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Letter to the Editor

Emergence of Dengue virus 4 genotypes II b and I in the city of Rio de Janeiro

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Dengue virus (DENV) is an arthropod-borne virus (arbovirus) within the family *Flaviviridae* and consists of four serotypes (DENV-1, DENV-2, DENV-3 and DENV-4). Human infection with DENV result in a spectrum of manifestations ranging from asymptomatic and subclinical (70% of all DENV infections) to dengue fever (DF) or dengue hemorrhagic fever (DHF).¹ There are an estimated 100 million infections occurring annually worldwide, of which ~500,000 are severe life-threatening infections.¹ The principal risk factors for DHF include the strain of virus, prior infection with a heterologous serotype, age and genetic background of the individual.² In Brazil, outbreaks of DF caused by DENV-1 occurred since the early 1980s of the last century and the number of DHF cases increased after 1994, as a result of the introduction of DENV-2 and DENV-3 and the spread of the main vector *Aedes aegypti*.³ DENV-4 was first detected in 1982 in the Amazon region of Brazil, co-circulating with DENV-1.⁴ However, DF or DHF caused by DENV-4 were not observed in Brazil until 2008, when three patients in the Amazon region suffered from DF caused by DENV-4 genotype I.³ In 2010, DF caused by DENV-4 genotype II was reported from the city of Roraima in the north of Brazil.⁵ During an outbreak of DENV-1 in the city of Niteroi (state of Rio de Janeiro) in March 2011, DENV-4 was detected in 7 patients. Thus, active DENV surveillance was implemented in the city of Rio de Janeiro in order to detect and characterize all DENV serotypes. Therefore, 10% of febrile patients that were assisted at health units designated as sentinels were tested for the presence of DENV NS1 antigen in serum (PlateliaTM NS1 Ag, Biorad, France). Reactive serum samples were further used to isolate DENV in C6/36 cells (ATCC CRL-1660) followed by RT-PCR and nucleotide sequencing in order to characterize the DENV strains.⁶ As a result of this surveillance, the first case of DENV-4 infection was detected in December 2011 in a febrile patient from the district of Padre Miguel,

west region of Rio de Janeiro. Since then, the number of cases of DF started to increase in the city of Rio de Janeiro and also in the state, reaching 10,042 laboratory confirmed cases until August 2012. During this period, DENV-1 was also found in the city of Rio de Janeiro. However, only 32% of DENV infections serotyped by Rio de Janeiro State Laboratory were caused by DENV-1. The majority of DENV infections were caused by DENV-4. Therefore, 10 selected DENV-4 isolates were characterized in more detail. Phylogenetic analysis of these 10 DENV-4 strains revealed the presence of DENV-4 genotype II b during the outbreak (Fig. 1) in the city of Rio de Janeiro. The DENV-4 genotype II b strains were closely related to strains detected in the city of Roraima in 2010 (strain Br246RR) and in the state of São Paulo State in 2011 (strain SPH317947).^{5,7} Moreover, DENV-4 genotype II strains have been also found to circulate in the neighboring countries of Brazil. Our strains are most closely related to those strains from Venezuela and Colombia (Fig. 1). Interestingly, one of our DENV-4 strains (RJ1243581) clusters with DENV-4 genotype I strains in our phylogenetic tree and network and is closely related with the strains AM1619 and AM750 from the city of Manaus (Fig. 1). Since the first detection of genotype I in Manaus in 2008 no other reports of this genotype in South or Central America have been published. Most likely, this DENV-4 genotype was introduced from Asia into Brazil.⁸ However, we cannot rule out the silent introduction of this genotype from the neighboring countries into Brazil. Traditionally, the E gene is the most frequently used gene to perform DENV genotyping. However, other genes were demonstrated to be suitable targets for genotyping as well.⁹ We sequenced a 322 nucleotide fragment covering partial regions of the C and prM genes. The sequence analysis resulted in the construction of a stable and reliable phylogenetic tree and network. The emergence of DENV-4 in the state of Rio de Janeiro in March 2011 forced us to implement a powerful DENV surveillance program in the city of Rio de Janeiro that led to the fast and reliable identification of DENV-4 infections in December 2011. The characterization of the circulating DENV-4 genotypes might help to improve our understanding of how DENV-4 was introduced and spread in the region. Moreover, DENV-4 maintenance in the environment and the possible association between a specific DENV-4 genotype and the severity of DF will be in the focus of our surveillance program. In addition, studies to perform an active mosquito-based surveillance for DENV and other arboviruses are in progress in the city of Rio de Janeiro. This might provide data about arbovirus activity and distribution before an outbreak occurs.¹⁰

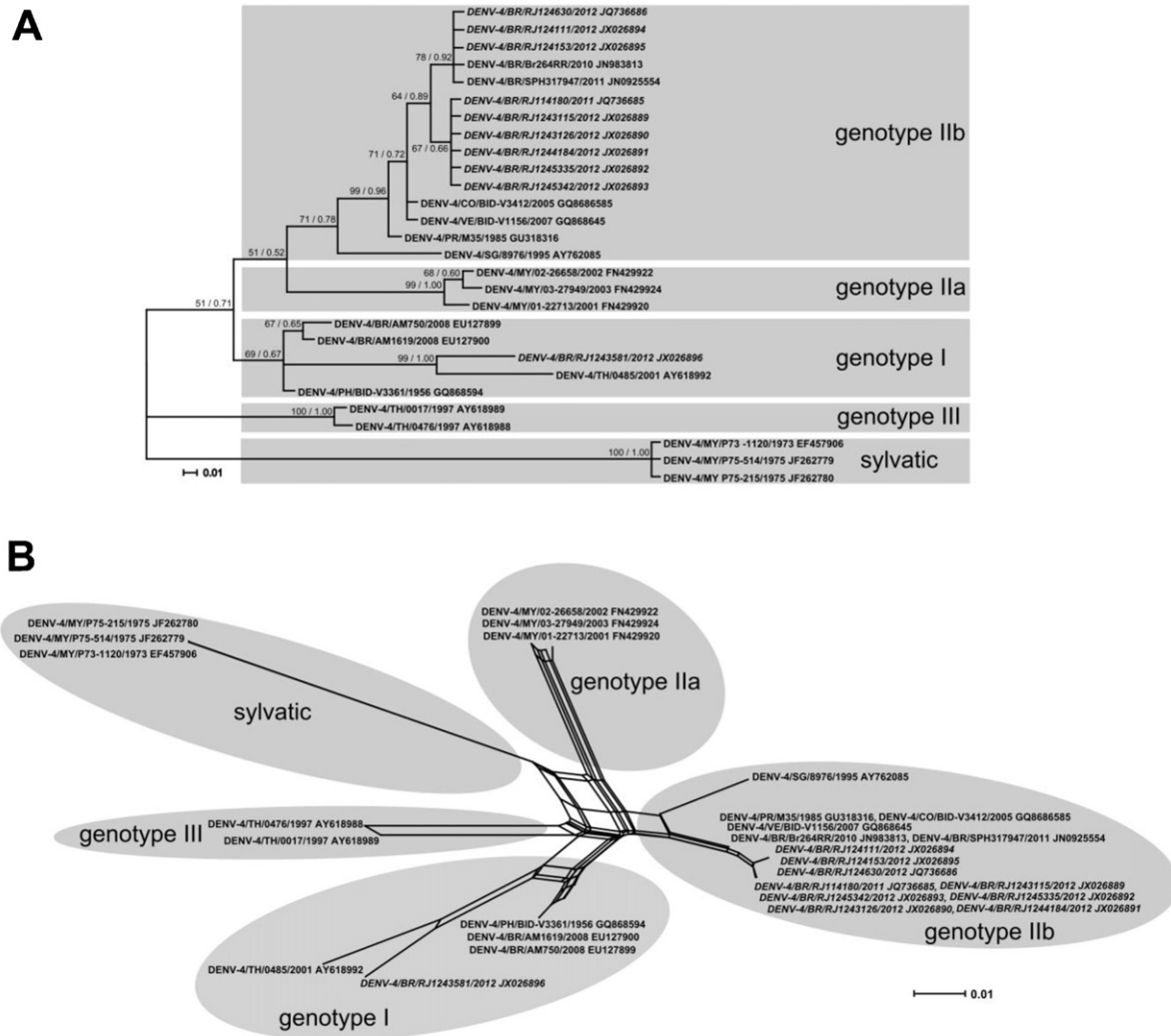


Fig. 1. Phylogenetic analysis of Dengue virus 4 (DENV-4) strains. The Bayesian phylogenetic tree (A) and a Neighbor-net network (B) were constructed by using partial nucleotide sequence of the C and prM gene (length, 322 nucleotides) of DENV-4 strains. For each sequence used, the standardized DENV strain nomenclature and GenBank accession number are shown. The multiple nucleotide sequence alignment was analyzed by the Markov Chain Monte Carlo method implemented in the program MrBayes (version 3.0) applying the general time-reversible (GTR) substitution model.¹¹ Posterior probabilities are shown at the nodes of the tree to the right of the vertical divider. In addition, the alignment was analyzed by the neighbor-joining method, resulting in the same tree topology (not shown). Bootstrap values >50%, calculated from 10,000 replicates are indicated at the nodes of the tree to the left of the vertical divider. The branch length is proportional to evolutionary distance (scale bar). The newly described DENV-4 sequences from the city of Rio de Janeiro are shown in italics.

Conflict of interest

The authors do not have commercial or other associations that might pose a conflict of interest (e.g., pharmaceutical stock ownership or consultancy).

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