

Genetic characterization of chikungunya virus in field-caught *Aedes aegypti* mosquitoes collected during the recent outbreaks in 2019, Thailand

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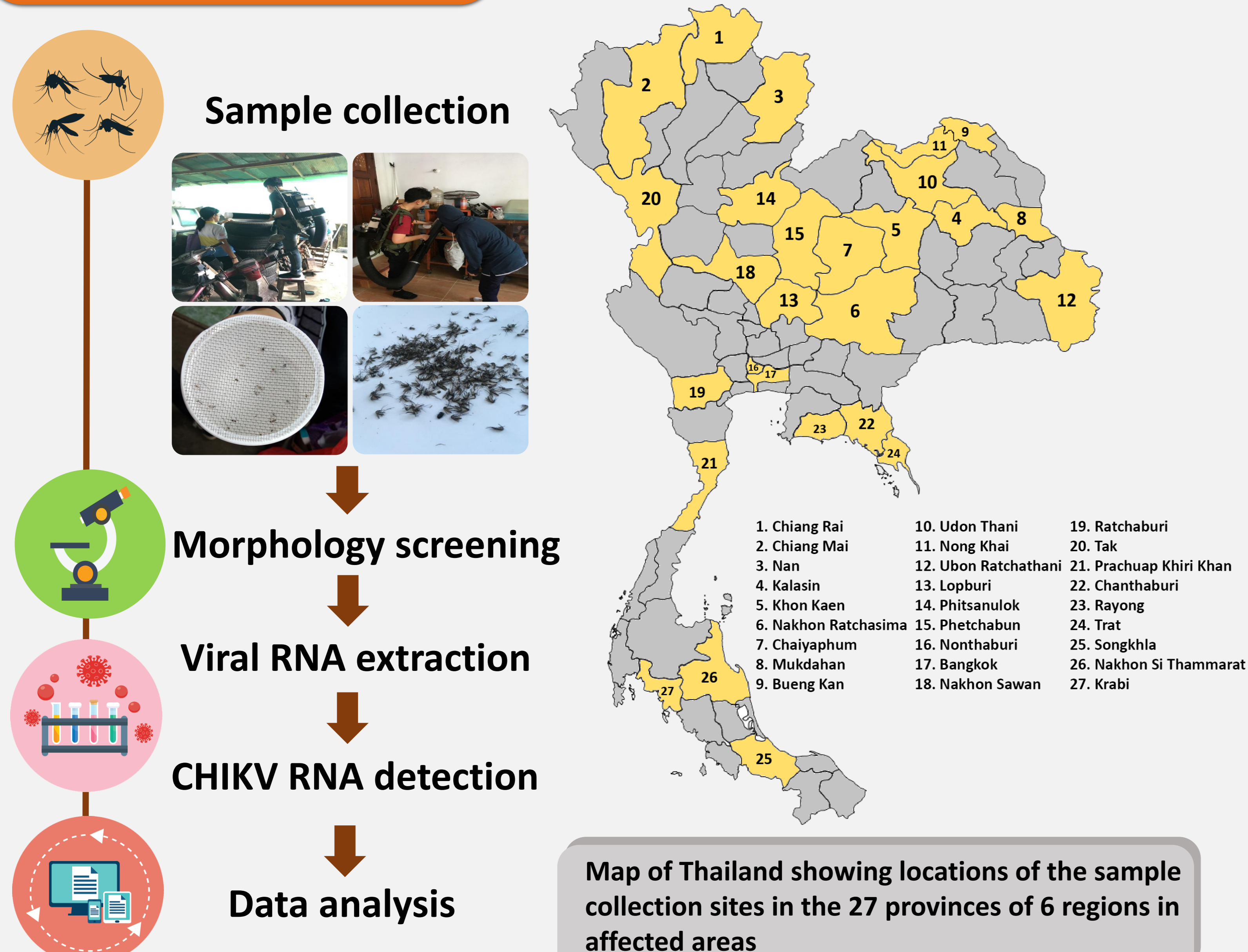
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Abstract Chikungunya virus (CHIKV) is a mosquito-borne virus belonging to the genus *Alphavirus*. The virus is transmitted to humans by the bite of infected female *Aedes* mosquitoes, primarily *Aedes aegypti*. Recently, there have been 3,794 chikungunya cases in 27 provinces reported by the Bureau of Epidemiology of Health Ministry, Thailand during 1 January-16 June 2019; however, the cause of the re-emergence of CHIKV outbreaks is uncertain. Therefore, the aims of this study were to detect and analyze the genetic diversity of CHIKV infection in field-caught mosquitoes. Both female and male *Ae. aegypti* were collected from endemic areas of Thailand, and CHIKV detection was done by using E1-nested RT-PCR and sequencing analysis. A total of 1,646 *Ae. aegypti* samples (900 females and 746 males) were tested. CHIKV was detected in 54 (3.28%) and 14 samples (0.85%) in female and male mosquitoes, respectively. Seventeen samples of female *Ae. aegypti* collected from the Ubon Ratchathani, Chiang Rai, Chiang Mai, Nakhon Sawan, and Songkhla provinces found mutation at E1: A226V. Interestingly, E1: K211E mutation was observed in 50 samples collected from Nong Khai, Bangkok, Prachuap Khiri Khan, and Krabi. In addition, the phylogenetic tree indicated that CHIKV in *Ae. aegypti* samples were from the Indian Ocean Clade and East/South African Clade. Both clades belong to the ECSA genotype. The information obtained from this study could be used for prediction, epidemiological study, prevention, and effective vector control of CHIKV.

Introduction

CHIKV infection is one of the emerging and re-emerging mosquito-borne viral diseases transmitted by *Aedes* mosquitoes such as *Ae. aegypti* and *Ae. albopictus*, the Asian tiger mosquito associated with several recent large-scale epidemics. CHIKV was initially transmitted through the vector *Ae. aegypti*, whereas *Ae. albopictus* showed a mutation in the E1-A226V of CHIKV for improving transmissibility to humans. In Thailand, the re-emergence of CHIKV outbreaks in Thailand is unpredictable, and effective disease control relies on vector mosquito control measures only. It is necessary to understand the transmission cycle of CHIKV associated with mosquito vectors in endemic areas. Therefore, in this study, we focused on the detection of CHIKV infection in both female and male *Ae. aegypti* around patients' homes in different regions of Thailand by using E1-nested RT-PCR. The data could be valuable for designing effective therapeutic strategies, vector control measures, and eradication of CHIKV associated with *Ae. aegypti* mosquitoes in Thailand.

Method



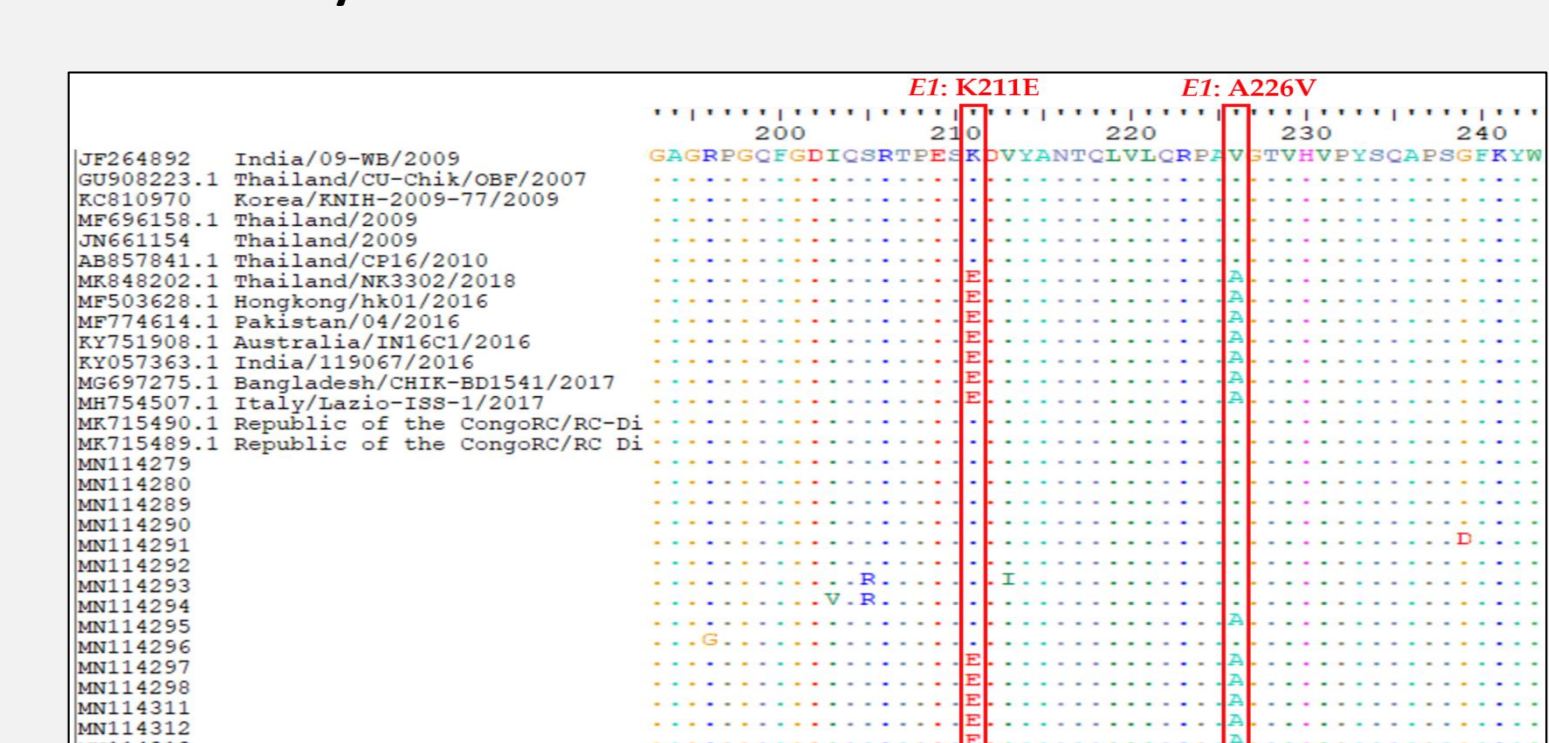
Result

Molecular detection of CHIKV RNA in *Ae. aegypti*

Chikungunya virus (CHIKV) detection in *Aedes aegypti* collected from various regions of Thailand

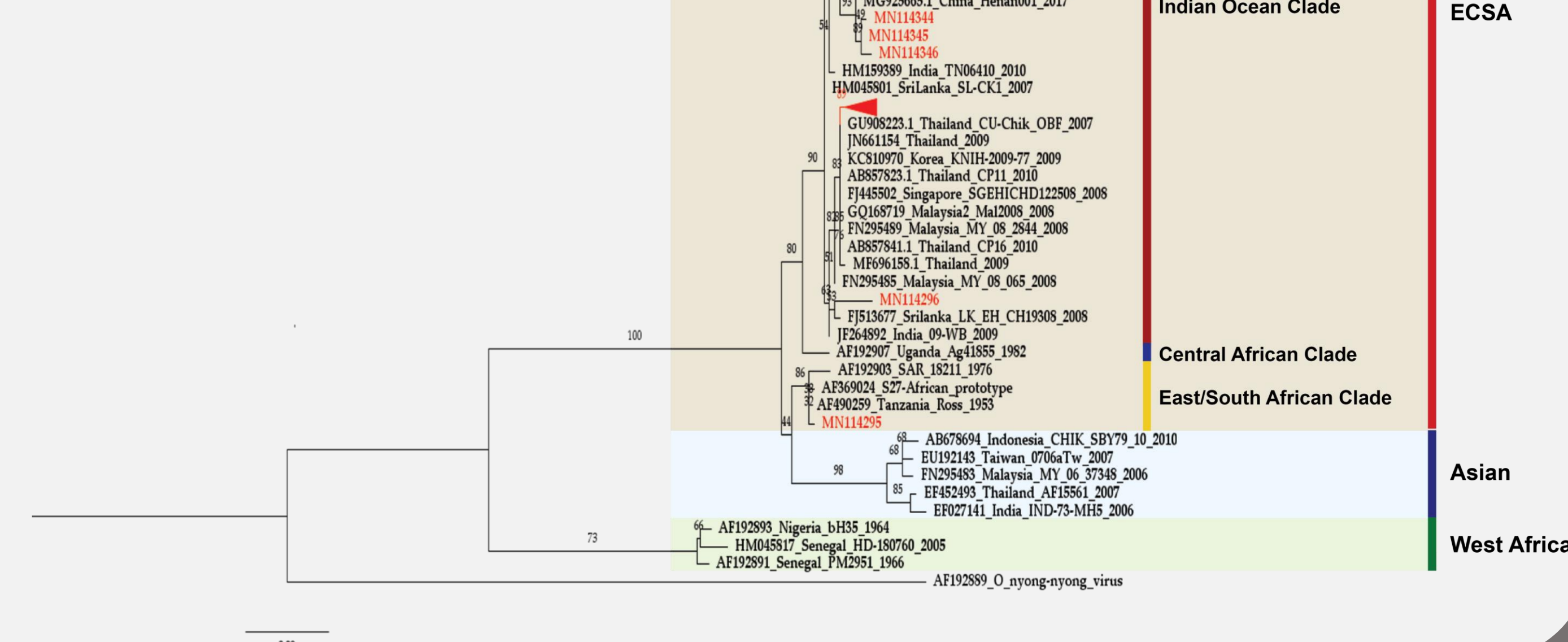
Regions	Provinces	Total Sample (n)	Chikungunya-Positive (n/total)	
			Female	Male
Northern	Chiang Rai	50	2/28	0/22
	Chiang Mai	64	2/37	0/27
	Nan	40	1/22	0/18
Northeastern	Kalasin	7	0/5	0/2
	Khon Kaen	63	0/33	0/30
	Nakhon Ratchasima	19	0/12	0/7
	Chaiyaphum	40	0/25	0/15
	Mukdahan	8	0/4	0/4
	Bueng Kan	10	0/7	0/3
	Udon Thani	60	0/32	0/28
Central	Nong Khai	258	0/145	3/113
	Ubon Ratchathani	75	10/42	0/33
	Lopburi	60	0/32	0/28
	Phitsanulok	51	0/27	0/24
	Phetchabun	51	0/32	0/19
Western	Nonthaburi	21	0/13	0/8
	Bangkok	89	11/51	3/38
	Nakhon Sawan	64	2/35	0/29
Eastern	Ratchaburi	85	0/47	0/38
	Tak	96	0/52	0/44
Southern	Prachuap Khiri Khan	93	24/46	8/47
	Chanthaburi	60	0/29	0/31
Southern	Rayong	45	0/22	0/23
	Trat	60	0/29	0/31
	Songkhla	80	1/38	0/42
	Nakhon Si Thammarat	62	0/28	0/34
Total		1646	54/900	14/746

The alignment of amino acid sequences of the E1 gene of CHIKV in *Ae. aegypti* at positions of the A226V and K211E mutations, indicated by red vertical columns



Sequencing and phylogenetic analysis

Phylogenetic tree of CHIKV in mosquitoes constructed from partial E1 sequences from all regions of Thailand



Conclusion

- CHIKV RNA was detected in female *Ae. aegypti* (3.28%) more than male *Ae. aegypti* (0.85%).
- All 17 sequences representative from this outbreak revealed E1: A226V, as observed in 2008-2010 isolates from the serum of Thai patients.
- The E1: K211E mutation was observed in 50 samples from both female and male *Ae. aegypti* collected from Nong Khai, Bangkok, Prachuap Khiri Khan, and Krabi.
- The widespread and renewed chikungunya fever epidemic in the current outbreak in Thailand may contribute to the molecular evolutionary adaptation acquired by CHIKV as a result of the A226V and K211E mutations in the E1 gene in *Ae. aegypti* in the different regions of Thailand.

References

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