

Lasiodiplodia syzygii sp. nov.

(Botryosphaeriaceae) causing post-harvest water-soaked brown lesions on *Syzygium samarangense* in Chiang Rai, Thailand

Chao-Rong Meng[‡], Qian Zhang[‡], Zai-Fu Yang[‡], Kun Geng[§], Xiang-Yu Zeng[‡], K. W. Thilini Chethana^{|,¶}, Yong Wang[‡]

[‡] Department of Plant Pathology, Agricultural College, Guizhou University, Guiyang, China

[§] Guiyang plant protection and inspection station, Guiyang, China

[|] Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai, Thailand

[¶] School of Science, Mae Fah Luang University, Chiang Rai, Thailand

Corresponding author: Yong Wang (yongwangbis@aliyun.com)

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Abstract

Background

Syzygium samarangense (Wax apple) is an important tropical fruit tree with high economic and nutrient value and is widely planted in the tropics or subtropics of Asia. Post-harvest water-soaked brown lesions were observed on mature fruits of ornamental wax apples in Chiang Rai Province, Thailand. A fungus with morphological characters, similar to *Lasiodiplodia*, was consistently isolated from symptomatic fruits. Phylogenetic analyses, based on ITS, LSU, TEF1-a and *tub2*, revealed that our isolates were closely related to, but phylogenetically distinct from, *Lasiodiplodia rubropurpurea*.

New information

Morphological comparisons indicated that pycnidia and conidiogenous cells of our strains were significantly larger than *L. rubropurpurea*. Comparisons of base-pair differences in the four loci confirmed that the species from wax apple was distinct from *L. rubropurpurea* and a new species, *L. syzygii* sp. nov., is introduced to accommodate it. Pathogenicity tests confirmed the newly-introduced species as the pathogen of this post-harvest water-soaked brown lesion disease on wax apples.

Keywords

Botryosphaeriaceae, fruit disease, new pathogen, wax apple

Introduction

Wax apple [*Syzygium samarangense* (Blume) Merrill and Perry] belongs to the *Myrtaceae* and was naturalised in the Philippines thousands of years ago (Lim 2012, Shen et al. 2012). As a kind of juicy tropical fruit like watermelon with economic importance, it has been commonly and widely cultivated in many Asian countries (Nesa et al. 2014). Every part of *S. samarangense* also has potential medicinal values (Shen et al. 2012).

Due to the fruit characteristics, such as thin peel and tender pulp with high respiratory intensity, wax apples are prone to damage by pathogens and cannot be stored for a long time (Yang et al. 2009). This causes a significant post-harvest loss. Many studies suggest that wax apple is mainly threatened by fungal diseases. For example, a new fruit rot of wax apple caused by *Phytophthora palmivora* was reported in southern Taiwan during the rainy periods in 1982 (Lin et al. 1984). Yang et al. (2009) and Che et al. (2015) reported *Lasiodiplodia theobromae* as the causal agent of black spot disease on harvested wax apple fruits. *Pestalotiopsis samarangensis* was isolated from the fruit rot in wax apples from markets in Thailand (Maharachchikumbura et al. 2013). *Chrysosporthe deuterocubensis* caused cankers on wax apple and branches in Taiwan (Fan et al. 2013).

The present study reports a new post-harvest water-soaked brown lesion disease on wax apples caused by *Lasiodiplodia* sp. in Chiang Rai, Thailand. Morphological and multi-locus phylogenetic analyses revealed that our strain represented a novel species. A pathogenicity test on fruits confirmed the pathogenic relationship between *L. syzygii* and *Syzygium samarangense*.

Materials and methods

Sample collection, isolation and morphology

Rotten wax apple fruits were occasionally collected from a food market near Mae Fah Luang University in Chiang Rai, Thailand. On the third day after the wax apple fruits were collected, it was observed that there were conidiomata bulges on the surface of the fruit, white hyphae and the fruit turned black, rotted and had cytoplasmic extravasation. Diseased samples were conserved in self-sealing bags and then taken back to the laboratory and photographed. Before isolation, diseased fruits were surface disinfected with 70% ethanol for 30 s, 1% sodium hypochlorite (NaClO) for 1 min and repeatedly twice rinsed in sterile distilled water for 30 s. Pure cultures were obtained by single-conidium isolation following a modified method outlined by Chomnunti et al. (2011) and Maharachchikumbura et al. (2013). The morphology of fungal colonies was recorded

following the method of Hu et al. (2007). Fungal mycelium and spores were observed under a light microscope and photographed. The holotype specimen is deposited in the Herbarium of the Department of Plant Pathology, Agricultural College, Guizhou University (HGUP). The ex-type and isotype cultures are deposited in the Culture Collection at the Department of Plant Pathology, Agriculture College, Guizhou University, P.R. China (GUCC) and the Mae Fah Luang University Culture Collection (MFLUCC) in Thailand.

DNA extraction, PCR reaction and sequencing

Fungal cultures were grown on PDA at 28°C. When colonies nearly covered the entire Petri dish (90 mm diam.), fresh mycelia were scraped from the agar surface with sterilised scalpels. Genomic DNA was extracted using a BIOMIGA Fungus Genomic DNA Extraction Kit (GD2416) following the manufacturer's protocol. DNA amplification was performed in a 25 µl reaction volume following Liang et al. (2018). Primers ITS1 and ITS4 (White et al. 1990) were used to amplify the internal transcribed spacer regions and intervening 5.8S rRNA region (ITS) and LR0R and LR5 for 28S rRNA (LSU) region (Vilgalys and Hester 1990, Rehner and Samuels 1994). Two protein-coding gene fragments, the β -tubulin (*tub2*) and translation elongation factor 1-alpha (TEF1-a) were amplified with primer pairs BT2A/BT2B (Glass and Donaldson 1995, O'Donnell and Cigelnik 1997) and EF1-688F/EF1-986R, respectively (Carbone and Kohn 1999, Alves et al. 2008). Purification and sequencing of the PCR amplicons were done by SinoGenoMax, Beijing. The DNA sequences are deposited in the GenBank and their accession numbers are provided in Table 1. The DNA base differences of the four loci amongst our strains and ex-type or representative strains of relative taxa are shown (Table 2).

Phylogenetic analyses

Sequences of 45 *Lasiodiplodia* isolates, representing all species known from culture, were aligned using the online version of MAFFT v. 7.307 (Katoh and Standley 2016) and manually improved, where necessary, using MEGA v. 6.06 (Koichiro et al. 2013). Mesquite v. 2.75 (Maddison 2008) was used to concatenate the aligned sequences of the different loci. Ambiguous regions were excluded from analyses using AliView (Larsson 2014), gaps were treated as missing data and optimised manually with *Botryosphaeria dothidea* (CMW8000) and *B. fabicerciana* (CBS 127193) as the outgroups (Table 1). The alignment document has been deposited in TreeBASE (www.treebase.org) and the accession number is 27461. Phylogenetic analyses were constructed by Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference methods. First, the ambiguous regions were excluded from the alignment and gaps were treated as missing data. The MP analysis was done with PAUP v. 4.0b10 (Swofford 2002), using the heuristic search option with 1,000 random taxa addition and tree bisection and reconnection (TBR) as the branch swapping algorithm. Maxtrees was set to 5000. Tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI) were calculated for each tree generated. The Maximum Likelihood (ML) analysis was performed using IQ-tree (Nguyen et al. 2015, Chernomor et al. 2016). Nucleotide substitution models were selected under the Akaike Information Criterion (AIC) by jModelTest2 (Darriba et al.

2012) on XSEDE in the CIPRES web portal (Miller et al. 2010). For the ITS dataset, the TPM3uf+I model was selected (-lnL = 1316.7068), for LSU, the TrN+I (-lnL = 1643.7273), for TEF1-a, the HKY+I+G (-lnL = 2399.0528) and for β -tubulin, the TIM3+G (-lnL = 1161.0392). ML was inferred under partitioned models. Non-parametric bootstrap analysis was implemented with 1000 replicates. Bayesian Inference (BI) analyses was conducted in MrBayes 3.2 (Ronquist et al. 2012). MrModeltest v.2.3 (Nylander 2004) was used to estimate the best evolutionary models under the Akaike Information Criterion (AIC). HKY+I was selected as the best model for ITS, for LSU, HKY+I+G, for TEF1-a, HKY+I+G and for β -tubulin, GTR+G was selected as the best model. Six Markov Chain Monte Carlo runs were launched with random starting trees for 1,000,000 generations and sampling every 1,000 generations. The first 25% resulting trees were discarded as burn-in.

Pathogenicity tests

One isolate of the new *Lasiodiplodia* species (GUCC 9719.1) was grown on PDA and when the cultures covered the entire surface of the Petri dish, mycelia were scraped off with a sterilised blade. Conidiomata were crushed with a glass rod to prepare a spore suspension of 1×10^5 spores/ml. Pathogenicity testing was carried out on five healthy fruits of wax apple bought from the market. Inoculations were carried out in April 2020. The surface of the fruits was wiped with 70% ethanol and allowed to air-dry. Three fruits were slightly wounded by pin-pricking and 3 ml of spores suspension was sprayed on to the wound. The other two wounded fruits were maintained as control and inoculated with 2 ml of sterile deionised water. All inoculated fruits were placed in plastic bags, labelled and a high level of humidity was maintained for seven days by the addition of wet sterile cotton wool in each bag in an illuminated incubator at $28 \pm 3^\circ\text{C}$. Daily observations were made on the development of disease symptoms. When fruits developed the symptoms, they were removed from the bags. Two isolates obtained from the diseased tissue were grown on PDA and then sequenced with primer pairs of the above four DNA markers to confirm the identity.

Taxon treatment

Lasiodiplodia syzygii C.R. Meng, Qian Zhang & Yong Wang bis sp. nov.

- MycoBank [837701](#)

Materials

Holotype:

- scientificName: *Lasiodiplodia syzygii*; kingdom: Fungi; class: Dothideomycetes; order: Botryosphaerales; family: Botryosphaeriaceae; genus: *Lasiodiplodia*; country: Thailand; stateProvince: Chiang Rai; catalogNumber: HGUP 9719; recordedBy: Wang Yong; identifiedBy: Chao-Rong Meng; dateIdentified: 2020; type: ex-type living culture GUCC 9719.1; MFLU 19-0565, isotype, isotype living culture MFLUCC 19-0257.; occurrenceID: 719AE4B1-FECC-5B8C-8CCE-502281673BBE

Other material:

- a. scientificName: *Lasiodiplodia syzygii*; kingdom: Fungi; class: Dothideomycetes; order: Botryosphaerales; family: Botryosphaeriaceae; genus: *Lasiodiplodia*; country: China; stateProvince: Guiyang; catalogNumber: HGUP 9720 and HGUP 9721; recordedBy: Wang Yong; identifiedBy: Chao-Rong Meng; dateIdentified: 2020; type: living cultures GUCC 9719.2, GUCC 9719.3 and GUCC 9719.4; occurrenceID: 80728FE6-B502-5E8A-87E6-BBF9413E1236

Description

Pathogenic on Syzygium samarangense. Sexual morph: Undetermined. **Asexual morph** (Fig. 2): *Conidiomata* up to 2 mm diam., pycnidial, covered with hyphae, black, globose, ostiolate, solitary, separate, uniloculate, immersed to semi-immersed. *Conidiomatal wall* composed of thick-walled, dark brown cells of *textura angularis*, becoming thin-walled and hyaline towards the inner region. *Paraphyses* cylindrical, aseptate, hyaline. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 10–14.5 × 3.5–4.5 µm (average = 11 × 3.7 µm, n = 20), hyaline, smooth, holoblastic forming conidia at their tips. *Conidia* thick-walled, wall up to 1 µm wide, ovoid with both ends rounded, hyaline and remaining so for a long time, becoming pale brown with obsolete striations and occasionally with 1-septate after discharging from the conidioma, (27–)30–32(–36) × (13–)15–17(–20) µm (average = 31.3 × 16.4 µm, n = 50), L/W = 1.9.

Culture characteristics: Conidia germinate on PDA within 24 hours at room temperature (25–30°C) with germ tubes produced from both ends of the conidia. Colonies with white fluffy mycelium on PDA, after 7 days become olivaceous-grey at the centre, white at the edge, raised, fluffy, dense filamentous.

Notes: *Lasiodiplodia syzygii* strains are closely related to *L. rubropurpurea*, but formed a distinct, well-supported clade in the phylogenetic analyses. Base-pairs comparisons between *L. syzygii* ex-type strain (GUCC 9719.1) and ex-type strain of *L. rubropurpurea* (WAC 12535) found seven base differences (1.3%) in ITS region and five differences (0.6%) on LSU, but nine differences (2.1%) in *tub2* and 34 in TEF1-a (10.4%) (Table 2). *Lasiodiplodia syzygii* produced larger pycnidia (up to 2 mm) and larger conidiogenous cells (10–14.5 × 3.5–4.5 µm) than *L. rubropurpurea* (0.5–1.5 mm and 7–13 × 3–5 µm) (Burgess et al. 2006).

Etymology

In reference to the host from which the fungus was first isolated.

Analysis

Phylogenetic analyses

Four *Lasiodiplodia* strains isolated from *Syzygium samarangense* were sequenced. The final alignment of ITS, LSU, TEF1-a and *tub2* comprised of 2177 characters, viz. ITS: 1–530, LSU: 533–1423, TEF1-a: 1426–1752 and β -tubulin: 1755–2183. Of these, 1843 characters were constant and 73 were parsimony-uninformative. Maximum parsimony analysis of the remaining 261 parsimony-informative characters resulted in 850 most parsimonious trees (TL = 676, CI = 0.64, RI = 0.81, RC = 0.52 and HI = 0.36) and the first one is shown as Fig. 1. The ML and Bayesian analyses resulted in trees with similar topologies. Strains GUCC 9719.1, GUCC 9719.2, GUCC 9719.3 and GUCC 9719.4 formed an independent well-supported clade sister to *Lasiodiplodia rubropurpurea* (MP: 100%, ML: 100% and Bayesian posterior probability: 1) Comparison of the DNA base-pair differences between our strains and *L. rubropurpurea* species in four gene regions (Table 2) confirmed the presence of two species; therefore, a new species is introduced for those isolates from wax apple.

Pathogenicity test on the fruits of wax apple

At the third day after inoculation, water-soaked areas with a few white hyphae began to appear on all inoculated fruits similar to the naturally-infected wax apples (Fig. 2a and Fig. 3a). The water-soaked symptom of diffusion with abundant hyphae producing mycelium further appeared on inoculated *Syzygium samarangense* fruits after five days (Fig. 3b). At the 7th day after inoculation, the symptoms spread throughout the fruit (Fig. 3c), together with many white mycelia and more hyphae accompanied by cytoplasmic exosmosis. The control fruits (Fig. 3d) did not show any symptom. The fungi were re-isolated from the lesions of inoculated wax apple fruits and the re-identified (GUCC 9719.3 and GUCC 9719.4) sequencing four gene regions.

Discussion

This study revealed a new species of *Lasiodiplodia*, *L. syzygi* from rotting fruits of *Syzygium samarangense*. Phylogenetic analyses, based on ITS, LSU, TEF1-a and *tub2*, showed that it is phylogenetically closer to *L. rubropurpurea*. Comparisons of DNA base-pair differences in the four loci, as well as morphological differences, confirmed the novelty of this species. The fungus was proved to be pathogenic and, therefore, it is the causal agent of the post-harvest water-soaked brown lesions on wax apple.

Wax apple (*Syzygium samarangense*) is known to be affected by many fungal pathogens that often cause economic losses. These include *Colletotrichum gloeosporioides* (Udayanga et al. 2013) and *Lasiodiplodia theobromae* which was the causal agent of black spot disease (Che et al. 2015), *Pestalotiopsis* spp. and *Phytophthora* spp. The fruit disease

of the current study did not show any typical symptoms of black spot caused by *L. theobromae*. Furthermore, the pink or orange spore masses, typical of anthracnose caused by *C. gloeosporioides* or epidermal to superficial, acervular conidiomata reported by Maharachchikumbura et al. (2013) for *Pestalotiopsis*, were not seen in the current study. The fruit rot caused by *Phytophthora* spp. spread more rapidly (only 2 or 3 days up to a whole fruit) and results in a sour taste on fruits. However, the *L. syzygii* needed about seven days to completely rot the fruit and did not cause any sour taste in the fruits. Thus, the study reports a new disease on wax apple.

Lasiodiplodia resides in Botryosphaeriaceae, Botryosphaerales (Hongsanan et al. 2020) and comprises several species known to cause important or potentially important diseases on woody hosts, mostly in the tropics or sub-tropics (Phillips et al. 2019). Very few species of this family appear to be host-specific (Dissanayake et al. 2016). In south-western China and adjoining areas, agriculture and forestry play an important role in the local economy, which might facilitate the spread of this wax apple disease. Thus, research needs to focus on the occurrence of this newly-discovered pathogen in other economically-important plants and in other locations, as well as how to manage it by biological or chemical control approaches. It is also remarkable to find a new disease on such an important commercial fruit indicating that there are numerous new taxa to be discovered in Thailand (Hyde et al. 2018) and Botryosphaeriaceae (Hyde et al. 2020).

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References

- Alves A, Crous PW, Correia A, Phillips AJL (2008) Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*. Fungal Diversity 28: 1-13.
- Burgess TI, Barber PA, Mohali S, Pegg G, de Beer W, Wingfield MJ (2006) Three new *Lasiodiplodia* spp. from the tropics, recognized based on DNA sequence comparisons and morphology. Mycologia 98 (3): 423-435. <https://doi.org/10.1080/15572536.2006.11832677>
- Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91 (3): 553-556. <https://doi.org/10.2307/3761358>
- Che J, Liu B, Ruan C, Tang J, Huang D (2015) Biocontrol of *Lasiodiplodia theobromae*, which causes black spot disease of harvested wax apple fruit, using a strain of

Brevibacillus brevis FJAT-0809-GLX. Crop Protection 67: 178-183. <https://doi.org/10.1016/j.cropro.2014.10.012>

- Chernomor O, Von Haeseler A, Minh BQ, et al. (2016) Terrace aware data structure for phylogenomic inference from supermatrices. Systematic Biology 65 (6): 997-1008. <https://doi.org/10.1093/sysbio/syw037>
- Chomnunti P, Schoch CL, Aguirre-Hudson B, Ko-Ko TW, Hongsanan S, Jones EBG, Kodsueb R, Phookamsak R, Chuksatiro E, Bahkali AH, Hyde KD (2011) Capnodiaceae. Fungal Diversity 51 (1): 103-134. <https://doi.org/10.1007/s13225-011-0145-6>
- Darriba D, Taboada GL, Doallo R, Posada D, et al. (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9 (8): 772-772. <https://doi.org/10.1038/nmeth.2109>
- Dissanayake AJ, Phillips AJL, Li XH, Hyde KD (2016) Botryosphaeriaceae: Current status of genera and species. Mycosphere 7 (7): 1001-1073. <https://doi.org/10.5943/mycosphere/si/1b/13>
- Fan M, Huang CC, Huang JS, Tsai SF, Yeh HC, Hong CF (2013) First report of *Chrysosporthe deuterocubensis* causing canker on *Syzygium samarangense* in Taiwan. Plant Disease 97 (11): 1508-1508. <https://doi.org/10.1094/PDIS-03-13-0345-PDN>
- Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology 61 (4): 1323-1330. <https://doi.org/10.1128/AEM.61.4.1323-1330.1995>
- Hongsanan S, Hyde KD, Phookamsak R, Wanasinghe DN, McKenzie EHC, Sarma VV, Boonmee S, Lücking R, Bhat DJ, Liu NG, Tennakoon DS, Pem D, Karunarathna A, Jiang SH, Jones EBG, Phillips AJL, Manawasinghe IS, Tibpromma S, Jayasiri SC, Sandamali DS, Jayawardena RS, Wijayawardene NN, Ekanayaka AH, Jeewon R, Lu YZ, Dissanayake AJ, Zeng XY, Luo ZL, Tian Q, Phukhamsakda C, Thambugala KM, Dai DQ, Chethana KWT, Samarakoon MC, Ertz D, Bao DF, Doilom M, Liu JK, Pérez-Ortega S, Suija A, Senwana C, Wijesinghe SN, Konta S, Niranjana M, Zhang SN, Ariyawansa HA, Jiang HB, Zhang JF, Norphanphoun C, de Silva NI, Thiyagaraja V, Zhang H, Bezerra JDP, Miranda-González R, Aptroot A, Kashiwadani H, Harishchandra D, Sérusiaux E, Aluthmuhandiram JVS, Abeywickrama PD, Devadatha B, Wu HX, Moon KH, Gueidan C, Schumm F, Bundhun D, Mapook A, Monkai J, Chomnunti P, Suetrong S, Chaiwan N, Dayarathne MC, Yang J, Rathnayaka AR, Bhunjun CS, Xu JC, Zheng JS, Liu G, Feng Y, Xie N (2020) Refined families of Dothideomycetes: Dothideomycetidae and Pleosporomycetidae. Mycosphere 11 (1): 1553-2107. <https://doi.org/10.5943/mycosphere/11/1/13>
- Hu HL, Jeewon R, Zhou DQ, Zhou TX, Hyde KD (2007) Phylogenetic diversity of endophytic *Pestalotiopsis* species in *Pinus armandii* and *Ribes* spp.: evidence from rDNA and β -tubulin gene phylogenies. Fungal Diversity 24: 1-22.
- Hyde KD, Norphanphoun C, Chen J, Dissanayake AJ, Doilom M, Hongsanan S, Jayawardena RS, Jeewon R, Perera RH, Thongbai B, Wanasinghe DN, Wisitrassameewong K, Tibpromma S, Stadler M (2018) Thailand's amazing diversity: up to 96% of fungi in northern Thailand may be novel. Fungal Diversity 93 (1): 215-239. <https://doi.org/10.1007/s13225-018-0415-7>
- Hyde KD, Jeewon R, Chen YJ, Bhunjun CS, Calabon MS, Jiang HB, Lin CG, Norphanphoun C, Sysouphanthong P, Pem D, Tibpromma S, Zhang Q, Doilom M,

- Jayawardena RS, Liu JK, Maharachchikumbura SSN, Phukhamsakda C, Phookamsak R, Al-Sadi AM, Thongklang N, Wang Y, Gafforov Y, Jones EBG, Lumyong S (2020) The numbers of fungi: is the descriptive curve flattening? *Fungal Diversity* 103: 219-271. <https://doi.org/10.1007/s13225-020-00458-2>
- Kato H, Standley DM, et al. (2016) A simple method to control over-alignment in the MAFFT multiple sequence alignment program. *Bioinformatics* 32 <https://doi.org/10.1093/bioinformatics/btw108>
 - Koichiro T, Stecher G, Peterson D, Filipowski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0.[J. Molecular Biology and Evolution 2725-2729. <https://doi.org/10.1093/molbev/mst197>
 - Larsson A (2014) AliView: a fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics* 30 (22).
 - Liang Y, Ran SF, Bhat J, Hyde KD, Wang Y, Zhao DG (2018) *Curvularia microspora* sp. nov. associated with leaf diseases of *Hippeastrum striatum* in China. *MycKeys* 29: 49-61. <https://doi.org/10.3897/mycokeys.29.21122>
 - Lim TK (2012) Edible medicinal and non-medicinal plants. Springer, Dordrecht. <https://doi.org/10.1007/978-94-007-4053-2>
 - Lin CC, Wang DN, Chang HS (1984) Fruit rot of wax apple caused by *Phytophthora palmivora*. *Plant Disease* 68: 351. <https://doi.org/10.1094/PD-68-351b>
 - Maddison WP (2008) Mesquite: a modular system for evolutionary analysis. *Evolution* 62: 1103-1118.
 - Maharachchikumbura SSN, Guo LD, Chuksatiro E, McKenzie EHC, Hyde KD (2013) A destructive new disease of *Syzygium samarangense* in Thailand caused by the new species *Pestalotiopsis samarangensis*. *Tropical Plant Pathology* 38 (3): 227-235. <https://doi.org/10.1590/S1982-56762013005000002>
 - Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceeding of the 2010 gateway computing environments workshop (GCE)*, New Orleans, Louisiana. 7 pp. [ISBN 1424497523]. <https://doi.org/10.1109/GCE.2010.5676129>
 - Nesa L, Munira S, Mollika S, Islam M (2014) Evaluation of analgesic, anti-inflammatory and CNS depressant activities of methanolic extract of *Lawsonia inermis* barks in mice. *Avicenna Journal of Phytomedicine* 4 (4): 287-296.
 - Nguyen L, Schmidt HA, Von Haeseler A, Minh BQ, et al. (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* 32 (1): 268-274. <https://doi.org/10.1093/molbev/msu300>
 - Nylander JAA (2004) MrModeltest v2. Evolutionary Biology Centre, Uppsala University, Uppsala.
 - O'Donnell K, Cigelnik E (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics and Evolution* 7 (1): 103-116. <https://doi.org/10.1006/mpev.1996.0376>
 - Phillips AJL, Hyde KD, Alves A, Liu JK (2019) Families in *Botryosphaeriales*: a phylogenetic, morphological and evolutionary perspective. *Fungal Diversity* 94 (1): 1-22. <https://doi.org/10.1007/s13225-018-0416-6>
 - Rehner SA, Samuels GJ (1994) Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. *Mycological Research* 98 (6): 625-634. [https://doi.org/10.1016/S0953-7562\(09\)80409-7](https://doi.org/10.1016/S0953-7562(09)80409-7)

- Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61 (3): 539-542. <https://doi.org/10.1093/sysbio/sys029>
- Shen SC, Chang WC, Chang CL (2012) Fraction from wax apple [*Syzygium samarangense* (Blume) Merrill and Perry] fruit extract ameliorates insulin resistance via modulating insulin signaling and inflammation pathway in tumor necrosis factor α -treated FL83B mouse hepatocytes. *International Journal of Molecular Sciences* 13 (7): 8562-8577. <https://doi.org/10.3390/ijms13078562>
- Swofford DL (2002) PAUP*: Phylogenetic analysis using parsimony (and other methods), version 4.0 b10. Sinauer Associates.
- Udayanga D, Manamgoda DS, Liu XZ, Chukeatirote E, Hyde KD (2013) What are the common anthracnose pathogens of tropical fruits? *Fungal Diversity* 61 (1): 165-179. <https://doi.org/10.1007/s13225-013-0257-2>
- Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172 (8): 4238-4246. <https://doi.org/10.1128/JB.172.8.4238-4246.1990>
- White TJ, Bruns T, Lee SJWT, 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*. 18. Academic Press, 315-322 pp. <https://doi.org/10.1016/B978-0-12-372180-8.50042-1>
- Yang FZ, Li M, Gao ZY, Hu MZ (2009) Progress in control technology of fruit diseases in wax-apple (*Syzygium samarangense* (Bl.) Merr. et Perry). *Journal of Zhejiang Agricultural Sciences* 5: 961-964.

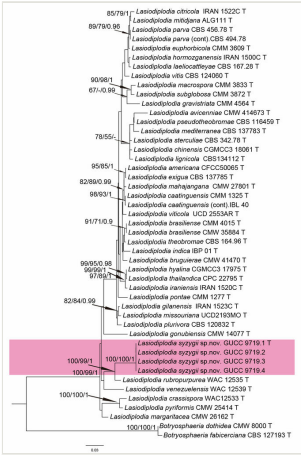


Figure 1. One of 850 most parsimonious trees obtained from a combined analyses of the ITS, LSU, TEF1-a and β -tubulin sequence dataset. Bootstrap values > 50% and BPP values > 0.90 are provided at the nodes and separated by “/”. Bootstrap values < 50% and Bayesian posterior probability (BPP) values < 0.90 were labelled with “-”. The tree was rooted with *Botryosphaeria fabriceana* (CBS 127193) and *B. dothidea* (CMW 8000). The branch of the new *Lasiodiplodia* species is highlighted with pink.

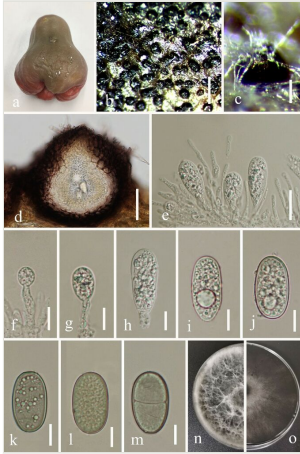


Figure 2.

Lasiodiplodia syzygii (MFLUCC 19-0257). **a.** infected fruit; **b, c.** Conidiomata on the host; **d.** Section through a conidioma; **e.** Conidia developing amongst paraphyses; **f-h.** Conidia formed on conidiogenous cells; **i-m.** Immature conidia; **n-o.** Colonies on PDA culture; **n.** From above; **o.** From below. Scale bars: b = 300 μ m, c = 140 μ m, d = 50 μ m, e = 20 μ m, f-m = 10 μ m.

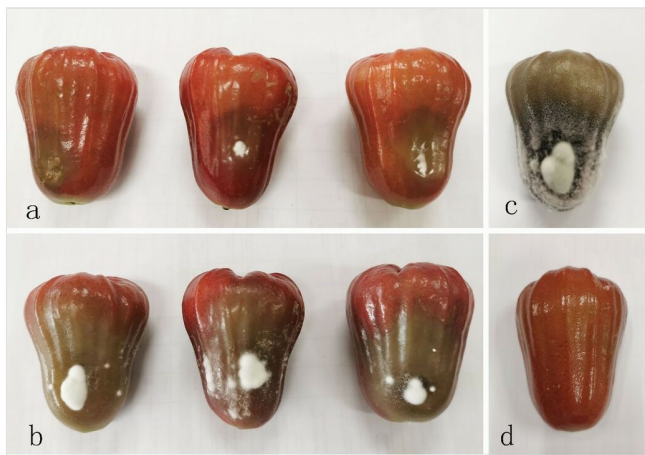


Figure 3.

Symptoms developing on *Syzygium samarangense* fruits inoculated with *Lasiodiplodia syzygii*.
a. Symptom at 3rd day; **b.** Symptom at 5th day; **c.** Symptom at 7th day; **d.** Control.

Table 1.

Table 1 GenBank accession numbers of isolates included in this study. Ex-type isolates are labelled with superscript T.

Species	Isolate no.	GenBank no.			
		ITS	LSU	<i>tef 1</i>	<i>tub2</i>
<i>Lasiodiplodia americana</i>	CFCC50065 ^T	KP217059	MF410052	KP217067	KP217075
<i>L. avicenniae</i>	CMW 414673 ^T	KP860835	–	KP860680	KP860758
<i>L. brasiliense</i>	CMM 4015 ^T	JX464063	–	JX464049	–
<i>L. brasiliense</i>	CMW 35884	KU887094	–	KU886972	KU887466
<i>L. bruguierae</i>	CMW 41470 ^T	KP860833	–	KP860678	KP860756
<i>L. caatinguensis</i>	CMM 1325 ^T	KT154760	–	KT008006	KT154767
<i>L. caatinguensis</i>	IBL 40	KT154762	–	KT154755	KT154769
<i>L. chinensis</i>	CGMCC3.18061 ^T	KX499889	–	KX499927	KX500002
<i>L. citricola</i>	IRAN 1522C ^T	GU945354	–	GU945340	KU887505
<i>L. crassispora</i>	WAC12533 ^T	DQ103550	DQ377901	EU673303	KU887506
<i>L. euphorbicola</i>	CMM 3609 ^T	KF234543	–	KF226689	KF254926
<i>L. exigua</i>	CBS 137785 ^T	KJ638317	–	KJ638336	KU887509
<i>L. gilanensis</i>	IRAN 1523C ^T	GU945351	–	GU945342	KU887511
<i>L. gonubiensis</i>	CMW 14077 ^T	AY639595	DQ377902	DQ103566	DQ458860
<i>L. gravistriata</i>	CMM 4564 ^T	KT250949	–	KT250950	–
<i>L. hormozganensis</i>	IRAN 1500C ^T	GU945355	–	GU945343	KU887515
<i>L. hyalina</i>	CGMCC3.17975 ^T	KX499879	–	KX499917	KX499992
<i>L. indica</i>	IBP 01 ^T	KM376151	–	–	–
<i>L. iraniensis</i>	IRAN 1520C ^T	GU945348	–	GU945336	KU887516
<i>L. laeliocattleyae</i>	CBS 167.28 ^T	KU507487	DQ377892	KU507454	–
<i>L. lignicola</i>	CBS134112	JX646797	JX646814	KU887003	JX646845
<i>L. macrospora</i>	CMM 3833 ^T	KF234557	–	KF226718	KF254941
<i>L. mahajangana</i>	CMW 27801 ^T	FJ900595	–	FJ900641	FJ900630
<i>L. margaritacea</i>	CMW 26162 ^T	EU144050	KX464354	EU144065	KU887520
<i>L. mediterranea</i>	CBS 137783 ^T	KJ638312	–	KJ638331	KU887521
<i>L. missouriana</i>	UCD2193MO ^T	HQ288225	–	HQ288267	HQ288304
<i>L. mitidjana</i>	ALG111 ^T	MN104115	–	MN159114	–
<i>L. parva</i>	CBS 456.78 ^T	EF622083	KF766362	EF622063	KU887523
<i>L. parva</i>	CBS 494.78	EF622084	EU673258	EF622064	EU673114
<i>L. plurivora</i>	CBS 120832 ^T	EF445362	KX464356	EF445395	KU887524
<i>L. pontae</i>	CMM 1277 ^T	KT151794	–	KT151791	KT151797
<i>L. pseudotheobromae</i>	CBS 116459 ^T	EF622077	EU673256	EF622057	EU673111

<i>L. pyriformis</i>	CMW 25414 ^T	EU101307	–	EU101352	KU887527
<i>L. rubropurpurea</i>	WAC 12535 ^T	DQ103553	DQ377903	DQ103571	EU673136
<i>L. sterculiae</i>	CBS 342.78 ^T	KX464140	JX681073	KX464634	KX464908
<i>L. subglobosa</i>	CMM 3872 ^T	KF234558	–	KF226721	KF254942
<i>L. syzygii</i>	MFLUCC 19-0219.1 ^T	MT990531	MT990548	MW016943	MW014331
<i>L. syzygii</i>	GUCC 9719.2	MW081991	MW081988	MW087101	MW087104
<i>L. syzygii</i>	GUCC 9719.3	MW081992	MW081989	MW087102	MW087105
<i>L. syzygii</i> sp. nov.	GUCC 9719.4	MW081993	MW081990	MW087103	MW087106
<i>L. thailandica</i>	CPC 22795 ^T	KJ193637	–	KJ193681	–
<i>L. theobromae</i>	CBS 164.96 ^T	AY640255	EU673253	AY640258	KU887532
<i>L. venezuelensis</i>	WAC 12539 ^T	DQ103547	DQ377904	DQ103568	KU887533
<i>L. viticola</i>	UCD 2553AR ^T	HQ288227	–	HQ288269	HQ288306
<i>L. vitis</i>	CBS 124060 ^T	KX464148	KX464367	KX464642	KX464917
<i>Botryo sphaeria dothidea</i>	CMW 8000 ^T	AY236949	AY928047	AY236898	AY236927
<i>B. fabierciana</i>	CBS 127193 ^T	HQ332197	MF410028	HQ332213	KF779068

Table 2.

DNA base pair differences between *Lasiodiplodia syzygii* and *L. rubropurpurea* in four separate loci. ^T = ex-type

<i>L. syzygiumae</i> strains	<i>Lasiodiplodia rubropurpurea</i> WAC 12535 ^T			
	ITS (1–530)	LSU (531–1421)	TEF1-a(1422–1748)	β -tubulin (1749–2177)
MFLUCC 19-0257=GUCC 9719.1 ^T	7	5	34	9
GUCC 9719.2	7	5	34	9
GUCC 9719.3	7	5	34	9
GUCC 9719.4	7	5	34	9
Total number of differences	55			