



Complete Nucleotide Sequence of a Novel Hibiscus-Infecting Cilevirus from Florida and Its Relationship with Closely Associated Cileviruses

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ABSTRACT The complete nucleotide sequence of a recently discovered Florida (FL) isolate of hibiscus-infecting cilevirus (HiCV) was determined by Sanger sequencing. The movement and coat protein gene sequences of the HiCV-FL isolate are more divergent than other genes of the previously sequenced HiCV-HI (Hawaii) isolate.

Hibiscus-infecting cilevirus (HiCV) was first described from Hawaii in 2013 (1). Other than *Hibiscus*, no other plant genus has been identified as a natural or experimental host for HiCV. Based on sequence identity and phylogenetic relationships, HiCV is most closely related to citrus leprosis virus cytoplasmic type 2 (CiLV-C2) (2, 3). Leprosis-like symptoms in citrus were first reported from Florida in 1911 and disappeared in the mid-1960s (4, 5). This previous outbreak was shown to be due to the nuclear rather than the cytoplasmic type of leprosis (6). HiCV was only recently characterized, and no serological or molecular diagnostic tests are available (1). Although *Brevipalpus* spp. are considered potential vectors of the genus *Cilevirus*, transmission of HiCV with *Brevipalpus* spp. has not yet been demonstrated (1, 7).

Hibiscus rosa-sinensis leaves with typical green ringspot symptoms on senescent leaves were collected in Tampa, FL, USA, in August 2016. Total RNA from HiCV symptomatic and asymptomatic hibiscus leaves was isolated using the RNeasy Plant minikit (Qiagen, Valencia, CA, USA). HiCV-HI (Hawaii) and CiLV-C2 sequences were aligned to design specific and generic primers for reverse transcription (RT)-PCR assay. RT-PCR with CiLV-HI-specific primers produced amplicons of HiCV-HI cilevirus using total RNA as the template. Overlapping RT-PCR amplicons were cloned, and the complete viral genomes were assembled using Sanger sequencing (8). In this study, we report the first complete genomic sequence of an HiCV isolate from Florida.

The complete genome of the HiCV-FL isolate is 13,718 nucleotides (nt) in length. The genome is composed of RNA1 and RNA2 of 8,727 and 4,991 nt, respectively, including the poly(A) tails at the 3' termini. RNA1 of HiCV-FL has two open reading frames (ORFs). ORF1 of RNA1 encodes a large polyprotein (p285) with methyl-transferase, protease, helicase, and RNA-dependent RNA polymerase domains that share 95 to 98% amino acid (aa) sequence identity with CiLV-C2 (GenBank accession number JX000024) and HiCV-HI (GenBank accession number KC626783), respectively. ORF2 encodes a putative coat protein gene (p29) that is distinct and shares only 92 to 93% aa sequence identity with HiCV-HI and CiLV-C2. RNA2 is organized into five ORFs that encode four hypothetical proteins and one movement protein (MP) in the order of 5'-p15-p9-p61-MP-p24-3'. ORF2 of RNA2 is 243 nt in length, longer than the lengths observed in the

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HiCV-HI (195 nt) and CiLV-C2 (204 nt) genome sequences. Three other hypothetical proteins of HiCV-FL RNA2, p15, p61, and p24, share 94 to 99% nt and 98 to 100% aa sequence identities with HiCV-HI. Most of the sequence divergence between HiCV-FL RNA2 and HiCV-HI is in the MP region. Interestingly, although HiCV-FL MP shares only 87% nt identity, it has 97% aa sequence identity with CiLV-C2 (GenBank accession number JX000025), followed by 91% nt and 94% aa sequence identities with HiCV-HI (GenBank accession number KC626784). Overall, the ORFs of HiCV-FL RNA1 and RNA2 have 97% and 95% nt sequence identities, respectively, with the HiCV-HI sequences. Further research is needed to determine if HiCV is capable of infecting citrus and producing any symptoms.

Accession number(s). The genome sequences of the HiCV isolate from Florida have been deposited in GenBank under the accession numbers [MG253805](#) (RNA1) and [MG253804](#) (RNA2).

REFERENCES

- Melzer MJ, Simbajon N, Carillo J, Borth WB, Freitas-Astúa J, Kitajima EW, Neupane KR, Hu JS. 2013. A cilevirus infects ornamental hibiscus in Hawaii. *Arch Virol* 158:2421–2424. <https://doi.org/10.1007/s00705-013-1745-0>.
- Roy A, Choudhary N, Guillermo LM, Shao J, Govindarajulu A, Achor D, Wei G, Picton DD, Levy L, Nakhla MK, Hartung JS, Brlansky RH. 2013. A novel virus of the genus *Cilevirus* causing symptoms similar to citrus leprosis. *Phytopathology* 103:488–500. <https://doi.org/10.1094/PHYTO-07-12-0177-R>.
- Roy A, Hartung JS, Schneider WL, Shao J, León MG, Melzer MJ, Otero-Colina G, Beard JJ, Bauchan GR, Ochoa R, Brlansky RH. 2015. Role bending: complex relationships between viruses, hosts and mite vectors related to citrus leprosis, an emerging disease. *Phytopathology* 105:1013–1025. <https://doi.org/10.1094/PHYTO-12-14-0375-FI>.
- Childers CC, Rodrigues JC, Derrick KS, Achor DS, French JV, Welbourn WC, Ochoa R, Kitajima EW. 2003. Citrus leprosis and its status in Florida and Texas: past and present. *Exp Appl Acarol* 30:181–202. <https://doi.org/10.1023/B:APPA.0000006548.01625.72>.
- Fawcett HS. 1911. Scaly bark or nail head rust of citrus. *Fla Agric Exp Stn Bull* 106:1–41.
- Hartung JS, Roy A, Fu S, Shao J, Schneider WL, Brlansky RH. 2015. History and diversity of *Citrus leprosis virus* recorded in herbarium specimens. *Phytopathology* 105:1277–1284. <https://doi.org/10.1094/PHYTO-03-15-0064-R>.
- Rodrigues JCV, Childers CC. 2013. *Brevipalpus* mites (Acari: Tenuipalpidae): vectors of invasive, non-systemic cytoplasmic and nuclear viruses in plants. *Exp Appl Acarol* 59:165–175. <https://doi.org/10.1007/s10493-012-9632-z>.
- Roy A, Brlansky RH. 2010. Genome analysis of an orange stem pitting *citrus tristeza virus* isolate reveals a novel recombinant genotype. *Virus Res* 151:118–130. <https://doi.org/10.1016/j.virusres.2010.03.017>.