Tropical Plant Pathology, vol. 37(3):165-174, 2012
Copyright by the Brazilian Phytopathological Society. Printed in Brazil
www.sbfito.com.br

RESEARCH ARTICLE / *ARTIGO*

New species and notes of *Colletotrichum* on daylilies (*Hemerocallis* spp.)

Youlian Yang[1, 2], Zuoyi Liu[1], Lei Cai[3] & Kevin D. Hyde[4]

[1]Guizhou Key Laboratory of Agricultural Biotechnology, Guizhou Academy of Agricultural Sciences, Guiyang, Guizhou 550006, P. R. China; [2]Department of Life Science, Liupanshui Normal University, Shuicheng, Guizhou 553006, P. R. China; [3]State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, No.10, North 4th Ring Road West, Beijing 100190, P. R. China; [4]Institute of Excellence in Fungal Research, School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand; Visiting Professor, Botany and Microbiology Department, College of Science, King Saud University, Riyadh, Saudi Arabia

Author for correspondence: Kevin D. Hyde, e-mail: kdhyde3@gmail.com

ABSTRACT

Nine *Colletotrichum* strains were isolated from diseased and dead stalks of *Hemerocallis* species (daylilies) from Guizhou, Guangxi, and Liaoning provinces in China. Morphological characteristics and multilocus phylogenetic analysis of ACT, CHS I, GPDH, ITS, and TUB 2 indicate that these strains represent four taxa. *Colletotrichum hemerocallidis* is a new species that is described, illustrated, and compared with similar species. *Colletotrichum gloeosporioides*, *C. liriopes*, and *C. spaethianum* are also recorded on *Hemerocallis* species.

Key words: Anthracnose, multilocus phylogeny, systematics.

INTRODUCTION

Hemerocallis species (including *H. fulva* (L.) Linn., *H. citrina* Baroni, and other species or cultivars) are economically important as food plants in China, Japan, Korea, Thailand, and Vietnam, being known as "yellow flower vegetables" or "golden needles" in China (Zhou et al., 1994; Staples & Kristiansen, 1999; Zhang & Chen, 2008). Species are also cultivated and bred worldwide for their showy flowers and ability to adapt to a wide range of soils and climates (Munson, 1989; Tomkins et al., 2010), and are used as Traditional Chinese Medicine (Zhu et al., 2008; Ma et al., 2010).

Hemerocallis production has often been limited by anthracnose disease (Jiang et al., 1993). Disease outbreaks can be severe with 100% of some ornamental *Hemerocallis* species being infected (Jiang et al., 1993). Six *Colletotrichum* species have previously been reported as causal agent of anthracnose of *Hemerocallis* including *C. dematium* (Pers.) Grove on *Hemerocallis* sp. in the United States (Farr & Rossman, 2011), *C. gloeosporioides* (Penz.) Penz. & Sacc. on *H. citrina* in China (Gu et al., 2007), *C. liliacearum* Ferraris on *H. fulva* var. *kwanso* Regel in China (Jiang et al., 1993; Farr & Rossman, 2011), *C. lilii* Plakidas ex Boerema & Hamers on *Hemerocallis* sp. in the United States (Farr & Rossman, 2011), *C. spaethianum* (Allesch.) Damm, P.F. Cannon, & Crous, on *Hemerocallis* sp. in New Zealand, and *Colletotrichum* sp. (CBS 125338) on *H. fulva* in Canada (Damm et al., 2009). There is, however, little knowledge concerning the *Colletotrichum* species associated with *Hemerocallis* in China. The objective of this paper was to characterize *Colletotrichum* species associated with these plants in China based on morphology and multilocus DNA sequence data.

MATERIALS AND METHODS

Isolation of *Colletotrichum*

Dead leaves and stalks of *Hemerocallis citrina*, *H. fulva,* and *H. fulva* var. *kwanso* with anthracnose lesions were collected in Guizhou, Guangxi, and Liaoning provinces in China from 2008 to 2011 (Table 1). Single-spore isolates were obtained using the procedure described by Choi et al. (1999) and Chomnunti et al. (2011). Pure cultures were stored at 4°C on PDA slants. Isolates are deposited in Guizhou Academy of Agricultural Sciences, China, and the China General Microbiological Culture Collection Center (CGMCC).

Morphological and cultural characterization

Starter cultures were prepared by growing each isolate on PDA at 25°C in darkness for five days. Five replicate cultures of each isolate were prepared by aseptically cutting disks from the actively growing edge of the starter culture using a sterile cork borer. Each plug was placed onto PDA plates (90 mm × 15 mm) and grown in alternating light and dark at 25ºC (Sutton, 1980). To induce sporulation, plugs of actively growing mycelium were placed on to the surface

TABLE 1 - Sources of strains of *Colletotrichum* spp. with GenBank accession numbers used in this study

Taxon	Strain no.	GenBank no.					Host	Site	Reference
		ITS	TUB2	CHS I	GPDH	ACT			
C. anthrisci	CBS125334▲	GU227845	GU228139	GU228335	GU228237	GU227943	*Anthriscus sylvestris*	Netherlands	Damm et al. (2009)
	CBS125335	GU227846	GU228240	GU228336	GU228238	GU227944	*Anthriscus sylvestris*	Netherlands	Damm et al. (2009)
C. boninense	MAFF305972▲	HM585399	HM585421	HM582032	HM585386	HM582001	*Crinum asiaticum* var. *sinicum*	Japan	Moriwaki et al. (2003)
	CSSX10	HM585401	HM585420	HM582031	HM585384	HM581999	*Oncidium flexuosum*	China	Yang et al. (2009)
C. chlorophyti	IMI103806▲	GU227894	GU228188	GU228384	GU228286	GU227992	*Chlorophytum* sp.	India	Damm et al. (2009)
	CBS142.79	GU227895	GU228189	GU228385	GU228187	GU227993	*Stylosanthes hamata*	Australia	Damm et al. (2009)
C. circinans	CBS221.81▲	GU227855	GU228149	GU228345	GU228247	GU227953	*Allium cepa*	Serbia	Damm et al. (2009)
	CBS125331	GU227861	GU228155	GU228351	GU228253	GU227959	*Anthriscus sylvestris*	Germany	Damm et al. (2009)
C. curcumae	IMI288937▲	GU227893	GU228187	GU228383	GU228285	GU227991	*Curcuma longa*	India	Damm et al. (2009)
C. dematium	CBS125.25▲	GU227819	GU228113	GU228309	GU228211	GU227917	*Eryngium campestre*	France	Damm et al. (2009)
	CBS115524	GU227826	GU228120	GU228316	GU228218	GU227924	*Vitis vinifera*	South Africa	Damm et al. (2009)
	IMI350847	GU227825	GU228119	GU228315	GU228217	GU227923	*Solanum tuberosum*	Australia	Damm et al. (2009)
C. fructi	CBS346.37▲	GU227844	GU228138	GU228334	GU228236	GU227942	*Malus sylvestris*	USA	Damm et al. (2009)
C. fructicola	MFLU090228▲	FJ972603	FJ907441		FJ972578	FJ907426	*Coffea arabica*	Thailand	Prihastuti et al. (2009)
	CSSX7	GQ485604	GQ849435	GQ856734	GQ856760	GQ856770	*Crinum asiaticum*	China	Yang et al. (2009)
C. gloeosporioides	CBS953.97▲	GQ485605	GQ849434	GQ856733	GQ856762	GQ856782	*Citrus sinensis*	Italy	Yang et al. (2009)
	CDLG 1	**JQ400008**	**JQ400022**	**JQ400001**	**JQ400015**	**JQ399994**	*Hemerocallis citrina*	China	This paper
	CDLG 4	**JQ400009**	**JQ400023**	**JQ400002**	**JQ400016**	**JQ399995**	*Hemerocallis fulva*	China	This paper
C. hymenocallidis	CBS125378▲	GQ485600	GQ849438	GQ856730	GQ856757	GQ856775	*Hymenocallis americana*	China	Yang et al. (2009)
	CBS125379	GQ485601	GQ849439	GQ856729	GQ856759	GQ856776	*Hymenocallis americana*	China	Yang et al. (2009)
C. hemerocallidis	**CDLG5▲**	**JQ400005**	**JQ400019**	**JQ399998**	**JQ400012**	**JQ399991**	*Hemerocallis fulva* var. *kwanso*	China	This paper
	CDLN6	**JQ400006**	**JQ400020**	**JQ399999**	**JQ400013**	**JQ399992**	*Hemerocallis fulva* var. *kwanso*	China	This paper
	CDLN7	**JQ400007**	**JQ400021**	**JQ400000**	**JQ400014**	**JQ399993**	*Hemerocallis fulva*	China	This paper
	CBS125338★	GU227828	GU228121	GU228318	GU228220	GU227926	*Hemerocallis fulva*	Canada	Damm et al. (2009)
C. lilii	CBS109214	GU227810	GU228104	GU228300	GU228202	GU227908	*Lilium* sp.	Japan	Damm et al. (2009)
	CBS186.30	GU227811	GU228105	GU228301	GU228203	GU227909	*Lilium* sp.	Netherlands	Damm et al. (2009)
C. lineola	CBS125337▲	GU227829	GU228123	GU228319	GU228221	GU227927	*Apiaceae*	Czech Republic	Damm et al. (2009)
	CBS282.85	GU227843	GU228137	GU228333	GU228235	GU227941	*Allium giganteum*	Netherlands	Damm et al. (2009)

cont.

cont.

TABLE 1 - Sources of strains of *Colletotrichum* spp. with GenBank accession numbers used in this study

Taxon	Strain no.	GenBank no.					Host	Site	Reference
		ITS	TUB2	CHS I	GPDH	ACT			
C. liriopes	CBS119444▲	GU227804	GU228098	GU228294	GU228196	GU227903	*Lirope muscari*	Mexico	Damm et al. (2009)
	CBS122747	GU227805	GU228099	GU228295	GU228197	GU227904	*Lirope muscari*	Mexico	Damm et al. (2009)
	CDLG3	**JQ400004**	**JQ400018**	**JQ399997**	**JQ400011**	**JQ399990**	*Hemerocallis fulva*	China	This paper
C. phaseolorum	CBS157.36	GU227896	GU228190	GU228386	GU228288	GU227994	*Phaseolus radiatus*	Japan	Damm et al. (2009)
	CBS158.36	GU227897	GU228191	GU228387	GU228289	GU227995	*Vigna sinensis*	Japan	Damm et al. (2009)
C. rusci	CBS119206▲	GU227818	GU228112	GU228308	GU228210	GU227916	*Ruscus* sp.	Italy	Damm et al. (2009)
C. siamense	MFLU090230▲	FJ 972613	FJ 907438		FJ 972575	FJ 907423	*Coffea arabica*	Thailand	Prihastuti et al. (2009)
	CSST4	GQ485603	GQ849443	GQ856732	GQ856761	GQ856780	*Hymenocallis* sp.	Thailand	Yang et al. (2009)
C. spaethianum	CBS167.49▲	GU227807	GU228101	GU228297	GU228199	GU227905	*Hosta sieboldiana*	Germany	Damm et al. (2009)
	CBS100063	GU227808	GU228102	GU228298	GU228200	GU227906	*Lilium* sp.	South Korea	Damm et al. (2009)
	CDLG2	**JQ400003**	**JQ400017**	**JQ399996**	**JQ400010**	**JQ399989**	*Hemerocallis fulva*	China	This paper
	CDLL1*						*Hemerocallis citrina*	China	This paper
	CDLL2*						*Hemerocallis citrina*	China	This paper
C. spinaciae	CBS125349	GU227852	GU228146	GU228342	GU228244	GU227950	*Chenopodium album*	USA	Damm et al. (2009)
	CBS128.57	GU227847	GU228141	GU228337	GU228239	GU227945	*Spinacia oleracea*	Netherlands	Damm et al. (2009)
C. tofieldiae	CBS495.85	GU227801	GU228095	GU228291	GU228193	GU227899	*Tofieldia calyculata*	Switzerland	Damm et al. (2009)
	IMI288810	GU227803	GU228097	GU228293	GU228195	GU227901	*Dianthus* sp.	UK	Damm et al. (2009)
C. trichellum	CBS102642	GU227816	GU228110	GU228306	GU228208	GU227914	*Hedera helix*	New Zealand	Damm et al. (2009)
	HKUCC10378	GQ485589	GQ849447	GQ856724	GQ856749	GQ856786	*Hedera helix*	Japan	Yang et al. (2009)
C. truncatum	CBS151.35▲	GU227862	GU228156	GU228352	GU228254	GU227960	*Phaseolus lunatus*	USA	Damm et al. (2009)
	CBS120709	GQ485593	GQ849429	GQ856739	GQ856753	GQ856783	*Capsicum frutescens*	India	Yang et al. (2009)
C. verruculosum	IMI 45525▲	GU227806	GU228100	GU228296	GU228198	GU227904	*Crotalaria juncea*	Zimbabwe	Damm et al. (2009)
Colletotrichum sp.	CBS125326	GU227827	GU228121	GU228317	GU228219	GU227925	*Rubus idaeus*	Canada	Damm et al. (2009)
Glomerella lindemuthiana (Outgroup)	CBS151.28	GU227800	GU8094	GU228290	GU228192	GU227898	*Phaseolus vulgaris*	UK	Damm et al. (2009)

Note: CBS - Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; HKUCC - The University of Hong Kong Culture Collection; IMI - Culture collection of CABI Europe UK Centre, Egham, UK; MFLU - Mae Fah Luang University, Thailand; ▲ - ex-type or ex-epitype cultures;***-** cited as *Colletotrichum* sp. 2 according to Damm et al. (2009); * - has not been sequenced. The isolated strains and newly generated sequences are shown in bold.

of synthetic nutrient-poor agar medium (SNA: 1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 0.2 g glucose, 0.2 g sucrose, 20 g agar, 1 L tap water) with autoclaved filter paper and double-autoclaved stems of *Sium suave* Walt. [(Apiaceae; comp. method proposed by Damm et al. (2009), using stems of *Anthriscus sylvestris* (L.) Hoffm., Apiaceae)] and incubated in the same conditions. Colony diameter was measured at day seven. After 7-10 days, the size and shape of 50 conidia harvested from the cultures were measured, and their mean and standard deviations (SD) were calculated. 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 $1 \times 10^{12\text{-}14}$conidia/mL) on a microscope slide, then placing the slide inside a Petri dish containing cotton moistened with distilled sterile water, and incubated at 25ºC in darkness. After incubation for 24 hours, conidial appressoria formed by germ tubes were characterized.

DNA extraction and sequencing

DNA was extracted from the isolates grown on PDA at 25ºC for 8-10 days using a modified protocol of Chen et al. (2007). The partial sequence of the actin (ACT), beta-tubulin (TUB2), 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), 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 9.5 µL 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 five gene regions cited 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) and manually adjusted to give the best fit with BioEdit 7.0.8.

A partition homogeneity test (PHT) was performed with 1000 replicates in PAUP 4.0b10 (Swofford, 2003) to evaluate statistical congruence among the five gene regions and 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, and gaps were treated as missing data. 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 visualized in Treeview. When analyzing single and combined sequences, some reference sequences were obtained from GenBank (Table 1). Sequences obtained in this study were submitted to GenBank (accession No: ACT, JQ399989- JQ399995; CHS I, JQ399996-JQ400002; ITS, JQ400003- JQ400009; GPDH, JQ400010- JQ400016; TUB 2, JQ400017-JQ400023), the alignment in TreeBASE (http://www.treebase.org/treebase/index.html, ID: 12294), and taxonomic novelties in MycoBank (Crous et al., 2004).

RESULTS

Isolation of *Colletotrichum* species

Nine isolates of *Colletotrichum* were obtained from recently dead or infected stalks and leaves of *Hemerocallis citrina*, *H. fulva*, and *H. fulva* var. *kwanso* in Guiyang, Nanning, and Dandong, China.

Phylogenetic analysis

The partition homogeneity test ($P = 0.01$) suggested that the individual gene partitions were not highly incongruent (Farris et al., 1995; Cunningham, 1997), thus the five gene datasets (ACT, CHS I, GPDH, ITS, TUB 2) from the *Colletotrichum* species plus datasets obtained from GenBank were combined for phylogenetic analysis. The combined datasets comprise 1797 characters after alignment, of which 700 characters are parsimony-informative, 991 constant, and 106 parsimony-uninformative. Parsimony analysis generated eight trees; SH test verified that they were similar, one of which (tree length = 2131 steps, CI = 0.605, RI = 0.88, RC = 0.532, HI = 0.395) is shown in Figure 1. Tree topologies obtained from the individual alignment of five genes and from the combined alignment are similar to each other, with only slight differences in bootstrap values, e. g. *Colletotrichum spaethianum* and *C. lilii* were not distinguished in two (ACT and ITS) of five phylogenies.

The phylogram constructed using combined datasets shows that the Chinese *Hemerocallis* isolates cluster into four distinct clades with high bootstrap support, presumably representing different *Colletotrichum* species. Sequences of the cultures CDLG2 and CDLG3 cluster with sequences of *Colletotrichum spaethianum* (CBS 167.49) and *C. liriopes*

FIGURE 1 - Maximum parsimony phylogram inferred from combined partial ACT, CHS I, GPDH, ITS, and TUB 2 sequence data, showing phylogenetic relationships of *Colletotrichum* species isolated from *Hemerocallis citrina*, *H. fulva*, and *H. fulva* var. *kwanso* in China (tree length = 2131 steps, CI = 0.605, RI = 0.88, RC = 0.532, HI = 0.395). Values above the branches are parsimony bootstrap data (equal or above 50%). The tree is rooted with *Glomerella lindemuthiana* (CBS 151.28). ▲, ex-type or ex-epitype.

(CBS 119444) with 100% bootstrap support, respectively. Sequences of CDLG1, CDLG4, and *Colletotrichum gloeosporioides* epitype (CBS 953.57) are nested in a clade with 100% bootstrap support. Sequences of CDLG5, CDLN6, CDLN7, and CBS 125338 form a distinct clade with 100% bootstrap value (Figure 1).

Taxonomy

The nine strains isolated from *Hemerocallis* spp. represent four species based on DNA sequence analysis and morphological characteristics. Three strains represent one new species. The other six isolates represent three known *Colletotrichum* species which are presented with comments.

Colletotrichum gloeosporioides (Penz.) Penz. & Sacc., *Atti Inst. Veneto Sci. lett., ed Arti*, Sér. 62: 670 (1884)

Colletotrichum gloeosporioides has been epitypified and can now be identified using sequence data (Cannon et al., 2008; Cai et al., 2009; Hyde et al., 2009; Phoulivong, 2011). In the present study this species was isolated from dead stalks of *Hemerocallis citrina* and *H. fulva*. Acervuli are black with pink conidia masses and setae are sparse. Based on morphological identification, Gu et al. (2007) reported that *C. gloeosporioides* caused severe anthracnose on *Hemerocallis citrina* leaves and this is confirmed here using morphological and molecular data. Fruit rots (anthracnose) have often been attributed to *C. gloeosporioides* with identifications based on morphological characteristics, but *C. gloeosporioides* is not a common pathogen on tropical fruits as shown by a recent study by Phoulivong et al. (2010).

Material examined: China, Guizhou province, Guizhou Academy of Agricultural Sciences, on recently dead flower stalk of *Hemerocallis citrina*, 10 June 2008, Y. L. Yang (GZAAS 080055, ex-living culture CDLG1); China, Guizhou province, Guiyang Botanical Garden of Medicinal Plants, on recently dead flower stalk of *H. fulva*, 1 July 2008, Y. L. Yang (GZAAS 080058, ex-living culture CDLG4).

Colletotrichum hemerocallidis Y. L. Yang, Zuo Y. Liu, K.D. Hyde & L. Cai, sp. nov.

MycoBank: MB 564162

Etymology: Named after its host, *Hemerocallis* sp.

Holotype: China, Guizhou Province: Guiyang. On dead stalk of *Hemerocallis fulva* var. *kwanso* Regel, 1 July 2008, Y. L. Yang (GZAAS 080059; ex-holotype living culture CDLG5 = CGMCC 3.14971, CBS 130642).

On host, *acervuli* elliptical to circular, arranged irregularly, subepidermal, disrupting outer epidermal cell wall of host, setae present (Figure 2A). *Setae* 71.5-130.5 × 7-12 µM, dark brown, opaque, 2- to 4-septated, base inflated, tip acute (Figure 3). *Conidiophores* hyaline, pale brown at base, cylindrical, 1- to 2-celled, branched, 12-19.5(-25.5) × 3-5 µM, mean ± SD = 15.6 ± 3.4 × 4.2 ± 0.5 µM (n = 20) (Figure 3), *conidiogenous cells* cylindrical to ampulliform, hyaline, 9-19 (-24) × 3.5-5 µM, mean ± SD = 14.1 ± 3.5 × 4.3 ± 0.5 µM (n = 20). *Conidia* slightly curved, often straight on one side and slightly curved on the other, hyaline, (17.5-) 20.5-27 × 3.5-5 µM, mean ± SD = 23.2 ± 2 × 4.1 ± 0.3 µM (n = 30), base truncate, apex acute (Figure 2C).

In culture: Colonies on PDA, attaining 4.9-6.7 cm, mean ± SD = 6.1 ± 0.5 cm (n = 15) diam. in seven days at 25°C. Aerial mycelium sparse, white to grey, flat with entire margin, reverse greenish black. Sclerotia present, globose to subglobose, without setae. *Conidia* not produced. Colonies on SNA, attaining 4.8-6.1 cm, mean ± SD = 5.5 ± 3.7 cm (n = 15) diam. in seven days at 25°C. Aerial very sparse, grey. Sclerotia absent; Conidia not produced.

On *Sium suave* stem: *acervuli* abundant (Figure 2B), *setae* dark brown to black, opaque, smooth, septation hardly visible, 76.5-152.5 × 5-11.5 µM, tapered from base to apex. *Conidiophores* pale brown, 1-to 3-septate, branched, 16.5-40 (-44.5) × 3.5-5 µM, mean ± SD = 28.1 ± 8.3 × 4.3 ± 0.5 µM (n = 20) (Figure 4). *Conidiogenous cells* pale brown, cylindrical to elongate ampulliform, 7-16.5 (-19) × 3.5-5.5 µM, mean ± SD = 12.3 ± 3.3 × 4.5 ± 0.6 µM (n = 20). *Conidia* in white to yellowish masses, hyaline, smooth-walled, aseptate, one side straight and the other slightly curved, apex acute or slightly rounded, base truncate, 23-31.5 (-33.5) × 3.5-5.5 µM, mean ± SD = 27.8 ± 2.3 × 4.6 ± 0.4 µM (n = 150) (Figure 2 I). *Mycelial appressoria* clavate, brown, margin entire, sometimes slightly lobed, 6.5-16 (-18.5) × 5-9 µM, mean ± SD = 11.7 ± 2.9 × 6.5 ± 1.1 µM (n = 50) (Figures 2D, E), usually in loose groups; *Conidial appressoria* clavate to irregular, brown, margin entire to crenate, sometimes deeply lobed, 6.5-13 × 4-9.5 µM, mean ± SD = 9.4 ± 1.4 × 6.4 ± 1.2 µM (n = 60) (Figures 2F, G, H).

Known hosts and distribution: *Hemerocallis fulva*, *Hemerocallis fulva* var. *kwanso*, Guizhou and Guangxi provinces, China.

Additional specimens examined: China, Guangxi province, Nanning, on leaf spot of *Hemerocallis fulva* var. *kwanso*, 19 June 2008, Y. L. Yang (GZAAS 080040, living culture CDLN6); China, Guangxi province, Nanning, on leaf spot of *Hemerocallis fulva*, 19 June 2008, Y. L. Yang (GZAAS 080041, living culture CDLN7).

Notes: The conidial shape of *C. hemerocallidis* is similar to that of *C. anthrisci* Damm, P.F. Cannon & Crous, and *C. lineola* Corda, while the conidial width and mycelial appressoria of *C. hemerocallidis* are different from those of *C. anthrisci* and *C. lineola*. The conidia of *C. hemerocallidis* are wider than those of the latter (3.5-5.5 µm vs. 3-4 µm). The mycelial appressoria of *C. hemerocallidis* are clavate with entire or sometimes slightly lobed margins, while those of *C. anthrisci* are navicular, bullet-shaped to clavate, and those of *C. lineola* ellipsoidal to clavate (Damm et al., 2009). In multilocus phylograms, sequence data of *C. anthrisci*, *C. hemerocallidis*, and *C. lineola* indicate positions nested in different clades (Figure 1).

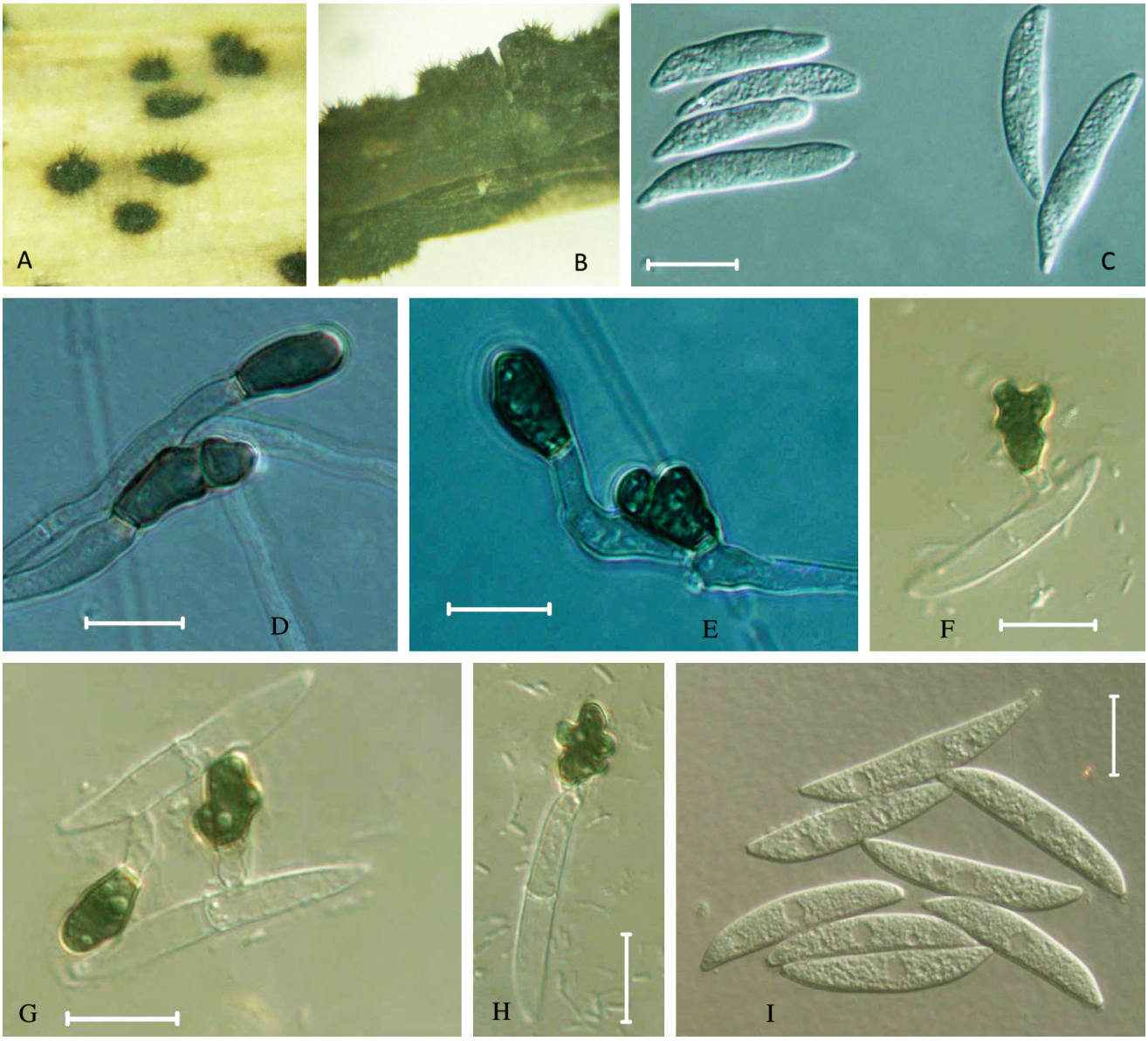

FIGURE 2 - *Colletotrichum hemerocallidis* (holotype). **A** and **B**, acervuli, **A.** on stem of *Hemerocallis fulva* var. *kwanso*; **B.** on a stem of *Sium suave*; **C** and **I.** conidia, **C.** on *H. fulva* var. *kwanso*; **I.** on *Sium suave*; **D** and **E.** mycelial appressoria; **F**, **G**, and **H.** conidial appressoria. Bars = 10 µM.

Colletotrichum liriopes Damm, P.F. Cannon & Crous

This taxon was isolated from a dead stalk of *Hemerocallis fulva*, acervuli are small with short black setae.

Material examined: China, Guizhou Province: Guiyang Botanical Garden of Medicinal Plants. On recently dead flower stalk of *Hemerocallis fulva*, 1 July 2008, Y. L. Yang (GZAAS 080057, living culture CDLG3).

Note: This taxon was first reported from *Liriopes muscari* (Decne.) L. H. Bailey in Mexico (Damm et al., 2009). We also collected this species from anthracnose on *Eria coronaria* (Lindl.) Rchb. F. (*Orchidaceae*) and a healthy root of *Pleione bulbocodioides* (Franch.) Rolfe (*Orchidaceae*) (Yang et al., 2011), so this species is not host-specific.

Colletotrichum spaethianum (Allesch.) Damm, P.F. Cannon & Crous

This species was isolated from anthracnose of *Hemerocallis fulva*, causing brown spots on leaves and having small acervuli containing black setae.

Material examined: China, Guizhou Province: Guiyang Botanical Garden of Medicinal Plants. On leaf spot of *Hemerocallis fulva*, 1 July 2008, Y. L. Yang (GZAAS 080056, living culture CDLG2). China, Liaoning Province: on leaf spot of *Hemerocallis citrina*,

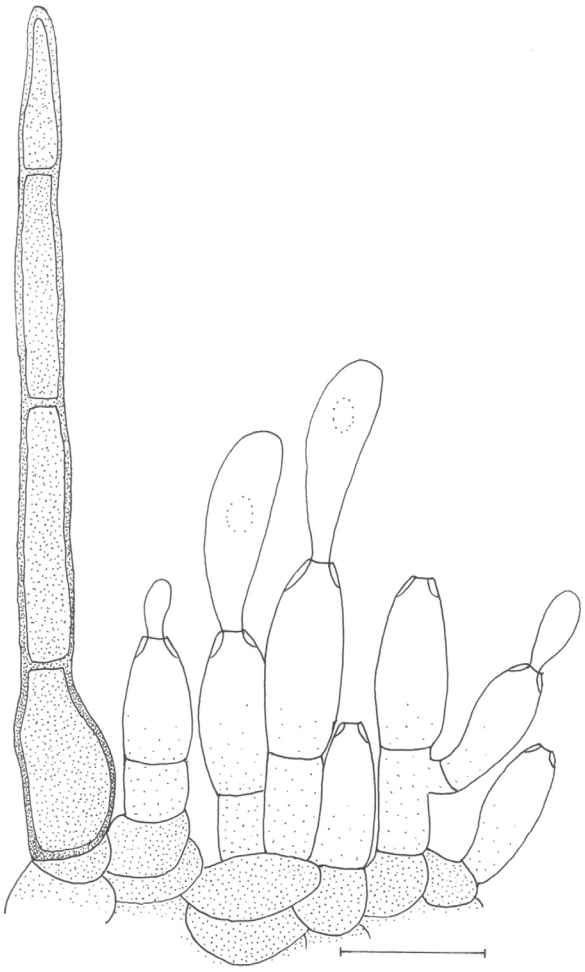

FIGURE 3 - Seta and conidiophores of *Colletotrichum hemerocallidis* (holotype) on *H. fulva* var. *kwanso*. Bar = 10 µM.

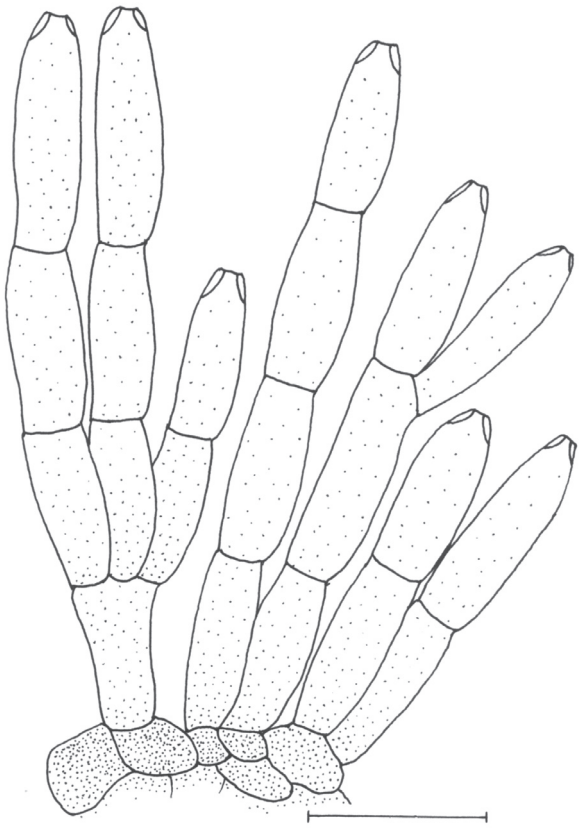

FIGURE 4 - Conidiophores of *Colletotrichum hemerocallidis* (holotype) on *Sium suave*. Bar = 10 µM.

4 July 2011, Y. L. Yang (GZAAS 110007, living culture CDLL1; GZAAS 110008, living culture CDLL2).

Note: Damm et al. (2009) reported *Colletotrichum spaethianum* from dead stems of *Hosta sieboldiana* (Lodd.) Engl., leaf spot of *Hemerocallis* sp., and infected leaf of *Lilium* sp. Yang et al. (2009) also isolated this species from a leaf spot of *Hymenocallis americana* (Jacq.) Salisb. This suggests a broad host range for this species.

DISCUSSION

Six species of *Colletotrichum* have previously been reported from *Hemerocallis* species, but with the exception of *C. spaethianum* (CBS 101631) and *Colletotrichum* sp. (CBS 125338) which have been sequenced, the identifications were based on morphological characteristics (Table 2). In the context of the present study, the species of *Colletotrichum* on *Hemerocallis* spp. in China are accurately identified and data are provided extending our knowledge on the host range and distribution of four species. One new species is proposed. Several studies have shown the importance of using sequence data when identifying *Colletotrichum* species, because wrong diagnosis may otherwise result (Phoulivong et al., 2010; Damm et al., 2010; Cai et al., 2011; Ko Ko et al., 2011).

Colletotrichum hemerocallidis apparently is saprobic and pathogenic on *H. fulva*. This suggests that *C. hemerocallidis* is similar to some other *Colletotrichum* species (e.g. *C. gloeosporioides*, *C. liriopes*, *C. spaethianum*) in having more than one biological life strategy (Damm et al., 2009; Rojas et al., 2010; Yang et al., 2011; Phoulivong, 2011). As we gain more knowledge on the distribution and host range of *Colletotrichum* species, it appears that many species may be saprobes, endophytes, or pathogens, having a wide host range and distribution. The new strains of *Colletotrichum gloeosporioides* obtained during this study were isolated from symptomatic tissues of *Hemerocallis* thus suggesting these strains are pathogens of this genus.

TABLE 2 - *Colletotrichum* species known from *Hemerocallis* spp.

Species	Host	Country	Strain	Reference
Colletotrichum dematium	*Hemerocallis* sp.	Zimbabwe	Unknown	Farr & Rossman (2011)
C. gloeosporioides/	*H. citrina*	China	Unknown	Gu et al. (2007)
Glomerella cingulata		China	CDLG1	This study
		Brunei Darussalam	Unknown	Farr & Rossman (2011)
	H. fulva	China	CDLG4	This study
C. hemerocallidis	*H. fulva* var. *kwanso*	China	CDLG5, CDLN6	This study
	H. fulva	China	CDLN7	This study
	H. fulva	Canada	CBS 125338★	Damm et al. (2009)
C. liliacearum▪	*Hemerocallis* sp.,	USA	Unknown	Farr & Rossman (2011)
	H. fulva var. *kwanso*	China	Unknown	Jiang et al. (1993)
C. lilii	*Hemerocallis* sp.	USA	Unknown	Farr & Rossman (2011)
C. liriopes	*H. fulva*	China	CDLG3	This study
C. spaethianum•	*H. citrina*	New Zealand	CBS 101631•	Damm et al. (2009)
		China	CDLL1, CDLL2	This study
	H. fulva	China	CDLG2	This study

Note: ▪, a synonym of *Colletotrichum spaethianum* according to Damm et al. (2009); •, previously reported as *C. capsici* (CBS 101631),★, cited as *Colletotrichum* sp. 2 by Damm et al. (2009).

ACKNOWLEDGEMENTS

This project was supported by the National Natural Science Foundation of China (No. 31070025) and Guizhou Science and Technology Department [No. (2010) 4002-1]. The National Research Council of Thailand awarded grant No. 5420102003 to study the genus *Colletotrichum*. Lei Cai acknowledges grants CAS KSCX2-YW-Z-1026 and NSFC 31110103906. This work was also supported by a grant from the National Plan of Science and Technology, King Abdulaziz City of Science and Technology, Riyadh, Saudi Arabia (10-Bio-965-02).

REFERENCES

Cai L, Hyde KD, Taylor PWJ, Weir BS, Waller J, Abang MM, Zhang JZ, Yang YL, Phoulivong S, Liu ZY, Prihastuti H, Shivas RG, McKenzie EHC, Johnston PR (2009) A polyphasic approach for studying *Colletotrichum*. Fungal Diversity 39:183-204.

Cai L, Udayanga D, Manamgoda DS, Maharachchikumbura SSN, Mckenzie EHC, Guo LD, Liu XX, Bahkali AH, Hyde KD (2011) The need to carry out a re-inventory of plant pathogens. Tropical Plant Pathology 36:205-213.

Cannon PF, Buddie AG, Bridge PD (2008) The epitypification of *Colletotrichum gloeosporioides*. Mycotaxon 104: 189-204.

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91:553-556.

Chen J, Xu LL, Liu B, Liu XZ (2007) Taxonomy of *Dactylella* complex and *Vermispora*. I. Generic concepts based on morphology and ITS sequences data. Fungal Diversity 26:73-83.

Choi YW, Hyde KD, Ho WWH (1999) Single spore isolation of fungi. Fungal Diversity 2:29- 38.

Chomnunti P, Schoch CL, Aguirre-Hudson B, Ko-Ko TW, Hongsanan S, Jones EB, Kodsueb R, Phookamsak R, Chukeatirote E, Bahkali AH, Hyde KD (2011) Capnodiaceae. Fungal Diversity 51:103-134.

Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G (2004) MycoBank: an online initiative to launch mycology into the 21st century. Studies in Mycology 50:19-22.

Cunningham CW (1997) Can three incongruence tests predict when data should be combined? Molecular Biology and Evolution 14:733-740.

Damm U, Woudenberg JHC, Cannon PF, Crous PW (2009) *Colletotrichum* species with curved conidia from herbaceous hosts. Fungal Diversity 39:45-87.

Damm U, Baroncelli R, Cai L, Kubo Y, O'Connell R, Weir B, Yoshino K, Cannon PF (2010) *Colletotrichum*: species, ecology and interactions. Journal of Microbiology and Antimicrobials - Fungus 2:161-165.

Farr DF, Rossman AY (2011) Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. Available at: http://nt.ars-grin.gov/fungaldatabases. Accessed on September 10, 2011.

Farris JS, Kallersjo M, Kluge AG, Bult C (1995) Testing significance of incongruence. Cladistics 10:315-319.

Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology 61:1323-1330.

Gu JY, Xi PG, Li MH, Jiang ZD (2007) Descriptions of pathogenic fungi on six wild vegetables in Guangzhou Region. Jounal of Huazhong Agricultural University 26:41-45. (*In Chinese*)

Guerber JC, Liu B, Johnston P, Correll JC (2003) Characterization of diversity in *Colletotrichum acutatum sensu lato* by sequence analysis of two introns, mtDNA and intron RFLPs, and mating compatibility. Mycologia 95:872-895.

Hyde KD, Cai L, Cannon PF, Crouch JA, Crous PW, Damm U, Goodwin PH, Chen H, Johnston PR, Jones EBG, Liu ZY, McKenzie EHC, Moriwaki J, Noireung P, Pennycook SR, Pfenning LH, Prihastuti H, Sato T, Shivas RG, Tan YP, Taylor PWJ, Weir BS, Yang YL, Zhang JZ (2009) *Colletotrichum* - names in current use. Fungal

Diversity 39:147-183.

Jiang FL, Niu YM, Gu WQ (1993) Studies on anthracnose of *Hemerocallis*. Journal of Zhejiang Forestry College 10:23-29. (*In Chinese*)

Ko Ko TW, Mckenzie EHC, Bahkali AH, To-Anun C, Chukeatirote E, Promputtha I, Abd-Elsalam KA, Soytong K, Wulandari NF, Sanoamuang N, Jonglaekha N, Kobsueb R, Cheewangkoon R, Wikee S, Chamyuang S, Hyde KD (2011) The need for re-inventory of Thai phytopathogens. Chiang Mai Journal of Science 38:1-13.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, GibsonTJ, Diggins DG (2007) Clustal W and Clustal X. version 2.0. Bioinformatics 23:2947-2948.

Ma HF, Meng SM, Zhao HD, Liu JL (2010). Study on extraction of flavonoids from *Hemerocallis fulva* by microwave. Shanxi Chemical Industry 30:28-33. (*In Chinese*)

Moriwaki J, Sato T, Tsukiboshi T (2003) Morphological and molecular characterization of *Colletotrichum boninense* sp. nov. from Japan. Mycoscience 44:47-53.

Munson RW (1989) Hemerocallis, the Daylily Journal. Portland OR. Timber Press.

O'Donnell K, Cigelnik E (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Molecular Phylogenetics and Evolution 7:103-116.

Phoulivong S, Cai L, Chen H, McKenzie Eric HC, Abdelsalam K, Chukeatirote E, Hyde KD (2010) *Colletotrichum gloeosporioides* is not a common pathogen on tropical fruits. Fungal Diversity 44:33-43.

Phoulivong S, Cai L, Parinn N, Chen H, Abd-Elsalam KA, Chukeatirote E, Hyde KD (2010) A new species of *Colletotrichum* from *Cordyline fruticosa* and *Eugenia javanica* causing anthracnose disease. Mycotaxon114:247-257.

Phoulivong S (2011) *Colletotrichum*, naming, control, resistance, biocontrol of weeds and current challenges. Current Research in Environmental & Applied Mycology 1:53-73.

Prihastuti H, Cai L, Chen H, McKenzie EHC, Hyde KD (2009) Characterization of *Colletotrichum* species associated with coffee berries in northern Thailand. Fungal Diversity 39:89-109.

Rojas EI, Rehner SA, Samuels GJ, Van Bael SA, Herre EA, Cannon P, Chen R, Pang JF, Wang RW, Zhang YP, Peng YQ, Sha T (2010) *Colletotrichum gloeosporioides* s. l. associated with *Theobroma cacao* and other plants in Panama: Multilocus phylogenies distinguish host-associated pathogens from asymptomatic endophytes. Mycologia 102:1318-1338.

Staples GW, Kristiansen MS (1999) Ethnic culinary herbs: A guide to identification and cultivation in Hawaii. Honolulu HI. University of Hawaii Press.

Sutton BC (1980) The coelomycetes. Fungi imperfecti with pycnidia, acervuli and stromata. Kew UK. Commonwealth Mycological Institute.

Swofford DL (2003) PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4b10. Sunderland MA. Sinauer Associates.

Than PP, Jeewon R, Hyde KD, Pongsupasamit S, Mongkolporn O, Taylor PWJ (2008) Characterization and pathogenicity of *Colletotrichum* species associated with anthracnose disease on chilli (*Capsicum* spp.) in Thailand. Plant Pathology 57:562-572.

Tomkins JP, Wood TC, Barnes LS, Westman A, Wing RA (2010) Evaluation of genetic variation in the daylily (*Hemerocallis* spp.) using AFLP markers. Plant Cell, Tissue and Organ Culture 102:199-204.

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds.) PCR Protocols: a guide to methods and applications. San Diego CA. Academic Press. pp. 315-322.

Yang YL, Liu ZY, Cai L, Hyde KD, Yu ZN, McKenzie EHC (2009) *Colletotrichum* anthracnose of *Amaryllidaceae.* Fungal Diversity 39:123-146.

Yang YL, Cai L, Yu ZN, Liu ZY, Hyde KD (2011) *Colletotrichum* species on *Orchidaceae* in southwest China. Cryptogamie Mycologie 32:229-253.

Zhang YZ, Chen T (2008) Study on growth and nutrient uptake characteristics of major cultivars of daylily in Hunan Province. Crop research 22:95-100. (*In Chinese*)

Zhou TL, Wang KZ, Zhang GZ, Du YS (1994) Ananlysis of isoperoxidase of 36 day lily kinds. Acta Botanica Boreali-Occidentalia Sinica 14:122-126. (*In Chinese*)

Zhu PF, Zhang LX, Liu L (2008) Compatibility and hybridus embryos culture in vitro between *Hemerocallis hybridus* and *H. citrina*. Northern Horticulture 8:190-191. (*In Chinese*)

TPP 487 - Received 16 January 2012 - Accepted 25 April 2012
Section Editor: Meike Piepenbring