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# Genetic characterization of the rare Bruconha virus (*Bunyavirales: Orthobunyavirus*) isolated in Vale do Ribeira (Atlantic Forest biome), Southeastern Brazil

**Antônio Charlys da Costa** [1], **Vanessa dos Santos Morais** [1], **Roberta Marcatti de Azevedo** [1], **Karolina Morales Barrio Nuevo** [2], **Mariana Sequetin Cunha** [1,2]

## ABSTRACT

Brazil is a great source of arbovirus diversity, mainly in the Amazon region. However, other biomes, especially the Atlantic Forest, may also be a hotspot for emerging 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 isolate of BRCV named Span321532 was obtained from an adult sentinel mouse placed in Iguape city in 2011, and a full-length genome was generated with nucleotide differences ranging between 1.5%, 5.3% and 5% (L, M and S segments, respectively) from the prototype isolated 35 years earlier. In addition, each segment placed BRCV into different clusters, showing the high variety within *Bunyavirales*. Although no evidence for reassortants was detected, this finding 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*.

**KEYWORDS**: Arbovirus. *Bunyavirales*. Active surveillance. Phylogenetics.

## 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 (NS), with an RNA of negative-sense. Within the order, the *Orthobunyavirus* genus, which belongs to the *Peribunyaviridae* family, contains 103 species divided into distinct serogroups[5,6].Many of them may have severe impacts on the health of humans and animals, such as Oropouche virus and Schmallenberg virus.

Several Orthobunyaviruses, mainly from groups C and Guama, have been isolated in the Atlantic Forest biome within Sao Paulo State (Southeastern Brazil) during active surveillance programs for arbovirus during the 1960s and 1970s,

[1]Universidade de São Paulo, Faculdade de Medicina, Instituto de Medicina Tropical de São Paulo, São Paulo, São Paulo, Brazil

[2]Instituto Adolfo Lutz, Núcleo de Doenças de Transmissão Vetorial, São Paulo, São Paulo, Brazil

**Correspondence to**: Mariana Sequetin Cunha
Instituto Adolfo Lutz, Núcleo de Doenças de Transmissão Vetorial, Av. Doutor Arnaldo, 355, CEP 01246-000, São Paulo, SP, Brazil
Tel: +55 11 30682901

**E-mail:** masequetin@gmail.com

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such as Bertioga virus (BERV), Boraceia virus (BORV), Cananeia virus (CNAV), Caraparu virus (CARV), Guaratuba virus (GTRV) and Itimirim virus (ITIV)[7-9]. In the Atlantic Forest, it is believed that these viruses are carried mainly by rodents (or other small mammals) and *Culex* mosquitoes[7]. As described for segmented genomes, Orthobunyaviruses are capable of reassortment, which can have epidemiological importance[10]. For instance, Ngari virus is a reassortant Bunyavirus associated with outbreaks of hemorrhagic fever in Africa[11]; Iquitos and Itaya viruses are emerging reassortant Bunyaviruses associated with human illness in Peru[12]. Reassortants are also described in Group C Orthobunyaviruses, once the phylogenetic analysis revealed that Caraparu virus, which circulates in Brazil, contained an S segment sequence that is nearly identical to Oriboca virus, indicating a natural reassortant virus[13]. However, as viruses belonging to the *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 in this particular biome. Here we describe a full-length genome of Bruconha virus (BRCV), a group C *Orthobunyavirus* isolated in 2011, in Iguape city, Sao Paulo State, Brazil.

## MATERIALS AND METHODS

### Ethics

This research was approved by CEUA under the 02/2011 protocol, Programa de vigilancia ecoepidemiologica de arbovirus no estado de Sao Paulo, by Dr. Luis Eloy Pereira. The laboratory animals used were euthanized in a $CO_2$ chamber.

### Area

In Sao Paulo State, during the arbovirus eco-epidemiological surveillance program, mosquito and bird blood samples were collected. Also, sentinel mice were placed in selected areas with water and food *ad libitum*. This program was conducted from 2011 to 2016 at Vale do Ribeira, a region of the Atlantic Forest located on the southern coast of the State (Figure 1A). The aim of this project was to identify hosts and vectors of arboviruses. The Vale do Ribeira region is occupied by the hydrographic basin of the Ribeira do Iguape river, and includes 16 counties. It contains over 21,000 km$^2$ of well-preserved forests (around 21% of the total remaining Atlantic Forest in Brazil). The region has high temperatures with high rainfall throughout the year (Figures 1B and 1C). The economy is based on subsistence agriculture. Besides the biological diversity of Vale do Ribeira, this region was selected thanks to the previous detection of several arbovirus, including the outbreak caused by the Flavivirus Rocio virus (1975-1977)[14]. It is known that deforestation has increased in Brazil over the last years, mainly in the Amazon region[15]. In order to check the conservation at Vale do Ribeira, we applied the Mapbiomas plug-in using QGIS v 3.16, to compare the Atlantic Forest biome in 1986 and 2011, showing the forest maintenance during the last 3 decades (Figure 2).

### Sample collection

In 2011, a total of 6 field trips, each lasting 3 days, with five taking place in Iguape city and one in Panorama city (March, April, May and November), were conducted. Nets were used for the capture of wild birds, while domestic birds were controlled by the owner. Also, each trip brought two cages of sentinel Swiss mice, with 1 mother and 6 newborns each (total = 12), as well as water and food *ad libitum*[16]. Blood samples from birds (0.2-1.0 mL, depending on the animal weight) were collected by venipuncture 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. The sentinel mice were brought back to the laboratory and observed for 21 days. Table 1 shows all bird captures made during 2011.

### 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 resulting suspension was centrifuged at 1800×g for 15 min. The supernatant was withdrawn and frozen at –70 °C before further processing. Each fragment was inoculated in cell tubes containing monolayer cultures of C6/36 cells. The culture tubes were incubated for nine days at 28 °C with an L-15 medium containing 2% FBS, penicillin (100 units/mL) and streptomycin (100 µg/mL). Indirect immune assay (IFA) tests were then performed using an in-house hyper immune serum for the detection of genus *Flavivirus* (antibody anti-Saint Louis virus), *Alphavirus* (antibody anti-Mayaro virus) and *Bunyavirus* (antibody anti-Caraparu virus).

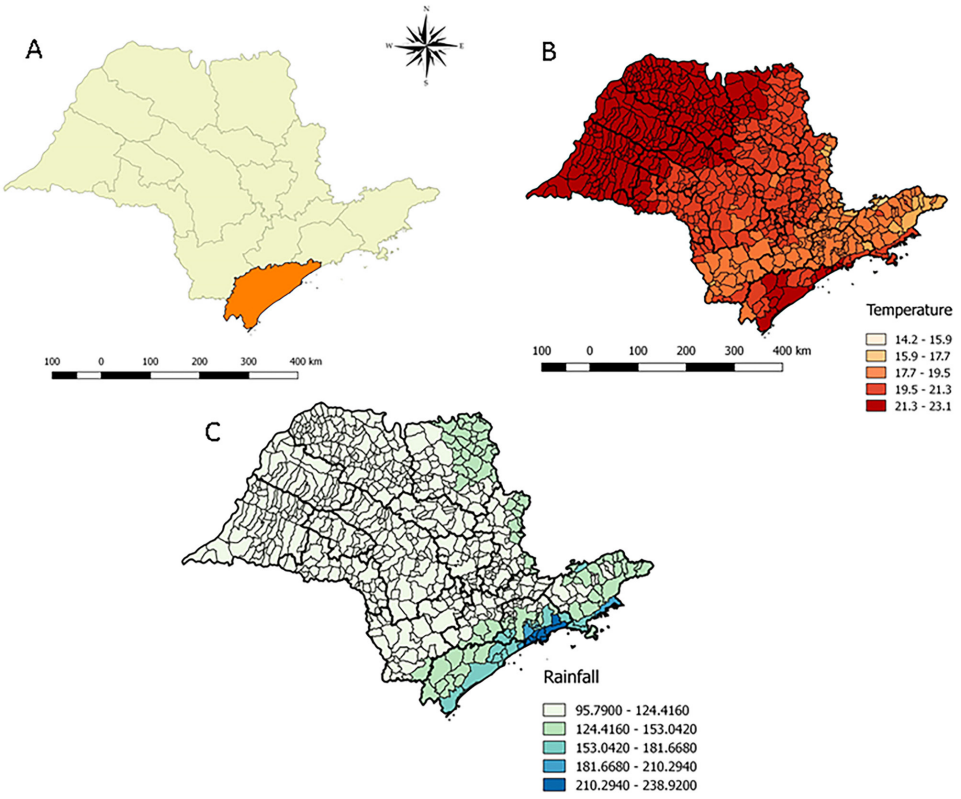


**Figure 1** - Sao Paulo State map showing the location of Vale do Ribeira (in orange) (A), average temperature (B), and rainfall (C).

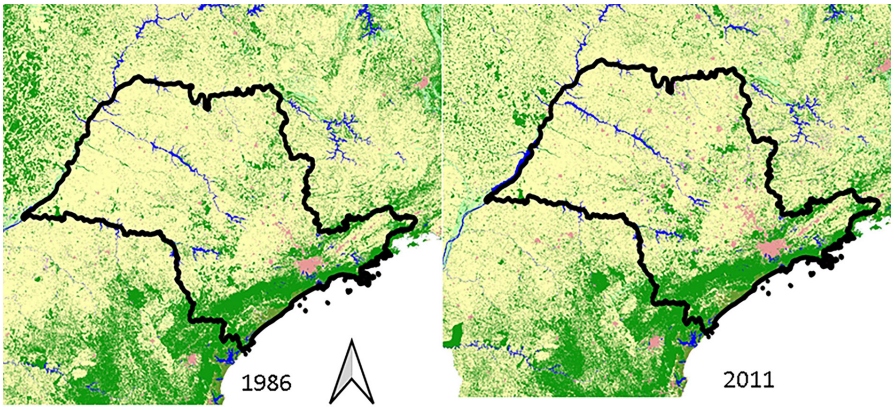


**Figure 2 -** Sao Paulo State vegetation in 1986 and 2011, revealing Atlantic Forest preservation at Vale do Ribeira. Dark green areas show forests, light yellow shows areas used for agriculture, and blue areas show rivers. Maps were constructed using QGIS Hannover version 3.16 with the Mapbiomas plug-in.

## Sequencing

RNA was extracted from isolate Span321532 C6/36 supernatant using the 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[17]. 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[18]. After *de novo* assembly of short sequence reads, longer contigs were made using the customized *de novo* assembly software described earlier[19]. Sequences were then used for similarity searches using BLASTx against the proteins of all viral

**Table 1 -** Species of birds captured during the arbovirus surveillance program in Iguape city, Sao Paulo State, Brazil, 2011.

| Family | Species | Number of individuals |
|---|---|---|
| *Anatidae* | *Anas platyrhynchos domesticus* | 23 |
| *Psittacidae* | *Brotogeris tirica* | 1 |
| *Coerebidae* | *Coereba flaveola* | 2 |
| *Columbidae* | *Columbina talpacoti* | 1 |
| *Conopophagidae* | *Conopophaga lineata* | 1 |
| *Tyrannidae* | *Fluvicola nengeta* | 1 |
| *Furnariidae* | *Furnarius rufus* | 4 |
| *Tyrannidae* | *Leptopogon amaurocephalus* | 1 |
| *Pipridae* | *Manacus manacus* | 2 |
| *Tyrannidae* | *Myiophobus fasciatus* | 1 |
| *Tyrannidae* | *Myozetetes similis* | 1 |
| *Hirundinidae* | *Notiochelidon cyanoleuca* | 2 |
| *Picidae* | *Picumnus temminckii* | 1 |
| *Thraupidae* | *Ramphocelus bresilius* | 3 |
| *Trochilidae* | *Ramphodon naevius* | 1 |
| *Thraupidae* | *Saltator similis* | 3 |
| *Thraupidae* | *Sicalis flaveola* | 4 |
| *Fringillidae* | *Sporophila caerulescens* | 14 |
| *Hirundinidae* | *Stelgidopteryx ruficollis* | 8 |
| *Thraupidae* | *Tangara seledon* | 6 |
| *Thraupidae* | *Thraupis sayaca* | 1 |
| *Troglodytidae* | *Troglodytes aedon* | 3 |
| *Turdidae* | *Turdus amaurochalinus* | 1 |
| *Turdidae* | *Turdus rufiventris* | 3 |
| *Fringillidae* | *Zonotrichia capensis* | 4 |

genomes in GenBank. The bioinformatics pipeline trimmed sequences of primers involved in the random RT-PCR reaction and kept only a single copy of repeated sequences. The residual sequences from the human genome were also removed to accelerate the analyses. Contigs of overlapping short reads were then generated using an in-house hybrid *de novo* assembly program specially designed for viral metagenomics, generating longer contigs to facilitate the recognition of highly divergent viral genomes[18,19]. A search was then performed for sequence similarity against all annotated viral genomes in GenBank. We used a computationally demanding protein step to reduce the signal noise by removing those sequences with higher levels of similarity to non-viral sequences (based on annotation) from the list of tentative viral hits 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 the length of contigs) to be recognized[18]. 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[18], no reads related to human, fungal, or bacterial sequences were obtained.

### Alignment and phylogenetic analysis

Sequences from segments S, M and L ORFs were aligned with different groups of Orthobunyavirus sequences using Muscle Codon in Mega v.7[20], and manually inspected. For segment S, only the nucleoprotein ORF (NP) was aligned. Sequences with excessive gaps in the alignment were removed. Rio Preto da Eva virus (*Pacuvirus*) was used as the outer group. The best model and phylogenetic analysis were performed using IQ-TREE with an ultrafast bootstrap (1,000 replicates)[21,22], and the tree generated was edited using FigTree v.14.3 with a mid-point root. Similarities between the segments and the other Orthobunyavirus were calculated using the BioEdit Sequence Alignment Editor[23]. Nucleotide sequences for segments S, M and L determined in this study have been deposited in the GenBank under accession Nº OK338018-OK338020.

## RESULTS

Samples of liver and spleen obtained from an adult sentinel mouse (named Span321532), who was placed on May 25th, 2011 in Iguape city (24° 38' 412'' S, 47° 29' 214 W) and was euthanized, were positive for anti-Caraparu virus hyperimmune sera after one passage in C6/36 cell lines. The brain sample was negative, suggesting that BRCV is not neurotropic. No virus was isolated from the birds. For phylogenetic analysis, for segment S the best model according to BIC was TVM+F+I+G4; for the M and L segments, GTR+F+I+G4 were used. All trees placed Span321532 within Group C of Orthobunyavirus with high bootstrap values, similarly to the BRCV prototype strain from 1976 (GenBank accession Nº MK896603-MK896605) (Figure 3), with nucleotide similarities of 98.5% for the L segment, 94.7% for M and 95% for S (Table 2). Phylogenetic trees reveal that 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

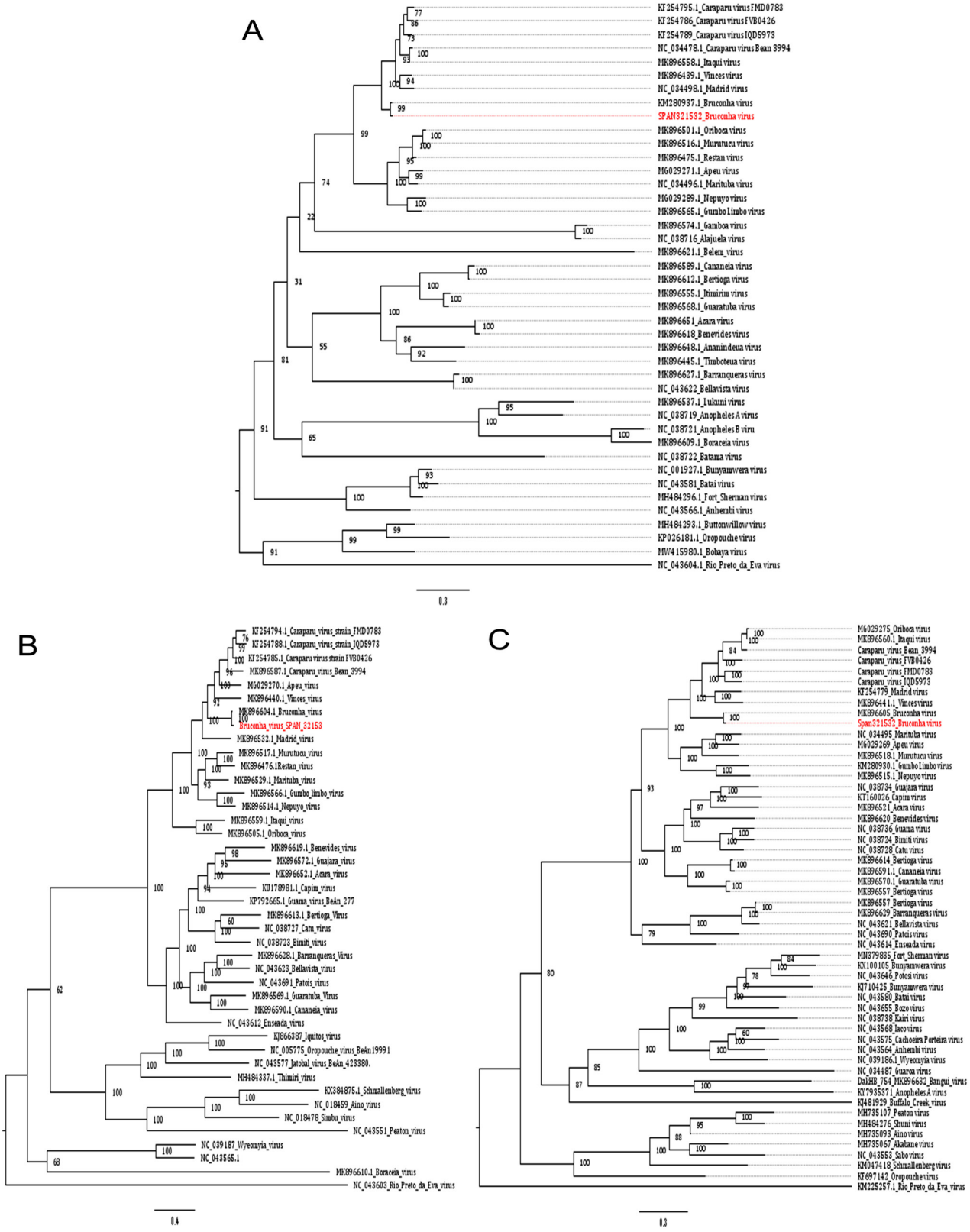


**Figure 3 -** Phylogenetic tree of the BRCV isolate Span321532. Sequences of Orthobunyavirus ORFs; whole genomes were aligned using the MUSCLE codon in MEGA 7 software (Kumar *et al*.[20]). Best model for each segment and the maximum likelihood tree were obtained and constructed using IQ-Tree (Nguyen *et al*.[21]): (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 outer group.

**Table 2 -** S, M and L segments' nucleotide 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 | NEPV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S | 95 | **82.9** | 68.7 | 69.7 | 69.3 | 82.5 | 82.2 | 81.5 | 81.7 | 69.5 | 81.7 | 69.7 | 80.8 | 70.7 | 68.3 |
| M | 94.7 | 62 | 68.6 | 67.2 | 63.5 | 72.4 | 72.5 | 72.7 | **73.1** | 67.4 | 71 | 68.4 | 71.2 | 71.6 | 67 |
| L | 98.5 | 77.2 | 73 | 71.9 | 77 | 77 | **78.1** | 77.9 | 77.6 | 73 | 77.6 | 73.4 | 77.3 | 72.5 | 72 |

strains, Madrid virus, Vinces virus and Itaqui virus; for the M segment, with Caraparu strains, Apeu virus, Vinces virus and Madrid virus; and for the 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.

## DISCUSSION

Here we describe a new coding-complete genome of a rare Bruconha Orthobunyavirus, isolated in 2011 in the Brazilian Atlantic Forest biome. In 1961, the Adolfo Lutz Institute started a program that aimed to investigate arbovirus epidemiology and diversity in Sao Paulo State, when several arboviruses, including new Bunyavirales, were discovered and characterized. For instance, 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 Sao Paulo city, in 1962[8], and later from *Phoniomyia pilicauda*[24]. This virus was considered the causative agent of an infectious illness among residents in Salesopolis city, 100 km away from Sao Paulo city, the capital of the state[24]. Strains of Bertioga virus (Guama group) and Anhembi virus (Bunyamera group) were isolated from sentinel mice, *Phoniomyia pilicauda*, *Trichoprosopon pailidiventer*, and from a spiny rat (*Proechimys iheringi*)[7]. BRCV was isolated from *Culex sacchettae* mosquitoes in Iguape city, in February, April and November, 1976. Almost 4 decades later, we describe a new isolate of BRCV from the same county, showing viral persistence within its hosts. We also found that the nucleotide identity diverged 1.5%, 5.3% and 5% (L, M and S segments, respectively) from the 1976 prototype. Unfortunately, coordinates from this strain are not available.

Reassortant events have shaped the evolution of several segmented viruses, including Group C Orthobunyaviruses[5,10], and it was proposed that BRCV obtained its S segment from CARV[13]. 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 segments had it with different Caraparu strains: one isolated in Bolivia, in 2008, and the other in Peru, in 2006, respectively. It is important to note that Caraparu isolates were obtained from the Brazilian Amazon basin, the Northern region, Peru, and also from Sao Paulo state, from *Culex* mosquitoes, humans and non-human primates[13]. BRCV was never isolated outside the Atlantic Forest, although intensive programs for virus discovery have been performed during the last decades in Brazil. These differences corroborate previous studies showing that the S and M segments have different evolutionary histories[13]. However, isolate Span321532 had no evidence of reassortants within other Orthobunyaviruses belonging to group C.

Only one human case of illness that was caused by Caraparu virus (or a close-related virus) was described at Vale do Ribeira: a 28 years old male biologist who worked with entomological investigations presented a mild febrile disease, and he fully recovered[9]. As this virus is quite similar to BRCV, it is likely that BRCV could cause an undiagnosed illness in the region. Interestingly, despite the high mutation rates of arbovirus and the great diversity of pathogenic Orthobunyaviruses at Vale do Ribeira, outbreaks were never reported in the Southeastern region of Brazil. In fact, the circulation of several arboviruses is often related with climate, habitat, presence of vectors and animal density and movement. A possible explanation regarding the low incidence of the disease at Vale do Ribeira is the conservation of the biome, where a dilution effect may occur[25]. This region has harbored several conservation units since the 1980s, with lower deforestation of the Atlantic Forest remnants when compared to other Brazilian biomes, which may decrease the risk of human infection. Moreover, BRCV is apparently restricted to the Atlantic Forest biome, where only two genomes were obtained until now within a 35-year interval, with high similarities and a lack of reassortant events. Increasing active surveillance of the viruses in order to obtain more sequences of Bunyavirales would generate improvements on information regarding viral genetics and evolution, shedding light on this issue. Furthermore, given the high biodiversity of the Vale do Ribeira, these studies would allow us to better understand the sylvatic hosts and vectors.

## CONCLUSION

Although it is known that Group C Orthobunyavirus circulates among rodents, marsupials and eventually humans in Sao Paulo State, epidemiological aspects regarding BRCV are scarce. Moreover, in order to understand the patterns of other cases of arbovirus circulation in the human population at Vale do Ribeira, it is necessary to perform differential diagnosis for Dengue, Zika and Chikungunya viruses during the acute phase, when methods for viral detection can be performed.

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## AUTHORS' CONTRIBUTIONS

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

## CONFLICT OF INTERESTS

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

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## REFERENCES

1. Olival KJ, Hosseini PR, Zambrana-Torrelio C, Ross N, Bogich TL, Daszak P. Host and viral traits predict zoonotic spillover from mammals. Nature. 2017;546:646-50.
2. Nunes MR, Faria NR, Vasconcelos JM, Golding N, Kraemer MU, Oliveira LF, et al. Emergence and potential for spread of Chikungunya virus in Brazil. BMC Med. 2015;13:102.
3. Faria NR, Azevedo RS, Kraemer MU, Souza R, Cunha MS, Hill SC, et al. Zika virus in the Americas: early epidemiological and genetic findings. Science. 2016;352:345-9.
4. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature. 2013;496:504-7.
5. Kapuscinski ML, Bergren NA, Russell BJ, Lee JS, Borland EM, Hartman DA, et al. Genomic characterization of 99 viruses from the bunyavirus families Nairoviridae, Peribunyaviridae, and Phenuiviridae, including 35 previously unsequenced viruses. PLoS Pathog. 2021;17:e1009315.
6. Walker PJ, Siddell SG, Lefkowitz EJ, Mushegian AR, Adriaenssens EM, Dempsey DM, et al. Changes to virus taxonomy and the Statutes ratified by the International Committee on Taxonomy of Viruses (2020). Arch Virol. 2020;165:2737-48.
7. Calisher CH, Coimbra TL, Lopez OS, Muth DJ, Sacchetta LA, Francy DB, et al. Identification of new Guama and Group C serogroup bunyaviruses and an ungrouped virus from Southern Brazil. Am J Trop Med Hyg. 1983;32:424-31.
8. Lopes OS, Forattini OP, Fonseca IE, Lacerda JP, Sacchetta LA, Rabello EX. Boracéia virus: a new virus related to anopheles B virus. Proc Soc Exp Biol Med Soc. 1966;123:502-4.
9. Iversson LB, Travassos da Rosa AP, Coimbra TL, Ferreira IB, Nassar ES. Human disease in Ribeira Valley, Brazil caused by Caraparu, a group C arbovirus: report of a case. Rev Inst Med Trop Sao Paulo. 1987;29:112-7.
10. Briese T, Calisher CH, Higgs S. Viruses of the family Bunyaviridae: are all available isolates reassortants? Virology. 2013;446:207-16.
11. Gerrard SR, Li L, Barrett AD, Nichol ST. Ngari virus is a Bunyamwera virus reassortant that can be associated with large outbreaks of hemorrhagic fever in Africa. J Virol. 2004;78:8922-6.
12. Aguilar PV, Barrett AD, Saeed MF, Watts DM, Russell K, Guevara C, et al. Iquitos virus: a novel reassortant Orthobunyavirus associated with human illness in Peru. PLoS Negl Trop Dis. 2011;5:e1315.
13. Nunes MR, Travassos da Rosa AP, Weaver SC, Tesh RB, Vasconcelos PF. Molecular epidemiology of group C viruses (Bunyaviridae, Orthobunyavirus) isolated in the Americas. J Virol. 2005;79:10561-70.
14. Lopes OS, Coimbra TL, Sacchetta LA, Calisher CH. Emergence of a new arbovirus disease in Brazil. I. Isolation and characterization of the etiologic agent, Rocio virus. Am J Epidemiol. 1978;107:444-9.
15. Silva Junior CH, Pessôa AC, Carvalho NS, Reis JB, Anderson LO, Aragão LE. The Brazilian Amazon deforestation rate in 2020 is the greatest of the decade. Nat Ecol Evol. 2021;5:144-5.
16. Causey OR, Causey CE, Maroja OM, Macedo DG. The isolation of arthropod-borne viruses, including members of two hitherto undescribed serological groups, in the Amazon region of Brazil. Am J Trop Med Hyg. 1961;10:227-49.

17. Costa AC, Thézé J, Komninakis SC, Sanz-Duro RL, Felinto MR, Moura LC, et al. Spread of Chikungunya virus East/Central/South African genotype in Northeast Brazil. Emerg Infect Dis. 2017;23:1742-4.
18. Deng X, Naccache SN, Ng T, Federman S, Li L, Chiu CY, et al. An ensemble strategy that significantly improves de novo assembly of microbial genomes from metagenomic next-generation sequencing data. Nucleic Acids Res. 2015;43:e46.
19. Wommack KE, Bhavsar J, Ravel J. Metagenomics: read length matters. Appl Environ Microbiol. 2008;74:1453-63.
20. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33:1870-4.
21. Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. 2015;32:268-74.
22. Minh BQ, Nguyen MA, von Haeseler A. Ultrafast approximation for phylogenetic bootstrap. Mol Biol Evol. 2013;30:1188-95.
23. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser. 1999;41:95-8.
24. Lopes OS, Sacchetta LA. Epidemiology of Boraceia virus in a forested area in São Paulo, Brazil. Am J Epidemiol. 1974;100:410-3.
25. Khalil H, Ecke F, Evander M, Magnusson M, Hörnfeldt B. Declining ecosystem health and the dilution effect. Sci Rep. 2016;6:31314.