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## Occurrence of '*Candidatus Phytoplasma aurantifolia*' (16SrII group) in Bolivia

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During a survey for phytoplasma at the PROINPA Foundation, Cochabamba, Bolivia in June 2008, plants of podocarpus (*Podocarpus macrophyllus*) showed symptoms of shortened internodes, leaf size reduction and proliferation compared to healthy ones, while those of rose (*Rosa* sp.) exhibited little leaf and yellowing. Both podocarpus and rose are widely distributed ornamental plants in the urban area of Cochabamba, and symptoms were observed in at least 20% of plants of each species surveyed at PROINPA. In addition, symptoms of leaf deformation, crinkling, and curling were displayed by wild plants of tomatillo (*Physalis ixocarpa*) growing nearby. Three plants with symptoms and one symptomless plant respectively of each species were collected. A nested PCR with universal 16S rRNA gene primers R16mF2/R1 and fU5/rU3 was carried out from total DNAs extracted from collected plant material. All plants with symptoms yielded PCR amplicons (~880 bp), whilst the symptomless plants did not. PCR products were purified (Wizard, Promega), cloned (pGEM T-Easy Vector, Promega) and sequenced (<http://www.dnaseq.co.uk>). A representative sequence was deposited in GenBank for podocarpus (FJ207457), rose (FJ207453) and tomatillo (FJ207452). BLAST comparisons indicated that the 16S rDNA sequences of these phytoplasmas showed the highest identity (99%) with those of phytoplasma members of

group 16SrII, '*Candidatus Phytoplasma aurantifolia*'. A disease in tomatillo was recently associated with a '*Ca. Phytoplasma asteris*'-related strain in Mexico (Santos-Cervantes *et al.*, 2007). However, no phytoplasmas have been reported associated with diseases in podocarpus or rose in the region.

This is the first report of phytoplasmas of group 16SrII in Bolivia and its association with diseases in podocarpus, tomatillo and rose plants.

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## First identification of '*Candidatus Phytoplasma aurantifolia*' infecting *Fallopia japonica* in the United Kingdom

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Japanese knotweed (*Fallopia japonica*), family Polygonaceae, is a tall, invasive, perennial weed, first introduced into the UK in the 1850's. It has since spread to occupy more than half of the 10 km squares used to map plant distribution in the British Isles. The plant causes problems in both rural and urban habitats due to its vigorous growth and ability to form dense thickets. In urban environments its growth can damage buildings and tarmac surfaces.

In July 2008, plants of *F. japonica* growing along a boundary fence in Egham were seen with symptoms of severe stunting and witches' brooms. Symptoms were most pronounced in the emerging shoots, which displayed proliferation, shortened internodes and small leaves. Infected plants also exhibited a pronounced silver flecking on the leaves, which superficially resembled thrip-damage. The knotweed stand occupied an area of approximately 35 m<sup>2</sup> and 50 of the 88 plants (56.8%) displayed obvious symptoms.

Leaves from four plants with symptoms and one healthy-looking plant were collected and the DNA extracted and indexed in a nested PCR assay with universal primers P1m (Hren *et al.*, 2007) /P7 (Gundersen & Lee, 1996) and fU5/rU3 (Lorenz *et al.*, 1995). PCR products of expected size (~880 bp) were obtained from all plants displaying symptoms, but not from the healthy-looking plant. Nested PCR products of a representative plant were purified, cloned (pGEM-T Easy Vector, Promega), and sequenced in both directions using M13 sequencing primers (<http://www.dnaseq.co.uk>). The 16S rDNA sequence was com-

pared with those held in GenBank using BLAST. The closest matches (99%) were to phytoplasma members of group 16SrII, '*Candidatus Phytoplasma aurantifolia*', including those associated with crotalaria witches' broom (EU650181, EF656454) and sweetpotato little leaf (EU170353). The new phytoplasma sequence was deposited in GenBank (FJ008924).

The severe stunting of Japanese knotweed has important implications for its competitive ability and it was noted that stinging nettles (*Urtica dioica*) were overgrowing many of the stunted plants. This is the first record of a phytoplasma infecting *F. japonica*, and the first identification of a phytoplasma from group 16SrII in the UK.

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