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Article

# Subterranean Clover Stunt Virus Revisited: Detection of Two Missing Genome Components

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**Abstract:** Subterranean clover stunt virus (SCSV) is a type species of the genus *Nanovirus* in the family *Nanoviridae*. It was the first single-stranded DNA plant virus with a multipartite genome, of which genomic DNA sequences had been determined. All nanoviruses have eight genome components except SCSV, for which homologs of two genome components present in all other nanovirus genomes, DNA-U2 and DNA-U4, were lacking. We analysed archived and more recent samples from SCSV-infected legume plants to verify its genome composition and found the missing genome components. These results indicated that SCSV also has eight genome components and is a typical member of the genus *Nanovirus*.

**Keywords:** nanovirus; virus evolution; rolling circle replication; high-throughput sequencing

## 1. Introduction

Nanovirids, members of the genera *Babuvirus* and *Nanovirus* of the *Nanoviridae* family, are plant viruses with a genome consisting of multiple circular single-stranded (ss) DNA molecules that are individually encapsidated in small isometric particles of about 20 nm in diameter [1]. These viruses are transmitted in a circulative persistent (non-propagative) manner by various aphid species [2]. Currently, there are eight species approved by the International Committee on Taxonomy of Viruses in the Genus *Nanovirus*: *Black medic leaf roll virus* [3], *Faba bean necrotic stunt virus* [4], *Faba bean necrotic yellows virus* [5,6], *Faba bean yellow leaf virus* [7], *Milk vetch dwarf virus* [8], *Pea necrotic yellow dwarf virus* [9], *Pea yellow stunt virus* [3], and *Subterranean clover stunt virus* [10]. Subterranean clover stunt virus (SCSV) is the type species of the genus *Nanovirus* [1,11] as it is the first nanovirus of which

genome components have been sequenced [12]. Recently, two new nanovirus species have been discovered, one from the wild perennial legume *Sophora alopecuroides* L. in Iran [13] and another one from *Vicia cracca* L. in France [14], providing further evidence for *Fabaceae* being the principal hosts of nanoviruses.

With the exception of SCSV, the genomes of all nanoviruses, including those of the two recently described nanoviruses Sophora yellow stunt associated virus (SYSaV) [13] and cow vetch latent virus (CvLV) [14] comprise eight characteristic ssDNA circles of about 1 kb in size. The genome components are named according to the functions of the respective encoded proteins: DNA-R (master replication initiator protein, M-Rep, required for replication initiation of all genomic DNAs) [15], DNA-S (structural = capsid protein, CP) [5], DNA-C (cell cycle link protein, Clink, modulates the host's cell cycle in favour of nanovirus replication) [16], DNA-M (movement protein, MP) and DNA-N (nuclear shuttle protein, NSP). The functional identities of MP and NSP were inferred from similarities with the corresponding geminivirus proteins [17–19]. In addition, three other DNA molecules, DNA-U1, DNA-U2 and DNA-U4, are typical constituents of a nanovirus genome, however, the function of the proteins they encode are hitherto not known. Only interaction data of the U1 and U4 proteins with other nanovirus proteins and subcellular localization data are available [20]. The U1 and U2 proteins of faba bean necrotic stunt virus (FBNSV) are essential symptom determinants whereas the U4 protein is dispensable for symptom formation and aphid transmission [21]. The NSP is also not required for FBNSV symptom development but is obligatory for the spread of the virus as it acts as mandatory helper factor for nanovirid aphid transmission [21]. Moreover, the NSP of pea necrotic yellow dwarf virus has been shown to interact with a plant G3BP-like protein, a constituent of stress granules [17].

Despite the wealth of biological data available for the subterranean clover stunt disease and its causative virus [22–28], SCSV remained the sole nanovirus with only six integral genome components, lacking DNA-U2 and DNA-U4 [1,10,15]. This prompted us to elucidate whether SCSV, thus far only found in Australia, might have a genome composition different from those of all other nanoviruses, implying that the two absent components might be dispensable in SCSV infections.

In this study, we have detected in various SCSV samples from three different hosts the missing genome components DNA-U2 and DNA-U4, providing evidence that SCSV has eight genome components, the same as all currently known members of the genus *Nanovirus*.

## 2. Materials and Methods

### 2.1. Virus Isolates and Field Materials

SCSV isolate F [29] and SCSV isolate SCS1 (unpublished) isolated from field-infected subterranean clover (*Trifolium subterraneum*) were maintained in the same host for several years by successive transmissions at CSIRO, Canberra, using the cowpea aphid (*Aphis craccivora*) [12] and were provided in 2001 to H.-J.V. In this study, the SCSV F provided in 2001 is designated SCSV-[AU;F\*] to differentiate it from the SCSV F isolate (designated as SCSV-[AU;F]) that was sampled in 1992 and whose genome sequence had been published in 1995 [10]. SCS1 is designated as SCSV-[AU;SCS1].

Two additional SCSV-infected field samples collected and deposited by the late M. Schwinghamer in the New South Wales Plant Pathology Herbarium (DAR), Orange, NSW, were SCSV-[AU;2534B] from faba bean (*Vicia faba*) (DAR collection number 76607), collected at Myall Vale in 1997, and SCSV-[AU;3771A] from pea (*Pisum sativum*) (DAR collection number 76849), collected at Quandialla in 2003. They were sent in 2012 to B.G. as dried leaf tissue.

To complement analyses of these archived samples, we collected in December 2018 at Harrison, ACT, seventy-two field samples of subterranean clover and checked fourteen of them for the presence of SCSV. SCSV from these samples is designated SCSV-[AU;Har].

## 2.2. DNA Extraction, RCA, PCR, Cloning, and Sequencing

Total DNA was extracted from SCSV-infected dried tissue according to a modified Edwards protocol as described previously [4] with an additional step of phenol-chloroform extraction. Total DNA preparations were subjected to rolling circle amplification (RCA) to increase the amount of circular DNA using an Illustra TempliPhi Amplification Kit (GE Healthcare, Little Chalfont, Buckinghamshire, UK) following the manufacturer's instructions. The isothermal incubation for RCA was at 30 °C for 18–20 h followed by a final incubation at 65 °C for 10 min.

RCA products were used as templates for PCR amplification of SCSV genome components using different combinations of primers (Supplementary Table S1) and Phusion DNA polymerase (Thermo Fisher, Waltham, MA, USA) or Taq II DNA polymerase (Eurobio Ingen, Les Ulis, France). For this, 1 µL of tenfold diluted RCA product was added to 20 or 50 µL of reaction mixture containing 10 pmol each of corresponding primers, 50 µM of each deoxynucleoside triphosphate, and 0.5 units of DNA polymerase in provided reaction buffer. In addition, PCR amplifications using degenerate primers were performed using Taq II DNA polymerase. Amplification conditions are specified in Supplementary Table S1.

PCR products were either sequenced directly using a commercial sequencing provider (Eurofins Genomics, Ebersberg, Germany) or inserted into plasmid pBluescript KSII(+) (pBKSII) (Stratagene/Agilent, Santa Clara, CA, USA) linearized by *EcoRV* or *HincII* restriction endonucleases. Insert DNAs of recombinant plasmids were sequenced, and sequences were analysed with DNASTAR Lasergene version 8.0.2 (DNASTAR, Inc. Madison, Wisconsin, USA) and Geneious version 8.1.8 (Biomatters, Auckland, New Zealand).

Libraries for deep sequencing were prepared from diluted (1:10 *v/v*) RCA products using the Nextera XT Library Kit (Illumina, San Diego, CA, USA). Quality checked libraries were sequenced using Illumina MiSeq (paired end reads 2 × 301 bp) (DSMZ, Braunschweig, Germany).

## 2.3. Sequence Assembly and Analysis

Illumina reads were trimmed, and de novo assembled using the Geneious assembler v. 10.2.3 (assembler settings: Low Sensitivity/Fast and circularized contigs). Local BlastN and BlastP against the plant virus reference databank from the National Center for Biotechnology Information (accessed on 11 June 2018) identified contigs matching nanovirus sequences. To generate the final consensus sequence for the genome molecules from a given isolate, all Illumina reads of the respective isolate were mapped in a single step to all its genome components (Low Sensitivity/Fastest [10%]).

Multi-alignments were done using MUSCLE [30] as implemented in Geneious or MEGA7 [31]. Phylogenetic analyses were conducted in MEGA7 and SDT v. 1.2 [32]. Maximum likelihood trees (100 bootstrap repetitions) were constructed using MEGA7 or PhyML [33], with nucleotide substitution models chosen by model test in MEGA7.

## 3. Results

### 3.1. Identification of SCSV DNA-U2 and DNA-U4

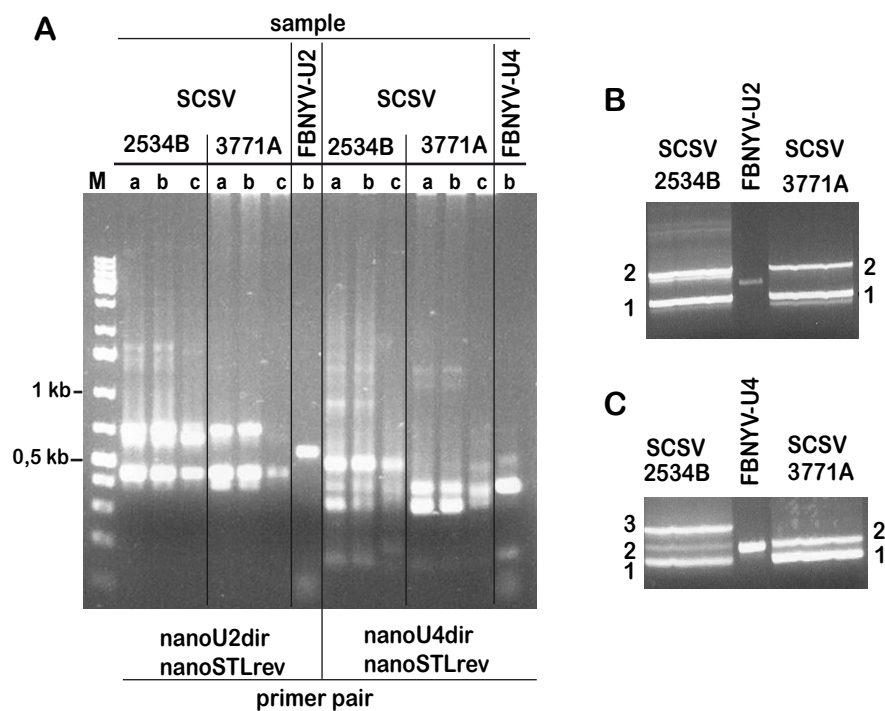
Alignment of the DNA-U2 and DNA-U4 sequences of all nanoviruses known in 2014 [3] indicated small regions of similarity in their respective coding parts (Supplementary Figures S1 and S2). This allowed the design of the degenerate primers nanoU2dir and nanoU4dir (Supplementary Table S1). Stem-loop regions of nanovirus DNA-U2 and DNA-U4 share longer stretches of sequence identity, which allowed the design of the primers nanoSTLdir and nanoSTLrev. We used these primers to potentially amplify SCSV DNA-U2 and DNA-U4 by gradient PCR with RC-amplified template DNA derived from isolates SCSV-[AU;3771A] Quandialla 2003 from pea and SCSV-[AU;2534B] Myall Vale 1997 from the faba bean.

Two major amplification products were obtained from the DNA samples of these isolates when targeting DNA-U2 (Figure 1A). By contrast, amplifications aimed at identifying DNA-U4 yielded three

products for isolate 2534B and two products for isolate 3771A, the proportion of the latter differed slightly depending on the annealing temperature during PCR. Only amplifications at three different annealing temperatures are shown in Figure 1A. Products obtained at different annealing temperatures from the respective isolates were pooled, size-separated by agarose gel electrophoresis (Figure 1B,C), gel-eluted, and directly sequenced using the same primers as for PCR.

The sequences of the amplification products marked “2” (Figure 1B) for DNA from both isolates were similar to published DNA-U2 sequences of other nanoviruses. By contrast, the sequences of the PCR-products of both isolates, marked “1” in Figure 1B, corresponded to the SCSV DNA-R sequence. The sequences of the PCR products from both isolates, marked “1” in Figure 1C, had similarity with published DNA-U4 sequences of nanoviruses. In contrast, the sequences of the products marked “2” (both isolates) and “3” (isolate 2534B only) all corresponded to SCSV DNA-C sequences. Based on the PCR-amplified DNA-U2 and DNA-U4 sequences we designed component-specific back-to-back primers (Supplementary Table S1) and used them to amplify full-length DNA-U2 and DNA-U4 of SCSV. This way we identified, cloned and sequenced DNA-U2 and DNA-U4 from isolates SCSV-[AU;3771A] and SCSV-[AU;2534B].

In addition, we designed specific primers for the thus far known SCSV components DNA-R, DNA-S, DNA-C, DNA-M, DNA-N, DNA-U1, based on sequences of SCSV-[AU;F] [10,34] (Supplementary Table S1). We used these primers to amplify the respective genome components of isolates SCSV-[AU;3771A] and SCSV-[AU;2534B], which we subsequently cloned and sequenced.



**Figure 1.** Amplification of subterranean clover stunt virus (SCSV) DNA-U2 and DNA-U4 fragments by PCR. (A) Gel electrophoresis of PCR products obtained from different nanovirus samples (indicated at the top) using primer nanoSTLrev and the degenerate primers specific for DNA-U2 (nanoU2dir) or DNA-U4 (nanoU4dir), as indicated at the bottom. Letters a, b, and c indicate annealing temperatures of the PCR reactions: 45.0, 53.7 and 65.0 °C respectively; (B) preparative gel-electrophoresis of the same PCR products as in (A) for DNA-U2 amplification; (C) preparative gel-electrophoresis of the same PCR products as in (A) for DNA-U4 amplification.

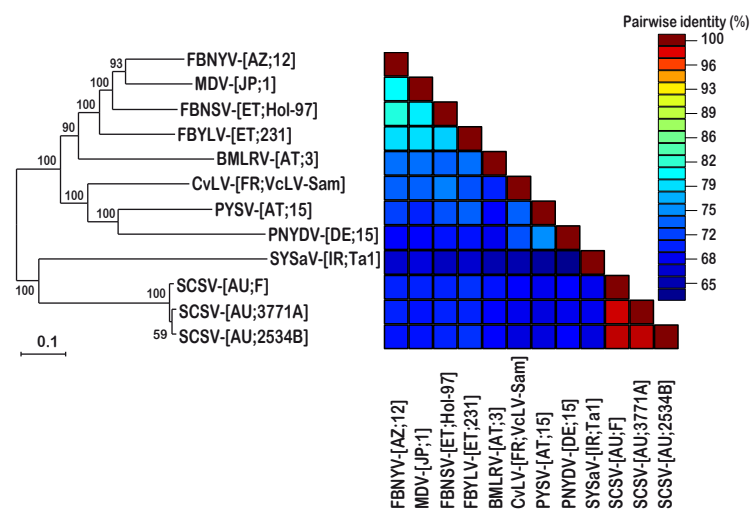
By the same way as described for SCSV-[AU;3771A] and SCSV-[AU;2534B] we also obtained a complete copy of DNA-U2 from isolate SCSV-[AU;F\*]. However, we never obtained any PCR products

from SCSV-[AU;F\*] DNA when using the various DNA-U4 primers listed in Supplementary Table S1. Remarkably, using RC-amplified DNA derived from another archived SCSV isolate, SCSV-[AU;SCS1], which had also been maintained in subterranean clover for a prolonged period, we did not detect any DNA-U4 either when using the U4-specific primers of Table S1 whereas other DNAs (e.g., DNA-U2, DNA-R, DNA-S) were detected by PCR.

### 3.2. DNA Sequence Analysis and Comparison of Three Different SCSV Isolates

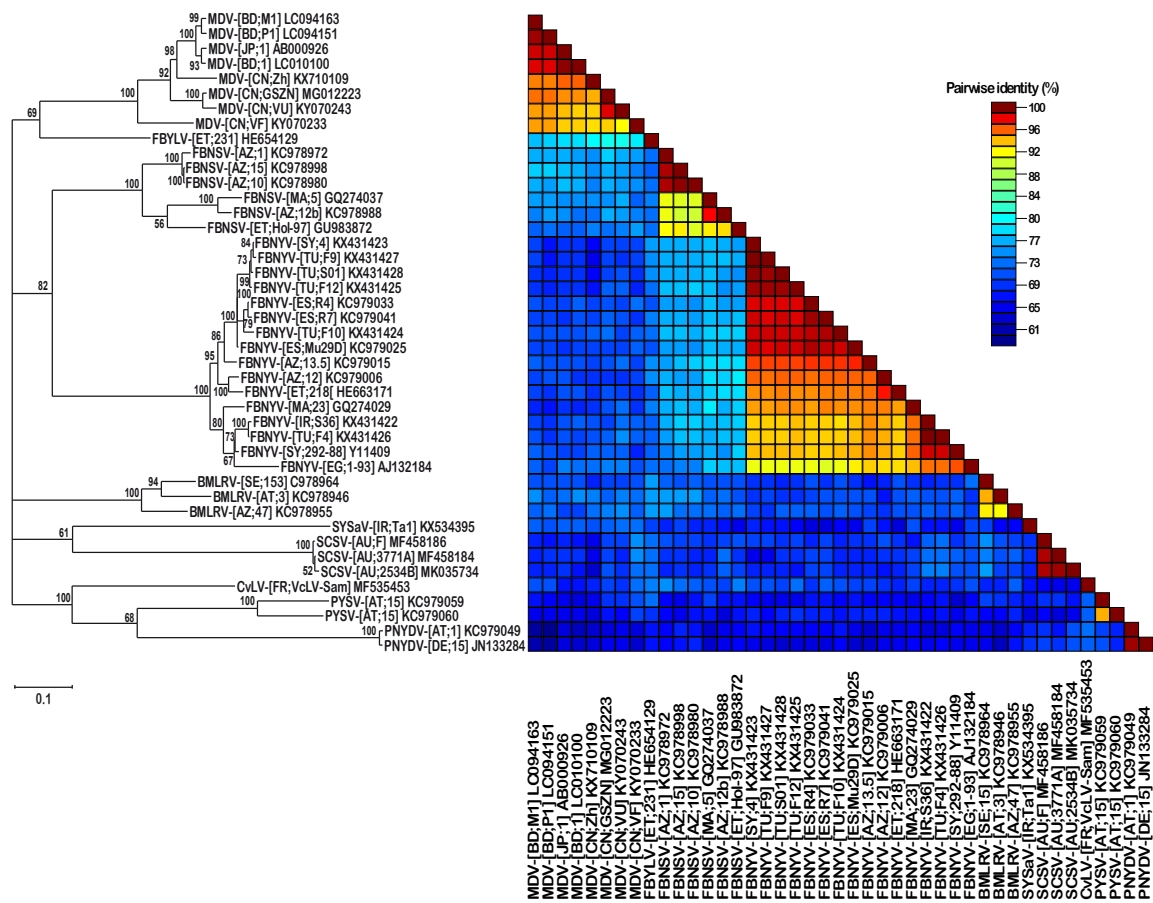
For an unbiased analysis of the SCSV genome and to assure that we did not miss any SCSV and SCSV-associated DNAs in our PCR and cloning experiments, RCA DNA from all three isolates were subjected to MiSeq sequencing, complementing any previous information obtained by Sanger-sequencing of the cloned eight integral nanovirus genome components of SCSV-[AU;3771A]. Thus, eight nanovirus genomic DNA components were identified for SCSV isolates Quandialla 2003 (SCSV-[AU;3771A]) and Myall Vale 1997, SCSV-[AU;2354B] while RCA preparations of SCSV-[AU;F\*] did not contain any DNA-U4. The number of reads and the average coverage per genome component by deep sequencing of DNA from the three SCSV isolates are shown in Supplementary Table S2.

The comparison of SCSV genome sequences with those of selected isolates of nanovirus species using concatenated genomic DNAs (in order of DNA-R, -S, -C, -M, -N, -U1, -U2, and -U4) showed sequence differences of 22–37% between them while the genomes of the three SCSV isolates were 97–98% identical (see Supplementary Table S4) and formed a separate clade from other nanovirus sequences upon phylogenetic analysis (Figure 2).



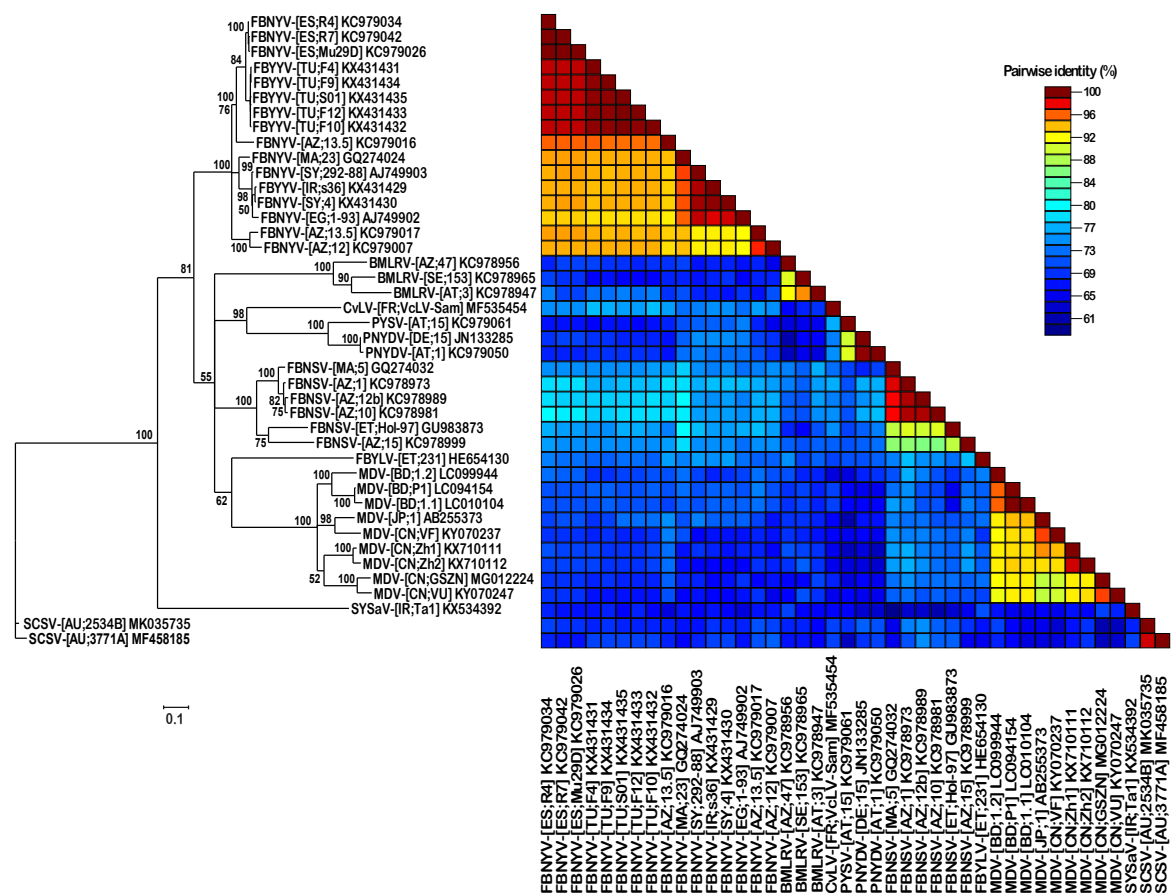
**Figure 2.** Maximum likelihood tree of nanovirus genome sequences. Individual genomic DNAs of faba bean necrotic yellows virus (FBNYV), milk vetch dwarf virus (MDV), faba bean necrotic stunt virus (FBNSV), faba bean yellow leaf virus (FBYL V), black medic leaf roll virus (BMLRV), cow vetch latent virus (CvLV), pea yellow stunt virus (PYSV), pea necrotic yellow dwarf virus (PNYDV), Sophora yellows associated virus (SYSaV), and subterranean clover stunt virus (SCSV) were concatenated in the order DNA-R, -S, -C, -M, -N, -U1, -U2, -U4 and aligned by MUSCLE. The tree was constructed in MEGA7 using the GTR+G+I nucleotide substitution model and is midpoint rooted. Branch support is shown as per cent bootstrap values. The scale bar represents 0.1 substitutions per site. On the right, a color-coded Sequence Demarcation Tool (SDT) matrix shows per cent pairwise sequence identities.

The SCSV DNA-U2 components identified here had highly similar sequences (98–99% sequence identity) and were clearly distinct (61–70% sequence identity) from the DNA-U2 sequences of all other nanoviruses (Figure 3). Similarly, DNA U4 sequences of SCSV were also distinct from those of other nanoviruses (Figure 4).



**Figure 3.** Maximum likelihood tree of nanovirus DNA-U2 sequences. Nanovirus DNA-U2 sequences were aligned by MUSCLE and the tree was constructed in MEGA7 using the TN93+G+I nucleotide substitution model and is midpoint rooted. Branch support is shown as per cent bootstrap values while low support (<50% bootstrap) branches were collapsed. The scale bar represents 0.1 substitutions per site. The right panel shows per cent pairwise sequence identities in a color-coded SDT matrix.

Interestingly, a second DNA-U4 molecule (DNA-U4.2; GenBank accession MK035736) was identified by deep sequencing in SCSV-[AU;2534B] (Table 1). It is eight nucleotides shorter and represents a recombination event, with nucleotides 934 to 948 of DNA-U4.2 being derived from DNA-C (or DNA-M or DNA-U1) of the same isolate (Supplementary Figure S3). In addition, deep sequencing of DNA of SCSV isolate [AU;3771A] uncovered an additional recombinant DNA-M, showing a recombination event with DNA-R at nucleotide 927 to 958 (Supplementary Figure S4). In both cases, the genome components DNA-M.1 and DNA-M.2 (recombinant, GenBank accession MK291271) and DNA-U4.1 and DNA-U4.2 (recombinant) were about equally abundant (see Supplementary Table S2). Remarkably, the DNA-N molecule identified by deep sequencing of isolate SCSV-[AU;F\*] differed from the original DNA-N of SCSV-[AU;F] [12], available at GenBank (accession U16733), as well as from DNA-N of SCSV-[AU;3771A] and SCSV-[AU;2534B] by a 22 nucleotide deletion (nt 294–316) and a 87 nt recombinant sequence derived from DNA-S (position 896–982) (Supplementary Figure S5). We had found the same deletion in a recombinant DNA-N by an earlier pyro-sequencing of SCSV-[AU;F\*] DNA and had verified its physical existence by sequencing a cloned molecule of DNA-N from SCSV-[AU;F\*].



**Figure 4.** Maximum likelihood tree of nanovirus DNA-U4 sequences. Nanovirus DNA-U4 sequences were aligned by MUSCLE and a midpoint rooted PhyML tree was constructed using the TN93+G+I nucleotide substitution model. Branch support is shown as per cent bootstrap values and low support (<50% bootstrap) branches were collapsed. The scale bar represents 0.1 substitutions per site. The right panel shows per cent pairwise sequence identities in a color-coded SDT matrix.

An overall comparison of the SCSV-[AU;F] and SCSV-[AU;F\*] genome sequences (including the two alpha-satellites) showed a total of 102 changes, counting deletions >20 nt and the recombination as single events (Table S3). This corresponds to a rate of substitutions per site and year of  $1.1 \times 10^{exp-3}$ , which fits well with the substitution rate of  $1.8 \times 10^{exp-3}$  per site and year determined for faba bean necrotic stunt virus (FBNSV) [35].

In addition to the eight SCSV genome components, we found in all three SCSV infected plant samples analysed here the two alpha-satellites described earlier [10], formerly named SCSV C2 (GenBank accession U16731) and SCSV C6 (GenBank accession U16735) and reclassified as subterranean clover stunt alpha-satellite 1 and subterranean clover stunt alpha-satellite 2, respectively [36]. A third alpha-satellite (GenBank accession MK291270), clearly distinct from the two aforementioned alpha-satellites but most closely related (77% sequence identity) to Sophora yellow stunt alpha-satellite 3 (GenBank accession KX534406) [13,36], was found associated with SCSV-[AU;3771A].

The absence of DNA-U4 in the historical isolate SCSV-[AU;F\*] when analysed by deep sequencing prompted us to analyse more recent samples of subterranean clover to assess any potential influence of this host on DNA-U4 maintenance upon infection. Of the seventy-two samples of subterranean clover displaying SCSV-like symptoms, collected in December 2018, fourteen were randomly selected for PCR analyses. When tested by PCR using SCSV component-specific primers ten out of fourteen samples analysed contained SCSV DNAs, in seven of them all eight integral genome components of nanoviruses, including DNA-U2 and DNA-U4, were detected (see Supplementary Table S5). When



RC-amplification preceded the PCR analyses, all eight SCSV components were detected in the ten previously SCSV-positive samples as well as in additional ones with an apparently lower virus content (Supplementary Table S6). We determined the DNA sequences of DNA-U4 components of three different virus isolates from subterranean clover. They were about 96% identical to the DNA-U4 molecules of the SCSV isolates from pea (SCSV-[AU;3771A]) and the faba bean (SCSV-[AU;2534B]) (see Supplementary Table S4).

**Table 1.** Accession numbers of genome DNAs of subterranean clover stunt virus isolates and their associated alpha-satellites.

	DNA	Virus Isolate			
		SCSV-[AU;3771A]	SCSV-[AU;2534B]	SCSV-[AU;F]	SCSV-[AU;F*] <sup>1</sup>
Genome components	DNA-R	MF458178	MK035728	AJ290434	
	DNA-S	MF458179	MK035729	U16734	
	DNA-C	MF458180	MK035730	U16732	
	DNA-M	MF458181 <sup>2</sup>	MK035731	U16730	
	DNA-M.2	MK291271			
	DNA-N	MF458182	MK035732	U16733	MK291272
	DNA-U1	MF458183	MK035733	U16736	
	DNA-U2	MF458184	MK035734	-	MF458186
	DNA-U4	MF458185	MK035735 <sup>3</sup>	-	-
	DNA-U4.2		MK035736		
Alphasatellites	SCSA 1	MK291268	MK035737	U16731	
	SCSA 2	MK291269	MK035738	U16735	
	SYSA 3	MK291270	-	-	

<sup>1</sup> SCSV-[AU;F\*] represents sequences of DNAs after about nine years propagation of the original SCSV-[AU;F] isolate in the laboratory. Only SCSV-[AU;F\*] DNAs with >30 differences from SCSV-[AU;F] were deposited in GenBank. All sequences determined in this study are provided as Supplementary data. <sup>2</sup> This molecule is named DNA-M.1 for isolate SCSV-[AU;3771A] <sup>3</sup> This molecule is named DNA-U4.1 for isolate SCSV-[AU;2534B].

#### 4. Discussion

Using degenerate primers designed on the basis of nanovirus DNA-U2 and DNA-U4 sequences, we were able to amplify and clone the corresponding genome components representing DNA-U2 and DNA-U4 from two SCSV genomes from virus-infected grain legume field samples, SCSV-[AU;2534B] from the faba bean and SCSV-[AU;3771A] from the pea.

A comparison of the SCSV genome with those of other members of the genus *Nanovirus* revealed that the SCSV genome is not so different after all, since it has the same eight integral genome components as other nanoviruses described to date. Like the other members of the genus, SCSV isolates are associated with alpha-satellites. A closer look by deep sequencing uncovered not only the two known alpha-satellites but also a third one, related to Sophora yellow stunt alpha-satellite 3.

We also found several examples of recombinant SCSV genome components, in two cases coexisting with non-recombinant counterparts, e.g., DNA-M.1 and DNA-M.2 in isolate [AU;3771A] as well as DNA-U4.1 and DNA-U4.2 in isolate [AU;2534B]. Another interesting and quite peculiar variant is the single DNA-N molecule found in SCSV-[AU;F\*], which combines a 22 nucleotide deletion 5' of the sequence coding for the transmission factor NSP with a 87 nucleotide recombinant stretch 5' of the common region stem-loop derived from DNA-S. Deletion and recombination occurred in the noncoding region, leaving the amino acid sequence of the NSP unaffected. At which moment during the passaging of SCSV-F such a quite drastic DNA-N mutant had arisen and became established remains open. Intra- and inter-genome recombination appears common among ssDNA viruses [37] and has been found in all nanovirid genomes analysed for recombination, including SCSV [3,38–41].

It remains a matter of speculation why no DNA-U2 and DNA-U4 molecules had been found in earlier molecular analysis of SCSV-[AU;F] [10] from subterranean clover. The most trivial explanation would be that in the cloning experiments described [10,29], the authors simply missed these two DNAs, as had also been the case for the essential DNA-R of SCSV [34].

Another possibility would be that upon prolonged SCSV passaging in subterranean clover by aphid transmission [12], DNA-U2 and DNA-U4 were lost. However, that is not true for DNA-U2 since we detected it in SCSV-[AU;F\*] by both PCR and deep sequencing. By contrast, an explanation for the absence of DNA-U4 in sample SCSV-[AU;F\*] is not as straightforward. Contrary to its presence in the two SCSV genomes from pea (SCSV-[AU;3771A]) and the faba bean (SCSV-[AU;2534B]), we were unable to detect any DNA-U4 in SCSV-[AU;F\*], both by DNA-U4-specific PCR analysis as well as by deep sequencing. Hence, DNA-U4 was most probably lost from the initial subterranean clover isolate of SCSV during initial separation from other viruses or during prolonged maintenance of the isolate by repeated aphid transmissions in the laboratory. Similarly, using component-specific PCR we also did not detect any DNA-U4 in SCSV-[AU;SCS1], an isolate that had been maintained in subterranean clover for a longer period in the laboratory.

To rule out the possibility that the subterranean clover host itself was unable to maintain DNA-U4 along with the other SCSV genome components early during the infection process, we collected recent samples of subterranean clover displaying SCSV-like symptoms. At least ten of the fourteen plants analysed were infected by SCSV, with all ten infected plants containing the eight genomic DNAs characteristic of legume-infecting nanoviruses. This clearly shows that field-infected subterranean clover maintains all eight integral genome components at least until the characteristic disease symptoms develop.

Loss of nanovirus genome components upon successive aphid transfer under laboratory conditions has been observed in several instances and has caused the loss of virus isolates; see for instance reference [4]. A similar loss of several isolates had also happened during laboratory maintenance of SCSV (unpublished).

In this context it should be mentioned that another nanovirus, FBNSV, intentionally devoid of DNA-U4, could be maintained for a prolonged period of more than two years by successive aphid transmissions without any obvious difference from the wild-type virus in symptom severity and transmissibility [21]. In addition, the relative abundance of individual genome components of FBNSV varies according to a host-specific genome formula between faba bean (*Vicia faba*) and barrel clover (*Medicago truncatula*) [42]. Yet, the reason for the absence of DNA-U4 in the historical SCSV-[AU;F\*] and SCSV-[AU;SCS1] appears to be an unintentional loss from subterranean clover.

Due to the lack of suitable diagnostic tools in early studies, the reservoir hosts of SCSV have not been identified. Among a variety of various leguminous species analysed, the woolly burr medic (*Medicago minima*) appears to be a plausible candidate for a reservoir host as it is preferred over subterranean clover by the cowpea aphid (*A. craccivora*), the principal and most effective vector aphid of SCSV, and also serves as an overwintering host of the vector [25,26]. In addition, the highly susceptible black medic (*Medicago lupulina*) and burr medic (*Medicago hispida*) would represent potential candidates of reservoir hosts [23,43]. Assessing the ecology and epidemiology of SCSV by modern molecular analysis tools may provide attractive new aspects of the subterranean clover stunt disease and its causative agent, the nanovirus SCSV.

## 5. Conclusions

We have shown that SCSV also possesses a genome that comprises eight distinct and integral DNA components, as observed for all members of the genus *Nanovirus*.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/1999-4915/11/2/138/s1>, Figure S1: Multialignment of nanovirus DNA-U2 sequences, Figure S2: Multialignment of nanovirus DNA-U4 sequences, Figure S3: Multi-alignment of SCSV DNA-U4 sequences, Figure S4: SCSV-[AU;3771A]\_DNA-M.1\_MF458181 vs. DNA-M.2\_MK291271\_alignment, Figure S5: SCSV-[AU;F]\_DNA-

N\_U16733 vs. SCSV-[AU;F\*]\_DNA-N\_MK291272 alignment; Table S1: Primers used for amplification of SCSV DNAs, Table S2: Summary of deep sequencing data from three SCSV samples, Table S3: SCSV nucleotide substitutions, Table S4: Pairwise sequence identities of selected nanovirus genomes and DNAs U2 and -U4, Table S5: Detection by PCR of SCSV DNAs in field samples of symptomatic subterranean clover, Table S6: Detection of SCSV DNAs in field samples after rolling circle amplification followed by PCR; Supplementary text file: DNA sequences determined and used for comparisons, in FastA format.

**Author Contributions:** Conceptualization, B.G. investigation, D.K., Q.B., I.G., M.S., J.T., P.C., B.G., T.T.; validation, D.K., B.G., T.T.; visualization, B.G., T.T.; writing—original draft, B.G., D.K., T.T.; writing—review and editing, D.K., Q.B., I.G., S.W., H.-J.V., J.T., P.C., B.G., T.T.; funding acquisition, B.G.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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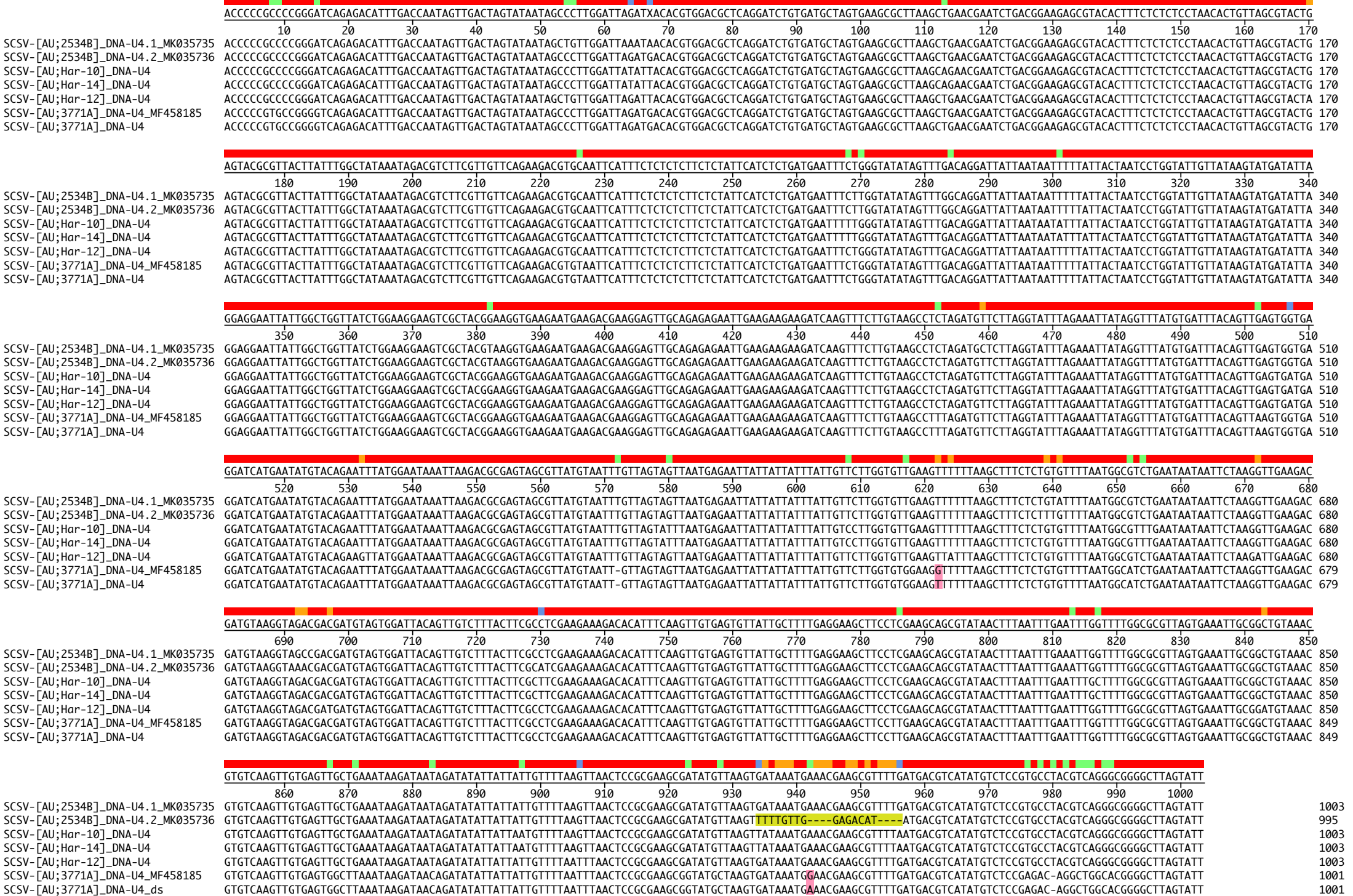
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BMLRV-[AT;3]_DNA-U2 KC978945 452-TCCAATGAAGATATGTTGGGTCAGTTCGTCAGACGCCATGGAAGAAGAGTCA-503
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BMLRV-[AZ;47]_DNA-U2 KC978955 425-TCTAACGAAGATATGTTGGGTCAGTTCGTCAGACGCCATGGAAGAAGGCTCA-476
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nanoU2dir primer TGYAGAYRYCATGGAAGRA

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**Figure S1.** Multialignment of nanovirus DNA-U2 sequences. Alignment of multiple sequences was performed with MultAlign (INRA, Toulouse, URL: <http://multalin.toulouse.inra.fr/multalin/>). Sequences displayed span nucleotides from the respective 5'- to 3'-coordinates indicated. The sequence of the degenerate primer nanoU2dir is shown below the alignment

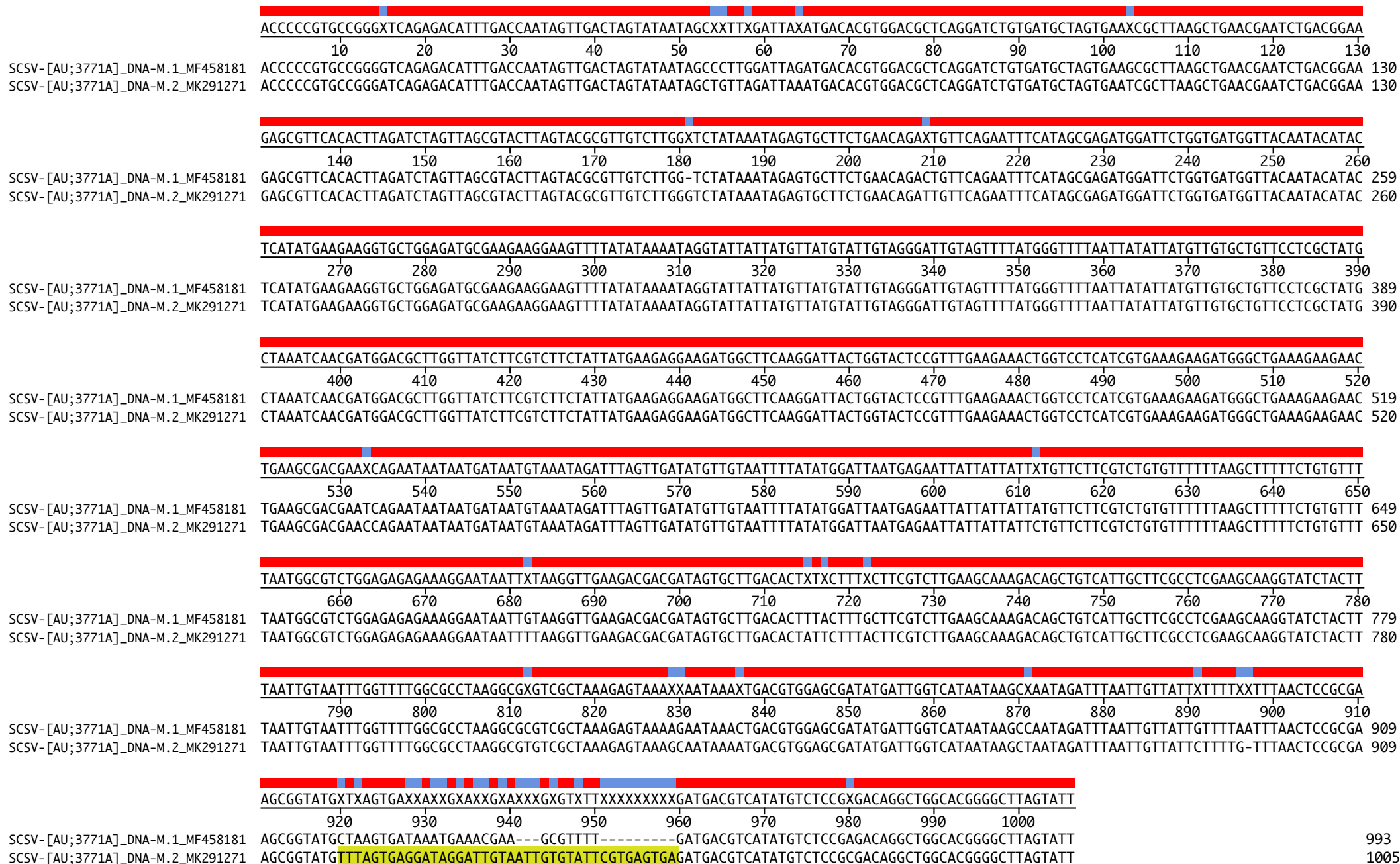
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FBNYV-[ES;R7]_DNA-U4 KC979042	601-ATTCAAAGGTTGATGAAGAAGATGTGGAGTATTTTAAACGGTCTGGCAA-650
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FBNSV-[AZ;10]_DNA-U4 KC978981	623-ACACGAAGGTTGAAGGTGATGATCTGGAATACTTGCAACGCCTATGGGAA-672
FBNSV-[MA;5]_DNA-U4 GQ274032	584-ACACGAAGGTTGAAGGAGATGATCTCGAATACTTGCAACGCCTATGGGAA-633
FBNSV-[ET;Ho1-97]_DNA-U4 GU983873	614-AAACAAAGGTTGAAGGCGATGATCTTGAATATTTACAGCGATTGTGGGAA-663
FBNSV-[AZ;15]_DNA-U4 KC978999	620-ATACGAAGGTCGAAGGTGATGATCTAGACTATCTTAAACGTATGTGGGAA-669
FBYLV-[ET;231]_DNA-U4 HE654130	590-ATACGAAGGTGGACGATGAAGATTTGGAATATTTGCAACGTGTCTGGAAA-639
PYSV-[AT;15]_DNA-U4 KC979061	598-ACGTGAAGGTTGAAGGTGAAGATTTAGAGTATTTACAGTCTATATGGAGA-647
PNYDV-[DE;15]_DNA-U4 JN133285	598-ATGTCAAGGTTGAAGGTGAAGACCTGGAGTATTTACAGTCTTTATGGAAA-647
PNYDV-[AT;1]_DNA-U4 KC979050	597-ATGTCAAGGTTGAAGGTGAAGACCTGGAGTATTTACAGTCTTTATGGAAA-646
MDV-[JP;1]_DNA-U4 AB255373	576-ATACCTCTCTAGACGGTGAAGACCTTGAATATTTGCAACGTTTCTGGGAA-625
BMLRV-[AT;3]_DNA-U4 KC978947	585-ATATGAAGATTGAAGACGATGACCTTGAGTATCTTCAAGGAATATGGGAT-634
BMLRV-[SE;153]_DNA-U4 KC978965	576-ATATGAAGATTGAAGACGATGACCTTGAGTATCTTCAAGGAATATGGGAT-625
BMLRV-[AZ;47]_DNA-U4 KC978956	577-ATATGAAGATTGAAGACGATGACATGGAGTACATCAAAGATTATGGGAG-626
nanoU4dir primer	GGTTGAHGRHGAHGAYSTGGARTA

**Figure S2.** Multialignment of nanovirus DNA-U4 sequences. Alignment of multiple sequences was performed with MultAlign (INRA, Toulouse, URL: <http://multalin.toulouse.inra.fr/multalin/>). Sequences displayed span nucleotides from the respective 5'- to 3'-coordinates indicated. The sequence of the degenerate primer nanoU4dir is shown below the alignment.

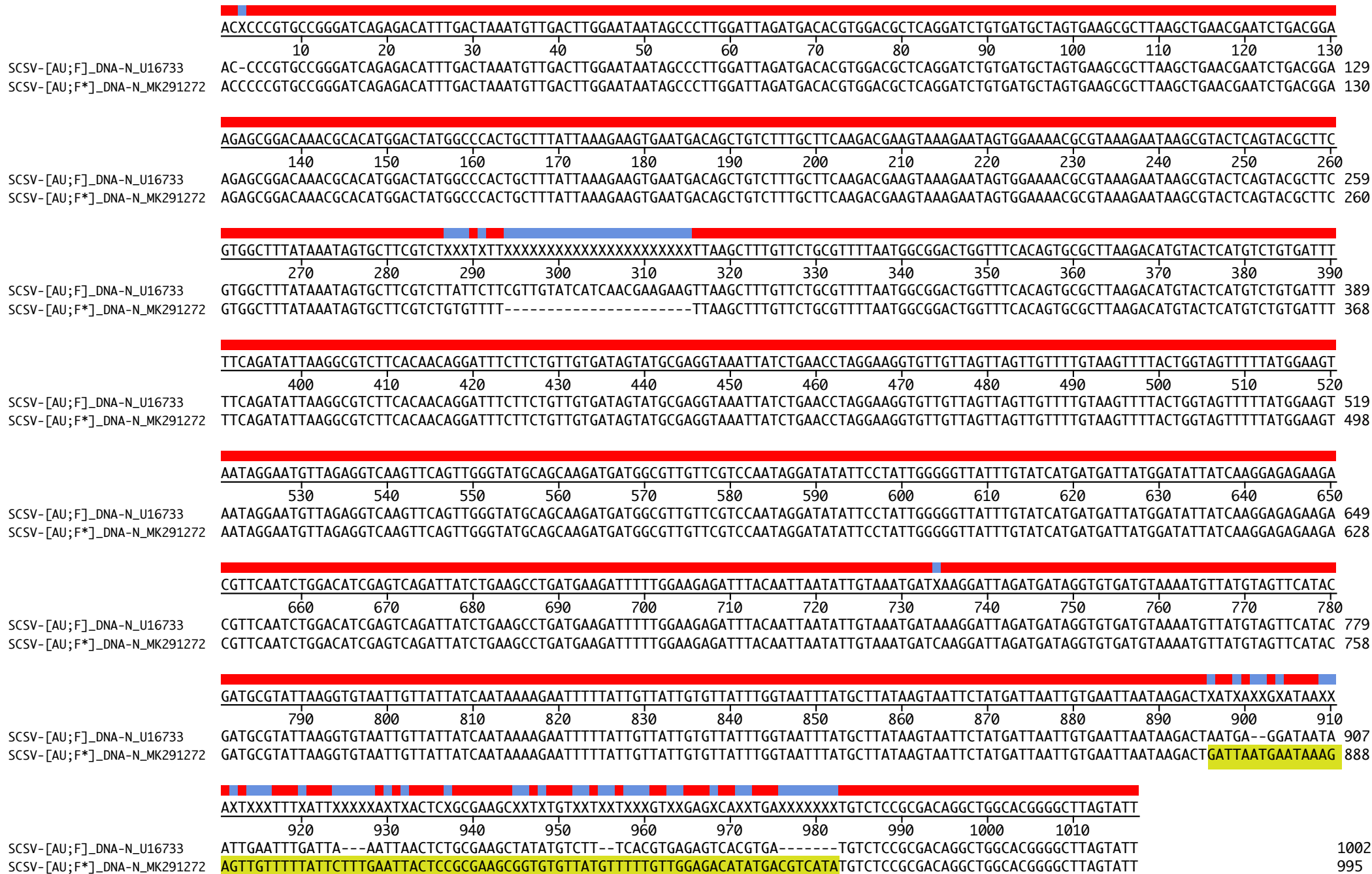


**Figure S3.** Multialignment of SCSV DNA-U4 sequences. Alignment was performed using MegAlign of DNA Star. The yellow box indicates sequence homologous to DNA-C. Pink boxes indicate nucleotide differences between two DNA-U4 sequences from SCSV-[AU;3771A], that of a cloned DNA (DNA-U4\_MF458185) and that of the DNA-U4 consensus sequence determined by deep sequencing (DNA-U4\_ds).





**Figure S4.** SCSV-[AU;3771A]\_DNA-M.1\_MF458181\_vs\_DNA-M.2\_MK291271 alignment. Alignment was performed using MegAlign of DNA Star. The yellow box indicates sequence homologous to DNA-R.



**Figure S5.** SCSV-[AU;F]\_DNA-N\_U16733 vs SCSV-[AU;F\*]\_DNA-N\_MK291272 alignment. Alignment was performed using MegAlign of DNA Star. The yellow box indicates sequence homologous to DNA-S.

**Table S1.** Primers used for amplification of SCSV DNAs

DNA	Primer name (direction)	Sequence (5'-3')	T <sub>m</sub> , C°	Use <sup>a, b</sup>
nanovirus DNAs <sup>c</sup>	nano-STL-dir	GGCTTAGATATTACCCC	49.2	2
	nano-STL-rev	GGTAATACTAAGCCCC	49.2	2 <sup>f</sup>
nanovirus DNA-U2 <sup>d</sup>	nano-U2-dir	TGYAGAYRYCATGGAAGRA	53.4	2 <sup>f</sup>
nanovirus DNA-U4 <sup>e</sup>	nano-U4-dir	GGTTGAHGRHGAHGAYSTGGARTA	61.8	2 <sup>f</sup>
R	SCSV-R-dir	TTCGTCTACAGTCCTTCGTCTCCATCAA	65.1	1, 4
	SCSV-R-rev	GTAGACGAATAATTTGGGTGTATGGCCC	65.1	1, 4
	SCSV-R790-dir	GAATACGTCAATTATGGAGTCATTGAACAGCT	64.4	1
	SCSV-R790-rev	ACGTATTCTCTCGAAGCTTCGAGGAAAGTCG	65.5	1
M	SCSV-M-dir	GAAGATGGGCTGAAAGAAGAAGTGAAGCG	66.7	1, 4
	SCSV-M-rev	CCATCTTCTTTACGATGAGGACCAGTTTC	66.8	1, 4
	SCSV-M390-dir	CTAAATCAACGATGGACGCTTGGTTATC	63.7	1
	SCSV-M390-rev	TGATTTAGCATAGCGAGGAACAGCACAAAC	65.3	1
N	SCSV-N-dir	GGAAGGTGTTGTTAGTTAGTTGTTTTGTAA	61.3	1, 4
	SCSV-N-rev	CACCTTCCTAGGTTTCAGATAATTTACCTCG	65.4	1, 4
	SCSV-N420-dir	GCTTCGTGGCTCTATAAATAGTGCTTCGTCTT	63.4	1
	SCSV-N420-rev	CACGAAGCGTACTGAGTACGCTTATTCTTTACG	65.0	1
C	SCSV-C-dir	GAGTTATGGAGGAGATGCATTACAAGCTT	63.9	1, 4
	SCSV-C-rev	CCATAACTCTCCATTTTCAGATTTA	55.9	1, 4
S	SCSV-S-dir	GGAGGTTAAGCCATTCGCTGATGGTTCAAG	68.1	1, 4
	SCSV-S-rev	TAACCTCCGCTCCAGTAACATCTCTCTTAT	65.4	1, 4
U1	SCSV-U1-dir	GTGATTAACGTGAAGGACTGAGGTT	61.6	1, 4
	SCSV-U1-rev	CGTTAATCACTGAACCTTCTC	56.5	1, 4
U2	SCSV_37-U2frDir	CTAAGATGCCTAGAGATGGACCTGCAAGGTG	69.5	1
	SCSV_37-U2frRev	CTTAGGATAAGCCTTCACTCTTCTTCCATG	65.4	1
	SCSV_37-U2fr2dir	AATTATAGATATCAAGTGTATTACTCTG	56.3	1, 4
	SCSV_37-U2fr2rev	CTATAATTACCATAATCATACAAATCA	54.3	1, 4
	SCSV-U2-224-dir	GATTTGTTCTGGGTTTTATATGAA	55.3	1
	SCSV-U2-224-rev	CAGAACAATCTTGTCTTCTT	54.2	1
U4	SCSV_37-U4fr2dir	GTGAGGATCATGAATATGTACAG	57.1	1, 4
	SCSV_37-U4fr2rev	TCCTCACCACCTAACTGTAAATCAC	59.7	1, 4
	SCSV-U4-340-dir	GGAGGAATTATTGGCTGGTTATCTGGAA	63.7	1,3, 4
	SCSV-U4-340-rev	ATTCTCCTAATATCATACTTATAACAATACCAGG	63.6	1, 4
	SCSV-U4.1-932-rev	CGTTTCATTTATCACTTAACATATCGC	58.9	3
	SCSV-U4.2-932-rev	TCTCCAACAAAACTTAACATATCGC	58.5	3

- a – Primers used for PCR amplification of the corresponding DNAs with the purpose of: (1) entire component amplification and cloning, (2) partial amplification, (3) specific amplification of DNA-U4.1 or DNA-U4.2 fragment by PCR, (4) detection of SCSV DNAs in field samples of symptomatic subterranean clover.
- b – PCR amplifications employing component-specific primers were performed at annealing temperatures corresponding to a minimal T<sub>m</sub> of a given primer pair.
- c – Primers designed based on the DNA sequence in the region with potential stem-loop structure (STL) of nanovirus DNAs
- d – Primers designed on the basis of the alignment of the nanovirusDNA-U2 sequences.
- e – Primers designed on the basis of the alignment of the nanovirus DNA-U4 sequences.
- f - PCR amplifications using nano-STL-rev in combination with a degenerate primer were performed with Taq II DNA polymerase as follows: 95°C, 5 min; 5 cycles of: 95°C, 30 s, 45°C, 45 s, 72°C, 2 min; followed by 35 cycles of: 95°C, 30 s, gradient 45°C to 65°C, 45 s, 72°C, 45 s; final elongation: 72°C, 4 min.

**Table S2.** Summary of deep sequencing data from three SCSV samples

Sample	SCSV-[AU;3771A] (1,519,544 total reads)			SCSV-[AU; 2534B] (1,560,076 total reads)			SCSV-[AU;-F*] (1,984,716 total reads)		
	Reads	% of reads	Cov. Mean	Reads	% of reads	Cov. Mean	Reads	% of reads	Cov. Mean
DNA									
DNA-R	4,273	0.28	6,878	32,450	2.08	6,156	26,548	1.34	4,881
DNA-S	10,419	0.69	1,849	73,136	4.69	14,378	33,825	1.70	6,496
DNA-M	27,366	1.80	4,394	68,165	4.37	12,132	400,292	20.17	72,184
DNA-M.2 <sup>a</sup>	12,979	0.85	2,074						
DNA-N	11,943	0.79	2,072	100,037	6.41	19,303	12,764	0.64	2,343
DNA-C	20,471	1.35	3,602	43,620	2.80	8,152	6,168	0.31	796
DNA-U1	5,727	0.38	929	40,129	2.57	7,436	121,430	6.12	22,534
DNA-U2	2,510	0.17	421	41,474	2.66	7,863	2,513	0.13	431
DNA-U4	86,676	5.70	14,167	109,090	6.99	19,932			
DNA-U4.2				123,375	7.91	22,640			
alphasatellites									
SCSA 1	169,370	11.15	29,851	293,801	18.83	57,449			
SCSA 1.1							91,250	4.60	17,207
SCSA 1.2							93,260	4.70	17,178
SCSA 2	125,028	8.23	22,230	192,094	12.31	38,112	460,008	23.18	89,133
SYSA 3	31,974	2.10	5,520						
total	508,736	33.48		1,117,371	71.62		1,248,058	62.88	

Circularized consensus sequences were generated from *de novo* assembly of deep sequencing reads.

Cov. Mean – average coverage.

a – recombinant with DNA-R

**Table S3.** Nucleotide substitutions

Comparison of two different SCSV isolates	Total changes	% divergence	C/A	G/A	T/A	G/C	T/C	T/G	indel	other
SCSV-[AU;3771A]_DNA-R_MF458178 SCSV-[AU;2534B]_DNA-R_MK035728	12	1,2	1	3	1	1	4	2	0	
SCSV-[AU;3771A]_DNA-S_MF458179 SCSV-[AU;2534B]_DNA-S_MK035729	38	3,8	1	8	8	4	11	5	1	
SCSV-[AU;3771A]_DNA-C_MF458180 SCSV-[AU;2534B]_DNA-C_MK035730	21	1,7	0	5	4	2	3	3	4	
SCSV-[AU;3771A]_DNA-M_MF458181 SCSV-[AU;2534B]_DNA-M_MK035731	50	3,7	2	6	8	6	10	4	14	
SCSV-[AU;3771A]_DNA-N_MF458182 SCSV-[AU;2534B]_DNA-N_MK035732	11	1	1	4	1	0	2	2	1	
SCSV-[AU;3771A]_DNA-U1_MF458183 SCSV-[AU;2534B]_DNA-U1_MK035733	26	2,5	2	9	2	4	5	2	2	
SCSV-AU;3771A]_DNA-U2_MF458184 SCSV-[AU;2534B]_DNA-U2_MK035734	15	1,4	1	4	1	2	3	3	1	
SCSV-[AU;3771A]_DNA-U4_MF458185 SCSV-[AU;2534B]_DNA-U4_MK035735	38	3,7	2	11	2	4	10	7	2	
<b>Sum</b>	<b>211</b>		<b>10</b>	<b>50</b>	<b>27</b>	<b>23</b>	<b>48</b>	<b>28</b>	<b>25</b>	
Comparison of cloned DNAs vs deep sequenced DNAs (_ds) of the same isolate (SCSV-[3771A])	Total changes	% divergence	C/A	G/A	T/A	G/C	T/C	T/G	indel	other
SCSV-[AU;3771A]_DNA-R_MF458178 SCSV-[AU;3771A]_DNA-R_ds	0	0	0	0	0	0	0	0	0	
SCSV-[AU;3771A]_DNA-S_MF458179 SCSV-[AU;3771A]_DNA-S_ds	5	0,5	0	0	1	0	3	1	0	
SCSV-[AU;3771A]_DNA-C_MF458180 SCSV-[AU;3771A]_DNA-C_ds	7	0,7	0	1	0	1	3	2	0	
SCSV-[AU;3771A]_DNA-M_MF458181 SCSV-[AU;3771A]_DNA-M_ds	2	0,2	0	0	0	0	0	2	0	
SCSV-[AU;3771A]_DNA-M_MF458181 SCSV-[AU;3771A]_DNA-M.2_ds (recombinant with DNA-R)	25	3,7	2	6	3	4	5	2	2 + 12†	† part of 32 nt derived from DNA-R
SCSV-[AU;3771A]_DNA-N_MF458182 SCSV-[AU;3771A]_DNA-N_ds	8	0,8	1	3	1	0	2	1	0	
SCSV-[AU;3771A]_DNA-U1_MF458183 SCSV-[AU;3771A]_DNA-U1_ds	15	1,4	1	5	1	3	3	1	1	
SCSV-AU;3771A]_DNA-U2_MF458184 SCSV-AU;3771A]_DNA-U2_ds	0	0	0	0	0	0	0	0	0	
SCSV-[AU;3771A]_DNA-U4_MF458185 SCSV-[AU;3771A]_DNA-U4_ds	2	0,2	0	1	0	0	0	1	0	
<b>Sum, counting recombination event as 1</b>	<b>64</b>		<b>4</b>	<b>16</b>	<b>6</b>	<b>8</b>	<b>16</b>	<b>10</b>	<b>1</b>	
Comparison of published SCSV-[AU;F] DNAs with deep sequenced SCSV-[AU;F*] progeny	Total changes	% divergence	C/A	G/A	T/A	G/C	T/C	T/G	indel	other
SCSV-[AU;F]_DNA-R_AJ290434 SCSV-[AU;F*]_DNA-R	0	0	0	0	0	0	0	0	0	
SCSV-[AU;F]_DNA-S_U16734 SCSV-[AU;F*]_DNA-S	12	1	1	1	2	1	2	3	2	
SCSV-[AU;F]_DNA-C_U16732 SCSV-[AU;F*]_DNA-C	3	0,3	0	0	0	2	1	0	0	
SCSV-[AU;F]_DNA-M_U16730 SCSV-[AU;F*]_DNA-M	29	1,8	2	4	1	2	6	3	11	
SCSV-[AU;F]_DNA-N_U16733 SCSV-[AU;F*]_DNA-N (Δ 22nt & 87nt recombinant with DNA-S)	36	3,6	2	9	8	0	6	8	1 + Δ22	87 nt recombinant with DNA-S
SCSV-[AU;F]_DNA-U1_U16736 SCSV-[AU;F*]_DNA-U1	11	0,8	1	3	1	0	3	0	3	
SCSV-[AU;F*]_DNA-U2_MF458186 (cloned DNA) SCSV-[AU;F*]_DNA-U2	3	0,3	2	1	0	0	0	0	0	
SCSA1-[AU;F]_alphasat_1_U16731 SCSA1-[AU;F*]_alphasat_1.1	3	0,3	0	0	1	0	1	1	0	
SCSA1-[AU;F]_alphasat_1_U16731 SCSA1-[AU;F*]_alphasat_1.2 (Δ24 nt)	4	0,3	1	0	1	0	1	0	Δ24	
SCSA2-[AU;F]_alphasat_2_U16735 SCSA2-[AU;F*]_alphasat_2	1	0,1	1	0	0	0	0	0	0	
<b>Sum, counting deletions &gt;20 nt and recombination event as 1</b>	<b>102</b>		<b>10</b>	<b>18</b>	<b>14</b>	<b>5</b>	<b>20</b>	<b>15</b>	<b>16</b>	

**Table S5.** Detection by PCR of SCSV DNAs in field samples of symptomatic subterranean clover

Sample	DNA	-R		-S		-M		-C		-N	-U1		-U2		-U4 <sup>a</sup>		-U4 <sup>b</sup>	
		x35 <sup>c</sup>	x35	x43 <sup>d</sup>	x35	x43	x35	x43	x35	x43	x35	x43	x35	x43	x35	x43	x35	x43
1		-	-	nt <sup>e</sup>	-	nt	-	nt	-	-	-	-	-	-	-	-	-	-
2		-	-	nt	-	nt	-	nt	-	-	-	-	-	-	-	-	-	-
3		++	+	++	++	nt	-	++	+	+	nt	+	+	-/+	++	-	-/+	-
4		++	-	+	-	+	-	++	++	-	++	-	+	-	-	-	-	-
5		++	-	++	-	+	-	-/+	-	-	-/+	-	+/-	-	-	-	-	-
6		+++	+++	nt	+++	nt	+++	nt	+++	+++	nt	+++	+++	+++	+++	+	++	+
7		++	+	nt	++	nt	++	nt	+	+/-	nt	+	+	-/+	+	-/+	-/+	-/+
8		-	-	nt	-	nt	-	nt	-	-	nt	-	-	-	-	-	-	-
9		-	-	nt	-	nt	-	nt	-	-	nt	-	-	-	-	-	-	-
10		++	-	++	++	nt	-	++	+	-	++	+	++	-	+	+	-/+	-/+
11		++	+	nt	++	nt	-	-	+	+	nt	+	++	-/+	++	-/+	+	+
12		++	++	nt	++	nt	++	nt	++	++	nt	+	++	+	+	+	+	+
13		++	-	-	-	++	-	-	-	-	+/-	-	+	-	+/-	-	-	-
14		++	++	nt	++	nt	++	nt	++	++	nt	++	++		++	+	+	+
Total SCSV-positive samples		10		9		10		8		8		10		10		8		7

Subterranean clover plants showing symptoms of potential SCSV infection were collected on December 24, 2018, in Harrison, ACT. After total DNA extractions, PCR reactions were performed using primers specific for SCSV DNA (indicated in Table S1), the amplified DNAs were subjected to agarose gel electrophoresis. The relative amounts of amplification products from different samples were estimated visually by staining intensity, ranging from traces (-/+) to very high amplification levels (+++).

a – primers SCSV\_37-U4fr2dir and SCSV\_37-U4fr2rev.

b – primers SCSV-U4-340-dir and SCSV-U4-340-rev.

c - 35 cycles of PCR amplification.

d - 43 cycles of PCR amplification.

e - if 35 PCR cycles yielded amplified DNA of a given genome component, no amplification using 43 cycles was done.

**Table S6.** Detection of SCSV DNAs in field samples after rolling circle amplification followed by PCR.

Sample	DNA	-S	-C	-N	-U4 <sup>b</sup>
1		+++	+++	+++	+++
4		+++	+++	+++	+++
5		+++	+++	+++	+++
11		+++	+++	+++	+++
13		+++	+++	+++	+++

Total DNA extractions of samples 1, 4, 5, 11 and 13 (same as in Table S5) were subjected to rolling circle amplification followed by PCR amplification (35 cycles) using the same SCSV component-specific primers as in Table S5.

Virus acronyms and accession numbers	MDV-[BD;M1] LC094163	MDV-[BD;P1] LC09415	MDV-[IP;1] AB000926	MDV-[BD;1] LC01010	MDV-[CN;Zh] KX710109	MDV-[CN;GSZN] MG012223	MDV-[CN;VU] KY070243	MDV-[CN;VF] KY070233	FBYV-[ET;231] HE654129	FBNV-[AZ;1] KC97897	FBNV-[AZ;15] KC978998	FBNV-[AZ;10] KC978980	FBNV-[MA;5] GQ274037	FBNV-[AZ;12b] KC978988	FBNV-[ET;Hol-97] GU983872	FBNV-[SY;4] KX431423	FBNV-[TU;F9] KX431427	FBNV-[TU;S01] KX431428	FBNV-[TU;F12] KX431425	FBNV-[ES;R4] KC979033	FBNV-[ES;R7] KC979041	FBNV-[TU;F10] KX431424
MDV-[BD;M1] LC094163	100																					
MDV-[BD;P1] LC094151	98,7	100																				
MDV-[IP;1] AB000926	96,8	96,8	100																			
MDV-[BD;1] LC010100	96,3	99,5		100																		
MDV-[CN;Zh] KX710109	89,6	89,4	91,0	90,8																		
MDV-[CN;GSZN] MG012223	90,5	90,3	89,0	88,6	87,6	100																
MDV-[CN;VU] KY070243	88,0	88,0	86,8	86,4	86,2	95,8	100															
MDV-[CN;VF] KY070233	88,1	88,2	86,5	86,1	85,8	86,3	84,5	100														
FBNV-[ET;231] HE654129	71,3	71,9	72,3	72,2	73,8	74,3	74,2	72,9	100													
FBNV-[AZ;1] KC97897	70,4	70,4	70,4	69,7	69,2	72,0	70,4	69,5	67,7	100												
FBNV-[AZ;15] KC978998	71,6	71,6	71,3	70,6	69,2	70,1	69,6	68,7	67,4	97,9	100											
FBNV-[AZ;10] KC978980	70,4	69,8	70,9	70,3	68,4	70,3	69,7	69,1	67,9	97,9	99,3	100										
FBNV-[MA;5] GQ274037	69,6	69,8	69,2	69,0	69,3	68,9	70,2	67,5	68,6	83,7	83,2	83,0	100									
FBNV-[AZ;12b] KC978988	68,7	69,5	69,2	68,1	67,6	70,1	69,8	68,2	68,0	83,4	82,3	82,0	95,0	100								
FBNV-[ET;Hol-97] GU983872	67,2	67,7	67,4	66,7	66,7	67,9	68,4	69,7	68,1	84,0	84,6	83,1	84,8	85,2	100							
FBNV-[SY;4] KX431423	65,7	64,1	65,2	65,2	62,9	66,0	65,8	65,9	69,9	70,6	70,6	70,3	71,0	70,6	69,9	100						
FBNV-[TU;F9] KX431427	65,7	64,1	65,0	65,0	62,8	65,9	65,5	66,1	69,2	70,3	70,6	70,3	71,1	71,2	70,4	99,7	100					
FBNV-[TU;S01] KX431428	65,0	64,4	64,9	64,9	63,5	66,1	66,4	65,5	69,0	70,4	70,5	70,3	70,9	69,8	69,0	99,1	98,8	100				
FBNV-[TU;F12] KX431425	65,0	64,4	65,3	65,2	64,0	67,1	66,9	64,9	67,7	71,0	70,9	71,6	71,4	69,4	70,0	98,8	98,5	98,9	100			
FBNV-[ES;R4] KC979033	66,5	66,1	65,5	66,9	66,4	66,1	67,6	66,2	69,5	69,6	69,2	71,2	70,1	70,1	70,1	96,3	96,0	96,2	96,2	100		
FBNV-[ES;R7] KC979041	65,7	66,0	65,4	66,5	65,8	67,0	66,4	65,5	68,2	70,3	70,2	69,9	71,1	70,6	70,8	96,6	96,3	96,5	96,3	98,6	100	
FBNV-[TU;F10] KX431424	66,2	66,4	66,5	66,4	66,9	67,5	66,2	67,1	67,9	70,8	70,9	70,6	71,9	71,6	71,7	96,9	97,0	96,8	97,6	98,0	98,0	100
FBNV-[ES;Mu29D] KC979025	65,5	65,9	64,9	65,6	66,0	65,9	65,8	67,3	66,6	69,6	69,2	71,1	70,3	70,6	66,6	96,6	96,3	96,7	96,7	98,3	97,1	96,9
FBNV-[AZ;13.3] KC979015	67,3	66,8	66,2	66,8	65,8	66,6	67,6	65,8	68,9	70,1	71,1	71,6	72,7	72,6	72,5	92,1	92,1	92,4	92,3	92,7	92,8	92,9
FBNV-[AZ;12] KC979006	66,0	66,3	66,2	65,5	65,1	65,4	65,3	65,6	68,9	70,4	69,9	70,0	72,1	71,6	72,8	90,6	90,3	90,9	90,7	90,4	90,5	90,5
FBNV-[ET;218] HE663171	65,6	65,4	66,2	66,6	65,2	66,3	65,8	65,1	67,2	71,1	70,6	70,6	71,9	72,2	72,7	88,9	88,9	89,2	89,0	88,9	89,2	89,3
FBNV-[MA;23] GQ274029	64,8	64,0	65,1	64,5	65,1	66,6	67,7	65,3	68,1	70,3	70,9	70,6	72,9	70,3	71,5	88,5	88,5	88,6	88,6	88,5	89,2	89,0
FBNV-[IR;S36] KX431422	65,9	64,9	66,6	66,7	66,2	67,8	66,9	67,0	70,4	71,8	71,8	70,8	70,9	71,9	86,4	86,1	86,5	86,6	86,0	86,2	86,2	85,9
FBNV-[TU;F4] KX431426	66,2	65,4	66,5	65,6	66,8	67,1	69,1	67,1	66,9	69,8	71,3	71,3	70,3	70,8	71,4	86,4	86,4	86,5	86,6	86,0	86,2	86,1
FBNV-[SY;292-381] Y11409	67,2	66,3	66,1	65,9	67,0	67,2	67,3	64,7	68,3	69,5	69,5	70,7	69,4	71,8	86,8	86,8	86,9	86,9	86,5	86,5	86,7	86,7
FBNV-[EG;1-93] A1132184	67,0	66,1	68,2	67,7	67,9	68,8	68,8	66,7	68,1	67,9	68,4	68,6	72,0	71,1	71,2	83,7	83,7	83,8	83,8	83,5	83,2	83,8
BMLRV-[SE;153] DNA-U2 KC978964	66,5	66,3	66,3	66,6	64,6	65,6	66,2	66,2	69,9	67,3	66,4	67,0	66,6	66,5	66,2	64,9	65,1	64,7	66,7	66,4	66,3	66,3
BMLRV-[AT;3] KC978946	68,9	67,2	67,8	69,5	68,2	67,1	68,4	68,1	70,0	69,6	69,7	70,0	67,1	66,1	66,5	65,5	65,6	65,5	65,0	65,9	66,7	66,2
BMLRV-[AZ;47] KC978955	67,2	67,6	67,3	66,0	67,9	68,6	69,4	67,4	69,9	69,6	67,6	67,9	67,7	68,9	67,9	64,0	67,1	66,9	67,3	67,3	66,3	67,4
SYSAV-[IR;9a1] KX534395	67,2	66,7	67,3	67,3	66,4	67,3	68,1	67,6	66,1	65,8	64,6	64,5	63,6	62,7	64,2	63,9	63,6	63,2	62,1	62,2	63,0	63,0
SCSV-[AU;F] MF458186	64,4	64,2	64,5	63,7	62,6	66,0	65,9	69,0	67,8	66,2	66,0	65,7	65,2	65,5	66,2	66,0	65,8	65,1	65,6	64,8	65,7	64,5
SCSV-[AU;3771A] MF458184	64,8	65,3	65,3	64,9	62,4	65,7	64,5	68,9	68,0	65,2	64,9	64,7	64,6	67,8	65,2	62,8	62,7	65,5	65,0	65,8	65,8	64,7
SCSV-[AU;2534B] MK035734	64,8	64,6	65,4	64,1	61,4	66,2	65,0	68,0	67,6	64,8	65,5	64,6	65,2	67,1	65,8	64,3	64,2	64,5	63,7	65,5	65,6	64,8
CVL-[PR;WtIV-Sam] MF535453	66,7	66,3	64,0	64,1	64,4	66,1	64,9	68,0	68,5	69,1	67,6	67,4	66,4	65,5	68,4	66,5	66,7	65,9	68,1	65,1	67,4	66,5
PYSV-[AT;15] KC979059	63,8	63,4	62,8	61,9	62,4	65,4	65,5	67,7	63,4	62,8	63,8	66,0	64,0	64,0	64,0	63,3	62,9	62,8	63,9	64,2	63,1	63,1
PYSV-[AT;15] KC979060	62,5	62,9	62,5	62,4	61,6	63,6	63,4	65,7	65,0	63,4	62,9	63,8	64,1	62,1	64,2	64,6	63,5	64,7	65,4	64,6	64,7	63,7
PNYDV-[AT;1] KC979049	59,1	58,6	61,6	60,7	61,0	62,1	62,8	62,0	64,3	64,8	64,2	61,5	62,5	63,2	63,9	63,1	63,4	63,5	63,4	63,3	63,6	63,6
PNYDV-[DE;15] JN133284	59,9	59,6	61,6	61,4	62,0	62,5	62,8	61,6	63,7	63,2	63,8	64,7	61,5	62,5	63,0	62,3	61,6	61,4	62,0	61,6	61,6	63,2

FBNVV-[ES;Mu29D] KC979025	FBNVV-[AZ;13.5] KC979015	FBNVV-[AZ;12] KC979006	FBNVV-[ET;218] HE663171	FBNVV-[MA;23] GQ274029	FBNVV-[IR;336] KX43142	FBNVV-[TU;F4] KX431426	FBNVV-[SY;292-88] Y11409	FBNVV-[EG;1-93] AJ132184	BMLRV-[SE;153] KC978964	BMLRV-[AT;3] KC978946	BMLRV-[AZ;47] KC978955	YSaV-[IR;Ta1] KX534395	SCSV-[AU;F] MF458186	SCSV-[AU;3771A] MF458184	SCSV-[AU;2534B] MK035734	CvLV-[FR;VclV-Sam] MF535453	PYSV-[AT;15] KC97905	PYSV-[AT;15] KC979060	PNYDV-[AT;1] KC979049	PNYDV-[DE;15] JN133284
100																				
93,2	100																			
90,8	90,7																			
89,3	90,0	94,3	100																	
88,8	89,2	89,2	88,2	100																
86,7	88,7	87,3	86,5	90,2	100															
86,7	88,7	87,4	86,5	90,1	99,9	100														
87,2	88,7	87,5	86,4	91,0	96,9	96,9														
84,7	86,3	85,5	85,6	87,2	90,4	90,4	100													
65,5	64,1	64,4	66,3	64,9	64,9	65,4	67,7	100												
66,2	66,7	64,0	66,8	66,9	66,4	66,3	67,3	65,3	87,6	100										
66,3	66,1	65,4	66,6	66,3	66,9	66,7	67,0	66,1	85,1	84,0	100									
62,5	65,7	64,2	61,9	66,5	64,2	63,9	62,8	65,7	65,7	63,0	63,4	100								
65,0	64,9	66,3	65,1	64,1	67,2	67,7	65,4	66,8	69,1	66,2	66,1	65,3	100							
64,6	64,7	65,4	64,9	66,1	67,9	68,0	66,9	66,3	68,6	66,3	67,0	65,0	97,8	100						
64,6	64,6	64,0	64,4	65,7	68,6	68,3	66,7	66,7	69,6	65,8	66,1	64,2	98,3	98,6	100					
65,5	65,8	65,2	64,4	65,8	64,2	64,2	65,5	66,7	64,4	64,8	67,5	63,6	62,9	62,9	62,7	100				
64,1	63,5	63,3	63,2	62,7	63,0	63,8	61,6	63,3	65,7	65,0	64,7	64,0	65,1	64,9	64,1	67,2				
64,5	64,6	64,5	63,7	63,8	63,4	63,6	62,5	63,9	62,9	63,5	64,6	62,0	63,0	62,2	62,6	67,4	100			
62,8	61,0	62,5	62,8	63,2	61,9	62,2	63,2	62,3	62,3	61,2	63,3	64,6	64,9	64,0	66,7	67,8	66,1	100		
61,2	61,1	62,6	62,8	63,0	62,8	63,0	62,5	62,7	62,3	60,6	64,1	65,0	65,9	64,9	66,8	67,0	66,5	64,6	100	100



Virus acronyms and accession numbers	FBNVV-[ES;R4] KC979034	FBNVV-[ES;R7] KC979042	FBNVV-[ES;Mu29D] KC979026	FBNVV-[TU;F4] KX431431	FBNVV-[TU;F9] KX431434	FBNVV-[TU;S01] KX431435	FBNVV-[TU;F12] KX431433	FBNVV-[TU;F10] KX431432	FBNVV-[AZ;13.5] KC979016	FBNVV-[MA;23] GQ274024	FBNVV-[SY;292-88] AJ749903	FBNVV-[IR;36] KX431429	FBNVV-[SY;4] KX431430	FBNVV-[EG;1-93] AJ749902	FBNVV-[AZ;13.5] GQ274024	FBNVV-[SY;292-88] AJ749903	FBNVV-[IR;36] KX431429	FBNVV-[SY;4] KX431430	FBNVV-[EG;1-93] AJ749902	FBNVV-[AZ;13.5] GQ274024	BMLRV-[AZ;47] KC978956	BMLRV-[AT;3] KC978947	BMLRV-[SE;153] KC978965	CVL-[FR;VCLV-[Sam] MFS535454	PYSV-[AT;15] KC979061	PNYDV-[DE;15] JN133285
FBNVV-[ES;R4] KC979034	100																									
FBNVV-[ES;R7] KC979042	99.8	100																								
FBNVV-[ES;Mu29D] KC979026	99.8	100	100																							
FBNVV-[TU;F4] KX431431	97.5	97.6	97.6	100																						
FBNVV-[TU;F9] KX431434	97.3	97.4	97.4	99.6	100																					
FBNVV-[TU;S01] KX431435	97.4	97.5	97.5	99.5	99.5	100																				
FBNVV-[TU;F12] KX431433	97.3	97.4	97.4	99.4	99.7	100																				
FBNVV-[TU;F10] KX431432	97.5	97.6	97.6	99.4	99.4	99.7	99.6	100																		
FBNVV-[AZ;13.5] KC979016	90.8	90.7	90.7	90.5	90.1	90.3	90.2	90.3	100																	
FBNVV-[MA;23] GQ274024	88.0	88.1	88.1	87.6	87.3	87.5	87.3	87.3	86.8	100																
FBNVV-[SY;292-88] AJ749903	87.8	87.9	87.9	87.2	87.1	87.3	87.3	87.3	86.1	91.8	100															
FBNVV-[IR;36] KX431429	87.7	87.8	87.8	87.0	87.1	87.3	87.3	87.3	85.9	98.0	100															
FBNVV-[SY;4] KX431430	87.8	87.9	87.9	87.1	87.2	87.4	87.3	87.4	86.1	91.6	98.4	99.4	100													
FBNVV-[EG;1-93] AJ749902	85.2	85.3	85.3	85.6	85.5	85.7	85.7	85.7	84.9	90.6	97.2	96.4	97.0													
FBNVV-[AZ;13.5] GQ274024	88.0	88.0	88.0	87.4	87.0	87.5	87.2	87.3	86.5	86.7	84.9	84.8	85.1	84.0												
FBNVV-[SY;292-88] AJ749903	87.8	87.7	87.7	87.2	87.2	87.4	87.4	87.5	85.4	87.0	85.0	84.9	85.1	84.0	93.5	100.0										
BMLRV-[AZ;47] KC978956	64.4	65.6	65.6	65.6	65.4	64.4	64.4	65.2	65.4	65.8	64.1	64.3	64.6	63.7	62.2	100										
BMLRV-[AT;3] KC978947	65.7	65.2	65.2	63.5	63.6	63.5	63.0	63.5	64.8	64.7	64.1	64.4	64.9	63.0	64.5	64.6	64.6	83.0	83.0	100						
BMLRV-[SE;153] KC978965	68.6	66.1	66.1	67.9	67.5	68.4	68.0	68.2	65.4	66.6	64.6	64.8	63.9	65.3	64.0	65.6	64.0	84.0	84.0			100				
CVL-[FR;VCLV-[Sam] MFS535454	69.1	69.3	69.3	70.8	70.4	69.8	69.8	71.0	69.7	69.7	68.7	69.0	69.4	67.6	69.9	69.2	62.9	64.6	65.2				100			
PYSV-[AT;15] KC979061	64.1	63.7	63.7	63.4	63.7	63.6	63.0	63.8	64.2	66.6	66.9	67.1	67.0	68.3	64.5	62.3	61.8	62.8	62.8					100		
PNYDV-[DE;15] JN133285	65.3	65.6	65.6	65.1	64.8	64.5	64.5	64.3	64.1	67.4	69.2	69.2	69.0	68.2	65.8	65.1	59.8	63.1	63.1						100	
PNYDV-[AT;1] KC979050	64.5	64.8	64.8	65.3	65.0	65.1	65.1	64.8	64.0	67.5	68.8	67.8	67.8	66.6	65.1	60.4	62.6	61.8	68.9					82.5		
FBNSV-[MA;5] GQ274032	69.1	69.2	69.2	69.1	69.0	69.1	69.5	69.6	69.1	71.7	69.4	68.6	69.0	68.0	68.4	67.4	70.2	68.4	67.8					65.6		99.4
FBNSV-[AZ;1] KC978973	72.2	72.2	72.2	70.6	70.5	70.9	70.8	71.1	72.2	72.1	70.5	70.8	71.2	71.1	70.6	69.5	68.5	67.4	69.8					70.1		70.3
FBNSV-[AZ;12b] KC978989	72.2	71.7	71.7	71.4	71.4	71.7	71.6	72.1	71.8	73.6	69.7	69.4	69.6	68.6	70.5	70.1	67.0	66.4	69.6					68.4		69.2
FBNSV-[AZ;10] KC978981	73.5	72.9	72.9	72.0	72.0	72.2	72.1	72.5	72.6	72.8	70.0	69.6	69.9	69.0	70.5	70.4	67.3	66.5	69.0					66.8		70.2
FBNSV-[ET;Ho97] GU983873	70.2	69.8	69.8	69.4	69.4	70.6	70.7	70.1	68.2	73.6	68.2	69.6	69.0	70.1	70.8	69.8	64.4	63.2	66.5					69.0		67.4
FBNSV-[AZ;15] KC978999	69.8	69.4	69.4	69.4	69.4	69.4	69.4	69.4	69.6	72.4	68.9	68.8	69.0	68.0	68.8	69.0	66.9	66.8	67.3					67.2		66.8
FBNV-[ET;21] HX54130	68.7	67.7	67.7	67.4	67.4	67.7	67.5	67.4	69.5	68.4	69.7	69.3	69.6	67.8	67.3	67.4	69.0	66.1	67.2					66.1		66.3
MDV-[BD;1.2] LC09944	67.7	67.1	67.1	65.3	65.0	66.1	66.1	66.2	66.3	67.6	65.6	64.8	65.1	65.8	67.9	66.4	66.3	64.5	64.4					61.9		64.6
MDV-[BD;P1] LC094154	66.7	66.4	66.4	66.9	67.1	66.6	66.6	67.0	66.2	67.0	67.1	66.8	67.0	67.1	66.6	68.3	64.4	65.3	65.8					65.8		62.7
MDV-[BD;1.1] LC010104	66.4	66.2	66.2	66.7	66.9	66.4	66.4	66.7	65.7	67.4	66.4	66.9	67.1	67.3	68.1	66.5	65.4	65.7	64.4					66.0		63.1
MDV-[IP;1] AB255373	66.1	66.4	66.4	65.3	65.5	67.5	67.5	67.7	68.9	66.5	68.4	67.3	64.5	64.1	67.1	67.1	65.8	67.3	64.5					63.3		63.6
MDV-[CN;F7] KY070237	65.2	64.9	64.9	64.6	64.8	64.6	64.5	64.8	66.6	63.0	64.0	64.0	63.7	64.5	64.0	65.3	64.3	63.9	63.0					62.3		63.3
MDV-[CN;2h1] KX710111	66.4	66.1	66.1	65.5	65.3	65.4	65.4	65.4	65.6	67.8	64.1	64.9	64.4	63.8	64.5	64.3	63.4	66.3	65.7					62.8		61.7
MDV-[CN;2h2] KX710112	65.7	65.5	65.5	65.6	65.4	65.5	65.5	65.7	67.6	63.9	63.3	63.2	63.2	63.1	64.5	65.2	67.3	65.0	62.5					65.4		62.0
MDV-[CN;GS2N] MG012224	65.1	65.2	65.2	64.4	64.9	64.3	64.3	64.4	67.2	64.6	64.3	63.5	63.8	65.0	63.2	63.7	63.3	66.0	63.3					66.0		63.4
MDV-[CN;VU] KY070247	64.8	65.0	65.0	63.6	64.2	63.5	63.5	63.6	66.7	63.1	63.9	63.0	63.4	64.0	65.2	62.6	62.9	66.2	65.5					62.8		62.9
SFSV-[IR;11] KX534392	64.2	64.1	64.1	63.8	64.6	64.7	63.3	63.8	63.3	63.6	63.3	61.6	63.8	63.5	65.5	62.9	63.2	63.3	63.0					63.3		61.4
SCSV-[AU;25348] MK035735	65.5	65.4	65.4	64.5	64.9	64.5	64.6	64.7	64.4	64.0	63.6	64.9	64.6	64.3	64.4	64.2	64.4	63.9	65.6					85.5		87.1
SCSV-[AU;3771A] MF458185	64.5	63.8	63.8	65.5	65.6	65.9	65.9	66.0	64.4	63.0	64.5	65.5	65.5	64.1	66.6	67.1	63.8	64.0	62.9					65.6		60.4
SCSV-[AU;Har-10]	65.8	65.7	65.7	66.0	66.2	66.5	66.5	66.5	65.7	65.2	64.1	65.9	66.5	66.7	66.2	67.8	67.0	65.2	63.3					64.9		64.3
SCSV-[AU;Har-14]	65.8	65.7	65.7	66.0	66.2	66.5	66.5	66.5	65.7	65.2	64.1	65.9	66.5	66.7	66.2	67.8	67.0	65.2	65.5					64.9		65.4
SCSV-[AU;Har-12]	66.0	66.2	66.2	65.8	65.9	66.4	66.2	66.3	65.9	63.6	65.3	66.4	65.6	65.8	69.3	69.0	63.6	65.2	62.8					61.8		66.9

PNYDV-[AT;1] KC979050	FBNSV-[MA;5] GG274032	FBNSV-[AZ;1] KC978973	FBNSV-[AZ;12b] KC978989	FBNSV-[AZ;10] KC978981	FBNSV-[ET;Hq;97] GU983873	FBNSV-[AZ;15] KC978999	FBYLV-[ET;231] HE654130	MDV-[BD;1.2] LC099944	MDV-[BD;P1] LC094154	MDV-[BD;1.1] LC010104	MDV-[JP;1] AB255373	MDV-[CN;VF] KY070237	MDV-[CN;Zh1] KX710111	MDV-[CN;Zh2] KX710112	MDV-[CN;GSZn] MG012224	MDV-[CN;VU] KY070247	SYSaV-[IR;Ta1] KX534392	SCSV-[AU;2534B] MK035735	SCSV-[AU;3771A] MF458185	SCSV-[AU;Har-10]	SCSV-[AU;Har-14]	SCSV-[AU;Har-12]
100																						
65.6	100																					
70.7	96.3																					
70.2	94.5	100																				
70.8	95.0	98.1	100																			
68.7	81.7	98.3	98.2	100																		
68.7	82.7	82.4	83.0	100																		
67.9	79.6	79.4	79.2	81.3	100																	
67.4	68.2	71.2	69.3	69.3	67.9	71.4	100															
65.5	66.6	70.4	68.1	66.3	65.2	67.4	68.4	100														
62.2	67.6	69.3	66.3	65.9	62.8	67.2	68.0	91.0	100													
62.9	69.5	69.3	66.7	66.4	62.8	68.7	67.2	90.8	99.8	100												
63.5	68.7	68.0	67.8	66.2	66.6	67.4	67.7	85.0	86.6	86.6	100											
62.4	68.9	68.5	68.4	67.5	67.0	68.3	65.4	84.4	85.8	85.6	92.3	100										
60.6	69.6	70.6	68.3	67.5	66.9	70.0	67.4	85.2	85.8	85.6	89.0	86.7	100									
60.9	69.7	70.5	68.8	68.1	67.2	70.1	67.2	84.9	85.9	85.7	85.3	96.9	100									
62.5	67.9	69.0	67.7	67.8	67.6	67.4	67.9	84.4	85.0	85.0	81.4	82.0	84.6	84.9	100							
62.2	67.1	67.9	66.4	66.8	66.7	66.3	66.0	83.5	85.1	84.8	81.5	82.2	84.8	85.4	92.0	100						
61.3	58.6	61.4	61.1	59.7	61.3	62.7	66.5	63.8	62.8	62.7	63.2	62.6	63.7	63.4	66.1	64.5						
66.5	62.4	68.5	68.9	67.3	66.4	65.4	63.7	65.0	63.3	62.3	62.7	61.0	63.7	65.4	59.9	61.7	64.7	100				
65.6	62.8	66.8	69.2	67.2	67.1	62.5	62.3	64.6	63.4	62.1	61.8	61.9	63.7	65.2	60.8	61.2	65.8	96.4	100			
65.6	63.9	69.0	69.5	67.4	66.8	62.4	61.5	63.9	63.0	61.9	62.6	62.9	62.4	61.9	62.0	60.5	65.0	97.7	96.1	100		
65.6	63.9	69.0	69.5	67.4	66.8	62.4	61.5	63.9	63.0	61.9	62.6	62.9	62.4	61.9	62.0	60.5	65.0	97.7	96.1	100		
66.1	63.1	68.8	68.3	67.3	66.6	65.5	64.0	64.9	63.1	61.9	62.3	61.4	64.9	66.0	59.3	61.0	66.1	98.3	96.6	97.9	100	100