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RESEARCH ARTICLE

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Investigating bark, ambrosia and nitidulid beetle (Coleoptera: Scolytinae and Nitidulidae) communities and their potential role in the movement of *Ceratocystis manginecans* in commercial forestry plantations in Riau, Indonesia

Kira M. T. Lynn[1] | Michael J. Wingfield[1] | Marthin Tarigan[2] | Alvaro Durán[2] | Samuel A. Santos[2] | Wilma J. Nel[1] | Irene Barnes[1]

[1]Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa

[2]Plant Health Program, Research and Development, Asia Pacific Resources International Holdings Ltd, Pangkalan Kerinci, Indonesia

Correspondence
Irene Barnes, University of Pretoria, Hilcrest, 74 Lunnon Road, Pretoria 0002, South Africa.
Email: irene.barnes@fabi.up.ac.za

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Abstract

1. Ceratocystis Canker and Wilt Disease (CCWD), caused by the fungal pathogen *Ceratocystis manginecans*, poses a significant threat to *Acacia* and *Eucalyptus* plantations in Indonesia. Infections typically occur through wounds on the main stems of trees, and infected trees, particularly *Acacia*, are often co-infested by various wood-associated beetles. The aim of this study was to identify the beetle species associated with *Ceratocystis*-infected trees and to assess their potential role in dispersing *C. manginecans*.
2. Beetle and frass samples were collected from *Ceratocystis*-infected compartments of *Acacia* and *Eucalyptus* propagated as monocultures in Riau, Indonesia, either by using traps or by direct collection from infected trees. The samples were screened for the presence and viability of *Ceratocystis* propagules using a quantitative polymerase chain reaction-high-resolution melting analysis (qPCR-HRMA) diagnostic tool and a carrot baiting technique, respectively.
3. Fourteen beetle species, predominantly collected from traps, representing nine genera of the *Scolytinae* and *Nitidulidae*, were identified from the two plantation types.
4. The qPCR tool detected the presence of *Ceratocystis* DNA on 13 of the 14 beetle species at concentrations as low as 0.015–0.0019 ng. However, viable *Ceratocystis* propagules could not be recovered from these beetles using culture-based methods.
5. Of the 105 frass samples screened, qPCR detected *Ceratocystis* DNA in 67 samples; however, only one *Ceratocystis* isolate was obtained using the carrot baiting method.
6. The results of this study suggest that the beetles and beetle frass can harbour *Ceratocystis* propagules, as indicated by the detection of fungal DNA. However, the

viability of the propagules and the ability of the beetles and/or beetle frass to spread *C. manginecans* requires further investigation.

KEYWORDS
Ceratocystidaceae, fungal pathogen detection, insect-fungus interactions, qPCR, tropical plantation forestry

INTRODUCTION

The fungal pathogen *Ceratocystis manginecans* is the causal agent of Ceratocystis Canker and Wilt Disease (CCWD) on a variety of economically important plant hosts (Al Adawi, Barnes, et al., 2013; Liu et al., 2021; Tarigan, Roux, et al., 2011; Wingfield et al., 2023). In Southeast Asia (SEA), this disease has resulted in the discontinuation of *Acacia mangium* as a profitable forestry species (Harwood & Nambiar, 2014) and is an emerging threat to *Acacia crassicarpa* and clonal *Eucalyptus pellita* planted to meet the demand for pulp and paper (Barnes et al., 2023; Wingfield et al., 2023). Disease symptoms resulting from *Ceratocystis* spp. infection of trees, particularly those in the Latin American Clade (LAC), include cankers, wilt, vascular tissue discoloration and, in severe cases, tree death (Al Adawi, Barnes, et al., 2013; Barnes et al., 2018; Tarigan, Roux, et al., 2011).

Ceratocystis is classified in *Ceratocystidaceae* as defined by de Beer et al. (2014) and species infect hosts via wounds, allowing them to colonize the vascular tissues (Al Adawi et al., 2006; Heath et al., 2009; Sun et al., 2020; Wingfield et al., 2023). In Indonesia, *C. manginecans* infection typically occurs on the main stems of *Acacia* and *Eucalyptus* trees via wounds caused by animal feeding and silvicultural practices or through natural openings (Tarigan, Wingfield, et al., 2011; Wingfield et al., 2023). Despite best management practices to reduce these points of infection, the movement of *C. manginecans* within and between *Acacia* and *Eucalyptus* plantations has continued in Indonesia (Hlongwane, 2022).

The long-necked ascomata and sticky ascospores of *Ceratocystis* spp., as well as their fruity aromas, make them well adapted to insect dispersal (Webber & Gibbs, 1989; Wingfield et al., 2017). This accounts for the fact that many *Ceratocystis* spp. have been reported to have associations with insects, particularly nitidulid beetles (Coleoptera: Nitidulidae) and flies (Diptera) (Al Adawi, Al Jabri, et al., 2013; Appel et al., 1990; Heath et al., 2009). Specifically, nitidulid beetles, commonly known as sap beetles, are generalist feeders attracted to the aromatic compounds produced by these fungi, or to the sugary exudates associated with freshly made wounds (Harrington, 2013; Harrington & Appel, 2009). These fresh wounds can occur on healthy uninfected trees, providing entry points for fungal infection. During these interactions, the exoskeletons and/or guts of the insects can become inoculated with fungal spores that are subsequently transmitted to new, susceptible hosts when they disperse in search of food (Gibbs & French, 1979; Harrington, 2013).

Ceratocystis species can also form opportunistic associations with other insects, such as ambrosia and bark beetles, which can act as vectors (Al Adawi, Al Jabri, et al., 2013; Klepzig et al., 2001; Roy et al., 2018; Roy et al., 2023; Wingfield et al., 2023). In these associations, *Ceratocystis* species may be transported as auxiliary fungi, neither directly attracting their vectors nor serving as their primary food source (Al Adawi, Al Jabri, et al., 2013; Roy et al., 2018). These loose associations occur because many bark and ambrosia beetles favour plant hosts with weakened defence mechanisms making them easier to colonize (Batra, 1967; Lieutier et al., 2009; Ranger et al., 2015, 2023). Plant hosts infected or weakened by pathogenic *Ceratocystis* species can thus be targeted by a variety of bark and ambrosia beetles and subsequently opportunistically aid in the dispersal of the fungi (Al Adawi, Al Jabri, et al., 2013; Roy et al., 2018, 2020, 2023; Wingfield et al., 2023).

Ambrosia beetles are a group of obligate fungus-feeding woodborers in the *Scolytinae*, that are largely economically unimportant since they favour dead or dying plant hosts (Hulcr et al., 2015). However, in some cases they may be aggressive primary pests that live in obligate associations with fungi, particularly in genera such as *Raffaelea* and *Fusarium*, and transmit these fungi to trees causing serious damage (Eskalen et al., 2013; Fraedrich et al., 2008; Ploetz et al., 2013). *Ceratocystis* spp. are not known to be obligate nutritional symbionts of ambrosia beetles (Mayers et al., 2020; Mayers et al., 2022). However, the spread of several *Ceratocystis* diseases has been linked to ambrosia beetle damage and the frass that they produce (Ocasio-Morales et al., 2007; Soulioti et al., 2015; Souza et al., 2013; Tsopelas et al., 2017). The best-studied example is in Hawai'i, where ambrosia beetles, together with their frass, have been shown to play a role in the dispersal of *Ceratocystis lukuohia* and *Ceratocystis huliohia* (Hughes et al., 2023; Roy et al., 2020, 2023), the causal agents of Rapid ʻŌhiʻa Death (Barnes et al., 2018). When diseased *Metrosideros polymorpha* is infested by these beetles, expelled frass inoculated with *Ceratocystis* propagules, particularly chlamydospores, acts as an inoculum source for future infections (Hughes et al., 2023). Additionally, the exoskeletons of infesting beetles can become contaminated with propagules, resulting in passive dispersal of the pathogen (Al Adawi, Al Jabri, et al., 2013; Peplinski, 1974; Roy et al., 2023). The boring activities of the beetles into healthy trees (Kuhnholz et al., 2001; Lynn et al., 2021) can also establish wounds necessary for subsequent new *Ceratocystis* infections (Harrington, 2013).

Unlike ambrosia beetles, bark beetles lack obligate associations with fungi but rather feed on the tissue beneath the bark of trees (Hulcr et al., 2015). Although they differ ecologically from ambrosia beetles, bark beetles play a similar role in spreading diseases caused by *Ceratocystis* spp. This is demonstrated by the role that the mango bark beetle *Cryphalus dilutus* (Johnson et al., 2017), has played in the spread of *C. manginecans* in commercial mango orchards in Oman and Pakistan (Al Adawi, Al Jabri, et al., 2013).

FIGURE 1 Compartments infected with Ceratocystis Canker and Wilt Disease (CCWD). (a) Symptoms of CCWD in infected *Eucalyptus* showing wilting, coppicing and mortality. (b) Symptoms of CCWD in infected *Acacia crassicarpa* showing stem canker malformations.

In Indonesia, the movement of *C. manginecans* within and between commercial plantations has raised questions regarding its infection biology in these environments. *Acacia crassicarpa* trees infected by *C. manginecans* (Tarigan, Roux, et al., 2011) are commonly co-infested by different species of ambrosia beetle, particularly *Euwallacea similis* and *E. perbrevis* (Lynn et al., 2020; Lynn et al., 2021). However, not all of these infested trees are infected with *C. manginecans* or show symptoms of disease, suggesting that beetle infestation can also occur in disease-free trees (Lynn et al., 2020; Lynn et al., 2021). In contrast, *Eucalyptus* trees wilting due to infection by *C. manginecans* are rarely found to be infested by ambrosia beetles. Because ambrosia, bark and nitidulid beetles can act as opportunistic vectors of fungal pathogens, and the expelled frass of beetles can act as a dispersal agent of the pathogen, their involvement in the dispersal of *C. manginecans* in Indonesia has been hypothesised. This is supported by indirect evidence from similar observations in Malaysia (Wingfield et al., 2023). The aim of this study was thus to (1) investigate the community of wood-dwelling beetles present in *A. crassicarpa* and clonal *E. pellita* plantations in Riau, Indonesia, and (2) to consider their possible role in the spread of *C. manginecans*.

MATERIALS AND METHODS

Collection of beetles and frass

Beetle specimens and frass samples were obtained from five *A. crassicarpa* (2–3 years-old) and five clonal *E. pellita* (5–6 years old) compartments showing symptoms of *C. manginecans* infection in Riau, Indonesia (Figure 1). All plantations were monocultures maintained and harvested to produce pulp and paper. A map of the sample sites and their proximity to natural greenbelts is shown in Figure 2. The clonal *E. pellita* compartments are located on mineral soil, 73.5 km from the *A. crassicarpa* compartments, which are mostly grown on peatland. Measured from the centre of the compartments, the *Eucalyptus* sampling sites were closer to the native forest greenbelts than the *Acacia* sampling sites (Figure 2).

Beetle specimens were collected by direct field sampling using modified Bambara beetle traps baited with a lure (Hulcr & McCoy, 2015; Nel et al., 2021). These traps were constructed from 2-L plastic bottles, with a lengthwise cavity (approximately 20 × 5 cm) cut from the middle of the bottle. A 60 mm Petri dish with a plastic 0.5 mm mesh insert, punctured with five circular holes (each 0.5 cm in diameter), was glued to the inside base of the bottle to prevent specimens from falling into the ethanol/anti-freeze lure. A thin plastic rain shield was placed above the bottle (Figure 3). Five traps were set up in each compartment. The first trap was placed 10 rows of trees in from the access road (approx. 30 m), and the subsequent traps were positioned diagonally, 10 trees apart (approx. 30 m), forming a V shape. Each trap was attached to a tree at a height of 1.5 m. The traps were filled to just below the base of the Petri dish with a mixture of nine parts ethanol (99%) to one-part anti-freeze (ethylene glycol based) and left for 4–5 days, after which beetles were collected and the traps refilled. Five collections of beetle specimens from traps were made from late April to early May 2022, which is considered the Indonesian dry season.

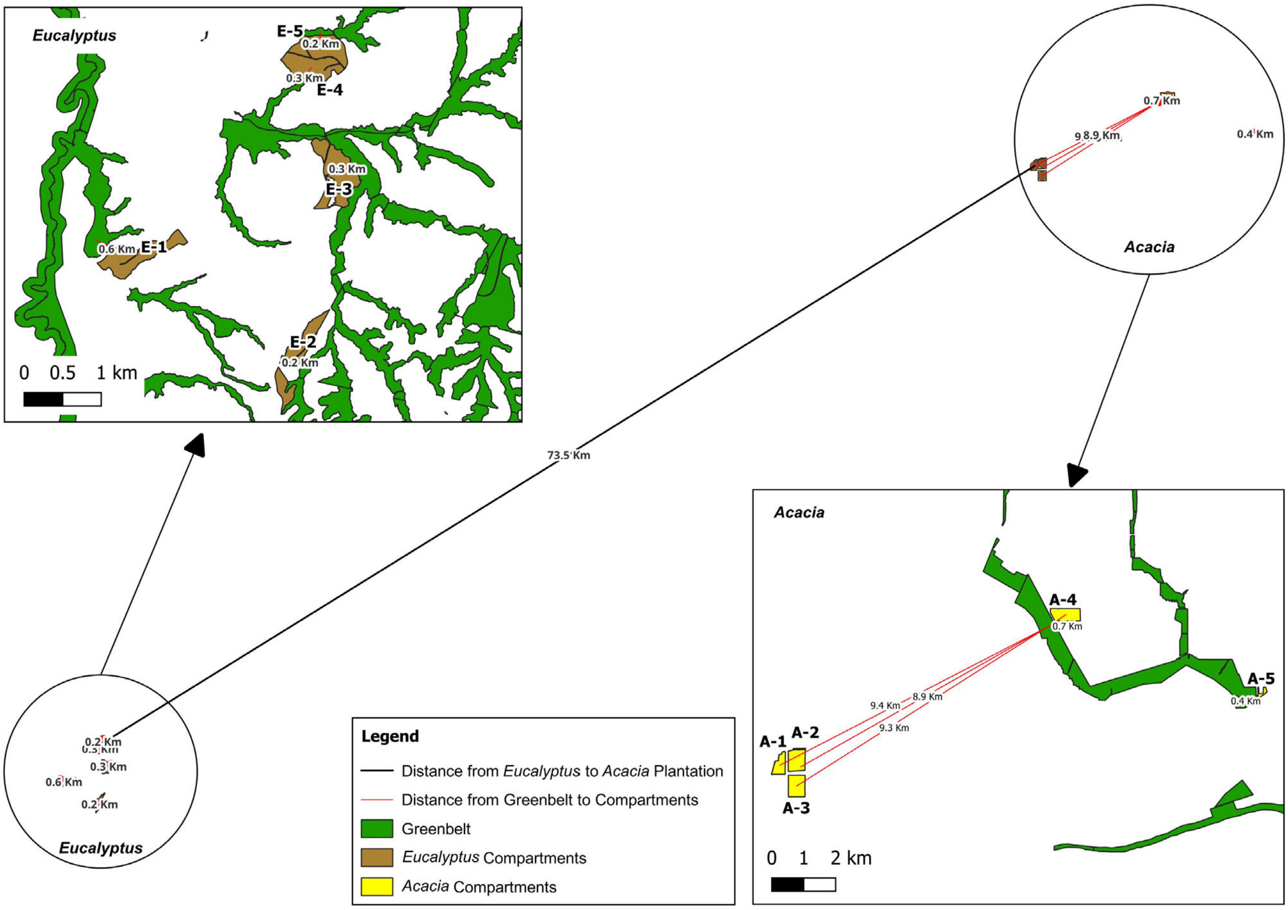

FIGURE 2 Map of sampling sites from commercial plantations in Riau, Indonesia. This map illustrates the locations of sampling sites, their distances from each other and their proximity to greenbelts. [Exact distance data are not included due to confidentiality agreements with the forestry partner, but approximate proximity can be inferred from the scale bar.]

Although ethanol is a well-documented attractant for many wood-associated beetles, including nitidulids, not all scolytine species are attracted to ethanol (Dodge et al., 2017). Therefore, manual collections of beetles were also conducted from three highly infested *A. crassicarpa* trees at each of the five collection sites. The clonal *E. pellita* showed few or no signs of beetle infestation, and manual beetle extractions from this host were not performed. The highly infested *A. crassicarpa* trees were felled 50 cm above ground level, cut into 50 cm billets, and split to reveal beetle galleries. Live beetle specimens were extracted and placed directly into individual 2 mL Eppendorf® tubes.

For frass collection, trees along the Bambara trap placement sites were inspected for extruding frass (Figure 4). Frass was collected from individual beetle entry holes and placed directly into 2 mL Eppendorf® tubes. Although care was taken to select fresh samples from trees showing typical symptoms of *C. manginecans* infection, such as depressed and discoloured bark and canker formations on stems, samples were collected predominantly based on availability. Therefore, the freshness of the samples and the degree of *C. manginecans* infection (assessed based on non-destructive visual inspection for infection symptoms) varied across the samples. Five frass collection trips were conducted throughout the study period, from late April to early May 2022.

Beetle collection and identification

All beetle specimens collected using both the modified Bambara beetle traps and manual extractions were tentatively identified by microscopic comparisons using an online bark and ambrosia beetle identification resource (https://www.barkbeetles.info/amer_links.php) and online nitidulid resource (https://bugguide.net/node/view/1117200/bgimage). Based on these initial identifications, the beetles were sorted into different morphotypes and stored individually in 2 mL Eppendorf® tubes for subsequent *Ceratocystis* screening and molecular identification. Representatives of each morphotype were sent to Dr. Andrew J. Johnson (Forest Entomology Laboratory, University of Florida, Gainesville) who aided in their morphological identification, to mostly species level. For each morphotype, the lateral and dorsal views of representative specimens were photographed using an AXIO Zoom.V16 microscope equipped with an AxioCaM 512 colour camera using Zen Blue 3.2 (Carl Zeiss CMP, Göttingen, Germany).

FIGURE 3 Modified Bambara beetle trap. (a) Modified trap placed in CCWD infected *Acacia crassicarpa* compartments. (b) This trap contained a punctured 60 mm Petri dish with a plastic 0.5 mm mesh insert glued to the lid to prevent beetle specimens from falling into the ethanol/anti-freeze lure.

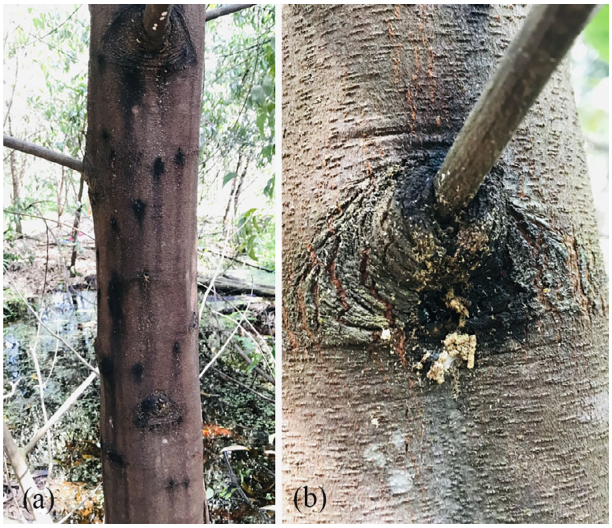

FIGURE 4 *Acacia crassicarpa* trees infested with wood-boring beetles expelling frass. (a) Heavily infested *A. crassicarpa* stem. (b) Frass noodle expelled from infested *A. crassicarpa* stem.

Only specimens identified in the families *Scolytinae* and *Nitidulidae* were used in subsequent analysis, as species in these families have previously been reported to be associated with the dispersal of *Ceratocystidaceae* species.

For further molecular based identification, beetle fragments left over after the *Ceratocystis* screening as described below, were used for DNA extraction using the Macherey Nagel NucleoSpin Tissue Kit (Macherey–Nagel, Dueren, Germany) following the manufacturer's protocol. Fragments of beetles directly macerated onto carrots could not be retrieved for DNA extraction and were only recorded based on their morphological identification. For molecular-based beetle identification, PCR amplification of the partial ribosomal large subunit (28S) (Belshaw & Quicke, 1997; Cognato, 2013) was performed using the primer pair 3665 and 4068. PCR amplification of the mitochondrial cytochrome oxidase c subunit I gene (COI) (Folmer et al., 1994) using the primer pairs LCOI490 and HCO2198 was performed on 20 representative specimens for additional confirmation of specimen identity. Each PCR reaction mixture consisted of 2.5 μL 5× MyTaq buffer (Bioline, London, UK), 0.25-μL MyTaq DNA polymerase (Bioline), 2-μL DNA template, 0.5 μL of each primer (10 mM), and 8.25 μL of sterile deionized water, for a 14-μL total reaction mixture. PCR amplification of the 28S region was performed according to Cognato (2013), and for the COI region according to Lynn et al. (2020).

PCR amplification was confirmed by staining 1-μL PCR product with 1-μL GelRed Nucleic Acid Gel Stain (Biotium, Hayward, California) and separation by electrophoresis on a 2% agarose gel, followed by visualization under ultraviolet (UV) light. Successful PCR amplicons were treated with ExoSAP-IT™ PCR Product Clean-up Reagent (ThermoFisher Scientific, Massachusetts, United States). Cleaned products were sequenced in both directions in 10 μL reactions using the same primer pairs as stated above, and the BigDye®Terminator v3.1 cycle sequencing kit (ThermoFisher Scientific). The sequencing annealing temperature for both primer pairs was 56°C. Sequencing products were cleaned using the sodium acetate/ethanol method and submitted to the University of Pretoria DNA sanger sequencing facility for analyses on the ABI Prism 3500xl autosequencer (Applied Biosystems, Foster City, California).

The forward and reverse sequencing reads were assembled into contigs using CLC Bio Main Workbench 6 (CLC Bio, www.clcbio.com), and the consensus sequences were extracted and exported. BLASTn of the consensus sequences against the National Centre for Biotechnology Information (NCBI) GenBank database (http://www.ncbi.nlm.nih.gov), was performed to confirm morphological species identification.

Detection and viability screening of *Ceratocystis* from beetles and frass

Beetle specimens (230) were macerated in individual 2 mL Eppendorf® tubes using sterile tweezers, after which 1 mL of ddH_20 was added. The same amount of ddH_2O was also added to the 2 mL Eppendorf® tubes containing the field-collected beetle frass (approx. 50 mg). The tubes were agitated for 10 s using a vortex mixer. The beetle and frass suspensions were then divided into two equal (500 μL) aliquots, retaining beetle fragments and frass particles in each aliquot. These were used for DNA detection and viability screening of *Ceratocystis* from the beetles and frass as described below. As a positive control (Pos 1 and Pos 2), two living beetles collected from Bambara traps, morphologically identified as *Xyleborus* spp., were surface sterilized by washing in 70% ethanol and allowed to walk across a fully grown pure culture of *C. manginecans* (Biolab, Midrand, South Africa) (Table S1). These beetles were then macerated and processed the same way as described above for the field-collected beetles.

Detection of *Ceratocystis* DNA via qPCR

To detect *Ceratocystis* from beetle specimens, fragments of the macerated beetles were removed from the beetle suspension aliquots using a centrifuge, after which the entire 500 μL beetle suspension supernatant was used for fungal DNA extraction using the Zymo Quick-DNA Fungal/Bacterial Kit, following the manufacturer's instructions. The fragments of macerated beetle were individually stored in ethanol (100%) at −20°C to be used for molecular based identification of the beetles as described above. For DNA detection of *Ceratocystis* from frass samples, the entire frass suspensions (500 μL), including the frass particles, was used for fungal DNA extraction using the DNeasy Plant Pro Kit (Qiagen, Germany), following the manufacturer's instructions. After extraction, the DNA was concentrated using an Eppendorf® centrifugal vacuum concentrator and resuspended in 20 μL ddH_20, to allow for optimal qPCR detection. The concentrated samples were screened for *Ceratocystis* using the real-time PCR method developed by Lynn et al. (2024). Samples were screened in duplicate, and each reaction was repeated. The standard curve generated using the pDNA for the *C. manginecans* isolate (CMW13851) in Lynn et al. (2024) was used to determine the target amplicon copy number of samples screened. Cq values are inversely related to DNA concentration (lower Cq values indicate higher template abundance). In this study, a Ct ≤30 was considered a strong signal for positive detection of *Ceratocystis* DNA. However, using the standard curve of the gDNA of the *C. manginecans* isolate (CMW13851) generated by Lynn et al. (2024) and the Ct values of the positive beetle controls, a Ct value of 30 ≤ 34 was considered to indicate the presence of *Ceratocystis* DNA at very low concentrations.

Screening for viable *Ceratocystis* propagules detected from beetles and frass

Beetles were sampled for viable *Ceratocystis* in two ways. Firstly, to test whether the beetles and frass harboured viable *Ceratocystis* propagules, the second suspension aliquots were re-agitated for 10 s using a vortex mixer and the entire contents of the suspensions, including macerated beetle fragments or frass particles, were placed on carrot baits (Moller & De Vay, 1968). Specifically, each suspension was divided into two equal amounts that were placed onto the surfaces of two 1 cm carrot discs that had been soaked overnight in ddH20 amended with 0.001 g/vol of streptomycin sulphate (SIGMA, Steinheim, Germany) to control bacterial contamination. The two carrot pieces were then attached to each other such that the suspensions were in contact with the inner sides of the carrot slices. Carrot baits were incubated in moist chambers at room temperature for up to 14 days. Secondly, an additional 66 baits were prepared by directly macerating beetles onto the carrot baits, omitting the water suspension and agitation steps. Baits containing no suspension or macerated beetle parts were set up as negative controls, and three baits containing discoloured wood shavings from known *Ceratocystis*-infected *Eucalyptus* trees were used as positive controls.

The carrot baits were inspected from day 5 to 14 of incubation for the presence of *Ceratocystis* perithecia. Where these were present, single ascospore drops were transferred to 2% Malt Extract Agar (MEA, 20 g/L malt, 20 g/L agar, Biolab, Midrand, South Africa) using a sterile needle. After 10 days at 25°C, the fully grown pure cultures were morphologically confirmed as *Ceratocystis* and single hyphal tip isolations were made onto 2% MEA to obtain pure cultures. After 10 days of incubation at 25°C, mycelium was scraped from the surfaces of the agar using a sterile scalpel and used to extract genomic DNA using the Zymo Quick-DNA Fungal/Bacterial Kit, following manufacturer's instructions. Species-level identifications were confirmed by DNA sequencing of the Internal Transcribed Spacer (ITS) rDNA gene region using the primers ITS1F and ITS4. PCR amplification, sequencing and sequence assembly were done following the methods described by Fourie et al. (2015). Consensus sequences were screened against the NCBI GenBank database using BLASTn, with type species selected, to confirm species identity.

Statistical analyses

A series of chi-squared tests were conducted to determine if there were statistically significant differences in the collection and screening

TABLE 1 Beetle species collected across *Ceratocystis* infected *Eucalyptus pellita* and *Acacia crassicarpa* compartments.

	Beetle specimens	*Eucalyptus* compartments							*Acacia* compartments							Grand total	
		E1	E2	E3	E4	E5	E Total	E Total no. of beetles	A1	A2	A3	A4	A5	A Total	A Total no. of beetles		Grand total no. of beetles
Ambrosia beetles	*Xyleborus affinis*	1	7	9	–	1	**18**	**112**	3	2	–	4	–	**9**	**67**	**27**	**179**
	Xyleborus perforans	1	11	13	–	3	**28**		1	7	4	3	4	**19**		**47**	
	Xyleborus bispinatus	–	–	1	–	–	**1**		–	–	–	–	–	–[c]		**1**	
	Xylosandrus crassiusculus	3	2	1	–	–	**6**		–	3	3	1	–	**7**		**13**	
	Euwallacea similis[a,b]	–	–	–	–	–	–		2	–	4	–	1	**7**		**7**	
	Euwallacea perbrevis[a,b]	–	–	–	–	–	–		4	–	2	–	4	**10**		**10**	
	Stictodex dimidiatus (syn. *Xyleborus decumans*)	10	7	16	–	2	**35**		–	–	–	–	–	–		**35**	
	Xyleborus spinulosus (syn. *Xyleborus artespinulosus*)	–	–	1	–	–	**1**		–	1	–	–	–	**1**		**2**	
	Xyleborinus exiguus	2	2	1	–	1	**6**		–	1	–	–	–	**1**		**7**	
	Xyleborinus sharpae	–	1	1	–	–	**2**		–	4	1	–	–	**5**		**7**	
	Xyleborus sp. (*X. affinis*/*X. perforans*)[a]	1	1	8	–	1	**11**		2	–	–	2	4	**8**		**19**	
	Xyleborinus sp. (*X. exiguus*/*X. sharpae*)[a]	3	1	–	–	–	**4**		–	–	–	–	–	–		**4**	
Bark beetles	*Hypothenemus* sp.	6	–	4	2	1	**13**	**13**	2	3	5	33	23	**66**	**68**	**79**	**81**
	Beaverium sp.	–	–	–	–	–	–		1	–	–	–	–	**1**		**1**	
	Glostatus sp. (syn. *Stephanopodius*)	–	–	–	–	–	–		–	–	–	1	–	**1**		**2**	
Nitidulid beetle	*Epuraea* sp.	4	2	10	–	1	**17**	**17**	1	9	1	8	2	**21**	**21**	**38**	**38**
	Total no. of beetles per compartment (abundance)	31	34	65	2	9	**142**	N/A	16	30	20	52	38	**156**	N/A	**298**	N/A
	Total no. of different beetle species (or morphotypes) per compartment (diversity)[c]	7	7	10	1	6	**10**	N/A	7	8	7	6	5	**12**	N/A	N/A	N/A

Note: Bold values represent grand totals of each species.
[a]Identified based on morphology.
[b]Collected from *Acacia crassicarpa* billets.
[c]Diversity of morpho-species present in the compartments, excluding ambrosia morphologically identified to genus level.

data. Chi-square tests were conducted using RStudio (RStudio Team, 2020), with the *dplyr* package (Wickham et al., 2023). The significance level (α) was set at 0.05.

Chi-squared goodness-of-fit tests were used to determine whether the observed frequencies of beetle species proportions deviate significantly from the expected frequencies. This test assessed for significant differences in (1) the proportion of the total number of ambrosia, bark and nitidulid beetles collected and (2) the proportion of ambrosia, bark and nitidulid beetles from *Eucalyptus* and *Acacia* compartments. Observed frequencies were compared against expected frequencies under the null hypothesis of equal proportions.

Chi-squared tests for independence were used to assess different variables related to beetle species, specimen counts and pathogen presence in the *Acacia* and *Eucalyptus* compartments. These tests assessed for significant differences in (1) the total number of specimens collected from *A. crassicarpa* and *E. pellita*, (2) the distribution of beetle types (ambrosia, bark and nitidulid) between *A. crassicarpa* and *E. pellita* compartments and (3) the number of specimens which tested positive for *Ceratocystis* DNA in *A. crassicarpa* and *E. pellita*.

RESULTS

Beetle collection and identification

Most beetle specimens obtained in this study were collected using the modified Bambara beetle traps (548 specimens) across the 10 compartments sampled (five clonal *E. pellita* and five *A. crassicarpa* compartments). An additional 16 specimens were manually extracted from the felled *A. crassicarpa* trees (Table S1). Based on morphological characteristics, these specimens were sorted into 36 morphotypes. Based on morphological characteristics, 298 specimens representing 14 morphotypes were identified as on-target species belonging to *Scolytinae* and *Nitidulidae*. Of these, ambrosia beetles were the most prevalent, with 179 (60%) specimens collected, followed by bark beetles with 81 (27%) specimens and a single nitidulid species with 38 (13%) specimens collected (Table 1). A Chi-squared goodness-of-fit test showed that these proportions deviated significantly from equal expectations ($\chi^2 = 105.15$, $p < 2.2e{-16}$). The remaining 266 specimens were excluded from this study as they resided in either *Laemophloeidae* or *Staphylinidae* that were not a focus of this study, or they could not be accurately identified.

For DNA-based identification of the collected beetle specimens, consensus sequences of the 28 s region were obtained for 206 of the specimens screened. The 16 specimens that were obtained by manual extractions from beetle galleries and the 66 specimens used for direct maceration onto carrot baits were not sequenced. These were identified based only on morphology, several of which could only be identified to genus level (Table S1). Consensus sequences of the COI region for all 20 specimens screened, were generated to aid in molecular identification. Results from the BLASTn search using the resulting 28 s and COI sequences successfully identified 12 of the 14 morphotypes to species level (Table S1), whereas the 13th (*Hypothenemus* sp.) and 14th one (*Epuraea* sp.) was identified to genus level. The sequence data of the 28S (LSU) region also revealed diversity among several species, with multiple haplotypes identified (Table S1). Figures of several of the morpho-species collected can be found in Figure S1.

Of the 14 morpho-species collected in this study, 10 were identified as ambrosia beetles, that is, *Euwallacea similis*, *E. perbrevis*, *Stictodex dimidiatus* (syn. *Xyleborus* [*Xbo.*] *decumans*), *Xyleborus* (*Xbo.*) *affinis*, *Xbo. bispinatus*, *Xbo. perforans*, *Xbo. spinulosus* (syn. *Xbo. artespinulosus*), *Xyleborinus* (*Xbi.*) *exiguus*, *Xbi. sharpae* and *Xylosandrus* (*Xs.*) *crassiusculus* (Figure 5, Table 1). With the exception of *E. perbrevis*, that was exclusively collected via manual extraction from beetle galleries, all morphological species identifications could be confirmed using DNA sequence data. *Xyleborus perforans* was the most prevalent ambrosia beetle species collected with 47 specimens collected (15.8%), whereas *Xbo. bispinatus* (0.3%) was the rarest with only one specimen collected from a *Eucalyptus* compartment.

Of the 14 morpho-species collected in this study, three species were identified as bark beetles, that is, *Hypothenemus* sp., *Beaverium* sp. and *Glostatus* sp. (syn. *Stephanopodius*; Jordal, 2023) (Figure 5, Table 1). The most abundant bark beetle species collected was *Hypothenemus* sp., represented by 79 (26.5%) specimens collected in both clonal *E. pellita* and *A. crassicarpa* compartments. The remaining specimens totalling 38 (12.8%) individuals were identified as the nitidulid beetle, *Epuraea* sp., based on DNA sequence data. Specimens of this nitidulid beetle were found in both clonal *E. pellita* and *A. crassicarpa* compartments (Figure 5, Table 1).

A total of 142 (47.7%) specimens were collected across the *Eucalyptus* compartments (Table 1). Of these, ambrosia beetles were the most abundant (112 specimens, 78.9%), followed by bark beetles (13 specimens, 9.2%) and the nitidulid *Epuraea* sp. (17 specimens, 12%). Based on the Chi-squared goodness-of-fit test, the proportions of ambrosia (112), bark (12) and nitidulid (17) beetles in the *Eucalyptus* compartments deviated significantly from the expected proportions ($\chi^2 = 132.69$, $p < 2.2e^{-16}$). Of the 14 different morpho-species collected in this study, 10 were collected from the sampled *Eucalyptus* compartments. Only four scolytine species, that is, *E. similis*, *E. perbrevis*, *Beaverium* sp. and *Glostatus* sp., were not collected from the *Eucalyptus* compartments, whereas *S. dimidiatus* and *Xbo. bispinatus* were exclusively collected from the *Eucalyptus* compartments. The lowest beetle abundance and species diversity was recorded for two compartments (E4 and E5) where traps had been damaged by monkeys. The greatest beetle abundance and diversity were recorded in compartment E3, which was located in close proximity to native forest greenbelts (Figure 2; approx. 0.3 km).

A total of 156 (52.3%) specimens were collected across the *Acacia* compartments (Table 1). The collections consisted of 67 (42.9%) ambrosia beetles, 68 (43.6%) bark beetles and 21 (13.5%) *Epuraea* sp. Based on the Chi-squared goodness-of-fit test, the proportions of ambrosia (67), bark (68) and nitidulid (38) beetles in *Acacia* compartments did not deviate significantly from the expected proportions ($\chi^2 = 10.069$, $p = 0.007$). Twelve of the 14 morpho-species were collected from the *Acacia* compartments (Figure 5). However, only *E.*

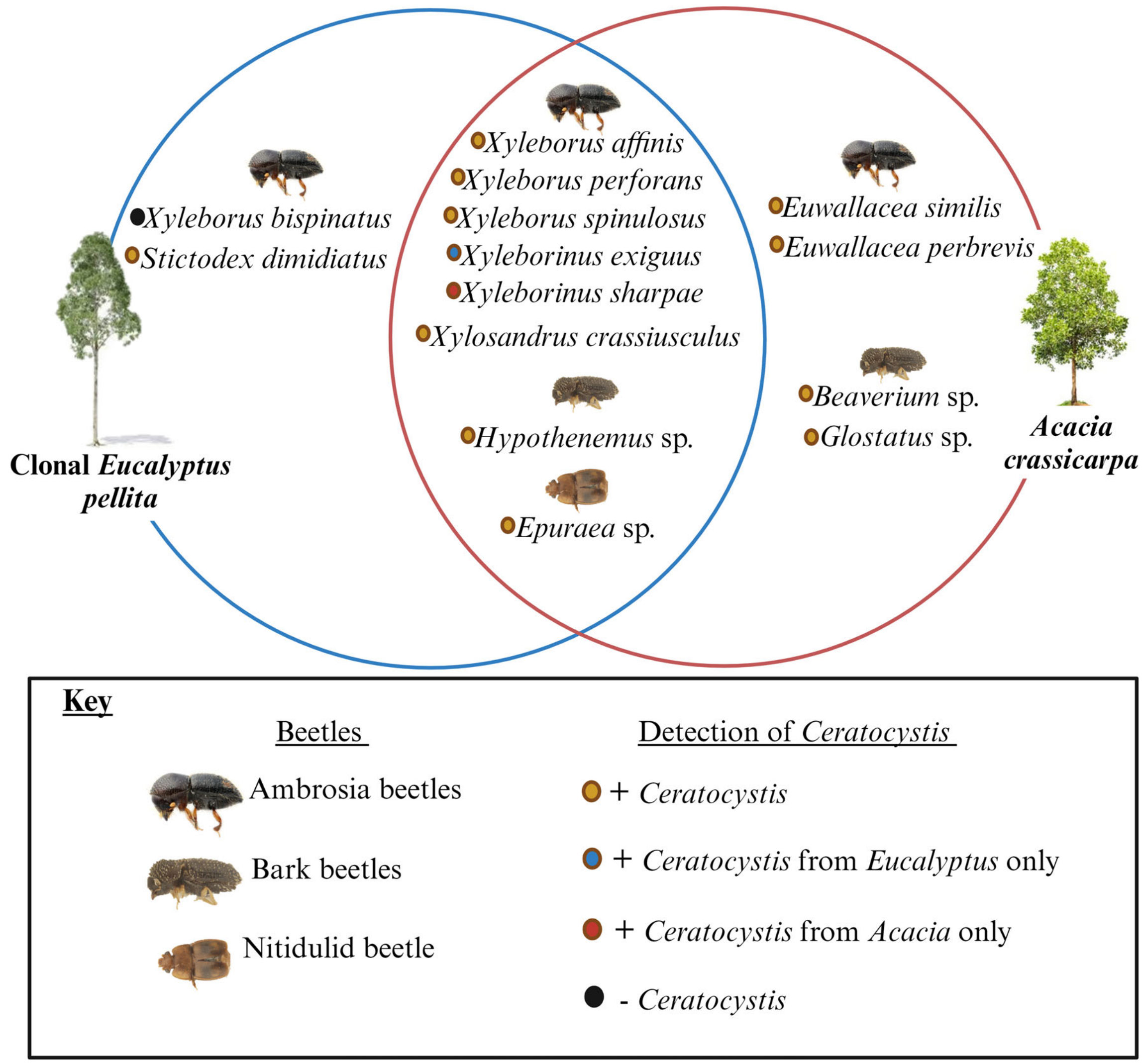

FIGURE 5 Venn diagram summarizing the beetle collections from the clonal *Eucalyptus pellita* and *Acacia crassicarpa* compartments screened, and the positive detection of *Ceratocystis* on the beetles. The blue and red circles represent beetle species collected from *Eucalyptus* and *A. crassicarpa* compartments respectively. Symbols next to species names indicate (1) whether species are ambrosia, bark or nitidulid beetles and (2) whether *Ceratocystis* DNA were detected on representatives of the species during screening. Symbols are representative and are not anatomically nor biologically accurate.

similis and *E. perbrevis* were found to be actively infesting *A. crassicarpa* in these compartments. Two compartments (A4 and A5) had the highest beetle abundance among the *Acacia* sites sampled, possibly due to their proximity to native forest greenbelts (Table 1; Figure 2: approx. 0.7 km and 0.5 km, respectively).

Based on the Chi-squared test for independence with a significance level set at 0.05, the total specimen abundance was not significantly different ($\chi^2 = 1.134$, $p = 0.287$) between the *A. crassicarpa* (156) and *E. pellita* (142) compartments. This suggests that there was no association between the tree host and specimen number (Table 1). Furthermore, a significant difference was found for both the number of ambrosia beetles (A) ($\chi^2 = 11.313$, $p = 0.001$) and the number of bark beetles (B) ($\chi^2 = 37.346$, $p = 9.89e^{-10}$) collected from the *A. crassicarpa* (A = 67, B = 68) and *E. pellita* (A = 112, B = 13). However, no significant difference was found in the number of nitidulid beetles ($\chi^2 = 0.421$, $p = 0.516$) collected from the *Acacia* (21) and *Eucalyptus* (17). This reflects a significant association between the species of tree and the types of beetles' present (excluding nitidulid beetles).

Detection and viability of *Ceratocystis* from beetles and frass

Detection of *Ceratocystis* DNA via qPCR

A total of 230 of the 298 beetle specimens were screened for *C. manginecans* using the qPCR method described by Lynn et al. (2024). Of these, 118 (51.3%) specimens, representing 13 of the 14 collected beetle species, tested positive for the presence of *Ceratocystis* DNA in low concentrations (0.015–0.0019 ng: Figure 5, Table 2, Table S1). Only *Xbo. bispinatus*, which was represented by a single specimen, did not show evidence of *Ceratocystis* DNA (Figure 4, Table 2, Table S1).

TABLE 2 Beetle specimens screened for *Ceratocystis* using a qPCR assay.

Identity	Total number of specimens screened for *Ceratocystis*	Ratio of specimens in *E. pellita* (E) and *A. crassicarpa* (A)	Percentage of specimens found positive for *Ceratocystis* DNA	Documented fungal genera associated with ambrosia beetles [nutritional ambrosial associates and auxiliary fungi]	Reference
Ambrosia beetles					
Xyleborus affinis	27	E = 18 A = 9	E = 38.9 A = 55.6	*Raffaelea lauricola* *Raffaelea arxii* *Ceratocystis* sp. *Cephalosporium pallidum* *Ambrosiella* sp. *Fusarium* sp. *Ceratocystis lukuohia* *Ceratocystis huliohia*	Ploetz et al. (2013) Nel et al. (2021) Herrera & Grillo (1989), Souza et al. (2013) Verrall (1943) Batra (1967) Roeper (1996) Roy et al. (2023) Roy et al. (2023)
Xyleborus perforans	47	E = 28 A = 19	E = 42.9 A = 42.1	*Ceratocystis lukuohia* *Ceratocystis huliohia* *Ambrosiella* sp.	Roy et al. (2023) Roy et al. (2023) Batra (1967)
Xyleborus bispinatus	1	E = 1 A = 0	E = 0 A = N/A	*Harringtonia lauricola* *Fusarium* sp. *Raffaelea subfusca* *Raffaelea arxii* *Ceratocystis lukuohia* *Ceratocystis huliohia*	Menocal et al. (2023) Roy et al. (2023) Roy et al. (2023)
Xylosandrus crassiusculus	9	E = 5 A = 4	E = 80 A = 75	*Ambrosiella roeperi* *Ceratocystiosps lunata*	Harrington et al. (2014) Nel et al. (2021)
Euwallacea similis	7	E = 0 A = 7	E = N/A A = 71.4	*Fusarium mekan* *Fusarium akasia* *Fusarium warna* *Fusarium rekanum* *Fusarium awan*	Lynn et al. (2021)
Euwallacea perbrevis	5	E = 0 A = 5	E = N/A A = 20	*Fusarium* sp. [AF-13, AF-14, AF-16, AF-17, AF-18] *Fusarium akasia* *Fusarium rekanum* *Fusarium ambrosium*	Lynn et al. (2021)
Stictodex dimidiatus (syn. *Xyleborus decumans*)	31	E = 31 A = 0	E = 61.3 A = N/A	Unknown	N/A
Xyleborus spinulosus (syn. *Xyleborus artespinulosus*)	2	E = 1 A = 1	E = 100 A = 100	Unknown	N/A
Xyleborinus exiguus	7	E = 6 A = 1	E = 66.7 A = 0	Unknown	N/A
Xyleborinus sharpae	7	E = 4 A = 3	E = 0 A = 66.7	Unknown	N/A
Total	143	E = 94 A = 49	E = 50 A = 51 T = 50.3		
Bark beetles					
Hypothenemus sp.	56	E = 9 A = 47	E = 33.3 A = 53.2	N/A	N/A
Beaverium sp.	1	E = 0 A = 1	E = 0 A = 100	N/A	N/A

TABLE 2 (Continued)

Identity	Total number of specimens screened for *Ceratocystis*	Ratio of specimens in *E. pellita* (E) and *A. crassicarpa* (A)	Percentage of specimens found positive for *Ceratocystis* DNA	Documented fungal genera associated with ambrosia beetles [nutritional ambrosial associates and auxiliary fungi]	Reference
Glostatus sp. (syn. *Stephanopodius)*	1	E = 0 A = 1	E = N/A A = 100	N/A	N/A
Total	58	E = 9 A = 49	E = 33.3 A = 76 T = 51.7		
Nitidulid beetles					
Epuraea sp.	29	E = 12 A = 17	E = 41.7 A = 64.7 T = 55.2	N/A	N/A
Overall total	230	E = 115 A = 115	E = 47.8 A = 54.8 T = 51.3		

Of the 115 specimens collected from the *A. crassicarpa* compartments screened for *Ceratocystis* DNA, 63 (54.8%) tested positive for *Ceratocystis* DNA (Figure 5, Table 2, Table S1). Of the other 115 specimens collected from the *E. pellita* compartments, 55 (47.8%) tested positive for *Ceratocystis* DNA (Figure 5, Table 2, Table S1). Based on the Chi-squared test for independence with a significance level set at 0.05, the number of specimens that tested positive for *Ceratocystis* DNA was not significantly different ($\chi^2 = 0.853$, $p = 0.356$), indicating that there was no association between the specimens collected from the compartments and the positive detection of *Ceratocystis* DNA from these specimens. Out of the specimens screened, 143 were ambrosia beetles, 58 were bark beetles, and 29 were *Epuraea* sp., of which 72 (50.3%), 30 (51.7%) and 16 (55.2%) tested positive for *Ceratocystis* DNA, respectively.

A total of 105 frass samples were collected from 105 beetle exit holes on *A. crassicarpa* (Figure 4, Table S2). For the 105 frass samples, 67 tested positive (~68%) for the presence of *Ceratocystis* DNA (Table S2). Nine of these samples had Cq values below 30, indicating higher DNA concentrations (approximately 0.125–0.015 ng), while the remaining samples had Cq values between 30 and 34, consistent with very low DNA concentrations (approximately 15–1.9 pg). These thresholds were determined based on the qPCR standard curve described in Lynn et al. (2024) and the Cq values of the positive controls tested in this study.

Screening for viable *Ceratocystis* propagules detected from beetles and frass

Ceratocystis manginecans was recovered from only one of the 105 frass samples screened using the carrot bait method (F12, Table S2). *Ceratocystis manginecans* was not recovered from any of the 230 beetles macerated in ddH20 and placed onto carrot baits or from the 66 specimens macerated and directly placed onto carrot baits (Table S1). Only the two positive *C. manginecans* contaminated beetle controls (Figure S2) and an additional positive control that consisted of infected wood shavings yielded *C. manginecans* growth and sporulation on the carrot baits.

DISCUSSION

In this study, 14 beetle species were found in *Eucalyptus* and *Acacia* plantations in Riau, Indonesia. These included 10 ambrosia, three bark, and one nitidulid beetle species. To the best of our knowledge, four of these beetles (*Xyleborus bispinatus*, *Xbo. spinulosus*, *Xbi. sharpae* and a *Glostatus* sp.) are reported from Indonesia for the first time. Although the abundance and species richness of beetles were similar in the traps across the *Eucalyptus* and *Acacia* compartments, active beetle infestations in the *Eucalyptus* compartments were uncommon, with specimens collected exclusively from traps. Similarly, although most specimens in the *Acacia* compartments were from traps, a high number of active infestations were observed, with several individuals (*E. similis* and *E. perbrevis*) manually extracted from galleries in infested trees. Using a qPCR assay, approximately 51% of all specimens screened, representing 13 beetle species, tested positive for the presence of *C. manginecans*. However, isolations using culture-based methods provided no evidence of viable *Ceratocystis* propagules on any of the beetles sampled. This was also true for the frass expelled by beetles boring into diseased trees, where viable propagules were recovered from only a single sample.

Many of the beetle species reported in this study are thought to be native to Southeast Asia (https://ambrosiasymbiosis.org/ambrosia-beetles/diversity/). Despite their presence in *Acacia* and *Eucalyptus* monocultures, their ability to infest and reproduce in these non-native hosts remains unclear. Evidence of beetle infestation, such as entry holes and exuding frass from living trees, was observed across *Acacia* compartments in this study. These findings are consistent with previous screening surveys conducted by the forestry company and reported in Lynn et al. (2020, 2021). Notably, infestations were recorded even in compartments far from surrounding greenbelts, suggesting that active beetle infestation occurs throughout the plantations.

Euwallacea similis and *E. perbrevis* were confirmed to infest *Acacia* in this study, consistent with earlier findings (Lynn et al., 2020, 2021), while *Xbi. exiguus*, *Xs. crassiusculus* and a *Hypothenemus* species have also been reported on *Acacia* in previous studies (Lynn et al., 2020; Wingfield et al., 2023). Several other species identified here (*Xbo. affinis*, *Xbo. perforans*, *Xbo. bispinatus*) are known from other pathosystems and are capable of infesting living plant material (Ploetz et al., 2017; Roy et al., 2023; Wingfield et al., 2023), although their infestation of living trees in the sampled compartments was not confirmed.

In contrast, only limited beetle infestation was observed in *Eucalyptus* compartments. While infestation of *Acacia* was widespread on both living and dead trees, beetle entry holes and frass in *Eucalyptus* were seen only on a few dead or dying trees. As such, the beetle diversity recorded in traps from *Eucalyptus* compartments may reflect spillover from surrounding greenbelts. To the best of our knowledge, none of the beetle species captured in this study have previously been reported infesting *Eucalyptus* plantations.

The higher infestation levels observed in *A. crassicarpa* compared to clonal *E. pellita* could be attributed to the historical widespread collapse of exotic, monoculture *A. mangium* (Tarigan, Roux, et al., 2011). The increase in compromised and genetically related hosts could have provided an ideal breeding ground for native beetle species, resulting in a subsequent surge in the beetle populations throughout these homogeneous environments (Lynn et al., 2020; Tarigan, Roux, et al., 2011; Wingfield et al., 2023). These large beetle populations, which exceed their natural thresholds, combined with *C. manginecans* infection and habitat fragmentation, may explain why the closely related species to *A. mangium* has been more vulnerable to infestation. Parallels of this expansion in beetle populations beyond their natural threshold and their impact are well recognized (Branco et al., 2015; Negrón & Cain, 2019; Ploetz et al., 2013; Raffa et al., 2008).

The collapse of the non-native *A. mangium* plantations could also explain the variation in the proportion of ambrosia and bark beetles collected across the *A. crassicarpa* and clonal *E. pellita* compartments. Hulcr et al. (2007) noted that bark beetles show a preference for closely related plant hosts, while ambrosia beetles are more polyphagous. In the present study, bark beetles were predominantly collected from *A. crassicarpa* compartments, whereas ambrosia beetles were predominantly collected from compartments of clonal *E. pellita*. Furthermore, the proportions of the ambrosia, bark and nitidulid beetles deviated significantly from expectation, with the more polyphagous ambrosia beetles being the most abundant in this study.

Ceratocystis spp. are not considered obligate nutritional symbionts of scolytine beetles, but the capacity of these insects to transmit *Ceratocystis* spp. has been previously reported (Roy et al., 2023; Soulioti et al., 2015; Tsopelas et al., 2017). This study represents the first detection of *Ceratocystis* DNA on several scolytine species, that is, *E. similis*, *E. perbrevis*, *S. dimidiatus*, *Xbo. spinulosus*, *Xbi. exiguus*, *Xbi. sharpae*, *Hypothenemus* sp., *Beaverium* sp., and *Glostatus* sp. Only three of the species collected, *Xbo. affinis*, *Xbo. perforans* and *Xbo. ferrugineus* have previously been shown to be associated with *Ceratocystis* spp., all of which have been found involved in Rapid ʻŌhiʻa Death (Roy et al., 2023), and *Xbo. affinis* involved in mango sudden decline disease (Soulioti et al., 2015). The association of these species with various *Ceratocystis* spp., particularly those in the LAC that include some of the most aggressive tree pathogens, is likely not due to specialized adaptations or coevolution. Instead, it may result from the broad distribution and polyphagous nature of these ambrosia beetles, combined with their preference for declining hosts (CABI, 2022; Sobel et al., 2015).

Culture-based screening using the carrot baiting method for *C. manginecans* was found to be inconsistent in this study. Although the method was successful for positive controls, viable propagules were not recovered from any field-collected beetles, and only one frass sample yielded a culture, despite over half testing positive using qPCR. This may reflect low spore abundance—as suggested by the high Cq values of many screened samples—loss of viability during the two-day trap period, or limitations of the method itself. In particular, the lack of continuous spore agitation in our approach likely reduced contact between spores and the carrot surface, which is critical when spore concentrations are low. In contrast, Roy et al. (2023) employed a modified aeration method with water agitation to enhance spore-carrot contact, which improved sensitivity. However, even with this refinement, viable recovery remained low (<3%) across multiple beetle collection methods (Roy et al., 2023), highlighting the broader challenge of isolating viable *Ceratocystis* propagules from insect samples. Furthermore, Roy et al. (2018) observed a similar trend, reporting that *C. lukuohia* was more frequently detected by qPCR than through carrot baiting of beetle frass samples. These findings underscore the need for improved viability detection methods for future studies.

The detection of varying concentrations of *Ceratocystis* DNA (high vs. low) in beetle frass samples could reflect differing levels of fungal load within the frass itself, which may influence its potential role in the dispersal of the pathogen. A previous study by Hughes et al. (2023) used statistical modelling to show that the viability of *C. lukuohia* chlamydospores in frass declined over time and was negatively affected by increased temperature. They further suggested that ultraviolet light exposure may also contribute to viability loss. In addition, variation in propagule abundance may arise from differences in frass age, time since host death and microbial competition under field conditions. While we did not assess propagule type in this study, the lack of viable spores detected in frass samples using culture-based methods could be explained by these factors, as samples were not always collected fresh and regional temperatures can exceed 30°C

(WeatherSpark.com). Although contaminated frass has been reported as an inoculum source for other pathogenic species in the LAC (Harrington, 2013; Hughes et al., 2023; Soulioti et al., 2015; Wingfield et al., 2023), its role in infectivity of *C. manginecans* in Indonesian plantations requires further investigation.

The varying degrees of beetle infestation in the two tree genera considered in this study suggests that their exposure to CCWD differs. For example, *E. perbrevis*, found only infesting *Acacia*, is known to infest healthy trees (Lynn et al., 2021), creating potential entry wounds for *Ceratocystis*. These, as well as naturally occurring wounds, attract many secondary borers (Lynn et al., 2021) and other insects, further increasing trees to pathogen exposure. Observational evidence of this includes *Ceratocystis* perithecia colonizing wood around borer gallery entrance wounds (Figure S3) and insects feeding on artificially wounded *A. crassicarpa* and *E. pellita* stems colonized by *C. manginecans* (Figure S4). In the case of *Eucalyptus*, beetle infestation was low overall, suggesting a potential spillover of these beetles from nearby greenbelts as well as from colonized *Eucalyptus* trees already infected with, or dying from, *C. manginecans*.

The areas of trees in monocultures affected by CCWD is increasing in Indonesia. As a consequence, populations of beetles are growing and the likelihood of exposure of trees to infestation and infection by *C. manginecans* is increasing. Given the importance of wood-dwelling beetles and frass in the dispersal of *Ceratocystis* species in other pathosystems, it was hypothesised in this study was that these factors would also be relevant for *C. manginecans*. However, our results failed to support this hypothesis, but they do suggest that an interaction exists between *C. manginecans*-infected trees and wood-associated beetles in plantations in Indonesia. We further hypothesize that spillover of beetles from surrounding green belts could also explain why beetles found in this study, but not confirmed to infest *Acacia* or *Eucalyptus* trees, were carrying *Ceratocystis* DNA. These beetles could be associated with other tree species in areas of native vegetation that may harbour the pathogen, especially considering that South East Asia has been proposed as a possible centre of origin for *C. manginecans* (Fourie et al., 2016), and the pathogen has been reported on numerous hosts in Indonesia (Pratama et al., 2021a; Pratama et al., 2021b; Pratama et al., 2024). While cross-contamination of beetles in traps cannot be entirely ruled out, we consider this unlikely, as care was taken to ensure beetles did not come into contact with each other during sampling. The impact of this interaction on the spread of CCWD in plantations of *Acacia* and *Eucalyptus* requires further investigation.

CONCLUSIONS

In this study, we investigated the diversity of wood-dwelling beetles and their potential role in the distribution and spread of *C. manginecans* causing Ceratocystis canker and wilt disease (CCWD) in *Acacia* and *Eucalyptus* plantations in Riau, Indonesia. The results of the study provided evidence of four beetle species apparently not previously recorded in Indonesia. Many of the beetle species collected appeared to carry *Ceratocystis* DNA at low concentrations. In contrast, we were not able to isolate viable *Ceratocystis* from any beetle tested. This may indicate that the beetles are not acting as vectors in this pathosystem, or alternatively, that the culture-based method used was not sensitive enough to detect viable propagules. As a result, the possible roles of beetles in the occurrence of CCWD requires further investigation. This includes a need to apply Leach's rules necessary to identify vectors in a disease pathosystem.

Active beetle infestation was observed only in *Acacia* compartments, suggesting that primary woodborers can induce wounds that could serve as entry points for *Ceratocystis*. In addition, frass expelled from diseased trees was found to be contaminated with *Ceratocystis*, although its role as a source of viable *C. manginecans* inoculum remains to be confirmed. In contrast, almost no active beetle infestation was observed in *Eucalyptus* compartments, and wood-dwelling beetles found in these plantations likely spill over from the surrounding greenbelts, infesting only those trees already diseased or dead. While many questions remain regarding the specific roles of these beetles in disease dispersal and the viability of the *Ceratocystis* propagules, a clear interaction between *Ceratocystis*-infected trees and wood-dwelling beetles exists and this interaction is different for *Acacia* and *Eucalyptus*. Further research is required to better understand this interaction and thus to improve disease management strategies.

AUTHOR CONTRIBUTIONS

Kira M. T. Lynn: Conceptualization; data curation; formal analysis; investigation; methodology; validation; visualization; writing – original draft. **Michael J. Wingfield:** Conceptualization; funding acquisition; project administration; resources; supervision; writing – review and editing. **Marthin Tarigan:** Funding acquisition; resources; writing – review and editing. **Alvaro Durán:** Funding acquisition; resources; writing – review and editing. **Samuel A. Santos:** Investigation; resources; writing – review and editing. **Wilma J. Nel:** Data curation; investigation; methodology; writing – review and editing. **Irene Barnes:** Conceptualization; funding acquisition; investigation; project administration; resources; supervision; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data are openly available in the supporting information. All consensus DNA sequences generated in this study have been deposited in the NCBI GenBank database (https://www.ncbi.nlm.nih.gov/). Accession numbers for every sequence are provided in Table S1.

ORCID

Kira M. T. Lynn https://orcid.org/0000-0003-0533-0458
Michael J. Wingfield https://orcid.org/0000-0001-9346-2009
Marthin Tarigan https://orcid.org/0000-0002-9128-2650
Alvaro Durán https://orcid.org/0000-0002-3035-9087
Samuel A. Santos https://orcid.org/0000-0002-4192-0829
Wilma J. Nel https://orcid.org/0000-0001-6368-2203
Irene Barnes https://orcid.org/0000-0002-4349-3402

REFERENCES

Al Adawi, A.O., Al Jabri, R.M., Deadman, M.L., Barnes, I., Wingfield, B. & Wingfield, M.J. (2013) The mango sudden decline pathogen, *Ceratocystis manginecans*, is vectored by *Hypocryphalus mangiferae* (Coleoptera: Scolytinae) in Oman. *European Journal of Plant Pathology*, 135(2), 243–251. Available from: https://doi.org/10.1007/s10658-012-0081-7

Al Adawi, A.O., Barnes, I., Khan, I.A., Al Subhi, A.M., Al Jahwari, A.A., Deadman, M.L. et al. (2013) *Ceratocystis manginecans* associated with a serious wilt disease of two native legume trees in Oman and Pakistan. *Australasian Plant Pathology*, 42(2), 179–193. Available from: https://doi.org/10.1007/s13313-012-0196-5

Al Adawi, A.O., Deadman, M.L., Al Rawahi, A.K., Al Maqbali, Y.M., Al Jahwari, A.A., Al Saadi, B.A. et al. (2006) Aetiology and causal agents of mango sudden decline disease in the Sultanate of Oman. *European Journal of Plant Pathology*, 116(4), 247–254. Available from: https://doi.org/10.1007/s10658-006-9056-x

Appel, D.N., Kurdyla, T. & Lewis, R. (1990) Nitidulids as vectors of the oak wilt fungus and other *Ceratocystis* spp. in Texas. *European Journal of Forest Pathology*, 20(6–7), 412–417. Available from: https://doi.org/10.1111/j.1439-0329.1990.tb01156.x

Barnes, I., Abdul Rauf, M., Fourie, A., Japarudin, Y. & Wingfield, M. (2023) *Ceratocystis manginecans* and not *C. fimbriata* a threat to propagated *Acacia* spp. in Sabah, Malaysia. *Journal of Tropical Forest Science*, 35, 16–26. Available from: https://doi.org/10.26525/jtfs2023.35S.SI.16

Barnes, I., Fourie, A., Wingfield, M.J., Harrington, T.C., McNew, D.L., Sugiyama, L.S. et al. (2018) New *Ceratocystis* species associated with rapid death of *Metrosideros polymorpha* in Hawai'i. *Persoonia – Molecular Phylogeny and Evolution of Fungi*, 40(1), 154–181. Available from: https://doi.org/10.3767/persoonia.2018.40.07

Batra, L.R. (1967) Ambrosia fungi: a taxonomic revision, and nutritional studies of some species. *Mycologia*, 59(6), 976–1017. Available from: https://doi.org/10.2307/3757271

Belshaw, R. & Quicke, D.L.J. (1997) A molecular phylogeny of the Aphidiinae (hymenoptera: Braconidae). *Molecular Phylogenetics and Evolution*, 7(3), 281–293. Available from: https://doi.org/10.1006/mpev.1996.0400

Branco, M., Brockerhoff, E.G., Castagneyrol, B., Orazio, C. & Jactel, H. (2015) Host range expansion of native insects to exotic trees increases with area of introduction and the presence of congeneric native trees. *Journal of Applied Ecology*, 52(1), 69–77. Available from: https://doi.org/10.1111/1365-2664.12362

CABI. (2022) *Xyleborus perforans (island pinhole borer)*. Wallingford, UK: CABI Compendium. Available from: https://doi.org/10.1079/cabicompendium.57169

Cognato, A.I. (2013) Molecular phylogeny and taxonomic review of Premnobiini Browne, 1962 (Coleoptera: Curculionidae: Scolytinae). *Frontiers in Ecology and Evolution*, 1, 1–12. Available from: https://doi.org/10.3389/fevo.2013.00001

de Beer, Z.W., Duong, T.A., Barnes, I., Wingfield, B.D. & Wingfield, M.J. (2014) Redefining *Ceratocystis* and allied genera. *Studies in Mycology*, 79(1), 187–219. Available from: https://doi.org/10.1016/j.simyco.2014.10.001

Dodge, C., Coolidge, J., Cooperband, M., Cossé, A., Carrillo, D. & Stouthamer, R. (2017) Quercivorol as a lure for the polyphagous and Kuroshio shot hole borers, *Euwallacea* spp. nr. Fornicatus (Coleoptera: Scolytinae), vectors of Fusarium dieback. *PeerJ*, 5, 1–19. Available from: https://doi.org/10.7717/peerj.3656

Eskalen, A., Stouthamer, R., Lynch, S.C., Rugman-Jones, P.F., Twizeyimana, M., Gonzalez, A. et al. (2013) Host range of Fusarium dieback and its ambrosia beetle (Coleoptera: Scolytinae) vector in Southern California. *Plant Disease*, 97(7), 938–951. Available from: https://doi.org/10.1094/PDIS-11-12-1026-RE

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *IMolecular Marine Biology and Biotechnology*, 3(5), 294–299. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0141813019349955

Fourie, A., Wingfield, M.J., Wingfield, B.D. & Barnes, I. (2015) Molecular markers delimit cryptic species in *Ceratocystis* sensu stricto. *Mycological Progress*, 14(1), 1020. Available from: https://doi.org/10.1007/s11557-014-1020-0

Fourie, A., Wingfield, M.J., Wingfield, B.D., Thu, P.Q. & Barnes, I. (2016) A possible centre of diversity in South East Asia for the tree pathogen, *Ceratocystis manginecans*. *Infection, Genetics and Evolution*, 41, 73–83. Available from: https://doi.org/10.1016/j.meegid.2016.03.011

Fraedrich, S.W., Harrington, T.C., Rabaglia, R.J., Ulyshen, M.D., Mayfield, A.E., Hanula, J.L. et al. (2008) A fungal symbiont of the redbay ambrosia beetle causes a lethal wilt in redbay and other Lauraceae in the southeastern United States. *Plant Disease*, 92(2), 215–224. Available from: https://doi.org/10.1094/PDIS-92-2-0215

Gibbs, J.N. & French, D.W. (1979) *The transmission of oak wilt*. North Central Forest: U.S. Department of Agriculture, Forest Service, p. 368. Available from: https://doi.org/10.2737/NC-RP-185

Harrington, T.C. (2013) *Ceratocystis* diseases. In: Gonthier, P. & Nicolotti, G. (Eds.) *Infectious forest diseases*. UK: CABI, pp. 230–255. Available from: https://doi.org/10.1079/9781780640402.0230

Harrington, T.C. & Appel, D.N. (2009) The genus *Ceratocystis*. Where does the oak wilt fungus fit? In: Billings, R.F. & Appel, D.N. (Eds.) *The proceedings of the 2nd National oak Wilt Symposium*. Austin, Texas: National Oak Wilt Symposium.

Harrington, T.C., McNew, D., Mayers, C., Fraedrich, S.W. & Reed, S.E. (2014) *Ambrosiella roeperi* sp. nov. is the mycangial symbiont of the granulate ambrosia beetle, *Xylosandrus crassiusculus*. *Mycologia*, 106(4), 835–845. Available from: https://doi.org/10.3852/13-354

Harwood, C.E. & Nambiar, E.K.S. (2014) Productivity of *acacia* and eucalypt plantations in Southeast Asia. *International Forestry Review*, 16(2), 249–260. Available from: https://doi.org/10.1505/146554814811724766

Heath, R.N., Wingfield, M.J., Van Wyk, M. & Roux, J. (2009) Insect associates of *Ceratocystis albifundus* and patterns of association in a native savanna ecosystem in South Africa. *Environmental Entomology*, 38(2), 356–364. Available from: https://doi.org/10.1603/022.038.0207

Herrera, I.L. & Grillo, R.H. (1989) *Spathodea campanulata* Beauv., new host plant of *Ceratocystis fimbriata* Hell & Halst and *Xyleborus* spp. *Centro Agrı´cola Agrı´cola*, 16, 91–93.

Hlongwane, G. (2022) *Identification and population genetic studies of Ceratocystis spp. infecting Eucalyptus and Acacia plantations in South Africa and Indonesia*. South Africa: University of Pretoria.

Hughes, M.A., Roy, K., Harrington, T.C., Brill, E. & Keith, L.M. (2023) *Ceratocystis lukuohia* -infested ambrosia beetle frass as inoculum for *Ceratocystis* wilt of ʻōhiʻa (*Metrosideros polymorpha*). *Plant Pathology*, 72(2), 232–245. Available from: https://doi.org/10.1111/ppa.13653

Hulcr, J., Atkinson, T.H., Cognato, A.I., Jordal, B.H. & McKenna, D.D. (2015) Morphology, taxonomy, and phylogenetics of bark beetles. In: Vega, F.E. & Hofstetter, R.W. (Eds.) *Bark beetles*. Cambridge, Massachusetts: Elsevier, pp. 41–84. Available from: https://doi.org/10.1016/B978-0-12-417156-5.00002-2

Hulcr, J. & McCoy, N. (2015) Catching beetles. Available from: http://www.ambrosiasymbiosis.org/ambrosia-beetles/catching-beetles/

Hulcr, J., Mogia, M., Isua, B. & Novotny, V. (2007) Host specicity of ambrosia and bark beetles (Col., Curculionidae: Scolytinae and Platypodinae) in a New Guinea rainforest. *Forest Research*, 32, 762–772. Available from: https://doi.org/10.1111/j.1365-2311.2007.00939.x

Johnson, A.J., Knížek, M., Atkinson, T.H., Jordal, B.H., Ploetz, R.C. & Hulcr, J. (2017) Resolution of a global mango and fig pest identity crisis. *Insect Systematics and Diversity*, 1(2), 1–10. Available from: https://doi.org/10.1093/isd/ixx010

Jordal, B.H. (2023) *Glostatina*, a new xyloctonine subtribe for *Glostatus* (Coleoptera: Curculionidae), based on clear genetic and morphological differences. *European Journal of Entomology*, 120, 199–232. Available from: https://doi.org/10.14411/eje.2023.025

Klepzig, K., Moser, J.C., Lombardero, F.J., Hofstetter, R. & Ayers, M. (2001) Symbiosis and competition: complex interactions among beetles, fungi and mites. *Symbiosis*, 30, 83–96.

Kuhnholz, S., Borden, J. & Uzunovic, A. (2001) Secondary ambrosia beetles in apparently healthy trees: adaptations, potential causes and suggested research. *Integrated Pest Management Reviews*, 6(3–4), 209–219. Available from: https://doi.org/10.1023/A:1025702930580

Lieutier, F., Yart, A. & Salle, A. (2009) Stimulation of defenses by Ophiostomatoid fungi can explain attack success of bark beetles on conifers. *Annals of Forest Science*, 66(8), 1–22. Available from: https://doi.org/10.1051/forest/2009066

Liu, F., Duong, T.A., Barnes, I., Wingfield, M.J. & Chen, S. (2021) Population diversity and genetic structure reveal patterns of host association and anthropogenic impact for the globally important fungal tree pathogen *Ceratocystis manginecans*. *Journal of Fungi*, 7(9), 759. Available from: https://doi.org/10.3390/jof7090759

Lynn, K.M.T., Wingfield, M.J., Durán, A., Marincowitz, S., Oliveira, L.S.S., de Beer, Z.W. et al. (2020) *Euwallacea perbrevis* (Coleoptera: Curculionidae: Scolytinae), a confirmed pest on *acacia Crassicarpa* in Riau, Indonesia, and a new fungal symbiont, *Fusarium rekanum* Sp. Nov. *Antonie Van Leeuwenhoek*, 113(6), 803–823. Available from: https://doi.org/10.1007/s10482-020-01392-8

Lynn, K.M.T., Wingfield, M.J., Durán, A., Oliveira, L.S.S., de Beer, Z.W. & Barnes, I. (2021) Novel *Fusarium* mutualists of two *Euwallacea* species infesting *Acacia crassicarpa* in Indonesia. *Mycologia*, 113(3), 536–558. Available from: https://doi.org/10.1080/00275514.2021.1875708

Lynn, K.M.T., Wingfield, M.J., Hammerbacher, A. & Barnes, I. (2024) High-resolution melting curve analysis: a detection assay for *Ceratocystis eucalypticola* and *C. manginecans* in infected *Eucalyptus*. *Fungal Biology*, 128(6), 2062–2072. Available from: https://doi.org/10.1016/j.funbio.2024.07.011

Mayers, C.G., Harrington, T.C. & Biedermann, P.H.W. (2022) Mycangia define the diverse ambrosia beetle–fungus symbioses. In: *The convergent evolution of agriculture in humans and insects*. Cambridge, Massachusetts: The MIT Press, pp. 105–142. Available from: https://doi.org/10.7551/mitpress/13600.003.0013

Mayers, C.G., Harrington, T.C., Masuya, H., Jordal, B.H., McNew, D.L., Shih, H.-H. et al. (2020) Patterns of coevolution between ambrosia beetle mycangia and the Ceratocystidaceae, with five new fungal genera and seven new species. *Persoonia – Molecular Phylogeny and Evolution of Fungi*, 44, 41–66. Available from: https://doi.org/10.3767/persoonia.2020.44.02

Menocal, O., Cruz, L.F., Kendra, P.E., Berto, M. & Carrillo, D. (2023) Flexibility in the ambrosia symbiosis of *Xyleborus bispinatus*. *Frontiers in Microbiology*, 14, 1110474. Available from: https://doi.org/10.3389/fmicb.2023.1110474

Moller, W.J. & De Vay, J.E. (1968) Carrot as species-selective isolation medium for *Ceratocystis fimbriata*. *Phytopathology*, 58, 123–126.

Negrón, J.F. & Cain, B. (2019) Mountain pine beetle in colorado: a story of changing forests. *Journal of Forestry*, 117(2), 144–151. Available from: https://doi.org/10.1093/jofore/fvy032

Nel, W.J., Wingfield, M.J., de Beer, Z.W. & Duong, T.A. (2021) Ophiostomatalean fungi associated with wood boring beetles in South Africa including two new species. *Antonie Van Leeuwenhoek*, 114(6), 667–686. Available from: https://doi.org/10.1007/s10482-021-01548-0

Ocasio-Morales, R.G., Tsopelas, P. & Harrington, T.C. (2007) Origin of *Ceratocystis platani* on native *Platanus orientalis* in Greece and its impact on natural forests. *Plant Disease*, 91(7), 901–904. Available from: https://doi.org/10.1094/PDIS-91-7-0901

Peplinski, J.D. (1974) Nonsurvival of *Ceratocystis fagacearum* in frass of oak bark beetles and ambrosia beetles. *Phytopathology*, 64(12), 1528. Available from: https://doi.org/10.1094/Phyto-64-1528

Ploetz, R.C., Hulcr, J., Wingfield, M.J. & de Beer, Z.W. (2013) Destructive tree diseases associated with ambrosia and bark beetles: Black swan events in tree pathology? *Plant Disease*, 97(7), 856–872. Available from: https://doi.org/10.1094/PDIS-01-13-0056-FE

Ploetz, R.C., Konkol, J.L., Narvaez, T., Duncan, R.E., Saucedo, R.J., Campbell, A. et al. (2017) Presence and prevalence of *Raffaelea lauricola*, cause of Laurel wilt, in different species of ambrosia beetle in Florida, USA. *Journal of Economic Entomology*, 110(2), 347–354. Available from: https://doi.org/10.1093/jee/tow292

Pratama, R., Muslim, A., Suwandi, S., Damiri, N. & Soleha, S. (2021a) Jackfruit (*Artocarpus heterophyllus*), a new host plant of *Ceratocystis* wilt in South Sumatra, Indonesia. *Australasian Plant Disease Notes*, 16, 1–6. Available from: https://doi.org/10.1007/s13314-021-00435-x

Pratama, R., Muslim, A., Suwandi, S., Damiri, N. & Soleha, S. (2021b) First report of bullet wood (*Mimusops elengi*) sudden decline disease caused by *Ceratocystis manginecans* in Indonesia. *Biodiversitas*, 22, 2636–2645. Available from: https://doi.org/10.13057/biodiv/d220522

Pratama, R., Muslim, A., Suwandi, S. & Shk, S. (2024) First report of *Ceratocystis fimbriata* causing wilt disease of soursop in South Sumatra, Indonesia. *Biodiversitas Journal of Biological Diversity*, 24, 6711–6721. Available from: https://doi.org/10.13057/biodiv/d241233

Raffa, K.F., Aukema, B.H., Bentz, B.J., Carroll, A.L., Hicke, J.A., Turner, M.G. et al. (2008) Cross-scale drivers of natural disturbances prone to anthropogenic amplification: the dynamics of bark beetle eruptions. *Bioscience*, 58(6), 501–517. Available from: https://doi.org/10.1641/B580607

Ranger, C.M., Parajuli, M., Gresham, S., Barnett, J., Villani, S., Walgenbach, J. et al. (2023) Type and duration of water stress influence host selection and colonization by exotic ambrosia beetles (Coleoptera: Curculionidae). *Frontiers in Insect Science*, 3, 1–11. Available from: https://doi.org/10.3389/finsc.2023.1219951

Ranger, C.M., Schultz, P.B., Frank, S.D., Chong, J.H. & Reding, M.E. (2015) Non-native ambrosia beetles as opportunistic exploiters of living but weakened trees. *PLoS One*, 10(7), 1–21. Available from: https://doi.org/10.1371/journal.pone.0131496

Roeper, R.A. (1996) Ambrosia beetles of the continental United States and Canada and status of knowledge of their associated primary symbiotic fungi. *Newsletter of the Michigan Entomological Society*, 41, 12–14.

Roy, K., Ewing, C.P., Hughes, M.A., Keith, L. & Bennett, G.M. (2018) Presence and viability of *Ceratocystis lukuohi* in ambrosia beetle frass from rapid ʻŌhiʻa death-affected *Metrosideros polymorpha* trees on Hawaiʻi Island. *Forest Pathology*, 49(1), 1–4. Available from: https://doi.org/10.1111/efp.12476

Roy, K., Jaenecke, K.A., Dunkle, E.J., Mikros, D. & Peck, R.W. (2023) Ambrosia beetles (Coleoptera: Curculionidae) can directly transmit the fungal pathogens responsible for rapid ʻŌhiʻa death. *Forest Pathology*, 53(3), e12812. Available from: https://doi.org/10.1111/efp.12812

Roy, K., Jaenecke, K.A. & Peck, R.W. (2020) Ambrosia beetle (Coleoptera: Curculionidae) communities and frass production in ʻŌhiʻa (Myrtales: Myrtaceae) infected with *Ceratocystis* (Microascales: Ceratocystidaceae) fungi responsible for rapid ʻŌhiʻa death. *Environmental Entomology*, 49(6), 1345–1354. Available from: https://doi.org/10.1093/ee/nvaa108

RStudio: Integrated Development Environment for R. RStudio, PBC, Boston. Available from http://www.rstudio.com/

Sobel, L., Lucky, A. & Hulcr, J. (2015) Ambrosia beetle *Xyleborus affinis* Eichhoff, 1868 (Insecta: Coleoptera: Curculionidae: Scolytinae). *EDIS*, 2015(5), 5. Available from: https://doi.org/10.32473/edis-in1094-2015

Soulioti, N., Tsopelas, P. & Woodward, S. (2015) *Platypus cylindrus*, a vector of *Ceratocystis platani* in *Platanus orientalis* stands in Greece. *Forest Pathology*, 45(5), 367–372. Available from: https://doi.org/10.1111/efp.12176

Souza, A.G.C., Maffia, L.A., Murta, H.M., Alves, Y.H., Pereira, R.M. & Picanço, M.C. (2013) First report on the association between *Ceratocystis fimbriata*, an agent of mango wilt, *Xyleborus affinis*, and the sawdust produced during beetle colonization in Brazil. *Plant Disease*, 97(8), 1116. Available from: https://doi.org/10.1094/PDIS-12-12-1204-PDN

Sun, Y., Li, M., Wang, Y., Li, L., Wang, M., Li, X. et al. (2020) *Ceratocystis fimbriata* employs a unique infection strategy targeting peltate glandular trichomes of sweetpotato (*Ipomoea batatas*) plants. *Phytopathology*, 110(12), 1923–1933. Available from: https://doi.org/10.1094/PHYTO-05-20-0165-R

Tarigan, M., Roux, J., Van Wyk, M., Tjahjono, B. & Wingfield, M.J. (2011) A new wilt and die-back disease of *Acacia mangium* associated with *Ceratocystis manginecans* and *C. acaciivora* sp. nov. in Indonesia. *South African Journal of Botany*, 77(2), 292–304. Available from: https://doi.org/10.1016/j.sajb.2010.08.006

Tarigan, M., Wingfield, M.J., van Wyk, M., Tjahjono, B. & Roux, J. (2011) Pruning quality affects infection of *Acacia mangium* and *A. crassicarpa* by *Ceratocystis acaciivora* and *Lasiodiplodia theobromae*. *Southern Forests: A Journal of Forest Science*, 73(3–4), 187–191. Available from: https://doi.org/10.2989/20702620.2011.639498

Tsopelas, P., Santini, A., Wingfield, M.J. & de Beer, Z.W. (2017) Canker stain: a lethal disease destroying iconic plane trees. *Plant Disease*, 101(5), 645–658. Available from: https://doi.org/10.1094/PDIS-09-16-1235-FE

Verrall, A.F. (1943) Fungi associated with certain ambrosia beetles. *Journal of Agricultural Research*, 66(3), 135–144.

Webber, J.F. & Gibbs, J.N. (1989) Insect dissemination of fungal pathogens of trees. In: *Insect-fungus interactions*. Cambridge, Massachusetts: The Royal Entomological Society of London, pp. 161–193. Available from: https://doi.org/10.1016/b978-0-12-751800-8.50013-6

Wickham, H., François, R., Henry, L., Müller, K. & Vaughan, D. (2023) *dplyr: A grammar of data manipulation. R package version 1.1.4*. California, USA: GitHub. Available from: https://github.com/tidyverse/dplyr, https://dplyr.tidyverse.org

Wingfield, M., Wingfield, B., Warburton, P., Japarudin, Y., Lapammu, M., Abdul Rauf, M. et al. (2023) Ceratocystis wilt of *Acacia mangium* in Sabah: understanding the disease and reducing its impact. *Journal of Tropical Forest Science*, 35, 51–66. Available from: https://doi.org/10.26525/jtfs2023.35S.SI.51

Wingfield, M.J., Barnes, I., de Beer, Z.W., Roux, J., Wingfield, B.D. & Taerum, S.J. (2017) Novel associations between ophiostomatoid fungi, insects and tree hosts: current status—future prospects. *Biological Invasions*, 19(11), 3215–3228. Available from: https://doi.org/10.1007/s10530-017-1468-3

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Beetle collections from clonal *Eucalyptus pellita* and *Acacia crassicarpa* compartments and the detection of *Ceratocystis* using carrot baiting and qPCR detection.

Table S2. Frass samples collected and detection of *Ceratocystis* using carrot baiting and qPCR detection.

Figure S1. Figures of several of the morpho-species collected in infected *Acacia crassicarpa* and clonal *Eucalyptus pellita* compartments (a) Mature *Beaverium* sp. collected from modified Bambara beetle trap in infected *A. crassicarpa* compartments. Bar = 500 μm. (b) Mature *Hypothenemus* sp. collected from modified Bambara beetle trap in infected *A. crassicarpa* and clonal *E. pellita* compartments. Bar = 200 μm. (c) Mature *Epuraea* sp. collected from modified Bambara beetle trap in infected *A. crassicarpa* and clonal *E. pellita* compartments. Bar = 500 μm. (d) Mature *Stictodex dimidiatus* (syn. *Xyleborus decumans*) collected from modified Bambara beetle trap in infected clonal *E. pellita* compartments. Bar = 200 μm. (e) Mature *Xyleborus affinis* collected from modified Bambara beetle trap in infected *A. crassicarpa* and clonal *E. pellita* compartments. Bar = 200 μm. (f) Mature *Xylosandrus crassiusculus* collected from modified Bambara beetle trap in infected *A. crassicarpa* and clonal *E. pellita* compartments. Bar = 200 and 500 μm. (g) Mature *Xyleborinus exiguus* collected from modified Bambara beetle trap in infected *A. crassicarpa* and clonal *E. pellita* compartments. Bar = 200 μm. (h) Mature *Xyleborus perforans* collected from modified Bambara beetle trap in infected *A. crassicarpa* and clonal *E. pellita* compartments. Bar = 200 μm. (i) Mature *Xyleborinus sharpae* collected from modified Bambara beetle trap in infected *A. crassicarpa* and clonal *E. pellita* compartments. Bar = 200 μm.

Figure S2. Macerated beetle mix susceptions when placed onto carrot baits yielded sporulation characteristic of *C. manginecans*.

Figure S3. Association of beetle wounding and *Ceratocystis* infection on *Acacia crassicarpa*. (a) Typical staining of vascular tissue due to *Ceratocystis* infection on *Acacia crassicarpa* surrounding a borer hole. (b) *Ceratocystis* perithecia colonizing wood from and below borer wound.

Figure S4. Artificially wounded *Acacia crassicarpa* stem colonized by *Ceratocystis*. *Ceratocystis* colonized tissue being fed upon by (a) a fly and (b) a nitidulid beetle.

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