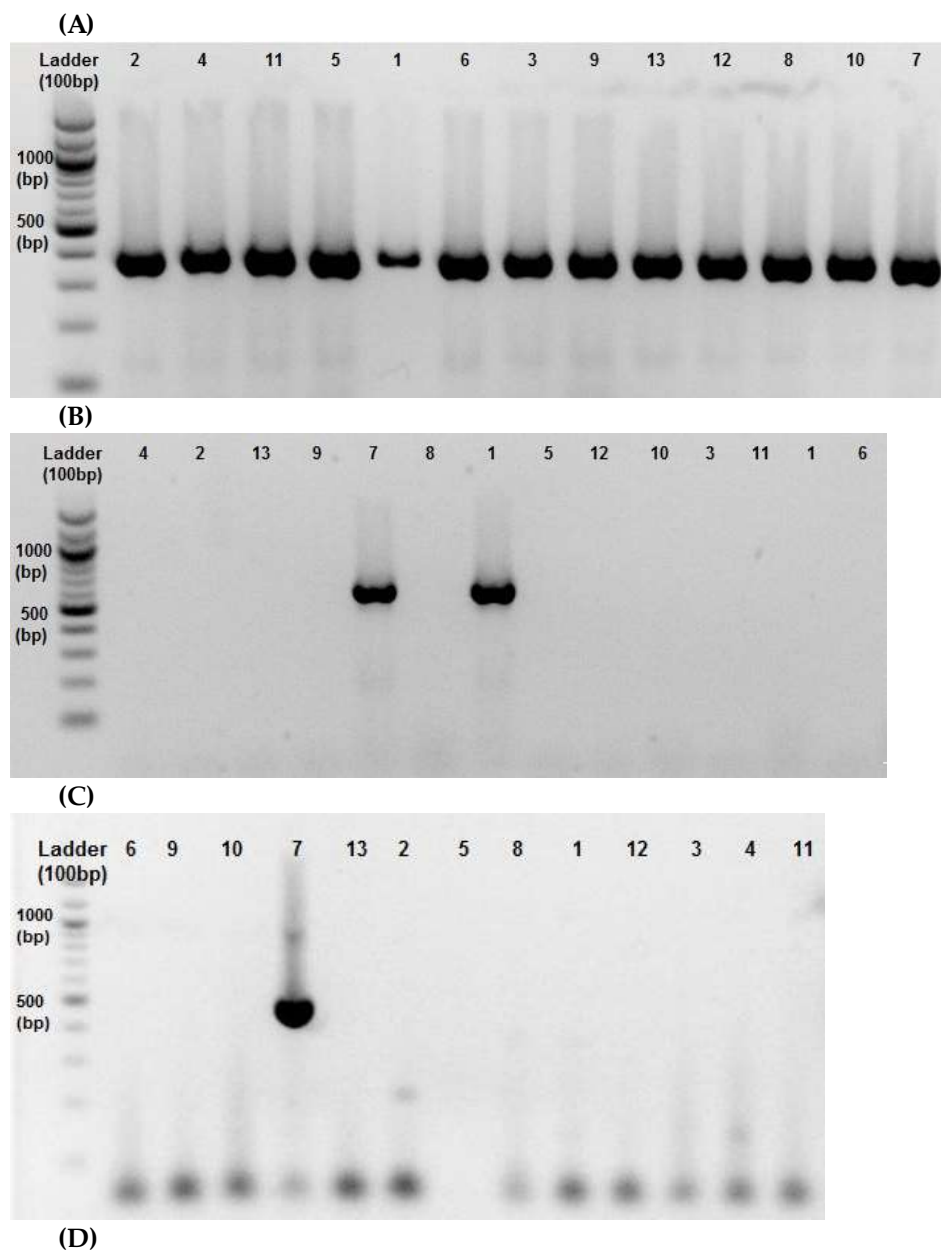
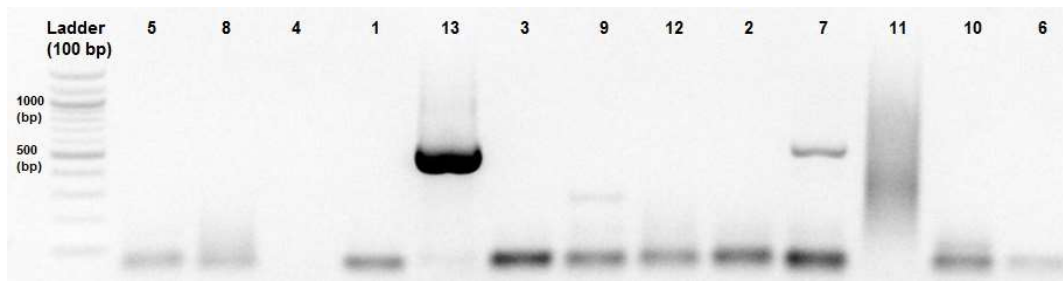


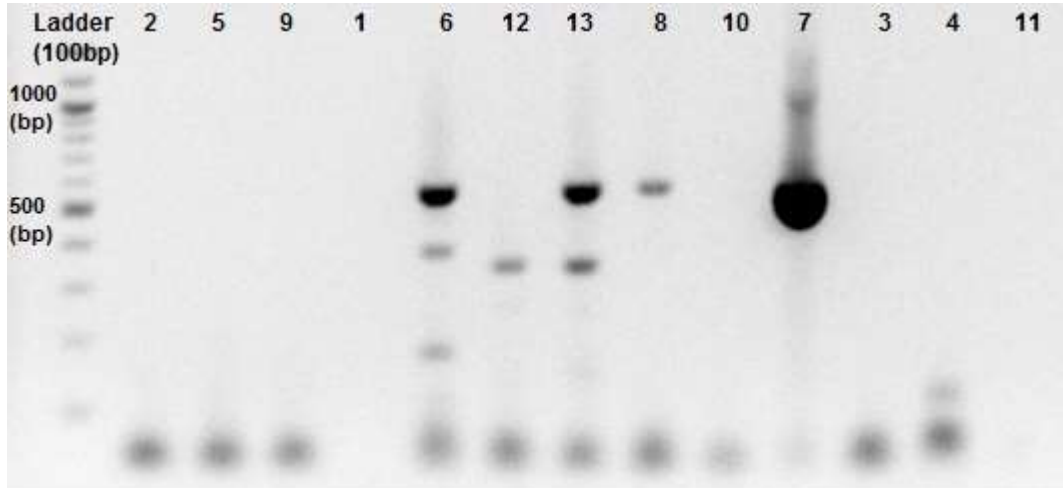
Figure S1. Amino acid alignment showing conserved motifs. **A**, Amino acid alignment of ambi-like viruses described in this study and related members. QPB44666 – *Tulesnella ambivirus* 2, QPB44664 – *Tulesnella ambivirus* 1, QMP84026 – *Rhizoctonia solani ambivirus* 2, QMP84024 – *Rhizoctonia solani 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.





(E)



(F)

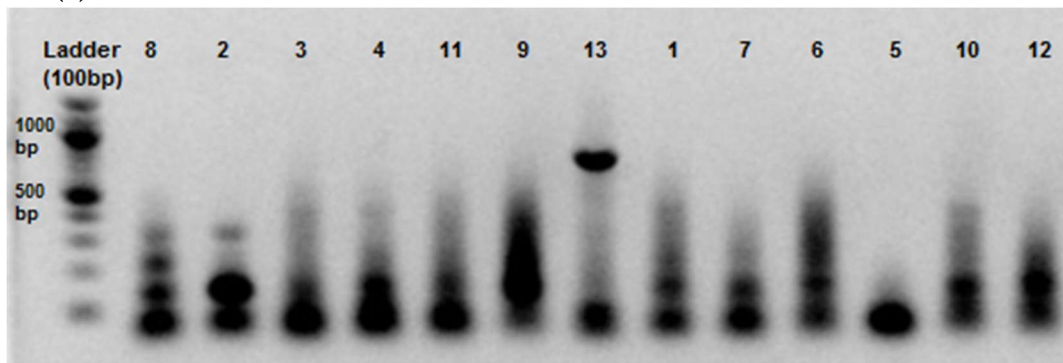


Figure S2. Resulting RT-PCR gels. **A**, Positive control with EF primers **B**, AALV1 **C**, AoALV2 **D**, AoALV3 **E**, AoALV4 **F**, AoTV1; 100bp DNA Ladder (Thermo Fisher Scientific) was used in all analyses.

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	CTCGCTGAGCCTCTCTACATCTT		
Armillaria ostoyae ambi-like virus 2	Forward	CGAGATGTTAGCCCTATAACCCATC	60	487
	Reverse	GGTGTAGGAACTGTCTGTACTCTG		
Armillaria ostoyae ambi-like virus 3	Forward	CCGACTATAGGATTGTCCCTTCAG	60	477
	Reverse	CATCTGTCTTATCAGCTCTCTGGG		
Armillaria ostoyae ambi-like virus 4	Forward	CTATCCCTTCCGACTTCTTTCCC	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				B			
	AoALV1	AoALV2	AoALV3		AoALV1	AoALV2	AoALV3
AoALV2	27.66			AoALV2	19.53		
AoALV3	31.40	31.90		AoALV3	77.46	18.65	
AoALV4	85.73	29.82	30.09	AoALV4	90.93	19.20	77.52

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

Virus Name	Contig Name	Ribozyme (+)	e-Value	Ribozyme (-)	e-Value
AoALV1	ambi_spadesMETA_NODE_2_length_6862	HHRz (4510-4564)	1.4e-07	HHRz (330-254)	4.6e-11
AoALV2	ambi_spadesMETA_NODE_4_length_6697	HPRz (4424-4531)	5,00E-08	HPRz (4987-4884)	5.7e-10
AoALV3	ambi_spadesMETA_NODE_8_length_4562	HHRz (1727-1803)	7.7e-09	HHRz (2068-2014)	1.1e-08
AoALV4	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).