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Diversity of *Brevipalpus*-transmitted plant viruses

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Only a handful of *Brevipalpus* (Acari, Tenuipalpidae)-transmitted viruses (BTV) were known until the turn of the 20th century, but since then up to 40 new cases have been reported. Molecular characterization accompanied by biological and morphological data significantly increased our knowledge on BTV. There are basically two types of BTV: (1) cytoplasmic, bacilliform particles inducing a cytoplasmic viroplasm, with a genome of (+) ssRNA either bipartite (*Cilevirus*) or tripartite (*Higrevirus*), represented respectively by *Citrus leprosis C cilevirus* and *Hibiscus green spot 2 higrevirus*; (2) nuclear, rodlike virions causing an intranuclear viroplasm, genome of (-) ssRNA, bipartite (*Dichorhavirus*), with *Orchid fleck dichorhavirus* as the type species.

In the last few years several new putative members were added to the genus *Cilevirus*: *Citrus leprosis virus C2*; *Passion fruit green spot virus*, *Ligustrum leprosis virus* and *Solanum violaeifolium ringspot virus*. Similarly, in addition to the two accepted species of genus *Dichorhavirus*, *Orchid fleck dichorhavirus* and *Coffee ringspot dichorhavirus*, putative members are *Clerodendrum chlorotic spot virus*, *Cestrum ringspot virus*, a recently characterized *Citrus leprosis virus N* and *Citrus chlorotic spot virus* from Brazil, among others. Overall, the new viruses show 50-70% nucleotide sequence identity with their cognates in the two genera. Nuclear type of citrus leprosis from Mexico and Colombia was demonstrated to be caused by citrus isolate of orchid fleck virus (OFV).

So far, except for OFV which has a worldwide distribution, and HGRSV-2 found in Hawaii, all reported cases of BTV occurred in the Americas, but BTV most likely will also be present in other parts of the globe.

NOTES:

Complete genome sequence of Clerodendrum chlorotic spot virus, a putative dichorhavirus

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Clerodendrum chlorotic spot virus (CICSV), which is considered a tentative member of the genus *Dichorhavirus* (family *Rhabdoviridae*, order *Mononegavirales*), causes non-systemic chlorotic and necrotic lesions in the leaves of several plant species of the genus *Clerodendrum*, family *Lamiaceae*. Rod-shaped particles of this virus (ca. 40 nm x 100-110 nm) occur in nuclear electro-lucent inclusions, and adjacent to the membranous systems of both the nuclear envelope and the endoplasmic reticulum of the infected cells. Leaves of a *Clerodendrum* sp. plant showing chlorotic and necrotic symptoms were collected in a residential garden in Piracicaba, State of São Paulo, Brazil, in 2015. Mites from that plant were anatomically identified as *Brevipalpus yothersi*. Presence of putative CICSV particles and the typical cytopathic effects associated to their infection were confirmed by transmission electron microscopy. CICSV RNA was detected by RT-PCR using primers that specifically amplify its *L* gene. Five hundred ng of RNA extracted from the leaf lesions were sequenced by NGS. *In silico* analyses revealed that the viral genome is split into two RNA molecules and, similar to other dichorhaviruses, it harbors six ORFs in the 3'-5' orientation. Five of these ORFs, *N*, *P*, *MP*, *M* and *G* genes are arranged in the RNA1, whereas the *L* gene is in the RNA2. CICSV RNA1 and RNA2 show 52-72% and 60-76% nucleotide sequence identity, respectively, with the cognate molecules of orchid fleck virus, coffee ringspot virus (CoRSV) and citrus leprosis virus N. Best identity scores of each ORF both at nucleotide and amino acid levels were always obtained in comparison with CoRSV. Molecular data confirm the results of previous biological tests that suggested CICSV as a putative dichorhavirus and support the assignation of this virus to a new species of the genus.

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Analysis of the NID domain of the capsid protein of banana streak virus and utilization for diagnostics

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The banana streak virus (BSV) capsid protein contains an N-terminal, intrinsically disordered (NID) domain that is surface-exposed on the virion and likely is multifunctional and plays important roles in viral replication and transmission. The immunodominant continuous epitopes on the virion are also located in the NID domain, and therefore this domain is of great interest from a diagnostics perspective. The BSV capsid protein is cleaved from a larger polyprotein through the action of the virus-encoded aspartic protease but the enzyme substrate sites, and hence the protein boundaries, have not yet been determined. Success has been achieved in expressing the cauliflower mosaic virus (CaMV) aspartic protease in *E. coli* using methods developed for mammalian-infecting retroviruses and enzyme activity is currently being investigated. Once methods are optimized for CaMV, then work will begin on characterizing the BSV AP.

Using chemically synthesized peptides to mimic the continuous epitopes in the BSV NID domain, antisera have been raised in rabbits, and shown to cross-react with the virus in a range of assay formats such as ELISA, immunosorbent electron microscopy and immunocapture PCR. This technology promises to provide a rapid and reproducible way of generating immunodiagnostic reagents for all plant-infecting pararetroviruses.