Table S1

Reaction Mix

Reagents	Volumen
RNA	5 µl
One step Buffer 2x	10 µl
Primer Mix	2 µl
Enzyme Mix	0.2 µl
PCR grade water	2. 8 µl
Total	20 µl

Thermal cycler program

- 42°C by 60 min.
- 94°C by 3 min.
- 35 cycles:
    - 94 ° C by 30 seg.
    - 60 ° C by 30 seg.
    - 68 ° C by 45 seg.
- 68 ° C by 5 min.
- Hold 4 ° C

Primer	Sequence
Chik_NSP3 Forward	5'- TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCCGTCCCGTCAGACCTG -3'
Chik_NSP3 Reverse	5'- GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTCGTCGTCCGTGTCTGAGC -3'
Chik_E1 Forward	5'- TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCAGGGCTCATACCGCATCCG -3'
Chik_E1 Reverse	5'- GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGCATGTTCCCTACGGCG -3'
Chik_E2 Forward	5'- TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCATGCCCCCAGACACCCC -3'
Chik_E2 Reverse	5'- GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCCCACGTGACCTCGAGC -3'

Table S1 supplement. PCR conditions and primers used for NSP3, E1 and E2 partial gene amplification.

Table S2

Adapters

Fordward	5’ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-[locus specific sequence]
Reverse	5’ GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-[locus specific sequence]

Index

Library	INDEX	Library	INDEX
CHIKV Pool 1	S517/N701	CHIKV Pool 13	S517/N704
CHIKV Pool 2	S502/N701	CHIKV Pool 14	S502/N704
CHIKV Pool 3	S503/N701	CHIKV Pool 15	S503/N704
CHIKV Pool 4	S504/N701	CHIKV Pool 16	S504/N704
CHIKV Pool 5	S517/N702	CHIKV Pool 17	S517/N705
CHIKV Pool 6	S502/N702	CHIKV Pool 18	S502/N705
CHIKV Pool 7	S503/N702	CHIKV Pool 19	S503/N705
CHIKV Pool 8	S504/N702	CHIKV Pool 20	S504/N705
CHIKV Pool 9	S517/N703	CHIKV Pool 21	S517/N706
CHIKV Pool 10	S502/N703	CHIKV Pool 22	S502/N706
CHIKV Pool 11	S503/N703	CHIKV Pool 23	S503/N706
CHIKV Pool 12	S504/N703	CHIKV Pool 24	S504/N706

Table S2. Oligonucleotide (primers) sequences of Illumina adapters and indexes used in library prep kits.

Table S3

Library	Location	Raw reads	After filtering	After trimming	After paired	Clusters	Clusters at Effective Sample Depth 95%	Secuences after collapse
1	Baja California	242552	206169	175244	87622	711	5	2
2	Baja California Sur	63730	56720	50481	25240	2401	12	3
3	Baja California Sur	774200	619360	495488	247744	4600	16	5
4	Baja California Sur	514036	436931	371391	185696	4470	9	3
5	Chiapas	797444	685802	589790	294895	3483	14	3
6	Chiapas	405064	340254	285813	142907	1725	5	2
7	Colima	776386	667692	574215	287108	2651	8	2
8	Colima	270730	235535	204916	102458	1276	5	3
9	Colima	265666	215189	174303	87152	1333	6	4
10	Mexico City	238756	198167	164479	82240	1399	5	3
11	Guerrero	621614	540804	470500	235250	2190	9	1
12	Mexico State	463504	393978	334882	167441	2708	13	1
13	Michoacan	556414	489644	430887	215444	2785	11	2
14	Nuevo Leon	694416	569421	466925	233463	4711	11	2
15	Oaxaca	340280	282432	234419	117209	2082	11	3
16	Oaxaca	536842	472421	415730	207865	3520	15	3
17	Quintana Roo	660062	547851	454717	227358	5228	17	3
18	Sinaloa	729816	623263	532266	266133	4166	8	3
19	Tabasco	689954	565762	463925	231963	2275	10	3
20	Veracruz	495530	424174	363093	181546	3279	8	2
21	Veracruz	648214	538018	446555	223277	3962	10	2
22	Veracruz	583714	478645	392489	196245	2750	9	2
23	Yucatan	479128	388094	314356	157178	1382	7	1
24	Yucatan	548152	465929	396040	198020	3385	14	1
Total		**12396204**	**10442256.74**	**8802902.687**	**4401451.344**	**68472**	**238**	**59**

Table S3. Data set construction workflow information. Adapters and poor-quality reads (minimum Phred quality of 30) were removed using Trimmomatic software. Each data set was trimmed to a common length and the amplicon sequence variants (ASVs) within each pool were determined by clustering centroids with 100% identity using the VSEARCH software.

Table S4

Statistic	observed mean CI (95%)	null mean (CI 95%)	significance
AI	**5.6592 (4.7279-6.5381)**	**7.291 (7.0579-7.395)**	**< 0.001**
PS	**55.322 (53-57)**	**65.7873 (64.289-67.139)**	**< 0.001**
MC (Baja California)	1 (1-1)	1 (1-1)	1
MC (BCS)	**2.4316 (2-4)**	**1.0563 (1.001-1.1809)**	**0.01**
MC (Chiapas)	**1.9985 (1.998-2)**	**1.0063 (1-1.0139)**	**0.01**
MC (Colima)	**1.9987 (1.998-2.1)**	**1.0052 (1-1.015)**	**0.01**
MC (Mexico City)	1.3261 (1-2)	1.0393 (1-1.1371)	1
MC (Guerrero)	**1.9987 (1.998-2.1)**	**1.0052 (1-1.015)**	**0.01**
MC (Mexico State)	1 (1-1)	1 (1-1)	1
MC (Michoacan)	1.4959 (1-3)	1.0407 (1-1.1357)	1
MC (Nuevo Leon)	1 (1-1)	1 (1-1)	1
MC (Oaxaca)	**1.9985 (1.998-2)**	**1.0063 (1-1.0139)**	**0.01**
MC (Quintana Roo)	**1.9987 (1.998-2.1)**	**1.0052 (1-1.015)**	**0.01**
MC (Sinaloa)	1.0127 (1-1.03)	1.0149 (1-1.0149)	1
MC (Tabasco)	1 (1-1)	1 (1-1)	1
MC (Veracruz)	**1.9985 (1.998-2)**	**1.0063 (1-1.0139)**	**0.01**
MC (Yucatan)	1 (1-1)	1 (1-1)	1
MC (Jalisco)	1.0431 (1-1.1)	1.0315 (1-1.1481)	1
MC (Tamaulipas)	1 (1-1)	1 (1-1)	1

Table S4 supplement. Phylogeny-trait association test. (AI, Association Index, and PS, Parsimony Score). Statistical analysis of the association between phylogeny and geographical locations of the sequence data.

Figure S1

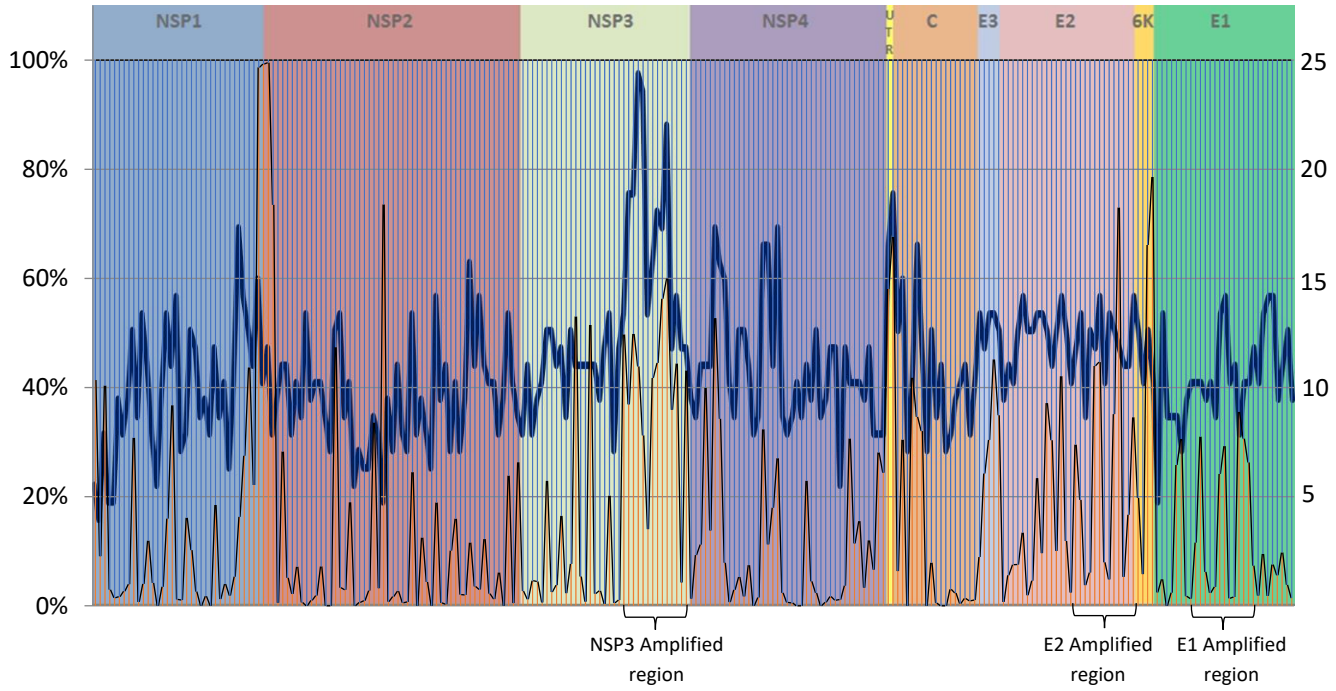


Figure S1. Hypervariable sites identified in 368 whole genome CHICKV sequences from the Virus Pathogen Database and Analysis Resource (ViPR) (NIH/DHHS) The “x” axis represents the position in the CHIKV CDS. Continuous blue line: Number of nucleotide changes in 45 nucleotides window (Right “y” axis). Red lines: Synonymous and non-synonymous mutation rate (Left “y” axis).

Figure S2

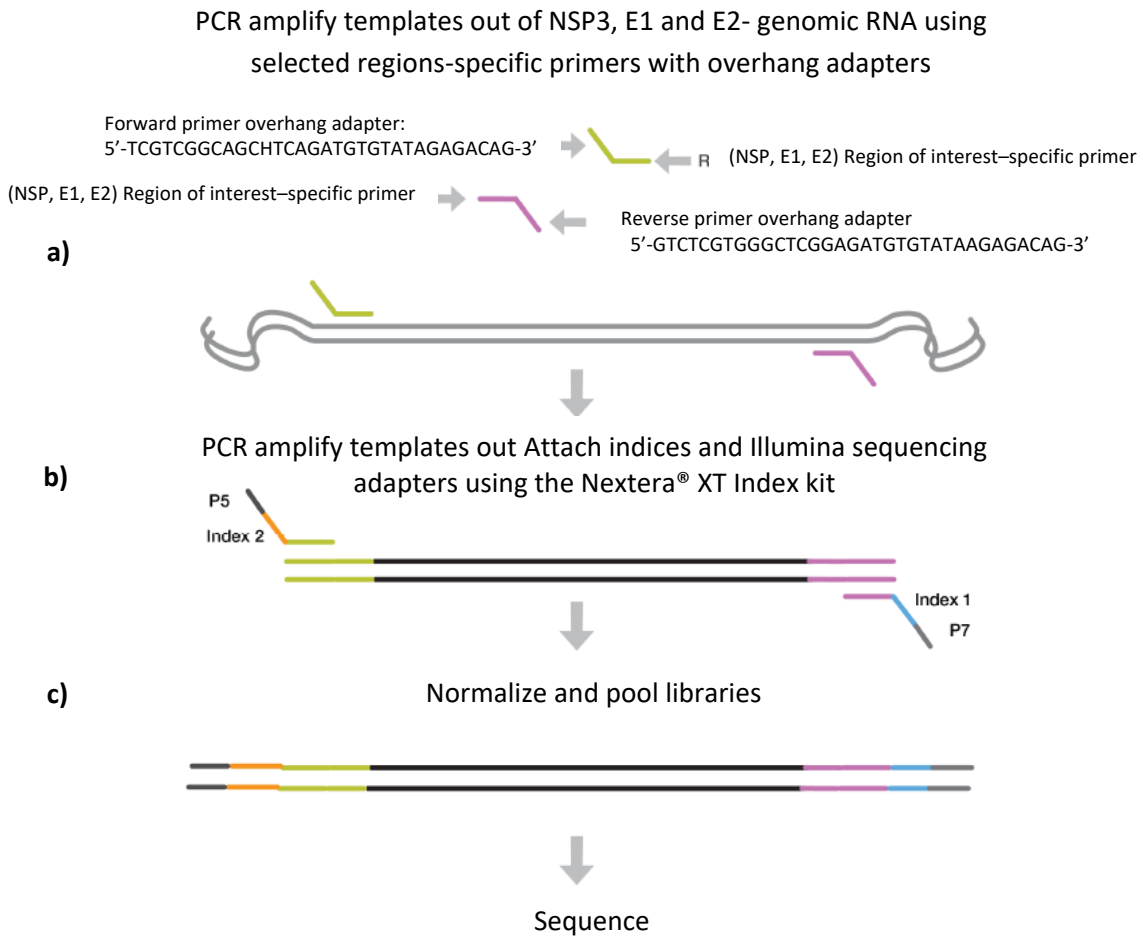


Figure S2 supplement. Amplicon workflow. **a)** Defined forward and reverse primers (Table S1) that are complementary upstream and downstream of the region of interest (NSP3, E1 and E2) were designed with overhang adapters and used to amplify templates from genomic RNA. **b)** A subsequent limited - cycle amplification step was performed to add multiplexing indices and Illumina sequencing adapters. **c)** Libraries were normalized, pooled and sequenced on the MiSeq system. Modified from reference [33].

Figure S3

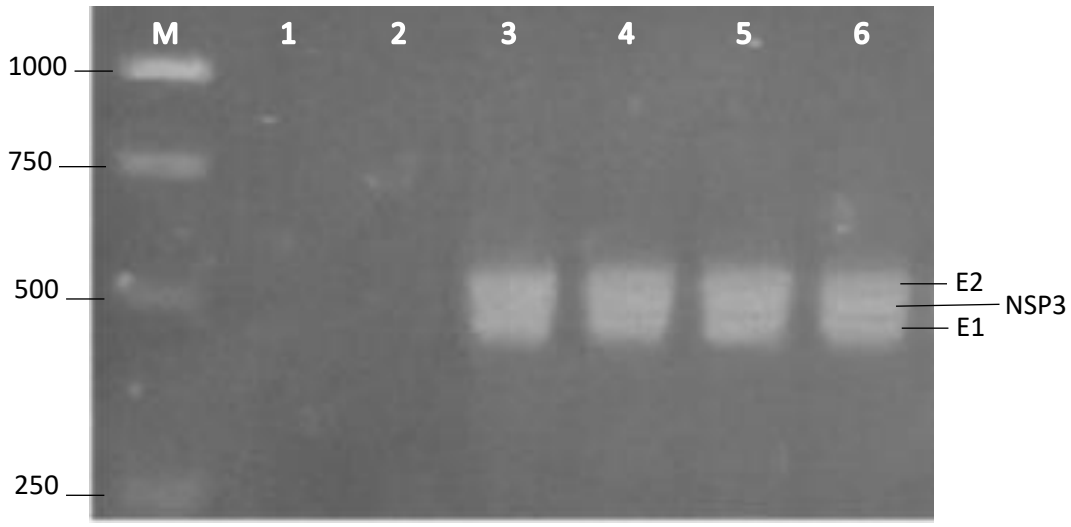


Figure S3 supplement. Agarose gel electrophoresis of PCR-amplified products from the CHIKV NSP3 (514 nt), E2 (547 nt) and E1 (479 nt) amplified regions in 4 of the serum samples selected for the study. Lane M = 1Kb ladder, lane 1 and 2 = negative control (distilled water). Lane 3 to 6 = RNA obtained from serum samples.

Figure S4

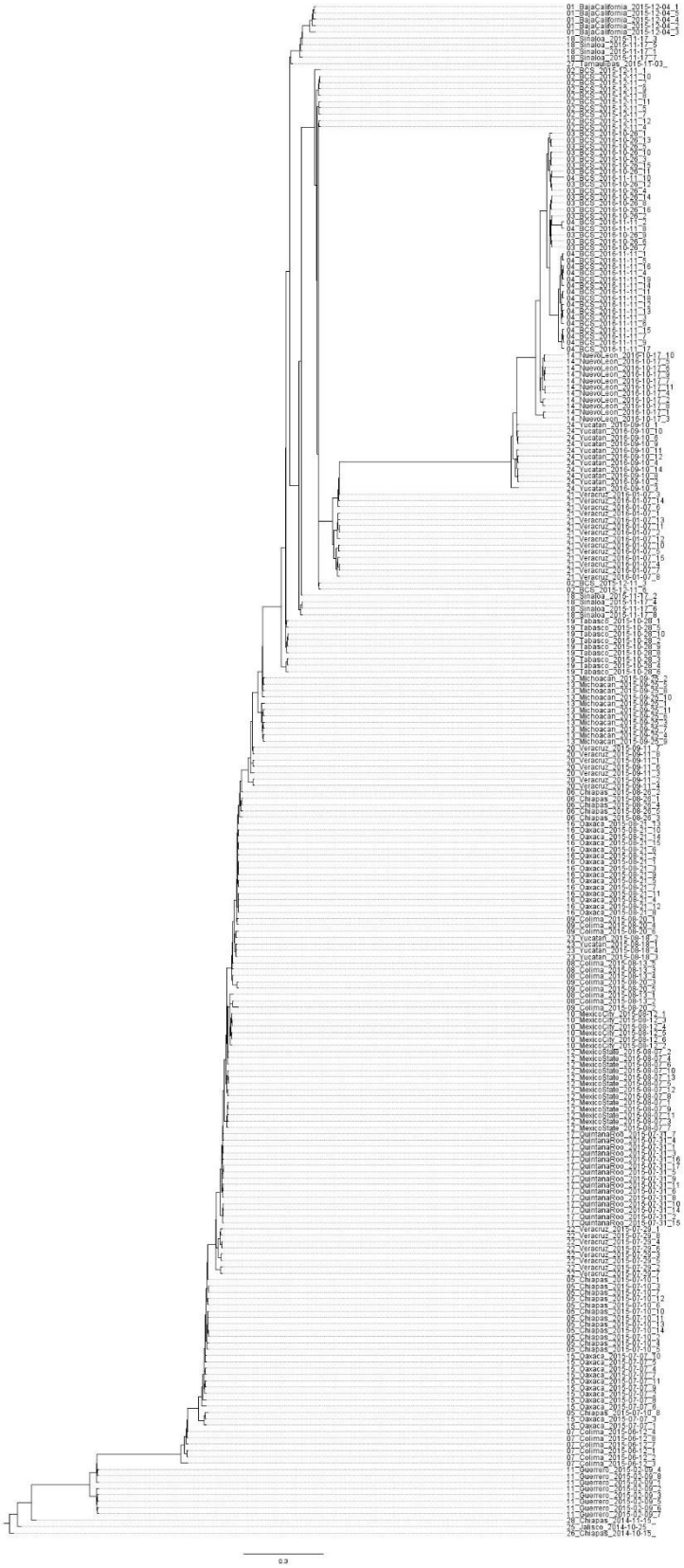


Figure S4 supplement. Preliminary MCC tree constructed with the 238 new sequences variants detected in our sequences.

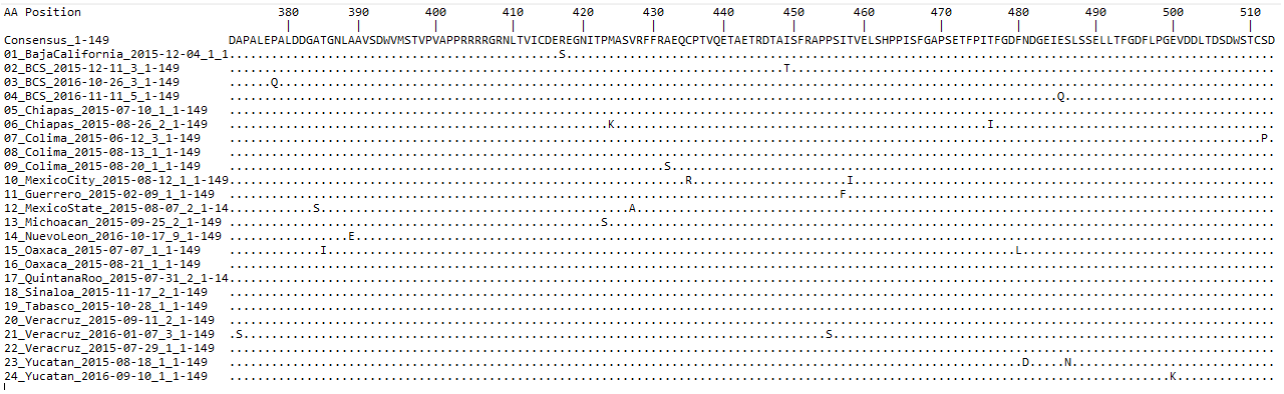
Figure S5

Location	2015	2016
Baja California (B.C.)	2	
Baja California Sur (B.C.S.)	3	8
Chiapas (CHI.)	5	
Colima (COL.)	9	
Mexico City (cdMX)	3	
Guerrero (GUE.)	1	
Mexico State (MEX.)	1	
Michoacan (MICH.)	2	
Nuevo Leon (N.L.)		2
Oaxaca (OAX.)	6	
Quintana Roo (Q.R.)	3	
Sinaloa (SIN.)	3	
Tabasco (TAB.)	3	
Veracruz (VER.)	4	2
Yucatan (YUC.)	1	1

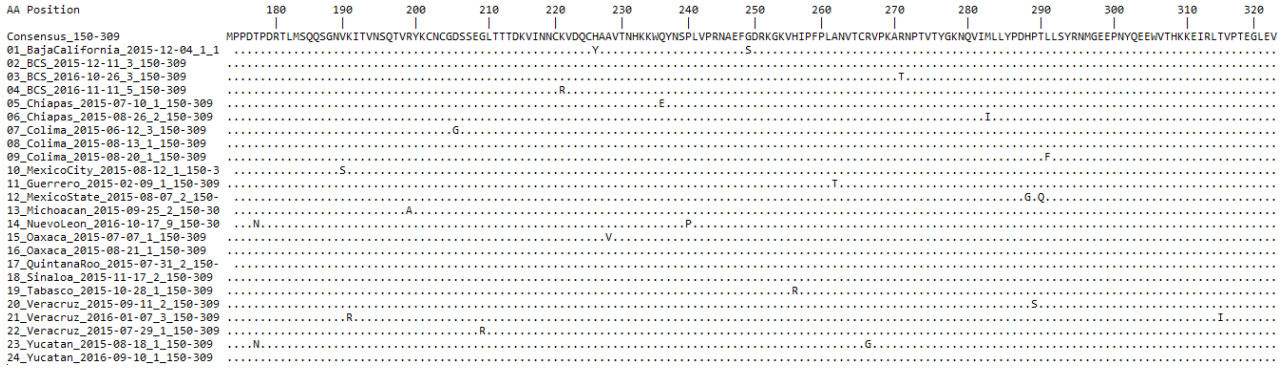
Figure S5 supplement. Left: Geographical distribution of CHIKV variant sequences used in this study. Black dots on the map indicate places where the original serum samples were obtained. Right: number of variant sequences obtained by location and year.

Figure S6

a)



b)



c)

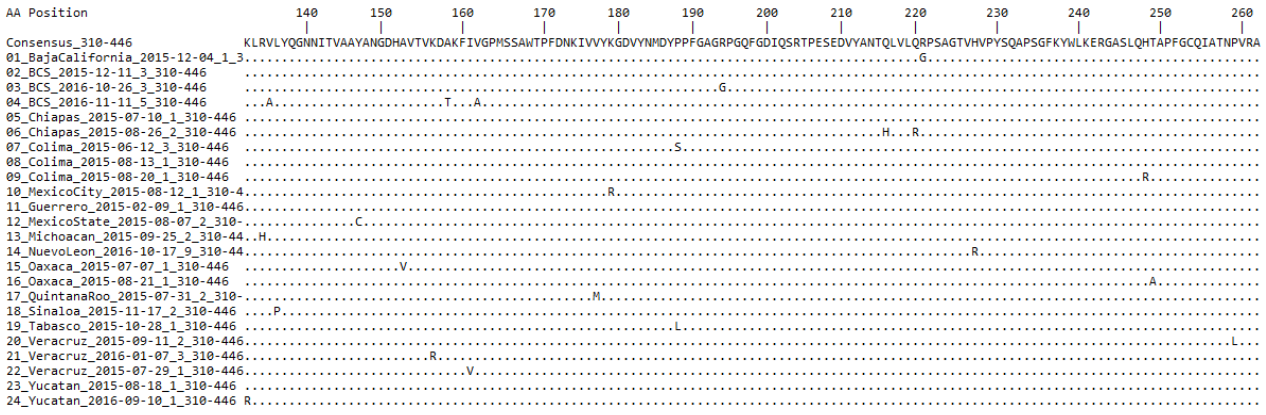


Figure S6. Non-synonymous mutations found in **a)** NSP3, **b)** E1 and **c)** E2. The first Mexican CHIKV sequence reported in October 2014 was used as the baseline sequence.