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## Group IV Getah Virus in *Culex* Mosquitoes, Malaysia

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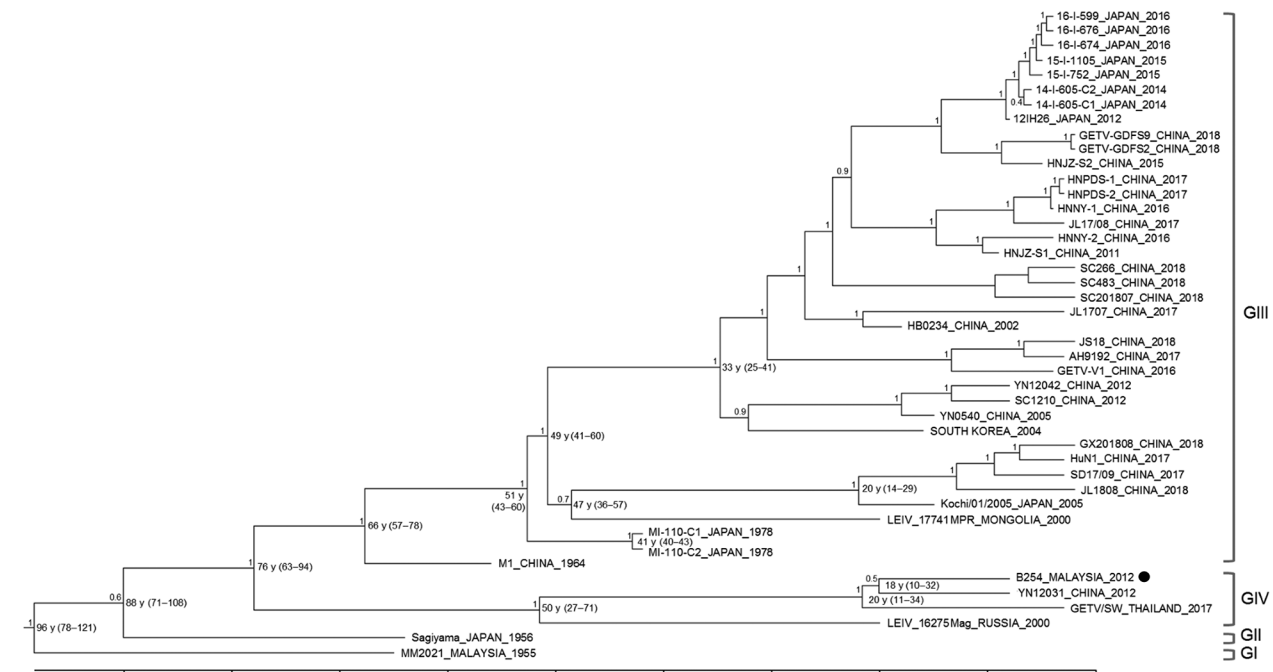
A new Getah virus (GETV) strain, B254, was isolated from *Culex fuscocephalus* mosquitoes captured at Mount Ophir, Malaysia, in 2012. Phylogenetic analyses revealed that GETV B254 is distinct from the old Malaysia GETV MM2021 strain but closely related to group IV GETV from Russia (LEIV16275Mag), China (YN12031), and Thailand (GETV/SW/Thailand/2017).

Getah virus (GETV) is an emerging mosquito-borne alphavirus of the family *Togaviridae*. The virus was first reported in 1955 from *Culex gelidus* mosquitoes collected near Kuala Lumpur, Malaysia (1). Serologic evidence of GETV infection was found in various large domestic mammals (2) and humans (3). GETV infections in these populations, however, were mostly inapparent. In other regions, reproductive failures in pregnant sows, death in piglets, and hind limb edema in racehorses, as well as neurologic symptoms and death in blue foxes, have been reported (4–7).

We conducted mosquito surveillance in the forests of 4 different states in Peninsular Malaysia, Perak, Pahang, Selangor, and Johor, during 2011–2014. We captured a total of 4,160 mosquitoes from the study sites by BG-Sentinel CO<sub>2</sub> trap (Biogents AG, <https://eu.biogents.com>) with the addition of UV light and sorted them into 208 pools according to their species, determined by morphologic keys, and collection sites. We homogenized the pooled mosquitoes, inoculated

them into C6/36 mosquito cells, and blind passaged for 5 passages. We obtained cytopathic effects from 1 of the pools on day 4 postinfection during the last passage. The pool comprised *Cx. fuscocephalus* mosquitoes collected from the forested area at Mount Ophir (Malay: Gunung Ledang) in Johor in 2012. We screened the culture supernatants by reverse transcription PCR (RT-PCR) using the in-house developed alphavirus generic primers targeting the nsP4 gene and flavivirus primers targeting the nonstructural 5–3' untranslated region junction. A similar sample pool that showed cytopathic effects was found positive for the presence of alphaviruses, as indicated by a 683 bp amplicon. Sanger sequencing of the amplicon revealed high sequence similarity to GETV. We obtained the complete coding sequence of the GETV isolate, designated as B254, using 10 pairs of RT-PCR amplification primers and genome sequencing. We deposited the genome in GenBank (accession no. LR990838).

We constructed a maximum clade credibility tree using the Bayesian Markov chain Monte Carlo analysis based on the complete coding regions of GETV B254 and other GETV isolates available in GenBank (Figure). The GETV phylogeny revealed 4 major groups of viruses: group I (GI), GII, GIII, and GIV (8). GI and GII consisted of early identified GETV isolates; GI contained the Malaysia MM2021 isolate (1955), and GII, Japan Sagiya (1956). The GIII and GIV comprised the



**Figure.** Maximum-clade credibility tree of complete coding sequences of Getah virus (GETV) strain B254 from Malaysia (black dot) and reference strains. Horizontal branches are drawn to a scale of estimated year of divergence. Times to the most recent common ancestor with 95% highest posterior density values (ranges in parentheses) are shown at nodes. Posterior probability values  $\geq 0.6$  of key nodes are shown. G, group.

most recent circulating virus strains. The newly isolated Malaysia GETV B254 is phylogenetically distinct from the ancestral Malaysia MM2021. It clustered within the GIV clade, which included the strains recovered in Russia (LEIV16275Mag, 2007), China (YN12031, 2012) and Thailand (GETV/SW/Thailand/2017). The B254 strain showed the highest nucleotide sequence homology with YN12031 (98.8%), isolated from the *Armigeres subalbatus* mosquito, and GETV/SW/Thailand/2017 (98.6%), isolated from pig serum.

Currently, only the GIII GETV lineage is associated with epidemic outbreaks. The GIV GETV, however, consisted of only a few virus strains. We calculated the mean evolutionary rate of GIV GETV at  $4.10 \times 10^{-4}$  substitutions/site/year and the rate for GIII at  $2.98 \times 10^{-4}$  substitutions/site/year. These results suggest that the GIV viruses may be under a different selection pressure, potentially involving a different host. It is also possible that GIV viruses are maintained in a yet-to-be-identified enzootic transmission cycle involving mosquitoes and asymptomatic hosts.

The *Culex* mosquitoes, particularly *Cx. gelidus* and *Cx. tritaeniorhynchus*, have been identified as the most predominant vector host for GETV in Malaysia (1,2). *Cx. fuscocephalus* mosquitoes, however, are not an uncommon vector; they have been known to carry the virus in China and Sri Lanka (9,10). *Cx. fuscocephalus* mosquitoes could be an emerging vector for GETV transmission in Malaysia along with the other *Culex* mosquitoes.

The high similarity of genome sequences of GIV GETV strains recovered thousands of kilometers apart raised the possibility that the virus may share a common dispersal route. One possible route linking the virus is the East Asian–Australasian flyway of migratory birds. GIV GETV could have been introduced to the regions through the migratory birds along the flyway, which included Malaysia as one of the stopover sites; the abundantly available *Culex* mosquitoes at the roosting sites could have maintained the transmission.

In summary, we identified a new GETV strain, B254, in Malaysia that is phylogenetically distinct from the old Malaysia MM2021 strain. The virus strain shares high similarities to the widely distributed GIV GETV. Although further surveillance studies are needed for confirmation, this finding suggests that GIV GETV strains could share a common dispersal origin, possibly through the bird trans migratory flyways.

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