ORIGINAL ARTICLE

Transmission of SARS-CoV-2 in northern Ghana: insights from whole-genome sequencing

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Abstract

Following the detection of the first imported case of COVID-19 in the northern sector of Ghana, we molecularly characterized and phylogenetically analysed sequences, including three complete genome sequences, of severe acute respiratory syndrome coronavirus 2 obtained from nine patients in Ghana. We performed high-throughput sequencing on nine samples that were found to have a high concentration of viral RNA. We also assessed the potential impact that long-distance transport of samples to testing centres may have on sequencing results. Here, two samples that were similar in terms of viral RNA concentration but were transported from sites that are over 400 km apart were analyzed. All sequences were compared to previous sequences from Ghana and representative sequences from regions where our patients had previously travelled. Three complete genome sequences and another nearly complete genome sequence with 95.6% coverage were obtained. Sequences with coverage in excess of 80% were found to belong to three lineages, namely A, B.1 and B.2. Our sequences clustered in two different clades, with the majority falling within a clade composed of sequences from sub-Saharan Africa. Less RNA fragmentation was seen in sample KATH23, which was collected 9 km from the testing site, than in sample TTH6, which was collected and transported over a distance of 400 km to the testing site. The clustering of several sequences from sub-Saharan Africa suggests regional circulation of the viruses in the subregion. Importantly, there may be a need to decentralize testing sites and build more capacity across Africa to boost the sequencing output of the subregion.

Abbreviations

CoVs	Coronaviruses
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
WHO	World Health Organization
GISAID	Global Initiative on Sharing Avian Influenza Data
KCCR	Kumasi Centre for Collaborative Research in Tropical Medicine
KNUST	Kwame Nkrumah University of Science and Technology
TTH	Tamale Teaching Hospital
KATH	Komfo Anokye Teaching Hospital
KSH	Kumasi South Hospital
GHS	Ghana Health Service
RT-PCR	Real-time polymerase chain reaction
PANGOLIN	Phylogenetic Assignment of Named Global Outbreak Lineages
Ct	Cycle threshold

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Introduction

Coronaviruses (CoVs) are minute in size (65–125 nm in diameter) and contain a single-stranded RNA ranging in length from 26 to 32 kb. The subfamily *Orthocoronavirinae* includes the genera *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*. Until 2002, when the world witnessed a severe acute respiratory syndrome (SARS) outbreak caused by SARS-CoV in Guangdong, China, coronaviruses, with the exception of 229E and OC43, were thought to infect only animals [1]. Only a

decade later, another pathogenic coronavirus, known as Middle East respiratory syndrome coronavirus (MERS-CoV), caused an endemic outbreak in Middle Eastern countries [2].

In December 2019, the World Health Organization (WHO) was informed about a cluster of patients who presented with pneumonia of unknown aetiology in the city of Wuhan (Hubei province) in China [3]. Shortly afterwards, a new type of coronavirus, now termed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was isolated and identified by scientists from China [4]. Sequencing results revealed that it belongs to the genus *Betacoronavirus* of the family *Coronaviridae* and has 96% genome sequence identity to a previously detected SARS-like bat coronavirus [5, 6]. The genetic sequence of this new virus was shared with the international community on January 10, 2020 [7, 8]. Infections caused by this virus have spread to all WHO regions and had an enormous adverse global impact. On March 11, 2020, WHO declared a global pandemic [9], which prompted increased and sustained international action and response. By August 27, 2020, over 24 million cases were confirmed, with more than 830,000 deaths worldwide (https://www.worldometers.info/coronavirus/).

The first confirmed case of COVID-19 in Africa was reported in Egypt on February 14, 2020 [10], and the first case in sub-Saharan Africa was in Nigeria on February 28, 2020 [11]. Ghana recorded its first confirmed case of COVID-19 on March 12, 2020 [12]. After this, all suspected cases of COVID-19 were confirmed by reverse transcription polymerase chain reaction (RT-PCR) as recommended by WHO [13]. While RT-PCR still remains the gold standard, issues relating to transport of samples from primary healthcare facilities to centralised testing laboratories, laboratory infrastructure, human resources, supply chain management, and stockpiling laboratory consumables and reagents have remained key challenges [14–16]. Beyond using RT-PCR to establish the presence of the virus, one big shortfall in several African countries is the inability to sequence the genome of the virus and to study its biology. Thankfully, the Africa Centres for Disease Control and Prevention (Africa CDC) is making a massive effort to establish genomic sequencing centres in Africa [17]. However, until this becomes a reality, the inability of several African countries to sequence viral isolates may result in deficits in our understanding of the distribution of the various SARS-CoV-2 strains circulating on the African continent as well as a lack of knowledge about its transmission dynamics [18].

The paucity of data is evidenced by the measure and proportion of sequences and genome data deposited in the Global Initiative on Sharing Avian Flu Data (GISAID) repository originating from Africa institutions (https://www.gisaid.org/). This means that Africa needs to build more capacity for genome sequencing in order to understand the distribution of the various strains circulating on the continent and

their transmission dynamics. This could eventually assist in the development of vaccines against COVID-19. It is likely that the African sequences are already relatively widely distributed globally [19]. In an effort to contribute to this initiative and following the detection of the first imported case in the northern sector of Ghana on March 13, 2020, we have now molecularly characterized and phylogenetically analyzed sequences, including three complete genome sequences, of SARS-CoV-2 obtained from nine of the first 200 patients observed in Ghana. Eight of these patients had a recent history of foreign travel, and one did not.

Materials and methods

Sample collection and transport

Between February 29 and March 28, 2020, samples were obtained from patients with suspected COVID-19 from the Tamale Teaching Hospital (TTH) in the Northern Region, and Komfo Anokye Teaching Hospital (KATH) and Kumasi South Hospital (KSH) in the Ashanti Region of Ghana (Fig. 1). Recruitment of cases at these facilities was done according to the Ghana National Surveillance Strategy protocol [20]. Suspected COVID-19 cases were defined as individuals presenting with fever (>38 °C) or a history of fever and symptoms of respiratory tract illness such as cough or shortness of breath, or individuals who were in close contact with a person who was suspected or confirmed to have COVID-19.

Nasopharyngeal and or oropharyngeal swabs were obtained using flocked swabs (Copan Group, Brescia, Italy) and kept in 500 µl of RNAlater (QIAGEN, Hilden, Germany) in 1.5-ml tubes (Eppendorf, Regensburg, Germany) and transported immediately at ambient temperature for confirmation at the Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR), Kumasi, in the Ashanti Region of Ghana.

The KCCR is one of the two main research laboratories in Ghana designated for COVID-19 testing. This is because of its longstanding experience and the expertise of its scientists in studies related to coronaviruses (http://kccr.org/). The Centre is currently Ghana's second largest testing site, which serves the northern sector of the country. During the early phase of the pandemic, the Centre received samples from 12 out of 16 regions in Ghana and tested approximately 1,200 samples daily.

Viral RNA extraction and PCR detection

Using a starting volume of 140 µl, both nasopharyngeal and oropharyngeal swabs from each patient were extracted together as a single sample using a QIAGEN Viral RNA
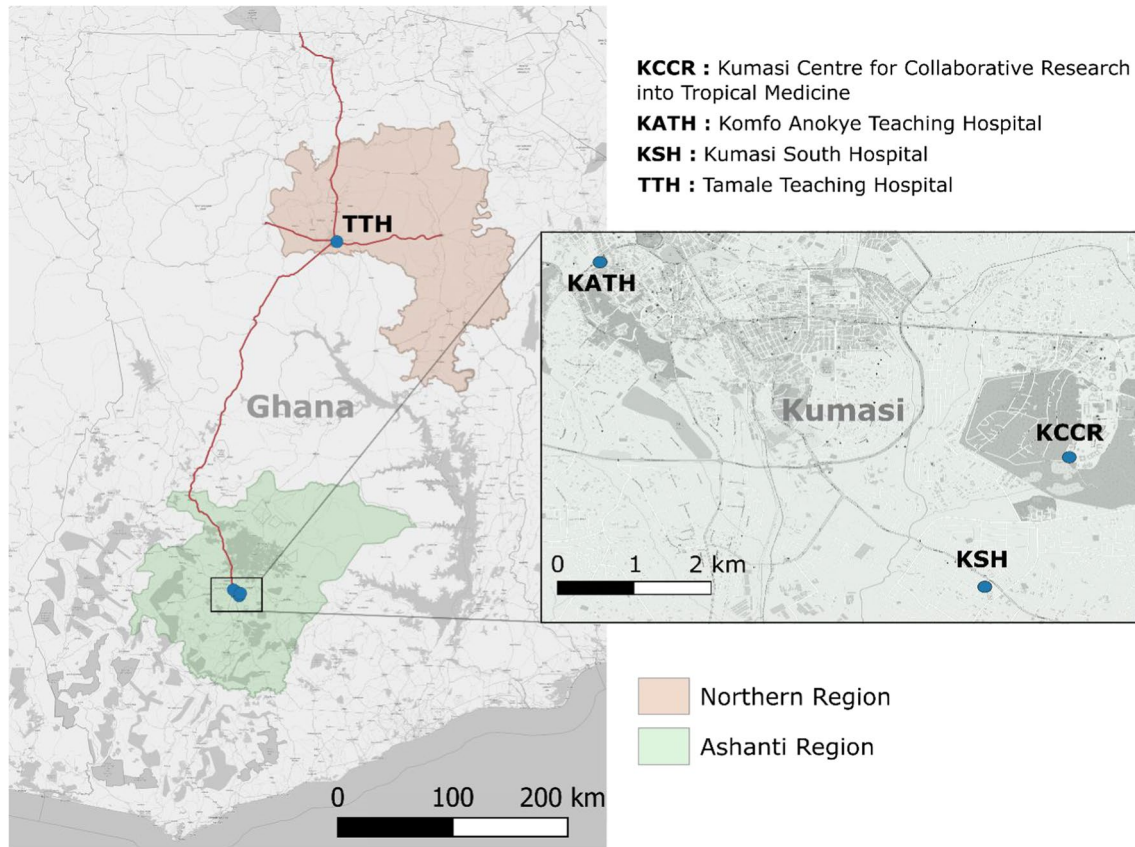
Fig. 1 Map of Ghana showing sample collection sites. The map was generated using Quantum GIS version 3.6.2 and data freely available from www.openstreetmaps.org. Samples were collected from the Northern Region (orange) and the Ashanti Region (green) and tested at the KCCR, also in Kumasi in the Ashanti Region of Ghana

Mini Kit (QIAGEN, Hilden, Germany) according to manufacturer's instructions. Samples were eluted in a 100-µl volume, and SARS-CoV-2 RNA was detected using a Real-Star® SARS-CoV-2 RT-PCR Kit (Altona, Germany) according to the manufacturer's instructions. Sample quantification was done using an externally generated standard curve based on serially diluted SARS-CoV-2 *in vitro* transcripts. All samples with a cycle threshold (Ct) of 40 or above were considered negative. All amplification runs were validated by including positive and negative controls.

Whole-genome sequencing

High-throughput sequencing for samples with sufficiently high RNA concentrations as determined by quantitative real-time PCR were sequenced using an Illumina Next-Seq platform (Illumina, San Diego, California, U.S.) and a KAPA RNA Hyper Prep kit (Roche Molecular Diagnostics, Basel, Switzerland) according to manufacturer's instructions. In order to estimate the potential impact that long-distance transport of samples to testing centers may have on sequencing results, the two samples that were closest in

terms of viral RNA concentration were tested using an Agilent 4200 TapeStation system (Agilent Technologies, CA, USA). These samples comprised one from TTH in the north, which is approximately 400 kilometers from the testing site, and another from KATH in Kumasi (in the same city as the testing site, approximately 9 km away).

Phylogenetic analysis

Sequences from this study, with the exception of three that had less than 80% of the genome sequenced, were compared to previous sequences from Ghana and representative sequences from regions where patients had previously travelled. These included sequences from the USA, Japan, France, and Guinea and available sequences from sub-Saharan Africa. All sequences from the region of interest as of April 2020 were obtained from GISAID (https://www.gisaid.org/), and duplicates were removed. The non-redundant sequences were then clustered at a minimum threshold of 99.9% using CD-HIT (http://weizhongli-lab.org/cd-hit/), and representative sequences from each cluster were selected. Multiple sequence alignments were done using the MAFFT

plugin in Geneious prime (http://www.geneious.com). Phylogenetic analysis was done by Bayesian inference using the MrBayes [21] plugin in Geneious prime with a chain length of 1.1 million and a subsampling frequency of 200. A general time-reversible substitution model with a gamma distribution and proportion of invariable sites (GTR+G+I) was used for the analysis. All sequences with genome coverage greater than 80% were analyzed using the Phylogenetic Assignment of Named Global Outbreak Lineages (PANGOLIN) online resource (https://pangolin.cog-uk.io/) for lineage assignment.

Ethical approval

We obtained ethical approval from the Committee on Human Research Publications and Ethics of the School of Medicine and Dentistry at the Kwame Nkrumah University of Science and Technology (CHPRE/AP/462/19) and the Institutional Review Board of the Ghana Health Service (GHS-ERC087/03/20).

Results

Sample description and comparison

A total of nine samples obtained from nine patients that tested positive for SARS-CoV-2 were analyzed. Six of the samples were from the Northern Region, while three were from the Ashanti Region of Ghana. Six patients were asymptomatic, and three were symptomatic, presenting with cough, headache, general weakness, sore throat, shortness of breath, and diarrhea. All but one of the patients had a history of travel to the USA, Japan, France or Guinea (Table 1).

Sample TTH6 was transported over 400 km by road and was presumably exposed to ambient temperature for a longer time than sample KATH23, which was transported over a distance of 9 km to the testing centre, where both samples underwent similar processing procedures.

Despite a difference of approximately only 576 copies/μL between samples KATH23 and TTH6 (Table 1), TapeStation analysis showed less RNA fragmentation in KATH23 (Supplementary Figs. S1 and S2), which yielded a complete genome sequence on the first attempt, whereas TTH6 yielded a sequence with approximately 82.8% genome coverage, which was increased to 95.6% with resequencing efforts.

Sequence description and phylogenetic analysis

The percent coverage of the available genome sequences in this study ranged from as low as 23.2% to complete coverage. Complete genome sequences were obtained for three samples, and another nearly complete genome sequence had a coverage of 95.6%. Sequences with coverage in excess of 80% were found to belong to three lineages, namely A, B.1, and B.2. The least number of nucleotide substitutions in comparison to the original SARS-CoV-2 isolate from China (NC_045512) was the one from a patient with no known travel history (KATH23). The one with the most nucleotide substitutions was from a patient known to have travelled to at least two locations in Asia and Europe before arriving in Ghana (KSH61). The D614G amino acid substitution in the spike protein (A23403G), which is purported to enhance viral infectivity [22], was found in three sequences from this study (Table 2). Our sequences clustered in two different clades, with the majority falling within a clade composed solely of sequences from sub-Saharan Africa (Fig. 2). All seven sequences that had greater than 50% coverage were submitted to the SARS-CoV-2 repository on

Table 1 Description of SARS-CoV-2-positive samples analyzed in this study

Sample ID	Sampling site	Region	Gender	Travel history	Symptoms at presentation	Viral RNA concentration (copies/μL)
SARS-CoV-2/Tamale/TTH7/2020	TTH	Northern	F	Guinea	Asymptomatic	1.90×10^2
SARS-CoV-2/Kumasi/KSH54/2020	KSH	Ashanti	F	USA	General weakness, sore throat, shortness of breath, diarrhea	7.34×10^{-1}
SARS-CoV-2/Tamale/TTH9/2020	TTH	Northern	F	Guinea	Asymptomatic	5.25×10^0
SARS-CoV-2/Tamale/TTH10/2020	TTH	Northern	F	Guinea	Asymptomatic	6.59×10^5
SARS-CoV-2/Kumasi/KSH61/2020	KSH	Ashanti	M	Japan, France	Cough and headache	2.91×10^1
SARS-CoV-2/Tamale/TTH6/2020	TTH	Northern	F	Guinea	Asymptomatic	6.24×10^2
SARS-CoV-2/Tamale/TTH11/2020	TTH	Northern	F	Guinea	Asymptomatic	1.38×10^2
SARS-CoV-2/Tamale/TTH8/2020	TTH	Northern	F	Guinea	Asymptomatic	6.16×10^{-1}
SARS-CoV-2/Kumasi/KATH23/2020	KATH	Ashanti	M	No travel history	Shortness of breath	1.20×10^3

TTH, Tamale Teaching Hospital; KSH, Kumasi South Hospital; KATH, Komfo Anokye Teaching Hospital

Table 2 Phylogenetic lineage description and nucleotide substitutions in available genome sequences in comparison to an early genome sequence from China

Sample ID	Percentage genome coverage	Nucleotide variations	PANGOLIN lineage (probability)
SARS-CoV-2/Ghana/Tamale_TTH7/2020	84.7	A14253G T22714C G25606T T28144C	A (0.55)
SARS-CoV-2/Kumasi/KSH54/2020	23.2	A1749T A23403G G25606T T28144C	-
SARS-CoV-2/Tamale/TTH9/2020	48.3	T380C C14408T G25606T T28144C	-
SARS-CoV-2/Tamale/TTH10/2020	Complete	G25606T T28144C	A (0.99)
SARS-CoV-2/Kumasi/KSH61/2020	82.7	A12121T C14408T G16027T T19920G T22385C A23403G G25563T G25606T T28564C	B.1 (1)
SARS-CoV-2/Tamale/TTH6/2020	95.6	G25606T T28144C	B.2 (0.98)
SARS-CoV-2/Tamale/TTH11/2020	Complete	G25606T T28144C	A (0.99)
SARS-CoV-2/Tamale/TTH8/2020	60.7	C14408T A23403G	-
SARS-CoV-2/Kumasi/KATH23/2020	Complete	T28144C	A (0.98)

the GISAID platform and assigned the accession numbers EPI_ISL_515181-515184 and EPI_ISL_515247-515249.

Discussion

Despite the impressive drive from different regions of the globe, including Africa, to sequence SARS-CoV-2 genomes for studying the epidemiology of the virus, Africa still lags behind in terms of its genome sequencing output, as was seen by searching the SARS-CoV-2 repository on GISAID in August 2020. Apart from a lack of availability of sequencing technology and expertise, which could account for this shortfall [23], contributory factors may be degradation of genetic material during transport to available diagnostic centres due to the mostly centralized testing system prevalent on the African continent [24]. Our study shows that although two samples had similar viral loads (difference = 576 copies/μL), the samples obtained from Kumasi with a viral load of 1.20×10^3 copies/μL (approximately 9 km from the testing laboratory) showed less fragmentation than the one from Tamale in the northern part of Ghana, with a viral load of $6.24 \times$

10^2 copies/μL, that had to be transported over a distance of approximately 400 km across the country before being processed. This highlights the importance of correct sample storage and transport conditions. Although some level of viral RNA fragmentation can be tolerated when diagnostic real-time PCR is used due to short amplicon sizes, the same cannot be said for downstream whole-genome sequencing [25]. Therefore, decentralization of diagnostic centres and proper sample storage are important for boosting the sequencing output of the subregion. In addition, as the world races towards development of a vaccine, the importance of sequence data from the African region cannot be overemphasized. Capacity building in Africa for this purpose is paramount.

The assigned PANGOLIN lineages are reflective of the sampling times of the sequences, as seen with the early circulating A, B.1, and B.2 lineages from January to May 2020 [26]. This supports the reported epidemiological links in terms of travel, which was associated with early introduction of the virus into Ghana, mainly from Asia and Europe. The sequence from the individual with no known travel history had the least number of nucleotide substitutions compared to the original sequence from China. This
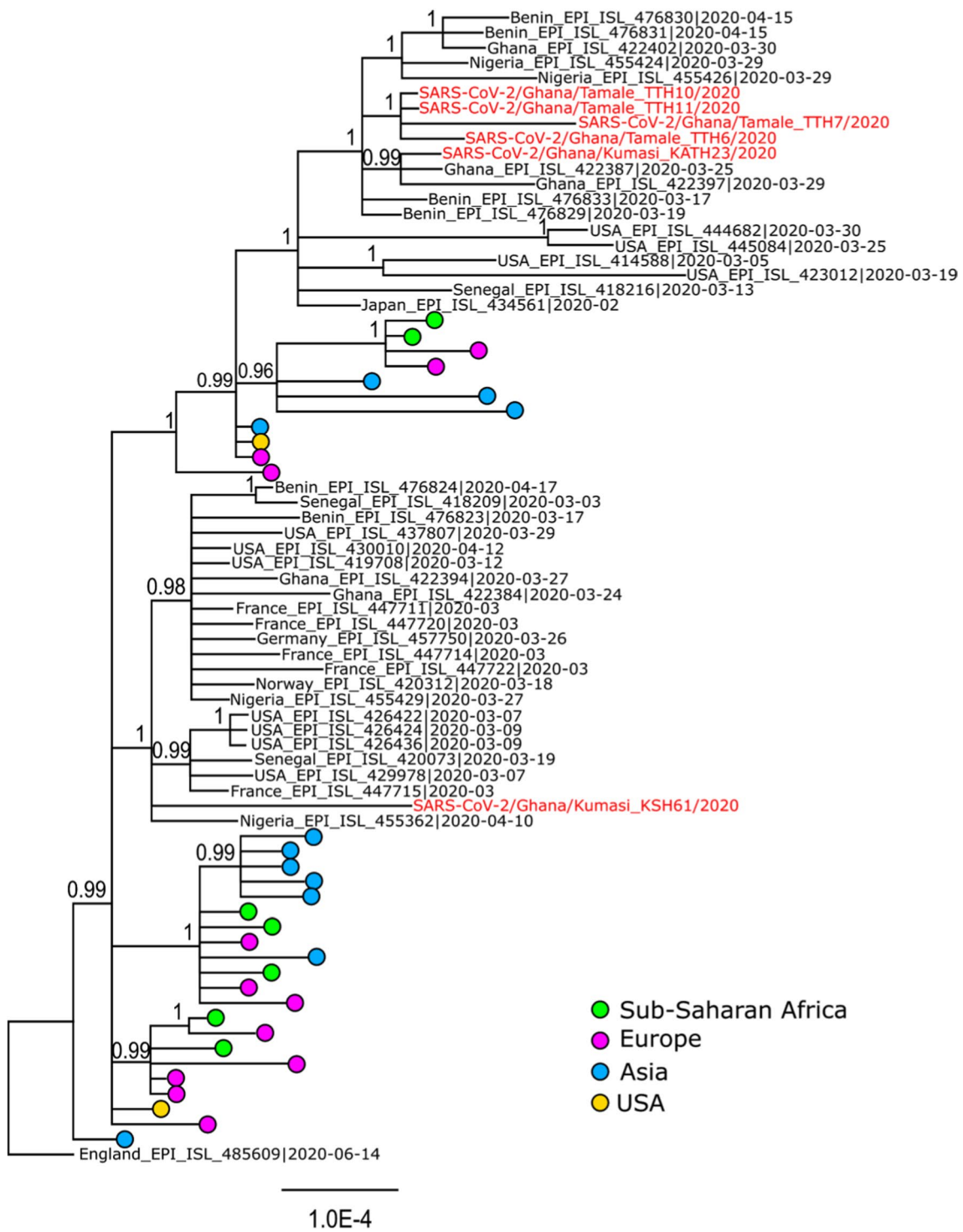
Fig. 2 Phylogenetic analysis of SARS-CoV-2 genome sequences Phylogenetic analysis was performed on 76 representative genome sequences by Bayesian inference using the GTR+G+I substitution model. Sequences in the tree are designated by location, GISAID accession numbers and date of collection. Sequences from this study are highlighted in red with sequence-specific names. The tree was rooted with randomly selected sequences from England collected in June 2020

may hint at earlier introductions and possible community spread than previously believed.

Sequences from sub-Saharan Africa, including previous ones from Ghana, were observed to cluster in different clades across the topology of the phylogenetic tree. However the subset of sequences clustering together only from sub-Saharan Africa may point to the circulation of viruses within the region due to movement through porous land borders [27, 28]. A lot of emphasis is placed on introductions from other regions such as Europe and Asia through air travel, but introductions from within the subregion also appear to play a major role in virus circulation through movement of people. The number of sequences included in the analysis, however, was limited, and interpretation of these data should therefore be done with caution.

Conclusion

Analysis of sequences from the early stages of the outbreak can provide important insights into the viral diversity present in regions where genomic data are lacking. There is the need for further studies on adequate sample storage and transportation when testing facilities are far from sample collection sites. The clustering of several sequences from sub-Saharan Africa suggests regional circulation of the viruses in the subregion.

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