

A

Phlebiopsis\_gigantea\_ambI-like\_virus\_1\_UJT31805  
Heterobasidion\_ambI-like\_virus\_1\_UHK02572  
Heterobasidion\_ambI-like\_virus\_2\_UHK02574  
Tulasnella\_ambivirius\_1\_QPB44664  
Tulasnella\_ambivirius\_2\_QPB44666  
Rhizoctonia\_solanii\_ambivirius\_1\_QMP84026  
Rhizoctonia\_solanii\_ambivirius\_1\_QMP84024  
Armillaria\_borealis\_ambI-like\_virus\_2\_OUD20363  
Armillaria\_mellea\_ambI-like\_virus\_2\_DAD54839  
Armillaria\_ambI-like\_virus\_3\_QUD20376  
Cryphonectria\_parasitica\_ambivirius\_1\_QMP84022  
Armillaria\_mellea\_ambI-like\_virus\_1\_DAD54837  
Phlebiopsis\_gigantea\_ambI-like\_virus\_2\_UJT31806  
Tulesnella\_ambivirius\_3\_QPB44670  
Ceratostomellum\_ambivirius\_1\_QPB44668  
Tulesnella\_ambivirius\_4\_QPB44672  
Tulasnella\_ambivirius\_5\_QPB44674  
Armillaria\_novae-zelandiae\_ambI-like\_virus\_1\_DAD54838  
Armillaria\_luteobubalina\_ambI-like\_virus\_1\_DAD5  
Armillaria\_borealis\_ambI-like\_virus\_1\_QUD20357  
Armillaria\_ecypa\_ambI-like\_virus\_1\_DAD54833  
Heterobasidion\_ambI-like\_virus\_3\_UHK02576  
Heterobasidion\_ambI-like\_virus\_4\_UHK02578  
Armillaria\_ambI-like\_virus\_1  
Armillaria\_ostoyae\_ambI-like\_virus\_2  
Armillaria\_ostoyae\_ambI-like\_virus\_3  
Armillaria\_ostoyae\_ambI-like\_virus\_4

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Armillaria\_borealis\_ambl-like\_virus\_1\_QUD20357  
Armillaria\_ecypa\_ambl-like\_virus\_1\_DAD54833  
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Tulasnella\_ambivirius\_5\_QPB44674  
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Armillaria\_ostoyae\_ambI-like\_virus\_3  
Armillaria\_ostoyae\_ambI-like\_virus\_4

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HETCYKANVEVBBMVRMPTTSGLBERTY-AAGTAHISKLHQFPNLNCELEDPFTIRN-VBNVPQRVINDEFDDDEPE-DPYLDTTLETSNSIAQVPIFIL  
ME-LGKLGVBVBBMVRMPTTSGLBERTY-AAGTAHGPFSCLNCELESDCQDFPFTIRN-KLPRQSRITY-D---EFG-EYVNRDYLAEASSRATL-RSMFV  
LNUITFCFOEGEYEVBBMVRMPTTSGLBERTY-AAGTAHGSVSAVLDGADWACDFCPSSRRS-KLPRTRIFRN-EN-ENG-YLRUQYDLELESSRSISI-RSFV  
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L E T Q V Y P H E P V D A B R Y M N Q D L E R Y A Y A R G S D V M H T S Q Y V Y S I A N H Y D A F P P E T R K D - F M P R O P L A - - - D D D - V E Y Y D O S L D S T V P Y R F T  
M C R M D R M B A T E P Y A R M Y P N N E R A B Y A R G P D V F A S S I D O A N F I L V D S L W S T H T R K D - F N I Y G D D I L L - - - P D F E L U F I Y D Y A S L S K R B R H F T  
M H T H E A E P M I B M A Y Y N D O L E R B Y A R G T V Y G K S K R V O E I F N I L D E C F P V B Y R N Y - F E L P I E N I L L - - - S A V R L A Y D I S R A E K Y F V  
M S T F I A T E P M I B M A Y Y N D O L E R B Y A R G T V Y G K S K R V O E I F N I L D E C F P V B Y R N Y - F E L P I E N I L L - - - S A V R L A Y D I S R A E K Y F V  
M Y E Y G O P V P M I B M A Y Y N D O L E R B Y A R G T V Y G K S K R V O E I F N I L D E C F P V B Y R N Y - F E L P I E N I L L - - - S A V R L A Y D I S R A E K Y F V  
M T E Y G O P V P M I B M A Y Y N D O L E R B Y A R G T V Y G K S K R V O E I F N I L D E C F P V B Y R N Y - F E L P I E N I L L - - - S A V R L A Y D I S R A E K Y F V  
M R D T V I S P V P M I B M A Y Y N D O L E R B Y A R G T V Y G K S K R V O E I F N I L D E C F P V B Y R N Y - F E L P I E N I L L - - - S A V R L A Y D I S R A E K Y F V  
M D S C H Q I Q P C M I B M A Y Y N D O L E R B Y A R G T V Y G K S K R V O E I F N I L D E C F P V B Y R N Y - F E L P I E N I L L - - - S A V R L A Y D I S R A E K Y F V  
M A M Y C I Q P C M I B M A Y Y N D O L E R B Y A R G T V Y G K S K R V O E I F N I L D E C F P V B Y R N Y - F E L P I E N I L L - - - S A V R L A Y D I S R A E K Y F V  
M E I C E A B V C E M B A R F T D L K B R L Y T C C G D Y W A S R F I R D L V T D C N M L P A C H M R G - F T N V D S I N H A H - - - V T H G S - R V T I V Y D S I N H A H - K F R I  
M S L Y C I Q P C M I B M A Y Y N D O L E R B Y A R G T V Y G K S K R V O E I F N I L D E C F P V B Y R N Y - F E L P I E N I L L - - - S A V R L A Y D I S R A E K Y F V  
M E G E T I Q V Q E T E L B A R Y T D L A B R Y A Y A A Y Y N H F A K R I T C L P V T P T G S A Y H N Y S I R N Y K Y - - - Q S H - T V L Y D S I N H A H - K F R I  
M A R C V Q A G T E T L B A R Y T D L A B R Y A Y A A Y Y N H F A K R I T C L P V T P T G S A Y H N Y S I R N Y K Y - - - Q S H - T V L Y D S I N H A H - K F R I  
M T H C G S S E R M B Y H G K B R E R Y A G A C R F C R F D P F M P T N H T K - R P D R I F L S S R - Y D K E D P - H F R Y D Y L - S N L S N H Q S R C B  
M T H C F R C S E R M B Y H G K B R E R Y A G A C R F C R F D P F M P T N H T K - R P D R I F L S S R - Y D K E D P - H F R Y D Y L - S N L S N H Q S R C B  
M T H C F R C S E R M B Y H G K B R E R Y A G A C R F C R F D P F M P T N H T K - R P D R I F L S S R - Y D K E D P - H F R Y D Y L - S N L S N H Q S R C B  
M T H C F R C S E R M B Y H G K B R E R Y A G A C R F C R F D P F M P T N H T K - R P D R I F L S S R - Y D K E D P - H F R Y D Y L - S N L S N H Q S R C B

NRISEMCSCGIIIEVDADEGFAARDIGMM-LSVYINQL-NDNPESMELBVGDGRYQDL---VCHHTAGFLGVYGNLATCIVHGCSMLVMSDPSRLNVA  
 YKISSEVRCHIIVYDPSYACMEQDINGEM-IEWAEY-NNKAEMEDLSQGLGEYGG---FLEOHGIAEFLGVYGNLACSMFLGAVBLQGRMDT-SKLNLIA  
 YELSEVRCHTGYVYDGHAGCMEQDINGEM-IWEAEY-NNKAEMEDLSQGFQDFGYGG---FLEOHGIAEFLGVYGNLACSMFLGAVBLQGRMDT-SKLNLIA  
 DHLAEFRCEPEFTYNDARECGIITTDIFGEL-PQEYNEECNKEPEIISYEFYKSQKGFRH---AHHYAGMCGVYGNLACSMFLGMLMGVTGND-MKFVFA  
 DFLADLCOTSTFTYDWRKLGITRDINGEM-IAEINQANRNPNTMPSWRDFSEKHFHPR---YHHHAGLGVYGNLACSMFLGMLMGVTGND-MKFVFA  
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 LKHTEENCPYVAPSVEBYPFGDKDVTIS---EHARASMLGFLGVNLSCEFAIIGQCGEID-TQGQW  
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 DNEAIERFRTYVWLHDIFRNGWVTPDINGEM-HARVNTENCYLTGDTDASVHLQGSA-GT---TLLQHTGCMGVNLSCEFAIAYHMDPSVPMRDE-EEVNA  
 GEIADWTCTIIEQVYDQGGMG-YMVGIVL-IIREYEVNCVDPPDASELLSVHE-A---VLSHNTGMGVNLSCEFAIAYHMDPSVPMRDE-EEVNA  
 RALAHLYETKETAYVFDVTRDVPWTRSVSVEY---ILEYETCTNEOSFEDICBVARSEP--GL---IIIHLNGCALGVNLSCEFAIAYHMDPSVPMRDE-EEVNA  
 AQTAGMFSGCKMIDVHMDHGVIQDIEGY-HENYVQNVINQIOPEDVSBLLETFSW-VP---YYKOLRSGLGVNLSCEFAIAYHMDPSVPMRDE-EEVNA  
 DALSRERFRCTIVKWDIFYQVWIEDIGY---ILAYNQTVNHPHAADBVSBLKEDLFW-FP---SYHOLRNGLGMGLGPNLGLSIIHRSFADGH-MHICCV  
 DALSRERFRCTIVKWDIFYQVWIEDIGY---ILAYNQTVNHPHAADBVSBLKEDLFW-FP---SYHOLRNGLGMGLGPNLGLSIIHRSFADGH-MHICCV  
 HXWALLTIVWPDLYDHPVHATIDVINGEM-IQDYNNAFQFCASMEBVIOKHLS---DR---VPHFMHGQCGCLGVNLSCEFAIAYHMDPSVPMRDE-EEVNA  
 FYIGORLGLIIPABDVKNGKIFDIEGY---IYVYEVNVSNSHCAFSDIBIAPDAF-ID---IYOTRSNGMGLGVNLSCEFAIAYHMDPSVPMRDE-EEVNA  
 HHISSEMRGIKBYTWOMREGRIRERDVGEL-ISEYNDISINIHAAKSBSMDAEQQ---ED---VWYOMANSGMGLGVNLSCEFAIABNICAACDD1-DLCNCW  
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[GDPGLVTTD-----REKFLFIRVRLGMEESKAY-]-VGS-EPGAIAKRPISYDVENL-H-|-IVTARILIPWPFERFAH  
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[GDPGCUDTRVR-----DQTYEIQHVN1LISQEEERTT-S-SPDRFT-TWCPVCPGGY-FEROF-|-VETEYUPPFPPNAAILDI  
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[GDPGAAULAKKV-----AKLQEVAYAKSLSVDAISERKS-F-MWFRPDYE-]-DYSSEFGQYKKRPIVSLAYAGE-|-VQMGYLPNPFFNKAJIGF  
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[GDPGAAULVHFDER-----VSRSYFIVNLB-GEVAWEKI-TI-W-NEWMTE-E-]-QRAEAGWHFKRPIVYVWE-Y-|-WVGTLPFPPCSAM-WER  
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[GDPGAAFLPQDN-----GYERTIMMEALNAPVMAEKVE-T-TWDGSRVVE-]-NDGDDWHAHTRKPITRLNLDNES-|-MYYGTQIPSPVRCQNL  
[GDPGDPGILPED-A-----ANPEFMRVPLDPLVLSCAMERTF-]-RSDESESAIAKRPIWEDLPH-|-RTQYNNIPFSPVRCWQS  
[GDPGDPGILPED-A-----NNPEFMRVPLDPLVLSCAMERTF-]-RSDESESAIAKRPIWEDLPH-|-HTAFLNIPFSPVRCQQS

B

	1	10	20	30	40	50	60
Armillaria_spp_tymovirus	W	NDFG-VRVKGQ-LKAKKL-E	ALALTEPKAGQTINVLPEWIA	TATYRYIMACIKKNSHIFP	GGEIS		
Lentinula_edodes_tyto-like_virus_1 QOX06053.1	W	QDFAA-VRVKGQ-LKAKKL-E	ALALDAPKAGOTINVMGEWIA	TATYRYIMVMNIRHKHRIFP	GGEIS		
Lolium_latent_virus_YP_001718499.1	W	QDERM-VYLLKAQ-NVTKA-S	KFNPLTAKAGOTISAFKQAV	VMKFRYLRITP-DNINCEMQ			
Alfalfa_virus_5_QJD13457.1	F	PANAI-LFLNKQ-SWVKKL-E	KFVKPGQTSIAFKSQSTVLLTL	YLERKKREREQHDNIVIMCEK	IT		
Botryosphaeriavirus_X_NS_932306.1	F	DPNVY-LFLFKQ-SWVKKL-E	KFVKGMQFKAGQTISFSKQV	LLTLYERACRCRNSHDNIVIMCEK	IT		
Alfalfa_virus_F_YP_009551972.1	W	RWSAV-IEFSKAQ-HKVNE	GSFLFG-DWKCQTLALMDHA	VILAKYQRLFDKQDSNITHASHI			

	MOTIF I				MOTIF II				
	70	80	90	100	110	120	130	140	
Armillaria_spp._tymovirus	- L S A F N D R V -	- K E N W Q T D P N L T D K F C T I N D F T A E G S S Q G G E S V -	- T M D L A W F R W V S M P E V I L E S V						
Lentinula_edodes_tymovirus-like_virus_1_QOX06053.1	- L S A F N D R V -	- K E N W Q T D P N L T D K F C T I N D F T A E G S S Q G G E S V -	- T M D L A W F R W V S M P E V I L E S V						
Luminot_lateral_virus_YP_01718499.1	- P Q D I S K W A L P T K O K W N - F E R P - - - A F A S D F E - Q S Q D G A M H I - F E H A L W A R H F N V B S S L I E E	- P Q N Q F N D F V - - L T R F D - F S Q P - - - S Y T S D Y E C Y D Q S Q D G A M H I - F E H A L W A R H F N V B S S L I E E	- P Q N Q F N D F V - - L T R F D - F S Q P - - - S Y T S D Y E C Y D Q S Q D G A M H I - F E H A L W A R H F N V B S S L I E E						
Alfalfa_virus_S_Q014357.1	- P Q N Q F N D F V - - L T R F D - F S Q P - - - S Y T S D Y E C Y D Q S Q D G A M H I - F E H A L W A R H F N V B S S L I E E	- P Q N Q F N D F V - - L T R F D - F S Q P - - - S Y T S D Y E C Y D Q S Q D G A M H I - F E H A L W A R H F N V B S S L I E E	- P Q N Q F N D F V - - L T R F D - F S Q P - - - S Y T S D Y E C Y D Q S Q D G A M H I - F E H A L W A R H F N V B S S L I E E						
Botryotinia_virus_XN_932306.2	- P Q N Q F N D F V - - L T R F D - F S Q P - - - S Y T S D Y E C Y D Q S Q D G A M H I - F E H A L W A R H F N V B S S L I E E	- P Q N Q F N D F V - - L T R F D - F S Q P - - - S Y T S D Y E C Y D Q S Q D G A M H I - F E H A L W A R H F N V B S S L I E E	- P Q N Q F N D F V - - L T R F D - F S Q P - - - S Y T S D Y E C Y D Q S Q D G A M H I - F E H A L W A R H F N V B S S L I E E						
Alfalfa_virus_F_YP_00951926.1	- P Q D L S I W C - - Q O H L L - F E R D - - - H Y A N D T Y D T Y K D S Q D G L F N L - F E H A L R K A R H F C V P E E V I L D A	- P Q D L S I W C - - Q O H L L - F E R D - - - H Y A N D T Y D T Y K D S Q D G L F N L - F E H A L R K A R H F C V P E E V I L D A	- P Q D L S I W C - - Q O H L L - F E R D - - - H Y A N D T Y D T Y K D S Q D G L F N L - F E H A L R K A R H F C V P E E V I L D A						
Botryotinia_virus_XN_93256174	- P Q D L S I W C - - Q O H L L - F E R D - - - H Y A N D T Y D T Y K D S Q D G L F N L - F E H A L R K A R H F C V P E E V I L D A	- P Q D L S I W C - - Q O H L L - F E R D - - - H Y A N D T Y D T Y K D S Q D G L F N L - F E H A L R K A R H F C V P E E V I L D A	- P Q D L S I W C - - Q O H L L - F E R D - - - H Y A N D T Y D T Y K D S Q D G L F N L - F E H A L R K A R H F C V P E E V I L D A						

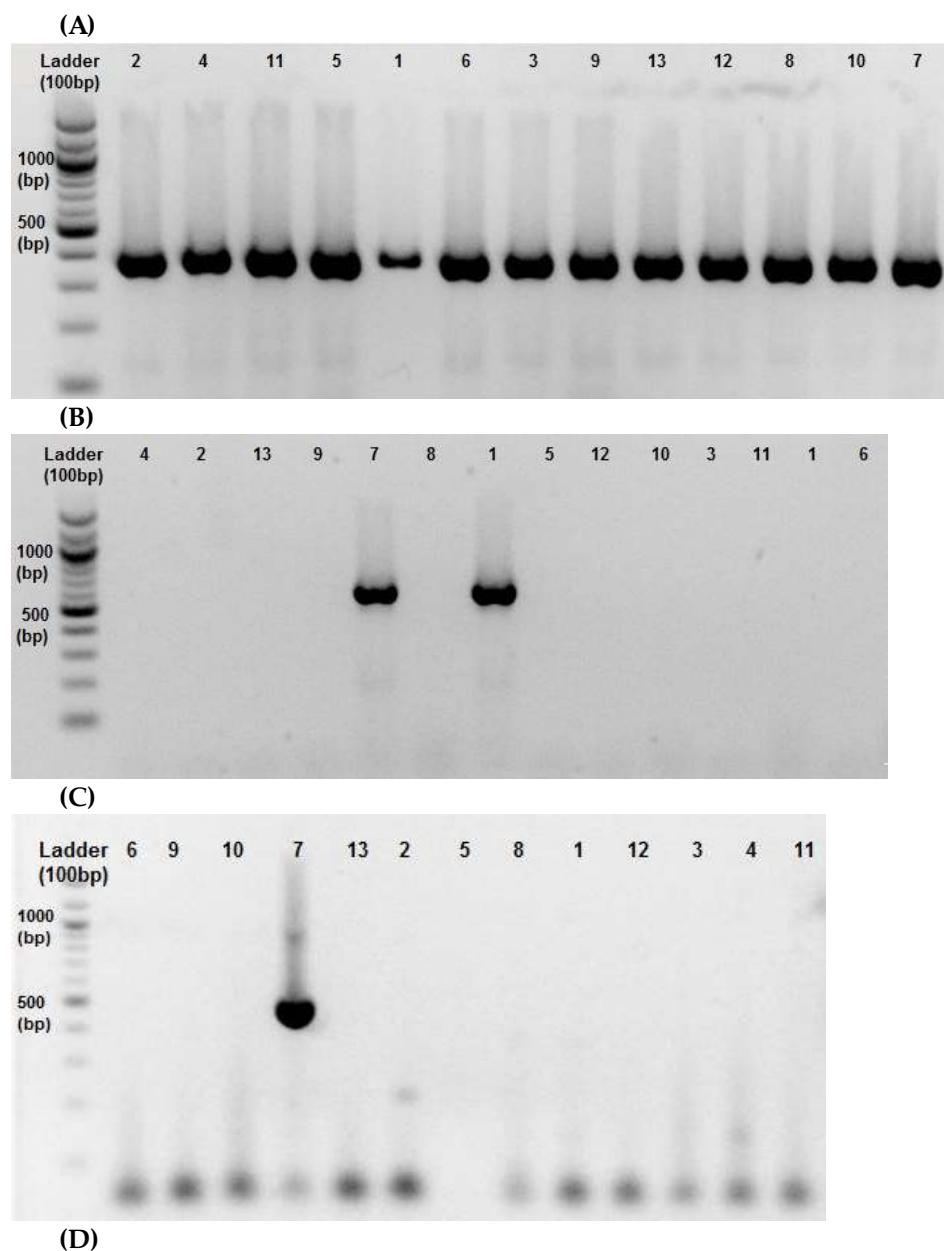
	200	210	220	230	235	MOTIF V	MOTIF VI
Armillaria spp._tymovirus	D H S V -	- P E H P Q W S K K W R A - T I S K L	Q Y V E E A D F C G W	L Q K H F G	G I		
Lentinula edodes_tymo-like virus_1_QOX06053.1	D H Y V -	- P E H P Q W S K R W R K - T I S K L	Q Y V E E A D F C G W	L Q E G V G	G I		
Armillaria tabescens_tymo-like virus_1_QXV18499.1	D O V Y -	- P E H P Q W S K R W R K - T I S K L	Q Y V E E A D F C G W	W M T P Y -	G L		
Alfalfa virus_1_QD13457.1	D Y C V -	- P E R S S W M Y S H Q - V A R K P D	F E Q G E F C C W	W M T P Y -	G L		
Bonnyt virus_X_NP_93206.1	D R N C -	- P E R A S S W M Y S H Q - V A R K P D	E Q G E F C C W	W M T P Y -	G L		
Alfalfa virus_YP_00951972.1	D S V P -	- P T N P S W H I A P L K I D R Y -	S L F - - C G Y	T K H - G I			
Rombxus mori latent virus_BAD35017.1	S P P P -	- P A H P D W D A V S P L - L T E K F	P V R N G I F C G Y	I G P A - G A			

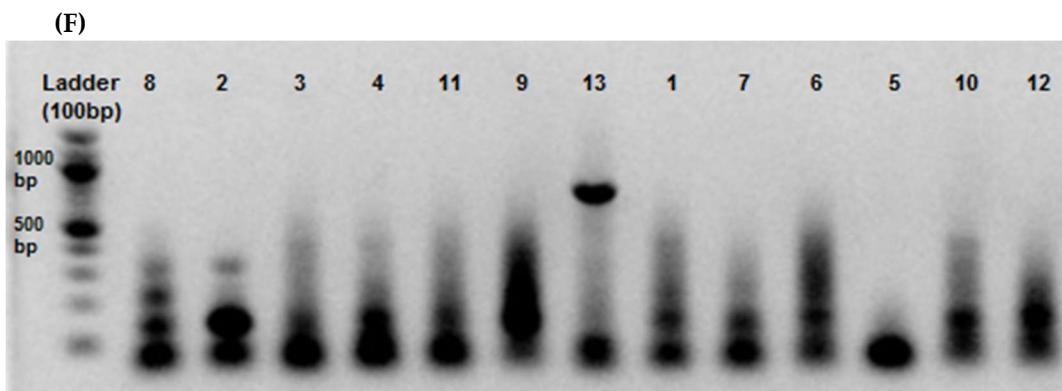
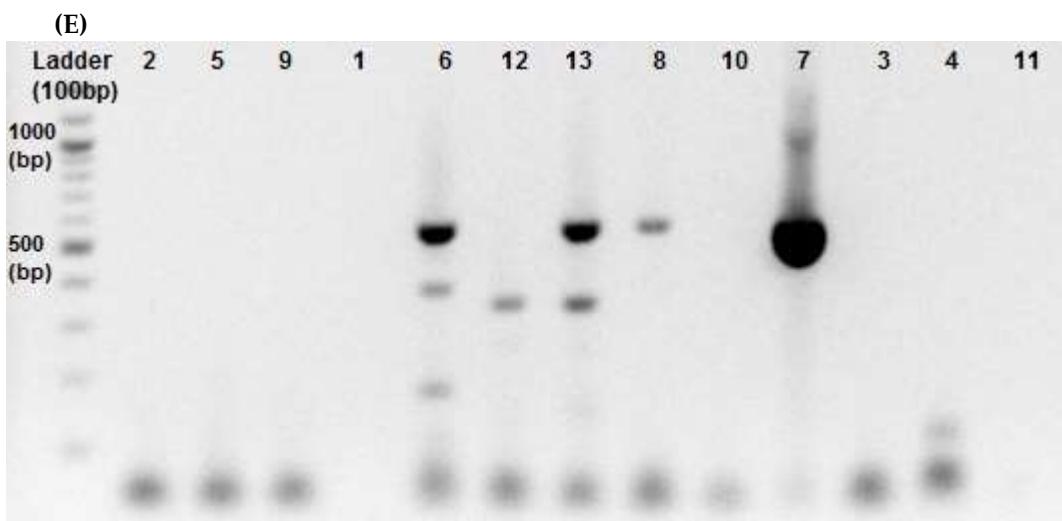
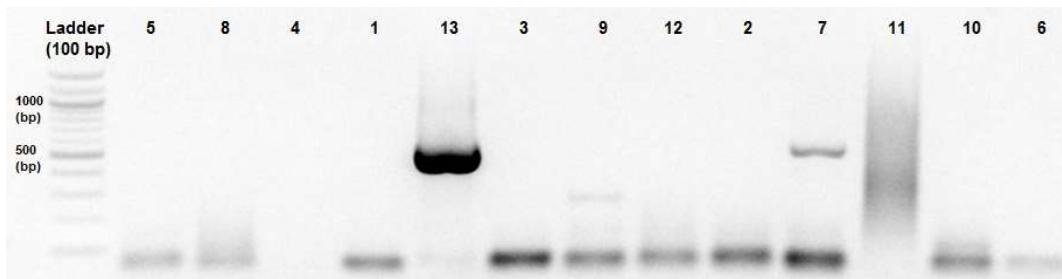
Legend

	A	100% similar
	A	80 to 100% similar
	A	60 to 80% similar
	A	Less than 60% similar

**Figure S1.** Amino acid alignment showing conserved motifs. **A**, Amino acid alignment of ambi-like viruses described in this study and related members. QPB44666 – Tulesnella ambivirus 2, QPB44664 – Tulesnella ambivirus 1, QMP84026 – Rhizoctonia solani ambivirus 2, QMP84024 – Rhizoctonia solani ambivirus 1, QUD20363 – Armillaria borealis ambi-like virus 2, AoALV3 – Armillaria ostoyae ambi-like virus 3\*, DAD54839 – Armillaria mellea ambi-like virus 2, AoALV4 – Armillaria ostoyae ambi-like virus 4\*, AoALV1 – Armillaria ambi-like virus 1\*, QUD20376 – Armillaria ambi-like virus 3, QMP84022 – Cryphonectria parasitica ambivirus 1, AoALV2 – Armillaria ostoyae ambi-like virus 2\*, DAD54837 – Armillaria mellea ambi-like virus 1, QPB44674 – Tulesnella ambivirus 5, DAD54841 – Armillaria novae-zelandiae ambi-like virus 1, DAD54835 – Armillaria luteobubalina ambi-like virus 1, QUD20357 – Armillaria borealis ambi-like virus 1, DAD54833 – Armillaria ectypa ambi-like virus 1, QPB44670 – Tulesnella ambivirus 3, QPB44672 – Tulesnella ambivirus 4, QPB44668 – Ceratobasidium ambivirus 1

**B**, Amino acid alignment of Armillaria tymovirus described in this study and related members. AoTV – Armillaria spp. tymovirus\*, QOX06053.1 - Lentinula edodes tymo-like virus 1, YP\_001718499.1 - Lolium latent virus, QJD13457.1 - Alfalfa virus S, NP\_932306.1 - Botrytis virus X, BAD35017.1 - Bombyx mori latent virus, YP\_009551972.1 - Alfalfa virus F.





**Figure S2.** Resulting RT-PCR gels. **A**, Positive control with EF primers **B**, AALV1 **C**, AoALV2 **D**, AoALV3 **E**, AoALV4 **F**, AoTV1; 100bp DNA Ladder (Thermo Fisher Scientific) was used in all analyses.

**Table S1.** List of primers used for direct virus RT-PCR detection

Virus	Direction	Sequences (5'-3')	Tm (°C)	Product size (nt)
Armillaria ambi-like virus 1	Forward	CCCCTCATGGTCACTATGGATATG	61	800
	Reverse	CTCGCTGAGCCTCTACATCTT		
Armillaria ostoyae ambi-like virus 2	Forward	CGAGATGTTAGCCCTATAACCCATC	60	487
	Reverse	GGTAGGAAGTGTCTGTACTCTG		
Armillaria ostoyae ambi-like virus 3	Forward	CCGACTATAGGATTGTCCCTTCAG	60	477
	Reverse	CATCTGTCTTATCAGCTCTGGG		
Armillaria ostoyae ambi-like virus 4	Forward	CTATTCCCTTCCGACTTCTTCCC	61	588
	Reverse	CTCTATCGAGACCAGATCCCAGATC		
Armillaria ostoyae tymovirus 1	Forward	GAGCTCTAGGTGAAGTGGAGTATG	60	731
	Reverse	CTCTCTTATAGGAACTCTAGGCGC		

**Table S2.** A, Pairwise Sequence Comparison (PASC) percentages (%) based on nucleotide sequence of ambi-like viruses hosted by *Armillaria cepistipes* and *ostoyae*. B, PASC % based on RdRP amino acid (aa) sequence of ambi-like viruses hosted by *Armillaria cepistipes* and *A. ostoyae*.

**A**

	AoALV1	AoALV2	AoALV3
AoALV2	27.66		
AoALV3	31.40	31.90	
AoALV4	85.73	29.82	30.09

**B**

	AoALV1	AoALV2	AoALV3
AoALV2	19.53		
AoALV3	77.46	18.65	
AoALV4	90.93	19.20	77.52

**Table S3.** Types and genomic position of the ribozymes detected in *Armillaria ambivirus*es.

Virus Name	Contig Name	Ribozyme (+)	e-Value	Ribozyme (-)	e-Value
<b>AoALV1</b>	ambi_spadesMETA_NODE_2_length_6862	HHRz (4510-4564)	1.4e-07	HHRz (330-254)	4.6e-11
<b>AoALV2</b>	ambi_spadesMETA_NODE_4_length_6697	HPRz (4424-4531)	5.00E-08	HPRz (4987-4884)	5.7e-10
<b>AoALV3</b>	ambi_spadesMETA_NODE_8_length_4562	HHRz (1727-1803)	7.7e-09	HHRz (2068-2014)	1.1e-08
<b>AoALV4</b>	ambi_spadesMETA_NODE_9_length_4549	HHRz (1-41)	0.021	HHRz (333-256)	2.5e-09

The (+) polarity is defined as the RNA strand coding for the polymerase (ORFA).