

Supplementary Information

Global evolutionary history and spatio-temporal dynamics of dengue virus type 2

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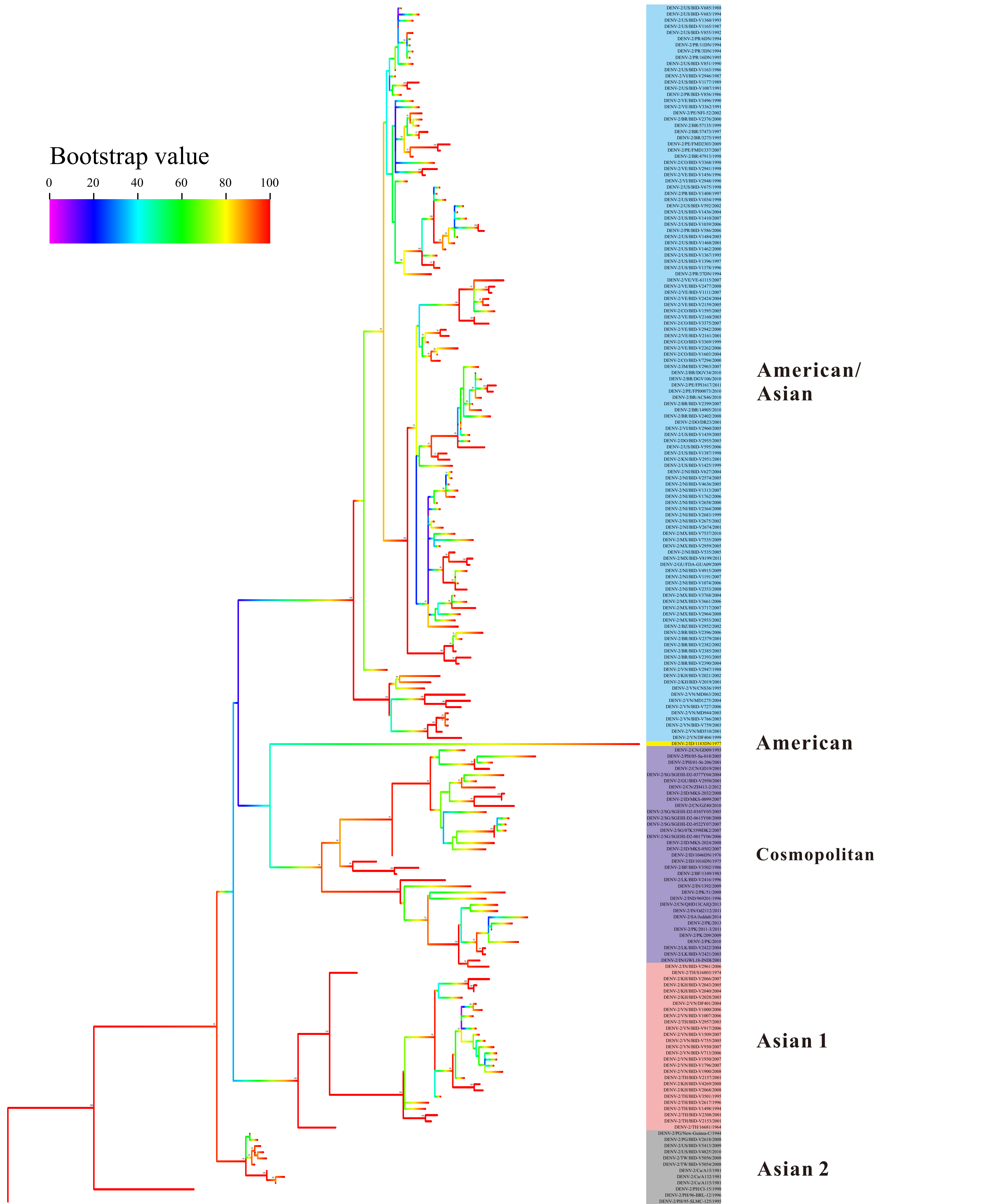


Figure S1. Maximum likelihood phylogeny of DENV-2 E gene sequences from 1944 to 2014 and the color gradient for the branches represents bootstrap values. The five subtypes are indicated on the right side of the tree.

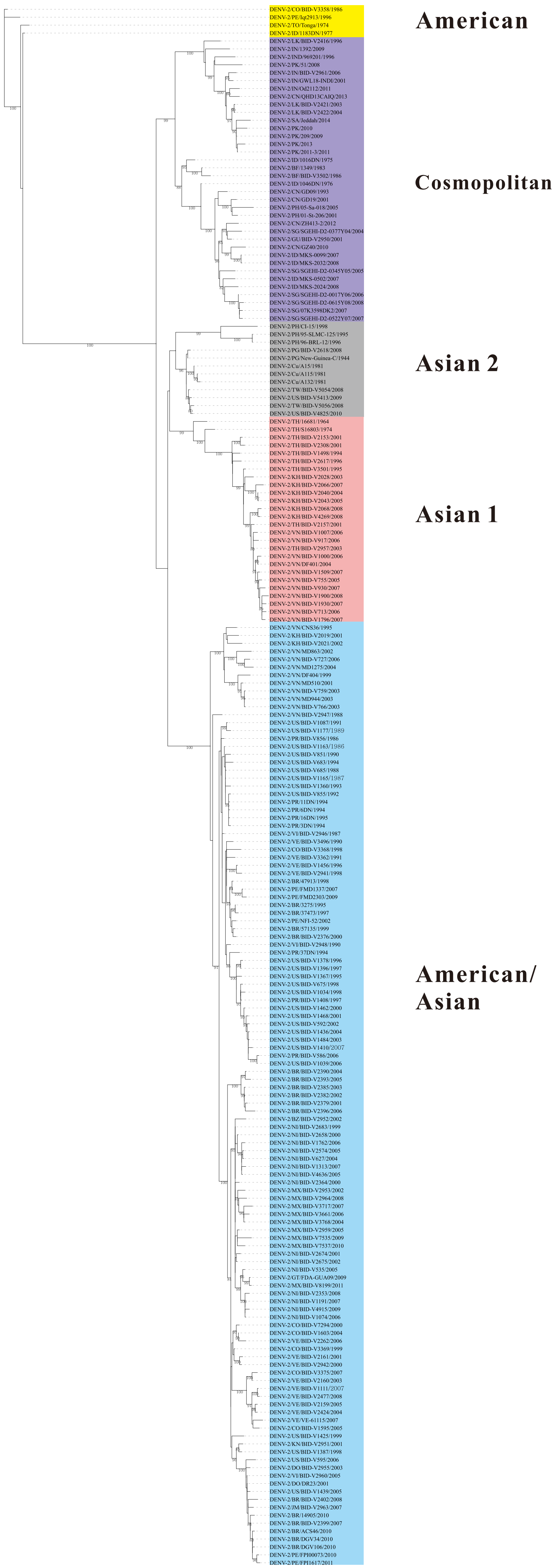


Figure S2. Maximum likelihood phylogeny including 194 isolates of DENV-2 E gene plus 3 American genotype reference sequences. The five subtypes are indicated on the right side of the tree.

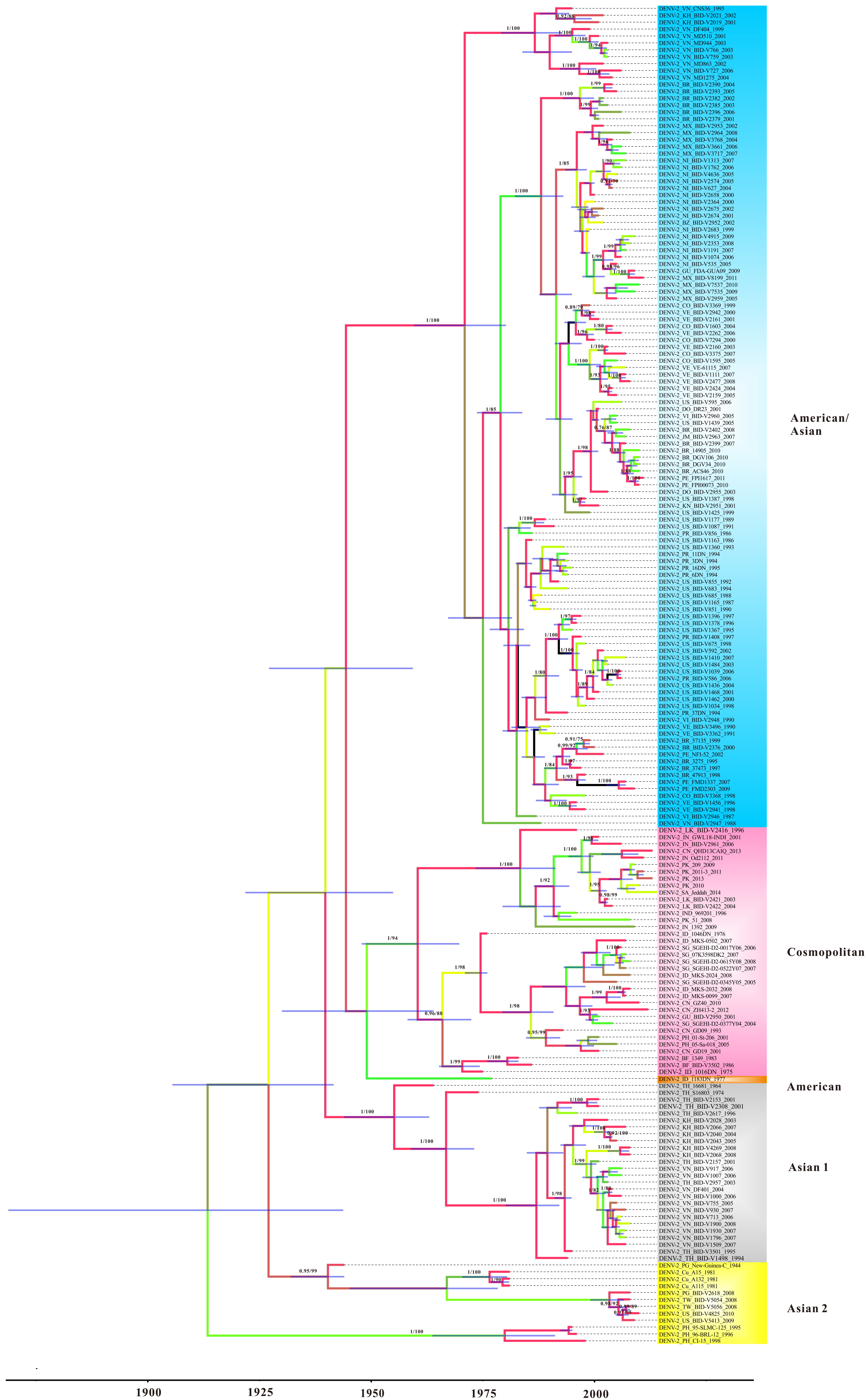


Figure S3. Maximum clade credibility (MCC) tree of Dengue virus type 2. Maximum clade credibility tree derived from the Bayesian analysis of the envelope protein of DENV-2 with the best fit model (relaxed exponential clock). DENV-2 genotypes (American, Asian 1 and 2, Cosmopolitan and American/Asian) are identified. The bootstrap (BS) values and posterior probability (PP) values for the key nodes are indicated as BS/PP.

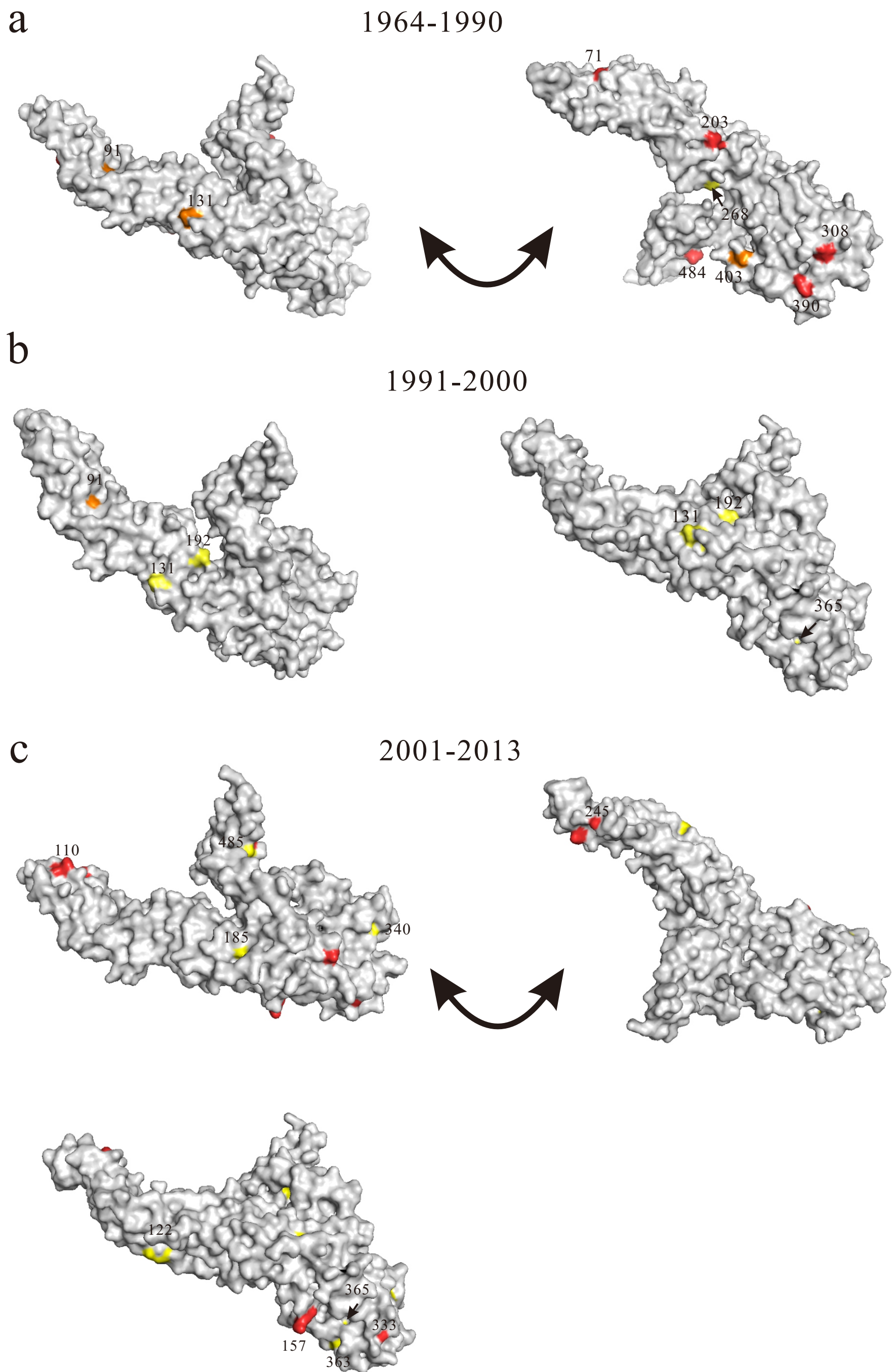


Figure S4. Mapping of positively selected amino-acid sites onto the three-dimensional structure of the E protein of DENV-2 during 1964-1990, 1991-2000 and 2001-2013, respectively. Yellow indicates positive selection site detected by the MEME method; The red color indicates positive selection site detected by the REL method; The orange color indicates positive selection sites that are detected by both methods.

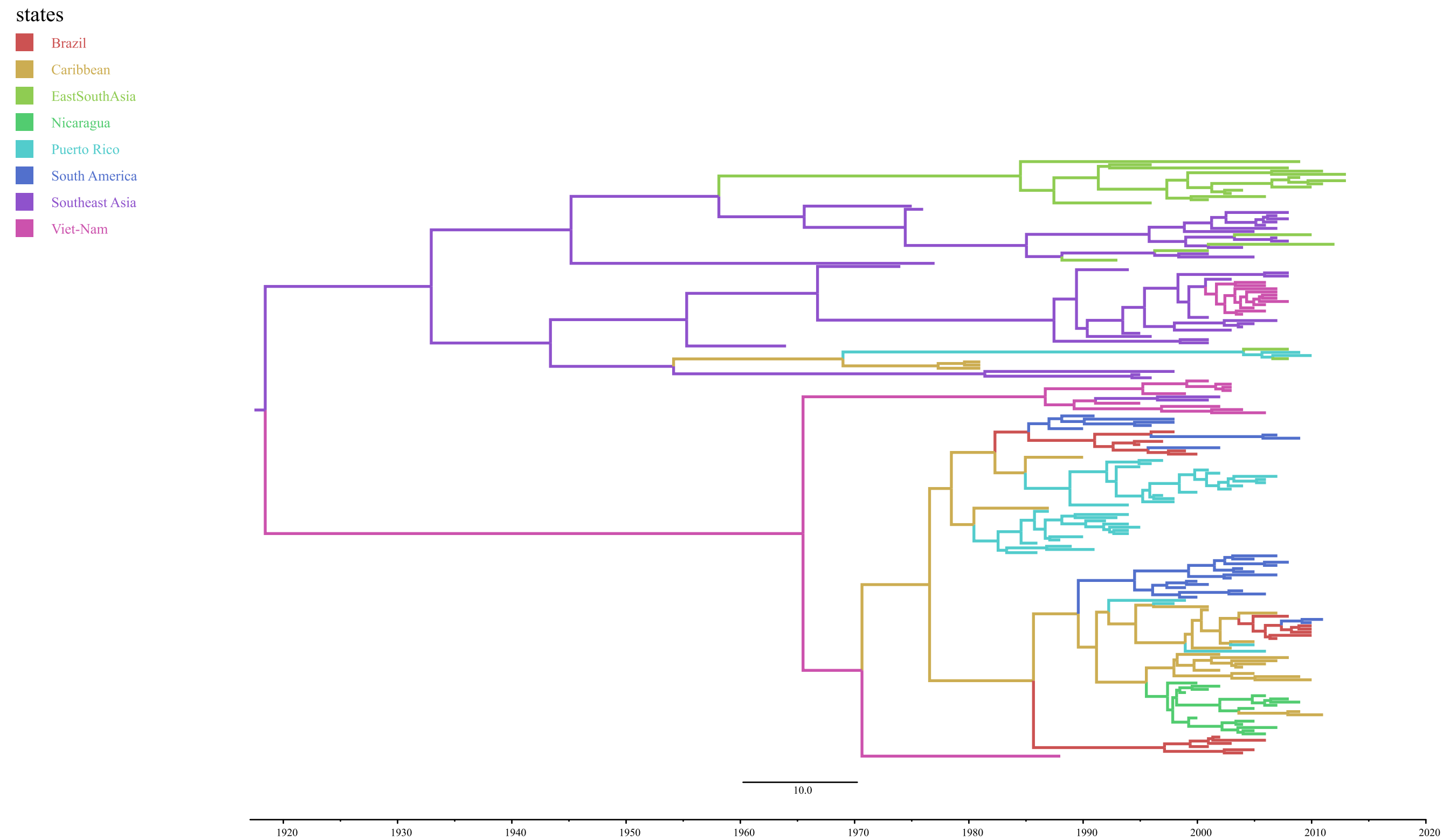
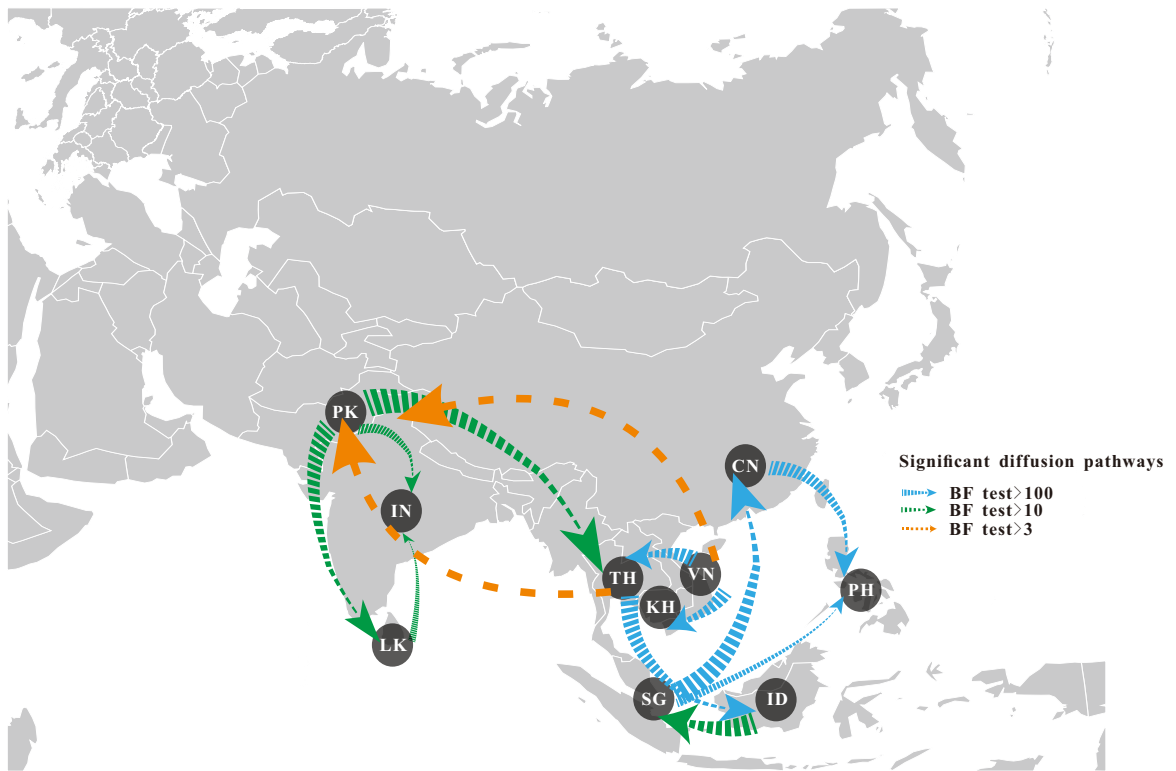


Figure S5. Bayesian phylogeographic tree of DENV-2 E gene sequences. Geographic locations showed with different colors in the tree are represent in the legend on the left.

a



b

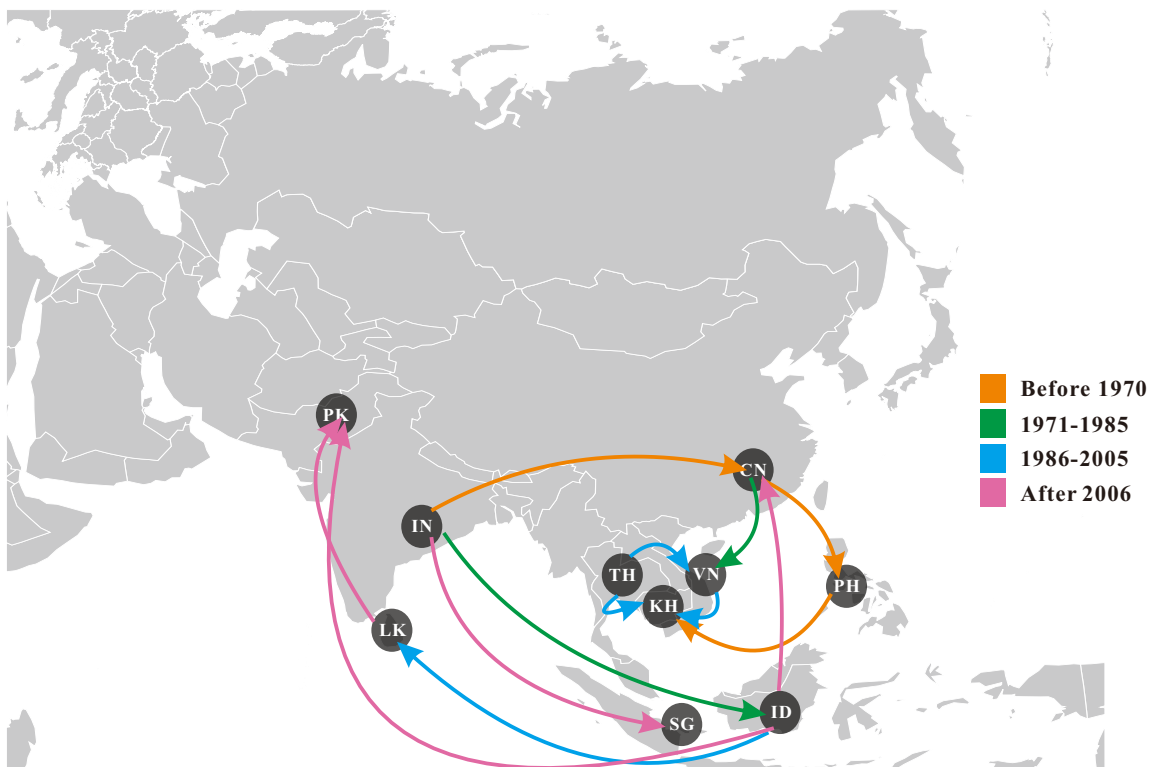


Figure S6. Spatiotemporal dynamics of dissemination of DENV-2. **a.** Significant pathways from one location to another are indicated on the maps. Blue arrow represents strongly supported rate with $100 \leq \text{BF} < 1000$; Dark green arrow, supported rate with $3 \leq \text{BF} < 100$; Orange arrow represents the support with $\text{BF} > 3$. PK, Pakistan; IN, India; LK, Sri Lanka; CN, China; PH, Philippines; TH, Thailand; VN, Viet Nam; KH, Cambodia; SG, Singapore; ID, Indonesia. **b.** Viral dispersal pattern between 1964 and 2013. Lines between locations represent branches in the Bayesian MCC tree along which location transitions occurs. The color of lines informs the date of the viral migrations among each pair of locations. The map source is downloaded freely from FREE WORLD MAPS (http://www.freeworldmaps.net/pdf/asia/asia_countries.pdf).

Table S1. Selection pressures of different periods for E gene of globally sampled DENV-2 virus.

Time	SLAC	FEL	REL	MEME	FUBAR
1964-1990	-	-	71,91,131,203,308,390,403,484	91,131,268,403	-
1991-2000	-	91	-	91,131,192,365	-
2001-2013	-	-	110,157,245,333	122,185,340,363,365,485	-

Table S2. A list of predicted the N-linked and O-linked glycosylation sites in E protein of DENV-2.

Glycosylation site	
N-linked	67 (NTTT), 153 (NDTG)
O-linked	S (32, 33, 40, 66, 69, 70, 76, 138, 142, 165, 236, 265, 353, 359, 405) T (168, 169, 186, 274, 363, 397, 431)

Table S3. Significant viral migration pathways between pairs of geographic locations. Links supported by Bayes Factor (BF) values.

Transition Between		Mean actual rate	Mean indicator	Bayes Factor
From	To			
South America	Caribbean	0.926	0.123	>1000
Southeast Asia	Viet-Nam	0.953	0.095	3-100
Southeast Asia	Brazil	0.939	0.066	3-100
Southeast Asia	Caribbean	0.965	0.065	3-100
Puerto Rico	Brazil	0.977	0.065	100-1000
East&South Asia	Nicaragua	0.985	0.057	100-1000
Southeast Asia	South America	1.964	1	3-100
Caribbean	South America	0.969	0.164	100-1000
Viet-Nam	Southeast Asia	0.97	0.071	3-100
East&South Asia	Southeast Asia	1.302	0.944	3-100
Nicaragua	Southeast Asia	0.983	0.032	3-100
Brazil	Puerto Rico	0.962	0.092	3-100
East&South Asia	Puerto Rico	0.991	0.076	>1000
Nicaragua	East&South Asia	0.872	0.34	100-1000

Table S4. Accession numbers of the sequences used in this study.

Accession	Strain name	Years	Country	Host
KF744397	DENV-2/PH/01-St-206/2001	2001	Philippines	Human
KF744398	DENV-2/PH/05-Sa-018/2005	2005	Philippines	Human
EU056810	DENV-2/BF/1349/1983	1983	Burkina-Faso	Human
JX475906	DENV-2/IN/1392/2009	2009	India	Human
JX669477	DENV-2/BR/14905/2010	2010	Brazil	Human
NC-001474	DENV-2/TH/16681/1964	1964	Thailand	Unknown
JX669480	DENV-2/BR/3275/1995	1995	Brazil	Human
JX669483	DENV-2/BR/37473/1997	1997	Brazil	Human
JX669484	DENV-2/BR/47913/1998	1998	Brazil	Human
JX669486	DENV-2/BR/57135/1999	1999	Brazil	Human
KF744403	DENV-2/PH/95-SLMC-125/1995	1995	Philippines	Human
KF744404	DENV-2/PH/96-BRL-12/1996	1996	Philippines	Human
KF744399	DENV-2/PH/CI-15/1998	1998	Philippines	Human
JX286516	DENV-2/BR/ACS46/2010	2010/3/1	Brazil	Human
JX649148	DENV-2/VN/CNS36/1995	1995	Viet-Nam	Human
KF704356	DENV-2/Cu/A115/1981	1981	Cuba	Human
KF704355	DENV-2/Cu/A132/1981	1981	Cuba	Human
KF704354	DENV-2/Cu/A15/1981	1981	Cuba	Human
KF041233	DENV-2/PK/2011-3/2011	2011	Pakistan	Human
KF041235	DENV-2/PK/209/2009	2009	Pakistan	Human
KF041236	DENV-2/PK/51/2008	2008	Pakistan	Human
GU131843	DENV-2/BF/BID-V3502/1986	1986	Burkina-Faso	Mosquito
FJ850072	DENV-2/BR/BID-V2376/2000	2000	Brazil	Human
FJ850074	DENV-2/BR/BID-V2379/2001	2001	Brazil	Human
FJ850076	DENV-2/BR/BID-V2382/2002	2002	Brazil	Human
GQ868640	DENV-2/BR/BID-V2385/2003	2003	Brazil	Human
FJ850082	DENV-2/BR/BID-V2390/2004	2004	Brazil	Human
FJ850085	DENV-2/BR/BID-V2393/2005	2005	Brazil	Human
FJ850088	DENV-2/BR/BID-V2396/2006	2006	Brazil	Human
FJ850091	DENV-2/BR/BID-V2399/2007	2007	Brazil	Human
GQ199890	DENV-2/BR/BID-V2402/2008	2008	Brazil	Human
FJ898461	DENV-2/BZ/BID-V2952/2002	2002	Belize	Human
FJ024474	DENV-2/CO/BID-V1595/2005	2005	Colombia	Human
FJ024477	DENV-2/CO/BID-V1603/2004	2004	Colombia	Human
GQ868552	DENV-2/CO/BID-V3368/1998	1998	Colombia	Human
GQ868553	DENV-2/CO/BID-V3369/1999	1999	Colombia	Human
GQ868558	DENV-2/CO/BID-V3375/2007	2007	Colombia	Human
KJ189305	DENV-2/CO/BID-V7294/2000	2000	Colombia	Human
FJ898451	DENV-2/DO/BID-V2955/2003	2003	Dominican-Republic	Human
HM488257	DENV-2/GU/BID-V2950/2001	2001	Guam	Human
HQ999999	DENV-2/GU/FDA-GUA09/2009	2009	Guatemala	Human
GQ398258	DENV-2/ID/1016DN/1975	1975	Indonesia	Human

GQ398264	DENV-2/ID/1046DN/1976	1976	Indonesia	Human
GQ398257	DENV-2/ID/1183DN/1977	1977	Indonesia	Human
FJ898454	DENV-2/IN/BID-V2961/2006	2006	India	Human
JQ922549	DENV-2/IN/969201/1996	1996	India	Human
GQ199892	DENV-2/JM/BID-V2963/2007	2007	Jamaica	Human
JF730044	DENV-2/KH/BID-V2019/2001	2001	Cambodia	Human
FJ639698	DENV-2/KH/BID-V2021/2002	2002	Cambodia	Human
GQ868620	DENV-2/KH/BID-V2028/2003	2003	Cambodia	Human
FJ639706	DENV-2/KH/BID-V2040/2004	2004	Cambodia	Human
FJ639709	DENV-2/KH/BID-V2043/2005	2005	Cambodia	Human
FJ639717	DENV-2/KH/BID-V2066/2007	2007	Cambodia	Human
FJ639718	DENV-2/KH/BID-V2068/2008	2008	Cambodia	Human
KF955401	DENV-2/KH/BID-V4269/2008	2008	Cambodia	Human
FJ898460	DENV-2/KN/BID-V2951/2001	2001	Saint-Kitts-and-Nevis	Human
FJ882602	DENV-2/LK/BID-V2416/1996	1996	Sri-Lanka	Human
GQ252676	DENV-2/LK/BID-V2421/2003	2003	Sri-Lanka	Human
GQ252677	DENV-2/LK/BID-V2422/2004	2004	Sri-Lanka	Human
FJ898438	DENV-2/MX/BID-V2953/2002	2002	Mexico	Human
GQ199894	DENV-2/MX/BID-V2959/2005	2005	Mexico	Human
FJ898439	DENV-2/MX/BID-V2964/2008	2008	Mexico	Human
GU131959	DENV-2/MX/BID-V3661/2006	2006	Mexico	Human
GU131975	DENV-2/MX/BID-V3717/2007	2007	Mexico	Human
GU131955	DENV-2/MX/BID-V3768/2004	2004	Mexico	Human
KJ189308	DENV-2/MX/BID-V7535/2009	2009	Mexico	Mosquito
KJ189309	DENV-2/MX/BID-V7537/2010	2010	Mexico	Mosquito
KJ189370	DENV-2/MX/BID-V8199/2011	2011	Mexico	Human
EU482620	DENV-2/NI/BID-V1074/2006	2006	Nicaragua	Human
EU482621	DENV-2/NI/BID-V1191/2007	2007	Nicaragua	Human
FJ882594	DENV-2/NI/BID-V1313/2007	2007	Nicaragua	Human
FJ744741	DENV-2/NI/BID-V1762/2006	2006	Nicaragua	Human
FJ810418	DENV-2/NI/BID-V2353/2008	2008	Nicaragua	Human
FJ744744	DENV-2/NI/BID-V2364/2000	2000	Nicaragua	Human
FJ850053	DENV-2/NI/BID-V2574/2005	2005	Nicaragua	Human
FJ850118	DENV-2/NI/BID-V2658/2000	2000	Nicaragua	Human
FJ850121	DENV-2/NI/BID-V2674/2001	2001	Nicaragua	Human
GQ199897	DENV-2/NI/BID-V2675/2002	2002	Nicaragua	Human
GQ199895	DENV-2/NI/BID-V2683/1999	1999	Nicaragua	Human
HQ541793	DENV-2/NI/BID-V4636/2005	2005	Nicaragua	Human
HQ705625	DENV-2/NI/BID-V4915/2009	2009	Nicaragua	Human
EU482757	DENV-2/NI/BID-V535/2005	2005	Nicaragua	Human
FJ898436	DENV-2/NI/BID-V627/2004	2004	Nicaragua	Human
KC294200	DENV-2/PE/FMD1337/2007	2007/3/28	Peru	Human
KC294201	DENV-2/PE/FMD2303/2009	2009/2/13	Peru	Human
KC294202	DENV-2/PE/FPI00073/2010	2010/12/13	Peru	Human

KC294210	DENV-2/PE/FPI1617/2011	2011/3/23	Peru	Human
KC294222	DENV-2/PE/NFI-52/2002	2002/3/22	Peru	Human
FJ906959	DENV-2/PG/BID-V2618/2008	2008	Papua-New-Guinea	Human
KF360005	DENV-2/PK/2010	2010/11/1	Pakistan	Human
KJ010186	DENV-2/PK/2013	2013/10/2	Pakistan	Human
GQ398313	DENV-2/PR/11DN/1994	1994	Puerto-Rico	Human
GQ398298	DENV-2/PR/16DN/1995	1995	Puerto-Rico	Human
GQ398283	DENV-2/PR/37DN/1994	1994	Puerto-Rico	Human
GQ398308	DENV-2/PR/3DN/1994	1994	Puerto-Rico	Human
GQ398292	DENV-2/PR/6DN/1994	1994	Puerto-Rico	Human
KF955373	DENV-2/PR/BID-V1408/1997	1997	Puerto-Rico	Human
KF955359	DENV-2/PR/BID-V586/2006	2006	Puerto-Rico	Human
KF955363	DENV-2/PR/BID-V856/1986	1986	Puerto-Rico	Human
GQ398266	DENV-2/SG/07K3598DK2/2007	2007	Singapore	Human
EU687246	DENV-2/TH/BID-V1498/1994	1994	Thailand	Human
FJ639828	DENV-2/TH/BID-V2153/2001	2001	Thailand	Human
FJ639832	DENV-2/TH/BID-V2157/2001	2001	Thailand	Human
FJ744722	DENV-2/TH/BID-V2308/2001	2001	Thailand	Human
FJ906958	DENV-2/TH/BID-V2617/1996	1996	Thailand	Human
FJ898452	DENV-2/TH/BID-V2957/2003	2003	Thailand	Human
GQ868543	DENV-2/TH/BID-V3501/1995	1995	Thailand	Human
HQ891023	DENV-2/TW/BID-V5054/2008	2008	Taiwan	Human
HQ891024	DENV-2/TW/BID-V5056/2008	2008	Taiwan	Mouse
EU482547	DENV-2/US/BID-V1034/1998	1998	USA	Human
EU482551	DENV-2/US/BID-V1039/2006	2006	USA	Human
EU529701	DENV-2/US/BID-V1087/1991	1991	USA	Human
EU569704	DENV-2/US/BID-V1163/1986	1986	USA	Human
EU482569	DENV-2/US/BID-V1165/1987	1987	USA	Human
EU482580	DENV-2/US/BID-V1177/1989	1989	USA	Human
EU482589	DENV-2/US/BID-V1360/1993	1993	USA	Human
EU569706	DENV-2/US/BID-V1367/1995	1995	USA	Human
EU596485	DENV-2/US/BID-V1378/1996	1996	USA	Human
EU687212	DENV-2/US/BID-V1387/1998	1998	USA	Human
EU569714	DENV-2/US/BID-V1396/1997	1997	USA	Human
EU596488	DENV-2/US/BID-V1410/2007	2007	USA	Human
EU677142	DENV-2/US/BID-V1425/1999	1999	USA	Human
EU687215	DENV-2/US/BID-V1436/2004	2004	USA	Human
EU687216	DENV-2/US/BID-V1439/2005	2005	USA	Human
EU687223	DENV-2/US/BID-V1462/2000	2000	USA	Human
EU687228	DENV-2/US/BID-V1468/2001	2001	USA	Human
EU687236	DENV-2/US/BID-V1484/2003	2003	USA	Human
HQ541799	DENV-2/US/BID-V4825/2010	2010	USA	Mouse
JF730054	DENV-2/US/BID-V5413/2009	2009	USA	Mouse
EU482723	DENV-2/US/BID-V592/2002	2002	USA	Human

EU482726	DENV-2/US/BID-V595/2006	2006	USA	Human
EU482732	DENV-2/US/BID-V675/1998	1998	USA	Human
EU482740	DENV-2/US/BID-V683/1994	1994	USA	Human
EU482742	DENV-2/US/BID-V685/1988	1988	USA	Human
EU482590	DENV-2/US/BID-V851/1990	1990	USA	Human
EU482594	DENV-2/US/BID-V855/1992	1992	USA	Human
EU482608	DENV-2/VE/BID-V1111/2007	2007	Venezuela	Human
EU687220	DENV-2/VE/BID-V1456/1996	1996	Venezuela	Human
FJ639733	DENV-2/VE/BID-V2159/2005	2005	Venezuela	Human
FJ639734	DENV-2/VE/BID-V2160/2003	2003	Venezuela	Human
JN819408	DENV-2/VE/BID-V2161/2001	2001	Venezuela	Human
FJ639822	DENV-2/VE/BID-V2262/2006	2006	Venezuela	Human
FJ850112	DENV-2/VE/BID-V2424/2004	2004	Venezuela	Human
FJ850107	DENV-2/VE/BID-V2477/2008	2008	Venezuela	Human
FJ898465	DENV-2/VE/BID-V2941/1998	1998	Venezuela	Human
FJ898466	DENV-2/VE/BID-V2942/2000	2000	Venezuela	Human
GQ868595	DENV-2/VE/BID-V3362/1991	1991	Venezuela	Human
GQ868540	DENV-2/VE/BID-V3496/1990	1990	Venezuela	Human
GQ868603	DENV-2/VI/BID-V2946/1987	1987	Virgin-Islands	Human
FJ898450	DENV-2/VI/BID-V2948/1990	1990	Virgin-Islands	Human
FJ898453	DENV-2/VI/BID-V2960/2005	2005	Virgin-Islands	Human
EU482445	DENV-2/VN/BID-V1000/2006	2006	Viet-Nam	Human
EU482451	DENV-2/VN/BID-V1007/2006	2006	Viet-Nam	Human
EU687248	DENV-2/VN/BID-V1509/2007	2007	Viet-Nam	Human
FJ859028	DENV-2/VN/BID-V1796/2007	2007	Viet-Nam	Human
FJ410237	DENV-2/VN/BID-V1900/2008	2008	Viet-Nam	Human
FJ547067	DENV-2/VN/BID-V1930/2007	2007	Viet-Nam	Human
JN819418	DENV-2/VN/BID-V2947/1988	1988	Viet-Nam	Human
EU482650	DENV-2/VN/BID-V713/2006	2006	Viet-Nam	Human
EU482664	DENV-2/VN/BID-V727/2006	2006	Viet-Nam	Human
EU482777	DENV-2/VN/BID-V755/2005	2005	Viet-Nam	Human
EU482781	DENV-2/VN/BID-V759/2003	2003	Viet-Nam	Human
EU482788	DENV-2/VN/BID-V766/2003	2003	Viet-Nam	Human
EU482463	DENV-2/VN/BID-V917/2006	2006	Viet-Nam	Human
EU482475	DENV-2/VN/BID-V930/2007	2007	Viet-Nam	Human
FM210216	DENV-2/VN/DF401/2004	2004	Viet-Nam	Human
FM210217	DENV-2/VN/DF404/1999	1999	Viet-Nam	Human
JX286524	DENV-2/BR/DGV106/2010	2010/4/15	Brazil	Human
JX286522	DENV-2/BR/DGV34/2010	2010/2/24	Brazil	Human
AB122020	DENV-2/DO/DR23/2001	2001	Dominican-Republic	Human
KC964094	DENV-2/CN/GD09/1993	1993	China	Human
KC964093	DENV-2/CN/GD19/2001	2001	China	Human
DQ448231	DENV-2/IN/GWL18-INDI/2001	2001	India	Human
JX470186	DENV-2/CN/GZ40/2010	2010	China	Human

KJ830750	DENV-2/SA/Jeddah/2014	2014/1/13	Saudi-Arabia	Human
FM210241	DENV-2/VN/MD1275/2004	2004	Viet-Nam	Human
FM210238	DENV-2/VN/MD510/2001	2001	Viet-Nam	Human
FM210227	DENV-2/VN/MD863/2002	2002	Viet-Nam	Human
FM210230	DENV-2/VN/MD944/2003	2003	Viet-Nam	Human
KC762676	DENV-2/ID/MKS-0099/2007	2007/7/17	Indonesia	Human
KC762656	DENV-2/ID/MKS-0502/2007	2007/12/11	Indonesia	Human
KC762663	DENV-2/ID/MKS-2024/2008	2008/2/20	Indonesia	Human
KC762675	DENV-2/ID/MKS-2032/2008	2008/3/8	Indonesia	Human
KM204118	DENV-2/PG/New-Guinea-C/1944	1944	Papua-New-Guinea	Human
JQ955624	DENV-2/IN/Od2112/2011	2011	India	Human
KF479233	DENV-2/CN/QHD13CAIQ/2013	2013/1/4	China	Human
GU289914	DENV-2/TH/S16803/1974	1974	Thailand	Human
JN851113	DENV-2/SG/SGEHI-D2-0017Y06/2006	2006	Singapore	Human
JN851125	DENV-2/SG/SGEHI-D2-0345Y05/2005	2005	Singapore	Human
JN851123	DENV-2/SG/SGEHI-D2-0377Y04/2004	2004	Singapore	Human
JN851114	DENV-2/SG/SGEHI-D2-0522Y07/2007	2007	Singapore	Human
JN851121	DENV-2/SG/SGEHI-D2-0615Y08/2008	2008	Singapore	Human
HQ332186	DENV-2/VE/VE-61115/2007	2007	Venezuela	Human
KC131142	DENV-2/CN/ZH413-2/2012	2012/9/1	China	Human

Text S1

Supplementary Methods:

Sequence dataset

E gene sequences for Dengue virus type 2 collected by the National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov/>) between 1944 and 2014. After removing duplicate strains and recombination sequences, the data set contained 946 DENV-2 sequences. Sampling locations for these sequences were parsed from strain names. Some regions in the original sequence data, such as Puerto Rico, are over-represented. In addition, the strains in recent years have a higher sample size compared to the beginning of the study period. To control these sampling biases, we subsampled the raw data randomly based on location and time to create a more equitable spatiotemporal distribution. Selected sequences to ensure the maximization of the available sequences within each region while still providing sufficient geographical diversity to ensure almost global coverage. From 1986 to 2010, Puerto Rico had many available sequences each year, so it was necessary to sample fewer sequences per year from the Puerto Rico in order to maintain a similar total number of sequences for each region throughout the study. We selected not more than 5 sequences per region per year for the virus. When selecting a subsampled sequence, we first selected the sequences with the complete year and then the longer sequences to exclude the incomplete sequences. Finally, E gene sequence data for 194 DENV-2 was obtained and used for the next analysis.

Phylogenetic Inference

Maximum likelihood (ML) phylogenetic trees were constructed in PhyML v 3.1¹ with 100 bootstrap replicates. A general time-reversible model of nucleotide substitution was implemented, with gamma distributed rate heterogeneity of 4 rate categories and a proportion of invariable sites. Then, the phylogeny of DENV-2 was generated using the Bayesian Monte Carlo Markov Chain (MCMC) method implemented in BEAST v.1.8.0². Different combinations of substitution models, clock models, and population size models were compared using a posterior simulation-based analogue of Akaike's information reiteration through MCMC (AICM), the AIC was measured from a posterior of each model in a Bayesian Monte Carlo context³. The comparisons were conducted in Tracer v1.5 (<http://beast.bio.ed.ac.uk>) using a log file of each model. For our dataset, the best-fit model incorporated a SRD06⁴ nucleotide substitution model with uncorrelated exponential relaxed

molecular clocks and an exponential growth population coalescent process prior over the phylogeny. This combined model is also used to estimate subsequent evolutionary rates and divergent times. MCMC chains were run for 300 million steps sampled every 10,000 states with 10% burn-in. MCMC convergence and effective sample size of parameter estimates were evaluated using Tracer 1.5. Maximum clade credibility (MCC) tree were summarized using Tree Annotator and visualized using FigTree v1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>). The overall rates of evolutionary change (nucleotide substitutions per site per year (subs/site/year)) and tree root ages were estimated simultaneously.

Discrete phylogeographic analyses

The phylogeographic analysis considered 8 locations: Nicaragua, Puerto Rico, Brazil, Caribbean, South America, Southeast Asia, Viet Nam and East&South Asia. To determine the viral dissemination network among geographical regions, an asymmetric discrete phylogeography model was applied. We employ a continuous time Markov chain (CTMC)⁵ phylogeographic model implemented in BEAST, coupled with model averaging using a Bayesian stochastic search variable selection (BSSVS) procedure⁵. BSSVS is a measure of statistical support for the rate of discrete traits and that enables us to compute a Bayes factor (BF) test for identifying the most parsimonious diffusion routes. In order to reduce the risk of model over-parameterization, the simplest model that the HKY nucleotide substitution model with strict clock and a constant-population coalescent process can be used. This tree was treated as independent draws from the posterior space of trees when subsequently used in the phylogeographic analyses. The resulting log files were used to calculate the BF for the diffusion between discrete locations, and to extract the actual non-zero rates and mean indicators for all statistically supported routes. Significant migration routes were summarized based on $BF > 3$. We defined the degree of rate support as follows: $BF > 1,000$ indicates decisive, $100 < BF < 1,000$ indicates very strong support, $10 < BF < 100$ indicates strong support and $3 < BF < 10$ indicates supported.

Recent advances in explicit calculation⁶ enable computation of the expected number of transitions (jumps) and waiting times in given locations (rewards). 'Markov jump' counts⁷ of the expected number of location state transitions along the phylogenetic branches provide a quantitative measure of gene flow between regions, representing successful viral introductions from one region to another. To quantify the dissemination process, we estimated the rate of DENV-2 transmission in the sampled

isolates using the 'Markov jump' counts of location state transitions for all possible states along the phylogeny. Then we used the time between state changes (Markov reward) to estimate the duration the ancestral population occupies a particular location. That is to say, the jumps quantify the transitions, whereas the rewards quantify the time spent in any particular state (on the phylogeny).

Phylogeny-trait association

We quantify spatial signal using Bayesian tip-association tests, implemented through the Bayesian tip-association significance testing (BaTS) software package. To determine the overall degree of geographical structure among the DENV-2 viruses, we assign each sequence to the eight geographical regions and compute association index (AI)⁸ and parsimony score (PS)⁹ using BaTS on a subset of 100 samples from the posterior distribution of topologies. The first two statistics are to assess the overall extent of clustering for the mutations of interest in the phylogenetic tree. Additionally, the monophyletic clade (MC)¹⁰ statistic allows the user to test for an association between individual geographical regions and viruses. The observed mean and its associated 95% confidence intervals (Upper and Lower CI) were obtained by analyzing trees sampled during the Bayesian phylogenetic reconstruction. In the present study, P values < 0.05 were considered significant for all statistics calculated by BaTS.

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