Genomic and epidemiological surveillance of Zika virus in the Amazon region

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Summary

Zika virus (ZIKV) has caused an explosive epidemic linked to severe clinical outcomes in the Americas. Until June 2018, 4,929 ZIKV suspected infections and 46 congenital syndrome cases were reported in Manaus city, Amazonas State, Brazil. Although Manaus is a key demographic hub in the Amazon region, little is known about the ZIKV epidemic there, both in terms of transmission and viral genetic diversity. Using portable virus genome sequencing we generated 59 ZIKV genomes in Manaus. Phylogenetic analyses indicated multiple introductions of ZIKV from northeast Brazil to Manaus. Spatial genomic analysis of virus movement among 6 areas in Manaus suggested that populous northern neighborhoods acted as sources of virus transmission to other neighborhoods. Our study revealed how the ZIKV epidemic was ignited and maintained within the largest urban metropolis in the Amazon. Those results might contribute to improve public health response to outbreaks in Brazil.

Introduction

Zika virus (ZIKV) is a flavivirus with a 11kb positive-sense RNA genome that has caused an explosive epidemic in the Americas linked to severe congenital syndromes, including microcephaly (Petersen et al., 2016). ZIKV transmission occurs via the bite of infected *Aedes aegypti* mosquitoes, although sexual and vertical transmission, as well as transmission through blood transfusion have been also reported (Petersen et a., 2016). Since its first detection in northeast Brazil in May 2015 (Zanluca et al., 2015; Campos et al., 2015), the country has reported nearly 1 million confirmed and suspected ZIKV infections, the greatest number among the 52 territories in the Americas that have reported ZIKV transmission (PAHO 2017; Zhanget al., 2017; Alex Perkins et al., 2016). In recent years Brazil has been gripped by a wave of severe and overlapping epidemics of mosquito-borne viruses, which together have challenged passive syndromic surveillance and led to increased morbidity and disability (de Oliveira et al., 2017; Cao-Lormeau et al., 2016; Grubaugh et al., 2017). The northeast and southeast regions of Brazil have been severely hit by the ZIKV epidemic and account for 75% ZIKV reported cases in Brazil between 2016 and 2018 (Ministério da Saúde 2016-2017; Ministério da Saúde 2018).

Although a lower number of ZIKV infections have been reported in the northern region of Brazil encompassing the Amazon, several studies suggest that the region may be a location of entry for *Aedes*-borne viruses to Brazil and increased epidemiological surveillance in the region is needed. For example, Brazilian dengue and chikungunya viruses seem to have emerged first in the Amazon region before spreading to other, more densely populated locations (Nunes et al., 2012; Nunes et al., 2014; Nunes et al., 2015). The Amazon region is also home to a high diversity of mosquito-borne viruses (Vasconcelos et al., 1992), including Mayaro (Azevedo et al.,

2009) and Oropouche (Azevedo et al., 2007), and an ecosystem that, under inadequate management, may facilitate the emergence and re-emergence of mosquito-borne virus epidemics (Faria et al., 2018; Vasconcelos et al., 2001). Moreover, climatic data suggests the possibility of year-round endemic transmission of arboviruses in the Amazon, which stands in contrast to seasonal epidemics in the southeast, south and center-west regions of Brazil (Messina et al., 2016; Obolski et al., 2019).

Between January 2015 and September 2018, Amazonas state, the largest federal unit in Brazil, reported 4,929 Zika cases, including 46 microcephaly cases in newborns. Most of these cases were reported in Manaus, the largest urban metropolis in the Amazon region, and cases were reported across several epidemic seasons (Ministério da Saúde 2015-2019). However, the epidemic transmission and genomics of Zika virus in the Amazon region remain poorly understood. It is also unclear how and where Zika may have persisted in the Amazon region across epidemic seasons. Following our previous experience of using a mobile laboratory to investigate the genomic epidemiology of ZIKV in Brazil (Faria et al., 2016), we used portable genome sequencing to locally generate ZIKV genomes from infected patients residing in Manaus city. Samples were collected from febrile cases between December 2015 and April 2017. We use molecular epidemiology analysis to uncover the diversity and persistence of Zika virus in Manaus, and its transmission in the Amazon region.

Results

Expansion of the Zika virus epidemic in Manaus

Until September 2018, 6,987 ZIKV cases were reported by the main public health laboratory in Amazonas state. The first PCR-confirmed case was identified in early November 2015 (vertical arrow in **Figure 1A**) and the first epidemic wave ("wave 1") peaked in mid-April 2016 (n=288 weekly number of cases) (**Figure 1A**). A second, smaller epidemic wave ("wave 2") peaked in early April 2017 (n=32 weekly number of cases) and was followed by a third epidemic wave ("wave 3") around mid-April 2018 (n=30 weekly number of cases). The estimated basic reproductive number of ZIKV from the first epidemic wave is $R_0 \sim 2.69$ (95% credible interval: 2.32 to 3.11), in line with previous estimates (Faria et al., 2016; Caminade et al., 2017).

Figure 1B shows the temporal distribution of microcephaly cases (n=46) reported in Manaus between 2015 and 2018. To investigate the temporal association between Zika confirmed cases and microcephaly cases in Manaus we use a Poisson regression model that accounts for cross-correlation. We find evidence of a possible temporal association between the ZIKV and microcephaly time series (p-value <0.001, cross-correlation coefficient = 0.43, for the period 19th Dec 2015 to 20th Jan 2018). This model estimates that the microcephaly time series lags the ZIKV cases by 29 weeks in Manaus (**Table S1**).

To better understand the epidemic transmission of ZIKV in Manaus, we compiled climatic data from a weather station in Manaus city center and evaluated ZIKV transmission potential using using the index P (Lourenço et al., 2017; Obolski et al., 2019). The estimated suitability index P consistently reveals high suitability (P>1) during the epidemic waves. According to the estimated P index, mosquitoes are able to contribute to transmission of ZIKV in Manaus throughout most of the year; i.e. each year includes one or more long periods of time during which P>1 (**Figure 1C**). The association between P and ZIKV cases was high (cross-correlation coefficient =0.815, coefficient p-value = 0.037). Moreover, we estimate that ZIKV cases lag the P index time series by 4.7 weeks on average (**Table S1**).

Highest ZIKV incidence in Centre-West Manaus region

We next explored the spatial distribution of ZIKV cases within individual neighborhoods of Manaus. Neighborhood-level yearly notified cases for ZIKV (2016 and 2017) and dengue virus (DENV; 2015 and 2016) were made available from the Brazilian Ministry of Health and case counts were grouped into six areas of Manaus city, North, West, East, Centre-West, Centre-South and South (**Figure 2**). In 2016, ZIKV incidence in Manaus was highest in the Centre-West area (5.3 cases per 1,000 inhabitants); within this city area, the Dom Pedro neighborhood had the highest incidence (10.5 cases per 1,000 inhabitants; **Table S2**). The lowest incidence was recorded in the East area of Manaus (1.5 cases per 1,000 inhabitants). Incidence in all Manaus neighborhoods in 2017 was negligible (**Figure 2A, Tables S2-3**). As expected, ZIKV case numbers and neighborhood population size were strongly positively associated (ρ= 0.91, p-value < 0.0001, summarized in **Figure 2B** and detailed in **Table S2**), with 24% of ZIKV cases in Manaus being reported in the most populous North area of the city. We found a moderate association between ZIKV and DENV incidence per area in 2016 (ρ = 0.47, p-value = 0.0002), although DENV incidence was on average 1.4-fold lower in 2016 compared to that of ZIKV, possibly due to previous circulation of DENV and therefore accumulation of herd-immunity to DENV (**Figure 3**).

Molecular diagnostics and genome sequencing from clinical samples

A total of 525 samples from patients (68% female, 359/525) visiting either local clinics or the main hospital in Manaus municipality between February 2014 and April 2017 were screened previously at the the Instituto Leonidas & Maria Deane (ILMD/FIOCRUZ) of Amazonas, the Central Laboratory of Public Health of

Amazonas (LACEN-Amazonas), and the Flavivirus Laboratory at FIOCRUZ Rio de Janeiro (LABFLA/FIOCRUZ) using an in-house RT-qPCR assay targeting the ZIKV envelope gene region (Naveca et al., 2017). Of the tested samples, 218 (42%) tested positive for ZIKV, of which 158 (72.5%) were from female patients. For positive samples, PCR cycle threshold (Ct) values were on average 34.18 (range: 15.19 to 41.01). We selected samples with Ct-values of 38 or less for genome sequencing, resulting in 106 samples with an average Ct of 31.38 (range: 15.19 to 38.00) (**Table S4**). These selected RT-qPCR positive samples were obtained on average 3 days (range: 0 to 14 days) after the onset of symptoms (**Table S4**) and were obtained from patients who resided in 40 different neighborhoods in Manaus. We used a MinION handheld nanopore sequencer to generate virus genome sequences from positive samples using our previously validated approach (Quick et al., 2017; Faria et al., 2017). We successfully generated 59 complete and near complete genome sequences (average coverage = 73%, see **Figure 4** and **Table S5**).

Genomic history of ZIKV in the capital city of Amazonas state

To better understand the establishment and transmission of ZIKV in Manaus, we added our newly generated consensus genome sequences to a global dataset of 423 ZIKV genomes including recently released ZIKV genomes from Angola and Cuba (Hill et al., 2019; Grubaugh et al., 2019) and we estimated an initial maximum likelihood (ML) phylogenetic tree (**Figure 5**). We find that 93% (55 of 59) of the novel Manaus isolates fall within a single large well-supported monophyletic clade (bootstrap score, BS = 94%) within the ZIKV American clade. This suggests that the ZIKV epidemic in Manaus was primarily caused by a single introduction, resulting in a large epidemic clade, named hereafter as Manaus clade.

We also identified four isolates from 2016 outside the main clade. Isolate AMA14, sampled in April 2016, falls basally to a clade containing 6 sequences from Chinese travelers infected in February 2016 in Venezuela (Sun et al., 2017) and a single sequence from the Dominican Republic. Isolate AMA59, sampled in January 2016, clusters with sequences from Southeast Brazil. A small clade containing isolates AMA53 and AMA20, sampled in January and April 2016 respectively, are closely related to a sequence from northeast Brazil and this resulting cluster is a sister clade to other sequences from Midwest, Southeast and Northeast regions of Brazil, and also to three isolates from Angola (which likely derived from Northeast Brazil; Hill et al. 2019). Taken together these data suggest at least four independent introductions into Manaus. Although one isolate from Venezuela cluster together within the Manaus clade we cannot make speculations about the transmission route between the two countries, because we don't have enough information about the epidemiological data of the sample as well as a higher samples number from Venezuela.

Spatio-temporal evolution of Zika virus in Manaus

We estimated a time scale for the evolution of the Manaus clade using the best-fitting molecular clock model. A regression of genetic divergence from root to tip against sampling dates confirmed sufficient temporal signal (r^2=0.62) (**Figure 6**). The evolutionary rate of the Manaus clade was calculated to be 1.09 x 10^{-3} substitutions/site/year (s/s/y; 95% Bayesian Credible Interval, BCI: 7.7 x 10^{-4} to 1.43 x 10^{-3} s/s/y). This is in line with previous analyses of other ZIKV datasets from the Americas (Faria et al., 2017; Faria et al., 2016). We estimate the date of the most recent common ancestor (MRCA) of the ZIKV Manaus clade to be around January 2015 (95% BCI: August 2014 to May 2015) (**Figure 7A**). Although this date represents a lower bound on the age of the Manaus clade, the estimated time of the MRCA of the Manaus clade coincides with a period of high ZIKV transmission potential in the city (**Figure 1C**).

In the Manaus clade, most of the sequences sampled from different city regions, are interspersed, suggesting a highly interconnected dispersion pattern. We thus investigated the movement of Zika virus among geographic areas in Manaus using a discrete trait phylogenetic model. We find strong statistical support that the Manaus clade originated in the North area of the city (location posterior support = 0.92; **Figure 7A**). Our analysis identifies the North and East areas as probable source locations of ZIKV transmission in Manaus, seeding most of the virus lineage movement events within the city. The North and East are the most populated and least economically developed areas of Manaus, which suggests a possible link between ZIKV transmission and socioeconomic factors at a within-city level. ZIKV genome sequences from the Centre-South area were not phylogenetically clustered, indicating a lack of local virus transmission there. In contrast, 6 of 10 strains from the West area of Manaus form a single monophyletic clade that resulted from an introduction during the peak of the epidemic in 2016 (**Figure 1** and **Figure 7**). These strains were isolated in April 2017, hence this lineage may have circulated unnoticed for 10 months before detection.

We also estimated the contributions of different geographic areas of Manaus to the persistence of ZIKV in the city by estimating the waiting times between virus lineage movements (Markov rewards) across the phylogeny of the Manaus clade. Our results support the hypothesis that the North area of Manaus acts as the main source location (42% of the total branch duration in the time-scaled phylogeny is inferred to be located in the North area). Finally, we used our spatial analysis to infer the location in Manaus of ZIKV lineages that persisted across epidemic waves (**Figure 1**, see also **Figure 7A**). Our results suggest that ZIKV was able to persist locally across the 2015 and 2017 epidemic seasons in the North, East, South and West areas of Manaus, which are also the four most populated areas of the city (**Table S3**).

Discussion

In this study we characterized disease transmission in the large ZIKV outbreak in Manaus city, Amazonas state, Northern Brazil, using a combination of portable genome sequencing and epidemiological analysis. We find that the ZIKV epidemic in Manaus, the largest metropolis in the Amazon region, was ignited by an introduction of a single virus lineage, most likely from northeast Brazil, which we infer was introduced around January 2015. This was a time of high climatic suitability for arbovirus transmission. We further show that the virus persisted locally until at least April 2017. Spatial genetic analysis indicates that the virus was introduced first to the northern neighborhoods of Manaus; from where the virus lineages seeded other nearby areas.

Analysis of the 59 ZIKV complete and partial genome sequences from 30 different neighborhoods in Manaus generated here provides a high-resolution contribution to our understanding of the introduction and progression of ZIKV in Brazil and to the transmission of ZIKV in tropical urban regions. Our analysis indicates that ZIKV was introduced to Manaus from the northeast region of Brazil on at least four occasions. This agrees with our previous work that has found that northeast Brazil played a significant role in the establishment and dissemination of ZIKV in the Americas (Faria et al., 2017; Faria et al., 2016).

Although, evidence of cross-border transmissions among locations that share a tropical climate is frequent and has been observed previously in the region, for example, for DENV serotype 4 (Nunes et al., 2011) and chikungunya virus (Naveca et al., 2019), and although our results show that one isolate from Venezuela, a country with a high suitability for *Ae*. *aegypti* that has direct river connections to Manaus, cluster within the Manaus clade (**Figure 5**), we cannot exclude that some of the ZIKV introductions to Manaus were from Venezuela, therefore we cannot make speculations about the direction of the transmissions between the two countries because the lack of epidemiological data linked to this sample as well as a higher samples number from Venezuela.

Our within-city phylogeographic reconstruction is consistent with a gravity-like model of ZIKV dissemination, with virus transmission being driven by the most populated areas which act as source locations, as shown previously for other infectious diseases (e.g. Kraemer et al., 2019; Kraemer et al., 2017). The North area of Manaus has had the highest rate of population growth in recent years and has the second lowest income of all areas in the city. This suggests that demographic and socioeconomic factors have likely determined the incidence and persistence of the virus across Manaus neighborhoods (Lindoso et al., 2009; Hagan et al., 2016; Wilder-Smith et al., 2017). Our within-city phylogeographic reconstruction (**Figure 7**) further indicates that ZIKV transmission persisted through multiple epidemic waves in several neighbourhoods.

It is important to note that phylogeographic analyses can be affected by sampling bias. In this study we compiled an updated dataset of ZIKV genome sequences dataset, comparatively few sequences from Brazil from 2017 and 2018 are available. This matches the small number of reported ZIKV cases in the country during this period, but under sampling may impact our conclusions concerning clustering with Manaus lineages after 2016. Regarding the within-city reconstructions, our sampling effort was successful in capturing ZIKV diversity in all main regions; the variation in sampling sizes obtained is approximately proportional to the number of ZIKV cases reported for each region in 2016-2017 (**Table S3**).

Epidemiological analysis of suspected ZIKV infections indicates a dominant epidemic wave of transmission in Manaus that peaked around mid-April 2016, followed by a second smaller wave in 2017. A third small epidemic peak in suspected cases can be noted around April 2018. We also find evidence that local microcephaly cases are correlated with local ZIKV cases and lag the latter by ~29 weeks. The introduction and spread of ZIKV over 2 or 3 consecutive waves in a given location has been observed previously and explained by the temporal accumulation of herd-immunity (Lourenço et al., 2017; Ferguson et al., 2016). It has been reported also that the vast majority of Zika infections go unnoticed and it is possible that the high similarity of case definitions for DENV, CHIKV and ZIKV, which co-circulate in the Amazon region (Nunes et al., 2012; Nunes et al., 2015; Vasconcelos et al., 1992; Naveca et al., 2019; da Costa et al., 2018) could have resulted in a significant number of ZIKV infections being classified as either dengue or chikungunya at the beginning of the epidemic.

We find that local among-season ZIKV transmission in the Brazilian Amazon is consistent with sustained local year-round ecological suitability for *Aedes* spp., as previously predicted from climatic data alone (Bogoch et al., 2016), and also with the indication of a possible ZIKV persistence through natural vertical transmission in *Aedes aegypti* populations in Manaus (da Costa et al., 2018; Izquierdo-Suzán et al., 2019; Chaves et al., 2019) although these cases require more caution because the nonspecific methodology used. Genetic and epidemiological analysis have indicated the northern region of Brazil has acted as a source region for DENV, or as stepping-stone for the dissemination of arboviruses to other areas of the country (Faria et al., 2018; Faria et al., 2019) these trends may have been influenced by increases in human mobility and vector suitability (Kraemer et al., 2019). Taken together, these results emphasize the ecological suitability of Manaus for the establishment of *Aedes*-borne viruses and highlight the need for continued arbovirus surveillance in Amazon urban areas.

In summary, we provide evidence for sustained local transmission of ZIKV in Manaus, Amazonas State, between 2015 and 2017, and we reveal the epidemiological connections between Manaus and other locations in South America.

The spread of ZIKV in Manaus was mediated by climatic, socioeconomic and demographic conditions, as well as by herd immunity (Lourenço et al., 2017) and our results shed light on the epidemiological dynamics of the virus urban tropical locations. Our work also provides an example of the relevance of integrating genetic and epidemiological surveillance when investigating arbovirus transmission (Kraemer et al., 2018). Ultimately such integration should aim for earlier detection of transmission of novel pathogens and for more real-time prediction of disease spread. The generation of genomic data by portable sequencing technology in local public health laboratories, as demonstrated here, can contribute substantially to these goals. Given the biodiversity of the Amazon basin, improving disease surveillance the region is crucial, both to improve public health responses and to increase our understanding of the diversity of known and unknown mosquito-borne viruses that co-circulate in the region.

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KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Virus strains		
Zika Virus strains from Amazon	This Study	
Biological Samples		
Serum, urine, cerebrospinal fluid samples from patients visiting either local clinics or the main hospital in Manaus municipality of Amazonas state	Amazonas State - Instituto Leonidas & Maria Deane (ILMD/FIOCRUZ) of Amazonas - The Central Laboratory of Public Health of Amazonas (LACEN-Amazonas) - The Flavivirus Laboratory at FIOCRUZ Rio de Janeiro (LABFLA/FIOCRUZ)	
Critical Commercial Assays		
QIAamp Viral RNA Mini Kit	Qiagen	Cat # 204443
TaqMan Fast Virus 1-Step Master Mix	Thermo-Fisher Scientific	Cat # 4444436
ProtoScript® II First Strand cDNA Synthesis Kit	New England Biolabs	Cat # E6560L
Q5 High-Fidelity DNA polymerase	New England Biolabs	Cat # M0491L
Agencourt AMPure XP	Beckman Coulter	Cat # A63880
Qubit dsDNA HS Assay Kit	Qiagen	Cat # Q32851
CDC monoplex assay	Lanciotti et al., 2016	N/A
Native Barcoding Expansion 1-12 (PCR-free)	Oxford Nanopore Technologies	Cat # EXP-NBD104
DNA Sequencing Kit SQK-MAP007/SQK-LSK108	Oxford Nanopore Technologies	Cat # SQK-LSK108
R9.4 flowcell	Oxford Nanopore Technologies	Cat # FLO-MIN106
Deposited Data		
59 Zika virus sequences 59 from Manaus, Amazonas State, Brazil have been deposited in the National Center for Biotechnology Information (NCBI) Genbank	This Study	National Center for Biotechnology Information (NCBI) Genbank: MK216687-MK216688; MK216690-MK216738; MK216740-MK216745; MK216747-MK216748.

423 publicly available Zika virus sequences obtained from the National Center for Biotechnology Information (NCBI) Genbank	N/A	National Center for Biotechnology Information (NCBI) Genbank: MK829154, MK829153, MK829152, MH544701, MH513600, MH513599, MH513598, MH157213, MH157208, MH157202, MH063265, MH063264, MH063263, MH063262, MH063261, MH063260, MH063259, MF783073, MF783072, MF073359, MF073358, MF073357, MG595216, MG494697, MF988743, KY441403, KY441402, KY441401, MF438286, MF384325, MF167360, MF159531, MF801426, MF801425, MF801424, KY606273, MF801423, MF801422, MF801421, MF801420, MF801419, MF801418, MF801417, KY606274, MF801416, MF801415, MF801414, MF801413, MF801412, MF801411, MF801410, MF801409, MF801408, MF801407, MF801406, MF801405, MF801404, MF801403, MF801402, MF801401, MF801400, MF801399, MF801398, MF801397, MF801396, KY606272, MF801395, MF801394, KY606271, MF801393, MF801392, MF801391, MF801390, MF801389, MF801388, MF801387, MF801386, MF801385, MF801384, MF801383, MF801382, MF801381, MF801380, MF801379, MF801378, MF801377, MF434522, MF434521, MF434520, MF434519, MF434518, MF434517, MF434516, KX446950, KX446951, KX856011, KU870645, KY785416, KY014319, KY785471, KY014310, KY014311, KY014312, KY785418, KY785414, KY785444, KY785461, KY785442, KY014315, KY785452, KY014327, KY014306, KY785448, KY785458, KY785431, KY785421, KX421195, MF098770, MF098771, KY785454, KU501216, KU501217, KX262887, KX766029, KY120349, KY325465, KY328289, KY631493, KY693676, KY693677, KY765317, KY765318, KY765323, KY765324, KY765325, KY927808, KX520666, KX830930, KY785437, KY785436, KY014317, KY785446, KY785451, KY014320, KY014308, KY785410, KY014307, KY014309, KY785411, KY785427, KY785479, KY785480, KY785467, KY785425, KY785426, KY785409, KY014305, KY785469, KY014313, KY785423, KY785449, KY785463, KY785455, KY785417, KY785475, KY785465, KY785483, KY785433, KY785439, KY014303, KY014321, KY785415, KY014297, KY785450, KY014301, KY785429, KY785456, KY014296, KY014304, KY785420, KY785476, KY014300, KY014302, KY014318, KY785466, KY785485, KY785477, KY785441, KY785419, KY785428, KY014314, KY014295, KY785445, KY785412, KY785443, KY785457, KY014325, KY785440, KY785474, KY014298, KY014326, KY014316, KY014324, KY014322, KY014323, KX247646, KY014299, KY785422, KY785472, KY785468, KY272991, KX156775, KX156774, KX156776, KU926309, MF098764, MF098765, MF098766, MF098769, MF098768, KX702400, KU926310, MF098767, KY785453, KY785478, KY785484, KY785473, KY785470, KY785460, KY785435, KY785434, KY785447, KY785413, KY785482, KY785424, KY785438, KY785430, KY785432, KY785481, KY785464, KY785462, KY785459, KX101065, KX101067, KX101064, KU940224, KU940227, KU940228, KX101063,

		KX101062, KX101061, KX101060, KX101066, KU312312, KU312313, KU312314, KU312315, KU501215, KU509998, KU646827, KU646828, KU647676, KU527068, KU707826, KU497555, KU729218, KU729217, KX197192, KU365780, KU365779, KU365778, KU365777, KX280026, KU321639, KU740184, KU758868, KU758869, KU758870, KU758871, KU758872, KU758873, KU758874, KU758875, KU758876, KU758877, KU761564, KU820897, KU820898, KU853012, KU922960, KU937936, KU955590, KU991811, KX051563, KX056898, KX087101, KX087102, KX197205, KX198135, KX212103, KX269878, KX377337, KX548902, KX601168, KX673530, KX766028, KX811222, KX879603, KX879604, KY003153, KY003154, KY003155, KY003156, KY003157, KY014328, KY014329, KY317936, KY317937, KY317938, KY317939, KY317940, KY348640, KY558989, KY558990, KY558991, KY558992, KY558993, KY558994, KY558995, KY558996, KY558997, KY558998, KY558999, KY559000, KY559001, KY559002, KY559003, KY559004, KY559005, KY559006, KY559007, KY559008, KY559009, KY559010, KY559011, KY559012, KY559013, KY559014, KY559015, KY559016, KY559017, KY559018, KY559019, KY559020, KY559021, KY559022, KY559023, KY559024, KY559025, KY559026, KY559027, KY559028, KY559029, KY559030, KY559031, KY559032, KY631492, KY693678, KY693679, KY693680, KY817930, KY989971, KX447521, KX447520, KX447519, KX447518, KX447517, KX447516, KX447515, KX447514, KX447513, KX447512, KX447511, KX447510, KX447509, KX369547, KJ776791, KU681081, KU681082, JN860885, EU545988, KY075932, KX922706, KX922707, KX832731, KX838904, KX838905, KX838906, KX922708, KY075937, KY075938, KY075939, KY075933, KY317936, KY317937, KY317939, KY317940, KY317938, MF159531, MH063260, MH063261, MH063262, MH063263, KX842449, MH063264, MH063265, FL257|/H, KY075934, KX922703, KY075935, KX922704, KX922705, KY075936.
Oligonucleotides		
ZIKV 1086 5’-CCGCTGCCCAACACAAG-3’	Lanciotti et al., 2016	N/A
ZIKV 1162c 5’-CCACTAACGTTCTTTTGCAGACAT-3’	Lanciotti et al., 2016	N/A
ZIKV 1107-FAM 5’-AGCCTACCTTGACAAGCAGTCA GACACTCAA /3IABkFQ -3’	Lanciotti et al., 2016	N/A
Software and Algorithms		
BEAST	Suchard et al., 2018	http://beast.community
jModelTest2	Darriba et al., 2012	https://github.com/ddarriba/jmodeltest2
MAFFT	Katoh and Standley, 2013	https://mafft.cbrc.jp/alignment/server/
RAxML v8	Stamatakis et al., 2014	https://github.com/stamatak/standard-RAxML
Zika Virus Typing tool	Fonseca et al., 2019	http://www.krisp.org.za/tools.php
PrimalScheme	Quick et al., 2017	http://primal.zibraproject.org
QGIS	QGIS Development Team	https://qgis.org
R Statistical Computing Software	The R Foundation	https://www.r-project.org/
R-package bdskytools	N/A	https://github.com/laduplessis/bdskytools
R-package ggplot2	Wickham, 2016	http://ggplot2.org/
R-package ggtree	Yu et al., 2016	https://github.com/GuangchuangYu/ggtree
TempEst	Rambaut et al., 2016	http://beast.community/tempest
Tracer	Rambaut et al., 2018	http://beast.community/tracer
Albacore	Loman et al., 2014	https://github.com/nanoporetech
Nanopolish	Loman et al., 2014	https://github.com/jts/nanopolish

Porechop	Loman et al., 2014	https://github.com/rrwick/Porechop
Other		
Alignment used in phylogenetic analyses, including 196 publicly available Zika virus sequences and 59 Zika virus sequences generated in this study	This Study	N/A

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for laboratory resources and reagents should be directed to and will be fulfilled by the corresponding author, Luiz Carlos Junior Alcantara (luiz.alcantara@ioc.fiocruz.br). Requests for computational resources and files should be directed to and will be fulfilled by the corresponding authors, Oliver G. Pybus (oliver.pybus@zoo.ox.ac.uk) and Nuno Rodrigues Faria (nuno.faria@zoo.ox.ac.uk). This study did not generate new unique reagents.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Sample collection

Samples (serum, urine, cerebrospinal fluid) from patients visiting either local clinics or the main hospital in Manaus municipality of Amazonas state were collected for molecular diagnostics and sent for testing at IMLD/FIOCRUZ, LACEN-Amazonas and LABFLA/FIOCRUZ. Sampled individuals that were subjected to molecular diagnostics presented exanthema accompanied by two or more of the following symptoms: fever, headache, conjunctivitis, arthralgia, myalgia, and edema. The majority of samples were linked to a digital record that collated epidemiological and clinical data such as date of sample collection, municipality of residence, neighborhood of residence, demographic characteristics (age and sex) and date of onset of clinical symptoms (**Table S4-S5**).

Ethical statement

The project was supported by the Pan American World Health Organization (PAHO) and the Brazilian Ministry of Health (MoH) as part of the arboviral genomic surveillance efforts within the terms of Resolution 510/2016 of CONEP (Comissão Nacional de Ética em Pesquisa, Ministério da Saúde; National Ethical Committee for Research, Ministry of Health). The diagnostic of ZIKV infection at ILMD was approved by the Ethics Committee of the State University of Amazonas (CAAE: 56.745.116.6.0000.5016).

METHOD DETAILS

Nucleic acid isolation and RT-qPCR

Most of the Zika-suspected clinical samples were screened for ZIKV RNA from serum (86%), urine (3.5%) and cerebrospinal fluid (CSF) (11%). Samples were obtained from 0 to 31 days after the onset of symptoms. Viral RNA was isolated from 140 µl samples using the QIAamp Viral RNA Mini kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. An internal positive control, the *Escherichia coli* bacteriophage MS2 (ATCC 15597-B1), was used during the RNA extraction as previously describe (Naveca et al., 2017). Cycle threshold (Ct) values were determined for all samples by probe-based reverse transcription quantitative real-time PCR (RT-qPCR) against the envelope (ENV) gene target for ZIKV detection (using 5′ FAM as the probe reporter dye) (Lanciotti et al., 2008) using the following primers 5'-CCGCTGCCCAACACAAG-3' (forward) 5'-CCACTAACGTTCTTTTGCAGACAT. 3' (reverse) and probe 5'- 6-FAM-AGCCTACCT/ZEN/TGACAAGCAGTCAGACACTCAA /3IABkFQ. RT-qPCR assays were performed with TaqMan Fast Virus 1-Step Master Mix in a reaction of

10μl using a final concentration of 0.3μM for primers and 0.1μM for probe in a StepOnePlus Real-Time PCR System (Applied Biosystems) installed at the Real-Time PCR Platform of ILMD-FIOCRUZ.

cDNA synthesis and whole genome nanopore sequencing

DNA amplification and sequencing were attempted on the 106 selected RT-PCR positive samples that exhibited Ct-values <38, in order to increase the genome coverage of clinical samples by nanopore sequencing (Quick et al., 2017). Extracted RNA was converted to cDNA using the Protoscript II First Strand cDNA synthesis Kit (New England Biolabs, Hitchin, UK) and random hexamer priming. Whole-genome amplification by multiplex PCR was attempted using the previously published Zika Asian primer scheme and 45 cycles of PCR using Q5 High-Fidelity DNA polymerase (NEB) as previously described (Quick et al., 2017). PCR products were cleaned-up using AmpureXP purification beads (Beckman Coulter, High Wycombe, UK) and quantified using fluorimetry with the Qubit dsDNA High Sensitivity assay on the Qubit 3.0 instrument (Life Technologies). PCR products for samples yielding sufficient material (more than 4ng/μL as determined using Qubit) were barcoded and pooled in an equimolar fashion using the Native Barcoding Kit (Oxford Nanopore Technologies, Oxford, UK). Sequencing libraries were generated from the barcoded products using the Genomic DNA Sequencing Kit SQK-MAP007/SQK-LSK208 (Oxford Nanopore Technologies) and were loaded onto a R9.4 flowcell. All sequencing was performed at ILMD-FIOCRUZ.

Generation of consensus sequences

Consensus sequences for each barcoded sample were generated following a previously published approach (Quick et al., 2017). Briefly, raw files were basecalled using Albacore (Loman et al., 2014), demultiplexed and trimmed using Porechop. Nanopolish variant calling was applied to the assembly to detect single nucleotide variants to the reference ZIKV genome (KJ776791). Only positions with ≥ 20x genome coverage were used to produce consensus alleles. Regions with lower coverage, and those in primer-binding regions were masked with N characters.

Collation of ZIKV complete genome datasets

Two complete or near-complete ZIKV genome datasets were generated. Dataset 1 (n = 482) comprised the data reported in this study (n = 59) plus a larger and updated dataset including recently released data from the ZIKV epidemic in Angola and Cuba (Hill et al., 2019; Grubaugh et al., 2019). Subsequently, to investigate the dynamic of the ZIKV infection within Manaus, genetic analyses were conducted on a smaller dataset including only sequences pertaining to the largest clade of virus strains circulating in Manaus (n = 56).

Maximum likelihood analysis and clock signal estimation

Maximum likelihood (ML) trees were estimated using RAxML v8 (Stamatakis et al., 2014) under an HKY nucleotide substitution model (Hasegawa et al., 1985), with a gamma distribution of among site rate variation (HKY + G + I) as selected by jModeltest.v.2 (Darriba et al., 2012). Statistical robustness of tree topology was inspected using 1000 bootstrap replicates; a bootstrap value >80% was considered notable. To estimate temporal signal in each dataset, sample collection dates were regressed against root-to-tip genetic distances obtained from the ML phylogenies using TempEst (Rambaut et al., 2016). When precise sampling dates were not available, a precision of 1 month or 1 year in the collection dates was considered.

Dated phylogenetics

To estimate time-calibrated phylogenies dated from time-stamped genome data, we conducted phylogenetic analysis using the Bayesian software package BEASTv.1.10.2 (Suchard et al., 2018). As previously (Thézé et al., 2018), we used the HKY nucleotide substitution model with codon partitions (Shapiro et al., 2006) and Bayesian Skygrid tree prior (Gill et al., 2013) with an uncorrelated relaxed clock with a lognormal distribution (Drummond et al., 2006). Analyses were run in duplicate in BEASTv.1.10.2 (Suchard et al., 2018) for 50 million MCMC steps, sampling parameters and trees every 5000th step. A non-informative continuous time Markov chain reference prior on the molecular clock rate was used (Ferreira et al., 2008). Convergence of MCMC chains was checked using Tracer v.1.7.1 (Rambaut et al., 2018). Maximum clade credibility trees were summarized using TreeAnnotator after discarding 10% as burn-in.

Phylogeographic analysis

We investigated the dynamics of ZIKV infection and virus lineage movements in Manaus using a sampled set of time-scaled phylogenies and the sampling location (area in Manaus) of each geo-referenced ZIKV sequence, as shown in **Table S6**. We discretised sequence sampling locations by considering 6 distinct geographic areas of the Manaus city: north (n=13), east (n=9), south (n=8), west (n=6), central-west (n=10), and centre-south (n=10), as shown in **Figure 7**. Phylogeographic reconstructions were conducted using the asymmetric discrete trait model implemented in BEASTv1.10.2 (Lemey et al., 2009). As part of the flexible discrete trait phylogeographic approach implemented in BEASTv1.10.2, we also estimated posterior expectations both the number of transitions among areas (Markov jumps) and the waiting times between transitions (Markov rewards) (Gill et al., 2013). Maximum clade credibility trees were summarized using TreeAnnotator after discarding 10% as burn-in. While the sampling is relatively homogeneous among sampled locations, the phylogeographic reconstruction will remain sampling dependent. For example, sampling effort could impact on the estimated transition frequencies among locations. However, with careful interpretation, phylogeographic analysis can provide valuable information about dispersal dynamics, including information about linkages that would not be evident without genomic data.

Epidemiological analysis

Number of weekly Zika virus cases in the municipality of Manaus were obtained from the Brazilian Ministry of Health. Cases were defined as suspected ZIKV infection when patients presented maculopapular rash and at least two of the following symptoms: fever, conjunctivitis, polyarthralgia or periarticular edema. Details and limitations of Zika virus surveillance approach based on notified or suspected cases have been described in more detail elsewhere (Faria et al., 2017). The epidemic basic reproductive number, R_0, was estimated as previously described (Faria et al., 2017). In brief, we fit a simple exponential growth rate model to weekly case counts from the first epidemic wave in Manaus. The period of exponential growth was selected, and a linear model was fitted to estimate the weekly exponential growth rate (r). We then derived reproductive number R_0 from r and a probability density distribution of the epidemic generation time. We assume a gamma-distribution function for the generation time with a mean of 20 days and a standard deviation of 7.4 days. We also explored other scenarios with generation time of 10 days.

Temporal association between Zika virus and microcephaly cases

The number of weekly microcephaly cases in the municipality of Manaus were obtained from the Brazilian Ministry of Health and are available. Zika virus and microcephaly case counts (*n*=46) were compared using a Poisson regression model with Akaike Information Criteria to find the best-fitting time-lagged model. In this case, p-value is the explanatory power of the Zika confirmed for microcephaly case counts to indicate the evidence for their association. Coefficients, cross-correlations and time-lags (in epidemiological weeks) for each comparison can be found in **Table S1**.

Daily Aedes-ZIKV transmission potential (P index)

Estimation of mosquito-borne virus suitability (P index) was calculated using a climate-driven method as previously described in (Obolski et al., 2019). The index P measures the reproductive (transmission) potential of an adult female mosquito for a given point in time. Manaus' average daily temperature and relative humidity (%) between 01/01/2014 to 21/01/2019 were obtained from the Instituto Nacional de Metereologia (INMET) weather station number 82331 (latitude: -3.11, longitude: -59.95). Climate data was downloaded from INMET's website (www.inmet.gov.br). Moreover, for a comparison between the suitability index P and Zika confirmed cases, we considered Zika non-negative counts as continuous and applied a log(x+1) transformation. We focus on the epidemic season 14th November 2015 to 10th August 2016. An autoregressive integrated moving average (ARIMA) model was used to account for any residual autocorrelation (P). In this case, the p-value reflects the explanatory power of suitability for Zika virus confirmed cases. We note that by using the index P as a proxy for transmission potential using climate data from a single weather station in Manaus, we do not take into account the possible effects of microclimate across the city. Although having been demonstrated to be highly correlated with mosquito-borne incidence in other cities of Brazil and elsewhere (Obolski et al., 2019; Perez-Guzman et al., 2018), the index P is not informed by factors that may play a role in transmission potential in Manaus, such as abundance of vegetation and human density or mobility.

QUANTIFICATION AND STATISTICAL ANALYSIS

Maximum Likelihood Phylogenetic Analysis

To assess the suitability of substitution models for our ZIKV alignment we performed a statistical model selection procedure based on the Akaike information criterion, using jModelTest2 (Darriba et al., 2012). This identified the best fitting substitution model (HKY + G + I) for ML phylogenetic analysis. A phylogenetic bootstrap analysis with 100 replicates using RAxML v8 (Stamatakis et al., 2014) was conducted to evaluate the statistical support for nodes of the ML phylogeny.

Dated phylogenetics and Phylogeographic analysis

To assess whether our data was suitable for a molecular clock phylogenetic analysis, we evaluated the temporal evolutionary signal in our ZIKV alignment using the statistical approaches in TempEst (Rambaut et al., 2016). A linear regression between sample collection dates and root-to-tip genetic distances obtained from the ML phylogeny indicated that the feasibility of a molecular clock approach. A Bayesian MCMC approach implemented in BEAST v1.10.2 (Suchard et al., 2018) was used to infer molecular clock.

Epidemiological analysis and Temporal association between Zika virus and microcephaly cases

A linear regression model developed and described in Faria et al., (2017), was used to assess the correlation between Zika virus and microcephaly cases, for each each distinct Manaus's neighbourhoods.

Daily Aedes-ZIKV transmission potential (P index)

Estimation of mosquito-borne virus suitability (P index) was calculated using a climate-driven method as previously described in (Obolski et al., 2019). An autoregressive integrated moving average (ARIMA) model was used to account for any residual autocorrelation (P). This model measures the reproductive (transmission) potential of an adult female mosquito for a given point in time and explains the variation in the ZIKV transmission potential.

DATA AND SOFTWARE AVAILABILITY

Data availability

New sequences have been deposited in GenBank under accession numbers MK216687-MK216688; MK216690-MK216738; MK216740-MK216745; MK216747- MK216748.

LEAD CONTACT AND MATERIAL AVAILABILITY

Further information and requests for material availability should be directed to and will be fulfilled by the corresponding author, Luiz Carlos Junior Alcantara (luiz.alcantara@ioc.fiocruz.br).

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Author's Contributions

Conception and design: M.G., N.R.F., N.L., O.G.P. and L.C.J.A.; Investigations: M.G., N. R. F., F.G.N, J.G.J., J.X., I.C., F.C.S.S., P.P.S., V.A.N., V.C.S., F.C.M.I., G.W., E.A.C.M., A.F., and F.L.; Data Curation: M.G., N.R.F. J.L., M.K., V.F., S.D., J.T., L.P., O.G.P., and L.C.J.A Formal Analysis: M.G., N.R.F. J.L., M.K., S.D., and L.P. Writing – Original Draft Preparation: M.G., N.R.F., J.L., M.K., F.G.N., O.G.P., and L.C.J.A Revision: M.G., N.R.F., J.L., M.K., F.G.N., O.G.P., T.G., M.R.T.N. T.O., and L.C.J.A Resources: L.C., M.C.C., FG.N., T.M.T., M.S.S., A.M.B.F., A.L.A., W.K.O., J.C., C.F.C.A. and L.C.J.A.

Declaration of Interest: The authors declare no competing interests.

Figures legend

Figure 1. Zika virus transmission in Manaus. A. ZIKV confirmed (dark orange bars) and suspected (light orange bars) cases per week in Manaus municipality notified from the Brazilian Ministry of Health (MoH), number of weekly RT-PCR positive cases (red line) tested in the Instituto Leonidas & Maria Deane (ILMD) FIOCRUZ, Amazonas Brazil. Below, the dates of sample collection of the virus genomes generated in this study are shown using grey bars with transparency, such that darker shading reflects more dense sampling. B. Number of microcephaly cases per week in Manaus municipality notified to the Brazilian Ministry of Health. C. Daily transmission potential of Aedes aegyti in Manaus inferred using MVSE R-package21 from Manaus' climatic data. Relative humidity and temperature (Celsius degrees) were collected by an INMET weather station (for exact location see **Figure 7B**).

Figure 2. **Spatial incidence of Zika virus in Manaus**. *A*. Circular plot shows ZIKV incidence (blue: 2016, and red: 2017) and DENV incidence (orange: 2015, green: 2016) in the different areas of Manaus. The units for the incidence are cases per 1,000 inhabitants per year. *B*. Number of yearly ZIKV cases plotted against number of inhabitants per Manaus area (detailed data on ZIKV cases per neighborhood can be found in **Table S3**). The names of the 4 neighborhoods with highest number of cases are shown. Circle sizes are proportional to the number of sequenced genomes per neighborhood, which are colored by region of Manaus. See **Table S2** for more details.

Figure 3. Number of yearly ZIKV cases plotted against number of DENV cases per 1,000 inhabitants per year per Manaus area (detailed data on ZIKV cases per neighborhood can be found in **Table S3**). The names of the neighborhoods with highest number of cases are shown. Circle sizes are proportional to the number of sequenced genomes per neighborhood, which are colored by region of Manaus. See **Table S2** for more details.

Figure 4. Zika virus sequencing statistics. The percentage of ZIKV genome sequenced plotted against RT–qPCR Ct value for each sample (n= 59). Each circle represents a sequence recovered from an infected individual in Manaus city.

Figure 5. **Maximum likelihood phylogeny of Zika virus in the Americas.** Maximum likelihood phylogeny was estimated with 482 complete or near complete genome sequences from Oceania and from the Americas. Sequences or clades from Manaus are numbered from (1) to (4), with the Manaus clade (4) being supported by

94% bootstrap score. Colors represent different locations. Scale bar represents expected substitutions per nucleotide site.

Figure 6. Root-to-tip plot. Regression of sequence sampling dates against root-to-tip genetic distances in a maximum likelihood phylogeny of the Manaus clade. Sequences are colored according to the six areas of Manaus city (North, West, East, Centre-West, Centre-South and South).

Figure 7. Phylogeography of ZIKV within Manaus. A Maximum clade credibility phylogeographic tree of the Manaus outbreak clade (n = 56). Branch colours represent most probable inferred locations. The black circles at internal nodes are sized in proportion to clade posterior probabilities. The branch thicknesses are sized in proportion to the most probable inferred locations. B. Map showing the inferred patterns of Zika virus transmission within areas of Manaus city. Circles are proportional to the population size of each area of the city. The arrows are sized in proportion to the diffusion dispersal rate. C. Violin plot showing the posterior distribution of the total duration of phylogeny branches that are inferred to be located in each region of Manaus (Markov rewards). Colours represent different areas in Manaus as indicated in part (A). The posterior distribution was calculated from 9000 sampled trees.

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Figure 1

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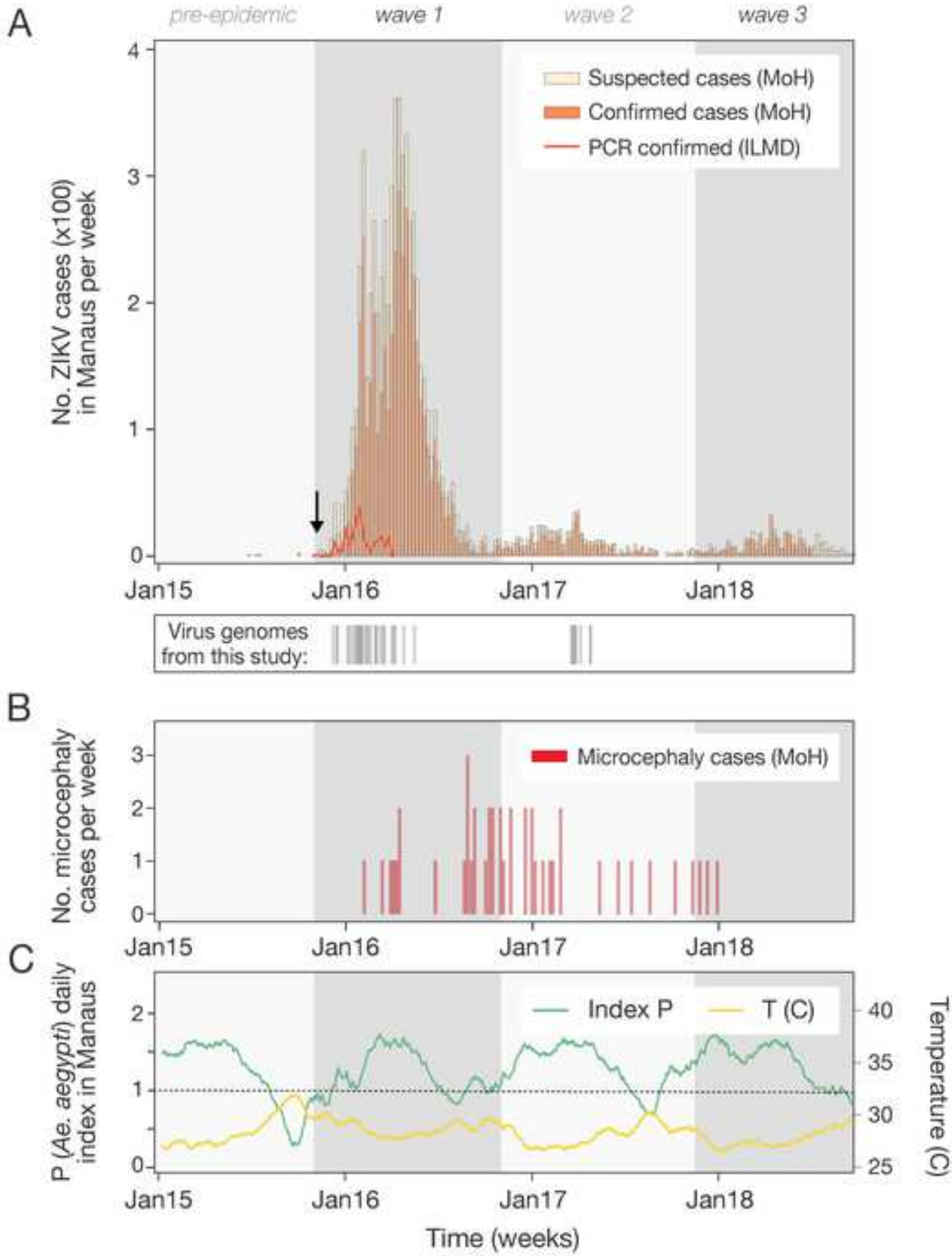

Figure 2

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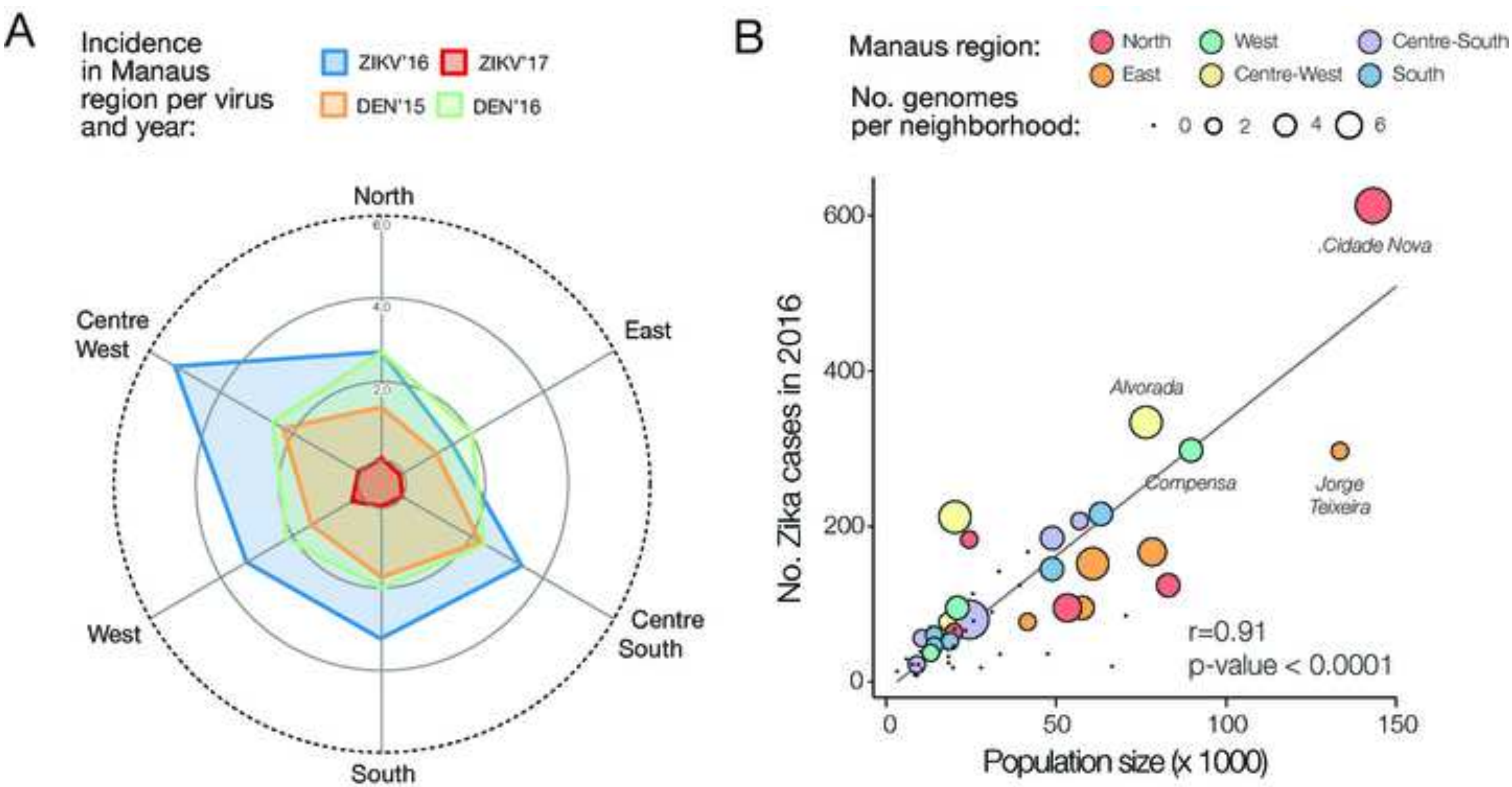

Figure 3

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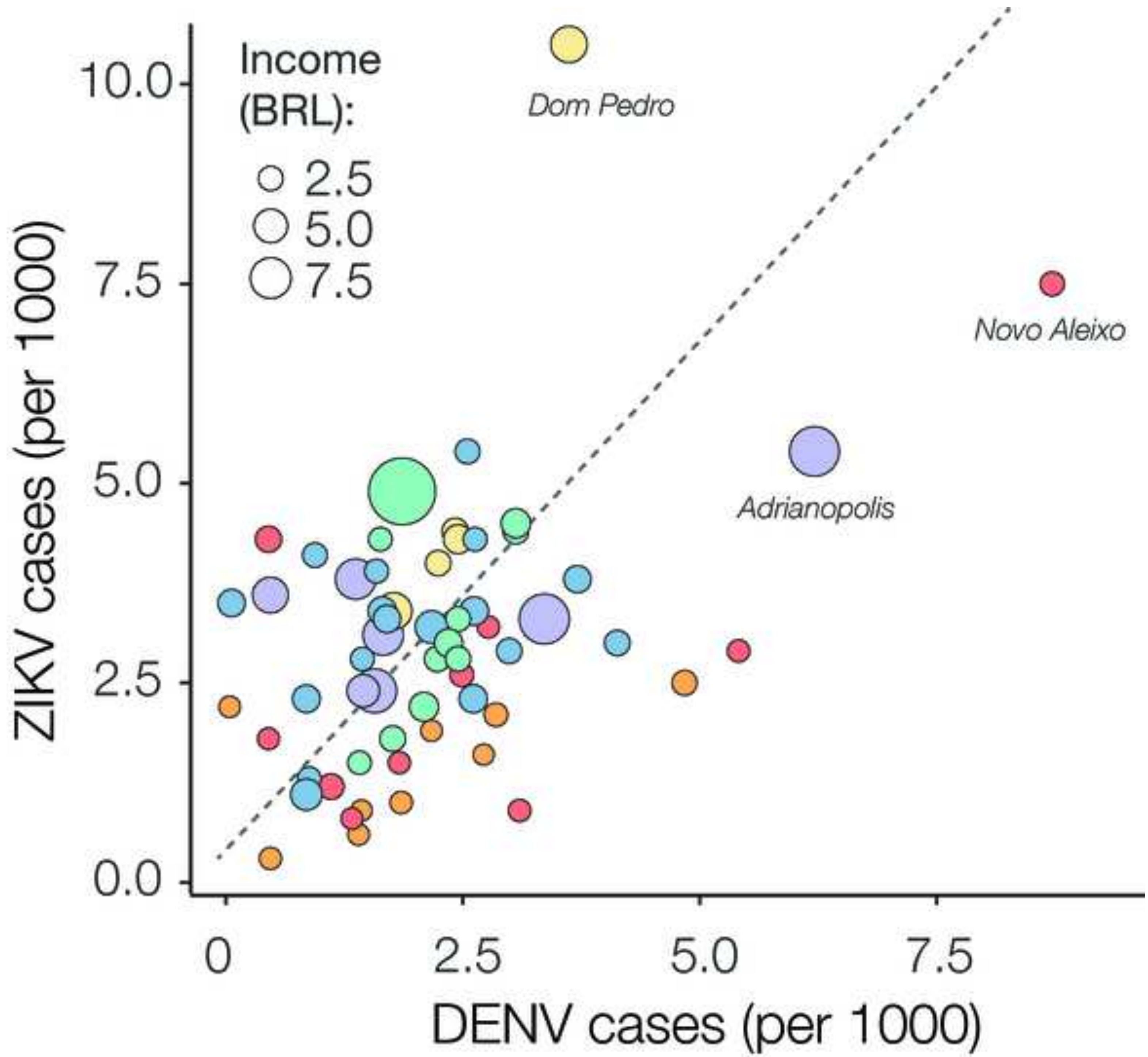

Figure 4

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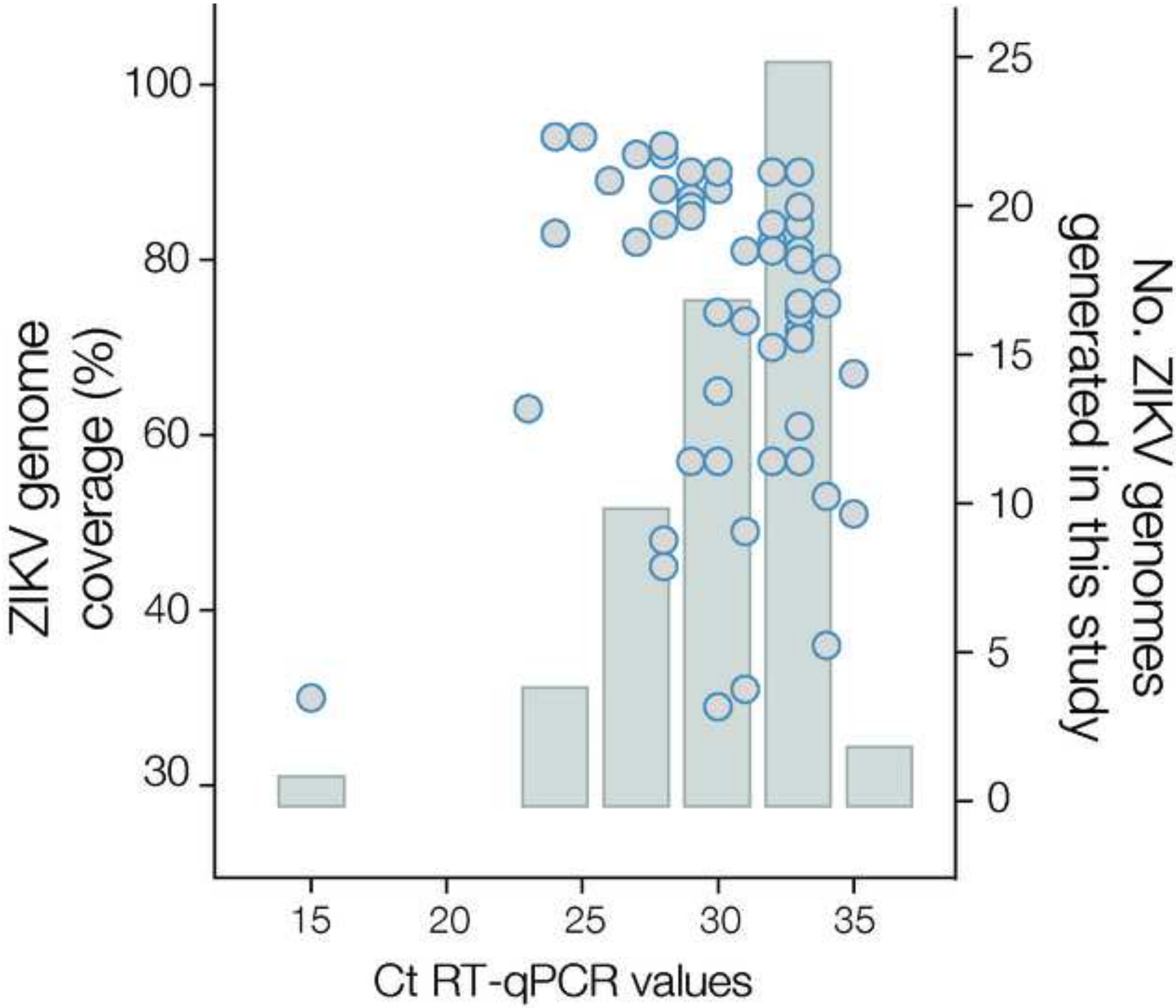

Figure 5

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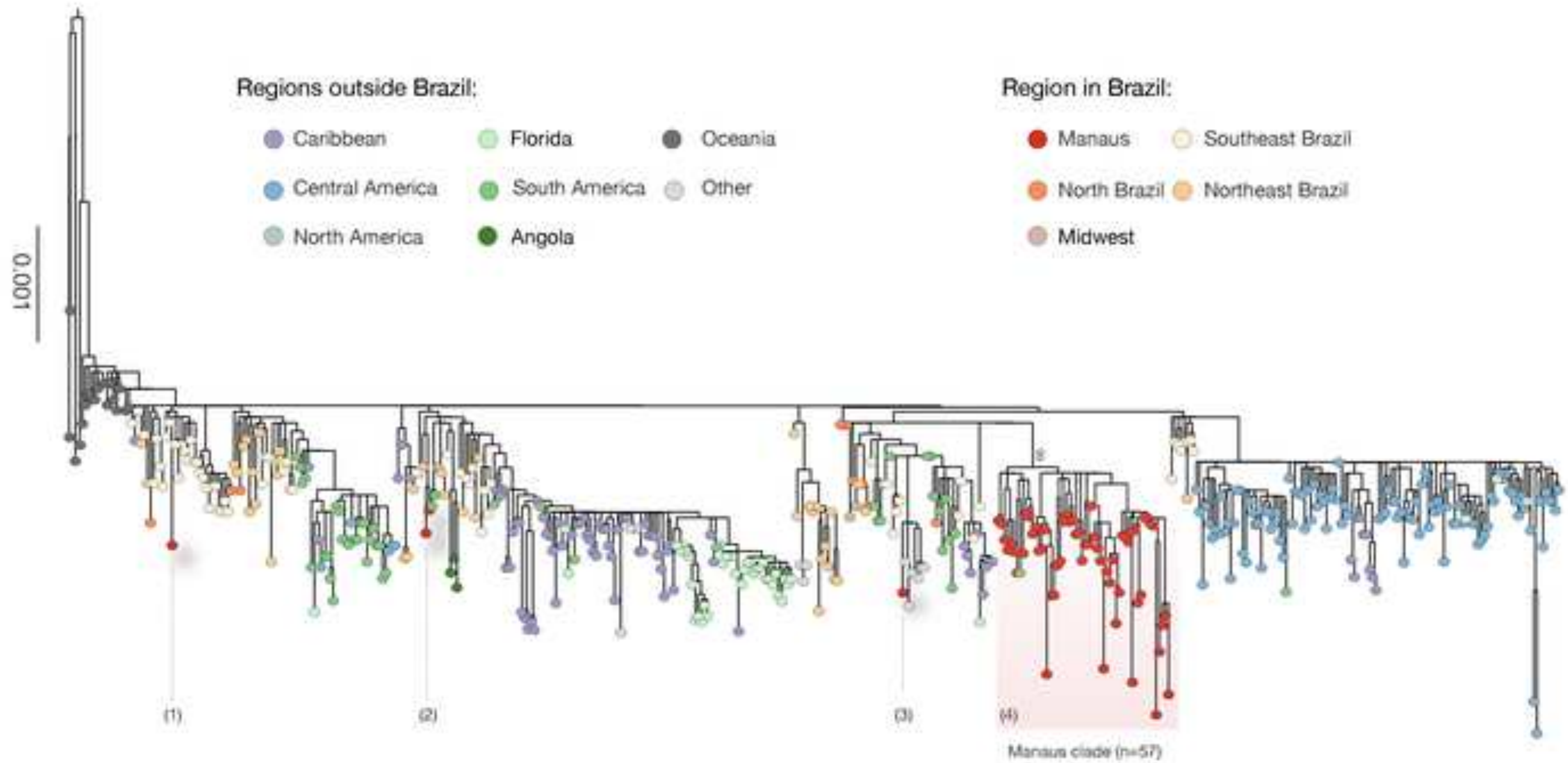
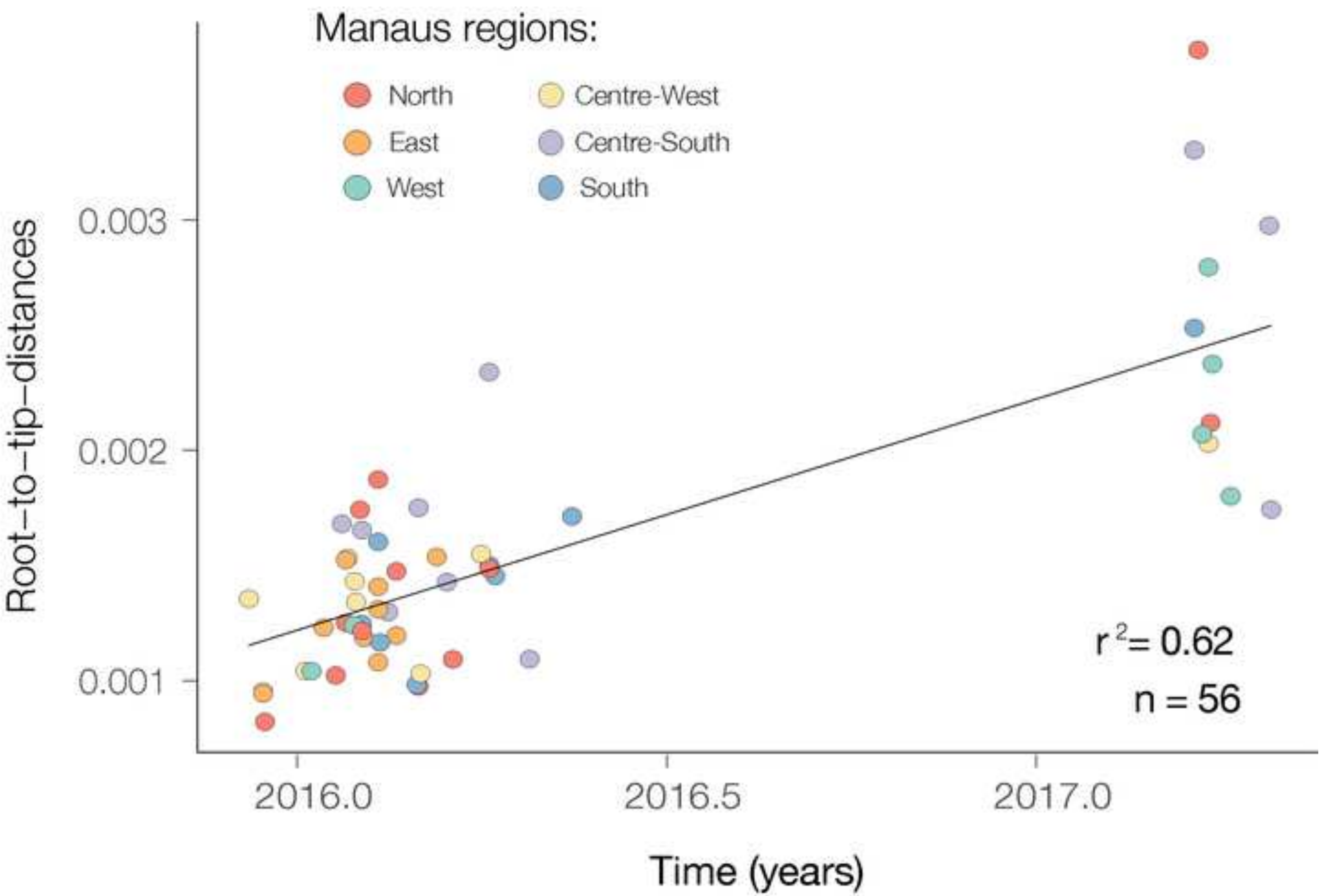


Figure 6



Manaus regions:
North
East
West
Centre-West
Centre-South
South
Root-to-tip-distances
0.003
0.002
0.001
2016.0
2016.5
2017.0
Time (years)
r 2= 0.62
n = 56

Figure 7

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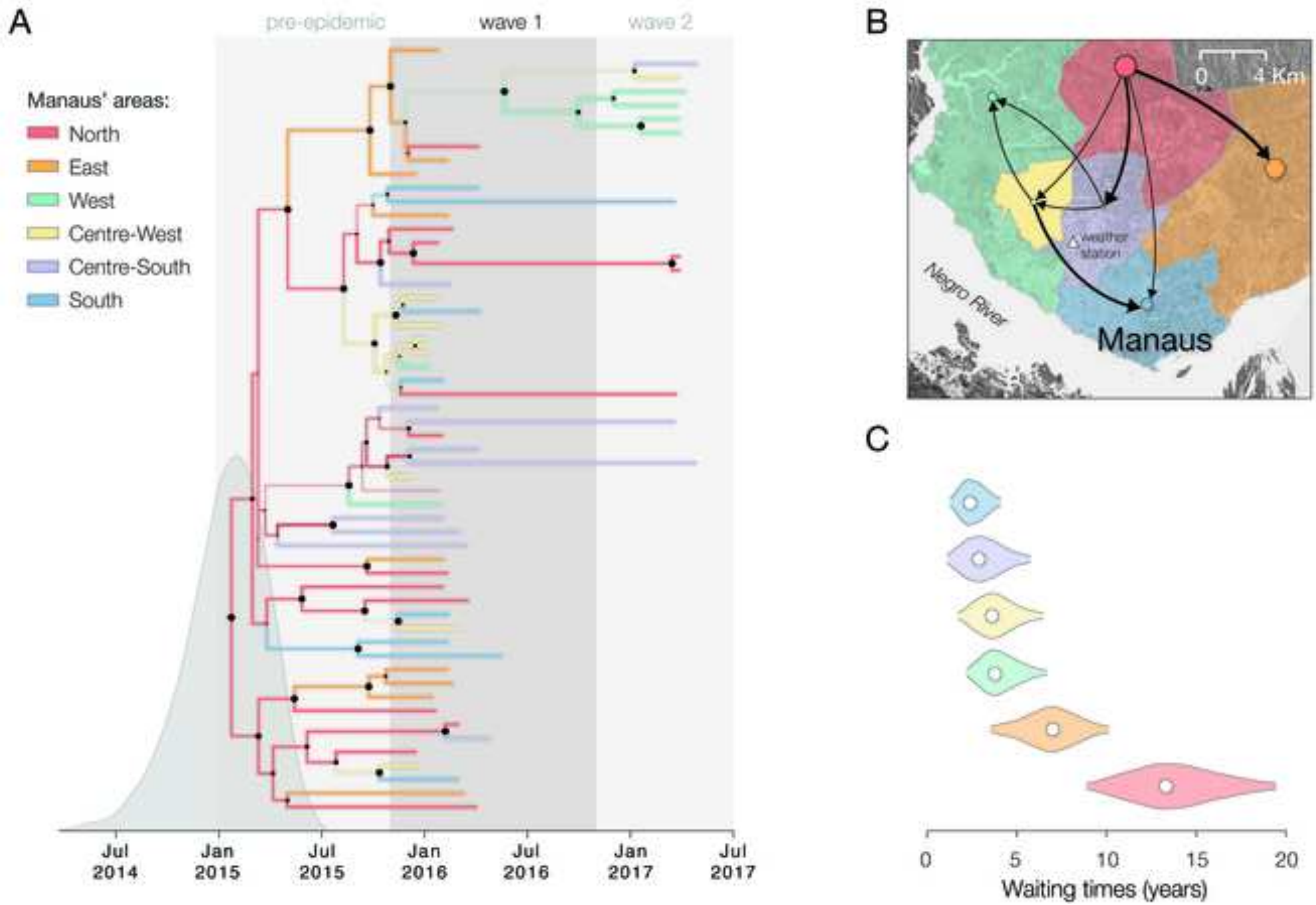

Supplementary Information

Supplementary table 1. Association between Zika virus cases and Microcephaly. The table provides the estimated correlated time period, Cross-correlation P-value, Coefficient P-value and time lag (in epidemiological weeks). Related to Figure 1.

Comparison	***Index P vs Zika confirmed***	***Zika confirmed vs Microcephaly counts***
Period	14th November 2015 - 10th August 2016	19th December 2015 - 20th Jan 2018
Cross-correlation P-value	1.59E-08	N/A
Coefficient P-value	0.037	6.92E-06
Cross-correlation	0.815	0.426
Time lag (Epi-Weeks)	Zika confirmed is 4.7 weeks behind index P	Microcephaly counts is 29.1 weeks behind Zika confirmed

Supplementary table 2. Epidemiological data from ZIKV and DENV incidence between 2015-2017 and density population calculated for each distinct Manaus's neighbourhoods. Related to Figures 2 and 3.

Neighbourhood	*Areas*	*Population 2017*	*IBGE 2010*	*ZIKV genomes*	*ZIKV 2016*	*ZIKV 2017*	*ZIKV 2016-2017*	*Incidence ZIKV 2016 1000*	*DENV 2015*	*DENV 2016*	*Incidence DENV 2015 1000*	*Incidence DENV 2016 1000*
Flores	Center-South	56859	2357	1	207	3	210	3.6	17	27	0.30	0.47
Parque 10 De Novembro	Center-South	48771	3112	2	185	3	188	3.8	43	67	0.88	1.37
São Geraldo	Center-South	8983	1898	1	22	0	22	2.4	6	13	0.67	1.45
Nossa Senhora Das Graças	Center-South	17869	3707	0	42	0	42	2.4	33	28	1.85	1.57
Chapada	Center-South	13219	3096	0	41	1	42	3.1	16	22	1.21	1.66
Aleixo	Center-South	24417	4930	6	80	4	84	3.3	76	82	3.11	3.36
Adrianópolis	Center-South	10459	4824	1	56	1	57	5.4	75	65	7.17	6.21
Planalto	Centre-West	19249	2500	0	66	2	68	3.4	42	34	2.18	1.77
Rendenção	Centre-West	41572	1090	0	167	2	169	4.0	118	93	2.84	2.24
Alvorada	Centre-West	76392	1171	4	334	9	343	4.4	146	185	1.91	2.42
Bairro Da Paz	Centre-West	17961	1464	1	77	2	79	4.3	25	44	1.39	2.45
Dom Pedro	Centre-West	20179	2456	4	212	3	215	10.5	49	73	2.43	3.62
Jorge Teixeira	East	133441	726	1	297	4	301	2.2	2	6	0.01	0.04
Gilberto Mestrinho	East	66429	784	0	20	0	20	0.3	17	31	0.26	0.47
Mauazinho	East	27852	734	0	18	0	18	0.6	25	39	0.90	1.40
Colônia Antônio Aleixo	East	19626	714	0	18	0	18	0.9	11	28	0.56	1.43
Armando Mendes	East	33441	802	0	35	0	35	1.0	46	62	1.38	1.85
Zumbi Dos Palmares	East	41563	725	1	77	3	80	1.9	56	90	1.35	2.17
Tancredo Neves	East	57728	700	2	95	3	98	1.6	68	157	1.18	2.72
Sao José	East	78222	920	3	167	1	168	2.1	139	223	1.78	2.85
Coroado	East	60709	1049	4	152	1	153	2.5	85	294	1.40	4.84
Cidade Nova	North	143201	1174	5	613	16	629	4.3	190	64	1.33	0.45
Colônia Terra Nova	North	53287	761	3	95	5	100	1.8	6	24	0.11	0.45
Nova Cidade	North	70428	1129	0	85	4	89	1.2	40	78	0.57	1.11
Monte das Oliveiras	North	47478	743	0	36	2	38	0.8	28	63	0.59	1.33
Cidade de Deus	North	82919	787	2	124	3	127	1.5	81	152	0.98	1.83

Colônia Santo Antonio	North	20851	925	0	54	2	56	2.6	40	52	1.92	2.49
Novo Israel	North	19887	792	1	64	1	65	3.2	16	55	0.80	2.77
Lago Azul	North	9022	816	0	8	1	9	0.9	2	28	0.22	3.10
Santa Etelvina	North	31034	814	0	90	4	94	2.9	68	168	2.19	5.41
Novo Aleixo	North	24417	973	1	183	2	185	7.5	105	213	4.30	8.72
Raiz	South	16694	1293	0	59	1	60	3.5	34	1	2.04	0.06
Nossa Senhora Aparecida	South	8270	1764	0	9	1	10	1.1	8	7	0.97	0.85
Presidente Vargas	South	9391	1327	0	22	0	22	2.3	16	8	1.70	0.85
Crespo	South	18266	908	0	24	0	24	1.3	23	16	1.26	0.88
Distrito Industrial	South	3201	1008	0	13	3	16	4.1	7	3	2.19	0.94
Educandos	South	18745	907	1	52	1	53	2.8	27	27	1.44	1.44
Colônia Oliveira Machado	South	10055	939	0	39	0	39	3.9	10	16	0.99	1.59
Cachoeirinha	South	20035	1414	0	68	2	70	3.4	29	33	1.45	1.65
São Lazaro	South	14108	1299	1	46	0	46	3.3	35	24	2.48	1.70
Centro	South	39228	1927	0	124	1	125	3.2	64	85	1.63	2.17
Betânia	South	12940	1028	0	70	2	72	5.4	32	33	2.47	2.55
São Francisco	South	19889	1385	0	46	0	46	2.3	56	52	2.82	2.61
Japiim	South	63092	1418	2	216	2	218	3.4	130	165	2.06	2.62
Morro da Liberdade	South	14078	931	1	60	0	60	4.3	19	37	1.35	2.63
Santa Luzia	South	7688	1075	0	22	0	22	2.9	5	23	0.65	2.99
Praça 14 De Janeiro	South	12117	1283	0	46	0	46	3.8	38	45	3.14	3.71
Petrópolis	South	48717	1094	2	145	2	147	3.0	130	201	2.67	4.13
Glória	West	10617	900	0	16	0	16	1.5	4	15	0.38	1.41
Tarumã	West	33168	914	0	142	10	152	4.3	46	54	1.39	1.63
São Raimundo	West	18199	1135	0	32	1	33	1.8	12	32	0.66	1.76
Ponta Negra	West	5919	9102	0	29	2	31	4.9	19	11	3.21	1.86
Santo Agostinho	West	19616	1483	0	43	3	46	2.2	39	41	1.99	2.09
Santo Antônio	West	23356	1120	0	66	6	72	2.8	40	52	1.71	2.23
São Jorge	West	25585	1367	0	78	3	81	3.0	52	60	2.03	2.35
Vila da Prata	West	13052	1049	1	37	4	41	2.8	18	32	1.38	2.45
Compensa	West	89645	1032	2	298	10	308	3.3	131	220	1.46	2.45
Nova Esperança	West	20919	1507	2	95	12	107	4.5	34	64	1.63	3.06
Lírio Do Vale	West	25457	1105	0	113	1	114	4.4	57	78	2.24	3.06

Supplementary table 3. Epidemiological data from ZIKV and DENV incidence between 2015-2017 and density population calculated for each distinct Manaus's areas. Related to Figures 2, 3 and 7.

Areas	*Pop 2017*	*IBGE 2010*	*ZIKV genomes*	*ZIKV2016*	*ZIKV 2017*	*ZIKV 2016 - 2017*	*Incidence ZIKV 2016 1000*	*Incidence ZIKV 2017 1000*	*DENV 2015*	*DENV 2016*	*Incidence DENV 2015_1000*	*Incidence DENV 2016 1000*
North	502524	891,4	14	1352	40	1392	2,7	0,1	576	897	1,3	2,8
East	519011	794,8888889	9	879	12	891	1,5	0,0	449	930	1,0	2,0
Center-South	180577	3417,714286	10	633	12	645	3,4	0,1	266	304	2,2	2,3
South	336514	1235,294118	8	1061	15	1076	3,2	0,0	663	776	1,8	2,0
West	285533	1883,090909	6	949	52	1001	3,2	0,2	452	659	1,6	2,2
Centre-West	175353	1736,2	8	856	18	874	5,3	0,1	380	429	2,2	2,5

Supplementary table 4. Sample information. ID = study identifier, Ct value = RT-qPCR quantification cycle threshold value. Related to Figure 4.

ID	*Sample type*	*Host*	*State*	*Municipality*	*Neighborhood*	*Collection date*	*Date onset*	*CT Values*	*Sex*	*Sequences obtained from Manaus*	*Sequences from Manaus-Clade*
AMA1	saliva	Human	Amazonas	Manaus	Japiim	11/02/16	07/02/16	32.27	M	no	no
AMA2	saliva	Human	Amazonas	Manaus	Colônia Terra Nova	19/02/16	17/02/16	28.97	F	yes	yes
AMA3	plasma	Human	Amazonas	Manaus	Morro da Liberdade	26/02/2016	23/02/16	32.74	F	no	no
AMA4	saliva	Human	Amazonas	Manaus	Adrianópolis	22/02/2016	17/02/16	29.61	F	no	no
AMA5	plasma	Human	Amazonas	Manaus	São José 3	10/03/16	05/03/16	32.79	F	yes	yes
AMA6	plasma	Human	Amazonas	Manaus	Aleixo	15/03/16	11/03/16	33.98	F	yes	yes
AMA7	saliva	Human	Amazonas	Manaus	Raiz	15/03/16	10/03/16	29.09	M	no	no
AMA8	plasma	Human	Amazonas	Manaus	Cidade Nova	15/03/16	11/03/16	32.55	M	no	no
AMA9	plasma	Human	Amazonas	Manaus	Cidade de Deus	18/03/16	16/03/16	28.31	F	yes	yes
AMA10	saliva	Human	Amazonas	Manaus	Japiim	23/03/2016	20/03/16	33.03	M	no	no
AMA11	plasma	Human	Amazonas	Manaus	Cidade Nova	31/03/2016	30/03/16	31.85	M	no	no
AMA12	saliva	Human	Amazonas	Manaus	Japiim	14/03/16	10/03/16	33.26	F	no	no
AMA13	plasma	Human	Amazonas	Manaus	Amazonino Mendes	01/04/16	30/03/16	33.46	M	yes	yes
AMA14	saliva	Human	Amazonas	Manaus	Coroado	06/04/16	02/04/16	32.82	F	yes	no
AMA15	plasma	Human	Amazonas	Manaus	Educandos	05/04/16	04/04/16	34.83	M	yes	yes
AMA16	plasma	Human	Amazonas	Manaus	Cidade Nova	05/04/16	02/04/16	32.43	F	yes	yes
AMA17	saliva	Human	Amazonas	Manaus	Japiim	05/04/16	02/04/16	30.49	F	no	no
AMA18	plasma	Human	Amazonas	Manaus	Japiim	06/04/16	03/04/16	35.37	M	no	no
AMA19	plasma	Human	Amazonas	Manaus	Petrópolis	08/04/16	04/04/16	28.52	M	yes	yes
AMA20	plasma	Human	Amazonas	Manaus	Aleixo	26/04/2016	25/04/16	26.81	F	yes	no
AMA21	plasma	Human	Amazonas	Manaus	Aleixo	25/04/2016	24/04/16	26.31	M	yes	yes
AMA22	plasma	Human	Amazonas	Manaus	Japiim	16/05/16	13/05/16	30.48	M	yes	yes
AMA23	saliva	Human	Amazonas	Manaus	Parque 10	26/04/17	20/04/17	30.09	M	yes	yes
AMA24	plasma	Human	Amazonas	Manaus	Flores	27/04/17	NA	33.5	M	yes	yes
AMA26	plasma	Human	Amazonas	Manaus	Japiim	11/02/16	NA	31.1	F	yes	yes
AMA27	plasma	Human	Amazonas	Manaus	Parque 10	15/02/16	12/02/16	29.97	F	yes	yes
AMA28	urine	Human	Amazonas	Manaus	Nossa Senhora das Graças	29/02/2016	23/02/16	32.19	M	no	no
AMA29	saliva	Human	Amazonas	Manaus	Cidade Nova	31/03/2016	26/03/16	34.37	F	no	no
AMA30	plasma	Human	Amazonas	Manaus	Grande Vitória	30/03/2016	28/03/16	35.48	F	no	no
AMA31	plasma	Human	Amazonas	Manaus	Aleixo	05/04/16	01/04/16	30.6	F	no	no

AMA32	plasma	Human	Amazonas	Manaus	Aleixo	05/04/16	01/04/16	32.33	F	yes	yes
AMA33	plasma	Human	Amazonas	Manaus	Tancredo Neves	19/02/16	15/02/16	34.4	F	yes	yes
AMA34	plasma	Human	Amazonas	Manaus	Flores	04/03/16	02/03/16	33.78	M	no	no
AMA35	plasma	Human	Amazonas	Manaus	São Lazaro	29/02/2016	25/02/16	22.85	F	yes	yes
AMA36	plasma	Human	Amazonas	Manaus	Cidade de Deus	01/03/16	27/02/16	29.8	F	yes	yes
AMA37	plasma	Human	Amazonas	Manaus	Adrianópolis	01/03/16	26/02/16	32,51	M	yes	yes
AMA38	plasma	Human	Amazonas	Manaus	Santos Dumont	02/03/16	29/02/16	32,29	M	yes	yes
AMA39	NA	control	Amazonas	Manaus	Na	NA	NA	-	-	no	no
AMA40	serum	Human	Amazonas	Manaus	João Paulo II	05/02/16	31/01/16	32.6	F	no	no
AMA41	serum	Human	Amazonas	Manaus	Coroado	03/02/16	31/01/16	29.32	F	yes	yes
AMA42	serum	Human	Amazonas	Manaus	Cidade Nova	10/02/16	07/02/16	32.85	M	yes	yes
AMA43	serum	Human	Amazonas	Manaus	Coroado	10/02/16	09/02/16	29.24	F	yes	yes
AMA44	serum	Human	Amazonas	Manaus	Nova Esperança	29/01/16	28/01/16	32.5	M	yes	yes
AMA45	serum	Human	Amazonas	Manaus	Dom Pedro	08/01/16	05/01/16	23.61	F	yes	yes
AMA46	serum	Human	Amazonas	Manaus	Morro da Liberdade	10/02/16	08/02/16	32.34	F	yes	yes
AMA47	serum	Human	Amazonas	Manaus	Petrópolis	02/02/16	31/01/16	28.2	M	yes	yes
AMA48	serum	Human	Amazonas	Manaus	Novo Aleixo	02/02/16	30/01/16	29.43	F	yes	yes
AMA49	serum	Human	Amazonas	Manaus	São José	10/02/16	08/02/16	26.77	M	yes	yes
AMA50	serum	Human	Amazonas	Manaus	Jorge Teixeira	10/02/16	10/02/16	30.7	F	yes	yes
AMA51	serum	Human	Amazonas	Manaus	Tancredo Neves	25/01/16	24/01/16	33.97	M	yes	yes
AMA52	serum	Human	Amazonas	Manaus	Aleixo	02/02/16	02/02/16	32.76	F	yes	yes
AMA53	urine	Human	Amazonas	Manaus	Alvorada	25/01/16	22/01/16	33.15	F	yes	no
AMA54	serum	Human	Amazonas	Manaus	Nossa Senhora das Graças	26/01/16	23/01/16	30.58	F	no	no
AMA55	serum	Human	Amazonas	Manaus	Dom Pedro	26/01/16	25/01/16	32.69	F	yes	yes
AMA56	serum	Human	Amazonas	Manaus	São José	04/01/16	02/01/16	33.43	F	no	no
AMA57	serum	Human	Amazonas	Manaus	São José	14/01/16	12/01/16	31.76	F	yes	yes
AMA58	serum	Human	Amazonas	Manaus	Colônia Terra Nova	20/01/16	18/01/16	32.24	F	yes	yes
AMA59	serum	Human	Amazonas	Manaus	Zumbi	14/01/16	11/01/16	28.31	F	yes	no
AMA60	serum	Human	Amazonas	Manaus	João Paulo II	27/01/16	22/01/16	33.91	F	no	no
AMA61	serum	Human	Amazonas	Manaus	Rio Piorini	16/12/15	12/12/15	33.6	F	yes	yes
AMA62	serum	Human	Amazonas	Manaus	Cidade Nova	25/01/16	21/01/16	30.33	F	yes	yes
AMA63	serum	Human	Amazonas	Manaus	Cidade Nova	28/01/16	23/01/16	-	F	no	no
AMA64	serum	Human	Amazonas	Manaus	Cidade Nova	25/01/16	20/01/16	33.78	F	no	no
AMA65	serum	Human	Amazonas	Manaus	Dom Pedro	30/01/16	29/01/16	24.89	F	yes	yes
AMA66	serum	Human	Amazonas	Manaus	São Geraldo	23/01/16	23/01/16	28.84	F	yes	yes
AMA67	serum	Human	Amazonas	Manaus	Dom Pedro	05/01/16	03/01/16	29.85	F	yes	yes
AMA68	serum	Human	Amazonas	Manaus	Novo Israel	01/02/16	27/01/16	31.00	F	yes	yes
AMA69	serum	Human	Amazonas	Manaus	Dom Pedro	24/01/16	20/01/16	33.66	M	no	no

AMA70	serum	Human	Amazonas	Manaus	Alvorada	29/01/16	27/01/16	33.31	F	yes	yes
AMA71	urine	Human	Amazonas	Manaus	Manoa	20/03/2017	17/03/17	27.90	F	yes	yes
AMA72	urine	Human	Amazonas	Manaus	Aleixo	20/03/2017	16/03/17	15.19	M	yes	yes
AMA73	urine	Human	Amazonas	Manaus	Alvorada 1	20/03/2017	15/03/17	28.65	M	no	no
AMA77	urine	Human	Amazonas	Manaus	Japiim 2	22/03/2017	18/03/17	28.06	F	no	no
AMA78	urine	Human	Amazonas	Manaus	Planalto	22/03/2017	19/03/17	28.72	F	no	no
AMA79	urine	Human	Amazonas	Manaus	Santo Antonio	22/03/2017	18/03/17	29.94	M	no	no
AMA80	serum	Human	Amazonas	Manaus	Colonia Terra Nova	22/03/17	NA	34.91	-	yes	yes
AMA81	urine	Human	Amazonas	Manaus	Lago Azul	23/03/2017	20/03/17	28.60	F	no	no
AMA82	plasma	Human	Amazonas	Manaus	Nova Esperança 2	24/03/17	21/03/17	31.0	F	yes	yes
AMA83	urine	Human	Amazonas	Manaus	Nossa Senhora das Graças	27/03/17	23/03/17	30.77	M	no	no
AMA84	plasma	Human	Amazonas	Manaus	Alvorada	27/03/17	23/03/17	33.0	F	yes	yes
AMA85	plasma	Human	Amazonas	Manaus	Vila Da Prata	27/03/17	25/03/17	32.0	F	yes	yes
AMA86	urine	Human	Amazonas	Manaus	Dom Pedro	27/03/17	26/03/17	31.83	F	no	no
AMA87	urine	Human	Amazonas	Manaus	Nova Cidade	27/03/17	23/03/17	30.96	M	no	no
AMA88	urine	Human	Amazonas	Manaus	Cidade Nova	28/03/17	26/03/17	24.15	M	yes	yes
AMA89	urine	Human	Amazonas	Manaus	Cidade Nova	28/03/17	23/03/17	27.51	F	yes	yes
AMA90	urine	Human	Amazonas	Manaus	Compensa	29/03/17	25/03/17	31.46	F	yes	yes
AMA91	urine	Human	Amazonas	Manaus	Compensa	07/04/17	24/03/17	27.21	F	yes	yes
AMA92	CSF	Human	Amazonas	Manaus	Na	22/01/16	NA	34.50	M	no	no
AMA93	CSF	Human	Amazonas	Manaus	Na	03/05/16	NA	36.30	F	no	no
AMA94	CSF	Human	Amazonas	Manaus	Na	20/05/16	NA	32.40	F	no	no
AMA95	CSF	Human	Amazonas	Manaus	Na	23/05/16	NA	36.10	M	no	no
AMA96	CSF	Human	Amazonas	Manaus	Na	15/06/16	NA	34.30	M	no	no
AMA97	CSF	Human	Amazonas	Manaus	Na	15/06/16	NA	36.60	M	no	no
AMA98	CSF	Human	Amazonas	Manaus	Na	28/03/17	NA	34.30	M	no	no
AMA99	CSF	Human	Amazonas	Manaus	Na	04/04/17	NA	33.0	M	no	no
AMA100	CSF	Human	Amazonas	Manaus	Na	15/04/17	NA	36.50	F	no	no
AMA101	CSF	Human	Amazonas	Manaus	Na	27/04/17	NA	36.0	F	no	no
AMA102	CSF	Human	Amazonas	Manaus	Na	28/04/17	NA	34.8	F	no	no
AMA284	serum	Human	Amazonas	Manaus	Na	11/07/16	NA	37.4	M	no	no
AMA285	urine	Human	Amazonas	Manaus	Na	11/07/16	NA	38.0	M	no	no
AMA289	urine	Human	Amazonas	Manaus	Na	18/12/15	11/12/15	34.94	F	no	no
AMA302	serum	Human	Amazonas	Manaus	Bairro_da_Paz	18/12/15	NA	28.19	F	yes	yes
AMA303	serum	Human	Amazonas	Manaus	Na	15/12/15	12/12/15	35.24	M	no	no
AMA304	serum	Human	Amazonas	Manaus	Na	15/12/15	12/12/15	35.89	M	no	no
Z02	serum	Human	Amazonas	Manaus	Coroado	15/12/15	NA	32.41	F	yes	yes
Z03	serum	Human	Amazonas	Manaus	Alvorada	15/12/15	NA	33.44	F	yes	yes

Supplementary Table 5. Epidemiological and sequencing statistics data for the sequenced samples. ID=study identifier; Accession number=NCBI accession number; QC= Quality control of a flow cell-number of available pores; Ct=RT-qPCR quantification cycle threshold value, d=Date onset. Related to Figure 4.

ID	State. Municipality	Neighbourhoods	Acc. Number	Sample type	CT Values	Sex	Collection date	d	Mapped reads	Average depth coverage	Bases covered >10x	Bases covered > 25x	Referenc e covered (%)
AMA2	AM. Manaus	Colônia Terra Nova	MK216687	saliva	28.97	F	19/02/16	2	13341	555	10038	9710	87.43
AMA5	AM. Manaus	São José 3	MK216688	plasma	32.79	F	10/03/16	5	15328	628	9837	9295	80.52
AMA6	AM. Manaus	Aleixo	MK216691	plasma	33.98	F	15/03/16	4	16995	708	9166	8987	78.73
AMA9	AM. Manaus	Cidade Deus	MK216690	plasma	28.31	F	18/03/16	2	5645	233	9764	9703	87.5
AMA13	AM. Manaus	Amazonino Mendes	MK216692	plasma	33.46	M	01/04/16	2	17191	687	9354	9044	80.07
AMA14	AM. Manaus	Coroado	MK216693	saliva	32.82	F	06/04/16	4	16521	726	9623	9251	83.72
AMA15	AM. Manaus	Educandos	MK216694	plasma	34.83	M	05/04/16	1	15863	672	8564	7924	67.25
AMA16	AM. Manaus	Cidade Nova	MK216695	plasma	32.43	F	05/04/16	3	10785	436	9719	9434	82.49
AMA19	AM. Manaus	Petrópolis	MK216696	plasma	28.52	M	08/04/16	4	9198	378	10176	9949	89.97
AMA20	AM. Manaus	Aleixo	MK216697	plasma	26.81	F	26/04/16	1	10525	427	10257	10060	91.74
AMA21	AM. Manaus	Aleixo	MK216698	plasma	26.31	M	25/04/16	1	58065	1794	10216	10043	89.49
AMA22	AM. Manaus	Japim	MK216699	plasma	30.48	M	16/05/16	3	219506	4636	10167	9915	87.54
AMA23	AM. Manaus	Parque 10	MK216700	saliva	30.09	M	26/04/17	6	356	20	5448	3335	28.75
AMA24	AM. Manaus	Flores	MK216701	plasma	33.5	M	27/04/17	4	525	40	8003	5298	36.2
AMA26	AM. Manaus	Japim	MK216702	plasma	31.1	F	11/02/16	NA	588	41	7247	4844	30.76
AMA27	AM. Manaus	Parque 10	MK216703	plasma	29.97	F	15/02/16	3	1983	121	9468	8761	74.39
AMA32	AM. Manaus	Aleixo	MK216705	plasma	32.33	F	05/04/16	4	20686	853	9237	8100	56.99
AMA33	AM. Manaus	Tancredo Neves	MK216704	plasma	34.4	F	19/02/16	4	48626	1690	9576	8929	53.27
AMA35	AM. Manaus	São Lazaro	MK216707	plasma	22.85	F	29/02/16	4	25509	1047	9873	8876	62.55
AMA36	AM. Manaus	Cidade de Deus	MK216706	plasma	29.8	F	01/03/16	3	25873	1088	9331	8585	56.98
AMA37	AM. Manaus	Adrianópolis	MK216709	plasma	32.51	M	01/03/16	4	38252	1573	9624	9196	72.49
AMA38	AM. Manaus	Santos Dumont	MK216708	plasma	32.29	M	02/03/16	2	34312	1415	9395	8689	57.01
AMA41	AM. Manaus	Coroado	MK216710	serum	29.32	F	03/02/16	3	17845	715	10104	9902	90.08
AMA42	AM. Manaus	Cidade Nova	MK216712	serum	32.85	M	10/02/16	3	27720	1127	9151	8912	79.74
AMA43	AM. Manaus	Coroado	MK216711	serum	29.24	F	10/02/16	1	6121	273	7325	6616	56.53
AMA44	AM. Manaus	Nova Esperança	MK216714	serum	32.5	M	29/01/16	1	27455	1117	9683	9631	86.46

AMA45	AM. Manaus	Dom Pedro	MK216713	serum	23.61	F	08/01/16	3	47788	1848	10424	10405	93.99
AMA46	AM. Manaus	Morro Da Liberdade	MK216715	serum	32.34	F	10/02/16	2	40955	1680	10187	10118	90.17
AMA47	AM. Manaus	Petropolis	MK216716	serum	28.2	M	02/02/16	2	52781	2139	10296	10240	91.8
AMA48	AM. Manaus	Novo Aleixo	MK216717	serum	29.43	F	02/02/16	3	53819	2176	9747	9632	86.46
AMA49	AM. Manaus	São José	MK216718	serum	26.77	M	10/02/16	2	48712	1969	10281	10248	91.78
AMA50	AM. Manaus	Jorge Teixeira	MK216719	serum	30.7	F	10/02/16	0	26840	1158	9576	9293	80.81
AMA51	AM. Manaus	Tancredo Neves	MK216720	serum	33.97	M	25/01/16	1	34012	1522	8833	8524	74.67
AMA52	AM. Manaus	Aleixo	MK216732	serum	32.76	F	02/02/16	0	32759	1364	7770	7581	56.97
AMA53	AM. Manaus	Alvorada	MK216721	urine	33.15	F	25/01/16	3	87106	3152	10313	10171	90.19
AMA55	AM. Manaus	Dom Pedro	MK216725	serum	32.69	F	26/01/16	1	93560	3017	8644	8441	71.14
AMA57	AM. Manaus	São José	MK216723	serum	31.76	F	14/01/16	2	54658	2386	9361	9308	83.84
AMA58	AM. Manaus	Colonia Terra Nova	MK216722	serum	32.24	F	20/01/16	2	71556	2893	9689	9432	83.84
AMA59	AM. Manaus	Zumbi	MK216724	serum	28.31	F	14/01/16	3	57671	2004	6372	6100	45.41
AMA61	AM. Manaus	Rio Piorini	MK216729	serum	33.6	F	16/12/15	4	69556	1919	7697	7006	52.86
AMA62	AM. Manaus	Cidade Nova	MK216726	serum	30.33	F	25/01/16	4	52960	2205	10084	10019	89.53
AMA65	AM. Manaus	Dom Pedro	MK216727	serum	24.89	F	30/01/16	1	43356	1769	10372	10185	93.63
AMA66	AM. Manaus	Sao Geraldo	MK216728	serum	28.84	F	23/01/16	0	59060	2393	9526	9425	85.49
AMA67	AM. Manaus	Dom Pedro	MK216730	serum	29.85	F	05/01/16	2	33535	1413	7954	7780	65.22
AMA68	AM. Manaus	Novo Israel	MK216733	serum	31.00	F	01/02/16	5	12246	549	7629	6547	49.13
AMA70	AM. Manaus	Alvorada	MK216731	serum	33.31	F	29/01/16	2	50127	2046	8590	8275	74.44
AMA71	AM. Manaus	Manoa	MK216734	urine	27.9	F	20/03/17	3	1022	50	7233	4789	48.13
AMA72	AM. Manaus	Aleixo	MK216735	urine	15.19	M	20/03/17	4	1191	73	4464	3537	29.96
AMA80	AM. Manaus	Colônia Terra Nova	MK216737	serum	34.91	-	22/03/17	-	2097	101	6776	5995	51.12
AMA82	AM. Manaus	Nova Esperança 2	MK216738	plasma	31.17	F	24/03/17	3	23014	999	9055	8679	73.46
AMA84	AM. Manaus	Alvorada	MK216736	plasma	32.98	F	27/03/17	4	71156	1748	8046	7462	61.34
AMA85	AM. Manaus	Vila Da Prata	MK216740	plasma	32.09	F	27/03/17	2	40434	1437	8554	7948	69.79
AMA88	AM. Manaus	Cidade Nova	MK216743	urine	24.16	M	28/03/17	2	18957	791	9979	9525	83.41
AMA89	AM. Manaus	Cidade Nova	MK216741	urine	27.52	F	28/03/17	5	17429	701	9652	9474	83.82
AMA90	AM. Manaus	Compensa	MK216742	urine	31.47	F	29/03/17	4	28933	1169	9554	9403	81.09
AMA91	AM. Manaus	Compensa	MK216744	urine	27.21	F	07/04/17	14	47445	1963	9702	9337	82.03
AMA302	AM. Manaus	Bairro da Paz	MK216745	serum	28.19	-	08/12/15	-	109246	3698	10480	10383	93.46
Z02	AM. Manaus	Coroado	MK216748	serum	32.41	F	15/12/15	-	261109	3731	9235	8956	81.37
Z03	AM. Manaus	Alvorada	MK216747	serum	33.44	F	15/12/15	4	26785	1023	8750	8619	75.14

Supplementary Table 6. Sampling location (Neighborhood, area in Manaus) of each geo-referenced ZIKV sequence. Related to Figure 7.

Taxa	Neighborhood	Latitude	Longitude
AMA13|Brazil_Amazonas_Manaus|Amazonino_Mendes|2016-04-01	Amazonino Mendes	-3.0592191	-60.0341907
AMA15|Brazil_Amazonas_Manaus|Educandos|2016-04-05	Educandos	-3.1412	-60.01
AMA16|Brazil_Amazonas_Manaus|Cidade_Nova|2016-04-05	Cidade Nova	-3.0363	-59.9855
AMA19|Brazil_Amazonas_Manaus|Petropolis|2016-04-08	Petropolis	-3.1089	-59.9942
AMA21|Brazil_Amazonas_Manaus|Aleixo|2016-04-25	Aleixo	-3.0978	-60.0087
AMA22|Brazil_Amazonas_Manaus|Japim|2016-05-16	Japim	-3.1127	-59.9768
AMA23|Brazil_Amazonas_Manaus|Parque_10|2017-04-26	Parque 10	-3.0776	-60.0084
AMA24|Brazil_Amazonas_Manaus|Flores|2017-04-27	Flores	-3.0545	-6.0086
AMA26|Brazil_Amazonas_Manaus|Japim|2016-02-11	Japim	-3.1127	-59.9768
AMA27|Brazil_Amazonas_Manaus|Parque_10|2016-02-15	Parque 10	-3.0776	-60.0084
AMA2|Brazil_Amazonas_Manaus|Colonia_Terra_Nova|2016-02-19	Colonia Terra Nova	-2.9839053	-60.0053343
AMA302|Brazil_Amazonas_Manaus|Bairro_da_Paz|2015-12-08	Bairro da Paz	-2.9864369	-60,0002129
AMA32|Brazil_Amazonas_Manaus|Aleixo|2016-04-05	Aleixo	-3.0978	-60.0087
AMA33|Brazil_Amazonas_Manaus|Tancredo_Neves|2016-02-19	Tancredo Neves	-3.0529204	-5.9305473
AMA35|Brazil_Amazonas_Manaus|Sao_Lazaro|2016-02-29	Sao Lazaro	-3.1407	-59.9955
AMA36|Brazil_Amazonas_Manaus|Cidade_de_Deus|2016-03-01	Cidade de Deus	-3.0219	-59.9475
AMA37|Brazil_Amazonas_Manaus|Adrianopolis|2016-03-01	Adrianopolis	-3.1017	-60.01
AMA38|Brazil_Amazonas_Manaus|Santos_Dumont|2016-03-02	Santos Dumont	-3.0549	-60.0291
AMA41|Brazil_Amazonas_Manaus|Coroado|2016-02-03	Coroado	-3.0906	-59.9811
AMA42|Brazil_Amazonas_Manaus|Cidade_Nova|2016-02-10	Cidade Nova	-3.0363	-59.9855
AMA43|Brazil_Amazonas_Manaus|Coroado|2016-02-10	Coroado	-3.0906	-59.9811
AMA44|Brazil_Amazonas_Manaus|Nova_Esperanca|2016-01-29	Nova Esperanca	-3.0834	-60.0591
AMA45|Brazil_Amazonas_Manaus|Dom_Pedro|2016-01-08	Dom Pedro	-3.0885	-60.0461
AMA46|Brazil_Amazonas_Manaus|Morro_da_Liberdade|2016-02-10	Morro da Liberdade	-3.1365	-60.0013
AMA47|Brazil_Amazonas_Manaus|Petropolis|2016-02-02	Petropolis	-3.1089	-59.9942

AMA48|Brazil_Amazonas_Manaus|Novo_Aleixo|2016-02-02	Novo Aleixo	-3.0622	-59.9739
AMA49|Brazil_Amazonas_Manaus|Sao_Jose|2016-02-10	Sao Jose	-3.068	-59.9479
AMA50|Brazil_Amazonas_Manaus|Jorge_Teixeira|2016-02-10	Jorge Teixeira	-3.0284	-59.9285
AMA51|Brazil_Amazonas_Manaus|Tancredo_Neves|2016-01-25	Tancredo Neves	-3.0529204	-5.9305473
AMA52|Brazil_Amazonas_Manaus|Aleixo|2016-02-02	Aleixo	-3.0978	-60.0087
AMA55|Brazil_Amazonas_Manaus|Dom_Pedro|2016-01-26	Dom Pedro	-3.0885	-60.0461
AMA57|Brazil_Amazonas_Manaus|Sao_Jose|2016-01-14	Sao Jose	-3.068	-59.9479
AMA58|Brazil_Amazonas_Manaus|Colonia_Terra_Nova|2016-01-20	Colonia Terra Nova	-2.9839053	-60.0053343
AMA5|Brazil_Amazonas_Manaus|Sao_Jose_3|2016-03-10	Sao Jose 3	-3.0746814	-60.041104
AMA61|Brazil_Amazonas_Manaus|Rio_Piorini|2015-12-16	Rio Piorini	-2.9647701	-59.997033
AMA62|Brazil_Amazonas_Manaus|Cidade_Nova|2016-01-25	Cidade Nova	-3.0363	-59.9855
AMA65|Brazil_Amazonas_Manaus|Dom_Pedro|2016-01-30	Dom Pedro	-3.0885	-60.0461
AMA66|Brazil_Amazonas_Manaus|Sao_Geraldo|2016-01-23	Sao Geraldo	-3.1078	-60.0267
AMA67|Brazil_Amazonas_Manaus|Dom_Pedro|2016-01-05	Dom Pedro	-3.0885	-60.0461
AMA68|Brazil_Amazonas_Manaus|Novo_Israel|2016-02-01	Novo Israel	-3.027249	-60.0129391
AMA6|Brazil_Amazonas_Manaus|Aleixo|2016-03-15	Aleixo	-3.0978	-60.0087
AMA70|Brazil_Amazonas_Manaus|Alvorada|2016-01-29	Alvorada	-3.0752	-60.0404
AMA71|Brazil_Amazonas_Manaus|Manoa|2017-03-20	Manoa	-3.0229109	-59.9963203
AMA72|Brazil_Amazonas_Manaus|Aleixo|2017-03-20	Aleixo	-3.0978	-60.0087
AMA80|Brazil_Amazonas_Manaus|Colonia_Terra_Nova|2017-03-22	Colonia Terra Nova	-2.9839053	-60.0053343
AMA82|Brazil_Amazonas_Manaus|Nova_Esperanca_2|2017-03-24	Nova Esperanca 2	-3.0834836	-60.043744
AMA84|Brazil_Amazonas_Manaus|Alvorada|2017-03-27	Alvorada	-3.0752	-60.0404
AMA85|Brazil_Amazonas_Manaus|Vila_da_Prata|2017-03-27	Vila_da_Prata	-3.1073	-60.0476
AMA88|Brazil_Amazonas_Manaus|Cidade_Nova|2017-03-28	Cidade Nova	-3.0363	-59.9855
AMA89|Brazil_Amazonas_Manaus|Cidade_Nova|2017-03-28	Cidade Nova	-3.0363	-59.9855
AMA90|Brazil_Amazonas_Manaus|Compenca|2017-03-29	Compenca	-3.1038	-60.0579
AMA91|Brazil_Amazonas_Manaus|Compenca|2017-04-07	Compenca	-3.1038	-60.0579
AMA9|Brazil_Amazonas_Manaus|Cidade_de_Deus|2016-03-18	Cidade de Deus	-3.0219	-59.9475
Z02|Brazil_Amazonas_Manaus|Coroado|2015-12-15	Coroado	-3.0906	-59.9811
Z03|Brazil_Amazonas_Manaus|Alvorada|2015-12-15	Alvorada	-3.0752	-60.0404
ZIKV|KY631492|BR_AM_16800005|Brazil_Manaus_Amazonas_State|2016-01-08	Manaus	-3,1189	-60,0215