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First report of *Columnea latent viroid* (CLVd)in *Gloxinia* gymnostoma, G. nematanthodes and G. purpurascens in a botanical garden in Denmark

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In 2009, a survey for pospiviroids in ornamental plants from the families Gesneriaceae and Solanaceae, conducted in the glasshouses of the Botanical Garden of Aarhus, identified *Columnea latent viroid* in *Gloxinia gymnostoma, G. nematanthodes* and *G. purpurascens.*

RNA was extracted from 100 mg fresh leaf material using the RNeasy Plant Mini Kit (Qiagen). RT-PCR was carried out using the Vid primers of Verhoeven et al. (2004) and the pCLVd4 and pCLVdR4 primers of Spieker (1996) with the OneStep RT-PCR Kit (Qiagen). An isolate of CLVd (9389007481), kindly provided by J.Th.J. Verhoeven, was used as positive control. A product of the expected size (c. 350 bp) was obtained from all three Gloxinia species and was subsequently sequenced. A BLAST search in the NCBI GenBank confirmed all three samples to be CLVd. However, as the sequences indicated presence of more than one genotype in the three Gloxinia species, the PCR products were cloned using the StratageneTM PCR Cloning Kit. Four clones from each Gloxinia species were sequenced and compared by alignment. A few nucleotide differences were observed in the CLVd sequences within and between hosts. The CLVd sequences have the following GenBank Accession Nos: G. purpurascens, HM043810 to HM0438113; G. gymnostoma, HM043814 to HM043816; and G. nematanthodes, HM043817 to HM043820.

This is the first record of *Gloxinia* species being hosts of CLVd. CLVd has previously been found in ornamentals *Columnea erythrophae* (Hammond *et al.*, 1989), *Nemathanthus wettsteini* (Singh *et al.*, 1992) and *Brunfelsia undulate* (Spieker, 1996), and in tomato (Verhoeven *et al.*, 2004; Steyer *at al.* 2009; Nixon *at al.*, 2009). The three *Gloxinia* species originate from South America, and *G. nematanthodes* and *G. purpurascens* more specifically from Bolivia . The plants came to the Botanical Garden of Aarhus in 1987/88 from a plant collection in USA . The three *Gloxinia* species stood close to each other in the glasshouse of the Botanical Garden of Aarhus. No other pospiviroids were detected in the nine other gesneriaceous and three solanaceous species in the same glasshouse, or in 110 more plants of the two families from neighbouring glasshouses, using the Vid and Posp1 primers of Verhoeven *et al.* (2004). This suggests that the viroid infection originated from the host plants' place of origin.

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