



Short Communication

Establishing a dengue genomic monitoring in Cuba: uncovering virus dynamics to enhance local response

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ABSTRACT

Background: A major dengue outbreak was reported by the Cuban Ministry of Health in 2022, highlighting the need for strong surveillance, with high pediatric cases showing warning signs. Using phylogenetic and epidemiologic approaches, we provide evidence toward a better understanding of dengue's recent transmission history. **Methods:** This study details the implementation of the genomic surveillance system for Dengue virus. Pre-screened DENV-3 positive samples (reverse transcription–polymerase chain reaction [RT-PCR] cycle threshold [Ct] <30) with epidemiologic metadata—including symptom onset, sample collection date, sex, age, residence, symptoms, and disease classification—underwent whole genome sequencing using Nanopore technology.

Results: Genome sequences from 2022 to 2023 confirmed DENV-3 genotype III circulation in western, central, and eastern Cuba, covering 15 provinces and one special municipality. All cases were autochthonous, with 93% classified as dengue without warning signs and 7% as severe dengue. Long-term analysis identified a seasonal window of heightened suitability from May to November. Phylogenetic analyses revealed that DENV-3 genotype III has been introduced into Cuba multiple times, primarily from Asia and the Americas. These independent introduction events, likely driven by international travel and trade, underscore the importance of continued viral importation monitoring. The newly sequenced Cuban DENV-3 genotype III genomes were assigned to two distinct sub-lineages within genotype III: 3III_C, corresponding to the older American lineage I (2001–2014), and 3III_B.3, representing a novel introduction into Cuba associated with the more recent American lineage II (2022–2023). Evidence suggests that 3III_C, previously circulating in the country, may have been replaced by 3III_B.3 around 2020.

Conclusions: This study outlines the development of a dengue genomic monitoring system in the country. The system will contribute to Cuba's public health response, facilitating timely interventions and potentially reducing the disease's impact on the local population.

Dengue, caused by the dengue virus (DENV) and transmitted by *Aedes* mosquitoes [1], is a single-stranded, positive-sense ribonucleic acid virus (~11,000 kb) of the genus *Orthoflavivirus* in the *Flaviviridae*

family [2]. DENV has four serotypes (DENV-1 to DENV-4), with multiple genotypes varying in distribution and epidemic potential [3]. Despite antigenic diversity, serotypes share high genetic similarity. Infection

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Table 1

Epidemiological, clinical, and sequencing data of the newly sequenced DENV-3 samples collected in Cuba during the 2022 outbreak. For each sample, the table reports the unique sample ID, RT-qPCR cycle threshold (CT) value, patient age and sex, clinical classification based on WHO criteria, and location and date of sample collection. Sequencing information includes the depth of coverage (average number of times each nucleotide position was sequenced), the percentage genome coverage, lineage assignment based on whole genome phylogenetic analysis, and the corresponding GenBank accession number.

IDs	CTs	Age	Sex	Dengue classification	Location	Collection date	Depth of Coverage	Coverage	Lineages	Accession numbers
D3-147	26	10	M	Dengue without warning sign	Matanzas	2022-07-03	5772.6	92.2	DENV-3 Genotype III	PQ553469
D3-181	26	26	M	Dengue without warning sign	La Habana	2022-07-03	6102.4	94.6	DENV-3 Genotype III	PQ553470
D3-234	24	13	F	Dengue without warning sign	Matanzas	2022-07-10	3656.6	92	DENV-3 Genotype III	PQ553471
D3-280	21	19	F	Dengue without warning sign	Guantanamo	2022-07-10	2509.4	93	DENV-3 Genotype III	PQ553472
D3-297	25	45	F	Dengue without warning sign	Cienfuegos	2022-07-10	5426.6	91.4	DENV-3 Genotype III	PQ553473
D3-318	27	23	M	Severe dengue	Matanzas	2022-07-17	4435.0	84.2	DENV-3 Genotype III	PQ553474
D3-344	26	26	F	Dengue without warning sign	Pinar del Rio	2022-07-17	8245.2	94.7	DENV-3 Genotype III	PQ553475
D3-350	27	7	F	Severe dengue	Santiago de Cuba	2022-07-17	2588.2	84.7	DENV-3 Genotype III	PQ553476
D3-415	20	22	M	Dengue without warning sign	La Habana	2022-07-24	3508.5	95.2	DENV-3 Genotype III	PQ553477
D3-419	20	36	M	Dengue without warning sign	La Habana	2022-07-24	1734.8	94.7	DENV-3 Genotype III	PQ553478
D3-502	20	50	M	Dengue without warning sign	Santiago de Cuba	2022-08-14	1995.0	99.3	DENV-3 Genotype III	PQ553479
D3-530	20	33	F	Dengue without warning sign	La Habana	2022-08-14	4531.8	97.4	DENV-3 Genotype III	PQ553480
D3-538	22	52	F	Severe dengue	Las Tunas	2022-08-14	4764.1	87	DENV-3 Genotype III	PQ553481
D3-629	21	57	M	Dengue without warning sign	La Habana	2022-08-28	8082.4	94	DENV-3 Genotype III	PQ553482
D3-643	25	78	M	Severe dengue	Villa Clara	2022-09-04	4782.5	94	DENV-3 Genotype III	PQ553483
D3-703	16	19	F	Dengue without warning sign	Artemisa	2022-09-18	6298.6	97.8	DENV-3 Genotype III	PQ553484
D3-705	24	19	F	Dengue without warning sign	Artemisa	2022-09-18	7720.6	93	DENV-3 Genotype III	PQ553485
D3-1033	24	29	M	Dengue without warning sign	La Habana	2022-10-09	5688.9	92	DENV-3 Genotype III	PQ553486
D3-1144	26	39	M	Severe dengue	Guantanamo	2022-10-30	9415.7	94.6	DENV-3 Genotype III	PQ553487
D3-1166	21	26	F	Dengue without warning sign	Sancti Spiritus	2022-11-06	5212.5	91.7	DENV-3 Genotype III	PQ553488
D3-1191	27	29	M	Dengue without warning sign	La Habana	2022-11-13	7167.9	84.5	DENV-3 Genotype III	PQ553489
D3-1206	25	38	M	Dengue without warning sign	Granma	2022-11-13	5936.4	89	DENV-3 Genotype III	PQ553490
D3-1255	24	23	M	Dengue without warning sign	Santiago de Cuba	2022-11-13	5821.3	90	DENV-3 Genotype III	PQ553491
D3-1261	17	29	M	Dengue without warning sign	La Habana	2022-11-13	3952.3	97	DENV-3 Genotype III	PQ553492
D3-1099	28	32	F	Dengue without warning sign	Ciego de Avila	2022-10-23	8085.5	82.2	DENV-3 Genotype III	PQ553493
D3-1052	29	17	F	Dengue without warning sign	La Habana	2022-10-16	6222.4	70	DENV-3 Genotype III	PQ553494
D3-1390	28	53	F	Dengue without warning sign	Camaguey	2023-10-15	5880.1	82.9	DENV-3 Genotype III	PQ553495
D3-1299	23	24	M	Dengue without warning sign	Granma	2023-10-01	5975.9	91.3	DENV-3 Genotype III	PQ553496
D3-1333	28	29	F	Dengue without warning sign	Villa Clara	2023-10-08	4989.0	84.5	DENV-3 Genotype III	PQ553497
D3-29	21	63	M	Dengue without warning sign	Camaguey	2023-01-22	3814.2	92	DENV-3 Genotype III	PQ553498
D3-101	17	85	M	Dengue without warning sign	Pinar del Rio	2023-02-12	5836.4	99	DENV-3 Genotype III	PQ553499
D3-116	20	33	M	Dengue without warning sign	Villa Clara	2023-02-12	6793.5	92.2	DENV-3 Genotype III	PQ553500
D3-121	20	25	M	Dengue without warning sign	Villa Clara	2023-02-12	6753.3	94.5	DENV-3 Genotype III	PQ553501
D3-123	20	56	M	Dengue without warning sign	Villa Clara	2023-02-12	5231.7	95	DENV-3 Genotype III	PQ553502
D3-124	17	50	F	Dengue without warning sign	Villa Clara	2023-02-12	8440.2	96	DENV-3 Genotype III	PQ553503
D3-138	18	49	F	Dengue without warning sign	Artemisa	2023-02-19	5088.2	96	DENV-3 Genotype III	PQ553504
D3-252	21	45	M	Dengue without warning sign	Pinar del Rio	2023-03-12	6467.9	93	DENV-3 Genotype III	PQ553505
D3-671	28	38	F	Dengue without warning sign	Holguin	2023-05-28	3419.1	86.7	DENV-3 Genotype III	PQ553506
D3-688	15	35	M	Dengue without warning sign	La Habana	2023-06-04	5885.2	98.3	DENV-3 Genotype III	PQ553507
D3-792	16	17	F	Dengue without warning sign	Cienfuegos	2023-07-02	4780.6	94	DENV-3 Genotype III	PQ553508
D3-793	17	47	M	Dengue without warning sign	Cienfuegos	2023-07-02	3869.6	95.2	DENV-3 Genotype III	PQ553509
D3-835	15	23	M	Dengue without warning sign	Pinar del Rio	2023-07-09	3278.5	97.2	DENV-3 Genotype III	PQ553510
D3-836	22	26	M	Dengue without warning sign	Pinar del Rio	2023-07-09	4725.2	94	DENV-3 Genotype III	PQ553511
D3-838	21	53	F	Dengue without warning sign	Pinar del Rio	2023-07-09	4645.8	94	DENV-3 Genotype III	PQ553512
D3-852	19	18	F	Dengue without warning sign	Villa Clara	2023-07-09	2037.1	98	DENV-3 Genotype III	PQ553513
D3-863	16	41	F	Dengue without warning sign	Camaguey	2023-07-09	2981.2	95.7	DENV-3 Genotype III	PQ553514
D3-953	18	37	M	Dengue without warning sign	La Habana	2023-07-30	2651.7	99.8	DENV-3 Genotype III	PQ553515
D3-1002	26	20	M	Dengue without warning sign	Holguin	2023-08-13	2359.0	95.1	DENV-3 Genotype III	PQ553516
D3-1006	28	73	F	Dengue without warning sign	Holguin	2023-08-13	2648.4	81.7	DENV-3 Genotype III	PQ553517
D3-1107	18	70	F	Dengue without warning sign	Villa Clara	2023-09-03	2487.1	96.4	DENV-3 Genotype III	PQ553518
D3-1108	16	60	F	Dengue without warning sign	Villa Clara	2023-09-03	2554.0	96	DENV-3 Genotype III	PQ553519
D3-1119	21	28	F	Dengue without warning sign	Ciego de Avila	2023-09-03	4180.9	95	DENV-3 Genotype III	PQ553520
D3-1120	21	16	M	Dengue without warning sign	Ciego de Avila	2023-09-03	3480.0	92	DENV-3 Genotype III	PQ553521
D3-1140	27	3	M	Dengue without warning sign	Sancti Spiritus	2023-09-10	2602.6	84.9	DENV-3 Genotype III	PQ553522
D3-1141	23	50	F	Dengue without warning sign	La Habana	2023-09-10	5184.5	92.8	DENV-3 Genotype III	PQ553523
D3-1151	23	33	M	Dengue without warning sign	Granma	2023-09-10	2576.1	93.2	DENV-3 Genotype III	PQ553524
D3-1208	21	42	M	Dengue without warning sign	Villa Clara	2023-09-17	3948.7	95	DENV-3 Genotype III	PQ553525
D3-1215	23	72	F	Dengue without warning sign	Granma	2023-09-17	5928.5	93	DENV-3 Genotype III	PQ553526
D3-1263	24	45	F	Dengue without warning sign	Villa Clara	2023-09-24	4947.0	94	DENV-3 Genotype III	PQ553527
D3-1273	15	81	F	Dengue without warning sign	Holguin	2023-09-24	2829.4	98	DENV-3 Genotype III	PQ553528
D3-1274	18	49	F	Dengue without warning sign	Holguin	2023-09-24	3460.0	95.6	DENV-3 Genotype III	PQ553529
D3-1286	20	36	F	Dengue without warning sign	La Habana	2023-10-01	3817.7	94.7	DENV-3 Genotype III	PQ553530
D3-1290	24	39	F	Dengue without warning sign	Holguin	2023-10-01	5871.0	95	DENV-3 Genotype III	PQ553531
D3-1304	17	28	M	Dengue without warning sign	Granma	2023-10-01	3253.6	95	DENV-3 Genotype III	PQ553532
D3-1321	22	16	F	Dengue without warning sign	Santiago de Cuba	2023-10-08	2997.6	95	DENV-3 Genotype III	PQ553533
D3-1330	28	18	M	Dengue without warning sign	Pinar del Rio	2023-10-08	2626.4	82.5	DENV-3 Genotype III	PQ553534
D3-1350	25	18	F	Dengue without warning sign	Artemisa	2023-10-08	4321.5	94	DENV-3 Genotype III	PQ553535
D3-1354	22	45	F	Dengue without warning sign	La Habana	2023-10-15	2707.1	93	DENV-3 Genotype III	PQ553536
D3-1364	24	25	M	Dengue without warning sign	La Habana	2023-10-15	3446.0	91.1	DENV-3 Genotype III	PQ553537
D3-1372	28	51	M	Dengue without warning sign	Matanzas	2023-10-15	3743.1	83.6	DENV-3 Genotype III	PQ553538
D3-1388	28	32	F	Dengue without warning sign	Granma	2023-10-15	9836.4	81.8	DENV-3 Genotype III	PQ553539

CT, cycle threshold, DENV, dengue virus.

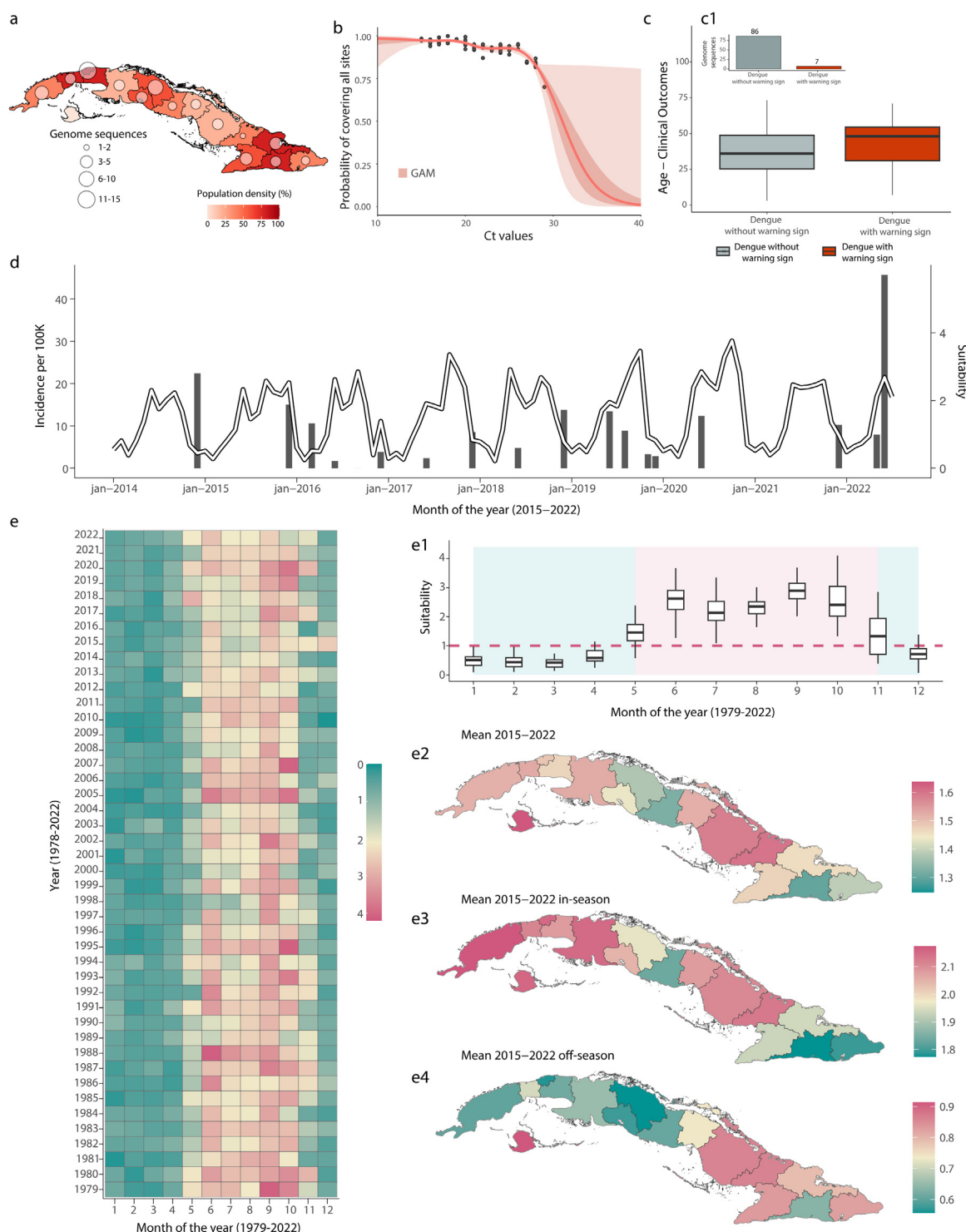


Figure 1. Spatiotemporal distribution and epidemiologic trends of dengue in Cuba. (a) Map of Cuba showing the number of DENV-3 genome sequences by provinces. The size of the circles indicates the number of new genomes generated in this study; (b) GAM of sample sequence coverage vs Ct. GAM predicted the probability of covering all genome sites, with sequencing depending on the Ct of each sample. Points are the samples, the red line is the mean predicted probability, the dark shaded area is the 50% CI, and the light shaded area is the 95% CI; (c) boxplot of the patient's (representing the study population) age and clinical outcomes value distribution; (c1) number of sequences per clinical outcome in the analyzed dataset; (d) time series of weekly (2014-2022) reported cases (black bars) normalized per 100,000 individuals (5) and daily mosquito viral suitability measure (index P - white lines); e, e1-4) temperature trends in Cuba between 1979 and 2022. CI, confidence interval; Ct, cycle threshold; DENV, dengue virus; GAM, generalized additive model.

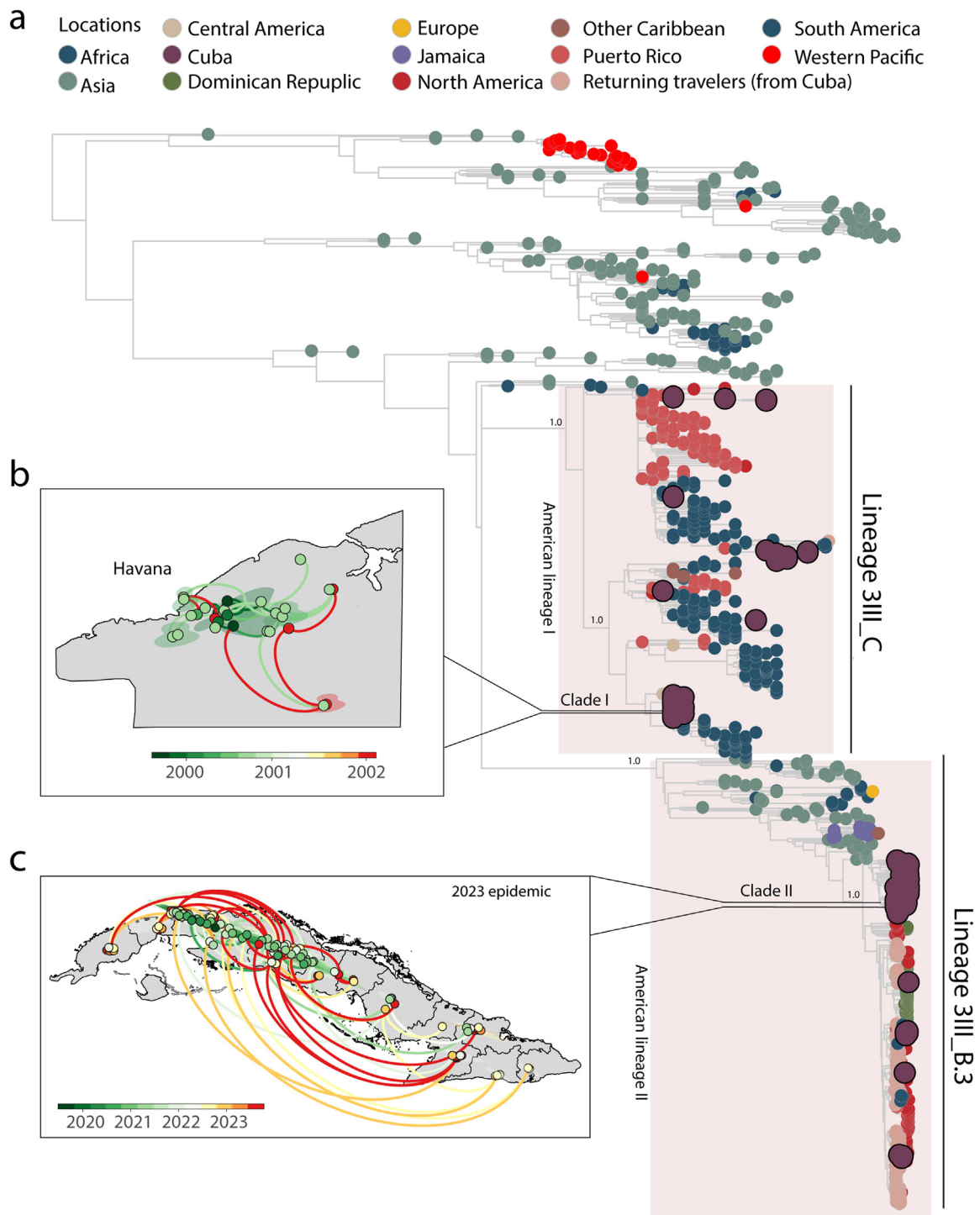


Figure 2. Dispersion dynamics of DENV3-III in Cuba. (a) Maximum likelihood phylogeny including the newly complete genome sequence from Cuba ($n = 71$) generated in this study combined with publicly available sequences ($n = 983$) from GenBank collected up to May 20, 2024. Colors indicate geographic location of sampling. Support for branching structure is shown by bootstrap values at key nodes; (b-c) spatiotemporal reconstruction of the spread of DENV3-III clade I and clade II in Cuba. Circles represent nodes of the maximum clade credibility phylogeny, colored according to their inferred time of occurrence (scale shown). 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. DENV, dengue virus.

grants long-lasting homologous but short-lived heterologous immunity [4], increasing severity upon reinfection due to antibody-dependent enhancement, complicating control. DENV infection can be asymptomatic or lead to severe dengue. By epidemiologic week 44 of 2024, the Americas reported 12,479,437 suspected cases, with an incidence of 1307 per

100,000. Of these, 6,652,683 (53%) were confirmed, 20,916 (0.17%) were severe, and 7575 deaths occurred [5]. Historically, DENV-1 and DENV-2 dominated in Latin America; however, in 2023, DENV-3 surged across the Caribbean and the Americas, shifting the epidemiologic landscape [6,7]. Cuba's surveillance efforts have provided insights into virus

evolution [8,9]. A major dengue outbreak was reported by the Cuban Ministry of Health in 2022, highlighting the need for strong surveillance [10], with high pediatric cases showing warning signs [11]. Molecular surveillance confirmed three circulating serotypes, with DENV-3 predominating. Genomic classification identified DENV-3 genotype III, specifically American lineage II [12]. Controlling dengue in the Caribbean is difficult due to limited data. To enhance surveillance, we collaborated with the Pan American Health Organization (PAHO) to strengthen response capacity at Cuba's National Reference Laboratory for arboviruses at Instituto Pedro Kouri. This study details the implementation of the surveillance system and provides ecologic, epidemiologic, and genetic data to improve preparedness and response.

In October 2023, the PAHO launched a genomic surveillance training program at the Instituto de Medicina Tropical “Pedro Kouri” in Havana, Cuba, introducing Nanopore sequencing for DENV investigations. The program covered sequencing, data analysis, and scientific dissemination through theoretical and practical sessions on mobile sequencing, bioinformatics, phylogenetics, epidemiology, and molecular techniques. Participants included postgraduate students, laboratory technicians, and health practitioners. The goal was to equip local experts with skills for real-time data analysis to improve early detection and response. Pre-screened DENV-3 positive samples (reverse transcription–polymerase chain reaction [RT-PCR] cycle threshold [Ct] <30) with epidemiologic metadata—including symptom onset, sample collection date, sex, age, residence, symptoms, and disease classification—underwent whole genome sequencing using Nanopore technology. This yielded 71 near-complete genome sequences (July 3, 2022 to October 15, 2023) deposited in GenBank (PQ553469–PQ553539, Table 1). In addition, 22 partial DENV-3 genomes (2001–2014, Sanger sequencing) and 961 global sequences from GenBank were analyzed. Phylogenetic trees explored relationships between Cuban and global DENV-3 isolates (Appendix).

Genome sequences from 2022 to 2023 confirmed DENV-3 genotype III circulation in western, central, and eastern Cuba, covering 15 provinces and one special municipality (Figure 1a). The average RT-PCR Ct value was 22 (range: 15–29) (Table 1, Figure 1b). Sample coverage analysis showed 93% average coverage, with Ct ≤28 achieving ≥90% coverage (Figure 1b). Patients' ages ranged from 3 to 85 years (median: 38), with 49% male (n = 35) and 51% female (n = 36) (Table 1, Figure 1c). All cases were autochthonous, with 93% classified as dengue without warning signs and 7% as severe dengue (Figure 1c1, Table 1). The PAHO [5] reported four major dengue outbreaks in Cuba (2015, 2016, 2019, and 2022). Climate-based mosquito viral suitability indexes indicated strong seasonality due to climate fluctuations (Figure 1d, white lines), consistent with previous Caribbean findings [10]. Low temporal resolution of incidence data limited correlation analyses. Retrospective suitability estimates (1979–2022) revealed key spatiotemporal patterns relevant to dengue transmission potential in Cuba (Figure 1e). Long-term analysis identified a seasonal window of heightened suitability from May to November (Figures 1e, e1). Between 2014 and 2022, suitability was highest in Isla de la Juventud, Camagüey, and Las Tunas and lowest in Villa Clara, Sancti Spiritus, Santiago de Cuba, and Guantánamo (Figure 1e2). During the in-season period (May–November), suitability peaked in western and central regions, particularly, in La Habana, whereas off-season suitability (December–April) was comparatively higher in the eastern provinces (Figure 1e3–e4). Although these patterns suggest a climatic influence, we acknowledge that the relationship between dengue transmission and meteorologic factors is complex and may involve temporal lags or additional ecological and socio-environmental drivers that warrant further investigation.

Phylogenetic analyses revealed that DENV-3 genotype III has been introduced into Cuba multiple times, primarily from Asia and the Americas (Figure 2a). These independent introduction events, likely driven by international travel and trade, underscore the importance of continued viral importation monitoring. Using the dynamic DENV lineage classification system proposed by Hill et al., [13] the newly sequenced

Cuban DENV-3 genotype III genomes (n = 71) were assigned to two distinct sub-lineages within genotype III: 3III_C, corresponding to the older American lineage I (2001–2014), and 3III_B.3, representing a novel introduction into Cuba associated with the more recent American lineage II (2022–2023). Evidence suggests that 3III_C, previously circulating in the country, may have been replaced by 3III_B.3 around 2020, although further sampling is required to confirm this transition. Within these lineages, we identified two main transmission clusters: clade I (n = 23) and clade II (n = 70), both composed of autochthonous cases, indicating sustained local transmission.

Phylogeographic analysis of clade I traced viral movements within La Habana (Figure 2b), suggesting a mean origin in late March 2000 (95% Higher Posterior Density (HPD): April 24, 1999 to December 28, 2000). This clade spread from the center toward the southeast and northeast, indicating intra-provincial transmission (Figure 2b). Clade II analysis traced viral movements across provinces (Figure 2c), suggesting a mean origin in early July 2019 (95% HPD: August 28, 2018 to January 5, 2021). Although no human DENV-3 cases were detected between 2019 and 2021, DENV-3 was identified by RT-PCR in *Aedes albopictus* larvae in Havana in 2019 (unpublished data). Clade II spread from northern to southern provinces, particularly, in late 2022–2023, when suitability shifted from north to south (Figure 2c).

Implementing the monitoring system and analyzing historical genomic and epidemiologic data improved the understanding of dengue transmission in Cuba. Retrospective analyses confirm conditions favoring DENV circulation. Strengthening genomic surveillance with advanced technologies and integrated clinical, epidemiologic, entomologic, and virologic monitoring will enhance prevention and control efforts. The new system represents a critical step toward improved dengue surveillance and response strategies.

Declarations of competing interest

The authors have no competing interests to declare.

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Ethics statement

This project was reviewed and approved by the ethical committee of the Institute of Tropical Medicine, Pedro Kouri (CEI-IPK 30-18). The sample processed in this study was obtained anonymously from material exceeding the routine diagnosis of arboviruses.

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Author contributions

Conception and design: MGG, JL, MG; Investigations: MGG, MG, MP, MA, AJB, SS; Data Analysis: MGG, MG, MP, MA, AJB, SS, SR, VK, LG, LF, JMR, JL, LCJA; Visualization: JL, MG; Writing – Original: MGG, JL, MG; Revision: MGG, MG, MP, MA, AJB, SS, SR, VK, LG, LF, JMR, JL, LCJA; Resources: MMG, MG, LCJA, JMR, LF.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijregi.2025.100683.

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