

Supporting Information

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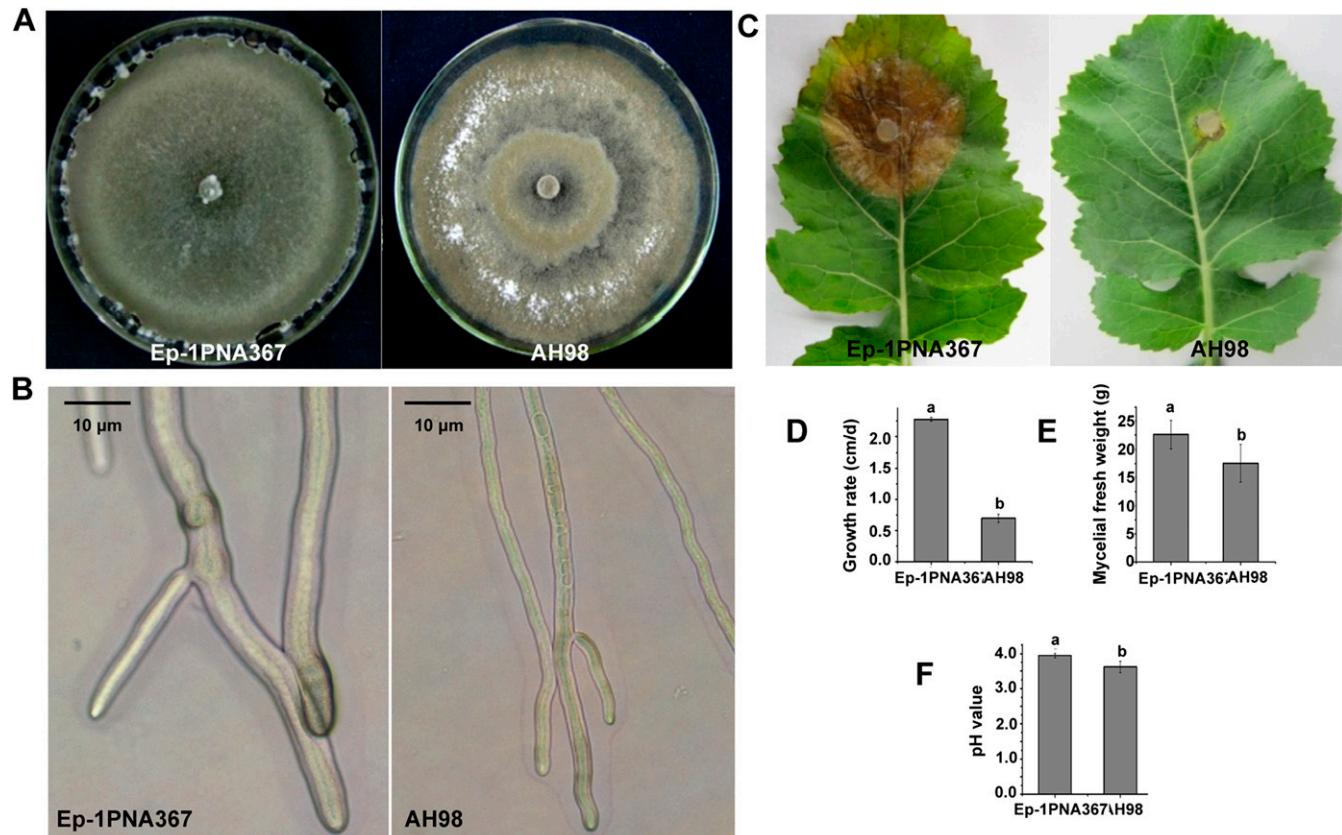


Fig. S1. Biological characterization of *Sclerotinia sclerotiorum* strain AH98. (A) Colony morphology of strain AH98 grown on potato dextrose agar (PDA) at 20 °C for 10 d. (B) Hyphal tips of strain AH98. (C) AH98 lost virulence on detached leaves of rapeseed. Photos were taken at 3 d postinoculation. (D) Growth rate of AH98 on PDA at 20 °C. (E) Fresh biomass of strain AH98 grown in 100 mL potato dextrose broth (PDB) in a 250-mL flask for 6 d. (F) pH values of filtrates of strain AH98 grown in 100 mL PDB in a 250-mL flask for 6 d. Error bars indicate the SD from five sample means. Means followed by the different letters on the top of each column are significantly different at the $P < 0.05$ level of confidence according to Duncan's multiple range test.

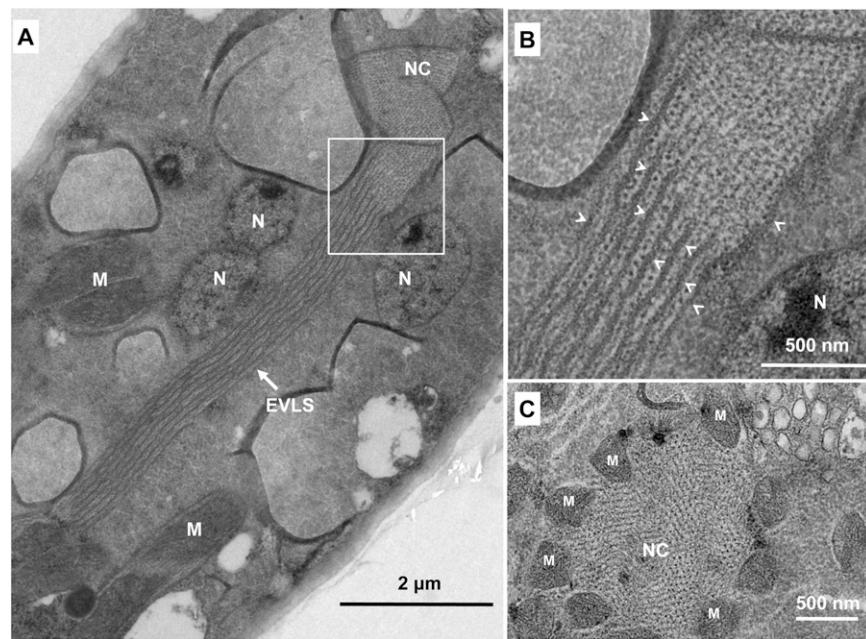


Fig. S2. Nucleocapsids and membrane EVLSs of SsNSRV-1 in ultrathin hyphal sections of virus-infected strain. (A) Intact EVLS (arrow) in original strain AH98. (B) Membranes of EVLS (white arrowheads). (C) Nucleocapsids in *Sclerotinia sclerotiorum* negative-stranded RNA virus 1 (SsNSRV-1)-infected strain Ep-1PNA367-PT2. EVLS, enveloped virion-like structures; M, mitochondrion; N, nucleus; NC, nucleocapsids.

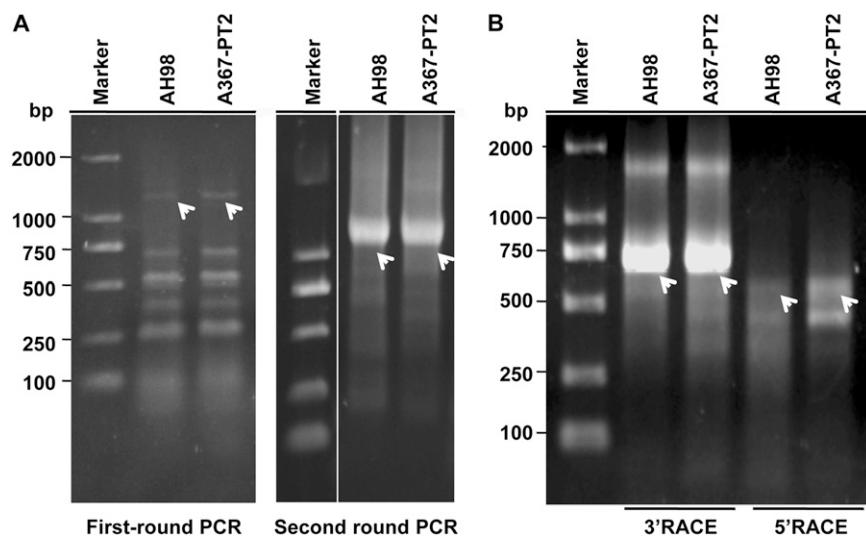


Fig. S3. (A) Viral genomic RNA of SsNSRV-1 was circularized and RT-PCR amplified with specific primers. (B) Viral genomic RNA of SsNSRV-1 was ligated to an oligonucleotide and 5' and 3' rapid amplification of cDNA ends analyzed. The primers and other oligonucleotides used for cDNA cloning are listed in Table S3. Both circularization and oligonucleotide ligation were catalyzed by T4 RNA ligase. The arrows indicate the specific DNA band of SsNSRV-1 amplified by PCR.

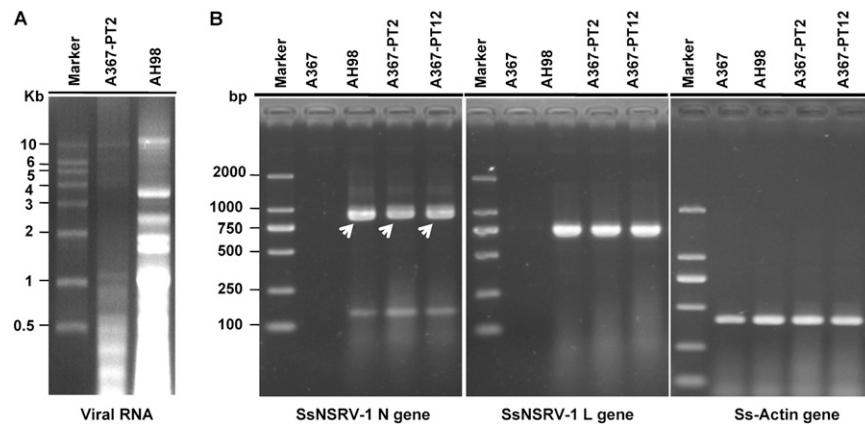


Fig. S4. (A) Viral RNA samples extracted from transfected strain Ep-1PNA367-PT2 and original strain AH98. RNAs were electrophoresed in 1.2% agarose gel containing 1% formaldehyde in 1× Mops buffer (pH 7.0). (B) RT-PCR detection of mycovirus SsNSRV-1 in transfected isolates (Ep-1PNA367-PT2 and Ep-1PNA367-PT12) using total RNA. The actin gene was used as an internal control. The sizes of the RT-PCR products for SsNSRV-1 are 721 and 921 nt, respectively.

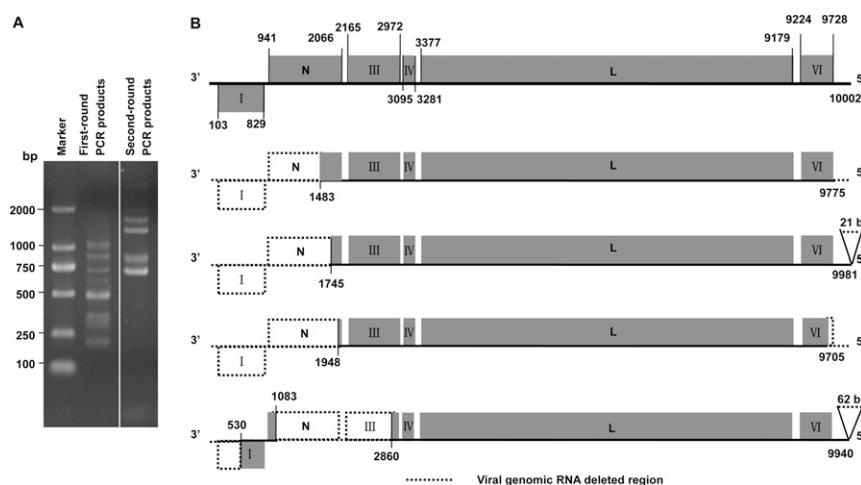


Fig. S5. (A) RT-PCR amplification of 5' and 3' terminus of defective viral RNAs. (B) Genome organization of defective viral RNAs of SsNSRV-1. A diagram of the genomic organization of SsNSRV-1 is shown in the first line.

Table S1. Sequencing analysis of the mRNAs of each viral gene of SsNrv-1

Table S1. Cont.

Viral genes

*PACE covariance

*Sequences highlighted in yellow are the universal primer CDSII-3. Sequence in blue is poly(A) sequence of the first-strand cDNA tailed by terminal deoxynucleotidyltransferase. Sequences in green are gene-specific primers. The start codons ATG are highlighted in purple.

AIG are highlighted in purple

Table S2. Peptide mass fingerprinting analysis of virions crude preparations, 20% and 25% sucrose gradient components, and p43 and p41 proteins

Amino acid sequence	Mass							
	Observed	Calculated	±Δ	Putative ORF	Position	Ion score	Frequency	
Virion crude preparations								
DRLPTIDLTR	1,198.6617	1,198.667	-0.0053	ORF II	27–36	21.69	1	
VIFGSAYIPIDR	1,349.7277	1,349.7343	-0.0066	ORF II	319–306	40.78	2	
NAISQFMPGELK	1,420.6941	1,420.702	-0.008	ORF II	183–195	31.48	2	
QPSPENLDAFNLK	1,454.6971	1,454.7041	-0.007	ORF II	168–180	58.88	3	
LTFGASLSPLLLITK	1,572.9415	1,572.9491	-0.0076	ORF II	251–265	59.31	1	
VFTDNSPYLSHAVLSK	1,776.8962	1,776.9046	-0.0084	ORF II	196–211	79.74	2	
LVIGAMISASGVNYSTAR	1,808.926	1,808.9454	-0.0194	ORF II	71–88	43.27	2	
EFSDETEYFAAAHALQR	2,096.9693	2,096.9803	-0.011	ORF II	276–293	109.06	3	
ALVMSSIVDKDDPMVSGTQR	2,148.0433	2,148.0555	-0.0122	ORF II	224–243	73.21	5	
FSHPSEDAEAEALPALPNSCVPK	2,580.1969	2,580.2166	-0.0197	ORF II	108–131	14.73	2	
QT'TADAISAASADLR	1,489.7315	1,489.7372	-0.0057	ORF I	156–170	68.9	1	
NIFPSSAALLTESTK	1,577.8209	1,577.8301	-0.0092	ORF I	93–107	55.58	2	
VEIQACHSSSVSLHK	1,593.7866	1,593.7933	-0.0067	ORF I	142–155	38.08	2	
LTAATNNILSMYYPEELK	1,906.9647	1,906.971	-0.0063	ORF I	190–206	59.01	1	
INALEALVHSQGQQISDLDQNSALR	2,847.4275	2,847.4475	-0.02	ORF I	116–141	3.09	1	
Sucrose gradient components, 20%								
DRLPTIDLTR	1,198.661	1,198.667	-0.006	ORF II	27–36	12.61	2	
VIFGSAYIPIDR	1,349.726	1,349.7343	-0.0083	ORF II	319–306	28.55	1	
NAISQFMPGELK	1,420.6931	1,420.702	-0.0089	ORF II	183–195	66.14	2	
QPSPENLDAFNLK	1,454.6958	1,454.7041	-0.0084	ORF II	168–180	14.12	4	
DKDTYGVNIEANR	1,493.7044	1,493.711	-0.0066	ORF II	14–26	25.06	1	
LTFGASLSPLLLITK	1,572.9414	1,572.9491	-0.0077	ORF II	251–265	90.24	1	
QPSPENLDAFNLR	1,610.7975	1,610.8053	-0.0078	ORF II	168–181	47.07	1	
VFTDNSPYLSHAVLSK	1,776.8925	1,776.9046	-0.0121	ORF II	196–211	72.36	3	
LVIGAMISASGVNYSTAR	1,808.9345	1,808.9454	-0.0109	ORF II	71–88	28.04	5	
EFSDETEYFAAAHALQR	2,096.969	2,096.9803	-0.0113	ORF II	276–293	46.81	4	
ALVMSSIVDKDDPMVSGTQR	2,148.0401	2,148.0555	-0.0154	ORF II	224–243	50.1	6	
FSHPSEDAEAEALPALPNSCVPK	2,580.2023	2,580.2166	-0.0143	ORF II	108–131	29.94	2	
Sucrose gradient components, 25%								
DRLPTIDLTR	1,198.6611	1,198.667	-0.0059	ORF II	27–36	2.69	1	
VIFGSAYIPIDR	1,349.7308	1,349.7343	-0.0035	ORF II	319–306	48.55	1	
NAISQFMPGELK	1,420.6941	1,420.702	-0.008	ORF II	183–195	54.25	1	
QPSPENLDAFNLK	1,454.6974	1,454.7041	-0.0068	ORF II	168–180	10.42	3	
LTFGASLSPLLLITK	1,572.9391	1,572.9491	-0.01	ORF II	251–265	73.49	1	
LVIGAMISASGVNYSTAR	1,808.9336	1,808.9454	-0.0119	ORF II	71–88	43.11	3	
EFSDETEYFAAAHALQR	2,096.9696	2,096.9803	-0.0107	ORF II	276–293	68.86	2	
ALVMSSIVDKDDPMVSGTQR	2,148.0365	2,148.0555	-0.019	ORF II	224–243	47.55	1	
FSHPSEDAEAEALPALPNSCVPK	2,580.1942	2,580.2166	-0.0224	ORF II	108–131	13.23	3	
p43 protein								
ALVMSSIVDK	1,077.57	1,077.57	-0.0023	ORF II	224–233	78.65	18	
ALVMSSIVDKDDPMVSGTQR	2,164.05	2,164.05	-0.004	ORF II	224–243	115.46	16	
DKDTYGVNIEANR	1,493.71	1,493.71	-0.0036	ORF II	14–26	107.32	8	
DRLPTIDLTR	1,198.66	1,198.67	-0.0049	ORF II	27–36	80.87	9	
DTSAEIK	762.38	762.38	0.001	ORF II	95–101	43.18	2	
DTSAEIKNWVISR	1,517.79	1,517.78	-0.0014	ORF II	95–107	66.63	1	
DTYGVNIEANR	1,250.58	1,250.59	-0.0051	ORF II	16–26	83.24	12	
EAAEETVPEAPAO	1,340.61	1,340.61	-0.0016	ORF II	363–375	104.85	11	
EFSDETEYFAAAHALQR	2,096.98	2,096.98	-0.0038	ORF II	276–293	81.54	6	
EKLIALLNIR	1,181.75	1,181.75	-0.0047	ORF II	353–362	47.28	4	
FLDTSTK	810.41	810.41	-0.0026	ORF II	294–300	37.37	4	
FSHPSEDAEAEALPALPNSCVPK	2,580.21	2,580.22	-0.0101	ORF II	108–131	58.75	1	
INKTFNSIIR	1,205.69	1,205.68	-0.012	ORF II	212–221	30.67	1	
LANVFKDTSAEIK	1,434.76	1,434.77	-0.0113	ORF II	89–101	53	1	
LPTIDLT	927.54	927.54	-0.0002	ORF II	29–36	55.25	34	
LTFGASLSPLLLITK	1,572.94	1,572.95	-0.0057	ORF II	251–265	69.61	16	
LVIGAMISASGVNYSTAR	1,808.94	1,808.95	-0.0008	ORF II	71–88	141.13	9	
MFYVMFR	1,008.45	1,008.46	-0.0029	ORF II	244–250	42.49	16	
NAISQFMPGELK	1,420.7	1,420.7	-0.0042	ORF II	183–195	86.58	42	
NWVISR	773.42	773.42	-0.0025	ORF II	102–107	30.34	13	

Table S2. Cont.

Amino acid sequence	Mass							
	Observed	Calculated	$\pm\Delta$	Putative ORF	Position	Ion score	Frequency	
QPS PENLDAFDNLK	1,471.73	1,471.73	-0.0056	ORF II	168–180	117.68	51	
QPS PENLDAFDNLKR	1,628.83	1,628.82	0.0166	ORF II	168–181	86.47	2	
RNAISQFMPGELK	1,593.78	1,593.78	0.0002	ORF II	182–195	51.77	4	
RTIPDVFK	974.55	974.55	-0.0045	ORF II	6.0–13	24.69	1	
TFNSIIR	849.47	849.47	-0.0018	ORF II	215–221	46.64	21	
TFNSIIRDR	1,120.62	1,120.6	0.0224	ORF II	215–223	33.65	2	
TIPDVFK	818.45	818.45	0.0008	ORF II	7.0–13	40.01	28	
TIPDVFKDK	1,061.57	1,061.58	-0.0037	ORF II	7.0–15	35.37	1	
VFTDNSPYLSHAVLSK	1,776.91	1,776.9	0.0009	ORF II	196–211	114.85	18	
VIFGSAYIPIDR	1,349.74	1,349.73	0.0011	ORF II	307–318	100.69	39	
YPQFFR	856.42	856.42	-0.0002	ORF II	270–275	38.32	8	
IVELTQKER	1,114.59	1,114.63	-0.0406	ORF V	539–547	14.12	1	
p41 protein								
ALVMSSIVDK	1,061.57	1,061.58	-0.0089	ORF II	224–233	57.34	4	
ALVMSSIVDKDDPMVSGTQR	2,180.03	2,180.05	-0.0121	ORF II	224–243	97.35	4	
DKDTYGVNIEANR	1,493.71	1,493.71	-0.0051	ORF II	14–26	44.44	3	
DRLPTIDLTR	1,198.66	1,198.67	-0.0082	ORF II	27–36	21.95	3	
DTYGVNIEANR	1,250.58	1,250.59	-0.0087	ORF II	16–26	60.88	3	
EAAEETVPEAPAQ	1,340.6	1,340.61	-0.008	ORF II	363–375	29.56	7	
EFSDELETEYFAAAHALQR	2,096.97	2,096.98	-0.0089	ORF II	276–293	66.41	1	
EKLIALLNIR	1,181.74	1,181.75	-0.0117	ORF II	353–362	37.07	1	
FLDTSTK	810.42	810.41	0.004	ORF II	294–300	15.99	2	
LIALLNIR	924.61	924.61	-0.0047	ORF II	355–362	48.51	1	
LPTIDLTR	927.53	927.54	-0.0061	ORF II	29–36	29.12	7	
LTFGASLSPLLITK	1,572.94	1,572.95	-0.0076	ORF II	251–265	85.58	3	
MFYVMFR	1,024.44	1,024.45	-0.0081	ORF II	244–250	19.79	4	
NAISQFMPGELK	1,436.69	1,436.7	-0.0117	ORF II	183–195	66.55	14	
NWVISR	773.41	773.42	-0.0038	ORF II	102–107	24.69	6	
QPS PENLDAFDNLK	1,471.72	1,471.73	-0.0115	ORF II	168–180	114.4	10	
RNAISQFMPGELK	1,593.78	1,593.78	0.001	ORF II	182–195	18.58	1	
TFNSIIR	849.47	849.47	-0.0051	ORF II	215–221	28.07	11	
TIPDVFK	818.45	818.45	-0.0029	ORF II	7.0–13	24.35	7	
VFTDNSPYLSHAVLSK	1,776.89	1,776.9	-0.012	ORF II	196–211	44.13	5	
VIFGSAYIPIDR	1,349.72	1,349.73	-0.0097	ORF II	307–318	57.38	5	
YPQFFR	856.42	856.42	-0.0053	ORF II	270–275	17.06	3	
LLESLIK	814.48	814.52	-0.035	ORF V	509–515	20.24	1	

The peptide sequences in bold are identified from virion crude preparations and from the mixture of p43 and p41 sample.

Table S3. Primers pairs used in this study

Primer name	Primer sequence (5'-3')	Genomic location	Product size, bp	Application
AH98-p1-F	GGACTCGGAAGTGTACAGAGAACG	175–190	582	(i) To make probe I; (ii) to detect the gene expression of ORF I; (iii) to detect the property of RNA-nucleoprotein complexes (RNPs)
AH98-p1-R	CGTCAATTCTACCTTCTTCAAACCTG	721–747		
AH98-p2-F	TGCCAAACTGGTTATCGGTGC	1143–1164	839	(i) To make probe II; (ii) to detect the gene expression of ORF II; (iii) to detect the property of viral RNA from AH98
AH98-p2-R	AGAGTTCCCTCTGCGTATTGTTCC	1957–1981		
AH98-p3-F	CTATCGAATAACTGCCAAGACCTCG	2174–2199	721	(i) To make probe III; (ii) to confirm transfectants that were infected by SsNSRV-1; (iii) to detect the gene expression of ORF III; (iv) to detect the property of RNPs
AH98-p3-R	GCATGTGATGTCGTAGCGAGTGTG	2870–2894		
98-Gene4-R	AACCAGATGTGACTCAACCTCTCG	3244–3270	176	(i) To make probe IV; (ii) to detect the gene expression of ORF IV
98-Gene4-F	TTGATTTTACTCGTTGTCGCG	3122–3147		
AH98-p5-F1	GAACGTCGTTCTGCGGCTCC	3415–3435	891	To detect the property of RNPs
AH98-p5-R1	AAGATTCGTTACGGATTCTGGACC	4280–4305		
AH98-p5-F2	ATTATGGCGGGGATTCTGG	5103–5124	873	(i) To make probe V; (ii) to detect the gene expression of ORF V; (iii) to detect the property of viral RNA from AH98
AH98-p5-R2	TCTGTGTTCTAACGGATAAGAGGGG	5949–5971		
AH98-p5-F3	ATTCTTACCACTTGCGGGATGC	6760–6783	940	To detect the property of RNPs
AH98-p5-R3	TGTAAACGGATTCTCACCCACTCTC	7674–7699		
AH98-p5-F4	AAGCCAACGACGCATCTCAC	6244–6266	722	To detect the distribution of SsNSRV-1
AH98-p5-R4	CGTCACGGAGAACGAGCAG	6945–6965		
AH98-P5-F5	TTCAATGGCGGGACAAGGA	5552–5572	1,051	To detect the distribution of SsNSRV-1
AH98-P5-R5	TCGTCACCAAGGCTTCTTGC	6581–6602		
AH98-p5-F6	AGACCACGATACGAAGATAAGAAAGAGA	8146–8174	921	To confirm transfectants that were infected by SsNSRV-1
AH98-P5-R6	CGGAAACTCTGTTGTCGTG	9044–9066		
98-Gene6-R	CGAGGACGGTTCTGCTGGTTC	9686–9709	358	(i) To make probe VI; (ii) to detect the gene expression of ORF VI
98-Gene6-F	ATCGTTTAACATGGAAAGCG	9260–9285		
AH98-p6-F	AGGAGGATGCCTCGTATAAAGAGAAG	9218–9245	682	To detect the property of RNPs
AH98-p6-R	CGTGTACCGTAACCTGTTGCT	9875–9900		
98-G1-RC5-1	TGATTCGCTTAGTGCCTCCGTT	408–431	413	5' RACE of ORF I
98-G1-RC5-2	CCGTAAAGAGCTGACTCGAAG	389–413		
98-G2-RC5-1	CTCGGCTTCGCTGGCATCTT	1281–1301	340	5' RACE of ORF II
98-G2-RC5-2	CAGACGGGACATGACTAAATCGAG	1257–1281		
98-G3-RC5-1	CGATCCCATTGATCCCGAGTC	2561–2582	414	5' RACE of ORF III
98-G3-RC5-2	CCCGAGTCTGACATATGAGTCAT	2544–2579		
98-Gene4-R	AACCAGATGTGACTCAACCTCTCG	3244–3270	165	(i) 5' RACE of ORF IV; (ii) to clone defective RNAs
98-G4-RC5-2	GACTCAACTCTTCGAATTACCAT	3234–3260		
98-G5-RC5-1	GTTCCCCATTCTAAACGCACAT	3793–3817	519	(i) 5' RACE of ORF V; (ii) to clone defective RNAs
98-G5-RC5-2	CGCACATAATCCGAGCGGT	3780–3800		
98-G6-RC5-1	GCATCCCTAACAAAGATACCGC	9515–9538	310	5' RACE of ORF VI
98-G6-RC5-2	CCCTCAAAAGATAACCGCATTAAT	9508–9534		
98-G1-RC3-1	GAAGCATTAGTTCACTCCAGTC	459–484	327	3' RACE of ORF I
98-G1-RC3-2	CAAGATCAGAACCTGCACTCCG	501–524		
98-G2-RC3-1	GTTATGTTGCTCTACTTTGGGC	1678–1704	366	3' RACE of ORF II
98-G2-RC3-2	GGGCATCCCTAACGCTCTCTTG	1700–1723		
98-G3-RC3-1	ATGTCAGACTCGGGATCAATGGG	2556–2577	398	3' RACE of ORF III
98-G3-RC3-2	GGGATCGGGAAATACTAACATCAC	2574–2598		
98-Gene4-F	TTGATTTTACTCGTTGTCGCG	3122–3147	149	3' RACE of ORF IV
98-G4-RC3-2	CTCGTTGCTGCGCACTAGTTT	3132–3155		
98-G5-RC3-1	GTCCTACGCTGGTCTTCTATGTG	8780–8807	367	3' RACE of ORF V

Table S3. Cont.

Primer name	Primer sequence (5'-3')	Genomic location	Product size, bp	Application
98-G5-RC3-2	GAGAATCAAGTATCAGATGTCGTCCTC	8812–8839		
98-G6-RC3-1	CTGCAAACCTCAGTTAACATAATCCGC	9337–9362	370	3' RACE of ORF VI
98-G6-RC3-2	CCGCAAAAAGAACCTCGATGAATT	9358–9381		
AH98-RC3-R1	GGTAGCTGCTGTCAACTCGCTC	664–685	>677	To clone genome termini
AH98-RC3-R2	CTGTCAATCGCTCAACTAACATCCTG	653–677		
AH98-RC5-F1	CCTCAATTCAATTACCCGCGC	9433–9455	>441	(i) To clone genome termini; (ii) to clone defective RNAs
AH98-RC5-F2	TTCGGACATTCCATCCAAACCT	9561–9583		
CDSIII-1	TAGAGACCGAGGCCGCCACATGTTTG– TTTTTTTTCTTTTTTTTVN			5' and 3' RACE
CDSIII-2	GACCGAGGCCGCCACATGTT			
CDSIII-3	CGAGGCGGCGACATGTT			
pC3-T7loop	GGATCCGGATTCTGTAATACGACTC– ACTATATTTTATAGTGAGTCGTATTA			Termini determination of genome
PC2-1	CGTATTACCGAATTCCCGGG			
PC2-2	CCGAATTCCCGGGATCC			

Table S4. Viruses or virus-like sequences selected for phylogenetic analysis in this study

Virus family	Genus	Virus name	Abbreviation	GenBank accession no. of L protein
Mononegavirales				
Filoviridae		Zaire ebolavirus	ZeboV	NP_066251.1
		Marburg virus	MarV	YP_001531159.1
Pneumoviridae	<i>Pneumovirus</i>	Human respiratory syncytial virus	HRSV	NP_056866.1
		Pneumonia virus of mice	PVM	YP_173335.1
	<i>Metapneumovirus</i>	Avian metapneumovirus	AMPV	YP_443845.1
		Human metapneumovirus	HMPV	YP_012613.1
Paramyxoviridae	<i>Morbillivirus</i>	Canine distemper virus	CDV	NP_047207.1
	<i>Henipavirus</i>	Nipah virus	NiV	NP_112028.1
	<i>Rubulavirus</i>	Mumps virus	MuV	NP_054714.1
		Avian parainfluenza virus type 4	APMV4	AFP89384.1
		Tioman virus	TioV	NP_665871.1
	<i>Avulavirus</i>	Newcastle disease virus	NDV	AFH08721.1
	Unassigned	Fer de Lance virus	FDLV	NP_899661.1
		J virus	JV	YP_338085.1
Bornaviridae	<i>Bornavirus</i>	Borna disease virus	BDV	NP_042024.2
		Avian bornavirus	ABV2	ADU05398.1
Rhabdoviridae	<i>Novirhabdovirus</i>	Infectious hemorrhagic necrosis virus	IHN	NP_042681.1
		Viral hemorrhagic septicemia virus	VHSV	NP_049550.1
	<i>Lyssavirus</i>	Rabies virus	RabV	BAL49594.1
	<i>Vesiculovirus</i>	Vesicular stomatitis Indiana virus	VSIV	NP_041716.1
	<i>Ephemerovirus</i>	Bovine ephemeral fever virus	BEFV	NP_065409.1
	<i>Cytorhabdovirus</i>	Lettuce necrotic yellows virus	LYNV	YP_425092.1
Nyamiviridae		Soybean cyst nematode midway virus1	SbCNV1	AEF56729.1
		Midway virus	MIDMV	YP_002905331.1
		Nyamanini virus	NYMV	YP_002905337.1
Segmented negative-stranded RNA viruses				
	<i>Dichorhabdovirus</i>	Orchid fleck virus (RNA2)	OFV	YP_001294929
	<i>Varicosavirus</i>	Lettuce big-vein associated virus (RNA1)	LBVaV	YP_002308576
		Mononegavirus L protein-like sequences		
		<i>Sclerotinia homoeocarpa</i> TSA3-L	ShTSA3-L	JW828891
		<i>Sclerotinia homoeocarpa</i> TSA2-L	ShTSA2-L	JW826636
		<i>Sclerotinia homoeocarpa</i> TSA1-L	ShTSA1-L	JU091016 JU091017