



# First report of a '*Candidatus Phytoplasma asteris*' isolate affecting a strawberry nursery in Cuba

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Strawberry (*Fragaria x ananassa*) is traditionally multiplied in Cuba by vegetative propagation without phytosanitary certification. During November 2011, surveys for phytoplasma to strawberry nurseries revealed plants showing symptoms of leaf yellowing and reddening, fruit deformation and stunting in an orchard of 'Ceiba del Agua', Artemisa province (Fig. 1). Similar symptoms were previously described in strawberry associated with phytoplasma infections (Jomantiene *et al.*, 1998; Valiunas *et al.*, 2006). Leaves and midribs collected from symptom-bearing and symptomless plants were subjected to total DNA extraction (Murray & Thompson, 1980). Total DNA was used as a template in a nested-PCR with generic primers targeting the phytoplasma 16S rRNA gene: P1/P7 (Deng & Hiruki, 1991) and R16F2n/R16R2 (Gundersen & Lee, 1996). R16F2n/R2 amplicons were purified, ligated in pGEM®-T Easy vector (Promega, Madison, WI, USA) and sequenced. The derived sequences were identified by comparison with phytoplasma reference sequences using BLAST ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) and RFLP analysis (determined by comparing the digestion sites map for the endonucleases *EcoRI*, *BfaI*, *HinfI*, *RsaI*, *AluI*, *MseI*, *HpaII* and *HaeIII* using VECTOR NTI v.8.0 from Invitrogen, CA, USA). Phylogenetic trees were constructed using Mega 4.0.

R16F2n/R2 amplicons of expected size (approx. 1200 bp) and similar to the reference control were amplified from 13/14 symptom-bearing plants. No amplification was yielded by symptomless plants (6/6). Two individual clones derived from four symptom-bearing plants were sequenced, and the partial consensus sequence of 1139 nt (GenBank Accession No. KC825053) exhibited 99% sequence identity with those of group 16SrI '*Ca. Phytoplasma asteris*', closer to members of subgroup 16SrI-F, including the apricot chlorotic leaf roll-aster yellow phytoplasma, ACLR-AY (AY265211), aster yellows phytoplasma strain CVB (AY265212), Bajgah periwinkle little leaf phytoplasma (DQ266089), and macadamia phytoplasma (KC513772). Nucleotide comparison with strawberry phytoplasma sequences from Lithuania (DQ086423; Valiunas *et al.*, 2006) and USA (U96614, U96616; Jomantiene *et al.*, 1998) showed identities lower than 98%. RFLP profiles derived from the R16F2n/R2 amplicon sequence were identical to those of the subgroup 16SrI-F mentioned above used as reference. A phylogenetic tree based on the 16S rDNA sequence of the strawberry phytoplasma under study and those of reference sequences supported PCR and RFLP results, and confirmed that the phytoplasma is a member of the 16SrI '*Ca. Phytoplasma asteris*' group, and possibly subgroup 16SrI-F (Fig. 2).

This is the first report of a '*Ca. Phytoplasma asteris*' isolate associated with symptoms in Cuban strawberry plants. Both RFLP and phylogeny analysis



Figure 1

show the closest relationship between the strawberry phytoplasma (KC825053) and the previously phytoplasma identified from Cuban macadamia trees (Pérez-López *et al.*, 2013) in Artemisa province. This suggests complex epidemiological implications for the two '*Ca. Phytoplasma asteris*' isolates in two different plant hosts within the same geographic location; indicating the possibility of significant risk of distributing possible phytoplasma-infected propagation material from strawberry nurseries to commercial strawberry fields. A more distant relationship was found between the Cuban strawberry phytoplasma sequence and those reported from strawberry in other countries, suggesting that this fruit crop supports the infection by a wide phytoplasma range.

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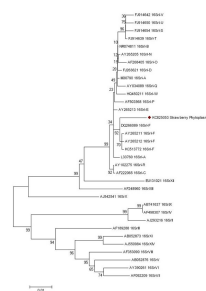


Figure 2

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