



## Correspondence

### Genetic characterization of dengue virus types 1 and 2 in India, with emphasis on the viruses circulating in Karnataka

Sir,

Dengue, a viral disease transmitted by *Aedes* mosquitoes, is a serious global public health problem, infecting 50 to 100 million people each year in >100 countries and resulting in at least 20,000 deaths annually<sup>1,2</sup>. Dengue is caused by one of the four antigenically related dengue virus serotypes (DENV-1 to 4) belonging to the family *Flaviviridae*<sup>3</sup>. The viral genome is a single-stranded, positive-sense RNA of about 10.7 kb which encodes three structural and seven non-structural (NS) proteins in the order 5'-C-prM(M)-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3'<sup>4</sup>. Based on the nucleotide sequence of the envelope (*E*), E/NS1 or capsid-premembrane (*CprM*) gene, the four DENV serotypes have been classified phylogenetically into three to five genotypes with certain genotypes being further classified into lineages or clades<sup>5</sup>.

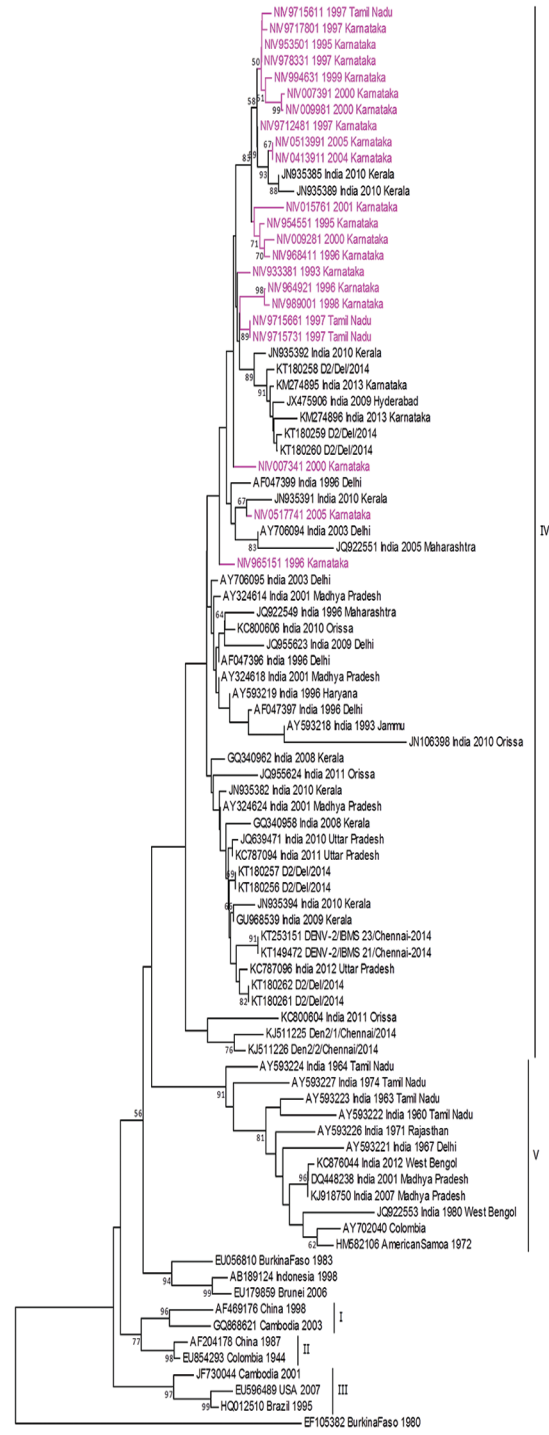
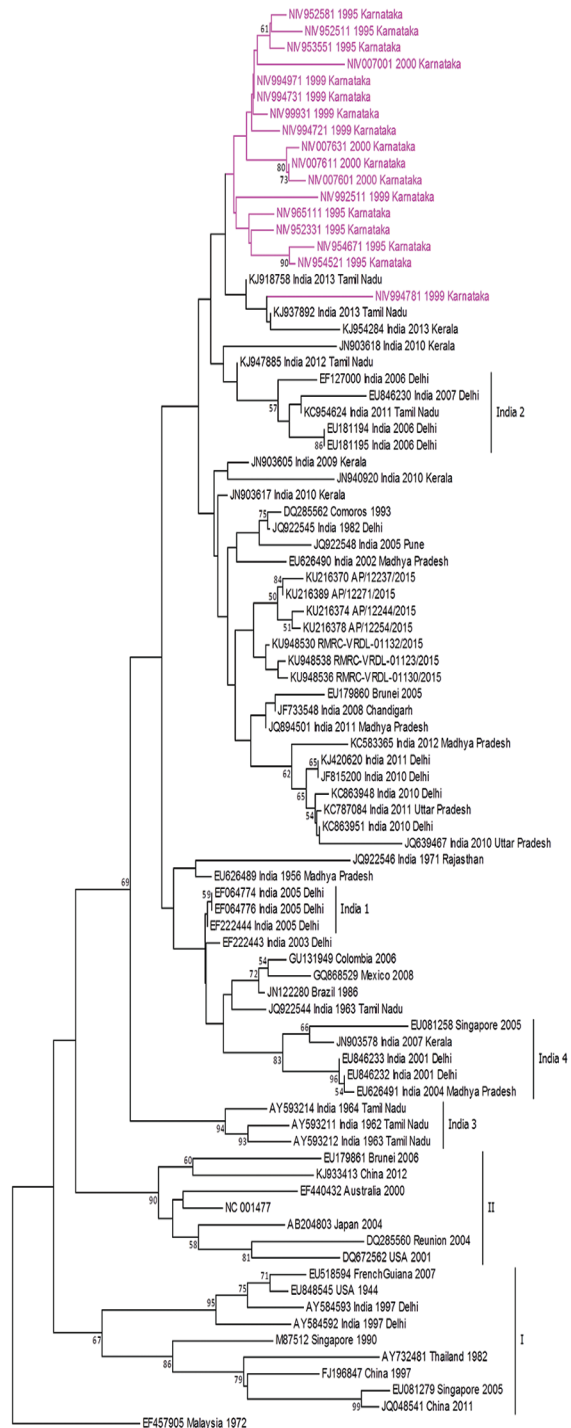
In India the incidence rate of dengue infection has grown steadily in the past few years<sup>6</sup>. Though DENV fevers have been recorded in India since the 1940s<sup>7</sup>, the first reported epidemic of dengue fever was during 1963-1964 from the Eastern Coast of India. Since then many outbreaks have been reported from all over the country<sup>8,9</sup>. The epidemiology of DENV and its prevalent serotypes has been ever changing in the country. With the increase in DENV cases, the need to understand the genetic nature of circulating DENV has become necessary. There are no reports of in-depth molecular genetic characterization of DENV in the State of Karnataka. *CprM* gene-based genotyping of DENV has proved to be useful for carrying out molecular epidemiology of these viruses<sup>10,11</sup>. Phylogenetic trees based on *CprM* and *E* gene regions are found to have a similar topology with no discrepancy between genotypes/sublineages. Hence, considering the availability of a larger dataset based on *CprM* gene sequences of Indian DENV isolates, the present study

was undertaken during January 2016-August 2016 to understand the genetic nature of circulating DENV in Karnataka by sequencing the *CprM* gene junction and comparing 504 bp of this region with other similar Indian DENV sequences reported till date and global reference sequences.

Being the diagnostic laboratory, blood/serum samples from suspected cases of DENV infection were referred to the ICMR-National Institute of Virology, Bengaluru unit, from multiple public health agencies. Samples were transported to the laboratory with the due precautions recommended for handling RNA viruses. Overall, 17 DENV-1 and 58 DENV-2 isolated from different outbreaks in Karnataka during 1993-2005 and three isolates from Tamil Nadu (1997) were used in this study, for sequencing of the *CprM* gene junction region. In brief, viral RNA was extracted from 140 µl of serum samples, using a QIAamp Viral RNA Mini kit (Qiagen, Germany), according to the manufacturer's instructions using published *CprM* gene-specific primers<sup>12</sup>. The PCR products were purified using QIAquick gel extraction Kit (Qiagen) and sequenced using BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA) in an automatic sequencer (ABI PRISM Genetic Analyzer 3100, Applied Biosystems). The *CprM* sequences of DENV-1 (nucleotide positions 137-640, with respect to DENV-1 sylvatic sequence Malaysia/1972) and DENV-2 (nucleotide positions 137-643, with respect to DENV-2 sylvatic sequence BurkinaFaso/1980) were compared with other *CprM* sequences of DENV-1 and -2, respectively, from India and other countries retrieved from the NCBI nucleotide database (<https://www.ncbi.nlm.nih.gov/nucleotide/>, as on 30 June, 2016). Multiple sequence alignment and phylogenetic trees were constructed using MEGA version 6.0<sup>13</sup>, employing the Neighbour-

Joining method<sup>14</sup> with the Kimura-2 parameter distance model and 1000 bootstrap replications, to determine the extent of genetic heterogeneity and trace the phylogeny of DENV-1 and DENV-2.

Phylogenetic analyses of the 17 DENV-1 isolates of this study and the other Indian and global sequences revealed that all the Karnataka isolates of the study period clustered in Genotype III (Fig. A). It was noted



**Figure.** Phylogenetic tree of selected representative sequences of *CprM* region of (A) DENV-1 and (B) DENV-2 by Neighbour-Joining method.

that these sequences could not be classified into any of the India 1-4 lineages that included the DENV-1 strains of several States in north and south India over the period from 1956 to 2015<sup>15</sup>. The per cent nucleotide divergence (PND) within the sequences from Karnataka was found to be 1.41 per cent. The amino acid alignment showed that there were a few mutations specific to some of the Karnataka isolates, namely, I127V in four isolates of 1995 and R97K in two isolates of 1999 (Data not shown). Analysis of DENV-2 isolates (Fig. B) showed that all the isolates of this study (which included three isolates of 1997 from Tamil Nadu) clustered within the Genotype IV (also referred to as the Cosmopolitan genotype) of DENV-2 along with majority of the other Indian isolates. The PND among the sequences of this study was found to be 1.7 per cent. A mutation A102T was found to be unique to many of the Karnataka isolates of the period 1995-2005. A few isolates from Karnataka were found to possess A102V as in some other strains from other parts of India. Co-circulation of both serotypes was noted during 1995-1996 and 1999-2000. At position 104, Karnataka sequences possessed M as in the Tamil Nadu sequences. The sequences of the later period from Kerala, Chennai and north India possessed a substitution M104V.

DENV-1 has been circulating in India since the 1940s<sup>7</sup> with earliest outbreaks reported from Vellore, Tamil Nadu, in 1950s-1960s. DENV-1 was also implicated during different outbreaks in Delhi, Rajasthan, Uttar Pradesh and Madhya Pradesh since 1970s<sup>8,16,17</sup> and recently (during 2015) in Arunachal Pradesh (GenBank accession numbers: KU216370, KU216374, KU216378 and KU216389). DENV-1 was associated with a dengue haemorrhagic fever (DHF) outbreak in Delhi in 1997<sup>18</sup>. Delineation of this serotype into three major genotypes designated as I-III<sup>19</sup> has been noted, with Genotype III (cosmopolitan genotype)<sup>20</sup>, being the most dominant one. Within this genotype, four distinct Indian sublineages have been identified, designated as India 1-4<sup>15,20</sup>. India-3 is extinct while the other three are the currently evolving lineages<sup>21</sup>. While the Delhi sequences exist in all the three lineages, during 2007-2008, Kerala also showed the circulation of the India-4 lineage of DENV-1<sup>22</sup>. A study based on the complete *E* gene has identified some more Indian clusters<sup>23</sup>. No change in the lineage of the Karnataka DENV-1 isolates has been observed during the period of more than a decade based on this study.

DENV-2 represents six genotypes including the sylvatic genotype<sup>5</sup>. The Genotype IV<sup>24</sup> (Cosmopolitan

genotype) having a wide geographical distribution and including majority of Indian DENV-2 strains circulating in northern India such as Delhi, Lucknow and Gwalior<sup>8,25</sup>; Gujarat and Haryana replaced the Genotype V (American genotype) of DENV-2 that predominantly circulated in India during the pre-1971 period. In Delhi, till 2003, the predominant serotype was DENV-2<sup>26</sup>. DENV-2 has also been reported from Southern India, in Kerala, along with DENV-3<sup>27</sup> and also in Chennai, Tamil Nadu (GenBank accession numbers: KT149472 and KT253151). The present study showed that the dominant serotype of DENV in Karnataka during the period 1993-2005 was DENV-2 of a single genotype (Genotype-IV). Many of the Karnataka sequences analyzed formed a separate cluster with a high bootstrap support value (82%) that was most likely due to the presence of a conserved amino acid T102. Overall, the present results revealed the involvement of two dengue serotypes, type 1 and 2, in Karnataka. The strains circulating in the State were genetically similar to those circulating in the other States of India during similar time periods. Further studies including continuation of DENV surveillance in the State are warranted to get the complete picture of the circulating serotypes and note any shift in dominance of these serotypes/genotypes.

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