Cryptogamie, Mycologie, 2011, 32 (3): 229-253

Colletotrichum species on *Orchidaceae* in southwest China

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Abstract – Twenty-two strains of *Colletotrichum*, representing eight species, were isolated from eight genera of *Orchidaceae* in Yunnan and Guizhou provinces in China. Fourteen strains were from lesions and are pathogens, four were from fallen/dead flowers or stems and four were isolated as endophytes. The strains are characterized through morphological studies and multilocus phylogenetic analysis (ACT, Tub 2, CAL, CHS I, GPDH and ITS). *Colletotrichum orchidearum* and the new species *Colletotrichum karstii* are described and illustrated, based on morphological characters and multilocus sequence data. Collections of the other *Colletotrichum* species are reported with notes.

Anthracnose / multilocus phylogeny / systematics / pathogenicity

INTRODUCTION

Orchidaceae is one of the largest plant families of the flowering plants (*Angiospermae*), containing an estimated 25,000 species (Jones, 2006). Many of the taxa are economically important ornamental plants, with more than 100,000 hybrids and cultivars produced to meet market demands for their showy flowers (http://en.wikipedia.org/wiki/Orchidaceae).

Anthracnose disease of *Orchidaceae* is a common problem worldwide (Li, 1999; Moriwaki *et al.*, 2003; Talubnak & Soytong 2010). The primary anthracnose disease fungi have been reported as *Colletotrichum boninense*

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Moriwaki, Toy. Sato & Tsukib., *C. crassipes* (Speg.) Arx, *C. crossandrae* Patel, Kamat & C.B. Pande, *C. gloeosporioides* (Penz.) Penz. & Sacc. (*Glomerella cingulata*), *C. lujae* Verpl. & Clem. *C. orchidearum* Allesch. (including its 13 forms and varieties), *C. stanhopeae* Henn. and *C. vanillae* Scalia (Allescher *et al.*, 1902; Patel *et al.*, 1953; von Arx, 1957; Sutton, 1980; Moriwaki *et al.*, 2003; Hyde *et al.*, 2009; Farr & Rossman, 2011). These anthracnose agents cause brown to black spot on leaves, flowers or stalks of a wide range of orchid plants (Teoh, 2005). With the recent changes in the understanding of species concepts in *Colletotrichum* (e.g. Cai *et al.*, 2009; Hyde *et al.*, 2009; Prihastuti *et al.*, 2009, 2010; Phouvilong *et al.*, 2010; Wikee *et al.*, 2011) it is not clear which species occur on orchid in China. The objective of this study was to characterize the species of *Colletotrichum* associated with lesions on flowers and leaves, plus four endophytes isolates from the roots of healthy *Pleione bulbocodioides* (*Orchidaceae*) in Guizhou and Yunnan provinces, China. In addition, the holotype of three forms, *Colletotrichum orchidearum* f. *cymbidii* Allesch., *C. orchidearum* f. *eriae* Allesch. and *C. orchidearum* f. *physosiphonis* Allesch. were investigated.

MATERIALS AND METHODS

Isolation of *Colletotrichum* from infected and healthy tissues

Leaves of *Arundina*, *Calanthe*, *Cattleya*, *Cymbidium*, *Eria*, *Oncidium* and *Vanda* (*Orchidaceae*) species with anthracnose lesions were collected in Guizhou and Yunnan provinces, China between June 2005 and October 2009 (Table 1). *Colletotrichum* associated with lesions and flowers were isolated via two methods depending on the status of fungal sporulation. Isolates were obtained from lesions without visible sporulation using the procedure described by Photita *et al.* (2005), while single-spore isolates from infected leaves or stems with sporulation were obtained using the procedure described by Choi *et al.* (1999). To obtain isolates from healthy roots of *Pleione bulbocodioides*, roots were cleaned in tap water, and dried with sterilized tissue paper. The samples were surface sterilized by dipping in 75% ethanol for 2-3 seconds, immersing in 0.1% mercuric chloride for 3-5 minutes and rinsing three times with sterilized water and finally drying on sterilized tissue paper. Dried roots were cut into about 1 cm segments, placed on potato dextrose agar (PDA), and then incubated at 25°C in darkness. The growing edges of any fungal hyphae developing from the segments were then transferred aseptically to PDA. The fungi were identified following sporulation. Single spore subcultures were obtained for each *Colletotrichum* isolate using the above-mentioned method (Choi *et al.*, 1999). Pure cultures were stored at 4°C on PDA slants. Isolates are deposited in Guizhou Academy of Agricultural Sciences, China, and China General Microbiological Culture Collection Center (CGMCC).

Morphological and cultural characterization

Starter cultures were prepared by growing each isolate on PDA at 25°C for 5 days. Five replicate cultures of each isolate were prepared by aseptically cutting actively growing areas near edge of starter culture using a sterile cork borer. Each plug was placed onto PDA plates (Petri dishes diameter: 90 × 15 mm) and grown in alternating 12 hours near UV/12 hours dark at 25°C (Sutton, 1980).

Colony diameter was measured at day six (for the fastest growing cultures, at day five). Growth rate was calculated as the 6-day or 5-day average of mean daily growth (mm per day). After 7-10 days, size and shape of 50 conidia harvested from the cultures were assessed. The colour of the conidial masses and zonation were recorded at day seven (Than *et al.*, 2008). Mycelial appressoria were produced and measured using a slide culture technique (Sutton, 1980). Conidial appressoria were also induced by placing conidia in two drops of distilled water (about $1.0 \times 10^{5\text{-}6}$conidia/ml) on a microscope slide, then placing the slide inside a Petri dish containing cotton moistened with distilled sterile water, and incubated at 25°C in darkness. After incubating 24-72 hours, conidial appressoria formed from germ tubes and were characterized

DNA extraction and sequencing

DNA was extracted from the isolates growing on PDA at 25°C for 8-10 days using a modified protocol of Chen *et al.* (2007). The partial sequence of the actin (ACT), beta-tubulin (Tub2), calmodulin (CAL), chitin synthase 1 (CHS I), glyceraldehyde-3-phosphate dehydrogenase (GPDH) gene and 5.8S nuclear ribosomal gene with the two flanking internal transcribed spacers (ITS) were amplified and sequenced using the primer pairs ACT-512F/ACT- 783R (Carbone & Kohn, 1999), T1/Bt-2b (O' Donnell & Cigelnik, 1997; Glass & Donaldson, 1995), CL1/CL2 (P. Johnston, personal communication), CHS-79F/CHS-354R (Carbone & Kohn, 1999), GDF1/GDR1 (Guerber *et al.*, 2003), and ITS-1/ITS-4 (White *et al.*, 1990), respectively. The PCR amplifications were performed in a 25 µl mixture containing 9.5µl ddH_2O, 12.5 µl 2×PCR Master Mix (TIANGEN Co. China), 1 µl of DNA template, 1 µl of each primer (10 µM). The reactions were performed with a thermal cycler (Mycler™, Bio-Rad, Hercules, CA, USA) using the thermal program described by Yang *et al.* (2009). PCR products were sequenced using the above-mentioned PCR primers and ABI BigDye v3.1 terminator sequencing chemistry according to the manufacturer's instructions of a BigDye® Terminator v3.1Cycle sequencing kit (Applied Biosystems, CA, USA) in an Applied Biosystems 3730xl DNA Analyzers at Sinomax Co., China.

Molecular phylogenetic analysis

Phylogenetic analysis was performed using the six gene regions described above. The accession numbers of sequences generated are listed in Table 1. Multiple sequence alignments were generated using ClustalX 2.0.10 (Larkin *et al.*, 2007). Gaps were treated as missing data.

Each of the single and combined sequence alignments were analyzed using maximum parsimony (MP) in PAUP* 4.0b10. Ambiguously aligned regions were excluded from all analyses. Trees were inferred using the heuristic search option with tree bisection-reconnection (TBR) branch swapping and 1000 random sequence additions. Maxtrees were unlimited, branches of zero length were collapsed and all multiple parsimonious trees were saved. Clade stability of the trees resulting from the parsimony analyses were assessed by bootstrap analysis with 1000 replicates. Trees were figured in Treeview. When analyzing single and combined sequences, some reference sequences were obtained from GenBank (Table 1). Sequences sequenced in this study were submitted at GenBank, the alignment in TreeBASE (ID 10825) (http://www.treebase.org/treebase/index.html), and taxonomic novelties in MycoBank (Crous *et al.*, 2004).

Table 1. Sources of isolates with GenBank accession numbers used in this study

Taxon	*Specimen no.*	*GenBank no*						*Host*	*Site*	*Reference*
		ITS	*Tub2*	*CAL*	*CHS I*	*GPDH*	*ACT*			
Colletotrichum boninense	**CORCS2**	**HM585400**	**HM585419**	**HM582005**	**HM582033**	**HM585385**	**HM582000**	*Pleione bulbocodioides*	Liupanshui, Guizhou	This paper
	CORCX10	**HM585401**	**HM585420**	**HM582006**	**HM582031**	**HM585384**	**HM581999**	*Oncidium flexuosum*	Jinghong, Yunnan	This paper
	[1]MAFF305972	**HM585399**	**HM585421**	**HM582004**	**HM582032**	**HM585386**	**HM582001**	*Crinum asiaticum* var. *sinicum*	Japan	This paper
	CSSN1	GQ485597	GQ849437	GQ849462	GQ856728	GQ856743	GQ856774	*Crinum asiaticum*	China	Yang *et al.*, 2009
	CSSX8	GQ485596	GQ849433	GQ849460	GQ856727	GQ856742	GQ856771	*Crinum asiaticum*	China	Yang *et al.*, 2009
C. cliviae	**CORCG2**	**HM585397**	**HM585422**	**HM582007**	**HM582024**	**HM585380**	**HM581985**	*Cymbidium hookerianum*	Guiyang, Guizhou	This paper
	CORCX9	**HM585398**	**HM585423**	**HM582008**	**HM582025**	**HM585381**	**HM581986**	*Arundina graminifolia*	Jinghong, Yunnan	This paper
	[1]CSSK4/CBS125375	GQ485607	GQ849440	GQ849464	GQ856722	GQ856756	GQ856777	*Clivia miniata*	China	Yang *et al.*, 2009
	CSSS2	GU109480	GU085870	GU085864	GU085866	GU085868	GU085862	*Clivia miniata*	China	Yang *et al.*, 2009
C. crassipes	**CORCS3**	**HM585410**	**HM585412**	**HM582002**	**HM582035**	**HM585379**	**HM581987**	*P. bulbocodioides*	Liupanshui, Guizhou	This paper
	STU-U4445	AY376530	AY376578					*Dryandra* sp.	Madeira	Lubbe *et al.*, 2004
	STE-U5302	AY376529	AY376577					*Dryas octopetala*	Switzerland	Lubbe *et al.*, 2004
C. fructicola	CSSX7	GQ485604	GQ849435	GQ849459	GQ856734	GQ856760	GQ856770	*Crinum asiaticum*	China	Yang *et al.*, 2009
	[1]MFLU090228	FJ972603	FJ907441	FJ917508		FJ972578	FJ907426	*Coffea arabica*	Thailand	Prihastuti *et al.*, 2009

Table 1. Sources of isolates with GenBank accession numbers used in this study *(continued)*

Taxon	*Specimen no.*	*GenBank no*						*Host*	*Site*	*Reference*
		ITS	*Tub2*	*CAL*	*CHS I*	*GPDH*	*ACT*			
C. gloeosporioides	**CORCG4**	**HM034808**	**HM034810**	**HM034802**	**HM034804**	**HM034806**	**HM034800**	*Vanda* sp., leaf	Luodian, Guizhou	This paper
	CORCG5	**HM034809**	**HM034811**	**HM034803**	**HM034805**	**HM034807**	**HM034801**	*Vanda* sp., leaf	Luodian, Guizhou	This paper
	[1]CBS953.97	GQ485605	GQ849434	GQ849452	GQ856733	GQ856762	GQ856782	*Citrus sinensis*	Italy	Yang *et al.*, 2009
C. hippeastri	CSSG1/CBS125376	GQ485599	GQ849446	GQ849469	GQ856725	GQ856764	GQ856788	*Hippeastrum vittatum*	China	Yang *et al.*, 2009
	CSSG2/CBS125377	GQ485598	GQ849445	GQ849470	GQ856726	GQ856765	GQ856789	*Hippeastrum vittatum*	China	Yang *et al.*, 2009
C. hymenocallidis	[1]CSSN2/CBS125378	GQ485600	GQ849438	GQ849463	GQ856730	GQ856757	GQ856775	*Hymenocallis americana*	China	Yang *et al.*, 2009
	CSSN3/CBS125379	GQ485601	GQ849439	GQ849451	GQ856729	GQ856759	GQ856776	*Hymenocallis americana*	China	Yang *et al.*, 2009
C. karstii	**[1]CORCG6(CGMCC3.14194)**	**HM585409**	**HM585428**	**HM582013**	**HM582023**	**HM585391**	**HM581995**	*Vanda* sp.	Luodian, Guizhou	This paper
	CORCK1	**HM585406**	**HM585424**	**HM582010**	**HM582019**	**HM585387**	**HM581991**	*Calanthe argenteo-striata*	Kunming, Yunnan	This paper
	CORCK3	**HM585407**	**HM585427**	**HM582011**	**HM582020**	**HM585388**	**HM581992**	*Eria coronaria*	Kunming, Yunnan	This paper
	CORCS4	**HM585405**	**HM585426**	**HM582012**	**HM582022**	**HM585390**	**HM581994**	*P. bulbocodioides*	Liupanshui, Guizhou	This paper
	CORCX7	**HM585408**	**HM585425**	**HM582009**	**HM582021**	**HM585389**	**HM581993**	*A. graminifolia.*	Jinghong, Yunnan	This paper
C. liriopes	**CORCK2**	**HM585396**	**HM585414**	**HM582018**	**HM582029**	**HM585382**	**HM581988**	*E. coronaria*	Kunming, Yunnan	This paper
	CORCS1	**HM585395**	**HM585415**	**HM582017**	**HM582030**	**HM585383**	**HM581989**	*P. bulbocodioides*	Liupanshui, Guizhou	This paper

Table 1. Sources of isolates with GenBank accession numbers used in this study *(continued)*

Taxon	*Specimen no.*	*GenBank no*						*Host*	*Site*	*Reference*
		ITS	*Tub2*	*CAL*	*CHS I*	*GPDH*	*ACT*			
	[1]CBS 119444	GU227804	GU228098		GU228294	GU228196	GU227903	*Lirope muscari*	Mexico	Damm *et al.*, 2009
	CBS 122747	GU227805	GU228099		GU228295	GU228197	GU227904	*Lirope muscari*	Mexico	Damm *et al.*, 2009
C. lupini	BBA 70385	AJ301935						*Lupinus angustifolius*	Germany	Nirenberg *et al.*, 2002
	BBA 63879	AJ301930						*Lupinus mutabilis*	Germany	Nirenberg *et al.*, 2002
C. orchidearum	**CORCG3**	**HM585402**	**HM585418**	**HM582014**	**HM582026**	**HM585392**	**HM581996**	*Cymbidium hookerianum*	Guiyang, Guizhou	This paper
	[2]CORCX1							*Cattleya* sp.	Jinghong, Yunnan	This paper
	[2]CORCX2							*Cattleya* sp.	Jinghong, Yunnan	This paper
	[2]CORCX3							*Cattleya* sp.	Jinghong, Yunnan	This paper
	[2]CORCX5							*Cattleya* sp.	Jinghong, Yunnan	This paper
	[1]CORCX6(CGMCC3.14195)	**HM585403**	**HM585416**	**HM582015**	**HM582027**	**HM585393**	**HM581997**	*Cattleya* sp.	Jinghong, Yunnan	This paper
	CORCX11	**HM585404**	**HM585417**	**HM582016**	**HM582028**	**HM585394**	**HM581998**	*O. flexuosum*	Jinghong, Yunnan	This paper
C. siamense	**COGCX8**	**HM585411**	**HM585413**	**HM582003**	**HM582034**	**HM585378**	**HM581990**	*A. graminifolia*	Jinghong, Yunnan	This paper
	CSST1	GQ485602	GQ849441	GQ849467	GQ856731	GQ856758	GQ856778	*Hymenocallis* sp.	Thailand	Yang *et al.*, 2009
	CSST4	GQ485603	GQ849443	GQ849465	GQ856732	GQ856761	GQ856780	*Hymenocallis* sp.	Thailand	Yang *et al.*, 2009

Table 1. Sources of isolates with GenBank accession numbers used in this study *(continued)*

Taxon	Specimen no.	GenBank no						Host	Site	Reference
		ITS	*Tub2*	*CAL*	*CHS I*	*GPDH*	*ACT*			
	[1]MFLU090230	FJ 972613	FJ 907438	FJ 917505		FJ 972575	FJ 907423	*Coffea arabica*	Thailand	Prihastuti *et al.*, 2009
C. simmondsii	[1]BRIP28519	GQ485606	GQ849430	GQ849454	GQ856735	GQ856763	GQ856784	*Carica papaya*	Australia	Yang *et al.*, 2009
C. spaethianum	CSSX3	GQ485584	GQ849432	GQ849456	GQ856719	GQ856745	GQ856767	*Hymenocallis americana*	China	Yang *et al.*, 2009
	CSSX5	GQ485586	GQ849426	GQ849448	GQ856718	GQ856747	GQ856769	*Hymenocallis americana*	China	Yang *et al.*, 2009
C. tofieldiae	CBS495.85	GU227801			GU228291	GU228193	GU227899	*Tofieldia calyculata*	Switzerland	Damm *et al.*, 2009
	IMI288810	GU227803			GU228293	GU228195	GU227901	*Dianthus* sp.	UK	Damm *et al.*, 2009
C. trichellum	HKUCC10378	GQ485589	GQ849447	GQ849466	GQ856724	GQ856749	GQ856786	Unknown	Unknown	Yang *et al.*, 2009
C. truncatum	CSSX2	GQ485595	GQ849424	GQ849457	GQ856736	GQ856750	GQ856766	*Crinum asiaticum*	China	Yang *et al.*, 2009
	CBS120709	GQ485593	GQ849429	GQ849453	GQ856739	GQ856753	GQ856783	*Capsicum frutescens*	India	Yang *et al.*, 2009
C. verruculosum	[1]IMI 4552	GU227806			GU228296	GU228198	GU227904	*Crotalaria juncea*	Zimbabwe	Damm *et al.*, 2009
Fusarium oxysporum	ATCC-MYA-3970	FJ614650						*Unknown*	USA	Unpublished

Note: ATCC, American Type culture collection; BRIP, Queensland Department of Primary Industries Plant Pathology Herbarium; CBS, Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; CGMCC, China General Microbiological Culture Collection Center; HKUCC, The University of Hong Kong Culture Collection; IMI, Culture collection of CABI Europe UK Centre, Egham, UK; MFLU, Mae Fah Luang University, Thailand; STE-U, Culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa. [1] ex-type or ex-epitype cultures; [2] strains not sequenced. The isolated strains and newly generated sequence are shown in bold.

Table 2. Synopsis of characters of *Colletotrichum* species from *Orchidaceae* in China

Taxon	*Conidia shape and size on PDA (μm)*	*Conidial appressoria shape and size (μm)*	*Mycelial appressoria shape and size (μm)*	*Ascospore shape and size (μm)*	*Colony characteristics on PDA*	*Mycelial growth rate(mm per day)*	*Life mode*
C. boninense (2 isolates)	Cylindrical, straight, hilum-like base, 12.5-18 × 6-8.5, $\bar{x}$ = 15.2 ± 1.2 × 7.1 ± 0.6, n = 100	Clavate to irregular, margin crenate, brown, 7.5-11 × 5-9, $\bar{x}$ = 9.4 ± 1.2 × 7.3 ± 1.2, n = 40	No data	Did not produce	White aerial mycelia; reverse reddish orange.	6.4-12.5 $\bar{x}$ = 9.6 ± 2.6, n = 10	Pathogen
C. cliviae (2 isolates)	Cylindrical, straight, obtuse at the ends, 14-20.5 × 5-6, $\bar{x}$ = 17.6±1.2 × 5.4±0.3, n = 50	Irregular, margin crenate to lobed, dark brown, 7.5-11.5 × 6.5-9, $\bar{x}$ = 9.7±1.4 × 7.4 ± 0.6, n = 20	Irregular, crenate or lobed, brown, 9.5-15.5 × 6.5-10.5, $\bar{x}$ = 12.4 ± 2.8 × 7.7 ± 1.8, n = 5	Falcate, fusiform, tapered towards ends, 12.5-21.5 × 5-7.5, $\bar{x}$ = 16.7 ± 2.2 × 6 ± 0.7, n = 100	White to grey, reverse brown to black.	13-14.8, $\bar{x}$ = 14 ± 0.6, n =10	Pathogen
C. crassipes (1 isolate)	Cylindrical, straight, obtuse at apex, truncate at base, 14.5-18.5 × 5.5-6.5, $\bar{x}$ = 15.6 ± 0.9 ×5.9 ±0.3, n = 50	Clavate to irregular, margin crenate, brown, 6-9 × 5-7.5, $\bar{x}$ = 7.9 ± 0.9 × 6.3 ± 0.6, n = 20	Clavate, margin entire to crenate, brown, 8.5-15.5 × 6.5-8 $\bar{x}$ = 11.9 ± 2.9 × 7.1 ± 0.7, n = 5	Did not produce	White to grey mycelia; reverse, dark chocolate brown.	14.8-15.8, $\bar{x}$ = 15.3 ± 0.4, n =5	Endophyte
C. gloeosporioides (2 isolates)	Cylindrical, straight, obtuse at ends, 13-20.5(-23) ± 4.5-6.5, $\bar{x}$ = 16.5 ± 1.6 × 5.4 ± 0.4, n = 80	Clavate to circular, margin entire, sepia brown, 6-10.5 × 5-8.5 $\bar{x}$ = 8.1 ± 0.8 × 6.5 ± 0.6, n = 40	Clavate, margin entire or pale brown, 8.5-13 ± 6-7.5, $\bar{x}$ = 10.5 ± 1.7 × 6.8 ± 0.5, n = 10	Did not produce	Greyish white, reverse pale yellow to grey.	10.3-11.5, $\bar{x}$ = 10.8 ± 0.4, n = 10	Pathogen
C. karstii (5 isolates)	Cylindrical, straight, obtuse at apex, base truncate, 12-19.5 × (5-)6-7.5, $\bar{x}$ = 15.4 ± 1.3 × 6.5 ± 0.5, n = 215	Circular to clavate, margin entire, sepia brown, 6.5-14.5 × 4-8.5, $\bar{x}$ = 9.3 ± 1.6 × 6.4 ± 0.9, n = 100	No data	Falcate, fusiform, 10.5-20 × 4.5-6.5, $\bar{x}$ =15.7 ± 1.7×5.3 ± 0.5, n = 60	White aerial mycelia; reverse reddish yellow.	8.3-11.4, $\bar{x}$ = 9.8 ± 0.9, n = 25	Pathogen Endophyte
C. liriopes (2 isolates)	Falcate, fusiform, 11-26 × 2.5-4.5 $\bar{x}$ = 17.7 ± 5.2 × 3.4 ± 0.4, n = 100	Clavate to irregular, margin crenate, sepia brown, 6.9-11.5 × 4.5-8.5 $\bar{x}$ = 8.6 ± 1.1 × 6.3 ± 0.9, n = 40	Clavate to irregular, margin crenate to lobbed, sepia brown 6.5-15 × 7-14, $\bar{x}$ = 11.7 ± 1.8 × 8.2 ± 1.3, n = 40	Did not produce	Grey, reverse dark brown to black.	8.4-12, $\bar{x}$ = 10.3 ± 1.6, n = 10	Pathogen Endophyte
C. orchidearum (7 isolates)	Cylindrical, straight, rounded at the ends. 15-23 × 5-6, $\bar{x}$ = 18.3 ± 1.5 × 5.4 ± 0.3, n = 150	Circular to irregular, margin entire to crenate, brown, 7-12 × 6.5-11, $\bar{x}$ = 10 ± 1.2 × 8.4 ±1.3, n = 60	No data	Falcate, fusiform, (15.5-)18-26 × 4.5-7, $\bar{x}$ = 21 ± 2.4 × 5.8 ± 0.7, n = 60	Pale grey to dark grey, reverse dark grey.	9.9-16.4 $\bar{x}$ = 14.2 ± 2, n = 15	Pathogen Saprotroph
C. siamense (1 isolates)	Fusiform to cylindrical, obtuse to slightly rounded ends, 13-16.5 × 4.5-5, $\bar{x}$ = 15.3 ± 0.9 × 4.7 ± 0.3, n = 50	Ovoid, margin entire, medium brown, 6.5-8.5 × 5.5-7, $\bar{x}$ = 7.1 ± 0.5 × 5.9 ± 0.4, n = 20	Ovate to clavate, margin entire, medium brown, 8-13 × 5-7.5, $\bar{x}$ = 10.2 ± 1.5 × 6.6 ± 0.8, n = 10	Did not produce	White, becoming pale grey with age, reverse pale yellowish to black.	14.3-17, $\bar{x}$ = 15.8 ± 1, n =5	Pathogen

Table 3. Pathogenicity testing of *Colletotrichum* isolates from *Orchidaceae*

Taxon		*Infection rating*[1] *(%)*			
		C. karstii		*C. orchidearum*	*C. siamense*
Strain		*CORCG6*	*CORCS4*	*CORCX6*	*CORCX8*
Capsicum annuum, fruit	Wound/drop	100	0	0	100
	Non wound/drop	0	0	0	0
Clivia miniata[3], leaf	Wound/drop	0	0	0	0
	Non wound/drop	0	0	0	0
Lycopersicon esculentum[3], fruit	Wound/drop	66.7	66.7	66.7	100
	Non wound/drop	0	0	0	66.7
Malus pumila[3], fruit	Wound/drop	88.9	22.2	56.7	100
	Non wound/drop	0	0	0	0

[1] Infection rating = the number of infected fruits or leaves divided by the number of total inoculated fruits or leaves; [2] = 11 replicates; c, [3], 9 replicates.

The model of evolution was estimated by using Mrmodeltest 2.2 (Nylander, 2004). Posterior probabilities (PP) (Rannala & Yang, 1996; Zhaxybayeva & Gogarten, 2002) were determined by Markov Chain Monte Carlo sampling (BMCMC) in MrBayes 3.0b4 (Huelsenbeck & Ronquist, 2001). Six simultaneous Markov chains were run for 1,000,000 generations and trees were sampled every 100th generations (resulting 10,000 total trees). The first 2,000 trees, which represented the burn-in phase of the analyses, were discarded and the remaining 8,000 trees were used for calculating posterior probabilities (PP) in the majority rule consensus tree.

Pathogenicity testing and host range

Four representative isolates from *Orchidaceae* were selected for pathogenicity testing (Table 3). Four plants were selected to test the host range (Table 3). These plants belong to 3 families, being host plants of *Colletotrichum* species that are morphologically similar to the isolates from *Orchidaceae*.

Single spore cultures of each isolate were grown on PDA for 7 days at 25°C. Spores were then harvested by adding 10 ml of sterilized distilled water onto the culture, which was then gently swirled to dislodge the conidia. The conidial suspension was filtered through two layers of muslin cloth and adjusted to 1×10^5 conidia/ml using a haemocytometer.

Healthy, freshly harvested, mature leaves or fruits were obtained and washed under running tap water for 60 seconds followed by surface sterilization by immersing the fruits or leaves in 70% ethanol for 3 minutes, 1% sodium hypochlorite solution for 3 minutes and then rinsing three times in sterilised distilled water for 2 minutes and drying with sterile tissue paper (Cai *et al.*, 2009; Montri *et al.*, 2009). The inoculation method has been described by Than *et al.* (2008). The wound/drop inoculation method involved pin pricking the leaf or fruit wall to about

1 mm depth; the non wound inoculation without pricking, and then placing 6 µl of conidia suspension (10^5 conidia/ml) onto the wound/non wound. Control fruits and leaves were inoculated with 6 µl of sterilized distilled water onto the wound/non wound. Each plant was inoculated with at least nine replicates per selected strain (Table 3). Inoculated leaves were kept individually in a glass bottle (350 ml, 12 height × 7 diameter cm) with 80 ml sterilized water and sealed with a fresh-keeping plastics bag. Inoculated fruits were placed individually in a plastic box (30 × 25 × 4 cm) with sterilized tissue paper containing sterilised distilled water to maintain approximately 95% relative humidity (Montri *et al.*, 2009). Boxes were covered with cling film and incubated at 25°C for 14 days. After 7 and 14 days, lesion development was assessed by estimating the percentage of disease on each fruit or leaf. Spore masses or acervuli on innoculated leaves or fruits were checked with a compound microscope and spores were transferred to PDA medium, and then incubated at 25°C for checking the colony and spore characters. All infected leaves or fruits were sterilized before disposal.

RESULTS

Collection of *Colletotrichum* species

Fourteen strains of *Colletotrichum* were isolated from anthracnose lesions or recently dead leaves of *Arundina*, *Calanthe*, *Cattleya, Cymbidium*, *Eria*, *Oncidium* and *Vanda* and four strains were isolated from flowers or stems of *Cattleya* sp. (Table 1). For comparison, four endophytic strains isolated from roots of *Pleione bulbocodioides* (*Orchidaceae*) and the ex-holotype of *Colletotrichum boninense* (MAFF 305972) were included in this study.

Phylogenetic analysis

The combined partial datasets of the six genes (ACT, CAL, CHS I, GPDH, ITS, Tub 2) of the *Colletotrichum* species from *Orchidaceae* plus datasets obtained from GenBank were comprised of 2797 characters after alignment, of which 1063 characters are parsimony-informative (38%), 1584 constant (56.6%), and 150 parsimony-uninformative (5.4%). Parsimony analysis generated two trees; KH test verified that two generated trees from parsimony analysis were similar, one of which (Tree length = 2653 steps) is shown in Figure 1.

The phylogram constructed using combined datasets showed that *Orchidaceae* isolates clustered into eight distinct clades with high bootstrap support (except in one case of 93%, all others are equal or above 99%), and Bayesian posterior probabilities (100%), thus presumably representing different *Colletotrichum* species.

Taxonomy

Based on DNA sequence data and morphological characteristics the 22 *Orchidaceae* isolates represent eight species of *Colletotrichum* (Tables 1, 2). Clade 1 represents an undescribed *Colletotrichum* species, Clade 2, 3, 4, 5, 6, 7 and 8 are *C. boninense*, *C. cliviae* Y.L. Yang, Zuo Y. Liu, K.D. Hyde & L. Cai, *C. orchidearum*, *C. siamense* Prihastuti, L. Cai & K.D. Hyde, *C. gloeosporioides*,

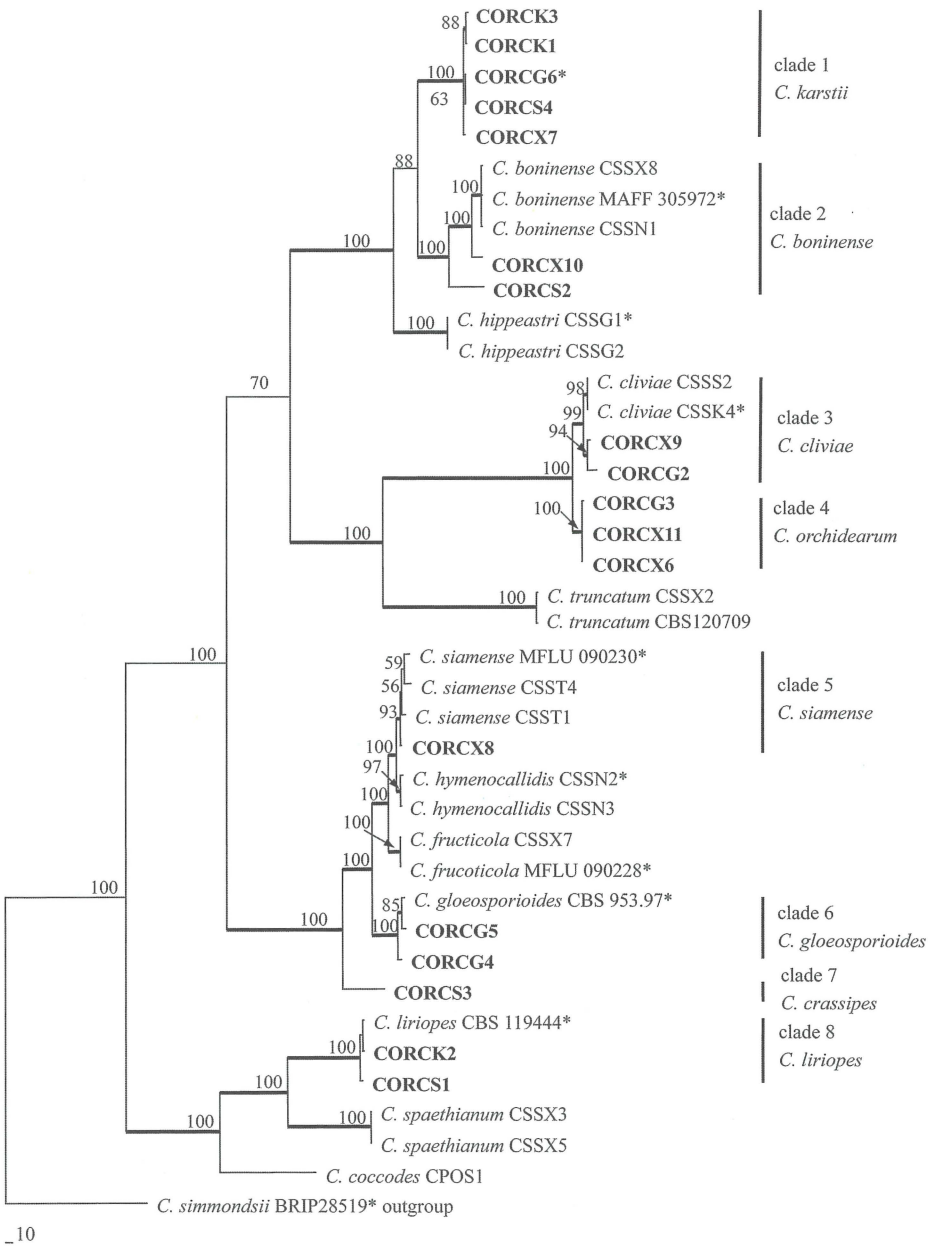

Fig. 1. Maximum parsimony phylograms inferred from combined partial ACT, Tub 2, CHS I, CAL, GPDH and ITS sequence data, showing phylogenetic relationships of *Colletotrichum* species isolated from *Orchidaceae* and selected sequences of *Colletotrichum* species (tree length = 2653 steps). Data analyzed with random addition sequence, unweighted parsimony and treating gaps as missing data. Values above the branches are parsimony bootstrap (equal or above 50%). The tree is rooted with *C. simmondsii* BRIP 28519). The thickened lines are Bayesian posterior probabilities (equal 100%). * ex-type or ex-epitype.

C. crassipes (Speg.) Arx and *C. liriopes* Damm, P. F. Cannon & Crous, respectively. *Colletotrichum orchidearum* and the new species are described and illustrated in this paper.

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

Material examined: CHINA, Yunnan Province, Jinghong, on leaf of *Oncidium flexuosum*, 3 August 2008, Y.L. Yang (GZAAS 080008, ex-living CORCX10); Guizhou Province, Shuicheng, endophyte from root of healthy *Pleione bulbocodioides*, 14 October 2004, Y.L. Yang (ex-living culture CORCS2).

Notes: This species was isolated as a pathogen and endophyte from orchids. Strain CORCX10 occurred on leaves of *Oncidium flexuosum* forming reddish brown, ellipsoid lesions with pale yellow conidial masses but rarely with setae. Strain CORCS2 was isolated as an endophyte from the roots of *Pleione bulbocodioides*. The conidia of strains CORCX10 and CORCS2 are much wider than those of ex-holotype and ex-paratype, but similar to those recorded on *Amaryllidaceae* (Yang *et al.*, 2009). In the individual and combined datasets, strains CORCX10, CORCS2 and MAFF 305972 (ex-holotype of *C. boninense*) nested in Clade 2 with high bootstrap support (Clade 2, Fig. 1).

Colletotrichum cliviae Y.L. Yang, Zuo Y. Liu, K.D. Hyde & L. Cai, *Fungal Diversity*. 39: 133 (2009) Fig. 2

Teleomorph: *Glomerella* sp.

Ascomata clustered, black, globose to subglobose, on surface or semi-immersed to completely immersed in PDA after about one month. **Peridium** of *textura angularis,* thick-walled. **Asci** 60-82 × 9.5-14 µm ($\bar{x}$ = 70.9 ± 8.3 × 11.7 ±1.1, n = 20), 8-spored, unitunicate, thin-walled, clavate (Figs 2D and G). **Ascospores** 12.5-21.5 × 5-7.5 µm ($\bar{x}$ = 16.7 ± 2.2 × 6 ± 0.7, n = 100), one-celled, hyaline, slightly curved to curved with obtuse to slightly rounded ends (Fig. 2H).

Material examined: CHINA, Yunnan Province, Jinghong, on leaf of *Arundina graminifolia*, 3 August 2008, Y.L. Yang (GZAAS 080011, ex-living CORCX9); Guizhou Province, Shuicheng, on leaf of *Cymbidium hookerianum*, 8 September 2008, Y.L. Yang (GZAAS 080013, ex-living culture CORCG2).

Notes: This species produced dark brown to black, ellipsoid lesions on leaves of *Cymbidium hookerianum* and *Arundina graminifolia* and lesions contained pale yellow conidial masses. This species formed both conidia and ascospores in culture (Fig. 2F and H). The conidia of the orchid isolates are shorter than those of the holotype (14-20.5 vs 19.5-24.5), but otherwise they are similar (Fig. 2B, C, E, F). The holotype of *C. cliviae* did not produce a sexual state under the same incubation conditions (Yang *et al.*, 2009), but the teleomorph was formed in the two isolates from *Orchidaceae* and is described here.

Colletotrichum crassipes (Speg.) Arx, *Verh. K. Akad. Wet.* , tweede sect. 51(3): 77 (1957)

Material examined: CHINA, Guizhou Province, Shuicheng, in root of healthy *Pleione bulbocodioides*, 14 October 2004, Y.L. Yang. (ex-living culture CORCS3).

Notes: This species was isolated from healthy roots of *Pleione bulbocodioides*. The taxon has been also reported as a pathogen on the orchids, *Bulbophylum cylindrum*, *Coelogyne cristata* and *Oncidium* spp. (Sutton 1980). The conidia and appressoria of strain CORCS3 are similar to *Colletotrichum crassipes* (Sutton 1992) (Table 2), and this strain, together with STE-U 4445 and

STE-U5302 (*C. crassipes*), clustered into a single clade with 100% bootstrap support based on the Tub2 squence (not shown).

Colletotrichum gloeosporioides (Penz.) Penz. & Sacc., *Atti Inst. Veneto Sci. lett., ed Arti*, Sér. 6 **2**: 670 (1884)

Material examined: CHINA, Guizhou Province, Luodian, on leaf of *Vanda* sp., 15 August 2009, Y.L. Yang (GZAAS 090004, ex-living culture CORCG4; GZAAS 090005, ex-living culture CORCG5).

Notes: This taxon causes ellipsoid, pale grey spots with black margins on leaves of *Vanda* sp. (Fig. 5A). Acervuli are subepidermal, and disrupt the outer epidermal cell wall of the host forming pink conidial masses without setae (Fig. 5B). *Colletotrichum gloeosporioides* has been also reported to cause anthracnose on several other orchid plants in several countries (Talubnak & Soytong, 2010; Farr & Rossman, 2011) although these identifications were based on morphology and must be treated with caution (Table 4).

Colletotrichum karstii Y.L. Yang, Zuo Y. Liu, K.D. Hyde & L. Cai, **sp. nov.** Fig. 3

MycoBank: MB 518609

Etymology: Karstii meaning karst, named after the geological feature of site where the species was collected.

Coloniae albae vel griseo. Conidia 12-19.5 × (5-) 6-8.5 µm, aseptata, hyalina, cylindrica, recta, ad basim truncata, ad apicem ***obtusa. Appressoris brunneis,*** *circularis* ***vel*** *clavatis, 6.5-14.5 × 4-8.5 µm. Asci 54-86 × 11-16.5 µm, unitunicati, tenuitunicati, clavati. Ascosporae 10.5-20 × 4.5-6.5 µm, unicellularae, hyalinae, leniter curvatae vel curvatae.*

Description: On host: **Acervuli** circular to elliptical, arranged irregularly, subepidermal, disrupting outer epidermal cell wall of host, setae sparse or absent, with yellow conidial masses (Fig. 3A). **Setae** 46-104 × 5-7 µm ($\bar{x}$ = 70.9 ± 19.2 × 6 ± 0.7, n = 7), dark brown, 4-8-septate, tapered towards the apex, base and apex paler (Fig. 3D). **Conidiophores** bell-shaped to cylindrical, hyaline, usually 1-celled, occasionally 2-3-celled, unbranched, 11-42 × 4-7.5 µm ($\bar{x}$ = 19.6 ± 7.3 × 5.7 ± 0.9, n = 30) (Fig. 3H). **Conidia** 12.5-19.5×6-8.5 µm ($\bar{x}$ = 15.9 ± 1.4 ×6.8 ± 0.5, n = 60), one-celled, smooth-walled, hyaline, straight, truncate at the base, obtuse at the apex.

Colonies on PDA: attaining 4.8-6.4 cm ($\bar{x}$ = 5.7 ± 0.5, n = 25) diam. in 6 days at 25°C, growth rate 8.3-11.4 mm per day ($\bar{x}$ = 9.8 ± 0.9, n = 25); flat with entire margin, at first white, becoming grey with age, usually with pink conidial masses, reverse yellow to dark brown (Fig. 3B, C), **Sclerotia** absent. **Setae** absent. **Conidia** in pink masses, 12-19.5 × (5-) 6-7.5 µm ($\bar{x}$ = 15.4 ± 1.3 × 6.5 ± 0.5, n = 215), one-celled, smooth-walled, hyaline, cylindrical, straight, truncate at the base, obtuse at the apex (Fig. 3I). **Appressoria** brown, circular to clavate, 6.5-14.5 × 4-8.5 µm ($\bar{x}$ = 9.3 ± 1.6 × 6.4 ± 0.9, n = 100) (Fig. 3E, F, G).

Teleomorph: *Glomerella* sp.

Ascomata dark brown, globose to subglobose, on surface or semi-immersed to completely immersed in PDA. **Peridium** of *textura angularis*, thick-walled. **Asci** 54-86 × 11-16.5 µm ($\bar{x}$ = 71.2 ± 9.2 ×12.7 ±1.4, n = 20), unitunicate, thin-walled, 8-spored, clavate (Figs. 3J and K). **Ascospores** 10.5-20 × 4.5-6.5 µm, ($\bar{x}$ =15.7 ± 1.7×5.3 ± 0.5, n = 60), one-celled, hyaline, slightly curved to curved with obtuse to slightly rounded ends (Fig. 3L).

Holotype: CHINA, Guizhou Province, Luodian, on leaf of *Vanda* sp., 15 August 2009, Y.L. Yang (GZAAS 090006; ex-holotype living culture CGMCC3.14194).

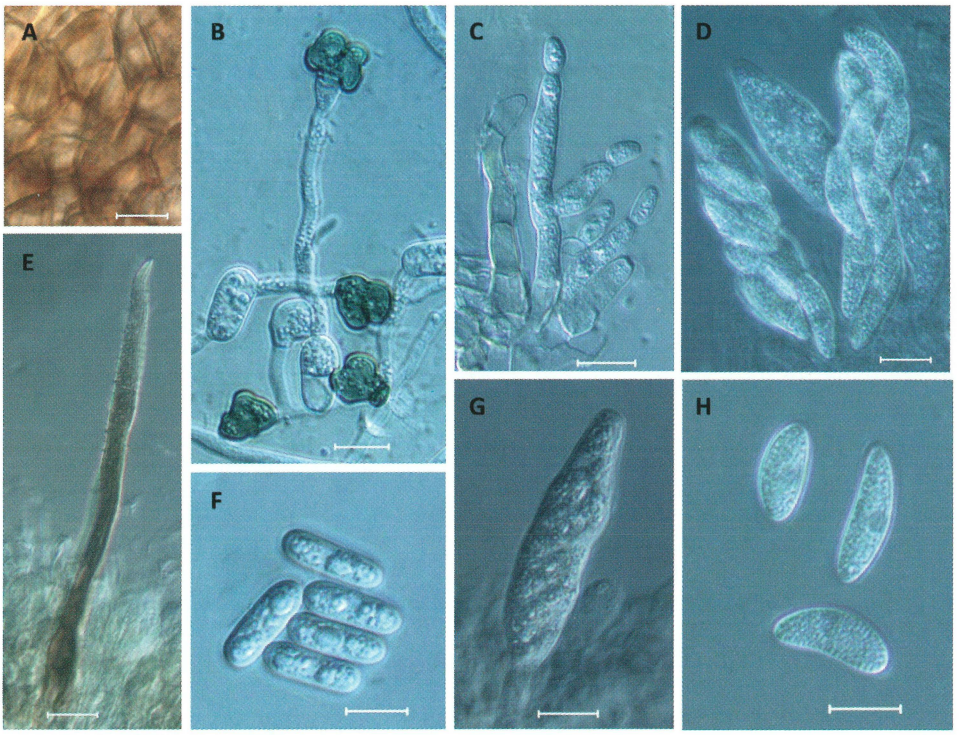

Fig. 2. *Colletotrichum cliviae* (from CORCX9). A, Squash of peridium; B, Conidial appressoria; C, Conidiophores; D, G, Asci; E, Seta; F, Conidia; H, Ascospores. (Bars: A-H = 10 μm).

Known host and distribution: Arundina graminifolia, *Calanthe argenteo-striata*, *Eria coronaria*, *Pleione bulbocodioides* and *Vanda* sp., Guizhou and Yunnan provinces, China.

Additional specimens examined: CHINA, Yunnan Province, Jinghong, on leaf of *Arundina graminifolia*, 1 August 2008, Y.L. Yang (GZAAS 080015, ex-living culture CORCX7); Yunnan Province, Kunming, on leaf of *Calanthe argenteo-striata* and *Eria Coronaria*, 10 August 2008, Y.L. Yang (GZAAS 080016, ex- living cultures CORCK1, GZAAS 080028, ex- living cultures CORCK3); Guizhou Province, Shuicheng, in root of *Pleione bulbocodioides*, 12 October 2004, Y.L. Yang (ex-living culture CORCS4).

Notes: The conidia and conidial appressoria of *C. karstii* overlap in size with those of *C. boninense*, while their shapes are clearly different (Table 2). The conidial appressoria of *C. karstii* are circular to clavate with an entire edge (Fig. 3E, F, G), but in *C. boninense* they are clavate to irregular with a crenate edge; the base of the conidium also differs (*C. boninense*: hilum-like vs *C. karstii*: truncate). In phylograms inferred from single and combined gene datasets, *C. karstii* nested into a monophyletic lineage with high bootstrap (except in the ITS phylogram with 66% bootstrap support, all others were 97% or above). *Colletotrichum karstii* was compared with other *Colletotrichum* species with cylindrical conidia formed on hosts in *Orchidaceae*. The conidia of *C. karstii* are clearly wider than those of *C. orchidearum* and *C. stanhopeae* (Allescher *et al.*,

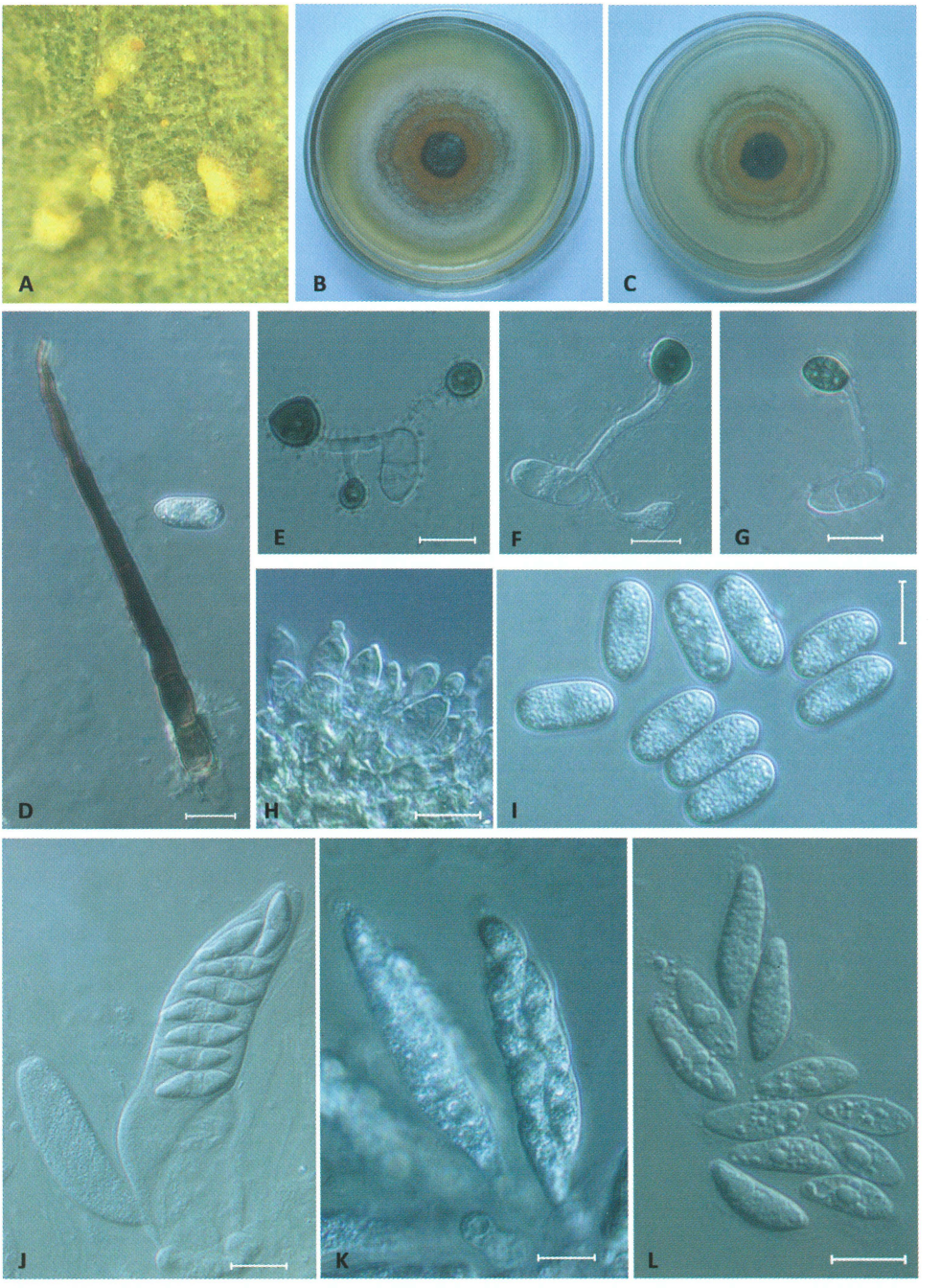

Fig. 3. *Colletotrichum karstii* (from holotype). A, Acervuli on leaf of *Vanda* sp.; B, C, Colony on PDA after 6 days, upper B and reverse C; D, Seta; E, F, G, Conidial appressoria; H, Conidiophores; I, Conidia; J, K, Asci; L, Ascospores. (Bars: D-L = 10 μm).

1902; Saccardo *et al.*, 1931). Although the conidia size of *C. karstii* overlaps with that of *C. vanillae*, their shapes are different. Conidia of *C. karstii* are truncate at the base and obtuse at the apex, while in *C. vanillae* the apex of the conidia are slightly wider than the base and rounded at ends (Saccardo & Saccardo, 1906).

Colletotrichum karstii was isolated from *Orchidaceae* as a pathogen (strains CORCK1, CORCK3, CORCX7, CORCG6) causing dark brown to black, ellipsoid lesions on leaves and was also isolated as an endophyte of roots (strain CORCS4). In addition, *Colletotrichum karstii* was isolated from grape (*Vitis vinifera*), chili (*Capsicum* spp.) and tomato (*Lycopersicon esculentum*) associated with anthracnose in China (unpublished data), this suggested this taxon have a wide range of hosts.

Colletotrichum liriopes Damm, P. F. Cannon & Crous, *Fungal Diversity* 39: 71 (2009)

Material examined: CHINA, Yunnan Province, Kunming, on leaf of *Eria Coronaria*, 10 August 2008, Y.L. Yang (GZAAS 080027, ex-living CORCK2); Guizhou Province, Shuicheng, endophyte of root of healthy *Pleione bulbocodioides*, 14 October 2004, Y.L. Yang (ex-living culture CORCS1).

Notes: Strain CORCK2 of this taxon is pathogenic on leaves of *Eria coronaria* causing ellipsoid, dark brown to black spots, containing pale yellow conidial masses, and with sparse setae. Strain CORCS1was isolated as an endophyte from the roots of *Pleione bulbocodioides*.

Colletotrichum orchidearum Allesch., *Rabenh. Krypt.-Fl.*, Edn 2 (Leipzig) **1**(7): 563 (1902) Fig. 4

On host: **Acervuli** circular to elliptical, dispersed or clustered, subepidermal, disrupting outer epidermal cell wall of host, with pale yellow conidial masses and dark brown setae (Fig. 4A). **Setae** 49.5-123.5 × 4.5-7 ($\bar{x}$ = 77.2 ± 21.5 × 5.5 ± 0.7, n = 20), dark brown to black, 2-7-septate (Fig. 4B, C), base inflated. **Conidiophores** hyaline, usually with a pale pigment at the base, 1-celled, occasionally 2-celled, 13-27.5 × 4-7 µm ($\bar{x}$ = 20.8 ± 4.8 × 5.8 ± 0.8, n = 20) (Figs. 4C and D). **Conidia** 13.5-22 × 4.5-6.5 µm ($\bar{x}$ = 17.6 ± 1.7 × 5.7 ± 0.6, n = 60), one-celled, smooth-walled, hyaline, rounded at the ends.

Colonies on PDA: attaining 5.7-7.2 cm ($\bar{x}$ = 6.8 ± 4.8, n = 15) diam. in 5 days at 25°C, growth rate 9.9-16.4 mm per day ($\bar{x}$ = 14.2 ± 2, n = 15); flat with entire margin; at first white, becoming grey with age, reverse pale yellow to dark brown with age (Fig. 4E, F), **Sclerotia** absent. **Setae** present. **Conidiophores** hyaline, 10-47 × 3.5-5.5 µm ($\bar{x}$ = 30.6 ± 10.8 × 4.5 ± 0.5, n = 10), branched at base or unbranched, 1-4-celled. **Conidia** in yellowish white masses, 15-23 × 5-6 µm ($\bar{x}$ = 18.3 ± 1.5 × 5.4 ± 0.3, n = 150), one-celled, smooth-walled, hyaline, cylindrical, straight, rounded at the ends (Fig. 4G, H). **Appressoria** dark brown, circular to irregular, margin entire to crenate, 7-12 × 6.5-11 µm ($\bar{x}$ = 10 ± 1.2 × 8.4 ±1.3, n = 60) (Fig. 4I, J).

Teleomorph: *Glomerella* sp.

Ascomata on PDA, dark brown, globose to subglobose. **Peridium** of *textura angularis*, thick-walled (Fig. 4K). **Asci** 54.5-100.5 × 9-13 µm ($\bar{x}$ = 77.9 ± 12.5×11.2 ± 1.3, n = 20), unitunicate, thin-walled, 6-8-spored, clavate (Figures 4L, M). **Ascospores** (15.5-) 18-26 × 4.5-7 µm, ($\bar{x}$ = 21 ± 2.4 × 5.8 ± 0.7, n = 60), one-celled, hyaline, slightly curved to curved with obtuse to slightly rounded ends (Fig. 4N).

Specimens examined: CHINA, Yunnan Province, Jinghong, on leaf of *Cattleya* sp., 1 August 2008, Y.L. Yang (GZAAS 080023; ex-holotype living culture CGMCC3.14195). Yunnan Province, Jinghong, on falling, dry flower of *Cattleya* sp., 1 August 2008, Y.L. Yang (GZAAS 080021, ex-living culture CORCX2); Yunnan Province, Jinghong, on dead scape of *Cattleya* sp., 1 August 2008, Y.L. Yang (GZAAS 080022, ex- living culture CORCX5); Yunnan Province, Jinghong, on leaf of *Oncidium flexuosum*, 1 August 2008, Y.L. Yang (GZAAS 080025, ex- living culture CORCX11); Guizhou Province, Guiyang, on leaf of *Cymbidium hookerianum*, 20 August 2008, Y.L. Yang (GZAAS 080029, ex- living culture CORCG3); GERMANY, on leaf of *Cymbidium pendulum* (M-0140830, holotype of *Colletotrichum orchidearum* f. *cymbidii*, 4, 1895, Allescher); on leaf of *Eria stellata* (M-0140831, holotype of *C. orchidearum* f. *eriae*), 4, 1895, Allescher; on leaf of *Physosiphon loddigesii* (M-0140832, holotype of *C. orchidearum* f. *physosiphonis*, 4, 1895, Allescher).

Notes: Allescher (1902) fist reported *Colletotrichum orchidearum* from Germany on leaves of *Orchidearum exoticarum*, *C. orchidearum* f. *cymbidii* on *Cymbidium pendulum*, and *C. orchidearum* f. *eriae* on *Eria stellata*. The holotype of *Colletotrichum orchidearum* appears to be lost. Fortunately, the three forms are held in M in Munich, Germany. The characters of conidia, setae and conidiophores in all three forms are similar (Table 4). This suggests that these form names are host related and may be a single species.

Colletotrichum orchidearum was isolated from dead scape, stem and fallen flower of *Cattleya* sp. and from leaves of *Cymbidium hookerianum* and *Oncidium flexuosum* with disease symptoms. This indicates that the taxon may also have a saprotrophic lifestyle. This species is in need of epitypification (Hyde *et al.*, 2009), but we are reluctant to use the specimen isolated here as it is not from Europe, but it can act as a voucher specimen until the species is epitypified with a specimen from Europe.

Colletotrichum siamense Prihastuti, L. Cai & K.D. Hyde, *Fungal Diversity* 39: 98 (2009).

Material examined: CHINA, Yunnan Province, Jinghong, on leaf of *Arundina graminifolia*, 2 August 2008, Y.L. Yang (GZAAS 080024, ex-living culture CORCX8).

Notes: This taxon causes ellipsoid to linear, pale brown to dark brown lesions on leaves of *Arundina graminifolia*.

Pathogenicity testing and host range

Pathogenicity testing was carried out on fruits of apple, chilli, tomato and leaves of *Clivia miniata* using spore suspensions of four strains (Table 3). The new species and *Colletotrichum orchidearum* do not appear to be host-specific to orchids based on the pathogenicity testing (Table 3). Each strain tested infected 2-3 fruits by wound/drop or non wound/drop inoculation forming water-sunken spots or mycelia on surface of fruits (Fig. 6). The symptoms on the same species of fruit infected by the different *Colletotrichum* species were similar. Both pathogenic (CORCG6) and endophytic (CORCS4) strains of *Colletotrichum karstii* infected *Malus pumila* fruits; strain CORCG6 caused anthracnose on fruits of *Capsicum annuum* by wound/drop inoculation, but the endophytic strain (CORCS4) did not. *Colletotrichum orchidearum* (strain CORCX6) was able to infect fruits of *Lycopersicon esculentum* and *M.*

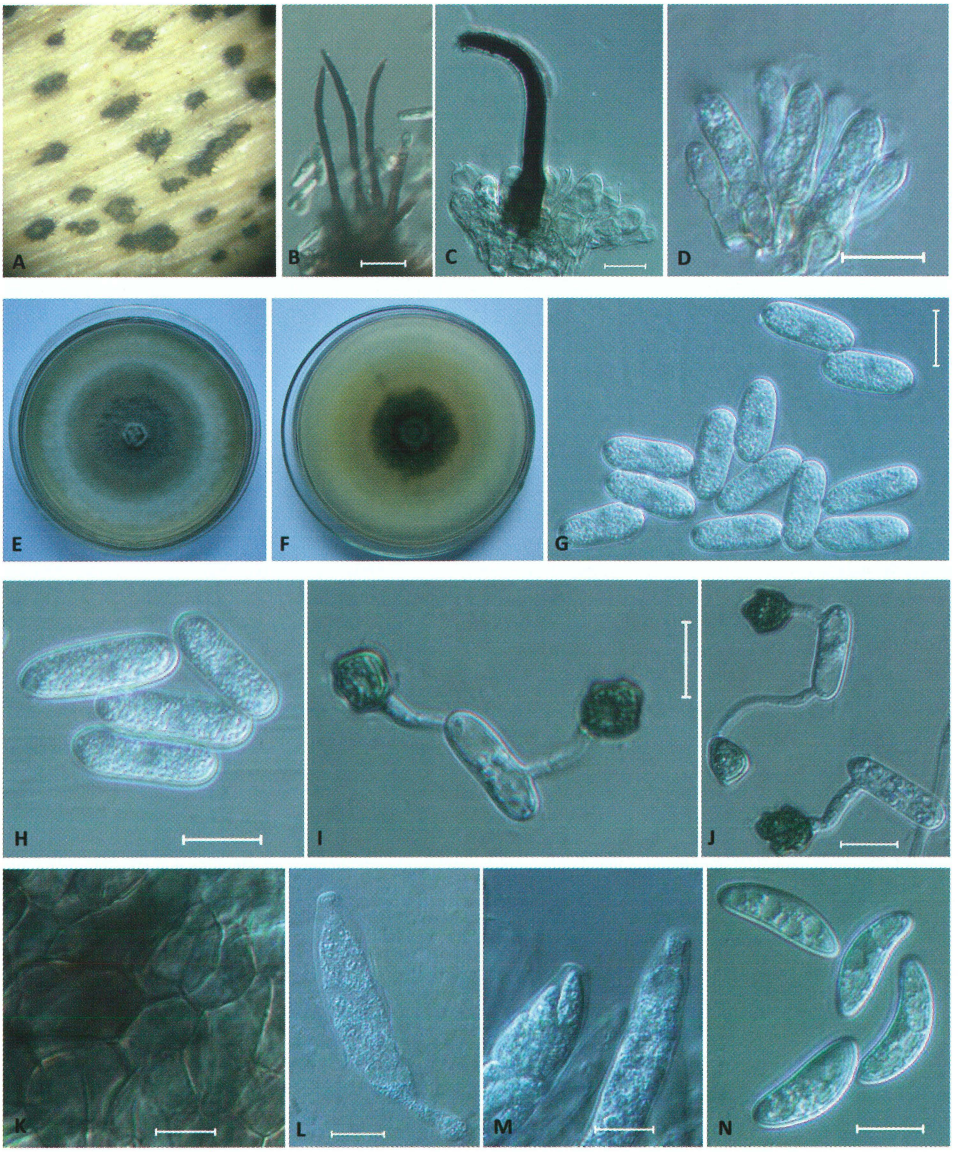

Fig. 4. *Colletotrichum orchidearum* (from CORCX6). A, Acervuli on leaf of *Cattleya* sp.; B, C. Setae; D, Conidiophores; E, F, Colony on PDA after 6 days, upper E and reverse F; G, H, Conidia; I, J, Conidial appressoria; K, Squash of peridium; L, M, Asci; N, Ascospores. (Bars: B = 20 μm, C, D, G-N = 10 μm).

pumila by wound/drop inoculation. *C. siamense* (strain CORCX8) was most aggressive, causing anthracnose on three kinds of fruits by wound/drop inoculation, and also infecting fruits of *L. esculentum* by non wound/drop inoculation.

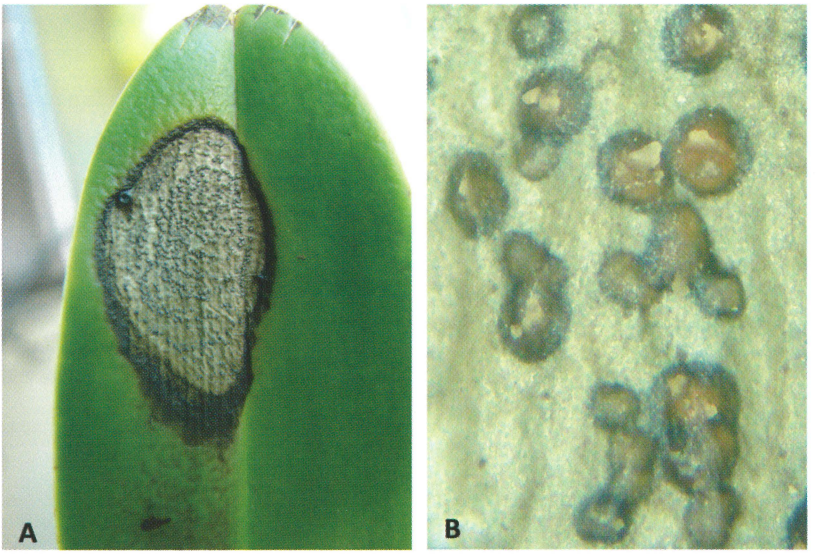

Fig. 5. Anthracnose symptoms and acervuli of *Colletotrichum gloeosporioides* on leaves of *Vanda* sp. A. Symptoms; B. Acervuli.

DISCUSSION

Colletotrichum species known from Orchidaceae

In this study we isolated eight species of *Colletotrichum*, of which *C. boninense, C. crassipes*, *C. gloeosporioides* and *C. orchidearum* have been previously recorded from orchids (Sutton, 1980; Li, 1999; Moriwaki *et al.*, 2003; Talubnak & Soytong, 2010). The other isolates belonged to *C. cliviae*, *C. liriopes*, *C. siamense* and a new species which is described above.

Thirteen forms/varieties of *Colletotrichum orchidearum* have previously been recorded on various orchids, namely, *C. orchidearum* f. *cochliodae* Verpl. & Claess., *C. orchidearum* f. *coelogynes* Marchal{?} & Verpl., *C. orchidearum* f. *cymbidii* Allesch., *C. orchidearum* f. *Eriae* Allesch., *C. orchidearum* f. *lycaste* Lebedeva., *C. orchidearum* f. *odontoglossi* Verpl. & Claess., *C. orchidearum* f. *orchidearum* Allesch., *C. orchidearum* f. *physosiphonis* Allesch., *C. orchidearum* f. *stanhopeae* Gutner., *C. orchidearum* var. *cochliodes* (Verpl. & Claess.) Trotter., *C. orchidearum* var. *odontoglossi* (Verpl. & Claess.) Trotter., *C. orchidearum* var. *orchidearum* Allesch. and *C. orchidearum* var. *stanhopeae* Gutner (Allescher *et al.*, 1902; Saccardo & Saccardo, 1906, Saccardo *et al.*, 1931; Trotter & Cash, 1972). These varieties or forms are based on host occurrence and would unlikely be supported as individual species, forms or varieties if molecular data could be applied. We therefore recommend the use of *Colletotrichum orchidearum* for all forms and varieties. *Colletotrichum crossandrae* Patel, Kamat & C.B. Pande, *C. lujae.* Verpl. & Clem., *C. stanhopeae* and *C. vanillae* Scalia have been also previously reported to infect *Orchidaceae* (Allescher *et al.*, 1902; Patel *et al.*, 1953; von Arx, 1957; Farr & Rossman, 2010) (Table 4), however, these names have not been used in the scientific literature over the past several decades (Sutton, 1980, 1992; Hyde *et al.*, 2009). Furthermore, ex-type cultures of these species are not

Table 4. Synopsis of characters of *Colletotrichum* species known from *Orchidaceae*

Species	*Conidial shape and size (μm)*	*Conidiophores (μm)*	*Setae (μm)*	*Host*	*Reference*
C. boninense	On *Oncidium flexuosum*, cylindrical, straight, with a hilum-like base, 12-17 × 5-7.5, $\bar{x}$ = 14.5 ± 1.7 ×6.8 ± 0.7, n = 30	Cylindrical, hyaline, pale brown at base,1-2 cells, 12- 26.5 × 4.5-7.5(- 9), $\bar{x}$ = 21.3 ± 3 ×6.4 ± 1.1, n = 10	No setae present on *Oncidium flexuosum*	*Cattleya* sp., *Cymbidium* sp. *Oncidium flexuosum, Pleione bulbocodioides*, other hosts belonging to various families	This paper; Farr & Rossman, 2011
C. cliviae	On *Cymbidium hookerianum*, cylindrical, straight, obtuse at the ends, 16-22.5 × 4-6.5, $\bar{x}$ = 19.6 ± 1.5 × 5.4 ± 0.4, n = 30	Cylindrical, hyaline, branched, 1-3 cells, 15.5-52 × 3.5-6.5, $\bar{x}$= 27.1 ± 13 × 5 ± 0.8, n = 10	Brown, 2-5 septa, slightly inflated at base, 97.5-125.5 × 5-8, $\bar{x}$ = 110.7 ± 9.7 × 6.3 ± 0.8, n = 10	Arundina graminifolia, Clivia miniata, Cymbidium hookerianum	This paper; Yang *et al.*, 2009
C. crassipes	On culture, cylindrical, straight, obtuse at apex, truncate at base, 14-28 × 5-7	No data	No data	*Aerangis koschyana, Bulbophylum cylindrum*, *Coelogyne cristata, Cymbidium* spp.,*Oncidium* spp., Orchids (*various*) *Vanda* sp., many other hosts belonging to various families	This paper; Sutton, 1992; Farr & Rossman, 2011
*C. crossandrae**	On culture, varying in shape, cylindrical to elongated, oval, sometimes slightly curved, 18-29 × 1-5 μm	Hyaline, cylindrical or slightly swollen, usually septated at base, round at apex, 12-28 × 3-6 μm	Dark greenish-brown, 3-5 septa, 97-147 × 3-5 μm	*Crossandra infundibuliformis*, *Vanda* sp.	Patel *et al.*, 1952
C. gloeosporioides	On *Vanda* sp., cylindrical, bluntly rounded ends and slightly flattened base, 12-20 × 4-6, $\bar{x}$ = 16 ± 1.9 × 5.1 ± 0.6, n = 30	Hyaline, cylindrical, branched, 1-2 cells, 14.5-29 × 3-5, $\bar{x}$ = 21.8 ± 5 × 3.9 ± 0.6, n = 10	No setae present on *Vanda* sp.	*Cattleya* sp., *Cymbidium* sp., *Dendrobium* sp., *Oncidium* sp., *Phalaenopsis* sp., *Stanhopea* sp., *Vanda* sp., *Vanilla planifolia*, other hosts belonging to various families	This paper; Cannon *et al.*, 2008; Talubnak & Soytong 2010; Farr & Rossman, 2011
C. karstii	On *Vanda* sp., cylindrical, straight, obtuse at apex, base truncate, 12.5- 19.5 × 6-8.5, $\bar{x}$= 15.9 ± 1.4 ×6.8 ± 0.5, n = 60	Bell-shaped to cylindrical, Hyaline, 1-3 cells, unbranched, 11-42 × 4-7.5, $\bar{x}$ = 19.6 ± 7.3 × 5.7 ± 0.9, n = 30	Sparse, dark brown, 4-8 septa, base and apex paler, 46-104 × 5-7, $\bar{x}$ = 70.9 ± 19.2 × 6 ± 0.7, n = 7	*Arundina graminifolia*, *Calanthe argenteo-striata*, *Eria coronaria, Pleione bulbocodioides, Vanda* sp., and other hosts belonging to various families	This paper
C. liriopes	On *Eria coronaria*, falcate, fusiform,19-25.5 × 3-5, $\bar{x}$ = 22.3 ± 1.5 × 4.1 ± 0.4, n = 30	Cylindrical, hyaline, 1-4 cells, branched, 14.5-28(-38) × 3.5- 5.5, $\bar{x}$= 21.8 ± 8.9 × 4.5 ± 0.7, n = 10	Brown, base and apex paler, 3-6 septa, often slightly inflated at base, 40.5-118 × 4-6.5, $\bar{x}$= 77.6 ± 24.5 × 5.2 ± 0.8, n = 10	*Eria coronaria*, *Lirope muscari, Pleione bulbocodioides*	This paper; Damm *et al.*, 2009

Table 4. Synopsis of characters of *Colletotrichum* species known from *Orchidaceae (continued)*

Species	*Conidial shape and size (μm)*	*Conidiophores (μm)*	*Setae (μm)*	*Host*	*Reference*
*C. lujae**	No data	No data	No data	*Lycaste* sp.	Ciferri, 1961; Farr & Rossman, 2011
C. orchidearum *	On *Cattleya* sp., cylindrical, straight, rounded at ends. 13.5-22 × 4.5-6.5, $\bar{x}$ = 17.6 ± 1.7 × 5.7 ± 0.6, n = 60	Hyaline, usually pale brown at base, 1-2 cells, 13- 27.5 × 4-7, $\bar{x}$ = 20.8 ± 4.8 × 5.8 ± 0.8, n = 20	Dark brown to black, usually inflated at base, 2-7 septa, 49.5-123.5 × 4.5-7, $\bar{x}$= 77.2 ± 21.5 × 5.5 ± 0.7, n = 20	*Cattleya* sp., *Cymbidium hookerianum*, *Cymbidium* sp., *Oncidium flexuosum*, *Bulbophyllum* sp., *Dendrochilum* sp., *Rhynchostylis* sp., *Orchidearum exoticarum*	This paper; Saccardo & Saccardo, 1906; Farr & Rossman, 2011
C. orchidearum f. *cymbidii* *	On *Cymbidium pendulum*, cylindrical, rounded at ends, 12-20 × 4-6.5, $\bar{x}$= 15.2 ± 2 ×5.3 ± 0.8, n = 30	Cylindrical, pale brown, 1-3 cells, branched, 14-25 × 5-6.5, $\bar{x}$ = 20.3 ± 4.2 × 5.5 ± 0.6, n = 10	Brown, inflated at base, 2-5 septa, 62-120 × 5.5-7.5, $\bar{x}$ = 87 ± 22.6 × 6.7 ± 0.5, n = 10	Cymbidium pendulum	This paper
C. orchidearum f. *eriae* *	On *Eria stellata*, cylindrical, rounded at ends, 13-18 × 4-6, $\bar{x}$= 16.5 ± 1.4 ×5.2 ± 0.5, n = 30	Cylindrical, pale brown, branched, 1-2 cells, 11-18 × 5-6, $\bar{x}$ = 14.8 ± 2.5 × 5.2 ± 0.4, n = 10	Brown, base inflated and paler, 60-130 × 5-6, $\bar{x}$ = 92.5 ± 25.4 × 5.7 ± 0.5, n = 10	*Eria stellata* (as syn. *Eria javanica*)	This paper
C. orchidearum f. *physosisphonis* *	On *Physosiphon loddigesii*, cylindrical, rounded at ends, 12-20 ×3.5-6, $\bar{x}$= 15.5 ± 2.3 ×5.2 ± 0.7, n = 30	Cylindrical, pale brown, 1-4 cells, branched, 13-28 × 4-6.5, $\bar{x}$ = 14 ± 2.2 × 5.2 ± 0.7, n = 10	Brown, base inflated and paler, 2-5 septa, 53-98 × 4.5-6, $\bar{x}$ = 86.5 ± 16.4 × 5.3 ± 0.7, n = 10	*Physosiphon loddigesii* (as syn. *Pleurothallis tubata*)	This paper
C. orchidearum f. *stanhopeae* *	On *Stanhopeae oculatae*, cylindrical, straight, rounded at ends, 15-18 × 4 μm	No data	Pale olive, 75-105 × 4.5 μm	Stanhopeae oculatae	Trotter & Cash, 1972
C. orchidearum var. *cochliodae**	On *Cochlioda* sp., oblong, hyaline, rounded at ends, 10.5-17.5 × 3.5-5 μm	Hyaline, 17.5-25 × 3.5-5 μm	Sparse, olive, obtuse at apex, 52.5-102.4 × 3.5-5 μm	*Cochiodae* sp.	Trotter & Cash, 1972
C. orchidearum var. *odontoglossi**	On *Odontoglossum* sp. cylindrical, straight to curved, obtuse at ends, 14-17.5 × 3.5-4.5 μm	Hyaline, 15-17.5 × 3.5-4.5 μm	Yellowish brown, 1-2 septa, 35-59.5 × 2-3 μm	*Odontoglossum* sp.	Trotter & Cash, 1972
C. siamense	On *Arundina graminifolia*, cylindrical to fusiform, rounded at ends, 12-14.5 × 4.5-6, $\bar{x}$ = 13.1 ± 0.7 × 5.5 ± 0.4, n = 30	Cylindrical, hyaline, 1-2 cells, branched, 11- 18 × 4-6, $\bar{x}$= 13.9 ± 2.2 × 4.7 ± 0.7, n = 10	Brown, 2-4 septa, inflated at base, 40-64 × 4-6, $\bar{x}$= 48.9.2 ± 8.1 × 5.1 ± 0.6, n = 10	*Arundina graminifolia*, *Coffea arabica*, *Hymenocallis* sp	This paper; Prihastuti *et al.*, 2009; Yang *et al.*, 2009
*C. vanillae**	On *Vanilla odorata*, oblong to cylindrical, apex slightly wider than base, rounded at ends, 18-21 × 5.6-7	Cylindrical, pale olive , 2 cells, 24-34 × 6.5-7	Brown, base slightly inflated and pale brown, 50-100 × 5-6	*Vanilla odorata*	Saccardo & Saccardo, 1906

* known from only *Orchidaceae*

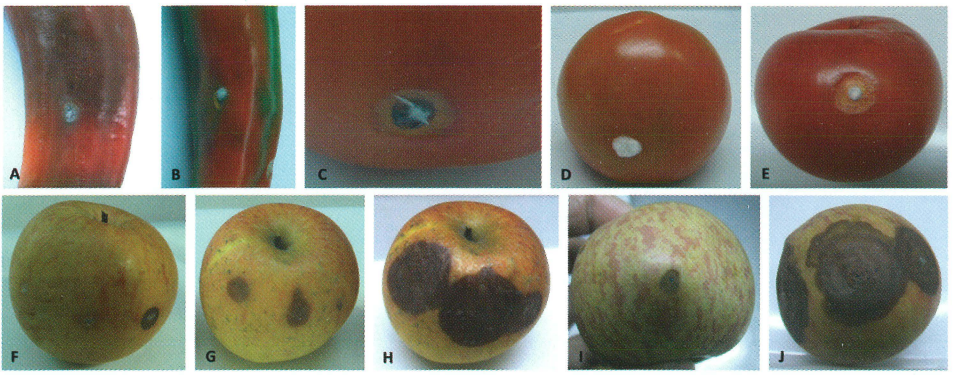

Fig. 6. Symptoms on fruit caused by *Colletotrichum* isolates from *Orchidaceae* through wound/drop inoculation. A, B, *Capsicum annuum* [A, Infected by *Colletotrichum karstii* (CORCG6) and B, *C. siamense* (CORCX8)]; C, D, E, *Lycopersicon esculentum* [C, infected by *C. orchidearum* (CORCX6), D, *C. karstii* (CORCG6) and E, *C. karstii* (CORCS4)]; F-J, *Malus pumila* [F, Infected by *Colletotrichum karstii* (CORCG6) with three inoculation points, G, H, Infected by *C. orchidearum* (CORCX6) with three inoculation points; I, Infected by *C. karstii* (strain CORCS4); J, Infected by *C. siamense* (CORCX8) with three inoculation points)]. A-G, I, 7 days after inoculation; H, J, after 14 days after inoculation.

available and thus they cannot be studied using modern concepts and these records should be treated as ambiguous. *Colletotrichum lujae* is very confusing because there is no record of this species in Index Fungorum or other references (Ciferri, 1961). The only information on this species is that it was isolated from *Lycaste* sp. in the Dominican Republic (Farr & Rossman, 2010); the record should be treated as ambiguous.

Host range and *Colletotrichum* species recognition

The understanding of host-specificity in *Colletotrichum* species may be better addressed using data on pathogenicity rather than occurrence on a host (Damm *et al.*, 2009). Some *Colletotrichum* species appear to be host-specific, e.g. *C. graminicola* (Ces.) G.W. Wilson may be specific on *Zea mays* (Politis, 1975; Sutton, 1966; Hyde *et al.*, 2009). However, most species do not appear to be host-specific and infect different hosts in different plant genera or families. For example, *C. cliviae* was described from *Amarillydaceae*, but pathogenicity testing has indicated that this species can also infect *Bletilla striata* (*Orchidaceae*) by wound/drop inoculation (Yang *et al.*, 2009). In the present study, two strains of *C. cliviae* were isolated from different genera of *Orchidaceae*, however *C. cliviae* was not found on *Bletilla striata* leaves with anthracnose symptoms. *Colletotrichum boninense*, *C. coccodes* and *C. siamense* can also infect several hosts (Lees & Hilton 2003; Moriwaki *et al.*, 2003; Lu *et al.*, 2004; Nitzan *et al.*, 2006; Ben-Daniel *et al.*, 2009; Prihastuti *et al.*, 2009; Yang *et al.*, 2009). *Colletotrichum* species can not be identified based on host occurrence, and molecular data is needed to reliably identify a species, however little is understood concerning the interaction between species and hosts and this requires extensive research, as also suggested in a recent study on *Pestalotiopsis* species on orchids (Tempesta et al., 2011).

Lifestyles of *Colletotrichum* species

Colletotrichum species such as *C. acutatum* J. H. Simmonds, *C. asianum* Prihastuti, L. Cai & K.D. Hyde, *C. boninense*, *C. fructicola* Prihastuti, L. Cai & K.D. Hyde, *C. gloeosporioides*, *C. siamense* and *C. yunnanense* Xiao Ying Liu & W.P. Wu, have been reported as endophytes from a wide range of plant species (Lu *et al.*, 2004; Promputtha *et al.*, 2007; Hyde *et al.*, 2009; Prihastuti *et al.*, 2009; Errasti *et al.*, 2010; Sanchez-M *et al.*, 2010; González & Tello, 2011). Of these, *C. asianum*, *C. fructicola* and *C. siamense* have been also isolated as epiphytes. This suggests that *Colletotrichum* species are able to survive within the healthy plant tissues as endophytes and are opportunistic pathogens (Prihastuti *et al.*, 2009). When humidity and temperature are optimal for disease development, these endophytic species may cause anthracnose of their hosts. The term 'endophyte' is used for those fungi that grow inside living plant tissues without causing apparent disease symptoms (Hyde Soytong 2008)—they may be latent pathogens (Brown *et al.*, 1998; Jumpponen, 2001; Photita *et al.*, 2004) and/or saprobes (Gardes, 2002; Promputtha *et al.*, 2007). The endophytic and pathogenic strains CORCS4 and CORCG6 isolated in this study infected *Lycopersicon esculentum* and *Malus pumila* fruits following wound/drop inoculation. This is slightly different from species isolated from coffee berries in Thailand (Prihastuti *et al.*, 2009), where *C. asianum, C. fructicola* and *C. siamense* were isolated as pathogens, endophytes, as well as epiphytic fungi, and all strains tested were able to infect non-wounded coffee berries in pathogenicity tests. In contrast, a putative strain of *C. gloeosporioides* isolated as an endophyte from healthy *Musa acuminata* did not cause any disease of banana leaves following pathogenicity testing (Photita *et al.*, 2004). Hence, the lifestyle of *Colletotrichum* species varies and is an area for further investigation.

Acknowledgements. The authors are indebted to Dagmar Triebel, Botanische Staatssammlung München, Germany, for supplying the holotype of *Colletotrichum orchidearum* f. *cymbidii*, *C. orchidearum* f. *eriae* and *C. orchidearum* f. *physosiphonis*, Shaun Pennycook, Landcare Research, Auckland, New Zealand for commenting on the forms/varieties of *Colletotrichum orchidearum*. The project was supported by the National Natural Science Foundation of China (No. 31070025) and Guizhou Science and Technology Department [No. (2010) 4002-1]. K.D. Hyde thanks the Global Research Network for Fungal Biology and King Saud University for support.

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