



First report of lethal yellowing disease associated with subgroup 16SrIV-A phytoplasmas in Antigua, West Indies

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Coconut (*Cocos nucifera*) is an important perennial crop. Lethal yellowing (LY), a phytoplasma-associated disease, has been devastating coconut production in parts of the Caribbean. Local officials in Antigua observed widespread mortality of palms due to an unknown disease. Coconut palm, Christmas palm (*Adonidia merrillii*) Chinese fan palm (*Livistona chinensis*), edible date palm (*Phoenix dactylifera*), and Fiji fan palm (*Pritchardia pacifica*) were most affected. This new development followed previous reports of palm mortality due to LY in St Kitts (Myrie *et al.*, 2012), an island in the West Indies with no prior history of the disease.

The present study was undertaken to ascertain whether widespread losses of palms currently under way in Antigua is a result of recent spread of LY disease to the island. Dead and declining coconut palms with foliar symptoms suggestive of LY disease (Fig. 1) were identified at several locations throughout Antigua. Twenty-nine palms exhibiting decline (Table 1) were sampled, DNA extracted (Myrie *et al.*, 2012) and assessed for infection by group 16SrIV phytoplasmas. Five trunk tissues from coconuts and a queen palm (*Syagrus romanzoffiana*) tested positive (20.7% of the sampled palms) for infection by group 16SrIV phytoplasmas by primary PCR using primer pair P1 (Deng & Hiruki, 1991) and P7 (Smart *et al.*, 1996) and in nested PCR using group 16SrIV-specific primer pair LY16Sf2/LY16-23Sr2 (Harrison *et al.*, 2008). Palms were reassessed by group 16SrIV-specific real-time PCR using primers 503LY16S-ANYF/503LY16S-ANYR and TaqMan® probe 503LY16S-ANYM (Myrie *et al.*, 2011) and with positive results in 20 palms (69%) including at least one representative of six palm species. Also, Bismarck palm (*Bismarckia*, royal palm (*Roystonea regia*) and foxtail palm (*Wodyetia bifurcata*), that had no prior history of susceptibility to LY, were infected. Nested PCR rDNA products (1.64 kb) in RFLP analysis produced fragment patterns that were indistinguishable (Fig. 2), but could be differentiated from Texas Phoenix palm decline phytoplasma, representative of 16SrIV-D.

Pairwise comparison between rDNA sequences (1.64 kb) derived from all six phytoplasma positive palms by nested PCRs yielded sequences that shared 99.9-100% identity with each other and 99-100% identity with comparable length rDNA sequences of phytoplasmas associated with coconut palms in St. Kitts (GenBank Accession No. JX0700937) and Jamaica (HQ613873). Representative sequences from coconut (AN6, AN28) and from *S. romanzoffiana* (AN21) were archived in GenBank (Accession Nos. JX560529, JX560530 and JX560531, respectively). Analysis of F2n/R2 portion of all six sequences using the PhyClassifier program (Zhao *et al.*, 2009), confirmed the group and subgroup affiliations

of Antiguan palm-associated phytoplasmas as members of group 16SrIV, subgroup A. A phylogenetic tree constructed from 16S rDNA sequences confirmed that all six phytoplasma strains examined clustered with other subgroup 16SrIV-A strains belonging to the coconut lethal yellows phytoplasma subclade (Fig. 3). This report will be used to inform the Antiguan authorities as to the spread of lethal yellowing disease, which will help in the establishment of phytosanitary measures.

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References

- Deng S, Hiruki C, 1991. Amplification of 16 S rRNA genes from culturable and non-culturable mollicutes. *Journal of Microbiological Methods* **14**, 53-61. [http://dx.doi.org/10.1016/0167-7012\(91\)90007-D](http://dx.doi.org/10.1016/0167-7012(91)90007-D)
- Harrison NA, Helmick EE, Elliott ML, 2008. Lethal yellowing-type diseases of palms associated with phytoplasmas newly identified in Florida, USA. *Annals of Applied Biology* **153**, 85-94. <http://dx.doi.org/10.1111/j.1744-7348.2008.00240.x>
- Myrie WA, Douglas L, Harrison NA, McLaughlin W, James M, 2012. First report of lethal yellowing disease associated with subgroup 16SrIV, a phytoplasma on St. Kitts in the Lesser Antilles. *New Disease Reports* **26**, 25. <http://dx.doi.org/10.5197/j.2044-0588.2012.026.025>
- Myrie W, Oropeza C, Sáenz L, Harrison N, Roca MM, Córdova I, Ku S, Douglas L, 2011. Reliable improved molecular detection of coconut lethal yellowing phytoplasma and reduction of associated disease through field management strategies. *Bulletin of Insectology* **64** (Supplement), S203-S204.
- Smart CD, Schneider B, Blomquist CL, Guerra LJ, Harrison NA, Ahrens U, Lorenz KH, Seemüller E, Kirpatrick BC, 1996. Phytoplasma-specific PCR primers based on sequences of the 16-23S rRNA spacer region. *Applied and Environmental Microbiology* **62**, 2988-2993.
- Zhao Y, Wei W, Lee IM, Shao J, Suo X, Davis RE, 2009. Construction of an interactive online phytoplasma classification tool, iPhyClassifier, and its application in analysis of the peach X-disease phytoplasma group (16SrIII). *International Journal of Systematic and Evolutionary Microbiology* **59**, 2582-2593. <http://dx.doi.org/10.1099/ijs.0.010249-0>



Figure 1

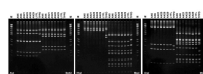


Figure 2



Figure 3

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