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Research Paper

Spread of two Zika virus lineages in Midwest Brazil

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ABSTRACT

Zika virus (ZIKV) has been intensively studied in South America and across the globe since 2015–2016 epidemics. However, in Brazil - the largest and the most affected country in terms of human infection by this virus, most of the viral molecular information is restricted to metropolitan centers distributed along the Brazilian coast and almost no information is known about the virus spread in most difficult access areas such as the Midwest region of the country. Here, we report two ZIKV complete genomes from samples obtained during arboviral surveillance at the Sinop city, southern border of the Amazonian forest, Midwest Brazil in 2015. Our results show that the virus was introduced in this region through two independent introductions: one occurred at the end of 2014, around the period that the virus was already distributed in other regions of the country and abroad, and a second at the end of 2015. Moreover, these genomes were clustered with other viral strains sampled at distant Brazilian states in line with other findings about the rapid spread of the virus throughout the country.

1. Introduction

Zika virus (ZIKV) is an arthropod-borne virus with a positive-sense single-stranded RNA genome. This virus belongs to the *Flaviviridae* family, *Flavivirus* genus, and has recently emerged as one of the most serious global public health threats (Baud et al., 2017; Musso and Gubler, 2016). Symptoms are usually mild and up to 80% of ZIKV infections in humans can be asymptomatic (Haby et al., 2018). However, ZIKV has been associated to neurological complications such as congenital Zika syndrome in newborns, Guillain-Barre syndrome and other neurological complications in adults (Depoux et al., 2018; Krauer et al., 2017; Miner and Diamond, 2017). The spread of ZIKV represents an additional challenge for public health systems, particularly because it can be transmitted by species from *Aedes* (*Ae. aegypti* and *Ae. albopictus*) (Wikan and Smith, 2016) and *Culex* (*Cx. quinquefasciatus*) (Guedes et al., 2017; Smartt et al., 2018) genera, which are abundant throughout tropical and subtropical regions (Guo et al., 2016; Kindhauser et al., 2016).

ZIKV was serendipitously discovered during a Yellow Fever surveillance project in Uganda in 1947 (Dick et al., 1952). Very few human infection cases were reported until a large outbreak occurred in the Micronesia and Pacific Yep Island (Duffy et al., 2009). Such outbreak was followed by its spread to other Pacific islands like French Polynesia, New Caledonia and Vanuatu in 2013–2014 and Eastern Island in early 2014 (Tognarelli et al., 2016). A recent study pointed that French Polynesia was the most probable source location of the ZIKV entering Americas, from where its spread through two simultaneous introductions to Americas and to other Pacific and Eastern islands (Delatorre et al., 2018). The virus then spread to the Americas entering through Central or South America (Campos et al., 2018) reaching the northeast of Brazil which was the epicenter of the Zika epidemics (Faria et al., 2017; Faria et al., 2016a; Thézé et al., 2018). Furthermore, Angola and Cape Verde have reported Zika outbreaks, which were associated with an increase in the microcephaly reported cases nationally (Kraemer et al., 2017; Lourenço et al., 2018). In Angola, it was later confirmed to be caused by the emergence of the ZIKV Asian lineage (Hill et al., 2019).

ZIKV has been intensively studied since the 2015 epidemics in South America (Campos et al., 2018; Faria et al., 2017; Lowe et al., 2018) but up to now there is a lack of available molecular genomic data from regions of difficult access in Brazil (Faria et al., 2017). Such data could shed light on the virus spread to other highly affected areas. Currently, there are several complete or draft genomes of ZIKV from three out of five Brazilian regions (North, Northeast and Southeast) with a higher

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concentration of data from the Northeast (the Zika epidemics epicenter) followed by the Southeast region (the largest populated and richest region of the country) while almost no information exists from the Midwest and South regions (https://www.viprbrc.org/ accessed 10 June 2019).

The study of full-length viral genomic sequences of clinical isolates during epidemic and inter epidemic situations can be used to improve our understanding of molecular epidemiology and virus evolution since it provides wealthy information about the virus variability and enough evolutionary markers that allows more robust evolutionary inferences than viral fragments normally used in arboviruses evolutionary studies (Gardy and Loman, 2018; Ladner et al., 2019). We report here a genomic investigation and phylogenetic study based on complete ZIKV genomes from patients living in Sinop, a city located in the southern border of the Amazonian forest, in the state of Mato Grosso, Midwest region of Brazil.

2. Material and methods

2.1. Sample collection

Serum samples were collected from 63 patients presenting clinical symptoms of dengue-like infection, during an arboviral surveillance from December 2015 to February 2016, in Sinop, State of Mato Grosso, Brazil. Samples collection and research use was approved by the Ethics Committee from Júlio Müller University Hospital – UFMT (288.172/2013). At the time of blood collection, patients filled out and signed an informed consent document.

2.2. Laboratory testing

Total RNA was extracted from 140 µL of patients' sera using QIAmp viral RNA kit, followed by a reverse transcription using random primer, and multiplex-nested RT-PCR for flaviviruses (dengue virus (DENV) 1–4, and ZIKV) and alphaviruses (mayaro virus, chikungunya virus (CHIKV), Eastern equine encephalitis virus, Venezuelan equine encephalitis virus, Western equine encephalitis virus and Aura virus) using specific primers targeting NS5 and nsP1 regions, respectively (de Morais Bronzoni et al., 2005). Briefly, we designed a new primer for ZIKV (CACTGGCCTCCTAGGCCCGTCCAT) and add it to the multiplex-nested RT-PCR for flaviviruses, along with DENV 1–4. We add CHIKV primer (TAGAGCAGGAAATTGATCCC) previously designed (Hasebe et al., 2002) to the multiplex-nested RT-PCR for alphaviruses. To confirm our results, ZIKV PCR-positive samples, were also amplified by quantitative RT PCR (RT-qPCR) using primers, probes, and cycling conditions for ZIKV detection as recommended by the Centers for Disease Control and Prevention (Lanciotti et al., 2008).

2.3. Genome sequencing

We used primers designed by the ZIBRA project (Faria et al., 2016b) following as described (Guedes et al., 2017) on the cDNA generated from the total RNA extracted directly from each sample. MiSeq (Illumina, San Diego, CA, USA) sequencing libraries were prepared with a Nextera XT Library Prep Kit (Illumina) using 2 ng of input cDNA derived from the ZIKV multiplex PCR, following manufacturer's instructions. Two samples were selected on the basis of DNA concentration after clean-up. MiSeq Reagent Kit V3 of 150 cycles (Illumina) was used in a paired-end strategy, resulting in 75 bp reads separated by ~350 bp. Samples were sequenced on the MiSeq (Illumina) platform at the Technological Platform Core at the Aggeu Magalhães Institute (IAM).

Trimming of primers sequences and quality filtering of reads were performed with Trimmomatic v 0.36 (Bolger et al., 2014) and quality checked with FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/ accessed 10 June 2019). These were subsequently mapped against the ZIKV BRPE243/2015 reference genome (KX197192) (Donald et al., 2016) using Bowtie 2 with default parameters (Langmead and Salzberg, 2012). Consensus sequences were obtained through the Integrated Genome Viewer software (Robinson et al., 2011) and the sequences have been deposited in GenBank with the accession number: MH513598 and MH513599 and the raw NGS reads in the European Nucleotide Archive under the project PRJEB30828.

2.4. Phylogenetic analyses

Multiple sequence alignment was performed using MAFFT version 7 (Katoh et al., 2017) through the MAFFT online server http://mafft.cbrc.jp/alignment/software/ (accessed 10 June 2019); maximum-likelihood analysis (ML) was performed using PhyML version 3.0 (Guindon et al., 2010) through the web site at http://www.atgc-montpellier.fr/phyml/ (accessed 10 June 2019). Coding regions corresponding to the complete genomes from Sinop were aligned with all published and available near-complete Zika virus genomes (> 9000 nucleotides), belonging to the African and Asian genotype, totalizing 408 genomes, collected from ViPR (Pickett et al., 2012) http://www.viprbrc.org/ (accessed 10 June 2019) (accession number of all genomes used in the analysis may be seen in Supplementary Table 1). Considering that ZIKV in the Americas belongs to the Asian genotype (Faria et al., 2016a), we have also performed phylogenetic analysis using only genomes belonging to Asian genotype. The ML phylogenies was reconstructed by using the best-fit general time-reversible (GTR) model with 4 gamma substitution rate categories (+ G) and invariant sites (+ I) (GTR + G + I) suggested by the Smart Model Selection implemented in PhyML online (Lefort et al., 2017); nucleotide substitution models were also accessed with jModelTest2 (Darriba et al., 2012) and the same (GTR + G + I) model was obtained for all alignment tested; for the tree search operation, we used SPR and statistical support for phylogenetic nodes were assessed by using aLRT SH-like metric. FigTree v1.4.2 (Rambaut, 2014) was used for visualization and figure generation.

2.5. Molecular clock analysis

We evaluated the clock signal with TempEst 1.5 (Rambaut et al., 2016) using as input the ML tree from a smaller set of ZIKV genomes (73 ZIKV genomes - Supplementary Table 1 and Supplementary Fig. 1). Bayesian molecular clock phylogenetic analysis was performed with BEAST 1.10.4 (Suchard et al., 2018) in three independent runs of 100 million MCMC steps and sampling parameter every 1000 steps. Ten percent of the sampled trees was removed to generate the final bayesian trees dataset. We used a Bayesian Skyline tree prior and a relaxed lognormal clock model. Molecular clock and demographic model were selected based on the best likelihood combination found with Path Sampling/Stepping-stone sampling comparing strict and uncorrelated lognormal clock models associated with three demographic models: Constant, Exponential Grown and Bayesian Skyline priors (Supplementary Table 2). Runs convergence was evaluated with Tracer 1.7.1 (Rambaut et al., 2018) and the final combined dataset showed an ESS > 200 for all parameters sampled. In order to evaluate if recombination took place between the analyzed genomes we performed the Pairwise Homoplasy Index (PHI) using the SplitsTree 4.10 (Bruen et al., 2006) with default parameters and the full set of tests implemented in the Recombination Detection Program - RDP v4.97 (Martin et al., 2015).

2.6. Spatial analysis

The data collected from ViPR (http://www.viprbrc.org/ accessed 10 June 2019) was integrated into an open source Geographic Information System (QGIS v2.18.24) software (http://www.qgis.org accessed 10 June 2019). The map showing the geographic distribution of ZIKV in Brazil and the virus possible route was developed at the state level.

Table 1
Sequencing data of two Zika viruses obtained from patients living in Sinop, MT, 2015.

Isolate ID	Collection date	ZIKV qRT-PCR Ct[a]	Genbank accession code	No. total reads[b]	No. mapped reads (%)[b]	Depth of coverage[b]	Genome length (nt)[b]	Amino acid mutations and protein location[c]
H355	09/12/2015	34.00	MH513598	676,945	40,478 (5.98%)	280.92	10,640	M1143 V NS1 I1398V NS2B T2068 M NS3 S2456 L NS4B
H366	11/12/2015	26.84	MH513599	1,454,943	1,337,597 (91.93%)	9282.85	10,658	M1143 V NS1

Depth of coverage = Number of mapped reads × 75 (read length)/reference genome length (10,807).
[a] Ct, cycle threshold; qRT-PCR, quantitative reverse transcription PCR.
[b] Genomic sequencing statistics (%) were calculated using KX197192 (10,807 nt long) as reference genome.
[c] Positions considering the reference genome polyprotein.

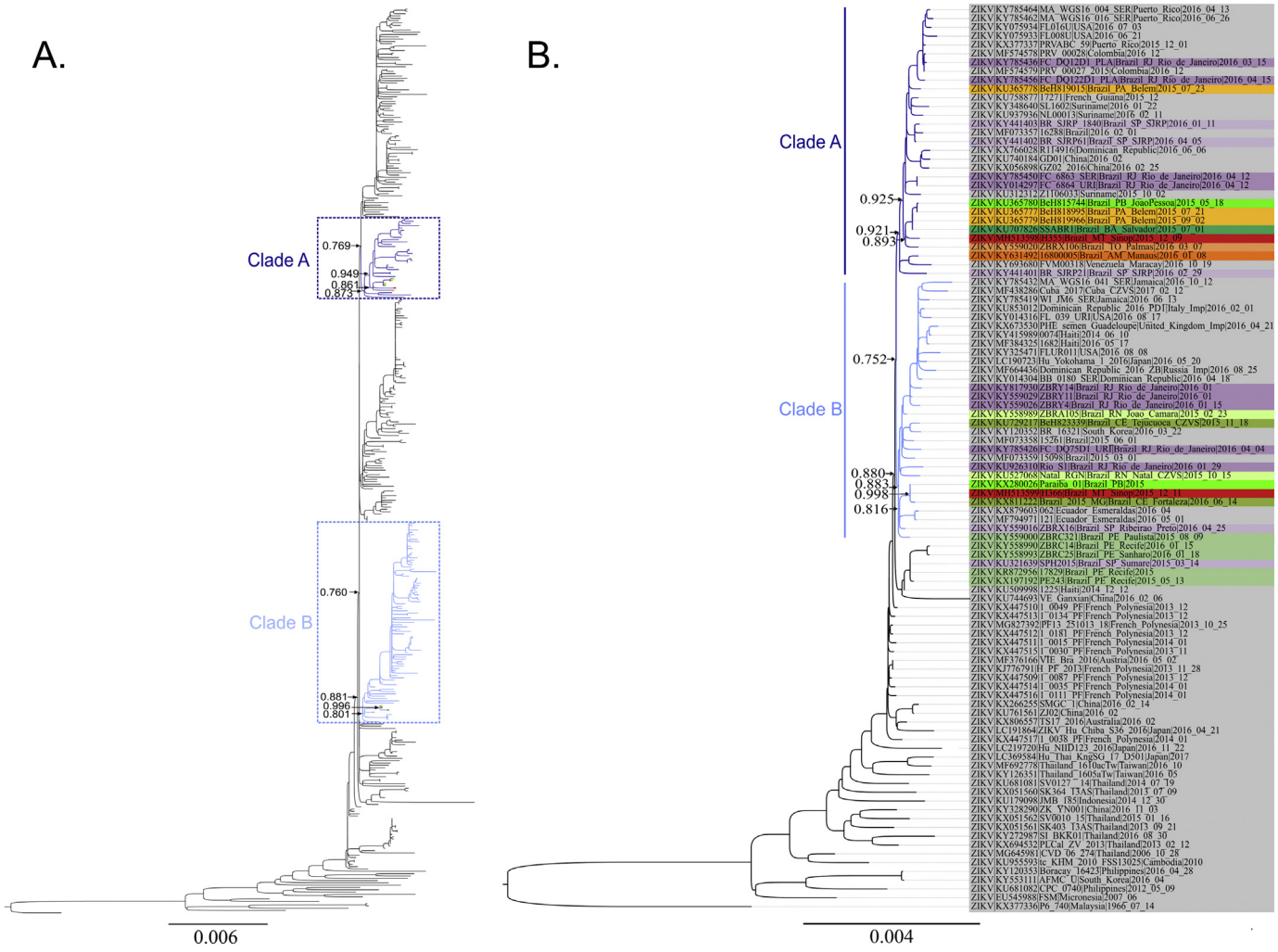

Fig. 1. Phylogenetic reconstruction and positioning of ZIKV genomes obtained in this study using maximum likelihood. A - Full phylogeny including all available Asian genotype ZIKV complete and draft genomes > 9 kb. B - Phylogenetic analysis of a subset of ZIKV sequences from the full ZIKV tree focusing on clades that the two ZIKV genomes obtained in this study clustered. aLRT SH-like branch support of key clades are depicted with arrows in panels A and B.

3. Results

We investigated 63 serum samples from which six samples were positive for ZIKV by Nested-PCR and RT-qPCR. The cycle thresholds (CTs) from ZIKV positive samples ranged from 25 to 36, and two of them were sequenced (Table 1). One sample (BR/Sinop/H355/2015) belonged to a 41-year-old female patient presenting petechial rash, back pain, arthralgia, normal leukocyte (4530/mm^3) and 314,000/mm^3 of platelet count. The other sample (BR/Sinop/H366/2015) was from a 30-year-old pregnant patient presenting fever, petechial rash, itch, diffuse myalgia, arthralgia, normal leukocyte (6390/mm^3) and 140,000/mm^3 of platelet count. Both patients did not report any recent history of travel and had their samples collected at the third day of symptoms, in December 2015. It is important to emphasize that the first evidence of human infection in the State of Mato Grosso occurred in November 2015 and, since then, autochthonous transmission of the virus was confirmed, with no imported cases reported (Ministério da Saúde, 2019).

The entire ZIKV genomes from these two samples were obtained with average coverage depth ranging from 280 to 9282× (Table 1).

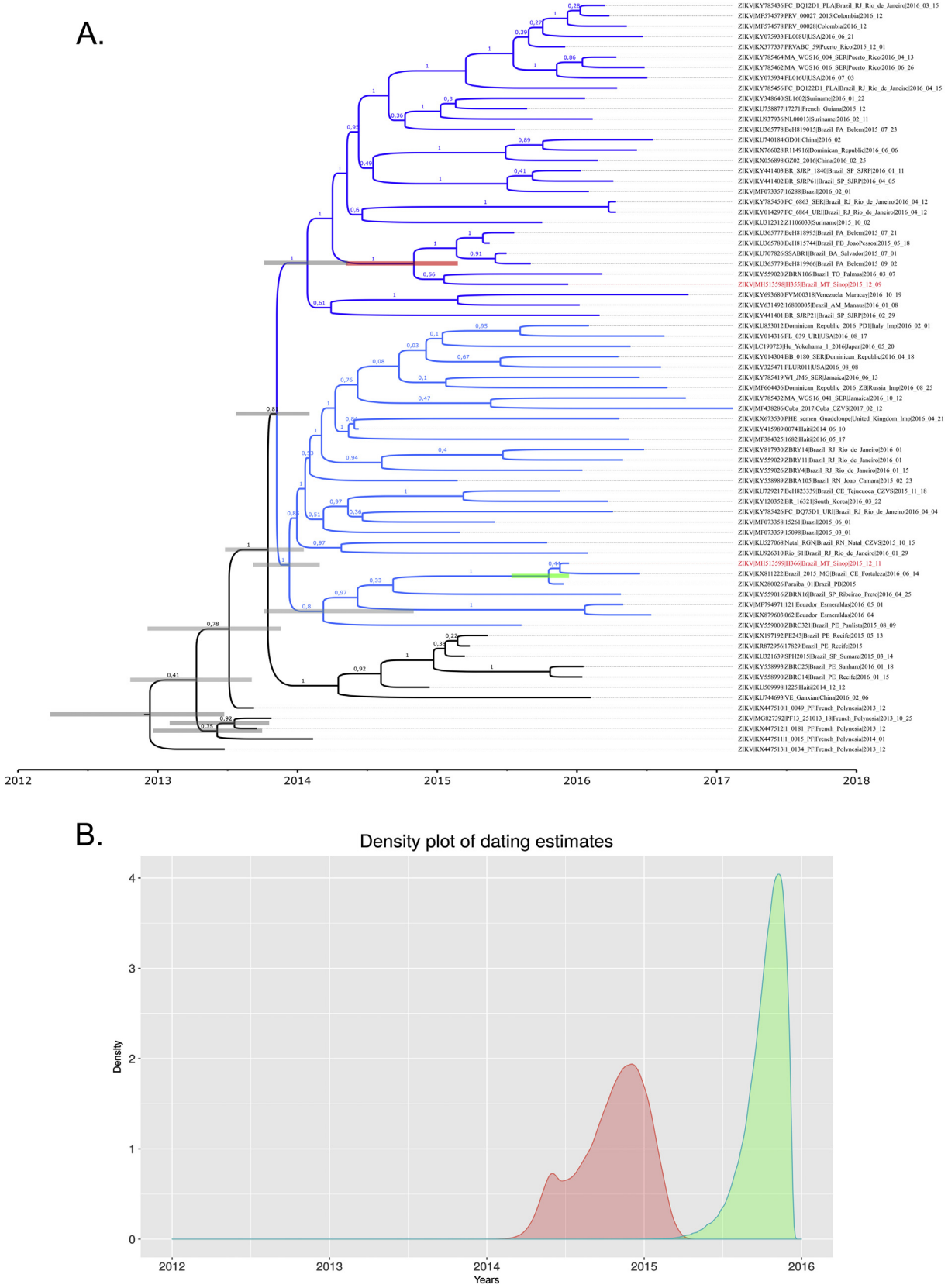

Fig. 2. Bayesian phylogenetic analysis using relaxed molecular clock and bayesian skyline demographic model. A) A subset of ZIKV genomes and the genomes sequenced in this study (red tip names). Node bars represent the HPD 95% dating estimates for each ancestral and red and green node bars are the HPD95% estimates for the clade ancestral in which the two genomes from Sinop clustered. B) Density distribution of the dating estimates for the same two tMRCAs depicted in red and green node bars in panel A. Colors of the branches follow the same pattern as Fig. 1 panel B: dark blue is clade A and light blue is clade B. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 3. Geographic distribution of ZIKV in Brazil and the virus possible route. Distribution of the number of ZIKV genomes sequenced per state and colored edges showing the possible route of ZIKV spread based on the most closely related ZIKV genome from the phylogenetic analysis shown on Fig. 1 panel B and Fig. 2 panel A. State colors follows tip colors in Fig. 1, panel B.

Maximum likelihood phylogenetic reconstruction of the obtained genomes and several available ZIKV genomes showed that strains infecting Sinop patients clustered within the Asian lineage (Fig. 1, panel A and Supplementary Fig. 2) along with several other draft ZIKV genomes from the 2015–2016 epidemics. More specifically, the H355 genome clustered in a clade along with genomes from the North and Southeast Brazilian regions and from Central America (Fig. 1 panel B, clade A), while the H366 genome clustered within another clade along with samples from Northeast and Southeast Brazilian regions as well as genomes from Central America (Fig. 1 panel B, clade B).

Using a relaxed molecular clock analysis we estimated the spread of ZIKV in the Midwest region of Brazil. We used the same genomic dataset from Fig. 1 panel B except that we kept only French Polynesian genomes as outgroup in order to compare with published estimates of the Asian genotype causing the 2015–2016 epidemics. We found no statistically significant signal of recombination in this dataset both with SpliTree ($p = .0985$) and RPD softwares. Our estimates showed that the dating of the most recent common ancestor (tMRCA) of the two genomes sequenced in this study differed: I - the most well supported branch leading to the Sinop genome from clade A was dated to October 2014 [95% highest posterior density (HPD) between June 2014 and February 2015, reddish node bar and violin plot]; II - while the tMRCA of the Sinop genome from clade B was dated to October 2015 [95% HPD between July 2015 and December 2015, greenish node bar and violin plot] (Fig. 2 panels A and B).

The two ZIKV genomes from Sinop were closely related ZIKV detected in patients from the state of Tocantins (TO) and Paraíba (PB) (Fig. 2) suggesting that two possible entry routes of ZIKV in Sinop, MT (Fig. 3).

We also characterized a number of nonsynonymous nucleotide mutations in the obtained genomes. We detected the previously reported amino acid mutations in one structural and four of the non-structural proteins, including the A983V mutation NS1, which has been implicated in immune evasion and is conserved in all Asian strains detected after the French Polynesia outbreak (Liu et al., 2017) and a pre-M mutation (S139 N) which has been associated with microcephaly

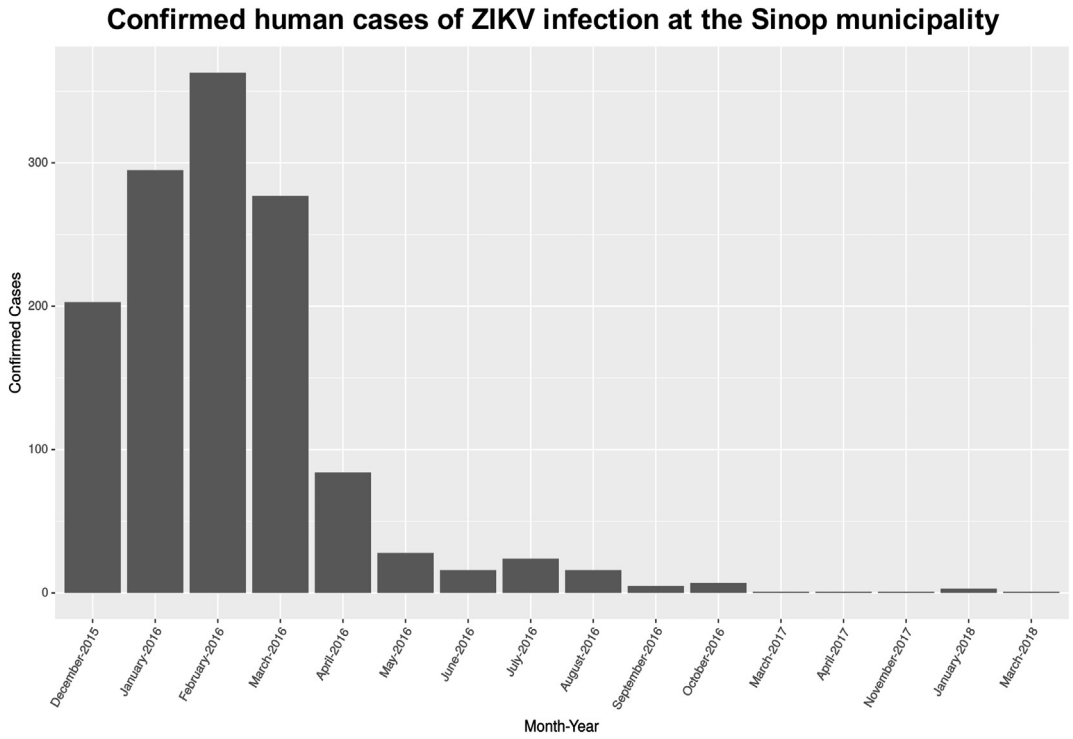

Fig. 4. Number of ZIKV confirmed cases at the Sinop municipality between 2015 and 2019 from the weekly epidemiological summaries of the Secretary of Health of the state of Mato Grosso.

in mice (Yuan et al., 2017). In addition, we also found mutations specific for our samples: NS2B (I1398V), NS3 (T2068 M) and NS4B mutation (S2456 L) (Table 1).

4. Discussion

ZIKV was a relatively unknown virus until its spread to Asian and Pacific islands and resulting epidemics after its arrival in the Americas (World Health Organization, 2015). Since the virus spread to South America, it was responsible for a large epidemic and several previously unknown pathogenic effect on newborns and adults (Cordeiro et al., 2016; Mlakar et al., 2016). Currently, the Asian lineage that entered South America is still spreading to different countries around the globe (Hill et al., 2019; Khongwichit et al., 2018; Woon et al., 2019; Yadav et al., 2019). Although several efforts have been made to study the virus biology, there are still knowledge gaps in several basic ZIKV research fronts such as the extent of its spread in affected areas. In Brazil, the large majority of the molecular genomic data about the ZIKV is restricted to large cities located in the coastline of the country and almost no information is available regarding the virus in the inner region of the country such as in the states of Mato Grosso, Mato Grosso do Sul and Goiás (Fig. 3). Here we report two ZIKV genomes from Sinop samples, a Southern Amazon city from the state of Mato Grosso.

Until late 2016, ZIKV surveillance had been performed only with ELISA assays and quantitative PCR, the gold standard for ZIKV molecular diagnostic (Xavier et al., 2017). The largest incidence of Zika in the Mato Grosso state occurred in 2016 (Ministério da Saúde, 2016). Weekly epidemiological summaries of Zika cases from Sinop city, published by the Secretary of Health of the State of Mato Grosso, shows that the first ZIKV confirmed case occurred in December 2015 with an incidence pick in February 2016 (363 cases) and the last reported case in March 2018 (Fig. 4, http://www.saude.mt.gov.br/dengue/arquivos/526/documentos accessed 10 June 2019). Molecular clock analysis suggested that the first ZIKV lineage was circulating at Sinop region around the end of 2014 and beginning of 2015 suggesting that human cases likely remained undetected until December 2015 (Fig. 2). Since neither patients traveled before reporting arbovirus-like symptoms, they likely become infected locally, supporting that local ZIKV transmission were occurring in the Sinop city before its first detection in December 2015. At this time, the second lineage was circulating in this region (tMRCA October 2015 - HPD95% July-December 2015 - Fig. 2) suggesting that the steady increase of case numbers until February 2016 pick (Fig. 4) occurred due to human infections likely derived from either strains. In addition, a recent study reported for the first time the circulation of the Zika virus in the state of Mato Grosso in the municipality of Tapurah in August 2015 (Costa et al., 2019). This municipality is located around 100 km distant from Sinop city. The detection of the ZIKV circulation in August 2015 in this region is in line with human cases infections derived from the either virus lineages sequenced in our study.

The tMRCA of the clade containing one strain from Sinop, three strain from North region (BeH818995, BeH819966, ZBRX106) and two strain from Northeast region (BeH815744, SSABR1) were dated around October 2014 much earlier than the first ZIKV notification case in Brazil which occurred between May and June 2015 (Kindhauser et al., 2016). In addition, a second lineage entered the region latter around October 2015 at the time that ZIKV strains already spread to several other Central and North America countries (Faria et al., 2017). The close clustering with ZIKV sequences available from other Brazilian states which are far apart from Mato Grosso state highlights the rapid expansion of these strain between distant Brazilian regions including the Midwest region. Lastly, we found two ZIKV amino acid mutations that were associated with the virus pathogenicity and conserved in all ZIKV genomes obtained after 2014. Several other amino acid changes were also found, but their role in the ZIKV evolution remains unknown.

In this study, we report for the first time the complete genome sequence of the Zika virus circulating in this region from two samples collected at Sinop city in a similar time frame (December 2015). Our data supports previous findings that the ZIKV circulating in this remote region of Brazil belongs to the Asian genotype and more specifically to two different clades found circulating in other Brazilian states. Although the two samples were obtained during December 2015,

phylogenetic analysis showed that two independent ZIKV lineages were circulating at this region and molecular clock analysis showed that they likely arrived at different time frames and from different regions of Brazil.

5. Conclusions

Our results show that two lineages of the Asian ZIKV genotype was circulating in the Midwest Brazil and that the last common ancestor of the clades containing such lineages have different time estimates. Hence, ZIKV was circulating in almost all Brazilian regions before its first detection and notification in May–June 2015. Such findings highlight the need of rapid and efficient deployment of molecular surveillance in difficult access areas in order to obtain curated data about the real extent of the epidemic in the human population.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2019.103974.

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Data statement

The datasets generated and analyzed during the current study are available at GenBank (MH513598-MH513598) and the European Nucleotide Archive (ENA - PRJEB30828).

Declaration of Competing Interest

The authors declare to have no competing interests.

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References

Baud, D., Gubler, D.J., Schaub, B., Lanteri, M.C., Musso, D., 2017. An update on Zika virus infection. Lancet 390, 2099–2109. https://doi.org/10.1016/S0140-6736(17)31450-2.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

Bruen, T.C., Philippe, H., Bryant, D., 2006. A simple and robust statistical test for detecting the presence of recombination. Genetics 172, 2665–2681. https://doi.org/10.1534/GENETICS.105.048975.

Campos, T.D.L., Durães-Carvalho, R., Rezende, A.M., de Carvalho, O.V., Kohl, A., Wallau, G.L., Pena, L.J., 2018. Revisiting key entry routes of human epidemic arboviruses into the mainland Americas through large-scale phylogenomics. Int. J. Genomics 2018, 6941735. https://doi.org/10.1155/2018/6941735.

Cordeiro, M.T., Pena, L.J., Brito, C.A., Gil, L.H., Marques, E.T., 2016. Positive IgM for Zika virus in the cerebrospinal fluid of 30 neonates with microcephaly in Brazil. Lancet 387, 1811–1812. https://doi.org/10.1016/S0140-6736(16)30253-7.

Costa, M.C.S., 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., Slhessarenko, R.D., 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.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9, 772. https://doi.org/10.1038/nmeth.2109.

de Morais Bronzoni, R.V.D., Baleotti, F.G., Nogueira, M.R.R., Nunes, M., Figueiredo, L.T.M., 2005. Duplex reverse transcription-PCR followed by nested PCR assays for detection and identification of Brazilian alphaviruses and Flaviviruses. J. Clin. Microbiol. 43, 696–702. https://jcm.asm.org/content/43/2/696.

Delatorre, E., Fernández, J., Bello, G., 2018. Investigating the role of easter island in migration of Zika virus from South Pacific to Americas. Emerg. Infect. Dis. 24, 2119–2121. https://doi.org/10.3201/eid2411.180586.

Depoux, A., Philibert, A., Rabier, S., Philippe, H.-J., Fontanet, A., Flahault, A., 2018. A multi-faceted pandemic: a review of the state of knowledge on the Zika virus. Public Health Rev. 39, 10. https://doi.org/10.1186/s40985-018-0087-6.

Dick, G.W., Kitchen, S., Haddow, A., 1952. Zika virus (I). Isolations and serological specificity. Trans. R. Soc. Trop. Med. Hyg. 46, 509–520. https://doi.org/10.1016/0035-9203(52)90042-4.

Donald, C.L., Brennan, B., Cumberworth, S.L., Rezelj, V., Clark, J.J., Cordeiro, M.T., Freitas, R., Franc, D.O., Pena, L.J., Wilkie, G.S., Davis, C., Owsianka, A.M., Hughes, J., Varjak, M., Selinger, M., Patel, A.H., Mclauchlan, J., Lindenbach, B.D., Fall, G., Sall, A.A., Biek, R., Rehwinkel, J., Schnettler, E., Kohl, A., 2016. Full genome sequence and sfRNA interferon antagonist activity of Zika virus from Recife, Brazil. PLoS Negl. Trop. Dis. 10, e0005048. https://doi.org/10.1371/journal.pntd.0005048.

Duffy, M.R., Chen, T.-H., Hancock, W.T., Powers, A.M., Kool, J.L., Lanciotti, R.S., Pretrick, M., Marfel, M., Holzbauer, S., Dubray, C., Guillaumot, L., Griggs, A., Bel, M., Lambert, A.J., Laven, J., Kosoy, O., Panella, A., Biggerstaff, B.J., Fischer, M., Hayes, E.B., 2009. Zika virus outbreak on Yap Island, Federated States of Micronesia. N. Engl. J. Med. 360, 2536–2543. https://doi.org/10.1056/NEJMoa0805715.

Faria, N.R., Azevedo, R. do S. da S., Kraemer, M.U.G., Souza, R., Cunha, M.S., Hill, S.C., Thézé, J., Bonsall, M.B., Bowden, T.A., Rissanen, I., Rocco, I.M., Nogueira, J.S., Maeda, A.Y., Vasami, F.G. da S., Macedo, F.L. de L., Suzuki, A., Rodrigues, S.G., Cruz, A.C.R., Nunes, B.T., Medeiros, D.B. de A., Rodrigues, D.S.G., Queiroz, A.L.N., da Silva, E.V.P., Henriques, D.F., da Rosa, E.S.T., de Oliveira, C.S., Martins, L.C., Vasconcelos, H.B., Casseb, L.M.N., Simith, D. de B., Messina, J.P., Abade, L., Lourenço, J., Alcantara, L.C.J., de Lima, M.M., Giovanetti, M., Hay, S.I., de Oliveira, R.S., Lemos, P. da S., de Oliveira, L.F., de Lima, C.P.S., da Silva, S.P., de Vasconcelos, J.M., Franco, L., Cardoso, J.F., Vianez-Júnior, J.L. da S.G., Mir, D., Bello, G., Delatorre, E., Khan, K., Creatore, M., Coelho, G.E., de Oliveira, W.K., Tesh, R., Pybus, O.G., Nunes, M.R.T., Vasconcelos, P.F.C., 2016a. Zika virus in the Americas: early epidemiological and genetic findings. Science 352, 345–349. https://doi.org/10.1126/science.aaf5036.

Faria, N.R., Sabino, E.C., Nunes, M.R.T., Alcantara, L.C.J., Loman, N.J., Pybus, O.G., 2016b. Mobile real-time surveillance of Zika virus in Brazil. Genome Med. 8, 97. https://doi.org/10.1186/s13073-016-0356-2.

Faria, N.R., Quick, J., Claro, I.M., Thézé, J., de Jesus, J.G., Giovanetti, M., Kraemer, M.U.G., Hill, S.C., Black, A., da Costa, A.C., Franco, L.C., Silva, S.P., Wu, C.-H., Raghwani, J., Cauchemez, S., du Plessis, L., Verotti, M.P., de Oliveira, W.K., Carmo, E.H., Coelho, G.E., Santelli, A.C.F.S., Vinhal, L.C., Henriques, C.M., Simpson, J.T., Loose, M., Andersen, K.G., Grubaugh, N.D., Somasekar, S., Chiu, C.Y., Muñoz-Medina, J.E., Gonzalez-Bonilla, C.R., Arias, C.F., Lewis-Ximenez, L.L., Baylis, S.A., Chieppe, A.O., Aguiar, S.F., Fernandes, C.A., Lemos, P.S., Nascimento, B.L.S., Monteiro, H.A.O., Siqueira, I.C., de Queiroz, M.G., de Souza, T.R., Bezerra, J.F., Lemos, M.R., Pereira, G.F., Loudal, D., Moura, L.C., Dhalia, R., França, R.F., Magalhães, T., Marques, E.T., Jaenisch, T., Wallau, G.L., de Lima, M.C., Nascimento, V., de Cerqueira, E.M., de Lima, M.M., Mascarenhas, D.L., Neto, J.P.M., Levin, A.S., Tozetto-Mendoza, T.R., Fonseca, S.N., Mendes-Correa, M.C., Milagres, F.P., Segurado, A., Holmes, E.C., Rambaut, A., Bedford, T., Nunes, M.R.T., Sabino, E.C., Alcantara, L.C.J., Loman, N.J., Pybus, O.G., 2017. Establishment and cryptic transmission of Zika virus in Brazil and the Americas. Nature 546, 406–410. https://doi.org/10.1038/nature22401.

Gardy, J.L., Loman, N.J., 2018. Towards a genomics-informed, real-time, global pathogen surveillance system. Nat. Rev. Genet. https://doi.org/10.1038/nrg.2017.88.

Guedes, D.R., Paiva, M.H., Donato, M.M., Barbosa, P.P., Krokovsky, L., Rocha, S.W.S., Saraiva La, K., Crespo, M.M., Rezende, T.M., Wallau, G.L., Barbosa, R.M., Oliveira, C.M., Melo-Santos, M.A., Pena, L., Cordeiro, M.T., Franca, R.F.D.O., De Oliveira, A.L.S., Peixoto, C.A., Leal, W.S., Ayres, C.F., 2017. Zika virus replication in the mosquito *Culex quinquefasciatus* in Brazil. Emerg. Microbes Infect. 6, e69. https://doi.org/10.1038/emi.2017.59.

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.

Guo, X.-X., Li, C.-X., Deng, Y.-Q., Xing, D., Liu, Q.-M., Wu, Q., Sun, A.-J., Dong, Y., Cao, W.-C., Qin, C.-F., Zhao, T.-Y., 2016. *Culex pipiens quinquefasciatus*: a potential vector to transmit Zika virus. Emerg. Microbes Infect. 5, e102. https://doi.org/10.1038/emi.2016.102.

Haby, M.M., Pinart, M., Elias, V., Reveiz, L., 2018. Prevalence of asymptomatic Zika virus infection: a systematic review. Bull. World Health Organ. 96, 402–413D. https://doi.org/10.2471/BLT.17.201541.

Hasebe, F., Parquet, M.C., Pandey, B.D., Mathenge, E.G.M., Morita, K., Balasubramaniam, V., Saat, Z., Yusop, A., Sinniah, M., Natkunam, S., Igarashi, A., 2002. Combined detection and genotyping of Chikungunya virus by a specific reverse transcription-polymerase chain reaction. J. Med. Virol. 67, 370–374. https://doi.org/10.1002/jmv.10085.

Hill, S.C., Vasconcelos, J., Neto, Z., Jandondo, D., Zé-Zé, L., Aguiar, R.S., Xavier, J., Thézé, J., Mirandela, M., Cândido, A.L.M., Vaz, F., Sebastião, C. dos S., Wu, C.-H., Kraemer, M., Melo, A., Schamber-Reis, B.L., de Azevedo, G.S., Tanuri, A., Higa, L.M., Clemente, C., da Silva, S.P., Candido, D. da S., Claro, I.M., Quibuco, D., Domingos, C., Pocongo, B., Watts, A.G., Khan, K., Alcantara, L.C., Sabino, E., Lackritz, E., Pybus, O.G., Alves, M.-J., Afonso, J., Faria, N.R., 2019. Emergence of the Zika virus Asian lineage in Angola. bioRxiv, 520437. https://doi.org/10.1101/520437.

Katoh, K., Rozewicki, J., Yamada, K.D., 2017. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Brief. Bioinform. bbx108. https://doi.org/10.1093/bib/bbx108.

Khongwichit, S., Wikan, N., Auewarakul, P., Smith, D.R., 2018. Zika virus in Thailand. Microbes Infect. 20, 670–675. https://doi.org/10.1016/J.MICINF.2018.01.007.

Kindhauser, M.K., Allen, T., Frank, V., Santhana, R.S., Dye, C., 2016. Zika: the origin and spread of a mosquito-borne virus. Bull. World Health Organ. 94, 675–686C. https://doi.org/10.2471/BLT.16.171082.

Kraemer, M.U.G., Brady, O.J., Watts, A., German, M., Hay, S.I., Khan, K., Bogoch, I.I., 2017. Zika virus transmission in Angola and the potential for further spread to other African settings. Trans. R. Soc. Trop. Med. Hyg. 111, 527–529. https://doi.org/10.1093/trstmh/try001.

Krauer, F., Riesen, M., Reveiz, L., Oladapo, O.T., Martínez-Vega, R., Porgo, T.V., Haefliger, A., Broutet, N.J., Low, N., Zika Causality, W.H.O., Working Group, W.Z.C.W, 2017. Zika virus infection as a cause of congenital brain abnormalities and Guillain-Barré syndrome: systematic review. PLoS Med. 14, e1002203. https://doi.org/10.1371/journal.pmed.1002203.

Ladner, J.T., Grubaugh, N.D., Pybus, O.G., Andersen, K.G., 2019. Precision epidemiology for infectious disease control. Nat. Med. 25, 206–211. https://doi.org/10.1038/s41591-019-0345-2.

Lanciotti, R.S., Kosoy, O.L., Laven, J.J., Velez, J.O., Lambert, A.J., Johnson, A.J., Stanfield, S.M., Duffy, M.R., 2008. Genetic and serologic properties of Zika virus associated with an epidemic, yap state, Micronesia, 2007. Emerg. Infect. Dis. 14, 1232–1239. https://doi.org/10.3201/eid1408.080287.

Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9, 357–359. https://doi.org/10.1038/nmeth.1923.

Lefort, V., Longueville, J.-E., Gascuel, O., 2017. SMS: smart model selection in PhyML. Mol. Biol. Evol. 34, 2422–2424. https://doi.org/10.1093/molbev/msx149.

Liu, Y., Liu, J., Du, S., Shan, C., Nie, K., Zhang, R., Li, X.-F., Zhang, R., Wang, T., Qin, C.-F., Wang, P., Shi, P.-Y., Cheng, G., 2017. Evolutionary enhancement of Zika virus infectivity in *Aedes aegypti* mosquitoes. Nature 545, 482–486. https://doi.org/10.1038/nature22365.

Lourenço, J., de Lourdes Monteiro, M., Valdez, T., Monteiro Rodrigues, J., Pybus, O., Rodrigues Faria, N., 2018. Epidemiology of the Zika virus outbreak in the Cabo Verde Islands, West Africa. PLoS Curr. 10. https://doi.org/10.1371/currents.outbreaks.19433b1e4d007451c691f138e1e67e8c.

Lowe, R., Barcellos, C., Brasil, P., Cruz, O., Honório, N., Kuper, H., Carvalho, M., 2018. The Zika virus epidemic in Brazil: from discovery to future implications. Int. J. Environ. Res. Public Health 15, 96. https://doi.org/10.3390/ijerph15010096.

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, vev003. https://doi.org/10.1093/ve/vev003.

Miner, J.J., Diamond, M.S., 2017. Zika virus pathogenesis and tissue tropism. Cell Host Microbe 21, 134–142. https://doi.org/10.1016/j.chom.2017.01.004.

Ministério da Saúde, 2016. Boletins epidemiológicos – secretaria de vigilância em saúde. Monitoramento dos casos de dengue, febre de chikungunya e febre pelo vírus Zika 47 (38), 1–10. http://portalms.saude.gov.br/boletins-epidemiologicos, Accessed date: 10 March 2019.

Mlakar, J., Korva, M., Tul, N., Popović, M., Poljšak-Prijatelj, M., Mraz, J., Kolenc, M., Resman Rus, K., Vesnaver Vipotnik, T., Fabjan Vodušek, V., Vizjak, A., Pižem, J., Petrovec, M., Avšič Županc, T., 2016. Zika virus associated with microcephaly. N. Engl. J. Med. 374, 951–958. https://doi.org/10.1056/NEJMoa1600651.

Musso, D., Gubler, D.J., 2016. Zika Virus. Clin. Microbiol. Rev. 29, 487–524. https://doi.org/10.1128/CMR.00072-15.

Pickett, B.E., Sadat, E.L., Zhang, Y., Noronha, J.M., Squires, R.B., Hunt, V., Liu, M., Kumar, S., Zaremba, S., Gu, Z., Zhou, L., Larson, C.N., Dietrich, J., Klem, E.B., Scheuermann, R.H., 2012. ViPR: an open bioinformatics database and analysis resource for virology research. Nucleic Acids Res. 40, D593–D598. https://doi.org/10.1093/nar/gkr859.

Rambaut, A., 2014. FigTree: Tree Figure Drawing Tool Version 1.4.3. University of Edinburgh, Edinburgh.

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, vew007. https://doi.org/10.1093/ve/vew007.

Rambaut, A., Drummond, A.J., Xie, D., Baele, G., Suchard, M.A., 2018. Posterior summarization in Bayesian phylogenetics using tracer 1.7. Syst. Biol. 67, 901–904. https://doi.org/10.1093/sysbio/syy032.

Robinson, J.T., Thorvaldsdóttir, H., Winckler, W., Guttman, M., Lander, E.S., Getz, G., Mesirov, J.P., 2011. Integrative genomics viewer. Nat. Biotechnol. 29, 24–26. https://doi.org/10.1038/nbt.1754.

Smartt, C.T., Shin, D., Kang, S., Tabachnick, W.J., 2018. *Culex quinquefasciatus* (Diptera: Culicidae) From Florida Transmitted Zika Virus. Front. Microbiol. 9, 768. https://doi.org/10.3389/fmicb.2018.00768.

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, vey016. https://doi.org/10.1093/ve/vey016.

Thézé, J., Li, T., du Plessis, L., Bouquet, J., Kraemer, M.U.G., Somasekar, S., Yu, G., de Cesare, M., Balmaseda, A., Kuan, G., Harris, E., Wu, C., Ansari, M.A., Bowden, R., Faria, N.R., Yagi, S., Messenger, S., Brooks, T., Stone, M., Bloch, E.M., Busch, M., Muñoz-Medina, J.E., González-Bonilla, C.R., Wolinsky, S., López, S., Arias, C.F., Bonsall, D., Chiu, C.Y., Pybus, O.G., 2018. Genomic epidemiology reconstructs the introduction and spread of Zika virus in Central America and Mexico. Cell Host Microbe 23, 855–864.e7. https://doi.org/10.1016/J.CHOM.2018.04.017.

Tognarelli, J., Ulloa, S., Villagra, E., Lagos, J., Aguayo, C., Fasce, R., Parra, B., Mora, J., Becerra, N., Lagos, N., Vera, L., Olivares, B., Vilches, M., Fernández, J., 2016. A report on the outbreak of Zika virus on Easter Island, South Pacific, 2014. Arch. Virol. 161, 665–668. https://doi.org/10.1007/s00705-015-2695-5.

Wikan, N., Smith, D.R., 2016. Zika virus: history of a newly emerging arbovirus. Lancet Infect. Dis. 16, e119–e126. https://doi.org/10.1016/S1473-3099(16)30010-X.

Woon, Y.L., Lim, M.F., Tg Abd Rashid, T.R., Thayan, R., Chidambaram, S.K., Syed Abdul Rahim, S.S., Mudin, R.N., Sivasampu, S., 2019. Zika virus infection in Malaysia: an epidemiological, clinical and virological analysis. BMC Infect. Dis. 19, 152. https://doi.org/10.1186/s12879-019-3786-9.

World Health Organization, 2015. Zika virus outbreaks in the Americas. Wkly Epidemiol. Rec. 90, 609–610.

Xavier, A.R., Kanaan, S., Bozzi, R.P., Amaral, L.V., Xavier, A.R., Kanaan, S., Bozzi, R.P., Amaral, L.V., 2017. Clinical and laboratory diagnosis of Zika fever: an update. J. Bras. Patol. e Med. Lab. 53, 252–257. https://doi.org/10.5935/1676-2444.20170039.

Yadav, P.D., Malhotra, B., Sapkal, G., Nyayanit, D.A., Deshpande, G., Gupta, N., Padinjaremattathil, U.T., Sharma, H., Sahay, R.R., Sharma, P., Mourya, D.T., 2019. Zika virus outbreak in Rajasthan, India in 2018 was caused by a virus endemic to Asia. Infect. Genet. Evol. 69, 199–202. https://doi.org/10.1016/J.MEEGID.2019.01.026.

Yuan, L., Huang, X.-Y., Liu, Z.-Y., Zhang, F., Zhu, X.-L., Yu, J.-Y., Ji, X., Xu, Y.-P., Li, G., Li, C., Wang, H.-J., Deng, Y.-Q., Wu, M., Cheng, M.-L., Ye, Q., Xie, D.-Y., Li, X.-F., Wang, X., Shi, W., Hu, B., Shi, P.-Y., Xu, Z., Qin, C.-F., 2017. A single mutation in the prM protein of Zika virus contributes to fetal microcephaly. Science 358, 933–936. https://doi.org/10.1126/science.aam7120.