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Rapid communication

Characterization of *Phyllosticta hostae* causing Phyllosticta leaf spot on spider lily in China

Run Hua Yi, Luo Jun Gan, Jing Chen, Xiao Ling Xu*

Department of Biotechnology, Agricultural College, Guangdong Ocean University, Zhanjiang 524088, Guangdong Province, China

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Abstract: Leaf spot disease on the spider lily [*Hymenocallis littoralis* (Jacq.) Salisb.] continues to cause serious problems in China. To confirm the pathogen, the pathogenicity of isolates from diseased leaves was tested according to Koch's postulates. The isolates were tentatively identified using morphological characteristics and confirmation was done by phylogenetic analysis of the translation elongation factor 1-alpha gene (TEF1), the actin gene (ACT), and internal transcibed spacer (ITS) sequences using neighbor-joining (NJ), maximum parsimony (MP), and Bayesian inference (BI) methods. The pathogen was identified as *Phyllosticta hostae*. Molecular analysis indicated very little diversity in the TEF1, ACT, and ITS gene. This is the first report of *P. hostae* causing leaf spot disease on spider lily in China.

Key words: *Hymenocallis littoralis*, leaf spot disease, *Phyllosticta hostae*, spider lily

Introduction

Hymenocallis littoralis (Jacq.) Salisb (members of the Amaryllidaceae), commonly named the spider lily, is a tropical bulbous herb with the staminal membrane surrounded by long and narrow tepals that characterize spider-like flowers (Martín *et al*. 2012). As a horticultural and medicinal plant, the spider lily was introduced to China in the early nineteen-eighties, and is widely cultivated in the provinces of Guangdong, Fujian, Yunnan, and Guangxi. The natural compounds of the spider lily display antineoplastic, antiviral properties, and potent cytotoxicity against human tumor cell lines (Renard-Nozaki *et al*. 1989; Griffin *et al*. 2007; Yew *et al*. 2010).

In June 2011, a new fungal disease, Phyllosticta leaf spot disease found on the spider lily, was noted on the Guangdong Ocean University campus, Zhanjiang, Guangdong Province, China. The disease occurred throughout the year and almost 100% of the plants were infected, which led to the decline of ornamental and medicinal value. Considering the seriousness and the lack of knowledge concerning the disease in China, the objective of this paper was to determine and characterize the pathogen of this disease based on morphology, and phylogenetic analyses.

Materials and Methods

From June 2011 to December 2014, leaves with spots were collected from different sites of Guangdong Province. Specimen which had typical symptoms were dried and preserved. To isolate the pathogen, sections from diseased leaf tissues were cut into pieces approximately 0.5 × 0.5 cm, surface-sterilised with 70% ethanol and 0.1% acid mercuric chloride, rinsed with sterile distilled water, blotted dry on sterile paper, and transferred to plates containing 20 ml Potato Dextrose Agar (PDA) medium amended with 20 $\mu g \cdot ml^{-1}$ ampicillin. Isolation plates were incubated at 28°C in the dark, for 3 days. The hyphae tips growing from the tissues were transferred onto PDA plates to get the axenic cultures. To obtain single spored isolates, conidia from pure cultures were suspended in sterile water and streaked onto PDA plates. The single-conidia forming colonies were obtained after an in-the-dark incubation at 28°C for 24 h. The isolates were incubated at 28°C in the dark for 7–10 days and stored at a 4°C refrigeration for the pathogenicity test, microscopic observation, and DNA extraction.

Isolates were incubated on PDA plates for 7 days at 25°C in the dark. Artificial inoculation was conducted to confirm the pathogenicity test, using the spore suspension and mycelial plugs according to Koch's postulate. Forceps were used to pick up the pycnidia and put them into a mortar. The pycnidia were squashed with sterile distilled water to release the pycnidiospores, the suspension was adjusted so the concentration was approximately 1×10^5 spores $\cdot ml^{-1}$. Approximately 0.5 ml of spore suspension was sprayed on the leaves, which were surface-disinfected with a 70% ethanol tampon three times, washed three times with the sterile distilled water, and acupunctured with sterile needles. Sterile distilled water was sprayed as a control. To keep the humidity level high, the leaves were covered with plastic bags for 48 h.

*Corresponding address:
scibyrh@163.com

Six millimeters mycelial plugs from the colony margin were inoculated onto the sterile surface of the leaves after the leaves had been acupunctured with sterile needles. The absorbent tampons were laid aside, and covered with transparent cellophane that was perforated with a needle for aeration. Sterile PDA plugs were used as the controls. The disease development was observed everyday.

Morphological characteristics on PDA were observed periodically until sporulation, by the methods described by Wulandari *et al*. (2009). Mycelial discs (5 mm diameter) were cut from the edges of the growing areas of the 7-day-old colony, and transferred to the center of Cornmeal Agar (CMA), Malt Extract Agar (MEA), PDA, and Oatmeal Agar (OA) plates, and incubated at 25°C under dark conditions. The mycelial growth rates were measured after incubation for two weeks. The pycnidia on the diseased leaf were examined and crushed to release spores in water mounts on a glass slide, under light microscope (Olympus BX51). Images were acquired with a digital camera DXM 1200F (Nikon). All microscopic characteristics were measured for at least 50 individuals, to calculate the mean size.

Pathogens were grown on Potato Dextrose Broth (PDB) and incubated in a 180 rpm rotatory shaker for 5 days at 25°C. Mycelia collection and genomic DNA exaction followed the protocol of Yi *et al*. (2003). Internal transcribed spacer (ITS) and small subunit (SSU) sequences were respectively amplified using the primer pair ITS1/ITS4 and NS1/NS4 (White *et al*. 1990). Primer pair EF1-728F (Carbone and Kohn 1999) and EF-2 (O'Donnell *et al*. 1998) were used to amplify the partial translation elongation factor 1-alpha gene (TEF1). The primer pair ACT-512F (Carbone and Kohn 1999) and ACT2Rd (Quaedvlieg *et al*. 2011) were used to amplify the partial actin gene (ACT).

To amplify the different loci, the polymerase chain reaction (PCR) mixture that was used consisted of template DNA (5–10 ng), 1.5 U Taq DNA polymerase, 1X PCR buffer, 0.25 mM of each dNTP, and 0.2 μM of each primer, and made up to a total reaction volume of 50 μl with sterile double-distilled water (ddH_2O). The cycling parameters were started with a long initial denaturation step at 95°C for 4 min, 35 cycles of denaturation at 94°C for 60 sec, annealing at 50°C for SSU primers and 55°C for ITS, ACT, and TEF primers for 60 sec, extension at 72°C for 60 sec followed by a final extension at 72°C for 10 min, and a soaking at 4°C for 3 h. Amplified fragments were purified and sequenced by the Sangon Biotech (Shanghai, China) Co., Ltd.

The sequences from the forward and reverse primers were assembled and deposited in the GenBank. The novel sequences of three isolates in this study, together with the additional reference sequences of representative *Phyllosticta* taxa were retrieved from GenBank (Table 1). The alignment of the obtained sequences was performed and truncated at the 5′- and 3′-ends using BioEdit (Hall 1999). Phylogenetic trees were constructed using neighbor joining (NJ), maximum parsimony (MP), and Bayesian inference (BI) methods. It should be noted, that the NJ, MP, and BI analyses were performed using MEGA5.0 (Tamura *et al*. 2011), PAUP4.0 (Swofford 2003), and MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003), respectively.

For NJ analyses, the evolutionary distances were computed using the Kimura 2-parameter model with 1,000 bootstrap replicates. Maximum-parsimony analyses were estimated using the heuristic searches with tree bisection reconnection (TBR) branch swapping and 1,000 random addition sequences. Branches of zero length were collapsed. Statistics calculated for parsimony included tree length (TL), consistency index (CI), retention index (RI), and the rescaled consistence index (RC). For Bayesian analyses, the best fit nucleotide substitution model was evaluated using jModelTest v2.1.4 (Santorum *et al*. 2014) according to the Bayesian information criterion (BIC). The model (GTR+I+G) and parameters lset apply to = (1/DNA) nst = 6 rates = invgamma; unlink shape = (all) pinvar = (all) statefreq = (all) revmat = (all); prset ratepr = variable statefreqpr = dirichlet (1,1,1,1); were applied. Posterior probabilities (PP) were determined by the Markov Chain Monte Carlo (MCMC) method (Mossel and Vigoda 2005), four MCMC chains were run simultaneously from random trees for ten million generations, sampled every 1,000 generations, and repeated twice. The first 25 percentages of trees were discarded as the burn-in phase of each analysis. The remaining trees were used for determining posterior probabilities and were shown in the majority rule consensus tree. The aligned data and phylogenetic trees were deposited in TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S14438).

Results

Phyllosticta leaf spot on spider lily occurred all year but became most serious during late September to early October in Guangdong Province. Small, circular or oval, brown-reddish to black spots occurred on the leaves in the early period. Spots sometime formed to be concave, oval spots, occasionally surrounded by a water-soaked, discolored halo. The spots gradually enlarged to be big patches. Subsequently, the diseased leaves became yellow with blighting at the tip (Fig. 1A). Pycnidia appeared on the infected positions or at the leaf tip (Fig. 1B).

The leaves with typical symptoms and with pycnidia were collected from the campus of the Guangdong Ocean University, Zhanjiang, Guangdong Province, China (E110°17′47″ N21°08′58″). The collected leaves were dried at 40°C for a week and deposited in the Mycological Herbarium of Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS). The numbers of the voucher herbarium specimens were HMAS 244379 and HMAS 244380.

From different sites, leaves with various degrees of severity and water-soaked discolored patches, brown-black patches, concave oval spots, and irregular big patches, were sampled so as to isolate the pathogens. The same morphological characteristics of isolates were obtained from all the diseased leaf tissues. On PDA, these isolates displayed the *Phyllosticta*-like characteristics of being lobate, and irregular with feathery margins.

Two representative isolates, CGMCC 3.15214 and CGMCC 3.15215, were selected for the pathogenicity test and deposited in the China General Microbiological Culture Collection Center (CGMCC). Three days after

Table 1. Sources of *Guignardia* and *Phyllosticta* isolates and GenBank accession numbers used in this study

Species	Strain voucher number	Host	Locality	GenBank accession number		
				ITS	TEF	ACT
Botryosphaeria obtusa	CMW 8232	Conifers	South Africa	AY972105	DQ280419	AY972111
Guignardia bidwellii	CBS 111645	*Parthenocissus quinquefolia* (Vitaceae)	USA	JN692542	JN692530	JN692518
G. mangiferae	IMI 260576*	*Mangifera indica*	India	JF261459	JF261501	JF343641
G. vaccinii	CBS 126.22	*Vaccinium macrocarpon* (Ericaceae)	USA	FJ538353	FJ538411	FJ538469
Phyllosticta aloeicola	CPC 21020	*Aloe ferox*	South Africa	KF154280	KF289193	KF289311
P. capitalensis	CBS 128856*	*Stanhopea* sp.	Brazil	JF261465	JF261507	JF343647
P. citriasiana	CBS 120486*	*Citrus maxima* (Rutaceae)	Thailand	FJ538360	FJ538418	FJ538476
P. citribraziliensis	CBS 100098*	*Citrus* sp. (Rutaceae)	Brazil	FJ538352	FJ538410	FJ538468
P. citricarpa	CBS 127454*	*Citrisx limon* (Rutaceae)	Australia	JF343583	JF343604	JF343667
P. citrichinaensis	CBS 130529*	*Citrus maxima* (Rutaceae)	China	JN791597	JN791452	JN791526
P. citrimaxima	CPC 20276*	*Citrus maxima*	Thailand	KF170304	KF289222	KF289300
P. concentrica	CBS 937.70*	*Hedera helix*	Italy	FJ538350	FJ538408	KF289257
P. cordylinophila	CPC 20261*	*Cordyline fruticosa* (*Asparagaceae*)	Thailand	KF170287	KF289172	KF289295
P. cussonia	CPC 14875*	*Cussonia* sp. (Araliaceae)	South Africa	JF343579	JF343600	JF343663
P. ericarum	CBS 132534*	*Erica gracilis* (Ericaceae)	South Africa	JX069865	KF289227	KF289291
P. foliorum	CBS 447.68 *	*Taxus baccata*	Netherlands	KF170309	KF289201	KF289247
P. gaultheriae	CBS 447.70*	*Gaultheria humifusa*	USA	JN692543	JN692531	KF289248
P. hostae	CGMCC 3.14355*	*Hosta plantaginea* (Liliaceae)	China	JN692535	JN692523	JN692511
P. hostae	CGMCC 3.14356	*Hosta plantaginea* (Liliaceae)	China	JN692536	JN692524	JN692512
P. hostae	CGMCC 3.15214	*Hymenocallis littoralis* (Amaryllidaceae)	China	JX524169	KJ094498	KJ094496
P. hostae	CGMCC 3.15215	*Hymenocallis littoralis* (Amaryllidaceae)	China	KC995113	KJ094499	KJ094495
P. hostae	GDOU 201203	*Hymenocallis littoralis* (Amaryllidaceae)	China	KC995114	KJ094500	KJ094497
P. hubeiensis	CGMCC3.14986*	*Viburnum odoratissimum* (*Adoxaceae*)	China	JX025037	JX025042	JX025032
P. hymenocallidicola	CBS 131309*	*Hymenocallis littoralis* (Amaryllidaceae)	Australia	JQ044423	KF289211	KF289242
P. hypoglossi	CBS 434.92*	*Ruscus aculeatus*	Italy	FJ538367	FJ538425	FJ538483
P. ilicis-aquifolii	CGMCC3.14358*	*Ilex aquifolium* (Aquifoliaceae)	UK	JN692538	JN692526	JN692514
P. paxistimae	CBS 112527*	*Paxistima mysinites*	USA	KF206172	KF289209	KF289239
P. philoprina	CBS 587.69	*Ilex aquifolium* (Aquifoliaceae)	Spain	KF154278	KF289206	KF289250
P. podocarpicola	CBS 728.79*	*Podocarpus maki*	USA	KF206173	KF289203	KF289252
P. rubra	CBS 111635*	*Acer rubrum*	USA	KF206171	KF289198	KF289233
P. spinarum	CBS 292.90	*Chamaecyparis pisifera* (Cupressaceae)	France	AF312009	JF343606	JF343669
P. styracicola	CGMCC3.14985*	*Styrax grandiflorus* (Styracaceae)	China	JX025040	JX025045	JX025035
P. telopeae	CBS 777.97*	*Telopea speciosissima*	Tasmania	KF206205	KF289210	KF289255
P. vacciniicola	CPC 18590*	*Vaccinium macrocarpum*	USA	KF170312	KF289229	KF289287
P. yuccae	CBS 117136	*Yucca elephantipes* (Asparagaceae)	New Zealand	JN692541	JN692529	JN692517

*indicates the ex-type cultures; CGMCC – China General Microbiological Culture Collection Center, China; CBS – Centra albureau voor Schimmel cultures, Utrecht, The Netherlands; MUCC – Lab. of Plant Pathology, Mie University; GDOU – Lab. of Plant Pathology, Guangdong Ocean University; CPC – Culture collection of P.W. Crous, housed at CBS

ITS – internal transcribed spacer; TEF – translation elongation factor; ACT – actin gene

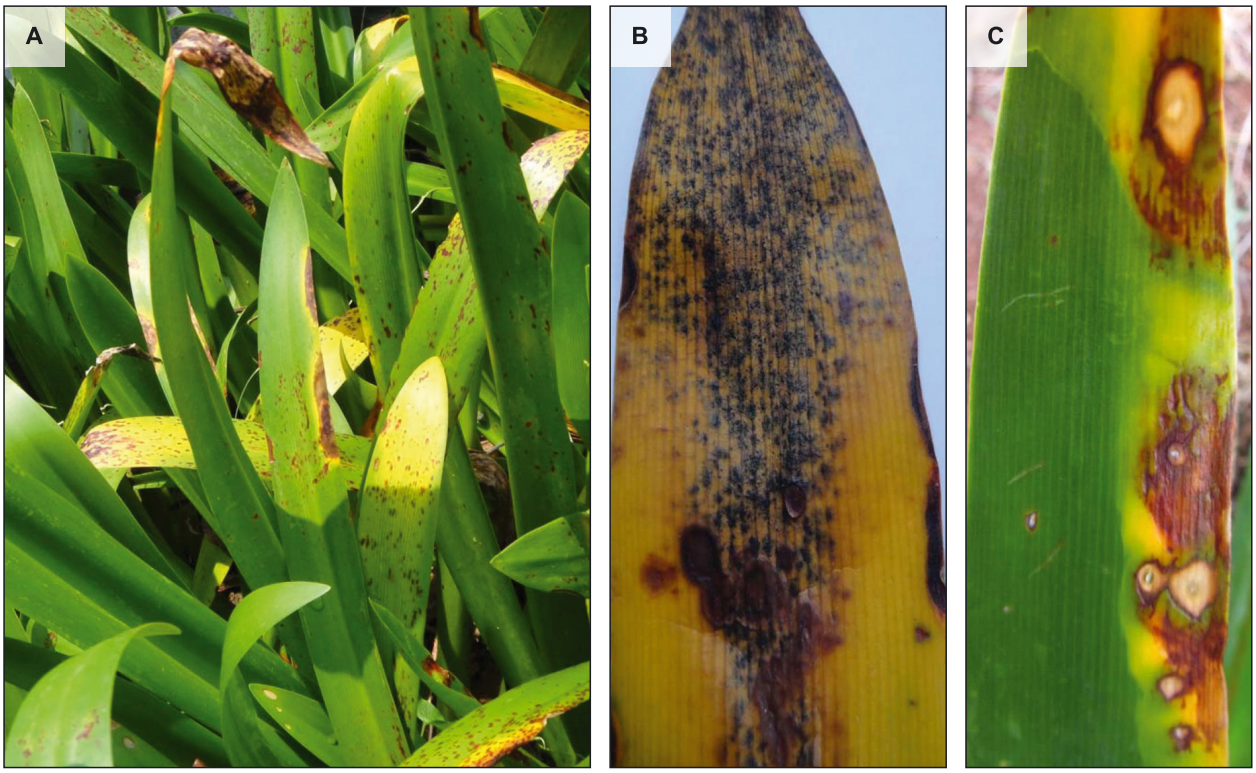

Fig. 1. Typical foliar symptom of Phyllosticta leaf spot on *Hymenocallis littoralis*: A – small, circular or oval, brown-reddish to black spots on leaves and the yellowing and blighting leaves at the tip; B – black pycnidia on diseased leaf; C – leaf with artificial inoculation

inoculation with spore suspension, tiny water-soaked spots appeared on the artificially inoculated positions. Gradually, the same symptoms developed as in nature (Fig. 1C), whereas the control leaves did not develop any symptoms. The water-soaked and discolored symptoms occurred later when using the mycelial plus inoculation, than with the spores suspension. The same morphological fungi were re-isolated from these diseased tissues.

The isolates morphology characteristics were described as below:

Phyllosticta hostae Y.Y. Su & L. Cai, Persoonia 28, 76–84 (2012) (Fig. 2).

Conidiomata pycnidial, solitary, black, erumpent, globose, separate or in small groups, embedded in a subepidermal stroma on host, stromata globose to ampulliform, forming an irregularly folded crust on PDA, exuding a colorless to opaque glossy conidial mass; pycnidia 41.6–150.3 (av. 92.3±22.4) μm in diam; pycnidial wall consisting of several layers of brown textura angularis, 8.7–24.7 (av. 16.1±3.3) μm thick; ostiole single, central, 15.1–36.0 (av. 25.5±5.2) μm wide, consisting of thickened, brown cells. Conidiophores subcylindrical to doliiform, frequently reduced to conidiogenous cells, coated in a mucoid layer. Conidiogenous cells termina terminal, subcylindrical to doliiform, hyaline, smooth, 6.3–23.8 (av. 14.3±3.3) × 2.3–6.8 (av. 4.4±0.9) μm. Conidia 5.2–12.5 (av. 8.5±2.5) × 3.8–8.6 (av. 6.4±1.8) μm, solitary, hyaline, aseptate, thin- and smooth- walled, smoothly guttulate, ellipsoid to obovoid, tapering toward a narrowly truncate base, enclosed in a mucilaginous sheath, 1.8–4.0 (av. 2.1±0.5) μm thick, and bearing a hyaline, mucoid apical appendage, 5.8–48.1 (av. 15.9±6.6) × 1.3–2.1 (av. 1.7±0.2) μm, straight to flexible, unbranched, tapering towards an acute apex; conidia length/width ratio 1.2–2.0 (av. 1.6±0.2).

Culture characteristics (in the dark, 25°C after 2 weeks): colonies reaching 74.2 mm diam. after 2 weeks on MEA, 76.8 mm on PDA and 71.3 mm on CMA, but only 55.3 mm on OA. Colonies on PDA were flat, the surface was black in the centre, spreading, lobate, irregular with feathery margin, sparse aerial mycelium, surface olivaceous grey, and reverse black, olivaceous at margin. Colonies on MEA were flat, spreading, irregular, with smooth, lobate margin, and sparse aerial mycelium. The surface was leaden to leaden-gray in colour. Colonies on OA were flat, with feathery, lobate margins and sparse aerial mycelium, olivaceous-black in the centre, pale olivaceous in the outer region. Colonies on CMA were flat, with feathery margins. The surface was dark in the centre, and an olive colour in the outer regions. No teleomorph was observed in agar (OA, PDA, MEA, and CMA).

Based on a megablast search of NCBIs GenBank nucleotide database, the closest hit using the ITS sequence of isolates CGMCC 3.15214 and CGMCC 3.15215 is *P. hostae* CGMCC 3.14357 [causing leaf spot of *Hosta plantaginea* in China, GenBank JN692537; Identities = = 549/552 (99.457%), Gaps = 2/552 (0.362%)]. A megablast search of the NCBIs GenBank nucleotide sequence database using the SSU sequence (GenBank JX524170) of CGMCC 3.15214 confirms its placement in the genus; closest hits included *Guignardia* sp. IFB-GLP-4 [GenBank GU380271; Identities = 1040/1063 (97.856%), Gaps = = 19/1063 (1.787%)].

The thirty-five combined sequences from 30 taxa of *Phyllosticta* (teleomorph: *Guignardia*) were phylogeneti-

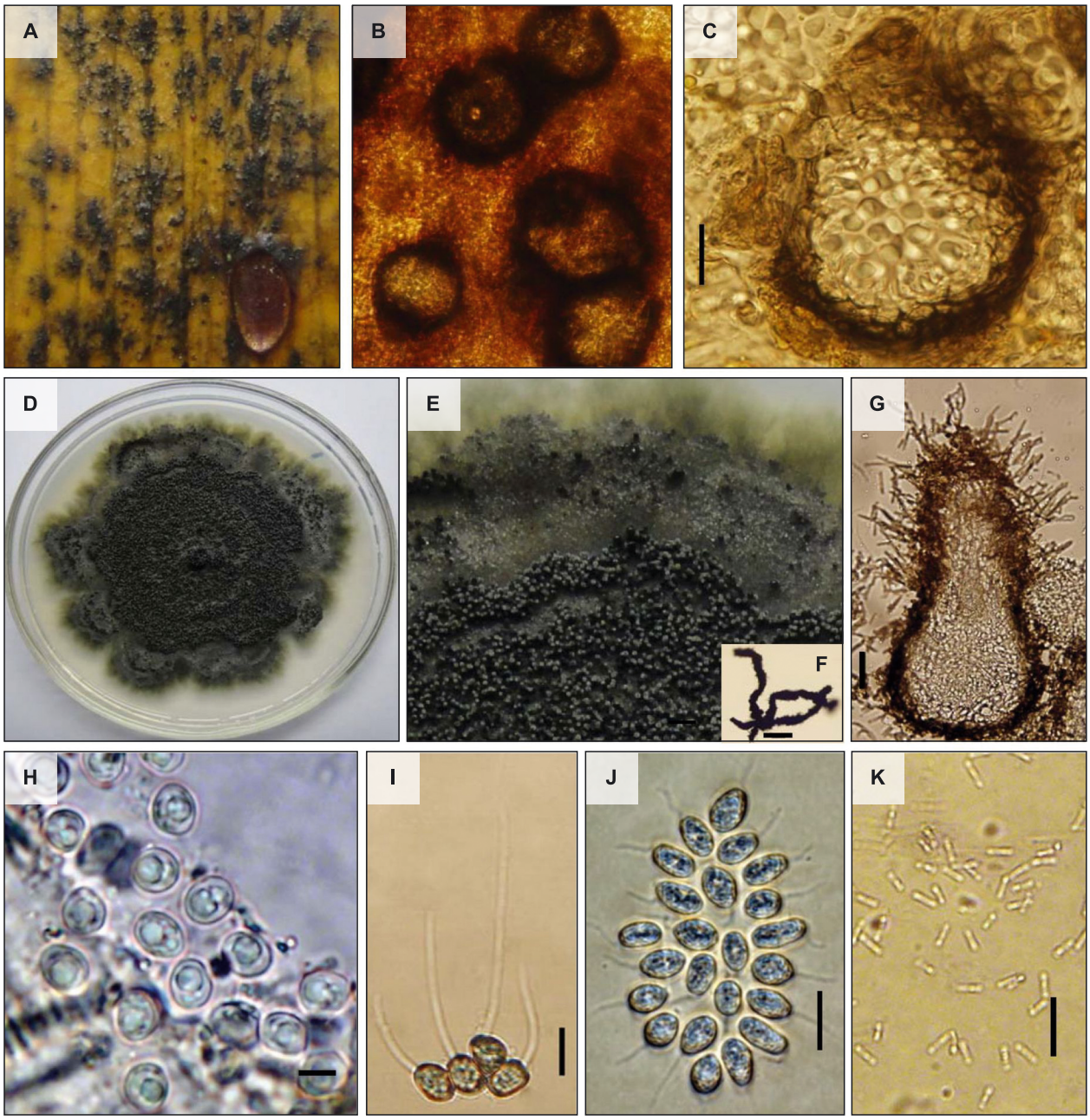

Fig. 2. Morphological characteristics of pathogen causing Phyllosticta leaf spot disease on spider lily: A – symptoms with pycnidia forming on leaf of *Hymenocallis littoralis*; B – an overlook of pycnidia with an ostiole in leaf tissue; C – vertical section of pycnidium in leaf tissue; D – colony on PDA in the dark, 25°C, after 15 d; E–F – pycnidia forming on PDA; G – vertical section of initial pycnidium on PDA; H–J – conidia with mucoid sheath and apical mucilaginous appendage, H–I – conidia forming on host; J – conidia forming on PDA; K – spermatium. Scale Bars: C = 20 μm; F = 500 μm; G = 50 μm; H = 10 μm; I, J, K = 20 μm

cally analysed using the combined ITS, ACT, and TEF dataset. *Botryosphaeria obtusa* CMW8232 was used as an outgroup. The combined dataset comprised 1106 total characters including gaps, of which 548 characters were constant, 338 were parsimony informative, and 220 were variable and parsimony-uninformative.

Phylogenetic analysis using NJ, MP, and Bayesian inference yielded evolutionary trees with identical topology, which represented the evolutionary history of *Phyllosticta* taxa. The isolates of CGMCC 3.15214, CGMCC 3.15215, and GDOU201203 formed a monophyletic group with *P. hostae* CGMCC3.14355 and CGMCC3.14356◎which was strong supported by the bootstrap value and Bayesian posterior probability (Fig. 3). The isolates also formed a heterogenous group with *P. hymenocallidicola* CBS 131309, which was isolated from *H. littoralis* and caused leaf spot and tip blight disease (Crous *et al*. 2011).

Phylogenetically, the isolates of CGMCC 3.15214, CGMCC 3.15215, and GDOU201203 clustered together with *Phyllosticta hostae* isolates based on all three of the gene regions sequenced. Three fixed nucleotide changes were observed over 548 nucleotides for ITS; whereas TEF1 only contained one fixed nucleotide change over 258 nucleotides, and ACT had only 3 fixed nucleotide changes and 3 indels over 232 nucleotides (Table 2).

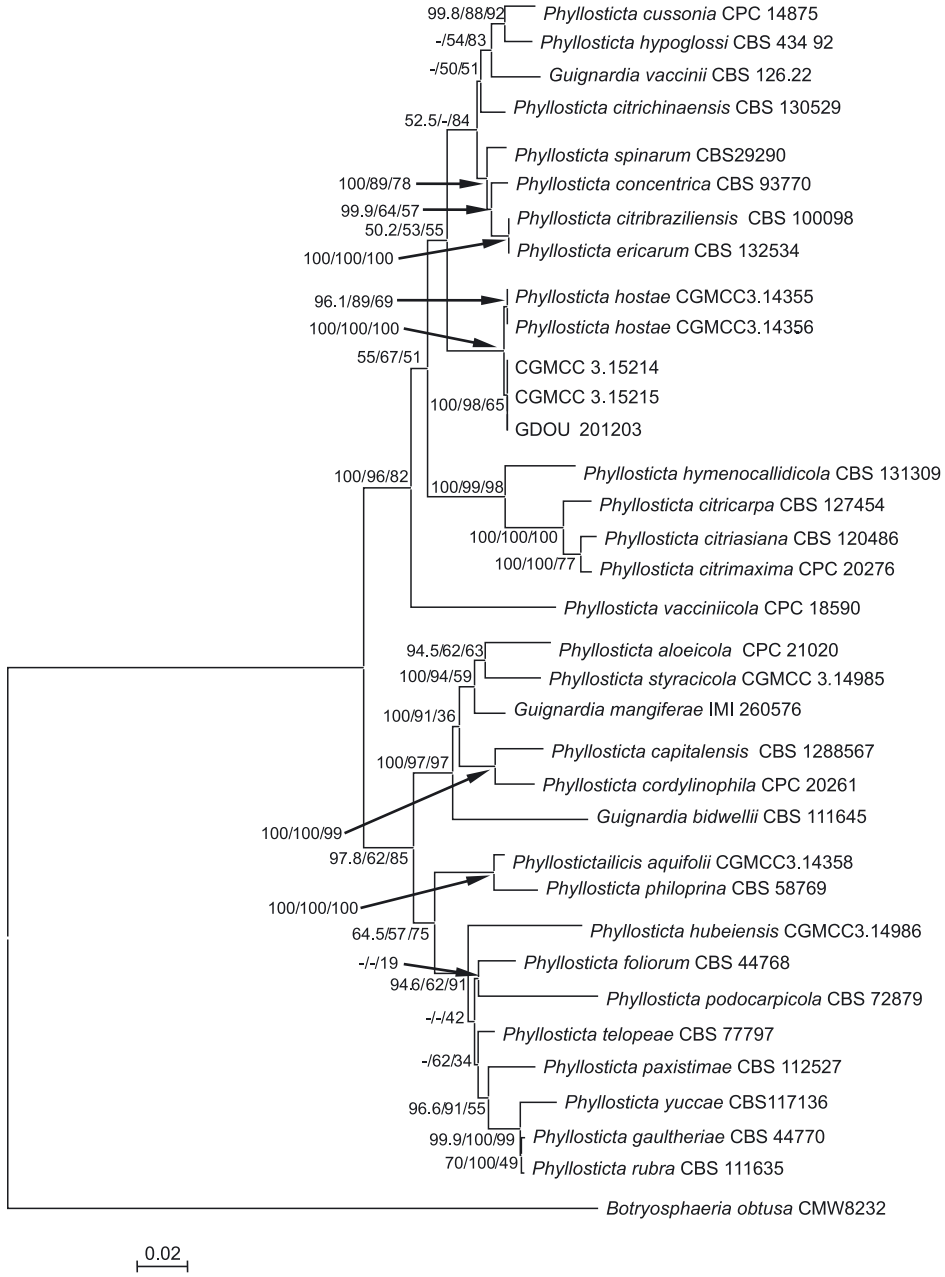

Fig. 3. Phylogenetic tree of *Phyllosticta* species reconstructed by neighbor-joining (NJ), maximum parsimony (MP) and Bayesian inference (BI) using the combined internal transcribed spacer (ITS), partial actin gene (ACT) and translation elongation factor (TEF) dataset. The numbers along the branches indicate posterior probability values (%) or bootstrap percentages resulting from different analyses in the order: BI/MP/NJ. The values lower than 50 are given as "–" except for NJ

Table 2. Nucleotide differences and their base positions observed in three loci among the isolates of *Phyllosticta hostae*. Sequences of CGMCC3.14355 were used as references to calculate base positions, which do not include spaces caused by alignment gaps

Isolates	ITS			ACT						TEF	References
	33	150	151	102	111	186	187	188	232	253	
CGMCC3.14355	A	C	T	A	C	C	G	C	G	T	Crous *et al.* 2011
CGMCC3.14356	A	C	T	A	C	C	G	C	G	T	Crous *et al.* 2011
CGMCC3.15215	G	T	C	G	T	–	–	–	A	C	this study
CGMCC3.15214	G	T	C	G	T	–	–	–	A	C	this study
GDOU 201203	G	T	C	G	T	–	–	–	A	C	this study

ITS – internal transcribed spacer; ACT – partial actin gene; TEF – translation elongation factor

Table 3. *Phyllosticta* spp. described from Amaryllidaceae and *Hosta plantaginea*

Phyllosticta species	Host plant	Pycnidia size [μm]	Conidiogenous cells [μm]	Conidia [μm]	Appendage size [μm]	References
P. amaryllidicola	*Amaryllis* sp.	120–150	2–6 × 1.5–4	9–12 × 5.5–7.5	4–7	Van der Aa 1973
P. cliviae	*Clivia nobilis*	105–150	–	3–6 × 2–3	–	Saccardo 1972
P. hymenocallidicola	*Hymenocallis littoralis*	200	7–15 × 3–4	(8–)9–10(–11) × × (6–)6.5–7	3–5(–8) × 1.5(–2)	Crous *et al*. 2011
P. hostae	*Hosta plantaginea*	40–150	7–22 × 2–5	8–15 × 5–9	4–8 × 1–3	Su and Cai 2012
P. hostae	*Hymenocallis littoralis*	41.6–150.3	6.3–23.8 × × 2.3–6.8	5.2–12.5 × × 3.8–8.5	5.8–48.1 × 1.3–2.1	this study

Discussion and Conclusions

Recognition of the *Phyllosticta* species was based on host and morphological characteristics. Because of its narrow host range, if two or more taxa were obtained from the same host, then the symptoms, ecological and pure culture characteristics under well-defined conditions, and molecular phylogenetic method were used for classification (Van der Aa 1973; Wikee *et al.* 2011).

The three isolates: CGMCC3.15214, CGMCC3.15215, and GDOU201203 can be distinguished morphologically or/and phylogenetically from other *Phyllosticta* spp. on Amaryllidaceae (Table 3). Nine *Phyllosticta* species had been recorded on Amaryllidaceae. The nine were: *P. amaryllidicola, P. amaryllidis, P. cliviae, P. crinicola, P. gemmipara, P. hymenocallidicola, P. hymenocallidis, P. narcissi*, and *P. oudemansii*. Six species, i.e. *P. amaryllidis, P. crinicola, P. gemmipara, P. hymenocallidis, P. narcissi*, and *P. oudemansii,* did not belong to *Phyllosticta.* Van der Aa and Vanev (2002) treated *P. amaryllidis* as a synonym of *Asteromella amaryllidis*. *Phyllosticta crinicola,* as a homotypic synonyms of *Phoma crinicola,* was renamed as *Boeremia crinicola* (Boerema 1978; Aveskamp *et al.* 2010). *Phyllosticta gemmipara* was a heterotypic synonym of *Phoma exigua* var. *exigua* and was transferred to *Boeremia exigua* var. *exigua* (Boerema 1993; Aveskamp *et al*. 2010). *Phyllosticta oudemansii* was a competing homonym of *P. narcissi*, while *P. narcissi* and *P. hymenocallidis*, as basionym of *Phoma narcissi,* whose current name is *Peyronellaea curtisii* (Boerema 1993; Boerema *et al*. 2004; Aveskamp *et al*. 2010).

Phyllosticta amaryllidicola, with the teleomorph *Guignardia sansevieriae* was changed to *G. mangiferae* (Anamorph: *Phyllosticta capitalensis*) (Punithalingam 1974; Wulandari *et al*. 2009), while *P. capitalensis* occurred on *Stanhopea* (Orchidaceae) causing leaf spot disease in glasshouses (Hennings 1908), and belonged to a different branch with CGMCC 3.15214, CGMCC 3.15215, and GDOU201203 in the phylogenetic tree (Fig. 3). *Phyllosticta cliviae*, on host of *Clivia nobilis,* produced smaller conidia than the three isolates, 3–6 × 2–3 μm *vs*. 5.2–12.5 (av. 8.5±2.5) × 3.8–8.6 (av. 6.4±1.8) μm (Saccardo 1972).

Only two species of *Phyllosticta* were known to occur on *H. littoralis*, namely *P. hymenocallidis* and *P. hymenocallidicola. Phyllosticta hymenocallidis,* formerly named as *P. narcissi*, *P. oudemansii*, and *Phoma narcissi*, was a synonym of *Peyronellaea curtisii* (Berk.) Aveskamp, Gruyter and Verkley 2010, which causes leaf scorch, neck rot, red spot disease, red leaf spot disease of *Narcissus* spp., *Hippeastrum* spp. and other Amaryllidaceae (Boerema 1993; Boerema *et al*. 2004; Aveskamp *et al*. 2010). *Phyllosticta hymenocallidicola*, which causes brown leaf spots and leaf tip blight of *H. littoralis*, differs from the characters of CGMCC 3.15214, CGMCC 3.15215, and GDOU201203. The pycnidia is smaller than *P. hymenocallidicola*, 41.6–150.3 (av. 92.1) μm *vs.* 200 μm in diameter, the ostiole is wider, 15.1–36.0 (av. 25.5) μm *vs.* 20 μm, the size of the conidiogenous cells are bigger, 6.3–23.8 (av. 14.3) × 2.3–6.8 (av. 4.4) μm *vs.* 7–15 × 3–4 μm, and the appendage is longer, 5.8–48.1 (av. 15.9±6.6) × 0.5–1.0 μm *vs*. 3–5(–8) × 1.5(–2) μm (Crous *et al*. 2011). The ITS sequence (GenBank JX524169) of CGMCC 3.15214 showed significant non-similarity with *P. hymenocallidicola* CBS 131310 [GenBank JQ044424, Identities = 584/630 (92.698%), Gaps = 13/630 (2.063%)]. Phylogenetic analysis using NJ, MP, and Bayesian showed that *P. hymenocallidicola* CBS 131310 was clustered into different groups with CGMCC 3.15214, CGMCC 3.15215, and GDOU201203.

Morphologically and phylogenetically, the isolates CGMCC 3.15214, CGMCC 3.15215, and GDOU201203 were closest to *P. hostae* on *H. plantaginea* (Liliaceae) (Table 3, Fig. 3). However, they were different from *P. hostae* by the longer appendage [5.8–48.1 (av.15.9±6.6) × 0.5–1.0 μm *vs.* 4–8 × 1–3 μm in *P. hostae*], by the smaller conidia, 5.2–12.5 (av. 8.5±2.5) × 3.8–8.6 (av. 6.4±1.8) μm *vs.* 8–15 (av. 10.9±1.4) × 5–9 (av. 7.6±0.8) μm in *P. hostae*, and by the color difference in the colony centre and at the margin on PDA, the black in the centre and the olivaceous color at the margin *vs. P. hostae* with a leaden-grey colour in the centre and lavender-grey at the margin (Su and Cai 2012).

In conclusion, Phyllosticta leaf spot disease on spider lily was caused by *P. hostae* Y.Y. Su & L. Cai (Su and Cai 2012).

References

Aveskamp M.M., de Gruyter J., Woudenberg J.H.C., Verkley G.J.M., Crous P.W. 2010. Highlights of the Didymellaceae: A polyphasic approach to characterise *Phoma* and related pleosporalean genera. Studies in Mycology 65 (1): 1–60.

Boerema G.H. 1978. Diagnostiek Mycologie. Verslagen en Mededelingen van de Plantenziektenkundige Dienst Wageningen 153 (18): 11–21.

Boerema G.H. 1993. Contributions towards a monograph of *Phoma* (*Coelomycetes*). II. Section *Peyronellaea*. Persoonia – Molecular Phylogeny and Evolution of Fungi 15 (2): 197–221.

Boerema G.H., De Gruyter J., Noordeloos M.E., Hamers M.E.C. 2004. *Phoma* identification manual: differentiation of specific and infra-specific taxa in culture. CABI Publishing, Wallingford, Oxfordshire, 470 pp.

Carbone I., Kohn L.M. 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91 (3): 553–556.

Crous P.W., Summerell B.A., Shivas R.G., Romberg M., Mel'nik V.A., Verkley G.J.M., Groenewald J.Z. 2011. Fungal Planet description sheets: 92–106. Persoonia – Molecular Phylogeny and Evolution of Fungi 27 (1): 130–162.

Griffin C., Sharda N., Sood D., Nair J., McNulty J., Pandey S. 2007. Selective cytotoxicity of *Pancratistatin*-related natural *Amaryllidaceae* alkaloids: evaluation of the activity of two new compounds. Cancer Cell International 7 (1): 10. DOI: 10.1186/1475-2867-1187-1110.

Hall T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Hennings P. 1908. Fungi S. Paulenses IV a cl. *Puttemans collecti*. Hedwigia 48: 481–420.

Martín J., Raymúndez M.B., Vallès J., Garnatje T., Raimúndez E. 2012. Palynological study of the Venezuelan species of the genus *Hymenocallis* (Amaryllidaceae). Plant Systematics and Evolution 298 (4): 695–701.

Mossel E., Vigoda E. 2005. Phylogenetic MCMC algorithms are misleading on mixtures of trees. Science 309 (5744): 2207–2209.

O'Donnell K., Kistler H., Cigelnik E., Ploetz R. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. Proceedings of the National Academy of Sciences of the United States of America 95 (5): 2044–2049.

Punithalingam E. 1974. Studies on *Sphaeropsidales* in culture. II. Mycological Papers 136: 1–63.

Quaedvlieg W., Kema G.H.J., Groenewald J.Z., Verkley G.J.M., Seifbarghi S., Razavi M., Gohari A.M., Mehrabi R., Crous P.W. 2011. *Zymoseptoria* gen. nov.: a new genus to accommodate *Septoria*-like species occurring on graminicolous hosts. Persoonia – Molecular Phylogeny and Evolution of Fungi 26 (1): 57–69.

Renard-Nozaki J., Kim T., Imakura Y., Kihara M., Kobayashi S. 1989. Effect of alkaloids isolated from *Amaryllidaceae* on herpes simplex virus. Research in Virology 140 (2): 115–128.

Ronquist F., Huelsenbeck J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19 (12): 1572–1574.

Saccardo P.A. 1972. Sylloge fungorum omnium hucusque cognitarum. XXVI., Johnson Reprint Corporation, New York, 1563 pp.

Santorum J.M., Darriba D., Taboada G.L., Posada D. 2014. jmodeltest.org: selection of nucleotide substitution models on the cloud. Bioinformatics 30 (9): 1310–1311.

Su Y.Y., Cai L. 2012. Polyphasic characterisation of three new *Phyllosticta* spp. Persoonia – Molecular Phylogeny and Evolution of Fungi 28 (1): 76–84.

Swofford D.L. (ed.) 2003. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sunderland, Massachusetts.: Sinauer Associates.

Tamura K., Peterson D., Peterson N., Stecher G., Nei M., Kumar S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28 (10): 2731–2739.

Van der Aa H.A. 1973. Studies in *Phyllosticta* I. Studies in Mycology 5: 1–110.

Van der Aa H.A., Vanev S. 2002. A revision of the species described in *Phyllosticta*, Utrech. Centraalbureau voor Schimmelcultures, The Netherlands, Utrecht, 510 pp.

White T., Bruns T., Lee S., Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. p. 315–322. In: "PCR Protocols: A Guide to Methods and Applications." (M. Innis, D. Gelfand, J. Shinsky, T. White, eds.). Academic Press, San Diego, CA, USA, 461 pp.

Wikee S., Udayanga D., Crous P.W., Chukeatirote E., McKenzie E.H.C., Bahkali A.H., Dai D., Hyde K.D. 2011. *Phyllosticta* – an overview of current status of species recognition. Fungal Diversity 51 (1): 43–61.

Wulandari N.F., To-anun C,. Hyde K.D., Duong L.M., de Gruyter J., Meffert J.P., Groenewald J.Z., Crous P.W. 2009. *Phyllosticta citriasiana* sp. nov., the cause of *Citrus* tan spot of *Citrus maxima* in Asia. Fungal Diversity 34 (1): 23–39.

Yew C.K., Balakrishnan B., Sundasekaran J., Subramaniam S. 2010. The effect of cytokinins on *in vitro* shoot length and multiplication of *Hymenocallis littoralis*. Journal of Medicinal Plants Research 4 (24): 2641–2646.

Yi R.H., Zhu X.R., Zhou E.X. 2003. Simplified CTAB method for quickly extracting the trace of filamentous fungal DNA. Journal of Zhanjiang Ocean University 23 (6): 72–73. (in Chinese)