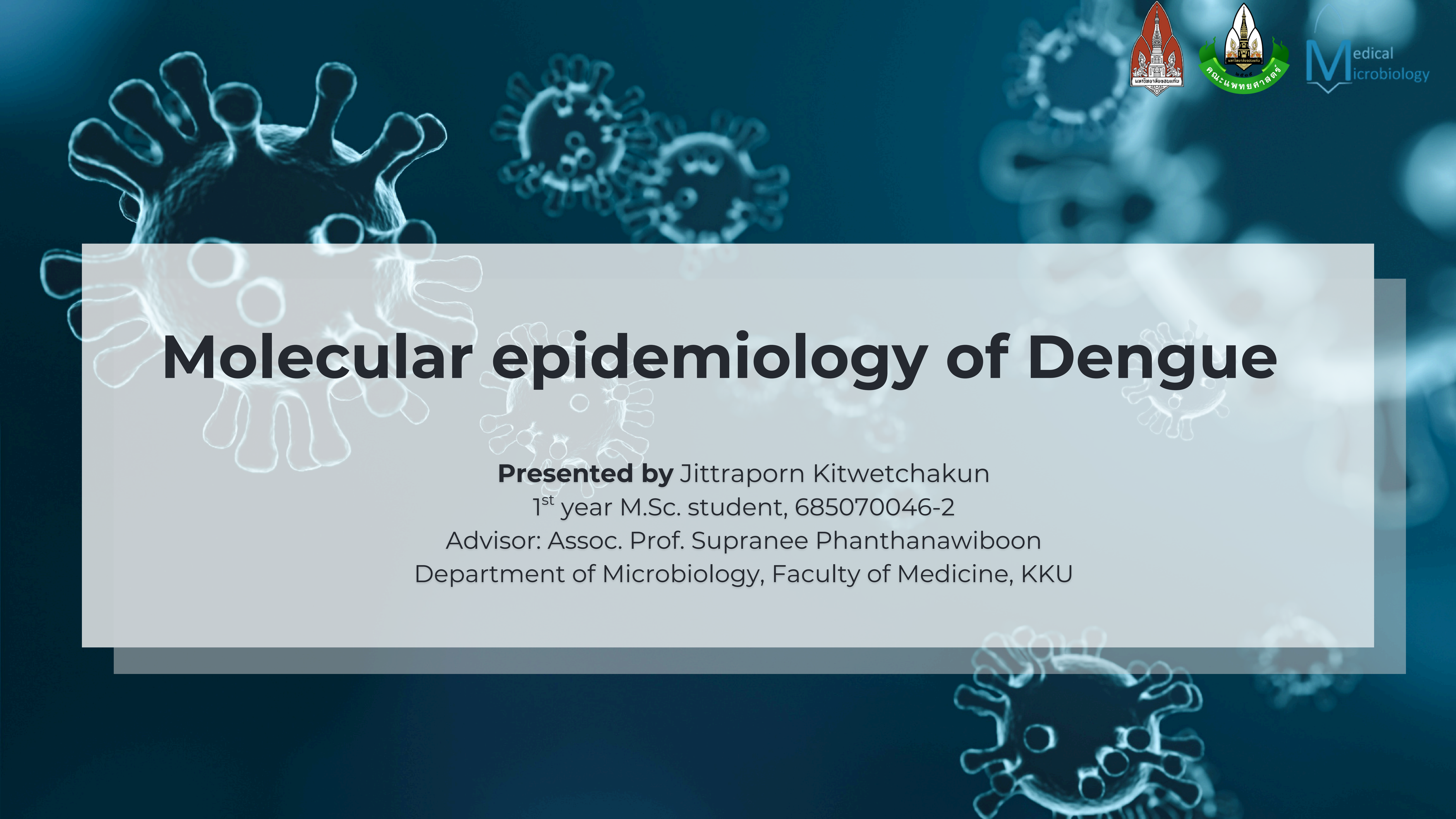
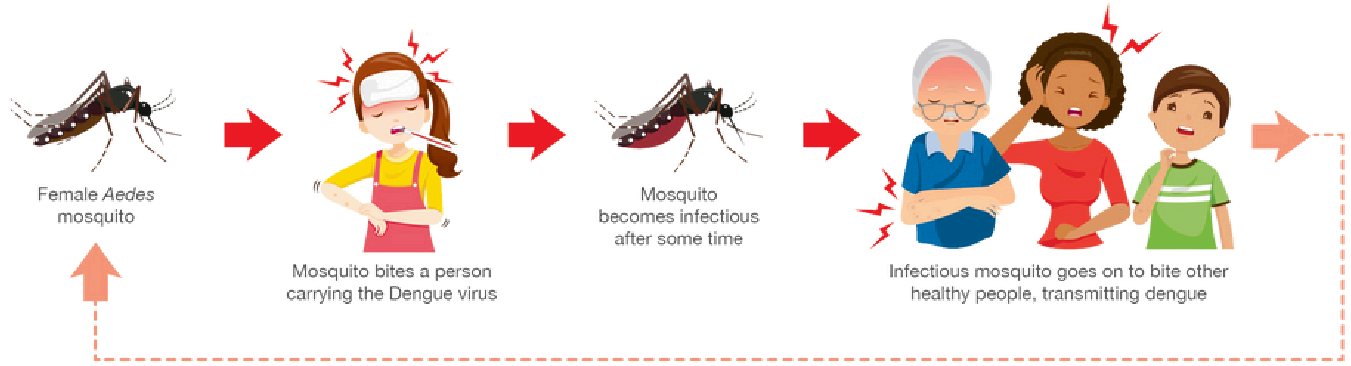


Molecular epidemiology of Dengue

Presented by Jittraporn Kitwetchakun
1st year M.Sc. student, 685070046-2
Advisor: Assoc. Prof. Supranee Phanthanawiboon
Department of Microbiology, Faculty of Medicine, KKU

Background on Dengue

DENGUE FEVER



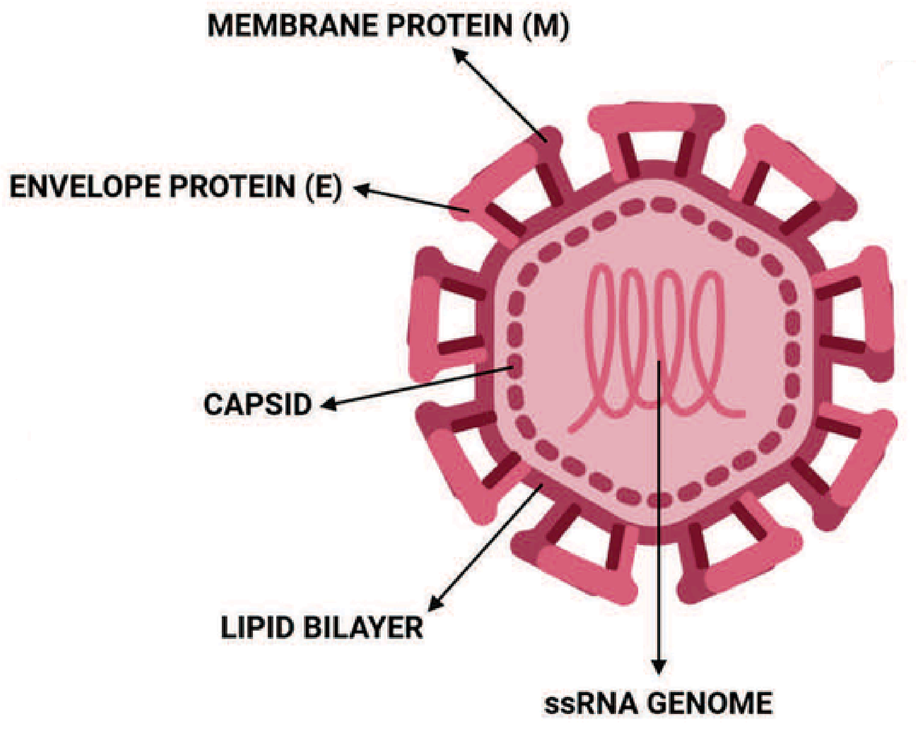
- Causes **a hundred million** dengue cases annually, with significant morbidity and mortality.
- **No specific antiviral drugs** and **vaccine provide limited** or incomplete.

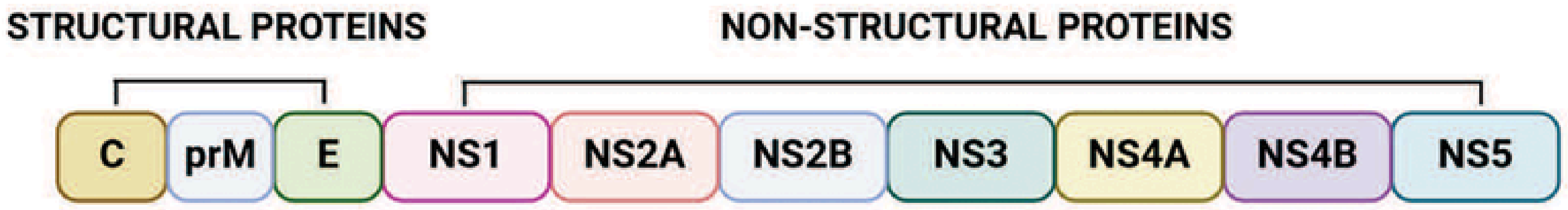
Factors

- ▶ Climate change
- ▶ Large urban center
- ▶ Globalization

DENGUE VIRUS

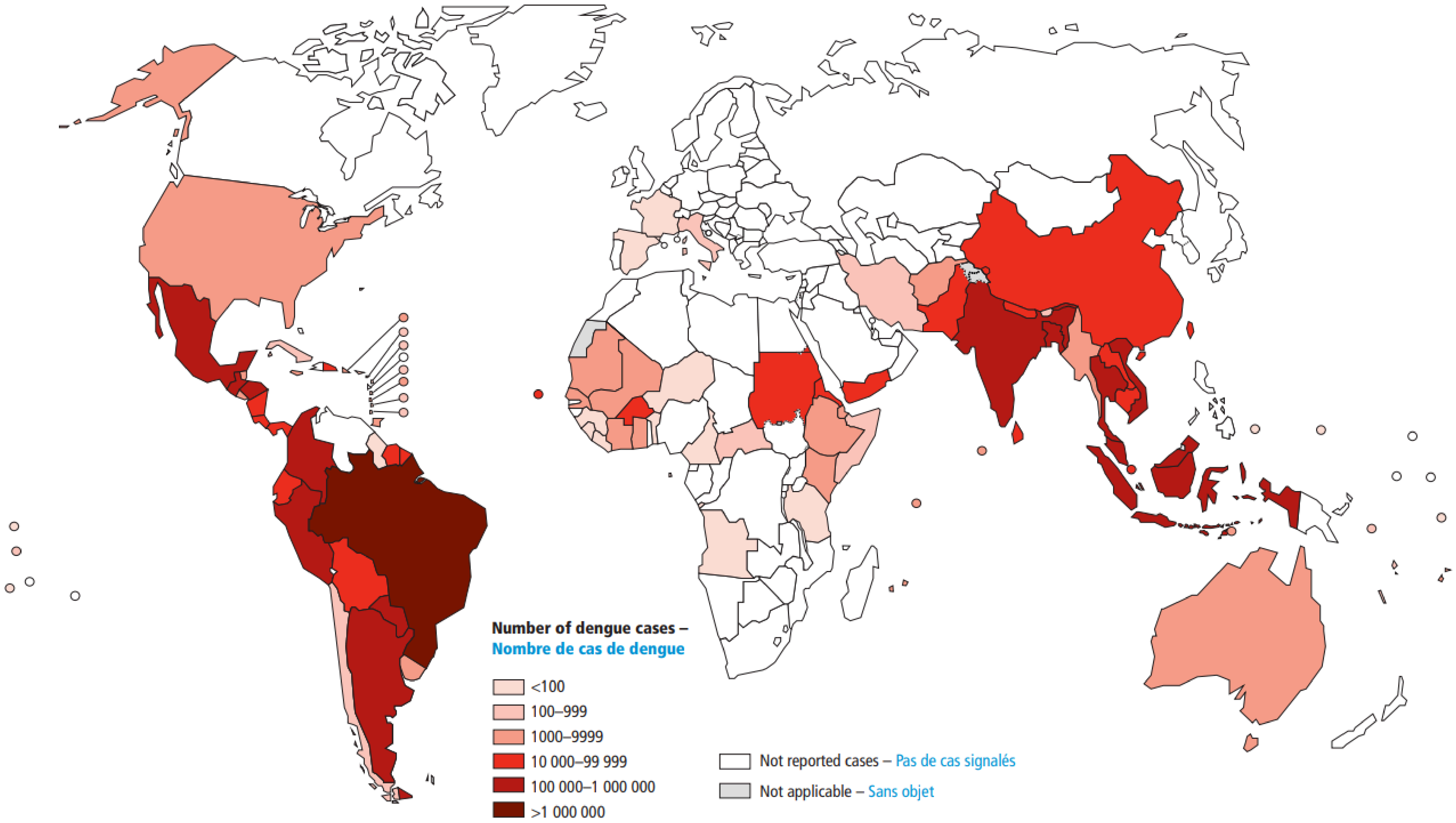
- Genus : Flavivirus
- Family : Flaviviridae
- Genome : +ssRNA enveloped virus
- 4 serotype : DENV-1 to DENV-4





Ruvalcaba-Hernández et al., 2025

Dengue burden trend



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

Uses genetic data alongside epidemiological information to understand transmission patterns, evolutionary changes, and the spread of pathogens across populations, locations, and time.

Transmission

Reveals how dengue spreads over time and space

Serotype shifts

Identify genotype replacement & emergence of new lineages

Outbreaks

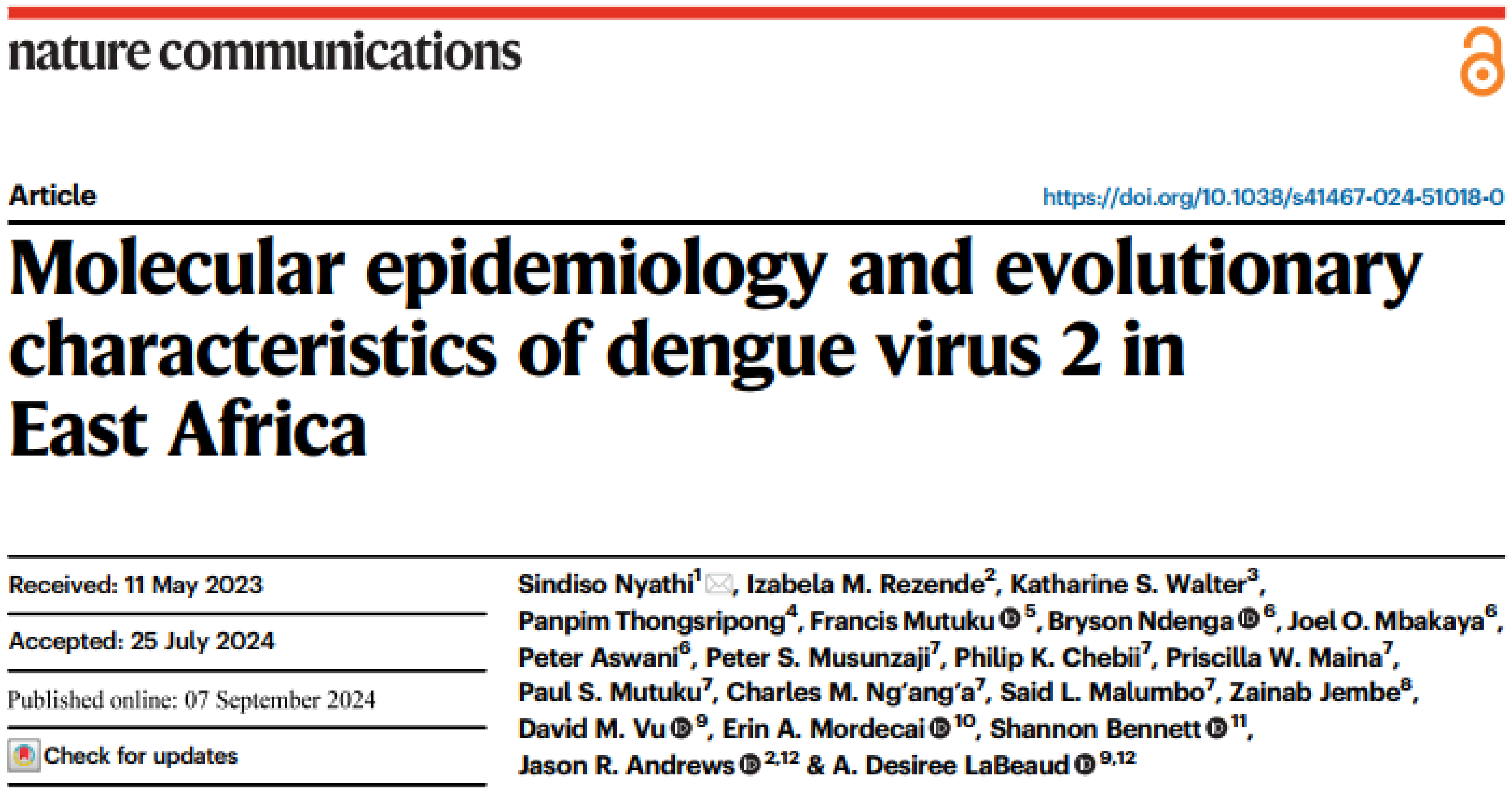
Improves outbreak prediction

Public health

Supports evidence-based public health interventions.

Examining the molecular epidemiology of DENV between Thailand and Laos provides a strong foundation for understanding regional transmission dynamics and informing targeted surveillance strategies.

Seminar papers



nature communications

Article

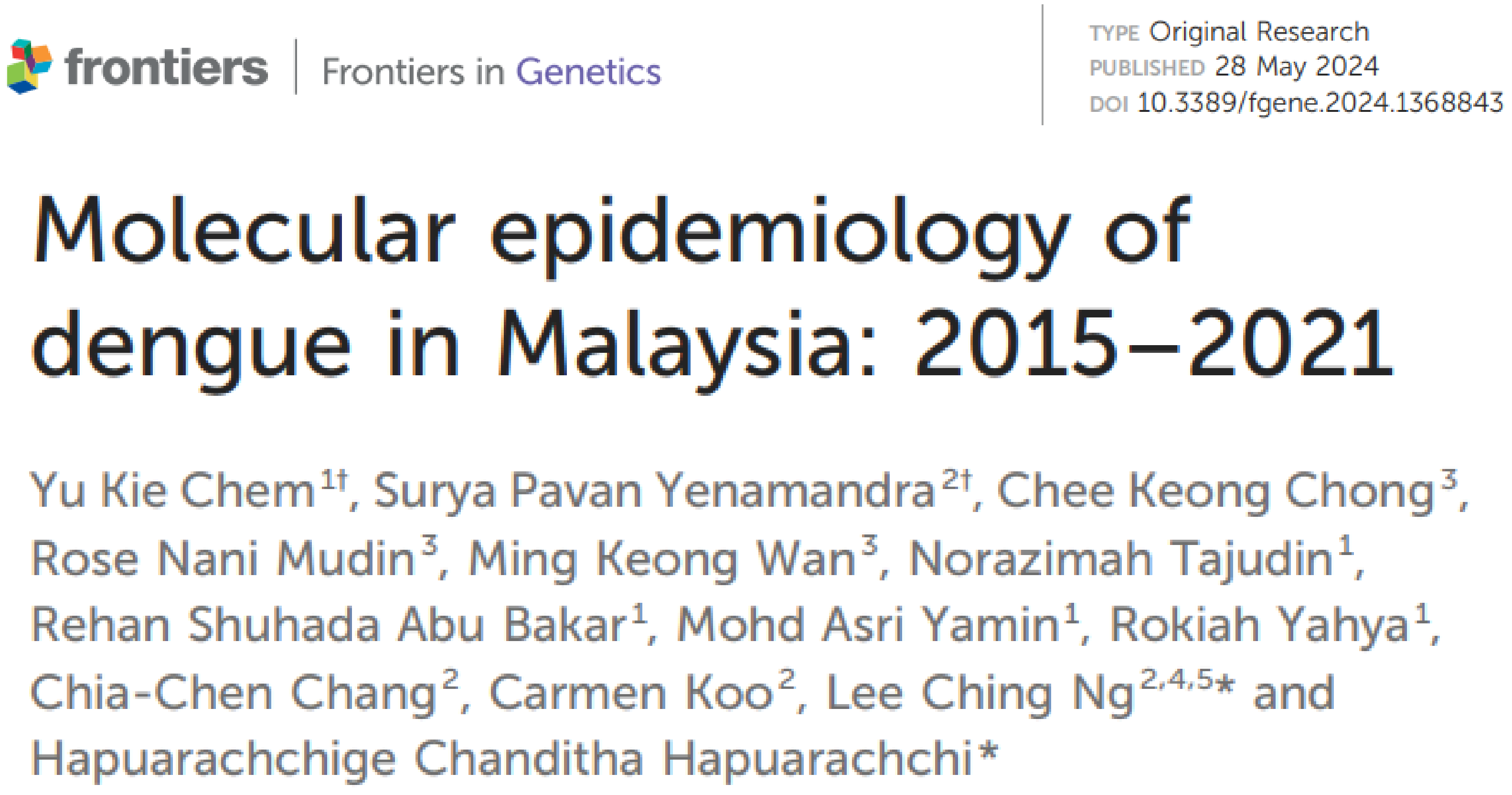
https://doi.org/10.1038/s41467-024-51018-0

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

Received: 11 May 2023

Accepted: 25 July 2024

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]



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*

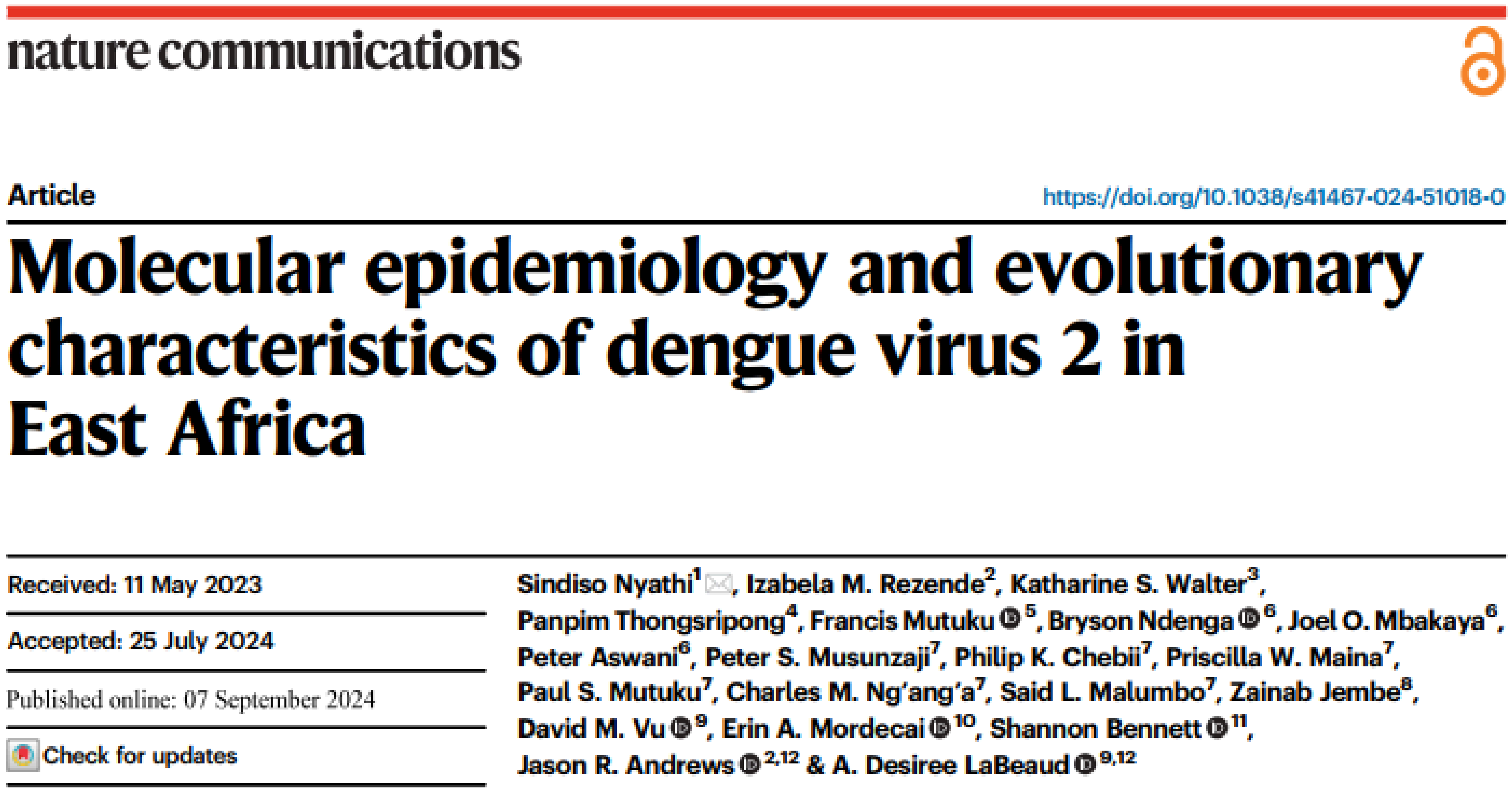
Nature communications

Published in 2023, IF 15.7 (Q1)

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

Received: 11 May 2023

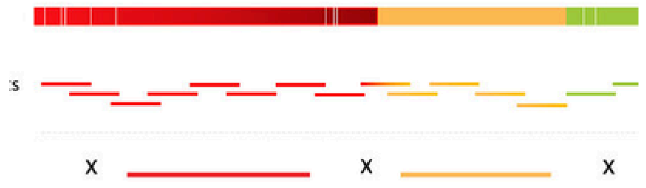
Accepted: 25 July 2024

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]

1 Objectives

- To characterize the genetic diversity of DENV-2 circulating in East Africa
- To determine viral origins, introduction events, and spread patterns
- To assess evolutionary rates and persistence of DENV-2 lineages in the region

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 virus 2 sequences isolated from Kenya

Objective To investigate dengue virus diversity distribution

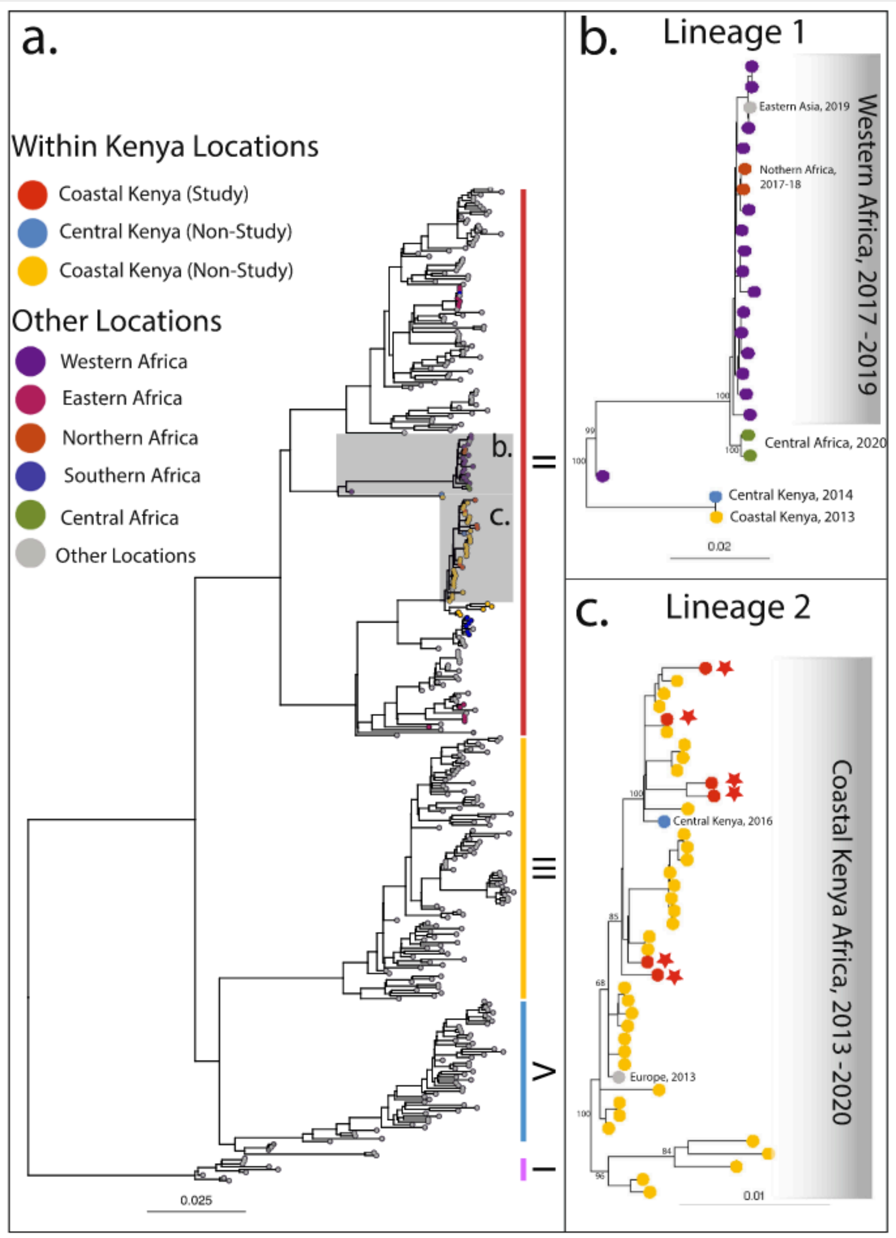
METHOD

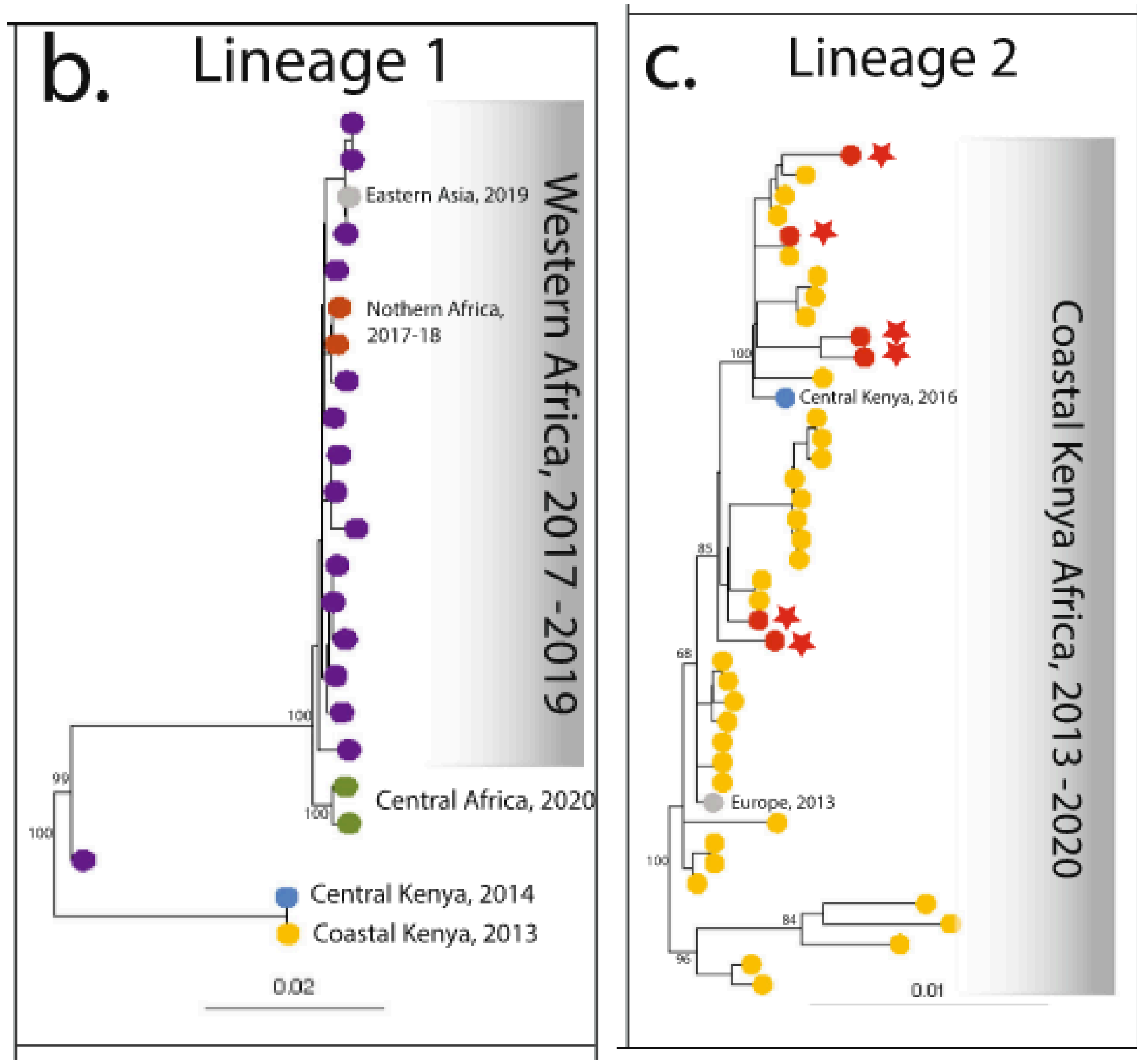
Dengue detection

Whole-genome NGS

Multiple sequence alignment with global sequence

ML phylogenetic reconstruction





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

Result 1: Dengue virus 2 sequences isolated from Kenya

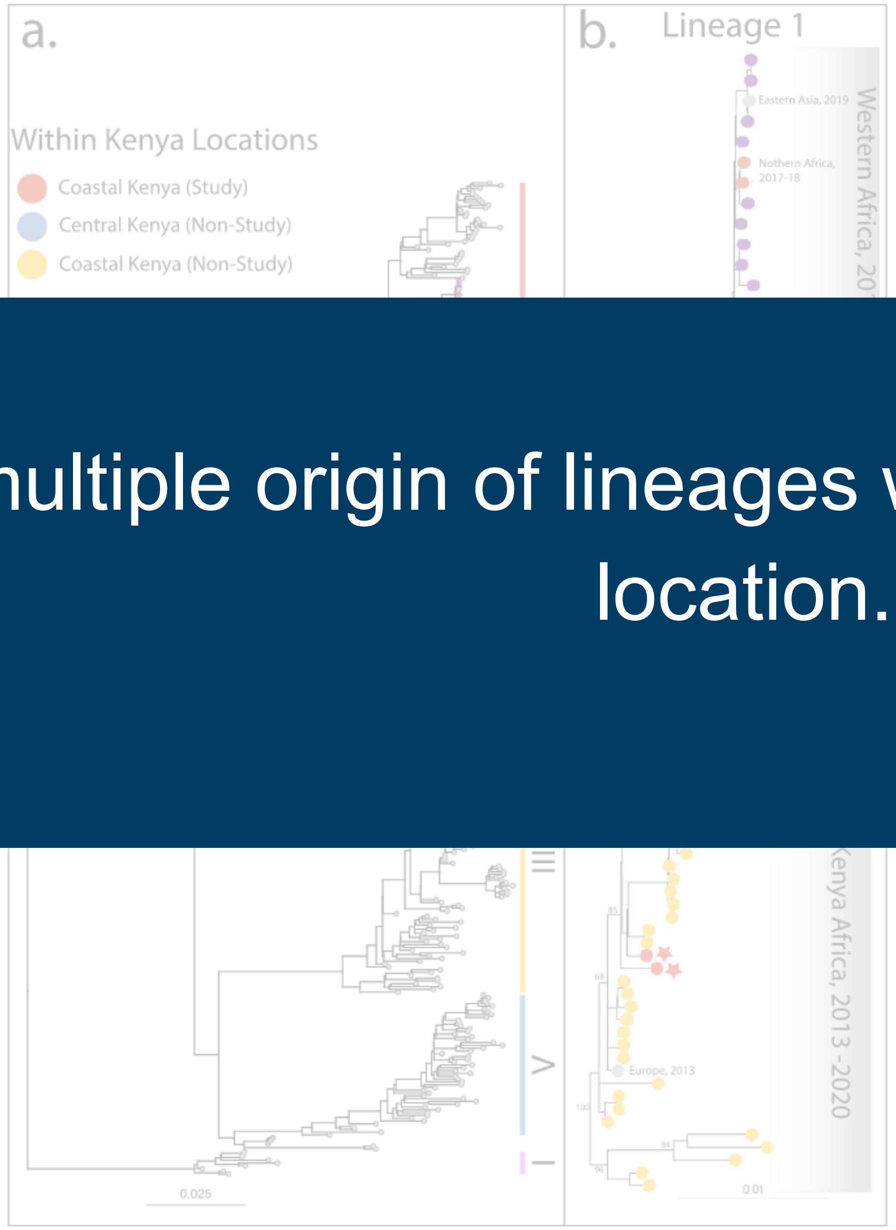
Objective To investigate circulating dengue virus diversity

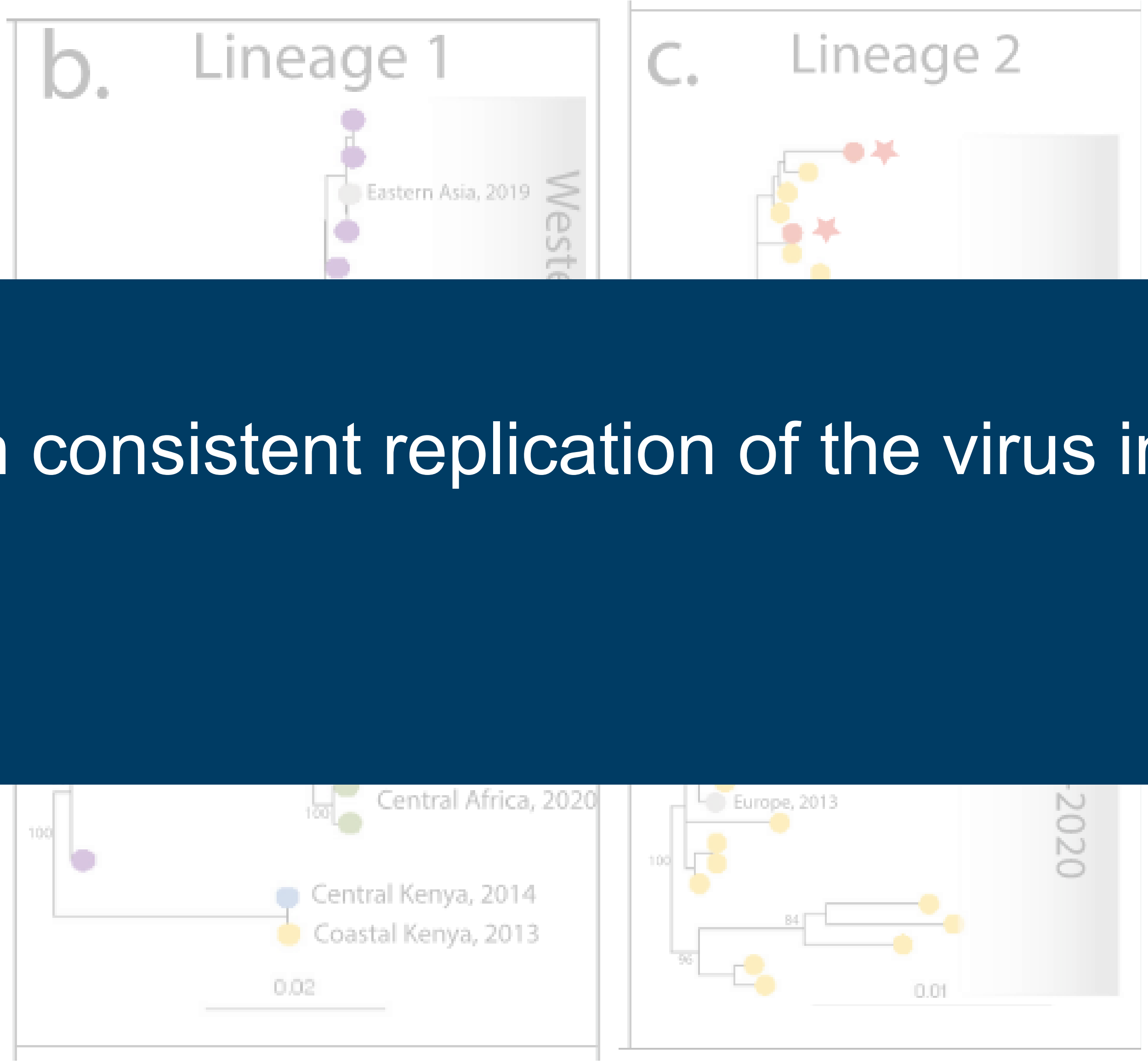
METHOD

Dengue detection

Multiple sequence alignment (MAFFT) with global

ML phylogenetic reconstruction (IQ-TREE)





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 in Kenya and assess their temporal emergence.

METHOD

temporal signal

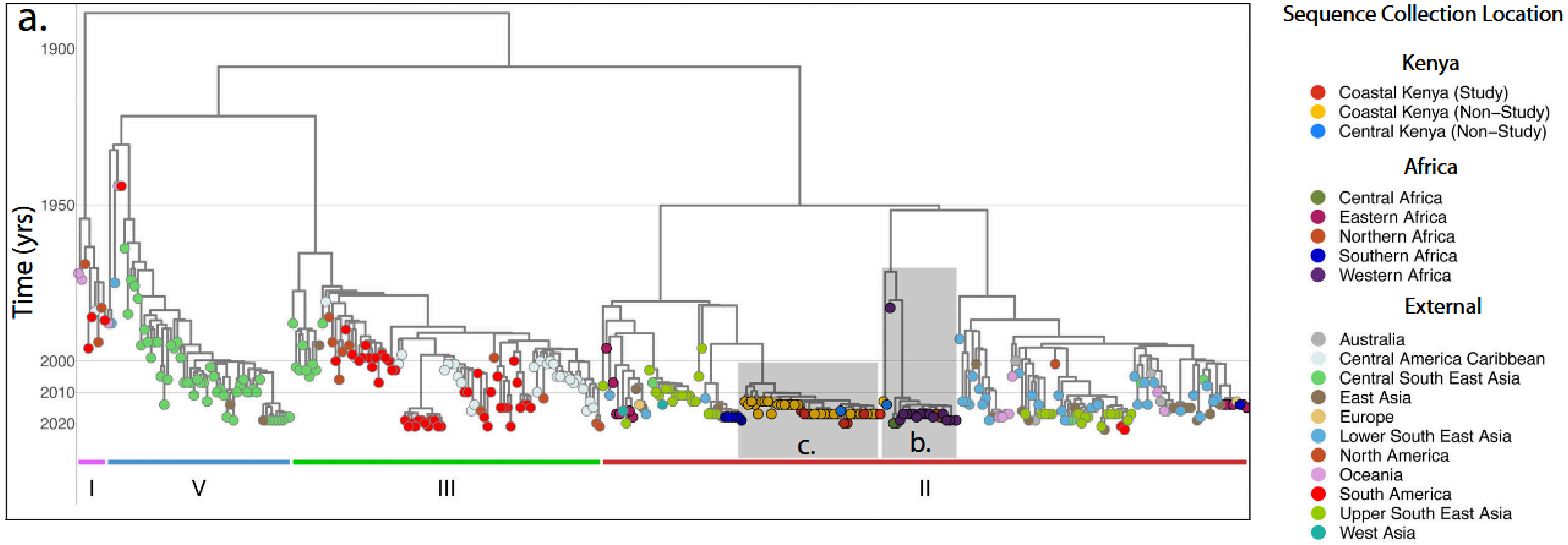
⬇

Bayesian phylogenetic analysis

BEAST X
Bayesian Evolutionary Analysis Sampling Trees

⬇

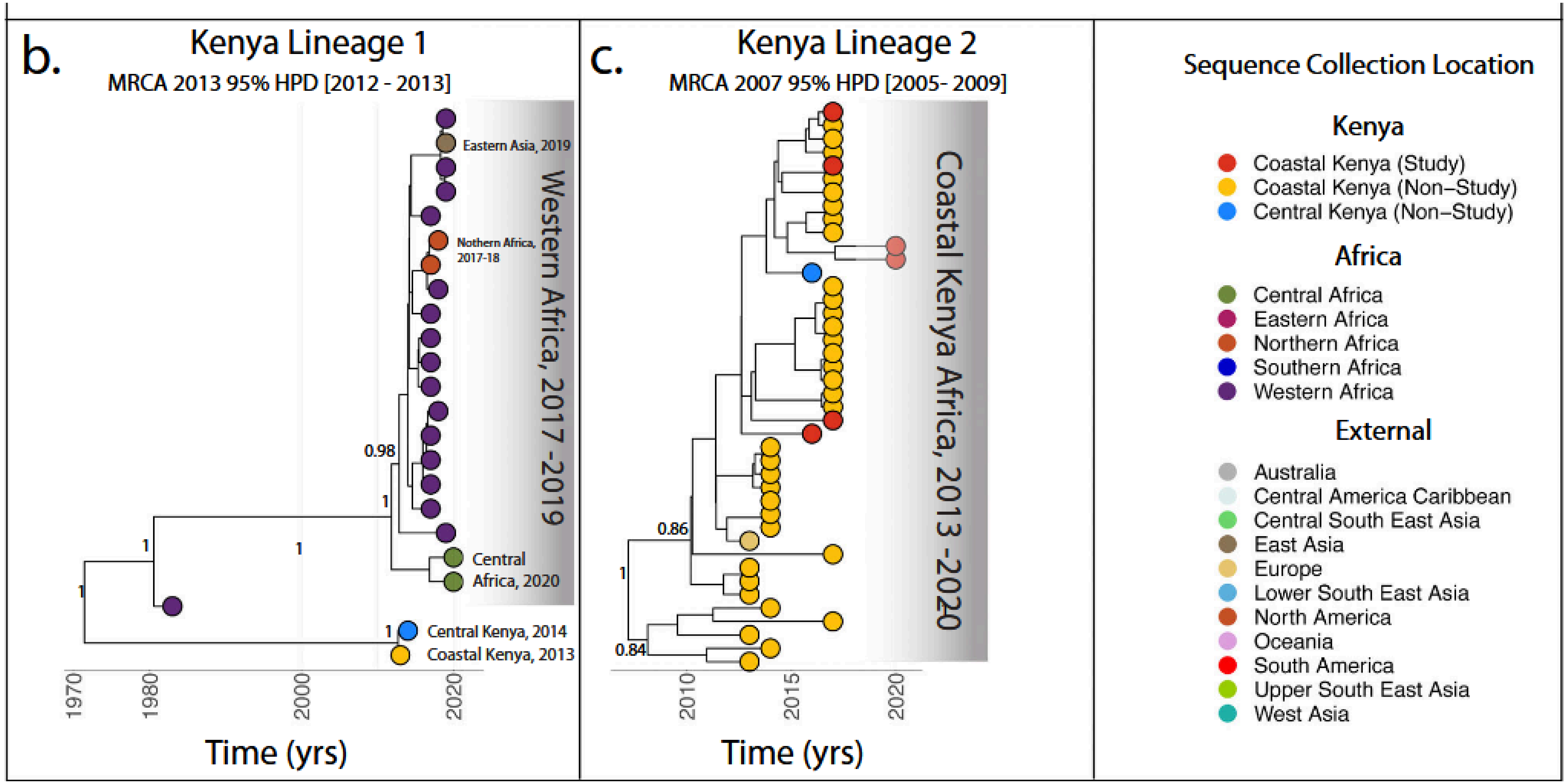
Time-scaled phylogenetic tree



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.



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.

Result 3: Dengue virus 2 phylogeography

Objective To determine the origins and introduction events of DENV-2 into Kenya.

METHOD

Time-scaled tree

⬇

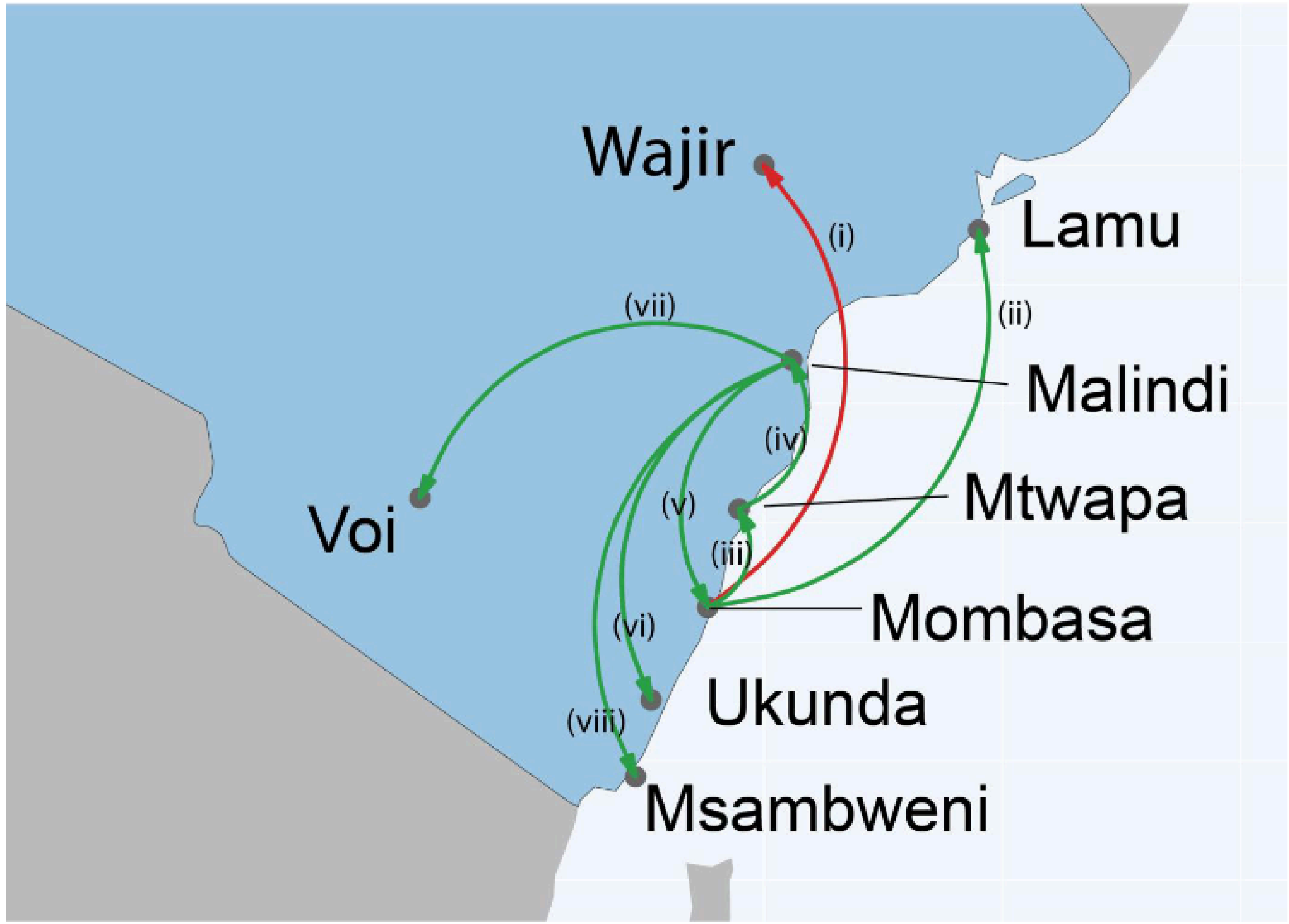
Discrete phylogeographic modeling

⬇

Markov process

⬇

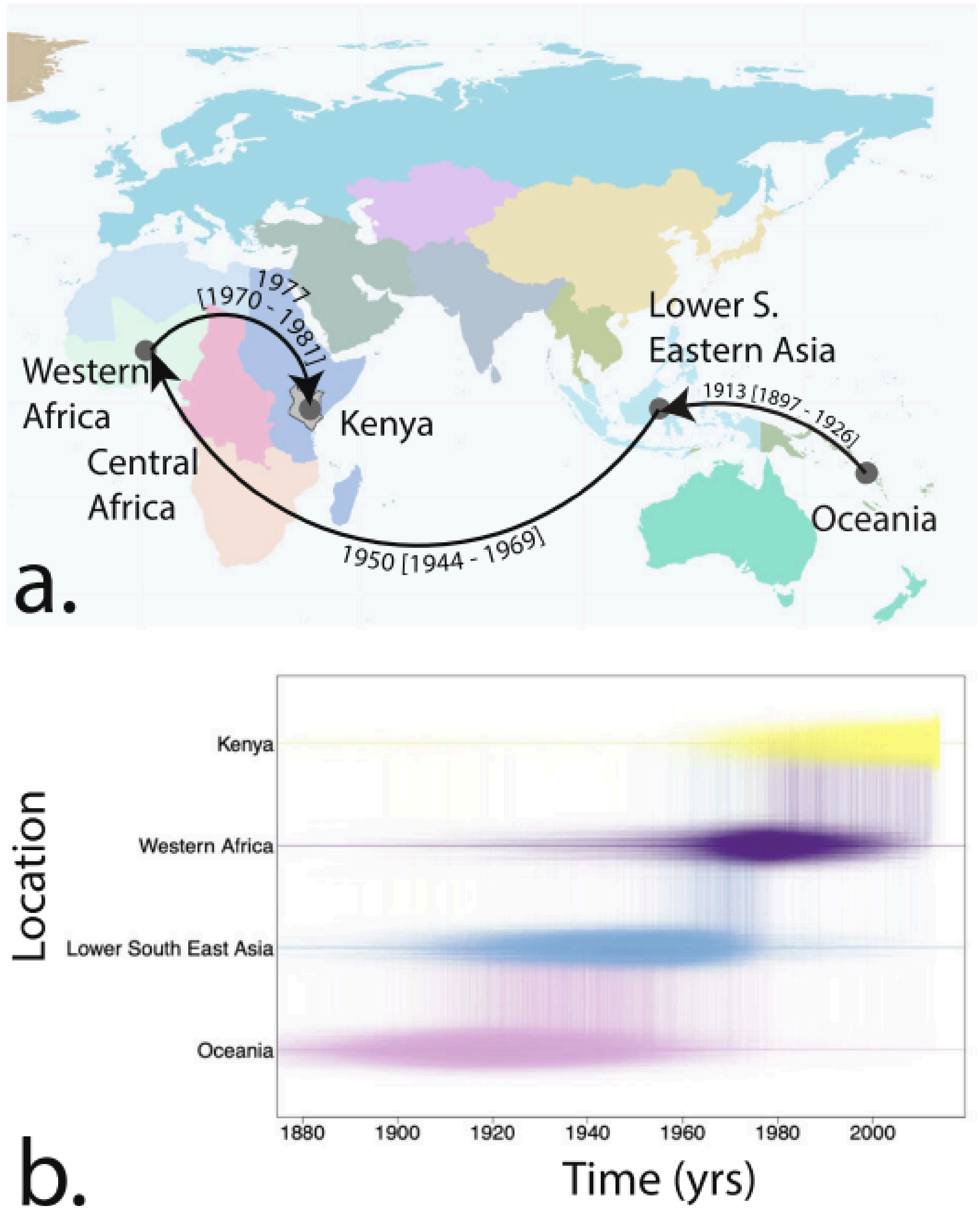
Bayesian Stochastic Search Variable Selection

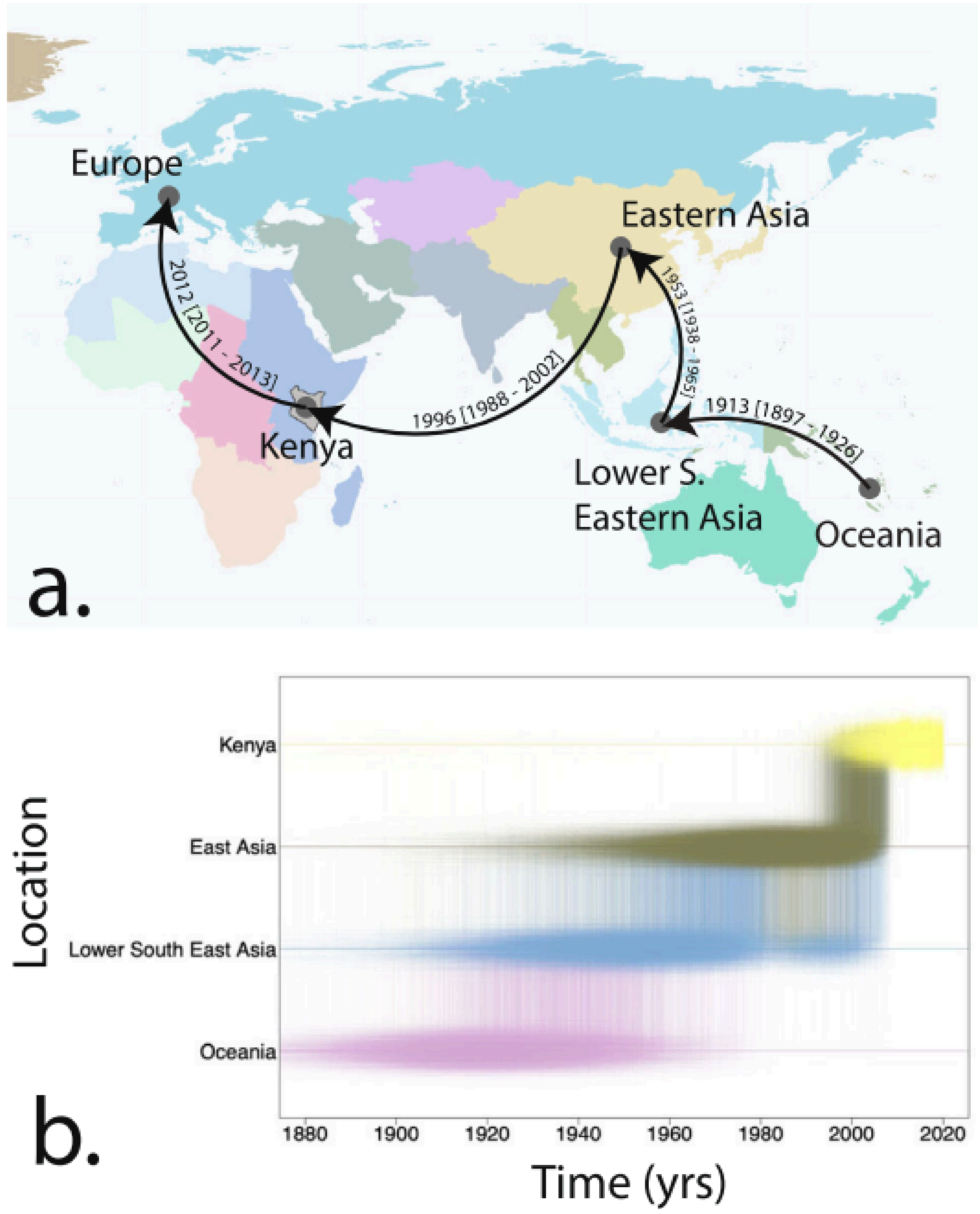


The viral movement is concentrated within coastal regions with coastal sites acting as hubs for local transmission.

Result 3: Dengue virus 2 phylogeography

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



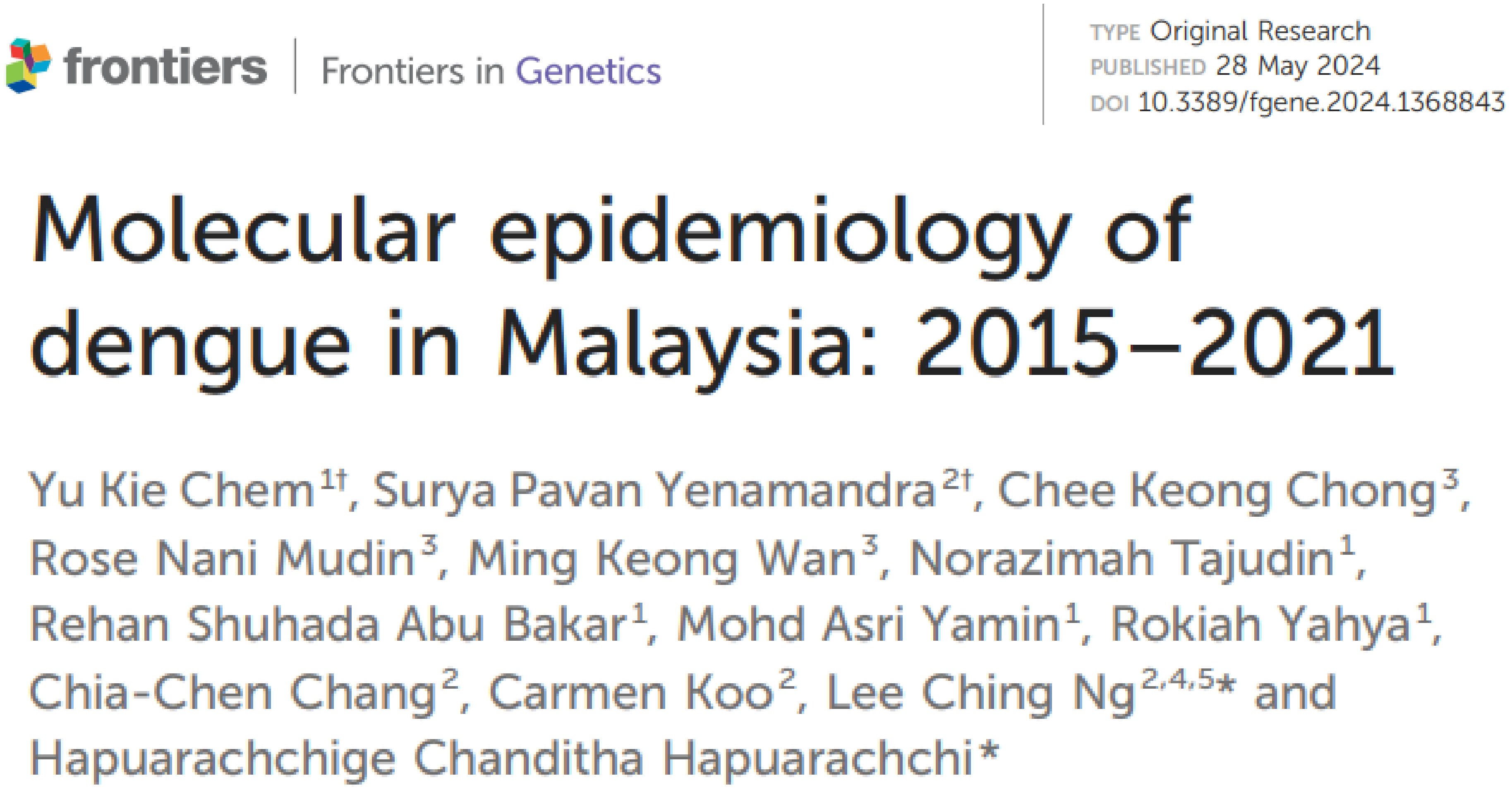


Despite these different entry points, both lineages trace their deeper evolutionary origins to Oceania and Lower Southeast Asia

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.

OVER VIEW

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

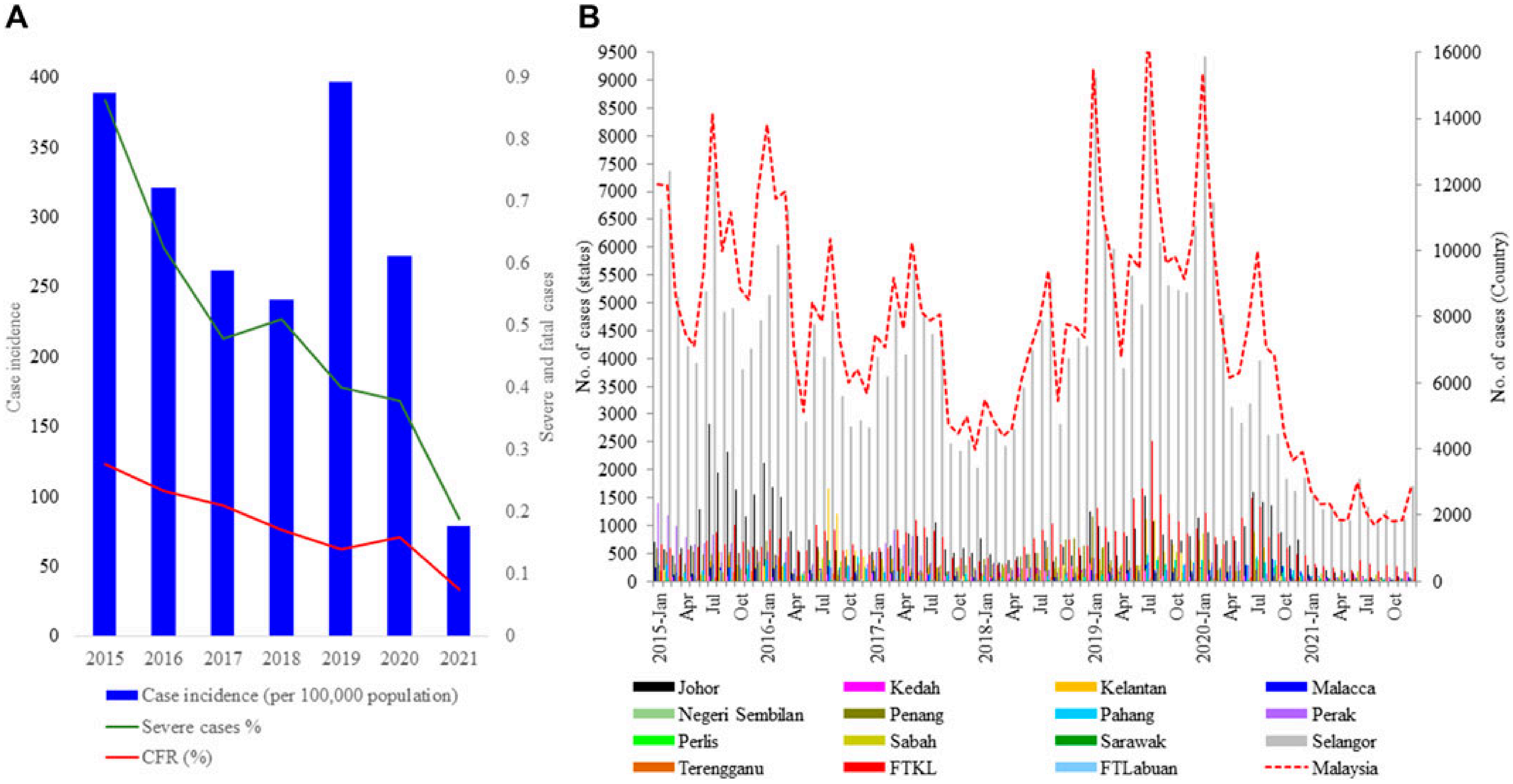
National reported cases

⬇

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.

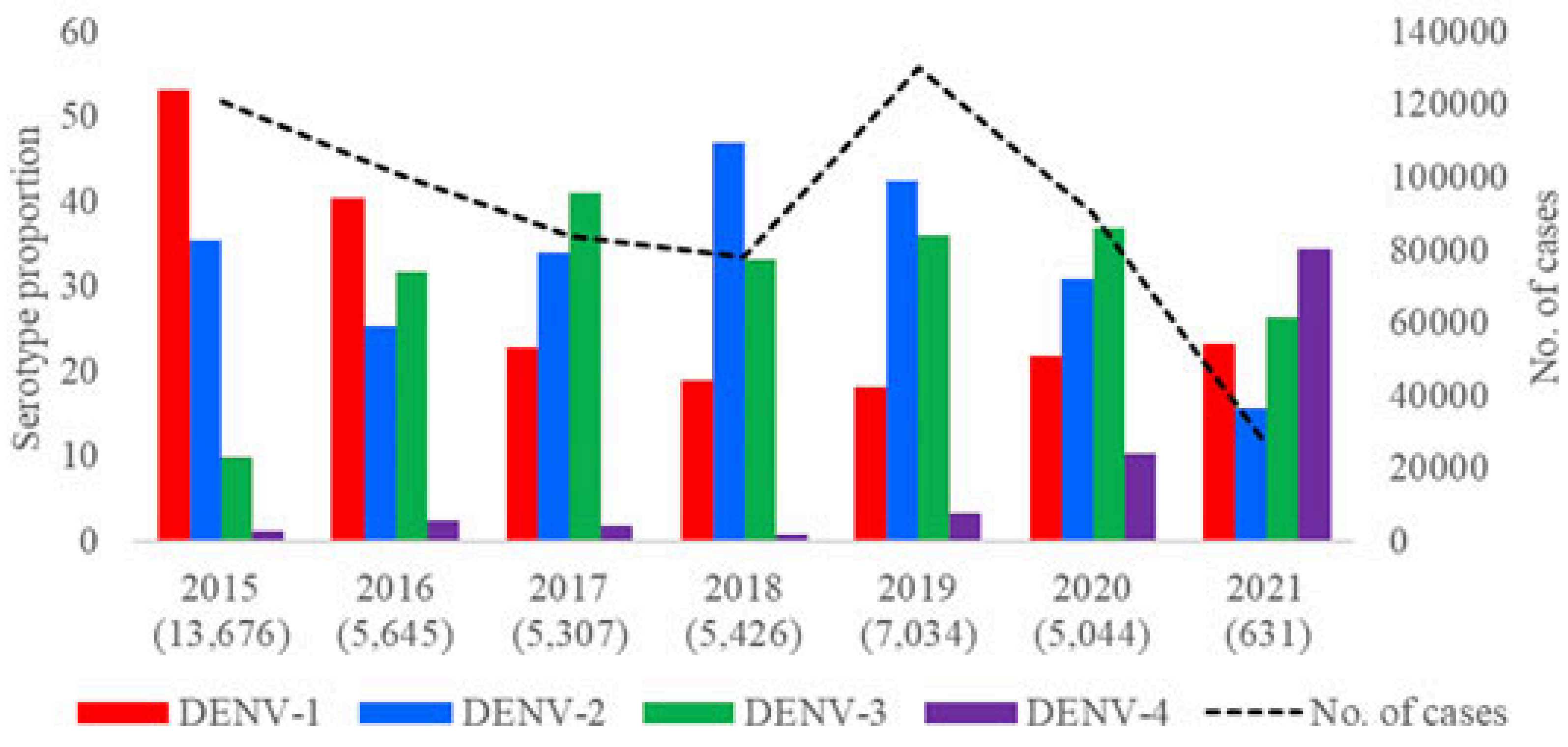
Result 2: Distribution of DENV serotype

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

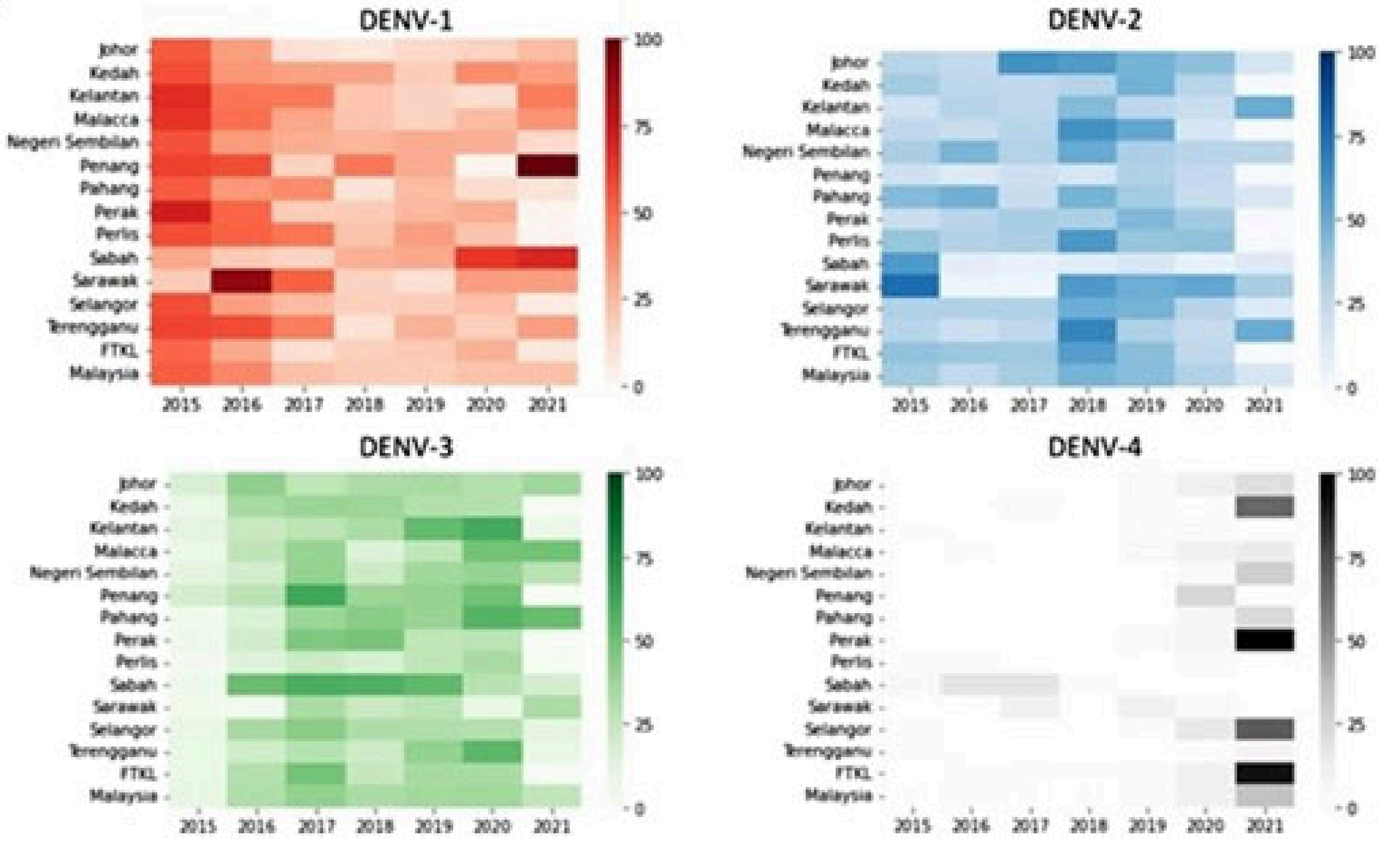
METHOD

Serum → RNA extraction → 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

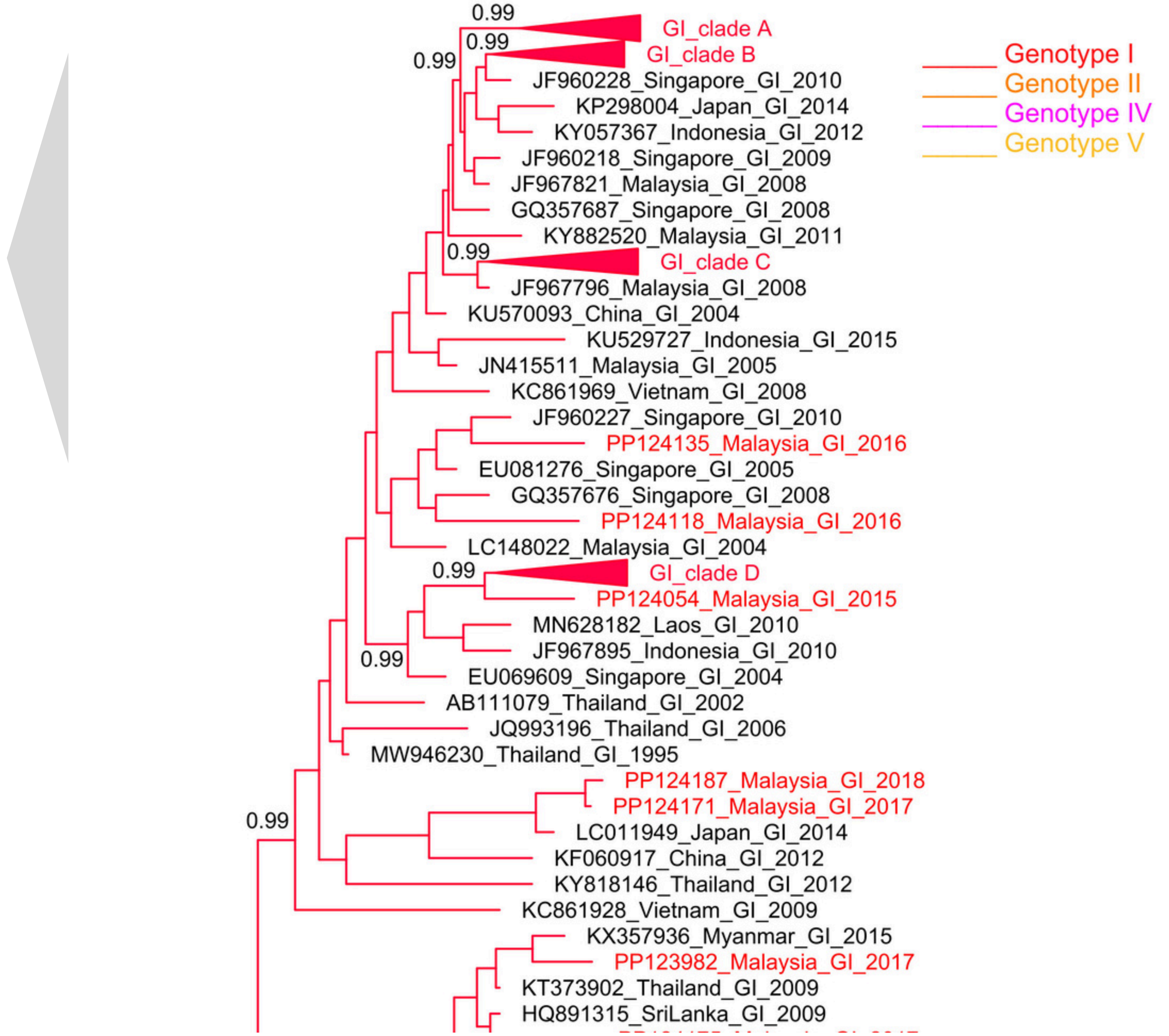
E protein sequence

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

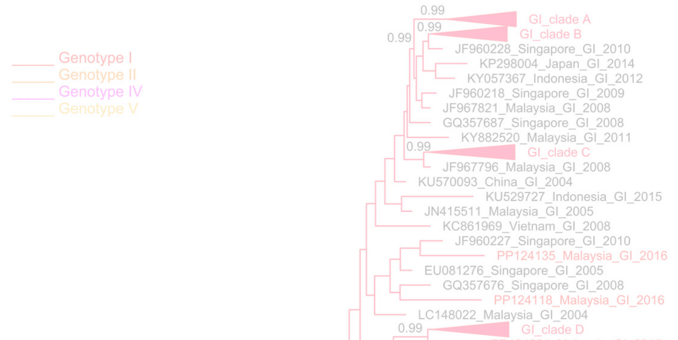
Result 3: Genotype and Lineage Dynamics

Objective

To assess dengue virus genetic diversity and infer lineage origins.

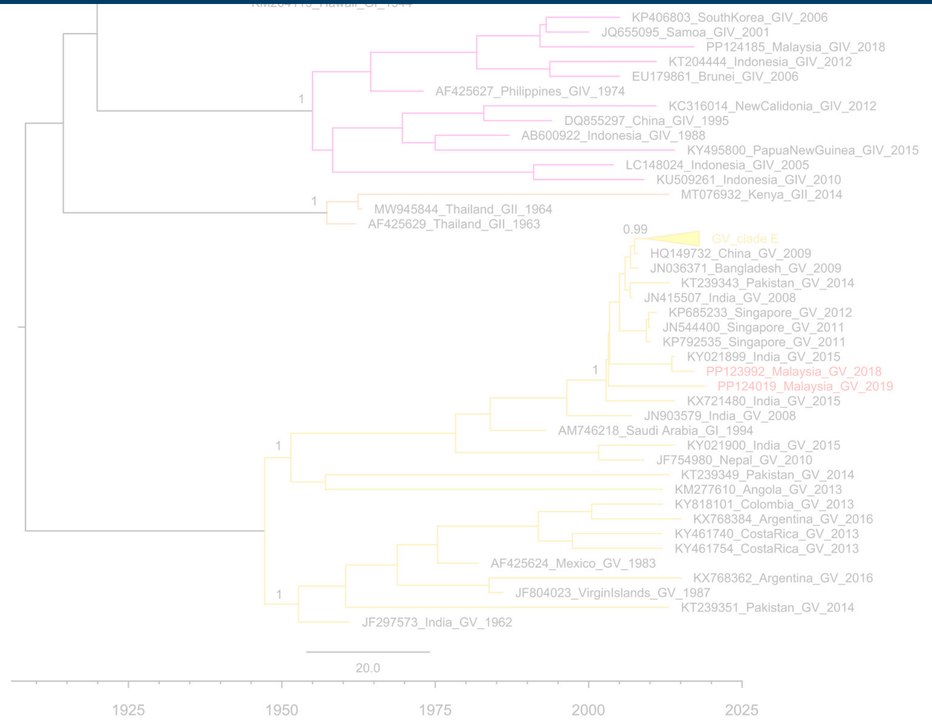
METHOD

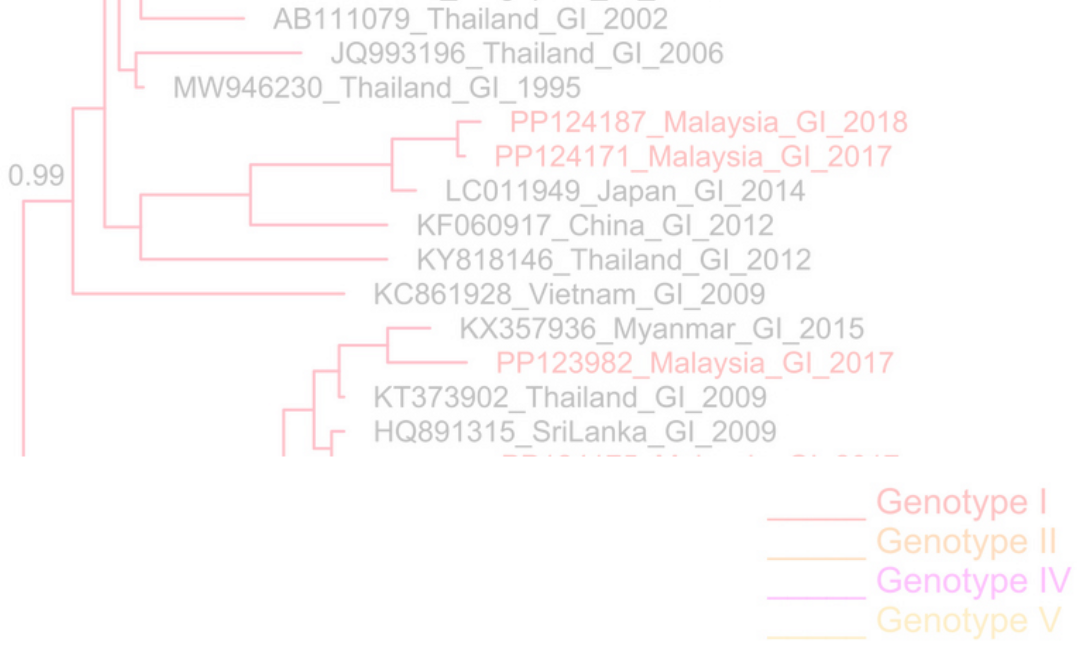
National reported cases



Each clade included sequences from many regional countries

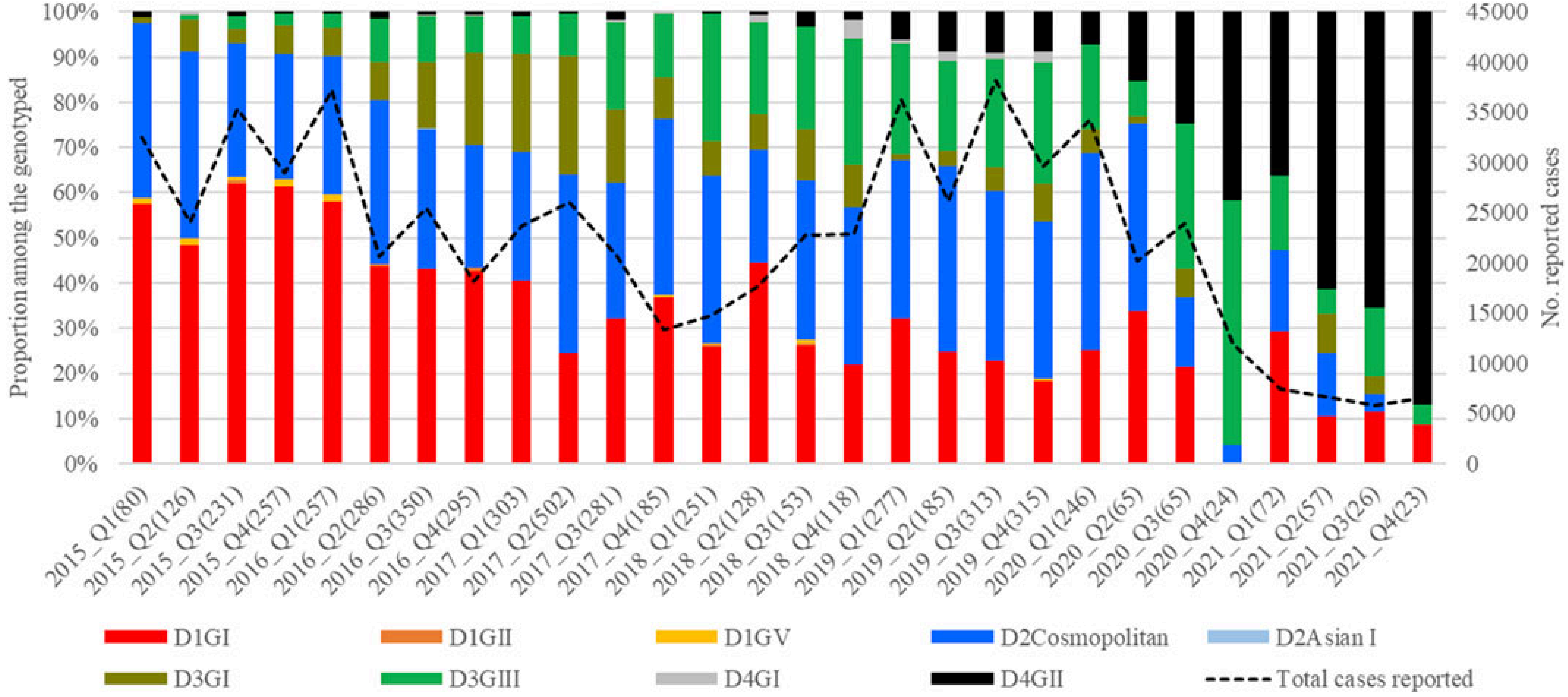
- Suggesting the widespread presence of respective clades in South/East Asia
- Indicating the potential sources of their introduction into Malaysia

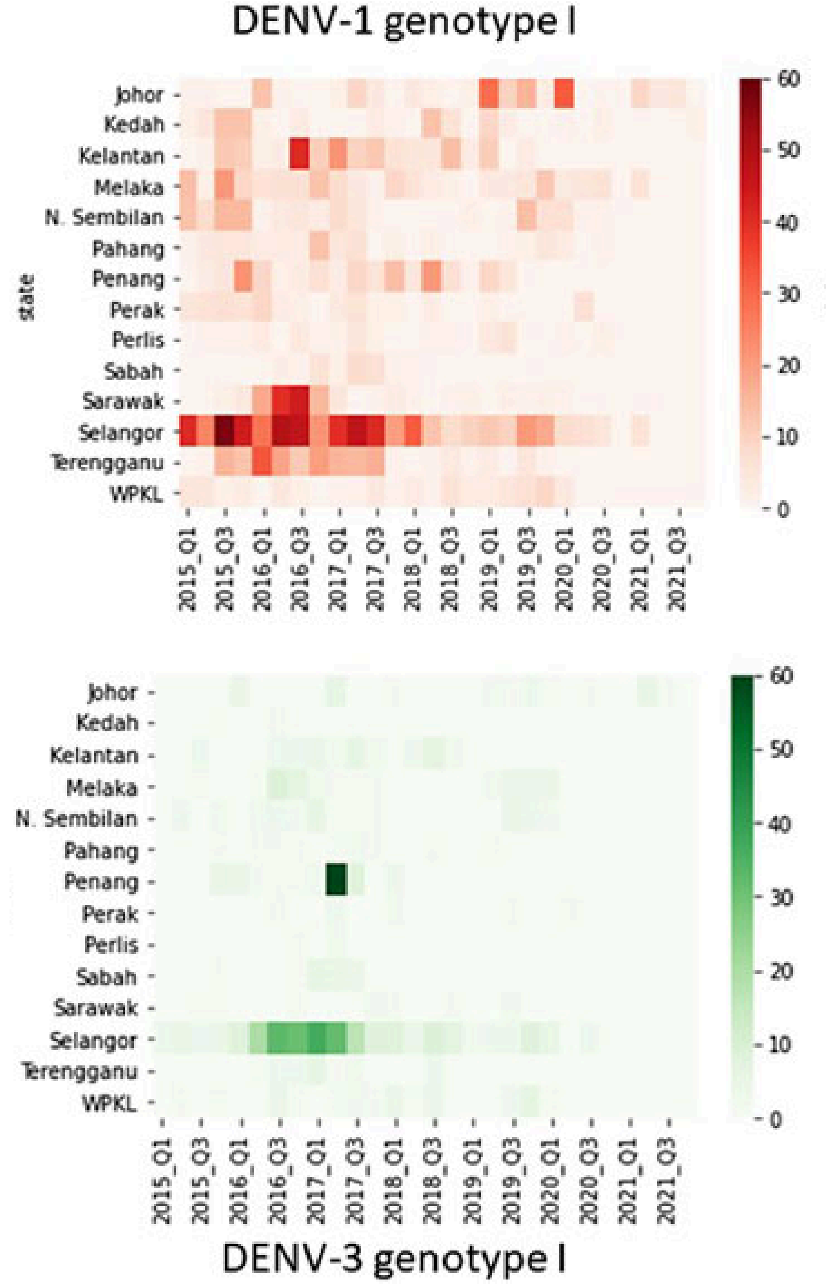




Result 3: Genotype and Lineage dynamics

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



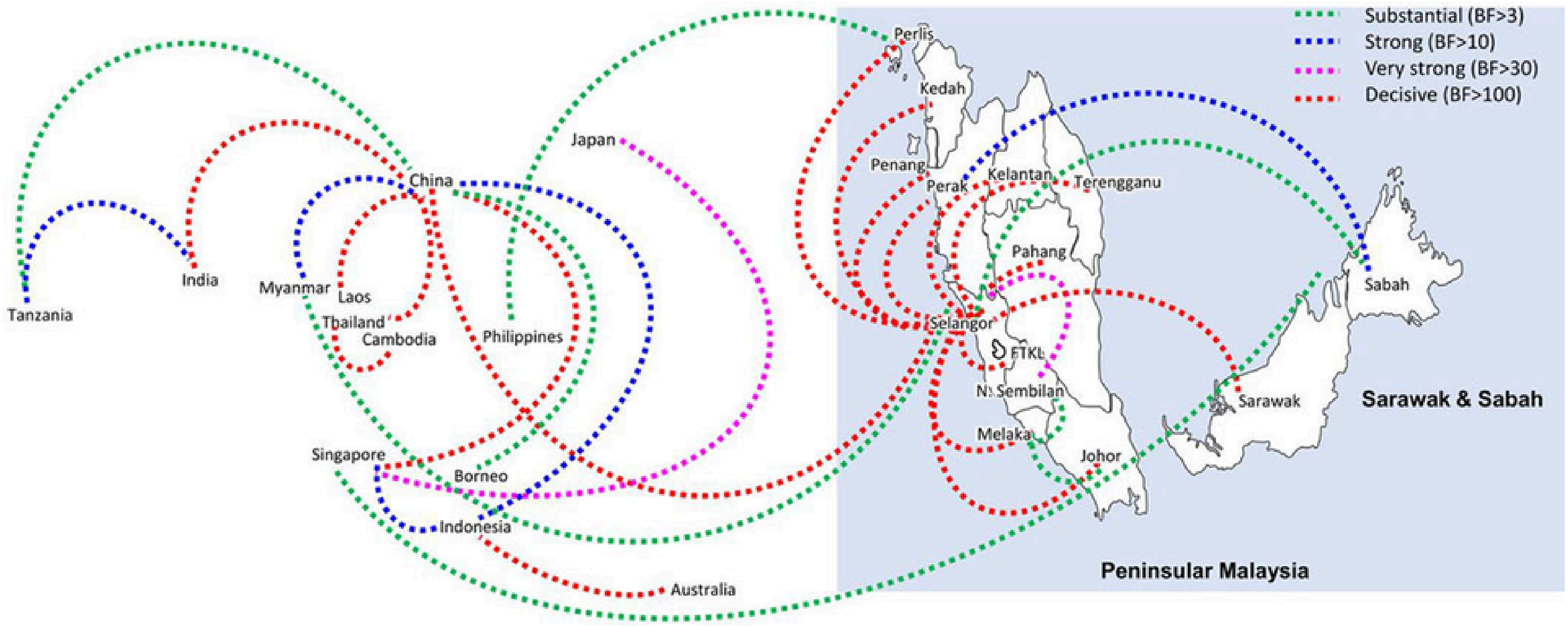

DENV-1 genotype I

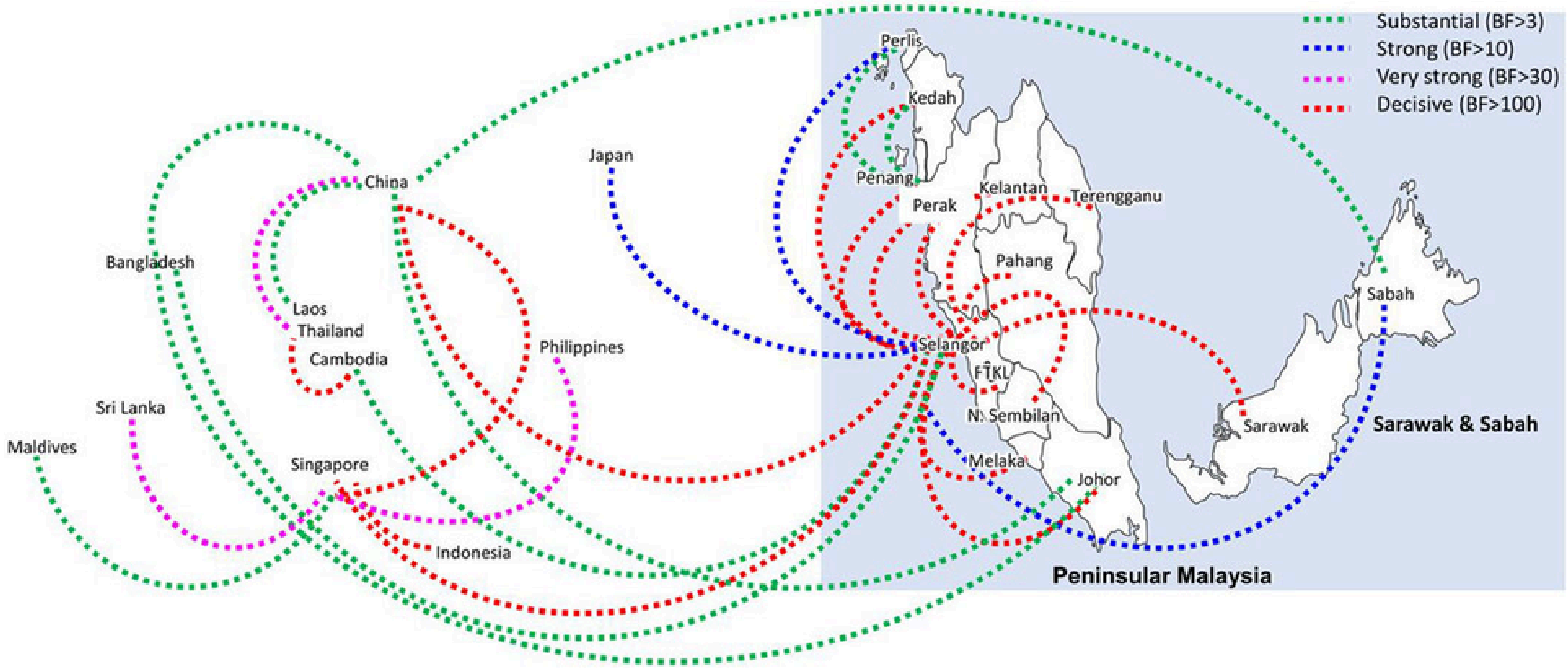
DENV-3 genotype I

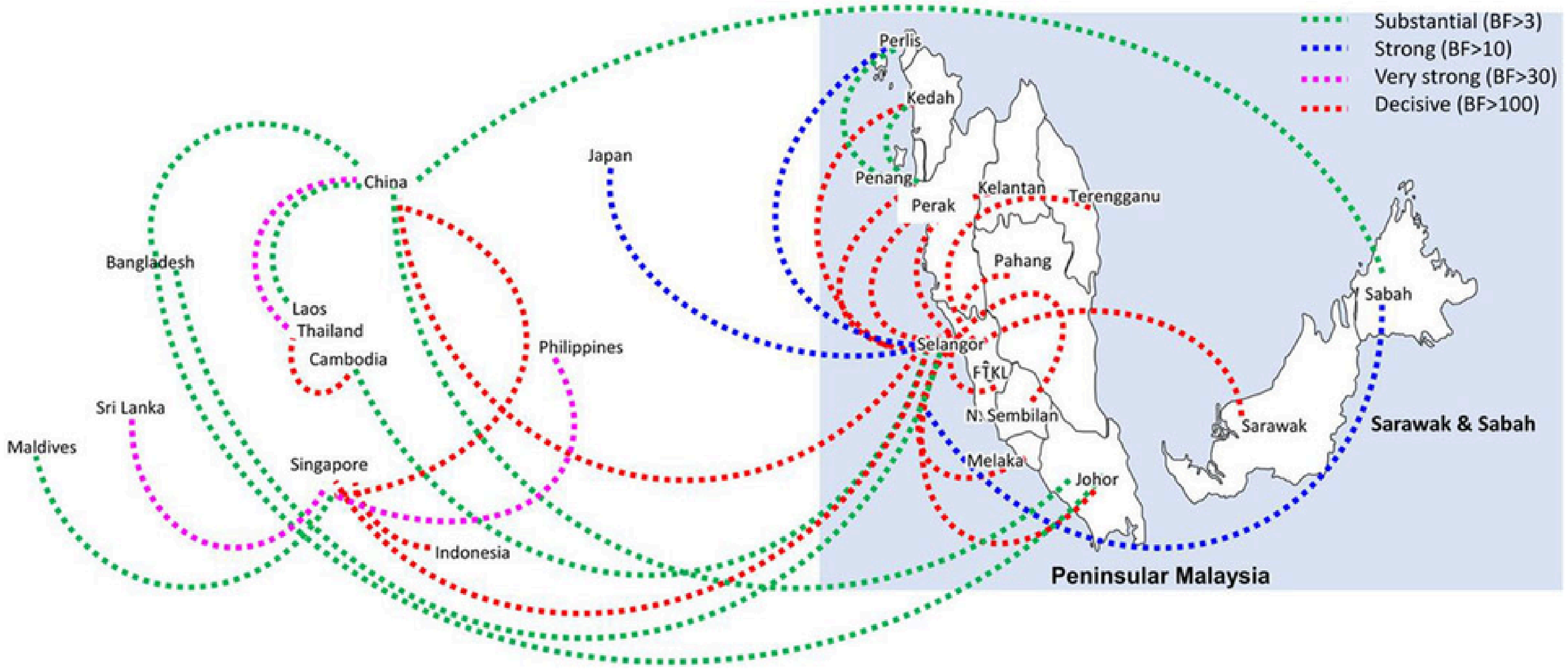
Lineage turnover was associated with changes in case burden and some introduced genotypes failed to sustain local transmission.

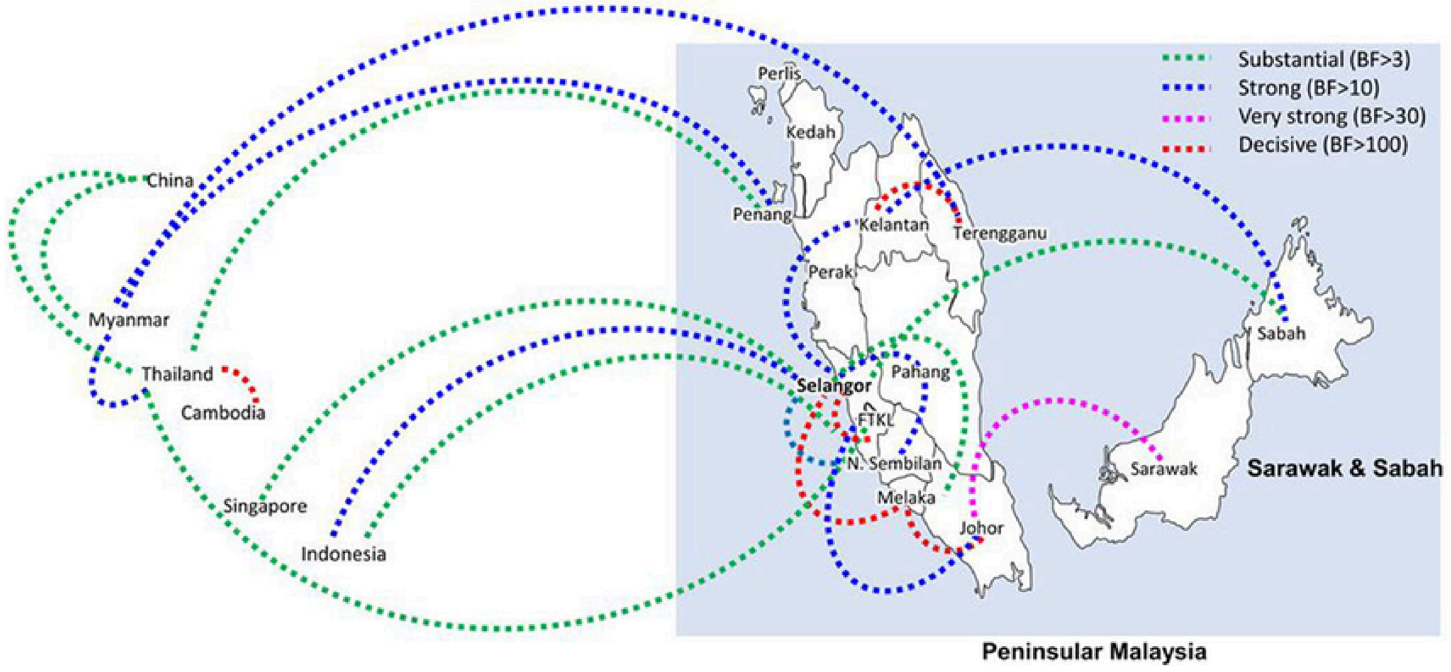
Result 4: Phylogeography analysis of E protein

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



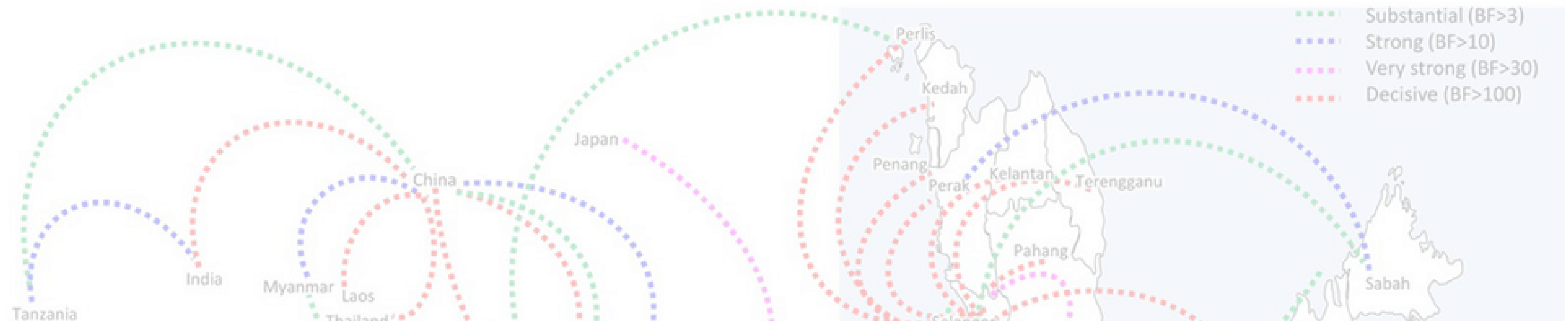


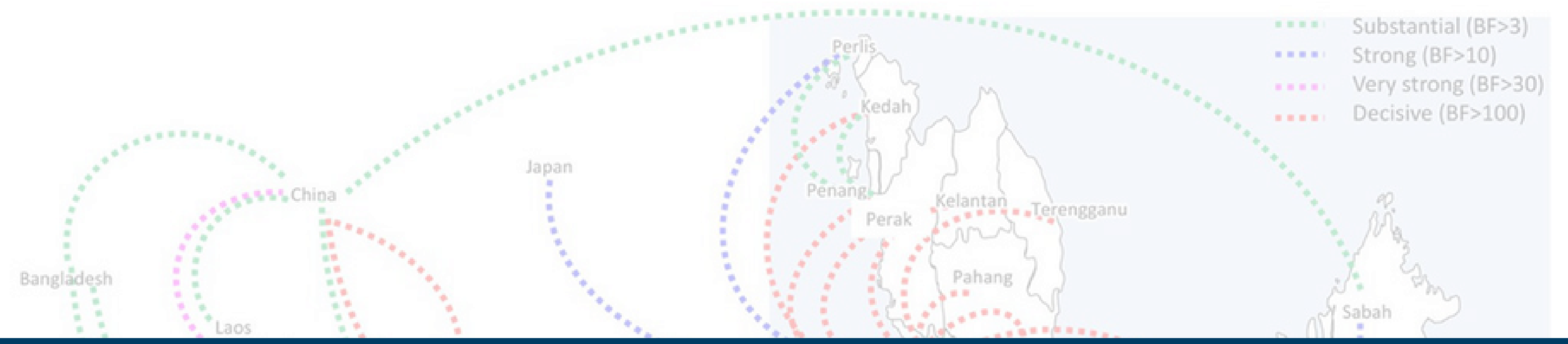




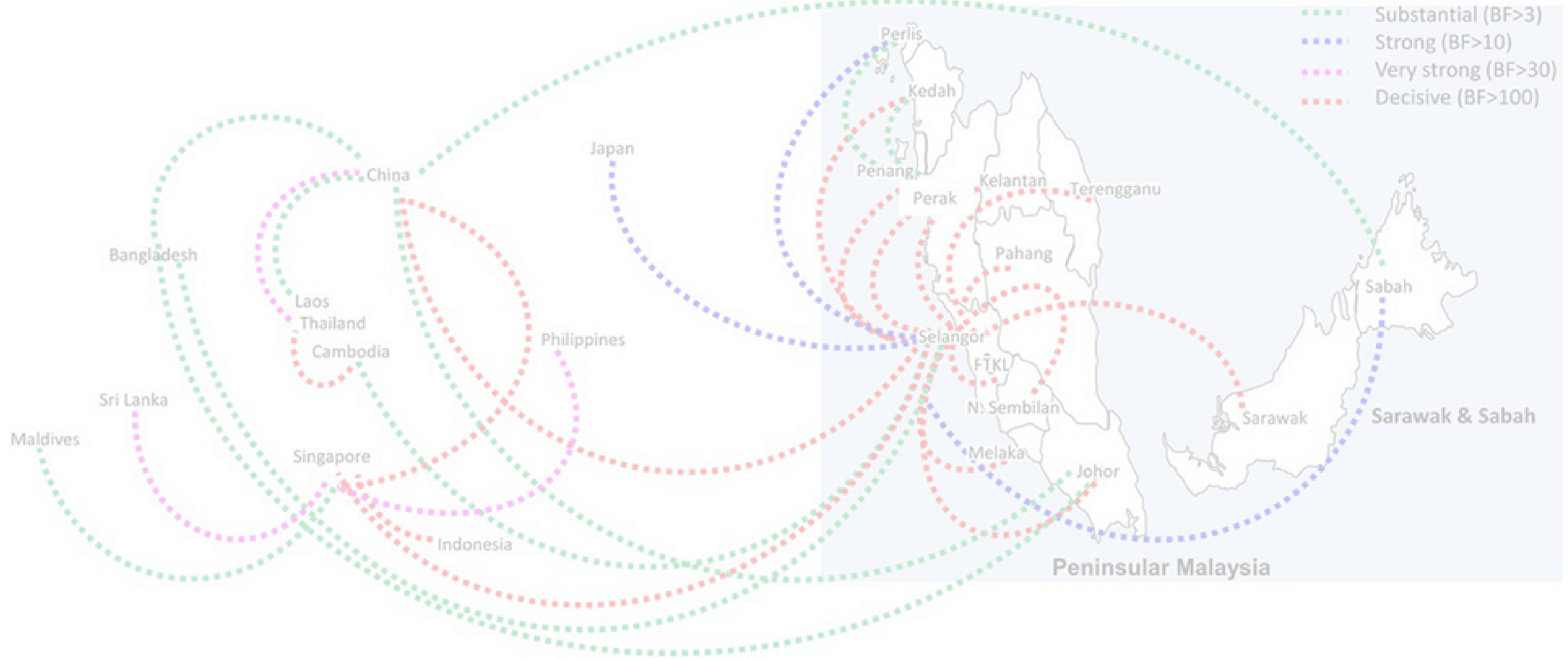
Result 2: -

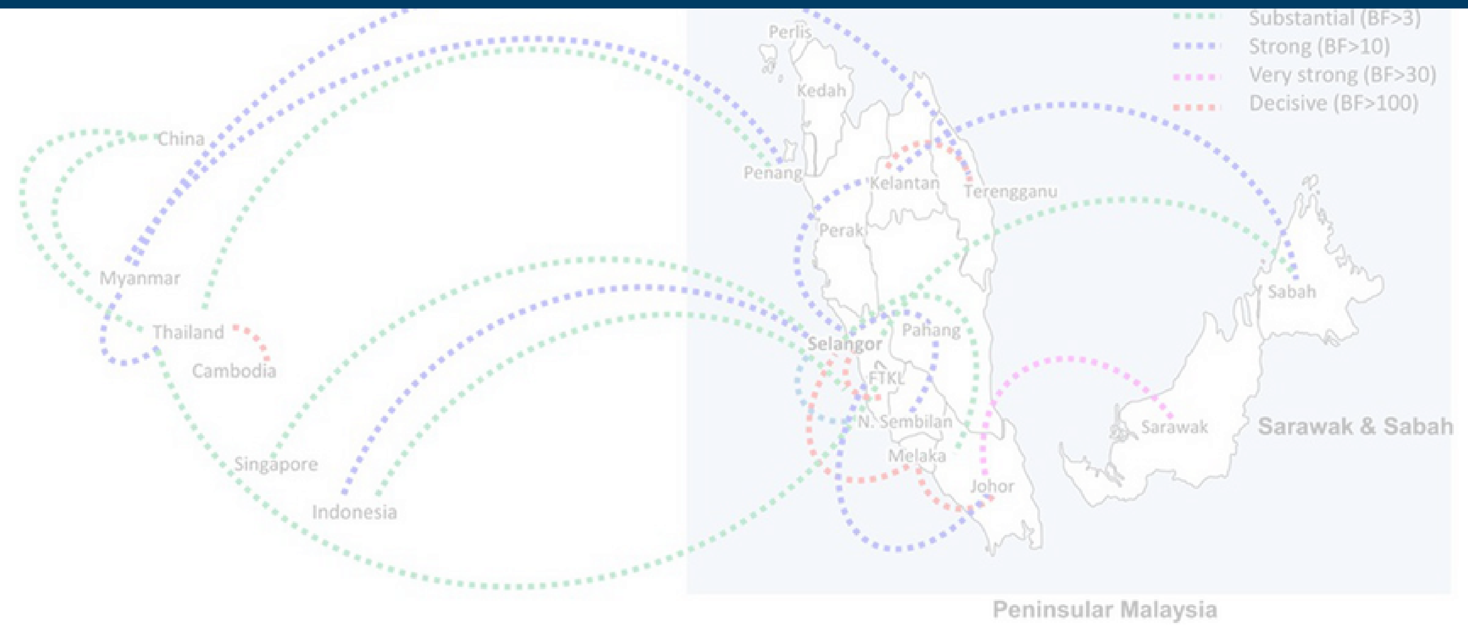
Objective





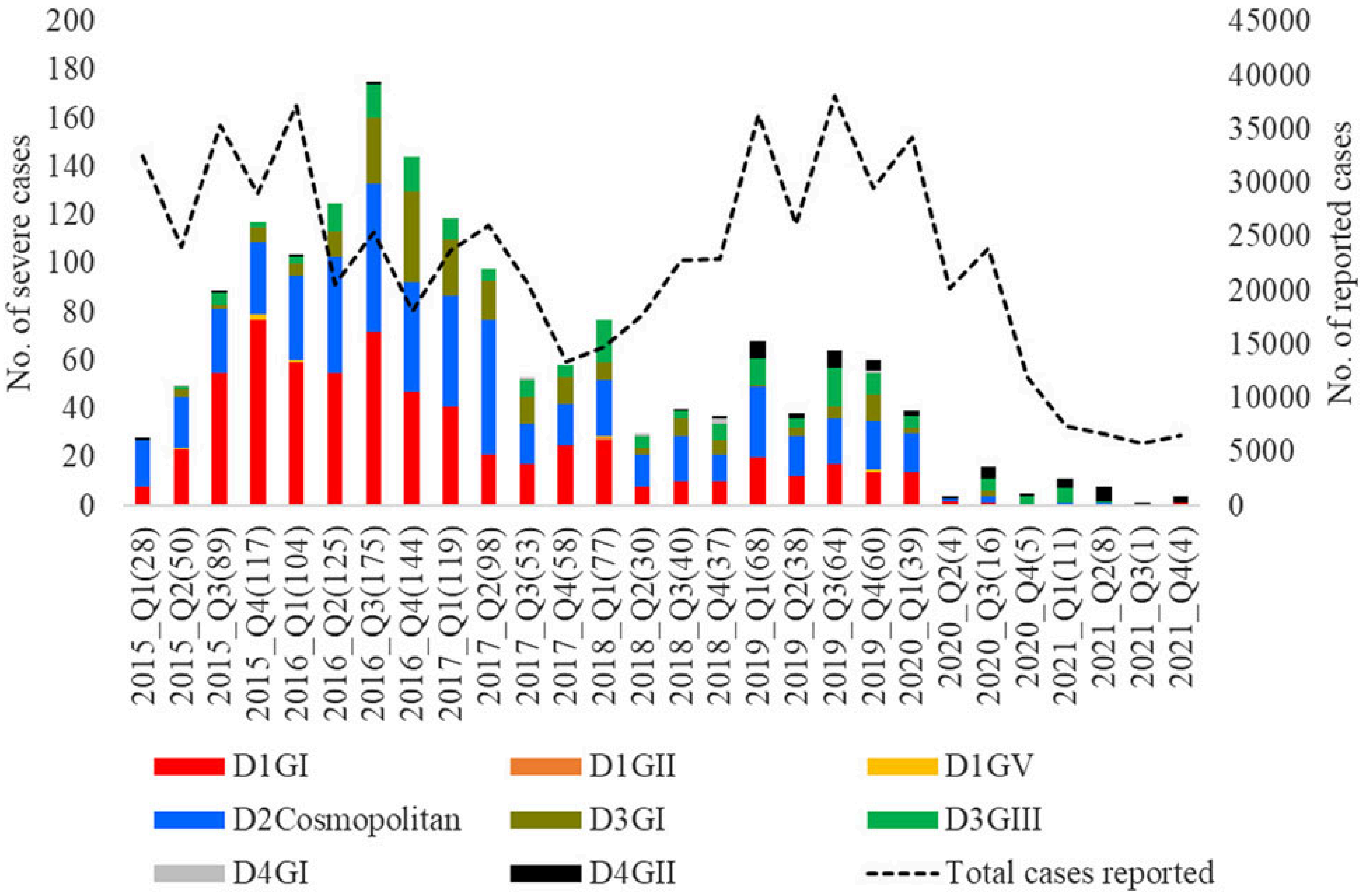
Dengue virus transmission occurs predominantly within Malaysia with **Selangor acting as a major hub linking multiple states** while international introductions play a limited role in sustaining transmission.





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 genotype of DENV-2 collectively contributed the highest number of severe infections in each year from 2015 to 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 histor	• Clearly captures long-term temporal dynamics. • 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

THANK YOU FOR YOUR ATTENTION