BMC Infectious Diseases

RESEARCH

Open Access

North–south pathways, emerging variants, and high climate suitability characterize the recent spread of dengue virus serotypes 2 and 3 in the Dominican Republic

Isaac Miguel[1†], Edwin P. Feliz[1†], Robinson Agramonte[1], Pedro V. Martinez[1], Carlos Vergara[1], Yvonne Imbert[1], Lucia De la Cruz[1], Nurys de Castro[1], Odalis Cedano[1], Yamilka De la Paz[1], Vagner Fonseca[2], Gilberto A. Santiago[3], Jorge L. Muñoz-Jordán[3], Armando Peguero[4], Robert Paulino-Ramírez[4], Nathan D. Grubaugh[5,6,7,8], Ana Maria Bispo de Filippis[9], Luiz Carlos Junior Alcantara[10], Jairo Mendez Rico[11†], José Lourenço[12*†], Leticia Franco[11†] and Marta Giovanetti[13,14*†]

Abstract

Background Dengue fever remains a significant public health challenge in tropical and subtropical regions, with its transmission dynamics being influenced by both environmental factors and human mobility. The Dominican Republic, a biodiversity hotspot in the Caribbean, has experienced recurrent dengue outbreaks, yet detailed understanding of the virus's transmission pathways and the impact of climatic factors remains limited. This study aims to elucidate the recent transmission dynamics of the dengue virus (DENV) in the Dominican Republic, utilizing a combination of genomic sequencing and epidemiological data analysis, alongside an examination of historical climate patterns.

Methods We conducted a comprehensive study involving the genomic sequencing of DENV samples collected from patients across different regions of the Dominican Republic over a two-year period. Phylogenetic analyses were performed to identify the circulation of DENV lineages and to trace transmission pathways. Epidemiological data were integrated to analyze trends in dengue incidence and distribution. Additionally, we integrated historical climate data spanning several decades to assess trends in temperature and their potential impact on DENV transmission potential.

Results Our results highlight a previously unknown north–south transmission pathway within the country, with the co-circulation of multiple virus lineages. Additionally, we examine the historical climate data, revealing long-term trends towards higher theoretical potential for dengue transmission due to rising temperatures.

[†]Isaac Miguel and Edwin P. Feliz contributed equally to this work.

[†]Jairo Mendez Rico, José Lourenço, Leticia Franco and Marta Giovanetti are co-senior authors.

*Correspondence:
José Lourenço
lourenco.jml@gmail.com
Marta Giovanetti
giovanetti.marta@gmail.com
Full list of author information is available at the end of the article

Conclusion This multidisciplinary study reveals intricate patterns of dengue virus transmission in the Dominican Republic, characterized by the co-circulation of multiple DENV lineages and a novel transmission pathway. The observed correlation between rising temperatures and increased dengue transmission potential emphasizes the need for integrated climate-informed strategies in dengue control efforts. Our findings offer critical insights for public health authorities in the Dominican Republic and similar settings, guiding resource allocation and the development of preparedness strategies to mitigate the impacts of climate change on dengue transmission.

Keywords Dengue virus, Genomic epidemiology, Dominican Republic, Caribbean

Background

Dengue fever, caused by dengue virus serotypes 1–4 (DENV-1–4), represents a significant public health challenge in tropical and subtropical regions [1]. A vector-borne disease predominantly transmitted by *Aedes aegypti* mosquitoes, annually affects millions with symptoms ranging from mild fever to severe conditions like dengue hemorrhagic fever and dengue shock syndrome [1]. In the past 3 years, dengue activity has surged in the Americas. Approximately 3.4 million cases were reported in 2023, exceeding the 2.8 million total for 2022 [2]. Situated in the Caribbean basin, the Dominican Republic has been frequently grappled with dengue outbreaks over the past two decades. The high incidence of dengue in this country is thought to impact the overall epidemiology of dengue in the region.

In 2023, 28,078 cases have been reported by the country's surveillance system which captures physicians' reporting of dengue cases [2]. Though this surveillance system does not provide a complete picture of dengue transmission, it allows health authorities to maintain awareness of, and response to, dengue transmission throughout the country. In fact, the number of RT-PCR positive samples normally obtained per year, and exceeding 7,000 in 2023, is sufficiently high to provide an opportunity to further characterize transmission using genomic surveillance approaches or through additional analysis of related epidemiological data. In this regard, we implemented a genome-based surveillance approach in partnership with the Pan-American Health Organization and the Central Public Health Laboratory in Santo Domingo to sequence and analyze DENV whole genome sequences from the 2023 epidemic. We aimed and identifying existing lineages within the context of recent emergence of novel DENV-2 and -3 variants in the Americas [3]. In addition, by integrating genomic, epidemiological, and climatic data, we provide a historical overview of the epidemiological trajectory of DENV in the Dominican Republic. This comprehensive analysis lays the groundwork for establishing a genomic surveillance model in the Caribbean, with potential to monitor and respond to dengue transmission in the region.

Material and methods

Sample collection and whole genome sequencing

A total of 85 samples were obtained from patients exhibiting clinical symptoms consistent with dengue viral infection. All cases were classified as mild, with the most common symptoms being fever and cutaneous rash. These samples were sent to the Laboratorio Central de Salud Pública of the Dominican Republic in Santo Domingo for genomic sequencing. Initially, nucleic acid extraction was performed using the QIAamp Viral RNA Mini Kit (Qiagen). Subsequently, real-time reverse transcription PCR (RT-qPCR) for DENV 1–4 was conducted on the samples, following previously described methods [4]. Samples that tested positive ($n=85$) with a cycle threshold (Ct) value of ≤ 36 underwent whole genome amplification using a set of tiled primers described elsewhere [5, 6]. The resulting DNA amplicons were purified using AMPure XP beads (Beckman Coulter, Brea, USA). Library preparation of the purified amplicons was carried out using the COVIDseq Kit (Illumina, San Diego, USA), originally designed for SARS-CoV-2 genomic research but adapted for other viruses [7, 8]. Subsequent sequencing was performed using the Illumina MiSeq platform (Illumina, San Diego, USA), following the manufacturer's recommended protocols. Consensus sequence generation was conducted using the ViralUnity pipeline (available at https://github.com/filiperomero2/ViralUnity) and typing assignment of dengue virus sequences was accomplished using the dengue virus typing tool available at https://www.genomedetective.com/app/typingtool/dengue/ [9].

Phylogenetic and phylodynamic inferences

We generated phylogenetic trees to investigate the relationship between the sequenced genomes from the Dominican Republic and those from other regions worldwide. Alignment of all sequences was performed using MAFFT [10] and manually adjusted using AliView [11]. Initial maximum likelihood phylogenies were constructed using IQ-TREE 2 software with the HKY + G4 substitution model [12]. Time-scaled trees were inferred using TreeTime [13] and BEAST software [14], preceded by TempEst [15] analysis to assess temporal signal. A

rigorous model selection process, employing path-sampling (PS) and steppingstone (SS) procedures, was utilized to determine the optimal molecular clock model for Bayesian phylogenetic analysis [16]. The uncorrelated relaxed molecular clock model was chosen based on estimation of marginal likelihoods, incorporating the codon-based SRD06 model of nucleotide substitution and the nonparametric Bayesian Skyline coalescent model. To simulate the geographic spread of the identified 2022–2023 transmission clade, we utilized a flexible relaxed random walk diffusion model [17, 18], accounting for variation in dispersal rates across branches with a Cauchy distribution and a jitter window site of 0.01 [19, 20]. Each sequence was assigned geographic coordinates of latitude and longitude. Bayesian phylogenetic inference analyses were performed in BEAST v1.10.4, with two runs of 50 million Markov Chain Monte Carlo chains (MCMC) each, and samples were taken every 10,000 steps in the chain. Convergence was assessed using Tracer, ensuring effective sample size for all significant model parameters was greater than 200. The maximum clade credibility (MCC) trees for each run were summarized using TreeAnnotator after discarding the initial 10% as burn-in. Finally, the R package 'seraphim' version 1.0 [20] was used to extract and visualize spatiotemporal data contained within the posterior trees.

Eco-epidemiological modelling

Weekly reported cases of DENV in the Dominican Republic from 2013 to 2023 were gathered and structured from the PAHO database for dengue [21]. Notified infections were defined as dengue cases where a diagnostic test was performed and yielded a positive result, as per the PAHO platform criteria. The theoretical suitability of climate-driven dengue virus transmission was assessed using the mathematical expression of index P, incorporating humidity (u) and temperature (t) variables [21]:

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

Briefly, the index utilizes mathematical formulations based on empirically established connections between DENV and Ae. aegypti characteristics alongside meteorological factors. Climate-dependent characteristics encompass the extrinsic incubation period (⟦γ⟧ ((t))^v), adult mosquito lifespan (⟦μ⟧ ((u,t))^v), adult mosquito biting rate (⟦a⟧ ((u))^v), transmission probability per mosquito bite from infected human to susceptible mosquito (⟦ϕ⟧ ((t))^(h→v)), and from infected mosquito to susceptible human (⟦ϕ⟧ _((t))^(v→h)). Climate-independent traits include intrinsic incubation period (⟦γ⟧ ^h), human lifespan (⟦μ⟧ ^h), and human infectious period (⟦σ⟧ ^h). Comprehensive methodological details and technical validation of Index P can be found in Nakase et al. [22, 23]. Monthly climate data for the Dominican Republic was sourced from Copernicus.eu satellite climate data [24]. Temperature data summary was derived by calculating yearly lowest, mean, and maximum values.

Results

A total of 85 complete genomes were obtained from 100 PCR positive samples with sufficient DNA (2 ng/L) for library preparation. The average cycle threshold (Ct) value for PCR was 24.6, with values ranging between 12.6 and 36 (Table S1). The ages of the patients sampled ranged from 2 to 48 years, with a median age of 17 years. Of these patients, 53% ($n=45$) were male (Table S1), and all cases examined were classified as autochthonous. The sequencing procedure yielded an average coverage of 84%, ranging from 60 to 99% (Table S1). This allowed for the identification of the DENV-2 genotype III variant ($n=29$) and the DENV-3 genotype III ($n=56$) variant. Genome sequences were obtained from all three macroregions of the Dominican Republic, which are further segmented into ten specific regions (Fig. 1A).

The distribution of the DENV-2-III and DENV-3-III genotypes reflected current DENV transmission patterns. DENV-2-III has been circulating predominantly in the north-central parts of the country since 2014. In contrast, DENV-3-III, recently introduced through population-dense areas, was found in both the north-central and southeastern regions (Fig. 1A).

While climate-based suitability for DENV transmission presented clear, yearly oscillations demarking time periods with potential for dengue activity, notified DENV cases (between 2014 and 2023) revealed only three waves, of cases (defined as increases in the number of cases above the historical median) occurring in 2015–2016, 2019–2020 and 2021–2022 (Fig. 1B). The reasons why transmission is low in some years are unclear, though we can consider a mix of local factors including accumulated herd-immunity to specific serotypes, yearly changes in mosquito populations not captured by the climate-based suitability measure, and population cross-immunity post Zika virus emergence after 2016 [25–28]. Nonetheless, during the three epidemic waves, Pearson's correlation between cases and suitability was high, at 0.88 for 2015–2016, 0.76 for 2019–2020 and 0.93 for 2021–2022. Together, these results suggest that, although climate suitability alone is insufficient to promote waves of DENV transmission, local temperature variation tends to be highly associated.

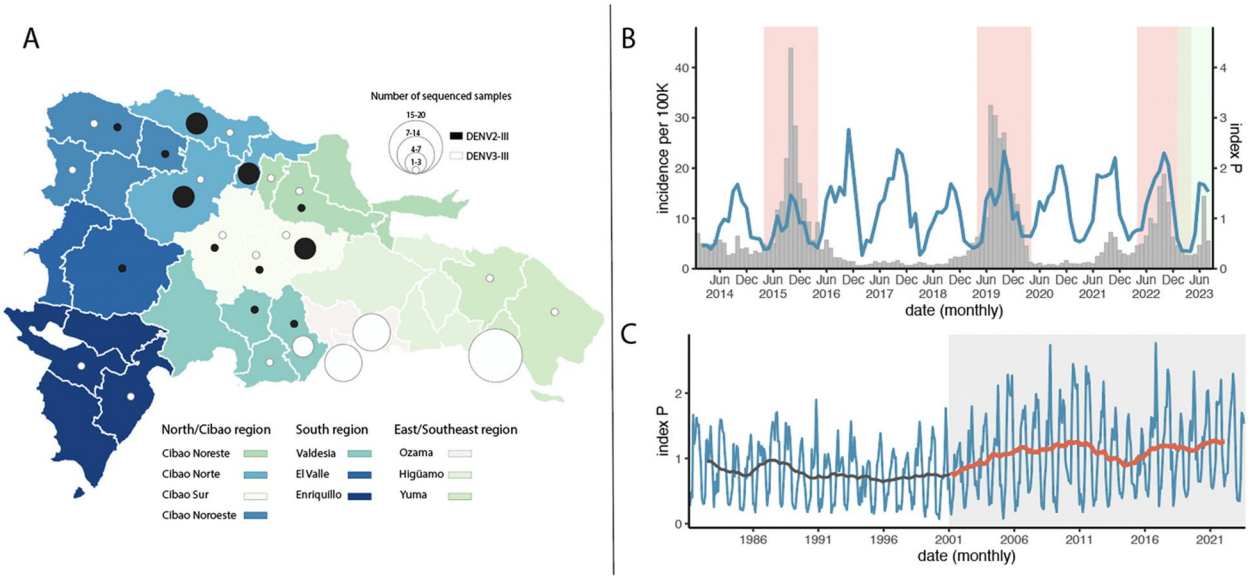

Fig. 1 Dynamics of DENV-2 and DENV-3 in the Dominican Republic. **A** Map of the Dominican Republic (DR) depicting the sampling of new DENV-2 and DENV-3 genome sequences by region and district. The color and size of the circles represent the number of new genomes generated in this study: black for DENV-2 and white for DENV-3. **B** Time series of dengue cases reported monthly in Dominican Republic, presented as incidence per 100,000 inhabitants (gray bars) versus the climate-based suitability (index P, blue line) for the period data where available. The shaded areas in pink mark visually identifiable waves of cases (between April of consecutive years, see main text) and green marks the year 2023 from which all genomic samples were obtained. **C** Long-term time series of monthly climate-based suitability (index P, 1981–2023, blue line). The moving average of suitability (plus-minus 18 months) is presented in black before the year 2001 and in red thereafter (the shaded gray area highlights this time period)

We also explored the historical trends in climate-based suitability in the Dominican Republic (over the past 40 years, Fig. 1C). This study revealed recent trends towards higher suitability for DENV transmission, particularly after the turn of the century, which derives from the emergence of much higher seasonal peaks of suitability; before 2001, the average yearly peak of suitability was 1.42 (min 0.95; max 1.89), while after 2001, the average peak was 2.08 (min 1.45; max 2.76). However, the time period analyzed is too short for a statistical assessment of significance. Local climate change, driven by rises in temperature, is theoretically favoring the transmission of DENV in the Dominican Republic, as in other countries of the region.

To understand the phylogenetic history of DENV-2-III, we combined our recently sequenced samples ($n=29$) from 2023 with an additional set of 14 DENV-2-III viral genomes that were sequenced in the Dominican Republic in 2022 [29]. This set included samples collected from the Southern, Eastern regions, and the National District (Santo Domingo) [29]. These sequences were then combined with other sequences of the same genotype ($n=647$) retrieved from GenBank for context. Our analysis revealed that the new genomes from this study cluster with sequences recently obtained by others [29] and form a distinct monophyletic clade with robust statistical support that is basal to the DENV-2-III BR4 variant that emerged in 2019 in Brazil (Fig. 2) [4].

Additionally, we reconstructed the viral movements of this clade ($n=43$) within the different regions in the Dominican Republic. We estimate that the mean time of origin of this variant was early-January 2014 with a 95% highest posterior density (HPD), ranging from mid-June 2013 to late-January 2014. These results suggest that the 2023 epidemic may have not been caused by a novel introduction but could be the result of continual transmission within the Dominican Republic of a viral strain that was introduced in the region in early 2014. This variant spread from the southern part of the country (Ozama region) toward the southeastern, northern and midwestern, as demonstrated in Fig. 2.

To explore the phylogenetic history of DENV-3-III in the Dominican Republic, we combined our new genome dataset ($n=56$) with 1,760 sequences from GenBank for global context. Our analysis revealed that the new genomes group within the recently reported American lineage II of genotype III; however, our sequences formed a distinct monophyletic clade that separates from the main lineage, which includes viral sequences recently identified from other Caribbean and Latin American countries, such as Cuba, Puerto Rico, Brazil, Suriname, and as well as in North America (specifically Florida)

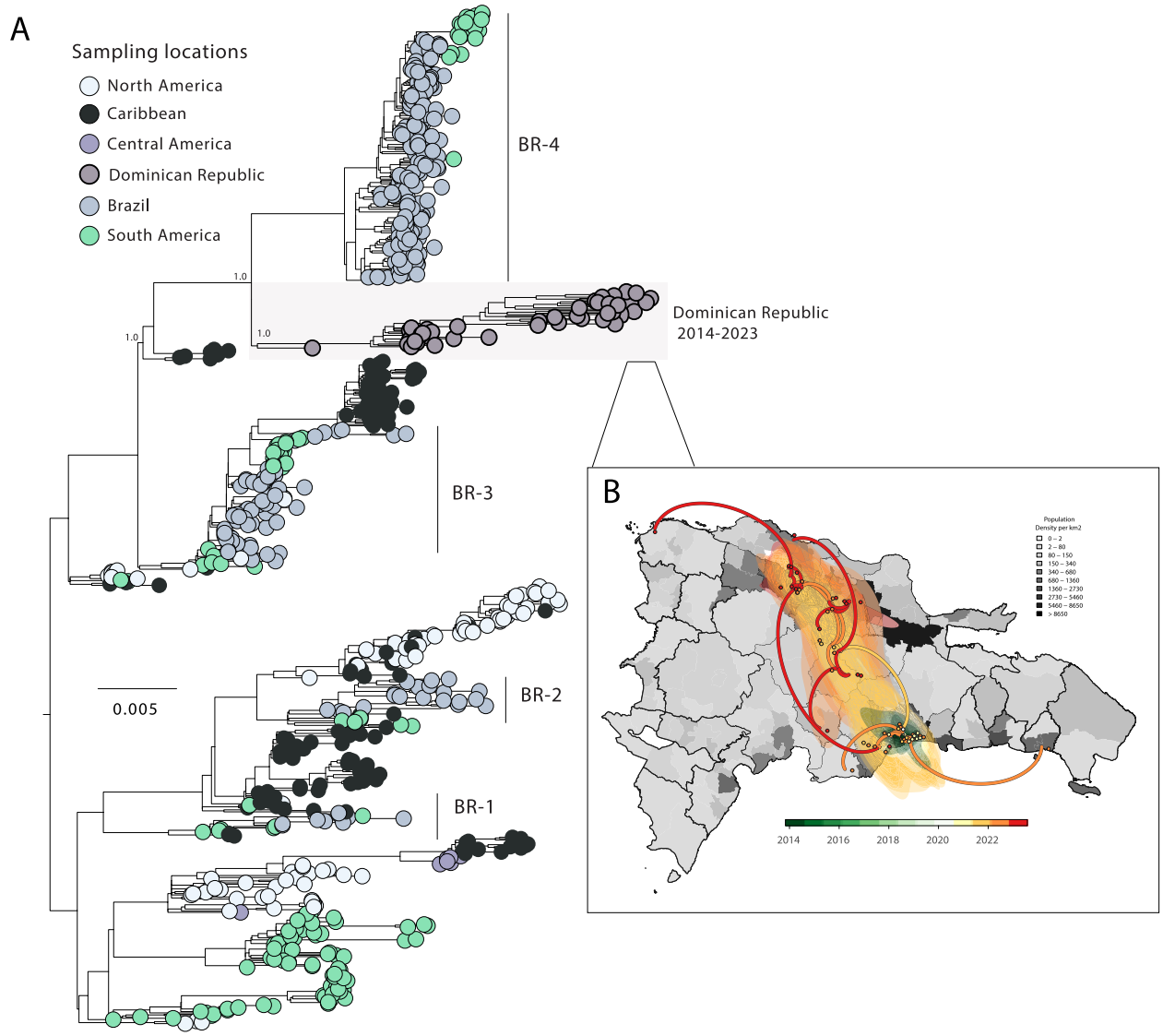

Fig. 2 Dispersion dynamics of DENV-2-III in the Dominican Republic. **A** Maximum likelihood (ML) phylogenetic analysis of 29 new complete genome sequences of DENV-2-III generated in this study combined with 661 sequences from GenBank. The scale bar represents units of nucleotide substitutions per site (s/s) and the tree is mid-pointed rooted. Colors represent geographic sampling locations. **B** The highlight on the right (panel B) shows the phylogeographic reconstruction of the Dominican Republic Clade ($n=43$). Solid curved lines denote the links between nodes and the directionality of movement. Circles represent nodes of the MCC phylogeny and are colored according to their inferred time of occurrence. Shaded areas represent the 80% highest posterior density interval and depict the uncertainty of the phylogeographic estimates for each node

[3, 29]. These results propose a complex transmission scenario, with introduction events likely mediated by trans-continental travel and underscores the significant influence of human movement in facilitating the spread and introduction of viral lineages to new regions [8] (Fig. 3).

We investigated the spatial–temporal dynamics of the Dominican Republic DENV-3-III variant in more detail using a smaller data set ($n=56$) derived from the American lineage II. Phylogeographic analyses allowed the reconstruction of viral movements across different regions in the Dominican Republic (Fig. 3) and suggested a mean time of origin in early April 2023 (95% highest posterior density (HPD): 2 to 27 April 2023). This variant spread from the south of the country (Yuma region) towards the north and later to the southern part of the country, as indicated by isolates from the Cibao Noroeste, El Valle, and Enriquillo regions (Fig. 3).

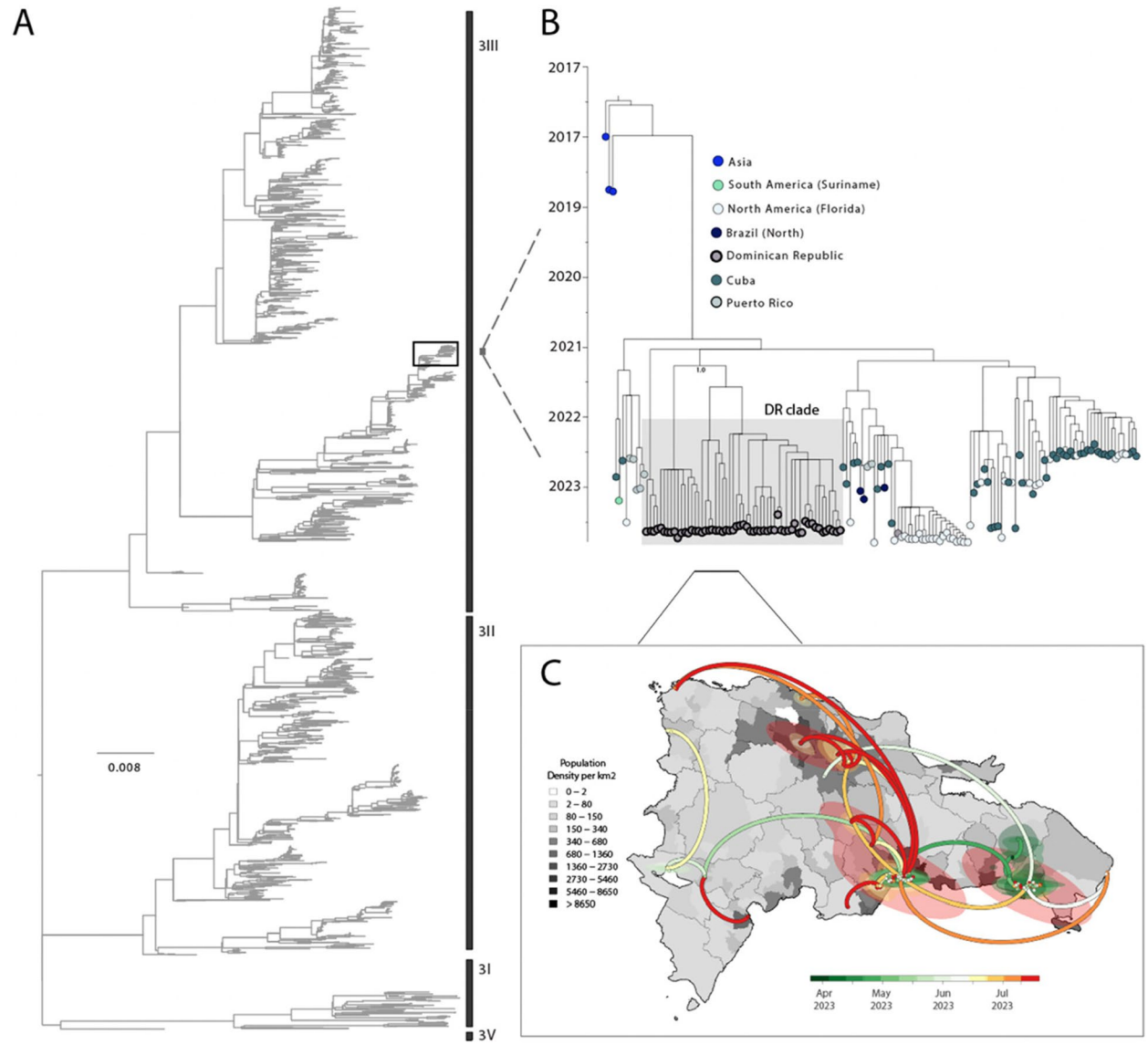

Fig. 3 Dispersion dynamics of DENV-3-III in the Dominican Republic. **A** Maximum Likelihood (ML) phylogenetic analysis of 56 new complete genome sequences of the DENV-3-III genotype from this study, in addition with 1,760 sequences from GenBank representing all different DENV-3 genotypes. The scale bar represents units of nucleotide substitutions per site (s/s) and the tree is mid-pointed rooted; **B** Maximum clade credibility tree (MCC) of the DENV-3-III emerging American lineage II from 2022–2023, including $n = 56$ complete genome sequences from the Dominican Republic from this study combined with 168 additional genomes for context. Sequences are colored according to sampling location. **C** Phylogeographic reconstruction of the Dominican Republic Clade ($n = 56$). Circles represent nodes of the MCC phylogeny and are colored according to their inferred time of occurrence. Shaded areas represent the 80% highest posterior density interval and depict the uncertainty of the phylogeographic estimates for each node. Solid curved lines denote the links between nodes and the directionality of movement

Conclusion

In conclusion, our genome-based surveillance system, integrated with other methodologies, provided a nuanced view of the recent dengue epidemiology in the Dominican Republic. By elucidating a key transmission pathway from the South to the North for both genotypes, identifying co-circulation of different DENV serotypes and genotypes, and highlighting the cumulative effect that local climate change appears to be having on the transmission potential of DENV, we underscore the importance of advanced monitoring techniques. The identification of the South as point of entry for viral variants requires further study, in particular given the presence of likely introduction points (international airports and cruise ports) being present both in the North and the South. Seroprevalence studies are also urgently needed to characterize

the local immunity landscape against different serotypes in search for a better understanding of the actual serotype spatial distribution in the island, and its possible impact on regional public health in future serotype switching events. Only through active tracking of viral evolution and spread can we enable timely interventions, ensuring communities are fortified against the persistent threat of dengue and fostering adaptability in response to the changing landscape of infectious diseases.

Limitations

In this study, we elucidate the recent transmission dynamics of the dengue virus in the Dominican Republic by combining genomic sequencing, epidemiological data analysis, and an examination of historical climate patterns. However, the scarcity of complete DENV genome sequences in Latin America limits our ability to characterize the molecular epidemiology of viral strains at a regional level, highlighting the importance of increasing sequencing efforts to improve real-time data generation, sharing, and representativeness. Despite this limitation, our phylodynamic analysis offers valuable insights into the dynamics of DENV in the country, providing a foundation for better-informed public health strategies and interventions.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-024-09658-6.

Supplementary Material 1.

Acknowledgements

This study was supported by PAHO, USAID, Dominican Republic Public Health Ministry, (funding for the genomics department of Defillo national Laboratory) and in part by the National Institutes of Health (NIH) USA grant U01 AI151698 for the United World Arbovirus Research Network (UWARN) and the CRP-ICGEB RESEARCH GRANT 2020 Project CRP/BRA20-03, Contract CRP/20/03. M. Giovanetti's funding is provided by PON "Ricerca e Innovazione " 2014-2020. The authors would also like to acknowledge the Global Consortium to Identify and Control Epidemics – CLIMADE (T.O., L.C.J.A., E.C.H., J.L., M.G.) (https://climade.health/) and National Institute of Allergy and Infectious Diseases of the NIH Award Number DP2AI176740 (NDG). The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the CDC and NIH.

Authors' contributions

Conception and design: I.M., J.L., and M.G.; Investigations: I.M., E.F., R.A., P.V.M., C.V., Y.I., L.d.l.C., N.d.C., O.C., Y.D.I.P., V.F., G.A.S., J.L.M.J., R.P.R., N.D.G., A.M.B.d.F., L.C.J.A., J.M.R., J.L., L.F., M.G.; Data Analysis: I.M., E.F., V.F., J.L., and M.G; Visualization: V.F., J.L., and M.G; Writing – Original: J.L., and M.G.; Revision: I.M., E.F., R.A., P.V.M., C.V., Y.I., L.d.l.C., N.d.C., O.C., Y.D.I.P., V.F., G.A.S., J.L.M.J., R.P.R., N.D.G., A.M.B.d.F., L.C.J.A., J.M.R., J.L., L.F., M.G.; Resources: I.M., R.P.R., N.D.G., J.M.R., and L.F.

Funding

This study received support from various organizations and institutions. Specifically, funding was provided by the Pan American Health Organization (PAHO), the United States Agency for International Development (USAID), and the Dominican Republic Public Health Ministry under the supervision of Isaac Miguel. Furthermore, the National Institutes of Health (NIH) in the United States contributed through grant U01 AI151698 for the United World Arbovirus Research Network (UWARN), under the supervision of Luiz Carlos Junior Alcantara. Moreover, support was also received from the CRP-ICGEB RESEARCH GRANT 2020 Project CRP/BRA20-03, Contract CRP/20/03, overseen by Marta Giovanetti. Marta Giovanetti's funding is provided by PON "Ricerca e Innovazione" 2014–2020.

Availability of data and materials

Newly generated DENV-2 and DENV-3 sequences have been deposited in GenBank under accession number OR616454-OR616538 (Table S1).

Declarations

Ethics approval and consent to participate

The Pan American Health Organization Ethics Review Committee (PAHOERC) thoroughly reviewed and approved our project (IRB Ref. No. PAHO-2016–08-0029). The samples utilized were de-identified residual samples obtained from routine arbovirus diagnoses conducted at the public health laboratory in the Dominican Republic, which is an integral component of the country's Ministry of Health public network. It's important to note that informed consent was not required as the samples used were residual specimens collected for diagnostic screening at the Reference Laboratory of Health in Santo Domingo. Consequently, the Pan American Health Organization (PAHO) has authorized under the terms of the 510/2016 Resolution the utilization of clinical samples collected at the Central Public Health Laboratory without the need for informed consent. This decision aims to expedite knowledge acquisition and enhance surveillance and response efforts during outbreaks.

Consent for publication

Not applicable.

Competing interests

Conflict of interests Nathan D. Grubaugh (NDG) is a paid consultant for BioNTech. All other authors declare that there are no conflicts of interests.

Author details

[1]Laboratorio Nacional de Referencia en Salud Pública Dr. Defilló, Santo Domingo, Dominican Republic. [2]Department of Exact and Earth Sciences, University of the State of Bahia, Salvador, Brazil. [3]Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, San Juan, Puerto Rico. [4]Universidad Iberoamericana (UNIBE), Instituto de Medicina Tropical & Salud Global, Santo Domingo, Dominican Republic. [5]Department of Epidemiology of Microbial Diseases, Yale School of Public Health, New Haven, Connecticut, USA. [6]Yale Institute for Global Health, Yale University, New Haven, Connecticut, USA. [7]Department of Ecology and Evolutionary Biology, Yale University, New Haven, Connecticut, USA. [8]Public Health Modeling Unit, Yale School of Public Health, New Haven, Connecticut, USA. [9]Laboratório de Arbovírus E Vírus Hemorrágicos, Instituto Oswaldo Cruz, Rio de Janeiro, Rio de Janeiro, Brazil. [10]Instituto Rene Rachou, Fundação Oswaldo Cruz, Belo Horizonte, Minas Gerais, Brazil. [11]Infectious Hazards Management, Health Emergencies Department (PHE), Pan American Health Organization / World Health Organization (PAHO/WHO), Washington, DC, USA. [12]Universidade Católica Portuguesa, Faculdade de Medicina, Biomedical Research Center, Lisbon, Portugal. [13]Department of Sciences and Technologies for Sustainable Development and One Health, Università Campus Bio-Medico diRoma, Rome, Italy. [14]Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil.

Received: 12 March 2024 Accepted: 24 July 2024
Published online: 29 July 2024

References

1. Guzman M, Gubler D, Izquierdo A, et al. Dengue infection. Nat Rev Dis Primers. 2016;2:16055. https://doi.org/10.1038/nrdp.2016.55.
2. Pan American Health Organization (PAHO), DENV Report 2023. https://www3.paho.org/data/index.php/en/mnu-topics/indicadores-dengue-en/dengue-regional-en/315-reg-dengue-incidence-en.html.

3. Naveca FG, Santiago GA, Maito RM, Ribeiro Meneses CA, do Nascimento VA, de Souza VC, do Nascimento FO, Silva D, Mejía M, Gonçalves L, de Figueiredo RMP, Ribeiro Cruz AC, DinizNunes BT, Presibella MM, Quallioarques NF, Riediger IN, de Mendonça MCL, de Bruycker-Nogueira F, Sequeira PC, de Filippis AMB, Resende P, Campos T, Wallau GL, Gräf T, Delatorre E, Kopp E, Morrison A, Muñozordán JL, Bello G. Reemergence of Dengue Virus Serotype 3, Brazil, 2023. Emerg Infect Dis. 2023;29(7):1482–4. https://doi.org/10.3201/eid2907.230595. PMID: 37347884; PMCID: PMC10310385.
4. Santiago GA, Vergne E, Quiles Y, Cosme J, Vazquez J, Medina JF, Medina F, Colón C, Mar-golis H, Muñoz-Jordán JL. Analytical and clinical performance of the CDC real time RT-PCR as-say for detection and typing of dengue virus. PLoS Negl Trop Dis. 2013;7(7):e2311.
5. Adelino TÉR, Giovanetti M, Fonseca V, et al. Field and classroom initiatives for portable sequence-based monitoring of dengue virus in Brazil. Nat Commun. 2021;12:2296. https://doi.org/10.1038/s41467-021-22607-0.
6. Guimarães NR, Tomé LR, Lamounier LO, Silva MF, Lima MT, da Costa AB, et al. Genomic Surveillance of Monkeypox Virus, Minas Gerais, Brazil, 2022. Emerg Infect Dis. 2023;29(6):1270–3. https://doi.org/10.3201/eid2906.230113.
7. Chen NFG, Chaguza C, Gagne L, Doucette M, Smole S, Buzby E, Hall J, Ash S, Harrington R, Cofsky S, Clancy S, Kapsak CJ, Sevinsky J, Libuit K, Park DJ, Hemarajata P, Garrigues JM, Green NM, Sierra-Patev S, Carpenter-Azevedo K, Huard RC, Pearson C, Incekara K, Nishimura C, Huang JP, Gagnon E, Reever E, Razeq J, Muyombwe A, Borges V, Ferreira R, Sobral D, Duarte S, Santos D, Vieira L, Gomes JP, Aquino C, Savino IM, Felton K, Bajwa M, Hayward N, Miller H, Naumann A, Allman R, Greer N, Fall A, Mostafa HH, McHugh MP, Maloney DM, Dewar R, Kenicer J, Parker A, Mathers K, Wild J, Cotton S, Templeton KE, Churchwell G, Lee PA, Pedrosa M, McGruder B, Schmedes S, Plumb MR, Wang X, Barcellos RB, Godinho FMS, Salvato RS, Ceniseros A, Breban MI, Grubaugh ND, Gallagher GR, Vogels CBF. Development of an amplicon-based sequencing approach in response to the global emergence of mpox. PLoS Biol. 2023;21(6):e3002151. https://doi.org/10.1371/journal.pbio.3002151. PMID:37310918;PMCID:PMC10263305.
8. Chantal B.F. Vogels et al. DengueSeq: A pan-serotype whole genome amplicon sequencing protocol for dengue virus. MedRxiv. 2023. https://doi.org/10.1101/2023.10.13.23296997.
9. Fonseca V, Libin PJK, Theys K, Faria NR, Nunes MRT, Restovic MI, Freire M, Giovanetti M, Cuypers L, Nowé A, Abecasis A, Deforche K, Santiago GA, Siqueira IC, San EJ, Machado KCB, Azevedo V, Filippis AMB, Cunha RVD, Pybus OG, Vandamme AM, Alcantara LCJ, de Oliveira T. A computational method for the identification of Dengue, Zika and Chikungunya virus species and genotypes. PLoS Negl Trop Dis. 2019;13(5):e0007231. https://doi.org/10.1371/journal.pntd.0007231. PMID:31067235;PMCID: PMC6527240.
10. Katoh K, Rozewicki J, Yamada KD. MAFFT online service: multiple sequence alignment, interac-tive sequence choice and visualization. Brief Bioinform. 2019;20(4):1160–6.
11. Larsson A. AliView: a fast and lightweight alignment viewer and editor for large datasets. Bio-informatics. 2014;30:3276–8.
12. Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: a fast and effective stochastic al-gorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. 2015;32(1):268–74.
13. Sagulenko P, Puller V, Neher RA. TreeTime: Maximum-likelihood phylodynamic analysis. Virus Evol. 2018;4(1):vex042. https://doi.org/10.1093/ve/vex042. PMID: 29340210; PMCID: PMC5758920.
14 Suchard MA, Lemey P, Baele G, Ayres DL, Drummond AJ, Rambaut A. Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. Virus Evol. 2018;4:vey016.
15 Rambaut A, Lam TT, Max Carvalho L, Pybus OG. Exploring the temporal structure of heter-ochronous sequences using TempEst (formerly Path-O-Gen). Virus Evol. 2016;2:vew007.
16. Baele G, Li WL, Drummond AJ, Suchard MA, Lemey P. Accurate model selection of re-laxed molecular clocks in bayesian phylogenetics. Mol Biol Evol. 2013;30:239–43.
17. Lemey P, Rambaut A, Welch JJ, Suchard MA. Phylogeography takes a relaxed random walk in continuous space and time. Mol Biol Evol. 2010;27:1877–85.
18. Pybus OG, Suchard MA, Lemey P, Bernardin FJ, Rambaut A, Crawford FW, Gray RR, Arinamin-pathy N, Stramer SL, Busch MP, Delwart EL. Unifying the spatial epidemiology and molec-ular evolution of emerging epidemics. Proc Natl Acad Sci. 2012;109:15066–71.
19. Dellicour S, et al. Relax, keep walking - a practical guide to continuous phylogeographic inference with BEAST. Mol Biol Evol. 2021;38:3486–93.
20. Dellicour S, Rose R, Faria NR, Lemey P, Pybus OG. SERAPHIM:studying environ-mental rasters and phylogenetically informed movements. Bioinformatics. 2016;32:3204–6.
21. Dengue weekly report, PAHO. https://www3.paho.org/data/index.php/en/mnu-topics/indicadores-dengue-en/dengue-regional-en/315-reg-dengue-incidence-en.html.
22. Nakase T, Giovanetti M, Obolski U, Lourenço J. Global transmission suitability maps for dengue virus transmitted by Aedes aegypti from 1981 to 2019. Scientific Data. 2023;10:1–11.
23. Nakase T, Giovanetti M, Obolski U, Lourenço J. A retrospective analysis of climate-based dengue virus transmission suitability and demographic changes over the last four decades. medRxiv. 2023. p. 2023.11.21.23298791. https://doi.org/10.1101/2023.11.21.
24. Essential climate variables for assessment of climate variability from 1979 to present. Copernicus Climate Data Store. https://cds.climate.copernicus.eu/cdsapp#!/dataset/ecv-for-climate-change.
25. Pan American Health Organization (PAHO), dengue cases, PAHO/WHO Data - Weekly Report. https://www3.paho.org/data/index.php/en/mnu-topics/chikv-en/550-chikv-weekly-en.html.
26. Nakase T, Giovanetti M, Obolski U, et al. Global transmission suitability maps for dengue virus transmitted by Aedes aegypti from 1981 to 2019. Sci Data. 2023;10:275. https://doi.org/10.1038/s41597-023-02170-7.
27. Pinotti, et al. Shifting patterns of dengue three years after Zika virus emergence in Brazil. MerXiv. 2023. https://doi.org/10.1101/2023.05.29.23290597.
28. Petrone ME, Earnest R, Lourenço J, et al. Asynchronicity of endemic and emerging mosquito-borne disease outbreaks in the Dominican Republic. Nat Commun. 2021;12:151. https://doi.org/10.1038/s41467-020-20391-x.
29. Taylor-Salmon E, Hill V, Paul LM, Koch RT, Breban MI, Chaguza C, et al. Travel surveillance uncovers dengue virus dynamics and introductions in the Caribbean. medRxiv. 2023. p. 2023.11.11.23298412. https://doi.org/10.1101/2023.11.11.23298412.

Publisher's Note