



# A new monopartite begomovirus species, *Chilli leaf curl Vellanad virus*, and associated betasatellites infecting chilli in the Vellanad region of Kerala, India

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*Capsicum annuum* (chilli) is one of the most important commercial crops cultivated in the Indian sub-continent for vegetable, spice and industrial purposes. Leaf curl disease of chilli has become a serious problem in the Vellanad district of Kerala, one of the major chilli growing areas of India. Disease incidence up to 100% was recorded during December 2008 in this region. The infected plants exhibited typical upward leaf curling, crinkling, puckering and reduction of leaf area along with stunting of whole plants (Fig. 1).

Total DNA was isolated from chilli plants and applied as template for PCR amplification of begomovirus-specific fragments using degenerate primer pairs (PAL1v1978/PAR1c496) for detection of *Begomovirus* DNA-A (Rojas *et al.*, 1993) and Beta01/Beta02 for DNA- (Briddon *et al.*, 2002). Specific fragments of 1.5 kb for DNA-A and 1.4 kb for DNA-, respectively, were obtained from samples associated with symptoms. Rolling circle amplification (RCA) using  $\phi$ 29 DNA polymerase was carried out on the total DNA preparation, followed by digestion with *Kpn* I. An amplicon of ~2.8 kb was gel-eluted and cloned into *Kpn* I-linearized pBluescript II KS(+). Restriction digestion of plasmid DNA from the resulting clones indicated the presence of one type of molecule. Betasatellite-specific amplicons of ~1.4 kb were cloned into pTZ57R/T. The corresponding satellite (sat) DNAs could be categorised into two groups based on their restriction pattern. In spite of repeated attempts, we failed to isolate DNA-B from the plants showing appropriate symptoms, indicating that the isolate was a monopartite begomovirus.

Full-length sequencing revealed a viral genome (HM007121) of 2,788 nt with seven predicted ORFs (AV1, AV2, AC1, AC2, AC3, AC4, AC5). Sequence alignment using MegAlign (DNASTAR, Madison, WI) showed the virus to have the highest levels of nucleotide sequence identity (77%) with a "Pakistan" strain of *Pepper leaf curl Bangladesh virus* (AF314531). With other chilli-infecting begomoviruses in the Indian sub-continent, there was between 64 and 76% sequence identity. The present virus is distinctly placed in a separate clade in the phylogenetic tree (Fig. 2). According to the ICTV guidelines (Fauquet *et al.*, 2008), it has to be considered a new begomovirus species for which the name *Chilli leaf curl Vellanad virus* (ChiLCVeV) is proposed. The associated betasatellites (GenBank Accession Nos. JN663876, 1355 nt and JN663877, 1372 nt) each contain a single ORF C1. They share maximum identity of 95.6% and 96.9% with *Radish leaf curl betasatellite* (EF175734) and *Tomato leaf curl Bangladesh betasatellite* (AY438558), respectively. Following ICTV guidelines (Briddon *et al.*, 2008) along with phylogenetic analysis (Fig. 3) and nucleotide sequence identity data, the two molecules are therefore identified as isolates of satDNAs described earlier. Old World begomoviruses have been shown to be widely associated with one or more

DNA betasatellites (Briddon *et al.*, 2003; Chattopadhyay *et al.*, 2008). Together, these findings indicate that a new begomovirus species, ChiLCVeV, along with two distinct betasatellites (*Radish leaf curl betasatellite* and *Tomato leaf curl Bangladesh betasatellite*) are associated with chilli leaf curl disease in Vellanad, Kerala.

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Figure 1

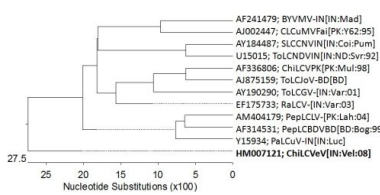


Figure 2

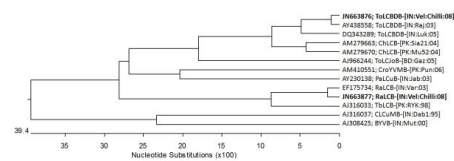


Figure 3

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