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First report of a phytoplasma affecting cassava orchards in Cote d'Ivoire.

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Cassava (*Manihot esculenta*) is one of the staple foods in Africa, cultivated in around 40 countries and producing more than three million tonnes every year (United Nations, 2005). In Côte d'Ivoire, cassava production is second to yams, and it is widely used as a typical food side dish called 'attieké'. Moreover, cassava-based flour or starch is widely used by private companies, which has made cassava one of the most important Ivorian industrial crops. Cassava has become an alternative income source for women in the south littoral of the coconut-growing area of Grand-Lahou, since the Côte d'Ivoire lethal yellowing phytoplasma (CILY, 16SrXXII-B, 'Candidatus Phytoplasma palmicola-related strains') devastated over 400 hectares of coconut plantations (Arocha *et al.*, 2017).

Recently, symptoms of leaf mosaic, curling and yellowing (Figs. 1-2) were observed in cassava orchards located in the coconut-growing villages of Badadon and Braffedon in Grand-Lahou, which are currently affected by CILY. Leaf samples were collected at random from 12 symptom-bearing and two symptomless cassava plants from the two villages. Total DNA was extracted and used as a template for a nested PCR assay with universal primers that target the phytoplasma 16S rRNA gene, R16mF2/R1 for the first PCR reaction, and R16F2n/R2 for the nested reaction (Gundersen & Lee, 1996). A second PCR assay was performed with primers P1 (Deng & Hiruki, 1991) and P7 (Schneider *et al.*, 1995) followed by a nested PCR with primers G813 and AwkaSR (Tymon *et al.*, 1998) that specifically target the 16S rRNA gene and part of the intergenic region 16/23S rRNA gene of the Cape St. Paul Wilt Disease phytoplasma (16SrXXII-B) from Ghana. R16F2n/R2 and G813/AwkaSR amplicons were produced from six of the 12 symptom-bearing samples but not from the two symptomless plants. R16F2n/R2 amplicons from each sample were purified (EZNA Cycle Pure Kit, Omega Bio-Tek, USA), cloned (pGEM-T Easy Vector, Promega), and sequenced (University of Toronto, Canada). A consensus sequence was deposited in GenBank (Accession No. KY563222), and was compared to those of other phytoplasmas in the database and analysed through *iPhyClassifier* (<http://plantpathology.ba.ars.usda.gov>). R16F2n/R16R2 sequences from all samples shared 100% sequence identity and were 99% similar to those of the 16SrXXII-B phytoplasmas, including the CILY phytoplasma (KC999037). Analysis of the R16F2n/R2 sequences with *iPhyClassifier* and the pDRAW32 software (<http://www.acaclone.com>) showed that the Côte d'Ivoire cassava phytoplasma and the CILY phytoplasma KC99937, exhibited identical *AluI*, *RsaI*, *TaqI*, *HaeIII*, *BstUI*, *KpnI*, *HhaI*, *HinfI*, *HpaI*, *HpaII*, *MseI*, *Sau3AI*, *DraI*, and *BfaI* RFLP profiles. Phylogenetic analysis (MEGA 4.0) based on the 16S rDNA sequence (Fig.

3) supported the grouping of the Côte d'Ivoire cassava phytoplasma within the 16SrXXII-B cluster, closely related to 'Ca. P. palmicola' (16SrXXII-A).

To our knowledge this is the first report of a phytoplasma affecting cassava in Côte d'Ivoire. The findings suggest that cassava may be a potential alternative host for the CILY phytoplasma, which poses a serious threat for the food security of the smallholder coconut and cassava farmers, especially women in Grand-Lahou, Côte d'Ivoire.



Figure 1: Symptoms of yellowing observed in leaves of a cassava plant in the village of Badadon, Grand-Lahou.



Figure 2: Symptoms of mosaic and curling (A) observed in leaves from cassava plants collected in Braffedon, Grand-Lahou compared to symptomless leaves (B).

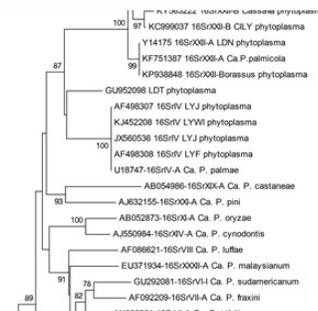


Figure 3: Phylogenetic tree based on the R16F2n/R2 sequences of the Côte d'Ivoire cassava phytoplasma and reference 16Sr phytoplasma groups. 'Ca. P. sp': 'Candidatus Phytoplasma sp.' Ribosomal group/subgroup when available are reported after GenBank accession numbers in parenthesis. LDG: Lethal Decline Ghana; LDN: Lethal Decline Nigeria; CSPWD: Cape St. Paul Wilt Disease; LYJ: Lethal Yellowing Jamaica; LYWI: Lethal Yellowing West Indies; LYF: Lethal Yellowing Florida; BVGY: Buckland Valley grapevine yellows. *Acholeplasma laidlawii* was used to root the tree. Bootstrap numbers >70 % shown above nodes.

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