Genomic Surveillance of Yellow Fever Virus Epidemic Waves in São Paulo, Brazil, 2017 – 2018

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Article Summary Line: Genomic surveillance of yellow fever in São Paulo during the yellow fever 2017-2018 epidemic reveals movement towards Atlantic coast.

Running title: Phylogeography of yellow fever virus in São Paulo

Keywords: Yellow fever, outbreak, Brazil

Abstract

São Paulo (SP), a densely populated state in southeast Brazil that contains one of the world's largest urban regions, has experienced its largest yellow fever virus (YFV) outbreak in decades. Surveillance in non-human primates (NHP) is important in order to detect YFV early during an epidemic or epizootic, to quantify the magnitude of the outbreak in NHP, and to evaluate the risk of YFV spillover infection in human populations. To better understand the genetic diversity and spatial distribution of YFV during the current outbreak in southeast Brazil, we generated 46 new virus genomes from YFV positive cases identified in 18 different municipalities in SP, mostly sampled from non-human primates between April 2017 and February 2018. Our data show that most NHP cases in São Paulo state were likely caused by the introduction of a single YFV lineage from Minas Gerais to São Paulo. Phylogenetic and phylogeographic analyses of these data indicate that YFV spread southwards from Minas Gerais into São Paulo state at a typical rate of <1km per day. These results shed light in the sylvatic transmission of yellow fever in highly fragmented forested regions in São Paulo state and highlight the importance of continued operational research and surveillance of zoonotic pathogens in sentinel populations.

Author´s Summary

Since July 2016, the southeast region of Brazil has experienced the largest yellow fever virus (YFV) outbreak in decades. São Paulo is the most densely populated state in southeast Brazil. YFV is not normally present in São Paulo state and therefore a large proportion of the 18 million inhabitants of the state have not been vaccinated against YFV. The presence of YFV in São Paulo state therefore represents a serious threat to public health. In Brazil, YFV typically circulates among non-human primates, with cases in humans representing isolated 'spillover' events from this predominantly sylvatic cycle. Understanding the epidemiological dynamics and spread of YFV in primates is therefore critical for contextualising human cases, and guiding vaccination strategies that can better protect local human populations. Here, we analyse the geographic and temporal distribution of observed cases of YFV in non-human primates in São Paulo state. We generate sequence data from 46 YFV positive cases, and perform phylogenetic and phylogeographic analyses aimed at understanding the spatial spread of YFV in São Paulo state. We show that most cases in non-human primates in the São Paulo state were likely caused by a single introduction of YFV from Minas Gerais to São Paulo. Analyses of these data indicate that YFV spread southwards from Minas Gerais into São Paulo state at a typical rate of <1 km per day, consistent with a scenario of continued spread in non-human primates and sylvatic vectors across forested patches, with occasional spillover to unvaccinated human populations.

Introduction

Yellow fever (YF) is an acute hemorrhagic disease caused by the yellow fever virus (YFV), an single-stranded positive-sense RNA virus from the Flavivirus genus. Clinical manifestations of YF in humans range from inapparent or mild disease in up to 80% of infected cases, to severe hepatitis and hemorrhagic disease. Among patients who develop visceral disease case fatality rate can range from 20% to 60% [1]. YFV is commonly classified into four genotypes, denoted West African, East African, South American genotype I (SA1) and South American genotype II (SA2) [2, 3]. To date, only the SA1 and the SA2 genotypes have been detected in the Americas [2, 4].YF is preventable in humans by administration of a single dose of an extremely effective vaccine (17D) that provides life-long protection against the disease.

Yellow fever is endemic to the American and African tropics, where nearly 400 million people are estimated to be at risk of infection [5]. In the Americas, YFV transmission is thought to occur through two main transmission cycles: (i) a sylvatic cycle that involves transmission between tree-dwelling mosquitoes (e.g. *Haemagogus janthinomys*, *Haemagogus leucocelaenus* and in some local areas, mosquitoes of the Sabethes genus) and non-human primates in forested areas ([6-8]); and (ii) an urban cycle, undetected since the 1940s with transmission between humans and *Aedes aegypti* mosquitoes in heavily populated areas [1, 9]. Alongside the urban and sylvatic cycles, in Africa, YFV transmission also occurs through (iii) an intermediary cycle, in which semi-domestic mosquitos that inhabit both urban and forested environment scan transmit YFV to both humans and non-human primates [1]. In Brazil, epizootics of YF in nonhuman primates (NHP) typically ignite in neotropical forests and subsequently cause human epidemics that mostly affect unvaccinated male adults living in rural areas. These events occur approximately every 5-14 years [10]. Importantly, surveillance of primate epizootics can be used as an indicator of epidemic risk (i.e. as a sentinel event), alerting us to the likelihood of future human cases [11]. Since 1999, most YFV cases were registered in Southeast region, outside the regions in North and Center-West where transmission of YFV predominated in the past, and indicating a recent and unprecedent expansion

of the virus towards highly populated urban centres in Southeast Brazil (e.g. São Paulo, Rio de Janeiro and Belo Horizonte,) where vaccination had not been recommended before.

Since July 2016, the southeast region of Brazil has experienced the largest YFV outbreak in decades. The epizootic/epidemic is caused by a rapidly-spreading lineage of the SA1 genotype that is thought to have originated from the Amazon region [12]. By March 2019 there were 2,204 confirmed cases of YF and 757 fatalities, according to the Brazilian Ministry of Health [13]. São Paulo state is a highly densely populated state of southeast Brazil that contains one of the world's largest urban agglomerations. From January 2017 to 12 April 2019, São Paulo state reported 656 human cases and 827 NHP cases of YF [14]. Despite the magnitude and expansion of the outbreak in São Paulo state, little is known about how fast the virus is spreading through NHP populations in the region. Concerningly, as of late September 2018, 46% of people in São Paulo state (12 million people) were still not vaccinated for YFV [13]. The risk of reestablishment of urban transmission of YFV remains uncertain, but if established, would have very serious consequences for public health.

Analytical insights into the spread of YFV among NHP populations in São Paulo state (SP) can help to guide public health efforts and targeted vaccination campaigns. Here, we report the screening of dead NHP in São Paulo state for YFV using a previously-developed portable sequencing protocol, resulting in the generation of 46 YFV genomes from positive samples. We analyse epidemiological case data and virus phylogeography to characterize the spread of YFV in São Paulo state during the first two waves of the current outbreak.

Methods

Sample collection and mapping of non-human primate cases

Notifications of dead NHP are made to the São Paulo's State Epidemiological Health Department by telephone, by fax, or electronically. Where multiple NHP were found dead at the same mortality event, the number of primates at each location was recorded but in most instances only one or a few animals from each event were tested. Despite the possibility of epidemiological association with positive, dead animals, these untested animals are not considered positive by current surveillance systems. Each YFV-confirmed NHP case was georeferenced according to the original case location using a handheld Garmin Etrex® GPS. Quantum GIS [15] was used to create a choropleth depicting the geographic distribution and number of confirmed cases across different municipalities in São Paulo state.

Sample collection of human cases

We tested 6 randomly selected samples from human cases collected in São Paulo state from four municipalities (**Table S1**). Samples were collected for molecular diagnostics in several municipalities of Sao Paulo state, and sent for testing at Instituto Adolfo Lutz.

Ethics statement

Research on human cases was supported by 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). Only already dead non-human primates were sampled for YFV surveillance. The surveillance protocol for dead non-human primates was approved by the Ethics Committee for the use of Animals In Research, Instituto Adolfo Lutz, under the numbers 0135D/2012 and 020G/2014.

Identification of YFV positive cases

YFV positive cases were detected using at least one of the three methods described below; immunofluorescence, immunohistochemistry, or reverse transcription quantitative PCR (RT-qPCR). In rare circumstances (n=12 cases), a case was also considered positive based on its close epidemiological association with tested, RT-qPCR positive cases. Here, a confirmed case is considered to be one that is positive by at least one method.

Indirect immunoflorescence

Samples of NHP blood or serum, and tissue material suspensions obtained from autopsies, were identified using a standardized indirect immunofluorescence technique [16]. An in-house polyclonal anti-flavivirus antibody (anti-DENV 1-4) and an anti-mouse IgG-FITC antibody (Sigma) were used. Positive samples were typed by indirect immunofluorescence with monoclonal antibodies for YFV (Biomanguinhos, Rio de Janeiro).

Histopathology and immunohistochemistry for NHP cases

Samples of brain, heart, lung, liver, spleen and kidney from NHP were fixed in formaldehyde and embedded in paraffin. Histological sections of these tissues were stained with hematoxylin and eosin and examined on a microscope. Where damage consistent with YFV infection was observed, indirect immune histochemistry was used to detect the presence of the yellow fever virus antigen. Sections of liver (0.3uM) were placed on slides coated with silane and treated with an in-house polyclonal anti-YFV antibody (produced in mice, and used at 1/30,000 dilution). The slides were treated with anti-mouse secondary antibodies, linked to either horseradish peroxidase (Reveal HRP Spring polymer System, Spring) or to alkaline phosphate (Link MACH4 universal AP Polymer and Polymer MACH4 universal AP, Biocare). Chromogenic detection of the presence of YFV was

171 subsequently conducted using the substrates 3,3'-diaminobenzidine or Fast Red (Warp Red,
172 Biocare), respectively.
173
174 ***RT-qPCR***
175 Total RNA was extracted from tissue and serum samples using two commercial kits:
176 QIAamp® RNA Blood for tissues and QIAamp® Viral RNA Kit for serum (Qiagen Inc., Germany)
177 according to the manufacturer's instructions. Viral RNA was detected using two previously
178 published RT-qPCR techniques [17, 18].
179
180 ***MinION genome sequencing***
181 A selection of positive samples was sequenced using a rapid whole-genome sequencing protocol
182 that has been previously validated and successfully applied in Brazil [19, 20]. In brief, cDNA was
183 produced from viral RNA using random hexamers and the Protoscript II First Strand cDNA
184 synthesis kit (NEB). The genome was amplified using a multiplex PCR scheme designed to produce
185 overlapping 500bp amplicons across the whole coding region of the recent South American
186 genotype I outbreak clade. PCR products were quantified, barcoded using the Oxford Nanopore
187 Technologies (ONT) Native Barcoding Kit (NBD103), and pooled in an equimolar fashion.
188 Sequencing libraries consisting of 10-12 samples per library were constructed using the Ligation
189 Sequencing kit (ONT, SQK-LSK108). Sequencing was performed on a ONT flow cell for up to 48h
190 as described previously [12].
191
192 ***Generation of consensus sequences***
193 Consensus sequences for each barcoded sample were generated following a previously published
194 approach [21]. Briefly, raw files were basecalled using Albacore, demultiplexed and trimmed using
195 Porechop, and then mapped with *bwa* to a reference genome (GenBank accession number
196 JF912190). Nanopolish variant calling was applied to the assembly to detect single nucleotide

197 variants to the reference genome. Consensus sequences were generated; non-overlapped primer
198 binding sites, and sites for which coverage was <20X were replaced with ambiguity code N.
199 Sequencing statistics can be found in **Table S2**. Accession numbers of newly generated sequences
200 can be found in **Table S1**.
201
202
203 ***Phylogenetic analyses***
204 Consensus sequences were aligned using MAFFT v.7 [22]. Maximum likelihood (ML)
205 phylogenetic trees were estimated using RAxML v.8 [23] under a GTR + Γ_4 nucleotide substitution
206 model. Statistical support for phylogenetic nodes was estimated using a ML bootstrap approach
207 with 100 replicates. The genome sequences generated here were combined with a previously
208 released alignment of genomes from the 2016-2018 YFV epidemic sampled elsewhere in Brazil
209 [12]. The full dataset analysed here contains 98 YFV sequences, 46 of which were isolated from SP
210 and generated in this study (alignments and ML phylogenetic tree of full dataset can be found in
211 GitHub repository (to be added).
212
213 ***Phylogeographic analyses***
214 To investigate the spread of YFV in São Paulo, we analysed in more detail Lineage 2, a
215 phylogenetic lineage that includes the 46 new genomes from SP and two basal sequences from
216 Minas Gerais. These 48 sequences are shown in a ML phylogeny in **Fig. 2A**. Georeferenced and
217 time-stamped sequences were analysed in BEAST v.1.8.4 [24] using the BEAGLE library to
218 enhance computational speed [25]. We used a skygrid coalescent tree prior [26] and a continuous
219 phylogeographic model that uses a relaxed random walk to model the spatial diffusion of lineages.
220 Dispersal velocity variation among lineages was represented using a Cauchy distribution [27].
221 Virus diffusion through time and space was summarised using 1000 phylogenies sampled at
222 regular intervals from the posterior distribution (after exclusion of burn-in). The R package

223 "seraphim" [28] was used to extract the spatio-temporal information contained in these phylogenies,
224 whose branches can be considered as movement vectors (each having start and end spatial
225 coordinates, and start and end dates, in decimal units). The package "seraphim" was also used to
226 estimate statistics of spatial dissemination, such as lineage dispersal velocity, and change inthe
227 maximal wavefront distance from epizootic origin (**FigureS2**) [28]. The results shown in Figure 2
228 were generated using the "spreadGraphic" R function [28].

229 ***Data availability***

230 Epidemiological data, genomic data and XML files analysed in this study are available in the
231 GitHub repository (XXX). New sequences have been deposited in GenBank under accession
232 numbers MH018064, MH018067, MH030049- MH030091 and MH193173- MH193175.

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234

235

Results

Liver, brain or blood samples from 591 NHP tested positive for YFV during the period from epidemiological week 29 of 2016 to week 4 of 2018. An additional 138 NHP were observed dead at the same time and location as identified positive cases, but were not tested for YFV. Samples were confirmed positive using immunohistochemistry (*n*=466) and/or RT-qPCR (*n*=311). Most confirmed cases (at least one positive diagnostic result) in NHP were from animals of the *Alouatta* genus (88%; 403 of 459 cases for which genus information was available), followed by *Callithrix* (8%; 35/459), *Callicebus* (2%; 9/459), *Cebus* (2%; 9/459) and *Sapajus* (0.7%; 3/459).

Our results reveal two main epizootic waves, in an area non-vaccinated against YFV, with no notified cases of YFV since the 1940s, preceded by a 'pre-epizootic' phase of low case counts, occurring in an area that have been previously vaccinated. The pre-epizootic phase runs from July 2016 to Jan 2017 (6%, 33/591 cases), phase 1 runs from Feb to Jul 2017 (20%, 119/591 cases) and phase 2 from Jul 2017 to Feb 2018 (74%, 439/591 cases) (**Fig. 1A**).The peaks of epizootic phases 1 and 2 were mid Apr 2017 and late Nov 2017, respectively.

YF cases until November 2018 were geographically widespread across 57 municipalities of SP, with most cases concentrated around the southeast region of the state (**Fig. 2**). The greatest number of confirmed cases were observed in the municipalities of Mairiporã (*n*=88), Jundiaí (*n*=68), São Paulo (*n*=62), Bragança Paulista (*n*=48), and Atibaia (*n*=36). There is a clear distinction between the geographic location of cases in the pre-epizootic and in the epizootic phases 1 and 2 (**Fig. 1B**). Specifically, almost all earlier cases that form part of the pre-epizootic wave occur in a geographically distinct cluster of municipalities in the north of São Paulo state (**Fig. 1B**; **Fig. 2**), where YFV has also been detected in 2000 and 2008 [29]. The two waves of epizootic transmission begin at the time that the virus began to be detected in municipalities in the south of São Paulo state (within 200km of São Paulo municipality) (**Figs. 1B and 2**).

To investigate the source and transmission of YFV during the two epizootic waves, and the genetic diversity of the virus circulating in NHP populations across SP state, we generated whole

genome for forty-one RT-qPCR positive NHP samples (median Ct-values of 13, range 9-25) from 14 municipalities using a previously described MinION sequencing protocol [12, 19]. We also sequenced 6 whole genomes from human samples collected in São Paulo state (median Ct-values of 35, range 32-37), representing an additional four municipalities. The most recent samples chosen for genome sequencing were from Jan-Feb 2018: isolate SA130 is from a human case in Mairiporã municipality, and isolates SA129 and SA131 are from *Alouatta* monkeys, from Guarulhos municipality and from the south of São Paulo city, respectively (**Fig. S1)**.

Newly generated genome sequences (*n*=46) were aligned to available outbreak genome data (*n*=52) and maximum likelihood methods were used to estimate phylogenetic relationships. Our genetic analyses indicate a strong phylogenetic spatial structure of the ongoing epizootic in SP state, with almost all (42/46) sequences from SP clustering in a single well-supported monophyletic clade (bootstrap score=91%). The most closely related sequences to the main SP outbreak clade are from Santa Rita de Caldas (M11), and Caldas (M5) (**Fig. 3**, see **Fig. 2** for locations). These data indicate that the majority of the enzootic infections of YFV in SP may have arisen from a single introduction, possibly from south of Minas Gerais. This single introduction was subsequently followed by sustained transmission within SP state giving rise to epizootic waves 1 and 2 (**Fig. 1**).

Finally we used a continuous diffusion model to investigate how rapidly the virus has been spreading over space and time. We find that that YFV disseminated rapidly from the south of Minas Gerais state through São Paulo municipality in the direction to the Atlantic Forest of São Paulo (**Fig. 4**), with the epidemic wavefront moving on average 0.84 (0.50-2.19) km/day (**Fig. S2**). To investigate whether virus lineages moved faster during the first epizootic season, we estimate mean phylogeny branch velocity for phase 1 of the epizootic to that of phase 2. Mean branch velocity for phase 1 was estimated to be around 0.97 (BCI: 0.5-3.0) km/day. For the second epizootic phase we find that the mean branch velocity was slightly slower at 0.78 (BCI: 0.46-2.22) km/day, although the difference is not statistically significant.

Discussion

In this study we present data on the temporal and geographic distribution of detected YFV cases in NHPs in São Paulo state, Brazil, during 2016-2018. Our results demonstrate the existence of several distinct phases of the outbreak. Specifically, we observe a 'pre-epizootic' period in which a small number of cases were identified in the northern region of SP state during late 2016. This pre-epizootic period was followed by two larger epizootic phases, the first from July 2016 – July 2017, and the second from August 2017 to at least Feb 2018 (the data of the most recent data available for this study). During these two phases, a large number of cases were observed in the south of SP state. We generated 46 novel genomes of YFV from samples collected in 18 different municipalities in São Paulo state. These majority of sequenced viruses were sampled from *Alouatta* monkeys during the late 2017 and early 2018, and are therefore largely representative of the second epizootic phase (**Figure S1, Fig. 1**). Phylogenetic and phylogeographic analyses of these data indicate that virus lineages move diffused southwards from Minas Gerais into São Paulo state with an invasion speed of<1km per day (**Fig. 4**).

Understanding the processes that led to the initiation of the two main epizootic waves in SP is important for preventing future YFV outbreaks. In this study were not able to sequence samples from cases identified in northern SP during the pre-epizootic phase. We are therefore unable to use genetic and evolutionary analysis to evaluate the spatial and epidemiological relationship between cases observed during this pre-epizootic phase in the north of SP state, and cases observed during the subsequent, larger outbreaks in the south of SP state. Sequencing of samples from the pre-epizootic wave would be important to determine whether the northern, pre-epidemic cases directly initiated the epizootic observed in Minas Gerais and southern São Paulo around February 2017, or whether the northern YFV lineage was successfully extinguished before onwards spread. We contend that longitudinal entomological data across different climatic regions in Sao Paulo state will help to disentangle seasonal and spatial differences in the availability of mosquito vectors and to better anticipate future YFV transmission.

As of January 2019, human and NHP cases of YFV have been reported in the south western regions of São Paulo state, and in neighbouring Paraná state(PAHO 2019). These cases may represent the beginning of a third wave of YFV in the region, and the continued expansion of YFV down the coastal region of southern Brazil. Interestingly, historical data from sylvatic YFV outbreaks in Brazil suggest a similar geographic pattern of viral spread during sylvatic outbreaks that occurred during the 1930s and 1950s [30, 31]. Detailed comparison of data from the current outbreak to historical outbreaks, or to YFV genome sequences recovered from archival samples, may help to identify key ecological corridors of YFV transmission in Brazil that have remained constant over time.

Using YFV genetic data, we estimate that the virus lineages moved at a mean rate of <1 km per day during the first and second epizootic phases in SP state. We observe no difference between the rate of viral lineage dissemination during the first (Feb 2017 – July 2017) and second (August 2017 - Feb 2018) phases (**Fig. S2**). Our estimate of the rate of YFV lineage movement in SP state is slightly lower than that previously estimated by ourselves and others for the states of Minas Gerais, Espírito Santo and Rio de Janeiro (~4km/day) [12]. Additional analyses of larger datasets than span state borders are necessary to test whether ecological drivers, such as NHP density and the degree of habitat fragmentation, or whether differences in surveillance efforts and sampling or differences in viral load found in NHP species might explain differences in the rate of YFV spread among different areas.

The vaccination strategies employed to combat the YFV in SP state during the ongoing outbreak have been designed on the basis of the georeferenced positive NHP occurrences (Adriano Pinter, personal communication). Human activities may have the potential to accelerate the dispersal of YFV virus lineages via the travel of asymptomatic patients, illegal trade in wildlife, or by encouraging the 'hitchhiking' of infected mosquitos in cargo or vehicles during agricultural or forestry work. A better understanding of the contribution of these factors is needed to achieve WHO's goals to eliminate YFV epidemics before 2026.

Understanding how YFV is introduced into highly urbanised areas, such as São Paulo city, is important for designing strategies that can effectively interrupt these introductions. Isolate SA131 was collected from an *Alouatta* sp. individual at the Parque Estadual das Fontes do Ipiranga (PEFI), a public park that forms part of the São Paulo city zoo and which comprises a 53 km^2 fragmented piece of Atlantic forest. The zoo, the largest in Brazil, attracts tens of thousands of visitors per week and was closed to visitors on the 22 Jan 2018 after YFV confirmation, and reopened on the 15 Mar 2018.

The area has ecological conditions for the maintenance of mosquitoes of the genus *Sabethes* and *Haemagogus*, vectors of forest mosquitoes commonly involved in the wild transmission cycle of the YFV, however entomological collections performed in the park did not show the presence of *Haemagogus* mosquitoes, two specimens of the genus *Sabethes* were collected, *Sa. albiprivus* and *Sa. chloropterus*. Among the most frequent mosquitoes were *Aedes scapularis* and *Aedes albopictus*. Phylogenetic analyses show that the YFV genome recovered from sample SA131 clusters together (bootstrap score=76%) with isolate Y37-244 collected in Piracaia, 120 km from the park, and not with more local isolates that were detected in the outskirts of SP or in surrounding municipalities. This result could be explained by: (i) incomplete sampling of a wave of continuous transmission among NHPs that live between the two locations; (ii) human-mediated transport of YFV infected NHP, (iii) human-mediated transport of mosquitos [32] or (iv) introduction of the virus by an asymptomatic human visitor, who carried the virus from Piracaia to PEFI. Scenario (i) seems unlikely since PEFI is an isolated forested fragment to the south of SP city with limited connectivity to other forested fragments; at this stage, scenarios (ii), (iii) and (iv) remain possible.

Our study sheds light on the spatial and temporal dynamics of yellow fever in different animal hosts across Sao Paulo state. A better understanding of the vectors and host species involved in the persistence of the virus both in epidemic seasons and in non-epidemic periods is critical to understand the drivers of yellow fever transmission and anticipate future outbreaks.

Biographical Sketch

Renato Pereira de Souza has a PhD in Epidemiology from the University of São Paulo, Brazil. He is currently a Scientific Researcher at the Instituto Adolfo Lutz, reference laboratory for arboviruses in São Paulo, where he combines epidemiological surveillance with studies on the molecular evolution of arboviruses, hantavirus and arenavirus. Sarah Hill has a PhD in viral genomic epidemiology from the University of Oxford. She is currently a postdoctoral researcher at the University, working on understanding the dynamics of mosquito-borne arboviruses.

Acknowledgments

We thank the IAL and the USPTM staff, in particular the crew from Núcleo de Doenças de Transmissão Vetorial and from the Centro de Patologia. This study was developed as a collaborative effort between the IAL, FIOCRUZ, and the University of Oxford, UK. The research was supported by a Wellcome Trust and Royal Society Sir Henry Dale Fellowship (grant 204311/Z/16/Z), internal HEFCE GCRF grant 005073, John Fell Research Fund Grant 005166, Medical Research Council and FAPESP CADDE partnership award (MR/S0195/1), CNPq #400354/2016-0 and FAPESP# 2016/01735-2, and by the Oxford Martin School. SD is supported by the Fund for Scientific Research (FWO) Flanders (Fonds voor Wetenschappelijk Onderzoek, Flanders, Belgium). This work was also supported by CNPq/MCTI Decit/SCTIE/MoH (440685/2016-8) and CAPES (88887.130716/2016-00).

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Supporting Information Legends

Technical Appendix. Contains additional Figure S1, Figure S2, Table S1 and Table S2. Figure S1: date of sample collection and number of genomes generated from each host genus. Figure S2. evolution of the wavefront distance from epidemic origin over time. Table S1: details of the YFV genomes generated in this study. Table S2: non-human primate yellow fever virus genome sequences from São Paulo, by municipality.

Figure Legends

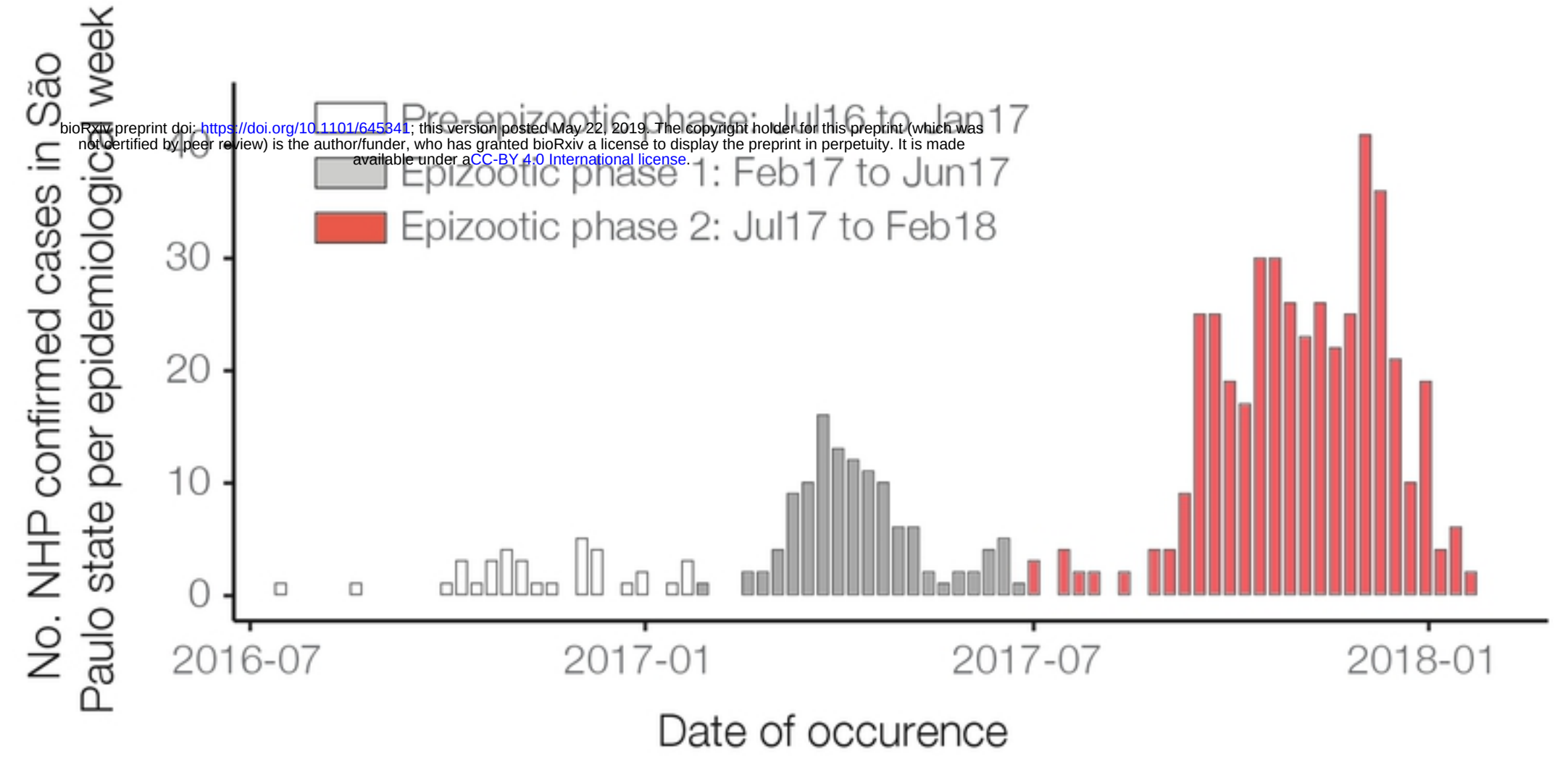
Figure 1. Distribution of weekly YFV cases. **A**. NHP YFV cases diagnosed by RT-qPCR per week. **B**. Distance in kilometres from the municipality of NHP YFV occurrence to São Paulo municipality is plotted against date of sample collection.

Figure 2. Choropleth map of the distribution of confirmed NHP cases per municipality in São Paulo state between July 2016 and February 2018. Triangles depict locations of human sequenced cases; diamonds depict 14 municipalities in São Paulo state from which non-human primate genome sequences have been generated. Red symbols correspond to earlier cases between July 2017 and February 2018. Names for key samples/isolates are shown (see also Figure 3).

Figure 3. Maximum likelihood phylogenetic tree of YFV in São Paulo, Brazil. The tree includes 46 newly generated isolates, and 2 previously published ones (M5 and M11 isolates). Bootstrap scores are provided for well-supported nodes.

Figure 4. Reconstructed spatiotemporal diffusion of the São Paulo YFV clade. Phylogenetic branches are mapped in space according to the location of phylogenetic nodes (circles). Arrows show the cross-state movement of the virus from Minas Gerais followed by movement to the Atlantic forest closer to present. Shaded regions show 95% credible regions of internal nodes. Nodes and uncertainty regions are coloured according to time.

A.



B.

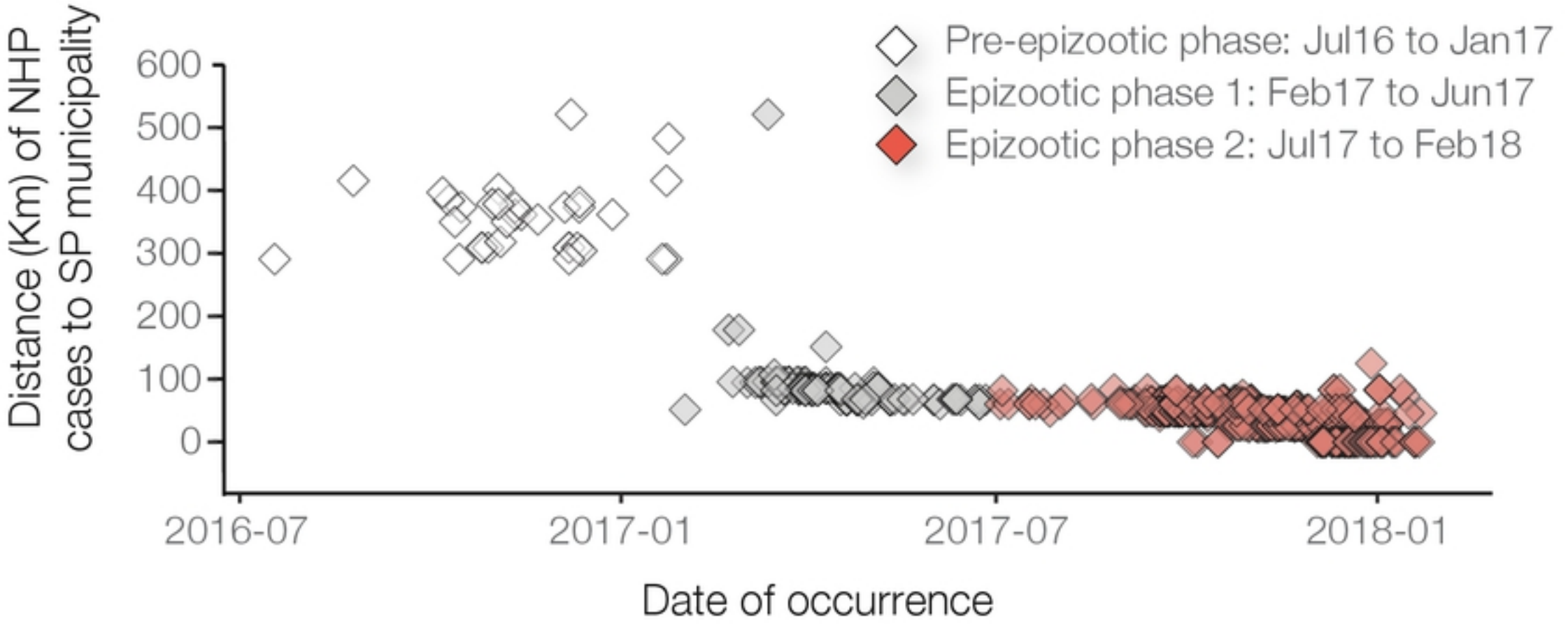


Figure 1

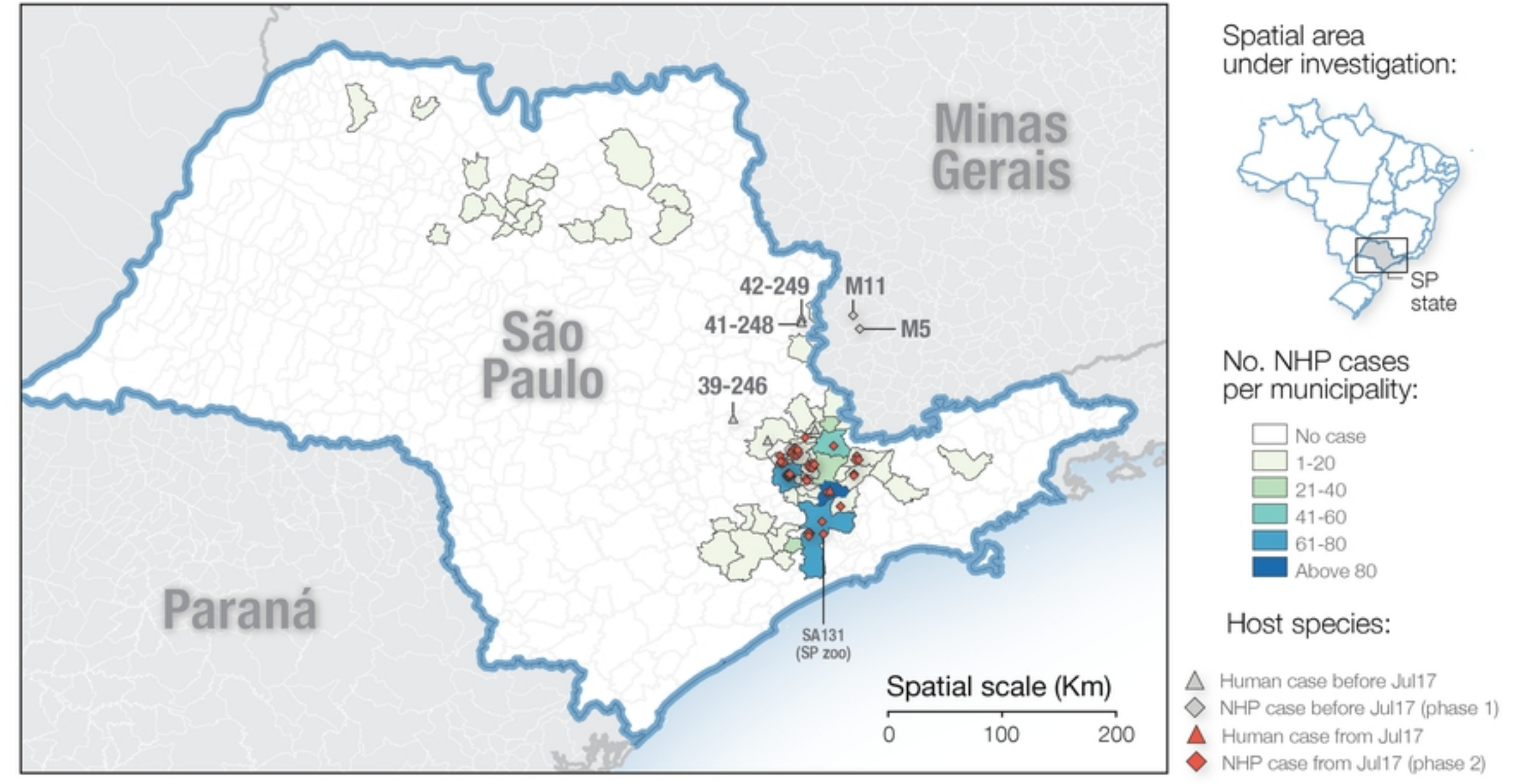

Minas Gerais
São Paulo
Paraná
42-249
M11
41-248
M5
39-246
SA131 (SP zoo)
Spatial scale (Km)
0
100
200
Spatial area under investigation:
SP state
No. NHP cases per municipality:
No case
1-20
21-40
41-60
61-80
Above 80
Host species:
Human case before Jul17
NHP case before Jul17 (phase 1)
Human case from Jul17
NHP case from Jul17 (phase 2)

Figure 2

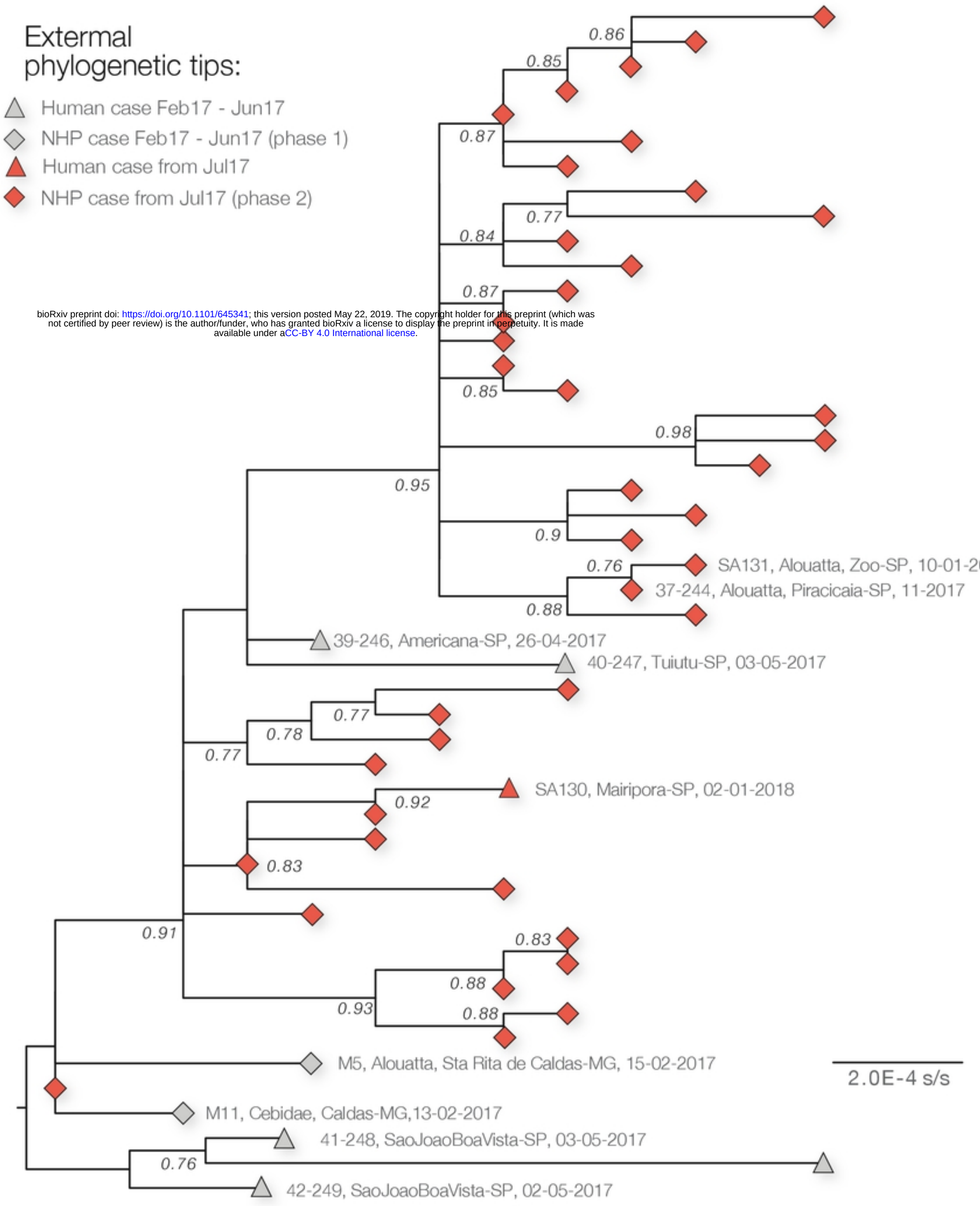


Figure 3

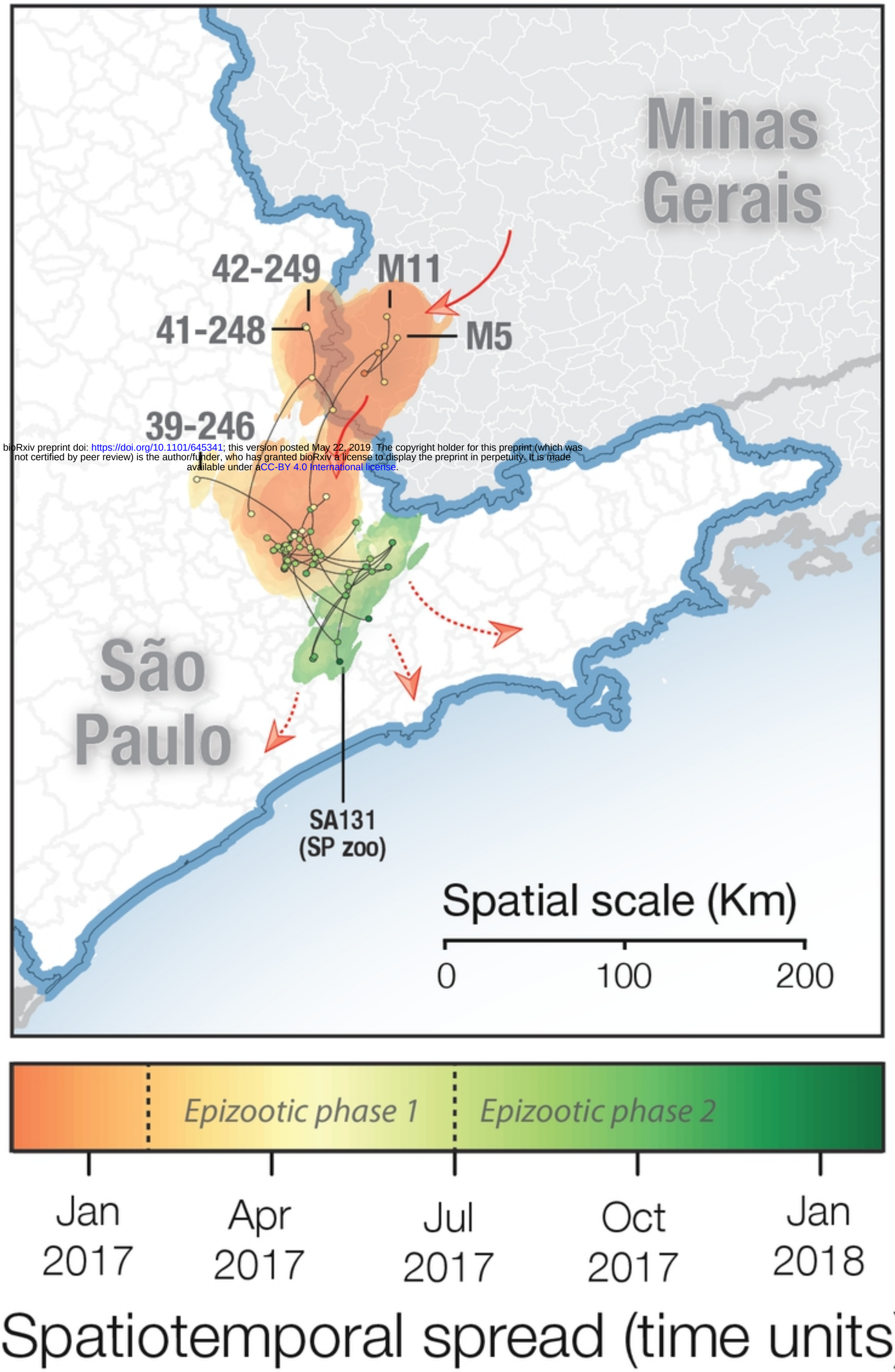


Figure 4