Diseases Caused by Bacteria and Phytoplasmas

First Report of Association of 16SrII-D Phytoplasma with *Cycas revoluta* in Oman

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Cycas revoluta Thunb. (Cycadaceae) is native to southern Japan and is used for production of sago and planted as an ornamental plant. In Oman, it is mainly used for ornamental purposes. In February 2020, typical symptoms of a phytoplasma disease were observed, and symptoms included yellowing, dryness, and abnormality of leaves with elongated internodes and malformed leaflets, with numerous, small leaves emerging from the trunks of plants at Sultan Qaboos University campus, Muscat, Oman (N 3°59'14", E 58°16'34"). The disease affected four (10%) out of approximately 40 trees grown on campus. Four symptomatic and two asymptomatic *Cycas* leaf samples were collected, and the total genomic DNA was isolated using the CTAB method with some modification (Doyle 1991). A nested-PCR assay was used to amplify the 16S rRNA region of the associated phytoplasma using the primer pairs P1/P7 (Gundersen and Lee 1996) and R16F2n/R16R2 (Smart et al. 1996). Sterile distilled water and alfalfa witches' broom phytoplasma were used as negative and positive controls, respectively. All symptomatic plants and the

positive control produced an expected amplicon size of ≈1,800 and 1,250 bp, and no such amplicons were detected in the negative control. The obtained fragments were sequenced bidirectionally and assembled, and our four sequences of 1,636 bp (accession nos. MT239343, MT239344, MT239345, and MT239346) showed 100% homology with alfalfa phytoplasma isolate from Sudan (KY449416). The presence of phytoplasma was further confirmed by amplifying the *tuf* gene using TUF-II-F1/TUF-II-R1 followed by seminested PCR (Al-Subhi et al. 2018). Two sequences were submitted to GenBank under accession numbers MT254086 and MT254087. The constructed phylogenetic tree of combined sequences of 16S rRNA and *tuf* genes showed clustering of the phytoplasma from the present study with phytoplasmas in 16SrII-D subgroup. The associated phytoplasma was designated as *Cycas* yellowing phytoplasma (CYP). Strains of 16SrII-D phytoplasmas are known to be associated with several economically important plants as well as weeds in Oman (Al-Subhi et al. 2018). Associations of phytoplasmas and *C. revoluta* have been previously reported in India and Egypt (Behiry 2018; Kumar et al. 2012). The present study is the first report of association of a 16SrII-D subgroup phytoplasma with *Cycas* yellowing in Oman. Given that the symptoms affect the aesthetics of *Cycas* species in gardens, there is a need to develop management practices to prevent the spread of the pathogen.

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