



# The *Colletotrichum gigasporum* species complex

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## Key words

Ascomycota  
morphology  
phylogeny  
systematics

**Abstract** In a preliminary analysis, 21 *Colletotrichum* strains with large conidia preserved in the CBS culture collection clustered with a recently described species, *C. gigasporum*, forming a clade distinct from other currently known *Colletotrichum* species complexes. Multi-locus phylogenetic analyses (ITS, ACT, TUB2, CHS-1, GAPDH) as well as each of the single-locus analyses resolved seven distinct species, one of them being *C. gigasporum*. *Colletotrichum gigasporum* and its close allies thus constitute a previously unknown species complex with shared morphological features. Five of the seven species accepted in the *C. gigasporum* species complex are described here as novel species, namely *C. arxii*, *C. magnisporum*, *C. pseudomajus*, *C. radidis* and *C. vietnamense*. A species represented by a single sterile strain, namely CBS 159.50, was not described as novel species, and is treated as *Colletotrichum* sp. CBS 159.50. Furthermore, *C. thailandicum* is reduced to synonymy with *C. gigasporum*.

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## INTRODUCTION

*Colletotrichum gigasporum* was originally reported from healthy leaves of *Centella asiatica* in Madagascar and *Stylosanthes guianensis* in Mexico, as well as from *Coffea arabica* in Colombia (Rakotoniriana et al. 2013). It has an endophytic growth habit and could be isolated from various host plants occurring in geographically distant areas.

The most distinctive morphological feature of *C. gigasporum* is the long straight conidia (up to 32 µm long, av. length 26 µm). Rakotoniriana et al. (2013) discussed the morphological differences between *C. gigasporum* and other species that produce large conidia, e.g. *C. crassipes*, *C. echinatum*, *C. macrosporum*, *C. taiwanense* and *C. vinosum*. Based on phylogenetic analyses of ITS and TUB2 sequence data, they showed *C. gigasporum* to belong to a distinct clade, distant from other currently accepted *Colletotrichum* species.

Numerous *Colletotrichum* isolates detected in a blastn search on GenBank have similar ITS sequences to that of the ex-type strain of *C. gigasporum*, e.g. isolates from *Coffea arabica* in Vietnam (Nguyen et al. 2010), *Hibiscus rosa-sinensis* in Thailand (Noireung et al. 2012), *Magnolia liliifera* in Thailand (Promputtha et al. 2007), *Taxus chinensis* var. *mairei* in China (Wu et al. 2013) and *Theobroma cacao*, *Trichilia tuberculata* and *Virola surinamensis* in Panama (Rojas et al. 2010). In our preliminary ITS analysis, 21 isolates retrieved from the CBS collection clustered with *C. gigasporum*, but showed considerable genetic variability, suggesting further species belonging to a previously unreported species complex.

The objectives of this study are to clarify the genetic and taxonomic relationships of *Colletotrichum* strains from various hosts and geographic areas thought to be closely related to *C. gigasporum*, and to describe the new species from this complex.

## MATERIALS AND METHODS

### Isolates

*Colletotrichum* isolates with large conidia were obtained from the culture collection of the CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands (CBS). All descriptions are based on ex-type cultures. Features of other strains are added if deviant. Cultures of additional isolates used for morphological and phylogenetic analyses are maintained in the CBS culture collection (Table 1).

### Morphological analysis

To enhance sporulation, 5-mm-diam plugs from the margin of actively growing cultures were transferred to the centre of 9-cm-diam Petri dishes containing synthetic nutrient-poor agar medium (SNA) (Nirenberg 1976) amended with autoclaved filter paper and double-autoclaved stems of *Anthriscus sylvestris* placed onto the agar surface. Strains were also studied after growth on oatmeal agar (OA). Cultures were incubated for 10 d at 20 °C under near UV light with a 12 h photoperiod. Measurements and photographs of characteristic structures were made according to methods described by Liu et al. (2012). Appressoria on hyphae were observed on the reverse side of colonies grown on SNA plates. Microscopic preparations were made in clear lactic acid, with 30 measurements per structure, and observed with a Nikon Eclipse 80i microscope using differential interference contrast (DIC) illumination. Colony characters and pigment production on SNA and OA incubated at 20 °C were noted after 10 d. Colony colours were scored according to Rayner (1970). Growth rates were measured after 7 and 10 d.

### Phylogenetic analyses

Genomic DNA of the isolates was extracted using the method of Damm et al. (2008). Eight loci including the 5.8S nuclear ribosomal gene with the two flanking internal transcribed spacers

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**Table 1** Strains of *Colletotrichum* studied in this paper with details about host/substrate and location, and GenBank accessions of the sequences generated. Strains studied in this paper are in **bold**.

Species	Accession number <sup>1</sup>	Host / Substrate	Locality	ITS	ACT	Tub2	CHS-1	GAPDH	HIS3 <sup>2</sup>	CAL <sup>2</sup>	GS <sup>2</sup>
<i>C. acutatum</i>	CBS 112996, ATCC 56816*	<i>Carica</i> sp.	Australia	JQ005776	JQ005839	JQ005860	JQ005797	JQ948677			
<i>C. anthracis</i>	CBS 125334*	<i>Anthriscus sylvestris</i>	Netherlands	GU227845	GU227943	GU228139	GU228335	GU228237			
	CBS 125335	<i>Anthriscus sylvestris</i>	Netherlands	GU227845	GU227943	GU228139	GU228335	GU228237			
<i>C. arxii</i>	<b>CBS 169.59, IMI 304050, IMI 309371</b>	<i>Oncidium excavatum</i>	Netherlands	KF687717	KF687784	KF687868	KF687781	KF687824	KF687846	–	KF687740
	<b>CBS 132511, Paphi 2-1*</b>	<i>Paphiopedilum</i> sp.	Germany	KF687716	KF687802	KF687881	KF687780	KF687843	KF687858	KF687819	KF687756
<i>C. boninense</i>	CBS 123755, MAFF 305972*	<i>Crinum asiaticum</i> var. <i>sinicum</i>	Japan	JQ005153	JQ005501	JQ005588	JQ005327	JQ005240			
	CBS 128526	<i>Dactylopusium dactyloides</i> , leaf endophyte	New Zealand	JQ005162	JQ005510	JQ005596	JQ005336	JQ005249			
<i>C. brevisporum</i>	BCC 38876*	<i>Neoregalia</i> sp.	Thailand	JN050238	JN050216	JN050244	KF687760	JN050227			
	MFLUCC 100182, BTL 23	<i>Pandanus pygmaeus</i>	Thailand	JN050239	JN050217	JN050245	–	JN050228			
<i>C. chlorophyti</i>	IMI 103806*	<i>Chlorophytum</i> sp.	India	GU227894	GU227992	GU228188	GU228384	GU228286			
<i>C. circinans</i>	CBS 111.21	<i>Allium cepa</i>	USA	GU227854	GU227952	GU228148	GU228344	GU228246			
	CBS 221.81*	<i>Allium cepa</i>	Serbia	GU227855	GU227953	GU228149	GU228345	GU228247			
	CBS 125375, CSSK4*	<i>Clivia miniata</i>	China	GQ485607	GQ856777	GQ849440	GQ856722	GQ856756			
<i>C. coccodes</i>	CBS 164.49	<i>Solanum tuberosum</i>	Netherlands	JQ005775	JQ005838	JQ005859	JQ005796	HM171672			
	CBS 369.75*	<i>Solanum tuberosum</i>	Netherlands	HM171679	HM171667	JX546873	JX546681	HM171673			
<i>C. dracaenophilum</i>	CBS 118199*	<i>Dracaena sanderana</i>	China	JX519222	JX519238	JX519247	JX519230	JX546707			
<i>C. fructi</i>	CBS 346.37*	<i>Malus sylvestris</i>	China	GU227844	GU227942	GU228138	GU228334	GU228236			
<i>C. gigasporum</i>	MAFF 242697	<i>Diospyros kaki</i>	Japan	242697 ITS <sup>3</sup>	242697 ACT <sup>1</sup>	242697 Tub <sup>2</sup>	–	242697 GAPDH <sup>4</sup>			
	<b>CBS 101881</b>	<i>Solanum betaceum</i>	New Zealand	KF687736	KF687798	KF687886	KF687777	KF687841	KF687861	KF687808	KF687745
	<b>CBS 109355, FMR 6728</b>	<i>Homo sapiens</i>	Brazil	KF687729	KF687798	KF687870	KF687774	KF687827	KF687849	KF687809	KF687746
	<b>CBS 124947</b>	<i>Theobroma cacao</i>	Panama	KF687731	KF687786	KF687871	KF687763	KF687828	KF687848	KF687810	KF687747
	<b>CBS 125385, E2452</b>	<i>Viola surinamensis</i>	Panama	KF687732	KF687787	KF687872	KF687764	KF687835	KF687850	KF687811	KF687748
	<b>CBS 125387, 4801</b>	<i>Theobroma cacao</i>	Panama	KF687733	KF687788	KF687873	KF687765	KF687834	KF687851	KF687812	KF687749
	<b>CBS 125475, LD30a(T4)</b>	<i>Coffea</i> sp.	Vietnam	KF687723	KF687789	KF687874	KF687766	KF687836	KF687852	KF687813	KF687750
	<b>CBS 125476, LD35b(B2)</b>	<i>Coffea</i> sp.	Vietnam	KF687728	KF687790	KF687875	KF687767	KF687833	KF687853	KF687814	KF687751
	<b>CBS 125730, 3386</b>	<i>Theobroma cacao</i>	Panama	KF687735	KF687793	KF687878	KF687770	KF687840	KF687856	KF687817	KF687754
	<b>CBS 125731, E1249</b>	<i>Trichilia tuberculata</i>	Panama	KF687727	KF687794	KF687879	KF687771	KF687837	KF687857	KF687818	KF687755
	<b>CBS 132881, CPC 12084</b>	<i>Acacia auriculiformis</i>	Thailand	KF687725	KF687795	KF687880	KF687772	KF687829	KF687859	KF687820	KF687757
	<b>CBS 132884, CPC 16323</b>	<i>Musa</i> sp.	Mexico	KF687720	KF687796	–	KF687773	KF687830	KF687860	–	KF687737
	<b>CBS 133266, MUCL 44947*</b>	<i>Centella asiatica</i>	Madagascar	KF687715	–	KF687866	KF687761	KF687822	KF687844	–	–
	<b>CBS 159.75</b>	air and stored grains	India	KF687726	KF687783	KF687884	KF687776	KF687839	KF687863	KF687821	KF687739
	<b>CBS 181.52</b>	<i>Theobroma cacao</i>	East Africa	KF687734	KF687799	KF687885	KF687775	KF687838	KF687862	KF687805	KF687741
(syn. <i>C. thailandicum</i> )	BCC 38879, LC0596, HR01MFU	<i>Hibiscus rosa-sinensis</i>	Thailand	JN050242	JN050220	JN050248	KF687758	JN050231			
	MFLUCC 100192, LC0958, CMSP34	<i>Alocasia</i> sp.	Thailand	JN050243	JN050221	JN050249	KF687759	JN050232			
<i>C. gloeosporioides</i>	CBS 953.97*	<i>Citrus sinensis</i>	Italy	GQ485605	GQ856782	GQ849434	GQ856733	GQ856762			
	CORCG5	<i>Vanda</i> sp.	China	HM034809	HM034801	HM034811	HM034805	HM034807			
<i>C. graminicola</i>	CBS 130836, M 1.001*	<i>Zea mays</i>	USA	JQ005767	JQ005830	JQ005851	JQ005788	–			
<i>C. karstii</i>	CBS 132134,										
	CGMCC 3.14194*	<i>Vanda</i> sp.	China	HM585409	HM581995	HM585428	HM582023	HM585391			
<i>C. lindernuthianum</i>	CBS 523.97	<i>Phaseolus coccineus</i>	Costa Rica	JX546815	JX546823	JX546861	JX546669	JX546719			
	CBS 144.31*	<i>Phaseolus vulgaris</i>	Germany	JQ005779	JQ005842	JQ005863	JQ005800	JX546712			
<i>C. lineola</i>	CBS 125339	<i>Apiaceae</i>	Czech Republic	GU227830	GU227928	GU228124	GU228320	GU228222			
	CBS 125337*	<i>Apiaceae</i>	Czech Republic	GU227829	GU227927	GU228123	GU228319	GU228221			
<i>C. liriopes</i>	CBS 122747	<i>Liriope muscari</i>	Mexico	GU227805	GU227903	GU228099	GU228295	GU228197			
	CBS 119444*	<i>Liriope muscari</i>	Mexico	GU227804	GU227902	GU228098	GU228294	GU228196			
<i>C. magnisporum</i>	<b>CBS 398.84*</b>	unknown	unknown	KF687718	KF687803	KF687882	KF687782	KF687842	KF687865	–	KF687742
<i>C. nigrum</i>	CBS 128507	<i>Capsicum annuum</i>	New Zealand	JX546843	JX546851	JX546890	JX546698	JX546747			
	CBS 169.48*	<i>Capsicum</i> sp.	Argentina	JX546838	JX546846	JX546885	JX546693	JX546742			
	CBS 129828*	<i>Oncidium</i> sp., leaf	Germany	JQ005169	JQ005517	JQ005603	JQ005343	JQ005256			
<i>C. oncidii</i>	CBS 130242	<i>Oncidium</i> sp., leaf	Germany	JQ005170	JQ005518	JQ005604	JQ005344	JQ005257			

Accession	Species	Country	GenBank ID	GenBank ID	GenBank ID	GenBank ID	GenBank ID
CBS 571.88*	<i>Camellia sinensis</i>	Taiwan	KF687722	KF687801	KF687883	KF687779	KF687826
CBS 529.93*	unknown	Costa Rica	KF687719	KF687785	KF687869	KF687762	KF687864
CBS 119206*	<i>Ruscus</i> sp.	Italy	GU227818	GU227916	GU228112	GU228308	KF687847
MAFF 239721*	<i>Sansevieria trifasciata</i>	Japan	AB212991	239721_ACT <sup>3</sup>	239721_Tub2 <sup>3</sup>	-	239721_GAPDH <sup>3</sup>
MAFF 239175	<i>Sansevieria trifasciata</i>	Japan	239175_ITS <sup>3</sup>	239175_ACT <sup>3</sup>	239175_Tub2 <sup>3</sup>	-	239175_GAPDH <sup>3</sup>
CBS 130421, BRIP 28519*	<i>Carica papaya</i>	Australia	GU183331	GO849454	GU183289	GO856735	GQ856763
CBS 168.49	<i>Lupinus polyphyllus</i>	Germany	GU227802	GU227900	GU228096	GU228292	GU228194
CBS 495.85	<i>Tofieldia calyculata</i>	Switzerland	GU227801	GU227899	GU228095	GU228291	GU228193
CBS 102667	<i>Passiflora edulis</i> , leaf blotch	New Zealand	JQ005165	JQ005513	JQ005599	JQ005339	JQ005252
CBS 128544*	<i>Solanum melongena</i>	New Zealand	JQ005164	JQ005512	JQ005598	JQ005338	JQ005251
CBS 217.64	<i>Hedera helix</i>	Germany	GU227812	GU227910	GU228106	GU228302	GU228204
CBS 118.98	<i>Hedera</i> sp.	UK	GU227813	GU227911	GU228107	GU228303	GU228205
BCC 38877, LC0598, L58*	<i>Citrus maxima</i>	Thailand	JN050240	JN050218	JN050246	-	JN050229
MFLUCC-100167, LC0957, BTL07	<i>Paphiopedicellum bellatulum</i>	Thailand	JN050241	JN050219	JN050247	-	JN050230
CBS 120709	<i>Capasicum frutescens</i>	India	GU227877	GU227975	GU228171	GU228367	GU228269
CBS 151.35*	<i>Phaseolus lunatus</i>	USA	GU227862	GU227960	GU228156	GU228352	GU228254
IMI 45525	<i>Crotalaria juncea</i>	Zimbabwe	GU227806	GU227904	GU228100	GU228296	GU228198
CBS 125477, BMT25(L3)	<i>Coffea</i> sp.	Vietnam	KF687720	KF687791	KF687876	KF687768	KF687831
CBS 125478, LD16(L2)*	<i>Coffea</i> sp.	Vietnam	KF687721	KF687792	KF687877	KF687769	KF687832
CBS 132135, AS 3.9617*	<i>Buxus</i> sp.	China	JX546804	JX519239	JX519248	JX519231	JX546706
CBS 159.50	<i>Dennis</i> sp.	Indonesia	KF687724	KF687800	KF687867	KF687778	KF687823
CBS 869.98*	<i>Ipomoea batatas</i>	South Africa	JQ005780	JQ005843	JQ005864	JQ005801	JX546612

<sup>1</sup> CBS, CGMCC; China General Microbiological Culture Collection; ATCC; American Type Culture Collection; BCC; BIOTEC Culture Collection; Thailand; 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; ICMP; International Collection of Microorganisms from Plants, Auckland, New Zealand; IMI; Culture collection of CAB International, Egham, UK; LC; Working collection of Lei Cai, housed at CAS, China; MAFF; MAFF Genebank Project, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan; MFLUCC; Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; MUCL; BCCM/MUCL collection, Universiteit catholique de Louvain, Belgium.

<sup>2</sup> HIS3, CAL, GS genes were not used in multi-locus phylogenetic analysis.

<sup>3</sup> sequences downloaded from NIAS GenBank ([http://www.gene.afrc.go.jp/index\\_en.php](http://www.gene.afrc.go.jp/index_en.php)).

\* indicate ex-type strains.

(ITS), a 200-bp intron of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a partial sequence of the actin (ACT), chitin synthase 1 (CHS-1), beta-tubulin (TUB2), calmodulin (CAL), glutamine synthetase (GS) and histon3 (HIS3) genes were amplified and sequenced using the primer pairs ITS1F (Gardes & Bruns 1993) + ITS4 (White et al. 1990), GDF1 + GDR1 (Guerber et al. 2003), ACT-512F + ACT-783R (Carbone & Kohn 1999), CHS-354R + CHS-79F (Carbone & Kohn 1999), T1 (O'Donnell & Cigelnik 1997) + Bt-2b (Glass & Donaldson 1995), CL1 + CL2A (O'Donnell et al. 2000), GSF1 + GSR1 (Stephenson et al. 1997) and CYLH3F + CYLH3R (Crous et al. 2004b), respectively. The PCR protocols were performed as described by Damm et al. (2009).

The DNA sequences obtained from forward and reverse primers were used to obtain consensus sequences using MEGA v. 5.1 (Tamura et al. 2011), and subsequent alignments were generated using MAFFT v. 6 (Kato & Toh 2010), and manually edited using MEGA v. 5.1.

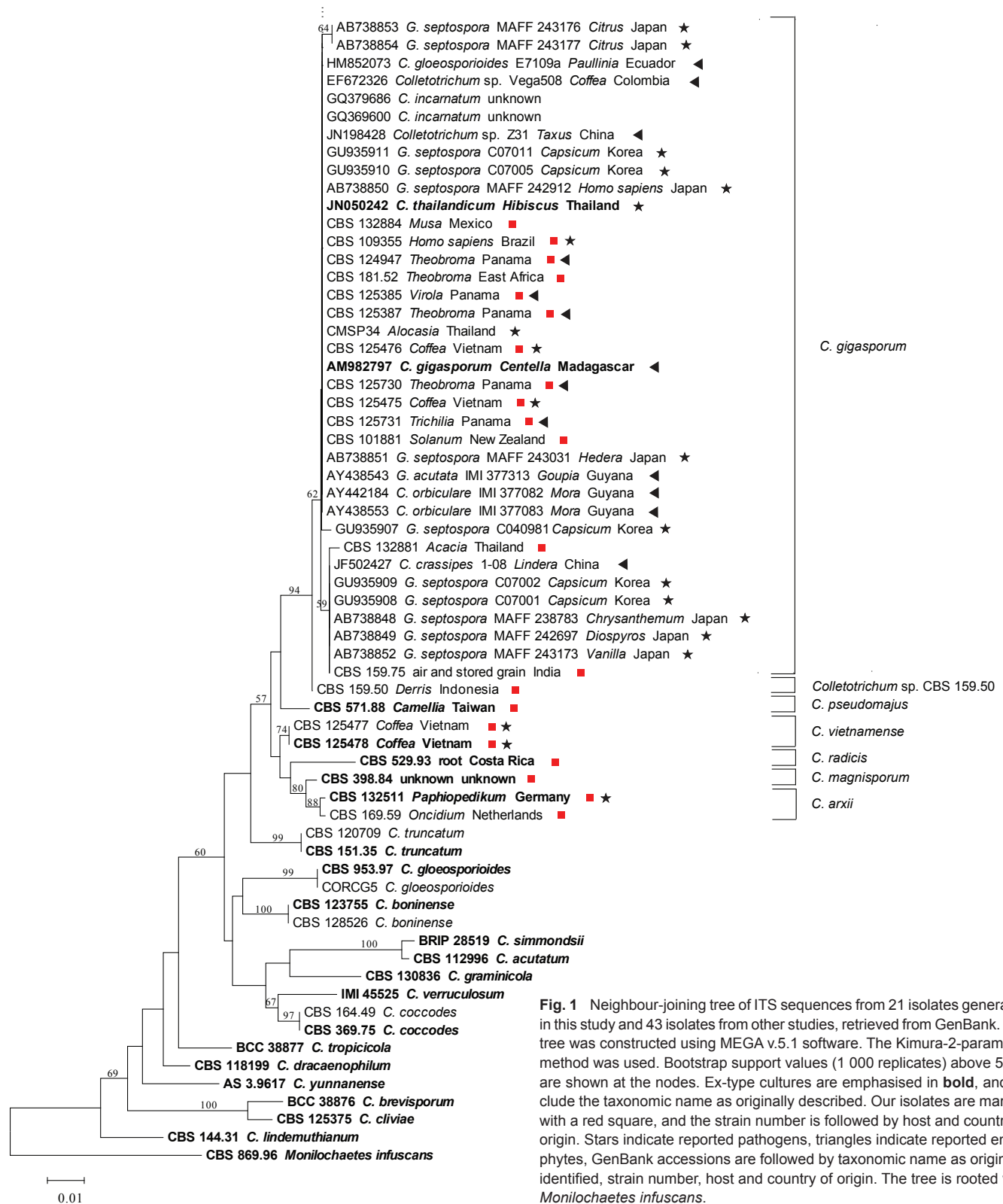
Sequences of the 21 *Colletotrichum* strains studied in this paper as well as sequences of 50 reference strains (Table 1) downloaded from GenBank ([www.ncbi.nlm.nih.gov/genbank/](http://www.ncbi.nlm.nih.gov/genbank/)) and NIAS GenBank ([www.gene.afrc.go.jp/about\\_en.php](http://www.gene.afrc.go.jp/about_en.php)) were included in individual alignments and eight single gene phylogenies were generated using a distance-based method. The ITS alignment included a further 22 sequences that were found in blastn searches in GenBank in addition to those in Table 1. Distance matrixes of the aligned sequences were calculated using the Kimura 2-parameter model (Kimura 1980), and analysed with the Neighbour-joining (NJ) algorithm (Saitou & Nei 1987) using MEGA v. 5.1, excluding positions with gaps. The reliability of the inferred trees was estimated by bootstrap analyses with 1 000 replicates.

A maximum parsimony analysis was performed on the multi-locus alignment including five of the eight loci (ACT, CHS-1, GAPDH, ITS, TUB2) of a total of 71 strains (Table 1) using PAUP v. 4.0b10 (Swofford 2002). Ambiguously aligned regions were excluded from all analyses. Unweighted parsimony (UP) analysis was performed. Trees were inferred using the heuristic search option with TBR branch swapping and 1 000 random sequence additions. Maxtrees were unlimited, branches of zero length were collapsed and all multiple parsimonious trees were saved. Clade stability was assessed in a bootstrap analysis with 1 000 replicates, each with 10 replicates of random stepwise addition of taxa. A second phylogenetic analysis of the concatenated alignment using a Markov Chain Monte Carlo (MCMC) algorithm was done to generate trees with Bayesian posterior probabilities in MrBayes v. 3.2.1 (Ronquist & Huelsenbeck 2003). Nucleotide substitution models were determined using MrModeltest v. 2.3 (Nylander 2004) for each gene region and included in the analyses. Two analyses of four MCMC chains were run from random trees for 10 000 000 generations and sampled every 1 000 generations. The first 25 % of trees were discarded as the burn-in phase of each analysis and posterior probabilities determined from the remaining trees. *Monilochaetes infuscans* strain CBS 869.96 was used as outgroup in all analyses. Sequences derived in this study were lodged in GenBank, the multi-locus alignment and tree in TreeBASE (<http://www.treebase.org/treebase-web/search/studySearch.html>) (S15175), and taxonomic novelties in MycoBank ([www.MycoBank.org](http://www.MycoBank.org); Crous et al. 2004a).

**RESULTS**

*Phylogeny*

The eight NJ trees derived from the single gene sequence alignments (ACT, CAL, CHS-1, GAPDH, GS, HIS3, ITS, TUB2)



**Fig. 1** Neighbour-joining tree of ITS sequences from 21 isolates generated in this study and 43 isolates from other studies, retrieved from GenBank. The tree was constructed using MEGA v.5.1 software. The Kimura-2-parameter method was used. Bootstrap support values (1 000 replicates) above 50 % are shown at the nodes. Ex-type cultures are emphasised in bold, and include the taxonomic name as originally described. Our isolates are marked with a red square, and the strain number is followed by host and country of origin. Stars indicate reported pathogens, triangles indicate reported endophytes, GenBank accessions are followed by taxonomic name as originally identified, strain number, host and country of origin. The tree is rooted with *Monilochaetes infuscans*.

confirmed that the 21 CBS isolates and the ex-type and other strains of *C. gigasporum* constituted a monophyletic lineage, distant from other known major clades of the genus *Colletotrichum* recognised by Cannon et al. (2012). The NJ trees are not shown in this study except for the phylogeny based on ITS data (Fig. 1). Isolates studied in this paper (marked with red squares) are separated into seven subclades, which could also be confirmed with the other seven single gene phylogenies.

The multi-locus phylogenetic analysis included 70 ingroup strains, with *Monilochaetes infuscans* (CBS 869.96) as outgroup. The dataset of five loci comprised 1 512 characters including

the alignment gaps, of which 699 characters were parsimony-informative, 85 parsimony-uninformative and 728 constant. Parsimony analysis resulted in 94 most parsimonious trees, one of them (length = 3417, CI = 0.438, RI = 0.798, RC = 0.349, HI = 0.562) is shown in Fig. 2, where the 21 strains studied belong to a major clade consisting of seven subclades. More than half of the strains clustered in the largest subclade (*C. gigasporum*) with a high bootstrap support and Bayesian posterior probability value (100/1.00). The Bayesian tree confirmed the tree topology of the trees obtained with maximum parsimony.

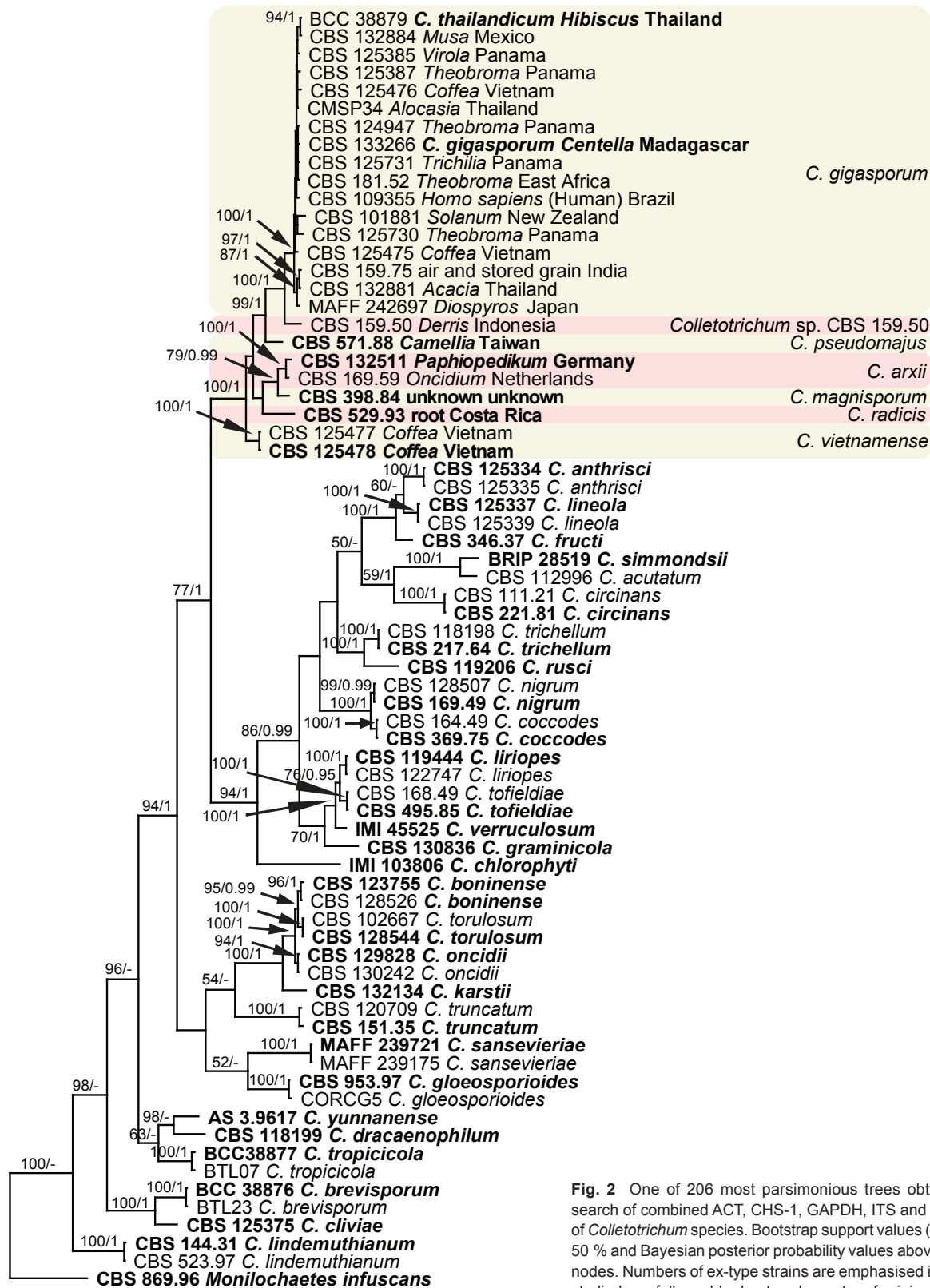


Fig. 2 One of 206 most parsimonious trees obtained from a heuristic search of combined ACT, CHS-1, GAPDH, ITS and TUB2 gene sequences of *Colletotrichum* species. Bootstrap support values (1 000 replicates) above 50 % and Bayesian posterior probability values above 0.95 are shown at the nodes. Numbers of ex-type strains are emphasised in bold. Strain numbers studied are followed by host and country of origin. The tree is rooted with *Monilochaetes infuscans*.

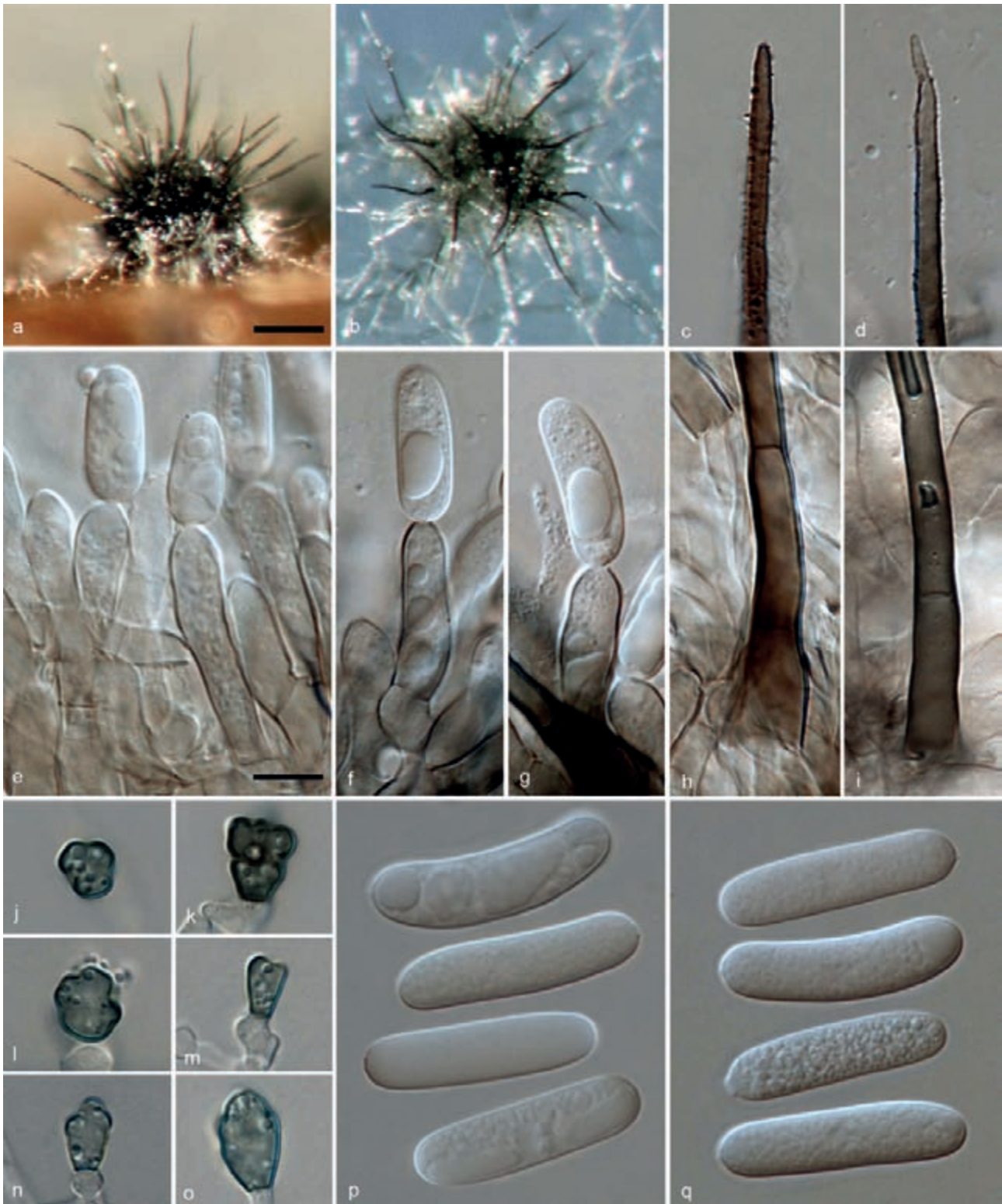
***Colletotrichum arxii*** F. Liu, L. Cai, Crous & Damm, sp. nov. — MycoBank MB807164; Fig. 3

*Etymology.* Named after Josef Adolf von Arx for his very substantial contribution to the classification of the genus *Colletotrichum*.

On *Anthriscus* stem. *Vegetative hyphae* hyaline, smooth-walled, septate, branched. *Conidiomata* acervular, conidiophores and setae formed on a cushion of roundish to angular brown cells. *Setae* pale to medium brown, smooth-walled to verruculose, 1–5-septate, 80–260 µm long, base cylindrical, 3.5–6 µm

**Taxonomy**

Based on the results of the single and multi-locus phylograms, we accept seven species within the *C. gigasporum* species complex, including six species that are new to science. In addition, two recently described species are shown to be synonymous. All novel species are characterised and illustrated below except for a species which is represented by a single strain, CBS 159.50. Since this strain is sterile, we designate it as *Colletotrichum* sp. CBS 159.50.



**Fig. 3** *Colletotrichum arxii* (CBS 132511). a, b. Acervuli; c, d. tips of setae; e–g. conidiophores; h, i. basal parts of setae; j–o. appressoria; p, q. conidia (a, d, f–g, i, q: from *Anthriscus* stem; b, c, e, h, j–p: from SNA. – a, b: DM; c–q: DIC). — Scale bars: a = 100  $\mu$ m (applies to a, b); e = 10  $\mu$ m (applies to c–q).

diam, tip acute to obtuse. *Conidiophores* pale brown, septate, branched. *Conidiogenous cells* pale brown, cylindrical to clavate, 17.5–24  $\times$  5–7  $\mu$ m, opening 1–2.5  $\mu$ m diam. *Conidia* hyaline, aseptate, smooth-walled, cylindrical to slightly curved, both ends rounded, 21–32  $\times$  5.5–7.5  $\mu$ m, av.  $\pm$  SD = 28.1  $\pm$  2.6  $\times$  6.8  $\pm$  0.5  $\mu$ m, L/W ratio = 4.1; the other isolate CBS 169.59 forms relatively shorter conidia, 20–26.5  $\times$  5.5–7.5  $\mu$ m, av.  $\pm$  SD = 23.1  $\pm$  2  $\times$  6.4  $\pm$  0.5  $\mu$ m, L/W ratio = 3.6.

On SNA. *Vegetative hyphae* hyaline to medium brown, smooth-walled, septate, branched. *Conidiomata* acervular. *Setae* pale to medium brown, smooth-walled to verruculose, 1–3-septate,

120–180  $\mu$ m long, base cylindrical to inflated, 4.5–7.5  $\mu$ m diam, tip acute. *Conidiophores* hyaline to pale brown, septate, branched. *Conidiogenous cells* hyaline to pale brown, cylindrical to clavate, 10–21.5  $\times$  5.5–7.5  $\mu$ m, opening 1.5–3  $\mu$ m diam. *Conidia* hyaline, aseptate, smooth-walled, cylindrical to slightly curved, both ends rounded, (20–)24.5–30  $\times$  5.5–7.5  $\mu$ m, av.  $\pm$  SD = 27.0  $\pm$  1.8  $\times$  6.7  $\pm$  0.5  $\mu$ m, L/W ratio = 4; the other isolate CBS 169.59 forms relatively shorter conidia, 15.5–24  $\times$  5–7.5  $\mu$ m, av.  $\pm$  SD = 21.4  $\pm$  2  $\times$  6.3  $\pm$  0.5  $\mu$ m, L/W ratio = 3.4. *Appressoria* (few observed) pale brown, aseptate, solitary, with a ellipsoidal to irregular outline and a crenate or

lobed margin, 4–11.5 × 4–9 µm, av. ± SD = 8.5 ± 2.5 × 6.0 ± 1.5 µm, L/W ratio = 1.4.

**Culture characteristics** — Colonies on OA flat with undulate margin, surface white, aerial mycelium lacking; reverse white; colonial diam 54–63 mm in 7 d, > 90 mm in 10 d. Colonies on SNA flat with erose or dentate margin, medium hyaline, buff around *Anthriscus* stem, aerial mycelium lacking; colonial diam 68–77 mm in 7 d, > 90 mm in 10 d.

**Specimens examined.** GERMANY, Berlin, glasshouse, on living leaves of *Paphiopedilum* sp., Dec. 2010, U. Damm (holotype CBS H-21492, culture ex-type CBS 132511 = Paphi 2-1). — NETHERLANDS, Baarn, Cantonspark, on *Oncidium excavatum*, unknown collection date and collector (isolated by J.A. von Arx in 1956), culture CBS 169.59 = IMI 304050 = IMI 309371.

**Notes** — Although there are many *Colletotrichum* species reported from orchids, which include *C. boninense* (s.lat.), *C. cinctum*, *C. cliviae*, *C. crassipes*, *C. cymbidiicola*, *C. gloeosporioides* (s.lat.), *C. liriopes*, *C. lujae*, *C. macrosporum*, *C. oncidii*, *C. orchidearum*, *C. orchidophilum*, *C. siamense*, *C. stanhopeae*, *C. vanillae* (Stoneman 1898, Allescher 1902, Patel et al. 1953, von Arx 1957, Sutton 1980, Li 1999, Moriwaki et al. 2003, Talubnak & Soyong 2010, Yang et al. 2011, Damm et al. 2012a), *C. arxii* can be distinguished from these species either from phylogenetic data or morphological characteristics. *Colletotrichum arxii* is phylogenetically distinct from the *C. acutatum*, *C. boninense* and *C. gloeosporioides* complexes, as well as *C. cliviae* and *C. liriopes* (Fig. 2), and could be morphologically distinguished from the other species that presently still lack molecular data.

*Colletotrichum arxii* differs from *C. macrosporum*, a species from an orchid from Brazil, by forming narrower conidia (*C. macrosporum* 28–32 × 8–10 µm) (Saccardo 1896). Although *C. orchidearum* was originally described by Allescher (1902) from Munich, Germany, the same location as our strain CBS 132511, they can be differentiated from each other based on conidial size, with *C. arxii* forming significantly longer conidia than *C. orchidearum* (*C. orchidearum* (13.5–)15.5–19.5 × 5–6 µm, av. ± SD = 17.2 ± 1.6 × 5.5 ± 0.3 µm) (Damm et al. 2012a).

*Colletotrichum cinctum* (Berk. & M.A. Curtis) Stoneman was originally described from orchids, *Oncidium* sp. and *Maxillaria* sp. (Stoneman 1898) and also identified from *Paphiopedilum insigne* (specimen BPI 397219) in the USA (collected by J. Rubinger on 14 July 1921, unpubl.). *Colletotrichum stanhopeae* was described from *Stanhopea* sp. in Brazil (Hennings 1908), *C. vanillae* from *Vanilla odorata* in Italy (Saccardo 1906) and *C. lujae* from Luja in Belgium (Verplancke 1935). However, the conidia of these four species, *C. cinctum* (12–15 × 3–4 µm), *C. stanhopeae* (10–16 × 3.5–4 µm), *C. vanillae* (18–21 × 5.5–7 µm), *C. lujae* (9.3–10.5 × 2–3.1 µm) are significantly smaller than those of *C. arxii* (20–30 × 5.5–7.5 µm).

Closest match in a blastn search with the ITS sequence of strain CBS 132511 (with 99 % identity, 8 bp differences) was an endophytic isolate (DQ780412) from *Magnolia liliifera* probably in Thailand (Promputtha et al. 2007) and an endophytic isolate (FJ205460) from an orchid in Taiwan (Wang et al. unpubl. data). The closest match with the TUB2 sequence (with 97 % identity, 16 bp differences) was isolate MUCL 41702 from *Orchis* in Singapore (FN599826; Rakotoniriana & Munaut, unpubl. data).

***Colletotrichum gigasporum*** E.F. Rakotoniriana & Munaut, Mycol. Progr. 12: 407. 2013

= *Colletotrichum thailandicum* Phouliv., Noireung, L. Cai & K.D. Hyde, Cryptog. Mycol. 33: 354. 2012.

**Notes** — *Colletotrichum gigasporum* is characterised by large conidia ((22–)25–29(–32) × (6–)7–9 µm). Phylogenetic analyses by Rakotoniriana et al. (2013) based on the ITS and TUB2 sequences placed it in a distinct clade far from the cur-

rently accepted *Colletotrichum* species. Another species with large conidia (27–30 × 9–10 µm), *C. thailandicum*, was described from diseased *Alocasia* sp. and *Hibiscus rosa-sinensis* from Thailand (Noireung et al. 2012). *Colletotrichum thailandicum* is morphologically similar to *C. gigasporum*; the ITS and β-tubulin sequences of both fungi are identical or near-identical (differed in two nucleotide position in β-tubulin). In addition, phylogenetic analyses of single locus data, including ITS (Fig. 1), and multi-locus data (Fig. 2), show that the ex-type strains of the two species cluster together in one strongly supported clade. Since *C. gigasporum* was published online earlier (8 August 2012) than *C. thailandicum* (September 2012), we regard *C. thailandicum* as a synonym of *C. gigasporum*.

Strain CBS 109355, isolated from a phaeohyphomycotic cyst from a Brazilian man, was originally identified as *C. crassipes*, mainly based on morphology of the appressoria with crenate or deeply lobed margins and its size of conidia (Castro et al. 2001). In addition, strains CBS 159.75 and IMI 302450, which were deposited as *C. crassipes* in the CBS and IMI culture collections, were compared morphologically with CBS 109355 by Castro et al. (2001). However, strains CBS 159.75 and CBS 109355 were reidentified as *C. gigasporum* in the present study (Fig. 2). Hitherto, the taxonomic status of *C. crassipes* as well as the genetic relationship between *C. gigasporum* and *C. crassipes* remain unclear due to the lack of an ex-type culture and DNA sequence data. Thus, an epitype is needed to stabilise the nomenclature of *C. crassipes*.

In addition to being a disease-causing agent of humans, *C. gigasporum* is also associated with *Musa* sp. (Fig. 1, 2), the anthracnose of which is commonly considered to be caused by *C. musae* that belongs to the *C. gloeosporioides* species complex (Weir et al. 2012). However, *C. gigasporum* is phylogenetically distinct from *C. musae*, and its conidia are significantly larger than those of *C. musae*. Additional *Colletotrichum* species associated with *Musa* spp. include *C. cavendishii*, *C. liukuensis* and *C. paxtonii*. *Colletotrichum gigasporum* differs from *C. liukuensis* (Sawada 1959), a species on leaves of *M. liukuensis* in Taiwan, and *C. cavendishii* (Pettrak 1925), a species on living leaves of *M. cavendishii* by producing larger conidia (20.5–25.5 × 6–9 µm vs 12–14 × 4.8–5.5 µm and 10–19 × 4.5–7 µm, respectively). *Colletotrichum paxtonii*, a species associated with banana in St. Lucia, belongs to the *C. acutatum* complex (Johnston & Jones 1997, Damm et al. 2012a) and is therefore not closely related to *C. gigasporum*.

Our 5-locus phylogram shows that several strains from diverse countries and hosts cluster with *C. gigasporum* (syn. *C. thailandicum*). Based on our blastn search in GenBank, the results of which are included in the ITS phylogeny, 22 additional ITS sequences from GenBank cluster with the ex-type strain of *C. gigasporum*, including sequences derived from strains isolated from plants as endophytes or pathogens and even strains that were isolated from human tissue (Fig. 1). This is in accordance with the conjecture that ecologically *C. gigasporum* can occur as either endophyte or pathogen (Rakotoniriana et al. 2013). The isolates from which most of these GenBank sequences were generated had been previously identified as *C. crassipes*, *C. gloeosporioides*, *C. incarnatum*, *C. orbiculare* or *C. taiwanense* (sexual morph *Glomerella septospora*) (Fig. 1).

The ascospores and conidia of *C. gigasporum* resemble those of *C. taiwanense* with respect to their size. However, *C. gigasporum* produces aseptate conidia and 0–1-septate ascospores (Rakotoniriana et al. 2013), while the conidia of *C. taiwanense* may become 1–5-septate with age and ascospores are mostly 3-septate and may become up to 6- or 8-septate when old (Sivanesan & Hsieh 1993). *Colletotrichum taiwanense*, originally described from *Styrax formosanus* in Taiwan, is currently poorly characterised using molecular methods (Hyde et al. 2009,



**Fig. 4** *Colletotrichum magnisporum* (CBS 398.84). a, b. Acervuli; c, d. conidiophores; e, i, j. setae; f–h. conidia (a, d, g–j: from *Anthriscus* stem; b, c, e, f: from SNA. – a, b: DM; c–m: DIC). — Scale bars: a = 100 µm (applies to a, b); f = 10 µm (applies to c–j).



Cannon et al. 2012). Unfortunately, a subculture from the ex-type isolate of *C. taiwanense* (IMI 353024) is contaminated; the original strain could not be recovered. Several plant pathogenic strains from various hosts (none of them from *Styrax*) that were previously identified as *C. taiwanense* were reidentified as *C. gigasporum* based on the ITS-rDNA phylogram in this study (Fig. 1). *Colletotrichum gigasporum* differs from *C. incarnatum* (Zimmermann 1901), a species first described from *Coffea liberica* in Java, by producing larger conidia (20.5–25.5 × 6–9 µm vs 14–19 × 5 µm).

Some strains from *Mora excelsa* in Guyana had been previously identified as *C. orbiculare* (Lu et al. 2004) and grouped with *C. gigasporum* in our ITS tree. However, *C. orbiculare* was recently redefined and shown to belong to a different species complex together with *C. lindemuthianum* (Damm et al. 2013).

Although the ITS-rDNA phylogram revealed that *C. gigasporum* strains formed two subclades (Fig. 1), the bootstrap values are too low to support two distinct species, which could also be verified by the multi-locus phylogram (Fig. 2).

***Colletotrichum magnisporum*** F. Liu, L. Cai, Crous & Damm, *sp. nov.* — MycoBank MB807163; Fig. 4

*Etymology.* Referring to the large size of its conidia.

On *Anthriscus* stem. *Vegetative hyphae* hyaline to brown, smooth-walled, septate, branched. *Conidiomata* acervular, conidiophores and setae formed on a cushion of angular brown cells. *Setae* medium to dark brown, smooth-walled to verruculose, 0–4-septate, 42.5–105 µm long, base cylindrical to inflated, 5.5–11.5 µm diam, tip acute to obtuse. *Conidiophores* hyaline to brown, septate, branched. *Conidiogenous cells* hyaline to medium brown, cylindrical or clavate, 18–33.5 × 5.5–10 µm, opening 1.5–2.5 µm diam. *Conidia* hyaline, aseptate, smooth-walled, cylindrical with rounded ends, 28–39 × 8.5–10.5 µm, av. ± SD = 33.8 ± 4.1 × 9.9 ± 0.6 µm, L/W ratio = 3.4.

On SNA. *Vegetative hyphae* hyaline to medium brown, smooth-walled, septate, branched. *Conidiomata* acervular. *Setae* medium to dark brown, smooth-walled to verruculose, 1–4-septate, 91.5–230.5 µm long, base cylindrical to inflated, 5–12.5 µm diam, tip ± acute. *Conidiophores* hyaline to medium brown, septate, branched. *Conidiogenous cells* hyaline to pale brown, cylindrical to clavate, 17.5–26.5 × 7.5–9.5 µm, opening 1.5–2.5 µm diam. *Conidia* hyaline, aseptate, smooth-walled, cylindrical with rounded ends, 28.5–40.5 × 8.5–11 µm, av. ± SD = 34.3 ± 2.7 × 9.7 ± 0.5 µm, L/W ratio = 3.5. *Appressoria* not observed.

Culture characteristics — Colonies on OA flat with entire margin, surface iron-grey with a white margin, aerial mycelium lacking; reverse olivaceous-grey to iron-grey; colonial diam 56–60 mm in 7 d, > 90 mm in 10 d. Colonies on SNA flat with entire margin, medium hyaline, buff around *Anthriscus* stem, aerial mycelium lacking; colonial diam 64–65 mm in 7 d, > 90 mm in 10 d.

*Specimen examined.* Unknown collection details (deposited in CBS culture collection in June 1984) (holotype CBS H-21491, culture ex-type CBS 398.84).

Notes — Although *C. magnisporum* is represented by only a single strain in this study, it could be distinguished from the related species *C. arxii* based on its phylogenetic distance (Fig. 2) and its morphology. The two species differ by 40 bp differences in five genes totally, as well as a long insertion (174 bp) in GAPDH sequences in *C. arxii* that is missing in *C. magnisporum*. In addition, the conidia of *C. arxii* (24.5–30 × 5.5–7.5 µm, av. = 27 × 6.7 µm) are shorter and narrower than *C. magnisporum* (28.5–40.5 × 8.5–11 µm, av. = 34.3 × 9.7 µm). For other comments see *C. radialis*.

The closest matches in a blastn search in GenBank with the ITS sequence of strain CBS 398.84 were with 100 % identity EF672323 from the endophytic isolate VegaE4-36 from *Coffea arabica* from Hawaii, USA (Vega et al. 2010), EU686812 from an endophytic isolate from *Rhipidoctadum racemiflorum* from Panama (Higgins et al. 2011), as well as KF436311 from the endophytic isolate TK780 from a tropical woody plant from Panama (Higginbotham et al. 2013). The closest match with the TUB2 sequence (with 96 % identity, 16 bp differences) was isolate MUCL 41702 from *Orchis* in Singapore (FN599826; Rakotoniriana & Munaut unpubl. data).

***Colletotrichum pseudomajus*** F. Liu, L. Cai, Crous & Damm, *sp. nov.* — MycoBank MB807165; Fig. 5

*Etymology.* Referring to its morphology, which resembles that of *Glomerella major*.

On OA. *Vegetative hyphae* medium brown, smooth-walled, septate, branched. *Conidiomata* acervular, conidiophores and setae formed on a cushion of roundish brown cells. *Setae* medium to dark brown, smooth-walled to verruculose, 0–3-septate, 100–215 µm long, base inflated to cylindrical, 4–8 µm diam, tip acute. *Conidiophores* hyaline to medium brown, septate, branched. *Conidiogenous cells* hyaline to pale brown, cylindrical to clavate, 12–18 × 4–8 µm, opening 1.5–2 µm diam. *Conidia* hyaline, aseptate, smooth-walled, cylindrical with rounded ends, occasionally slightly curved, 21.5–27 × 6–9 µm, av. ± SD = 24.3 ± 1.5 × 7.8 ± 0.6 µm, L/W ratio = 3.1.

Sexual morph developed on OA. *Ascomata* globose, sometimes subconical, black, surrounded with brown hairs, 95–165 µm diam, ostiolate; neck, when present, 35–60 µm long; outer wall composed of angular brown cells, 6–20 µm diam. *Interascal tissue* composed of paraphyses, thin-walled, hyaline, septate, the apex rounded. *Asci* cylindrical, 93–123.5 × 10.5–12.5 µm, 8-spored. *Ascospores* uni- or biserially arranged, hyaline, aseptate, smooth-walled, lunate, tip ± acute, 20–27.5 × 5–7 µm, av. ± SD = 24.2 ± 1.6 × 6.2 ± 0.4 µm, L/W ratio = 3.9.

On *Anthriscus* stem. Remaining sterile.

On SNA. *Vegetative hyphae* hyaline to medium brown, smooth-walled, septate, branched. *Conidiomata* acervular. *Setae* dark brown, smooth-walled to verruculose, 0–3-septate, 125–190 µm long, base cylindrical to inflated, 5.5–8 µm diam, tip acute. *Conidiophores* pale brown, septate, branched. *Conidiogenous cells* pale brown, cylindrical, clavate to bullet-shaped, 14.5–18 × 4–8 µm, opening 1.5–2 µm diam. *Conidia* hyaline, aseptate, smooth-walled, cylindrical with rounded ends, 22–30.5 × 6.5–9.5 µm, av. ± SD = 26.3 ± 1.7 × 8.1 ± 0.5 µm, L/W ratio = 3.2. *Appressoria* not observed.

Sexual morph developed on SNA. *Ascomata* globose, subconical to obpyriform, black, surrounded with hyaline to medium brown hairs, 260–360 µm diam, ostiolate; neck when present, 60–200 µm long; outer wall composed of angular brown cells, 5–15 µm diam. *Interascal tissue* composed of paraphyses, thin-walled, hyaline, septate, the apex rounded. *Asci* cylindrical, 73.5–98.5 × 10–12.5 µm, 8-spored. *Ascospores* uni- or biserially arranged, hyaline, aseptate, smooth-walled, lunate, tip ± acute, 18.5–25 × 4.5–7.5 µm, av. ± SD = 21.2 ± 1.5 × 6.0 ± 0.7 µm, L/W ratio = 3.5.

Culture characteristics — Colonies on OA umbonate with entire margin, surface iron-grey to greenish black, white aerial mycelium; reverse olivaceous-grey; colonial diam 42–45 mm in 7 d, 65–68 mm in 10 d. Colonies on SNA flat with entire margin, medium hyaline; colonial diam 40–47 mm in 7 d, 66–74 mm in 10 d.

*Specimen examined.* TAIWAN, on twig of *Camellia sinensis*, unknown collection date and collector (isolated by J. Chen) (holotype CBS H-21493, culture ex-type CBS 571.88).



**Fig. 5** *Colletotrichum pseudomajus* (CBS 571.88). a, f. Acervuli; b, c. tips of setae; d, i. conidiophores; e. paraphyses; g, h. basal parts of setae; j. outer surface of peridium; k, l. conidia; m, q, r. ascospores; n. ascomata; o, p. asci (a, b, d, e, g, j, k, m, n, p: from OA; c, f, h, i, l, o, q, r: from SNA. – a, f, n: DM; b–e, g–m, o–r: DIC). — Scale bars: f = 100 µm (applies to a, f, n); k = 10 µm (applies to b–e, g–m, o–r).

Notes — Several *Colletotrichum* species have been reported from tea plants, which include *C. camelliae* described on living leaves of tea plants (*Camellia sinensis*) from Sri Lanka (Massee 1899), *Glomerella cingulata* f. sp. *camelliae* described from ornamental camellia from New Zealand (Dickens & Cook 1989) and *Glomerella major* described from healthy wood in the vicinity of rotting lesions on *Camellia sinensis* from North-East India (Tunstall 1934).

Weir et al. (2012) clarified the taxonomic status of *G. cingulata* f. sp. *camelliae* based on molecular analysis and pathogenicity tests, showing it to belong to the *C. gloeosporioides* complex. The phylogenetic analysis shows that strain CBS 571.88 (here referred as *C. pseudomajus*) is phylogenetically distinct from the *C. gloeosporioides* complex. Additionally, *C. pseudomajus* differs from *G. cingulata* f. sp. *camelliae* in producing much larger conidia and ascospores (*C. pseudomajus*: conidia 22–30.5 × 6.5–9.5 µm and ascospores 18.5–25 × 4.5–7.5 µm vs *G. cingulata* f. sp. *camelliae*: conidia 11.3–21.8 × 3.5–6.9 µm and ascospores 10–13 × 3.5–4.5 µm) (Dickens & Cook 1989).

The name *C. camelliae*, although not listed by Hyde et al. (2009) and Cannon et al. (2012), is widely used for the causal agent of the brown blight disease of tea (Sosa de Castro et al. 2001, Muraleedharan & Baby 2007). However, the status of *C. camelliae* and its taxonomic relationship with *G. cingulata* f. sp. *camelliae* remain unresolved (Weir et al. 2012). There are 11 ITS sequences of *Colletotrichum* sp. from tea in GenBank (EF063686, FJ515007, EU732732, FJ216456, HQ832797, JQ809665, HQ832801, AB548281, AB218993, GQ916544, HE655519), of which sequence HQ832801 associated strain nested within the *C. boninense* complex in the ITS phylogenetic tree, while the others belong to several clades within the *C. gloeosporioides* complex (data not shown). Appropriate fresh collections associated with brown blight symptoms of tea from Sri Lanka are needed for epitypification to clarify the phylogenetic relationships of this taxon. *Colletotrichum pseudomajus* can be distinguished from *C. camelliae* by its significantly larger conidia (22–30.5 × 6.5–9.5 µm vs 15–17 × 4–5 µm).

*Colletotrichum pseudomajus* is morphologically similar to *G. major* except for the presence of paraphyses and the shape of its ascospores. Paraphyses were reported to be absent in *G. major*, but thin-walled, hyaline and septate paraphyses are present in *C. pseudomajus*; ascospores of *G. major* are ellipsoid, not allantoid, with obtuse or subacute tips (Tunstall 1935), while those of *C. pseudomajus* are lunate, with more or less acute tips (Fig. 5). Currently, the phylogenetic position of *G. major* is unresolved due to the lack of an ex-type isolate. Thus, an epitype is needed to stabilise the nomenclature of *G. major* and to clarify the relationship between *C. pseudomajus* and *G. major*.

The closest matches in a blastn search with the ITS sequence of CBS 571.88 with 100 % identity were JX009424, the sequence generated from the same isolate by Weir et al. (2012), and JQ809667 from the endophytic isolate JD08-18 from *Camellia sinensis* in China (Fang et al. 2013), as well as JN418782 from the endophytic isolate E10202g from *Otoba parvifolia* in Ecuador (Barba et al. unpubl. data). Closest match with the TUB2 sequence (with 93 % identity, 32 bp differences) was isolate MUCL 41702 from *Orchis* in Singapore (FN599826; Rakotoniriana & Munaut unpubl. data). The blastn search with the GAPDH sequence of CBS 571.88 showed similarity with JN050231 (85 % identity, 34 bp differences) from isolate BCC 38879 from *Hibiscus rosa-sinensis* in Thailand (Noireung et al. 2012) which is here referred to *C. gigasporum*, and JX009422 (99 % identity, 1 bp difference), a sequence generated from the same isolate. The only base difference in the end of the sequence was due to sequencing error by Weir et al. (2012).

***Colletotrichum radialis*** F. Liu, L. Cai, Crous & Damm, *sp. nov.*  
— MycoBank MB807166; Fig. 6

*Etymology.* Referring to the host organ, a plant root, from which it was isolated.

On *Anthriscus* stem. *Vegetative hyphae* hyaline to medium brown, smooth-walled, septate, branched. *Conidiomata* acervular, conidiophores and setae formed on a cushion of angular brown cells. *Setae* brown, smooth-walled, 0–3-septate, 77–192 µm long, base cylindrical to inflated, 5.5–6.5 µm diam, tip acute to obtuse. *Conidiophores* hyaline to brown, septate, branched. *Conidiogenous cells* hyaline to medium brown, cylindrical to clavate, 14–23 × 5.5–8.5 µm, opening 1.5–2 µm diam. *Conidia* hyaline, aseptate, smooth-walled, cylindrical to slightly curved, both ends rounded, 15.5–28 × 5.5–9.5 µm, av. ± SD = 22.6 ± 3.4 × 7.8 ± 0.7 µm, L/W ratio = 2.9.

On SNA. *Vegetative hyphae* hyaline to medium brown, smooth-walled, septate, branched. *Chlamydospores* not observed (but see below). *Conidiomata* acervular. *Setae* medium to dark brown, smooth-walled, 0–3-septate, 43–230 µm long, base cylindrical to inflated, 3.5–8.5 µm diam, tip acute to obtuse. *Conidiophores* brown, septate, branched. *Conidiogenous cells* medium brown, cylindrical to clavate, 11.5–24 × 5–9 µm, opening 1–2.5 µm diam. *Conidia* hyaline, aseptate, smooth-walled, cylindrical to slightly curved, 25.5–32.5 × 6.5–9.5 µm, av. ± SD = 28.2 ± 1.7 × 7.9 ± 0.6 µm, L/W ratio = 3.6. *Appressoria* not observed on the undersurface of the medium, but in old cultures appressoria-like structures that possibly function as chlamydospores were observed within the medium; these are single or in small dense clusters, light to medium brown, smooth-walled, globose, subglobose, elliptical to clavate in outline, with an entire or undulate margin, 4–8.5 µm diam.

Culture characteristics — Colonies on OA flat with entire margin, aerial mycelium lacking; colonial diam 64–71 mm in 7 d, > 90 mm in 10 d. Colonies on SNA flat with entire margin, aerial mycelium lacking, medium hyaline, buff around *Anthriscus* stem; colonial diam 64–75 mm in 7 d, > 90 mm in 10 d.

*Specimen examined.* COSTA RICA, La Selva, host plant unknown (isolated from a plant root), unknown collection date and collector (isolated by G. Weber in Mar. 1993) (holotype CBS H-21494, culture ex-type CBS 529.93).

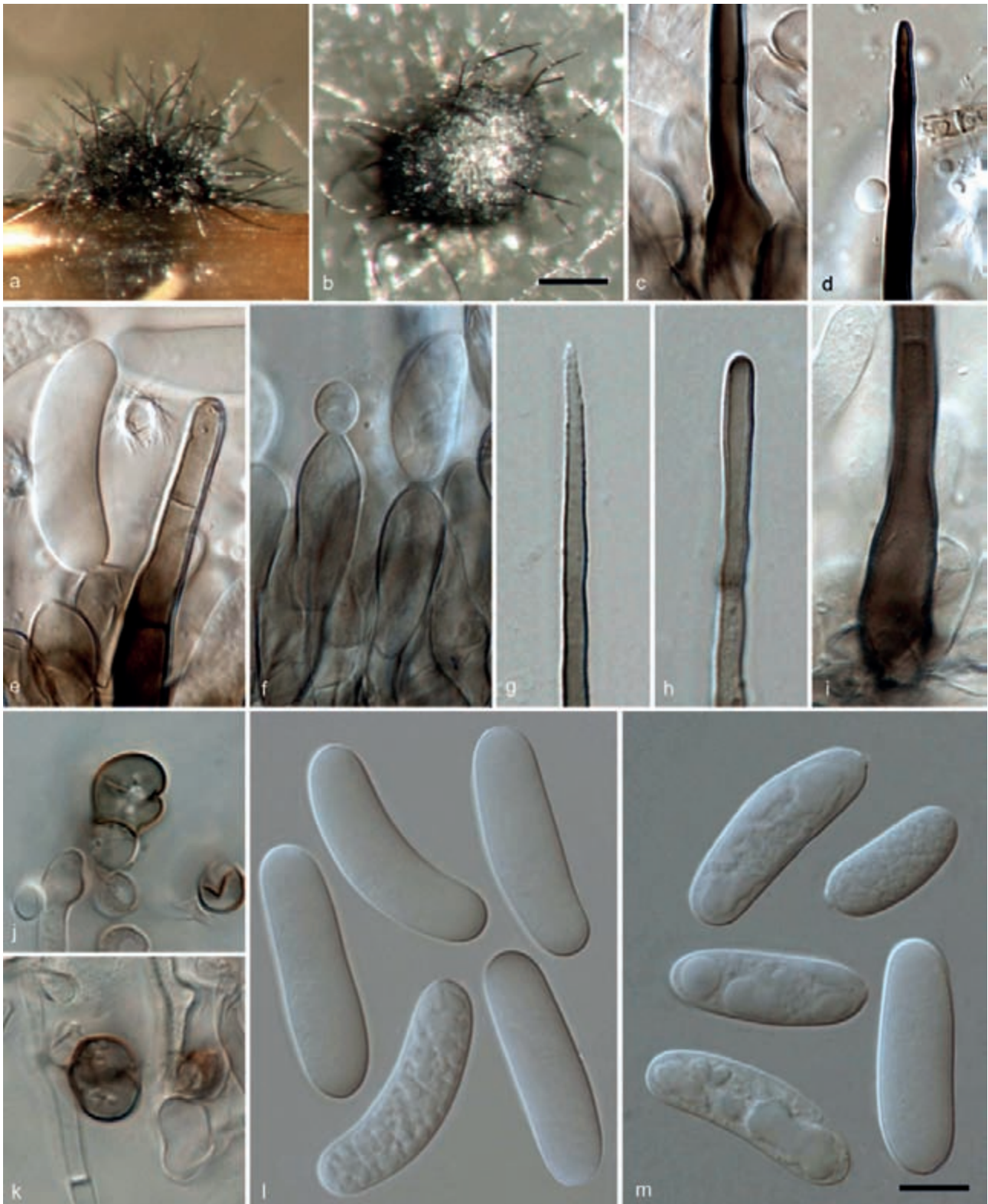
Notes — *Colletotrichum radialis* is phylogenetically close to but clearly differentiated from *C. magnisporum* based on multi-locus and single gene phylogenetic analyses (Fig. 1, 2). Furthermore, *C. radialis* produces relatively short and narrow conidia (25.5–32.5 × 6.5–9.5 µm, av. = 28.2 × 7.9 µm) compared to those of *C. magnisporum* (28.5–40.5 × 8.5–11 µm, av. = 34.3 × 9.7 µm). In addition, many conidia of *C. radialis* are slightly curved, while those of *C. magnisporum* are straight.

The closest match in a blastn search with the ITS sequence of CBS 529.93 was FJ205460 (with 97 % identity, 18 bp differences) from a root associated isolate from an orchid in Taiwan (Wang et al. unpubl. data). Closest matches with the TUB2 sequence were FN599817 (with 95 % identity, 22 bp differences) from isolate CBS 169.59 from *Oncidium excavatum* in the Netherlands, which is here referred to as *C. arxii* (Munaut et al. unpubl. data) and FN599826 (with 95 % identity, 23 bp differences; Rakotoniriana & Munaut unpubl. data) from isolate MUCL 41702 from *Orchis* in Singapore.

***Colletotrichum vietnamense*** F. Liu, L. Cai, Crous & Damm, *sp. nov.* — MycoBank MB807167; Fig. 7

*Etymology.* Referring to the country where the fungus was collected.

On *Anthriscus* stem. *Vegetative hyphae* hyaline to medium brown, smooth-walled, septate, branched. *Conidiomata* acervular, conidiophores and setae formed on a cushion of angular



**Fig. 6** *Colletotrichum radicans* (CBS 529.93). a, b. Acervuli; c, i. basal parts of setae; d, g, h. tips of setae; e. conidiogenous cells with conidia; f. conidiophores; j, k. appressoria-like structures; l, m. conidia (a, f–i, m: from *Anthriscus* stem; b–e, j–l: from SNA. — a, b: DM; c–m: DIC). — Scale bars: b = 100  $\mu$ m (applies to a, b); m = 10  $\mu$ m (applies to c–m).

brown cells. *Setae* medium to dark brown, smooth-walled to verruculose, 1–3-septate, 100–180  $\mu$ m long, base cylindrical to inflated, 6–9.5  $\mu$ m diam, tip subacute to rounded. *Conidiophores* hyaline to brown, septate, branched. *Conidiogenous cells* hyaline to medium brown, cylindrical, clavate to pyriform, 17–26.5  $\times$  7–9.5  $\mu$ m, opening 2–3.5  $\mu$ m diam, collarete (few observed) 0.5  $\mu$ m long. *Conidia* hyaline, aseptate, smooth-walled, cylindrical, occasionally slightly curved, both ends rounded, 19.5–40  $\times$  8–10.5  $\mu$ m, av.  $\pm$  SD = 32.3  $\pm$  4.9  $\times$  9.5  $\pm$  0.6  $\mu$ m, L/W ratio = 3.4.

On SNA. *Vegetative hyphae* hyaline to medium brown, smooth-walled, septate, branched. *Conidiomata* acervular. *Setae* medium to dark brown, smooth-walled to verruculose, 1–7-septate, 118–176  $\mu$ m long, base cylindrical to inflated, 7.5–9.5  $\mu$ m diam, tip subacute. *Conidiophores* hyaline to brown, septate, branched. *Conidiogenous cells* hyaline to medium brown, cylindrical, clavate, to pyriform, 13–20.5  $\times$  7.5–10  $\mu$ m, opening 2–3  $\mu$ m diam, collarete 0.5  $\mu$ m long. *Conidia* hyaline, aseptate, smooth-walled, cylindrical, occasionally slightly curved, both ends



**Fig. 7** *Colletotrichum vietnamense* (CBS 125478). a, b. Acervuli; c, d. tips of setae; e, f. conidiophores; g, h. basal parts of setae; i–l. appressoria; m, n. conidia (a, d, f, h, n: from *Anthriscus* stem; b, c, e, g, i–m: from SNA. a, b: DM; c–n: DIC). — Scale bars: b = 100  $\mu$ m (applies to a, b); m = 10  $\mu$ m (applies to c–n).

rounded,  $24\text{--}39 \times 7.5\text{--}11.5 \mu\text{m}$ , av.  $\pm$  SD =  $31.2 \pm 3.6 \times 9.6 \pm 0.7 \mu\text{m}$ , L/W ratio = 3.3. *Appressoria* (only few observed) pale brown, solitary, irregular outline with crenate or lobed margin,  $9\text{--}17 \times 5.5\text{--}12.5 \mu\text{m}$ , av.  $\pm$  SD =  $13.2 \pm 2.7 \times 9.1 \pm 2.7 \mu\text{m}$ , L/W ratio = 1.2.

**Culture characteristics** — Colonies on OA flat with entire margin, rosy-buff pigmented, aerial mycelium white to grey, sparse; reverse olivaceous-grey; colonial diam 56–61 mm in 7 d, > 90 mm in 10 d. Colonies on SNA flat with entire margin, medium hyaline, buff around *Anthriscus* stem, aerial mycelium lacking; colonial diam 61–63 mm in 7 d, > 90 mm in 10 d.

**Specimens examined.** VIETNAM, Lam Dong Province, Dalat, from anthracnose on leaf of *Coffea* sp., unknown collection date, P. Nguyen & E. Lijeroth (holotype CBS H-21512, culture ex-type CBS 125478 = LD16(L2)); Dak Lac Province, Buon Ma Thout, from anthracnose on leaf of *Coffea* sp., unknown collection date, P. Nguyen & E. Lijeroth, culture CBS 125477 = BMT25(L3).

**Notes** — Anthracnose of *Coffea* sp. can be caused by various *Colletotrichum* species, e.g., *C. acutatum* (Damm et al. 2012a), *C. asianum* (Prihastuti et al. 2009), *C. coffeanum* (Noack 1901), *C. coffeophilum* (Spegazzini 1919), *C. costaricense* (Damm et al. 2012a), *C. fructicola* (Prihastuti et al. 2009), *C. incarnatum* (Zimmermann 1901), *C. kahawae* (Waller et al. 1993), *C. queenslandicum* (Weir et al. 2012), *C. siamense* (Prihastuti et al. 2009) and *C. walleri* (Damm et al. 2012a). The newly described species *C. vietnamense* is morphologically and phylogenetically different from these species. *Colletotrichum asianum*, *C. fructicola*, *C. kahawae*, *C. queenslandicum* and *C. siamense*, belong to the *C. gloeosporioides* complex, and *C. acutatum*, *C. costaricense* and *C. walleri*, belong to the *C. acutatum* complex, all of them have much smaller conidia (Shivas & Tan 2009, Damm et al. 2012a, Weir et al. 2012).

*Colletotrichum coffeanum* was characterised by 1–2-septate setae; pyriform hyaline conidiophores,  $18\text{--}20 \times 4 \mu\text{m}$ ; smooth,

oblong with rounded ends, often curved conidia, 12–18 × 4–5 µm (Noack 1901). *Colletotrichum coffeophilum* produces aseptate setae, 25–50 × 4–6 µm; conidia ellipsoidal and hyaline, 1-guttulate, 13–15 × 6–8 µm (Spegazzini 1919). *Colletotrichum incarnatum* has dark brown setae, flat tipped, base cylindrical or somewhat swollen, 85 × 4–5 µm; conidia oblong, 14–19 × 5 µm (Zimmermann 1901). In contrast, *C. vietnamense* differs from these three species in forming much larger conidia and longer setae.

Another species known to occur on *Coffea* sp. from Vietnam in this complex is *C. gigasporum* (CBS 125476 and CBS 125475), which can be distinguished from *C. vietnamense* by each of the eight genes used in this study, including ITS (Fig. 1).

The closest matches with the ITS sequence of CBS 125478 were FJ968584 (with 100 % identity), a sequence generated from the same isolate by Nguyen et al. (2010), and EF672327 (with 100 % identity) from the endophytic isolate PR61F2, also from *Coffea arabica*, but from coffee berries in Puerto Rico, a country in Central America (Vega et al. unpubl. data). Closest match with the TUB2 sequence was KC293665 (with 96 % identity, 20 bp differences) from isolate gnqczg15 from China (Huang et al. unpubl. data).

## DISCUSSION

Many of the strains included in the present study were deposited in the CBS culture collection as *C. crassipes* (Speg.) Arx. However, *C. crassipes* is a species with uncertain taxonomic status. There is significant confusion regarding its morphology in the literature. Spegazzini (1878) originally described this fungus as *Gloeosporium crassipes* from *Vitis vinifera* from Conegliano, Italy with conidia measuring 20–30 × 7–8 µm. Subsequently, von Arx (1957) combined *Gloeosporium crassipes* in *Colletotrichum* as *C. crassipes* along with 17 synonyms. The conidial size of *C. crassipes* was reported as 22–31 × 6–8 µm, broadly matching the original description; and the appressoria as irregular, usually lobed, measuring 8–12 µm (von Arx 1957). Sutton (1980) presented a different morphological concept of *C. crassipes*, which was characterised by conidia measuring 10–15 × 4.5–6.5 µm, long clavate or circular appressoria with crenate or deeply divided edges, 10.5–14 × 7–9.5 µm, and reduced another two names to synonymy with it. However, when Sutton summarised an accepted taxa list of *Colletotrichum* species, *C. crassipes* was characterised with conidia again with a different size (14–28 × 5–7 µm), and he suspected that this species may consist of a number of separate taxa (Sutton 1992). Moreover, several isolates identified as *C. crassipes* that have sequences lodged in GenBank actually belong to *C. gloeosporioides* s.lat. (Weir et al. 2012). Recollecting and epitypification of this taxon is required to stabilise the phylogenetic position of *C. crassipes*.

Although morphological features are not stable and change under different growth conditions and with repeated subculturing, species of the *C. gigasporum* species complex form larger conidia than most of the other species in the genus *Colletotrichum*, which provides a valuable character for species complex level diagnosis. Conidia of two other species with large conidia, *C. euphorbiae* and *C. sansevieriae*, differ in shape; they are slightly clavate with a round apex tapering to a truncate to slightly acute base (Nakamura et al. 2006, Crous et al. 2013). These two species do not belong to the *C. gigasporum* complex.

While single gene data, especially ITS data, are usually not sufficient for species recognition in most of the *Colletotrichum* species complexes or groups (Cannon et al. 2012) and multi-locus phylogenies are therefore now routinely used as the primary basis on which to describe new *Colletotrichum* species (Damm et al. 2012a, b, Weir et al. 2012, Liu et al. 2013a, b),

species of the *C. gigasporum* species complex can be easily distinguished from each other using the individual gene data included in this study (Fig. 1).

*Colletotrichum gigasporum* appears to have a wide host range and geographic distribution. Isolates treated in this paper and those deposited in GenBank originate mainly from Africa (East Africa, Madagascar), Central and South America (Brazil, Chile, Columbia, Ecuador, Guyana, Mexico, Panama), Asia (China, India, Japan, Korea, Thailand, Vietnam) and New Zealand (Fig. 1). Besides, this species is associated with various host plants as pathogens and endophytes, from air and stored grain, indicating that it is not host-specific and apparently has different life styles. This character is not unique to *C. gigasporum*, many other *Colletotrichum* species have been reported as both pathogens and endophytes, e.g. *C. boninense*, *C. karstii* and *C. liriopes* (Yang et al. 2011, Damm et al. 2012b, Tao et al. 2013). For instance, *C. boninense* causes diseases of *Crinum asiaticum* var. *sinicum* and *Solanum lycopersicum*, and is also an endophyte of *Bletilla ochracea* and *Dacrycarpus dacrydioides* (Damm et al. 2012b, Tao et al. 2013). The relationship between plant endophytic and pathogenic isolates of the same *Colletotrichum* species needs more research, as some endophytes may be latent pathogens (Lu et al. 2004).

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