First report of '*Candidatus* Phytoplasma palmae' (16SrIV-A subgroup) associated with palm Lethal Yellowing disease on *Cocos nucifera* and *Pritchardia sp.* in Guadeloupe, French West Indies

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Keywords: *Cocos nucifera*, *Pritchardia sp.*, phytoplasma, lethal yellowing, palm, Guadeloupe

Lethal Yellowing (LY) disease causes major damage to palms in Central America and the Caribbean. It has been reported as far south as Antigua (Myrie et al., 2014). LY affects over forty palm species, seriously impacts the coconut industry and alters the landscapes on islands with a tourist-based economy.

In March 2021, the presence of LY disease was regularly monitored in Guadeloupe. Two palm species (*Cocos nucifera* and *Pritchardia sp.)* died on a private property in Saint-Anne, Grande Terre. Yellowing of lower fronds and necrosis of inflorescences were reported on some neighboring palms. One symptomatic *Cocos nucifera* (GP21-007) and four symptomatic *Pritchardia sp.* (GP21-005, GP21-006, GP21-008 and GP21-009) were sampled by stem drilling. Samples from four asymptomatic coconut trees (GP21-001 to GP21-004) were collected in the locality of Deshaies.

DNA was extracted from the nine sawdust samples following a cetyltrimethylammonium bromide (CTAB) modified protocol (Doyle and Doyle, 1990). A quantitative polymerase chain reaction (PCR), following the protocol described by Christensen et al. (2004), was performed on DNA to diagnose the presence of phytoplasmas. An exponential amplification was observed for all DNA extracts from symptomatic palm samples (threshold number of PCR cycles (Ct) ranged from 18.50 to 23.58). DNA from asymptomatic samples yielded negative results (undetermined Ct). To identify the phytoplasma associated with LY, DNA samples were subjected to PCR, based on the 16SrRNA gene, plus internal transcribed spacers (ITS) using P1-1T (Pilet et al., 2021)/P7 (Schneider et al., 1995) primers, and *sec*A gene using the primer pair secAFor1/secARev1 (Hodgetts et al. 2008). Amplicons of 1.8 kb covering the 16S ribosomal operon and 830 bp for the *sec*A gene were produced using DNA from symptomatic trees. All amplicons

were double strand sequenced (Genewiz, UK). The corresponding sequences were deposited in GenBank and subjected to BLASTn on NCBI.

Sequences of the ribosomal operon gene (accession no. ON521114 to ON521118) were identical for the five positive samples. Sequencing revealed two distinct ribosomal operons with heterozygous peaks on the DNA chromatogram. The first aMino ambiguity (M = Adenine or Cytosine) was observed in the 16Sr RNA gene. The second was observed in the first intergenic transcript spacer. The 16S rDNA sequence (M = Cytosine) presented 100% identity with accession no. HQ613874 and 99.93% with accession no. U18747, the reference sequence for '*Candidatus* Phytoplasma palmae'. The virtual RFLP pattern derived from the 16S rDNA F2nR2 fragment and identified using iPhyclassifier (Zhao et al. 2009) was identical to the reference pattern for the 16SrIV-A subgroup. A unique sequence was obtained for the partial *sec*A gene (OP136139 to OP136143), sharing 100% identity with EU267187 for the palm LY phytoplasma preprotein translocase subunit (*sec*A) gene. This is the first report of '*Ca.* Phytoplasma palmae' (subgroup 16SrIV-A) associated with palm LY disease on *Cocos nucifera* and *Pritchardia sp.* in Guadeloupe.

Measures to eradicate LY were implemented as soon as its presence was confirmed in Guadeloupe. LY phytoplasmas continue to spread in the Caribbean and are approaching South America, where the known vector, *Haplaxius crudus,* has already been reported (Silva et al., 2019). This poses a major threat to the coconut economy and the diversity of palm trees.

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Figure S1. Symptoms of Lethal Yellowing disease observed on *Cocos nucifera* in Sainte-Anne, Guadeloupe (Credit: C. Diman).

225x169mm (72 x 72 DPI)

Figure S2. Symptoms of Lethal Yellowing disease observed on *Pritchardia sp.* in Sainte-Anne, Guadeloupe (Credit: C. Diman).

225x169mm (72 x 72 DPI)