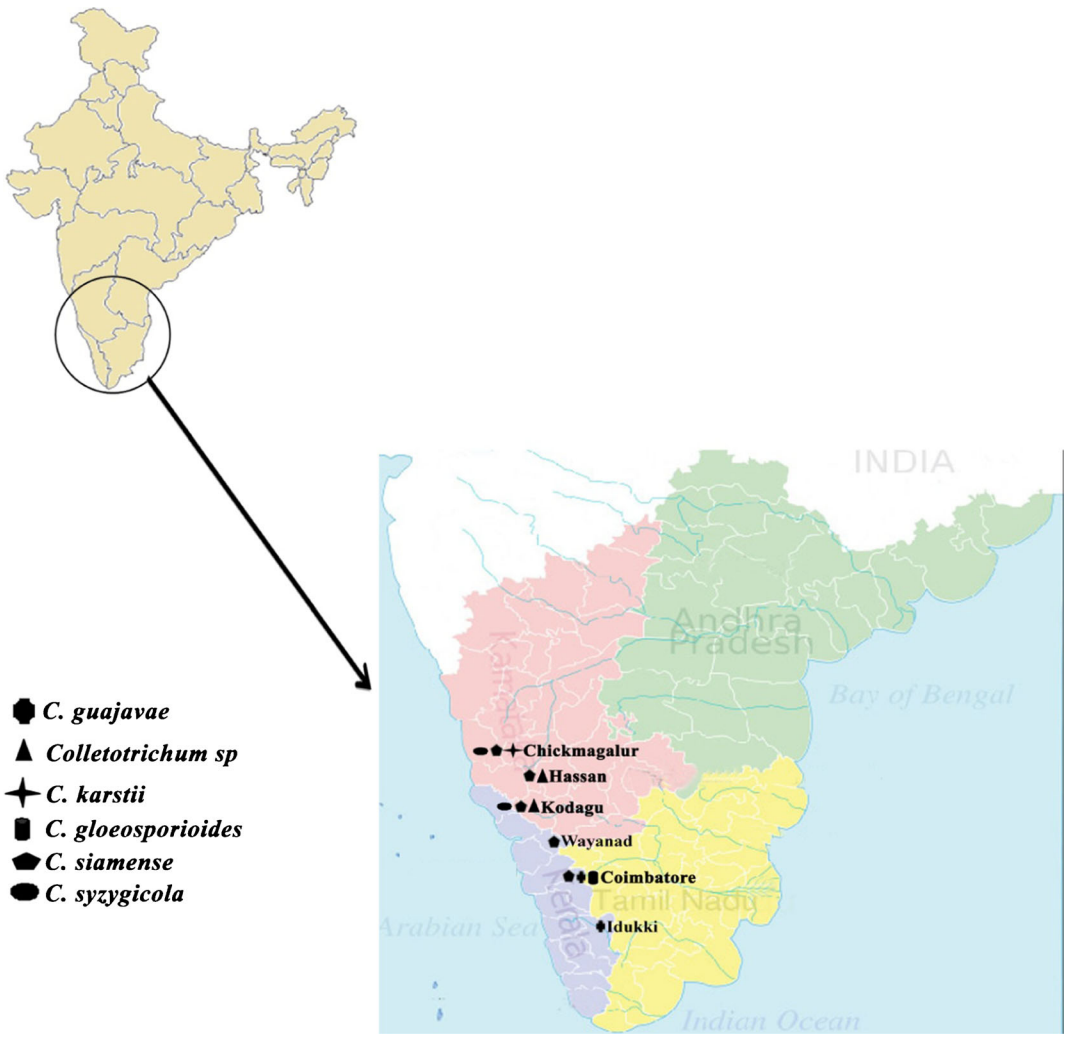

Fig. 2 A map showing the regions of anthracnose-affected small cardamom in South India

Table 1 Details of *Colletotrichum* isolates used in this study with the 25 new isolates of small cardamom from India included in bold

Species	Culture accession no.	Host	Location	Gen bank accession no.								Reference
				ITS	GAPDH	ACT	CHS-1	GS	TUB2	CYLH3	AP-MAT	
C. acerbum	CBS 128530, ICMP 12921	*Malus domestica, bitter rot of fruit*	New Zealand	JQ948459	JQ948790	JQ949780	JQ949120	–	JQ950110	JQ949450	–	Damm et al. 2012
C. acutatum	CBS 110735	*Pinus radiata*	South Africa	JQ948354	JQ949675	JQ948685	JQ949345	–	JQ949015	JQ950005	–	Damm et al. 2012
C. aenigma	ICMP 18686	*Pyrus pyrifolia*	Japan	JX010243	JX009913	JX009519	JX009789	JX010079	JX010390	–	–	Weir et al. 2012
C. aeschynomenes	ICSMP 17673	*Aeschynomene virginica*	USA	JX010176	JX009930	JX009483	JX009799	JX010081	JX010392	–	–	Weir et al. 2012
C. alatae	ICMP 18122	*Dioscorea alata*	Nigeria	JX010191	JX010011	JX009470	JX009846	JX010136	JX010449	–	–	Weir et al. 2012
C. alienum	ICMP 12068	*Malus domestica*	New Zealand	JX010255	JX009925	JX009492	JX009889	–	–	–	–	Weir et al. 2012
C. annellatum	CBS 129826	*Hevea brasiliensis, leaf*	Colombia	JQ005222	JQ005309	JQ005570	JQ005396	–	JQ005656	JQ005483	–	Damm et al. 2012
C. aotearoa	ICMP 17324	*Kunzea ericoides*	New Zealand	JX010198	JX009991	JX009538	JX009770	JX010109	JX010418	–	–	Weir et al. 2012
C. asianum	ICMP 18696	*Mangifera indica*	Australia	JX010192	JX009915	JX009576	JX009753	JX010073	JX010384	–	–	Weir et al. 2012
C. austral	CBS 116478	*Trachycarpus fortunei*	South Africa	JQ948455	JQ948786	JQ949776	JQ949116	–	JQ950106	JQ949446	–	Damm et al. 2012
C. beeveri	CBS 128527, ICMP 18594	*Brachyglottis repanda*	New Zealand	JQ005171	JQ005258	JQ005345	JQ005519	–	JQ005605	JQ005432	–	Damm et al. 2012
C. boninense	CBS 112115	*Leucospermum sp.*	Australia	JQ005160	JQ005247	JQ005508	JQ005334	–	JQ005594	JQ005421	–	Damm et al. 2012
C. brasiliense	CBS 128501, ICMP 18607	*Passiflora edulis, fruit anthracnose*	Brazil	JQ005235	JQ005322	JQ005583	JQ005409	–	JQ005669	JQ005496	–	Damm et al. 2012
C. brassicicola	CBS 101059	*Brassica oleracea var. gemmifera, leaf spot*	New Zealand	JQ005172	JQ005259	JQ005520	JQ005346	–	JQ005606	JQ005433	–	Damm et al. 2012
C. brisbanense	CBS 292.67	*Capsicum annuum*	Australia	JQ948291	JQ948621	JQ949612	JQ948952	–	JQ949942	JQ949282	–	Damm et al. 2012
C. chrysanthemi	CBS 126519	*Chrysanthemum coronarium, vascular discoloration*	Netherlands	JQ948272	JQ948602	JQ949593	JQ948933	–	JQ949923	JQ949263	–	Damm et al. 2012
C. clidemiae	ICMP 18706	*Vitis sp.*	USA	JX010274	JX009909	JX009476	JX009777	JX010128	JX010439	–	–	Weir et al. 2012
C. colombiense	CBS 129817	*Passiflora edulis, leaf*	Colombia	JQ005173	JQ005260	JQ005521	JQ005347	–	JQ005607	JQ005434	–	Damm et al. 2012
C. constrictum	CBS 128503, ICMP 12936	*Citrus limon*, fruit rot	New Zealand	JQ005237	JQ005324	JQ005585	JQ005411	–	JQ005671	JQ005498	–	Weir et al. 2012
C. cordylinicola	ICMP 18579	*Cordyline fruticosa*	Thailand	JX010226	JX009975	HM470235	JX009864	JX010122	JX010440	–	JQ899274	Weir et al. 2012
C. cordylinicola	LC856/ BCC 38872	Codyline fruticosa	–	HM470246	HM470240	HM470234	–	HM470243	HM47029	–	–	Phoulivong et al. 2010
C. cosmic	CBS 853.73	*Cosmos* sp., seed	Netherlands	JQ948274	JQ948604	JQ949595	JQ948935		JQ949925	JQ949265	–	Damm et al. 2012
C. costaricense	CBS 330.75	*Coffea arabica,* cv. Typica, berry	Costa Rica	JQ948180	JQ948510	JQ949501	JQ948841	–	JQ949831	JQ949171	–	Damm et al. 2012
C. cuscutae	IMI 304802	*Cuscuta* sp.	Dominica	JQ948195	JQ948525	JQ949516	JQ948856	–	JQ949846	JQ949186		Damm et al. 2012
C. cymbidiicola	CBS 123757 MAFF 306100	*Cymbidium* sp.	Japan	JQ005168	JQ005255	JQ005516	JQ005342	–	JQ005602	JQ005429	–	Damm et al. 2012
C. dacrycarpi	CBS 130241 ICMP 19107	*Dacrycarpus dacrydioides*, leaf endophyte	New Zealand	JQ005236	JQ005323	JQ005584	JQ005410	–	JQ005670	JQ005497	–	Damm et al. 2012
C. fioriniae	ATCC 12097, CPC 19392	*Rhododendron* sp.	USA	JQ948307	JQ948637	JQ949628	JQ948968	–	JQ949958	JQ949298	–	Damm et al. 2012
C. fructicola	ICMP 12568	*Persea americana*	Australia	JX010166	JX009946	JX009529	JX009762	–			–	Weir et al. 2012

Table 1 (continued)

Species	Culture accession no.	Host	Location	Gen bank accession no.								Reference
				ITS	GAPDH	ACT	CHS-1	GS	TUB2	CYLH3	AP-MAT	
C. gloeosporioides	IMI 356878	*Citrus sinensis*	Italy	JX010152	JX010056	JX009531	JX009818	JX010085	JX010445	–	–	Weir et al. 2012
C. gloeosporioides	CBS 119204, ICMP 18678	*Pueraria lobata*	USA	JX010150	JX010013	JX009502	JX009790	–	–	–	–	Weir et al. 2012
C. gloeosporioides	**OCAC24**	***Elettaria cardamomum***	**Tamil nadu, India**	**KJ813602**	**KJ813552**	**KJ813452**	**KJ813502**	**KJ813577**	**KJ813477**	**KJ813527**	**KP743487**	**This study**
C. godetiae	CBS 125972, PD 85/456	*Fragaria × ananassa*	Ireland	JQ948423	JQ948754	JQ949744	JQ949084	–	JQ950074	JQ949414	–	Damm et al. 2012
C. graminicola	CBS 130836, M 1.001	*Zea mays*	USA	JQ005767	–	JQ005830	JQ005788	–	JQ005851	HQ005809	DQ002857	Cannon et al. 2012
C. guajavae	IMI 350839	*Psidium guajava*, fruit	India	JQ948270	JQ948600	JQ949591	JQ948931	–	JQ949921	JQ949261	–	Damm et al. 2012
C. guajavae	**OCAC23**	***Elettaria cardamomum***	**Tamil nadu, India**	**KJ813590**	**KJ813540**	**KJ813440**	**KJ813490**	**KJ813565**	**KJ813465**	**KJ813515**	**–**	**This study**
C. guajavae	**OCAC25**	***Elettaria cardamomum***	**Tamil nadu, India**	**KJ813591**	**KJ813541**	**KJ813441**	**KJ813491**	**KJ813566**	**KJ813466**	**KJ813516**	**–**	**This study**
C. guajavae	**OCAC9**	***Elettaria cardamomum***	**Kerala, India**	**KJ813592**	**KJ813542**	**KJ813442**	**KJ813492**	**KJ813567**	**KJ813467**	**KJ813517**	**–**	**This study**
C. guajavae	**OCAC1**	***Elettaria cardamomum***	**Kerala, India**	**KJ813593**	**KJ813543**	**KJ813443**	**KJ813493**	**KJ813568**	**KJ813468**	**KJ813518**	**–**	**This study**
C. hippeastri	CBS 125376	*Hippeastrum vittatum*, leaf	China	JQ005231	JQ005318	JQ005579	JQ005405	–	JQ005665	JQ005492	–	Damm et al. 2012
C. horii	ICMP 12942	*Diospyros kaki*	New Zealand	GQ329687	GQ329685	JX009533	JX009748	JX010072	JX010375	–	–	Weir et al. 2012
C. indonesiense	CBS 127551	*Eucalyptus* sp.	Indonesia	JQ948288	JQ948618	JQ949609	JQ948949	–	JQ949939	JQ949279	–	Damm et al. 2012
C. johnstonii	IMI 357027	*Citrus* sp.	New Zealand	JQ948443	JQ948774	JQ949764	JQ949104	–	JQ950094	JQ949434	–	Damm et al. 2012
C. kahawae subsp. Ciggaro	ICMP 19122	*Vaccinium sp.*	USA	JX010228	JX009950	JX009536	JX009902	JX010134	JX010433	–	–	Weir et al. 2012
C. kahawae subsp. Ciggaro	ICMP 18728	*Miconia* sp.	Brazil	JX010239	JX010048	JX009525	JX009850	–	–	–	–	Weir et al. 2012
C. kahawae subsp. Kahawae	ICMP 17816	*Coffea arabica*	Kenya	JX010231	JX010012	JX009452	JX009813	JX010130	JX010444	–	–	Weir et al. 2012
C. kahawae subsp. Kahawae	CBS 982.69	*Coffea arabica*	Kenya	JX010234	JX010040	JX009474	JX009829	JX010125	JX010435	–	–	Weir et al. 2012
C. kahawae	IMI 319418	*Coffea arabica*	Kenya	FJ 972608	FJ 972583	FJ 907432	–	FJ 972588	FJ 907446	–	–	Prihastuti et al. 2009
C. kahawae	IMI 363578	*Coffea arabica*	Kenya	FJ 972607	FJ 972584	FJ 907433	–	FJ 972587	FJ 907447	–	–	Prihastuti et al. 2009
C. karstii	CBS 106.91	*Carica papaya, fruit spots*	Brazil	JQ005220	JQ005307	JQ005568	JQ005394	–	JQ005654	JQ005481	–	Damm et al. 2012
C. karstii	CBS 111998	*Leucospermum sp.*	Australia	JQ005212	JQ005299	JQ005560	JQ005386	–	JQ005646	JQ005473	–	Damm et al. 2012
C. karstii	**OCAC10**	***Elettaria cardamomum***	**Karnataka, India**	**KJ813594**	**KJ813544**	**KJ813444**	**KJ813494**	**KJ813569**	**KJ813469**	**KJ813519**	**–**	**This study**
C. karstii	**OCAC4**	***Elettaria cardamomum***	**Karnataka, India**	**KJ813595**	**KJ813545**	**KJ813445**	**KJ813495**	**KJ813570**	**KJ813470**	**KJ813520**	**–**	**This study**
C. kinghornii	CBS 198.35	*Phormium* sp.	UK	JQ948454	JQ948785	JQ949775	JQ949115	–	JQ950105	JQ949445	–	Damm et al. 2012
C. laticiphilum	CBS 112989	*Hevea brasiliensis*	India	JQ948289	JQ948619	JQ949610	JQ948950	–	JQ949940	JQ949280	–	Damm et al. 2012
C. limetticola	CBS 114.14	*Citrus aurantifolia*, young twig	USA, Florida	JQ948193	JQ948523	JQ949514	JQ948854	–	JQ949844	JQ949184	–	Damm et al. 2012

Table 1 (continued)

Species	Culture accession no.	Host	Location	Gen bank accession no.								Reference
				ITS	GAPDH	ACT	CHS-1	GS	TUB2	CYLH3	AP-MAT	
C. lupine	CBS 109216	*Lupinus mutabilis*	Bolivia	JQ948156	JQ948486	JQ949477	JQ948817	–	JQ949807	JQ949147	–	Damm et al. 2012
C. musae	ICMP 12930	*Musa* sp.	New Zealand	JX010141	JX009986	JX009566	JX009881	–	–	–	–	Weir et al. 2012
C. novae-zelandiae	CBS 130240, ICMP 12064	*Citrus* sp. (grapefruit)	New Zealand	JQ005229	JQ005316	JQ005577	JQ005403	–	JQ005663	JQ005490	–	Damm et al. 2012
C. nupharicola	ICMP 17938	*Nuphar lutea subsp. Polysepala*	USA	JX010189	JX009936	JX009486	JX009834	JX010087	JX010397	–	–	Weir et al. 2012
C. nymphaeae	CBS 100064	*Anemone* sp.	Netherlands	JQ948224	JQ948554	JQ949545	JQ948885	–	JQ949875	JQ949215	–	Damm et al. 2012
C. oncidii	CBS 129828	*Oncidium* sp., leaf	Germany	JQ005169	JQ005256	JQ005517	JQ005343	–	JQ005603	JQ005430	–	Damm et al. 2012
C. parsonsiae	CBS 128525, ICMP 18590	*Parsonsia capsularis*, leaf endophyte	New Zealand	JQ005233	JQ005320	JQ005581	JQ005407	–	JQ005667	JQ005494	–	Damm et al. 2012
C. paxtonii	IMI 165753	*Musa* sp.	Saint Lucia	JQ948285	JQ948615	JQ949606	JQ948946	–	JQ949936	JQ949276	–	Damm et al. 2012
C. petchii	CBS 125957	*Dracaena*, leaf spots	Netherlands	JQ005226	JQ005313	JQ005574	JQ005400	–	JQ005660	JQ005487	–	Damm et al. 2012
C. phormii	CBS 118191	*Phormium* sp., leaf	South Africa	JQ948453	JQ948784	JQ949774	JQ949114	–	JQ950104	JQ949444	–	Damm et al. 2012
C. phyllanthi	CBS 175.67	*Phyllanthus acidus*	India	JQ005221	JQ005308	JQ005569	JQ005395	–	JQ005655	JQ005482	–	Damm et al. 2012
C. psidii	ICMP 19120	*Psidium sp.*	Italy	JX010219	JX009967	JX009515	JX009901	JX010133	JX010443	–	KC888931	Weir et al. 2012
C. pyricola	CBS 128531, ICMP 12924	*Pyrus communis*, fruit rot	New Zealand	JQ948445	JQ948776	JQ949766	JQ949106	–	JQ950096	JQ949436	–	Damm et al. 2012
C. rhombiforme	CBS 129953	*Olea europaea*	Portugal	JQ948457	JQ948788	JQ949778	JQ949118	–	JQ950108	JQ949448	–	Damm et al. 2012
C. salicis	CBS 113.14	*Malus domestica, cv. Manks Küchenapfel, fruit*	Germany	JQ948478	JQ948809	JQ949799	JQ949139	–	JQ950129	JQ949469	–	Damm et al. 2012
C. scovillei	CBS 120708	*Capsicum annuum*	Thailand	JQ948269	JQ948599	JQ949590	JQ948930	–	JQ949920	JQ949260	–	Damm et al. 2012
C. scovillei	CBS 126529	*Capsicum sp.*	Indonesia	JQ948267	JQ948597	JQ949588	JQ948928	–	JQ949918	JQ949258	–	Damm et al. 2012
C. siamense	ICMP 12567	*Persea americana*	Australia	JX010250	JX009940	JX009541	JX009761	JX010076	JX010387	–	–	Weir et al. 2012
C. siamense	ICMP 17795	*Malus domestica*	USA	JX010162	JX010051	JX009506	JX009805	JX010082	JX010393	–	–	Weir et al. 2012
C. siamense	**OCAC8**	***Elettaria cardamomum***	**Tamil nadu, India**	**KJ813608**	**KJ813558**	**KJ813458**	**KJ813508**	**KJ813583**	**KJ813483**	**KJ813533**	**KP743480**	**This study**
C. siamense	**OCAC14**	***Elettaria cardamomum***	**Karnataka, India**	**KJ813609**	**KJ813559**	**KJ813459**	**KJ813509**	**KJ813584**	**KJ813484**	**KJ813534**	**KP743481**	**This study**
C. siamense	**OCAC3**	***Elettaria cardamomum***	**Karnataka, India**	**KJ813610**	**KJ813560**	**KJ813460**	**KJ813510**	**KJ813585**	**KJ813485**	**KJ813535**	**KP743482**	**This study**
C. siamense	**OCAC19**	***Elettaria cardamomum***	**Karnataka, India**	**KJ813611**	**KJ813561**	**KJ813461**	**KJ813511**	**KJ813586**	**KJ813486**	**KJ813536**	**KP743483**	**This study**
C. siamense	**OCAC22**	***Elettaria cardamomum***	**Karnataka, India**	**KJ813612**	**KJ813562**	**KJ813462**	**KJ813512**	**KJ813587**	**KJ813487**	**KJ813537**	**KP743484**	**This study**
C. siamense	**OCAC21**	***Elettaria cardamomum***	**Karnataka, India**	**KJ813613**	**KJ813563**	**KJ813463**	**KJ813513**	**KJ813588**	**KJ813488**	**KJ813538**	**KP743485**	**This study**
C. siamense	**OCAC16**	***Elettaria cardamomum***	**Kerala, India**	**KJ813614**	**KJ813564**	**KJ813464**	**KJ813514**	**KJ813589**	**KJ813489**	**KJ813539**	**KP743486**	**This study**
C. simmondsii	CBS 111531	*Protea cynaroides*	USA	JQ948282	JQ948612	JQ949603	JQ948943	–	JQ949933	JQ949273	–	Damm et al. 2012
C. sloanei	IMI 364297	*Theobroma cacao*, leaf	Malaysia	JQ948287	JQ948617	JQ949608	JQ948948	–	JQ949938	JQ949278	–	Damm et al. 2012

Table 1 (continued)

Species	Culture accession no.	Host	Location	Gen bank accession no.								Reference
				ITS	GAPDH	ACT	CHS-1	GS	TUB2	CYLH3	AP-MAT	
C. syzygicola	DNCL021/ MFLUCC 10-0624	Syzygium samarangense	Thailand	KF242094	KF242156	KF157801	–	KF242125	KF254880	–	–	Udayanga et al. 2013
C. syzygicola	DNCL018/ MFLUCC 10-0621	Citrus aurantifolia	Thailand	KF242093	KF242155	KF157800	–	KF242124	KF254879	–	–	Udayanga et al. 2013
C. syzygicola	**OCAC20**	***Elettaria cardamomum***	**Karnataka, India**	**KJ813596**	**KJ813546**	**KJ813446**	**KJ813496**	**KJ813571**	**KJ813471**	**KJ813521**	**KP743474**	**This study**
C. syzygicola	**OCAC18**	***Elettaria cardamomum***	**Karnataka, India**	**KJ813597**	**KJ813547**	**KJ813447**	**KJ813497**	**KJ813572**	**KJ813472**	**KJ813522**	**KP743475**	**This study**
C. syzygicola	**OCAC15**	***Elettaria cardamomum***	**Karnataka, India**	**KJ813598**	**KJ813548**	**KJ813448**	**KJ813498**	**KJ813573**	**KJ813473**	**KJ813523**	**KP743476**	**This study**
C. syzygicola	**OCAC7**	***Elettaria cardamomum***	**Karnataka, India**	**KJ813599**	**KJ813549**	**KJ813449**	**KJ813499**	**KJ813574**	**KJ813474**	**KJ813524**	**KP743477**	**This study**
C. syzygicola	**OCAC2**	***Elettaria cardamomum***	**Karnataka, India**	**KJ813600**	**KJ813550**	**KJ813450**	**KJ813500**	**KJ813575**	**KJ813475**	**KJ813525**	**KP743478**	**This study**
C. syzygicola	**OCAC12**	***Elettaria cardamomum***	**Karnataka, India**	**KJ813601**	**KJ813551**	**KJ813451**	**KJ813501**	**KJ813576**	**KJ813476**	**KJ813526**	**KP743479**	**This study**
C. tamarilloi	CBS 129811	*Solanum betaceum*, fruit	Colombia	JQ948185	JQ948515	JQ949506	JQ948846	–	JQ949836	JQ949176	–	Damm et al. 2012
C. theobromicola	ICMP 15445	*Acca sellowiana*	New Zealand	JX010290	JX010027	JX009509	JX009893	–	–	–	–	Weir et al. 2012
C. ti	ICMP 4832	*Cordyline* sp	New Zealand	JX010269	JX009952	JX009520	JX009898	JX010123	JX010442	–	–	Weir et al. 2012
C. torulosum	CBS 102667	*Passiflora edulis*, leaf blotch	New Zealand	JQ005165	JQ005252	JQ005513	JQ005339	–	JQ005599	JQ005426	–	Damm et al. 2012
C. tropicale	CBS 124943, ICMP 18651	*Annona muricata*	Panama	JX010277	JX010014	JX009570	JX009868	–	–	–	–	Weir et al. 2012
C. walleri	CBS 125472	*Coffea* sp., leaf tissue	Vietnam	JQ948275	JQ948605	JQ949596	JQ948936	–	JQ949926	JQ949266	–	Damm et al. 2012
C. xanthorrhoeae	IMI 350817	*Xanthorrhoea* sp.	Australia	JX010260	JX010008	JX009479	JX009814	–	–	–	–	Weir et al. 2012
Glomerella cingulata "*f.sp. camelliae*"	ICMP 18542	*Camellia sasanqua*	USA	JX010223	JX009994	JX009488	JX009857	JX010118	JX010429	–	–	Weir et al. 2012
Colletotrichum sp.	**OCAC13**	***Elettaria cardamomum***	**Karnataka, India**	**KJ813603**	**KJ813553**	**KJ813453**	**KJ813503**	**KJ813578**	**KJ813478**	**KJ813528**	**KP743488**	**This study**
Colletotrichum sp.	**OCAC17**	***Elettaria cardamomum***	**Karnataka, India**	**KJ813604**	**KJ813554**	**KJ813454**	**KJ813504**	**KJ813579**	**KJ813479**	**KJ813529**	**KP743489**	**This study**
Colletotrichum sp.	**OCAC11**	***Elettaria cardamomum***	**Karnataka, India**	**KJ813605**	**KJ813555**	**KJ813455**	**KJ813505**	**KJ813580**	**KJ813480**	**KJ813530**	**KP743490**	**This study**
Colletotrichum sp.	**OCAC6**	***Elettaria cardamomum***	**Karnataka, India**	**KJ813606**	**KJ813556**	**KJ813456**	**KJ813506**	**KJ813581**	**KJ813481**	**KJ813531**	**KP743491**	**This study**
Colletotrichum sp.	**OCAC5**	***Elettaria cardamomum***	**Karnataka, India**	**KJ813607**	**KJ813557**	**KJ813457**	**KJ813507**	**KJ813582**	**KJ813482**	**KJ813532**	**KP743492**	**This study**

produced using the slide culture technique (Johnston and Jones 1997). PDA pieces of 3 cm square were kept on microscope slides and placed in an empty Petri dish containing filter paper moistened with sterile distilled water. Spores were inoculated on the edge of the agar on all four sides and a sterile cover slip was placed over the inoculated agar. The Petri plates were incubated at 25 ±1 °C on a laboratory bench sealed with parafilm. Appressoria were formed under the cover slip after 4-7 days. For each isolate, length and width of 100 randomly chosen conidia and appressoria were determined at × 400 magnification with a Zeiss bright field microscope using Axio Vision soft ware. For each isolate, three replicates were maintained and each replicate contained six Petri plates. The experiments were repeated thrice.

DNA extraction

Fungal isolates were grown in potato dextrose broth at 25 ± 1 °C for 7 days. Mycelia were harvested from liquid cultures through Whatman no.3 filter paper, dried and subsequently ground into a fine powder in a mortar and pestle using liquid nitrogen. DNA was extracted from mycelial powder according to the method of Raeder and Broda (1985) and slightly modified by Chowdappa et al. (2003) by incubating at 37 °C for 10 min after the phenol: chloroform: isoamyl alcohol (25:24:1) precipitation. This was followed by precipitation with 0.54 volumes of isopropyl alcohol and centrifugation at 10,000 rpm for 2 min. The DNA pellet was washed with 70 % cold ethanol, dried at room temperature overnight (16 h) and then re-suspended in 30 µl of 10 mM TE buffer (pH.8). DNA was stored at -20 °C.

PCR amplification and DNA sequencing

Eight loci including the partial rDNA-ITS (*ITS*), actin (*ACT*), chitin synthase 1 (*CHS-1*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), β-tubulin (*TUB2*), Histone (*CYLH3*), glutamine synthetase (*GS*) and *ApMat* were amplified and sequenced using the primer pairs *ITS-1 F* (Gardes and Bruns 1993) /*ITS-4* (White et al. 1990), *ACT512F/ACT783R* (Carbone and Kohn 1999), *CHS-79 F/CHS-354R* (Carbone and Kohn 1999), *GDF1/GDR1* (Templeton et al. 1992), *Bt2a/Bt2b* (Glass and Donaldson 1995), *CYLH3F/CYLH3R* (Crous et al. 2004), *GSF1/GSR1* (Stephenson et al. 1997) and *AM-F/AM-R* (Silva et al. 2012a), respectively.

Each 50-µl PCR mixture included 39.75 µl of PCR-grade water, 5 µl of reaction buffer, 1 µl of 2.5 mM of each dNTP, 1 µl of each primer (10pmol/µl), 1 µl of DNA template and 0.25 µl of Taq DNA polymerase. PCR reactions were carried out in a thermal cycler (Eppendorf Master cycler). The cycling parameters for *ITS* consisted of a denaturation step at 95 °C for 3 min followed by 35 cycles at 95 °C for 30 s, 55 °C for 45 s, 72 °C for 45 s and a final step at 72 °C for 15 min. The cycling parameters for *ACT* were initiated at 95 °C for 3 min followed by 35 cycles at 95 °C for 30 s, 54 °C for 45 s, 72 °C for 1 min and a final step at 72 °C for 15 min. The cycling parameters for partial *CHS* and *CYLH3* region consisted of a 4 min denaturing step at 95 °C followed by 35 cycles at 95 °C for 30 s, 58 °C for 45 s, 72 °C for 45 s and a final cycle of 15 min at 72 °C. *GAPDH* and *GS* consisted of 94 °C for 4 min, followed by 35 cycles at 94 °C for 45 s, 60 °C for 45 s, 72 °C for 1 min and a final cycle at 72 °C for 15 min. The cycling parameters for *TUB2* consisted of 95 °C for 4 min followed by 35 cycles at 95 °C for 30 s, 54 °C for 45 s, 72 °C for 60 s and a final step at 72 °C for 15 min. The cycling parameters for *ApMat* were initiated at 94 °C for 3 min followed by 30 cycles at 94 °C for 45 s, 62 °C for 45 s, 72 °C for 1 min and a final step at 72 °C for 7 min. PCR amplification products were separated by 1.5 % agarose electrophoresis gels in 1.0 × TBE buffer and were observed under UV light after staining with ethidium bromide (0.5 µg/ ml). Amplified DNA products of *ITS, ACT, CHS-1, GAPDH, TUB2, CYLH3, GS* and *ApMat* were purified using a Nucleospin® gel and PCR Clean-up (Macherey-nagel, Germany). Products were sequenced using the sequencing service from Merck Specialities Pvt Ltd, Bangalore. Sequencing of each PCR product was performed in both directions. Sequencing reactions contained the same primers of all genes that were used in the PCR.

Phylogenetic analyses

Reference sequences downloaded from the Fungal Biodiversity Centre (CBS-KNAW) (http://www.cbs.

knaw.nl/*Colletotrichum*/) were included in the analyses along with the combined dataset of ITS, *ACT, CHS-1, GAPDH, TUB2, CYLH3, GS* and *ApMat* gene regions of our isolates (Table 1). Sequences were aligned with Clustal W, in Bioedit v.7.0.5.3 (Hall 1999) and manually adjusted where necessary, the alignment gaps were treated as missing data for each gene, then nucleotide alignments of all genes were concatenated in MEGA v.5 (Tamura et al. 2011). Nucleotide substitution models were determined using Mr-Model-test v.2.3 (Nylander 2004) for each gene region under the Akaike Information Criterion (AIC) and included in the analyses. Phylogenetic reconstructions of concatenated and individual gene trees were performed using Bayesian (BI) Markov Chain Monte Carlo (MCMC) and Maximum Likelihood (ML) criteria. Bayesian analyses (BI) were performed using Mr-Bayes v.3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). MCMC were run for 20 million generations and for every 1000 generations sampling was done with the first 50 % of samples discarded as burn-in. Maximum likelihood (ML) analyses were performed in RAxML v7.0.4 (Stamatakis 2006), all free modal parameters were estimated by RAxML for the concatenated dataset with ML estimate of 25 per site rate categories. In the RAxML platform, the concatenated dataset was partitioned by locus. The RAxML software accommodated the GTR model of nucleotide substitution with the additional options of modeling rate heterogeneity (Γ) and proportion invariable sites (I) and the thorough bootstrap algorithm of RAxML with 1000 replications was implemented with nodal support. Phylogenetic trees and data files were viewed in MEGA v. 5 (Tamura et al. 2011) and Fig-Tree v1.2.2 (Rambaut and Drummond 2008). The sequences of all small cardamom isolates used in multi-gene analyses were deposited in GenBank (Table 1).

Pathogenicity testing

The pathogenicity of the isolates was determined by inoculating intact small cardamom leaves of variety Appangala1 according to the method of Silva et al. (2012b) with slight modifications. Fully matured second leaves from the spindle leaf of one-year-old plants were inoculated. The leaves were surface sterilized by smearing with 1 % sodium hypochlorite solution before inoculation, then washed three times in sterile distilled water and subsequently air dried. The middle portions of the leaves were then pinpricked and placed on wire mesh platforms. Inoculation was carried out on the wounded leaves by placing 20 μl of a conidial suspension (10^6/ml) prepared from 20-day-old PDA cultures. The fungal inoculum was produced in Petri dishes containing PDA, which were incubated for 20 days at 28 °C under a 12-h photoperiod. Spore suspensions were prepared by adding 20 ml of sterile distilled water to the surface of the cultures, brushing with a soft bristle brush, and filtering through a double layer of cheesecloth. Spore concentration was determined using a haemocytometer and adjusted to 10^6 conidia ml^{-1} with sterile water. Leaves used as control were inoculated with 20 μl of sterile distilled water. After inoculation, the leaves were placed in moist chambers and maintained at 25 °C ± 1 °C, RH 90 % under a 14-h photoperiod for 7 days. The lesion size was recorded on each leaf seven days after the inoculation.

Results

Collection of isolates

Twenty-five isolates of *Colletotrichum* were obtained from small cardamom showing typical symptoms of anthracnose in Karnataka, Kerala and Tamil Nadu states of India (Figs. 1 & 2). The detailed morphological and multilocus phylogenetic analyses (*ITS, ACT, CHS-1, GAPDH, TUB2, CYLH3, GS* and *ApMat* genes) revealed six species: *C. karstii* (2 isolates), *C. gloeosporioides* (1), *C. siamense* (7), *C. syzygicola* (6), *Colletotrichum* sp (5), and *C. guajavae* (4).

Morphological characters

The *Colletotrichum* isolates clustered into six morphological groups based on colony characteristics, growth rate and conidial morphology (Fig. 3 and Table 2).

In the morphological group 1 (*C. karstii*) isolates, the upper surface color of the colony is pale olivaceous gray with a growth rate ranging from 8.7 to 11.1 mm with an average of 10.0 ± 0.7 mm/day. The conidia length and width varied from 14.2 to 16.1 × 5.1–7.0 μm (15.3 ± 0.8 × 6.0 ± 0.6 μm) respectively and were cylindrical, hyaline, straight and obtuse at both apexes. Appressoria were circular or clavate measuring 6.0–10.0 × 5.0–7.0 μm (7.8 ± 1.7 × 6.1 ± 0.9 μm).

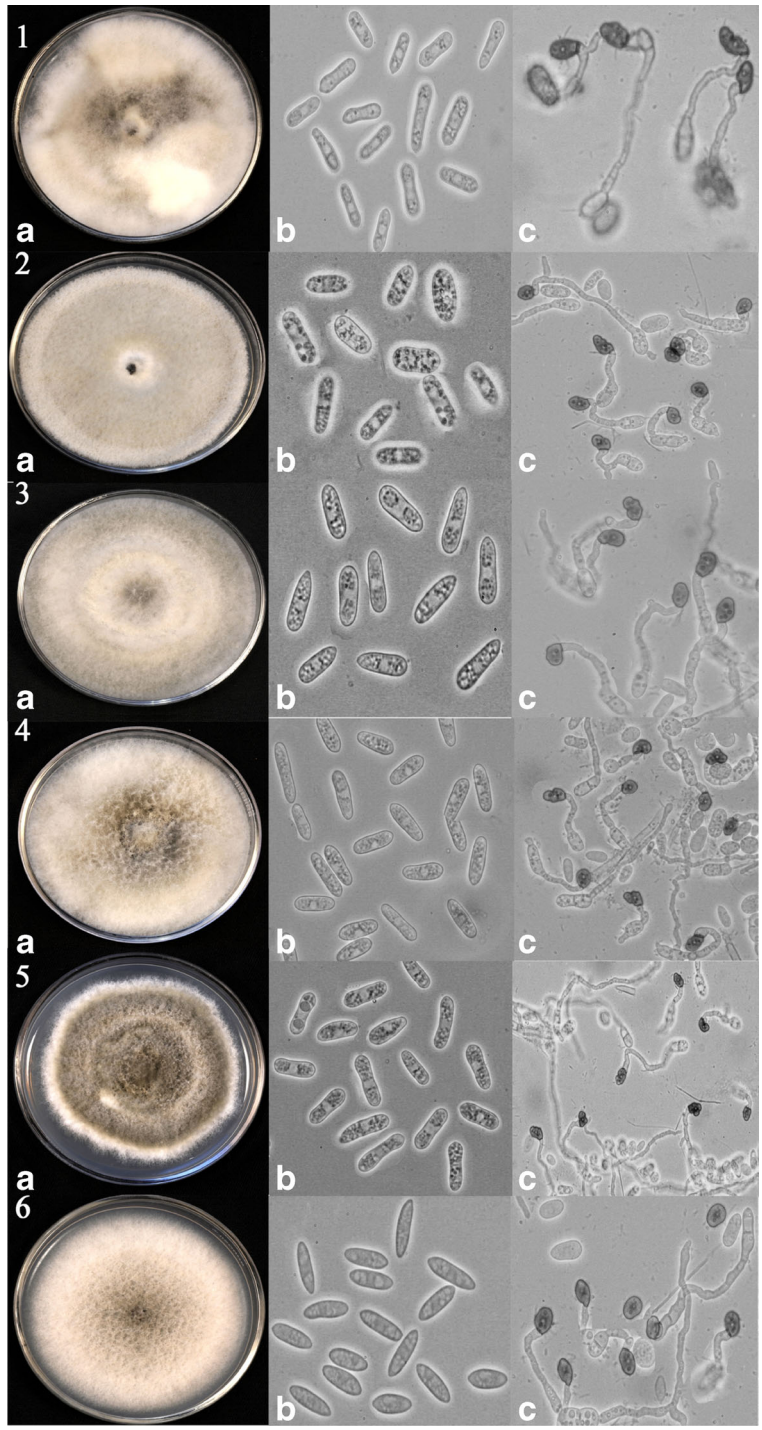

Fig. 3 Morphology of *Colletotrichum* species isolated from small cardamom in India 1) *C. karstii* 2) *C. gloeosporioides* 3) *C. siamense* 4) *C. syzygicola* 5) Unknown *Colletotrichum* sp. 6) *C. guajavae* **a**) Culture on PDA, **b**) conidia, **c**) appressoria

The isolates of morphological group 2 (*C. gloeosporioides*) produced pale olivaceous grey colonies on PDA with an average growth rate of 11.1 ±0.2 mm/day ranging from 7.8 to 11.1 mm. The conidia were all sub cylindrical with blunt round ends. The average length and width of the conidia were 12.5–2×4.5–6.1 μm (14.7±1.6×5.3±0.6 μm), respectively. Appressoria were clavate, regular or irregular in outline

Table 2 Morphological data of *Colletotrichum* isolates from small cardamom (*Elettaria cardamomum*) in India

Species	Colony morphology		Conidial morphology			Appressoria morphology	
	Growth rate (mm/day)	Colony color	Length (μm)	Width (μm)	Shape	Length x Width (μm)	Shape
C. karstii	10.0 ± 0.7 (8.7–11.1)	Pale olivaceous grey	15.3 ± 0.8 (14.2–16.1)	6.0 ± 0.6 (5.1–7.0)	Cylindrical, straight, obtuse at both apexes	7.8 ± 1.7 × 6.1 ± 0.9 (6.0–10.0 × 5.0–7.0)	Circular to clavate
C. gloeosporioides	11.1 ± 0.2 (7.8–11.1)	Olivaceous grey	14.7 ± 1.6 (12.5–17.2)	5.3 ± 0.6 (4.5–6.1)	Sub cylindrical with blunt round ends	9.4 ± 0.8 × 6.0 ± 0.6 (8.2–11.0 × 5.2–7.0)	Clavate, regular or irregular in outline and weakly lobed
C. siamense	11.0 ± 0.9 (7.5–11.0)	Greyish white, pale yellowish to pinkish	13.8 ± 3.1 (9.0–17.1)	4.7 ± 0.9 (3.0–6.0)	Fusiform to cylindrical	8.5 ± 2.7 × 6.0 ± 0.9 (4.8–12.3 × 4.9–7.1)	Ovoid
C. syzygicola	10.8 ± 1.3 (7.2–11.0)	White to grey	13.8 ± 1.2 (6.0–12.5)	5.6 ± 0.3 (5.2–6.1)	Ovoid to cylindrical with rounded apices	18.6 ± 0.7 × 7.4 ± 0.3 (17.7–19.7 × 7.1–7.9)	Circular to clavate and lobed
Unknown *Colletotrichum sp.*	10.7 ± 2.4 (7.0–11.0)	Grey to dark grey	12.1 ± 3.3 (8.5–16.7)	5.9 ± 1.0 (4.3–7.2)	Cylindrical, straight, apex rounded, often tapering slightly towards the base	8.0 ± 1.9 × 6.0 ± 0.8 (5.1–10.2 × 4.8–7.1)	Variable in shape, clavate to irregular and lobed
C. guajavae	10.7 ± 1.8 (7.2–11.0)	Pale olivaceous grey	8.8 ± 2.2 (6.0–12.5)	3.7 ± 0.7 (2.6–4.7)	Cylindrical to fusiform with both ends slightly acute with or without guttules	8.8 ± 0.5 × 5.2 ± 0.6 (8.1–9.5 × 4.5–6.1)	Subglobose or elliptical

and weakly lobed measuring 8.2–11.0 × 5.2–7.0 μm (9.4 ± 0.8 × 6.0 ± 0.6 μm).

The morphological group 3 isolates (*C. siamense*) on PDA exhibited dense grayish-white aerial mycelium, pale yellowish to pinkish colony with visible orange conidial masses at the inoculum point with a growth rate ranging from 7.5 to 11.0 mm with an average of 11.0 ± 0.9 mm/day. Setae were not found. Conidia were single-celled, smooth-walled, guttulate, hyaline, fusiform with obtuse to slightly rounded ends, sometimes cylindrical with 9.00–17.1 × 3.0–6.0 μm (13.8 ± 3.1 × 4.7 ± 0.9 μm) in size. Appressoria were brown and ovoid measuring 4.8–12.3 × 4.9–7.1 μm (8.5 ± 2.7 × 6.0 ± 0.9 μm).

Colonies of the morphological group 4 (*C. syzygicola*) isolates exhibited white to grey with orange conidial mass on the upper surface and grey on the reverse side with an average growth rate of 10.8 ± 1.3 mm/day ranging from 7.2 to 11.0 mm. Conidia were hyaline, ovoid to cylindrical with rounded apices 6.0–12.5 × 5.2–6.1 μm (13.8 ± 1.2 × 5.6 ± 0.3 μm). Appressoria were brown to dark brown, circular to clavate and lobed measuring 17.7–19.7 × 7.1–7.9 μm (18.6 ± 0.7 × 7.4 ± 0.3 μm).

The morphological group 5 isolates (*Colletotrichum* sp) produced light grey to dark grey colonies on PDA with a growth rate ranging from 7.0 to 11.0 mm with an average of 10.7 ± 2.4 mm/day. The conidia were cylindrical, straight, with each apex rounded, often tapering slightly towards the base, measuring 8.5–16.7 × 4.3–7.2 μm (12.1 ± 3.3 × 5.9 ± 1.0 μm). Appressoria were variable in shape, clavate to irregular and lobed 5.1–10.2 × 4.8–7.1 μm (8.0 ± 1.9 × 6.0 ± 0.8 μm).

The morphological group 6 (*C. guajavae*) isolates had flat colonies with white or pale olivaceous grey to rosy buff color on the upper side while on the reverse side, the color was grey olivaceous to olivaceous black; growth rate ranged from 7.2 to 11.0 mm with an average of 10.7 ± 1.8 mm/day. Conidia were hyaline, smooth-walled, aseptate, straight, cylindrical to fusiform with both ends slightly acute, with or without guttules 6.0–12.5 × 2.6–4.7 μm (8.8 ± 2.2 × 3.7 ± 0.7 μm). Appressoria formed singly, were medium brown, smooth-walled, subglobose or elliptical to clavate, measuring 8.1–9.5 × 4.5–6.1 μm (8.8 ± 0.5 × 5.2 ± 0.6 μm).

Phylogenetic analyses

The trimmed sequences of the *ITS* region ranged from 597 to 618 bp, the *ACT* gene ranged from 259 to 281 bp, *CHS1* gene ranged from 289 to 299 bp, *GAPDH* ranged between 262 and 277 bp, *TUB2* gene ranged from 447 to 701 bp, *CYLH3* varied from 392 to 411 bp, *GS* ranged between 789 and 865 bp and the *ApMat* gene ranged from 863 to 906 bp. The dataset of combined eight genes used for phylogenetic analyses included 4454 characters including the alignment gaps, the gene boundaries are *ITS*: 1–621 bp, *ACT*: 622–906 bp, *CHS1*: 907–1203 bp, *GAPDH*: 1204–1511 bp, *TUB2*: 1512–2230 bp, *CYLH3*: 2231–2653 bp, *GS*: 2654–3523 bp and *ApMat*: 3524–4454 bp, of which 2564 characters were conserved, 1866 characters variable and 1254 characters were parsimony informative. For Bayesian analysis, a GTR + G + I model was selected for combined multi-gene data analysis and incorporated in the analysis. The consensus tree obtained from Bayesian analyses confirmed the tree topology of rapid bootstrapping estimations of RAxML (Fig. 4). The combined analyses resulted in the detection of six well separated clades: two isolates included in group 1 corresponded to *C. karstii* with a bootstrap support of 98 % and a Bayesian posterior probability value of 1; group 2 had one isolate, representing *C. gloeosporioides* with a bootstrap support/Bayesian posterior probability value of 100/1; group 3 had seven isolates representing *C. siamense* with 91/1; group 4 had six isolates representing *C. syzygicola* with 100/1; group five had five isolates of *Colletotrichum* sp. with 97/1; and the group six had four isolates representing *C. guajavae* with 90/0.97.

Pathogenicity testing

Typical symptoms of sunken and oval streaks with reddish margin surrounded by yellow halo were observed on the leaves of the small cardamom variety Appangala1 inoculated with isolates of: *C. karstii* (2 isolates), *C. gloeosporioides* (1), *C. siamense* (7), *C. syzygicola* (6), *Colletotrichum* sp (5), and *C. guajavae* (4), after seven days. All of the isolates were re-isolated from the leaves and showed the same morphological characteristics that were observed upon the initial isolation.

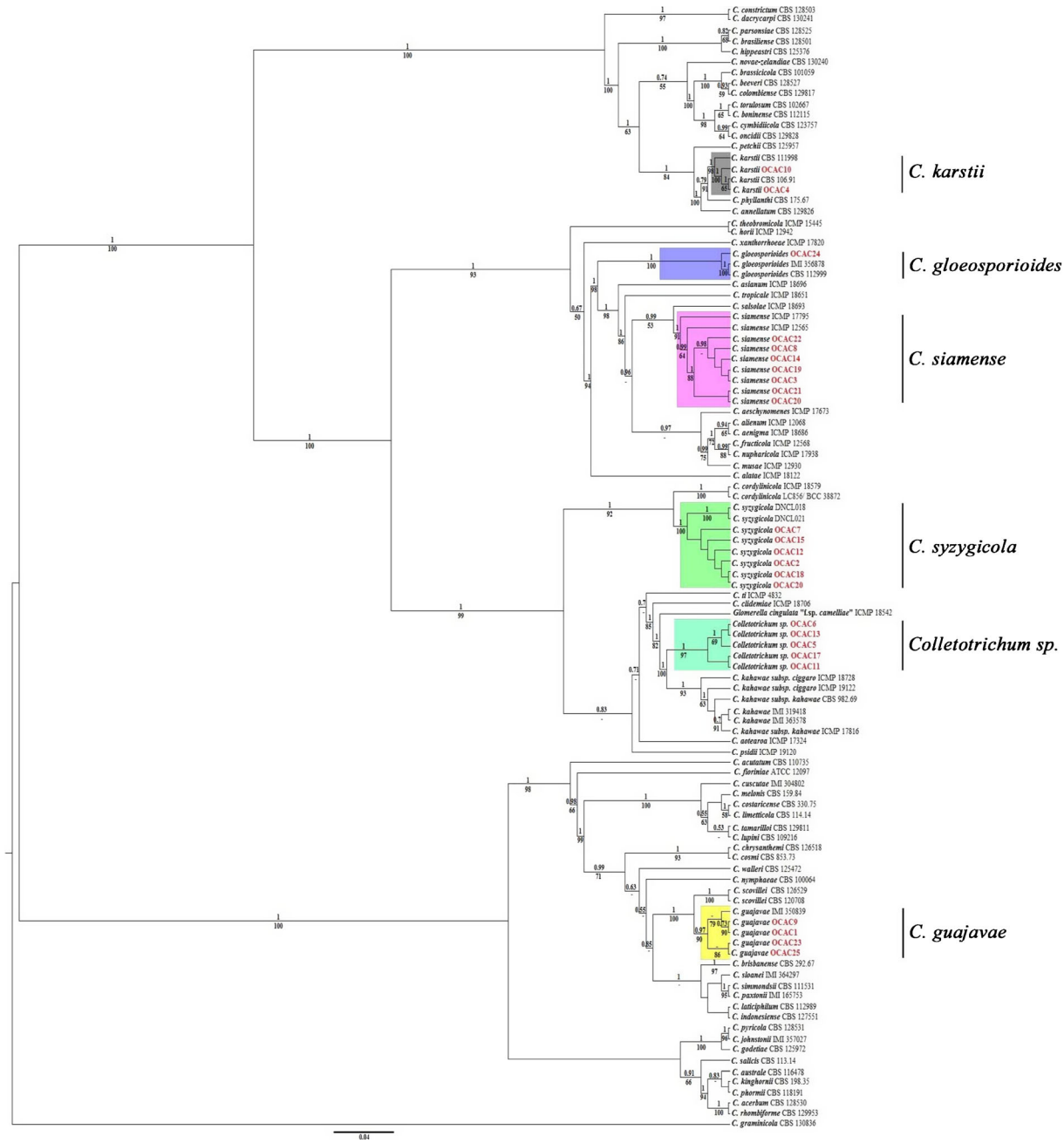

Fig. 4 Phylogenetic tree of *Colletotrichum* species, isolated from small cardamom in India, constructed with concatenated sequences of the ACT, TUB2, CHS-1, CYLH3, GAPDH, GS and ITS genes. Bayesian posterior probability values above 0.50 are shown at the nodes and bootstrap support values (500 replicates) above 50 % below BPP values. The *colour shadings* indicate the detection of six well separated *Colletotrichum* species from small cardamom. *Red letters* indicate the isolate numbers of *Colletotrichum* species obtained from small cardamom

Discussion

This study represents the first attempt to characterize *Colletotrichum* species associated with anthracnose of small cardamom in India using a polyphasic approach. *Colletotrichum gloeosporioides* was previously considered as the only causal organism of the disease based on morphological characters (Govindaraju et al. 1998;

Praveena and Biju 2012; Saju et al. 2013). No *Colletotrichum* species reported on small cardamom have been analyzed previously using molecular approaches. Detailed morphological and molecular phylogenetic analyses of twenty-five isolates revealed six species as the causal agent of small cardamom anthracnose, i.e., *C. karstii* (*C. boninnense* species complex), *C. gloeosporioides*, *C. siamense*, *C. syzygicola, Colletotrichum* sp. (*C. gloeosporioides* species complex), and *C. guajavae* (*C. acutatum* species complex).

Colletotrichum karstii a species in the *C. boninense* species complex was reported on several host plants in the world, including orchid plants (Yang et al. 2011; Damm et al. 2012a; Jadrane et al. 2012), *Citrus* (Peng et al. 2012), *Mangifera indica, Carica papaya, Eugenia uniflora*, *Bombax aquaticum* (Damm et al. 2012a; Weir et al. 2012; Lima et al. 2013) and apple (Velho and Stadnik 2014). Some isolates from *Passiflora edulis* in Brazil that were initially identified as *C. boninense* (Tozze et al. 2010) were subsequently revealed to be *C. karstii* based on *GAPDH* phylogeny (Damm et al. 2012a). *Colletotrichum karstii* isolates identified in this study are fitted to the description of Damm et al. (2012a).

Colletotrichum gloeosporioides was epitypified by Cannon et al. (2008) and it has been possible to compare strains of *Colletotrichum* with that of the epitype, using multi-locus analysis (Cannon et al. 2012; Damm et al. 2012a, b; Weir et al. 2012; Doyle et al. 2013; Sharma et al. 2013; Hyde et al. 2014; Yan et al. 2014). Our *C. gloeosporioides* isolate fit the description of *C. gloeosporioides* based on morphology and phylogeny (Cannon et al. 2008; Weir et al. 2012). Previously, there were a few reports of occurrence of *C. gloeosporioides* on large cardamom based on morphology (Saju et al. 2013) but those isolates are not available for comparison to confirm whether they belong to *C. gloeosporioides*.

Colletotrichum siamense was originally described as a causal agent of anthracnose on coffee berries in northern Thailand (Prihastuti et al. 2009). *C. siamense* is thought to be geographically diverse with a wide host range (Weir et al. 2012) and is considered to be a species complex on several hosts (Doyle et al. 2013; Sharma et al. 2013; Udayanga et al. 2013; Lima et al. 2013). The *ApMat* marker has been reported to be highly useful in resolving the *C. siamense* species complex (Silva et al. 2012a; Sharma et al. 2013). Our isolates of *C. siamense* fit within the morphological description of *C. siamense* (Prihastuti et al. 2009) and this was further confirmed by multi-gene analysis including of the *ApMat* gene (Sharma et al. 2013, 2014). There are no previous reports of the association of *C. siamense* as a pathogen of small cardamom.

Colletotrichum syzygicola was first reported from northern Thailand on *Citrus aurantifolia* and *Syzygium samarangense* (Udayanga et al. 2013). This *C. syzygicola* was earlier closely related to *C. cordylinicola* originally described from *Cordyline fruticosa,* an ornamental plant in the *Asparagaceae* in Thailand ((Phoulivong et al. 2010). Our isolates fit the description of *C. syzygicola* (Udayanga et al. 2013) based on morphology and multi-locus phylogeny .

The unknown *Colletotrichum* sp., forming a separate clade which is closely related to the *C. kahawae* clade within a group with high bootstrap support (Fig. 4), needs to be examined in detail.

Colletotrichum guajavae was described within the *C. acutatum* species complex. Peres et al. (2002) identified *C. acutatum* (*s. lat.*) from a guava fruit in Brazil based on *ITS* sequences which was identical to that of *C. guajavae* in the *C. acutatum* complex identified by Damm et al. (2012b). Later, Guerber et al. (2003) identified *C. acutatum* (*s. lat.*) based on a phylogeny from combined *GAPDH* and *GS* sequences. But it belongs to the clade of *C. guajavae* where the GAPDH sequence generated by that study differs at 5 bp from that of the *C. guajavae* ex-holotype strain IMI 350839 of Damm et al. (2012b). Our *C. guajavae* isolates corresponded to the description of Damm et al. (2012b).

The results of the pathogenecity studies clearly indicate that six species of *Colletotrichum* produced typical symptoms of anthracnose on leaves of small cardamom and the fungal isolates were re-isolated. Thus, this study revealed that six species of *Colletotrichum* are responsible for small cardamom anthracnose in India. The present study provides basic information for developing management strategies which, in the absence of resistant or tolerant small cardamom cultivars, should include looking for effective bio-control agents and green fungicides with acceptable levels of residue contamination for use in commercial small cardamom production in India as it is an export-oriented crop.

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Compliance with ethical standards We hereby state that this manuscript has not been submitted to any other journal other than European Journal of Plant Pathology. The Co- authors have no conflicts of interest to declare; and no human participants or animals were used in the current research. All the authors provided the informed consent for the submission of the manuscript.

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