Molecular epidemiology of Dengue

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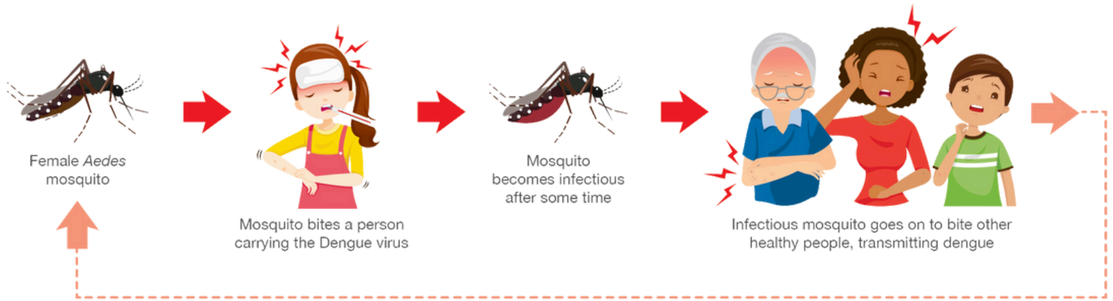
Background on Dengue

DENGUE FEVER



- Causes **a hundred million** dengue cases annually, with significant morbidity and mortality.

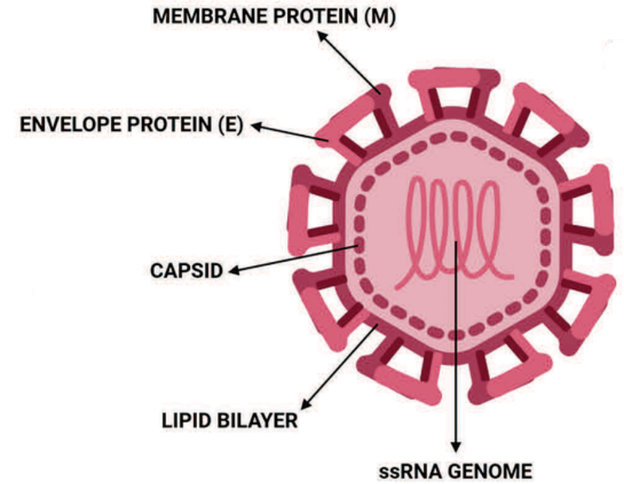
- **No specific antiviral drugs** and **vaccine provide limited** or incomplete.

Factors

- Climate change
- Large urban center
- Globalization
- Limited in surveillance and reporting

DENGUE VIRUS

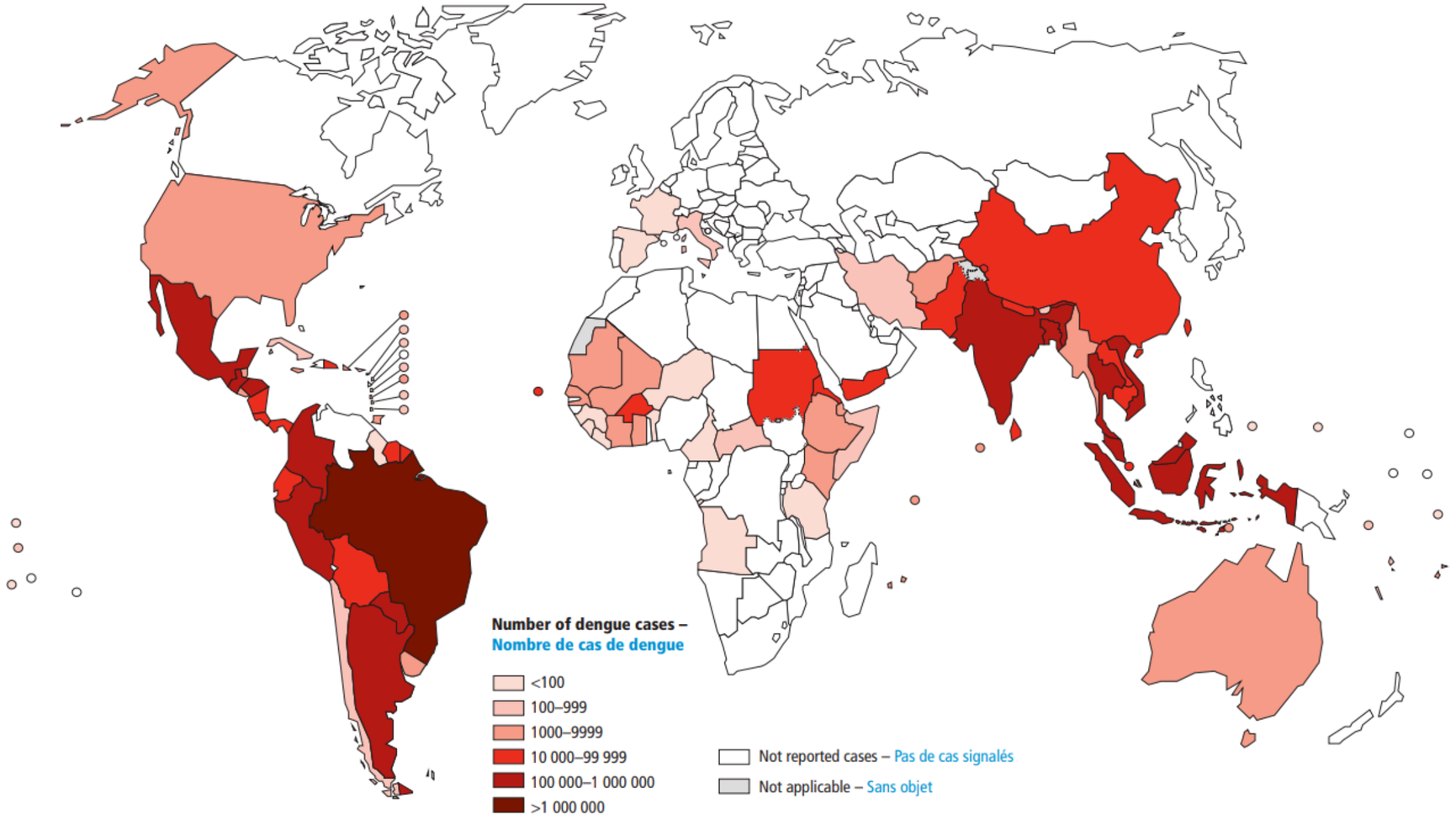
- Genus : *Flavivirus*
- Family : *Flaviviridae*
- Genome : +ssRNA enveloped virus
- 4 serotype : DENV-1 to DENV-4



Ruvalcaba-Hernández et al., 2025

Dengue burden trend

Global distribution of dengue cases, by country, 2024



Region of the Americas

South-East Asia Region

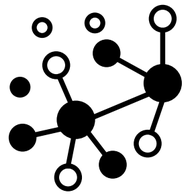
Western Pacific Region

Dengue is endemic in **tropical and subtropical regions**, with **recurrent outbreaks** reported in many areas. More than half of the global population now lives in regions at risk of dengue infection.

Molecular epidermiology

Genetic data + **Epidemiological information**

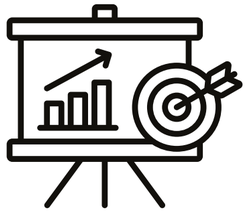
Relationships



Identify genetic relatedness among viral strains, define clusters and transmission networks.

01

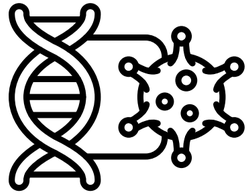
Strains shifts



Detect genotype replacement over time, reflect viral fitness and population immunity

02

Evolutionary



Estimate divergence times and MRCA, reconstruct viral evolutionary history.

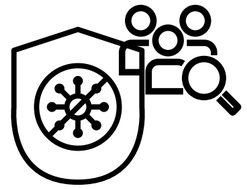
03

Transmission

Infer viral movement, identify transmission routes, hubs and directionality

04

Outbreak



Distinguish repeated introductions vs local spread and link viral genetics to epidemic events

05

Seminar papers

nature communications

Article

https://doi.org/10.1038/s41467-024-51018-0

Molecular epidemiology and evolutionary characteristics of dengue virus 2 in East Africa

Received: 11 May 2023

Accepted: 25 July 2024

Published online: 07 September 2024

Check for updates

Sindiso Nyathi[1], Izabela M. Rezende[2], Katharine S. Walter[3], Panpim Thongsripong[4], Francis Mutuku[5], Bryson Ndenga[6], Joel O. Mbakaya[6], Peter Aswani[6], Peter S. Musunzaji[7], Philip K. Chebii[7], Priscilla W. Maina[7], Paul S. Mutuku[7], Charles M. Ng'ang'a[7], Said L. Malumbo[7], Zainab Jembe[8], David M. Vu[9], Erin A. Mordecai[10], Shannon Bennett[11], Jason R. Andrews[2,12] & A. Desiree LaBeaud[9,12]

Nature communications

Published in 2023, IF 15.7 (Q1)

frontiers | Frontiers in Genetics

TYPE Original Research
PUBLISHED 28 May 2024
DOI 10.3389/fgene.2024.1368843

Molecular epidemiology of dengue in Malaysia: 2015–2021

Yu Kie Chem[1†], Surya Pavan Yenamandra[2†], Chee Keong Chong[3], Rose Nani Mudin[3], Ming Keong Wan[3], Norazimah Tajudin[1], Rehan Shuhada Abu Bakar[1], Mohd Asri Yamin[1], Rokiah Yahya[1], Chia-Chen Chang[2], Carmen Koo[2], Lee Ching Ng[2,4,5*] and Hapuarachchige Chanditha Hapuarachchi*

Frontiers

Published in 2024, IF 2.8 (Q2)

First paper

nature communications

Article

https://doi.org/10.1038/s41467-024-51018-0

Molecular epidemiology and evolutionary characteristics of dengue virus 2 in East Africa

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1 Objectives

- To assess the underlying genetic structure of circulating DENV-2.
- To evaluate the extent of gene flow within Kenya
- To contribute global and local circulation patterns to disease spread.

OVERVIEW

DENV detection and whole-genome NGS

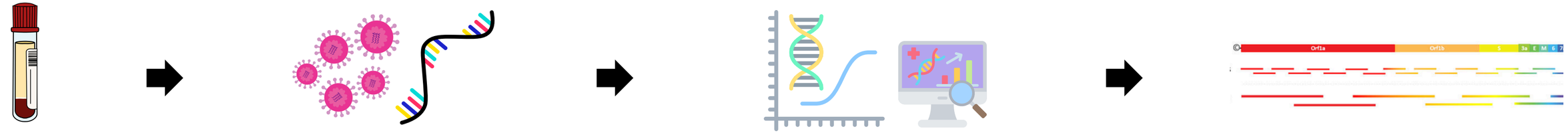
Phylogenetic and phylogeographic of Kenyan DENV-2 in a global context.

To investigate the drivers of dengue outbreaks in Kenya

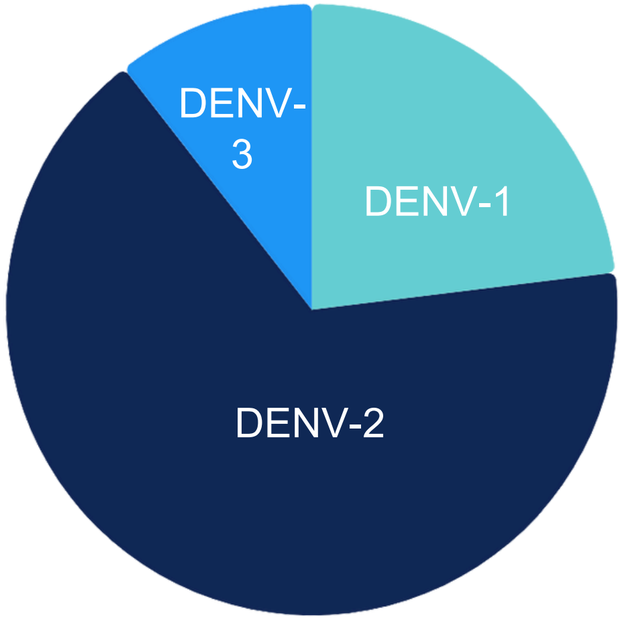
Result 1: Dengue proportion in Kenya

Objective To identify the circulating dengue virus serotypes.

METHOD



Blood → RNA extraction → DENV detection and serotyping → Amplicon-based WGS



DENV-2 Only six samples were successfully sequenced.

Result 1: Dengue virus 2 sequences isolated from Kenya

Objective

To investigate dengue virus genetics diversity distribution in Kenya.

METHOD

Newly 6 sequences combine with global sequence

⬇

Multiple sequence alignment

⬇

Recombinant detection

⬇

ML phylogenetic reconstruction

Kenya

Site	City	N
Study	Msambweni	1
	Ukunda	5
Total		**6**
Non-study	Lamu	1
	Malindi	14
	Mombasa	13
	Mtwapa	7
	Voi	1
	Wajir	1
Total		**37**
Grand Total		**43**

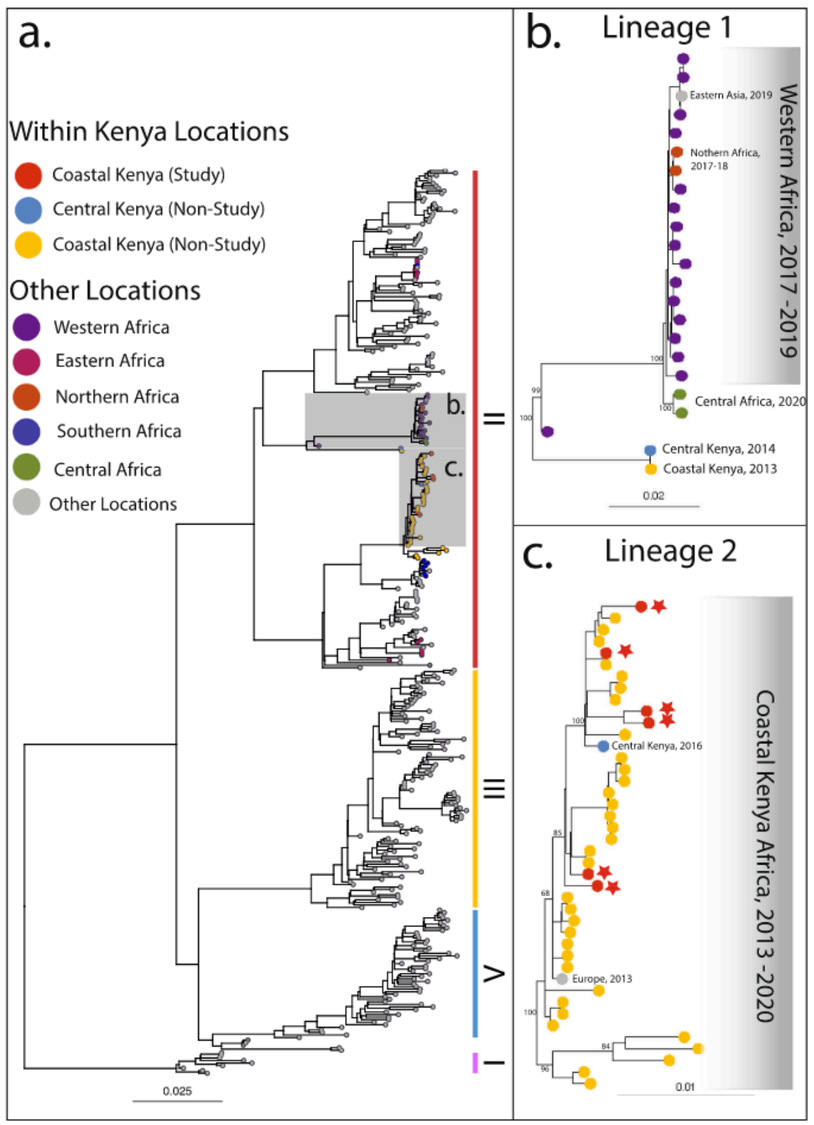
Global region

Region	Study Region	N
Oceania	Australia	8
	Oceania (Melanesia/Polynesia)	11
Eurasia	Europe	3
	Central South-Eastern Asia	63
	Eastern Asia	22
	Lower South-Eastern Asia	33
	Upper South-Eastern Asia	36
	Western Asia	2
Americas	Central America and Caribbean	31
	North America	14
	South America	50
Africa	Eastern Africa	12
	Northern Africa	2
	Southern Africa	7
	Western Africa	16
	Central Africa	2
Total		**312**

A total of 355 sequences were included in phylogenetic analyses.
(1944-2022)

Result 1: Dengue virus 2 sequences isolated from Kenya

Objective To investigate dengue virus genetics diversity distribution in Kenya.



Genotype II (Cosmopolitan)

- Over 50% of included sequences
- The broadest geographic distribution

Genotype V (Asian I)

- Only 51 sequences
- The most geographically restricted

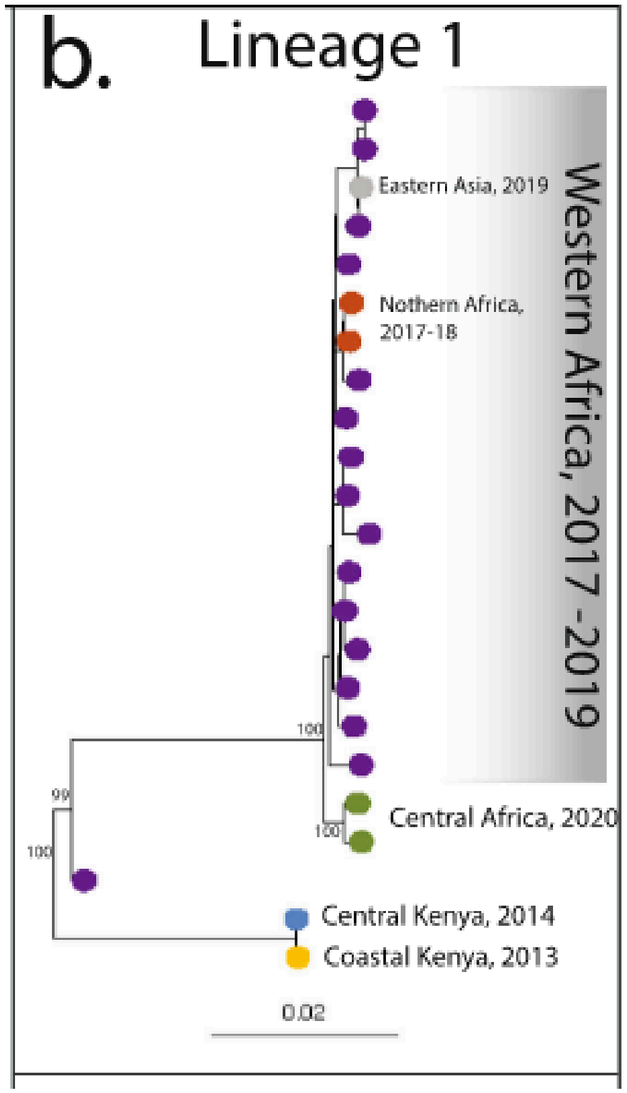
Non-sylvatic genotypes

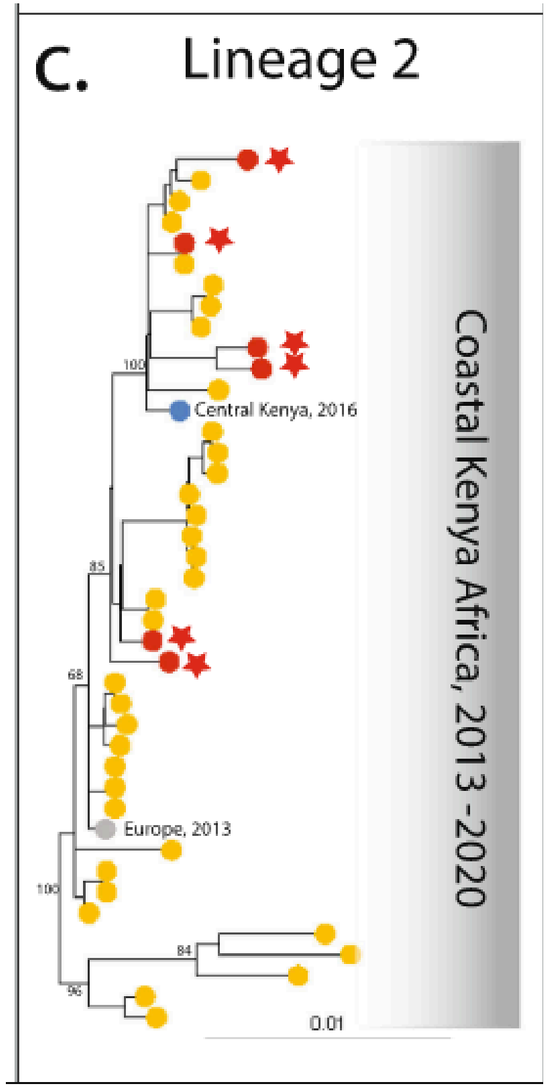
Sylvatic genotypes

Result 1: Dengue virus 2 sequences isolated from Kenya

Objective

To investigate dengue virus genetics diversity distribution in Kenya.





- **The L1** clustered closely with sequences from **West and Central Africa**
- **The L2** clade clustered closely with sequences from **Southern Africa and Eastern Asia**

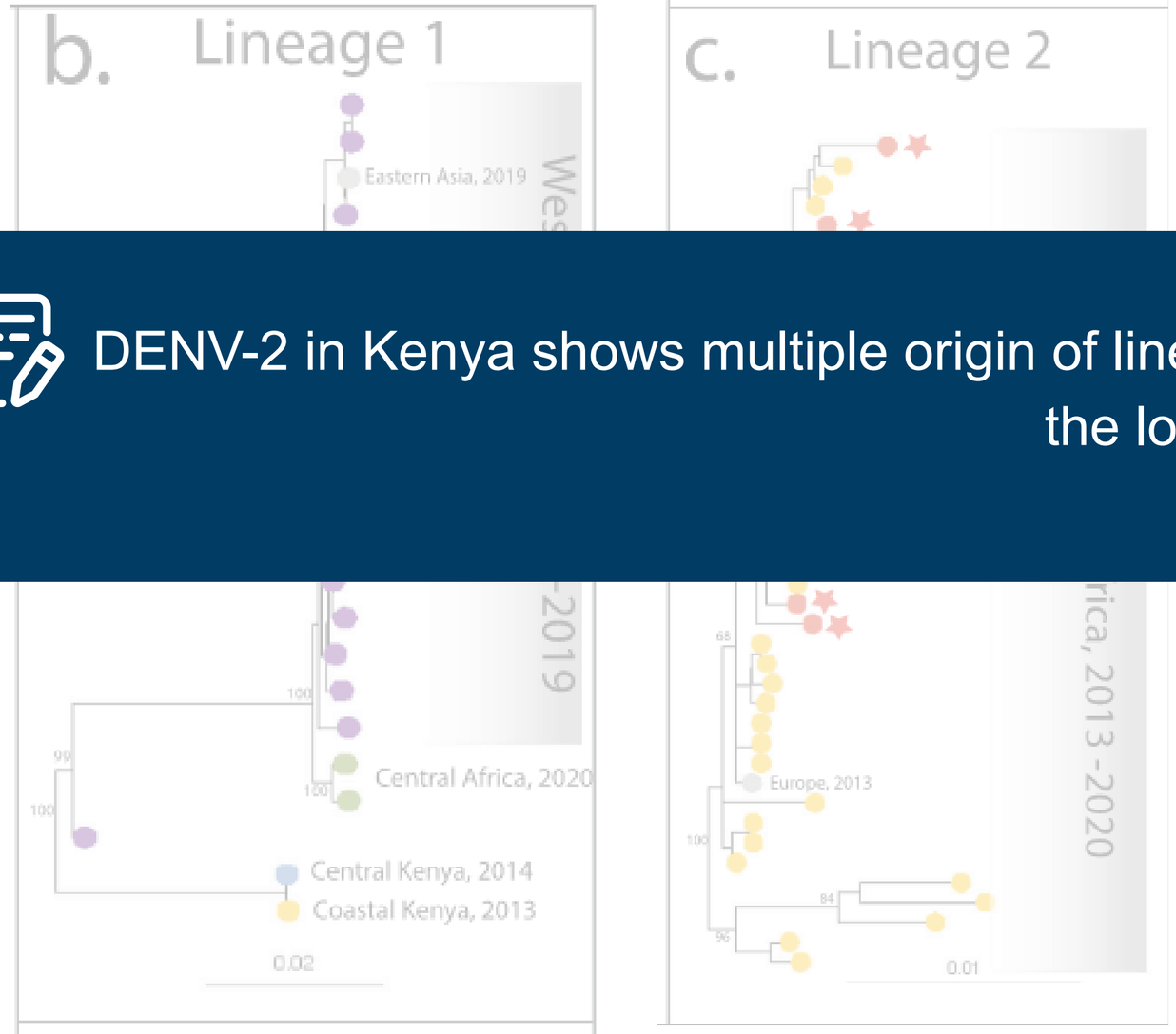
Within Kenya Locations
- Coastal Kenya (Study)
- Central Kenya (Non-Study)
- Coastal Kenya (Non-Study)

Other Locations
- Western Africa
- Eastern Africa
- Northern Africa
- Southern Africa
- Central Africa
- Other Locations

Result 1: Dengue virus 2 sequences isolated from Kenya

Objective

To investigate dengue virus genetics diversity distribution in Kenya.



- The L1 clustered closely with sequences from

DENV-2 in Kenya shows multiple origin of lineages with consistent replication of the virus in the location.

Result 2: Bayesian phylogenetic analyses

Objective To estimate divergence times among DENV-2 lineages and assess their temporal emergence.

METHOD

Temporal signal

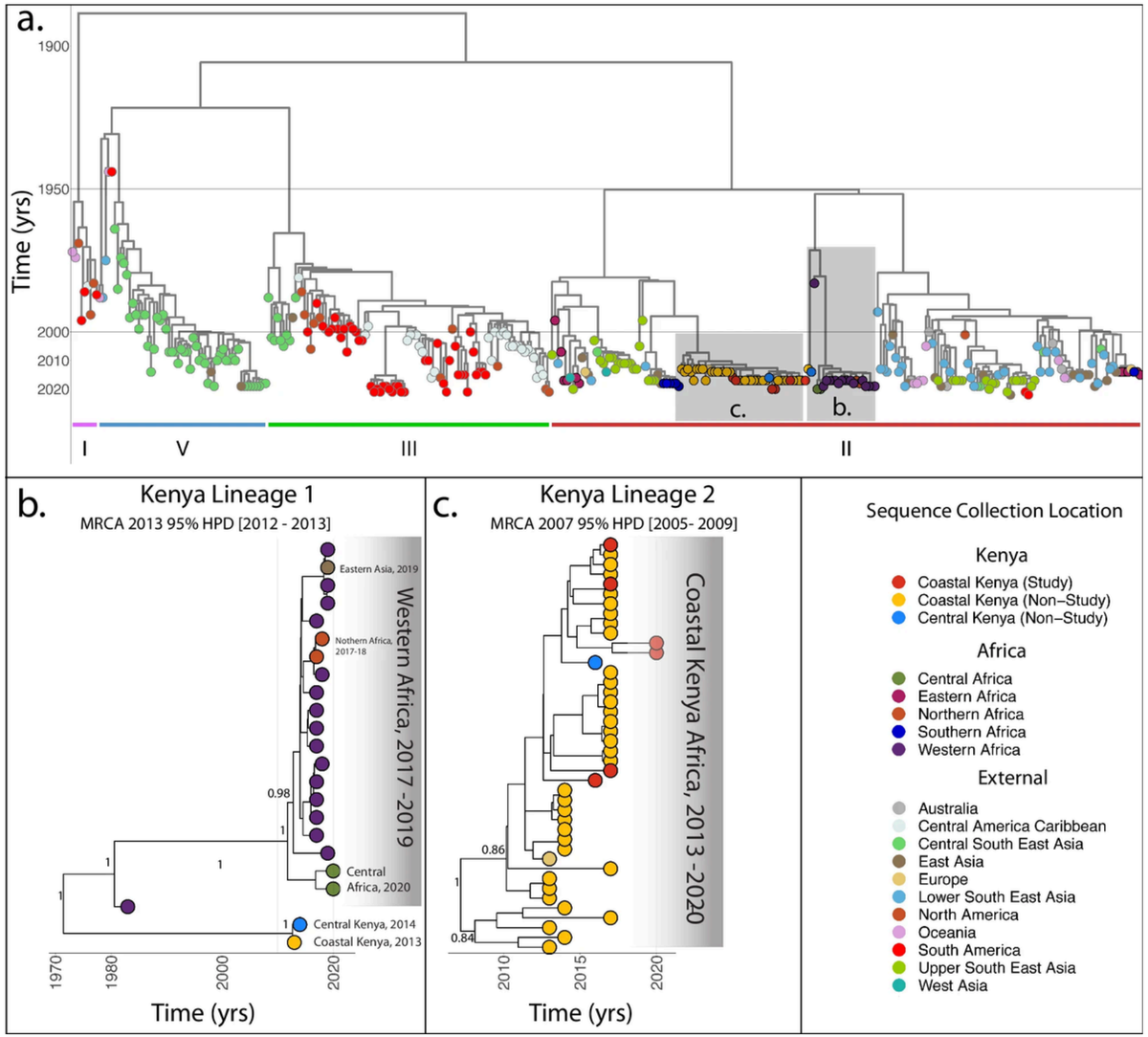
⬇

Bayesian phylogenetic analysis

BEAST X
Bayesian Evolutionary Analysis Sampling Trees

⬇

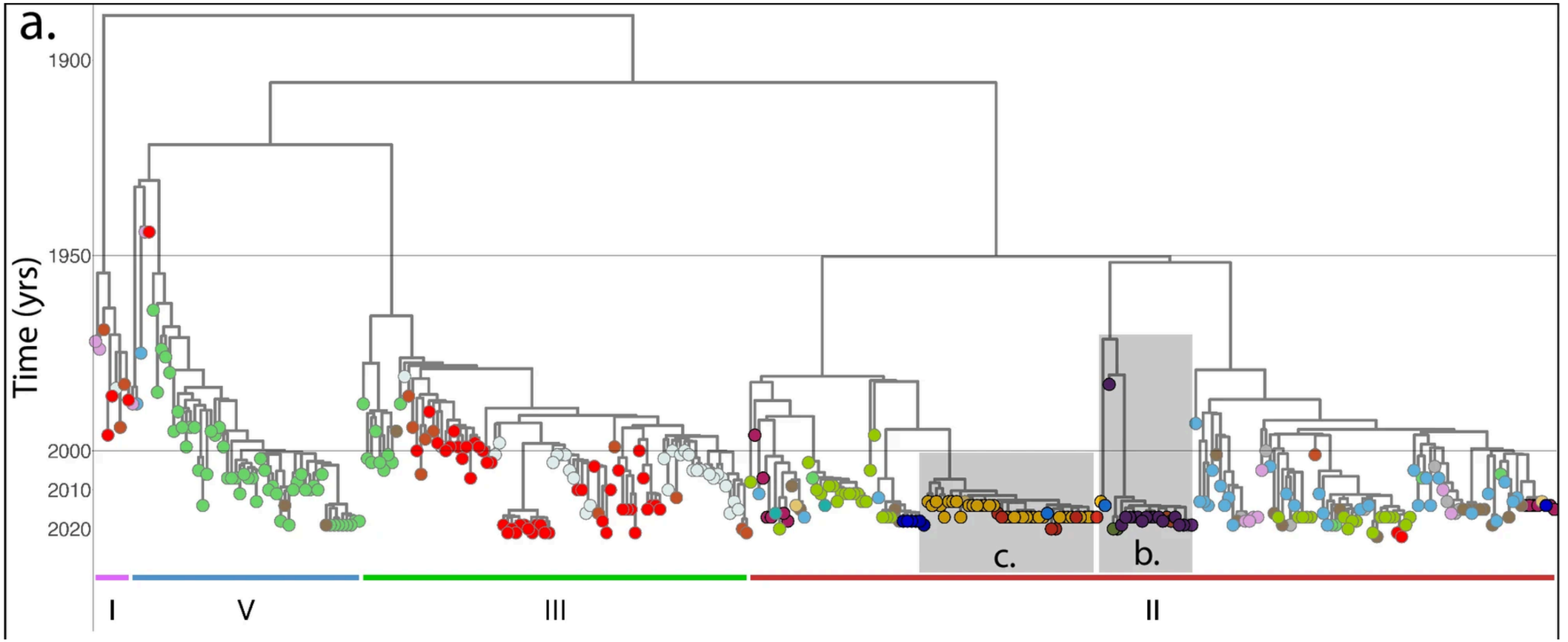
Time-scaled phylogenetic tree



Result 2: Bayesian phylogenetic analyses

Objective

To estimate divergence times among DENV-2 lineages and assess their temporal emergence.

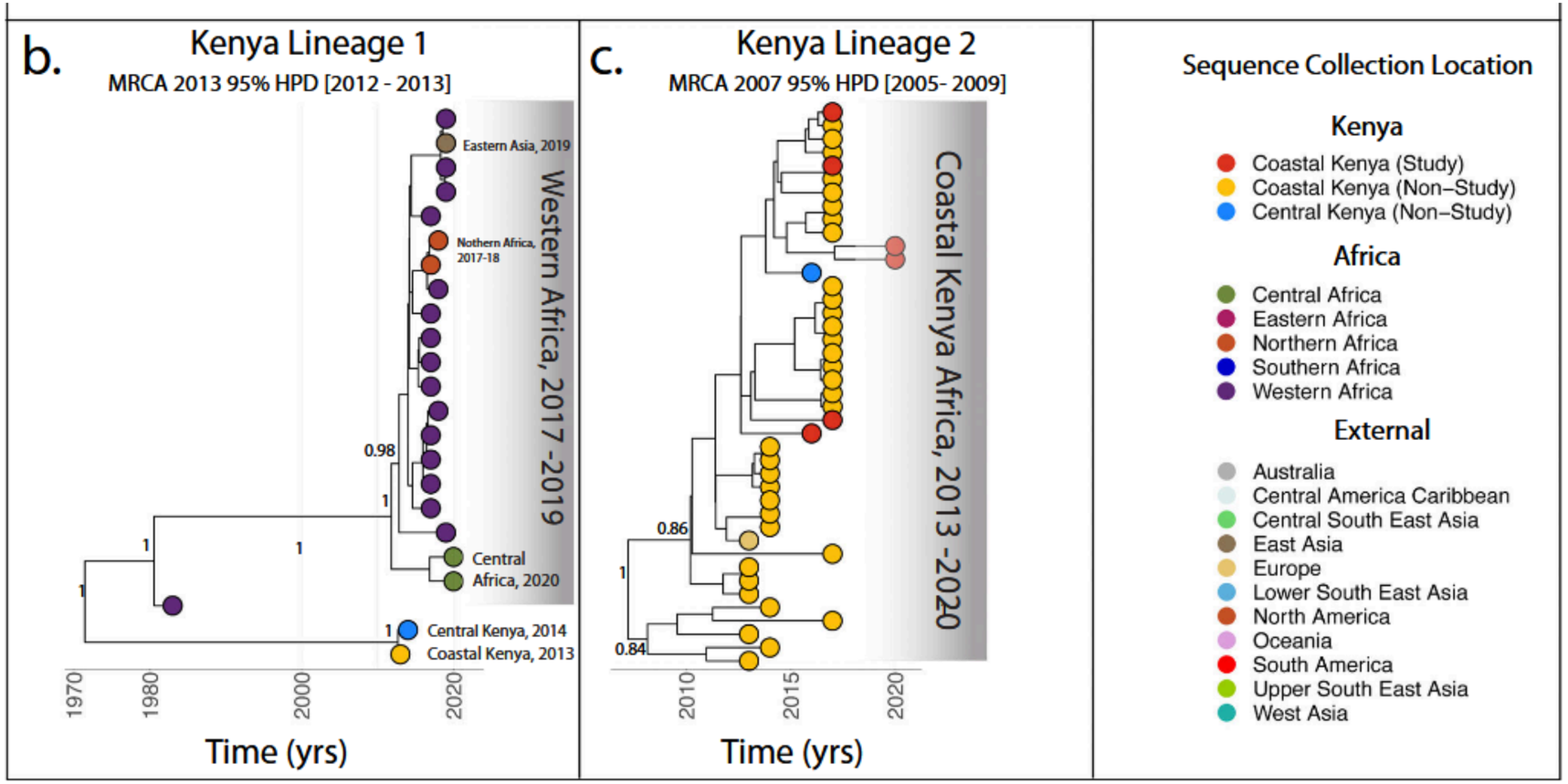


The MCC tree confirmed that DENV-2 isolates collected in Kenya are **not monophyletic** consisting of at least **two separate lineages.**

Result 2: Bayesian phylogenetic analyses

Objective

To estimate divergence times among DENV-2 lineages in Kenya and assess their temporal emergence.

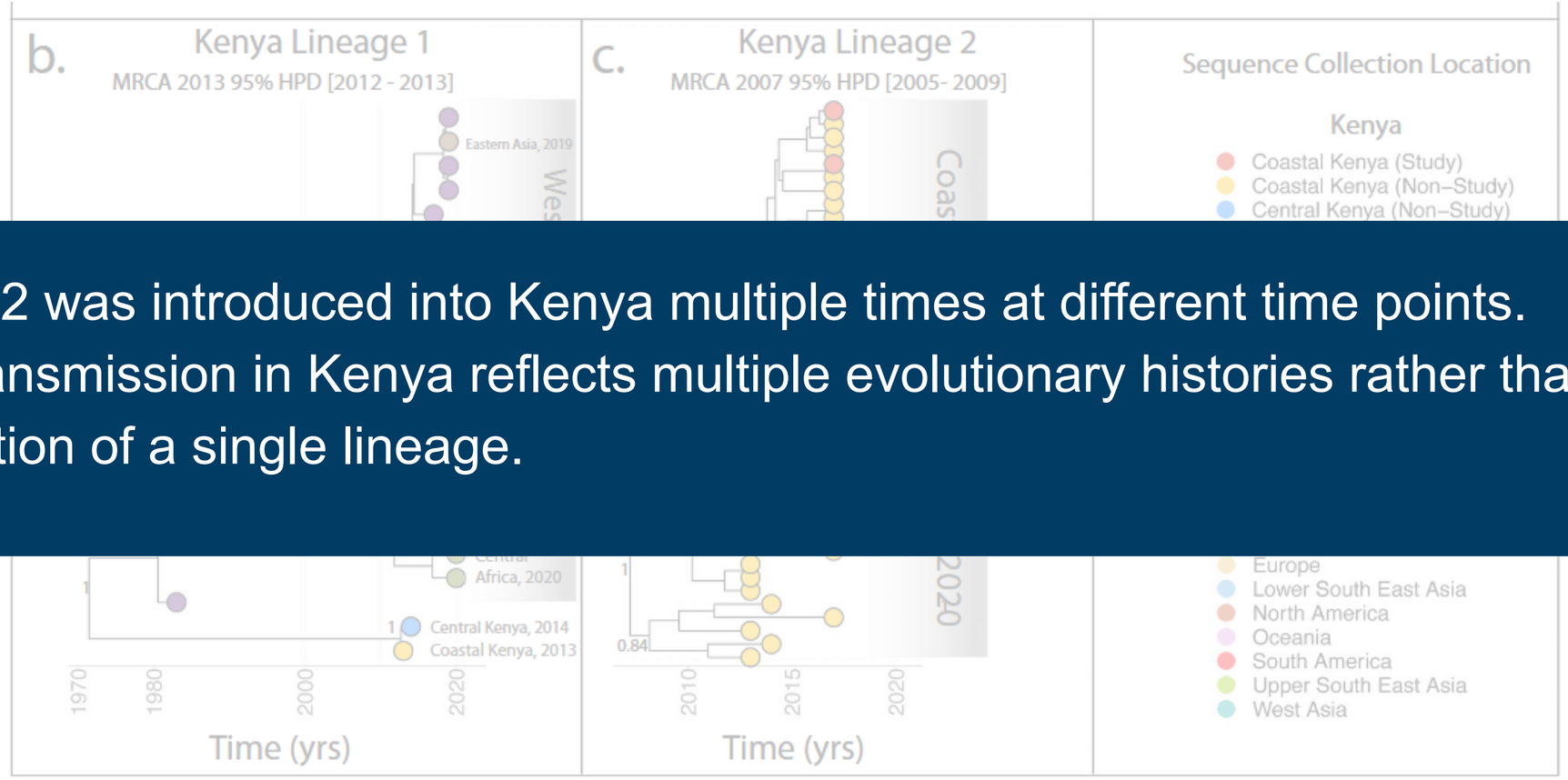


L2 has an estimated MRCA around 2007 whereas L1 has a more recent MRCA in Kenya in 2013.

Result 2: Bayesian phylogenetic analyses

Objective

To estimate divergence times among DENV-2 lineages in Kenya and assess their temporal emergence.



- DENV-2 was introduced into Kenya multiple times at different time points.
- The transmission in Kenya reflects multiple evolutionary histories rather than sustained circulation of a single lineage.

L2 has an estimated MRCA around 2007 whereas L1 has a more recent MRCA in Kenya in 2013.

Result 3: Geographic spread pattern of DENV-2 within Kenya

Objective To determine the origins and geneflow of DENV-2 in Kenya.

METHOD

Time-scaled tree

⬇

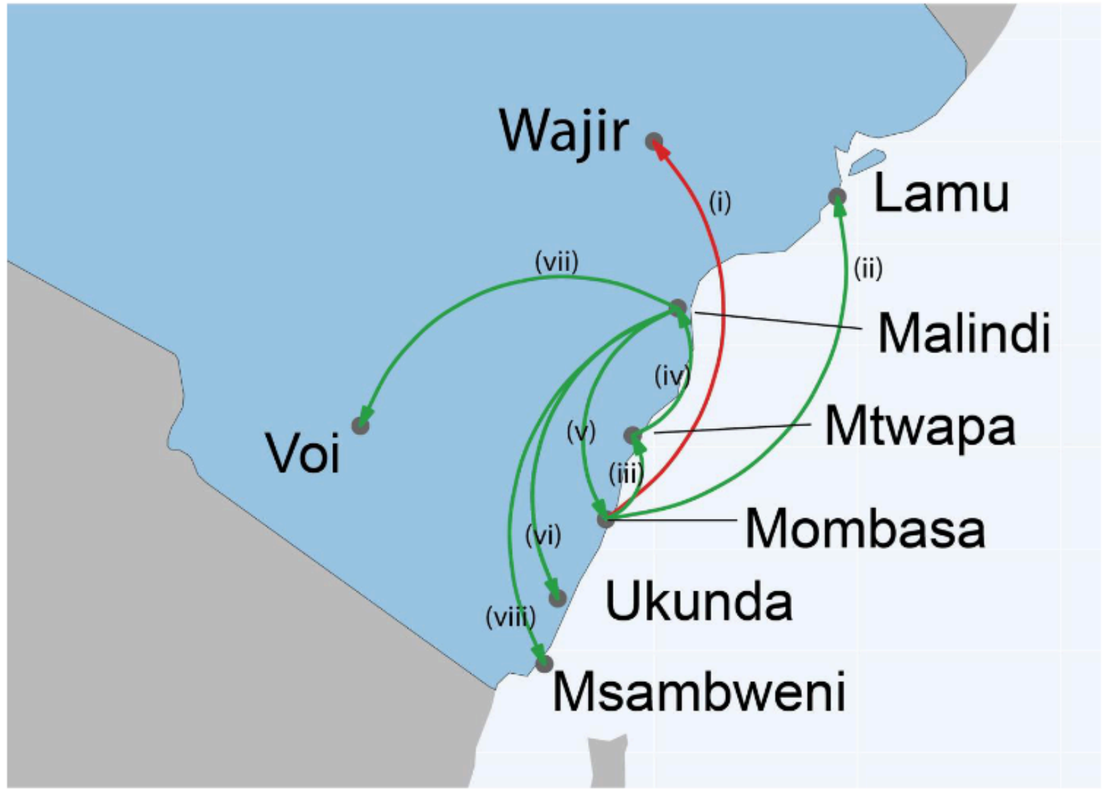
Discrete phylogeographic modeling

⬇

Markov process

⬇

Bayesian Stochastic Search Variable Selection



The viral movement is concentrated within coastal regions with **coastal sites acting as hubs for local transmission.**

Result 3: Geographic spread pattern of DENV-2 within Kenya

Objective

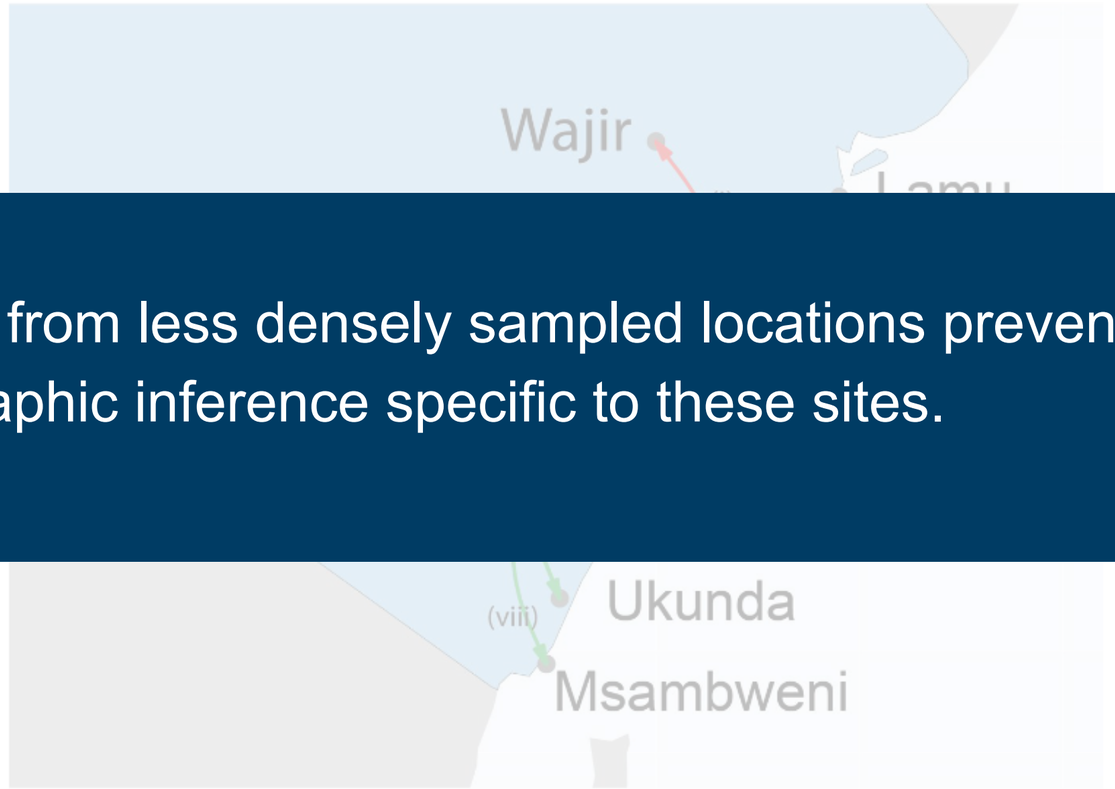
To determine the origins and geneflow of DENV-2 in Kenya.

METHOD

Time-scaled tree

Markov process

Bayesian Stochastic Search Variable Selection



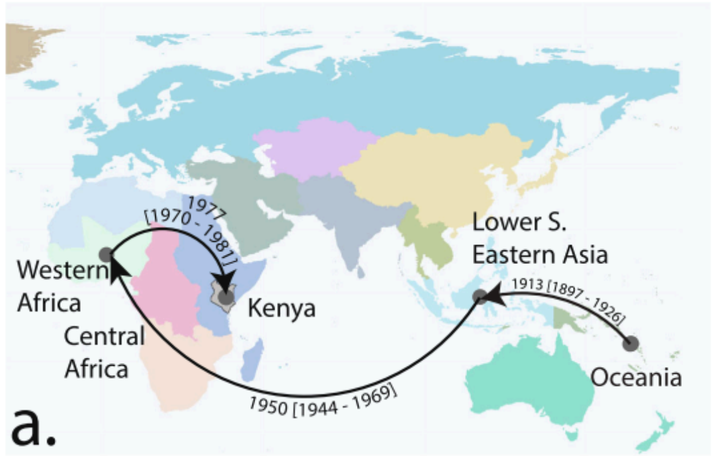
Limited sequencing data collected from less densely sampled locations prevent more accurate phylogeographic inference specific to these sites.

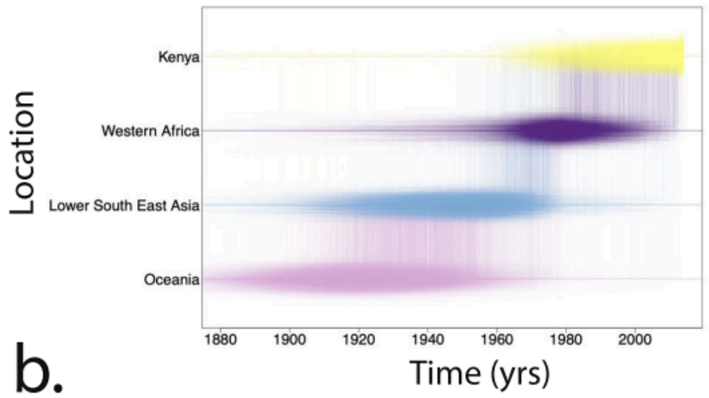
The viral movement is concentrated within coastal regions with **coastal sites acting as hubs for local transmission.**

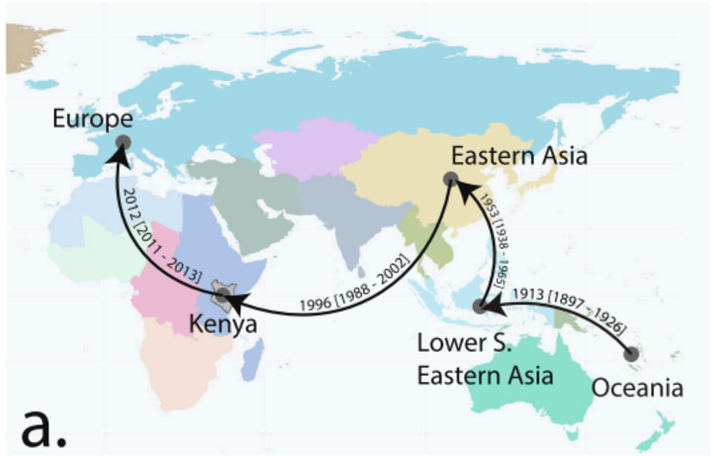
Result 3: Geographic history of DENV-2 detected in Kenya.

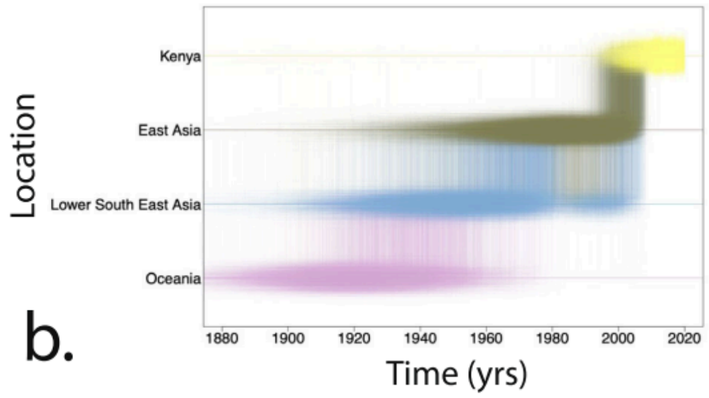
Objective

To determine the origins and introduction events of DENV-2 into East Africa.









Despite these different entry points, **both lineages** trace their deeper evolutionary origins to **Oceania and Lower Southeast Asia.**

Conclusion

- **DENV-2 in Kenya** is not locally derived but the result of **multiple historical introductions**.
- **East and Southeast Asia functions as a global source** region which corresponds to the substantial trade and travel flows between Asia and Africa.
- Introduction into Kenya appears to be followed by **regional amplification, especially along the coast** rather than widespread export.

Second paper

frontiers | Frontiers in Genetics

TYPE Original Research
PUBLISHED 28 May 2024
DOI 10.3389/fgene.2024.1368843

Molecular epidemiology of dengue in Malaysia: 2015–2021

Yu Kie Chem[1†], Surya Pavan Yenamandra[2†], Chee Keong Chong[3], Rose Nani Mudin[3], Ming Keong Wan[3], Norazimah Tajudin[1], Rehan Shuhada Abu Bakar[1], Mohd Asri Yamin[1], Rokiah Yahya[1], Chia-Chen Chang[2], Carmen Koo[2], Lee Ching Ng[2,4,5]* and Hapuarachchige Chanditha Hapuarachchi*

2 Objectives

- To characterize genetic diversity, temporal dynamics, and phylogeographic spread of DENV in Malaysia
- To assess spatiotemporal transmission in Malaysia and across Asia.

OVERVIEW

Patient samples

+

Dengue surveillance data

↓

Dengue detection and E protein sequencing

↓

Molecular epidemiological analyses

↓

Understanding outbreak patterns and disease severity

Result 1: Dengue Burden and Distribution in Malaysia

Objective To describe the dengue disease burden in Malaysia

METHOD

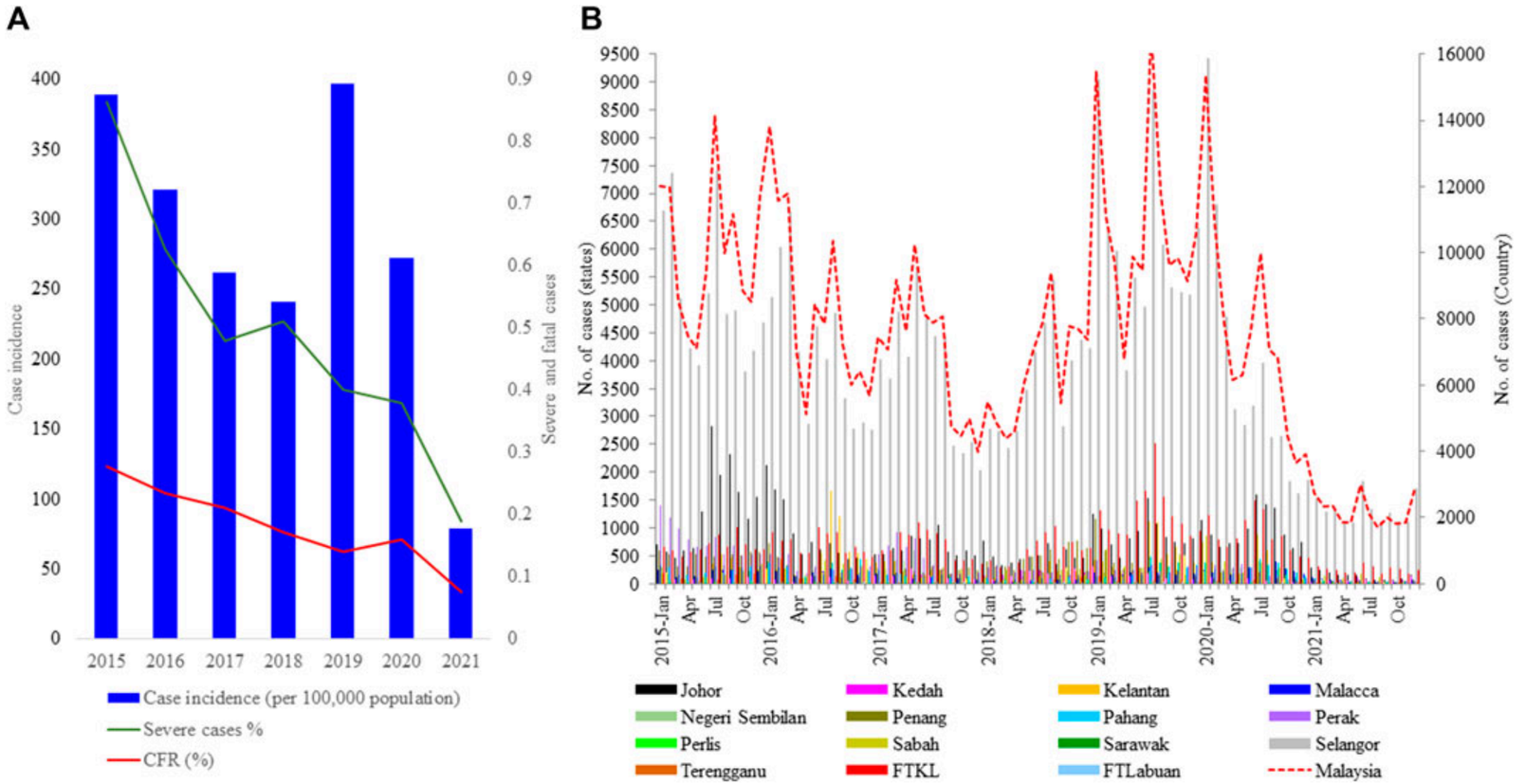
National reported cases

⬇

State-level and temporal aggregation

⬇

National dengue surveillance data analysis



Most of dengue-related fatalities were highest in the same states with the severe and greatest disease burden.

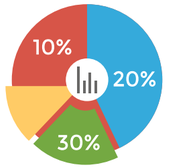
Result 2: Distribution of DENV serotype

Objective To describe patterns of DENV serotype circulation over time and states.

METHOD

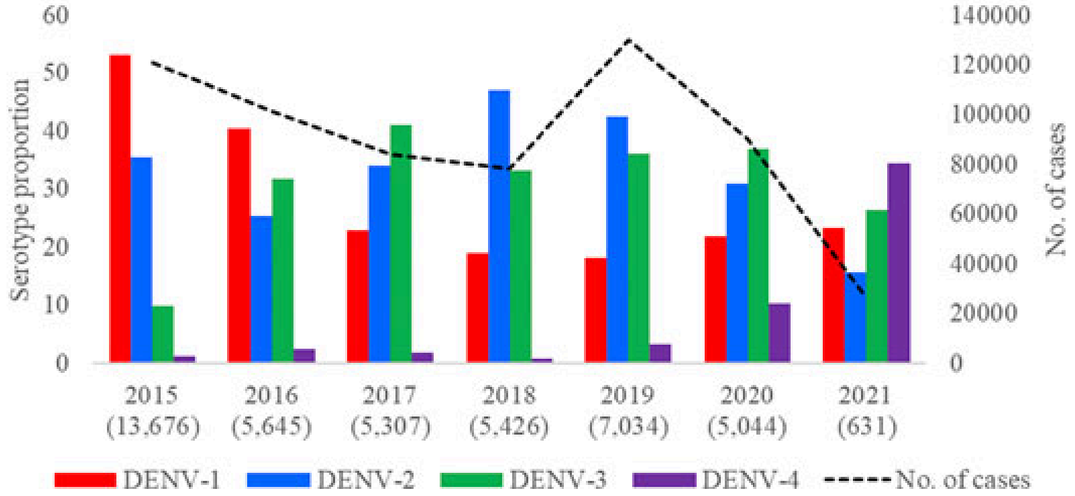
Serum

RNA extraction

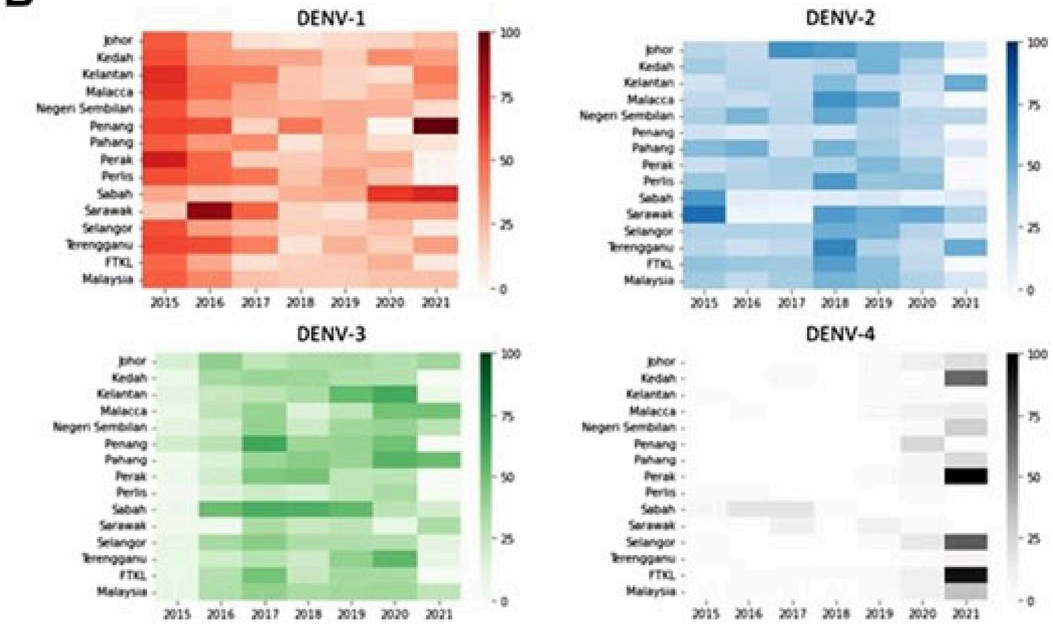


Detection and Serotyping

A



B



The serotype proportion fluctuated over time, dominant periodically and no clear dominance of a single serotype across Malaysia from 2016 to 2021

Result 3: Genotype and Lineage dynamics

Objective To assess dengue virus genetic diversity and infer lineage origins.

METHOD

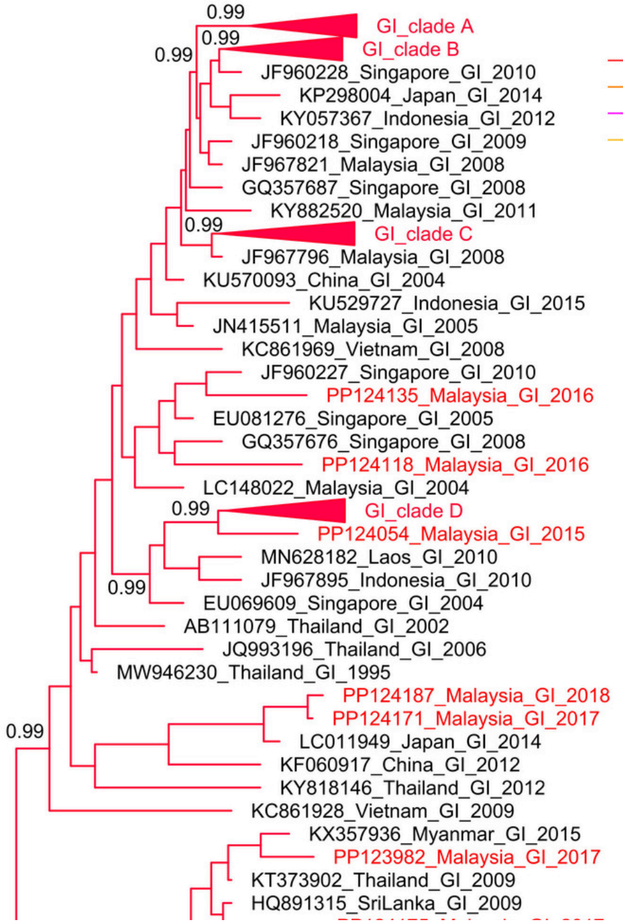
E protein sequence

Malaysian
n = 5,471

Asian
n = 17,732

Maximum likelihood analysis

MEGA



- 18 monophyletic clades across genotypes
- Basal sequences from South/East Asia and Australia

Result 3: Genotype and Lineage dynamics

Objective

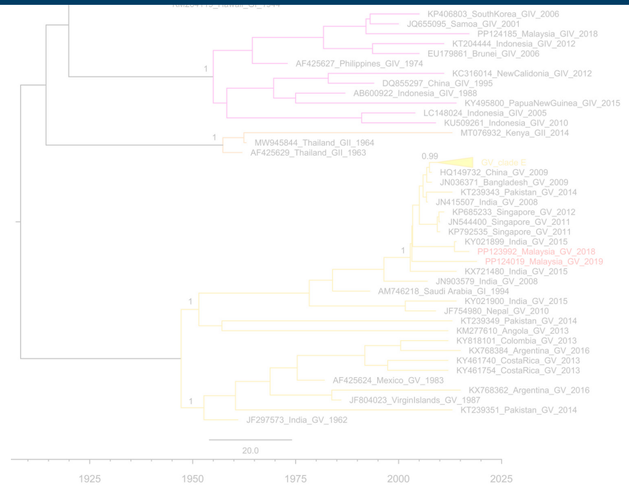
To assess dengue virus genetic diversity and infer lineage origins.

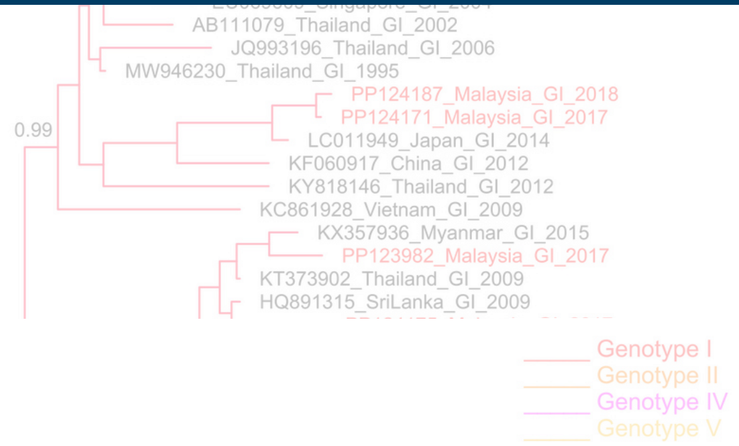
METHOD

National reported cases

Each clade included sequences from many regional countries

- Suggesting the widespread presence of respective clades in South/East Asia
- Indicating the potential sources of their introduction into Malaysia

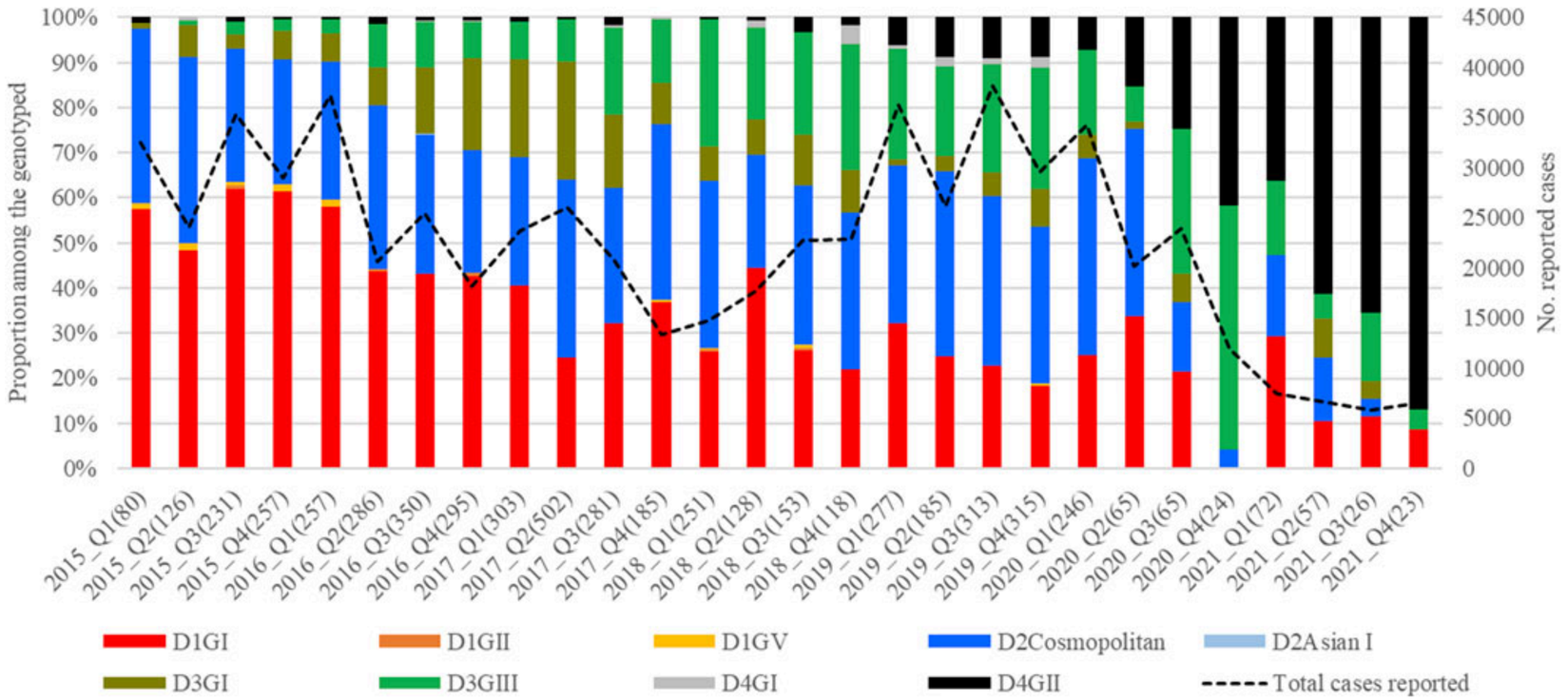


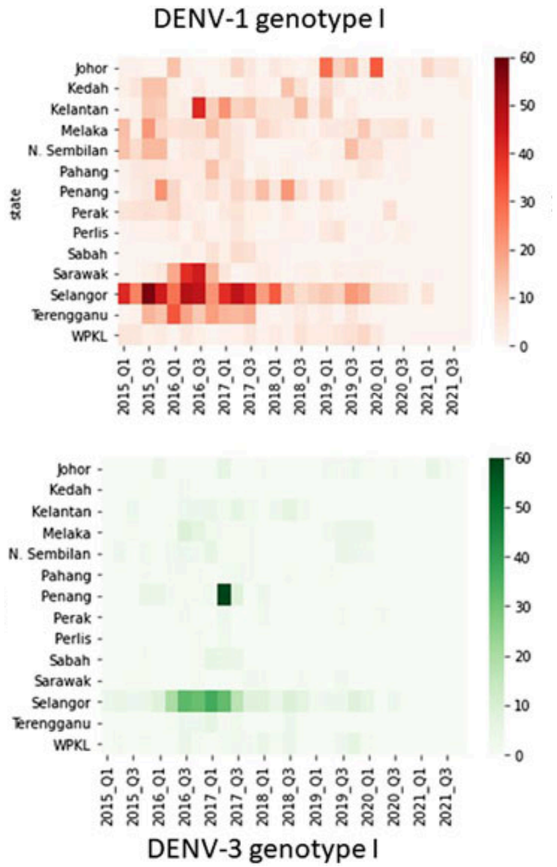


Result 3: Genotype and Lineage dynamics

Objective

To identify evidence of lineage turnover over time and across regions.



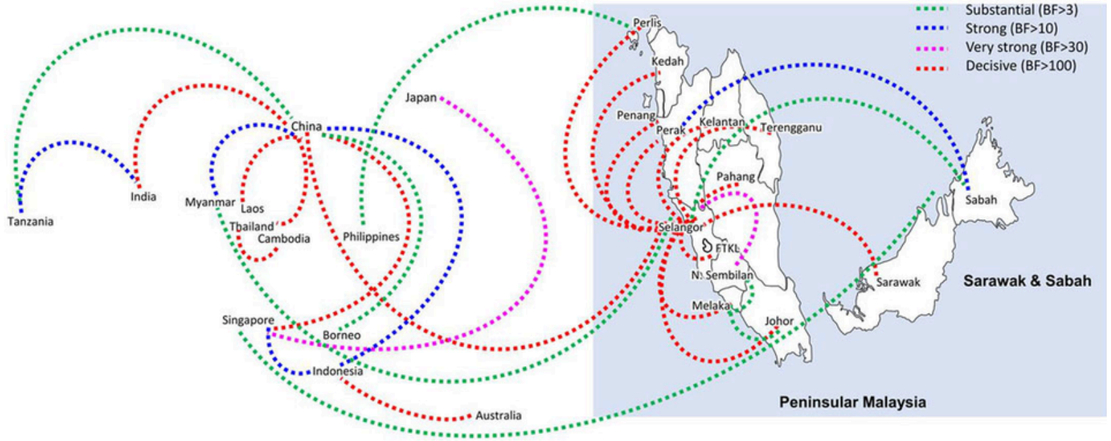


Lineage turnover was associated with changes in case burden and some introduced genotypes failed to sustain local transmission.

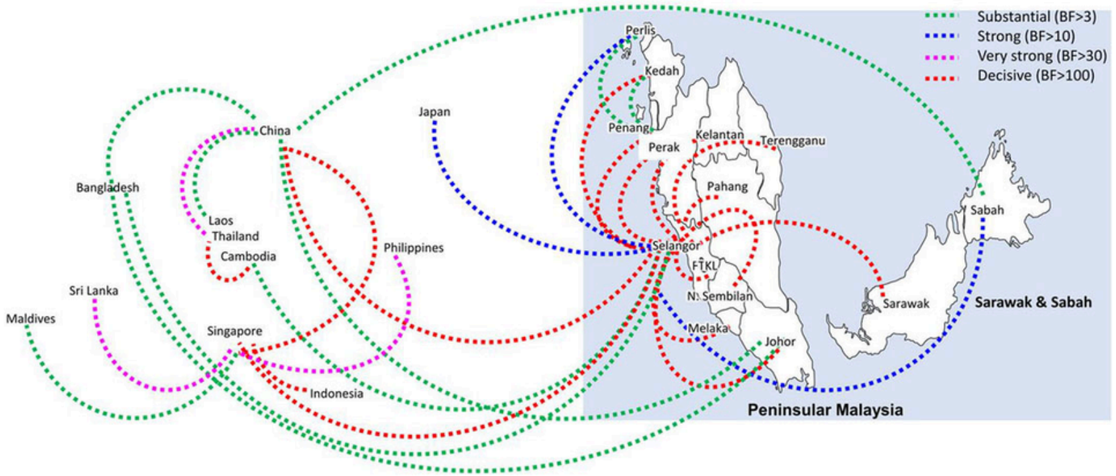
Result 4: Phylogeography analysis of E protein

Objective

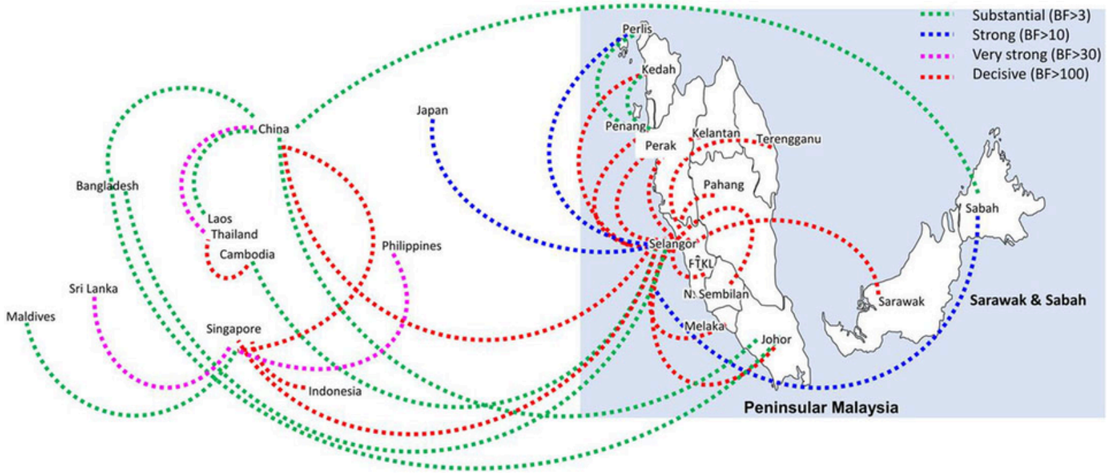
To reconstruct dengue virus spread and identify key domestic and regional hubs.



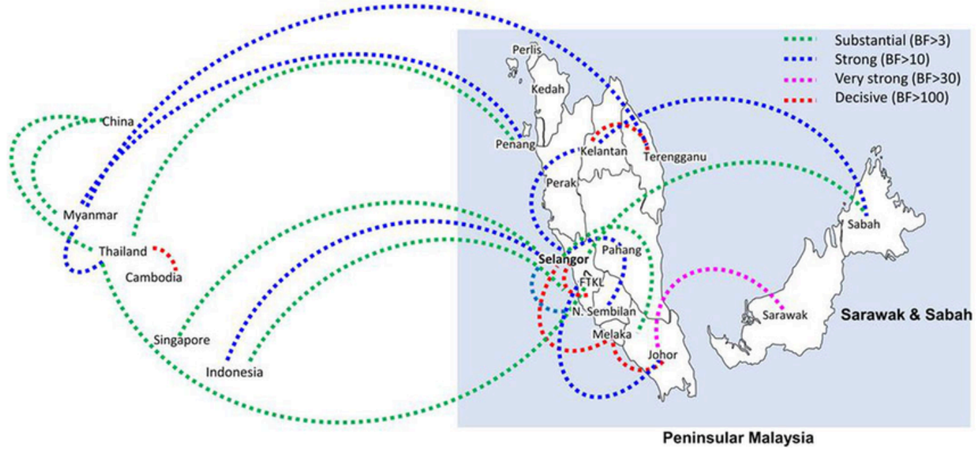
DENV-1



DENV-2



DENV-3

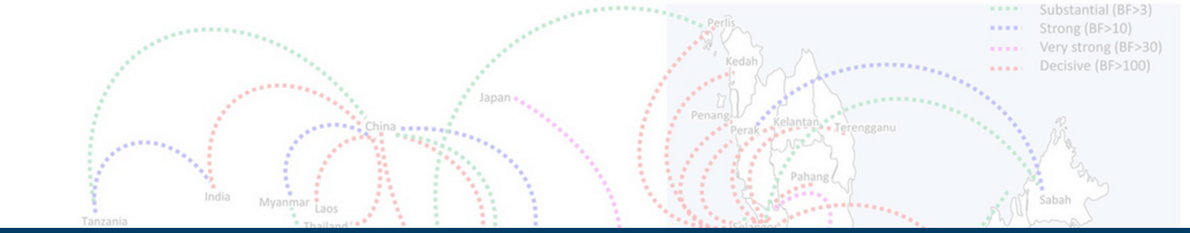


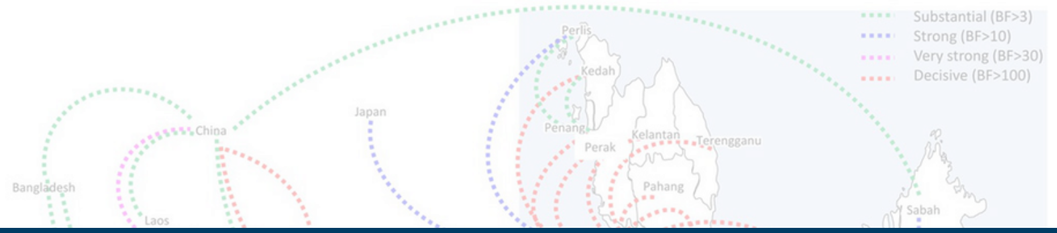
DENV-4

Result 4: Phylogeography analysis of E protein

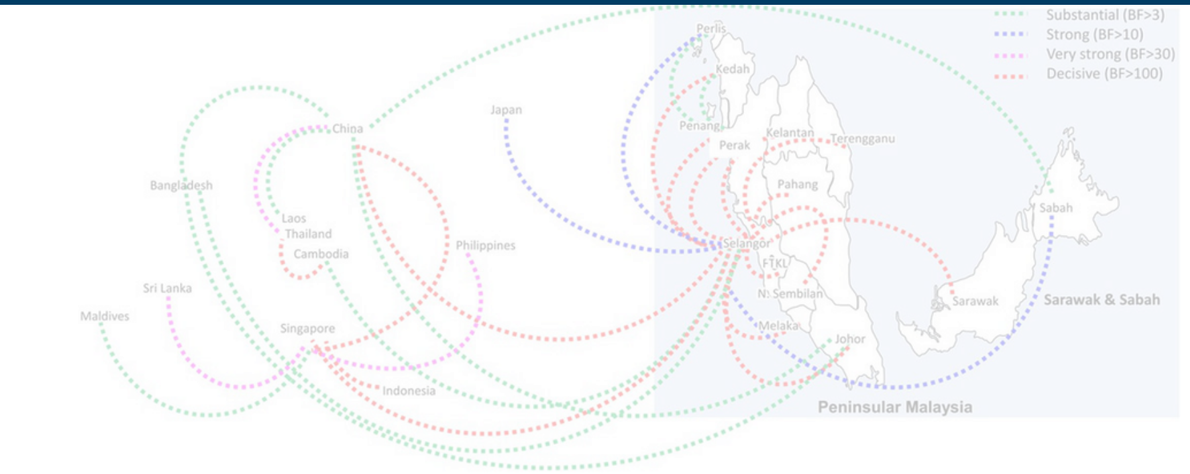
Objective

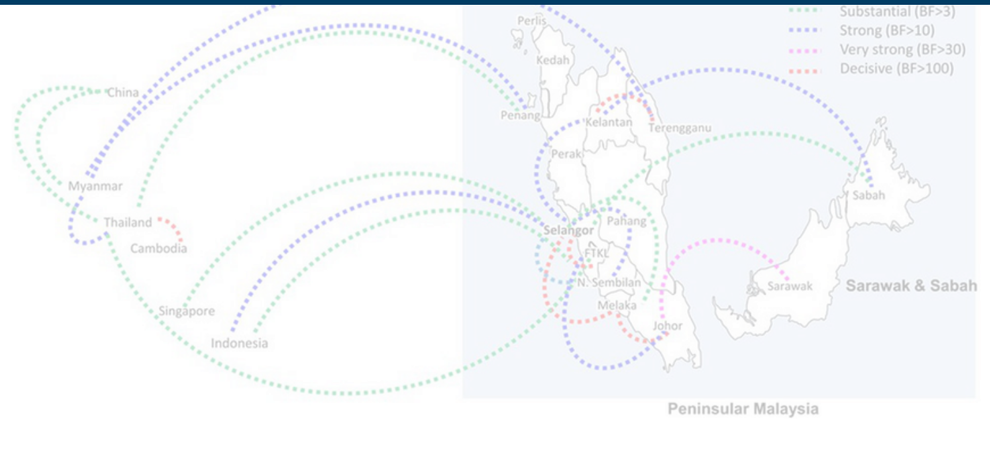
To reconstruct dengue virus spread and identify key domestic and regional hubs.





Dengue virus transmission occurs predominantly within Malaysia with **Selangor acting as a major hub linking multiple states** while international introductions play a limited role in sustaining transmission.

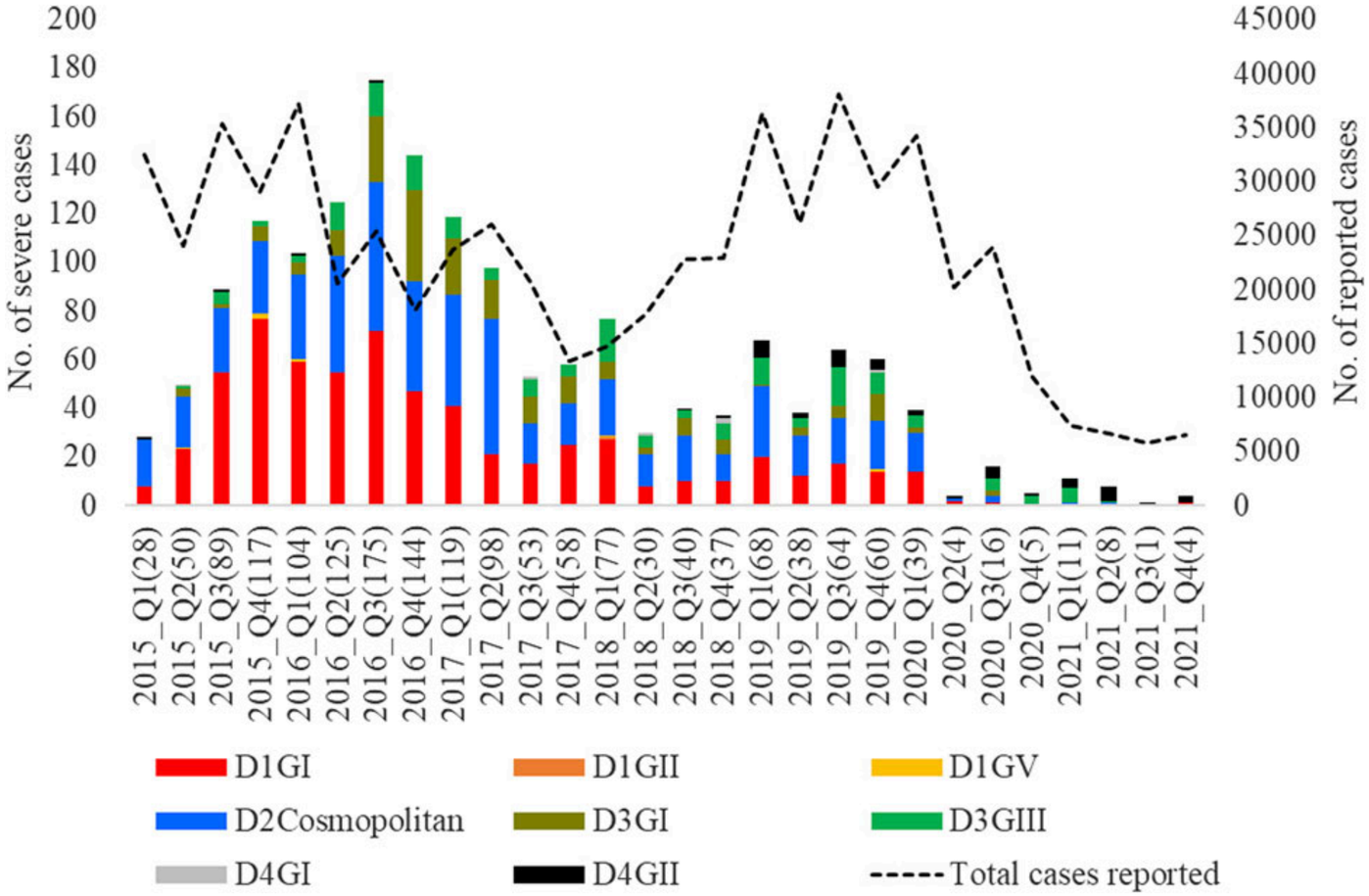




Result 5: Genotype and dengue severity

Objective

To evaluate whether specific dengue virus genotypes are associated with increased disease severity.



Genotype I of DENV-1 and **cosmopolitan of DENV-2** collectively **contributed the highest number of severe infections** in each year from 2015-2020.

Conclusion

- Viral lineages were repeatedly introduced but **only a subset successfully sustained local transmission** highlighting the importance of domestic spread through **highly connected urban hubs.**

- **Severe dengue is associated with viral genotypes** but severity patterns largely reflect genotype prevalence highlighting the importance of host and epidemiological factors.

Criticisms

	First Paper	Second paper
Strong point	• Provides inference of both the **direction** and **relative rates of viral movement** between regions • Enables **more accurate reconstruction** of evolutionary history.	• Comprehensive comparison **across serotypes.** • Links viral genotypes with **disease severity.**
Weak point	• **Limited sampling density and focus on DENV-2** constrain inference of sustained local transmission and broader dengue dynamics.	• Phylogeographic analyses infer gene flow between locations but **cannot reconstruct exact transmission pathways** or directional movement with certainty. • **Lower molecular resolution** for individual lineages.

Acknowledgement

Advisor

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THANK YOU FOR YOUR ATTENTION