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RESEARCH ARTICLE

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Unravelling *Colletotrichum* species associated with *Camellia*: employing ApMat and GS loci to resolve species in the *C. gloeosporioides* complex

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Key words

Camellia
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Abstract We investigated the phylogenetic diversity of 144 *Colletotrichum* isolates associated with symptomatic and asymptomatic tissues of *Camellia sinensis* and other *Camellia* spp. from seven provinces in China (Fujian, Guizhou, Henan, Jiangxi, Sichuan, Yunnan, Zhejiang), and seven isolates obtained from other countries, including Indonesia, UK, and the USA. Based on multi-locus (ACT, ApMat, CAL, GAPDH, GS, ITS, TUB2) phylogenetic analyses and phenotypic characters, 11 species were distinguished, including nine well-characterised species (*C. alienum*, *C. boninense*, *C. camelliae*, *C. cliviae*, *C. fioriniae*, *C. fructicola*, *C. gloeosporioides*, *C. karstii*, *C. siamense*), and two novel species (*C. henanense* and *C. jiangxiense*). Of these, *C. camelliae* proved to be the most dominant and probably host specific taxon occurring on *Camellia*. An epitype is also designated for the latter species in this study. *Colletotrichum jiangxiense* is shown to be phylogenetically closely related to the coffee berry pathogen *C. kahawae* subsp. *kahawae*. Pathogenicity tests and the pairwise homoplasy index test suggest that *C. jiangxiense* and *C. kahawae* subsp. *kahawae* are two independent species. This study represents the first report of *C. alienum* and *C. cliviae* occurring on *Camellia sinensis*. In addition, our study demonstrated that the combined use of the loci ApMat and GS in a phylogenetic analysis is able to resolve all currently accepted species in the *C. gloeosporioides* species complex.

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INTRODUCTION

Camellia, a genus of flowering plants in the family *Theaceae*, is cultivated in eastern and southern Asia, from the Himalayas east to Japan and Indonesia. Many species of *Camellia* (*Ca*.) are of major commercial importance. For example, leaves of *Ca. sinensis* are processed to produce tea, a popular beverage, while *Ca. japonica*, *Ca. oleifera*, and *Ca. sasanqua* and their hybrids are cultivated as ornamentals. *Camellia* production is affected by a large number of diseases, of which anthracnose, caused by species of the genus *Colletotrichum*, is one of the most important (Copes & Thomson 2008, Farr & Rossman 2014, Guo et al. 2014). Several *Colletotrichum* species have been reported from *Camellia*, e.g. *C. boninense* (Damm et al. 2012b), *C. camelliae* (Thompson & Johnston 1953, Tai 1979, Alfieri et al. 1984), *C. carveri* (Cash 1952), *C. coccodes* (Thaung 2008), *C. gloeosporioides* (Alfieri et al. 1984, Shivas 1989, Lu et al. 2000, Chen 2003, Guo et al. 2014), *C. pseudomajus* (Liu et al. 2014), *C. queenslandicum* (Simmonds 1966; syn. *C. gloeosporioides* var. *minor*, Weir et al. 2012), and *Glomerella major* (Tunstall 1934).

The genus *Colletotrichum* was also considered as one of the dominant endophytic genera in *Camellia* plants (Lu et al. 2007, Dai et al. 2008, Osono 2008, Fang et al. 2013). *Colletotrichum acutatum* and *C. gloeosporioides* were recognised as frequently occurring endophytic species in *Ca. japonica* based on morphological characteristics (Osono 2008). Fang et al. (2013) also found that *C. gloeosporioides* was one of the dominant endophytic species in *Ca. sinensis* based on ITS sequence data. Other reports of endophytic isolates of *Colletotrichum* on *Camellia* were, however, only identified to genus level.

Because of the commercial yield losses experienced in tea plantations due to *Colletotrichum* infections, as well as the limited knowledge of their identity and endophytic growth in *Camellia* plants, accurate identification of the causal organisms is of extreme importance. Most of the recent taxonomic treatments have primarily focused on the study of different *Colletotrichum* species complexes, for example *C. acutatum* (Damm et al. 2012a), *C. boninense* (Damm et al. 2012b), *C. caudatum* (Crouch 2014), *C. destructivum* (Damm et al. 2014), *C. gigasporum* (Liu et al. 2014), *C. gloeosporioides* (Weir et al. 2012), *C. graminicola* (Crouch et al. 2009), and *C. orbiculare* (Damm et al. 2013). Robust identification of *Colletotrichum* species relies on multi-locus sequence data (Cai et al. 2009, Cannon et al. 2012, Weir et al. 2012, Damm et al. 2013, Liu et al. 2013a, Crouch 2014). However, previous phylogenetic studies have rarely included isolates from *Camellia.* Thus far only a few strains of *C. boninense*, *C. fioriniae*, *C. lupini*, and *Glomerella cingulata* 'f. sp. *camelliae*' from *Camellia* were

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included in multi-locus phylogenies (Damm et al. 2012a, b, Weir et al. 2012, Sharma et al. 2014). In contrast, most of the studies that focused on the identification of *Colletotrichum* species associated with *Camellia* were only based on host, morphology or ITS sequence data (Tai 1979, Alfieri et al. 1984, Copes & Thomson 2008, Thaung 2008, Fang et al. 2013, Guo et al. 2014). Published reports of *C. acutatum* and *C. gloeosporioides* on *Camellia* should therefore be interpreted with care. Furthermore, although *C. camelliae* is regarded as the causal agent of brown blight disease of tea, the taxonomic and phylogenetic status of this pathogen remains unresolved (Weir et al. 2012).

The aim of the present study was thus to investigate the taxonomic and phylogenetic diversity of *Colletotrichum* spp. associated with *Ca. sinensis* and other *Camellia* spp. based on sequence data of six loci (ACT, CAL, GAPDH, GS, ITS, TUB2). A further aim was to test the usefulness of the ApMat locus in resolving taxa in the *C. gloeosporioides* complex (Crouch et al. 2009, Rojas et al. 2010, Silva et al. 2012b, Doyle et al. 2013, Sharma et al. 2013a, 2014) in combination with the other loci listed above.

MATERIALS AND METHODS

Collection and isolates

Diseased and healthy leaves of tea plants (*Ca. sinensis*) and other *Camellia* spp. were collected from seven provinces in China (Fujian, Guizhou, Henan, Jiangxi, Sichuan, Yunnan, and Zhejiang). Plant pathogenic fungi were isolated from leaf spots using both single spore and tissue isolation methods. Single spore isolation following the protocol of Choi et al. (1999) was adopted for collections with visible foliar sporulation, while tissue isolation was used for sterile isolates. Fungal endophytes were isolated by cutting four fragments (4 mm^2) per leaf from the apex, base and lateral sides, surface sterilised with 70 % ethanol for 1 min, 0.5 % NaClO for 3 min, 70 % ethanol for 1 min, rinsed in sterile water, and then transferred to quarter-strength potato dextrose agar (1/4 PDA; 9.75 g Difco PDA, 15 g Difco agar and 1 L distilled water). After 3–21 d, mycelial transfers were made from the colony periphery onto PDA. *Colletotrichum* colonies were primarily identified based on cultural characteristics on PDA, morphology of the spores, and ITS sequence data.

Type specimens of new species from this study were deposited in the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS), and ex-type living cultures deposited in the China General Microbiological Culture Collection centre (CGMCC). A further seven isolates from *Camellia* originating from other countries including Indonesia, UK, and the USA used in this study were obtained from the culture collection of the International Collection of Microorganisms from Plants, Landcare Research, Auckland, New Zealand (ICMP) and the CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands (CBS).

Morphological analysis

Agar plugs (5-mm-diam) were taken from the periphery of actively growing cultures and transferred to the centre of 9-cm-diam Petri dishes containing PDA or synthetic nutrient-poor agar medium (SNA; Nirenberg 1976) amended with double-autoclaved stems of *Anthriscus sylvestris* placed onto the agar surface. Cultures were incubated at room temperature (c. 25 °C) for 7 d. Colony characters and pigment production on PDA were noted after 7 d. Colony colours were rated according to Rayner (1970). Colony diameters were measured after 7 and 10 d.

Conidia were taken from acervuli on PDA and mounted in clear lactic acid. Cultures were examined periodically for the development of ascomata. Ascospores were described from ascomata crushed in lactic acid. If a fungus was not sporulating on PDA, morphological characters were described from SNA or from inoculated stems of *Anthriscus sylvestris*. Hyphal appressoria were observed on the reverse side of colonies grown on SNA plates. At least 30 measurements per structure were noted and observed with a Nikon Eclipse 80i microscope using differential interference contrast (DIC) illumination. Descriptions and illustrations of taxonomic novelties were deposited in MycoBank (www.MycoBank.org; Crous et al. 2004).

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from axenic cultures with a modified CTAB protocol as described in Guo et al. (2000). Seven loci including the 5.8S nuclear ribosomal gene with the two flanking internal transcribed spacers (ITS), an intron of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a partial sequence of the actin (ACT), beta-tubulin (TUB2), glutamine synthetase (GS), calmodulin (CAL) and Apn2-Mat1-2 intergenic spacer and partial mating type (Mat1-2) gene (ApMat) were amplified and sequenced using the primer pairs ITS1 + ITS4 (White et al. 1990), GDF1 + GDR1 (Guerber et al. 2003), ACT-512F + ACT-783R (Carbone & Kohn 1999), T1 + Bt-2b (Glass & Donaldson 1995, O'Donnell & Cigelnik 1997), GSF1 + GSR1 (Stephenson et al. 1997), CL1C + CL2C (Weir et al. 2012), and AMF1 + AMR1 (Silva et al. 2012b), respectively. PCR amplification protocols were performed as described by Liu et al. (2012), but the denaturing temperatures were adjusted to 52 °C for ITS, GAPDH, ACT, GS, CAL, and ApMat, and 55 °C for TUB2. Purification and sequencing of PCR amplicons were carried out by the SinoGenoMax Company, Beijing, China. DNA sequences generated with forward and reverse primers were used to obtain consensus sequences using MEGA v. 5.1 (Tamura et al. 2011). All novel sequences were deposited in NCBIs GenBank database (www.ncbi.nlm.nih.gov/; KJ954359–KJ955371, KM360143–KM360146, KM610172–KM610185, Table 1, 2), and the alignments and trees in TreeBASE (www.treebase.org/treebase-web/home.html; study S16761).

Phylogenetic analyses

Multiple sequence alignments were generated using MAFFT v. 7 (Katoh & Standley 2013), and if necessary, manually edited in MEGA v. 5.1. Bayesian analyses were performed on concatenated alignments using MrBayes v. 3.2.2 (Ronquist et al. 2012) as described by Crous et al. (2006) using nucleotide substitution models that were selected by MrModeltest v. 2.3 (Nylander 2004), with critical values for the topological convergence diagnostic set to 0.01. Maximum likelihood (ML) analyses were implemented using the CIPRES Science Gateway v. 3.3 (www.phylo.org), and the RAxML-HPC BlackBox was selected with default parameters. Six loci (ACT, CAL, GAPDH, GS, ITS, and TUB2) were concatenated for the multi-locus analysis of *C. gloeosporioides* s.l., while four loci (ACT, GAPDH, ITS, TUB2) were used for the multi-locus analysis of other *Colletotrichum* species. Due to the lack of available ApMat gene sequences of most of the recently identified *Colletotrichum* isolates, the ApMat locus could not be included in the concatenated alignment. Therefore, a single ApMat phylogeny was generated including sequences of 136 *C. gloeosporioides* s.l. isolates obtained from *Camellia* in this study, and 181 reference sequences that were retrieved from NCBI-GenBank. An additional phylogeny using a concatenated ApMat and GS sequence alignment was constructed which included 126 *C. gloeosporioides* s.l. isolates from *Camellia* and 33 reference isolates.

Table 1 Strains of the *C. gloeosporioides* s.l. species studied in this paper with details about host and location, and GenBank accessions of the sequences generated.

Species	Accession number[a]	Host	Locality	GenBank accessions						
				ITS	GAPDH	ACT	TUB2	CAL	GS	ApMat
C. aenigma	ICMP 18608*	*Persea americana*	Israel	JX010244	JX010044	JX009443	JX010389	JX009683	JX010078	KM360143
	ICMP 18686	*Pyrus pyrifolia*	Japan	JX010243	JX009913	JX009519	JX010390	JX009684	JX010079	
C. aeschynomenes	ICMP 17673, ATCC 201874*	*Aeschynomene virginica*	USA	JX010176	JX009930	JX009483	JX010392	JX009721	JX010081	KM360145
C. alatae	CBS 304.67, ICMP 17919*	*Dioscorea alata*	India	JX010190	JX009990	JX009471	JX010383	JX009738	JX010065	KC888932
	ICMP 18122	*Dioscorea alata*	Nigeria	JX010191	JX010011	JX009470	JX010449	JX009739	JX010136	
C. alienum	ICMP 12071*	*Malus domestica*	New Zealand	JX010251	JX010028	JX009572	JX010411	JX009654	JX010101	KM360144
	ICMP 18621	*Persea americana*	New Zealand	JX010246	JX009959	JX009552	JX010386	JX009657	JX010075	
	IMI 313842, ICMP 18691	*Persea americana*	Australia	JX010217	JX010018	JX009580	JX010385	JX009664	JX010074	
	LC3114, LF322	*Ca. sinensis*, endophyte	China	**KJ955131**	**KJ954832**	**KJ954411**	**KJ955279**	**KJ954684**	**KJ954982**	**KJ954545**
C. aotearoa	ICMP 17324	*Kunzea ericoides*	New Zealan	JX010198	JX009991	JX009538	JX010418	JX009619	JX010109	
	ICMP 18532	*Vitex lucens*	New Zealand	JX010220	JX009906	JX009544	JX010421	JX009614	JX010108	
	ICMP 18537*	*Coprosma* sp.	New Zealand	JX010205	JX010005	JX009564	JX010420	JX009611	JX010113	KC888930
C. asianum	GM595, MTCC 11680	*Mangifera indica*	India	JQ894679	JQ894623	JQ894545	JQ894601	KC790789		JQ894554
	ICMP 18580, CBS 130418*	*Coffea arabica*	Thailand	FJ972612	JX010053	JX009584	JX010406	FJ917506	JX010096	FR718814
	IMI 313839, ICMP 18696	*Mangifera indica*	Australia	JX010192	JX009915	JX009576	JX010384	JX009723	JX010073	
C. boninense	MAFF 305972, CBS 123755*	*Crinum asiaticum* var. *sinicum*	Japan	JQ005153	JQ005240	JQ005501	JQ005588	JQ005674		
C. camelliae	**CBS 125502**	*Camellia* sp., pathogen	unknown	**KJ955077**	**KJ954778**	**KJ954359**		**KJ954630**	**KJ954928**	
	ICMP 10643, LF897, LC3667	*Camellia* × *williamsii*	UK	JX010224	JX009908	JX009540	JX010436	JX009630	JX010119	**KJ954625**
	ICMP 10646, LF898, LC3668	*Ca. sasanqua*	USA	JX010225	JX009993	JX009563	JX010437	JX009629	JX010117	**KJ954626**
	ICMP 18542, LF899, LC3669	*Ca. sasanqua*	USA	JX010223	JX009994	JX009488	JX010429	JX009628	JX010118	**KJ954627**
	CGMCC 3.14924, LC1363	*Ca. sinensis*, pathogen	China	**KJ955080**	**KJ954781**	**KJ954362**	**KJ955229**	**KJ954633**	**KJ954931**	**KJ954496**
	CGMCC 3.14925, LC1364*	*Ca. sinensis*, pathogen	China	**KJ955081**	**KJ954782**	**KJ954363**	**KJ955230**	**KJ954634**	**KJ954932**	**KJ954497**
	CGMCC 3.14926, LC1365	*Ca. sinensis*, pathogen	China	**KJ955082**	**KJ954783**	**KJ954364**	**KJ955231**	**KJ954635**	**KJ954933**	**KJ954498**
	LC2944, LF152	*Camellia* sp., pathogen	China	**KJ955090**	**KJ954791**	**KJ954372**	**KJ955239**	**KJ954643**	**KJ954941**	**KJ954506**
	LC2962, LF170	*Camellia* sp., pathogen	China	**KJ955091**	**KJ954792**	**KJ954373**	**KJ955240**	**KJ954644**	**KJ954942**	**KJ954507**
	LC2998, LF206	*Ca. sinensis*, pathogen	China	**KJ955094**	**KJ954795**	**KJ954376**	**KJ955243**	**KJ954647**	**KJ954945**	**KJ954510**
	LC2999, LF207	*Ca. sinensis*, pathogen	China	**KJ955095**	**KJ954796**	**KJ954377**	**KJ955244**	**KJ954648**	**KJ954946**	**KJ954511**
	LC3000, LF208	*Ca. sinensis*, pathogen	China	**KJ955096**	**KJ954797**	**KJ954378**	**KJ955245**	**KJ954649**	**KJ954947**	
	LC3001, LF209	*Ca. sinensis*, pathogen	China	**KJ955097**	**KJ954798**	**KJ954379**	**KJ955246**	**KJ954650**	**KJ954948**	**KJ954512**
	LC3002, LF210	*Ca. sinensis*, pathogen	China	**KJ955098**	**KJ954799**	**KJ954380**	**KJ955247**	**KJ954651**	**KJ954949**	**KJ954513**
	LC3004, LF212	*Ca. sinensis*, pathogen	China	**KJ955099**	**KJ954800**	**KJ954381**	**KJ955248**	**KJ954652**	**KJ954950**	**KJ954514**
	LC3005, LF213	*Ca. sinensis*, pathogen	China	**KJ955100**	**KJ954801**	**KJ954382**	**KJ955249**	**KJ954653**	**KJ954951**	**KJ954515**
	LC3006, LF214	*Ca. sinensis*, pathogen	China	**KJ955101**	**KJ954802**	**KJ954383**	**KJ955250**	**KJ954654**	**KJ954952**	**KJ954516**
	LC3007, LF215	*Ca. sinensis*, pathogen	China	**KJ955102**	**KJ954803**	**KJ954384**	**KJ955251**	**KJ954655**	**KJ954953**	**KJ954517**
	LC3008, LF216	*Ca. sinensis*, pathogen	China	**KJ955103**	**KJ954804**	**KJ954385**	**KJ955252**	**KJ954656**	**KJ954954**	**KJ954518**
	LC3014, LF222	*Ca. sinensis*, pathogen	China	**KJ955104**	**KJ954805**	**KJ954386**	**KJ955253**	**KJ954657**	**KJ954955**	**KJ954519**
	LC3015, LF223	*Ca. sinensis*, pathogen	China	**KJ955105**	**KJ954806**	**KJ954387**		**KJ954658**	**KJ954956**	**KJ954520**
	LC3017, LF225	*Ca. sinensis*, pathogen	China	**KJ955106**	**KJ954807**	**KJ954388**	**KJ955254**	**KJ954659**	**KJ954957**	**KJ954521**
	LC3018, LF226	*Ca. sinensis*, pathogen	China	**KJ955107**	**KJ954808**	**KJ954389**	**KJ955255**	**KJ954660**	**KJ954958**	**KJ954522**
	LC3019, LF227	*Ca. sinensis*, pathogen	China	**KJ955108**	**KJ954809**	**KJ954390**	**KJ955256**	**KJ954661**	**KJ954959**	**KJ954523**
	LC3054, LF262	*Ca. sinensis*, pathogen	China	**KJ955110**	**KJ954811**	**KJ954391**	**KJ955258**	**KJ954663**	**KJ954961**	**KJ954525**
	LC3057, LF265	*Ca. sinensis*, pathogen	China	**KJ955111**	**KJ954812**	**KJ954392**	**KJ955259**	**KJ954664**	**KJ954962**	**KJ954526**
	LC3070, LF278	*Ca. sinensis*, pathogen	China	**KJ955112**	**KJ954813**	**KJ954393**	**KJ955260**	**KJ954665**	**KJ954963**	**KJ954527**
	LC3071, LF279	*Ca. sinensis*, pathogen	China	**KJ955113**	**KJ954814**		**KJ955261**	**KJ954666**	**KJ954964**	**KJ954528**
	LC3076, LF284	*Ca. sinensis*, endophyte	China	**KJ955114**	**KJ954815**	**KJ954394**	**KJ955262**	**KJ954667**	**KJ954965**	**KJ954529**
	LC3089, LF297	*Ca. sinensis*, endophyte	China	**KJ955115**	**KJ954816**	**KJ954395**	**KJ955263**	**KJ954668**	**KJ954966**	**KJ954530**
	LC3091, LF299	*Ca. sinensis*, endophyte	China	**KJ955116**	**KJ954817**	**KJ954396**	**KJ955264**	**KJ954669**	**KJ954967**	**KJ954531**
	LC3092, LF300	*Ca. sinensis*, endophyte	China	**KJ955117**	**KJ954818**	**KJ954397**	**KJ955265**	**KJ954670**	**KJ954968**	**KJ954532**
	LC3095, LF303	*Ca. sinensis*, endophyte	China	**KJ955118**	**KJ954819**	**KJ954398**	**KJ955266**	**KJ954671**	**KJ954969**	**KJ954533**
	LC3096, LF304	*Ca. sinensis*, endophyte	China	**KJ955119**	**KJ954820**	**KJ954399**	**KJ955267**	**KJ954672**	**KJ954970**	**KJ954534**

Table 1 (cont.)

Species	Accession number[a]	Host	Locality	GenBank accessions						
				ITS	GAPDH	ACT	TUB2	CAL	GS	ApMat
C. camelliae (cont.)	**LC3100, LF308**	*Ca. sinensis*, endophyte	China	**KJ955120**	**KJ954821**	**KJ954400**	**KJ955268**	**KJ954673**	**KJ954971**	**KJ954535**
	LC3101, LF309	*Ca. sinensis*, endophyte	China	**KJ955121**	**KJ954822**	**KJ954401**	**KJ955269**	**KJ954674**	**KJ954972**	**KJ954536**
	LC3102, LF310	*Ca. sinensis*, endophyte	China	**KJ955122**	**KJ954823**	**KJ954402**	**KJ955270**	**KJ954675**	**KJ954973**	**KJ954537**
	LC3103, LF311	*Ca. sinensis*, endophyte	China	**KJ955123**	**KJ954824**	**KJ954403**	**KJ955271**	**KJ954676**	**KJ954974**	**KJ954538**
	LC3107, LF315	*Ca. sinensis*, endophyte	China	**KJ955124**	**KJ954825**	**KJ954404**	**KJ955272**	**KJ954677**	**KJ954975**	**KJ954539**
	LC3109, LF317	*Ca. sinensis*, endophyte	China	**KJ955126**	**KJ954827**	**KJ954406**	**KJ955274**	**KJ954679**	**KJ954977**	**KJ954540**
	LC3111, LF319	*Ca. sinensis*, endophyte	China	**KJ955128**	**KJ954829**	**KJ954408**	**KJ955276**	**KJ954681**	**KJ954979**	**KJ954542**
	LC3112, LF320	*Ca. sinensis*, endophyte	China	**KJ955129**	**KJ954830**	**KJ954409**	**KJ955277**	**KJ954682**	**KJ954980**	**KJ954543**
	LC3113, LF321	*Ca. sinensis*, endophyte	China	**KJ955130**	**KJ954831**	**KJ954410**	**KJ955278**	**KJ954683**	**KJ954981**	**KJ954544**
	LC3116, LF324	*Ca. sinensis*, endophyte	China	**KJ955132**	**KJ954833**	**KJ954412**	**KJ955280**	**KJ954685**	**KJ954983**	**KJ954546**
	LC3117, LF325	*Ca. sinensis*, endophyte	China	**KJ955133**	**KJ954834**	**KJ954413**	**KJ955281**	**KJ954686**	**KJ954984**	**KJ954547**
	LC3123, LF331	*Ca. sinensis*, endophyte	China	**KJ955134**	**KJ954835**	**KJ954414**	**KJ955282**	**KJ954687**	**KJ954985**	**KJ954548**
	LC3128, LF336	*Ca. sinensis*, pathogen	China	**KJ955135**	**KJ954836**	**KJ954415**	**KJ955283**	**KJ954688**	**KJ954986**	**KJ954549**
	LC3129, LF337	*Ca. sinensis*, pathogen	China	**KJ955136**	**KJ954837**	**KJ954416**	**KJ955284**	**KJ954689**	**KJ954987**	**KJ954550**
	LC3130, LF338	*Ca. sinensis*, pathogen	China	**KJ955137**	**KJ954838**	**KJ954417**	**KJ955285**	**KJ954690**	**KJ954988**	**KJ954551**
	LC3131, LF339	*Ca. sinensis*, pathogen	China	**KJ955138**	**KJ954839**		**KJ955286**	**KJ954691**	**KJ954989**	**KJ954552**
	LC3142, LF350	*Ca. sinensis*, pathogen	China	**KJ955139**	**KJ954840**	**KJ954418**	**KJ955287**	**KJ954692**	**KJ954990**	**KJ954553**
	LC3143, LF351	*Ca. sinensis*, pathogen	China	**KJ955140**	**KJ954841**	**KJ954419**	**KJ955288**	**KJ954693**	**KJ954991**	**KJ954554**
	LC3147, LF355	*Ca. sinensis*, pathogen	China	**KJ955141**	**KJ954842**	**KJ954420**	**KJ955289**	**KJ954694**	**KJ954992**	**KJ954555**
	LC3148, LF356	*Ca. sinensis*, pathogen	China	**KJ955142**	**KJ954843**	**KJ954421**	**KJ955290**	**KJ954695**	**KJ954993**	**KJ954556**
	LC3158, LF367	*Ca. sinensis*, endophyte	China	**KJ955144**	**KJ954845**	**KJ954423**	**KJ955292**	**KJ954697**	**KJ954995**	**KJ954558**
	LC3173, LF383	*Ca. sinensis*, endophyte	China	**KJ955147**	**KJ954848**	**KJ954425**	**KJ955295**		**KJ954998**	**KJ954560**
	LC3269, LF491	*Ca. sinensis*, pathogen	China	**KJ955150**	**KJ954851**		**KJ955297**	**KJ954702**	**KJ955001**	**KJ954562**
	LC3270, LF492	*Ca. sinensis*, pathogen	China	**KJ955151**	**KJ954852**	**KJ954428**	**KJ955298**	**KJ954703**	**KJ955002**	**KJ954563**
	LC3274, LF496	*Ca. sinensis*, pathogen	China	**KJ955153**	**KJ954854**	**KJ954430**	**KJ955300**	**KJ954705**	**KJ955004**	**KJ954564**
	LC3279, LF501	*Ca. sinensis*, pathogen	China	**KJ955154**	**KJ954855**	**KJ954431**	**KJ955301**	**KJ954706**	**KJ955005**	**KJ954565**
	LC3282, LF504	*Ca. sinensis*, pathogen	China	**KJ955155**	**KJ954856**	**KJ954432**	**KJ955302**	**KJ954707**	**KJ955006**	**KJ954566**
	LC3319, LF541	*Ca. sinensis*, pathogen	China	**KJ955160**	**KJ954861**	**KJ954436**	**KJ955307**	**KJ954712**		**KJ954571**
	LC3322, LF544	*Ca. sinensis*, pathogen	China	**KJ955161**	**KJ954862**	**KJ954437**	**KJ955308**	**KJ954713**	**KJ955011**	**KJ954572**
	LC3323, LF545	*Ca. sinensis*, pathogen	China	**KJ955162**	**KJ954863**		**KJ955309**	**KJ954714**	**KJ955012**	**KJ954573**
	LC3328, LF550	*Ca. sinensis*, pathogen	China	**KJ955163**	**KJ954864**		**KJ955310**	**KJ954715**	**KJ955013**	**KJ954574**
	LC3330, LF552	*Ca. sinensis*, pathogen	China	**KJ955164**	**KJ954865**	**KJ954438**	**KJ955311**	**KJ954716**	**KJ955014**	**KJ954575**
	LC3335, LF557	*Ca. sinensis*, pathogen	China	**KJ955165**	**KJ954866**	**KJ954439**	**KJ955312**	**KJ954717**	**KJ955015**	**KJ954576**
	LC3350, LF572	*Ca. sinensis*, pathogen	China	**KJ955166**	**KJ954867**	**KJ954440**	**KJ955313**	**KJ954718**	**KJ955016**	**KJ954577**
	LC3352, LF574	*Ca. sinensis*, pathogen	China	**KJ955167**	**KJ954868**	**KJ954441**	**KJ955314**	**KJ954719**	**KJ955017**	**KJ954578**
	LC3355, LF577	*Ca. sinensis*, pathogen	China	**KJ955168**	**KJ954869**	**KJ954442**	**KJ955315**	**KJ954720**	**KJ955018**	**KJ954579**
	LC3367, LF589	*Ca. sinensis*, pathogen	China	**KJ955170**	**KJ954871**	**KJ954444**	**KJ955317**	**KJ954722**	**KJ955020**	
	LC3374, LF596	*Ca. sinensis*, pathogen	China	**KJ955173**	**KJ954874**	**KJ954447**	**KJ955320**	**KJ954725**	**KJ955023**	**KJ954582**
	LC3379, LF601	*Ca. sinensis*, pathogen	China	**KJ955174**	**KJ954875**	**KJ954448**	**KJ955321**	**KJ954726**	**KJ955024**	**KJ954583**
	LC3385, LF607	*Ca. sinensis*, pathogen	China	**KJ955178**	**KJ954879**	**KJ954451**	**KJ955325**	**KJ954730**	**KJ955028**	**KJ954586**
	LC3387, LF609	*Ca. sinensis*, pathogen	China	**KJ955179**	**KJ954880**	**KJ954452**	**KJ955326**	**KJ954731**	**KJ955029**	**KJ954587**
	LC3389, LF611	*Ca. sinensis*, pathogen	China	**KJ955180**	**KJ954881**	**KJ954453**	**KJ955327**	**KJ954732**	**KJ955030**	**KJ954588**
	LC3395, LF617	*Ca. sinensis*, pathogen	China	**KJ955181**	**KJ954882**	**KJ954454**	**KJ955328**	**KJ954733**	**KJ955031**	**KJ954589**
	LC3398, LF620	*Ca. sinensis*, pathogen	China	**KJ955182**	**KJ954883**	**KJ954455**	**KJ955329**	**KJ954734**	**KJ955032**	**KJ954590**
	LC3401, LF623	*Ca. sinensis*, pathogen	China	**KJ955183**	**KJ954884**	**KJ954456**	**KJ955330**	**KJ954735**	**KJ955033**	**KJ954591**
	LC3403, LF625	*Ca. sinensis*, pathogen	China	**KJ955185**	**KJ954886**	**KJ954458**	**KJ955332**	**KJ954737**	**KJ955035**	**KJ954593**
	LC3408, LF630	*Ca. sinensis*, pathogen	China	**KJ955186**	**KJ954887**	**KJ954459**	**KJ955333**	**KJ954738**	**KJ955036**	**KJ954594**
	LC3469, LF694	*Ca. sinensis*, pathogen	China	**KJ955204**	**KJ954905**	**KJ954474**	**KJ955350**	**KJ954755**	**KJ955054**	**KJ954610**
	LC3488, LF715	*Ca. sinensis*, pathogen	China	**KJ955206**	**KJ954907**	**KJ954476**	**KJ955352**	**KJ954757**	**KJ955056**	**KJ954612**
	LC3492, LF720	*Ca. sinensis*, pathogen	China	**KJ955208**	**KJ954909**	**KJ954478**	**KJ955354**	**KJ954759**	**KJ955058**	**KJ954614**

	LC3506, LF734	*Ca. sinensis*, pathogen	China	**KJ955209**	**KJ954910**	**KJ954479**	**KJ955355**	**KJ954760**	**KJ955059**	**KJ954615**
	LC3513, LF741	*Camellia* sp., pathogen	China	**KJ955210**	**KJ954911**		**KJ955356**	**KJ954761**	**KJ955060**	**KJ954616**
	LC3514, LF742	*Camellia* sp., pathogen	China	**KJ955211**	**KJ954912**	**KJ954480**	**KJ955357**	**KJ954762**	**KJ955061**	**KJ954617**
	LC3515, LF743	*Camellia* sp., pathogen	China	**KJ955212**	**KJ954913**	**KJ954481**	**KJ955358**	**KJ954763**	**KJ955062**	**KJ954618**
	LC3516, LF744	*Camellia* sp., pathogen	China	**KJ955213**	**KJ954914**		**KJ955359**	**KJ954764**	**KJ955063**	**KJ954619**
	LC3561, LF789	*Ca. sinensis*, pathogen	China	**KJ955217**	**KJ954918**	**KJ954485**	**KJ955363**	**KJ954768**	**KJ955067**	**KJ954621**
	LC3562, LF790	*Ca. sinensis*, pathogen	China	**KJ955218**	**KJ954919**	**KJ954486**		**KJ954769**	**KJ955068**	**KJ954622**
C. clidemiae	ICMP 18658*	*Clidemia hirta*	USA, Hawaii	JX010265	JX009989	JX009537	JX010438	JX009645	JX010129	KC888929
	ICMP 18706	*Vitis* sp.	USA	JX010274	JX009909	JX009476	JX010439	JX009639	JX010128	
C. cordylinicola	LC0886, ICMP 18579*	*Cordyline fruticosa*	Thailand	JX010226	JX009975	HM470235	JX010440	HM470238	JX010122	JQ899274
C. dianesei	CMM4083, MFLU 1300058*	*Mangifera indica*	Brazil	KC329779	KC517194	KC517298	KC517254	KC517209	KC430894	
	CMM4088, MFLU 1300059	*Mangifera indica*	Brazil	KC329781	KC517162	KC517300	KC517255	KC517210	KC430900	
	CMM4089, MFLU 1300060	*Mangifera indica*	Brazil	KC329783	KC517163	KC517302	KC517256	KC517211	KC430879	
C. endophytica	MFLUCC 130417, LC1216	*Pennisetum purpureum*	Thailand	KC633853	KC832853	KC692467		KC810017		
	MFLUCC 130418, LC0324*	*Pennisetum purpureum*	Thailand	KC633854	KC832854	KF306258		KC810018		
	MFLUCC 130419, LC0327	*Pennisetum purpureum*	Thailand	KC633855	KC832846	KC692468		KC810016		
C. fructicola	CBS 125395, ICMP 18645	*Theobroma cacao*	Panama	JX010172	JX009992	JX009543	JX010408	JX009666	JX010098	
	CBS 238.49, ICMP 17921	*Ficus edulis*	Germany	JX010181	JX009923	JX009495	JX010400	JX009671	JX010090	
	GM567, MTCC 11679	*Mangifera indica*	India	JQ894676	JQ894630	JQ894543	JQ894600	KC790787		JQ894576
	ICMP 18581, CBS 130416*	*Coffea arabica*	Thailand	JX010165	JX010033	FJ907426	JX010405	FJ917508	JX010095	JQ807838
	ICMP 18646, CBS 125397, MTCC 10906	*Tetragastris panamensis*	Panama	JX010173	JX010032	JX009581	JX010409	JX009674	JX010099	
	LC2923, LF130	*Ca. sinensis*, pathogen	China	**KJ955083**	**KJ954784**	**KJ954365**	**KJ955232**	**KJ954636**	**KJ954934**	**KJ954499**
	LC2924, LF131	*Ca. sinensis*, pathogen	China	**KJ955084**	**KJ954785**	**KJ954366**	**KJ955233**	**KJ954637**	**KJ954935**	**KJ954500**
	LC2925, LF132	*Ca. sinensis*, pathogen	China	**KJ955085**	**KJ954786**	**KJ954367**	**KJ955234**	**KJ954638**	**KJ954936**	**KJ954501**
	LC2926, LF133	*Ca. sinensis*, pathogen	China	**KJ955086**	**KJ954787**	**KJ954368**	**KJ955235**	**KJ954639**	**KJ954937**	**KJ954502**
	LC3155, LF364	*Ca. sinensis*, endophyte	China	**KJ955143**	**KJ954844**	**KJ954422**	**KJ955291**	**KJ954696**	**KJ954994**	**KJ954557**
	LC3167, LF376	*Ca. sinensis*, endophyte	China	**KJ955145**	**KJ954846**		**KJ955293**	**KJ954698**	**KJ954996**	**KJ954559**
	LC3284, LF506	*Ca. sinensis*, pathogen	China	**KJ955156**	**KJ954857**	**KJ954433**	**KJ955303**	**KJ954708**	**KJ955007**	**KJ954567**
	LC3288, LF510	*Ca. sinensis*, pathogen	China	**KJ955157**	**KJ954858**		**KJ955304**	**KJ954709**	**KJ955008**	**KJ954568**
	LC3315, LF537	*Ca. sinensis*, pathogen	China	**KJ955159**	**KJ954860**	**KJ954435**	**KJ955306**	**KJ954711**	**KJ955010**	**KJ954570**
	LC3368, LF590	*Ca. sinensis*, pathogen	China	**KJ955171**	**KJ954872**	**KJ954445**	**KJ955318**	**KJ954723**	**KJ955021**	**KJ954580**
	LC3370, LF592	*Ca. sinensis*, pathogen	China	**KJ955172**	**KJ954873**	**KJ954446**	**KJ955319**	**KJ954724**	**KJ955022**	**KJ954581**
	LC3384, LF606	*Ca. sinensis*, pathogen	China	**KJ955177**	**KJ954878**	**KJ954450**	**KJ955324**	**KJ954729**	**KJ955027**	**KJ954585**
	LC3402, LF624	*Ca. sinensis*, pathogen	China	**KJ955184**	**KJ954885**	**KJ954457**	**KJ955331**	**KJ954736**	**KJ955034**	**KJ954592**
	LC3417, LF639	*Ca. sinensis*, endophyte	China	**KJ955188**	**KJ954889**	**KJ954461**	**KJ955335**	**KJ954740**	**KJ955038**	**KJ954595**
	LC3425, LF647	*Ca. sinensis*, endophyte	China	**KJ955190**	**KJ954891**	**KJ954463**	**KJ955337**	**KJ954741**	**KJ955040**	**KJ954596**
	LC3427, LF649	*Ca. sinensis*, endophyte	China	**KJ955191**	**KJ954892**	**KJ954464**	**KJ955338**	**KJ954742**	**KJ955041**	**KJ954597**
	LC3430, LF652	*Ca. sinensis*, endophyte	China	**KJ955192**	**KJ954893**	**KJ954465**	**KJ955339**	**KJ954743**	**KJ955042**	**KJ954598**
	LC3433, LF655	*Ca. sinensis*, endophyte	China	**KJ955193**	**KJ954894**	**KJ954466**	**KJ955340**	**KJ954744**	**KJ955043**	**KJ954599**
	LC3434, LF656	*Ca. sinensis*, endophyte	China	**KJ955194**	**KJ954895**	**KJ954467**	**KJ955341**	**KJ954745**	**KJ955044**	**KJ954600**
	LC3447, LF670	*Ca. sinensis*, endophyte	China	**KJ955195**	**KJ954896**		**KJ955342**	**KJ954746**	**KJ955045**	**KJ954601**
	LC3451, LF674	*Ca. sinensis*, endophyte	China	**KJ955196**	**KJ954897**		**KJ955343**	**KJ954747**	**KJ955046**	**KJ954602**
	LC3457, LF681	*Ca. sinensis*, endophyte	China	**KJ955197**	**KJ954898**	**KJ954468**	**KJ955344**	**KJ954748**	**KJ955047**	**KJ954603**
	LC3461, LF685	*Ca. sinensis*, pathogen	China	**KJ955199**	**KJ954900**		**KJ955346**	**KJ954750**	**KJ955049**	**KJ954605**
	LC3462, LF686	*Ca. sinensis*, pathogen	China	**KJ955200**	**KJ954901**	**KJ954470**	**KJ955347**	**KJ954751**	**KJ955050**	**KJ954606**
	LC3464, LF689	*Ca. sinensis*, pathogen	China	**KJ955202**	**KJ954903**	**KJ954472**		**KJ954753**	**KJ955052**	**KJ954608**
	LC3465, LF690	*Ca. sinensis*, pathogen	China	**KJ955203**	**KJ954904**	**KJ954473**	**KJ955349**	**KJ954754**	**KJ955053**	**KJ954609**
	LC3471, LF696	*Ca. sinensis*, pathogen	China	**KJ955205**	**KJ954906**	**KJ954475**	**KJ955351**	**KJ954756**	**KJ955055**	**KJ954611**
	LC3489, LF716	*Ca. sinensis*, endophyte	China	**KJ955207**	**KJ954908**	**KJ954477**	**KJ955353**	**KJ954758**	**KJ955057**	**KJ954613**
	LC3545, LF773	*Ca. sinensis*, endophyte	China	**KJ955214**	**KJ954915**	**KJ954482**	**KJ955360**	**KJ954765**	**KJ955064**	**KJ954620**
	LC3569, LF797	*Ca. sinensis*, pathogen	China	**KJ955219**	**KJ954920**	**KJ954487**	**KJ955364**	**KJ954770**	**KJ955069**	**KJ954623**
	LC3666, LF896, ICMP 18656	*Ca. sinensis*, pathogen	Indonesia	**KJ955221**	**KJ954922**	**KJ954489**	**KJ955366**	**KJ954772**	**KJ955071**	**KJ954624**
	LC3670, LF900, ICMP 10642	*Camellia* sp., pathogen	UK	**KJ955225**	**KJ954926**	**KJ954492**	**KJ955370**	**KJ954776**	**KJ955075**	**KJ954628**
C. fructivorum	Coll1092, BPI 884114, CBS 133135	*Rhexia virginica*	USA	JX145133			JX145184			
	Coll1414, BPI 884103, CBS 133125*	*Vaccinium macrocarpon*	USA	JX145145			JX145196			

Table 1 (cont.)

Species	Accession number[a]	Host	Locality	GenBank accessions						
				ITS	GAPDH	ACT	TUB2	CAL	GS	ApMat
C. gloeosporioides	IMI 356878, ICMP 17821, CBS 112999*	*Citrus sinensis*	Italy	JX010152	JX010056	JX009531	JX010445	JX009731	JX010085	JQ807843
	LC3110, LF318	*Ca. sinensis*, endophyte	China	**KJ955127**	**KJ954828**	**KJ954407**	**KJ955275**	**KJ954680**	**KJ954978**	**KJ954541**
	LC3312, LF534	*Ca. sinensis*, pathogen	China	**KJ955158**	**KJ954859**	**KJ954434**	**KJ955305**	**KJ954710**	**KJ955009**	**KJ954569**
	LC3382, LF604	*Ca. sinensis*, pathogen	China	**KJ955176**	**KJ954877**	**KJ954450**	**KJ955323**	**KJ954728**	**KJ955026**	**KJ954584**
	LC3686, LF916	*Ca. sinensis*, pathogen	China	**KJ955226**	**KJ954927**	**KJ954493**	**KJ955371**	**KJ954777**	**KJ955076**	**KJ954629**
C. grevilleae	CBS 132879, CPC 15481*	*Grevillea* sp.	Italy	KC297078	KC297010	KC296941	KC297102	KC296963	KC297033	
C. henanense	**LC3030, CGMCC 3.17354, LF238 ***	*Ca. sinensis*, pathogen	China	**KJ955109**	**KJ954810**	**KM023257**	**KJ955257**	**KJ954662**	**KJ954960**	**KJ954524**
	LC2820, LF24	*Cirsium japonicum*, pathogen	China	**KM610182**	**KM610178**	**KM610172**	**KM610184**	**KM610176**	**KM610180**	**KM610174**
	LC2821, LF25	*Cirsium japonicum*, pathogen	China	**KM610183**	**KM610179**	**KM610173**	**KM610185**	**KM610177**	**KM610181**	**KM610175**
C. horii	ICMP 17968	*Diospyros kaki*	China	JX010212	GQ329682	JX009547	JX010378	JX009605	JX010068	
	NBRC 7478, ICMP 10492, MTCC 10841*	*Diospyros kaki*	Japan	GQ329690	GQ329681	JX009438	JX010450	JX009604	JX010137	JQ807840
C. jiangxiense	**LC3266, CGMCC 3.17361, LF488**	*Ca. sinensis*, pathogen	China	**KJ955149**	**KJ954850**	**KJ954427**		**KJ954701**	**KJ955000**	**KJ954561**
	LC3460, CGMCC 3.17362, LF684	*Ca. sinensis*, endophyte	China	**KJ955198**	**KJ954899**	**KJ954469**	**KJ955345**	**KJ954749**	**KJ955048**	**KJ954604**
	LC3463, CGMCC 3.17363, LF687*	*Ca. sinensis*, pathogen	China	**KJ955201**	**KJ954902**	**KJ954471**	**KJ955348**	**KJ954752**	**KJ955051**	**KJ954607**
C. kahawae subsp. *ciggaro*	ICMP 12952	*Persea americana*	New Zealand	JX010214	JX009971	JX009431	JX010426	JX009648	JX010126	
	ICMP 18534	*Kunzea ericoides*	New Zealand	JX010227	JX009904	JX009473	JX010427	JX009634	JX010116	HE655657
	ICMP 18539*	*Olea europaea*	Australia	JX010230	JX009966	JX009523	JX010434	JX009635	JX010132	
C. kahawae subsp. *kahawae*	IMI 319418, ICMP 17816*	*Coffea arabica*	Kenya	JX010231	JX010012	JX009452	JX010444	JX009642	JX010130	JQ894579
	CBS 982.69, ICMP 17915	*Coffea arabica*	Angola	JX010234	JX010040	JX009474	JX010435	JX009638	JX010125	
	IMI 361501, ICMP 17905	*Coffea arabica*	Cameroon	JX010232	JX010046	JX009561	JX010431	JX009644	JX010127	
C. melanocaulon	Coll126, BPI 884101, CBS 133123	*Vaccinium macrocarpon*	USA	JX145142			JX145193			JX145309
	Coll131, BPI 884113, CBS 133251*	*Vaccinium macrocarpon*	USA	JX145144			JX145195			JX145313
C. musae	CBS 116870, ICMP 19119, MTCC 11349*	*Musa* sp.	USA	JX010146	JX010050	JX009433	HQ596280	JX009742	JX010103	KC888926
	IMI 52264, ICMP 17817	*Musa sapientum*	Kenya	JX010142	JX010015	JX009432	JX010395	JX009689	JX010084	
C. nupharicola	CBS 469.96, ICMP 17938	*Nuphar lutea* subsp. *polysepala*	USA	JX010189	JX009936	JX009486	JX010397	JX009661	JX010087	
	CBS 470.96, ICMP 18187*	*Nuphar lutea* subsp. *polysepala*	USA	JX010187	JX009972	JX009437	JX010398	JX009663	JX010088	JX145319
	CBS 472.96, ICMP 17940	*Nymphaea ordorata*	USA	JX010188	JX010031	JX009582	JX010399	JX009662	JX010089	
C. proteae	CBS 132882, CPC 14859*	*Protea* sp.	South Africa	KC297079	KC297009	KC296940	KC297101	KC296960	KC297032	
	CBS 134301, CPC 14860	*Protea* sp.	South Africa	KC842385	KC842379	KC842373	KC842387	KC842375	KC842387	
C. psidii	CBS 145.29, ICMP 19120*	*Psidium* sp.	Italy	JX010219	JX009967	JX009515	JX010443	JX009743	JX010133	KC888931
C. queenslandicum	ICMP 1778*	*Carica papaya*	Australia	JX010276	JX009934	JX009447	JX010414	JX009691	JX010104	KC888928
	ICMP 18705	*Coffea* sp.	Fiji	JX010185	JX010036	JX009490	JX010412	JX009694	JX010102	
C. rhexiae	Coll1026, BPI 884112, CBS 133134*	*Rhexia virginica*	USA	JX145128			JX145179			JX145290
	Coll877, BPI 884110, CBS 133132	*Vaccinium macrocarpon*	USA	JX145157			JX145209			JX145302
C. salsolae	ICMP 19051*	*Salsola tragus*	Hungary	JX010242	JX009916	JX009562	JX010403	JX009696	JX010093	KC888925
C. siamense	DAR 76934, ICMP 18574	*Pistacia vera*	Australia	JX010270	JX010002	JX009535	JX010391	JX009707	JX010080	
	GM018, MTCC 11672	*Mangifera indica*	India	JQ894653	JQ894624	JQ894533	JQ894594	KC790778		
	GM057, MTCC 11590	*Mangifera indica*	India	JQ894658	JQ894620	JQ894534	JQ894590	KC790780		JQ894551
	GM172, MTCC 11591	*Mangifera indica*	India	JQ894662	JQ894621	JQ894535	JQ894591	KC790781		JQ894562
	GM385	*Mangifera indica*	India	JQ894668	JQ894626	JQ894536	JQ894596	KC790782		JQ894568
	GM390, MTCC 11677	*Mangifera indica*	India	JQ894670	JQ894627	JQ894537	JQ894597	KC790783		JQ894570
	GM473, MTCC 11589	*Mangifera indica*	India	JQ894673	JQ894622	JQ894539	JQ894592	KC790785		JQ894553
	GM529, MTCC 11592	*Mangifera indica*	India	JQ894675	JQ894629	JQ894540	JQ894599	KC790786		JQ894575
	GZAAS 5.09538	*Murraya* sp.	China	JQ247632	JQ247608	JQ247656	JQ247645	JQ247597	JQ247620	
	ICMP 12567	*Persea americana*	Australia	JX010250	JX009940	JX009541	JX010387	JX009697	JX010076	
	ICMP 18121	*Dioscorea rotundata*	Nigeria	JX010245	JX009942	JX009460	JX010402	JX009715	JX010092	
	ICMP 18578, CBS 130417*	*Coffea arabica*	Thailand	JX010171	JX009924	FJ907423	JX010404	FJ917505	JX010094	JQ899289
	LC0148	*Camellia* sp., pathogen	China	**KJ955078**	**KJ954779**	**KJ954360**	**KJ955227**	**KJ954631**	**KJ954929**	**KJ954494**
	LC0149	*Camellia* sp., pathogen	China	**KJ955079**	**KJ954780**	**KJ954361**	**KJ955228**	**KJ954632**	**KJ954930**	**KJ954495**
	LC2931, CGMCC 3.17353, LF139	*Camellia* sp., pathogen	China	**KJ955087**	**KJ954788**	**KJ954369**	**KJ955236**	**KJ954640**	**KJ954938**	**KJ954503**
	LC2940, LF148	*Camellia* sp., pathogen	China	**KJ955088**	**KJ954789**	**KJ954370**	**KJ955237**	**KJ954641**	**KJ954939**	**KJ954504**

C. siamense (cont.)	**LC2941, LF149**	*Camellia* sp., pathogen	China	**KJ955089**	**KJ954790**	**KJ954371**	**KJ955238**	**KJ954642**	**KJ954940**	**KJ954505**
	LC2969, LF177	*Camellia oleifera*, pathogen	China	**KJ955092**	**KJ954793**	**KJ954374**	**KJ955241**	**KJ954645**	**KJ954943**	**KJ954508**
	LC2974, LF182	*Camellia* sp., endophyte	China	**KJ955093**	**KJ954794**	**KJ954375**	**KJ955242**	**KJ954646**	**KJ954944**	**KJ954509**
	LC3409, LF631	*Ca. sinensis*, pathogen	China	**KJ955187**	**KJ954888**	**KJ954460**	**KJ955334**	**KJ954739**	**KJ955037**	
	MTCC 9660	*Mangifera indica*	India	JQ894649	JQ894619	JQ894532	JQ894589	KC790790		JQ894548
	NK24, MTCC 11599	*Mangifera indica*	India	JQ894681	JQ894632	JQ894546	JQ894602	KC790791		JQ894582
	NK28, MTCC 11593	*Mangifera indica*	India	JQ894687	JQ894633	JQ894547	JQ894603	KC790792		
C. siamense (syn. *C. hymenocallidis*)	CBS 125378, ICMP 18642, LC0043	*Hymenocallis americana*	China	JX010278	JX010019	JX009441	JX010410	JX009709	JX010100	JQ899283
C. siamense (syn. *C. jasmini-sambac*)	CBS 130420, ICMP 19118	*Jasminum sambac*	Vietnam	HM131511	HM131497	HM131507	JX010415	JX009713	JX010105	JQ807841
C. siamense (syn. *C. murrayae*)	GZAAS 5.09506	*Murraya* sp.	China	JQ247633	JQ247609	JQ247657	JQ247644	JQ247596	JQ247621	
C. temperatum	Coll1103, BPI 884098, CBS 133120	*Vaccinium macrocarpon*	USA	JX145135			JX145186			JX145297
	Coll883, BPI 884100, CBS 133122*	*Vaccinium macrocarpon*	USA	JX145159			JX145211			JX145298
C. theobromicola	MTCC 11350, CBS 124945, ICMP 18649*	*Theobroma cacao*	Panama	JX010294	JX010006	JX009444	JX010447	JX009591	JX010139	KC790726
C. theobromicola (syn. *C. fragariae*)	CBS 142.31, ICMP 17927, MTCC 10325	*Fragaria* × *ananassa*	USA	JX010286	JX010024	JX009516	JX010373	JX009592	JX010064	JQ807844
C. ti	ICMP 4832*	*Cordyline* sp.	New Zealand	JX010269	JX009952	JX009520	JX010442	JX009649	JX010123	KM360146
	ICMP 5285	*Cordyline australis*	New Zealand	JX010267	JX009910	JX009553	JX010441	JX009650	JX010124	
C. tropicale	CBS 124949, ICMP 18653, MTCC 11371*	*Theobroma cacao*	Panama	JX010264	JX010007	JX009489	JX010407	JX009719	JX010097	KC790728
	MAFF 239933, ICMP 18672	*Litchi chinensis*	Japan	JX010275	JX010020	JX009480	JX010396	JX009722	JX010086	
C. viniferum	GZAAS 5.08601, yg1*	*Vitis vinifera* cv. *Shuijing*	China	JN412804	JN412798	JN412795		JQ309639	JN412787	
	GZAAS 5.08608, yg4	*Vitis vinifera* cv. *Hongti*	China	JN412802	JN412800	JN412793		JN412782	JN412784	
C. xanthorrhoeae	BRIP 45094, ICMP 17903, CBS 127831*	*Xanthorrhoea preissii*	Australia	JX010261	JX009927	JX009478	JX010448	JX009653	JX010138	KC790689
	IMI 350817a, ICMP 17820	*Xanthorrhoea* sp.	Australia	JX010260	JX010008	JX009479		JX009652		

[a] AS, CGMCC: China General Microbiological Culture Collection; ATCC: American Type Culture Collection; BPI: U.S. National Fungus Collections, USA; BRIP: Plant Pathology Herbarium, Department of Employment, Economic, Development and Innovation, Queensland, Australia; CBS: Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; CPC: Working collection of Pedro W. Crous, housed at CBS, The Netherlands; DAR: Plant pathology Herbarium, Australia; GZAAS: Guizhou Academy of Agricultural Sciences Herbarium, China; ICMP: International Collection of Microorganisms from Plants, Auckland, New Zealand; IMI: Culture collection of CABI Europe UK Centre, Egham, UK; LC: Working collection of Lei Cai, housed at CAS, China; LF: Working collection of Fang Liu, housed at CAS, China; MAFF: Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan; MFLUCC: Mae Fah Luang University Culture Collection, ChiangRai, Thailand; MTCC: Microbial type culture collection and gene bank, India; NBRC: NITE Biological Resource Centre, Japan.

* = ex-type culture. Strains/sequences studied in this paper are in **bold** font.

Genealogical concordance phylogenetic species recognition analysis

Phylogenetically related but ambiguous species were analysed using the Genealogical Concordance Phylogenetic Species Recognition (GCPSR) model by performing a pairwise homoplasy index (PHI) test as described by Quaedvlieg et al. (2014). The PHI test was performed in SplitsTree4 (Huson 1998, Huson & Bryant 2006) in order to determine the recombination level within phylogenetically closely related species using a 6-locus concatenated dataset (ACT, CAL, GAPDH, GS, ITS, and TUB2). If the pairwise homoplasy index results were below a 0.05 threshold ($\Phi_w < 0.05$), it was indicative for significant recombination present in the dataset. The relationship between closely related species was visualised by constructing a splits graph.

Pathogenicity

Koch's postulates were conducted as described in Cai et al. (2009). Six *Colletotrichum* isolates were selected for pathogenicity tests: *C. camelliae* CGMCC 3.14925, *C. henanense* CGMCC 3.17354, *C. jiangxiense* CGMCC 3.17362 and CGMCC 3.17363, *C. kahawae* subsp. *kahawae* IMI 319418 and IMI 363578. Healthy leaves of intact 2-yr-old tea plants were washed with sterilised water, and then inoculated using the wound/drop and non-wound/drop inoculation methods. Plants inoculated with sterile water were used as control. The inoculated samples were incubated at room temperature in normal light regimes in the greenhouse for 14 d.

RESULTS

Isolates

In total, 144 *Colletotrichum* isolates were obtained from *Camellia* tissues from the main tea growing regions in China. Of these, 102 isolates were isolated from diseased tissues, and 42 from asymptomatic tissues (Table 1, 2).

Phylogenetic analyses of the combined datasets

Based on the BLAST search results of the NCBI database with the ITS sequences, all *Colletotrichum* isolates in this study were preliminarily allocated to species complexes: 141 isolates belonged to the *C. gloeosporioides* species complex, eight isolates belonged to the *C. boninense* species complex, one isolate belonged to *C. acutatum* species complex, and one isolate was identified as *C. cliviae*.

The 6-locus (ACT, CAL, GAPDH, GS, ITS, TUB2) phylogenetic analysis of the *C. gloeosporioides* species complex included 229 isolates from *Camellia* and other hosts, with *C. boninense* (CBS 123755) as the outgroup (see Fig. 1 for a version of this phylogeny with selected identical isolates removed; the complete alignment and tree, as Fig. S1, is available from TreeBASE). The dataset comprised 3 522 characters including the alignment gaps. For the Bayesian inference, a GTR+I+G model with inverse gamma-distributed rate was selected for ACT, HKY+G with gamma-distributed rates for CAL and ITS, GTR+G with gamma-distributed rates for GAPDH, GS, and TUB2. The maximum likelihood tree confirmed the tree topology and posterior probabilities of the Bayesian consensus tree. Isolates from *Camellia* in the *C. gloeosporioides* complex clustered in seven clades (data present in TreeBASE as Fig. S1): one *Camellia* isolate clustered with the ex-type isolate of *C. alienum*, 32 isolates clustered with *C. fructicola*, four isolates clustered with *C. gloeosporioides*, 91 isolates clustered with *C. camelliae* (syn. *Glomerella cingulata* 'f. sp. *camelliae*'), and eight isolates clustered with the ex-type isolates of *C. siamense*, *C. dianesei*, and *C. melanocaulon* in one clade. Three *Camellia* isolates formed a distinct clade (posterior probability = 1),

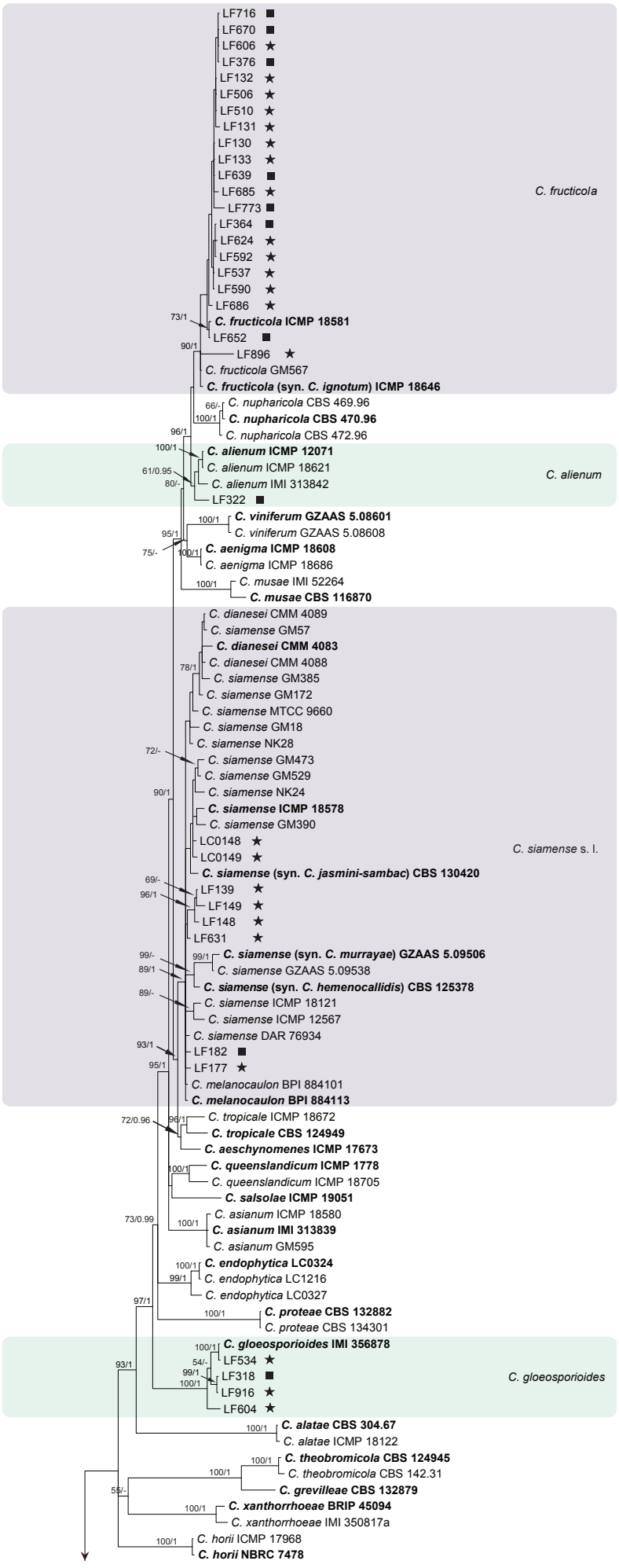

Fig. 1 Fifty percent majority rule consensus tree from a Bayesian analysis based on a 6-gene combined dataset (ACT, CAL, GAPDH, GS, ITS, TUB2) showing phylogenetic affinities of a reduced set of *Colletotrichum* isolates from *Camellia* isolated in this study with species of the *C. gloeosporioides* species complex. The RAxML bootstrap support values (ML > 50) and Bayesian posterior probabilities (PP > 0.95) are displayed at the nodes (ML/PP). The tree was rooted to *C. boninense* (CBS 123755). The scale bar indicates 0.9 expected changes per site. Ex-type cultures are emphasised in **bold**, and include the taxonomic name as originally described. Coloured blocks are used to indicate clades containing Chinese isolates from *Camellia*; stars indicate pathogens, squares indicate endophytes.

Fig. 1 (cont.)

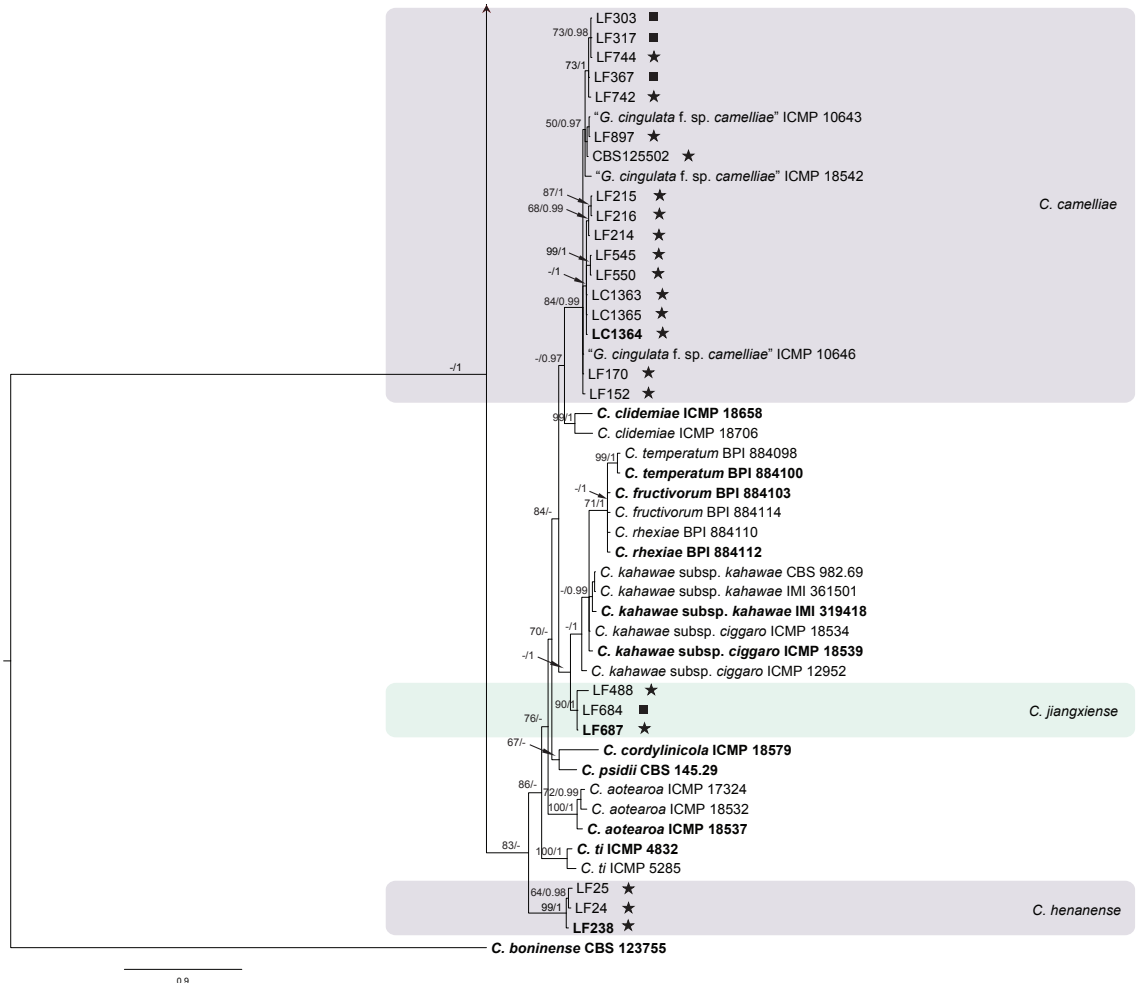

Fig. 2 Fifty percent majority rule consensus tree from a Bayesian analysis based on a 4-gene combined dataset (ITS, GAPDH, ACT, TUB2) showing phylogenetic affinities of *Colletotrichum* isolates from *Camellia* with members of the *Colletotrichum* species outside of the *C. gloeosporioides* species complex. The RAxML bootstrap support values (ML > 50) and Bayesian posterior probabilities (PP > 0.95) are displayed at the nodes (ML/PP). The tree was rooted to *Monilochaetes infuscans* (CBS 869.96). The scale bar indicates 0.2 expected changes per site. Ex-type cultures are emphasised in **bold**. Coloured blocks are used to indicate clades containing Chinese isolates from *Camellia*; stars indicate pathogens, squares indicate endophytes.

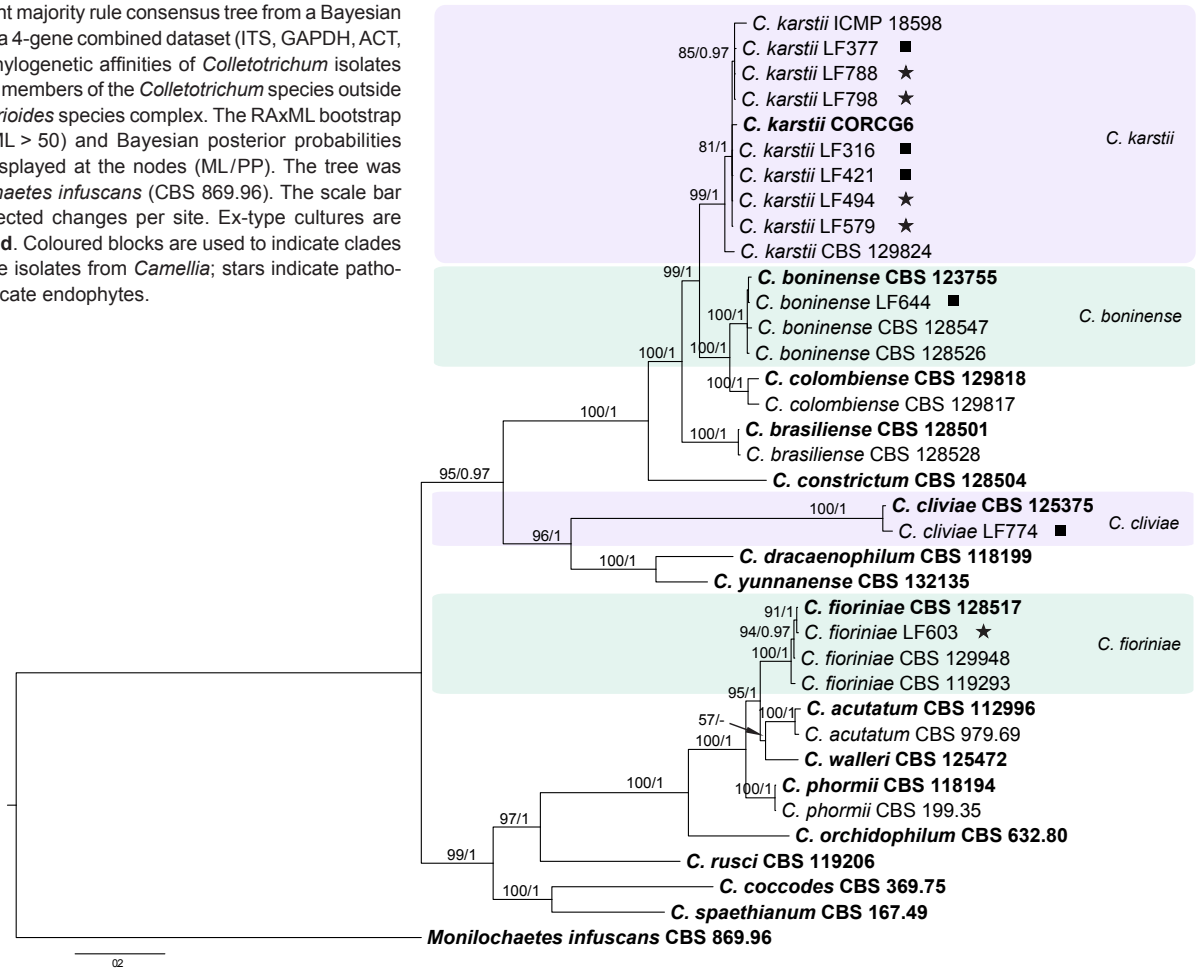

Table 2 Strains of *Colletotrichum* excluded from the *C. gloeosporioides* species complex. Details are provided about host and location, and GenBank accessions of the sequences generated.

Species	Association number[a]	Host	Locality	GenBank accessions			
				ITS	GAPDH	ACT	TUB2
C. acutatum	CBS 112996, ATCC 56816*	*Carica papaya*	Australia	JQ005776	JQ948677	JQ005839	JQ005860
	CBS 979.69	*Coffea arabica*	Kenya	JQ948400	JQ948731	JQ949721	JQ950051
C. boninense	CBS 123755, MAFF 305972*	*Crinum asiaticum* var. *sinicum*	Japan	JQ005153	JQ005240	JQ005501	JQ005588
	CBS 128526, ICMP 18591	*Dacrycarpus dacrydioides*	New Zealand	JQ005162	JQ005249	JQ005510	JQ005596
	CBS 128547, ICMP 10338	*Camellia* sp.	New Zealand	JQ005159	JQ005246	JQ005507	JQ005593
	LC3422, CGMCC 3.14356, LF644	*Camellia sinensis*, endophyte	China	**KJ955189**	**KJ954890**	**KJ954462**	**KJ955336**
C. brasiliense	CBS 128501, ICMP 18607*	*Passiflora edulis*	Brazil	JQ005235	JQ005322	JQ005583	JQ005669
	CBS 128528, ICMP 18606	*Passiflora edulis*	Brazil	JQ005234	JQ005321	JQ005582	JQ005668
C. cliviae	CBS 125375*	*Clivia miniata*	China	JX519223	JX546611	JX519240	JX519249
	LC3546, CGMCC 3.17358, LF774	*Camellia sinensis*, endophyte	China	**KJ955215**	**KJ954916**	**KJ954483**	**KJ955361**
C. coccodes	CBS 369.75*	*Solanum tuberosum*	Netherlands	HM171679	HM171673	HM171667	JX546873
C. colombiense	CBS 129817	*Passiflora edulis*	Colombia	JQ005173	JQ005260	JQ005521	JQ005607
	CBS 129818*	*Passiflora edulis*	Colombia	JQ005174	JQ005261	JQ005522	JQ005608
C. constrictum	CBS 128504, ICMP 12941*	*Citrus limon*	New Zealand	JQ005238	JQ005325	JQ005586	JQ005672
C. dracaenophilum	CBS 118199*	*Dracaena sanderana*	China	JX519222	JX546707	JX519238	JX519247
C. fioriniae	CBS 119293	*Vaccinium corymbosum*	New Zealand	JQ948314	JQ948644	JQ949635	JQ949965
	CBS 128517*	*Fiorinia externa*	USA	JQ948292	JQ948622	JQ949613	JQ949943
	CBS 129948	*Tulipa* sp.	UK	JQ948344	JQ948674	JQ949665	JQ949995
	LC3381, CGMCC 3.17357, LF603	*Camellia sinensis*, pathogen	China	**KJ955175**	**KJ954876**	**KJ954449**	**KJ955322**
C. karstii	CBS 129824	*Musa* sp.	Colombia	JQ005215	JQ005302	JQ005563	JQ005649
	CBS 132134, CORCG6, CGMCC 3.14194*	*Vanda* sp.	China	HM585409	HM585391	HM581995	HM585428
	LC3108, LF316	*Camellia sinensis*, endophyte	China	**KJ955125**	**KJ954826**	**KJ954405**	**KJ955273**
	LC3168, LF377	*Camellia sinensis*, endophyte	China	**KJ955146**	**KJ954847**	**KJ954424**	**KJ955294**
	LC3210, LF421	*Camellia sinensis*, endophyte	China	**KJ955148**	**KJ954849**	**KJ954426**	**KJ955296**
	LC3272, LF494	*Camellia sinensis*, pathogen	China	**KJ955152**	**KJ954853**	**KJ954429**	**KJ955299**
	LC3357, LF579	*Camellia sinensis*, pathogen	China	**KJ955169**	**KJ954870**	**KJ954443**	**KJ955316**
	LC3560, LF788	*Camellia sinensis*, pathogen	China	**KJ955216**	**KJ954917**	**KJ954484**	**KJ955362**
	LC3570, CGMCC 3.17359, LF798	*Camellia sinensis*, pathogen	China	**KJ955220**	**KJ954921**	**KJ954488**	**KJ955365**
	MAFF 305973, ICMP 18598	*Passiflora edulis*	Japan	JQ005194	JQ005281	JQ005542	JQ005628
C. orchidophilum	CBS 632.80*	*Dendrobium* sp.	USA	JQ948151	JQ948481	JQ949472	JQ949802
C. phormii	CBS 118194*	*Phormium* sp.	Germany	JQ948446	JQ948777	JQ949767	JQ950097
	CBS 199.35	*Phormium* sp.	UK	JQ948447	JQ948778	JQ949768	JQ950098
C. rusci	CBS 119206*	*Ruscus* sp.	Italy	GU227818	GU228210	GU227916	GU228112
C. spaethianum	CBS 167.49*	*Funkia sieboldiana*	Germany	GU227807	GU228199	GU227905	GU228101
C. walleri	CBS 125472*	*Coffea* sp.	Vietnam	JQ948275	JQ948605	JQ949596	JQ949926
C. yunnanense	AS 3.9167, CBS 132135*	*Buxus* sp.	China	JX546804	JX546706	JX519239	JX519248
Monilochaetes infuscans	CBS 869.96*	*Ipomoea batatas*	South Africa	JQ005780	JX546612	JQ005843	JQ005864

[a] AS, CGMCC: China General Microbiological Culture Collection; ATCC: American Type Culture Collection; CBS: Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; 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; MAFF: Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan.

* = ex-type culture. Strains/sequences studied in this paper are in **bold** font.

most closely related to *C. kahawae* s.l. A simplified tree was subsequently generated by removing 87 isolates of *C. camelliae* and *C. fructicola* (Fig. 1).

Fig. 2 shows the identity of the *Camellia* isolates that fell outside of the *C. gloeosporioides* species complex. The concatenated alignment (ACT, GAPDH, ITS, TUB2) contained 37 isolates, with *Monilochaetes infuscans* (CBS 869.96) as outgroup. The dataset comprised 1 559 characters including the alignment gaps. For the Bayesian inference, a HKY+G model with gamma-distributed rate was selected for ACT, HKY+I+G with inverse gamma-distributed rate for GAPDH, GTR+I+G with inverse gamma-distributed rates for ITS and TUB2. The maximum likelihood tree confirmed the tree topology and posterior probabilities of the Bayesian consensus tree. Seven *Camellia* isolates clustered with the ex-type isolate of *C. karstii*, one isolate clustered with *C. boninense*, one isolate clustered with *C. fioriniae* and one isolate clustered with *C. cliviae*.

The pathogenic and endophytic isolates of *Colletotrichum* studied here were labelled with stars and squares, respectively, on the multi-locus phylogenetic trees (Fig. 1, 2). Isolates from symptomatic *Camellia* leaves belong to eight clades, representing *C. camelliae*, *C. fioriniae*, *C. fructicola*, *C. gloeosporioides*, *C. henanense*, *C. jiangxiense*, *C. karstii*, and *C. siamense*. Isolates from asymptomatic tissues belong to nine clades representing *C. alienum*, *C. boninense*, *C. camelliae*, *C. cliviae*, *C. fructicola*, *C. gloeosporioides*, *C. henanense*, *C. karstii*, and *C. siamense*.

ApMat-based phylogenetic analysis

The phylogenetic analysis of the *C. gloeosporioides* species complex using the ApMat locus included 317 isolates from *Camellia* and other hosts (rooted with *C. xanthorrhoeae*), and 785 characters with alignment gaps were involved in the dataset. All isolates included in this analysis were separated into 15 main clades and 12 single-isolate lineages (see Fig. 3 for a cartoon version of this phylogeny; the complete alignment and tree, as Fig. S2, is available from TreeBASE). One of the clades is represented by an assemblage of more than one species, including

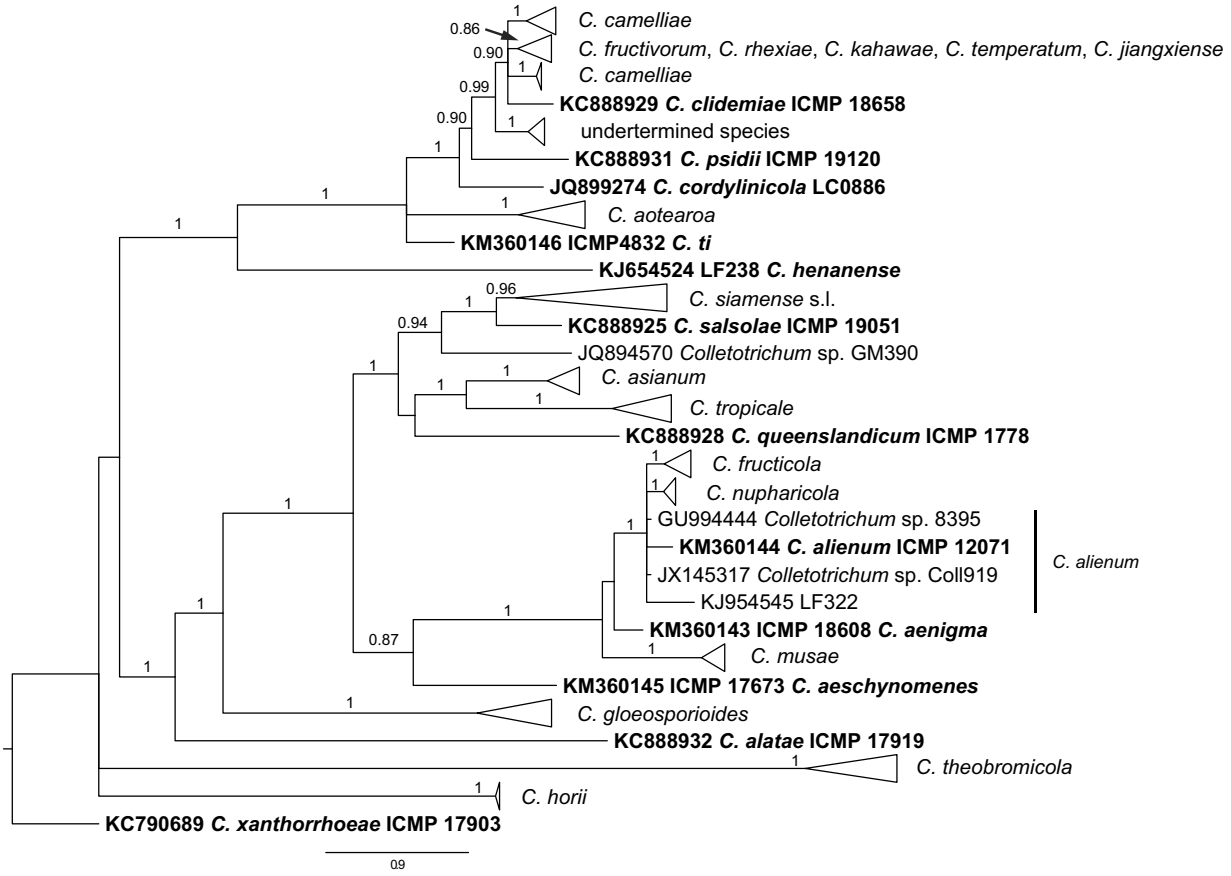

Fig. 3 Collapsed cartoon of the 50 % majority rule consensus tree from a Bayesian analysis based on the ApMat dataset showing phylogenetic affinities of *Colletotrichum* isolates from *Camellia* with members of the *C. gloeosporioides* species complex. Bayesian posterior probabilities values are displayed at the node. The tree was rooted to *C. xanthorrhoeae* (ICMP 17903). The scale bar indicates 0.6 expected changes per site. Ex-type cultures are emphasised in **bold**, and include the taxonomic name as originally described.

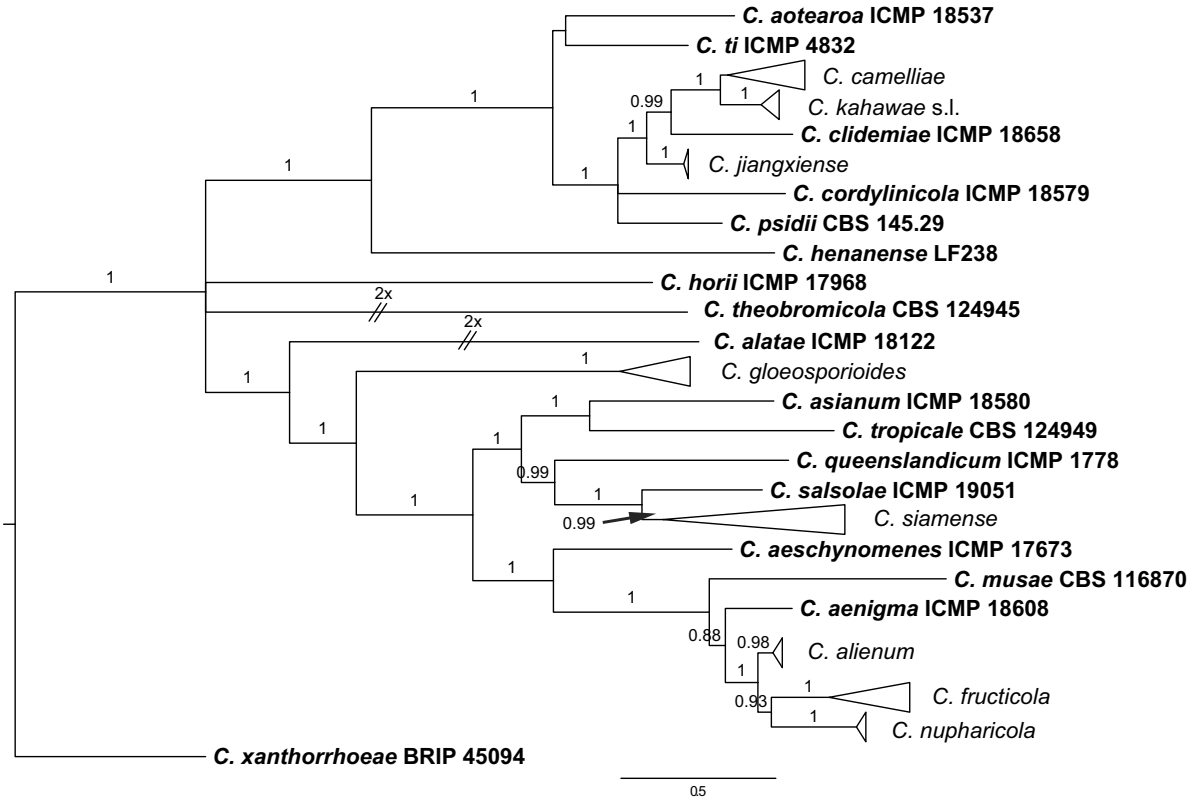

Fig. 4 Collapsed cartoon of the 50 % majority rule consensus tree from a Bayesian analysis based on the combined ApMat and GS alignment showing phylogenetic affinities of *Colletotrichum* isolates from *Camellia* with species of the *C. gloeosporioides* species complex. Bayesian posterior probabilities values are displayed at the node. The tree was rooted to *C. xanthorrhoeae* (ICMP 17903). The scale bar indicates 0.5 expected changes per site. Ex-type cultures are emphasised in **bold**. Extremely long branches were halved in length (indicated with 2× above two diagonal lines) to better fit the tree to the page.

C. fructivorum, *C. jiangxiense*, *C. kahawae*, *C. rhexiae*, and *C. temperatum* (Fig. 3, S2). Of these five species, *C. fructivorum*, *C. rhexiae*, and *C. temperatum* formed monophyletic species clades. However, strains from *C. jiangxiense* and *C. kahawae* were intermingled in one clade and the two species could not be differentiated from each other. The *C. camelliae* isolates were separated into two distinct clades, while the other species formed monophyletic clades.

ApMat & GS-based phylogenetic analysis

Colletotrichum jiangxiense and *C. kahawae* subsp. *kahawae* cannot be separated on the basis of the ApMat locus. They are mainly distinguished from one another based on the GS gene (see also notes under *C. jiangxiense*); the two species formed distinct clades in the GS gene phylogeny (not shown). The potential of the concatenated ApMat and GS genes to serve as a barcode for the *C. gloeosporioides* species complex was demonstrated by re-constructing a phylogenetic tree using the sequences listed in Table 1 (Fig. 4). All species of the *C. gloeosporioides* species complex included in the analysis could be delimited clearly based on the concatenated ApMat & GS gene tree.

Pairwise homoplasy index (PHI) test

A pairwise homoplasy index (PHI) test using a 6-gene dataset (ACT, CAL, GAPDH, GS, ITS, TUB2) was further performed to determine the recombination level between *C. jiangxiense* and its phylogenetically closely related species, *C. kahawae* subsp. *ciggaro* and *C. kahawae* subsp. *kahawae*. Based on the result no significant recombination events could be detected between *C. kahawae* s.l. and *C. jiangxiense* (Φ_w = 1) (Fig. 5).

Pathogenicity

The tea plant leaves inoculated with a conidial suspension of *Colletotrichum* isolates from symptomatic tea leaves (*C. camelliae* CGMCC 3.14925, *C. henanense* CGMCC 3.17354, *C. jiangxiense* CGMCC 3.17363) developed typically brown lesions around the leaf wounds after 14 d (Fig. 6). The inoculated *Colletotrichum* isolates could be re-isolated from the periphery of these lesions, thereby fulfilling Koch's postulates. Leaves of the control plants were inoculated with sterile water, and leaves inoculated with isolates of *C. kahawae* subsp. *kahawae* did not develop any symptoms after 14 d past inoculation (Fig. 6).

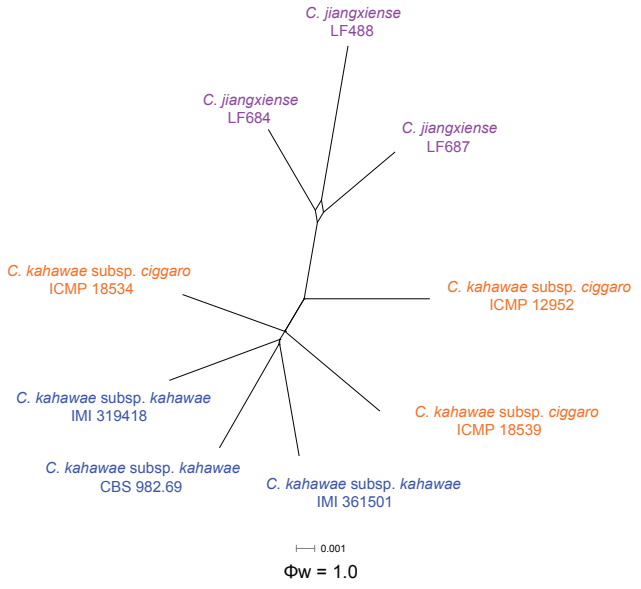

Fig. 5 The result of the pairwise homoplasy index (PHI) test of closely related species using both LogDet transformation and splits decomposition. PHI test results (Φ_w) < 0.05 indicate significant recombination within the dataset.

Taxonomy

Based on the multi-locus phylogenies (Fig. 1–4 and Fig. S1, S2 in TreeBASE), the 151 *Colletotrichum* isolates from *Camellia sinensis* and other *Camellia* spp. belonged to 11 species, including two species that proved to be new to science.

Colletotrichum alienum B. Weir & P.R. Johnst, Stud. Mycol. 73: 139. 2012

Description and illustrations — See Weir et al. (2012) and Liu et al. (2013b).

Material examined. China, Jiangxi Province, Ganzhou, Yangling National Forest Park, on living leaf of *Ca. sinensis*, Apr. 2013, *F. Liu*, culture CGMCC 3.17355 = LC3114 = LF322.

Notes — *Colletotrichum alienum* was previously only known from Australia, New Zealand, Portugal, and South Africa (Weir et al. 2012, Liu et al. 2013b). In the present study, one endophytic isolate CGMCC 3.17355 from a tea leaf clustered together with the ex-type culture of *C. alienum* (ICMP 12071) in the multi-locus phylogenetic tree (Fig. 1); this is the first reported occurrence of *C. alienum* on *Ca. sinensis* and in China.

Both conidia and ascospores of the tea isolate (CGMCC 3.17355) are slightly shorter than that of the ex-type (ICMP 12071) of *C. alienum* (conidia 14.5 × 4.6 µm vs 16.5 × 5 µm, ascospores 16.3 × 4.4 µm vs 18.1 × 4.6 µm; Weir et al. 2012).

Colletotrichum boninense Moriwaki, Toy. Sato & Tsukib., Mycoscience 44: 48. 2003

Description and illustrations — See Moriwaki et al. (2003) and Damm et al. (2012b).

Material examined. China, Jiangxi Province, Ganzhou, Fengshan Mountain, on living leaf of *Ca. sinensis*, Sept. 2013, *Y. Zhang*, culture CGMCC 3.14356 = LC3422 = LF644.

Notes — The endophytic isolate (LF644) from a tea leaf evaluated in this study was identified as *C. boninense* based on the multi-locus phylogenetic analyses (Fig. 2). This species was previously reported on *Camellia* sp. from New Zealand (Damm et al. 2012b).

Conidia of the tea isolate (CGMCC 3.14356) on PDA are wider, and the L/W ratio is smaller than that of the ex-type culture (CBS 123755) of *C. boninense* on *Anthriscus* stem and SNA (CGMCC 3.14356: 10–15 × 6.5–8 µm, mean = 13.7 × 7.3 µm, L/W ratio = 1.9 vs CBS 123755: on *Anthriscus* stem (9–)12–14.5(–16.5) × (4–)5.5–6.5 µm, av = 13.2 × 5.8 µm, L/W ratio = 2.3, on SNA (8.5–)11–14.5(–17.5) × (4–)5–6(–6.5) µm, av = 12.8 × 5.4 µm, L/W ratio = 2.4). Conidia of CBS 123755 often contain two large polar guttules, which were absent in the tea isolate.

Colletotrichum camelliae Massee, Bull. Misc. Inform. Kew 1899: 91. 1899. — Fig. 7

= *Glomerella cingulata* 'f. sp. *camelliae*' Dickens & R.T.A. Cook, Pl. Pathol. 38: 85. 1989.

On PDA: *Colonies* 69–71 mm diam in 7 d, > 90 mm diam in 10 d, flat with entire edge, aerial mycelium white, cottony, sparse; reverse white at first, then grey to black at the centre. *Conidiomata* not observed, conidiophores formed directly on aerial mycelium, hyaline, septate. *Conidiogenous cells* hyaline, cylindrical, 16–42 × 1.5–4.5 µm. *Conidia* hyaline, smooth-walled, guttulate, cylindrical with obtuse ends, sometimes narrowed at the centre or towards the base, 9–25 × 3.5–7.5 µm, av ± SD = 15.5 ± 3.3 × 5.0 ± 0.9 µm, L/W ratio = 3.1. *Appressoria* irregularly shaped, clavate, crenate, lobed, brown to dark brown, solitary, branched, catenate, with age sometimes

Fig. 6 Pathogenicity test of selected isolates on tea plant leaves after 14 d. a. *C. jiangxiense* (CGMCC 3.17363); b, c. *C. henanense* (CGMCC 3.17354); d. *C. kahawae* subsp. *kahawae* (IMI 363578); e. *C. camelliae* (CGMCC 3.14925); f. control.

complex chlamydospore-like structures develop, 6.5–13.5 × 5.0–10.5 µm, av ± SD = 10.0 ± 1.8 × 7.5 ± 1.3, L/W ratio = 1.3.

Materials examined. China, Fujian Province, Zhangzhou, on *Ca. sinensis*, Nov. 2012, *L. Cai*, culture LF214; Guizhou Province, Huishui District, on *Ca. sinensis*, 11 Nov. 2010, *P. Tan* (HMAS 243126 epitype designated here MBT178292, culture ex-epitype CGMCC 3.14925 = LC1364); ibid., HMAS 243127, culture CGMCC 3.14924 = LC1363; ibid., HMAS 243128, culture CGMCC 3.14926 = LC1365; Jiangxi Province, Yangling National Forest Park, on *Ca. sinensis*, Apr. 2013, *F. Liu*, culture LC3095 = LF303; ibid., culture LC3109 = LF317. – Sri Lanka, on leaves of *Camellia* sp., 8 Apr. 1899, *J.C. Willis*, K(M) 173540 holotype. – USA, South Carolina, on *Ca. sasanqua*, 1982, unknown collector, culture LC3668 = LF898 = ICMP 10646.

Notes — To our knowledge, the earliest known record of tea anthracnose was described in 1899 by Massee (in Willis 1899) from living leaves of *Ca. sinensis* from Sri Lanka. The holotype sample is preserved in K(M) 173540 and labelled *C. camelliae* (Fig. 8). Although it was subsequently synonymised with *C. gloeosporioides* (von Arx 1957), the name *C. camelliae* is still widely used in fungaria, websites, trade and semi-popular literature as the causal agent of the brown blight disease of tea plants (Weir et al. 2012). In 1989, *Glomerella cingulata* 'f. sp. *camelliae*' was proposed as the causal agent of disease on ornamental *Ca. saluenensis* hybrids, but without distinguishable morphological characteristics compared to *G. cingulata* (Dickens & Cook 1989). Weir et al. (2012) revealed *G. cingulata* 'f. sp. *camelliae*' to belong to the *C. gloeosporioides* complex. However, due to the lack of an ex-type culture of *C. camelliae*, the genetic relationship between *C. camelliae* and *G. cingulata* 'f. sp. *camelliae*' remained unresolved.

We evaluated the holotype specimen of *C. camelliae* from K, but very few morphological characters could be observed on

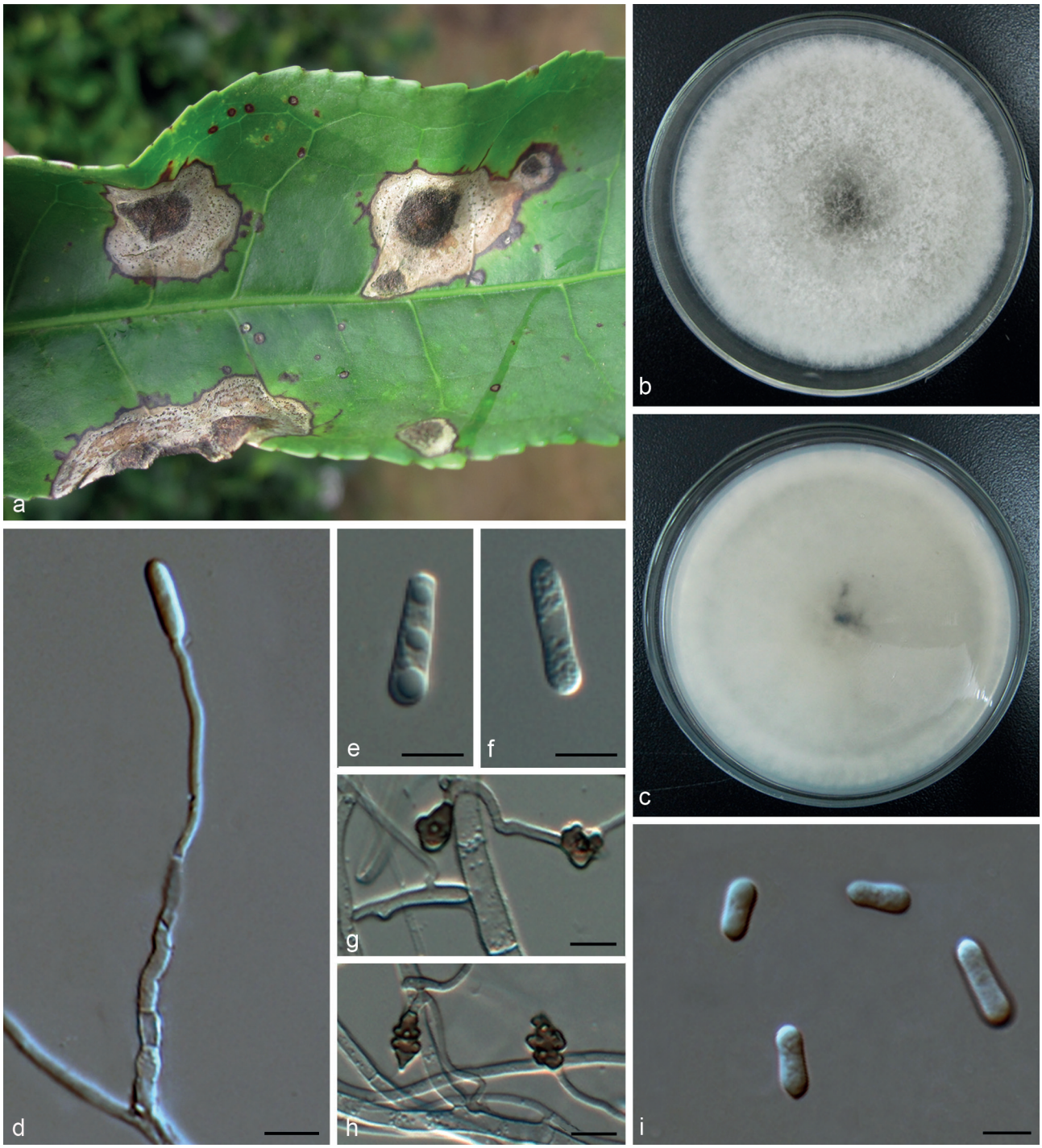

Fig. 7 *Colletotrichum camelliae* (CGMCC 3.14925). a. Symptom on tea leaf; b, c. forward and reverse view of culture 7 d after inoculation; d. conidiophores; e, f, i. conidia; g, h. appressoria (b–f, i from PDA; g, h from SNA). — Scale bar: d–i = 10 µm.

this old specimen, and DNA extraction was unsuccessful. Conidia on the holotype specimen are hyaline and cylindrical (Fig. 8), 14.5–20 × 4–6 µm, av ± SD = 17.2 ± 1.2 × 4.9 ± 0.4 µm. Conidial dimensions of isolates in this study on PDA (9–25 × 3.5–7.5 µm, av ± SD = 15.5 ± 3.3 × 5.0 ± 0.9 µm) are in accordance with the holotype specimen.

Several efforts to obtain a fresh culture from tea plants from Sri Lanka, the original location from where *C. camelliae* was reported, proved to be unsuccessful. However, we collected many anthracnose diseased samples in the tea fields from different provinces in China. Leaf lesions were dark brown and circular at first, then enlarged to become more irregular, with many of the lesions coalescing; raised black circular masses were found at the centre of lesions, bordered by a discoloured margin (Fig. 7a). Isolates from these samples clustered together with authentic isolates of *G. cingulata* 'f. sp. *camelliae*' (cited by Dickens & Cook 1989) in the 6-gene and ApMat phylogenetic trees (Fig. 1 and Fig. S2 in TreeBASE). Inoculations using conidial suspensions were performed on tea plants under controlled environmental conditions to test whether this fungus was the causal agent of tea anthracnose disease. The inoculations resulted in leaf infection of *Ca. sinensis* consistent with the original natural infections. Re-isolation and re-sequencing confirmed that the culture was identical to the one used for inoculation. No symptoms were produced in the negative control plants. A pathogenicity test with isolates of *G. cingulata* 'f. sp. *camelliae*' from ornamental *Camellia* on detached tea (*Ca. sinensis*) leaves was performed by Weir et al. (2012) and the isolates proved to be highly virulent. The *Colletotrichum* isolates from tea brown blight symptoms from India, showing affinities to *G. cingulata*

Fig. 8 Holotype of *C. camelliae* (K (M) 173540). a. Label of the specimen; b. tea leaf with *C. camelliae* colonisation from above and below; c–g. conidia. — Scale bars: c–g = 10 µm.

'f. sp. *camelliae*', were also pathogenic to detached tea leaves (Sharma et al. 2014). All the tests and analyses demonstrated that the isolates collected from typical brown blight symptoms on tea in the field and those from ornamental varieties are the same species. Since *C. camelliae* was published earlier than *G. cingulata* 'f. sp. *camelliae*' (1899 vs 1989), and there is no nomenclatural priority for formae speciales (Art. 4, http://www.iapt-taxon.org/nomen/main.php?page=art4), the name *C. camelliae* is adopted for the anthracnose pathogen of tea and is epitypified in this study, and *G. cingulata* 'f. sp. *camelliae*' is synonymised with *C. camelliae*.

Colletotrichum cliviae Y.L. Yang et al., Fung. Diversity 39: 133. 2009 — Fig. 9

On PDA: *Colonies* 65–69 mm diam in 7 d, > 90 mm diam in 10 d, flat with entire edge. Cultures on PDA and SNA are sterile, but a sexual morph developed on *Anthriscus* stem. *Ascomata* globose, brown to black, covered by sparse and white aerial mycelium, outer wall composed of flattened angular cells. *Asci* cylindrical, 62–92 × 8–12 µm, 8-spored. *Ascospores* uni- or biseriately arranged, hyaline, aseptate, smooth-walled, allantoid, ellipsoidal or ovoid with rounded ends, 11–16.5 × 4–6.5

µm, av ± SD = 13.8 ± 1.6 × 5.8 ± 0.5 µm, L/W ratio = 2.4. No asexual morph was observed in this study. Yang et al. (2009) provided a description of the asexual morph of this species.

Material examined. China, Guangxi Province, Guilin, on living leaf of *Ca. sinensis*, Sept. 2013, *T.W. Hou*, culture CGMCC 3.17358 = LC3546 = LF774.

Notes — *Colletotrichum cliviae* was reported to cause anthracnose diseases on *Clivia miniata*, *Arundina graminifolia* and *Cymbidium hookerianum* in China (Yang et al. 2009, 2011). The host range was recently extended to include *Cattleya*, *Calamus thwaitesii*, *Phaseolus*, and *Saccharum* (Sharma et al. 2013b). In the present study, a single isolate (CGMCC 3.17358) of *Colletotrichum* from a healthy tea leaf proved to belong to *C. cliviae*, but the asexual morph was not observed. Conversely, this is the first report of a sexual morph of *C. cliviae*, and the first report of this species on *Ca. sinensis*.

Colletotrichum fioriniae (Marcelino & Gouli) R.G. Shivas & Y.P. Tan, Fung. Diversity 39: 117. 2009

Basionym. *Colletotrichum acutatum* var. *fioriniae* Marcelino & Gouli, Mycologia 100: 362. 2008.

Description and illustration — See Damm et al. (2012a).

Materials examined. China, Jiangxi Province, Ganzhou, Yangling National Forest Park, on *Ca. sinensis*, Apr. 2013, *F. Liu*, culture CGMCC 3.17357 = LC3381 = LF603.

Notes — *Colletotrichum fioriniae* was previously reported from *Ca. reticulata* in Kunming, Yunnan Province and from *Ca. sinensis* in Fujian Province in China (Damm et al. 2012a, Liu 2013).

Colletotrichum fructicola Prihast., L. Cai & K.D. Hyde, Fung. Diversity 39: 158. 2009 — Fig. 10

On PDA: *Colonies* 74–79 mm diam in 7 d, > 90 mm diam in 10 d, flat with entire edge, aerial mycelium dense, cottony, grey to dark grey in the centre, white at the margin; reverse greyish green with white halo. *Chlamydospores* not observed. *Conidiomata* acervular, only one seta was observed, brown, smooth-walled, 1-septate, 64 µm long, base inflated, 4 µm diam, tip more or less acute. *Conidiophores* hyaline, septate, branched. *Conidiogenous cells* hyaline, cylindrical or ampulliform, 7.5–18.5 µm, apex 1–3 µm diam. *Conidia* hyaline, aseptate, smooth-walled, cylindrical, both ends rounded, 11.5–17.5 × 3–5.5 µm, av ± SD = 14.9 ± 1.3 × 4.4 ± 0.4 µm, L/W ratio = 3.4. *Appressoria* not observed.

Materials examined. China, Guangxi Province, Guilin, on *Ca. sinensis*, Sept. 2013, *T.W. Hou*, culture LC3545 = LF773; ibid., culture LC3489 = LF716; Hangzhou, on *Ca. sinensis*, Oct. 2013, *F. Liu*, culture LC3569 = LF797; on *Ca. sinensis*, Sept. 2012, *L. Cai*, culture CGMCC 3.17352 = LC2923 = LF130; Jiangxi Province, Ganzhou, Fengshan Mountain, on *Ca. sinensis*, Sept. 2013, *Y. Zhang*, culture LC3462 = LF686; ibid., culture LC3451 = LF674; Yangling National Forest Park, on *Ca. sinensis*, Apr. 2013, *F. Liu*, culture LC3284 = LF506. – Indonesia, on *Ca. sinensis*, Jan. 1979, *H. Semangun*, culture LC3666

Fig. 9 *Colletotrichum cliviae* on *Anthriscus* stem (CGMCC 3.17358). a. Ascomata; b. ascospores; c, d. asci and ascospores. — Scale bar: b = 10 µm, scale bar of b applies to b–d.

= LF896 = ICMP 18656. UK, on a shipment of *Camellia* flowers from New Zealand, on *Camellia* sp., 1982, staff of Ministry of Agriculture, Fisheries & Food, culture LC3670 = LF900 = ICMP 10642.

Notes — This study supplements the morphological characteristics of setae of *C. fructicola* that were not observed in the previous studies. *Colletotrichum fructicola* was reported to cause anthracnose diseases on several varieties of *Ca. sinensis* in many regions in Fujian Province, China (Liu 2013). In the present study, the species was found to be widely distributed throughout China, although there appears to be some variation in sequence data among isolates from *Ca. sinensis.* Conidia of the tea isolates (LC2923, av = 14.9 × 4.4 µm and LC3451, av = 15.03 × 4.35 µm) are longer than that of the ex-type (MFLU 090228, av = 11.53 × 3.55) of *C. fructicola.*

Colletotrichum gloeosporioides (Penz.) Penz. & Sacc., Atti Reale Ist. Veneto Sci. Lett. Arti., ser. 6, 2: 670. 1884 — Fig. 11

Basionym. *Vermicularia gloeosporioides* Penz., Michelia 2: 450. 1882.

On PDA: *Colonies* 56–58 mm diam in 7 d, > 90 mm diam in 10 d, flat with erose edge, scattered acervuli with orange conidial ooze near centre, fuscous black pigment near the edge; reverse honey with fuscous black near the edge. *Chlamydospores* not observed. *Conidiomata* acervular, conidiophores formed on a cushion of roundish and medium brown cells. *Setae* not observed. *Conidiophores* hyaline to pale brown, septate, branched. *Conidiogenous cells* hyaline, cylindrical to ampulliform, 5.5–17.5 µm, apex 1–2 µm diam. *Conidia* hyaline, aseptate, smooth-walled, cylindrical, both ends bluntly rounded, 11–15.5 × 4.5–6 µm, av ± SD = 13.5 ± 1.2 × 5.5 ± 0.3 µm, L/W ratio = 2.5. *Appressoria* medium to dark brown, aseptate, solitary or in groups, variable in shape, circular, clavate, ellipsoidal or irregular in outline, crenate or slightly lobed at edge, 7.5–13.5 × 5–9.5 µm, av ± SD = 9.5 ± 1.4 × 6.5 ± 0.9 µm, L/W ratio = 1.5.

Materials examined. China, Jiangxi Province, on *Ca. sinensis*, Sept. 2013, *Y.H. Gao*, culture CGMCC 3.17360 = LC3686 = LF916; Ganzhou, Yangling National Forest Park, on *Ca. sinensis*, Apr. 2013, *F. Liu*, culture LC3110 = LF318; ibid., culture LC3312 = LF534; ibid., culture LC3382 = LF604.

Notes — *Colletotrichum gloeosporioides* is listed as a pathogen of *Camellia* in Australia, Brazil, China, Hong Kong, Japan, and the USA (Farr & Rossman 2014). However, many

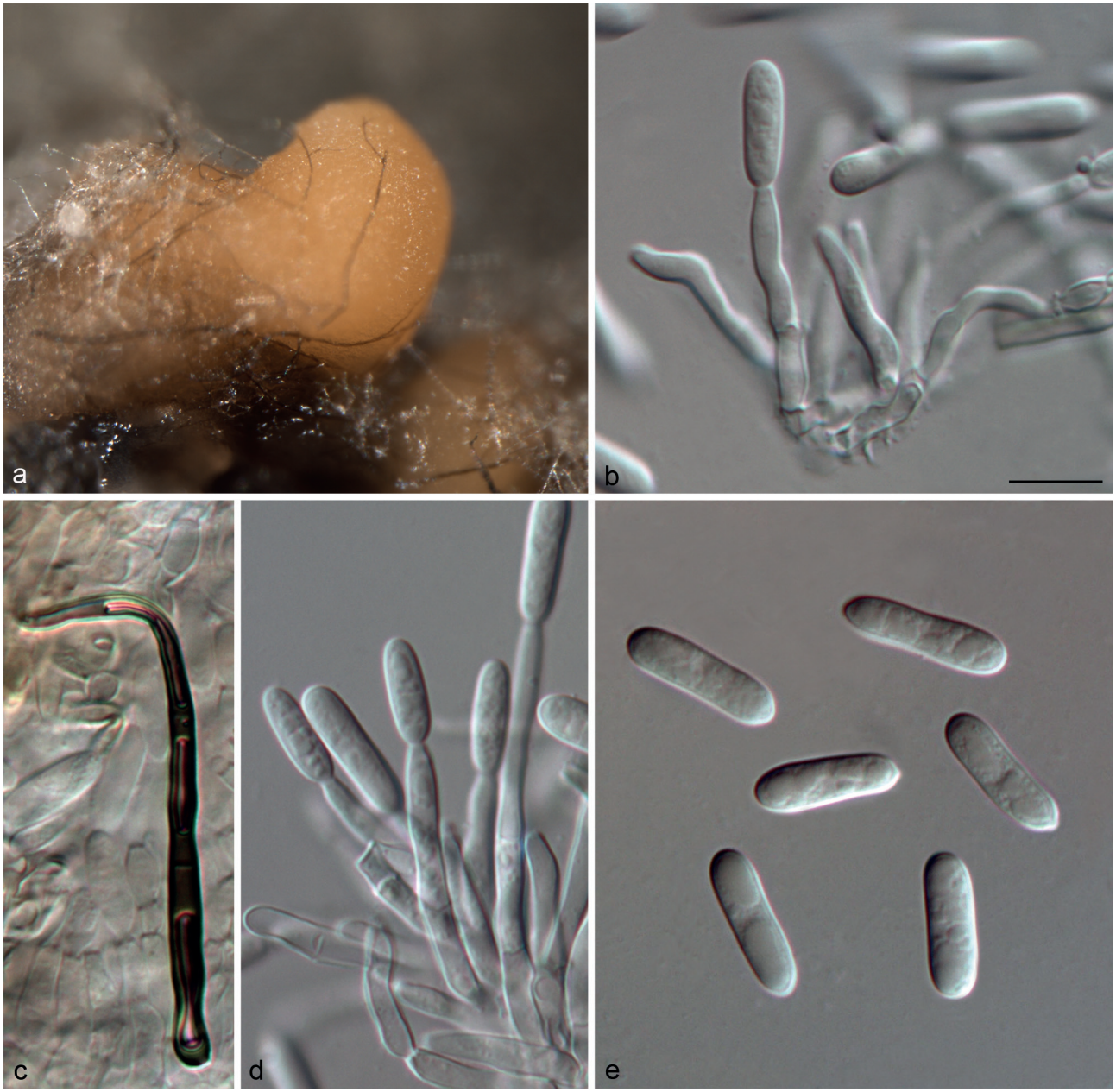

Fig. 10 *Colletotrichum fructicola* on PDA (a, b, d, e from LC2923; c from LC3451). a. Acervulus; b, d. conidiophores; c. seta; e. conidia. — Scale bar: b = 10 µm, scale bar of b applies to b–e.

of these reports probably refer to this species in its broader sense as a species complex and need to be further verified (Watson 1950, Shivas 1989, Osono 2008, Guo et al. 2014). For example, the anthracnose pathogen *C. gloeosporioides* was recently detected in 30–60 % of the *Ca. sinensis* fields in the Yellow Mountain region in China during 2011 to 2012 (Guo et al. 2014), the identification of which, however, was solely based on morphology and NCBI BLAST searches with ITS sequences, and was not based on the presently accepted classification system in *Colletotrichum* (Cannon et al. 2012). *Colletotrichum gloeosporioides* was also considered to be one of the dominant endophytic taxa of *Camellia* in the study of Fang et al. (2013) based on ITS analysis, the identification of which needs to be verified by multi-locus analysis. In our investigation, four isolates of *C. gloeosporioides* were associated with *Camellia*, confirming this species to occur on this host. However, *C. gloeosporioides* is not the dominant *Colletotrichum* species on *Camellia* spp. at the localities where we sampled.

Colletotrichum henanense F. Liu & L. Cai, *sp. nov.* — MycoBank MB809160; Fig. 12

Etymology. Named after the collection site, Henan province, China.

On PDA: *Colonies* 53–59 mm diam in 7 d, > 90 mm diam in 10 d, aerial mycelium pale olivaceous-grey to olivaceous-grey; reverse sulphur-yellow to straw with pale olivaceous-grey to iron-grey in the centre. *Chlamydospores* not observed. *Conidiomata* acervular, conidiophores formed on a cushion of roundish and medium brown cells. *Setae* not observed. *Conidiophores* hyaline to pale brown, septate, branched. *Conidiogenous cells* hyaline to pale brown, cylindrical to ovoid or

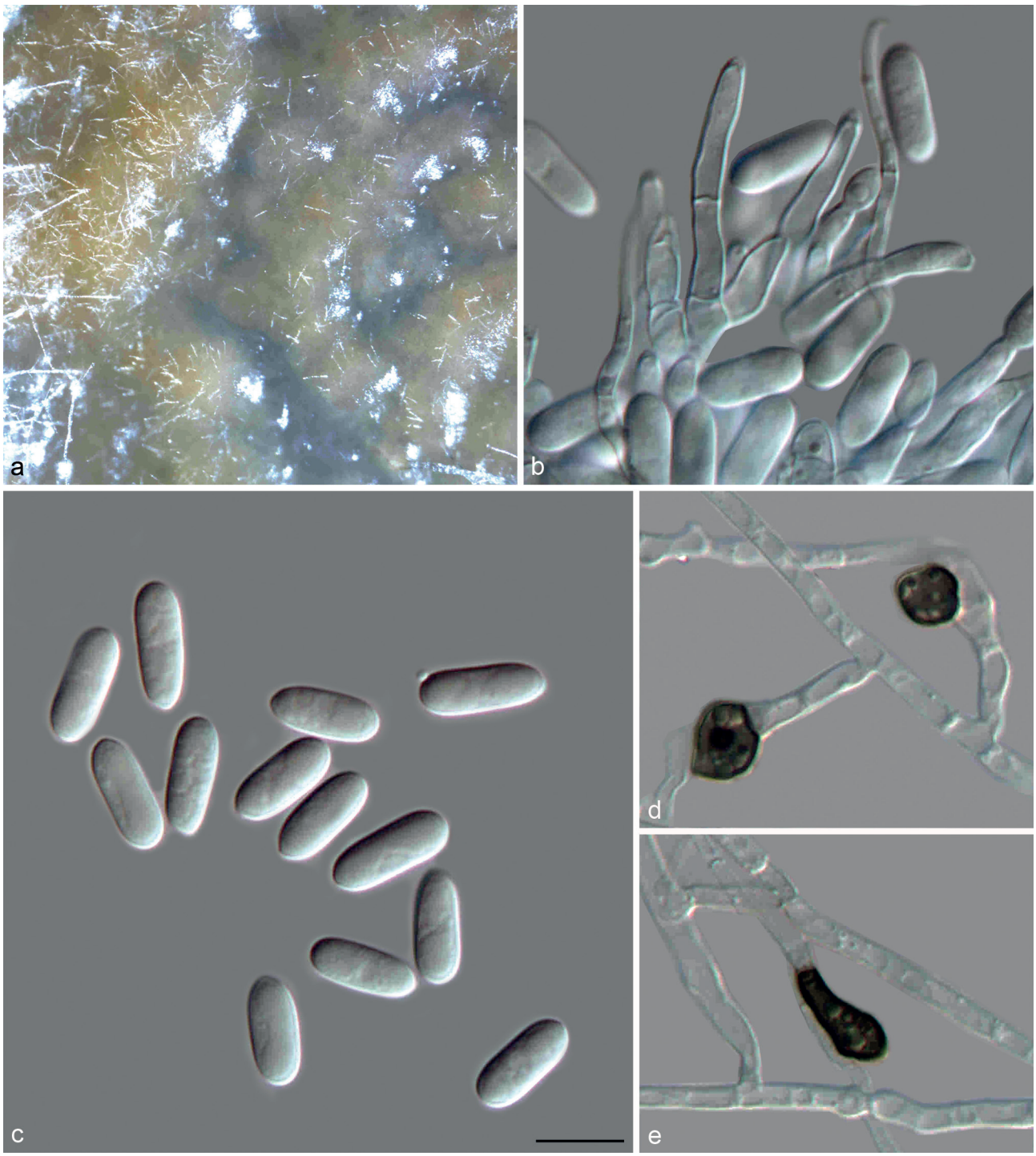

Fig. 11 *Colletotrichum gloeosporioides* (LC3686). a. Acervulus; b. conidiophores; c. conidia; d, e. appressoria (a–c from PDA; d, e from SNA). — Scale bar: c = 10 µm, scale bar of c applies to b–e.

ampulliform, 5.5–12.5 µm, apex 1–2 µm diam. *Conidia* hyaline, usually aseptate, sometimes becoming 1-septate with age, smooth-walled, cylindrical, both ends obtusely rounded, contents sometimes with guttulae, 8–17 × 3–5.5 µm, av ± SD = 12.5 ± 1.8 × 4.5 ± 0.6 µm, L/W ratio = 2.8. *Appressoria* single or in small groups, medium brown, outline mostly clavate or elliptical, rarely lobate, 7–14.5 × 5–9 µm, av ± SD = 11.2 ± 3.7 × 6.7 ± 2 µm, L/W ratio = 1.7.

Materials examined. China, Henan Province, Xinyang, on *Ca. sinensis*, 23 Sept. 2012, *M. Zhang & R. Zang* (holotype HMAS 245381, culture ex-type CGMCC 3.17354 = LC3030 = LF238 = CSBX001); Beijing, Water Great Wall, on *Cirsium japonicum*, 2010, *L. Cai*, culture LC2820 = LF24; ibid., culture LC2821 = LF25.

Notes — The isolates of *C. henanense* isolated from tea plants and *Cirsium japonicum* formed a distinct clade that could be clearly distinguished from other species in the *C. gloeosporioides* species complex (Fig. 1). A BLASTn search of NCBI GenBank with the ITS sequence of CGMCC 3.17354 showed 99 % similarity to quite a number of sequences from isolates previously identified as *C. gloeosporioides* in other studies. The closest match in a BLASTn search in GenBank with the GAPDH sequence of CGMCC 3.17354 was GenBank JX009967 (99 % identity, 3 bp differences), the sequence generated from an authentic isolate of *C. psidii* CBS 145.29 (Weir et al. 2012), and with 98 % identity (5–6 bp differences) to some sequences of

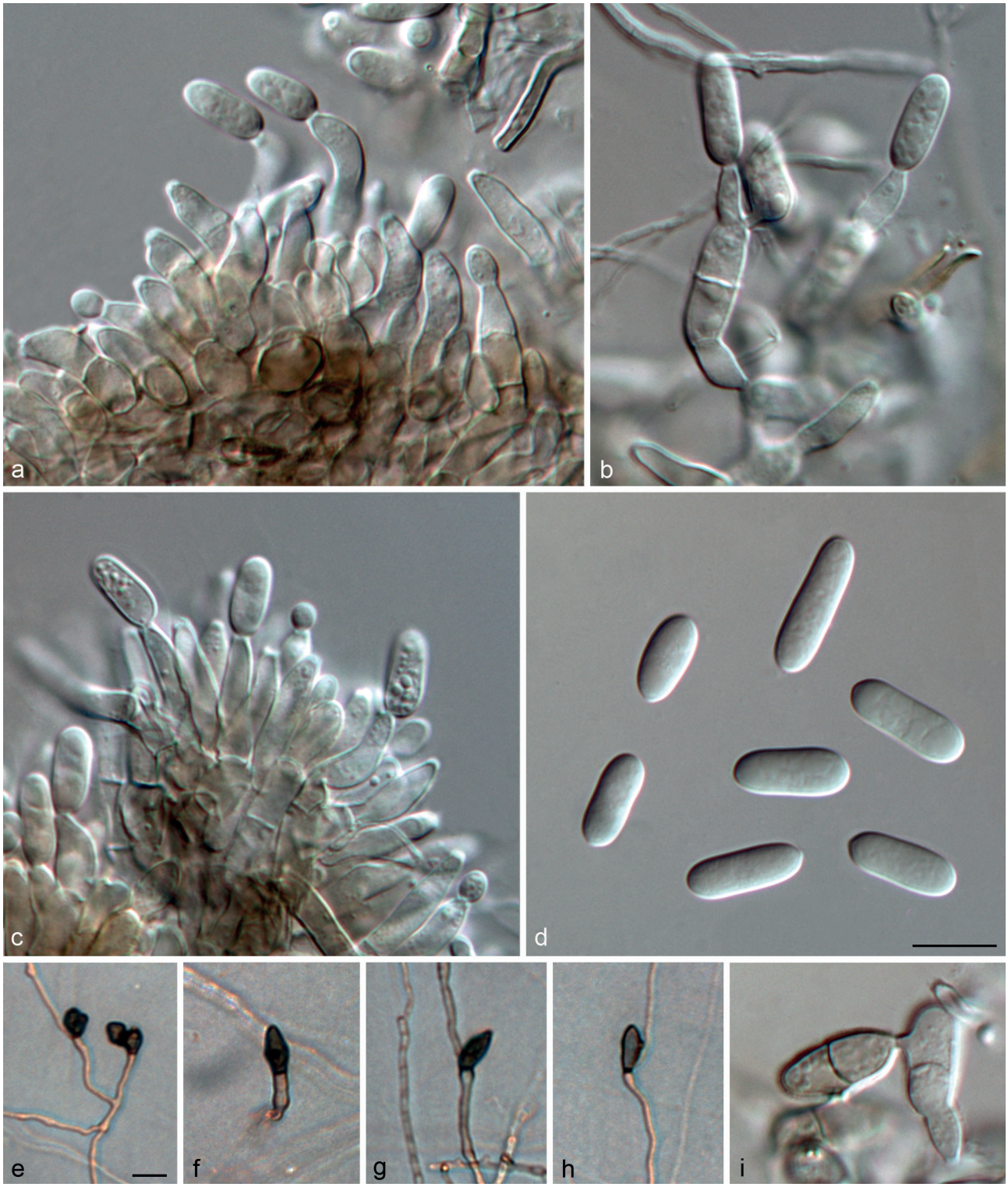

Fig. 12 *Colletotrichum henanense* (CGMCC 3.17354). a–c. Conidiophores; d, i. conidia; e–h. appressoria (a–d, i from PDA; e–h from SNA). — Scale bars: d, e = 10 µm, scale bar of d applies to a–d, i; scale bar of e applies to e–h.

C. aotearoa, *C. ti*, and *Glomerella cingulata* 'f. sp. *camelliae*' isolates (Weir et al. 2012). The top 10 closest matches with the TUB2 sequence (with 97 % identity, 20–23 bp differences) were the isolates of *C. aotearoa* and *C. kahawae* subsp. *ciggaro* analysed in the study of Weir et al. (2012).

Colletotrichum jiangxiense F. Liu & L. Cai, *sp. nov.* — MycoBank MB809161; Fig. 13

Etymology. Named after the collection site, Jiangxi Province, China.

On PDA: *Colonies* 50–53 mm diam in 7 d, > 90 mm diam in 10 d, flat with entire edge, aerial mycelium dense, cottony, white to grey, numerous small acervuli with orange conidial masses near the margin; reverse olivaceous with pale orange near the margin. Appressoria-like structures pale brown to brown, circular, ellipsoidal or irregular. *Conidiomata* acervular, conidiophores formed on a cushion of roundish and medium brown cells. *Setae* not observed. *Conidiophores* hyaline to pale brown, branched. *Conidiogenous cells* hyaline to pale brown, cylindrical, 11.5–20 µm, apex 1–2.5 µm diam. *Conidia* hyaline, aseptate, smooth-walled, cylindrical, both ends bluntly rounded, or one end bluntly rounded and one end acutely rounded, 13–19 × 4–6 µm, av ± SD = 15.2 ± 1.0 × 5.2 ± 0.4 µm, L/W ratio = 2.9. *Appressoria* not observed.

Materials examined. China, Jiangxi Province, Ganzhou, Fengshan Mountain, on *Ca. sinensis*, Sept. 2013, *Y. Zhang* (holotype HMAS 245382, culture ex-type CGMCC 3.17363 = LC3463 = LF687); ibid., culture CGMCC 3.17362 = LC3460 = LF684; Yangling National Forest Park, on *Ca. sinensis*, Apr. 2013, *F. Liu*, culture CGMCC 3.17361 = LC3266 = LF488.

Notes — Based on multi-locus sequence data (ACT, CAL, GAPDH, GS, ITS, TUB2), *C. jiangxiense* is phylogenetically closely related to the devastating coffee berry pathogen *C. kahawae* subsp. *kahawae*, and up to four other taxa, namely *C. kahawae* subsp. *ciggaro*, *C. temperatum*, *C. fructivorum*, and *C. rhexiae* (Fig. 1). All of the *C. jiangxiense* isolates differ from both *C. kahawae* subsp. *kahawae* and *C. kahawae* subsp. *ciggaro* by 1 bp change in CAL, 2 bp changes in ITS, and 17 bp changes and 1 bp indel in GS. Additionally, the 22 bp deletion in the GS sequence used to distinguish *C. kahawae* subsp. *ciggaro* from *C. kahawae* subsp. *kahawae* (Weir et al. 2012) is also lacking in the sequences of the *C. jiangxiense* isolates. Phylogenetic analyses based on single genes (except GS) could not clearly separate *C. jiangxiense* from the above listed species (results not shown). Comparisons of morphological

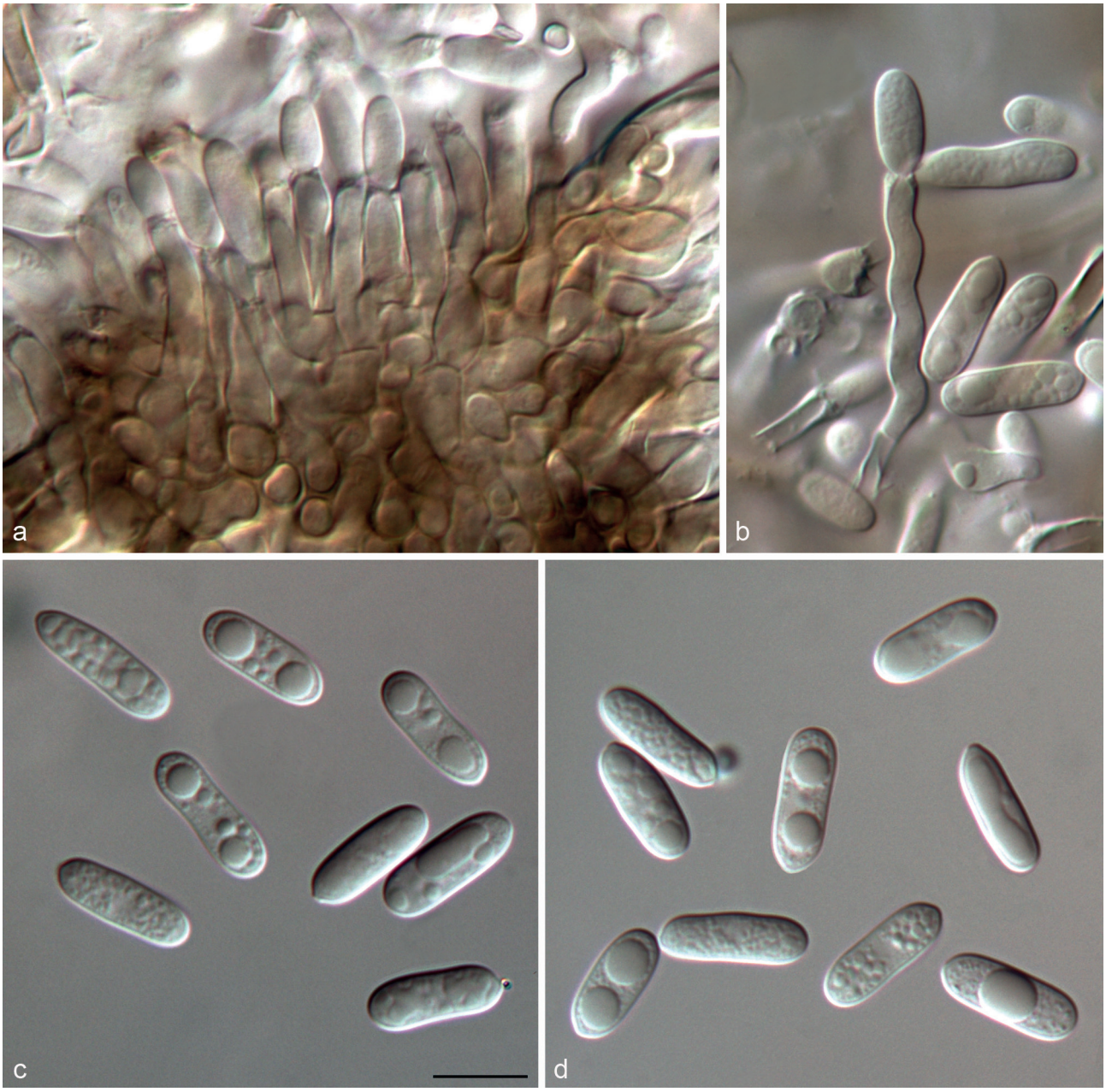

Fig. 13 *Colletotrichum jiangxiense* on PDA (CGMCC 3.17363). a, b. Conidiophores; c, d. conidia. — Scale bar: c = 10 µm, scale bar of c applies to a–d.

and ecological characteristics were also made between these species. Conidia of the tea isolate (CGMCC 3.17363, av = 15.2 × 5.2 µm) are shorter than that of the ex-type culture (ICMP 18539, av = 17.8 × 5.1) of *C. kahawae* subsp. *ciggaro*. *Colletotrichum kahawae* subsp. *kahawae* is host-specific to *Coffea* and was confirmed causing no disease symptoms on *Camellia sinensis* by cross infection experiments (Fig. 6). In conclusion, the pathogenicity test, PHI test (Φ_w = 1) and phylogenetic analyses all suggested that *C. jiangxiense* is distinct from *C. kahawae* s.l.

The closest match in a BLASTn search with the ITS sequences of CGMCC 3.17363 was GenBank JN715848 (with 100 % identity) from isolate R046 from a fruit of *Rubus glaucus* in Colombia, which was identified as *C. kahawae* subsp. *ciggaro* (Afanador-Kafuri et al. unpubl. data). Closest matches with the TUB2 sequence were GenBank KC297083 and KC297082

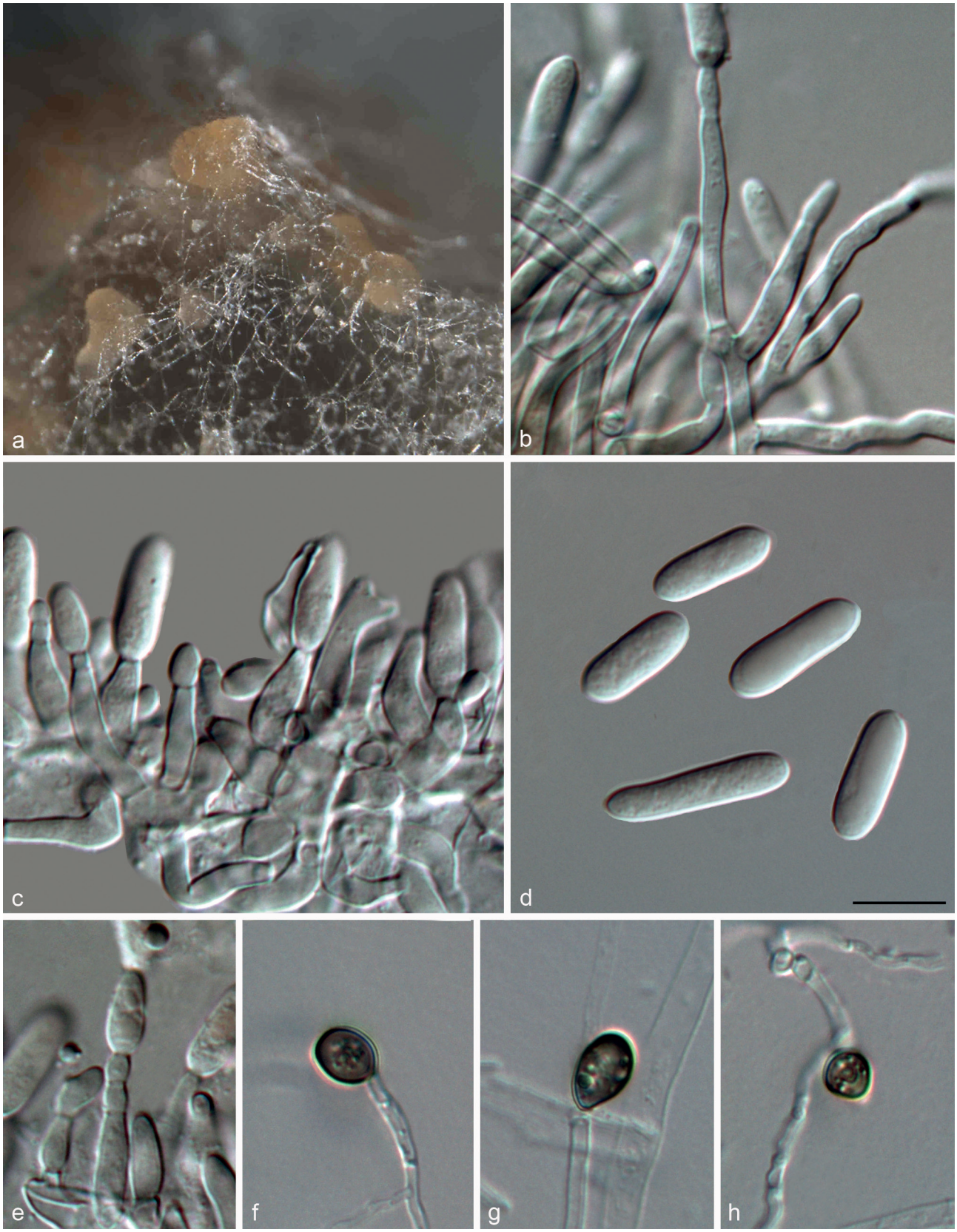

Fig. 14 *Colletotrichum siamense* on PDA (CGMCC 3.17353). a. Acervulus; b, c, e. conidiophores; d. conidia; f–h. appressoria. — Scale bar: d = 10 µm, scale bar of d applies to b–h.

(with 100 % identity) from isolate CBS 115194 and CBS 112984 from *Banksia* sp., both of which are *C. kahawae* subsp. *ciggaro* (Liu et al. 2013b). The GAPDH blast result showed that the sequence of CGMCC 3.17363 was identical to those of the *C. kahawae* subsp. *ciggaro* isolates ICMP 18534 (GenBank JX009904) and ICMP 18544 (GenBank JX009920) (Weir et al. 2012), while CGMCC 3.17363 could be distinguished from ICMP 18534 in the multi-locus tree (Fig. 1).

Colletotrichum karstii Y.L. Yang et al., Cryptog. Mycol. 32: 241. 2011

Description and illustrations — See Yang et al. (2011) and Damm et al. (2012b).

Materials examined. China, Hangzhou, on *Ca. sinensis*, Oct. 2013, *F. Liu*, culture CGMCC 3.17359 = LC3570 = LF798; on *Ca. sinensis*, Oct. 2013, *F. Liu*, culture LC3560 = LF788.

Notes — *Colletotrichum karstii* is a common and geographically diverse species, occurring on various host plants. It was previously reported to be pathogenic to *Ca. sinensis* in China (Liu 2013) and *Camellia* in Italy (Schena et al. 2013). Comparing it to the available TUB2 sequences from *Camellia* in Schena et al. (2013), 4 bp differences were detected between the Italian *C. karstii* and the Chinese isolates.

Colletotrichum siamense Prihast., L. Cai & K.D. Hyde, Fung. Diversity 39: 98. 2009 — Fig. 14

On PDA: *Colonies* 79 mm diam in 7 d, > 90 mm diam in 10 d, aerial mycelium white, cottony, sparse, surface of colony with numerous small acervuli with orange conidial ooze; reverse pale yellowish. *Chlamydospores* not observed. *Conidiomata* acervular, conidiophores formed on a cushion of roundish and medium brown cells. *Setae* not observed. *Conidiophores* hyaline, branched. *Conidiogenous cells* hyaline, cylindrical to ampulliform, 6.5–16 µm, apex 1–2 µm diam. *Conidia* hyaline, aseptate, smooth-walled, cylindrical, both ends bluntly rounded, 12–15.5 × 4–5.5 µm, mean ± SD = 13.8 ± 0.9 × 4.7 ± 0.35 µm, L/W ratio = 2.9. *Appressoria* medium brown, aseptate, solitary, circular, clavate or ellipsoidal, 5.5–9.5 × 5–7.5 µm, mean ± SD = 7.5 ± 1.32 × 5.8 ± 0.7 µm, L/W ratio = 1.3.

Materials examined. China, Sichuan Province, Chengdu Botanical Garden, on *Ca. oleifera*, Oct. 2012, *F. Liu*, culture LC2969 = LF177; on *Camellia* sp., Oct. 2012, *F. Liu*, culture LC2974 = LF182; ibid., culture CGMCC 3.17353 = LC2931 = LF139; Yunnan Province, Pu'er, on *Camellia* sp., 2010, *D.M. Hu*, culture LC0149 = PE007-2.

Notes — Conidiogenous cells of *C. siamense* were not well-illustrated in the original publication (Prihastuti et al. 2009), but are illustrated here based on our isolate from *Camellia* (Fig. 14). *Colletotrichum melanocaulon* was proposed as a novel species closely related to *C. siamense* based on the sequence data of ITS, TUB2, DNA lyase (APN2) and an intergenic spacer between the 3' end of the DNA lyase and the mating type locus MAT1-2 (apn2mat/IGS) (Doyle et al. 2013). Since ACT, CAL, GAPDH and GS gene sequences of *C. melanocaulon* were unavailable, only ITS and TUB2 sequences of the ex-type culture (BPI 884101) were included in our genetic analysis. Another recently published new species *C. dianesei* (Lima et al. 2013), phylogenetically related to *C. siamense*, was also included in the study. The multi-locus phylogenetic analysis result showed that both *C. melanocaulon* and *C. dianesei* clustered together with the ex-type isolate of *C. siamense* (CBS 18578), and its synonyms *C. murrayae* (GZAAS 5.09506), *C. jasmini-sambac* (CBS 130420) and *C. hymenocallidis* (CBS 125378) (Fig. 1). As the ex-type of these species and isolates from tea plants formed a robust clade with high posterior probability (1, Fig. 1, and 0.96, Fig. 3), we suspect *C. melanocaulon* and *C. dianesei* to be synonyms of *C. siamense*. Further studies are needed to confirm if these taxa are synonymous, or if *C. siamense* is a species complex (Sharma et al. 2013a).

DISCUSSION

Colletotrichum species on Camellia

In this study, pathogenic and endophytic *Colletotrichum* isolates associated with *Ca. sinensis* and other *Camellia* spp. were allocated to different species complexes and further assigned to 11 species, including nine known and two new species. Furthermore, this study also represents the first report of *C. alienum*, *C. cliviae*, *C. jiangxiense*, and *C. henanense* from tea plants. Six species were isolated from both symptomatic and asymptomatic leaves tissues, namely *C. camelliae*, *C. fructicola*, *C. gloeosporioides*, *C. jiangxiense*, *C. karstii*, and *C. siamense.* This indicates that they could switch their lifestyle from endophytic to plant pathogenic in nature, and provides additional support for the hypothesis that endophytes can be latent pathogens (Photita et al. 2001, Romero et al. 2001). Some *Colletotrichum* species were collected only once from this host; *C. fioriniae* and *C. henanense* were obtained from symptomatic tea leaves, while *C. alienum*, *C. boninense* and *C. cliviae* were only encountered as endophytes in tea plants. Previous pathogenicity tests showed that *C. fructicola* isolates from symptomless tissues could cause disease on *Citrus* fruits (Huang et al. 2013). Consequently, we hypothesise that endophytic species in *Camellia* could also be potential latent pathogens. Further investigations are therefore required to clarify the ecological relationships of the pathogenic and endophytic *Colletotrichum* species on *Camellia*.

Based on this study, *C. camelliae* is the dominant *Colletotrichum* species on *Camellia* in China and is probably host-specific to *Camellia*. These findings make *C. camelliae* an appropriate model for addressing questions of population structure and dispersal at broad geographical and landscape level. Knowledge of molecular demographic parameters, such as rates of gene flow, levels of species divergence and migration patterns between populations will elucidate the biogeographic history, and the evolutionary and adaptive mechanisms. Information on the genetic structure of the populations can also assist in the development of disease management strategies (Rampersad et al. 2013). Additional collections from *Camellia* growing regions across the world would therefore aid us to characterise the population structure of this important pathogen and to confirm whether this species is indeed the dominant *Colletotrichum* species globally.

Colletotrichum acutatum and *C. gloeosporioides* were previously reported as the dominant endophytic species in *Camellia* based on morphological characteristics or ITS sequence data (Osono 2008, Fang et al. 2013). However, we did not isolate any *C. acutatum* s.str. isolates in our study, and only a single isolate of *C. fioriniae*, belonging to the *C. acutatum* species complex, was obtained from symptomatic tissue. In addition, although the majority of strains from *Camellia* in this study belong to the *C. gloeosporioides* species complex, only four of them are *C. gloeosporioides* s.str., including three pathogenic and one endophytic isolates. This indicates that many of the previous identifications of *Colletotrichum* species on *Camellia* were probably incorrect.

Apart from the *Colletotrichum* species found in this study, *Camellia* spp. could also be infected or colonised by a few other species, i.e. *C. lupini* (Damm et al. 2012a), *C. acutatum*, *C. carveri*, *C. coccodes*, and *C. queenslandicum* (syn. *C. gloeosporioides* var. *minor*, Weir et al. 2012) (Farr & Rossman 2014). These reports (except *C. lupini*), however, need to be

verified based on the presently accepted classification system in *Colletotrichum*.

Combined use of ApMat and GS in the C. gloeosporioides species complex

The Apn2-Mat1 locus was introduced for differentiation of *Colletotrichum* species in the *C. graminicola* species complex by Crouch et al. (2009), while Rojas et al. (2010) applied it to the *C. gloeosporioides* species complex. Following this, a new marker in the intergenic region of APN2 and MAT1-2-1 was specifically designed to improve the systematics of the *C. gloeosporioides* species complex (Silva et al. 2012b), and the locus was renamed as ApMat, which has subsequently been used in molecular phylogenetic analyses of this group (Sharma et al. 2013a, 2014, Vieira et al. 2014).

In the study of Silva et al. (2012a), the ApMat locus proved to be the most informative marker compared to other standard markers, and could resolve species in the *C. gloeosporioides* species complex and provide a similar amount of information and support as the concatenated tree based on seven loci (ApMat, Apn25L, MAT5L, MAT1-2-1, ITS, β-tub2, GS). However, it is noteworthy that the sample size in their study was rather limited, including only 22 isolates belonging to six divergent species from *Coffea*. Subsequently, the ApMat marker was employed to analyse species in the *C. gloeosporioides* complex that are associated with *Mangifera indica* using a larger sample size, in which 39 *Colletotrichum* isolates were separated into nine lineages, namely *C. fragariae*, *C. fructicola*, *C. jasmini-sambac*, *C. melanocaulon* and five unnamed lineages (Sharma et al. 2013a). In that study, only 15 of the *Colletotrichum* isolates used in the ApMat gene analysis were also included in a multilocus phylogenetic tree (ACT, CAL, CHS, GAPDH, ITS, TUB2) where they were separated into four clades corresponding to *C. theobromicola*, *C. asianum*, *C. siamense* and *C. fructicola*. However, no comparison was made between the results of the single-locus ApMat and the multi-locus phylogenetic analysis.

In order to determine if the ApMat sequences provide adequate phylogenetic information compared to that of a multi-locus dataset, we constructed both single-locus ApMat and combined 6-marker (ACT, CAL, GAPDH, GS, ITS, TUB2) trees using the same *Colletotrichum* isolates associated with *Camellia* collected in this study. All ApMat reference sequences used in Sharma et al. (2013a) were incorporated in our ApMat analysis, except for GenBank KC888927 from *C. alienum* isolate ICMP 12071 (incorrect sequence deposited by the original author). The ApMat sequence of isolate ICMP 12071 was re-sequenced and submitted to GenBank as GenBank KM360144 in this study. Our study demonstrated that 22 species (*C. aenigma*, *C. aeschynomenes*, *C. alatae*, *C. alienum*, *C. asianum*, *C. aotearoa*, *C. camelliae*, *C. clidemiae*, *C. cordylinicola*, *C. fructicola*, *C. gloeosporioides*, *C. henanense*, *C. horii*, *C. musae*, *C. nupharicola*, *C. psidii*, *C. queenslandicum*, *C. salsolae*, *C. siamense*, *C. theobromicola*, *C. ti*, and *C. tropicale*) could be clearly delimitated with ApMat (Fig. 3 and Fig. S2 in TreeBASE). Although *C. fructivorum*, *C. jiangxiense*, *C. kahawae* subsp. *kahawae*, *C. rhexiae*, and *C. temperatum* clustered together in one big clade, the species *C. fructivorum*, *C. rhexiae*, and *C. temperatum* could be delimitated by forming three small subclades with high posterior probabilities (Fig. S2 in TreeBASE). However, *C. jiangxiense* and *C. kahawae* subsp. *kahawae* could not be distinguished from each other. Furthermore, isolates of *C. camelliae* were separated into two subclades (Fig. 3 and Fig. S2 in TreeBASE). Although *C. jiangxiense* could be distinguished from *C. kahawae* s.l. by the GS marker, the other species in the *C. gloeosporioides* species complex could not be delimited very well, e.g. *C. camelliae*, *C. fructicola*, *C. siamense*, and *C. queenslandicum* (data not shown). This study demonstrates that the ApMat marker provides superior phylogenetic information compared to other used loci and can distinguish most species in the *C. gloeosporioides* species complex. A further phylogenetic analysis using the concatenated ApMat and GS alignment showed that all species could be delimited, including *C. jiangxiense* and *C. kahawae* subsp. *kahawae*. We therefore recommend a combination of ApMat and GS as an effective way of identifying species in the *C. gloeosporioides* species complex.

In the present study we mainly focused on the taxonomy and biodiversity of *Colletotrichum* species associated with tea plants in China as plant pathogens and/or endophytes. Further attention should be given to surveys from different geographical regions to help resolve the life cycles and ecology of these species, especially of *C. camelliae*. Because of the important commercial value of tea plantations, appropriate disease management strategies in tea plantations should also be developed to control infection by *Colletotrichum* species.

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