The 2013 outbreak of DENV in Guarujá

Life Cycle of DENV

Endemic/Epidemic

Transovarial transmission

Ae. furcifer (West Africa)
Ae. albopictus (Southeast Asia)

Intrinsic incubation period (6.5 days)

Ae. aegypti
Ae. albopictus
Ae. polynensiensis

Zone of Emergence

???

Extrinsic incubation period

25°C (15.0 days)

30°C (5.9 days)

Primates

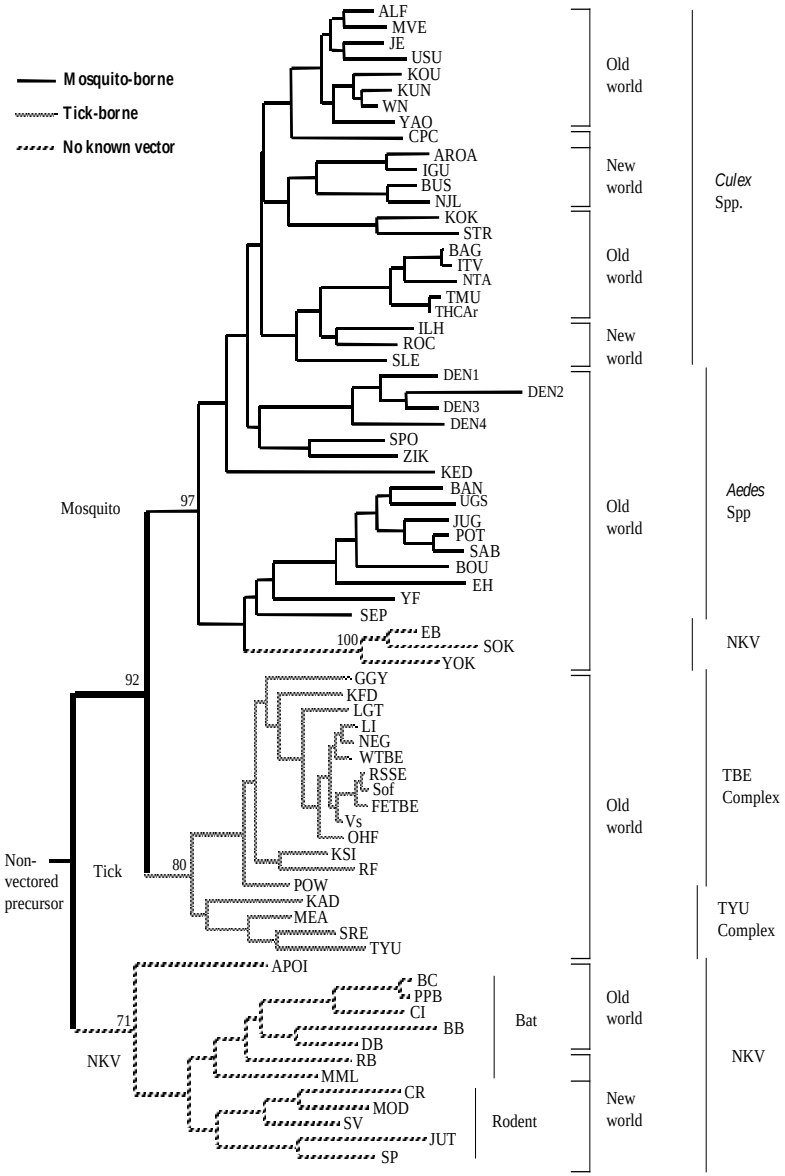
Ae. luteochephalus (West Africa)
Ae. furcifer (West Africa)
Ae. niveus (Southeast Asia)

Humans

Transovarial transmission

Sylvatic

Flavivirus vector associations



Sean Nee & Phylodynamics

Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 545–546, January 1996

Commentary

Evolving virus plagues

John J. Holland

Department of Biology, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0116

Recently, the AIDS pandemic and other new or emerging viruses have focused attention on emerging infectious diseases (1, 2). The factors involved in emergence are diverse and include global transportation, urban crowding and poverty, changing behavioral patterns, rapid virus evolution, human population growth, etc. Until now, no studies have clearly linked human population expansion with in- of Europe and Asia are mainly zoonoses. Tick-borne encephalitis (TBE) viruses exist almost exclusively in "forest cycles" involving ticks and various vertebrate hosts. Humans play little if any role in these cycles, and human disease is accidental and dead-end (albeit sometimes severe). In work described in this issue of the *Proceedings* (pages 548–553), Zanotto *et al.* (6) have compared the molecular

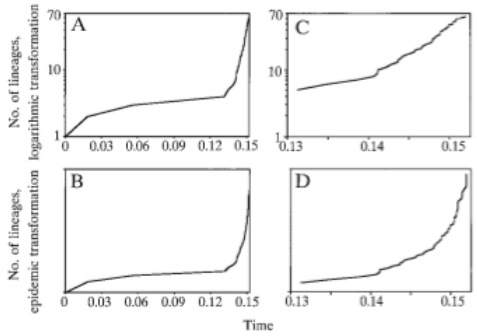


FIG. 2. (*A*) Lineages through time of 66 dengue viruses. The time at which each lineage division occurs on a constant-rate (KITSCH) phylogenetic tree is plotted with the time axis scaled as the number of nucleotide substitutions from the root of the tree to the tips. (*B*) Epidemic transformation of the same data. This transformation of the *y* axis determines whether the rate of population growth has been constant through time (straight line), increasing (upward curvature, as here), or decreasing (downward curvature). (*C*) Lineages through time of the most recent 62 nodes of dengue viruses. (*D*) Epidemic transformation of the same data.

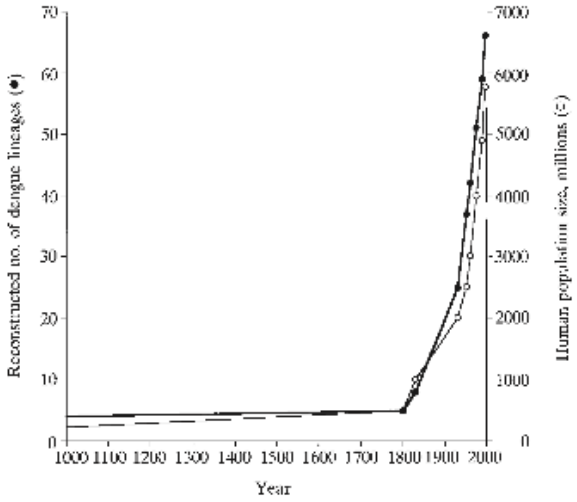
Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 548–553, January 1996
Evolution

Population dynamics of flaviviruses revealed by molecular phylogenies

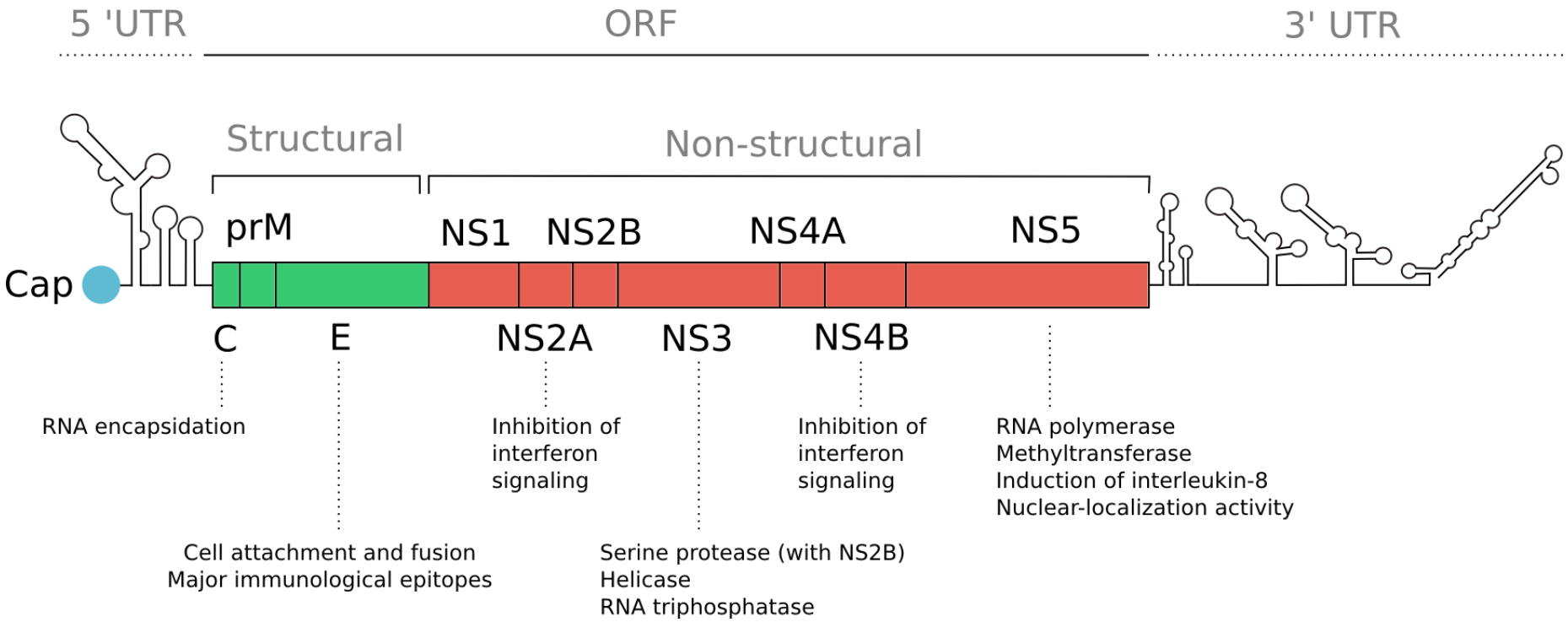
PAOLO M. DE A. ZANOTTO*, ERNEST A. GOULD*, GEORGE F. GAO*, PAUL H. HARVEY†, AND EDWARD C. HOLMES†‡

*National Environment Research Council Institute of Virology and Environmental Microbiology, Mansfield Road, Oxford, OX1 3SR, United Kingdom; and †Wellcome Centre for the Epidemiology of Infectious Disease, Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3PS, United Kingdom

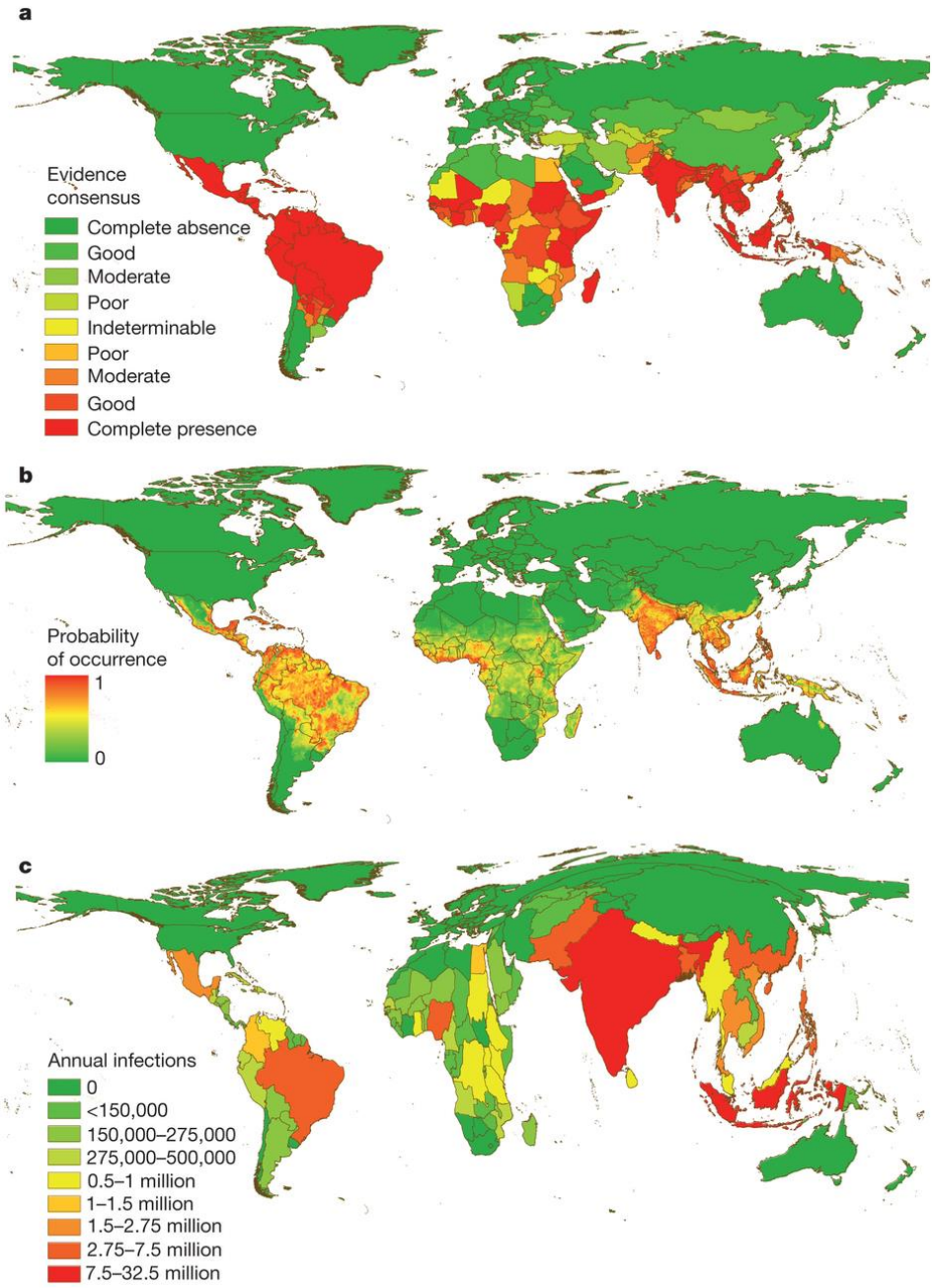
Communicated by Robert M. May, University of Oxford, Oxford, United Kingdom, October 20, 1995 (received for review August 1, 1995)

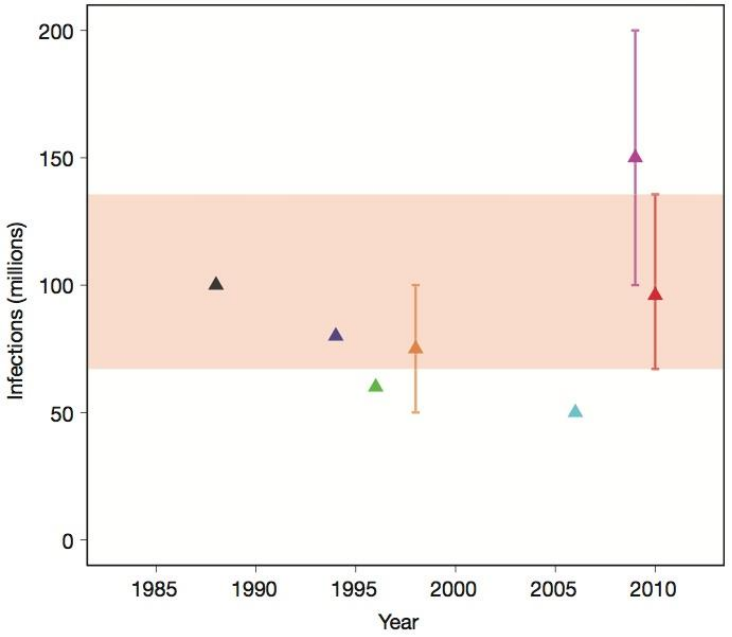


DENV Genome

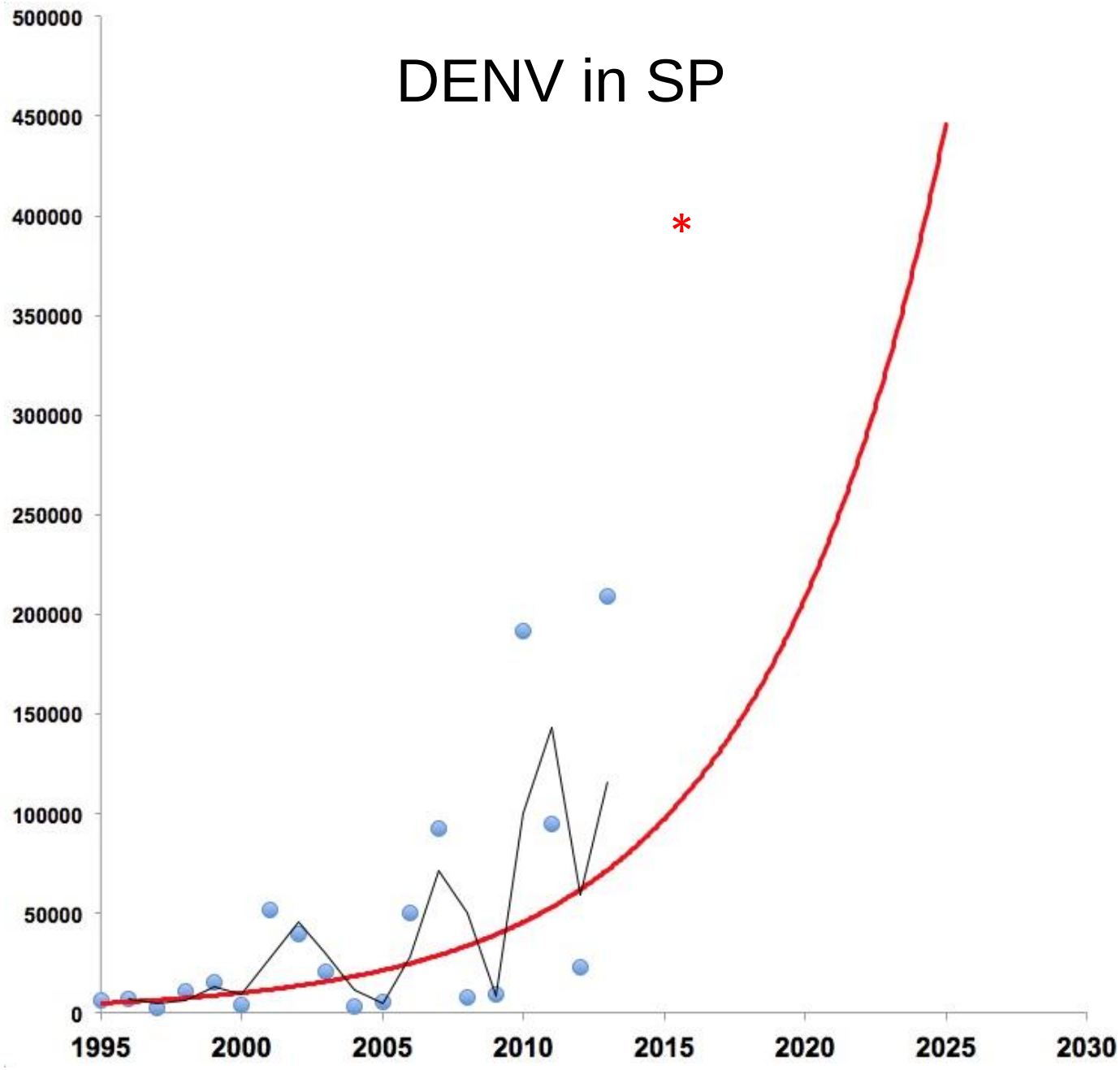

5 'UTR
ORF
3' UTR
Structural
Non-structural
prM
NS1
NS2B
NS4A
NS5
Cap
C
E
NS2A
NS3
NS4B
RNA encapsidation
Cell attachment and fusion
Major immunological epitopes
Inhibition of interferon signaling
Serine protease (with NS2B)
Helicase
RNA triphosphatase
Inhibition of interferon signaling
RNA polymerase
Methyltransferase
Induction of interleukin-8
Nuclear-localization activity

DENV Burden


a
Evidence consensus
Complete absence
Good
Moderate
Poor
Indeterminable
Poor
Moderate
Good
Complete presence
b
Probability of occurrence
1
0
c
Annual infections
0
<150,000
150,000–275,000
275,000–500,000
0.5–1 million
1–1.5 million
1.5–2.75 million
2.75–7.5 million
7.5–32.5 million


Infections (millions)
200
150
100
50
0
1985
1990
1995
2000
2005
2010
Year

DENV in SP


500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
1995
2000
2005
2010
2015
2020
2025
2030
*

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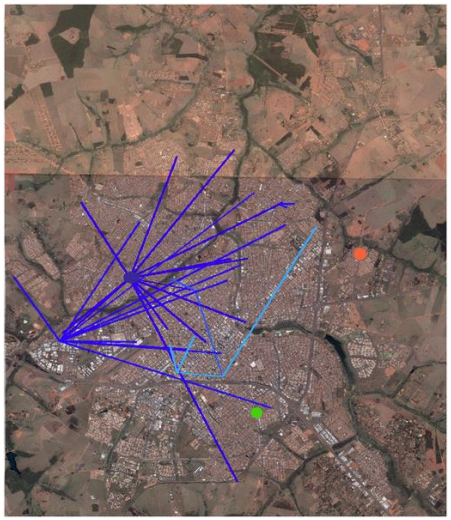
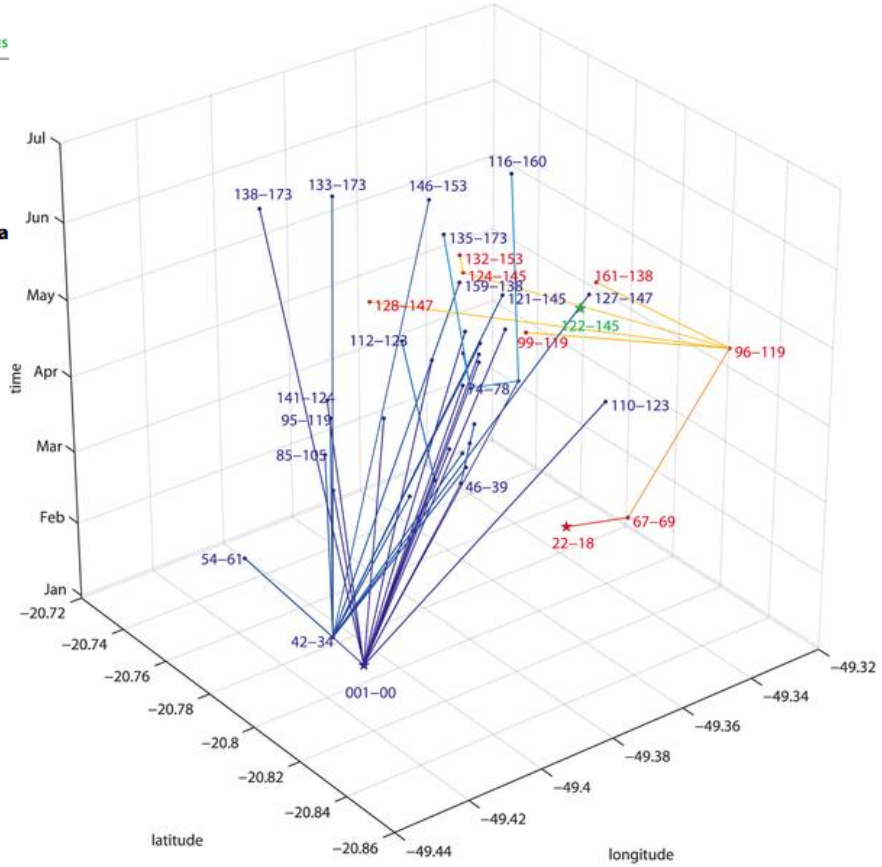
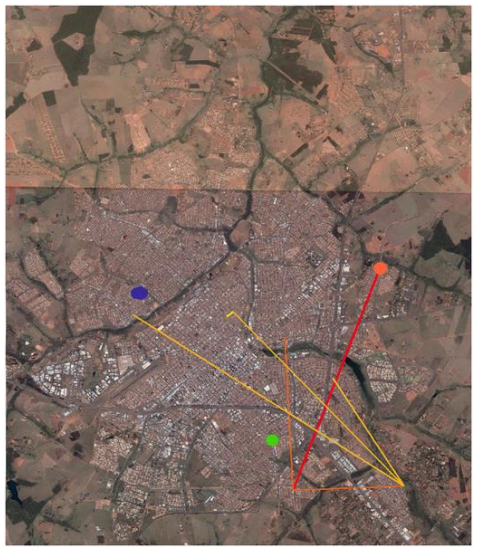
PLoS NEGLECTED TROPICAL DISEASES

Spatio-Temporal Tracking and Phylodynamics of an Urban Dengue 3 Outbreak in São Paulo, Brazil

Adriano Mondini[1], Roberta Vieira de Moraes Bronzoni[1], Silvia Helena Pereira Nunes[1], Francisco Chiaravalloti Neto[1,2], Eduardo Massad[3], Wladimir J. Alonso[4], Eduardo S. M. Lázzaro[5], Amena Alcântara Ferraz[5], Paolo Marinho de Andrade Zanotto[6], Maurício Lacerda Nogueira[1]*







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PLOS | NEGLECTED TROPICAL DISEASES

Viewpoints

Detection of Four Dengue Serotypes Suggests Rise in Hyperendemicity in Urban Centers of Brazil

Christian Julián Villabona-Arenas[1], Jessica Luana de Oliveira[1], Carla de Sousa Capra[2], Karime Balarini[3], Mauricio Loureiro[4], Celso Ricardo Theoto P. Fonseca[3], Saulo Duarte Passos[4], Paolo Marinho de Andrade Zanotto[1]*

1 Laboratório de Evolução Molecular e Bioinformática, Departamento de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, São Paulo, Brazil, **2** Laboratório de Saúde Pública, Secretaria da Saúde, Prefeitura Municipal de Guarujá, Guarujá, São Paulo, Brazil, **3** Itapema Laboratório de Análises Clínicas, Guarujá, São Paulo, Brazil, **4** Laboratório de Infectologia Pediátrica, Faculdade de Medicina de Jundiaí, Jundiaí, São Paulo, Brazil

DENV-3 samples were from genotype V

- Genotype III has been the most prevalent in Brazil. However, genotypes I and V were associated with dengue outbreaks in Brazil.

- Cryptic circulation of DENV-3 in the region?

- A new introduction from Asia?

- Q: “What is the *A. aegypti* lineage in Santos?”
  R: “Singapore”!

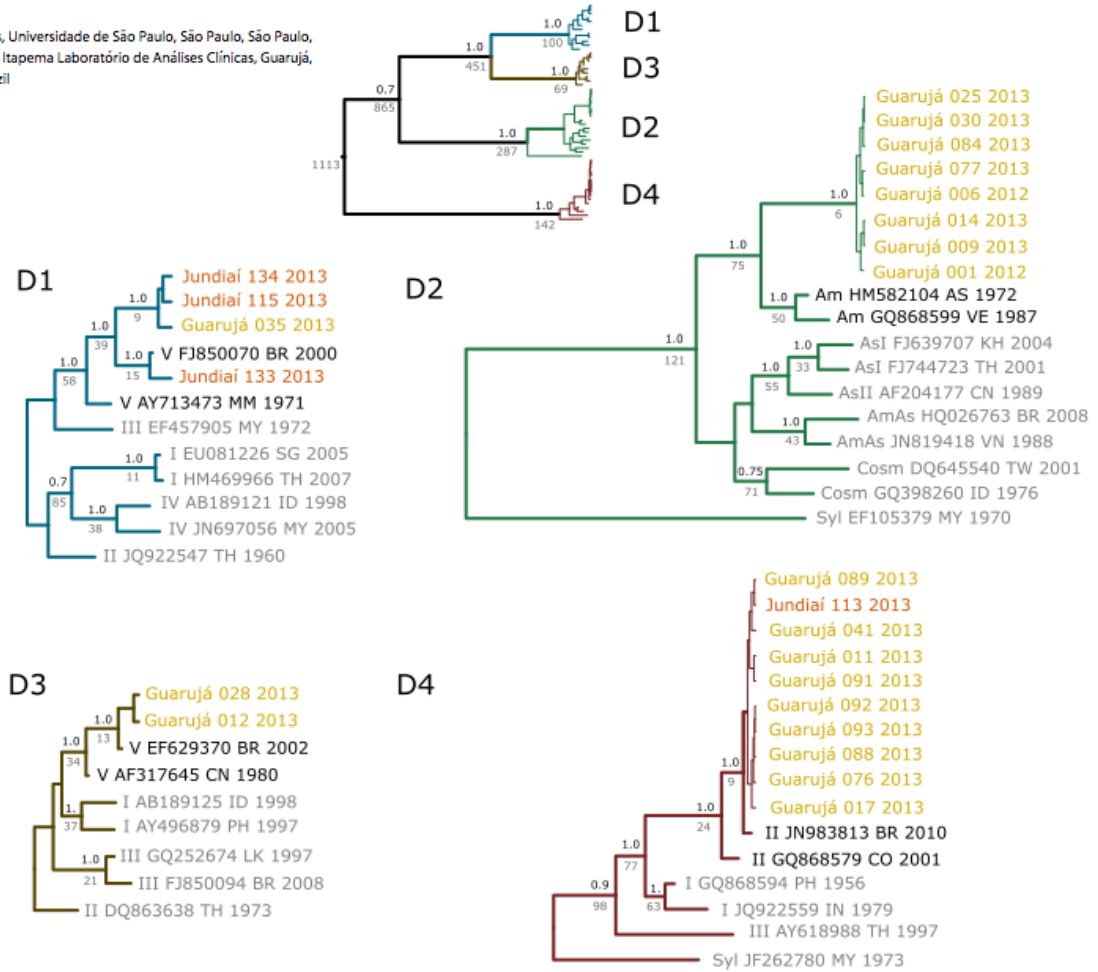


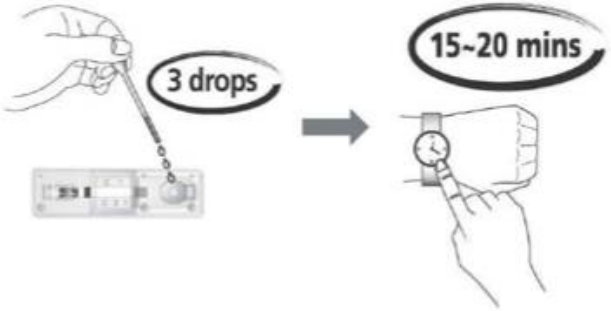
Figure 1. Maximum credibility tree (and its four serotype subtrees) showing the evolutionary relationships among the capsid/premembrane junction sequences of 55 strains. Posterior probability support values are shown over the nodes and TMRCAs values below the nodes. Reference sequences abbreviated by serotype (D1–4), genotype (*Am*: American, *AmAs*: American/Asian, *As*: Asian, *Cosm*: Cosmopolitan, *Syl*: Sylvatic, *I*, *II*, *III*, *IV*, or *V*), GenBank accession, ISO code for country, and isolation year.
doi:10.1371/journal.pntd.0002620.g001

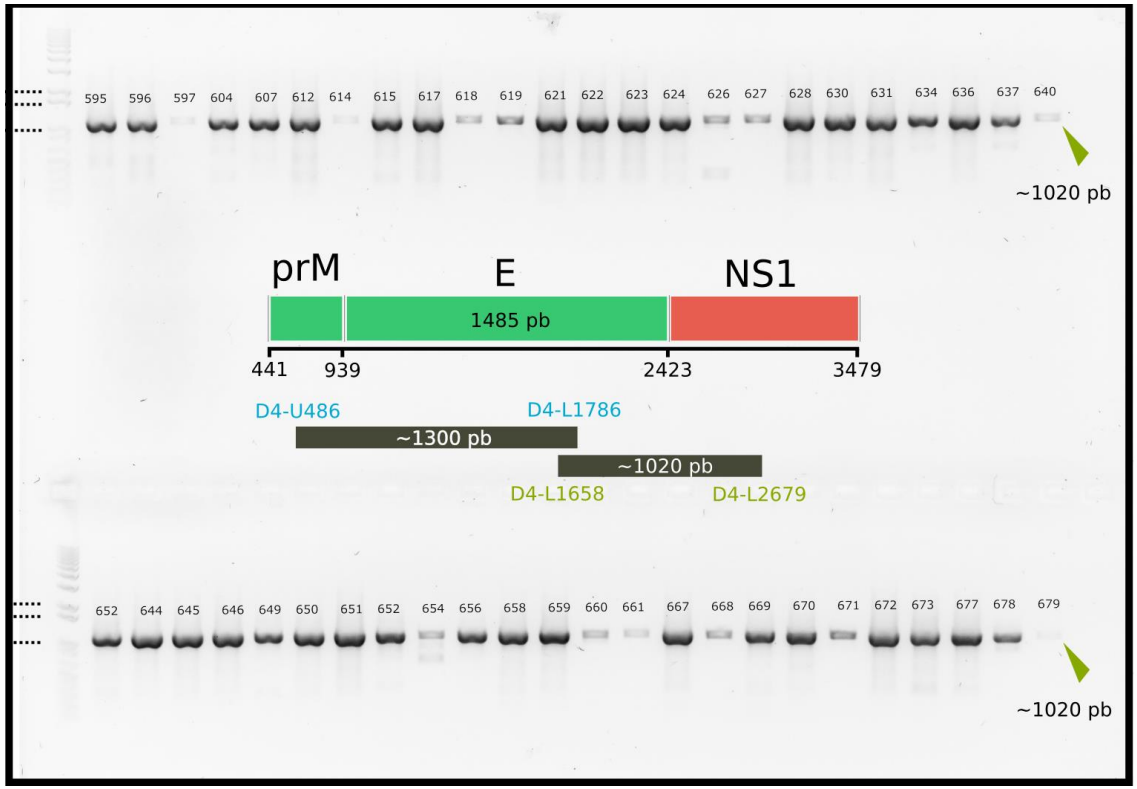
DENV-4 Outbreak in Guarujá

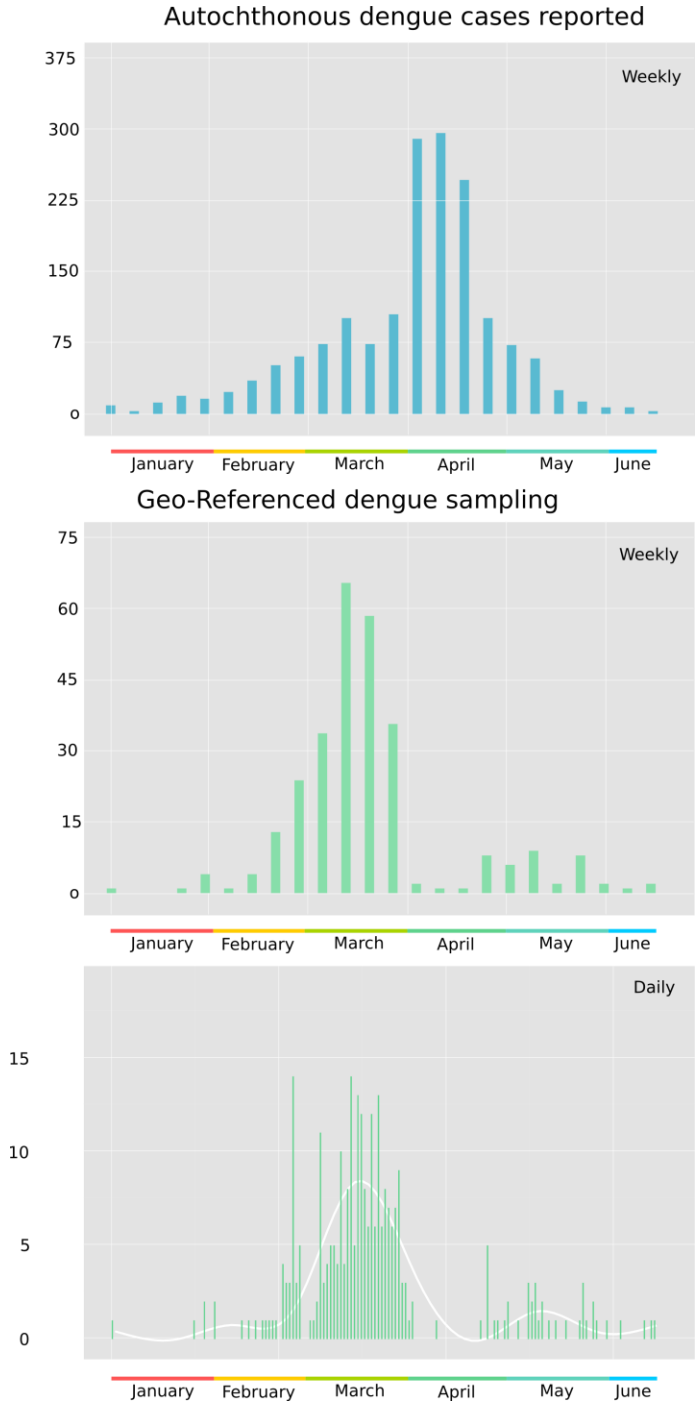
- 1216 serum samples from January to June 2013.
- 879 samples were tested in our laboratory for dengue: 525 (60% of processed samples) were positive for dengue.
- 505 (96% of positive samples) were positive for DENV-4 and 20 belong to any of the other three serotypes (10 DENV-1, 8 DENV-2 and 2 DENV-3).
- 354 DENV-4 (70% of positive) were sequenced1485 bp-long envelope gene from viral genomic RNA amplified directly.
- 285 patients (81%) geo-locate based on the addresses provided, from the cohort of 354 patients.

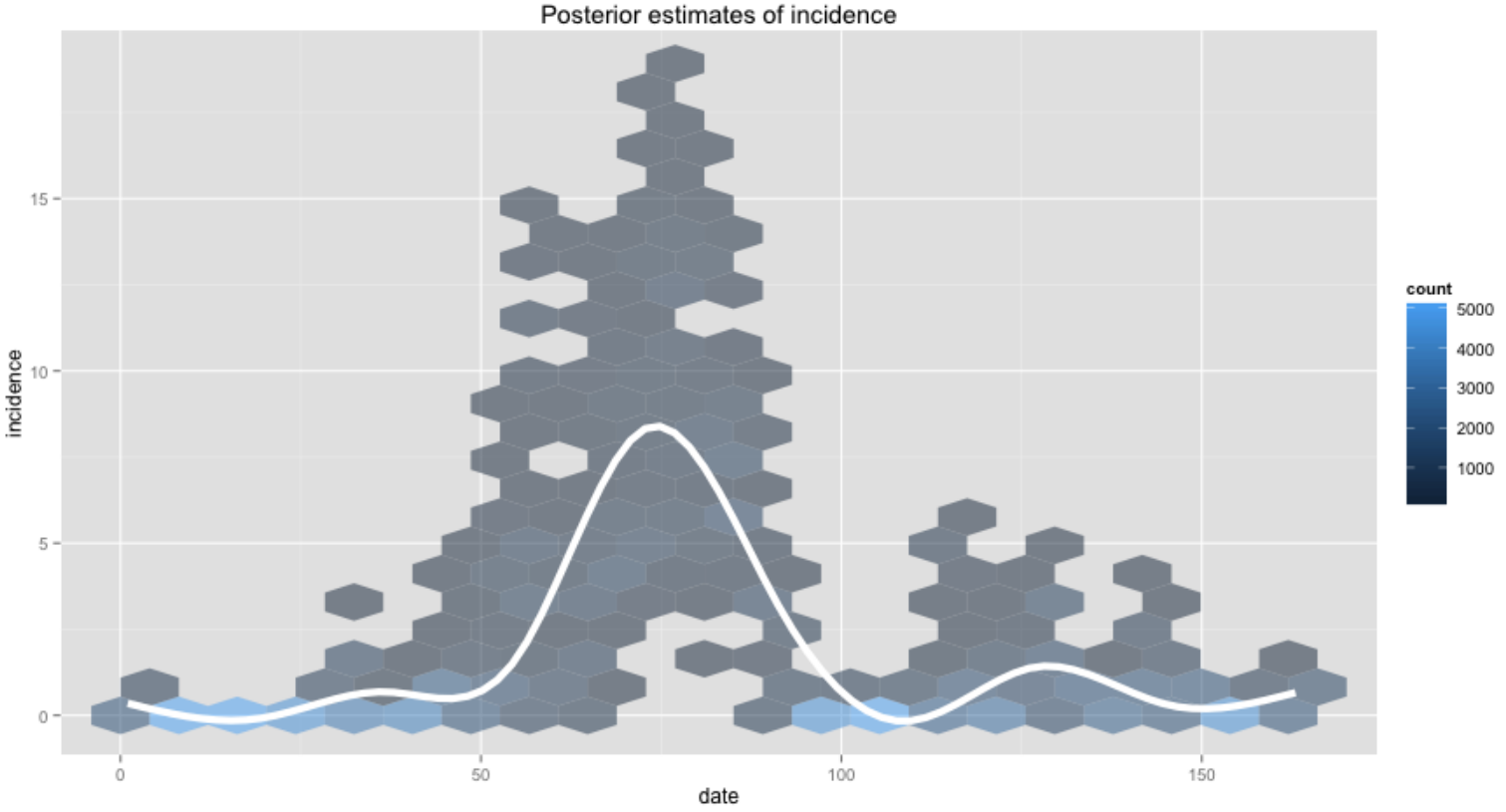

São Paulo state
Bertioga
Santos
Santos
Guarujá

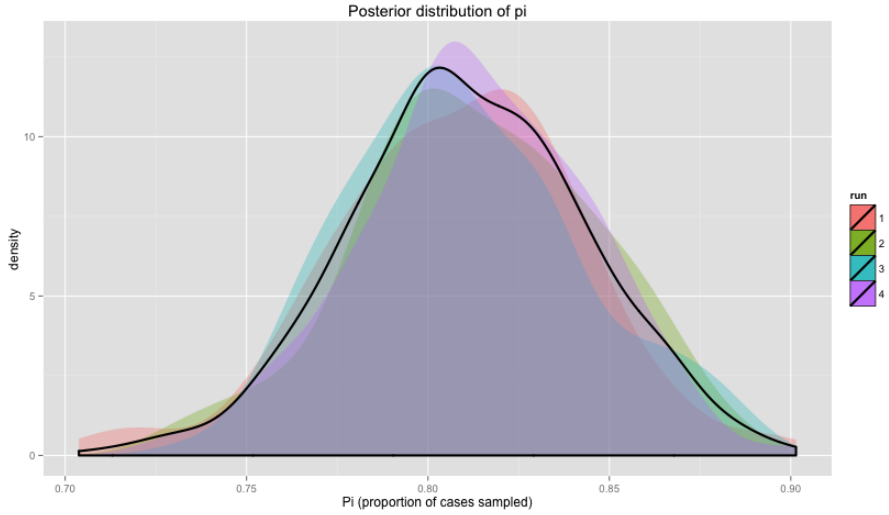
Immunochromatography for IgM, IgG & NS1





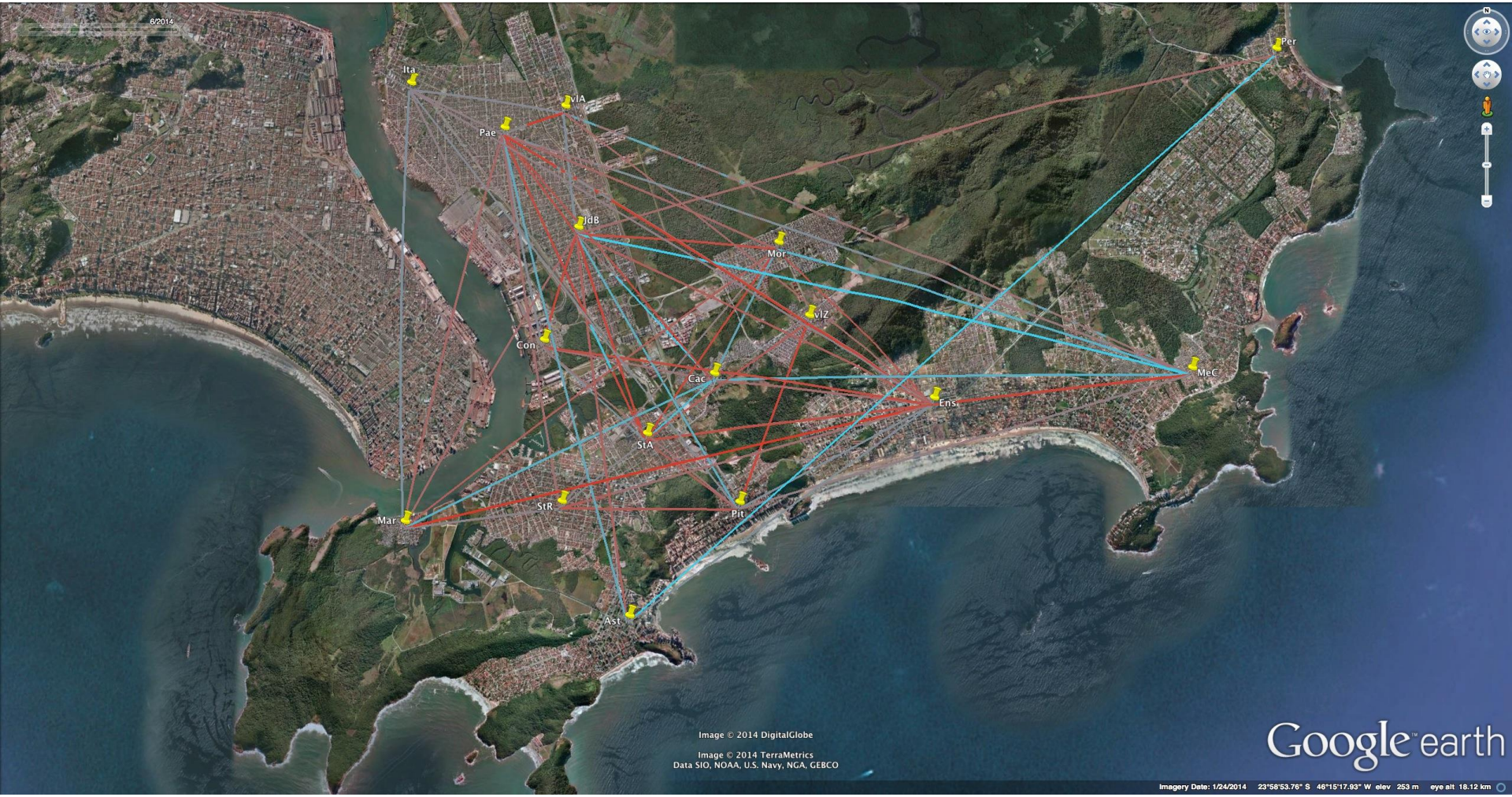

Autochthonous dengue cases reported
Weekly
375
300
225
150
75
0
January
February
March
April
May
June
Geo-Referenced dengue sampling
Weekly
75
60
45
30
15
0
January
February
March
April
May
June
Daily
15
10
5
0
January
February
March
April
May
June

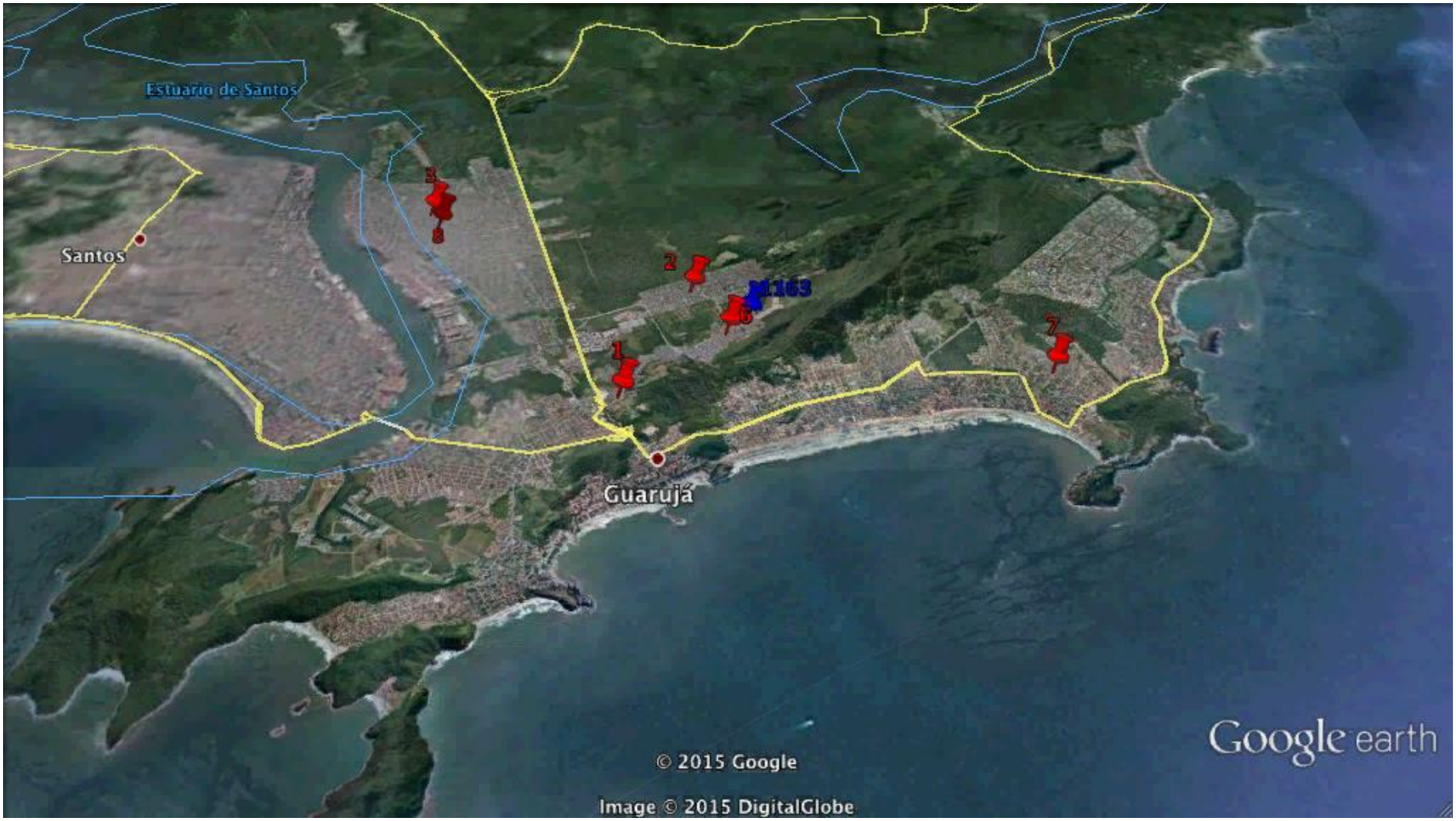

Posterior estimates of incidence
incidence
15
10
5
0
0
50
100
150
date
count
5000
4000
3000
2000
1000


Posterior distribution of pi
density
10
5
0
0.70
0.75
0.80
0.85
0.90
Pi (proportion of cases sampled)
run
1
2
3
4

Widely dispersed “outset” of the DENV 4 Outbreak

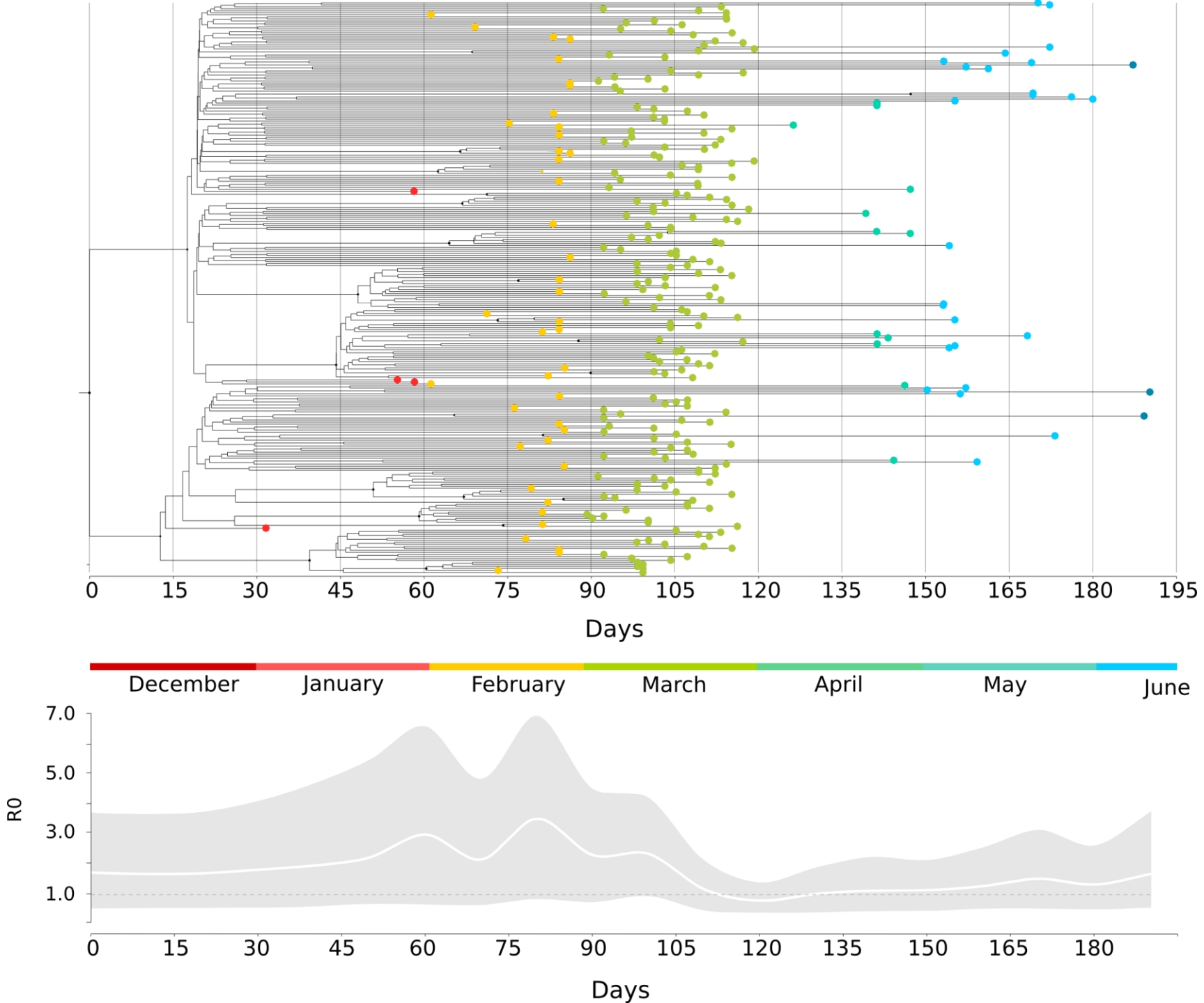
Widely-dispersed “end” of the DENV 4 outbreak

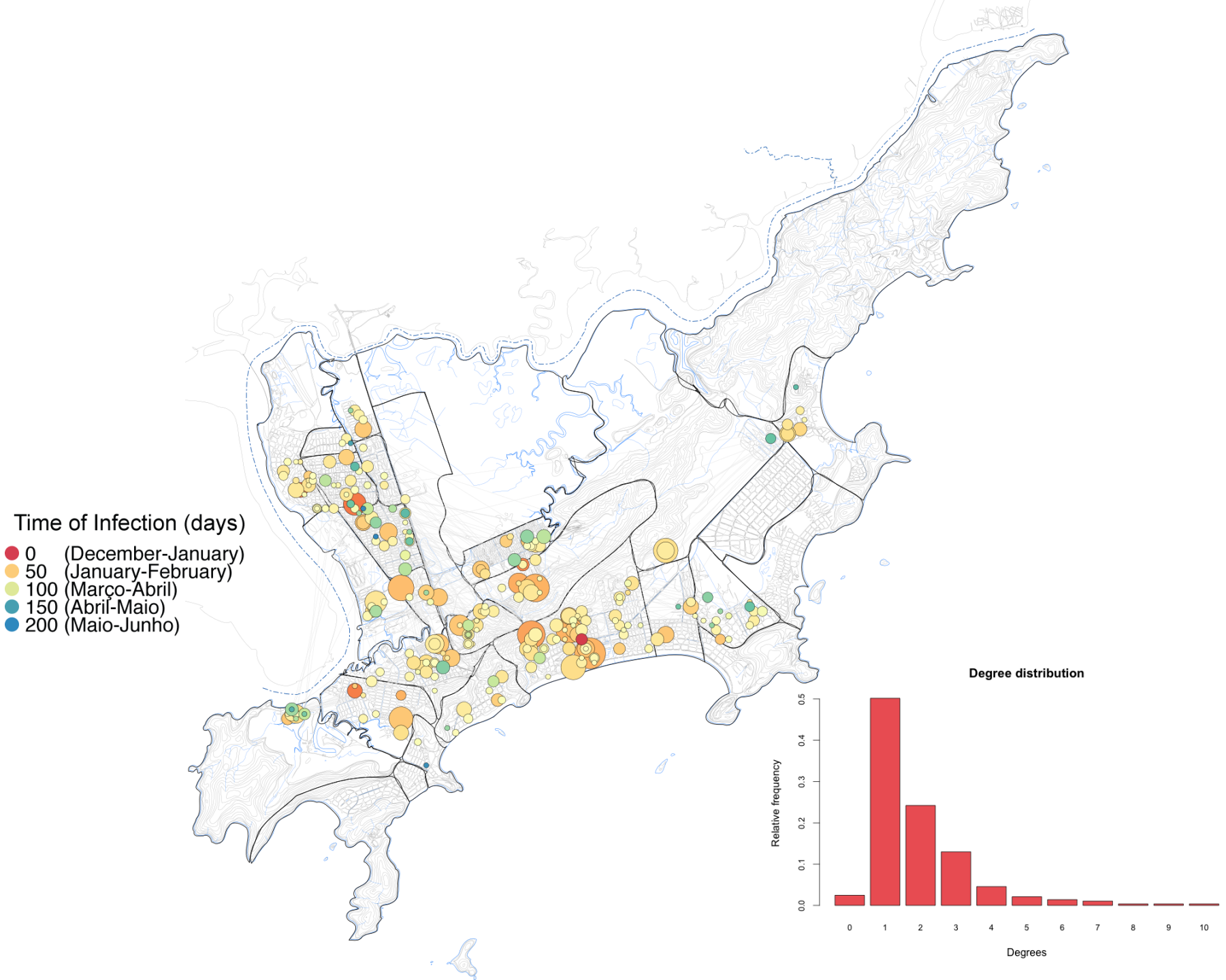


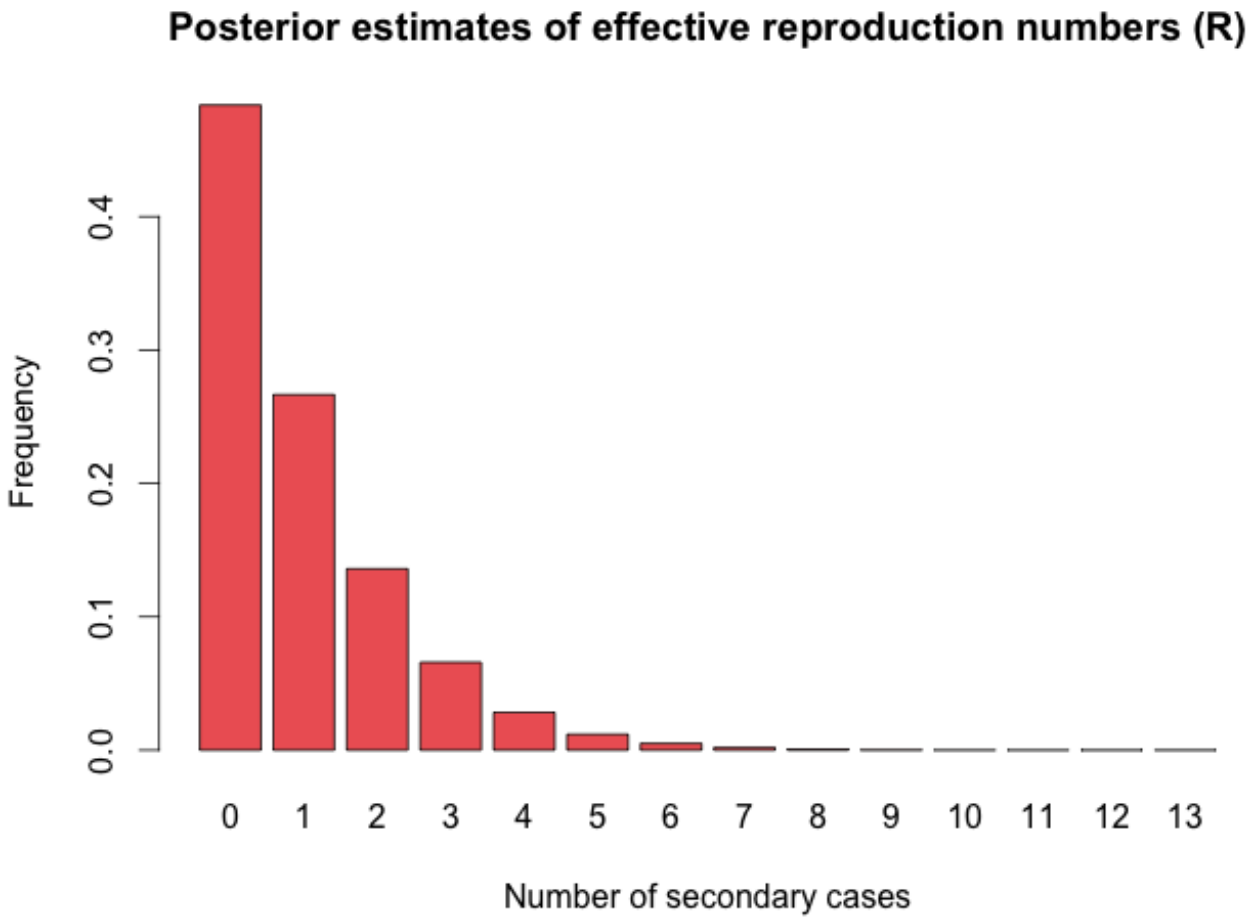


Days

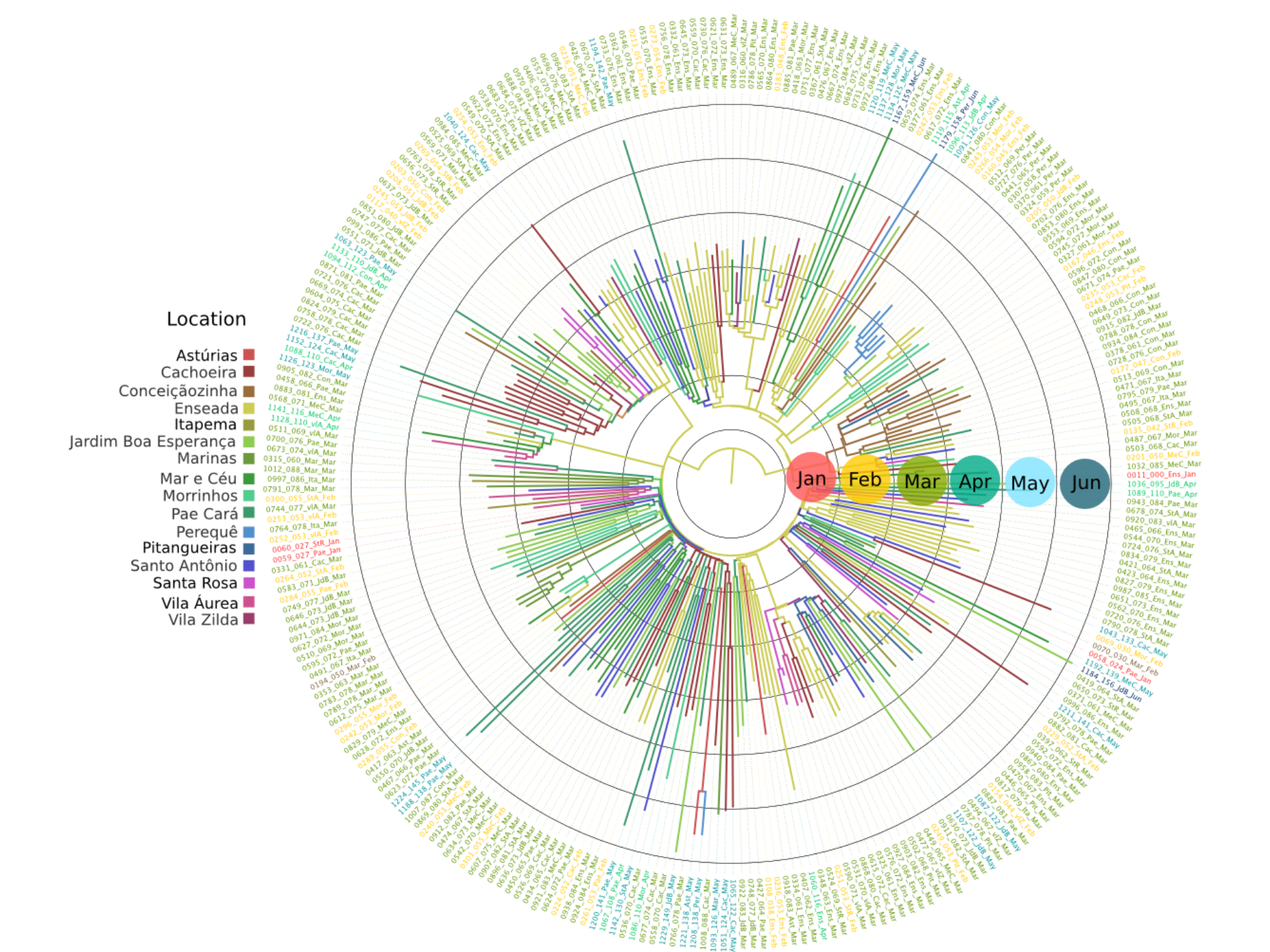
December January February March April May June


0
15
30
45
60
75
90
105
120
135
150
165
180
195
Days
December
January
February
March
April
May
June
7.0
5.0
3.0
1.0
R0
0
15
30
45
60
75
90
105
120
135
150
165
180
Days

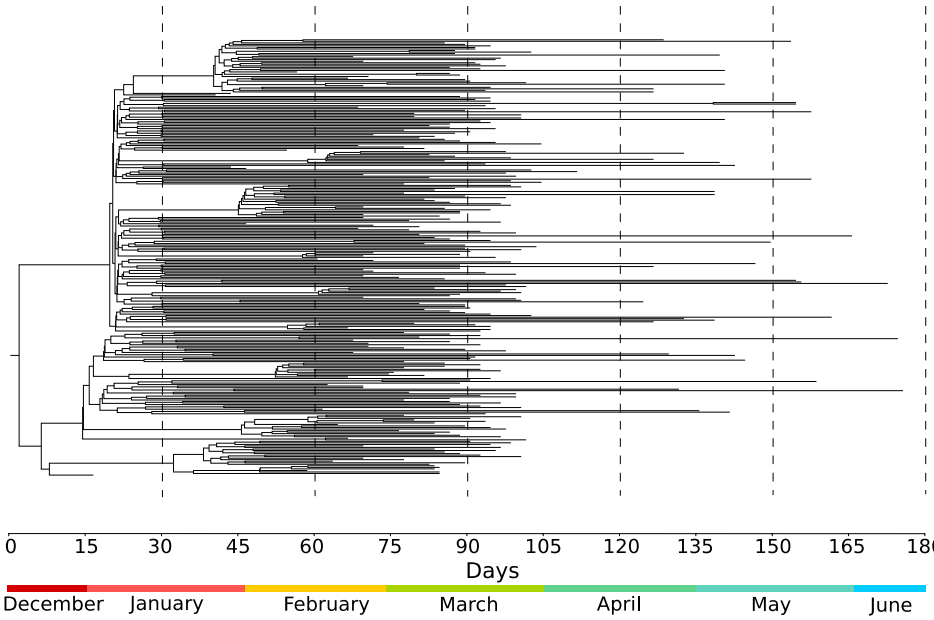

Time of Infection (days)
0 (December-January)
50 (January-February)
100 (Março-Abril)
150 (Abril-Maio)
200 (Maio-Junho)
Degree distribution
Relative frequency
0.0
0.1
0.2
0.3
0.4
0.5
0
1
2
3
4
5
6
7
8
9
10
Degrees

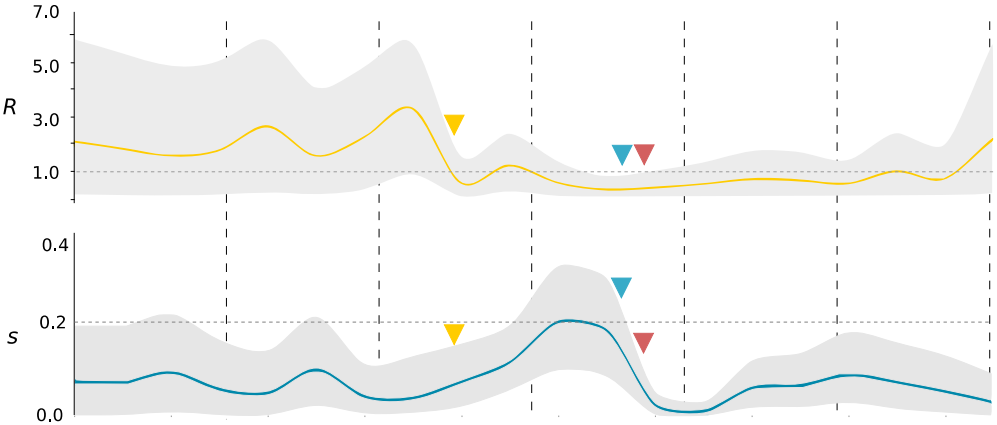


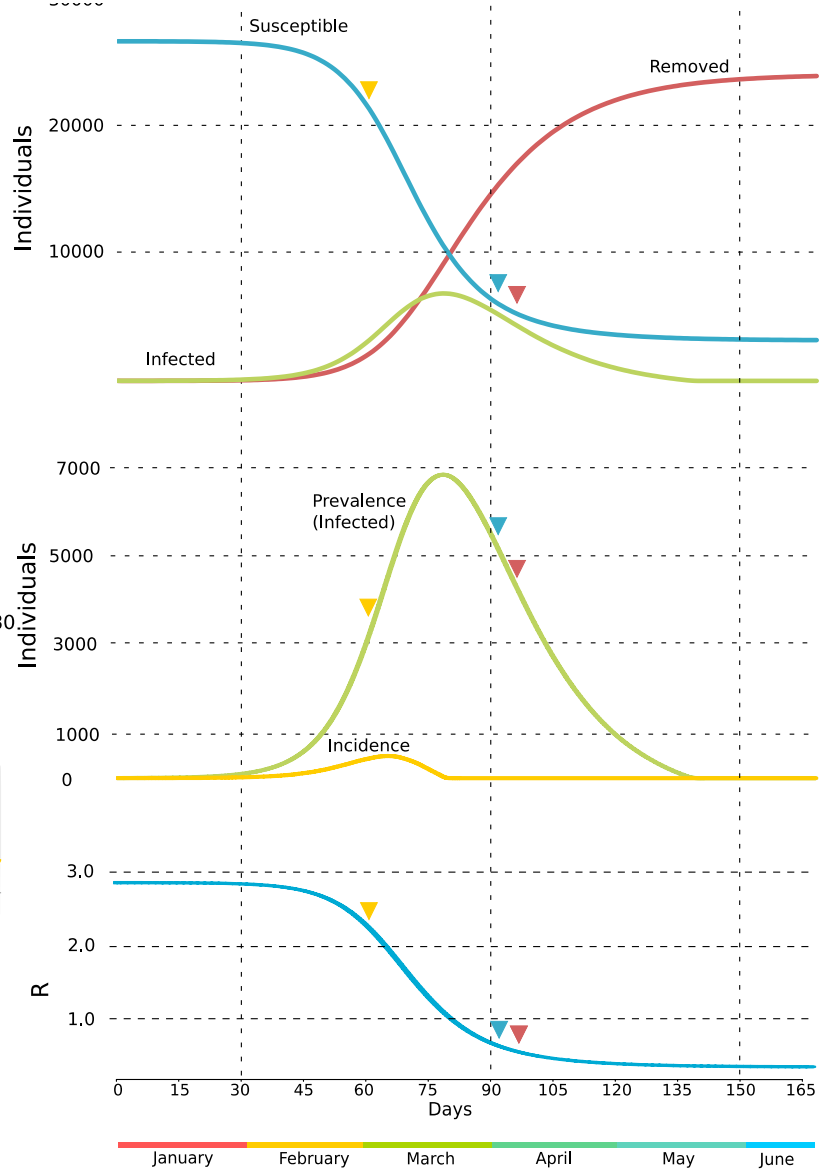
The basic reproduction number, R, is the number of secondary infections that results from a single infected human. Basic reproduction numbers estimated for dengue range between 1.33 and 11.6 (Halstead, 2008). Favier *et al.* and Massad *et al.* analyzed epidemic data from several cities in Brazil and developed values ranging from 3.8 to 5.1 and from 2.7 to 11.6, respectively.


Location
Astúrias
Cachoeira
Conceiçãozinha
Enseada
Itapema
Jardim Boa Esperança
Marinas
Mar e Céu
Morrinhos
Pae Cará
Perequê
Pitangueiras
Santo Antônio
Santa Rosa
Vila Áurea
Vila Zilda
Jan
Feb
Mar
Apr
May
Jun

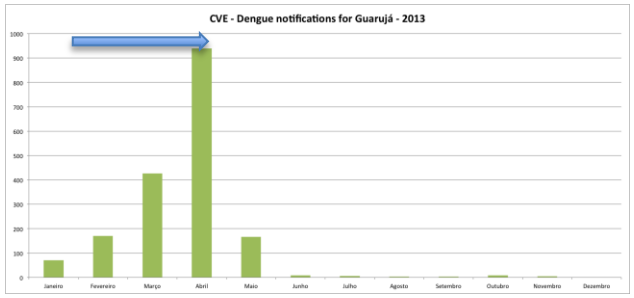
BDSKY, R & SP & SIR model







Blue: epidemiological situation.
Yellow: source reduction initiatives in shantytowns started.
Red: insecticide



Bertioga

Santos

Santos

Atlantic Ocean

Oldest

Newest

Santo Antônio

Marinas

Pitangueiras

Santa Rosa

Astúrias

Morrinhos

Cachoeira

Vila Zilda

Itapema

Pae Cará

Jardim Boa Esperança

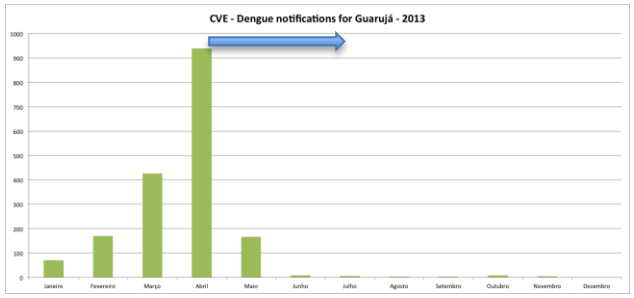
Conceiçãozinha

Vila Áurea

Perequê

Enseada

Mar e Céu



Bertioga

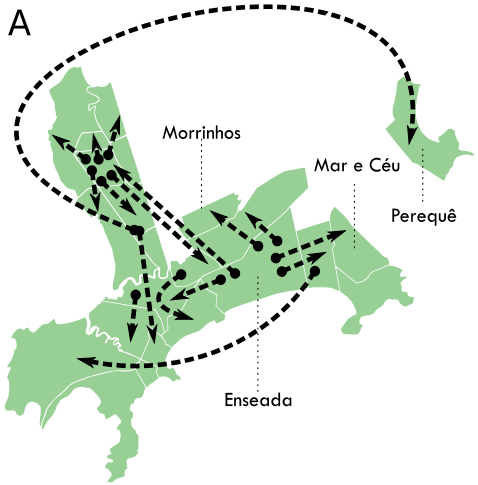
Santos

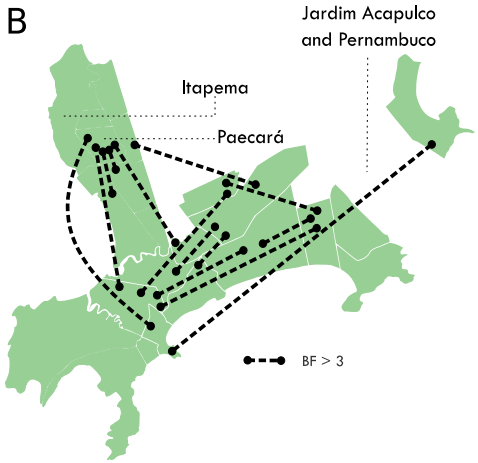
Santos

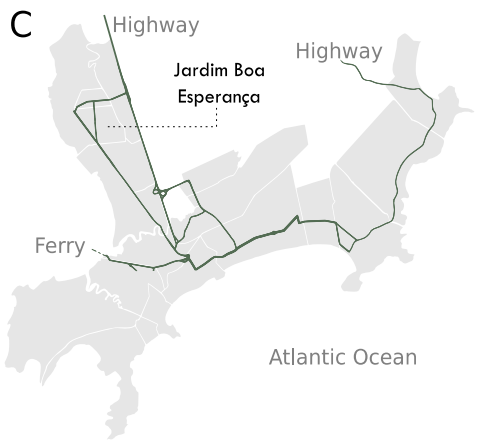
Atlantic Ocean

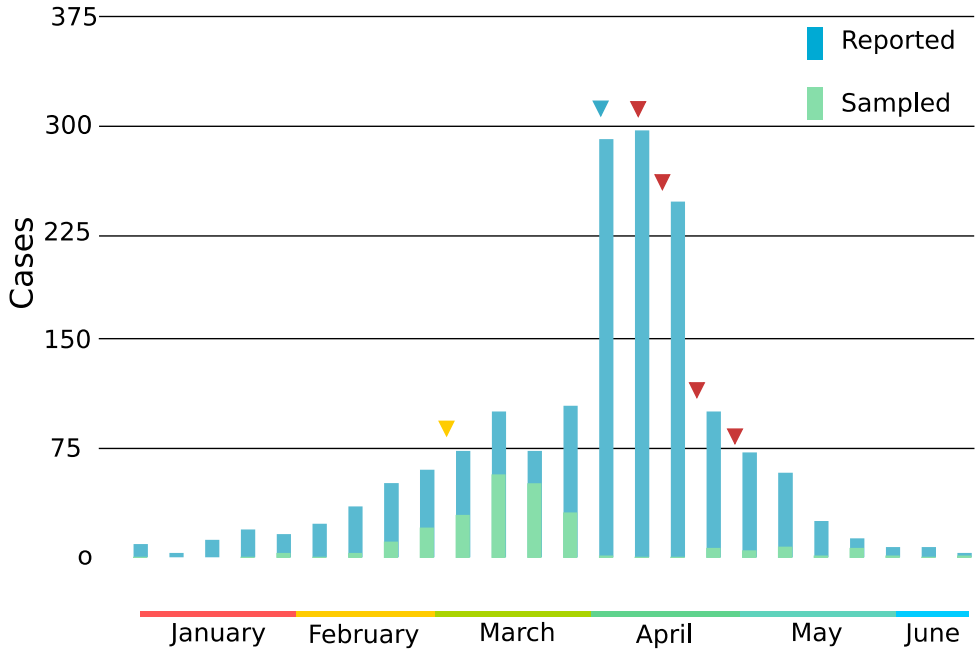
Santo Antônio
Marinas
Pitangueiras
Santa Rosa
Astúrias
Morrinhos
Cachoeira
Vila Zilda
Itapema
Pae Cará
Jardim Boa Esperança
Conceiçãozinha
Vila Áurea
Perequê
Enseada
Mar e Céu

BF > 3

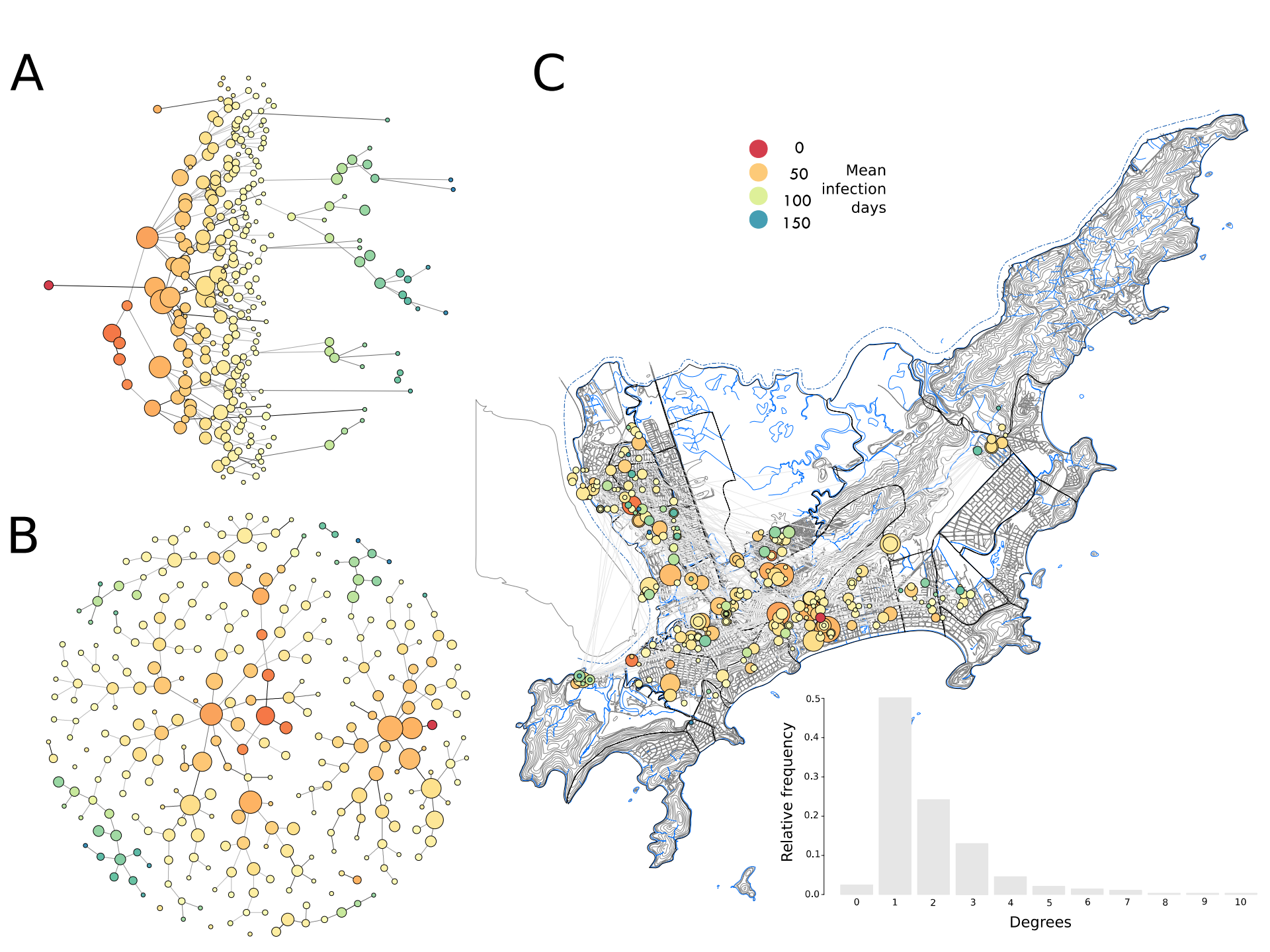




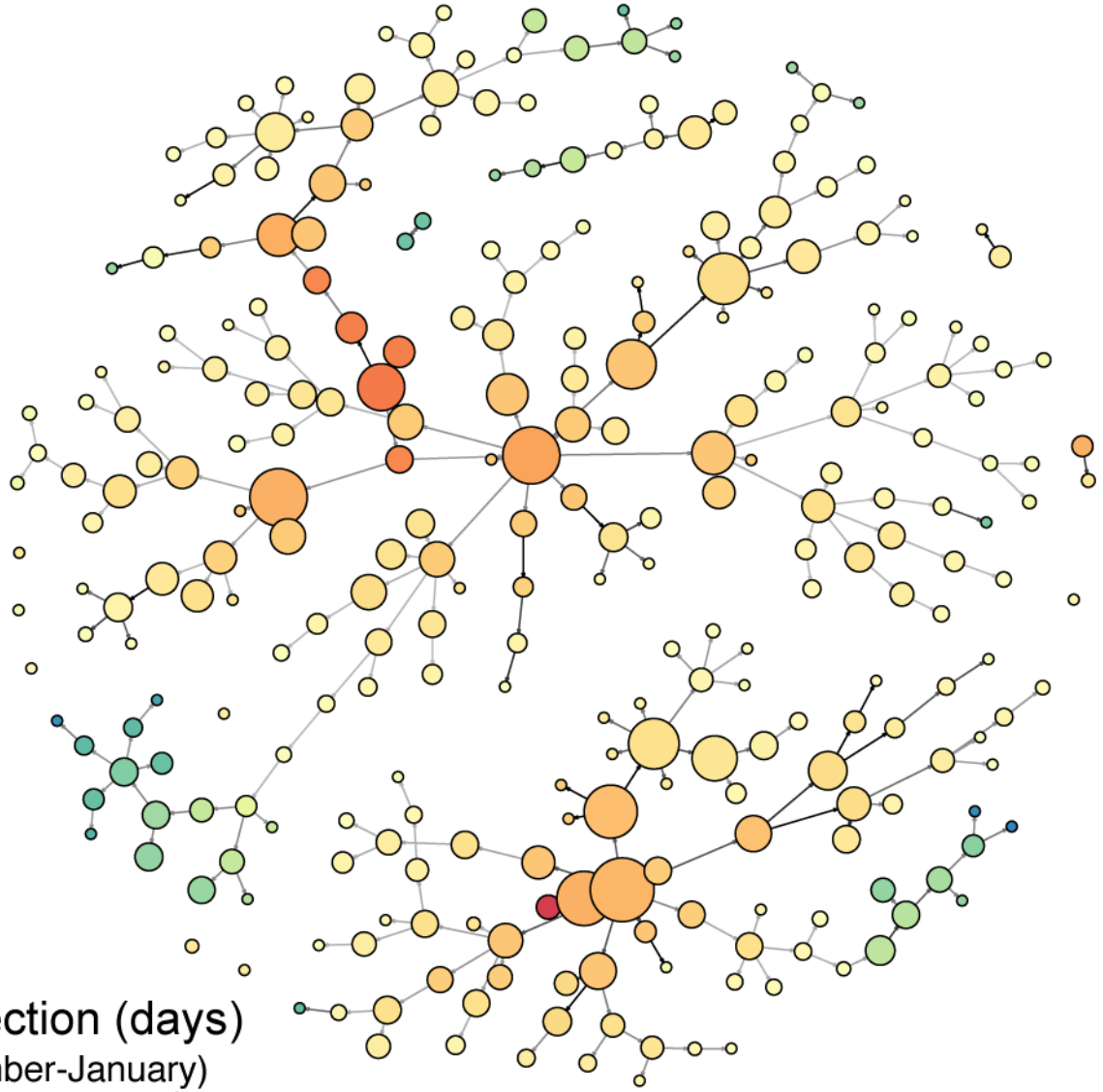




Blue: epidemiological situation.
Yellow: source reduction initiatives in shantytowns started.
Red: insecticide

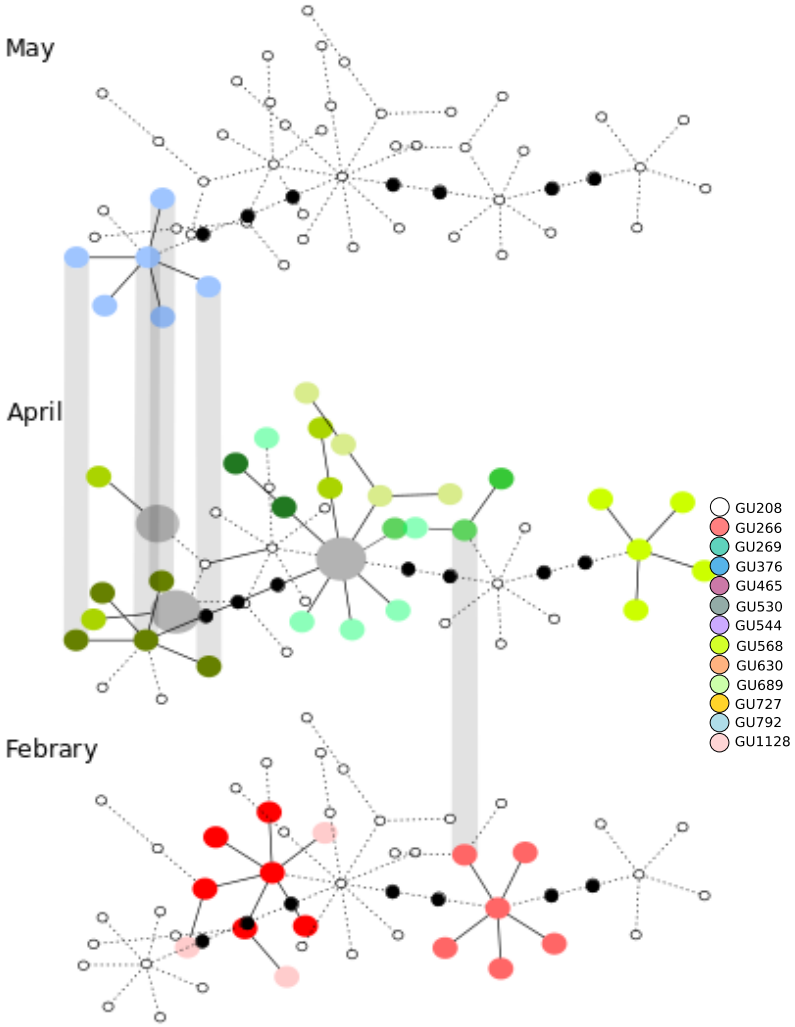

A
B
C
0
50
100
150
Mean infection days
Relative frequency
0.0
0.1
0.2
0.3
0.4
0.5
0
1
2
3
4
5
6
7
8
9
10
Degrees

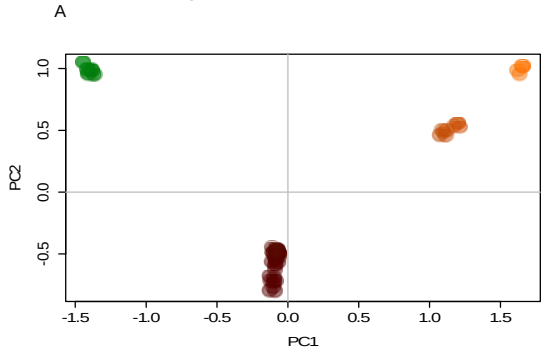
DENV4 - Transmission Network

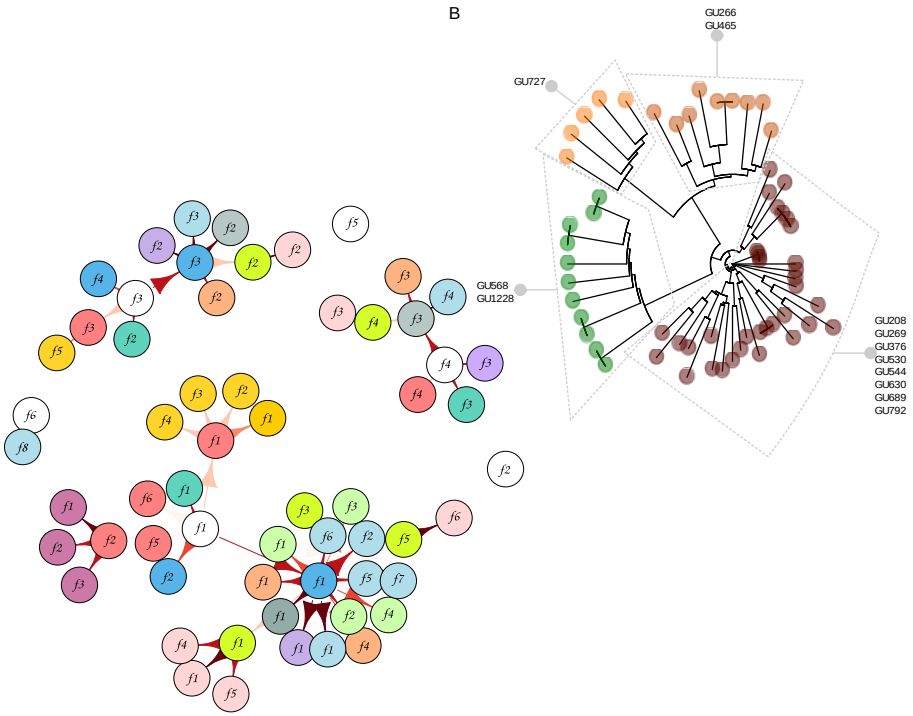


Time of Infection (days)
- 0 (December-January)
- 50 (January-February)
- 100 (Março-Abril)
- 150 (Abril-Maio)
- 200 (Maio-Junho)

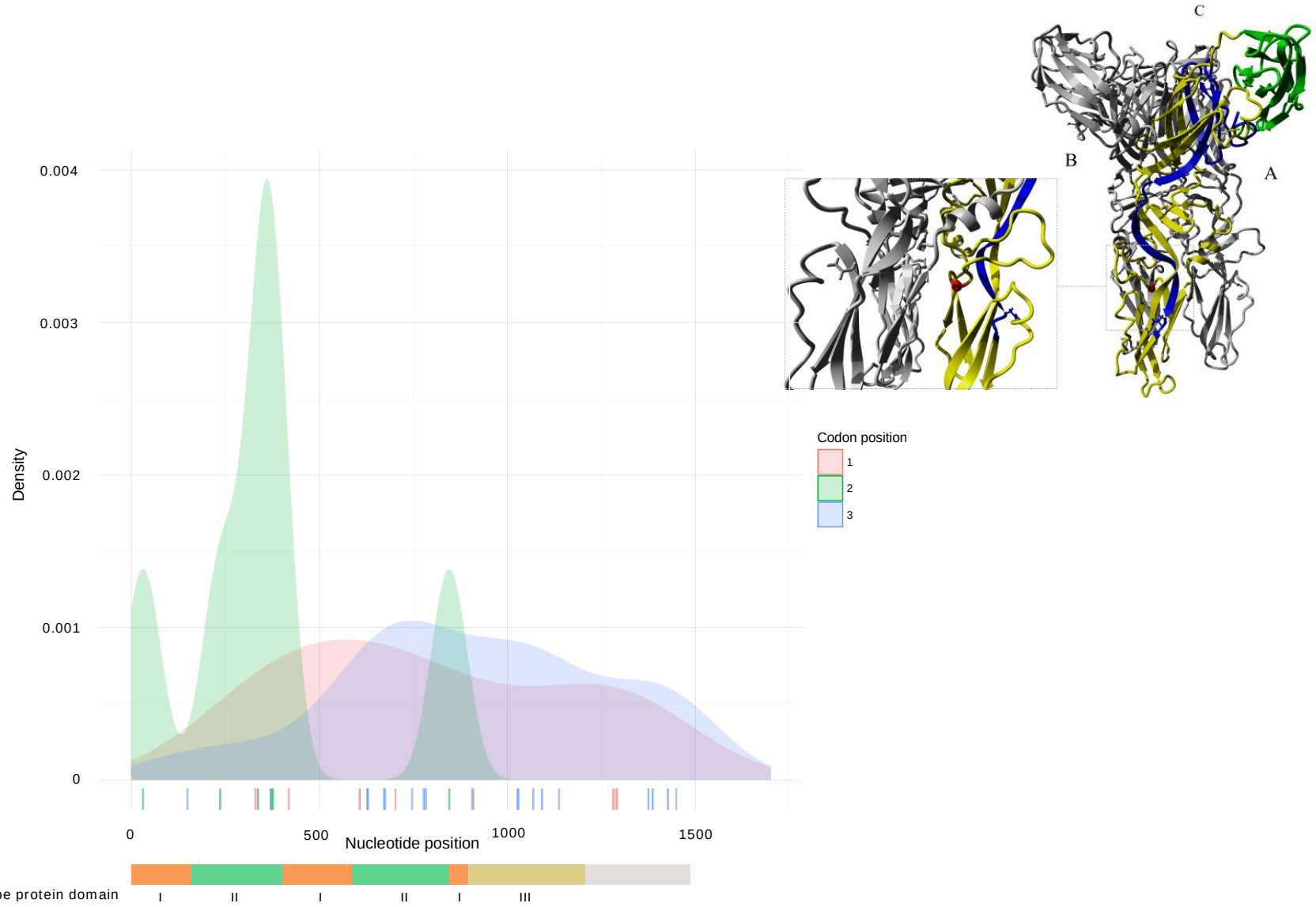
Temporal network showing the genetic relationships of intra-host variants of the DENV-4 E region from different samples



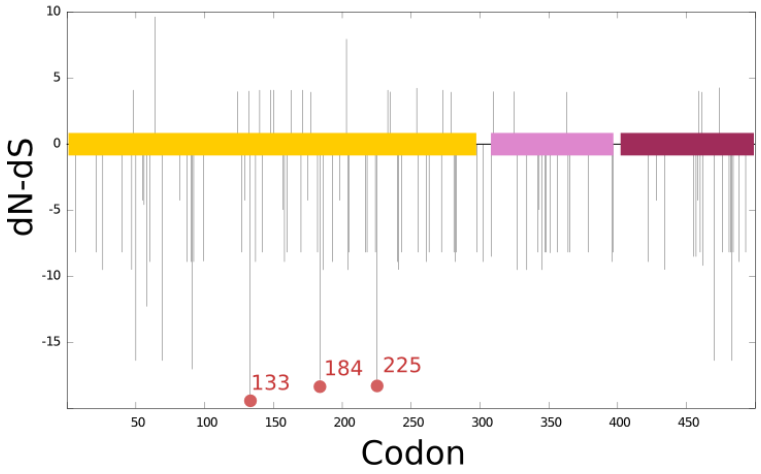




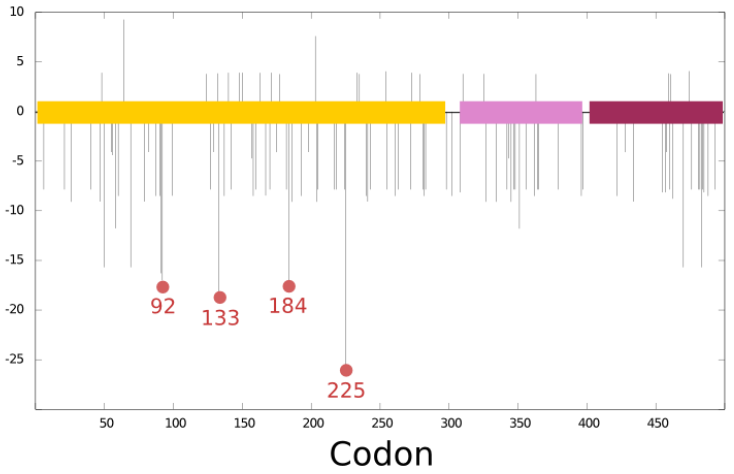
Distribution of SNPs in the envelope gene of DENV-4. Linear representation of the DENV-4 envelope is showed at the bottom. Codon positions are represented by colors (residue 235)



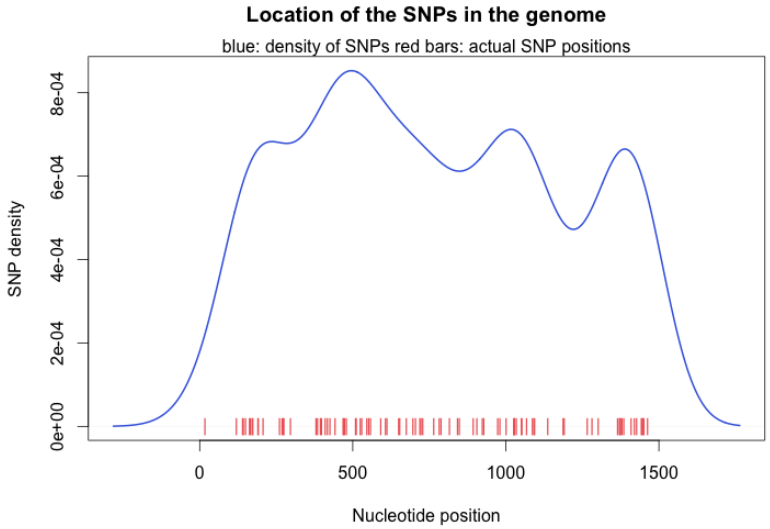
SLAC

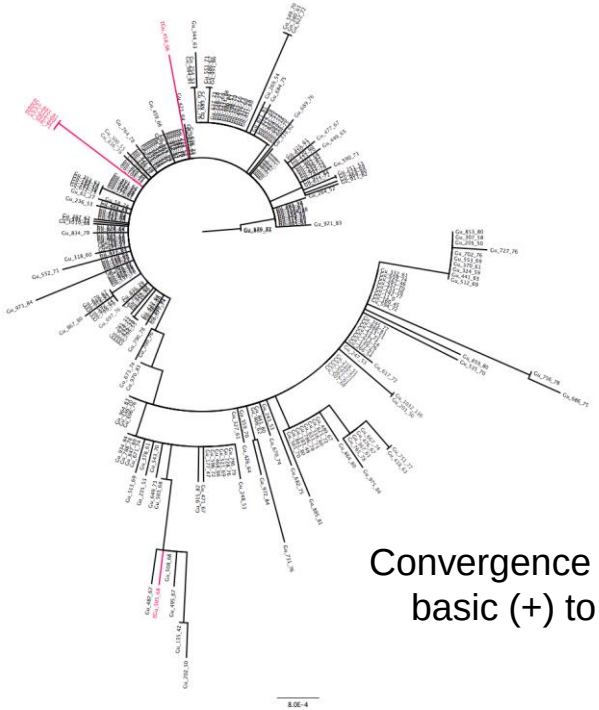


FEL

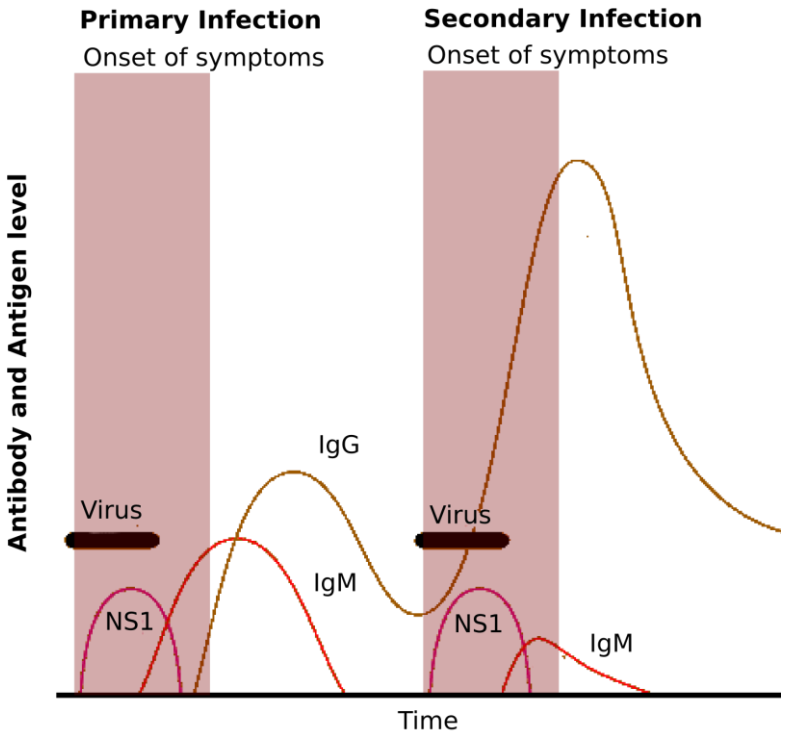


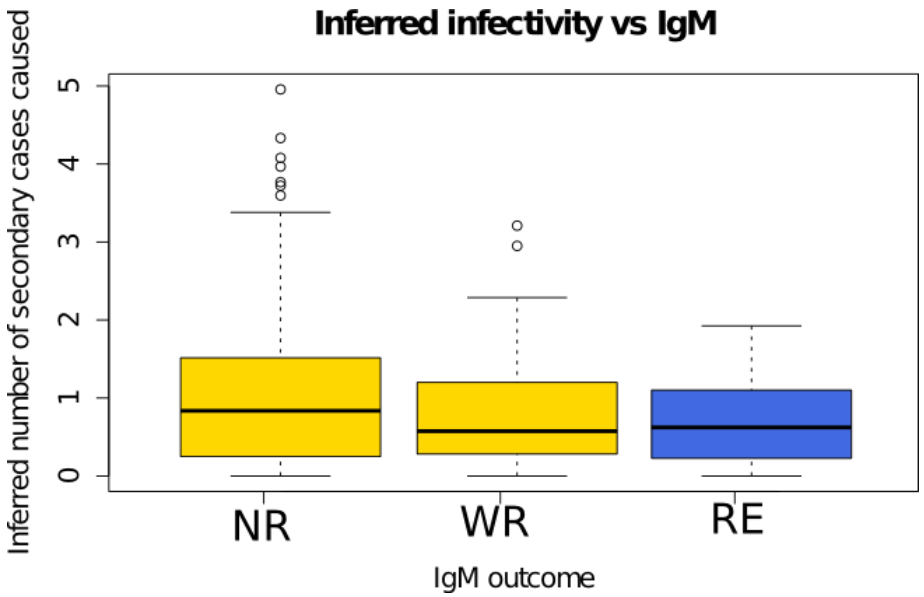
E gene domains

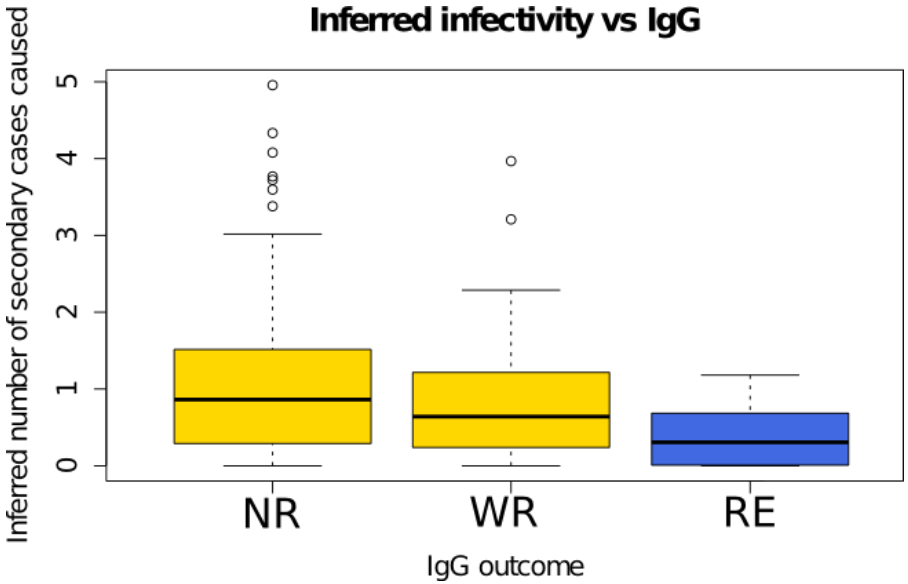


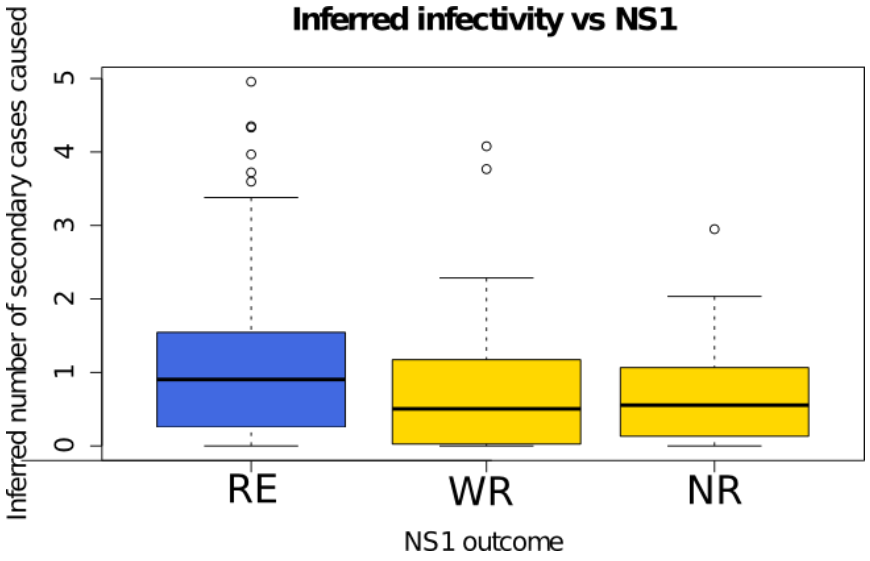


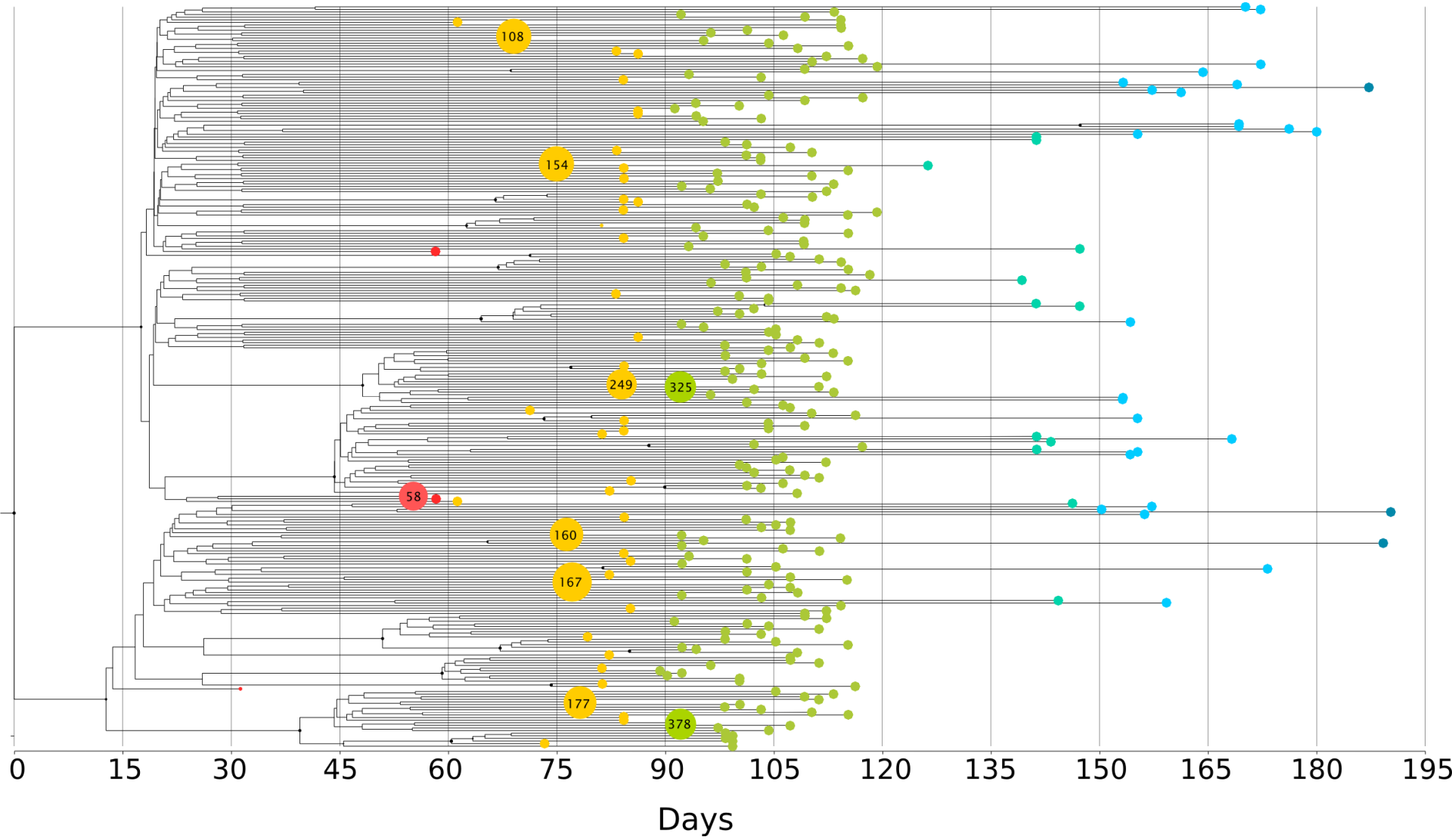
Convergence on K203E
basic (+) to acid (-)


Primary Infection
Onset of symptoms
Secondary Infection
Onset of symptoms
Antibody and Antigen level
IgG
Virus
Virus
IgM
NS1
NS1
IgM
Time

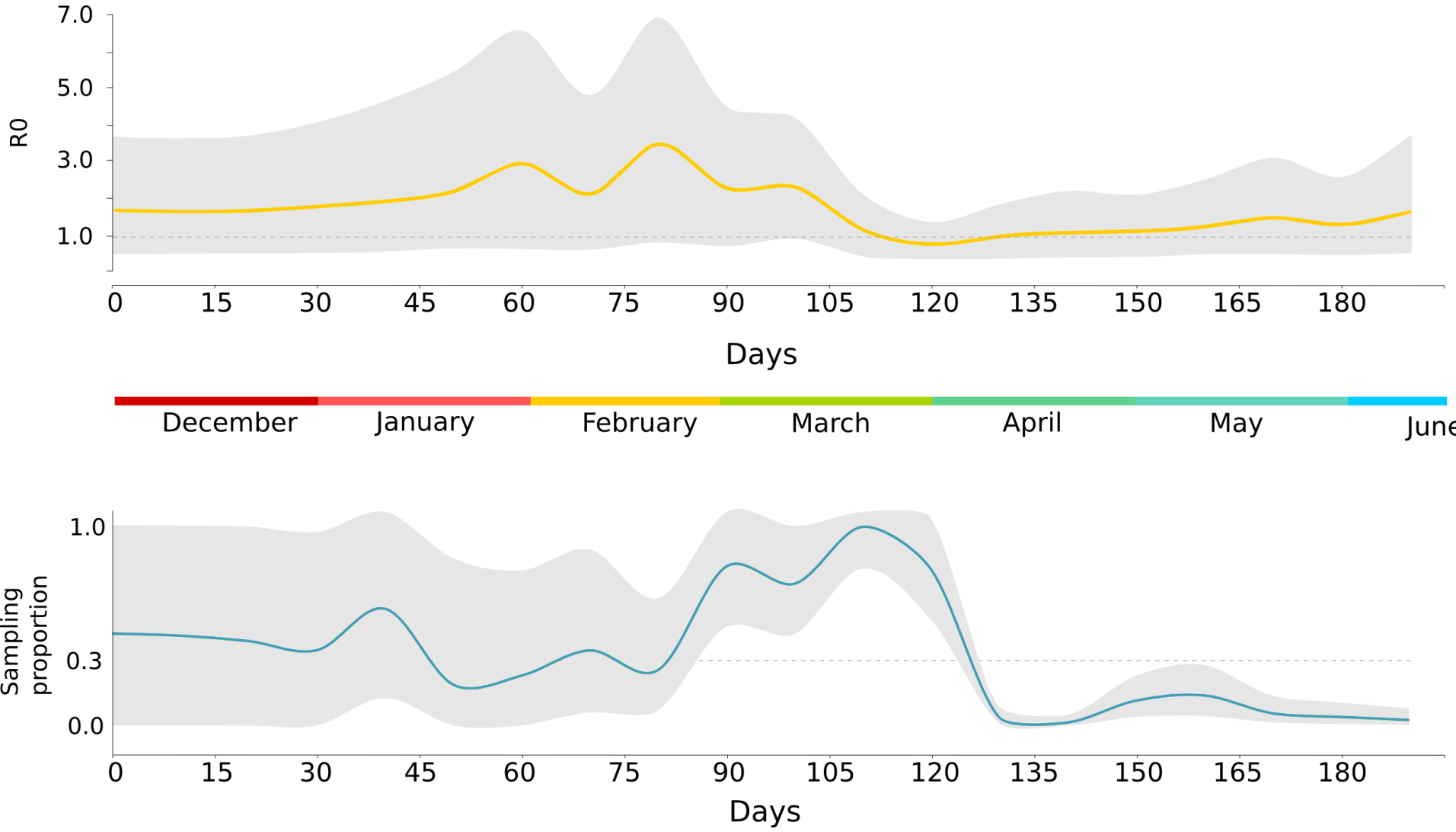

Inferred infectivity vs IgM
Inferred number of secondary cases caused
0
1
2
3
4
5
NR
WR
RE
IgM outcome


Inferred infectivity vs IgG
Inferred number of secondary cases caused
0
1
2
3
4
5
NR
WR
RE
IgG outcome

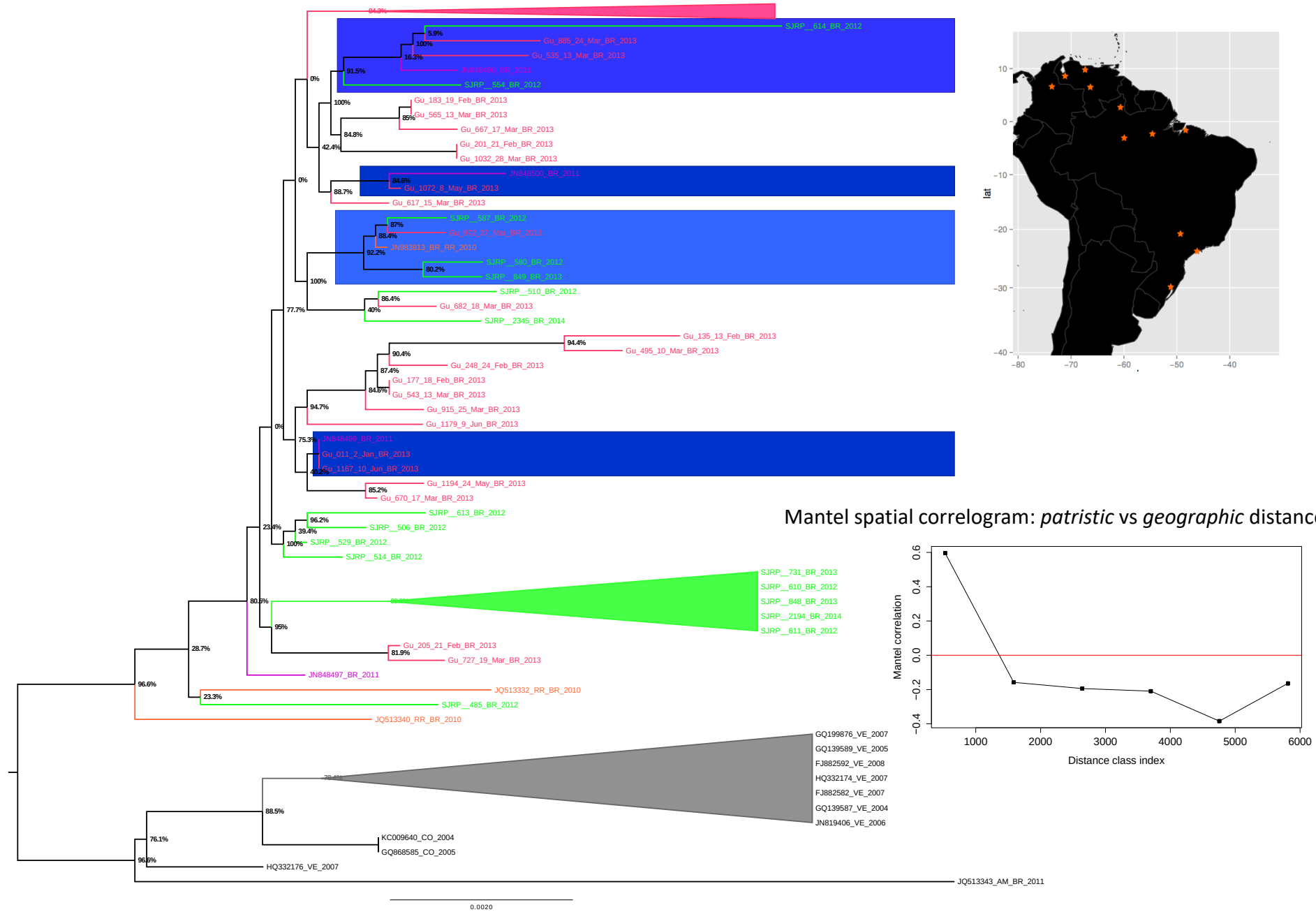

Inferred infectivity vs NS1
Inferred number of secondary cases caused
0
1
2
3
4
5
RE
WR
NR
NS1 outcome


108
154
249
325
58
160
167
177
378
0
15
30
45
60
75
90
105
120
135
150
165
180
195
Days

R & SP BDSKY

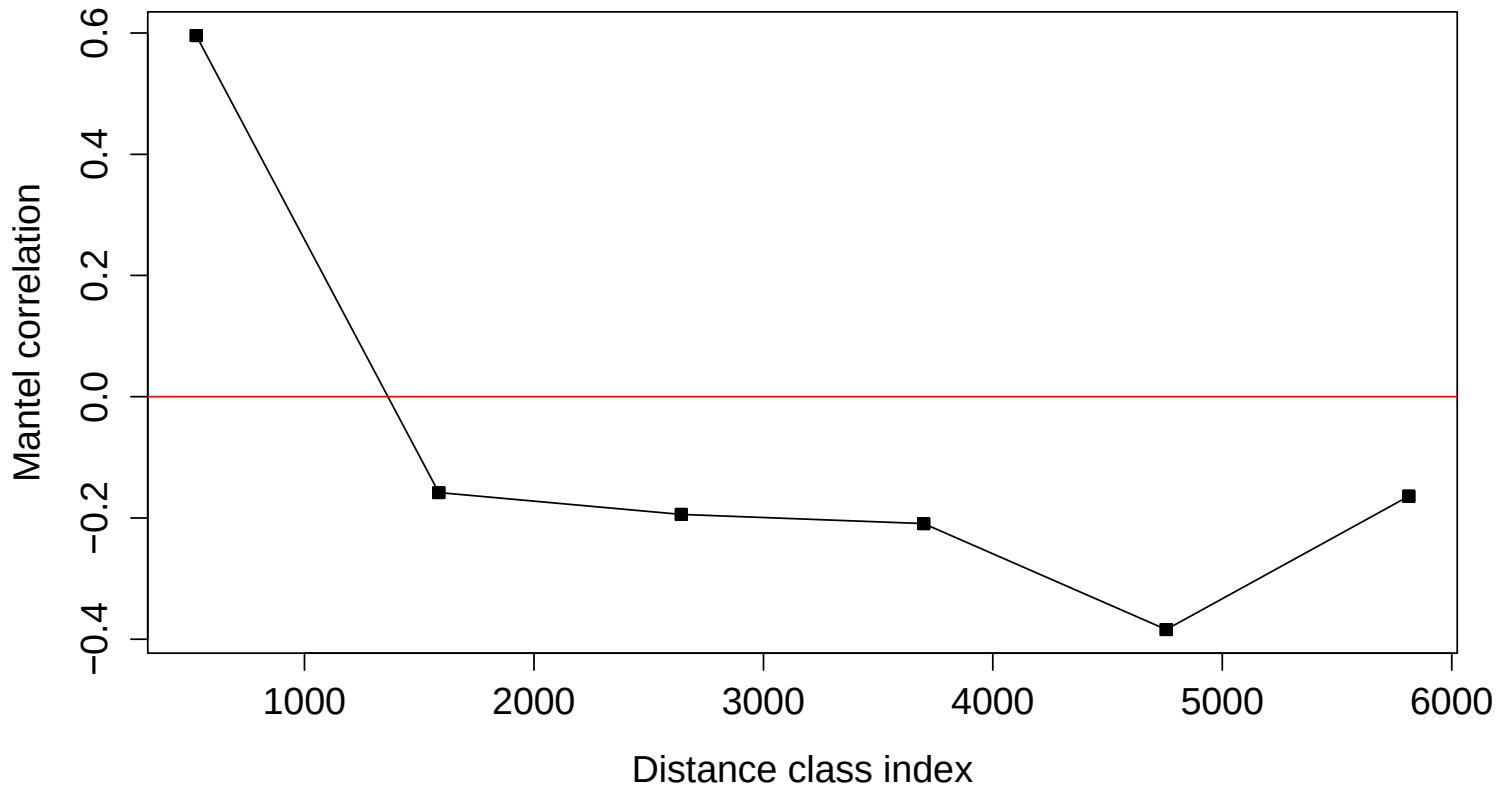

7.0
5.0
3.0
1.0
R0
0
15
30
45
60
75
90
105
120
135
150
165
180
Days
December
January
February
March
April
May
June
1.0
0.3
0.0
Sampling proportion
0
15
30
45
60
75
90
105
120
135
150
165
180
Days

Detecting wide & fast spatial DENV dispersal

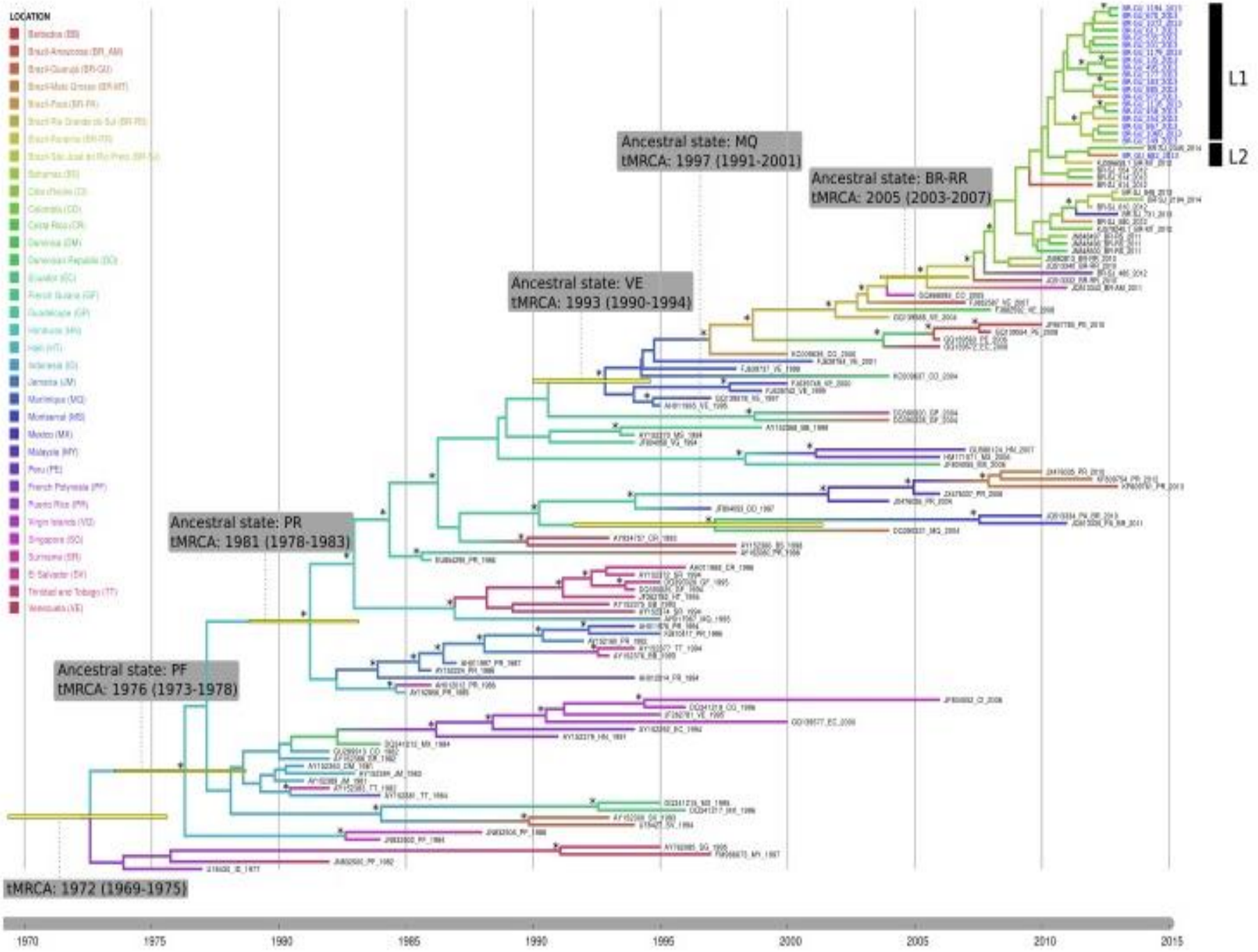


Mantel spatial correlogram: *patristic* vs *geographic* distances

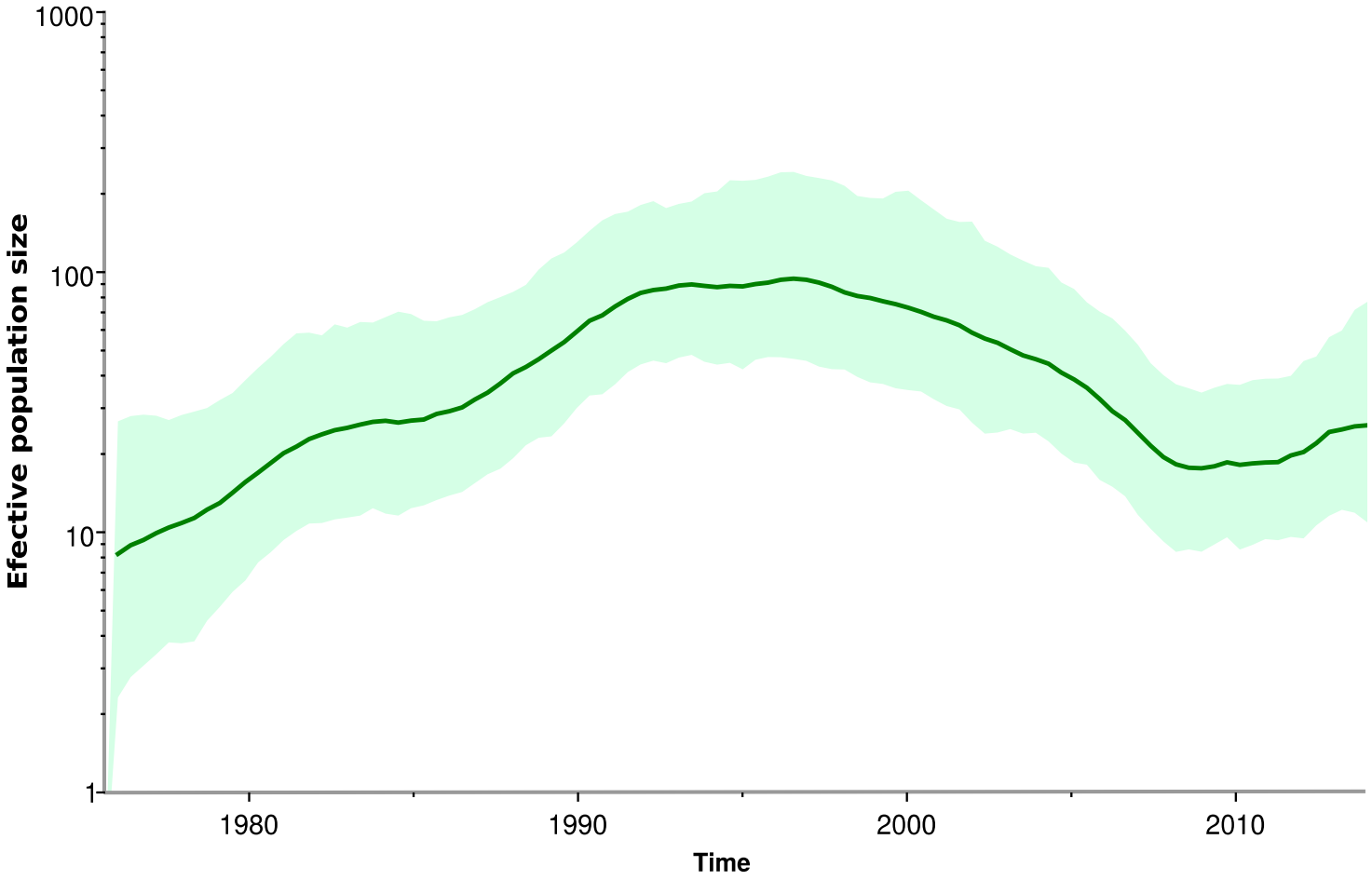
Mantel spatial correlogram: *patristic* vs *geographic* distances



Based on Carrel *et al.*, 2010


LOCATION
Barbados (BB)
Brazil-Amazonas (BR_AM)
Brazil-Goiania (BR-GO)
Brazil-Mato Grosso (BR-MT)
Brazil-Para (BR-PA)
Brazil-Rio Grande do Sul (BR-RS)
Brazil-Roraima (BR-RR)
Brazil-Sao Jose do Rio Preto (BR-SJ)
Bahamas (BS)
Cabo Verde (CI)
Colombia (CO)
Costa Rica (CR)
Dominica (DM)
Dominican Republic (DO)
Ecuador (EC)
French Guiana (GF)
Guadeloupe (GP)
Honduras (HN)
Haiti (HT)
Indonesia (ID)
Jamaica (JM)
Martinique (MQ)
Montserrat (MS)
Mexico (MX)
Malaysia (MY)
Peru (PE)
French Polynesia (PF)
Puerto Rico (PR)
Virgin Islands (VI)
Singapore (SG)
Suriname (SR)
El Salvador (SV)
Trinidad and Tobago (TT)
Venezuela (VE)
L1
L2
Ancestral state: MQ
tMRCA: 1997 (1991-2001)
Ancestral state: BR-RR
tMRCA: 2005 (2003-2007)
Ancestral state: VE
tMRCA: 1993 (1990-1994)
Ancestral state: PR
tMRCA: 1981 (1978-1983)
Ancestral state: PF
tMRCA: 1976 (1973-1978)
tMRCA: 1972 (1969-1975)
1970
1975
1980
1985
1990
1995
2000
2005
2010
2015

BDSKY of DENV4: recent growth in Brazil!



Conclusions

- Phylodynamics of DENV works! *i.e.*, E gene good maker, stable rates of change, stable nucleotide composition, no significant recombination systematic error.

- We need to incorporate viral genealogy & patient data to better understand the evolution of an outbreak.

- Real time follow-up of dengue outbreaks can be useful for control: Guarujá stands as a proof of concept.

- “Where there is no overlap there is gap” – Mark Miller, MD.

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Laboratório de Evolução Molecular e Bioinformática, Departamento de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo

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Laboratório de Saúde Pública, Secretaria da Saúde, Prefeitura Municipal de Guarujá

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Itapema Laboratório de Análises Clínicas, Guarujá

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Laboratório de Infectologia Pediátrica, Faculdade de Medicina de Jundiaí

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Bolsa PQ CNPq