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Study of Dengue virus E proteingene of clinical isolates of Andhra Pradesh: in the contest to Epidemiological features

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Abstract

In this current article, we showed the dengue virus serotype 2 protein E gene from the clinical samples of Andhra Pradesh in an acute phase infection. The models showed positive for the protein E gene with RT-PCR techniques and cellular isolates. Two unique sequences are identified with new substrains, which are similar to the hermits of the earlier reports. This study provided the epidemiology insight of the isolated strain through the phylogenetic analyses.

Keywords: Dengue Virus, Endemic, PCR, Viral Protein E, Viral serotyping

Introduction

Dengue fever has emerged as one of the most extraordinary arboviral infections in recent history, owing to the rapid epidemiological evolution over the previous two decades. It is a severe public health threat worldwide, with tropical and subtropical regions being particularly vulnerable. (Wilder-Smith et al. 2017). This disease is widespread in tropical nationals include with severe manifestations in various age groups. Dengue fever (DF) is characterized by a range of disease intensities ranging from mild to life-threatening dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS)(Guha-Sapir and Schimmer 2005; Tian et al. 2018). From past epidemiology data represents the seroprevalence of the dengue virus serotypes include DENV1, DENV 2(Fried et al. 2010), DENV 3(Gupta et al. 2012), DENV 4(Costa et al. 2012) and DENV

5(da Silva Voorham 2014; Mustafa et al. 2015) in which are more prevent evolutionary forms based on the geographic structures (Roy and Bhattacharjee 2021; da Silva Voorham 2014).

Dengue virus substitution rates have been evaluated using a variety of methodological approaches and are comparable to the evolutionary rates of other RNA viruses through the envelope protein E(Costa et al. 2012).We believe that dengue serotypes should be studied in comparative fashion because there is increasing evidence that their epidemic potentials differ and that episodes of secondary infection with different serotypes frequently result in higher morbidity rates due to the more severe forms of the infection. (Pawitan 2011; Guo et al. 2017). Furthermore, the reasons for dengue serotype diversification remain unknown. It has been proposed that the five serotypes arose via separate zoonotic transmission to humans. If this is the case, we can expect DENV lineages to have adapted to the new host and, as a result, positive selection signatures along the DENV genome(Fried et al. 2010; Soo et al. 2016). Due to the intrinsically high mutation rate associated with RNA-dependent RNA polymerase, their quick replication rates, and their enormous population sizes, RNA viruses exhibit immense genetic variety. This genetic variability is most visibly manifested in dengue virus by the presence of five antigenically different serotypes. Prior to the availability of gene sequence data, it was recognized that genetic variation occurred within each serotype. However, the introduction of comparative gene sequence analysis has enabled the dissection of the genetic structure of dengue virus populations and the elucidation of the processes regulating viral evolution (Holmes and Twiddy 2003; Murrell et al. 2011; Lin et al. 2017).

One of the objectives of this work was to undertake a detailed comparison of the evolution of a dengue virus serotype isolate's E gene sequence with the protein E gene sequence of dengue virus serotype 2. Based on a large number of publicly available sequences collected over the previous few decades, we examined the demographic dynamics of epidemic gene sequences collected over the previous few decades and computed evolutionary parameters important for understanding the development of the DENV 2 serotype. We also looked at the impact of adaptive molecular evolution on DENV serotype emergence and the heterogeneity of selective factors along the envelope gene.

Experimental methodology

Clinical Sample and virus extraction

The clinical samples with acute-phase dengue infections (patents infected within a few days) were obtained from clinically diagnosed dengue fever patients who presented to Government Hospital, Anantapuramu, Andhra Pradesh, India. All samples are collections are sought out through proper ethical standards as per the institute and the university norms. The samples were procured aseptically through venipuncture and transported to the working lab in clinical standards to the Deparment of Virology laboratory, Sri Venkateshwara University , Tirupati, India. The viral particles are isolated by centrifugation of the collected induvial blood for 10 minutes at 2000 rpm at 4^{o}C. Plasma samples were kept at –80°C until they were tested further. The WHO case definitions were used to develop clinical criteria for diagnosing dengue fever.

Cell culture and Virus cultivation

The viral isolates were cultured on the host cells such as Vero cell lines (African green monkey kidney cells) collected from American type culture collection with ATCC CCL-81. These cells are cultured in Eagle's minimum essential medium (MEM) supplemented with 5% fetal bovine serum (GIBCO, USA). The serum content was lowered to 1.5 % in maintenance medium (MM) for further usage. These cells incubated in a humidified incubator at 37^{o}C, 5% CO_2 flow for 24 hours of period. As the cells reach to a maximum confluency, the extracted viral particleswere transinfected to the cell lines, the inoculation was done at80-90% confluency of the cell lines with a 1:10 dilution of viral stock Vero cells of $5x10^4$ ml^{-1} and grown for seven days. The virus particles from the cell lines were harvested by scraping and stored at -80^{o}C. The virus was passaged 3 times under eachcondition with a 1:10 dilution of viral stock from the previous passage to check the consistency of the infection. Viral RNA was extracted from cell scrapped samples using the QIAamp Viral RNA minikit (Qiagen, Germany) as per manufacturers' instructions. Extracted RNA samples was stored at -70°C or used for RT-PCR immediately.

Viral gene extraction and Complementary DNA preparation

Once the RNA was isolated form the viral particles, the samples are allowed to Reverse transcriptase Polymerase chain reaction(RT-PCR) expression-based analysis. Complementary DNA (cDNA) was prepared by using RT-PCR method, by using random universal primers under optimized PCR conditions. Once the cDNA obtained was stored at -20^{o}C and further proceed for the Polymerase chain reaction (PCR)

PCR and Sequencing the E Protein genes

Primers were developed and obtained from the Sandor life sciences Resources, Hyderabad, India which are listed in the table 1. These primers are gene specific, which are used to amplify the protein E genes from the viral cDNA and its flanking regions using PCR. Both forward and reverse primers are used for the PCR run under optimized conditions. Once the amplified sequences are obtained, they are subject to DNA electrophoresis for conformations of the PCR. Once the amplification product obtained, they are subjected to Exo SAP purification. These PCR products are allowed to sequencing using the ABI Big Dye Terminator V 3.0 Kit as per manufactures instructions. All these products are resolved by DNA electrophoresis on an ABI 3730xl capillary sequencer.

Epidemiology analysis

The obtained sequences were further analyzed for the assessment of the epidemiology studies. Using NCBI nucleotide BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi), the sequences are aligned for the conservative and varied nucleotides compared with the previous sequences listed in the ncbi database (https://www.ncbi.nlm.nih.gov/) for highly similar sequences. The obtained alignments were further assed for the construction for the phylogenetic tree based on the fast minimum evolutions. All the sequences are aligned further using MEGA software package with crustal W program, for the minimum variation and conservative sequence assessment and representations. The obtained tree was further assed for the genetic distances and representation using network format file from the ncbi blast data and incorporated in the ITOL (https://itol.embl.de/login.cgi).

Results and Discussions

Here in this reserch we collected blood from the 230 patients who are in the age inbetween 10-18 years, who are showd the dengue symptoms at thte time of the sample collection. Where when we done the diagnosis of these pateinets, we obtained 33 patetints are positive for the DENV-2. Based on the severity of the personlas, we later opted the most sever DENV-2 for this study followed by the characterization of the enevelop protein E. The collected samples are allowed to get the viral particles through a series of purification steps, these viral particles are allowed to incubate in the Vero cell lines for a period of seven days for the individual cultures. We successfully infected the cell lines, later we extracted the viral RNA from the induvial samples for the respective cell line cultures. The isolated and purified RNA was analyzed through the nanodrop for the concentrations, as the viral load was considered to be isolated in lower yield which around < 10 pM. Hence, we further amplified the gene using the RT-PCR, based on the customized forward and reverses primers as shown in the table-1, we successfully obtained the two products respective for the primers utilized. As figure -1 showed the DNA gel electrophoresis for the complementary DNA (cDNA) amplified from the PCR, the band ~248bps represents the amplified cDNA of the DENV-2. We further assessed the characteristics of the obtained cDNA by sanger-based sequencing, where the NCBI data set based sequence alignments shows that, the amplified cDNA would belong to the DENV-2 (dengue virus serotype-2) variant, the sequences are showed in the figure-2. These sequences based on the reverse and forward primers as designated DF-1 (Forward) and DR-1 (Reverse) are further submitted tot eh NCBI data base, were we got accession for DF-1 was OL440937 and DR-1 was OL445422 was successfully published. These sequences are further allowed to BLAST -ed in the NCBI and the EMBL data bases, where we got the confirmation of the sequences are the genes encode to DENV viral protein E, the sequences characteristics for the conservation and variable sequences for the top 5 sequences are represented in the figure 3(a) and 3(b).

Later we performed the epidemiological assessments for the respective sequences include DF-1 and DR-1, where we obtained the promising results where DF-1 showed the clade which was similar to the 99.8% which is closely related the clade sequences for the protein E of the DENV serotype 2 viral characteristics. While the DR-2 showed the quite new variant respond by forming new clade which was quite consistent with sequence data about 99% with the DENV-2. Our data reveled there would be new strains form the DNEV-2 based on the mutations in the gene isolated sequences, which include 12T, 37C, 36G, and 38G were seen in the variable region of the DF-1 viral sequences and for the DR-1 we seen the mutations at 10G, 24T, 45C respectively.

2.5 billion people are expected to be at risk of contracting dengue fever(Alhaeli et al. 2016; Barde et al. 2012). Dengue viruses are responsible for a variety of well-described clinical diseases include, DF which is a clinical illness that is often characterized by increased capillary permeability, which can progress to DSS and DHF if the capillaries become dilated(Lin et al. 2017; Wu et al. 2021). Dengue fever outbreaks can have a substantial economic and health impact on any country(Buddhari et al. 2014; Edgerton et al. 2021). Dengue virus serotypes 1–5

was now circulating in Asia, Africa, and the Americas, a major change from the situation 20 or 30 years ago(Ferreira-de-Lima and Lima-Camara 2018). When creating an efficient surveillance and prevention programme for any disease, it is vital to account for epidemiological variation(Roy and Bhattacharjee 2021).

It was observed that all of the viruses studied belonged to the Indian subcontinent and other tropical countries lineage of the cosmopolitan genotype after a phylogenetic study of 27DENV-2 E gene sequences acquired between 2007 and 2017(Dhal et al. 2020; Thao et al. 2021). However, despite the fact that there were much more DF samples among the samples collected, there was no distinguishing characteristic in the E gene sequences that could distinguish between DF and DSS samples. As illustrated in figure-4 (a), with the exception of 27 isolates that were closely linked to a prior Asian strain and included southern India, the remaining 2 isolates formed a unique monophyletic clade with 78 percent bootstrap support, which is distinct from the previous Asian strain(Zheng et al. 2010). As illustrated in Figure 4 (b), the same clade obtained 100 percent bootstrap support in the DENV-2 whole genome analysis, further enhancing the credibility of the clade for the other sequence. There were 27 DENV-2 isolates in this monophyletic clade, all of which were recovered from sera collected in the southern portion of India (Anantapuramu, Andhra Pradesh State) in 2017 and 2018. According to a comparison of complete E gene sequences available in the GenBank database, these two viruses shared the highest nucleotide similarity (98.8-99.5 percent) with viruses that had been reported in India and other parts of the world in the period between 2007 and 2017, as well as with viruses that had been reported in other parts of the world in the same period(dos Santos Soares et al. 2017).

Conclusions

Dengue fever is a major public health concern in our country, and effective control measures must be put in place immediately to prevent a new pandemic of epidemic proportions. We have all established tolerance to DENV-1 to 5 to the community and has the potential to become an epidemic strain. In order to avoid a future pandemic, it is essential to implement adequate and timely preventative measures. The study of such difficulties may be of interest to epidemiologists and virologists in order to plan effective control of the virus or the development of future vaccines. Future efforts should be directed toward the development of enhanced laboratory-based surveillance systems that can detect the onset of approaching dengue outbreaks. As a result, the study emphasizes the role played by virus evolution in the spread of dengue to locations where the disease has not previously been a major public health problem, as well as the creation of novel strains of the virus that may be associated with severe outbreaks.

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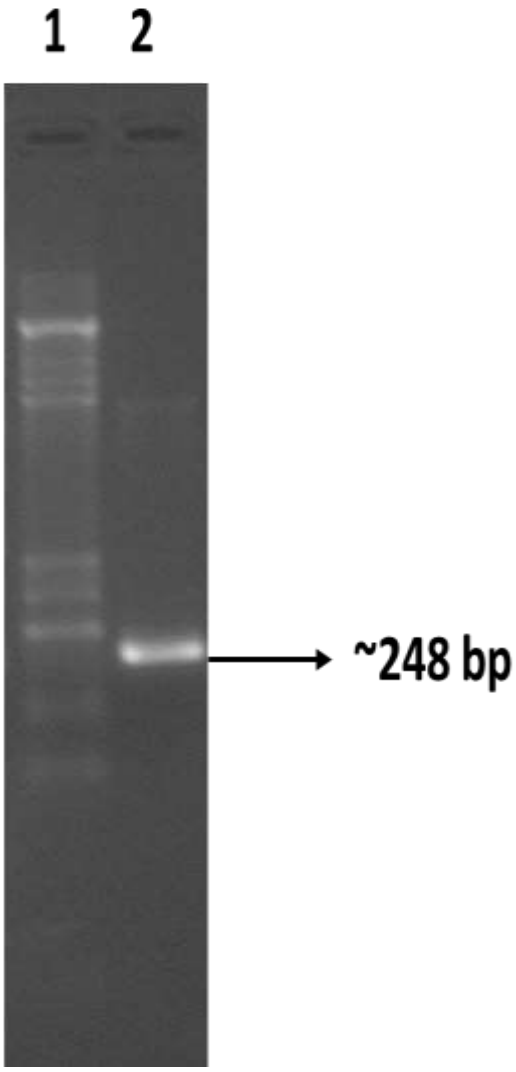
Figure 1: DNA electrophoretic gel images showing (1) The 1kbp DNA ladder and (2) Query sample sequences showing ~248bp



(a)


lcl|Query_50526:889-1366
lcl|Query_50528:867-1358
lcl|Query_50527:889-1380
lcl|Query_50529:889-1366
lcl|Query_50531:889-1380
lcl|Query_50530:889-1380

(b)


lcl|Query_16092:863-1336
lcl|Query_16094:841-1314
lcl|Query_16097:863-1336
lcl|Query_16095:863-1336
lcl|Query_16093:863-1336
lcl|Query_16096:863-1336

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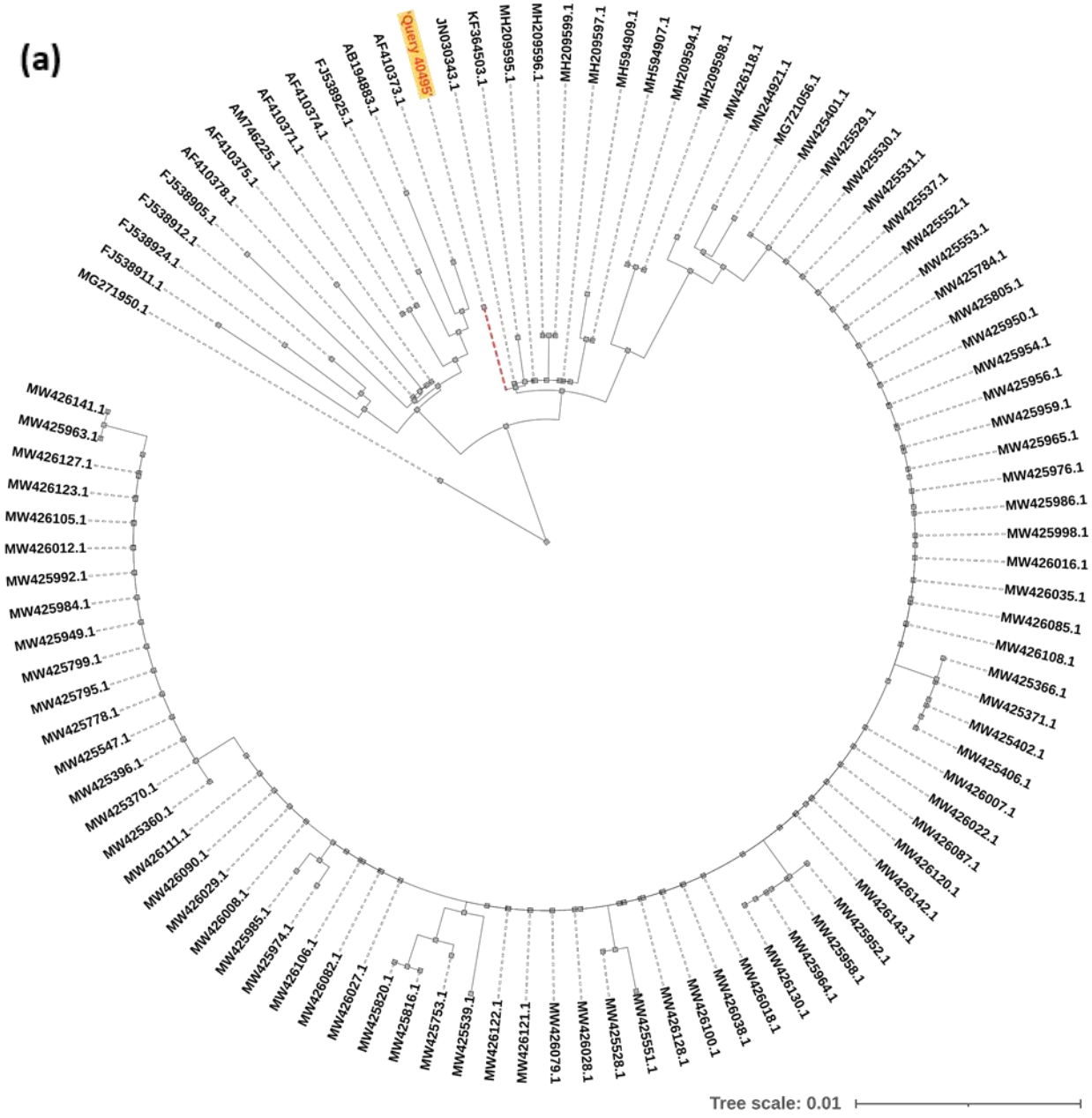
se


(a)
Query 40495
JN030343.1
KF364503.1
MH209595.1
MH209596.1
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MH209598.1
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MG721056.1
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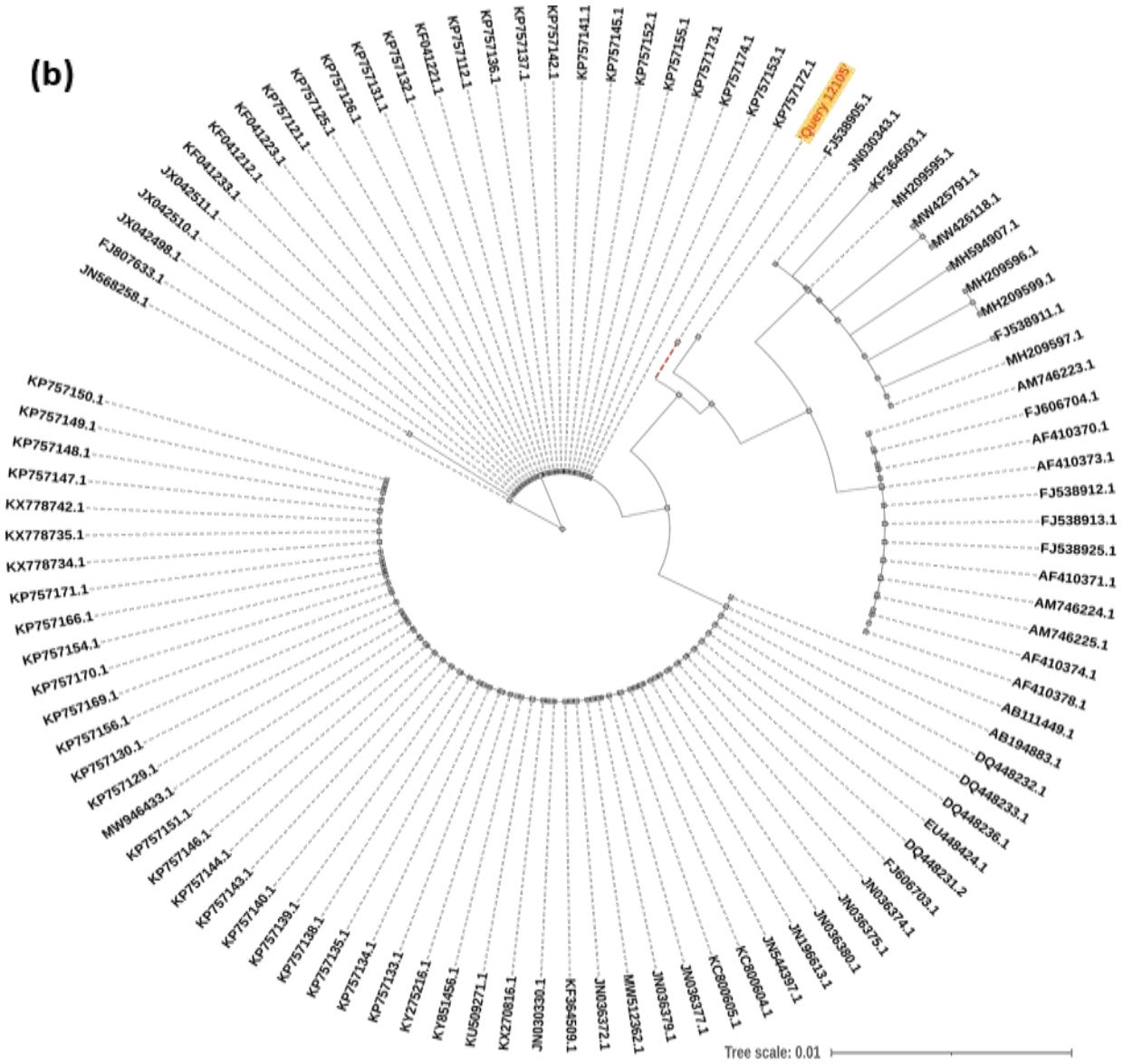

Figure 3: Phylogenetic tree of DENV-2. (a) DF-1 and (b) DR-1The maximum-likelihood tree was constructed based on the complete envelope gene sequences generated during this study and retrieved from GenBank. Sequences from query sequence are highlighted in red. Figures on branches are bootstrap percentages. Only bootstrap values more than 95% are shown on the major nodes.

Table 1: Primer details for Polymerase Chain Reaction

Primer Code	**Orientation**	**5' to 3' direction**	**Location of genome**
D2ES1F	Forward	GCAATCCTGGATACCCATAGG	850-872
D2ES1R	Reverse	CTTCCCCTGAATGAGGTGTT	1359-1378