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Colletotrichum aracearum and *C. camelliae-japonicae*, two holomorphic new species from China and Japan

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Abstract

Colletotrichum aracearum sp. nov. from *Monstera delociosa* and *Philodenron selloum*, and *C. camelliae-japonicae* sp. nov. from *Camellia japonica*, are described in this paper. Strains were isolated using single spore isolation technique from the spore masses formed on leaf lesions, and successfully induced for sexual states in cultivation. Morphological comparisons were made with related species. Phylogenetic relationships were inferred based on ITS, ACT, TUB2, and GAPDH sequences. *C. camelliae-japonicae* belongs to the *C. boninense* species complex, while *C. aracearum* does not nest within any currently known species complex but forms a sister clade to *C. cliviae*. This is the first report of *Colletotrichum* species from hosts *Monstera delociosa* and *Philodenron selloum*.

Key words – Anthracnose – *Camellia* – *Monstera* – Morphology – *Philodenron* – Phylogeny – Taxonomy

Introduction

Colletotrichum is one of the most important genera among plant pathogenic fungi, mainly causing anthracnose on a wide range of crops or ornamental plants (Sutton 1980, Bailey et al. 1992, Freeman et al. 1998, Ureña-Padilla et al. 2002, Farr et al. 2006, Than et al. 2008a, Yang et al. 2009, Wikee et al. 2011, Noireung et al. 2012). Plant diseases associated with *Colletotrichum* species often caused significant economic losses (Sutton et al. 1992, Farr et al. 2006, Prihastuti et al. 2009). For example, *Colletotrichum* spp. cause extensive pre- and postharvest damage to chilli fruits, with yield losses up to 50% (Manandhar et al. 1995, Pakdeevaraporn et al. 2005, Than et al. 2008b). In addition, many *Colletotrichum* species have also been recorded as endophytes, epiphytes, or saprobes (Photita et al. 2001, Kumar & Hyde 2004, Liu et al. 2007, Prihastuti et al. 2009, Hyde et al. 2009, Rojas et al. 2010).

Studies of *Colletotrichum* could be traced back to Corda (1831), who first described *Colletotrichum lineola*. However, the taxonomy of *Colletotrichum* has been uncertain for centuries, until recently polyphasic characters, especially the molecular data were employed to delimitate species (Cannon et al. 2000, Hyde et al. 2009, Cai et al. 2009, Cannon et al. 2012, Damm et al. 2012b, Weir et al. 2012, Crouch 2014, Liu et al. 2015). Polyphasic characterization employing morphology and multi-locus phylogeny has contributed to the successful identification and epitypification of many *Colletotrichum* species which significantly stabilized the taxonomy (Damm

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et al. 2012a). Currently, *Colletotrichum* comprises of 11 major species complexes and several independent species. However, most species were only observed for their asexual morphs.

The objective of this study was to describe two novel *Colletotrichum* species from China and Japan for their holomorphic morphological characters. Phylogenetic relationships were inferred based on combined multi-locus sequence data.

Materials & Methods

Isolates and morphology

Strains of *Colletotrichum* were isolated from the anthracnose lesions on *Camellia japonica* (camellia), *Monstera delociosa* (ceriman) and *Philodendron selloum* (lacy tree philodendron) from China and Japan. Single spore isolation technique was applied to plant tissue where spore masses were formed. Spore masses were picked off with a fine forceps and suspended in sterilized water. The spore suspension was diluted to a reasonable concentration and spread onto the surface potato dextrose agar (PDA), flowed by incubation overnight at room temperature (25 ºC). Single germinating spores were picked up with a sterilized needle and transferred to new PDA plate for morphological and molecular study (Zhang et al. 2013).

Each isolate was plated onto PDA to prepare actively growing cultures. 4-mm-diam. plugs from the actively growing edge of a 5-day-old culture were transferred to the centre of fresh synthetic nutrient-poor agar medium (SNA) plates (Nirenberg 1976). The *Anthriscus sylvestris* stems were double-autoclaved and placed onto the surface of the SNA plate to promote sporulation (Damm et al. 2012a). Morphological descriptions were made after 7 days growth under alternating 12 hours near UV/12 hours dark at 25 ºC (Sutton 1980). Colony characters were observed and the diameter was measured at day seven. The growth rate was calculated as the 5-day average of mean daily growth (mm per day). Mycelial appressoria were produced using a slide culture technique (Sutton 1980). Microscopic preparations were made in clear lactic acid, with 30 measurements per structure as observed under a Nikon Eclipse 80i (Nikon Instech Company Limited, Kawasaki, Kanagawa, Japan) compound microscope using differential interference contrast (DIC) illumination. Taxonomic descriptions and nomenclature were deposited in Index Fungorum (Index Fungroum 2016).

DNA extraction, PCR amplification and sequencing

Genomic DNA of the isolates was extracted using the method of Guo et al. (2000). Four loci were amplified, including the internal transcribed spacer regions and intervening 5.8S rRNA gene (ITS), partial beta-tubulin (TUB2), actin (ACT), the glyceraldehyde-3-phosphate dehydrogenase (GAPDH), using primer pairs ITS1/ITS4, T1/Bt-2b, ACT-512F/ACT-783R, GDF1/GDR1, respectively. Amplification mixtures and conditions were followed as described by Liu et al. (2012). Purification and sequencing of PCR amplicons were carried out by the Biomed Sequencing Company, Beijing, China.

Sequence alignment and molecular phylogenetic analysis

Sequences of references and outgroup (*Monilochaetes infuscans*) were downloaded from GenBank and are listed in Table 1. Single gene and concatenated gene datasets were aligned with MAFFT v.7 (Katoh & Frith 2012), and manually edited in MEGA v.6.0 when necessary (Tamura et al. 2013). Bayesian inference (BI) and Maximum Likelihood (ML) methods were implemented in this study. Bayesian analyses were performed using MrBayes v.3.2.2 (Ronquist et al. 2012) as described by Liu et al. (2014). Evolutionary models were selected by MrModeltest v.2.3 (Nylander 2004), with critical values for the topological convergence diagnostic set to 0.01. Posterior probabilities (PP) were determined by Markov Chain Monte Carlo sampling (BMCMC) in MrBayes v.3.2.2 (Zhaxybayeva & Gogarten 2002, Ronquist et al. 2012). Four simultaneous Markov chains were run for 1 000 000 generations and trees were sampled every 100th generation

Table 1 Strains of *Colletotrichum* used in this study. Details are provided about hosts, location and GenBank accessions of the sequences generated

Species	Association number	Host	Locality	GenBank accessions			
				ITS	GAPDH	ACT	TUB2
C. annellatum	CBS 129826*	*Hevea indica*	Colombia	JQ005222	JQ005309	JQ005570	JQ005656
C. araceaerum	**CGMCC 3.14982, LC1033***	*Monstera deliciosa*	China	**KX853166**	**KX893585**	**KX893577**	**KX893581**
C. araceaerum	**CGMCC 3.14983, LC1041**	*Philodendron selloum*	China	**KX853167**	**KX893586**	**KX893578**	**KX893582**
C. beeveri	CBS 128527, ICMP 18594*	*Brachyglottis repanda*	New Zealand	JQ005171	JQ005258	JQ005519	JQ005605
C. boninense	CBS 123755, MAFF 305972*	*Crinum asiaticum*	Japan	JQ005153	JQ005240	JQ005501	JQ005588
C. boninense	CBS 128547, ICMP 10338	*Camellia* sp.	New Zealand	JQ005159	JQ005246	JQ005507	JQ005593
C. boninense	CBS 128526, ICMP 18591	*Dacrycarpus dacrydioides*	New Zealand	JQ005162	JQ005249	JQ005510	JQ005596
C. brasiliense	CBS 128501, ICMP 18607*	*Passiflora edulis*	Brazil	JQ005235	JQ005322	JQ005583	JQ005669
C. brasiliense	CBS 128528, ICMP 18606	*Passiflora edulis*	Brazil	JQ005234	JQ005321	JQ005582	JQ005668
C. brassicicola	CBS 101059, LYN 16331*	*Brassica oleracea*	New Zealand	JQ005172	JQ005259	JQ005520	JQ005606
C. brevisporum	BCC 38876*	*Neoregalia* sp.	Thailand	JN050238	JN050227	JN050216	JN050244
C. brevisporum	MFLUCC 100182	*Pandanus pygmaeus*	Thailand	JN050239	JN050228	JN050217	JN050245
C.camelliae-japonicae	**CGMCC3.18117, LC6415**	*Camellia japonica*	Japan	**KX853164**	**KX893583**	**KX893575**	**KX893579**
C.camelliae-japonicae	**CGMCC3.18118, LC6416***	*Camellia japonica*	Japan	**KX853165**	**KX893584**	**KX893576**	**KX893580**
C. citricola	CBS 134228, CGMCC 3.15227*	*Citrus unshiu*	China	KC293576	KC293736	KC293616	KC293656
C. citricola	CBS 134229	*Citrus unshiu*	China	KC293577	KC293737	KC293617	KC293657
C. citricola	CBS 134230	*Citrus unshiu*	China	KC293578	KC293738	KC293618	KC293658
C. cliviae	CBS 125375*	*Clivia miniata*	China	JX519223	JX546611	JX519240	JX519249
C. colombiense	CBS 129818*	*Passiflora edulis*	Colombia	JQ005174	JQ005261	JQ005522	JQ005608
C. colombiense	CBS 129817	*Passiflora edulis*	Colombia	JQ005173	JQ005260	JQ005521	JQ005607
C. constrictum	CBS 128504, ICMP 12941*	*Citrus limon*	New Zealand	JQ005238	JQ005325	JQ005586	JQ005672
C. constrictum	CBS 128503, ICMP 12936	*Solanum betaceum*	New Zealand	JQ005237	JQ005324	JQ005585	JQ005671
C. curcumae	IMI 288937	*Curcuma longa*	India	GU227893	GU228285	GU227991	GU228187
C. cymbidiicola	IMI 347923*	*Cymbidium* sp.	Australia	JQ005166	JQ005253	JQ005514	JQ005600
C. cymbidiicola	CBS 128543, ICMP 18584	*Cymbidium* sp.	New Zealand	JQ005167	JQ005254	JQ005515	JQ005601
C. cymbidiicola	CBS 123757, MAFF 306100	*Cymbidium* sp.	Japan	JQ005168	JQ005255	JQ005516	JQ005602
C. dacrycarpi	CBS 130241, ICMP 19107*	*Dacrycarpus dacrydioides*	New Zealand	JQ005236	JQ005323	JQ005584	JQ005670
C. dracaenophilum	CBS 118199*	*Buxus* sp.	China	JX519222	JX546707	JX519238	JX519247

Table 1 (continued)

C. excelsum-altitudum	CGMCC 3.15130, LC2344	*Bletilla ochracea*	China	HM751815	KC843502	KC843548	JX625211
C. excelsum-altitudum	CGMCC 3.15131, LC2345	*Bletilla ochracea*	China	JX625182	KC843503	KC843549	JX625212
C. gloeosporioides	IMI 356878*, CBS 112999	*Citrus sinensis*	Italy	JX010152	JX010056	JX009531	JX010445
C. karstii	CBS 129833	*Musa* sp.	Mexico	JQ005175	JQ005262	JQ005523	JQ005609
C. karstii	CBS 127591	*Sapium integerrimium*	Australia	JQ005186	JQ005273	JQ005534	JQ005620
C. karstii	CBS 132134, CGMCC 3.14194*	*Vanda* sp.	China	HM585409	HM585391	HM581995	HM585428
C. novae-zelandiae	CBS 128505, ICMP 12944*	*Capsicum annuum*	New Zealand	JQ005228	JQ005315	JQ005576	JQ005662
C. novae-zelandiae	CBS 130240, ICMP 12064	*Citrus grapefruit*	New Zealand	JQ005229	JQ005316	JQ005577	JQ005663
C. oncidii	CBS 129828*	*Oncidium* sp.	Germany	JQ005169	JQ005256	JQ005517	JQ005603
C. oncidii	CBS 130242	*Oncidium* sp.	Germany	JQ005170	JQ005257	JQ005518	JQ005604
C. parsonsiae	CBS 128525, ICMP 18590*	*Parsonsia capsularis*	New Zealand	JQ005233	JQ005320	JQ005581	JQ005667
C. petchii	CBS 118193, AR 3658	*Dracaena sanderana*	China	JQ005227	JQ005314	JQ005575	JQ005661
C. petchii	CBS 125957	*Dracaena* sp.	Netherlands	JQ005226	JQ005313	JQ005574	JQ005660
C. petchii	CBS 378.94*	*Dracaena marginata*	Italy	JQ005223	JQ005310	JQ005571	JQ005657
C. phyllanthi	CBS 175.67, MACS 271*	*Phyllanthus acidus*	India	JQ005221	JQ005308	JQ005569	JQ005655
C. sp.	CBS 123921, MAFF 238642	*Dendrobium kingianum*	Japan	JQ005163	JQ005250	JQ005511	JQ005597
C. torulosum	CBS 128544, ICMP 18586*	*Solanum melongena*	New Zealand	JQ005164	JQ005251	JQ005512	JQ005598
C. torulosum	CBS 102667	*Passiflora edulis*	New Zealand	JQ005165	JQ005252	JQ005513	JQ005599
C. tropicicola	BCC 38877*	*Citrus maxima*	Thailand	JN050240	JN050229	JN050218	JN050246
C. tropicicola	MFLUCC 100167	*Panphiopedilum bellatolum*	Thailand	JN050241	JN050230	JN050219	JN050247
C. truncatum	CBS 151.35*	*Phaseolus lunatus*	USA	GU227862	GU228254	GU227960	GU228156
C. truncatum	CBS 120709	*Capsicum frutescens*	India	GU227877	GU228269	GU227975	GU228171
C. yunnanense	AS 3.9167, CBS 132135*	*Buxus* sp.	China	JX546804	JX546706	JX519239	JX519248
M. infuscans	CBS 869.96	*Ipomoea batatas*	South Africa	JQ005780	JX546612	JQ005843	JQ005864

[a] AS, CGMCC: China General Microbiological Culture Collection; BCC: BIOTEC Culture Collection, Thailand; CBS: Culture collection of the Centraal bureau 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; MACS: MACS Collection of Microorganisms, India; MAFF: Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand.

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

(resulting in 10 000 total trees). The first 25% of the trees were discarded as the burn-in phase of each analysis, and the remaining trees were used to calculate posterior probabilities (Cai et al. 2006, Liu et al. 2012). Maximum-likelihood analyses including 1000 bootstraps replicates were conducted using RAxML v.7.2.6 (Stamatakis et al. 2010). A general time reversible model (GTR) was applied with a gamma-distributed rate variation. Trees were visualized in FigTree 1.4.0 (Rambaut 2012).

Genealogical concordance phylogenetic species recognition analysis

Phylogenetically closely related species were analyzed 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 SplitsTree v.4 (Huson 1998, Huson & Bryant 2006) in order to determine the recombination level within phylogenetically closely related species using a 4-locus concatenated dataset (ACT, GAPDH, ITS and TUB2). Pairwise homoplasy index below a 0.05 threshold ($\Phi_w < 0.05$) indicates significant recombination present in the dataset. The relationship between closely related species was visualized by constructing a splits graph.

Results

Phylogenetic analyses

Fifty-two combined ACT, GAPDH, ITS and TUB2 sequences were aligned, comprising 1730 characters including gaps after alignment (295 for ACT; 350 for GAPDH; 577 for ITS; 508 for TUB2). The concatenated alignment was deposited in TreeBASE (Study Accession URL: http://purl.org/phylo/treebase/phylows/study/TB2:S19933). For the Bayesian inference, a HKY+G model with inverse gamma distributed rate was selected for ACT; a HKY+I+G model with inverse gamma distributed rate was selected for TUB2 and GAPDH; a GTR+I+G model with inverse gamma distributed rate was selected for ITS.

Two strains isolated from *Camellia japonica* clustered within the *C. boninense* complex, and the other two strains isolated from *Philodenron selloum* and *Monstera delociosa* formed a sister clade to *C. cliviae*, which do not belong to any previously known species complexes (Fig. 1).

Pairwise homoplasy index (PHI) test

A pairwise homoplasy index (PHI) test using a 4-gene dataset (ACT, GAPDH, ITS and TUB2) was performed to determine the recombination level between *C. camelliae-japonicae* and its phylogenetically closely related species, *C. citricola* (Fig. 2). No significant recombination events could be detected between *C. camelliae-japonica* and *C. citricola* ($\Phi w = 1$).

Taxonomy

Colletotrichum aracearum LW. Hou & L. Cai, **sp. nov.** Fig. 3

Index Fungorum number: IF552557

Etymology – named after the host plant family, *Araceae*.

Description – Asexual morph: *Conidiomata* acervular, pale yellow colored. Conidiophores and setae formed on a pale brown cushion. *Setae* 135–155 µm long, medium brown, smooth to verruculose, 3–4-septate, bacilliform, sometimes slightly inflated at base, 5–7 µm diam. at the widest part. *Conidiophores* hyaline or pale brown; mostly septate, branched or unbranched at the base, up to 52 µm long. *Conidiogenous cells* hyaline or pale brown, cylindrical, ovoid, ampulliform or lageniform, 8.5–18 × 3–5 µm. *Conidia* hyaline, oblong, apex and base rounded, often containing scattered small granular, wall smooth, aseptate, straight, 14–19 × 4.5–6 µm (mean ± SD = 16.5 ± 1.4 × 5.7 ± 0.3 µm, n=30), L/W ratio = 2.8. *Appressoria* solitary, dark greenish, thick-walled, entire edge or crenate, rarely lobate, smooth-walled, ellipsoidal or irregular in shape, 7.5–12.5 × 5.5–9.5 µm (mean ± SD = 9.5 ± 1.5 × 7.4 ± 1.2 µm, n=20), L/W ratio = 1.3.

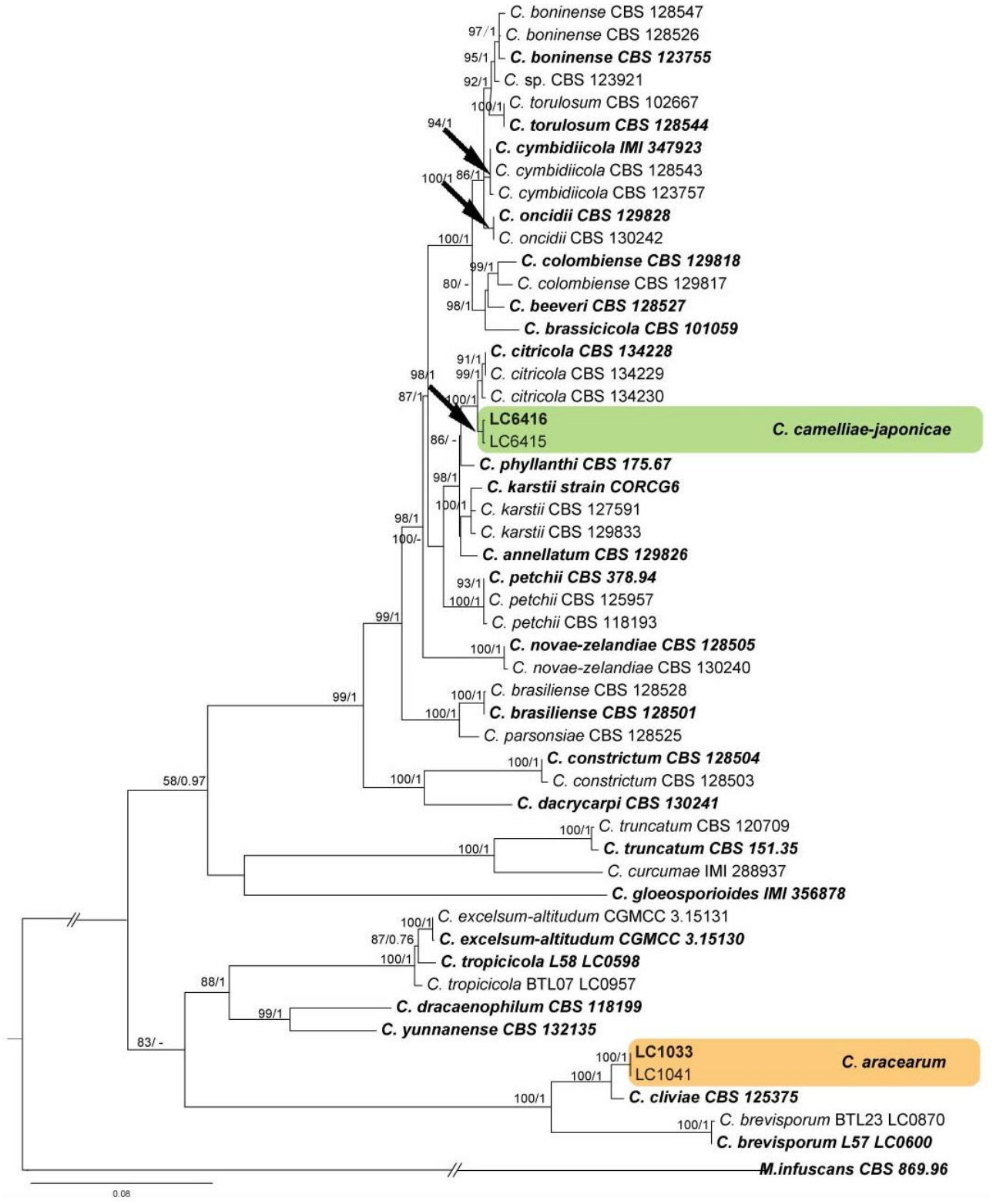

Fig. 1 – Phylogenetic tree from a Bayesian analysis based on combined gene sequences (ACT, GAPDH, ITS and TUB2) showing the phylogenetic relationships among the *C. boninense* complex, the *C. truncatum* complex, and independent units in *Colletotrichum*. The RAxML bootstrap support values (ML > 50) and Bayesian posterior probabilities (PP > 0.95) are displayed at the nodes (PP/ML). The tree is rooted with *Monilochaetes infuscans* (CBS 869.96). The scale bar indicates 0.08 expected changes per site. Ex-type cultures are emphasized in bold.

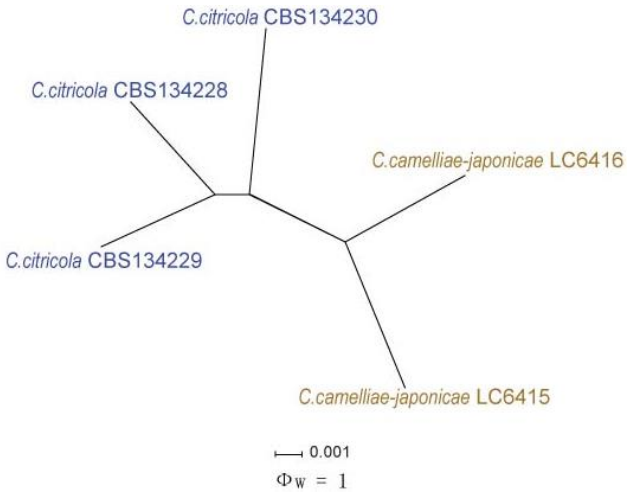

Fig. 2 – The result of the pairwise homoplasy index (PHI) test of closely related species using both LogDet transformation and splits decomposition. PHI test results (Φ_w) < 0.05 indicate significant recombination within the dataset.

Sexual morph – *Ascomata* perithecia, oval, globose or obpyriform; medium brown, 175–260 ×125–165 µm; the outer wall composed of flattened angular cells 2.5–8.5 µm diam. *Interascal tissue* composed of rather irregular thin-walled hyaline septate paraphyses. *Asci* in basal fascicle, clavate, with a truncated apex and a small refractive apical ring, 32.5–70 × 6–14 µm, 8-spored. *Ascospores* hyaline, aseptate, allantoid, 8.5–11.5 × 2–3 µm, mean ± SD = 10.2 ± 0.9 × 2.5 ± 0.2 µm, L/W ratio = 4.1.

Culture characters – Colonies on SNA flat with entire margin, surface covered with dark brown or black ascomata and scattered pale yellow conidiomata. Surface of *Anthriscus* covered with ascomata and partly with white mycelia. Reverse hyaline. Colonies on PDA attaining 6–6.2 cm diam. in 5 days at 25 ºC, growth rate 12–13 mm per day; at first white, becoming pale grayish, finally become dark greenish with white edges; surface with dark ascomata. Reverse brown or greenish.

Materials examined – China, Guangdong Province, botanical garden, on *Monstera deliciosa*, 25 November 2010, Yuanying Su, holotype HMAS 243485, ex-holotype living culture CGMCC 3.14982 (= LC1033). China, Guangdong Province, botanical garden, on *Philodenron selloum*, Yuanying Su, 25 November 2010, living culture CGMCC 3.14983 (= LC1041).

Notes – Two strains of *C. araceaerum* were obtained from different hosts, i.e. *Monstera delociosa* and *Philodenron selloum*, both belonging to *Araceae*. Both strains presented similar growth rates and morphological characters. This is the first report of *Colletotrichum* from these two host plants (Farr & Rossman 2016). The clade representing *C. araceaerum* is closely related to *C. cliviae*, a species that has not been observed for sexual morph (Yang et al. 2009). *C. araceaerum* differs from *C. cliviae* in producing shorter conidia (14–19 × 4.5–6 µm vs. 19.5–24.5 × 4.5–7 µm), smaller appresorria (9.5 ± 1.5 × 7.4 ± 1.2 µm vs. 11.7 ± 1.2 × 8.6 ± 1.2 µm), and slower growth rate (12–13 mm/day vs. 15.2–16 mm/day).

Colletotrichum camelliae-japonicae LW. Hou & L. Cai, **sp. nov.** Fig. 4

Index Fungorum Number: IF552558

Etymology – named after the epithet of its host plants, *Camellia japonica*.

Description – Asexual morph: *Vegetative hyphae* 1.5–3 µm diam.; hyaline, smooth-walled, branched with septate. *Conidiomata* acervular, conidiophores formed on a pale brown cushion; conidiophores hyaline, septate, occasionally branched at base, 35.5–46 µm long. *Setae* unobserved. *Conidiogenous cells* hyaline, 7–21.5 × 3–4.5 µm, oblong to ampulliform, often extending to form new conidiogenous loci. *Conidia* hyaline, oblong, single-celled, apex and base rounded, with a

Fig. 3 – *Colletotrichum araceaerum* (from ex-holotype strain LC1033) on SNA. A, B Conidiomata. C, D, E Setae. F, G, H Conidiophores. I, J, K, L, M, N Appressoria. O, P Conidia from SNA. Q Conidia from the *Anthriscus sylvestris* stems. R, S Ascomata. T Outer surface of apical regions of asci. U Peridium in cross section. V Ascospores. W, X, Y Asci. – Scale bars = 10 µm.

Fig. 4 – *Colletotrichum camelliae-japonicae* (from holotype strain LC6416) on SNA. A, B Conidiomata. C, D, E Conidiophores. F, G, H, I, K Appressoria. L Conidia from SNA. M Conidia from the *Anthriscus sylvestris* stems. N, O Ascomata. P Outer surface of apical regions of asci. Q Peridium in cross section. R Ascospores. S, T, U Asci. – Scale bars = 10 µm.

prominent scar; smooth-walled, aseptate, most contents granular or guttulate; 11–14.5 × 5–6.5 µm, mean ± SD = 12.5 ± 0.8 × 5.5 ± 0.3 µm, L/W ratio = 2.3. *Appressoria* solitary, dark greenish, thick-walled, entire edge, subglobose, ellipsoidal or obpyriform, sometimes irregular in shape, smooth-walled, 5–11 × 4–7 µm (mean ± SD = 8.5 ± 1.6 × 5.5 ± 0.9 µm, n=20), L/W ratio = 1.5.

Sexual morph – *Ascomata* perithecia, ellipsoidal, globose, ovoid, obpyriform, aggregated or scattered, 190–250 × 155–250 µm, glabrous, brown, abundant but mostly sterile. The outer wall of ascomata composed of flattened angular cells, 3–5.5 µm diam. *Interascal tissue* composed of rather irregular thin-walled hyaline septate paraphyses. *Asci* clavate, 58.5–79 × 11.5–14.5 µm long, 8-spored; apex with a truncated and a small apical ring. *Ascospores* hyaline, one-celled, allantoid or fusiform, slightly curved, 13.5–18.5 × 4–5.5 µm, mean ± SD = 16.5 ± 1.1 × 5 ± 0.4 µm, L/W ratio = 3.3.

Culture characters – Colonies on SNA flat, lacking aerial mycelium; surface covered with orange or pale yellow conidiomata; surface of *Anthriscus* covered with ascomata and white mycelium. Reverse hyaline. Colonies on PDA attaining 4–4.2 cm diam. in 7 days at 25 °C, growth rate 8–9 mm per day; at first white, becoming grayish and finally covered with orange conidia mass. Reverse pale brown or grayish.

Material examined – Japan, intercepted by Ningbo Entry-Exit Inspection and Quarantine Bureau when exporting to China, on *Camellia japonica*, Weijun Duan, 25 November 2013, HMAS 247042 (Holotype designated here), ex-holotype living culture CGMCC 3.18118 (=LC6416); *ibid*. CGMCC 3.18117 (=LC6415).

Notes – Two strains of *C. camelliae-japonicae* were isolated from *Camellia japonica* imported from Japan and intercepted by Ningbo Entry-Exit Inspection and Quarantine Bureau. *Camellia japonica* is a commonly cultivated economic crop in China and other Asian countries. Several species of *Colletotrichum* have been reported as pathogens and endophytes from *Camellia* (Liu et al. 2015), but *C. camelliae-japonicae* is distinct from known species in morphological and phylogenetic characters. Based on multi-locus sequence data (ACT, GAPDH, ITS and TUB2), the clade representing *C. camelliae-japonicae* did not nest in any known species complexes but formed a sister clade to *C. citricola* with highly supported bootstrap value and posterior probability (Fig. 1). *Colletotrichum camelliae-japonicae* differs from *C. citricola* in producing narrower ascospores (4.0–5.5 µm, mean ± SD = 5.0 ± 0.4 µm vs. 5.3–6.7 µm, mean = 6.1 µm, L/W ratio 2.6 vs. 3.3) and shorter conidia (11–14.5 µm, mean ± SD = 12.5 ± 0.8 µm vs. 13.7–16.1 µm, mean = 15.1 µm).

Discussion

Although the ITS has been proposed as a universal barcode for fungi (Begerow et al. 2010, Druzhinina et al. 2005, Eberhardt et al. 2010, Schoch et al. 2011, Schoch et al. 2012), it is evolutionarily too conserved to distinguish taxa in *Colletotrichum* (Du et al. 2005, Crouch et al. 2009, Begerow et al. 2010). In this study, polyphasic approach was used to identify strains of *Colletotrichum*. It is very interesting that both new species described in this study have been successfully induced for the sexual stages and described.

Clades representing two new species were well separated from known species in the phylogenetic tree. Both species also present distinct morphological differences from their closely related species. Apart from 11 major lineages in this genus, previous studies also revealed several independently evolved small clusters, which have been named *C. dracaenophilum*, *C. yunnanense* and *C. cliviae* (Cannon et al. 2012). *Colletotrichum araceaerum* appeared to be an additional distinct species that do not belong to the 11 major clades.

Plants belongs to the *Araceae* are frequently infected by *Colletotrichum* species (Farr & Rossman 2016). This is the first report of *Colletotrichum* species from hosts *Monstera delociosa* and *Philodenron selloum* in *Araceae*. As economically important crops in Asia (Wachira et al. 1995), *Camellia* spp. have been widely cultivated from the Himalayas to Japan and Indonesia. As a potential quarantine object, accurate identification of *C. camelliae-japonicae* contributes to the prevention of the outbreak of camellia anthracnose.

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