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Tracing and tracking the emergence, epidemiology and dispersal of dengue virus to Africa during the 20th century

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ABSTRACT

The four mosquito-borne dengue virus serotypes (DENV1–DENV4) cause a high burden of disease throughout the tropical and sub-tropical regions of the world. Nevertheless, their precise epidemiological history in Africa, including when and where they originated and were distributed during the 20th century, remains unclear stressing the need for *One Health* focused research. Accordingly, we conducted a time-scaled molecular epidemiological reconstruction using publicly available and newly sequenced dengue virus genomes of African origin representing all four serotypes to deduce the most likely temporal and spatial transmission routes of each DENV serotype from their ancestral regions to, within and from Africa.

Our analyses suggest that during the 20th century, serotypes DENV1–DENV3 were introduced to Africa from South East Asia on multiple occasions. The earliest evidence recorded indicates introduction of DENV2 during the early-1940s and of DENV1 during the mid-1940s to Western Africa from South East Asia. The analysis also implies an early introduction of DENV4 during the mid-1940s to Western Africa, alongside DENV1, probably originating in South East Asia. Establishment of DENV3 in Africa appears to have occurred later in the 1960s, apparently originating from South East Asia. However, with the re-establishment of DENV in the Americas, following the cessation of the PAHO mosquito control programme during the mid-20th century, evidence of introductions of DENV1 and DENV2 from the Americas to Western Africa was also observed. The data also identify intra-regional circulation of DENV, but also inter-regional dispersal of all four serotypes within Africa, which has led to a high degree of geographical overlap among serotypes. It is also noteworthy that DENV from both Eastern and Western Africa, have been introduced into Central Africa but there is no support for the converse relationship. For serotypes DENV1–DENV3, we observed probable exports from within established African DENV clusters (≥2 sequences) primarily to Eastern and Southern Asia.

Collectively, our findings support the view that all DENV serotypes, apart from DENV4, have been introduced on multiple occasions to Africa, primarily originating from South East Asia, and subsequently to neighbouring regions within Africa.

1. Introduction

Epidemiological records, dating from the 16th century onwards, in the Caribbean Islands and coastal towns of Southern, Central and North America describe a dengue-like disease associated with incoming commercial vessels from the Old World with Africa as the primary origin of the cargo on these ships [1]. During the same period, epidemiologically similar disease outbreaks were described in Zanzibar, India, Egypt, Japan, and throughout South East Asia [2–4]. Thus, for centuries, a disease fitting the clinical and epidemiological characteristics of

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currently recognized dengue fever, was likely being introduced westwards from Africa to the Americas, alongside yellow fever virus, largely via the slave trading ships and eastwards on the returning commercial routes throughout the southern European temperate regions and the Asian tropical and sub-tropics [1,5,6]. Today, the aetiological agent is known to be a flavivirus (genus *Flavivirus,* family *Flaviviridae*), i.e. dengue virus (DENV) comprising four distinct serotypes (DENV1–DENV4) existing in an enzootic cycle infecting primates and an epidemic cycle affecting humans [7]. However, despite efforts to develop safe and effective vaccines and antiviral treatments, the prospects for control of these four DENV serotypes looks bleak.

During the 20th century, the four genetically related serotypes of dengue virus (DENV1–DENV4) have primarily been transmitted to humans by *Aedes aegypti aegypti* (Aaa), a descendant urban domestic mosquito variant of the African forest-associated *A. aegypti formosus* (Aaf) species. The four serotypes have become established globally throughout the tropics and sub-tropics [8,9], causing up to 500 million cases of dengue fever each year [8,10–12] with an astonishing four billion people at risk of infection [13]. Moreover, with the impact of climate change, increased urbanisation and a continuously expanding commercial transportation industry, these numbers are predicted to continue to grow [14]. Despite being the most common reported human pathogenic arthropod-borne virus (arbovirus) infection and likely endemic in many African countries [15], our understanding of the epidemiology and possible origins of DENV in Africa are largely undefined, mainly due to the lack of adequate surveillance and a coordinated diagnostic and reporting infrastructure. For example, in some parts of Africa, dengue fever is frequently misdiagnosed as being due to other diseases such as malaria. Consequently, the true burden of dengue fever/haemorrhagic fever and its dispersal in Africa is largely unknown [10,16,17].

Although dengue-like illness has been described in the Americas since the late 16th century, the first reported outbreaks of dengue fever in Africa occurred at the end of the 18th century in Zanzibar, Senegal and Gambia [18,19]. However, the possibility that early dengue-like urban epidemics in Africa may have been caused by other arboviruses cannot be entirely ruled out [20,21]. The first DENV epidemic in Africa, confirmed with retrospective serological studies (DENV1 antibodies), occurred in 1927 in South Africa [22], and the first isolates of African strains belonged to serotypes DENV1 and DENV2 recovered from febrile patients in Nigeria in the 1960s [23]. Since the beginning of the 20th century, local scattered outbreaks, presumed to be due to DENV, were reported, with a steep increase in dengue fever incidence and from the 1980s, onwards [18], outbreaks of dengue fever involving all four serotypes had been reported in most countries on the African continent [15,24–27]. Recently, dengue outbreaks have been reported in Angola [28], Gabon [29], Ghana [30] and Senegal [31]. However, many outbreaks probably go unnoticed as indicated by the high burden in children with undifferentiated fever in Kenya [32]. Overall, there is a relatively high seroprevalence across the entire African continent [17,33], reaching more than 50% in some regions [34]. Whether or not this high-level background immunity can explain the relatively low incidence of dengue haemorrhagic fever in Africa remains to be determined.

Nevertheless, despite the probable high endemicity of DENV throughout the populated regions of Africa and the need to increase surveillance and sequencing efforts [16,35], there are few DENV sequences of African origin available in the public databases when compared with DENV sequences of Latin American and Asian origin. In this study, we conducted a comprehensive genome-based molecular epidemiological reconstruction of all four DENV serotypes using available published DENV whole genome (or near-complete) sequences of African origin and 21 newly sequenced complete DENV genomes of introduced cases from travellers who recently visited an African country. Specifically, our study focuses on (i) identifying whether these extant DENV emerged in Africa from enzootic African viruses or were introduced into Africa, (ii) the connectivity of DENV dispersal within Africa, (iii) defining the main routes of import and export of DENV to and from Africa.

2. Materials and methods

2.1. Sequencing of samples from Swedish patients

RNA isolated from serum from 29 dengue virus positive Swedish patients returning from travel abroad were included in this study (Supplementary Table S2). KAPA RNA HyperPrep (Roche) was used to prepare sequencing libraries following the manufacturer's instructions with the following adjustments; fragmentation at 85 °C for 1 min, and 18 cycles of library amplification. Nextflex 96 DNA barcodes (PerkinElmer) were used as adapters. Capture-based target enrichment was performed using bespoke myBaits probes (Arbor Biosciences) following the manufacturer's instructions using pools of up to eight DNA libraries. The probes were designed by Arbor Biosciences using all available whole genome sequences of the dengue virus available in the NCBI database at the time (2017). The capture-based target enrichment briefly involved: hybridization of the pooled DNA libraries together with the baits at 65 °C for 22 h, followed by capture bead binding, washes, 25 cycles of amplification using the KAPA HiFi HotStart ReadyMix and the (KAPA) library amplification primer mix, and finally 1× bead-based clean-up using KAPA Pure Beads. The enriched DNA libraries were sequenced on the MiSeq platform (Illumina) following the manufacturer's protocol. Adapters were removed and sequence reads were trimmed using Trim galore v.0.6.4 (https://github.com/FelixKrueger/TrimGalore), a wrapper around Cutadapt and FastQ, using default settings with the following adjustments low-quality end trimming set to Phred 30 and minimum read length 50 bp. Trimmed reads were aligned using Bowtie2 v.2.4.1 [36] using the library of sequences used to design the enrichment probes (above) as reference. Sequences were called using the best-hit (most reads aligned) reference sequence (see supplementary Table S3), calling nucleotides with a minimum coverage of 30 reads and majority variants (>50%) using Geneious v.2020.1 by Biomatters Ltd. The sequences have been deposited to GenBank (see accession numbers in Supplementary Table S3).

2.2. Reconstructing the emergence of DENV in Africa

Sequences were obtained with an exhaustive search in databases NCBI with specific emphasis on African dengue virus samples (Supplementary Table S2). In order to test the correct model for the time-based phylogeny we analysed three different clock models on our data set (Supplementary Table S4). Generalised time reversible (GTR) with empirical base frequencies, four gamma variables and invariant sites, were selected as site model based on the ModelFinder model selector as a part of the IQ-TREE v1.6.9 [37]. As the aim of the study did not include any demographic modelling, the tree prior was set to a flexible non-parametric Gaussian Markov random field (GMRF) Bayesian Skyride [38]. Marginal likelihood estimation (MLE) using both path sampling (PS) and stepping-stone sampling (SS) are compared in Supplementary Table S4; uncorrelated relaxed clock with a lognormal distribution was selected for DENV1, DENV2, and DENV3, whereas uncorrelated relaxed clock with an exponential distribution was selected for DENV4. The following taxa outgroups were defined; for DENV1 one sample of sylvatic origin from Brunei in 2014 (KR919820), for DENV2 one sample of sylvatic origin from Malaysia in 2015 (KY923048), for DENV3 the clade including L11433, L11434 and L11439 from Latin American and Oceania, and for DENV4 three samples isolated from higher primates (Simiiformes) (JF262780, JF262779, EF457906). Remaining taxa were defined as a monophyletic ingroup. All serotypes were run with starting UPGMA tree. Countries were grouped into regions using the geoscheme of the United Nations Statistical Division (M49) (merging some of the non-African regions). Regions were included as traits with an

asymmetric substitution model with Bayesian Stochastic Search Variable Selection (BSSVS) procedure, using the same clock setting and the partition tree as the corresponding sequences, with ancestral state reconstruction. The aligned sequences for each serotype were run in Beast v1.10.1 [39] three separate times, apart from DENV2 that was run four times, for at least 150 M Markov chain Monte Carlo (MCMC) generations, logging every 5 k chain. The log and tree files from the three runs were concatenated using logcombiner, parameter log files were down-sampled every 10 k chain applying a burn-in for each file when steady state was reached. Rates log and tree files were concatenated without any modifications. Parameter log files were examined in Tracer [40] to ensure ESS > 100. Treeannotator v1.10.1 was used to identify the best trees from the MCMC runs of each serotype based on maximum clade credibility (calculating median node heights). Reconstructed regions of the ancestral nodes were set to minimum 90% probability (noteworthy exceptions are described with coloured stars and described in the text). Clusters of ≥2 samples from an African region were assigned a cluster definition and are described in the results. All tip labels (samples), posterior and 95% highest posterior density (HPD) are shown in supplementary Figs. S1–S4. SpreaD3 v0.9.6 [41] was used to calculate Bayes factor (BF) support and posterior probability (PP) using default settings (Poisson prior mean: log [2], prior offset: n-1) for the rates between regions (our discrete trait) of each of the four serogroups using the concatenated rates log files from the three runs described above (Supplementary Table S1) [42]. Only rates with BF > 10 were included, where transmissions with BF 10–100 were considered to be strongly

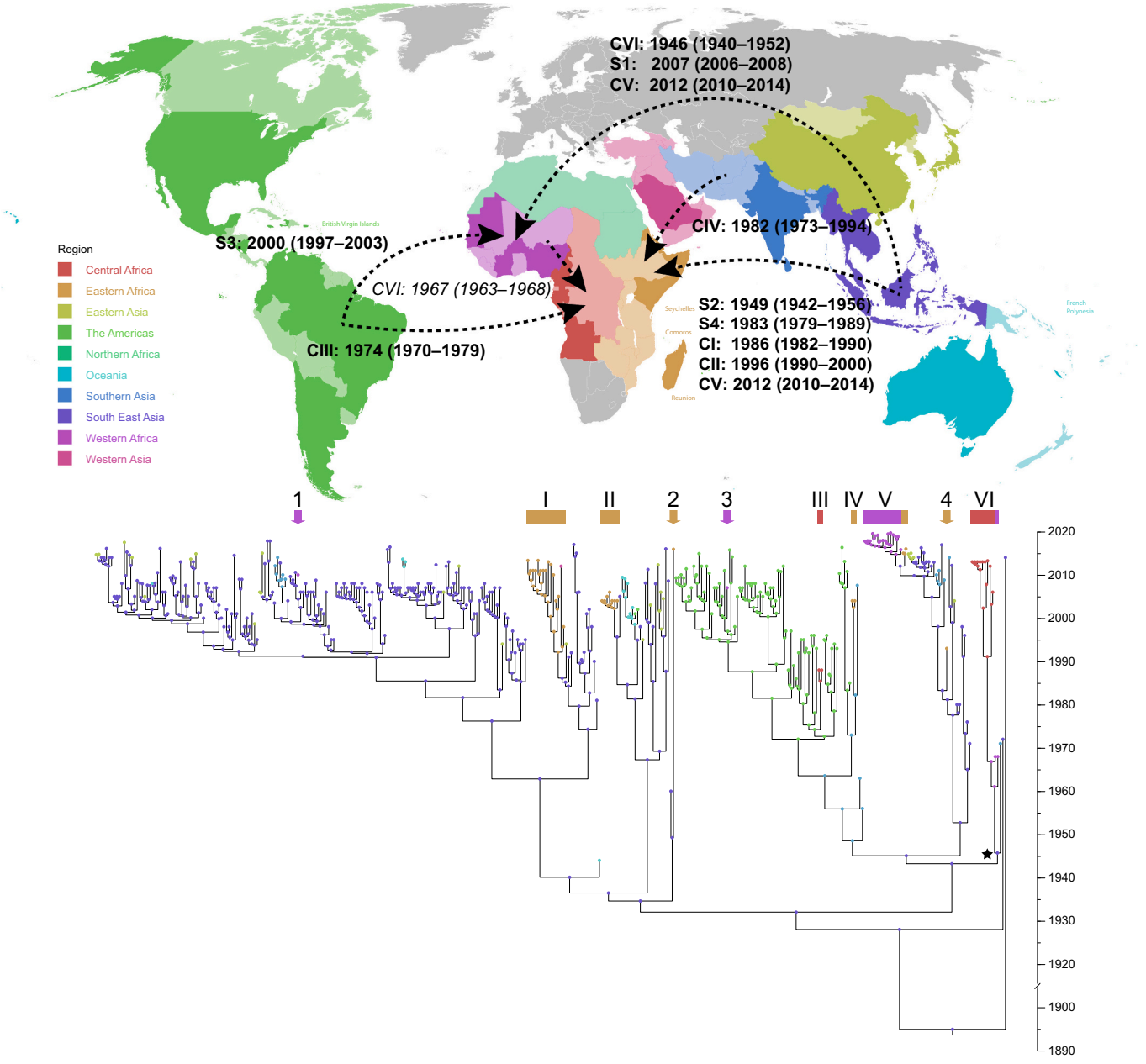

Fig. 1. Global map showing the distribution of the samples included in the present study representing serotype DENV1. Countries are colour-coded by regions (where light shade represents countries not represented with samples). Countries not included in this study are colour-coded grey. Tips and nodes in the time-based phylogeny are colour-coded by geographical regions, nodes are coloured by most likely ancestral location. Shown on top of the phylogeny and colour-coded by the African region in which they were isolated, clusters of ≥2 samples are labelled with Roman numerals and singletons are labelled with Arabic numerals. Suggested transmission events to, from and within the African continent are shown with dashed arrows on the global map, with time of divergence of MRN-AA of the different clusters and singletons shown with 95% highest posterior density in parentheses.

supported and BF > 100 were very strongly supported.

2.3. Ethical statement

Handling and sequencing the samples from 29 dengue virus positive Swedish patients was in accordance with the ordinance from the Swedish Parliament 2013:1020:§3.

3. Results

3.1. DENV in Africa

Overall, our time-scaled phylogeographic analysis, based predominantly on extant and currently circulating DENV variants, suggests that all four DENV serotypes were introduced, on multiple occasions, to Africa primarily from Southern or South East Asia since the 1940s (Fig. 1–4). In other words, the currently circulating known diversity of variant DENV strains in Africa predominantly represents those introduced from Asia during the 20th century. For each African cluster or singleton, the time of divergence to the most recent non-African ancestor

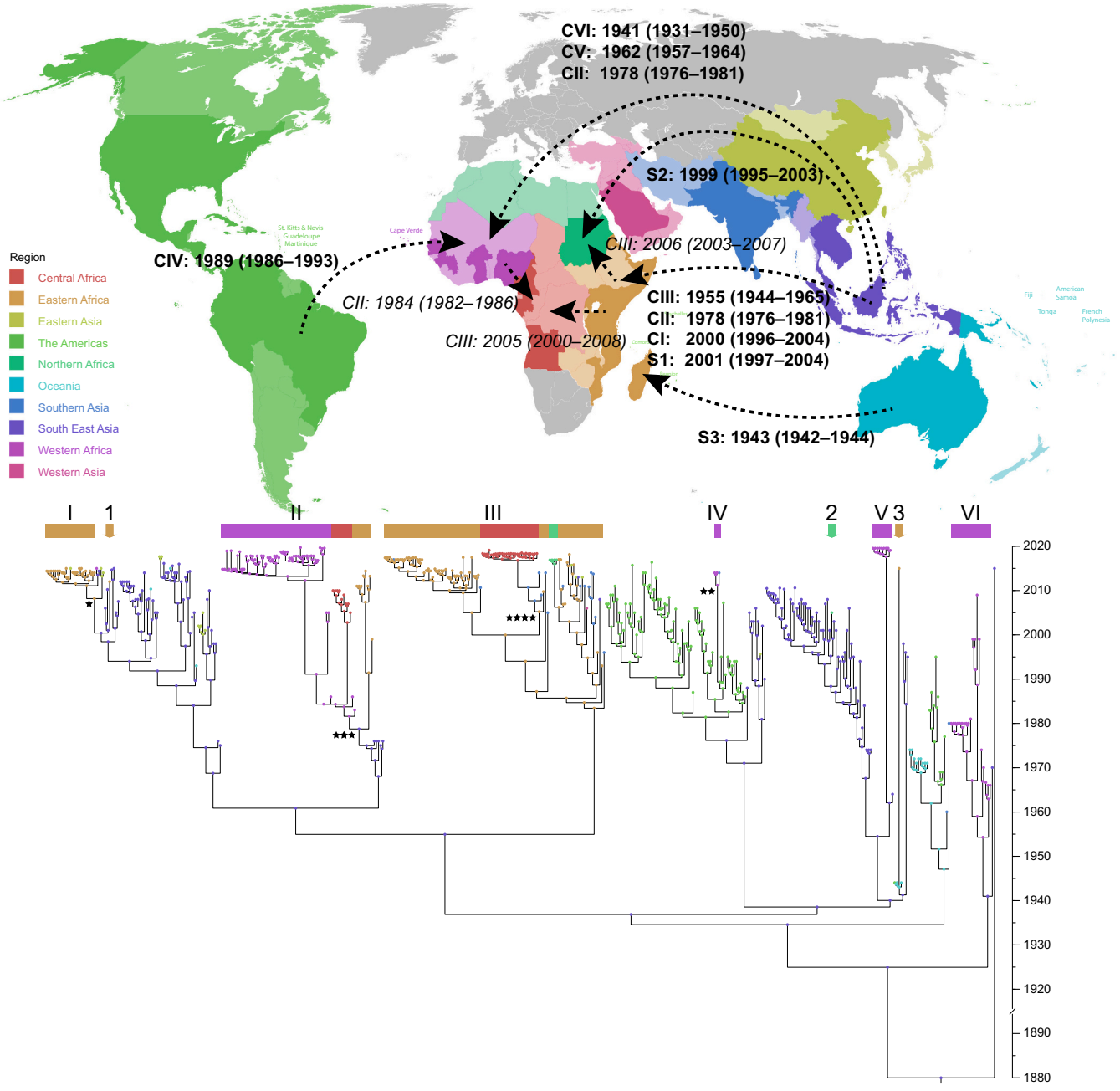

Fig. 2. Global map showing the distribution of the samples included in the present study representing serotype DENV2. Countries are colour-coded by regions (where light shade represents countries not represented with samples). Countries not included in this study are colour-coded grey. Tips and nodes in the time-based phylogeny are colour-coded by geographical regions, nodes are coloured by most likely ancestral location. Shown on top of the phylogeny and colour-coded by the African region in which they were isolated, clusters of ≥2 samples are labelled with Roman numerals and singletons are labelled with Arabic numerals. Suggested transmission events to, from and within the African continent are shown with dashed arrows on the global map (internal transmissions in italics), with time of divergence of MRN-AA of the different clusters and singletons shown with 95% highest posterior density in parentheses.

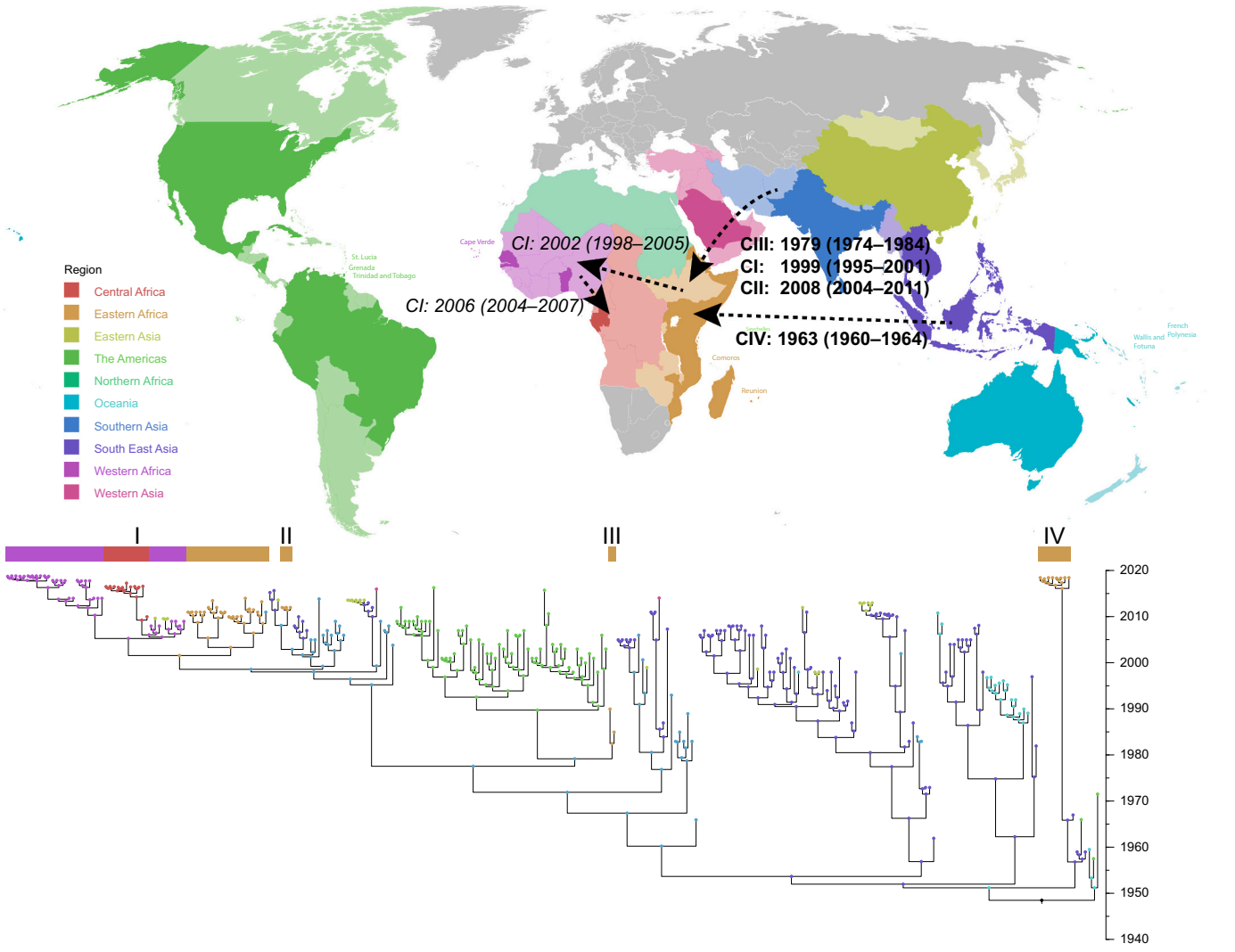

Fig. 3. Global map showing the distribution of the samples included in the present study representing serotype DENV3. Countries are colour-coded by regions (where light shade represents countries not represented with samples). Countries not included in this study are colour-coded grey. Tips and nodes in the time-based phylogeny are colour-coded by geographical regions, nodes are coloured by most likely ancestral location. Shown on top of the phylogeny and colour-coded by the African region in which they were isolated, clusters of ≥2 samples are labelled with Roman numerals and singletons are labelled with Arabic numerals. Suggested transmission events to, from and within the African continent are shown with dashed arrows on the global map (internal transmissions in italics), with time of divergence of MRN-AA of the different clusters and singletons shown with 95% highest posterior density in parentheses.

(MRN-AA), i.e. the most recent phylogenetic node predicted (>90% probability) to be of non-African origin, was re-construed from the time-scaled phylogeny. The analyses also imply that there has been significant intra-African dispersal of DENV, which is the most likely source of regional ongoing endemics, with low indication of exportation to regions outside of Africa, as shown for DENV1–DENV3 serotypes (Fig. 1–3).

Our analysis also reveals that (i) clusters of DENV1–3 overlapped geographically in Eastern Africa in the 2010s, (ii) clusters of DENV2 and DENV3 overlapped in Western Africa in the 2010s, and (iii) clusters of DENV1 and DENV2 are both found in Central Africa. However, DENV1 was present from the early 2000s whereas DENV2 was present from the 2010s.

3.2. Serotype DENV1

The earliest time to divergence from the most recent non-African ancestor (MRN-AA) for DENV1, i.e., the first introduction, was estimated to be ca. 1946 (95% highest posterior density [HPD]: 1940–1952), following an introduction from Southern Asia (location probability [LP]: 55%) into Western Africa (node with black star in Fig. 1). This particular lineage later formed a cluster, defined herein as the well-supported phylogenetic grouping of two or more sequences from an African region, around year 1961 (HPD: 1954–1966) which expanded to include Central Africa around 1967 (HPD: 1963–1968) (Fig. 1, cluster VI). The cluster is represented with sequences from Nigeria in Western Africa, and from Gabon, Cameroon and Angola in Central Africa (Supplementary Fig. S1). A single sequence (i.e. singleton) from Kenya in Eastern Africa diverged from its closest ancestor in South East Asia (LP: >99%) around year 1949 (HPD: 1942–1956) (Fig. 1, singleton 2). Two sequences from Angola in Central Africa can be traced to probable introduction from Latin America (LP: >99%) around the year 1974 (HPD: 1970–1979) (Fig. 1, cluster III). Another two sequences from the island of La Réunion off the East-African coast sampled in 2004 can be traced back to an MRN-AA in Southern Asia (LP: 93%) in 1982 (HPD: 1973–1994) (Fig. 1, cluster IV). A singleton from the islands of Comoros collected in 1993 was also shown to have diverged from an MRN-AA in South East Asia (LP: 94%) in 1983 (HPD: 1979–1989) (Fig. 1, singleton 4).

Sequences of African origin in serotype DENV1 formed two

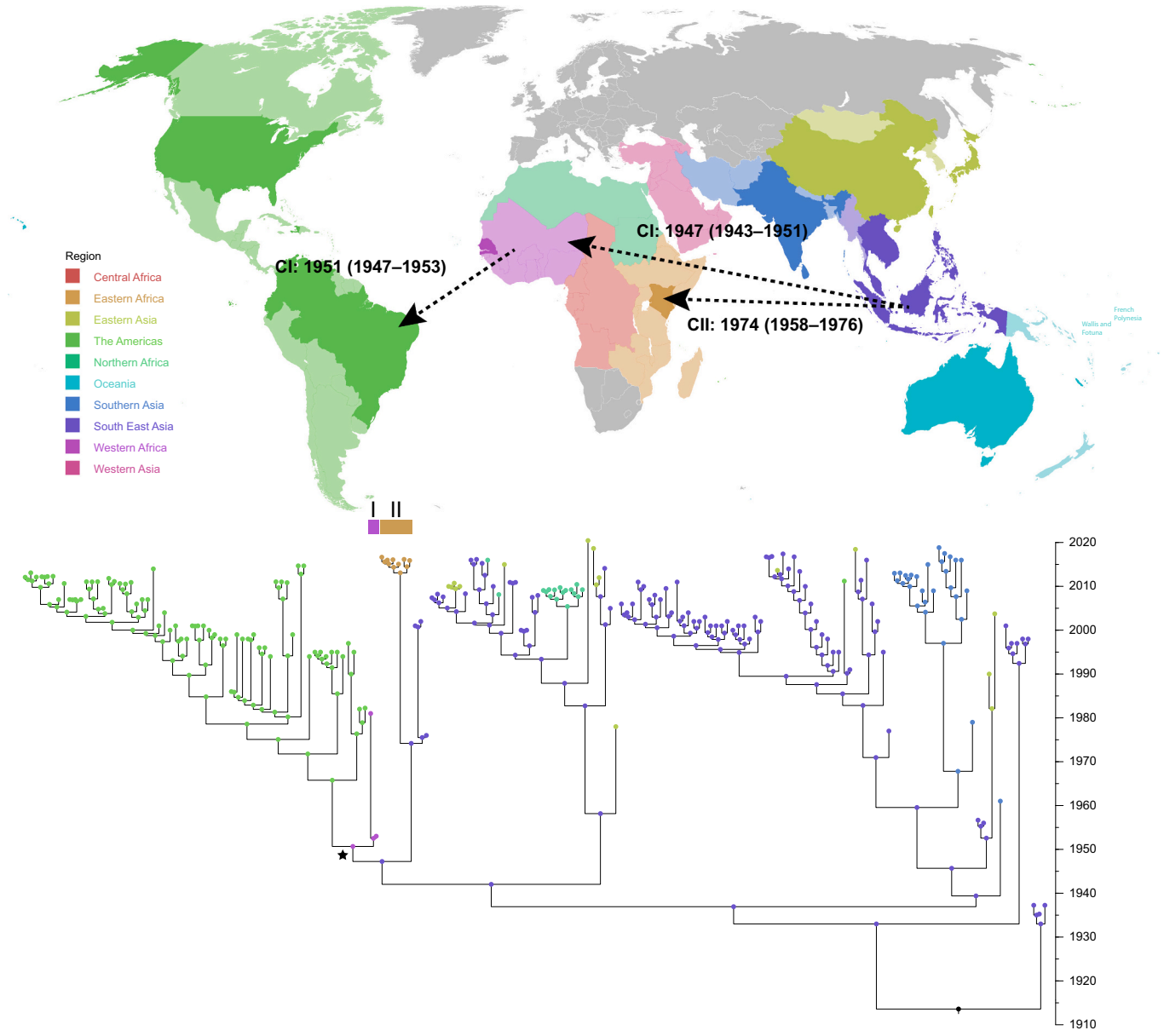

Fig. 4. Global map showing the distribution of the samples included in the present study representing serotype DENV4. Countries are colour-coded by regions (where light shade represents countries not represented with samples). Countries not included in this study are colour-coded grey. Tips and nodes in the time-based phylogeny are colour-coded by geographical regions, nodes are coloured by most likely ancestral location. Shown on top of the phylogeny and colour-coded by the African region in which they were isolated, clusters of ≥2 samples are labelled with Roman numerals and singletons are labelled with Arabic numerals. Suggested transmission events to, from and within the African continent are shown with dashed arrows on the global map (internal transmissions in italics), with time of divergence of MRN-AA of the different clusters and singletons shown with 95% highest posterior density in parentheses.

additional clusters (Fig. 1, clusters I and II), that diverged from their MRN-AA in South East Asia (LP: 92% and > 99%, respectively) on two separate occasions in 1986 (HPD: 1982–1990) and 1996 (HPD: 1990–2000). Subsequently, clusters I and II were formed around 1992 (95% HPD: 1988–1996) and 2002 (HPD: 2001–2003), respectively. Cluster I is represented by sequences until 2013, whereas the most recent sequence belonging to cluster II, can be traced back to 2006. Geographically, cluster I is restricted to the northern countries of Eastern Africa (Somalia, Kenya, Eritrea, Djibouti), whereas cluster II is restricted to the eastern islands of Eastern Africa (La Réunion, Seychelles, Madagascar). Two additional singletons, both sampled in Western Africa, were estimated with MRN-AA from South East Asia (LP: >99%) and Latin America (LP: >99%), with estimated divergence in 2007 (HPD: 2006–2008) and 2000 (HPD: 1997–2003) respectively (Fig. 1, singletons 1 and 3). Finally, clusters of Eastern African (from Eritrea and Kenya) and Western African sequences (Mauritania, Burkina Faso, Cote d'Ivoire, Senegal and Benin) were estimated to be of relatively recent origin, 2012 (HPD: 2010–2014) with an MRN-AA in South East Asia (LP: 80%) (Fig. 1, cluster V).

The discrete trait model points to two main routes of introduction of DENV1 to Africa with very strong support (BF > 100), from the Americas to Western Africa (BF: 167, PP: 96%) and from South East Asia to Eastern Africa (BF: 148; PP: 95%) (Fig. 5 and supplementary Table S1). The model proposes one additional route of introduction with strong support (BF: 10–100), from South East Asia to Western Africa (BF: 42; PP: 85%). The model also implies one internal route within Africa, from Western Africa to Central Africa (BF: 144; PP: 95%) and one exportation route, from Eastern Africa to Western Asia (BF: 191; PP: 96%) (Fig. 5).

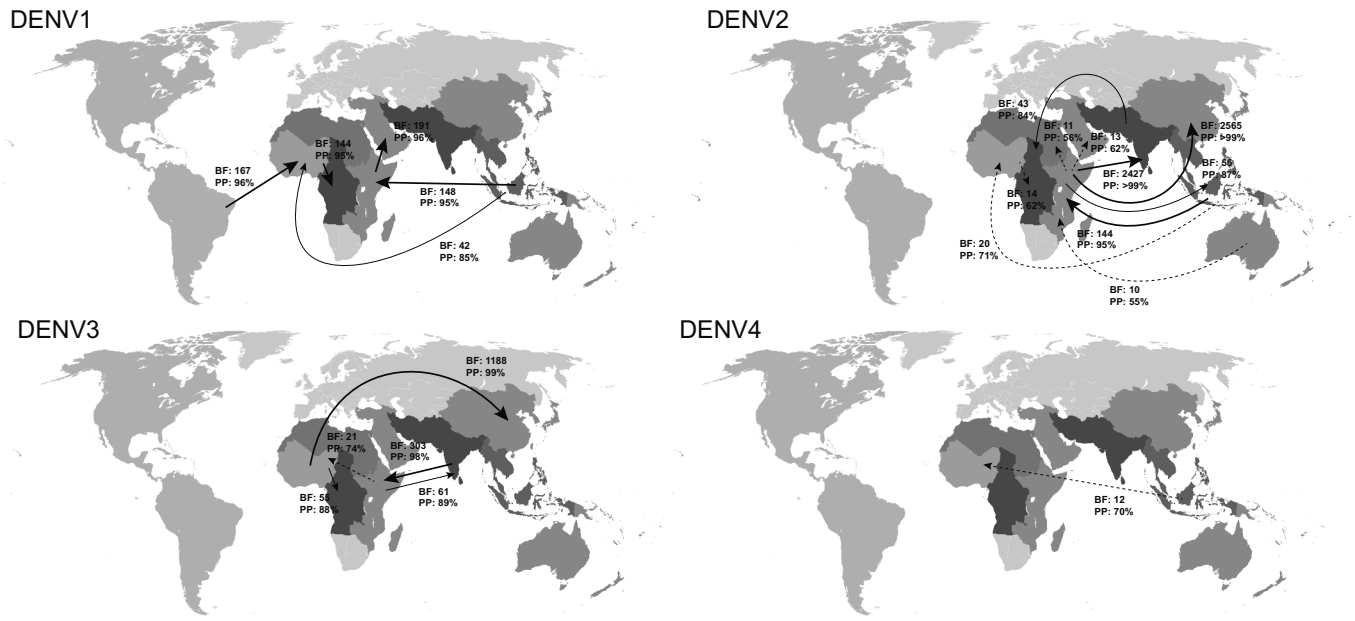

Fig. 5. Global maps showing the discrete trait model showing the proposed routes of transmissions to, within, and from Africa for the four different serotypes. Solid thick lines represent transmissions with extreme support (Bayes Factor [BF] > 100), solid thin lines represent transmissions with very strong support (BF: 30–100), and dashed lines represent transmissions with strong support (BF: 10–30). Suggested transmissions with lower support (BF < 10) are not shown (Supplementary Table S1).

3.3. Serotype DENV2

Following the divergence from the MRN-AA from South East Asia (LP: 97%) in 1941 (HPD: 1931–1950), the emergence of the first monophyletic African DENV2 cluster was seen in Western Africa (Fig. 2, cluster VI), with earliest cases showing up in Nigeria and Senegal (Supplementary Fig. S2). Following its establishment in the region, the cluster has remained active for almost 70 years, with the last observed case from around the year 2009 (in Guinea Bissau).

Cluster III was possibly introduced to Eastern Africa as early as 1955 (HPD: 1944–1965) following dispersal from South East Asia (LP: 98%) (Fig. 2). Possible exports from Eastern Africa to Eastern Asia (China), Southern Asia (India), and some cases of possible exportation to South East Asia (Malaysia, Singapore) and Western Asia (Saudi Arabia) were observed multiple times within this cluster. We note that the Northern African sequences in cluster III were probably introduced from Eastern Africa (LP: 97%) around 2006 (HPD: 2003–2007) (Fig. 2). The Central African sequences in cluster III are indicated to have diverged from an MRN-AA from Southern Asia (LP: 98%) around 2010 (HPD: 2008–2013), of which the most recent African ancestor can be traced back to Eastern Africa in 2005 (HPD: 2000–2008) (Fig. 2, four stars).

Cluster V represents nine relatively recent and closely related sequences from Nigeria, comprising a single monophyletic group that diverged from their MRN-AA in South East Asia (LP: >99%) around 1962 (HPD: 1957–1964) (Fig. 2). Thus, potentially this DENV lineage was present but remained undetected in Nigeria for almost 60 years.

The MRN-AA of cluster II appears to have originated in 1978 (HPD: 1976–1981) from South East Asia (LP: 58%), becoming established in Western Africa (LP: >99%) around 1982 (HPD: 1979–1983) and Eastern Africa (LP: 96%) around 1991 (HPD: 1983–1998). Alternatively, this cluster was established in Eastern Africa (LP: 34%) (Fig. 2, three black stars), pushing the MRN-AA from South East Asia back to 1975 (HPD: 1973–1976). Dispersal from Western Africa (LP: >99%) to Central Africa is estimated to have occurred around 1984 (HPD: 1982–1986), forming a monophyletic Central African cluster in 2003 (HPD: 1996–2005). Cluster II was then maintained in each of these three regions at least until 2010 (Fig. 2).

There is some uncertainty over the first establishment of cluster IV in Africa following the divergence from the MRN-AA from the Americas (LP: >99%) around 1989 (HPD: 1986–1993), the reconstructed phylogeny placed the ancestral node of cluster IV to Western Africa (LP: 50%) or Southern Asia (LP: 27%) (Fig. 2, node marked with two black stars). Similarly, there is some uncertainty as to the MRN-AA of cluster I (Eastern Africa LP: 62%) (Fig. 2, node marked with one black star), most likely due to divergence from South East Asia (LP: 98%) around 2000 (HPD: 1996–2004). Most of the sequences in this cluster have been isolated from patients in Tanzania, with a few sequences from Mozambique, Kenya, and Cape Verde in Western Africa (Supplementary Fig. S2). However, a few non-African sequences from the Philippines and Taiwan can be found nested within this cluster, indicative of possible exportation cases (Supplementary Fig. S2). A singleton (also from Tanzania) outside of cluster I can be traced to a separate introduction from South East Asia (LP: 97%) around the same time as cluster I (2001, HPD: 1997–2004) (Fig. 2, singleton 1).

A singleton introduction from South East Asia (LP: >99%) to Northern Africa was also noted to have occurred in the late 1990s (1999, HPD: 1995–2003), marking one of the few cases of direct introduction to Northern Africa (Sudan) (Fig. 2, singleton 2). Another singleton observed in Eastern Africa can be traced back to a likely MRN-AA in Oceania (Papua New Guinea) (LP: 98%) around 1943 (HPD: 1942–1944) (Fig. 2, singleton 3).

Out of the larger clusters (>2 African sequences), clusters I, V and VI are mainly restricted to single regions in Eastern Africa (with exceptions in Cape Verde and Taiwan) and in Western Africa (respectively), whereas clusters II and III are composed of more than one African region. Clusters I and III coincide in the Eastern African region during the 2010s. However, a closer inspection reveals that most of the sequences belonging to cluster I were found in Tanzania and Mozambique (southern reaches of the region), whereas sequences in cluster III were mainly found in Kenya and Sudan (northern reaches of the region) (Supplementary Fig. S2). All these four clusters probably originated independently from South East Asia (LP: >90%).

The discrete trait model points to one route of introduction of DENV2 to Africa with robust support (BF > 100), from South East Asia to Eastern Africa (BF: 144, PP: 95%) (Fig. 5 and supplementary Table S1). The model implies three other routes of introduction with strong support,

from Southern Asia to Central Africa (BF: 43; PP: 84%), from South East Asia to Western Africa (BF: 20; PP: 71%), and from Oceania to Eastern Africa (BF: 10; PP: 55%). Two routes of transmission within Africa were identified with strong support; from Western Africa to Central Africa (BF: 14; PP: 62%) and from Eastern to Northern Africa (BF: 11; PP: 56%). The model implies two routes of export from Africa with robust support, both from Eastern Africa, to Eastern Asia (BF: 2565; PP: >99%) and to Southern Asia (BF: 2427; PP: >99%). One additional route of exportation is implied from Africa with strong support, from Eastern Africa to Western Asia (BF: 13; PP: 62%) (Fig. 5 and Supplementary Table S1).

3.4. Serotype DENV3

The most ancient sequence of DENV 3 in Africa comes from Mozambique in 1985, with an MRN-AA from Southern Asia (LP: 67%) around 1979 (HPD: 1974–1984) (Fig. 3, cluster III). The reconstructed phylogeny points to a possible earlier introduction date, tracing back to a relatively recent collection of sequences from Kenya in 2015, cluster IV, with an MRN-AA divergence event occurring in South East Asia (LP: >99%) in 1963 (HPD: 1960–1964) (Fig. 3, cluster IV). However, the most recent Eastern African ancestor (LP: >99%) of the monophyletic cluster IV is much more recent (2013, HPD: 2012–2014).

Two clusters, one representing many sequences from multiple African regions (cluster I) and one represented with only three (cluster II), appear to have originated from the same non-African ancestor somewhere in Southern Asia. The MRN-AA of cluster I can be traced back to Southern Asia (LP: >99%) around 1999 (HPD: 1995–2001). Subsequently, the cluster appears to have established in Eastern Africa (LP: 73%) around 2002 (HPD: 1998–2005), then transferred to and established in Western Africa (LP: 98%) around 2005 (HPD: 2003–2007) (Fig. 3, cluster I). The group of sequences from Central Africa appears to have originated in Western Africa (LP: >99%) around 2006 (HPD: 2004–2007) (Fig. 3, cluster I). Three sequences from Eastern Asia (China) were located within cluster I, probably being exported from Western Africa on separate occasions (both LP: >99%). The MRN-AA of the three sequences from Eastern Africa (Reunion) making up cluster II can be traced back to Southern Asia (LP: 71%) around 2012 (HPD: 2011–2012) (Fig. 3 and Supplementary Fig. S3).

The discrete trait model points to one single route of introduction to Africa with robust support, from Southern Asia to Eastern Africa (BF: 303; PP: 98%) (Fig. 5 and supplementary Table S1). The model implies two internal routes of spread within Africa, from Western Africa to Central Africa (BF: 55; PP: 88%) and from Eastern Africa to Western Africa (BF: 21, PP: 74%). Two routes of export from Africa are proposed, one with very strong support from Western Africa to Eastern Asia (BF: 1188; PP: 99%) and one with strong support from Eastern Africa to Southern Asia (BF: 61; PP: 89%) (Fig. 5).

3.5. Serotype DENV4

DENV4 are represented with two complete genome sequences of Western African origin, and six of Eastern African origin. DENV4 appears to have dispersed following divergence from an MRN-AA in South East Asia (LP: 99%) in the late 1940s (1947, HPD: 1943–1951) and emerged in Western Africa in the early 1950s (1953, HPD: 1951–1953) (Fig. 4, cluster I). This cluster may then have further dispersed around 1951 (HPD: 1951–1953) to the Americas (LP: 48%) (Fig. 4, black star). Alternatively, but less likely, the American cluster may have been introduced directly from South East Asia (LP: 30%). Another cluster of sequences from Eastern Africa (Kenya) also originated from a South East Asian ancestor (LP: >99%) as cluster I, albeit more recently in the mid-1970s (1974, HPD: 1958–1976) (Fig. 4, cluster II).

The discrete trait model points to one route of import to Africa with strong support, from South East Asia to Western Africa (BF: 12; PP: 70%) (Fig. 5 and Supplementary Table S1). No other routes of transmission with strong or very strong support could be identified in the analyses. Transmission routes with weaker support are shown for DENV4 and the other serotypes are summarized in Supplementary Table S1.

4. Discussion

Despite being recognized globally as the most common arboviral infection of humans throughout the tropics, the pre-20th century and more recent history, epidemiology and dispersal of DENV throughout the African continent remains poorly defined. Furthermore, whilst at least 25% of all annual global dengue infections are estimated to occur in Africa [43], the number of complete genome sequences in public databases obtained from African sources currently represents less than 2% of the total DENV genome diversity. To expand our knowledge of DENV epidemiology and dispersal in Africa, we conducted a large-scale phylo-epidemiological study based on both newly sequenced DENV genomes of 20th century African origin ($n = 21$), together with previously published representative 20th century sequences of African and non-African origin. Our study specifically focused on (i) identifying the geographical sources of DENV in Africa, (ii) examining evidence for regional intra-connectivity of DENV within Africa and (iii) assessing the interconnectivity, i.e., the relative extent of introduction/exportation to/from Africa.

4.1. Emergence of DENV in Africa

The analyses imply overall that the current extant lineages present in Africa were introduced from Asian, rather than emerging directly from earlier ancestral African lineages (Figs. 1–5). Furthermore, the phylogenies suggest that the earliest introduction of DENV to Africa during the 20th century probably occurred in the early 1940s, when DENV2 was exported from South East Asia and introduced to Western Africa (Fig. 2). Subsequently, during the 1940s introduction of DENV1 and DENV4 occurred from South East Asia to Western Africa, and for DENV1, also to Eastern Africa (Figs. 1 and 4). There have also been several separate introduction events to Africa at regular intervals, as seen especially for DENV2 from South East Asia to Eastern and Western Africa during the 1950s, 1960s and the 1970s (Fig. 2). Since the 1950s and onwards, there was a general shift in the African region to which DENV was introduced, from Western Africa to Eastern Africa. The establishment of DENV3 in Eastern Africa appears to have originated in the 1960s from South East Asia, followed by westward transmission to Western Africa and subsequent establishment in Central Africa (Fig. 3). Transmission from Asia is also supported by previous studies of DENV2 and DENV1 outbreaks in Africa [25,26]. Potentially, the shift in regions of establishment of DENV on the African continent may have been caused by changes in global trade and travel following the world wars. Alternatively, detection bias could explain why more cases have been described and sequenced from Eastern Africa, and hence recent introduction events from the Asian region to Western Africa may simply have escaped detection and characterisation.

The cultural-commercial connections between the Americas (particularly Brazil) and several Western African countries may account for the apparent transmission of DENV1 and DENV2 from the Americas to Western and Central Africa. Similarly, trade and exchange of workforces in the latter half of the 20th century between Eastern Africa and countries in South East Asia and Eastern Asia may have been responsible for sporadic transfer of DENV (specifically DENV2 and DENV3) out of Africa. Finally, increasing tourism from Europe, North America, and emerging economies in Asia, have all contributed to the dispersal and transmission both out of and into Africa during recent decades.

4.2. African Intra-connectivity of DENV

No evidence of dispersal of DENV from Western to Eastern Africa was detected. Thus, the establishment of DENV in Eastern Africa after the second world war is most likely to have been caused by separate

introduction events from Asia, rather than transmission from other African regions). During the late 1960s to 1980s, the phylogenies predict the introduction of DENV1 and DENV2 to Central Africa from Western Africa (Figs. 1 and 2). Then in the 1980s to 2000s, DENV2 and DENV3 appear to have dispersed from Eastern to Western Africa (Figs. 2 and 3), and DENV2 from Eastern Africa also dispersed into Northern Africa during the early 21st century. With the exception of the Saharan region and other dry regions in South Africa, *Ae aegypti* is the primary transmission vector of the four DENV serotypes in tropical and sub-tropical Africa [15]. However, before the middle of the 20th century, *Aedes albopictus*, was generally considered to be a secondary vector of DENV, and was largely confined to Asia, but was not significantly present in Africa [44,45]. Thus, the distribution of *Ae albopictus* was not a limiting factor for transmission within the continent. The propensity for trade and interaction between countries in Western and Central Africa probably explains the transmission between these two regions. However, relatively low human population densities in the African interior may also explain why no evidence of DENV transmission was observed between Eastern and Central Africa.

4.3. Exportation of DENV out of Africa

Exportation of DENV out of Africa can be assessed by the detection of nested sequences of non-African origin within African clusters. Examples can be seen within two relatively large clusters of African sequences in DENV2 (clusters I and III) (Fig. 2), in DENV1 (cluster I) and in DENV3 (cluster I) (Figs. 1 and 3 respectively). These patterns of transfer are confirmed by the discrete trait models. Economic and cultural interactions, e.g. religious pilgrimages, may have provided the conditions for the exportation of DENV2 from Eastern Africa to Northern Africa (Sudan) and of DENV1 and DENV2 from Eastern Africa to Western Asia (Saudi Arabia). However, exportation of DENV2 and DENV3 may also be linked to visiting workers in Africa from Eastern Asia (China). Several sequences originating from Southern Asia are nested within a large cluster of sequences of African origin for DENV2 (cluster II), the reconstructed phylogeny is consistent with multiple exportation events starting in the 1980s.

The reconstructed phylogeny of DENV4 is consistent with a Western African origin (location probability 48%) of the ancestor of a large cluster of sequences of American origin, vs. a South East Asia origin (location probability 30%), pointing to a possible transfer of DENV4 from South East Asia via Western Africa and the Atlantic Ocean, to the Americas (Fig. 4). However, in contrast the discrete trait model lends slightly stronger support to direct transmission between South East Asia and the Americas (BF: 8; PP: 59%), i.e., across the Pacific Ocean, compared with transmission via Western Africa (BF: 7; PP: 57%) (Supplementary Table S1).

Earlier introduction and exportation events from and to Asia and localized outbreaks in Africa cannot be ruled out in the present study. It seems unlikely that any of the included sequences originate from earlier dispersal to Africa, e.g. the strain causing the suggested 1927 South African outbreak [22], as most of the ancestral African nodes emerge around the 1940s and onwards. As shown in the reconstructed phylogeny, several clusters are not represented with recent sequences (<10 years ago), e.g. cluster II in DENV1 and the Central African part of cluster II in DENV2, Figs. 1 and 2). This may be due to lack of sampling, or extinction of the virus or even replacement of another strain/serogroup. Clearly, the African continent as a whole is severely undersampled in the context of DENV and other arboviruses [20]. Indeed, it is possible that viruses of these ancestral lineages and clusters may be circulating unnoticed in the human populations and/or in sylvatic reservoirs [7,46], as has been observed for DENV2 in Senegal [47–49]. In the present study, two sequences isolated almost 30 years apart in Senegal were identified as genetically closely related in the DENV4 phylogeny (cluster I, Fig. 4). This may be explained if DENV4 had established a sylvatic reservoir, despite the lack of definitive evidence of sylvatic circulation of DENV4 in Africa. Furthermore, it should be noted that most of the sequences included in this study indicated to be of African origin do form phylogenetic clusters, supporting a 'true' African origin of that group of sequences. Conversely, the geographical origin of the singletons, i.e. the single branching sequences, remains more uncertain as the geographical origin of infection of these sequences may be difficult to verify. Thus caution should be exercised when analysing these sequences.

4.4. The future of DENV in Africa

Despite the relatively limited evidence from Africa, when compared with the Asian and South American/Caribbean regions, dengue virus is becoming a more important public health problem in Africa. To a large extent this can be attributed to increasing commercial activities between African countries and also with countries outside Africa. Moreover, the threat of malaria, as a major human pathogen in Africa is gradually being reduced, largely through major and coordinated insect control measures. Thus, with a projected WHO target date of 2030 to reduce significantly the threat from malaria in Africa, the scale of insect control measures is likely to be significantly reduced, potentially creating suitable conditions for DENV to replace malaria.

The observation that a DENV lineage appears to have been present but undetected in Nigeria for almost 60 years (DENV2 cluster V, Fig. 2), may be of significance for our understanding of future dengue virus epidemiology in Africa, perhaps directly indicating that DENV does survive long-term in the sylvatic forest environment. Thus, to understand the epidemiology of DENV, one must apply a One Health approach to examine the environmental characteristics that promote suitable living-conditions for the mosquito-vector and its interaction with animal reservoirs and endemic transmission to humans [7]. Historically, it has been believed for many years that people of African ethnicity may be more resistant than Asians and Europeans, to infection by dengue viruses and this was recently supported by studies of dengue fever, in Colombia [50,51]. For example, in areas of the Colombian city, Cali, where citizens of African-ethnicity represent a high proportion of the population, mild or asymptomatic dengue infections are more commonly recorded than in areas where Colombians of European-ethnicity represent the predominant population. Importantly, despite having lower levels of viraemia, the relatively asymptomatic African Colombians can transmit the virus to susceptible European and/or Asian-ethnicity individuals [52]. Furthermore, *A. albopictus* is now widespread in South America and continuously expanding its geographic boundaries. Unlike *Ae. aegypti,* which has a strong preference for the urban environment and particularly being indoors among humans as a rich source of human blood and a variety of water-retaining sources, *A. albopictus* prefers the outdoor environment where it can obtain water and sap from vegetation. Thus, acting as a secondary vector of DENV but also being more widely distributed than domestic *Ae. aegypti*, *A. albopictus* is more likely to introduce the viruses into the suburban/rural regions and even the neighbouring forests. Moreover, *Ae. albopictus* could contribute to the future risk of spreading DENV further afield to warm temperate climates as Africans, Europeans and Asians resume travelling after the impact of COVID-19, and malarial mosquito control programmes subside. Consequently, Africa remains an important region of the tropical world for the continued evolution and dispersal of DENV. However, although the recorded resistance/immunity of Africans to infection by DENV may be beneficial for Africans in the presence of DENV, its relatively benign presence but continued transmissibility throughout most of Africa could still present an important threat to non-ethnic visitors, with the subsequent risk of exporting the virus to temperate regions.

5. Conclusions

In the present phylogeographical study, several novel DENV

sequences of African origin have been added, increasing the available number of sequences from the severely under-sampled continent. Our findings point to multiple introductions of DENV1–DENV3 into Africa, most of these originating in South East Asia in the post-world war era. Furthermore, the study also implies transmission within Africa of these serogroups, from Western to Central Africa, and from Eastern to Western Africa. These routes of transfer likely reflect mosquito migration and socio-economic interactions between the different African countries. The discrete trait models confirm these findings, and point to possible exportation events to Eastern Asia – probably reflecting the increased influence and interaction with Asian countries. Identifying routes of transmission offers a valuable tool to access efforts to monitor, modulate and ultimately eradicate DENV as a public health threat in Africa.

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Declaration of Competing Interest

All authors have read the manuscript and have no conflict of interest relating to the manuscript.

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