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Morpho-phylogenetic evidence reveals *Lasiodiplodia chiangraiensis* sp. nov. (*Botryosphaeriaceae*) associated with woody hosts in northern Thailand

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Abstract

Lasiodiplodia species are commonly as endophytes, saprobes and pathogens in tropics and subtropics. During an investigation of *Botryosphaeriaceae* in Thailand, two *Lasiodiplodia* taxa were isolated. Morphological characteristics and phylogenetic analyses based on combined ITS, *tef* and *tub2* sequence data support the establishment of a novel species, *Lasiodiplodia chiangraiensis*, isolated from woody hosts. *Lasiodiplodia chiangraiensis* is phylogenetically close to *L. iraniensis* and *L. thailandica*, but represents a distinct lineage. The new species could be distinguished from extant *Lasiodiplodia* species by its mature conidial dimensions. A detailed description and illustration are provided, as well as an updated phylogenetic tree (ITS, *tef* and *tub2*) including all species (with available molecular data) of *Lasiodiplodia*. In addition, the accepted genera in *Botryosphaeriaceae* based on recent studies are given.

Keywords: 1 new taxon, asexual morph, multi-gene, phylogeny, taxonomy

Introduction

The family *Botryosphaeriaceae* was introduced by Theissen & Sydow (1918) with three genera, *Botryosphaeria* (type genus), *Dibotryon* and *Phaeobotryon*. In recent years, genera in this family have been subjected to continuous revisions (Arx & Müller 1954, 1975, Barr 1987, Crous *et al.* 2006, Liu *et al.* 2012, Phillips *et al.* 2013, 2019, Dissanayake *et al.* 2016, Wijayawardene *et al.* 2020). The subsequent study by Hongsanan *et al.* (2020) based on morphology and phylogeny, accepted 22 genera (TABLE 1) in *Botryosphaeriaceae*, consisting of more than 170 species. Among all the families within Botryosphaeriales, *Botryosphaeriaceae* is the largest with a broad host range. Fungi in this family are well-known as plant endophytes, saprobes and pathogens, causing ulceration, dieback and stem-end rot of plants or fruits, e.g., *Botryosphaeria*, *Diplodia*, *Dothiorella*, *Lasiodiplodia* and *Neofusicoccum* (Smith *et al.* 1996, Phillips *et al.* 2006, 2013, Slippers & Wingfield 2007, Liu *et al.* 2012, Li *et al.* 2014, Dissanayake *et al.* 2016, Zhang *et al.* 2021). The sexual morphs of *Botryosphaeriaceae* are characterized by solitary or clustered ascostromata, often with two-layers of dark brown to hyaline cells; 8-spored, short-stipitate, clavate asci and hyaline or pigmented, aseptate or septate, ellipsoid or ovoid ascospores, and its asexual morphs are coelomycetous, characterized by ovoid, hyaline or brown, aseptate, one- or multi-septate conidia, and conidiophores mostly reduced to conidiogenous cells (Denman *et al.* 2000, Crous *et al.* 2006, Phillips *et al.* 2006, 2013, 2019, Liu *et al.* 2012, Slippers *et al.* 2013).

TABLE 1. Genera accepted in *Botryosphaeriaceae* for the past 100 years.

Theissen & Sydow (1918)	Arx & Müller (1954)	Barr (1987)	Liu *et al.* (2012)	Phillips *et al.* (2013)	Yang *et al.* (2017)	Phillips *et al.* (2019)	Hongsanan *et al.* (2020)
Botryosphaeria	*Auerswaldia*	*Auerswaldia*	*Aplosporella*	*Barriopsis*	Alanphillipsia	*Alanphillipsia*	*Alanphillipsia* (*A. aloes*)*
Dibotryon	*Auerswaldiella*	*Auerswaldiella*	*Auerswaldia*	*Botryobambusa*	*Barriopsis*	*Barriopsis*	*Barriopsis* (*B. stevensiana*)
Phaeobotryon	*Bagnisiella*	*Botryosphaeria*	*Auerswaldiella*	*Botryosphaeria*	*Botryosphaeria*	*Botryobambusa*	*Botryobambusa* (*B. fusicoccum*)
	Botryosphaeria	*Discochora*	*Barriopsis*	*Cophinforma*	*Cophinforma*	*Botryosphaeria*	*Botryosphaeria* (*B. dothidea*)
	Cleistosphaeria	*Dothidotthia*	*Botryobambusa*	*Diplodia*	*Diplodia*	*Cophinforma*	*Cophinforma* (*C. eucalypti*)
	Ellisiodothis	*Homostegia*	*Botryosphaeria*	*Dothiorella*	*Eutiarosporella*	*Diplodia*	*Diplodia* (*D. mutila*)
	Guignardia	*Leptoguignardia*	*Cophinforma*	*Endomelanconiopsis*	*Lasiodiplodia*	*Dothiorella*	*Dothiorella* (*D. pyrenophora*)
	Montagnellina	*Neodeightonia*	*Diplodia*	*Lasiodiplodia*	*Macrophomina*	*Endomelanconiopsis*	*Endomelanconiopsis* (*E. endophytica*)
	Microdothiella	*Phyllachorella*	*Dothiorella*	*Macrophomina*	*Marasasiomyces*	*Lasiodiplodia*	*Eutiarosporella* (*E. tritici*)
	Muyocopron		*Endomelanconiopsis*	*Neodeightonia*	*Neodeightonia*	*Macrophomina*	*Lasiodiplodia* (*L. theobromae*)
	Parastigmatea		*Lasiodiplodia*	*Neofusicoccum*	*Neoscytalidium*	*Neodeightonia*	*Macrophomina* (*M. philippinensis*)
	Pilgeriella		*Leptoguignardia*	*Neoscytalidium*	*Oblongocollomyces*	*Neofusicoccum*	*Marasasiomyces* (*M. karoo*)
	Pyrenostigme		*Macrophomina*	*Phaeobotryon*	*Phaeobotryon*	*Neoscytalidium*	*Mucoharknessia* (*M. cortaderiae*)
	Trabutia		*Macrovalsaria*	*Pseudofusicoccum*	*Sakireeta*	*Oblongocollomyces*	*Neodeightonia* (*N. subglobosa*)
	Vestergrenia		*Melanops*	*Spencermartinsia*	*Sphaeropsis*	*Phaeobotryon*	*Neofusicoccum* (*N. parvum*)
			Neodeightonia	*Sphaeropsis*	*Tiarosporella*	*Sphaeropsis*	*Neoscytalidium* (*N. dimidiatum*)
			Neofusicoccum	*Tiarosporella*		*Tiarosporella*	*Oblongocollomyces* (*O. variabilis*)
			Neoscytalidium				*Phaeobotryon* (*P. cercidis*)
			Phaeobotryon				*Sakireeta* (*S. madreeya*)
			Phaeobotryosphaeria				*Sardiniella* (*S. urbana*)
			Phyllachorella				*Sphaeropsis* (*S. visci*)
			Phyllosticta				*Tiarosporella* (*T. paludosa*)
			Pseudofusicoccum				
			Pyrenostigme				
			Saccharata				
			Sivanesania				
			Spencermartinsia				
			Tiarosporella				
			Vestergrenia				

*The generic type species are given between brackets.

The genus *Lasiodiplodia* was formally established by Clendenin (1896), and typified by *L. tubericola* Ellis & Everhart (= *L. theobromae*; Liu *et al.* 2012). *Lasiodiplodia* species mainly occur on many woody hosts in the tropics and subtropics, causing fruit or root rots, cankers, stem blight or dieback and sap staining (Punithalingam 1980, Mohali *et al.* 2002, Slippers & Wingfield 2007, Ismail *et al.* 2012, Marques *et al.* 2013, Phillips *et al.* 2013, Dissanayake *et al.* 2016, Zhao *et al.* 2019). There are 72 *Lasiodiplodia* epithets listed in Index Fungorum (May 2021), of which 37 ex-type/isotype/neotype species entries have been accepted and uploaded to the Botryosphaeriales website (https://botryosphaeriales.org/), including colour illustrations, descriptions and notes. *Lasiodiplodia* species have subglobose or oval, smooth, thick-walled, initially hyaline conidia that become dark brown and striated when matured (Phillips *et al.* 2013). Generally, conidiophores are reduced to conidiogenous cells (Phillips *et al.* 2013, Wang *et al.* 2019). The typical features of sexual morphs are globose to subglobose, often ostiolate ascomata with 4–5 individual locules, and clavate, stipitate asci with hyaline to dark brown aseptate ascospores (Phillips *et al.* 2013). Colonies of *Lasiodiplodia* are fast-growing, white at first, becoming black or dark brown with age (Jiang *et al.* 2018, Wang *et al.* 2019, Zhao *et al.* 2019, Dayarathne *et al.* 2020).

During investigations of *Botryosphaeriaceae* in northern Thailand, a new species *Lasiodiplodia chiangraiensis* was found and is described below. Its typical morphology fits well with *Lasiodiplodia* and a phylogenetic analysis based on multi-gene (ITS, *tef* and *tub2*) confirm its phylogenetic placement. A detailed description and illustration are provided, as well as an updated phylogenetic tree of *Lasiodiplodia*.

Materials and methods

Collection and examination of specimens

Dead wood samples were collected in July and December 2019 from Mae Fah Luang University in Chiang Rai, Thailand. Samples were taken to the laboratory, stored in paper bags, and the sampling information (date, place, GPS, etc.) were recorded. The specimens were examined using a LEICA EZ4 microscope following the method described in Chomnunti *et al*. (2014). Hand-sectioning of conidiomata was carried out using a razor blade. The fungus was removed with a sterile needle and transferred to a small drop of double distilled water on a clean slide and covered with a cover glass. Photomicrographs of the fungal specimens were captured using a Nikon ECLIPSE Ni compound microscope fitted with a Nikon DS-Ri2 digital camera. All measurements were made with the Tarosoft (R) Image Frame Work (IFW) program (Liu *et al.* 2010). Photo plates were made with Adobe Photoshop CC Extended version 20.0.1. Herbarium materials were deposited in the Herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand, and duplicated in the herbarium of the Guizhou Academy of Agricultural Sciences (GZAAS), Guiyang, P. R. China.

Single spore isolations were made on potato dextrose agar (PDA) following the method of Chomnunti *et al.* (2014), and germinated spores were transferred to malt extract agar (MEA) or PDA. Cultures were deposited at Mae Fah Luang University Culture Collection (MFLUCC), Thailand and Guizhou Culture Collection (GZCC), China.

DNA extraction, PCR amplification and sequencing

In a sterile environment, a sterilized toothpick or scalpel was used to scrape off fresh mycelium after one week on PDA or MEA media (about 50–100 mg), and then transferred to a sterilized 1.5 ml micro-centrifuge tube. Ezup Column Fungi Genomic DNA Purification Kit (Sangon Biotech, P. R. China) was used to extract DNA, according to the manufacturer's instructions. Polymerase chain reaction (PCR) amplification and sequencing of the ITS rDNA region was conducted using the primer pair ITS4/ITS5 (White *et al.* 1990). The *tef* and *tub2* regions were amplified using the primer pairs EF1-728F/EF1-986R (Carbone & Kohn 1999) and Bt2a/Bt2b (Glass & Donaldson 1995), respectively. The final volume (25 µl) contained 2 µl DNA, 12.5 µl PCR mix, 8.5 µl distilled water and 1 µl of each primer. The PCR thermal cycle program for ITS and *tub2* amplification was: initial denaturation at 94 °C for 3 mins, followed by 40 cycles of denaturation at 94 °C for 45 seconds, annealing at 56 °C for 50 seconds, elongation at 72 °C for 1 min, and a final extension at 72 °C for 10 mins. The *tef* amplification was: initial denaturation at 94 °C for 5 mins, followed by 34 cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 50 seconds, elongation at 72 °C for 1 min, and a final extension at 72 °C for 5 mins. The PCR products were sequenced at Sangon Biotechnology Co. (Shanghai, P. R. China).

TABLE 2. GenBank accession numbers of the isolates included in this study.

Species	Isolate number	Host	Location	GenBank accession number		
				ITS	***tef***	***tub2***
Lasiodiplodia acaciae	CBS 136434*	*Acacia* sp.	Indonesia	MT587421	MT592133	MT592613
L. aquilariae	CGMCC 3.18471*	*Aquilaria crassna*	Laos	KY783442	KY848600	N/A
L. avicenniae	CMW 41467*	*Avicennia marina*	South Africa	KP860835	KP860680	KP860758
L. avicenniae	LAS 199	*Avicennia marina*	South Africa	KU587957	KU587947	KU587868
L. avicenniarum	MFLUCC 17-2591*	*Avicennia marina*	Thailand	MK347777	MK340867	N/A
L. brasiliensis	CMM 4015*	*Mangifera indica*	Brazil	JX464063	JX464049	N/A
L. brasiliensis	CMM 4469	*Anacardium occidentale*	Brazil	KT325574	KT325580	N/A
L. bruguierae	CMW 41470*	*Bruguiera gymnorrhiza*	South Africa	KP860832	KP860677	KP860755
L. bruguierae	CMW 42480	*Bruguiera gymnorrhiza*	South Africa	KP860834	KP860679	KP860757
L. chiangraiensis	**MFLUCC 21-0003***	**Unknown host**	**Thailand**	**MW760854**	**MW815630**	**MW815628**
L. chiangraiensis	**GZCC 21-0003**	**Unknown host**	**Thailand**	**MW760853**	**MW815629**	**MW815627**
L. chonburiensis	MFLUCC 16-0376*	*Pandanus* sp.	Thailand	MH275066	MH412773	MH412742
L. cinnamomi	CFCC 51997*	*Cinnamomum camphora*	China	MG866028	MH236799	MH236797
L. cinnamomi	CFCC 51998	*Cinnamomum camphora*	China	MG866029	MH236800	MH236798
L. citricola	CBS 124707*	*Citrus* sp.	Iran	GU945354	GU945340	KU887505
L. citricola	CBS 124706	*Citrus* sp.	Iran	GU945353	GU945339	KU887504
L. crassispora	CBS 118741*	*Santalum album*	Australia	DQ103550	DQ103557	KU887506
L. crassispora	CMW 13488	*Eucalyptus urophylla*	Venezuela	DQ103552	DQ103559	KU887507
L. crassispora (*L. pyriformis*)	CBS 121770	*Acacia mellifera*	Namibia	EU101307	EU101352	KU887527
L. euphorbiaceicola	CMM 3609*	*Jatropha curcas*	Brazil	KF234543	KF226689	KF254926
L. euphorbiaceicola	CMW 33268	*Adansonia* sp.	Senegal	KU887131	KU887008	KU887430
L. gilanensis	CBS 124704*	*Citrus* sp.	Iran	GU945351	GU945342	KU887511
L. gilanensis	CBS 124705	*Citrus* sp.	Iran	GU945352	GU945341	KU887510
L. gilanensis (*L. missouriana*)	CBS 128311	*Vitis vinifera*	USA	HQ288225	HQ288267	HQ288304
L. gonubiensis	CMW 14077*	*Syzygium cordatum*	South Africa	AY639595	DQ103566	DQ458860
L. gonubiensis	CMW 14078	*Syzygium cordatum*	South Africa	AY639594	DQ103567	EU673126
L. gravistriata	CMM 4564*	*Anacardium humile*	Brazil	KT250949	KT250950	N/A
L. gravistriata	CMM 4565	*Anacardium humile*	Brazil	KT250947	KT266812	N/A
L. hormozganensis	CBS 124709*	*Olea* sp.	Iran	GU945355	GU945343	KU887515
L. hormozganensis	CBS 124708	*Mangifera indica*	Iran	GU945356	GU945344	KU887514
L. iranensis	CBS 124710*	*Salvadora persica*	Iran	GU945348	GU945336	KU887516
L. iranensis	CBS 124711	*Juglans* sp.	Iran	GU945347	GU945335	KU887517
L. iranensis (*L. jatrophicola*)	CMM 3610	*Jatropha curcas*	Brazil	KF234544	KF226690	KF254927
L. krabiensis	MFLUCC 17-2617*	*Bruguiera* sp.	Thailand	MN047093	MN077070	N/A
L. laeliocattleyae	CBS 130992*	*Mangifera indica*	Egypt	KU507487	KU507454	KU887508
L. laeliocattleyae	BOT 29	*Mangifera indica*	Egypt	JN814401	JN814428	N/A
L. lignicola	CBS 134112*	Dead wood	Thailand	JX646797	KU887003	JX646845
L. lignicola (*L. chinensis*)	CGMCC 3.18061	Woody branch	China	KX499889	KX499927	KX500002
L. macrospora	CMM 3833*	*Jatropha curcas*	Brazil	KF234557	KF226718	KF254941
L. mahajangana	CMW 27801*	*Terminalia catappa*	Madagascar	FJ900595	FJ900641	FJ900630
L. mahajangana	CMW 27818	*Terminalia catappa*	Madagascar	FJ900596	FJ900642	FJ900631
L. mahajangana (*L. caatinguensis*)	CMM 1325	*Citrus sinensis*	Brazil	KT154760	KT008006	KT154767
L. mahajangana (*L. exigua*)	CBS 137785	*Quercus ilex*	Tunisia	KJ638317	KJ638336	KU887509
L. margaritacea	CBS 122519*	*Adansonia gibbosa*	Australia	EU144050	EU144065	KU887520

.....continued on the next page

TABLE 2 (Continued**)**

Species	Isolate number	Host	Location	GenBank accession number		
				ITS	***tef***	***tub2***
L. mediterranea	CBS 137783*	*Quercus ilex*	Italy	KJ638312	KJ638331	KU887521
L. mediterranea	CBS 137784	*Vitis vinifera*	Italy	KJ638311	KJ638330	KU887522
L. microcondia	CGMCC 3.18485*	*Aquilaria crassna*	Laos	KY783441	KY848614	N/A
L. parva	CBS 456.78*	Cassava-field soil	Colombia	EF622083	EF622063	KU887523
L. parva	CBS 494.78	Cassava-field soil	Colombia	EF622084	EF622064	EU673114
L. plurivora	STE-U 5803*	*Prunus salicina*	South Africa	EF445362	EF445395	KP872421
L. plurivora	STE-U 4583	*Vitis vinifera*	South Africa	AY343482	EF445396	KU887525
L. pontae	CMM 1277*	*Spondias purpurea*	Brazil	KT151794	KT151791	KT151797
L. pseudotheobromae	CBS 116459*	*Gmelina arborea*	Costa Rica	EF622077	EF622057	EU673111
L. pseudotheobromae	CBS 116460	*Acacia mangium*	Costa Rica	EF622078	EF622058	KU198428
L. rubropurpurea	WAC 12535*	*Eucalyptus grandis*	Australia	DQ103553	DQ103571	EU673136
L. rubropurpurea	WAC 12536	*Eucalyptus grandis*	Australia	DQ103554	DQ103572	KU887530
L. subglobosa	CMM 3872*	*Jatropha curcas*	Brazil	KF234558	KF226721	KF254942
L. subglobosa	CMM 4046	*Jatropha curcas*	Brazil	KF234560	KF226723	KF254944
L. syzygii	GUCC 9719.1*	Wax apple	Thailand	MT990531	MW016943	MW014331
L. thailandica	CBS 138760*	*Mangifera indica*	Thailand	KJ193637	KJ193681	N/A
L. thailandica	CBS 138653	*Phyllanthus acidus*	Thailand	KM006433	KM006464	N/A
L. thailandica (*L. hyalina*)	CGMCC 3.17975	*Acacia confusa*	China	KX499879	KX499917	KX499992
L. thailandica (*L. swieteniae*)	MFLUCC 18-0244	*Swietenia mahagoni*	Thailand	MK347789	MK340870	MK412877
L. theobromae	CBS 164.96*	Fruit along coral reef coast	Papua	AY640255	AY640258	KU887532
L. theobromae	CBS 111530	*Leucospermum* sp.	USA	EF622074	EF622054	KU887531
L. tropica	CGMCC 3.18477*	*Aquilaria crassna*	Laos	KY783454	KY848616	KY848540
L. venezuelensis	WAC 12539*	*Acacia mangium*	Venezuela	DQ103547	DQ103568	KU887533
L. venezuelensis	WAC 12540	*Acacia mangium*	Venezuela	DQ103548	DQ103569	KU887534
L. viticola	CBS 128313*	*Vitis vinifera*	USA	HQ288227	HQ288269	HQ288306
L. viticola	UCD 2604MO	*Vitis vinifera*	USA	HQ288228	HQ288270	HQ288307
L. vitis	CBS 124060*	*Vitis vinifera*	Italy	KX464148	KX464642	KX464917
Diplodia mutila	CMW 7060	*Fraxinus excelsior*	Netherlands	AY236955	AY236904	AY236933
D. seriata	CBS 112555*	*Vitis vinifera*	Portugal	AY259094	AY573220	DQ458856

***** Indicates ex-type/ex-epitype isolates. The new species is indicated in bold.

Abbreviations of isolates and culture collections: **BOT**—Personal number of S. Denman; **CBS**—Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; **CFCC**—China Forestry Culture Collection Center, Beijing, China; **CGMCC**—China General Microbiological Culture Collection Center; **CMM**—Culture Collection of Phytopathogenic Fungi "Prof. Maria Menezes", Universidade Federal Rural de Pernambuco, Recife, Brazil; **CMW**—Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI) of the University of Pretoria, Pretoria South Africa; **GZCC**—Guizhou Culture Collection, Guiyang, China; **MFLUCC**—Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **STE-U**—Culture Collection of the Department of Plant Pathology, University of Stellenbosch, South Africa; **UCD**—University of California, Davis, Plant Pathology Department Culture Collection; **WAC**—Department of Agriculture Western Australia Plant Pathogen Collection, Perth, Australia.

Sequence alignment and phylogenetic analysis

According to blast results and previous literature, all the type and reference sequences of *Lasiodiplodia* were selected and downloaded from GenBank for phylogenetic analysis. All sequences used in this study are listed in TABLE 2. The MAFFT v7.307 online tool (https://mafft.cbrc.jp/alignment/server/) and MEGA 5 (Tamura *et al.* 2013) were used to align the sequence data. Phylogenetic analyses of the combined sequence data were performed using maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI) methods as detailed in Dissanayake *et al.*

(2020). The best model of evolution was determined using MrModeltest v2 (Nylander *et al.* 2004). The BI analysis was conducted in MrBayes v 3.2.6 (Ronquist *et al.* 2012), and ML analysis was performed in raxmlGUI v 1.3.1 (Silvestro & Michalak 2012). The MP analysis was performed in PAUP*4.0b10 (Swofford 2002). Phylogenetic trees were drawn with FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). The DNA sequences generated in this study were deposited in GenBank (TABLE 2) and the alignments were submitted to TreeBase (submission ID: 27962). The taxonomic novelty was submitted to the Faces of Fungi database (Jayasiri *et al.* 2015) and MycoBank.

Results

Phylogenetic analysis

The combined ITS, *tef* and *tub2* data set comprised 74 taxa with *Diplodia mutila* (CMW 7060) and *D. seriata* (CBS 112555) as the outgroup taxa. The dataset comprised 1,214 characters (ITS: 1–509; *tef*: 510–831; *tub2:* 832–1,214) after alignment, including gaps. The maximum parsimonious dataset consisted of 1,214 characters, of which 922 characters were constant, and 213 characters were parsimony informative, while 79 variable characters were parsimony-uninformative. The MP analysis resulted with tree length of 608 steps [consistency index (CI) = 0.637, retention index (RI) = 0.863, relative consistency index (RC) = 0.549, homoplasy index (HI) = 0.363], and the result of MP analysis is shown in FIG. 1. In the ML analyses, the best scoring RAxML tree with a final likelihood value of -5246.586923 is presented. The matrix had 369 distinct alignment patterns, with 12.54% of undetermined characters or gaps. Estimated base frequencies were: A = 0.205394, C = 0.308282, G= 0.256189, T = 0.230136; substitution rates AC = 1.118735, AG = 3.649904, AT = 1.583682, CG = 1.229656, CT = 4.928610, GT = 1.000000; gamma distribution shape parameter (alpha) = 0.176473. The maximum likelihood (ML), maximum parsimony (MP) and Bayesian methods (BI) for phylogenetic analyses resulted in trees with similar topologies. Phylogenetic results (FIG. 1) showed that two isolates (MFLUCC 21-0003 and GZCC 21-0003), representing *Lasiodiplodia chiangraiensis*, clustered together and formed a distinct lineage within *Lasiodiplodia*. They have close phylogenetic relationship with *L. iranensis*, represented by three isolates (one of which was previously known as *L. jatrophicola*), but can be recognized as a phylogenetically distinct species (FIG. 1).

Taxonomy

Lasiodiplodia chiangraiensis N. Wu, A.J. Dissanayake & Jian K. Liu ***sp. nov.*** (FIG. 2)
MycoBank number: MB839203, *Facesoffungi number*: FoF09518.

Etymology:—Named after Chiang Rai Province in Thailand, where the fungus was collected.

Holotype:—MFLU 21-0003.

Saprobic on the bark of an unidentified host, forming conspicuous, black spots on the host surface. **Sexual morph:** not observed. **Asexual morph:** *Conidiomata* 170–190 µm diam., 160–190 µm high, semi-immersed or immersed in the substrate, solitary, gregarious or confluent, globose to subglobose, short neck, dark brown. *Peridium* up to 21–35 µm wide, consisting of brown, small cells of *textura angularis*, becoming thin-walled and hyaline towards the inner region. *Ostiole* 30–70 µm diam., centrally located, papillate. *Paraphyses* 2–5 µm wide, hyaline, cylindrical, aseptate, not branched, rounded at apex. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 7–11 µm long, 3.5–5 µm wide, hyaline, cylindrical. *Conidia* (21–)22–27(–30) × (12–)13–15(–17) µm (av. = 25 × 14 µm, n = 30), subglobose to oval, rounded at the apex, frequently constricted in the middle, hyaline, aseptate or one-septate, guttulate, without longitudinal striations or mucilaginous sheath.

Culture characteristics:—Conidia germinating on PDA within 12 h. Colonies reaching 90 mm diam. after 4–5 days at 20–23 °C, circular, white during the first few days, sparse, aerial, surface smooth with crenate edge, filamentous, after 2 weeks becoming black.

Material examined:—THAILAND. Chiang Rai: Amphoe Mueang, Tambon Nang Lae, Mae Fah Luang University, Botanical Garden, 20°02’22.7’’N, 99°53’38.1’’E, on unidentified dead wood, 17 July 2019, Na Wu, YW113 (MFLU 21-0003, holotype; GZAAS 21-0003, isotype), ex-type living culture MFLUCC 21-0003; *ibid.,* on decaying wood, 12 December 2019, Na Wu, YW401 (GZAAS 21-0014), living culture GZCC 21-0003.

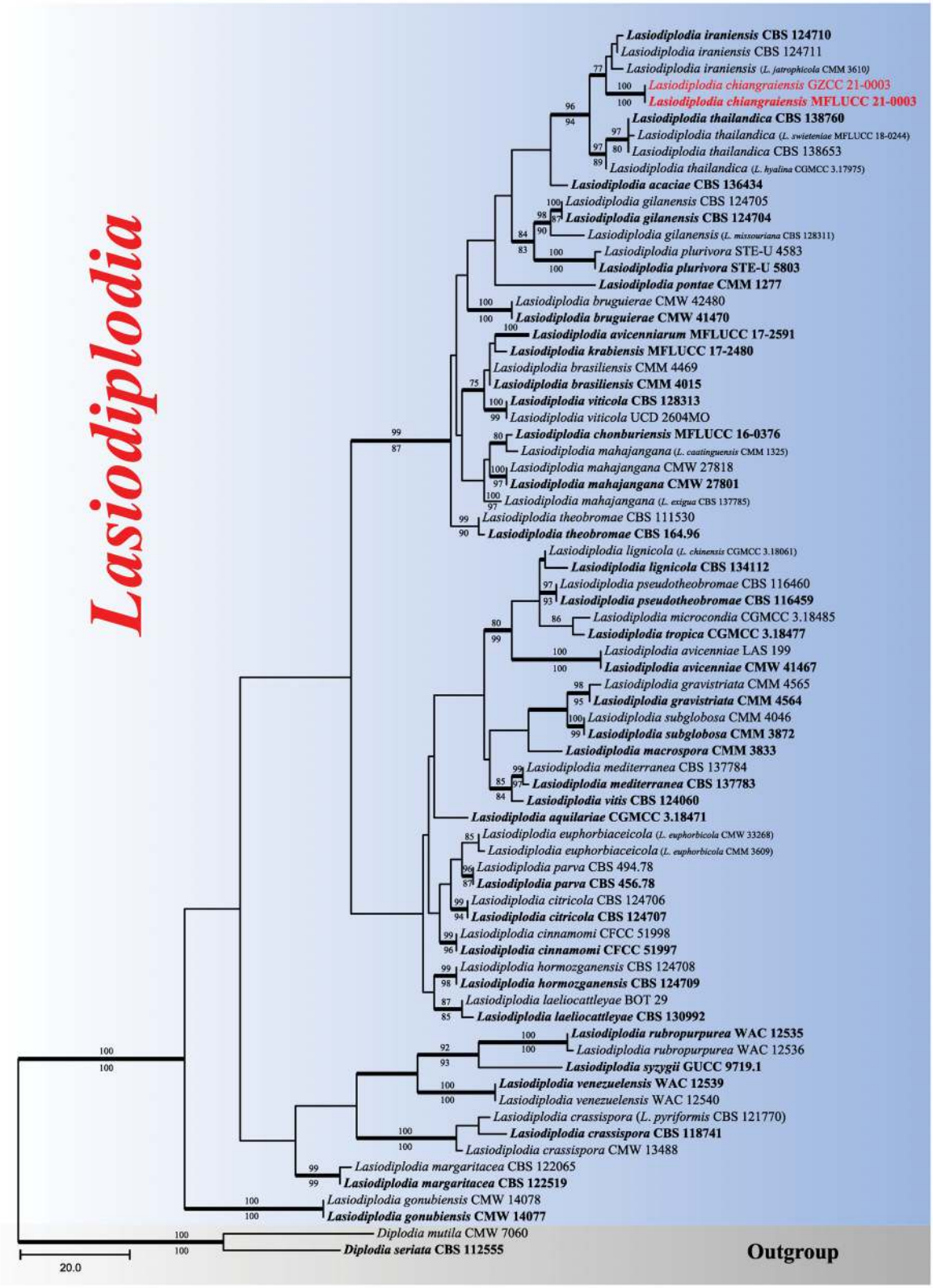

FIGURE 1. Phylogenetic tree generated from maximum parsimony (MP) analysis based on combined ITS, *tef* and *tub2* sequence data of *Lasiodiplodia*. Bootstrap values for maximum likelihood (ML) and maximum parsimony (MP) equal to or greater than 75% are placed above and below the branches, respectively. Branches with Bayesian posterior probabilities (BYPP) equal or greater than 0.95 are thickened. The new isolates are indicated in red and ex-type strains are in bold. The tree is rooted to *Diplodia mutila* (CMW 7060) and *D. seriata* (CBS 112555). The scale bar shows 20 changes.

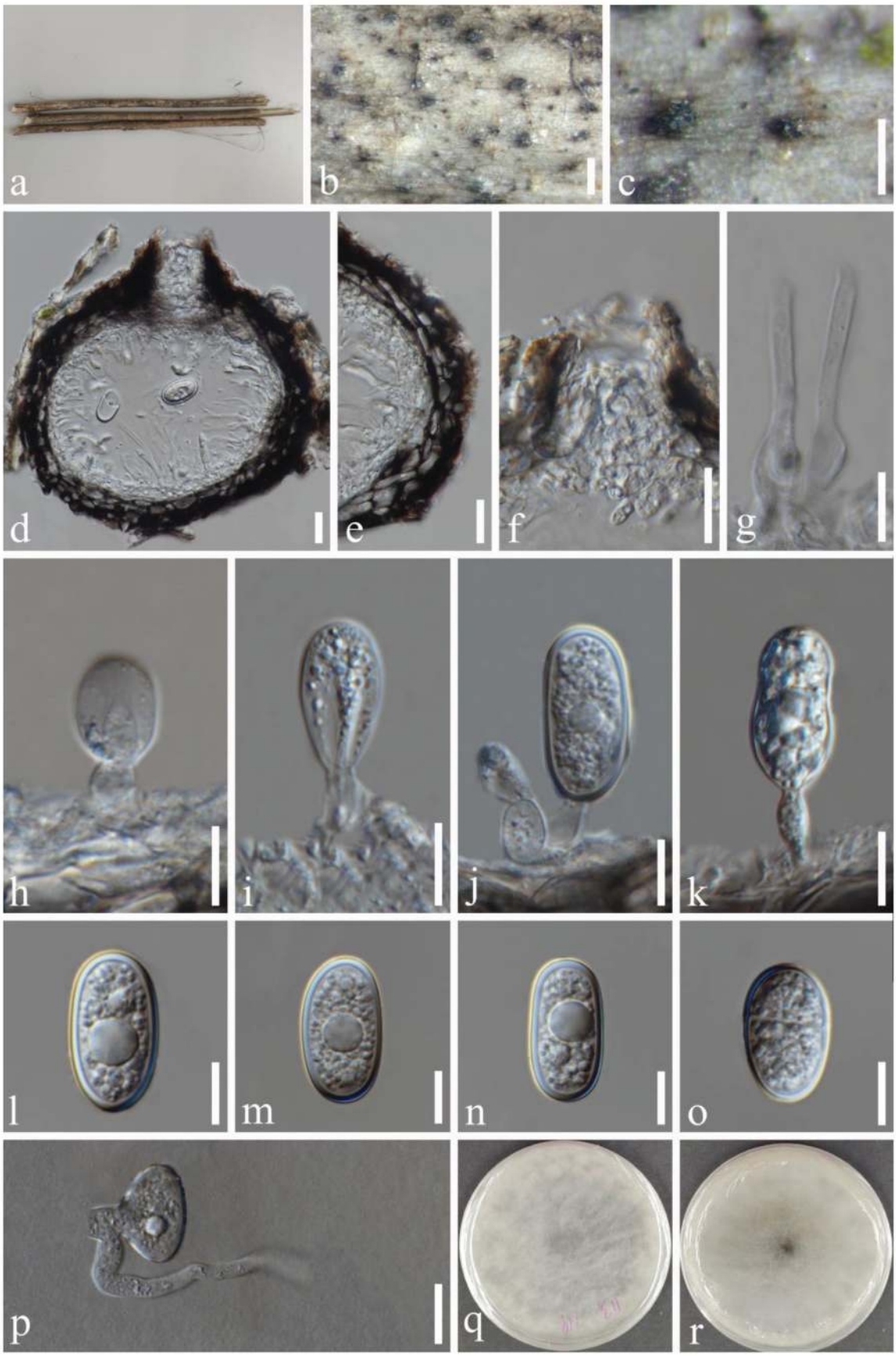

FIGURE 2. *Lasiodiplodia chiangraiensis* (MFLU 21-0003, holotype). a–c. Conidiomata on host surface. d. Section through conidiomata. e. Peridium. f. Ostiolar region with periphyses. g. Paraphyses. h–k. Conidia developing on conidiogenous cells. l–o. Hyaline, aseptate conidia. p. Germinating conidium. q, r. Colonies after 7 days on PDA (q from above, r from below). Scale bars: b = 500 μm, c = 200 μm, d–e = 10 μm, f = 20 μm, g–p = 10 μm.

Known distribution:—Chiang Rai, Thailand.

Notes:—*Lasiodiplodia chiangraiensis* is phylogenetically closely related to *L. iranensis* but formed a distinct linage (FIG. 1), and can be recognized as a new species. Morphologically, these species can be distinguished from the dimensions of their conidia (TABLE 3). In addition, conidia of *L. chiangraiensis* are hyaline without longitudinal striations, while those of *L. iraniensis* become dark brown with age. In terms of the nucleotides comparison, *L. chiangraiensis* (MFLUCC 21-0003) and *L. iraniensis* (CBS 124710, ex-type) differed in one base pair (bp) in ITS region, seven in *tef* region and two in *tub2*.

TABLE 3. A morphological comparison of conidial dimensions of *Lasiodiplodia chiangraiensis* and its phylogenetically closely related species.

Species	Conidial dimensions (µm)	L/W ratio	Reference
L. chiangraiensis	(21–)22–27(–30) × (12–)13–15(17)	1.9	This study
L. iraniensis	(15.3–)17–23 (–29.7) × 11–14	1.6	Abdollahzadeh *et al.* (2010)
L. iraniensis (*L. jatrophicola*)	22–26 × 14–17	-	Machado *et al.* (2014)

Discussion

Lasiodiplodia is one of the largest genera in the family *Botryosphaeriaceae*. In recent years, many *Lasiodiplodia* species have been found globally confirming their cosmopolitan distribution, e.g., Algeria, Australia, Botswana, Brazil, China, Colombia, Costa Rica, Egypt, Germany, India, Iran, Italy, Laos, Madagascar, Namibia, Netherlands, Papua New Guinea, Portugal, South Africa, Thailand, Tunisia, USA, Venezuela (Abdollahzadeh *et al.* 2010, Ismail *et al.* 2012, Marques *et al.* 2013, Machado *et al.* 2014, Netto *et al.* 2014, Prasher & Singh 2014, Linaldeddu *et al.* 2015, Trakunyingcharoen *et al.* 2015, Dou *et al.* 2017, Rodríguez-Gálveza *et al.* 2017). *Lasiodiplodia theobromae*, the type species of the genus, is one of the most common pathogens that causes various diseases in woody plants (Punithalingam 1980, Burgess *et al.* 2006, Burruano *et al.* 2008, Wright & Harmon 2009, Luo *et al.* 2011, Fan *et al.* 2013, Sinha *et al.* 2018). Zhang *et al.* (2021) evaluated the species in Botryosphaeriales, and over 20 members of *Lasiodiplodia* were synonymized to avoid inaccurate species introductions. To date, 38 species are accepted in *Lasiodiplodia*, including *L. chiangraiensis* described in this study with strict protocol followed. The establishment of the new species is justified with both morphology and phylogeny evidence, and an updated phylogenetic tree of *Lasiodiplodia* following the latest treatment is provided; this can be a reference for future taxonomy and phylogeny study of *Lasiodiplodia*.

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