Epidemiological dynamics of an urban Dengue 4 outbreak in São Paulo, Brazil

Christian Julián Villabona Arenas, Jessica Luana de Oliveira, Carla de Sousa-Capra, Karime Balarini, Celso Ricardo Theoto Pereira da Fonseca, Paolo M de A Zanotto

Background Dengue studies at the urban scale are scarce and required for guiding control efforts. In Brazil, the burden of dengue is high and challenges city public health administrations with limited resources. Here we studied the dynamics of a dengue epidemic in a single city. **Methods** Serum samples from dengue suspected cases were collected and tested, from December 2012 and July 2013 in Guarujá, Brazil. We use incidence series analysis to provide a detailed view of the reproduction number dynamics and a Bayesian analysis to infer the spread of the serotype using geographic and temporal data. **Results** We obtained nucleotide sequences from 354 envelope genes and georeferenced 286 samples during the course of the outbreak. Serotype 4 was responsible for the epidemic. We identified at least two major lineages that overlapped in distribution. We observed high Reproduction numbers and high cladogenesis prior to the escalation of clinical case notifications. Three densely populated non-adjacent neighborhoods played a pivotal role during the onset and/or course of the epidemic. **Discussion** Our findings point to high dengue virus transmission with a substantial proportion of unapparent cases that led to a late recognition of an outbreak. Usually source reductions initiatives tend to be insufficient once an epidemic has been established. Nevertheless, health authorities in Guarujá prioritized vector control on specific places with clusters of georeferenced viremic patients, which appear to have diminished the epidemic impact.

Epidemiological dynamics of an urban Dengue 4 outbreak in São Paulo, Brazil

Authors

Christian Julián Villabona-Arenas[1-3], Jessica Luana de Oliveira[1,4], Carla de Sousa-Capra[5], Karime Balarini[6], Celso Ricardo Theoto Pereira da Fonseca[6], Paolo Marinho de Andrade Zanotto[1]

Affiliations

[1] Laboratory of Molecular Evolution and Bioinformatics, Department of Microbiology, Biomedical Sciences Institute, University of São Paulo, São Paulo, Brazil.

[2] UMI233, Institut de Recherche pour le Développement (IRD), Université de Montpellier, Montpellier, France

[3] Laboratoire d'Informatique, de Robotique et de Microélectronique de Montpellier (LIRMM), Institut de Biologie Computationnelle (IBC), Université de Montpellier, Montpellier, France

[4] Department of Biomedicine, University of Mogi das Cruzes, Mogi das Cruzes, São Paulo, Brazil

[5] Office of Epidemiological Surveillance, Department of Health, Guarujá, São Paulo, Brazil.

[6] ITAPEMA, clinical laboratory analysis center, Guarujá, Brazil.

***Corresponding author**:

Paolo Marinho de Andrade Zanotto

Av. Prof. Lineu Prestes, 1374, Sao Paulo, SP, 05508-000, Brazil

pzanotto@usp.br

Abstract

Background

Dengue studies at the urban scale are scarce and required for guiding control efforts. In Brazil, the burden of dengue is high and challenges city public health administrations with limited resources. Here we studied the dynamics of a dengue epidemic in a single city.

Methods

Serum samples from dengue suspected cases were collected and tested, from December 2012 and July 2013 in Guarujá, Brazil. We use incidence series analysis to provide a detailed view of the reproduction number dynamics and a Bayesian analysis to infer the spread of the serotype using geographic and temporal data.

Results

We obtained nucleotide sequences from 354 envelope genes and georeferenced 286 samples during the course of the outbreak. Serotype 4 was responsible for the epidemic. We identified at least two major lineages that overlapped in distribution. We observed high Reproduction numbers and high cladogenesis prior to the escalation of clinical case notifications. Three densely populated non-adjacent neighborhoods played a pivotal role during the onset and/or course of the epidemic.

Discussion

Our findings point to high dengue virus transmission with a substantial proportion of unapparent cases that led to a late recognition of an outbreak. Usually source reductions initiatives tend to be insufficient once an epidemic has been established. Nevertheless, health authorities in Guarujá prioritized vector control on specific places with clusters of georeferenced viremic patients, which appear to have diminished the epidemic impact.

Introduction:

The dengue viruses exist as four antigenically distinct serotypes named DENV-1, DENV-2, DENV-3 and DENV-4. Dengue fever (DF) is a disease caused by any of the DENV (Chen & Vasilakis 2011). Following cartographic approaches there is an estimated 390-million (95%

54 credible interval 284–528) dengue infections worldwide per year, of which 96 million (67–136)
55 manifest apparently (any level of clinical or subclinical severity) (Bhatt et al. 2013).
56 Dengue is endemic in Brazil. This means that the disease occurs every year, usually
57 during the wet season when *Aedes* mosquitoes' populations are high and the rainfall is optimal
58 for breeding. People not only provide the mosquitoes with blood meals but also water-holding
59 containers where the mosquito lays their eggs. In addition, this country is at periodic risk for
60 epidemic dengue (*i.e.*, when large numbers of people become infected during a short period),
61 which requires a coincidence of large numbers of vector mosquitoes, large numbers of people
62 with no immunity to one or more of the four serotypes (CDC 2014).
63 Dengue virus serotype 4 (DENV-4) reemerged in the northern Brazil in 2010, 28 years
64 after it was last detected in the country in 1982, and has been responsible for several outbreaks
65 since then (Nunes et al. 2012). In 2013, "1,468,873 million" dengue cases were reported
66 countrywide, including 6,969 severe cases and 545 deaths. These numbers entail a challenge
67 for public health authorities, which in a timely manner need to allocate resources and trained
68 personnel to try diminishing the health impact of the disease. Programs to control populations
69 of mosquitoes strain public resources, especially in resource-limited settings (Shepard et al.
70 2011; Stahl et al. 2013). In this context, understanding epidemic spread in urban settings is
71 crucial because the results may guide the allocation of scarce resources toward future vector
72 control.
73 The spatiotemporal patterns of dengue spread in Brazilian settings are limited and
74 mostly based on serological prevalence and incidence data (Barreto & Teixeira 2008; Teixeira
75 Mda et al. 2002; Teixeira et al. 2013). Some recent studies address this topic in a larger scale
76 (Nunes et al. 2012; Nunes et al. 2014). So far, only one work address the spatial dynamics of an
77 urban dengue outbreak in the city of São Jose de Rio Petro using viral genetic data (Mondini et
78 al. 2009). These studies are imperative because socio-demographic and ecological factors affect
79 diffusion dynamics (Cuong et al. 2013; Jeefoo et al. 2011; Raghwani et al. 2011; Rasmussen et al.
80 2014; Schreiber et al. 2009; Vazquez-Prokopec et al. 2010). In the present work we describe an
81 outbreak of DENV-4 during 2013 in the city of Guarujá, Brazil, following Bayesian phylogenetic

analysis of envelope gene sequences. Our results emphasize the importance of real-time follow up and early actions to achieve better control during epidemics.

Methods:

Study Site

Guarujá (23° 59′ 37″ S 46° 15′ 23″ W) is a coastal city in Santo Amaro Island, situated at the shore of the State of São Paulo, Brazil (Fig. S1A). The city is embedded in a tropical rain forest. It has a tropical humid climate that is characterized by having high average air temperature and rainfall. The average annual temperature is 24.7 °C (Min 18 °C | Max 31.3 °C) and the annual rainfall is 3,413 mm; February is the wettest month (average rainfall of 412.8 mm) and August is the driest one (average rainfall of 155.6 mm). The city main economic sources are seasonal tourism and port related activities. The estimated population in 2013 was 306,683 and the population density was 2,026.80 inh/km². Official dengue reports, to the Epidemiological Surveillance Center of the State (CVE), date back to 1997 and there are around 24000 cumulated cases up to date. The disease is an important public health problem. Unfortunately, there is no detailed information concerning the previous exposure to distinct DENV serotypes; the municipality relies on any feedback provided by the Central Public Health laboratory from The State of São Paulo.

Sample Collection:

In late 2012, our group at University of São Paulo initiated a joint effort with the Guarujá Municipality's office of epidemiological surveillance and with a local clinical laboratory analysis center (Itapema) to map the incidence of dengue in the city and obtain viral genetic data. Both institutions contributed with the collection of samples citywide and by performing preliminary immunochromatographic diagnostic tests for dengue.

Patients of any age with symptoms and signs of dengue disease that were examined in Primary Health Care facilities and Emergency Care Units were considered for the study.

Symptoms included fever, frontal or retro-orbital headache, severe pain (muscles, bones, legs, joints, lower back or abdominal), nausea, vomiting, taste disturbance and anorexia. Signs included high fever (usually between 38.5 and 41°C) persisting for 24 hours, rash, hemorrhagic manifestations, hypotension and narrow pulse pressure. In addition, patients were tested using a Dengue Duo Test, a qualitative immunoassay for the simultaneous detection of the NS1 antigen as well as both the IgG and IgM antibodies. Dengue NS1 antigen, as reviewed by (Kassim 2011), is a highly conserved glycoprotein produced in both membrane-associated and secretion forms. It is abundant in the serum of viremic patients during the early stages of DENV infection and can be detected before the formation of antibodies. Therefore, to increase the chances of obtaining the virus from the samples, only serums samples from patients who signed consent forms and had positive IgM and/or NS1 antigen outcomes were referred to our laboratory.

Ethics Statement

The Ethical Review Board of the Biomedical Science Institute at University of São Paulo approved this study (Statement 933/CEP). All adult subjects provided an informed written consent, and a parent or guardian of any child participant provided the written informed consent on their behalf.

Molecular Testing

Viral RNA was extracted from the serum samples with the QIAmp viral RNA mini kit (Qiagen, Venlo, Limburg, Netherlands) and the complementary DNA was synthesized using the SuperScript® VILO™ cDNA Synthesis Kit (Life Technologies, Carlsbad, California, United States). The GoTaq® Green Master Mix (Promega, Madison, Wisconsin, United States) was used for PCR amplifications of the envelope gene using the primers of (Bennett et al. 2003). The ExoSAP-IT reagent was used for PCR Product Cleanup (Affymetrix, Santa Clara, California, United States) and the sequencing reaction was performed using the BigDye® Terminator v3.1 Cycle

Sequencing Kit (Life Technologies). Sequencing reaction products were purified using the BigDye XTerminator Purification Kit (Life Technologies) and sequenced on an ABI PRISM ® 3130 Genetic Analyzer (Life Technologies). Contigs were assembled using the program Codon Code aligner.

Genetic Analysis

Sequences were aligned using Muscle 3.8.31 (Edgar 2004a; Edgar 2004b) followed by visual inspection and manual editing with Mesquite 2.75 (Maddison & Maddison 2014). Polymorphisms were analyzed with DNASP 5 (Librado & Rozas 2009). The Tajima D statistic test was used to evaluate deviations from the neutral expectation of molecular evolution. The package HyPhy v2.2 was used to screen for recombination (SBP-Single Breakpoint Recombination and GARD-Genetic Algorithms for Recombination Detection) and for positive selection. The dengue strain H780090 isolated in Boa Vista, RR-Brazil (29 of November of 2010) was used as a reference. Both genealogy-based, codon-site models Single Likelihood Ancestor Counting (SLAC) and the Fixed Effects Likelihood (FEL) methods were used to estimate the non-synonymous (dN) and synonymous (dS) rates of substitution (Delport et al. 2010; Kosakovsky Pond & Frost 2005; Pond et al. 2005).

Phylogenetic analysis

The sequences obtained in this study were combined with a DENV-4 database from a previous study (Villabona-Arenas & Zanotto 2011) in order to identify the genotype. This was achieved using high-throughput clustering with the UCLUST algorithm in the package USEARCH (Edgar 2010).

The JModeltest software was used for the statistical selection of the best-fit model of nucleotide substitution under the Akaike information criterion (Darriba et al. 2012; Guindon & Gascuel 2003). Sequences were dated according to the day of sampling. The dataset generated was used for phylogenetic reconstruction and the estimation of the rate of evolutionary change

(μ) (subs/site/year) using Bayesian Inference (IB) in Beast v2.3.1 (Bouckaert et al. 2014). A Bayesian maximum clade credibility tree was inferred from a set of plausible trees sampled at the stationary phase of four independent Markov Chain Monte Carlo (MCMC) runs with 200 million generations each using a relaxed (uncorrelated lognormal) molecular clock (Drummond et al. 2006).

Time-varying reproduction numbers

We estimated the transmission dynamics of R using the incidence time series (weeks) for the epidemiological year of 2012-2013 (Cori et al. 2013; Salje et al. 2012). The serial interval distribution parameters (2.0, 0.5) reflected the human infectious period plus the extrinsic incubation period in the mosquito. A censored Bayesian time-to-event model estimated the dengue intrinsic incubation period (the time between a human being infected and the onset of symptoms due to the infection) around six days (95% CI 3 -10) and the best-fitting temperature-dependent extrinsic incubation period model estimated of 6.5 days (95% CI 2-15) at 30°C (Chan & Johansson 2012). For *R*, we used a prior distribution (2.0, 5.0) that reflected previous dengue basic reproduction values estimates (between 1.33 and 11.6) (Halstead 2008).

The Birth–death skyline (BDSKY) model was also used to estimate epidemiological parameters (Kuhnert et al. 2014; Stadler et al. 2013). The BDSKY parameterization consisted of 3 correlated parameters that can have a different number of changes, specified through the dimension option: (*i*) the effective reproduction number (*R*), (*ii*) the become un-infectious rate (*y*) and, (*iii*) the sampling proportions (*s*). For parameters *R* and *y* (the inverse of the infectious period) we used a lognormal (1.0, 1.0) and a lognormal (-2.0, 0.5) distributions, respectively, that reflected the same rationale behind the priors of the incidence time series analysis. We had information of how densely the samples were relative to the overall number of notifications at the end of the epidemic by the Public health authorities (0.2%, see Fig. 1D) but we use a Beta (2.0, 20.0) prior that assumed that the proportion of observed/sampled infections was even smaller. This is because dengue infection is characterized by an iceberg effect, in which most cases are asymptomatic with documented symptomatic-to-unapparent

199 ratios as large as 1:18 (Balmaseda et al. 2010; Endy et al. 2011; Yap et al. 2013). We used one
200 dimension for parameters *s* and *y* and six dimensions for parameter *R* to avoid over-
201 parameterization. The analyses were done with Beast v2.3.1 (Bouckaert et al. 2014) and the
202 convergence of parameters was assessed using Tracer v1.6 program
203 (http://tree.bio.ed.ac.uk/software/tracer/) until all parameters estimates showed Effective
204 Sample Size (ESS) values over 200.
205
206 Spatiotemporal dispersion pattern
207
208 We used the Bayesian stochastic search variable selection (BSSVS) approach, which
209 assumes exchange rates in the CTMC to be zero with some prior probability, to find the most
210 parsimonious set of rates explaining the diffusion process along the phylogenies for a
211 geolocated dataset (Lemey et al. 2009). Locations were represented by discrete groups of
212 adjacent neighborhoods (discrete phylogeography) and a Bayes factor (BF) test was run to
213 identify the rates contributing to the migration path with the software Spread v1.0.4 (Bielejec
214 et al. 2011). The number of neighborhoods was reduced to a maximum of 10 localities (chosen
215 by vicinity a by number of samples reported) in order to diminish sample-size bias.
216
217 **Results:**
218
219 Sampling
220
221 The year 2013 coincided with a steep rise in the confirmed cases of dengue fever in the
222 State of São Paulo (Fig. S1B). Public Health authorities of Guarujá reported a total of 1805
223 autochthonous dengue cases during this year. Figure 1D compares the actual number of official
224 cases reported and our DENV-4 geolocated sampling.
225 We studied 505 PCR dengue-positive patients during the study In Guarujá. These
226 samples were collected between December 2012 and July 2013. Serotyping determined 10
227 (1.9%) to be DENV-1, eight (1.5%) to be DENV-2, two to be DENV-3 (0.4%) and 505 to be DENV-

4 (96.2%). Preliminary results reporting the documentation of the co-circulation of the four serotypes was published elsewhere (Villabona-Arenas et al. 2014).

Complete envelope (E) gene sequences were obtained for 354 DENV-4 (1485 bp-long). The remaining DENV-4 was not completed processed due to technical problems (*e.g.*, did not yield sufficient viral RNA). These sequences were deposited in GenBank under the accessions KP703864 - KP704217.

DENV-4 genetic diversity

A total of 109 sites (7.3%) of the envelope gene were polymorphic; 32 sites (2.1%) fell in the first and second codon position and 77 (5.2%) fell in the third codon position. There were a total of 95 haplotypes and 75 (79%) of them represented one unique sequence; the three most frequent haplotypes had 119 (34%), 38 (11%) and 30 (8.5%) sequences (Haplotype diversity, the probability that two haplotypes drawn uniformly at random from the population are not the same, was 0.86). The Tajima's D value was of −2.56 ($P < 0.001$) (-2.48 for the combination of both first and second codon positions, -2.26 for the third codon position) evidenced an excess of low frequency polymorphisms relative to expectation, indicating population size expansion and purifying selection. In agreement with this, the overall rate of non-synonymous over synonymous changes *dN*/*dS* value of 0.15 (95% CI 0.10-0.21) for the entire gene suggested purifying selection. Only a few codons, which fell in the central and dimerization gene domains, showed significant purifying selection (codons 92, 133, 184 and 225). Although a few sites experienced an elevation on *dN*/*dS* there was no statistical evidence for adaptive evolution at the significant level of 0.05.

Evolutionary history and epidemiological dynamics

To determine the genotype of DENV-4 in Guarujá, sequences were compared with available worldwide E gene sequences. The 354 local sequences fell into the Latin-American cluster of viruses of Genotype II together with other Brazilian samples (see Data S1). This

257 serotype was relatively new to the country and outbreaks had been reported throughout the
258 country since it was first detected in Brazil in 2011.
259 The substitution model selected for phylogenetic inference using only the sequences
260 from the city was Tamura-Nei (TrN) with invariables sites. The mean evolutionary rate was
261 $2.79x10^{-3}$ (95% HPDs: $2.06x10^{-3}$ – $3.77x10^{-3}$). The estimates for the epidemiological parameters
262 were: *s* of 0.014 (95% HPDs: 0.003-0.031), *y* of 0.15 (95% HPDs: 0.1-0.2) and an estimated origin
263 of the epidemic around the 21th of December-2012 (95% HPDs: 16th December-2012 – 26th
264 December-2012). The value for *y* implies a coupled people/mosquito infectious period of 6.7
265 days. We set informative epidemiological priors because dengue virus diversity does not change
266 much in the time-scale of our study. Figure S3 show the extent to which prior information
267 match the posterior. Sampling from the prior analysis indicated that the posterior and prior
268 traces were the same and that the overall constraints were not forcing the results.
269 Fig. 1A shows two clades early on in the epidemic. The mean time to the most recent
270 common ancestor of these clades did not differ significantly, suggesting that both viral lineages
271 diverged over similar time-scales, and then co-circulated.
272 The dynamic of *R* is presented in Fig. 1B and Fig. 1C. A value of the parameter *R* over 1.0
273 indicates that the disease will be able to spread in a population. For the birth-death analysis,
274 the estimates were high during January, February (the thick of the summer season) and May;
275 there is an abrupt dip during the transition of March to April. For the time series analysis, the
276 estimates are high (with some fluctuations) during the first four months of the year and the
277 curve decrease rapidly by the end of April with values below 1.0 in May and June.
278
279 Phylogeography of DENV-4 over the city
280
281 We were able to geolocate 286 patients (81%) (Fig. S1.A) based on the addresses
282 recorded by the Guarujá Municipal Health Department (Records were not available for the
283 remaining patients). Our first geolocated sample was collected in January the 2nd 2013 at the
284 neighborhood Enseada. This location has a high number of residents (20.883 based on the 2010
285 census records) and is home to the largest beach concentrating bathers from all over the city.

Pae-Cará together with its neighbor Itapema (here condensed as the location Pae-cará), are the neighborhoods with more residents (26,054 and 26,070 respectively) followed by Morrinhos (24,387), Enseada and Jardim Boa Esperança (20,753). The digital map was provided by the Municipality's office and represents the master plan for development and urban planning in the city. Figure 2 illustrates the overall discrete spatial diffusion over the urban area. These results were gauged from a full location-annotated MCC tree, available as Fig. S2, that evidenced an early widespread distribution of the virus in January. The introduction events into each discrete unit are depicted in Fig. 2A; these represent viral diffusion during the onset of the epidemic. These figures suggest that two localities, Enseada and Pae-Cará, were key virus sources. Later on, all regions become interconnected in terms of viral diffusion. The adjacent high-income, low-population density neighborhoods (Jardim Acapulco and Pernambuco) had no cases sampled. This is not explained by distance or lack of connection, because low-income areas nearby (the shantytowns in Mar e Céu, to the South, and Pereque Beach, to the North) had several cases during the epidemic. Bayes factor test of significant diffusion rates shows that another two localities (Morrinhos and Jardim Boa Esperança) played an important role during the course of the epidemic (Fig. 2B). The initial diffusion pattern reproduced to some degree the main access highways of the island: a north-south axis with Pae-Cará and a west-east axis over the littoral with Enseada. (Fig. 2C)

Discussion:

In the present study we described the outbreak of DENV-4 during 2013 in the city of Guarujá, Brazil. We obtained a substantial georeferenced sequence data from this single outbreak; these sequences represents 16% (286/1805) of the dengue notifications done by local public health authorities.

During the outbreak we documented purifying selection and found no statistical evidence of adaptive evolution. Nonetheless, we should take into consideration that the observed differences between our sequences do not represent necessarily fixation events along independent lineages, but polymorphisms segregating in the population; Kryazhimskiy and

Plotkin highlighted that the hallmark signature of positive selection is violated within a population and dN/dS<1 can occur under both negative and positive selection (Kryazhimskiy & Plotkin 2008). Similarly, segregating sites in the population may be responsible for the high-inferred evolutionary rate. Given so, inferences about selection and rates drawn from the analyses should be interpreted with caution.

Following the agreement with the Municipality's office of epidemiological surveillance and the clinical laboratory analysis center, there was a steady collection of samples during the first trimester of the year. Nonetheless, on April 4th 2013, the Public health authorities of Guarujá announced an epidemic alert. In Brazil, the epidemic alter in cities with over 250,000 inhabitants is set when a city reaches the incidence of 100 cases per 100 thousand inhabitants (the estimated population of Guarujá was of 308,000 inhabitants). This means that, during April, the city's authorities strengthened control measures and directed human and hospital resources to be prepared for the threat. Also, confirmatory diagnoses for dengue became clinical and epidemiological (*i.e.*, all patients who experienced acute febrile illness followed by two or more dengue-like symptoms were considered confirmed cases).

The number of cases increased dramatically after the epidemic alert was announced. It is important to note that this surveillance measure ignores the possibility for infection from other pathogens and Dengue is difficult to distinguish from other acute febrile illnesses (Messina et al. 2014; Potts & Rothman 2008; Wiwanitkit 2012); besides population awareness and the distress of the situation might encourage mildly symptomatic people to look for assistance that otherwise would not. In out study, the switch to clinical diagnosis impacted our sampling scheme that relied in serological testing. Municipality health authorities directed resources thereafter to mosquito control and hospital care expenditures. Also, samples had to been sent to the Central Public Health laboratory from The State of São Paulo (Adolfo Lutz Institute) as a norm. Following further agreement with the local office of epidemiological surveillance, a reduced number of samples were collected during the second trimester of the year.

Both reproduction number dynamics (Fig 1B and 1C) and the timing of the coalescent events (concentrated around February) point to an epidemic that started much earlier than the

case report records. A comparable observation was done in another Brazilian setting (Mondini et al. 2009): the epidemic peak by demographic skyline methods took place around two months before the epidemic peak by case report data. The authors argue that such finding resulted from an increase in false positives after the epidemic alert.

Higher values in reproduction numbers (or relative genetic diversity estimates) preceding the peak of laboratory-confirmed cases may reflect virus population spread in a large unreported infected population. Dengue virus infection results in more asymptomatic cases than symptomatic ones and this make very hard any early detection of increased incidence. This phenomenon has been documented for Brazilian urban settings (Endy 2002; Poblap et al. 2006; Teixeira Mda et al. 2002). The spatial diffusion analysis shows that when a significant number of clinical cases began to appear, the virus was practically distributed throughout the city (Fig. S2). Indeed, by looking at the available data on dengue from 2012 (Fig. S1C) it is clear that there was an ongoing transmission early on. Unfortunately, given the limited resources in most dengue endemic areas, control interventions are triggered in response to a significant increase of occurrences.

Following the dynamics of the reproduction number using incidence time series, we observed the last peak of the epidemic during the epidemiological alert period and the decrease thereafter. In contrast, the birth-death skyline pointed to a low reproduction number that coincided with the increase in case report. There is not much data available to elucidate what may be driving R below 1.0 around this period. Even though we suspect that this sudden decrease may be related with sampling bias, additional analyses considering the effect of sampling reproduced similar outcomes (Fig S1B and FigS1C). Phylodynamics methods should be robust to sampling only a fraction of the infected individuals but nonparametric models, such as skyline plots, require enough phylogenetic diversity within the sampled genetic data (du Plessis & Stadler 2015). Major constraints, imposed by a life cycle that alternates infection in mosquitoes and humans, act on the evolution of DENV and the sampling time-scale results in a substantial proportion of identical sequences during the outbreak; in this analysis, collection dates were crucial for drawing phylogenetic inferences. More importantly, it has been shown that these methods not always reconstruct complex dynamics when other factors such as

seasonality, spatial structure and vector dynamics are not incorporated (Rasmussen et al. 2014).

During November of 2012, the city led the ranking of mosquito infestation in the State of São Paulo and the averaged Breteau index for *Aedes aegypti* during 2103 was still high enough (3.12) to sustain epidemics. Nonetheless, soon after the source reduction initiatives were highly encouraged in shantytowns (March), we observed a steadily decrease in the reproduction number estimates (Fig. 1C). It may be the case that these initiatives were initially effective but the flow of people and of mosquitoes from other areas may have offset their contribution. Municipality health authorities considered the use of massive insecticide nebulization when extensive symptomatic cases were recognized. The use of mosquito fogging trucks is costly and relies on availability from the Central Public Health laboratory from The State of São Paulo; given so, this type of control is not executed on a greater number of neighborhoods and strategic areas have to be selected. Crucially, following the surveillance done with the Dengue Duo test, mosquito control actions were planned and directed by the Coordination for Dengue Prevention and Control in those neighborhoods that were reporting clusters of reactive NS1 antigen people. Three locations were selected (Enseada, Paé-Cara and Morrinhos) and four rounds of insecticide nebulization were applied during April. In Guarujá, massive insecticide nebulization coincided with a rapid reduction in the number of cases over time.. Moreover, in the State of São Paulo there is a tendency for the number of cases in each epidemic to rise dramatically when compared to the previous outbreak (in this case, the 2010 epidemic) (Fig. S1B) and the population of Guarujá was a *naïve immune population* to DENV-4. Nevertheless, Guarujá was among the municipalities (103 out of 429) that showed a statistically significant reduction of notification when considering the burden of 2010 (more than 10000 clinical cases were presented) and the total number of cases for the State. This suggests that control strategy achieved some degree of control.

As a caveat, we would argue that Interpreting the temporal trends is not always straightforward and changes in R can not only be due to the impact of control measures but also to the depletion of the size of the susceptible population or changes in seasonality. It has been argued that the turning point of a dengue epidemic frequently occurs before large-scale

intervention measures are implemented and then vector control may has little impact because transmission is usually near its peak (Egger et al. 2008). Nonetheless, by looking at the case notification in the months that followed the sampling period (Fig S1C) it is clear that there are enough susceptible people to sustained transmission all over the year.

The effectiveness of vector control approaches is scarce and almost nothing is known about how well it reduces DENV transmission (Achee et al. 2015). However, the two high-income neighborhoods that had privately-owned and hired vector control services (as informed by the health authorities) showed a discontinuity when pinpointing the dengue incidence over the city. This further suggests that control actions, when applied in a timely and sustained manner, are useful. These actions are costly; rapid and unplanned urbanization (*e.g.* shantytowns) has provided appropriate circumstances (high population density and high contact rates between humans and mosquitos) for substantial vector breeding in Guarujá and several municipalities of Brazil. Moreover, nearby cities, such as Santos and São Vicente (Fig 1B), also have important number of dengue notifications throughout the year and therefore a continuous flux of infected people and mosquitoes is expected. Under this scenario, city public health administrations with limited resources encounter a big challenge. In Guarujá, local authorities prioritized vector control on specific places with clusters of georeferenced viremic patients and the epidemic did not reach higher expected numbers. To achieve full control however, this may be not enough, especially because unapparent infections also contribute to DENV persistent circulation, but it is a first coherent step (Duong et al. 2015). Concerted efforts and resources from the central and local governments and the public remain vital to fight the disease. Besides, there are limited studies quantifying dengue underreporting; studies with active and passive dengue surveillance figures and entomological data are needed in order to fill these important research gaps (Toan et al. 2015).

Conclusion:

Studying urban outbreaks is important; successful public health interventions require detailed knowledge of the disease dynamics and how it spread within the population. It is very

difficult to eliminate *Aedes* mosquitoes because they bounce back to initial numbers even after vector population disturbances resulting from human interventions. Nonetheless, in the absence of a vaccine, source reduction initiatives and massive control actions are the options that city public health administrations have. We have evidenced that a delayed response may result in an epidemic that grow beyond the capabilities of local health authorities but that sound efforts may diminish its effect. (du Plessis & Stadler 2015).

Acknowledgments:

We thank all the participants in this study for generously helping us in this research. We also thank all the medical staff and administrative staff from the Primary Health Care Facilities, the Emergency Care Units and the ITAPEMA clinical laboratory analysis center who have kindly assisted us by collecting blood samples from participants. We thank the Guarujá Municipality's office of epidemiological surveillance for the discussions and the practical and technical advice.

References:

Achee NL, Gould F, Perkins TA, Reiner RC, Jr., Morrison AC, Ritchie SA, Gubler DJ, Teyssou R, and Scott TW. 2015. A critical assessment of vector control for dengue prevention. *PLoS Negl Trop Dis* 9:e0003655. 10.1371/journal.pntd.0003655

Balmaseda A, Standish K, Mercado JC, Matute JC, Tellez Y, Saborio S, Hammond SN, Nunez A, Aviles W, Henn MR, Holmes EC, Gordon A, Coloma J, Kuan G, and Harris E. 2010. Trends in patterns of dengue transmission over 4 years in a pediatric cohort study in Nicaragua. *J Infect Dis* 201:5-14. 10.1086/648592

Barreto ML, and Teixeira MG. 2008. Dengue in Brazil: Epidemiological situation and Contribution to a Research Agenda. *estudos avançados* 22:53-72.

Bennett SN, Holmes EC, Chirivella M, Rodriguez DM, Beltran M, Vorndam V, Gubler DJ, and McMillan WO. 2003. Selection-driven evolution of emergent dengue virus. *Mol Biol Evol* 20:1650-1658. 10.1093/molbev/msg182

Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS, Hoen AG, Sankoh O, Myers MF, George DB, Jaenisch T, Wint GR, Simmons CP, Scott TW, Farrar JJ, and Hay SI. 2013. The global distribution and burden of dengue. *Nature* 496:504-507. 10.1038/nature12060

464 Bielejec F, Rambaut A, Suchard MA, and Lemey P. 2011. SPREAD: spatial phylogenetic
465 reconstruction of evolutionary dynamics. *Bioinformatics* 27:2910-2912.
466 10.1093/bioinformatics/btr481
467 Bouckaert R, Heled J, Kuhnert D, Vaughan T, Wu CH, Xie D, Suchard MA, Rambaut A, and
468 Drummond AJ. 2014. BEAST 2: a software platform for Bayesian evolutionary
469 analysis. *PLoS Comput Biol* 10:e1003537. 10.1371/journal.pcbi.1003537
470 Chan M, and Johansson MA. 2012. The incubation periods of Dengue viruses. *PLoS One*
471 7:e50972. 10.1371/journal.pone.0050972
472 Chen R, and Vasilakis N. 2011. Dengue--quo tu et quo vadis? *Viruses* 3:1562-1608.
473 10.3390/v3091562
474 Cori A, Ferguson NM, Fraser C, and Cauchemez S. 2013. A new framework and software to
475 estimate time-varying reproduction numbers during epidemics. *Am J Epidemiol*
476 178:1505-1512. 10.1093/aje/kwt133
477 Cuong HQ, Vu NT, Cazelles B, Boni MF, Thai KT, Rabaa MA, Quang LC, Simmons CP, Huu TN,
478 and Anders KL. 2013. Spatiotemporal dynamics of dengue epidemics, southern
479 Vietnam. *Emerg Infect Dis* 19:945-953. 10.3201/eid1906.121323
480 Darriba D, Taboada GL, Doallo R, and Posada D. 2012. jModelTest 2: more models, new
481 heuristics and parallel computing. *Nat Methods* 9:772. 10.1038/nmeth.2109
482 Delport W, Poon AF, Frost SD, and Kosakovsky Pond SL. 2010. Datamonkey 2010: a suite of
483 phylogenetic analysis tools for evolutionary biology. *Bioinformatics* 26:2455-2457.
484 10.1093/bioinformatics/btq429
485 Drummond AJ, Ho SY, Phillips MJ, and Rambaut A. 2006. Relaxed phylogenetics and dating
486 with confidence. *PLoS Biol* 4:e88. 10.1371/journal.pbio.0040088
487 du Plessis L, and Stadler T. 2015. Getting to the root of epidemic spread with phylodynamic
488 analysis of genomic data. *Trends Microbiol* 23:383-386. 10.1016/j.tim.2015.04.007
489 Duong V, Lambrechts L, Paul RE, Ly S, Lay RS, Long KC, Huy R, Tarantola A, Scott TW,
490 Sakuntabhai A, and Buchy P. 2015. Asymptomatic humans transmit dengue virus to
491 mosquitoes. *Proc Natl Acad Sci U S A*. 10.1073/pnas.1508114112
492 Edgar RC. 2004a. MUSCLE: a multiple sequence alignment method with reduced time and
493 space complexity. *BMC Bioinformatics* 5:113. 10.1186/1471-2105-5-113
494 Edgar RC. 2004b. MUSCLE: multiple sequence alignment with high accuracy and high
495 throughput. 32.
496 Edgar RC. 2010. Search and clustering orders of magnitude faster than BLAST.
497 *Bioinformatics* 26:2460-2461. 10.1093/bioinformatics/btq461
498 Egger JR, Ooi EE, Kelly DW, Woolhouse ME, Davies CR, and Coleman PG. 2008.
499 Reconstructing historical changes in the force of infection of dengue fever in
500 Singapore: implications for surveillance and control. *Bull World Health Organ*
501 86:187-196. 10.2471/blt.07.040170
502 Endy TP. 2002. Epidemiology of Inapparent and Symptomatic Acute Dengue Virus
503 Infection: A Prospective Study of Primary School Children in Kamphaeng Phet,
504 Thailand. *American Journal of Epidemiology* 156:40-51. 10.1093/aje/kwf005
505 Endy TP, Anderson KB, Nisalak A, Yoon IK, Green S, Rothman AL, Thomas SJ, Jarman RG,
506 Libraty DH, and Gibbons RV. 2011. Determinants of inapparent and symptomatic
507 dengue infection in a prospective study of primary school children in Kamphaeng
508 Phet, Thailand. *PLoS Negl Trop Dis* 5:e975. 10.1371/journal.pntd.0000975

509 Guindon S, and Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large
510 phylogenies by maximum likelihood. *Syst Biol* 52:696-704.
511 10.1080/10635150390235520
512 Halstead SB. 2008. Dengue virus-mosquito interactions. *Annu Rev Entomol* 53:273-291.
513 10.1146/annurev.ento.53.103106.093326
514 Jeefoo P, Tripathi NK, and Souris M. 2011. Spatio-temporal diffusion pattern and hotspot
515 detection of dengue in Chachoengsao province, Thailand. *Int J Environ Res Public*
516 *Health* 8:51-74. 10.3390/ijerph8010051
517 Kassim F. 2011. Use of dengue NS1 antigen for early diagnosis of dengue virus infection.
518 *Southeast asian J trop Med public health* 42.
519 Kosakovsky Pond SL, and Frost SD. 2005. Not so different after all: a comparison of
520 methods for detecting amino acid sites under selection. *Mol Biol Evol* 22:1208-1222.
521 10.1093/molbev/msi105
522 Kryazhimskiy S, and Plotkin JB. 2008. The population genetics of dN/dS. *PLoS Genet*
523 4:e1000304. 10.1371/journal.pgen.1000304
524 Kuhnert D, Stadler T, Vaughan TG, and Drummond AJ. 2014. Simultaneous reconstruction
525 of evolutionary history and epidemiological dynamics from viral sequences with the
526 birth-death SIR model. *J R Soc Interface* 11:20131106. 10.1098/rsif.2013.1106
527 Lemey P, Rambaut A, Drummond AJ, and Suchard MA. 2009. Bayesian phylogeography
528 finds its roots. *PLoS Comput Biol* 5:e1000520. 10.1371/journal.pcbi.1000520
529 Librado P, and Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA
530 polymorphism data. *Bioinformatics* 25:1451-1452. 10.1093/bioinformatics/btp187
531 Maddison WP, and Maddison DR. 2014. Mesquite: a modular system for evolutionary
532 analysis. . Version 3.01 ed: http://mesquiteproject.org.
533 Messina JP, Brady OJ, Scott TW, Zou C, Pigott DM, Duda KA, Bhatt S, Katzelnick L, Howes RE,
534 Battle KE, Simmons CP, and Hay SI. 2014. Global spread of dengue virus types:
535 mapping the 70 year history. *Trends Microbiol* 22:138-146.
536 10.1016/j.tim.2013.12.011
537 Mondini A, de Moraes Bronzoni RV, Nunes SH, Chiaravalloti Neto F, Massad E, Alonso WJ,
538 Lazzaro ES, Ferraz AA, de Andrade Zanotto PM, and Nogueira ML. 2009. Spatio-
539 temporal tracking and phylodynamics of an urban dengue 3 outbreak in Sao Paulo,
540 Brazil. *PLoS Negl Trop Dis* 3:e448. 10.1371/journal.pntd.0000448
541 Nunes MR, Faria NR, Vasconcelos HB, Medeiros DB, Silva de Lima CP, Carvalho VL, Pinto da
542 Silva EV, Cardoso JF, Sousa EC, Jr., Nunes KN, Rodrigues SG, Abecasis AB, Suchard
543 MA, Lemey P, and Vasconcelos PF. 2012. Phylogeography of dengue virus serotype 4,
544 Brazil, 2010-2011. *Emerg Infect Dis* 18:1858-1864. 10.3201/eid1811.120217
545 Nunes MR, Palacios G, Faria NR, Sousa EC, Jr., Pantoja JA, Rodrigues SG, Carvalho VL,
546 Medeiros DB, Savji N, Baele G, Suchard MA, Lemey P, Vasconcelos PF, and Lipkin WI.
547 2014. Air travel is associated with intracontinental spread of dengue virus serotypes
548 1-3 in Brazil. *PLoS Negl Trop Dis* 8:e2769. 10.1371/journal.pntd.0002769
549 Poblap T, Nitatpattana N, Chaimarin A, Barbazan P, Chauvancy G, Yoksan S, and Gonzalez JP.
550 2006. Silent transmission of virus during a Dengue epidemic, Nakhon Pathom
551 Province, Thailand 2001. *Southeast asian J trop Med public health* 37:899-903.
552 Pond SL, Frost SD, and Muse SV. 2005. HyPhy: hypothesis testing using phylogenies.
553 *Bioinformatics* 21:676-679. 10.1093/bioinformatics/bti079

554 Potts JA, and Rothman AL. 2008. Clinical and laboratory features that distinguish dengue
555 from other febrile illnesses in endemic populations. *Trop Med Int Health* 13:1328-
556 1340. 10.1111/j.1365-3156.2008.02151.x
557 Raghwani J, Rambaut A, Holmes EC, Hang VT, Hien TT, Farrar J, Wills B, Lennon NJ, Birren
558 BW, Henn MR, and Simmons CP. 2011. Endemic dengue associated with the co-
559 circulation of multiple viral lineages and localized density-dependent transmission.
560 *PLoS Pathog* 7:e1002064. 10.1371/journal.ppat.1002064
561 Rasmussen DA, Boni MF, and Koelle K. 2014. Reconciling phylodynamics with
562 epidemiology: the case of dengue virus in southern Vietnam. *Mol Biol Evol* 31:258-
563 271. 10.1093/molbev/mst203
564 Salje H, Lessler J, Endy TP, Curriero FC, Gibbons RV, Nisalak A, Nimmannitya S,
565 Kalayanarooj S, Jarman RG, Thomas SJ, Burke DS, and Cummings DA. 2012.
566 Revealing the microscale spatial signature of dengue transmission and immunity in
567 an urban population. *Proc Natl Acad Sci U S A* 109:9535-9538.
568 10.1073/pnas.1120621109
569 Schreiber MJ, Holmes EC, Ong SH, Soh HS, Liu W, Tanner L, Aw PP, Tan HC, Ng LC, Leo YS,
570 Low JG, Ong A, Ooi EE, Vasudevan SG, and Hibberd ML. 2009. Genomic epidemiology
571 of a dengue virus epidemic in urban Singapore. *J Virol* 83:4163-4173.
572 10.1128/JVI.02445-08
573 Shepard DS, Coudeville L, Halasa YA, Zambrano B, and Dayan GH. 2011. Economic impact of
574 dengue illness in the Americas. *Am J Trop Med Hyg* 84:200-207.
575 10.4269/ajtmh.2011.10-0503
576 Stadler T, Kuhnert D, Bonhoeffer S, and Drummond AJ. 2013. Birth-death skyline plot
577 reveals temporal changes of epidemic spread in HIV and hepatitis C virus (HCV).
578 *Proc Natl Acad Sci U S A* 110:228-233. 10.1073/pnas.1207965110
579 Stahl HC, Butenschoen VM, Tran HT, Gozzer E, Skewes R, Mahendradhata Y, Runge-
580 Ranzinger S, Kroeger A, and Farlow A. 2013. Cost of dengue outbreaks: literature
581 review and country case studies. *BMC Public Health* 13:1048. 10.1186/1471-2458-
582 13-1048
583 Teixeira Mda G, Barreto ML, Costa Mda C, Ferreira LD, Vasconcelos PF, and Cairncross S.
584 2002. Dynamics of dengue virus circulation: a silent epidemic in a complex urban
585 area. *Trop Med Int Health* 7:757-762.
586 Teixeira MG, Siqueira JB, Jr., Ferreira GL, Bricks L, and Joint G. 2013. Epidemiological trends
587 of dengue disease in Brazil (2000-2010): a systematic literature search and analysis.
588 *PLoS Negl Trop Dis* 7:e2520. 10.1371/journal.pntd.0002520
589 Toan NT, Rossi S, Prisco G, Nante N, and Viviani S. 2015. Dengue epidemiology in selected
590 endemic countries: factors influencing expansion factors as estimates of
591 underreporting. *Tropical Medicine & International Health* 20:840-863.
592 10.1111/tmi.12498
593 Vazquez-Prokopec GM, Kitron U, Montgomery B, Horne P, and Ritchie SA. 2010. Quantifying
594 the spatial dimension of dengue virus epidemic spread within a tropical urban
595 environment. *PLoS Negl Trop Dis* 4:e920. 10.1371/journal.pntd.0000920
596 Villabona-Arenas CJ, de Oliveira JL, Capra Cde S, Balarini K, Loureiro M, Fonseca CR, Passos
597 SD, and Zanotto PM. 2014. Detection of four dengue serotypes suggests rise in
598 hyperendemicity in urban centers of Brazil. *PLoS Negl Trop Dis* 8:e2620.
599 10.1371/journal.pntd.0002620

600 Villabona-Arenas CJ, and Zanotto PM. 2011. Evolutionary history of Dengue virus type 4:
601 insights into genotype phylodynamics. *Infect Genet Evol* 11:878-885.
602 10.1016/j.meegid.2011.02.007
603 Wiwanitkit V. 2012. The importance of accurate diagnosis of dengue fever. *Future Virology*
604 7:53-62. Doi 10.2217/Fvl.11.129
605 Yap G, Li C, Mutalib A, Lai YL, and Ng LC. 2013. High rates of inapparent dengue in older
606 adults in Singapore. *Am J Trop Med Hyg* 88:1065-1069. 10.4269/ajtmh.12-0150
607

1

Phylogenetic relationships and reproduction numbers of DENV-4 genotype II isolated in the municipality of Guarujá from January-June 2013.

A) Maximum clade credibility (MCC) tree inferred using envelope gene sequences. Branch tips were removed for simplicity. B) Median estimates and 95% HPD intervals for the effective reproductive number using Birth-Death skyline methods. C) Median estimates and 95% IC for the effective reproductive number using incidence time series data. For B and C official dengue reports done by epidemiological week and sampling done in a daily basis are presented. The band represents the period in which the epidemic alert was announced. The gray triangle informs when the neighboring city of Santos announced its own epidemic alert.

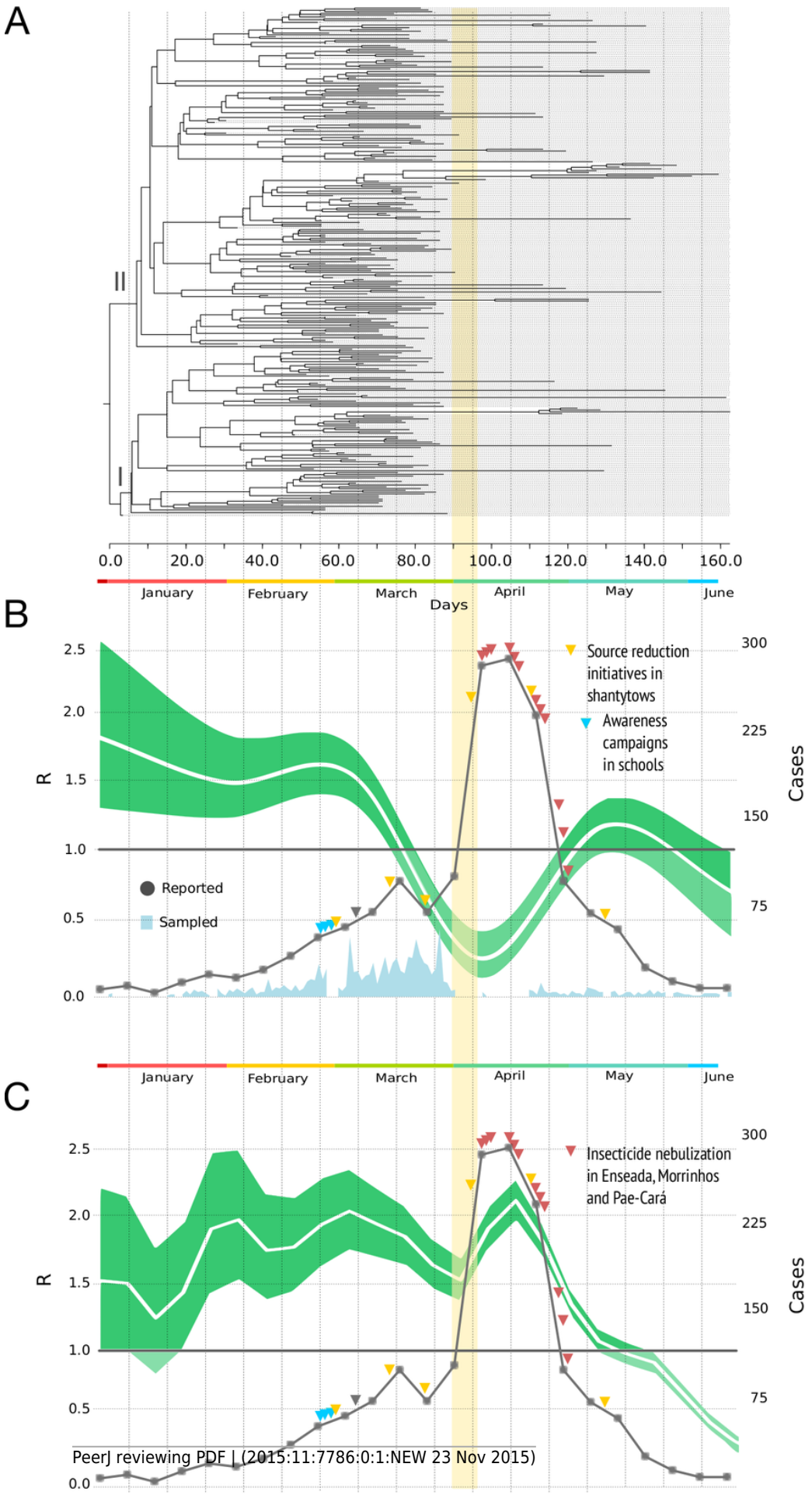
A
II
I
0.0
20.0
40.0
60.0
80.0
100.0
120.0
140.0
160.0
January
February
March
April
May
June
Days
B
R
Cases
Source reduction initiatives in shantytows
Awareness campaigns in schools
Reported
Sampled
C
Insecticide nebulization in Enseada, Morrinhos and Pae-Cará

Figure 2(on next page)

Diffusion of DENV-4 genotype II in the municipality of Guarujá from January-June 2013.

Discontinuous green areas represent discrete areas. A) Introduction routes into each area. B) Routes that best explain virus diffusion all over the city. The reconstruction was done following a location-annotated MCC tree available as Fig. S2. C) The main avenues and highways of Guarujá. Names are given for the areas that are quoted in the text.