



First report of a phytoplasma associated with witches' broom symptoms in *Waltheria indica* in Brazil

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Waltheria indica (Malvaceae) is a short-lived shrub, native to the neotropics but with a pantropical distribution, which is frequently used for medicinal purposes (Zongo *et al.*, 2013). In January 2016, *W. indica* plants located in a coastal area of Vila Velha (state of Espírito Santo, Brazil, S20°31'42.51", W40°22'24.1356") were found exhibiting symptoms of witches' broom and yellowing (Fig. 1).

Leaf samples were collected from branches of three symptom-bearing and two symptomless *W. indica* plants. Total DNA was extracted from each sample and subjected to nested polymerase chain reaction (nPCR) amplification of the phytoplasma 16S rRNA gene with primers P1/P7 (Deng & Hiruki, 1991) and R16F2n/R2 (Gundersen & Lee, 1996). Amplicons of the expected size (1.2 kb) were obtained from all plants showing symptoms but not from symptomless plants. The amplicons were purified using an Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare, UK), cloned using the pGEM®-T Easy Vector System I (Promega, USA) and sequenced by Macrogen Inc. (Korea). BLAST searches confirmed that the consensus 16S rDNA sequence of the *Waltheria indica* witches' broom phytoplasma (WIWB) (GenBank Accession No. KX691443) shared 99.4% sequence identity with those of 'Candidatus Phytoplasma asteris' (16SrI group) species (e.g. HM067755, KP864664, AF222066). The WIWB phytoplasma was preliminary classified as a member of the 16SrI-S subgroup, with a similarity coefficient of 0.99 based on RFLP analysis when compared to 16SrI phytoplasmas using iPhyClassifier (Zhao *et al.*, 2013). Phylogenetic analysis using the neighbour-joining method in MEGA7 software (www.megasoftware.net/) supported the sequencing and iPhyClassifier results (Fig. 2).

The occurrence of phytoplasmas in *W. indica* has been previously reported in Brazil and Australia (Kitajima & Costa, 1971; Schneider *et al.*, 1999), associated with phytoplasmas of group 16SrII 'Ca. P. aurantifolia'. To our knowledge, this is the first report of a member of 16SrI phytoplasma

associated with *W. indica* plants in Brazil, which may pose a threat to other nearby plant species.

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Figure 1

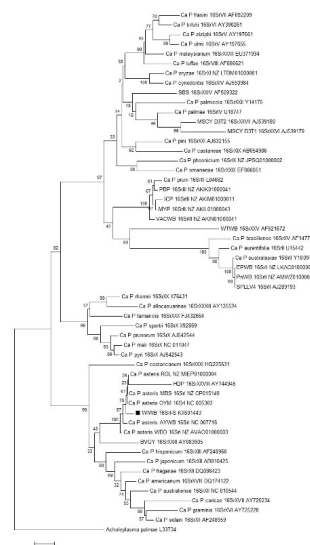


Figure 2

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