Table S1. DNA oligonucleotide probes for RNA and DNA blot hybridization and primers for RCA.

Sequence Analysis S1A. Nucleotide and amino acid sequence analysis.

Table summarizes comparison of the complete genome nucleotide sequences of reconstructed BSV species and their gene products (P1, P2 and P3) with those of the Genbank reference sequences of previously isolated and sequenced BSV species. Below the table, **the CLUSTAL alignments** of nucleotide sequences of three selected regions of the BSV genomes reconstructed in this study are shown.

Sequence Analysis S1B. Conservation of the *cis*-acting elements that drive ribosome shunt and leaky scanning in six BSV species. The conserved shunt configuration and short ORFs (sORFs) in the pgRNA of BSOLV, BSGFV, BSIMV, BSMYV, BSVNV, BSCAV, RTBV and CaMV are shown. The pgRNA is depicted as a thick line, large ORFs (I, II, and III) as thick line boxes, sORF as thin line boxes and numbers are the position in the virus genome (starting from the PBS / the transcription start site). The conserved sORF 1/1’ is boxed in red. Secondary structure of the pgRNA leader, as predicted using MFold, is depicted schematically above the pgRNA. In all but CaMV, the first two large ORFs are devoid of sORFs. The stability of the structure (*dG*) in kcal/mole is given. 5- and 3’-sequences flanking the main structure are shown in open conformation, since they do not form any extensive structures. The stable structural element (circled at the stem base) and adjacent regions are enlarged alongside. The short ORF 1/1’ and ORF I are boxed. AUG codons are in bold; potential non-AUG initiator codons within the shunt landing sequence are underlined. Below the pgRNA, the sequences at the junctions of ORFs I and ORFs II and III are shown with genome positions of the corresponding stop and start codons indicated.

Table S1. DNA oligonucleotide probes for RNA and DNA blot hybridization and primers for RCA

Name	Sequence	Detects
Degenerate primers for RCA:		
BSV_33_s	5' TKATGGCWTTYATGGGGTAAWWCC	
BSV_1175_as	5' ACHARBTCYTCHACGTCYTYYTT	
BSV_2275_s	5' ATGGARGYKGAYCTNKCHSAAGG	
BSV_3320_as	5' CKYTCRAAKACYTTDRBRTCAAA	
BSV_4373_s	5' ARWWBHWHTGYHWRCAYVMDTGG	
BSV_5141_as	5' GCYCCWGTGTCHARDATNGCRTT	
BSV_6055_s	5' TAYGARTGGCTHGTHATGCCVTT	
BSV_6882_as	5' CCAYCKDRYHCKNGAKGGYTTRT	
Probes for sRNA blot hybridization:		
miR160a_as	5’tggcatacagggagccaggca	miR160

Gf7166_s	5′ CCAGTGTTTTCCAAAGCAGCTGAA	Leader_up antisense; BSGFV, upstream of PBS
Gf7192_s	5′ TTTCAGTCTGTGAGTAGAAAGCAA	Leader_up antisense; BSGFV, upstream of PBS
Gf152_s	5′ ACTGGCATGATTATTGAATGAAAA	Leader_down antisense; BSGFV, downstream of PBS
Gf410_s	5′ AAACCCTCAGATAACGTGTCTCAT	Leader_down antisense; BSGFV, downstream of PBS
Gf768_s	5′ AAACGATCTGAAGCTGCTGCTCTC	ORF antisense; BSGFV, ORF I
Gf1226_s	5′ TAAGCTCACAGAACAATTGGACAA	ORF antisense; BSGFV, ORF II
Gf7166_as	5′ TTCAGCTGCTTTGGAAAACACTGG	Leader_up sense; BSGFV, upstream of PBS
Gf7192_as	5′ TTGCTTTCTACTCACAGACTGAAA	Leader_up sense; BSGFV, upstream of PBS
Gf152_as	5′ TTTTCATTCAATAATCATGCCAGT	Leader_down sense; BSGFV, downstream of PBS
Gf410_as	5′ ATGAGACACGTTATCTGAGGGTTT	Leader_down sense; BSGFV, downstream of PBS
Gf768_as	5′ GAGAGCAGCAGCTTCAGATCGTTT	ORF sense; BSGFV, ORF I
Gf1226_as	5′ TTGTCCAATTGTTCTGTGAGCTTA	ORF sense; BSGFV, ORF II
Vn7718_s	5′ CCTCTTACATTCTGTTCTTGAAAG	Leader_up antisense; BSVNV, upstream of PBS
Vn7759_s	5′ GAGTAGAGAACAAGATCTTTTGGG	Leader_up antisense; BSVNV, upstream of PBS
Vn199_s	5′ TGTGTTTCCAAGTTCTACTGAGAA	Leader_down antisense; BSVNV, downstream of PBS
Vn659_s	5′ TCTGGAACTGGTTATAATCTTGAA	Leader_down antisense; BSVNV, downstream of PBS
Vn1154_s	5′ TGGTTGTCCGCATCTCTGAACAAC	ORF antisense; BSVNV, ORF I
Vn1258_s	5′ ACTTCGAACAGAAGTTACTGGGAT	ORF antisense; BSVNV, ORF I
Vn7718_as	5′ CTTTCAAGAACAGAATGTAAGAGG	Leader_up sense; BSVNV, upstream of PBS
Vn7759_as	5′ CCCAAAAGATCTTGTTCTCTACTC	Leader_up sense; BSVNV, upstream of PBS
Vn199_as	5′ TTCTCAGTAGAACTTGGAAACACA	Leader_down sense; BSVNV, downstream of PBS
Vn659_as	5′ TTCAAGATTATAACCAGTTCCAGA	Leader_down sense; BSVNV, downstream of PBS
Vn1154_as	5′ GTTGTTCAGAGATGCGGACAACCA	ORF sense; BSVNV, ORF I
Vn1258_as	5′ ATCCCAGTAACTTCTGTTCGAAGT	ORF sense; BSVNV, ORF I
Cav7357_s	5′ AGTGTGAGTAGAGCGCAAGATCCG	Leader_up antisense; BSCAV, upstream of PBS
Cav7391_s	5′ TCCGTGAAATCCGTGCCGTTCTGG	Leader_up antisense; BSCAV, upstream of PBS
Cav114_s	5′ GTTTTATGATAGATAAGTCGAATG	Leader_down antisense; BSCAV, downstream of PBS
Cav299_s	5′ ATAGATAGACAGTGAACTCCGTGA	Leader_down antisense; BSCAV, downstream of PBS
Cav811_s	5′ TGAAGCGGACTCTCGAGAAGTTGG	ORF antisense; BSCAV, ORF I
Cav873_s	5′ TATCTGAAGAGACGACCCTTGAAC	ORF antisense; BSCAV, ORF I
Cav7357_as	5′ CGGATCTTGCGCTCTACTCACACT	Leader_up sense; BSCAV, upstream of PBS
Cav7391_as	5′ CCAGAACGGCACGGATTTCACGGA	Leader_up sense; BSCAV, upstream of PBS
Cav114_as	5′ CATTCGACTTATCTATCATAAAAC	Leader_down sense; BSCAV, downstream of PBS
Cav299_as	5′ TCACGGAGTTCACTGTCTATCTAT	Leader_down sense; BSCAV, downstream of PBS
Cav811_as	5′ CCAACTTCTCGAGAGTCCGCTTCA	ORF sense; BSCAV, ORF I
Cav873_as	5′ GTTCAAGGGTCGTCTCTTCAGATA	ORF sense; BSCAV, ORF I
OL7332_s	5′ TGTGCGAGTTCACTTTGTGCGAGT	Leader_up antisense; BSOLV, upstream of PBS
OL7364_s	5′ AGATCCTAGTTCCGCGAGCGTAGA	Leader_up antisense; BSOLV, upstream of PBS
OL120_s	5′ GAGCTGTATTCAGTCTTTCTTAGA	Leader_down antisense; BSOLV, downstream of PBS
OL379_s	5′ TATAAGACTGACTGAACTACAAGC	Leader_down antisense; BSOLV, downstream of PBS
OL777_s	5′ TGCAGAACTTAAGTAAGATTGTGC	ORF antisense; BSOLV, ORF I
OL2166_s	5′ CTCACCAATGTTCCCAACGTCGGA	ORF antisense; BSOLV, ORF III
OL7332_as	5′ ACTCGCACAAAGTGAACTCGCACA	Leader_up sense; BSOLV, upstream of PBS
OL7364_as	5′ TCTACGCTCGCGGAACTAGGATCT	Leader_up sense; BSOLV, upstream of PBS
OL120_as	5′ TCTAAGAAAGACTGAATACAGCTC	Leader_down sense; BSOLV, downstream of PBS
OL379_as	5′ GCTTGTAGTTCAGTCAGTCTTATA	Leader_down sense; BSOLV, downstream of PBS
OL777_as	5′ GCACAATCTTACTTAAGTTCTGCA	ORF sense; BSOLV, ORF I
OL2166_as	5′ TCCGACGTTGGGAACATTGGTGAG	ORF sense; BSOLV, ORF III
Mys7551_s	5′ AAGAATCCTCAGTTTTCTTCATCC	Leader_up antisense; BSMYV, upstream of PBS
Mys7587_s	5′ ACTTCCTGAAATTGGGCAAGCCCC	Leader_up antisense; BSMYV, upstream of PBS
Mys127_s	5′ CTCTGAAGCGAATTTCTGGCAAGT	Leader_down antisense; BSMYV, downstream of PBS
Mys374_s	5′ TCCGGACATGATAGTAAAACTCTG	Leader_down antisense; BSMYV, downstream of PBS
Mys885_s	5′ TTGAGGACTTAAACAGGGAACTGC	ORF antisense; BSMYV, ORF I
Mys909_s	5′ AAGCCCTGCGTTCTGACTATCTCT	ORF antisense; BSMYV, ORF I
Mys7551_as	5′ GGATGAAGAAAACTGAGGATTCTT	Leader_up sense; BSMYV, upstream of PBS
Mys7587_as	5′ GGGGCTTGCCCAATTTCAGGAAGT	Leader_up sense; BSMYV, upstream of PBS
Mys127_as	5′ ACTTGCCAGAAATTCGCTTCAGAG	Leader_down sense; BSMYV, downstream of PBS
Mys374_as	5′ CAGAGTTTTACTATCATGTCCGGA	Leader_down sense; BSMYV, downstream of PBS
Mys885_as	5′ GCAGTTCCCTGTTTAAGTCCTCAA	ORF sense; BSMYV, ORF I
Mys909_as	5′ AGAGATAGTCAGAACGCAGGGCTT	ORF sense; BSMYV, ORF I
Im7686_s	5′ CTTTTCTTACAGATGTTCTTGAAA	Leader_up antisense; BSIMV, upstream of PBS
Im7710_s	5′ TCTCTATGAGCAATCATTAGACGA	Leader_up antisense; BSIMV, upstream of PBS
Im133_s	5′ AGAAAGACTAAGAAGAGTATTCTA	Leader_down antisense; BSIMV, downstream of PBS
Im154_s	5′ CTATGTTATGATACTACTTGTGAA	Leader_down antisense; BSIMV, downstream of PBS
Im859_s	5′ CGTGAACTACTAATCCAGAACAGG	ORF antisense; BSIMV, ORF I
Im881_s	5′ GGAAGCTTCTGGAAGAACAAGAGC	ORF antisense; BSIMV, ORF I
Im7686_as	5′ TTTCAAGAACATCTGTAAGAAAAG	Leader_up sense; BSIMV, upstream of PBS

Im7710_as	5′ TCGTCTAATGATTGCTCATAGAGA	Leader_up sense; BSIMV, upstream of PBS
Im133_as	5′ TAGAATACTCTTCTTAGTCTTTCT	Leader_down sense; BSIMV, downstream of PBS
Im154_as	5′ TTCACAAGTAGTATCATAACATAG	Leader_down sense; BSIMV, downstream of PBS
Im859_as	5′ CCTGTTCTGGATTAGTAGTTCACG	ORF sense; BSIMV, ORF I
Im881_as	5′ GCTCTTGTTCTTCCAGAAGCTTCC	ORF sense; BSIMV, ORF I

Southern probes for BSV titers:

Cav_1	5′ GGACCAGAAAAAGCAATTGGAAGAATTGCAACACCTCAGT	BSCAV
Cav_2	5′ TTTGATCGTTAGGCTGCAACAAGAAAATCTTGCTCTCAGG	BSCAV
Ol_1	5′ CAGGAGGCTCAAAAGAAGGACTTATTAGAGTTGCAGAACT	BSOLV
Ol_2	5′ TGTGTACAAAGACACAACAGGAAGTCTTAGCCTTAAAAGAC	BSOLV
Im_1	5′ CTGGAAGAACAAGAGCTTCAACTTCAAGAAGTTCACAAGC	BSIMV
Im_2	5′ AGTGATCAAGCAGAATCAGCTCATCACTACGTTATCTGAG	BSIMV
Vn_1	5′ GGAAGAACAAAAGCAGCAGCTGTCTGAAATTAACAACCTAT	BSVNV
Vn_2	5′ TGATCAAACAAAATCAGCAGATCACCACCATCTCGGAAGA	BSVNV
GF_1	5′ AGCAGGAATTACAGCTCCAGGAGATTGGAAAACTTTCCAA	BSGFV
GF_2	5′ CTGCAATTACGTATAGAAGTTGCTAGTTTGCAGGAAGAGC	BSGFV
Mys_1	5′ GAGCTTGAAAAACTTCACAAGAAGCTTGAACTGTTTGGAGA	BSMYV
Mys_2	5′ CGGTGGTCAAAATCAAAGACGACATCGAAGGACTAAAAGA	BSMYV

Supplemental analysis S1A. Nucleotide and amino acid sequence analysis. Table summarizes comparison of the complete genome nucleotide sequences of reconstructed BSV species and their gene products (P1, P2 and P3) with those of the Genbank reference sequences of previously isolated and sequenced BSV species. Below the table, the CLUSTAL alignments of nucleotide sequences of three selected regions of the BSV genomes reconstructed in this study are shown.

I. Sequence analysis of the isolates of the six BSV species

New BSV isolate	**Length in nts**	**GenBank access. number**	**Old isolate acc. No.**	**Length in nts**	**Nucleotide identity in % Indels**	**Number of amino acid substitutions**	**Comments**
BSOLV	7,393	KJ013506	AJ002234	7,389	99.6% one 4-nt ins in IGR	one in P2, ten in P3	
BSGFV	7,263	KJ013507	AY493509	7,263	99.2%	one in P1, one in P2, 13 in P3	
BSIMV	7,768	KJ013508	HQ659760	7,768	99.7% one 1-nt del and one 1-nt ins in IGR	one in P1, five in P3	
BSMYV	7,652	KJ013509	AY805074	7,650	98.9% two 1-nt ins in IGR	two in P1, three in P2, 14 in P3	
BSVNV	7,797	KJ013510	AY750155	7,801	99.7% one 1-nt ins in IGR two 1-nt deletions in ORF I, on 3-nt del in ORFIII	six in P3, one aa del in P3 and 31 aa extention in P1	Elongated ORF I, similar to other BSVs
BSCAV	7,421	KJ013511	HQ593111	7,408	93.8% several short indels in IGR mostly upstream of PBS, three 1-nt and one 3-nt ins in a short region of ORF III	seven in P1, six in P2, 37 substitutions and two single aa deletions in P3	The isolates of two distinct strains of the viruses

IGR, intergenic region; **PBS**, Met-tRNA primer binding site; **P1**, product of ORF I; **P2**, product of ORF II; **P3**, product of ORF III; **aa**, amino acid; **del**, deletion; **ins**, insertion

II. Multiple sequence alignment of the reconstructed BSV genomes using CLUSTAL

CLUSTAL 2.0.12 multiple sequence alignment of a BSV region upstream of the primer binding site

* consensus nucleotides

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                                                                       CCAAT-box
BSOLV           GTTAGG-----------------CCAGAGACATGTGATGATGCTTATCT-GCATTATTGG
BSCAV           GTGTGT-----------------CCAGAGGTGTGCTGTGACGCGTCCCTTGCATTATTGG
BSIMV           ATTGGTGCCTGCGTGCATCACTACTCTAGCTTTATTAAAGTTTGTCGGTTGCATTATTGG
BSVNV           ATTG--------------------------------------TGTCGGTTGCATTATTGG
BSMYV           CCT-------GGTATTGGCGCTGCGGTTTTAAGCCCACGGTTTTCGGACTCCATGAGTTT
BSGFV           CCTAAA----------------GTTAGTGCGTTCCAACGCACATCTGTGTGTAAAGGTAT

                                                           TATA-box
BSOLV           -TGGATGCCACCTAACGATGC-TAGAAAGCTCCACAACTC-TCTATATAAGGAG--CCTT
BSCAV           -TGGGTGC-ACCTAACGATGC-GGGAAGCCGAACTCCC---TCTATAAATAGGACCCCGT
BSIMV           -TGCCTGCGTGCATCCGAAGC-AACCTTTGCCTTTCTCTA-TCTATAAGAGAAGG-CCTT
BSVNV           ATGCCTGCGTGCACCCTAAGC-AATCCCCGGCCCTCTTC--TCTATAAGAGGAG-CCCTT
BSMYV           -TGAAATCCGACGGCTTTAGTCTGAGAAGGCTCAGCCTTTCTCTATATAAGGG-TTTGTA
BSGFV           CTGGCTGTTTCCAGACGCTACCTCCCTCTTTTCTCCTCCCGTCTATATAAGGAGG-CAGA
                 **                                      ******
                                 +1 (transcription start site)
BSOLV           GTATTCAGGTTGCAAACACGCAC----CACAACGCGAGTTTACTCCTGATTTG------A
BSCAV           GTATTCAG-TTGCAAGCACGCAA----CACAACGCGAGCTTACTTCTGA--GA------A
BSIMV           GTAACTCAGTTGCAAGCATGCAA--TTCCCAC-GCAAGCTTACTTCTGAATTC---TCCA
BSVNV           GCAA-TCAGTTGCAAGCATGCAAGTTTCCCACTGCAAGCTTACTTCTGAGTTTGAGTTCA
BSMYV           ACCCCTCG-TTGCAAGCATAGTCGGAAATACCAGACTGCTTACTTCGAGTTTTGA----A
BSGFV           ACCTAAGTGTTTCAGGCATCGAGGGAAATACCCATCTGCCTAATCCACTTCCAG-----T
                         ** **  **                   *  ** * *

BSOLV           GAAATAAAAACT-TCTGTGCTTGAAACACACTTTGTGCGAGTTCACTTTGTGCGAGTA--
BSCAV           GAAATAAGAACAATTTGTGCTTGAAATACACCTTGTGTCAAGA------GTGTGAGTA--
BSIMV           GAAATCAAAGTCAATAGAATCCTTTTCTTACAGA-TGTTCTTGAAATCTCTATGAGCAA-
BSVNV           AGTTCAATAAAAATTCAAGCTTTCCTCTTACATTCTGTTCTTGAAAGGTTC--GATC---
BSMYV           ATCCCAATAAGAATCCTCAGTTTTCTTCATCCTTCTTTCGGTTCACTTCCTGAAATTGGG
BSGFV           GTTTTCCAAAGCAGCTGAAGTTTT------CAGTCTGTGAGTA--------GAAAGCAA-
                        *                     *    *                  *

BSOLV           -GAGCGCAAGATCCTAGTTCCGCGAGC-GT-AGACCTATC----------------
BSCAV           -GAGCGCAAGATCCGTGTTGGGAAATCCGTGAAATCCGTGCCGTTCTGGAAATCCG
BSIMV           TCATTAGACGAGTAAAGAACAAGATCCGCAAAGCCCCCCCCCTATA----------
BSVNV           TGATCGAGCGAGTAGAGAACAAGATCTTTTGGGATTTCCGCCGTTCCG--------
BSMYV           CAAGCCCCATAGTAAGGA--AAGATCCATTTGGCATAATTCCGCTGCATTCC----
BSGFV           -GATCCTTGTAAGAATTTTTGAGAAGT-TTATATTCGATTTCTCCTCATC------
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CLUSTAL 2.0.12 multiple sequence alignment of the pgRNA leader from six BSVs

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                +1 (transcription start site)                   PolyA signal
BSOLV_L         ACGCAC----CACAACGCGAGTTTACTCCTGA---------TTTGAGAAATAAAAACT-TCTG
BSCAV_L         ACGCAA----CACAACGCGAGCTTACTTCTGA--GA---------AGAAATAAGAACAATTTG
BSIMV_L         ATGCAA--TTCCCAC-GCAAGCTTACTTCTGAATTC------TCCAGAAATCAAAGTCAATAG
BSVNV_L         ATGCAAGTTTCCCACTGCAAGCTTACTTCTGAGTTTGAGTTCAAGTTCAATAAAAATTCA-AG
BSMYV_L         ATAGTCGGAAATACCAGACTGCTTACTTCGAGTTTTGA----AATCCCAATAAGAATCCTCAG
BSGFV_L         ATCGAGGGAAATACCCATCTGCCTAATCCACTTCCAGTGTTTTTTTTCCA-AAGCAGCTGAAG
                *                   *  ** * *

BSOLV_L         TGCTTGAAACACACTTTGTGCGAGTTCACTTTGTGCGAGTAGAGCGCA-AGATCCTAGTT
BSCAV_L         TGCTTGAAATACACCTTGTGTCAAGA------GTGTGAGTAGAGCGCA-AGATCCGTGTT
BSIMV_L         -------AATCCTTTTCTTACAGA-TGTTCTTGAAATCTCTATGAGCAATCATTAGACGA
BSVNV_L         ---------CTTTCCTCTTACATTCTGTTCTTGAAAGGTT--CGATC--TGATCGAGCGA
BSMYV_L         TTTTCTTCATCCTTCTTTCG-GTTCACTTCCTGAAAT-----TGGGCAAGCCCCATAGTA
BSGFV_L         TTTTC--------------------AGTCTGTGAGTAG----AAAGCA-AGATCCTTGTA
                                                *             *
                                                      Primer binding site
BSOLV_L         -------CCGCGA-----GCGT-AGACCT----ATC--TGGTATCAGAGCAAGGTT----
BSCAV_L         GGGAAATCCGTGAAATCCGTGC-CGTTCTGGAAATCCGTGGTATCAGAGCAAGGTT----
BSIMV_L         GTAAAGAACAAGATCCGCAAAG-CCCCCCCCC--TATATGGTATCAGAGCTAAGTTTTCT
BSVNV_L         GTAGAGAACAAGATCTTTTGGG-ATTTCCGCCGTTCCGTGGTATCAGAGCAAGGTTTTCT
BSMYV_L         AGGAAAGATCCATTTGGCATAA-TTCCGCTGCATTCC-TGGTATCAGAGCAAGGTTCACT
BSGFV_L         AGAATTTTTGAGAAGTTTATATTCGATTTCTCCTCATCTGGTATCAGAGCAAGGTT----
                                                      ************ * ***
                            Take-off site for the shunting ribosomes
                            sORF1/1’            Conserved motif
BSOLV_L         ---CGTT--TTTATGGCTTTCATGGGGTAATTCCTTTAGATAGGAGCCGAAGGGCTCTGC
BSCAV_L         ---AGTT--CTTATGGCTTTCATGGGGTAAAACCCTTAGGTAGGAGCCGATGGGCCCTGC
BSIMV_L         GTAAGTT--TTTATGGCTTTTATGGGGTAATTCCCTTAGATAGGAGCCTAAGGGCTCTGC
BSVNV_L         GTAAGTAA-TTTATGGCTTTCATGGGGTAAAACCCTTAGATAGGAGCCTGAGGGCTCTGC
BSMYV_L         GTAAGTAAATTTATGGCTTTCATGGGGTAATTCCCTAGGATAGGAGCCTAAGGGCTCTTT
BSGFV_L         ---AAGA--TTGATGGCATTCATGGGGTAAAACCCTAAGATAGGGGCCTGATTGAGTTCT
                          * ***** ** *********  ** *  * **** ***     *   *
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BSOLV_L         TTTTCTCTAATT-GAGTTACAAGTT---TATGA-----TTTAAATTGTTTAAATTGGAGC
BSCAV_L         TATTTTTGATTT-GGGTTAATGGTTGTACAAGT-----TTTATGATAGATAAGTCGAA--
BSIMV_L         TATGCTTCATTTTTAGAAAATAGCTGTGTAAGTAGACTTGCATAATGATTCTAGAAAGAC
BSVNV_L         TATGTTCTATTTTGAGAAAATAACTGTGTAAGTAGTAATGCATGATAAATCGAATAAGTT
BSMYV_L         TTATGCATATTTCTGAATTTCTGTT--AAGAACTATGCTTTATATACTCTGAAGCGAATT
BSGFV_L         ACGTTTCTGTTAAGGTAAGATTTCCTTTCCTGATAAGTCGAATAATTTTTA---TGTATC
                          *                              *       *

BSOLV_L         TGTATTCAGTCTTTCTTA-GAAAAATGAGCATGATT--TCGATTATAGCTGGTCAAGGCT
BSCAV_L         TGGAGCAACACTTATGTATGAAAAATGATTGCT-TT--ATGATAATAGATGGGCTAGGGT
BSIMV_L         TAAGAAGAGTATTCT--ATGTTATGATACTA--CTT--GTGAAAGGAAAAAGGATTTCTT
BSVNV_L         CTTTACGGATGGTCCTAAATTTATGAAATCATTCTT--TTACTATCTGAGTGAGACCCTT
BSMYV_L         TCTGGCAAGTAAGTTTTAAGAGGTA-GATGG---TA--ATACCTTATGAGGAGTAATACT
BSGFV_L         TTTCTCAAGTAATCTTGAAAAACTGGCATGATTATTGAATGAAAAATGAGCTATTACCAA
                                 *         *      *

BSOLV_L         GTAGGGAAAAATGATTAT--GTTTTATGCTAGTTGGT--CCAAGAGAGCATGCCTACCCA
BSCAV_L         GTAAGGAAAAATGATCACAAGGTCGATTCTATTTGTTTTCCAAAAGATCATGCCTACCCA
BSIMV_L         GCGTTTCCAAGTTCTACT--GAAATA-----------------------ATGTCCATCTG
BSVNV_L         GTGTTTCCAAGTTCTACT--GAGAAATCATGAGTAAAT-GGACGTTGACACACTGACTTA
BSMYV_L         ACATATCTAGACTAAGGAACGTCTTAGGA------------AAGAGGCGATGAGAAGATA
BSGFV_L         AAACAAAAAAATGGAGTTGGGAAGACAGAGGGGTTGTTTGCAGAAGGCCGTTCTAAGCCA
                        *           *                                  *

BSOLV_L         AGAAAA---AGTACCCGAAGAGAATGGGGAAAAAATTGGTTCTT---CGCATGTATGAGG
BSCAV_L         AGA------AGTACCC----ATGAGGGGGAAGATAGTGGTTCT----CGCATGAGTGAGG
BSIMV_L         AGAAA----------TGCCTAATATATAGT------------------GAAAGG----GA
BSVNV_L         AGGAAAAGAAAGATGTGCCTAGGATGAAGTCGAGCACTGTTCTAGGCTGAAGGGTGAAAA
BSMYV_L         AGGTGA--------CTACTGGGAATAGACGTAAGCCCATT--------GTAAGTCCCAAG
BSGFV_L         AGGTTGGGCGTCAAAGGCCGTGTCAGACCCGGAGATCTTCTAATATCCTCCTGAGGTAAT
                **                                                  *

BSOLV_L         ATAATATCCTAGAAACAGAACATAAACTGCGTGAAGTACTTACAAAGCCAAGATAATATC
BSCAV_L         AAGAAATATCATAGATAGACAGTGAACTCCGTGAAGTACTTACAT-GCTTAGCTGATATC
BSIMV_L         GAAAAATGACTTGAGTTGGGAGTGTTAGTTTATCGGACTTGGAGCAAAAGAACCAGAACC
BSVNV_L         GAAAAATGAGTTGAGTTGGGAGTAAGACTCTGAGAGGCTTGAGGCAAAAGAACCGGAACC
BSMYV_L         GAAGGGCAGCTTGTGGCAAAAAGGTGACTCAGTATAACCTGAAGATATCGCGTCTCTGTC
BSGFV_L         AAGCGCTTCGTGAAGTAGTTCAATAATTATGACTAGTTATTAAGA----GAGCTTGGGTA
                                                       *

BSOLV_L         CTATATAGAAACATGGGCATCGTGAA----GTATAAGACTG-ACTGAACTA-CAAGCTCC
BSCAV_L         CTATTTAGTGAGGTAGACACCGTGAA----GTAGTACATAA-AAAGCTCCAGTAAGACGT
BSIMV_L         TTACCTGGCAT-GTAAGACCCAAGGG----GCAGAAGCTGG-AGAACCCTGGTGTGAGTT
BSVNV_L         TTACCTAGCAAAGTAAGACCCAAGGGAGG-GCAGTAGCTGG-AGAACCCCGGTGTGAGTT
BSMYV_L         TAATTTAATATCCTGAGACTCCTAAAATCCGGACATGATAGTAAAACTCTGATAAAG---
BSGFV_L         AAAGGTAGCATCAAAAGTTAGTAAGACGTAAAGGAAGGAAGGAAAACCCTCAGATAACGT
                  *  *                                    *     *

BSOLV_L         TTTATAAACAAAAAGATCATAGACCT----CTGTAGGTACGTCAATACGGGTTAAGCATC
BSCAV_L         AATATCATTAGAACCTCTGTGA--CT----GACCAGGTACGCCAATAGAAAATA-GTATC
BSIMV_L         TCTT-ATTCGGTATTATTAAAACTCTAGTATCCTCAAAACTTCGTGAAGTATT------C
BSVNV_L         TCTTTACCTAGCAGAAGATATACTCTAGTATCCTCAAAACACCGTGAAGTATTTACAACC
BSMYV_L         -CAAAAGCCTGAAGCTTCGAAATACCTGCTAGCCAGAGATAAACTAAGGAAATTATAAAG
BSGFV_L         -GTCTCATGAAGGTTACCCCTATCCTTCTATTCTGAAATTACTTCTACCTAAC------C
                                        *                     *

BSOLV_L         CTGGAGGAGTACTCCTATCTAGTTTACGAAAAGAAAGTCATTCACC-----------TTG
BSCAV_L         CCGGAGGACTTCTCCTACCTTGTTTATCATAAGAAAGTCATCCACC-----------TTG
BSIMV_L         CTT---GAA-ACTATAATATTAGCAATTTGATGGTGTTCACTAGA------------TCA
BSVNV_L         CTTTATGAA-CAAGTATTTTCTGGAGTTAGATGTCAGCCACCAAAGATCATGATCTATCA
BSMYV_L         CCGTG-----CTCTTAACTTTAATTTACAAGAAAAATGCATAAATAACCCGGAGGTTCTT
BSGFV_L         TTGTATG-----------------------------------------------------


BSOLV_L         CATA-CAATTTATG----------------------------------------------
BSCAV_L         CAAAACGTTTTATG----------------------------------------------
BSIMV_L         CAAAAAAATTTGAGTTGTGCAAGAATGAAAGATTCCGAT-CACATGATAGTTGACTAAAC
BSVNV_L         CAAACAAACTTGAGTCATACAGA---GAAGAACTCAGATGTAACTGAATGACTAATGATC
BSMYV_L         TATCCTGCTTCAAAGCCTTTAGTTTACTTAAACCTTGAACCAAAGCTAATTCTG------
BSGFV_L         ------------------------------------------------------------


BSOLV_L         ------------------------------------------------------------
BSCAV_L         ------------------------------------------------------------
BSIMV_L         TGGAAGACCCTTTTAAGT-CTGAAAGAAAAACAGATGCATGAGCTACCTCTAGAACTGGC
BSVNV_L         AGGGACCTCTATTAAAGAGCTTAGTAAGAAGTAAGTGCATAAACTACCTCTGGAACTGGT
BSMYV_L         ------------------------------------------------------------
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BSGFV_L         ------------------------------------------------------------

BSOLV_L         ------------------------------------------------------------
BSCAV_L         ------------------------------------------------------------
BSIMV_L         TACAATCT-ATTATGAGCATG-GCATCCCAAAGGACTACTCCTATCTGTCTCAAGAATTT
BSVNV_L         TATAATCTTGAAATGAGCATATGTATCCCAAAGG-TAACTCCTATCTGTC-CGTGACCAT
BSMYV_L         ------------------------------------------------------------
BSGFV_L         ------------------------------------------------------------

BSOLV_L         --------------------------------
BSCAV_L         --------------------------------
BSIMV_L         GGTTTAAAAACCTTTGTTAA-------CTCTG
BSVNV_L         A----AGAAACCTTCTTTAAAGAAAAACTCTG
BSMYV_L         --------------------------------
BSGFV_L         --------------------------------
```

CLUSTAL 2.0.12 multiple sequence alignment

The sequence just upstream of the OFR I

```
BSOLV_L         -------ACAAAAAGATCATAGACCTCTGTAGGTACGTCAATACGGGTTAAGCATCCTGG
BSCAV_L         ------CATTAGAACCTC-TGTGACTGACCAGGTACGCCAATAGAAAATA-GTATCCCGG
BSIMV_L         -AGATGCATGAGCTACCTCTAGAACTGGCTACAATCT-ATTATGAGCATG-GCATCCCAA
BSVNV_L         ----TGCATAAACTACCTCTGGAACTGGTTATAATCTTGAAATGAGCATATGTATCCCAA
BSMYV_L         ----------AGCCGTGCTCTTAACT-TTAATTTACAAGAAAAATGCATAAATAACCCGG
BSGFV_L         ATCAAAAGTTAGTAAGACGTAAAGGAAGGAAGGAAAACCCTCAGATAACGTGTCTCATGA
                          *                   *                        *

BSOLV_L         AGGAGTACTCCTATCTAGTT--TACGAAAAGAA---AG--TCATTCACCTTGCATA-CAA
BSCAV_L         AGGACTTCTCCTACCTTGTT--TATCATAAGAA---AG--TCATCCACCTTGCAAAACGT
BSIMV_L         AGGACTACTCCTATCTGTCT--CAAGAATTTGGTTTAA--AAACCTTTGTTAA-------
BSVNV_L         AGG-TAACTCCTATCTGTC---CGTGACCATA----AG--AAACCTTCTTTAAAGA-AAA
BSMYV_L         AGG--TTCTTTATCCTGCTT--CAAAGCCTTTAGTTTACTTAAACCTTGAACCAAAGCTA
BSGFV_L         AGG-TTACCCCTATCCTTCTATTCTGAAATTACTTCTACCTAACCTTGTATG--------
                ***    *      *                           *

BSOLV_L         TTTATG
BSCAV_L         TTTATG
BSIMV_L         -CTCTG
BSVNV_L         ACTCTG
BSMYV_L         ATTCTG
BSGFV_L         ------
```

Sequence Analysis S1B. Conservation of the *cis*-acting elements that drive ribosome shunt and leaky scanning in six BSV species. The conserved shunt configuration and short ORFs (sORFs) in the pgRNA of BSOLV, BSGFV, BSIMV, BSMYV, BSVNV, BSCAV, RTBV and CaMV are shown. The pgRNA is depicted as a thick line, large ORFs (I, II, and III) as thick line boxes, sORF as thin line boxes and numbers are the position in the virus genome (starting from the PBS / the transcription start site). The conserved sORF 1/1’ is boxed in red. Secondary structure of the pgRNA leader, as predicted using MFold, is depicted schematically above the pgRNA. In all but CaMV, the first two large ORFs are devoid of sORFs. The stability of the structure (*dG*) in kcal/mole is given. 5- and 3’-sequences flanking the main structure are shown in open conformation, since they do not form any extensive structures. The stable structural element (circled at the stem base) and adjacent regions are enlarged alongside. The short ORF 1/1’ and ORF I are boxed. AUG codons are in bold; potential non-AUG initiator codons within the shunt landing sequence are underlined. Below the pgRNA, the sequences at the junctions of ORFs I and ORFs II and III are shown with genome positions of the corresponding stop and start codons indicated.

Sequence Analysis S1B. Conservation of the cis-acting elements that drive ribosome shunt and leaky scanning in six BSV species.