



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

Hou LW^{1,2}, Liu F¹, Duan WJ³, Cai L^{1*}

¹State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China

²University of Chinese Academy of Sciences, Beijing 100049, China

³Ningbo Academy of Inspection and Quarantine, Ningbo 315012, China

Hou LW, Liu F, Duan WJ, Cai L 2016 – *Colletotrichum aracearum* and *C. camelliae-japonicae*, two holomorphic new species from China and Japan. *Mycosphere* 7(8) 1111–1123, Doi 10.5943/mycosphere/si/2c/4

Abstract

Colletotrichum aracearum sp. nov. from *Monstera delociosa* and *Philodendron 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 *Philodendron selloum*.

Key words – Anthracnose – *Camellia* – *Monstera* – Morphology – *Philodendron* – 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, Pakdeevaporn 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

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

Table 1 (continued)

<i>C. excelsum-altitudum</i>	CGMCC 3.15130, LC2344	<i>Bletilla ochracea</i>	China	HM751815	KC843502	KC843548	JX625211
<i>C. excelsum-altitudum</i>	CGMCC 3.15131, LC2345	<i>Bletilla ochracea</i>	China	JX625182	KC843503	KC843549	JX625212
<i>C. gloeosporioides</i>	IMI 356878*, CBS 112999	<i>Citrus sinensis</i>	Italy	JX010152	JX010056	JX009531	JX010445
<i>C. karstii</i>	CBS 129833	<i>Musa</i> sp.	Mexico	JQ005175	JQ005262	JQ005523	JQ005609
<i>C. karstii</i>	CBS 127591	<i>Sapium integerrimum</i>	Australia	JQ005186	JQ005273	JQ005534	JQ005620
<i>C. karstii</i>	CBS 132134, CGMCC 3.14194*	<i>Vanda</i> sp.	China	HM585409	HM585391	HM581995	HM585428
<i>C. novae-zelandiae</i>	CBS 128505, ICMP 12944*	<i>Capsicum annuum</i>	New Zealand	JQ005228	JQ005315	JQ005576	JQ005662
<i>C. novae-zelandiae</i>	CBS 130240, ICMP 12064	<i>Citrus grapefruit</i>	New Zealand	JQ005229	JQ005316	JQ005577	JQ005663
<i>C. oncidii</i>	CBS 129828*	<i>Oncidium</i> sp.	Germany	JQ005169	JQ005256	JQ005517	JQ005603
<i>C. oncidii</i>	CBS 130242	<i>Oncidium</i> sp.	Germany	JQ005170	JQ005257	JQ005518	JQ005604
<i>C. parsonsiae</i>	CBS 128525, ICMP 18590*	<i>Parsonsia capsularis</i>	New Zealand	JQ005233	JQ005320	JQ005581	JQ005667
<i>C. petchii</i>	CBS 118193, AR 3658	<i>Dracaena sanderana</i>	China	JQ005227	JQ005314	JQ005575	JQ005661
<i>C. petchii</i>	CBS 125957	<i>Dracaena</i> sp.	Netherlands	JQ005226	JQ005313	JQ005574	JQ005660
<i>C. petchii</i>	CBS 378.94*	<i>Dracaena marginata</i>	Italy	JQ005223	JQ005310	JQ005571	JQ005657
<i>C. phyllanthi</i>	CBS 175.67, MACS 271*	<i>Phyllanthus acidus</i>	India	JQ005221	JQ005308	JQ005569	JQ005655
<i>C. sp.</i>	CBS 123921, MAFF 238642	<i>Dendrobium kingianum</i>	Japan	JQ005163	JQ005250	JQ005511	JQ005597
<i>C. torulosum</i>	CBS 128544, ICMP 18586*	<i>Solanum melongena</i>	New Zealand	JQ005164	JQ005251	JQ005512	JQ005598
<i>C. torulosum</i>	CBS 102667	<i>Passiflora edulis</i>	New Zealand	JQ005165	JQ005252	JQ005513	JQ005599
<i>C. tropicicola</i>	BCC 38877*	<i>Citrus maxima</i>	Thailand	JN050240	JN050229	JN050218	JN050246
<i>C. tropicicola</i>	MFLUCC 100167	<i>Panphiopedilum bellatolum</i>	Thailand	JN050241	JN050230	JN050219	JN050247
<i>C. truncatum</i>	CBS 151.35*	<i>Phaseolus lunatus</i>	USA	GU227862	GU228254	GU227960	GU228156
<i>C. truncatum</i>	CBS 120709	<i>Capsicum frutescens</i>	India	GU227877	GU228269	GU227975	GU228171
<i>C. yunnanense</i>	AS 3.9167, CBS 132135*	<i>Buxus</i> sp.	China	JX546804	JX546706	JX519239	JX519248
<i>M. infuscans</i>	CBS 869.96	<i>Ipomoea batatas</i>	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/phyloids/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 *Philodendron 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-japonicae* 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 \times 3–5 μm . *Conidia* hyaline, oblong, apex and base rounded, often containing scattered small granular, wall smooth, aseptate, straight, 14–19 \times 4.5–6 μm (mean \pm SD = 16.5 \pm 1.4 \times 5.7 \pm 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 \times 5.5–9.5 μm (mean \pm SD = 9.5 \pm 1.5 \times 7.4 \pm 1.2 μm , n=20), L/W ratio = 1.3.

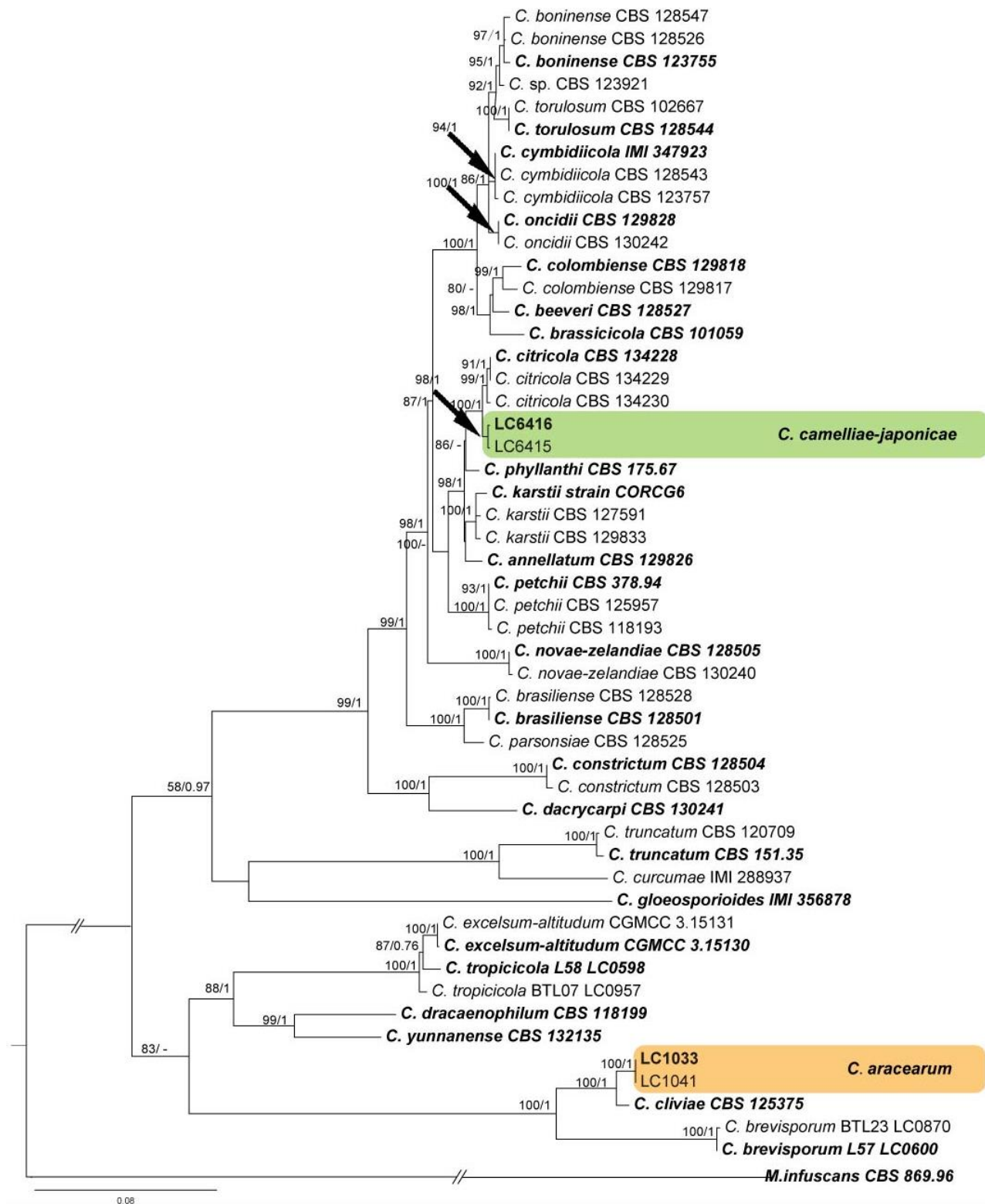


Fig. 1 – Phylogenetic tree from a Bayesian analysis based on combined gene sequences (ACT, GAPDH, ITS and TUB2) showing the phylogenetic relationships among the *C. boninense* complex, the *C. truncatum* complex, and independent units in *Colletotrichum*. The RAxML bootstrap support values (ML > 50) 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.

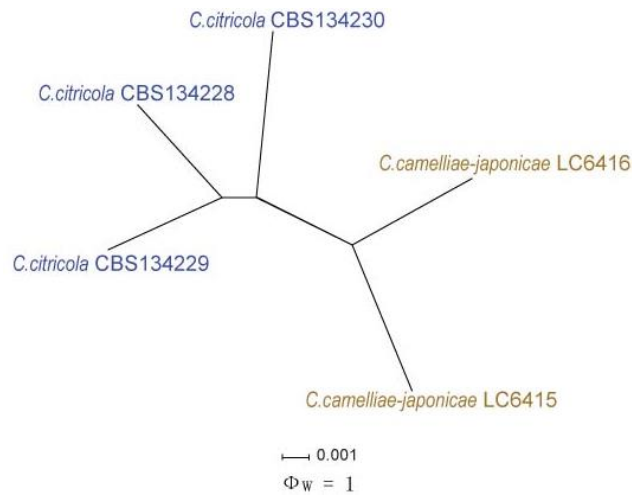


Fig. 2 – The result of the pairwise homoplasy index (PHI) test of closely related species using both LogDet transformation and splits decomposition. PHI test results (Φ_w) < 0.05 indicate significant recombination within the dataset.

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 °C, 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 *Philodendron selloum*, Yuanying Su, 25 November 2010, living culture CGMCC 3.14983 (= LC1041).

Notes – Two strains of *C. aracearum* were obtained from different hosts, i.e. *Monstera deliciosa* and *Philodendron 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. aracearum* is closely related to *C. cliviae*, a species that has not been observed for sexual morph (Yang et al. 2009). *C. aracearum* differs from *C. cliviae* in producing shorter conidia (14–19 × 4.5–6 µm vs. 19.5–24.5 × 4.5–7 µm), smaller appressoria (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

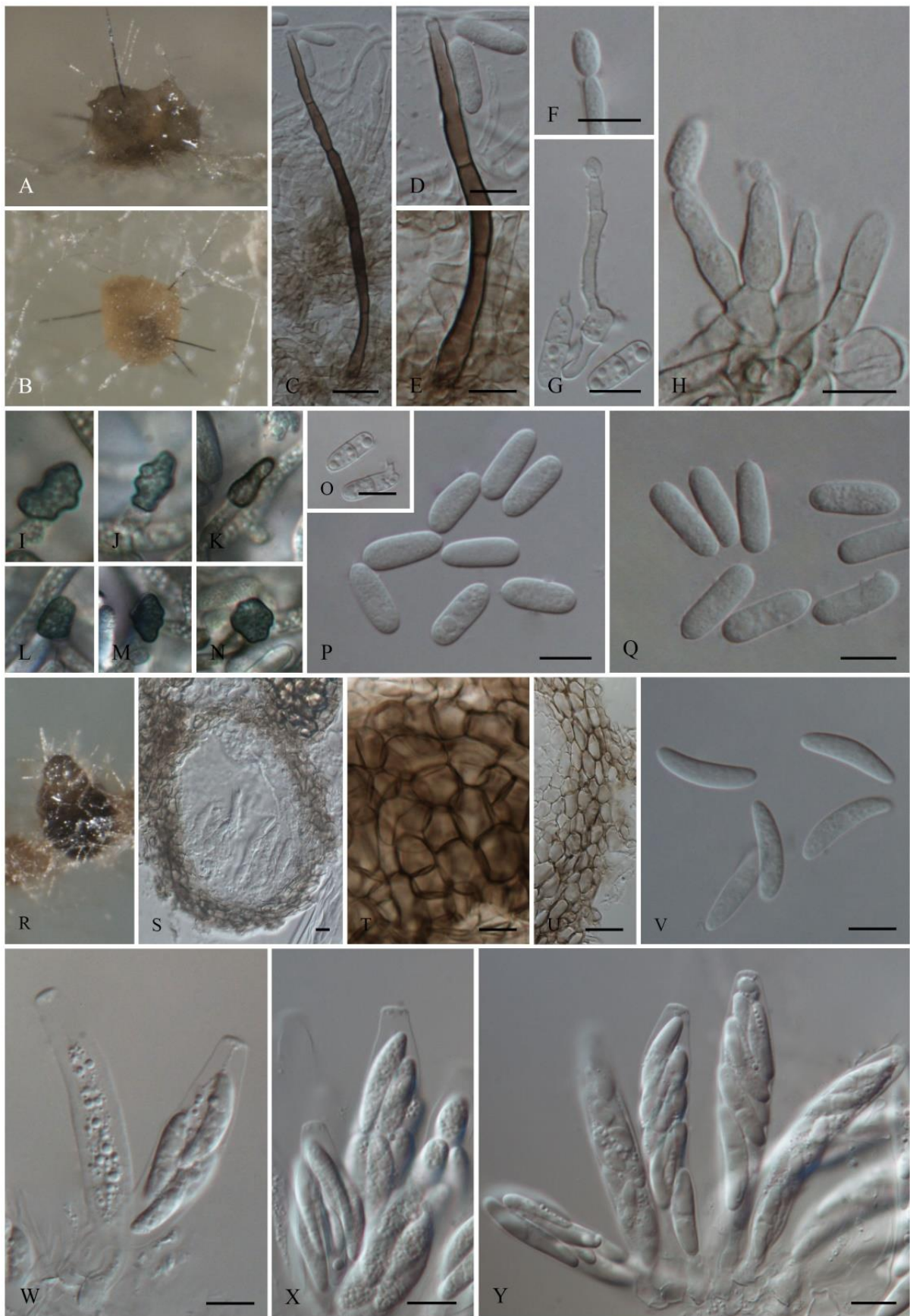


Fig. 3 – *Colletotrichum aracearum* (from ex-holotype strain LC1033) on SNA. A, B Conidiomata. C, D, E Setae. F, G, H Conidiophores. I, J, K, L, M, N Appressoria. O, P Conidia from SNA. Q Conidia from the *Anthriscus sylvestris* stems. R, S Ascomata. T Outer surface of apical regions of asci. U Peridium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

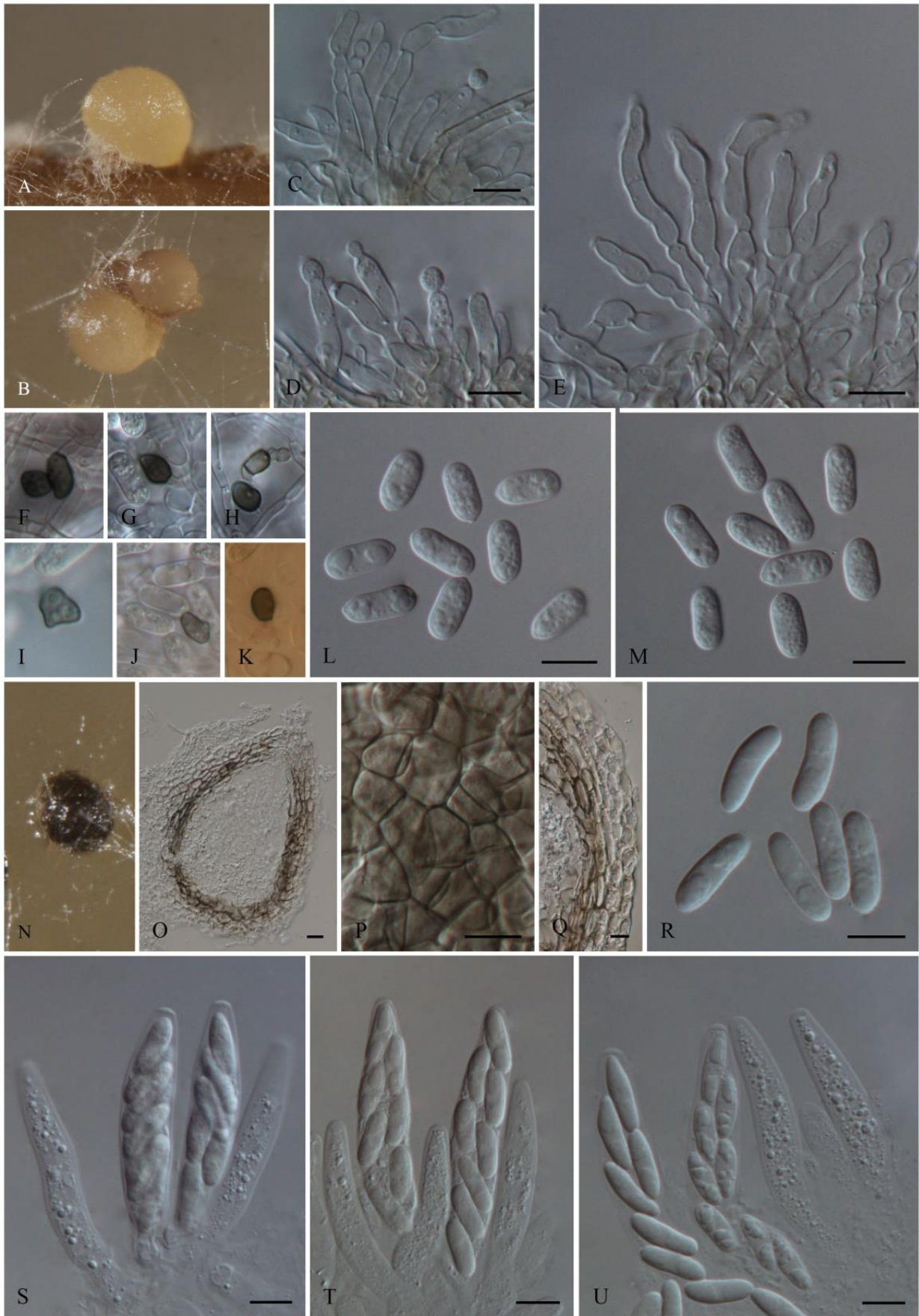


Fig. 4 – *Colletotrichum camelliae-japonicae* (from holotype strain LC6416) on SNA. A, B Conidiomata. C, D, E Conidiophores. F, G, H, I, K Appressoria. L Conidia from SNA. M Conidia from the *Anthriscus sylvestris* stems. N, O Ascomata. P Outer surface of apical regions of asci. Q Peridium in cross section.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

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

Sexual morph – *Ascomata* perithecia, ellipsoidal, globose, ovoid, obpyriform, aggregated or scattered, $190\text{--}250 \times 155\text{--}250 \mu\text{m}$, glabrous, brown, abundant but mostly sterile. The outer wall of ascomata composed of flattened angular cells, $3\text{--}5.5 \mu\text{m}$ diam. *Interascal tissue* composed of rather irregular thin-walled hyaline septate paraphyses. *Asci* clavate, $58.5\text{--}79 \times 11.5\text{--}14.5 \mu\text{m}$ long, 8-spored; apex with a truncated and a small apical ring. *Ascospores* hyaline, one-celled, allantoid or fusiform, slightly curved, $13.5\text{--}18.5 \times 4\text{--}5.5 \mu\text{m}$, mean \pm SD = $16.5 \pm 1.1 \times 5 \pm 0.4 \mu\text{m}$, 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 ($4.0\text{--}5.5 \mu\text{m}$, mean \pm SD = $5.0 \pm 0.4 \mu\text{m}$ vs. $5.3\text{--}6.7 \mu\text{m}$, mean = $6.1 \mu\text{m}$, L/W ratio 2.6 vs. 3.3) and shorter conidia ($11\text{--}14.5 \mu\text{m}$, mean \pm SD = $12.5 \pm 0.8 \mu\text{m}$ vs. $13.7\text{--}16.1 \mu\text{m}$, mean = $15.1 \mu\text{m}$).

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 *Philodendron 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.

Acknowledgements

Yuanying Su is thanked for providing strains. This study was financially supported by the National Natural Science Foundation of China (NSFC 31322001). L.W. Hou acknowledges CAS QYZDB-SSW-SMC044 for supporting her postgraduate studentship.

References

- Bailey JA, O'Connell RJ, Pring RJ, Nash C, Jeger MJ. 1992 – Infection strategies of *Colletotrichum* species. In: *Colletotrichum: Biology, Pathology and Control* (Bailey JA, Jeger MJ, eds), CAB International, Wallingford, The United Kingdom 88–120.
- Begerow D, Nilsson H, Unterseher M, Maier W. 2010 – Current state and perspectives of fungal DNA barcoding and rapid identification procedures. *Applied Microbiology and Biotechnology* 87, 99–108.
- Cai L, Hyde KD, Taylor PWJ, Weir B, Waller J, Abang MM, Zhang JZ, Yang YL, Phoulivong S, Liu ZY, Prihastuti H, Shivas RG, McKenzie EHC, Johnston PR. 2009 – A polyphasic approach for studying *Colletotrichum*. *Fungal Diversity* 39, 183–204.
- Cai L, Jeewon R, Hyde KD. 2006 – Phylogenetic investigations of Sordariaceae based on multiple gene sequences and morphology. *Mycological Research* 110, 137–150.
- Cannon PF, Bridge PD, Monte E. 2000 – Linking the past, present and future of *Colletotrichum* systematics. In: *Colletotrichum : Host Specificity, Pathology, and Host Pathogen Interaction* (eds. D. Prusky, S. Freeman and M.B. Dickman). APS Press, St. Paul, Minnesota, USA: 1–20.
- Cannon PF, Damm U, Johnston PR, Weir BS. 2012 – *Colletotrichum*—current status and future directions. *Studies in Mycology* 73, 181–213.
- Cordeiro ACI. 1831 – Die Pilze Deutschlands. In: *Deutschlands Flora in Abbildung nach der Natur mit Beschreibungen* (Sturm, J). Sturm, Nürnberg vol. 3, Abt. 12: 33–64, tab. 21–32.
- Crouch JA, Clarke BB, Hillman BI. 2009 – What is the value of ITS sequence data in *Colletotrichum* systematics and species diagnosis? A case study using the falcate-spored gramminicolous *Colletotrichum* group. *Mycologia* 101, 648–656.
- Crouch JA. 2014 – *Colletotrichum caudatum* s. l. is a species complex. *IMA Fungus* 5, 17–30.
- Damm U, Cannon PF, Woudenberg JHC, Crous PW. 2012a – The *Colletotrichum acutatum* species complex. *Studies in Mycology* 73, 37–113.
- Damm U, Cannon PF, Woudenberg JHC, Johnston PR, Weir BS, Tan YP, Shivas RG, Crous PW. 2012b – The *Colletotrichum boninense* species complex. *Studies in Mycology* 73, 1–36.
- Druzhinina IS, Kopchinskiy AG, Komoń M, Bissett J, Szakacs G, Kubicek CP. 2005 – An oligonucleotide barcode for species identification in *Trichoderma* and *Hypocrea*. *Fungal Genetics and Biology* 42, 813–828.
- Du M, Schardl CL, Nuckles EM, Vaillancourt LJ. 2005 – Using mating-type gene sequences for improved phylogenetic resolution of *Collectotrichum* species complexes. *Mycologia* 97, 641–658.
- Eberhardt U. 2010 – A constructive step towards selecting a DNA barcode for fungi. *New Phytologist* 187, 265–268.
- Farr DF, Aime MC, Rossman AY, Palm ME. 2006 – Species of *Colletotrichum* on *Agavaceae*. *Mycology Research* 110, 1395–1408.
- Farr DF, Rossman AY. 2016 – Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. <http://nt.ars-grin.gov/fungal-databases/> (Retrieved August 7, 2016).
- Freeman S, Katan T, Shabi E. 1998 – Characterization of *Colletotrichum* species responsible for anthracnose diseases of various fruits. *Plant Disease*, American Phytopathological Society, St. Paul, Minnesota, USA 82, 596–605.
- Guo LD, Hyde KD, Liew ECY. 2000 – Identification of endophytic fungi from *Livistona chinensis* based on morphology and rDNA sequences. *New Phytologist* 147, 617–630.
- Huson DH, Bryant D. 2006 – Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution* 23, 254–267.

- Huson DH. 1998 – SplitsTree: analyzing and visualizing evolutionary data. *Bioinformatics* 14, 68–73.
- Hyde KD, Cai L, McKenzie EHC, Yang YL, Zhang JZ, Prihastuti H. 2009 – *Colletotrichum*: a catalogue of confusion. *Fungal Diversity* 39, 1–17.
- Index Fungorum. 2016 – Index Fungorum. Available from: <http://www.indexfungorum.org/names/Names.asp> (accessed 14 July 2016).
- Katoh K, Frith MC. 2012 – Adding unaligned sequences into an existing alignment using MAFFT and LAST. *Bioinformatics* 28, 3144–3146.
- Kumar DSS, Hyde KD. 2004 – Biodiversity and tissue-recurrence of endophytic fungi from *Tripterygium wilfordii*. *Fungal Diversity* 17, 69–90.
- Liu F, Cai L, Crous PW, Damm U. 2014 – The *Colletotrichum gigasporum* species complex. *Persoonia* 33, 83–97.
- Liu F, Hu DM, Cai L. 2012 – *Conlarium duplumascospora* gen. et. sp. nov. and *Jobellisia guangdongensis* sp. nov. from freshwater habitats in China. *Mycologia* 104, 1178–1186.
- Liu F, Weir BS, Damm U, Crous PW, Wang Y, Liu B, Wang M, Zhang M, Cai L. 2015 – Unravelling *Colletotrichum* species associated with *Camellia*: employing *ApMat* and *GS* loci to resolve species in the *C. gloeosporioides* complex. *Persoonia* 35, 63–86.
- Liu XY, Xie XM, Duan JX. 2007 – *Colletotrichum yunnanense* sp. nov., a new endophytic species from *Buxus* sp. *Mycotaxon* 100, 137–144.
- Manandhar JB, Hartman GL, Wang TC. 1995 – Anthracnose development on pepper fruits inoculated with *Colletotrichum gloeosporioides*. *Plant Disease*, American Phytopathological Society, St. Paul, Minnesota, USA 79, 380–383.
- Nirenberg HI. 1976 – Untersuchungen über die morphologische und biologische Differenzierung in der Fusarium-Sektion Liseola. *Mitt. Biol. Bundesanst. Land-u. Forstwirtschaft. Berlin-Dahlem*, 169: 1–117.
- Noireung P, Phoulivong S, Liu F, Cai L, McKenzie EHC, Chukeatirote E, Jones EBG, Ali H Bahkalif, Hyde KD. 2012 – Novel species of *Colletotrichum* revealed by morphology and molecular analysis. *Cryptogamie, Mycologie* 33, 347–362.
- Nylander JAA. 2004 – MrModeltest v2.3 software. Evolutionary Biology Center, Uppsala University, Sweden.
- Pakdeevaporn P, Wasee S, Taylor PWJ, Mongkolporn O. 2005 – Inheritance of resistance to anthracnose caused by *Colletotrichum capsici* in Capsicum. *Plant Breeding* 124, 206–208.
- Photita W, Lumyong S, Lumyong P, Ho WH, McKenzie EHC, Hyde KD. 2001 – Fungi on *Musa acuminata* in Hong Kong. *Fungal Diversity* 6, 99–106.
- Prihastuti H, Cai L, Chen H, McKenzie EHC, Hyde KD. 2009 – Characterization of *Colletotrichum* species associated with coffee berries in northern Thailand *Fungal Diversity* 39, 89–109.
- Quaedvlieg W, Binder M, Groenewald JZ, Summerell BA, Carnegie AJ, Burgess TI, Crous PW. 2014 – Introducing the Consolidated Species Concept to resolve species in the *Teratosphaeriaceae*. *Persoonia-Molecular Phylogeny and Evolution of Fungi* 33, 1–40.
- Rambaut AA. 2012 – FigTree, version 1.4.0. <http://tree.bio.ed.ac.uk/software/figtree/> (accessed 2012, December 5).
- Rojas EI, Rehner SA, Samuels GJ, Van Bael SA, Herre EA, Cannon P, Chen R, Pang JF, Wang RW, Zhang YP, Peng YQ, Sha T. 2010 – *Colletotrichum gloeosporioides* sl. associated with *Theobroma cacao* and other plants in Panama: multilocus phylogenies distinguish host-associated pathogens from asymptomatic endophytes. *Mycologia* 102, 1318–1338.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Bret L, Liang L, Marc AS, Huelsenbeck JP. 2012 – MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61, 539–542.
- Schoch C, Seifert K, Crous PW. 2011 – Progress on DNA barcoding of Fungi. *IMA Fungus* 2, 5–9.
- Schoch C, Seifert K, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Miller AN. 2012 – Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences* 109, 6241–6246.

- Stamatakis A, Alachiotis N. 2010 – Time and memory efficient likelihood-based tree searched on phylogenomic alignments with missing data. *Bioinformatics* 26, i132–i139.
- Sutton BC, Bailey JA, Jeger MJ. 1992 – The genus *Glomerella* and its anamorph *Colletotrichum*. *Colletotrichum: biology, pathology and control*, CAB International, Wallingford, The United Kingdom 1–26.
- Sutton BC. 1980 – The Coelomycetes: Fungi Imperfecti with *Pycnidia Acervuli* and *Stomata*. Commonwealth Mycological Institute, Kew, England.
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013 – MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30, 2725–2729.
- Than PP, Jeewon R, Hyde KD, Pongsupasamit S, Mongkolporn O, Taylor PWJ. 2008a – Characterization and pathogenicity of *Colletotrichum* species associated with anthracnose on chilli (*Capsicum* spp.) in Thailand. *Plant Pathology* 57, 562–572.
- Than PP, Prihastuti H, Phoulivong S, Taylor PW, Hyde KD. 2008b – Chilli anthracnose disease caused by *Colletotrichum* species. *Journal of Zhejiang University Science B* 9, 764–778.
- Ureña-Padilla AR, MacKenzie SJ, Bowen BW, Legard DE. 2002 – Etiology and population genetics of *Colletotrichum* spp. causing crown and fruit rot of strawberry. *Phytopathology* 92, 1245–1252.
- Wachira FN, Waugh R, Powell W, Hackett CA. 1995 – Detection of genetic diversity in tea (*Camellia sinensis*) using RAPD markers. *Genome* 38, 201–210.
- Weir BS, Johnston PR, Damm U. 2012 – The *Colletotrichum gloeosporioides* species complex. *Studies in Mycology* 73, 115–180.
- Wikee S, Cai L, Pairin N, McKenzie EH, Su YY, Chukeatirote E, Thi HN, Bahkali AH, Moslem MA, Abdelsalam K, Hyde KD. 2011 – *Colletotrichum* species from Jasmine (*Jasminum sambac*). *Fungal Diversity* 46, 171–182.
- Yang YL, Liu ZY, Cai L, Hyde KD, Yu ZN, McKenzie EHC. 2009 – *Colletotrichum anthracnose* of Amaryllidaceae. *Fungal Diversity* 39, 123–146.
- Zhang K, Su YY, Cai L. 2013 – An optimized protocol of single spore isolation for fungi. *Cryptogamie, Mycologie* 34, 349–356.
- Zhaxybayeva O, Gogarten JP. 2002 – Bootstrap, Bayesian probability and maximum likelihood mapping: exploring new tools for comparative genome analyses. *Genomics* 3, 1–15.