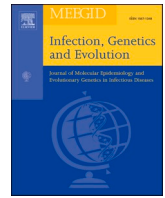




Contents lists available at ScienceDirect

# Infection, Genetics and Evolution

journal homepage: [www.elsevier.com/locate/meegid](http://www.elsevier.com/locate/meegid)

Research paper



## Molecular epidemiological investigation of Mayaro virus in febrile patients from Goiania City, 2017–2018

Elisângela de Paula Silveira-Lacerda<sup>a,1</sup>, Alice Laschuk Herlinger<sup>b,1</sup>, Amilcar Tanuri<sup>b,2</sup>, Giovanni Rezza<sup>c,2</sup>, Carlos Eduardo Anunciação<sup>a</sup>, Juliana Pires Ribeiro<sup>d</sup>, Isabele Pereira Tannous<sup>d</sup>, Gabrielly Regis Abrantes<sup>a</sup>, Elisângela Gomes da Silva<sup>a</sup>, Kauana Ferreira Arruda<sup>d</sup>, Adriano Roberto Vieira de Sousa<sup>a</sup>, Filipe Romero Rebelo Moreira<sup>b</sup>, Renato Santana Aguiar<sup>b,e</sup>, Jordana Faria Corrêa<sup>a</sup>, Marina Machado dos Santos<sup>a</sup>, Hugo Delleon Silva<sup>a</sup>, Marco Tulio A. Garcia-Zapata<sup>f</sup>, Natália Santana do Nascimento<sup>f</sup>, Mariane Talon de Menezes<sup>b</sup>, Richard Araujo Maia<sup>b</sup>, Orlando Ferreira C.<sup>b</sup>, Rafael Barbosa<sup>a</sup>, Rodrigo Brindeiro<sup>b</sup>, Clever Cardoso<sup>a</sup>, Sandra Maria Brunini<sup>d,\*</sup>

<sup>a</sup> Instituto de Ciências Biológicas, Universidade Federal de Goiás, Goiania. Av. Esperança s/n, Campus Samambaia - Prédio da Reitoria, 74690-900 Goiania/GO, Brazil

<sup>b</sup> Laboratório de Virologia Molecular, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Av. Carlos Chagas Filho, 373 – Centro de Ciências da Saúde, Ilha do Fundão, 21941-970 Rio de Janeiro/RJ, Brazil

<sup>c</sup> Department of Infectious Diseases, Istituto Superiore di Sanità, Viale Regina Elena, 299-00161 Roma, Italy

<sup>d</sup> Faculdade de Enfermagem, Universidade Federal de Goiás, Rua 227 – Setor Leste Universitário, 74605-080 Goiania/GO, Brazil

<sup>e</sup> Departamento de Genética, Ecologia e Evolução, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, 31270-901 Belo Horizonte/MG, Brazil

<sup>f</sup> Instituto de Patologia Tropical, Universidade Federal de Goiás, Rua 227 – Setor Leste Universitário, 74605-080, Goiania/GO, Brazil

### ARTICLE INFO

#### Keywords:

Mayaro virus  
Dengue virus  
Codetection

### ABSTRACT

Mayaro virus (MAYV) has historically been associated with sylvatic transmission; however, urban outbreaks have been reported in Brazil, including cases of co-detection with dengue virus (DENV). Therefore, we performed a molecular survey to investigate MAYV circulation and cocirculation with DENV within Goiania, a major city in Central-West Brazil. Among 375 subjects with arbovirus-like symptoms, 259 were positive for DENV and 26 for MAYV. Of these, 17 were coinfecting with DENV-2, suggesting co-transmission of the viruses. The most common complaints at the time of inclusion were myalgia, headache, fever, arthralgia, retro-orbital pain, and skin rash. No specific symptoms were associated with MAYV when either detected alone or co-detected with DENV, compared to that when DENV was detected alone. Most MAYV-infected subjects were women with no recent travel history to rural/sylvatic areas. Phylogenetic reconstruction indicated that the MAYV identified in this study is closely related with a lineage observed in Peru, belonging to genotype D. Our results corroborate the growing circulation of MAYV in urban environments in Brazil and reinforce the need to implement laboratory diagnosis in the Unified Health System, considering that the clinical manifestations of Mayaro fever are similar to those of other arboviruses, particularly dengue. Furthermore, most cases occurred in association with DENV-2. Further phylogenetic studies are needed to evaluate MAYV, which has not been widely examined.

### 1. Introduction

Mayaro virus (MAYV; *Alphavirus*, *Togaviridae*) is an arbovirus transmitted in the sylvatic cycle via the bite of mosquitoes in the genus

*Haemagogus* and sustained by non-human primates, with humans acting as occasional hosts. Phylogenetic analyses of MAYV led to the identification of three genotypes: L, limited to the central-north region of Brazil; D, widespread in the Pan-Amazon region of South America and the

\* Corresponding author at: Faculdade de Enfermagem, Universidade Federal de Goiás, Rua 227 – Setor Leste Universitário, CEP 74605-080, Goiania, GO, Brazil.  
E-mail address: [sandr brunini@hotmail.com](mailto:sandr brunini@hotmail.com) (S.M. Brunini).

<sup>1</sup> These authors contributed equally to this article.

<sup>2</sup> These authors contributed equally to this article.

<https://doi.org/10.1016/j.meegid.2021.104981>

Received 19 March 2021; Received in revised form 20 June 2021; Accepted 24 June 2021

Available online 29 June 2021

1567-1348/© 2021 Elsevier B.V. All rights reserved.

Caribbean; and N, represented by a single sequence isolated from Peru (Auguste et al., 2015). Additionally, a hybrid L/D genotype was described more recently in Brazil and Haiti (Mavian et al., 2017).

Similar to Chikungunya virus (CHIKV) fever, MAYV fever is a nonspecific febrile illness involving cephalgia, skin rash, and arthralgia, which may persist for months in severe cases (Acosta-Ampudia et al., 2018; Esposito and da Fonseca, 2017). MAYV was first isolated in 1954 from febrile rural workers in Trinidad (Anderson et al., 1957). Subsequently, sporadic outbreaks of MAYV were reported across Central and South America, in forest, rural, and peri-urban areas (Acosta-Ampudia et al., 2018; Esposito and da Fonseca, 2017). In Brazil, MAYV is considered as endemic to the Amazon Region, including states from the North and Central-West part of the country, with the first MAYV human cases described in 1953 (Causey and Maroja, 1957). Sporadic outbreaks were reported thereafter in rural, peri-urban, and sylvatic areas within endemic regions (Acosta-Ampudia et al., 2018; Brunini et al., 2017). Additionally, a serological survey based on MAYV-specific IgM antibody capture enzyme-linked immunosorbent assay, conducted between June 2014 and June 2015 in Goiania, revealed that 15 individuals initially suspected of having CHIKV infection were, in fact, positive for MAYV (Brunini et al., 2017), indicating that clinical misdiagnosis of MAYV can occur because it shares clinical manifestations with CHIKV. Notably, all MAYV cases were related to rural workers who worked in forest areas within 15 days prior to symptom onset, which correlates with spill-over events from the sylvatic cycle of MAYV transmission within the Amazon region (Abad-Franch et al., 2012).

Although MAYV is described as being restricted to forest/rural areas, its recent dispersion has been reported beyond endemic areas. In Brazil, states never before associated with the virus have been highlighted as being at high risk for experiencing an MAYV epidemic (Lorenz et al., 2019). Moreover, in the central region, the distribution patterns of MAYV cases indicate that the virus has likely shifted vectors, or its vector has adapted to densely populated (urban) landscapes; these factors increase the likelihood of MAYV emergence within these areas (Abad-Franch et al., 2012). In this context, a study carried out in Manaus (northern Brazil) between January 2007 and December 2008 used an enzyme immunoassay of infected culture cells to identify MAYV-specific IgM antibodies in 33 patients presenting with febrile illness for at least five days. Additionally, the MAYV genome was detected in one of these patients (Mourão et al., 2012). Similarly, MAYV cases were identified during a DENV outbreak in a primarily urban city in the state of Mato Grosso (central-west Brazil) (Vieira et al., 2015), and more recently in a city in the state of Pará (northern Brazil) (Saatkamp et al., 2021). Additionally, a study in Cuiabá (Central-West Brazil) identified the MAYV genome in 15 patients by quantitative reverse transcription polymerase chain reaction (RT-qPCR) targeting non-structural protein 1 (NSP1). Sequencing and phylogenetic analysis of a 270-nucleotide (nt) fragment revealed that MAYV circulating in Cuiabá belongs to the L genotype. Notably, 12 of 15 patients were coinfecting with DENV-4 (Zuchi et al., 2014). Similar results were observed in Haiti in 2015, where MAYV/DENV-1 was co-detected in a child from a rural area (Lednicky et al., 2016) and more recently, in the predominantly urban district of Piura, in Peru, where 54 of 86 reported MAYV-cases were coinfections with DENV (Aguilar-Luis et al., 2021). Additionally, two studies performed in Cuiabá in 2019 described the vertical transmission of MAYV in *Aedes aegypti* (de Souza Costa et al., 2019; Maia et al., 2019), attributing the spread of the virus in Central-West Brazil to this urban-adapted mosquito, which is also the main DENV vector (Scott and Morrison, 2010).

MAYV surveillance in Latin America and the Caribbean region is limited (Ganjan and Riviere-Cinnamond, 2020). However, some advances were observed in Brazil; after the increase in MAYV cases outside the Amazon region, Mayaro fever became notifiable. However, the Brazilian protocol recommends that MAYV tests for patients with arbovirus-like symptoms should only be performed if the patient is negative for dengue and chikungunya and if the patient has visited rural

or wild areas in the last 15 days. Thus, the Mayaro fever load can still be underreported and underestimated due to the Brazilian recommendation, especially in urban cases.

The accumulating evidence that MAYV is increasing across urban areas suggests its potential as a public health threat in Brazil (Acosta-Ampudia et al., 2018; Esposito and da Fonseca, 2017). To evaluate whether MAYV dissemination into urban areas has also occurred in other municipalities in Central-West Brazil, we performed a prospective study between 2017 and 2018 in Goiania, the capital of the state of Goias, where the rural MAYV incidence is high. Additionally, as MAYV/DENV codetection has already been described and DENV is also prevalent in Goias, we further evaluated the occurrence of MAYV/DENV coinfection in Goiania.

## 2. Material and methods

### 2.1. Study design and eligibility

This was a cross-sectional study involving prospective data collection in the municipality of Goiania, Central-West, Brazil. All study participants were interviewed, and all biological samples were collected exclusively for the present study.

For each participant, interviews and blood samples collections were conducted using two of the six emergency care services (ECS) in primary care units with laboratory support from the Brazilian Unified Health System. The inclusion criteria were as follows: subjects  $\geq 18$  years of age, with fever ( $\geq 37.5$  °C) and/or rash, and at least two of the following symptoms: arthralgia, myalgia, and headache up to seven days prior to sample collection, in June 2017 and from January to July 2018 (periods of higher and lower incidences of dengue in the city, respectively).

To estimate the sample size, we used the "Sample Size Proportion" tool in OpenEpi (<http://www.openepi.com>). The estimated sample size was 369 patients with arbovirus-like symptoms, considering a statistical power of 80% ( $\beta = 20\%$ ), significance level of 95% ( $\alpha = 0.05$ ), design effect of 1.5, and estimated MAYV fever prevalence of 20% in Goiania City (Brunini et al., 2017). We used a convenience-type sampling procedure, selecting patients with arbovirus-like symptoms who attended two of six ECS in primary care units.

The study protocol was approved by the ethical review board of the Hospital das Clínicas of the Federal University of Goias (#66469917.1.000.5078). All study participants signed an informed consent form.

Subjects who agreed to participate were interviewed to determine the following information: age, sex, whether they were living in urban areas or near parks/protected areas, and whether they had visited any rural/sylvatic areas 15 days prior to symptom onset. Additionally, they were interviewed to determine the presence of the following symptoms: fever, skin rash, myalgia, arthralgia, headache, retro-orbital pain, prostration, maculopapular rash, pruritic rash, abdominal pain, nausea, vomiting, somnolence, irritability, oliguria, respiratory distress, cyanosis, photosensitivity, epigastric pain, back pain, asthenia, dizziness, chills, and fatigue. We also collected 10 mL blood sample from each patient using the Vacuette® Tube Serum Separator Gel (Greiner Bio-One, Kremsmünster, Austria) to detect DENV, zika virus (ZIKV), CHIKV, and MAYV.

### 2.2. Laboratory diagnostic

#### 2.2.1. Sample processing and RNA extraction

Blood samples were stored at 4 °C until centrifugation (3000  $\times$ g, 10 min) for serum preparation. Serum samples were used for RNA extraction or stored at  $-80$  °C until further analyses. Hemolysed samples were discarded.

Virus RNA extraction was performed using serum samples (200  $\mu$ L) with the Quibase Bio Gene DNA/RNA Viral Extraction Kit® (cat. # K204; Bioclin-Quibasa, Belo Horizonte, Brazil) according to the

manufacturer's instructions.

### 2.2.2. Mayaro virus RNA detection

To detect MAYV RNA, a primer set designed based on the alignment of 34 complete MAYV genomes available from GenBank was used. The alignment was performed in BioEdit (version 7.0.5.2) to generate a consensus sequence. This sequence was then used to design a primer set using the Primer-BLAST tool (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>). This primer set (forward: 5'-GACGACCTGCAGTCAGT-GAT-3'; reverse, 5'-GTCTTAAAGGCCACAGGCA-3') targeted the NSP1 gene (mapping at position 424–1349 on Mayaro isolate BeAr20290, KT754168.1), generating a 925-base pair (bp) amplicon. The specificity of the primers was tested against samples positive for CHIKV ( $n = 10$ ), DENV ( $n = 10$ ), and ZIKV ( $n = 10$ ).

Complementary DNA (cDNA) was synthesised from 1.0  $\mu$ L of RNA using M-MLV Reverse Transcriptase (cat. # M1302; Sigma-Aldrich, St. Louis, MO, USA) prior to PCR amplification.

PCR was performed using the Platinum Taq DNA Polymerase (cat. # 10966034; Thermo Fisher, MA, USA), 1.0  $\mu$ L of cDNA, 0.5  $\mu$ L 10 mM deoxynucleotides (dNTPs); 2.5  $\mu$ L of Buffer 10 $\times$  (200 nM Tris-HCl pH 8.4 and 500 mM KCl), 1.0  $\mu$ L 50 mM MgCl<sub>2</sub>, and 5.0  $\mu$ L of each primer at 2.5  $\mu$ M (final volume of 25  $\mu$ L). A Veriti Dx thermo cycler (Thermo Fisher Scientific, Waltham, MA, USA) was used (95 °C for 2 min; 35 cycles at 95 °C for 30 s, 65 °C for 1 min, and 72 °C for 2 min; and maintained at 4 °C). Amplification was evaluated by 1% agarose gel electrophoresis using a 100 bp ladder (cat. # 15628019; Thermo Fisher Scientific) and UniSafe dye (cat. # R01031; Uniscience, Sao Paulo, Brazil).

The results were confirmed by RT-qPCR using a GoTaq® Probe qPCR Master Mix kit (cat. # A6101; Promega, Madison, WI, USA) according to the manufacturer's instructions, as well as a custom-made PrimeTime (Integrated DNA Technologies, Coralville, IA, USA) generating a 107 bp fragment targeting the NSP1 gene (forward: 5'-ATA-GACGACCTGCAGTC-3'; reverse: 5'-TGATAGACTGCCACCTC-3'; probe: 5'-/56FAM/TCCTGCATGTCTGATCTGTGTAAGGC/3iABkFQ/3'). Nuclease-free water and cDNA from negative samples were used as negative controls.

The MAYV BR/SJRP/LPV01/2015 strain (GenBank accession number KT818520.1), provided by Rafael Elias Marques from the National Reference Laboratory for Arbovirus of Campinas (Unicamp), was used as a positive control.

### 2.2.3. Dengue virus RNA detection

To determine whether DENV could be detected in MAYV-positive samples, RT-qPCR (cat. # K201; Bioclin-Quibase, Belo Horizonte, Brazil) was performed to diagnose DENV according to the manufacturer's instructions.

Additionally, serotype-specific nested PCR was performed using primer sets targeting non-structural protein 5, which has been described previously (Bronzoni et al., 2004), to further characterise DENV co-detected with MAYV. In the first round, a SuperScript III One-step RT-PCR System with Platinum Taq Polymerase (cat. # 12574026; Thermo Fisher Scientific) and primers specific for the *Flavivirus* genus were used. The second round was performed with primer sets specific for each DENV serotype (1–4) and Platinum Taq DNA Polymerase (cat. # 10966034; Thermo Fisher Scientific). Amplification was evaluated by 1% agarose gel electrophoresis.

### 2.2.4. Virus sequencing

To characterise MAYV circulating in Goiania, the 11 amplicons (925 bp) for which sufficient DNA was available ( $\geq 9$  ng/ $\mu$ L) were sequenced using a BigDye™ Terminator v3.1 Cycle Sequencing kit (cat. # 4337458, Thermo Fisher Scientific) in an ABI3130 instrument (Thermo Fisher Scientific). The sequences were compared with those in the GenBank database using the Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990).

High-quality sequences (query sequences) were compared to subject sequences deposited in the NCBI GenBank database (<http://www.ncbi.nlm.gov/BLAST/>) using BLASTn. Multiple sequence alignment and editing were performed using ClustalX2 (Thompson et al., 1997) and BioEdit (version 7.0.5.2), respectively. The obtained DENV amplicons were sequenced to confirm the results of nested PCR.

### 2.2.5. Phylogenetic reconstruction

The obtained NSP1 sequences were aligned with all 71 MAYV genome sequences available on NCBI with MAFFT v7.407 (Katoh and Standley, 2013). Prior to phylogenetic analysis, a recombination scan was conducted using RDP4 (Martin et al., 2015). Next, a

maximum likelihood (ML) phylogeny was constructed using IQ-Tree v1.6.2 (Nguyen et al., 2015) under the GTR + I +  $\Gamma$ 4 model (Tavaré, 1986; Yang, 1994), as suggested by the built-in model selection algorithm (Kalyaanamoorthy et al., 2017). Ultrafast bootstrap approximation (UFBoot) and Shimodaira–Hasegawa-like approximate likelihood ratio test (SH-aLRT) were used to assess the statistical support of the clades (Guindon et al., 2010; Hoang et al., 2018).

Next, we inferred a time-scaled phylogenetic tree and performed formal phylogeographic reconstruction using BEAST v1.10.4 (Suchard et al., 2018). As our initial assessment revealed that the new sequences belonged to genotype D, we assembled a dataset composed of all sequences with available dates and those generated in this study. We re-inferred the ML tree in the same manner as described above and accessed the temporal signal of these sequences using TempEst v1.5.3 (Rambaut et al., 2016). Subsequently, three independent Markov chain Monte Carlo runs were performed using: (1) the uncorrelated relaxed clock model with a log normal rate distribution (Drummond et al., 2006), (2) the coalescent skyride tree prior (Minin et al., 2008), and (3) the GTR + I +  $\Gamma$ 4 nucleotide substitution model (Tavaré, 1986; Yang, 1994). Each chain consisted of 20 million generations sampled every 2000 steps, and all data were combined with a 10% burn-in threshold. Furthermore, a formal discrete symmetric phylogeographic model was inferred considering each country as a possible state (Lemey et al., 2009). Our dataset included sequences from Brazil ( $n = 28$ ), Peru ( $n = 11$ ), Venezuela ( $n = 7$ ), Bolivia ( $n = 6$ ), Trinidad and Tobago ( $n = 3$ ), Haiti ( $n = 3$ ), and French Guiana ( $n = 1$ ). In contrast, the genotyping tool available on Genome Detective (Vilsker et al., 2019) was used to classify DENV sequences from cases in which MAYV/DENV were co-detected.

All alignments, logs, trees, and beast xml files are provided in Appendix A.

### 2.2.6. Serological diagnosis

MAYV and CHIKV IgM detection was performed using anti-MAYV virus IgM (cat. # EI 295c-9601 M, Euroimmun, Lübeck, Germany) and anti-CHIKV virus IgM (cat. # EI 293a-9601 M, Euroimmun) enzyme-linked immunosorbent assay kits, respectively, according to the manufacturer's instructions. Briefly, serum samples were diluted 1:101 in sample diluent containing anti-human IgG and transferred (100  $\mu$ L) to a microplate coated with mixtures of MAYV and CHIKV recombinant antigens, respectively. The plate was then incubated for 1 h at 37 °C, washed, and peroxidase-labelled anti-human IgM was added. After incubation at room temperature for 30 min, the wells were washed again and reacted with chromogen-substrate solution (tetramethylbenzidine and hydrogen peroxidase) for 15 min at room temperature. The colour reaction was stopped by adding 0.5 M sulphuric acid. Optical densities were measured at 450 and 620 nm (reference wavelength) using a spectrophotometer reader (Asys Hitech GMBH, Eugendorf, Austria).

### 2.2.7. Statistical analysis

Data were analysed using Stata Statistical Software, version 14.0 (StataCorp LP, College Station, TX, USA). Initially, the Anderson-Darling test was performed to verify the normality of the quantitative variables (Razali and Wah, 2011). In descriptive analysis, the quantitative variables were presented as the median and interquartile range because of



the absence of normality in the Anderson-Darling test, and qualitative variables were presented as absolute and relative frequencies. The chi-square test ( $\chi^2$ ) or Fisher's exact test was used to compare categorical variables, and the Mann Whitney test was used for continuous variables. Statistical significance was set at  $p < 0.05$ . The spatial distribution of the patients tested was based on their place of residence, and the software used was ArcGIS (ESRI, Redlands, CA, USA) with a simple geocoding method and direct conversion of occurrences.

### 3. Results

Of the 375 tested subjects, 259 (69.1%; 95% confidence interval [CI]: 64.2–73.5) were positive for DENV, and 26 (6.9%; 95% CI: 4.8–10.0) were positive for MAYV. Of the MAYV-positive cases, nine (2.4%; 95% CI: 1.3–4.5) patients were infected only with MAYV, whereas in 17 (4.5%; 95% CI: 2.8–7.1) DENV-2 was also detected. The overall incidence of MAYV/DENV co-detection was 65.4% (95% CI: 46.2–80.6) (Table 1).

Most MAYV-infected patients were female (73.1%). The median age was 39 years (interquartile range, 29–48.8 years). Only 11.5% of MAYV-infected subjects reported living in forest areas, and none of these cases were MAYV mono-detection. Regarding travelling patterns prior to disease onset, 55.6% of patients in whom MAYV was mono-detected had visited rural/forest areas up to 15 days prior to disease onset, which was higher than the number of negative (22.7%), MAYV/DENV co-detected (23.5%), and DENV (23.0%) cases. The clinical characteristics of the arbovirus-positive patients are shown in Table S1. The main complaints of MAYV-positive patients at the time of inclusion in the study were myalgia, cephalgia, fever, arthralgia, retro-orbital pain, and skin rash (Table 1). No specific symptoms were associated with MAYV detected either alone or in combination with DENV compared to in DENV mono-detected patients.

The case distribution by epidemiological week and season is shown in Fig. 1. Most MAYV-positive cases occurred during the Brazilian wet season when the number of dengue cases was also increased. In addition, patients were from several districts in the area covered by the ECU, as shown in Fig. 2.

ML phylogeny analysis to characterise MAYV circulating in Goiania indicated that all of our samples belonged to genotype D (Fig. S1). More specifically, these viruses were grouped in a monophyletic clade sister to a sequence sampled in Peru in 1997 (Accession number: MK070491) with good statistical support (UFBoot = 95.4; SH-aLRT = 100). In contrast, support for the relationships among the new sequences was substantially low (mean UFBoot = 0; mean SH-aLRT = 67.4), as expected based on their high identity (average identity  $\geq 99\%$ ). No recombination signal was detected in the dataset. Considering these results, we evaluated the temporal signal present in the genotype D sequences by estimating the correlation between root-to-tip distances and sampling times ( $R^2 = 0.53$ ;  $p < 0.05$ ). This result was significant, and we inferred a time-stamped phylogeny and performed formal inference of the spatial diffusion phylogenetic process. The results suggested that our sequences diverged from MK070491 in around 1996 (95% highest posterior density, HPD: September 1988–August 1997) and that the most recent common ancestor of this clade was from Peru (posterior probability = 0.98; Fig. 3). Moreover, the evolutionary rate was estimated as  $1.0 \times 10^{-4}$  nucleotide substitutions per site per year (95%

**Table 1**

Laboratory diagnosis of outpatients with arbovirus-like symptoms. Goiania, Central-West Brazil.

Dengue	Mayaro		Total
	Positive	Negative	
Positive	17	259	276
Negative	9	90	99
Total	26	349	375

highest posterior density:  $1.15 \times 10^{-4}$ – $1.04 \times 10^{-4}$ ), and the age of the root was inferred as 1892 (95% highest posterior density: August 1871–July 1913), with Brazil as the inferred location (posterior probability = 0.73). For DENV found in codetection cases, serotype-specific nested PCR revealed that the virus was serotype 2. The genome detection typing tool suggested that all sequences belonged to genotype III (Southern Asian/American) of DENV-2.

### 4. Discussion

Our results showed that most MAYV cases occurred with DENV, were concentrated in the wet season (January to March) and occurred mostly among urban residents who did not visit rural areas. Recently, such occurrence of MAYV within urban settings has been described in the North Region of Brazil, Amazon biome (Mourão et al., 2012), and Central-West Region, in the “cerrado” biome (Zuchi et al., 2014; de Souza Costa et al., 2019). In contrast to the population in our study, most cases occurred in municipalities with up to 600,000 inhabitants, indicating the growing importance of introduction of the “cerrado” biome in the MAYV cycle, which can be favoured by climatic conditions, the simultaneous presence of other arboviruses epidemics, and the high abundance of urban vectors mosquito species such as *Ae. aegypti* during the rainy season (Lorenz et al., 2017). Moreover, natural infection of *Aedes* mosquitoes by MAYV has been detected by Serra et al. (2016). Therefore, our results corroborate those of previous studies identifying MAYV as a potential candidate for the next major urban arbovirus epidemic (Acosta-Ampudia et al., 2018; Esposito and da Fonseca, 2017).

We identified 26 subjects with positive RT-PCR results for MAYV, three of which were MAYV IgM-positive and none of which were CHIKV IgM-positive (data not shown). A previous study in Manaus (Northern Brazil) described 33/631 (5.2%) subjects reactive to MAYV, as indicated by IgM detection in an enzyme immunoassay of infected culture cells; only one subjected showed positive RT-PCR results for MAYV RNA (Mourão et al., 2012). This finding differs from ours, as we found that most of our cases were RT-PCR-positive for MAYV RNA despite IgM detection. This difference may be related to the time required for sample collection, as we included patients who had symptoms for up to seven days, unlike a previous study that included patients with up to five days of symptoms (Mourão et al., 2012).

In contrast to the study from Manaus, performed before the introduction of CHIKV in Brazil (Nunes et al., 2015), our survey was performed when CHIKV was already circulating in the country. Notably, MAYV fever is easily mistaken for CHIKV and DENV (de Mota et al., 2015), and cross-reactivity is observed between CHIKV and MAYV, as they are both members of the Semliki Forest Antigenic Serocomplex (Caglioti et al., 2013; Martins et al., 2019). Consequently, serological tests are not specific enough to differentiate between viruses, and molecular diagnosis appears to be the best choice for accurately identifying MAYV. In fact, all 375 subjects included in this study were negative for CHIKV according to RT-qPCR targeting CHIKV (data not shown). However, the molecular detection of viruses is limited to the acute infection period when viremia is high. In this regard, the MAYV incidence described here is similar to that reported in other cities, such as Manaus (5.2%) and Cuiabá (2.5%), in which MAYV urbanisation is suspected (Mourão et al., 2012; Zuchi et al., 2014); however, the incidence is lower than that determined in a serological survey performed in a rural area adjacent to Goiania (20%) (Brunini et al., 2017). Therefore, we cannot rule out that our study design may have resulted in underestimation of the MAYV incidence within the Goiania municipality, as MAYV viremia is typically short (Esposito and da Fonseca, 2017; Napoleão-Pego et al., 2014). However, molecular diagnosis is specific and supports the aims of our study.

Interestingly, both this study and that from Cuiabá showed similarly high rates of MAYV and DENV codetection (Zuchi et al., 2014). Whereas a study from 2011/2012 reported that MAYV was coinfecting with DENV-4, herein DENV-2 serotype was codetected with MAYV. These

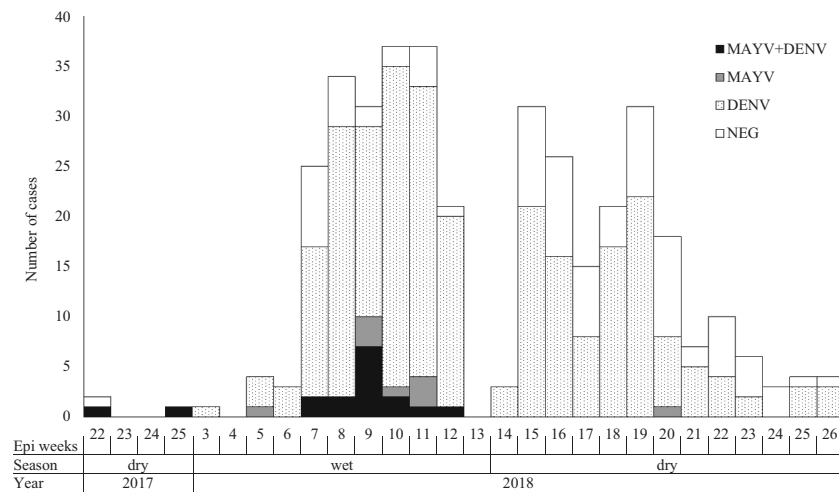


Fig. 1. Distribution of detection patients with arbovirus-like symptoms by laboratorial diagnosis and epidemiological week. Goiania, Goiás, Central-West Brazil, 2019.

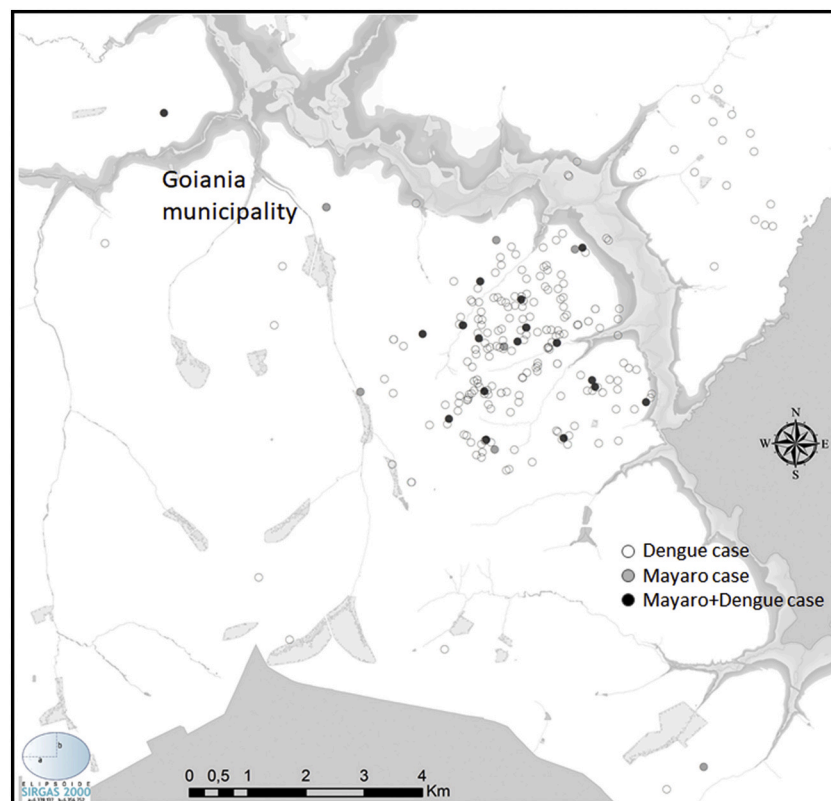
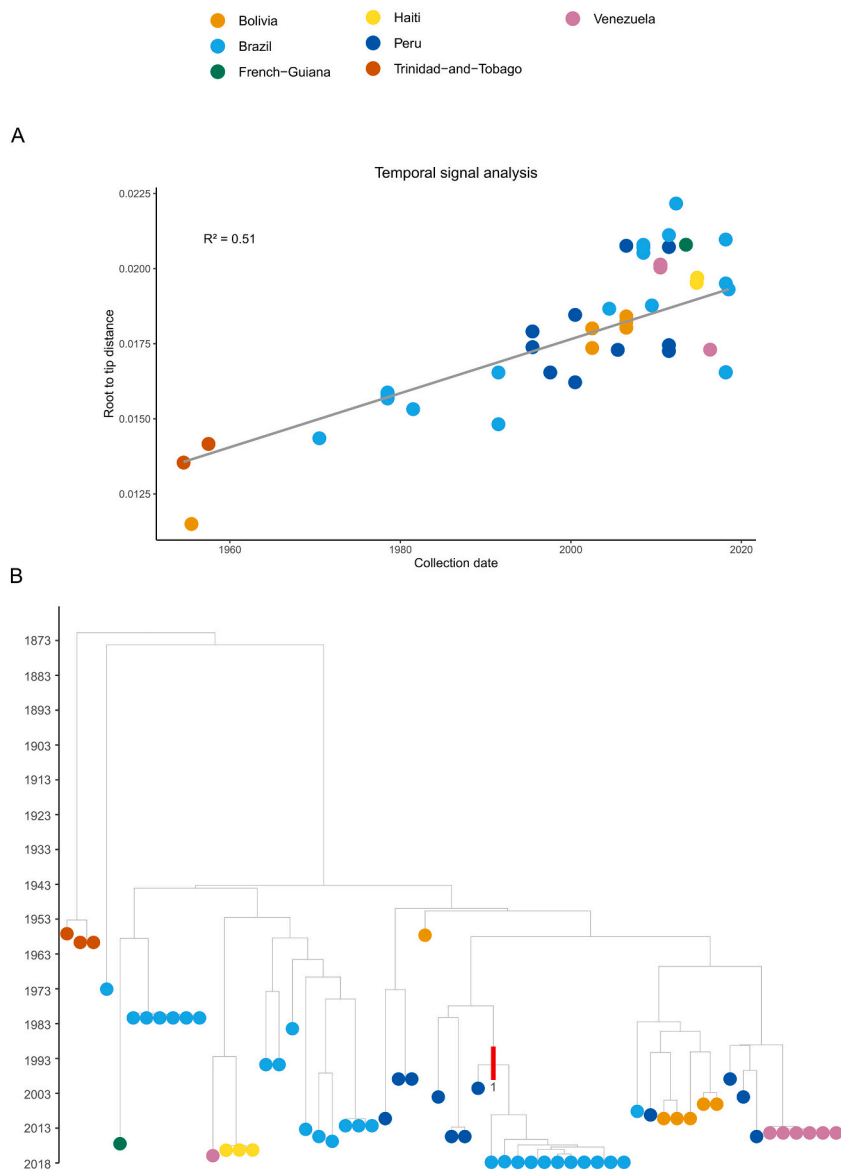


Fig. 2. Spatial distribution of detection patients with arbovirus-like symptoms by laboratorial diagnosis. Goiania, Goiás, Central-West Brazil, 2019.

findings agree with epidemiological data showing that DENV-4 was the predominant serotype during the former MAYV outbreak, whereas DENV-2 was the predominant serotype during the latter (Brazil, 2018). Notably, in contrast to the study from Cuiabá, which observed circulation of MAYV genotype L (de Souza Costa et al., 2019), we identified MAYV genotype D circulating in Goiania. Specifically, our sequences were in a monophyletic clade sister to the IQT 4235 strain from Iquitos (Peru). Interestingly, a study comparing the transmission efficiency of two MAYV strains, TRVL 4675 (from Trinidad) and IQT 4235 (from Peru), to which the isolates from Goiania are sisters, in two laboratory-adapted *Ae. aegypti* lineages, showed that both are transmitted. However, the IQT 4235 strain was more efficiently transmitted than the

TRVL 4675 strain, as revealed by evaluation of the virus titre in head tissue and saliva by plaque assay (Kantor et al., 2019). Our data brings further evidence that MAYV might have switched vectors, corroborating those of previous reports for the natural transmission of MAYV in *Ae. aegypti* (de Souza Costa et al., 2019; Maia et al., 2019), and the increasing number of reports of MAYV cases co-detected with DENV, both in rural and urban settings (Zuchi et al., 2014; Lednicky et al., 2016; de Souza Costa et al., 2019; Aguilar-Luis et al., 2021), reinforcing that a shift occurred in the MAYV sylvatic transmission cycle towards urban transmission. Nonetheless, further entomological surveillance should be performed to confirm this hypothesis.

Our phylogeographic reconstruction suggests that MAYV strains



**Fig. 3.** Time scaled phylogenetic reconstruction of selected Mayaro virus genome sequences. Prior to temporal analysis, a maximum likelihood tree was inferred with a selected set of samples to estimate the temporal information contained within MAYV genomes. For this purpose, a root to tip distances plot was performed, revealing reasonable correlation ( $R^2 = 0.52$ ) between sequences collection dates and genetic distances (A). A time scaled phylogenetic tree was then inferred on a fully Bayesian framework, that integrates over all parameters uncertainty, joint to a discrete symmetric phylogeographic model (B). This estimate supports that the isolates herein described cluster in a monophyletic clade, which diverged from the most closely related sequence from Peru between 1988 and 1997 (Posterior probability = 1; 95% HPD marked in the red bar). Colour codes on tree tips mark sequences country of origin. Despite this estimate, the sparse availability of MAYV genetic data hampers the interpretation of the inferred model, so no direct conclusion on phylogeographic dispersion patterns can be drawn. Notwithstanding, this model supports that the Goiânia outbreak viruses are not related to more recently sampled MAYV strains circulating in Brazil, suggesting a cryptic pattern of viral circulation may exist across South America, as observed for Dengue virus. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

from Goiania diverged from MK070491 in Peru between 1988 and 1997. However, given the scarcity of MAYV sequences in public databases, we could not confirm the epidemiological link between these locations, and thus our results may be considered as speculative. Further studies aimed at identification and sequencing of heterochronous MAYV samples from distinct geographic regions should re-evaluate the spatial spread history reported here. Additionally, increased sampling efforts for MAYV are required for more precise phylogeographic analyses.

Finally, the clinical diagnosis of MAYV fever is not specific, as the symptoms are similar to those of other mosquito-borne diseases such as DENV and CHIKV fever (de Mota et al., 2015). Our data corroborate these observations, as symptoms presented by MAYV-infected patients did not differ from those of patients negative for this virus. Nonetheless, a high rate of MAYV/DENV-2 coinfection was observed, providing a new layer of complexity for the clinical diagnosis of MAYV, as the symptoms likely overlap.

## 5. Conclusion

Herein, we describe MAYV cases occurring within the urban area of Goiania city, reinforcing the urgency of including MAYV in the

differential diagnosis of arboviruses in such areas, as well as the endemic rural/sylvatic areas nearby. These cases were identified among patients with dengue-like symptoms and were oligosymptomatic, suggesting that they would otherwise not be reported to the epidemiological surveillance system as MAYV cases. Therefore, it is important to develop sensitive and specific molecular and serological tests that can discriminate different arboviruses occurring in the same area. In addition, the high rate of MAYV/DENV codetection indicates that MAYV diagnosis should be performed in DENV-positive subjects outside of the endemic area, as DENV/MAYV co-transmission may occur. These findings agree with previous data from other regions in Brazil, indicating that MAYV has invaded urban areas, possibly by shifting vector mosquito species. Despite being important for inferring the origin of the epidemic described herein, our phylogenetic analysis may be limited by the current low number of MAYV sequences available, indicating that MAYV circulating in Goiania City belongs to genotype D, and that it diverged from the Peruvian strain IQT 4235 in around 1996. Although we cannot assure an epidemiological link between these regions of South America, further studies with larger sample numbers collected at different times and locations will allow for better characterisation of these complex transmission patterns.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.meegid.2021.104981>.

## Author Statement

**Elisângela de Paula Silveira-Lacerda:** Investigation, Writing-Original Draft, Funding acquisition; **Alice Laschuk Herlinger:** Investigation, Methodology, Formal analysis, Writing-Original Draft, Writing-Review & Editing; **Amilcar Tanuri:** Formal Analysis, Writing-Original Draft, Writing-Review & Editing, Funding acquisition; **Giovanni Rezza:** Formal analysis, Writing- Review & Editing; **Carlos Eduardo Anunciação:** Investigation; **Juliana Pires Ribeiro:** Investigation, Formal Analysis; **Isabele Pereira Tannous:** Investigation, Writing – Original Draft; **Gabrielly Regis Abrantes:** Investigation; **Elisângela Gomes da Silva:** Investigation; **Kauana F. Arruda,** Writing – Review & Editing; **Adriano Roberto Vieira de Sousa,** Investigation; **Filipe Romero Rebelo Moreira:** Investigation, Formal Analysis, Writing - Original Draft, Writing – Review & Editing; **Renato Santana Aguiar:** Formal analysis, Writing – Review & Editing; **Jordana Faria Corrêa:** Investigation; **Marina Machado dos Santos:** Investigation; **Hugo Delleon Silva,** Investigation; **Marco Tulio A Garcia-Zapata:** Investigation; **Natália Santana do Nascimento:** Investigation; **Mariane Talon de Menezes:** Investigation; **Richard Araujo Maia:** Investigation; **Orlando C. Ferreira:** Formal Analysis, Writing –Review & Editing; **Rafael Barbosa Pinto:** Investigation; **Rodrigo Brindeiro,** Writing- Review & Editing, Funding Acquisition; **Cleber Cardoso,** Investigation; **Sandra Maria Brunini,** Investigation, Data curation, Conceptualization, Writing – Review & Editing, Funding Acquisition.

## Funding

This work was supported by the Financiadora de Estudos e Projetos; Fundo Nacional de Desenvolvimento Tecnológico (FINEP/FNDCT) n° 01/2016 – Zika (Quantizika); Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro; Conselho Nacional de Desenvolvimento Científico e Tecnológico [grant number 440900/2016–6] and CAPES [grant number 88881.130757/2016–1]. GRA; ARVS; EGS; JFC; IPT are fellows from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

## Declaration of Competing Interest

The authors have no conflicts of interest to declare.

## Acknowledgments

The authors would like to acknowledge the dedicated efforts of the team of the Municipal Health Department of Goiania for conducting this research. Particularly, we thank Frederico Ferreira Costa of the Final Systems Management (SEDETEC – Goiania/GO) and Ruth Minamisava (UFG) for their contributions to construction of the spatial distribution graph. We also thank Rafael Elias Marques from the National Reference Laboratory for Arbovirus of Campinas for providing the MAYV strain, and Harrison James Westgarth (UFRJ) for English language review. We would also like to thank all volunteers and researchers involved in this study.

## References

Abad-Franch, F., Grimmer, G.H., de Paula, V.S., Figueiredo, L.T.M., Braga, W.S.M., Luz, S.L.B., 2012. Mayaro virus infection in Amazonia: A multimodel inference approach to risk factor assessment. *PLoS Negl. Trop. Dis.* 6, e1846 <https://doi.org/10.1371/journal.pntd.0001846>.

Acosta-Ampudia, Y., Monsalve, D.M., Rodríguez, Y., Pacheco, Y., Anaya, J.-M., Ramírez-Santana, C., 2018. Mayaro: an emerging viral threat? *Emerg. Microbes Infect.* 7, 163–173. <https://doi.org/10.1038/s41426-018-0163-5>.

Aguiar-Luis, M.A., Del Valle-Mendoza, J., Sandoval, I., Silva-Caso, W., Mazulis, F., Carrillo-Ng, H., Tarazona-Castro, Y., Martins-Luna, J., Aquino-Ortega, R., Peña-Tuesta, I., Comejo-Tapia, A., Del Valle, L.J., 2021. A silent public health threat:

emergence of Mayaro virus and co-infection with Dengue in Peru. *BMC Res. Notes* 14 (1), 29. <https://doi.org/10.1186/s13104-021-05444-8>.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. *J. Mol. Biol.* 215 (3), 403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2).

Anderson, C.R., Wattley, G.H., Ahin, N.W., Downs, W.G., Reese, A.A., 1957. Mayaro virus: a new human disease agent. *Am. J. Trop. Med. Hyg.* 6, 1012–1016. <https://doi.org/10.4269/ajtmh.1957.6.1012>.

Auguste, A.J., Liria, J., Forrester, N.L., Giambalvo, D., Moncada, M., Long, K.C., Morón, D., de Manzione, N., Tesh, R.B., Halsey, E.S., Kochel, T.J., Hernandez, R., Navarro, J.-C., Weaver, S.C., 2015. Evolutionary and ecological characterization of Mayaro virus strains isolated during an outbreak, Venezuela, 2010. *Emerg. Infect. Dis.* 21, 1742–1750. <https://doi.org/10.3201/eid2110.141660>.

Brazil, 2018. Ministério da Saúde. Secretaria de Vigilância em Saúde Boletim Epidemiológico [WWW Document]. vol. 49, n. 49, Nov. URL <http://portalarquivos2.saude.gov.br/images/pdf/2018/novembro/13/boletim-epidemiologico.pdf> (accessed 6.18.19).

Bronzoni, R.V.M., Moreli, M.L., Cruz, A.C.R., Figueiredo, L.T.M., 2004. Multiplex nested PCR for Brazilian Alphavirus diagnosis. *Trans. R. Soc. Trop. Med. Hyg.* 98, 456–461. <https://doi.org/10.1016/j.trstmh.2003.09.002>.

Brunini, S., França, D.D.S., Silva, J.B., Silva, L.N., Silva, F.P.A., Spadoni, M., Rezza, G., 2017. High frequency of Mayaro virus IgM among febrile patients, Central Brazil. *Emerg. Infect. Dis.* 23, 1025–1026. <https://doi.org/10.3201/eid2306.160929>.

Caglioti, C., Lalle, E., Castilletti, C., Carletti, F., Capobianchi, M.R., Bordin, L., 2013. Chikungunya virus infection: an overview. *New Microbiol.* 36 (3), 211–227.

Causey, O.R., Maroja, O.M., 1957. Mayaro virus: a new human disease agent. III. Investigation of an epidemic of acute febrile illness on the river Guama in Pará, Brazil, and isolation of Mayaro virus as causative agent. *Am. J. Trop. Med. Hyg.* 6, 1017–1023.

de Mota, M.T.O., Ribeiro, M.R., Vedovello, D., Nogueira, M.L., 2015. Mayaro virus: a neglected arbovirus of the Americas. *Futur. Virol.* 10, 1109–1122. <https://doi.org/10.2217/fvl.15.76>.

de Souza Costa, M.C., Siqueira Maia, L.M., Costa de Souza, V., Gonzaga, A.M., Correa de Azevedo, V., Ramos Martins, L., Chavez Pavoni, J.H., Gomes Naveca, F., Dezengrini Shlessarenko, R., 2019. Arbovirus investigation in patients from Mato Grosso during Zika and Chikungunya virus introduction in Brazil, 2015–2016. *Acta Trop.* 190, 395–402. <https://doi.org/10.1016/j.actatropica.2018.12.019>.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biol.* 4, 699–710. <https://doi.org/10.1371/journal.pbio.0040088>.

Esposito, D.L.A., da Fonseca, B.A.L., 2017. Will Mayaro virus be responsible for the next outbreak of an arthropod-borne virus in Brazil? *Brazilian J. Infect. Dis. Ther.* 21, 540–544. <https://doi.org/10.1016/j.bjid.2017.06.002>.

Ganjian, N., Riviere-Cinnamond, A., 2020. Mayaro virus in Latin America and the Caribbean. *Rev. Panam. Salud Publica* 44. <https://doi.org/10.26633/RPSP.2020.14.e14>.

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst. Biol.* 59, 307–321. <https://doi.org/10.1093/sysbio/syq010>.

Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q., Vinh, L.S., 2018. UFBoot2: improving the ultrafast bootstrap approximation. *Mol. Biol. Evol.* 35, 518–522. <https://doi.org/10.5281/zenodo.854445>.

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A., Jermin, L.S., 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat. Methods* 14, 587–589. <https://doi.org/10.1038/nmeth.4285>.

Kantor, A.M., Lin, J., Wang, A., Thompson, D.C., Franz, A.W.E., 2019. Infection pattern of Mayaro virus in *Aedes aegypti* (Diptera: Culicidae) and transmission potential of the virus in mixed infections with chikungunya virus. *J. Med. Entomol.* 56, 832–843. <https://doi.org/10.1093/jme/tjy241>.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* 30, 772–780. <https://doi.org/10.1093/molbev/mst010>.

Lednický, J., Beau De Rochars, V.M., Elbadry, M., Loeb, J., Telisma, T., Chavannes, S., Anilis, G., Cella, E., Ciccozzi, M., Okech, B., Salemi, M., Morris, J.G., 2016. Mayaro virus in child with acute febrile illness, Haiti, 2015. *Emerg. Infect. Dis.* 22, 2000–2002. <https://doi.org/10.3201/eid2211.161015>.

Lemey, P., Rambaut, A., Drummond, A.J., Suchard, M.A., 2009. Bayesian phylogeography finds its roots. *PLoS Comput. Biol.* 5 <https://doi.org/10.1371/journal.pcbi.1000520>.

Lorenz, C., Azevedo, T.S., Virginio, F., Aguiar, B.S., Chiaravalloti-Neto, F., Suesdek, L., 2017. Impact of environmental factors on neglected emerging arboviral diseases. *PLoS Negl Trop Dis* 11 (9). <https://doi.org/10.1371/journal.pntd.0005959>.

Lorenz, C., Freitas Ribeiro, A., Chiaravalloti-Neto, F., 2019. Mayaro virus distribution in South America. *Acta Trop.* 198, 105093. <https://doi.org/10.1016/j.actatropica.2019.105093>.

Maia, L.M.S., Bezerra, M.C.F., Costa, M.C.S., Souza, E.M., Oliveira, M.E.B., Ribeiro, A.L.M., Miyazaki, R.D., Shlessarenko, R.D., 2019. Natural vertical infection by dengue virus serotype 4, Zika virus and Mayaro virus in *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia) albopictus*. *Med. Vet. Entomol.* 33, 437–442. <https://doi.org/10.1111/mve.12369>.

Martin, D.P., Murrell, B., Golden, M., Khoosal, A., Muhire, B., 2015. RDP4: Detection and analysis of recombination patterns in virus genomes. *Virus Evol* 1 (1), <https://doi.org/10.1093/ve/vev003>.

Martins, K., Gregory, M., Valdez, S., Sprague, T., Encinales, L., Pacheco, N., Cure-Cure, C., Porras-Ramirez, A., Mendoza, A., Chang, A.L., Pitt, M., Nasar, F., 2019.



- Neutralizing antibodies from convalescent chikungunya virus patients can cross-neutralize Mayaro and Una viruses. *Am. J. Trop. Med. Hyg.* 100 (6), 1541–1544. <https://doi.org/10.4269/ajtmh.18-0756>.
- Mavian, C., Rife, B.D., Dollar, J.J., Cella, E., Ciccozzi, M., Prosperi, M.C.F., Lednicky, J., Morris, J.G., Capua, I., Salemi, M., 2017. Emergence of recombinant Mayaro virus strains from the Amazon basin. *Sci. Rep.* 7 (1), 8718. <https://doi.org/10.1038/s41598-017-07152-5>.
- Minin, V.N., Bloomquist, E.W., Suchard, M.A., 2008. Smooth skyride through a rough skyline: Bayesian coalescent-based inference of population dynamics. *Mol. Biol. Evol.* 25, 1459–1471. <https://doi.org/10.1093/molbev/msn090>.
- Mourão, M.P.G., Bastos, M.D.S., De Figueiredo, R.P., Gimaque, J.B.L., Dos Santos Galusso, E., Kramer, V.M., De Oliveira, C.M.C., Naveca, F.G., Figueiredo, L.T.M., 2012. Mayaro fever in the city of Manaus, Brazil, 2007–2008. *Vector-Borne Zoo Dis.* 12, 42–46. <https://doi.org/10.1089/vbz.2011.0669>.
- Napoleão-Pego, P., Gomes, L.P., Procvance-Jr, D.W., De-Simone, S.G., 2014. Mayaro virus disease. *J. Hum. Virol. Retrovirol.* 1 (3), 00018. <https://doi.org/10.15406/jhvr.2014.01.00018>.
- Nguyen, L.T., Schmidt, H.A., Von Haeseler, A., Minh, B.Q., 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol.* 32, 268–274. <https://doi.org/10.1093/molbev/msu300>.
- Nunes, M.R.T., Faria, N.R., de Vasconcelos, J.M., Golding, N., Kraemer, M.U.G., de Oliveira, L.F., da Silva Azevedo, R. do S., da Silva, D.E.A., da Silva, E.V.P., da Silva, S.P., Carvalho, V.L., Coelho, G.E., Cruz, A.C.R., Rodrigues, S.G., da Silva Gonçalves Vianez, J.L., Nunes, B.T.D., Cardoso, J.F., Tesh, R.B., Hay, S.I., Pybus, O. G., da Costa Vasconcelos, P.F., 2015. Emergence and potential for spread of Chikungunya virus in Brazil. *BMC Med.* 13, 102. <https://doi.org/10.1186/s12916-015-0348-x>.
- Rambaut, A., Lam, T.T., Max Carvalho, L., Pybus, O.G., 2016. Exploring the temporal structure of heterochronous sequences using TempEst (formerly Path-O-Gen). *Virus Evol.* 2 <https://doi.org/10.1093/ve/vew007>.
- Razali, N.M., Wah, Y.B., 2011. Power comparisons of Shapiro-Wilk, Kolmogorov-Smirnov, Lilliefors and Anderson-Darling tests. *J. Stat. Model. Anal.* 2, 21–33. <https://doi.org/10.1515/bile-2015-0008>.
- Saatkamp, C.J., Rodrigues, L., Pereira, A., Coelho, J.A., Marques, R., Souza, V.C., Nascimento, V., Saatkamp, J., Naveca, F.G., Figueiredo, R., 2021. Mayaro virus detection in the western region of Pará state, Brazil. *Rev. Soc. Bras. Med. Trop.* 54, e0055–e2020. <https://doi.org/10.1590/0037-8682-0055-2020>.
- Scott, T.W., Morrison, A.C., 2010. Vector dynamics and transmission of dengue virus: implications for dengue surveillance and prevention strategies. *Curr. Top. Microbiol. Immunol.* 338, 115–128. [https://doi.org/10.1007/978-3-642-02215-9\\_9](https://doi.org/10.1007/978-3-642-02215-9_9).
- Serra, O.P., Cardoso, B.F., Ribeiro, A.L.M., Santos, F.A.L., Silhessarenko, R.D., 2016. Mayaro virus and dengue virus 1 and 4 natural infection in culicids from Cuiabá, state of Mato Grosso, Brazil. *Mem. Inst. Oswaldo Cruz* 111 (11). <https://doi.org/10.1590/0074-02760150270>.
- Suchard, M.A., Lemey, P., Baele, G., Ayres, D.L., Drummond, A.J., Rambaut, A., 2018. Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evol.* 4, 1–5. <https://doi.org/10.1093/ve/vey016>.
- Tavaré, S., 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. *Am. Math. Soc. Lect. Math. Life Sci.* 17, 57–86.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 25 (24), 4876–4882. <https://doi.org/10.1093/nar/25.24.4876>.
- Vieira, C.J., Silva, D.J., Barreto, E.S., Siqueira, C.E., Colombo, T.E., Ozanic, K., Schmidt, D.J., Drummond, B.P., Mondini, A., Nogueira, M.L., Bronzoni, R.V., 2015. Detection of Mayaro virus infections during a dengue outbreak in Mato Grosso, Brazil. *Acta Trop.* 147, 12–16. <https://doi.org/10.1016/j.actatropica.2015.03.020>.
- Vilsker, M., Moosa, Y., Nooij, S., Fonseca, V., Ghysens, Y., Dumon, K., Pauwels, R., Alcantara, L.C., Vanden Eynden, E., Vandamme, A.M., Deforche, K., De Oliveira, T., 2019. Genome detective: an automated system for virus identification from high-throughput sequencing data. *Bioinformatics* 35, 871–873. <https://doi.org/10.1093/bioinformatics/bty695>.
- Yang, Z., 1994. Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. *J. Mol. Evol.* 39, 306–314. <https://doi.org/10.1007/BF00160154>.
- Zuchi, N., da Heinen, L.B.S., dos Santos, M.A.M., Pereira, F.C., Silhessarenko, R.D., 2014. Molecular detection of Mayaro virus during a dengue outbreak in the state of Mato Grosso, Central-West Brazil. *Mem. Inst. Oswaldo Cruz* 109, 820–823. <https://doi.org/10.1590/0074-0276140108>.