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Lasiodiplodia chinensis, a new holomorphic species from China

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Abstract

A new species of *Lasiodiplodia* (*L. chinensis*) is described and illustrated from several hosts collected from Hainan and Shandong Province in China. Both sexual and asexual states of *L. chinensis* were observed, which is characterized by its broadly clavate to clavate asci, fusiform, hyaline and aseptate ascospores, and initially hyaline, aseptate, ovoid to ellipsoid conidia that become pigmented and 1–2-septate with longitudinal striations when mature. Phylogenetically, *L. chinensis* is closely related to *L. pseudotheobromae*, *L. sterculiae* and *L. lignicola*. Morphological comparisons of these four species lead to the conclusion that the collected taxon is new to science

Key words – Botryosphaeriaceae – phylogeny – sexual morph – taxonomy

Introduction

Lasiodiplodia was formally introduced by Clendenin, (1896), and typified by *L. theobromae* (Phillips et al. 2013). Species of *Lasiodiplodia* are mostly distributed in tropical and subtropical regions where they can cause cankers, die-back, fruit or root rot, branch blight or discoloration on a wide range of woody hosts (Punithalingam 1980, Ismail et al. 2012, Phillips et al. 2013, Cruywagen et al. 2016). Forty-two species have been included in *Lasiodiplodia* (http://www.mycobank.org, Oct. 2016), of which thirty species have been described since 2004 (Phillips et al. 2013, Machado et al. 2014, Netto et al. 2014). Presently 31 species are known from culture (Dissanakake et al. 2016). However, the sexual morphs of *Lasiodiplodia* species have rarely been recorded and are known for only four *Lasiodiplodia* species, namely, *L. gonubiensis* Pavlic, Slippers & M.J. Wingf., *L. lignicola* (Ariyaw., J.K. Liu & K.D Hyde) A.J.L. Phillips, A. Alves & Abdollahz, *L. theobromae* (Pat.) Griffon & Maubl. and *L. pseudotheobromae* A.J.L. Phillips, A. Alves & Crous (Liu et al. 2012, Phillips et al. 2013, Trakunyingcharoen et al. 2015, Tennakoon et al. 2016).

Lasiodiplodia pseudotheobromae was introduced by Alves et al. (2008) as a cryptic species in *L. theobromae* (Pat.) Griffon & Maubl, which is a cosmopolitan pathogen that occurs on a large number of hosts in the tropics and subtropics (Alves et al. 2008, Machado et al. 2014, Wei et al. 2014, Dissanayake et al. 2015, Awan et al. 2016). In the course of an ongoing survey of Botryosphaeriaceae in China, a new species of *Lasiodiplodia* was obtained from woody plants in Hainan and Shandong Province. This species is closely related to but differs from *L. pseudotheobromae*. In this paper, we name and describe this species based on its sexual and asexual morphology, and resolve its phylogenetic position within *Lasiodiplodia*.

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Materials & Methods

Isolates and morphology

Twigs were air-dried at room temperature and examined with an Olympus SZ 61 dissecting microscope without prior incubation in a moist chamber. Photomicrographs were taken with a Nikon Coolpix 995 digital camera fitted with an eyepiece adapter to the dissecting microscope. Microscopic observations of ascostromatal contents were carried out from material mounted in water. Thin vertical free-hand sections were made with a razor blade under the dissecting microscope and mounted in water. Photomicrographs were taken with a Nikon Coolpix 995 digital camera connected to a trinocular Leitz Orthoplan microscope and processed with Adobe Photoshop Elements 10 software. Measurements of ascospores, asci, hamathecial elements, conidia, paraphyses and conidiogenous cells were made from water mounts.

Isolations were made from ascomata or conidiomata on dead or dying twigs of different hosts and grown on 2 % water agar (WA) (Biolab, S.A.), and subsequently transferred to synthetic nutrient-poor agar (SNA) with sterilized pine needles. Isolates grown on malt extract agar (MEA, Biolab, S.A.) were kept at ambient temperatures (about 26–28 °C) in the dark to establish colony characteristics. Fungal isolates and herbarium specimens were deposited at Beijing Forestry University (BJFU) with duplicates in the China General Microbiological Culture Collection Center (CGMCC) and the Mycological Herbarium of the Institute of Microbiology, Chinese Academy of Sciences (HMAS).

DNA extraction, PCR amplification

Mycelium was grown on MEA plates and DNA extracted with CTAB plant genome DNA fast extraction kit (Aidlab Biotechnologies Co., Ltd, Beijing, China). The internal transcribed spacer of rDNA (ITS) was amplified and sequenced with primers ITS-1 and ITS-4 (White et al. 1990). The translation elongation factor-1α (*tef1-α*) was amplified and sequenced with primers EF1-688F and EF1-1251R (Alves et al. 2008). The β-tubulin gene (*TUB*) was amplified and sequenced with primers Bt2a and Bt2b (Glass & Donaldson 1995). The *RPB2* sequences were amplified and sequenced using primers RPB2-LasF and RPB2-LasR (Cruywagen et al. 2016). PCR amplification and sequencing followed the protocol of Zhang et al. (2009).

Sequence alignment and phylogenetic analysis

The combined loci of ITS, *RPB2*, *tef1-α* and *TUB* were used to infer the phylogenetic relationships of the new species and another species of *Lasiodiplodia*. Sequences generated were analyzed with other sequences obtained from GenBank (Table 1). Alignments were conducted in MEGA v. 6 (Tamura et al. 2013) and phylogenetic analyses performed in PAUP v. 4.0b10 (Swofford 2002) and MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003). Prior to phylogenetic analysis, ambiguous sequences at the start and end were deleted and gaps manually adjusted to optimize the alignments. Maximum Parsimony (MP) was used to conduct heuristic searches as implemented in PAUP with the default options method (Zhang et al. 2008). Analyses were done under different parameters of maximum parsimony criteria as outlined in Zhang et al. (2008). Clade stability was assessed in a bootstrap analysis with 1 000 replicates, random sequence additions with maxtrees set to 1 000 and other default parameters as implemented in PAUP. For the MrBayes analysis, the best-fit model of nucleotide evolution (GTR+I+G) was selected by Akaike information criterion (AIC; Posada & Buckley 2004) in MrModeltest v. 2.3. The metropolis-coupled Markov Chain Monte Carlo (MCMCMC) approach was used to calculate posterior probabilities (Huelsenbeck & Ronquist 2005). A preliminary Bayesian inference (BI) analysis using MrBayes software revealed that the Markov Chain Monte Carlo (MCMC; Huelsenbeck & Ronquist, 2001) steady state was reached after less than 10,000 generations (the average standard deviation of split frequencies was constantly below 0.01). A conservative burn-in of 100 trees was chosen and a full analysis of 10,000,000 generations was carried out with sampling every 100

generations. Trees were viewed in TREEVIEW. The nucleotide sequences generated in this paper were deposited in GenBank (Table 1). Trees and alignments were deposited in TreeBase (S20597).

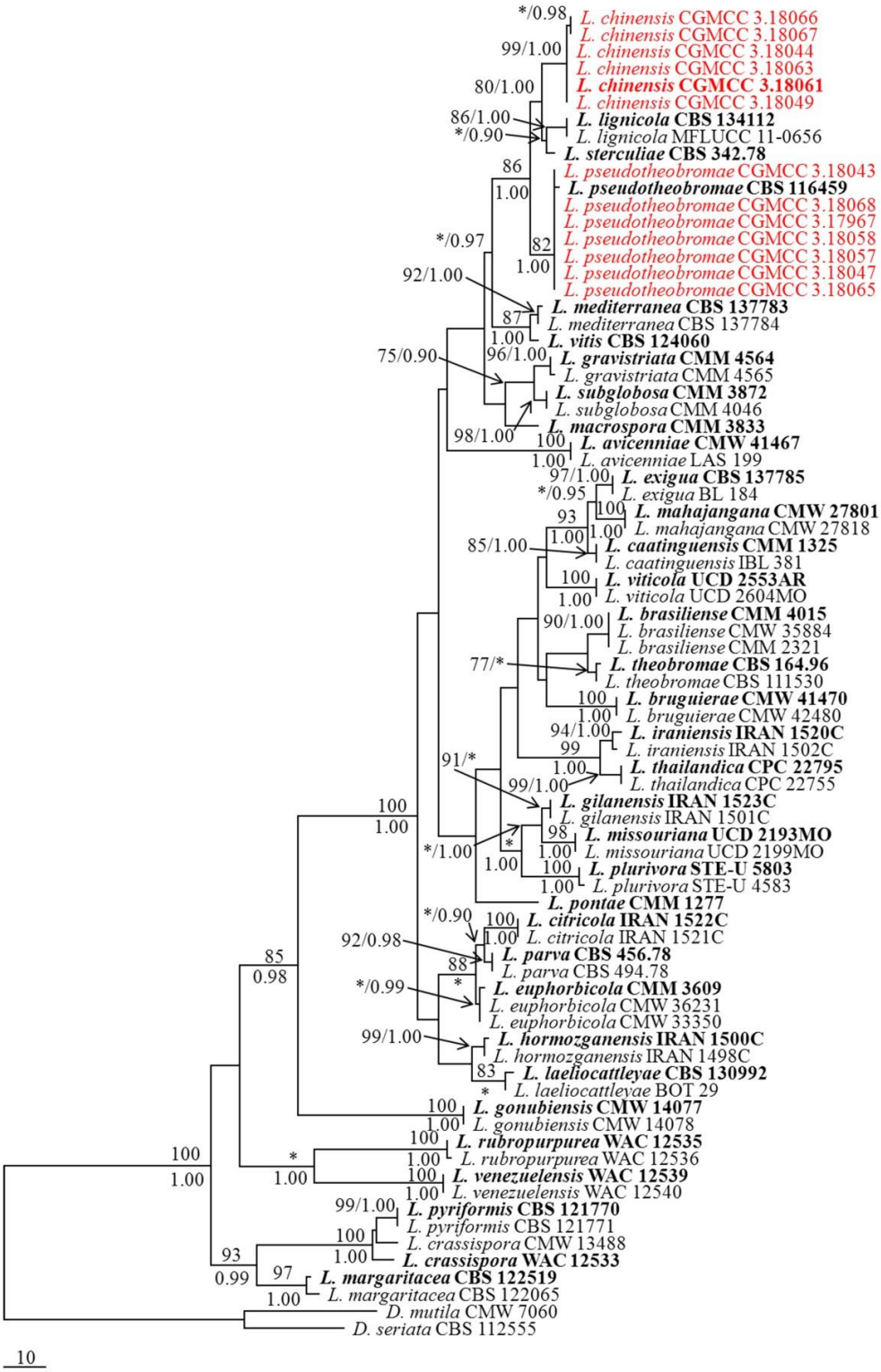

Fig. 1 – Maximum parsimony tree generated from sequence analysis of the combined ITS, tef1-α, TUB and RPB2 dataset. Designated out group taxon is Diplodia mutila and D. seriata. Bootstrap support values for maximum parsimony (MP) greater than 75% are shown above the nodes, and Bayesian bootstrap (BP) support values above 0.90 are shown under the branches (* = MP value less than 75% or BP value less than 0.90). The species characterized in this study are in red, and the ex-type strains are in boldface.

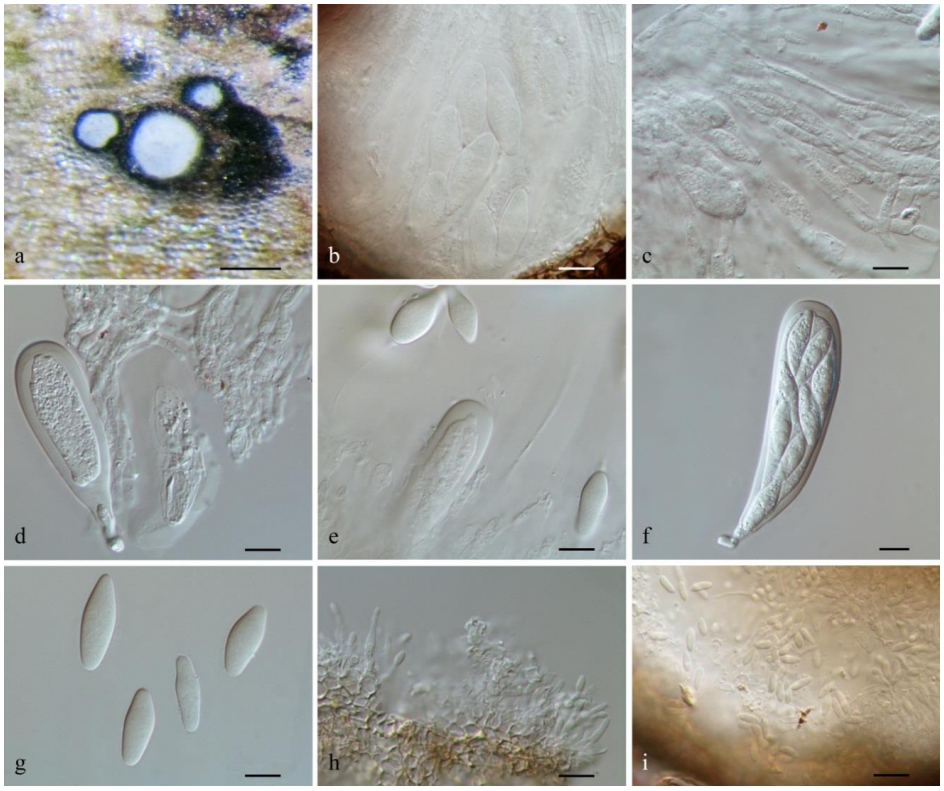

Fig. 2 – *Lasiodiplodia chinensis* (from holotype: HMAS247143) a Immersed ascomata on the host. b A section of ascoma with asci inside. c, d Cellular pseudoparaphyses and immature asci. e Ascus tip showing apical chamber and released ascospores. f A mature ascus. g Released ascospores. h Spermatogenous cells. i A section with spermatia inside. Scale bars: a = 200 µm, b–i = 10 µm.

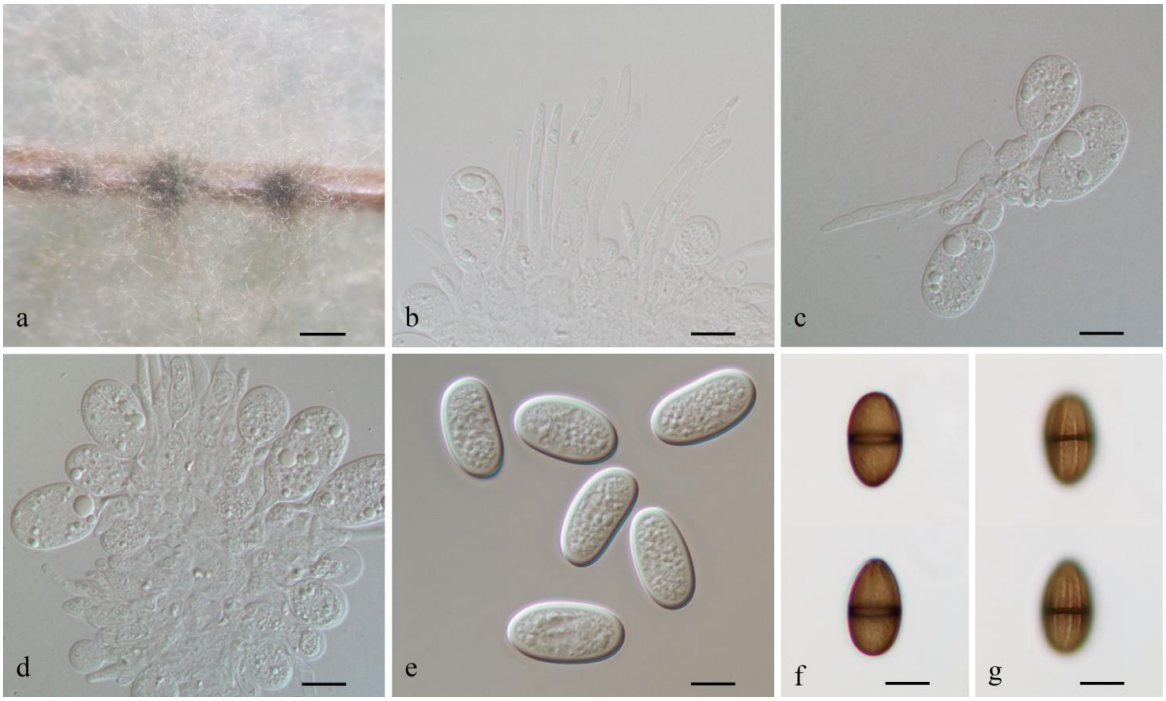

Fig. 3 – *Lasiodiplodia chinensis* (From ex-type: CGMCC3.18061) a. Conidiomata formed on pine needles in culture. b–d. Conidia developing on conidiogenous cells among paraphyses. e. Hyaline, immature conidia with granular content. f–g. Mature conidia in two different focal planes to show the longitudinal striations. Scale bars: a = 500 µm; b–g = 10 µm.

Table 1 GenBank and culture collection accession numbers of species included in the phylogenetic study. Newly deposited sequences are shown in bold.

Species	**Cultures**	**Host**	**Locality**	**GenBank**			
				ITS	**tef1-a**	**TUB**	**RPB2**
L. avicenniae	CMW 41467	*Avicennia marina*	South Africa	KP860835	KP860680	KP860758	KU587878
L. avicenniae	LAS 199	*Avicennia marina*	South Africa	KU587957	KU587947	KU587868	KU587880
L. brasiliense	CMM 4015	*Mangifera indica*	Brazil	JX464063	JX464049	N/A	N/A
L. brasiliense	CMM 2321	*Carica papaya*	Brazil	KC484797	KC481528	N/A	N/A
L. brasiliense	CMW 35884	*Adansonia madagascariensis*	Madagascar	KU887094	KU886972	KU887466	KU696345
L. bruguierae	CMW 41470	*Bruguiera gymnorrhiza*	South Africa	KP860833	KP860678	KP860756	KU587875
L. bruguierae	CMW 42480	*Bruguiera gymnorrhiza*	South Africa	KP860832	KP860677	KP860755	KU587876
L. caatinguensis	CMM 1325	*Citrus sinensis*	Brazil	KT154760	KT008006	KT154767	N/A
L. caatinguensis	IBL 381	*Spondias purpurea*	Brazil	KT154757	KT154751	KT154764	N/A
L. chinensis	CGMCC3.18061	unknown	China	**KX499889**	**KX499927**	**KX500002**	**KX499965**
L. chinensis	CGMCC3.18044	*Vaccinium uliginosum*	China	**KX499875**	**KX499913**	**KX499988**	**KX499951**
L. chinensis	CGMCC3.18049	*Rhodomyrtus tomentosa*	China	**KX499878**	**KX499916**	**KX499991**	**KX499954**
L. chinensis	CGMCC3.18063	*Canarium parvum*	China	**KX499891**	**KX499929**	**KX500004**	**KX499967**
L. chinensis	CGMCC3.18066	*Hevea brasiliensis*	China	**KX499899**	**KX499937**	**KX500012**	**KX499974**
L. chinensis	CGMCC3.18067	*Sterculia lychnophora*	China	**KX499901**	**KX499939**	**KX500014**	**KX499976**
L. citricola	IRAN 1522C	*Citrus* sp.	Iran	GU945354	GU945340	KU887505	KU696351
L. citricola	IRAN 1521C	*Citrus* sp.	Iran	GU945353	GU945339	KU887504	KU696350
L. crassispora	WAC 12533	*Santalum album*	Australia	DQ103550	DQ103557	KU887506	KU696353
L. crassispora	CMW 13488	*Eucalyptus urophylla*	Venezuela	DQ103552	DQ103559	KU887507	KU696352
L. euphorbiicola	CMM 3609	*Jatropha curcas*	Brazil	KF234543	KF226689	KF254926	N/A
L. euphorbiicola	CMW 33350	*Adansonia digitata*	Botswana	KU887149	KU887026	KU887455	KU696346
L. euphorbiicola	CMW 36231	*Adansonia digitata*	Zimbabwe	KU887187	KU887063	KU887494	KU696347
L. exigua	CBS 137785	*Retama raetam*	Tunisia	KJ638317	KJ638336	KU887509	KU696355
L. exigua	BL 184	*Retama raetam*	Tunisia	KJ638318	KJ638337	N/A	N/A
L. gilanensis	IRAN 1523C	Unknown	Iran	GU945351	GU945342	KU887511	KU696357
L. gilanensis	IRAN 1501C	Unknown	Iran	GU945352	GU945341	KU887510	KU696356
L. gonubiensis	CMW 14077	*Syzygium cordatum*	South Africa	AY639595	DQ103566	DQ458860	KU696359
L. gonubiensis	CMW 14078	*Syzygium cordatum*	South Africa	AY639594	DQ103567	EU673126	KU696358

Table 1 (cont.)

Species	Cultures	Host	Locality	GenBank			
				ITS	tef1-a	TUB	RPB2
L. gravistriata	CMM 4564	*Anacardium humile*	Brazil	KT250949	KT250950	N/A	N/A
L. gravistriata	CMM 4565	*Anacardium humile*	Brazil	KT250947	KT266812	N/A	N/A
L. hormozganensis	IRAN 1500C	*Olea* sp.	Iran	GU945355	GU945343	KU887515	KU696361
L. hormozganensis	IRAN 1498C	*Mangifera indica*	Iran	GU945356	GU945344	KU887514	KU696360
L. iraniensis	IRAN 1520C	*Salvadora persica*	Iran	GU945348	GU945336	KU887516	KU696363
L. iraniensis	IRAN 1502C	*Juglans* sp.	Iran	GU945347	GU945335	KU887517	KU696362
L. laeliocattleyae	CBS 130992	*Mangifera indica*	Egypt	JN814397	JN814424	KU887508	KU696354
L. laeliocattleyae	BOT 29	*Mangifera indica*	Egypt	JN814401	JN814428	N/A	N/A
L. lignicola	CBS 134112	dead wood	Thailand	JX646797	KU887003	JX646845	KU696364
L. lignicola	MFLUCC 11-0656	dead wood	Thailand	JX646798	N/A	JX646846	N/A
L. macrospora	CMM 3833	*Jatropha curcas*	Brazil	KF234557	KF226718	KF254941	N/A
L. mahajangana	CMW 27801	*Terminalia catappa*	Madagascar	FJ900595	FJ900641	FJ900630	KU696365
L. mahajangana	CMW 27818	*Terminalia catappa*	Madagascar	FJ900596	FJ900642	FJ900631	KU696366
L. margaritacea	CBS 122519	*Adansonia gibbosa*	Western Australia	EU144050	EU144065	KU887520	KU696367
L. margaritacea	CBS 122065	*Adansonia gibbosa*	Western Australia	EU144051	EU144066	N/A	N/A
L. mediterranea	CBS 137783	*Quercus ilex*	Italy	KJ638312	KJ638331	KU887521	KU696368
L. mediterranea	CBS 137784	*Vitis vinifera*	Italy	KJ638311	KJ638330	KU887522	KU696369
L. missouriana	UCD 2193MO	*Vitis* sp.	USA	HQ288225	HQ288267	HQ288304	KU696370
L. missouriana	UCD 2199MO	*Vitis* sp.	USA	HQ288226	HQ288268	HQ288305	KU696371
L. parva	CBS 456.78	*Cassava field-soil*	Colombia	EF622083	EF622063	KU887523	KU696372
L. parva	CBS 494.78	*Cassava field-soil*	Colombia	EF622084	EF622064	EU673114	KU696373
L. plurivora	STE-U 5803	*Prunus salicina*	South Africa	EF445362	EF445395	KU887524	KU696374
L. plurivora	STE-U 4583	*Vitis vinifera*	South Africa	AY343482	EF445396	KU887525	KU696375
L. pontae	CMM 1277	*Spondias purpurea*	Brazil	KT151794	KT151791	KT151797	N/A
L. pseudotheobromae	CBS 116459	*Gmelina arborea*	Costa Rica	EF622077	EF622057	EU673111	KU696376
L. pseudotheobromae	CGMCC3.18043	*Morus alba Linn*	China	**KX499872**	**KX499910**	**KX499985**	**KX499948**
L. pseudotheobromae	CGMCC3.18047	*Pteridium aquilinum*	China	**KX499876**	**KX499914**	**KX499989**	**KX499952**
L. pseudotheobromae	CGMCC3.18057	*Lagerstroemia indica*	China	**KX499885**	**KX499923**	**KX499998**	**KX499961**

Table 1 (cont.)

Species	Cultures	Host	Locality	GenBank			
				ITS	tef1-a	TUB	RPB2
L. pseudotheobromae	CGMCC3.18058	unknown	China	**KX499887**	**KX499925**	**KX500000**	**KX499963**
L. pseudotheobromae	CGMCC3.18065	unknown	China	**KX499895**	**KX499933**	**KX500008**	**N/A**
L. pseudotheobromae	CGMCC3.17967	unknown	China	**KX499897**	**KX499935**	**KX500010**	**KX499972**
L. pseudotheobromae	CGMCC3.18068	unknown	China	**KX499902**	**KX499940**	**KX500015**	**KX499977**
L. pyriformis	CBS 121770	*Acacia mellifera*	Namibia	EU101307	EU101352	KU887527	KU696378
L. pyriformis	CBS 121771	*Acacia mellifera*	Namibia	EU101308	EU101353	KU887528	KU696379
L. rubropurpurea	WAC 12535	*Eucalyptus grandis*	Australia	DQ103553	DQ103571	EU673136	KU696380
L. rubropurpurea	WAC 12536	*Eucalyptus grandis*	Australia	DQ103554	DQ103572	KU887530	KU696381
L. sterculiae	CBS 342.78	*Sterculia oblonga*	Germany	KX464140	KX464634	KX464908	KX463989
L. subglobosa	CMM 3872	*Jatropha curcas*	Brazil	KF234558	KF226721	KF254942	N/A
L. subglobosa	CMM 4046	*Jatropha curcas*	Brazil	KF234560	KF226723	KF254944	N/A
L. thailandica	CPC 22795	*Mangifera indica*	Thailand	KJ193637	KJ193681	N/A	N/A
L. thailandica	CPC 22755	*Phyllanthus acidus*	Thailand	KM006433	KM006464	N/A	N/A
L. theobromae	CBS 164.96	Fruit along coral reef coast	Papua New Guinea	AY640255	AY640258	KU887532	KU696383
L. theobromae	CBS 111530	Unknown	Unknown	EF622074	EF622054	KU887531	KU696382
L. venezuelensis	WAC 12539	*Acacia mangium*	Venezuela	DQ103547	DQ103568	KU887533	KU696384
L. venezuelensis	WAC 12540	*Acacia mangium*	Venezuela	DQ103548	DQ103569	KU887534	N/A
L. viticola	UCD 2553AR	*Vitis* sp.	USA	HQ288227	HQ288269	HQ288306	KU696385
L. viticola	UCD 2604MO	*Vitis* sp.	USA	HQ288228	HQ288270	HQ288307	KU696386
L. vitis	CBS 124060	*Vitis vinifera*	Italy	KX464148	KX464642	KX464917	KX463994
Diplodia mutila	CMW 7060	*Fraxinus excelsior*	Netherlands	AY236955	AY236904	AY236933	EU339574
D. seriata	CBS 112555	*Vitis vinifera*	Portugal	AY259094	AY573220	DQ458856	N/A

Results

Phylogenetic analyses

Phylogenetic analysis of the combined ITS, tef1-α, TUB and RPB2 sequence dataset comprising 1957 bp revealed 301 parsimony-informative characters. The outgroup taxa were *Diplodia mutila* and *D. seriata*. The heuristic search with random addition of taxa (1,000 replicates) generated 504 most parsimonious trees of 762 steps (CI = 0.646, RI = 0.866, RC = 0.559, HI = 0.354). In the phylogenetic tree, the clade comprising *L. chinensis*, *L. lignicola*, *L. sterculiae* and *L. pseudotheobromae* as well as the subclades comprising individual species of *L. chinensis* and *L. pseudotheobromae* all received high bootstrap support (Fig. 1). The clade comprising *L. chinensis*, *L. sterculiae* and *L. lignicola* also received high support for Bayesian analysis, while moderate support for MP analysis (Fig. 1).

Taxonomy

Lasiodiplodia chinensis Z. P. Dou, Y. Zhang ter., **sp. nov.** Figs 2, 3

MycoBank 819527; *Facesoffungi number*: FoF02831

Etymology – The epithet chinensis refers to China, the country from which it is described.

Saprobic or *pathogen*, associated with woody branches. **Sexual morph**: *Ascomata* immersed under bark, sometimes erumpent, globose to subglobose, solitary or in small groups of up to 4, black, 140–290 µm diam., 170–185 µm high. *Papilla* up to 28–43 µm diam. wide, with a central ostile. *Peridium* 12–49 µm wide, thin to thick-walled, composed of several layers of dark brown to black cells of *textura angularis*. *Pseudoparaphyses* hyaline, cellular, septate, constricted at the septum, 3–4 µm wide. *Asci* bitunicate with thick endotunica and well-developed apical chamber best seen in water, with a short, furcate pedicel, broadly clavate to clavate, 53–90(–116) × 14–23(–25) µm, 8-spored. *Ascospores* overlapping bi- to tri-seriate, fusiform to broadly fusiform, with rounded ends, the upper half often broader than the lower half, hyaline, aseptate, straight or slightly curved, smooth-walled, 19–25 × 6–9 µm (av. of 30 conidia = 22.1 × 7.6 µm). **Asexual morph**: *Conidiomata* stromatic, produced on pine needles on SNA within 1–2 wk, semi-immersed, sometimes superficial, solitary, papillate, uniloculate, dark brown to black, covered with dense mycelium, 210–320 µm diam. *Paraphyses* cylindrical, initially aseptate, becoming up to 9-septate when mature, unbranched, occasionally basal cells swollen, hyaline, up to 99 µm long, 3–7 µm wide. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, hyaline, cylindrical to ampulliform, proliferating percurrently, (8–)10–15(–18) × 4–6(–7) µm (av. = 12.4 × 5.0 µm, n = 20). *Conidia* produced in culture initially hyaline, unicellular, ovoid to ellipsoid, thick-walled with granular content, round at apex, occasionally truncate at base, becoming pigmented while did not attach to the conidiogenous cell, with one septum when mature, developing longitudinal striations when mature, (18–)19–25 ×12–14 µm (av. of 30 conidia = 21.9 × 12.6 µm, L/W ratio = 1.75, range from 1.43 to 2.08). *Spermatogenous cells* discrete or integrated, hyaline, smooth, fusiform to broadly cylindrical to ampulliform, holoblastic, proliferating percurrently or proliferating internally resulting in annellations, 8–15 × 2–3 µm. *Spermatia* hyaline, smooth, aseptate, ellipsoidal to allantoid with rounded ends, 5–7 × 2 µm.

Culture characteristics – *Colonies* on MEA at 28 °C in darkness, initially white with dense aerial mycelia, becoming pale olivaceous grey to olivaceous grey on the surface within 10 d, with the reverse sides of the colonies olivaceous grey to olivaceous black. Colonies reaching an average of 79 mm on MEA after 2 d in the dark at 28 °C.

Specimens examined – CHINA, Hainan Province, Wanning City, Xinglong medical plants garden, from branch of an unknown woody plant, 4 November 2015, W. He & Z.P. Dou (HMAS247143, holotype), ex-type living culture, CGMCC3.18061; Haikou City, Meilan District, from branch of *Canarium parvum*, 6 November 2015, Y. Zhang & Y.P. Zhou (CGMCC3.18063); from branch of *Hevea brasiliensis*, 6 November 2015, Y. Zhang & Y.P. Zhou (CGMCC3.18066); Danzhou City, the Danzhou Tropical Botanical Garden, from branch of *Rhodomyrtus tomentosa*, 3

November 2015, Y. Zhang & Y.P. Zhou (CGMCC3.18049); from branch of *Sterculia lychnophora*, 3 November 2015, W. He & Z.P. Dou (HMAS247144, CGMCC3.18067); Shandong province, Qingdao City, Huangdao District, from branch of *Vaccinium uliginosum*, 13 December 2014, J.H. Zhao (Paratype, HMAS247145, CGMCC3.18044).

Notes — Phylogenetic analyses based on combined ITS, *RPB2*, *TUB* and *tef1-a* sequences indicated that *L. chinensis* is closely related to *L. pseudotheobromae*, *L. sterculiae* and *L. lignicola*, but the golden to dark brown mature ascospores of *L. pseudotheobromae* and *L. lignicola* differentiate these two species from *L. chinensis* (Tennakoon et al. 2016). Only the asexual morph has been reported for *L. sterculiae*, which differs from *L. chinensis* by its hyaline and smaller conidia ((12–)14–16 (–17) × (8–)10–11 (–12) μm) (Yang et al 2016).

Discussion

Of the five species of *Lasiodiplodia* with known sexual morphs, namely *L. chinensis, L. pseudotheobromae, L. lignicola, L. gonubiensis* and *L. theobromae*, golden to dark brown ascospores have been reported for *L. pseudotheobromae* and *L. lignicola* (Liu et al. 2012, Tennakoon et al. 2016). The ascospores of *L. gonubiensis* are initially hyaline, and then turn pale brown, 1–2-septate within ascoma or shortly after discharge (Trakunyingcharoen et al. 2015). No pigmented or septate ascospores of *L. chinensis* were observed in the present study. Although *L. theobromae* have been reported to have hyaline ascospores, the connection between *L. theobromae* and its sexual morph has not been proven conclusively (Phillips et al. 2013). However, *L. theobromae* is phylogenetically separated from *L. chinensis* (Fig. 3). So far, the sexual morphs of *Lasiodiplodia* species can be defined as having hyaline to dark brown aseptate ascospores, which can develop one or two septa when aged. Interestingly, of the five species with sexual stage reported, four of the reports are from Asia, i.e. *L. pseudotheobromae* and *L. chinensis* from China (Tennakoon et al. 2016, this study), *L. lignicola* and *L. gonubiensis* from Thailand (Liu et al. 2012, Trakunyingcharoen et al. 2015). Six collections of *Lasiodiplodia chinensis* have been obtained from both tropical (Hainan Province) and temperate regions (Shandong Province) in China, and the hosts include *Canarium parvum* (Burseraceae), *Hevea brasiliensis* (Euphorbiaceae), *Rhodomyrtus tomentosa* (Myrtaceae), *Sterculia lychnophora* (Malvaceae) and *Vaccinium uliginosum* (Vaccinieae). This indicates the wide geographic distribution and broad host spectrum of *L. chinensis.*

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