

Fig. S1

Characterization of Dak Nong virus, a insect nidovirus isolated from *Culex* mosquitoes in Vietnam in Archives of Virology by Kuwata *et al.*. Corresponding author; Haruhiko Isawa, Ph. D. of National Institute of Infectious Diseases. E-mail, hisawa@nih.go.jp

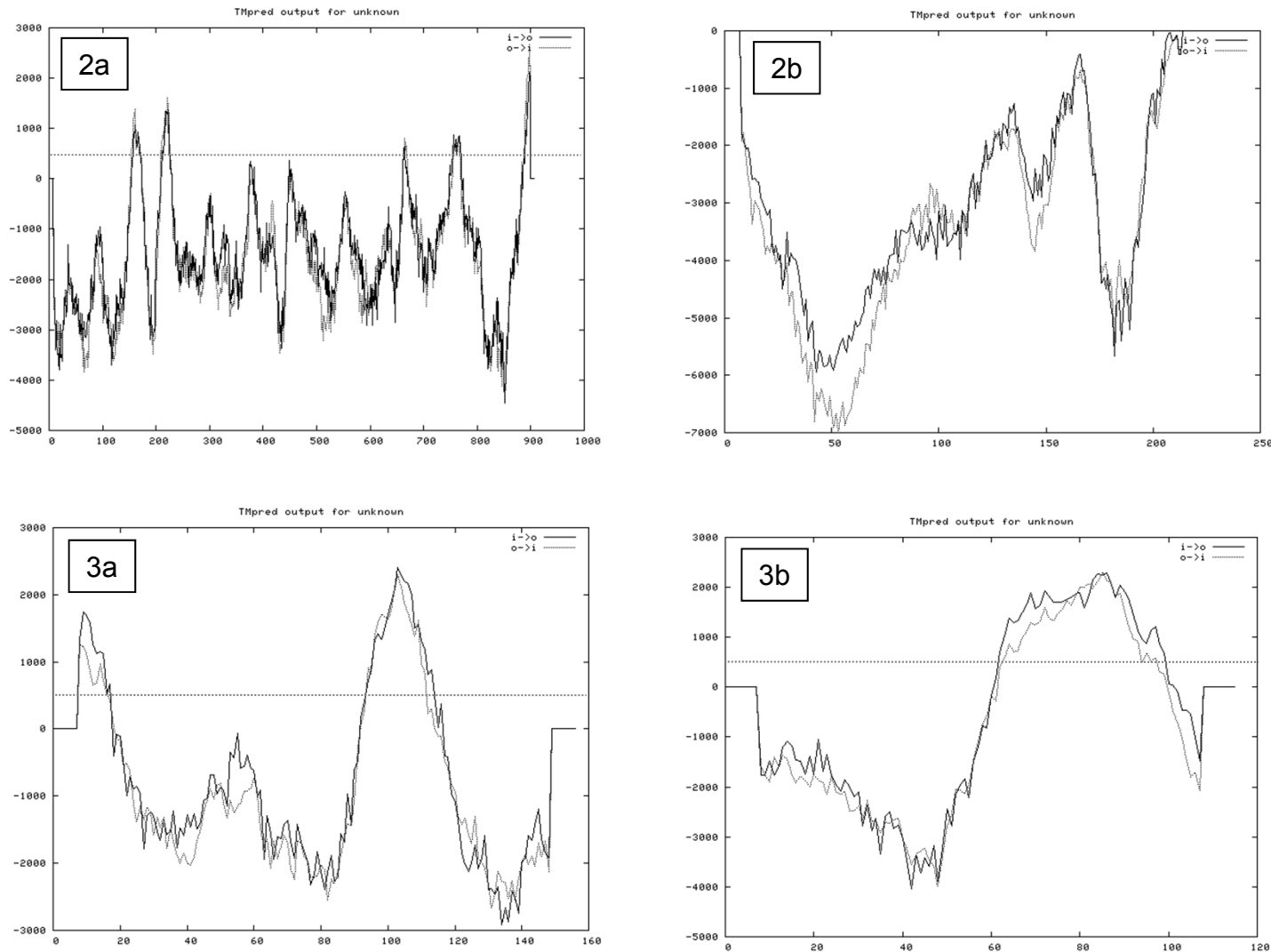


Fig. S1 Hydrophobicity plots for the predicted DKNV structural proteins encoded by ORF2a, ORF2b, ORF3a, and ORF3b. Hydrophobicities of the four putative structural proteins were calculated using TMpred [20]. Horizontal dashed lines depict the threshold (value of 500) for significant association with transmembrane helices.

Fig. S2

Characterization of Dak Nong virus, a insect nidovirus isolated from *Culex* mosquitoes in Vietnam in Archives of Virology by Kuwata *et al.*. Corresponding author; Haruhiko Isawa, Ph. D. of National Institute of Infectious Diseases. E-mail,hisawa@nih.go.jp

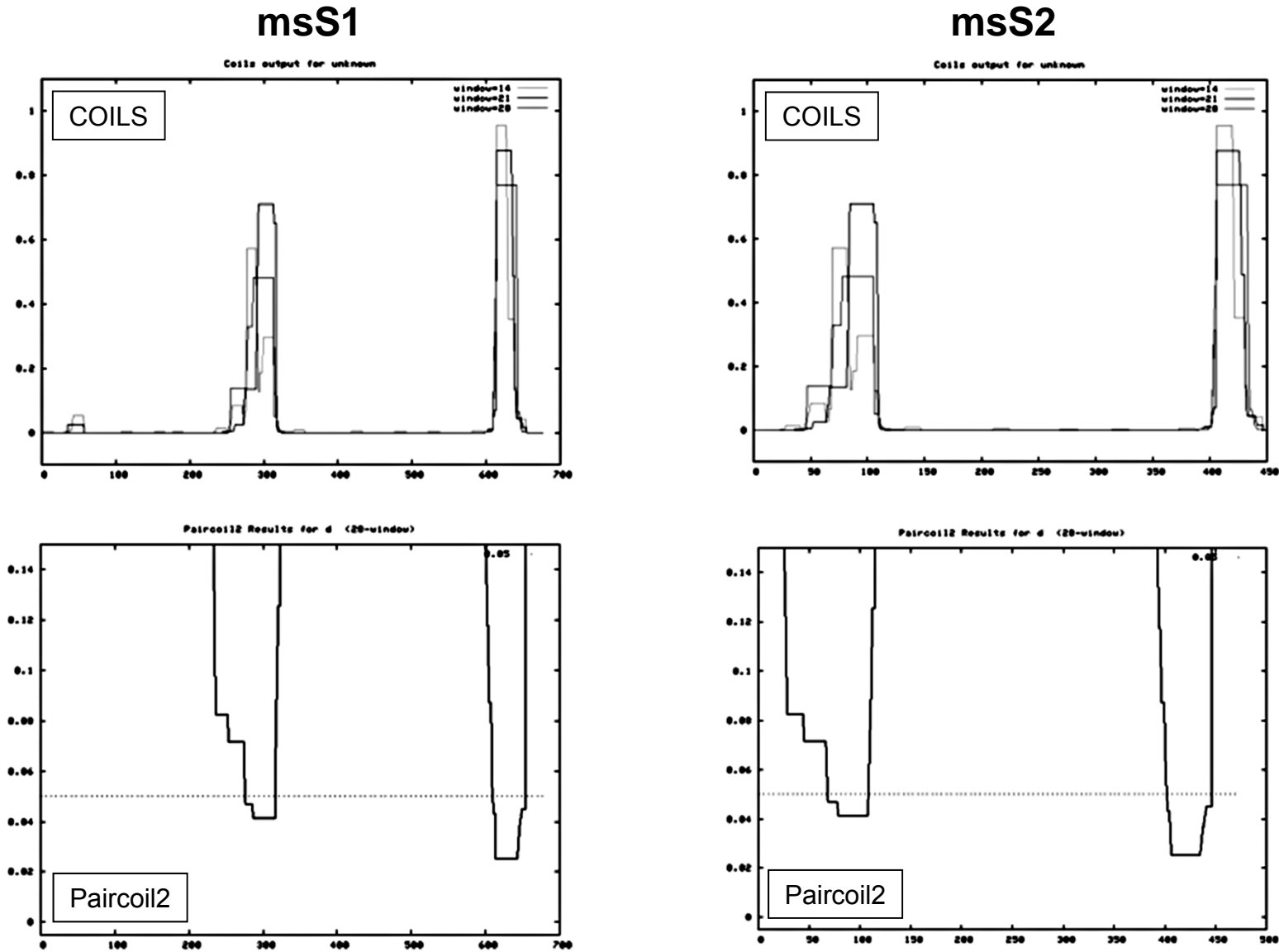


Fig. S2 Regions of coiled-coil structure in the two types of DKNV spike glycoprotein, msS1 and msS2, predicted using the COILS [24] and Paircoil2 [25] programs.

Fig. S3

Characterization of Dak Nong virus, a insect nidovirus isolated from *Culex* mosquitoes in Vietnam in Archives of Virology by Kuwata *et al.*. Corresponding author; Haruhiko Isawa, Ph. D. of National Institute of Infectious Diseases. E-mail, hisawa@nih.go.jp

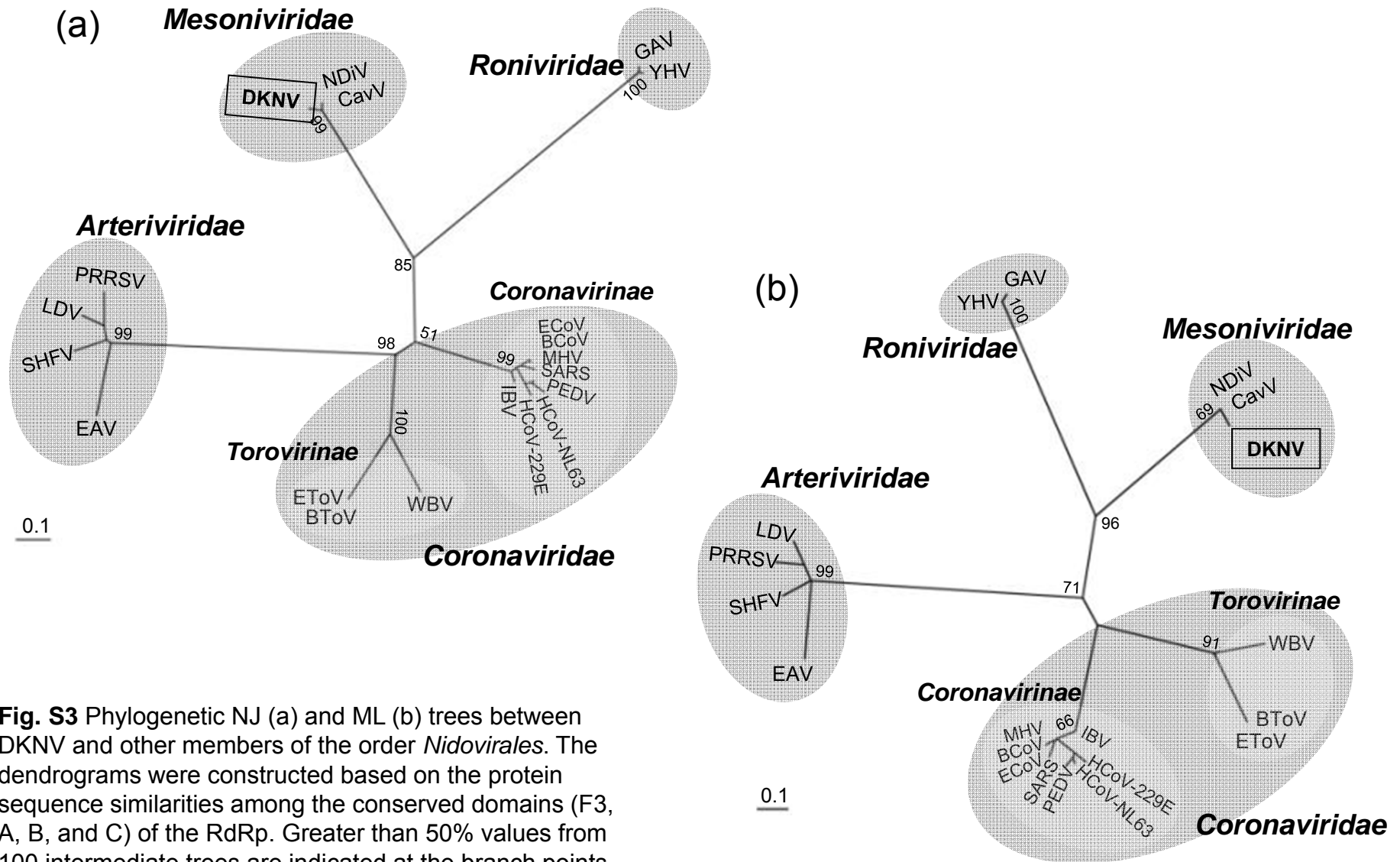


Fig. S3 Phylogenetic NJ (a) and ML (b) trees between DKNV and other members of the order *Nidovirales*. The dendrograms were constructed based on the protein sequence similarities among the conserved domains (F3, A, B, and C) of the RdRp. Greater than 50% values from 100 intermediate trees are indicated at the branch points. Bar represents 0.1 substitutions per site.

Fig. S4

Characterization of Dak Nong virus, a insect nidovirus isolated from *Culex* mosquitoes in Vietnam in Archives of Virology by Kuwata *et al.*. Corresponding author; Haruhiko Isawa, Ph. D. of National Institute of Infectious Diseases. E-mail, hisawa@nih.go.jp

(a)

```
DKNV 301 NELEDDTEAEETLKSKRKGKSGEFSKTSKSRKETHIQNSNDAETPETLKTATAENVAQNGLQNASASTKQKRKGK-LTPQTQLLDHTL-NQTRLAHRTPY 398
NDiV 300 S.QD.....D.Q.N.....-----Q.K.....Q...AK..KF.R.Q.F 344
CavV 300 L.I.....Q.T.....-----Q.....Q...AK.SKP.R.QSH 343
```

(b)

```
DKNV 1695 RLLSDFNITAEQYVQHGYNVDYNNFV-----SCIKYGLMDNKNVTYNNQSATSVSVVDAPTR-LDYILDVLYVLLYMFTHTHPAFYIA 1776
NDiV 1644 .....A.Y...I.....INNENRYTTTTIGTKSFET.....K.E.Y..T..IFNPPEHSSSGF.NTM.....FV..... 1743
CavV 1640 .....N.Y..YI.....NNENRYTTTTIGTKSFET.....KPD.Y.....IFNSPEHSSSGF.NTM.....FV..... 1739
```

Fig. S4 Alignment of the partial ORF1a sequences among DKNV, NDiV, and CavV. DKNV ORF1a protein contain a 55-aa insertion (₃₁₉K to ₃₇₄G) (a) and a 22-aa deletion (₁₇₂₀V to ₁₇₂₁S) (b) compared with NDiV and CavV.

Fig. S5

Characterization of Dak Nong virus, a insect nidovirus isolated from *Culex* mosquitoes in Vietnam in Archives of Virology by Kuwata *et al.*. Corresponding author; Haruhiko Isawa, Ph. D. of National Institute of Infectious Diseases. E-mail, hisawa@nih.go.jp

3CL	DKNV	1	SSLRGAIVNGYIYVQRHLFGSKKQFEFEACYNNGKGLLNCHNLERSKYDIDS AELIGTLIRIPLLNDHSPFKIKLHPAPLTYNGPVTLYLSRYDTELQKDV
	NDiV	1V.....I.....K..D.....HDKQ.I.H.S...D..S.....N...
	CavV	1V.....I.....K.....HDK..I.H.SI..D..S.....N...
	DKNV		LCVHTGFISEGHHDIKTVFGDCGGMLFDPKGRLLGLHCAG 140
	NDiV	M..... 140
	CavV	M..... 140
HEL1	DKNV	1	TNYCYVCGENAYLTCATCERAFNCNSADTDHGSHIEQHLQYSGHTCLYLNCKTVKCKHCYTNDINLLYTTGREHYCEAHKPKHAARI 86
	NDiV	1N.....M.....Q..F.M.....D...S...N.V.. 86
	CavV	1N.....M.....H..F.T.....D.....N.V.. 86
EXON	DKNV	1	FPINIVMNDYICFDAEFLNPKDNLQEPVMLS YGFSSKYGKRR IAGIPVRYIKDRFDKIVPQ EYNYRDN NKPLTSTYNCEW MRKQYPDQYKHLHSVTQGI
	NDiV	1	..M...D.C.....R..V.....K.NR...K..K.....T.D....H.E.....T..M...
	CavV	1	..M...D.C.....R.....K.NR..I.HK...K.....S.D..K..H.....T..L...
	DKNV		RNSTLVDLKP LLNFCVDMHVKPVI VTWAGASDH CFLKANTLYPDISTVCNITTRCSSQP VYAS PQGLHTYYLCQYHAHHLQGYINITHFVNLEIIDLKL
	NDiV		..D.T.....S.....H.....A.....I..T..I.....R.....Q.KDHV.....V
	CavV		..D.T..R.....S.....R.H.....T..I.....R.....Q.KDH.....V
	DKNV		NYNQYTGERILNVFHENLKLKLENVASNSLTDCHARYCRTVHAPITPHDPLDSSIMTQCIYQ 265
	NDiV		DR...D..T.R.Y..DY...T.D.D.....T.....A.....A..... 265
	CavV		DR...N..T.R.Y..DY...T.D.D.....T.....I..P.....A..... 265
N-MT	DKNV	1	EMCKLRRELQQVWYKQYVDHNITHCNMGCGKDHLKHALHNI DILQ GK-NPQNNMNTHTCD AEEHIYFDSHWYKEGNFTKPSYIFSDINKEHYYNLGT TGL
	NDiV	1K...S..TNT.K.....EP.QQ.....V.....S.....S.....A.G.....K.....
	CavV	1K...EK..I.TKK.....EP.QQ.....V.....S.....S.....D.G.K.....K.....
	DKNV		SLYLNSKCAKYVHEYRTINGTDVFKTLYAQYCALERQPHQAAIKPSCSIPDCIITSNIGENFQTLVCNVHKDQMDIIGKISQATGYGYQFIYTG 193
	NDiV		C.....Y.....VS.N...S..SP..D.G.K...E.E.....R.....S.....K..... 194
	CavV		C.....Y.....VS.N...S..SP..D.G.K...V.E.....R.....Q...EL.S.A..K..... 194
O-MT	DKNV	1	SALSKAPHNWDHLEIPGYNTRKQHSGHMTTKALGILHILQDSMLYTNRKTLPKLPVIMPGSASF LGETVLANEMSKYLKQTKFIHVDPRLKIDNQTT
	NDiV	1	A.....S.....N...L...Y..D.....A.T.....I.....N...
	CavV	1	A..A..LD.....S.....N...L...Y..D.....T.....V.I.....N...
	DKNV		HYRKTLMEMLDIGYTTTELIISDIHNNTSPWIPELVSYTQKYLIDTGTLMKITSRGATEDNLALLETL SKDFTYVRCNLNAVITYSSELWIVFADKRKPP
	NDiV		.H.....D.KN.....MT..L..V.....V.QQ..D..N.....F.....N.....
	CavV		.H.....D.NN.....ME..L.....AV.QQ..HMA.N.....F.....N.....
	DKNV		VQGWTSHELKSELRKHWYAMTRSIIQPIMRSRTC VFRYSPK 241
	NDiV	RA.....S.....L...QS..... 241
	CavV	RA.....S.....L..A.QS..... 241

Fig. S5 Alignment of the deduced amino acid sequences of the conserved functional domains of ORF1a/b (3CL, HEL1, EXON, N-MT, and O-MT) among DKNV, NDiV, and CavV.

Fig. S6

Characterization of Dak Nong virus, a insect nidovirus isolated from *Culex* mosquitoes in Vietnam in Archives of Virology by Kuwata *et al.*. Corresponding author; Haruhiko Isawa, Ph. D. of National Institute of Infectious Diseases. E-mail, hisawa@nih.go.jp

DKNV	1	-ACUAUUGAAAAU <u>UUGUUU</u> CACCC <u>AAUAAUACUACUACU</u> ACAAGUAUAACCCCCAGUCC	59
NDiV	1	-.....AA.....C.U.....U.....C.A.....-.....	58
CavV	1	G.....A.....U.....U.A.....-.....	59
DKNV	60	GUCUGCUAGAGACGCUAAACUCU-AGCAAUUA <u>UACUAGCC</u> ACAUCAGUUGCUUAAAGAAC	118
NDiV	59UC.....G.UA.C.A..C.....	118
CavV	60AUC.....G.U..CCA..C.....	119
DKNV	119	CAC <u>UUUGAGACACUCU</u> CCCCACUUAAC <u>AUCUUUU</u> AGGAAUCUUUGAUGCUACAACAACUUGG	178
NDiV	119	.U.....C.....	178
CavV	120	.U.....C.....	179
DKNV	179	CUAGUAUAACAGCAA <u>UUCGU</u> AUUUUUA-AGUUUAAGUGGUUUUUACGCCCAUACUU-	236
NDiV	179-.....AU.....CG.A..C..C.....G....A..-CCA..G.U...A...U	236
CavV	180-.....AU.....CA.AC.A..C.C.....G....A..-CCA..G.U...A...U	237
DKNV	237	AAUUGAUUUUGUUUGCUAUUGUUACAACUAUUUCAUCAUUAACAGAGGCUAUAUUCUAGUG	296
NDiV	237	G.AA.G.....-.....GU...AC.C..-G-.....--.....	291
CavV	238	G.AA.G.....-.....G..UG.GC..G..--.....-..A.....	293
DKNV	297	CUCAGCGCGUAAUAGAAGUA--CUUUUACAGUAGCAUGAGUUUGUUGUAAAUCGUCUUG	354
NDiV	292U.A.....C.-.....AU...GCG..A...U.....A.....	350
CavV	294U.A.....-...ACG..GCG.....U.....A.....	352
DKNV	355	AUACGACACAUCUAAA <u>AUG</u>	373
NDiV	351-----C.....	363
CavV	353-----C.....	365

Start codon

Fig. S6 Alignment of the 5'-UTR sequences among DKNV, NDiV, and CavV. TRSs for subgenomic mRNA2 and mRNA3 are enclosed by dotted and solid lines, respectively. Sources for TRSs in CavV and NDiV are obtained from [8] and [9], respectively.

Fig. S7

Characterization of Dak Nong virus, a insect nidovirus isolated from *Culex* mosquitoes in Vietnam in Archives of Virology by Kuwata *et al.*. Corresponding author; Haruhiko Isawa, Ph. D. of National Institute of Infectious Diseases. E-mail, hisawa@nih.go.jp

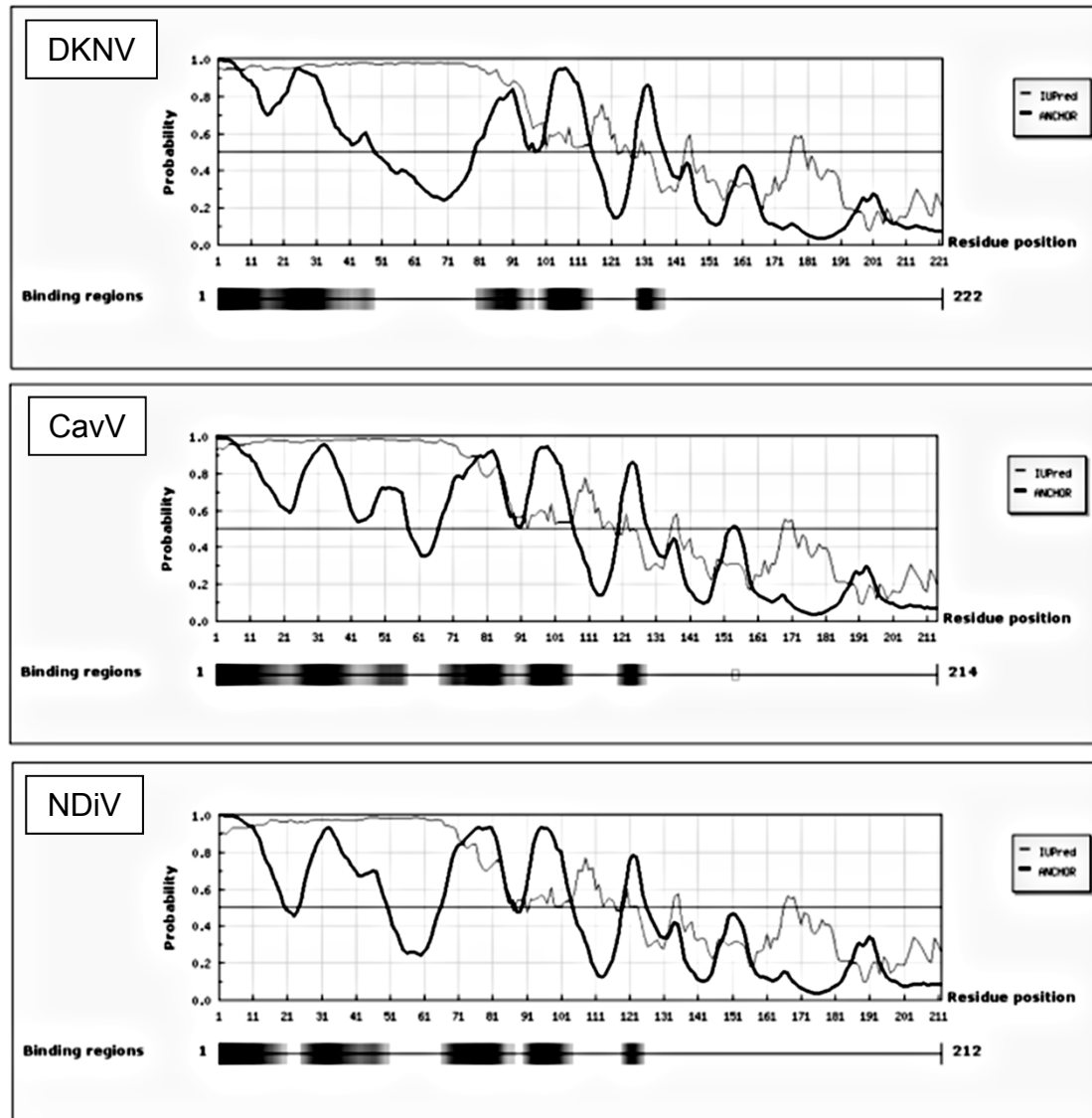


Fig. S7 Order/Disorder prediction for the putative nucleocapsid proteins of DKNV, CavV, and NDIV. The patterns of the three graphs are quite similar to each other; the anterior half of the peptides exhibits regional disorder, while the posterior half exhibits regional order.