

Autochthonous Japanese encephalitis with Yellow fever coinfection in Africa

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To the Editor: Japanese encephalitis virus and yellow fever virus are mosquito-borne flaviviruses that circulate in disjunct geographic areas with different mosquito vectors. Japanese encephalitis is endemic to most of Asia and the Western Pacific, whereas yellow fever occurs in tropical areas of Africa and South America. Both viruses lead to a wide spectrum of disease severities that include asymptomatic infection and mild illness with influenza-like symptoms. However, severe yellow fever disease can be fatal in 20 to 60% of cases¹, whereas symptomatic Japanese encephalitis virus can progress to severe encephalitis, with case fatality rates of up to 30%. Survivors often have long-term neuropsychological sequelae². In March 2016, during the yellow fever outbreak in Angola, a 19-year-old man was admitted to the Cunene Provincial Hospital with a 5-day history of fever, headache, and jaundice. The patient, who survived, worked in the capital city of Luanda at the onset of disease and had not traveled abroad. A blood sample was obtained, and a test for yellow fever virus was positive. The sample was later processed for high-throughput RNA sequencing. Because the protocol followed made use of randomly primed cDNA synthesis, it provided a more complete and quantitative view of all RNA present in the sample than would have otherwise been possible, enabling the characterization of potential coinfecting viruses³. Surprisingly, *de novo* assembly of the sample revealed a Japanese encephalitis virus genome in addition to the expected yellow fever virus genome. Phylogenetic analysis revealed that this Japanese encephalitis virus variant belongs to genotype III, clustering closely with variants sampled in Asia (Fig. 1A, and Supplementary Appendix). The yellow fever virus sequence was closely related to both a sequence from the 1971 yellow fever virus outbreak in Angola and the recently reported yellow fever virus sequence detected in a sample from a Chinese traveler returning from Angola⁴ (Fig. 1B). The likelihood of sample contamination is small, since neither the Institut Pasteur of Dakar, Senegal, where the RNA extracted from the samples in Angola was delivered, nor the laboratory at the Institut Pasteur, Paris, where the libraries were constructed and sequenced, has ever had any material containing Japanese encephalitis virus. In addition, none of the 15 additional RNA samples from patients with yellow fever virus who were treated at the same time provided reads that

corresponded to Japanese encephalitis virus. Leftover sera from the Angola samples were later extracted at Institut Pasteur of Dakar, and only sample C17 was positive for Japanese encephalitis virus RT-qPCR. We found evidence of locally acquired infection with Japanese encephalitis virus in Africa in addition to coinfection with yellow fever virus, which raises the issue of the risk of circulation of the Japanese encephalitis virus and human infection in Africa. Since both the vectors and suitable hosts (e.g., pigs) for Japanese encephalitis virus are present in Angola, more studies of this virus in this locality, including serosurveillance, are needed. Increased levels of population movement between Asia and Africa may provide opportunities for pathogens to expand their geographic range. These data also highlight the potential usefulness of high-throughput sequencing, particularly untargeted approaches, for pathogen surveillance⁵.

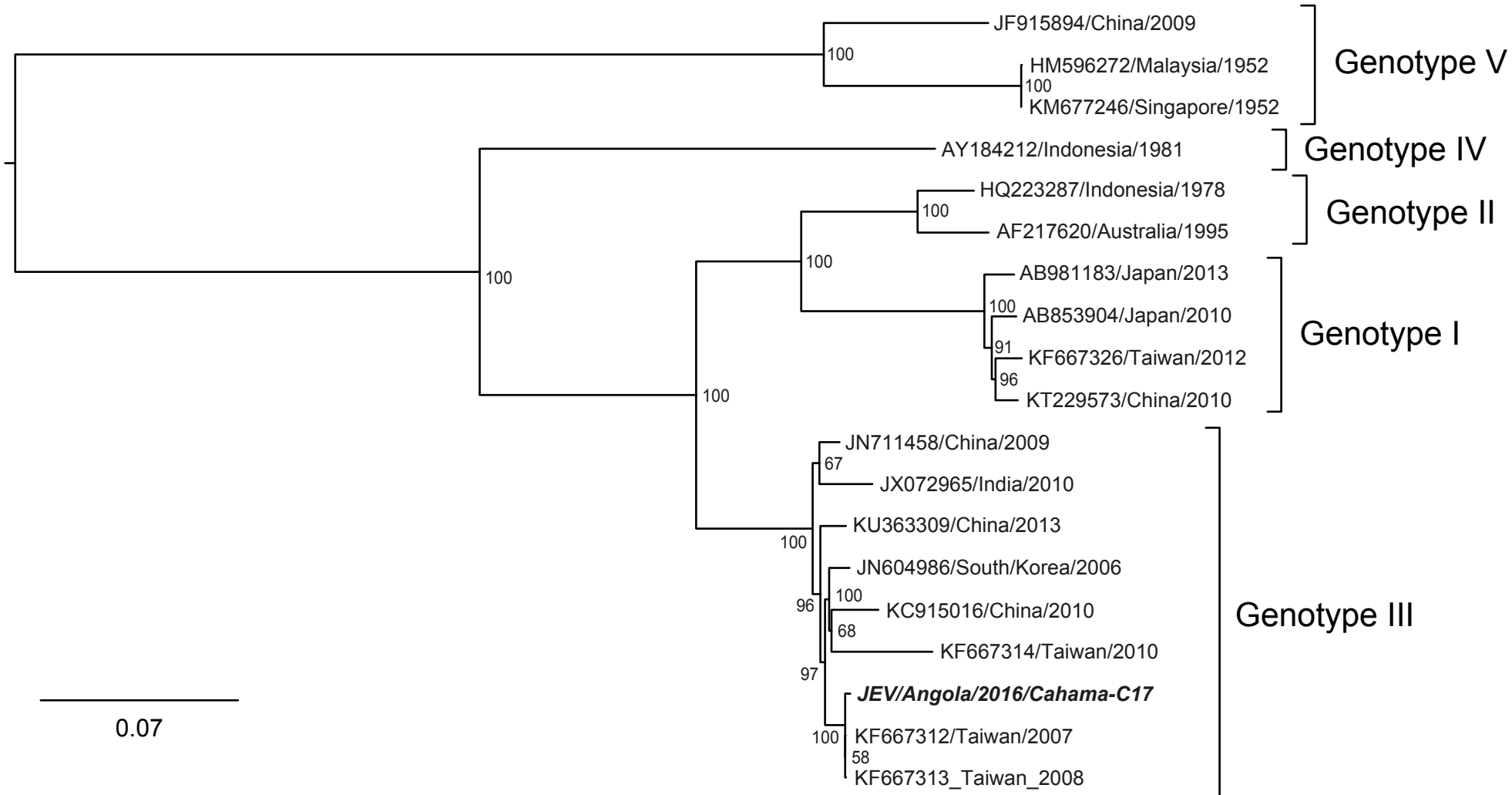
Figure legends

Figure 1. Maximum likelihood phylogenetic trees of representative full-length (A) JEV and (B) YFV sequences. The isolates newly sequenced here are shown in bold, italic font. Viruses are identified by GenBank accession numbers, country of sampling and isolation date and viral genotypes are indicated. All horizontal branch lengths are scaled to the number of nucleotide substitutions per site and the trees were mid-point rooted for clarity only. Bootstrap values are shown for key nodes.

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A



B

