New Disease Reports

First report of the identification of a '*Candidatus* Phytoplasma pruni'-related strain of phytoplasma in *Melothria pendula*

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Melothria pendula, also known as the creeping cucumber or the Guadeloupe cucumber, is a plant in the Cucurbitacea family with exceptional laxative properties. It is known as pepininho in Brazil where it is widespread throughout the southern, central and northern regions, growing wild in household gardens and backyards. Symptoms of chlorosis, little leaf and leaf malformation were observed in *M. pendula* plants grown in private gardens of the Seropédica area in the state of Rio de Janeiro during 2016 (Fig 1.).

Leaves, petioles and flowers were collected from four symptomatic and two symptomless *M. pendula* plants. Total DNA was extracted (FastDNA Spin Kit, MP Biomedicals, USA) and tested for phytoplasmas in a nested PCR assay with universal primers that target the phytoplasma 16S rRNA gene. Primers R16mF2/R1 were used for the first PCR reaction, and R16F2n/R2 for the nested reaction (Gundersen & Lee, 1996). R16F2n/R2 PCR amplicons were obtained for all symptomatic samples but not from the symptomless plants. Four R16F2n/R2 amplicons were purified (EZNA Cycle Pure Kit, Omega Bio-Tek, USA), cloned (pGEM-T Easy Vector, Promega), and sequenced (University of Toronto, Canada).

The 16S rDNA sequences from the four symptomatic *M. pendula* plants were identical and the consensus sequence was deposited in GenBank (Accession No. MK108032). The 16S rDNA consensus sequence shared between 98 to 99% identity with those of members of the 16SrIII phytoplasma group (Western X-disease) as confirmed by phylogenetic analysis (MEGA v 4.0) (Fig. 2). The phytoplasma isolated from *M. pendula* was placed within the 16SrIII group cluster as a '*Candidatus* Phytoplasma pruni'-related strain showing the highest sequence identities with subgroups -J (99.5%; AF147706), -U (99.03%, HM589213), -Q (99.03%, AF302841), -B (99.02%, AF189288) and -R (99%, AF373105). The *i* PhyClassifier analysis (https://plantpathology.ba.ars.usda.gov) indicated that the virtual RFLP pattern derived from the 16S rDNA F2nR2 fragment of the *M. pendula* phytoplasma (MK108032) was most similar to the reference pattern of the group 16SrIII, subgroup U (HM589213), with a pattern similarity coefficient of 0.98, which suggested that this strain is a variant of the phytoplasma subgroup 16SrIII-U.

M. pendula was confirmed as a new host for phytoplasmas worldwide. It was also added to the Brazilian inventory of alternative phytoplasma hosts. In Brazil, the phytoplasma group 16SrIII causes disease in numerous host species. Notably, a severe disease in cassava called 'frog skin' that may also be associated with asymptomatic infections (Montano *et al*., 2011), and was recently found in citrus in São Paulo and Minas Gerais States (Wulff *et al*., 2018) in some cases in mixed infection with '*Candidatus* Liberibacter asiaticus'. The group 16SrIII has been also reported affecting *Melia azedarach* (Duarte *et al*., 2009) and Leonurus sibiricus (Flôres & Bedendo, 2013). The presence of a '*Ca*. P. pruni'-related strain in *M. pendula* poses a threat not only for *Melothria*-related species but also for other plant species grown in nearby private gardens and backyards in the state of Rio de Janeiro in Brazil.

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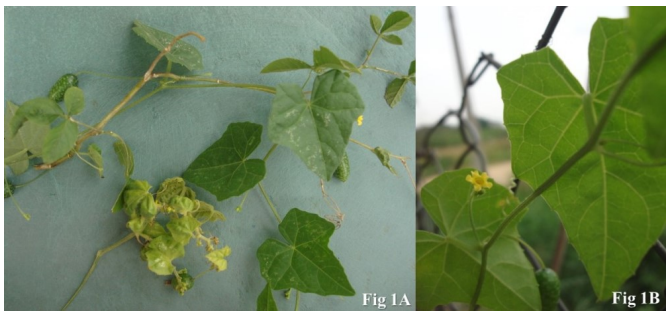

Figure 1

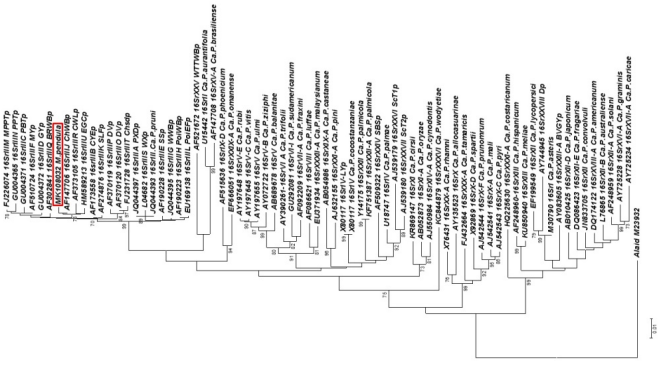

Figure 2

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