

## Diseases Caused by Bacteria and Phytoplasmas

### First Report of ‘*Candidatus Phytoplasma trifolii*’-Related Strain Associated with Flower Abortion and Necrosis in Prickly Pear Cactus in Zacatecas, Mexico

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Mexico is the most important producer of prickly pear cactus (*Opuntia ficus-indica*) worldwide with a total of 471,637 tons on 46,555 ha in 2018. In July 2019, the presence of symptoms of flower abortion, foliar necrosis, loss of thorns, yellowing, deformation, and proliferation of cladodes was documented in approximately 8% of prickly pear plants from 20 ha of commercial fields in the municipality of Pinos in Zacatecas, Mexico (22°18'0" N, 101°34'0" W). Total DNA was extracted from 10 symptomatic and five symptomless prickly pear plants. Direct and nested PCR assays targeting the 16S rRNA gene were used to confirm the association of phytoplasma with this new symptomatology. The primers used for direct PCR were P1 (5'-AAGAGTTTGATCCTGGCTCAG GATT-3') and Tint (5'-TCAGGCGTGTGCTCTAACCAGC-3') (Smart et al. 1996), and for nested PCR, R16F2n (5'-GAAACGACTGCTAA GACTGG-3') and R16R2 (5'-TGACGGGCGGTGTACAAACCCCG-3') (Gundersen and Lee 1996). No PCR products were obtained from the symptomless plants. The nested PCR amplicons (1.2 kb) amplified from all symptomatic plants were cloned separately and directly sequenced. BLAST analysis of the 16S rDNA sequences revealed that they shared 100% sequence identity to each other and 99.0% sequence identity with those of

the 16SrVI group, ‘*Candidatus Phytoplasma trifolii*’ strains. Computer-simulated restriction fragment length polymorphism (RFLP) analysis of the prickly pear phytoplasma sequence (GenBank accession no. MT507114) was performed using iPhyClassifier (<https://plantpathology.ba.ars.usda.gov/cgi-bin/resource/iphyclassifier.cgi>), and RFLP profiles were compared with each phytoplasma group and subgroup (Zhao et al. 2013), confirming that the analyzed sequence shared 99.0% identity with those of the group 16SrVI (reference strain AY390261), and also it was classified into a new subgroup (16SrVI-K). There are reports of phytoplasmas of the 16SrI (Fucikovsky-Zak et al. 2011), II (Hernández-Pérez et al. 2009), and XIII (Suaste et al. 2012) groups associated with effects in prickly pear cactus in Mexico. However, there are no reports of 16SrVI group phytoplasmas infecting Cactaceae species. ‘*Ca. P. trifolii*’ has been related to other diseases in many important crops in Mexico (Reveles-Torres et al. 2018), and its proliferation in prickly pear cactus fields has increased due to application of mineral and organic fertilizers (Santiago-Lorenzo et al. 2016), which reduce the use of insecticides and increase the presence of insects that can act as possible vectors of phytoplasmas. This is the first report of ‘*Ca. P. trifolii*’ associated with a new disease in prickly pear cactus, and the identification of a phytoplasma sequence belongs to a new subgroup (16SrVI-K). The showed results establish the importance of implementing an integrated management program to reduce the proliferation and incidence of ‘*Ca. P. trifolii*’ in economically important crops in Mexico and other countries.

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