



Molecular detection and phylogeny of phytoplasmas affecting cycads in India

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Cycads are an ancient group of gymnosperms, often considered to be living fossils. Recently, abnormal yellowing symptoms were observed on two cycad species of the order Cycadales: *Cycas revoluta* (family Cycadaceae), and *Zamia furfuracea* (family Zamiaceae), grown as ornamental plants in gardens of the North Delhi region, India. *C. revoluta* exhibited yellowing symptoms on fully developed leaves in all the whorls on the rachis and the leaf lamina (Fig. 1a). *Z. furfuracea* showed a pale yellow colour in the leaf lamina and little leaf symptoms (Fig. 1b). Genomic DNA was isolated from the leaf midribs of five symptom-bearing and two symptomless *C. revoluta* plants, and three symptom-bearing and one symptomless *Z. furfuracea* plants, using the CTAB method (Saghai-Marooof *et al.*, 1987). DNA was used as a template in nested-PCR with phytoplasma universal 16S ribosomal DNA primers P1/P7 (Deng & Hiruki, 1991) and fU5/rU3 primers (Lorenz *et al.*, 1995). The amplicons were resolved on a 1.2% agarose gel, extracted, purified (QIAquick Gel Extraction Kit, QIAGEN, USA) and sequenced. Phylogenetic relationships were evaluated using the 16S rDNA sequences of phytoplasmas identified from *C. revoluta* (Cycas yellowing phytoplasma) and *Z. furfuracea* (Zamia yellowing phytoplasma), and from 18 other 16S rDNA sequences from GenBank reference phytoplasmas using the maximum parsimony method of MEGA version 4.01. The bootstrap consensus tree was inferred from 1000 replicates using *Acholeplasma laidlawii* as the outgroup.

Amplicons of desired size (~880bp) were obtained in all symptom-bearing plants while no amplification was observed for the symptomless ones. BLAST analysis of the phytoplasma 16S rDNA sequences from both *Cycas* and *Zamia* yellowing phytoplasmas showed 99% sequence identity with those of members of phytoplasma group 16SrII ('*Candidatus* Phytoplasma aurantifolia'). The phylogenetic tree supported the sequence comparison analysis since the 16S rDNA sequences of *Cycas* and *Zamia* yellowing phytoplasmas (GenBank Accession Nos. HQ625434 and HQ625433, respectively) clustered in the same phylogenetic branch of 16SrII phytoplasmas (Fig. 2). Previous reports indicate that gymnosperms in India are highly sensitive to phytoplasma infection. '*Ca. Phytoplasma pini*' (AJ632155, AJ310849), group 16SrV (Schneider *et al.*, 2005) and a '*Ca. Phytoplasma phoenicium*'-related phytoplasma (AF515637), group 16SrIX-E (Davis *et al.*, 2010) have been associated with leaf abnormalities. Moreover, a '*Ca. Phytoplasma trifolii*'-related strain

(FJ002570), group 16SrVI (Gupta *et al.*, 2010) has been associated with *Araucaria heterophylla* little leaf and stunting. This is the first record of cycads (Cycadales) being infected by phytoplasmas of group 16SrII, the most widespread phytoplasma group in the region. The fact that gymnosperms are increasingly grown as ornamentals in India and have been identified as natural hosts for phytoplasmas, including those of 16SrII, has epidemiological impact possibly important for future disease control in the country.

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Figure 1

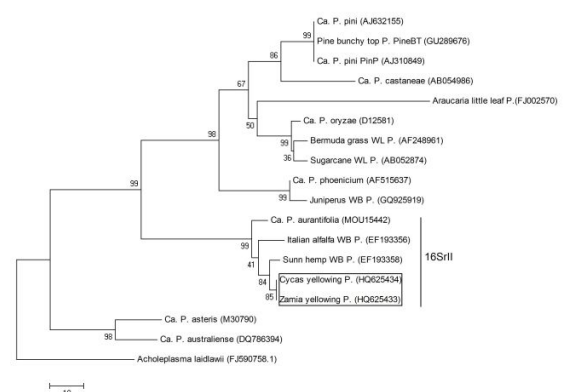


Figure 2

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