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GENOME SEQUENCES

Complete Coding Sequences of 22 East/Central/South African Genotype Chikungunya Virus Isolates from Thailand (2018 to 2019)

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ABSTRACT The coding-complete genome sequences of 22 chikungunya virus strains collected from the 2018–2019 outbreak in Thailand are reported. All sequences belong to the East/Central/South African (ECSA) genotype and contain two mutations, E1:K211E and E2:V264A, which were previously shown to be associated with increased viral infectivity, dissemination, and transmission in *Aedes aegypti*.

Chikungunya virus (CHIKV) is a mosquito-borne alphavirus, belonging to the family *Togaviridae*, that causes febrile illness associated with high fever, rash, and arthralgia (1). CHIKV is considered a global threat to public health (2). Currently, there are no licensed vaccines or treatments (3).

A 2008–2009 CHIKV outbreak in southern Thailand was caused by an East/Central/South African (ECSA) genotype CHIKV strain harboring the E1:A226V mutation (4). This mutation is associated with increased viral transmission in *Aedes albopictus* (5). The most recent CHIKV outbreak in Thailand was reported in 2018 to 2019 (6, 7). To investigate the CHIKV genomes from this outbreak, we sequenced 22 existing CHIKV isolates from Thailand. The CHIKV isolates were previously propagated in C6/36 cell lines (8). RNA was extracted from the culture supernatant using the QIAamp viral RNA minikit (Qiagen). Seven primer pairs were designed (available upon request) and used to amplify the near-complete genome in seven overlapping fragments using the SuperScript III One-Step reverse transcription-PCR (RT-PCR) system with Platinum *Taq* high-fidelity DNA polymerase (Invitrogen). The fragments were pooled at the same concentration (10 ng/μl). DNA libraries were constructed using the QIAseq FX DNA library preparation kit (Qiagen) and subjected to paired-end sequencing on a MiSeq system using the MiSeq reagent kit v2 (2 $\times$ 250 nucleotides [nt]). A total of 48 million reads (1.2 to 2.6 million reads per sample) between 100 and 250 nt long were generated. Raw sequence reads were trimmed and mapped to a reference genome (GenBank accession number MN630017), and a consensus sequence was generated using ngs_mapper v1.2.4 (https://doi.org/10.5281/zenodo.594756). We obtained a mean coverage of $\geq$500$\times$, and the GC contents ranged from 50.2% to 50.8%.

The assembled genome sequences obtained ranged from 11,376 to 11,811 nt, consisting of two open reading frames (ORFs) and 5′ and 3′ untranslated regions (UTRs). The phylogenetic analysis (Fig. 1) revealed that all new sequences belong to the ECSA genotype and are in the same group as those with GenBank accession numbers

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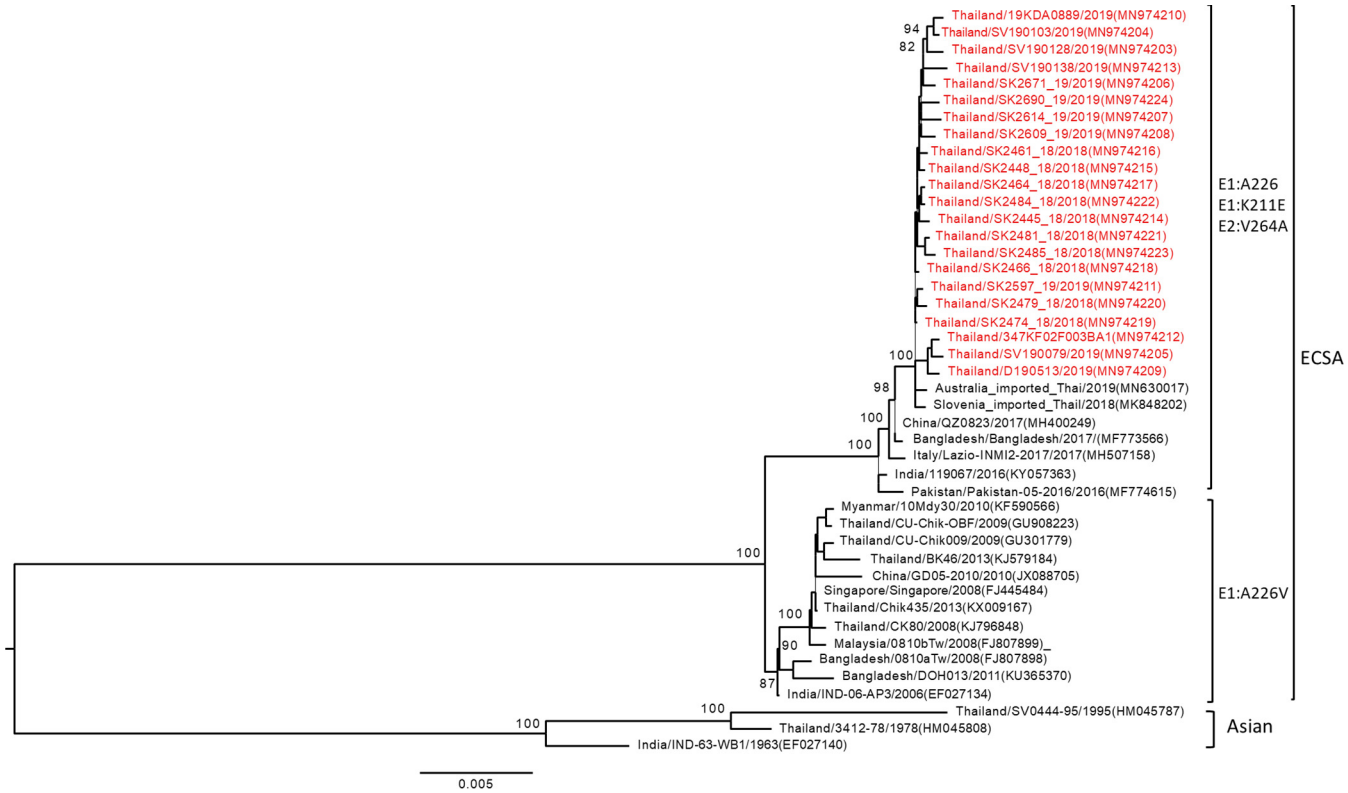

FIG 1 Maximum likelihood phylogenetic tree of 44 CHIKV genome sequences (excluding 5′ and 3′ UTRs) determined using IQ-TREE v1.6.12 and nucleotide substitution model GTR+F+R2 (14). Multiple sequence alignments were performed using Multiple Alignment using Fast Fourier Transform (MAFFT) v7.407 with default settings (15). The phylogenetic tree was drawn with FigTree v1.4.4. The two CHIKV genotypes (Asian and ECSA) are shown, including grouping of 22 Thai sequences from 2018 to 2019 from this study (GenBank accession numbers MN974203 to MN974224) (red) within the ECSA genotype.

MK848202 and MN630017, which were reported as cases imported from Thailand to Slovenia (7) and Australia.

The first ORF encodes a 2,475-amino-acid (aa)-long nonstructural (NS) polyprotein that is intracellularly processed into proteins NS1, NS2, NS3, and NS4. Nineteen of our sequences (GenBank accession numbers MN974203, MN974204, MN974206 to MN974213, and MN974216 to MN974224) contain an opal readthrough termination codon between NS3 and NS4 (TGA at nucleotide positions 5645 to 5647). The other 3 sequences (GenBank accession numbers MN974205, MN974214, and MN974215) contain CGA at the same positions, encoding arginine as the 1,857th amino acid. The opal codon is important for viral maintenance in vertebrate and invertebrate hosts (9), as well as downstream translation (10).

The second ORF is a 1,249-aa-long structural polyprotein coding for proteins C, E3, E2, 6K, and E1. The mutation E1:A226V was found in none of the new sequences. Instead, the E1:K211E and E2:V264A mutations were found in all new sequences. These mutations were previously found in India and Kenya in 2016 (11, 12) and in Thailand in 2018 (13). These findings suggest that CHIKV strains might have been introduced into Thailand, potentially initiating the epidemics.

Data availability. The 22 assembled CHIKV sequences were submitted to GenBank (accession numbers MN974203 to MN974224). The raw reads were deposited in the SRA (accession numbers SAMN13926320 to SAMN13926341).

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