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Does morphology matter in taxonomy of *Lasiodiplodia*? An answer from *Lasiodiplodia hyalina* sp. nov.

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Abstract

A new species of *Lasiodiplodia* (*L. hyalina*) is described and illustrated from *Acacia confusa* and an unidentified woody plant collected in Southern China. Only asexual states of *L. hyalina* were observed, which is characterized by most conidia remaining hyaline with only about 10% conidia becoming pigmented after three months in culture. Phylogenetically, *L. hyalina* is closely related to *L. thailandica*. Morphologically, the larger conidiogenous cells and paraphyses of *L. hyalina* are distinct from those of *L. thailandica*, which leads to the conclusion that the collected taxon is new to science. *Lasiodiplodia thailandica* is reported as a new record in China with *Podocarpus macrophyllus* and *Albizia chinensis* as its new hosts.

Key words – Botryosphaeriaceae – China – hyaline conidia – phylogeny

Introduction

Lasiodiplodia Ellis & Everh. was formally introduced in Clendenin (1896), and was typified by *L. theobromae* (Pat.) Griffon & Maubl. (Phillips et al. 2013). *Lasiodiplodia* had been considered as a possible synonym of *Diplodia* Fr. (Denman et al. 2000), while the presence of pycnidial paraphyses, longitudinal striations on mature conidia, and the results of phylogenetic studies suggest that it separates from *Diplodia* as a well-defined genus (Sutton 1980, Zhou & Stanosz 2001, Slippers et al. 2004, Phillips et al. 2008, 2013, Prasher & Singh 2014). Although the morphological characteristics of *Lasiodiplodia* spp. are quite comparable, features of pycnidia, conidia and paraphyses have been widely used in distinguishing *Lasiodiplodia* from other genera of Botryosphaeriaceae as well as distinguishing different species within *Lasiodiplodia* (Phillips et al. 2013, Slippers et al. 2017).

So far, five *Lasiodiplodia* species have been reported in China, namely *L. chinensis* Z. P. Dou, Y. Zhang ter., *L. hormozganensis* Abdollahz., Zare & A.J.L. Phillips, *L. iraniensis* Abdollahz., Zare & A.J.L. Phillips, *L. pseudotheobromae* A.J.L. Phillips, A. Alves & Crous and *L. theobromae* (Zhao et al. 2010, Luo et al. 2011, Li et al. 2015, Dou et al. 2017). During an opportunistic collection of ascomycetous fungi in Southern China, one new taxon with general characteristics of *Lasiodiplodia* was collected. Combined ITS, *tef1-α*, *TUB* and *RPB2* DNA sequence comparisons verified its new status within *Lasiodiplodia*. Based on the combination of morphological and molecular differences, a new species, *L. hyalina*, is introduced. *Lasiodiplodia*

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thailandica is collected from *Podocarpus macrophyllus* D. Don and *Albizia chinensis* Merr., which is reported as a new record in China herein.

Materials & Methods

Isolates and morphology

Cankered branches of *Acacia* spp., *Podocarpus* spp., *Albizia* spp. as well as some unidentified tree species were collected in Guangdong and Hainan Province, China during November 2015 and January 2016. Wood segments of 0.5 cm × 0.5 cm × 0.2 cm were cut from the canker lesion boundary. The wood segments were then surface sterilized (Pavlic et al. 2004) and cultured on malt extract agar (MEA) for fungal strains. Plates were incubated at 28 °C under continuous near-UV light for two weeks and colonies resembling *Lasiodiplodia* spp. were selected and transferred to synthetic nutrient-poor agar (SNA). Isolates were maintained on 2 % MEA at 28 °C and stored at 4 °C. Isolates grown on MEA were kept at ambient temperatures (about 28 °C) in the dark to establish colony characteristics. Fungal isolates 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).

To induce sporulation, isolates were grown on 2 % water agar (WA) (Biolab, S.A.) with sterilized pine needles placed onto the medium, at 28 °C under near-UV light. Released conidia and squash mounts of pycnidia formed on the pine needles, were mounted in water on microscope slides and examined microscopically. Measurements and digital photographs were made using a Nikon Coolpix 995 digital camera connected to a trinocular Leitz Orthoplan microscope and processed with Adobe Photoshop Elements 10 software. Measurements of conidia, paraphyses and conidiogenous cells were made from water mounts.

DNA extraction, PCR amplification

DNA was extracted from mycelium grown on MEA plates 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. 2017). PCR amplification and sequencing followed the protocol of Zhang et al. (2009).

Sequence alignment and phylogenetic analysis

The combined loci of ITS, *tef1-α*, *TUB* and *RPB2* were used to infer the phylogenetic relationships among different species of *Lasiodiplodia* by maximum parsimony (MP) and MrBayes analyses. 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 the 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 12,000,000 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 (S20654).

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 taxon was *Diplodia mutila* and *D. seriata*. The heuristic search with random addition of taxa (1,000 replicates) generated 5000 most parsimonious trees of 771 steps (CI = 0.638, RI = 0.864, RC = 0.551, HI = 0.362). In the phylogenetic tree, the clade comprising *L. thailandica*, *L. hyalina* and *L. iraniensis* received high support for both Bayesian and MP analysis (Fig. 1). The subclades comprising individual species of *L. thailandica* and *L. iraniensis* both received high Bayesian analysis support, and moderate support in MP analysis.

Taxonomy

Lasiodiplodia hyalina Z. P. Dou, Y. Zhang, **sp. nov.** Fig 2

Mycobank No.: MB 817651; *Facesoffungi number*: FoF03151.

Etymology – from the Latin "hyaline", in reference to the hyaline conidia.

Sexual morph unknown. **Asexual morph**: *Conidiomata* stromatic, produced on pine needles on SNA within 1–2 wk, solitary, immersed or semi-immersed, iron grey to black, covered with dense mycelium, mostly uniloculate, 255–500 μm diam, solitary, globose, thick-walled, with a central ostiole. *Paraphyses* hyaline, cylindrical, thin-walled, initially aseptate, becoming up to 1–7 septate when mature, sometimes branched or connected to the ladder shaped or H form, rounded at apex, occasionally basal or apical cells swollen, 24–82 × 3–7 μm. *Conidiophores* absent. *Conidiogenous cells* holoblastic, discrete, hyaline, smooth, thin-walled, cylindrical to ampulliform, proliferating percurrently, (8–)9–18(–20) × 4–7 μm (av. = 12.6 × 5.3 μm, n = 60). *Conidia* initially hyaline, aseptate, ellipsoid to ovoid, occasionally with a median or submedian constriction, including granular content, both ends broadly rounded, thick-walled, verruculose, (19–)20–27(–28) × 12–16 μm (av. of 30 conidia = 24 × 13.6 μm, L/W ratio = 1.77, range from 1.36 to 2.00), a few conidia turning pale brown with a single median septum and longitudinal striations after three months, but most conidia remain hyaline.

Culture characteristics – *Colonies* on MEA initially white with woolly aerial mycelia, becoming iron grey to black on the surface after 2 weeks; reverse side of the colonies olivaceous-grey to dark black. Colonies reaching 76.5 mm on MEA after 48 h in the dark at 28 °C.

Specimens examined – CHINA, Hainan Province, Danzhou City, the Danzhou Tropical Botanical Garden, from cankered stems of *Acacia confusa* Merr., 3 November 2015, Y. Zhang & Y. P. Zhou (HMAS 255216, holotype), ex-type living culture, CGMCC 3.17975; Guangdong Province, Guangzhou City, Sculpture Park, from cankered branches of an unidentified woody plant, 21 January 2016, Z.P. Dou & Z.C. Liu (CGMCC 3.18383).

Note – The conidia of *L. hyalina* keep hyaline until three months' growing on SNA, then only a small proportion (ca. 10%) of conidia become pigmented with striations on the surface, which looks senescent. Phylogenetically, *L. hyalina* is closely related to *L. thailandica* and *L. iraniensis* (Fig.1). The *tef1-α* region of *L. hyalina* is distinguishable from those of *L. thailandica* (CPC 22795, GenBank Accession No. KJ193681) and *L. iraniensis* (IRAN 1520C, GenBank Accession No. GU945336) with identity of 97.0% and 97.1%, respectively. In addition, the larger conidiogenous cells ((8–)9–18(–20) × 4–7 μm *vs* 8–9 × 2–4 μm) and broader (3–7 μm *vs* 1–1.5 μm), branching and

anastomosing paraphyses of *L. hyalina* are distinguishable from those of *L. thailandica* (Trakunyingcharoen et al. 2015). The smaller conidiomata (255–500 μm *vs* up to 980 μm) and shorter paraphyses (24–82 μm *vs* up to 127 μm) of *L. hyalina* differs from those of *L. iraniensis* (Abdollahzadeh et al. 2010). Furthermore, some hyaline conidia of *L. hyalina* show a median or submedian constriction (Fig.2), which also distinguishes it from the other two species.

Lasiodiplodia thailandica T. Trakunyingcharoen, L. Lombard & Crous, in Trakunyingcharoen, Lombard, Groenewald, Toanun & Crous, *Persoonia* 34: 95 (2015)

Specimens examined – CHINA, Guangdong Province, Guangzhou City, Baiyun Mountain, from cankered branch of *Podocarpus macrophyllus*, 19 January 2016, Z.P. Dou & Z.C. Liu (CGMCC 3.18382). Yangchun City, Kongtongyan Scenic Area, from cankered branch of *Albizia chinensis*, 23 January 2016, Z.P. Dou & Z.C. Liu (CGMCC 3.18384).

Discussion

Morphological characteristics of sexual or asexual stage have their weakness in taxonomy of Botryosphaeriaceae, while their significance cannot be ignored (Phillips et al. 2013, Slippers et al. 2014, 2017). Morphologically, the striations on the pigmented conidia and the presence of conidiomatal paraphyses distinguish *Lasiodiplodia* from all other genera of Botryosphaeriaceae, while the morphology of pycnidia or conidia (especially dimensions), as well as morphology of the paraphyses can be used in species identification of *Lasiodiplodia* spp (Table 2, Phillips et al. 2013). For instance, the large-sized, 1–3-septate mature conidia and aseptate paraphyses of *L. gonubiensis* Pavlic, Slippers & M.J. Wingf. could be distinguishable from other reported species of *Lasiodiplodia*, and *L. rubropurpurea* T.I. Burgess, Barber & Pegg could be recognized based on its unique livid red to dark vinaceous pycnidia (Pavlic et al. 2004, Burgess et al. 2006, Alves et al. 2008). Although the morphology of *Lasiodiplodia* species differ from each other in some degree, the identification of *Lasiodiplodia* species cannot be safely applied without the help of related DNA sequence comparisons.

Phylogenetically, *L. hyalina* forms a robust clade with *L. thailandica* (Fig. 1). Conidia of both *L. hyalina* and *L. thailandica* tend to keep hyaline, and only a small proportion of the discharged conidia getting pigmented with age (Trakunyingcharoen et al. 2015), which differs from most other reported species of *Lasiodiplodia*. Although no pigmented conidia were produced in *L. sterculiae* Tao Yang & Crous after being cultured for two months in SNA medium, it lacks of the description about aged conidia for this species (Yang et al. 2017). Thus, the conidia pigmentation of *L. sterculiae* cannot be determined until information about the aged conidia was provided after longer incubation. So far, the one (or rarely up to three) septum, pigmented conidia with striations on its surface (sometimes for aged conidia) can serve as diagnosing characteristics for *Lasiodiplodia* yet.

Lasiodiplodia thailandica was first described from symptomless twigs of *Mangifera indica* from Thailand (Trakunyingcharoen et al. 2015), and it was retrieved from the cankered branches of *Podocarpus macrophyllus* and *Albizia chinensis* in tropical region of China. It seems that *L. thailandica* is a tropical species with a wide range of host spectrum. *Podocarpus* spp. seem to be good hosts for fungi, as many new fungal taxa has been reported from the genus (Dai et al. 2009, 2010, 2011, Zhou & Dai 2013).

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***
Diplodia mutila	CMW 7060	*Fraxinus excelsior*	Netherlands	AY236955	AY236904	AY236933	EU339574
D. seriata	CBS 112555	*Vitis vinifera*	Portugal	AY259094	AY573220	DQ458856	N/A
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	CGMCC 3.18061	unknown	China	KX499889	KX499927	KX500002	KX499965
L. chinensis	CGMCC 3.18044	*Vaccinium uliginosum*	China	KX499875	KX499913	KX499988	KX499951
L. chinensis	CGMCC 3.18066	*Hevea brasiliensis*	China	KX499899	KX499937	KX500012	KX499974
L. chinensis	CGMCC 3.18067	*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
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. hyalina	CGMCC 3.17975	*Acacia confusa*	China	**KX499879**	**KX499917**	**KX499992**	**KX499955**
L. hyalina	CGMCC 3.18383	unknown tree	China	**KY767661**	**KY751302**	**KY751299**	**KY751296**
L. iraniensis	IRAN 1520C	*Salvadora persica*	Iran	GU945348	GU945336	KU887516	KU696363
L. iraniensis	IRAN 1502C	*Juglans* sp.	Iran	GU945347	GU945335	KU887517	KU696362
L. iraniensis	CMM 3610	*Jatropha curcas*	Brazil	KF234544	KF226690	KF254927	N/A
L. iraniensis	CMW 36237	*Adansonia digitata*	Mozambique	KU887121	KU886998	KU887499	KU696348
L. iraniensis	CMW 36239	*Adansonia digitata*	Mozambique	KU887123	KU887000	KU887501	KU696349
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	CGMCC 3.18047	*Pteridium aquilinum*	China	KX499876	KX499914	KX499989	KX499952
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. thailandica	CGMCC 3.18382	*Podocarpus macrophyllus*	China	**KY767662**	**KY751303**	**KY751300**	**KY751297**
L. thailandica	CGMCC 3.18384	*Albizia chinensis*	China	**KY767663**	**KY751304**	**KY751301**	**KY751298**
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

Table 2 A morphological comparison of *Lasiodiplodia* spp.

Species	**Paraphyses**			**Conidiogenous cells**		**Conidia**					**References**
	B[1]	**Se**[2]	**Size (µm)**	**An**[3]	**Size (µm)**	**Se**	**Size (µm)**	**L**[4]**/W**[4]	**Cl**[5]	**PT**[6]	
L. avicenniae	UN[1]	S[2]	⩽170, 2–4	1–2	6–15×3–6	1	19–30×9–15	UN	Br[5]	UN	Osorio et al. (2017)
L. brasiliense	UN	A[2]	UN	UN	UN	1	22.7–29.2×11.7–17.0	UN	UN	UN	Netto et al. (2014)
L. bruguierae	NO[1]	NO	NO	UN	11–23×2.7–5	1	19–32×11–15	UN	DB[5]	UN	Osorio et al. (2017)
L. caatinguensis	B	UN	31–60×2–5	UN	7–15×2–6	1	13–20.2×10.1–12.5	1.54	DB	UN	Coutinho et al. (2017)

L. chinensis	NB[1]	1–9	⩽99, 3–7	NO	10–15×4–6	1	19–25×12–14	1.75	UN	UN	Dou et al. (2017)
L. citricola	OB[1]	1–5	⩽125, 3–4	1–2	11–16×3–5	1	20–31×11–19	1.6	UN	UN	Abdollahzadeh et al. (2010)
L. crassispora	UN	S	21–66×2–4	UN	6–19×3–7	1	27–33×14–17	1.8	UN	UN	Burgess et al. (2006)
L. euphorbiicola	OB	S	⩽76, 2−4	UN	5–15×3–4	1	15–23×9–12	UN	DB	UN	Machado et al. (2014)
(*L. marypalme*)	UN	A	UN	UN	UN	1	19.1–28.5×10–15.3	UN	UN	UN	Netto et al. (2014)
L. exigua	UN	MS[2]	61–99×2–3	UN	12–19×3–5	1	19.6−24.3×10.8−13.3	1.8	DB	UN	Linaldeddu et al. (2015)
(*L. americana*)	OB	1–3	⩽90, 2–3.5	1–2	10–18×3–5	1	14.0–24.5×10.5–15.0	1.57	DB	UN	Chen et al. (2015)
L. gilanensis	OB	1–3	⩽95, 2–4	UN	11–18×3–5	1	25–39×14.5–19	1.9	UN	UN	Abdollahzadeh et al. (2010)
L. gonubiensis	UN	A	14–65×1.5–3	UN	6.5–18×1–4.5	1–3	28–39×14–21	1.9	Ci[5]–Se[5]	UN	Pavlic et al. (2004)
L. gravistriata	UN	A	UN	UN	9–14×3–5	1	24.5–28.5×10.5–16	UN	UN	UN	Netto et al. (2017)
L. hormozganensis	OB	1–7	⩽83, 2–4	UN	9–15×3–5	1	15.3–25.2×11–14	1.7	UN	UN	Abdollahzadeh et al. (2010)
L. hyalina	OB	1–7	24–82 ×3–7	NO	8–20×4–7	1	19–28 ×12–16	1.77	PB[5]	3 MO[6]	Present study
L. indica	OB	S	⩽120,1.5–3.5	UN	8.5–17.5×1.5–4	1–2	20–38×11–20.5	UN	DB	UN	Prasher & Singh (2014)
L. iraniensis	OB	1–6	⩽127, 2–4	UN	9–16×3–5	1	15.3–29.7×11–14	1.6	UN	UN	Abdollahzadeh et al. (2010)
(*L. jatrophicola*)	OB	S	⩽70, 3	UN	7–15×2–5	1	22−26×14−17	UN	DB	UN	Machado et al. (2014)
L. laeliocattleyae	UN	A	⩽95,2–3	UN	11–14×3–4	1	18–27.4×11.7–17.2	1.6	DB	UN	Rodríguez-Gálvez et al. (2017)
(*L. egyptiacae*)	UN	A	⩽57, 2–3	1–2	5–11×3–5	1	17−27×11−13	2	Br	UN	Ismail et al. (2012)
L. lignicola	UN	A	⩽15	UN	10–15×2.5–3.5	UN	15–17.5×8–11	1.7	DB	UN	Phillips et al. (2013)
L. macrospora	NB	S	⩽105, 3−4	UN	8–20×2.5–4	1–3 young,1 mature	28−35×15−17	UN	DB	UN	Machado et al. (2014)
L. mahajangana	UN	A	27.5–66×2–5	UN	10–26×3–6	1	13.5–21.5×10–14	1.4	UN	UN	Begoude et al. (2010)
L. margaritacea	UN	1–2	19–54×1.5–3	UN	6–19.5×2–4.5	1	12–19×10–12.5	1.3	Ci–Se	UN	Pavlic et al. (2008)
L. mediterranea	OB	S	66–107×2–3	UN	11–16×3–5	1–2	26.3−37×13.5−18	1.9	DB	LT[6]	Linaldeddu et al. (2015)
L. missouriana	NB	A	⩽55, 2–3	UN	UN	1	16.1–21×8.1–11.8	1.89	DB	UN	Urbez-Torres et al. (2012)
L. parva	UN	S	⩽105, 3–4	1–2	UN	1	15.5–24.5×10–14.5	1.8	dark	LT	Alves et al. (2008)
L. plurivora	OB	1–6	⩽130, 2–10	UN	8–13×4–7	1	22–35×13–18.5	1.9	Br	UN	Damm et al. (2007)
L. pontae	B	UN	19–46×2–3	UN	6–16×3–5	1	16–26×9.6–15	1.74	Br	UN	Coutinho et al. (2017)
L.	OB	RS[2]	⩽58, 3–4	1–2	UN	1	22.5–33×13.5–20	1.7	DB	LT	Alves et al. (2008)

pseudotheobromae											
L. pyriformis	UN	A	27–33.5×1.5–2	UN	7–16×2.5–6.5	A	19–28×13.5–21.5	1.3	Se	4 wk[6]	Slippers et al. (2014)
L. rubropurpurea	UN	A	30–58×1.5–3.5	1	7–15×3–5	1	24–33×13–17	1.9	UN	UN	Burgess et al. (2006)
L. sterculiae	NO	NO	NO	1–2	7–12 × 2.5–3.5	UN	14–16×10–11	UN	H[5]	UN	Yang et al. (2017)
L. subglobosa	NB	A	≤41, 2−3	UN	8−18×3−4.5	1	16−23×11−17	UN	DB	UN	Machado et al. (2014)
L. thailandica	UN	1–3	25–51× 1–1.5	UN	8–9×2–4	1	20–26×12–16	UN	PB	UN	Trakunyingcharoen et al.(2015)
L. theobromae	OB	S	≤55, 3–4	1–2	UN	1	19–32.5×12–18.5	1.9	DB	LT	Alves et al. (2008)
L. venezuelensis	UN	S	12–45×1.5–5	UN	5–15×3–5	1	26–33×12–15	2.1	UN	UN	Burgess et al. (2006)
L. viticola	NB	A	≤60, 2–3	UN	UN	1	17–23×8–11	2.05	DB	UN	Urbez-Torres et al. (2012)
L. vitis	NB	A	≤60, 2–3	1–3	5–15 × 5–8	1	26–28×15–16	UN	DB	UN	Yang et al. (2017)
L. sp.	OB	RS	≤61, 2–3	UN	11–15×3–4	1	16–26×9–16	1.7	Br	SA[6]	Rodríguez-Gálvez et al. (2017)

Note: [1] B=branch, UN=unknown, NO=not observed, OB=occasionally branched=rarely branched=sometimes branched, NB=not branched.

[2] Se=septation, S=septate, A=aseptate, MS=mostly septate, RS=rarely septate=mostly aseptate.

[3] An=annellations.

[4] L=Length, W=Width.

[5] Cl=colour, Br=brown, DB=dark brown, Ci=cinnamon, Se=sepia, PB=pale brown, H=hyaline.

[6] PT=schedule of getting pigmentation, MO= month, LT=a long time, wk= week, SA=soon after being formed.

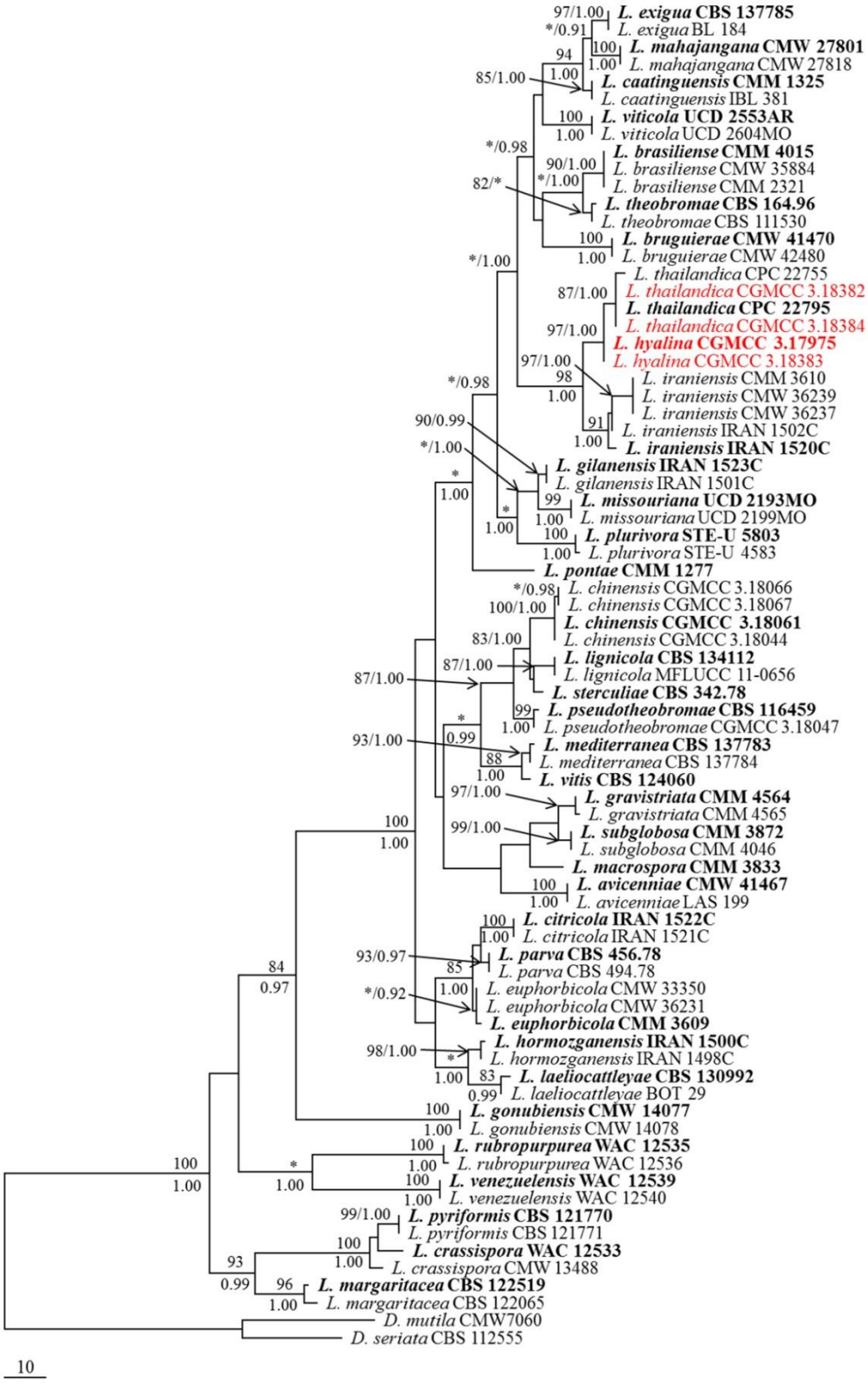

Figure 1 – Maximum parsimony tree generated from sequence analysis of the combined ITS nrDNA, *tef1*-α, *TUB* and *RPB2* dataset. Designated out group taxon is *Diplodia mutila and D. seriata*. Bootstrap support values for maximum parsimony (MP) greater than 80% are shown above at the nodes. Bayesian bootstrap (BP) posterior probability scores above 0.90 are shown under the branches (* = MP value less than 80% or BP value less than 0.90). The species characterized in this study are in red, and the ex-type strains are in boldface.

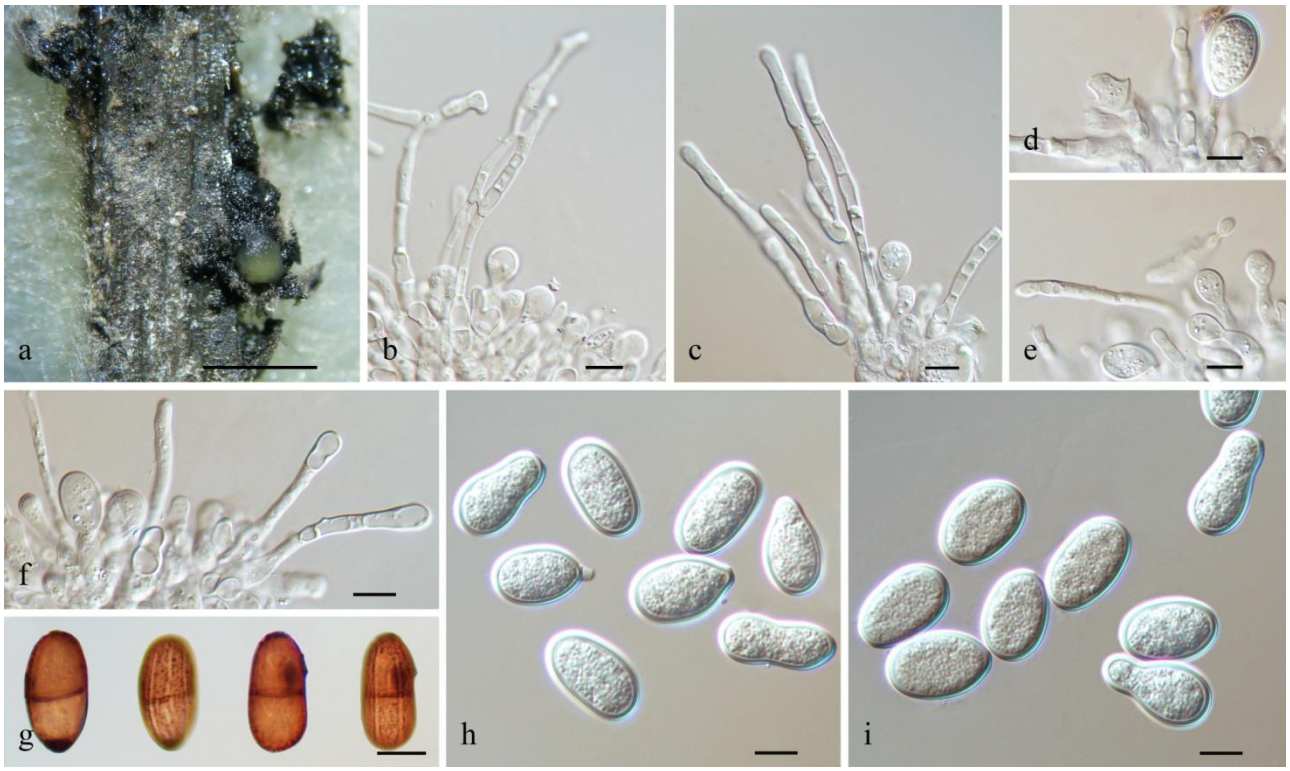

Figure 2 – *Lasiodiplodia hyalina* (From holotype, CGMCC 3.17975) a. Conidiomata formed on pine needles in culture. b. The ladder shaped paraphyses. c–f. Conidia developing on conidiogenous cells between paraphyses. g. Mature, 1-septate conidia with longitudinal striations. h, i. Hyaline conidia. Scale bars: a = 1mm; b–i = 10 µm.

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References

Abdollahzadeh J, Javadi A, Goltapeh EM, Zare R et al. 2010 – Phylogeny and morphology of four new species of *Lasiodiplodia* from Iran. Persoonia 25, 1–10.

Alves A, Crous PW, Correia A, Phillips AJL. 2008 – Morphological and molecular data reveal cryptic species in *Lasiodiplodia theobromae*. Fungal Diversity 28, 1–13.

Begoude BAD, Slippers B, Wingfield MJ, Roux J. 2010 – Botryosphaeriaceae associated with *Terminalia catappa* in Cameroon, South Africa and Madagascar. Mycological Progress 9, 101–123.

Burgess TI, Barber PA, Mohali S, Pegg G et al. 2006 – Three new *Lasiodiplodia* spp. from the tropics, recognized based on DNA sequence comparisons and morphology. Mycologia 98, 423–435.

Chen SF, Li GQ, Liu FF, Michailides TJ. 2015 – Novel species of Botryosphaeriaceae associated with shoot blight of pistachio. Mycologia 107, 780–792.

Clendenin I. 1896 – *Lasiodiplodia* Ellis & Everh. n. gen. Botanical Gazette Crawfordsville 21, 92–93.

Coutinho IBL, Freire FCO, Lima CS, Lima JS et al. 2017 – Diversity of genus *Lasiodiplodia*, associated with perennial tropical fruit plants in northeastern Brazil. Plant Pathology 66, 90–104.

Cruywagen EM, Slippers B, Roux J, Wingfield MJ. 2017 – Phylogenetic Species Recognition and hybridisation in *Lasiodiplodia*: A case study on species from baobabs. Fungal Biology 121, 420–436.

Dai YC, Cui BK, Liu XY. 2010 – *Bondarzewia podocarpi,* a new and remarkable polypore from tropical China. Mycologia 102, 881–886.

Dai YC, Cui BK, Yuan HS, 2009 – *Trichaptum* (Basidiomycota, Hymenochaetales) from China with a description of three new species. Mycological Progress 8, 281–287.

Dai YC, Cui BK, Yuan HS, He SH et al. 2011 – Wood-inhabiting fungi in southern China 4. Polypores from Hainan Province. Annales Botanici Fennici 48, 219–231.

Damm U, Crous PW, Fourie PH. 2007 – Botryosphaeriaceae as potential pathogens of *Prunus* species in South Africa, with descriptions of *Diplodia africana* and *Lasiodiplodia plurivora* sp. nov. Mycologia 99, 664–680.

Denman S, Crous PW, Taylor JE, Kang JC et al. 2000 – An overview of the taxonomic history of *Botryosphaeria,* and a re-evaluation of its anamorphs based on morphology and ITS rDNA phylogeny. Studies in Mycology 45, 129–140.

Dou ZP, He W, Zhang Y. 2017 – *Lasiodiplodia chinensis* sp. nov., a new holomorphic species from China. Mycosphere 8(2), 521–532.

Glass NL, Donaldson GC. 1995 – Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous Ascomycetes. Applied & Environmental Microbiology 61, 1323–1330.

Huelsenbeck JP, Ronquist F. 2001 – MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Huelsenbeck JP, Ronquist F. 2005 – Bayesian analysis of molecular evolution using MrBayes. In: *Statistical methods in molecular evolution* (ed Nielsen R.), Springer, New York: 183–232.

Ismail AM, Cirvilleri G, Polizzi G, Crous PW et al. 2012 – *Lasiodiplodia* species associated with dieback disease of mango (*Mangifera indica*) in Egypt. Australasian Plant Pathology 41, 649–660.

Li GQ, Arnold RJ, Liu FF, Li JQ et al. 2015 – Identification and Pathogenicity of *Lasiodiplodia* Species from *Eucalyptus urophylla* × *grandis*, *Polyscias balfouriana* and *Bougainvillea spectabilis* in Southern China. Journal of Phytopathology 163, 956–967.

Linaldeddu BT, Deidda A, Scanu B, Franceschini A et al. 2015 – Diversity of Botryosphaeriaceae species associated with grapevine and other woody hosts in Italy, Algeria and Tunisia, with descriptions of *Lasiodiplodia exigua* and *Lasiodiplodia mediterranea* sp. nov. Fungal Diversity 71, 201–214.

Luo M, Dong ZY, Bin SY, Lin JT. 2011 – First report of fruit rot disease on pomelo caused by *Lasiodiplodia theobromae* in China. Plant Disease 95, 1190–1190.

Machado AR, Pinho DB, Pereira OL. 2014 – Phylogeny, identification and pathogenicity of the Botryosphaeriaceae associated with collar and root rot of the biofuel plant *Jatropha curcas* in Brazil, with a description of new species of *Lasiodiplodia*. Fungal Diversity 67, 1–17.

Netto MSB, Assunção IP, Lima GSA, Marques MW et al. 2014 – Species of *Lasiodiplodia* associated with papaya stem-end rot in Brazil. Fungal Diversity 67, 127–141.

Netto MSB, Lima WG, Correia KC, Silva CFBD et al. 2017 – Analysis of phylogeny, distribution, and pathogenicity of Botryosphaeriaceae species associated with gummosis of *Anacardium* in Brazil, with a new species of *Lasiodiplodia*, Fungal Biology 121, 437–451.

Osorio JA, Crous CJ, Beer ZWD, Wingfield MJ et al. 2017 – Endophytic Botryosphaeriaceae, including five new species, associated with mangrove trees in South Africa. Fungal Biology 121, 361–393.

Pavlic D, Slippers B, Coutinho TA, Gryenhout M et al. 2004 – *Lasiodiplodia gonubiensis* sp. nov., a new *Botryosphaeria* anamorph from native *Syzygium cordatum* in South Africa. Studies in Mycology 50, 313–322.

Pavlic D, Wingfield MJ, Barber P, Slippers B et al. 2008 – Seven new species of the Botryosphaeriaceae from baobab and other native trees in Western Australia. Mycologia 100(6), 851–866.
Phillips AJL, Alves A, Abdollahzadeh J, Slippers B et al. 2013 – The Botryosphaeriaceae: genera and species known from culture. Studies in Mycology 76, 51–167.
Phillips AJL, Alves A, Pennycook SR, Johnston PR et al. 2008 – Resolving the phylogenetic and taxonomic status of dark-spored teleomorph genera in the *Botryosphaeriaceae*. Persoonia 21, 29–55.
Posada D, Buckley TR. 2004 – Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Systematic Biology 53, 793–808.
Prasher IB, Singh G. 2014 – *Lasiodiplodia indica* -A new species of coelomycetous mitosporic fungus from India. Kavaka 43, 64–69.
Rodríguez-Gálvez E, Guerrero P, Barradas C, Crous PW et al. 2017 – Phylogeny and pathogenicity of *Lasiodiplodia*, species associated with dieback of mango in Peru. Fungal Biology 121, 437–451.
Ronquist F, Huelsenbeck JP. 2003 – MrBayes3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Slippers B, Crous PW, Denman S, Coutinho TA et al. 2004 – Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. Mycologia 96, 83–101.
Slippers B, Crous PW, Jami F, Groenewald JZ et al. 2017 –Diversity in the Botryosphaeriales: Looking back, looking forward. Fungal Biology 121, 307–321.
Slippers B, Roux J, Wingfield MJ, Walt FJJVD et al. 2014 – Confronting the constraints of morphological taxonomy in the Botryosphaeriales. Persoonia 33, 155–168.
Sutton BC, 1980. *The Coelomycetes: Fungi Imperfecti with Pycnidia, Acervuli and Stromata*. Kew, UK: Commonwealth Mycological Institute.
Swofford DL. 2002. PAUP*. *Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Version 4. Sinauer Associates, Sunderland, Massachusetts.
Tamura K, Stecher G, Peterson D, Filipski A et al. 2013 – MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology & Evolution 30, 2725–2729.
Trakunyingcharoen T, Lombard L, Groenewald JZ, Cheewangkoon R et al. 2015 – Caulicolous Botryosphaeriales from Thailand. Persoonia 34, 87–99.
Urbez-Torres JR, Peduto F, Striegler RK, Urrea-Romero KE et al. 2012 – Characterization of fungal pathogens associated with grapevine trunk diseases in Arkansas and Missouri. Fungal Diversity 52(1), 169–189.
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*. Academic Press, New York, pp. 315–322.
Yang T, Groenewald JZ, Cheewangkoon R, Jami F et al. 2017 – Families, genera and species of Botryosphaeriales. Fungal Biology 121, 322–346.
Zhang Y, Jeewon R, Fournier J, Hyde KD. 2008 – Multi-gene phylogeny and morphotaxonomy of *Amniculicola lignicola*: a novel freshwater fungus from France and its relationships to the Pleosporales. Mycological Research 112, 1186–1194.
Zhang Y, Wang HK, Fournier J, Crous PW et al. 2009 – Towards a phylogenetic clarification of *Lophiostoma* / *Massarina* and morphologically similar genera in the Pleosporales. Fungal Diversity 38, 225–251.
Zhao JP, Lu Q, Liang J, Decock C et al. 2010 – *Lasiodiplodia pseudotheobromae*, a new record of pathogenic fungus from some subtropical and tropical trees in southern China. Cryptogamie Mycologie 31, 431–439.
Zhou LW, Dai YC. 2013 – Taxonomy and phylogeny of wood-inhabiting hydnoid Russulales: two new genera, three new species and two new combinations. Mycologia 105, 636–649.

Zhou S, Stanosz GR. 2001 – Relationships among *Botryosphaeria* species and associated anamorphic fungi inferred from the analysis of ITS and 5.8S rDNA sequences. Mycologia 93, 516–527.