Dokumen Bukti Korespondensi untuk karya penelitian dengan judul artikel : **Jackfruit (Artocarpus heterophyllus), a new host plant of Ceratocystis wilt in South Sumatra, Indonesia**.

Penulis : Rahmat Pratama, ***Ahmad Muslim*(sebagai penulis korespondensi)***, Suwandi, Nurhayati D., Soleha, Nama Jurnal : Australasian Plant Disease Notes, Penerbit : CSIRO Netherlands, Volume Jurnal : 16, Nomor Jurnal : 24, TahunTerbit Jurnal : 2021, Halaman : 1-6, Online-ISSN : 1833-928X, Yang terdiri dari :

1) Submission Process
   - Submission Confirmation and Draft Submit (20 Februari 2021)
   - Revise Before Review 1 (22 Februari 2021)
   - Draft Perbaikan
   - Revise Before Review 2 (01 Maret 2021)
   - Draft Perbaikan

2) Review Process :
   - Revisi Pertama (17 Mei 2021)
   - Draft Perbaikan Revisi Pertama (22 Mei 2021)
   - Revisi Kedua (12 Juni 2021)
   - Draft Perbaikan Revisi Kedua (14 Juni 2021)
   - Revisi Ketiga (18 Juni 2021)
   - Draft Perbaikan Revisi Ketiga (28 Juni 2021)
   - Revisi Keempat (25 Juli 2021)
   - Draft Perbaikan Revisi Keempat (31 Juli 2021)

3) Proofread and Publishing Process
   - Letter of Accepted (05 Agustus 2021)
   - Proofread (27 Agustus 2021)
   - Draft Terbit dan Naskah Akhir (Final Manuscript) Untuk Diterbitkan (Published) pada Australasian Plant Disease Notes, Journal of the Australasian Plant Pathology Society (11 September 2021)

SUBMISSION PROCESS

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%S_NUMBER% Submission Confirmation

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Australasian Plant Disease Notes

Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia

--Manuscript Draft--

Manuscript Number:	APDN-D-21-00015
Full Title:	Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia
Article Type:	Plant Disease Note
Keywords:	Sudden death; Pathogenicity; Jackfruit; Indonesia; Wilt disease
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Funding Information:	Kementerian Riset Teknologi Dan Pendidikan Tinggi Republik Indonesia (068/SP2H/AMD/LT/DRPM/2020) — Dr. A. Muslim
Abstract:	Artocarpus heterophyllus (jackfruit) is an economically important fruit crop in Indonesia and widely grown throughout the country. Growers in South Sumatra recently reported the death of jackfruit trees with symptoms of vascular stain, yellowing and browning leaves until wilting on several lateral branches and trees, which in later stages of the disease lost most of their leaves and suddenly died. The causal agent of this disease was identified based on both morphological appearance and comparisons of DNA sequence data for the β-tubulin and ITS gene regions as Ceratocystis manginecans. Pathogenicity tests were conducted by inoculating wounds on the stems of A. heterophyllus with mycelial plugs (4 mm diam.). After 45 days of inoculation, the fungi produced lesions of significant length and severe foliar symptoms in inoculated plants. Koch's postulates were fulfilled by reisolation of C. manginecans from the inoculated plants. This is the first report of C. manginecans causing wilt and die-back disease on A . heterophyllus in Indonesia.

Jackfruit (*Artocarpus heterophyllus*), a New Host Plant of *Ceratocystis* Wilt in South Sumatra, Indonesia

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Abstract

Artocarpus heterophyllus (jackfruit) is an economically important fruit crop in Indonesia and widely grown throughout the country. Growers in South Sumatra recently reported the death of jackfruit trees with symptoms of vascular stain, yellowing and browning leaves until wilting on several lateral branches and trees, which in later stages of the disease lost most of their leaves and suddenly died. The causal agent of this disease was identified based on both morphological appearance and comparisons of DNA sequence data for the β-tubulin and ITS gene regions as *Ceratocystis manginecans.* Pathogenicity tests were conducted by inoculating wounds on the stems of *A. heterophyllus* with mycelial plugs (4 mm diam.). After 45 days of inoculation, the fungi produced lesions of significant length and severe foliar symptoms in inoculated plants. Koch's postulates were fulfilled by reisolation of *C. manginecans* from the inoculated plants. This is the first report of *C. manginecans* causing wilt and die-back disease on *A. heterophyllus* in Indonesia.

Keywords: Sudden death · Pathogenicity · Jackfruit · Indonesia · Wilt disease

Jackfruit (*A. heterophyllus*) belongs to the family Moraceae, and it is known in Indonesian as "Nangka". Jackfruit is grown widely in Indonesia and many countries with tropical and subtropical climates. Jackfruit is among the most exported fruits worldwide, and one of the most important fruit crops in Indonesia. It is popular because it is delicious and has considerable nutrition and health benefits (Ranasinghe et al. 2019).

In a recent study of jackfruit in 2019, wilt and die-back symptoms were observed for the first time on *A. heterophyllus* in the agricultural field of Sriwijaya University (Indralaya), Plaju (Palembang) and Gelumbang (Prabumulih), Indonesia. Jackfruit trees were reported to

die within a period from July to September 2019. Leaves of dying trees had yellowing symptoms, followed by the wilting of the leaves on several lateral branches, drying of twigs and the wilt of the whole tree. This type of wilting was termed as sudden death or wilt (Pornsuriya and Sunpapao 2015). This study aimed to identify the cause of a new wilt disease-causing sudden death of *A. heterophyllus* plants in Indonesia. Sap stain moulds were isolated from infected Jackfruit, and identified based on morphological characteristics and molecular analysis.

Artocarpus heterophyllus wood produced grey lesions resulting from growth of *Ceratocystis* on the stem (Fig. 1a). Wilt disease changed leaf colour from green to yellow until wilting on several lateral branches and loss of most of their fruits and sudden died (Fig. 1b). Affected 2 to 5 year-old trees were mainly observed in plantations, but trees with wilt disease were also present in home gardens. Wilt symptoms in the field were observed resulting in high lesion length development which caused discoloration of bark and wood, presence of sap flow from lesion surfaces, discoloration of leaves and extended foliar wilting or loss until tree death. Trees took up to five months to completely die after the first symptoms were observed.

Samples of diseased trunks were collected from three to five year-old trees, approximately ten trees in location sampling, in August and October 2019. Wood samples were taken from lesions of wilted trees using a knife sterilised in 70% ethanol. Wood samples were collected from *A. heterophyllus* showing brown to black streaking in the woody xylem. Each sample was wrapped in tissue paper and placed in a cool box. The same day, the wood samples (1–20 mm length, 1–2 mm thick) were sandwiched between two slices of fresh carrot and placed on sterile dry paper in plastic boxes at 25 °C following the method of Moller and DeVay (1968) (Fig. 1c). After 5–10 days, hat-shaped spores of putative *Ceratocystis* pathogens were placed on 2% (w/v) malt extract agar (MEA) (Merck, Germany), and incubated at 25 °C in a laboratory. The isolated fungi were initially identified based on morphological characteristics of a 14 day old culture. Mycelium on MEA grey, reverse side of colony olivaceous grey; submerged mycelium darkening as the ascomata develop forming fine, radiating fibrils (Fig. 1d).

Morphological traits of fruiting bodies and spores were observed under an optical Olympus CX33 microscope. Ascomata developing within seven days and mature within ten days, superficial or partly embedded in the agar, dark brown to black. Ascomata of *Ceratocystis* with necks supporting sticky masses of ascospores on the carrot slices. Ascomatal bases dark brown to black, base subglobes to globes and measured (n=100), 131.5 to 250.7 × 101.6 to 236.5 µm (length/width) (Fig. 2a). Ascomata necks erect, occasionally curved, black at the base

becoming subhyaline towards the apex, smooth to crenulate, 324.7 to 579.1 µm long including ostiolar hyphae (Fig.2b). Phialides pale brown to hyaline (Fig.2c). Ascospores hat-shaped, 3.4 to 6.8 × 2.1 to 6.2 µm (length/width) (Fig.2d). Bacilliform conidia 11.1 to 36.1 × 2.1 to 7.4 µm (length/width) (Fig.2e). Barrel conidia 4.4 to 16.1 × 2.7 to 6.9 µm (length/width) (Fig.2f). Chlamydospores oval, thick walled, smooth, 6.7 to 16.5 × 5.9 to 12.9 µm (length/width) (Fig.2g).

Based on morphological characters, the fungus was identified as *C.manginecans.* A culture of the fungus was deposited in Culture Collection of Phytopathology Laboratory, Department of Plant Protection, Faculty of Agriculture, Sriwijaya University, Indralaya, South Sumatera, Indonesia. To confirm the species identification, isolates were cultured on potato dextrose broth (PDB) at room temperature for one week. Mycelial mat was filtered through Whatman filter paper and genomic DNA was extracted from fungal mycelial mat using YeaStar Genomic DNA Kit (Zymo Research Corporation, California, USA).

PCR conditions and reactions for two gene regions were used to identify the *Ceratocystis* isolates; the Internal Transcribed Spacer (ITS) and part of the β-tubulin (βt) gene. Amplifications were carried out in 50 µl reactions containing 20 µl DreamTaq Green PCR Master Mix (Eppendorf, Germany) (DreamTaq DNA Polymerase, 2X DreamTaq Green buffer, dNTPs, and 4 mM $MgCl_2$), 1,5 µl of each forward and reverse primer, 4 µl of DNA template and 23 µl sterilised water. The PCRs were performed with a C1000 Touch™ thermal cycler (Bio-Rad, USA). The PCR cycling parameters were as follows: initial denaturation for 5 min at 95 °C, followed by 35 cycles at 95 °C for 30 s, 56 °C for 45 s and 72 °C for 1 min. Amplification was completed at 72 °C for 10 min and the PCR product was stored at 10 °C. The PCR amplicons were sequenced at 1st BASE (Malaysia). Raw sequence data were assembled, examined, and manually edited using Genestudio 2.1.1.5 (Genestudio, Suwanee, Georgia) and BioEdit software (Hall 1999).

The DNA sequences were compared to the GenBank database via the nucleotide-nucleotide BLAST search interface located at the National Center for Biotechnology Information, Bethesda, USA. Relevant sequences were transferred NoteTab Light v7.2. Sequences from different gene regions were aligned using Mesquite v3.5 (Maddison and Maddison 2018) (http://mesquiteproject.org) and corrected manually.

For the ITS and β-tubulin, amplification resulted in fragments of ~550 base pairs (bp) in size. The sequences of the amplified products were then deposited in the GenBank database and assigned accession numbers isolate CAAW31171 (MT355410; MT412106), isolate CAAW30817 (MT355413, MT412109), and isolate CAAW30268 (MT355412; MT412108)

for the ITS and β-tubulin, respectively, and they were compared with the sequences of *C.manginecans* available at GenBank. Blast searches in GenBank indicated that our isolates grouped within *C.manginecans* species with 99% identity of the sequences. The two gene regions (ITS and βt) were combined and analysed as a single dataset. Maximum Parsimony (MP) analyses were performed in MEGA v. 10 (Kumar et al. 2016; Paul et al. 2018) with 1000 bootstrap replications. According to the phylogenetic relationships derived from the Maximum Parsimony (MP) analyses, our *C. manginecans* isolates (CAAW31171, CAAW30817, CAAW30268) in *Artocarpus heterophyllus* was closely related to *C. manginecans* in *A. mangium* (Fig.3). This sequence similarity to prior cases of *C. manginecans* corroborates the identification by phenotypic characteristics, suggesting that the causal agent of sudden death disease on *A. heterophyllus* in Indonesia, represented by the CAAW31171, CAAW30817, CAAW30268 isolates, should be regarded as *C. manginecans*.

To determine the pathogenicity of fungi isolated, The pathogenic potential of isolates was evaluated by the under bark inoculation method described by O'Gara et al. (1997) using Five-month-old *A. heterophyllus* seedlings with stem diameters of 6-8 mm and heights <1.5 m were prepared for pathogenicity test. Wounds were made on the stems of the seedlings using a cork borer (4 mm diam.), and mycelial discs (4 mm diam.) taken from an actively growing colony on 2% MEA (14 days) (Tarigan et al. 2010; Tarigan et al. 2011; Chi et al. 2019a) were placed in the wounds with the mycelium facing downwards. These were covered with Parafilm (Pechiney, Menasha, Wisconsin) to reduce contamination and desiccation. Ten plants of each tree species were inoculated with sterile MEA plugs to serve as controls. Fungal isolates were re-isolated and re-identified using morphological characteristics for Koch's postulates confirmation. The fungi were shown to be pathogenic in young *A. heterophyllus*, with plants exhibiting wilt symptoms 45 days after inoculation (data not shown). When re-isolated, the fungus was phenotypically identical to the prior isolate of *C. manginecans* (CAAW31171, CAAW30817, CAAW30268).

In this study, *A. heterophyllus* were found to represent new diseases and hosts for *C. manginecans*. Confirmation by morphological characteristics and molecular identification was *C. manginecans*. This is the first report of *C. manginecans* causing wilt and die-back in Jackfruit in the world. This fungus is similar to those in descriptions given for *C. manginecans* isolated from diseased *Acacia* trees (Tarigan et al., 2010; Tarigan et al., 2011). *C. manginecans* forms part of the *C. fimbriata* s. l. complex, which is typified by *C. fimbriata* sensu stricto that causes black rot of sweet potato (Engelbrecht and Harrington 2005). High levels of genetic

variation found for isolates of *C. manginecans* from Vietnam suggest a possible Southeast Asia origin for the pathogen (Fourie et al. 2016).

The symptoms of *C. manginecans* wilt disease in *A. heterophyllus* are stems cankers, the stems become chapped as though torn apart, fruit rot and progressive loss of the canopy resulting in tree death. *A. heterophyllus* trees showed typical symptoms of infection by the *Ceratocystis* fungus; the same was true of a serious wilt pathogen of *Acacia mangium* and *Acacia crassicarpa* in Indonesia and Vietnam (Tarigan et al. 2010; Tarigan et al. 2011; Chi et al. 2019b), a serious pathogen of mango trees in Oman and Pakistan (Van Wyk et al., 2007) and *Dalbergia sissoo* wilt caused by *C. manginecans,* previously reported in Pakistan (Al-Adawi et al. 2013). *C. manginecans* infecting native trees in these countries is serious and could potentially lead to the devastation of important components of the natural biodiversity of Indonesia.

Acknowledgement

This research was funded by PMDSU scholarship with budget year of 2019-2021 according to the Director of Research and Community Service, Directorate of Research and Community Service (DRPM), Directorate General for Research and Development, Ministry of Research, Technology, and Higher Education, Number: 068/SP2H/AMD/LT/DRPM/2020 chaired by Ahmad Muslim.

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Table

223 **Table 1** *Ceratocystis* isolates considered in the phylogenetic analyses

Species	Isolates no.	GenBank accession no.	Gene regions	Host	Geographical origin	Collector
C. manginecans	**CAAW31171**	**MT355410**	**ITS**	***Artocarpus heterophyllus***	**Indonesia**	**R.Pratama**
		MT412106	**βt**			
C. manginecans	**CAAW30268**	**MT355412**	**ITS**	***A. heterophyllus***	**Indonesia**	**R.Pratama**
		MT412108	**βt**			
C. manginecans	**CAAW30817**	**MT355413**	**ITS**	***A. heterophyllus***	**Indonesia**	**R.Pratama**
		MT412109	**βt**			
C. atrox	CMW19383	EF070414	ITS	*Eucalyptus grandis*	Australia	M.J. Wingfield
	CBS120517	EF070430	βt			
C. atrox	CMW19385	EF070415	ITS			
	CBS120518	EF070431	βt	*E. grandis*	Australia	M.J. Wingfield
C. caryae	CMW14808	EF070423	ITS			
	CBS115168	EF070440	βt	*Carya ovata*	U.S.A	J. Johnson
C. caryae	CMW14793	EF070424	ITS			
	CBS114716	EF070439	βt	*C. cordiformis*	U.S.A	J. Johnson
C. manginecans	CMW22621	EU588661	ITS	*A. mangium*	Indonesia	M. Tarigan
		EU588640	βt			
C. manginecans	CMW22595	EU588660	ITS	*A. mangium*	Indonesia	M. Tarigan
		EU588639	βt			
C. manginecans	CMW22564	EU588657	ITS	*A. mangium*	Indonesia	M. Tarigan
		EU588637	βt			
C. manginecans	CMW22563	EU588656	ITS	*A. mangium*	Indonesia	M. Tarigan
		EU588636	βt			
C. manginecans	CMW22562	EU588655	ITS	*Acacia mangium*	Indonesia	M. Tarigan
		EU588635	βt			
C. manginecans	CMW13851	AY953383	ITS	*Mangifera indica*	Oman	M. Deadman
		EF433308	βt			
C. manginecans	CMW13851	AY953383	ITS	*Mangifera indica*	Oman	M. Deadman
		EF433308	βt			
C. manginecans	CMW13852	AY953384	ITS	*Hypocryphalus mangifera*	Oman	M. Deadman
		EF433309	βt			
C. manginecans	CMW13854	AY953385	ITS	*M. indica*	Oman	M. Deadman
		EF433310	βt			
C. manginecans	CMW22579	EU588658	ITS	*A. mangium*	Indonesia	M. Tarigan
		EU588638	βt			
C. manginecans	CMW22581	EU588659	ITS	*A. mangium*	Indonesia	M. Tarigan
		EU604671	βt			
C. manginecans	CMW21123	EU588662	ITS	*A. crassicarpa*	Indonesia	M. Tarigan
		EU588641	βt			
C. manginecans	CMW21125	EU588663	ITS	*A. crassicarpa*	Indonesia	M. Tarigan
		EU588642	βt			
C. manginecans	CMW21127	EU588664	ITS	*A. crassicarpa*	Indonesia	M. Tarigan
		EU588643	βt			
C. manginecans	CMW21132	EU588665	ITS	*A. crassicarpa*	Indonesia	M. Tarigan
		EU588644	βt			
C. manginecans	CMW23628	EF433303	ITS	*Hypocryphalus mangifera*	Pakistan	A. Al-Adawi
		EF433312	βt			
C. manginecans	CMW23634	EF433302	ITS	*M. indica*	Pakistan	A. Al-Adawi
		EF433311	βt			
C. manginecans	CMW23641	EF433305	ITS	*M. indica*	Pakistan	A. Al-Adawi
		EF433314	βt			
C. manginecans	CMW23643	EF433304	ITS	*M. indica*	Pakistan	A. Al-Adawi
		EF433313	βt			
C. obpyriformis	CMW23807	EU245004	ITS	*A. mearnsii*	South Africa	R.N. Heath
		EU244976	βt			
C. obpyriformis	CMW23808	EU245003	ITS	*A. mearnsii*	South Africa	R.N. Heath
		EU244975	βt			
C. pirilliformis	CMW6569	AF427105	ITS	*E. nitens*	Australia	M.J.Wingfield
		DQ371652	βt			
C. pirilliformis	CMW6579	AF427105	ITS	*E. nitens*	Australia	M.J. Wingfield
		DQ371653	βt			

C. polyconidia	CMW23809	EU245006	ITS	*A. mearnsii*	South Africa	R.N. Heath
		EU244978	βt			
C. polyconidia	CMW23818	EU245007	ITS	*A. mearnsii*	South Africa	R.N. Heath
		EU244979	βt			
C. polycroma	CMW11424	AY528970	ITS	*Syzygium aromaticum*	Indonesia	M.J. Wingfield
	CBS115778	AY528966	βt			
C. polycroma	CMW11436	AY528971	ITS	*S. aromaticum*	Indonesia	M.J. Wingfield
	CBS115777	AY528967	βt			
C. populicola	CMW14789	EF070418	ITS	*Populus* sp.	Poland	J. Gremmen
	CBS119.78	EF070434	βt			
C. populicola	CMW14819	EF070419	ITS	*Populus* sp.	U.S.A	T. Hinds
	CBS114725	EF070435	βt			
C. smalleyi	CMW14800	EF070420	ITS	*C. cordiformis*	U.S.A	G. Smalley
	CBS114724	EF070436	βt			
C. variospora	CMW20935	EF070421	ITS	*Quercus alba*	U.S.A	J. Johnson
	CBS114715	EF070437	βt			
C. variospora	CMW20936	EF070422	ITS	*Q. robur*	U.S.A	J. Johnson
	CBS114714	EF070438	βt			
C. virescens	CMW3276	AY528984	ITS	*Quercus* sp.	USA	T. Hinds
		AY528990	βt			
C. zombamontana	CMW15235	EU245002	ITS	*Eucalyptus* sp.	Malawi	R.N. Heath & J. Roux
		EU244974	βt			
C. zombamontana	CMW15236	EU245000	ITS	*Eucalyptus* sp.	Malawi	R.N. Heath & J. Roux
		EU244972	βt			

Colour figure

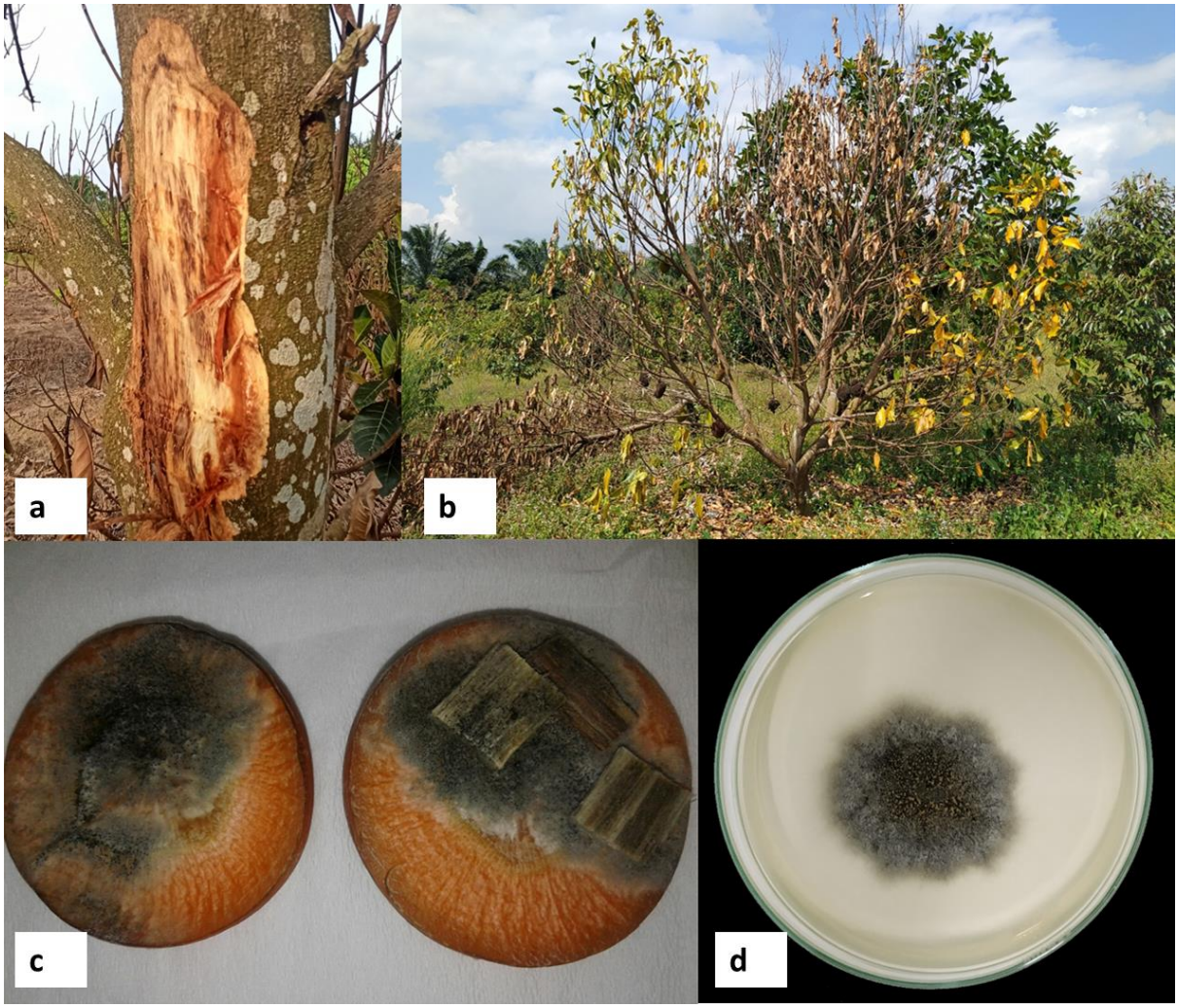

Fig. 1 Symptoms of *Ceratocystis manginecans* wilt disease in *Artocarpus heterophyllus:* **a**. vascular discoloration of infected tree; **b.** three-year-old tree with wilted, yellowing leaves and rotten fruit; **c.** isolation of the fungus from discoloured xylem showing dark mycelium and sporulation on the carrot slices after 7 days; **d.** view of the colony of *Ceratocystis manginecans* on malt extract agar (MEA) incubated for 7 days at 25 °C.

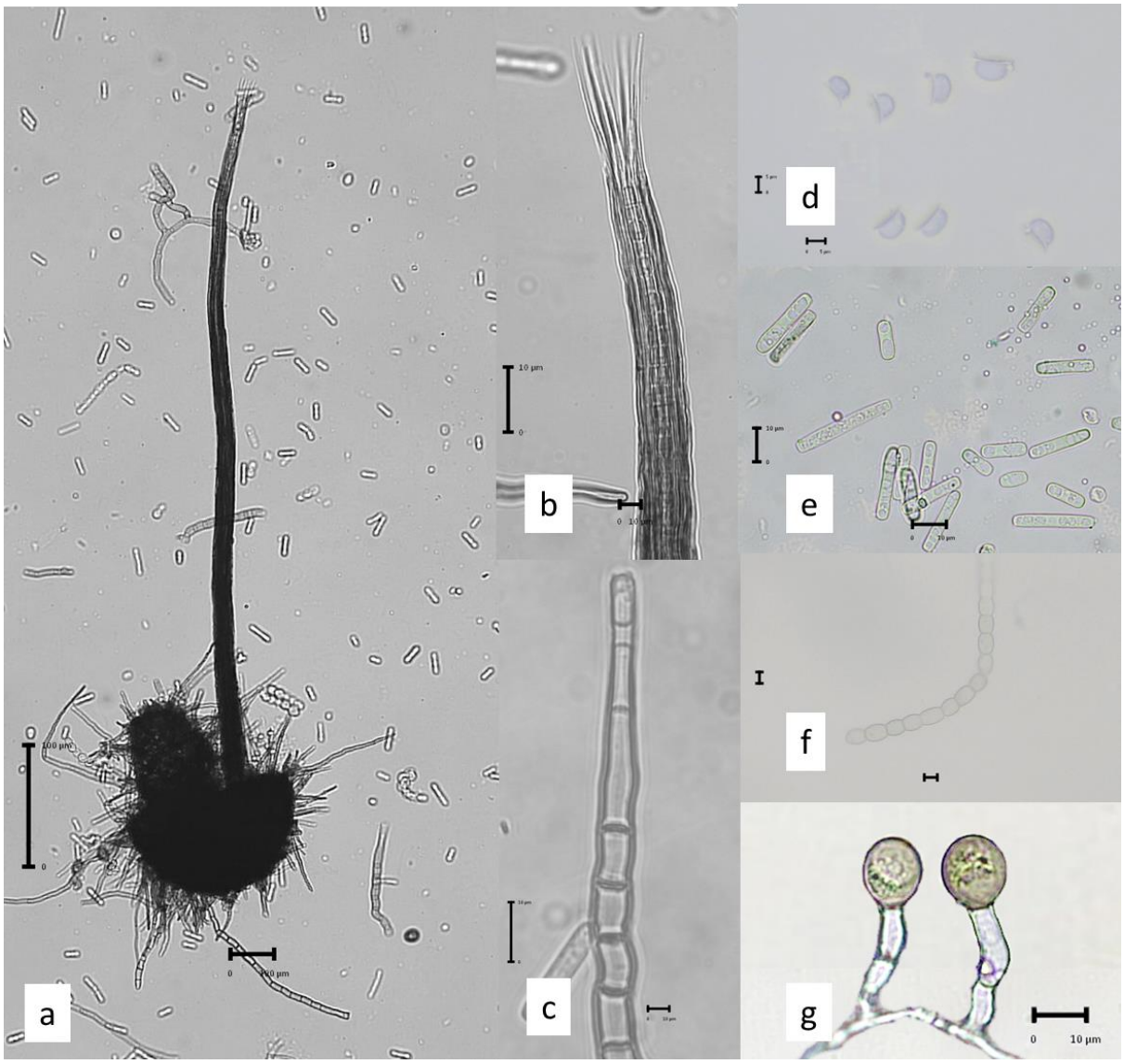

Fig. 2. Morphological characteristics of *Ceratocystis manginecans* isolated from *Artocarpus heterophyllus* stem lesion: **a.** ascomata with pirilliform base, **b.** conidiophore/phialide; **c.** Divergent ostiolar hyphae; **d.** chlamydospores of various shapes; **e.** cylindrical conidia; **f.** hat-shaped ascospores. Scale bars: a = 100 µm; b,c,e,f,g = 10 µm; d = 5 µm.

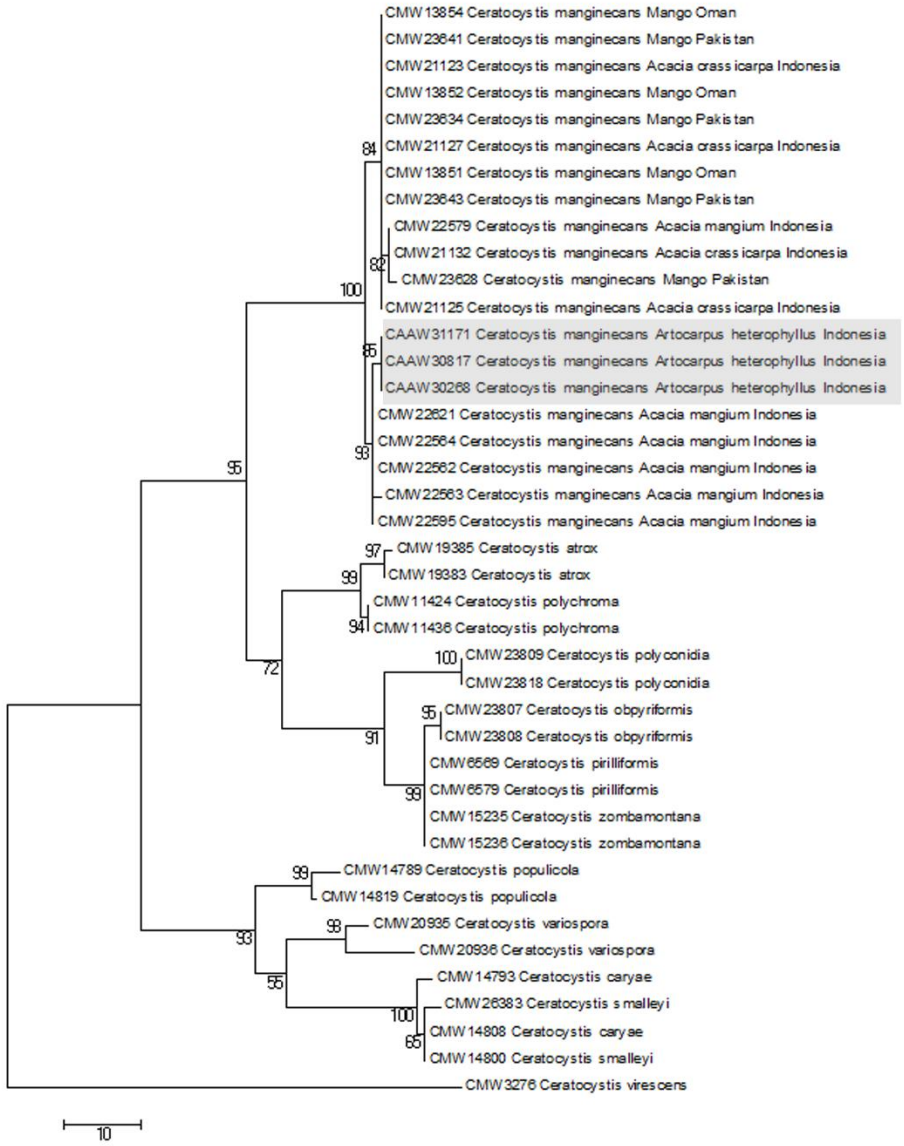

Fig. 3 Phylogenetic tree constructed by MEGA with Maximum Parsimony (MP) search for the combined sequence data of the ITS region and β-tubulin gene (CAAW31171, CAAW30268, and CAAW30817) and their related species from GenBank. Consistency (CI), retention (RI), and composite indexes (CoI) are 0.725275, 0.935733, and 0.734932 for all sites and parsimony-informative sites. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Bootstrap values >50% are indicated above the branches. The analysis involved 41 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were 856 positions in the final dataset. *Ceratocystis virescens* was used as the out-group.

Revise before review 1

a. muslim unsri <a_muslim@unsri.ac.id>

APDN-D-21-00015 Please approve changes to your submission Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia

1 message

APDN <em@editorialmanager.com> Mon, Feb 22, 2021 at 4:34 AM
Reply-To: APDN <jude.estrera@springernature.com>
To: "A. Muslim" <a_muslim@unsri.ac.id>

Submission ID: APDN-D-21-00015

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Your submission entitled "Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia" has been sent back to you:

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Australasian Plant Disease Notes

Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia

--Manuscript Draft--

Manuscript Number:	APDN-D-21-00015R1
Full Title:	Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia
Article Type:	Plant Disease Note
Keywords:	Sudden death; Pathogenicity; Jackfruit; Indonesia; Wilt disease
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Funding Information:	Kementerian Riset Teknologi Dan Pendidikan Tinggi Republik Indonesia (068/SP2H/AMD/LT/DRPM/2020) — Dr. A. Muslim
Abstract:	In 2019, wilt and sudden death were observed on Artocarpus heterophyllus (jackfruit) has been noted. Identification was performed by sequence analysis of the concatenated β-tubulin and ITS gene regions. Sequencing of the PCR product confirmed this pathogen was Ceratocystis manginecans . C. manginecans causing sudden death disease in A. heterophyllus is being reported for the first time in Indonesia and worldwide.
Response to Reviewers:	Associate Editor Alistair McTaggart, Ph.D Australasian Plant Disease Notes Journal Centre for Horticultural Science The University of Queensland Australia Dear Associate Editor, We have re-submit our journal with corresponding improvements suggested. Please address all correspondence concerning this manuscript to me at: a_muslim@unsri.ac.id Laboratory of Phytopathology, Department of Plant Protection, Faculty of Agriculture, Sriwijaya University, Indralaya, South Sumatera, 30662, Indonesia. Telephone +628117826119. Thank you for your consideration of the manuscript.

	Sincerely, A. Muslim, Ph.D

Author(s) details

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Dear Editor in Chief

We wish to submit an manuscript titled "Jackfruit (*Artocarpus heterophyllus*), a New Host Plant of Ceratocystis Wilt in South Sumatra, Indonesia" in Australasian Plant Disease Notes Journal.

We have submitted Our manuscript in Australian Plant Pathology Journal on July 2020. Our manuscript with application number AUPP-D-20-00215, has been recommended by the journal editorial office of AUPP (Australian Plant Pathology Journal) for transfer to the journal Australasian Plant Disease Note. Due to the interest of this study about a new diseases in Jak trees, the reviewer strongly adviced to resubmit our manuscript as a "Research Note" in Australasian Plant Disease Note.

According Journals Editorial Office of Springer/AUPP and reviewer suggestions. We have revised our manuscriptas the two reviewers of AUPP comments, in particular to change pathogen name as *Ceratocystis manginecans* and writing adjustments.
Our manuscript has been done proofreading in London Proofreaders with order # 5048.

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Thank you for your consideration of the manuscript.

Sincerely,
A. Muslim, Ph.D

Jackfruit (*Artocarpus heterophyllus*), a New Host Plant of *Ceratocystis* Wilt in South Sumatra, Indonesia

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Abstract

In 2019, wilt and sudden death were observed on *Artocarpus heterophyllus* (jackfruit) has been noted. Identification was performed by sequence analysis of the concatenated β-tubulin and ITS gene regions. Sequencing of the PCR product confirmed this pathogen was *Ceratocystis manginecans*. *C. manginecans* causing sudden death disease in *A. heterophyllus* is being reported for the first time in Indonesia and worldwide.

Keywords: Sudden death · Pathogenicity · Jackfruit · Indonesia · Wilt disease

Jackfruit (*A. heterophyllus*) belongs to the family Moraceae, and it is known in Indonesian as "Nangka". Jackfruit is grown widely in Indonesia and many countries with tropical and subtropical climates. Jackfruit is among the most exported fruits worldwide and has considerable nutrition and health benefits (Ranasingheet al. 2019).

In a recent study of jackfruit in 2019, wilt and die-back symptoms were observed for the first time on *A. heterophyllus* in the agricultural field of Sriwijaya University (Indralaya), Plaju (Palembang) and Gelumbang (Prabumulih), Indonesia. Jackfruit trees were reported to die within a period from July to September 2019. Jackfruit wood produced grey lesions resulting from growth of *Ceratocystis* on the stem (Fig. 1a). Leaves of dying trees had yellowing symptoms, followed by the wilting of the leaves on several lateral branches, drying of twigs and the wilt of the whole tree (Fig. 1b). This type of wilting was termed as sudden death or wilt (Pornsuriya and Sunpapao 2015).

Wood samples were taken from lesions of wilted trees using a knife sterilised in 70% ethanol. Wood samples were collected from *A. heterophyllus* showing brown to black streaking in the woody xylem. Each sample was wrapped in tissue paper and placed in a cool

box. The same day, the wood samples (1–20 mm length, 1–2 mm thick) were sandwiched between two slices of fresh carrot and placed on sterile dry paper in plastic boxes at 25 °C following the method of Moller and DeVay (1968) (Fig. 1c). After 5–10 days, hat-shaped spores of putative *Ceratocystis* pathogens were placed on 2% (w/v) malt extract agar (MEA) (Merck, Germany), and incubated at 25 °C in a laboratory. The isolated fungi were initially identified based on morphological characteristics of a 14 day old culture. Mycelium on MEA grey, reverse side of colony olivaceous grey; submerged mycelium darkening as the ascomata develop forming fine, radiating fibrils (Fig. 1d).

Morphological traits of fruiting bodies and spores were observed under an optical Olympus CX33 microscope. Ascomatal bases dark brown to black, base subglobes to globes and measured (n=100), 131.5 to 250.7×101.6 to 236.5 µm (length/width) (Fig. 2a). Ascomata necks erect, occasionally curved, black at the base becoming subhyaline towards the apex, smooth to crenulate, 324.7 to 579.1 µm long including ostiolar hyphae (Fig. 2b). Phialides pale brown to hyaline (Fig. 2c). Ascospores hat-shaped, 3.4 to 6.8×2.1 to 6.2 µm (length/width) (Fig. 2d). Bacilliform conidia 11.1 to 36.1×2.1 to 7.4 µm (length/width) (Fig. 2e). Barrel conidia 4.4 to 16.1×2.7 to 6.9 µm (length/width) (Fig. 2f). Chlamydospores oval, thick walled, smooth, 6.7 to 16.5×5.9 to 12.9 µm (length/width) (Fig. 2g). Based on morphological characters, the fungus was identified as *C. manginecans.*

To confirm the species identification, isolates were cultured on potato dextrose broth (PDB) at room temperature for one week. Mycelial mat was filtered through Whatman filter paper and genomic DNA was extracted from fungal mycelial mat usingYeaStar Genomic DNA Kit (Zymo Research Corporation, California, USA). PCR conditions and reactions for two gene regions were used to identify the *Ceratocystis* isolates; the Internal Transcribed Spacer (ITS) and part of the β-tubulin (βt) gene. Amplifications were carried out in 50 µl reactions containing 20 µl DreamTaq Green PCR Master Mix (Eppendorf, Germany) (DreamTaq DNA Polymerase, 2X DreamTaq Green buffer, dNTPs, and 4 mM $MgCl_2$), 1,5 µl of each forward and reverse primer, 4 µl of DNA template and 23µl sterilised water. The PCRs were performed with a C1000 Touch™ thermal cycler (Bio-Rad, USA). The PCRcycling parameters were as follows: initial denaturation for 5 min at 95 °C, followed by 35 cycles at 95 °C for 30 s, 56 °C for 45 s and 72 °C for 1 min. Amplification was completedat 72 °C for 10 min and the PCR product was stored at 10 °C. The PCR amplicons were sequenced at 1st BASE (Malaysia).

For the ITS and β-tubulin, amplification resulted in fragments of ~550 base pairs (bp) in size. The sequences of the amplified products were then deposited in the GenBank

database and assigned accession numbers isolate CAAW31171 (MT355410; MW717653), isolate CAAW30817 (MT355413, MW717656), and isolate CAAW30268 (MT355412; MW717655) for the ITS and β-tubulin, respectively, and they were compared with the sequences of *C. manginecans* available at GenBank. Blast searches in GenBank indicated that our isolates grouped within *C. manginecans* species with 99% identity of the sequences.The two gene regions (ITS and βt) were combined and analysed as a single dataset. Maximum Parsimony (MP) analyses were performed in MEGA v. 10 (Kumar et al. 2016; Paul et al. 2018) with 1000 bootstrap replications. According to the phylogenetic relationships derived from the Maximum Parsimony (MP) analyses, our *C. manginecans* isolates (CAAW31171, CAAW30817, CAAW30268) in *A. heterophyllus* was closely related to *C. manginecans* in *Mangifera indica* and *Acacia mangium* (Fig. 3). This sequence similarity to prior cases of *C. manginecans* corroborates the identification by phenotypic characteristics, suggesting that the causal agent of suddendeath disease on *A. heterophyllus* in Indonesia, represented by the CAAW31171, CAAW30817, CAAW30268 isolates, should be regarded as *C. manginecans*.

The pathogenic potential of isolates was evaluated by the under bark inoculation method described by O'Gara et al. (1997) using Five-month-old *A. heterophyllus* seedlings with stem diameters of 6-8 mm and heights <1.5 m were prepared for pathogenicity test. Wounds were made on the stems of the seedlings using a cork borer (4 mm diam.), and mycelial discs (4 mm diam.) taken from an actively growing colony of *C. manginecans* on 2% MEA (14 days) (Tarigan et al. 2010; Tarigan et al. 2011; Chi et al. 2019a) were placed in the wounds with the mycelium facing downwards. These were covered with Parafilm (Pechiney, Menasha, Wisconsin) to reduce contamination and desiccation. Ten plants of each tree species were inoculated with sterile MEA plugs to serve as controls. Fungal isolates were re-isolated and re-identified using morphological characteristics for Koch's postulates confirmation. The fungi were shown to be pathogenic in young *A. heterophyllus*, with plants exhibiting wilt symptoms 45 days after inoculation (data not shown). When re-isolated, the fungus was phenotypically identical to the prior isolate of *C. manginecans* (CAAW31171, CAAW30817, CAAW30268).

This is the first report of *C. manginecans* causing wilt and die-back in Jackfruit in Indonesia and worldwide. The symptoms of *C. manginecans* wilt disease in *A. heterophyllus* are stems cankers, the stems become chapped as though torn apart, fruit rot and progressive loss of the canopy resulting in tree death. *A. heterophyllus* trees showed typical symptoms of infection by the *Ceratocystis* fungus; the same was true of a serious wilt pathogen of *A. mangium* and *A. crassicarpa* in Indonesia and Vietnam (Tarigan et al. 2010; Tarigan et al.

2011; Chi et al. 2019b), a serious pathogen wilt of mango trees in Oman and Pakistan (Van Wyk et al., 2007) caused by *C. manginecans*, previously reported in Pakistan (Al-Adawi et al. 2013). *C. manginecans* infecting native trees in these countries is serious and could potentially lead to the devastation of important components of the natural biodiversity of Indonesia.

Acknowledgement

This research was funded by PMDSU scholarship with budget year of 2019-2021 according to the Director of Research and Community Service, Directorate of Research and Community Service (DRPM), Directorate General for Research and Development, Ministry of Research, Technology, and Higher Education, Number: 068/SP2H/AMD/LT/DRPM/2020 chaired by Ahmad Muslim.

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Table

Table 1 *Ceratocystis* isolates considered in the phylogenetic analyses

Species	Isolates no.	Host	Geographical origin	GenBank accession no.	
				ITS	βt
C. manginecans	**CAAW31171**	***Artocarpus heterophyllus***	**Indonesia**	**MT355410**	**MW717653**
	CAAW30268	***A. heterophyllus***	**Indonesia**	**MT355412**	**MW717655**
	CAAW30817	***A. heterophyllus***	**Indonesia**	**MT355413**	**MW717656**
	CMW22621	*Acacia mangium*	Indonesia	EU588661	EU588640
	CMW22595	*A. mangium*	Indonesia	EU588660	EU588639
	CMW22564	*A. mangium*	Indonesia	EU588657	EU588637
	CMW22563	*A. mangium*	Indonesia	EU588656	EU588636
	CMW22562	*A. mangium*	Indonesia	EU588655	EU588635
	CMW13851	*Mangifera indica*	Oman	AY953383	EF433308
C. fimbriatomima	CMW24376	*Eucalyptus*	Venezuela	NR166018	EF190953
C. obpyriformis	CMW23807	*Acacia mearnsii*	South Africa	EU245004	EU244976
	CMW23808	*A. mearnsii*	South Africa	EU245003	EU244975
C. papillata	CMW8856	*Lemon tree*	Colombia	NR119486	AY233874
C. pirilliformis	CMW6569	*Eucalyptus nitens*	Australia	AF427105	DQ371652
	CMW6579	*E. nitens*	Australia	AF427105	DQ371653
C. polyconidia	CMW23809	*A. mearnsii*	South Africa	EU245006	EU244978
	CMW23818	*A. mearnsii*	South Africa	EU245007	EU244979
C. virescens	CMW3276	*Quercus* sp.	USA	AY528984	AY528990
C. zombamontana	CMW15235	*Eucalyptus* sp.	Malawi	EU245002	EU244974
	CMW15236	*Eucalyptus* sp.	Malawi	EU245000	EU244972

Colour figure

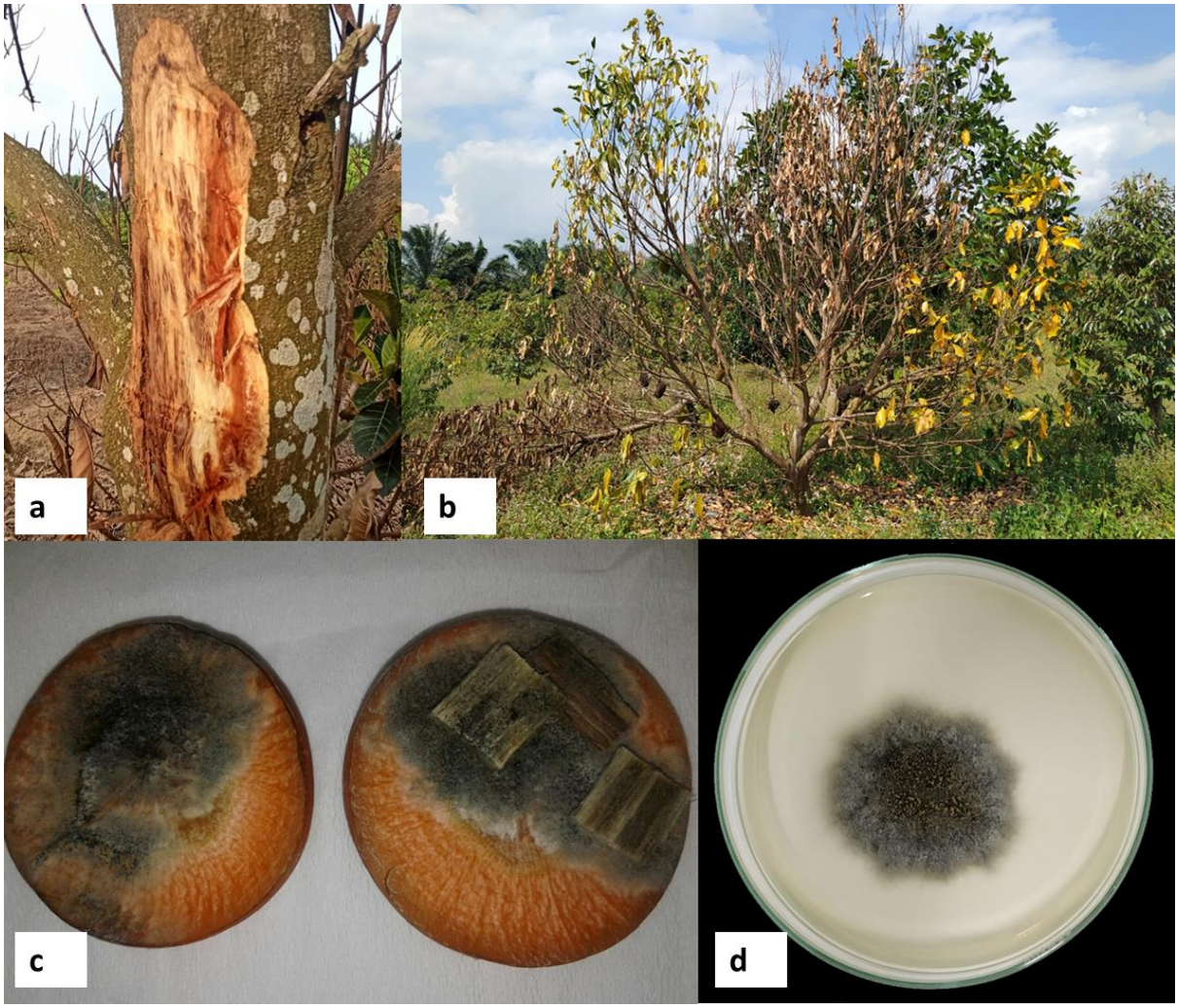

Fig. 1 Symptoms of *Ceratocystis manginecans* wilt disease in *Artocarpus heterophyllus:* **a**. vascular discoloration of infected tree; **b.** three-year-old tree with wilted, yellowing leaves and rotten fruit; **c.** isolation of the fungus from discoloured xylem showing dark mycelium and sporulation on the carrot slices after 7 days; **d.** view of the colony of *Ceratocystis manginecans* on malt extract agar (MEA) incubated for 7 days at 25 °C.

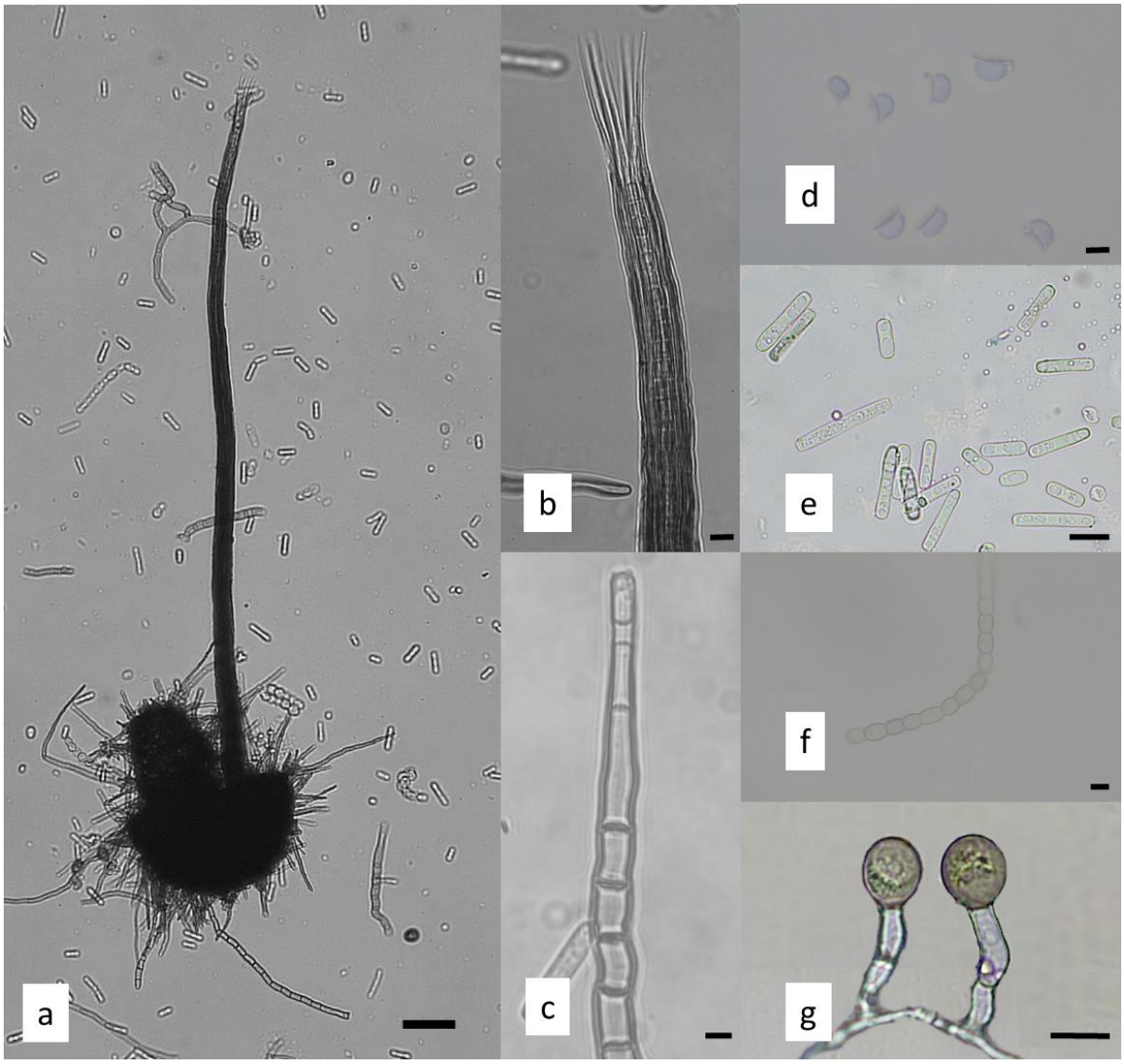

Fig. 2. Morphological characteristics of *Ceratocystis manginecans* isolated from *Artocarpus heterophyllus* stem lesion: **a.** ascomata with pirilliform base, **b.** divergent ostiolar hyphae; **c.** conidiophore/phialide; **d.** hat-shaped ascospores; **e.** cylindrical conidia; **f.** Chain of barrel-shaped conidia; **g.** chlamydospores of various shapes. Scale bars: a = 100 µm; b,c,e,f,g = 10 µm; d = 5 µm.

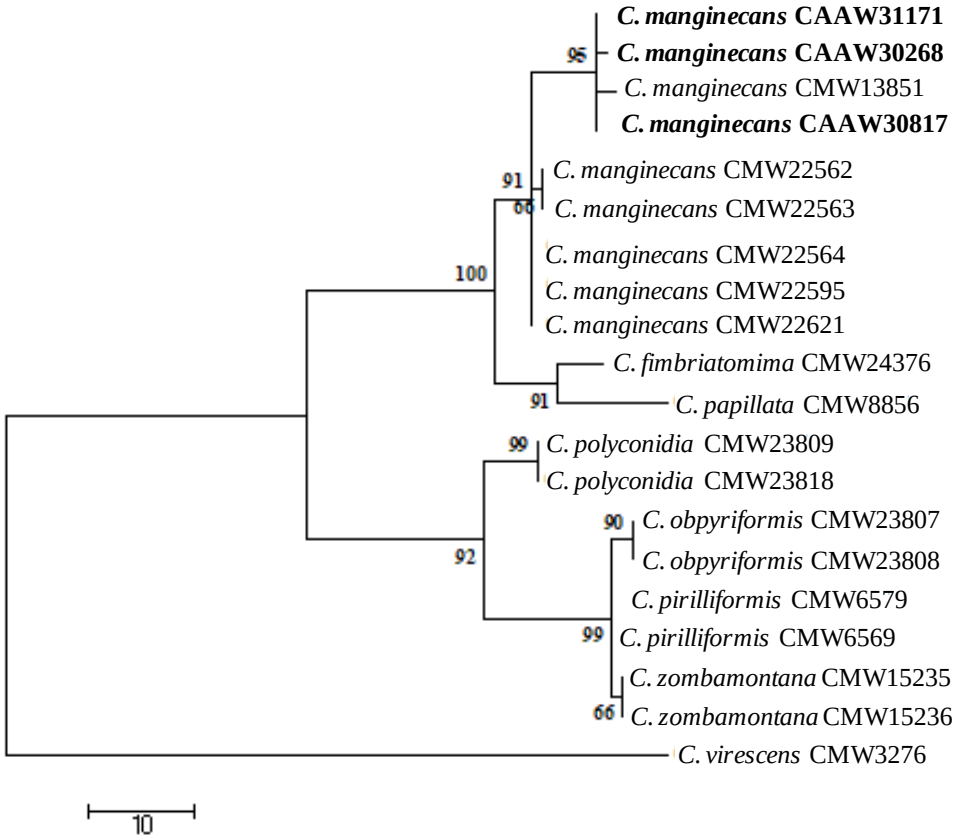

Fig. 3 Phylogenetic tree constructed by MEGA with Maximum Parsimony (MP) search for the combined sequence data of the ITS region and β-tubulin gene (CAAW31171, CAAW30268, and CAAW30817) and their related species from GenBank. Consistency (CI), retention (RI), and composite indexes (CoI) are 0.819149, 0.952113, and 0.861689 for all sites and parsimony-informative sites. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Bootstrap values >50% are indicated above the branches. The analysis involved 20 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were 831 positions in the final dataset. *Ceratocystis virescens* was used as the out-group.

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Thank you for your approval - [EMID:040bb87487b633bd]

1 message

APDN <em@editorialmanager.com> Mon, Feb 22, 2021 at 8:26 PM
Reply-To: APDN <jude.estrera@springernature.com>
To: "A. Muslim" <a_muslim@unsri.ac.id>

Dear Dr. Muslim,

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a. muslim unsri <a_muslim@unsri.ac.id>

Revise before review

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CC: dagmar.hanold@adelaide.edu.au, dhanold@gmail.com

Dear Dr. Muslim,

We have received the reports from our advisors on your manuscript, "Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia" (APDN-D-21-00015), submitted to Australasian Plant Disease Notes.

The decision is to revise before review.

Below, please find the editor's comments for your perusal.
You are kindly requested to also check the website for possible reviewer attachment(s).

Please submit your revised manuscript online by using
the Editorial Manager system which can be accessed at:

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Please submit you revised manuscript before 29 Mar 2021 or request an extension of the deadline. If we do not hear from you by then, the manuscript will be automatically withdrawn.

With kind regards,

Alistair McTaggart, Ph.D
Associate Editor

COMMENTS FOR THE AUTHOR:

The manuscript contains adequate methods and an interesting story reporting Ceratocystis on Jackfruit.
There are some improvements needed before this is sent to a reviewer, and in its current state would likely be rejected based on the quality of writing. The writing needs thorough proof reading. Begin by shortening the length of the manuscript, it is too long for the information it contains. The reader does not need background information that Jackfruits are delicious. Provide information that will help the reader understand the context of the new knowledge. Although the word limit is 1,500 words, aim for less, as a new disease report should not need large amounts of background information.
Check caption for Figure 2, there are images yet to be explained.
The tree can be improved. Firstly in its presentation, Latin binomials should be italicised, remove BS values that do not provide support. The taxon selection needs thought, show the reader other species in the Ceratocystis fimbriata species complex as these will be most informative for your identification. Use ex-type sequences where you can, these can be searched for on GenBank and usually start with 'NR_'.
We look forward to receive an improved version of this manuscript.

Our flexible approach during the COVID-19 pandemic

If you need more time at any stage of the peer-review process, please do let us know. While our systems will continue to remind you of the original timelines, we aim to be as flexible as possible during the current pandemic.

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a. muslim unsri <a_muslim@unsri.ac.id> Mon, Mar 1, 2021 at 9:15 PM
To: APDN <jude.estrera@springernature.com>

Dear Alistair McTaggart, Ph.D, Associate Editor of APDN

Thank you very much for your response to our manuscripts submitted to Australasian Plant Disease Notes.
We are going to revise our manuscripts as your comment, and send it as soon as possible.
We hope we can submit our revised manuscript before 29 Mar 2021.

Best regard
A. Muslim
[Quoted text hidden]

DRAFT PERBAIKAN

Australasian Plant Disease Notes

Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia

--Manuscript Draft--

Manuscript Number:	APDN-D-21-00015R1
Full Title:	Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia
Article Type:	Plant Disease Note
Keywords:	Sudden death; Pathogenicity; Jackfruit; Indonesia; Wilt disease
Corresponding Author:	A. Muslim, Ph.D. Universitas Sriwijaya Fakultas Pertanian Palembang, Sumatera Selatan INDONESIA
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Funding Information:	Kementerian Riset Teknologi Dan Pendidikan Tinggi Republik Indonesia (068/SP2H/AMD/LT/DRPM/2020) — Dr. A. Muslim
Abstract:	In 2019, wilt and sudden death were observed on Artocarpus heterophyllus (jackfruit) has been noted. Identification was performed by sequence analysis of the concatenated β-tubulin and ITS gene regions. Sequencing of the PCR product confirmed this pathogen was Ceratocystis manginecans . C. manginecans causing sudden death disease in A. heterophyllus is being reported for the first time in Indonesia and worldwide.
Response to Reviewers:	Associate Editor Alistair McTaggart, Ph.D Australasian Plant Disease Notes Journal Centre for Horticultural Science The University of Queensland Australia Dear Associate Editor, We have re-submit our journal with corresponding improvements suggested. Please address all correspondence concerning this manuscript to me at: a_muslim@unsri.ac.id Laboratory of Phytopathology, Department of Plant Protection, Faculty of Agriculture, Sriwijaya University, Indralaya, South Sumatera, 30662, Indonesia. Telephone +628117826119. Thank you for your consideration of the manuscript.

	Sincerely, A. Muslim, Ph.D

Author(s) details

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Jackfruit (*Artocarpus heterophyllus*), a New Host Plant of *Ceratocystis* Wilt in South Sumatra, Indonesia

Abstract

In 2019, wilt and sudden death were observed on *Artocarpus heterophyllus* (jackfruit) has been noted. Identification was performed by sequence analysis of the concatenated β-tubulin and ITS gene regions. Sequencing of the PCR product confirmed this pathogen was *Ceratocystis manginecans*. *C. manginecans* causing sudden death disease in *A. heterophyllus* is being reported for the first time in Indonesia and worldwide.

Keywords: Sudden death · Pathogenicity · Jackfruit · Indonesia · Wilt disease

Jackfruit (*A. heterophyllus*) belongs to the family Moraceae, and it is known in Indonesian as "Nangka". Jackfruit is grown widely in Indonesia and many countries with tropical and subtropical climates. Jackfruit is among the most exported fruits worldwide and has considerable nutrition and health benefits (Ranasingheet al. 2019).

In a recent study of jackfruit in 2019, wilt and die-back symptoms were observed for the first time on *A. heterophyllus* in the agricultural field of Sriwijaya University (Indralaya), Plaju (Palembang) and Gelumbang (Prabumulih), Indonesia. Jackfruit trees were reported to die within a period from July to September 2019. Jackfruit wood produced grey lesions resulting from growth of *Ceratocystis* on the stem (Fig. 1a). Leaves of dying trees had yellowing symptoms, followed by the wilting of the leaves on several lateral branches, drying of twigs and the wilt of the whole tree (Fig. 1b). This type of wilting was termed as sudden death or wilt (Pornsuriya and Sunpapao 2015).

Wood samples were taken from lesions of wilted trees using a knife sterilised in 70% ethanol. Wood samples were collected from *A. heterophyllus* showing brown to black streaking in the woody xylem. Each sample was wrapped in tissue paper and placed in a cool box. The same day, the wood samples (1–20 mm length, 1–2 mm thick) were sandwiched between two slices of fresh carrot and placed on sterile dry paper in plastic boxes at 25 °C following the method of Moller and DeVay (1968) (Fig. 1c). After 5–10 days, hat-shaped spores of putative *Ceratocystis* pathogens were placed on 2% (w/v) malt extract agar (MEA) (Merck, Germany), and incubated at 25 °C in a laboratory. The isolated fungi were initially identified based on morphological characteristics of a 14 day old culture. Mycelium on MEA grey, reverse side of colony olivaceous grey; submerged mycelium darkening as the ascomata develop forming fine, radiating fibrils (Fig. 1d).

Morphological traits of fruiting bodies and spores were observed under an optical Olympus CX33 microscope. Ascomatal bases dark brown to black, base subglobes to globes and measured (n=100), 131.5 to 250.7×101.6 to 236.5 µm (length/width) (Fig. 2a). Ascomata necks erect, occasionally curved, black at the base becoming subhyaline towards the apex, smooth to crenulate, 324.7 to 579.1 µm long including ostiolar hyphae (Fig. 2b). Phialides pale brown to hyaline (Fig. 2c). Ascospores hat-shaped, 3.4 to 6.8×2.1 to 6.2 µm (length/width) (Fig. 2d). Bacilliform conidia 11.1 to 36.1×2.1 to 7.4 µm (length/width) (Fig. 2e). Barrel conidia 4.4 to 16.1×2.7 to 6.9 µm (length/width) (Fig. 2f). Chlamydospores oval, thick walled, smooth, 6.7 to 16.5×5.9 to 12.9 µm (length/width) (Fig. 2g). Based on morphological characters, the fungus was identified as *C. manginecans.*

To confirm the species identification, isolates were cultured on potato dextrose broth (PDB) at room temperature for one week. Mycelial mat was filtered through Whatman filter paper and genomic DNA was extracted from fungal mycelial mat usingYeaStar Genomic DNA Kit (Zymo Research Corporation, California, USA). PCR conditions and reactions for two gene regions were used to identify the *Ceratocystis* isolates; the Internal Transcribed Spacer (ITS) and part of the β-tubulin (βt) gene. Amplifications were carried out in 50 µl reactions containing 20 µl DreamTaq Green PCR Master Mix (Eppendorf, Germany) (DreamTaq DNA Polymerase, 2X DreamTaq Green buffer, dNTPs, and 4 mM $MgCl_2$), 1,5 µl of each forward and reverse primer, 4 µl of DNA template and 23µl sterilised water. The PCRs were performed with a C1000 Touch™ thermal cycler (Bio-Rad, USA). The PCRcycling parameters were as follows: initial denaturation for 5 min at 95 °C, followed by 35 cycles at 95 °C for 30 s, 56 °C for 45 s and 72 °C for 1 min. Amplification was completedat 72 °C for 10 min and the PCR product was stored at 10 °C. The PCR amplicons were sequenced at 1st BASE (Malaysia).

For the ITS and β-tubulin, amplification resulted in fragments of ~550 base pairs (bp) in size. The sequences of the amplified products were then deposited in the GenBank database and assigned accession numbers isolate CAAW31171 (MT355410; MW717653), isolate CAAW30817 (MT355413, MW717656), and isolate CAAW30268 (MT355412; MW717655) for the ITS and β-tubulin, respectively, and they were compared with the sequences of *C. manginecans* available at GenBank. Blast searches in GenBank indicated that our isolates grouped within *C. manginecans* species with 99% identity of the sequences.The two gene regions (ITS and βt) were combined and analysed as a single dataset. Maximum Parsimony (MP) analyses were performed in MEGA v. 10 (Kumar et al. 2016; Paul et al. 2018) with 1000 bootstrap replications. According to the phylogenetic relationships derived from the Maximum Parsimony (MP) analyses, our *C. manginecans* isolates (CAAW31171, CAAW30817,

CAAW30268) in *A. heterophyllus* was closely related to *C. manginecans* in *Mangifera indica* and *Acacia mangium* (Fig. 3). This sequence similarity to prior cases of *C. manginecans* corroborates the identification by phenotypic characteristics, suggesting that the causal agent of suddendeath disease on *A. heterophyllus* in Indonesia, represented by the CAAW31171, CAAW30817, CAAW30268 isolates, should be regarded as *C. manginecans*.

The pathogenic potential of isolates was evaluated by the under bark inoculation method described by O'Gara et al. (1997) using Five-month-old *A. heterophyllus* seedlings with stem diameters of 6-8 mm and heights <1.5 m were prepared for pathogenicity test. Wounds were made on the stems of the seedlings using a cork borer (4 mm diam.), and mycelial discs (4 mm diam.) taken from an actively growing colony of *C. manginecans* on 2% MEA (14 days) (Tarigan et al. 2010; Tarigan et al. 2011; Chi et al. 2019a) were placed in the wounds with the mycelium facing downwards. These were covered with Parafilm (Pechiney, Menasha, Wisconsin) to reduce contamination and desiccation. Ten plants of each tree species were inoculated with sterile MEA plugs to serve as controls. Fungal isolates were re-isolated and re-identified using morphological characteristics for Koch's postulates confirmation. The fungi were shown to be pathogenic in young *A. heterophyllus*, with plants exhibiting wilt symptoms 45 days after inoculation (data not shown). When re-isolated, the fungus was phenotypically identical to the prior isolate of *C. manginecans* (CAAW31171, CAAW30817, CAAW30268).

This is the first report of *C. manginecans* causing wilt and die-back in Jackfruit in Indonesia and worldwide. The symptoms of *C. manginecans* wilt disease in *A. heterophyllus* are stems cankers, the stems become chapped as though torn apart, fruit rot and progressive loss of the canopy resulting in tree death. *A. heterophyllus* trees showed typical symptoms of infection by the *Ceratocystis* fungus; the same was true of a serious wilt pathogen of *A. mangium* and *A. crassicarpa* in Indonesia and Vietnam (Tarigan et al. 2010; Tarigan et al. 2011; Chi et al. 2019b), a serious pathogen wilt of mango trees in Oman and Pakistan (Van Wyk et al., 2007) caused by *C. manginecans,* previously reported in Pakistan (Al-Adawi et al. 2013). *C. manginecans* infecting native trees in these countries is serious and could potentially lead to the devastation of important components of the natural biodiversity of Indonesia.

Acknowledgement

This research was funded by PMDSU scholarship with budget year of 2019-2021 according to the Director of Research and Community Service, Directorate of Research and Community Service (DRPM), Directorate General for Research and Development,

Ministry of Research, Technology, and Higher Education, Number: 068/SP2H/AMD/LT/DRPM/2020 chaired by Ahmad Muslim.

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Table

Table 1 *Ceratocystis* isolates considered in the phylogenetic analyses

Species	Isolates no.	Host	Geographical origin	GenBank accession no.	
				ITS	βt
C. manginecans	**CAAW31171**	***Artocarpus heterophyllus***	**Indonesia**	**MT355410**	**MW717653**
	CAAW30268	***A. heterophyllus***	**Indonesia**	**MT355412**	**MW717655**
	CAAW30817	***A. heterophyllus***	**Indonesia**	**MT355413**	**MW717656**
	CMW22621	*Acacia mangium*	Indonesia	EU588661	EU588640
	CMW22595	*A. mangium*	Indonesia	EU588660	EU588639
	CMW22564	*A. mangium*	Indonesia	EU588657	EU588637
	CMW22563	*A. mangium*	Indonesia	EU588656	EU588636
	CMW22562	*A. mangium*	Indonesia	EU588655	EU588635
	CMW13851	*Mangifera indica*	Oman	AY953383	EF433308
C. fimbriatomima	CMW24376	*Eucalyptus*	Venezuela	NR166018	EF190953
C. obpyriformis	CMW23807	*Acacia mearnsii*	South Africa	EU245004	EU244976
	CMW23808	*A. mearnsii*	South Africa	EU245003	EU244975
C. papillata	CMW8856	*Lemon tree*	Colombia	NR119486	AY233874
C. pirilliformis	CMW6569	*Eucalyptus nitens*	Australia	AF427105	DQ371652
	CMW6579	*E. nitens*	Australia	AF427105	DQ371653
C. polyconidia	CMW23809	*A. mearnsii*	South Africa	EU245006	EU244978
	CMW23818	*A. mearnsii*	South Africa	EU245007	EU244979
C. virescens	CMW3276	*Quercus* sp.	USA	AY528984	AY528990
C. zombamontana	CMW15235	*Eucalyptus* sp.	Malawi	EU245002	EU244974
	CMW15236	*Eucalyptus* sp.	Malawi	EU245000	EU244972

Colour figure

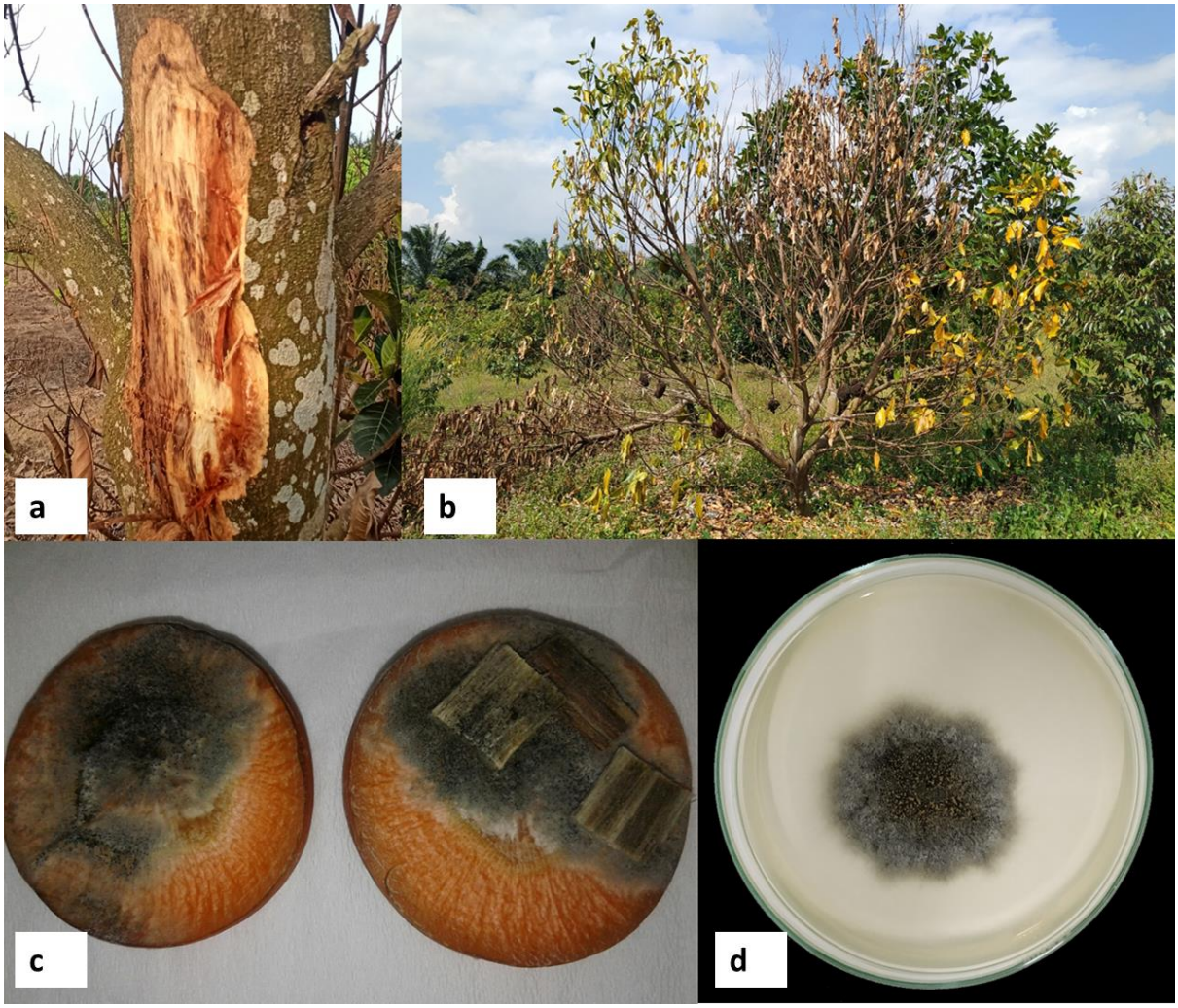

Fig. 1 Symptoms of *Ceratocystis manginecans* wilt disease in *Artocarpus heterophyllus:* **a**. vascular discoloration of infected tree; **b.** three-year-old tree with wilted, yellowing leaves and rotten fruit; **c.** isolation of the fungus from discoloured xylem showing dark mycelium and sporulation on the carrot slices after 7 days; **d.** view of the colony of *Ceratocystis manginecans* on malt extract agar (MEA) incubated for 7 days at 25 °C.

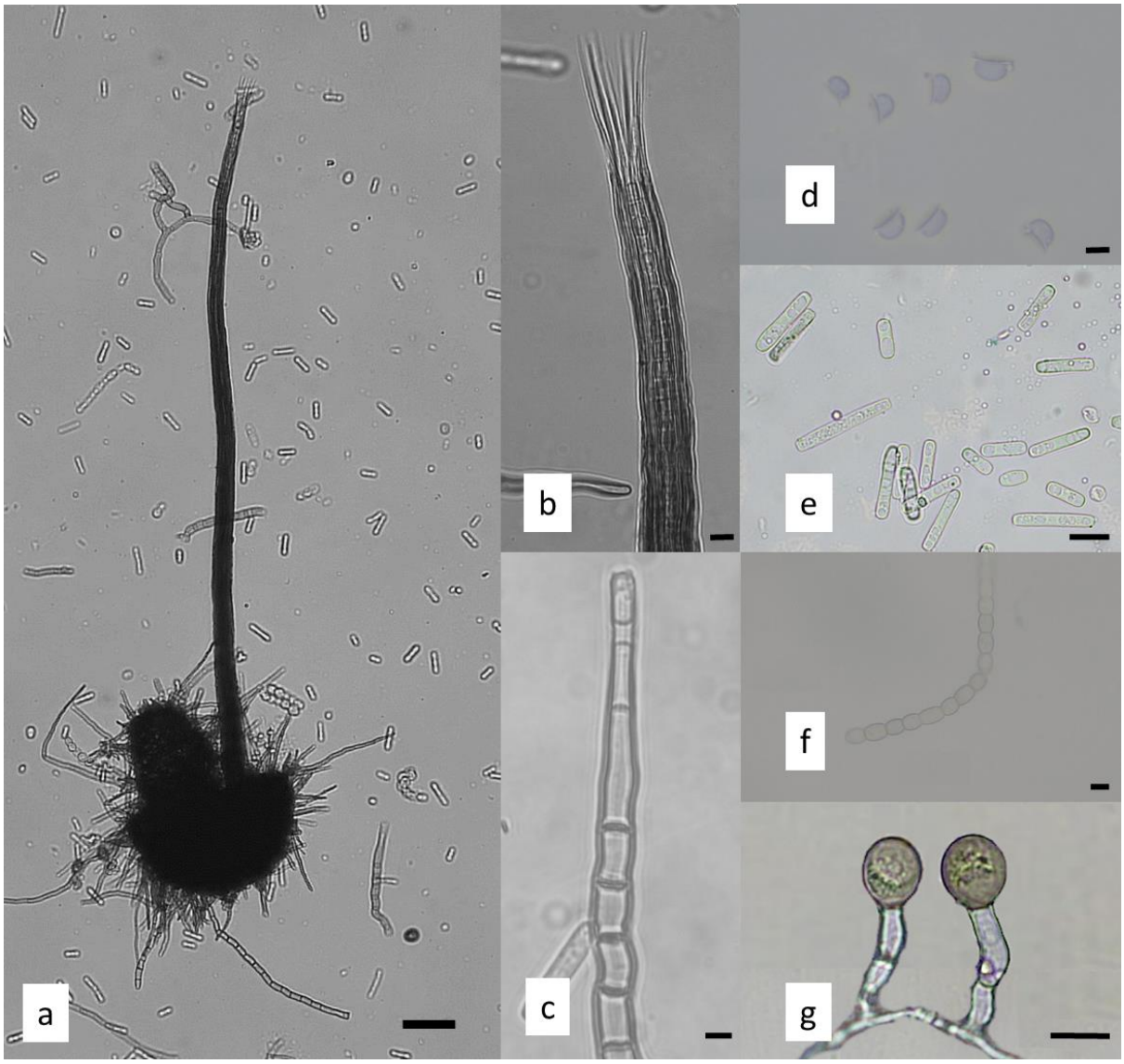

Fig. 2. Morphological characteristics of *Ceratocystis manginecans* isolated from *Artocarpus heterophyllus* stem lesion: **a.** ascomata with pirilliform base, **b.** divergent ostiolar hyphae; **c.** conidiophore/phialide; **d.** hat-shaped ascospores; **e.** cylindrical conidia; **f.** Chain of barrel-shaped conidia; **g.** chlamydospores of various shapes. Scale bars: a = 100 µm; b,c,e,f,g = 10 µm; d = 5 µm.

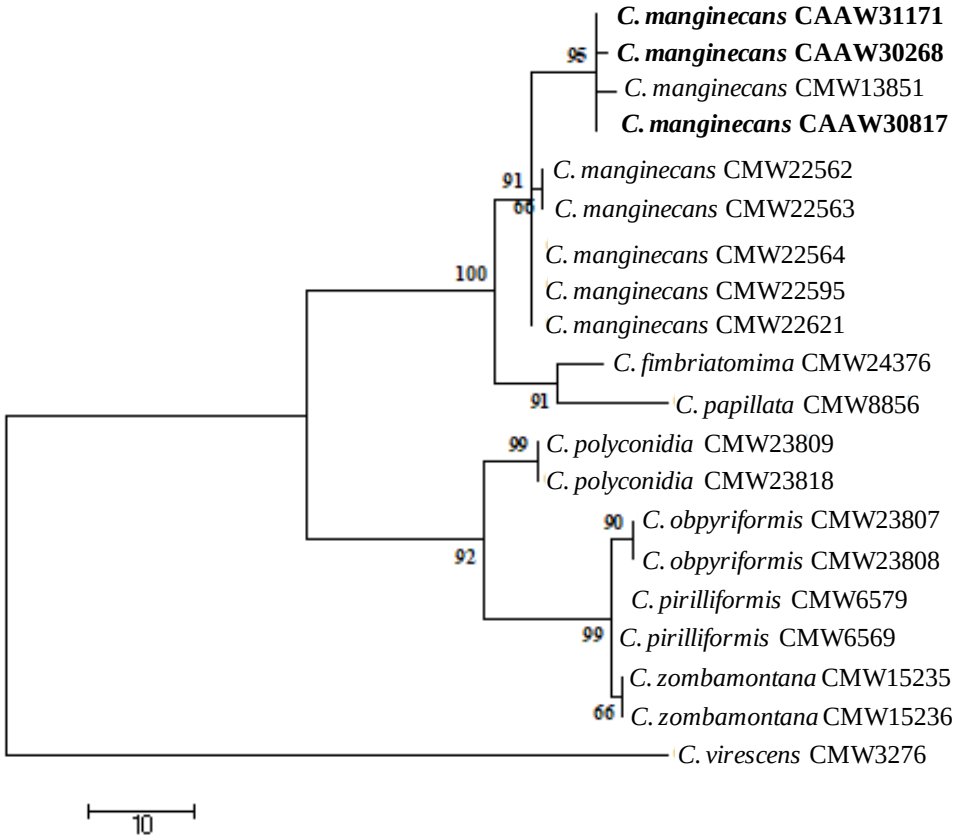

Fig. 3 Phylogenetic tree constructed by MEGA with Maximum Parsimony (MP) search for the combined sequence data of the ITS region and β-tubulin gene (CAAW31171, CAAW30268, and CAAW30817) and their related species from GenBank. Consistency (CI), retention (RI), and composite indexes (CoI) are 0.819149, 0.952113, and 0.861689 for all sites and parsimony-informative sites. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Bootstrap values >50% are indicated above the branches. The analysis involved 20 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were 831 positions in the final dataset. *Ceratocystis virescens* was used as the out-group.

a. muslim unsri <a_muslim@unsri.ac.id>

Thank you for your approval - [EMID:a73d107a34960eb0]

1 message

APDN <em@editorialmanager.com> Mon, Mar 22, 2021 at 5:14 AM
Reply-To: APDN <jude.estrera@springernature.com>
To: "A. Muslim" <a_muslim@unsri.ac.id>

Dear Dr. Muslim,

Thank you for approving the changes that we made to your submission entitled "Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia".

You will be able to check on the progress of your paper by logging on to Editorial Manager as an author. The URL is https://www.editorialmanager.com/apdn/.

Thank you for submitting your work to this journal.

Kind regards,

Our flexible approach during the COVID-19 pandemic

If you need more time at any stage of the peer-review process, please do let us know. While our systems will continue to remind you of the original timelines, we aim to be as flexible as possible during the current pandemic.

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REVIEW PROCESS

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a. muslim unsri <a_muslim@unsri.ac.id>

Major Revisions requested APDN-D-21-00015R1

2 messages

APDN <em@editorialmanager.com> Mon, May 17, 2021 at 11:45 PM
Reply-To: APDN <jude.estrera@springernature.com>
To: "A. Muslim" <a_muslim@unsri.ac.id>

CC: dagmar.hanold@adelaide.edu.au, dhanold@gmail.com

Dear Dr. Muslim,

We have received the reports from our advisers on your manuscript, "Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia" (APDN-D-21-00015R1), submitted to Australasian Plant Disease Notes.

Based on the advice received, I have decided that your manuscript can be accepted for publication after you have carried out the corrections as suggested by the reviewer(s).

Below, please find the reviewers' comments for your perusal.
You are kindly requested to also check the website for possible reviewer attachment(s).

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Please submit you revised manuscript before 14 Jun 2021 or request an extension of the deadline. If we do not hear from you by then, the manuscript will be automatically withdrawn.

With kind regards,

Eduardo Guatimosim, PhD
Associate Editor

COMMENTS FOR THE AUTHOR:

Reviewer: In this Note, the authors describe for the first time the Ceratocystis wilt on Artocarpus heterophyllus (Jackfruit) in Indonesia. The note needs a major revision for publication. Please, find below my comments:

1. There are currently different approaches on defining the boundaries of species identification on Ceratocystis fimbriata complex. However, many descriptions were based on ITS variation alone and some of them have already been synonymized based on solid studies (Harrington et al., (2014) Mycologia 106:224-242, Oliveira et al (2015), Phytopath 105:1229 - 1244). According to the Harrington et al., (2014) Mycologia 106:224-242, Oliveira et al (2015), Phytopath 105:1229 - 1244, C. manginecans reported on mango are in fact genotypes of C. fimbriata.
2. The phylogenetic analysis conducted in the current study derived from a poor dataset that did not consider the Ceratocystis species diversity. Therefore, to provide a better characterization of Ceratocystis on Jackfruits, isolates from Latin American Clade (LAC) and Asian-Australian clade (AAC) must be taken into consideration.
3. Regardless the approach used by the authors, relevant publications should not be ignored. References provided do not represent fully the current context considered on Ceratocystis research.
4. In which substrate the plants for inoculation were grown? In which condition the inoculated plants were kept? How was the climate variation during the 45 days after inoculation? How was the plant response in the inoculation point? Did the authors measure the lesion size of inoculated plants? Please provide this information and compare with control plants.
5. Line 10: Avoid repetition of keywords in the title.

6. Line 19-20: Please, provide a better description from symptoms on the woods.
7. Line 49-50: Which primers were used?
8. The writing requires minor revision.

Our flexible approach during the COVID-19 pandemic

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a. muslim unsri <a_muslim@unsri.ac.id> Mon, May 24, 2021 at 7:33 AM
To: APDN <jude.estrera@springernature.com>

Dear Prof. Eduardo Guatimosim, PhD
Associate Editor
Australasian Plant Disease Notes

Thank you very much for corrections to reviewers' comments of our manuscript No. APDN-D-21-00015R1 entitled “Jackfruit (*Artocarpus heterophyllus*), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia" by Rahmat Pratama, Ahmad Muslim, Suwandi Suwandi, Nurhayati Damiri, Soleha Soleha.

We are really appreciating the corrections.

We have revised and made some modified corrections as suggested by the reviewer(s).
We will submit revised our manuscript through process review in the Springer System.

We hope we can send you our revision today.

Thank you very much for your kindness and excellent cooperation.

Best regard

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DRAFT PERBAIKAN

Australasian Plant Disease Notes

Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia

--Manuscript Draft--

Manuscript Number:	APDN-D-21-00015R2
Full Title:	Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia
Article Type:	Plant Disease Note
Keywords:	Sudden death disease; Moraceae; Ceratocystis fimbriata sensu stricto
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Funding Information:	Kementerian Riset Teknologi Dan Pendidikan Tinggi Republik Indonesia (068/SP2H/AMD/LT/DRPM/2020) — Dr. A. Muslim
Abstract:	In 2019, wilt and sudden death were observed on Artocarpus heterophyllus (jackfruit) has been noted. Identification was performed by sequence analysis of the concatenated β-tubulin and ITS gene regions. Sequencing of the PCR product confirmed this pathogen was Ceratocystis fimbriata sensu stricto. C. fimbriata causing sudden death disease in A. heterophyllus is being reported for the first time in Indonesia and worldwide.
Response to Reviewers:	May 22, 2021 Dear Eduardo Guatimosim, PhD Associate Editor Australasian Plant Disease Notes Thank you very much for corrections to reviewers' comments of our manuscript. We are really appreciating the corrections. We have revised and make some modified the corrections as suggested by the reviewer(s) Here, we enclose revised version of the manuscript No. APDN-D-21-00015R1 entitled “Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia" by Rahmat Pratama, Ahmad Muslim, Suwandi Suwandi, Nurhayati Damiri, Soleha Soleha. Below is a summary of our response to the reviewers' comments.

Comment [1]: There are currently different approaches on defining the boundaries of species identification on Ceratocystis fimbriata complex. However, many descriptions were based on ITS variation alone and some of them have already been synonymized based on solid studies (Harrington et al., (2014) Mycologia 106:224-242, Oliveira et al (2015), Phytopath 105:1229 - 1244). According to the Harrington et al., (2014) Mycologia 106:224-242, Oliveira et al (2015), Phytopath 105:1229 - 1244, C. manginecans reported on mango are in fact genotypes of C. fimbriata.
Our response: We agree with the currently approaches on species identification of Ceratocystis fimbriata complex. Our Isolates from Jackfruit was identified by ITS as C. fimbriata ITS5 haplotype of C. fimbriata based on alignment according Harrington et al. (2014) and Li et al. (2016). β-tubulin sequence of our isolates confirmed the assignment to LAC of C. fimbriata sensu lato and they are phylogenetically clustered closely with ex-type and ex-paratype of C. manginecans and C. fimbriata. C. manginecans is considered synonym or conspecific of C. fimbriata sensu stricto (Harrington et al. 2014; Oliveira et al. 2015). We have made major changes on the species identification in the manuscript.

Comment [2]: The phylogenetic analysis conducted in the current study derived from a poor dataset that did not consider the Ceratocystis species diversity. Therefore, to provide a better characterization of Ceratocystis on Jackfruits, isolates from Latin American Clade (LAC) and Asian-Australian clade (AAC) must be taken into consideration.
Our response: We have included Tubulin sequences of references isolates from Latin American Clade (LAC) and Asian-Australian clade (AAC) to implement the new phylogenetic analysis. We found that Jackfruit isolates are grouped within the LAC along with other references sequences of C. fimbriata sensu stricto.

Comment [3]: Regardless the approach used by the authors, relevant publications should not be ignored. References provided do not represent fully the current context considered on Ceratocystis research.
Our response: We have inserted the relevant publications regarding recent taxonomy on Ceratocystis fimbriata complex.

Comment [4]: In which substrate the plants for inoculation were grown? In which condition the inoculated plants were kept? How was the climate variation during the 45 days after inoculation? How was the plant response in the inoculation point? Did the authors measure the lesion size of inoculated plants? Please provide this information and compare with control plants.
Our response:
a.The substrate used for growing are soil mix (field soil + peat + chicken manure) in a 10 cm diam. plastic pots
b.Pots were placed under a 50 percent shading nets in experimental field of faculty of agriculture Sriwijaya university
c.Inoculated plants were grown in the end of dry seasons (July to September 2019) and to maintain humidity the plants were watered twice a day.
d.The plant response in the inoculation point: initial symptoms appeared two weeks post inoculation as brown lesions on the wood of inoculation site.
e.Lesion length after 45 days from inoculation was 17.88-34.74 cm.

We have add all above informations in our manuscript

Comment [5]: Line 10: Avoid repetition of keywords in the title.
Our response: We agree and change keywords in the title to be “Sudden death disease · Moraceae · Ceratocystis fimbriata sensu stricto”

Comment [6]: Line 19-20: Please, provide a better description from symptoms on the woods.
Our response: Detail description on symptoms has been described in the result section. The sentence is “Wood of wilted trees showed a brown to black streaking in the woody xylem. Symptoms on the dying Jackfruit wood produced grey to brown lesions and included a streaking pattern of discoloration in the sapwood (Fig. 1a) and in some cases the lesions extended to heartwood (Fig. 1b). The lesion could be found partially or totally affected the sapwood from the basal stem until the branches.”

	Comment [7]: Line 49-50: Which primers were used? Our response: We used both ITS and β-tubulin primers. ITS primer has been performed to describe and group ITS genotypes (Harrington et al. 2014) and β-tubulin applied to describe and group Latin American Clade (LAC) and Asian-Australian clade (AAC). Comment [8]: The writing requires minor revision. Our response: Thank you very much, We have revised and make some modified the corrections as suggested by the reviewer(s). We feel that these changes have adequately addressed the comments and suggestions of reviewer(s). Please feel free to contact me if you need any additional information or clarification. Thank you very much for your consideration of the manuscript and excellent cooperation Yours sincerely, Ahmad Muslim Associate Professor Faculty of Agriculture, Sriwijaya University Jl. Palembang-Prabumulih Km.32, Indralaya, Palembang, Indonesia E-mail: a_muslim@unsri.ac.id

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Jackfruit (*Artocarpus heterophyllus*), a New Host Plant of *Ceratocystis* Wilt in South Sumatra, Indonesia

Abstract

In 2019, wilt and sudden death were observed on *Artocarpus heterophyllus* (jackfruit) has been noted. Identification was performed by sequence analysis of the concatenated β-tubulin and ITS gene regions. Sequencing of the PCR product confirmed this pathogen was *Ceratocystis fimbriata sensu stricto*. *C. fimbriata* causing sudden death disease in *A. heterophyllus* is being reported for the first time in Indonesia and worldwide.

Keywords: Sudden death disease · Moraceae · *Ceratocystis fimbriata sensu stricto* ·

Jackfruit (*A. heterophyllus*) belongs to the family Moraceae, and it is known in Indonesian as "Nangka". Jackfruit is grown widely in Indonesia and many countries with tropical and subtropical climates. Jackfruit is among the most exported fruits worldwide and has considerable nutrition and health benefits (Ranasinghe et al. 2019).

In a recent study of jackfruit in 2019, wilt and die-back symptoms were observed for the first time on *A. heterophyllus* in the agricultural field of Sriwijaya University (Indralaya), Plaju (Palembang) and Gelumbang (Prabumulih), Indonesia. Jackfruit trees were reported to die within a period from July to September 2019. Wood of wilted trees showed a brown to black streaking in the woody xylem. Symptoms on the dying Jackfruit wood produced grey to brown lesions and included a streaking pattern of discoloration in the sapwood (Fig. 1a) and in some cases the lesions extended to heartwood (Fig. 1b). The lesion could be found partially or totally affected the sapwood from the basal stem until the branches. Leaves of dying trees had yellowing symptoms, followed by the wilting of the leaves on several lateral branches, drying of twigs and the wilt of the whole tree (Fig. 1c). This type of wilting was termed as sudden death or wilt (Pratama et al. 2021).

Wood samples were taken from lesions of wilted trees using a knife sterilised in 70% ethanol. Each sample was wrapped in tissue paper and placed in a cool box. The same day, the wood samples (1–20 mm length, 1–2 mm thick) were sandwiched between two slices of fresh carrot and placed on sterile dry paper in plastic boxes at 25 °C following the method of Moller and DeVay (1968) (Fig. 1d). After 5–10 days, hat-shaped spores of putative *Ceratocystis* pathogens were placed on 2% (w/v) malt extract agar (MEA) (Merck, Germany), and incubated at 25 °C in a laboratory. The isolated fungi were initially identified based on morphological characteristics of a 14 day old culture. Mycelium on MEA grey, reverse side of colony

olivaceous grey; submerged mycelium darkening as the ascomata develop forming fine, radiating fibrils.

Morphological traits of fruiting bodies and spores were observed under an optical Olympus CX33 microscope. Ascomatal bases dark brown to black, base subglobes to globes and measured (n=100), 131.5 to 250.7×101.6 to 236.5 µm (length/width) (Fig. 2a). Ascomata necks erect, occasionally curved, black at the base becoming subhyaline towards the apex, smooth to crenulate, 324.7 to 579.1 µm long including ostiolar hyphae (Fig. 2b). Phialides pale brown to hyaline (Fig. 2c). Ascospores hat-shaped, 3.4 to 6.8×2.1 to 6.2 µm (length/width) (Fig. 2d). Bacilliform conidia 11.1 to 36.1×2.1 to 7.4 µm (length/width) (Fig. 2e). Barrel conidia 4.4 to 16.1×2.7 to 6.9 µm (length/width) (Fig. 2f). Chlamydospores oval, thick walled, smooth, 6.7 to 16.5×5.9 to 12.9 µm (length/width) (Fig. 2g). Based on morphological characters, the fungus was identified as *C. fimbriata.*

To confirm the species identification, isolates were cultured on potato dextrose broth (PDB) at room temperature for one week. Mycelial mat was filtered through Whatman filter paper and genomic DNA was extracted from fungal mycelial mat using YeaStar Genomic DNA Kit (Zymo Research Corporation, California, USA). PCR conditions and reactions for two gene regions were used to identify the *Ceratocystis* isolates; the Internal Transcribed Spacer (ITS) and part of the β-tubulin (βt) gene. Amplifications were carried out in 50 µl reactions containing 20 µl DreamTaq Green PCR Master Mix (Eppendorf, Germany) (DreamTaq DNA Polymerase, 2X DreamTaq Green buffer, dNTPs, and 4 mM $MgCl_2$), 1,5 µl of each forward and reverse primer, 4 µl of DNA template and 23µl sterilised water. The PCRs were performed with a C1000 Touch™ thermal cycler (Bio-Rad, USA). The PCR cycling parameters were as follows: initial denaturation for 5 min at 95 °C, followed by 35 cycles at 95 °C for 30 s, 56 °C for 45 s and 72 °C for 1 min. Amplification was completed at 72 °C for 10 min and the PCR product was stored at 10 °C. The PCR amplicons were sequenced at 1st BASE (Malaysia).

For the ITS and β-tubulin, amplification resulted in fragments of ~550 base pairs (bp) in size. The sequences of the amplified products were then deposited in the GenBank database and assigned accession numbers isolate CAAW31171 (MT355410; MW717653), isolate CAAW30817 (MT355413, MW717656), and isolate CAAW30268 (MT355412; MW717655) for the ITS and β-tubulin. β-tubulin datasets were generated using ex-type and ex-paratype sequences representing species in the Latin American (LAC) and Asian clade (AC) of the *C. fimbriata* species complex (Fourie et al. 2015; Oliveira et al. 2015; Barnes et al. 2018). To determine relatedness of isolates from jackfruit with known *C. fimbriata* populations, the ITS sequence was manually aligned with known ITS haplotypes as designated by Harrington et al.

(2014); Li et al. (2016) and phylogenetic analyses were performed. Maximum Parsimony (MP) analyses were performed in MEGA v. 10 (Kumar et al. 2016; Paul et al. 2018) with 1000 bootstrap replications. β-tubulin sequence of our isolates confirmed the assignment to LAC of *C. fimbriata sensu lato* (Fig. 3a). Manual alignment of the ITS sequences with previously described ITS genotypes (Harrington et al. 2014; Li et al. 2016) grouped the isolates into ITS5 haplotype of *C. fimbriata sensu stricto* (Fig. 3b).

The pathogenic potential of isolates was evaluated by the under bark inoculation method described by O'Gara et al. (1997) using Five-month-old *A. heterophyllus* seedlings with stem diameters of 6-8 mm and heights <1.5 m were prepared for pathogenicity test. Seedlings were grown in 10 cm diameter plastic pots containing a soil mix (topsoil + peat + chicken manure) under a 50% shading net. Plants were watered daily to maintain humidity, and any mortality occurring before the end of the experiment was recorded. Wounds were made on the stems of the seedlings using a cork borer (4 mm diam.), and mycelial discs (4 mm diam.) taken from an actively growing colony of *C. fimbriata* on 2% MEA (14 days) (Pratama et al. 2021) were placed in the wounds with the mycelium facing downwards. These were covered with Parafilm (Pechiney, Menasha, Wisconsin) to reduce contamination and desiccation. Ten plants of each tree species were inoculated with sterile MEA plugs to serve as controls (Fig. 4a). Fungal isolates were re-isolated and re-identified using morphological characteristics for Koch's postulates confirmation. In pathogenicity tests, initial symptoms appeared two weeks post-inoculation as brown lesions on the wood of inoculation site (Fig. 4b). Forty-five days after inoculation, plants exhibited wilt symptoms, lesions of wood discoloration extended to heartwood (Fig. 4c) and length (downward + upward) was 17.88 until 34.74 cm. When re-isolated, the fungus was phenotypically identical to the prior isolate of *C. fimbriata* (CAAW31171, CAAW30817, CAAW30268).

This is the first report of *C. fimbriata* causing wilt and die-back in Jackfruit in Indonesia and worldwide. The symptoms of *C. fimbriata* wilt disease in Jackfruit are stems cankers, the stems become chapped as though torn apart, fruit rot and progressive loss of the canopy resulting in tree death. Jackfruit trees showed typical symptoms of infection by the *Ceratocystis* fungus; the same was true of a serious wilt pathogen of *A. mangium* and *A. crassicarpa* in Indonesia (Tarigan et al. 2011), *Lansium domesticum* in Indonesia (Suwandi et al. 2021) and on Sweet Potato and Pomegranate in China (Li et al. 2016). *C. fimbriata* infecting native trees in these countries is serious and could potentially lead to the devastation of important components of the natural biodiversity of Indonesia.

Acknowledgement

This research was funded by PMDSU scholarship with budget year of 2019-2021 according to the Director of Research and Community Service, Directorate of Research and Community Service (DRPM), Directorate General for Research and Development, Ministry of Research, Technology, and Higher Education, Number: 068/SP2H/AMD/LT/DRPM/2020 chaired by Ahmad Muslim.

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Table

156 **Table 1** *Ceratocystis* isolates considered in the phylogenetic analyses

Species	Haplotype	Isolates no.	Host	Origin	GenBank accession no.	
					ITS	β-tubulin
C. fimbriata	ITS1a	C1418	*Ipomoea batatas*	USA	AY157956	-
	ITS1	C1857	*Ficus carica*	Brazil	HQ157542	-
	ITS1b	CMW4797	*Eucalyptus* sp.	Congo	FJ236733	-
	ITSb	CMW9998	*Eucalyptus* sp.	South Africa	FJ236721	-
	ITS2	C1655	*Mangifera indica*	Brazil	HQ157546	-
	ITS3	C1440	*Eucalyptus* sp.	Brazil	HQ157544	-
	ITS3	CMW5328	*E. grandis*	Uganda	AF395686	-
	ITS4	C1442	*Eucalyptus* sp.	Brazil	HQ157545	-
	ITS5	**CAAW31171**	***Artocarpus heterophyllus***	**Indonesia**	**MT355410**	**MW717653**
	ITS5	**CAAW30268**	***A. heterophyllus***	**Indonesia**	**MT355412**	**MW717655**
	ITS5	**CAAW30817**	***A. heterophyllus***	**Indonesia**	**MT355413**	**MW717656**
	ITS5	CMW38737	*E. grandis*	Zimbabwe	KF878326	KF878335
	ITS5	C1345	*Eucalyptus* sp.	Brazil	AY157966	-
	ITS5	A59662	*Camellia sinensis*	China	KF650948	-
	ITS5	YM061	*Colocasia esculenta*	China	AM712445	-
	ITS5	P20053	*Punica granatum*	China	AM292204	-
	ITS5	C1	*Acacia* sp.	Vietnam	MF033455	MF040712
	ITS5	CMW22563	*A. mangium*	Indonesia	EU588656	EU588636
	ITS5	WRC	*Lansium domesticum*	Indonesia	MT229127	MW013766
	ITS6	C2055	*Mangifera* sp.	Brazil	HQ157548	-
	ITS6z	CMW13582	*Hypocryphalus mangifera*	Oman	KC261853	-
	ITS6z	WBC	*L. domesticum*	Indonesia	MT229128	MW013767
	ITS7b	CMW13851	*M. indica*	Oman	AY953383	EF433308
	ITS7b	CMW23634	*M. indica*	Pakistan	EF433302	EF433311
	ITS7b	CMW22579	*A. mangium*	Indonesia	EU588658	-
	ITS8a	CMW8856	*Citrus* sp.	Colombia	AY233867	-
	ITS8c	CMW17808	*Eucalyptus* sp	Colombia	EF127990	-
	ITS8e	CMW22092	*E. deglupta*	Ecuador	FJ151432	-
	ITS9	C1558	*M. indica*	Brazil	AY157965	-
	ITS9	C1914	*C. esculenta*	Brazil	HQ157540	-
	ITS10	C994	*M. indica*	Brazil	AY157964	-
	ITS10a	Cf4	*M. indica*	Brazil	EF042605	-
	ITS11	C1865	*C. esculenta*	Brazil	AY526286	-
	ITS12	C1926	*C. esculenta*	Brazil	HQ157541	-
	ITS14	C1688	*M. indica*	Brazil	AY526291	-
	ITS15	C925	*Gmelina arborea*	Brazil	AY157967	-
	ITS16	C924	*G. arborea*	Brazil	HQ157539	-
C. pirilliformis	Asian clade (AC)	CMW6569	*E. nitens*	Australia	-	DQ371652
	AC	CMW6579	*E. nitens*	Australia	-	DQ371653
C. polychroma	AC	CMW11424	*Syzygium aromaticum*	Indonesia	-	AY528966
	AC	CMW11436	*S. aromaticum*	Indonesia	-	AY528967
C. atrox	AC	CMW19383	*E. grandis*	Australia	-	EF070430
	AC	CMW19385	*E. grandis*	Australia	-	EF070431
C. neglecta	Latin American clade (LAC)	CMW17808	*E. grandis*	Colombia	-	EU881898
	LAC	CMW18194	*E. grandis*	Colombia	-	EU881899
C. colombiana	LAC	CMW5751	*Coffea arabica*	Colombia	-	AY177225
	LAC	CMW5761	*C. arabica*	Colombia	-	AY177224
C. cacaofunesta	LAC	CMW14803	*Theobroma cacao*	Ecuador	-	KJ631108
	LAC	CMW15051	*T. cacao*	Costa Rica	-	KJ601510
C. papillata	LAC	CMW8850	*Citrus* × *Tangelo hybrid*	Colombia	-	AY233875
	LAC	CMW8856	*Citrus limon*	Colombia	-	AY233874
C. fimbriata	LAC	CMW14797	*M. indica*	Brazil	-	EF433307
	LAC	CMW28907	*M. indica*	Brazil	-	FJ200270

	LAC	CMW1547	*I. batatas*	Papua New Guinea	-	EF070443
	LAC	C1421	*I. batatas*	USA	-	KF302689
C. fimbriatomima	LAC	CMW24174	*Eucalyptus hybrid*	Venezuela	-	EF190951
	LAC	CMW24176	*Eucalyptus hybrid*	Venezuela	-	EF190952
C. fimbriata	LAC	CMW21127	*A. crassicarpa*	Indonesia	-	EU588643
	LAC	CMW24664	*Eucalyptus hybrid*	China	-	JQ862720
	LAC	CBS115173	*Gmelina arborea*	Brazil	-	KF302700
	LAC	CBS14653	*C. arabica*	Suriname	-	KF302702
C. platani	LAC	CMW14802	*Platanus occidentalis*	USA	-	EF070425
	LAC	CMW23450	*P. occidentalis*	Greece	-	KJ601513

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Colour figure

Fig. 1 Symptoms of *Ceratocystis fimbriata* wilt disease in *Artocarpus heterophyllus:* **a**. vascular discoloration of infected tree; **b.** The discolored wood extended to the heartwood of the basal stem; **c.** three-year-old tree with wilted, yellowing leaves and rotten fruit; **d.** isolation of the fungus from discoloured xylem showing dark mycelium and sporulation on the carrot slices after 7 days.

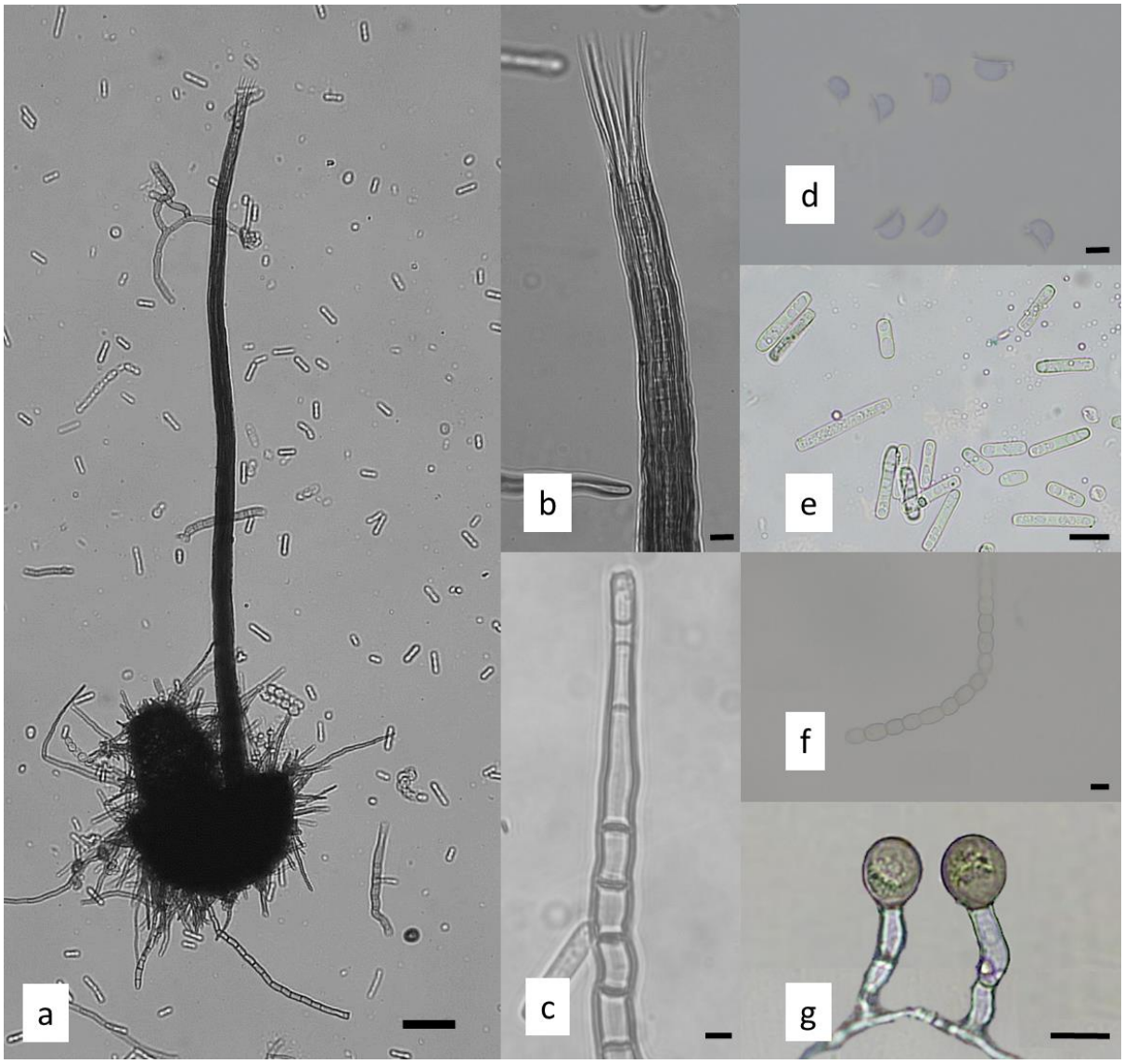

Fig. 2. Morphological characteristics of *Ceratocystis fimbriata* isolated from *Artocarpus heterophyllus* stem lesion: **a.** ascomata with pirilliform base, **b.** divergent ostiolar hyphae; **c.** conidiophore/phialide; **d.** hat-shaped ascospores; **e.** cylindrical conidia; **f.** Chain of barrel-shaped conidia; **g.** chlamydospores of various shapes. Scale bars: a = 100 µm; b,c,e,f,g = 10 µm; d = 5 µm.

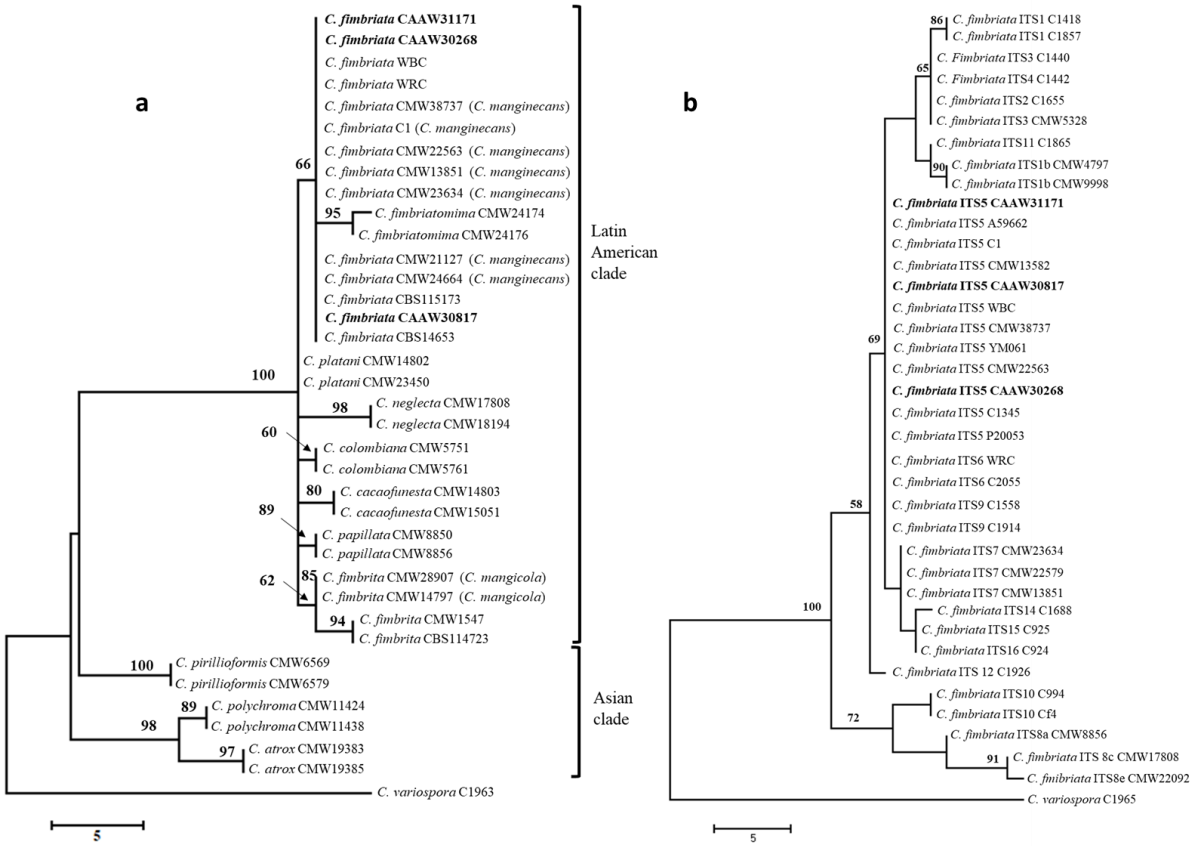

Fig. 3 Phylogenetic tree constructed by MEGA with Maximum Parsimony (MP) analysis of the **a**. β-tubulin sequences from Jackfruit tree in Indonesia (marked in bold) and other species in the Latin American and Asian clade of the *C. fimbriata* species complex. Species names considered to be synonyms of *C. fimbriata sensu stricto* are in parentheses (Harrington et al. 2014; Oliveira et al. 2015). **b.** ITS sequences from Jackfruit tree in Indonesia (marked in bold) and genotypes (sequences) of the *C. fimbriata sensu stricto*. The ITS haplotypes of *C. fimbriata* are numbered following the numerical designations of Harrington et al. (2014). Consistency (CI), retention (RI), and composite indexes (CoI) for β-tubulin were 0.566667, 0.845238, 0.668011, respectively and ITS was 0.933333, 0.976563, 0.932836, respectively. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Bootstrap values >50% are indicated above the branches. The analysis involved 38 (β-tubulin) and 37 (ITS) nucleotide sequences. All positions containing gaps and missing data were eliminated. There were 408 (β-tubulin) and 518 (ITS) positions in the final dataset. *Ceratocystis variospora* was used as the out-group.

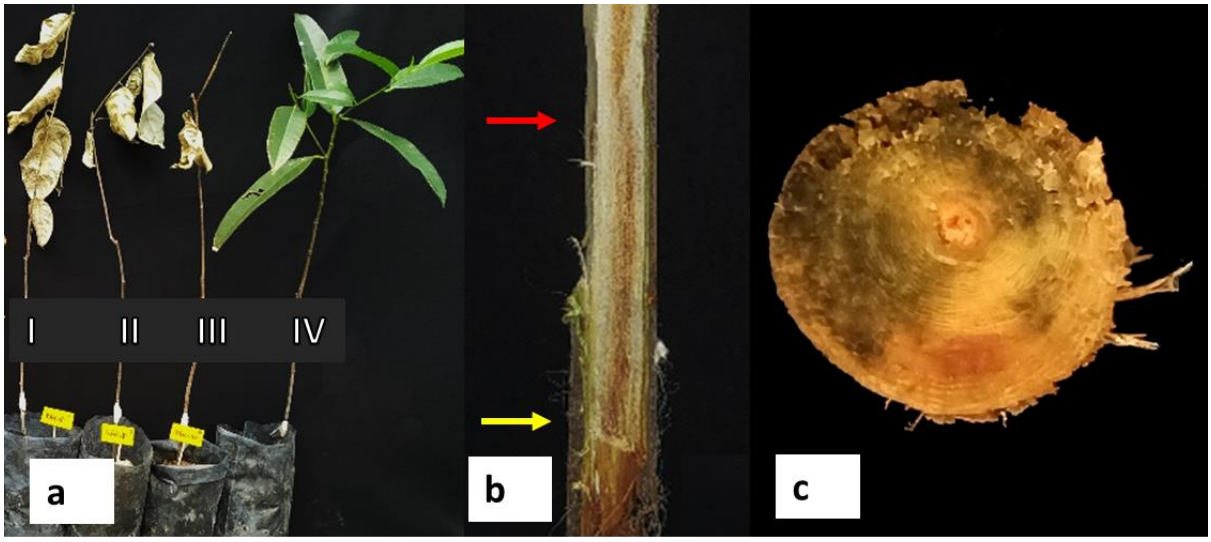

Fig. 4 Response after 45 days of *Artocarpus heterophyllus* seedlings to under-bark inoculation with mycelium of *Ceratocystis*. **a.** total wilting of plant inoculated with CAAW31171 (I), CAAW30817 (II), CAAW30268 (III) and the control seedling appeared healthy (IV); **b.** yellow arrow indicates the point of inoculation and red arrow the lesion boundary; **c.** The discoloured seedlings wood extended to the heartwood of the basal stem.

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Submission Confirmation

1 message

APDN <em@editorialmanager.com> Mon, May 24, 2021 at 2:04 PM
Reply-To: APDN <jude.estrera@springernature.com>
To: "A. Muslim" <a_muslim@unsri.ac.id>

Dear Dr. Muslim,

We acknowledge, with thanks, receipt of the revised version of your manuscript, "Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia", submitted to Australasian Plant Disease Notes

The manuscript number is APDN-D-21-00015R2.

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a. muslim unsri <a_muslim@unsri.ac.id>

Your Submission APDN-D-21-00015R2

1 message

APDN <em@editorialmanager.com> Sat, Jun 12, 2021 at 12:49 AM
Reply-To: APDN <jude.estrera@springernature.com>
To: "A. Muslim" <a_muslim@unsri.ac.id>

CC: dagmar.hanold@adelaide.edu.au, dhanold@gmail.com

Dear Dr. Muslim,

We have received the reports from our advisors on your manuscript, 'Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia' (APDN-D-21-00015R2), submitted to Australasian Plant Disease Notes.

Based on the advice received, I have decided that your manuscript can be accepted for publication after you have carried out the corrections as suggested by the reviewer(s).

Below, please find the reviewers' comments for your perusal.
You are kindly requested to also check the website for possible reviewer attachment(s).

Please submit your revised manuscript online by using
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Please submit you revised manuscript before 09 Jul 2021 or request an extension of the deadline. If we do not hear from you by then, the manuscript will be automatically withdrawn.

With kind regards,

Eduardo Guatimosim, PhD
Associate Editor

COMMENTS FOR THE AUTHOR:

Dear authors
Thank you for uploading the revised manuscript. It seems to me that it is quite better than on its first version. However, I believe that there are still corrections to be done.

line 8-9. Never start a sentence with an abreviation. So, please correct “C. fimbriata” to “Ceratocystis fimbriata”. Alternatively, you can change the last sentence to be read as follows: "This is the first report of C. fimbiriata causing sudden death disease in A. heterophyllus in Indonesia and worldwide.
line 12-13. The first time that a scientific name is presented in the main text, it should not be abbreviated. Please change the phrase "Jackfruit (A. heterophyllus) belongs to the family Moraceae, and it is known in Indonesian as “Nangka”. Jackfruit is grown..." to read as follows: "Jackfruit (Artocarpus heterophyllus, Moraceae) is known in Indonesian as “Nangka”, and is cultivated ..."
line 13. remove "Indonesia and"
line 16. The first sentence mention a recent study of 2019, but fails to present the reference. Please present the literature at the end of the sentence (at line 18).
line 38. replace "subglobes to globes" by "subglobose to globose"
lines 39-45 . Range measurements should be done using en-dashes.
line 39-45. remove "(length/width)"

line 46. The first time that a scientific name is presented in the main text, it should not be abbreviated.
line 48. Replace "Mycelial mat" by "Mycelium"
line 52. As requested by the reviewer, please provide the primers names used on the study. Also provide the references of each primer
line 99. Never start a sentence with an abbreviation
line 175. Replace "b,c,e,f,g" by "b-c, e-g" using en-dashes
line 186. The caption is too long. Lot of the information presented here should be at the main text, and not on the figure caption. Please make it short.
line 186, 187. Replace "of the..." by "of:..."

In accordance with APDN Submission guidelines, it is mandatory the deposition of a pure culture of the pathogen in a culture collection registered by the World Federation for Culture Collections (http://www.wfcc.info/collections). Please provide the culture collections codes, the collection name, and where it is housed.

Our flexible approach during the COVID-19 pandemic

If you need more time at any stage of the peer-review process, please do let us know. While our systems will continue to remind you of the original timelines, we aim to be as flexible as possible during the current pandemic.

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DRAFT PERBAIKAN

Australasian Plant Disease Notes

Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia

--Manuscript Draft--

Manuscript Number:	APDN-D-21-00015R3
Full Title:	Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia
Article Type:	Plant Disease Note
Keywords:	Sudden death disease; Moraceae; Ceratocystis fimbriata sensu stricto
Corresponding Author:	A. Muslim, Ph.D. Universitas Sriwijaya Fakultas Pertanian Palembang, Sumatera Selatan INDONESIA
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Funding Information:	Kementerian Riset Teknologi Dan Pendidikan Tinggi Republik Indonesia (068/SP2H/AMD/LT/DRPM/2020) — Dr. A. Muslim
Abstract:	In 2019, wilt and sudden death were observed on Artocarpus heterophyllus (jackfruit) has been noted. Identification was performed by sequence analysis of the concatenated β-tubulin and ITS gene regions. Sequencing of the PCR product confirmed this pathogen was Ceratocystis fimbriata sensu stricto. This is the first report of C. fimbriata causing sudden death disease in A. heterophyllus in Indonesia and worldwide.
Response to Reviewers:	June 14, 2021 Dear Eduardo Guatimosim, PhD Associate Editor Australasian Plant Disease Notes Thank you very much for corrections to reviewers' comments of our manuscript. We are really appreciating the corrections. We have revised and make some modified the corrections as suggested by the reviewer(s) Here, we enclose revised version of the manuscript No. APDN-D-21-00015R1 entitled “Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia" by Rahmat Pratama, Ahmad Muslim, Suwandi Suwandi, Nurhayati Damiri, Soleha Soleha. Below is a summary of our response to the reviewers' comments.

Comment [1]: line 8-9. Never start a sentence with an abreviation. So, please correct “C. fimbriata” to “Ceratocystis fimbriata”. Alternatively, you can change the last sentence to be read as follows: "This is the first report of C. fimbriata causing sudden death disease in A. heterophyllus in Indonesia and worldwide.
Our response: We agree and change sentence to be "This is the first report of C. fimbriata causing sudden death disease in A. heterophyllus in Indonesia and worldwide”.

Comment [2]: line 12-13. The first time that a scientific name is presented in the main text, it should not be abbreviated. Please change the phrase "Jackfruit (A. heterophyllus) belongs to the family Moraceae, and it is known in Indonesian as “Nangka”. Jackfruit is grown..." to read as follows: "Jackfruit (Artocarpus heterophyllus, Moraceae) is known in Indonesian as “Nangka”, and is cultivated ..."
Our response: Thank you very much. We agree and change sentence to be “Jackfruit (Artocarpus heterophyllus, Moraceae) is known in Indonesian as “Nangka”, and is cultivated”.

Comment [3]: line 13. remove "Indonesia and"
Our response: We agree and removed "Indonesia and" in sentence

Comment [4]: line 16. The first sentence mention a recent study of 2019, but fails to present the reference. Please present the literature at the end of the sentence (at line 18).
Our response:
We agree and change sentence to be “In July 2019, wilt and die-back symptoms were observed for the first time on A. heterophyllus in the agricultural field of Sriwijaya University (Indralaya), Plaju (Palembang) and Gelumbang (Prabumulih), Indonesia.”

Comment [5]: line 38. replace "subglobes to globes" by "subglobose to globose"
Our response:
We agree and change sentence to be "subglobose to globose"

Comment [6]: lines 39-45 . Range measurements should be done using en-dashes.
Our response:
Thank you very much. We agree and change sentence to be “measured (n=100), 131.5-250.7×101.6-236.5 µm (Fig. 2a). Ascomata necks erect, occasionally curved, black at the base becoming subhyaline towards the apex, smooth to crenulate, 324.7-579.1 µm long including ostiolar hyphae (Fig. 2b). Phialides pale brown to hyaline (Fig. 2c). Ascospores hat-shaped, 3.4-6.8×2.1-6.2 µm (Fig. 2d). Bacilliform conidia 11.1-36.1×2.1-7.4 µm (Fig. 2e). Barrel conidia 4.4-16.1×2.7-6.9 µm (Fig. 2f). Chlamydospores oval, thick walled, smooth, 6.7-16.5×5.9-12.9 µm (Fig. 2g)”.

Comment [7]: line 39-45. remove "(length/width)"
Our response:
Thank you very much. We agree and remove "(length/width)"

Comment [8]: line 46. The first time that a scientific name is presented in the main text, it should not be abbreviated.
Our response:
We agree and change sentence to be "Ceratocystis fimbriata”

Comment [9]: line 48. Replace "Mycelial mat" by "Mycelium"
Our response:
We agree and replace "Mycelial mat" by "Mycelium"

Comment [10]: line 52. As requested by the reviewer, please provide the primers names used on the study. Also provide the references of each primer
Our response:
We agree and change sentence to be "PCR conditions and reactions for two gene regions were used to identify the Ceratocystis isolates; the Internal Transcribed Spacer (ITS) with primers ITS 1 and ITS4 (White et al. 1990) and part of the β-tubulin (βt) gene with primers βt1a and βt1b (Glass and Donaldson 1995)”

Comment [11]: line 99. Never start a sentence with an abbreviation
Our response:
We agree and change sentence to be "Ceratocystis fimbriata”

Comment [12]: line 175. Replace "b,c,e,f,g" by "b-c, e-g" using en-dashes
Our response:
We agree and change sentence to be "Scale bars: a = 100 μm; b-c, e-g = 10 μm; d = 5 μm.”

Comment [13]: line 186. The caption is too long. Lot of the information presented here should be at the main text, and not on the figure caption. Please make it short.
Our response:
We agree and change sentence “Phylogenetic tree constructed by MEGA with Maximum Parsimony (MP) analysis by a. β-tubulin sequences from Jackfruit tree in Indonesia (marked in bold) and other species in the Latin American and Asian clade of the C. fimbriata species complex. Species names considered to be synonyms of C. fimbriata sensu stricto are in parentheses (Harrington et al. 2014; Oliveira et al. 2015). b. ITS sequences from Jackfruit tree in Indonesia (marked in bold) and genotypes (sequences) of the C. fimbriata sensu stricto. The ITS haplotypes of C. fimbriata are numbered following the numerical designations of Harrington et al. (2014). Consistency (CI), retention (RI), and composite indexes (CoI) for β-tubulin were 0.566667, 0.845238, 0.668011, respectively and ITS was 0.933333, 0.976563, 0.932836, respectively. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Bootstrap values >50% are indicated above the branches. The analysis involved 38 (β-tubulin) and 37 (ITS) nucleotide sequences. All positions containing gaps and missing data were eliminated. There were 408 (β-tubulin) and 518 (ITS) positions in the final dataset. Ceratocystis variospora was used as the out-group.” to be "Phylogenetic tree constructed by MEGA with Maximum Parsimony (MP) analysis by a. β-tubulin sequences from Jackfruit tree in Indonesia (marked in bold) and other species in the Latin American and Asian clade of the C. fimbriata species complex. b. ITS sequences from Jackfruit tree in Indonesia (marked in bold) and genotypes (sequences) of the C. fimbriata sensu stricto.”

Comment [14]: line 186, 187. Replace "of the..." by "of:..."
Our response:
We agree and change sentence to be "analysis by”

Comment [15]: In accordance with APDN Submission guidelines, it is mandatory the deposition of a pure culture of the pathogen in a culture collection registered by the World Federation for Culture Collections (http://www.wfcc.info/collections). Please provide the culture collections codes, the collection name, and where it is housed.
Our response:
Specimens were deposited in the culture collection of the Phytopathology Laboratory of Sriwijaya University (Indralaya, Indonesia) as HPTUnsri-2101. Currently our isolates are being processed for deposit in the Indonesian Culture Collection (InaCC), under the management of Microbiology Division, Research Center for Biology, the Indonesian Institute of Sciences (Cibinong, Indonesia).

We feel that these changes have adequately addressed the comments and suggestions of reviewer(s). Please feel free to contact me if you need any additional information or clarification.

Thank you very much for your consideration of the manuscript and excellent cooperation

Yours sincerely,

Ahmad Muslim
Associate Professor
Faculty of Agriculture, Sriwijaya University
Jl. Palembang-Prabumulih Km.32, Indralaya, Palembang, Indonesia

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*Corresponding Author: a_muslim@unsri.ac.id

Jackfruit (*Artocarpus heterophyllus*), a New Host Plant of *Ceratocystis* Wilt in South Sumatra, Indonesia

Abstract

In 2019, wilt and sudden death were observed on *Artocarpus heterophyllus* (jackfruit) has been noted. Identification was performed by sequence analysis of the concatenated β-tubulin and ITS gene regions. Sequencing of the PCR product confirmed this pathogen was *Ceratocystis fimbriata sensu stricto*. This is the first report of *C. fimbriata* causing sudden death disease in *A. heterophyllus* in Indonesia and worldwide.

Keywords: Sudden death disease · Moraceae · *Ceratocystis fimbriata sensu stricto* ·

Jackfruit (*Artocarpus heterophyllus*, Moraceae) is known in Indonesian as "Nangka", and is cultivated widely in many countries with tropical and subtropical climates. Jackfruit is among the most exported fruits worldwide and has considerable nutrition and health benefits (Ranasinghe et al. 2019).

In July 2019, wilt and die-back symptoms were observed for the first time on *A. heterophyllus* in the agricultural field of Sriwijaya University (Indralaya), Plaju (Palembang) and Gelumbang (Prabumulih), Indonesia. Wood of wilted trees showed a brown to black streaking in the woody xylem. Symptoms on the dying Jackfruit wood produced grey to brown lesions and included a streaking pattern of discoloration in the sapwood (Fig. 1a) and in some cases the lesions extended to heartwood (Fig. 1b). The lesion could be found partially or totally affected the sapwood from the basal stem until the branches. Leaves of dying trees had yellowing symptoms, followed by the wilting of the leaves on several lateral branches, drying of twigs and the wilt of the whole tree (Fig. 1c). This type of wilting was termed as sudden death or wilt (Pratama et al. 2021).

Wood samples were taken from lesions of wilted trees using a knife sterilised in 70% ethanol. Each sample was wrapped in tissue paper and placed in a cool box. The same day, the wood samples (1–20 mm length, 1–2 mm thick) were sandwiched between two slices of fresh carrot and placed on sterile dry paper in plastic boxes at 25 °C following the method of Moller and DeVay (1968) (Fig. 1d). After 5–10 days, hat-shaped spores of putative *Ceratocystis* pathogens were placed on 2% (w/v) malt extract agar (MEA) (Merck, Germany), and incubated at 25 °C in a laboratory. The isolated fungi were initially identified based on morphological characteristics of a 14 day old culture. Mycelium on MEA grey, reverse side of colony

olivaceous grey; submerged mycelium darkening as the ascomata develop forming fine, radiating fibrils.

Morphological traits of fruiting bodies and spores were observed under an optical Olympus CX33 microscope. Ascomatal bases dark brown to black, base subglobose to globose and measured (n=100), 131.5–250.7×101.6–236.5 μm (Fig. 2a). Ascomata necks erect, occasionally curved, black at the base becoming subhyaline towards the apex, smooth to crenulate, 324.7–579.1 μm long including ostiolar hyphae (Fig. 2b). Phialides pale brown to hyaline (Fig. 2c). Ascospores hat-shaped, 3.4–6.8×2.1–6.2 μm (Fig. 2d). Bacilliform conidia 11.1–36.1×2.1–7.4 μm (Fig. 2e). Barrel conidia 4.4–16.1×2.7–6.9 μm (Fig. 2f). Chlamydospores oval, thick walled, smooth, 6.7–16.5×5.9–12.9 μm (Fig. 2g). Based on morphological characters, the fungus was identified as *Ceratocystis fimbriata*. Specimens were deposited in the culture collection of the Phytopathology Laboratory of Sriwijaya University (Indralaya, Indonesia) as HPTUnsri-2101.

To confirm the species identification, isolates were cultured on potato dextrose broth (PDB) at room temperature for one week. Mycelium was filtered through Whatman filter paper and genomic DNA was extracted from fungal mycelial mat using YeaStar Genomic DNA Kit (Zymo Research Corporation, California, USA). PCR conditions and reactions for two gene regions were used to identify the *Ceratocystis* isolates; the Internal Transcribed Spacer (ITS) with primers ITS 1 and ITS4 (White et al. 1990) and part of the β-tubulin (βt) gene with primers βt1a and βt1b (Glass and Donaldson 1995). Amplifications were carried out in 50 μl reactions containing 20 μl DreamTaq Green PCR Master Mix (Eppendorf, Germany) (DreamTaq DNA Polymerase, 2X DreamTaq Green buffer, dNTPs, and 4 mM $MgCl_2$), 1,5 μl of each forward and reverse primer, 4 μl of DNA template and 23μl sterilised water. The PCRs were performed with a C1000 Touch™ thermal cycler (Bio-Rad, USA). The PCR cycling parameters were as follows: initial denaturation for 5 min at 95 °C, followed by 35 cycles at 95 °C for 30 s, 56 °C for 45 s and 72 °C for 1 min. Amplification was completed at 72 °C for 10 min and the PCR product was stored at 10 °C. The PCR amplicons were sequenced at 1st BASE (Malaysia).

For the ITS and β-tubulin, amplification resulted in fragments of ~550 base pairs (bp) in size. The sequences of the amplified products were then deposited in the GenBank database and assigned accession numbers isolate CAAW31171 (MT355410; MW717653), isolate CAAW30817 (MT355413, MW717656), and isolate CAAW30268 (MT355412; MW717655) for the ITS and β-tubulin. β-tubulin datasets were generated using ex-type and ex-paratype sequences representing species in the Latin American (LAC) and Asian clade (AC) of the *C.*

fimbriata species complex (Fourie et al. 2015; Oliveira et al. 2015; Barnes et al. 2018). To determine relatedness of isolates from jackfruit with known *C. fimbriata* populations, the ITS sequence was manually aligned with known ITS haplotypes as designated by Harrington et al. (2014); Li et al. (2016) and phylogenetic analyses were performed. Maximum Parsimony (MP) analyses were performed in MEGA v. 10 (Kumar et al. 2016; Paul et al. 2018) with 1000 replications. The analysis involved 38 (β-tubulin) and 37 (ITS) nucleotide sequences. All positions containing gaps and missing data were eliminated. There were 408 (β-tubulin) and 518 (ITS) positions in the final dataset. *Ceratocystis variospora* was used as the out-group. β-tubulin sequence of our isolates confirmed the assignment to LAC of *C. fimbriata sensu lato* (Fig. 3a). Manual alignment of the ITS sequences with previously described ITS genotypes (Harrington et al. 2014; Li et al. 2016) grouped the isolates into ITS5 haplotype of *C. fimbriata sensu stricto* (Fig. 3b). Consistency (CI), retention (RI), and composite indexes (CoI) for β-tubulin were 0.566667, 0.845238, 0.668011, respectively and ITS was 0.933333, 0.976563, 0.932836, respectively.

The pathogenic potential of isolates was evaluated by the under bark inoculation method described by O'Gara et al. (1997) using Five-month-old *A. heterophyllus* seedlings with stem diameters of 6-8 mm and heights <1.5 m were prepared for pathogenicity test. Seedlings were grown in 10 cm diameter plastic pots containing a soil mix (topsoil + peat + chicken manure) under a 50% shading net. Plants were watered daily to maintain humidity, and any mortality occurring before the end of the experiment was recorded. Wounds were made on the stems of the seedlings using a cork borer (4 mm diam.), and mycelial discs (4 mm diam.) taken from an actively growing colony of *C. fimbriata* on 2% MEA (14 days) (Pratama et al. 2021) were placed in the wounds with the mycelium facing downwards. These were covered with Parafilm (Pechiney, Menasha, Wisconsin) to reduce contamination and desiccation. Ten plants of each tree species were inoculated with sterile MEA plugs to serve as controls (Fig. 4a). Fungal isolates were re-isolated and re-identified using morphological characteristics for Koch's postulates confirmation. In pathogenicity tests, initial symptoms appeared two weeks post-inoculation as brown lesions on the wood of inoculation site (Fig. 4b). Forty-five days after inoculation, plants exhibited wilt symptoms, lesions of wood discoloration extended to heartwood (Fig. 4c) and length (downward + upward) was 17.88 until 34.74 cm. When re-isolated, the fungus was phenotypically identical to the prior isolate of *C. fimbriata* (CAAW31171, CAAW30817, CAAW30268).

This is the first report of *C. fimbriata* causing wilt and die-back in Jackfruit in Indonesia and worldwide. The symptoms of *C. fimbriata* wilt disease in Jackfruit are stems cankers, the

stems become chapped as though torn apart, fruit rot and progressive loss of the canopy resulting in tree death. Jackfruit trees showed typical symptoms of infection by the *Ceratocystis* fungus; the same was true of a serious wilt pathogen of *A. mangium* and *A. crassicarpa* in Indonesia (Tarigan et al. 2011), *Lansium domesticum* in Indonesia (Suwandi et al. 2021) and on Sweet Potato and Pomegranate in China (Li et al. 2016). *Ceratocystis fimbriata* infecting native trees in these countries is serious and could potentially lead to the devastation of important components of the natural biodiversity of Indonesia.

Acknowledgement

This research was funded by PMDSU scholarship with budget year of 2019-2021 according to the Director of Research and Community Service, Directorate of Research and Community Service (DRPM), Directorate General for Research and Development, Ministry of Research, Technology, and Higher Education, Number: 068/SP2H/AMD/LT/DRPM/2020.

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169 **Table 1** *Ceratocystis* isolates considered in the phylogenetic analyses

Species	Haplotype	Isolates no.	Host	Origin	GenBank accession no.	
					ITS	β-tubulin
C. fimbriata	ITS1a	C1418	*Ipomoea batatas*	USA	AY157956	-
	ITS1	C1857	*Ficus carica*	Brazil	HQ157542	-
	ITS1b	CMW4797	*Eucalyptus* sp.	Congo	FJ236733	-
	ITSb	CMW9998	*Eucalyptus* sp.	South Africa	FJ236721	-
	ITS2	C1655	*Mangifera indica*	Brazil	HQ157546	-
	ITS3	C1440	*Eucalyptus* sp.	Brazil	HQ157544	-
	ITS3	CMW5328	*E. grandis*	Uganda	AF395686	-
	ITS4	C1442	*Eucalyptus* sp.	Brazil	HQ157545	-
	ITS5	**CAAW31171**	***Artocarpus heterophyllus***	**Indonesia**	**MT355410**	**MW717653**
	ITS5	**CAAW30268**	***A. heterophyllus***	**Indonesia**	**MT355412**	**MW717655**
	ITS5	**CAAW30817**	***A. heterophyllus***	**Indonesia**	**MT355413**	**MW717656**
	ITS5	CMW38737	*E. grandis*	Zimbabwe	KF878326	KF878335
	ITS5	C1345	*Eucalyptus* sp.	Brazil	AY157966	-
	ITS5	A59662	*Camellia sinensis*	China	KF650948	-
	ITS5	YM061	*Colocasia esculenta*	China	AM712445	-
	ITS5	P20053	*Punica granatum*	China	AM292204	-
	ITS5	C1	*Acacia* sp.	Vietnam	MF033455	MF040712
	ITS5	CMW22563	*A. mangium*	Indonesia	EU588656	EU588636
	ITS5	WRC	*Lansium domesticum*	Indonesia	MT229127	MW013766
	ITS6	C2055	*Mangifera* sp.	Brazil	HQ157548	-
	ITS6z	CMW13582	*Hypocryphalus mangifera*	Oman	KC261853	-
	ITS6z	WBC	*L. domesticum*	Indonesia	MT229128	MW013767
	ITS7b	CMW13851	*M. indica*	Oman	AY953383	EF433308
	ITS7b	CMW23634	*M. indica*	Pakistan	EF433302	EF433311
	ITS7b	CMW22579	*A. mangium*	Indonesia	EU588658	-
	ITS8a	CMW8856	*Citrus* sp.	Colombia	AY233867	-
	ITS8c	CMW17808	*Eucalyptus* sp	Colombia	EF127990	-
	ITS8e	CMW22092	*E. deglupta*	Ecuador	FJ151432	-
	ITS9	C1558	*M. indica*	Brazil	AY157965	-
	ITS9	C1914	*C. esculenta*	Brazil	HQ157540	-
	ITS10	C994	*M. indica*	Brazil	AY157964	-
	ITS10a	Cf4	*M. indica*	Brazil	EF042605	-
	ITS11	C1865	*C. esculenta*	Brazil	AY526286	-
	ITS12	C1926	*C. esculenta*	Brazil	HQ157541	-
	ITS14	C1688	*M. indica*	Brazil	AY526291	-
	ITS15	C925	*Gmelina arborea*	Brazil	AY157967	-
	ITS16	C924	*G. arborea*	Brazil	HQ157539	-
C. pirilliformis	Asian clade (AC)	CMW6569	*E. nitens*	Australia	-	DQ371652
	AC	CMW6579	*E. nitens*	Australia	-	DQ371653
C. polychroma	AC	CMW11424	*Syzygium aromaticum*	Indonesia	-	AY528966
	AC	CMW11436	*S. aromaticum*	Indonesia	-	AY528967
C. atrox	AC	CMW19383	*E. grandis*	Australia	-	EF070430
	AC	CMW19385	*E. grandis*	Australia	-	EF070431
C. neglecta	Latin American clade (LAC)	CMW17808	*E. grandis*	Colombia	-	EU881898
	LAC	CMW18194	*E. grandis*	Colombia	-	EU881899
C. colombiana	LAC	CMW5751	*Coffea arabica*	Colombia	-	AY177225
	LAC	CMW5761	*C. arabica*	Colombia	-	AY177224
C. cacaofunesta	LAC	CMW14803	*Theobroma cacao*	Ecuador	-	KJ631108
	LAC	CMW15051	*T. cacao*	Costa Rica	-	KJ601510
C. papillata	LAC	CMW8850	*Citrus* × *Tangelo hybrid*	Colombia	-	AY233875
	LAC	CMW8856	*Citrus limon*	Colombia	-	AY233874
C. fimbriata	LAC	CMW14797	*M. indica*	Brazil	-	EF433307
	LAC	CMW28907	*M. indica*	Brazil	-	FJ200270

	LAC	CMW1547	*I. batatas*	Papua New Guinea	-	EF070443
	LAC	C1421	*I. batatas*	USA	-	KF302689
C. fimbriatomima	LAC	CMW24174	*Eucalyptus hybrid*	Venezuela	-	EF190951
	LAC	CMW24176	*Eucalyptus hybrid*	Venezuela	-	EF190952
C. fimbriata	LAC	CMW21127	*A. crassicarpa*	Indonesia	-	EU588643
	LAC	CMW24664	*Eucalyptus hybrid*	China	-	JQ862720
	LAC	CBS115173	*Gmelina arborea*	Brazil	-	KF302700
	LAC	CBS14653	*C. arabica*	Suriname	-	KF302702
C. platani	LAC	CMW14802	*Platanus occidentalis*	USA	-	EF070425
	LAC	CMW23450	*P. occidentalis*	Greece	-	KJ601513

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Colour figure

Fig. 1 Symptoms of *Ceratocystis fimbriata* wilt disease in *Artocarpus heterophyllus:* **a**. vascular discoloration of infected tree; **b.** The discolored wood extended to the heartwood of the basal stem; **c.** three-year-old tree with wilted, yellowing leaves and rotten fruit; **d.** isolation of the fungus from discoloured xylem showing dark mycelium and sporulation on the carrot slices after 7 days.

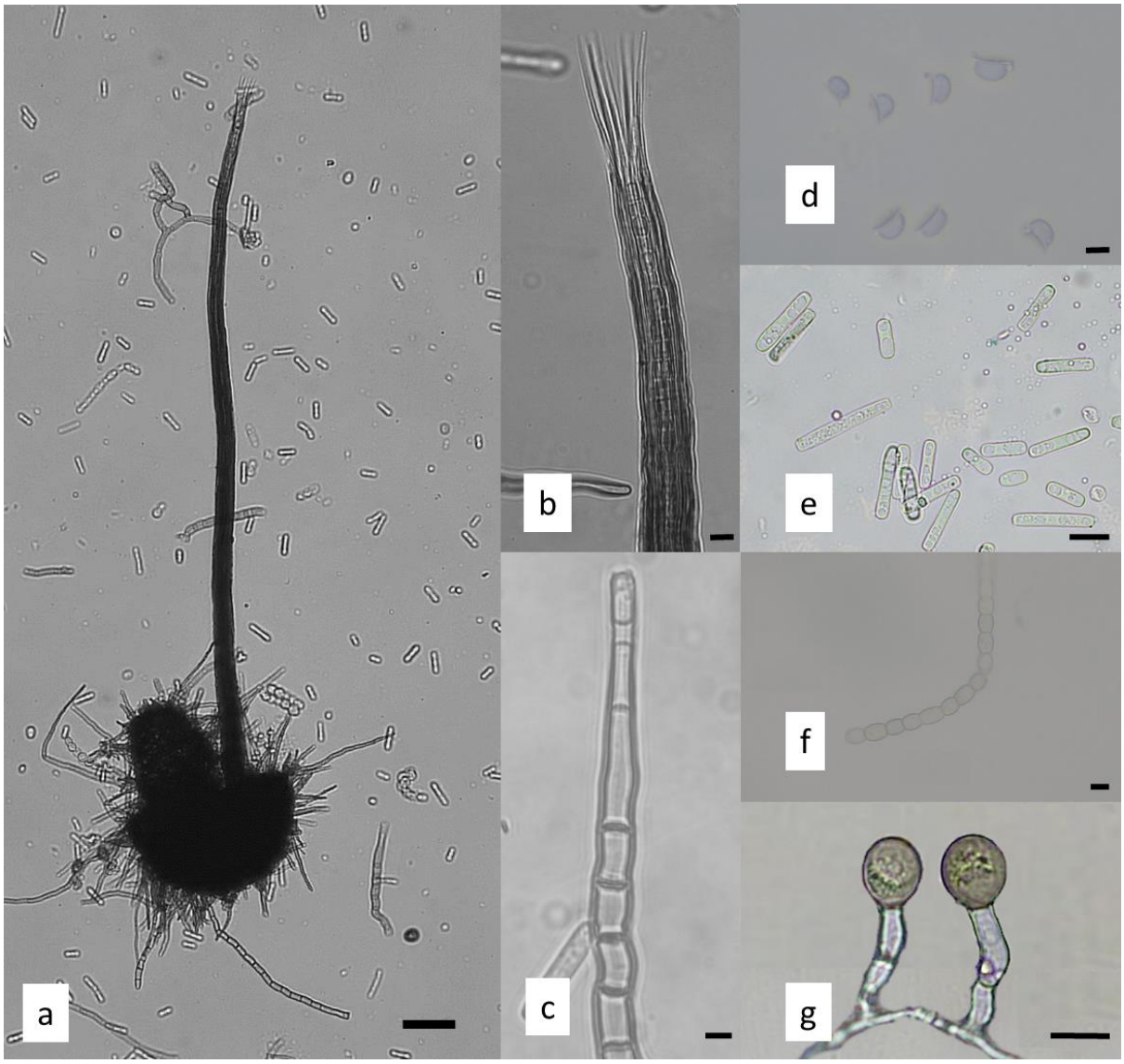

Fig. 2. Morphological characteristics of *Ceratocystis fimbriata* isolated from *Artocarpus heterophyllus* stem lesion: **a.** ascomata with pirilliform base, **b.** divergent ostiolar hyphae; **c.** conidiophore/phialide; **d.** hat-shaped ascospores; **e.** cylindrical conidia; **f.** Chain of barrel-shaped conidia; **g.** chlamydospores of various shapes. Scale bars: a = 100 µm; b–c, e–g = 10 µm; d = 5 µm.

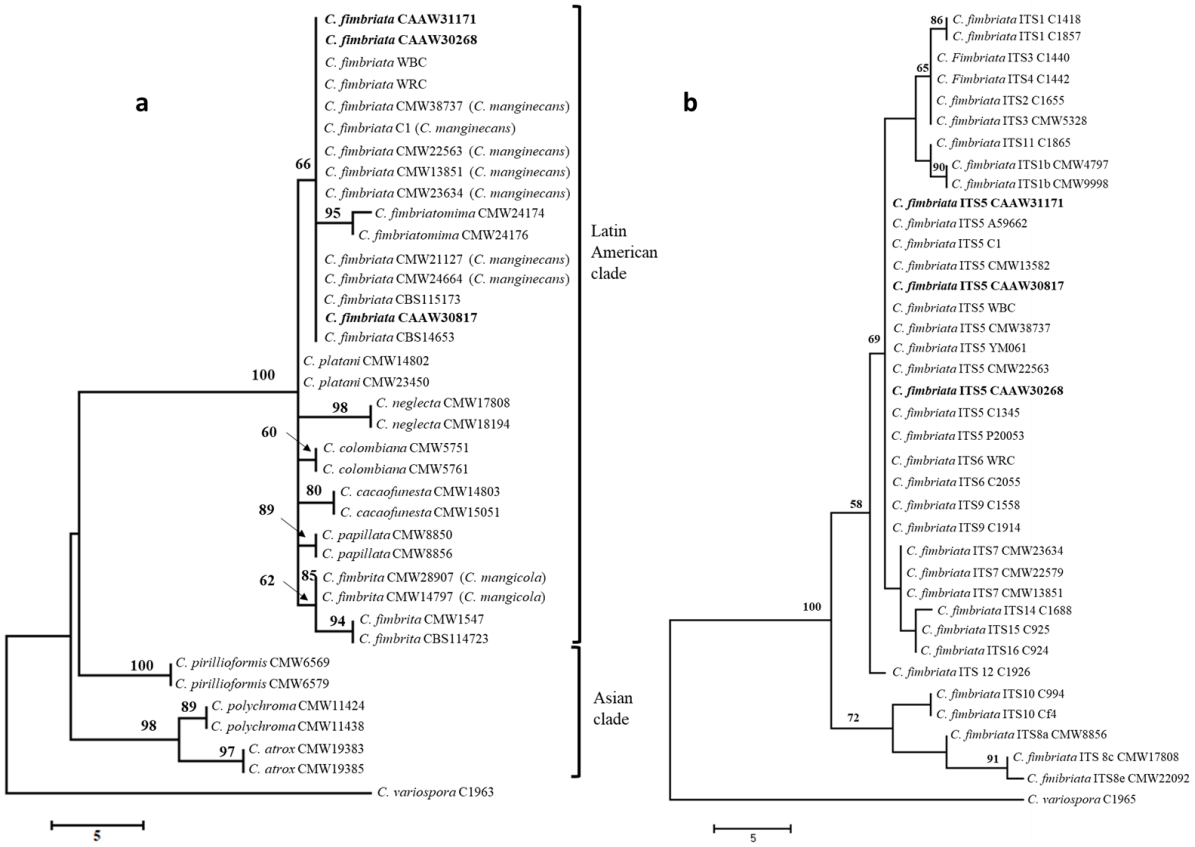

Fig. 3 Phylogenetic tree constructed by MEGA with Maximum Parsimony (MP) analysis by **a**. β-tubulin sequences from Jackfruit tree in Indonesia (marked in bold) and other species in the Latin American and Asian clade of the *C. fimbriata* species complex. **b.** ITS sequences from Jackfruit tree in Indonesia (marked in bold) and genotypes (sequences) of the *C. fimbriata sensu stricto*.

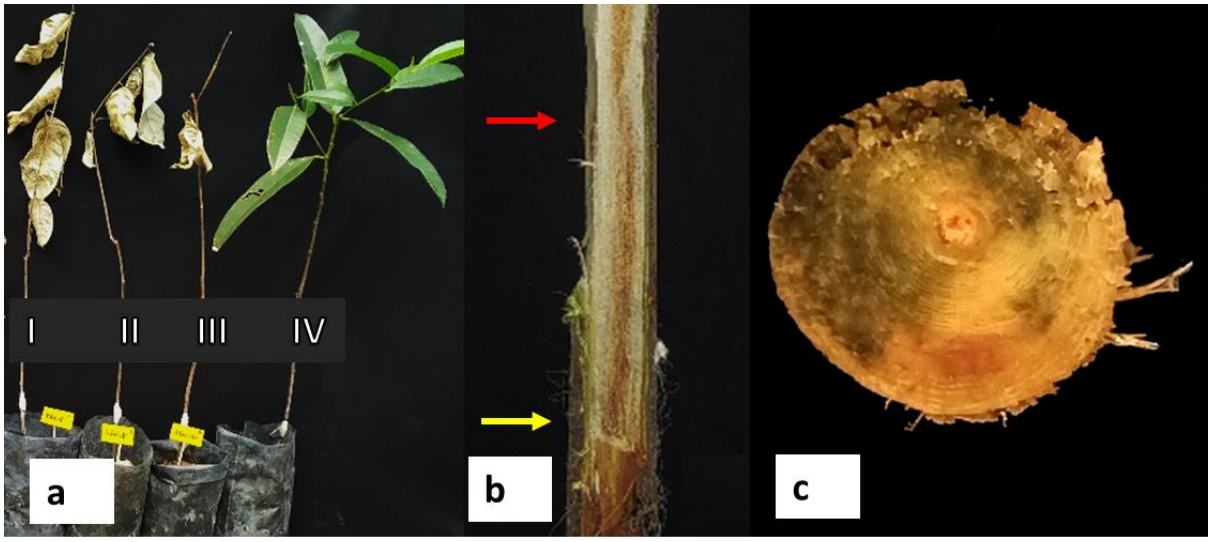

Fig. 4 Response after 45 days of *Artocarpus heterophyllus* seedlings to under-bark inoculation with mycelium of *Ceratocystis*. **a.** total wilting of plant inoculated with CAAW31171 (I), CAAW30817 (II), CAAW30268 (III) and the control seedling appeared healthy (IV); **b.** yellow arrow indicates the point of inoculation and red arrow the lesion boundary; **c.** The discoloured seedlings wood extended to the heartwood of the basal stem.

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1 message

APDN <em@editorialmanager.com> Mon, Jun 14, 2021 at 11:29 PM
Reply-To: APDN <jude.estrera@springernature.com>
To: "A. Muslim" <a_muslim@unsri.ac.id>

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Your Submission APDN-D-21-00015R3

3 messages

APDN <em@editorialmanager.com> Fri, Jun 18, 2021 at 12:59 AM
Reply-To: APDN <jude.estrera@springernature.com>
To: "A. Muslim" <a_muslim@unsri.ac.id>

CC: dagmar.hanold@adelaide.edu.au, dhanold@gmail.com

Dear Dr. Muslim,

We have received the reports from our advisors on your manuscript, 'Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia' (APDN-D-21-00015R3), submitted to Australasian Plant Disease Notes.

Based on the advice received, I have decided that your manuscript can be accepted for publication after you have carried out the corrections as suggested by the reviewer(s).

Below, please find the reviewers' comments for your perusal.
You are kindly requested to also check the website for possible reviewer attachment(s).

Please submit your revised manuscript online by using
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With kind regards,

Eduardo Guatimosim, PhD
Associate Editor

COMMENTS FOR THE AUTHOR:

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Thank you for submitting the reviewed manuscript.
However, I do not think you understood the question raised regarding the submission into a culture collection.
As postulated in the guidelines, authors must provide the culture collection information and their accession numbers, of a culture collection registered on WFCC, within the text. Without these informations, your manuscript cannot be further processed.
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a. muslim unsri <a_muslim@unsri.ac.id> Tue, Jun 29, 2021 at 3:38 PM
To: APDN <jude.estrera@springernature.com>

Dear Eduardo Guatimosim, PhD
Associate Editor
Australasian Plant Disease Notes

Thank you very much for corrections to reviewers' comments of our manuscript. We are really appreciating the corrections. We have revised and make some modified the corrections as suggested by the reviewer(s)

Here, we enclose revised version of the manuscript No. APDN-D-21-00015R1 entitled "Jackfruit (*Artocarpus heterophyllus*), a New Host Plant of *Ceratocystis* Wilt from South Sumatra, Indonesia" by Rahmat Pratama, Ahmad Muslim, Suwandi Suwandi, Nurhayati Damiri, Soleha Soleha.

Below is a summary of our response to the reviewers' comments.

Comment [1]: authors must provide the culture collection information and their accession numbers, of a culture collection registered on WFCC, within the text. please add the name of the collection at lines 45-46, and replace the private collection codes in the text, tables and figures (trees included).
Our response: We agree and specimens were deposited in the ICBB Culture Collection for Microorganisms and Cell Culture, Indonesian Center for Biodiversity and Biotechnology, (Bogor, Indonesia) as ICBB9852 and ICBB9853. Our private collection codes replaced in the text, tables and figures (trees included).

We feel that these changes have adequately addressed the comments and suggestions of reviewer(s). Please feel free to contact me if you need any additional information or clarification.

Thank you very much for your consideration of the manuscript and excellent cooperation

Yours sincerely,

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a. muslim unsri <a_muslim@unsri.ac.id> Wed, Jul 14, 2021 at 9:26 AM
To: APDN <jude.estrera@springernature.com>
Cc: dagmar.hanold@adelaide.edu.au, dhanold@gmail.com

Dear Eduardo Guatimosim, PhD
Associate Editor
Australasian Plant Disease Notes

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Thank you very much for your consideration of the manuscript and excellent cooperation

Yours sincerely,

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On Fri, Jun 18, 2021 at 1:00 AM APDN <em@editorialmanager.com> wrote:
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DRAFT PERBAIKAN

Australasian Plant Disease Notes

Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia

--Manuscript Draft--

Manuscript Number:	APDN-D-21-00015R4
Full Title:	Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia
Article Type:	Plant Disease Note
Keywords:	Sudden death disease; Moraceae; Ceratocystis fimbriata sensu stricto
Corresponding Author:	A. Muslim, Ph.D. Universitas Sriwijaya Fakultas Pertanian Palembang, Sumatera Selatan INDONESIA
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Funding Information:	Kementerian Riset Teknologi Dan Pendidikan Tinggi Republik Indonesia (068/SP2H/AMD/LT/DRPM/2020) — Dr. A. Muslim
Abstract:	In 2019, wilt and sudden death were observed on Artocarpus heterophyllus (jackfruit) has been noted. Identification was performed by sequence analysis of the concatenated β-tubulin and ITS gene regions. Sequencing of the PCR product confirmed this pathogen was Ceratocystis fimbriata sensu stricto . This is the first report of C. fimbriata causing sudden death disease in A . heterophyllus in Indonesia and worldwide.
Response to Reviewers:	June 28, 2021 Dear Eduardo Guatimosim, PhD Associate Editor Australasian Plant Disease Notes Thank you very much for corrections to reviewers' comments of our manuscript. We are really appreciating the corrections. We have revised and make some modified the corrections as suggested by the reviewer(s) Here, we enclose revised version of the manuscript No. APDN-D-21-00015R1 entitled “Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia" by Rahmat Pratama, Ahmad Muslim, Suwandi Suwandi, Nurhayati Damiri, Soleha Soleha. Below is a summary of our response to the reviewers' comments.

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Jackfruit (*Artocarpus heterophyllus*), a New Host Plant of *Ceratocystis* Wilt in South Sumatra, Indonesia

Abstract

In 2019, wilt and sudden death were observed on *Artocarpus heterophyllus* (jackfruit) has been noted. Identification was performed by sequence analysis of the concatenated β-tubulin and ITS gene regions. Sequencing of the PCR product confirmed this pathogen was *Ceratocystis fimbriata sensu stricto*. This is the first report of *C. fimbriata* causing sudden death disease in *A. heterophyllus* in Indonesia and worldwide.

Keywords: Sudden death disease · Moraceae · *Ceratocystis fimbriata sensu stricto* ·

Jackfruit (*Artocarpus heterophyllus*, Moraceae) is known in Indonesian as "Nangka", and is cultivated widely in many countries with tropical and subtropical climates. Jackfruit is among the most exported fruits worldwide and has considerable nutrition and health benefits (Ranasinghe et al. 2019).

In July 2019, wilt and die-back symptoms were observed for the first time on *A. heterophyllus* in the agricultural field of Sriwijaya University (Indralaya), Plaju (Palembang) and Gelumbang (Prabumulih), Indonesia. Wood of wilted trees showed a brown to black streaking in the woody xylem. Symptoms on the dying Jackfruit wood produced grey to brown lesions and included a streaking pattern of discoloration in the sapwood (Fig. 1a) and in some cases the lesions extended to heartwood (Fig. 1b). The lesion could be found partially or totally affected the sapwood from the basal stem until the branches. Leaves of dying trees had yellowing symptoms, followed by the wilting of the leaves on several lateral branches, drying of twigs and the wilt of the whole tree (Fig. 1c). This type of wilting was termed as sudden death or wilt (Pratama et al. 2021).

Wood samples were taken from lesions of wilted trees using a knife sterilised in 70% ethanol. Each sample was wrapped in tissue paper and placed in a cool box. The same day, the wood samples (1–20 mm length, 1–2 mm thick) were sandwiched between two slices of fresh carrot and placed on sterile dry paper in plastic boxes at 25 °C following the method of Moller and DeVay (1968) (Fig. 1d). After 5–10 days, hat-shaped spores of putative *Ceratocystis* pathogens were placed on 2% (w/v) malt extract agar (MEA) (Merck, Germany), and incubated at 25 °C in a laboratory. The isolated fungi were initially identified based on morphological characteristics of a 14 day old culture. Mycelium on MEA grey, reverse side of colony

olivaceous grey; submerged mycelium darkening as the ascomata develop forming fine, radiating fibrils.

Morphological traits of fruiting bodies and spores were observed under an optical Olympus CX33 microscope. Ascomatal bases dark brown to black, base subglobose to globose and measured (n=100), 131.5–250.7×101.6–236.5 µm (Fig. 2a). Ascomata necks erect, occasionally curved, black at the base becoming subhyaline towards the apex, smooth to crenulate, 324.7–579.1 µm long including ostiolar hyphae (Fig. 2b). Phialides pale brown to hyaline (Fig. 2c). Ascospores hat-shaped, 3.4–6.8×2.1–6.2 µm (Fig. 2d). Bacilliform conidia 11.1–36.1×2.1–7.4 µm (Fig. 2e). Barrel conidia 4.4–16.1×2.7–6.9 µm (Fig. 2f). Chlamydospores oval, thick walled, smooth, 6.7–16.5×5.9–12.9 µm (Fig. 2g). Based on morphological characters, the fungus was identified as *Ceratocystis fimbriata*. Two representative isolates were deposited at the ICBB Culture Collection for Microorganisms and Cell Culture, Indonesian Center for Biodiversity and Biotechnology, (Bogor, Indonesia) as ICBB9852 and ICBB9853.

To confirm the species identification, isolates were cultured on potato dextrose broth (PDB) at room temperature for one week. Mycelium was filtered through Whatman filter paper and genomic DNA was extracted from fungal mycelial mat using YeaStar Genomic DNA Kit (Zymo Research Corporation, California, USA). PCR conditions and reactions for two gene regions were used to identify the *Ceratocystis* isolates; the Internal Transcribed Spacer (ITS) with primers ITS 1 and ITS4 (White et al. 1990) and part of the β-tubulin (βt) gene with primers βt1a and βt1b (Glass and Donaldson 1995). Amplifications were carried out in 50 µl reactions containing 20 µl DreamTaq Green PCR Master Mix (Eppendorf, Germany) (DreamTaq DNA Polymerase, 2X DreamTaq Green buffer, dNTPs, and 4 mM $MgCl_2$), 1,5 µl of each forward and reverse primer, 4 µl of DNA template and 23µl sterilised water. The PCRs were performed with a C1000 Touch™ thermal cycler (Bio-Rad, USA). The PCR cycling parameters were as follows: initial denaturation for 5 min at 95 °C, followed by 35 cycles at 95 °C for 30 s, 56 °C for 45 s and 72 °C for 1 min. Amplification was completed at 72 °C for 10 min and the PCR product was stored at 10 °C. The PCR amplicons were sequenced at 1st BASE (Malaysia).

For the ITS and β-tubulin, amplification resulted in fragments of ~550 base pairs (bp) in size. The sequences of the amplified products were then deposited in the GenBank database and assigned accession numbers isolate ICBB9852 (MT355410; MT412106), isolate ICBB9853 (MT355412; MT412108), and isolate CAAW30817 (MT355413, MT412109) for the ITS and β-tubulin. β-tubulin datasets were generated using ex-type and ex-paratype

sequences representing species in the Latin American (LAC) and Asian clade (AC) of the *C. fimbriata* species complex (Fourie et al. 2015; Oliveira et al. 2015; Barnes et al. 2018). To determine relatedness of isolates from jackfruit with known *C. fimbriata* populations, the ITS sequence was manually aligned with known ITS haplotypes as designated by Harrington et al. (2014); Li et al. (2016) and phylogenetic analyses were performed. Maximum Parsimony (MP) analyses were performed in MEGA v. 10 (Kumar et al. 2016; Paul et al. 2018) with 1000 replications. The analysis involved 38 (β-tubulin) and 37 (ITS) nucleotide sequences. All positions containing gaps and missing data were eliminated. There were 408 (β-tubulin) and 518 (ITS) positions in the final dataset. *Ceratocystis variospora* was used as the out-group. β-tubulin sequence of our isolates confirmed the assignment to LAC of *C. fimbriata sensu lato* (Fig. 3a). Manual alignment of the ITS sequences with previously described ITS genotypes (Harrington et al. 2014; Li et al. 2016) grouped the isolates into ITS5 haplotype of *C. fimbriata sensu stricto* (Fig. 3b). Consistency (CI), retention (RI), and composite indexes (CoI) for β-tubulin were 0.566667, 0.845238, 0.668011, respectively and ITS was 0.933333, 0.976563, 0.932836, respectively.

The pathogenic potential of isolates was evaluated by the under bark inoculation method described by O'Gara et al. (1997) using Five-month-old *A. heterophyllus* seedlings with stem diameters of 6-8 mm and heights <1.5 m were prepared for pathogenicity test. Seedlings were grown in 10 cm diameter plastic pots containing a soil mix (topsoil + peat + chicken manure) under a 50% shading net. Plants were watered daily to maintain humidity, and any mortality occurring before the end of the experiment was recorded. Wounds were made on the stems of the seedlings using a cork borer (4 mm diam.), and mycelial discs (4 mm diam.) taken from an actively growing colony of *C. fimbriata* on 2% MEA (14 days) (Pratama et al. 2021) were placed in the wounds with the mycelium facing downwards. These were covered with Parafilm (Pechiney, Menasha, Wisconsin) to reduce contamination and desiccation. Ten plants of each tree species were inoculated with sterile MEA plugs to serve as controls (Fig. 4a). Fungal isolates were re-isolated and re-identified using morphological characteristics for Koch's postulates confirmation. In pathogenicity tests, initial symptoms appeared two weeks post-inoculation as brown lesions on the wood of inoculation site (Fig. 4b). Forty-five days after inoculation, plants exhibited wilt symptoms, lesions of wood discoloration extended to heartwood (Fig. 4c) and length (downward + upward) was 17.88 until 34.74 cm. When re-isolated, the fungus was phenotypically identical to the prior isolate of *C. fimbriata* (ICBB9852, ICBB9853, CAAW30817).

This is the first report of *C. fimbriata* causing wilt and die-back in Jackfruit in Indonesia and worldwide. The symptoms of *C. fimbriata* wilt disease in Jackfruit are stems cankers, the stems become chapped as though torn apart, fruit rot and progressive loss of the canopy resulting in tree death. Jackfruit trees showed typical symptoms of infection by the *Ceratocystis* fungus; the same was true of a serious wilt pathogen of *A. mangium* and *A. crassicarpa* in Indonesia (Tarigan et al. 2011), *Lansium domesticum* in Indonesia (Suwandi et al. 2021) and on Sweet Potato and Pomegranate in China (Li et al. 2016). *Ceratocystis fimbriata* infecting native trees in these countries is serious and could potentially lead to the devastation of important components of the natural biodiversity of Indonesia.

Acknowledgement

This research was funded by PMDSU scholarship with budget year of 2019-2021 according to the Director of Research and Community Service, Directorate of Research and Community Service (DRPM), Directorate General for Research and Development, Ministry of Research, Technology, and Higher Education, Number: 068/SP2H/AMD/LT/DRPM/2020.

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Table

Table 1 *Ceratocystis* isolates considered in the phylogenetic analyses

Species	Haplotype	Isolates no.	Host	Origin	GenBank accession no.	
					ITS	β-tubulin
C. fimbriata	ITS1a	C1418	*Ipomoea batatas*	USA	AY157956	-
	ITS1	C1857	*Ficus carica*	Brazil	HQ157542	-
	ITS1b	CMW4797	*Eucalyptus* sp.	Congo	FJ236733	-
	ITSb	CMW9998	*Eucalyptus* sp.	South Africa	FJ236721	-
	ITS2	C1655	*Mangifera indica*	Brazil	HQ157546	-
	ITS3	C1440	*Eucalyptus* sp.	Brazil	HQ157544	-
	ITS3	CMW5328	*E. grandis*	Uganda	AF395686	-
	ITS4	C1442	*Eucalyptus* sp.	Brazil	HQ157545	-
	ITS5	**ICBB9852**	***Artocarpus heterophyllus***	**Indonesia**	**MT355410**	**MT412106**
	ITS5	**ICBB9853**	***A. heterophyllus***	**Indonesia**	**MT355412**	**MT412108**
	ITS5	**CAAW30817**	***A. heterophyllus***	**Indonesia**	**MT355413**	**MT412109**
	ITS5	CMW38737	*E. grandis*	Zimbabwe	KF878326	KF878335
	ITS5	C1345	*Eucalyptus* sp.	Brazil	AY157966	-
	ITS5	A59662	*Camellia sinensis*	China	KF650948	-
	ITS5	YM061	*Colocasia esculenta*	China	AM712445	-
	ITS5	P20053	*Punica granatum*	China	AM292204	-
	ITS5	C1	*Acacia* sp.	Vietnam	MF033455	MF040712
	ITS5	CMW22563	*A. mangium*	Indonesia	EU588656	EU588636
	ITS5	WRC	*Lansium domesticum*	Indonesia	MT229127	MW013766
	ITS6	C2055	*Mangifera* sp.	Brazil	HQ157548	-
	ITS6z	CMW13582	*Hypocryphalus mangifera*	Oman	KC261853	-
	ITS6z	WBC	*L. domesticum*	Indonesia	MT229128	MW013767
	ITS7b	CMW13851	*M. indica*	Oman	AY953383	EF433308
	ITS7b	CMW23634	*M. indica*	Pakistan	EF433302	EF433311
	ITS7b	CMW22579	*A. mangium*	Indonesia	EU588658	-
	ITS8a	CMW8856	*Citrus* sp.	Colombia	AY233867	-
	ITS8c	CMW17808	*Eucalyptus* sp	Colombia	EF127990	-
	ITS8e	CMW22092	*E. deglupta*	Ecuador	FJ151432	-
	ITS9	C1558	*M. indica*	Brazil	AY157965	-
	ITS9	C1914	*C. esculenta*	Brazil	HQ157540	-
	ITS10	C994	*M. indica*	Brazil	AY157964	-
	ITS10a	Cf4	*M. indica*	Brazil	EF042605	-
	ITS11	C1865	*C. esculenta*	Brazil	AY526286	-
	ITS12	C1926	*C. esculenta*	Brazil	HQ157541	-
	ITS14	C1688	*M. indica*	Brazil	AY526291	-
	ITS15	C925	*Gmelina arborea*	Brazil	AY157967	-
	ITS16	C924	*G. arborea*	Brazil	HQ157539	-
C. pirilliformis	Asian clade (AC)	CMW6569	*E. nitens*	Australia	-	DQ371652
	AC	CMW6579	*E. nitens*	Australia	-	DQ371653
C. polychroma	AC	CMW11424	*Syzygium aromaticum*	Indonesia	-	AY528966
	AC	CMW11436	*S. aromaticum*	Indonesia	-	AY528967
C. atrox	AC	CMW19383	*E. grandis*	Australia	-	EF070430
	AC	CMW19385	*E. grandis*	Australia	-	EF070431
C. neglecta	Latin American clade (LAC)	CMW17808	*E. grandis*	Colombia	-	EU881898
	LAC	CMW18194	*E. grandis*	Colombia	-	EU881899
C. colombiana	LAC	CMW5751	*Coffea arabica*	Colombia	-	AY177225
	LAC	CMW5761	*C. arabica*	Colombia	-	AY177224
C. cacaofunesta	LAC	CMW14803	*Theobroma cacao*	Ecuador	-	KJ631108
	LAC	CMW15051	*T. cacao*	Costa Rica	-	KJ601510
C. papillata	LAC	CMW8850	*Citrus* × *Tangelo hybrid*	Colombia	-	AY233875
	LAC	CMW8856	*Citrus limon*	Colombia	-	AY233874
C. fimbriata	LAC	CMW14797	*M. indica*	Brazil	-	EF433307
	LAC	CMW28907	*M. indica*	Brazil	-	FJ200270

	LAC	CMW1547	*I. batatas*	Papua New Guinea	-	EF070443
	LAC	C1421	*I. batatas*	USA	-	KF302689
C. fimbriatomima	LAC	CMW24174	*Eucalyptus hybrid*	Venezuela	-	EF190951
	LAC	CMW24176	*Eucalyptus hybrid*	Venezuela	-	EF190952
C. fimbriata	LAC	CMW21127	*A. crassicarpa*	Indonesia	-	EU588643
	LAC	CMW24664	*Eucalyptus hybrid*	China	-	JQ862720
	LAC	CBS115173	*Gmelina arborea*	Brazil	-	KF302700
	LAC	CBS14653	*C. arabica*	Suriname	-	KF302702
C. platani	LAC	CMW14802	*Platanus occidentalis*	USA	-	EF070425
	LAC	CMW23450	*P. occidentalis*	Greece	-	KJ601513

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Colour figure

Fig. 1 Symptoms of *Ceratocystis fimbriata* wilt disease in *Artocarpus heterophyllus:* **a**. vascular discoloration of infected tree; **b.** The discolored wood extended to the heartwood of the basal stem; **c.** three-year-old tree with wilted, yellowing leaves and rotten fruit; **d.** isolation of the fungus from discoloured xylem showing dark mycelium and sporulation on the carrot slices after 7 days.

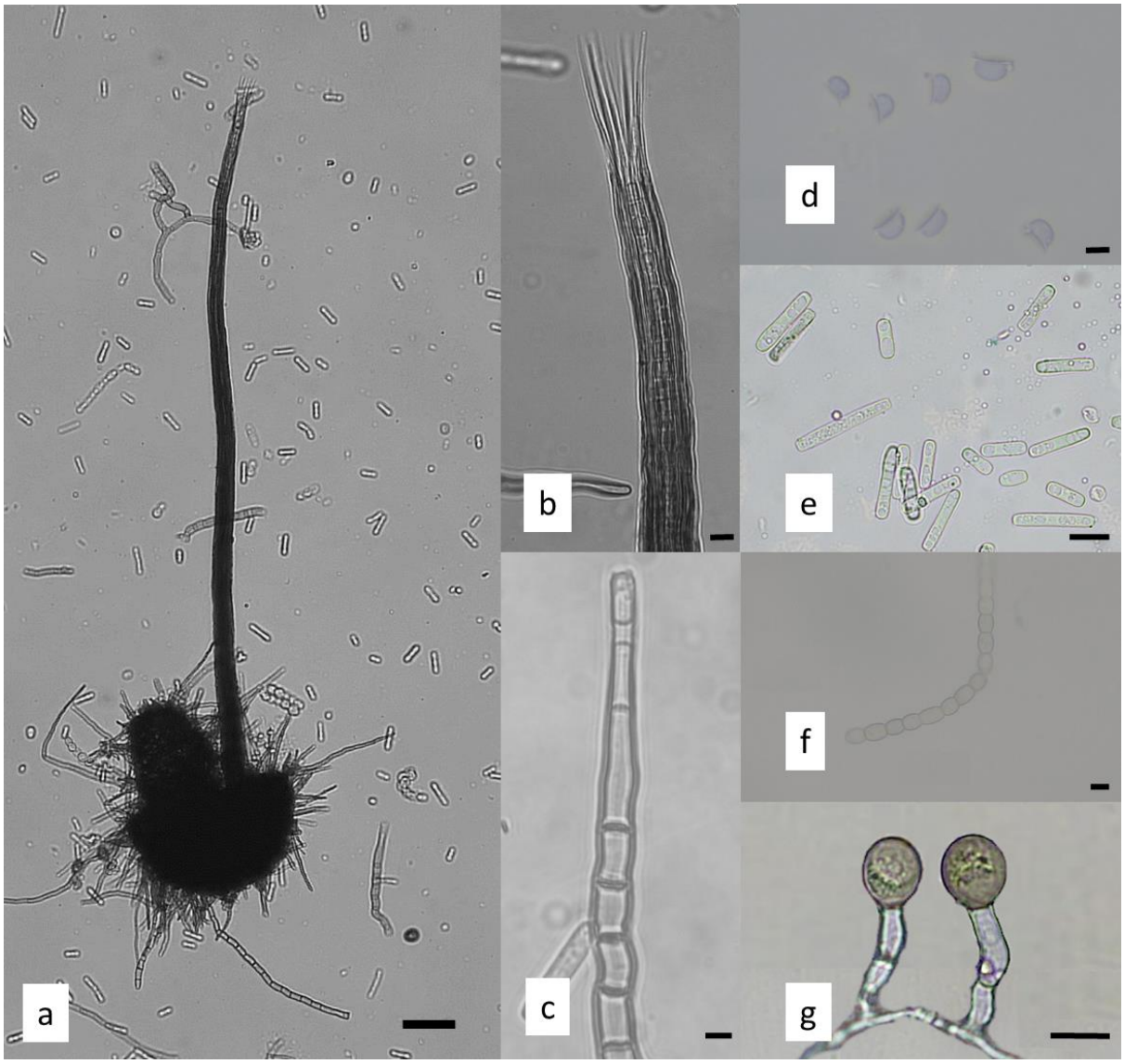

Fig. 2. Morphological characteristics of *Ceratocystis fimbriata* isolated from *Artocarpus heterophyllus* stem lesion: **a.** ascomata with pirilliform base, **b.** divergent ostiolar hyphae; **c.** conidiophore/phialide; **d.** hat-shaped ascospores; **e.** cylindrical conidia; **f.** Chain of barrel-shaped conidia; **g.** chlamydospores of various shapes. Scale bars: a = 100 µm; b–c, e–g = 10 µm; d = 5 µm.

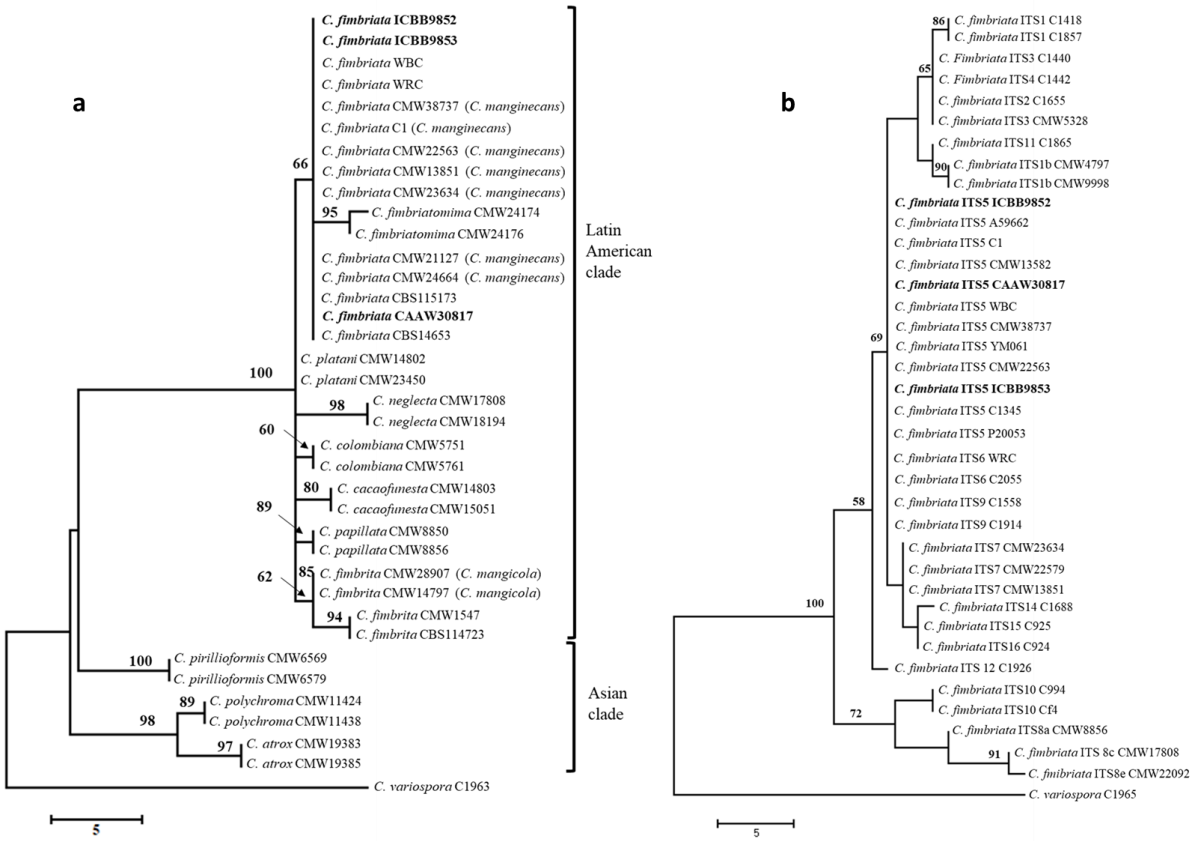

Fig. 3 Phylogenetic tree constructed by MEGA with Maximum Parsimony (MP) analysis by **a**. β-tubulin sequences from Jackfruit tree in Indonesia (marked in bold) and other species in the Latin American and Asian clade of the *C. fimbriata* species complex. **b.** ITS sequences from Jackfruit tree in Indonesia (marked in bold) and genotypes (sequences) of the *C. fimbriata sensu stricto*.

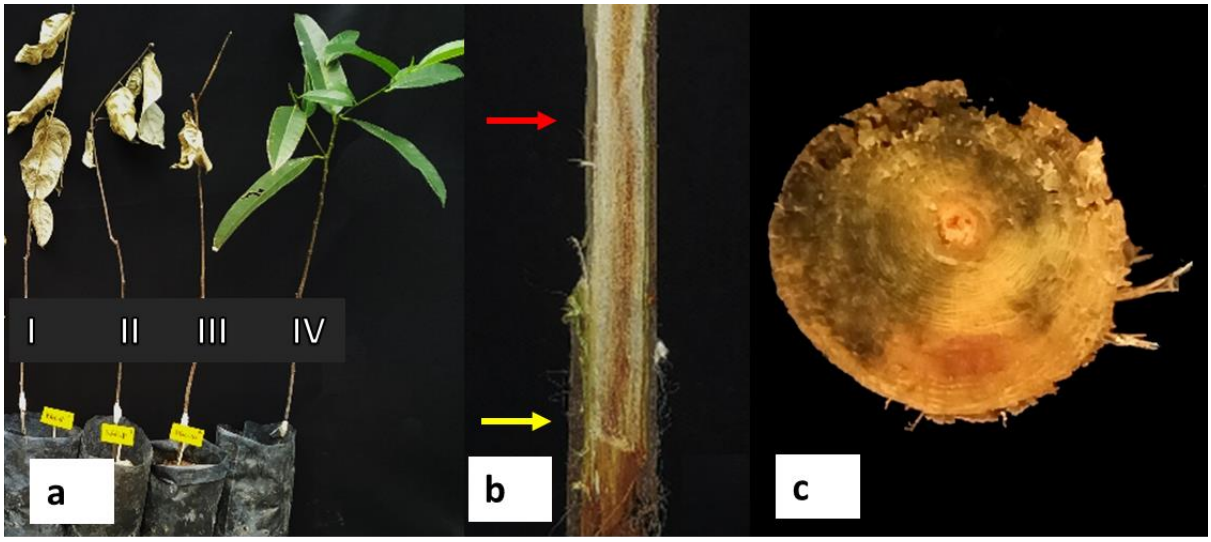

Fig. 4 Response after 45 days of *Artocarpus heterophyllus* seedlings to under-bark inoculation with mycelium of *Ceratocystis.* **a.** total wilting of plant inoculated with ICBB9852 (I), CAAW30817 (II), ICBB9853 (III) and the control seedling appeared healthy (IV); **b.** yellow arrow indicates the point of inoculation and red arrow the lesion boundary; **c.** The discoloured seedlings wood extended to the heartwood of the basal stem.

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Re: Your Submission APDN-D-21-00015R3 [APDN] [AU] [REVSUB] [R]

1 message

Jude Estrera <Jude.Estrera@springernature.com> Wed, Jul 14, 2021 at 11:47 PM
To: "a. muslim unsri" <a_muslim@unsri.ac.id>
Cc: "dagmar.hanold@adelaide.edu.au" <dagmar.hanold@adelaide.edu.au>, "dhanold@gmail.com" <dhanold@gmail.com>

Dear Dr. Muslim,

Thank you for your email.

This is to confirm that your response to review comments has been uploaded accordingly in the EM. Your paper has been assigned to the Editor and rest assured that it will be process accordingly.

Should you have further concerns, please feel free to let me know.

Kind Regards,

Jude Estrera
(he/him/his)
JEO Assistant
Journals Editorial Office (JEO)

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From: a. muslim unsri <a_muslim@unsri.ac.id>
Sent: Wednesday, July 14, 2021 10:26 AM
To: Jude Estrera <Jude.Estrera@springernature.com>
Cc: dagmar.hanold@adelaide.edu.au <dagmar.hanold@adelaide.edu.au>; dhanold@gmail.com <dhanold@gmail.com>
Subject: Re: Your Submission APDN-D-21-00015R3

[External - Use Caution]

Dear Eduardo Guatimosim, PhD
Associate Editor
Australasian Plant Disease Notes

Thank you very much for corrections to reviewers' comments of our manuscript. We are really appreciating the corrections. We have revised and make some modified the corrections as suggested by the reviewer(s)

Here, we enclose revised version of the manuscript No. APDN-D-21-00015R1 entitled “Jackfruit (*Artocarpus heterophyllus*), a New Host Plant of *Ceratocystis* Wilt from South Sumatra, Indonesia" by Rahmat Pratama, Ahmad Muslim, Suwandi Suwandi, Nurhayati Damiri, Soleha Soleha.

Below is a summary of our response to the reviewers' comments.

Comment [1]: authors must provide the culture collection information and their accession numbers, of a culture collection registered on WFCC, within the text. please add the name of the collection at lines 45-46, and replace the private collection codes in the text, tables and figures (trees included).
Our response: We agree and specimens were deposited in the ICBB Culture Collection for Microorganisms and Cell Culture, Indonesian Center for Biodiversity and Biotechnology, (Bogor, Indonesia) as ICBB9852 and ICBB9853. Our private collection codes replaced in the text, tables and figures (trees included).

We feel that these changes have adequately addressed the comments and suggestions of reviewer(s). Please feel free to contact me if you need any additional information or clarification.

Thank you very much for your consideration of the manuscript and excellent cooperation

Yours sincerely,

Ahmad Muslim
Associate Professor
Faculty of Agriculture, Sriwijaya University
Jl. Palembang-Prabumulih Km.32, Indralaya, Palembang, Indonesia
E-mail: a_muslim@unsri.ac.id

On Fri, Jun 18, 2021 at 1:00 AM APDN <em@editorialmanager.com> wrote:

CC: dagmar.hanold@adelaide.edu.au, dhanold@gmail.com

Dear Dr. Muslim,

We have received the reports from our advisors on your manuscript, 'Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia' (APDN-D-21-00015R3), submitted to Australasian Plant Disease Notes.

Based on the advice received, I have decided that your manuscript can be accepted for publication after you have carried out the corrections as suggested by the reviewer(s).

Below, please find the reviewers' comments for your perusal.
You are kindly requested to also check the website for possible reviewer attachment(s).

Please submit your revised manuscript online by using
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Please submit you revised manuscript before 15 Jul 2021 or request an extension of the deadline. If we do not hear from you by then, the manuscript will be automatically withdrawn.

With kind regards,

Eduardo Guatimosim, PhD
Associate Editor

COMMENTS FOR THE AUTHOR:

Dear authors
Thank you for submitting the reviewed manuscript.
However, I do not think you understood the question raised regarding the submission into a culture collection.
As postulated in the guidelines, authors must provide the culture collection information and their accession numbers, of a culture collection registered on WFCC, within the text. Without these informations, your manuscript cannot be further processed.
When you get the accession codes of InaCC, please add the name of the collection at lines 45-46, and replace the private collection codes by those of InaCC in the text, tables and figures (trees included).
Then, resubmit so we can continue to analyze.

Our flexible approach during the COVID-19 pandemic

If you need more time at any stage of the peer-review process, please do let us know. While our systems will continue to remind you of the original timelines, we aim to be as flexible as possible during the current pandemic.

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Your Submission APDN-D-21-00015R4

1 message

APDN <em@editorialmanager.com> Sun, Jul 25, 2021 at 4:30 PM
Reply-To: APDN <jude.estrera@springernature.com>
To: "A. Muslim" <a_muslim@unsri.ac.id>

CC: dagmar.hanold@adelaide.edu.au, dhanold@gmail.com

Dear Dr. Muslim,

We have received the reports from our advisors on your manuscript, 'Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia' (APDN-D-21-00015R4), submitted to Australasian Plant Disease Notes.

Based on the advice received, I have decided that your manuscript can be accepted for publication after you have carried out the corrections as suggested by the reviewer(s).

Below, please find the reviewers' comments for your perusal.
You are kindly requested to also check the website for possible reviewer attachment(s).

Please submit your revised manuscript online by using
the Editorial Manager system which can be accessed at:

https://www.editorialmanager.com/apdn/

Your username is: a.muslim
If you forgot your password, you can click the 'Send Login Details' link on the EM Login page.

Please submit you revised manuscript before 20 Aug 2021 or request an extension of the deadline. If we do not hear from you by then, the manuscript will be automatically withdrawn.

With kind regards,

Kerrie Ann Davies, PhD
Associate Editor

COMMENTS FOR THE AUTHOR:

Having read through your MS, please make the following changes:

Lines 5-6 : delete 'has been noted'. This simply repeats 'were observed', and is not needed.

Line 22: 'affected' should be 'affecting'; and 'until' should be replaced with 'to'

Line 23: delete 'the' between 'by' and 'wilting

Line 43: insert 'these' between 'Based on' and 'morphological'

Line 46: add the word 'accessions' before the numbers on Line 47

Line 50: insert 'the' between 'extracted from' and 'fungal ....'

Line 56: should 1.5 not 1,5

Line 57: add a space between 23 and ul

Line 83: replace F on 'Five' with a lower case 'f'

Line 90: 'downwards' should be 'inwards'

Lines 93-94: Sentence should read '....morphological characteristics for confirmation of Koch's postulates."

Line 95: should read "...lesions at the inoculation site on the wood (Fig 4b).'

line 97: should read '...and length of discolouration (downward .....'

Line 101-102: should read '...Jackfruit include cankers on stems, with the stems becoming chapped as.....'

Line 103 - 106: I do not understand why this sentence is important for the story your MS is trying to tell. Whu is it important to know that jackfruit in Indonesia also showed symptoms typical of other pathogens? Are you trying to say that C. fimbriata is not the only potentially serious pathogen for Jackfruit in Indonesia, or that the symptoms of the fungi you list are similar to those of Ceratocystis? Please amend to clarify.

Line106-108: This final sentence should read '.....infections of native trees in these countries could potentially lead to devastation of .... components of the natural biodiversity in Indonesia'.

Caption for Table 1: replace 'considered' with 'included'

Note that figure captions should all be listed at the end of the list of references - please move

The caption for Fig 4 should read: 'Response of Artocarpus heterophyllous seedlings 45 days after under-bark ..... ', and on line 211 ....(III), and the healthy control seedling (IV); and line 213 should read '..discoloured wood extended ..... of the basal stem of the seedling.'

Please make any comments in the Response to Reviewers box. Thank you.

Our flexible approach during the COVID-19 pandemic

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DRAFT PERBAIKAN

Australasian Plant Disease Notes

Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia

--Manuscript Draft--

Manuscript Number:	APDN-D-21-00015R5
Full Title:	Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia
Article Type:	Plant Disease Note
Keywords:	Sudden death disease; Moraceae; Ceratocystis fimbriata sensu stricto
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Funding Information:	Kementerian Riset Teknologi Dan Pendidikan Tinggi Republik Indonesia (068/SP2H/AMD/LT/DRPM/2020) — Dr. A. Muslim
Abstract:	In 2019, wilt and sudden death were observed on Artocarpus heterophyllus (jackfruit) has been noted. Identification was performed by sequence analysis of the concatenated β-tubulin and ITS gene regions. Sequencing of the PCR product confirmed this pathogen was Ceratocystis fimbriata sensu stricto . This is the first report of C. fimbriata causing sudden death disease in A . heterophyllus in Indonesia and worldwide.
Response to Reviewers:	July 31, 2021 Dear Kerrie Ann Davies, PhD Associate Editor Australasian Plant Disease Notes Thank you very much for corrections to reviewers' comments of our manuscript. We are really appreciating the corrections. We have revised and make some modified the corrections as suggested by the reviewer(s) Here, we enclose revised version of the manuscript No. APDN-D-21-00015R4 entitled “Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia" by Rahmat Pratama, Ahmad Muslim, Suwandi Suwandi, Nurhayati Damiri, Soleha Soleha. Below is a summary of our response to the reviewers' comments.

Comment [1]: Lines 5-6 : delete 'has been noted'. This simply repeats 'were observed', and is not needed.
Our response: We agree and change sentence to be " In 2019, wilt and sudden death were observed on Artocarpus heterophyllus (jackfruit)".

Comment [2]: Line 22: 'affected' should be 'affecting'; and 'until' should be replaced with 'to'
Our response: Thank you very much. We agree and change 'affected' to be 'affecting' dan replaced 'until' to be 'to'.

Comment [3]: Line 23: delete 'the' between 'by' and 'wilting
Our response: We agree and delete 'the' between 'by' and 'wilting.

Comment [4]: Line 43: insert 'these' between 'Based on' and 'morphological'
Our response:
We agree and insert 'these' between 'Based on' and 'morphological'.

Comment [5]: Line 46: add the word 'accessions' before the numbers on Line 47
Our response:
We agree and added the word 'accessions' before the numbers on Line 47.

Comment [6]: Line 50: insert 'the' between 'extracted from' and 'fungal ....'
Our response:
Thank you very much. We agree and insert 'the' between 'extracted from' and 'fungal ....'

Comment [7]: Line 56: should 1.5 not 1,5
Our response:
Thank you very much. We agree and change 1,5 to be 1.5.

Comment [8]: Line 57: add a space between 23 and ul
Our response:
We agree and add a space between 23 and ul.

Comment [9]: Line 83: replace F on 'Five' with a lower case 'f'
Our response:
We agree and replace F on 'Five' with a lower case 'f'

Comment [10]: Line 90: 'downwards' should be 'inwards'
Our response:
We agree and change 'downwards' to be 'inwards'.

Comment [11]: Lines 93-94: Sentence should read '....morphological characteristics for confirmation of Koch's postulates."
Our response:
We agree and change sentence to be "…..re-identified using morphological characteristics for confirmation of Koch's postulates."

Comment [12]: Line 95: should read "...lesions at the inoculation site on the wood (Fig 4b).'
Our response:
We agree and change sentence to be "...lesions at the inoculation site on the wood (Fig 4b).'

Comment [13]: line 97: should read '...and length of discolouration (downward .....'
Our response:
We agree and change sentence '...and length of discolouration (downward .....'.

Comment [14]: Line 101-102: should read '...Jackfruit include cankers on stems, with the stems becoming chapped as.....'
Our response:
We agree and change sentence to be '...Jackfruit include cankers on stems, with the

stems becoming chapped as.....'.

Comment [15]: Line 103 - 106: I do not understand why this sentence is important for the story your MS is trying to tell. Whu is it important to know that jackfruit in Indonesia also showed symptoms typical of other pathogens? Are you trying to say that C. fimbriata is not the only potentially serious pathogen for Jackfruit in Indonesia, or that the symptoms of the fungi you list are similar to those of Ceratocystis? Please amend to clarify.
Our response:
We explain that C. fimbriata is also a serious wilt pathogen of Acacia mangium, Acacia crassicarpa, Lansium domesticum in Indonesia and Pomegranate in China. The symptoms of the fungi we list are similar of Ceratocystis at those plants. We change sentence to be “Ceratocystis fimbriata is a serious wilt pathogen of jackfruit, as well as of A. mangium and A. crassicarpa in Indonesia (Tarigan et al. 2011), Lansium domesticum in Indonesia (Suwandi et al. 2021) and Pomegranate in China (Li et al. 2016)”.

Comment [16]: Line106-108: This final sentence should read '.....infections of native trees in these countries could potentially lead to devastation of .... components of the natural biodiversity in Indonesia'.
Our response:
We agree and change sentence to be " Ceratocystis fimbriata infections of native trees in these countries could potentially lead to devastation of important components of the natural biodiversity in Indonesia”.

Comment [17]: Caption for Table 1: replace 'considered' with 'included'
Our response:
We agree and change 'considered' to be 'included'

Comment [18]: Note that figure captions should all be listed at the end of the list of references - please move
Our response:
We agree and move all figure captions at the end of the list of references

Comment [19]: The caption for Fig 4 should read: 'Response of Artocarpus heterophyllous seedlings 45 days after under-bark ..... ', and on line 211 ....(III), and the healthy control seedling (IV); and line 213 should read '..discoloured wood extended ..... of the basal stem of the seedling.'
Our response:
We agree and change sentence to be " Response of Artocarpus heterophyllous seedlings 45 days after under-bark inoculation with mycelium of Ceratocystis. a. total wilting of plant inoculated with ICBB9852 (I), CAAW30817 (II), ICBB9853 (III) and the healthy control seedling (IV); b. yellow arrow indicates the point of inoculation and red arrow the lesion boundary; c. The discoloured wood extended to the heartwood of the basal stem of the seedling”.

We feel that these changes have adequately addressed the comments and suggestions of reviewer(s). Please feel free to contact me if you need any additional information or clarification.

Thank you very much for your consideration of the manuscript and excellent cooperation

Yours sincerely,

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Jackfruit (*Artocarpus heterophyllus*), a New Host Plant of *Ceratocystis* Wilt in South Sumatra, Indonesia

Abstract

In 2019, wilt and sudden death were observed on *Artocarpus heterophyllus* (jackfruit). Identification was performed by sequence analysis of the concatenated β-tubulin and ITS gene regions. Sequencing of the PCR product confirmed this pathogen was *Ceratocystis fimbriata sensu stricto*. This is the first report of *C. fimbriata* causing sudden death disease in *A. heterophyllus* in Indonesia and worldwide.

Keywords: Sudden death disease · Moraceae · *Ceratocystis fimbriata sensu stricto* ·

Jackfruit (*Artocarpus heterophyllus*, Moraceae) is known in Indonesian as "Nangka", and is cultivated widely in many countries with tropical and subtropical climates. Jackfruit is among the most exported fruits worldwide and has considerable nutrition and health benefits (Ranasinghe et al. 2019).

In July 2019, wilt and die-back symptoms were observed for the first time on *A. heterophyllus* in the agricultural field of Sriwijaya University (Indralaya), Plaju (Palembang) and Gelumbang (Prabumulih), Indonesia. Wood of wilted trees showed a brown to black streaking in the woody xylem. Symptoms on the dying Jackfruit wood produced grey to brown lesions and included a streaking pattern of discoloration in the sapwood (Fig. 1a) and in some cases the lesions extended to heartwood (Fig. 1b). The lesion could be found partially or totally affecting the sapwood from the basal stem to the branches. Leaves of dying trees had yellowing symptoms, followed by wilting of the leaves on several lateral branches, drying of twigs and the wilt of the whole tree (Fig. 1c). This type of wilting was termed as sudden death or wilt (Pratama et al. 2021).

Wood samples were taken from lesions of wilted trees using a knife sterilised in 70% ethanol. Each sample was wrapped in tissue paper and placed in a cool box. The same day, the wood samples (1–20 mm length, 1–2 mm thick) were sandwiched between two slices of fresh carrot and placed on sterile dry paper in plastic boxes at 25 °C following the method of Moller and DeVay (1968) (Fig. 1d). After 5–10 days, hat-shaped spores of putative *Ceratocystis* pathogens were placed on 2% (w/v) malt extract agar (MEA) (Merck, Germany), and incubated at 25 °C in a laboratory. The isolated fungi were initially identified based on morphological characteristics of a 14 day old culture. Mycelium on MEA grey, reverse side of colony

olivaceous grey; submerged mycelium darkening as the ascomata develop forming fine, radiating fibrils.

Morphological traits of fruiting bodies and spores were observed under an optical Olympus CX33 microscope. Ascomatal bases dark brown to black, base subglobose to globose and measured (n=100), 131.5–250.7×101.6–236.5 µm (Fig. 2a). Ascomata necks erect, occasionally curved, black at the base becoming subhyaline towards the apex, smooth to crenulate, 324.7–579.1 µm long including ostiolar hyphae (Fig. 2b). Phialides pale brown to hyaline (Fig. 2c). Ascospores hat-shaped, 3.4–6.8×2.1–6.2 µm (Fig. 2d). Bacilliform conidia 11.1–36.1×2.1–7.4 µm (Fig. 2e). Barrel conidia 4.4–16.1×2.7–6.9 µm (Fig. 2f). Chlamydospores oval, thick walled, smooth, 6.7–16.5×5.9–12.9 µm (Fig. 2g). Based on these morphological characters, the fungus was identified as *Ceratocystis fimbriata*. Two representative isolates were deposited at the ICBB Culture Collection for Microorganisms and Cell Culture, Indonesian Center for Biodiversity and Biotechnology, (Bogor, Indonesia) as accessions ICBB9852 and ICBB9853.

To confirm the species identification, isolates were cultured on potato dextrose broth (PDB) at room temperature for one week. Mycelium was filtered through Whatman filter paper and genomic DNA was extracted from the fungal mycelial mat using YeaStar Genomic DNA Kit (Zymo Research Corporation, California, USA). PCR conditions and reactions for two gene regions were used to identify the *Ceratocystis* isolates; the Internal Transcribed Spacer (ITS) with primers ITS 1 and ITS4 (White et al. 1990) and part of the β-tubulin (βt) gene with primers βt1a and βt1b (Glass and Donaldson 1995). Amplifications were carried out in 50 µl reactions containing 20 µl DreamTaq Green PCR Master Mix (Eppendorf, Germany) (DreamTaq DNA Polymerase, 2X DreamTaq Green buffer, dNTPs, and 4 mM $MgCl_2$), 1.5 µl of each forward and reverse primer, 4 µl of DNA template and 23 µl sterilised water. The PCRs were performed with a C1000 Touch™ thermal cycler (Bio-Rad, USA). The PCR cycling parameters were as follows: initial denaturation for 5 min at 95 °C, followed by 35 cycles at 95 °C for 30 s, 56 °C for 45 s and 72 °C for 1 min. Amplification was completed at 72 °C for 10 min and the PCR product was stored at 10 °C. The PCR amplicons were sequenced at 1st BASE (Malaysia).

For the ITS and β-tubulin, amplification resulted in fragments of ~550 base pairs (bp) in size. The sequences of the amplified products were then deposited in the GenBank database and assigned accession numbers isolate ICBB9852 (MT355410; MT412106), isolate ICBB9853 (MT355412; MT412108), and isolate CAAW30817 (MT355413, MT412109) for the ITS and β-tubulin. β-tubulin datasets were generated using ex-type and ex-paratype

sequences representing species in the Latin American (LAC) and Asian clade (AC) of the *C. fimbriata* species complex (Fourie et al. 2015; Oliveira et al. 2015; Barnes et al. 2018). To determine relatedness of isolates from jackfruit with known *C. fimbriata* populations, the ITS sequence was manually aligned with known ITS haplotypes as designated by Harrington et al. (2014); Li et al. (2016) and phylogenetic analyses were performed. Maximum Parsimony (MP) analyses were performed in MEGA v. 10 (Kumar et al. 2016; Paul et al. 2018) with 1000 replications. The analysis involved 38 (β-tubulin) and 37 (ITS) nucleotide sequences. All positions containing gaps and missing data were eliminated. There were 408 (β-tubulin) and 518 (ITS) positions in the final dataset. *Ceratocystis variospora* was used as the out-group. β-tubulin sequence of our isolates confirmed the assignment to LAC of *C. fimbriata sensu lato* (Fig. 3a). Manual alignment of the ITS sequences with previously described ITS genotypes (Harrington et al. 2014; Li et al. 2016) grouped the isolates into ITS5 haplotype of *C. fimbriata sensu stricto* (Fig. 3b). Consistency (CI), retention (RI), and composite indexes (CoI) for β-tubulin were 0.566667, 0.845238, 0.668011, respectively and ITS was 0.933333, 0.976563, 0.932836, respectively.

The pathogenic potential of isolates was evaluated by the under bark inoculation method described by O'Gara et al. (1997) using five-month-old *A. heterophyllus* seedlings with stem diameters of 6-8 mm and heights <1.5 m were prepared for pathogenicity test. Seedlings were grown in 10 cm diameter plastic pots containing a soil mix (topsoil + peat + chicken manure) under a 50% shading net. Plants were watered daily to maintain humidity, and any mortality occurring before the end of the experiment was recorded. Wounds were made on the stems of the seedlings using a cork borer (4 mm diam.), and mycelial discs (4 mm diam.) taken from an actively growing colony of *C. fimbriata* on 2% MEA (14 days) (Pratama et al. 2021) were placed in the wounds with the mycelium facing inwards. These were covered with Parafilm (Pechiney, Menasha, Wisconsin) to reduce contamination and desiccation. Ten plants of each tree species were inoculated with sterile MEA plugs to serve as controls (Fig. 4a). Fungal isolates were re-isolated and re-identified using morphological characteristics for confirmation of Koch's postulates. In pathogenicity tests, initial symptoms appeared two weeks post-inoculation as brown lesions at the inoculation site on the wood (Fig. 4b). Forty-five days after inoculation, plants exhibited wilt symptoms, lesions of wood discoloration extended to heartwood (Fig. 4c) and length of discolouration (downward + upward) was 17.88 until 34.74 cm. When re-isolated, the fungus was phenotypically identical to the prior isolate of *C. fimbriata* (ICBB9852, ICBB9853, CAAW30817).

This is the first report of *C. fimbriata* causing wilt and die-back in Jackfruit in Indonesia and worldwide. The symptoms of *C. fimbriata* wilt disease in Jackfruit include cankers on stems, with the stems becoming chapped as though torn apart, fruit rot and progressive loss of the canopy resulting in tree death. *Ceratocystis fimbriata* is a serious wilt pathogen of jackfruit, as well as of *A. mangium* and *A. crassicarpa* in Indonesia (Tarigan et al. 2011), *Lansium domesticum* in Indonesia (Suwandi et al. 2021) and Pomegranate in China (Li et al. 2016). *Ceratocystis fimbriata* infections of native trees in these countries could potentially lead to devastation of important components of the natural biodiversity in Indonesia.

Acknowledgement

This research was funded by PMDSU scholarship with budget year of 2019-2021 according to the Director of Research and Community Service, Directorate of Research and Community Service (DRPM), Directorate General for Research and Development, Ministry of Research, Technology, and Higher Education, Number: 068/SP2H/AMD/LT/DRPM/2020.

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Table

Table 1 *Ceratocystis* isolates included in the phylogenetic analyses

Species	Haplotype	Isolates no.	Host	Origin	GenBank accession no.	
					ITS	β-tubulin
C. fimbriata	ITS1a	C1418	*Ipomoea batatas*	USA	AY157956	-
	ITS1	C1857	*Ficus carica*	Brazil	HQ157542	-
	ITS1b	CMW4797	*Eucalyptus* sp.	Congo	FJ236733	-
	ITSb	CMW9998	*Eucalyptus* sp.	South Africa	FJ236721	-
	ITS2	C1655	*Mangifera indica*	Brazil	HQ157546	-
	ITS3	C1440	*Eucalyptus* sp.	Brazil	HQ157544	-
	ITS3	CMW5328	*E. grandis*	Uganda	AF395686	-
	ITS4	C1442	*Eucalyptus* sp.	Brazil	HQ157545	-
	ITS5	**ICBB9852**	***Artocarpus heterophyllus***	**Indonesia**	**MT355410**	**MT412106**
	ITS5	**ICBB9853**	***A. heterophyllus***	**Indonesia**	**MT355412**	**MT412108**
	ITS5	**CAAW30817**	***A. heterophyllus***	**Indonesia**	**MT355413**	**MT412109**
	ITS5	CMW38737	*E. grandis*	Zimbabwe	KF878326	KF878335
	ITS5	C1345	*Eucalyptus* sp.	Brazil	AY157966	-
	ITS5	A59662	*Camellia sinensis*	China	KF650948	-
	ITS5	YM061	*Colocasia esculenta*	China	AM712445	-
	ITS5	P20053	*Punica granatum*	China	AM292204	-
	ITS5	C1	*Acacia* sp.	Vietnam	MF033455	MF040712
	ITS5	CMW22563	*A. mangium*	Indonesia	EU588656	EU588636
	ITS5	WRC	*Lansium domesticum*	Indonesia	MT229127	MW013766
	ITS6	C2055	*Mangifera* sp.	Brazil	HQ157548	-
	ITS6z	CMW13582	*Hypocryphalus mangifera*	Oman	KC261853	-
	ITS6z	WBC	*L. domesticum*	Indonesia	MT229128	MW013767
	ITS7b	CMW13851	*M. indica*	Oman	AY953383	EF433308
	ITS7b	CMW23634	*M. indica*	Pakistan	EF433302	EF433311
	ITS7b	CMW22579	*A. mangium*	Indonesia	EU588658	-
	ITS8a	CMW8856	*Citrus* sp.	Colombia	AY233867	-
	ITS8c	CMW17808	*Eucalyptus* sp	Colombia	EF127990	-
	ITS8e	CMW22092	*E. deglupta*	Ecuador	FJ151432	-
	ITS9	C1558	*M. indica*	Brazil	AY157965	-
	ITS9	C1914	*C. esculenta*	Brazil	HQ157540	-
	ITS10	C994	*M. indica*	Brazil	AY157964	-
	ITS10a	Cf4	*M. indica*	Brazil	EF042605	-
	ITS11	C1865	*C. esculenta*	Brazil	AY526286	-
	ITS12	C1926	*C. esculenta*	Brazil	HQ157541	-
	ITS14	C1688	*M. indica*	Brazil	AY526291	-
	ITS15	C925	*Gmelina arborea*	Brazil	AY157967	-
	ITS16	C924	*G. arborea*	Brazil	HQ157539	-
C. pirilliformis	Asian clade (AC)	CMW6569	*E. nitens*	Australia	-	DQ371652
	AC	CMW6579	*E. nitens*	Australia	-	DQ371653
C. polychroma	AC	CMW11424	*Syzygium aromaticum*	Indonesia	-	AY528966
	AC	CMW11436	*S. aromaticum*	Indonesia	-	AY528967
C. atrox	AC	CMW19383	*E. grandis*	Australia	-	EF070430
	AC	CMW19385	*E. grandis*	Australia	-	EF070431
C. neglecta	Latin American clade (LAC)	CMW17808	*E. grandis*	Colombia	-	EU881898
	LAC	CMW18194	*E. grandis*	Colombia	-	EU881899
C. colombiana	LAC	CMW5751	*Coffea arabica*	Colombia	-	AY177225
	LAC	CMW5761	*C. arabica*	Colombia	-	AY177224
C. cacaofunesta	LAC	CMW14803	*Theobroma cacao*	Ecuador	-	KJ631108
	LAC	CMW15051	*T. cacao*	Costa Rica	-	KJ601510
C. papillata	LAC	CMW8850	*Citrus* × *Tangelo hybrid*	Colombia	-	AY233875
	LAC	CMW8856	*Citrus limon*	Colombia	-	AY233874
C. fimbriata	LAC	CMW14797	*M. indica*	Brazil	-	EF433307
	LAC	CMW28907	*M. indica*	Brazil	-	FJ200270

	LAC	CMW1547	*I. batatas*	Papua New Guinea	-	EF070443
	LAC	C1421	*I. batatas*	USA	-	KF302689
C. fimbriatomima	LAC	CMW24174	*Eucalyptus hybrid*	Venezuela	-	EF190951
	LAC	CMW24176	*Eucalyptus hybrid*	Venezuela	-	EF190952
C. fimbriata	LAC	CMW21127	*A. crassicarpa*	Indonesia	-	EU588643
	LAC	CMW24664	*Eucalyptus hybrid*	China	-	JQ862720
	LAC	CBS115173	*Gmelina arborea*	Brazil	-	KF302700
	LAC	CBS14653	*C. arabica*	Suriname	-	KF302702
C. platani	LAC	CMW14802	*Platanus occidentalis*	USA	-	EF070425
	LAC	CMW23450	*P. occidentalis*	Greece	-	KJ601513

212

Colour figure

Fig. 1 Symptoms of *Ceratocystis fimbriata* wilt disease in *Artocarpus heterophyllus:* **a**. vascular discoloration of infected tree; **b.** The discolored wood extended to the heartwood of the basal stem; **c.** three-year-old tree with wilted, yellowing leaves and rotten fruit; **d.** isolation of the fungus from discoloured xylem showing dark mycelium and sporulation on the carrot slices after 7 days.

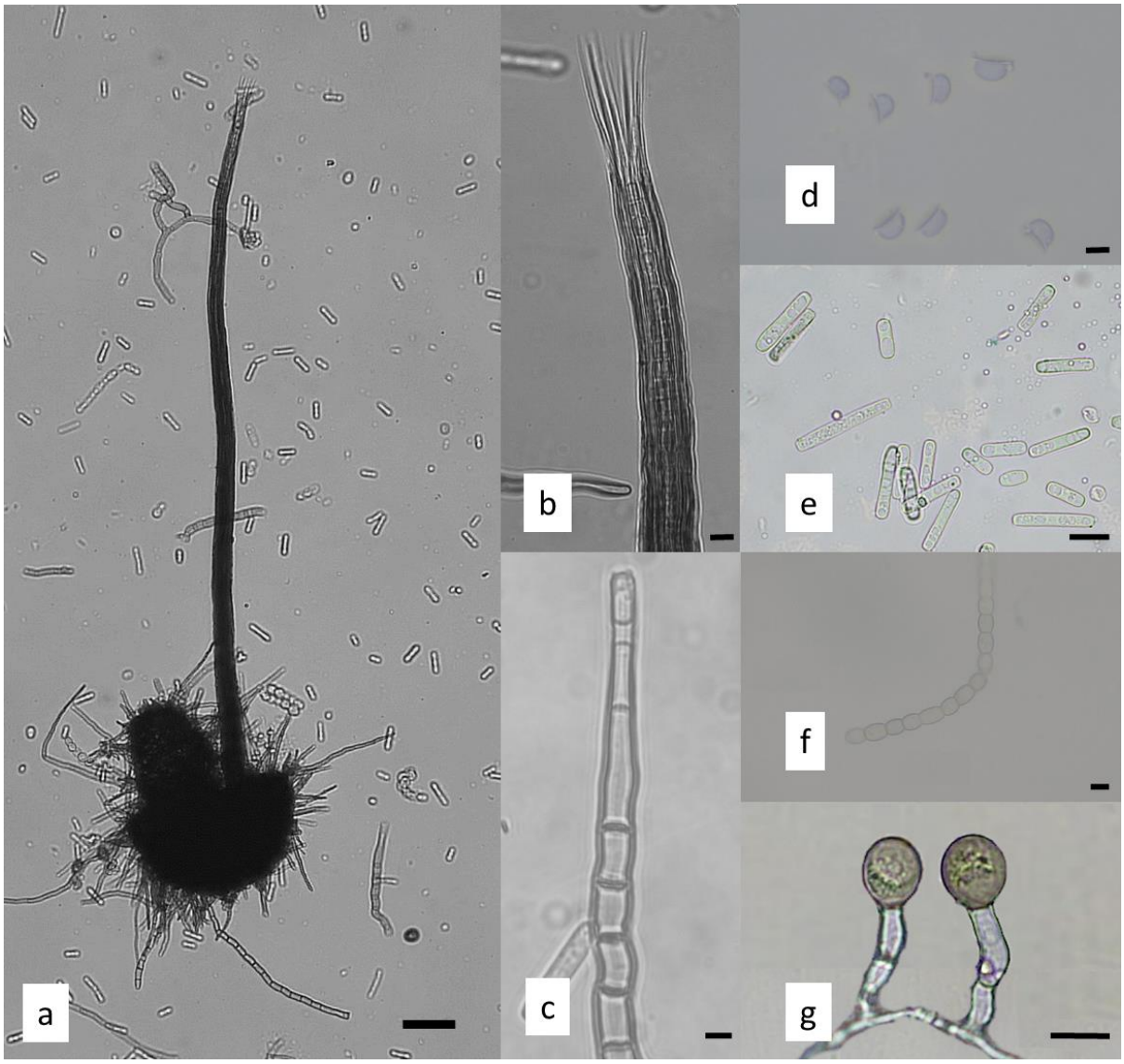

Fig. 2 Morphological characteristics of *Ceratocystis fimbriata* isolated from *Artocarpus heterophyllus* stem lesion: **a.** ascomata with pirilliform base, **b.** divergent ostiolar hyphae; **c.** conidiophore/phialide; **d.** hat-shaped ascospores; **e.** cylindrical conidia; **f.** Chain of barrel-shaped conidia; **g.** chlamydospores of various shapes. Scale bars: a = 100 µm; b–c, e–g = 10 µm; d = 5 µm.

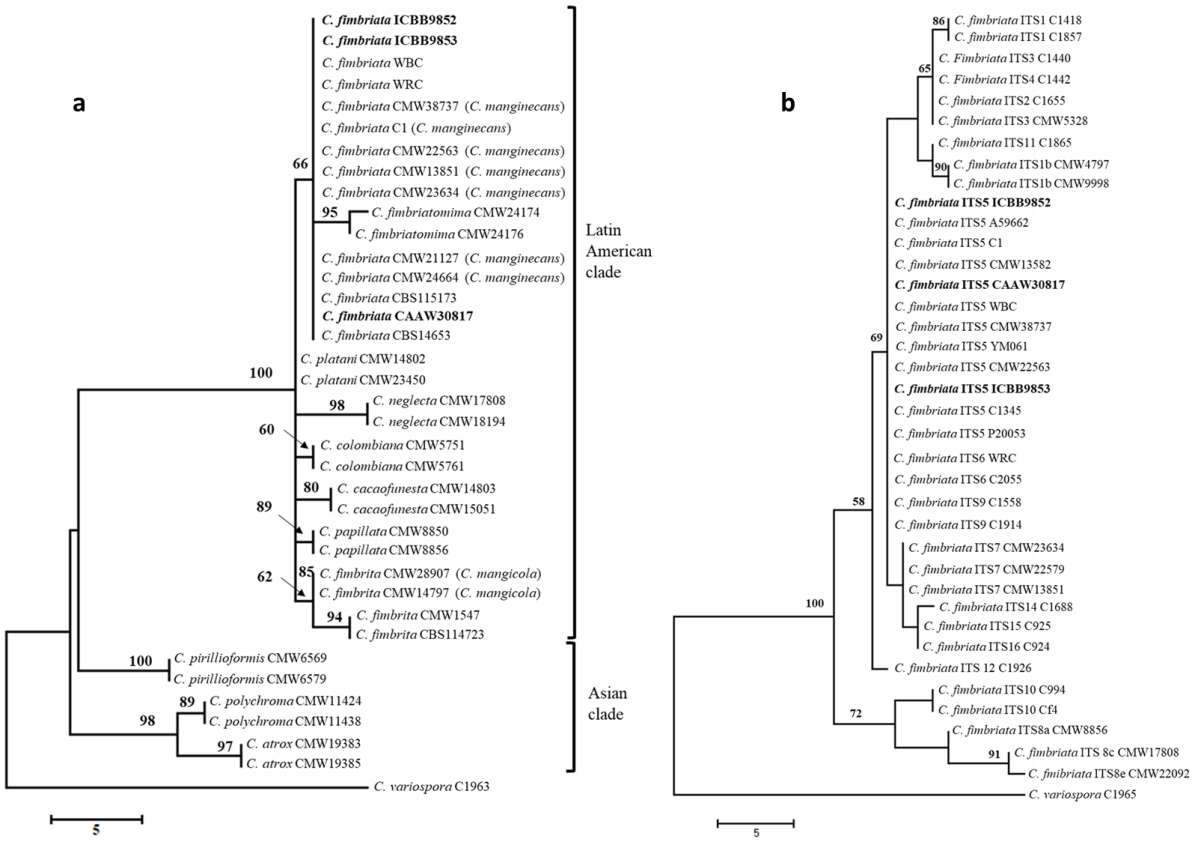

Fig. 3 Phylogenetic tree constructed by MEGA with Maximum Parsimony (MP) analysis by **a**. β-tubulin sequences from Jackfruit tree in Indonesia (marked in bold) and other species in the Latin American and Asian clade of the *C. fimbriata* species complex. **b.** ITS sequences from Jackfruit tree in Indonesia (marked in bold) and genotypes (sequences) of the *C. fimbriata sensu stricto*.

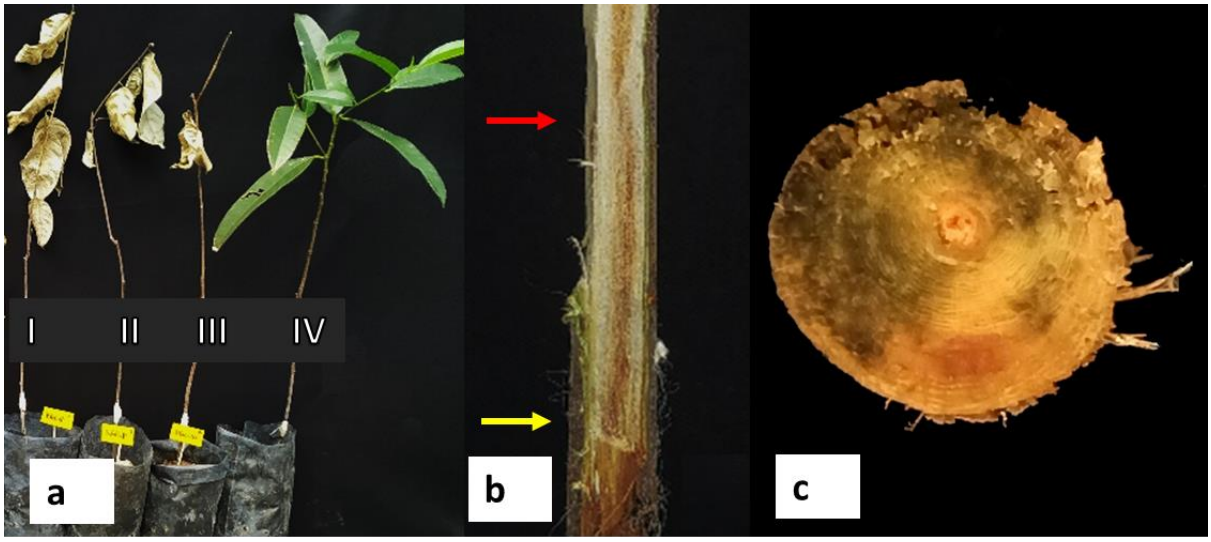

Fig. 4 Response of *Artocarpus heterophyllous* seedlings 45 days after under-bark inoculation with mycelium of *Ceratocystis.* **a.** total wilting of plant inoculated with ICBB9852 (I), CAAW30817 (II), ICBB9853 (III) and the healthy control seedling (IV); **b.** yellow arrow indicates the point of inoculation and red arrow the lesion boundary; **c.** The discoloured wood extended to the heartwood of the basal stem of the seedling.

a. muslim unsri <a_muslim@unsri.ac.id>

Your Submission APDN-D-21-00015R4

1 message

APDN <em@editorialmanager.com> Sun, Jul 25, 2021 at 4:30 PM
Reply-To: APDN <jude.estrera@springernature.com>
To: "A. Muslim" <a_muslim@unsri.ac.id>

CC: dagmar.hanold@adelaide.edu.au, dhanold@gmail.com

Dear Dr. Muslim,

We have received the reports from our advisors on your manuscript, 'Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia' (APDN-D-21-00015R4), submitted to Australasian Plant Disease Notes.

Based on the advice received, I have decided that your manuscript can be accepted for publication after you have carried out the corrections as suggested by the reviewer(s).

Below, please find the reviewers' comments for your perusal.
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With kind regards,

Kerrie Ann Davies, PhD
Associate Editor

COMMENTS FOR THE AUTHOR:

Having read through your MS, please make the following changes:

Lines 5-6 : delete 'has been noted'. This simply repeats 'were observed', and is not needed.

Line 22: 'affected' should be 'affecting'; and 'until' should be replaced with 'to'

Line 23: delete 'the' between 'by' and 'wilting

Line 43: insert 'these' between 'Based on' and 'morphological'

Line 46: add the word 'accessions' before the numbers on Line 47

Line 50: insert 'the' between 'extracted from' and 'fungal ....'

Line 56: should 1.5 not 1,5

Line 57: add a space between 23 and ul

Line 83: replace F on 'Five' with a lower case 'f'

Line 90: 'downwards' should be 'inwards'

Lines 93-94: Sentence should read '....morphological characteristics for confirmation of Koch's postulates."

Line 95: should read "...lesions at the inoculation site on the wood (Fig 4b).'

line 97: should read '...and length of discolouration (downward .....'

Line 101-102: should read '...Jackfruit include cankers on stems, with the stems becoming chapped as.....'

Line 103 - 106: I do not understand why this sentence is important for the story your MS is trying to tell. Whu is it important to know that jackfruit in Indonesia also showed symptoms typical of other pathogens? Are you trying to say that C. fimbriata is not the only potentially serious pathogen for Jackfruit in Indonesia, or that the symptoms of the fungi you list are similar to those of Ceratocystis? Please amend to clarify.

Line106-108: This final sentence should read '.....infections of native trees in these countries could potentially lead to devastation of .... components of the natural biodiversity in Indonesia'.

Caption for Table 1: replace 'considered' with 'included'

Note that figure captions should all be listed at the end of the list of references - please move

The caption for Fig 4 should read: 'Response of Artocarpus heterophyllous seedlings 45 days after under-bark ..... ', and on line 211 ....(III), and the healthy control seedling (IV); and line 213 should read '..discoloured wood extended ..... of the basal stem of the seedling.'

Please make any comments in the Response to Reviewers box. Thank you.

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Your Submission APDN-D-21-00015R5 - [EMID:4e8494bedda6eb9f]

2 messages

APDN <em@editorialmanager.com> Thu, Aug 5, 2021 at 7:49 AM
Reply-To: APDN <jude.estrera@springernature.com>
To: "A. Muslim" <a_muslim@unsri.ac.id>

CC: dagmar.hanold@adelaide.edu.au, dhanold@gmail.com

Dear Dr. Muslim,

We are pleased to inform you that your manuscript, "Jackfruit (Artocarpus heterophyllus), a New Host Plant of Ceratocystis Wilt from South Sumatra, Indonesia" (APDN-D-21-00015R5), has been accepted for publication in Australasian Plant Disease Notes.

You will receive an e-mail from Springer in due course with regards to the Transfer of Copyright.

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With best regards,

Dagmar Hanold
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a. muslim unsri <a_muslim@unsri.ac.id> Fri, Aug 20, 2021 at 5:30 AM
To: APDN <jude.estrera@springernature.com>

Dear Prof. Dagmar Hanold
Editor in Chief
Australasian Plant Disease Notes

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We are really happy to hear that our paper has been accepted for publishing in your Journal " Australasian Plant Disease Notes".
We hope we can contribute to submit another paper in your journal namely, in the aspect of new diseases in plants.

We are waiting for the next information from you and also from springer regarding the process of our paper for publishing in your Journal.

Thank you very much for your kindness dan excellent cooperation

Best Regard
Ahmad Muslim
Sriwijaya University
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Article Title : Jackfruit (*Artocarpus heterophyllus*), a new host plant of *Ceratocystis* wilt in South Sumatra, Indonesia
DOI : 10.1007/s13314-021-00435-x
APDN-D-21-00015R5

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Cc: Jennyca.Parcon@springernature.com

JACKFRUIT (ARTOCARPUS HETEROPHYLLUS), A NEW HOST PLANT OF CERATOCYSTIS WILT FROM SOUTH SUMATRA, INDONESIA

Article DOI: 10.1007/s13314-021-00435-x

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JACKFRUIT (ARTOCARPUS HETEROPHYLLUS), A NEW HOST PLANT OF CERATOCYSTIS WILT FROM SOUTH SUMATRA, INDONESIA

Article DOI: 10.1007/s13314-021-00435-x

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- Published: 11 September 2021

Jackfruit (*Artocarpus heterophyllus*), a new host plant of *Ceratocystis* wilt in South Sumatra, Indonesia

- Rahmat Pratama,
- Ahmad Muslim✉,
- Suwandi Suwandi,
- Nurhayati Damiri &
- Soleha Soleha

Australasian Plant Disease Notes **16**,
Article number: 24 (2021)

Abstract

In 2019, wilt and sudden death were observed on *Artocarpus heterophyllus* (jackfruit). Identification was performed by sequence analysis of the concatenated β-tubulin and ITS gene regions. Sequencing of the PCR product confirmed this pathogen was *Ceratocystis fimbriata* sensu stricto. This is the first report of *C. fimbriata* causing sudden death disease in *A. heterophyllus* in Indonesia and worldwide.

Jackfruit (*Artocarpus heterophyllus*, Moraceae) is known in Indonesian as “Nangka”, and is cultivated widely in many countries with tropical and subtropical climates. Jackfruit is among the most exported fruits worldwide and has considerable nutrition and health benefits (Ranasinghe et al. 2019).

In July 2019, wilt and die-back symptoms were observed for the first time on *A. heterophyllus* in the agricultural field of Sriwijaya University (Indralaya), Plaju (Palembang) and Gelumbang (Prabumulih), Indonesia. Wood of wilted trees showed a brown to black streaking in the woody xylem. Symptoms on the dying Jackfruit wood produced grey to brown lesions and included a streaking pattern of discoloration in the sapwood (Fig. 1a) and in some cases the lesions extended to heartwood (Fig. 1b). The lesion could be found partially or totally affecting the sapwood from the basal stem to the branches. Leaves of dying trees had yellowing symptoms, followed by wilting of the leaves on several lateral branches, drying of twigs and the wilt of the whole tree (Fig. 1c). This type of wilting was termed as sudden death or wilt (Pratama et al. 2021).

Fig. 1

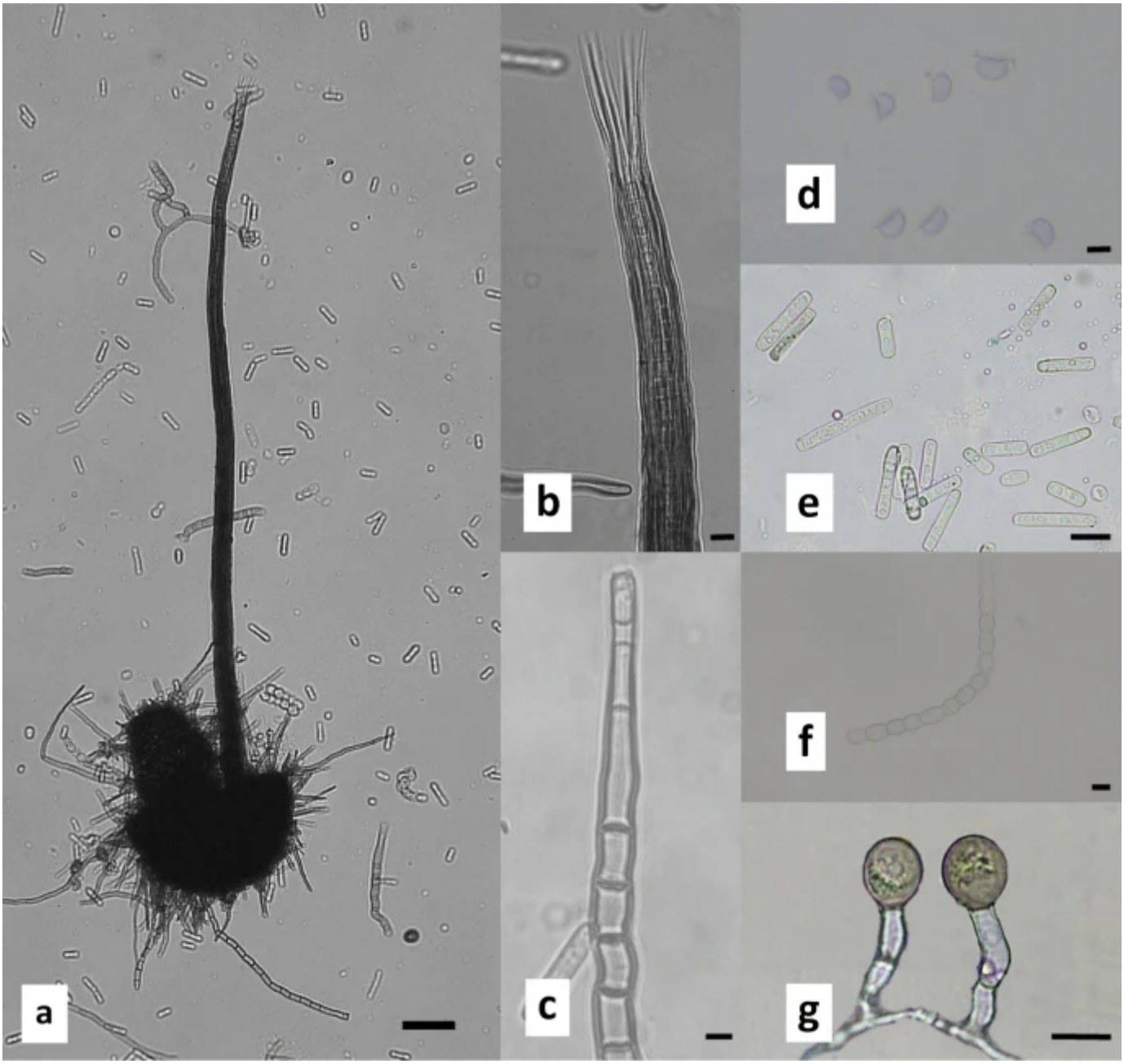
Symptoms of *Ceratocystis fimbriata* wilt disease in *Artocarpus heterophyllus:* **a** vascular discoloration of infected tree; **b** the discolored wood extended to the heartwood of the basal stem; **c** three-year-old tree with wilted, yellowing leaves and rotten fruit; **d** isolation of the fungus from discoloured xylem showing dark mycelium and sporulation on the carrot slices after 7 days

Wood samples were taken from lesions of wilted trees using a knife sterilised in 70% ethanol. Each sample was wrapped in tissue paper and placed in a cool box. The same day, the wood samples (1–20 mm length, 1–2 mm thick) were sandwiched between two slices of fresh carrot and placed on sterile dry paper in plastic boxes at 25 °C following the method of Moller and DeVay (1968) (Fig. 1d). After 5–10 days, hat-shaped spores of putative *Ceratocystis* pathogens were placed on 2% (w/v) malt extract agar (MEA) (Merck, Germany), and incubated at 25 °C in a laboratory. The isolated fungi were initially identified based on morphological characteristics of a 14 day old culture. Mycelium on MEA grey, reverse side of colony olivaceous grey; submerged mycelium darkening as the ascomata develop forming fine, radiating fibrils.

Morphological traits of fruiting bodies and spores were observed under an optical Olympus CX33 microscope. Ascomatal bases dark brown to black, base subglobose to globose and measured (n = 100), 131.5–250.7 × 101.6–236.5 µm (Fig. 2a). Ascomata necks erect, occasionally curved, black at the base becoming subhyaline towards the apex, smooth to crenulate, 324.7–579.1 µm long including ostiolar hyphae (Fig. 2b). Phialides pale brown to hyaline (Fig. 2c). Ascospores hat-shaped, 3.4–6.8 × 2.1–6.2 µm (Fig. 2d). Bacilliform conidia 11.1–36.1 × 2.1–7.4 µm (Fig. 2e). Barrel conidia 4.4–16.1 × 2.7–6.9 µm (Fig. 2f). Chlamydospores oval, thick walled, smooth, 6.7–16.5 × 5.9–12.9 µm (Fig. 2g). Based on these morphological characters, the fungus was identified as *Ceratocystis fimbriata*. Two representative isolates were deposited at the

ICBB Culture Collection for Microorganisms and Cell Culture, Indonesian Center for Biodiversity and Biotechnology, (Bogor, Indonesia) as accessions ICBB9852 and ICBB9853.

Fig. 2



Morphological characteristics of *Ceratocystis fimbriata* isolated from *Artocarpus heterophyllus* stem lesion: **a** ascomata with pirilliform base, **b** divergent ostiolar hyphae; **c** conidiophore/phialide; **d** hat-shaped ascospores; **e** cylindrical conidia; **f** Chain of barrel-shaped conidia; **g** chlamydospores

of various shapes. Scale bars: a = 100 µm; b–c, e–g = 10 µm; d = 5 µm

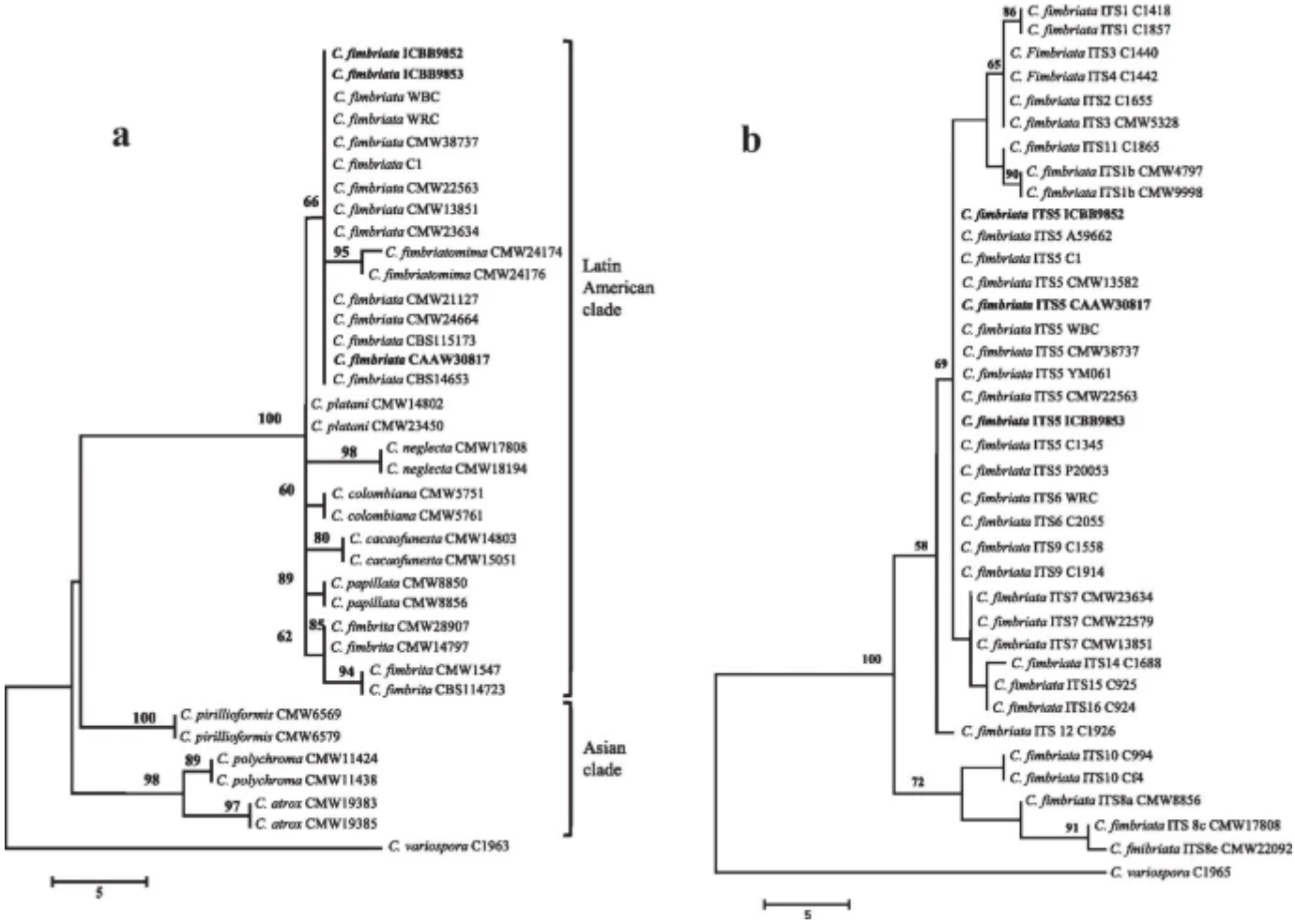
To confirm the species identification, isolates were cultured on potato dextrose broth (PDB) at room temperature for one week. Mycelium was filtered through Whatman filter paper and genomic DNA was extracted from the fungal mycelial mat using YeaStar Genomic DNA Kit (Zymo Research Corporation, California, USA). PCR conditions and reactions for two gene regions were used to identify the *Ceratocystis* isolates; the Internal Transcribed Spacer (ITS) with primers ITS 1 and ITS4 (White et al. 1990) and part of the β-tubulin (βt) gene with primers βt1a and βt1b (Glass and Donaldson 1995). Amplifications were carried out in 50 µl reactions containing 20 µl DreamTaq Green PCR Master Mix (Eppendorf, Germany) (DreamTaq DNA Polymerase, 2X DreamTaq Green buffer, dNTPs, and 4 mM $MgCl_2$), 1.5 µl of each forward and reverse primer, 4 µl of DNA template and 23 µl sterilised water. The PCRs were performed with a C1000 Touch™ thermal cycler (Bio-Rad, USA). The PCR cycling parameters were as follows: initial denaturation for 5 min at 95 °C, followed by 35 cycles at 95 °C for 30 s, 56 °C for 45 s and 72 °C for 1 min. Amplification was completed at 72 °C for 10 min and the PCR product was stored at 10 °C. The PCR amplicons were sequenced at 1st BASE (Malaysia).

For the ITS and β-tubulin, amplification resulted in fragments of ~ 550 base pairs (bp) in size. The sequences of the amplified products were then deposited in the GenBank database and assigned accession numbers isolate ICBB9852 (MT355410;

MT412106), isolate ICBB9853 (MT355412; MT412108), and isolate CAAW30817 (MT355413, MT412109) for the ITS and β-tubulin. β-tubulin datasets were generated using ex-type and ex-paratype sequences representing species in the Latin American (LAC) and Asian clade (AC) (Table 1) of the *C. fimbriata* species complex (Fourie et al. 2015; Oliveira et al. 2015; Barnes et al. 2018). To determine relatedness of isolates from jackfruit with known *C. fimbriata* populations, the ITS sequence was manually aligned with known ITS haplotypes as designated by Harrington et al. (2014), Li et al. (2016) and phylogenetic analyses were performed. Maximum Parsimony (MP) analyses were performed in MEGA v. 10 (Kumar et al. 2016; Paul et al. 2018) with 1000 replications. The analysis involved 38 (β-tubulin) and 37 (ITS) nucleotide sequences. All positions containing gaps and missing data were eliminated. There were 408 (β-tubulin) and 518 (ITS) positions in the final dataset. *Ceratocystis variospora* was used as the out-group. β-tubulin sequence of our isolates confirmed the assignment to LAC of *C. fimbriata* sensu lato (Fig. 3a). Manual alignment of the ITS sequences with previously described ITS genotypes (Harrington et al. 2014; Li et al. 2016) grouped the isolates into ITS5 haplotype of *C. fimbriata* sensu stricto (Fig. 3b). Consistency (CI), retention (RI), and composite indexes (CoI) for β-tubulin were 0.566667, 0.845238, 0.668011, respectively and ITS was 0.933333, 0.976563, 0.932836, respectively.

Table 1 *Ceratocystis* isolates included in the phylogenetic analyses

Fig. 3



Phylogenetic tree constructed by MEGA with Maximum Parsimony (MP) analysis by **a** β-tubulin sequences from Jackfruit tree in Indonesia (marked in bold) and other species in the Latin American and Asian clade of the *C. fimbriata* species complex. **b** ITS sequences from Jackfruit tree in Indonesia (marked in bold) and genotypes (sequences) of the *C. fimbriata* sensu stricto

The pathogenic potential of isolates was evaluated by the under bark inoculation method described by O'Gara et al. (1997) using five-month-old *A. heterophyllus* seedlings with stem diameters of 6–8 mm and heights < 1.5 m were prepared for pathogenicity test. Seedlings were grown in 10 cm diameter plastic pots containing a soil mix (topsoil + peat + chicken manure) under a 50% shading net. Plants were watered daily to maintain humidity, and any mortality occurring before the end of the

experiment was recorded. Wounds were made on the stems of the seedlings using a cork borer (4 mm diam.), and mycelial discs (4 mm diam.) taken from an actively growing colony of *C. fimbriata* on 2% MEA (14 days) (Pratama et al. 2021) were placed in the wounds with the mycelium facing inwards. These were covered with Parafilm (Pechiney, Menasha, Wisconsin) to reduce contamination and desiccation. Ten plants of each tree species were inoculated with sterile MEA plugs to serve as controls (Fig. 4a). Fungal isolates were re-isolated and re-identified using morphological characteristics for confirmation of Koch's postulates. In pathogenicity tests, initial symptoms appeared two weeks post-inoculation as brown lesions at the inoculation site on the wood (Fig. 4b). Forty-five days after inoculation, plants exhibited wilt symptoms, lesions of wood discoloration extended to heartwood (Fig. 4c) and length of discolouration (downward + upward) was 17.88 until 34.74 cm. When re-isolated, the fungus was phenotypically identical to the prior isolate of *C. fimbriata* (ICBB9852, ICBB9853, CAAW30817).

Fig. 4

Response of *Artocarpus heterophyllous* seedlings 45 days after under-bark inoculation with mycelium of *Ceratocystis*. **a** total wilting of plant inoculated with ICBB9852 (I), CAAW30817 (II), ICBB9853 (III) and the healthy control seedling (IV); **b** yellow arrow indicates the point of inoculation and red arrow the lesion boundary; **c** The discoloured wood extended to the heartwood of the basal stem of the seedling

This is the first report of *C. fimbriata* causing wilt and die-back in Jackfruit in Indonesia and worldwide. The symptoms of *C. fimbriata* wilt disease in Jackfruit include cankers on stems, with the stems becoming chapped as though torn apart, fruit rot and progressive loss of the canopy resulting in tree death. *Ceratocystis fimbriata* is a serious wilt pathogen of jackfruit, as well as of *A. mangium* and *A. crassicarpa* in Indonesia (Tarigan et al. 2011), *Lansium domesticum* in Indonesia (Suwandi et al. 2021) and Pomegranate in China (Li et al. 2016). *Ceratocystis fimbriata* infections of native trees in these countries could potentially lead to devastation of important components of the natural biodiversity in Indonesia.

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Acknowledgements

This research was funded by PMDSU scholarship with budget year of 2019-2021 according to the Director of Research and Community Service, Directorate of Research and Community Service (DRPM), Directorate General for Research and Development, Ministry of Research, Technology, and Higher Education, Number: 068/SP2H/AMD/LT/DRPM/2020.

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Pratama, R., Muslim, A., Suwandi, S. *et al.* Jackfruit (*Artocarpus heterophyllus*), a new host plant of *Ceratocystis* wilt in South Sumatra, Indonesia. *Australasian Plant Dis. Notes* **16,** 24 (2021). https://doi.org/10.1007/s13314-021-00435-x

- Received22 February 2021
- Accepted04 August 2021
- Published11 September 2021
- DOIhttps://doi.org/10.1007/s13314-021-00435-x

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Keywords

- Sudden death disease
- Moraceae
- *Ceratocystis fimbriata* sensu stricto

Australasian Plant Disease Notes (2021) 16:24
https://doi.org/10.1007/s13314-021-00435-x

Jackfruit (*Artocarpus heterophyllus*), a new host plant of *Ceratocystis* wilt in South Sumatra, Indonesia

Rahmat Pratama[1] · Ahmad Muslim[2] · Suwandi Suwandi[2] · Nurhayati Damiri[2] · Soleha Soleha[1]

Received: 22 February 2021 / Accepted: 4 August 2021

Abstract
In 2019, wilt and sudden death were observed on *Artocarpus heterophyllus* (jackfruit). Identification was performed by sequence analysis of the concatenated β-tubulin and ITS gene regions. Sequencing of the PCR product confirmed this pathogen was *Ceratocystis fimbriata* sensu stricto. This is the first report of *C. fimbriata* causing sudden death disease in *A. heterophyllus* in Indonesia and worldwide.

Keywords Sudden death disease · Moraceae · *Ceratocystis fimbriata* sensu stricto

Jackfruit (*Artocarpus heterophyllus*, Moraceae) is known in Indonesian as "Nangka", and is cultivated widely in many countries with tropical and subtropical climates. Jackfruit is among the most exported fruits worldwide and has considerable nutrition and health benefits (Ranasinghe et al. 2019).

In July 2019, wilt and die-back symptoms were observed for the first time on *A. heterophyllus* in the agricultural field of Sriwijaya University (Indralaya), Plaju (Palembang) and Gelumbang (Prabumulih), Indonesia. Wood of wilted trees showed a brown to black streaking in the woody xylem. Symptoms on the dying Jackfruit wood produced grey to brown lesions and included a streaking pattern of discoloration in the sapwood (Fig. 1a) and in some cases the lesions extended to heartwood (Fig. 1b). The lesion could be found partially or totally affecting the sapwood from the basal stem to the branches. Leaves of dying trees had yellowing symptoms, followed by wilting of the leaves on several lateral branches, drying of twigs and the wilt of the whole tree (Fig. 1c). This type of wilting was termed as sudden death or wilt (Pratama et al. 2021).

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Wood samples were taken from lesions of wilted trees using a knife sterilised in 70% ethanol. Each sample was wrapped in tissue paper and placed in a cool box. The same day, the wood samples (1–20 mm length, 1–2 mm thick) were sandwiched between two slices of fresh carrot and placed on sterile dry paper in plastic boxes at 25 °C following the method of Moller and DeVay (1968) (Fig. 1d). After 5–10 days, hat-shaped spores of putative *Ceratocystis* pathogens were placed on 2% (w/v) malt extract agar (MEA) (Merck, Germany), and incubated at 25 °C in a laboratory. The isolated fungi were initially identified based on morphological characteristics of a 14 day old culture. Mycelium on MEA grey, reverse side of colony olivaceous grey; submerged mycelium darkening as the ascomata develop forming fine, radiating fibrils.

Morphological traits of fruiting bodies and spores were observed under an optical Olympus CX33 microscope. Ascomatal bases dark brown to black, base subglobose to globose and measured (n = 100), 131.5–250.7 × 101.6–236.5 µm (Fig. 2a). Ascomata necks erect, occasionally curved, black at the base becoming subhyaline towards the apex, smooth to crenulate, 324.7–579.1 µm long including ostiolar hyphae (Fig. 2b). Phialides pale brown to hyaline (Fig. 2c). Ascospores hat-shaped, 3.4–6.8 × 2.1–6.2 µm (Fig. 2d). Bacilliform conidia 11.1–36.1 × 2.1–7.4 µm (Fig. 2e). Barrel conidia 4.4–16.1 × 2.7–6.9 µm (Fig. 2f). Chlamydospores oval, thick walled, smooth, 6.7–16.5 × 5.9–12.9 µm (Fig. 2g). Based on these morphological characters, the fungus was identified as *Ceratocystis fimbriata.* Two representative isolates were deposited at the ICBB Culture Collection

Published online: 11 September 2021

Fig. 1 Symptoms of *Ceratocystis fimbriata* wilt disease in *Artocarpus heterophyllus:* **a** vascular discoloration of infected tree; **b** the discolored wood extended to the heartwood of the basal stem; **c** three-year-old tree with wilted, yellowing leaves and rotten fruit; **d** isolation of the fungus from discoloured xylem showing dark mycelium and sporulation on the carrot slices after 7 days

for Microorganisms and Cell Culture, Indonesian Center for Biodiversity and Biotechnology, (Bogor, Indonesia) as accessions ICBB9852 and ICBB9853.

To confirm the species identification, isolates were cultured on potato dextrose broth (PDB) at room temperature for one week. Mycelium was filtered through Whatman filter paper and genomic DNA was extracted from the fungal mycelial mat using YeaStar Genomic DNA Kit (Zymo Research Corporation, California, USA). PCR conditions and reactions for two gene regions were used to identify the *Ceratocystis* isolates; the Internal Transcribed Spacer (ITS) with primers ITS 1 and ITS4 (White et al. 1990) and part of the β-tubulin (βt) gene with primers βt1a and βt1b (Glass and Donaldson 1995). Amplifications were carried out in 50 µl reactions containing 20 µl DreamTaq Green PCR Master Mix (Eppendorf, Germany) (DreamTaq DNA Polymerase, 2X DreamTaq Green buffer, dNTPs, and 4 mM $MgCl_2$), 1.5 µl of each forward and reverse primer, 4 µl of DNA template and 23 µl sterilised water. The PCRs were performed with a C1000 Touch™ thermal cycler (Bio-Rad, USA). The PCR cycling parameters were as follows: initial denaturation for 5 min at 95 °C, followed by 35 cycles at 95 °C for 30 s, 56 °C for 45 s and 72 °C for 1 min. Amplification was completed at 72 °C for 10 min and the PCR product was stored at 10 °C. The PCR amplicons were sequenced at 1st BASE (Malaysia).

For the ITS and β-tubulin, amplification resulted in fragments of ~ 550 base pairs (bp) in size. The sequences of the amplified products were then deposited in the GenBank database and assigned accession numbers isolate ICBB9852 (MT355410; MT412106), isolate ICBB9853 (MT355412; MT412108), and isolate CAAW30817 (MT355413, MT412109) for the ITS and β-tubulin. β-tubulin datasets were generated using ex-type and ex-paratype sequences representing species in the Latin American (LAC) and Asian clade (AC) (Table 1) of the *C. fimbriata* species complex (Fourie et al. 2015; Oliveira et al. 2015; Barnes et al. 2018). To determine relatedness of isolates from jackfruit with known *C. fimbriata* populations, the ITS sequence was manually aligned with known ITS haplotypes as designated by Harrington et al. (2014), Li et al. (2016) and phylogenetic analyses were performed. Maximum Parsimony

Table 1 *Ceratocystis* isolates included in the phylogenetic analyses

Species	Haplotype	Isolates no	Host	Origin	GenBank accession no	
					ITS	β-Tubulin
C. fimbriata	ITS1a	C1418	*Ipomoea batatas*	USA	AY157956	–
	ITS1	C1857	*Ficus carica*	Brazil	HQ157542	–
	ITS1b	CMW4797	*Eucalyptus* sp.	Congo	FJ236733	–
	ITSb	CMW9998	*Eucalyptus* sp.	South Africa	FJ236721	–
	ITS2	C1655	*Mangifera indica*	Brazil	HQ157546	–
	ITS3	C1440	*Eucalyptus* sp.	Brazil	HQ157544	–
	ITS3	CMW5328	*E. grandis*	Uganda	AF395686	–
	ITS4	C1442	*Eucalyptus* sp.	Brazil	HQ157545	–
	ITS5	**ICBB9852**	***Artocarpus heterophyllus***	**Indonesia**	**MT355410**	**MT412106**
	ITS5	**ICBB9853**	***A. heterophyllus***	**Indonesia**	**MT355412**	**MT412108**
	ITS5	**CAAW30817**	***A. heterophyllus***	**Indonesia**	**MT355413**	**MT412109**
	ITS5	CMW38737	*E. grandis*	Zimbabwe	KF878326	KF878335
	ITS5	C1345	*Eucalyptus* sp.	Brazil	AY157966	–
	ITS5	A59662	*Camellia sinensis*	China	KF650948	–
	ITS5	YM061	*Colocasia esculenta*	China	AM712445	–
	ITS5	P20053	*Punica granatum*	China	AM292204	–
	ITS5	C1	*Acacia* sp.	Vietnam	MF033455	MF040712
	ITS5	CMW22563	*A. mangium*	Indonesia	EU588656	EU588636
	ITS5	WRC	*Lansium domesticum*	Indonesia	MT229127	MW013766
	ITS6	C2055	*Mangifera* sp.	Brazil	HQ157548	–
	ITS6z	CMW13582	*Hypocryphalus mangifera*	Oman	KC261853	–
	ITS6z	WBC	*L. domesticum*	Indonesia	MT229128	MW013767
	ITS7b	CMW13851	*M. indica*	Oman	AY953383	EF433308
	ITS7b	CMW23634	*M. indica*	Pakistan	EF433302	EF433311
	ITS7b	CMW22579	*A. mangium*	Indonesia	EU588658	–
	ITS8a	CMW8856	*Citrus* sp.	Colombia	AY233867	–
	ITS8c	CMW17808	*Eucalyptus* sp.	Colombia	EF127990	–
	ITS8e	CMW22092	*E. deglupta*	Ecuador	FJ151432	–
	ITS9	C1558	*M. indica*	Brazil	AY157965	–
	ITS9	C1914	*C. esculenta*	Brazil	HQ157540	–
	ITS10	C994	*M. indica*	Brazil	AY157964	–
	ITS10a	Cf4	*M. indica*	Brazil	EF042605	–
	ITS11	C1865	*C. esculenta*	Brazil	AY526286	–
	ITS12	C1926	*C. esculenta*	Brazil	HQ157541	–
	ITS14	C1688	*M. indica*	Brazil	AY526291	–
	ITS15	C925	*Gmelina arborea*	Brazil	AY157967	–
	ITS16	C924	*G. arborea*	Brazil	HQ157539	–
C. pirilliformis	Asian clade (AC)	CMW6569	*E. nitens*	Australia	–	DQ371652
	AC	CMW6579	*E. nitens*	Australia	–	DQ371653
C. polychroma	AC	CMW11424	*Syzygium aromaticum*	Indonesia	–	AY528966
	AC	CMW11436	*S. aromaticum*	Indonesia	–	AY528967
C. atrox	AC	CMW19383	*E. grandis*	Australia	–	EF070430
	AC	CMW19385	*E. grandis*	Australia	–	EF070431
C. neglecta	Latin American clade (LAC)	CMW17808	*E. grandis*	Colombia	–	EU881898
	LAC	CMW18194	*E. grandis*	Colombia	–	EU881899
C. colombiana	LAC	CMW5751	*Coffea arabica*	Colombia	–	AY177225
	LAC	CMW5761	*C. arabica*	Colombia	–	AY177224
C. cacaofunesta	LAC	CMW14803	*Theobroma cacao*	Ecuador	–	KJ631108

Table 1 (continued)

Species	Haplotype	Isolates no	Host	Origin	GenBank accession no	
					ITS	β-Tubulin
	LAC	CMW15051	*T. cacao*	Costa Rica	–	KJ601510
C. papillata	LAC	CMW8850	*Citrus*×*Tangelo hybrid*	Colombia	–	AY233875
	LAC	CMW8856	*Citrus limon*	Colombia	–	AY233874
C. fimbriata	LAC	CMW14797	*M. indica*	Brazil	–	EF433307
	LAC	CMW28907	*M. indica*	Brazil	–	FJ200270
	LAC	CMW1547	*I. batatas*	Papua New Guinea	–	EF070443
	LAC	C1421	*I. batatas*	USA	–	KF302689
C. fimbriatomima	LAC	CMW24174	*Eucalyptus hybrid*	Venezuela	–	EF190951
	LAC	CMW24176	*Eucalyptus hybrid*	Venezuela	–	EF190952
C. fimbriata	LAC	CMW21127	*A. crassicarpa*	Indonesia	–	EU588643
	LAC	CMW24664	*Eucalyptus hybrid*	China	–	JQ862720
	LAC	CBS115173	*Gmelina arborea*	Brazil	–	KF302700
	LAC	CBS14653	*C. arabica*	Suriname	–	KF302702
C. platani	LAC	CMW14802	*Platanus occidentalis*	USA	–	EF070425
	LAC	CMW23450	*P. occidentalis*	Greece	–	KJ601513

Isolates from jackfruit in Indonesia are marked in bold

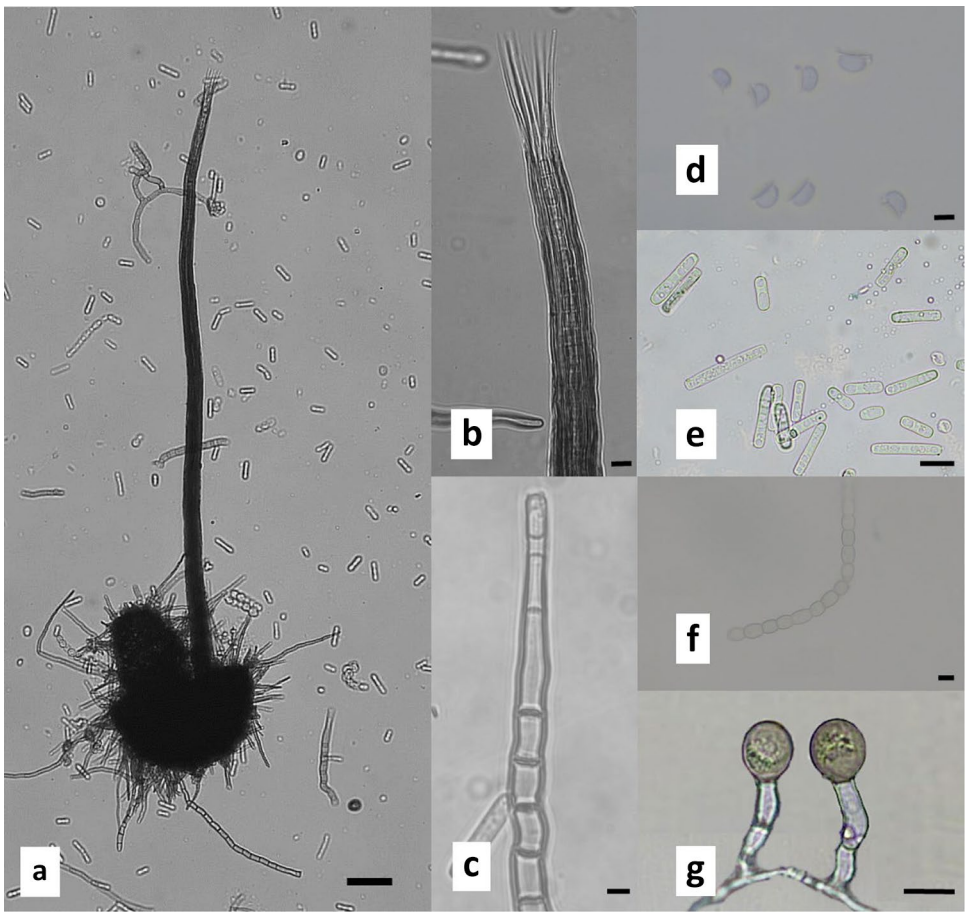

Fig. 2 Morphological characteristics of *Ceratocystis fimbriata* isolated from *Artocarpus heterophyllus* stem lesion: **a** ascomata with pirilliform base, **b** divergent ostiolar hyphae; **c** conidiophore/phialide; **d** hat-shaped ascospores; **e** cylindrical conidia; **f** Chain of barrel-shaped conidia; **g** chlamydospores of various shapes. Scale bars: a = 100 µm; b–c, e–g = 10 µm; d = 5 µm

(MP) analyses were performed in MEGA v. 10 (Kumar et al. 2016; Paul et al. 2018) with 1000 replications. The analysis involved 38 (β-tubulin) and 37 (ITS) nucleotide sequences. All positions containing gaps and missing data were eliminated. There were 408 (β-tubulin) and 518 (ITS) positions in the final dataset. *Ceratocystis variospora* was

used as the out-group. β-tubulin sequence of our isolates confirmed the assignment to LAC of *C. fimbriata* sensu lato (Fig. 3a). Manual alignment of the ITS sequences with previously described ITS genotypes (Harrington et al. 2014; Li et al. 2016) grouped the isolates into ITS5 haplotype of *C. fimbriata* sensu stricto (Fig. 3b). Consistency (CI), retention (RI), and composite indexes (CoI) for β-tubulin were 0.566667, 0.845238, 0.668011, respectively and ITS was 0.933333, 0.976563, 0.932836, respectively.

The pathogenic potential of isolates was evaluated by the under bark inoculation method described by O'Gara et al. (1997) using five-month-old *A. heterophyllus* seedlings with stem diameters of 6–8 mm and heights < 1.5 m were prepared for pathogenicity test. Seedlings were grown in 10 cm diameter plastic pots containing a soil mix (topsoil + peat + chicken manure) under a 50% shading net. Plants were watered daily to maintain humidity, and any mortality occurring before the end of the experiment was recorded. Wounds were made on the stems of the seedlings using a cork borer (4 mm diam.), and mycelial discs (4 mm diam.) taken from an actively growing colony of *C. fimbriata* on 2% MEA (14 days) (Pratama et al. 2021) were placed in the wounds with the mycelium facing inwards. These were covered with Parafilm (Pechiney, Menasha, Wisconsin) to reduce contamination and desiccation. Ten plants of each tree species were inoculated with sterile MEA plugs to serve as controls (Fig. 4a). Fungal isolates were re-isolated and re-identified using morphological characteristics for confirmation of Koch's postulates. In pathogenicity tests, initial symptoms appeared two weeks post-inoculation as brown lesions at the inoculation site on the wood (Fig. 4b). Forty-five days after inoculation, plants exhibited wilt symptoms, lesions of wood discoloration extended to heartwood (Fig. 4c) and length of discolouration (downward + upward) was 17.88 until 34.74 cm. When re-isolated, the fungus was phenotypically identical to the prior isolate of *C. fimbriata* (ICBB9852, ICBB9853, CAAW30817).

This is the first report of *C. fimbriata* causing wilt and dieback in Jackfruit in Indonesia and worldwide. The symptoms

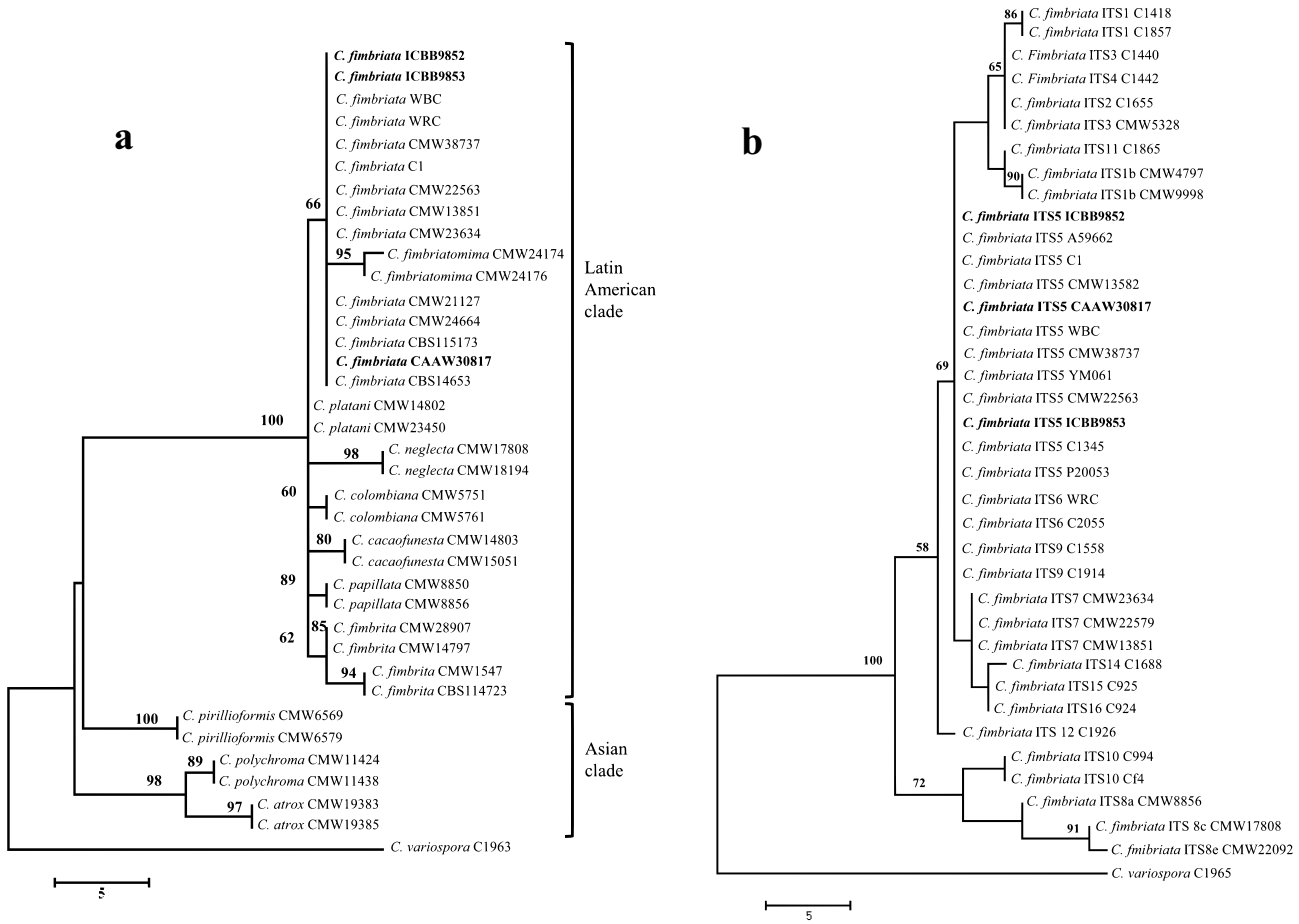

Fig. 3 Phylogenetic tree constructed by MEGA with Maximum Parsimony (MP) analysis by **a** β-tubulin sequences from Jackfruit tree in Indonesia (marked in bold) and other species in the Latin American and Asian clade of the *C. fimbriata* species complex. **b** ITS sequences from Jackfruit tree in Indonesia (marked in bold) and genotypes (sequences) of the *C. fimbriata* sensu stricto

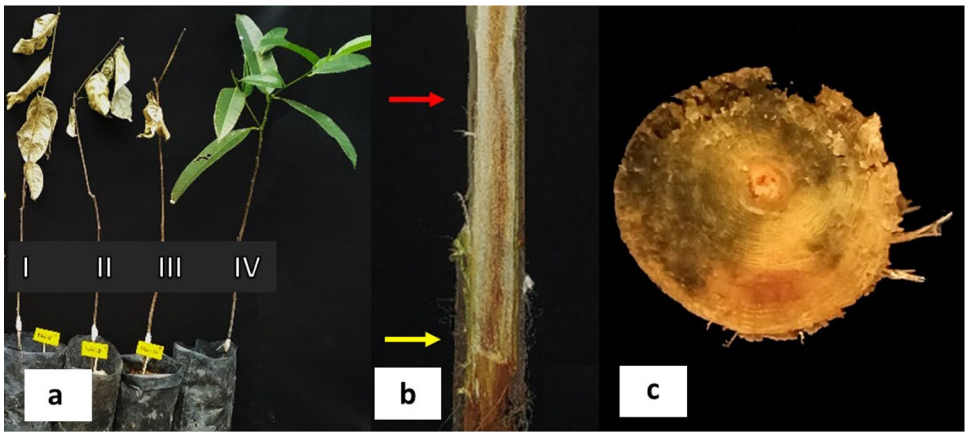

Fig. 4 Response of *Artocarpus heterophyllous* seedlings 45 days after under-bark inoculation with mycelium of *Ceratocystis*. **a** total wilting of plant inoculated with ICBB9852 (I), CAAW30817 (II), ICBB9853 (III) and the healthy control seedling (IV); **b** yellow arrow indicates the point of inoculation and red arrow the lesion boundary; **c** The discoloured wood extended to the heartwood of the basal stem of the seedling

of *C. fimbriata* wilt disease in Jackfruit include cankers on stems, with the stems becoming chapped as though torn apart, fruit rot and progressive loss of the canopy resulting in tree death. *Ceratocystis fimbriata* is a serious wilt pathogen of jackfruit, as well as of *A. mangium* and *A. crassicarpa* in Indonesia (Tarigan et al. 2011), *Lansium domesticum* in Indonesia (Suwandi et al. 2021) and Pomegranate in China (Li et al. 2016). *Ceratocystis fimbriata* infections of native trees in these countries could potentially lead to devastation of important components of the natural biodiversity in Indonesia.

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