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 Cycadaeae), 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 whors 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 Pt/P7 (Deng & Hiruki, 1991) and JU5/JU3 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 laidlawaii* 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 phytoplasms showed 99% sequence identity with those of members of phytoplasma group 16SrIV (‘*Candidatus Phytoplasma aurantifolia*’). The phylogenetic tree supported the sequence comparison analysis since the 16S rDNA sequences of *Cycas* and *Zamia* yellowing phytoplasms (GenBank Accession Nos. HQ625434 and HQ625433, respectively) clustered in the same phylogenetic branch of 16SrIV phytoplasms (Fig. 2). Previous reports indicate that gymnosperms, including those of 16SrI and 16SrII, have been identified as natural hosts for phytoplasmas, including those of 16SrI, and have been identified as natural hosts for phytoplasmas, including those of 16SrII. The fact that yellowing phytoplasmas have been associated with *Araucaria heterophylla* leaf little 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 16SrI, has epidemiological impact possibly important for future disease control in the country.

**References**


