Universidade de Brasília
Instituto de Ciências Biológicas
Departamento de Fitopatologia
Programa de Pós-Graduação em Biologia Microbiana

Doctoral Thesis

Viral diversity in tree species

FLÁVIA MILENE BARROS NERY

Brasília - DF, 2020

FLÁVIA MILENE BARROS NERY

Viral diversity in tree species

Thesis presented to the University of Brasília as a partial requirement for obtaining the title of Doctor in Microbiology by the Post - Graduate Program in Microbiology.

Advisor

Dra. Rita de Cássia Pereira Carvalho

Co-advisor

Dr. Fernando Lucas Melo

BRASÍLIA, DF - BRAZIL

FICHA CATALOGRÁFICA

NERY, F.M.B

Viral diversity in tree species

Flávia Milene Barros Nery

Brasília, 2025

Pages number: 126

Doctoral Thesis - Programa de Pós-Graduação em Biologia Microbiana, Universidade de Brasília, DF.

I - Virus, tree species, metagenomics, High-throughput sequencing

II - Universidade de Brasília, PPBM/ IB

III - Viral diversity in tree species

A minha mãe Ruth

Ao meu noivo Neil

Dedico

Agradecimentos

A Deus, gratidão por tudo e por ter me dado uma família e amigos que me amam e me apoiam em todas as minhas escolhas.

Minha mãe Ruth e meu noivo Neil por todo o apoio e cuidado durante os momentos mais difíceis que enfrentei durante minha jornada. Aos meus irmãos André, Diego e meu sobrinho Bruno Kawai, gratidão.

Aos meus amigos de longa data Rafaelle, Evanessa, Chênia, Tati, Leo, Suzi, Camilets, Ricardito, Jorgito e Diego, saudade da nossa amizade e dos bons tempos. Amo vocês com todo o meu coração!

Minha orientadora e grande amiga Profa Rita de Cássia Pereira Carvalho, a quem escolhi e fui escolhida para amar e fazer parte da família.

Ao meu co-orientador dr. Fernando Lucas Melo por todos os ensinamentos nesses quatro anos de dedicação.

Minha nova família Reginaldo e Thiago por me receberem de braços abertos.

Aos colegas do mestrado e doutorado Caroline, Karina, Adriana, Josiane, Micheline, Luciane e Juliana, pelos bons tempos.

Minhas “filhas” de laboratório Jordan, Maria Luiza, Letícia e colegas de laboratório Felipe Fochat e Vinícius.

Companhia Urbanizadora da Nova Capital (NOVACAP) e seu funcionário Silomar.

A Estação Experimental Biológica (EEB-UnB) e os funcionários Fábio, Wasington, Deusdete, Francisco e sr. Chico.

A Lu Medeiros, por sempre me ajudar com simpatia e dedicação em ajudar todos os momentos.

A Embrapa Hortaliças, aos pesquisadores Mirtes Lima e Leonardo Boiteux, pelo apoio e incentivo nesses quatro anos, em especial ao pesquisador L. Boiteux pelas sugestões e colaborações valiosas.

A Fundação de Apoio à Pesquisa do Distrito Federal (FAP-DF).

Ao Programa de Pós-Graduação em Biologia Microbiana da Universidade de Brasília (PPG-BioMicro).

A Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela bolsa.

"The success of life is not measured by the path you have conquered, but by the difficulties you have overcome on the path".

Abraham Lincoln

Work carried out in the Department of Plant Pathology of the Institute of Biological Sciences of the University of Brasília (UnB), under the guidance of Dr. Rita de Cássia Pereira Carvalho. Financial Support by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Viral diversity in tree species

FLÁVIA MILENE BARROS NERY

Thesis approved __/__/____by

Dr. Eliane Ferreira Noronha
Department of Cell Biology (UnB)
(Internal Examiner)

Érico de Campos Dianese
Universidade Federal de Goiás (UFG)
(External Examiner)

Raquel Neves Mello
Embrapa Arroz e Feijão
(External Examiner)

Tatsuya Inoue Nagata
Department of Cell Biology (UnB)
(External Examiner - Surrogate)

Dra. Rita de Cássia Pereira Carvalho
Department of Plant Pathology (UnB)
(President)

BRASÍLIA, DF - BRAZIL

2020

Contents

,

List of Figures

CHAPTER 1 - Literature Review

CHAPTER 2 - Molecular characterization of Hovenia dulcis-associated virus (HDaV1) and (HDaV2): new tentative species within the order *Picornavirales*

CHAPTER 3 - Two new *Circoviridae* associated with tree species

List of Tables

CHAPTER 1 - Literature Review

CHAPTER 2 - Molecular characterization of Hovenia dulcis-associated virus (HDaV1) and (HDaV2): new tentative species within the order *Picornavirales*

CHAPTER 3 -Two new *Circoviridae* associated with tree species

Resumo Geral

O Brasil possui uma das maiores coberturas vegetais do mundo, composta por florestas plantadas e naturais distribuídas em todos os biomas. As espécies florestais e arbóreas são afetadas por vários patógenos, incluindo vírus, e representam relevantes reservatórios da diversidade microbiana. No entanto, pesquisas de vírus ocorrendo em árvores são escassas. Desde 2002, avanços na descoberta da diversidade microbiana em diferentes comunidades foram alcançados por sequenciamento de alto rendimento (*High-throughput sequencing* - HTS). Para determinar os vírus que ocorrem em espécies arbóreas cultivadas na NOVACAP (Companhia Urbanizadora da Nova Capital do Brasil), 60 mudas correspondendo a 27 espécies botânicas de espécies de árvores exibindo sintomas típicos de vírus de plantas foram coletadas em 2014. Essas amostras foram processadas para formar três *pools* contendo 20 amostras em cada. Inicialmente essas amostras foram enriquecidas para vírus por semipurificação de partículas virais, a partir das quais foi realizada a extração do RNA e enviada para o sequenciamento *Illumina* (MiSeq). O *pool* de DNA, obtido após o enriquecimento via RCA (*Rolling Circle Amplification*) de amostras individuais, foi enviado para o sequenciamento por *Illumina* Hiseq. Os *reads* foram usados para montar os *contigs* que foram comparados a um banco de dados por BLASTx. Como resultado da análise de RNA, duas novas espécies denominadas Hovenia dulcis-associated virus 1 (HDaV1) e Hovenia dulcis-associated virus 2 (HDaV2) foram detectadas em *Hovenia dulcis* Thunb. As extremidades 3' do genoma de HDaV1 e HDaV2 foram recuperadas por *Rapid Amplification of cDNA Ends* (RACE). As duas espécies virais (HDaV1 e HDaV2) foram classificadas em grupos distintos na ordem *Picornavirales*. Como resultado da análise de DNA, 13 *contigs* foram obtidos com dois deles, representando vírus de genoma circular. Esses vírus apresentaram baixa identidade de nucleotídeos (nt) e aminoácidos (aa) com sequências disponíveis no GenBank e foram então selecionados para uma caracterização mais refinada. Estes novos *contigs* representaram duas potencialmente novas espécies, sendo classificados na família *Circoviridae* [gêneros *Circovirus* (*contig* 11404) e *Cyclovirus* (*contig* 126)]. *Primers* específicos para os *contigs* 126 e 11404 foram usados para detecção viral em amostras individuais. Os *amplicons* das amostras positivas foram clonados em pGEM-T Easy e as sequências dos genomas foram recuperadas por *Sanger*. Seis plantas correspondendo a cinco espécies botânicas *Amburana cearensis* (Allemao) A. C. Sm. (amburana), *Erythrina mulungu* Mart. ex Benth (eritrina), *Eucalyptus urophylla* S. T. Blake (eucalipto vermelho), *Caesalpinia ferrea* Mart. ex Tul. (pau-ferro) e *Samanea tubulosa* (Benth.) Barneby & J. W. Grimes (sete cascas) foram positivas para o *contig* 126 e esta espécie viral foi nomeada Caesalpinia ferrea-associated virus (CFaV). Uma amostra de *Astronium fraxinifolium* foi a única que apresentou amplicon esperado ao usar *primers* para o *contig* 11404. Essa provável nova espécie foi denominada Astronium fraxinifolium-associated virus (AFaV).

Palavras-chave: espécies florestais, metagenômica, sequenciamento de alto rendimento

General Abstract

O Brasil possui uma das maiores coberturas vegetais do mundo, composta por florestas plantadas e naturais distribuídas em todos os biomas. As espécies florestais e arbóreas são afetadas por vários patógenos, incluindo vírus, e representam relevantes reservatórios da diversidade microbiana. No entanto, pesquisas de vírus ocorrendo em árvores são escassas. Desde 2002, avanços na descoberta da diversidade microbiana em diferentes comunidades foram alcançados por sequenciamento de alto rendimento (*High-throughput sequencing* - HTS). Para determinar os vírus que ocorrem em espécies arbóreas cultivadas na NOVACAP (Companhia Urbanizadora da Nova Capital do Brasil), 60 mudas correspondendo a 27 espécies botânicas de espécies de árvores exibindo sintomas típicos de vírus de plantas foram coletadas em 2014. Essas amostras foram processadas para formar três *pools* contendo 20 amostras em cada. Inicialmente essas amostras foram enriquecidas para vírus por semipurificação de partículas virais, a partir das quais foi realizada a extração do RNA e enviada para o sequenciamento *Illumina* (MiSeq). O *pool* de DNA, obtido após o enriquecimento via RCA (*Rolling Circle Amplification*) de amostras individuais, foi enviado para o sequenciamento por *Illumina* Hiseq. Os *reads* foram usados para montar os *contigs* que foram comparados a um banco de dados por BLASTx. Como resultado da análise de RNA, duas novas espécies denominadas Hovenia dulcis-associated virus 1 (HDaV1) e Hovenia dulcis-associated virus 2 (HDaV2) foram detectadas em *Hovenia dulcis* Thunb. As extremidades 3' do genoma de HDaV1 e HDaV2 foram recuperadas por *Rapid Amplification of cDNA Ends* (RACE). As duas espécies virais (HDaV1 e HDaV2) foram classificadas em grupos distintos na ordem *Picornavirales*. Como resultado da análise de DNA, 13 *contigs* foram obtidos com dois deles, representando vírus de genoma circular. Esses vírus apresentaram baixa identidade de nucleotídeos (nt) e aminoácidos (aa) com sequências disponíveis no GenBank e foram então selecionados para uma caracterização mais refinada. Estes novos *contigs* representaram duas potencialmente novas espécies, sendo classificados na família *Circoviridae* [gêneros *Circovirus* (*contig* 11404) e *Cyclovirus* (*contig* 126)]. *Primers* específicos para os *contigs* 126 e 11404 foram usados para detecção viral em amostras individuais. Os *amplicons* das amostras positivas foram clonados em pGEM-T Easy e as sequências dos genomas foram recuperadas por *Sanger*. Seis plantas correspondendo a cinco espécies botânicas *Amburana cearensis* (Allemao) A. C. Sm. (amburana), *Erythrina mulungu* Mart. ex Benth (eritrina), *Eucalyptus urophylla* S. T. Blake (eucalipto vermelho), *Caesalpinia ferrea* Mart. ex Tul. (pau-ferro) e *Samanea tubulosa* (Benth.) Barneby & J. W. Grimes (sete cascas) foram positivas para o *contig* 126 e esta espécie viral foi nomeada Caesalpinia ferrea-associated virus (CFaV). Uma amostra de *Astronium fraxinifolium* foi a única que apresentou amplicon esperado ao usar *primers* para o *contig* 11404. Essa provável nova espécie foi denominada Astronium fraxinifolium-associated virus (AFaV).

Keywords: forest species, metagenomic, High-throughput sequencing

General Introduction

Forests are responsible for covering just over 30% of the entire surface of the planet (KEENAN et al., 2015). According to IBÁ (2017), the forestry sector contributed 132 billion dollars to the global Gross Domestic Product (GDP). Forests are important economically (creating jobs) and environmentally since they provide and preserve crucial natural resources. Brazil is among the countries with the largest forest coverage (≈ 497 million ha) (SFB, 2019). Currently, the estimate is that Brazil has over 488 million ha of native forests and more than 9 million ha of planted areas (SBF, 2019). Most of the planted forests are used for the extraction of cellulose and timber (IBÁ, 2017).

Studies of viral species in tree plants are incipient (FRAILE; GARCÍA-ARENAL, 2016). However, with High-throughput sequencing (HTS) technology combined with viral enrichment methodologies, several viral species have been detected and characterized across a wide array of environments, (REAVY et al., 2015;CASTRIGNANO; NAGASSE-SUGAHARA, 2015), including plants (FONSECA et al., 2018).

Tree species can offer a rich environment for the discovery and relationship of many viruses and their hosts. In addition, the growing human activity has expanded cultivated areas close to natural ecosystems, favoring the spread of viruses from cultivated species to species present in natural systems and vice versa (ROOSSINCK; GARCÍA-ARENAL, 2015). As a consequence, several plant/pathogen interactions have occurred and new viruses have emerged under these environmental conditions (JONES, 2009).

Hypotheses

There is an overall lack of information about viral diversity associated with commercial tree plants as well as in native forest species. It is believed that a great viral diversity and a new species of viruses may be associated with plants grown in Central Brazil. This diversity can be explored using HTS.

General Objective

- The major objective of the present work was to investigate viral diversity associated with commercial tree plants as well as in native forest species maintained in the NOVACAP (Companhia Urbanizadora da Nova Capital do Brasil) - Nursery II (Brasília - DF) using metagenomics.

Specifics Objectives

- Identify viral species associated with tree seedlings at NOVACAP (Companhia Urbanizadora da Nova Capital do Brasil) - Nursery II;
- Characterize viral species using High-throughput sequencing (HTS) and Sanger dideoxy sequencing;
- To carry out phylogenetic analyses of viruses occurring in a subgroup of commercial tree plants as well as in native forest species.

Chapter 1

1. Literature Review

1.1. Forestry importance in Brazil and in the world

Forests across the world occupied a total area of approximately 4 billion hectares (ha)., Russia occupies the first position among the countries with the largest area covered with natural vegetation (≈ 815 million ha), followed by Brazil with more than 497 million ha. Canada occupies the third position with more than 347 million ha (FAO, 2020) (**Figure 1**).

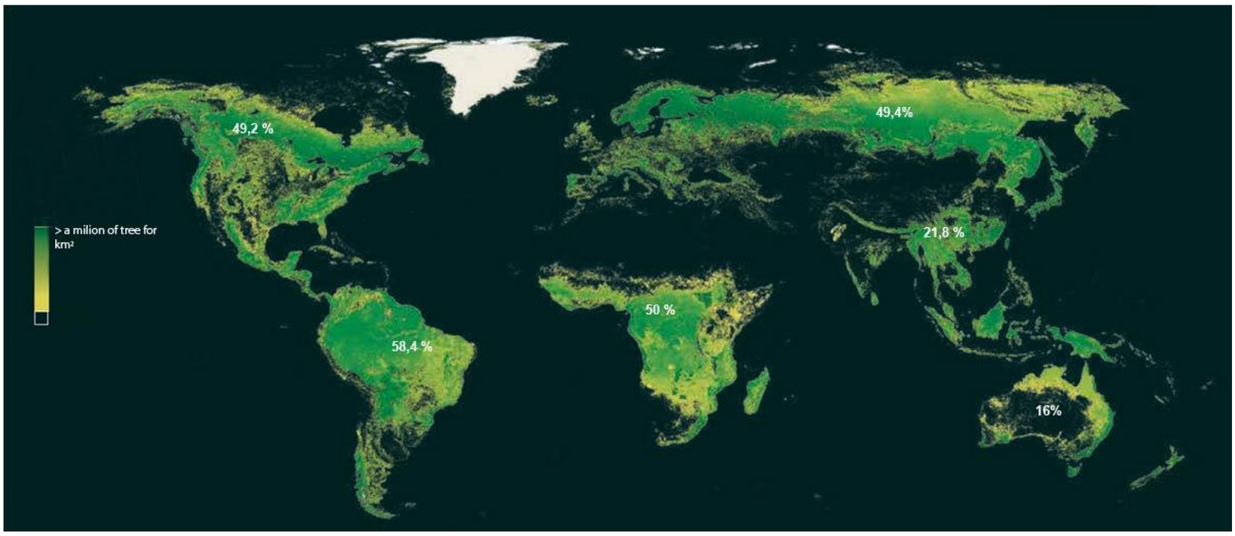


Figure 1. Areas covered with natural vegetation in the world (in green). Source: data.worldbank.org/forests (adapted). Russian Federation, Brazil, Canada, Democratic Republic of the Congo, China, and Australia are the major countries with covered with natural vegetation with 49.4, 58.4, 49.2, 50, 21.8 and 16 %, respectively.

The forestry sector is represented by two segments: **(a)** wood forest products (WFPs), and **(b)** non-wood forest products (NWFPs) composed by vines, oils, seeds, waxes, rubbers, fibers, and oilseeds (SIVIERO et al., 2016).

The most important forest species employed by the wood industry in Brazil are from the genus *Eucalyptus* (occupying 7.1 million ha) and from the genus *Pinus* with 2.0 million ha ("SNIF", 2018). According to Indústria Brasileira de Árvores are also important forest species in Brazil *Acacia mangium* Willd., *Acacia mearnsii* De Wild., rubber tree (*Hevea brasiliensis*), "parica" *(Schizolobium amazonicum* Herb.), teak (*Tectona grandis* L. f.), paraná pine [(*Araucaria angustifolia* (Bertol. Kuntze) and *Populus*. These species are employed for industrial, commercial, and pharmaceutical purposes and they also contributes to the sustainable economic development (IBÁ, 2017).

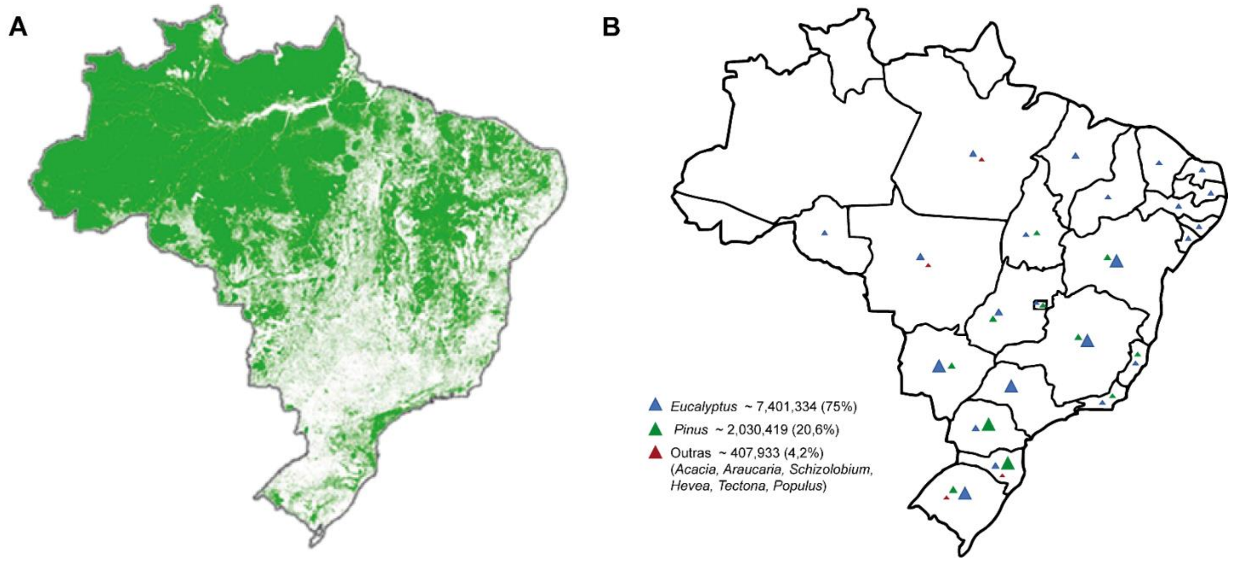


Figure 2. Native and planted forest species in Brazil in hectare (ha). **(A)** Areas covered with natural forests and (**B**) Areas with planted forests. Source: SINIF - Boletim de Notícias (2019).

Among the Brazilian biomes, the Cerrado is considered a global biodiversity hotspot with more than 10,000 plant species. This biome covers an area of approximately two million km^2, equivalent to 21% of the area with native vegetation in the Brazilian territory (DAMASCO et al., 2018).

In this biome, *Eucalyptus* species have been widely used due to their rapid growth. The cultivation of these species is considered a promising economic activity due to their use in the cosmetic, pharmaceutical, and timber industries (SAID et al., 2016).

1.2 Viral diversity in plants

The increasing globalization as well as the expansion of agricultural frontiers (including areas formerly occupied by the Cerrado biome) favor the transit of plant materials and their pathogens, which poses a threat to native species (VINCENT; COUTTS; JONES, 2014). According to Silva (2018), due to agricultural expansion and urban growth, a significant decrease in forest areas was observed.

Tree species can be reservoirs of unknown viruses or even viral species that occur in field and vegetable crops grown in the vicinity of natural ecosystem areas. In this scenario, new hosts can provide the possibility of a wide array of events that generate variability for the viruses (such as mutations and recombination), which may give rise to new species. In fact, a wide array of pathogens has been detected infecting tree species. However, viruses are the most underestimated group of pathogens, especially in trees (ROOSSINCK, 2019).

Viruses are considered the most abundant biological entities on the planet (ROOSSINCK; BAZÁN, 2017), being able to cause disease and economic losses in different cultures worldwide (RYBICKI, 2015). According to ICTV (2020) there are 6.590 species of virus either associated or infecting different organisms, including plants, mammals, insects, bacteria, archaea, fungi, and protist. However, estimates suggest there are over 1 million virus species found in mammals alone. Half of this total poses a threat to human health (CARLSON et al., 2019). Although studies involving viruses focus on diseases in humans, these pathogens can interact with different organisms, , modifying the behavior of their hosts to benefit them such as inducing tolerance to extreme temperatures (MORSY et al., 2010), droughts (MÁRQUEZ et al., 2007), and insect vector (PEÑAFLOR et al., 2016; MAUCK et al., 2018). Likewise, molecular studies suggest that viruses can move across different environments, allowing several tree species to function as alternatives hosts, allowing virus survival and spread (ALABI et al., 2008; ALVES, 2018; ROOSSINCK; GARCÍA-ARENAL, 2015).

In this context, the knowledge of plant viruses is increasing since the last decade (ICTV 2020), with several viruses have been described and characterized via HTS (KREUZE et al., 2009; ADAMS et al., 2012; LI et al., 2012), However, most of these studies are related to viruses of agronomic importance with little knowledge about the diversity in tree species, although there are some reports about viruses that have broken the barrier and moved between agricultural environments to forest environments ((BERNARDO et al, 2018, ROOSINCK, 2014; 2015; VICENT et al. 2014).

1.3. Detection plant viruses

Electron Microscopy, host range, serological and molecular techniques are being used together with Sanger sequencing for the diagnosis of plant viruses. In the last 10 years, new approaches have been used to study diversity of microorganisms, including virus. Different sequencing platforms have been applied to determine the viral diversity. Among the commonly used platforms are the following: **(a)** 454 platform (Roche Genome Sequencer FLX) – this approach is based on pyrosequencing where the DNA is fragmented and adapters are added to the ends, the polymerase enzyme performs the amplification of the fragments. When a nucleotide is incorporated, a pyrophosphate is released, which is then combined with a molecule by the enzyme ATP-sulfurylase, resulting in the production of ATP. This reaction is associated with the emission of light in each well (RONAGHI, 2001), ***(b)*** Sequencing by Oligonucleotide Ligation and Detection *(*SOLiD) (Applied Biosystems) (ANSORGE, 2009), **c)** IonTorrent (Thermo Scientific) consists on the amplification of DNA from the release of H^+ ions powered by semiconductor chips (MERRIMAN; R&D TEAM; ROTHBERG, 2012), **d)** Nanopore (Oxford) – this strategy based on the detection of the DNA sequence from an electrical signal generated by the DNA fragment through a protein pore (DEAMER; AKESON; BRANTON, 2016) and **e)** Illumina Genome Analyzer (GA) – this platform is based on synthesis sequencing and features two sequencing lines: Miseq (produces reads with sizes of 300 bp) and Hiseq (reads with sizes of 150 bp) These platforms have different advantages compared to the first generation platforms, such as Sanger, allowing the sequencing of several genomes and generating an enormous amount of information (CARVALHO; SILVA, 2010; KEHOE et al., 2014).

High-throughput sequencing techniques (known as HTS) are capable of generating millions of reads and even entire genomes in a single run (CARVALHO; SILVA, 2010; VON BUBNOFF, 2008). These sequencing platforms have allowed the study and characterization of many microbiota communities (ROOSSINCK; MARTIN; ROUMAGNAC, 2015). The first work on HTS in tree species was carried out by Loconsole et al. (2012) in this work, five viral species had the complete genome recovered in the different tree genera: *Prunus*, *Citrus*, *Vitis*, *Ficus*, *Corylus*, *Diospyros*, and *Morus*. Currently, high-throughput sequencing allowed the discovery of other new viruses. According to Malathi, Renuka; Devi (2019) more than 120, 000 viral genomes have been already been detected. However, most of the viral sequences obtained by metagenomics do not correspond to previously known viruses, corroborating the information of Mokili et al. (2012) that little is known about the viral diversity of the planet.

Several viral species have been detected and characterized in different environments (REAVY et al., 2015 CASTRIGNANO; NAGASSE-SUGAHARA, 2015), including plants (FONSECA et al., 2018).using HTS in combination with viral RNA and DNA enrichment methodologies such as dsRNA (double-stranded RNA), sRNA (small RNA), siRNA (short interfering RNA), miRNA (microRNA), rRNA (ribosomal RNA), and RCA (Rolling Circle

Amplification) (FONSECA et al., 2018; GAAFAR; ZIEBELL, 2020; INOUE-NAGATA et al., 2004; WANG; GERSTEIN; SNYDER, 2009).

In summary, the use of HTS has allowed major advances in the discovery of novel plant-associated viruses (ADAMS et al., 2009; AL RWAHNIH et al., 2009; KREUZE et al., 2009a). However, for viruses in tree species in according to Roossinck et al. (2010) these studies are mainly concentrated in Europe.

1.4. Virus in forest trees around the world

Nienhaus and Castello (1989) compiled works of viral species in forest trees in Europe (**Table 1**). Among forest trees, some birch species (*Betula pendula* Roth and *B. pubescens* Ehrh. family Betulaceae) are hosts of viral species classified into different families. Viruses occurring in tree species can also be observed in *Sorbus* (*Sorbus aucuparia* L.) This information is listed in **Table 1**.

Table 1. Virus species, classified by family and/or genus, reported in association with tree species in worldwide (updated and adapted from Nienhaus and Castello, 1989; Büttner, 2013).

Tree species	Family or Genus	Virus species or particle types	location	Reference
Abies alba Mill.		icosahedral particles	Germany	(FLACHMANN et al., 1990; FRENZEL, 1988)
Acer spp.	*Nepovirus*	Maple leaf perforation virus	United Kingdom	(BLATTNY, 1965);(ŠUBÍKOVÁ, 1973)
		Arabis mosaic virus	United Kingdom	(THOMAS, 1970)
		Maple mosaic virus	United Kingdom	(SZIRMAI, 1972)
		Peach rosette virus	United States	(KENIKNIGHT, 1960)
	Tobamovirus		Canada	(LANA; THOMAS; PETERSON, 1980)
	Cucumovirus	Cucumber mosaic virus	Turkey	(ERDILLER, 1986)
	Emaravirus	Maple mottle-associated virus	Germany	(RUMBOU et al., 2020a)
Adansonia digitata L.	*Badnavirus*	Cacao swollen shoot virus	United Kingdom	(BRUNT, 1996)
Aesculus spp.	*Secoviridae*	Strawberry latent ringspot virus	Germany	(SCHIMANSKI; SCHMELZER; ALBRECHT, 1976)
	Nepovirus	Cherry leaf roll virus	Germany	(SWEET; CAMPBELL, 1976)
	Trichovirus	Apple chlorotic leaf spot virus	Germany	(HENTSCH; FUCHS; GRÜNTZIG, 1997)
	Ilarvirus	Apple mosaic virus	Slovakia	(POLAK; ZIEGLEROVA, 1997; SWEET; BARBARA, 1979)
Betula spp.	*Alphanecrovirus*	Tobacco necrosis virus	United Kingdom	(COOPER, 1979a)
		Prune dwarf virus	United States	(HAMACHER, 1986)/ Germany;(COOPER, 1979b)
	Nepovirus	Cherry leaf roll virus	Germany Finland United Kingdom; France and Germany	(SCHIMANSKI et al., 1980; SCHMELZER, 1972) (JALKANEN; BÜTTNER; VON BARGEN, 2007) (COOPER; ATKINSON, 1975); (RUMBOU et al., 2019)/
	Ilarvirus	Prunus necrotic ringspot virus	United States	(COOPER; MASSALSKI, 1984)
	Tobravirus	Tobacco rattle virus	United Kingdom	(COOPER; MASSALSKI, 1984)
	Ilarvirus	Apple mosaic virus	United States	(GOTLIEB, 1975; GOTLIEB; BERBEE, 1973)
	Badnavirus		United States	(HARDCASTLE; GOTLIEB, 1980)
		Birch leaf roll-associated virus	Germany	(RUMBOU et al., 2018)
		Particles	Germany	(HAMACHER; GIERSIEPEN, 1989)
	Carlavirus,	Birch carlavirus,	Finland and Germany	(RUMBOU et al., 2020b)
	Idaeovirus	Birch idaeovirus	Finland and Germany	(RUMBOU et al., 2020b)
	Capillovirus	Birch capillovirus	Finland and Germany	(RUMBOU et al., 2020b)
Carpinus spp.	*Ilarvirus*	Apple mosaic virus	Czech Republic	(POLAK; ZIEGLEROVA, 1997)
Castanea sativa Mill.	*Tobamovirus*	Tobacco mosaic virus	Hungary	(HORVÁTHI et al., 1975)

Table 1. Virus species, classified by family and/or genus, reported in association with tree species in worldwide (updated and adapted from Nienhaus and Castello, 1989; Büttner, 2013).

Tree species	Family or Genus	Virus species or particle types	location	Reference
Ceiba pentandra (L.) Gaertn.	*Badnavirus*	Cacao swollen shoot virus	United Kingdom	(BRUNT, 1996)
Cercis sp.		particles	United States	(KIM; MARTIN, 1978)
Cola spp.	*Badnavirus*	Cacao swollen shoot virus	United Kingdom	(BRUNT et al. 1996)
Corynocarpus laevigatus J. R. Forst. & G. Forst.	*Cucumovirus*	Cucumber mosaic virus	New Zealand	(ASHBY, 1977)
Cupressus arizonica Greene	*Nepovirus*	Tobacco ringspot virus	United States	(FULTON, 1969)
Eucalyptus spp.		particles	India	(SASTRY et al., 1971)
		icosahedral particles	Australia	(BRZOSTOWSKI; GRACE, 1974)
Fagus sp.	*Nepovirus*	Tomato blacking virus	United Kingdom	(SCHMELZER, K.; SCHMIDT, E. S.; SCHMIDT, 1966)
	Potex/ Potyvirus		France	(NIENHAUS, 1985)
	Potexvirus	Potato virus X	France	(WINTER; NIENHAUS, 1989)
	Potyvirus	Bean yellow mosaic virus	France	WINTER; NIENHAUS (1989)
	Nepovirus	Cherry leaf roll virus	United Kingdom	(JONES et al., 1990)
	Ilarvirus	Apple mosaic virus	Slovakia	(POLAK; ZIEGLEROVA, 1997)
	Alphanecrovirus	Tobacco necrosis virus	Germany	(BÜTTNER; NIENHAUS, 1989)
Fraxinus spp.	*Nepovirus*	Cherry leaf roll virus	France, Germany	(WINTER; NIENHAUS, 1989), (HAMACHER; QUADT, 1991)/ (GIERSIEPEN, 1993), (NIENHAUS, F.; HAMACHER, 1989)
	Alphanecrovirus	Tobacco necrosis virus	Italy	(CASALICCHIO, 1965)
		particles	Italy	(CIFERRI; CORTE; RUI, 1961)
	Bromovirus	Brome mosaic virus	Germany	(BARNETT, 1971)
	Nepovirus	Arabis mosaic virus	United Kingdoms	(COOPER, 1979c); (COOPER, 1975); (COOPER; SWEET, 1976)
	Tobamovirus	Tobacco mosaic virus	United States	(AMICO et al., 1985); (CASTELLO; AMICO; SHIEL, 1983) (FERRIS; CASTELLO; SINCLAIR, 1987)/ (CASTELLO; AMICO; SHIEL, 1983), (FERRIS; CASTELLO; SINCLAIR, 1989); (LANA; AGRIOS, 1974)
	Nepovirus	Tobacco ringspot virus	United States	(FERRIS; CASTELLO; SINCLAIR, 1987, 1989), (AMICO et al., 1985), (HIBBEN; HAGAR, 1975; HIBBEN; REESE; CASTELLO, 1988)

Table 1. Virus species, classified by family and/or genus, reported in association with tree species in worldwide (updated and adapted from Nienhaus and Castello, 1989; Büttner, 2013).

Tree species	Family or Genus	Virus species or particle types	location	Reference
Fraxinus spp.	*Caulimoviridae* *Partitiviridae*		United States	(BRATSCH et al., 2016)
		White ash mosaic virus	United States	(MACHADO-CABALLERO et al., 2013)
Hevea brasiliensis (Wild. ex A. Juss) Müll. Arg.	*Capillovirus*	Rubber tree virus 1	China	(LI et al., 2019)
Juglans sp.	*Nepovirus*	Cherry leaf roll virus	United Kingdom United States	(COOPER, 1979b); (MASSALSKI; COOPER, 1984)/ (ROWHANI et al., 1985)
	Unassigned genus	Walnut yellow mosaic virus	Italy	(SAVINO et al., 1976)
Lagerstroemia indica L.	*Potyvirus*	Watermelon mosaic virus	China	(WU; CHENG; LI, 2018)
Malus spp.	*Trichovirus*	Apple chlorotic leaf spot virus	Germany	(CADMAN, 1963)/(LISTER; BANCROFT; NADAKAVUKAREN, 1965)
	Nepovirus	Tomato ringspot virus	Iran	(MOINI et al., 2010)
	Nepovirus	Cherry leaf roll virus	New Zealand	(WOO; PEARSON, 2014)/; (WOO; CLOVER; PEARSON, 2012)
Paulownia sp.	*Ilarvirus*	Apple mosaic virus	Germany	(SWEET, 1980)
		Particles	China	(HUNG, et al., 1981))
Picea sp.	*Tobamovirus*	Tomato mosaic virus	United States	(JACOBI; CASTELLO, 1992); (CASTELLO et al., 1995), (BACHAND; CASTELLO, 1998)
Pinus spp.		Particles	Germany	(BIDDLE; TINSLEY, 1968)
	Unassigned genus	Scots pine mosaic virus	Germany	(SCHMELZER, K.; SCHMIDT, E. S.; SCHMIDT, 1966)
	Alphanecrovirus	Tobacco necrosis virus	Germany	(BÜTTNER; NIENHAUS, 1989)
	Caulimoviridae	Pinus nigra virus 1	Madrid	(RASTROJO et al., 2018)
	Cryptovirus		Germany and Hungary	(VELICEASA et al., 2006)
	Orthotospovirus	Tomato spotted wilt virus	Georgia	(MULLIS et al., 2006)
Populus spp.	*Carlavirus*	Poplar mosaic virus	Grã-Bretanha, Italy and Germany	(BIDDLE; TINSLEY, 1971); (BOCCARDO, G.; LUISONI, E.; LOVISOLO, 1973); (LUISONI; BOCCARDO; MILNE, 1976); (SCHMELZER, K.; SCHMIDT, E. S.; SCHMIDT, 1966); (SMITH; CAMPBELL, 2004)
	Rhabdovirus		Czech Republic	(NAVRATIL, 1981)
	Potyvirus		Canada	(MARTIN; BERBEE; OMUEMU, 1982)
	Nepovirus	Tomato black ring virus	United Kingdom	(COOPER; SWEET, 1976)

Table 1. Virus species, classified by family and/or genus, reported in association with tree species in worldwide (Adapted from Nienhaus and Castello, 1989; Büttner, 2013, with updated).

Tree species	Family or Genus	Virus species or particle types	location	Reference
Populus spp.	*Nepovirus*	Tobacco necrosis ringspot virus Strawberry latent ringspot virus	United States Israel	(CASTELLO; BERBEE, 1978)/ (COHEN et al., 1995)
	Nepovirus	Arabis mosaic virus	United Kingdom	(COOPER; SWEET (1976)
	Emaravirus	Cherry leaf roll virus	Germany	(LÖW, 1995)
		Aspen mosaic-associated virus	Germany	(VON BARGEN et al., 2020)
	Trichovirus	Apple chlorotic leaf spot virus	India	(RANA et al., 2008)
	Tobravirus	Tobacco rattle virus	United Kingdom	COOPER; SWEET (1976)
Prunus spp.	*Ilarvirus*	Apple mosaic virus	Australia	(KINOTI et al., 2018)/
	Ilarvirus	Prune dwarf virus	Germany United States	(SCHIMANSKI; SCHMELZER; ALBRECHT, 1976) (NÉMETH et al., 2010) (MEGAHED; MOORE, 1967) (MEGAHED; MOORE, 1969);
	Ilarvirus	Prunus necrotic ringspot virus	Germany	(FULTON, 1970)
	Potyvirus	Plum pox virus	Japan, United State and Germany	(MAEJIMA et al., 2010); (DAMSTEEGT et al., 2007); (ADAMS, 1978)/
	Alphanecrovirus	Tobacco necrosis virus	United States	(CASTELLO; BERBEE, 1978)/
	Nepovirus	Cherry leaf roll virus	Germany	(LÖW, 1995); (SCHIMANSKI, H. H.; FUNK, 1968); (CIFERRI; CORTE; RUI, 1961);
	Tombusvirus	Petunia asteroid mosaic virus	Germany	(GRUENTZIG et al., 1989)
	Trichovirus	Apple chlorotic leaf spot virus	India	(RANA et al., 2008)
	Secoviridae	Strawberry latent ringspot virus	Israel	(COHEN et al., 1995)
	Prunevirus	Apricot vein clearing-associated virus	Australia	(KINOTI et al., 2017)
	Cucumovirus	Cucumber mosaic virus	China	(YU et al., 2020)
Quercus spp.	*Tobamovirus*	Tobacco mosaic virus	Germany	(NIENHAUS; YARWOOD, 1972) (YARWOOD; HECHT-POINAR, 1973) (NIENHAUS, 1975, 1985)
	Alphanecrovirus	Tobacco necrosis virus	France	(BÜTTNER; NIENHAUS, 1989)
	Potexvirus/Potyvirus		France	(NIENHAUS,1985)
		particles particles/rigid particles	United States Germany	(KIM; FULTON, 1973); FRISCHMUCH et al. (1990)
		Icosahedral Particles	France	NIENHAUS (1985)
	Emaravirus		Germany	(BANDTE et al., 2020)
Robinia spp.	*Nepovirus*		Poland and United States	(BANG; CHOI; LEE, 2006); (BORODYNKO et al., 2007); (DELIBASIC et al., 2013);
	Cucumovirus	Peanut stunt virus	Poland	(MINICKA et al., 2020)
Salix spp.	*Alphanecrovirus*	Tobacco necrosis virus	Germany	(KOPP; CASTELLO; ABRAHAMSON, 1999)
	Tobamovirus	Tomato mosaic virus		
	Bromovirus	Brome mosaic virus		

Table 1. Virus species, classified by family and/or genus, reported in association with tree species in worldwide (Adapted from Nienhaus and Castello, 1989; Büttner, 2013, with updated).

Tree species	Family or Genus	Virus species or particle types	location	Reference
Sambucus spp.	*Nepovirus*	Cherry leaf roll virus	Ukraine, Yugoslavian, and United States	(ELLIS; CONVERSE; STACE-SMITH, 1991); (MILIČIĆ et al., 1987)/ (VILLAMOR; EASTWELL, 2016)
	Pelarspovirus	Elderberry latent virus	Ukraine	(JONES, 1972); (ELLIS; CONVERSE; STACE-SMITH, 1991)
	Nepovirus	Tomato black ring virus	Poland	(POSPIESZNY; BORODYNKO; JOŃCZYK, 2004)
	Nepovirus		Canada and United States	(HANSEN; STACE-SMITH, 1971)
	Emaravirus	European mountain ash ringspot-associated virus	Finland	(ARNDT et al., 2009)
	Carlavirus	Elderberry Carlavirus	Netherlands	(VAN LENT; WIT; DIJKSTRA, 1980)
	Aureusvirus	Elderberry aureusvirus 1	Czech Republic	(SAFÁVROVÁ et al., 2018)
	Carlavirus	Blueberry scorch virus	Poland	(KALINOWSKA; PADUCH-CICHAL; CHODORSKA, 2013)
	Bromoviridae	Sambucus virus S	Czech Republic	(ŠAFÁŘOVÁ; CANDRESSE; NAVRÁTIL, 2018)
Senna spp.	*Potyvirus*	Telosma mosaic virus	China	(YAO et al., 2019)
	Potyvirus	Bean yellow mosaic virus	China	(QIU et al., 2019)
	Begomovirus	African cassava mosaic virus	United States	(ALABI; WHANGER; WU, 1985)
	Closteroviridae		Venezuela	(MARYS et al., 2000)
	Begomovirus	Senna leaf curl virus	India	(KUMAR et al., 2016)
Sorbus spp.	*Nepovirus*	Tomato blackring virus	Germany	(MILINKO, I.; SCHMELZER, 1961)
	Secoviridae	Strawberry latent ringspot virus	Germany and New Zealand	(SCHMELZER, 1968),(TANG; WARD; CLOVER, 2013)
	Ilarvirus	Apple mosaic virus	New Zealand	(SCHMELZER, 1977)
	Trichovirus	Apple chlorotic leafspot virus	United Kingdom	(SWEET, 1980)
	Betaflexiviridae	Stem pitting virus	United Kingdom	(SWEET, 1980)
	Emaravirus	European mountain ash ringspot-associated virus	Poland and Germany	(KALLINEN et al., 2009)/ (MIELKE-EHRET et al., 2010; MIELKE et al., 2008; MIELKE; MUEHLBACH, 2007); (DRUCIAREK; LEWANDOWSKI; TZANETAKIS, 2019)/ (VON BARGEN et al., 2019)

Table 1. Virus species, classified by family and/or genus, reported in association with tree species in worldwide (Adapted from Nienhaus and Castello, 1989; Büttner, 2013, with updated).

Tree species	Family or Genus	Viral species or particle type	location	Reference
Sorbus spp.	*Nepovirus*	Cherry leaf roll virus	Germany	(REBENSTORF et al., 2006)
	Emaravirus	Aspen mosaic-associated virus.	Germany	(VON BARGEN et al., 2020)
Sterculia tragacantha Lindl.	*Badnavirus*	Cacao swollen shoot virus	United Kingdom	(BRUNT et al., 1996)
Tecoma stans	*Begomovirus*		India	(MARWAL; SAHU; GAUR, 2013)
Ulmus spp.	*Ilarvirus*	Elm mottle virus	Germany	(JONES, 1974);(SCHMELZER, 1966, 1969); (JURKE et al., 2017)
	Nepovirus	Arabis mosaic virus	United Kingdom	(MCNAMARA, 1980)
	Nepovirus	Cherry leaf roll virus	United States	(FORD et al., 1972); (FULTON, 1970)
	Nepovirus	Tobacco ringspot virus	United States	(SHIEL, P. J.; CASTELLO, 1985)/ United States
	Potyvirus		Germany	(SCHMELZER, 1966)
	Nepovirus	Tomato ringspot virus	United States	(VARNEY; MOORE, 1952); (1965) /
	Carlavirus		Germany	(EISOLD, 2017)
		Particles	Germany	(BANDTE et al., 2004)/
Zanha africana (Radlk.) Exell	*Ipomovirus*	Cassava brown streak virus	Mozambique	(AMISSE et al., 2019)
Ziziphus jujuba Mill.	*Emaravirus*	Jujube yellow mottle-associated virus	China	(YANG et al., 2019)
	Caulimoviridae	Jujube mosaic-associated virus	China	(DU et al., 2017)

1.5. Viruses in forest trees in Brazil

In Brazil, the first study on tree species was carried out in 1979, when Lin et al. (1979) detected an isolate of Cassia mild mosaic virus (family *Betaflexiviridae*, genus *Carlavirus*) in *Cassia sylvestris* Vell. (LIN; KITAJIMA; COSTA, 1980). Tree species classified in the genera *Cassia* and *Senna* are part of the family Fabaceae, one of the largest plants genera, and interestingly in Brazil, most of the studies dealing with viruses in tree species were carried out with samples from this family. The genus *Cassia* presents only two virus reports, one classified within the order *Tymovirales* and the other within the genus *Potyvirus* (NICOLINI et al., 2012) **(Table 2**). The *Senna* genus is already possible to observe five virus reports classified in the following genera: Five virus reports were made with samples from the genus *Senna*, including viruses of the following genera: *Carlavirus*, *Potyvirus*, *Tymovirus*, *Potexvirus*, and *Allexvirus* **(Table 2)** (ALMEIDA et al., 2002a; ALVES, 2018; BESERRA JR. et al., 2011; LIN; KITAJIMA; COSTA, 1980; REZENDE et al., 2016; SEABRA; et al., 2001)**.** Also noteworthy is the species *Hevea brasiliensis* that presents a *Carlavirus* (JUNQUEIRA et al., 1985, 1987) and recently detected a *Tymovirus* named Hevea brasiliensis virus (FONSECA et al., 2018).

Batista (2014) detected 12 viruses classified in the genera *Cucumovirus* (n=1), *Potyvirus* (n=5), *Tobamovirus* (n=1) and Ortho*tospovirus* (n=4) in trees from different environments environments in Central Brazil: **a)** NOVACAP (Companhia Urbanizadora da Nova Capital do Brasil) - Nursery II; **b)** APA/EEB - UnB (Área de Preservação Ambiental da Estação Experimental de Biologia da Universidade de Brasília) and **c)** CRAD - UnB (Centro de Conservação da Natureza e Recuperação em Áreas Degradadas do Bioma Cerrado).

Table 1. Virus species, classified by family and/or genus, reported in tree species in Brazil.

Tree species	Order or Genus	Viral species	Reference
Amburana cearensis (Allemão) A. C. Sm.	*Orthotospovirus*	Groundnut ringspot virus Tomato spotted wilt virus	(BATISTA, 2014)
Anadenanthera colubrina (Vell.) Brenan.	*Begomovirus*	Bean golden mosaic virus	(BATISTA, 2020)
Caesalpinia echinata Lam.	*Orthotospovirus*	Groundnut ringspot virus Tomato chlorotic spot virus Zucchini lethal chlorosis virus	(BATISTA, 2014)
Caesalpinia pluviosa D.C	*Potyvirus*	Papaya ringspot virus, Watermelon mosaic virus Pepper yellow mosaic virus, Zucchini yellow mosaic virus	(BATISTA, 2014)
	Cucumovirus	Cucumber mosaic virus	
	Orthotospovirus	Tomato spotted wilt virus	
Cassia hoffmannseggii Mart. ex Benth.	*Potyvirus*	Cowpea aphid-borne mosaic virus	(NICOLINI et al., 2012)
Cardiopetalum calophyllum (Schltdl.)	*Begomovirus*	-	(ROCHA, 2017)
Enterolobium contortisiliquum (Vell.) Morong	*Orthotospovirus*	Groundnut ringspot virus	(BATISTA, 2014)
Eriotheca pubescens (Mart.) Schott & Endl.	*Tobamovirus*	Pepper mild mottle virus	(BATISTA, 2014)
Hevea brasiliensis (Wild. ex A. Juss) Müll. Arg.	*Carlavirus*	-	(GAMA et al., 1983)
	Potexvirus	-	(JUNQUEIRA et al., 1985, 1987)
		Hevea brasiliensis virus	(FONSECA et al., 2018)
Hymenaea courbaril L.	*Orthotospovirus*	Zucchini lethal chlorosis virus	(BATISTA, 2014)
Miconia albicans (Sw.) Triana	*Potyvirus*	Watermelon mosaic virus	(BATISTA, 2014)
	Orthotospovirus	Tomato spotted wilt virus	
Mimosa caesalpinifolia Benth.	*Begomovirus*		(FARIAS, 2012)
Ouratea duparquetiana (Baill.) Baill.	*Gemykibivirus*	Ouratea duparquetiana-associated gemykibivirus	(BATISTA, 2020)
Senna spp.	*Carlavirus*	Cassia mild mosaic virus	(LIN et al., 1979);
	Potyvirus	-	(BESERRA JR et al., 2012); (ALMEIDA et al., 2002b)
		-	(LIN et al., 1979)
	Tymovirales		(BESERRA JR et al., 2012)
	Carlavirus	Cassia mild mosaic virus	(SEABRA et al., 2001); (LIN et al., 1979)
	Potexvirus	Senna mosaic virus	(REZENDE et al., 2016)
	Allexivirus		(ALVES, 2018)
Tecoma stans (L.) Juss. ex Kunth	*Gemykolovirus*	Tecoma stans-associated gemykolovirus	(BATISTA, 2020)
Trembleya parviflora	*Gemykrogvirus*	Gila monster-associated gemycircularvirus 1	(BATISTA, 2020)
Zeyheria tuberculosa (Vell.) Bureau	*Potyvirus*	Potato virus Y Papaya ringspot virus	(BATISTA, 2014)
	Orthotospovirus	Tomato chlorotic spot virus, Groundnut ringspot virus Tomato spotted wilt virus	
	Cucumovirus	Cucumber mosaic virus	

- Species were not identified.

In this context, viruses have been reported and characterized, causing disease and economic losses in different cultures worldwide or in association corroborating to the diversity of the planet for many years, techniques used for viral detection depended on prior virus knowledge. However, with the advance of new sequencing technologies and metagenomics, unknown viruses to the scientific community have been discovered (PECMAN et al., 2017), and the number of new viruses has increased significantly (ADAMS; FOX, 2016).

In this context, the present work has as main objective to identify the occurrence of viral species in seedlings used in afforestation of Brasília and the surrounding region from the NOVACAP (Companhia Urbanizadora da Nova Capital do Brasil) - Nursery II. The chapters 2 and 3 will be presented in a manuscript format. **Chapter 2:** Molecular characterization of Hovenia dulcis-associated virus 1 (HDaV1) and 2 (HDaV2): new tentative species within the order *Picornavirales*. This article was published in **Viruses** (available: https://www.mdpi.com/1999-4915/12/9/950). The **Chapter 3:** Two new *Circoviridae* associated with tree species (will be submitted to Archives of Virology). A general discussion will be presented.

2. References

ADAMS, A. N. The detection of *Plum pox virus* species by enzyme-linked immunosorbent assay (ELISA). **Annals of Applied Biology**, v. 90, n. 2, p. 215-221, 1978.

ADAMS, I.; FOX, A. Diagnosis of plant viruses using next-generation sequencing and metagenomic analysis. In: **Current Research Topics in Plant Virology**. [s.l.] Springer, 2016. p. 323-335.

ADAMS, I. P. et al. Next-generation sequencing and metagenomic analysis: a universal diagnostic tool in plant virology. **Molecular Plant Pathology**, v. 10, n. 4, p. 537-545, 2009.

AL RWAHNIH, M. et al. Deep sequencing analysis of RNAs from a grapevine showing syrah decline symptoms reveals a multiple virus infection that includes a novel virus. **Virology**, v. 387, n. 2, p. 395-401, 2009.

ALABI, N. S.; WHANGER, P. D.; WU, A. S. H. Interactive effects of organic and inorganic Selenium with cadmium and mercury on spermatozoal oxygen consumption and motility in vitro. **Biology of Reproduction**, v. 33, n. 4, p. 911-919, 1985.

ALABI, O. J. et al. Alternate hosts of *African cassava mosaic virus* and East African cassava mosaic Cameroon virus in Nigeria. **Archives of Virology**, v. 153, n. 9, p. 1743-1747, 2008.

ALMEIDA, Á. M. R. et al. Mosaic in *Senna occidentalis* in southern Brazil induced by a new strain of *soybean mosaic virus*. **Fitopatologia Brasileira**, v. 27, p. 151-156, 2002.

ALVES, T. M. **Caracterização biológica e molecular do agente causal do mosaico em *Senna rizzinii***. [s.l.] UNIVERSIDADE ESTADUAL DO SUDOESTE DA BAHIA, 2018.

AMICO, L. A. et al. Transmission of Tobacco mosaic and Tobacco ringspot viruses from Moraine ash in New York. **Plant Disease**, v. 69, p. 542, 1985.

AMISSE, J. J. G. et al. First report of cassava brown streak viruses on wild plant species in Mozambique. **Physiological and Molecular Plant Pathology**, v. 105, p. 88-95, 2019.

ANSORGE, W. J. Next-generation DNA sequencing techniques. **New Biotechnology**, v. 25, n. 4, p. 195-203, 2009.

ARNDT, N. et al. **Occurrence of EMARAV and CLRV in tree species native to Finland.** Crop plant resistance to biotic and abiotic factors. Proceedings of the 3rd international symposium on plant protection and plant health in Europe/Ed. Feldmann, F., Alford, DV & Furk, C. **Anais...** Finland: 2009.

ASHBY, J. W. Infection of karaka (*Corynocarpus laevigatus* JR & G. Forst.) by *Cucumber mosaic virus*. **New Zealand Journal of Agricultural Research**, v. 20, n. 4, p. 533-534, 1977.

BACHAND, G. D.; CASTELLO, J. D. Seasonal pattern of tomato mosaic *Tobamovirus* infection and concentration in red spruce seedlings. **Applied and Environmental Microbiology**, v. 64, n. 4, p. 1436-1441, 1998.

BANDTE, M. et al. Virus-diseased *Ulmus* laevis in eastern Germany. **Forest Systems**, v. 13, n. 1, p. 65-69, 2004.

BANG, J.-H.; CHOI, J.-K.; LEE, S.-Y. Characterization of *Peanut stunt virus* isolated from black locust tree (*Robinia pseudo-acacia* L.). **The Plant Pathology Journal**, v. 22, n. 2, p. 125-130, 2006.

BARNETT, O. W. Mosaic of blackjack oak. **Plant Disease Reporter**, 1971.

BATISTA, J. G. **Ocorrência natural de espécies virais e avaliação de potenciais hospedeiras experimentais de *Begomovirus, Potyvirus* e *Tospovirus* em plantas arbóreas e berinjela (*Solanum melongena*)**. [s.l.] Universidade de Brasília, 2014.

BATISTA, J. G. **Viroma de plantas em áreas nativas e cultivadas do Distrito Federal**. [s.l.]

Universidade de Brasília, 2020.

BERNARDO, P. et al. Geometagenomics illuminates the impact of agriculture on the distribution and prevalence of plant viruses at the ecosystem scale. **The EMBO Journal**, v. 12, n. 1, p. 173, 2018.

BESERRA JR., J. E. A. et al. Partial genome sequence of a *Potyvirus* and of a virus in the order Tymovirales found in *Senna macranthera* in Brazil. **Tropical Plant Pathology**, v. 36, n. July 2006, p. 116-120, 2011.

BESERRA JR, J. E. A. et al. Partial genome sequence of a *Potyvirus* and of a virus in the order *Tymovirales* found in *Senna macranthera* in Brazil. **Tropical Plant Pathology**, v. 36, n. 2, p. 116-120, 2012.

BIDDLE, P. G.; TINSLEY, T. W. Virus diseases of conifers in Great Britain. **Nature**, v. 219, n. 5161, p. 1387, 1968.

BIDDLE, P. G.; TINSLEY, T. W. *Poplar mosaic virus* in Great Britain. **New Phytologist**, v. 70, n. 1, p. 61-66, 1971.

BLATTNY, C. Further information on Poplar mosaic. Lesn.Cas. **Praha**, v. 11, p. 637-648, 1965.

BOCCARDO, G.; LUISONI, E.; LOVISOLO, D. **Characterization of an Italian isolate of *Poplar mosaic virus*.** In: 2nd International Congress of Plant Pathology. **Anais**… Minnesota, Minneapolis, Minnesota, U.S.A,: 1973.

BORODYNKO, N. et al. Identification of the new strain of *Strawberry latent ringspot virus* isolated from black locust (*Robinia pseudoacacia* L.). **Journal of Phytopathology**, v. 155, n. 11-12, p. 738-742, 2007.

BRATSCH, S. A. et al. **Partial characterization of two new viruses in ash belonging to the families *Partitiviridae* and *Caulimoviridae***. III International Symposium on Woody Ornamentals of the Temperate Zone 1191. **Anais...** 2016.

BRUNT, A. A. et al. Plant Viruses Online: descriptions and Lists from the VIDE database. Version: 20th August 1996.‘URL hm. **Biology**, 1996.

BRZOSTOWSKI, H. W.; GRACE, T. D. C. Viruslike particles isolated from diseased eucalypts. 1974.

BÜTTNER, C.; NIENHAUS, E. Virus contamination of soils in forest ecosystems of the Federal Republic of Germany. **Forest Pathology**, v. 19, n. 1, p. 47-53, 1989.

CADMAN, C. H. Biology of soil-borne viruses. **Annual Review of Phytopathology**, v. 1, n. 1, p. 143-172, 1963.

CARLSON, C. J. et al. Global estimates of mammalian viral diversity accounting for host sharing. **Nature Ecology & Evolution**, v. 3, n. 7, p. 1070-1075, 2019.

CARVALHO, M. C. D. C. G. DE; SILVA, D. C. G. DA. Sequenciamento de DNA de nova geração e suas aplicações na genômica de plantas. **Ciência Rural**, v. 40, p. 735-744, 2010.

CASALICCHIO, G. La punteggiatura cloronecrotica de *Fragsino maggiore*. **Monti Boschi**, v. 16, p. 39-46, 1965.

CASTELLO, J. D. et al. *Tomato mosaic virus* infection of red spruce on whiteface mountain, New York: prevalence and potential impact. **Canadian Journal of Forest Research**, v. 25, n. 8, p. 1340-1345, 1995.

CASTELLO, J. D.; AMICO, L. A.; SHIEL, P. **Seasonal distribution of *Tobacco mosaic-virus* in moraine ash**. Phytopathology. **Anais**...1983.

CASTELLO, J. D.; BERBEE, J. G. The detection and elimination of viruses from hybrid poplar clones. **Phytopathology News**, v. 12, p. 186, 1978.

CASTRIGNANO, S. B.; NAGASSE-SUGAHARA, T. K. The metagenomic approach and causality in virology. **Revista de Saúde Pública**, v. 49, p. 1-5, 2015.

CIFERRI, R.; CORTE, A.; RUI, D. Two viruses of *Fraxinus*: necrotic leaf curl and leaf marbling.

Rivista di Patologia Vegetale, v. 3, p. 241-250, 1961.

COHEN, J. et al. Lisianthus leaf curl-a new disease of lisianthus caused by *Tomato yellow leaf curl virus*. **Plant Disease**, v. 79, n. 4, p. 416-420, 1995.

COOPER, J. I. *Arabis mosaic vims*, a cause of chlorotic symptoms in Leaves of *Fraxinus excelsior* L. **Plant Pathology**, v. 24, n. 2, p. 114-116, 1975.

COOPER, J. I. Virus diseases of trees and shrubs. Institute of Terrestrial Ecology. **Unit of Invertebrate Virology. Oxford UK**, 1979a.

COOPER, J. I. **The prevalence of cherry leaf roll virus in *Juglans regia* in the United Kingdom.** XI International Symposium on Fruit Tree Virus Diseases 94. **Anais**… 1979b.

COOPER, J. I. Virus diseases of trees and shrubs. Institute of terrestrial ecology. **Cambrian News Aberystwyth Cambridge**, 1979c.

COOPER, J. I.; ATKINSON, M. A. *Cherry leaf roll virus* causing a disease of *Betula* spp. in the United Kingdom. **Forestry: An International Journal of Forest Research**, v. 48, n. 2, p. 193-203, 1975.

COOPER, J. I.; MASSALSKI, P. R. Viruses and virus-like diseases affecting *Betula* spp. **Proceedings of the Royal Society of Edinburgh, Section B: Biological Sciences**, v. 85, n. 1-2, p. 183-195, 1984.

COOPER, J. I.; SWEET, J. B. The detection of viruses with nematode vectors in six woody hosts. **Forestry: An International Journal of Forest Research**, v. 49, n. 1, p. 73-78, 1976.

DAMASCO, G. et al. The Cerrado biome: a forgotten biodiversity hotspot. **Frontiers for Young Minds**, v. 6, n. June, p. 1-9, 2018.

DAMSTEEGT, V. D. et al. *Prunus* host range of *Plum pox virus* (PPV) in the United States by aphid and graft inoculation. **Plant Disease**, v. 91, n. 1, p. 18-23, 2007.

DEAMER, D.; AKESON, M.; BRANTON, D. Three decades of nanopore sequencing. **Nature Biotechnology**, v. 34, n. 5, p. 518-524, 2016.

DELIBASIC, G. et al. First report of the natural infection of *Robinia pseudoacacia* with *Alfalfa mosaic virus*. **Plant Disease**, v. 97, n. 6, p. 851, 2013.

DRUCIAREK, T.; LEWANDOWSKI, M.; TZANETAKIS, I. E. First Report of *European mountain ash ringspot-associated Emaravirus* in *Sorbus aucuparia* in Poland. **Plant Disease**, v. 103, n. 1, p. 166, 2019.

DU, K. et al. Full genome sequence of Jujube mosaic-associated virus, a new member of the family *Caulimoviridae*. **Archives of Virology**, v. 162, n. 10, p. 3221-3224, 2017.

EISOLD, A.-M. E. Molecular characterization of a new *Carlavirus* detected in German *Ulmus laevis* (Pall.) by Illumina high throughput sequencing. **Communications in Agricultural and Applied Biological Sciences**, v. 82, p. 337-346, 2017.

ELLIS, P. J.; CONVERSE, R. H.; STACE-SMITH, R. **Viruses of *Sambucus canadensis* in North America**. XV International Symposium on Small Fruit Virus Diseases 308. **Anais…** 1991.

ERDILLER, G. Acer virus diseases in Turkey. **Journal of Turkish Phytopathology**, v. 15, n. 2, p. 46-59, 1986.

FAO. Food and Agriculture Organization. In: FAO (Ed.). **Global Forest Resources Assessment 2020**. Rome: FAO, 2020. p. 23.

FARIAS, P. C. **Avaliação da resposta de espécies florestais do Cerrado a espécies virais dos gêneros *Potyvirus* e *Tospovirus* e estudos de caracterização de vírus em *Mimosa caesalpiniifolia* . Avaliação da resposta de espécies florestais do Cerrado**. [s.l.] Universidade de Brasília, 2013.

FERRIS, M. A.; CASTELLO, J. D.; SINCLAIR, W. A. **Symptom development on white ash and green ash seedlings inoculated with viruses and a mycoplasma-like organism. phytopathology.** Phytopathology. **Anais...** 1987.

FERRIS, M. A.; CASTELLO, J. D.; SINCLAIR, W. A. Effects of virus and mycoplasmalike organism infection on green and white ash. **Phytopathology**, v. 79, n. 5, p. 579-583, 1989.

FLACHMANN, M. et al. Isometric virus-like particles in *Abies alba* Mill. and other *Abies* species: partial purification and improved detection by means of immunoelectron microscopy. **Journal of Phytopathology**, v. 129, n. 3, p. 193-202, 1990.

FONSECA, P. L. C. et al. Virome analyses of *Hevea brasiliensis* using small RNA deep sequencing and PCR techniques reveal the presence of a potential new virus. **Virology Journal**, v. 15, n. 1, p. 184, 2018.

FORD, R. E. et al. Discovery and characterization of *Elm mosaic virus* in Iowa. **Phytopathology**, v. 62, n. 9, p. 987-992, 1972.

FRAILE, A.; GARCÍA-ARENAL, F. Environment and evolution modulate plant virus pathogenesis. **Current Opinion in Virology**, v. 17, p. 50-56, 2016.

FRENZEL, B. Studies on the hormone balance of coniferous trees in the North black forest being healthy, diseased by air pollution or effected by sulphur. 1988.

FULTON, J. P. Transmission of *Tobacco ringspot virus* to roots of a conifer by a Nematode. **Phytopathology**, v. 59, n. 2, p. 236, 1969.

FULTON, R. W. P*runus necrotic ringspot virus*. CMI/AAB Descript. **Plant Viruses No. 5**, 1970.

GAAFAR, Y. Z. A.; ZIEBELL, H. Comparative study on three viral enrichment approaches based on RNA extraction for plant virus/viroid detection using high-throughput sequencing. **PloS One**, v. 15, n. 8, p. e0237951, 2020.

GAMA, M. et al. Um *Carlavirus* em seringueira (*Hevea brasiliensis*). **Fitopatologia Brasileira**, v. 8, p. 621, 1983.

GIERSIEPEN, R. Untersuchungen zur charakterisierung des kirschenblattrollvirus (CLRV) als Ursache einer Eschenvirose. 1993.

GOTLIEB, A. R. ***Apple mosaic virus* infecting yellow birch in Vermont**. Proc. American Phytopathological Society **Anais**...1975.

GOTLIEB, A. R.; BERBEE, J. G. Line pattern of birch caused by *Apple mosaic virus.* **Phytopathology**, v. 63, n. 12, p. 1470-1477, 1973.

GRUENTZIG, M. et al. On the causal agent of detrimental canker of sweet and mazzard cherry in the German Democratic Republic. **Archiv fuer Phytopathologie und Pflanzenschutz (German DR)**, 1989.

HAMACHER, J. Viruskontamination von forstgehOlzen unter besonderer Beriicksichtigung der Birke. **Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft**, 1986.

HAMACHER, J.; GIERSIEPEN, R. Histologisch-cytologische veranderungen in kirschenblattrollvirus-infizierten und durch luftschadstoffe gestressten *Betula-arten*. **Nachrichtenblatt dt. Pflanzenschutzdienstes**, v. 41, n. 8-9, p. 124-130, 1989.

HAMACHER, J.; QUADT, A. Light-and electron microscopic studies of *Cherry leaf roll virus* (CLRV) on European Ash (*Fraxinus excelsior* L.). **Journal of Phytopathology**, v. 131, n. 3, p. 215-226, 1991.

HANSEN, A. J.; STACE-SMITH, R. Properties of a virus isolated from golden elderberry, *Sambucus nigra* aurea. **Phytopathology**, v. 61, n. 10, p. 1222-1229, 1971.

HARDCASTLE, T.; GOTLIEB, A. R. An enzyme-linked immunosorbent assay for the detection of *Apple mosaic virus* in yellow birch. **Canadian Journal of Forest Research**, v. 10, n. 3, p. 278-283, 1980.

HENTSCH, T.; FUCHS, E.; GRÜNTZIG, M. Das Strawberry latent ringspot (?) *Nepovirus* (SLRSV) an *Aesculus hippocastanum* L. **Archives of Phytopathology & Plant Protection**, v. 31, n. 1, p. 13-23, 1997.

HIBBEN, C. R.; HAGAR, S. S. Pathogenicity of an ash isolate of *Tobacco ringspot virus*. **Plant**

Disease Reporter, 1975.

HIBBEN, C. R.; REESE, J. A.; CASTELLO, J. D. **Identification of *Tomato ringspot virus* in ash in New-York.Plant Disease** Amer Phytopathological SOC 3340 PILOT KNOB ROAD, ST PAUL, MN 55121, 1988.

HORVÁTHI, J. et al. Demonstration of virus-like particles in sweet chestnut and oak with leaf deformations in Hungary/Nachweis virusähnlicher Partikeln in Edelkastanien und Eichen mit Sichelblättrigkeit-Symptomen in Ungarn. **Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and Protection**, p. 498-502, 1975.

HUNG, J. F. et al. Observation o f rodshaped virus particles in the trees infected with Paulownia witches’ broom. **Nature Magazine**, v. 4, p. 79-80, 1981.

IBÁ. **Indústria Brasileira de Árvores**, 2017.

INOUE-NAGATA, A. K. et al. A simple method for cloning the complete *Begomovirus* genome using the bacteriophage phi29 DNA Polymerase. **Journal of Virological Methods**, v. 116, n. 2, p. 209-211, 2004.

JACOBI, V.; CASTELLO, J. D. Infection of red spruce, black spruce, and balsam fir seedlings with *Tomato mosaic virus*. **Canadian Journal of Forest Research**, v. 22, n. 7, p. 919-924, 1992.

JALKANEN, R.; BÜTTNER, C.; VON BARGEN, S. *Cherry leaf roll virus* abundant on *Betula pubescens* in Finland. **Silva Fennica**, v. 41, n. 4, p. 755, 2007.

JONES, A. T. Purification and properties of *Elderberry latent virus*, one of four sap-transmissible viruses obtained from American elder (*Sambucus canadensis* L.). **Annals of Applied Biology**, v. 70, n. 1, p. 49-58, 1972.

JONES, A. T. ***Elm mottle virus*. Commonwealth mycol**. 1974.

JONES, A. T. et al. Serological comparison of isolates of *Cherry leaf roll virus* from diseased beech and birch trees in a forest decline area in Germany with other isolates of the virus. **Journal of Phytopathology**, v. 129, n. 4, p. 339-344, 1990.

JONES, R. A. C. Plant virus emergence and evolution: origins, new encounter scenarios, factors driving emergence, effects of changing world conditions, and prospects for control. **Virus Research**, v. 141, n. 2, p. 113-130, 2009.

JUNQUEIRA, N. T. V. et al. **New diseases caused by virus, fungi and also a bacterium on rubber from Brazil and their impact on international quarantine.** Embrapa Amazônia Ocidental-Artigo em anais de congresso (ALICE). **Anais**...1985.

JUNQUEIRA, N. T. V. et al. **Virose da seringueira: uma nova enfermidade**. [s.l.] EMBRAPA-CNPSD, 1987.

JURKE, I. et al. Detection of *Elm mottle virus* (EMoV) and novel putative *Carlavirus* in the genus *Ulmus* in northern Germany. **Cearing**, v. 25, n. 4, p. 15, 2017.

KALINOWSKA, E.; PADUCH-CICHAL, E.; CHODORSKA, M. First report of Blueberry scorch virus in elderberry in Poland. **Plant Disease**, v. 97, n. 11, p. 1515, 2013.

KALLINEN, A. K. et al. Detection, distribution, and genetic variability of *European mountain ash ringspot-associated virus*. **Phytopathology**, v. 99, n. 4, p. 344-352, 2009.

KEENAN, R. J. et al. Dynamics of global forest area: Results from the FAO Global Forest Resources Assessment 2015. **Forest Ecology and Management**, v. 352, p. 9-20, 2015.

KEHOE, M. A. et al. Plant virology and next generation sequencing: experiences with a *Potyvirus*. **PLoS One**, v. 9, n. 8, p. e104580, 2014.

KENIKNKNIGHT, G. Prunus injucunda small and *Acer rubrum* L., natural hosts of *Peach rosette virus*. **Plant Disease Reporter**, v. 44, n. 3, 1960.

KIM, K. S.; FULTON, J. P. Association of virus-like particles with a ringspot disease of oak. **Plant Disease Reporter**, 1973.

KIM, K. S.; MARTIN, E. M. Virus-like particles associated with yellow ringspot of redbud.

Phytopathol. News, v. 12, p. 119, 1978.

KINOTI, W. M. et al. First Report of *Apricot vein clearing-associated virus* (AVCaV) in Australia and in a new host, *Prunus cerasifera*. **Plant Disease**, v. 101, n. 7, p. 1337, 2017.

KINOTI, W. M. et al. The Incidence and genetic diversity of *Apple mosaic virus* (ApMV) and *Prune dwarf virus* (PDV) in *Prunus* species in Australia. **Viruses**, v. 10, n. 3, p. 136, 2018.

KOPP, R. F.; CASTELLO, J. D.; ABRAHAMSON, L. P. Viruses in *Salix* grown for bioenergy. **Forest Pathology**, v. 29, n. 2, p. 117-122, 1999.

KREUZE, J. F. et al. Complete viral genome sequence and discovery of novel viruses by deep sequencing of small RNAs: A generic method for diagnosis, discovery and sequencing of viruses. **Virology**, v. 388, n. 1, p. 1-7, 2009.

KUMAR, J. et al. Senna leaf curl virus: a novel *Begomovirus* identified in *Senna occidentalis*. **Archives of Virology**, v. 161, n. 9, p. 2609-2612, 2016.

LANA, A. F.; THOMAS, O. T.; PETERSON, J. F. A virus isolated from sugar maple. **Journal of Phytopathology**, v. 97, n. 3, p. 214-218, 1980.

LANA, A. O.; AGRIOS, G. N. Properties of a strain of *Tobacco mosaic virus* isolated from white ash trees [*Fraxinus americana,* host range]. **Phytopathology (USA)**, 1974.

LI, Z. et al. Complete genome sequence of a novel *Capillovirus* infecting *Hevea brasiliensis* in China. **Archives of Virology**, p. 1-4, 2019.

LIN, M. T. et al. Properties of a possible *Carlavirus* isolated from a cerrado native plant, *Cassia sylvestris*. **Plant Disease**, v. 63, p. 501-505, 1979.

LIN, M. T.; KITAJIMA, E. W.; COSTA, C. L. Association of *Cassia mild mosaic virus* with dieback of *Cassia macranthera* in central Brazil. **Plant Disease**, v. 64, n. 6, p. 587-589, 1980.

LISTER, R. M.; BANCROFT, J. B.; NADAKAVUKAREN, M. J. Some sap-transmissible viruses from apple. **Phytopathology**, v. 55, n. 8, p. 859, 1965.

LOCONSOLE, G. et al. Next generation sequencing and metagenomic analysis advances plant virus diagnosis and discovery. **Journal of Plant Pathology**, v. 94, p. 48-49, 2012.

LÖW, A. **Untersuchungen zum ganzjährigen serologischen und immuncytologischen Nachweis von *Cherry leaf roll virus* (CLRV) und *Prune dwarf virus* (PDV) in Wildkirschen (*Prunus avium*)**. [s.l.], 1995.

LUISONI, E.; BOCCARDO, G.; MILNE, R. G. Purification and some properties of an Italian isolate of *Poplar mosaic virus*. **Journal of Phytopathology**, v. 85, n. 1, p. 65-73, 1976.

MACHADO-CABALLERO, J. E. et al. Identification, transmission, and partial characterization of a previously undescribed Flexivirus causing a mosaic disease of ash (*Fraxinus* spp.) in the USA. **Plant Health Progress, Plant Management Network**, 2013.

MAEJIMA, K. et al. First report of *Plum pox virus* in *Prunus* sinfecting Japanese apricot (*Prunus mume* Sieb. et Zucc.) in Japan. **Journal of General Plant Pathology**, v. 76, n. 3, p. 229-231, 2010.

MALATHI, V. G.; RENUKA DEVI, P. ssDNA viruses: Key players in global virome. **Virus Disease**, v. 30, n. 1, p. 3-12, 2019.

MÁRQUEZ, L. M. et al. A virus in a fungus in a plant: three-way symbiosis required for thermal tolerance. **Science**, v. 315, n. 5811, p. 513-515, 2007.

MARTIN, R. R.; BERBEE, J. G.; OMUEMU, J. O. Isolation of a *Potyvirus* from declining clones of Populus. **Phytopathology**, v. 72, n. 9, p. 1158-1162, 1982.

MARWAL, A.; SAHU, A. K.; GAUR, R. K. First report of molecular characterization of *Begomovirus* infecting an ornamental plant *Tecoma stans* and a medicinal plant *Justicia adhatoda*. **Science International**, v. 25, n. 4, 2013.

MARYS, E. E. et al. Partial characterisation of a new virus in brusca from the Venezuelan lowlands. **Annals of Applied Biology**, v. 136, n. 2, p. 125-130, 2000.

MASSALSKI, P. R.; COOPER, J. I. The location of virus-like particles in the male gametophyte of birch, walnut and cherry naturally infected with *Cherry leaf roll virus* and its relevance to vertical transmission of the virus. **Plant Pathology**, v. 33, n. 2, p. 255-262, 1984.

MCNAMARA, D. G. The spread of *Arabis mosaic virus* through non-cultivated vegetation. **Plant Pathology**, v. 29, n. 4, p. 173-176, 1980.

MEGAHED, E.-S.; MOORE, J. D. Differential mechanical transmission of *Prunus* viruses from seed of various *Prunus* spp. and from different parts of the same seed. **Phytopathology**, v. 57, p. 821-822, 1967.

MEGAHED, E.-S.; MOORE, J. D. Inactivation of necrotic ringspot and prune dwarf viruses in seeds of some *Prunus* spp. **Phytopathology**, 1969.

MERRIMAN, B.; R&D TEAM, I. T.; ROTHBERG, J. M. Progress in ion torrent semiconductor chip based sequencing. **Electrophoresis**, v. 33, n. 23, p. 3397-3417, 2012.

MIELKE-EHRET, N. et al. Detection of *European mountain ash ringspot-associated virus*-specific RNA and protein P3 in the pear leaf blister mite *Phytoptus pyri* (Eriophyidae). **Archives of Virology**, v. 155, n. 6, p. 987-991, 2010.

MIELKE, N. et al. Detection of *European mountain ash ringspot-associated virus* (EMARAV) in *Sorbus aucuparia* L. by a specific antiserum and reverse transcription-PCR. **Forest Pathology**, v. 38, n. 6, p. 371-380, 2008.

MIELKE, N.; MUEHLBACH, H.-P. A novel, multipartite, negative-strand RNA virus is associated with the ringspot disease of European mountain ash (*Sorbus aucuparia* L.). **Journal of General Virology**, v. 88, n. 4, p. 1337-1346, 2007.

MILIČIĆ, D. et al. *Cherry leafroll virus* and elderberry *Carlavirus* on *Sambucus nigra* L. in South-East Europe. **Acta Botanica Croatica**, v. 46, n. 1, p. 1-8, 1987.

MILINKO, I.; SCHMELZER, K. Zur kenntnis der mosaikkrankheit der *Robinia* (*Robinia pseudo-acacia*). **Phytopathology**, v. 4, p. 36-41, 1961.

MINICKA, J. et al. High-throughput sequencing facilitates discovery of new plant viruses in Poland. **Plants**, v. 9, n. 7, p. 820, 2020.

MOINI, A. A. et al. Widespread occurrence of *Tomato ring spot virus* in deciduous fruit trees in Iran. **Julius-Kühn-Archiv**, n. 427, p. 127, 2010.

MOKILI, J. L.; ROHWER, F.; DUTILH, B. E. Metagenomics and future perspectives in virus discovery. **Current Opinion in Virology**, v. 2, n. 1, p. 63-77, 2012.

MORSY, M. R. et al. Teasing apart a three-way symbiosis: transcriptome analyses of *Curvularia protuberata* in response to viral infection and heat stress. **Biochemical and Biophysical Research Communications**, v. 401, n. 2, p. 225-230, 2010.

MULLIS, S. W. et al. First report of pinaceae in Georgia naturally infected with *Tomato spotted wilt virus*. **Plant Disease**, v. 90, n. 3, p. 376, 2006.

NAVRATIL, S. **A *Rhabdovirus* associated with vein yellowing and vein necrosis of balsam *poplar***. Phytopathology. **Anais**...1981

NÉMETH, M. et al. Surveying viruses on ornamental trees and shrubs in two Hungarian botanical gardens and an arboretum. **Julius-Kühn-Archiv**, n. 427, p. 293, 2010.

NICOLINI, C. et al. A distinct *Tymovirus* infecting *Cassia hoffmannseggii* in Brazil. **Virus Genes**, v. 45, n. 1, p. 190-194, 2012.

NIENHAUS, F.; HAMACHER, J. Virosen in Eschen.Allg. **Forstzeitg**, v. 16, p. 385-386, 1989.

NIENHAUS, F. Viren und virusverdächtige erkrankungen in eichen (*Quercus robur* und *Quercus sessiliflora*)/Viruses and virus-like diseases in oaks (*Quercus robur* and *Quercus sessiliflora)*. **Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and Protection**, p. 739-749, 1975.

NIENHAUS, F. Infectious diseases in forest trees caused by viruses, mycoplasma-like organisms and primitive bacteria. **Experientia**, v. 41, n. 5, p. 597-603, 1985.

NIENHAUS, F.; CASTELLO, J. D. Viruses in forest trees. **Annual Review of Phytopathology**, v. 27, n. 1, p. 165-186, 1989.

NIENHAUS, F.; YARWOOD, C. E. Transmission of virus from oak leaves fractionated with s. **Phytopathology**, v. 62, p. 313-315, 1972.

PECMAN, A. et al. Next generation sequencing for detection and discovery of plant viruses and viroids : Comparison of two approaches. v. 8, n. October, p. 1-10, 2017.

PEÑAFLOR, M. F. G. V. et al. Effects of single and mixed infections of *Bean pod mottle virus* and *Soybean mosaic virus* on host-plant chemistry and host-vector interactions. **Functional Ecology**, v. 30, n. 10, p. 1648-1659, 2016.

POLAK, Z.; ZIEGLEROVA, J. **Spontaneous occurrence of Apple mosaic virus in some forest and ornamental woody species.** Proc., XIVth Slovak and Czech Plant Protection Conference, Nitra. **Anais**...1997.

POSPIESZNY, H.; BORODYNKO, N.; JOŃCZYK, M. First report of *Tomato black ring virus* (TBRV) in the natural infection of *Sambucus nigra* in Poland. **Journal of Plant Protection Research**, v. 44, n. 4, p. 373-376, 2004.

QIU, Y. et al. *Senna bicapsularis*: a new natural host of *Bean yellow mosaic virus* in China. **Plant Disease**, v. 103, n. 8, p. 2144, 2019.

RANA, T. et al. Characterisation of *Apple chlorotic leaf spot virus* infecting almonds in India. **Australasian Plant Disease Notes**, v. 3, n. 1, p. 65-67, 2008.

RASTROJO, A. et al. A new putative *Caulimoviridae* genus discovered through air metagenomics. **Microbiology Resource Announcements**, v. 7, n. 14, p. e00955-18, 2018.

REAVY, B. et al. Distinct circular single-stranded DNA viruses exist in different soil types. **Applied and Environmental Microbiology**, v. 81, n. 12, p. 3934-3945, 2015.

REBENSTORF, K. et al. Host species-dependent population structure of a pollen-borne plant virus, *Cherry leaf roll virus*. **Journal of Virology**, v. 80, n. 5, p. 2453-2462, 2006.

REZENDE, J. A. M. et al. Biological and molecular characterization of a putative new *Potexvirus* infecting *Senna occidentalis.* **Archives of Virology**, v. 162, n. 2, p. 529-533, 2016.

ROCHA, G. A. ***Begomovirus* em áreas de cerrado: de plantas herbáceas cultivadas a arbóreas selvagens**. [s.l.] Universidade Federal de Goiás, 2017.

RONAGHI, M. Pyrosequencing sheds light on DNA sequencing. n. 650, p. 3-11, 2001.

ROOSSINCK, M. J. et al. Ecogenomics: using massively parallel pyrosequencing to understand virus ecology. **Molecular Ecology**, v. 19, n. s1, p. 81-88, 2010.

ROOSSINCK, M. J. Metagenomics of plant and fungal viruses reveals an abundance of persistent lifestyles. **Frontiers in Microbiology**, v. 5, p. 767, 2015.

ROOSSINCK, M. J. Viruses in the phytobiome. **Current Opinion in Virology**, v. 37, p. 72-76, 2019.

ROOSSINCK, M. J.; BAZÁN, E. R. Symbiosis: viruses as intimate partners. **Annual Review of Virology**, v. 4, n. 1, p. 123-139, 2017.

ROOSSINCK, M. J.; GARCÍA-ARENAL, F. Ecosystem simplification, biodiversity loss and plant virus emergence. **Current Opinion in Virology**, v. 10, p. 56-62, 2015.

ROOSSINCK, M. J.; MARTIN, D. P.; ROUMAGNAC, P. Plant virus metagenomics: advances in virus discovery. **Phytopathology**, v. 105, n. 6, p. 716-727, 2015.

ROWHANI, A. et al. Serological detection of *Cherry leafroll virus* in English walnut trees. **Phytopathology**, v. 75, n. 1, p. 48-52, 1985.

RUMBOU, A. et al. A novel *Badnavirus* discovered from Betula sp. affected by birch leaf-roll. **PloS One**, v. 13, n. 3, p. e0193888, 2018.

RUMBOU, A. et al. Unravelling the virome in birch: RNA-Seq reveals a complex of known and novel viruses. **bioRxiv**, p. 740092, 2019.

RUMBOU, A. et al. A novel *Emaravirus* identified in maple with leaf mottle symptoms by deep. **bioRxiv**, 2020a.

RYBICKI, E. P. A Top Ten list for economically important plant viruses. **Archives of Virology**, v. 160, n. 1, p. 17-20, 2015.

ŠAFÁŘOVÁ, D.; CANDRESSE, T.; NAVRÁTIL, M. Complete genome sequence of a novel *Bromovirus* infecting elderberry (*Sambucus nigra* L.) in the Czech Republic. **Archives of Virology**, v. 163, n. 2, p. 567-570, 2018.

SAFÁVROVÁ, D. et al. Molecular characterization of a novel *Aureusvirus* infecting elderberry (*Sambucus nigra* L.). **PloS One**, v. 13, n. 8, p. e0200506, 2018.

SAID, Z. B.-O. S. et al. Essential oils composition, antibacterial and antioxidant activities of hydrodistillated extract of *Eucalyptus globulus* fruits. **Industrial Crops and Products**, v. 89, p. 167-175, 2016.

SASTRY, K. S. M. et al. Three virus diseases of *Eucalyptus citriodora*. **Indian Phytopathology**, 1971.

SAVINO, V. et al. **Occurence of two sap-transmissible viruses in walnuts**. X International Symposium on Fruit Tree Virus Diseases 67. **Anais**...1976.

SCHIMANSKI, H. H.; FUNK, T. Studies on the extent of transfer of *Cherry ringspot virus* by the seeds of wild cherry (*Prunus avium* L.). **Plant Breeding Reviews, Archiv Gartenbau 16**, v. 16, p. 143-150, 1968.

SCHIMANSKI, H.-H.; SCHMELZER, K.; ALBRECHT, H.-J. Seed transmission of cherry leaf roll virus in black cherry (*Prunus serotina* Ehrh.). **Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, II**, v. 131, n. 2, p. 117-119, 1976.

SCHIMANSKI, H. H.; ALBRECHT, H. J.; KEGLER, H. Seed transmission of cherry leaf roll virus in silver birch (*Betula pendula)*. **Archiv fur Phytopathologie und Pflanzenschutz**, v. 16, n. 3, p. 231-232, 1980.

SCHMELZER, K.; SCHMIDT, E. S.; SCHMIDT, H. B. Viruskrankheiten und virusverdächtige Erscheinungen a Forstgehölzen. **Archiv für Forstwesen**, v. 15, p. 107-120, 1966.

SCHMELZER, K. **Untersuchungen an viren der zier- und wildgeholze. 5. mitteilung: virosen a n Populus und Sambucus**. Phytopathol. Z. **Anais**...1966.

SCHMELZER, K. Neue befunde iiber die geographische verbreitung des *Robinien mosaiks* und iiber seine ursachlichen Viren. **Forstwes**, v. 17:62, p. 1-28, 1968.

SCHMELZER, K. **Das Ulmenscheckungs- Virus**. Phytopathol. **Anais...**1969

SCHMELZER, K. *Cherry leaf roll virus* from birch (*B. pendula*). **Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene**, v. 127, n. 1, 1972.

SCHMELZER, K. **Zicr-, forst- , und fildgehOlze. In pjlanzliche virologie**. ed. M. Klinkowski, D. Spaar. **Anais**...Berlin: 1977.

SEABRA;, P. V. et al. Detecção de *Carlavirus* em *Senna macranthera*. **Revista Brasileira de Horticultura Ornamental**, v. 7, n. 1, p. 67-71, 2001.

SERVIÇO FLORESTAL BRASILEIRO, SFB. **Florestas do Brasil em resumo**. 1. ed. Brasília: MAPA, 2019.

SHIEL, P. J.; CASTELLO, J. D. Detection of Tobacco mosaic and Tobacco ringspot viruses in herbaceous and woody plants near virus-infected white ash trees in central New York. **Plant Disease**, p. 791, 1985.

SILVA, C. M. DA. Entre Fênix e Ceres: A grande aceleração e a fronteira agrícola no Cerrado. **Varia Historia**, v. 34, n. 65, p. 409-444, 2018.

SIVIERO, A. et al. **Etnobotânica e Botânica Econômica do Acre**, 2016.

SMITH, C. M.; CAMPBELL, M. M. *Populus* genotypes differ in infection by, and systemic spread of, *Poplar mosaic virus*. **Plant Pathology**, v. 53, n. 6, p. 780-787, 2004.

ŠUBÍKOVÁ, V. The mechanical transmission of Euonymus mosaic virus, maple leaf perforation by leaf extract or leaf nucleic acid to herbaceous plants. **Biologia Plantarum**, v. 15, n. 3, p. 166-170, 1973.

SWEET, J. B. **Fruit tree virus infections of woody exotic and indigenous plants in Britain**. XI International Symposium on Fruit Tree Virus Diseases 94. **Anais**...1980.

SWEET, J. B.; BARBARA, D. J. A yellow mosaic disease of horse chestnut (*Aesculus* spp.) caused by *Apple mosaic virus*. **Annals of Applied Biology**, v. 92, n. 3, p. 335-341, 1979.

SWEET, J. B.; CAMPBELL, A. I. Pome fruit virus infections of some woody ornamental and indigenous species of Rosaceae. **Journal of Horticultural Science**, v. 51, n. 1, p. 91-97, 1976.

SZIRMAI, J. Acer virus disease in maple trees planted in avenues. **Acta phytopathologica**, 1972.

TANG, J.; WARD, L. I.; CLOVER, G. R. G. The diversity of *Strawberry latent ringspot virus* in New Zealand. **Plant Disease**, v. 97, n. 5, p. 662-667, 2013.

THOMAS, P. R. Host status of some plants for *Xiphinema diversicaudatum* (Micol.) and their susceptibility to viruses transmitted by this species. **Annals of Applied Biology**, v. 65, n. 1, p. 169-178, 1970.

VAN LENT, J. W. M.; WIT, A. J.; DIJKSTRA, J. Characterization of a *Carlavirus* in elderberry (*Sambucus* spp.). **Netherlands Journal of Plant Pathology**, v. 86, n. 2, p. 117-134, 1980.

VARNEY, E. H.; MOORE, J. D. Strain of *Tomato ringspot virus* from American Elm. **Phytopathology**, v. 42, n. 9, p. 476-477, 1952.

VELICEASA, D. et al. Searching for a new putative Cryptic virus in *Pinus sylvestris* L. **Virus Genes**, v. 32, n. 2, p. 177-186, 2006.

VILLAMOR, D. E. V; EASTWELL, K. C. *Sambucus nigra* subsp. caerulea and *Malva* spp.: newly identified hosts of *Cherry rasp leaf virus*. **Plant Disease**, v. 100, n. 4, p. 867, 2016.

VINCENT, S. J.; COUTTS, B. A.; JONES, R. A. C. Effects of introduced and indigenous viruses on native plants: exploring their disease causing potential at the agro-ecological interface. **PloS One**, v. 9, n. 3, p. e91224, 2014.

VON BARGEN, S. et al. Determination of the complete genome sequence of *European mountain ash ringspot-associated emaravirus* from *Sorbus intermedia* reveals two additional genome segments. **Archives of Virology**, v. 164, n. 7, p. 1937-1941, 2019.

VON BARGEN, S. et al. Characterisation of a novel *Emaravirus* identified in mosaic-diseased Eurasian aspen (*Populus tremula*). **Annals of Applied Biology**, 2020.

VON BUBNOFF, A. Next-generation sequencing: the race is on. **Cell**, v. 132, n. 5, p. 721-723, 2008.

WANG, Z.; GERSTEIN, M.; SNYDER, M. RNA-Seq: a revolutionary tool for transcriptomics. **Nature Reviews Genetics**, v. 10, n. 1, p. 57, 2009.

WINTER, S.; NIENHAUS, F. Identification of viruses from European beech (*Fagus sylvatica* L.) of declining forests in Northrhine-Westfalia (FRG). **Forest Pathology**, v. 19, n. 2, p. 111-118, 1989.

WOO, E. N. Y.; CLOVER, G. R. G.; PEARSON, M. N. First report of *Cherry leaf roll virus* (CLRV) in *Malus domestica*. **Australasian Plant Disease Notes**, v. 7, n. 1, p. 151-156, 2012.

WOO, E. N. Y.; PEARSON, M. N. Biological and molecular variation of *Cherry leaf roll virus* isolates from *Malus domestica, Ribes rubrum, Rubus idaeus, Rumex obtusifolius* and *Vaccinium darrowii*. **Plant Pathology**, v. 63, n. 4, p. 838-845, 2014.

WU, X.; CHENG, X.; LI, W. First report of natural infection of *Watermelon mosaic virus* on

Lagerstroemia indica in China. **Plant Disease**, v. 102, n. 5, p. 1048, 2018.

YANG, C. et al. Identification and characterization of a novel *Emaravirus* associated with jujube (*Ziziphus jujuba* Mill.) yellow mottle disease. **Frontiers in Microbiology**, v. 10, p. 1417, 2019.

YAO, L. Z. et al. First report of Telosma mosaic virus infecting emperor’s candlesticks (*Senna alata*) in China. **Plant Disease**, v. 103, n. 3, p. 594, 2019.

YARWOOD, C. E.; HECHT-POINAR, E.; VON BARGEN, S. Viruses from rusts and mildews. **Phytopathology**, v. 63, n. 9, p. 1111-1115, 1973.

YU, C. et al. First identification of *Cucumber mosaic virus* in *Prunus serrulata, Photinia serrulata, Begonia semperflorens* and *Osmanthus matsumuranus*. **Journal of Plant Pathology**, p. 1-4, 2020.

CHAPTER 2 - Molecular characterization of Hovenia dulcis-associated virus 1 (HDaV1) and 2 (HDaV2): new tentative species within the order *Picornavirales*

Flávia M. B. Nery[1], Fernando L. Melo[1*], Leonardo S. Boiteux[3], Simone G. Ribeiro[4], Renato O. Resende[2], Anelise F. Orílio[2], Josiane G. Batista[1], Mirtes F. Lima[3] and Rita C. Pereira-Carvalho[1]*.

[1] Departamento de Fitopatologia, Universidade de Brasília (UnB), Campus Universitário Darcy Ribeiro, Brasília-DF, 70910-900, Brazil. flamilene.nery@gmail.com (F.M.B.N.); 05josiane@gmail.com (J.G.B.)

[2] Departamento de Biologia Celular, Universidade de Brasília (UnB), Campus Universitário Darcy Ribeiro, Brasília, DF 70910-900, Brazil; rresende@unb.br (R.O.R.); aneliseorilio@gmail.com (A.F.O.)

[3] Embrapa Hortaliças, Brasília, DF 70275-970, Brazil; leonardo.boiteux@embrapa.br (L.S.B.); mirtes.lima@embrapa.br (M.F.L.)

[4] Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF 70770-017, Brazil; simone.ribeiro@embrapa.br (S.G.R)

***** Correspondence: flmelo@unb.br (F.L.M.); rcpcarvalho@unb.br (R.C.P.-C.)

VIRUSES, 2020

Received: 22 July 2020 / Revised: 8 August 2020 / Accepted: 15 August 2020 / Published: 27 August 2020

Abstract: In a systematic field survey for plant-infecting viruses, leaf tissues were collected from trees showing virus-like symptoms in Brazil. After viral enrichment, total RNA was extracted and sequenced using the MiSeq platform (Illumina). Two nearly full-length picorna-like genomes of 9534 and 8158 nucleotides were found associated with *Hovenia dulcis* (*Rhamnaceae* family). Based upon their genomic information, specific primers were synthetized and used in RT-PCR assays to identify plants hosting the viral sequences. The larger contig was tentatively named as Hovenia dulcis-associated virus 1 (HDaV1), and it exhibited low nucleotide and amino acid identities with *Picornavirales* species. The smaller contig was related to insect-associated members of the *Dicistroviridae* family but exhibited a distinct genome organization with three non-overlapping open reading frames (ORFs), and it was tentatively named as Hovenia dulcis-associated virus 2 (HDaV2). Phylogenetic analysis using the amino acid sequence of RNA-dependent RNA polymerase (RdRp) revealed that HDaV1 and HDaV2 clustered in distinct groups, and both viruses were tentatively assigned as new members of the order *Picornavirales*. HDaV2 was assigned as a novel species in the *Dicistroviridae* family. The 5’ ends of both viruses are incomplete. In addition, a nucleotide composition analysis (NCA) revealed that HDaV1 and HDaV2 have similarities with invertebrate-infecting viruses, suggesting that the primary host(s) of these novel virus species remains to be discovered.

Keywords: virome; metagenomics; *Hovenia dulcis*; HDaV1; HDaV2; *Picornavirales*

1. Introduction

Natural forest ecosystems and cultivated forest plantations are responsible for covering around 30% of the entire surface of the Earth (KEENAN et al., 2015). The economic exploitation of forests provides a wide range of benefits, including the production of food, timber wood, charcoal, and pharmaceutical and cosmetic products, among other items. Importantly, the conservation and expansion of native forests generate positive impacts in carbon sequestration as well as in preserving fauna and flora diversity and mitigating the deleterious effects of climate change (KEENAN et al., 2015).

Tree species are affected by various pathogens, which are responsible for extensive economic and ecological damages (BEŁKA, 2015). Native and cultivated tree species may serve as alternative sources of inoculum of viruses that can infect other economically important crop species.

Therefore, information about the viral diversity associated with forest host species is critical and provides the basis for the establishment of effective disease management and control strategies. Several studies of viruses occurring in the temperate forests of Europe have been carried out (NIENHAUS; CASTELLO, 1989). However, the characterization of viruses infecting trees, especially in Neotropical areas, is yet scarce (BÜTTNER et al., 2013).

In Brazil, the pioneering studies on the characterization of viruses on natural forest ecosystems started in the late 1970s (LIN; KITAJIMA; COSTA, 1980), allowing the identification of virus species classified in the genera *Carlavirus*, *Orthotospovirus*, *Potyvirus*, and *Tymovirus* (BESERRA JR et al., 2012; GAMA et al., 1983; LIN et al., 1979; NICOLINI et al., 2012).

The metagenomic/ecological genomic strategies, coupled with large-scale sequencing platforms, increased the knowledge about the microbial diversity across a wide range of natural environments (MOROZOVA; MARRA, 2008) and has contributed to detect, identify, and characterize several new plant-associated new viruses and viroids without prior knowledge of their genomes (DE BRUIJN, 2011; PRABHA; BARANWAL; JAIN, 2013).

A further advantage of the metagenomic strategies is the detection of plant-associated viral sequences, even at low concentrations in their host tissues (BARBA; CZOSNEK; HADIDI, 2014). A wide range of protocols for enriching virus particles have been used, and several viruses have been detected and characterized after employing these approaches (CANDRESSE et al., 2014; MELCHER et al., 2008; PARDINA et al., 2012; ROOSSINCK; MARTIN; ROUMAGNAC, 2015; SHEVELEVA et al., 2013).

However, the metagenomic characterization of viruses and viroids in tree species is limited to species into the genera of high economic relevance such as *Prunus*, *Pyrus*, *Malus*, *Citrus*, *Actidinia*, *Diospyros*, *Morus*, and *Vitis* (FAJARDO et al., 2017; MALIOGKA et al., 2018; ROTT et al., 2017). Here, we describe the near full-length genomes of two putative novel virus species within *Picornavirales* associated with leaf samples from *Hovenia dulcis* Thumb. (Rhamnaceae family).

2. Material and Methods

2.1. Plant Material

Leaves of tree seedlings displaying virus-like symptoms were collected at the NOVACAP II (Companhia Urbanizadora da Nova Capital do Brasil) - Nursery II. At the time of collection, the seedlings were about seven months old.

A total of 60 plant samples were obtained from 27 native and exotic species from 14 botanical families. Before the enrichment of viral particles, leaf samples were collected from all the seedlings, gently cleaned with a brush, and stored at -80 °C.

2.2. Enrichment of Viral Particles

Three individual pools (including 20 samples each, with a total weight of 10 g for each pool) were ground on liquid nitrogen. Afterward, 100 mL of 0.1 M sodium phosphate buffer, pH 8.0 containing 1 mM EDTA, and 0.2% β-mercaptoethanol were added to each sample pool. The samples were macerated, filtered using cheesecloth, and centrifuged (at 2800× *g* for 20 min).

The aqueous layer was transferred to ultracentrifuge tubes, and with a long needle, a layer of a sucrose solution (20%) was added into the tube bottom to form a sucrose cushion. Subsequently, the samples were submitted to analytical ultracentrifugation at 4 °C and 33,000× *g* for 2 h. RNA extraction was carried out from the pellet fraction, employing TRIzol reagent (Life Technologies, Carlsbad, CA, USA). Pellets were resuspended in 1 mL of TRIzol and transferred to a fresh tube containing 200 μL of chloroform. All samples were mixed to form a single pool (including all 60 samples) and vigorously vortexed for 15 s and left at room temperature for 3 min. Subsequently, the samples were centrifuged at 12,000× *g* for 15 min at 4 °C.

The aqueous layer (1 mL) was transferred to a new tube, and 500 μL of isopropanol was added. The samples were kept at room temperature for 10 min. After that, the samples were again centrifuged (at 12,000× *g* for 10 min at 4 °C). The supernatant was discarded, and 1 mL of 75% ethanol was added to wash the pellet. After centrifuging at 7500× *g* for 5 min, the ethanol was carefully discarded, and the samples left on ice for 2 min. Finally, the RNA was resuspended in RNase-free DEPC (Diethyl pyrocarbonate)-treated water, left on ice for 15 min, aliquoted and stored at -80 °C.

2.3. High-throughput sequencing and Analysis

The pooled RNA was sequenced using the MiSeq sequencing platform (Illumina, San Diego, CA, USA) at the Universidade Católica de Brasília (UCB). Total RNA was converted to cDNA using random hexamers, the library was prepared with Nextera™ DNA Sample Prep Kit and sequenced using MiSeq Reagent Kits v2 (2 × 150 bp) (Illumina, San Diego, CA, USA). The raw reads were quality trimmed and assembled de novo using the CLC Genomics Workbench (v 8.0, Qiagen, CA, USA).

The resulting contigs were compared to the complete viral RefSeq database using Blastx and Blastp algorithms (ALTSCHUL et al., 1990) implemented in Geneious program v. 9.1.3 (KEARSE et al., 2012).

All sequences with hits matching the viral database were then subjected to a Blastx search against the complete nr database to exclude false positives. To confirm the assembly results and further extend incomplete genomes, trimmed reads were mapped back to the viral contigs and reassembled until genome completion or no further extension.

Genomic regions covered by less than three sequence reads were amplified by RT-PCR (see **Table 1** for primer sequences), and Sanger sequenced.

The final contigs were annotated using Geneious program (v. 9.1.3, Biomatters, Auckland, New Zealand) (KEARSE et al., 2012). The 3’ends secondary structure were predicted using the RNAfold web server (http://rna.tbi.univie.ac.at//cgi-bin/RNAWebSuite/RNAfold.cgi) (GRUBER; BERNHART; LORENZ, 2015; LORENZ et al., 2008, 2011). The sequences were deposited at the GenBank under the accession numbers MT079817 and MT079818.

Table 1. Specific primers sequences used in PCR, RT-PCR, and 3′ RACE.

Primer Name	**Primer Sequence 5′-3′**	**AT [1] (°C)**	**Amplicon Size (bp)**	**Application/Target Genomic Regions**
HDaV1_7626_F [2]	AGTCACTGGTGCGTTAGGTG	57	993	Detection/Capsid
HDaV1_8618_R [3]	GTAAGCATACCTCCACGCGA			
HDaV2_6983_F	GAATGAACTGCGTGCTACAC	59	757	Detection/Capsid
HDaV2_7739_R	CCGGGGGAAAACAGCAGT			
C1622_254_F [4]	TTAATGGGGTTGCAGGGCTT	60	519	Detection/RdRp
C1622_772_R [4]	TCATGACTCCTATGCGCCAC			
C1177_207_F [5]	GTGTCGTTTGTATCGCAGGC	59	674	Detection/RdRp
C1177_880_R [5]	CGCGCTCATAGCCAAACAAA			
C_1797_31_F [6]	ATTGAAAACGCGACCTGCAC	59	571	Detection/RdRp
C1797_601_R [6]	GCGGGATAAGCTCACCAAGT			
HDaV2_1630_F	TGCAAGAGTACCAGGAACAGAATAAT	54	608	Low coverage region 1/ORF1
HDaV2_2236_R	GCAAGGCCATGATACATGACCA			
HDaV2_3431_F	AGAAAGTGTTTACTATGTAGCACCAACT	59	549	Low coverage region 2/ORF1b
HDaV2_3981_R	CTATTCCTTGGCAGGCTTGACG			
HDaV2_6422_F	GTCTGCTCCTGATGCTAATCCG	58	540	Low coverage region 3
HDaV2_6961_R	GCTGGGACATCATCAAGGGAAC			
Oligod50TM4	GTTTTCCCAGTCACGACTTAATTAA(T)50	65	-	Race cDNA
M4	GTTTTCCCAGTCACGACT	56	-	Race 3′ PCR
HDaV1_9041_F	CCTCAGAAGTTTTCGAGACTGC	56	-	Race 3′ PCR
HDaV2_7256_F	ACCTCACAAATATACTGTTGGTGAGG	60	-	Race 3′ PCR
HDaV2_7518_F	CCTGAACTTGGTATATTGGATGTTCCC	60	-	Race 3′ PCR

[1] Annealing temperature; [2] F: Forward; [3] R: Reverse; [4] contig1622: *Fabavirus*; [5] contig1177: *Fabavirus*; **[6]** contig1797: *Comovirus.*

2.4. RNA Extraction and Virus Detection by RT-PCR

Based on the assembled contigs, a set of specific primers were designed to determine the presence or absence of each of the five viruses and used to assay each sample by RT-PCR (**Table 1**). The total RNA was individually extracted from each of the 60 original samples using the Hot Phenol protocol (VERWOERD; DEKKER; HOEKEMA, 1989). All centrifugation steps were carried out at 4 °C. The purity and integrity of the RNA were confirmed by electrophoresis on a 1% agarose gel. Complementary DNA (cDNA) was synthesized with the viral-specific reverse primers. The reaction was performed using the Moloney Murine leukemia Virus Reverse Transcriptase (M-MLV) (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. Initially, a mixture of 3.5 µL of RNAse-free water, 4.5 µL of RNA, 1 µL of reverse primer (10 µM), and 1 µL of dNTP (10 mM) was incubated at 70 °C for 5 min. Then 3 µL of M-MLV 5X buffer [250 mM Tris-HCl (pH 8.3); 375 mM KCl; 15 mM $MgCl_2$, and 0.1 M DTT], 1 µL of M-MLV enzyme (200 U/µL), 1 µL of RNase OUT (40 U/µL) (Invitrogen, Carlsbad, CA, USA) 1 µL of 100 mM DTT, and 4 µL of RNAse-free water were added to, in a total volume of 20 µL. Samples were incubated at 37 °C for 60 min and 15 min at 70 °C. The PCR assays were performed in a total volume of 12.5 µL. The reaction was composed by 8.0 µL of DNAse-free water, 1.25 µL of buffer 10X, 0.4 µL of $MgCl_2$ (50 Mm), 0.25 µL of dNTP (10 mM), 0.25 µL reverse primer (10 µM), 0.25 µL of forward primer (10 µM), 0.1 µL of *Taq* DNA polymerase (500 U/µL) (Invitrogen, Carlsbad, CA, USA), and 2 µL of cDNA. The PCR parameters were as follows: initial denaturation of 94 °C for 2 min followed by 34 cycles of denaturation (94 °C for 30 s), annealing (58 °C for 45 s), and extension (72 °C for 1 min). A final extension step (72 °C for 7 min) was employed. The correct size PCR products were identified by gel electrophoresis (1%), gel-purified, cloned into pGEM-T Easy Vector (Promega, Madison, WI, USA), and Sanger sequenced at CNPH (Centro Nacional de Pesquisa de Hortaliças, Brasília, Brazil).

2.5. 3′ RACE

The 3′ end of the genomes was amplified using the 3′ RACE, as described by (CHEN; ADAMS, 2001; SILVA et al., 2013). Briefly, the cDNA was synthesized using an oligo(dT) primer with an anchor sequence (Oligod50TM4) (**Table 1**) and SuperScript™ III Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA), according to the manufacturer’s instructions. The PCR was performed using this cDNA with virus-specific forward primers (**Table 1**) and the anchor reverse primer M4. The PCR products were identified by gel electrophoresis (1%), gel-purified, and Sanger sequenced at CNPH.

2.6 Phylogenetic Analyses

Phylogenetic analyses were carried out with RNA-dependent-RNA-polymerase (RdRP) protein sequences from members belonging to the order *Picornavirales*. The amino acid sequences were aligned using ClustalW (THOMPSON; GIBSON; HIGGINS, 2003) implemented in Geneious (KEARSE et al., 2012). The maximum likelihood tree was inferred with the FastTree algorithm (PRICE; DEHAL; ARKIN, 2009) implemented in Geneious, with JTT+CAT (JONES; TAYLOR; THORNTON, 1992). Branch support was estimated using the non-parametric Shimodaira-Hasegawa-like approximate likelihood ratio test (SH-aLRT) (GUINDON et al., 2010). The genome organization of each of the viruses was annotated on the tree using the Evolview v3 server (SUBRAMANIAN et al., 2019). The accession numbers of sequences used in the alignment are displayed in Table S1.

2.7. Nucleotide Composition Analysis (NCA)

The nucleotide composition analysis (NCA) method was used to infer the most likely virus host in this study (KAPOOR et al., 2010). For NCA, a dataset of 278 complete genomes sequences with defined host origins (e.g., insects, vertebrates, plants, algae, protozoans, and environmental samples) and also comprising genomes from species, genera, and families within the order *Picornavirales* were retrieved from the NCBI/GenBank (https://www.ncbi.nlm.nih.gov/). In viruses with bipartite genomes, the sequences of the components were concatenated, and these were considered as the complete genome. A Linear Discriminant Analysis (LDA) was performed to identify the most likely host species of the viruses reported in the present work. Dinucleotide frequencies for each sequence were determined using the program simple sequence editor (SSE), version 1.3 (SIMMONDS, 2012). LDA was performed using the R program (www.R-project.org) (version 3.4.2) (TEAM, 2013), implementing the MASS, LDA function, and ggplot2 package. The accession numbers and hosts of the genomes employed in the NCA are presented in Table S2.

3. Results

As part of a field survey for plant-infecting viruses, samples from a variety of plant species were collected in 2014 at NOVACAP Nursery II, located in Brasília-DF, Brazil. This nursery is responsible for the production of tree seedlings for urban reforestation purposes. We processed and sequenced one pool of samples containing viral enriched RNA from 60 plants, including two plants of *H. dulcis* showing virus-like symptoms (interveinal chlorosis) (**Figure 1A**). After MiSeq sequencing (Illumina), a total of 5,005,110 raw reads were generated. The raw reads were trimmed and de novo assembled using CLC Genomics Workbench v.8.0 (Quiagen program). The resulting 2162 contigs were compared against a viral RefSeq database using Blastx algorithm (ALTSCHUL et al., 1990) and five contigs were initially assigned to the *Picornavirales* order. While two large contigs (9529 and 8126 nucleotides-nts) were related to unclassified members of *Picornavirales* and *Dicistroviridae*, the remaining three contigs with small sizes (1096, 781, and 635 nts) and relatively low coverage (≤50 reads) were related to Secoviridae members. The presence of these putative novel viruses was investigated by RT-PCR and Sanger sequencing in all samples individually. All sixty samples were negative to the *Secoviridae* related contigs (data not shown); therefore, they were not further investigated. The two large contigs were detected only in one *H. dulcis* leaf sample. After 3′ RACE, five additional nucleotides from 3′-terminal sequence of the larger contig were recovered, resulting in a final contig with 9534 nts plus the poly(A) tail. Moreover, the minor contig was increased in 32 nts, resulting in a final contig with 8158 nts plus the poly(A) tail.

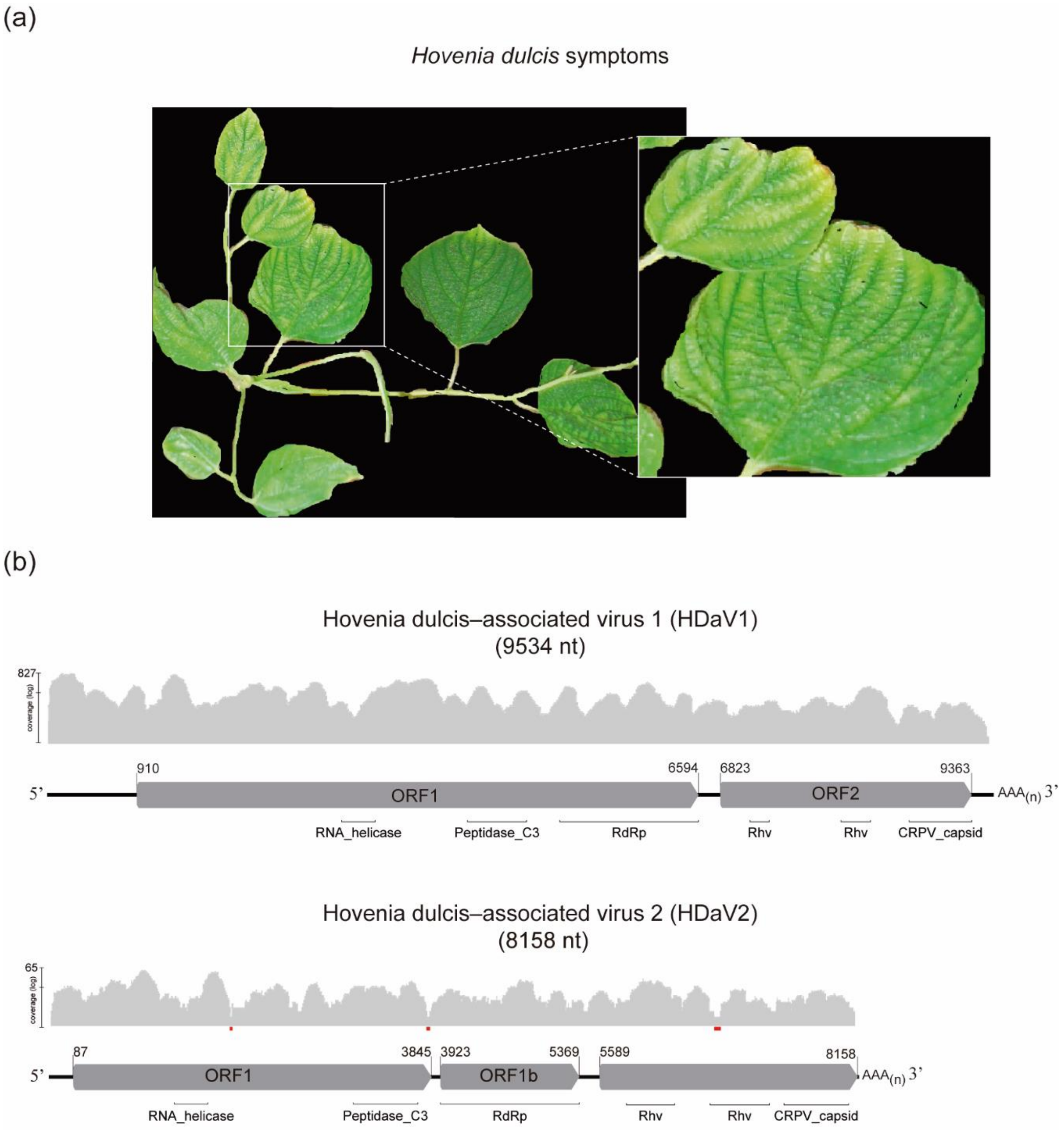


Figure 1. *Hovenia dulcis* symptoms and viral genomic organization. **(A)** Virus-like symptoms observed in leaves of *Hovenia dulcis*: interveinal chlorosis and **(B)** Schematic representation of Hovenia dulcis-associated virus 1 (HDaV1) and Hovenia dulcis-associated virus 2 (HDaV2) genomic organization and sequencing coverage. All open reading frames (ORFs) are represented as arrows pointing from the 5' to the 3' end and are colored in grey. Nucleotide positions indicate the start and end of ORFs. ORF 1 and 1b encodes non-structural polyproteins, including putative functional domains (RNA Helicase; 3C peptidase protease; and RdRp: RNA-dependent RNA polymerase). ORF 2 encodes a structural polyprotein with capsid protein domains [Rhv: picornavirus (*Rhinovirus*) and capsid protein-like, CrPV capsid: cricket paralysis virus capsid protein-like]. Regions with low coverage are highlighted in red, and the numbered regions were confirmed by RT-PCR and Sanger sequencing

The nearly full-length genome of 9534 nts (assembled from 8669 reads) displayed two non-overlapping ORFs (**Figure 1B).** The first ORF (spanning from the nucleotide 910 to the nucleotide 6594) encodes the replication proteins (one helicase, a tyrosine-like serine protease, and RNA-dependent RNA polymerase domains) and the second ORF (spanning from the nucleotide 6823 to the nucleotide 9363) codes for a structural protein (CP domain). The two ORFs are separated by a 228 nts intergenic region (IGR). However, characteristic IRES-like structures were not identified. The 5′-UTR and 3′-UTR ends contain 909 and 171 nts, respectively. Although it is likely that the 5′-UTR is still incomplete, this is one of the largest 5′UTR (909 nts) when compared to other genetically related viruses, while two insect-infecting viruses, Hubei picorna-like virus and Hubei picorna-like virus 79 each have reported 5′-UTR of only 211 nts, suggesting that the 5′-UTR size varies significantly among the order *Picornavirales*.

Pairwise identity comparisons of this contig sequence with those of representative *Picornavirales* members indicated that it shares the highest degree of nucleotide identity (71%) with the Darwin bee virus 6 isolate NT-8 (9123 nts) (MG995696), a yet unclassified *Picornavirales* member reported infecting honey bees (*Apis mellifera*) (ROBERTS; ANDERSON; DURR, 2018). Additionally, the putative proteins, encoded by ORF 1 and ORF 2, share 65.5% and 70% aa identity with Darwin bee virus polyproteins (AWK77846 and AWK77847), supporting its classification as a new species according to the species demarcation criteria proposed by the International Committee on Taxonomy of Viruses (ICTV) (i.e., protein identity of less than 90% with its closest relatives) (BOURGAREL et al., 2019). The name *Hovenia dulcis-associated virus* 1 (HDaV1) is proposed for this virus.

Interestingly, the contig of 8158 nts (assembled from 689 reads) presented three non-overlapping ORFs of 3758 nts (ORF1), 1446 (ORF1b) and 2571 nts (ORF2) (**Figure 1B**). Since low coverage contigs are more susceptible to sequencing errors and spurious assembly, three pairs of primers were designed to confirm regions with insufficient coverage (**Figure 1B),** highlighted in red). The sequence generated by Sanger sequencing was identical to the sequence obtained by HTS, except for one Illumina read with an insertion in a T homopolymer (position 3827 to 3830), which would reconstitute the longer ORF1 frame typical of members of the family *Dicistroviridae* (data not shown). To further investigate this result, we performed a new HTS using total RNA from *H. dulcis* leaves, and no insertion was observed in the reads mapped in this genomic region. However, only a limited number of reads (226 reads) mapped to the HDaV2 genome.

The first two ORFs were separated by a short IGR of 78 nts, and they encode the non-structural protein precursors. The ORF1 putative protein presented the RNA_helicase and 3C peptidase protease domains, whereas ORF1b putative protein presented the RNA-dependent RNA polymerase domain. ORF2 is separated from ORF1b by an IGR of 219 nts and encodes a structural polyprotein, which contains the three capsid domains (**Figure 1B**). All ORFs are predicted to initiate translation at canonical AUG codons. Moreover, the 5′-UTR and IGR motifs, typical of dicistrovirus (BONNING; MILLER, 2010), were not observed. Unfortunately, some nucleotides at the 5′ end of the genome are missing compared with other related viruses (described below). Interestingly, the poly(A) tail was located immediately downstream of the stop codon, which is an uncommon feature among members of the order *Picornavirales*. A hairpin structure was predicted at nucleotide positions 8124-8158 (**Figure S1**). However, its functionality remains to be evaluated.

The pairwise identity comparisons indicated that it shares the highest degree of nucleotide identity with two viruses reported in *Bemisia tabaci* samples from Brazil: Bemisia-associated dicistrovirus 2 (MN231041, unpublished) (BaDV-2) and Bemisia-associated dicistrovirus 1 (BaDV-1) (MH459180) (NAKASU et al., 2019). Crucially, BaDV-2 presented the same unusual genomic organization (three non-overlapping ORFs) observed above. A comparison of these genomes revealed a single nucleotide deletion that produces two ORFs (ORF1 and ORF1b), which do not occur in BaDV-1 ORF1 (**Figure S2**). Based on Blastp analysis, BaDV-2 shared 48%, 73%, and 69% of an amino acid identity, the putative proteins, encoded by ORF1, ORF1b, and ORF 2, respectively. Moreover, the BaDV-1 polyproteins (AZB50980 and AZB50981) shared 44% and 69% aa identity with ORF1/ORF1b and ORF2, respectively. Overall, these results confirm that HDaV2 represents a new species within the family *Dicistroviridae*, which we have tentatively named *Hovenia dulcis-associated virus* 2 (HDaV2).

The phylogenetic analysis based on the conserved RdRp domain of the two novel viruses (HDaV1 and HDaV2) and representative viruses in the order *Picornavirales* confirmed the Blastx and pairwise identity results (**Figure 2**). HDaV1 showed close relationships to a several unclassified bicistronic picorna-like arthropod-infecting viruses: Darwin bee virus 6 (ROBERTS; ANDERSON; DURR, 2018), Hubei picorna-like-79, Hubei picorna-like-78, Hubei picorna-like-80 (SHI et al., 2016), and Tetranychus urticae-associated picorna-like virus 2 (FRANÇOIS et al., 2019), forming a potential new family within the *Picornavirales* order (**Figure 2**). The genomes of Hubei picorna-like virus 80 and Tetranychus urticae-associated picorna-like virus 2 are probably incomplete given the absence of an ORF encoding the structural polyproteins (ORF2). Additionally, HDaV2 clustered with BaDV-1 and BaDV-2 in a monophyletic clade, supporting the notion that these viruses represent new species within a new genus in the family *Dicistroviridae* (NAKASU et al., 2017).

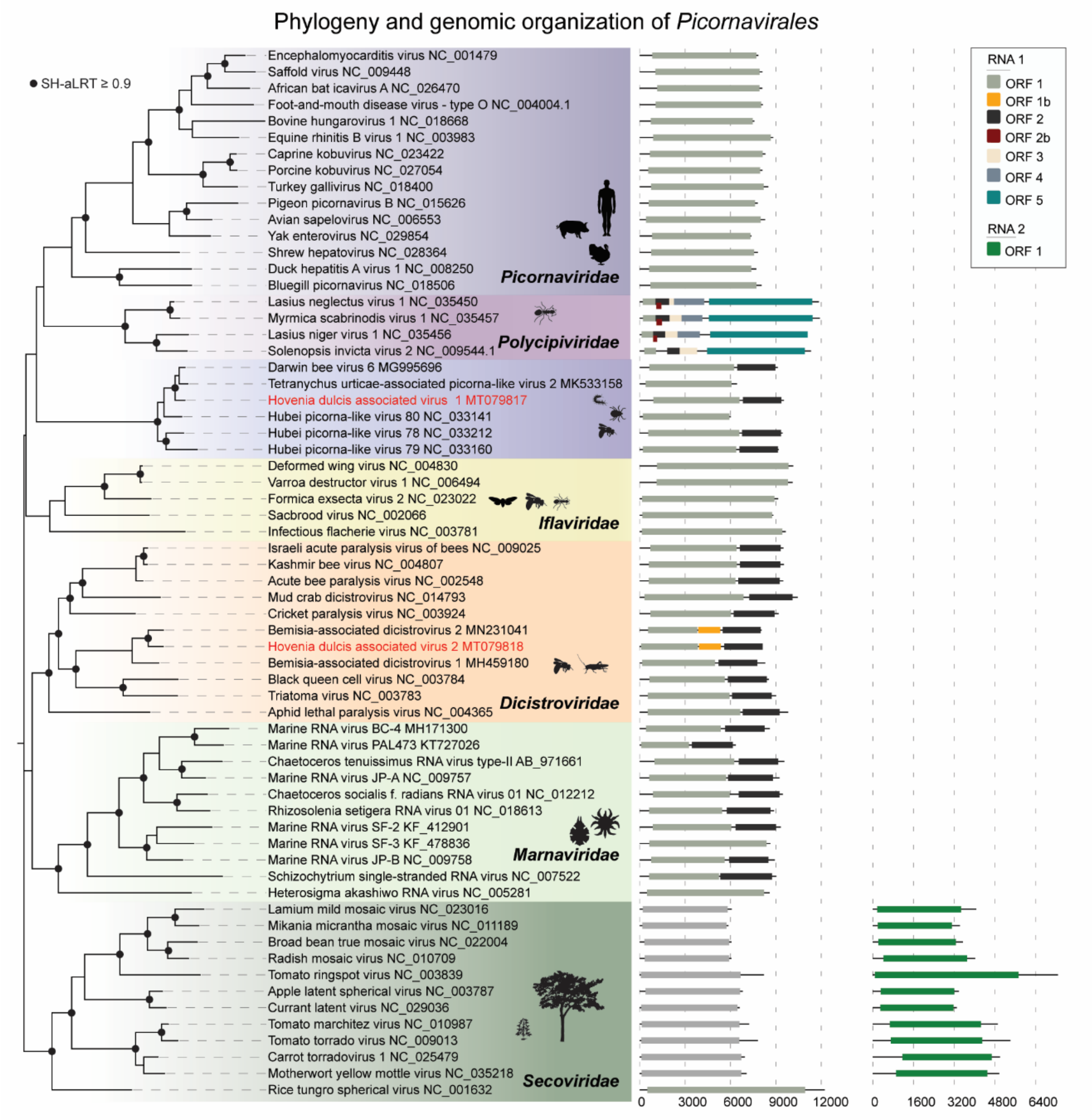


Figure 2. Phylogeny and genomic organization of representative members of order *Picornavirales*. Phylogenetic analysis based on the amino acid sequence of RdRp (RNA-dependent RNA polymerase) of members from six families within the order *Picornavirales*. Sequences were aligned using ClustalW (THOMPSON; GIBSON; HIGGINS, 2003) and the maximum likelihood tree inferred with FastTree (PRICE; DEHAL; ARKIN, 2009). The black circles represent nodes with aLRT ≥0.9. Hovenia dulcis-associated virus 1 (HDaV1) and Hovenia dulcis-associated virus 2 (HDaV2) are highlighted in red. The genome organization was plotted with the Evolview v3 program (SUBRAMANIAN et al., 2019)

A nucleotide composition analysis (NCA) was performed in an attempt to identify the most likely host(s) of the HDaV1 and HDaV2. A total of 278 sequences from the order *Picornavirales* were used, and four pre-defined categories of hosts were used for NCA (viz. invertebrates, others (algae, protozoa, and environmental samples), plants, and vertebrates). As shown in **Figure 3**, three groups were formed after linear discriminant analysis: plant-infecting viruses (in green), vertebrate-infecting viruses (in purple), and a third mixed group formed by invertebrate- and protist-infecting viruses (red and black). HDaV1 and HDaV2 clustered with invertebrate infecting viruses (**Figure 3**).

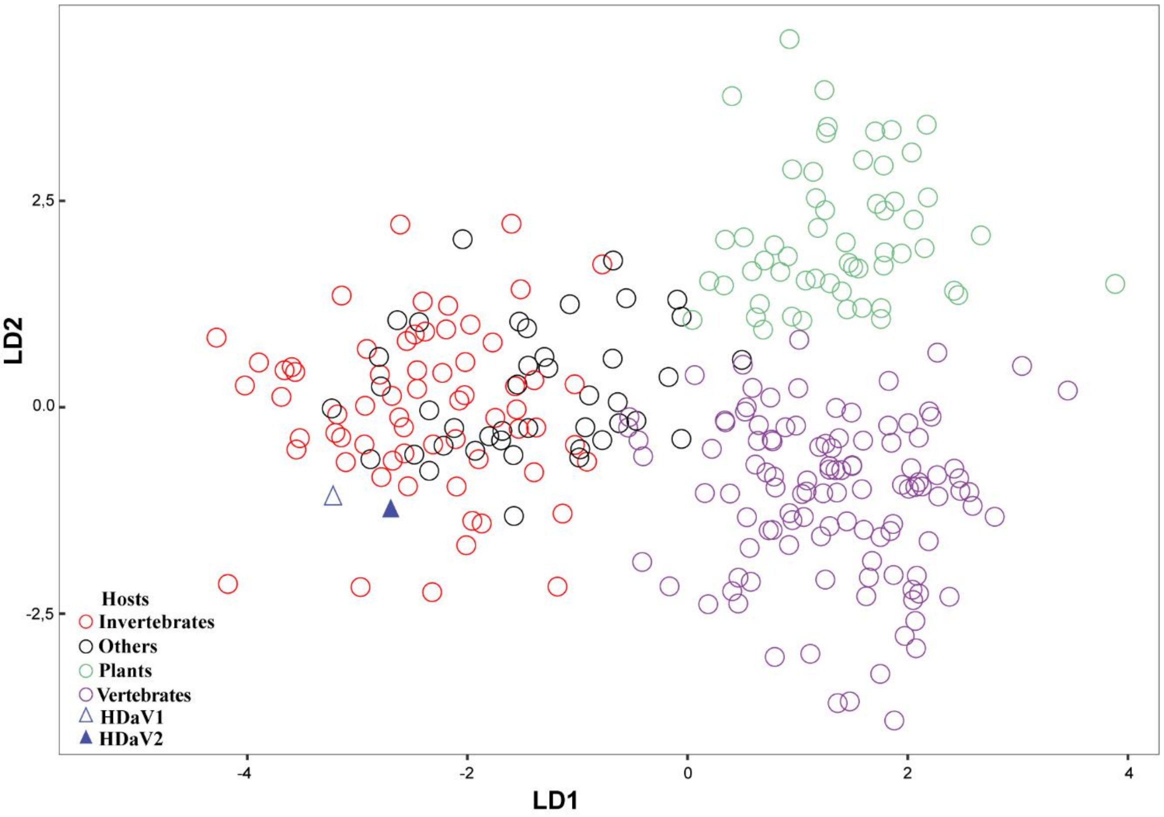


Figure 3. Linear discriminant analysis (LDA) used to classify viral sequences into host groups. Linear discriminant analysis comparing nucleotide composition from members of order *Picornavirales* with known hosts. Invertebrates-infecting viruses are shown in open red circles, plant-infecting viruses are in open green circles, and vertebrate-infecting viruses in open purple circles; Hovenia dulcis-associated virus 1 (HDaV1) is indicated by an open blue triangle, and Hovenia dulcis-associated virus 2 (HDaV2) is indicated by a solid blue triangle. Other picornaviruses (algae, protozoa, and environmental samples) are shown in open black circles.

4. Discussion

In a systematic field survey for plant-infecting viruses, leaf tissues were collected from trees showing virus-like symptoms in Brazil. Two putative new ssRNA+ viruses were found in leaves of *H. dulcis* seedlings grown under nursery conditions, confirming that the viral enrichment protocol followed by HTS is a sensible and economic strategy for discovering new viruses, even with the dilution effect of sample-pooling. Based upon the genomic organization and phylogenetic analyzes, these two viruses were tentatively classified as novel viral species within the order *Picornavirales*. We proposed the names Hovenia dulcis-associated virus 1 (HDaV1) and Hovenia dulcis-associated virus 2 (HDaV2).

The order *Picornavirales* harbors viruses with ssRNA+ genomes, spherical particles with a diameter around 30 nm, distinct genomic organization and segmentation (mono or bipartite), as well as distinct host organisms (algae, insects, protists, plants, and vertebrates) (OLENDRAITE et al., 2017). Currently, the combined analyses of these features allow for the allocation of viral species into six families (*Dicistroviridae*, *Iflaviridae*, *Marnaviridae*, *Secoviridae*, *Picornaviridae*, and *Polycipiviridae*) (LANG; VLOK; SUTTLE, 2018; LE GALL et al., 2008). HDaV1 and HDaV2 shared many of the key characteristics of the *Picornavirales* members, including the genomic organization with conserved regions with HEL/PRO/RdRp motifs (KING et al., 2011). However, HDaV2 presented a novel genome organization within the family *Dicistroviridae*, with three non-overlapping ORFs.

Importantly, HDaV1 and HDaV2 clustered with invertebrate-infecting viruses, suggesting that they might be (i) invertebrate-infecting viruses derived from some undetected invertebrate that was contaminating our plant samples, (ii) they are bona fide yet unknown plant-infecting viruses, or (iii) they can infect both invertebrates and plants. The presumed relationships with insect-infecting viruses were supported by the NCA results, which grouped HDaV1 and HDaV2 with picornaviruses infecting invertebrate and “other” hosts rather than those infecting either vertebrates or plants (**Figure 3**). Therefore, an invertebrate or invertebrates are the most likely primary hosts of both HDaV1 and HDaV2. However, it is important to highlight that during sample collection, it was not possible to determine the conspicuous presence of insects or mites. Besides, before viral enrichment process, leaf tissues were carefully cleaned with a brush under a stereo-microscope. Significantly, no reads/contigs were related to *Bemisia tabaci* genes, which was the only insect observed during our surveys. Moreover, dual tropism (invertebrates/plants) have already been described in both invertebrate-infecting viruses or plant-infecting viruses [45-49]. Dual tropism could explain, for example, the presence of virus-like symptoms in the *H. dulcis* leaf samples. For instance, Rhopalosiphum padi virus - RhPV (genus *Cripavirus*; family *Dicistroviridae*) is commonly reported infecting aphids, which are well-characterized plant-pests. In this context, plants might also serve as secondary hosts or reservoirs for RhPV, contributing to its horizontal transmission in aphids (GILDOW; D’ARCY, 1990; JIWAJI et al., 2019; REGELIN, 2010). In some cases, virus replication in both plants and insects has also been confirmed. Tobacco ringspot virus (genus *Nepovirus*, family *Secoviridae*) is a main example, which was found causing systemic infection in *Apis mellifera* [46-48]. Recently, the ability of an insect-infecting RNA virus from Lepidoptera to establish infection in cowpea [*Vigna unguiculata* (L.) Walp] as well as in mammalian cell culture lines has been demonstrated, providing evidence of a virus that can infect hosts of distinct kingdoms (JIWAJI et al., 2019). We also found three contigs related to family *Secoviridae*, but they were not detected in any plant within the pool, probably due to their relatively low coverage or due to index hopping, which may result in the assignment of sequencing reads to the wrong index during demultiplexing (MA et al., 2019).

Therefore, the virus detection exclusively in leaf samples of *H. dulcis* allowed us to speculate that the two new viruses described here could have a close relationship with this plant, even though, no viral movement protein was identified in the HDaV1 and HDaV2 genomes. In addition, no other plant sampled in the same area was found to be positive for either HDaV1 or HDaV2, reinforcing the hypothesis that these viruses might be exclusively associated with either *H. dulcis* or with some yet unidentified arthropod pest of this plant species. In this context, further biological assays should be performed to elucidate the interaction among *H. dulci*s and both viruses.

Supplementary Materials: Supplementary materials can be found at www.mdpi.com/xxx/s1. **Figure S1**: The 3′ ends of HDaV1 and HDaV2 genomes. **Figure S2**: Alignment of a selected region of HDaV2 and two related viruses (BaDV-1 and BaDV-2). Table S1: The accession numbers of the sequences used in the phylogeny. Table S2: The accession numbers of the virus sequences and their hosts used in the nucleotide composition analysis (NCA).

Author Contributions: Conceptualization, F.M.B.N., F.L.M., and R.C.P.-C.; data curation, F.M.B.N. and F.L.M.; formal analysis, F.M.B.N., F.L.M., and R.C.P.-C.; funding acquisition, S.G.R., R.C.P.-C., and F.L.M.; investigation, F.M.B.N., J.G.B., M.F.L., and A.F.O.; project administration, S.G.R.; resources, R.O.R. and M.F.L.; supervision, R.C.P.-C. and F.L.M.; visualization, F.M.B.N. and F.L.M.; writing-original draft, F.M.B.N., F.L.M., L.S.B., and R.C.P.-C.; writing-review and editing, F.M.B.N., F.L.M., L.S.B., S.G.R., R.O.R., A.F.O., J.G.B., M.F.L., and R.C.P.-C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by grants from Embrapa, Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq and Fundação de Apoio à Pesquisa do Distrito Federal-FAP/DF. The APC was partially funded by Universidade de Brasília.

Acknowledgments: The authors are grateful to the financial support of Capes (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), FAP-DF (Fundação de Apoio à Pesquisa do DF) and Embrapa (Empresa Brasileira de Pesquisa Agropecuária). The authors would like to thank Maria Esther de Noronha Fonseca and Antonio Francisco Costa (CNPH-Embrapa Hortaliças) and Giovana Santos Caleiro (Instituto de Medicina Tropical-IMT) for their help and support in Sanger dideoxy sequencing. We are deeply grateful to three anonymous reviewers for their invaluable suggestions.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Supplementary Materials: Supplementary materials can be found at www.mdpi.com/xxx/s1. **Figure S1**: The 3′ ends of HDaV1 and HDaV2 genomes. **Figure S2:** Alignment of a selected region of HDaV2 and two related viruses (BaDV-1 and BaDV-2). Table S1: The accession numbers of the sequences used in the phylogeny. Table S2: The accession numbers of the virus sequences and their hosts used in the nucleotide composition analysis (NCA).

Author Contributions: Conceptualization, F.M.B.N., F.L.M., and R.C.P.-C.; data curation, F.M.B.N. and F.L.M.; formal analysis, F.M.B.N., F.L.M., and R.C.P.-C.; funding acquisition, S.G.R., R.C.P.-C., and F.L.M.; investigation, F.M.B.N., J.G.B., M.F.L., and A.F.O.; project administration, S.G.R.; resources, R.O.R. and M.F.L.; supervision, R.C.P.-C. and F.L.M.; visualization, F.M.B.N. and F.L.M.; writing-original draft, F.M.B.N., F.L.M., L.S.B., and R.C.P.-C.; writing-review and editing, F.M.B.N., F.L.M., L.S.B., S.G.R., R.O.R., A.F.O., J.G.B., M.F.L., and R.C.P.-C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by grants from Embrapa, Conselho Nacional de Desenvolvimento Científico e tecnológico - CNPq and Fundação de Apoio à Pesquisa do Distrito Federal - FAP/ DF. The APC was partially funded by Universidade de Brasília.

Acknowledgments: The authors are grateful to the financial support of Capes (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), FAP-DF (Fundação de Apoio à Pesquisa do DF) and Embrapa (Empresa Brasileira de Pesquisa Agropecuária). The authors would like to thank Maria Esther de Noronha Fonseca and Antonio Francisco Costa (CNPH-Embrapa Hortaliças) and Giovana Santos Caleiro (Instituto de Medicina Tropical-IMT) for their help and support in Sanger dideoxy sequencing. We are deeply grateful to three anonymous reviewers for their invaluable suggestions.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

5. Conclusions

- Two new species were identified in *Hovenia dulcis* and were named Hovenia dulcis-associated virus - HDaV 1 and Hovenia dulcis-associated virus 2 - HDaV2, classified within the order *Picornavirales.*
- HDaV 1 and HDaV2 could have a close relationship with this plant, even though, no movement protein has been found in the HDaV1 and HDaV2 genomes.

6. References

ALTSCHUL, S. F. et al. Basic local alignment search tool. **Journal of Molecular Biology**, v. 215, n. 3, p. 403-410, 1990.

BARBA, M.; CZOSNEK, H.; HADIDI, A. Historical perspective, development and applications of next-generation sequencing in plant virology. **Viruses**, v. 6, n. 1, p. 106-136, 2014.

BEŁKA, M. Gonthier, P.; Nicolotti, G., 2013: Infectious forest diseases. Ed. by Gonthier, P.; Nicolotti, G. CABI: Wallingford, UK; Boston, MA, 641. 2015.

BESERRA JR, J. E. A. et al. Partial genome sequence of a *Potyvirus* and of a virus in the order *Tymovirales* found in *Senna macranthera* in Brazil. **Tropical Plant Pathology**, v. 36, n. 2, p. 116-120, 2012.

BONNING, B. C.; MILLER, W. A. Dicistroviruses. **Annual Review of Entomology**, v. 55, p. 129-150, 2010.

BOURGAREL, M. et al. Next-generation sequencing on insectivorous bat guano: An accurate tool to identify arthropod viruses of potential agricultural concern. **Viruses**, v. 11, n. 12, p. 1-14, 2019.

BÜTTNER, C. et al. Forest diseases caused by viruses. In: **Infectious Forest Diseases**. [s.l.] CABI: Wallingford, Oxfordshire, UK, 2013. p. 50-75.

CANDRESSE, T. et al. Appearances can be deceptive: revealing a hidden viral infection with deep sequencing in a plant quarantine context. **PloS One**, v. 9, n. 7, p. e102945, 2014.

CHEN, J.; ADAMS, M. J. A universal PCR primer to detect members of the *Potyviridae* and its use to examine the taxonomic status of several members of the family. **Archives of Virology**, v. 146, n. 4, p. 757-766, 2001.

DE BRUIJN, F. J. Handbook of molecular microbial ecology II: metagenomics in different habitats. In: [s.l.] John Wiley & Sons, 2011. p. 15-24.

FAJARDO, T. V. M. et al. High-throughput sequencing applied for the identification of viruses infecting grapevines in Brazil and genetic variability analysis. **Tropical Plant Pathology**, v. 42, n. 4, p. 250-260, 2017.

FLENNIKEN, M. L. Honey bee-infecting plant virus with implications on honey bee colony health. **MBio**, v. 5, n. 2, p. e00877-14, 2014.

FRANÇOIS, S. et al. A new prevalent densovirus discovered in Acari. Insight from metagenomics in viral communities associated with two-spotted mite (*Tetranychus urticae*) populations. **Viruses**, v. 11, n. 3, p. 233, 2019.

GALBRAITH, D. A. et al. Investigating the viral ecology of global bee communities with high-throughput metagenomics. **Scientific Reports**, v. 8, n. 1, p. 8879, 2018.

GAMA, M. et al. Um *Carlavirus* em seringueira (*Hevea brasiliensis*). **Fitopatologia Brasileira**, v. 8, p. 621, 1983.

GILDOW, F. E.; D'ARCY, C. J. Cytopathology and experimental host range of *Rhopalosiphum padi virus*, a small isometric RNA virus infecting cereal grain aphids. **Journal of Invertebrate Pathology**, v. 55, n. 2, p. 245-257, 1990.

GRUBER, A. R.; BERNHART, S. H.; LORENZ, R. The ViennaRNA web services. In: **RNA Bioinformatics**. [s.l.] Springer, 2015. p. 307-326.

GUINDON, S. et al. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. **Systematic Biology**, v. 59, n. 3, p. 307-321, 2010.

JIWAJI, M. et al. *Providence virus*: An animal virus that replicates in plants or a plant virus that infects and replicates in animal cells? **PloS One**, v. 14, n. 6, p. e0217494, 2019.

JONES, D. T.; TAYLOR, W. R.; THORNTON, J. M. The rapid generation of mutation data matrices from protein sequences. **Bioinformatics**, v. 8, n. 3, p. 275-282, 1992.

KAPOOR, A. et al. Use of nucleotide composition analysis to infer hosts for three novel picorna-like viruses. **Journal of Virology**, v. 84, n. 19, p. 10322-10328, 2010.

KEARSE, M. et al. Geneious Basic: an integrated and etendable desktop software platform for the organization and analysis of sequence data. **Bioinformatics**, v. 28, n. 12, p. 1647-1649, 2012.

KEENAN, R. J. et al. Dynamics of global forest area: Results from the FAO Global Forest Resources Assessment 2015. **Forest Ecology and Management**, v. 352, p. 9-20, 2015.

KING, A. M. Q. et al. Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses. In: **Virus taxonomy: ninth report of the International Committee on Taxonomy of Viruses**. [s.l.] Elsevier, 2011. p. 881-899.

LANG, A. S.; VLOK, M.; SUTTLE, C. A. Assigning 4 new and 2 unassigned genera to the family *Marnaviridae*. **International Committee on Taxonomy of Viruses - ICTV**, v. 2, n. June, p. 1-6, 2018.

LE GALL, O. et al. *Picornavirales*, a proposed order of positive-sense single-stranded RNA viruses with a pseudo-T= 3 virion architecture. **Archives of Virology**, v. 153, n. 4, p. 715, 2008.

LI, J. L. et al. Systemic spread and propagation of a plant-pathogenic virus in European honey bees, *Apis mellifera.* **MBio - American Society for Microbiology**, v. 5, n. 1, p. e00898-13, 2014.

LIN, M. T. et al. Properties of a possible *Carlavirus* isolated from a cerrado native plant, Cassia sylvestris. **Plant Disease**, v. 63, p. 501-505, 1979.

LIN, M. T.; KITAJIMA, E. W.; COSTA, C. L. Association of *Cassia mild mosaic virus* with dieback of *Cassia macranthera* in central Brazil. **Plant Disease**, v. 64, n. 6, p. 587-589, 1980.

LORENZ, A. R. et al. The Vienna RNA Websuite. **Nucleic Acids Research**, v. 36, p. W70-4, 2008.

LORENZ, R. et al. Vienna RNA Package 2.0. **Algorithms for Molecular Biology**, v. 6, n. 1, p. 26, 2011.

MA, Y. et al. Phytovirome analysis of wild plant populations: comparison of double-stranded RNA and virion-associated nucleic acid metagenomic approaches. **Journal of Virology**, v. 94:e01462-, n. 1, 2019.

MALIOGKA, V. I. et al. Recent advances on detection and characterization of fruit tree viruses using high-throughput sequencing technologies. **Viruses**, v. 10, n. 8, p. 436, 2018.

MELCHER, U. et al. Evidence for novel viruses by analysis of nucleic acids in virus-like particle fractions from *Ambrosia psilostachya*. **Journal of Virological Methods**, v. 152, n. 1-2, p. 49-55, 2008.

MOROZOVA, O.; MARRA, M. A. Applications of next-generation sequencing technologies in functional genomics. **Genomics**, v. 92, n. 5, p. 255-264, 2008.

NAKASU, E. Y. T. et al. Erratum to: Discovery of two small circular ssDNA viruses associated with the whitefly *Bemisia tabaci*. **Archives of Virology**, v. 162, n. 11, p. 3563-3566, 2017.

NAKASU, E. Y. T. et al. Complete genome sequence and phylogenetic analysis of a novel *Dicistrovirus* associated with the whitefly *Bemisia tabaci.* **Virus Research**, v. 260, p. 49-52, 2019.

NICOLINI, C. et al. A distinct *Tymovirus* infecting *Cassia hoffmannseggii* in Brazil. **Virus Genes**, v. 45, n. 1, p. 190-194, 2012.

NIENHAUS, F.; CASTELLO, J. D. Viruses in forest trees. **Annual Review of Phytopathology**, v. 27, n. 1, p. 165-186, 1989.

OLENDRAITE, I. et al. *Polycipiviridae*: A proposed new family of polycistronic picorna-like

RNA viruses. **Journal of General Virology**, v. 98, n. 9, p. 2368-2378, 2017.

PARDINA, P. E. R. et al. Complete nucleotide sequence of an Argentinean isolate of *Sweet potato virus* G. **Virus Genes**, v. 45, n. 3, p. 593-595, 2012.

PRABHA, K.; BARANWAL, V. K.; JAIN, R. K. Applications of next generation hgh throughput sequencing technologies in characterization, discovery and molecular interaction of plant viruses. **Indian Journal of Virology**, v. 24, n. 2, p. 157-165, 2013.

PRICE, M. N.; DEHAL, P. S.; ARKIN, A. P. FastTree: Computing large minimum evolution trees with profiles instead of a distance matrix. **Molecular Biology and Evolution**, v. 26, n. 7, p. 1641-1650, 2009.

REGELIN, Z. T. **Translation and replication of *Rhopalosiphum padi virus* RNA in a plant cellular environment**. [s.l.] Master of Science. Digital Repository Iowa State University, Ames, Iowa. 2010-04-30, 2010.

ROBERTS, J. M. K.; ANDERSON, D. L.; DURR, P. A. Metagenomic analysis of Varroa-free Australian honey bees (*Apis mellifera*) shows a diverse *Picornavirales* virome. **Journal of General Virology**, v. 99, n. 6, p. 818-826, 2018.

ROOSSINCK, M. J.; MARTIN, D. P.; ROUMAGNAC, P. Plant virus metagenomics: Advances in virus discovery. **Phytopathology**, v. 105, n. 6, p. 716-727, 2015.

ROTT, M. et al. Application of next generation sequencing for diagnostic testing of tree fruit viruses and viroids. **Plant Disease**, v. 101, n. 8, p. 1489-1499, 2017.

SHEVELEVA, A. et al. Complete genome sequence of a novel *Plum pox virus* strain W isolate determined by 454 pyrosequencing. **Virus Genes**, v. 47, n. 2, p. 385-388, 2013.

SHI, M. et al. Redefining the invertebrate RNA virosphere. **Nature**, v. 540, n. 7634, p. 539-543, 2016.

SILVA, K. N. et al. First report of *Johnsongrass mosaic virus* (JGMV) infecting *Pennisetum purpureum* in Brazil. **Plant Disease**, v. 97, n. 7, p. 1003, 2013.

SIMMONDS, P. SSE: A nucleotide and amino acid sequence analysis platform. **BMC Research Notes**, v. 5, n. 1, p. 50, 2012.

SUBRAMANIAN, B. et al. Evolview v3: a webserver for visualization, annotation, and management of phylogenetic trees. **Nucleic Acids Research**, v. 47, n. W1, p. W270-W275, 2019.

TEAM, R. C. **R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria**Vienna, AustriaR Foundation for Statistical Computing, , 2013. Disponível em: <http://www.r-project.org/>

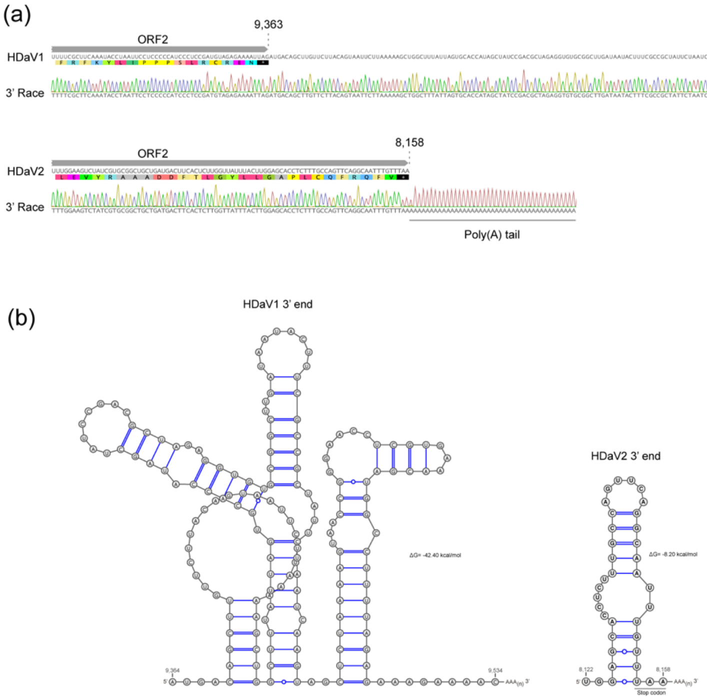
THOMPSON, J. D.; GIBSON, T. J.; HIGGINS, D. G. Multiple sequence alignment using ClustalW and ClustalX. **Current Protocols in Bioinformatics**, v. 1, n. 1, p. 2-3, 2003.

VERWOERD, T. C.; DEKKER, B. M.; HOEKEMA, A. A small-scale procedure for the rapid isolation of plant RNAs. **Nucleic Acids Research**, v. 17, n. 6, p. 2362, 1989.

Supplementary materials of paper entitled “Molecular characterization of Hovenia dulcis-associated virus 1 (HDaV1) and 2 (HDaV2): new tentative species within the order *Picornavirales*”

Materials can be also found at https://www.mdpi.com/1999-4915/12/9/950/s1. **Figure S1**: The 3′ ends of HDaV1 and HDaV2 genomes. **Figure S2**: Alignment of a selected region of HDaV2 and two related viruses (BaDV-1 and BaDV-2). Table S1: The accession numbers of the sequences used in the phylogeny. Table S2: The accession numbers of the virus sequences and their hosts used in the nucleotide composition analysis (NCA).

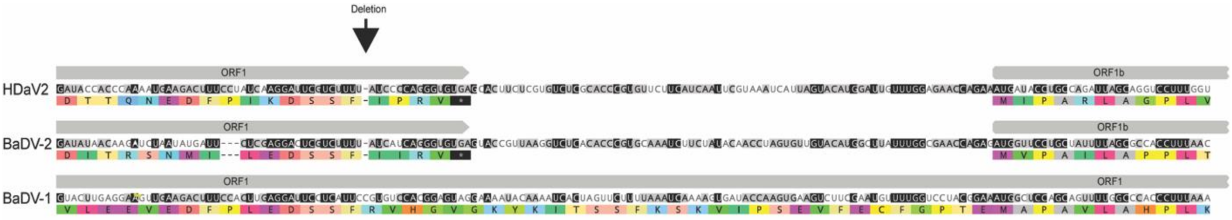
Flávia M. B. Nery, Fernando L. Melo, Leonardo S. Boiteux, Simone G. Ribeiro, Renato O. Resende, Anelise F. Orílio, Josiane G. Batista, Mirtes F. Lima and Rita C. Pereira-Carvalho.



Supplementary Figure 1. The 3’ ends of HDaV1 and HDaV2 genomes.

(a) Sanger sequencing of the 3’ RACE products.

The ORF2 is presented as a grey arrow. (b) The optimal secondary structure predicted for the 3’ends of HDaV1 and HDaV2 using the RNAfold web server (http://rna.tbi.univie.ac/cgibin/RNAWebSuit?RNAfold.cgi) (MA et al., 2019; REGELIN, 2010).



Supplementary Figure 2. Alignment of a selected region of HDaV2 and two related viruses (BaDV-1 and BaDV-2).

All ORFs are represented as arrows pointing from the 5′ to the 3′ end and are colored in grey. The protein translation is below the nucleotide sequence. The arrow shows a single nucleotide deletion that generates the two ORFs (ORF1 and ORF1b) in HDaV2 and BaDV-2.

Supplementary Table 1. Accession numbers of the sequences used in the phylogeny.

Family	**Accession**	**Organism**	**Sequence Length**
Dicistroviridae	NC_002548	Acute bee paralysis virus - ABPV	9491
Dicistroviridae	MH459180	Bemisia-associated dicistrovirus 1 - BaDV-1	8293
Dicistroviridae	MN231041	Bemisia-associated dicistrovirus 2 - BaDV-2	8052
Dicistroviridae	NC_003784	Black queen cell virus - BQCV	8550
Dicistroviridae	NC_003924	Cricket paralysis virus - CrPV	9185
Dicistroviridae	NC_009025	Israeli acute paralysis virus - IAPV	9499
Dicistroviridae	NC_004807	Kashmir bee virus - KBV	9524
Dicistroviridae	NC_014793	Mud crab dicistrovirus - MCDV	10436
Dicistroviridae	NC_003783	Triatoma virus - TrV	9010
Dicistroviridae	NC_004365	Aphid lethal paralysis virus - ALPV	9812
Iflaviridae	NC_004830	Deformed wing virus - DWV	10140
Iflaviridae	NC_023022	Formica exsecta virus 2	9160
Iflaviridae	NC_003781	Infectious flacherie virus - BmIFV	9650
Iflaviridae	NC_002066	Sacbrood virus - SBV	8832
Iflaviridae	NC_006494	Varroa destructor virus 1 - VDV-1	10112
Marnaviridae	NC_007522	Schizochytrium single-stranded RNA virus - SssRNAV	9035
Marnaviridae	NC_012212	Chaetoceros socialis f. radians RNA virus 01 - CsfrRNAV	9467
Marnaviridae	AB971661	Chaetoceros tenuissimus RNA virus type-II - CtenRNAVII	9562
Marnaviridae	NC_005281	Heterosigma akashiwo RNA virus - HaRNAV	8587
Marnaviridae	MH171300	Marine RNA virus BC-4	8593
Marnaviridae	NC_009757	Marine RNA virus JP-A	9236
Marnaviridae	NC_009758	Marine RNA virus JP-B	8926
Marnaviridae	KT727026	Marine RNA virus PAL473	6360
Marnaviridae	KF412901.2	Marine RNA virus SF-2	9321
Marnaviridae	KF478836.2	Marine RNA virus SF-3	8648
Marnaviridae	NC_018613	Rhizosolenia setigera RNA virus 01 - RsRNAV	8877
Picornaviridae	NC_026470	African bat icavirus A	8096
Picornaviridae	NC_006553	Avian sapelovirus	8289
Picornaviridae	NC_018506	Bluegill picornavirus	8050
Picornaviridae	NC_018668	Bovine hungarovirus 1 BHuV	7583
Picornaviridae	NC_023422	Caprine kobuvirus	8305
Picornaviridae	NC_008250	Duck hepatitis A virus 1 - DHAV-	7711
Picornaviridae	NC_001479	Encephalomyocarditis virus - EMCV	7835
Picornaviridae	NC_003983	Equine rhinitis B virus 1 - ERBV-1	8828
Picornaviridae	NC_004004	Foot-and-mouth disease virus - type O	8134
Picornaviridae	NC_015626	Pigeon picornavirus B - FMD	7801
Picornaviridae	NC_027054	Porcine kobuvirus - PKoV	8121
Picornaviridae	NC_009448	Saffold virus - SAFV	8115
Picornaviridae	NC_028364	Shrew hepatovirus - SrHAV	7810
Picornaviridae	NC_018400	Turkey gallivirus	8496

Picornaviridae	NC_029854	Yak enterovirus	7382
Polycipiviridae	NC_035450	Lasius neglectus virus 1 - LniV-1	11851
Polycipiviridae	NC_035456	Lasius niger virus 1 - LniV-1	11092
Polycipiviridae	NC_035457	Myrmica scabrinodis virus 1	11900
Polycipiviridae	NC_009544	Solenopsis invicta virus 2 - SINV-2	11319
Secoviridae	NC_003787	Apple latent spherical virus RNA 1 - ALSV	6812
Secoviridae	NC_003788	Apple latent spherical virus RNA 2 - ALSV	3384
Secoviridae	NC_022004	Broad bean true mosaic virus RNA 1 -	6068
Secoviridae	NC_022006	Broad bean true mosaic virus RNA 2	3532
Secoviridae	NC_025479	Carrot torradovirus 1 RNA 1	6944
Secoviridae	NC_025480	Carrot torradovirus 1 RNA 2	4995
Secoviridae	NC_029036	Currant latent virus RNA 2	3292
Secoviridae	NC_029038	Currant latent virus RNA 1	6603
Secoviridae	NC_023016	Lamium mild mosaic virus RNA 1 ´- LMMV	6080
Secoviridae	NC_023017	Lamium mild mosaic virus RNA 2 - LMMV	4065
Secoviridae	NC_011189	Mikania micrantha mosaic virus RNA 2 - MMMV	3418
Secoviridae	NC_011190	Mikania micrantha mosaic virus RNA 1 - MMMV	5862
Secoviridae	NC_035218	Motherwort yellow mottle virus RNA 1 - MYMoV	7068
Secoviridae	NC_035220	Motherwort yellow mottle virus RNA 2 - MYMoV	4963
Secoviridae	NC_010709	Radish mosaic virus RNA 1 - RaMV	6064
Secoviridae	NC_010710	Radish mosaic virus RNA 2 - RaMV	4020
Secoviridae	NC_013076	Tomato chocolate spot virus - ToCSV	5109
Secoviridae	NC_010987	Tomato marchitez virus RNA 1 - ToChV	7229
Secoviridae	NC_010988	Tomato marchitez virus RNA 2 - ToChV	4906
Secoviridae	NC_003839	Tomato ringspot virus RNA 2 - TomRSV	7271
Secoviridae	NC_003840	Tomato ringspot virus RNA 1- TomRSV	8214
Secoviridae	NC_009013	Tomato torrado virus RNA 1	7808
Secoviridae	NC_009032	Tomato torrado virus RNA 2	5403
Secoviridae	NC_001632	Rice tungro spherical virus RTSV	12226
unknow	MT079817	Hovenia dulcis-associated virus 1 HDaV1	9534
unknow	MT079818	Hovenia dulcis-associated virus 2 HDaV2	8126
unknow	MG995696	Darwin bee virus 6 - DBV	9123
unknow	NC_033212	Hubei picorna-like virus 78	9440
unknow	NC_033160	Hubei picorna-like virus 79	9186
unknow	NC_033141	Hubei picorna-like virus 80	6021
	MK533158	Tetranychus urticae-associated picorna-like virus 2	6432

Supplementary Table 2. The accession numbers of the virus sequences and their hosts used in the nucleotide composition analysis (NCA).

Acession Number	Virus	Host	ApA	ApC	ApG	ApT	CpA	CpC	CpG	CpT	GpA	GpC	GpG	GpT	TpA	TpC	TpG	TpT
NC_009013/ NC_009032	Tomato torrado virus	Plants	0.0792	0.0446	0.0592	0.0744	0.0540	0.0460	0.0201	0.0837	0.0596	0.0451	0.0471	0.0517	0.0646	0.0682	0.0771	0.1253
AB971661	Chaetoceros tenuissimus RNA virus	Others	0.0842	0.0516	0.0689	0.0749	0.0712	0.0387	0.0371	0.0578	0.0912	0.0473	0.0501	0.0549	0.0332	0.0671	0.0874	0.0844
African_bat_	African bat icavirus A	Vertebrates	0.0776	0.0649	0.0458	0.0594	0.0784	0.0773	0.0274	0.0862	0.0551	0.0494	0.0508	0.0484	0.0367	0.0778	0.0797	0.0850
KF412901	Marine RNA virus SF-2	Others	0.0643	0.0547	0.0582	0.0706	0.0676	0.0467	0.0444	0.0540	0.0722	0.0540	0.0520	0.0745	0.0437	0.0573	0.0980	0.0880
KT727026	Marine RNA virus PAL473	Others	0.0816	0.0613	0.0612	0.0804	0.0727	0.0426	0.0374	0.0554	0.0653	0.0469	0.0480	0.0588	0.0649	0.0572	0.0723	0.0940
MG995692	Darwin bee virus 5	Others	0.1022	0.0514	0.0527	0.0997	0.0583	0.0339	0.0282	0.0499	0.0643	0.0374	0.0413	0.0590	0.0814	0.0475	0.0797	0.1133
MG995693	Darwin bee virus 1	Others	0.1205	0.0536	0.0571	0.1081	0.0614	0.0224	0.0234	0.0493	0.0710	0.0326	0.0330	0.0531	0.0865	0.0478	0.0763	0.1039
MG995694	Darwin bee virus 2	Others	0.1045	0.0534	0.0679	0.1027	0.0601	0.0295	0.0237	0.0489	0.0687	0.0343	0.0456	0.0531	0.0950	0.0450	0.0645	0.1030
MG995695	Darwin bee virus 8	Others	0.0844	0.0516	0.0689	0.0749	0.0712	0.0387	0.0371	0.0578	0.0912	0.0473	0.0501	0.0549	0.0332	0.0671	0.0873	0.0844
MG995696	Darwin bee virus 6	Others	0.0981	0.0488	0.0582	0.1064	0.0535	0.0274	0.0206	0.0561	0.0729	0.0284	0.0369	0.0568	0.0872	0.0529	0.0793	0.1164
MG995697	Darwin bee virus 3	Others	0.0853	0.0484	0.0686	0.1004	0.0512	0.0329	0.0309	0.0495	0.0712	0.0382	0.0420	0.0687	0.0949	0.0451	0.0785	0.0942
MG995698	Darwin bee virus 7	Others	0.1091	0.0668	0.0685	0.0927	0.0834	0.0344	0.0256	0.0503	0.0700	0.0415	0.0394	0.0495	0.0745	0.0510	0.0669	0.0763
MG995699	Darwin bee virus 4	Others	0.0849	0.0560	0.0697	0.0884	0.0652	0.0345	0.0335	0.0484	0.0775	0.0435	0.0593	0.0562	0.0715	0.0476	0.0739	0.0898
MH171300	Marine RNA virus BC-4	Others	0.1034	0.0518	0.0655	0.0934	0.0695	0.0352	0.0170	0.0447	0.0731	0.0406	0.0479	0.0584	0.0681	0.0387	0.0897	0.1032
MH459180	Bemisia-associated dicistrovirus 1	Insects	0.1008	0.0572	0.0599	0.0893	0.0686	0.0355	0.0346	0.0454	0.0710	0.0359	0.0415	0.0580	0.0668	0.0555	0.0704	0.1095

MK533158	Tetranychus urticae-associated picorna-like virus 2	Insects	0.1074	0.0537	0.0610	0.1030	0.0527	0.0258	0.0247	0.0475	0.0840	0.0286	0.0454	0.0517	0.0809	0.0428	0.0786	0.1122
MN231041	Bemisia-associated_dicistrovirus_2	Insects	0.1067	0.0574	0.0529	0.0925	0.0645	0.0406	0.0288	0.0558	0.0666	0.0356	0.0389	0.0507	0.0719	0.0560	0.0712	0.1099
NC_001366	Theilovirus	Vertebrates	0.0694	0.0681	0.0480	0.0541	0.0698	0.0844	0.0389	0.0815	0.0620	0.0551	0.0511	0.0486	0.0385	0.0670	0.0788	0.0847
NC_001430	Enterovirus D	Vertebrates	0.0926	0.0662	0.0763	0.0769	0.0867	0.0544	0.0129	0.0557	0.0683	0.0459	0.0517	0.0524	0.0643	0.0433	0.0773	0.0749
NC_001479	Encephalomyocarditis virus	Vertebrates	0.0725	0.0582	0.0679	0.0583	0.0712	0.0892	0.0314	0.0673	0.0711	0.0516	0.0606	0.0522	0.0421	0.0601	0.0757	0.0705
NC_001489	Hepatovirus A	Vertebrates	0.0926	0.0396	0.0690	0.0915	0.0655	0.0318	0.0052	0.0582	0.0786	0.0306	0.0543	0.0543	0.0559	0.0587	0.0893	0.1248
NC_001490	Rhinovirus B14	Vertebrates	0.0994	0.0684	0.0674	0.0857	0.0892	0.0474	0.0122	0.0523	0.0639	0.0338	0.0501	0.0569	0.0684	0.0514	0.0750	0.0785
NC_001612	Enterovirus A	Vertebrates	0.0698	0.0668	0.0672	0.0693	0.0858	0.0646	0.0289	0.0622	0.0642	0.0549	0.0634	0.0553	0.0533	0.0553	0.0784	0.0606
NC_001617	Rhinovirus A	Vertebrates	0.0951	0.0610	0.0656	0.0917	0.0762	0.0471	0.0095	0.0604	0.0611	0.0337	0.0432	0.0592	0.0810	0.0515	0.0789	0.0849
NC_001632	Rice tungro spherical virus	Plants	0.0793	0.0510	0.0744	0.0739	0.0690	0.0409	0.0357	0.0510	0.0843	0.0569	0.05	0.0602	0.0461	0.0479	0.0912	0.0788
NC_001834	Drosophila C virus	Insects	0.1014	0.0486	0.0516	0.0978	0.0519	0.0302	0.0273	0.0530	0.0706	0.0315	0.0416	0.0601	0.0755	0.0521	0.0833	0.1234
NC_001874	Rhopalosiphum padi virus	Insects	0.0949	0.0579	0.0523	0.0951	0.0563	0.0374	0.0409	0.0509	0.0615	0.0433	0.0374	0.0601	0.0875	0.0471	0.0716	0.1057
NC_001897	Human parechovirus	Vertebrates	0.1022	0.0630	0.0672	0.0904	0.0815	0.0437	0.0091	0.0534	0.0683	0.0340	0.0535	0.0512	0.0709	0.0470	0.0772	0.0874
NC_001918	Aichi virus 1	Vertebrates	0.0495	0.0737	0.0328	0.0389	0.0785	0.1436	0.0605	0.0949	0.0450	0.0642	0.0587	0.0435	0.0219	0.0961	0.0594	0.0387
NC_002066	Sacbrood virus	Insects	0.0866	0.0502	0.0705	0.0912	0.0498	0.0349	0.0339	0.0445	0.0738	0.0402	0.0628	0.0662	0.0882	0.0378	0.0759	0.0934
NC_002548	Acute bee paralysis virus	Insects	0.1295	0.0557	0.0688	0.1029	0.0636	0.0295	0.0197	0.0411	0.0813	0.0280	0.0414	0.0500	0.0825	0.0406	0.0707	0.0947
NC_003003/ NC_003004	Broad bean wilt virus 2	Plants	0.0907	0.0396	0.0721	0.0795	0.0700	0.0339	0.0257	0.0466	0.0739	0.0547	0.0624	0.0597	0.0473	0.0480	0.0906	0.1053
NC_003005	Taura syndrome virus	Insects	0.0739	0.0536	0.0680	0.0845	0.0679	0.0450	0.0359	0.0533	0.0710	0.0480	0.0454	0.0661	0.0672	0.0554	0.0811	0.0838

NC_003113	Perina nuda virus	Insects	0.0735	0.0644	0.0703	0.0748	0.0602	0.0427	0.0385	0.0502	0.0781	0.0443	0.0632	0.0642	0.0712	0.0403	0.0778	0.0862
NC_003445/ NC_003446	Strawberry mottle virus	Plants	0.0767	0.0560	0.0571	0.0690	0.0637	0.0571	0.0346	0.0642	0.0717	0.0422	0.0627	0.0592	0.0467	0.0642	0.0815	0.0934
NC_003496/ NC_003495	Bean pod mottle virus	Plants	0.1028	0.0373	0.0624	0.0843	0.0663	0.0294	0.0124	0.0618	0.0672	0.0410	0.0450	0.0615	0.0505	0.0622	0.0949	0.1208
NC_003509/ NC_003502	Blackcurrant reversion virus	Plants	0.0667	0.0473	0.0587	0.0718	0.0598	0.0515	0.0377	0.0666	0.0605	0.0574	0.0592	0.0648	0.0575	0.0594	0.0864	0.0947
NC_003545/ NC_003544	Cowpea severe mosaic virus	Plants	0.0912	0.0512	0.0656	0.0827	0.0748	0.0327	0.0193	0.0580	0.0690	0.0479	0.0564	0.0587	0.0557	0.0531	0.0906	0.0930
NC_003549/ NC_003550	Cowpea mosaic virus	Plants	0.0824	0.0427	0.0669	0.0758	0.0651	0.0351	0.0184	0.0652	0.0709	0.0479	0.0576	0.0620	0.0494	0.0581	0.0955	0.1069
NC_003615/ NC_003623	Grapevine Fanleaf virus	Plants	0.0789	0.0442	0.0721	0.0650	0.0586	0.0496	0.0271	0.0646	0.0695	0.0566	0.0677	0.0596	0.0531	0.0495	0.0866	0.0973
NC_003622/ NC_003621	Grapevine chrome mosaic virus	Plants	0.0811	0.0435	0.0559	0.0754	0.0638	0.0561	0.0285	0.0673	0.0642	0.0558	0.0639	0.0543	0.0469	0.0603	0.0899	0.0931
NC_003626	Maize chlorotic dwarf virus	Plants	0.0992	0.0548	0.0769	0.0803	0.0687	0.0372	0.0273	0.0538	0.0849	0.0414	0.0537	0.0555	0.0584	0.0537	0.0775	0.0767
NC_003628	Parsnip yellow fleck virus	Plants	0.0901	0.0491	0.0768	0.0715	0.0733	0.0453	0.0202	0.0633	0.0774	0.0491	0.0585	0.0468	0.0468	0.0586	0.0764	0.0969
NC_003693/ NC_003694	Beet ringspot virus	Plants	0.0820	0.0449	0.0551	0.0749	0.0589	0.0533	0.0272	0.0702	0.0663	0.0533	0.0626	0.0548	0.0497	0.0581	0.0922	0.0963
NC_003741/ NC_003738	Red clover mottle virus	Plants	0.0889	0.0510	0.0722	0.0793	0.0738	0.0357	0.0139	0.0609	0.0679	0.0461	0.0543	0.0593	0.0607	0.0516	0.0872	0.0973
NC_003779	Plautia stali intestine virus	Insects	0.1018	0.0492	0.0672	0.0945	0.0556	0.0364	0.0210	0.0572	0.0666	0.0294	0.0387	0.0592	0.0887	0.0553	0.0670	0.1123
NC_003781	Infectious flacherie virus	Insects	0.0764	0.0500	0.0626	0.0900	0.0484	0.0374	0.0431	0.0405	0.0833	0.0459	0.0637	0.0661	0.0708	0.0362	0.0896	0.0960
NC_003782	Himetobi P virus	Insects	0.0959	0.0460	0.0644	0.0860	0.0547	0.0443	0.0247	0.0630	0.0727	0.0355	0.0358	0.0650	0.0691	0.0609	0.0840	0.0980
NC_003783	Triatoma virus	Insects	0.0937	0.0400	0.0585	0.0949	0.0427	0.0294	0.0189	0.0700	0.0679	0.0293	0.0410	0.0599	0.0827	0.0624	0.0798	0.1289
NC_003784	Black queen cell virus	Insects	0.0901	0.0536	0.0655	0.0829	0.0546	0.0358	0.0332	0.0616	0.0733	0.0333	0.0482	0.0615	0.0740	0.0626	0.0695	0.1001
NC_003785/ NC_003786	Satsuma dwarf virus	Plants	0.0670	0.0436	0.0605	0.0695	0.0507	0.0552	0.0474	0.0717	0.0699	0.0612	0.0643	0.0522	0.0530	0.0650	0.0755	0.0935

NC_003791/ NC_003792	Cycas necrotic stunt virus	Plants	0.0829	0.0447	0.0645	0.0687	0.0635	0.0510	0.0257	0.0626	0.0676	0.0600	0.0600	0.0584	0.0468	0.0472	0.0957	0.1007
NC_003799/ NC_003800	Squash mosaic virus	Plants	0.0846	0.0475	0.0634	0.0736	0.0645	0.0426	0.0233	0.0617	0.0676	0.0510	0.0554	0.0587	0.0523	0.0511	0.0906	0.1121
NC_003840/ NC_003839	Tomato ringspot virus	Plants	0.0650	0.0403	0.0626	0.0647	0.0561	0.0575	0.0427	0.0670	0.0586	0.0664	0.0626	0.0610	0.0527	0.0592	0.0807	0.1029
NC_003924	Cricket paralysis virus	Insects	0.1112	0.0647	0.0673	0.0830	0.0672	0.0404	0.0286	0.0480	0.0801	0.0367	0.0414	0.0507	0.0676	0.0425	0.0716	0.0990
NC_003976	Ljungan virus	Vertebrates	0.0904	0.0551	0.0598	0.0797	0.0772	0.0502	0.0137	0.0614	0.0684	0.0452	0.0593	0.0498	0.0490	0.0522	0.0897	0.0988
NC_003982	Porcine enterovirus 9	Vertebrates	0.0755	0.0575	0.0601	0.0563	0.0746	0.0553	0.0292	0.0652	0.0623	0.0613	0.0582	0.0587	0.0370	0.0503	0.0930	0.1054
NC_003983	Equine rhinitis B virus 1	Vertebrates	0.0649	0.0709	0.0549	0.0492	0.0784	0.0687	0.0403	0.0702	0.0604	0.0593	0.0532	0.0573	0.0363	0.0589	0.0817	0.0954
NC_003985	Teschovirus A	Vertebrates	0.0772	0.0632	0.0640	0.0642	0.0779	0.0562	0.0211	0.0630	0.0720	0.0558	0.0563	0.0533	0.0414	0.0433	0.0960	0.0951
NC_003987	Porcine sapelovirus 1	Vertebrates	0.0842	0.0619	0.0724	0.0864	0.0756	0.0537	0.0095	0.0523	0.0681	0.0381	0.0563	0.0567	0.0769	0.0375	0.0810	0.0893
NC_003988	Enterovirus H	Vertebrates	0.0921	0.0541	0.0669	0.0860	0.0753	0.0488	0.0229	0.0486	0.0669	0.0490	0.0612	0.0568	0.0650	0.0435	0.0828	0.0800
NC_003990	Tremovirus A	Vertebrates	0.0692	0.0533	0.0709	0.0764	0.0713	0.0430	0.0241	0.0520	0.0744	0.0447	0.0672	0.0707	0.0550	0.0495	0.0948	0.0835
NC_004004	Foot-and-mouth disease virus	Vertebrates	0.0674	0.0884	0.0574	0.0321	0.0773	0.0784	0.0723	0.0627	0.0734	0.0642	0.0679	0.0566	0.0273	0.0598	0.0644	0.0504
NC_004365	Aphid lethal paralysis virus	Insects	0.1014	0.0689	0.0479	0.0943	0.0592	0.0349	0.0408	0.0594	0.0673	0.0407	0.0338	0.0497	0.0846	0.0498	0.0690	0.0983
NC_004421	Aichivirus B	Vertebrates	0.0506	0.0717	0.0370	0.0423	0.0726	0.1047	0.0516	0.0998	0.0502	0.0614	0.0571	0.0486	0.0282	0.0911	0.0715	0.0615
NC_004439/ NC_004440	Tomato black ring virus	Plants	0.0815	0.0438	0.0570	0.0750	0.0590	0.0540	0.0272	0.0693	0.0669	0.0538	0.0614	0.0541	0.0500	0.0580	0.0906	0.0986
NC_004441	Equine rhinitis A virus	Vertebrates	0.0755	0.0673	0.0758	0.0690	0.0841	0.0582	0.0225	0.0566	0.0711	0.0494	0.0605	0.0546	0.0570	0.0464	0.0768	0.0753
NC_004451	Simian sapelovirus	Vertebrates	0.0868	0.0506	0.0640	0.0889	0.0639	0.0496	0.0106	0.0644	0.0684	0.0346	0.0554	0.0571	0.0711	0.0535	0.0855	0.0956
NC_004807	Kashmir bee virus	Insects	0.1133	0.0619	0.0663	0.0964	0.0642	0.0354	0.0272	0.0478	0.0824	0.0327	0.0369	0.0501	0.0780	0.0446	0.0717	0.0913

NC_004830	Deformed wing virus	Insects	0.0858	0.0426	0.0626	0.1037	0.0504	0.0290	0.0336	0.0452	0.0699	0.0404	0.0444	0.0706	0.0888	0.0462	0.0845	0.1024
NC_005092	Ectropis obliqua picorna-like virus	Insects	0.0771	0.0620	0.0674	0.0780	0.0637	0.0451	0.0384	0.0496	0.0758	0.0476	0.0633	0.0617	0.0679	0.0422	0.0793	0.0808
NC_005097/ NC_005096	Tobacco ringspot virus	Plants	0.0722	0.0440	0.0560	0.0651	0.0601	0.0586	0.0324	0.0693	0.0606	0.0608	0.0638	0.0617	0.0444	0.0572	0.0947	0.0992
NC_005266/ NC_005267	Raspberry ringspot virus	Plants	0.0643	0.0468	0.0576	0.0679	0.0611	0.0538	0.0359	0.0672	0.0641	0.0564	0.0682	0.0676	0.0470	0.0611	0.0947	0.0863
NC_005281	Heterosigma akashiwo RNA virus	Others	0.0777	0.0544	0.0594	0.0652	0.0621	0.0468	0.0516	0.0515	0.0692	0.0592	0.0599	0.0685	0.0478	0.0517	0.0858	0.0893
NC_006057/ NC_006056	Arabis mosaic virus	Plants	0.0802	0.0429	0.0656	0.0726	0.0585	0.0499	0.0272	0.0672	0.0666	0.0572	0.0616	0.0573	0.0559	0.0527	0.0883	0.0963
NC_006271/ NC_006272	Cherry rasp leaf virus	Plants	0.0706	0.0452	0.0654	0.0655	0.0550	0.0510	0.0234	0.0810	0.0599	0.0531	0.0533	0.0573	0.0613	0.0611	0.0815	0.1154
NC_006271/ NC_006272	Cherry rasp leaf virus	Plants	0.0706	0.0522	0.0634	0.0575	0.0592	0.0539	0.0313	0.0852	0.0630	0.0541	0.0579	0.0543	0.0510	0.0696	0.0768	0.0998
NC_006494	Varroa destructor virus-1	Insects	0.0842	0.0434	0.0631	0.1015	0.0491	0.0297	0.0368	0.0443	0.0695	0.0412	0.0432	0.0720	0.0894	0.0455	0.0829	0.1042
NC_006553	Avian sapelovirus	Vertebrates	0.0960	0.0609	0.0742	0.0766	0.0826	0.0434	0.0103	0.0537	0.0802	0.0376	0.0596	0.0595	0.0490	0.0479	0.0929	0.0754
NC_006964/ NC_006965	Strawberry latent ringspot virus	Plants	0.0633	0.0483	0.0645	0.0646	0.0593	0.0534	0.0225	0.0814	0.0685	0.0483	0.0656	0.0570	0.0495	0.0665	0.0869	0.1006
NC_007522	Aurantiochytrium single-stranded RNA virus	Others	0.0601	0.0705	0.0573	0.0544	0.0670	0.0636	0.0594	0.0710	0.0705	0.0583	0.0527	0.0547	0.0447	0.0685	0.0667	0.0805
NC_008029	Homalodisca coagulata virus	Insects	0.0875	0.0750	0.0633	0.0640	0.0843	0.0528	0.0392	0.0632	0.0731	0.0472	0.0437	0.0498	0.0450	0.0644	0.0676	0.0800
NC_008182/ NC_008183	Black raspberry necrosis virus	Plants	0.0711	0.0553	0.0698	0.0708	0.0732	0.0499	0.0323	0.0618	0.0779	0.0492	0.0748	0.0518	0.0448	0.0628	0.0768	0.0775
NC_008250	Duck hepatitis A virus 1	Vertebrates	0.0853	0.0593	0.0652	0.0747	0.0792	0.0518	0.0141	0.0607	0.0691	0.0429	0.0613	0.0559	0.0510	0.0519	0.0886	0.0888
NC_008714	Possum enterovirus W1	Vertebrates	0.0762	0.0669	0.0648	0.0712	0.0774	0.0636	0.0298	0.0597	0.0674	0.0516	0.0566	0.0544	0.0581	0.0485	0.0788	0.0752
NC_009013/ NC_003788	Apple latent spherical virus segment	Plants	0.0792	0.0520	0.0675	0.0716	0.0747	0.0466	0.0210	0.0626	0.0626	0.0519	0.0595	0.0625	0.0540	0.0543	0.0885	0.0914
NC_009025	Israel acute paralysis virus	Insects	0.1135	0.0552	0.0635	0.0939	0.0563	0.0363	0.0253	0.0535	0.0798	0.0322	0.0397	0.0563	0.0765	0.0477	0.0795	0.0908

NC_009448	Saffold virus	Vertebrates	0.1033	0.0690	0.0573	0.0629	0.0767	0.0544	0.0269	0.0695	0.0638	0.0410	0.0461	0.0504	0.0488	0.0630	0.0711	0.0959
NC_009530	Brevicoryne brassicae picorna-like virus	Insects	0.1070	0.0420	0.0587	0.1061	0.0416	0.0343	0.0221	0.0610	0.0514	0.0288	0.0309	0.0655	0.1141	0.0538	0.0648	0.1178
NC_009544	Solenopsis invicta virus 2	Others	0.0780	0.0539	0.0617	0.0865	0.0691	0.0438	0.0336	0.0546	0.0722	0.0475	0.0535	0.0571	0.0609	0.0558	0.0816	0.0903
NC_009757	Marine RNA virus JP-A	Others	0.0787	0.0457	0.0554	0.0909	0.0620	0.0415	0.0277	0.0624	0.0670	0.0447	0.0486	0.0594	0.0629	0.0617	0.0880	0.1032
NC_009758	Marine RNA virus JP-B	Others	0.1063	0.0529	0.0485	0.0999	0.0608	0.0315	0.0311	0.0558	0.0636	0.0351	0.0409	0.0571	0.0770	0.0598	0.0762	0.1033
NC_009891	Seal picornavirus type 1	Vertebrates	0.0727	0.0503	0.0552	0.0747	0.0615	0.0573	0.0234	0.0729	0.0615	0.0503	0.0456	0.0645	0.0575	0.0572	0.0977	0.0978
NC_009996	Rhinovirus C	Vertebrates	0.0897	0.0772	0.0648	0.0773	0.0897	0.0561	0.0141	0.0606	0.0587	0.0409	0.0514	0.0565	0.0710	0.0464	0.0772	0.0683
NC_010354	Bovine rhinitis B virus	Vertebrates	0.0847	0.0888	0.0637	0.0474	0.0835	0.0552	0.0289	0.0727	0.0672	0.0398	0.0499	0.0584	0.0492	0.0563	0.0729	0.0814
NC_010354	Bovine rhinitis B virus	Vertebrates	0.0847	0.0754	0.0688	0.0676	0.0874	0.0587	0.0289	0.0585	0.0687	0.0468	0.0596	0.0548	0.0558	0.0526	0.0727	0.0590
NC_010415	Enterovirus J	Vertebrates	0.0856	0.0690	0.0709	0.0702	0.0878	0.0558	0.0221	0.0525	0.0672	0.0493	0.0584	0.0600	0.0553	0.0441	0.0834	0.0682
NC_010709/ NC_010710	Radish mosaic virus	Plants	0.0839	0.0513	0.0738	0.0726	0.0671	0.0365	0.0281	0.0535	0.0795	0.0493	0.0570	0.0610	0.0510	0.0481	0.0880	0.0994
NC_010987/ NC_010988	Tomato marchitez virus	Plants	0.0867	0.0469	0.0711	0.0811	0.0776	0.0405	0.0178	0.0550	0.0682	0.0452	0.0547	0.0622	0.0532	0.0583	0.0868	0.0947
NC_011190/ NC_011189	Mikania micrantha mosaic virus	Plants	0.1058	0.0432	0.0658	0.0822	0.0620	0.0277	0.0211	0.0582	0.0737	0.0496	0.0541	0.0546	0.0557	0.0485	0.0909	0.1068
NC_011349	Seneca valley virus	Vertebrates	0.0699	0.0694	0.0498	0.0493	0.0681	0.0830	0.0480	0.0817	0.0644	0.0565	0.0638	0.0486	0.0360	0.0720	0.0717	0.0679
NC_011829	Porcine kobuvirus swine/S-1-HUN/2007/Hungary	Vertebrates	0.0514	0.0736	0.0350	0.0447	0.0703	0.0954	0.0403	0.1093	0.0514	0.0535	0.0557	0.0482	0.0316	0.0929	0.0778	0.0689
NC_012212	Chaetoceros socialis f. radians RNA virus	Others	0.0817	0.0474	0.0568	0.0934	0.0514	0.0363	0.0344	0.0600	0.0714	0.0377	0.0452	0.0593	0.0747	0.0608	0.0771	0.1122
NC_012798	Cosavirus E	Vertebrates	0.1032	0.0682	0.0599	0.0673	0.0768	0.0514	0.0178	0.0682	0.0684	0.0410	0.0474	0.0483	0.0505	0.0534	0.0801	0.0980
NC_012801	Human cosavirus B	Vertebrates	0.0876	0.0689	0.0600	0.0644	0.0761	0.0621	0.0174	0.0740	0.0640	0.0419	0.0507	0.0517	0.0533	0.0567	0.0803	0.0911

NC_012957	Salivirus NG-J1	Vertebrates	0.0405	0.0680	0.0287	0.0415	0.0732	0.1260	0.0522	0.1085	0.0408	0.0639	0.0549	0.0474	0.0242	0.1020	0.0712	0.0570
NC_012986	Salivirus A	Vertebrates	0.0401	0.0680	0.0299	0.0398	0.0705	0.1268	0.0527	0.1101	0.0424	0.0639	0.0528	0.0477	0.0248	0.1014	0.0712	0.0577
NC_013075/ NC_013076	Tomato chocolate spot virus	Plants	0.0864	0.0515	0.0684	0.0717	0.0783	0.0410	0.0221	0.0597	0.0665	0.0491	0.0557	0.0633	0.0469	0.0594	0.0884	0.0913
NC_013218/ NC_013219	Turnip ringspot virus	Plants	0.0868	0.0526	0.0754	0.0783	0.0717	0.0320	0.0231	0.0509	0.0778	0.0465	0.0518	0.0608	0.0567	0.0467	0.0865	0.1024
NC_013695	Enterovirus J	Vertebrates	0.0869	0.0623	0.0691	0.0775	0.0866	0.0518	0.0180	0.0529	0.0663	0.0453	0.0537	0.0594	0.0561	0.0499	0.0838	0.0805
NC_014137	Slow bee paralysis virus	Insects	0.0867	0.0386	0.0719	0.1057	0.0497	0.0296	0.0248	0.0470	0.0698	0.0411	0.0453	0.0674	0.0969	0.0418	0.0816	0.1020
NC_014411	Passerivirus A1	Vertebrates	0.0523	0.0668	0.0365	0.0424	0.0713	0.1479	0.0558	0.0968	0.0497	0.0672	0.0497	0.0410	0.0249	0.0899	0.0656	0.0423
NC_014412	Oscivirus A1	Vertebrates	0.0665	0.0445	0.0580	0.0653	0.0579	0.0772	0.0217	0.0747	0.0609	0.0513	0.0644	0.0611	0.0492	0.0584	0.0936	0.0953
NC_014413	Oscivirus A2	Vertebrates	0.0718	0.0535	0.0612	0.0679	0.0693	0.0617	0.0186	0.0802	0.0619	0.0537	0.0681	0.0525	0.0516	0.0610	0.0881	0.0789
NC_014793	Mud crab dicistrovirus	Insects	0.0671	0.0471	0.0474	0.0839	0.0498	0.0402	0.0250	0.0829	0.0558	0.0413	0.0435	0.0682	0.0729	0.0694	0.0928	0.1126
NC_015414/ NC_015415	Cherry leaf roll virus	Plants	0.0602	0.0458	0.0635	0.0588	0.0573	0.0667	0.0464	0.0669	0.0583	0.0661	0.0726	0.0639	0.0524	0.0588	0.0785	0.0838
NC_015492/ NC_015493	Grapevine Bulgarian latent virus	Plants	0.0728	0.0499	0.0633	0.0660	0.0596	0.0544	0.0398	0.0591	0.0680	0.0574	0.0695	0.0626	0.0517	0.0511	0.0849	0.0900
NC_015626	Pigeon picornavirus B	Vertebrates	0.0703	0.0684	0.0660	0.0725	0.0839	0.0548	0.0285	0.0524	0.0707	0.0490	0.0622	0.0606	0.0524	0.0472	0.0859	0.0752
NC_015934	Bat picornavirus 3	Vertebrates	0.0683	0.0683	0.0570	0.0607	0.0867	0.0780	0.0373	0.0675	0.0622	0.0556	0.0620	0.0529	0.0372	0.0676	0.0764	0.0623
NC_015936	Mouse kobuvirus	Vertebrates	0.0538	0.0669	0.0387	0.0416	0.0760	0.1385	0.0511	0.0910	0.0481	0.0610	0.0582	0.0472	0.0232	0.0903	0.0664	0.0482
NC_015940	Bat picornavirus 1	Vertebrates	0.0728	0.0428	0.0569	0.0770	0.0654	0.0642	0.0231	0.0602	0.0640	0.0578	0.0550	0.0587	0.0475	0.0481	0.1004	0.1062
NC_015941	Bat picornavirus 2	Vertebrates	0.0784	0.0464	0.0644	0.0840	0.0663	0.0575	0.0174	0.0564	0.0673	0.0472	0.0592	0.0598	0.0612	0.0465	0.0924	0.0956
NC_016405	Spodoptera exigua iflavirus	Insects	0.0854	0.0555	0.0416	0.0916	0.0474	0.0498	0.0283	0.0769	0.0631	0.0388	0.0311	0.0555	0.0781	0.0584	0.0875	0.1111

NC_016443/ NC_016444	Chocolate lily virus A	Plants	0.0767	0.0512	0.0542	0.0751	0.0696	0.0469	0.0302	0.0663	0.0669	0.0521	0.0524	0.0570	0.0442	0.0627	0.0916	0.1028
NC_016769	Porcine kobuvirus SH-W-CHN/2010/China	Vertebrates	0.0510	0.0737	0.0358	0.0463	0.0714	0.0959	0.0395	0.1073	0.0514	0.0517	0.0534	0.0501	0.0330	0.0929	0.0778	0.0688
NC_016964	Canine picornavirus	Others	0.0905	0.0401	0.0700	0.0915	0.0698	0.0493	0.0091	0.0542	0.0736	0.0379	0.0521	0.0605	0.0581	0.0552	0.0930	0.0950
NC_017939/ NC_017938	Grapevine deformation virus	Plants	0.0769	0.0466	0.0698	0.0665	0.0582	0.0500	0.0276	0.0645	0.0670	0.0549	0.0682	0.0610	0.0576	0.0488	0.0855	0.0971
NC_018226	Pasivirus A1	Vertebrates	0.0678	0.0463	0.0525	0.0804	0.0609	0.0435	0.0252	0.0629	0.0599	0.0531	0.0557	0.0697	0.0586	0.0496	0.1048	0.1092
NC_018383/ NC_018384	Grapevine Anatolian ringspot virus	Plants	0.0673	0.0456	0.0610	0.0652	0.0622	0.0629	0.0305	0.0686	0.0678	0.0595	0.0739	0.0539	0.0417	0.0563	0.0897	0.0937
NC_018400	Turkey gallivirus	Vertebrates	0.0650	0.0533	0.0413	0.0673	0.0589	0.1001	0.0306	0.0890	0.0569	0.0504	0.0465	0.0505	0.0463	0.0747	0.0859	0.0833
NC_018506	Bluegill picornavirus	Vertebrates	0.0844	0.0704	0.0762	0.0723	0.0806	0.0429	0.0224	0.0451	0.0975	0.0440	0.0533	0.0544	0.0408	0.0337	0.0974	0.0847
NC_018570	Macrobrachium rosenbergii Taihu virus	Insects	0.0747	0.0414	0.0613	0.0852	0.0511	0.0404	0.0277	0.0622	0.0627	0.0424	0.0414	0.0763	0.0743	0.0571	0.0925	0.1093
NC_018613	Rhizosolenia setigera RNA virus 01	Others	0.1033	0.0506	0.0576	0.1004	0.0538	0.0260	0.0281	0.0595	0.0702	0.0344	0.0368	0.0541	0.0846	0.0563	0.0730	0.1112
NC_018668	Bovine hungarovirus 1	Vertebrates	0.0772	0.0566	0.0618	0.0742	0.0772	0.0600	0.0173	0.0684	0.0698	0.0527	0.0516	0.0510	0.0458	0.0534	0.0944	0.0885
NC_020898/ NC_020897	Arracacha virus B	Plants	0.0817	0.0459	0.0585	0.0731	0.0602	0.0467	0.0220	0.0772	0.0626	0.0490	0.0564	0.0508	0.0549	0.0644	0.0819	0.1148
NC_021178	Canine picodicistrovirus	Vertebrates	0.0763	0.0551	0.0488	0.0783	0.0460	0.0552	0.0246	0.0859	0.0642	0.0358	0.0441	0.0602	0.0721	0.0656	0.0868	0.1010
NC_021220	Enterovirus F	Vertebrates	0.0645	0.0679	0.0656	0.0577	0.0799	0.0723	0.0434	0.0615	0.0680	0.0581	0.0653	0.0567	0.0433	0.0588	0.0738	0.0631
NC_021482	Sebokele virus 1	Vertebrates	0.0717	0.0551	0.0598	0.0718	0.0681	0.0596	0.0196	0.0790	0.0625	0.0560	0.0657	0.0471	0.0561	0.0556	0.0860	0.0864
NC_021566	Nilaparvata lugens honeydew virus-2	Insects	0.0948	0.0485	0.0659	0.0974	0.0634	0.0453	0.0246	0.0515	0.0647	0.0354	0.0439	0.0591	0.0836	0.0556	0.0687	0.0973
NC_022004/ NC_022006	Broad bean true mosaic virus	Plants	0.0878	0.0427	0.0674	0.0752	0.0610	0.0368	0.0202	0.0585	0.0678	0.0475	0.0579	0.0639	0.0565	0.0496	0.0916	0.1155
NC_022332	Eel picornavirus 1	Vertebrates	0.0999	0.0438	0.0649	0.0912	0.0639	0.0415	0.0119	0.0552	0.0739	0.0325	0.0583	0.0569	0.0620	0.0546	0.0864	0.1032

NC_022611	Halyomorpha halys virus	Insects	0.0965	0.0687	0.0687	0.0974	0.0697	0.0388	0.0186	0.0524	0.0666	0.0334	0.0402	0.0536	0.0987	0.0385	0.0662	0.0918
NC_022798 / NC_022799	Potato black ringspot virus	Plants	0.0696	0.0478	0.0544	0.0643	0.0624	0.0615	0.0344	0.0700	0.0590	0.0595	0.0606	0.0629	0.0451	0.0595	0.0927	0.0962
NC_022802	Feline sakobuvirus	Vertebrates	0.0377	0.0676	0.0395	0.0378	0.0680	0.0994	0.0510	0.0966	0.0479	0.0580	0.0735	0.0606	0.0290	0.0901	0.0761	0.0673
NC_023016/ NC_023017	Lamium mild mosaic virus	Plants	0.0936	0.0520	0.0757	0.0702	0.0783	0.0385	0.0254	0.0523	0.0696	0.0548	0.0615	0.0567	0.0500	0.0493	0.0799	0.0922
NC_023021	Formica exsecta virus 1	Insects	0.1154	0.0584	0.0629	0.0937	0.0596	0.0445	0.0309	0.0502	0.0793	0.0348	0.0346	0.0485	0.0762	0.0475	0.0687	0.0948
NC_023022	Formica exsecta virus 2	Insects	0.1036	0.0439	0.0627	0.1123	0.0511	0.0260	0.0255	0.0428	0.0640	0.0403	0.0436	0.0633	0.1039	0.0353	0.0794	0.1023
NC_023162	Carp picornavirus 1	Vertebrates	0.0787	0.0594	0.0507	0.0711	0.0674	0.0508	0.0339	0.0652	0.0739	0.0491	0.0568	0.0530	0.0398	0.0581	0.0915	0.1006
NC_023422	Caprine kobuvirus	Vertebrates	0.0421	0.0658	0.0368	0.0406	0.0702	0.1162	0.0493	0.0978	0.0477	0.0625	0.0605	0.0515	0.0254	0.0890	0.0756	0.0690
NC_023437	Fathead minnow picornavirus	Vertebrates	0.0785	0.0580	0.0504	0.0726	0.0678	0.0424	0.0411	0.0609	0.0745	0.0542	0.0521	0.0594	0.0388	0.0576	0.0966	0.0952
NC_023483	Antheraea pernyi iflavirus	Insects	0.0976	0.0438	0.0591	0.1070	0.0542	0.0265	0.0299	0.0437	0.0609	0.0350	0.0410	0.0698	0.0948	0.0491	0.0767	0.1108
NC_023627	Laodelphax striatella honeydew virus 1	Insects	0.0983	0.0514	0.0502	0.0880	0.0581	0.0522	0.0358	0.0587	0.0565	0.0461	0.0381	0.0614	0.0752	0.0551	0.0780	0.0969
NC_023637	Posavirus 1	Vertebrates	0.0877	0.0373	0.0300	0.0992	0.0359	0.0306	0.0176	0.1010	0.0439	0.0304	0.0282	0.0677	0.0868	0.0868	0.0944	0.1226
NC_023638	Posavirus 2	Vertebrates	0.0720	0.0605	0.0619	0.0764	0.0651	0.0598	0.0343	0.0725	0.0676	0.0538	0.0423	0.0516	0.0662	0.0575	0.0767	0.0816
NC_023676	Spodoptera exigua iflavirus 2	Insects	0.0707	0.0569	0.0679	0.0811	0.0592	0.0519	0.0366	0.0551	0.0732	0.0449	0.0625	0.0622	0.0737	0.0489	0.0757	0.0795
NC_023857	Chicken megrivirus	Vertebrates	0.0657	0.0491	0.0535	0.0708	0.0565	0.0618	0.0233	0.0904	0.0665	0.0462	0.0496	0.0545	0.0505	0.0749	0.0903	0.0965
NC_023858	Melegrivirus A	Vertebrates	0.0686	0.0499	0.0512	0.0694	0.0596	0.0670	0.0222	0.0916	0.0670	0.0467	0.0496	0.0529	0.0441	0.0767	0.0931	0.0905
NC_023861	Sicinivirus 1	Vertebrates	0.0449	0.0575	0.0408	0.0539	0.0677	0.1149	0.0525	0.0867	0.0468	0.0741	0.0512	0.0491	0.0377	0.0754	0.0765	0.0704
NC_023985	Duck picornavirus GL	Vertebrates	0.0875	0.0579	0.0773	0.0751	0.0836	0.0460	0.0077	0.0565	0.0773	0.0391	0.0654	0.0562	0.0496	0.0507	0.0876	0.0826

NC_023987	Mosavirus A2	Vertebrates	0.0825	0.0720	0.0613	0.0693	0.0837	0.0514	0.0216	0.0645	0.0791	0.0470	0.0545	0.0529	0.0399	0.0506	0.0961	0.0734
NC_023988	Tortoise picornavirus	Vertebrates	0.0969	0.0575	0.0651	0.0905	0.0786	0.0400	0.0101	0.0540	0.0675	0.0396	0.0521	0.0521	0.0671	0.0458	0.0839	0.0994
NC_024016	Heliconius erato iflavirus	Insects	0.0993	0.0413	0.0648	0.1104	0.0448	0.0268	0.0308	0.0462	0.0649	0.0333	0.0368	0.0697	0.1068	0.0471	0.0725	0.1045
NC_024070	Rosavirus A2	Vertebrates	0.0654	0.0616	0.0590	0.0458	0.0672	0.0851	0.0401	0.0763	0.0580	0.0608	0.0626	0.0640	0.0412	0.0613	0.0837	0.0679
NC_024073	Enterovirus sp.	Vertebrates	0.0805	0.0658	0.0724	0.0705	0.0860	0.0532	0.0205	0.0542	0.0651	0.0503	0.0582	0.0606	0.0576	0.0446	0.0831	0.0774
NC_024120	Duck megrivirus	Vertebrates	0.0773	0.0537	0.0567	0.0676	0.0688	0.0639	0.0180	0.0820	0.0663	0.0422	0.0577	0.0537	0.0430	0.0729	0.0874	0.0887
NC_024489	Asterionellopsis glacialis RNA virus	Others	0.0883	0.0473	0.0546	0.0855	0.0527	0.0344	0.0330	0.0594	0.0653	0.0414	0.0449	0.0642	0.0696	0.0564	0.0831	0.1198
NC_024497	Lymantria dispar iflavirus	Insects	0.1008	0.0409	0.0593	0.1114	0.0507	0.0279	0.0277	0.0454	0.0624	0.0331	0.0354	0.0668	0.0985	0.0500	0.0753	0.1143
NC_024765	Chicken picornavirus	Vertebrates	0.0421	0.0605	0.0406	0.0486	0.0683	0.1186	0.0539	0.0920	0.0460	0.0725	0.0543	0.0498	0.0354	0.0811	0.0740	0.0623
NC_024766	Chicken picornavirus 2	Vertebrates	0.0646	0.0472	0.0676	0.0643	0.0721	0.0661	0.0207	0.0768	0.0590	0.0555	0.0773	0.0577	0.0480	0.0668	0.0840	0.0724
NC_024768	Chicken picornavirus 4	Vertebrates	0.0710	0.0454	0.0508	0.0697	0.0547	0.0632	0.0198	0.0978	0.0672	0.0454	0.0444	0.0547	0.0440	0.0815	0.0966	0.0938
NC_024770	Chicken gallivirus 1	Vertebrates	0.0664	0.0550	0.0381	0.0790	0.0576	0.0350	0.0255	0.0785	0.0577	0.0445	0.0489	0.0702	0.0567	0.0622	0.1088	0.1158
NC_024770	Chicken gallivirus 1	Vertebrates	0.0664	0.0386	0.0357	0.0702	0.0405	0.0814	0.0216	0.1035	0.0533	0.0450	0.0428	0.0634	0.0508	0.0821	0.1042	0.1007
NC_025114	Salivirus FHB	Vertebrates	0.0443	0.0670	0.0293	0.0379	0.0680	0.1290	0.0546	0.1070	0.0410	0.0646	0.0571	0.0509	0.0251	0.0980	0.0727	0.0533
NC_025219	Cripavirus NB-1/2011/HUN	Insects	0.1172	0.0496	0.0704	0.1022	0.0594	0.0248	0.0223	0.0397	0.0727	0.0293	0.0379	0.0543	0.0901	0.0427	0.0635	0.1237
NC_025432	Chicken orivirus 1	Vertebrates	0.0571	0.0658	0.0645	0.0581	0.0780	0.0726	0.0361	0.0668	0.0566	0.0593	0.0667	0.0621	0.0539	0.0559	0.0773	0.0692
NC_025479/ NC_025480	Carrot torradovirus	Plants	0.0937	0.0493	0.0692	0.0820	0.0695	0.0356	0.0165	0.0656	0.0684	0.0418	0.0457	0.0574	0.0626	0.0606	0.0819	0.1003
NC_025675	Norway rat hunnivirus	Vertebrates	0.0815	0.0690	0.0701	0.0646	0.0849	0.0571	0.0307	0.0544	0.0661	0.0531	0.0658	0.0602	0.0528	0.0479	0.0786	0.0632

NC_025675	Norway rat hunnivirus	Vertebrates	0.0810	0.0657	0.0645	0.0635	0.0865	0.0629	0.0239	0.0606	0.0716	0.0570	0.0603	0.0533	0.0358	0.0483	0.0933	0.0718
NC_025835	Dinocampus coccinellae paralysis virus	Insects	0.1111	0.0443	0.0669	0.1076	0.0519	0.0315	0.0215	0.0537	0.0695	0.0288	0.0405	0.0539	0.0974	0.0541	0.0637	0.1037
NC_025890	Tortoise picornavirus	Vertebrates	0.1279	0.0684	0.0560	0.0866	0.0761	0.0333	0.0056	0.0630	0.0651	0.0266	0.0396	0.0510	0.0697	0.0498	0.0812	0.1000
NC_026314	Rabovirus A	Vertebrates	0.1028	0.0555	0.0681	0.0830	0.0888	0.0554	0.0079	0.0562	0.0753	0.0429	0.0581	0.0479	0.0428	0.0544	0.0900	0.0709
NC_026315	Lesavirus 1	Vertebrates	0.0668	0.0717	0.0574	0.0636	0.0817	0.0496	0.0230	0.0760	0.0613	0.0509	0.0580	0.0522	0.0497	0.0582	0.0841	0.0958
NC_026733	Graminella nigrifrons virus 1	Insects	0.0952	0.0396	0.0726	0.0943	0.0453	0.0362	0.0311	0.0492	0.0663	0.0429	0.0429	0.0683	0.0949	0.0431	0.0738	0.1041
NC_026921	Falcovirus A1	Vertebrates	0.0664	0.0643	0.0697	0.0623	0.0807	0.0719	0.0359	0.0608	0.0688	0.0618	0.0623	0.0513	0.0469	0.0513	0.0762	0.0693
NC_027054	Porcine kobuvirus	Vertebrates	0.0499	0.0728	0.0352	0.0461	0.0709	0.0947	0.0417	0.1073	0.0516	0.0547	0.0539	0.0474	0.0315	0.0926	0.0767	0.0729
NC_027126	Kilifi Virus	Others	0.1121	0.0649	0.0662	0.0976	0.0800	0.0454	0.0292	0.0514	0.0767	0.0381	0.0390	0.0413	0.0720	0.0576	0.0607	0.0677
NC_027128	La Jolla virus	Insects	0.0943	0.0593	0.0583	0.0869	0.0748	0.0456	0.0315	0.0559	0.0640	0.0500	0.0414	0.0519	0.0654	0.0529	0.0763	0.0915
NC_027214	Ampivirus A1	Vertebrates	0.0824	0.0628	0.0421	0.0730	0.0710	0.0646	0.0440	0.0794	0.0661	0.0431	0.0425	0.0408	0.0409	0.0886	0.0638	0.0950
NC_027713	Bombyx mori iflavirus	Insects	0.0801	0.0426	0.0618	0.0982	0.0444	0.0275	0.0334	0.0460	0.0689	0.0383	0.0496	0.0790	0.0894	0.0428	0.0909	0.1071
NC_027818	Phopivirus strain NewEngland	Vertebrates	0.1167	0.0476	0.0653	0.0941	0.0684	0.0322	0.0070	0.0525	0.0745	0.0294	0.0500	0.0541	0.0641	0.0507	0.0856	0.1077
NC_027915	Bellflower vein chlorosis virus	Plants	0.0839	0.0397	0.0733	0.0783	0.0591	0.0348	0.0216	0.0554	0.0797	0.0418	0.0568	0.0693	0.0525	0.0547	0.0958	0.1034
NC_027918	Kobuvirus cattle/Kagoshima-2-24-KoV/2015/JPN	Vertebrates	0.0480	0.0653	0.0343	0.0446	0.0662	0.1158	0.0625	0.0925	0.0486	0.0669	0.0614	0.0489	0.0295	0.0890	0.0676	0.0589
NC_027926/ NC_027927	Tomato necrotic dwarf virus	Plants	0.0877	0.0512	0.0677	0.0762	0.0787	0.0418	0.0168	0.0596	0.0677	0.0475	0.0555	0.0600	0.0488	0.0563	0.0908	0.0939
NC_028139/ NC_028146	Bean rugose mosaic virus	Plants	0.0913	0.0493	0.0687	0.0814	0.0729	0.0331	0.0177	0.0533	0.0707	0.0462	0.0538	0.0622	0.0560	0.0484	0.0927	0.1025
NC_028240	Posavirus 3	Vertebrates	0.0622	0.0546	0.0533	0.0689	0.0474	0.0695	0.0378	0.0811	0.0731	0.0493	0.0324	0.0674	0.0563	0.0623	0.0987	0.0855

NC_028363	Rodent hepatovirus	Vertebrates	0.1031	0.0413	0.0635	0.0948	0.0519	0.0319	0.0057	0.0613	0.0733	0.0298	0.0529	0.0537	0.0745	0.0478	0.0877	0.1270
NC_028364	Shrew hepatovirus	Vertebrates	0.1024	0.0391	0.0693	0.0949	0.0554	0.0337	0.0087	0.0488	0.0811	0.0305	0.0538	0.0551	0.0667	0.0435	0.0886	0.1284
NC_028365	Hedgehog hepatovirus	Vertebrates	0.1041	0.0446	0.0647	0.0993	0.0665	0.0300	0.0065	0.0502	0.0820	0.0261	0.0505	0.0541	0.0600	0.0524	0.0912	0.1176
NC_028366	Bat hepatovirus	Vertebrates	0.1128	0.0456	0.0656	0.0962	0.0667	0.0303	0.0065	0.0546	0.0805	0.0271	0.0464	0.0513	0.0601	0.0552	0.0866	0.1146
NC_028380	Chicken sicinivirus	Vertebrates	0.0438	0.0606	0.0397	0.0494	0.0666	0.1225	0.0524	0.0879	0.0447	0.0702	0.0572	0.0513	0.0385	0.0761	0.0740	0.0652
NC_028479	Rabbit picornavirus	Vertebrates	0.0396	0.0576	0.0505	0.0408	0.0689	0.0900	0.0524	0.0837	0.0520	0.0721	0.0741	0.0585	0.0281	0.0752	0.0798	0.0768
NC_028964	Gallivirus Pf	Vertebrates	0.0673	0.0413	0.0348	0.0668	0.0432	0.0812	0.0232	0.1063	0.0522	0.0467	0.0423	0.0617	0.0475	0.0847	0.1028	0.0982
NC_028981	Tupaia hepatovirus	Vertebrates	0.0999	0.0647	0.0564	0.0707	0.0809	0.0515	0.0241	0.0593	0.0695	0.0462	0.0486	0.0559	0.0415	0.0533	0.0910	0.0865
NC_029038/ NC_029036	Currant latent virus	Plants	0.0774	0.0517	0.0616	0.0712	0.0609	0.0453	0.0233	0.0717	0.0582	0.0451	0.0491	0.0615	0.0652	0.0591	0.0798	0.1189
NC_029052	Goose dicistrovirus	Insects	0.1210	0.0535	0.0536	0.1118	0.0620	0.0237	0.0209	0.0485	0.0682	0.0291	0.0333	0.0525	0.0886	0.0490	0.0754	0.1089
NC_029131	Delisea pulchra RNA virus	Vertebrates	0.0973	0.0421	0.0697	0.0891	0.0547	0.0335	0.0289	0.0372	0.0884	0.0370	0.0531	0.0697	0.0578	0.0416	0.0966	0.1032
NC_029854	Yak enterovirus	Vertebrates	0.0660	0.0691	0.0606	0.0579	0.0786	0.0711	0.0516	0.0587	0.0656	0.0648	0.0641	0.0568	0.0434	0.0550	0.0749	0.0621
NC_029905	Enterovirus SEV	Vertebrates	0.0895	0.0563	0.0710	0.0823	0.0809	0.0546	0.0167	0.0505	0.0691	0.0462	0.0585	0.0548	0.0595	0.0458	0.0824	0.0820
NC_030115	Anopheles C virus	Insects	0.0902	0.0640	0.0621	0.0748	0.0690	0.0403	0.0456	0.0529	0.0763	0.0469	0.0549	0.0558	0.0556	0.0566	0.0713	0.0837
NC_030232	Antarctic picorna-like virus 1	Others	0.0631	0.0502	0.0590	0.0699	0.0599	0.0457	0.0355	0.0597	0.0621	0.0552	0.0510	0.0653	0.0570	0.0497	0.0882	0.1286
NC_030233	Antarctic picorna-like virus 2	Others	0.0709	0.0597	0.0512	0.0785	0.0599	0.0600	0.0433	0.0692	0.0659	0.0503	0.0415	0.0530	0.0637	0.0621	0.0748	0.0961
NC_030234	Antarctic picorna-like virus 3	Others	0.0737	0.0412	0.0698	0.0928	0.0503	0.0400	0.0400	0.0379	0.0843	0.0420	0.0592	0.0706	0.0691	0.0451	0.0871	0.0967
NC_030235	Antarctic picorna-like virus 4	Others	0.0808	0.0519	0.0595	0.0835	0.0629	0.0391	0.0321	0.0508	0.0672	0.0445	0.0511	0.0635	0.0648	0.0494	0.0837	0.1153

NC_030454	Human enterovirus	Vertebrates	0.0733	0.0486	0.0513	0.0683	0.0577	0.0556	0.0188	0.0970	0.0661	0.0445	0.0458	0.0530	0.0443	0.0804	0.0935	0.1019
NC_030454	Human enterovirus	Vertebrates	0.0733	0.0702	0.0713	0.0642	0.0876	0.0614	0.0274	0.0644	0.0650	0.0519	0.0601	0.0579	0.0531	0.0572	0.0762	0.0588
NC_030697	Picornavirales Bu-3	Vertebrates	0.0546	0.0412	0.0771	0.0614	0.0456	0.0456	0.0483	0.0558	0.0975	0.0562	0.0759	0.0771	0.0366	0.0522	0.1054	0.0696
NC_030744	Picornavirales Tottori-HG1	Vertebrates	0.0432	0.0630	0.0402	0.0441	0.0616	0.1007	0.0725	0.0901	0.0576	0.0697	0.0429	0.0606	0.0282	0.0915	0.0751	0.0590
NC_030745	Picornavirales Bu-1	Vertebrates	0.1209	0.0414	0.0529	0.1175	0.0406	0.0217	0.0110	0.0643	0.0668	0.0229	0.0233	0.0568	0.1045	0.0515	0.0825	0.1214
NC_031105	Rosavirus B	Vertebrates	0.0629	0.0552	0.0633	0.0516	0.0694	0.0888	0.0323	0.0760	0.0637	0.0578	0.0612	0.0600	0.0371	0.0647	0.0857	0.0703
NC_031338	Moku virus isolate Big Island	Insects	0.0967	0.0459	0.0698	0.1038	0.0561	0.0266	0.0253	0.0481	0.0692	0.0388	0.0437	0.0608	0.0941	0.0449	0.0737	0.1027
NC_031687	Centovirus AC	Insects	0.0971	0.0706	0.0515	0.0809	0.0648	0.0458	0.0411	0.0549	0.0770	0.0457	0.0416	0.0481	0.0611	0.0448	0.0781	0.0970
NC_031688	Mosquito dicistrovirus	Insects	0.0968	0.0695	0.0572	0.0796	0.0630	0.0479	0.0349	0.0567	0.0771	0.0421	0.0404	0.0494	0.0662	0.0429	0.0764	0.0998
NC_031749	King virus	Insects	0.1023	0.0439	0.0679	0.1015	0.0496	0.0277	0.0247	0.0397	0.0689	0.0316	0.0420	0.0762	0.0946	0.0387	0.0842	0.1066
NC_031750	Riptortus pedestris virus-1	Others	0.0953	0.0386	0.0440	0.0942	0.0587	0.0309	0.0115	0.0703	0.0560	0.0310	0.0315	0.0686	0.0621	0.0710	0.1001	0.1362
NC_031750	Riptortus pedestris virus-1	Vertebrates	0.0953	0.0649	0.0755	0.0633	0.0775	0.0751	0.0229	0.0693	0.0630	0.0452	0.0464	0.0455	0.0632	0.0597	0.0552	0.0783
NC_031766/ NC_031763	Dioscorea mosaic-associated virus	Plants	0.0980	0.0495	0.0669	0.0804	0.0642	0.0295	0.0224	0.0616	0.0837	0.0450	0.0494	0.0527	0.0490	0.0536	0.0921	0.1020
NC_032087	Euscelidius variegatus virus 1	Insects	0.0847	0.0411	0.0617	0.0850	0.0447	0.0401	0.0357	0.0571	0.0653	0.0521	0.0455	0.0686	0.0779	0.0443	0.0887	0.1074
NC_032112	Bivalve RNA virus G1	Insects	0.0911	0.0614	0.0548	0.0789	0.0650	0.0406	0.0348	0.0649	0.0659	0.0392	0.0403	0.0614	0.0644	0.0640	0.0769	0.0965
NC_032113	Bivalve RNA virus G2	Insects	0.0749	0.0611	0.0588	0.0614	0.0701	0.0508	0.0475	0.0555	0.0627	0.0550	0.0604	0.0693	0.0484	0.0569	0.0809	0.0863
NC_032114	Bivalve RNA virus G3	Insects	0.0922	0.0444	0.0636	0.1012	0.0581	0.0332	0.0275	0.0490	0.0740	0.0373	0.0435	0.0536	0.0771	0.0529	0.0738	0.1185
NC_032115	Bivalve RNA virus G5	Insects	0.1016	0.0528	0.0477	0.0860	0.0564	0.0458	0.0301	0.0627	0.0675	0.0392	0.0375	0.0540	0.0627	0.0573	0.0827	0.1158

NC_032126	Livupivirus A	Others	0.0833	0.0677	0.0491	0.0617	0.0684	0.0729	0.0276	0.0847	0.0592	0.0502	0.0578	0.0470	0.0510	0.0627	0.0798	0.0770
NC_032222	Hubei picorna-like virus 82	Others	0.1286	0.0552	0.0744	0.0994	0.0889	0.0274	0.0217	0.0281	0.0755	0.0329	0.0406	0.0478	0.0645	0.0505	0.0603	0.1042
NC_032270/ NC_032271	soybean latent spherical virus	Plants	0.0799	0.0420	0.0662	0.0792	0.0561	0.0471	0.0247	0.0580	0.0667	0.0508	0.0634	0.0615	0.0645	0.0461	0.0881	0.1058
NC_032480	Husavirus sp.	Vertebrates	0.0471	0.0556	0.0626	0.0548	0.0515	0.0572	0.0695	0.0592	0.0855	0.0616	0.0680	0.0756	0.0362	0.0631	0.0905	0.0618
NC_032804	Biomphalaria virus 1	Others	0.0970	0.0355	0.0465	0.0867	0.0633	0.0317	0.0114	0.0706	0.0608	0.0337	0.0337	0.0732	0.0444	0.0761	0.1098	0.1256
NC_032840	Biomphalaria virus 3	Others	0.0835	0.0472	0.0790	0.0734	0.0567	0.0341	0.0171	0.0500	0.0908	0.0393	0.0565	0.0759	0.0522	0.0372	0.1099	0.0968
NC_032978	Shuangao insect virus 8	Others	0.1125	0.0504	0.0467	0.1019	0.0540	0.0393	0.0314	0.0545	0.0691	0.0374	0.0351	0.0467	0.0759	0.0520	0.0752	0.1177
NC_033053	Hubei picorna-like virus 71	Others	0.1149	0.0719	0.0714	0.0856	0.0864	0.0413	0.0230	0.0472	0.0731	0.0403	0.0430	0.0457	0.0695	0.0444	0.0646	0.0777
NC_033116	Hubei picorna-like virus 77	Others	0.0987	0.0542	0.0377	0.0942	0.0605	0.0474	0.0206	0.0767	0.0480	0.0318	0.0308	0.0544	0.0777	0.0717	0.0758	0.1199
NC_033141	Hubei picorna-like virus 80	Others	0.1014	0.0553	0.0645	0.1012	0.0533	0.0278	0.0281	0.0505	0.0844	0.0282	0.0415	0.0530	0.0831	0.0482	0.0734	0.1060
NC_033152	Hubei picorna-like virus 81	Others	0.1035	0.0518	0.0654	0.0934	0.0695	0.0352	0.0170	0.0447	0.0731	0.0406	0.0479	0.0584	0.0681	0.0387	0.0897	0.1032
NC_033160	Hubei picorna-like virus 79	Others	0.1090	0.0631	0.0447	0.0954	0.0623	0.0438	0.0224	0.0766	0.0586	0.0290	0.0266	0.0391	0.0824	0.0692	0.0596	0.1182
NC_033212	Hubei picorna-like virus 78	Others	0.1052	0.0553	0.0629	0.0974	0.0637	0.0414	0.0188	0.0501	0.0750	0.0256	0.0341	0.0500	0.0769	0.0516	0.0690	0.1230
NC_033214	Hubei picorna-like virus 10	Others	0.1169	0.0590	0.0521	0.0944	0.0647	0.0343	0.0229	0.0497	0.0703	0.0305	0.0401	0.0553	0.0706	0.0478	0.0810	0.1105
NC_033456	Biomphalaria virus 2	Others	0.0896	0.0627	0.0538	0.1065	0.0566	0.0286	0.0289	0.0545	0.0642	0.0319	0.0338	0.0610	0.1022	0.0452	0.0745	0.1061
NC_033492/ NC_033493	Petunia chlorotic mottle virus	Plants	0.0942	0.0413	0.0713	0.0824	0.0601	0.0439	0.0213	0.0587	0.0735	0.0476	0.0603	0.0549	0.0614	0.0511	0.0834	0.0946
NC_033619	Helicoverpa armigera iflavirus	Insects	0.0993	0.0380	0.0617	0.1066	0.0387	0.0239	0.0249	0.0484	0.0618	0.0296	0.0351	0.0762	0.1059	0.0443	0.0810	0.1245
NC_033695	Enterovirus AN12	Vertebrates	0.0666	0.0677	0.0626	0.0596	0.0791	0.0750	0.0460	0.0623	0.0666	0.0619	0.0653	0.0526	0.0442	0.0577	0.0726	0.0600

NC_033793	Goose megrivirus	Vertebrates	0.0773	0.0567	0.0559	0.0638	0.0693	0.0689	0.0220	0.0836	0.0684	0.0426	0.0563	0.0509	0.0387	0.0755	0.0840	0.0860
NC_033818	Bat kunsagivirus	Vertebrates	0.0591	0.0607	0.0497	0.0574	0.0793	0.0875	0.0278	0.0784	0.0542	0.0560	0.0509	0.0595	0.0343	0.0690	0.0921	0.0842
NC_033820	Bat sapelovirus	Vertebrates	0.0688	0.0520	0.0781	0.0737	0.0826	0.0581	0.0099	0.0425	0.0683	0.0399	0.0621	0.0622	0.0531	0.0431	0.0822	0.1232
NC_033823	Bat iflavirus	Insects	0.0783	0.0474	0.0592	0.0915	0.0511	0.0394	0.0419	0.0592	0.0702	0.0524	0.0442	0.0605	0.0770	0.0524	0.0818	0.0936
NC_034206	Bakunsa virus	Vertebrates	0.0547	0.0556	0.0512	0.0590	0.0730	0.0905	0.0256	0.0840	0.0522	0.0548	0.0556	0.0545	0.0405	0.0722	0.0849	0.0918
NC_034214/ NC_034215	Peach rosette mosaic virus	Plants	0.0658	0.0425	0.0661	0.0748	0.0604	0.0502	0.0260	0.0661	0.0645	0.0548	0.0643	0.0571	0.0586	0.0553	0.0841	0.1095
NC_034217	Pityohyphantes rubrofasciatus iflavirus	Insects	0.1059	0.0555	0.0523	0.1010	0.0468	0.0375	0.0279	0.0551	0.0851	0.0317	0.0331	0.0522	0.0769	0.0426	0.0888	0.1077
NC_034245	Bovine rhinovirus 1	Vertebrates	0.0623	0.0801	0.0607	0.0445	0.0782	0.0598	0.0378	0.0600	0.0723	0.0549	0.0740	0.0638	0.0349	0.0410	0.0925	0.0831
NC_034267	Enterovirus goat	Vertebrates	0.0751	0.0625	0.0679	0.0649	0.0787	0.0710	0.0311	0.0545	0.0686	0.0529	0.0660	0.0569	0.0482	0.0491	0.0792	0.0735
NC_034381	Miniopterus schreibersii picornavirus 1	Vertebrates	0.0829	0.0755	0.0566	0.0513	0.0758	0.0699	0.0309	0.0814	0.0655	0.0477	0.0566	0.0465	0.0420	0.0650	0.0723	0.0801
NC_034384	Diamondback moth iflavirus	Insects	0.0727	0.0644	0.0717	0.0715	0.0634	0.0509	0.0402	0.0521	0.0776	0.0501	0.0623	0.0604	0.0667	0.0411	0.0761	0.0787
NC_034385	Cosavirus F	Vertebrates	0.0860	0.0666	0.0651	0.0685	0.0736	0.0663	0.0176	0.0696	0.0699	0.0448	0.0541	0.0482	0.0568	0.0494	0.0801	0.0835
NC_034453	Ferret parechovirus	Vertebrates	0.0964	0.0604	0.0546	0.0780	0.0838	0.0403	0.0150	0.0688	0.0654	0.0464	0.0463	0.0529	0.0439	0.0606	0.0953	0.0919
NC_034568	Picorna-like virus AWando15	Others	0.1013	0.0478	0.0475	0.0998	0.0490	0.0297	0.0218	0.0650	0.0604	0.0254	0.0364	0.0577	0.0857	0.0626	0.0743	0.1356
NC_034617	Harrier picornavirus 1	Vertebrates	0.0629	0.0560	0.0570	0.0679	0.0578	0.0649	0.0249	0.0799	0.0664	0.0438	0.0631	0.0543	0.0567	0.0629	0.0826	0.0989
NC_034971	Canine kobuvirus	Vertebrates	0.0567	0.0719	0.0354	0.0381	0.0791	0.1422	0.0550	0.0945	0.0461	0.0645	0.0543	0.0439	0.0203	0.0921	0.0642	0.0415
NC_035110	Fur seal picorna-like virus	Vertebrates	0.0968	0.0540	0.0451	0.0814	0.0609	0.0492	0.0393	0.0694	0.0595	0.0423	0.0414	0.0533	0.0601	0.0731	0.0707	0.1035
NC_035115	Apis dicistrovirus	Insects	0.0926	0.0435	0.0456	0.0947	0.0535	0.0322	0.0207	0.0617	0.0647	0.0343	0.0389	0.0630	0.0656	0.0581	0.0957	0.1352

NC_035184	Big Sioux River virus	Insects	0.0969	0.0653	0.0586	0.0870	0.0584	0.0386	0.0397	0.0537	0.0690	0.0401	0.0363	0.0575	0.0836	0.0462	0.0683	0.1006
NC_035198	Aimelvirus 2	Vertebrates	0.0821	0.0722	0.0556	0.0607	0.0744	0.0589	0.0222	0.0823	0.0710	0.0394	0.0540	0.0488	0.0428	0.0675	0.0814	0.0869
NC_035214/ NC_035219	Lettuce necrotic leaf curl virus	Plants	0.0687	0.0577	0.0701	0.0649	0.0790	0.0469	0.0270	0.0614	0.0667	0.0525	0.0560	0.0622	0.0471	0.0572	0.0842	0.0982
NC_035218/ NC_035220	Motherwort yellow mottle virus	Plants	0.0853	0.0538	0.0600	0.0727	0.0743	0.0444	0.0233	0.0645	0.0668	0.0485	0.0521	0.0556	0.0454	0.0599	0.0877	0.1056
NC_035455	Solenopsis invicta virus 4	Others	0.1215	0.0537	0.0521	0.1102	0.0632	0.0304	0.0193	0.0517	0.0727	0.0321	0.0328	0.0416	0.0802	0.0483	0.0750	0.1149
NC_035457	Myrmica scabrinodis virus 1	Others	0.1129	0.0556	0.0563	0.1068	0.0682	0.0464	0.0252	0.0517	0.0696	0.0306	0.0331	0.0414	0.0808	0.0590	0.0601	0.1023
NC_035779	Falcon picornavirus	Vertebrates	0.0861	0.0402	0.0594	0.0844	0.0599	0.0575	0.0185	0.0595	0.0687	0.0502	0.0595	0.0490	0.0554	0.0476	0.0900	0.1140
NC_035798	Tioga picorna-like virus 1	Others	0.0762	0.0634	0.0543	0.0798	0.0825	0.0493	0.0426	0.0589	0.0698	0.0470	0.0432	0.0560	0.0452	0.0736	0.0759	0.0824
NC_036389	Chequa iflavirus	Insects	0.0725	0.0305	0.0639	0.0951	0.0311	0.0287	0.0273	0.0628	0.0632	0.0410	0.0453	0.0800	0.0952	0.0497	0.0928	0.1208
MT079817	Hovenia dulcis-associated virus 1	Unknow	0.0914	0.0560	0.0679	0.0973	0.0559	0.0363	0.0277	0.0509	0.0769	0.0336	0.0376	0.0566	0.0885	0.0448	0.0714	0.1071
MT079818	Hovenia dulcis-associated virus 2	Unknow	0.0966	0.0541	0.0523	0.0929	0.0557	0.0421	0.0269	0.0621	0.0693	0.0313	0.0377	0.0533	0.0743	0.0592	0.0748	0.1175

CHAPTER 3 - Two new *Circoviridae* associated with tree species

Flávia M. B. Nery[1], Fernando L. Melo[1], Leonardo S. Boiteux[2], Maria Esther N. Fonseca[2], Simone G. Ribeiro[3], Rita de Cássia Pereira-Carvalho[1]

Archives of Virology

[1]Departamento de Fitopatologia, Universidade de Brasília (UnB), Campus Universitário Darcy Ribeiro, Brasília-DF, 70910-900, Brazil.

[2]National Center for Vegetable Crops Research (CNPH) Embrapa Hortaliças, Brasília, DF 70275-970, Brazil;

[3] Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF, 70770-017, Brazil

Correspondence author: rcpcarvalho@unb.br

Abstract

The *Circoviridae* family is composed of viruses with small (≈ 1.8-2.1 kb), circular, single-stranded DNA (ssDNA) genomes, which are packed into non-enveloped. This family is represented by two genera (*Circovirus* and *Cyclovirus*). Thus far, *Circovirus* and *Cyclovirus* have been detected in association with animals. However, we recovered via High-throughput sequencing two novel *Circoviridae*-like genomes associated with tree species. The low identity in comparison with other species ascribed in *Circoviridae* suggests that they may represent novel members of this family. We proposed the names: Caesalpinia ferrea-associated virus - CFaV and Astronium fraxinifolium-associated virus - AFaV.

1. Introduction

Advances in High-throughput sequencing (HTS) technologies together with the availability of robust bioinformatics tools are leading to the discovery of a huge diversity of cryptic viruses across a wide array of hosts and environmental conditions (JONES et al., 2017;

MINICKA et al., 2020; NERY et al., 2020; REIS, 2020). Several circular single-stranded DNA (ssDNA) viruses have been detected and characterized in a wide range of samples using HTS approaches (KRABERGER et al., 2019; LABONTÉ; SUTTLE, 2013; RICHET et al., 2019). DNA viruses are classified into double-stranded (dsDNA) and single-stranded (ssDNA) (KRUPOVIC et al., 2020). Eukaryotic ssDNA viruses have been classified into ten families. However, many viruses remain unclassified (SIMMONDS et al., 2017).

Among them, seven families (Realm: *Monodnaviria*, Kingdom *Shotokuvirae*, Phylum *Cressdnaviricota*, Class *Arfiviricetes*) comprise viral species sharing the feature of encoding two proteins [replication-associated protein (REP) and capsid protein (CP)]. This group of viruses has been reported infecting algae (*Bacilladnaviridae*), animals (*Circoviridae*), plants (*Nanoviridae* and *Geminiviridae*), and fungi (*Genomoviridae*). Species of *Genomoviridae* are also associated with different organisms, like animals and plants (ABBAS et al., 2019; ICTV, 2020; KRUPOVIC et al., 2020; MALATHI; RENUKA DEVI, 2019). For viruses classified in *Smacoviridae* and "*Redondoviridae*" animals as the most likely hosts, although smacovirids have been already associated with archaea (DÍEZ-VILLASEÑOR; RODRIGUEZ-VALERA, 2019).

The *Circoviridae* family is composed of viruses with small (≈ 1.8-2.1 kb), circular, single-stranded DNA (ssDNA) genomes, which are packed into non-enveloped, icosahedral particles (BREITBART et al., 2017; ROSARIO et al., 2017). The genome organization of the circoviruses consists of two ambisense open reading frames (ORFs), that encode the replication-associated protein (REP) and the capsid protein (CP). This family is represented by two genera: *Circovirus* (n = 43) and *Cyclovirus* (n = 51) (ICTV, 2020).

Circovirus genomes have the *Rep* gene in the viral sense and the *CP* gene in the complementary sense, whereas *Cyclovirus* displays the opposite order in terms of gene organization (ROSARIO et al., 2017).

Viruses occurring in forest trees are poorly studied and HTS technology can be very efficient to expand our knowledge about viral species present in association with this environment. Here, we recovered via HTS two novel *Circoviridae*-like genomes associated with two Neotropical tree species (*Caesalpinia ferrea* Mart. ex Tul. and *Astronium fraxinifolium* Schott) from Central Brazil. Thus far, *Circovirus* and *Cyclovirus* have been detected in association with vertebrates and invertebrates (ROSARIO et al., 2017; SADEGHI et al., 2018; WANG et al., 2018). Therefore, to our knowledge, this is the first report of circoviruses associated with plants.

2. Materials and Methods

2.1. Leaf samples, DNA extraction and enrichment of circular ssDNA viruses

Leaf samples of the native tree species *Astronium fraxinifolium* and Brazilian ironwood (*Caesalpinia ferrea*) displaying virus-like symptoms were collected in a nursery belonging to the NOVACAP II (Companhia Urbanizadora da Nova Capital do Brazil II) in 2014 **(Supplementary Table 1).** The symptoms were documented, and then the leaf surfaces were carefully cleaned with a camel’s hairbrush, aiming to eliminate potential contamination by arthropods. Genomic DNA was individually extracted from leaf samples using a CTAB modified protocol with organic solvents (BOITEUX; FONSECA; SIMON, 1999). The purified circular DNAs were subsequently used as a template in Rolling Circle Amplification (RCA) assays (INOUE-NAGATA et al., 2004). The RCA was performed in a total volume of 10 µL using the following reagents: 1.0 µL of 10X phi29 DNA polymerase reaction buffer (NEB, Ipswich, MA, USA), 1.0 µL of Bovine serum albumin - BSA (10 mg/mL), 1.0 µL of dNTP mixture (10 mM) (Invitrogen, Carlsbad, CA, USA), 1.0 µL of random primers (10 µM), 1,0 µL of DNA, 4.9 µL of DNAse-free water was mixed with 0.1 µL of phi29 DNA Polymerase (10,000 U/mL).

2.2. High-throughput sequencing (HTS), bioinformatic and metagenomic analyses

The RCA products were sequenced in an Illumina HiSeq 2500 platform (2 × 125 paired ends) at Macrogen Inc. (South Korea). Sequence analyzes were performed essentially as previously described (ADAMS; FOX, 2016; KREUZE et al., 2009b). The following steps were performed: (*i*) elimination of low-quality sequences and adapters; (*ii*) reassembly of sequences and organization into contigs, using the CLC Genomics Workbench 10 program; (*iii*) validation of contigs by local alignments using Blastx algorithm against a virus database (Viral RefSeq-GenBank) (https://blast.ncbi.nlm.nih.gov/); (*iv*) Blastx results were compared to ViralRefSeq and another round of Blastx analyses was performed with the general database (only contigs with higher coverages were selected); and (*v*) the contigs showing identity to viruses after alignments were then analyzed with the assistance of the Geneious R11 program (KEARSE et al., 2012). Contigs were mapped back using the reads in order to obtain the complete genome sequences. Then, a Blastn analysis was performed with the entire genome and the most similar sequence was used for transferring the viral annotation.

Contigs were mapped back using the reads in order to obtain the complete genome sequences. Then, a Blastn analysis was performed with the entire genome and the most similar sequence was used for viral annotation transferring.

2.3. Design of *Circovirus*-specific PCR primers, detection of contigs obtained by HTS and cloning of amplicons

To confirm the HTS data, overlapping and abutting primers were obtained (**Table 1**). The PCR assays were performed in a total volume of 12,5 µL using the following reagents: 1 µL of RCA product (dilute in 1:100 of DNAse-free water) was mixed with 0.1 µL of *Taq* DNA polymerase (5 U/ µL) (Invitrogen, Carlsbad, CA, USA), 1.25 µL of 10 X PCR buffer, 0.4 µL of Magnesium Chloride (50 mM), 0.25 mM of each primer (**Table 1**), 0.25 µL of dNTP mixture (10 mM) (Invitrogen, Carlsbad, CA, USA) and 9.0 µL of DNAse-free water. The PCR products were resolved in 1% agarose-gel electrophoresis. The purification of DNA fragments obtained from PCR was performed with the GFX (GE Healthcare) purification kit following manufacturers' guidelines and cloned into pGEM T Easy vector (Promega). These clones were sequenced by Sanger at Myleus (Belo Horizonte, Minas Gerais-MG, Brazil) and at Embrapa Hortaliças (Brasília-DF, Brazil). Internal primers were designed to recovery the complete genome sequence.

Table 1. Primer sequences employed for detection and sequencing of two novel *Circovirus*-like genomes.

Accession Number	**Contigs**	**Primer name/ Primers Sequence (5’-3’) ***	**Product Length (bp)****	**Reads**	**AT[+] (°C)**	**Application/ Target**
MT707946	AFaV_11404[1]	AFaV_321F CAGAGAACTAACCCAACATCACGT [a] AFaV_322R-GTATATGCTCGCGCCATGTGB	2543	128	57	Detection/ CP
		F_AFaVPINT - GGTCGTCGAGGTCTGTT AFaVPINT_R- GGTTTCGGAGCAGTTA	-	-	-	Recover genome
MT707947	CFaV_126[2]	CFaV_858F-TTCTGGGCAGGCGTTC [b] CFaV_867R-TAGAATTCAATCCTCGGAGTGGCT	2342	1029	55	Detection/REP
		F_CFaVPINT-TTCTGGGCAGGCGTTC CFaVPINT _R-AGTGGATCAAAAGCCTT	-	-	-	Recover genome

[1] AFaV - Astronium fraxinifolium-associated virus; [2] CFaV - Caesalpinia ferrea-associated virus; [a] Overlapping primers and [b] abutting primers. * F: Forward, R: Reverse. **bp: base pairs. CP: coat protein, REP: Replicase. [+]AT: annealing temperature.

2.4. Phylogenetic Analysis

Phylogenetic analysis was performed using the 60 replicase protein (REP) sequences from members of the *Circovirus* family (**Supplementary Table 2**). The analysis was carried out using the Geneious V.11.1 program (KEARSE et al., 2012). Alignment was performed with the MUSCLE software and trees were developed using the maximum likelihood (ML) method Fast Tree algorithm (PRICE; DEHAL; ARKIN, 2009) with 1,000 repetitions bootstrap.

2.5. Linear Discriminant Analysis (LDA)

Linear Discriminant Analysis was performed to infer the host, for these 60 sequences of the *Circoviridae* members. These sequences were downloaded from NCBI/GenBank [www.ncbi.nlm.nih.gov/ (**Supplementary Table 2**)]. Dinucleotide frequencies for each sequence were determined using the program Simple Sequence Editor (SSE), version 1.3 (SIMMONDS, 2012) . LDA was performed using R program (www.R-project.org) (version 3.4.2) (TEAM, 2013) implementing the MASS, LDA function, and ggplot2 package.

3. Results and Discussion

The HTS procedure recovered 35,035 reads, following Blastx analyses and map-to-reference. Two reads were selected (11404 and 126) and used as a reference for assembling the complete genomes, through Geneious (San Diego, CA, USA) (KEARSE et al., 2012). The amplicon expected for contig 11404 was obtained from the sample *A. fraxinifolium* (**Figures 1A**

and 1B) using primers: AFaV_321F/ AFaV_322R. Also, amplicons of expected size were detected for contig 126 in leaf samples from the Brazilian ironwood (**Figures 1C and D**) using primers: CFaV_858F/ CFaV_867R.

The amplicons obtained for contigs 126 and 11404 were cloned into pGEM-T Easy (Promega, Madison, WI, USA) as recommended by the manufacturer and sequenced by Sanger.

The full genomes obtained through PCR with primers F_CFaVPINT/ CFaVPINT_R (contig 126) and F_AFaVPINT/ AFaVPINT-R (contig 11404) (**Table 1**) were recovered via Sanger and then analyzed using Geneious v. 11.1 (San Diego, CA, USA) (KEARSE et al., 2012) and NCBI tools (www.ncbi.nlm.gov/). The two contigs 11404 (*Astronium fraxinifolium*-associated virus - AFaV) (**Figure 1C**) and 126 (Caesalpinia ferrea-associated virus - CFaV (**Figure 1D**) showed identity with species already classified in the family *Circoviridae*.

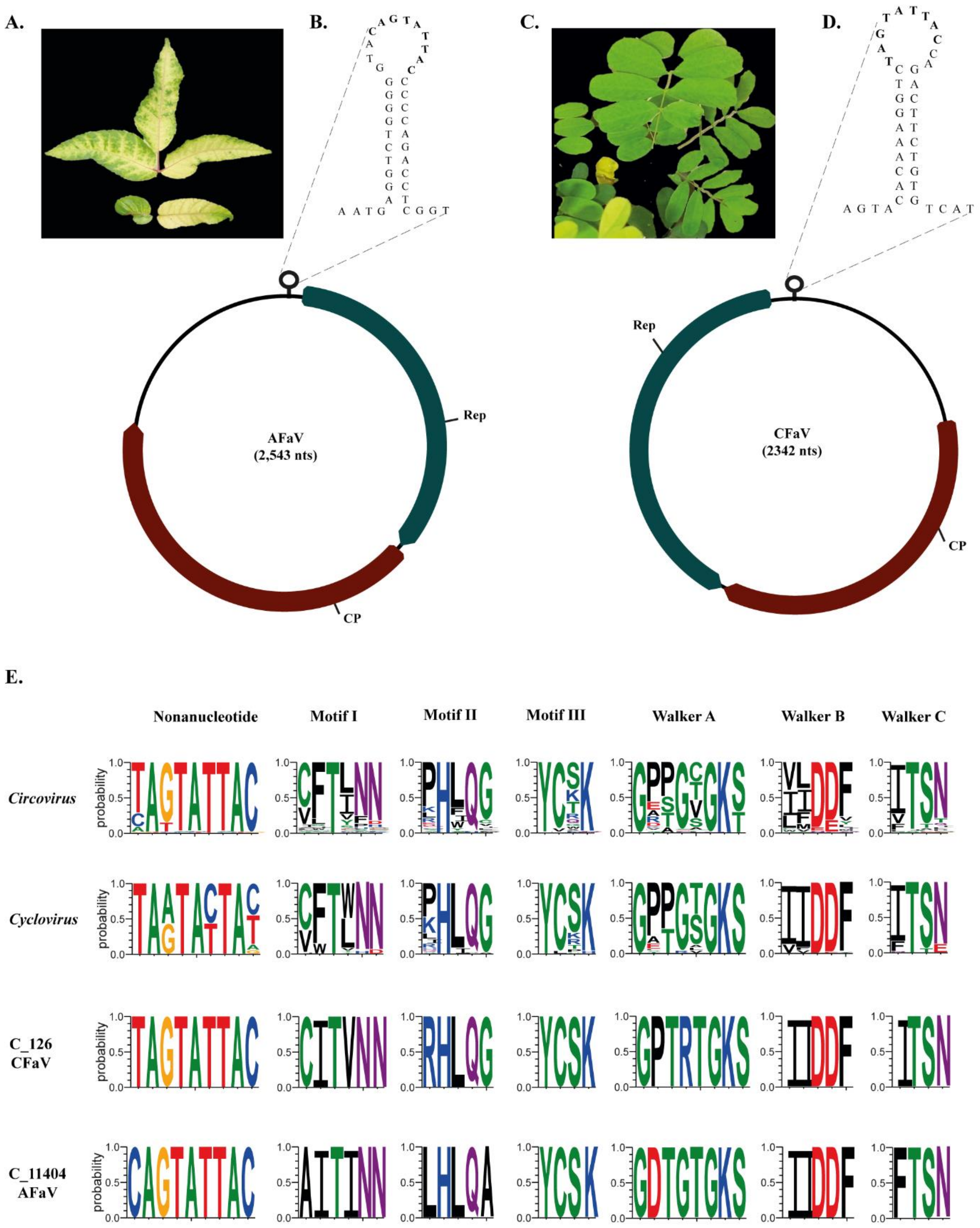


Figure 1 A-E. Characterization of two new *Circovirus*-like genomes species detected in association with two Neotropical tree species in Central Brazil; **A**. Symptomatic leaves of *Astronium fraxinifolium* Schott from which Astronium fraxinifolium-associated virus (AFaV) was isolated. **B**. Genome organization of AFaV in red the region encoding the CP (Coat Protein) and in blue, the Rep region (Replicase). **C**. Symptomatic leaves of *Caesalpinia ferrea* Mart. ex Tul. from which the Caesalpinia ferrea-associated virus (CFaV) was obtained. **D.** Genome organization of CFaV, in red the region encoding the CP (Coat Protein)

and in blue, the Rep region. In bold the sequence of nonanucleotide and **E.** Summary of conserved nonanucleotides, rolling circle replication (RCR) and superfamily 3 (SF3) Helicase Motifs of the *Circoviridae* family. The logos were generated using Weblogo 3 (CROOKS et al., 2004) with representative sequences from members of *Cyclovirus* (*n*= 49) and *Circovirus* (*n*= 78), including Astronium fraxinifolium-associated virus (AFaV) and Caesalpinia ferrea-associated virus (CFaV) **(Supplementary Table 3)**.

AFaV circular genome (GenBank accession MT707946) comprised 2543 nts in length and exhibited amino acid identity values of 45% and 27% coverage with Anguilla anguilla circovirus (APZ87906). The full CFaV circular genome (MT707947) displayed 2342 nts and showed 56% amino acid identity with Bat-associated Cyclovirus 7 (genus *Cyclovirus*) (YP009506292). According to the criteria for the classification of a new species within the family *Circoviridae,* sequences with pairwise nucleotide identity lower than 80% are classified as a distinct species (ICTV, 2019; ROSARIO et al., 2017).

Viruses classified in the family *Circoviridae* exhibit in the intergenic region a potential stem-loop structure, including the nonanucleotide sequence: NANTAT(C)TAC(T/A) (LOIKO et al., 2018; ROSARIO et al., 2017).

The putative rolling circle replication (RCR) encodes a REP protein with high conserved endonuclease domains including: Motifs I, II, and III as well as the helicase function with the conserved Walker A, B and C domains (**Figure 1E**) (MALATHI; RENUKA DEVI, 2019; ROSARIO et al., 2017).

These species and other *Circovirus* members display two ORFs: the first one (in the viral sense) encodes the REP protein, and the second (in a complementary sense) encodes the CP. The nonanucleotide CAGTATTAC is observed in species of the family and may display variations (CAG(T)TATTAC) in the 3rd position (ROSARIO et al., 2017).

Thus far, the genus *Circovirus* has 51 accepted species. All members display the CAGTATTAC (**Figure 1E**) as their nonanucleotide sequence (ROSARIO et al., 2017) as well as the two previously described ORFs. *Circovirus* species have been detected in association with vertebrates (MALE et al., 2016) and invertebrates (DAYARAM et al., 2013; MALATHI; RENUKA DEVI, 2019).

It was observed in contig 11404 (corresponding to the AFaV genome) the nonanucleotide sequence CAGTATTAC, which is identical to that found in the members of the genus *Circovirus* (**Figure 1E**).

On the other hand, the genus *Cyclovirus* is represented by 43 species (ICTV, 2019; LECIS et al., 2020; ROSARIO et al., 2017). In addition to the two ORFs, it was possible to observe the intergenic region with the stem-loop structure in the CFaV genome (**Figure 1E**), containing the nonanucleotide (TAGTATTAC) region (BIAGINI et al., 2012).

The species classified in this genus have been detected in a wide array of vertebrates such as bats (LECIS et al., 2020; LI et al., 2010a), pigs (ALLAN et al., 1998; KRAKOWKA et al., 2001; ZHANG et al., 2020), birds (CHANG et al., 2020; HALAMI et al., 2008; HATTERMANN et al., 2003; JOHNE et al., 2006; KASZAB et al., 2018), fishes (LORINCZ et al., 2011; MATSUMOTO et al., 2019) as well as in humans and others primates (LI et al., 2010b).

The putative motifs rolling circle replication initiator (RCR I, II, and III) and helicase domains (Walker A, B, and C) were found in both AFaV and CFaV genomes. However, AFaV displayed some genetic variations in motifs I, II, and Walker A (**Figure 1E**).

Two ORFs were observed in both CFaV and AFav genomes. In CFaV, the ORF encoding the CP (in the viral sense) displayed 822 nts in length. In the complementary sense, the ORF coding the REP protein displayed 924 nts. However, the region containing the introns (a common feature across cycloviruses) was not identified in this contig, reinforcing the notion that CFaV may represent a new species. On the other hand, AFav displayed (in the viral sense) the ORF coding for the REP, and in the complementary sense the ORF coding the CP.

Therefore, the two putative novel viruses (AFaV and CFaV) reported here, displayed several characteristics that are also present in the genomes of the members of the *Circoviridae* family, including genomic organization, ORF orientation, and the nonanucleotide sequence (**Figure 1E**). After phylogenetic analyzes (with representative isolates of the family *Circoviridae* family) grouped CFaV with members of the genus *Cyclovirus* (**Figure 2**). However, AFaV did not position itself with any of the species already classified in the two genera of the family *Circoviridae* (**Figure 2)**.

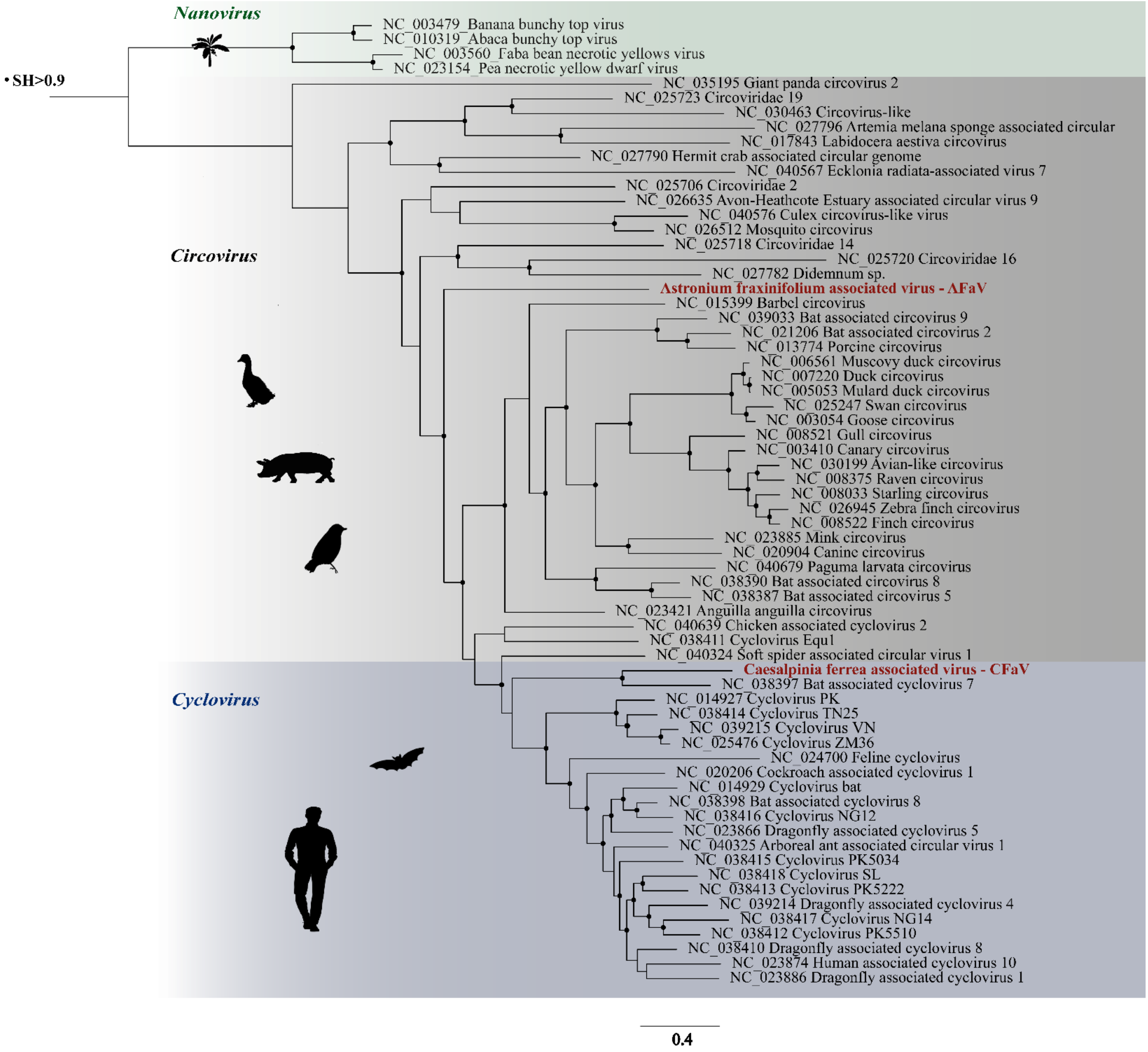


Figure 2. Phylogenetic tree based on REP protein sequence comparisons with members of the *Circoviridae* and *Nanoviridae* families. The amino acid sequences were aligned with the MUSCLE software. For the construction of the phylogenetic tree, Maximum Likelihood (ML) was derived with a bootstrap value with 1,000 repetitions using Fasttree (JTT+CAT). *Nanoviridae* sequences were used as outgroups. The black dots represent nodes with Shimodaira-Hasegawa (SH) value > 0.9.

The LDA analysis showed that the viruses detected in this study did not group with any virus belonging to the family *Circoviridae* described until now (**Figure 3**). These results suggest that the plants may be new hosts for these two novel circoviruses. The genetic diversity of these two new viruses may result in their assignment to a potential new genus within the family *Circoviridae.*

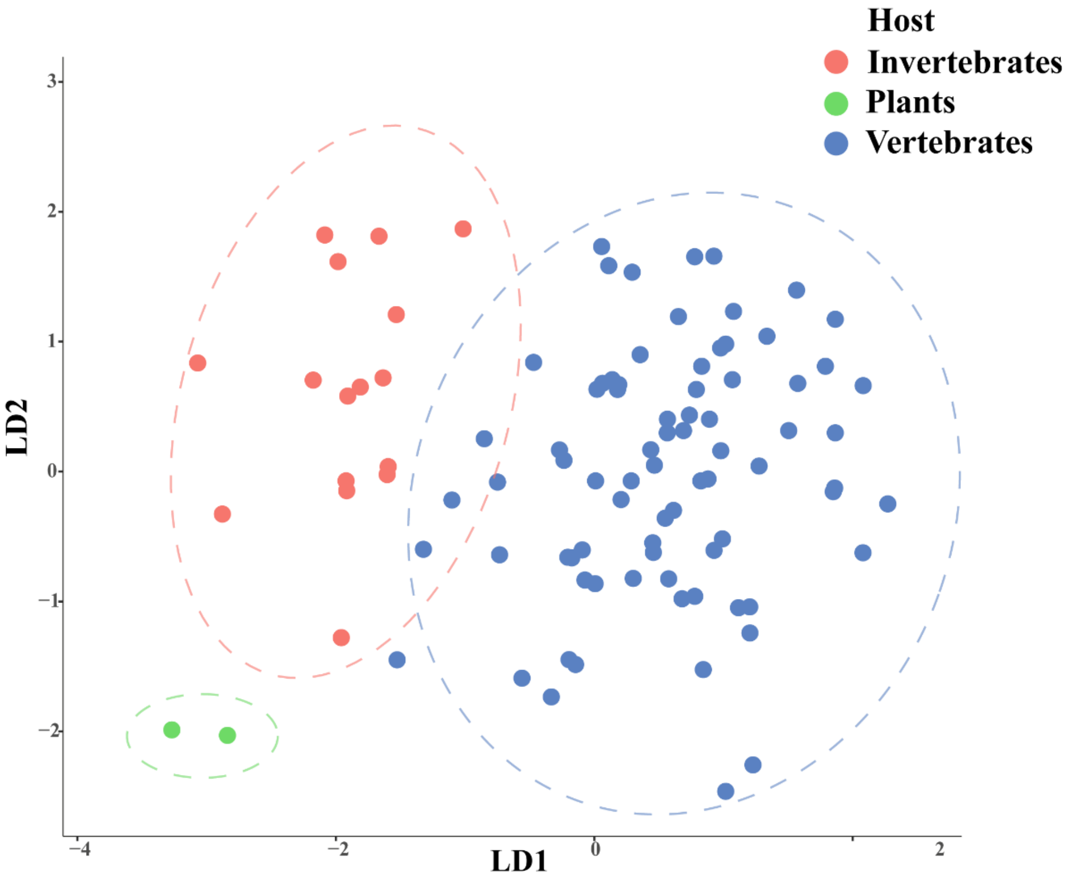


Figure 3. Linear discriminant analysis (LDA). LDA used to classify viral sequences into host groups. Linear discriminant analysis comparing nucleotide composition from members of *Circoviridae* family with known hosts. Invertebrates-infecting viruses are shown in pink circles, vertebrates-infecting viruses are shown in blue circles and the new viruses in this study (Astroniun fraxinifolim- associated virus - AFaV and Caesalpinia ferrea- associated virus - CFaV) detected in plants are shown in green circles.

Previous studies indicated that the REP protein sequence of the plant-associated members of the genus *Nanovirus* is similar to those found in vertebrate and invertebrate-associated *Circovirus* (GIBBS; WEILLER, 1999), Phylogenetic analyzes with the genetic information of these members gave strong support to the notion that they have the same ancestor (GIBBS:WEILLER, 1999). Therefore, an herbivore eating a plant infected with *Nanovirus* acquired the virus that underwent evolutionary processes that made it able to infect other hosts. So far, there are no reports of members of the *Circoviridae* infecting plants. In this context, more information is necessary to be obtained about these putative plant-associated new species that were classified in the family *Circoviridae*. The unique genomic features of these two viruses make us hypothesize that plants could also serve as hosts for circoviruses. Future efforts will be concentrated on the production of infectious clones for CFaV and AFaV aiming to demonstrate their infectivity in plants.

Supplementary materials of paper "Two new *Circoviridae* associated with tree species"

Supplementary Table 1. Identification of tree species collected at NOVACAP (Companhia Urbanizadora da Nova Capital do Brasil) - Nursery II.

Botanical family	**Tree species** **Common name**	 **Scientific name**	**Number of plants sampled**	**Symptoms** [1]
Anacardiaceae	Locust wood	*Astronium fraxinifolium* Schott	3	CL/ LD
Apocynaceae	“Guatambu”	*Aspidosperma parvifolium* A. DC.	2	CS
Bignoniaceae	Pink trumpet tree	*Handroanthus impetiginosus* Mart. ex DC.	2	B/ N
	Yellow poui	*Handroanthus serratifolius* (Vahl) S. Grose	4	Y/ LD / NL
	“Bolsa de pastor”	*Zeyheria tuberculosa* (Vell.) Bureau ex Verl.	2	Y / CL
Cecropiaceae	Pumpwoods	*Cecropia hololeuca* Miq.	2	LD / WS
Fabaceae	Brazil red wood	*Paubrasilia echinata* Lam.	3	PC
	Faveira	*Parkia pendula* Willd.	3	Y / LD
	Mulungu	*Erythrina mulungu* Mart. ex Benth.	3	R
	“Pau pereira”	*Platycyamus regnelli* Benth.	3	Y / LD
	“Sete cascas”	*Samanea tubulosa* (Benth.) Barneby & J. W. Grime.	3	CS / M/ MO
	Brazilian ironwood	*Caesalpinia ferrea* Mart. ex Tul.	1	CL
	Indian rosewood	*Dalbergia violacea* Vogel	2	CL
	Copaiba	*Copaifera langsdorffii* Desf.	2	IW / MO
	“Pau-sangue”	*Pterocarpus violaceus* Vogel	2	CL/ LD
	Cerejeira	*Amburana cearensis* Schwacke & Taub	2	CS
	silk tree	*Albizia hasslerii* Chodat	1	Y
	Grapia	*Apuleia leiocarpa* Vogel	2	Y

[1] Y: yellowing; B: blistering; CL: chlorosis; CS: chlorotic spots; LD: leaf deformation; M: mosaic; MO: mottle; NL: necrotic lesion; N: necrosis; R: rugose and WS: without symptom.

Supplementary Table 1. Identification of tree species collected at NOVACAP (Companhia Urbanizadora da Nova Capital do Brasil) - Nursery II.

Botanical family	Tree species		Number of plants sampled	Symptoms [1]
	Common name	Scientific name		
Guttiferae	"Pau santo"	*Kielmeyera variabilis* Mart. & Zucc	1	MO
Lecythidaceae	"jequitibá branco"	*Cariniana estrellensis* (Raddi) Kuntze *(*Raddi	3	LD
Malvaceae	West Indian-elm	*Guazuma ulmifolia* Lam.	3	CL
Melastomataceae	Brazilian glorytree	*Tibouchina granulosa* (Desr.) Cogn.	2	M; NL
Meliaceae	Mahogany	*Swietenia macrophylla* King	2	CS / LD
Myrtaceae	Timor white gum	*Eucalyptus urophylla* S.T. Blake	2	DF/ WS
Rhamnaceae	Japanese raisintree	*Hovenia dulcis* Thunb. Voucher.	2	IC / M
Rutaceae	Brazilian Boxwood	*Esenbeckia leiocarpa* Engl.	2	CL/R
Sapindaceae	Southern soapberry	*Sapindus saponaria* L.	1	NL

[1] Y: yellowing; B: blistering; CL: chlorosis; CS: chlorotic spots; IC: interveinal chlorosis; LD: leaf deformation; M: mosaic; MO: mottle; NL: necrotic lesion; N: necrosis; R: rugose and WS: without symptom.

Supplementary Table 2. The accession of the sequences used in the phylogeny and the nucleotide frequence composition analysis (NCA).

Name	Organism	Host	ApA	ApC	ApG	ApT	CpA	CpC	CpG	CpT	GpA	GpC	GpG	GpT	TpA	TpC	TpG	TpT
NC_040325	Arboreal ant associated circular virus 1	Invertebrate	0.0690	0.0532	0.0605	0.0724	0.0617	0.0566	0.0617	0.0509	0.0566	0.0571	0.0424	0.0707	0.0684	0.0639	0.0622	0.0928
NC_038400	Bat-associated cyclovirus 11	Vertebrate	0.0808	0.0743	0.0515	0.0743	0.0705	0.0477	0.0722	0.0564	0.0689	0.0586	0.0553	0.0440	0.0608	0.0662	0.0477	0.0705
NC_038393	Bat-associated cyclovirus 2	Vertebrate	0.1012	0.0752	0.0531	0.0859	0.0774	0.0413	0.0554	0.0560	0.0627	0.0582	0.0396	0.0424	0.0735	0.0560	0.0548	0.0673
NC_025791	Bat circovirus POA/2012/II	Vertebrate	0.0582	0.0479	0.0525	0.0804	0.0559	0.0542	0.0576	0.0536	0.0587	0.0536	0.0473	0.0724	0.0661	0.0656	0.0747	0.1015
NC_040639	Chicken-associated cyclovirus 2	Vertebrate	0.0895	0.0518	0.0355	0.0974	0.0625	0.0512	0.0456	0.0636	0.0529	0.0416	0.0388	0.0523	0.0698	0.0782	0.0653	0.1041
NC_020206	Cockroach-associated cyclovirus 1	Invertebrate	0.0554	0.0484	0.0390	0.0775	0.0431	0.0542	0.0676	0.0577	0.0466	0.0484	0.0542	0.0851	0.0763	0.0717	0.0728	0.1020
NC_038407	Cyclovirus Chimp11	Vertebrate	0.1098	0.0858	0.0526	0.0789	0.0749	0.0532	0.0532	0.0526	0.0589	0.0521	0.0538	0.0360	0.0830	0.0435	0.0412	0.0704
NC_038411	Cyclovirus Equ1	Vertebrate	0.0983	0.0674	0.0511	0.0793	0.0467	0.0418	0.0500	0.0619	0.0733	0.0456	0.0435	0.0554	0.0771	0.0456	0.0733	0.0896
NC_014930	Cyclovirus NGchicken15/NGA/2009	Vertebrate	0.1035	0.0836	0.0489	0.0716	0.0682	0.0574	0.0597	0.0534	0.0620	0.0415	0.0546	0.0483	0.0733	0.0569	0.0432	0.0739
NC_038405	Cyclovirus PKbeef23/PAK/2009	Vertebrate	0.0964	0.0599	0.0631	0.0795	0.0539	0.0523	0.0626	0.0506	0.0719	0.0555	0.0550	0.0512	0.0768	0.0517	0.0528	0.0670
NC_014928	Cyclovirus PKgoat11/PAK/2009	Vertebrate	0.1057	0.0766	0.0509	0.0789	0.0674	0.0486	0.0566	0.0509	0.0663	0.0486	0.0549	0.0440	0.0726	0.0497	0.0514	0.0771
NC_027530	Cyclovirus TsCyV-1_JP-NUBS-2014	Vertebrate	0.0716	0.0543	0.0526	0.0772	0.0616	0.0560	0.0610	0.0582	0.0582	0.0565	0.0481	0.0560	0.0644	0.0699	0.0571	0.0974
NC_025476	Cyclovirus ZM36a	Vertebrate	0.0756	0.0585	0.0504	0.0740	0.0655	0.0638	0.0660	0.0628	0.0494	0.0617	0.0413	0.0569	0.0687	0.0740	0.0515	0.0799
NC_040826	Dragonfly-associated cyclovirus	Invertebrate	0.0732	0.0453	0.0674	0.0738	0.0464	0.0416	0.0611	0.0406	0.0738	0.0501	0.0590	0.0796	0.0669	0.0527	0.0748	0.0938

NC_023886	Dragonfly-associated cyclovirus 1	Invertebrate	0.1156	0.0748	0.0391	0.0978	0.0742	0.0558	0.0587	0.0477	0.0535	0.0523	0.0368	0.0385	0.0840	0.0535	0.0472	0.0707
NC_023868	Dragonfly-associated cyclovirus 3	Invertebrate	0.0755	0.0761	0.0579	0.0667	0.0731	0.0737	0.0573	0.0650	0.0562	0.0439	0.0386	0.0538	0.0714	0.0761	0.0386	0.0761
NC_023866	Dragonfly-associated cyclovirus 5	Invertebrate	0.0662	0.0522	0.0488	0.0763	0.0522	0.0611	0.0589	0.0538	0.0482	0.0471	0.0499	0.0752	0.0768	0.0656	0.0628	0.1049
NC_034977	Duck-associated cyclovirus 1	vertebrate	0.0878	0.0768	0.0605	0.0594	0.0763	0.0721	0.0626	0.0615	0.0658	0.0615	0.0468	0.0479	0.0547	0.0621	0.0521	0.0521
NC_024700	Feline cyclovirus	Vertebrate	0.1569	0.0875	0.0570	0.0999	0.0818	0.0435	0.0395	0.0344	0.0734	0.0305	0.0293	0.0327	0.0892	0.0378	0.0401	0.0666
NC_023874	Human-associated cyclovirus 10	Vertebrate	0.0743	0.0581	0.0559	0.0811	0.0553	0.0503	0.0570	0.0587	0.0587	0.0520	0.0380	0.0643	0.0805	0.0609	0.0620	0.0928
NC_021568	Human cyclovirus VS5700009	Vertebrate	0.0852	0.0661	0.0754	0.0666	0.0524	0.0333	0.0623	0.0508	0.0874	0.0579	0.0666	0.0541	0.0677	0.0415	0.0617	0.0710
NC_032682	Indian encephalitis associated cyclovirus	Vertebrate	0.1226	0.0877	0.0564	0.0750	0.0726	0.0523	0.0587	0.0529	0.0639	0.0500	0.0476	0.0395	0.0819	0.0465	0.0389	0.0535
NC_031755	Mouse associated cyclovirus 1	Vertebrate	0.1189	0.0898	0.0422	0.0736	0.0808	0.0687	0.0548	0.0673	0.0597	0.0462	0.0305	0.0346	0.0646	0.0673	0.0435	0.0575
NC_038402	Pacific flying fox-associated cyclovirus-1	Vertebrate	0.0515	0.0447	0.0453	0.0697	0.0432	0.0775	0.0661	0.0583	0.0520	0.0552	0.0624	0.0786	0.0645	0.0676	0.0749	0.0884
NC_040324	Soft spider-associated circular virus 1	Invertebrate	0.0847	0.0630	0.0548	0.0728	0.0770	0.0718	0.0563	0.0573	0.0568	0.0604	0.0424	0.0506	0.0568	0.0677	0.0568	0.0708
NC_020099	Acartia tonsa copepod circovirus	Invertebrate	0.0695	0.0773	0.0677	0.0569	0.0839	0.0803	0.0557	0.0575	0.0749	0.0587	0.0569	0.0521	0.0425	0.0611	0.0623	0.0425
NC_023421	Anguilla anguilla circovirus	Vertebrate	0.0770	0.0581	0.0625	0.0618	0.0610	0.0596	0.0451	0.0625	0.0770	0.0574	0.0654	0.0596	0.0436	0.0531	0.0865	0.0698
NC_040528	Apteryx rowi circovirus-like virus	Vertebrate	0.0979	0.0643	0.0441	0.0681	0.0677	0.0864	0.0547	0.0648	0.0513	0.0485	0.0437	0.0441	0.0571	0.0749	0.0451	0.0873
NC_030199	Avian-like circovirus	Invertebrate	0.0785	0.0496	0.0749	0.0578	0.0578	0.0650	0.0459	0.0578	0.0774	0.0568	0.0671	0.0630	0.0465	0.0552	0.0764	0.0702
NC_015399	Barbel circovirus	Vertebrate	0.0839	0.0604	0.0634	0.0655	0.0476	0.0522	0.0680	0.0450	0.0767	0.0532	0.0563	0.0752	0.0645	0.0471	0.0737	0.0675
NC_038385	Bat-associated circovirus 1	Vertebrate	0.0656	0.0446	0.0709	0.0661	0.0527	0.0462	0.0339	0.0656	0.0634	0.0629	0.0666	0.0704	0.0656	0.0451	0.0913	0.0892

NC_021206	Bat-associated circovirus 2	Vertebrate	0.0818	0.0462	0.0702	0.0568	0.0557	0.0651	0.0228	0.0657	0.0663	0.0484	0.0835	0.0657	0.0512	0.0496	0.0869	0.0841
NC_038387	Bat-associated circovirus 5	Vertebrate	0.0607	0.0397	0.0812	0.0545	0.0511	0.0545	0.0463	0.0463	0.0760	0.0683	0.1147	0.0674	0.0483	0.0358	0.0841	0.0712
NC_038388	Bat-associated circovirus 6	Vertebrate	0.0703	0.0474	0.0883	0.0543	0.0612	0.0490	0.0250	0.0495	0.0809	0.0538	0.1011	0.0761	0.0479	0.0346	0.0974	0.0633
NC_038389	Bat-associated circovirus 7	Vertebrate	0.0674	0.0504	0.0859	0.0473	0.0612	0.0556	0.0319	0.0504	0.0751	0.0618	0.1055	0.0715	0.0473	0.0314	0.0906	0.0664
NC_038390	Bat-associated circovirus 8	Vertebrate	0.0508	0.0406	0.0725	0.0508	0.0459	0.0546	0.0532	0.0517	0.0745	0.0667	0.1238	0.0750	0.0430	0.0435	0.0909	0.0624
NC_039033	Bat-associated circovirus 9	Vertebrate	0.0522	0.0511	0.0806	0.0501	0.0801	0.0954	0.0237	0.0669	0.0638	0.0717	0.0911	0.0522	0.0374	0.0479	0.0838	0.0522
NC_035799	Bat circovirus	Vertebrate	0.0540	0.0422	0.0696	0.0516	0.0483	0.0696	0.0611	0.0507	0.0810	0.0753	0.0905	0.0625	0.0341	0.0426	0.0881	0.0786
NC_001944	Beak and feather disease virus	Vertebrate	0.0588	0.0432	0.0598	0.0522	0.0422	0.0683	0.0668	0.0512	0.0648	0.0683	0.0944	0.0743	0.0482	0.0487	0.0814	0.0773
NC_003410	Canary circovirus	Vertebrate	0.0720	0.0437	0.0740	0.0535	0.0530	0.0807	0.0452	0.0586	0.0761	0.0540	0.0812	0.0643	0.0416	0.0586	0.0756	0.0679
NC_020904	Canine circovirus	Vertebrate	0.0509	0.0451	0.0597	0.0635	0.0475	0.0684	0.0509	0.0674	0.0761	0.0606	0.0829	0.0650	0.0441	0.0606	0.0912	0.0660
NC_038391	Chimpanzee stool avian-like circovirus Chimp17	Vertebrate	0.0683	0.0445	0.0714	0.0579	0.0564	0.0832	0.0496	0.0667	0.0729	0.0641	0.0848	0.0522	0.0450	0.0636	0.0683	0.0512
NC_023888	Circo-like virus-Brazil hs1	Vertebrate	0.1612	0.0713	0.0871	0.0931	0.0653	0.0230	0.0190	0.0392	0.0939	0.0202	0.0455	0.0412	0.0919	0.0321	0.0491	0.0669
NC_034210	Circovirus sp.	Vertebrate	0.0551	0.0513	0.0754	0.0545	0.0700	0.0861	0.0321	0.0689	0.0732	0.0738	0.0839	0.0518	0.0379	0.0460	0.0909	0.0492
NC_002361	Columbid circovirus	Vertebrate	0.0663	0.0476	0.0643	0.0496	0.0589	0.0825	0.0697	0.0565	0.0688	0.0810	0.0771	0.0619	0.0334	0.0565	0.0776	0.0481
NC_040576	Culex circovirus-like virus	Invertebrate	0.0685	0.0671	0.0430	0.0777	0.0700	0.0569	0.0560	0.0705	0.0492	0.0613	0.0362	0.0478	0.0681	0.0681	0.0594	0.1004
NC_040833	Culex circovirus-like virus	Invertebrate	0.0642	0.0460	0.0710	0.0482	0.0592	0.0678	0.0692	0.0551	0.0665	0.0774	0.0833	0.0642	0.0410	0.0605	0.0669	0.0596
NC_023854	Dragonfly orbiculatusvirus	Invertebrate	0.0821	0.0366	0.0591	0.0904	0.0387	0.0225	0.0376	0.0507	0.0585	0.0376	0.0533	0.0821	0.0889	0.0528	0.0815	0.1275
NC_007220	Duck circovirus	Vertebrate	0.0578	0.0533	0.0563	0.0558	0.0482	0.0668	0.0633	0.0633	0.0598	0.0623	0.0678	0.0779	0.0568	0.0593	0.0804	0.0709
NC_040363	Farfantepenaeus duorarum circovirus	Invertebrate	0.0619	0.0691	0.0430	0.0640	0.0670	0.0732	0.0763	0.0558	0.0640	0.0640	0.0681	0.0553	0.0445	0.0660	0.0640	0.0640
NC_040381	Feline stool-associated circular virus KU14	Vertebrate	0.0411	0.0509	0.0680	0.0421	0.0577	0.0924	0.0729	0.0792	0.0528	0.0866	0.0778	0.0577	0.0509	0.0724	0.0562	0.0416

NC_008522	Finch circovirus	Vertebrate	0.0673	0.0428	0.0709	0.0464	0.0566	0.0882	0.0571	0.0576	0.0688	0.0714	0.0796	0.0653	0.0342	0.0571	0.0775	0.0592
NC_035200	Giant panda circovirus 1	Vertebrate	0.0802	0.0525	0.0609	0.0689	0.0539	0.0437	0.0507	0.0579	0.0707	0.0488	0.0620	0.0754	0.0576	0.0612	0.0835	0.0722
NC_035194	Giant panda circovirus 3	Vertebrate	0.1041	0.0562	0.0630	0.0811	0.0679	0.0309	0.0366	0.0438	0.0758	0.0438	0.0430	0.0656	0.0562	0.0483	0.0860	0.0977
NC_003054	Goose circovirus	Vertebrate	0.0610	0.0462	0.0533	0.0621	0.0330	0.0571	0.0604	0.0654	0.0692	0.0560	0.0703	0.0808	0.0588	0.0566	0.0923	0.0775
NC_008521	Gull circovirus	Vertebrate	0.0684	0.0438	0.0615	0.0615	0.0561	0.0689	0.0605	0.0635	0.0659	0.0762	0.0561	0.0635	0.0443	0.0600	0.0836	0.0664
NC_024694	Human circovirus VS6600022	Vertebrate	0.0953	0.0850	0.0561	0.0780	0.0939	0.0692	0.0568	0.0480	0.0681	0.0438	0.0490	0.0427	0.0572	0.0699	0.0416	0.0455
NC_038392	Human stool-associated circular virus NG13	Vertebrate	0.0937	0.0477	0.0719	0.0742	0.0560	0.0513	0.0395	0.0454	0.0666	0.0536	0.0631	0.0595	0.0707	0.0395	0.0684	0.0990
NC_017843	Labidocera aestiva circovirus	Invertebrate	0.0856	0.0681	0.0919	0.0528	0.0970	0.0652	0.0556	0.0380	0.0749	0.0630	0.0641	0.0533	0.0403	0.0596	0.0437	0.0471
NC_040362	Meles meles circovirus-like virus	Vertebrate	0.0668	0.0740	0.0451	0.0469	0.0794	0.0799	0.0830	0.0672	0.0596	0.0745	0.0596	0.0460	0.0271	0.0812	0.0519	0.0578
NC_023885	Mink circovirus	Vertebrate	0.0702	0.0474	0.0685	0.0680	0.0474	0.0514	0.0457	0.0428	0.0765	0.0628	0.0817	0.0691	0.0600	0.0257	0.0937	0.0891
NC_040383	Molossus molossus circovirus 1	Vertebrate	0.0883	0.0813	0.0599	0.0769	0.0813	0.0435	0.0542	0.0580	0.0731	0.0467	0.0492	0.0454	0.0631	0.0656	0.0511	0.0624
NC_040380	Molossus molossus circovirus 3	Vertebrate	0.1027	0.0774	0.0613	0.0871	0.0803	0.0365	0.0477	0.0570	0.0696	0.0380	0.0453	0.0424	0.0755	0.0696	0.0409	0.0686
NC_026512	Mosquito circovirus	Invertebrate	0.0666	0.0472	0.0467	0.0689	0.0569	0.0677	0.0620	0.0660	0.0518	0.0643	0.0444	0.0518	0.0535	0.0734	0.0598	0.1190
NC_005053	Mulard duck circovirus	Vertebrate	0.0562	0.0522	0.0562	0.0542	0.0441	0.0712	0.0662	0.0657	0.0647	0.0627	0.0662	0.0747	0.0532	0.0612	0.0797	0.0717
NC_006561	Muscovy duck circovirus	Vertebrate	0.0639	0.0544	0.0493	0.0609	0.0458	0.0679	0.0554	0.0710	0.0619	0.0503	0.0584	0.0770	0.0564	0.0674	0.0845	0.0755
NC_040679	Paguma larvata circovirus	Vertebrate	0.0973	0.0424	0.0665	0.0718	0.0458	0.0482	0.0183	0.0434	0.0684	0.0280	0.0887	0.0795	0.0660	0.0371	0.0911	0.1075
NC_022897	Penaeus monodon circovirus VN11	Invertebrate	0.0676	0.0648	0.0586	0.0468	0.0580	0.0654	0.0704	0.0648	0.0699	0.0704	0.0823	0.0608	0.0428	0.0580	0.0715	0.0479
NC_001792	Porcine circovirus 1	Vertebrate	0.0717	0.0415	0.0768	0.0558	0.0472	0.0581	0.0347	0.0558	0.0689	0.0558	0.0956	0.0677	0.0581	0.0404	0.0803	0.0916
NC_005148	Porcine circovirus 2	Vertebrate	0.0764	0.0510	0.0702	0.0583	0.0566	0.0578	0.0379	0.0549	0.0713	0.0498	0.0917	0.0657	0.0510	0.0487	0.0787	0.0798

NC_023877	Porcine stool-associated circular virus 4	Vertebrate	0.0551	0.0460	0.0537	0.0698	0.0537	0.0574	0.0443	0.0698	0.0571	0.0490	0.0655	0.0826	0.0584	0.0729	0.0907	0.0739
NC_008375	Raven circovirus	Vertebrate	0.0606	0.0453	0.0659	0.0548	0.0448	0.0590	0.0712	0.0559	0.0680	0.0659	0.0738	0.0791	0.0527	0.0606	0.0759	0.0664
NC_038386	Rhinolophus ferrumequinum circovirus 1	Vertebrate	0.0875	0.0449	0.0722	0.0722	0.0557	0.0517	0.0256	0.0529	0.0762	0.0443	0.0898	0.0648	0.0580	0.0443	0.0875	0.0722
NC_025246	Silurus glanis circovirus	Vertebrate	0.0769	0.0591	0.0575	0.0687	0.0698	0.0835	0.0433	0.0601	0.0606	0.0535	0.0662	0.0535	0.0555	0.0601	0.0667	0.0652
NC_008033	Starling circovirus	Vertebrate	0.0834	0.0500	0.0771	0.0553	0.0635	0.0795	0.0422	0.0572	0.0703	0.0504	0.0810	0.0630	0.0480	0.0626	0.0645	0.0519
NC_025247	Swan circovirus	Vertebrate	0.0545	0.0539	0.0573	0.0606	0.0348	0.0640	0.0724	0.0528	0.0809	0.0517	0.0713	0.0814	0.0556	0.0545	0.0842	0.0702
NC_028045	Tadarida brasiliensis circovirus 1	Vertebrate	0.0946	0.0419	0.0691	0.0657	0.0527	0.0595	0.0385	0.0510	0.0730	0.0606	0.0929	0.0572	0.0510	0.0396	0.0832	0.0696
NC_026945	Zebra finch circovirus	Vertebrate	0.0646	0.0449	0.0697	0.0449	0.0545	0.0853	0.0717	0.0444	0.0777	0.0732	0.0843	0.0707	0.0268	0.0525	0.0803	0.0545
MT707946	Astronium fraxinifolium-associated virus	Plants	0.0846	0.0830	0.0578	0.0653	0.0913	0.0590	0.0574	0.0543	0.0622	0.0586	0.0504	0.0464	0.0531	0.0610	0.0519	0.0637
MT707947	Caesalpinia ferrea-associated virus	Plants	0.0598	0.0419	0.0607	0.0726	0.0551	0.0431	0.0585	0.0513	0.0624	0.0440	0.0658	0.0786	0.0577	0.0790	0.0662	0.1034

Supplementary Table 3. Conserved of nonanucleotide and motifs from *Circoviridae* family

NAME	MOTIF I	MOTIF II	MOTIF II	Walker A	Walker B	Motif C
NC_030465_Circovirus-like genome DCCV-2	SLTYSN	THFHA	ICKD	GDTGCGKT	VFDDM	FTAN
NC_030468_Circovirus-like genome DCCV-12	CWTYHD	RHLQG	YCQK	GEPFSGKT	ICDDV	FTSN
NC_030456_Circovirus-like genome DHCV-5	CFTIFD	EHFQG	YCSK	GVPGAGKS	ILEDF	VTSN
NC_017843_Labidocera aestiva circovirus	TGTVNN	PHIQW	YCGK	GESGTGKS	IIEEM	VTSN
NC_030471_Circovirus-like genome DHCV-1	CFTVNN	PHLQG	YCSK	GPAGTGKS	LIEEW	ITSN
NC_030463_Circovirus-like genome DCCV-1	VFTINN	PHLQG	YCKK	GEAGVGKT	IINDL	ITSQ
NC_013028_Circovirus-like genome CB-A	CFTYNN	PHLQG	YCQK	GPSGTGKS	LIDDV	VTSN
NC_030475_Circovirus-like genome DHCV-6	CFTYNN	RHLQG	YCSK	GATGTGKS	YLEDF	VTSN
NC_030473_Circovirus-like genome DCCV-5	VFTYNN	PHLQG	YCSK	GRTGSGKS	LLDDV	VTSQ
NC_035194_Giant panda circovirus 3 -	CFTSYH	RHWQC	YCKK	GKTNLNKT	LFDEF	IISN
NC_013025_Circovirus-like genome RW-C -	CFTGFN	EHWQG	YCKK	GPTGCGKS	IIDDF	ITSS
NC_040382_Molossus molossus circovirus 2 -	CFTLNN	PHLQG	YCKK	GETGTGKT	IIDEF	ITSN
NC_030477_Circovirus-like genome DHCV-3 -	CFTVNN	KHLQC	YCKK	GRSGIGKT	LIDDV	LTSL
NC_030460_Circovirus-like genome DCCV-10 -	CFTLNN	PHYQG	YCTK	GEPGAGKT	LFDDL	ITAP
NC_040576_Culex circovirus-like virus -	CFTYNN	PHLQG	YCKK	GPSGSGKT	VLDDF	ITSI
NC_026512_Mosquito circovirus	CWTLNN	PHLQG	YCKK	GASGTGKT	LLDDY	VTSI
NC_030464_Circovirus-like genome DCCV-11 -	VYTLFA	VHLQG	YCTK	GDAGVGKT	VIDDF	ITSN
NC_023888_Circo-like virus-Brazil hs1	CFTE	LHIQG	YCQK	GRSGVGKS	IIDDF	ITSN
NC_023421_Anguilla anguilla circovirus	CFTLNN	PHSQG	YCSK	GIPGCGKS	ILDDF	ITSN

NC_006561_Muscovy duck circovirus	VFTINN	PHLQG	YCSK	GPPGTGKS	VMDDF	TTSN
NC_007220_Duck circovirus	VFTINN	PHLQG	YCAK	GPPCTGKS	VMDDF	ITSN
NC_005053_Mulard duck circovirus	VFTLNN	PHLQG	YCAK	GPPGTGKS	VMDDF	ITSN
NC_025247_Swan circovirus -	VFTINN	PHLQG	YCSK	GPPGSGKS	VMDDF	ITSN
NC_003054_Goose circovirus	VFTINN	PHLQG	YCSK	GRPGSGKS	VMDDF	ITSN
NC_001944_Beak and feather disease virus -	CFTLNN	PHLQG	YCSK	GPPGCGKS	ILDDF	ITSN
NC_008521_Gull circovirus -	CFTLNN	PHLQG	YCGK	GPPGCGKS	IIDDF	FTSN
NC_003410_Canary circovirus	CFTLNN	PHLQG	YCSK	GPSGVGKS	IMDDF	ITSN
NC_008033_Starling circovirus -	CFTLNN	PHLQG	YCSK	GPSGVGKS	IIDDF	ITSN
NC_026945_Zebra finch circovirus -	VFTLNN	PHLQG	YCSK	GPSGVGKS	VIDDF	ITSN
NC_008522_Finch circovirus -	CFTLNN	PHLQG	YCGK	GPSGCGKS	IIDDF	ITSN
NC_008375_Raven circovirus -	CFTLNN	PHLQG	YCTK	GPSGVGKS	VIDDF	VTSN
NC_030199_Avian-like circovirus -	CFTLNN	PHLQG	YCSK	GPSGVGKS	VIDDF	ITSN
NC_038391_Chimpanzee stool avian-like circovirus Chimp17	CFTLNN	PHLQG	YCSK	GPSGVGKS	VIDDF	ITSN
NC_002361_Columbid circovirus	CFTLNN	PHLQG	YCSK	GPPGCGKS	IIDDF	VTSN
NC_015399_Barbel circovirus	CFTLNN	PHLQG	YCTK	GDPGCGKS	IVDDF	VTSN
NC_038392_Human stool-associated circular virus NG13	CFTLNN	PHLQG	YCSK	GPPGCGKS	IIDDF	LTSN
NC_025246_Silurus glanis circovirus	VFTLNN	PHLQG	YCSK	GPPGCGKS	ILDDF	ITSN
NC_039033_Bat-associated circovirus 9	CFTINN	PHIQG	YCSK	GPPGCGKS	ILDDF	ITSN
NC_021206_Bat-associated circovirus 2	VFTLNN	PHLQG	YCTK	GPPGCGKS	ILDDF	ITSN
NC_013774_Porcine circovirus type 1/2a	VFTLNN	PHLQG	YCSK	GPPGCGKS	VLDDF	ITSN
NC_001792_Porcine circovirus 1	VFTLNN	PHLQG	YCSK	GPPGCGKS	VLDDF	ITSN
NC_005148_Porcine circovirus 2	VFTLNN	PHLQG	YCSK	GPPGCGKS	VIDDF	ITSN
NC_038385_Bat-associated circovirus 1	CFTINN	PHLQG	YCSK	GVPGVGKS	CLDDY	ITSN
NC_023885_Mink circovirus	CFTINN	PHLQG	YCSK	GPPGVGKS	VLDDF	ITSN
NC_035799_Bat circovirus	CFTLNN	PHLQG	YCSK	GPPGVGKS	VIDDF	ITSN
NC_020904_Canine circovirus	CFTINN	PHLQG	YCSK	GPPGCGKS	ILDDF	ITSN
NC_040679_Paguma larvata circovirus	CFTLNN	QHLQG	YCKK	GPPGCGKT	CLDDF	ITSN

NC_038390_Bat-associated circovirus 8	CFTLNN	PHLQG	YCRK	GRPGVGKT	VFDDF	FTSN
NC_038387_Bat-associated circovirus 5	CFTINN	PHLQG	YCRK	GEPGVGKT	IFDDF	FTSN
NC_038389_Bat-associated circovirus 7	CFTINN	PHLQG	YCRK	GPPGVGKT	VLDDF	ITSN
NC_038388_Bat-associated circovirus 6	CFTLNN	PHLQG	YCQK	GPPGVGKT	VLDDF	ITSN
NC_038386_Rhinolophus ferrumequinum circovirus 1	CFTINN	KHLQG	YCTK	GEPGSGKS	VLDDF	ITSN
NC_028045_Tadarida brasiliensis circovirus 1	CFTINN	PHLQG	YCSK	GEPGSGKS	VLDDF	ITSN
NC_040383_Molossus molossus circovirus 1	CFTLNN	NHFQG	YCRK	GASGTGKS	VLDDF	ITST
NC_040380_Molossus molossus circovirus 3	CFTLNN	YHFQG	YCRK	GASGTGKS	VLDDF	ITST
NC_040363_Farfantepenaeus duorarum circovirus	CFTVFN	SHIQG	YCQK	GDTETGKT	LLDDF	ITSN
NC_040386_Molossus molossus circovirus 4	CFTSFD	LHAQG	YCKK	GPAGCGKT	LFDDI	FTSN
NC_020099_Acartia tonsa copepod circovirus	CFTLNN	LHIQG	YCTK	GPTGSGKS	ILDDY	ITCD
NC_040528_Apteryx rowi circovirus-like virus	CFTLNN	PHFQG	YCTK	GPPGTGKS	IFDDF	ITTN
NC_040362_Meles meles circovirus-like virus	CFTLNN	PHFQG	YCTK	GTPGTGKS	LFDDF	ITTN
NC_040381_Feline stool-associated circular virus KU14	CGTLHL	RHFQF	YCSK	GPSGGGKS	ILDDF	ITSN
NC_013023_Circovirus-like genome RW-A	CFTHNN	PHFQG	YCSR	GPTGVGKT	LFDDF	VTSN
NC_030467_Circovirus-like genome DCCV-3	CFTINN	QHIQG	YCSK	GEPGVGKT	LIDDF	ITSN
NC_013026_Circovirus-like genome RW-D	VITVNN	PHLQI	YCTK	GPAGSGKT	IIDDL	ITCQ
NC_030469_Circovirus-like genome DCCV-13	MLTIPH	KHWQL	YVWK	GPTGTGKS	VFDEF	ITSN
NC_013027_Circovirus-like genome RW-E	LLTIPE	LHWQV	YVWK	GPTGTGKS	VVDEF	ITSN
NC_030474_Circovirus-like genome DHCV-2	ILTIPH	LHWQI	YVWK	GATGTGKS	VIDEF	ITSN
NC_035200_Giant panda circovirus 1	ILTISC	KHWQV	YCRK	GRTGTGKS	VIDEF	ITSN

NC_030457_Circovirus-like genome DCCV-8	ICTIPR	LHWQV	YVHK	GRTGTGKS	VIDEF	ITSN
NC_035196_Giant panda circovirus 4	CFTINN	DHLQG	YCMK	GPPGVGKS	LLDEF	ITSN
NC_030470_Circovirus-like genome DCCV-4	VFTING	KHIQG	YCTK	GSTGVGKT	VLDDY	ITTP
NC_024694_Human circovirus VS6600022	FVTSFN	IHYHL	YIKK	GKSGAGKS	WFDEF	ISTT
NC_023877_Porcine stool-associated circular virus 4	CITAWN	RHLQC	YCKK	GASGSGKS	WVDEF	ISTT
NC_013020_Circovirus-like genome BBC-A - putative Rep protein CDS	AFTDFN	PHLQG	YCKK	GPPGCGKS	IFDDQ	FIDN
NC_022897_Penaeus monodon circovirus VN11	VFTWNN	PHLQG	YCTK	GPTGTGKT	VLDEI	ITST
NC_030459_Circovirus-like genome DCCV-9	VLTVFD	KHYQC	YCSK	GAAGCGKT	LFNNV	VTSL
NC_038397_Bat-associated cyclovirus 7	CFTVNN	QHLQG	YCTK	GPTRTGKS	IIDDF	ITSN
NC_031755_Mouse-associated cyclovirus 1	CFTTNN	RHLQG	YCTK	GPTRTGKS	IIDDF	ITSN
NC_038411_Cyclovirus Equ1	CFTYNN	KHLQG	YCTK	GETGTGKS	IIDDF	ITSN
NC_040324_Soft spider-associated circular virus 1	CFTLNN	RHLQG	YCCK	GPPGSGKS	VVDDF	ITSN
NC_038405_Cyclovirus PKbeef23/PAK/2009 -	CWTLNN	KHLQG	YCSK	GPTGAGKS	IFDDF	FTSN
NC_014927_Cyclovirus PKgoat21/PAK/2009	CWTLNN	KHLQG	YCSK	GPTGAGKS	IFDDF	FTSN
NC_038414_Cyclovirus TN25	CWTLNN	KHLQG	YCSK	GPTGSGKS	IIDDF	ITSE
NC_038404_Pacific flying fox-associated cyclovirus-3	CWTLNN	KHLQG	YCSK	GATGLGKS	VIDEF	ITSN
NC_021568_Human cyclovirus VS5700009	CWTLNN	KHLQG	YCSK	GATGLGKS	VIDDF	ITSN
NC_039215_Cyclovirus VN	CWTLNN	KHLQG	YCSK	GPTGTGKS	VIDDF	ITSE
NC_025476_Cyclovirus ZM36a	CWTLNN	KHLQG	YCSK	GSTGTGKS	VIDDF	ITSE
NC_040826_Dragonfly-associated cyclovirus	VFTLHN	PHIQA	YCRK	GPPGSGKS	IIDDF	ITSN
NC_023869_Dragonfly-associated cyclovirus 2	CFTVNN	PHLQG	YCSK	GPPGSGKS	IIDDF	ITSN
NC_023874_Human-associated cyclovirus 10	CFTWHD	IHLQG	YCSK	GPPGSGKS	IIDDF	ITSN
NC_038396_Bat-associated cyclovirus 6	CFTWND	PHIQG	YCRK	GPPGSGKS	IIDDF	FTSN

NC_038400_Bat-associated cyclovirus 11	CFTWNN	PHLQG	YCSK	GPPGSGKS	IIDDF	ITSN
NC_038395_Bat-associated cyclovirus 4	CFTWNN	LHLQG	YCSK	GPPGTGKS	VIDDF	ITSN
NC_038417_Cyclovirus NG14	CFTWNN	HHLQG	YCSK	GPPGSGKS	IIDDF	ITSN
NC_038401_Bat-associated cyclovirus 13	CFTWNN	NHLQG	YCSK	GPPGSGKS	IIDDF	ITSN
NC_023866_Dragonfly-associated cyclovirus 5	CFTLNN	IHLPG	YCRK	GPTGSGKS	IIDDF	ITSN
NC_038407_Cyclovirus Chimp11	CFTWNN	PHLQG	YCRK	GPPGSGKS	IIDDF	FTSN
NC_038418_Cyclovirus SL-108277	CFTWNN	IHLQG	YCSK	GPPGSGKS	IIDDF	ITSN
NC_038409_Dragonfly-associated cyclovirus 7	CFTWNN	LHLQG	YCSK	GAPGTGKS	IIDDF	ITSN
NC_038406_Cyclovirus NGchicken8/NGA/2009	VFTWNN	PHLQG	YCSK	GPPGTGKS	IIDDF	ITSN
NC_014930_Cyclovirus NGchicken15/NGA/2009	VFTWNN	PHLQG	YCSK	GPPGTGKS	IIDDF	ITSN
NC_038413_Cyclovirus PK5222 -	CFTWNN	PHLQG	YCSK	GPPGTGKS	IIDDF	ITSN
NC_036877_Cyclovirus PK5006 -	CFTWND	PHLQG	YCSK	GPPGTGKS	IIDDF	ITSN
NC_014928_Cyclovirus PKgoat11/PAK/2009	CFTWNN	LHLQG	YCSK	GPPGSGKS	IIDDF	ITSN
NC_040325_Arboreal ant-associated circular virus 1	VFTLNN	PHLQG	YCSK	GPPGTGKS	IIDDF	ITSN
NC_038410_Dragonfly-associated cyclovirus 8	CFTWNN	PHLQG	YCSK	GPPGSGKS	IIDDF	ITSN
NC_023886_Dragonfly-associated cyclovirus 1 - replication associated protein CDS	VFTWNN	PHIQG	YCSK	GPPGTGKS	IIDDF	ITSN
NC_038412_Cyclovirus PK5510	CFTWNN	PHLQG	YCSK	GPPGSGKS	IIDDF	ITSN
NC_039214_Dragonfly-associated cyclovirus 4	CFTWNN	PHLQG	YCSK	GLPGTGKS	IIDDF	ITTN
NC_038408_Dragonfly-associated cyclovirus 6	VFTLNN	PHLQG	YCSK	GEPGTGKS	IIDDF	ITSN
NC_027530_Cyclovirus TsCyV-1_JP-NUBS-2014	VFTWNN	PHLQG	YCSK	GEPGTGKS	IIDDF	ITSN
NC_038415_Cyclovirus PK5034	CFTWNN	PHLQG	YCKK	GPPGSGKS	IIDDF	FTSN
NC_034547_Bat cyclovirus GF-4c	IFTWNN	PHLQG	YCSK	GEPGTGKS	IIDDF	ITSN
NC_038394_Bat-associated cyclovirus 3	VFTWNN	RHLQG	YCKK	GPPGSGKS	IVDDF	FTSN

NC_014929_Cyclovirus bat/USA/2009	VFTWNN	PHLQG	YCKK	GPPGTGKS	IIDDF	FTSN
NC_038398_Bat-associated cyclovirus 8	CFTLNN	LHLQG	YCSK	GPTGSGKS	IIDDF	ITSE
NC_038416_Cyclovirus NG12	VFTLNN	PHLQG	YCSK	GPTGSGKS	IIDDF	ITSE
NC_038399_Bat-associated cyclovirus 9	VFTLNN	SHLQG	YCSK	GPTGSGKS	IIDDF	ITSE
NC_038393_Bat-associated cyclovirus 2	VFTLNN	PHLQG	YCSK	GPPGSGKS	IIDDF	ITSN
NC_020206_Cockroach-associated cyclovirus 1	VFTLNN	PHLQG		GPTGTGKS	IIDDF	ITSN
NC_025792_Bat circovirus POA/2012/VI	CFTWNN	PHLQG	YCKK	GPPGTGKS		NTTN
NC_038403_Pacific flying fox-associated cyclovirus-2	VFTLNN	KHLQG	YLPH	GTPGVGKS	IIDDY	ITTN
NC_038402_Pacific flying fox-associated cyclovirus-1	VFTLNN	KHLQG	YLPH	GAPGVGKS	IIDDF	ITSN
Astronium fraxinifolium-associated virus	**AITINN**	**LHLQA**	**YCSK**	**GDTGTGKS**	**IIDDF**	**FTSN**
Caesalpinia ferrea-associated virus	**CITVNN**	**RHLQG**	**YCSK**	**GPTRTGKS**	**IIDDF**	**ITSN**

4. Conclusions

Our results showed the presence of two new viruses associated with tree species classified in *Circoviridae* family. Astronium fraxinifolium-associated virus was detected (AFaV) in *Astronium fraxinifolium* and Caesalpinia ferrea-associated virus (CFaV) in *Caesalpinia ferrea*. The present information in this study suggests that tree species may serve as a reservoir for viruses. This is the first report of circoviruses associated with plants detected by HTS.

5. References

ABBAS, A. A. et al. *Redondoviridae*, a family of small, circular DNA viruses of the human oro-respiratory tract associated with periodontitis and critical illness. **Cell Host & Microbe**, v. 25, n. 5, p. 719-729, 2019.

ADAMS, I.; FOX, A. Diagnosis of plant viruses using next-generation sequencing and metagenomic analysis. In: **Current Research Topics in Plant Virology**. [s.l.] Springer, 2016. p. 323-335.

ALLAN, G. M. et al. Isolation of Porcine circovirus-like viruses from pigs with a wasting disease in the USA and Europe. **Journal of Veterinary Diagnostic Investigation**, v. 10, n. 1, p. 3-10, 1998.

BIAGINI, P. et al. *Circoviridae*, p 343-349. **Virus taxonomy: classification and nomenclature of viruses. Ninth report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, New York, NY**, v. c, 2012.

BOITEUX, L.; FONSECA, M. E. N.; SIMON, P. W. Effects of plant tissue and DNA purification method on randomly amplified polymorphic DNA-based genetic fingerprinting analysis in carrot. **Journal of the American Society for Horticultural Science**, v. 124, n. 1, p. 32-38, 1999.

BREITBART, M. et al. ICTV Virus Taxonomy Profile: *Circoviridae*. **The Journal of General Virology**, v. 98, n. 8, p. 1997, 2017.

CHANG, W.-S. et al. Meta-transcriptomic analysis of virus diversity in urban wild birds with paretic disease. **bioRxiv**, 2020. Conferir

CROOKS, G. E. et al. WebLogo: a sequence logo generator. **Genome Research**, v. 14, n. 6, p. 1188-1190, 2004.

DAYARAM, A. et al. High global diversity of cycloviruses amongst dragonflies. **Journal of General Virology**, v. 94, n. 8, p. 1827-1840, 2013.

REIS, LNA. et al. Metagenomics of neotropical single-stranded DNA viruses in Tomato cultivars with and without the *Ty*-1 gene. **Viruses**, v. 12, n. 8, p. 819, 2020.

DÍEZ-VILLASEÑOR, C.; RODRIGUEZ-VALERA, F. CRISPR analysis suggests that small circular single-stranded DNA smacoviruses infect archaea instead of humans. **Nature Communications**, v. 10, n. 1, p. 1-9, 2019.

GIBBS, M. J.; WEILLER, G. F. Evidence that a plant virus switched hosts to infect a vertebrate and then recombined with a vertebrate-infecting virus. **Proceedings of the National Academy of Sciences**, v. 96, n. 14, p. 8022-8027, 1999.

HALAMI, M. Y. et al. Detection of a novel *Circovirus* in mute swans (*Cygnus olor*) by using nested broad-spectrum PCR. **Virus Research**, v. 132, n. 1-2, p. 208-212, 2008.

HATTERMANN, K. et al. Cloning and sequencing of *Duck circovirus* (DuCV). **Archives of Virology**, v. 148, n. 12, p. 2471-2480, 2003.

ICTV. **ICTV online. International Committee on Taxonomy of Viruses**. Disponível em: <https://talk.ictvonline.org/>. Acesso em: 21 dez. 2019.

ICTV. **ICTV Online Report. International Committee on Taxonomy of Viruses ICTV**. Disponível em: <https://talk.ictvonline.org/taxonomy/>. Acesso em: 27 ago. 2020.

INOUE-NAGATA, A. K. et al. A simple method for cloning the complete *Begomovirus* genome using the bacteriophage phi29 DNA Polymerase. **Journal of Virological Methods**, v. 116, n. 2, p. 209-211, 2004.

JOHNE, R. et al. Genome of a novel *Circovirus* of starlings, amplified by multiply primed Rolling-Circle Amplification. **Journal of General Virology**, v. 87, n. 5, p. 1189-1195, 2006.

JONES, S. et al. Viral diagnostics in plants using next generation sequencing: computational analysis in practice. **Frontiers in Plant Science**, v. 8, p. 1770, 2017.

KASZAB, E. et al. Characterization of the genomic sequence of a novel CRESS DNA virus identified in Eurasian jay (*Garrulus glandarius*). **Archives of Virology**, v. 163, n. 1, p. 285-289, 2018.

KEARSE, M. et al. Geneious basic: An integrated and etendable desktop software platform for the organization and analysis of sequence data. **Bioinformatics**, v. 28, n. 12, p. 1647-1649, 2012.

KRABERGER, S. et al. Unravelling the single-stranded DNA virome of the New Zealand blackfly. **Viruses**, v. 11, n. 6, p. 532, 2019.

KRAKOWKA, S. et al. Activation of the immune system is the pivotal event in the production of wasting disease in pigs infected with Porcine circovirus-2 (PCV-2). **Veterinary Pathology**, v. 38, n. 1, p. 31-42, 2001.

KREUZE, J. F. et al. Complete viral genome sequence and discovery of novel viruses by deep sequencing of Small RNAs: A generic method for diagnosis, discovery and sequencing of viruses. **Virology**, v. 388, n. 1, p. 1-7, 2009.

KRUPOVIC, M. et al. Cressdnaviricota: A virus phylum unifying 7 families of rep-encoding viruses with single-stranded, circular DNA genomes. **Journal of Virology**, 2020.

LABONTÉ, J. M.; SUTTLE, C. A. Previously unknown and highly divergent ssDNA viruses populate the oceans. **The ISME Journal**, v. 7, n. 11, p. 2169-2177, 2013.

LECIS, R. et al. Genomic characterization of a novel Bat-associated circovirus detected in european miniopterus schreibersii bats. **Virus Genes**, p. 1-4, 2020.

LI, L. et al. Bat guano virome: Predominance of dietary viruses from insects and plants plus novel mammalian viruses. **Journal of Virology**, v. 84, n. 14, p. 6955-6965, 2010a.

LI, L. et al. Multiple diverse circoviruses infect farm animals and are commonly found in human and chimpanzee feces. **Journal of Virology**, v. 84, n. 4, p. 1674-1682, 2010b.

LOIKO, M. R. et al. Columbid circoviruses detected in free ranging pigeons from southern Brazil: Insights on PiCV evolution. **Archives of Virology**, v. 163, n. 11, p. 3083-3090, 2018.

LORINCZ, M. et al. First detection and analysis of a *Fish circovirus*. **Journal of General Virology**, v. 92, n. 8, p. 1817-1821, 2011.

MALATHI, V. G.; RENUKA DEVI, P. ssDNA viruses: Key players in global virome. **Virus Disease**, v. 30, n. 1, p. 3-12, 2019.

MALE, M. F. et al. Cycloviruses, gemycircularviruses and other novel replication-associated protein encoding circular viruses in pacific flying fox (*Pteropus tonganus*) faeces. **Infection, Genetics and Evolution**, v. 39, p. 279-292, 2016.

MATSUMOTO, T. et al. A novel Bat-associated circovirus identified in Northern Hokkaido, Japan. **Archives of Virology**, v. 164, n. 8, p. 2179-2182, 2019.

MINICKA, J. et al. High-throughput sequencing facilitates discovery of new plant viruses in Poland. **Plants**, v. 9, n. 7, p. 820, 2020.

NERY, F. et al. Molecular characterization of Hovenia dulcis-associated virus 1 (HDaV1) and 2 (HDaV2): new tentative species within the order *Picornavirales*. **Viruses**, v. 12, n. 9, p. 950, 2020.

PRICE, M. N.; DEHAL, P. S.; ARKIN, A. P. FastTree: Computing large minimum evolution trees with profiles instead of a distance matrix. **Molecular Biology and Evolution**, v. 26, n. 7, p. 1641-1650, 2009.

RICHET, C. et al. Novel circular DNA viruses associated with *Apiaceae* and *Poaceae* from South Africa and New Zealand. **Archives of Virology**, v. 164, n. 1, p. 237-242, 2019.

ROSARIO, K. et al. Revisiting the taxonomy of the family *Circoviridae*: establishment of the genus *Cyclovirus* and removal of the genus *Gyrovirus*. **Archives of Virology**, v. 162, n. 5, p. 1447-1463, 2017.

SADEGHI, M. et al. Virome of> 12 thousand Culex mosquitoes from throughout California. **Virology**, v. 523, p. 74-88, 2018.

SIMMONDS, P. SSE: A nucleotide and amino acid sequence analysis platform. **BMC Research Notes**, v. 5, n. 1, p. 50, 2012.

SIMMONDS, P. et al. Consensus statement: virus taxonomy in the age of metagenomics. **Nature Reviews Microbiology**, v. 15, n. 3, p. 161, 2017.

TEAM, R. C. **R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria**Vienna, AustriaR Foundation for Statistical Computing, , 2013. Disponível em: <http://www.r-project.org/>

WANG, B. et al. Molecular detection of novel circoviruses in ticks in Northeastern China. **Ticks and tick-borne diseases**, v. 9, n. 4, p. 836-839, 2018.

ZHANG, H.-H. et al. Novel *Circovirus* species identified in farmed pigs designated as *Porcine circovirus* 4, hunan province, China. **Transboundary and Emerging Diseases,** v. 67, n. 3, p. 1057-1061, 2020.

General Discussion

Forests cover approximately 30% of the surface of the planet. Forest species are economically important (SILBERNAGEL et al., 2013) and they can serve as a habitat for several microorganisms. According to Roossinck et al. (2010), native tree species in close proximity to cultivated crop agroecosystems can function as potential hosts for viruses and could, favor the emergence of new species in the cultivated plants.

Virus studies in trees have been carried out mainly in Europe (NIENHAUS; CASTELLO, 1989; BANG et a., 2006; JALKANEN et al.,2007; BÜTTNER et al., 2013; RUMBOU et al., 2018; 2020;) with only few cases described in Brazil (LIN et. al., 1979; GAMA et al., 1983; ALMEIDA et al., 2002; BATISTA, 2014; REZENDE, 2016; FONSECA et al., 2018).

The advent of HTS, metagenomics in association with bioinformatics analysis has increase the rate of the discovery and characterization of new viral species, facilitating the complete recover of viral genomes (ADAMS; FOX, 2016; PECMAN et al., 2017). However, most sequences generated by HTS are not previously known viruses, corroborating with the statements of Roossinck; Bazán (2017) that viruses are the most abundant biological entities on the planet and with the observation made by Mokili et al. (2012) that only 1% of viral diversity is currently known. Here, metagenomic analyzes were performed on symptomatic samples in order to investigate the viral diversity in tree species that have been cultivated at NOVACAP - nursery II in Brazil. These analyzes were performed for both RNA and DNA viruses using HTS, resulting in the identification and characterization of a new set of viruses.

In the RNA analysis, two new putative species named Hovenia dulcis- associated virus (HDaV1 and HDaV2) were detected and characterized in a single sample from japanise raisintree (*Hovenia dulcis* Thumb. Voucher) and were classified within the order *Picornavirales* (**Chapter 2**).

In the DNA analysis, two viral sequences were obtained and classified within the *Circoviridae* family (**Chapter 3**). The first sequence was named as Astronium fraxinifolium-associated virus (AFaV) and the second as Caesalpinia ferrea-associated virus (CFaV). So far, circoviruses have been described only in association with vertebrates and invertebrates. The findings reported here are, therefore, suggesting that members of this viral family may also be in association with plants, which was never described before.

The order *Picornavirales* presents viruses with the genomic constitution of RNA (+), with monopartite (non-segmented) genome and, in some cases, segmented, bipartite genomes. The members classified in that order are capable of infecting different organisms such as: algae, insects, birds, men, and plants. HDaV1 displayed a genomic organization similar to members classified within the *Dicistroviridae* family. however, when performing a Blast, low identity was observed, with viruses does not have a defined family. However, HDaV2 presented a new genomic organization (with three non-overlapping ORFs) differently found among dicistroviruses (only two ORFs). In addition, both viruses showed conserved domains for helicase, protease, and polymerase proteins commonly found within order. Although phylogenetic analyzes and NCA clustered with invertebrate-infecting viruses we believed that HDaV1 and HDaV2 can be insect viruses that use plants as an alternative host, or they are plant viruses or plant and invertebrate viruses. However, it is possible to observe some examples as *Rhopalosiphum padi virus* is a virus that infects insets and capable of using plants as a host but not replicating (GILDOW; D'ARCY, 1990; REGELIN, 2010), and Providence virus capable of replicating in both plant cells and animal cells (JIWAJI, M. et al., 2019).

With the massive employment of metagenomics (after 2002), the number of described and characterized viruses has increased considerably. Currently, this number reached 6590 (ICTV, 2020). According to He et al. (2020) among them, 1100 are viruses that infect plants. Furthermore, metagenomics has revealed new circular ssDNA viruses (within previously known families) as well as many viruses that remain yet unclassified (SIMMONDS et al., 2017).

The ssDNA viruses encoding only two proteins [REP (replication-associated protein) (Rep) and CP (capsid protein)] are associated with several organisms, including algae (*Bacilladnaviridae),* fungi (*Genomoviridae),* plants (*Nanoviridae* and *Geminiviridae*), and animals (*Circoviridae and Smacoviridae*) (ABBAS et al., 2019; ICTV, 2020).

The novel Astronium fraxinifolium-associated virus (AFaV) was isolated here from *Astronium fraxinifolium* Schott and Caesalpinia ferrea-associated virus (CFaV) was obtained from *Caesalpinia ferrea* Mart. ex Tul. Both viruses were obtained from symptomatic leaves. AFaV and CFaV displayed two ORFs (Rep and Cp) structurally similar to that of the circoviruses (ROSARIO et al., 2018). (Chapter 3). Furthermore, CFaV showed CP in viral sense and Rep in complementary sense as observed in members of the *Cyclovirus*. Interestingly, AFaV showed CP and Rep in viral sense differing in the genomic organization of the members belonging to this family.

Concomitantly, a potential nonanucleotide sequence (CAGTATTAC/ TAGTATTAC) and conserved domains for RCR (Rolling circle replication) which appears to be quite conserved among ssDNA viruses include the helicase motifs (Motifs I, II, and III) and endonuclease (Walker A, B and C) (MALATHI; RENUKA DEVI, 2019; ROSARIO et al., 2018) were found in AFaV and CFaV.

AFaV displayed 77% nucleotide identity (with only 4% coverage) with Anguilla anguilla (APZ87906) and CFaV 70% nucleotide identity (10% coverage) with Bat-associated Cyclovirus 7 (YP009506292). The established threshold criterium for new species within the family *Circoviridae* is 80% pairwise-nucleotide identity (ICTV, 2019; ROSARIO et al., 2017).

Phylogenetic analysis grouped CFaV in genus *Cyclovirus*, whereas AFaV did not cluster with any of the currently characterized species. Likewise, NCA analyzes were not able to cluster AFaV with any member of the *Circoviridae* family. Therefore, the results of the present work suggest that the family Circoviridae may be composed by a potential new plant-associated genus. Studies carried out by Gibbs; Weiller (1999) showed that circoviruses probably evolved from a *Nanovirus* since both share similarities in the Rep protein, which suggests a commom ancestor. However, more information must be produced about these putative new species associated with plants classified in the family *Circoviridae*.

References

ABBAS, A. A. et al. *Redondoviridae*, a family of small, circular DNA viruses of the human oro-respiratory tract associated with periodontitis and critical illness. **Cell Host & Microbe**, v. 25, n. 5, p. 719-729, 2019.

ADAMS, I.; FOX, A. Diagnosis of plant viruses using next-generation sequencing and metagenomic analysis. In: **Current Research Topics in Plant Virology**. [s.l.] Springer, 2016. p. 323-335.

ALMEIDA, Á. M. R. et al. Mosaic in *Senna occidentalis* in southern Brazil induced by a new strain of *soybean mosaic virus*. **Fitopatologia Brasileira**, v. 27, p. 151-156, 2002.

BANG, J.-H.; CHOI, J.-K.; LEE, S.-Y. Characterization of *Peanut stunt virus* isolated from black locust tree (*Robinia pseudo-acacia* L.). **The Plant Pathology Journal**, v. 22, n. 2, p. 125-130, 2006.

BATISTA, J. G. **Ocorrência natural de espécies virais e avaliação de potenciais hospedeiras experimentais de *Begomovirus*, *Potyvirus* e *Tospovirus* em plantas arbóreas e berinjela (*Solanum melongena*)**. [s.l.] Universidade de Brasilia, 2014.

BÜTTNER, C. et al. Forest diseases caused by viruses. In: **Infectious Forest Diseases**. [s.l.] CABI: Wallingford, Oxfordshire, UK, 2013. p. 50-75.

FONSECA, P. L. C. et al. Virome analyses of *Hevea brasiliensis* using small RNA deep sequencing and PCR techniques reveal the presence of a potential new virus. **Virology Journal**, v. 15, n. 1, p. 184, 2018.

GAMA, M. et al. Um *Carlavirus* em seringueira (*Hevea brasiliensis*). **Fitopatologia Brasileira**, v. 8, p. 621, 1983.

GIBBS, M. J.; WEILLER, G. F. Evidence that a plant virus switched hosts to infect a vertebrate and then recombined with a vertebrate-infecting virus. **Proceedings of the National Academy of Sciences**, v. 96, n. 14, p. 8022-8027, 1999.

GILDOW, F. E.; D'ARCY, C. J. Cytopathology and experimental host range of *Rhopalosiphum padi virus*, a small isometric RNA virus infecting cereal grain aphids. **Journal of Invertebrate Pathology**, v. 55, n. 2, p. 245-257, 1990.

HE, Y.-Z. et al. A plant DNA virus replicates in the salivary glands of its insect vector via recruitment of host DNA synthesis machinery. **Proceedings of the National Academy of Sciences**, v. 117, n. 29, p. 16928-16937, 2020.

ICTV. **ICTV Online Report. International Committee on Taxonomy of Viruses ICTV**. Disponível em: <https://talk.ictvonline.org/taxonomy/>. Acesso em: 27 ago. 2020.

JALKANEN, R.; BÜTTNER, C.; BARGEN, S. VON. Pubescens in Finland. **Silva Fennica**, v. 41, n. 4, p. 755-762, 2007.

JIWAJI, M. et al. *Providence virus:* an animal virus that replicates in plants or a plant virus that infects and replicates in animal cells? **PloS One**, v. 14, n. 6, p. e0217494, 2019.

LIN, M. T. et al. Properties of a possible *Carlavirus* isolated from a cerrado native plant, *Cassia sylvestris*. **Plant Disease**, v. 63, p. 501-505, 1979.

MALATHI, V. G.; RENUKA DEVI, P. ssDNA viruses: Key players in global virome. **Virus Disease**, v. 30, n. 1, p. 3-12, 2019.

MOKILI, J. L.; ROHWER, F.; DUTILH, B. E. Metagenomics and future perspectives in virus discovery. **Current Opinion in Virology**, v. 2, n. 1, p. 63-77, 2012.

NIENHAUS, F.; CASTELLO, J. D. Viruses in forest trees. **Annual Review of Phytopathology**, v. 27, p. 165-186, 1989.

PECMAN, A. et al. Next generation sequencing for detection and discovery of plant viruses and viroids : Comparison of two approaches. v. 8, n. October, p. 1-10, 2017.

REGELIN, Z. T. **Translation and replication of *Rhopalosiphum padi virus* RNA in a plant cellular environment**. [s.l.] Master of Science. Digital Repository Iowa State University, Ames, Iowa. 2010-04-30, 2010.

REZENDE, J. A. M. et al. Biological and molecular characterization of a putative new *Potexvirus* infecting *Senna occidentalis.* **Archives of Virology**, v. 162, n. 2, p. 529-533, 2016.

ROOSSINCK, M. J. et al. Ecogenomics: using massively parallel pyrosequencing to understand virus ecology. **Molecular Ecology**, v. 19, n. s1, p. 81-88, 2010.

ROOSSINCK, M. J.; BAZÁN, E. R. Symbiosis: viruses as intimate partners. **Annual Review of Virology**, v. 4, n. 1, p. 123-139, 2017.

ROSARIO, K. et al. Virus discovery in all three major lineages of terrestrial arthropods highlights the diversity of single-stranded DNA viruses associated with invertebrates. **Peer J**, v. 6, p. e5761, 2018.

RUMBOU, A. et al. A novel *Badnavirus* discovered from *Betula* sp. affected by birch leaf-roll. **PloS One**, v. 13, n. 3, p. e0193888, 2018.

RUMBOU, A. et al. Unravelling the virome in birch: RNA-Seq reveals a complex of known and novel viruses. **Plos One**, v. 15, n. 6, p. e0221834, 2020.

SILBERNAGEL, E. U. A política de floresta plantada na administração pública federal. 2013.

SIMMONDS, P. et al. Consensus statement: virus taxonomy in the age of metagenomics. **Nature Reviews Microbiology**, v. 15, n. 3, p. 161, 2017.