

Table S1: Number of Nile tilapia collected from five sampling sites in Egypt, tissue type, and number of pooled samples collected for metagenomic analysis following RNA and/or DNA extraction

Sampling site	Liver	Kidney	Spleen	Brain	Gills
Husseiniya	5 fish* RNA=2** DNA=2***	5 fish RNA=2 DNA=2	5 fish RNA=2 DNA=1	5 fish RNA=2 DNA=2	5 fish RNA=2 DNA=2
Mariout	5 fish RNA=2	ND****	4 fish RNA=1	5 fish RNA=1 DNA=2	ND
Ward Island	3 fish RNA=1 DNA=1	3 fish RNA=1 DNA=1	3 fish RNA=1 DNA=1	3 fish RNA=1	ND
Om Khalaf	3 fish RNA=1	ND	3 fish RNA=1	3 fish RNA=2	ND
Bahr Yusef	2 fish RNA=1 DNA=1	2 fish RNA=1 DNA=1	2 fish RNA=1 DNA=1	2 fish RNA=2	2 fish DNA=2
Total	18 fish RNA=7 DNA=4	10 fish RNA=4 DNA=4	17 fish RNA=6 DNA=3	18 fish RNA=8 DNA=4	7 fish RNA=2 DNA=4

*Number of fish sampled

**Number of pooled samples extracted for RNA

***Number of pooled samples extracted for DNA

****Not done

Table S2: Pairwise nucleotide sequence distances based on the PB1 gene between AmnoonvirusEGY1 isolate (of this study) and representative members of the order Articulavirales. Distances were calculated using the *p*-distance model and are expressed as the percentage of differing nucleotide sites between sequences. Lower values indicate higher sequence similarity.

	AmnoonvirusEGY1	TiLV*_Index_strain	Rainbowtrout_orthomyxovirus1	Infectious_Salmon_Anemia_virus	Yancheng_orthomyxo-like_virus	Wenling_orthomyxo-like_virus2	Wenling_hagfish_influenza_virus	Wuhan_asiatric_toad_influenza_virus	Wuhan_spiny_eel_influenza_virus	Wuhan_carp_Isavirus1	Xibalbanus_thogotovirus1	Influenza_A	Influenza_B	Influenza_C	Influenza_D	Pilchard_orthomyxovirus	Lautavirus	TiLV_like_virus1	Fancy_Tailed_Guppy_Virus_B2	Flavolineata_virus	Dolomieu_virus	Namensis_virus	Stewartii_virus	Przewalskiivirus	Asotusvirus1	Asotusvirus2
AmnoonvirusEGY1																										
TiLV*_Index_strain	13.38																									
Rainbowtrout_orthomyxovirus1	74.23	74.49																								
Infectious_Salmon_Anemia_virus	75.51	75.26	45.66																							
Yancheng_orthomyxo-like_virus	76.55	76.03	73.45	69.07																						
Wenling_orthomyxo-like_virus2	73.71	73.45	68.04	70.62	71.39																					
Wenling_hagfish_influenza_virus	68.70	68.19	73.98	74.49	76.80	69.33																				
Wuhan_asiatric_toad_influenza_virus	66.92	64.90	69.90	71.17	76.29	71.91	55.98																			
Wuhan_spiny_eel_influenza_virus	75.70	76.96	66.07	62.76	73.20	71.65	73.79	66.33																		
Wuhan_carp_Isavirus1	70.92	72.45	48.21	44.39	71.65	70.36	67.86	70.66	63.27																	
Xibalbanus_thogotovirus1	73.82	74.35	71.99	73.56	79.32	70.16	68.06	71.20	68.59	70.68																
Influenza_A	75.44	76.71	61.73	59.44	71.91	68.56	68.70	67.09	42.28	57.91	69.63															
Influenza_B	74.43	74.43	61.48	58.16	72.68	69.59	71.50	66.58	33.42	59.44	67.80	40.00														
Influenza_C	73.16	74.18	62.50	65.82	65.21	69.85	67.43	63.80	50.13	61.22	65.97	46.08	47.34													
Influenza_D	75.44	75.70	61.22	60.20	70.36	67.78	68.45	62.53	52.66	58.16	67.54	48.86	48.10	34.18												
Pilchard_orthomyxovirus	73.21	73.72	47.70	48.21	70.88	69.07	73.47	71.43	66.84	46.94	77.75	61.99	65.31	63.78	62.76											
Lautavirus	69.21	69.21	76.79	76.79	72.68	73.20	73.03	73.28	75.57	75.00	75.92	75.06	75.57	74.30	74.30	75.26										
TiLV_like_virus1	23.48	17.42	74.23	72.96	75.26	76.03	66.92	61.87	75.19	70.92	74.08	75.70	73.16	72.66	75.19	75.00	68.96									
Fancy_Tailed_Guppy_Virus_B2	22.47	17.68	73.72	73.47	75.52	76.03	67.43	63.64	75.70	70.15	74.35	76.20	74.18	71.65	74.43	74.23	68.96	7.58								
Flavolineata_virus	58.88	59.39	74.49	73.98	72.16	78.09	65.90	68.53	67.77	70.66	74.61	71.57	70.81	70.81	73.35	72.45	70.99	59.14	58.63							
Dolomieu_virus	78.32	80.06	76.59	73.70	73.99	78.90	76.30	74.57	71.39	72.83	76.59	74.28	71.39	77.46	76.88	75.72	74.57	79.77	79.19	77.17						
Namensis_virus	59.54	60.05	73.98	72.45	72.16	74.23	67.68	68.70	77.35	71.43	75.13	72.77	72.77	71.50	72.01	74.23	71.50	56.74	57.51	58.52	79.48					
Stewartii_virus	70.95	71.72	78.15	77.89	75.26	76.55	77.12	76.09	75.32	75.32	74.87	74.04	75.84	73.52	75.58	78.15	73.78	72.24	73.26	70.18	75.14	77.12				
Przewalskiivirus	75.51	76.02	69.13	69.39	69.33	75.52	70.66	73.98	67.60	69.13	72.51	71.17	69.64	70.92	69.13	66.07	76.53	74.74	76.02	72.70	76.01	73.47	73.01			
Asotusvirus1	72.45	73.98	70.92	71.17	76.55	75.52	75.77	73.98	76.28	72.45	78.27	72.45	74.49	75.00	73.21	69.64	71.68	75.26	73.98	72.70	71.68	71.94	75.58	70.66		
Asotusvirus2	69.95	71.24	74.35	73.32	70.21	75.91	77.72	73.58	74.61	75.65	77.75	71.50	73.83	74.87	76.17	74.87	70.21	70.73	70.47	73.32	76.88	73.32	73.32	75.39	71.50	

Table S3: Pairwise amino acid sequences distances based on the PB1 gene between the Egyptian Amnoonvirus isolate (this study) and representative members of the order Articulavirales. Distances were calculated using the *p*-distance model and are expressed as the percentage of differing amino acid sites between sequences. Lower values indicate higher sequence similarity.

	AmnoonvirusEGY1	TiLV*_Index_strain	Rainbowtrout_orthomyxovirus1	Infectious_Salmon_Anemia_virus	Yancheng_orthomyxo-like_virus	Wenling_orthomyxo-like_virus2	Wenling_hagfish_influenza_virus	Wuhan_asiatic_toad_influenza_virus	Wuhan_spiny_eel_influenza_virus	Wuhan_carp_Isavirus1	Xibalbanus_thogotovirus1	Influenza_A	Influenza_B	Influenza_C	Influenza_D	Pilchard_orthomyxovirus	Lautavirus	TiLV_like_virus1	Fancy_Tailed_Guppy_Virus_B2	Flavolineata_virus	Dolomieu_virus	Namensis_virus	Stewartii_virus	Przewalskiivirus	Asotusvirus1	Asotusvirus2
AmnoonvirusEGY1																										
TiLV*_Index_strain	16.33																									
Rainbowtrout_orthomyxovirus1	95.83	95.83																								
Infectious_Salmon_Anemia_virus	97.92	97.92	70.83																							
Yancheng_orthomyxo-like_virus	95.83	97.92	89.58	91.67																						
Wenling_orthomyxo-like_virus2	95.83	95.83	91.67	91.67	93.75																					
Wenling_hagfish_influenza_virus	93.75	93.75	89.58	93.75	100	85.42																				
Wuhan_asiatic_toad_influenza_virus	95.92	95.92	91.67	97.92	95.83	89.58	85.42																			
Wuhan_spiny_eel_influenza_virus	97.92	97.92	91.67	91.67	91.67	95.83	93.75	87.50																		
Wuhan_carp_Isavirus1	93.75	95.83	72.92	60.42	89.58	95.83	85.42	91.67	85.42																	
Xibalbanus_thogotovirus1	89.58	91.67	93.75	97.92	95.83	95.83	87.50	91.67	87.50	97.92																
Influenza_A	91.67	93.75	91.67	87.50	91.67	89.58	91.67	89.58	58.33	89.58	91.67															
Influenza_B	89.58	93.75	81.25	83.33	83.33	91.67	89.58	85.42	54.17	87.50	85.42	64.58														
Influenza_C	87.50	89.58	93.75	93.75	87.50	95.83	89.58	77.08	77.08	91.67	87.50	70.83	72.92													
Influenza_D	89.58	89.58	85.42	87.50	85.42	93.75	93.75	89.58	77.08	83.33	91.67	66.67	66.67	52.08												
Pilchard_orthomyxovirus	97.92	97.92	66.67	68.75	93.75	95.83	93.75	89.58	91.67	68.75	93.75	91.67	85.42	93.75	89.58											
Lautavirus	91.67	89.58	93.75	89.58	89.58	95.83	93.75	95.83	91.67	87.50	93.75	87.50	95.83	89.58	85.42	93.75										
TiLV_like_virus1	44.90	42.86	93.75	93.75	97.92	95.83	95.83	93.88	97.92	89.58	85.42	93.75	97.92	89.58	91.67	95.83	89.58									
Fancy_Tailed_Guppy_Virus_B2	40.82	38.78	93.75	93.75	100	100	97.92	95.92	95.83	89.58	87.50	91.67	97.92	89.58	91.67	95.83	91.67	22.45								
Flavolineata_virus	77.08	75.00	89.58	93.75	95.83	93.75	87.50	95.83	93.75	95.83	95.83	97.92	89.58	89.58	93.75	93.75	93.75	81.25	75.00							
Dolomieu_virus	97.67	97.67	90.70	90.70	100	95.35	93.02	95.35	93.02	90.70	97.67	93.02	95.35	95.35	95.35	93.02	90.70	97.67	95.35	93.02						
Namensis_virus	85.42	85.42	89.58	91.67	93.75	89.58	93.75	85.42	91.67	93.75	95.83	85.42	85.42	89.58	85.42	91.67	95.83	79.17	81.25	89.58	100					
Stewartii_virus	87.50	93.75	95.83	95.83	87.50	93.75	95.83	93.75	93.75	93.75	93.75	89.58	95.83	89.58	91.67	97.92	91.67	91.67	95.83	91.67	93.02	93.75				
Przewalskiivirus	95.83	97.92	95.83	97.92	91.67	95.83	93.75	93.75	87.50	91.67	89.58	95.83	91.67	95.83	93.75	91.67	93.75	91.67	91.67	95.83	100.00	91.67	87.50			
Asotusvirus1	95.83	95.83	91.67	93.75	95.83	93.75	91.67	91.67	97.92	93.75	95.83	89.58	100	97.92	95.83	95.83	91.67	93.75	93.75	93.75	95.35	95.83	87.50	87.50		
Asotusvirus2	91.67	95.83	89.58	91.67	91.67	93.75	97.92	81.25	97.92	89.58	89.58	93.75	91.67	89.58	91.67	91.67	91.67	91.67	95.83	97.92	97.67	87.50	93.75	91.67	91.67	

Table S4: Pairwise nucleotide sequences distances based on the PB1 gene between AmnoonvirusEGY1 isolate (of this study) and representative members of the *Amnoonviridae* family (classified, and unclassified). Distances were calculated using the *p*-distance model and are expressed as the percentage of differing nucleotide sites between sequences. Lower values indicate higher sequence similarity.

	AmnoonvirusEGY1	TilapiaLakeVirus	Flavolineatavirus	Dolomieuvirus	Namensisvirus	Stewartiivirus	Plagiosomusvirus	Przewalskiivirus	Asotusvirus1	Asotusvirus2	Fancy-TailedGuppyVirus	TilapiaLakeVirus-LikeVirus
AmnoonvirusEGY1												
TilapiaLakeVirus	13.13											
Flavolineatavirus	61.32	59.03										
Dolomieuvirus	69.11	68.29	60.83									
Namensisvirus	58.78	57.76	58.52	69.17								
Stewartiivirus	70.98	72.41	77.10	73.15	72.17							
Plagiosomusvirus	71.21	71.21	62.12	66.67	69.70	71.21						
Przewalskiivirus	68.17	70.27	70.91	73.17	67.58	74.25	69.70					
Asotusvirus1	71.22	68.42	76.11	78.86	77.29	71.61	74.60	75.65				
Asotusvirus2	71.30	69.28	69.01	73.17	67.54	72.06	69.70	69.97	69.88			
Fancy-TailedGuppyVirus	22.47	18.43	56.74	63.41	57.00	72.41	71.21	70.87	70.76	71.59		
TilapiaLakeVirus-LikeVirus	23.23	17.42	58.27	65.04	55.73	70.98	71.21	71.47	71.64	73.91	8.08	

Table S5: Pairwise amino acid sequences distances based on the PB1 gene between AmnoonvirusEGY1 isolate (this study) and representative members of the *Amnoonviridae* family (classified, and unclassified). Distances were calculated using the *p*-distance model and are expressed as the percentage of differing amino acid sites between sequences. Lower values indicate higher sequence similarity.

	AmnoonvirusEGY1	TilapiaLakeVirus	Flavolineatavirus	Dolomieuvirus	Namensisvirus	Stewartiivirus	Plagiosomusvirus	Przewalskiivirus	Asotusvirus1	Asotusvirus2	Fancy-TailedGuppyVirus	TilapiaLakeVirus-LikeVirus
AmnoonvirusEGY1												
TilapiaLakeVirus	19.19											
Flavolineatavirus	87.76	82.65										
Dolomieuvirus	92.86	89.29	81.48									
Namensisvirus	84.69	82.65	81.63	92.59								
Stewartiivirus	87.06	85.88	96.43	95.83	90.48							
Plagiosomusvirus	82.35	76.47	64.71	100.00	88.24	94.12						
Przewalskiivirus	86.08	86.08	88.46	85.71	80.77	94.29	94.12					
Asotusvirus1	91.67	88.10	95.18	89.29	92.77	82.05	93.75	84.93				
Asotusvirus2	82.14	80.95	87.95	89.29	84.34	92.11	88.24	83.10	84.52			
Fancy-TailedGuppyVirus	41.41	36.36	80.61	82.14	82.65	89.41	76.47	88.61	91.67	89.29		
TilapiaLakeVirus-LikeVirus	40.40	35.35	85.71	85.71	81.63	89.41	76.47	88.61	94.05	89.29	14.14	

Table S6: Pairwise nucleotide sequence distances between AmnoonvirusEGY1 isolate (this study) and different *Amnoonviridae* members, including the TiLV, TiLV-like, and FTGV by segment 5. Distances were calculated using the p-distance model and are expressed as the percentage of differing nucleotide sites between sequences. Lower values indicate higher sequence similarity.

[illegible]

Table S8: Pairwise nucleotide sequence distances between AmnoonvirusEGY1 isolate (this study) and different *Amnoonviridae* members, including the TiLV and TiLV-like by segment 7. Distances were calculated using the *p*-distance model and are expressed as the percentage of differing nucleotide sites between sequences. Lower values indicate higher sequence similarity.

	AmnoonvirusEGY1	KU751820/TiLV/Israel/Til-4-2011*Index_strian	OM469315/TiLV/India/S27/SRLAAH/2021	OR101705/TiLV/India/KR-7/S2/SRLAAH/2021	OR101707/TiLV/India/CC-7/S4/SRLAAH/2022	OR101706/TiLV/India/RV-7/S3/SRLAAH/2021	OQ437060/TiLV/Hong_Kong/Israel-HK	KU552137/TilapiaVirus/Israrl/AD-2016	ON376578/TiLV/Viet_Nam/HB196-VN-2020	PV356103/TiLV/Colombia/ICA2023	MN939378/TiLV/Bangladesh/BD-2017	BK063216/TiLV-like_virus/Caribbean/Maracas_2015-2	BK063206/TiLV-like_virus/Caribbean/Maracas_2015-1
AmnoonvirusEGY1													
KU751820/TiLV/Israel/Til-4-2011*Index_strian	53.26												
OM469315/TiLV/India/S27/SRLAAH/2021	55.17	4.71											
OR101705/TiLV/India/KR-7/S2/SRLAAH/2021	54.79	2.90	1.81										
OR101707/TiLV/India/CC-7/S4/SRLAAH/2022	54.02	4.35	1.81	3.62									
OR101706/TiLV/India/RV-7/S3/SRLAAH/2021	54.79	2.90	4.71	2.90	3.62								
OQ437060/TiLV/Hong_Kong/Israel-HK	53.26	0.00	4.71	2.90	4.35	2.90							
KU552137/TilapiaVirus/Israrl/AD-2016	53.26	2.54	3.62	2.54	3.26	2.54	2.54						
ON376578/TiLV/Viet_Nam/HB196-VN-2020	52.11	5.43	6.16	5.43	5.80	5.43	5.43	4.35					
PV356103/TiLV/Colombia/ICA2023	54.02	3.62	5.07	3.62	4.71	2.90	3.62	2.54	4.71				
MN939378/TiLV/Bangladesh/BD-2017	54.79	4.35	1.09	2.90	1.45	4.35	4.35	3.26	5.80	4.71			
BK063216/TiLV-like_virus/Caribbean/Maracas_2015-2	52.87	19.05	20.15	19.78	19.78	19.78	19.05	19.41	19.78	19.41	19.78		
BK063206/TiLV-like_virus/Caribbean/Maracas_2015-1	52.87	19.05	20.15	19.78	19.78	19.78	19.05	19.41	19.78	19.41	19.78	0.00	

Table S9: Pairwise amino acid sequence distances between AmnoonvirusEGY1 isolate (this study) and different *Amnoonviridae* members, including the TiLV and TiLV-like by segment 7. Distances were calculated using the *p*-distance model and are expressed as the percentage of differing amino acid sites between sequences. Lower values indicate higher sequence similarity.

	AmnoonvirusEGY1	KU751820/TiLV/Israel/Til-4-2011*Index_strian	OM469315/TiLV/India/S27/SRLAAH/2021	OR101705/TiLV/India/KR-7/S2/SRLAAH/2021	OR101707/TiLV/India/CC-7/S4/SRLAAH/2022	OR101706/TiLV/India/RV-7/S3/SRLAAH/2021	OQ437060/TiLV/Hong_Kong/Israel-HK	KU552137/TilapiaVirus/Israrl/AD-2016	ON376578/TiLV/Viet_Nam/HB196-VN-2020	PV356103/TiLV/Colombia/ICA2023	MN939378/TiLV/Bangladesh/BD-2017	BK063216/TiLV-like_virus/Caribbean/Maracas_2015-2	BK063206/TiLV-like_virus/Caribbean/Maracas_2015-1
AmnoonvirusEGY1													
KU751820/TiLV/Israel/Til-4-2011*Index_strian	62.03												
OM469315/TiLV/India/S27/SRLAAH/2021	63.29	2.41											
OR101705/TiLV/India/KR-7/S2/SRLAAH/2021	62.03	0.00	2.41										
OR101707/TiLV/India/CC-7/S4/SRLAAH/2022	63.29	2.41	0.00	2.41									
OR101706/TiLV/India/RV-7/S3/SRLAAH/2021	63.29	2.41	4.82	2.41	4.82								
OQ437060/TiLV/Hong_Kong/Israel-HK	62.03	0.00	2.41	0.00	2.41	2.41							
KU552137/TilapiaVirus/Israrl/AD-2016	62.03	1.20	1.20	1.20	1.20	3.61	1.20						
ON376578/TiLV/Viet_Nam/HB196-VN-2020	62.03	2.41	2.41	2.41	2.41	4.82	2.41	1.20					
PV356103/TiLV/Colombia/ICA2023	63.29	1.20	2.41	1.20	2.41	3.61	1.20	2.41	3.61				
MN939378/TiLV/Bangladesh/BD-2017	63.29	2.41	0.00	2.41	0.00	4.82	2.41	1.20	2.41	2.41			
BK063216/TiLV-like_virus/Caribbean/Maracas_2015-2	65.82	15.66	16.87	15.66	16.87	18.07	15.66	15.66	15.66	16.87	16.87		
BK063206/TiLV-like_virus/Caribbean/Maracas_2015-1	65.82	15.66	16.87	15.66	16.87	18.07	15.66	15.66	15.66	16.87	16.87	0.00	

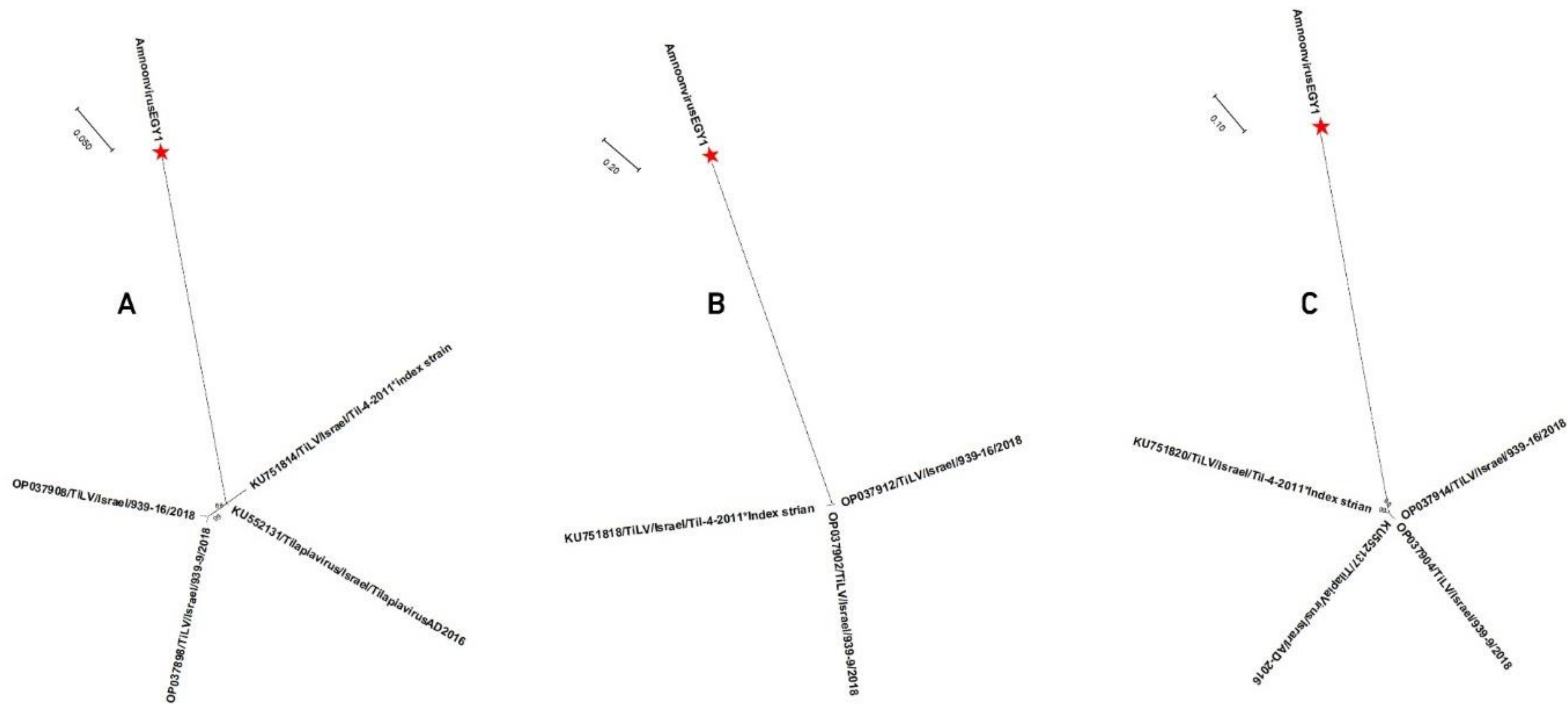


Figure S1. An unrooted maximum likelihood phylogenetic trees illustrating the phylogenetic relationships between AmnoonvirusEGY1 nucleotide sequences (indicated by red ★) and TiLV strains from Israel for genomic segments 1, 5, and 7. A. Segment 1 was aligned with four Israeli strains (GenBank Accession Nos. OP037908, OP037898, KU552131, and KU751814). B. Segment 5 was compared with three Israeli strains (OP037912, OP037902, and KU751818). C. Segment 7 was analyzed against four Israeli strains (OP037914, OP037904, KU552137, and KU751820). Each viral isolate is labeled with its GenBank accession number, virus name, country of origin, and isolate designation. Bootstrap values $\geq 70\%$ are displayed at corresponding nodes to indicate clade support. Scale bars represent the number of nucleotide substitutions per site: 0.050 for panel A, 0.20 for panel B, and 0.10 for panel C.