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## Australasian Plant Disease Notes

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

--Manuscript Draft--

<b>Manuscript Number:</b>	APDN-D-21-00015	
<b>Full Title:</b>	Jackfruit ( <i>Artocarpus heterophyllus</i> ), a New Host Plant of <i>Ceratocystis</i> Wilt from South Sumatra, Indonesia	
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<b>Abstract:</b>	<p><i>Artocarpus heterophyllus</i> (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 <math>\beta</math>-tubulin and ITS gene regions as <i>Ceratocystis manginecans</i>. Pathogenicity tests were conducted by inoculating wounds on the stems of <i>A. heterophyllus</i> 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 <i>C. manginecans</i> from the inoculated plants. This is the first report of <i>C. manginecans</i> causing wilt and die-back disease on <i>A. heterophyllus</i> in Indonesia.</p>	

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

3  
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14 **Abstract**

15 *Artocarpus heterophyllus* (jackfruit) is an economically important fruit crop in Indonesia and  
16 widely grown throughout the country. Growers in South Sumatra recently reported the death  
17 of jackfruit trees with symptoms of vascular stain, yellowing and browning leaves until wilting  
18 on several lateral branches and trees, which in later stages of the disease lost most of their  
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20 morphological appearance and comparisons of DNA sequence data for the  $\beta$ -tubulin and ITS  
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22 wounds on the stems of *A. heterophyllus* with mycelial plugs (4 mm diam.). After 45 days of  
23 inoculation, the fungi produced lesions of significant length and severe foliar symptoms in  
24 inoculated plants. Koch's postulates were fulfilled by reisolation of *C. manginecans* from the  
25 inoculated plants. This is the first report of *C. manginecans* causing wilt and die-back disease  
26 on *A. heterophyllus* in Indonesia.

27 **Keywords:** Sudden death · Pathogenicity · Jackfruit · Indonesia · Wilt disease  
28

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

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

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

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

52 Samples of diseased trunks were collected from three to five year-old trees,  
53 approximately ten trees in location sampling, in August and October 2019. Wood samples were  
54 taken from lesions of wilted trees using a knife sterilised in 70% ethanol. Wood samples were  
55 collected from *A. heterophyllus* showing brown to black streaking in the woody xylem. Each  
56 sample was wrapped in tissue paper and placed in a cool box. The same day, the wood samples  
57 (1–20 mm length, 1–2 mm thick) were sandwiched between two slices of fresh carrot and  
58 placed on sterile dry paper in plastic boxes at 25 °C following the method of Moller and DeVay  
59 (1968) (Fig. 1c). After 5–10 days, hat-shaped spores of putative *Ceratocystis* pathogens were  
60 placed on 2% (w/v) malt extract agar (MEA) (Merck, Germany), and incubated at 25 °C in a  
61 laboratory. The isolated fungi were initially identified based on morphological characteristics  
62 of a 14 day old culture. Mycelium on MEA grey, reverse side of colony olivaceous grey;  
63 submerged mycelium darkening as the ascomata develop forming fine, radiating fibrils (Fig.  
64 1d).

65 Morphological traits of fruiting bodies and spores were observed under an optical  
66 Olympus CX33 microscope. Ascomata developing within seven days and mature within ten  
67 days, superficial or partly embedded in the agar, dark brown to black. Ascomata of *Ceratocystis*  
68 with necks supporting sticky masses of ascospores on the carrot slices. Ascomatal bases dark  
69 brown to black, base subglobes to globes and measured (n=100), 131.5 to 250.7 × 101.6 to  
70 236.5 µm (length/width) (Fig. 2a). Ascomata necks erect, occasionally curved, black at the base

71 becoming subhyaline towards the apex, smooth to crenulate, 324.7 to 579.1  $\mu\text{m}$  long including  
72 ostiolar hyphae (Fig.2b). Phialides pale brown to hyaline (Fig.2c). Ascospores hat-shaped, 3.4  
73 to  $6.8 \times 2.1$  to  $6.2 \mu\text{m}$  (length/width) (Fig.2d). Bacilliform conidia  $11.1$  to  $36.1 \times 2.1$  to  $7.4 \mu\text{m}$   
74 (length/width) (Fig.2e). Barrel conidia  $4.4$  to  $16.1 \times 2.7$  to  $6.9 \mu\text{m}$  (length/width) (Fig.2f).  
75 Chlamydospores oval, thick walled, smooth,  $6.7$  to  $16.5 \times 5.9$  to  $12.9 \mu\text{m}$  (length/width)  
76 (Fig.2g).

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

84 PCR conditions and reactions for two gene regions were used to identify the  
85 *Ceratocystis* isolates; the Internal Transcribed Spacer (ITS) and part of the  $\beta$ -tubulin ( $\beta\text{t}$ ) gene.  
86 Amplifications were carried out in  $50 \mu\text{l}$  reactions containing  $20 \mu\text{l}$  DreamTaq Green PCR  
87 Master Mix (Eppendorf, Germany) (DreamTaq DNA Polymerase, 2X DreamTaq Green buffer,  
88 dNTPs, and  $4 \text{ mM MgCl}_2$ ),  $1.5 \mu\text{l}$  of each forward and reverse primer,  $4 \mu\text{l}$  of DNA template  
89 and  $23 \mu\text{l}$  sterilised water. The PCRs were performed with a C1000 Touch™ thermal cycler  
90 (Bio-Rad, USA). The PCR cycling parameters were as follows: initial denaturation for 5 min  
91 at  $95 \text{ }^\circ\text{C}$ , followed by 35 cycles at  $95 \text{ }^\circ\text{C}$  for 30 s,  $56 \text{ }^\circ\text{C}$  for 45 s and  $72 \text{ }^\circ\text{C}$  for 1 min.  
92 Amplification was completed at  $72 \text{ }^\circ\text{C}$  for 10 min and the PCR product was stored at  $10 \text{ }^\circ\text{C}$ .  
93 The PCR amplicons were sequenced at 1st BASE (Malaysia). Raw sequence data were  
94 assembled, examined, and manually edited using Genestudio 2.1.1.5 (Genestudio, Suwanee,  
95 Georgia) and BioEdit software (Hall 1999).

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

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

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

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

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



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

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

150

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157

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

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

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

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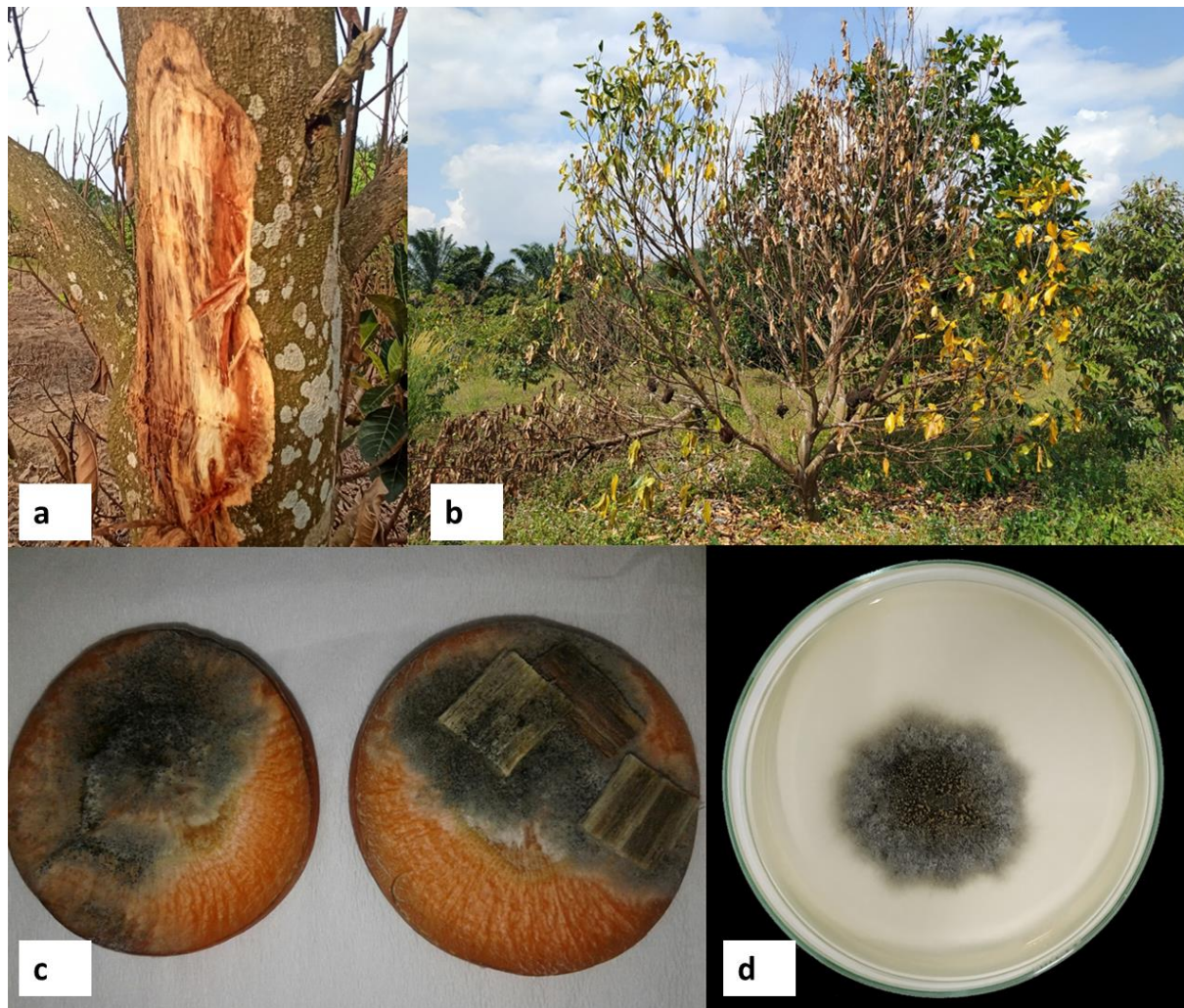
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245 **Fig. 1** Symptoms of *Ceratocystis manginecans* wilt disease in *Artocarpus heterophyllus*:  
246 **a.** vascular discoloration of infected tree; **b.** three-year-old tree with wilted, yellowing leaves  
247 and rotten fruit; **c.** isolation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

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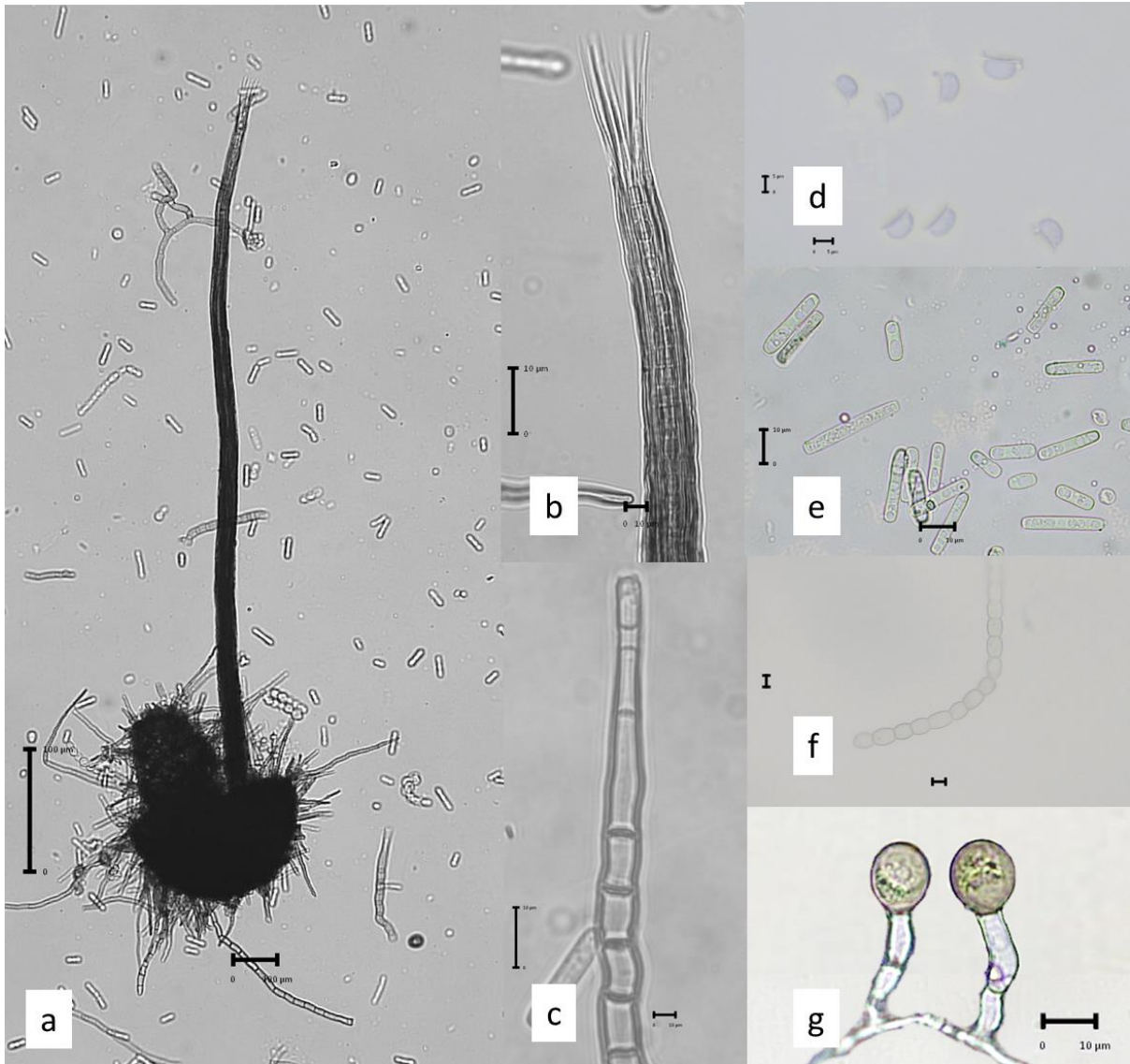
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261 **Fig. 2.** Morphological characteristics of *Ceratocystis manginecans* isolated from *Artocarpus*  
 262 *heterophyllus* stem lesion: **a.** ascomata with pirilliform base, **b.** conidiophore/phialide; **c.**  
 263 Divergent ostiolar hyphae;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

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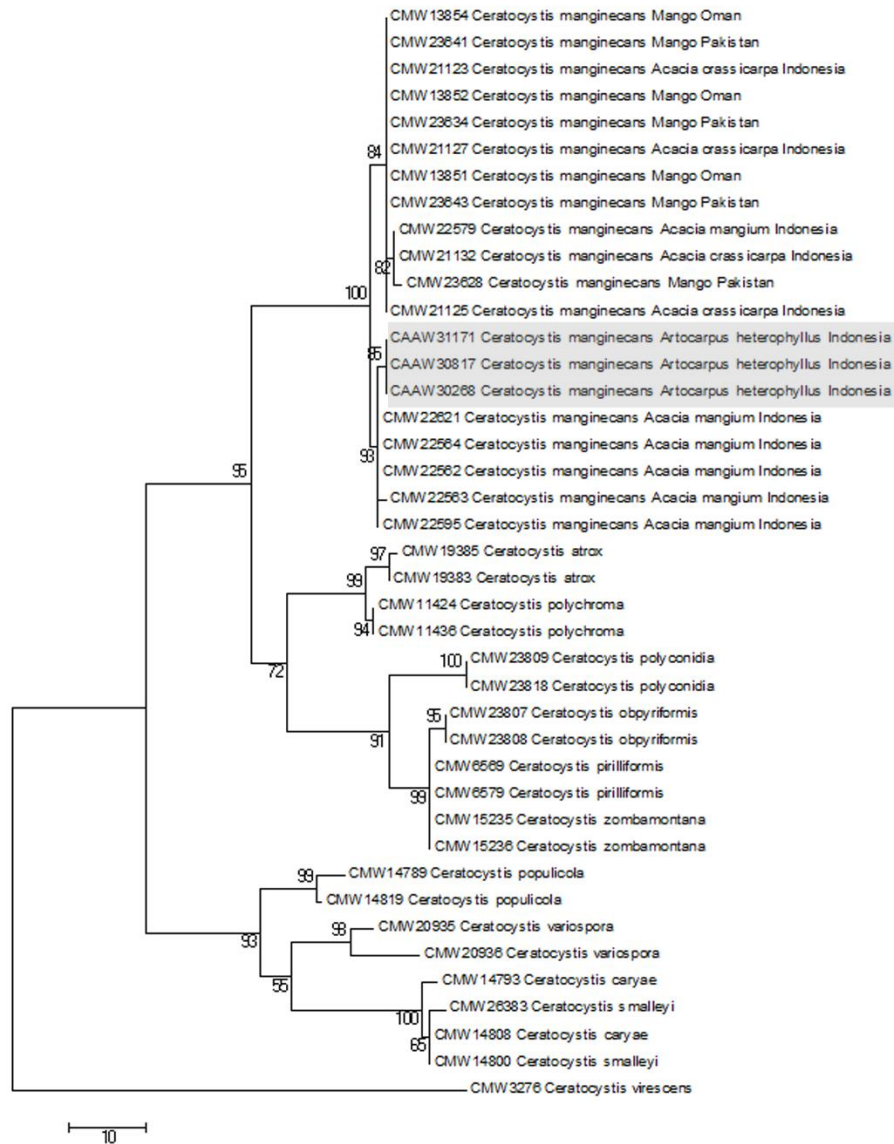
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275 **Fig. 3** Phylogenetic tree constructed by MEGA with Maximum Parsimony (MP) search for the  
 276 combined sequence data of the ITS region and  $\beta$ -tubulin gene (CAAW31171, CAAW30268,  
 277 and CAAW30817) and their related species from GenBank. Consistency (CI), retention (RI),  
 278 and composite indexes (CoI) are 0.725275, 0.935733, and 0.734932 for all sites and parsimony-  
 279 informative sites. The percentage of replicate trees in which the associated taxa clustered  
 280 together in the bootstrap test (1000 replicates) is shown next to the branches. Bootstrap values  
 281  $>50\%$  are indicated above the branches. The analysis involved 41 nucleotide sequences. All  
 282 positions containing gaps and missing data were eliminated. There were 856 positions in the  
 283 final dataset. *Ceratocystis virescens* was used as the out-group.

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**Revise before review 1**

a. muslim unsri &lt;a\_muslim@unsri.ac.id&gt;

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**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

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APDN <em@editorialmanager.com>  
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To: "A. Muslim" <a\_muslim@unsri.ac.id>

Mon, Feb 22, 2021 at 4:34 AM

Submission ID: APDN-D-21-00015

Dear Dr. Muslim,

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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--

<b>Manuscript Number:</b>	APDN-D-21-00015R1	
<b>Full Title:</b>	Jackfruit ( <i>Artocarpus heterophyllus</i> ), a New Host Plant of <i>Ceratocystis</i> Wilt from South Sumatra, Indonesia	
<b>Article Type:</b>	Plant Disease Note	
<b>Keywords:</b>	Sudden death; Pathogenicity; Jackfruit; Indonesia; Wilt disease	
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<b>Funding Information:</b>	Kementerian Riset Teknologi Dan Pendidikan Tinggi Republik Indonesia (068/SP2H/AMD/LT/DRPM/2020)	Dr. A. Muslim
<b>Abstract:</b>	In 2019, wilt and sudden death were observed on <i>Artocarpus heterophyllus</i> (jackfruit) has been noted. Identification was performed by sequence analysis of the concatenated $\beta$ -tubulin and ITS gene regions. Sequencing of the PCR product confirmed this pathogen was <i>Ceratocystis manginecans</i> . <i>C. manginecans</i> causing sudden death disease in <i>A. heterophyllus</i> is being reported for the first time in Indonesia and worldwide.	
<b>Response to Reviewers:</b>	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 Sumatra, 30662, Indonesia. Telephone +628117826119.  Thank you for your consideration of the manuscript.	

Sincerely,  
A. Muslim, Ph.D



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Editor in Chief  
Prof. Dagmar Hanold  
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Australia

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 advised 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 manuscripts 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.

Please address all correspondence concerning this manuscript to me at: [a.muslim@unsri.ac.id](mailto: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

# 1 **Jackfruit (*Artocarpus heterophyllus*), a New Host Plant of *Ceratocystis* Wilt** 2 **in South Sumatra, Indonesia**

3  
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## 14 **Abstract**

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

20 **Keywords:** Sudden death · Pathogenicity · Jackfruit · Indonesia · Wilt disease  
21

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

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

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

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

45 Morphological traits of fruiting bodies and spores were observed under an optical  
46 Olympus CX33 microscope. Ascumatal bases dark brown to black, base subglobes to globes  
47 and measured (n=100), 131.5 to 250.7×101.6 to 236.5 µm (length/width) (Fig. 2a). Ascomata  
48 necks erect, occasionally curved, black at the base becoming subhyaline towards the apex,  
49 smooth to crenulate, 324.7 to 579.1 µm long including ostiolar hyphae (Fig. 2b). Phialides  
50 pale brown to hyaline (Fig. 2c). Ascospores hat-shaped, 3.4 to 6.8×2.1 to 6.2 µm  
51 (length/width) (Fig. 2d). Bacilliform conidia 11.1 to 36.1×2.1 to 7.4 µm (length/width) (Fig.  
52 2e). Barrel conidia 4.4 to 16.1×2.7 to 6.9 µm (length/width) (Fig. 2f). Chlamydospores oval,  
53 thick walled, smooth, 6.7 to 16.5×5.9 to 12.9 µm (length/width) (Fig. 2g). Based on  
54 morphological characters, the fungus was identified as *C. manginecans*.

55 To confirm the species identification, isolates were cultured on potato dextrose broth  
56 (PDB) at room temperature for one week. Mycelial mat was filtered through Whatman filter  
57 paper and genomic DNA was extracted from fungal mycelial mat using YeaStar Genomic  
58 DNA Kit (Zymo Research Corporation, California, USA). PCR conditions and reactions for  
59 two gene regions were used to identify the *Ceratocystis* isolates; the Internal Transcribed  
60 Spacer (ITS) and part of the  $\beta$ -tubulin ( $\beta$ t) gene. Amplifications were carried out in 50 µl  
61 reactions containing 20 µl DreamTaq Green PCR Master Mix (Eppendorf, Germany)  
62 (DreamTaq DNA Polymerase, 2X DreamTaq Green buffer, dNTPs, and 4 mM MgCl<sub>2</sub>), 1.5 µl  
63 of each forward and reverse primer, 4 µl of DNA template and 23 µl sterilised water. The  
64 PCRs were performed with a C1000 Touch™ thermal cycler (Bio-Rad, USA). The  
65 PCR cycling parameters were as follows: initial denaturation for 5 min at 95 °C, followed by  
66 35 cycles at 95 °C for 30 s, 56 °C for 45 s and 72 °C for 1 min. Amplification was  
67 completed at 72 °C for 10 min and the PCR product was stored at 10 °C. The PCR amplicons  
68 were sequenced at 1st BASE (Malaysia).

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

71 database and assigned accession numbers isolate CAAW31171 (MT355410; MW717653),  
72 isolate CAAW30817 (MT355413, MW717656), and isolate CAAW30268 (MT355412;  
73 MW717655) for the ITS and  $\beta$ -tubulin, respectively, and they were compared with the  
74 sequences of *C. manginecans* available at GenBank. Blast searches in GenBank indicated that  
75 our isolates grouped within *C. manginecans* species with 99% identity of the sequences. The  
76 two gene regions (ITS and  $\beta$ t) were combined and analysed as a single dataset. Maximum  
77 Parsimony (MP) analyses were performed in MEGA v. 10 (Kumar et al. 2016; Paul et al.  
78 2018) with 1000 bootstrap replications. According to the phylogenetic relationships derived  
79 from the Maximum Parsimony (MP) analyses, our *C. manginecans* isolates (CAAW31171,  
80 CAAW30817, CAAW30268) in *A. heterophyllum* was closely related to *C. manginecans* in  
81 *Mangifera indica* and *Acacia mangium* (Fig. 3). This sequence similarity to prior cases of *C.*  
82 *manginecans* corroborates the identification by phenotypic characteristics, suggesting that the  
83 causal agent of suddendead disease on *A. heterophyllum* in Indonesia, represented by the  
84 CAAW31171, CAAW30817, CAAW30268 isolates, should be regarded as *C. manginecans*.

85 The pathogenic potential of isolates was evaluated by the under bark inoculation  
86 method described by O’Gara et al. (1997) using Five-month-old *A. heterophyllum* seedlings  
87 with stem diameters of 6-8 mm and heights <1.5 m were prepared for pathogenicity test.  
88 Wounds were made on the stems of the seedlings using a cork borer (4 mm diam.), and  
89 mycelial discs (4 mm diam.) taken from an actively growing colony of *C. manginecans* on  
90 2% MEA (14 days) (Tarigan et al. 2010; Tarigan et al. 2011; Chi et al. 2019a) were placed in  
91 the wounds with the mycelium facing downwards. These were covered with Parafilm  
92 (Pechiney, Menasha, Wisconsin) to reduce contamination and desiccation. Ten plants of each  
93 tree species were inoculated with sterile MEA plugs to serve as controls. Fungal isolates were  
94 re-isolated and re-identified using morphological characteristics for Koch’s postulates  
95 confirmation. The fungi were shown to be pathogenic in young *A. heterophyllum*, with plants  
96 exhibiting wilt symptoms 45 days after inoculation (data not shown). When re-isolated, the  
97 fungus was phenotypically identical to the prior isolate of *C. manginecans* (CAAW31171,  
98 CAAW30817, CAAW30268).

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

105 2011; Chi et al. 2019b), a serious pathogen wilt of mango trees in Oman and Pakistan (Van  
106 Wyk et al., 2007) caused by *C. manginecans*, previously reported in Pakistan (Al-Adawi et  
107 al. 2013). *C. manginecans* infecting native trees in these countries is serious and could  
108 potentially lead to the devastation of important components of the natural biodiversity of  
109 Indonesia.

110

### 111 **Acknowledgement**

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114 Research and Community Service (DRPM), Directorate General for Research and  
115 Development, Ministry of Research, Technology, and Higher Education, Number:  
116 068/SP2H/AMD/LT/DRPM/2020 chaired by Ahmad Muslim.

117

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

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

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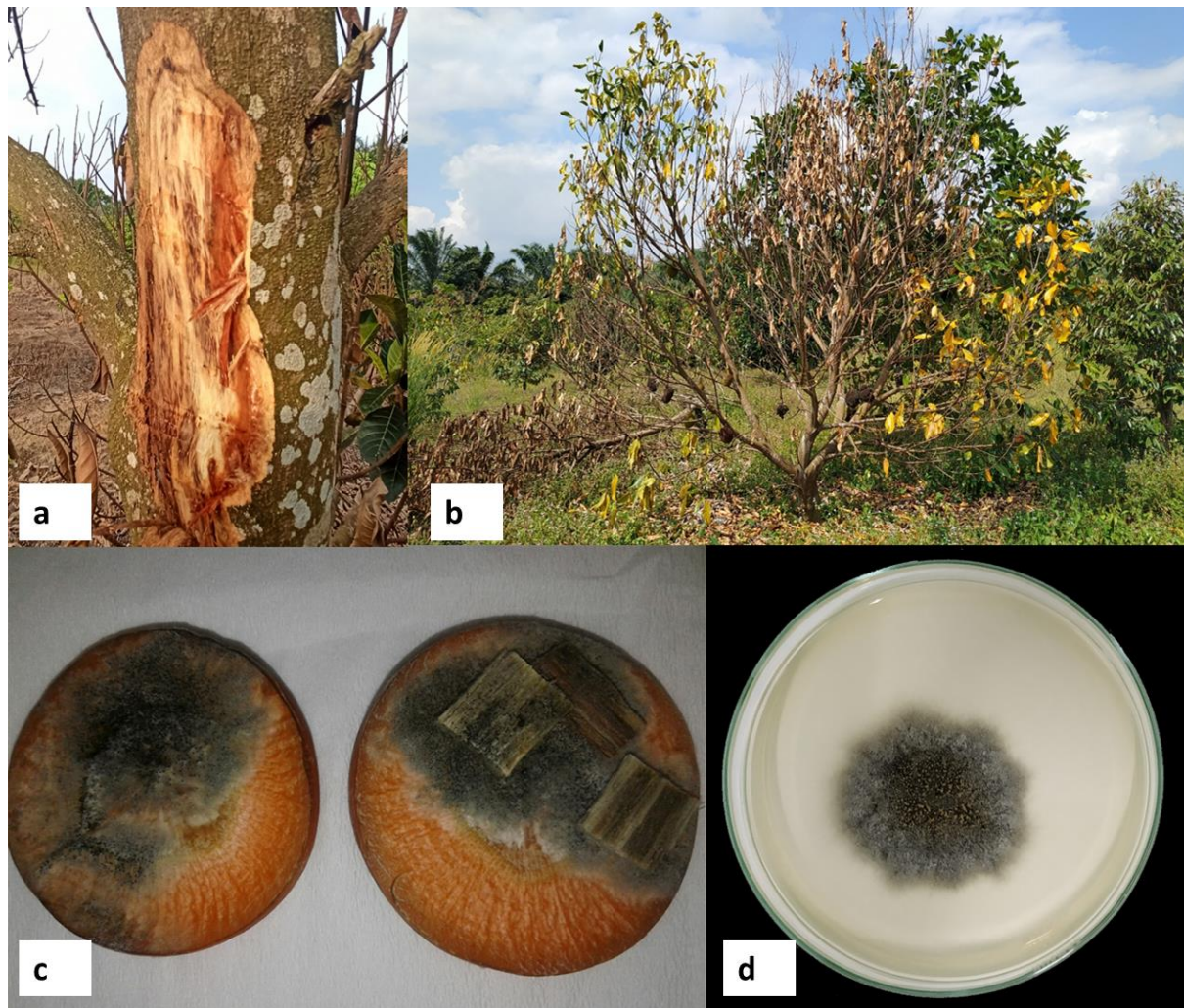
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207 **Fig. 1** Symptoms of *Ceratocystis manginecans* wilt disease in *Artocarpus heterophyllus*: **a.**  
208 vascular discoloration of infected tree; **b.** three-year-old tree with wilted, yellowing leaves  
209 and rotten fruit; **c.** isolation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

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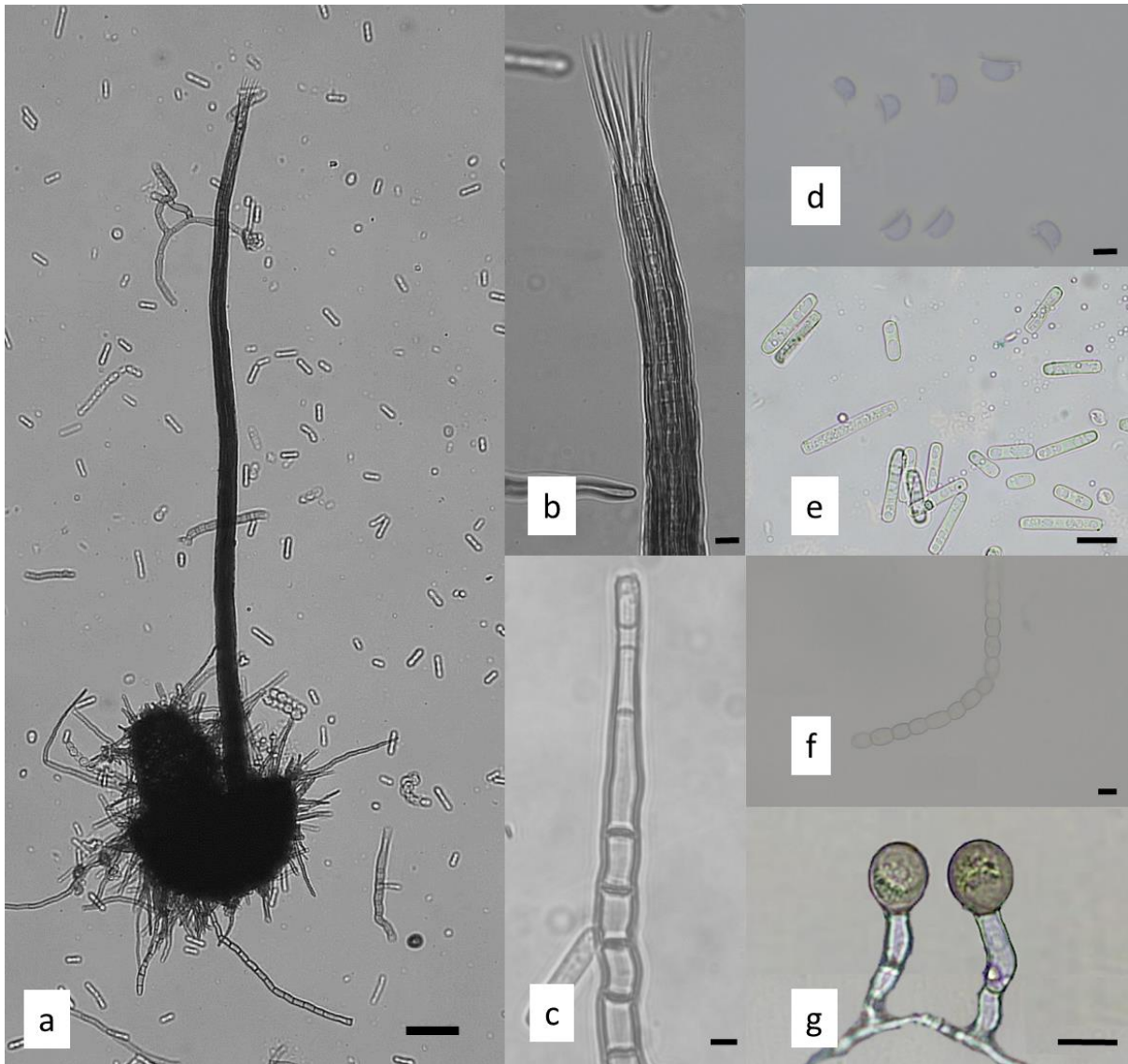
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223 **Fig. 2.** Morphological characteristics of *Ceratocystis manginecans* isolated from *Artocarpus*  
 224 *heterophyllus* stem lesion: **a.** ascomata with pirilliform base, **b.** divergent ostiolar hyphae; **c.**  
 225 conidiophore/phialide;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

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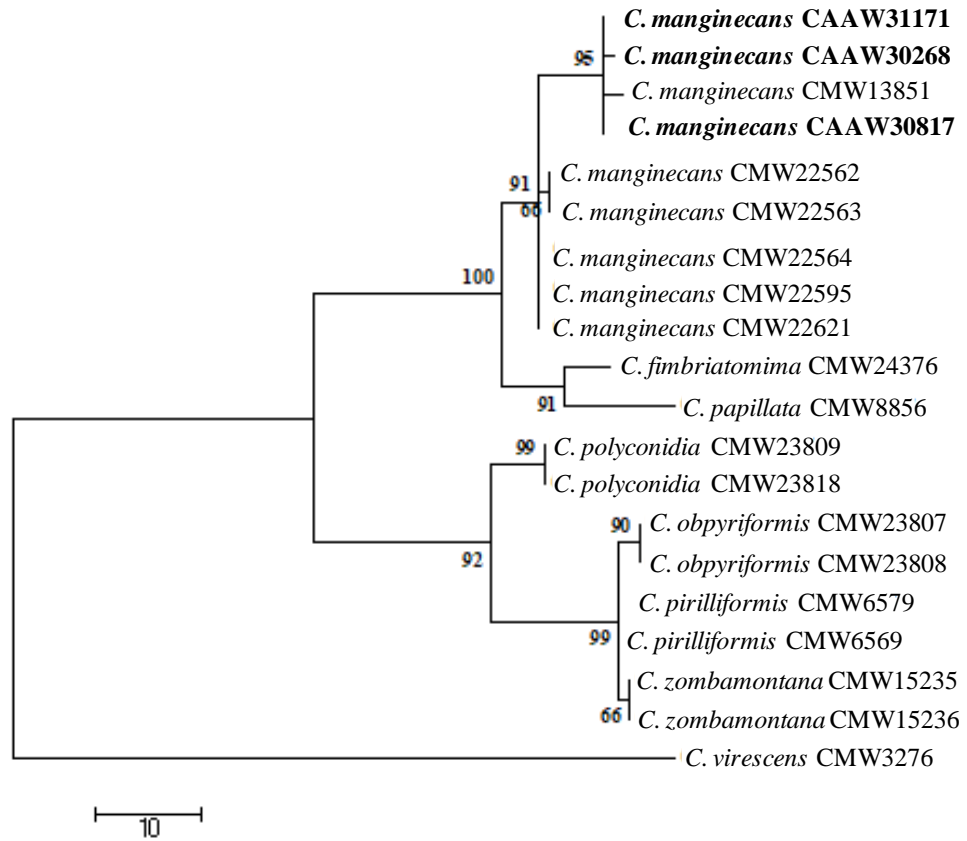
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237 **Fig. 3** Phylogenetic tree constructed by MEGA with Maximum Parsimony (MP) search for  
 238 the combined sequence data of the ITS region and  $\beta$ -tubulin gene (CAAW31171,  
 239 CAAW30268, and CAAW30817) and their related species from GenBank.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



a. muslim unsri &lt;a\_muslim@unsri.ac.id&gt;

---

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

1 message

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**APDN** <em@editorialmanager.com>  
Reply-To: APDN <jude.estrera@springernature.com>  
To: "A. Muslim" <a\_muslim@unsri.ac.id>

Mon, Feb 22, 2021 at 8:26 PM

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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## Revise before review 2

a. muslim unsri &lt;a\_muslim@unsri.ac.id&gt;

---

### Revise before review

2 messages

APDN <em@editorialmanager.com>  
Reply-To: APDN <jude.estrera@springernature.com>  
To: "A. Muslim" <a\_muslim@unsri.ac.id>

Mon, Mar 1, 2021 at 1:06 PM

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:

<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 your 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>  
To: APDN <jude.estrera@springernature.com>

Mon, Mar 1, 2021 at 9:15 PM

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]

## Australasian Plant Disease Notes

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

--Manuscript Draft--

<b>Manuscript Number:</b>	APDN-D-21-00015R1	
<b>Full Title:</b>	Jackfruit ( <i>Artocarpus heterophyllus</i> ), a New Host Plant of <i>Ceratocystis</i> Wilt from South Sumatra, Indonesia	
<b>Article Type:</b>	Plant Disease Note	
<b>Keywords:</b>	Sudden death; Pathogenicity; Jackfruit; Indonesia; Wilt disease	
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	Suwandi Suwandi, PhD	
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	Soleha Soleha, S.P	
<b>Order of Authors Secondary Information:</b>		
<b>Funding Information:</b>	Kementerian Riset Teknologi Dan Pendidikan Tinggi Republik Indonesia (068/SP2H/AMD/LT/DRPM/2020)	Dr. A. Muslim
<b>Abstract:</b>	In 2019, wilt and sudden death were observed on <i>Artocarpus heterophyllus</i> (jackfruit) has been noted. Identification was performed by sequence analysis of the concatenated $\beta$ -tubulin and ITS gene regions. Sequencing of the PCR product confirmed this pathogen was <i>Ceratocystis manginecans</i> . <i>C. manginecans</i> causing sudden death disease in <i>A. heterophyllus</i> is being reported for the first time in Indonesia and worldwide.	
<b>Response to Reviewers:</b>	<p>Associate Editor Alistair McTaggart, Ph.D Australasian Plant Disease Notes Journal Centre for Horticultural Science The University of Queensland Australia</p> <p>Dear Associate Editor,</p> <p>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 Sumatra, 30662, Indonesia. Telephone +628117826119.</p> <p>Thank you for your consideration of the manuscript.</p>	



Sincerely,  
A. Muslim, Ph.D

**R. Pratama<sup>1</sup> · A. Muslim<sup>2\*</sup> · S. Suwandi<sup>2</sup> · N. Damiri<sup>2</sup> · S. Soleha<sup>1</sup>**

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# 1 **Jackfruit (*Artocarpus heterophyllus*), a New Host Plant of *Ceratocystis* Wilt** 2 **in South Sumatra, Indonesia**

## 3 4 **Abstract**

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

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

11

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

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

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

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

45 To confirm the species identification, isolates were cultured on potato dextrose broth  
46 (PDB) at room temperature for one week. Mycelial mat was filtered through Whatman filter  
47 paper and genomic DNA was extracted from fungal mycelial mat using YeaStar Genomic DNA  
48 Kit (Zymo Research Corporation, California, USA). PCR conditions and reactions for two gene  
49 regions were used to identify the *Ceratocystis* isolates; the Internal Transcribed Spacer (ITS)  
50 and part of the  $\beta$ -tubulin ( $\beta$ t) gene. Amplifications were carried out in 50 µl reactions containing  
51 20 µl DreamTaq Green PCR Master Mix (Eppendorf, Germany) (DreamTaq DNA Polymerase,  
52 2X DreamTaq Green buffer, dNTPs, and 4 mM MgCl<sub>2</sub>), 1,5 µl of each forward and reverse  
53 primer, 4 µl of DNA template and 23µl sterilised water. The PCRs were performed with a  
54 C1000 Touch™ thermal cycler (Bio-Rad, USA). The PCR cycling parameters were as follows:  
55 initial denaturation for 5 min at 95 °C, followed by 35 cycles at 95 °C for 30 s, 56 °C for 45 s  
56 and 72 °C for 1 min. Amplification was completed at 72 °C for 10 min and the PCR product  
57 was stored at 10 °C. The PCR amplicons were sequenced at 1st BASE (Malaysia).

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

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

74 The pathogenic potential of isolates was evaluated by the under bark inoculation  
75 method described by O’Gara et al. (1997) using Five-month-old *A. heterophyllum* seedlings  
76 with stem diameters of 6-8 mm and heights <1.5 m were prepared for pathogenicity test.  
77 Wounds were made on the stems of the seedlings using a cork borer (4 mm diam.), and mycelial  
78 discs (4 mm diam.) taken from an actively growing colony of *C. manginecans* on 2% MEA (14  
79 days) (Tarigan et al. 2010; Tarigan et al. 2011; Chi et al. 2019a) were placed in the wounds  
80 with the mycelium facing downwards. These were covered with Parafilm (Pechiney, Menasha,  
81 Wisconsin) to reduce contamination and desiccation. Ten plants of each tree species were  
82 inoculated with sterile MEA plugs to serve as controls. Fungal isolates were re-isolated and re-  
83 identified using morphological characteristics for Koch’s postulates confirmation. The fungi  
84 were shown to be pathogenic in young *A. heterophyllum*, with plants exhibiting wilt symptoms  
85 45 days after inoculation (data not shown). When re-isolated, the fungus was phenotypically  
86 identical to the prior isolate of *C. manginecans* (CAAW31171, CAAW30817, CAAW30268).

87 This is the first report of *C. manginecans* causing wilt and die-back in Jackfruit in  
88 Indonesia and worldwide. The symptoms of *C. manginecans* wilt disease in *A. heterophyllum*  
89 are stems cankers, the stems become chapped as though torn apart, fruit rot and progressive  
90 loss of the canopy resulting in tree death. *A. heterophyllum* trees showed typical symptoms of  
91 infection by the *Ceratocystis* fungus; the same was true of a serious wilt pathogen of *A.*  
92 *mangium* and *A. crassicarpa* in Indonesia and Vietnam (Tarigan et al. 2010; Tarigan et al.  
93 2011; Chi et al. 2019b), a serious pathogen wilt of mango trees in Oman and Pakistan (Van  
94 Wyk et al., 2007) caused by *C. manginecans*, previously reported in Pakistan (Al-Adawi et al.  
95 2013). *C. manginecans* infecting native trees in these countries is serious and could potentially  
96 lead to the devastation of important components of the natural biodiversity of Indonesia.

97

## 98 **Acknowledgement**

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

102 Ministry of Research, Technology, and Higher Education, Number:  
103 068/SP2H/AMD/LT/DRPM/2020 chaired by Ahmad Muslim.

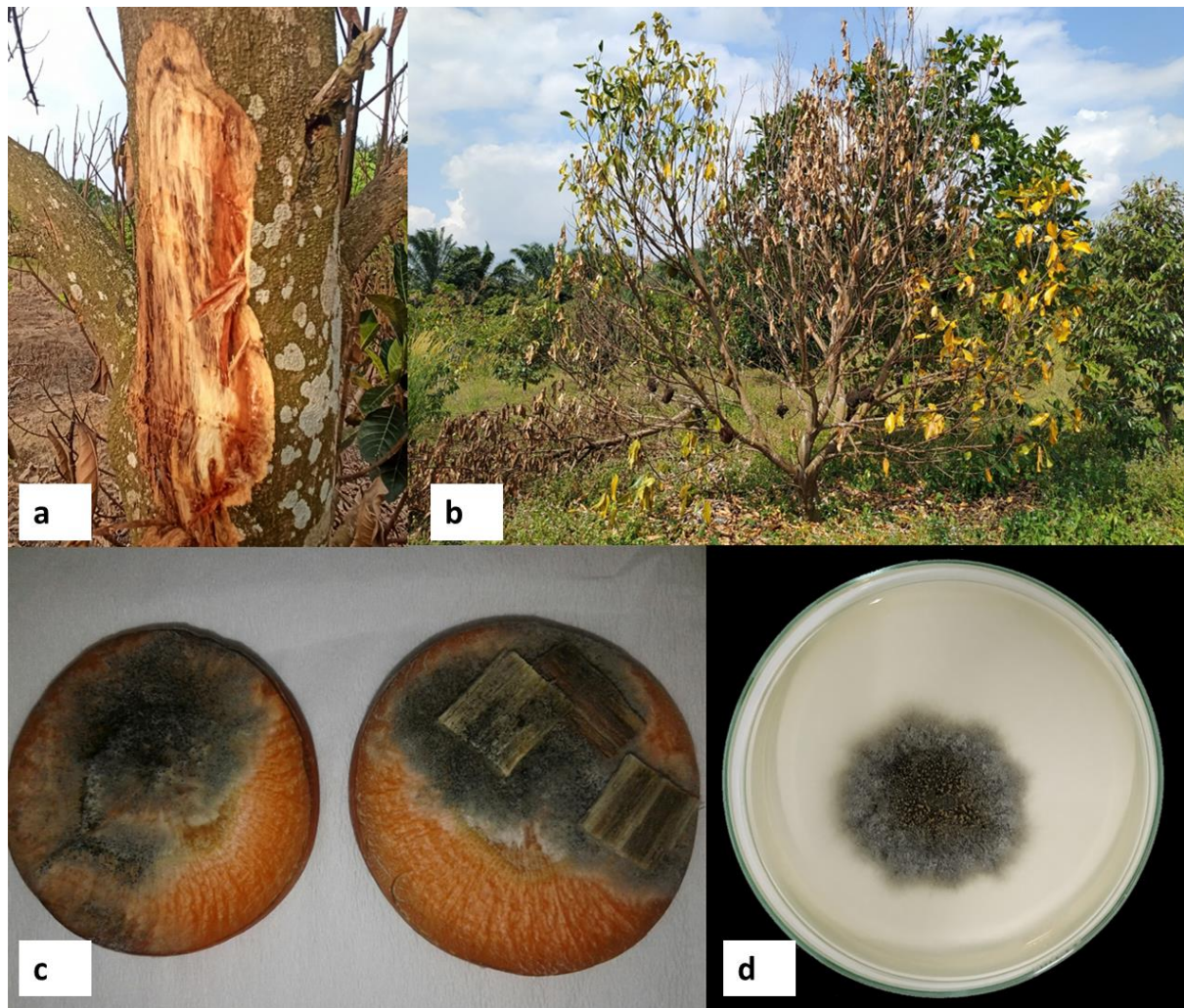
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138 disease of *Acacia mangium* associated with *Ceratocystis manginecans* and *C. acaciivora*  
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141 R, Wingfield MJ (2007) *Ceratocystis manginecans* sp. nov., causal agent of a destructive  
142 mango wilt disease in Oman and Pakistan. *Fung Div* 27: 213–230

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

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



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

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

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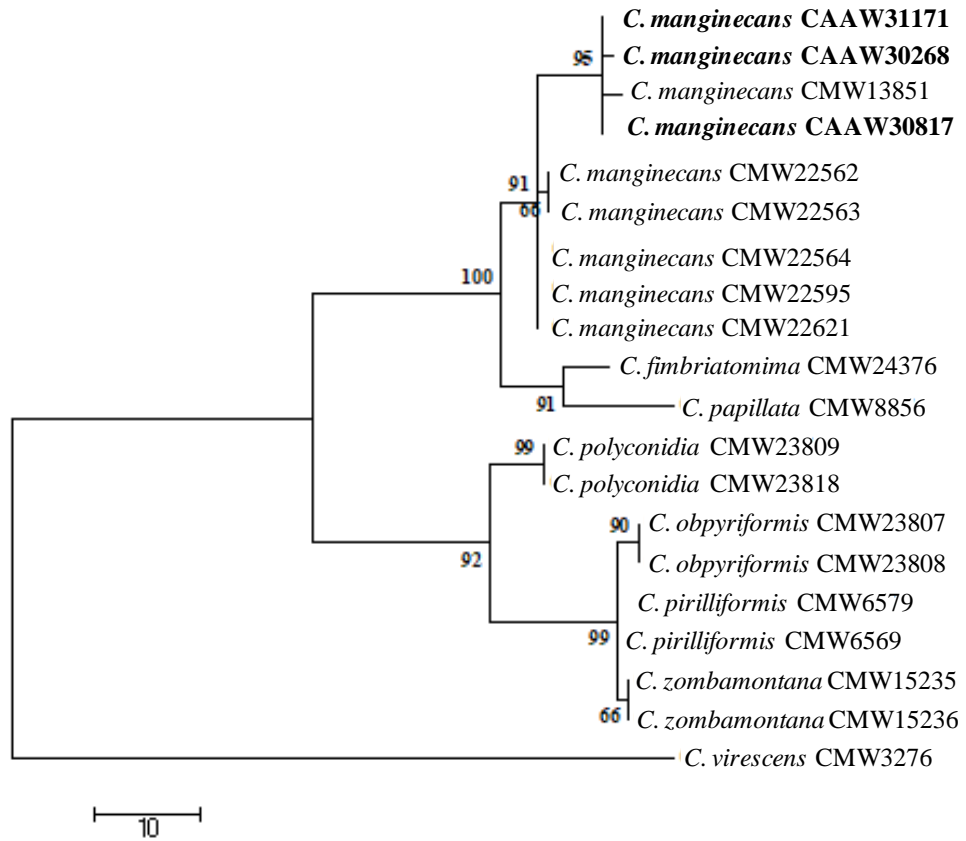
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176 **Fig. 3** Phylogenetic tree constructed by MEGA with Maximum Parsimony (MP) search for the  
 177 combined sequence data of the ITS region and  $\beta$ -tubulin gene (CAAW31171, CAAW30268,  
 178 and CAAW30817) and their related species from GenBank. Consistency (CI), retention (RI),  
 179 and composite indexes (CoI) are 0.819149, 0.952113, and 0.861689 for all sites and parsimony-  
 180 informative sites. The percentage of replicate trees in which the associated taxa clustered  
 181 together in the bootstrap test (1000 replicates) is shown next to the branches. Bootstrap values  
 182 >50% are indicated above the branches. The analysis involved 20 nucleotide sequences. All  
 183 positions containing gaps and missing data were eliminated. There were 831 positions in the  
 184 final dataset. *Ceratocystis virescens* was used as the out-group.



a. muslim unsri &lt;a\_muslim@unsri.ac.id&gt;

---

**Thank you for your approval - [EMID:a73d107a34960eb0]**

1 message

---

**APDN** <em@editorialmanager.com>  
Reply-To: APDN <jude.estrera@springernature.com>  
To: "A. Muslim" <a\_muslim@unsri.ac.id>

Mon, Mar 22, 2021 at 5:14 AM

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**



# REVISI PERTAMA

a. muslim unsri &lt;a\_muslim@unsri.ac.id&gt;

## Major Revisions requested APDN-D-21-00015R1

2 messages

APDN <em@editorialmanager.com>  
Reply-To: APDN <jude.estrera@springernature.com>  
To: "A. Muslim" <a\_muslim@unsri.ac.id>

Mon, May 17, 2021 at 11:45 PM

CC: [dagmar.hanold@adelaide.edu.au](mailto:dagmar.hanold@adelaide.edu.au), [dhanold@gmail.com](mailto: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).

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

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Please submit your 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\*\***

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>  
To: APDN <jude.estrera@springernature.com>

Mon, May 24, 2021 at 7:33 AM

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

Ahmad Muslim  
Associate Professor  
Faculty of Agriculture, Sriwijaya University  
Jl. Palembang-Prabumulih Km.32, Indralaya, Palembang, Indonesia  
E-mail: [a\\_muslim@unsri.ac.id](mailto:a_muslim@unsri.ac.id)

[Quoted text hidden]

## Australasian Plant Disease Notes

### Jackfruit (*Artocarpus heterophyllus*), a New Host Plant of *Ceratocystis* Wilt from South Sumatra, Indonesia --Manuscript Draft--

<b>Manuscript Number:</b>	APDN-D-21-00015R2	
<b>Full Title:</b>	Jackfruit ( <i>Artocarpus heterophyllus</i> ), a New Host Plant of <i>Ceratocystis</i> Wilt from South Sumatra, Indonesia	
<b>Article Type:</b>	Plant Disease Note	
<b>Keywords:</b>	Sudden death disease; Moraceae; <i>Ceratocystis fimbriata</i> sensu stricto	
<b>Corresponding Author:</b>	A. Muslim, Ph.D. Universitas Sriwijaya Fakultas Pertanian Palembang, Sumatera Selatan INDONESIA	
<b>Corresponding Author Secondary Information:</b>		
<b>Corresponding Author's Institution:</b>	Universitas Sriwijaya Fakultas Pertanian	
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<b>First Author:</b>	Rahmat Pratama, S.Si	
<b>First Author Secondary Information:</b>		
<b>Order of Authors:</b>	Rahmat Pratama, S.Si A. Muslim, Ph.D. Suwandi Suwandi, PhD Nurhayati Damiri, Professor Soleha Soleha, S.P	
<b>Order of Authors Secondary Information:</b>		
<b>Funding Information:</b>	Kementerian Riset Teknologi Dan Pendidikan Tinggi Republik Indonesia (068/SP2H/AMD/LT/DRPM/2020)	Dr. A. Muslim
<b>Abstract:</b>	<p>In 2019, wilt and sudden death were observed on <i>Artocarpus heterophyllus</i> (jackfruit) has been noted. Identification was performed by sequence analysis of the concatenated <math>\beta</math>-tubulin and ITS gene regions. Sequencing of the PCR product confirmed this pathogen was <i>Ceratocystis fimbriata</i> sensu stricto. <i>C. fimbriata</i> causing sudden death disease in <i>A. heterophyllus</i> is being reported for the first time in Indonesia and worldwide.</p>	
<b>Response to Reviewers:</b>	<p>May 22, 2021</p> <p>Dear Eduardo Guatimosim, PhD Associate Editor Australasian Plant Disease Notes</p> <p>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)</p> <p>Here, we enclose revised version of the manuscript No. APDN-D-21-00015R1 entitled "Jackfruit (<i>Artocarpus heterophyllus</i>), a New Host Plant of <i>Ceratocystis</i> Wilt from South Sumatra, Indonesia" by Rahmat Pratama, Ahmad Muslim, Suwandi Suwandi, Nurhayati Damiri, Soleha Soleha.</p> <p>Below is a summary of our response to the reviewers' comments.</p>	

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).  $\beta$ -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  $\beta$ -tubulin primers. ITS primer has been performed to describe and group ITS genotypes (Harrington et al. 2014) and  $\beta$ -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  
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E-mail: a\_muslim@unsri.ac.id

**Rahmat Pratama<sup>1</sup> · Ahmad Muslim<sup>2\*</sup> · Suwandi Suwandi<sup>2</sup> · Nurhayati Damiri<sup>2</sup> · Soleha Soleha<sup>1</sup>**

<sup>1</sup>Agriculture Sciences Graduate Program, Faculty of Agriculture, Universitas Sriwijaya. Jl. Padang Selasa No. 524, Bukit Besar, Palembang 30139, South Sumatra, Indonesia

<sup>2</sup>Laboratory of Phytopathology, Department of Plant Protection, Faculty of Agriculture, Sriwijaya University, Indralaya, South Sumatera, 30662, Indonesia

\*Corresponding Author: a\_muslim@unsri.ac.id

# 1 **Jackfruit (*Artocarpus heterophyllus*), a New Host Plant of *Ceratocystis* Wilt** 2 **in South Sumatra, Indonesia**

## 3 4 **Abstract**

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

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

11  
12 Jackfruit (*A. heterophyllus*) belongs to the family Moraceae, and it is known in  
13 Indonesian as “Nangka”. Jackfruit is grown widely in Indonesia and many countries with  
14 tropical and subtropical climates. Jackfruit is among the most exported fruits worldwide and  
15 has considerable nutrition and health benefits (Ranasinghe et al. 2019).

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

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

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

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

47 To confirm the species identification, isolates were cultured on potato dextrose broth  
48 (PDB) at room temperature for one week. Mycelial mat was filtered through Whatman filter  
49 paper and genomic DNA was extracted from fungal mycelial mat using YeaStar Genomic DNA  
50 Kit (Zymo Research Corporation, California, USA). PCR conditions and reactions for two gene  
51 regions were used to identify the *Ceratocystis* isolates; the Internal Transcribed Spacer (ITS)  
52 and part of the  $\beta$ -tubulin ( $\beta$ t) gene. Amplifications were carried out in 50 µl reactions containing  
53 20 µl DreamTaq Green PCR Master Mix (Eppendorf, Germany) (DreamTaq DNA Polymerase,  
54 2X DreamTaq Green buffer, dNTPs, and 4 mM MgCl<sub>2</sub>), 1,5 µl of each forward and reverse  
55 primer, 4 µl of DNA template and 23µl sterilised water. The PCRs were performed with a  
56 C1000 Touch™ thermal cycler (Bio-Rad, USA). The PCR cycling parameters were as follows:  
57 initial denaturation for 5 min at 95 °C, followed by 35 cycles at 95 °C for 30 s, 56 °C for 45 s  
58 and 72 °C for 1 min. Amplification was completed at 72 °C for 10 min and the PCR product  
59 was stored at 10 °C. The PCR amplicons were sequenced at 1st BASE (Malaysia).

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

69 (2014); Li et al. (2016) and phylogenetic analyses were performed. Maximum Parsimony (MP)  
70 analyses were performed in MEGA v. 10 (Kumar et al. 2016; Paul et al. 2018) with 1000  
71 bootstrap replications.  $\beta$ -tubulin sequence of our isolates confirmed the assignment to LAC of  
72 *C. fimbriata sensu lato* (Fig. 3a). Manual alignment of the ITS sequences with previously  
73 described ITS genotypes (Harrington et al. 2014; Li et al. 2016) grouped the isolates into ITS5  
74 haplotype of *C. fimbriata sensu stricto* (Fig. 3b).

75 The pathogenic potential of isolates was evaluated by the under bark inoculation  
76 method described by O’Gara et al. (1997) using Five-month-old *A. heterophyllum* seedlings  
77 with stem diameters of 6-8 mm and heights <1.5 m were prepared for pathogenicity test.  
78 Seedlings were grown in 10 cm diameter plastic pots containing a soil mix (topsoil + peat +  
79 chicken manure) under a 50% shading net. Plants were watered daily to maintain humidity,  
80 and any mortality occurring before the end of the experiment was recorded. Wounds were made  
81 on the stems of the seedlings using a cork borer (4 mm diam.), and mycelial discs (4 mm diam.)  
82 taken from an actively growing colony of *C. fimbriata* on 2% MEA (14 days) (Pratama et al.  
83 2021) were placed in the wounds with the mycelium facing downwards. These were covered  
84 with Parafilm (Pechiney, Menasha, Wisconsin) to reduce contamination and desiccation. Ten  
85 plants of each tree species were inoculated with sterile MEA plugs to serve as controls (Fig.  
86 4a). Fungal isolates were re-isolated and re-identified using morphological characteristics for  
87 Koch’s postulates confirmation. In pathogenicity tests, initial symptoms appeared two weeks  
88 post-inoculation as brown lesions on the wood of inoculation site (Fig. 4b). Forty-five days  
89 after inoculation, plants exhibited wilt symptoms, lesions of wood discoloration extended to  
90 heartwood (Fig. 4c) and length (downward + upward) was 17.88 until 34.74 cm. When re-  
91 isolated, the fungus was phenotypically identical to the prior isolate of *C. fimbriata*  
92 (CAAW31171, CAAW30817, CAAW30268).

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

103 **Acknowledgement**

104 This research was funded by PMDSU scholarship with budget year of 2019-2021  
105 according to the Director of Research and Community Service, Directorate of Research  
106 and Community Service (DRPM), Directorate General for Research and Development,  
107 Ministry of Research, Technology, and Higher Education, Number:  
108 068/SP2H/AMD/LT/DRPM/2020 chaired by Ahmad Muslim.

109

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

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



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

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158

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

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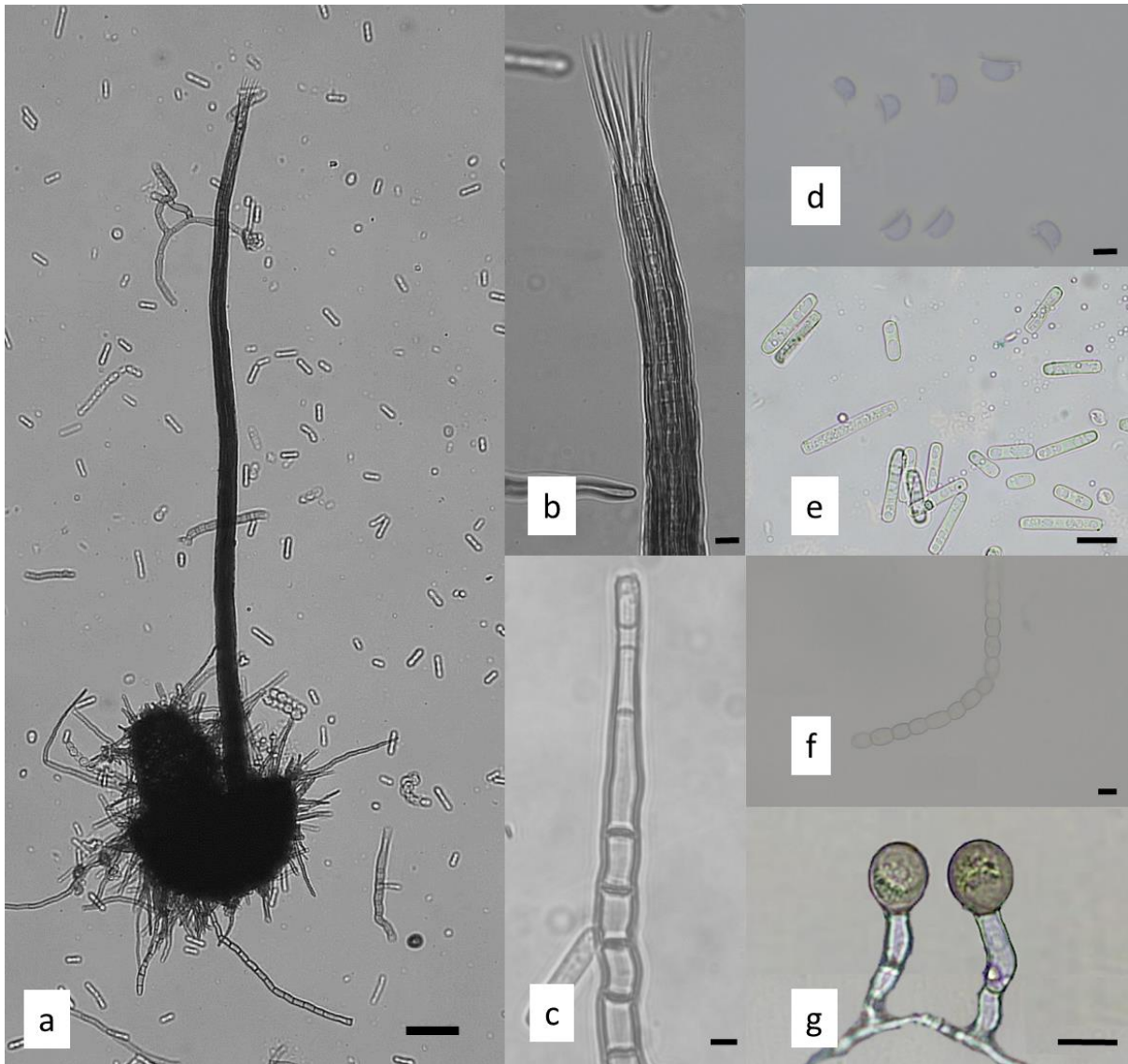
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172 **Fig. 2.** Morphological characteristics of *Ceratocystis fimbriata* isolated from *Artocarpus*  
 173 *heterophyllus* stem lesion: **a.** ascomata with pirilliform base, **b.** divergent ostiolar hyphae; **c.**  
 174 conidiophore/phialide; **d.**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

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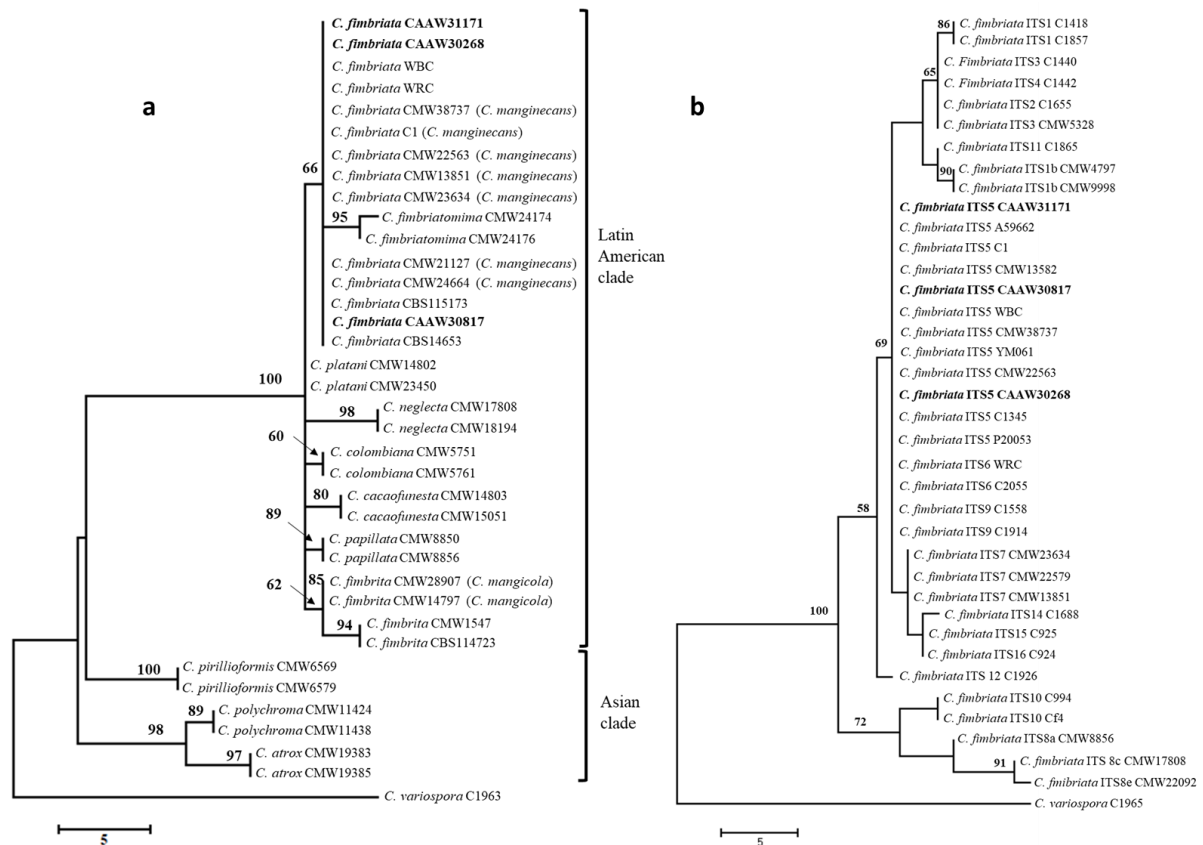
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186 **Fig. 3** Phylogenetic tree constructed by MEGA with Maximum Parsimony (MP) analysis of

187 the **a.**  $\beta$ -tubulin sequences from Jackfruit tree in Indonesia (marked in bold) and other species

188 in the Latin American and Asian clade of the *C. fimbriata* species complex. Species names

189 considered to be synonyms of *C. fimbriata sensu stricto* are in parentheses (Harrington et al.

190 2014; Oliveira et al. 2015). **b.** ITS sequences from Jackfruit tree in Indonesia (marked in bold)

191 and genotypes (sequences) of the *C. fimbriata sensu stricto*. The ITS haplotypes of *C. fimbriata*

192 are numbered following the numerical designations of Harrington et al. (2014). Consistency

193 (CI), retention (RI), and composite indexes (CoI) for  $\beta$ -tubulin were 0.566667, 0.845238,

194 0.668011, respectively and ITS was 0.933333, 0.976563, 0.932836, respectively. The

195 percentage of replicate trees in which the associated taxa clustered together in the bootstrap

196 test (1000 replicates) is shown next to the branches. Bootstrap values >50% are indicated above

197 the branches. The analysis involved 38 ( $\beta$ -tubulin) and 37 (ITS) nucleotide sequences. All

198 positions containing gaps and missing data were eliminated. There were 408 ( $\beta$ -tubulin) and

199 518 (ITS) positions in the final dataset. *Ceratocystis variospora* was used as the out-group.

200



201

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



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## Submission Confirmation

1 message

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**APDN** <em@editorialmanager.com>  
Reply-To: APDN <jude.estrera@springernature.com>  
To: "A. Muslim" <a\_muslim@unsri.ac.id>

Mon, May 24, 2021 at 2:04 PM

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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**Your Submission APDN-D-21-00015R2**

1 message

**APDN** <em@editorialmanager.com>  
Reply-To: APDN <jude.estrera@springernature.com>  
To: "A. Muslim" <a\_muslim@unsri.ac.id>

Sat, Jun 12, 2021 at 12:49 AM

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 the Editorial Manager system which can be accessed at:

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Please submit your 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 abbreviation. 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.

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.

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## Australasian Plant Disease Notes

### Jackfruit (*Artocarpus heterophyllus*), a New Host Plant of *Ceratocystis* Wilt from South Sumatra, Indonesia --Manuscript Draft--

<b>Manuscript Number:</b>	APDN-D-21-00015R3	
<b>Full Title:</b>	Jackfruit ( <i>Artocarpus heterophyllus</i> ), a New Host Plant of <i>Ceratocystis</i> Wilt from South Sumatra, Indonesia	
<b>Article Type:</b>	Plant Disease Note	
<b>Keywords:</b>	Sudden death disease; Moraceae; <i>Ceratocystis fimbriata</i> sensu stricto	
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<b>Order of Authors:</b>	Rahmat Pratama, S.Si A. Muslim, Ph.D. Suwandi Suwandi, PhD Nurhayati Damiri, Professor Soleha Soleha, S.P	
<b>Order of Authors Secondary Information:</b>		
<b>Funding Information:</b>	Kementerian Riset Teknologi Dan Pendidikan Tinggi Republik Indonesia (068/SP2H/AMD/LT/DRPM/2020)	Dr. A. Muslim
<b>Abstract:</b>	<p>In 2019, wilt and sudden death were observed on <i>Artocarpus heterophyllus</i> (jackfruit) has been noted. Identification was performed by sequence analysis of the concatenated <math>\beta</math>-tubulin and ITS gene regions. Sequencing of the PCR product confirmed this pathogen was <i>Ceratocystis fimbriata</i> sensu stricto. This is the first report of <i>C. fimbriata</i> causing sudden death disease in <i>A. heterophyllus</i> in Indonesia and worldwide.</p>	
<b>Response to Reviewers:</b>	<p>June 14, 2021</p> <p>Dear Eduardo Guatimosim, PhD Associate Editor Australasian Plant Disease Notes</p> <p>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)</p> <p>Here, we enclose revised version of the manuscript No. APDN-D-21-00015R1 entitled "Jackfruit (<i>Artocarpus heterophyllus</i>), a New Host Plant of <i>Ceratocystis</i> Wilt from South Sumatra, Indonesia" by Rahmat Pratama, Ahmad Muslim, Suwandi Suwandi, Nurhayati Damiri, Soleha Soleha.</p> <p>Below is a summary of our response to the reviewers' comments.</p>	

Comment [1]: line 8-9. Never start a sentence with an abbreviation. 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. heterophyllum 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. heterophyllum 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. heterophyllum) belongs to the family Moraceae, and it is known in Indonesian as "Nangka". Jackfruit is grown..." to read as follows: "Jackfruit (Artocarpus heterophyllum, 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 heterophyllum, 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. heterophyllum 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.  $\beta$ -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 (Col) for  $\beta$ -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 ( $\beta$ -tubulin) and 37 (ITS) nucleotide sequences. All positions containing gaps and missing data were eliminated. There were 408 ( $\beta$ -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.  $\beta$ -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,

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# 1 **Jackfruit (*Artocarpus heterophyllus*), a New Host Plant of *Ceratocystis* Wilt** 2 **in South Sumatra, Indonesia**

## 3 4 **Abstract**

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

10 **Keywords:** Sudden death disease · Moraceae · *Ceratocystis fimbriata sensu stricto* ·  
11

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

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

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

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

36 Morphological traits of fruiting bodies and spores were observed under an optical  
37 Olympus CX33 microscope. Ascumatal bases dark brown to black, base subglobose to globose  
38 and measured (n=100), 131.5–250.7×101.6–236.5  $\mu\text{m}$  (Fig. 2a). Ascomata necks erect,  
39 occasionally curved, black at the base becoming subhyaline towards the apex, smooth to  
40 crenulate, 324.7–579.1  $\mu\text{m}$  long including ostiolar hyphae (Fig. 2b). Phialides pale brown to  
41 hyaline (Fig. 2c). Ascospores hat-shaped, 3.4–6.8×2.1–6.2  $\mu\text{m}$  (Fig. 2d). Bacilliform conidia  
42 11.1–36.1×2.1–7.4  $\mu\text{m}$  (Fig. 2e). Barrel conidia 4.4–16.1×2.7–6.9  $\mu\text{m}$  (Fig. 2f).  
43 Chlamyospores oval, thick walled, smooth, 6.7–16.5×5.9–12.9  $\mu\text{m}$  (Fig. 2g). Based on  
44 morphological characters, the fungus was identified as *Ceratocystis fimbriata*. Specimens were  
45 deposited in the culture collection of the Phytopathology Laboratory of Sriwijaya University  
46 (Indralaya, Indonesia) as HPTUnsri-2101.

47 To confirm the species identification, isolates were cultured on potato dextrose broth  
48 (PDB) at room temperature for one week. Mycelium was filtered through Whatman filter paper  
49 and genomic DNA was extracted from fungal mycelial mat using YeaStar Genomic DNA Kit  
50 (Zymo Research Corporation, California, USA). PCR conditions and reactions for two gene  
51 regions were used to identify the *Ceratocystis* isolates; the Internal Transcribed Spacer (ITS)  
52 with primers ITS 1 and ITS4 (White et al. 1990) and part of the  $\beta$ -tubulin ( $\beta\text{t}$ ) gene with primers  
53  $\beta\text{t}1\text{a}$  and  $\beta\text{t}1\text{b}$  (Glass and Donaldson 1995). Amplifications were carried out in 50  $\mu\text{l}$  reactions  
54 containing 20  $\mu\text{l}$  DreamTaq Green PCR Master Mix (Eppendorf, Germany) (DreamTaq DNA  
55 Polymerase, 2X DreamTaq Green buffer, dNTPs, and 4 mM  $\text{MgCl}_2$ ), 1,5  $\mu\text{l}$  of each forward  
56 and reverse primer, 4  $\mu\text{l}$  of DNA template and 23 $\mu\text{l}$  sterilised water. The PCRs were performed  
57 with a C1000 Touch™ thermal cycler (Bio-Rad, USA). The PCR cycling parameters were as  
58 follows: initial denaturation for 5 min at 95 °C, followed by 35 cycles at 95 °C for 30 s, 56 °C  
59 for 45 s and 72 °C for 1 min. Amplification was completed at 72 °C for 10 min and the PCR  
60 product was stored at 10 °C. The PCR amplicons were sequenced at 1st BASE (Malaysia).

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

67 *fimbriata* species complex (Fourie et al. 2015; Oliveira et al. 2015; Barnes et al. 2018). To  
68 determine relatedness of isolates from jackfruit with known *C. fimbriata* populations, the ITS  
69 sequence was manually aligned with known ITS haplotypes as designated by Harrington et al.  
70 (2014); Li et al. (2016) and phylogenetic analyses were performed. Maximum Parsimony (MP)  
71 analyses were performed in MEGA v. 10 (Kumar et al. 2016; Paul et al. 2018) with 1000  
72 replications. The analysis involved 38 ( $\beta$ -tubulin) and 37 (ITS) nucleotide sequences. All  
73 positions containing gaps and missing data were eliminated. There were 408 ( $\beta$ -tubulin) and  
74 518 (ITS) positions in the final dataset. *Ceratocystis variospora* was used as the out-group.  $\beta$ -  
75 tubulin sequence of our isolates confirmed the assignment to LAC of *C. fimbriata sensu lato*  
76 (Fig. 3a). Manual alignment of the ITS sequences with previously described ITS genotypes  
77 (Harrington et al. 2014; Li et al. 2016) grouped the isolates into ITS5 haplotype of *C. fimbriata*  
78 *sensu stricto* (Fig. 3b). Consistency (CI), retention (RI), and composite indexes (CoI) for  $\beta$ -  
79 tubulin were 0.566667, 0.845238, 0.668011, respectively and ITS was 0.933333, 0.976563,  
80 0.932836, respectively.

81 The pathogenic potential of isolates was evaluated by the under bark inoculation  
82 method described by O’Gara et al. (1997) using Five-month-old *A. heterophyllum* seedlings  
83 with stem diameters of 6-8 mm and heights <1.5 m were prepared for pathogenicity test.  
84 Seedlings were grown in 10 cm diameter plastic pots containing a soil mix (topsoil + peat +  
85 chicken manure) under a 50% shading net. Plants were watered daily to maintain humidity,  
86 and any mortality occurring before the end of the experiment was recorded. Wounds were made  
87 on the stems of the seedlings using a cork borer (4 mm diam.), and mycelial discs (4 mm diam.)  
88 taken from an actively growing colony of *C. fimbriata* on 2% MEA (14 days) (Pratama et al.  
89 2021) were placed in the wounds with the mycelium facing downwards. These were covered  
90 with Parafilm (Pechiney, Menasha, Wisconsin) to reduce contamination and desiccation. Ten  
91 plants of each tree species were inoculated with sterile MEA plugs to serve as controls (Fig.  
92 4a). Fungal isolates were re-isolated and re-identified using morphological characteristics for  
93 Koch’s postulates confirmation. In pathogenicity tests, initial symptoms appeared two weeks  
94 post-inoculation as brown lesions on the wood of inoculation site (Fig. 4b). Forty-five days  
95 after inoculation, plants exhibited wilt symptoms, lesions of wood discoloration extended to  
96 heartwood (Fig. 4c) and length (downward + upward) was 17.88 until 34.74 cm. When re-  
97 isolated, the fungus was phenotypically identical to the prior isolate of *C. fimbriata*  
98 (CAAW31171, CAAW30817, CAAW30268).

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



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

108

### 109 **Acknowledgement**

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111 according to the Director of Research and Community Service, Directorate of Research  
112 and Community Service (DRPM), Directorate General for Research and Development,  
113 Ministry of Research, Technology, and Higher Education, Number:  
114 068/SP2H/AMD/LT/DRPM/2020.

115

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

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

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

---



171

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

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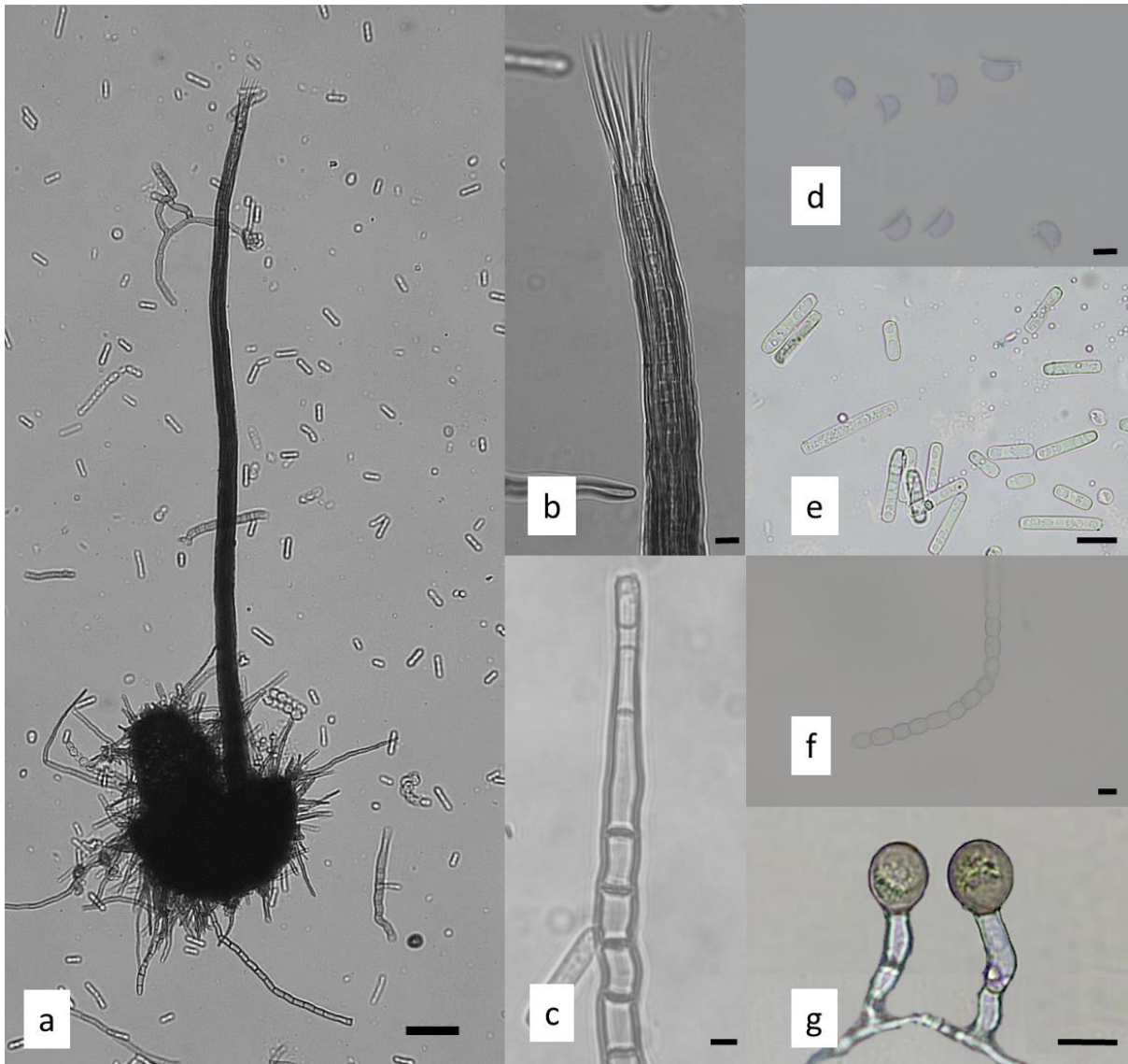
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185 **Fig. 2.** Morphological characteristics of *Ceratocystis fimbriata* isolated from *Artocarpus*  
 186 *heterophyllus* stem lesion: **a.** ascomata with pirilliform base, **b.** divergent ostiolar hyphae; **c.**  
 187 conidiophore/phialide; **d.**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

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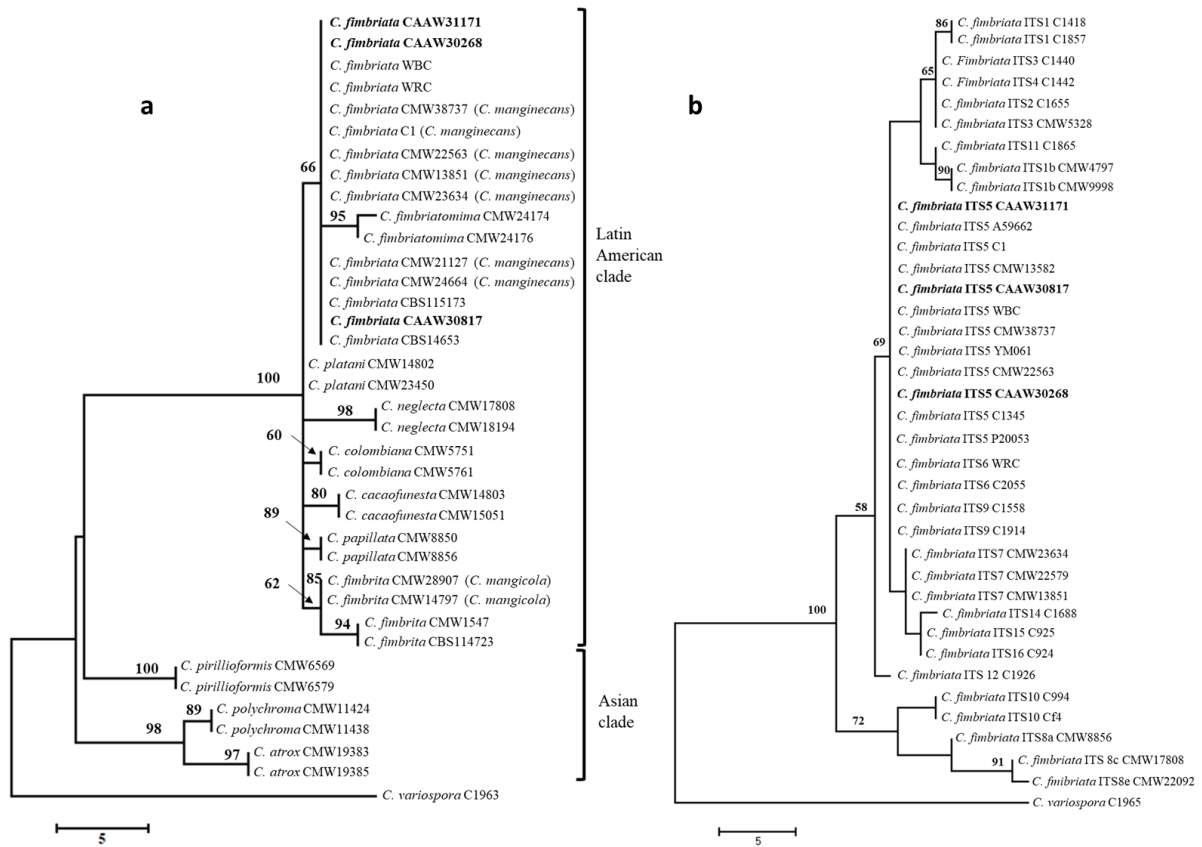
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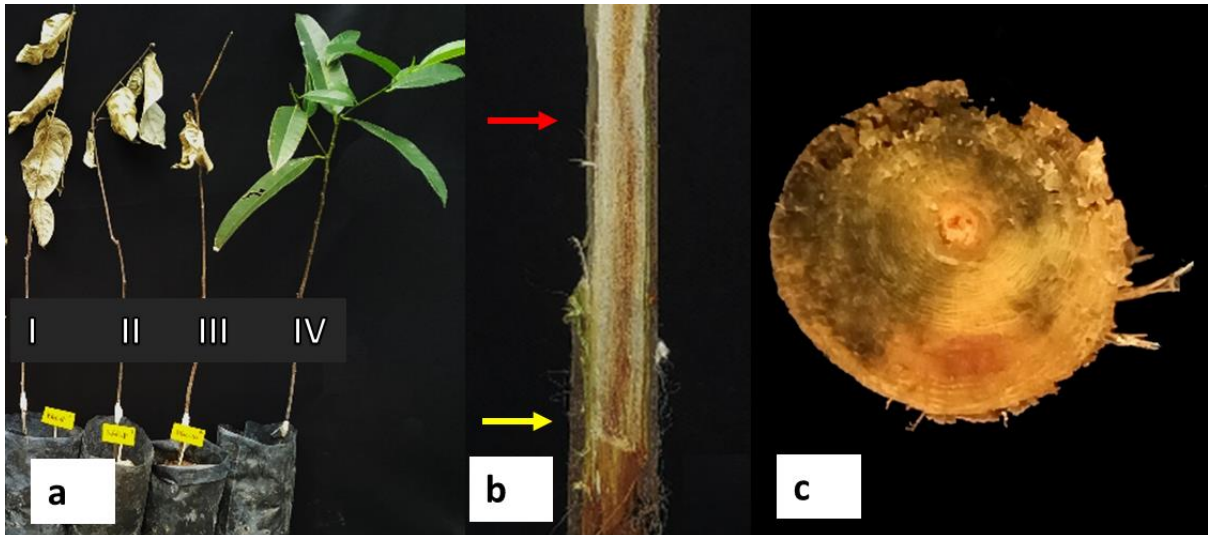
199 **Fig. 3** Phylogenetic tree constructed by MEGA with Maximum Parsimony (MP) analysis by  
 200 **a.**  $\beta$ -tubulin sequences from Jackfruit tree in Indonesia (marked in bold) and other species in  
 201 the Latin American and Asian clade of the *C. fimbriata* species complex. **b.** ITS sequences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

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





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1 message

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**APDN** <em@editorialmanager.com>  
Reply-To: APDN <jude.estrera@springernature.com>  
To: "A. Muslim" <a\_muslim@unsri.ac.id>

Mon, Jun 14, 2021 at 11:29 PM

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## REVISI KETIGA

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---

### Your Submission APDN-D-21-00015R3

3 messages

**APDN** <em@editorialmanager.com>  
Reply-To: APDN <jude.estrera@springernature.com>  
To: "A. Muslim" <a\_muslim@unsri.ac.id>

Fri, Jun 18, 2021 at 12:59 AM

CC: [dagmar.hanold@adelaide.edu.au](mailto:dagmar.hanold@adelaide.edu.au), [dhanold@gmail.com](mailto: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 the Editorial Manager system which can be accessed at:

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Please submit your 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.

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Tue, Jun 29, 2021 at 3:38 PM

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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Associate Professor  
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To: APDN <jude.estrera@springernature.com>  
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Wed, Jul 14, 2021 at 9:26 AM

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)

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Ahmad Muslim  
Associate Professor  
Faculty of Agriculture, Sriwijaya University  
Jl. Palembang-Prabumulih Km.32, Indralaya, Palembang, Indonesia  
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On Fri, Jun 18, 2021 at 1:00 AM APDN <[em@editorialmanager.com](mailto:em@editorialmanager.com)> wrote:

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## Australasian Plant Disease Notes

### Jackfruit (*Artocarpus heterophyllus*), a New Host Plant of *Ceratocystis* Wilt from South Sumatra, Indonesia --Manuscript Draft--

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

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

Yours sincerely,

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**Rahmat Pratama<sup>1</sup> · Ahmad Muslim<sup>2\*</sup> · Suwandi Suwandi<sup>2</sup> · Nurhayati Damiri<sup>2</sup> · Soleha Soleha<sup>1</sup>**

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# 1 **Jackfruit (*Artocarpus heterophyllus*), a New Host Plant of *Ceratocystis* Wilt** 2 **in South Sumatra, Indonesia**

## 3 4 **Abstract**

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

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

11

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

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

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



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

36 Morphological traits of fruiting bodies and spores were observed under an optical  
37 Olympus CX33 microscope. Ascumal bases dark brown to black, base subglobose to globose  
38 and measured (n=100), 131.5–250.7×101.6–236.5 μm (Fig. 2a). Ascumata necks erect,  
39 occasionally curved, black at the base becoming subhyaline towards the apex, smooth to  
40 crenulate, 324.7–579.1 μm long including ostiolar hyphae (Fig. 2b). Phialides pale brown to  
41 hyaline (Fig. 2c). Ascospores hat-shaped, 3.4–6.8×2.1–6.2 μm (Fig. 2d). Bacilliform conidia  
42 11.1–36.1×2.1–7.4 μm (Fig. 2e). Barrel conidia 4.4–16.1×2.7–6.9 μm (Fig. 2f).  
43 Chlamydospores oval, thick walled, smooth, 6.7–16.5×5.9–12.9 μm (Fig. 2g). Based on  
44 morphological characters, the fungus was identified as *Ceratocystis fimbriata*. Two  
45 representative isolates were deposited at the ICBB Culture Collection for Microorganisms and  
46 Cell Culture, Indonesian Center for Biodiversity and Biotechnology, (Bogor, Indonesia) as  
47 ICBB9852 and ICBB9853.

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

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

67 sequences representing species in the Latin American (LAC) and Asian clade (AC) of the *C.*  
68 *fimbriata* species complex (Fourie et al. 2015; Oliveira et al. 2015; Barnes et al. 2018). To  
69 determine relatedness of isolates from jackfruit with known *C. fimbriata* populations, the ITS  
70 sequence was manually aligned with known ITS haplotypes as designated by Harrington et al.  
71 (2014); Li et al. (2016) and phylogenetic analyses were performed. Maximum Parsimony (MP)  
72 analyses were performed in MEGA v. 10 (Kumar et al. 2016; Paul et al. 2018) with 1000  
73 replications. The analysis involved 38 ( $\beta$ -tubulin) and 37 (ITS) nucleotide sequences. All  
74 positions containing gaps and missing data were eliminated. There were 408 ( $\beta$ -tubulin) and  
75 518 (ITS) positions in the final dataset. *Ceratocystis variospora* was used as the out-group.  $\beta$ -  
76 tubulin sequence of our isolates confirmed the assignment to LAC of *C. fimbriata sensu lato*  
77 (Fig. 3a). Manual alignment of the ITS sequences with previously described ITS genotypes  
78 (Harrington et al. 2014; Li et al. 2016) grouped the isolates into ITS5 haplotype of *C. fimbriata*  
79 *sensu stricto* (Fig. 3b). Consistency (CI), retention (RI), and composite indexes (CoI) for  $\beta$ -  
80 tubulin were 0.566667, 0.845238, 0.668011, respectively and ITS was 0.933333, 0.976563,  
81 0.932836, respectively.

82 The pathogenic potential of isolates was evaluated by the under bark inoculation  
83 method described by O’Gara et al. (1997) using Five-month-old *A. heterophyllum* seedlings  
84 with stem diameters of 6-8 mm and heights <1.5 m were prepared for pathogenicity test.  
85 Seedlings were grown in 10 cm diameter plastic pots containing a soil mix (topsoil + peat +  
86 chicken manure) under a 50% shading net. Plants were watered daily to maintain humidity,  
87 and any mortality occurring before the end of the experiment was recorded. Wounds were made  
88 on the stems of the seedlings using a cork borer (4 mm diam.), and mycelial discs (4 mm diam.)  
89 taken from an actively growing colony of *C. fimbriata* on 2% MEA (14 days) (Pratama et al.  
90 2021) were placed in the wounds with the mycelium facing downwards. These were covered  
91 with Parafilm (Pechiney, Menasha, Wisconsin) to reduce contamination and desiccation. Ten  
92 plants of each tree species were inoculated with sterile MEA plugs to serve as controls (Fig.  
93 4a). Fungal isolates were re-isolated and re-identified using morphological characteristics for  
94 Koch’s postulates confirmation. In pathogenicity tests, initial symptoms appeared two weeks  
95 post-inoculation as brown lesions on the wood of inoculation site (Fig. 4b). Forty-five days  
96 after inoculation, plants exhibited wilt symptoms, lesions of wood discoloration extended to  
97 heartwood (Fig. 4c) and length (downward + upward) was 17.88 until 34.74 cm. When re-  
98 isolated, the fungus was phenotypically identical to the prior isolate of *C. fimbriata*  
99 (ICBB9852, ICBB9853, CAAW30817).

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

109

### 110 **Acknowledgement**

111 This research was funded by PMDSU scholarship with budget year of 2019-2021  
112 according to the Director of Research and Community Service, Directorate of Research  
113 and Community Service (DRPM), Directorate General for Research and Development,  
114 Ministry of Research, Technology, and Higher Education, Number:  
115 068/SP2H/AMD/LT/DRPM/2020.

116

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

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

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

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172

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

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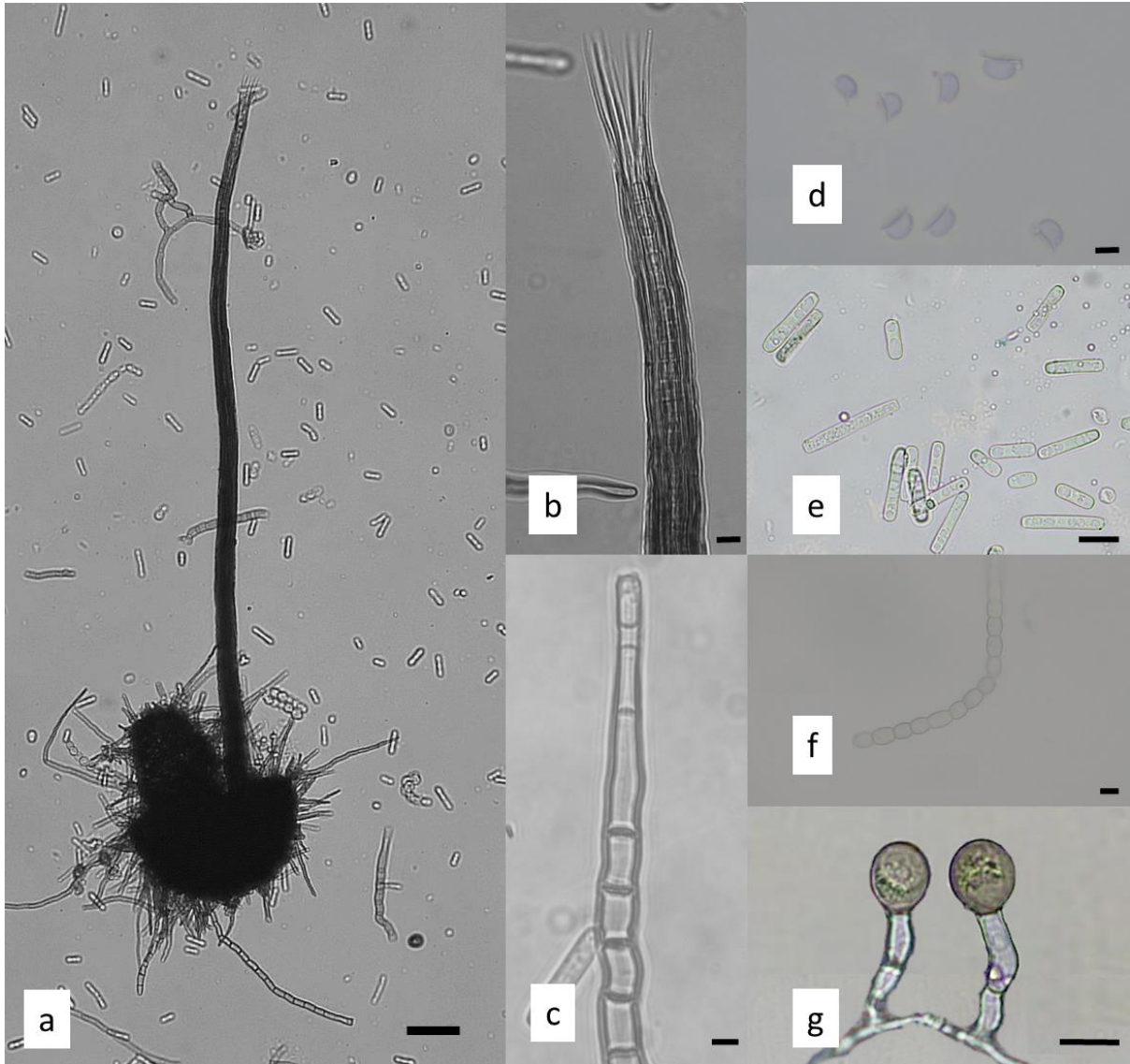
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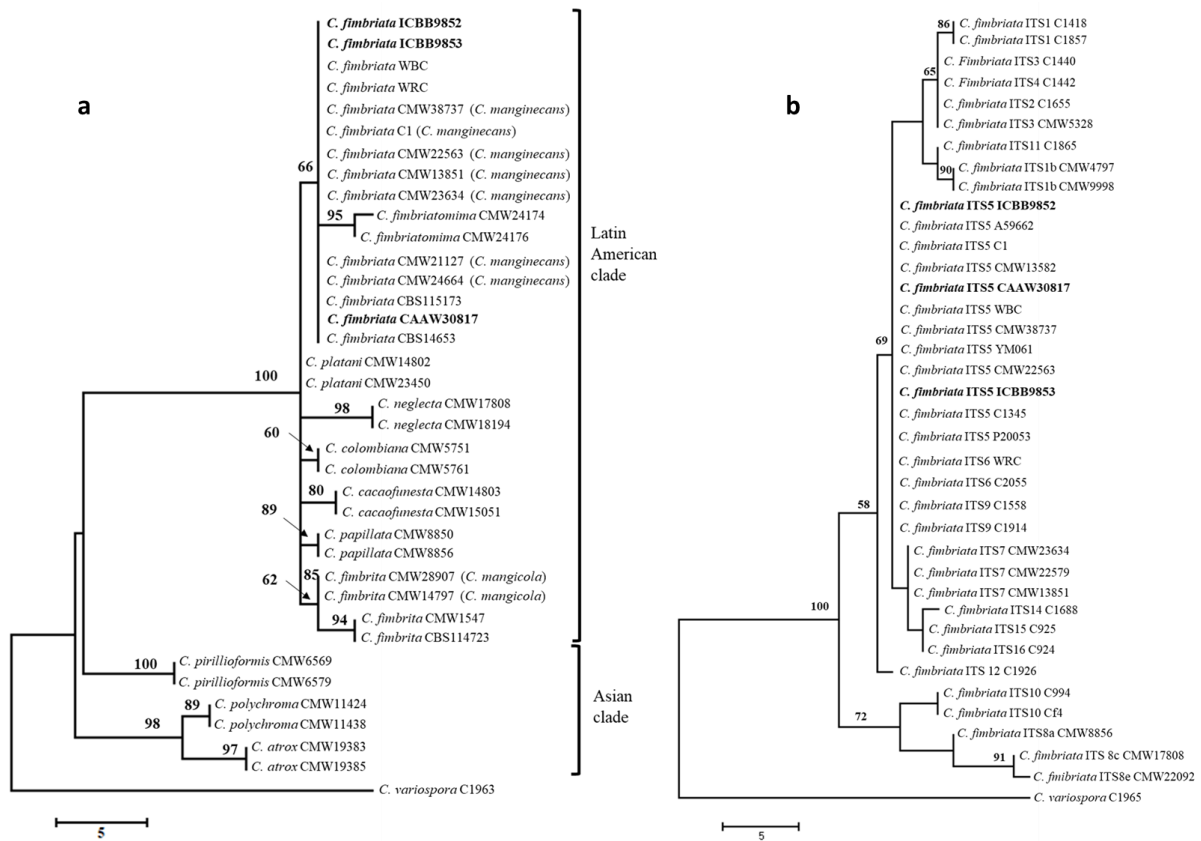
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 186 **Fig. 2.** Morphological characteristics of *Ceratocystis fimbriata* isolated from *Artocarpus*  
 187 *heterophyllus* stem lesion: **a.** ascomata with pirilliform base, **b.** divergent ostiolar hyphae; **c.**  
 188 conidiophore/phialide;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

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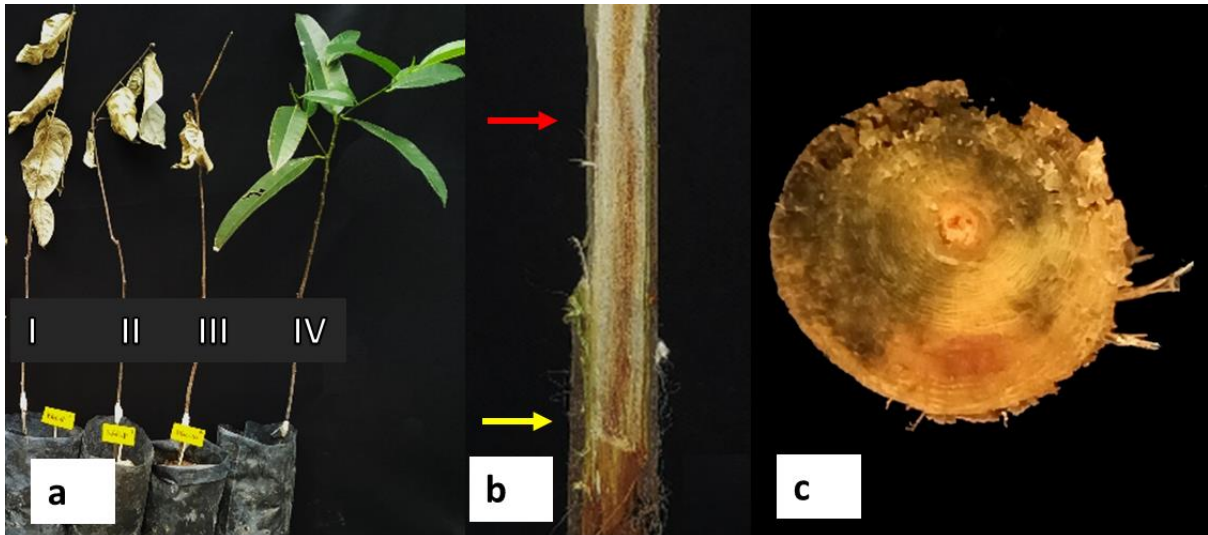
199 **Fig. 3** Phylogenetic tree constructed by MEGA with Maximum Parsimony (MP) analysis by  
200 **a.**  $\beta$ -tubulin sequences from Jackfruit tree in Indonesia (marked in bold) and other species in  
201 the Latin American and Asian clade of the *C. fimbriata* species complex. **b.** ITS sequences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

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



a. muslim unsri &lt;a\_muslim@unsri.ac.id&gt;

---

**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" &lt;a\_muslim@unsri.ac.id&gt;

Cc: "dagmar.hanold@adelaide.edu.au" &lt;dagmar.hanold@adelaide.edu.au&gt;, "dhanold@gmail.com" &lt;dhanold@gmail.com&gt;

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

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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](mailto:a_muslim@unsri.ac.id)

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

CC: [dagmar.hanold@adelaide.edu.au](mailto:dagmar.hanold@adelaide.edu.au), [dhanold@gmail.com](mailto: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).

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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\*\***

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**Your Submission APDN-D-21-00015R4**

1 message

**APDN** <em@editorialmanager.com>  
Reply-To: APDN <jude.estrera@springernature.com>  
To: "A. Muslim" <a\_muslim@unsri.ac.id>

Sun, Jul 25, 2021 at 4:30 PM

CC: [dagmar.hanold@adelaide.edu.au](mailto:dagmar.hanold@adelaide.edu.au), [dhanold@gmail.com](mailto: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).

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Please submit your 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. Who 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.

Line 106-108: 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\*\***

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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## Australasian Plant Disease Notes

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

--Manuscript Draft--

<b>Manuscript Number:</b>	APDN-D-21-00015R5	
<b>Full Title:</b>	Jackfruit ( <i>Artocarpus heterophyllus</i> ), a New Host Plant of <i>Ceratocystis</i> Wilt from South Sumatra, Indonesia	
<b>Article Type:</b>	Plant Disease Note	
<b>Keywords:</b>	Sudden death disease; Moraceae; <i>Ceratocystis fimbriata sensu stricto</i>	
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<b>Abstract:</b>	<p>In 2019, wilt and sudden death were observed on <i>Artocarpus heterophyllus</i> (jackfruit) has been noted. Identification was performed by sequence analysis of the concatenated <math>\beta</math>-tubulin and ITS gene regions. Sequencing of the PCR product confirmed this pathogen was <i>Ceratocystis fimbriata sensu stricto</i>. This is the first report of <i>C. fimbriata</i> causing sudden death disease in <i>A. heterophyllus</i> in Indonesia and worldwide.</p>	
<b>Response to Reviewers:</b>	<p>July 31, 2021</p> <p>Dear Kerrie Ann Davies, PhD Associate Editor Australasian Plant Disease Notes</p> <p>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)</p> <p>Here, we enclose revised version of the manuscript No. APDN-D-21-00015R4 entitled "Jackfruit (<i>Artocarpus heterophyllus</i>), a New Host Plant of <i>Ceratocystis</i> Wilt from South Sumatra, Indonesia" by Rahmat Pratama, Ahmad Muslim, Suwandi Suwandi, Nurhayati Damiri, Soleha Soleha.</p> <p>Below is a summary of our response to the reviewers' comments.</p>	



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. Why 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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# 1 **Jackfruit (*Artocarpus heterophyllus*), a New Host Plant of *Ceratocystis* Wilt** 2 **in South Sumatra, Indonesia**

## 3 4 **Abstract**

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

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

11

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

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

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

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

36 Morphological traits of fruiting bodies and spores were observed under an optical  
37 Olympus CX33 microscope. Ascomatal bases dark brown to black, base subglobose to globose  
38 and measured (n=100), 131.5–250.7×101.6–236.5 μm (Fig. 2a). Ascomata necks erect,  
39 occasionally curved, black at the base becoming subhyaline towards the apex, smooth to  
40 crenulate, 324.7–579.1 μm long including ostiolar hyphae (Fig. 2b). Phialides pale brown to  
41 hyaline (Fig. 2c). Ascospores hat-shaped, 3.4–6.8×2.1–6.2 μm (Fig. 2d). Bacilliform conidia  
42 11.1–36.1×2.1–7.4 μm (Fig. 2e). Barrel conidia 4.4–16.1×2.7–6.9 μm (Fig. 2f).  
43 Chlamydospores oval, thick walled, smooth, 6.7–16.5×5.9–12.9 μm (Fig. 2g). Based on these  
44 morphological characters, the fungus was identified as *Ceratocystis fimbriata*. Two  
45 representative isolates were deposited at the ICBB Culture Collection for Microorganisms and  
46 Cell Culture, Indonesian Center for Biodiversity and Biotechnology, (Bogor, Indonesia) as  
47 accessions ICBB9852 and ICBB9853.

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

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

67 sequences representing species in the Latin American (LAC) and Asian clade (AC) of the *C.*  
68 *fimbriata* species complex (Fourie et al. 2015; Oliveira et al. 2015; Barnes et al. 2018). To  
69 determine relatedness of isolates from jackfruit with known *C. fimbriata* populations, the ITS  
70 sequence was manually aligned with known ITS haplotypes as designated by Harrington et al.  
71 (2014); Li et al. (2016) and phylogenetic analyses were performed. Maximum Parsimony (MP)  
72 analyses were performed in MEGA v. 10 (Kumar et al. 2016; Paul et al. 2018) with 1000  
73 replications. The analysis involved 38 ( $\beta$ -tubulin) and 37 (ITS) nucleotide sequences. All  
74 positions containing gaps and missing data were eliminated. There were 408 ( $\beta$ -tubulin) and  
75 518 (ITS) positions in the final dataset. *Ceratocystis variospora* was used as the out-group.  $\beta$ -  
76 tubulin sequence of our isolates confirmed the assignment to LAC of *C. fimbriata sensu lato*  
77 (Fig. 3a). Manual alignment of the ITS sequences with previously described ITS genotypes  
78 (Harrington et al. 2014; Li et al. 2016) grouped the isolates into ITS5 haplotype of *C. fimbriata*  
79 *sensu stricto* (Fig. 3b). Consistency (CI), retention (RI), and composite indexes (CoI) for  $\beta$ -  
80 tubulin were 0.566667, 0.845238, 0.668011, respectively and ITS was 0.933333, 0.976563,  
81 0.932836, respectively.

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

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

108

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113 Ministry of Research, Technology, and Higher Education, Number:  
114 068/SP2H/AMD/LT/DRPM/2020.

115

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211 **Table 1** *Ceratocystis* isolates included in the phylogenetic analyses

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

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

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169

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

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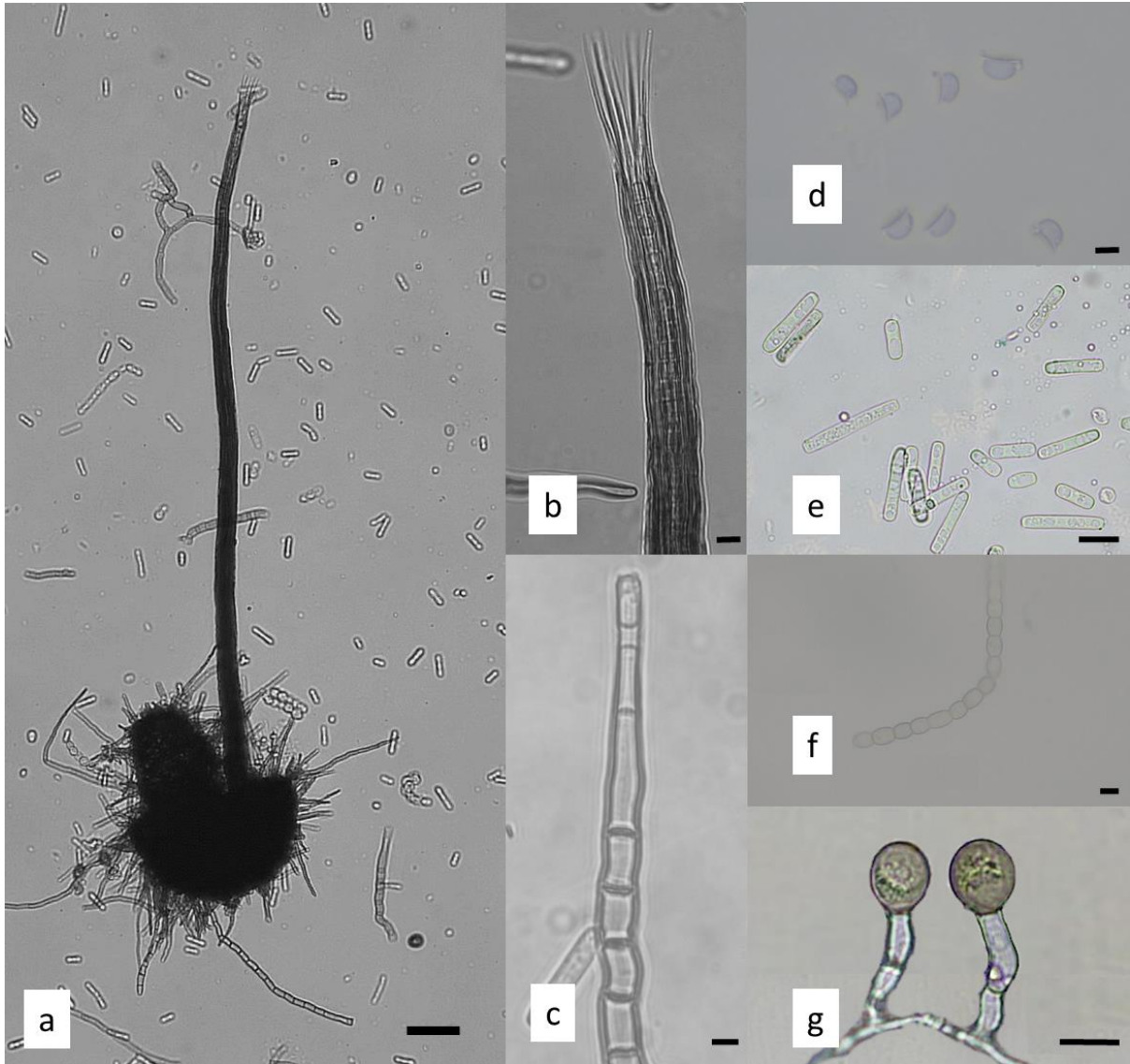
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183 **Fig. 2** Morphological characteristics of *Ceratocystis fimbriata* isolated from *Artocarpus*  
 184 *heterophyllus* stem lesion: **a.** ascomata with pirilliform base, **b.** divergent ostiolar hyphae; **c.**  
 185 conidiophore/phialide; **d.**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

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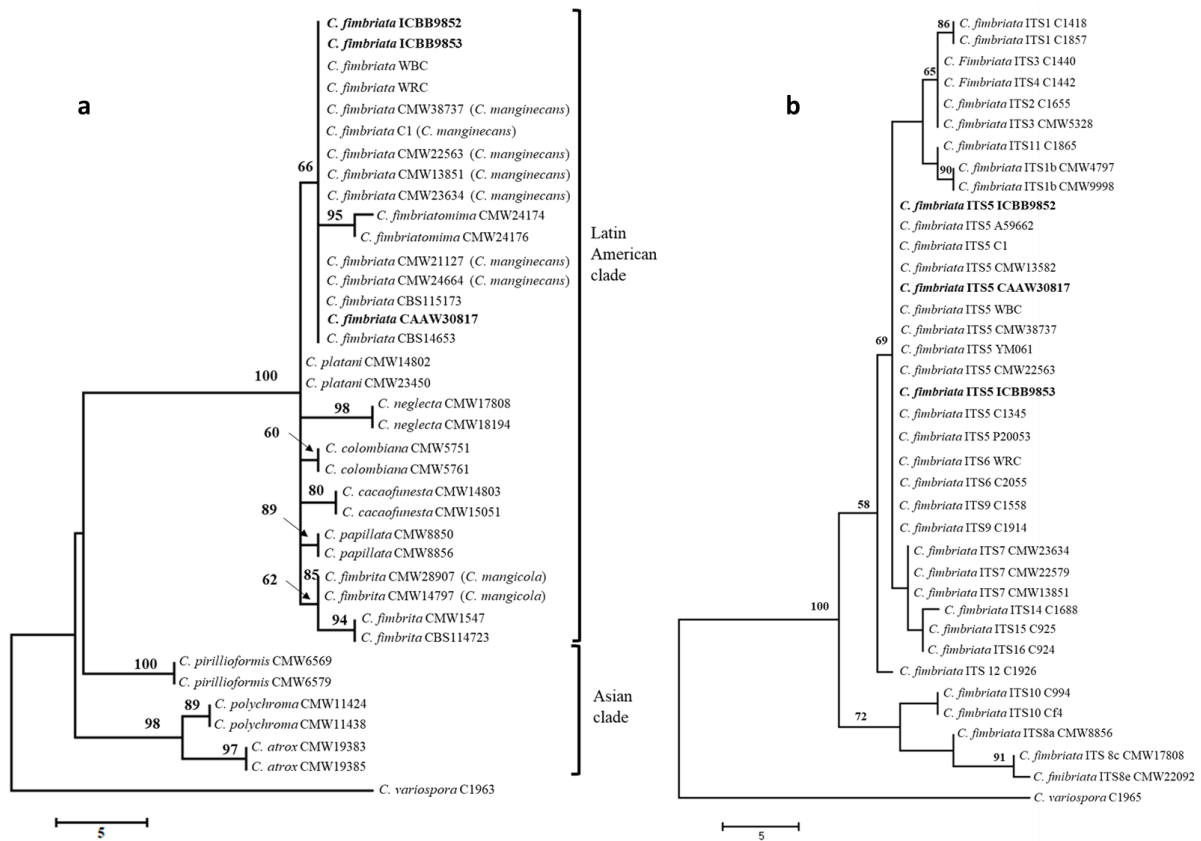
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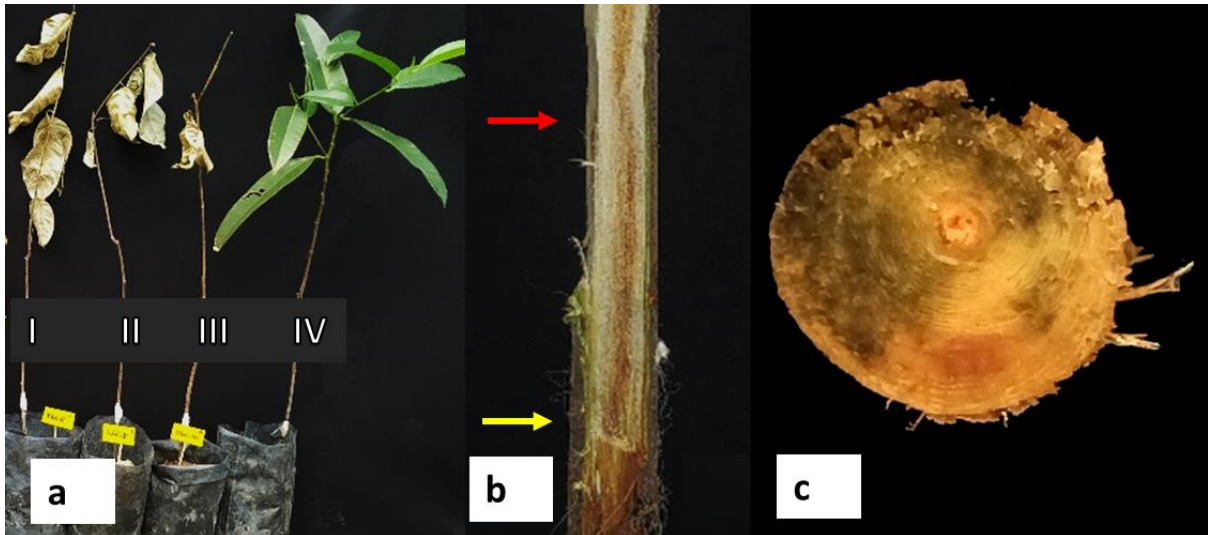
196 **Fig. 3** Phylogenetic tree constructed by MEGA with Maximum Parsimony (MP) analysis by  
 197 **a.**  $\beta$ -tubulin sequences from Jackfruit tree in Indonesia (marked in bold) and other species in  
 198 the Latin American and Asian clade of the *C. fimbriata* species complex. **b.** ITS sequences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

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**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 &lt;a\_muslim@unsri.ac.id&gt;

---

**Your Submission APDN-D-21-00015R4**

1 message

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**APDN** <em@editorialmanager.com>  
Reply-To: APDN <jude.estrera@springernature.com>  
To: "A. Muslim" <a\_muslim@unsri.ac.id>

Sun, Jul 25, 2021 at 4:30 PM

CC: [dagmar.hanold@adelaide.edu.au](mailto:dagmar.hanold@adelaide.edu.au), [dhanold@gmail.com](mailto: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).

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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. Who 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.

Line 106-108: 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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Thu, Aug 5, 2021 at 7:49 AM

CC: [dagmar.hanold@adelaide.edu.au](mailto:dagmar.hanold@adelaide.edu.au), [dhanold@gmail.com](mailto: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.

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With best regards,

Dagmar Hanold  
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Fri, Aug 20, 2021 at 5:30 AM

Dear Prof. Dagmar Hanold  
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Australasian Plant Disease Notes

Thank you very much for your email regarding our paper entitled "Jackfruit (*Artocarpus heterophyllus*), a New Host Plant of *Ceratocystis* Wilt from South Sumatra, Indonesia" (APDN-D-21-00015R5).

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Best Regard  
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# 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)

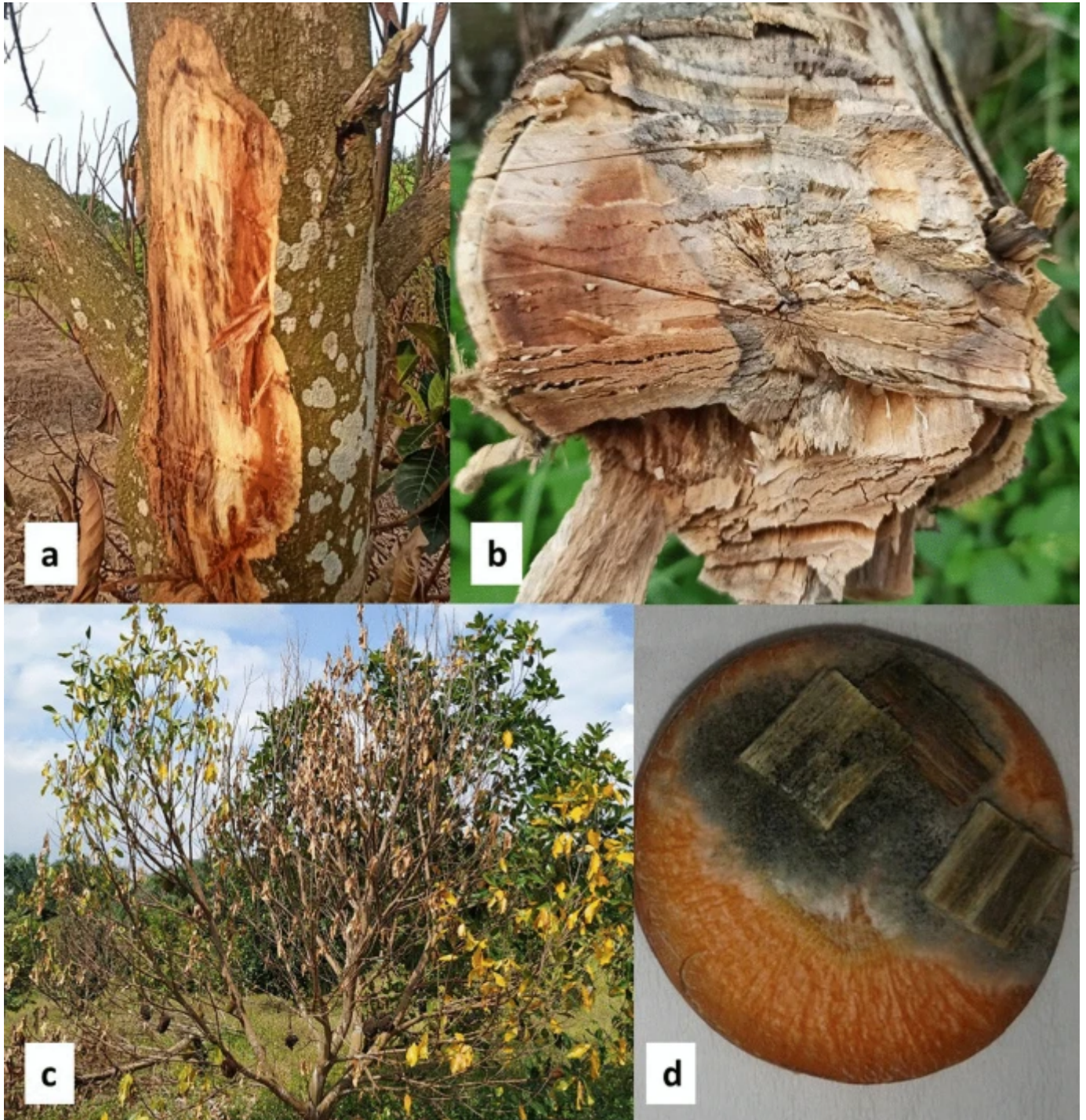
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## Abstract

In 2019, wilt and sudden death were observed on *Artocarpus heterophyllus* (jackfruit). Identification was performed by sequence analysis of the concatenated  $\beta$ -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**

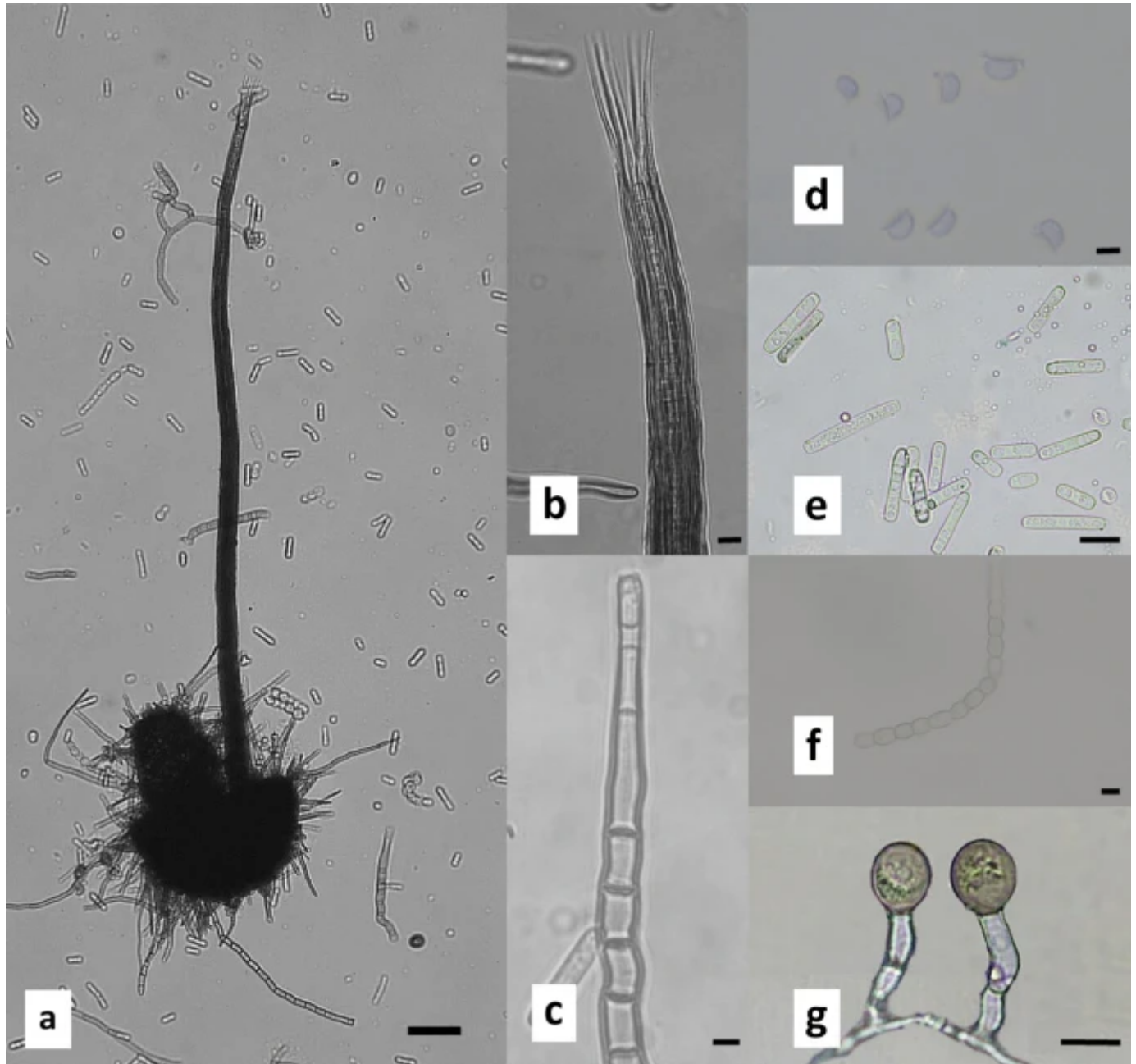
Symptoms of *Ceratocystis fimbriata* wilt disease in *Artocarpus heterophyllus*: **a** vascular discoloration of infected tree; **b** the discolored wood extended to the heartwood of the basal stem; **c** three-year-old tree with wilted, yellowing leaves and rotten fruit; **d** isolation of the fungus 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  $\mu\text{m}$ ; b–c, e–g = 10  $\mu\text{m}$ ; d = 5  $\mu\text{m}$

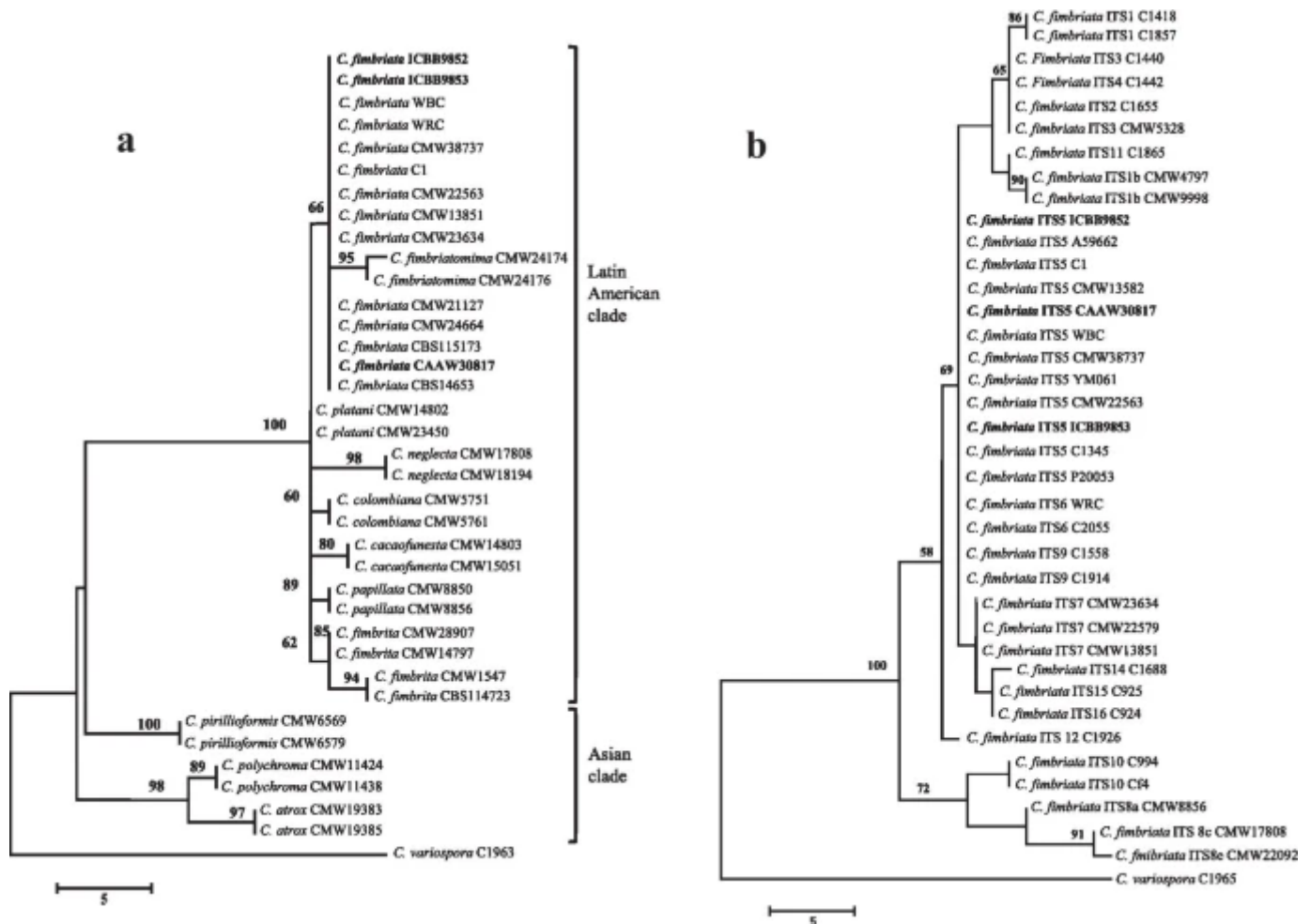
To confirm the species identification, isolates were cultured on potato dextrose broth (PDB) at room temperature for one week. Mycelium was filtered through Whatman filter paper and genomic DNA was extracted from the fungal mycelial mat using YeaStar Genomic DNA Kit (Zymo Research Corporation, California, 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  $\beta$ -tubulin ( $\beta\text{t}$ ) gene with primers  $\beta\text{t}1\text{a}$  and  $\beta\text{t}1\text{b}$  (Glass and Donaldson [1995](#)). Amplifications were carried out in 50  $\mu\text{l}$  reactions containing 20  $\mu\text{l}$  DreamTaq Green PCR Master Mix (Eppendorf, Germany) (DreamTaq DNA Polymerase, 2X DreamTaq Green buffer, dNTPs, and 4 mM  $\text{MgCl}_2$ ), 1.5  $\mu\text{l}$  of each forward and reverse primer, 4  $\mu\text{l}$  of DNA template and 23  $\mu\text{l}$  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  $\beta$ -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  $\beta$ -tubulin.  $\beta$ -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 ( $\beta$ -tubulin) and 37 (ITS) nucleotide sequences. All positions containing gaps and missing data were eliminated. There were 408 ( $\beta$ -tubulin) and 518 (ITS) positions in the final dataset. *Ceratocystis variospora* was used as the out-group.  $\beta$ -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  $\beta$ -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**  $\beta$ -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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### Keywords

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

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# 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). Identification was performed by sequence analysis of the concatenated  $\beta$ -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. Ascum bases dark brown to black, base subglobose to globose and measured (n = 100), 131.5–250.7 × 101.6–236.5  $\mu$ m (Fig. 2a). Ascum necks erect, occasionally curved, black at the base becoming subhyaline towards the apex, smooth to crenulate, 324.7–579.1  $\mu$ m long including ostiolar hyphae (Fig. 2b). Phialides pale brown to hyaline (Fig. 2c). Ascospores hat-shaped, 3.4–6.8 × 2.1–6.2  $\mu$ m (Fig. 2d). Bacilliform conidia 11.1–36.1 × 2.1–7.4  $\mu$ m (Fig. 2e). Barrel conidia 4.4–16.1 × 2.7–6.9  $\mu$ m (Fig. 2f). Chlamydospores oval, thick walled, smooth, 6.7–16.5 × 5.9–12.9  $\mu$ m (Fig. 2g). Based on these morphological characters, the fungus was identified as *Ceratocystis fimbriata*. Two representative isolates were deposited at the ICBB Culture Collection

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**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  $\beta$ -tubulin ( $\beta$ t) gene with primers  $\beta$ t1a and  $\beta$ t1b (Glass and Donaldson 1995). Amplifications were carried out in 50  $\mu$ l reactions containing 20  $\mu$ l DreamTaq Green PCR Master Mix (Eppendorf, Germany) (DreamTaq DNA Polymerase, 2X DreamTaq Green buffer, dNTPs, and 4 mM  $MgCl_2$ ), 1.5  $\mu$ l of each forward and reverse primer, 4  $\mu$ l of DNA template and 23  $\mu$ l 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  $\beta$ -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  $\beta$ -tubulin.  $\beta$ -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

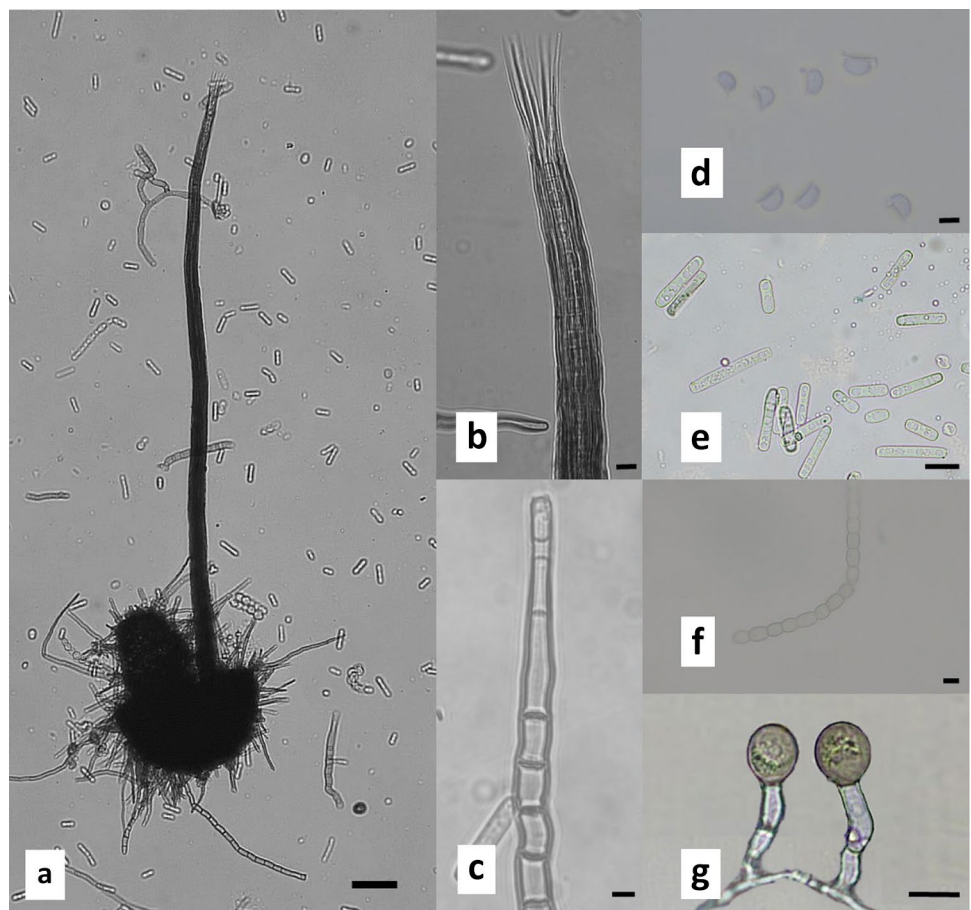
Species	Haplotype	Isolates no	Host	Origin	GenBank accession no	
					ITS	$\beta$ -Tubulin
<i>C. fimbriata</i>	ITS1a	C1418	<i>Ipomoea batatas</i>	USA	AY157956	–
	ITS1	C1857	<i>Ficus carica</i>	Brazil	HQ157542	–
	ITS1b	CMW4797	<i>Eucalyptus</i> sp.	Congo	FJ236733	–
	ITSb	CMW9998	<i>Eucalyptus</i> sp.	South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

**Table 1** (continued)

Species	Haplotype	Isolates no	Host	Origin	GenBank accession no	
					ITS	$\beta$ -Tubulin
<i>C. papillata</i>	LAC	CMW15051	<i>T. cacao</i>	Costa Rica	–	KJ601510
	LAC	CMW8850	<i>Citrus</i> × <i>Tangelo hybrid</i>	Colombia	–	AY233875
	LAC	CMW8856	<i>Citrus limon</i>	Colombia	–	AY233874
<i>C. fimbriata</i>	LAC	CMW14797	<i>M. indica</i>	Brazil	–	EF433307
	LAC	CMW28907	<i>M. indica</i>	Brazil	–	FJ200270
	LAC	CMW1547	<i>I. batatas</i>	Papua New Guinea	–	EF070443
<i>C. fimbriatomima</i>	LAC	C1421	<i>I. batatas</i>	USA	–	KF302689
	LAC	CMW24174	<i>Eucalyptus hybrid</i>	Venezuela	–	EF190951
	LAC	CMW24176	<i>Eucalyptus hybrid</i>	Venezuela	–	EF190952
<i>C. fimbriata</i>	LAC	CMW21127	<i>A. crassicarpa</i>	Indonesia	–	EU588643
	LAC	CMW24664	<i>Eucalyptus hybrid</i>	China	–	JQ862720
	LAC	CBS115173	<i>Gmelina arborea</i>	Brazil	–	KF302700
<i>C. platani</i>	LAC	CBS14653	<i>C. arabica</i>	Suriname	–	KF302702
	LAC	CMW14802	<i>Platanus occidentalis</i>	USA	–	EF070425
	LAC	CMW23450	<i>P. occidentalis</i>	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  $\mu$ m; b–c, e–g = 10  $\mu$ m; d = 5  $\mu$ m



(MP) analyses were performed in MEGA v. 10 (Kumar et al. 2016; Paul et al. 2018) with 1000 replications. The analysis involved 38 ( $\beta$ -tubulin) and 37 (ITS) nucleotide

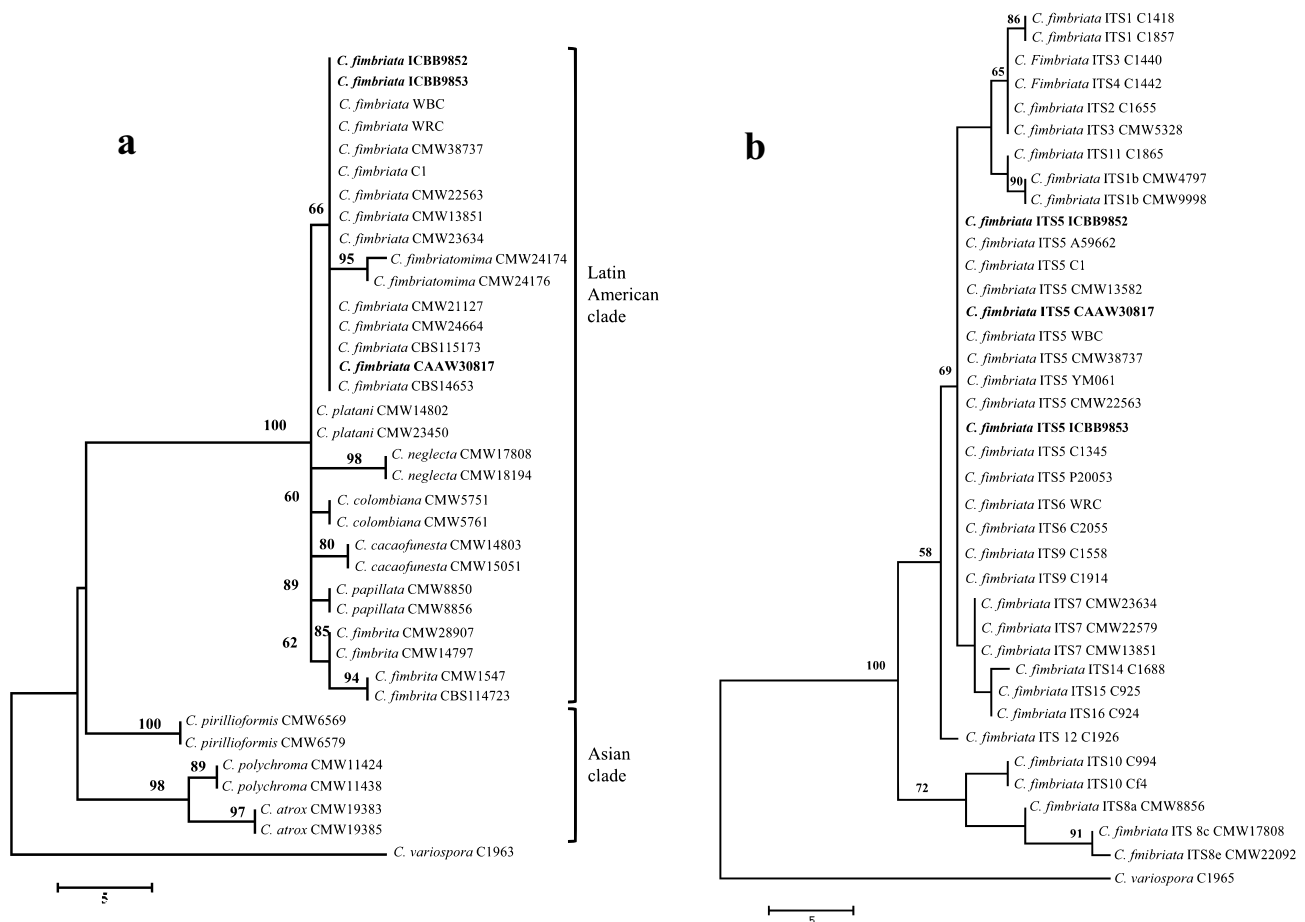
sequences. All positions containing gaps and missing data were eliminated. There were 408 ( $\beta$ -tubulin) and 518 (ITS) positions in the final dataset. *Ceratocystis variospora* was

used as the out-group.  $\beta$ -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  $\beta$ -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. heterophyllum* 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 discoloration (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).

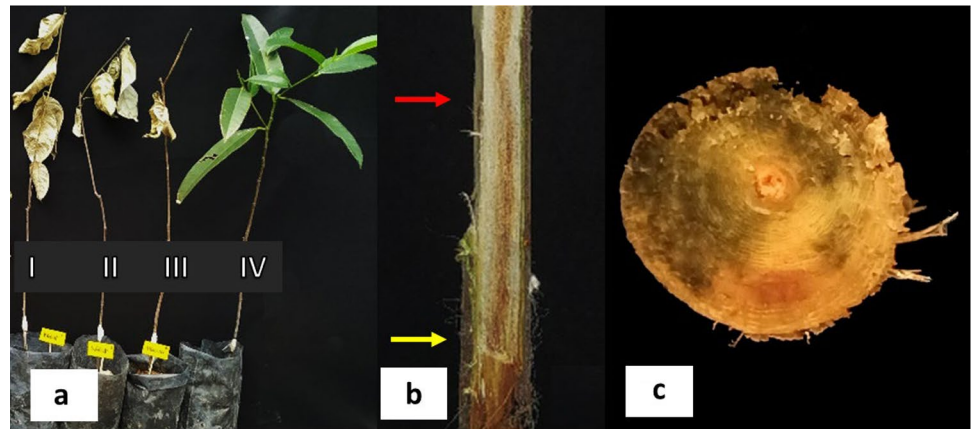
This is the first report of *C. fimbriata* causing wilt and die-back in Jackfruit in Indonesia and worldwide. The symptoms



**Fig. 3** Phylogenetic tree constructed by MEGA with Maximum Parsimony (MP) analysis by **a**  $\beta$ -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 heterophyllus* 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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