Research Square

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Genetic Characterization of Rare Bruconha Virus (Bunyavirales: *Orthobunyavirus*) Isolated in Vale Do Ribeira (Atlantic Forest Biome), Southeastern Brazil

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Abstract

Brazil is a great source of arbovirus diversity, mainly in the Amazon Region. However, other biomes, especially Atlantic Forest, may be also a hotspot for emergent viruses, including Bunyaviruses (Negarnaviricota: Bunyavirales). For instance, Vale do Ribeira, located in the Southeastern region, has been widely studied for virus surveillance, where Flavivirus, Alphavirus and Bunyaviruses were isolated during the last decades, including Bruconha virus (BRCV), a member of Orthobunyavirus genus Group C, in 1976. Recently, a new isolated of BRCV, named Span321532, was obtained from an adult sentinel mice placed in Iguape county, in 2011, and a full-length genome was generated, with nucleotide differences that varied from 1.5%, 5.3% and 5% (L, M and S segments respectively) from the prototype isolated 35 years earlier. Although no evidence of reassortants were detected, this findings reiterates the need for new surveillance and genomic studies in the area considering the high mutation rates of arbovirus and also to identify the hosts capable of supporting the continuous circulation of Orthobunyavirus.

1. Introduction

Central and South America are a great source of arbovirus diversity, and hotspots of emergent zoonoses [1]. In Brazil, outbreaks of *Flavivirus* and *Alphavirus*, such as Dengue, Chikungunya, Yellow Fever and Zika are described all over the country [2–4]. On the other hand, despite its lower incidence, Bunyaviruses (Negarnaviricota: Bunyavirales) are a large and diverse group of viruses that are important pathogens. Their genome is segmented and composed of three segments: a large (L) segment encoding a protein that functions as an RNA-directed RNA polymerase (RdRp); a medium (M) segment encoding glycoproteins (Gn and Gc); and a small (S) segment encoding a nucleoprotein (NP) and, in some clades, a non-structural protein, NSs, with a RNA of negative-sense. Within the order, *Orthobunyavirus* genus, belonging to the *Peribunyaviridae* family, contains 103 species divided into distinct serogroups [5, 6], of which several can have impact in humans and animals health, such as Oropouche virus and Schmallenberg virus.

Several orthobunyaviruses, mainly from groups C and Guama, have been isolated at Atlantic Forest biome within São Paulo State (southeastern Brazil) during active surveillance programs for arbovirus during the 60's and 70's, such as Bertioga virus (BERV), Boraceia virus (BORV), Cananeia virus (CNAV), Caraparu virus (CARV), Guaratuba virus (GTRV) and Itimirim virus (ITIV) (4–7). In the Atlantic Forest, it is believed that these viruses are maintained mainly by rodents (or other small mammals) and *Culex* mosquitoes [10]. As described for segmented genomes, orthobunyaviruses are capable of reassortment, which can have epidemiological importance [11]. For instance, Ngari virus is a reassortant bunyavirus associated with outbreaks of hemorrhagic fever in Africa [12]; Iquitos and Itaya viruses are emerging reassortant bunyaviruses associated with human illness in Peru [13]. Reassortants are also described in Group C *Orthobunyaviruses*, once phylogenetic analysis revealed that Caraparu virus, that circulates in Brazil, contained an S segment sequence that is nearly identical to Oriboca virus, indicating a natural reassortant virus [14]. However, as viruses belonging to *Peribunyaviridae* family are rarely found in the Southeastern region of Brazil, where Oropouche virus has never been detected, little is known about the epidemiological cycle and virus evolution at this particular biome. Here we describe a full length genome of Bruconha virus (BRCV), a group C *Orthobunyavirus* isolated in 2011, in Iguape city, São Paulo, Brazil.

2. Methods

2.1 Area

In São Paulo State, during the arbovirus eco-epidemiology surveillance program, mosquitoes and blood samples from birds and rodents were collected. Also, sentinel mice were placed in selected areas with water and food *ad libitum*. This program was conducted from 2011 until 2016 at Vale do Ribeira, an Atlantic Florest region located at the south coast of the State (Figure 1). The aim of this project was to identify arbovirus hosts and vectors, and also the circulation of these viruses in human and animal populations. Vale do Ribeira region is occupied by the hydrographic basin of the Ribeira do Iguape river, and includes 16 counties. It has over 21,000 Km^2 of well-preserved forests (about 21% of the total remaining Atlantic Forest in Brazil). The region has high temperatures with high rainfall during the year as depicted in Figure 2. Economy is based on subsistence agriculture. Besides the biological diversity of Vale do Ribeira, this region was selected thanks to previous detection of several arbovirus, including the outbreak caused by the flavivirus Rocio virus (1975-1977)[9]. It is known that deforestation has increased in Brazil during the last years, mainly in the Amazon region [15]. In order to check the conservation at Vale do Ribeira, we have used mapbiomas plug in in QGIS v 3.16 (https://mapbiomas.org/), comparing Atlantic Forest biome in 1986 and 2011. More, we have calculated the Normalized Difference Vegetation Index (NDVI) in Iguape County (from November 1980 until April 2019) using Landsat 8 in Google Earth Engine plataform (available at https://developers.google.com/earth-engine/datasets/catalog/landsat-8), with values that show a forest maintenance during the last 3 decades (Supplementary material).

2.2 Sample Collection

In 2011, a total of 6 field trips of 3-day long, being 5 to Iguape city and one to Panorama (months of March, April, May and November) were conducted. Nets were used for the capture of wild birds, while Tomahawk traps were placed for small mammals. Also, each trip had two sentinel Swiss mice cages with 1 mother and 6 newborns each (total=12)[16]. Blood samples (0.2-0.3ml) were collected by venipuncture (birds) and via retro-orbital (small mammals) and diluted in a phosphate-buffered saline solution with 0.75% bovine albumin, penicillin (100 units/mL) and streptomycin (100 µg/mL). All free range animals were released. Sentinel mice were brought back to the laboratory and observed for 21 days. Table 1 shows all bird captures performed during 2011. Due to the low sample amount, blood from small mammals was used only for Hantavirus ELISA IgG detection, and are not showed.

Table 1
Total of birds captured during arbovirus surveillance program in Iguape city, SP, Brazil, 2011

Family	Specie	Age	Sex
Turdidae	*Turdus rufiventris*	A	NI
Thraupidae	*Ramphocelus bresilius*	A	NI
Tyrannidae	*Myiophobus fasciatus*	A	NI
Picidae	*Picumnus temminckii*	A	NI
Fringillidae	*Sporophila caerulescens*	A	M
Funariidae	*Furnarius rufus*	A	NI
Funariidae	*Furnarius rufus*	A	NI
Tyrannidae	*Myozetetes similis*	A	NI
Fringillidae	*Sporophila caerulescens*	A	F
Pipridae	*Manacus manacus*	A	F
Fringillidae	*Sporophila caerulescens*	A	F
Funariidae	*Furnarius rufus*	A	NI
Fringillidae	*Sporophila caerulescens*	A	F
Funariidae	*Furnarius rufus*	A	NI
Troglodytidae	*Troglodytes aedon*	A	NI
Troglodytidae	*Troglodytes aedon*	A	NI
Tyrannidae	*Leptopogon amaurocephalus*	A	NI
Thraupidae	*Saltator similis*	A	NI
Thraupidae	*Saltator similis*	A	NI
Fringillidae	*Sporophila caerulescens*	Y	NI
Coerebidae	*Coereba flaveola*	A	NI
Thraupidae	*Ramphocelus bresilius*	Y	NI
Columbidae	*Columbina talpacoti*	A	M
Thraupidae	*Sicalis flaveola*	Y	NI
Thraupidae	*Sicalis flaveola*	Y	NI
Pipridae	*Manacus manacus*	Y	NI
Fringillidae	*Sporophila caerulescens*	Y	NI
Fringillidae	*Sporophila caerulescens*	Y	NI
Fringillidae	*Sporophila caerulescens*	Y	NI
Fringillidae	*Sporophila caerulescens*	Y	NI
Turdidae	*Turdus rufiventris*	A	NI
Trochilidae	*Ramphodon naevius*	A	M
Fringillidae	*Sporophila caerulescens*	Y	NI
Fringillidae	*Sporophila caerulescens*	Y	NI
Troglodytidae	*Troglodytes aedon*	A	NI
Fringillidae	*Zonotrichia capensis*	Y	NI
Trochilidae	*Ramphodon naevius*	A	M
Conopophagidae	*Conopophaga lineata*	A	M
Hirundinidae	*Stelgidopteryx ruficollis*	A	NI
Fringillidae	*Zonotrichia capensis*	A	NI

Legend: A=Adult; Y=young; F=Female; M=Male, NI=not identified

Family	Specie	Age	Sex
Hirundinidae	*Notiochelidon cyanoleuca*	A	NI
Hirundinidae	*Stelgidopteryx ruficollis*	A	NI
Hirundinidae	*Stelgidopteryx ruficollis*	A	NI
Hirundinidae	*Stelgidopteryx ruficollis*	A	NI
Tyrannidae	*Fluvicola nengeta*	A	NI
Hirundinidae	*Stelgidopteryx ruficollis*	A	NI
Fringillidae	*Zonotrichia capensis*	Y	NI
Hirundinidae	*Stelgidopteryx ruficollis*	A	NI
Hirundinidae	*Stelgidopteryx ruficollis*	A	NI
Thraupidae	*Sicalis flaveola*	Y	NI
Hirundinidae	*Stelgidopteryx ruficollis*	A	NI
Hirundinidae	*Notiochelidon cyanoleuca*	Y	NI
Fringillidae	*Sporophila caerulescens*	Y	NI
Fringillidae	*Zonotrichia capensis*	A	NI
Thraupidae	*Thraupis sayaca*	A	NI
Thraupidae	*Sicalis flaveola*	Y	NI
Fringillidae	*Sporophila caerulescens*	Y	NI
Psittacidae	*Brotogeris tirica*	Y	NI
Fringillidae	*Sporophila caerulescens*	Y	NI
Anatidae	*Anas platyrhynchos domesticus*	A	NI
Anatidae	*Anas platyrhynchos domesticus*	A	NI
Anatidae	*Anas platyrhynchos domesticus*	A	NI
Anatidae	*Anas platyrhynchos domesticus*	A	NI
Anatidae	*Anas platyrhynchos domesticus*	A	NI
Anatidae	*Anas platyrhynchos domesticus*	A	NI
Anatidae	*Anas platyrhynchos domesticus*	A	NI
Anatidae	*Anas platyrhynchos domesticus*	A	NI
Anatidae	*Anas platyrhynchos domesticus*	A	NI
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Anatidae	*Anas platyrhynchos domesticus*	A	NI
Anatidae	*Anas platyrhynchos domesticus*	A	NI
Anatidae	*Anas platyrhynchos domesticus*	A	NI
Anatidae	*Anas platyrhynchos domesticus*	A	NI
Anatidae	*Anas platyrhynchos domesticus*	A	NI
Anatidae	*Anas platyrhynchos domesticus*	A	NI
Anatidae	*Anas platyrhynchos domesticus*	A	NI
Legend: A=Adult; Y=young; F=Female; M=Male, NI=not identified			

Family	Specie	Age	Sex
Turdidae	*Turdus amaurochalinus*	A	NI
Thraupidae	*Saltator similis*	A	NI
Thraupidae	*Ramphocelus bresilius*	A	M
Thraupidae	*Tangara seledon*	Y	NI
Thraupidae	*Tangara seledon*	A	M
Coerebidae	*Coereba flaveola*	A	NI
Thraupidae	*Tangara seledon*	A	M
Thraupidae	*Tangara seledon*	A	M
Thraupidae	*Tangara seledon*	A	F
Thraupidae	*Tangara seledon*	Y	M
Turdidae	*Turdus rufiventris*	A	NI
Legend: A=Adult; Y=young; F=Female; M=Male, NI=not identified			

2.3 Virus isolation and Indirect immune assay (IFA)

Blood samples from birds were inoculated intracerebrally into newborn mice, and checked daily. Tissues (spleen, liver and brain) from the sentinel mice that died within 21 days or showed symptoms of viral infection (paralysis, ataxia) were triturated in sterile grinders containing 1 mL of phosphate-buffered saline solution with 0.75% bovine albumin, penicillin (100 units/mL) and streptomycin (100 µg/mL). The resultant suspension was centrifuged at 1800×g for 15 min. The supernatant was withdrawn and frozen at -70ºC until further processing. Each fragment was inoculated into cell tubes containing monolayer cultures of C6/36 cells. Culture tubes were incubated for nine days at 28°C with L-15 medium containing 2% FBS, penicillin (100units/mL) and streptomycin (100µg/mL). Indirect immune assay (IFA) tests were then performed using in house hyper immune serum for detection of genus *Flavivirus, Alphavirus* and *Bunyavirus* [17].

2.4 Sequencing

RNA was extracted from isolate Span321532 C6/36 supernatant using QIAamp Viral RNA Mini Kit following the manufacturer's instructions (QIAGEN, Hilden, Germany).

The protocol used to perform deep sequencing was a combination of several protocols normally applied to viral metagenomics and/or virus discovery (24). In summary, 50 mg of each triturated brain supernatant was diluted in 500 µL of Hanks' buffered salt solution (HBSS), added to a 2 mL impact-resistant tube containing lysing matrix C (MP Biomedicals, USA), and homogenized in a FastPrep-24 5G Homogenizer (MP biomedicals, USA). The homogenized sample was centrifuged at 12,000×g for 10 min, and approximately 300 µL of the supernatant was then percolated through a 0.45 µm filter (Merck Millipore, Billerica, MA, USA) to remove eukaryotic and bacterial cell-sized particles. One hundred microliters of cold PEG-it Virus Precipitation Solution (System Biosciences, CA, USA), roughly equivalent to one-fourth of the volume of the tube, was added to the filtrate, the contents of the tubes were gently mixed and then incubated at 4ºC for 24 h. The mixture was subsequently centrifuged at 10,000×g for 30 min at 4ºC and the supernatant (~350 µL) was discarded. The viral particle-rich pellet was treated with a mixture of nuclease enzymes (14 uni TURBO Dnase and 7 uni RNase Cocktail Enzyme Mix-Thermo Fischer Scientific, CA, USA; 9 uni Baseline-ZERO DNase — Epicentre, WI, USA; 25 Benzonase - Darmstadt, Germany; and 9 RQ1 RNase- Free DNase and 0.09mg RNase A Solution — Promega, WI, USA) to digest unprotected nucleic acids. The resulting mixture was subsequently incubated at 37ºC for 2 h [18].

After incubation, viral nucleic acids were extracted using the ZR & ZR-96 Viral DNA/RNA Kit (Zymo Research, CA, USA) according to the manufacturer's protocol. The cDNA synthesis was performed using AMV reverse transcriptase (Promega, WI, USA). A second strand of cDNA was synthetized using DNA Polymerase I Lar e (Klenow) Fragment (Promega, WI, USA). Subsequently, a Nextera XT Sample Preparation Kit (Illumina, CA, USA) was used to construct a DNA library, identified using dual barcodes. For size range, Pippin Prep (Sage Science, Inc.) was used to select a 300 bp insert (range 200-400 bp). The library was deep-sequenced using the HiSeq 2500 Sequencer (Illumina, CA, USA) with 126 bp ends [19]. Following de novo assembly of short sequence reads longer contigs were made using the customized de novo assembly software previously described [20]. Viral sequences were recognized by translating them in silico in all six possible reading frames. These virtual protein sequences were then used for similarity searches using BLASTx against the proteins of all viral genomes in GenBank. The bioinformatics pipeline trimed sequences of primers involved in the random RT-PCR reaction and kept only a single copy of repeated sequences. Residual sequences from the human genome were also removed to accelerate analyses. Contigs of overlapping short reads were then generated using an in-house hybrid de novo assembler program specially designed for viral metagenomics, generating longer contigs to facilitate the recognition of highly divergent viral genomes [19, 20]. A search was then performed for sequence similarity against all annotated viral genomes in GenBank. We used a protein computationally demanding step to reduce signal noise by removing from the list of tentative viral hits those sequences with higher levels of similarity to non-viral sequences (based on annotation) in the large NR (nonredundant) GenBank database. This NR database shows the family/genus/species of viruses with similarity to the generated data set (with adjustable E score ranges). The pipeline was fast and sensitive, allowing even highly divergent viruses with only ~15-20% protein identity (depending on length of contigs) to be recognized [19]. The final genome analysis was performed using Geneious software v9.1.8 (Biomatters Ltd., Auckland, New Zealand). Open reading frames were predicted with the Geneious ORF finder. Based on the bioinformatics pipeline used [19], no reads related to human, fungal, or bacterial sequences were obtained.

2.5 Aligment and Phylogenetic Analysis

Sequences from segments S, M and L ORFs were aligned with different groups of Orthobunyavirus on amino acid sequences using the Muscle algorithm and manually inspected using Mega v.7 [21]. For segment S, only the nucleoprotein ORF (NP) was aligned. After alignment, sequences were then converted back to their original nucleotide sequences for phylogenetic analysis. Sequences with excessive gaps in the alignment were removed. Rio Preto da Eva virus (*Pacuvirus*) was used as the outergroup. The best model and phylogenetic analysis were performed using IQ- TREE with a ultrafast bootstrap (1000 replicates) [22, 23], and the tree generated was edited using FigTree v.14.3 with a mid-point root. Similarities between the segments and other Orthobunyavirus were calculated using BioEdit Sequence Alignment Editor [24]. Nucleotide sequences for segments S, M and L determined in this study have been deposited in GenBank under accession numbers OK338018-OK338020.

3. Results

Samples of liver and spleen obtained from an adult sentinel mice (named Span321532) placed on May 25th 2011 in Iguape city (24º 38’ 412” S, 47º 29’ 214 W) that was euthanized was positive for anti-Caraparu virus hyperimune sera after one passage in C6/36 cell lines. Brain sample was negative, suggesting that BRCV is not neurotropic For phylogenetic analysis, for segment S the best model according to BIC was TVM+F+I+G4; for M and L segments, GTR+F+I+G4 were used. All trees placed SPAN321532 within Group C of Orthobunyavirus with high bootstrap values, in close proximity with BRCV prototype strain from 1976 (GenBank accession numbers MK896603-MK896605) (Figure 3A-C), with similarities of 98.5% for L segment, 94.7% for M and 95% for S (Table 2). No reassortant events were detected for this new isolate. However, each segment placed BRCV Span321532 within different clusters. For segment S (NP), BRCV clustered with all Caraparu virus strains, Madrid virus, Vinces virus and Itaqui virus; for M segment, with Caraparu strains, Apeu virus, Vinces virus and Madrid virus, and for L segment, with Caraparu strains, Oriboca virus, Itaqui virus, Madrid virus and Vinces virus, which were similar to those previously detected [5]. Similarities within members of Orthobunyavirus Group C are depicted in Table 2.

Table 2

S, M and L segments similarities between Bruconha virus Span321532 and Group C orthobunyavirus

Span 321532	BRCV	ITQV	MURV	GLV	ORIV	CARV Bean3994	CARV FMD0783	CARV IQD5973	CARV FVB0426	RESV	MADV	MTBV	VINV	APEUV
S	0,95	**0,829**	0,687	0,697	0,693	0,825	0,822	0,815	0,817	0,695	0,817	0,697	0,808	0,707
M	0,947	0,62	0,686	0,672	0,635	0,724	0,725	0,727	**0,731**	0,674	0,71	0,684	0,712	0,716
L	0,985	0,772	0,73	0,719	0,77	0,77	**0,781**	0,779	0,776	0,73	0,776	0,734	0,773	0,725

Legend: (BRCV) Bruconha virus; (ITQV): Itaqui virus; (MURV): Murutucu virus; (GLV): Gumbo limbo virus; (ORIV): Oriboca virus; (CARV): Caraparu virus; (RESV Restan virus; (MADV): Madrid virus; (MTBV): Marituba virus; (VINV): Vinces virus; (APEUV): Apeu vírus; (NEPV): Nepuyo virus. Values for higher similarities ar shown in bold.

4. Discussion

Here we describe a new genome of a rare Bruconha orthobunyavirus, isolated in 2011 at Brazilian Atlantic Forest biome. In 1961, Adolfo Lutz Institute began a program that aimed to investigate arbovirus diversity and epidemiology in São Paulo State, when several arboviruses, including new Bunyavirales, were discovered and characterized. Boraceia virus, a member of the Anopheles B group, was isolated from a pool of *Anopheles cruzii* at Serra do Mar, a forest area near São Paulo city, in 1962 [7], and later from *Phoniomyia pilicauda* [25]. This virus was considered as the causative agent of an infectious illness among residents in Salesópolis, distant 100 Km away from São Paulo city, the capital of the State [25]. Strains of Bertioga virus (Guama group) and Anhembi virus (Bunyamera group) were isolated from sentinel mice, and *Phoniomyia pilicauda*, *Trichoprosopon pailidiventer*, and from a spiny rat (*Proechimys iheringi*) [10]. BRCV was isolated from *Culex sacchettae* mosquitoes in Cananéia city, located in the south coast of São Paulo State, on February, April and November, 1976. Almost 4 decades later, we describe a new isolate of BRCV from Iguape county. This new isolate was obtained 60Km from the original one, showing that it may be dispersed through this region. We also found that nucleotide differences varied from 1.5%, 5.3% and 5% (L, M and S segments respectively) from the 1976 prototype.

Reassortant events have shaped the evolution of several segmented viruses, including that of Group C orthobunyaviruses [5, 11], and it was proposed that BRCV obtained its S segment from CARV [14]. On the other hand, Caraparu and Itaqui viruses had nearly identical L and S segments, but different M segments. Interestingly, within group C, Span321532 had the highest S segment (N protein) similarity with Itaqui virus (82.9%), while M and L with different Caraparu strains, one isolated in Bolivia in 2008, and the other in Peru, in 2006, respectively. It is important to note that other Caraparu isolates were obtaine from the Brazilian Amazon basin, at the North region, Peru, and also in São Paulo state, from *Culex* mosquitoes, humans and non-human primates [14]. However, BRCV was never isolated outside Atlantic Forest, although intensive programs for virus discovery have been performed during the last decades in Brazil. These differences corroborates with previous studies which demonstrates that the S and M segments have different evolutionary histories [14]. However, isolate Span321532 had no evidence of reassortants within other Orthobunyavirus belonging to group C.

Only one human case that was caused by Caraparus virus (or a close-related virus) was described at Vale do Ribeira, when a 28 year old male biologist that worked with entomological investigation presented a mild febrile disease, and fully recovered [8]. As this virus is quite similar to BRCV, it is likely that it may cause an undiagnosed illness at the region. For instance, several orthobunyaviruses have recently emerged in different parts of the world, causing disease in human or animal diseases, with many associated with CNS disease, such as Jamestown Canyon virus (JCV), a mosquito-borne orthobunyavirus that causes an acute febrile illness, meningitis, or meningoencephalitis [26].

Interestingly, despite arbovirus high mutation rates and the great diversity of pathogenic orthobunyavirus at Vale do Ribeira, outbreaks caused by this genus were never reported in the southeastern region of Brazil. In fact, the circulation of several arbovirus are often related with climate, habitat, presence of vectors and animal density and movement. A possible explanation regarding the low disease incidence at Vale do Ribeira is the conservation of the biome, where one may observe a dilution effect [27]. This region harbors several conservation units since the 1980's, with lower deforestation of Atlantic Forest remnants when compared to other Brazilian biomes, which may decrease the risk of human infection. More, BRCV apparently is restricted to Atlantic Forest biome, where only two genomes were obtained until now within a 35 years interval, with high similarities and lack of reassortant events. Increasing virus active surveillance in order to obtain more sequences of Bunyavirales would generate improvements on information regarding viral genetics and evolution, shedding light in this question. More, given the high biodiversity in Vale do Ribeira, these studies would allow us to better known sylvatic hosts and vectors. Although it is known that Group C Orthobunyavirus circulate among rodents, marsupials and eventually humans in São Paulo State, epidemiological aspects regarding BRCV are scarce. More, in order to understand the patterns of other arbovirus circulation in the human population at Vale do Ribeira, it is urged to perform differential diagnosis for Dengue, Zika and Chikungunya viruses during the acute phase, when one can perform direct methods for viral detection.

Declarations

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Ethics:

this research was approved by CEUA under the protocol 02/2011 "Programa de vigilância ecoepidemiológica de arbovirus no estado de São Paulo", by Dr. Luis Eloy Pereira. Laboratory animals used herein were euthanised using CO^2 chamber

Author Contributions:

Manuscript preparation: MSC, ACC. Obtained funding and study supervision: MSC. Experiments of viral detection and NGS: ACC, VSM, RM. Performed the analyses: MSC, KMBN. All authors reviewed, contributed to, and approved the final version of the manuscript.

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Competing Interests:

The authors have no relevant financial or non-financial interests to disclose.

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Figures

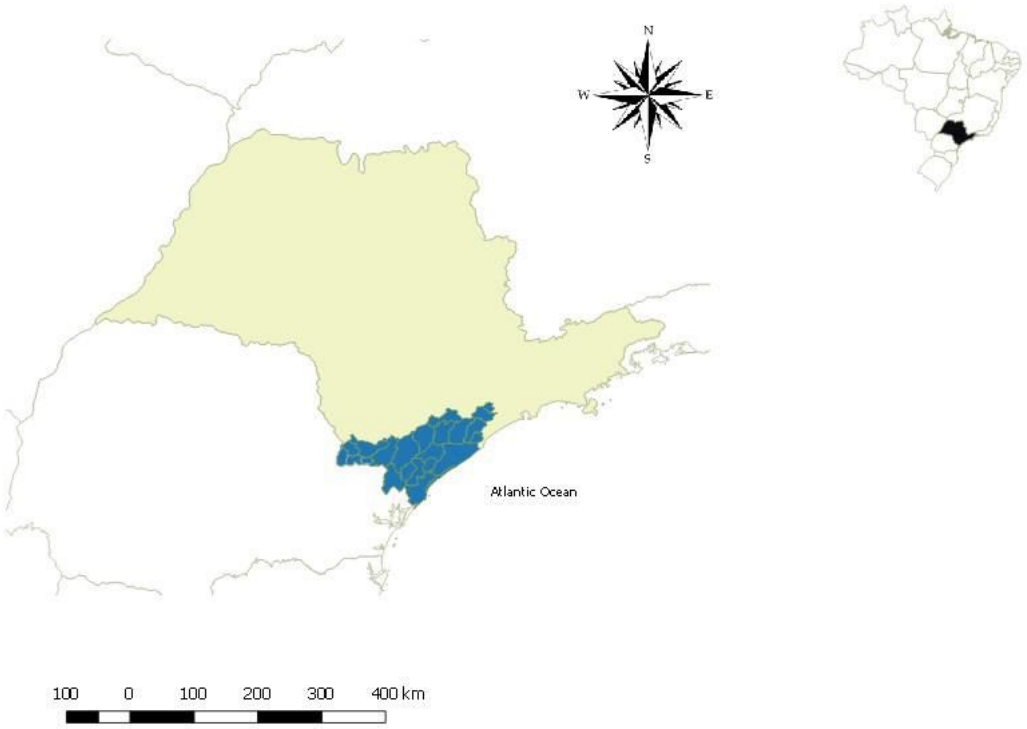

Figure 1

Map showing the location of Vale do Ribeira (in blue), SP, Brazil

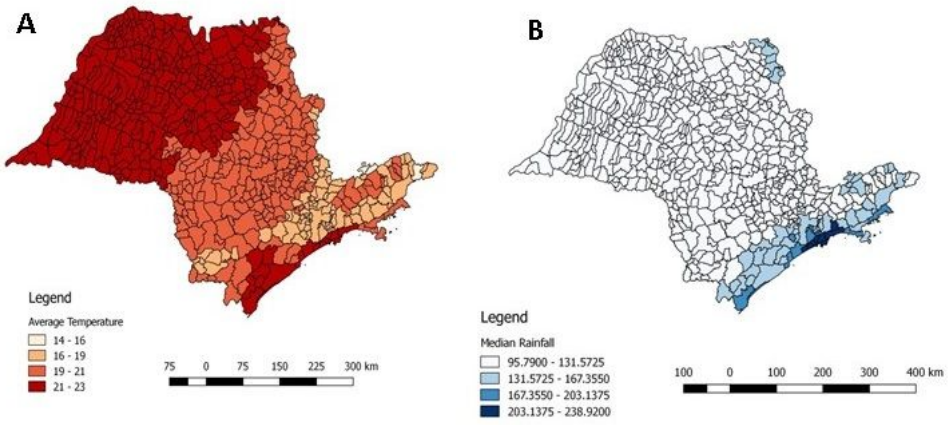

Figure 2

Maps of São Paulo State showing average temperature (2A) and rainfall (2B)

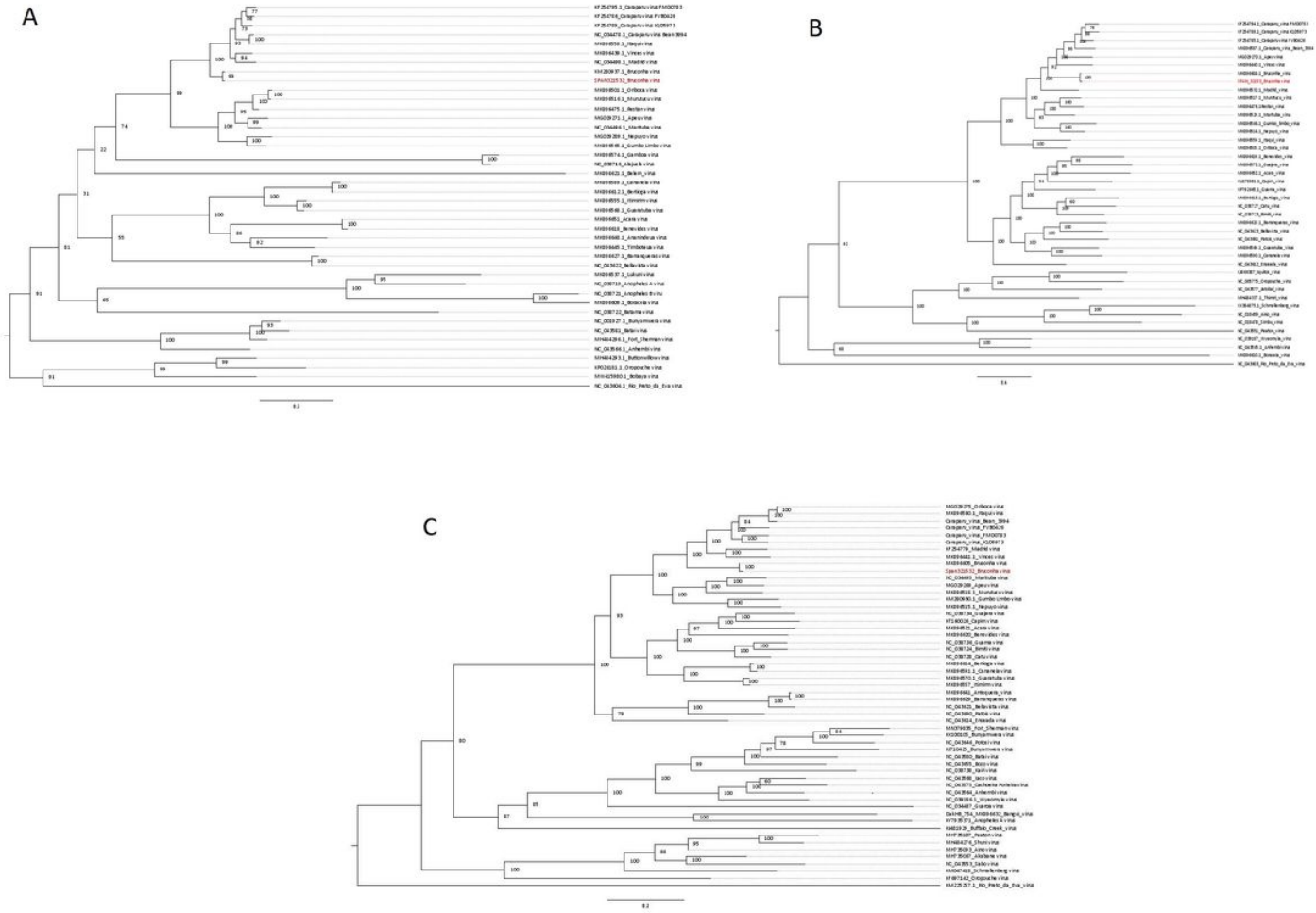

Figure 3

Phylogenetic tree of the BRCV isolate Span321532. Sequences of Orthobunyavirus ORFs whole genomes were aligned using MUSCLE codon in MEGA 7 software [21]. Best model for each segment and the maximum likelihood tree were obtained and constructed using IQ-Tree [22]. (A) Phylogenetic tree of S segment. (B) Phylogenetic tree of M segment. (C) Phylogenetic tree of L segment. BRCV is depicted in red. Tree was constructed using FigTree v.1.4.3 with an automatic scale and mid-point root. Scale in nucleotide substitutions per site. Numbers at nodes indicate bootstrap values. Rio Preto da Eva virus was used as outergroup.

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