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Colletotrichum species on *Citrus* leaves in Guizhou and Yunnan provinces, China

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Abstract – Thirty-eight strains of *Colletotrichum* were isolated from diseased *Citrus* leaves in Guizhou and Yunnan provinces in China. Based on morphological characters and multilocus sequence data, the strains were identified as *C. boninense* (1 strain), *C. brevispora* (1), *C. fructicola* (2), *C. gloeosporioides* (29), *C. karstii* (2), *C. simmondsii* (1) and *Colletotrichum murrayae*; the latter represents a new species which is described in this paper. *Colletotrichum gloeosporioides* was originally isolated from *Citrus sinensis* in southern Italy and was the most common species associated with diseased leaves of *Citrus* in China.

Anthracnose / morphology / multilocus phylogeny / plant disease

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INTRODUCTION

Citrus species originated in tropical and subtropical Southeast Asia and are probably the most widely planted fruit types worldwide (Katz & Weaver, 2003). In Guizhou and Yunnan provinces of China, citrus is one of the most widely grown subtropical fruit plants. According to the Department of Agriculture of Guizhou Province, in 2008, citrus plantations occupied up to 38,000 hm^2 with an output of 191,050,000 kg (Anon. 2009). However, many diseases attack citrus of which anthracnose, caused by *Colletotrichum gloeosporioides*, is a common disease of citrus in Guizhou Province, characteristically causing spots on leaves or rots on fruits (Hou *et al*., 2010).

Colletotrichum gloeosporioides (Penz.) Penz. & Sacc. was originally collected from *Citrus sinensis* in southern Italy (Cannon *et al.*, 2008) and since then has been recorded on a wide range of hosts including papaya (*Carica papaya*) (Kadir *et al.*, 2008; Rahman, 2007), mango (*Mangifera indica*) (Abd-Alla & Wafaa, 2010; Sangeetha & Rawal, 2008;), strawberry (*Fragaria* × *ananassa*) (Gullino *et al.*, 1985; Mackenzie *et al.*, 2006, 2007), king protea (*Protea cynaroides*) (Lubbe *et al.*, 2004), citrus (*Citrus sinensis*) (Benyahia *et al.*, 2003; Timmer *et al.*, 1994) and dragon fruit (*Hylocereus* spp.) (Masanto *et al.*, 2009). It has also been isolated from symptomless plant parts as endophytic strains (Cannon & Simmons, 2002; Errasti *et al.*, 2010; Gangadevi *et al.*, 2008; Rivera-Orduña *et al.*, 2011).

Following epitypification of *Colletotrichum gloeosporioides* (Cannon *et al.*, 2008), Phoulivong *et al.* (2010a) and Wikee *et al.* (2011) were able to show that *C. gloeosporioides* was actually not a common pathogen in the tropics and it was not the cause of anthracnoses of chilli (*Capsicum annuum*), guava (*Psidium guajava*), jujube (*Ziziphus jujuba*), mango (*Mangifera indica*), rose apple (*Syzygium jambos*), longan (*Dimocarpus longana*), banana (*Musa* sp.) or jasmine leaves in Laos, Thailand and Vietnam.

The present study aimed to establish the species of *Colletotrichum* associated with anthracnose of *Citrus* leaves in Guizhou and Yunnan provinces of China. The *Colletotrichum* strains were collected from diseased leaves of *Citrus*. The strains were characterized morphologically and six gene regions, *i.e.* partial actin (ACT), β-tubulin-2 (TUB2), glyceraldehyde-3-phosphate dehydrogenase (GPDH) genes, calmodulin (CAL), glutamine synthetase (GS) and the complete rDNA-ITS (ITS) were sequenced to identify the strains isolated from diseased leaves.

MATERIALS AND METHODS

Isolation of *Colletotrichum:* *Colletotrichum* samples were collected from anthracnose lesions on leaves of *Citrus* and related plants in Guizhou and Yunnan provinces of China during June and September 2009 (Table 1). Single-spore isolations from infected leaves with sporulation were carried out using the procedure described by Choi *et al*. (1999) and Chomnunti *et al*. (2011).

Morphological and cultural characterization: Mycelial discs (5 mm diam.) were taken from actively sporulating areas near the growing edge of 5-day-old cultures and transferred onto potato dextrose agar (PDA) in 9 cm diameter Petri dishes and incubated in the dark at 25°C. Four replicate cultures of each isolate were

Table 1. *Colletotrichum* species isolated from leaf lesions on *Citrus* and related plants in Guizhou and Yunnan provinces, China

Species	*Specimen no.**	*Host*	*Location*
C. boninense	GZAAS5.09505	*Citrus medica* L.	Puer City, Yunnan
C. brevispora	GZAAS5.09545	*C. medica*	Puer City, Yunnan
C. fructicola	GZAAS5.09507	*Citrus bergamia* Risso & Poit	Puer City, Yunnan
	GZAAS5.09521	*Citrus grandis* L.	Yuping County, Guizhou
C. gloeosporioides	GZAAS5.09503	*Citrus limon* (L.) Burm. f.	Jinghong City, Yunnan
	GZAAS5.09504	*C. grandis*	Jinghong City, Yunnan
	GZAAS5.09508	*Fortunella* sp.	Jinghong City, Yunnan
	GZAAS5.09511	*Citrus reticulata* Blanco	Puer City, Yunnan
	GZAAS5.09512	*C. grandis*	Puer City, Yunnan
	GZAAS5.09509	*C. reticulata*	Kunming City, Yunnan
	GZAAS5.09513	*C. reticulata*	Guiyang City, Guizhou
	GZAAS5.09514	*C. reticulata* cv. bendizao	Guiyang City, Guizhou
	GZAAS5.09515	*Fortunella* sp.	Guiyang City, Guizhou
	GZAAS5.09516	*C. reticulata*	Huishui County, Guizhou
	GZAAS5.09517	*C. reticulata*	Bijie City, Guizhou
	GZAAS5.09518	*C. reticulata*	Yinjiang County, Guizhou
	GZAAS5.09524	*C. reticulata*	Duyun City, Guizhou
	GZAAS5.09525	*C. grandis*	Duyun City, Guizhou
	GZAAS5.09527	*C. reticulata*	Libo County, Guizhou
	GZAAS5.09528	*C. grandis*	Libo County, Guizhou
	GZAAS5.09529	*C. reticulata*	Luodian County, Guizhou
	GZAAS5.09530	*C. sinensis* (L.) Osb.	Luodian County, Guizhou
	GZAAS5.09519	C. grandis	Tongren City, Guizhou
	GZAAS5.09520	*C. sinensis*	Yuping County, Guizhou
	GZAAS5.09522	*C. grandis* cv. huyou	Yuping County, Guizhou
	GZAAS5.09523	*C. grandis*	Cengong County, Guizhou
	GZAAS5.09526	*C. reticulata*	Danzai County, Guizhou
	GZAAS5.09531	*C. sinensis*	Anlong County, Guizhou
	GZAAS5.09532	*C. grandis*	Xingyi City, Guizhou
	GZAAS5.09533	*C. grandis*	Zunyi City, Guizhou
	GZAAS5.09534	*C. reticulata*	Zunyi City, Guizhou
	GZAAS5.09536	*Fortunella* sp.	Zhijin County, Guizhou
	GZAAS5.09537	*C. reticulata*	Zhijin County, Guizhou
C. karstii	GZAAS5.09501	*C. sinensis*	Dali City, Yunnan
	GZAAS5.09502	*C. reticulata*	Dali City, Yunnan
C. simmondsii	GZAAS5.09510	*Murraya* sp.	Puer City, Yunnan
Colletotrichum murrayae	GZAAS5.09506	*Murraya* sp.	Jinghong City, Yunnan
	GZAAS5.09538	*Murraya* sp.	Jinghong City, Yunnan

*: GZAAS: Guizhou Academy of Agricultural Sciences, Guizhou Province, China.

investigated. Colony diameter was measured daily for 8 days and growth rate (mm per day) was calculated. Colony colour, the conidial masses and zonation were recorded. Conidial appressoria were induced by inoculating conidia into two drops of distilled water on a glass slide. The slides were then placed inside Petri dishes containing cotton moistened with distilled sterile water, and incubated at 25°C in

darkness. After 24-48 hours, conidial appressoria, formed from germ tubes, were measured and photographed. Morphological data, such as conidia and appressoria size, were analyzed using analysis of variance ($P < 0.05$) with equal variance assumed (Duncan's test).

A synopsis of morphological characters of *Colletotrichum* species on *Citrus* and other *Rutaceae* plants are listed (Table 3).

DNA extraction and sequencing: Isolates were grown on PDA for 8 days at 25°C and mycelium was scraped from the surface. The method of DNA extraction followed a modified protocol of Chen *et al.* (2007) and Than *et al.* (2007). All strains in this study were amplified with the complete rDNA-ITS (ITS). Twelve *Colletotrichum* strains were selected and partial actin (ACT), β-tubulin (TUB2), calmodulin (CAL), glutamine synthetase (GS), glyceraldehyde-3-phosphate dehydrogenase (GPDH) genes and ITS regions were amplified by PCR reaction. The primers and PCR reaction systems were performed according to Prihastuti *et al.* (2009). The reactions were performed with a thermal cycler (PTC-200 DNA Engine, Bio-Rad Laboratories, USA). The PCR products were examined by electrophoresis stained with ethidium bromide on 1.2% agarose electrophoresis gels and purified according to the manufacturer's instructions of a TIANgel Midi Purification Kit (TIANGEN China). DNA sequencing was performed by Sino GenoMax, Beijing, China.

Molecular phylogenetic analysis: Phylogenetic analysis was performed using six gene regions. The GenBank accession numbers of all sequences are listed in Table 2. The methods used for single and multiple phylogenetic analysis were similar to those used by Cai *et al.* (2006). All sequence alignments have been deposited in TreeBASE (submission ID 12190).

RESULTS

Phylogenetic analysis

Thirty-eight *Colletotrichum* strains were isolated from anthracnose lesions from leaves of six species of *Rutaceae* in Guizhou and Yunnan provinces in China. The combined datasets of partial ACT, TUB2, CAL, GPDH, GS and the complete rDNA-ITS region were analysed and the phylogenetic relationships of the 38 strains were inferred using Maximum Parsimony (MP) and Bayesian analysis methods. Sequence data from six gene fragments comprised 3678 characters after alignment, of which 1472 characters were parsimony informative. One of 12 equally parsimonious trees (TL = 3448, CI = 0.744, RI = 0.868, RC = 0.646, HI = 0.256) is shown in Fig. 1. The phylogram showed that the isolates used in the study clustered into seven different lineages, which includes six species and one new species. These comprised *Colletotrichum boninense* (1 isolate), *C. brevispora* (1), *C. fructicola* (2), *C. gloeosporioides* (29), *C. karstii* (2), *C. simmondsii* (1), and *Colletotrichum murrayae* sp. nov.

Morphological analysis: The isolated species are presented in alphabetical order.

Table 2. Sequences obtained from GenBank used in the analysis

Species	*Strain*	*Reference*	*GenBank Accession Number*					
			ACT	*TUB-2*	*CAL*	*GPDH*	*GS*	*ITS*
C. asianum	BPD-I4 holotype	Prihastuti *et al*., 2009	FJ 907424	FJ 907439	FJ 917506	FJ 972595	FJ 972576	FJ 972612
C. asianum	BML-I14	Prihastuti *et al*., 2009	FJ 907421	FJ 907436	FJ 917503	FJ 972598	FJ 972573	FJ 972615
C. brevispora	Lca845	Cai *et al*., Pers comm.	–	–	–	–	–	–
C. brevispora	**GZAAS5.09545**	**This paper**	**JQ247647**	**JQ247635**	**JQ247589**	**JQ247599**	**JQ247611**	**JQ247623**
C.boninense	CORCX10	Yang *et al*., 2009	HM581999	HM585420	HM582006	HM585384	–	HM585401
C.boninense	MAFF305972 = LCA884	Yang *et al*., 2009	HM582001	HM585421	HM582004	HM585386	–	HM585399
C.boninense	**GZAAS5.09505**	**This paper**	**JQ247646**	**JQ247634**	–	**JQ247598**	**JQ247610**	**JQ247622**
C. cordylinicola	BCC 38872 holotype	Phoulivong *et al*., 2010 b	HM470234	HM470249	HM470237	HM470240	HM470243	HM470246
C. cordylinicola	BCC 38864	Phoulivong *et al*., 2010 b	HM470233	HM470248	HM470236	HM470239	HM470242	HM470245
C. fragariae	LC0220 = ICMP17927	Cai *et al*., Pers comm.	–	–	–	–	–	–
C. fructicola	BPD-I12	Prihastuti *et al*., 2009	FJ 907425	FJ 907440	FJ 917507	FJ 972594	FJ 972577	FJ 972611
C. fructicola	BPD-I18	Prihastuti *et al*., 2009	FJ 907427	FJ 907442	FJ 917509	FJ 972592	FJ 972579	FJ 972602
C. fructicola	BPD-I16 holotype	Prihastuti *et al*., 2009	FJ 907426	FJ 907441	FJ 917508	FJ 972593	FJ 972578	FJ 972603
C. fructicola	**GZAAS5.09507**	**This paper**	**JQ247649**	**JQ247637**	**JQ247590**	**JQ247601**	**JQ247612**	**JQ247625**
C. fructicola	**GZAAS5.09521**	**This paper**	**JQ247648**	**JQ247636**	**JQ247591**	**JQ247600**	**JQ247613**	**JQ247624**
C. *gloeosporioides*	CBS 953.97 epitype	Prihastuti *et al*., 2009	FJ 907430	FJ 907445	FJ 917512	FJ 972589	FJ 972582	FJ972609
C. *gloeosporioides*	CORCG5	Yang *et al*., 2009	HM034801	HM034811	HM034803	HM034807	–	HM034809
C. *gloeosporioides*	CORCG4	Yang *et al*., 2009	HM034800	HM034810	HM034802	HM034806	–	HM034808
C. gloeosporioides	**GZAAS5.09509**	**This paper**	**JQ247650**	**JQ247640**	**JQ247594**	**JQ247603**	**JQ247615**	**JQ247628**
C. gloeosporioides	**GZAAS5.09513**	**This paper**	**JQ247651**	**JQ247638**	**JQ247592**	**JQ247602**	**JQ247614**	**JQ247626**
C. gloeosporioides	**GZAAS5.09516**	**This paper**	**JQ247652**	**JQ247639**	**JQ247593**	**JQ247604**	**JQ247616**	**JQ247627**
C. horii	TSG001 neotype	Wikee *et al*., 2011	GU133374	GU133375	GU133376	GQ329682	GU133377	AY787483
C. horii	TSG002	Wikee *et al*., 2011	GU133379	GU133380	GU133381	GQ329680	GU133382	AY791890
C. horii	LC0218	Cai *et al*., Pers comm.	–	–	–	–	–	–
C. hymenocallidis	CSSN2 ex-type	Yang *et al*., 2009	GQ856775	GQ849438	GQ849463	GQ856757	–	GQ485600
C. hymenocallidis	CSSN3	Yang *et al*., 2009	GQ856776	GQ849439	GQ849451	GQ856759	–	GQ485601
C. jasmini-sambac	LLTA-01 ex-holotype	Wikee *et al*., 2011	HM131507	HM153768	HM131492	HM131497	HM131502	HM131511
C. jasmini-sambac	HLTX-01	Wikee *et al*., 2011	–	HM153769	HM131493	HM131498	HM131503	HM131512
C. jasmini-sambac	CLTA-01	Wikee *et al*., 2011	HM131510	HM153772	HM131496	–	HM131506	HM131515
C. kahawae	IMI319418 holotype	Prihastuti *et al*., 2009	FJ 907432	FJ 907446	FJ 917514	FJ 972588	FJ 972583	FJ 972608
C. kahawae	IMI 363578	Prihastuti *et al*., 2009	FJ 907433	FJ 907447	FJ 917515	FJ 972587	FJ 972584	FJ 972607
C. karstii	CORCG6 ex-type	Yang *et al*., 2011	HM581995	HM585428	HM582013	HM585391	–	HM585409

Table 2. Sequences obtained from GenBank used in the analysis *(continued)*

Species	*Strain*	*Reference*	*GenBank Accession Number*					
			ACT	*TUB-2*	*CAL*	*GPDH*	*GS*	*ITS*
C. karstii	CORCS4	Yang *et al*., 2011	HM581994	HM585426	HM582012	HM585390	–	HM585405
C. karstii	**GZAAS5.09501**	**This paper**	**JQ247653**	**JQ247641**	–	**JQ247605**	**JQ247618**	**JQ247629**
C. karstii	**GZAAS5.09502**	**This paper**	**JQ247654**	**JQ247642**	–	**JQ247606**	**JQ247617**	**JQ247630**
C. murrayae	**GZAAS5.09506** ex-type	**This paper**	**JQ247657**	**JQ247644**	**JQ247596**	**JQ247609**	**JQ247621**	**JQ247633**
C. murrayae	**GZAAS5.09538**	**This paper**	**JQ247656**	**JQ247645**	**JQ247597**	**JQ247608**	**JQ247620**	**JQ247632**
C. musae	CBS116870 epitype	Su *et al*., 2011	HQ596284	HQ596280	–	HQ596299	HQ596288	HQ596292
C. musae	MFLUCC 10-0977	Su *et al*., 2011	HQ596286	HQ596282	HQ596297	HQ596301	HQ596290	HQ596294
C. musae	MFLUCC 10-0978	Su *et al*., 2011	HQ596287	HQ596283	HQ596298	HQ596302	HQ596291	HQ596295
C. siamense	BML-I15	Prihastuti *et al*., 2009	FJ 907422	FJ 907437	FJ 917504	FJ 972597	FJ 972574	FJ 972614
C. siamense	BPD-I2 ex-holotype	Prihastuti *et al*., 2009	FJ 907423	FJ 907438	FJ 917505	FJ 972596	FJ 972575	FJ 972613
C. simmondsii	BRIP28519 ex-type	Shivas and Tan, 2009	FJ 907428	FJ 907443	FJ 917510	FJ 972580	FJ 972591	GU183331
C. simmondsii	CBS 294.67	Shivas and Tan, 2009	FJ 907429	FJ 907444	FJ 917511	FJ 972581	FJ 972590	FJ 972610
C. simmondsii	**GZAAS5.09510**	**This paper**	**JQ247655**	**JQ247643**	**JQ247595**	**JQ247607**	**JQ247619**	**JQ247631**
C. trichellum	HKUCC 10378	Yang *et al*., 2009	GQ856786	GQ849447	GQ849466	GQ856749	–	GQ485589

Note: ACT, actin; TUB-2, partial ß-tubulin; CAL, calmoudulin; GS, glutamine synthetase; GPDH, glyceraldehydes-3-phosphate dehydrogenase; ITS, complete rDNA-ITS region. The newly generated sequences in this study are shown in bold.

Table 3. Summary of morphological data of *Colletotrichum* species on *Citrus*

Species and Isolate numbers	*Conidia*			*Appresoria*			*Growth rate (mm/day)*
	Length (µm)	*Width (µm)*	*Shape*	*Length (µm)*	*Width (µm)*	*Shape*	
C. boninense GZAAS5.09505	14.53 ± 1.15 D (11.79 – 17.73)	6.63 ± 0.33 A (5.90 – 7.37)	Cylindrical	10.03 ± 1.76 A (7.44 – 14.05)	6.78 ± 0.84 B (5.05 – 8.45)	Irregularly lobed	8.94 ± 0.61 AB
C. brevispora GZAAS5.09545	16.19 ± 1.18 B (14.19 – 18.24)	5.71 ± 0.42 C (4.74 – 6.70)	Cylindrical	7.63 ± 2.40 DE (4.22 – 10.83)	6.35 ± 1.62 B (3.27 – 9.39)	Irregularly lobed and ovoid	8.17 ± 0.48 BC
C. fructicola GZAAS5.09521	12.53 ± 1.34 F (9.71 – 15.05)	5.31 ± 0.58 D (4.02 – 6.43)	Cylindrical	7.83 ± 1.27 CD (5.96 – 12.37)	5.13 ± 0.38 E (4.04 – 5.85)	Ovoid, clavate and slightly irregular	9.51 ± 0.40 A
C. *gloeosporioides* GZAAS5.09509	14.84 ± 0.91 CD (12.954 – 17.02)	5.32 ± 0.32 D (4.32 – 6.00)	Cylindrical	7.72 ± 1.07 D (5.44 – 10.40)	5.53 ± 0.59 CDE (4.21 – 7.59)	Clavateregular in outline or irregular and weakly lobed, the walls thickened	8.20 ± 0.33 BC
C. *gloeosporioides* GZAAS5.09516	17.17 ± 1.359 A (12.70 – 20.74)	5.50 ± 0.32D (4.73 – 6.29)	Cylindrical	7.68 ± 0.87 D (5.77 – 11.31)	5.70 ± 0.58 CD (4.34 – 6.70)		8.32 ± 0.36 BC
C. *gloeosporioides* GZAAS5.09513	15.38 ± 1.02 C 12.95 – 19.23	4.61 ± 0.32 E (3.65 – 5.25)	Cylindrical	8.09 ± 1.32 CD (6.28 – 13.51)	5.79 ± 0.79 C (4.24 – 7.89)		8.18 ± 0.23 BC
C. karstii GZAAS5.09502	15.19 ± 1.13 C (12.08 – 17.23)	6.20 ± 0.30 B (5.13 – 6.88)	Cylindrical	8.55 ± 0.86 BC (6.65 – 10.11)	8.50 ± 0.92 A (6.16 – 10.19)	Circular to clavate	7.19 ± 0.19 D
C. simmondsii GZAAS5.09510	16.22 ± 0.90 B (14.42 – 18.43)	5.51 ± 0.35CD (4.41 – 6.22)	Cylindrical	8.94 ± 0.98 B (6.89 – 11.19)	5.77 ± 0.87 C (4.45 – 10.24)	Ovoid, or slightly irregular	7.76 ± 0.09 CD
C. murrayae GZAAS5.09506	13.60 ± 1.42 E (9.71 – 15.05)	4.76 ± 0.46 E (3.80 – 5.75)	Cylindrical	6.80 ± 1.84 E (4.66 – 12.12)	5.24 ± 1.03 DE (3.88 – 7.70)	Ovoid, long clavate and slightly irregularly lobed	7.92 ± 0.89 CD
LSD (between group)	1.37	0.15		2.14	0.85		0.21

Least Significant Difference (LSD) means with the same letter in each column are not significantly different from each other based on DMRT test in Sirichai statistics version 6.

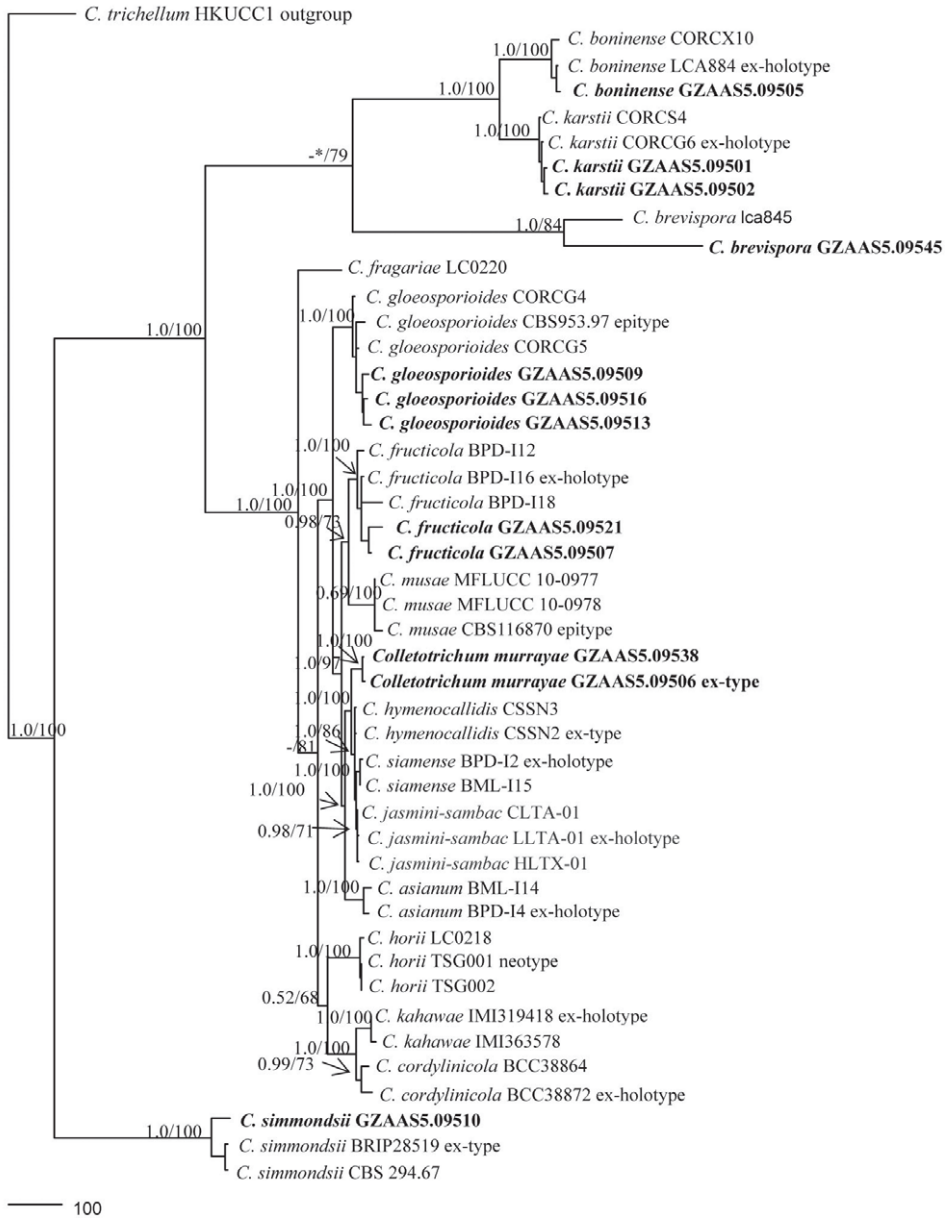

Fig. 1. Phylogram of tree generated maximum parsimony analysis based on combined ACT, TUB2, GPDH, ITS, CAL and GS sequences. Clade stabilities were calculated from Bayesian posterior probability (> 0.50), maximum parsimony (> 50%). The tree is rooted with *Colletotrichum trichellum*. Strains isolated in this study are shown in bold. *-: No data.

Colletotrichum boninense J. Moriwaki, T. Sato & T. Tsukiboshi, *Mycoscience* 44: 48-49 (2003) **Fig. 3**

One strain was isolated. Ascomata formed within 2-3 months on PDA (Fig. 3 d, e).

Material examined: China, Yunnan Province, Puer City, on living leaves of *C. medica*, June 2009, L.J. Peng (GZAAS5H.09505), living culture GZAAS5.09505.

Colletotrichum brevispora Phoulivong, Noireung, L. Cai & K.D. Hyde, *Cryptogamie, Mycologie* (32)3: this issue (2012) **Fig. 2**

Only one strain was isolated and clustered with the type strain of this species. *Appressoria* in slide cultures, brown, two types of appressoria produced, one is larger (9.5 – 12 × 6.5 – 8.15 µm, $\bar{x}$ = 11.05 µm ± 1.52 × 7.28 µm ± 1.25, n = 10), irregularly lobed; the other is smaller (4.6 – 8 × 3.8 – 6 µm, $\bar{x}$ = 7.77 µm ± 2.21 × 4.75 µm ± 1.36, n = 30), ovoid.

Material examined: China, Yunnan Province, Puer City, on living leaves of *C. medica*, June 2009, L.J. Peng (GZAAS5H.09545), living culture GZAAS5.09545.

Colletotrichum fructicola Prihastuti, L. Cai & K.D. Hyde, *Fungal Diversity* 39: 96, 98 (2009)

Material examined: China, Guizhou Province, Yuping County, on living leaves of *C. grandis*, August 2009, L.J. Peng (GZAAS5H.09521), living culture GZAAS5.09521; Yunnan Province, Puer City, on living leaves of *Citrus bergamia*, June 2009, L.J. Peng (GZAAS5H.09507), living culture GZAAS5.09507.

Colletotrichum gloeosporioides (Penz.) Penz. & Sacc., *Atti del Reale Istituto Veneto di Scienze, Lettere ed Arti*, Serie 6, 2: 670 (1884), *sensu* epitypification by P.F. Cannon, *Mycotaxon* 104:196 (2008) **Fig. 4 a-f**

Twenty-nine of the 38 strains isolated clustered with the epitype of *C. gloeosporioides* and were from five different host plants from 18 sites. The symptoms on *Citrus* plants are yellowish brown leaf spots, irregular or rounded. Acervuli can be sometimes seen on leaves (Fig. 4 g, h).

Material examined: China, Yunnan Province, Jinghong City, on living leaves of *C. limon*, June 2009, L.J. Peng (GZAAS5H.09503), living culture GZAAS5.09503; *ibid.*, on living leaves of *C. grandis*, June 2009, L.J. Peng (GZAAS5H.09504), living culture GZAAS5.09504; *ibid.*, on living leaves of *Fortunella* sp., June 2009, L.J. Peng (GZAAS5H.09508), living culture GZAAS5.09508; Yunnan Province, Puer City, on living leaves of *C. reticulata*, June 2009, L.J. Peng (GZAAS5H.09511), living culture GZAAS5.09511; *ibid.*, on living leaves of *C. grandis*, June 2009, L.J. Peng (GZAAS5H.09512), living culture GZAAS5.09512; Yunnan Province, Kunming City, on living leaves of *C. reticulata*, June 2009, L.J. Peng (GZAAS5H.09509), living culture GZAAS5.09509; Guizhou Province, Guiyang City, on living leaves of *C. reticulata* and *C. reticulata* cv. bendizao, July 2009, L.J. Peng (GZAAS5H.09513, GZAAS5H.09514), living cultures GZAAS5.09513, GZAAS5.09514; *ibid.*, on living leaves of *Fortunella* sp., July 2009, L.J. Peng (GZAAS5H.09515), living culture GZAAS5.09515; Huishui County, on living leaves of *C. reticulata*, July 2009, L.J. Peng (GZAAS5H.09516), living culture GZAAS5.09516; *ibid.*, Bijie City, on living leaves of *C. reticulata*, July 2009, L.J. Peng (GZAAS5H.09517), living culture GZAAS5.09517; *ibid.*, Yinjiang County, on living leaves of

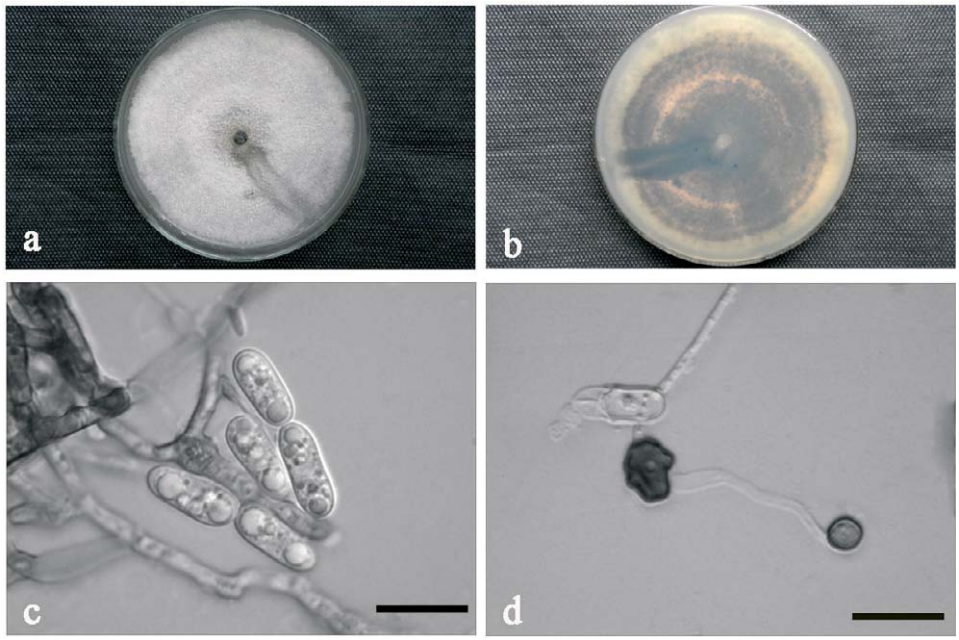

Fig. 2. *Colletotrichum brevispora* strain GZAAS5.09545. **a.** Colony upper; **b.** Colony reverse; **c.** Conidia; **d.** Appressoria produced from conidia. (Bars = 10 μm)

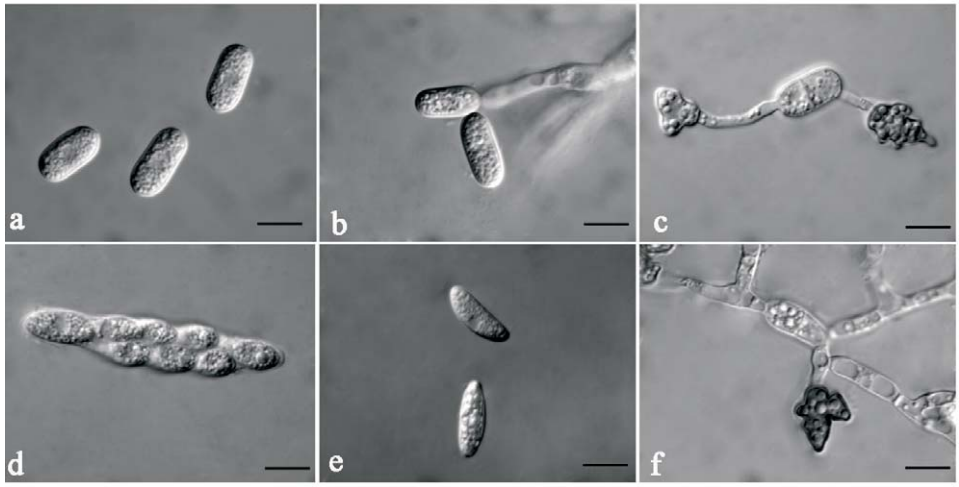

Fig. 3. *Colletotrichum boninense* strain GZAAS5.09505. **a.** Conidia; **b.** Conidia produced from conidiophore; **c.** Appressoria produced from conidia; **d.** Ascus produced on PDA; **e.** Ascospores released from asci; **f.** Appressoria produced from ascospore. (Bars = 10 μm)

C. reticulata, July 2009, L.J. Peng (GZAAS5H.09518), living culture GZAAS5.09518; *ibid.*, Duyun City, on living leaves of *C. reticulata* and *C. grandis*, July 2009, T. Sun (GZAAS5H.09524 and GZAAS5H.09525), living cultures

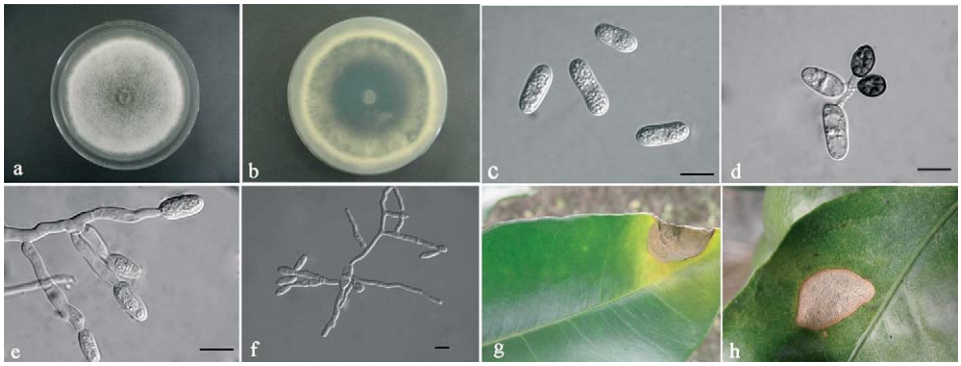

Fig. 4. *Colletotrichum gloeosporioides* strain GZAAS5.09513. **a.** Colony upper; **b.** Colony reverse; **c.** Conidia; **d.** Appressoria produced from conidia; **e, f.** Secondary condia; **g, h.** Symptoms on *C. reticulata* (mandarin orange) leaves. (Bars = 10 μm)

GZAAS5.09524 and GZAAS5.09525; *ibid.*, Libo County, on living leaves of *C. reticulata* and *C. grandis*, July 2009, L.J. Peng (GZAAS5H.09527 and GZAAS5H.09528), living cultures GZAAS5.09527 and GZAAS5.09528; *ibid.*, Luodian County, on living leaves of *C. reticulata* and *C. sinensis*, August 2009, T. Sun (GZAAS5H.09529 and GZAAS5H.09530), living cultures GZAAS5.09529 and GZAAS5.09530; *ibid.*, Tongren City, on living leaves of *C. grandis*, July 2009, L.J. Peng (GZAAS5H.09519), living culture GZAAS5.09519; *ibid.*, Yuping County, on living leaves of *C. sinensis* and *C. grandis* cv. huyou, August 2009, L.J. Peng (GZAAS5H.09520 and GZAAS5H.09522), living cultures GZAAS5.09520 and GZAAS5.09522; *ibid.*, Cengong County, on living leaves of *C. grandis*, August 2009, L.J. Peng (GZAAS5H.09523), living culture GZAAS5.09523; *ibid.*, Danzai County, on living leaves of *C. reticulata*, August 2009, L.J. Peng (GZAAS5H.09526), living culture GZAAS5.09526; *ibid.*, Anlong County, on living leaves of *C. sinensis*, August 2009, L.J. Peng (GZAAS5H.09531), living culture GZAAS5.09531; *ibid.*, Xingyi City, on living leaves of *C. grandis*, August 2009, L.J. Peng (GZAAS5H.09532), living culture GZAAS5.09532; *ibid.*, Zunyi City, on living leaves of *C. grandis* and *C. reticulata*, August 2009, L.J. Peng (GZAAS5H.09533 and GZAAS5H.09534), living cultures GZAAS5.09533 and GZAAS5.09534; *ibid.*, Zhijin County, on living leaves of *Fortunella* sp. and *C. reticulata*, July 2009, L.J. Peng (GZAAS5H.09536 and GZAAS5H.09537), living cultures GZAAS5.09536 and GZAAS5.09537.

Colletotrichum karstii Y.L. Yang, Z.Y. Liu, K.D. Hyde & L. Cai, *Cryptogamie*, *Mycologie* 32: 241, 243 (2011) **Fig. 5**

Two strains clustered with the type of *C. karstii*.

Material examined: China, Yunnan Province, Dali City, on living leaves of *C. sinensis* and *C. reticulata*, June 2009, L.J. Peng (GZAAS5.09501 and GZAAS5.09502), living cultures GZAAS5.09501 and GZAAS5.09502.

Colletotrichum simmondsii R.G. Shivas & Y.P. Tan, *Fungal Diversity* 39: 119-120 (2009) **Fig. 6 a-c**

One strain clustered with the type of *C. simmondsii*.

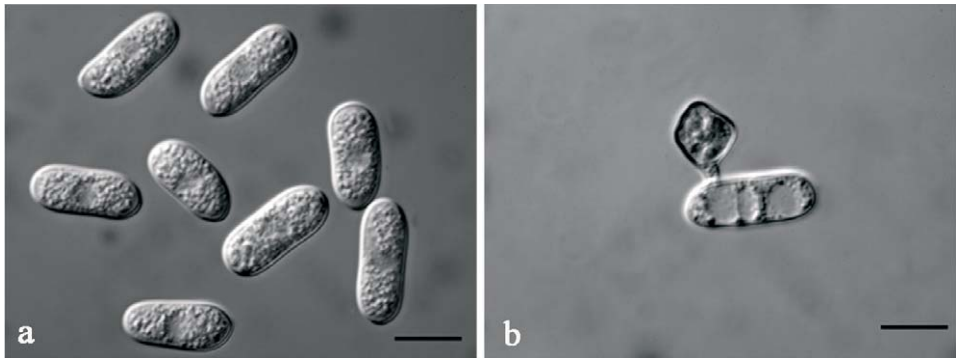

Fig. 5. *Colletotrichum karstii* strain GZAAS5.09502. **a.** Conidia; **b.** Appressoria produced from conidia. (Bars = 10 μm)

Material examined: China, Yunnan Province, Puer City, on living leaves of *Murraya* sp., June 2009, L.J. Peng (GZAAS5H.09510), living culture GZAAS5.09510.

Colletotrichum murrayae L.J. Peng & K.D. Hyde, **sp. nov.** **Fig. 6 d-f, 7 a-h**

MycoBank: MB 564951

Etymology: in reference to the host *Murraya* sp.

Description: Colonies on PDA at first white and becoming grey, reverse grey, reaching a maximum of 75 mm diam. in 7 days at 25°C in dark, growth rate 2-12.5 mm/day ($\bar{x}$ = 8.17 ± 0.48, n = 5) (Fig. 6 e, f). *Mycelium* white, dense, cottony. *Sclerotia* absent. *Setae* absent. *Conidiophores* hyaline, 1-celled, not branching, 8.5-13.5 × 2.5-4.5 μm (x =10.75 ± 2.8 × 3.2 ± 0.5, n = 20). *Conidia* not produced in sporodochia but common in mycelium, 10-15 μm long ($\bar{x}$ = 13.60 ± 1.42 μm, n = 100) × 4-6 μm wide ($\bar{x}$ = 4.76 ± 0.46 μm, n = 100), one-celled, smooth-walled, guttulate, hyaline, cylindrical with obtuse to slightly rounded ends, sometimes oblong, when conidia germinate a septum may be formed (Fig. 7 a). *Appressoria* 4.5-12 × 4-8 μm ($\bar{x}$ = 6.8 μm ± 1.84 × 5.24 μm ± 1.03, n = 50) in slide cultures, brown, ovoid, long clavate and sometimes slightly irregularly lobed, usually containing guttules (Fig. 7 b-h).

Teleomorph: not produced on PDA.

Material examined: China, Yunnan Province, Jinghong City, on living leaves of *Murraya* sp., June 2009, L.J. Peng (GZAAS5H.09506, **holotype**), ex-type culture GZAAS5.09506 and CBS; *ibid.*, (GZAAS5H.09538, **paratype**), ex-living culture GZAAS5.09538 and CBS.

Commentary: Two strains were isolated from leaf spots of *Murraya* sp. at Jinghong City, Yunnan Province of China. The strains were not well resolved in the ITS gene tree, but were distinguished from other *Colletotrichum* species in the six genes tree. The symptoms on *Murraya* sp. leaves caused by *Colletotrichum murrayae* are yellowish brown leaf spots, irregular on leaf margin. Acervuli can be sometimes seen on leaves (Fig. 6 d).

DISCUSSION

Yunnan Province is known as the “Kingdom of Plants” as the number of plant species, including tropical, sub-tropical, temperate and cool-temperate

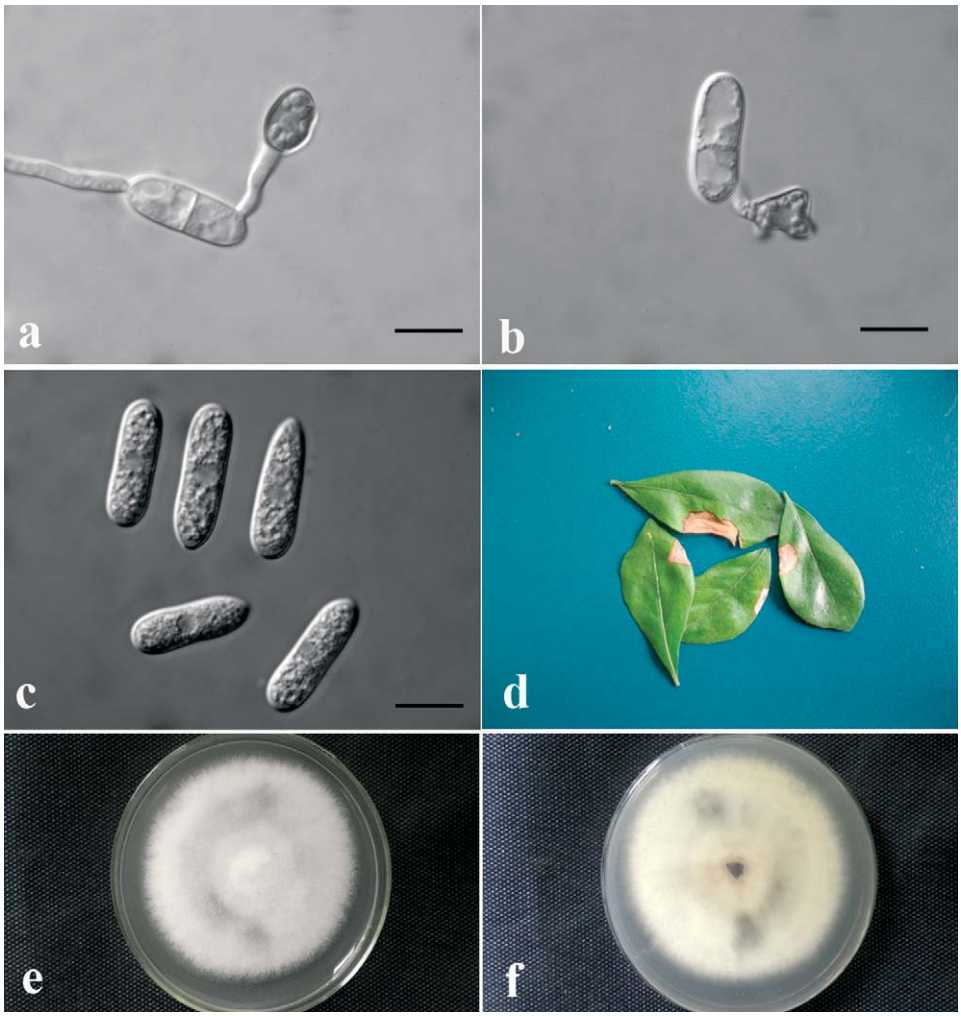

Fig. 6. *Colletotrichum simmondsii* strain GZAAS5.09510. **a, b.** Appressoria produced from conidia; **c.** Conidia; **d.** Symtoms on *Murraya* sp. leaves; **e.** *Colletotrichum murrayae* strain GZAAS5.09506 (holotype) colony upper; **f.** Colony reverse. (Bars = 10 μm)

plants, accounts for over 50% of the total plant biodiversity in China (Chai 2011). We isolated seven *Colletotrichum* species from eight host plants in Yunnan Province which would suggest that there is a great variety of *Colletotrichum* species in the province and that *Colletotrichum* species are not necessarily host-specific.

Various *Colletotrichum* species have been reported to cause disease of *Citrus*. Symptoms caused by *C. gloeosporioides* on *Citrus* are reported as, leaf anthracnose (Agostini *et al.*, 1992; Agostini & Timmer 1994; Brown *et al.*, 1996), postbloom fruit drop (Agostini *et al.*, 1992; Agostini & Timmer 1994; Brown *et al.,* 1996; Chung *et al.*, 2002; Lima *et al.*, 2011), withertip on twigs and tear stain on fruits (Benyahia *et al.*, 2003), fruit rot (Kaur *et al.*, 2007). *Colletotrichum acutatum*

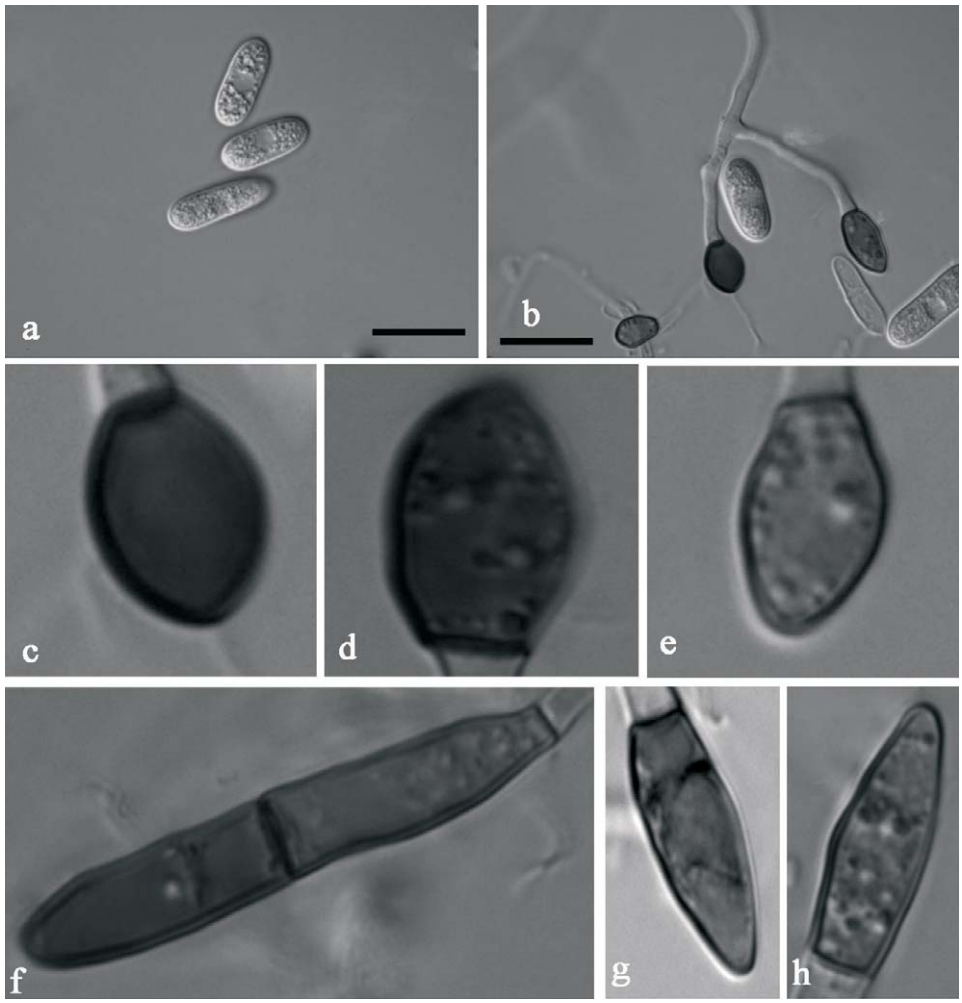

Fig. 7. *Colletotrichum murrayae* strain GZAAS5.09506 (holotype). **a.** Conidia; **b.** Conidia and approsoria; **c-h.** Approsoria. (Bars = 10 μm)

also can infect Key lime (*Citrus aurantifolia*) resulting in leaf anthracnose and post bloom fruit drop of sweet orange (*C. sinensis*) (Agostini *et al.*, 1992; Brown *et al.*, 1996; Chung *et al.*, 2002). *C. acutatum* was not detected in this study.

Colletotrichum boninense, which was isolated from a leaf lesions of *C. medica* has been also isolated from yellow passion fruits (*Passiflora edulis* f. *flavicarpa*) in Brazil (Júnior *et al.*, 2010), avocado fruits (*Persea americana*) in Mexican (Silva-Rojas & Ávila-Quezada 2011), spindle tree (*Euonymus japonica*) leaves in Korea (Lee *et al.*, 2005). These records, however, have not been confirmed based on molecular data and should be treated with caution. It has also been isolated as a pathogen (confirmed by molecular data) from leaves of *Oncidium flexuosum* and as an endophyte from roots of healthy *Pleione bulbocodioides* (Yang *et al.,* 2011).

Colletotrichum simmondsii has been reported to cause fruit rot or fruit anthracnose on chili pepper (*Capsicum frutescens*), strawberry (*Fragaria* × *ananassa*), papaya (*Carica papaya*), chinese pulsatilla (*Anemone chinensis*), litchi (*Litchi chinensis)*, tree tomato (*Cyphomandra betacea)*, avocado (*Persea americana*), mango (*Mangifera indica*), high bush blueberry (*Vaccinium corymbosum*) and tomato (*Lycopersicon esculentum)* (Shivas & Tan 2009). It also infected celery (*Apium graveolens* var. *dulce)* causing stunt anthracnose (Fujinaga *et al.*, 2011). We isolated one strain of *C. simmondsii* from a *Murraya* sp. leaf lesion.

Colletotrichum fructicola was isolated from coffee berries (*Coffea arabica)* as a pathogen and endophyte (Prihastuti *et al.*, 2009), leaves of spider lily (*Crinum asiaticum*) (Yang *et al.*, 2009) and fruits of table grape (*Vitis vinifera*).

Colletotrichum gloeosporioides is the most common species on *Citrus* plants, comprising 29 (76%) of 38 strains isolated in this study. Some species of *Colletotrichum* produce secondary conidia in culture directly from germinated primary conidia (Cannon *et al.,* 2000; Slade *et al.*, 1987). Leng *et al.* (1984) observed secondary conidia forming in eight isolates of *C. gloeosporioides* from *Citrus* spp. in China. Secondary conidia also can be formed in *C. acutatum* (Barbosa *et al.,* 2006; Leandro *et al.*, 2001). When inducing conidial appressoria in *C. gloeosporioides* on a glass slide, there were three ways in which conidia germinated, forming conidial appressoria, secondary conidia or both. We selected eight strains of *C. gloeosporioides* to observe the formation of secondary conidia (Fig. 4 e, f). All strains produced this phenomenon. Thus secondary conidia formation may be an important character of *C. gloeosporioides*.

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