Shifting Dynamics of Dengue Virus Serotype 2 and Emergence of Cosmopolitan Genotype, Costa Rica, 2024

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Dengue remains a major public health challenge. In Costa Rica, we implemented nationwide genomic surveillance to track dengue virus serotype 2 cosmopolitan genotype emergence. Phylogenetic and eco-epidemiologic analyses revealed early detection, climate-driven spread, and spatial heterogeneity. Our findings underscore the need for integrated surveillance to guide adaptive responses to emerging arboviral threats.

Dengue fever, caused by mosquitoborne dengue virus (DENV), remains a major public health threat. DENV is primarily transmitted by *Aedes aegypti* mosquitoes (*1*). Rising global dengue incidence has been linked to climate change and urbanization (*2*).

In Costa Rica, DENV transmission has become increasingly complex. Dengue cases rose from 30,649 in 2023 (*2*) to 31,259 in 2024 (*1*); San José, Alajuela, and Puntarenas reported the highest incidence rates. Inciensa launched a nationwide DENV sequencing program in 2023, which confirmed simultaneous circulation of all 4 serotypes (DENV-1–4). That study was approved by the Pan-American Health Organization Ethics Review Committee (reference no. PAHO-2024-08-0029) and was conducted as part of routine arbovirus surveillance at Inciensa. In February 2024, that surveillance detected DENV-2 genotype II (cosmopolitan); by September genotype II had fully replaced genotype III, and the earliest cases were reported in coastal districts (*3*,*4*). Genotype II is associated with more severe clinical outcomes (*4*) and has been reported in Peru and Brazil since 2019 (*3*,*4*), raising concerns for regional spread. We investigated whether ecologic factors were contributing to DENV shifts in Costa Rica.

The Study

To assess ecologic drivers, we compared dengue incidence with a climate-driven suitability index, which integrates temperature- and humidity-dependent mosquito traits, such as biting rate, lifespan, and extrinsic incubation. Before 2022, dengue activity was irregular in Costa Rica but surged during 2022–2024 (Figure 1, panel A); we noted a moderate correlation (r = 0.38) between suitability and incidence during the 2022–2023 epidemic (Appendix 1, https://wwwnc.cdc.gov/EID/article/31/11/25-0746-App1.pdf). At the province level, correlations in 2022 were broadly consistent

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(Figure 1, panel B), but in 2023, we observed stronger associations in Puntarenas and Limón, where the cosmopolitan genotype first appeared, suggesting ecologic and virologic factors converged to intensify local transmission (Figure 1, panel C).

Historically, DENV-1 and DENV-2 have been the predominant serotypes in Costa Rica, fluctuating in relative proportions. However, we observed a major shift in 2023–2024, characterized by co-circulation of all 4 serotypes, mirrored by emergence of DENV-4 in late 2022 and reemergence of DENV-3 in early 2023 after a 6-year absence (Appendix 1 Figure 1, panel A). The reemergence of DENV-3 aligns with the known ubiquitous serotype cycles observed every 7–9 years, and DENV-4 emergence aligns with its recent expansion in South America (*1*).

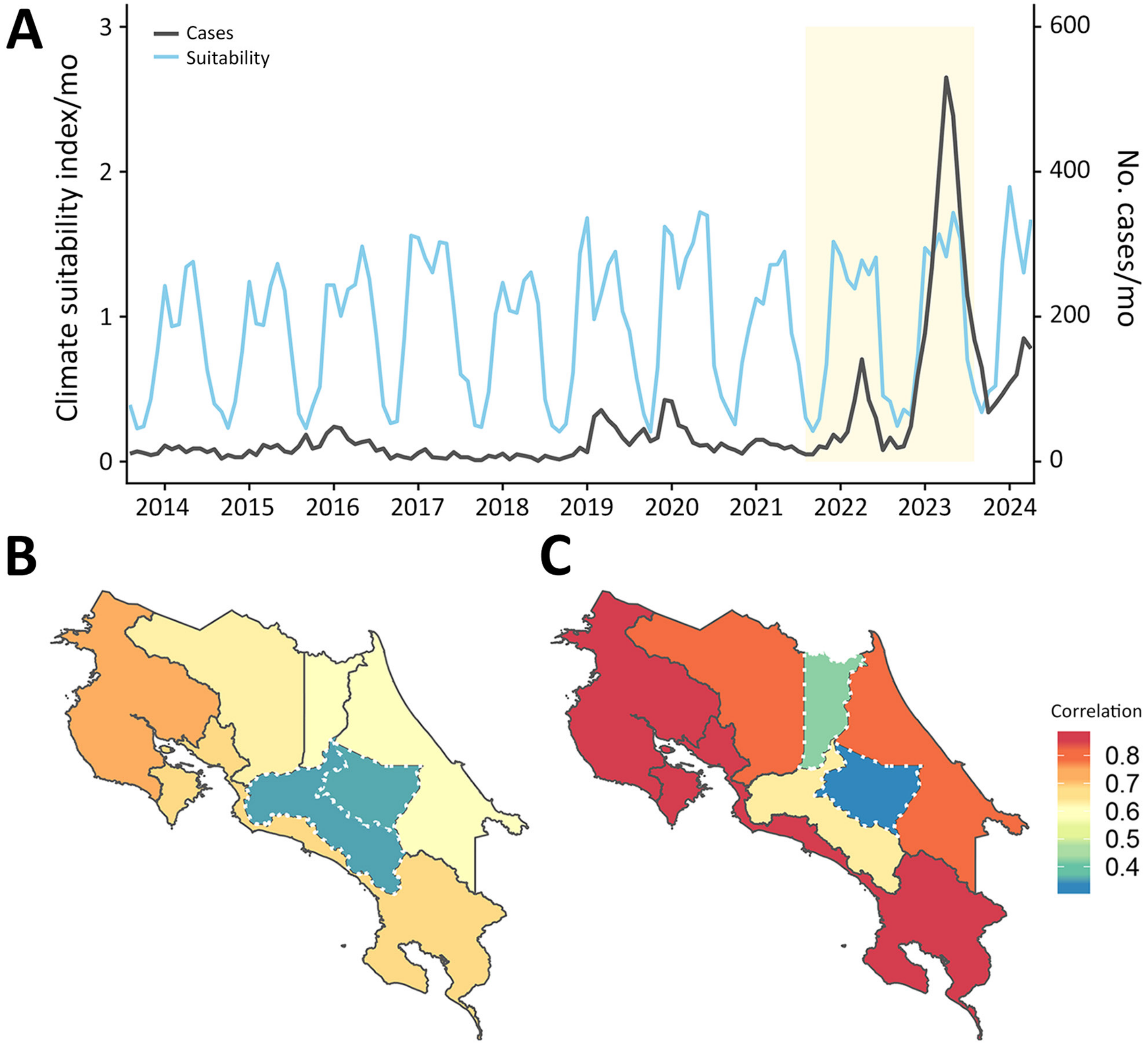

Figure 1. Temporal and spatial correlation between climate-driven suitability and dengue incidence from a study of shifting dynamics of dengue virus serotype 2 and emergence of cosmopolitan genotype, Costa Rica, 2024. A) Time series of monthly dengue cases and climate-driven suitability index for transmission during June 2014–November 2024. Shaded area (yellow) indicates the epidemic period during which enough cases with a clear seasonal signal were reported to enable an estimate correlation between suitability and incidence (Spearman r = 0.38; p<0.05). Scale bars for the y-axes differ substantially to underscore patterns. B, C) Province-level Spearman correlation values between monthly dengue incidence and climate suitability during 2022 (B) and 2023 (C). Warmer colors indicate stronger correlations. White dashed boundaries mark provinces with nonsignificant correlation (p>0.05) and solid dark gray boundaries indicate provinces with statistically significant correlation. In 2023, higher correlations were observed in eastern and coastal provinces where early cases of the dengue virus serotype 2 cosmopolitan genotype were detected.

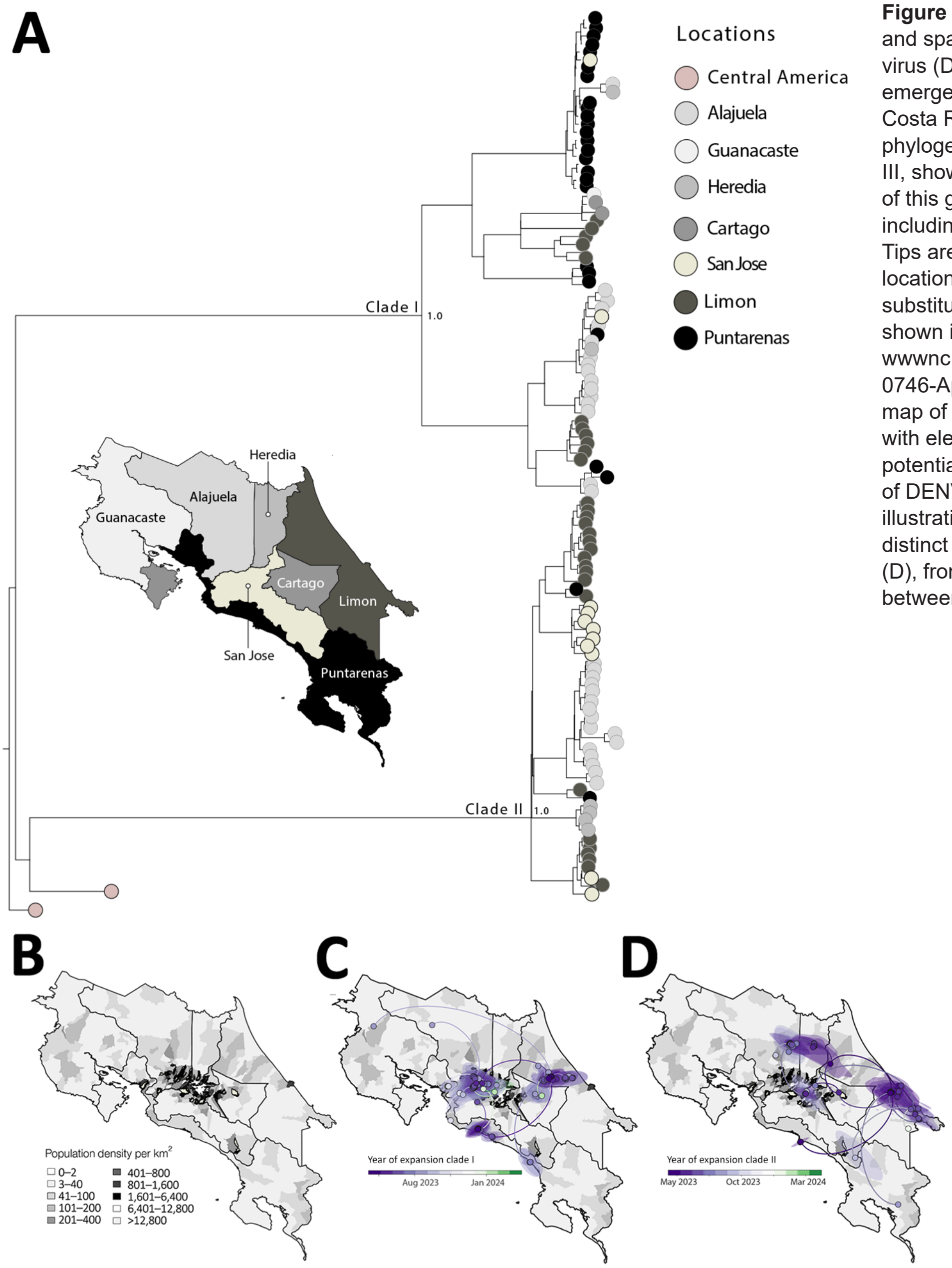

Figure 2. Maximum-likelihood phylogeny and spatiotemporal expansion of dengue virus (DENV) serotype 2 (DENV-2) and emergence of cosmopolitan genotype, Costa Rica, 2024. A) Time-stamped phylogenetic tree of DENV-2 genotype III, showing 2 independent introductions of this genotype into Costa Rica, including 1 major transmission cluster. Tips are colored according to sampling location. Scale bar indicates nucleotide substitutions per site. Full phylogeny shown in Appendix 1 Figure 2 (https://wwwnc.cdc.gov/EID/article/31/11/25-0746-App1.pdf). B) Population density map of Costa Rica, highlighting areas with elevated DENV transmission potential. C, D) Spatiotemporal expansion of DENV-2 genotype III lineage D.1.2, illustrating the progressive spread of 2 distinct clades, clade I (C) and clade II (D), from urban centers to coastal regions between May 2023 and March 2024.

To assess whether those serotype shifts were associated with longer-term changes in age distribution, we analyzed dengue case data spanning 2014–2024, the entire period of available national dengue surveillance. During the years with available dengue reports, age ranges among infected persons changed slightly, but we saw no quantifiably significant change over time (linear slope 0.17; p = 0.048) (Appendix 1 Figure 1, panel B). That estimate did not strongly support a substantial increase in the force-of-infection over time, which was supported by the relatively stable climate-driven suitability estimates (Figure 1). Force-of-infection should be mirrored by a decreasing age of reported infections; however, the age of infection increased slightly by 1.3 years for every extra circulating serotype ($p<0.001$), independent of year (Appendix Figure 1, panel B). That finding potentially indicates that serotype mixing increased the prevalence for secondary infections, which then occurred in older persons who were already seropositive.

Concurrently, we observed a marked change in circulating DENV-2 strains; the previously dominant genotype III was replaced by genotype II in early 2024 (Appendix 1 Figure 1, panel C). During May 2023–

August 2024, DENV-2 genotype III was more prevalent, particularly in Alajuela, San José, Puntarenas, and Limón, regions historically associated with high DENV transmission. Over time, however, genotype II became increasingly dominant, especially in San José, Cartago, and Alajuela, and genotype III declined. That pattern suggests a gradual replacement, potentially driven by selective advantage, immune escape, or repeated introductions from external sources.

After launching a nationwide genomic surveillance program, Inciensa generated 133 DENV-2 whole-genome sequences during 2023–2024. Using the dynamic DENV lineage classification system (*5*), we assigned 110 genotype III (Asian–American)

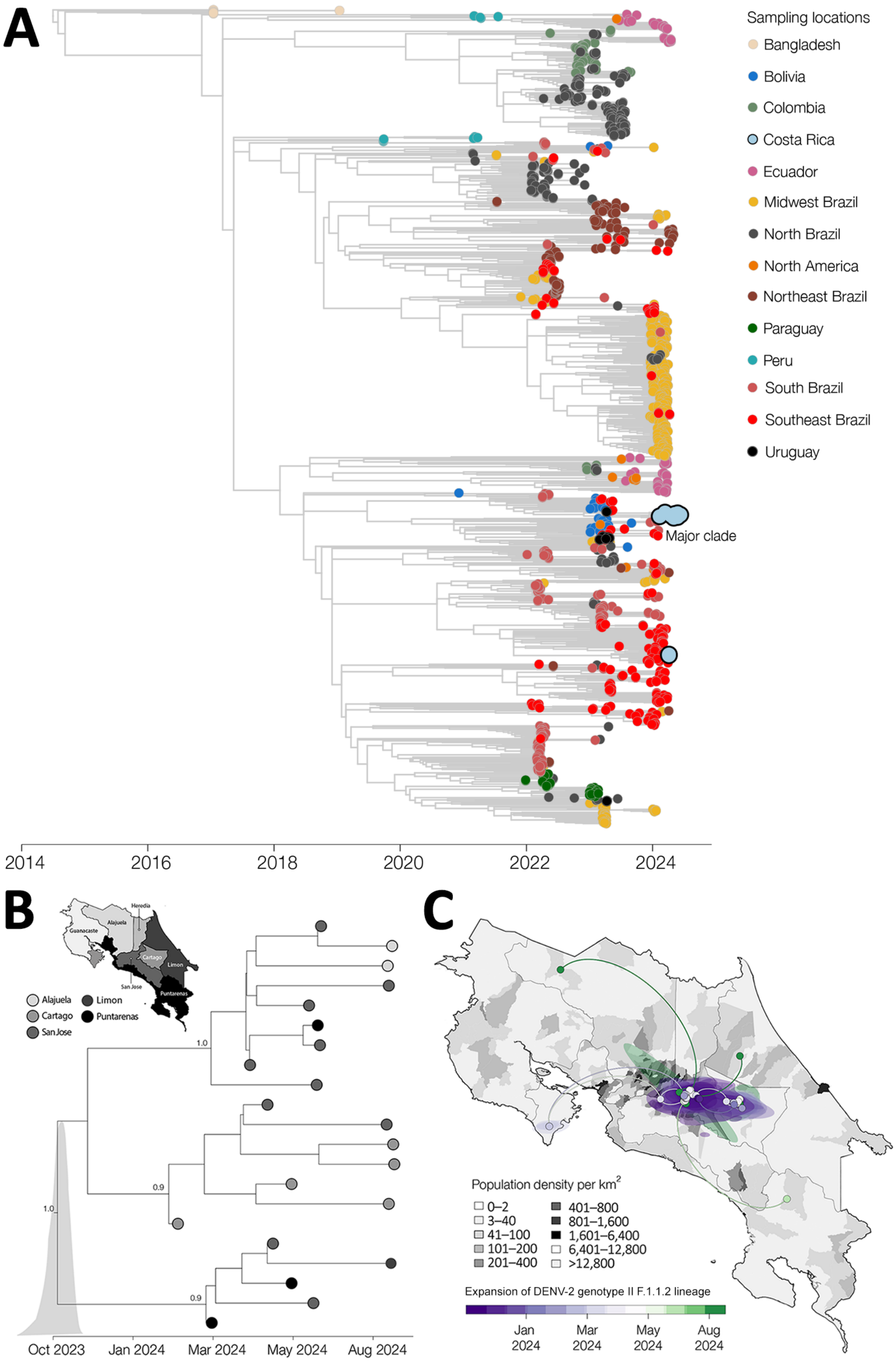

Figure 3. Time-scaled and spatiotemporal dynamics of dengue virus serotype 2 (DENV-2) and emergence of cosmopolitan genotype, Costa Rica, 2024. A) Time-scaled phylogenic reconstruction of global DENV-2 showing major clade in Costa Rica in 2024. Full phylogeny shown in Appendix 1 Figure 3 (https://wwwnc.cdc.gov/EID/article/31/11/25-0746-App1.pdf). B) Time-scaled phylogenetic reconstruction of the major DENV-2 genotype II clade, illustrating its expansion from Puntarenas, Limón, and Cartago in early 2024 (map inset). C) Spatiotemporal dynamics of the major DENV-2 genotype II clade in Costa Rica demonstrating its spread among provinces. DENV, dengue virus.

genomes to lineage D.1.2 and 23 genotype II (cosmopolitan) genomes to lineage F.1.1.2. Genotype III sequences were from 110 patients (mean age 38 years) across 7 provinces (Appendix 2 Table 1, https://wwwnc.cdc.gov/EID/article/31/11/25-0746-App2.xlsx); mean genome coverage was 92.6%, and mean cycle threshold was 22. Genotype II sequences were from 23 patients (mean age 38 years) in 5 provinces (Appendix 2 Table 2); mean coverage was 80%, and mean cycle threshold was 24. Phylodynamic analyses showed a well-supported monophyletic clade of DENV-2 genotype III in Costa Rica (Figure 2, panel A; Appendix 1 Figure 2), consistent with sustained local persistence after introduction events from Central America over the previous decade.

Maximum-likelihood phylogenetic reconstruction revealed cocirculation of 2 distinct clades within the DENV-2 genotype III D.1.2 lineage, here referred to as clades I and II (Figure 2, panel B). Although phylogenetically distinct, both clades belong to the same lineage. Phylogeographic analysis showed that early circulation was concentrated in Alajuela, Cartago, and San José, followed by expansion toward the coastal regions of Puntarenas and Limón. Clade I likely emerged in May 2023 (95% highest posterior density [HPD] April–late May 2023) (Figure 2, panel D) and spread from San José and Cartago to Puntarenas and Limón by early 2024. Clade II (Figure 2, panel E), detected as early as June 2023 with a similar HPD, displayed broader dispersal, including to the densely populated areas of Alajuela and San José. The spatial overlap of those sublineages with high-population regions (Figure 2, panel C) underscores the role of urban centers as transmission hubs enabling spread of DENV-2.

Further phylogenetic resolution of DENV-2 genotype II sequences revealed a distinct evolutionary trajectory compared with DENV-2 genotype III (Figure 3, panel A; Appendix 1 Figure 3), supporting the hypothesis of recent introduction followed by rapid establishment in Costa Rica. The time-stamped phylogenetic tree indicated that $\geq$2 independent introductions of the DENV-2 genotype II F.1.1.2 lineage likely occurred, potentially mediated by regional viral flow from countries in Latin America, including Bolivia and Brazil, and resulted in establishment of a well-supported monophyletic clade. Bayesian time-scaled phylogenetic analysis of that clade suggests emergence around October 2023, with a 95% HPD interval spanning from September to late November 2023. Early circulation was primarily concentrated in Puntarenas, Limón, and Cartago, then subsequently disseminated into San José, Alajuela, and Heredia (Figure 3, panel B). Reconstruction of viral dispersal for that major clade further highlighted its rapid establishment across densely populated areas (Figure 3, panel C). Initially detected in coastal and central provinces, the virus quickly spread into high-transmission hubs, particularly those characterized by high population density.

Conclusions

We used nationwide DENV genomic data and a suitability index to conduct an eco-epidemiologic assessment of dengue in Costa Rica. We documented replacement of DENV-2 Asian–American genotype by DENV-2 cosmopolitan genotype. Using sequencing, phylodynamics, and climate modeling, we showed how viral introductions, ecologic factors, and human mobility shaped transmission. Unlike other settings where genotype shifts were driven by immunity or fitness (*6*–*8*), we found no evidence of climate- or age-related increases. DENV-2 II, detected in early 2024, rapidly replaced DENV-2 genotype III despite declining circulation and did not show increased severity or deaths. At least 2 introduction events seeded widespread dissemination, consistent with patterns in Brazil and Southeast Asia (*9*,*10*). Now globally dominant, the cosmopolitan genotype has also been reported in Peru, Brazil, and Colombia (*3*,*4*,*11*). Its moderate correlation with climate suitability (r = 0.38) (*2*,*12*) and spread into urban centers (*13*–*15*) highlight how ecologic and mobility factors can amplify transmission. Those findings underscore the urgent need for real-time genomic surveillance integrated with environmental and mobility data to strengthen early dengue detection and targeted interventions.

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Author contributions: M.G.-E., M.G., J.L., and L.F. conceptualized and designed the study; M.G.-E., M.G., J.L., E.C.-L., C.S.-G., D.P.-S., L.A., V.F., F.D.-M., J.M., and L.F. conducted investigations; M.G., J.L., M.G.-E., and E.C.-L. performed data analysis; M.G. and J.L. created visualizations; M.G.-E., M.G., J.L., and L.F. wrote the first draft; and M.G., M.G.-E., E.C.-L., C.S.-G., D.P.-S., and J.L. revised and finalized the manuscript.

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Appendix 1

Material and Methods

Ethics Statement

The Pan American Health Organization Ethics Review Committee (PAHOERC) reviewed and approved this project (Ref. No. PAHO-2024–08–0029). The samples used in this study were analyzed as part of the routine epidemiologic surveillance of arboviruses at the Virology National Reference Center (CNRV, in Spanish) at Inciensa, which is the official laboratory of the Costa Rican Ministry of Health.

Sample Collection and Whole-Genome Sequencing

Samples were collected from patients exhibiting clinical symptoms consistent with dengue virus infection. Nucleic acid extraction was performed using the QIAamp Viral RNA Mini Kit (Qiagen). Subsequently, the extracted RNA was subjected to real-time reverse transcription PCR (RT-qPCR) targeting DENV serotypes 1–4, as previously described (*1*). Samples that tested positive (n = 133) and presented a cycle threshold (Ct) value of ≤30 were selected for whole-genome amplification using the CDC Next Generation Sequencing Protocol for DENV 1–4 with the Illumina MiSeq platform (*2*). This protocol, developed by the CDC, was transferred to the Arbovirus Diagnosis Laboratory Network of the Americas (RELDA) through the VIGENDA program coordinated by the Pan-American Health Organization (PAHO). Library preparation was carried out using the COVIDseq Kit (Illumina, San Diego, USA), originally developed for SARS-CoV-2 genomic studies but subsequently adapted for other viral targets (*3*,*4*). Sequencing was then conducted on the Illumina MiSeq platform (Illumina, San Diego, USA), following the manufacturer's recommendations, using V2 cartridges with a 2 × 150 cycles paired-end run. Adaptor trimming and quality filtering of the raw reads were performed using Trim-Galore v0.6.5 (*5*) with default parameters for Illumina paired-end reads. Processed reads were mapped to the reference genome using BWA-MEM v0.7.17–4 (*6*) with default settings. The reference genome used for alignment was the serotype-specific DENV2_Reference_Genome_PR1994, provided by the CDC's Dengue Branch in Puerto Rico. Primer sequences were removed using iVar v1.3.1 (*7*). Consensus sequences were then generated from the aligned reads using Samtools v1.10–13 (*8*) and iVar consensus v1.3.1, applying a minimum coverage depth threshold of 10 reads and the default frequency threshold for consensus base calling. Initial genotyping was performed using the Flavivirus Genotyping Tool (Version 0.1; https://www.rivm.nl/mpf/typingtool/flavivirus/). Subsequently, lineage classification was conducted using either the Genome Detective Dengue Typing Tool or Nextclade (*9*,*10*), employing the DENV-2 reference dataset for lineage determination.

Phylogenetic and Phylodynamic Inferences

We constructed phylogenetic trees to investigate the evolutionary relationship of sequenced DENV2 genomes from Costa Rica (DENV2-III n = 110 and DENV2-II n = 23) in comparison to globally sampled sequences (n = 654 and n = 2213 respectively). Sequence alignment was performed using MAFFT (*11*) and manually curated in AliView (*12*). Preliminary maximum likelihood phylogenies were reconstructed using IQ-TREE 2 under the HKY+G4 substitution model (*13*). Time-scaled phylogenies were inferred with TreeTime (*14*), while Bayesian phylogenetic analyses were conducted using BEAST (*15*). To ensure the robustness of the temporal framework, TempEst (*16*) was employed to assess the presence of a temporal signal. For Bayesian inference, we implemented a rigorous model selection approach, using both path-sampling (PS) and stepping-stone (SS) methods to identify the most appropriate molecular clock model (*17*). The uncorrelated relaxed molecular clock was selected for all datasets based on marginal likelihood estimation, in combination with the codon-based SRD06 nt substitution model and the Bayesian Skyline coalescent model. To reconstruct the geographic dissemination of the identified 2022–2023 transmission clade, we employed a relaxed random walk diffusion model (*18*,*19*), which accommodates heterogeneity in dispersal rates among branches, incorporating a Cauchy distribution and a jitter window site of 0.01 (*20*). Each sequence was georeferenced with latitude and longitude coordinates to enable spatiotemporal analyses.

Bayesian phylogenetic inference was performed using BEAST v1.10.4, with two independent Markov Chain Monte Carlo chains (MCMC) runs of 50 million interactions, sampling every 10,000 steps. Convergence was assessed in Tracer, ensuring effective sample size (ESS) >200 for all key parameters. Maximum clade credibility (MCC) trees were summarized using TreeAnnotator after discarding the initial 10% os samples as burn-in. Finally, we employed the R package 'seraphim' version 1.0 (*21*) to extract and visualize spatiotemporal patterns embedded within the posterior tree distribution.

Eco-epidemiologic modeling

The epidemiologic data on weekly confirmed cases of DENV in Costa Rica, from 2013 to 2024, were obtained from the CNRV and aggregated at a monthly level. We calculated the theoretical climate-based transmission potential of the dengue virus using the following mathematical expression (index P), where u stands for humidity and t for temperature:

$$P_{(u,t)} = \frac{{a^{v}_{(u)}}^{2} \phi^{v\to h}_{(t)} \phi^{h\to v}_{(t)} \gamma^{v}_{(t)} \gamma^{h}}{\mu^{v}_{(u,t)} (\sigma^{h}+\mu^{h})(\gamma^{h}+\mu^{v}_{(u,t)})} \; (22)$$

Briefly, the index uses mathematical expressions of empirically demonstrated relationships between DENV and *Ae. aegypti* traits and meteorological variables. Climate-dependent traits include the extrinsic incubation period ($\gamma^{v}_{(t)}$), adult mosquito lifespan ($\mu^{v}_{(u,t)}$), adult mosquito biting rate ($a^{v}_{(u)}$transmission probability per mosquito bite from infected human to susceptible mosquito ($\phi^{h\to v}_{(t)}$) and from infected mosquito to susceptible human ($\phi^{v\to h}_{(t)}$). Traits that are climate-independent include intrinsic incubation period (γ^{h}), human lifespan (μ^{h}) and human infectious period (σ^{h}), which are calibrated from published reports. Full methodological details, technical validation and parameterization of Index P can be found in Nakase et al (*22*). Monthly climate data for Costa Rica was obtained from Copernicus.eu satellite climate data (*23*).

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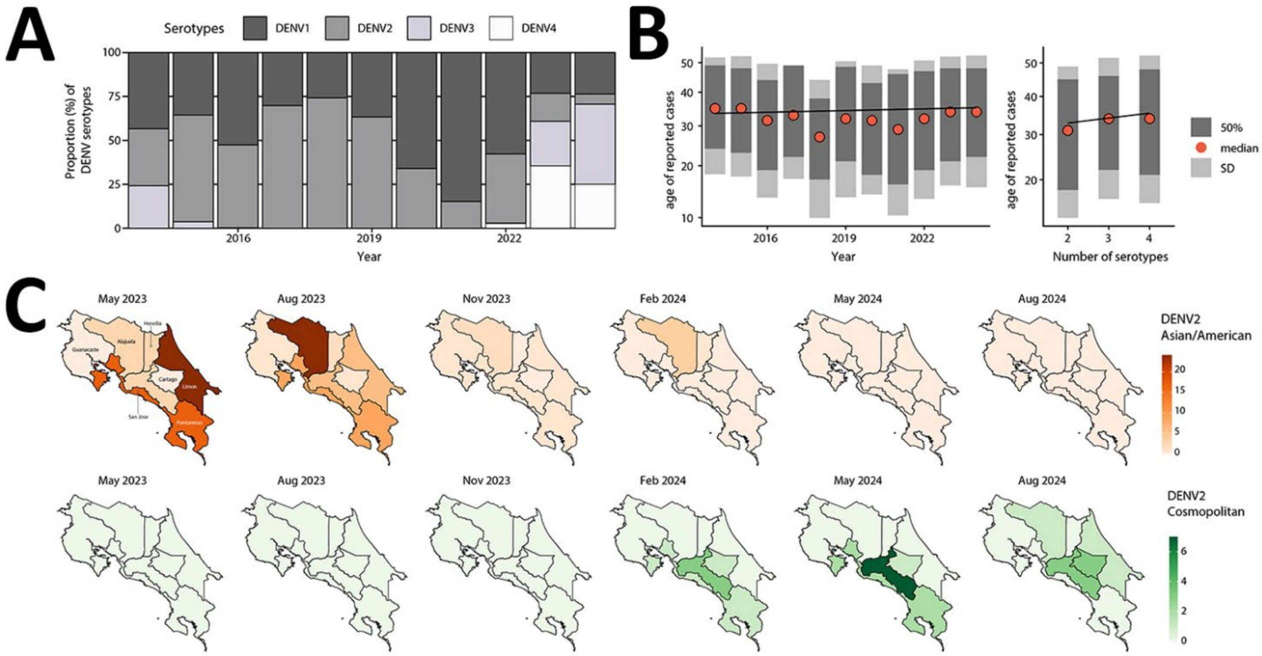
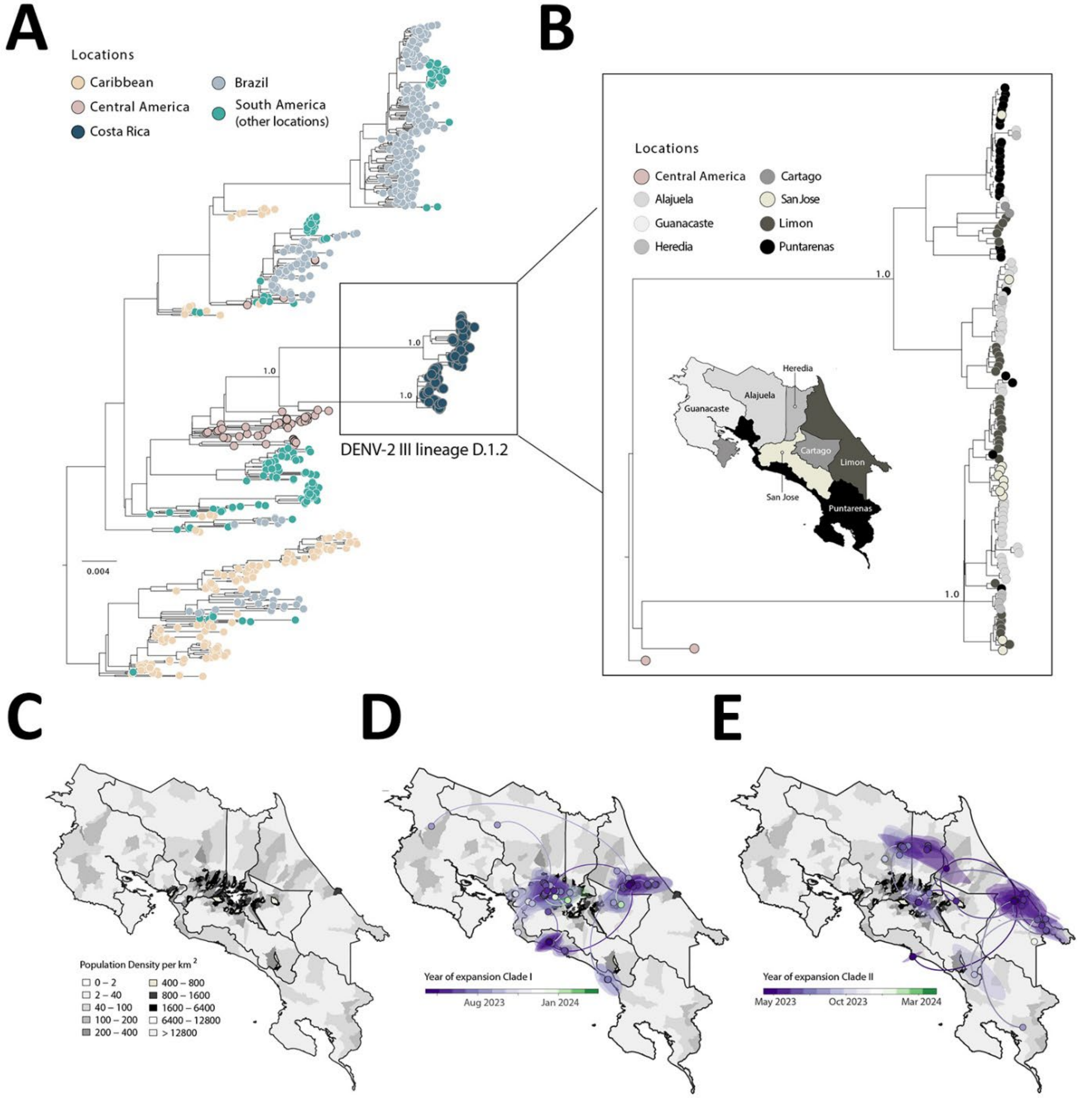
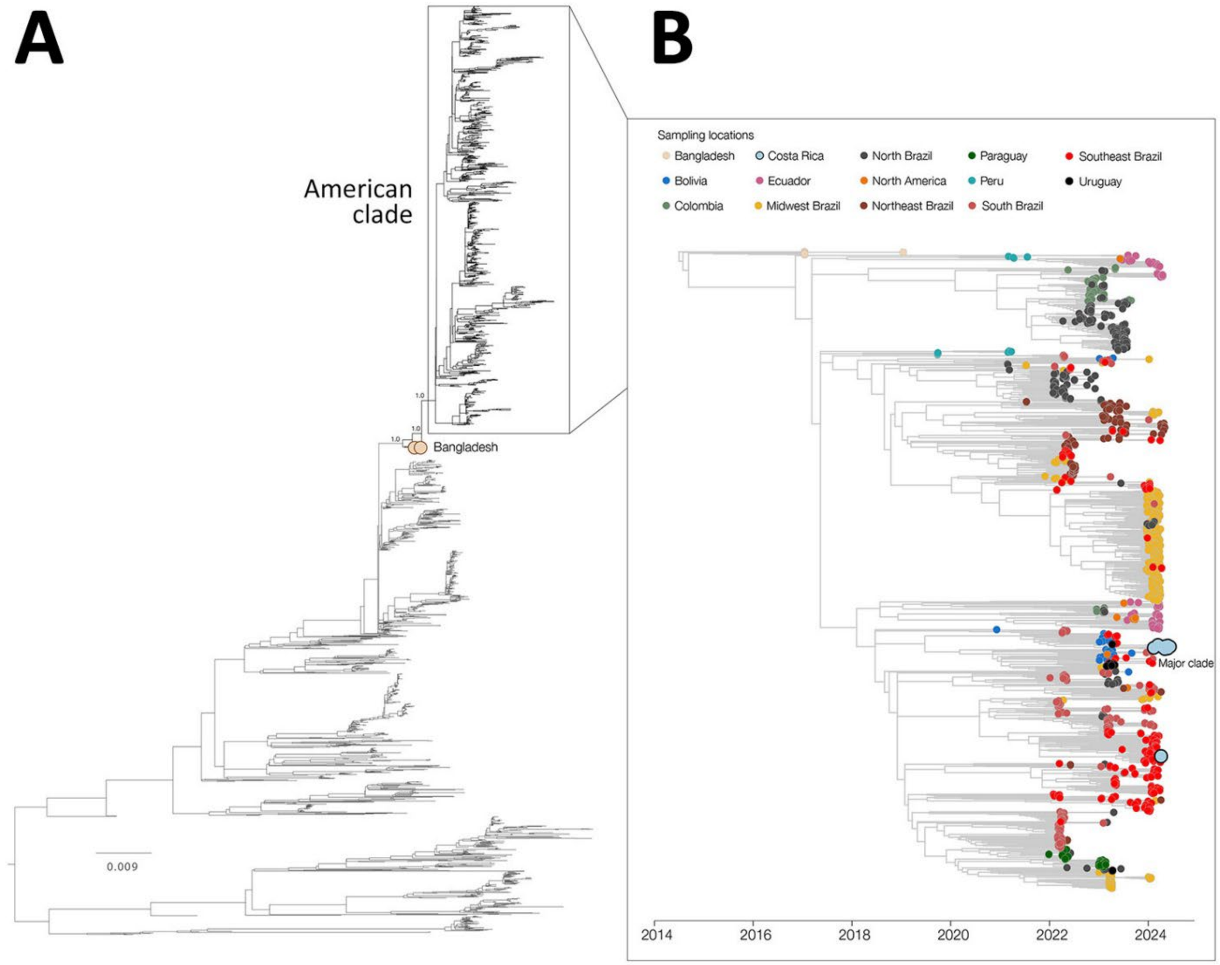


Appendix 1 Figure 1. Dengue serotype dynamics in Costa Rica. A) Proportional distribution of DENV serotypes over time; B) Age of dengue cases over time (left) and by number of circulating serotypes (right). Median (red), interquartile range (dark gray), and standard deviation (light gray) are shown; C) Geographic distribution of DENV2 genotypes in Costa Rica from May 2023 to August 2024.



Appendix 1 Figure 2. Evolutionary and spatiotemporal expansion of DENV-2 III in Costa Rica. A) Maximum likelihood (ML) phylogeny of DENV-2 III, showing Costa Rican sequences (blue) within the broader Central and South American clade. Phylogenetic support is indicated at key nodes. B) ML reconstruction of DENV-2 III lineage D.1.2 dispersal within Costa Rica; C) Population density map of Costa Rica, highlighting areas with elevated transmission potential. D, E) Spatiotemporal expansion of DENV-2 III lineage D.1.2, illustrating the progressive spread of two distinct clades—Clade I and II—from urban centers to coastal regions between May 2023 and March 2024.



Appendix 1 Figure 3. Emergence and spread of DENV-2 II in Costa Rica. A) Maximum-likelihood phylogeny of DENV-2 II, highlighting the American clade. B) Time-stamped phylogenetic tree, showing 2 independent introductions of this genotype into Costa Rica, including 1 major transmission cluster. Tips are colored according to sampling location. Scale bar indicates nucleotide substitutions per site. DENV, dengue virus.