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# Citrus Bright Spot Virus: A New Threat to Brazil's Citrus Industry

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**Summary**

*Citrus leprosis* (CL) is the primary viral disease impacting Brazilian citrus cultivation, particularly affecting sweet orange trees (*Citrus sinensis L*. Osbeck) in Southern Brazil. Infected trees exhibited rod-like viral particles (40×100 nm) and electron lucent viroplasm in the nuclei of plant cells. Initial RNA based diagnostics tests for known CL-causing viruses were negative, prompting further investigation through high throughput and Sanger sequencing. Researchers identified the genomes of bi-segmented single stranded negative RNA viruses, characteristic of the genus *Dichorhavirus*. This virus shares 98-99 % nucleotide sequence identity. Three haplotypes of CiBSV are clustered with CLV-N in phylogenetic analysis, named citrus bright spot virus (CiBSV). CiBSV is transmitted by flat mite species *B. azore*s. This study is the first to identify *B. azores* as a viral vector and proposes CiBSV as a new species, *Dichorhavirus australis.*

## Introduction

The genus *Dichorhavirus*, belongs to family *Rhabdoviridae* (order *Mononegavirales*). The plant-infecting viruses transmitted by flat mites. The genome of these viruses is bi-segmented negative-sense, single-stranded RNA (-ssRNA). Approximately (RNA-1 and RNA-2) 13 kb size. RNA-1 (7 kb) contains five ORFs encoding the nucleocapsid protein (N), phosphoprotein (P), movement protein (MP), matrix protein (M), and glycoprotein (G). RNA-2 (6 kb) base only, single ORF that encodes for the RNA-dependent RNA polymerase (RdRp), also known as the L protein.

The International Committee on Taxonomy of Viruses (ICTV) recognizes five species of dichorhavirus, which infect citrus (*Citrus spp*.), coffee (*Coffea spp.*), and ornamentals such

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as glory-bower, orchids, *Cestrum nocturnum, Solanum violifolium*, and *Vinca major*, but could not be detected by RT-PCR with specific primers for known *dichorhaviruses*, suggest a larger, undiscovered diversity within this genus.

*Dichorhaviruses* are transmitted plant-to-plant by certain species of flat mites belonging to genus *Brevipalpus (Acari: Tenuipalpidae*), in persistence propogative manner. For instance, *Orchid fleck virus* transmitted by *Brevipalpus californicus*, infects orchids and citrus, causing citrus leprosis (CL) disease in regions like Mexico, Colombia, South Africa, and Hawaii (USA). In Brazil, while OFV is found in orchids, it does not affect citrus, likely due to the absence of *B. californicus* in citrus orchards. However, other *dichorhaviruses* such as *Citrus leprosis virus* N (CiLV-N, *Dichorhavirus leprosis*) and *Citrus chlorotic spot virus* (CiCSV, *Dichorhavirus citri*), transmitted by *Brevipalpus phoenicis sensu stricto* (s.s.) and *Brevipalpus yothersi/Brevipalpus aff. yothersi* respectively, do cause CL in Brazil. The close relationship between Brevipalpus mites and dichorhaviruses likely results from coevolution, as suggested by phylogenetic data.

Citrus leprosis (CL) is the most significant viral disease affecting citrus production in Brazil. This disease can be caused by at least five different virus species within the genera Dichorhavirus and Cilevirus. Mainly commercial orchards are affected by *Citrus leprosis virus* C or *Cilevirus leprosis*, (genus Cilevirus, family Kitaviridae) in Brazil. Symptoms of CL including premature leaf drop, dieback and finally plant death. The management of virus transmiting mites alone constitute approximately 5 percent (54 million USD) of total management cost of orchard in Brazil annually. During 2020/2021, premature fruit drop due to CL amounted nearly 5.82 million boxes in southeastern Brazil.

In a recent study, researchers analyzed sweet orange (*Citrus sinensis* L. Osbeck) samples showing typical CL symptoms from three small citrus orchards in southern Brazil. They identified the genomic sequences of three new viral isolates, likely belonging to a new species of plant-infecting rhabdovirus within the genus *Dichorhavirus*. Additionally, they presented evidence of viral transmission by a previously unreported mite species (*Brevipalpus azores*) and described an RT-PCR-based detection method for this new dichorhavirus. This

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discovery marks a significant advancement in understanding the diversity and transmission dynamics of dichorhaviruses, with important implications for managing CL in Brazilian citrus orchards.

**Characterization of New Dichorhavirus causing *Citrus Leprosis virus***

Recent studies have identified rod-like particles associated with citrus leprosis (CL) symptoms in sweet orange plants collected from the states of Santa Catarina (SC) and Rio Grande do Sul (RS) in Brazil. These lesions, although brighter, resembled the chlorotic spots typically described in citrus plants affected by CL disease. Notably, necrotic lesions were frequently observed on the fruits but not on the affected leaves.

Transmission electron microscopy (TEM) analyses of ultrathin sections from symptomatic leaves collected in Passo Fundo and Marquês de Souza, RS, and Seara, SC, revealed cytopathic effects indicative of dichorhavirus infection. These effects included electron-lucent viroplasm in the nucleus and the presence of rod-like particles approximately 40×100 nm in size. These particles were found either dispersed or aggregated in the nucleoplasm and formed the characteristic “spoke wheel” arrangement in the cytoplasm of parenchymal cells in the foliar lesions.

Despite these observations, molecular tests for all known dichorhaviruses and cileviruses produced negative results. This suggests the possibility of a new or previously unidentified virus being responsible for the observed symptoms. High throughput and Sanger sequencing of sweet orange RNA extract and data analysis revealed three haplotypes of CiBSV. The bisegmented negative sense ssRNA genome (nearly 13 kb in length) of these virus isolates comprises of RNA-1 (7 kb) and RNA-2 (6 kb). They have nucleotide sequence identity value of more than 97 % ranging between 98-100 % among them. They are believed to be the strain of same virus named as CiBSV. The nucleotide sequence identity value of reference strains CiBSV-Pfdol with other D*ichorhavirus* strain ranging between 47-74 %. The preliminary transmission study of these viruses suggests the Brevipalpus as potential vector in which transmit the virus in propogative manner. (Fig 1)

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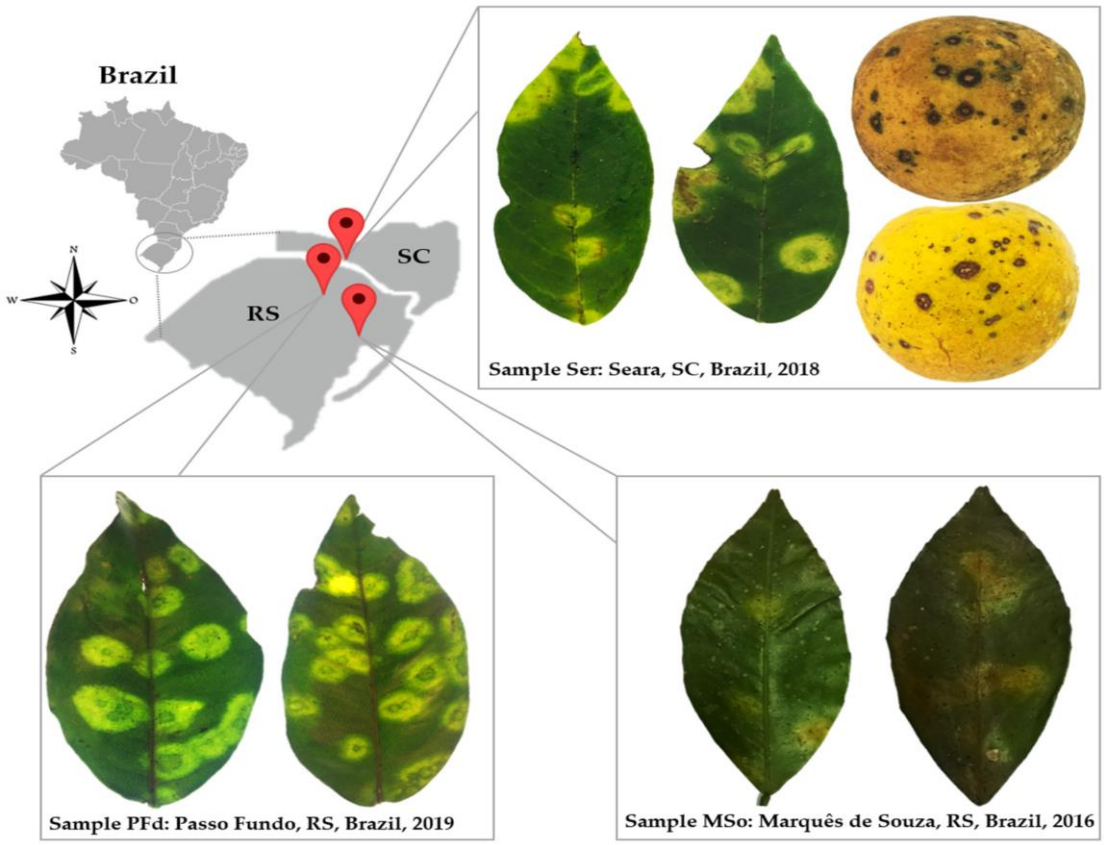


**Fig 1: Chlorotic and necrotic symptoms in leaves and fruits of sweet orange trees (*Citrus sinensis)* collected in three localities of the southern region of Brazil. SC: State of *Santa Catarina*; RS: State of *Rio Grande do Sul*. (Source: Camila Chabi-Jesus et al., 2023)**

## Conclusion and Future Prospect

The identification of rod-like particles associated with citrus leprosis symptoms in sweet orange plants from Santa Catarina and Rio Grande do Sul underscores the complexity of CL disease in Brazil. Despite the resemblance to known CL symptoms and the presence of cytopathic effects typical of dichorhaviruses, molecular tests failed to detect any known viruses, indicating the potential presence of a novel pathogen. This discovery calls for extensive research to isolate and characterize this new virus, which could significantly impact citrus disease management.

Future research should focus on advanced genomic techniques to identify the virus and understand its transmission mechanisms. Developing specific diagnostic tools and exploring integrated pest management strategies will be crucial in mitigating the spread of this potential

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new threat. Collaboration between researchers, citrus growers, and policymakers will be essential to safeguard Brazil's citrus industry from emerging viral diseases.

In India, the citrus industry is a significant contributor to the agricultural sector, with states like Assam, Maharashtra, Andhra Pradesh and Punjab being major producers. The introduction of a novel virus similar to the one affecting citrus in Brazil could have devastating effects on Indian citrus production. Therefore, implementing strict quarantine measures is essential to prevent the entry and spread of this potential pathogen. Control measures thorough inspections of imported citrus plants, enforcement of biosecurity protocols, and awareness campaigns for growers about the importance of disease-free planting material are critical. Additionally, establishing a network for early detection and rapid response to emerging citrus diseases will help protect India's citrus industry from significant economic losses.

**Reference**

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