

Supplementary Information

Analysis of DNAs associated with coconut foliar decay disease implicates a unique single-stranded DNA virus representing a new taxon

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Figures S1, S2, S3, S4; Tables S1, S2, S3, S4, S5, S6, S7

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MDV_alphasat_C10_Rep_BAA34048 LKTIIEQEPDDRTIIWVYGPXGEGKSTFAKYLTLKGGWFFYTAGGKTQDILYMYAQD--PTCHVCDIPRCKT--EYINYSVIEALKDRVIESTKYKPKVIEIEN--VHVIVSNVLPDY-----FKISEDRIKINI 283
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PNYDV_alphasat_3_Rep_AHC72280 LEAIQLPPNYRDLVWVYGPXGEGKSTFAKHL--IKYGWFFYTAGGKTQDILYMYAQD--PTCHVCDIPRCKT--EYINYSVIEALKDRVIESTKYKPKVIEIEN--VHVIVSNVLPDY-----FKISEDRIKINI 284
SCSV_alphasat_C6_Rep_AAA68022 MHLLEEEPPDRSIIWVYGPXGEGKSTFAKHL--IKYGWFFYTAGGKTQDILYMYAQD--PTCHVCDIPRCKT--EYINYSVIEALKDRVIESTKYKPKVIEIEN--VHVIVSNVLPDY-----FKISEDRIKINI 285
BMLRV_alphasat_2_Rep_AHC72185 EEELEEKIASPRSIWVYGPXGEGKSTFAKEL--ITRGWFFYTRGGKKNILFSYVDEGSKHIVFDIPRCN--QDYLNDVIEALKDRVIESTKYKPKVIEIEN--VHVIVSNVLPDY-----FKISEDRIKINI 281
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FBNSV_alphasat_2_Rep_AHC72218 EKELEKVPSPRSIIWVYGPXGEGKSTFAKEL--ITRGWFFYTRGGKKNILFSYVDEGSKHIVFDIPRCN--QDYLNDVIEALKDRVIESTKYKPKVIEIEN--VHVIVSNVLPDY-----FKISEDRIKINI 279
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GDS_alphasat_Rep_ALB26259 QTLLERAPDDRTIIWVYGPXGEGKSTFARDL YRSGTWFFYTRGGKADNVAYQYIGQ--LGNIVFDIPRDK--KDYLYSLEIEMFKDRILVSNKYEPIMAPLINC--IHVVMSNFLPDF-----EKISQDRVHVICPCGVCKQHVGKCEDEYV 315
SiLCuV_alphasat_Rep_CBY89002 QTLLERAPDDRTIIWVYGPXGEGKSTFARDL YRSGTWFFYTRGGKADNVAYQYIGQ--LGNIVFDIPRDK--KDYLYSLEIEMFKDRILVSNKYEPIMAPLINC--IHVVMSNFLPDF-----EKISQDRVHVICPCGVCKQHVGKCEDEYV 315
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FBYLV-[ET;231]_M_Rep_CCF74113 DALLME--RDGRRIIWVYGPXGEGKSTFAKHLVKTADFYSTGGKADIAFAWDHQ--ELVLFDFPRSF--EYVNYGVIEIQLKNGI IQSGKYQSVIKYSDY--VEVIFANFTPRS-----GMFSDRIVVYVA 286
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ABTV-[PH]_M_Rep_ABP96960 EEI MAE--PCHRRIIWVYGPXGEGKSTFAKHLVKTADFYSTGGKADIAFAWDHQ--ELVLFDFPRSF--EYVNYGVIEIQLKNGI IQSGKYQSVIKYSDY--VEVIFANFTPRS-----GMFSDRIVVYVA 286

B

Figure S1. Comparison of the master Rep protein sequences of nano- and babuviruses with the Rep protein sequences of selected alphasatellites associated with nanovirids, geminiviruses and CFDV as well as two alphasatellites isolated from insects.

Alignments were done using ClustalW in MegAlign of DNASTAR. Blue and green boxes A and B indicate the supposed M-Rep signature, i.e. two perfectly conserved sequence signatures characteristic of the M-Rep proteins of nanovirids. Names and accession numbers of the proteins are indicated on the left.

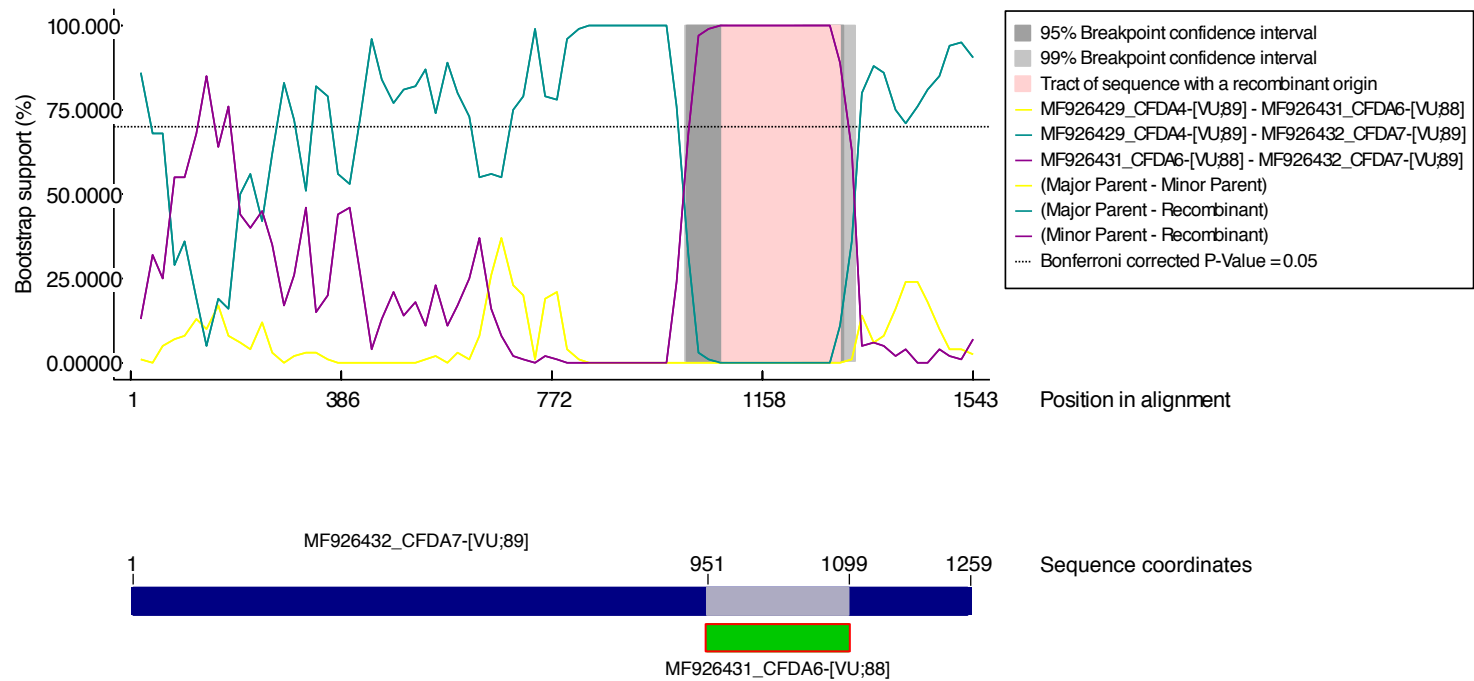
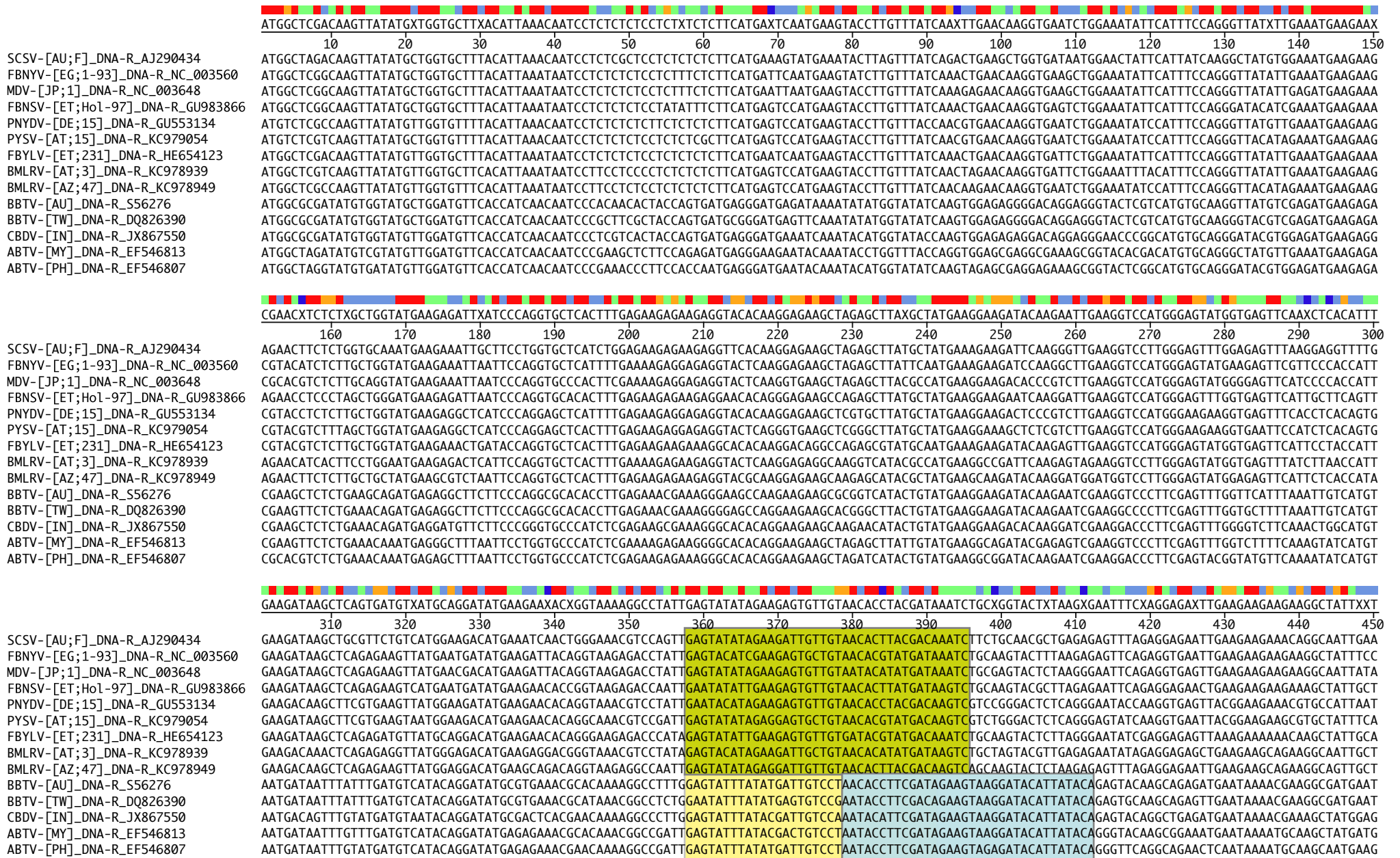


Figure S2. Recombination analysis

Sequences of CFD alphasatellites were aligned with sequences of ten banana bunchy top and two cardamom bushy dwarf alphasatellites using Muscle (UPGMB clustering, 8 iterations). A recombination analysis by RDP 4²⁶ identified CFDA7 as a potential recombinant. The recombination event was supported by RDP (P value 7.35×10^{-11}), GENECONV (P 1.3×10^{-10}), BootScan (P 1.09×10^{-12}), MaxChi (P 2.77×10^{-9}), Chimaera (P 3.51×10^{-9}), SiScan (P 3.0×10^{-11}) and 3Seq (P 1.54×10^{-5}). Top panel: Bootscan plot as implemented in RDP 4 showing the recombinant region flanked by potential recombination breakpoint regions.

A graphic representation of CFDA7 with the recombinant region (position 951 to 1099) possibly derived from CFDA6 (minor parent) is shown below the plot.

Figure S3



A



B

Figure S3. Alignment of coding sequences of nanovirus DNA-R components.

Alignments were done using ClustalW in MegAlign of DNASTAR. Coloured boxes indicate regions corresponding to the M-Rep amino acid signature regions identified in Fig. S1, boxes A and -B, respectively. These sequences were used to design degenerate oligonucleotide primers (1 to 6, Table S3). Green boxes: fully complementary primers designed for the corresponding region, yellow and blue boxes: overlapping reverse and direct primer, respectively. Names and accession numbers of the respective nano- and babuvirus DNA-R components are indicated on the left

Figure S4

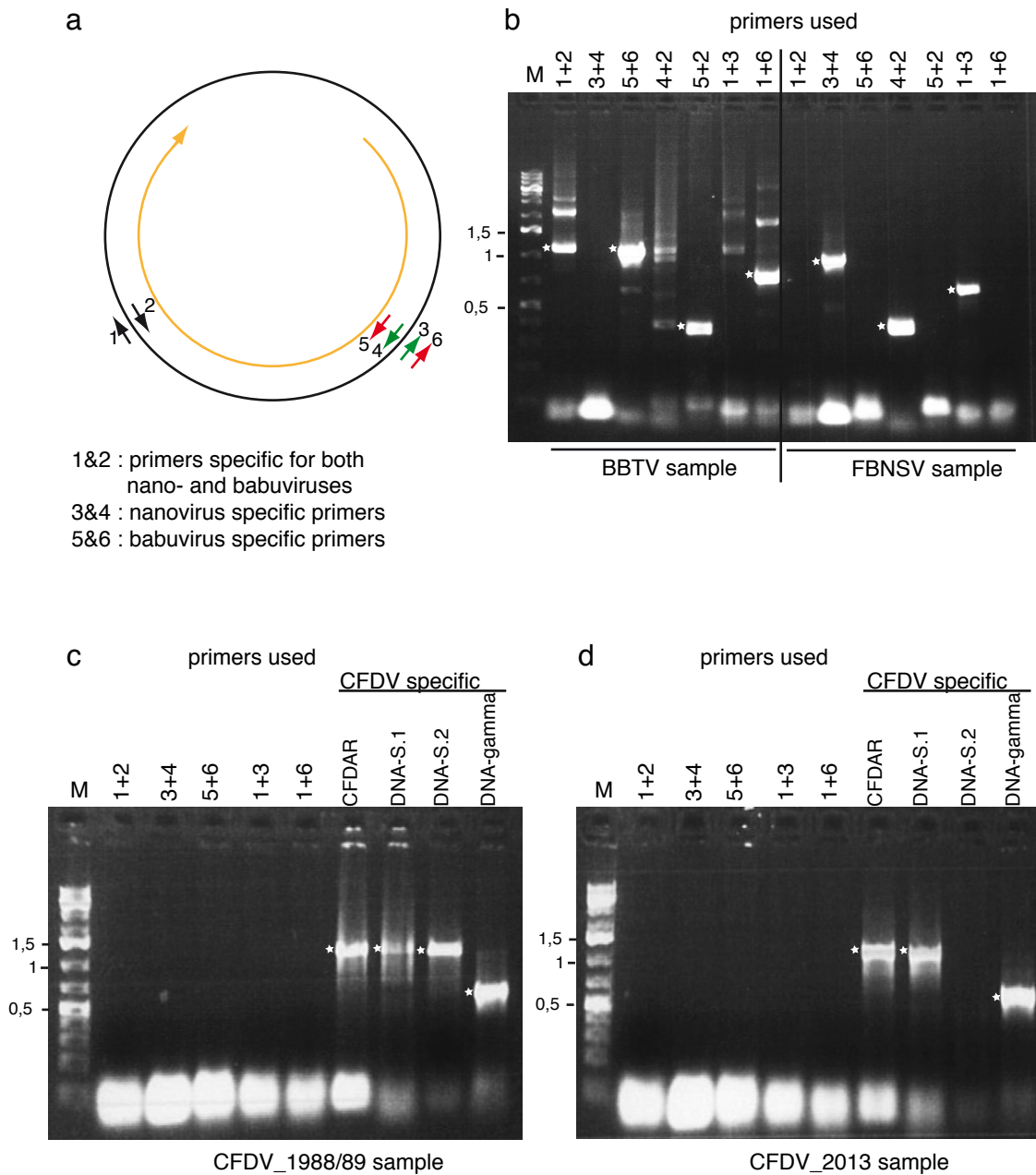


Figure S4. PCR amplification of nanovirus DNA-R components.

Degenerate primers (1 to 6, see Table S3) were designed based on the alignments of several babu- and nanovirus DNA-R sequences (see Fig. S3).

a - position and orientation of the primers in relation to nanovirus DNA-R.

b - control amplifications using total DNA extract of BBTV infected banana leaves (Hawaii-2013 sample, this work) or using the plasmid carrying a dimer of FBNSV DNA-R³⁰.

c - and d - PCR amplifications using CFDV samples. PCRs were performed on 1:10 diluted RCA products from a mixture of 23 different virion preparations of the CFDV_88/89 sample pool (c) or from a mixture of total DNA extracts from eleven different leaves of one symptomatic coconut palm for CFDV_2013 sample (d). CFDV specific primers were designed based on the cloned CFDV DNAs (the primer pairs used amplify a full-length DNA component as indicated, see Table S3).

M- 1 kb plus DNA ladder, the size (K bp) of several bands is indicated on the left of the marker, amplified DNA fragments of expected size are indicated by an asterisk.

Table S1. CFD samples prepared from symptomatic leaves of coconut palms showing date sampled at VARTC, palm identity, virus preparation date, and characteristics of the buoyant density zone (g/ml) in a Nycodenz® isopycnic density gradient from which the sample was collected

Sample No (CFD)	Sampling date	Palm ID (Number of palms)	Prep Date	Gradient density	DNA by PAGE	Virions by EM
1		cf2 (1)	28.02.89	1.23	+	+
2		cf2 (1)	28.02.89	1.24	+	+
3^{a,b}	11.1988	cf2 + cf3 (2)	06.03.89	1.24	+	+
4		cf2 + cf3 (2)	06.03.89	1.24	+	+
5		cf3 (1)	04.04.89	1.24	+	n.t.
6		cf3 (1)	10.04.89	1.24-1.28	+	n.t.
7^b	11.1988	cf4 (1)	18.04.89	1.23	+	n.t.
8	04.1989	MRD25-21A (1)	13.06.89	1.24-1.29	+	n.t.
9^{a,b}		MRD19 + MRD20 (2)	16.10.89	1.25	+	+
29		MRD19 + MRD20 (2)	16.10.89	1.26	+	n.t.
30	09.1989	MRD19 + MRD20 (2)	16.10.89	1.24	+	+
31		MRD19 + MRD20 (2)	16.10.89	1.28	-	-
32		MRD19 + MRD20 (2)	16.10.89	1.23	+	+
10	09.1989	MRD37.14 (1)	30.10.89	1.27	+	n.t.
11^b		MRD37.14 (1)	30.10.89	1.26	+	n.t.
21 ^c		MRD caged (unknown)	16.08.88	1.21-1.29	+	n.t.
22^{b,c}	07.1988	MRD caged (unknown)	23.08.88	1.27-1.29	+	n.t.
23 ^c		MRD caged (unknown)	23.08.88	1.23	+	n.t.
24		TT17-7 + 12-86-14 + 12-86-15 (3)	07.11.88	1.27-1.29	+	n.t.
25		TT17-7 + 12-86-14 + 12-86-15 (3)	07.11.88	1.21-1.23	+	n.t.
26^b	09.1988	TT17-7 + 12-86-14 + 12-86-15 (3)	14.11.88	1.23-1.27	+	+
27		TT17-7 + 12-86-14 + 12-86-15 (3)	14.11.88	1.29	+	+
28		TT17-7 + 12-86-14 + 12-86-15 (3)	14.11.88	1.23	+	+

Samples in which DNA or virions were detected by polyacrylamide gel electrophoresis (PAGE) or electron microscopy (EM), respectively, are indicated by (+), or (-) if not detected; n.t. = not tested. MRD, Malayan Red Dwarf; cf2 and other identifiers indicate that variety or hybrid source was not recorded

a - CFD samples from which RCA-DNA was used for cloning;

b - CFD samples from which RCA-DNA was pooled for deep sequencing;

c - CFD samples from caged MRD plants inoculated with viruliferous *Myndus taffini*.

Table S2. Viral DNAs identified in two CFD samples on the basis of similar RFLP patterns and sequencing

Sample	DNA	Size nt	Enzyme ^a					
			<i>AatI</i>	<i>EcoRI</i>	<i>BamHI</i>	<i>KpnI</i>	<i>AgeI</i>	<i>SaI</i>
CFD9	DNA-S.1	1286	12 ^b	7				
	DNA-S.2	1263	3	3				
	DNA-gamma	641				22		
	CFDAR	1271			6			
	CFDA2	1277			14			
	CFDA3	1252			3			
	CFDA4	1276			2	6		
	CFDA7	1259	3					
CFD3	CFDA1	1291					3	
	CFDA6	1264						3

a – DNA fragment obtained upon digestion of RCA DNA by a given enzyme was cloned into Litmus28 (for *AatI* and *AgeI* fragments) or pBluescript KSII (+)

b - number of clones showing similar RFLP pattern: insert DNAs of recombinant plasmids were amplified by PCR using M13 direct and reverse primers, and the PCR products were digested by *HaeIII* or *Sau3A* enzymes.

Table S3. Oligonucleotides used

DNA	Primer name (direction)	Sequence (5'-3')	T _m , C°	Use ^a
CFDV DNA-S.1	CFDV_S1-HindIII-dir	CCCATTAAGCTTAAAGCCCATTAAGCGATGAC	66.9	1
	CFDV_S1-HindIII-rev	GGCTTTAAGCTTAATGGGCTTCACGCAATTAC	68.2	1
	CFDV_S1-590_dir	CATTGGCGTTTTGTGCTCGATAGAG	62.7	2
	CFDV_S1-1160-rev	CTGCTCATCTATGCCCGCCA	63.7	2
CFDV DNA-S.2	CFDV_S2-810-BspEI-dir	TTCCGGAGATACTGCCG	55.2	1, 2
	CFDV_S2-810-BspEI-rev	TATCTCCGGAATACTTCA	49.1	1, 2
	CFDV_S2-590-dir	CATTGGCCTTGGTGTGGACAAAAT	61.0	2
CFDV DNA-S.1 & DNA-S.2	CFDV_S2-1160-rev	TCTGCTCAAGCTCTGCTCATCC	62.1	2
	CFDV-CP1&2-dir	CCGGAGGAATAATGTACACGAAGAAG	63.2	
	CFDV-CP1&2-dir	CCTCCGGCATATAAAGCCGGTCTTC	66.3	
DNA-S.1 related ^b	CFDV_STL1-dir	CGGCCTAGTATTACCCGCCACGCTC	71.1	1
	CFDV_STL1-rev	ACTAGGCCGCCACGCTTATATACAGAGC	69.5	1
DNA-S.2 related ^c	CFDV_STL2-dir	TGCTCCCTACCTCTGCTCATCCTG	66.1	1
	CFDV_STL2-rev	CAGAGCAGAGGTAGTGAGC	58.8	1
CFDV DNA- gamma	CFDV-gamma-dir	TGGCGCTGCTCGCCCGCTTCGCTCTGGAGC	75	1, 2
	CFDV-gamma-rev	GCAGCGCCATTAGCTTCTTCCAATTCACCTC	69.5	1, 2
CFDAR	CFDAR-BamHI-dir	CATAGAGGATCCGAAAAGAAATTTGATTCTCG	64.4	1, 2
	CFDAR-BamHI-rev	CTTTTCGGATCCTCTATGTACTGATACAGGAC	66.9	1, 2
CFDA1	CFDA1-dir	CTTGGCTATAAATGGGTTCTCTCC	60.6	2
	CFDA1-rev	CTTCTCCAGCAAGCTACTCACC	62.1	2
CFDA2	CFDA2-392-SstI-dir	TGCTGAGCTCCGCTTGAAGAACCTGG	69.5	3
	CFDA2-696-BamHI-rev	CTTCGGATCCTCTATGTACTGGTACAGTAC	66.8	3
	CFDA2-510-dir	ATTGGCAACTTGAGTTGCTCAG	58.4	2
	CFDA2-930-rev	ACAAATGCTAATTGATTATTCAAATATCAA	55.8	2
CFDA3	CFDA3-398-SstI-dir	ATTTGAGCTCGTTCCCCGGTTGTAGTTG	68.1	2, 3
	CFDA3-789-BamHI-rev	TTTCGGATCCTTCATCGATATAGGAAAATA	61.3	2, 3
CFDA4	CFDA4-262-BamHI-dir	GACAGGATCCACCTCGAACAGGCACAC	69.5	2, 3
	CFDA4-563-BamHI-rev	TCGTCAGCTTGCTTTGGATTTGAGACAGC	68.1	2, 3
CFDA5	CFDA5-dir	AGCAGCCGTGCTCGACGA	60.5	1, 2
	CFDA5-rev	CCGATGAGCAGGGGTATG	58.2	1, 2
CFDA6	CFDA6-dir	GTGGGTCCCACCTTGTTGGACTTTGTGGG	69.5	1
	CFDA6-rev	TGGGACCCACAAGGAGCGTCTGTGCGAC	71.0	1
	CFDA6-KpnI-1159-dir	TGTGGGTACCACTTGTTGGACTTTGTGGGTA	68.1	1,3
	CFDA6-KpnI-1172-rev	AAGTGGTACCCACAAGGAGCGTCTGTGCGA	69.5	1, 2,3
	CFDA6-402-SstI-dir	ATTGGAGCTCGTTCCCCGGTTCTTGTT	68.0	2, 3
	CFDA6-789-BamHI-rev	TTTCGGATCCTTCATCGATATAGGAAAATA	61.3	3
CFDA7	CFDA7-319-SstI-dir	CATGGAGCTCCGACGCGTCTGGTT	69.5	2, 3
	CFDA7-523-rev	ACCACAGCTGTAAAACCACTTTTCATTAAG	62.7	2, 3
CFDA8	CFDA8-210-rev	TTGTAAGTCCCGCCACCGGAC	65.8	2
	CFDA8-540-dir	CTTGAATTGTGTCTCAAATCGACGGA	63.4	2
	CFDA8-200-dir	AAGTCCGGTGGCCGGACAGTACAA	66.1	1
	CFDA8-200-rev	CCACCGGACTTCAGATGGAGGAAC	66.1	1
nanovirid DNA-R ^d	1 - b-KNG-dir	AARATGGnATnaTnCArAGCGGrAARtA	61.7	1
	2 - b-KNG-rev	TAYTTyCCGCTyTgnATnCCATTyTT	61.7	1
	3 - nanoEYIE-rev	GAYTTrTCrTAnGTGTTACAACACTCmTCdATrTAYtC	67.1	1
	4 - nanoEYIE-dir	GArTAYATHGAKGAGTGTGTAAACACnTAYGAYAArTC	67.1	1
	5 - babu-EYL-dir	AATACCTTCGAYAGAAGTArAGATACATTATACAG	63.6	1
	6 - babuEYL-rev	rTCGAAGGTATThGGACArTCrTATAAATACTC	64.3	1

a – a pair of primers indicated by the same number was used for PCR amplification of the corresponding DNA for the following purposes: (1) full-length cloning, (2) component-specific detection by PCR, (3) amplification of the fragment for construction of redundant copies of the component;

b – primers designed based on the DNA sequence in the region with potential stem-loop structure (STL) of CFDV DNA-S.1;

c – primers designed on the basis of the DNA sequence of STL region of CFDV DNA-S.2;

d – primers designed on the basis of the alignment of the nanovirid DNA-R sequences.

Table S4. Mapping of primary reads from deep sequencing data of three BBTV samples

Sample	BBTV-Hawaii_2013 (26,758,442 total reads)			BBTV-Nigeria_2013 (25,761,084 total reads)			BBTV-Vietnam_2013 (34,536,988 total reads)		
	Reads	% of reads	Cov. Mean	Reads	% of reads	Cov. Mean	Reads	% of reads	Cov. Mean
DNA-R	1,671,973	6.3	149,484	562,089	2.2	50,015	731,405	2.1	65,039
DNA-S	571,790	2.1	50,314	67,470	0.3	5,952	3,042,114	8.8	271,294
DNA-M	1,306,112	4.9	122,536	264,262	1	24,898	1,302,658	3.8	124,545
DNA-N	7,240,846	27.1	657,758	6,323,995	24.6	565,927	5,100,799	14.8	460,561
DNA-C	5,137,818	19.2	502,970	893,580	3.5	87,631	850,602	2.5	82,171
DNA-U3	8,579,130	32.1	797,873	5,282,003	20.5	487,589	3,922,111	11.4	363,683
BBTA2 ^a							3,161,344	9.2	279,229
total	24,507,669	91.6		13,393,399	52		18,111,033	52.4	

Geneious mapping of total reads against all identified (*de novo* assembled) BBTV DNAs, performed with the settings allowing 10% mismatch per read. Cov. Mean – average coverage.
a - a variant of the proposed alphasatellite species BBTA2²⁵

Table S5. CFDV mutant spectra

DNA	Year	Total	Type of base changes													transitions	transversions	deletions	insertions
			A C	A G	A T	C A	C G	C T	G A	G C	G T	T A	T C	T G					
DNA-R	1989														0	0			
	2013	2							1						0	1		1 (+1)	
DNA-S.1	1989																		
	2013	1											1						
	2015	0													0	0			
DNA-S.2	1989							1					1	1	2	1			
	2015	3													0	0			
CFDA1	1985														0	0			
	1988	2						1	1						2	0			
	1989	1											1		1	0			
	2013	20	1	1			2	3	3	2	3		1	2	7	11	1 (-3)	1 (+1)	

Number and type of changes between the most recent and respective previous sampling dates are shown. For calculation of the nucleotide substitution rates per site per year the deletion of three nucleotides is counted as one event.

Table S6. Cloning of redundant copies of CFD associated DNAs used for replication assays

DNA	Primers used for PCR amplification of monomer ^a	Monomer ^b	Primers used for PCR amplification of 0,2 mer ^c	Redundant copy in pBin19
CFDV DNA-S.1	CFDV_S1-HindIII-dir CFDV_S1-HindIII-rev	pBluescriptIIKS(+)-HindIII-S1-5		Dimer first obtained by cloning a monomer into pBluescriptIIKS(+) HindIII site and then transferred into pBin19 as BamHI-KpnI fragment
CFDV DNA-S.2	CFDV_S2-810-BspEI-dir CFDV_S2-810-BspEI-rev	pBluescriptIIKS(+)-BspEI-S2-4		Dimer first obtained by cloning a monomer into pBluescriptIIKS(+) BspEI site and then transferred into pBin19 as BamHI-KpnI fragment
CFDV DNA-gamma		pBluescriptIIKS(+)-KpnI-mini-6		Direct cloning of a monomer into KpnI site of pBin19 with selection of dimer insertion
CFDAR	CFDAR-BamHI-dir CFDAR-BamHI-rev	pBluescriptIIKS(+)-BamHI-AR-1		Direct cloning of a monomer into BamHI site of pBin19 with selection of dimer insertion
CFDA1		Litmus28-AgeI-A1-6 ^d		Dimer first obtained by cloning a monomer into Litmus28 AgeI site and then transferred into pBin19 as BamHI-KpnI fragment
CFDA2		pBluescriptIIKS(+)-BamHI-A2-2	CFDA2-392-SstI-dir CFDA2-696-BamHI-rev	302 bp fragment was PCR amplified using indicated primers, digested by SstI and inserted into pBin19-(SstI-HindIIIblunt) ^e plasmid. CFDA2 monomer was consequently inserted into BamHI site of this plasmid thus giving rise to CFDA2 1,2 mer.
CFDA3		pBluescriptIIKS(+)-BamHI-A3-24	CFDA3-398-SstI-dir CFDA3-789-BamHI-rev	390 bp fragment was PCR amplified using indicated primers, digested by SstI and inserted into pBin19-(SstI-HindIIIblunt) plasmid. CFDA3 monomer was subsequently inserted into BamHI site of this plasmid thus giving rise to CFDA3 1,2 mer.
CFDA4		pBluescriptIIKS(+)-BamHI-A4-14	CFDA4-262-BamHI-dir CFDA4-563-BamHI-rev	300 bp fragment was PCR amplified using indicated primers, digested by BamHI and inserted into pBin19-(BamHI-HindIIIblunt) ^f plasmid. CFDA4 monomer was consequently inserted into BamHI site of this plasmid thus giving rise to CFDA2 1,2 mer.
CFDA6	CFDA6-KpnI-1159-dir CFDA6-KpnI-1172-rev	pBluescriptIIKS(+)-HincII-A6-7	CFDA6-402-SstI-dir CFDA6-789-BamHI-rev	360 bp fragment was PCR amplified using indicated primers, digested by SstI and inserted into pBin19-(SstI-HindIIIblunt) plasmid. CFDA6 monomer, liberated as KpnI fragment, was ligated, ligation product digested by BamHI and inserted into BamHII site of the plasmid with cloned 360 fragment, thus giving rise to CFDA6 1,2 mer.

CFDA7	Litmus28-AatII-A7-15	CFDA7-319-SstI-dir CFDA7-523-rev	320 bp fragment was PCR amplified using indicated primers, digested by SstI and inserted into pBin19-(SstI-HindIIIblunt) plasmid. CFDA7 monomer, liberated as AatI fragment, was ligated, ligation product digested by HindIII and inserted into HindIII site of the plasmid with cloned 320 fragment, thus giving rise to CFDA7 1,2 mer
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- a - PCR performed on RCA DNA of sample CFD_88/89
- b - plasmids used to clone a given DNA in the restriction site indicated, numbers at the end identify the recombinant plasmids
- c - viral DNA from the plasmid shown under "Monomer" was used for PCR amplification
- d - if primers are not indicated in the second column, viral DNA was cloned as monomer after restriction of RCA DNA into the corresponding plasmid, see Table S2 for enzymes used.
- e - pBluescriptIIS(+) plasmid was digested by *HindIII*, the ssDNA ends resulting after digestion were filled using dNTPs and Klenow polymerase. After purification by phenol extraction and ethanol precipitation the plasmid DNA was digested by *SstI*.
- f - pBluescriptIIS(+) plasmid was digested by *HindIII*, the ssDNA ends resulting after digestion were filled using dNTPs and Klenow polymerase. After purification by phenol extraction and ethanol precipitation the plasmid DNA was digested by *BamHI*.

Table S7. GenBank accession numbers of CFD associated DNAs

Year \ DNA	1985	1988	1989	2013	2015
DNA-S.1			MF926436	MF926437	MF926438
DNA-S.2			MF926439		MF926440
DNA-gamma			MF926441	MF926442	MF926443
CFDAR			MF926434	MF926435	
CFDA1	M29963	MF926423	MF926424	MF926425	
CFDA2			MF926426		
CFDA3			MF926427		
CFDA4			MF926429	MF926444	
CFDA5			MF926430		
CFDA6		MF926431			
CFDA7			MF926432		
CFDA8					MF926433