International Journal of Infectious Diseases 133 (2023) 46–52

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Phylogeographic analysis of dengue virus serotype 1 and cosmopolitan serotype 2 in Africa

Philippe Selhorst[1,2,*], Sebastian Lequime[3], Gytis Dudas[4], Sam Proesmans[5], Pascal Lutumba[6,7], Freddy Katshongo[8], Kadrie Ramadan[9], Isabel Micalessi[9], Steve Ahuka-Mundeke[6,12], Veerle Vanlerberghe[10], Marjan Van Esbroeck[9], Kevin K. Ariën[1,11]

[1] Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium
[2] The Outbreak Research Team, Institute of Tropical Medicine, Antwerp, Belgium
[3] Microbial Ecology Cluster, Groningen Institute for Evolutionary Life Sciences, University of Groningen, Groningen, The Netherlands
[4] Institute of Biotechnology, Life Sciences Centre, Vilnius University, Vilnius, Lithuania
[5] Department of Internal Medicine, Infectious Diseases & Tropical Medicine, University of Antwerp, Antwerp, Belgium
[6] National Institute of Biomedical Research, Kinshasa, Democratic Republic of Congo
[7] University of Kinshasa, Kinshasa, Democratic Republic of Congo
[8] Higher Institute of Medical Techniques, Kinshasa, Democratic Republic of Congo
[9] Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium
[10] Department of Public Health, Institute of Tropical Medicine, Antwerp, Belgium
[11] Departement Biomedische Wetenschappen, University of Antwerp, Antwerp, Belgium
[12] Biomedical Department, Kinshasa Teaching Hospital, School of Medicine, Kinshasa University, Kinshasa, Democratic Republic of Congo

ARTICLE INFO

Article history:
Received 26 January 2023
Revised 24 March 2023
Accepted 9 April 2023

Keywords:
Dengue
DENV
Phylogenetics
Africa
Whole genome sequencing

ABSTRACT

Objectives: The origin and spread of dengue virus (DENV) circulating in Africa remain poorly characterized, with African sequences representing <1% of global sequence data.
Methods: Whole genome sequencing was performed on serum samples (n = 29) from an undifferentiated fever study in 2016 in the Democratic Republic of Congo (DRC), and from febrile travelers returning from Africa. The evolutionary history of the newly acquired African DENV-1 (n = 1) and cosmopolitan genotype DENV-2 (n = 18) genomes was reconstructed using a phylogeographic, time-scaled Bayesian analysis on a curated DENV panel including all known African sequences.
Results: A minimum of 10 and eight introductions could be identified into Africa for DENV-1 and cosmopolitan DENV-2, respectively, almost all originating from Asia. Three introductions were previously unknown. The currently circulating virus comprises mainly the recently introduced clades and one long-established African clade. Robust geographical clustering suggests limited spread of DENV after each introduction. Our data identified the DRC as the source of the 2018 Angolan DENV-2 epidemic, and similarly, the 2013 Angolan DENV-1 outbreak as the origin of our DRC study.
Conclusion: Active genomic surveillance of DENV in Africa at the portals of entry might help early outbreak response and limit sero- and genotype spread and human disease burden.

Introduction

Dengue is the most widespread mosquito-borne viral disease, which threatens nearly 40% of the world population, causing an estimated 36,000 deaths per year worldwide [1]. Symptoms can range from asymptomatic to mild fever to severe dengue, frequently with hemorrhagic presentation in about 1% of cases. The disease is caused by a single-stranded RNA virus belonging to the Flaviviridae and is transmitted between humans by the bite of female *Aedes* mosquitoes, primarily *Aedes aegypti* and, to a lesser extent, by the more globally dispersed *Aedes albopictus* [2]. With no current treatment available and the first commercially available vaccine (CYD-TDV or Dengvaxia) being only recommended in the exposed population [3], public health measures have mainly focused on clinical management and vector control. However, a new

* Corresponding author: Tel: +32(0)32476546.
E-mail address: pselhorst@itg.be (P. Selhorst).

https://doi.org/10.1016/j.ijid.2023.04.391

vaccine (TAK-003 or DENVax), which has been shown to reduce hospitalizations in the general population by 84%, is in the final stages of regulatory approval [4].

Dengue virus (DENV) comprises four closely related yet antigenically and genetically distinct serotypes (DENV-1, DENV-2, DENV-3, and DENV-4), which can differ by 30-35% in amino acid sequence [5]. Although DENV reinfections have been reported [6], infection by one serotype usually provides life-long immunity against homologous challenge but only briefly protects against a different serotype [7,8]. Life-threatening syndromes mainly occur after heterotypic secondary infection. This has been attributed to antibody-dependent enhancement, whereby pre-existing non- or subneutralizing antibodies to the primary infection facilitate DENV entry into Fc receptor-bearing cells [7].

DENV is considered a zoonotic disease because of the discovery of ecologically and evolutionary distinct DENV lineages that occur in a sylvatic transmission cycle between nonhuman primates and canopy-dwelling *Aedes* mosquitoes [9]. Interestingly, although more sampling is needed, the existence of sylvatic strains in an enzootic transmission cycle has so far only been shown for DENV-2 in the forests of West Africa and in Southeast Asia, where, in sentinel monkeys, the sylvatic lineages of DENV-1, -2, and -4 were found, as well as antibodies to DENV-3 [10].

Although DENV epidemics were first identified in the 40s and 50s in Asia [11], the disease has been around for much longer because descriptions of a DENV-like illness date back to at least 10th century China [12]. By the late 18th century, historical reports suggest an already widespread geographical dissemination of DENV, coinciding with the global increase in maritime trade. The increase in human movement through migration, slave trade, and commerce also led to the worldwide export of the African mosquito *Ae. aegypti* [2]. Facilitated by the domestication and establishment of this urban vector, DENV became endemic in most of the tropics by the late 19th century [13]. Over the last century, the continued spread of the virus, fueled by the advent of commercial air travel, resulted in overlapping epidemics of all four serotypes (*i.e.*, hyperendemicity) and an increase in severe dengue throughout most of the (sub)tropics [5]. This recent history of human DENV corresponds with its evolutionary genetics, with human endemic DENV-1, DENV-2, and DENV-4 diverging from their sylvatic progenitors around, respectively, 1660 (95% highest posterior density interval (HPD) 1470-1819), 1733 (95% HPD 1550-1844), and 1384 (95% HPD 702-1902) [14], whereas the most recent common ancestor of all serotypes in an older study was estimated around 1115 years ago [15].

Relatively little is known about DENV in Africa, and although the first historical records date back to the 19th century, DENV was first isolated in the 1960s in Nigeria. Retrospective neutralizing antibody testing in the 1950s, however, suggests that the earliest confirmed outbreak occurred in South Africa in 1926-1927 [16]. Even today, suboptimal surveillance limits our understanding of DENV prevalence, origins, and epidemiology on the continent. African dengue sequences represent <1% of the global DENV sequence data and even fewer data exist for the Democratic Republic of Congo (DRC). To fill some of these gaps, we performed a phylogenetic analysis on partial and/or complete African DENV-1 and DENV-2 sequences collected from returning travelers and from patients in a 2016 study on the etiologies of acute fever in the DRC [17].

Materials and methods

Serum collection

We collected all available DENV-1 and -2 serum samples with a cycle threshold value <32 stored at the Institute of Tropical Medicine, Antwerp. These included samples from travelers (n = 16) returning to Belgium from Africa that were collected for diagnosis between 2014 and 2020, as well as a sample series (n = 13) obtained during an undifferentiated fever study in 2016 in the DRC (Resfandi, ClinicalTrials.gov NCT02656862) (Table 1).

RNA extraction and real-time quantitative reverse transcription-polymerase chain reaction

At the Institute of Tropical Medicine, Antwerp, RNA was extracted using either a Maxwell RSC Instrument or Qiagen viral RNA minikit. Phocine distemper virus was added to all samples as an internal extraction and polymerase chain reaction (PCR) inhibition control [18]. A multiplex real-time quantitative reverse transcription PCR, which can distinguish between the four DENV serotypes, was then performed using primers and probes, as previously described [19]. Briefly, a 112bp fragment of the NS1 gene of DENV-1 or a 77bp fragment of the E gene of DENV-2 was amplified in 45 cycles by adding 5 µl of RNA to a 25-µl reaction using the Bioline SensiFAST mix, Reverse Transcriptase, and RiboSafe RNase inhibitor.

Whole genome sequencing using MinION

Whole genome sequencing was performed on an Oxford Nanopore MinION device using R9.4 flow cells (Oxford Nanopore Technologies, UK) based on a protocol from Quick *et al.*, 2017 [6]. Briefly, 7 μl of RNA was reverse transcribed to complimentary DNA using random hexamers and SuperScript IV (ThermoFisher). Subsequently, a serotype-specific multiplex PCR was performed with Q5 High-Fidelity DNA polymerase (New England Biolabs) in six reactions, using either a 400 bp DENV-1 or DENV-2 primer scheme (Supplementary Table 1). Primer schemes were designed using PrimalScheme (https://primalscheme.com/) and empirically refined. Cycling conditions were 30 seconds at 98°C, followed by 25-30 cycles at 98°C for 10 seconds, 65°C for 1.5 minutes, 72°C for 2 minutes, and a final elongation step at 72°C for 2 minutes. The resulting 400 bp PCR products were pooled, cleaned using AmpureXP magnetic beads (Beckman Coulter, UK), and quantified using a Qubit dsDNA High Sensitivity assay on a Qubit 3.0 instrument (Thermo Fisher Scientific, USA). The samples were then barcoded using the Ultra II End Repair/dA-Tailing Module (New England Biolabs) and the native barcoding kits NBD104 and NBD114 (Oxford Nanopore Technologies), cleaned with magnetic beads, and pooled at equimolar ratios before ligation of the AMII adapters with blunt/TA ligase master mix (New England Biolabs). Sequencing libraries were loaded onto the R9.4 flow cell using the ligation sequencing kit LSK109 (Oxford Nanopore Technologies), and sequencing data were collected overnight. Sequence reads were base-called in high accuracy mode and demultiplexed using the Guppy algorithm v3.6 (Oxford Nanopore Technologies). The consensus genome sequences were produced using the Artic network's bioinformatics pipeline (https://artic.network/ncov-2019/ncov2019-bioinformatics-sop.html), which incorporates primer removal, read alignment to a reference genome (AF309641.1_DENV1 or GQ398268.1_DENV2) with minimap2, and the nanopolish algorithm (https://github.com/jts/nanopolish) to improve the consensus sequence. Regions with insufficient coverage were masked with N characters.

Phylogenomic analysis of DENV-1 sequences

To reconstruct the origin of the 18 newly sequenced African DENV-1 genomes, we assembled a curated dataset, including all genotypes, consisting of the full genome DENV-1 Nextstrain

Table 1
DENV genomes sequenced in this study from travelers returning to Belgium from Africa and from our 2016 Resfandi study in the DRC.

Accession number	Serotype	Genotype	Country of origin	Collection date	Study
MZ130518	DENV-2	Cosmopolitan	Reunion	2019-05-14	Traveler
MZ130519	DENV-2	Cosmopolitan	Mozambique	2019-03-05	Traveler
MZ130520	DENV-2	Cosmopolitan	DRC	2016-04-11	Resfandi
MZ130521	DENV-2	Cosmopolitan	DRC	2016-06-20	Resfandi
MZ130522	DENV-2	Cosmopolitan	Egypt	2017-11-07	Traveler
MZ130524	DENV-2	Cosmopolitan	Benin	2019-07-09	Traveler
MZ130525	DENV-2	Cosmopolitan	Cameroon	2019-12-29	Traveler
MZ130526	DENV-2	Cosmopolitan	Tanzania	2014-05-21	Traveler
MZ130527	DENV-2	Cosmopolitan	Reunion	2019-01-05	Traveler
MZ130528	DENV-2	Cosmopolitan	Djibouti	2018-06-24	Traveler
MZ130529	DENV-2	Cosmopolitan	DRC	2017-04-30	Traveler
MZ130530	DENV-1	Genotype V	DRC	2016-06-17	Resfandi
MZ130531	DENV-1	Genotype V	DRC	2015-05-29	Traveler
MZ130532	DENV-1	Genotype V	DRC	2016-04-06	Resfandi
MZ130533	DENV-1	Genotype V	DRC	2016-06-17	Resfandi
MZ130534	DENV-1	Genotype V	DRC	2019-05-28	Resfandi
MZ130535	DENV-1	Genotype V	DRC	2016-05-09	Resfandi
MZ130536	DENV-1	Genotype V	DRC	2016-01-07	Resfandi
MZ130537	DENV-1	Genotype V	DRC	2016-06-14	Resfandi
MZ130538	DENV-1	Genotype V	DRC	2016-06-14	Resfandi
MZ130539	DENV-1	Genotype V	DRC	2016-06-20	Resfandi
MZ130540	DENV-1	Genotype V	DRC	2016-06-23	Resfandi
MZ130541	DENV-1	Genotype V	DRC	2016-02-26	Resfandi
MZ130542	DENV-1	Genotype V	DRC	2016-06-20	Resfandi
MZ130543	DENV-1	Genotype V	Seychelles	2016-07-25	Traveler
MZ130544	DENV-1	Genotype V	Africa	2020-01-02	Traveler
MZ130545	DENV-1	Genotype V	Djibouti	2020-01-12	Traveler
MZ130546	DENV-1	Genotype V	Tanzania	2020-01-08	Traveler
MZ130547	DENV-1	Genotype V	Tanzania	2020-01-31	Traveler

DENV, dengue virus; DRC, Democratic Republic of Congo.

panel (https://nextstrain.org/dengue/denv1) [20], which we downloaded from the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/genbank/), as well as all African DENV-1 sequences with a minimal length of 1400 bp available in the VIPR database (https://www.viprbrc.org/) on July 2021. Hence, this length filter included all full-length E gene sequences. Sequences were trimmed to the coding sequence (CDS) and aligned using MAFFT v7 [21]. We screened for recombinant sequences using RDP 4 [22] and removed the suspected recombinants if detected by more than three methods. To check for a temporal signal in the dataset, a maximum likelihood phylogenetic tree was inferred with IQ-TREE v1.6.12 [23] using the GTR+F+R5 model and used to perform a root-to-tip regression in Tempest. Eight outliers were removed. The location info of all sequences was double-checked in GenBank to reflect the country of sampling rather than the country of sequencing. To reduce the number of sequences for time-scaled analysis, we downsampled this dataset manually by using only one sequence per monophyletic group from the same country and sampled within the same year. This led to a total dataset of 730 sequences, including 18 new complete genomes, which were codon-aligned. Time-scaled Bayesian analysis was performed using the GTR + gamma nucleotide model with three codon partitions, an uncorrelated log-normal relaxed clock assumption, a skyline demographic model, and an ancestral geographic state reconstruction. Multiple seeds of a 600 million Markov chain Monte Carlo were run, sampling every 100,000 states with 10% burn-in, and combined using LogCombiner v1.10.4 to a total chain length of 3.78 billion. Statistical convergence was determined in Tracer v1.7.2 by examining the trace plots in addition to ESS values, which exceeded 200 for all parameters. A maximum clade credibility tree was finally constructed from the combined tree files using TreeAnnotator v1.10.4 and visualized in iTOL v6.5.6 (Figures 1 and 2). Trees displaying only the introductions into Africa (Figures 1 and 2) were visualized as an 'exploded tree' using the python package Baltic (https://github.com/evogytis/baltic and https://phylo-baltic.github.io/baltic-gallery/). The sequence, xml, and tree files are provided in the Supplementary materials.

Phylogenomic analysis of DENV-2 sequences

To reconstruct the origin of the 11 newly sequenced African DENV-2 Cosmopolitan genomes, we downloaded all available epidemic DENV-2 sequences from the VIPR database, with a minimum length of 1400 bp and aligned them to Nextstrain's DENV-2 genotype panel to identify the sequences belonging to the cosmopolitan genotype by constructing an machine learning tree in FastTree v2.1.10. The suspected recombinant sequences were removed from this cosmopolitan dataset, as well as sequences from animals or for which no date or location could be found. To limit the amount of data, we then selected only the sequences larger than 9000 bp, unless they were sampled in Africa, for which no second length filter was applied. This dataset was then further processed in a similar way as described previously for DENV-1 using the IQ-TREE and the GTR+F+R5 model for root-to-tip regression. A total of 13 outliers were identified in Tempest and after monophyletic downsampling, this resulted in a total of 366 sequences for time-scaled analysis using the GTR + gamma nucleotide substitution model. The combined Markov chain Monte Carlo length from multiple seeds was 1.35 billion. The sequence, xml, and tree files are provided in the Supplementary materials.

Results

To reconstruct the evolutionary history of the newly acquired African DENV genomes described in Table 1, a phylogeographic, time-scaled Bayesian analysis was performed using a curated panel of 730 and 366 near-complete genomes of, respectively, DENV-1 (all genotypes) and DENV-2 (cosmopolitan genotype).

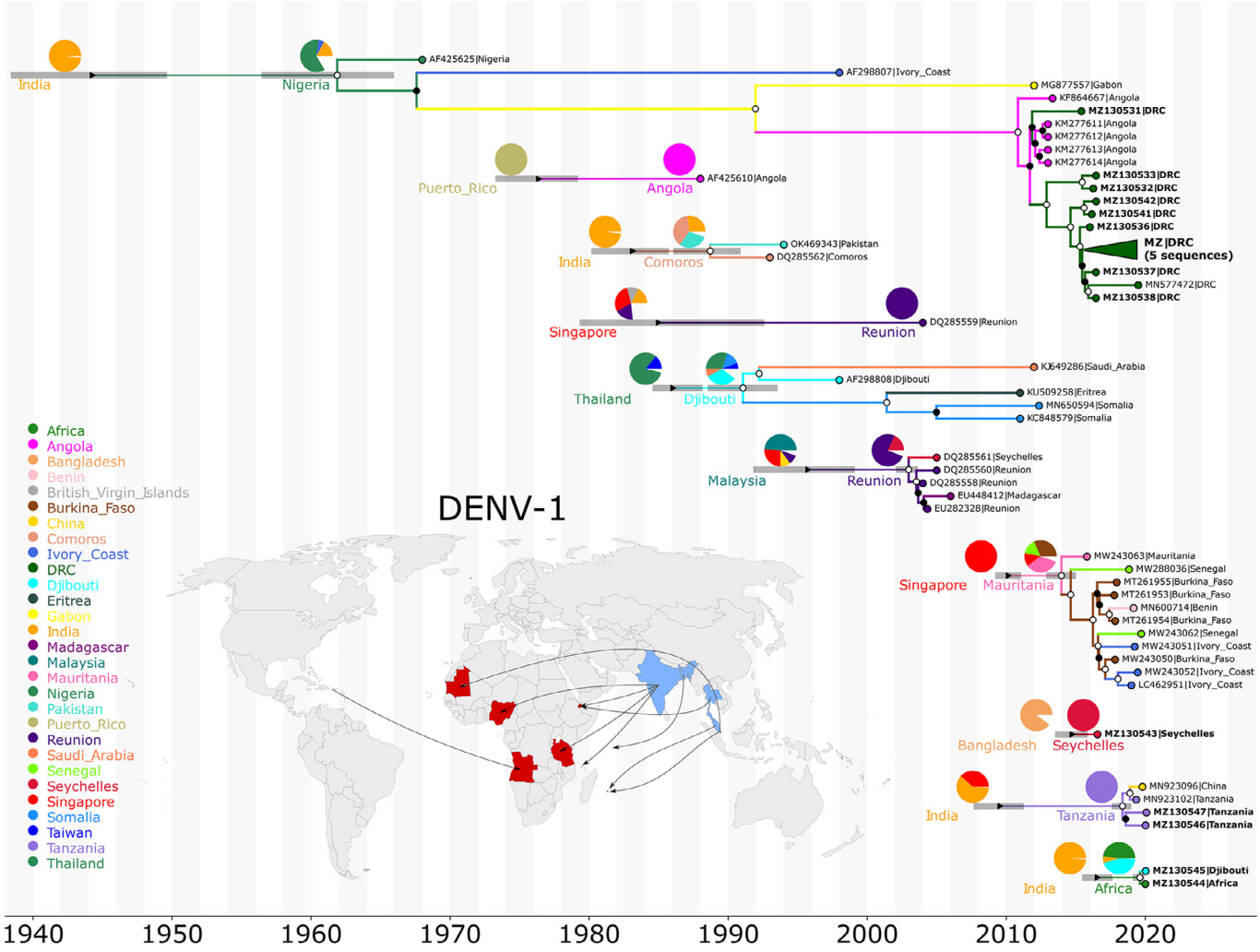

Figure 1. Bayesian phylogeographic analysis of DENV-1 whole genome sequences. Only the introductions to Africa are depicted. The triangles at the root of each subtree represent the country of origin for each introduction, whereas a circle represents tips. The names of the sequences generated in this study are shown in bold. Internal nodes are depicted as either open circles with full support (posterior probability >0.95) or black circles with less support (posterior probability ≤0.95). Colors indicate the most likely country of each branch and tip. For each introduction, pie charts and horizontal gray bars show, respectively, the country posterior probabilities and the 95% HPD interval at both the exporting and importing node. If an introduction is represented by only one sequence, a 95% HPD interval could not be estimated. For visual reasons, posterior probabilities <0.05 were left out of the pie charts. The map of the world summarizes the introductions from Asian countries (blue) into African countries (red). DENV, dengue virus; HPD, highest posterior density interval.

DENV-1 introductions into Africa

All new DENV-1 genomes (n = 18, Supplementary Figure 1, red names) belonged to genotype V and mostly confirm previously known introductions of DENV-1 into Africa because they cluster with African panel sequences (Supplementary Figure 1, blue names). From the limited amount of data available, a minimum of 10 introductions could be identified by estimating the time to the African clades' most recent common ancestor (Figure 1 and Supplementary Table 2). The oldest introduction occurred between 1938 and 1966 from India into Nigeria and gave rise to an African subtype V DENV-1 clade, which further spread to Ivory Coast, Gabon, Angola, and the DRC. All of the DRC genomes from our 2016 study belong to this clade and seemingly find their origin in the 2013 Angolan outbreak. The other nine introductions happened from the 1970s onward, of which eight originated from Asian countries, including India, Thailand Malaysia, Singapore, and Bangladesh. Three introductions, one from Puerto Rico to Angola, one from Singapore into Reunion Island, and one from Bangladesh into the Seychelles, have only one representative, which might suggest that they failed to establish. Finally, three of the new sequences represent two previously undetected introductions, one from India into Djibouti (est. 2016-2020) and the one from Bangladesh into the Seychelles (est. 2013-2017). Overall, the descending sequences of each introduction primarily cluster according to the geographical region within Africa.

DENV-2 cosmopolitan introductions into Africa

All African DENV-2 genomes (n = 11, Supplementary Figure 2, red names) obtained in this study belonged to the cosmopolitan genotype. To limit the amount of data, we only compared them against a panel of 366 cosmopolitan sequences. A minimum of eight cosmopolitan DENV-2 introductions could be identified (Figure 2 and Supplementary Table 2). Most new sequences confirm previously detected introductions into Africa, clustering with known African sequences (Supplementary Figure 2, blue names). However, a single sequence from a traveler returning from Egypt represents a new introduction (est. 2015-2018) and belongs to a

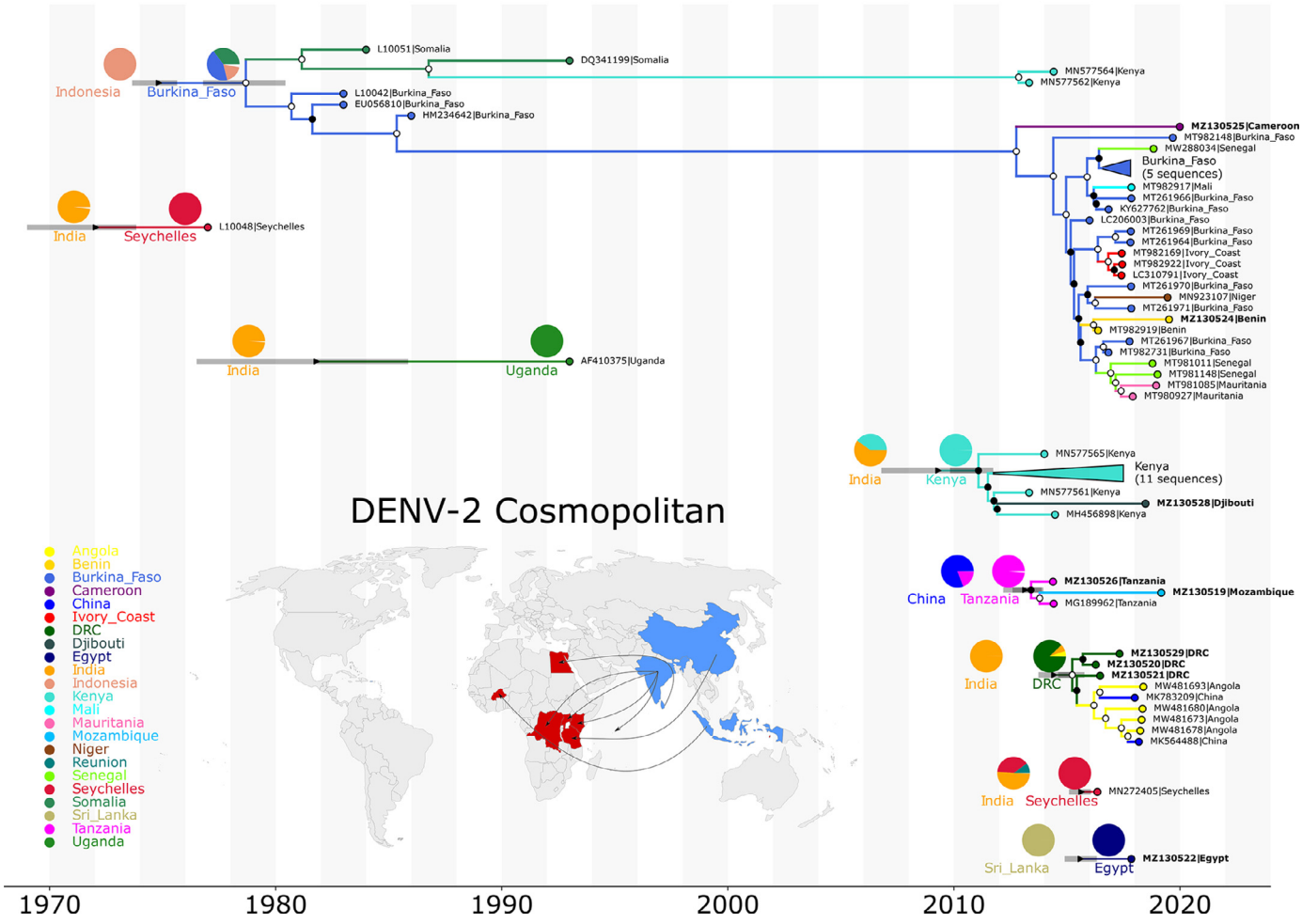

Figure 2. Bayesian phylogeographic analysis of cosmopolitan DENV-2 whole genome sequences.
DENV, dengue virus.

DENV-2 cosmopolitan clade that, until now, has only been detected in Malaysia and Sri Lanka. Again, the sequences within each African cluster often stem from the same geographical region. There were five introductions from India, one into Uganda (est. 1976-1993), one into Kenya (est. 2007-2012) that spread to Djibouti, one into the DRC (est. 2014-2016) that spread to China and Angola in 2018, and finally, two singleton introductions into the Seychelles (est. 1969-1977 and 2015-2016). In addition, one introduction from China into Tanzania (est. 2012-2014) is now also being detected in Mozambique. In contrast to these relatively recent introductions, an early introduction from Indonesia into Burkina Faso gave rise to a major West African cosmopolitan clade, which has since dispersed to Somalia, Kenya, Senegal, Benin, Ivory Coast, Cameroon, Mali, Niger, and Mauritania. We estimate this introduction to have occurred between 1973 and 1981.

Demographic history

The demographic history of DENV-1 and cosmopolitan DENV-2 was estimated using a Bayesian skyline plot (Figure 3). Overall, it showed an increasing trend consistent with the exponential growth and global spread of DENV observed over the last 70 years [11]. For DENV-1, the effective population size remained relatively stable until 1985, when a sharp increase could be observed. Hereafter, the population remained stable again until a second increase between 2005 and 2012, followed by a drop and flattening out. For cosmopolitan DENV-2, we observe a similar stable population until roughly 1985, after which an expansion occurred over 17 years, with a sharper increase around 2000. After that, however, growth has stalled in the last 20 years, with a temporary dip observed around 2011.

Discussion

DENV cases have exponentially grown over the past decades; yet, little is known about the introduction and spread of dengue in Africa. Therefore, we explored the evolutionary history of DENV-1 and the cosmopolitan genotype of DENV-2 in Africa using whole genome sequences available in public databases and by adding 29 new African DENV-1 and -2 sequences.

Almost all these genomes originate in Asia, specifically in India and its neighboring countries. Multiple introductions have occurred and, consistent with the divergence time of human DENV from its sylvatic progenitor, DENV-1 introductions into Africa date back earlier than the cosmopolitan DENV-2 introductions. We identified four previously unknown introductions of DENV-1 or -2 into Africa out of 18. No recent descendants were detected for some introductions, suggesting they did not establish locally, whereas other introductions diversified into clades. However, the strong geographical clustering observed within each African clade suggests that limited mixing occurred after each introduction in Africa.

Apart from confirming the already identified or new introductions, our sequence data also revealed the DRC as the most

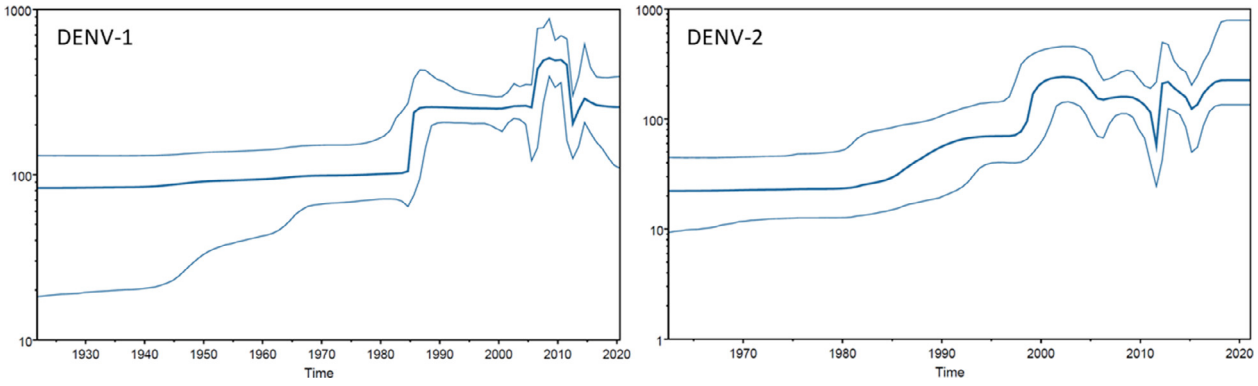

Figure 3. Demographic evolutionary history of DENV-1 and cosmopolitan DENV-2 based on whole genome sequences. The mean posterior values of the effective population size in log are plotted by the bold blue line, whereas the 95% HPD intervals are depicted by the light blue lines.
DENV, dengue virus; HPD, highest posterior density interval.

likely source of the 2018 Angolan DENV-2 epidemic and not East Africa or directly India, as was previously suggested [24,25]. Similarly but in the other direction, our DENV-1 2016 DRC sequences cluster within the 2013 Angolan outbreak. This is in line with samples from travelers returning from the DRC in 2015 [26] and 2019 [27]. Interestingly, such an epidemiological link between the two countries was also been shown in a yellow fever outbreak in 2015 [28].

Both DENV-1 and cosmopolitan DENV-2 datasets contain a long-established, and in that sense African, clade. For DENV-2, this clade was potentially established by an introduction from Indonesia into Burkina Faso or Somalia in the late 70s, whereas for DENV-1, it was potentially an import from India into Nigeria before 1962. This link might not be surprising because both countries were colonies of the British Empire, and soon after Nigerian independence in 1960, India provided strategic support and expanded trade with the country [29]. The fact that the remaining DENV-1 introductions almost all happened since the 1980s from exclusively Asian countries also corresponds with African and Asian countries strengthening commercial ties in the postcolonization period, which might have led to increased travel. Similarly, for cosmopolitan DENV-2, most introductions happened in the last 15 years, seemingly reflecting the existing economic relationships between, *e.g.*, China and DRC and India and East Africa.

This study is limited by the paucity of African sequences, by the predominance of recent sequences that can impact the time calibrations, and by sampling bias toward countries that generate more genomic sequences, which can impact the phylogeographic reconstructions. We attempted to minimize such bias by manually downsampling each monophyletic group from the same country and the same year to one sequence. Nonetheless the results in this study need to be interpreted with caution, mainly due to an overall lack of African sequence data.

In conclusion, we showed that DENV-1 and -2 cosmopolitan circulating in Africa consist of a mix of an older African clade and mostly recent introductions. The ongoing importation from DENV in Africa from a limited number of Asian countries, the continued increase in trade and human travel between Asia and Africa, and the limited geographical spread in Africa after importation call for active surveillance. As such, interventions at the portals of entry can potentially prevent further spread of different DENV geno- and serotypes, which would otherwise result in hyperendemicity and an increased disease burden. In addition, whole genome sequencing studies (on retrospective samples) will further enhance our understanding of DENV diversity and evolution in Africa.

Declaration of competing interest

The authors have no competing interests to declare.

Funding

PS is a member of the Institute of Tropical Medicine's Outbreak Research Team which is financially supported by the Department of Economy, Science and Innovation of the Flemish government.

Author contributions

Conceptualization: PS, SL; Investigation and Methodology: PS, SL, KR, IM, FK, SP; Formal analysis: PS, SL, GD; Resources: KA, MVE,SAM; Writing: PS, SL, KA, VV,MVE,GD; Funding acquisition and Supervision: KA, VV, LP, SAM. All authors have read and agreed to the final version of the manuscript.

Ethical approval

Sample collection and laboratory analysis were performed in view of diagnosis and standard of care under presumed consent or as part of a registered clinical trial (Resfandi, ClinicalTrials.gov NCT02656862) under informed consent and in full compliance with local laws and regulations and the principles of the Declaration of Helsinki and the International Conference Harmonization.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2023.04.391.

References

[1] Yang X, Quam MBM, Zhang T, et al. Global burden for dengue and the evolving pattern in the past 30 years. *J Travel Med* 2021;**28**:taab146. doi:10.1093/jtm/taab146.
[2] Weaver SC, Vasilakis N. Molecular evolution of dengue viruses: contributions of phylogenetics to understanding the history and epidemiology of the preeminent arboviral disease. *Infect Genet Evol* 2009;**9**:523–40. doi:10.1016/j.meegid.2009.02.003.
[3] Paz-Bailey G, Adams L, Wong JM, et al. Dengue vaccine: recommendations of the Advisory Committee on Immunization Practices, United States, 2021. *MMWR Recomm Rep* 2021;**70**:1–16. doi:10.15585/mmwr.rr7006a1.
[4] Rivera L, Biswal S, Sáez-Llorens X, et al. Three-year efficacy and safety of Takeda's dengue vaccine candidate (TAK-003). *Clin Infect Dis* 2022;**75**:107–17. doi:10.1093/cid/ciab864.

[5] Holmes EC, Twiddy SS. The origin, emergence and evolutionary genetics of dengue virus. *Infect Genet Evol* 2003;**3**:19–28. doi:10.1016/s1567-1348(03)00004-2.
[6] Waggoner JJ, Balmaseda A, Gresh L, et al. Homotypic dengue virus reinfections in Nicaraguan children. *J Infect Dis* 2016;**214**:986–93. doi:10.1093/infdis/jiw099.
[7] Guzman MG, Harris E. Dengue. *Lancet* 2015;**385**:453–65. doi:10.1016/S0140-6736(14)60572-9.
[8] Sabin AB. Research on dengue during World War II. *Am J Trop Med Hyg* 1952;**1**:30–50. doi:10.4269/ajtmh.1952.1.30.
[9] Vasilakis N, Weaver SC. Chapter 1 the history and evolution of human dengue emergence. *Adv Virus Res* 2008;**72**:1–76.
[10] Rudnick A, Lim TW, Ireland JL. *Dengue fever studies in Malaysia*. Kuala Lumpur: Institute for Medical Research; 1986.
[11] Messina JP, Brady OJ, Scott TW, et al. Global spread of dengue virus types: mapping the 70 year history. *Trends Microbiol* 2014;**22**:138–46. doi:10.1016/j.tim.2013.12.011.
[12] Gubler DJ. Dengue and dengue hemorrhagic fever. *Clin Microbiol Rev* 1998;**11**:480–96. doi:10.1128/CMR.11.3.480.
[13] Smith CEG. The history of dengue in tropical Asia and its probable relationship to the mosquito Aedes aegypti. *J Trop Med Hyg* 1956;**59**:243–51.
[14] Stica CJ, Barrero RA, Murray RZ, et al. Global evolutionary history and dynamics of dengue viruses inferred from whole genome sequences. *Viruses* 2022;**14**:703. doi:10.3390/v14040703.
[15] Twiddy SS, Holmes EC, Rambaut A. Inferring the rate and time-scale of dengue virus evolution. *Mol Biol Evol* 2003;**20**:122–9. doi:10.1093/molbev/msg010.
[16] Amarasinghe A, Kuritsk JN, Letson GW, et al. Dengue virus infection in Africa. *Emerg Infect Dis* 2011;**17**:1349–54. doi:10.3201/eid1708.101515.
[17] Proesmans S, Katshongo F, Milambu J, et al. Dengue and chikungunya among outpatients with acute undifferentiated fever in Kinshasa, Democratic Republic of Congo: a cross-sectional study. *PLoS Negl Trop Dis* 2019;**13**:e0007047. doi:10.1371/journal.pntd.0007047.
[18] Clancy A, Crowley B, Niesters H, et al. The development of a qualitative real-time RT-PCR assay for the detection of hepatitis C virus. *Eur J Clin Microbiol Infect Dis* 2008;**27**:1177–82. doi:10.1007/s10096-008-0556-9.
[19] Johnson BW, Russell BJ, Lanciotti RS. Serotype-specific detection of dengue viruses in a fourplex real-time reverse transcriptase PCR assay. *J Clin Microbiol* 2005;**43**:4977–83. doi:10.1128/JCM.43.10.4977-4983.2005.
[20] Hadfield J, Megill C, Bell SM, et al. Nextstrain: real-time tracking of pathogen evolution. *Bioinformatics* 2018;**34**:4121–3. doi:10.1093/bioinformatics/bty407.
[21] Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 2013;**30**:772–80.
[22] Martin DP, Murrell B, Golden M, et al. RDP4: detection and analysis of recombination patterns in virus genomes. *Virus Evol* 2015;**1**:vev003. doi:10.1093/ve/vev003.
[23] Nguyen LT, Schmidt HA, von Haeseler A, et al. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol* 2015;**32**:268–74. doi:10.1093/molbev/msu300.
[24] Hill S, Neto de Vasconcelos J, Granja BG, et al. Early genomic detection of cosmopolitan genotype of dengue virus Serotype 2, Angola, 2018. *Emerg Infect Dis* 2019;**25**:784–7.
[25] Neto Z, Martinez PA, Hill SC, et al. Molecular and genomic investigation of an urban outbreak of dengue virus serotype 2 in Angola, 2017–2019. *PLoS Negl Trop Dis* 2022;**16**:e0010255. doi:10.1371/journal.pntd.0010255.
[26] Yamamoto SP, Kasamatsu Y, Kanbayashi D, et al. Dengue virus in traveler returning to Japan from the Democratic Republic of the Congo, 2015. *Jpn J Infect Dis* 2019;**72**:426–8. doi:10.7883/yoken.JJID.2018.554.
[27] Colavita F, Vairo F, Carletti F, et al. Full-length genome sequence of a dengue serotype 1 virus isolate from a traveler returning from Democratic Republic of Congo to Italy, July 2019. *Int J Infect Dis* 2020;**92**:46–8. doi:10.1016/j.ijid.2019.12.023.
[28] Kraemer MUG, Faria NR, Reiner RC, et al. Spread of yellow fever virus outbreak in Angola and the Democratic Republic of the Congo 2015–16: a modelling study. *Lancet Infect Dis* 2017;**17**:330–8. doi:10.1016/S1473-3099(16)30513-8.
[29] Osondu-Oti A. An appraisal of India-Nigeria historical and contemporary relations. *Alternation Special Edition* 2015;**15**:102–9.