Discoveries of dual-host affiliated Insect-specific flaviviruses in Zambia

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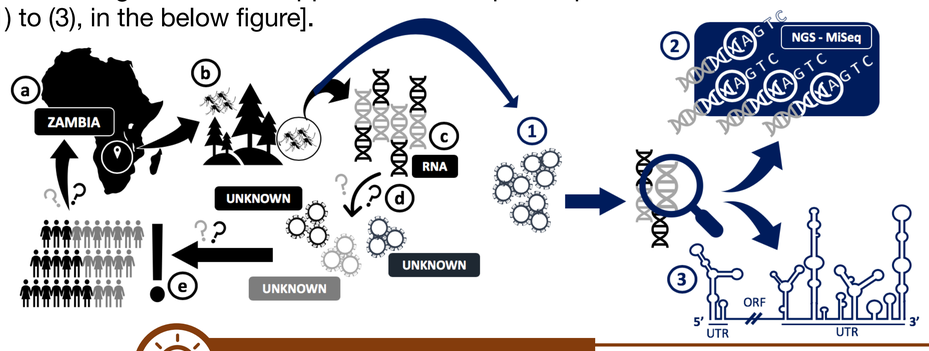
BACKGROUND

Surveillance of arboviruses in mosquito was conducted in Zambia to provide information for disease control and prevention and to make an informed decision on future disease outbreaks [(a) to (e), in the below figure]. To date, we have collected 15,198 female mosquitoes from various sites in Zambia consisting of several species. We screened for flaviviruses and virus isolation was performed on the positive samples. Whole genome sequencing was performed on the isolated viruses using Ilumina Miseq platform and rapid amplification cDNA ends method [(1) to (3), in the below figure].

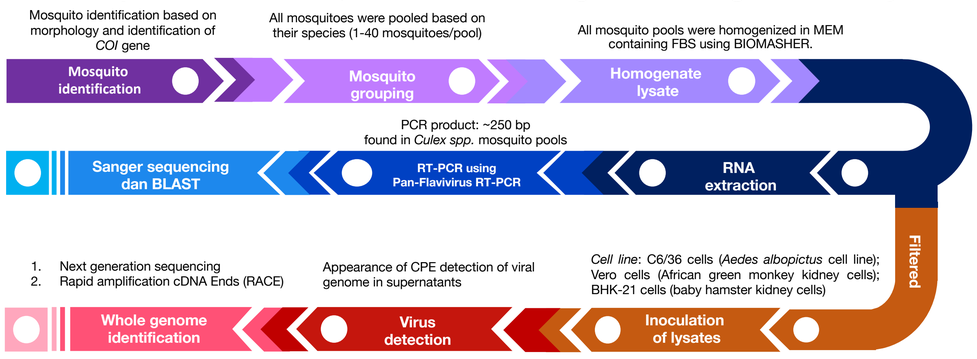


OBJECTIVES

To identify and characterize flaviviruses isolated from mosquitoes collected in several places in Zambia

MATERIALS AND METHODS

Mosquito identification, flavivirus screening and whole genome analysis



Construction of phylogenetic tree

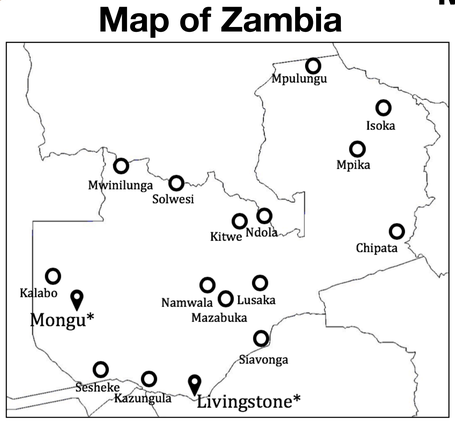
Maximum Likelihood tree was constructed based on full-genome sequence using MEGA 7 software. The phylogenetic tree was tested with bootstrap analysis with 1000 replications. All genome sequences were aligned using MUSCLE program.

Analysis of 5′- and 3′-untranslated regions (UTRs)

We used Covariance Models (CMs) to characterize and find evolutionary conserved elements in viral UTRs. CMs are statistical models that extend Hidden Markov Models (HMMs) by structure information. We built on previously published CMs for dISFVs (Ochsenreiter et al., 2019) and characterize novel elements based on structural alignments of dISFV 5'-UTR and 3'UTR regions. *RNAalifold* (from the *ViennaRNA* package) was performed to compute consensus structures of locally stable RNA elements and iteratively build novel CMs that are specific for structured RNAs in dISFVs UTRs.

RESULTS

Map of Zambia

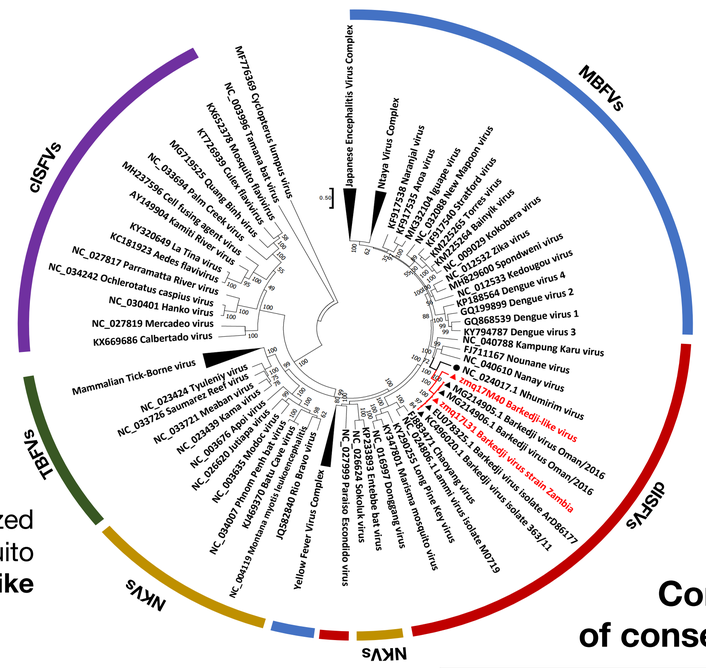


Mosquito pools and the prevalence

Year	Month	Place	Species	Mosquito no.	Pool no.	No. positive pools	NS5 gene nucleotide identity with BJV Israel
2017	April	Livingstone	*Culex quinquefasciatus*	780	34	1 (2.9%)	99%
			Culex univittatus	15	3		
			Culex nebulosus	5	3		
			Culex tigripes	11	3		
			Culex bitaenorhynchus	1	1		
			Aedes aegypti	5	5		
			Aedes spp.	7	3		
			Mansonia sp.	2	2		
			Anopheles spp.	72	15		
			Total	**898**	**69**	**1**	
2017	May	Mongu	*Culex quinquefasciatus*	285	22		
			Culex univittatus	309	15	2 (13.3%)	81%
			Culex annulioris	41	5	1 (20%)	81%
			Culex tigripes	2	2		
			Culex spp.	109	5		
			Aedes michintoshi	10	3		
			Aedes aegypti	1	1		
			Coquillettidia aurites	9	2		
			Coquillettidia metarika	7	2		
			Coquillettidia fuscopenata	46	5		
			Mansonia sp.	132	8		
			Uranotaenia sp.	1	1		
			Aedeomyia sp.	10	2		
			Anopheles spp.	1444	65		
			Total	**2406**	**138**	**3**	

3,304 female mosquitoes collected from Zambia in 2017 were pooled (n=207), homogenized and RNA extracted. We identified NS5 gene sequence of **Barkedji virus (BJV)** in four mosquito pools (table above). The full genome analysis revealed two different viruses, BJV and **BJV-like virus (BJLV)** with nucleotide identity with BJV isolate Oman 96.2% and 76.2%, respectively.

Circular phylogenetic tree of Flaviviruses



MBFVs: Mosquito-borne flaviviruses
dISFVs: dual-host insect-specific flaviviruses
cISFVs: classical insect-specific flaviviruses
NKVs: No-known vector viruses
TBFVs: Tick-borne flaviviruses

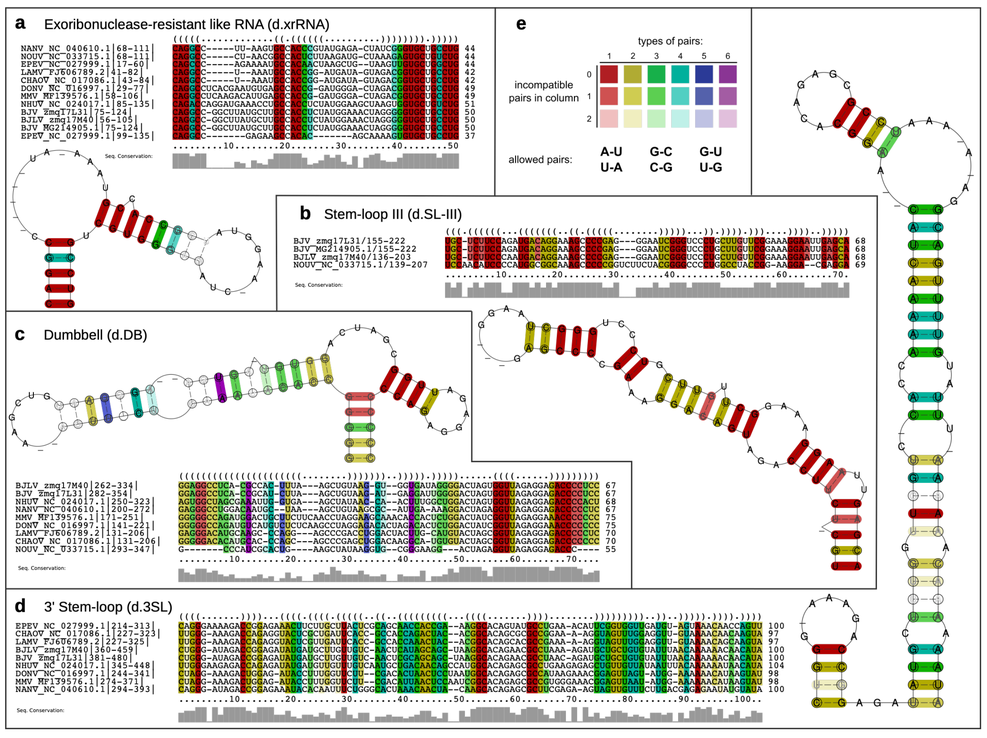
Ochsenreiter, Roman, et al. *Viruses* 11.3 (2019): 298.
Liu, Zhong-Yu, et al. *ELIFE* 5 (2016): e17636.
Ziv, Omer, et al. *Nat Methods* 15.10 (2018): 785-788.
Ng, Wy Ching, et al. *Viruses* 9.6 (2017): 137.
Kenney, Joan L., et al. *J Gen Virol* 95.0 12 (2014): 2796.

References

The importance of 5′- and 3′-UTRs for flavivirus:

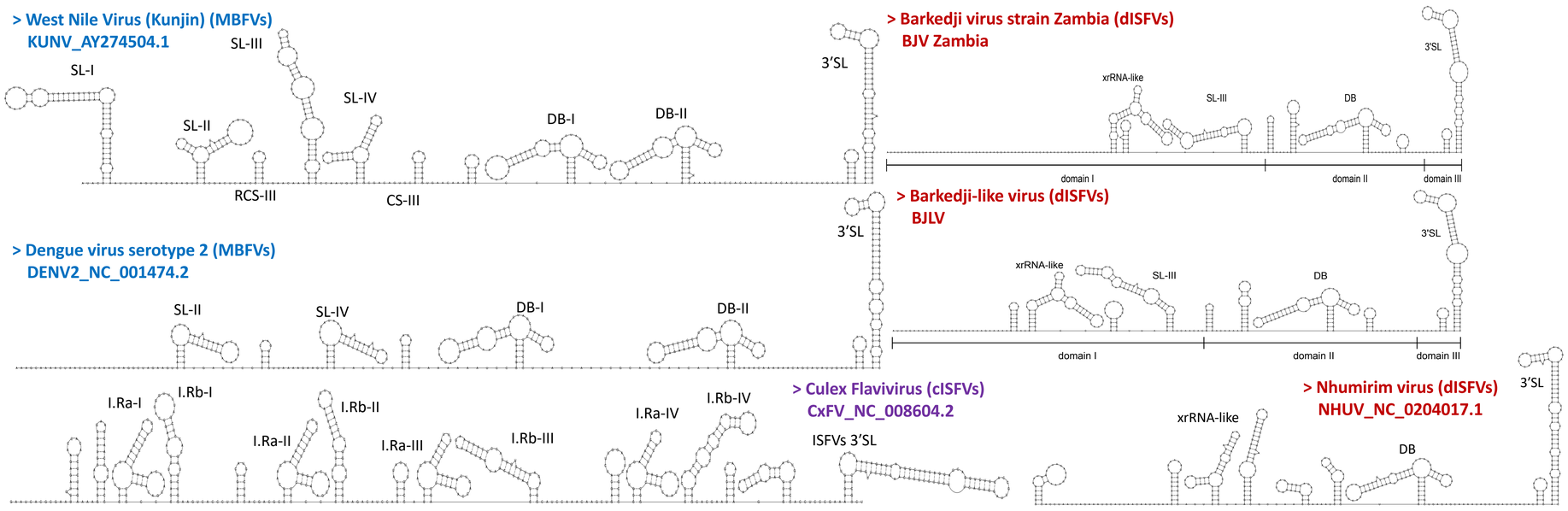
1. Facilitate the viral genome circularization for replication and translation (Liu, et al., *ELIFE,* 2016).
2. Facilitate the virus-host interaction (Ziv O, et al., *Nat Methods*, 2018).
3. Host's immune modulation by develop the sub-genomic flavivirus RNA (sfRNA) (Ng WC, et al., *Viruses*, 2017).

Consensus structure prediction of conserved elements in dISFVs 3′-UTRs

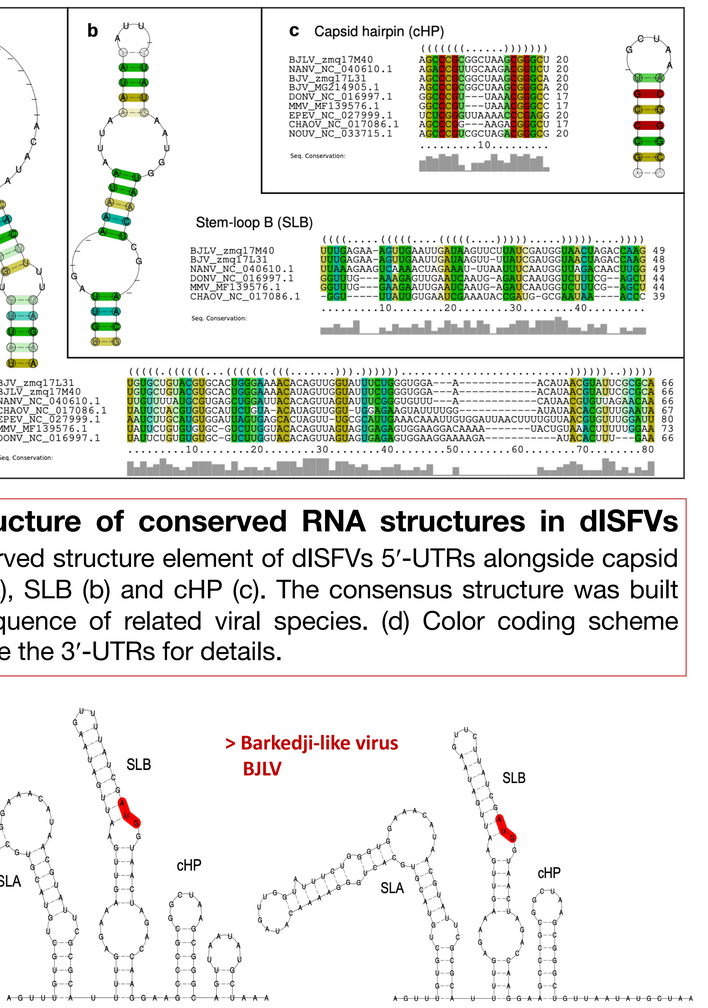


← Consensus structure of conserved RNA structures in dISFVs 3′-UTRs. The structural alignments and consensus structure of d.xrRNA (a), d.SL-III (b), d.DB (c) and d.3′SL (e). The grey bars shown below the alignment indicate the sequence conservation. (e) Coloring coding scheme used in *RNAalifold* for paired column in alignment. Circled nucleotides in the consensus structure indicate the compensatory mutations in the corresponding column of the alignment.

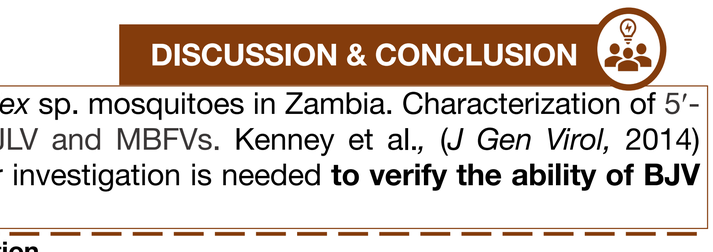
⬇Comparison of 3′-UTRs between dISFVs (BJV Zambia, BJLV and NHUV), MBFVs (KKUNV and DENV2) and cISFVs (CxFV). The 3′-UTRs of BJV Zambia and BJLV revealed homologous structures to xrRNA, SL-III, DB and terminal 3′-SL. In comparison, the secondary structure of dISFVs were more similar to MBFVs rather than cISFVs.



Consensus structure prediction of conserved elements in dISFVs 5′-UTRs



⬆Consensus structure of conserved RNA structures in dISFVs 5′-UTRs. The conserved structure element of dISFVs 5′-UTRs alongside capsid protein region, SLA (a), SLB (b) and cHP (c). The consensus structure was built from homologous sequence of related viral species. (d) Color coding scheme used in *RNAalifold;* see the 3′-UTRs for details.



⬆The 5′-UTRs prediction of BJV Zambia and BJLV. The 5′-UTRs of BJV Zambia and BJLV displayed the canonical structure of 5′-UTRs such as SLA, SLB and, as part of capsid protein nucleotide sequence, cHP. The start codon is highlighted in red.

STRUCTURE ABBREVIATION

SL: stem loop
DB: dumbbell
xrRNA-like: exoribonuclease resistance-like
RCS: repeat conserved-sequence
CS: conserved-sequence
I.Ra/I.Rb: Insect Repeat Element a/b

DISCUSSION & CONCLUSION

In this study, we identified and characterized a new strain of **BJV (10,899 nt)** and a novel flavivirus, **BJLV (10,885 nt)** isolated from *Culex* sp. mosquitoes in Zambia. Characterization of 5′- and 3′-UTRs revealed the similarity between dISFVs and MBFVs through their structure homologs, specifically for BJV Zambia, BJLV and MBFVs. Kenney et al., (*J Gen Virol,* 2014) hypothesized that dISFVs might be able to replicate in yet to be determined non-insect secondary host. Based on these results, further investigation is needed **to verify the ability of BJV and BJLV to infect and replicate mammalian host.**

COI disclosure: We declare that we have no conflict of interest in relation to this presentation