

Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Ceratocystis manginecans* Causing Udeuid (*Manilkara udoido*) Wilt in Palau

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Udeuid (*Manilkara udoido*) is a culturally and ecologically important forest tree endemic to Babeldaob Island, Palau (Kitalong et al. 2014). Extensive mortality of mature udeuid trees was noted in late 2021. Dying trees had wilting and yellowing leaves, with main stems showing necrotic inner bark, black-stained xylem, and attacks by bark and ambrosia beetles. Losses exceeded 50% in several areas within the core range of udeuid. Because symptoms were suggestive of *Ceratocystis* wilt, samples of necrotic and stained inner bark and xylem, as well as beetle frass, were placed between fresh carrot discs and incubated at RT (Moller and DeVay 1968). *Ceratocystis*-like perithecia formed after 7 days, individual ascospore drops were transferred to agar media, and colonies were hyphal-tipped. From January to July 2024, 23 isolates were obtained. Cultures on malt extract agar at 18°C formed black perithecia with globose bases, long necks, and sticky

drops of hat-shaped ascospores. Chains of cylindrical conidia from tapering phialides, doliform conidia from wide-mouthed phialides, and darkly pigmented aleuriiconidia were also produced. This morphology and DNA sequences of four gene regions were consistent for *Ceratocystis manginecans*, a Brazilian species that has been associated with major disease outbreaks in Asia and elsewhere. Recently, the species concept of *C. manginecans* was expanded to include some strains that were formerly considered as *C. fimbriata* (Harrington et al. 2024). Direct sequencing of ITS-rDNA resulted in overlapping electropherograms, suggesting intragenomic variation among rDNA repeats, and partial sequences (GenBank nos. PV978653 to PV978655; ITS types 6 and 7b), which are also typical for *C. manginecans* isolates from Oman, Pakistan, India, and other Asian countries (Harrington et al. 2014, 2024). All isolates had mating type gene sequences (*MAT1-1-2*, PV567692; *MAT1-2-1*, PV567694) of 100% identity with *C. manginecans* from *Eucalyptus* (KF482985 and OP921563, respectively) and various other hosts in Brazil, South Africa, India, and China (Harrington et al. 2024). Ceratoplatenin sequences (PV942056) were 100% identical with the *CP1a* sequence from *Punica* in India (OR654997) as well as *Mangifera* and other hosts in Pakistan (OR654996) and Oman. In each of two trials, eight wild, udeuid seedlings (50 to 120 cm tall) in containers were inoculated with either isolate DS02-PIII or 5759, and three seedlings served as controls (Hughes et al. 2020). Whatman filter paper disks were soaked in a conidial suspension, incubated on potato dextrose agar plates for 3 days at 24°C, and inserted into a shallow incision into the xylem. Control seedlings received a sterile disk. A wet cotton ball was wrapped around the wound before sealing with Parafilm. Plants were held in a covered nursery (28 ± 3°C, 80 to 90% RH) for 33 days in Trial 1 (DS02-PII) and 37 days in Trial 2 (5759). In Trial 1, 75% of inoculated seedlings wilted or died, compared with 62% in Trial 2. Controls showed no symptoms. Lesions in inoculated seedlings ranged from 8.1 to 32.6 cm long versus 0.4 to 3.2 cm in controls. The pathogen (with the *MAT* sequences of the originals) was recovered from all inoculated seedlings but none of the controls, thus fulfilling Koch's postulates. This is the first report of *C. manginecans* on the genus *Manilkara* and its first detection in the Pacific Islands. Typhoon Surigae caused extensive damage to large udeuid trees in April 2021 and may have contributed to the outbreak by creating wounds for infection. Udeuid mortality has continued in Palau, and the pathogen poses a serious threat to native forests and cultivated crops in Oceania.

References:

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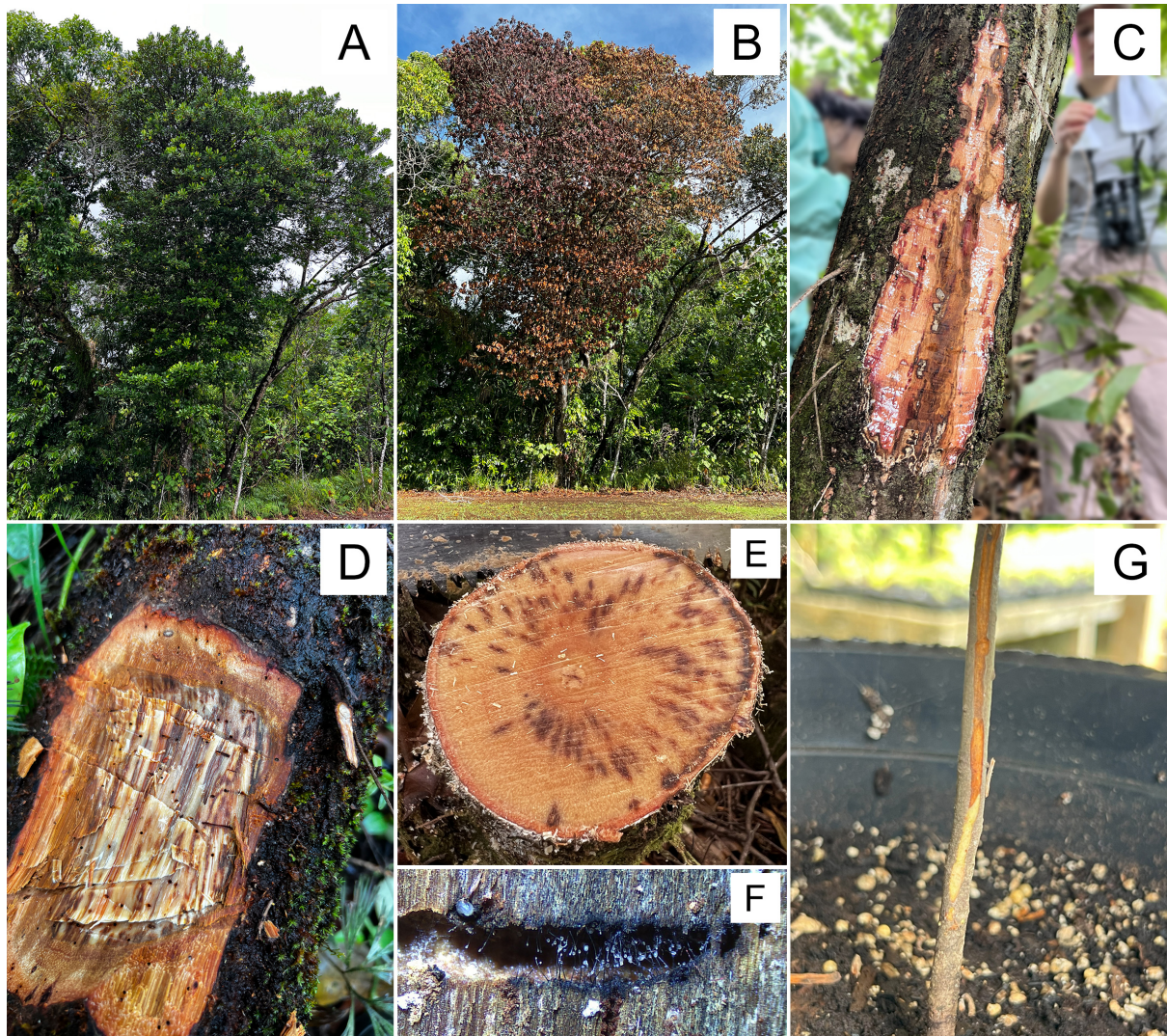


Figure S1. Symptoms and signs of wilt disease in udeuid tree (*Manilkara udoido*): (A) healthy-appearing udeuid tree in a mixed species stand (January 2024); (B) the same tree exhibiting wilt symptoms, including foliar browning and desiccation 3 months later; (C) necrosis of inner bark of a symptomatic tree; (D) discolored xylem with ambrosia beetle galleries; (E) transverse section showing radial xylem discoloration; (F) bark beetle gallery containing *Ceratocystis* perithecia; and (G) udeuid seedling artificially inoculated with *Ceratocystis manginecans* displaying inner bark necrosis below inoculation point.

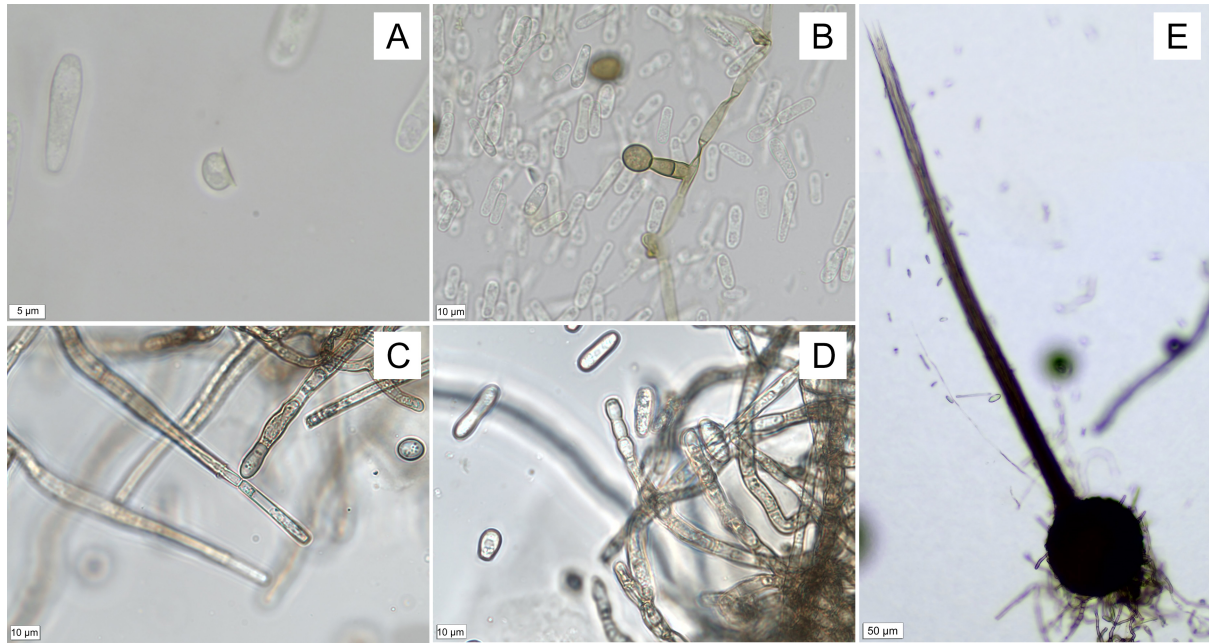


Figure S2. Morphological characteristics of *Ceratocystis manginecans* isolates collected from symptomatic udeuid trees (*Manilkara udoido*): (A) hyaline hat-shaped ascospore (4 to 6 µm diameter). Scale bar = 5 µm; (B) aleurioconidium (11 to 18 µm x 5 to 7 µm). Scale bar = 10 µm; (C) conidiophores with tapered phialides bearing cylindrical conidia (14 to 24 µm x 5 to 6 µm). Scale bar = 10 µm (D) conidiophore with widemouthed phialide bearing doliform (barrel-shaped) conidia (9 to 12 µm x 8 to 11 µm). Scale bar = 10 µm; (E) Perithecium (125 to 215 µm x 125 to 180 µm) with long black necks (600 to 785 µm) tapering from 30 to 34 µm wide at the base to 19 to 24 µm at the top. Scale bar = 50 µm. The white boxes in the lower left of each micrograph represent the scale bars.