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## Whole genome sequencing of outbreak strains from 2017 to 2018 reveals an endemic clade of dengue 1 virus in Cameroon

Bright Agbodzi<sup>a</sup>, Francine Berlangue Sado Yousseu<sup>b</sup>, Fredy Brice Nemg Simo<sup>b</sup>, Selassie Kumordjie<sup>a</sup>, Clara Yeboah<sup>a</sup>, Mba-Tihssommah Mosore<sup>a</sup>, Ronald E. Bentil<sup>a</sup>, Heather G. Coatsworth<sup>c</sup>, Naiki Attram<sup>a</sup>, Shirley Nimo-Paintsil<sup>a</sup>, Anne T. Fox<sup>a</sup>, Joseph H. K. Bonney<sup>d</sup>, William Ampofo<sup>d</sup>, Rhoel R. Dinglasan<sup>c</sup>, Terrel Sanders<sup>a</sup>, Michael R. Wiley<sup>e</sup>, Maurice Demanou<sup>b</sup> and Andrew G. Letizia<sup>a</sup>

<sup>a</sup>U.S. Naval Medical Research Unit EURAFCENT, Accra, Ghana; <sup>b</sup>Virology Department, Centre Pasteur, Yaounde, Cameroon; <sup>c</sup>Department of Infectious Diseases & Immunology and Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida, Gainesville, Florida, USA; <sup>d</sup>Virology Department, Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana; <sup>e</sup>Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE, USA

### ABSTRACT

Dengue fever is expanding as a global public health threat including countries within Africa. For the past few decades, Cameroon has experienced sporadic cases of arboviral infections including dengue fever. Here, we conducted genomic analyses to investigate the origin and phylogenetic profile of Cameroon DENV-1 outbreak strains and predict the impact of emerging therapeutics on these strains. Bayesian and maximum-likelihood phylogenetic inference approaches were employed in virus evolutionary analyses. An *in silico* analysis was performed to assess the divergence in immunotherapeutic and vaccine targets in the new genomes. Six complete DENV-1 genomes were generated from 50 samples that met a clinical definition for DENV infection. Phylogenetic analyses revealed that the strains from the current study belong to a sub-lineage of DENV-1 genotype V and form a monophyletic taxon with a 2012 strain from Gabon. The most recent common ancestor (TMRCA) of the Cameroon and Gabon strains was estimated to have existed around 2008. Comparing our sequences to the vaccine strains, 19 and 15 amino acid (aa) substitutions were observed in the immuno-protective prM-E protein segments of the Dengvaxia<sup>®</sup> and TetraVax-DV-TV003 vaccines, respectively. Epitope mapping revealed mismatches in aa residues at positions E155 and E161 located in the epitope of the human anti-DENV-1 monoclonal antibody HMAb 1F4. The new DENV strains constitute a conserved genomic pool of viruses endemic to the Central African region that needs prospective monitoring to track local viral evolution. Further work is needed to ascertain the performance of emerging therapeutics in DENV strains from the African region.

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**KEYWORDS** Dengue virus 1; Cameroon; phylogenetics; whole genome sequencing; vaccine; monoclonal antibodies; Africa

### Introduction

Dengue fever caused by dengue virus (DENV) poses the greatest human disease burden of any arbovirus, with an estimated 100–400 million global infections annually (1). The World Health Organization (WHO) has recorded more than an 8-fold increase in reported dengue cases in the past two decades (1). About half of the world's population is at risk of dengue infection, of which a large proportion live in Africa (2). DENV is a single-stranded positive-sense RNA virus with an 11-kb genome, and classified into four serotypes according to their distinctive antigenicity (DENV-1, DENV-2, DENV-3, and DENV-4) with diverse genotypes within each serotype (3). DENV genotypes continue to evolve due to mutations, recombination, lineage turnover and/or replacement that may occur as a result of selection pressure (4).

DENV is primarily transmitted by the anthropophilic *Aedes aegypti* and *Aedes albopictus* mosquito

vectors (5). The global spread of DENV has been attributed to economic, socio-demographic and climatic factors (6,7). After an extensive global expansion in recent decades, mosquito-associated transmission has been reported in urban and peri-urban areas in tropical and sub-tropical climates around the world (6,7). Infection with any of the DENV serotypes may be asymptomatic, as occurs in the majority of cases, or may result in a wide spectrum of clinical symptoms ranging from a mild flu-like syndrome (known as dengue fever) to the most severe forms of the disease, which are characterized by coagulopathy, increased vascular fragility and permeability (dengue haemorrhagic fever) that can lead to death (8). There is one currently licenced vaccine, CYD-TDV (Dengvaxia<sup>®</sup>; Sanofi Pasteur, Lyon, France). However, the general roll-out of this vaccine is limited because pre-screening for dengue virus serostatus or confirmation of prior infection is required (9). Other vaccines in advanced development

**CONTACT** Bright Agbodzi  [bagbodzi@noguchi.ug.edu.gh](mailto:bagbodzi@noguchi.ug.edu.gh); [bright.agbodzi@gmail.com](mailto:bright.agbodzi@gmail.com)

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include DENVax and TetraVax-DV-TV003 (TV003) which are in phase three trials (10,11).

In Africa, the first recorded dengue case was in 1779 (3). Since then, a series of outbreaks across the continent involving all four DENV serotypes have been reported in Benin, Cape Verde, Angola, Tanzania, Somalia, Egypt, Gabon, Burkina-Faso, and the Comoros (12–15). The genetic diversity of dengue virus (DENV) circulating in Africa remains poorly understood (16), with few studies having documented complete DENV genomic analyses in Africa (17–19).

In Cameroon, numerous studies have reported DENV seroprevalence (IgG) in rural and urban areas despite no reported outbreak, with rates as high as 80% (20,21). Studies on the serotypes and genotypes circulating in West Africa in general, and Cameroon specifically, are lacking, and could represent unique evolutionary patterns which remain unexplored. Some studies conducted in Yaoundé, Kribi, and Douala showed the circulation of DENV serotypes 1, 2, and more recently 3 in Cameroon (22–25). One genotype has been described for each serotype circulating in the country, genotype V for DENV-1, genotype II for DENV-2, and genotype III for DENV-3 (25). In this study, we conducted a genomic analysis to investigate the origin and phylogenetic profile of the novel Cameroon DENV-1 strains isolated between 2017 and 2018 and predicted the potential impact of emerging therapeutics and preventive measures against these novel strains.

## Methodology

### Ethical review

This study was reviewed and approved by the Naval Medical Research Command (NMRC) Institutional Review Board (IRB), approval number NAM-RU3.PJT.19.01 in compliance with all applicable Federal regulations governing the protection of human subjects and the National Ethical Committee of Research in Humans Health, Cameroon, under the approval numbers 2017/03/881/LCNERSH/SP and 1447 IEC-UD/06/2018/T.

### Samples

This retrospective study used human plasma samples originally collected as part of dengue public health outbreak responses in Cameroon (23,24). A total of 50 samples from suspected dengue fever cases or close contacts were analyzed. The samples were stored at  $-80^{\circ}\text{C}$  at the Centre Pasteur Cameroon in Yaoundé prior to next generation sequencing analysis at the Noguchi Memorial Institute for Medical Research (NMIMR) located in Accra, Ghana.

Samples were collected as part of sentinel site surveillance at community-based facilities, Medico-Social

Health Center of Yaoundé, and Mfou District Hospital in the Centre region, as well as Londjii Health Center in the South region, and New Bell District Hospital in the Littoral region. The enrolment procedure has been previously described (23,24).

### Polymerase chain reaction (PCR) detection of dengue virus

Viral RNA extraction was done using QIAamp Viral RNA mini kits (QIAGEN, Hilden, Germany) following manufacturer's instructions. DENV detection and data interpretation/validation were per the United States Centers for Disease Control and Prevention (US-CDC) Triplex real time RT-PCR protocol (26) on an ABI 7500 system using the SuperScript III Platinum One-step qRT-PCR enzyme kit (Invitrogen, Waltham, Massachusetts, USA). The PCR positive samples were selected for whole genome library preparation and sequencing.

### Whole genome sequence (WGS)

Sequencing libraries were prepared using the KAPA Hyper Prep Kit (Kapa Biosystems, Wilmington, Massachusetts, USA) according to the manufacturer's instructions. Viral enrichment was done using custom target capture probes (Twist Bioscience, San Francisco, USA). In brief, extracted RNA was fragmented, spiked with HELA RNA to increase ligation efficiency, and reverse transcribed to cDNA. Dual indexing of cDNA libraries was achieved using IDT unique dual indexes (IDT, Coralville, Iowa, USA). Libraries were enriched using the 1-plex pooling strategy described previously by quartering the amount of reagents for the enrichment step (27). Bar-coded pooled libraries were sequenced on an Illumina MiSeq, generating  $2 \times 150$  paired-end reads.

### Whole genome sequencing analysis

Demultiplexed raw fastq files were quality filtered to Phred score  $\geq 20$ , filtered for minimum read length of 40 base pairs (bp) and adaptor trimmed using BBduk (BBMap – Bushnell B. – sourceforge.net/projects/bbmap/). Read quality was confirmed using the FastQC tool. The resultant high-quality reads were used for *de novo* assembly using the SPAdes assembler v 3.15.2. Contigs were scaffolded against dengue 1 virus reference sequence and the consensus was used to query GenBank to obtain the best matching reference sequence. The retrieved reference (GenBank accession: MG877557.1), was used for reference-based assembly using Bowtie2.

### Phylogenetic analysis

The sequences from the current study were submitted to the online Genome detective virus tool (<https://>

[www.genomedetective.com/](http://www.genomedetective.com/)) for genotyping. To put our genomes into a global context, we randomly sub-sampled approximately 10% of DENV-1 genomes (>1000 bp) present at the ViPR database (<http://www.viprbrc.org>), together with all genomes of African origin, yielding a total dataset of 198 genomes. Sequences were aligned using MAFFT v1.5.0 implemented in Geneious Prime. To correct for the effects of ambiguous alignments due to the polymorphisms in 5' and 3' untranslated regions, the sequences were trimmed to the open reading frames (ORFs), and all subsequent phylogenetic analyses were conducted on the ORFs. A maximum-likelihood phylogenetic analysis was conducted using IQ-TREE under the GTR + F + R4 model and ultrafast bootstrapping ( $N = 1000$ ).

To investigate the origins and better understand the evolutionary dynamics of the sequenced strains, a Bayesian inference phylogenetic approach based on the Bayesian Markov chain Monte Carlo (MCMC) method was implemented in BEAST v1.10.4 (28) to determine the time of the most recent common ancestor (TMRCA) of the study strains. The MAFFT aligned complete ORFs described above were used for the BEAST analysis. The presence of temporal signal in the complete and subdivided datasets was determined by performing a root-to-tip genetic divergence and sampling date correlation analysis using maximum-likelihood trees generated as described above and implemented in TempEst v1.5.3 (<http://tree.bio.ed.ac.uk/software/tempest/>). An uncorrelated relaxed molecular clock with lognormally distributed rate categories, along with the nonparametric Bayesian Sky-Grid tree prior with 52 parameters was implemented (29). BEAST was run for three independent 120 million MCMC steps using a GTR + F + R4 substitution model. Parameter convergence was inspected in TRACER v1.7.1 to achieve an effective sample size ( $ESS > 200$ ). Tree files were combined with Logcombiner v1.10.4. A maximum clade credibility (MCC) phylogeny was computed from the posterior distributions of the complete dataset, excluding 10% as burn-in using TreeAnnotator (via BEAST). Tree visualization and annotation was done with FigTree v1.4.4 and Inkscape v1.2. With sequence availability from the African region skewed towards the envelope (E) protein, a maximum-likelihood phylogeny based on E sequence of all genomes analyzed above as well as all E sequences of African origin greater than 200 base pairs retrieved from the ViPR database was generated. Tree model inference and phylogeny were simultaneously conducted in IQ-TREE v1.6.1, executing 1000 bootstrap replicates.

### ***In silico PCR diagnostics evaluation***

We aligned primers and probes used in the CDC Trioplex assay (patent no. WO2018169550A1), CDC

DENV-1-4 RT-PCR (26), Universal Single-Probe RT-PCR Assay (30) and the DENV RT-PCR by (31) with the new Cameroon genomes using Geneious Prime v 2022.1.1.

### ***Vaccine strain and monoclonal antibody divergence analysis***

The Cameroon genomes presented here were compared to the Dengvaxia® (CYD-TDV) and TetraVax-DV-TV003 vaccine strains using sequence alignment. Analysis was restricted to the immuno-protective pre-membrane (prM) and the envelope (E) protein region of the vaccine strains. Sequences were aligned using MAFFT v1.5.0, implemented in Geneious Prime v2022.01. The sequences were grouped into clades as observed in Figure 1. Only amino acid (aa) substitutions present in two or more strains of a particular clade were recorded. Epitope mapping was done to compare the aa divergence of our strains to relevant epitopes that had been previously identified as potential targets for antiviral human monoclonal antibodies, HMAb 1F4 and HMAb 14c10 (32,33).

### ***Data availability***

The consensus sequences that were generated from the six Cameroon DENV-1 strains have been deposited at GenBank under accession numbers OQ593388–OQ593393.

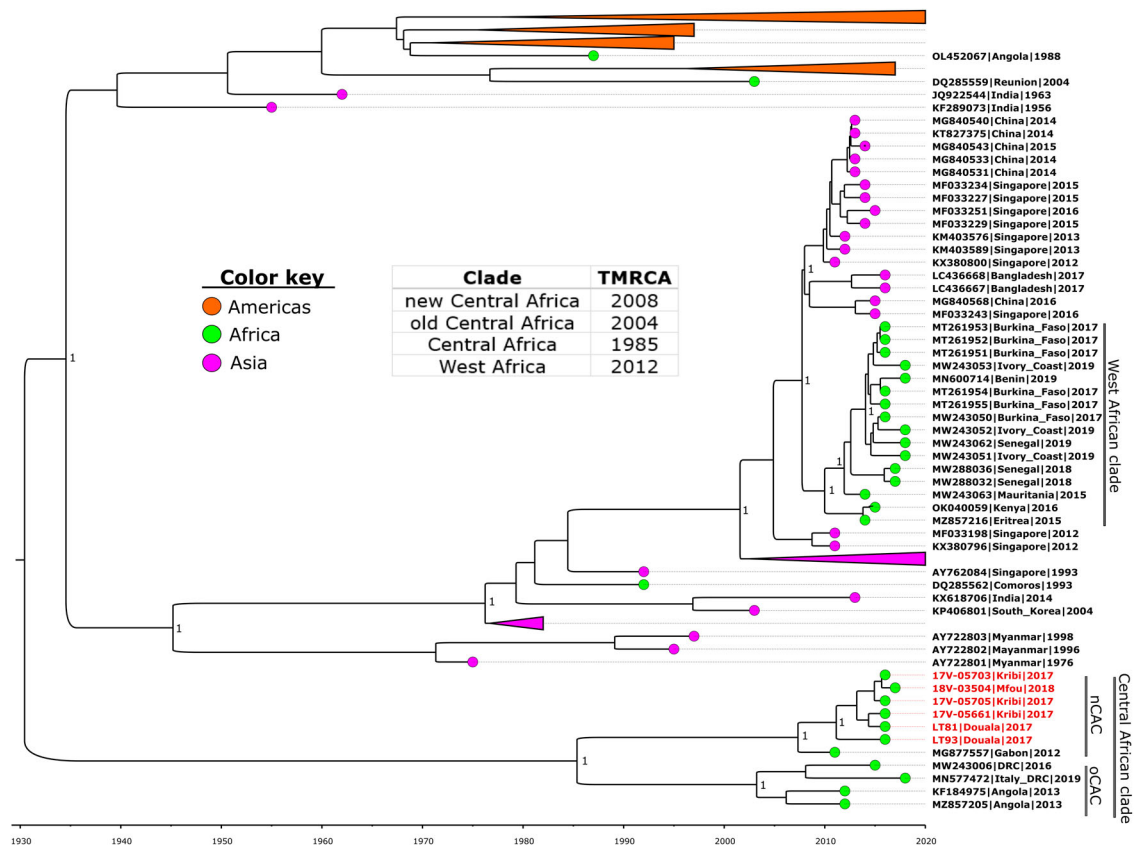
## **Results**

### ***Sample background***

Of the 50 samples tested, 6 (12%) were confirmed dengue positive by RT-PCR. Two of the samples were from patients seeking care through regular consultation, while the remaining four were from case contacts and symptom information is not available. The samples were from patients aged between 2 and 47, three of whom were female (Table 1).

### ***Whole genome sequencing and analysis***

From these six samples, we generated six DENV-1 genomes (1 from each sample) that ranged between 10,676 and 10,722 bp in length with an ORF of 10,179 bp. Amino acid divergence analysis revealed the presence of 110 aa substitutions across the ORFs, distributed into 30 structural and 80 non-structural proteins, when compared to DENV-1 standard strain (EU848545-Hawaii-1944) (Table 2). The segment with the highest proportion of substitutions was NS1 (4.82%), followed by C-prM (4.64%), while NS5 recorded the least number (2.0%).



**Figure 1.** Time-calibrated phylogeny of a subset of the global dengue 1 virus genomes alongside the Cameroon 2017–2018 outbreak strains (in red text). Coloured circles indicate geographic origins. Dates of the most recent common ancestor (TMRCA) for major clades are provided in the table inset. Posterior probabilities are shown at major nodes. Taxon labels include GenBank accession number, country, and year of isolation for reference sequences, while new sequences are labelled with strain names. nCAC: new Central African clade; oCAC: old Central African clade.

### Phylogenetic analysis

The genotypes of the six genomes were unverifiable using the Genome Detective tool, however, further phylogenetic analysis using Bayesian and maximum-likelihood approaches revealed that they belong to genotype V and were closely related to a 2012 strain from Gabon (GenBank accession no. MG877557.1). Molecular clock analysis revealed that the time of the most recent common ancestor (TMRCA) of the Cameroon strains was around 2013 (Figure 1). However, they formed a monophyletic taxon with the Gabon strain (the new Central African clade-nCAC)

with a TMRCA of 2008 (95% highest posterior density [HPD] 2005–2010). The nCAC is closely related to a monophyletic taxon with strains from DRC and Angola (the old Central African clade-oCAC) with a TMRCA of 2003 (95% [HPD] 1999–2007). The nCAC and oCAC formed the Central African clade (CAC) which shared a recent common ancestor in 1985 (95% [HPD] 1969–1997). The partial E protein analysis revealed the presence of four main clusters of DENV-1 in Africa (Figure 2). These clusters can be grouped into West African, Central African, Islands of East Africa, and Eastern Africa strains.

**Table 1.** Demographics and clinical background of the positive samples.

Sample ID	Sex	Location	Age	Collection year	PCR results (CT)	Clinical manifestations	Temperature (°C)	Type of case
LT81	Female	Douala	29	2017	39.72	Fever, arthralgia, myalgia, vomiting, headache	38.5	Consultation
LT93	Female	Douala	47	2017	35.31	Fever, arthralgia, myalgia, headache	37.5	Consultation
17V-05661	Female	Kribi	24	2017	32.35	-	-	Case contact
17V-05703	Male	Kribi	10	2017	35.7	-	-	Case contact
17V-05705	Male	Kribi	2	2017	36.76	-	-	Case contact
18V-03504	-	Mfou	-	2018	36.92	-	-	Case contact

Dashes (-) indicated data was not collected.

**Table 2.** Amino acid substitutions in ORFs of nCAC.

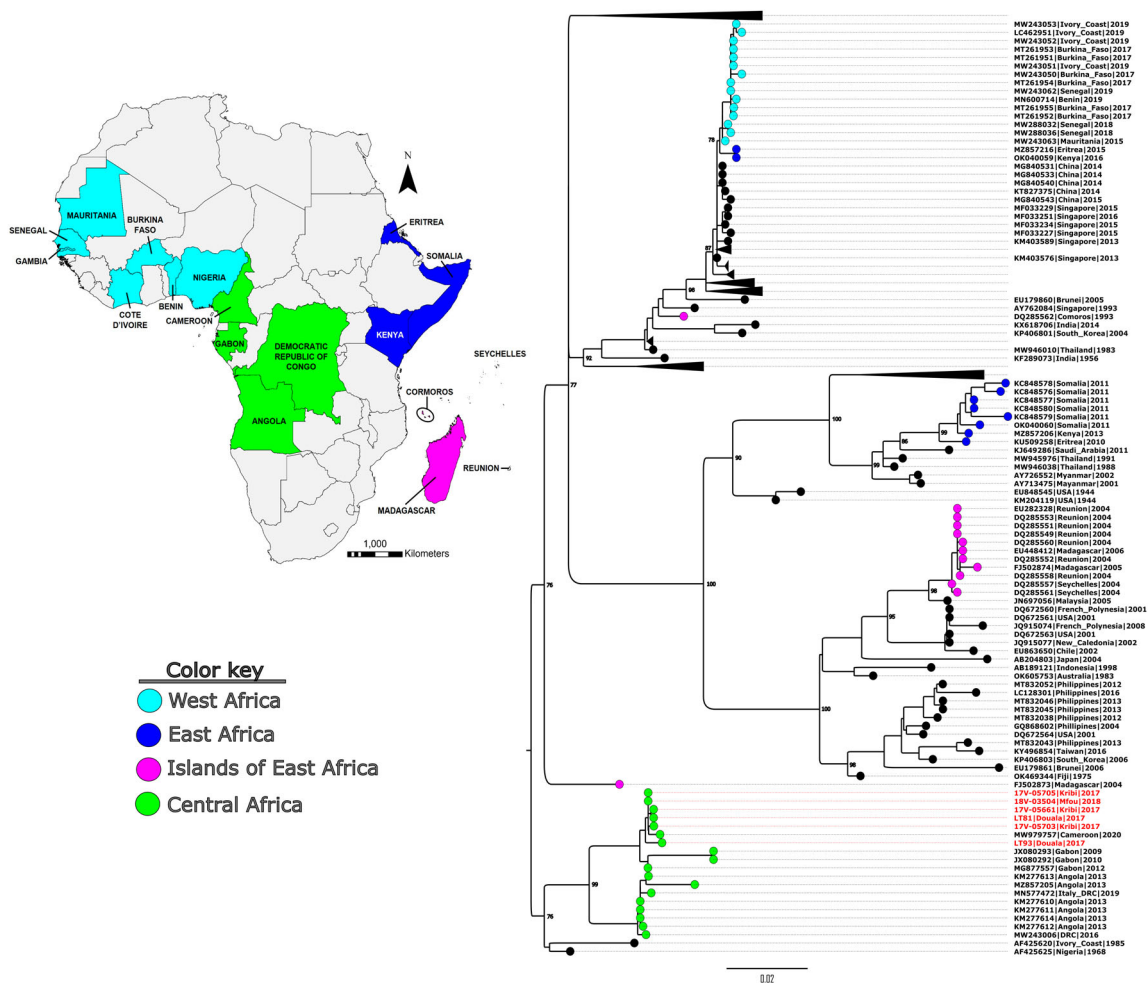
Structural proteins				Non-structural proteins									
C-prM		E		NS1		NS2		NS3		NS4		NS5	
AA substitution	N	AA substitution	N	AA substitution	N	AA substitution	N	AA substitution	N	AA substitution	N	AA substitution	N
V26S	1	D37N	7	G56V	7	I15V	7	V13A	7	Q19L	7	M34T	7
V26G	7	K52N	7	I66V	7	F87Y	2	E43D	7	Q36K	3	T135I	7
M51I	7	55V	1	S94N	7	I106V	7	S88L	7	V63S	1	E281K	2
G70S	7	I114L	7	I96V	6	I133M	7	K107R	7	V63A	2	K364R	7
L72F	7	P227S	7	T98A	7	A147T	7	A112T	7	L70H	1	R378K	7
K86R	7	E234Q	7	R101K	7	A148T	7	K185R	7	R76K	7	G382S	2
N90S	7	K277T	7	G105R	7	I155V	7	S250T	7	V93I	7	H435R	7
I150L	7	F279L	1	V128I	7	F159L	7	V332A	1	V93T	6	R436K	5
K172E	7	N290D	7	N139D	7	H162D	7	Y349H	2	M144V	6	N585S	7
A173V	1	T339I	7	V178M	7	V168M	7	N401S	1	V170A	7	I627V	1
R196Q	7	T346I	7	P181H	7	I174M	7	I474V	7	H174Q	7	S629L	7
D203E	7	A369T	7	V224I	7	T185A	1	G515E	7	I259T	7	T635N	7
A221T	7	A386T	6	I251T	7	L199F	7	K562R	7	A313T	7	P636S	1
		L402F	6	S261F	7	A210T	7	Q606L	7			V642A	7
		I439V	7	D340E	7	I277V	7					Q704L	7
		A473T	7	K347R	7	V324I	1					V782I	7
		V485D	1	L349M	7	Y328F	7					I784V	7
						I330M	7					L894P	7

AA, amino acid; N, number of strains.

### PCR diagnostics

In silico mapping of the primers and probes from the three RT-PCR assays showed no nucleotide

mismatches between the Cameroon genomes and the primers and probes of the CDC Triplex assay and the Universal Single-Probe RT-PCR Assay. However,



**Figure 2.** Molecular phylogenetic analysis of a subset of global DENV-1 partial E sequences alongside the Cameroon 2017–2018 outbreak strains (in red text). Tree model inference and phylogeny were simultaneously conducted in IQ-TREE v1.6.1, executing 1000 bootstrap replicates. The geographical origins of the major African clusters are colour coded and indicated in the inset map. Critical nodes are labelled with bootstrap values. The tree was visualized in FigTree v1.4.4.

in the case of the DENV RT-PCR by Johnson et al., there was a single nucleotide mismatch in both the forward and reverse primers.

### Vaccine strain divergence and monoclonal antibody epitope analysis

The nCAC and oCAC showed identical aa substitution patterns compared to the vaccine strains, with 19 and 15 aa substitutions recorded in the prM-E protein segments of the Dengvaxia® and TetraVax-DV-TV003 vaccine strains, respectively (Figure 3). This represented a 2.87% and 2.27% divergence from the Dengvaxia® and TetraVax-DV-TV003 vaccine strains, respectively. The West African clade showed similar results, with 19 and 17 aa substitutions observed for the Dengvaxia® and TetraVax-DV-TV003 vaccine strains, respectively. However, eight aa substitutions were not shared between the CAC and West African clade. Comparing CAC to the vaccine strains revealed that for the Dengvaxia® vaccine strain, the majority of the aa substitutions occurred in domain III (5/17) and the stem-transmembrane region (6/17) of the E protein. However, for the TetraVax-DV-TV003 vaccine strain, the majority of the aa substitutions were observed in domain II (5/13) and domain III (4/13).

The epitope mapping among known monoclonal antibody targets revealed mismatches in aa residues at positions E155 and E161 for HMAb 1F4 (Figure 4). At position E155, Ser was replaced with a similar residue, Thr, while at position E161 the residue Thr was replaced with Ile, a residue with different physico-chemical properties. These aa substitutions have previously been reported in strains from West Africa, Southeast Asia and the Americas (19,33). No mismatch was identified for HMAb 14c10.

### Discussion

Emerging and re-emerging viruses continue to pose a major global health burden. With the ever-evolving nature of viruses, it is practically impossible to predict the next outbreak, hence the need for proactive strategies to track, treat, and prevent them. Cameroon remains a hot spot for arboviral outbreaks (15,23,34). The wet-dense tropical ecology of the

	155	161	171
HMAb 14C10	T	I	S
HmAb 1F4	S	T	T
CYD1	S	T	S
TV003	T	T	S
nCAC	T	I	S
oCAC	T	I	S
WA	T	I	S

**Figure 4.** Amino acid divergence comparison between nCAC and epitopes of DENV-1 neutralizing human monoclonal antibodies (HMAb 1F4 and HMAb 14c10) and vaccine strains. Numerals represent the E protein amino acid position. The other African clades were included for comparison. CYD1: Dengvaxia®; TV003: TetraVax-DV-TV003; nCAC: new Central African clade; oCAC: old Central African clade; WA: West African clade.

southern Guinean-subequatorial region of the country provides an ideal environment for sylvatic and urban populations of competent arthropod vectors, hence the persistence of stable transmission cycles (35–37). The recent report of concurrent circulation of multiple DENV serotypes (25), an indicator of impending outbreaks, and the likelihood of severe disease outcomes highlights the ominous nature of the situation (38). Even though past studies have identified the circulation of three different serotypes (23–25), no complete genome data is available for the comprehensive characterization of these viruses. Here we describe six complete DENV-1 genomes isolated during the 2017–2018 dengue outbreak and investigate how they phylogenetically fit into the global DENV-1 landscape, as well as describe the homology to emerging vaccines and therapeutics.

Phylogenetic analyses revealed that the six Cameroon DENV-1 strains belong to genotype V and formed a monophyletic taxon, nCAC, with a 2012 Gabon strain (Gabon2012) (39). The time-calibrated phylogeny revealed that the common recent ancestor of the nCAC existed around 2008, suggesting the possible cryptic circulation of this strain in the Central African region over the last decade. The nCAC and oCAC shared a common ancestor in the mid-80s,

	prM(1-166)										E(167-661)																	
	15	59	72	118	122	203	254	280	321	327	368	369	370	391	463	503	505	512	517	535	546	552	563	602	605	627	650	660
KX239894 CYD1	T	A	D	K	R	D	A	I	S	T	K	E	R	T	M	F	T	T	V	A	V	A	T	V	I	V	L	G
DENV-1 TV003	S	T	E	K	K	D	T	I	T	T	E	K	R	S	M	F	S	T	L	A	V	A	S	I	I	I	M	Q
nCAC	S	A	D	K	K	N	A	L	T	I	K	E	K	S	M	F	I	I	L	T	V	T	S	V	V	I	M	Q
oCAC	S	V	D	K	K	N	A	L	T	I	K	E	K	S	M	F	I	I	L	T	V	T	S	V	V	I	M	Q
WA	S	A	D	R	K	N	A	L	T	I	K	E	K	S	V	I	T	T	L	T	I	A	S	V	V	I	M	Q

**Figure 3.** Dengvaxia® and TetraVax-DV-TV003 vaccine strain divergence analysis. The analysis was restricted to the immuno-protective pre-membrane (prM) and the envelope (E) protein region of CYD1. Numerals indicate amino acid positions. Only amino acid positions with disagreements are shown. Single-point disagreements are highlighted. CYD1: Dengvaxia®; TV003: TetraVax-DV-TV003; nCAC: new Central African clade; oCAC: old Central African clade; WA: West African clade.

suggesting that the Central African strains have been endemic in the region for many years, and constitute a unique genomic pool of DENV-1 viruses that remain unexplored. Other DENV-1 viruses isolated in Africa (mainly West Africa) seem to have been introduced more recently from Asia (19), confirming the circulation of diverse strains even with limited surveillance. The African continent could serve as a crucible for unique genetic divergence in the future of DENV 1 genomes. The E protein phylogeny, with more sequence representation, gave insights into the outcomes of major DENV-1 outbreaks and endemic circulation on the African continent (Figure 2). The phylogeny corroborated the endemicity of the Central African strains as revealed by the BEAST analysis. On the other hand, the other clusters which include the West African, Islands of East Africa, and Eastern Africa strains seem to have been introduced into Africa from Asia on separate occasions; perhaps through trade activities involving the transport of infected mosquitoes and/or humans (40). Interestingly, all these clusters belong to different genotypes, highlighting the diversity of DENV-1 strains that have caused major outbreaks on the continent.

Amino acid divergence analysis across the complete coding region revealed 110 aa substitutions occurring in the nCAC when compared to the DENV-1 standard strain. The number of aa substitutions is among the highest reported for similar studies (41–43). Although many of these mutations might be associated with virulence, pathogenicity, as well as vector competence (43), these functional studies were outside the scope of this study. The results from the *in silico* PCR diagnostic assessment suggests that assays evaluated should be able to detect the Cameroon strains.

Currently, vaccines remain the most promising preventive intervention targeting humans to curb severe disease (9). The live attenuated tetravalent dengue vaccine CYD-TDV or Dengvaxia®, developed by Sanofi Pasteur, is the only approved vaccine for dengue fever (9). Previous studies revealed that vaccine efficacy varied with respect to genotype diversity, which is usually influenced by geographical location (33). A comparison of the immuno-protective prM-E protein regions of the Dengvaxia® and TetraVax-DV-TV003 vaccine strains revealed noteworthy aa substitutions that need functional characterization to ascertain their effects on emerging preventive and therapeutic products. For both vaccine strains, a high proportion of the aa substitutions occurred in domain III (DIII) of the E protein. DIII is the most immuno-potent domain of the E protein, which is involved in host cell surface receptor recognition, and harbours conformation-dependent neutralizing epitopes (44–47). Mutations in DIII were reported to have led to escape from neutralization by conventional monoclonal neutralizing antibodies, thus the

divergence observed in our strains might affect the efficacy of vaccines, and the potency of neutralizing antibodies (44,46,48), although further functional studies are required to validate this.

The epitope mapping analysis revealed mismatches in residues at positions E155 and E161 for HMAb 1F4, however, at position E155, Ser was replaced with a similar residue, Thr and therefore, unlikely to affect binding. At position E161 on the other hand, the residue Thr was replaced with Ile, a residue with different physico-chemical properties, however, this change has been noted not to affect binding in a previous study (32). The two monoclonal antibodies are likely to be effective in neutralizing the Cameroon strains presented here based upon this *in silico* analysis, however, functional validation is warranted. In general, the African strains have shown greater diversity from the vaccine strains (19) and therefore, there is a need for more work to assess the efficacy of vaccines in the African region. A starting point will be considering African countries in clinical trials to gain contemporary results of vaccine efficacy in the region, as well as including viruses from Africa in designing and validating vaccines and other preventive therapeutics.

There are some limitations to this study. First, the long sample storage time prior to processing (>1 year) could have contributed to sample degradation over time, and consequently affecting the ratio of DENV positives detected, as well the quality of WGS data generated. Furthermore, important epidemiological parameters like clinical features and socio-demographical information were not captured for all samples such as those collected as part of contact tracing. We were not able to functionally characterize the mutations seen which prevented us from making assertive conclusions. The limited number of genomes from Africa also presented a bottleneck in deciphering the evolutionary relationships among African strains.

In conclusion, we report the first complete DENV genomes from Cameroon. These six DENV-1 genomes constitute a conserved genomic pool endemic to the Central African region. The unexplored nature of DENV in this region in the setting of unique genotypic evolution poses not only a local but global public health threat. The high genome-wide amino acid divergence observed is a testament to the need for prospective monitoring to track local viral evolution by investigating selection pressures imposed by hosts (mammalian and vectors) between endemic and imported DENV. These genomes offer an avenue for the expansion of preventive and therapeutic targets ensuring wider coverage. This study highlights the importance of ongoing surveillance efforts to provide accurate and actionable data for the global public health community and to enhance global health security by coordinating a surveillance network for vector-borne diseases.

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## Disclosure statement

No potential conflict of interest was reported by the author(s).

## Declarations

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## Author contributions

Conceptualization: BA, FBSY, MRW, MD, AGL; Writing – original draft: BA, FBSY; Sampling: FBSY, FBNS, MD; Molecular assays: BA, FBSY, FBNS, SK, CY, M-TM, REB; Whole genome sequencing: BA, FBSY, SK, CY, M-TM, REB; Bioinformatics analysis and data interpretation: BA, MRW; Writing – review and editing: BA, FBSY, FBNS, SK, CY, M-TM, REB, HGC, NA, SNP, ATF, JHKB, WA, RRD, TS, MRW, MD, AGL; Coordination: NA, SNP, ATF, JHKB, WA, MD, AGL. All authors have read and agreed to the published version of the manuscript.

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