

Deciphering Flavivirus Secrets: UTRs and xrRNAs

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Article
Functional RNA Structures in the 3'UTR of Tick-Borne, Insect-Specific and No-Known-Vector Flaviviruses

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Abstract: Untranslated regions (UTRs) of flaviviruses contain a large number of RNA structural elements involved in mediating the viral life cycle, including cyclisation, replication, and encapsidation. Here we report on a comparative genomics approach to characterize evolutionarily conserved RNAs in the 3'UTR of tick-borne, insect-specific and no-known-vector flaviviruses *in silico*. Our data support the wide distribution of previously experimentally characterized exoribonuclease-resistant RNAs (xrRNAs) within tick-borne and no-known-vector flaviviruses and provide evidence for the existence of a cascade of duplicated RNA structures within insect-specific flaviviruses. On a broader scale, our findings indicate that viral 3'UTRs represent a flexible scaffold for evolution to come up with novel xrRNAs.

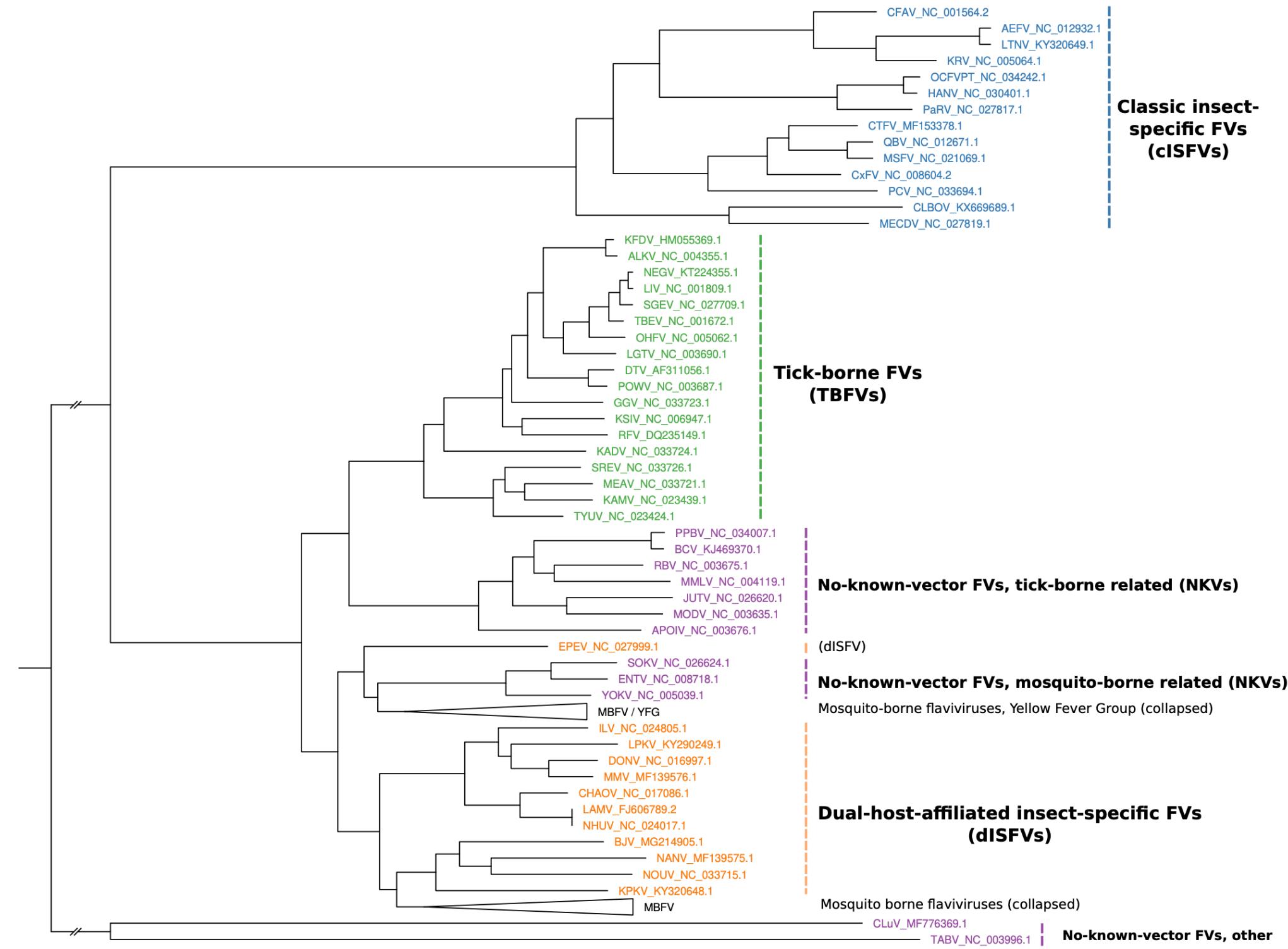
Keywords: flavivirus; non-coding RNA; secondary structure 

1. Introduction

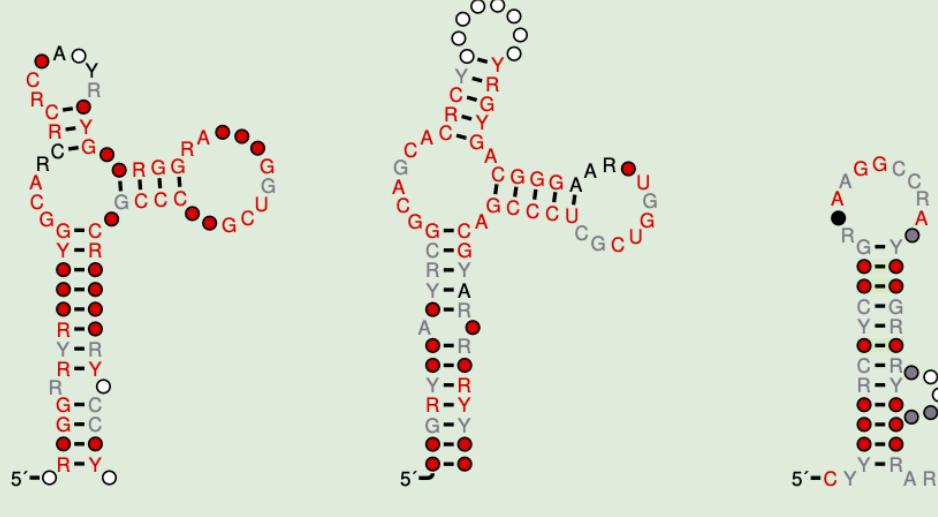
Flaviviruses are small, single-stranded positive-sense RNA viruses that are typically transmitted between arthropod vectors and vertebrate hosts. They are endemic in tropic and sub-tropic regions and represent a global health threat, although humans are considered dead end hosts in many cases. The genus *Flavivirus* within the *Flaviviridae* family comprises more than 70 species, which are organized into four groups, each with a specific host association: Mosquito-borne flaviviruses (MBFVs) and tick-borne flaviviruses (TBFs) spread between vertebrate (mammals and birds) and invertebrate (mosquitoes and ticks) hosts, while insect-specific flaviviruses (ISFVs) replicate specifically in mosquitoes and no-known-vector flaviviruses (NFKVs) have only been found in rodents and bats, respectively. This natural host-range-based classification is in good agreement with sequence-based phylogenetic clustering, mainly because all flaviviruses share a common genome organization [1]. Conversely, epidemiology, disease association [2] and transmission cycles [3] are fundamentally different among different flavivirus groups.

Emerging and re-emerging MBFVs such as Dengue virus (DENV), Japanese encephalitis virus (JEV), West Nile virus (WNV), Yellow fever virus (YFV) or Zika virus (ZIKV) are the causative agents of large-scale outbreaks that result in millions of human and veterinary infections every year [4]. Likewise, tick-borne encephalitis virus (TBEV), Powassan virus (POW) and other members of the tick-borne serocomplex are neuropathogenic agents that cause a large number of infections every year, resulting in a massive incidence increase since the 1970s [5]. Consequently, much research effort has gone into studying MBFV and TBFV biology, biochemistry and phylogeny [6]. The two remaining groups, ISFVs and NFKVs, however, have received limited attention in the research community, mainly

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a Tick-borne flaviviruses



T.xrRNA1

(TBFV xrRNA 1)

T.xrRNA2

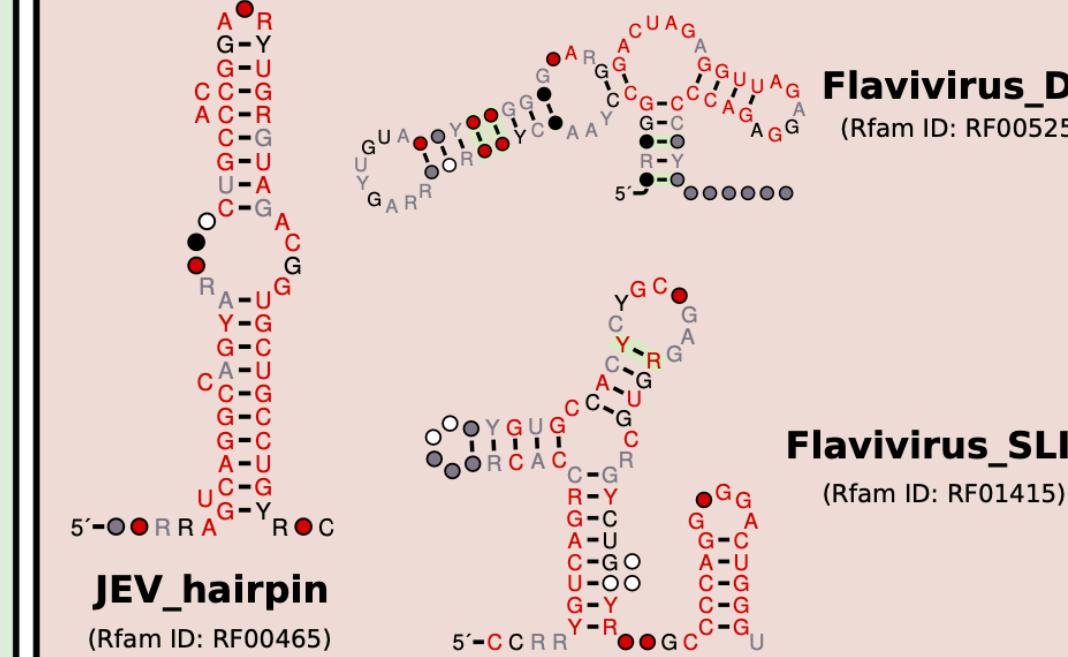
(TBFV xrRNA 2)

T.SL6

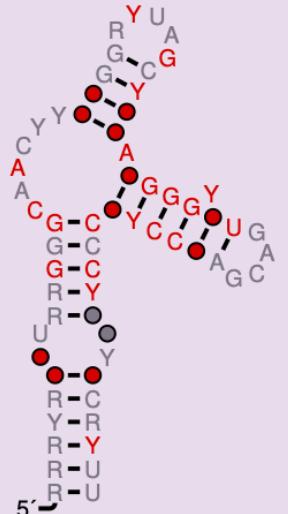
(TBFV SL-shaped element 6) (TBFV 3' terminal stem-loop)

T.3SL

d Mosquito-borne flaviviruses

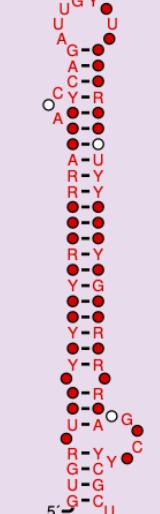


b No-known-vector FVs



N.xrRNA1

(NKV xrRNA 1)

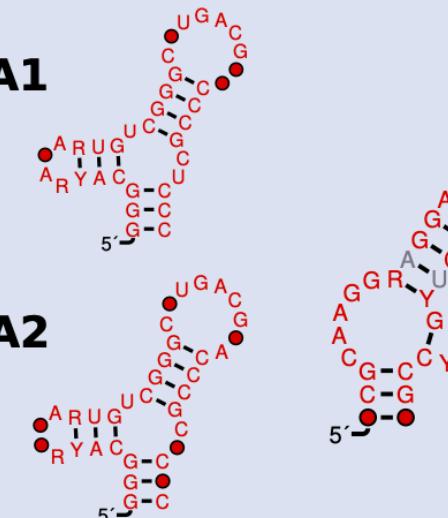


N.3SL

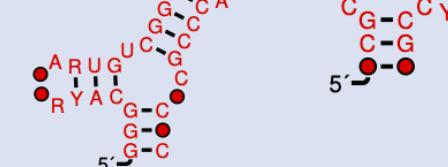
(NKV 3' terminal stem-loop)

c Classic insect-specific FVs

I.xrRNA1



I.xrRNA2



(ISFV xrRNA 1/2)

I.Ra

(ISFV repeat element a)

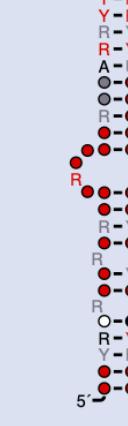
G



I.Rb

(ISFV repeat element b)

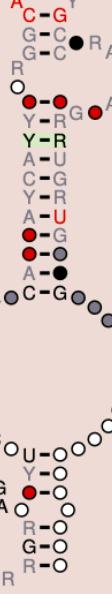
C



I.3SL

(ISFV 3' terminal stem-loop)

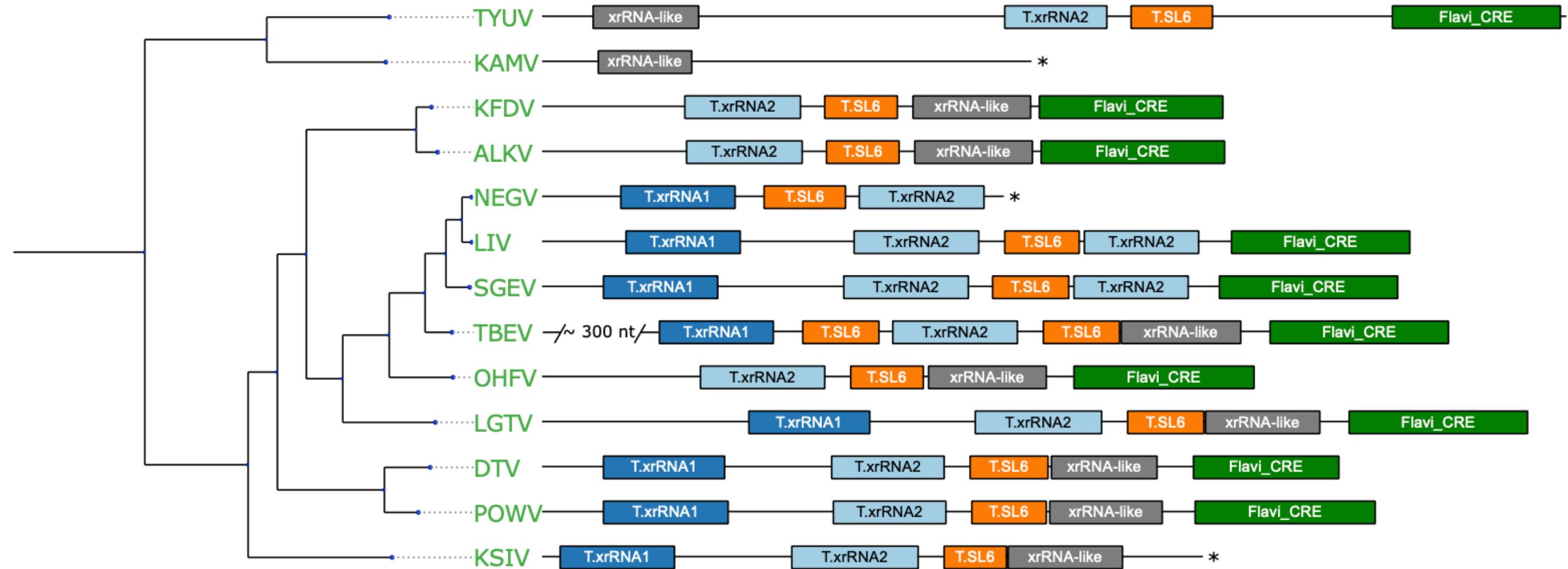
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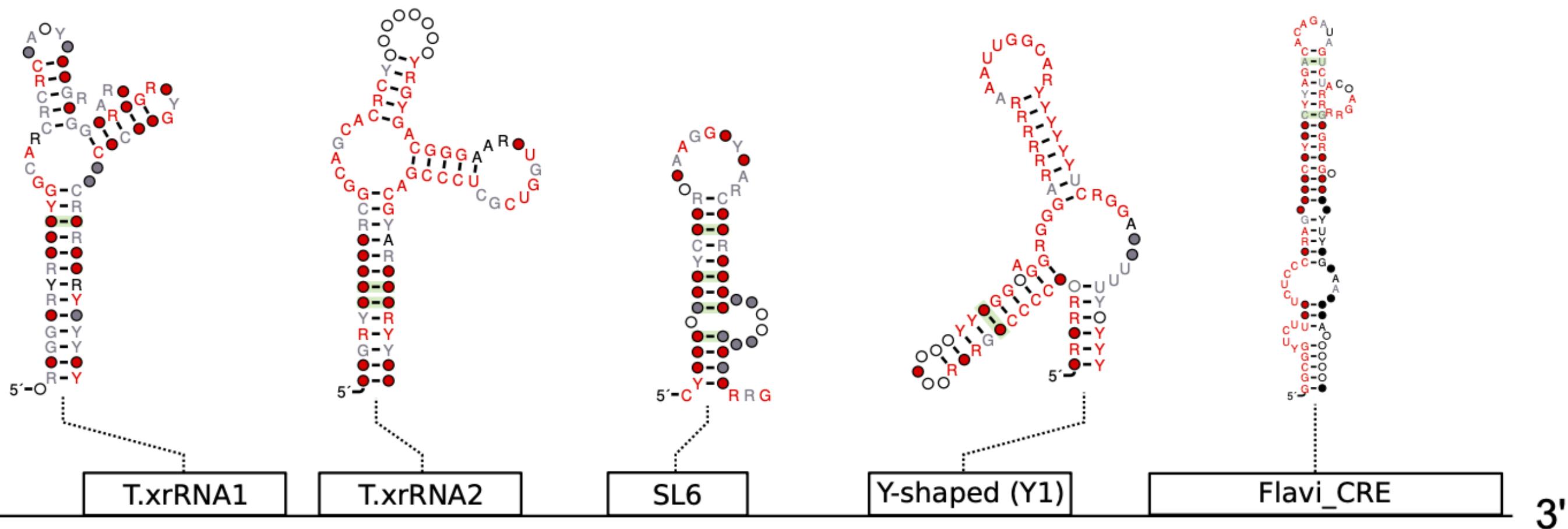


Flavi_CRE

(Rfam ID: RF00185)

a Tick-borne flaviviruses



b**c**

d**SL6**

SGEV | 261-306
 TBEV | 532-577
 LIV | 268-312
 LGTV | 339-381
 OHFV | 179-222
 KFDV | 164-204
 ALKV | 165-205
 DTV | 248-294
 POWV | 249-293
 TYUV | 341-386
 NEGV | 129-174
 KSIV | 233-270

. (((((.....((((((.....))))))).....))....)).
 CCCCCC-GCACCAUGACAAGGCCAACAUUGUGCACCAAAGGGAG-G 46
 CCCCCU-GCAUCAUUAAGGCCAACAUUGUGCAUGAAAGGGAG-G 46
 CCCCCC-GCCCAUGACAAGGCCAACAUUGAGCAUUAAGG-GAG-G 45
 CCCCUU-GCGUCCAGAGAAGGCCAACUUGGGCGU---UAUAAGGAG-G 43
 CCCCCC-GCACCAUG-GAAGGCCAACAUUGUGCAUG-AAGGAAA-G 44
 CCAAAA-CCUCCCAGAGAAGGCCAACUUGGGAGGCC---AUGAA-G 41
 CCAAAG-CCUCCCAGAGAAGGCCAACUUGGGAGGCC---AUGAA-G 41
 CAAAAGGCCUCUG-GAAGGCUCACCAGGAGGUUAGGCCAUUCUAGAG 47
 CAAAAGGCCUCUG-GAAGGCUCACCAGGAGGUUAGGCCUU-UAG-G 45
 CUUUC--CAUGAGAGGAGACGGUCAACUCUCAUGGAACAAAGAACCG 46
 CCCCCCCCUGGCCAGAAAAAGGGGGGGCAAACAGGCC--AGGGUGAAG 46
 CUGAC--CAUCCU-CAAGGCCGAGUGGGAUGC---GUAUGAAG 38
10.....20.....30.....40.....

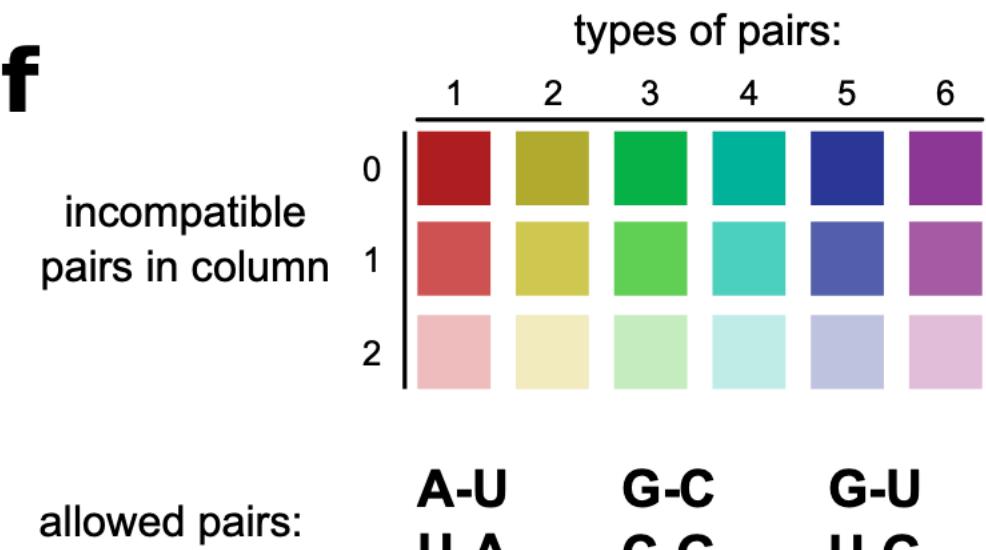
Seq. Conservation:

**e****Y1**

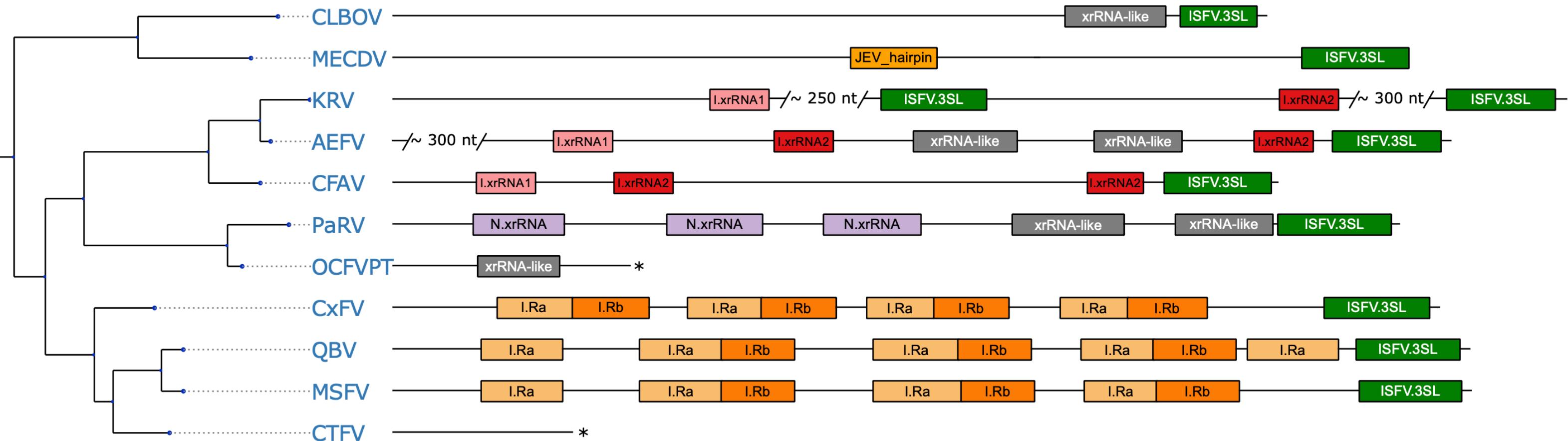
POWV | 291-361
 DTV | 290-354
 LGTV | 377-449
 TBEV | 573-645
 SGEV | 300-372
 LIV | 306-378
 KSIV | 264-335

(((((.....((((((.....))))))).....))....((((.....)))).....)).
 AGGAG--CCCCCGAGCAUAA---CUCGGGAGGAGGGAGGAAGA-AAA
 UAGAG--CCCCGGCAUAA---CUCGGGAGGAGGGAGGAAGA-CAAU
 GGAGG--CCCCAGGGAAACCCUUGGGAGGAGGGAGGAAGAGAG-AAA
 GGAGG--CCCCCGGAAGCACGUUCCGGAGGGAGGAAGAGAG-AAA
 GGGGAGGCCCGGAAGCACGUUCCGGAGGGAGGAAGAGAG-AAA
 AGGGAGGCCCGGAAGCAUGCUUCCGGAGGGAGGAAGAGAG-AAA
 UAUGAAGGCCUGGAG--AGAUCAGG-AGGGGGAGAGAGAGGA
10.....20.....30.....40.....50.....60.....70.....

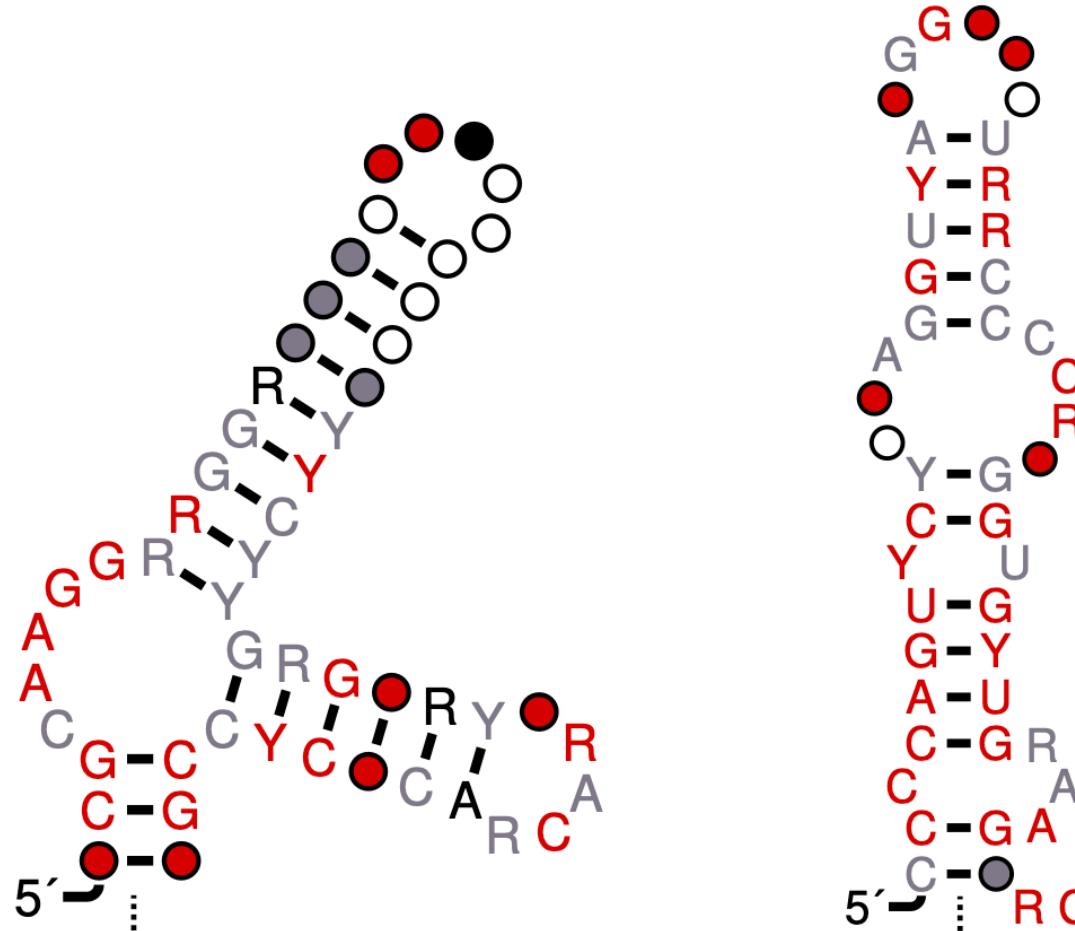
Seq. Conservation:

**f**

a Classic Insect-specific flaviviruses



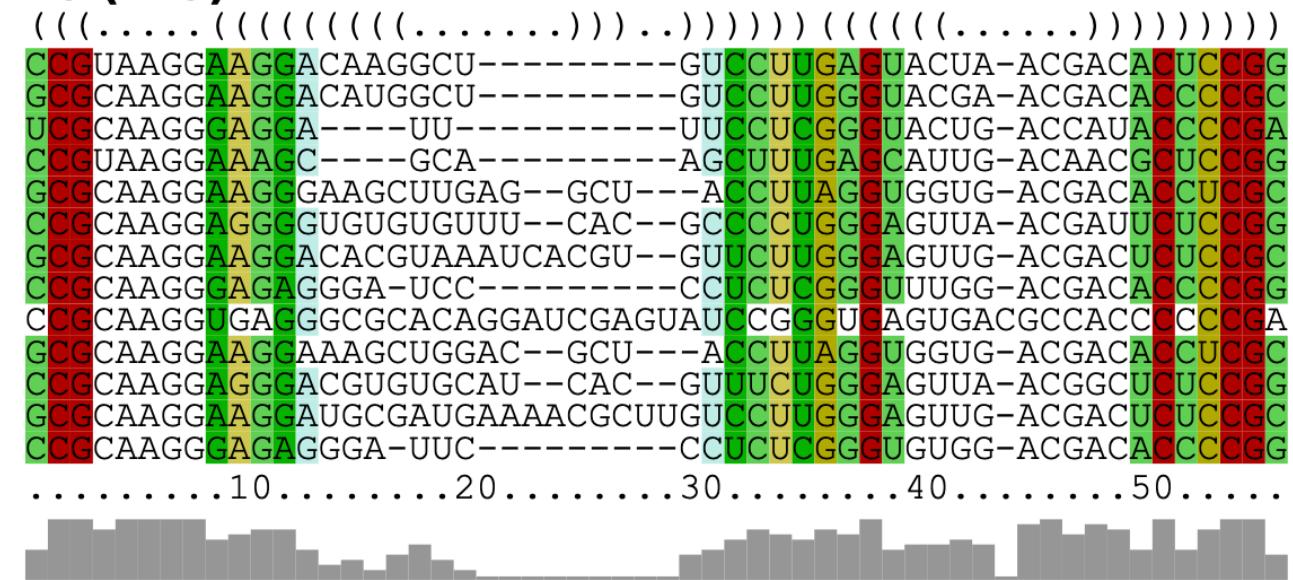
b CxFV, QBV, MSFV



Repeat element a (I.Ra)

CxFV 66-111	(((.....((((((.....))))....))))(((((.....)))))))	46
CxFV 185-230	CGCUAAGGAAGGACAAGGU-----GUCCUUGAGUACUA-ACGACACUCGGG	46
CxFV 298-338	GCGCAAGGAAGGACAUGGU-----GUCCUUGGUACUG-ACCAUACCCCGA	41
CxFV 418-459	UCGCAAGGGAGGA---UU-----UUCCUCGGGUACUG-ACCAUACCCCGA	42
QBV 56-105	CCGUAAGGAAAGC---GCA-----AGCUUUGAGCAUUG-ACAACGCCUCGG	50
QBV 155-205	GCGCAAGGAAGGGAAAGCUUGAG--GCU---ACCUUAGGUUGG-ACGACACUCGG	51
QBV 301-353	CCGCAAGGAGGGUGUGGUUU--CAC--GC CCCUUGGGAGUUA-ACGAUUCUCGG	53
QBV 431-475	GCGCAAGGAAGGACACGUAAAUCACGU--GUUCUUGGGAGUUG-ACGACACUCGG	45
QBV 535-590	CCGCAAGGAGAGGGGUUUGG-ACGACACUCGG	56
MSFV 56-105	CCGCAAGGUGAGGGCACAGGAUCGAGUAUCCGGGUAGUGACGCCACCCCGA	50
MSFV 155-205	GCGCAAGGAAGGAAAGCUGGAC--GCU---ACCUUAGGUUGG-ACGACACUCGG	51
MSFV 301-355	CCGCAAGGAGGGACGUGUGCAU--CAC--GUUCUUGGGAGUUA-ACGGCUCUCGG	55
MSFV 433-477	GCGCAAGGAAGGAUGCGAUGAAAACGCUUGUCCUUGGGAGUUG-ACGACACUCGG	45

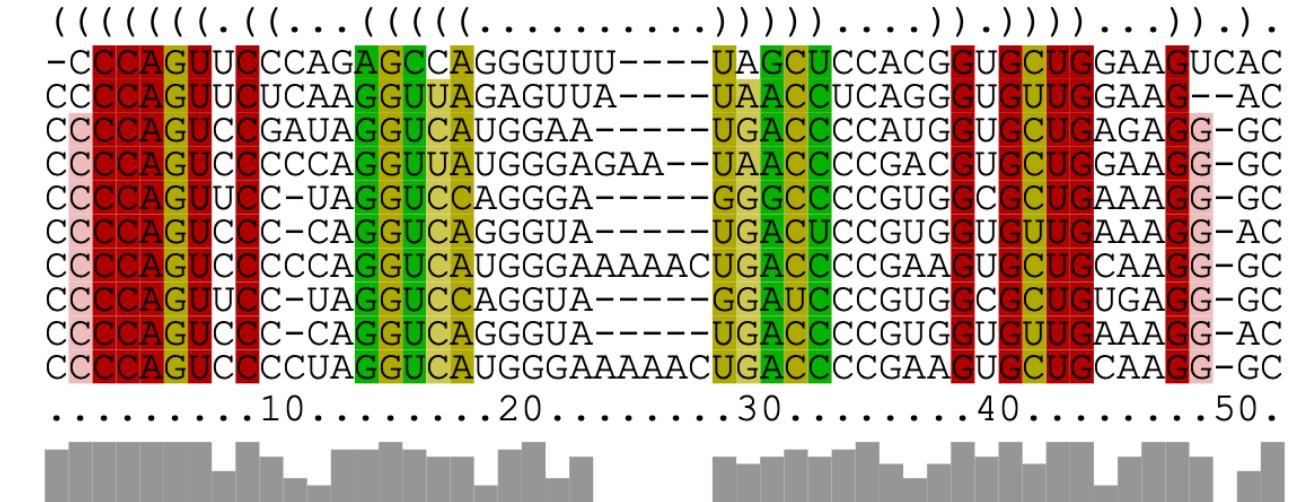
Seq. conservation



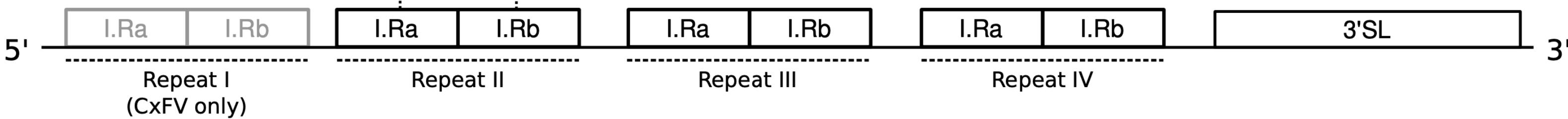
Repeat element b (I.Rb)

CxFV 113-159	(((((.((.(((((.....))))....))))....))))....).	47
CxFV 231-276	-CCCAAGUUCCAGAGCCAGGGUUU---UAGCUCCACCGUCCUGGAACUCAC	46
CxFV 339-384	CCCCAGUUCUCAAGGUAGAGUUA---UAACUCAGGGUGUUGGAAG--AC	46
CxFV 460-508	CCCCAGUCCCAGGUUAUGGGAGAA---UAACCCGACGUCCUGGAAGG-GC	49
QBV 206-250	CCCCAGUUCUCC-UAGGUCCAGGGA---GGGCCCGUGGCCUGAAAGG-GC	45
QBV 354-398	CCCCAGUCCC-CAGGUCAAGGGUA---UGACUCCGUGGUUGAAACGG-AC	45
QBV 476-526	CCCCAGUUCUCC-CAGGUCAUGGGAAAAACUGACCCGAAGUGCUGCAAGG-GC	51
MSFV 206-250	CCCCAGUUCUCC-UAGGUCCAGGU---GGAUUCCGUGGCCUGUGAGG-GC	45
MSFV 356-400	CCCCAGUCCC-CAGGUCAAGGGUA---UGACUCCGUGGUUGAAACGG-AC	45
MSFV 478-528	CCCCAGUUCUCCUAGGUCAUGGGAAAAACUGACCCGAAGUGCUGCAAGG-GC	51

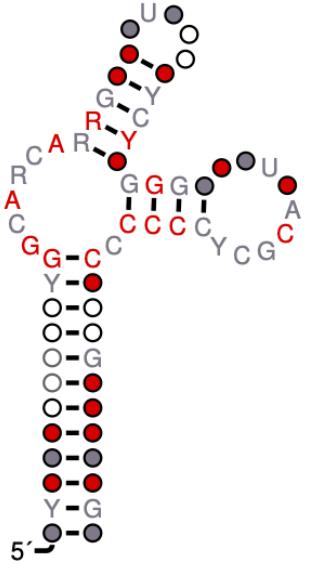
Seq. conservation



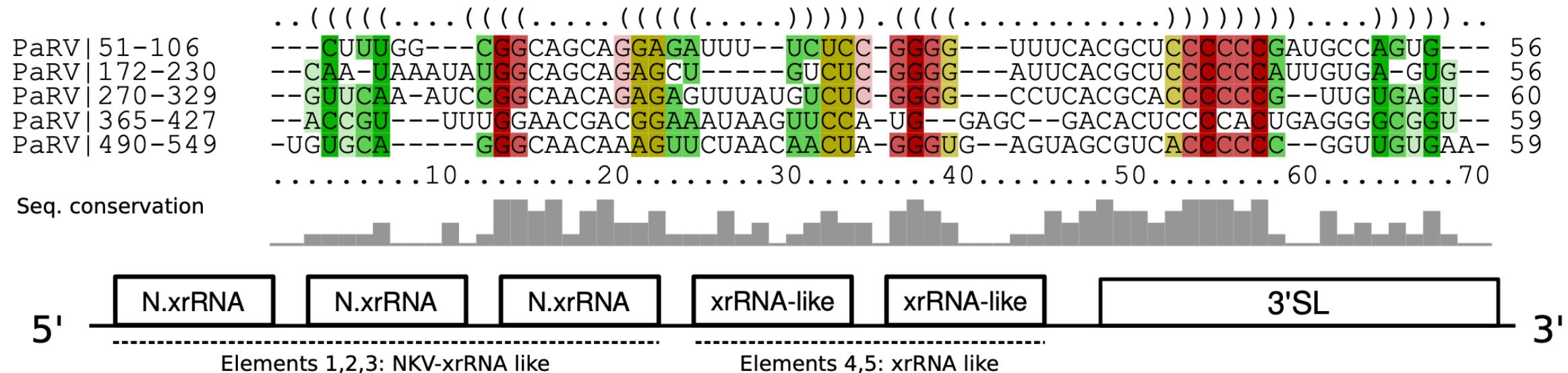
c



d PaRV



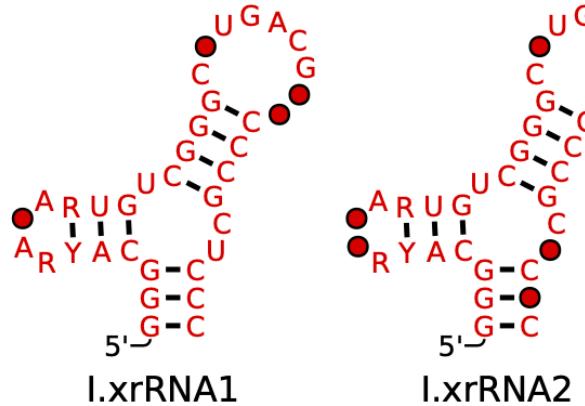
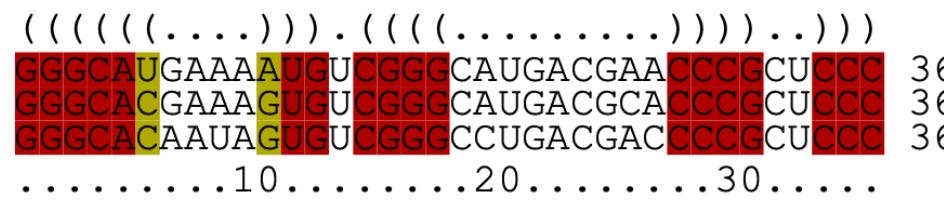
PaRV xrRNA-like element



e KRV, AEFV, CFAV

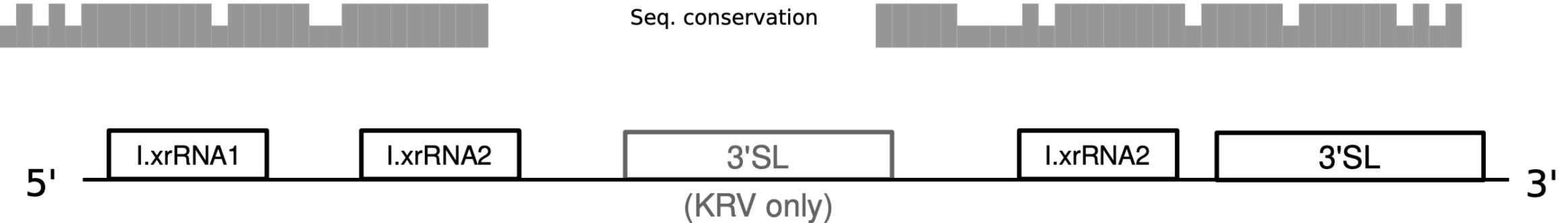
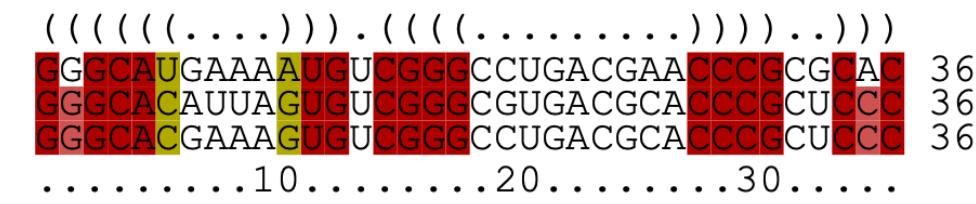
I.xrRNA1

CFAV | 53-88
KRV | 199-234
AE.FV 382-417

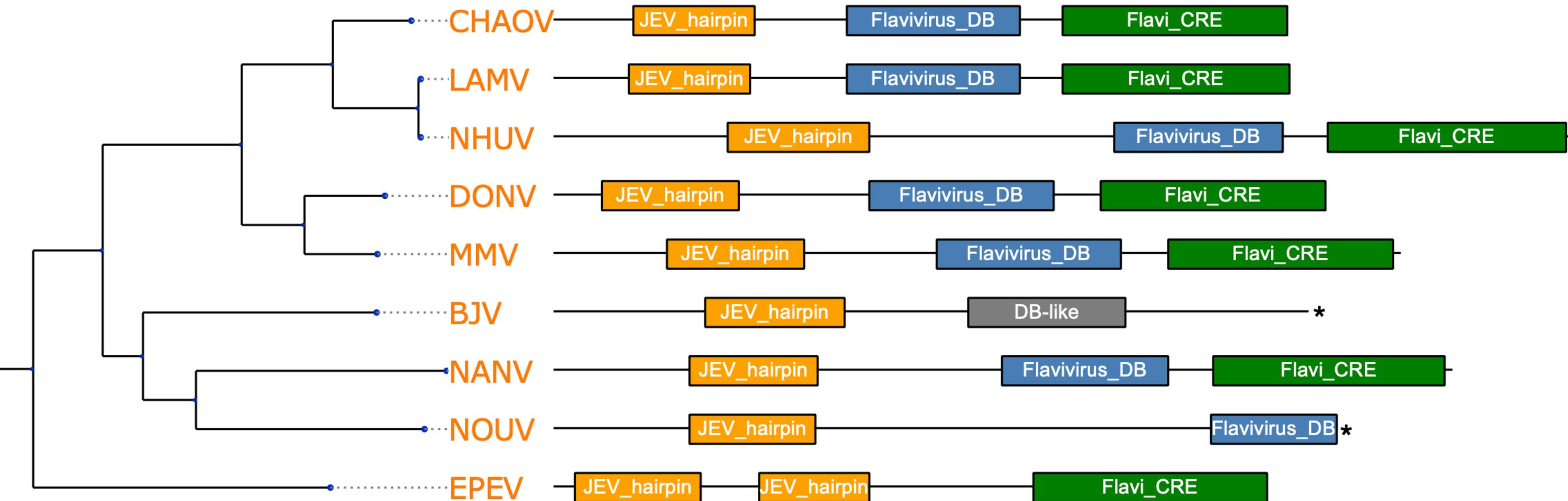


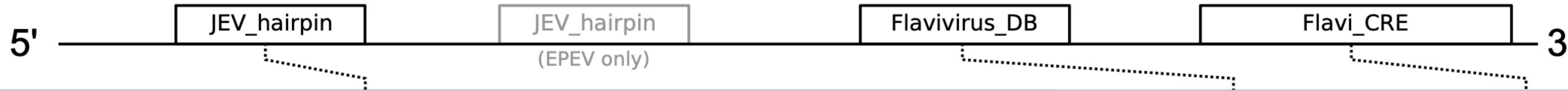
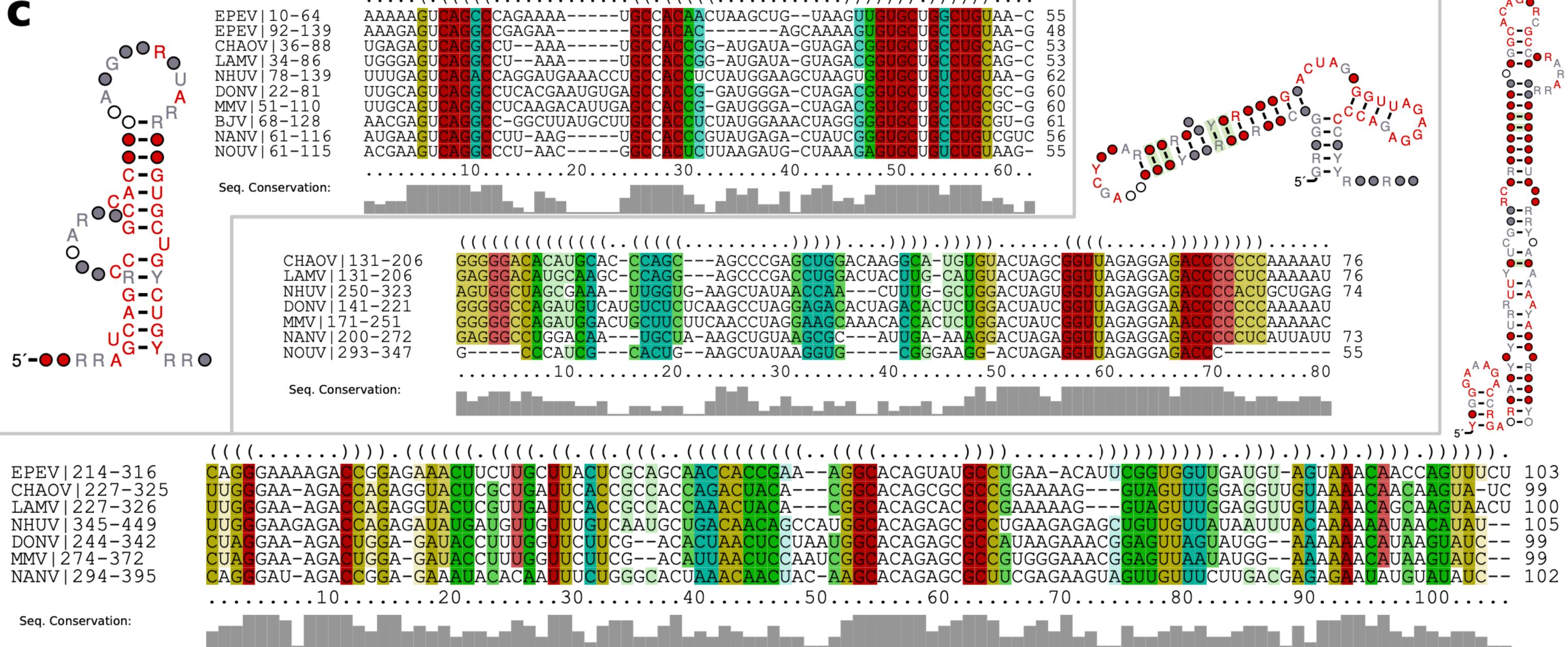
I.xrRNA2

AEFV | 520-555
CFAV | 139-174
KBV | 795-830

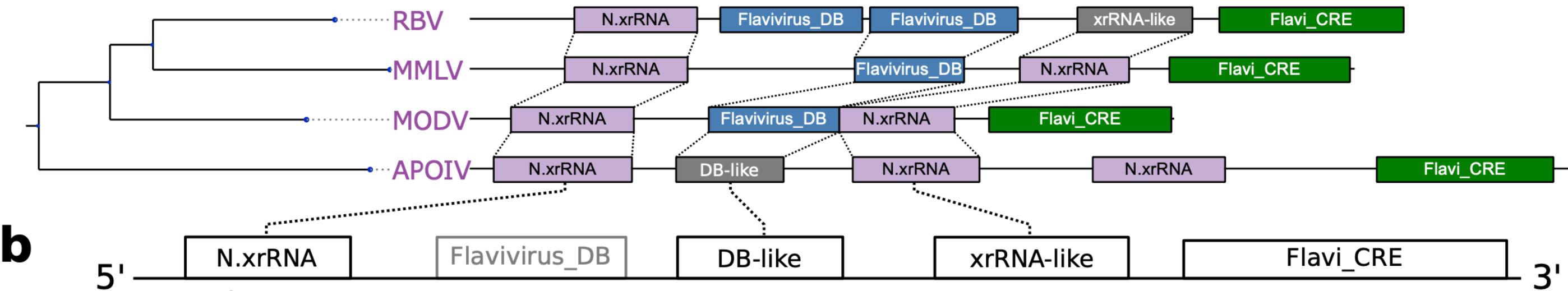


a Dual-host affiliated Insect-specific flaviviruses

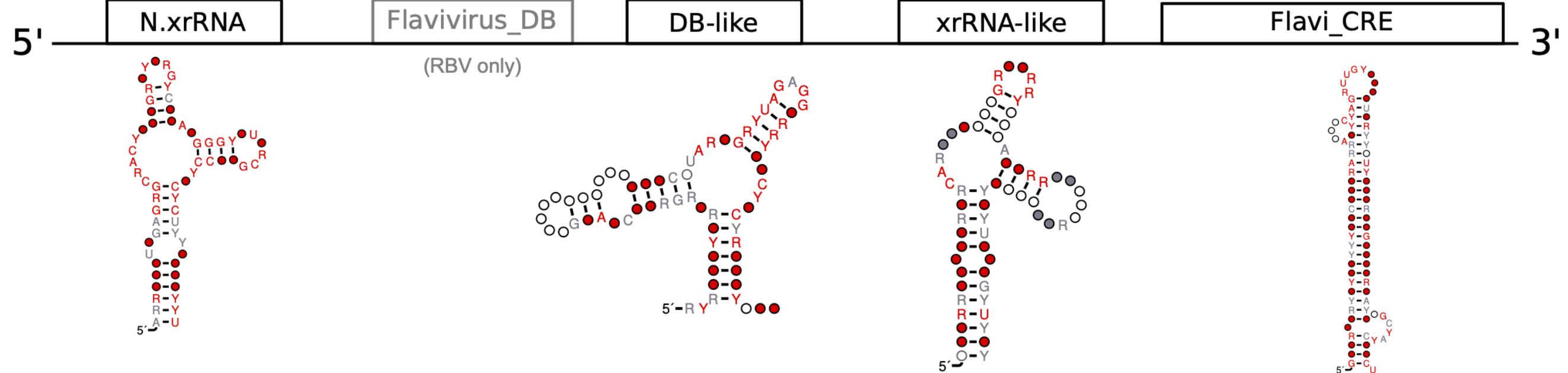


b**c**

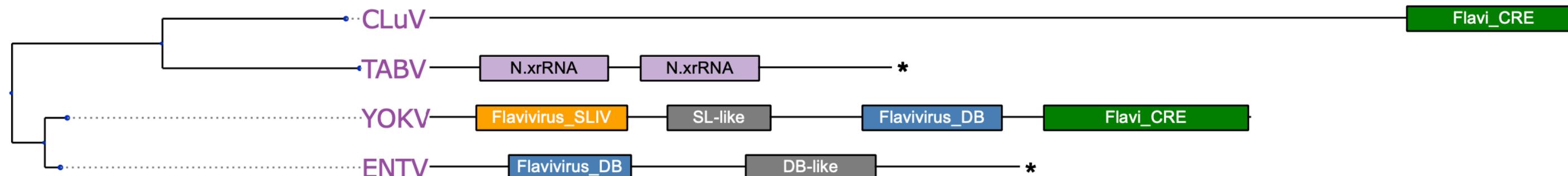
a Tick-borne related NKVs

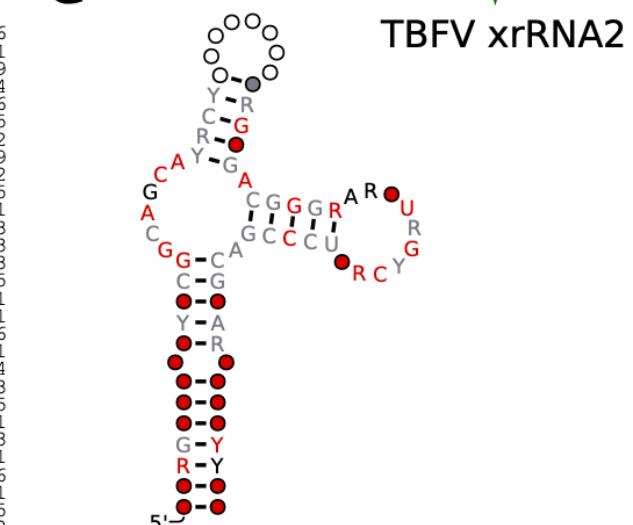
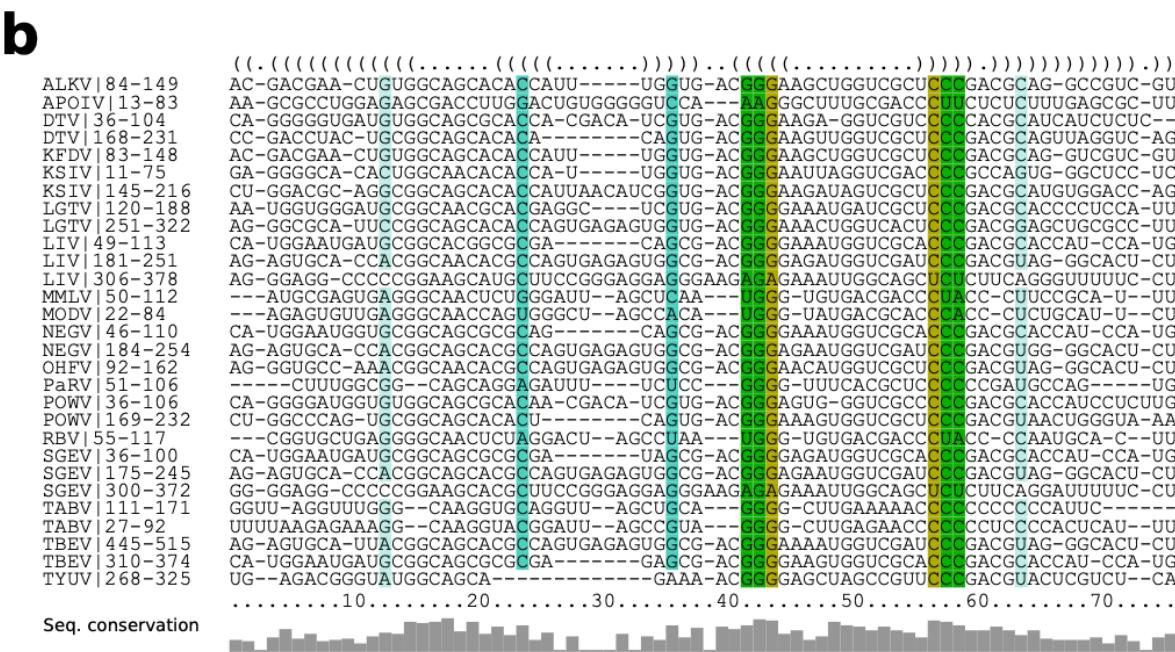
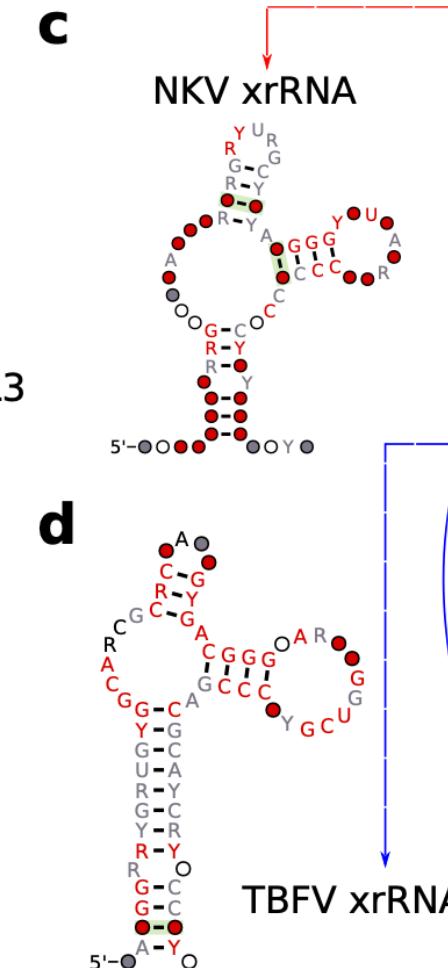
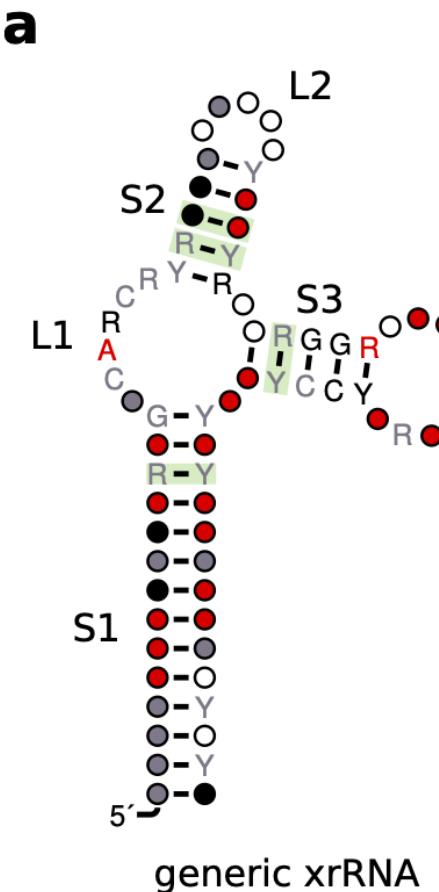


b



c Mosquito-borne related NKVs, TABV, CLuV





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Figure from doi:10.3390/y11030298