

Supplementary Materials Figures

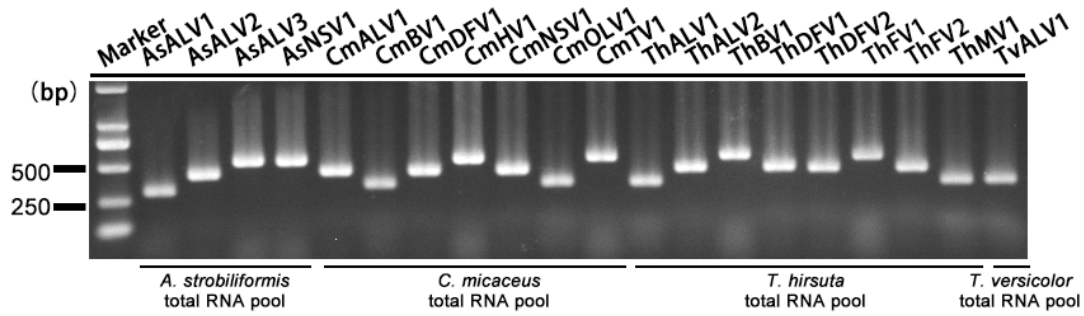


Figure S1: PCR confirmation of mycovirus contigs in four total RNA pools of macrofungi strains (including *Amanita strobiliformis*, *Coprinellus micaceus*, *Trametes hirsuta*, and *Trametes versicolor*, respectively). The viral primers were designed according to the contig sequences, and the primers used are listed in Table S3.

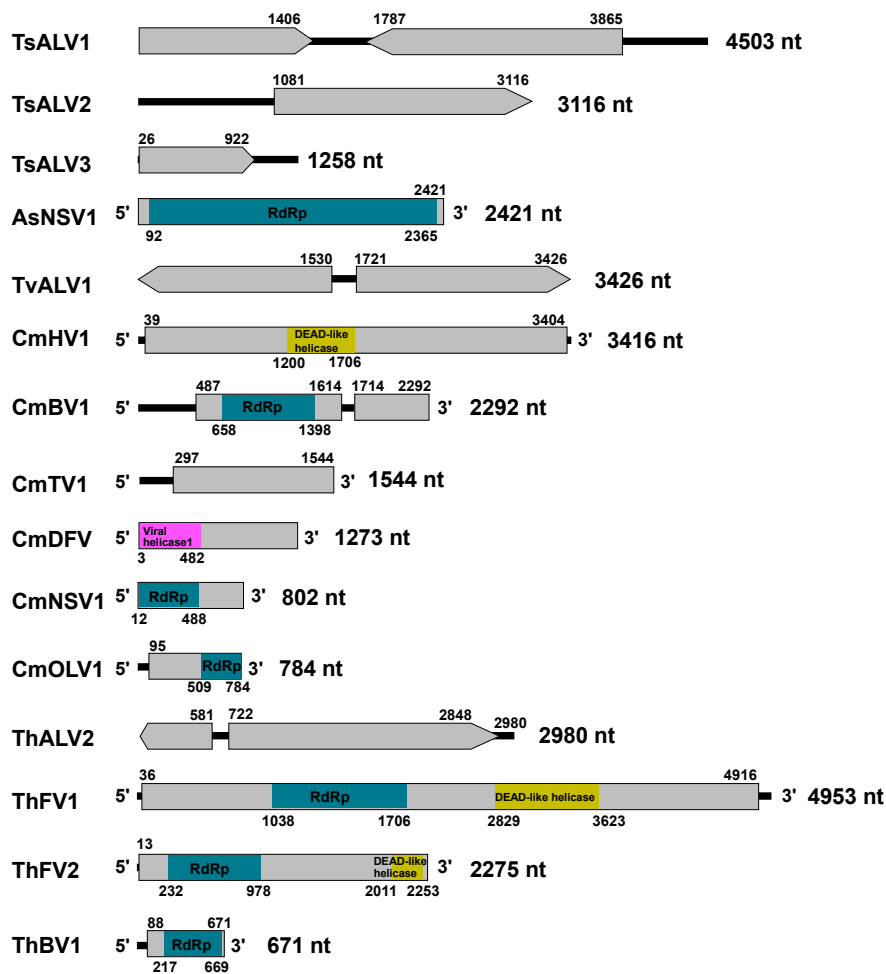


Figure S2: A schematic diagram shows the structure of other viral sequences. The conserved domains include RNA-dependent RNA polymerase (RdRp) and viral helicase (Hel), which are indicated on their fragments.

ThMV1	RTFWITNNTWEGTQIKDSTLKMISLTNNQTLSESWARTVSYKDKLISYKT--KDQMGFI	287
OnuMV1a	----NNTGWLTAIRRLK-----HLFTWGEITSIL----DPYKEGKWEKSDFY	230
ShMV	-GIWKDIYAWYISPLFPTL---LSF-IGRMNR---GNVL---IDLLRSEVSHWEATGVK	251
OnuMV3a	-GIWKDIYAWYISPLFPTL---LSF-IGRMNR---GNVL---IDLLRAEVSYWEATGVK	250
CcMV2a	-TSLLDALAFWSDPLRVIHF--IWF-NIRCYGYFWGLIWSMWLIFIMIISL---PYYLIA	73
CpMV1-NB631	-TSSVDALSFITQPTKIFTY--LDF-SVRVYKFR-GLLLWMHMMICILLITL---PYAIVS	317
I		
ThMV1	NEFIGNGIRMKHKTGKLTSTKEEAAGKVRVFAFMDPFTQWATEPLHRNTFSILKKMETDG	347
OnuMV1a	SFWASIQPLTDNVPYIGKLAAG-EEPGKVRIFAMVDVAVTQWLLKPLHEAIFKLLNIFAFDG	289
ShMV	---PSVSP--LELKGKLAKEEAAGKARVFAMADSIQSVMAPLNSWVFSKLGKLPMDG	306
OnuMV3a	---PSVSP--LDLKGKLAKEEAAGKARVFAMADSIQSVMAPLNSWVFSKLGKLPMDG	305
CcMV2a	---LCLGA--RAPVMGQLATVYDQAGKARIVASTNSWIQCSLFGLHNKIFSLRSPQDG	128
CpMV1-NB631	---FHLGA--LIPIMGLSVVYDQAGKARIVASTNSWIQAFYSLHLHVEKLLKNIQDG	372
*: *: : *: *: *		
II		
ThMV1	TFNQMKPISRIPW-----GSVPIYSFDTSAATDRTPISTQEDITSILYKDFSGAWRA	400
OnuMV1a	TFDQIGQLEKFMDRMKD---KAPYYYSFDLSAATDRPLPSIQILILKHLVSVPFSEAWGR	346
ShMV	TFNQQAPLNRVLQLYKEGLLDHVEFYSDLSAATDRPLMAFQKQIISVLFGSDADDWAT	366
OnuMV3a	TFNQQAPLNRVLQLYQDGLLDHVEFYSDLSAATDRPLMAFQKQIISVLFGSKFAKWAT	365
CcMV2a	TFDQNKPFDLLESL-Q---PGYMLYGFDLSAATDRPLIAFQKDI LNHL--GYPGGPWR	182
CpMV1-NB631	TFDQERPFKLLIKWLE---PTQKFYGFDTAATDRPLIDLQVDILNIIFKNPSSWRS	429
*: *: : *: *: *		
III		
ThMV1	TTVDRDYKTPKQSFLDWTFTKGMFVSRYKVGQPMGATSSWAMTAMTHHYMVHYSAWIS	460
OnuMV1a	LLTGRTYHL-----FYKKTSYEVQYQVQPMGALSSWGLALTHHLVVQYSAYLA	396
ShMV	LLVGRDWYL-----KDIPYRYSVGQPMGALSSWAHLALSHHVIVQIAA-MR	411
OnuMV3a	LLVGRDWYL-----KDIPYRYSVGQPMGALSSWAHLALSHHVIVQIAA-MR	410
CcMV2a	LLG-IKYN-----PCGFISYAVGQPMGAYSSFAHLALTHHVIVQVAA-QK	226
CpMV1-NB631	LLR-IKYK-----PQGFLTYAVGQPMGAYSSFAHLALTHHVIVQVAA-LN	473
: * ***** *: * *: *: *		
IV V VI		
ThMV1	GVVPIGTKFSQYAVTGDDVVIWNSTVAEAYTKVMQ-DTGVEVGLAKSVTPKKGLEFAK	519
OnuMV1a	F--GKVNLFKDYIVLGGDIVIGNHEVSIHYHLMTEILKVKINPTKGIHSP--HSLEFAK	452
ShMV	V--GK-LSFTNYALLGDDIVIAADKAVATSYHMIMTQILGVEINLSKSLVSS--NSFEFAK	466
OnuMV3a	V--GK-LPFTNYALLGDDIVIAADKAVATSYHMIMTQILGVEINLSKSLVSN--NSFEFAK	465
CcMV2a	A--GFSDRFTDYCILGDDIVIAANSLVAEAYKSLIF-DLGLSEISKSVISG--TFTEFAK	281
CpMV1-NB631	S--GFTTRFTDYCILGDDIVIAHDTVASEYKLME-TLGLSISGKSVISS--EFTEFAK	528
*: *: : *: *: *		
ThMV1	RTTVNGMDVSPMPFKEQMSAHQSTYNM-----RNFCDKYNTSPLAVLRFLGYGYQV	570
OnuMV1a	RFYVQYQDCSPLSLKEFS-SF-----G---TVYSSFTASLRKLDISVPNL-LTLVGRGGR-	502
ShMV	RLVTMDGEVSAGAKNLLVALKSRWGISVILDLYNKGLALSEQDLRQR-FSSIPTVSKQ	525
OnuMV3a	RLVTMDGEVSAGAKNLLVALKSRWGISVILDLYNKGLALSEQDLRQR-FSSIPTVSKQ	524
CcMV2a	KLRGPLMDISPIGAGLILYSLRNKYICVLVFEILERGLCMWYDVYPQL-LSLLPKIYRR	340
CpMV1-NB631	KLKGRNFDIFYRSWFSIIHFEKQILHLCTVFELLRRGVCELYDLPQY-INKLPIYLR	587
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Figure S3: Conserved amino acid sequence motifs of the putative RNA-dependent RNA polymerases of *Trametes hirsuta* mitovirus 1 (ThMV1), *Cryphonectria cubensis* mitovirus 2a (CcMV2a), *Ophiostoma* mitovirus 1a (OnuMV1a), *Sclerotinia homoeocarpa* mitovirus (ShMV), *Ophiostoma* mitovirus 3a (OnuMV3a), and *Cryphonectria parasitica* mitovirus 1-NB631 (CpMV1-NB631). “*” indicates identical amino acid residues; and “.” indicates low chemically similar amino acid residues.

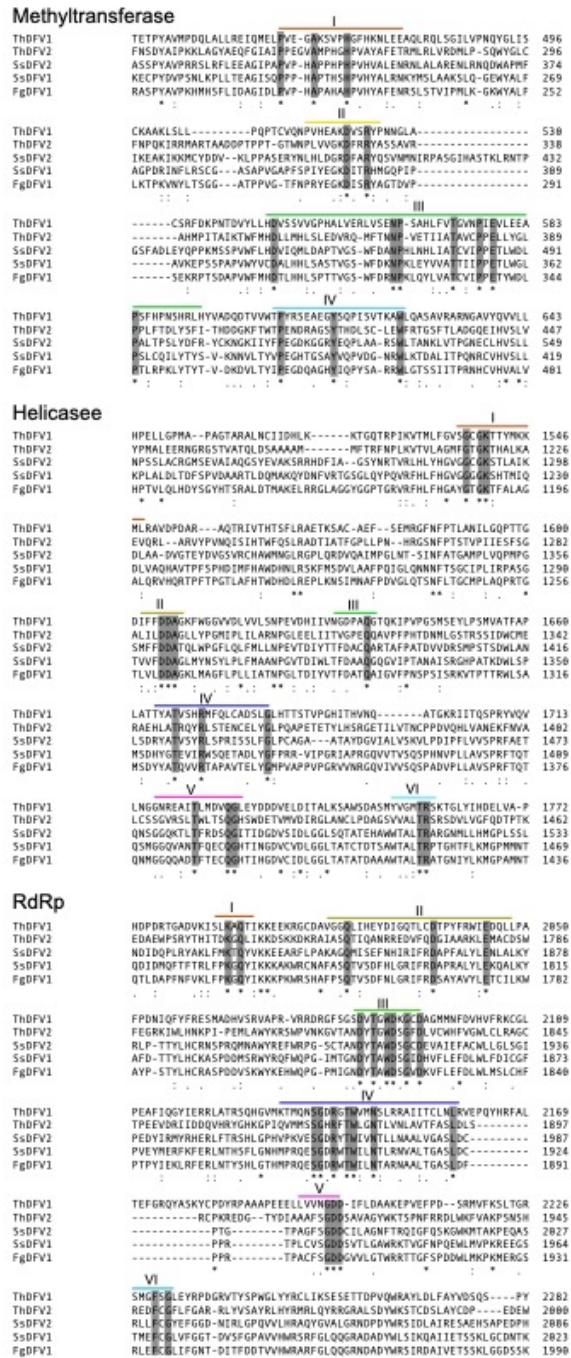


Figure S4: Multiple alignment of amino acid sequences of conserved domains including methyltransferase (Mtr), viral RNA Helicase (Hel), and RNA-dependent RNA polymerase (RdRp) domains of *Trametes hirsuta* deltaflexivirus 1 (ThDFV1), ThDFV2, *Sclerotinia sclerotiorum* deltaflexivirus 1 (SsDFV1), *Sclerotinia sclerotiorum* deltaflexivirus 2 (SsDFV2), and *Fusarium graminearum* deltaflexivirus 1 (FgDFV1). “*” indicates identical amino acid residues, and “.” indicates low chemically similar amino acid residues.

Supplementary Materials Tables

Table S1. Macrofungi strains from China used in this study

Sample name	Collected data	Location	Species	Total RNA pools
BFS-1	2021	Shizi Mountain	<i>Trametes versicolor</i>	sequencing library #1
BFS-2	2021	(30° 27' N, 114°	<i>Trametes versicolor</i>	
BFS-3	2021	20' E), Huazhong	<i>Trametes versicolor</i>	
BFS-4	2021	Agricultural	<i>Trametes versicolor</i>	
BFS-5	2021	University, Wuhan	<i>Trametes versicolor</i>	
BFS-6	2021	City, Hubei	<i>Trametes versicolor</i>	
BFS-7	2021	Province, China	<i>Trametes versicolor</i>	
BFS-8	2021		<i>Trametes versicolor</i>	
BFS-9	2021		<i>Trametes versicolor</i>	
BFS-10	2021		<i>Trametes versicolor</i>	
BFS-11	2021		<i>Trametes versicolor</i>	
BFS-12	2021		<i>Trametes versicolor</i>	
BFS-13	2021		<i>Trametes versicolor</i>	
BFS-14	2021		<i>Trametes versicolor</i>	
BFS-15	2021		<i>Trametes versicolor</i>	
d-1	2021	Shizi Mountain,	<i>Trametes hirsuta</i>	sequencing library #2
d-2	2021	Huazhong	<i>Trametes hirsuta</i>	
d-3	2021	Agricultural	<i>Trametes hirsuta</i>	
d-4	2021	University, Wuhan	<i>Trametes hirsuta</i>	
d-5	2021	City, Hubei	<i>Trametes hirsuta</i>	
d-6	2021	Province, China	<i>Trametes hirsuta</i>	
d-7	2021		<i>Trametes hirsuta</i>	
d-8	2021		<i>Trametes hirsuta</i>	
d-9	2021		<i>Trametes hirsuta</i>	
d-10	2021		<i>Trametes hirsuta</i>	
d-11	2021		<i>Trametes hirsute</i>	
d-12	2021		<i>Trametes hirsuta</i>	

d-13	2021		<i>Trametes hirsuta</i>	
d-14	2021		<i>Trametes hirsuta</i>	
d-15	2021		<i>Trametes hirsuta</i>	
d-16	2021		<i>Trametes hirsuta</i>	
d-17	2021		<i>Trametes hirsuta</i>	
d-18	2021		<i>Trametes hirsuta</i>	
d-19	2021		<i>Trametes hirsuta</i>	
d-20	2021		<i>Trametes hirsuta</i>	
DS-1	2021	Shizi Mountain,	<i>Amanita strobiliformis</i>	sequencing library #3
DS-2	2021	Huazhong	<i>Amanita strobiliformis</i>	
DS-3	2021	Agricultural	<i>Amanita strobiliformis</i>	
DS-4	2021	University, Wuhan	<i>Amanita strobiliformis</i>	
DS-5	2021	City, Hubei	<i>Amanita strobiliformis</i>	
DS-6	2021	Province, China	<i>Amanita strobiliformis</i>	
DS-7	2021		<i>Amanita strobiliformis</i>	
DS-8	2021		<i>Amanita strobiliformis</i>	
DS-9	2021		<i>Amanita strobiliformis</i>	
DS-10	2021		<i>Amanita strobiliformis</i>	
DS-11	2021		<i>Amanita strobiliformis</i>	
DS-12	2021		<i>Amanita strobiliformis</i>	
DS-13	2021		<i>Amanita strobiliformis</i>	
DS-14	2021		<i>Amanita strobiliformis</i>	
DS-15	2021		<i>Amanita strobiliformis</i>	
G-1	2021	Shizi Mountain,	<i>Coprinellus micaceus</i>	sequencing library #4
G-2	2021	Huazhong	<i>Coprinellus micaceus</i>	
G-3	2021	Agricultural	<i>Coprinellus micaceus</i>	
G-4	2021	University, Wuhan	<i>Coprinellus micaceus</i>	
G-5	2021	Province, Hubei	<i>Coprinellus micaceus</i>	
G-6	2021	Province, China	<i>Coprinellus micaceus</i>	
G-7	2021		<i>Coprinellus micaceus</i>	
G-8	2021		<i>Coprinellus micaceus</i>	
G-9	2021		<i>Coprinellus micaceus</i>	

G-10	2021		<i>Coprinellus micaceus</i>	
G-11	2021		<i>Coprinellus micaceus</i>	
G-12	2021		<i>Coprinellus micaceus</i>	
G-13	2021		<i>Coprinellus micaceus</i>	
G-14	2021		<i>Coprinellus micaceus</i>	
G-15	2021		<i>Coprinellus micaceus</i>	
G-16	2021		<i>Coprinellus micaceus</i>	
G-17	2021		<i>Coprinellus micaceus</i>	
G-18	2021		<i>Coprinellus micaceus</i>	
G-19	2021		<i>Coprinellus micaceus</i>	
G-20	2021		<i>Coprinellus micaceus</i>	
G-21	2021		<i>Coprinellus micaceus</i>	
G-22	2021		<i>Coprinellus micaceus</i>	
G-23	2021		<i>Coprinellus micaceus</i>	
G-24	2021		<i>Coprinellus micaceus</i>	
G-25	2021		<i>Coprinellus micaceus</i>	
G-26	2021		<i>Coprinellus micaceus</i>	
G-27	2021		<i>Coprinellus micaceus</i>	
G-28	2021		<i>Coprinellus micaceus</i>	
G-29	2021		<i>Coprinellus micaceus</i>	
G-30	2021		<i>Coprinellus micaceus</i>	
G-31	2021		<i>Coprinellus micaceus</i>	
G-32	2021		<i>Coprinellus micaceus</i>	
G-33	2021		<i>Coprinellus micaceus</i>	
G-34	2021		<i>Coprinellus micaceus</i>	
G-35	2021		<i>Coprinellus micaceus</i>	
G-36	2021		<i>Coprinellus micaceus</i>	
G-37	2021		<i>Coprinellus micaceus</i>	
G-38	2021		<i>Coprinellus micaceus</i>	
G-39	2021		<i>Coprinellus micaceus</i>	
G-40	2021		<i>Coprinellus micaceus</i>	

Table S2 The blastp, PCR confirmation and reads of mycovirus contigs in four species of macrofungi

Short name (Viral contigs)	Viral reads	Host strains	Best match	E-values	Query cover
AsALV1	17777	DS-1, DS-3, DS-4, DS-5, DS-9, DS-10	Phlebiopsis gigantea ambi-like virus 1	2e-104	36%
AsALV2	9839	DS-1, DS-2, DS-8, DS-14	Rhizoctonia solani ambivirus 1	3e-91	56%
AsALV3	4353	DS-6, DS-15	Tulasnella ambivirus 2	2e-14	63%
AsNSV1	22938	DS-1, DS-2, DS-4, DS-5, DS-14	Soybean leaf-associated negative-stranded RNA virus 4	3e-116	96%
CmALV1	958	G-2, G-20	Tulasnella ambivirus 5	4e-123	37%
CmBV1	288	G-3	Sclerotinia sclerotiorum barnavirus 1	8e-48	45%
CmDFV1	96	G-1	Pestalotiopsis deltaflexivirus 1	5e-57	54%
CmHV1	357	G-2, G-15, G-35	Rhizoctonia cerealis hypovirus	5e-173	74%
CmNSV1	50	G-36	Phytophthora condilina negative stranded RNA virus 2	3e-24	62%
CmOLV1	23	G-11	Armillaria mellea ourmia-like virus 1	2e-61	99%
CmTV1	110	G-14	Rhizoctonia solani toti-like virus 1	5e-144	98%
ThALV1	1755	d-5, d-8, d-16, d-17	Fusarium graminearum ambivirus 1	5e-82	44%
ThALV2	345	d-1, d-3	Downy mildew lesion associated ambivirus 2	9e-59	63%
ThBV1	11	d-1	Bemisia tabaci beny-like virus 3	1e-41	99%
ThDFV1	7023	d-1, d-2, d-3, d-16	Lentinula edodes deltaflexivirus 1	0.0	75%
ThDFV2	3494	d-10, d-13, d-17	Cat Tien Macrotermes Deltaflexi-like virus	7e-106	32%
ThFV1	414	d-3, d-9	Phlebiopsis gigantea fusarivirus 1	0.0	70%
ThFV2	221	d-7	Sclerotium rolfsii fusarivirus 2	0.0	99%
ThMV1	7199	d-1, d-2, d-4, d-5, d-7, d-13, d-16, d-20	Lentinula edodes mitovirus 1	0.0	57%
TvALV1	3201	BFS-2, BFS-3, BFS-14	Heterobasidion ambi-like virus 15	2e-94	46%

Table S3 Primers used to detect viral contigs

Name of primers	Sequence from 5' to 3'	Lengths of PCR products (bp)
AsALV1-f	GAGAGAAAGAGCCATTGTG	300
AsALV1-r	GCCTATGGGGCGGTGTATT	
AsALV2-f	GACGCATGACTGCTTGTCCTG	400
AsALV2-r	ATGCAAGTTGATACTACCGC	
AsALV3-f	GGGTCAGCCATTTGTGGCTCC	510
AsALV3-r	CACAACATTTTGCCTGATTGG	
ASNSV1-f	GTGCCTTGATACTCAAGCACCG	510
ASNSV1-r	CGGAGAACGGACCTGACTTCACG	
CmALV1-f	TGGTCATCTCGGGCTATGGGAA	410
CmALV1-r	GAGGAACTCGAGCGTCAGGCTA	
CmBV1-f	TACCCTCGAAACAAGAACCTGC	320
CmBV1-r	AGACGCTGCCTCTCGGTTCTCTC	
CmDFV1-f	ATGCGCGGAAGACGATGAGT	410
CmDFV1-r	GGCAAGACGACGAAGATATTGGAGAT	
CmHV1-f	TGTGTCTTTTAGAGAGTGGTTAA	511
CmHV1-r	GCTCTATCTATGGCTCGCATGA	
CmNSV1-f	CTGATGATAAAAATGAGTTATTC	413
CmNSV1-r	GTGAGGTGAGTCTCTGAAACCA	
CmOLV1-f	CCAATAAGGAGCTAGCGAGAA	338
CmOLV1-r	GCAACCCTTCCCTGAGTTTG	
CmTV1-f	CATTGAGTGGATGAAATGGCATG	527
CmTV1-r	CTGCTTGTTTACTGGGCTGTAGA	
ThALV1-f	ACAACTCGTAGGCAACCTCC	338
ThALV1-r	TAAATGATAAAGAGCAACACA	
ThALV2-f	CTCCGACCGCTCGATGAGG	455
ThALV2-r	TCTGACAACCACACTCCAACCAC	
ThBV1-f	GGTTCGTCAGGCAGAGGGCC	526
ThBV1-r	GATGGACCTCGGACACGATA	
ThDFV1-f	GCAGAAGAGGTCTGAACAACGG	455
ThDFV1-r	GAGTGACGCGAGCGGAGCCTGG	
ThDFV2-f	GCAGCTTTCTGCTTGCGTAC	454
ThDFV2-r	CGGTGAGCAGTGTCCGAAAACAT	
ThFV1-f	AGGGCTGGTTCTTGCTTGGGTA	546
ThFV1-r	CCTCTTTAGAGGCAGTGGGTA	
ThFV2-f	TGCTTTTGTATAGATGCAAACTA	506
ThFV2-r	TTGACCGGGTCGTGATAGACAA	
ThMV1-f	AAACAATAAGACCACTAGAATCT	329
ThMV1-r	GATTACTCTAACCTCTATTATTTT	
TvALV1-f	TGCGGAAAGTGTGGATTCAATG	330
TvALV1-r	TACAACTCACACGATGATGCGCT	