Supplementary Material 1

Metagenomics detection and characterisation of viruses in faecal samples from Australian wild birds

Running Title of the Supplementary Material

Results of method optimisation for metagenomics of viruses.

Authors

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Table S1. The marker viruses, their properties and source used for spiking the faecal samples.

Virus	Family	Characteristics	Sample type	Reference genome NCBI ID[#]
Bluetongue virus, Serotype 1 (BTV)*	*Reoviridae*	dsRNA virus; segmented linear genome; non-enveloped and icosahedral about 80 nm in diameter.	Tissue culture isolate	KM099511
Bovine viral diarrhoea virus (BVDV)*	*Flaviviridae*	Monopartite, linear, ssRNA (+) genome; enveloped, spherical, about 50 nm in diameter.	Tissue culture isolate	NC 001461
Porcine circovirus 2 (PCV2) *	*Circoviridae*	Monopartite, circular, ssDNA genome; non-enveloped, icosahedral about 20 nm in diameter.	Frozen infected swine spleen	NC 005148
Infectious laryngotracheitis virus (ILTV)*	*Herpesviridae*	Monopartite, linear, dsDNA genome; enveloped, spherical to pleomorphic, 120-200 nm in diameter, nucleocapsid present	Pooled tracheal swabs in viral transport medium	NC006623
Infectious bronchitis virus (IBV)[+]	*Coronaviridae*	Monopartite, linear ssRNA (+) genome; enveloped, spherical, about 120 nm in diameter, nucleocapsid present	Tissue culture isolate	KF460437

*Source: Elizabeth Macarthur Agriculture Institute, NSW, Australia
[+]Source: AAHL, Victoria, Australia
#The reference genomes from NCBI listed served to map the NGS reads in IGV.

The table gives the details of the viruses that acted as marker viruses for protocol optimisation. Each of these viruses was isolated/present from/in different samples.

Table S2. Primers for marker virus real-time PCR assays.

Virus	Primers	Reference
BTV	FP: GCGTTCGAAGTTTACATCAAT RP: CAGTCATCTCTCTAGACACTCTATAATTACG	[1]
BVDV	FP: GRAGTCGTCARTGGTTCGAC RP: TCAACTCCATGTGCCATGTAC	[2]
PCV2	FP: TGGCCCGACGTATTCTGATT RP: CAGCTGGGACAGCAGTTGAG	[3]
ILTV	FP: GCACGTCACGGACGATTGT RP: GCCGCCTCGTCTTTTGC	Courtesy of Dr P. D. Kirkland, Elizabeth Macarthur Agricultural Institute, NSW
IBV	FP: CAAGCAGATGCTCAAGTGGA RP: CTCACGCTGTTGTGACACCT	This study. NCBI reference sequence used DQ490206.1

The table displays the primers used for all the marker viruses and the origin of those primers. Both forward and reverse primers for each assay are shown. Primers for BTV, BVDV and PCV2 were taken from the literature. Primer sequences for ILTV were kindly provided by Dr P D Kirkland from the Elizabeth Macarthur Agricultural Institute. We designed the forward and reverse primers for IBV.

Table S3. Ct values of marker viruses during the start of the protocol and after the nucleic acid extraction step.

Virus	Variation A		Variation B		Variation C		Variation D		Variation E		Variation F	
Step	Start*	End[#]	Start	End	Start	End	Start	End	Start	End	Start	End
PCV2	16	16	16	12	17	13.5	17	16.5	17	15	19	14
IBV	28	39	28	36	28.5	26	28	26	27	24	27	21
ILTV	28	29	28	25	31	26	31	29	32	29	32	31
BVDV	31	30	31	35	31	28	31	27	30	28	30	28
BTV	29	28	29	29	30	26	30	24.5	31	26	31	25

*Nucleic acid isolated after homogenization of the spiked sample
[#] Nucleic acid isolated at the end of the variations

The table displays the Ct value of marker viruses, which were used as a reference for identifying how different virus enrichment combinations affected each virus type. For the “Start” Ct value, we took the spiked sample after homogenization and isolated the nucleic acid as per the described method. The “End” Ct value was determined using the nucleic acids isolated at the end stage of each variation. As there was an increase in more than 3Ct values, for one of the marker virus (IBV), we discontinued variation A and B. All other variations were processed for NGS.

Table S4: Number of high-quality NGS reads generated for the marker viruses with a minimum mapping quality threshold of 20

No. of reads / Virus	Variation C	Variation D	Variation E	Variation F
BTV	14	33	8	13
BVDV	13	50-100	16	5
PCV2	50-150	50-150	50-150	50-150
ILTV	37	39	39	33
IBV	13	50-100	18	9

The table displays the number of high quality (Q20 or higher) NGS reads obtained for each marker virus from the spiked sample variations C to F. This was calculated manually from IGV. We used the reference genome mentioned in Table S1 for each of the marker viruses.

References

1. Shaw, A. E. *et al.* Development and initial evaluation of a real-time RT-PCR assay to detect bluetongue virus genome segment 1. *J. Virol. Methods* **145,** 115–126 (2007).

2. Hoffmann, B., Depner, K., Schirrmeier, H. & Beer, M. A universal heterologous internal control system for duplex real-time RT-PCR assays used in a detection system for pestiviruses. *J. Virol. Methods* **136,** 200–9 (2006).

3. Opriessnig, T. *et al.* Effect of vaccination with selective bacterins on conventional pigs infected with type 2 porcine circovirus. *Vet. Pathol.* **40,** 521–9 (2003).

Supplementary Material 2

Metagenomics detection and characterisation of viruses in faecal samples from Australian wild birds

Running Title of the Supplementary Material

Representative phylogenetic trees of the consensus sequences generated and analysed using MEGA 6 or 7 software

Authors

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1. Avian Paramyxovirus 6 from MAD faecal sample

Figure and Table	Long Name (Format: Sample-virus-protein-length-quality-coverage-year)	Short name (Format: Sample-virus-protein-length)	Coverage	No. of nucleotides	Mapping quality threshold	NCBI accession number
S1.1	MAD-Avian-paramyxovirus-6-nucleocapsid-protein-153nt-Q32-C-8-20-2016	MAD-APMV6-NCP-153nt	8-20	153	32	MH000403
S1.2	MAD-Avian-paramyxovirus-6-nucleocapsid-protein-187nt-Q32-C-92-204-2016	MAD-APMV6-NCP-187nt	92-204	187	32	MH000404
S1.3	MAD-Avian-paramyxovirus-6-nucleocapsid-protein-270nt-Q32-C-4-26-2016	MAD-APMV6-NCP-270nt	4-26	270	32	MH000405
S1.4	MAD-Avian-paramyxovirus-6-nucleocapsid-protein-410nt-Q32-C-3-20-2016	MAD-APMV6-NCP-410nt	3-20	410	32	MH000406
S1.5	MAD-Avian-paramyxovirus-6-phosphoprotein-211nt-Q32-C-6-72-2016	MAD-APMV6-PP-211nt	6-72	211	32	MH000407
S1.6	MAD-Avian-paramyxovirus-6-phosphoprotein-167nt-Q32-C-3-17-2016	MAD-APMV6-PP-167nt	3-17	167	32	MH000408
S1.7	MAD-Avian-paramyxovirus-6-matrix-protein-297nt-Q32-C-4-23-2016	MAD-APMV6-MP-297nt	4-23	297	32	MH000409
S1.8	MAD-Avian-paramyxovirus-6-matrix-protein-241nt-Q32-C-3-24-2016	MAD-APMV6-MP-241nt	3-24	241	32	MH000410
S1.9	MAD-Avian-paramyxovirus-6-matrix-protein-132nt-Q32-C-16-24-2016	MAD-APMV6-MP-132nt	16-24	132	32	MH000411
S1.10	MAD-Avian-paramyxovirus-6-	MAD-APMV6-FP-276nt	3-8	276	32	MH000413

	fusion-protein-276nt-Q32-C-3-8-2016					
S1.11	MAD-Avian-paramyxovirus-6-small-hydrophobic-protein-351nt-Q32-C-4-19-2016	MAD-APMV6-SHP-351nt	4-19	351	32	MH000414
S1.12	MAD-Avian-paramyxovirus-6-large-polymerase-protein-617nt-Q32-C-16-115-2016	MAD-APMV6-Pol-617nt	16-115	617	32	MH000416
S1.13	MAD-Avian-paramyxovirus-6-large-polymerase-protein-496nt-Q32-C-11-53-2016	MAD-APMV6-Pol-496nt	11-53	496	32	MH000417
S1.14	MAD-Avian-paramyxovirus-6-large-polymerase-protein-702nt-Q32-C-4-37-2016	MAD-APMV6-Pol-702nt	4-37	702	32	MH000418
S1.15	MAD-Avian-paramyxovirus-6-large-polymerase-protein-199nt-Q32-C-3-19-2016	MAD-APMV6-Pol-199nt	3-19	199	32	MH000420
S1.16	MAD-Avian-paramyxovirus-6-large-polymerase-protein-380nt-Q32-C-20-52-2016	MAD-APMV6-Pol-380nt	20-52	380	32	MH000421
S1.17	MAD-Avian-paramyxovirus-6-large-polymerase-protein-204nt-Q32-C-6-19-2016	MAD-APMV6-Pol-204nt	6-19	204	32	MH000422
S1.18	MAD-Avian-paramyxovirus-6-large-polymerase-protein-241nt-Q32-C-3-9-2016	MAD-APMV6-Pol-241nt	3-9	241	32	MH000423
S1.19	MAD-Avian-paramyxovirus-6-large-polymerase-protein-284nt-Q32-C-4-18-2016	MAD-APMV6-Pol-284nt	4-18	284	32	MH000424

S1.20	MAD-Avian-paramyxovirus-6-large-polymerase-protein-383nt-Q32-C-21-187-2016	MAD-APMV6-Pol-383nt	21-187	383	32	MH000425
S1.21	MAD-Avian-paramyxovirus-6-large-polymerase-protein-507nt-Q32-C-3-35-2016	MAD-APMV6-Pol-507nt	3-35	507	32	MH000426
S1.22	MAD-Avian-paramyxovirus-6-large-polymerase-protein-343nt-Q32-C-3-19-2016	MAD-APMV6-Pol-343nt	3-19	343	32	MH000427
S1.23	MAD-Avian-paramyxovirus-6-large-polymerase-protein-156nt-Q32-C-3-31-2016	MAD-APMV6-Pol-156nt	3-31	156	32	MH000428

NCBI sequences taken for phylogenetic analysis

Long Name	Short Name (Format: NCBI accession number-virus-country/state)	Country of collection	Collection date
AB759118-Avian-paramyxovirus-6-viral-cRNA-complete-genome-strain:red-necked-stint/Japan/8KS0813/2008	AB759118-APMV6-JP	Japan	2008
GQ406232-Avian-paramyxovirus-6-strain-duck/Italy/4524-2/07-complete-genome	GQ406232-APMV6-IT	Italy	2007
KP762799-Avian-paramyxovirus-6-isolate-red-crested-pochard/Balkhash/5842/2013-complete-genome	KP762799-APMV6-KZ	Kazakhstan	2013
AY029299-Avian-paramyxovirus-6-complete-genome	AY029299-APMV6-TW	Taiwan	-
KT962980-Avian-paramyxovirus-6-isolate-teal/Novosibirsk_region/455/2009-complete-genome	KT962980-APMV6-RU	Russia	2009
JN571486-Avian-paramyxovirus-6-strain-APMV6/mallard/Belgium/12245/07-nucleoprotein(NP)-phosphoprotein(P)-matrix-protein(M)-fusion-protein(F)-	JN571486-APMV6-BE	Belgium	2007

small-hydrophobic-protein(SH)-hemagglutinin-neuramis			
KF267717-Avian-paramyxovirus-6-isolate-mallard/Jilin/127/2011-complete-genome	KF267717-APMV6-CN	China	2011

The table points towards the molecular phylogenetic analysis by Maximum Likelihood method of APMV6. The first half of the table provides details to identify the sample from which the virus was isolated, protein that encodes the consensus sequences that are being analysed, the length of the consensus sequences thus providing the depth of the gene being analysed, minimum mapping quality threshold used in IGV for the generation of the consensus sequences, the coverage of the consensus sequences from IGV, the year of collection of the sample and the NCBI accession number assigned to the particular consensus sequence. Corresponding short names have been used in the phylogenetic trees which contain the sample, virus, protein and the number of nucleotides. The second half of the table provides the details of the sequences that were used for the molecular phylogenetic analysis. They were found to be the most closely related sequences to the consensus sequences generated using BLASTN. Corresponding short names have been used in the phylogenetic trees which contain the NCBI accession number, virus and the country of collection. The collection date is given as retrieved from the corresponding NCBI nucleotide reference dataset which together with the country of collection can provide an insight into the possible evolution of the virus through the years.

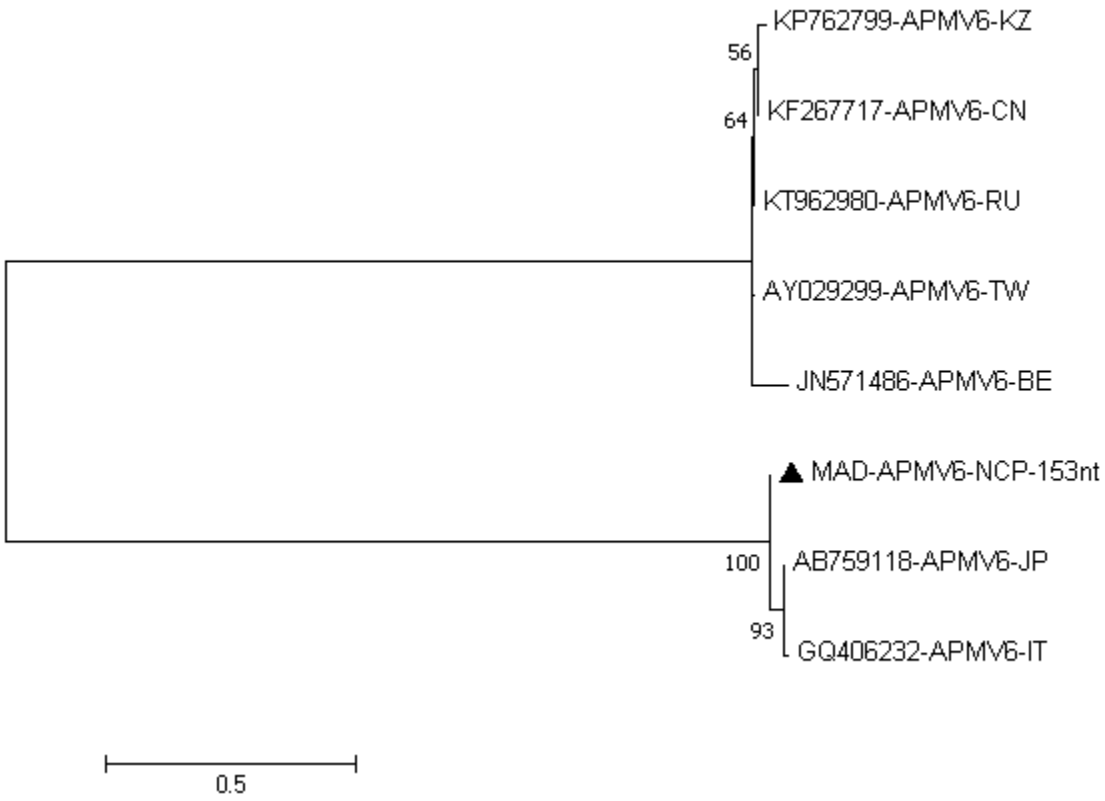


Figure S1.1. Molecular Phylogenetic analysis by Maximum Likelihood method of APMV6 partial NCP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-427.58) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.4716)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 63.10% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 153 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S1.1. Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 153 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-APMV6-NCP-153nt							
AB759118-APMV6-JP	4						
GQ406232-APMV6-IT	5	1					
KP762799-APMV6-KZ	34	37	36				
AY029299-APMV6-TW	35	38	37	5			
KT962980-APMV6-RU	36	39	38	3	2		
JN571486-APMV6-BE	36	37	38	13	10	10	
KF267717-APMV6-CN	35	38	37	2	3	1	11

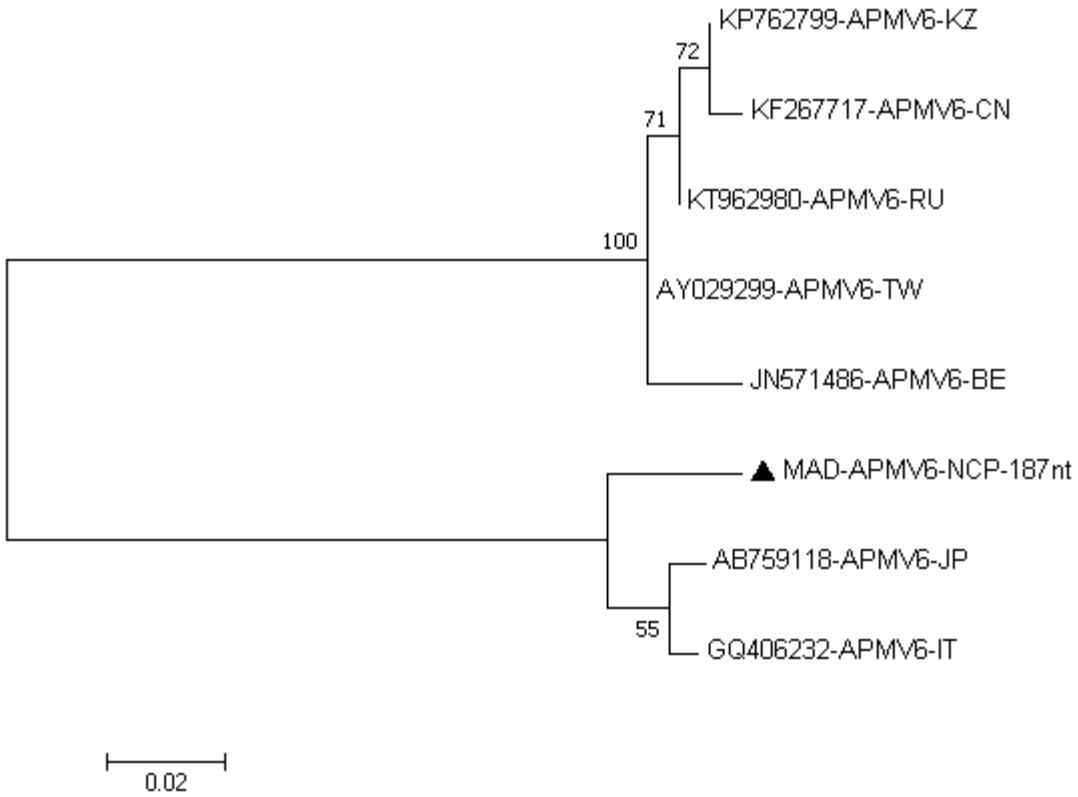


Figure S1.2. Molecular Phylogenetic analysis by Maximum Likelihood method of APMV6 partial NCP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-466.22) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 187 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S1.2: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 187 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-APMV6-NCP-187nt							
AB759118-APMV6-JP	7						
GQ406232-APMV6-IT	7	2					
KP762799-APMV6-KZ	39	37	36				
AY029299-APMV6-TW	37	35	34	2			
KT962980-APMV6-RU	38	36	35	1	1		
JN571486-APMV6-BE	38	38	37	5	3	4	
KF267717-APMV6-CN	40	38	37	1	3	2	6

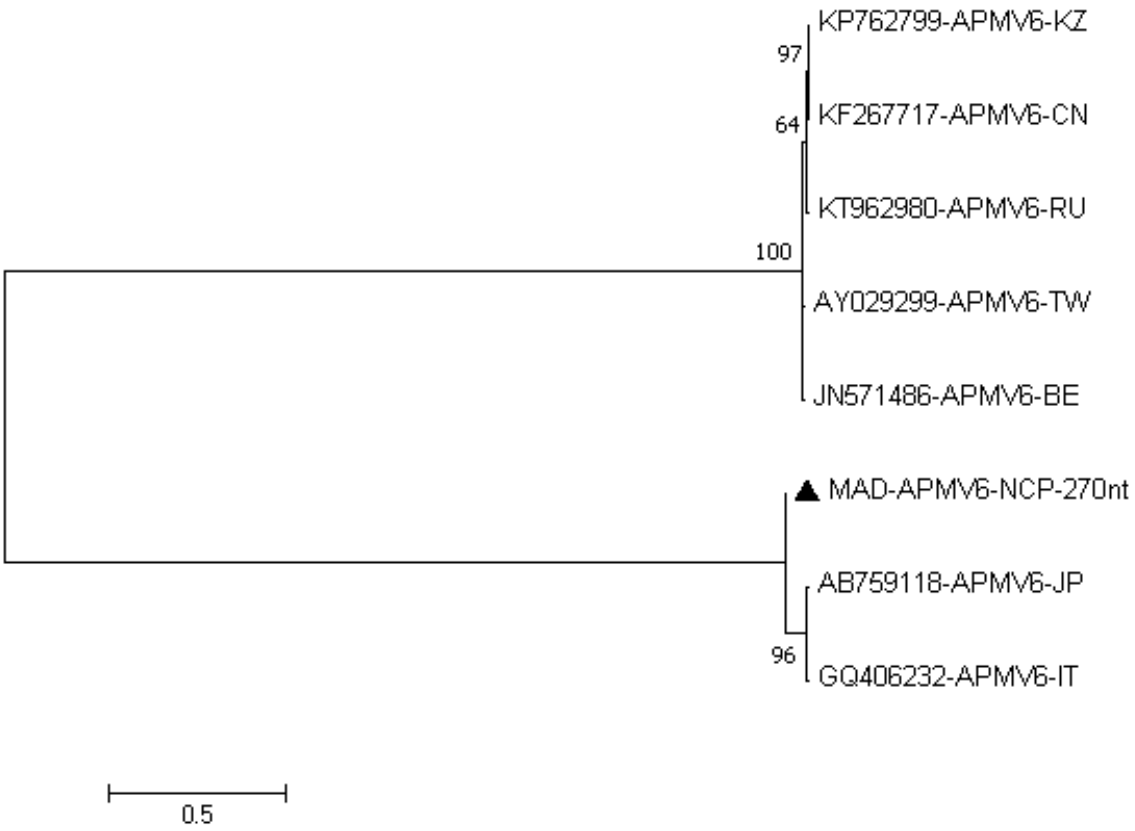


Figure S1.3. Molecular Phylogenetic analysis by Maximum Likelihood method of APMV6 partial NCP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-733.10) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.4529)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 64.25% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 270 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S1.3. Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 270 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-APMV6-NCP-270nt							
AB759118-APMV6-JP	15						
GQ406232-APMV6-IT	15	2					
KP762799-APMV6-KZ	64	66	67				
AY029299-APMV6-TW	62	64	65	5			
KT962980-APMV6-RU	63	63	64	4	5		
JN571486-APMV6-BE	61	63	64	5	2	5	
KF267717-APMV6-CN	64	66	67	0	5	4	5

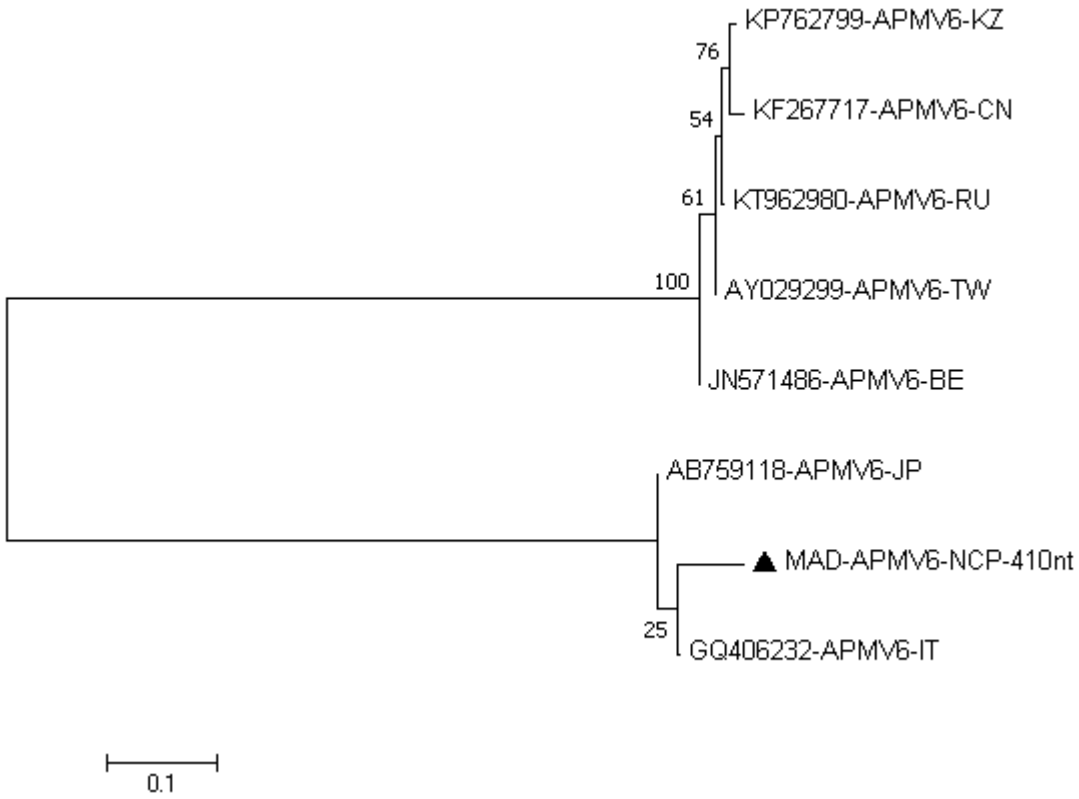


Figure S1.4. Molecular Phylogenetic analysis by Maximum Likelihood method of APMV6 partial NCP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-1125.76) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.3877)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 410 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S1.4. Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 410 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-APMV6-NCP-410nt							
AB759118-APMV6-JP	26						
GQ406232-APMV6-IT	22	8					
KP762799-APMV6-KZ	100	92	94				
AY029299-APMV6-TW	98	94	96	7			
KT962980-APMV6-RU	100	92	94	6	3		
JN571486-APMV6-BE	95	92	94	13	6	9	
KF267717-APMV6-CN	102	94	96	7	10	9	16

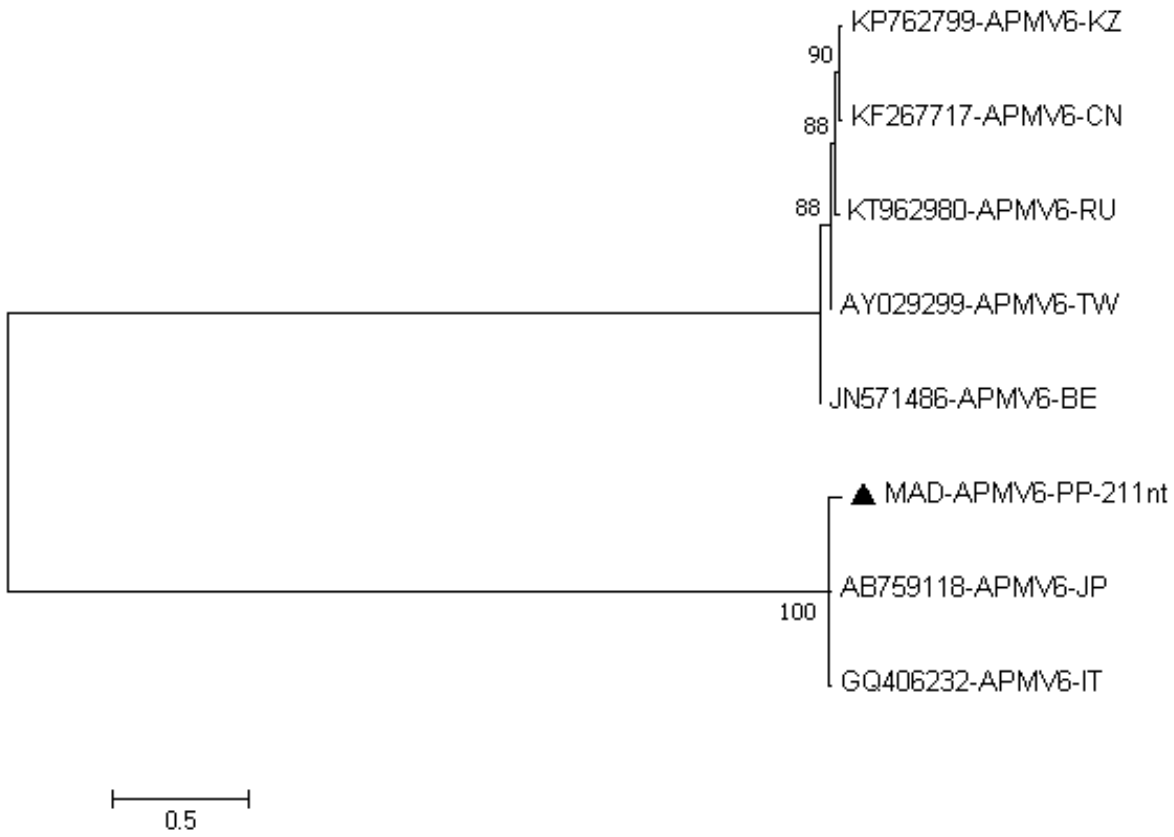


Figure S1.5. Molecular Phylogenetic analysis by Maximum Likelihood method of APMV6 partial PP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model[3]. The tree with the highest log likelihood (-682.39) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 57.35% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 211 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S1.5. Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 211 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-APMV6-PP-211nt							
AB759118-APMV6-JP	10						
GQ406232-APMV6-IT	10	4					
KP762799-APMV6-KZ	63	65	64				
AY029299-APMV6-TW	62	62	61	7			
KT962980-APMV6-RU	64	64	63	6	5		
JN571486-APMV6-BE	60	58	61	13	7	12	
KF267717-APMV6-CN	63	65	64	4	7	6	13

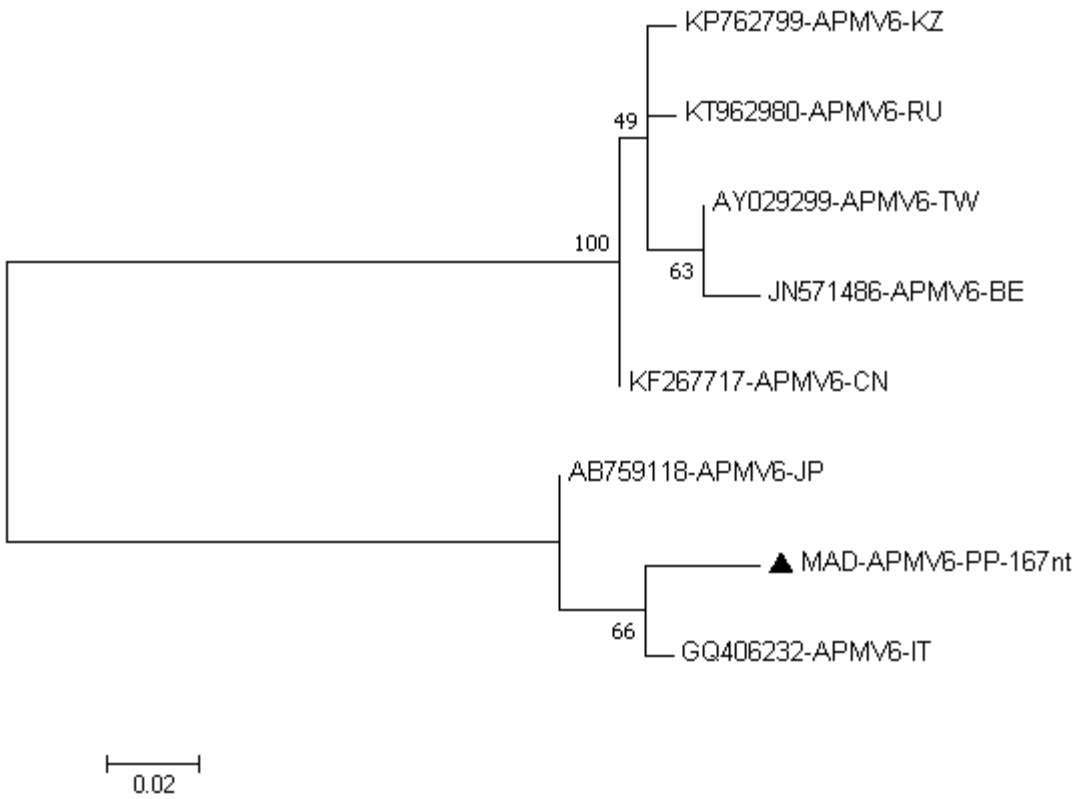


Figure S1.6. Molecular Phylogenetic analysis by Maximum Likelihood method of APMV6 partial PP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-436.43) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 167 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S1.6. Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 167 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-APMV6-PP-167nt							
AB759118-APMV6-JP	7						
GQ406232-APMV6-IT	5	4					
KP762799-APMV6-KZ	38	36	38				
AY029299-APMV6-TW	38	36	38	3			
KT962980-APMV6-RU	38	36	38	2	3		
JN571486-APMV6-BE	38	36	38	5	2	5	
KF267717-APMV6-CN	36	34	36	2	3	2	5

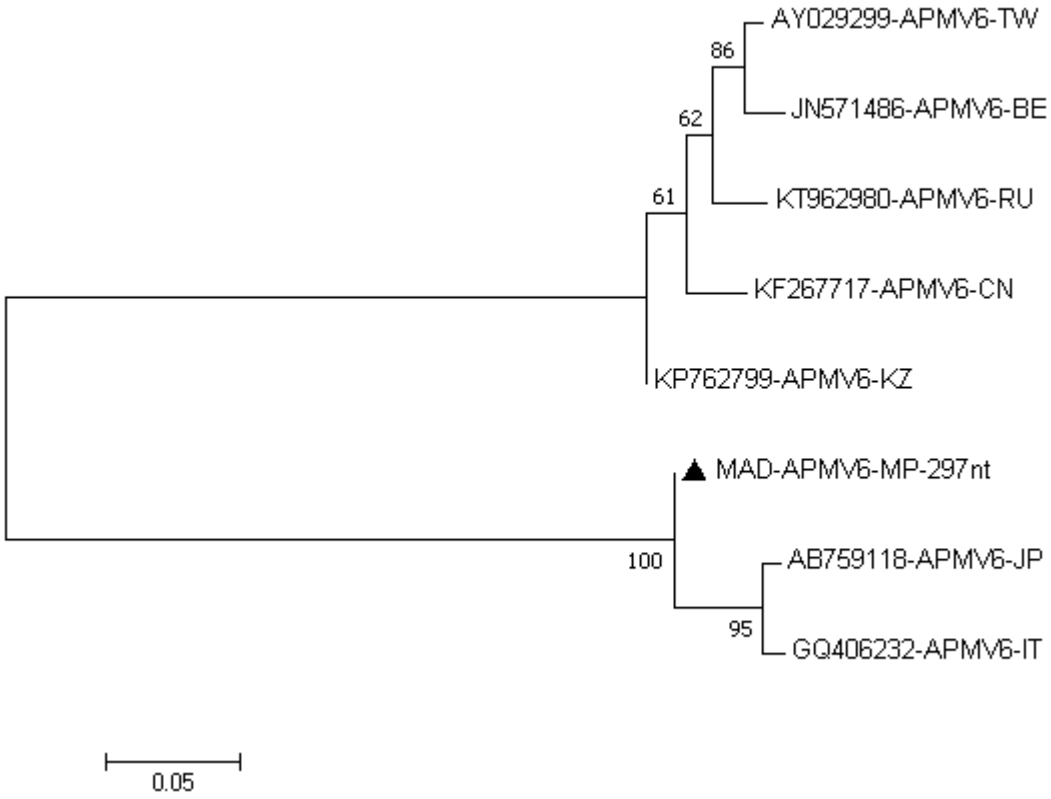


Figure S1.7. Molecular Phylogenetic analysis by Maximum Likelihood method of APMV6 partial MP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-855.36) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.3705)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 297 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S1.7. Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 297 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-APMV6-MP-297nt							
AB759118-APMV6-JP	10						
GQ406232-APMV6-IT	11	4					
KP762799-APMV6-KZ	59	63	64				
AY029299-APMV6-TW	68	68	71	11			
KT962980-APMV6-RU	64	64	67	12	10		
JN571486-APMV6-BE	67	67	70	12	6	12	
KF267717-APMV6-CN	66	66	69	10	13	13	15

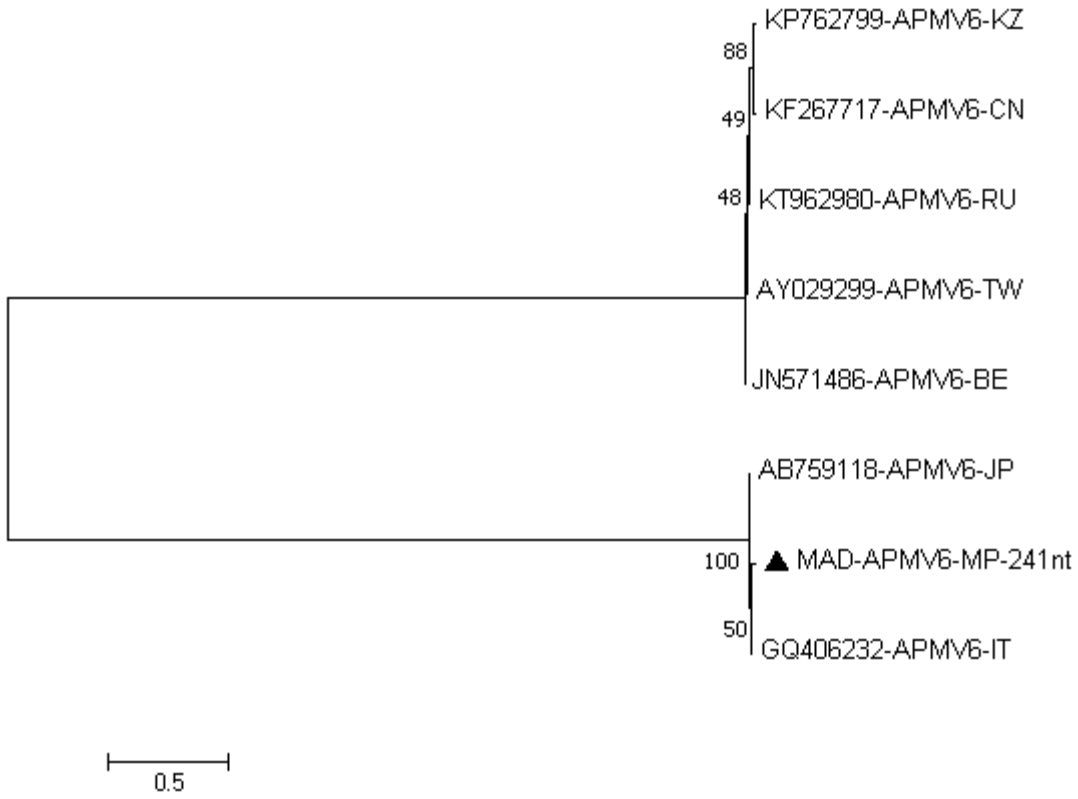


Figure S1.8. Molecular Phylogenetic analysis by Maximum Likelihood method of APMV6 partial MP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-656.08) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.5414)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 0.00% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 241 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S1.8. Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 241 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-APMV6-MP-241nt							
AB759118-APMV6-JP	7						
GQ406232-APMV6-IT	4	3					
KP762799-APMV6-KZ	61	60	61				
AY029299-APMV6-TW	60	59	60	8			
KT962980-APMV6-RU	61	60	61	6	2		
JN571486-APMV6-BE	59	58	59	9	3	5	
KF267717-APMV6-CN	62	61	62	3	7	5	10

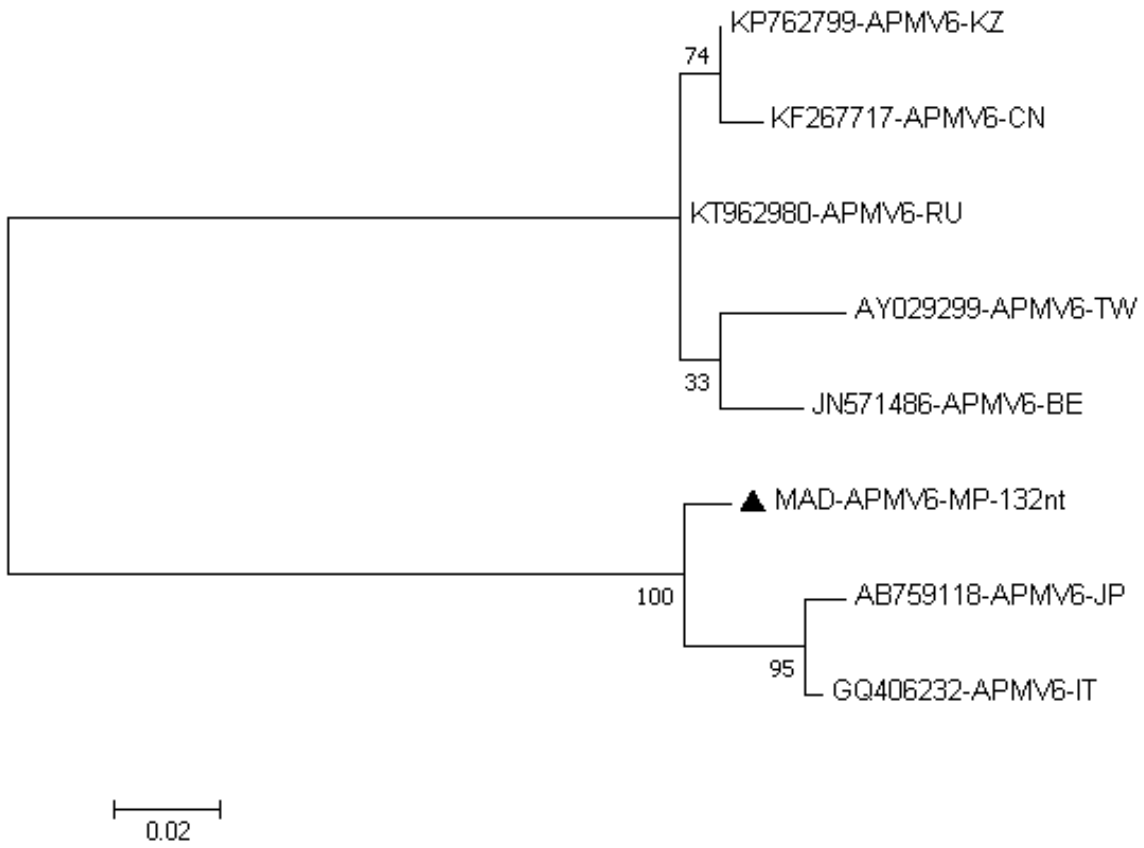


Figure S1.9. Molecular Phylogenetic analysis by Maximum Likelihood method of APMV6 partial MP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-357.56) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 132 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S1.9. Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 132 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-APMV6-MP-132nt							
AB759118-APMV6-JP	5						
GQ406232-APMV6-IT	4	1					
KP762799-APMV6-KZ	29	30	30				
AY029299-APMV6-TW	30	31	31	5			
KT962980-APMV6-RU	28	29	29	1	4		
JN571486-APMV6-BE	29	30	30	4	5	3	
KF267717-APMV6-CN	30	31	31	1	6	2	5

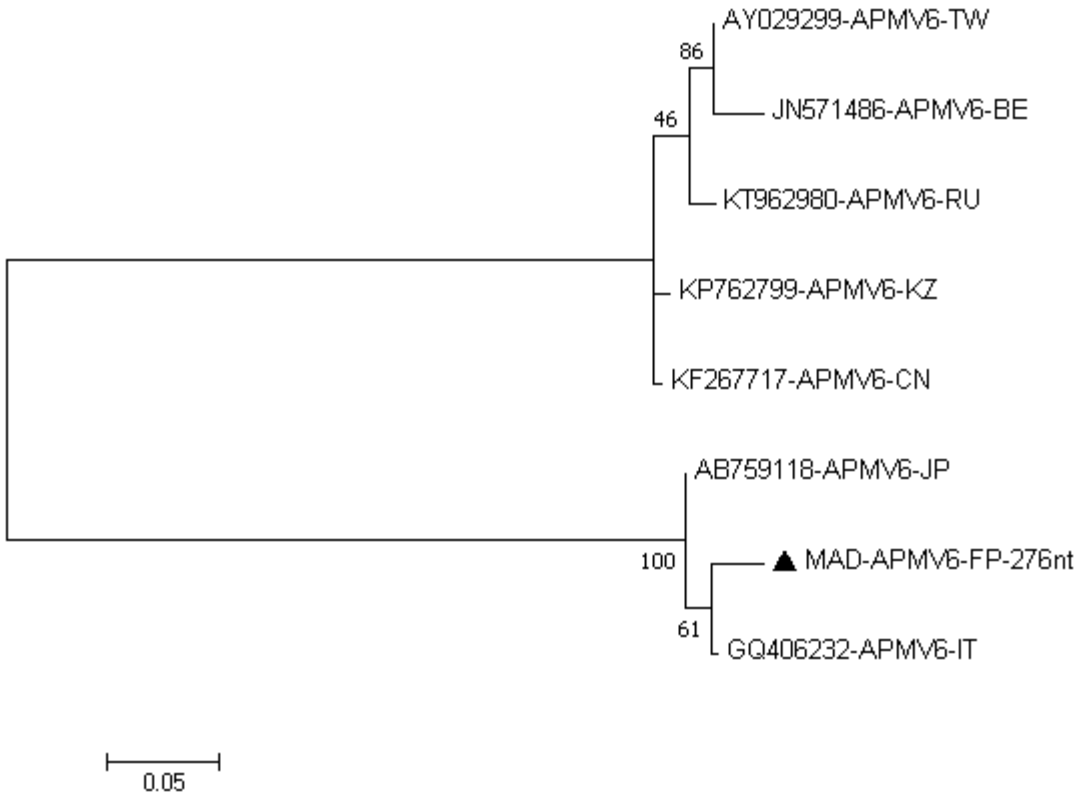
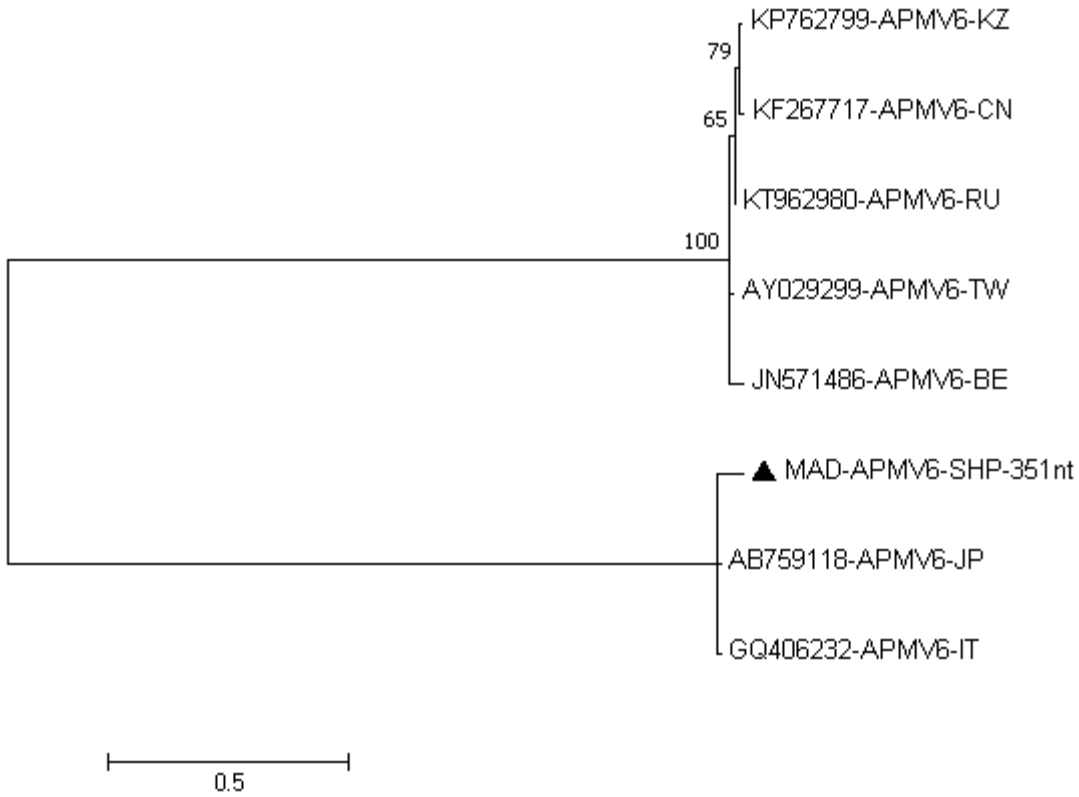


Figure S1.10. Molecular Phylogenetic analysis by Maximum Likelihood method of APMV6 partial FP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model[4]. The tree with the highest log likelihood (-744.95) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.4490)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 276 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S1.10. Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 276 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-APMV6-FP-276nt							
AB759118-APMV6-JP	9						
GQ406232-APMV6-IT	7	4					
KP762799-APMV6-KZ	63	61	63				
AY029299-APMV6-TW	63	61	63	9			
KT962980-APMV6-RU	61	59	61	9	6		
JN571486-APMV6-BE	66	62	66	15	6	12	
KF267717-APMV6-CN	63	61	63	3	8	8	14



FigureS 1.11. Molecular Phylogenetic analysis by Maximum Likelihood method of APMV6 partial SHP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-1186.85) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 33.12% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 344 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S1.11. Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 344 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-APMV6-SHP-351nt							
AB759118-APMV6-JP	19						
GQ406232-APMV6-IT	19	4					
KP762799-APMV6-KZ	166	155	155				
AY029299-APMV6-TW	164	153	153	10			
KT962980-APMV6-RU	166	155	155	5	5		
JN571486-APMV6-BE	167	156	156	15	11	12	
KF267717-APMV6-CN	165	154	154	5	11	6	15

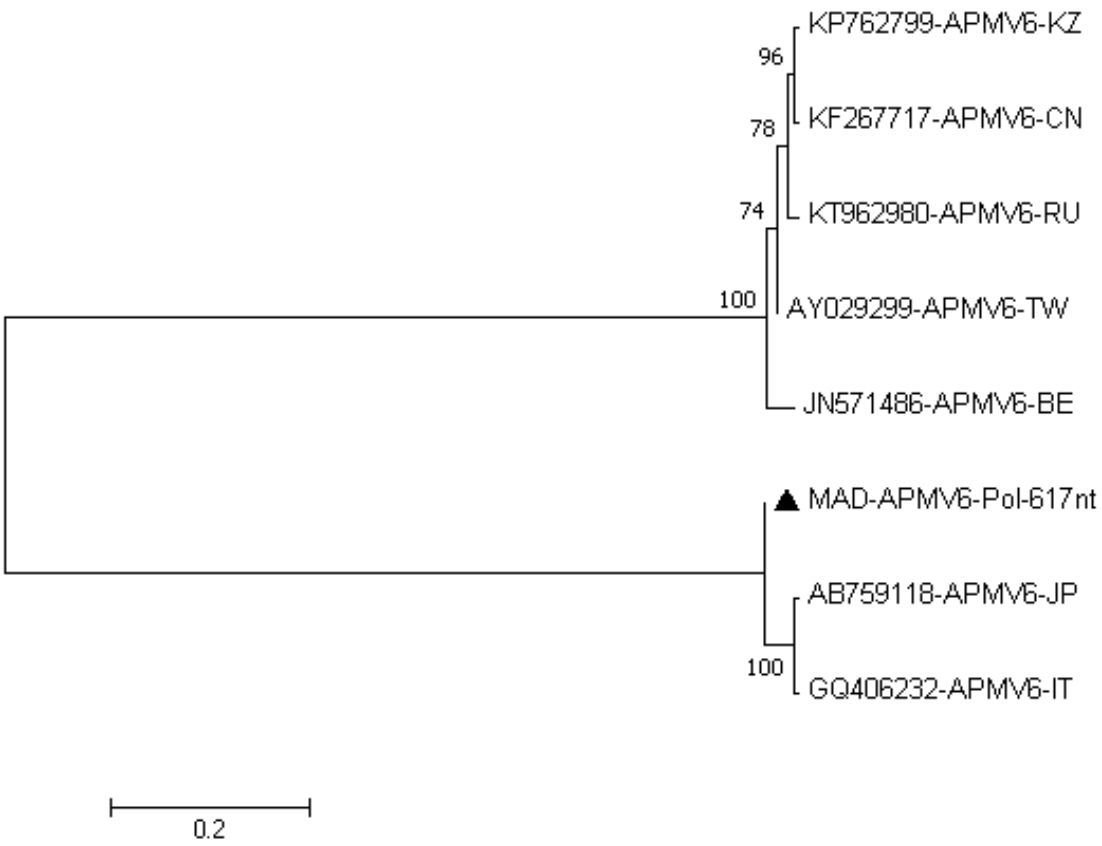


Figure S1.12: Molecular Phylogenetic analysis by Maximum Likelihood method of APMV6 partial Pol gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-1870.04) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 32.66% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 8 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 617 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S1.12: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 617 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-APMV6-Pol-617nt							
AB759118-APMV6-JP	20						
GQ406232-APMV6-IT	20	6					
KP762799-APMV6-KZ	188	188	190				
AY029299-APMV6-TW	193	191	193	14			
KT962980-APMV6-RU	190	188	190	14	14		
JN571486-APMV6-BE	193	193	193	33	23	33	
KF267717-APMV6-CN	188	188	190	6	14	14	33

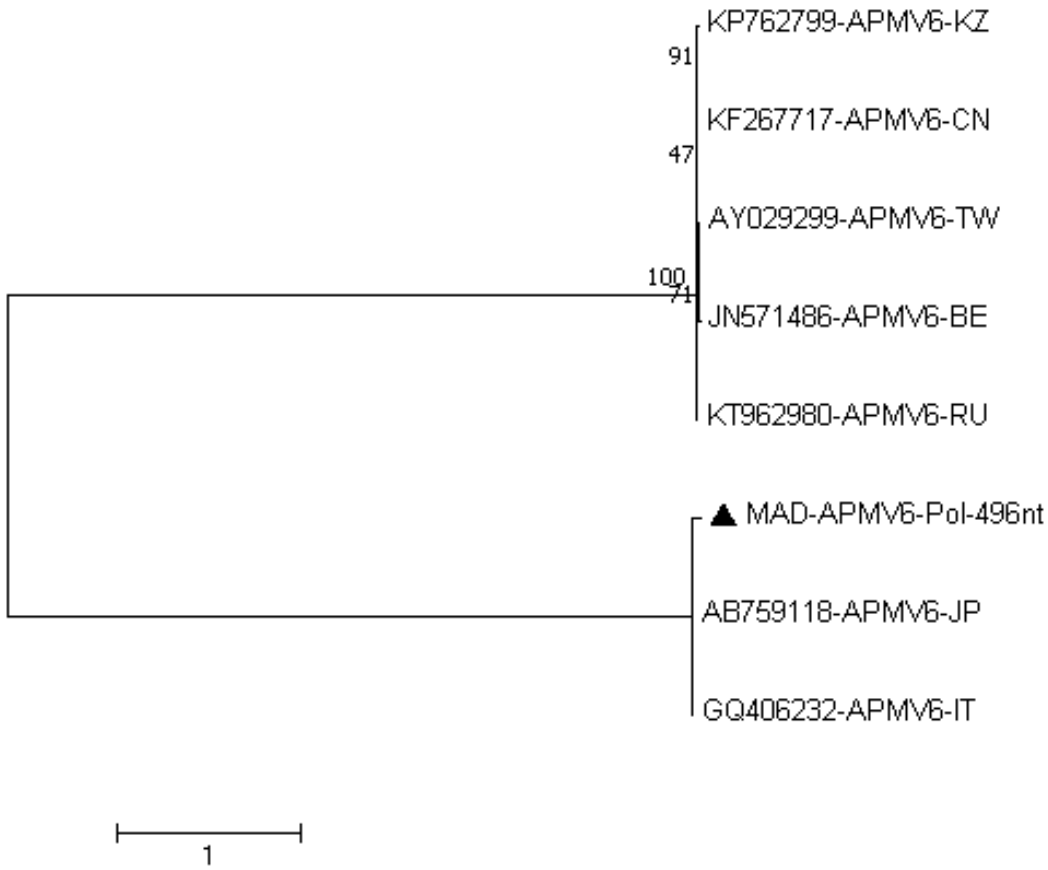


Figure S1.13: Molecular Phylogenetic analysis by Maximum Likelihood method of APMV6 partial Pol gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-1392.34) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 35.38% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 8 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 496 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S1.13: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 496 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-APMV6-Pol-496nt							
AB759118-APMV6-JP	22						
GQ406232-APMV6-IT	20	2					
KP762799-APMV6-KZ	131	129	127				
AY029299-APMV6-TW	131	129	127	8			
KT962980-APMV6-RU	129	127	125	7	7		
JN571486-APMV6-BE	134	132	130	13	7	10	
KF267717-APMV6-CN	131	129	127	2	6	5	11

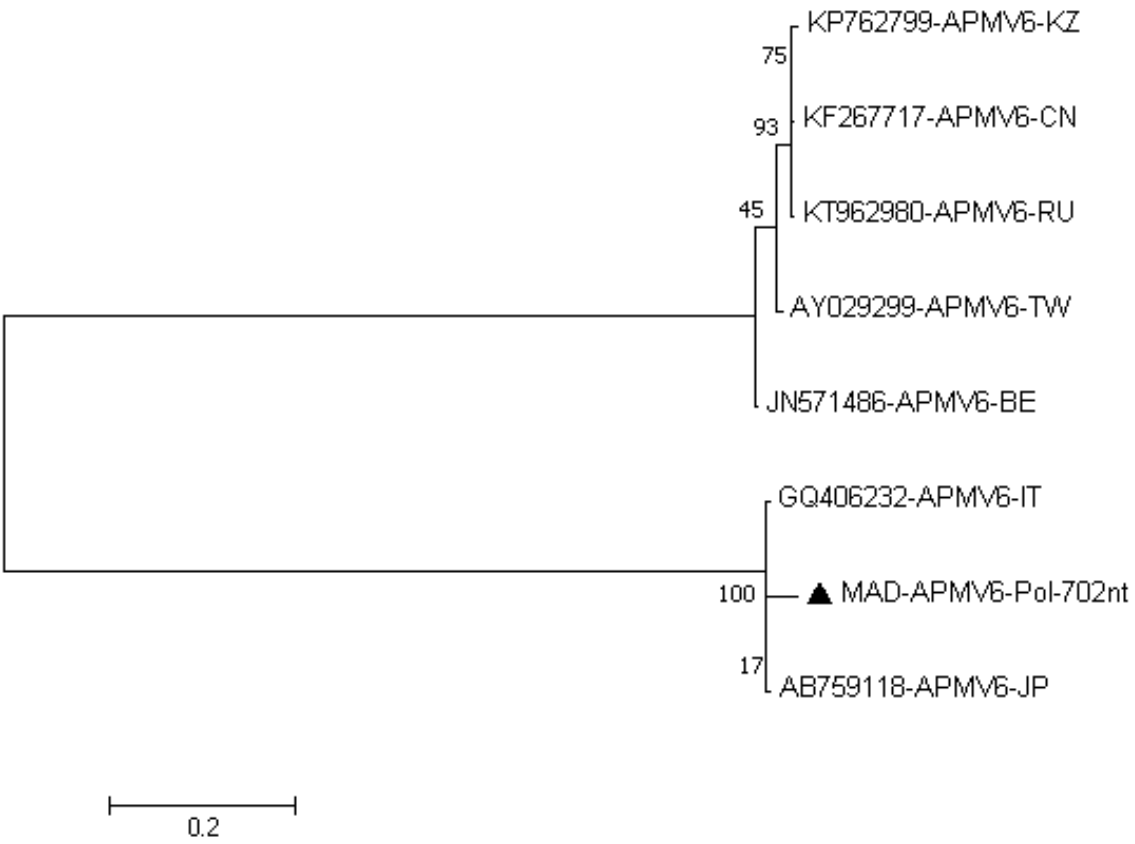


Figure S1.14: Molecular Phylogenetic analysis by Maximum Likelihood method of APMV6 partial Pol gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-1924.36) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.4751)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 8 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 702 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S1.14: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 702 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-APMV6-Pol-702nt							
AB759118-APMV6-JP	24						
GQ406232-APMV6-IT	24	6					
KP762799-APMV6-KZ	176	181	178				
AY029299-APMV6-TW	173	171	170	21			
KT962980-APMV6-RU	170	175	172	8	17		
JN571486-APMV6-BE	177	175	176	29	20	27	
KF267717-APMV6-CN	171	176	173	7	18	5	28

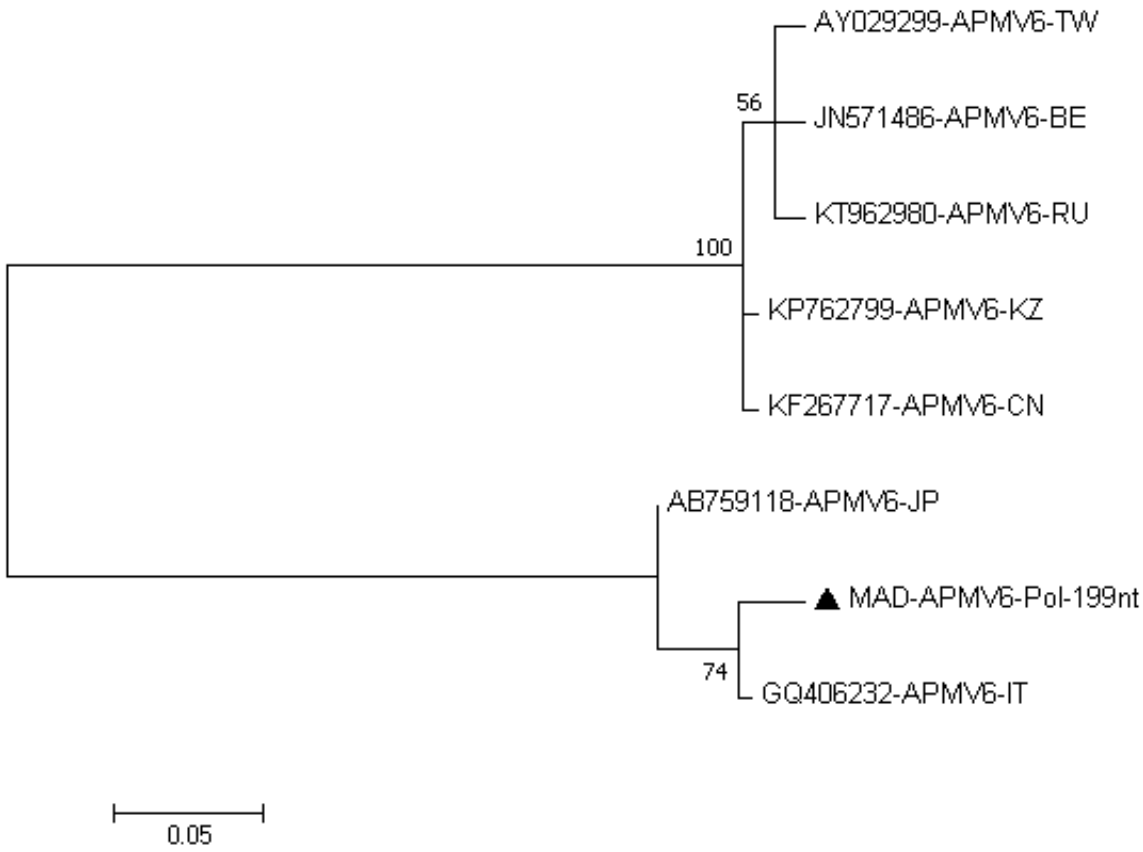


Figure S1.15: Molecular Phylogenetic analysis by Maximum Likelihood method of APMV6 partial Pol gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-572.97) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 36.68% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 8 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 199 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S1.15: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 199 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-APMV6-Pol-199nt							
AB759118-APMV6-JP	9						
GQ406232-APMV6-IT	5	6					
KP762799-APMV6-KZ	46	43	47				
AY029299-APMV6-TW	48	47	47	5			
KT962980-APMV6-RU	46	45	47	5	4		
JN571486-APMV6-BE	46	45	47	5	4	4	
KF267717-APMV6-CN	47	44	48	2	5	5	5

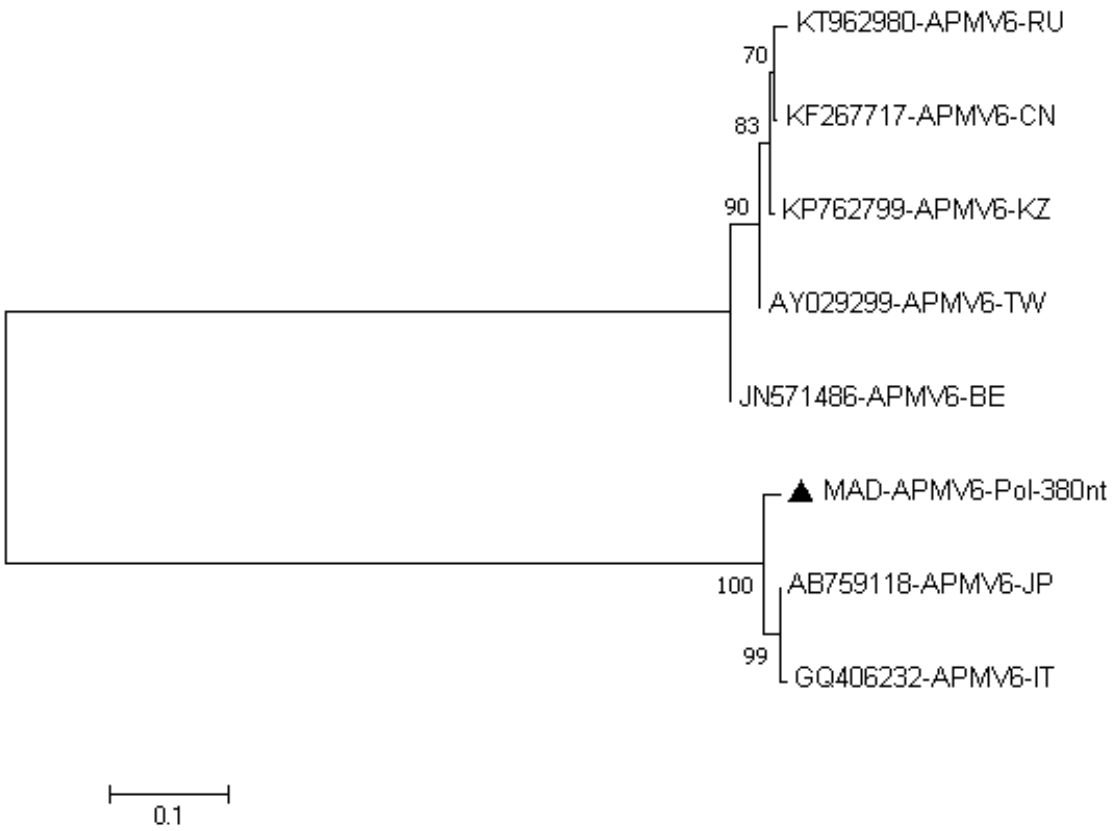


Figure S1.16: Molecular Phylogenetic analysis by Maximum Likelihood method of APMV6 partial Pol gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-1025.97) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 36.45% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 8 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 380 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S1.16: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 380 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-APMV6-Pol-380nt							
AB759118-APMV6-JP	9						
GQ406232-APMV6-IT	11	2					
KP762799-APMV6-KZ	89	91	92				
AY029299-APMV6-TW	91	92	93	4			
KT962980-APMV6-RU	93	95	96	6	8		
JN571486-APMV6-BE	88	89	90	13	9	15	
KF267717-APMV6-CN	92	94	95	3	5	5	14

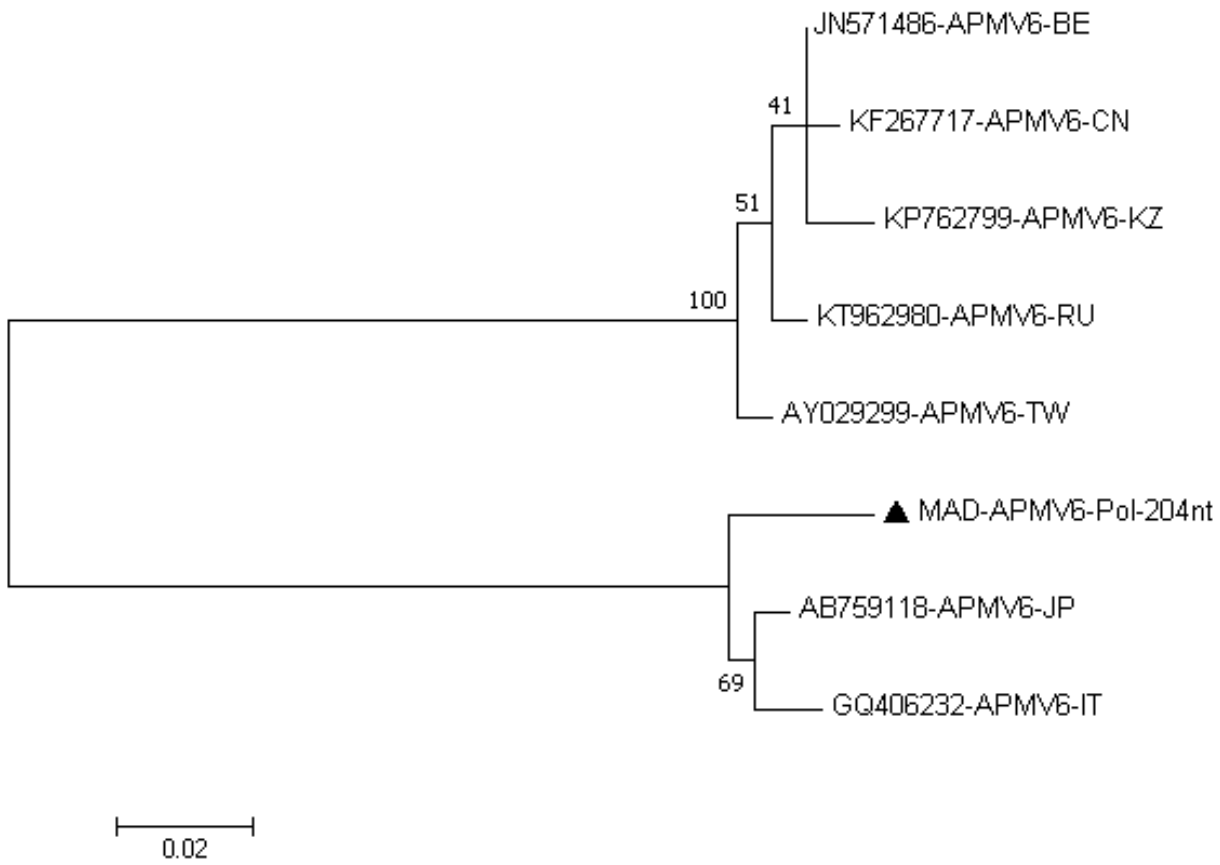


Figure S1.17: Molecular Phylogenetic analysis by Maximum Likelihood method of APMV6 partial Pol gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-509.35) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 8 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 204 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S1.17: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 204 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-APMV6-Pol-204nt							
AB759118-APMV6-JP	6						
GQ406232-APMV6-IT	7	3					
KP762799-APMV6-KZ	40	39	39				
AY029299-APMV6-TW	40	39	37	5			
KT962980-APMV6-RU	41	40	40	4	3		
JN571486-APMV6-BE	40	39	39	2	3	2	
KF267717-APMV6-CN	41	40	40	3	4	3	1

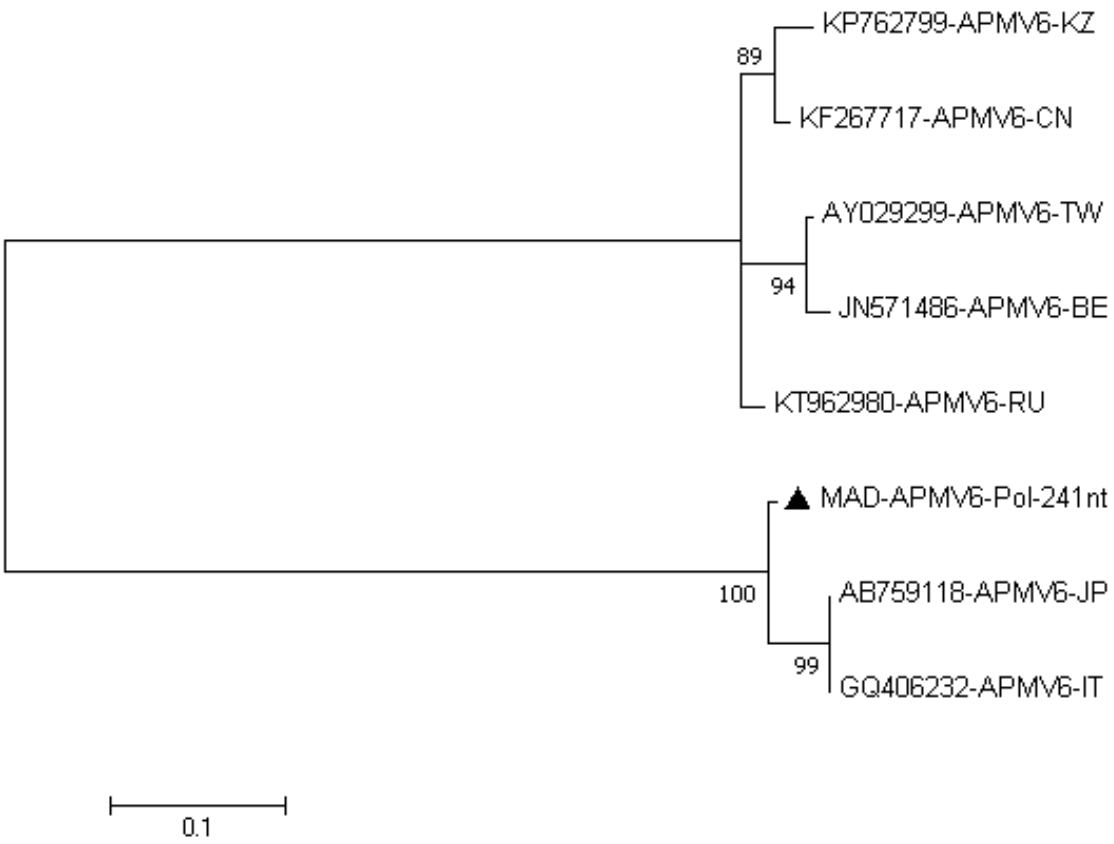


Figure S1.18: Molecular Phylogenetic analysis by Maximum Likelihood method of APMV6 partial Pol gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-720.37) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.5353)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 8 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 241 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S1.18: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 241 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-APMV6-Pol-241nt							
AB759118-APMV6-JP	9						
GQ406232-APMV6-IT	9	0					
KP762799-APMV6-KZ	64	63	63				
AY029299-APMV6-TW	66	66	66	15			
KT962980-APMV6-RU	61	61	61	12	12		
JN571486-APMV6-BE	64	64	64	17	4	14	
KF267717-APMV6-CN	65	64	64	7	15	9	17

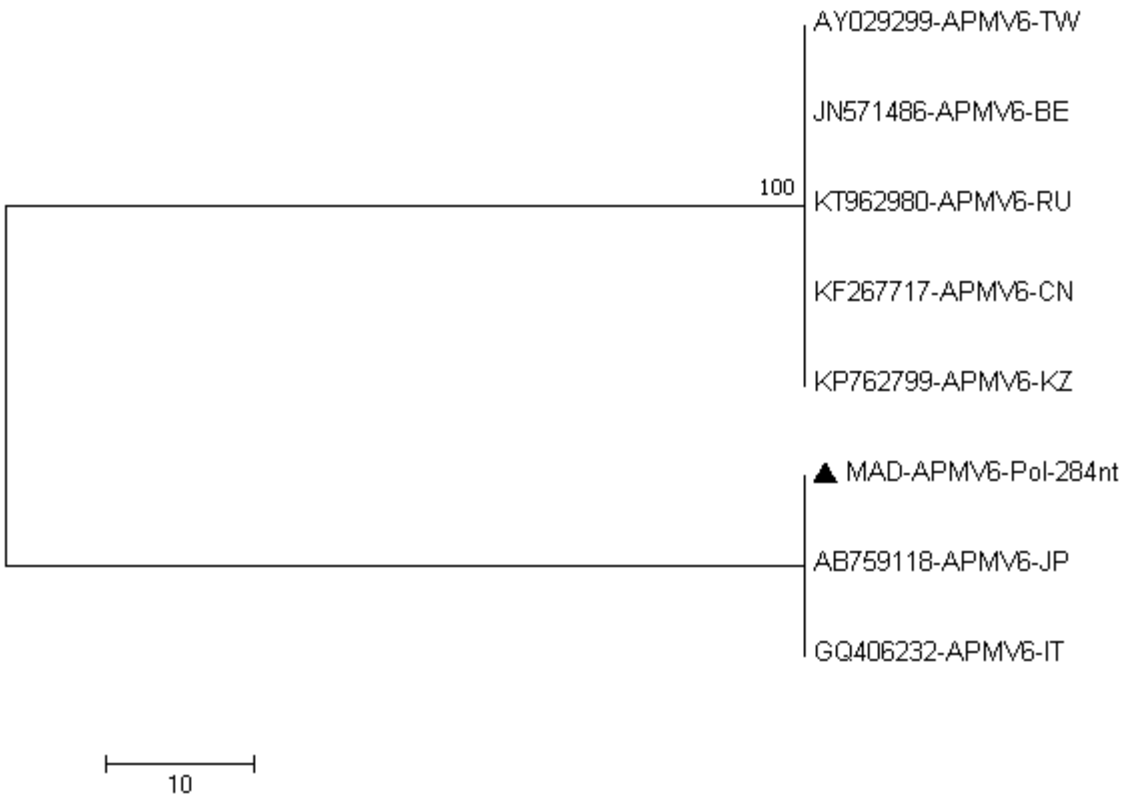


Figure S1.19: Molecular Phylogenetic analysis by Maximum Likelihood method of APMV6 partial Pol gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-816.02) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.5244)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 8 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 284 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S1.19: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 284 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-APMV6-Pol-284nt							
AB759118-APMV6-JP	3						
GQ406232-APMV6-IT	4	1					
KP762799-APMV6-KZ	77	75	75				
AY029299-APMV6-TW	81	79	79	6			
KT962980-APMV6-RU	80	78	78	5	5		
JN571486-APMV6-BE	78	78	78	7	5	6	
KF267717-APMV6-CN	79	77	77	2	6	5	7

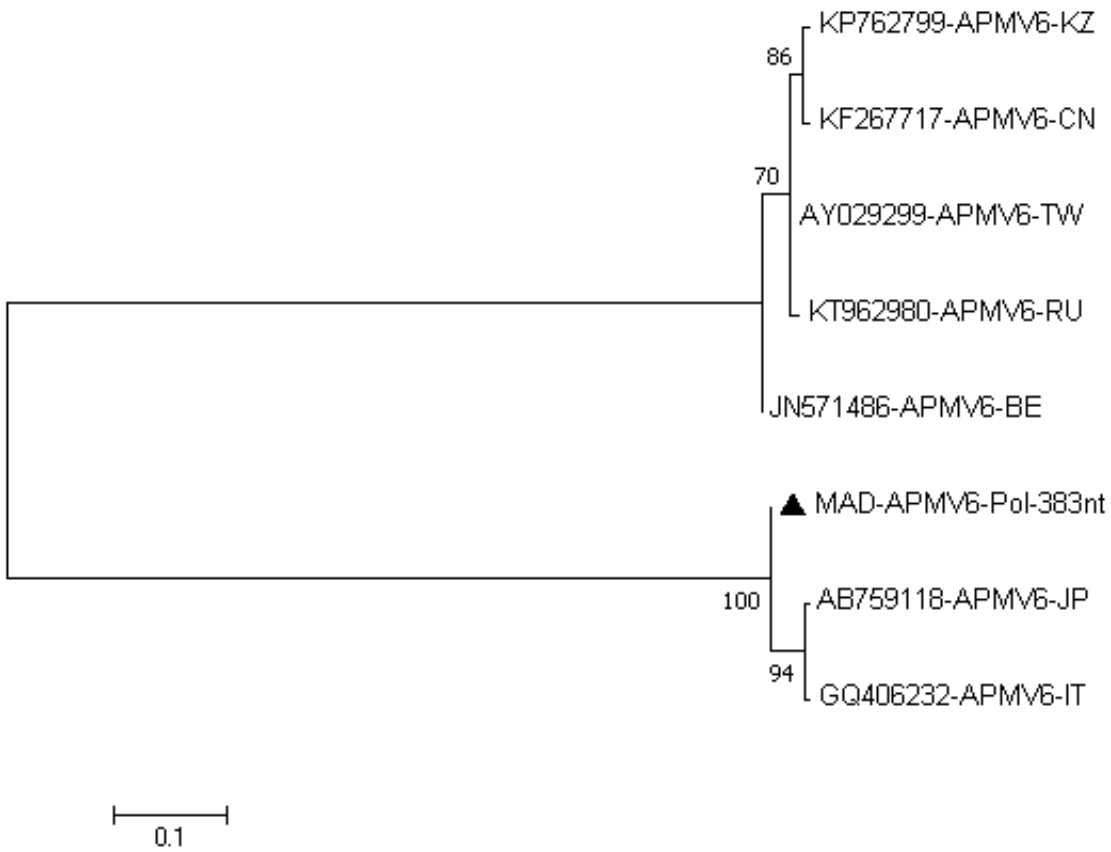


Figure S1.20: Molecular Phylogenetic analysis by Maximum Likelihood method of APMV6 partial Pol gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model[4]. The tree with the highest log likelihood (-1044.49) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 36.68% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 8 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 383 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S1.20: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 383 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-APMV6-Pol-383nt							
AB759118-APMV6-JP	12						
GQ406232-APMV6-IT	12	2					
KP762799-APMV6-KZ	90	88	88				
AY029299-APMV6-TW	89	87	87	6			
KT962980-APMV6-RU	90	88	88	9	3		
JN571486-APMV6-BE	91	89	89	13	9	12	
KF267717-APMV6-CN	91	89	89	4	6	9	15

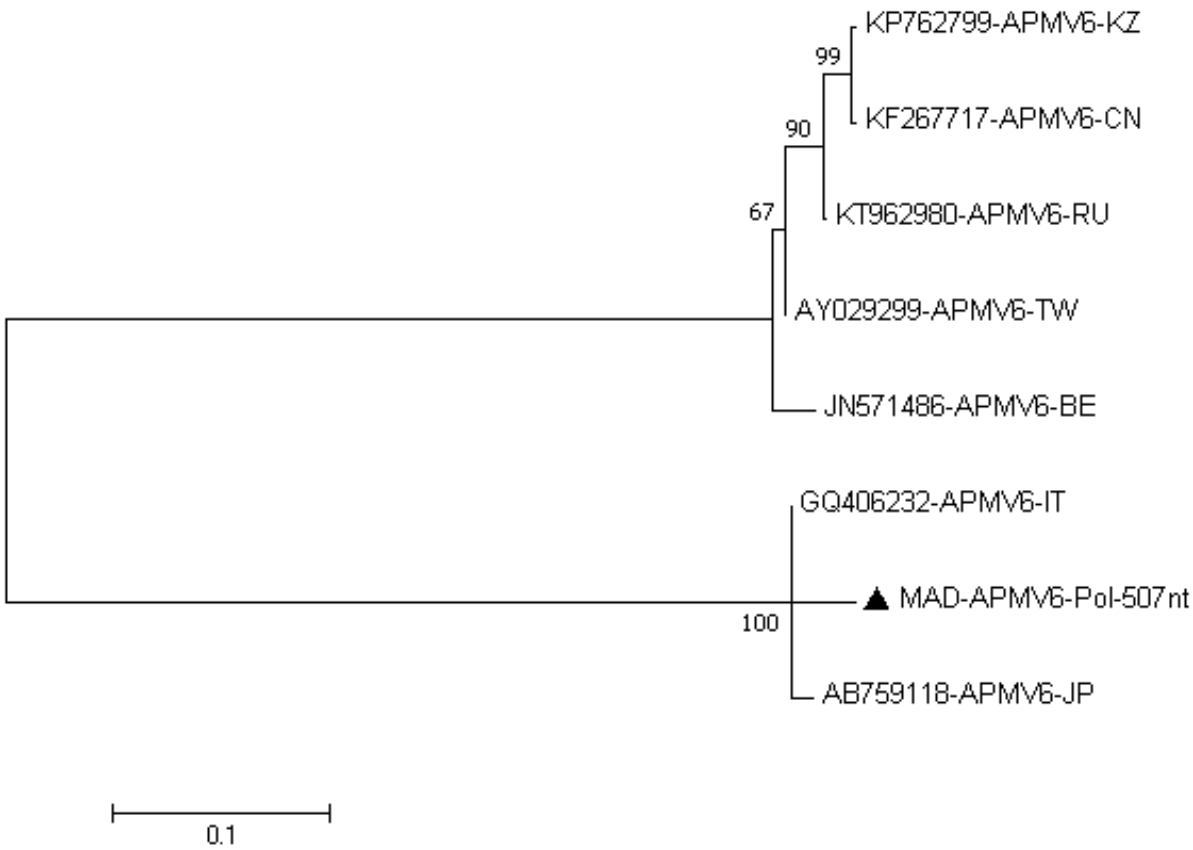


Figure S1.21: Molecular Phylogenetic analysis by Maximum Likelihood method of APMV6 partial Pol gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-1352.37) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.4413)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 8 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 507 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S1.21: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 507 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-APMV6-Pol-507nt							
AB759118-APMV6-JP	19						
GQ406232-APMV6-IT	14	5					
KP762799-APMV6-KZ	127	119	119				
AY029299-APMV6-TW	124	116	116	15			
KT962980-APMV6-RU	124	116	116	8	9		
JN571486-APMV6-BE	122	116	116	25	12	21	
KF267717-APMV6-CN	128	120	120	2	15	8	25

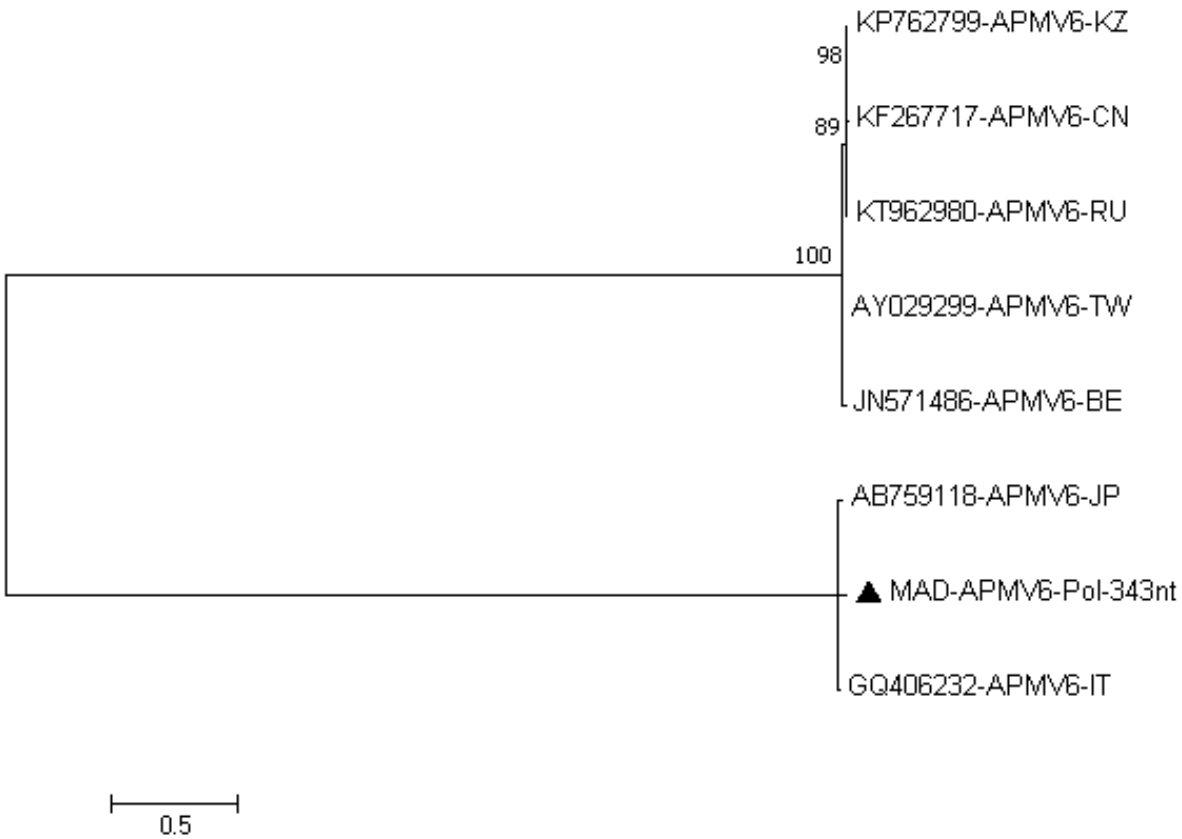


Figure S1.22: Molecular Phylogenetic analysis by Maximum Likelihood method of APMV6 partial Pol gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-947.92) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.5111)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 8 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 343 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S1.22: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 343 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-APMV6-Pol-343nt							
AB759118-APMV6-JP	14						
GQ406232-APMV6-IT	11	5					
KP762799-APMV6-KZ	94	94	93				
AY029299-APMV6-TW	90	90	89	6			
KT962980-APMV6-RU	92	92	91	4	6		
JN571486-APMV6-BE	91	91	90	10	4	10	
KF267717-APMV6-CN	95	95	94	1	7	5	11

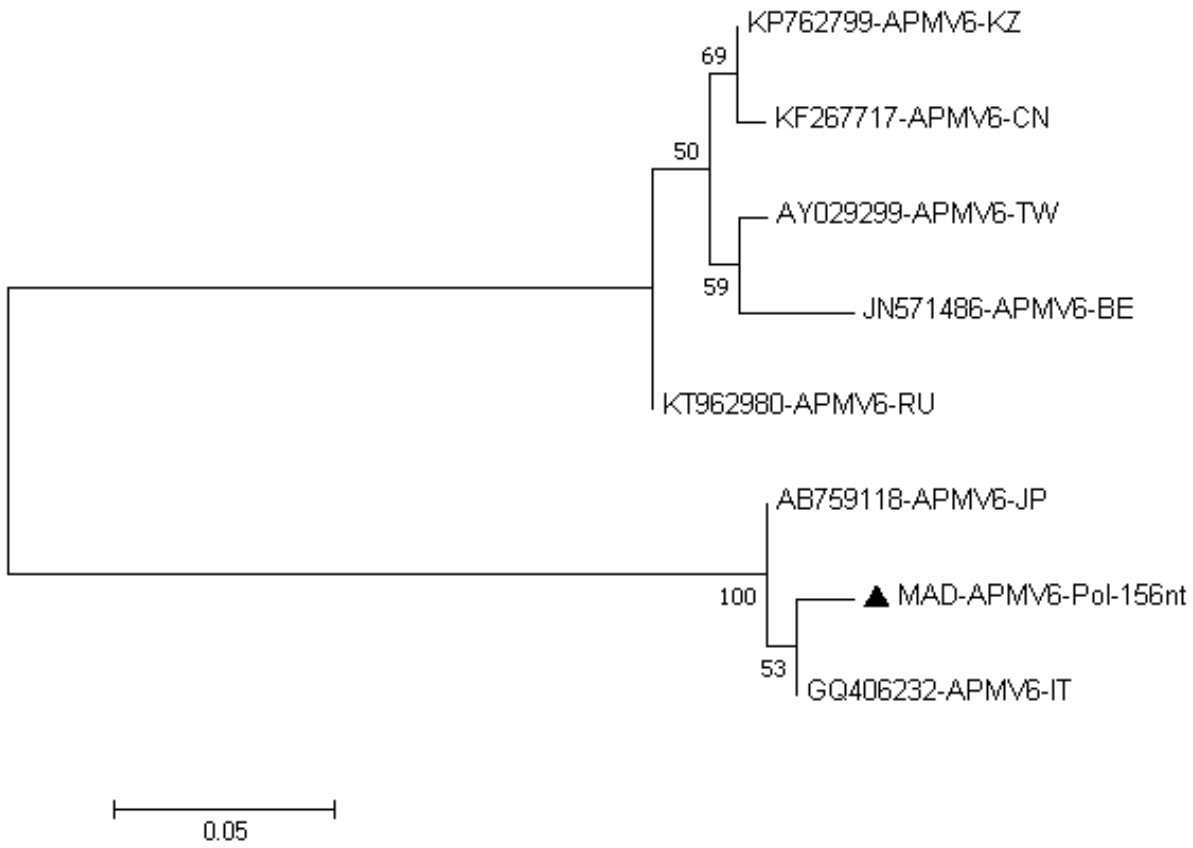


Figure S1.23: Molecular Phylogenetic analysis by Maximum Likelihood method of APMV6 partial Pol gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model[4]. The tree with the highest log likelihood (-402.62) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 8 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 156 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S1.23: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 156 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-APMV6-Pol-156nt							
AB759118-APMV6-JP	3						
GQ406232-APMV6-IT	2	1					
KP762799-APMV6-KZ	41	40	41				
AY029299-APMV6-TW	40	39	40	3			
KT962980-APMV6-RU	39	38	39	3	4		
JN571486-APMV6-BE	44	43	44	6	5	7	
KF267717-APMV6-CN	40	39	40	1	4	4	7

2. Gammacoronavirus from MAD faecal sample

Figure and Table	Long Name (Format: Sample-virus-protein-length-quality-coverage-year)	Short name (Format: Sample-virus-protein-length-year)	Coverage	No. of nucleotides	Mapping quality threshold	NCBI accession number
S2.1	MAD-Gammacoronavirus-NSP12-1071nt-Q32-C-4-65-2016	MAD-GCoV-NSP12-1071nt	4-65	1071	32	MG991677
S2.2	MAD-Gammacoronavirus-Spike-protein-524nt-Q32-C-3-19-2016	MAD-GCoV-SP-524nt	3-19	524	32	MG991678
S2.3	MAD-Gammacoronavirus-Spike-protein-469nt-Q32-C-3-19-2016	MAD-GCoV-SP-469nt	3-13	469	32	MG991679

NCBI sequences taken for phylogenetic analysis			
Long Name	Short Name (Format: NCBI accession number-virus-country/state-year)	Country of collection	Collection date
KM454473-Duck-coronavirus-isolate-DK/GD/27/2014-complete-genome	KM454473-DCoV-CN	China	2014
LN610099-Guinea-fowl-coronavirus-GfCoV/FR/2011-complete-genome	LN610099-GfCoV-FR	France	-
KR822424-European-turkey-coronavirus-080385d-complete-genome	KR822424-TCoV-FR	France	2008
EU022526-Turkey-coronavirus-isolate-TCoV-ATCC-complete-genome	EU022526-TCoV-US	United States of America	-
KT254279-Pigeon-dominant-Coronavirus-isolate-PdCoV/PG/Guangdong/1418/2014-NSP12-(1ab)-gene-partial-cds	KT254279-PdCoV-CN	China	2014
FN430415-Infectious-bronchitis-virus-NGA/A116E7/2006-complete-genome	FN430415-IBV-NG	Nigeria	2006
KF652223-Turkey-coronavirus-isolate-NC/20/09-spike-	KF652223-TCoV(P)-US	United States of America	2009

glycoprotein-(S)-gene-complete-cds			

The table points towards the molecular phylogenetic analysis by Maximum Likelihood method of GCoV. The first half of the table provides details to identify the sample from which the virus was isolated, protein that encodes the consensus sequences that are being analysed, the length of the consensus sequences thus providing the depth of the gene being analysed, minimum mapping quality threshold used in IGV for the generation of the consensus sequences, the coverage of the consensus sequences from IGV, the year of collection of the sample and the NCBI accession number assigned to the particular consensus sequence. Corresponding short names have been used in the phylogenetic trees which contain the sample, virus, protein and the number of nucleotides. The second half of the table provides the details of the sequences that were used for the molecular phylogenetic analysis. They were found to be the most closely related sequences to the consensus sequences generated using BLASTN. Corresponding short names have been used in the phylogenetic trees which contain the NCBI accession number, virus and the country of collection. The collection date is given as retrieved from the corresponding NCBI nucleotide reference dataset which together with the country of collection can provide an insight into the possible evolution of the virus through the years.

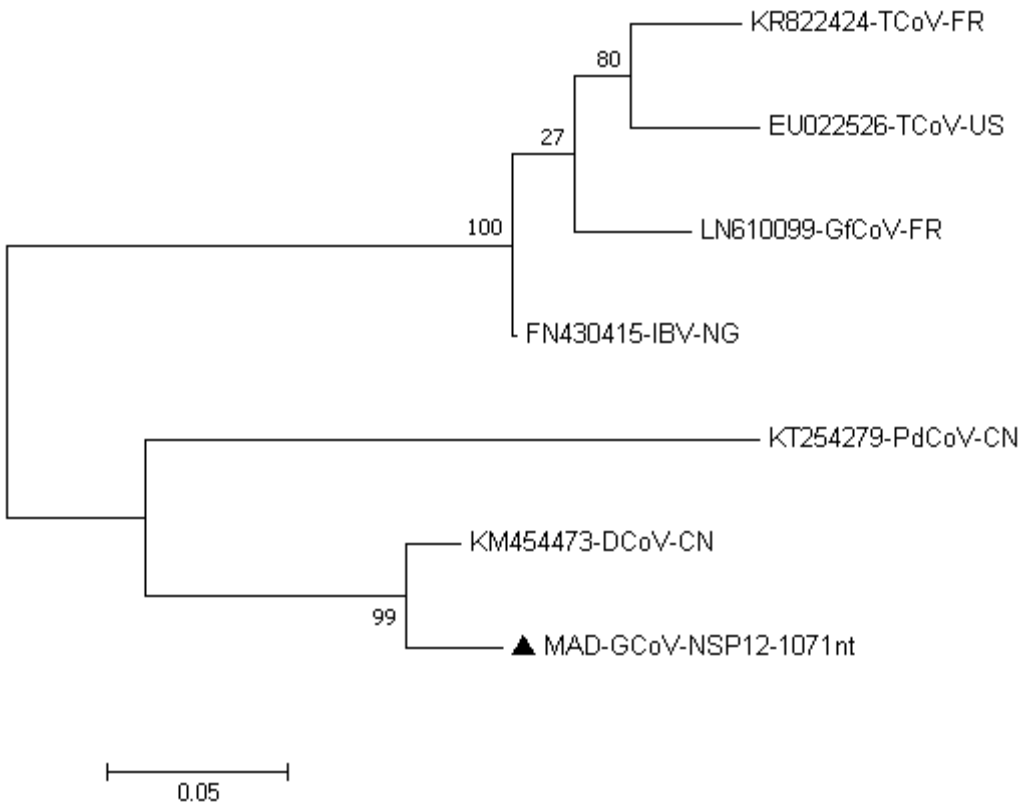


Figure S2.1. Molecular Phylogenetic analysis by Maximum Likelihood method of GCoV partial OLPP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model[4]. The tree with the highest log likelihood (-3293.18) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.2729)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 7 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 1071 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S2.1. Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 7 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 1071 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

KM454473-DCoV-CN						
LN610099-GfCoV-FR	163					
KR822424-TCoV-FR	177	67				
EU022526-TCoV-US	178	72	59			
KT254279-PdCoV-CN	152	186	170	187		
FN430415-IBV-NG	158	47	58	62	181	
MAD-GCoV-NSP12-1071nt	41	167	185	186	154	166

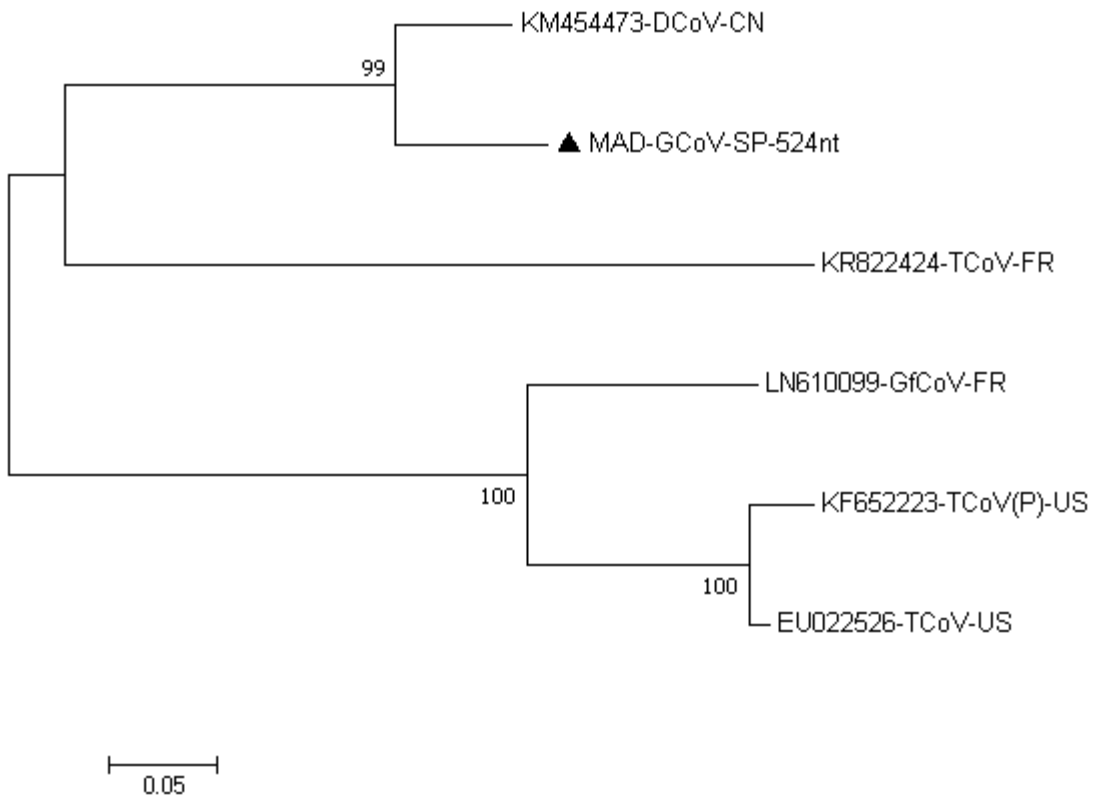


Figure S2.2: Molecular Phylogenetic analysis by Maximum Likelihood method of GCoV partial SP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model[5]. The tree with the highest log likelihood (-2146.14) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.6568)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 6 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 524 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S2.2: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 6 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 524 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

KM454473-DCoV-CN					
LN610099-GfCoV-FR	143				
KR822424-TCoV-FR	149	172			
KF652223-TCoV(P)-US	160	88	167		
EU022526-TCoV-US	153	83	160	19	
MAD-GCoV-SP-524nt	54	143	151	159	156

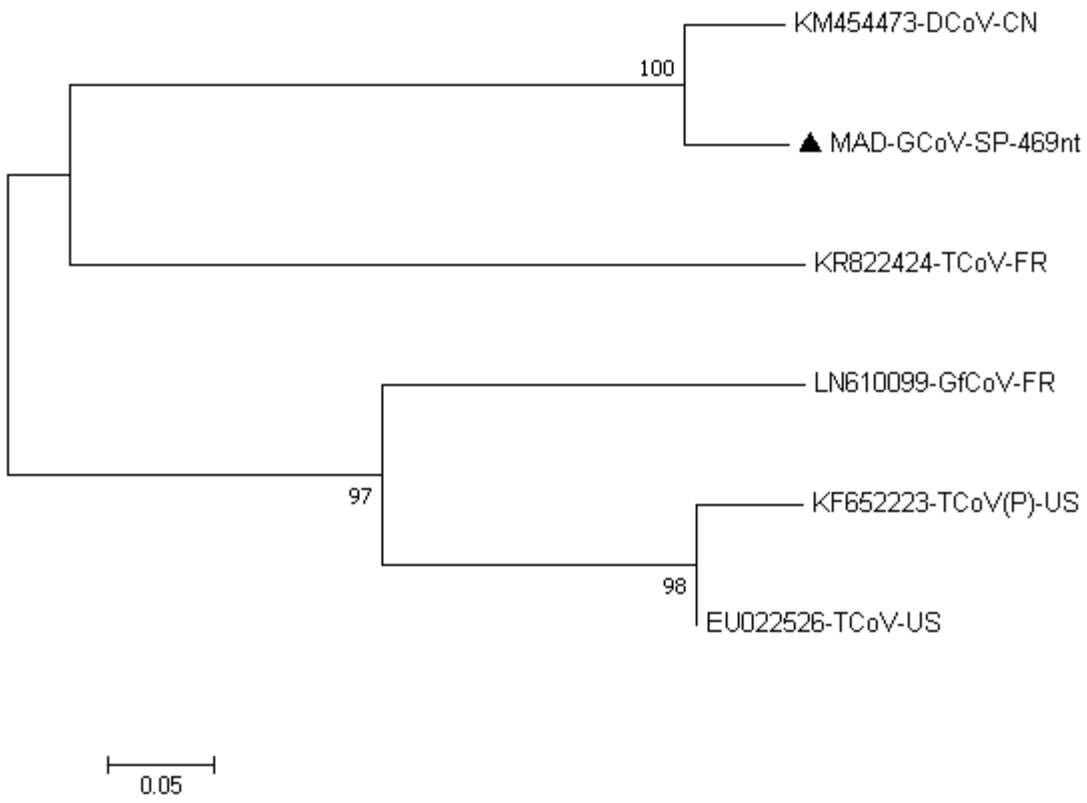


Figure S2.3: Molecular Phylogenetic analysis by Maximum Likelihood method of GCoV partial SP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model[4]. The tree with the highest log likelihood (-1893.27) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.5370)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 6 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 469 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S2.3: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. Standard error estimate(s) are shown above the diagonal. The analysis involved 6 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 469 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

KM454473-DCoV-CN					
LN610099-GfCoV-FR	126				
KR822424-TCoV-FR	127	129			
KF652223-TCoV(P)-US	132	101	126		
EU022526-TCoV-US	127	93	125	21	
MAD-GCoV-SP-469nt	38	130	131	133	126

3. Deltacoronavirus from MAD faecal sample

Figure and Table	Long Name (Format: Sample-virus-protein-length-quality-coverage-year)	Short name (Format: Sample-virus-protein-length-year)	Coverage	No. of nucleotides	Mapping quality threshold	NCBI accession number
S3.1	MAD-Deltacoronavirus-spike-glycoprotein-201nt-Q20-C-3-8-2016	MAD-DCoV-SP-201nt	3-8	201	20	Part of MH013337
S3.2	MAD-Deltacoronavirus-spike-glycoprotein-201nt-Q20-C-3-8-2016	MAD-DCoV-SP-aa-201nt	3-8	201	20	Part of MH013337
S3.3	MAD-Deltacoronavirus-replicase-polyprotein-205nt-Q20-C-3-30-2016	MAD-DCoV-RPP-205nt	3-30	205	20	Part of MH013331
S3.4	MAD-Deltacoronavirus-replicase-polyprotein-117nt-Q20-C-4-21-2016	MAD-DCoV-RPP-117nt	4-21	117	20	Part of MH013335
S3.5	MAD-Deltacoronavirus-spike-glycoprotein-1131nt-Q20-C-290-1700-2016	MAD-DCoV-SP-1131nt	290-1700	1131	20	Part of MH013337
S3.6	MAD-Deltacoronavirus-spike-glycoprotein-1131nt-Q20-C-290-1700-2016	MAD-DCoV-SP-aa-1131nt	290-1700	1131	20	Part of MH013337
S3.7	MAD-Deltacoronavirus-replicase-polyprotein-2121nt-Q20-C-39-1266-2016	MAD-DCoV-RPP-2121nt	39-1266	2121	20	Part of MH013332
S3.8	MAD-Deltacoronavirus-replicase-polyprotein-2121nt-Q20-C-39-1266-2016	MAD-DCoV-RPP-aa-2121nt	39-1266	2121	20	Part of MH013332
S3.9	MAD-Deltacoronavirus-envelope-protein-261nt-Q20-C-36-6274-2016	MAD-DCoV-EP-261nt	36-6274	261	20	MH013336

S3.10	MAD-Deltacoronavirus-envelope-protein-261nt-Q20-C-36-6274-2016	MAD-DCoV-EP-aa-261nt	36-6274	261	20	MH013336
S3.11	MAD-Deltacoronavirus-membrane-protein-654nt-Q20-C-36-6274-2016	MAD-DCoV-MP-654nt	36-6274	654	20	MH013338
S3.12	MAD-Deltacoronavirus-membrane-protein-654nt-Q20-C-36-6274-2016	MAD-DCoV-MP-aa-654nt	36-6274	654	20	MH013338
S3.13	MAD-Deltacoronavirus-orf1bstart-1095nt-Q20-C-5-336-2016	MAD-DCoV-RPP-1095nt	5-336	1095	20	MH013333
S3.14	MAD-Deltacoronavirus-orf1bstart-1095nt-Q20-C-5-336-2016	MAD-DCoV-RPP-aa-1095nt	5-336	1095	20	MH013333
S3.15	MAD-Deltacoronavirus-NS6-protein-276nt-Q20-C-36-6274-2016	MAD-DCoV-NS6-276nt	36-6274	276	20	MH013339
S3.16	MAD-Deltacoronavirus-NS6-protein-276nt-Q20-C-36-6274-2016	MAD-DCoV-NS6-aa-276nt	36-6274	276	20	MH013339
S3.17	MAD-Deltacoronavirus-Orf1bNSP11-13-2619nt-Q20-C-7-1923-2016	MAD-DCoV-RPP-2619nt	7-1923	2619	20	MH013334
S3.18	MAD-Deltacoronavirus-Orf1bNSP11-13-2619nt-Q20-C-7-1923-2016	MAD-DCoV-RPP-aa-2619nt	7-1923	2619	20	MH013334
S3.19	MAD-Deltacoronavirus-Orf1bNSP11-1914nt-Q20-C-14-290-2016	MAD-DCoV-RPP-1914nt	14-290	1914	20	MH013335
S3.20	MAD-Deltacoronavirus-	MAD-DCoV-RPP-aa-1914nt	14-290	1914	20	MH013335

	Orf1bNSP11-1914nt-Q20-C-14-290-2016					
S3.21	MAD-Deltacoronavirus-Orf1a-10650nt-Q20-C-6-2326-2016	MAD-DCoV-Orf1a-aa-10650nt	6-2326	10650	20	MH013332
S3.22	MAD-Deltacoronavirus-Orf1b-polymerase-2076nt-Q20-C-5-475-2016	MAD-DCoV-RPP-aa-2076nt	5-475	2076	20	MH013331
S3.23	MAD-Deltacoronavirus-spike-glycoprotein-3702nt-Q20-C-36-6274-2016	MAD-DCoV-SP-aa-3702nt	36-6274	3702	20	MH013337

NCBI sequences taken for phylogenetic analysis			
Long Name	Short Name (Format: NCBI accession number-virus-country/state-year)	Country of collection	Collection date
JQ065049-Common-moorhen coronavirus-HKU21-strain-HKU21-8295-complete-genome	JQ065049-MCoV-CN	China	2007
JQ065046-Magpie-robin-coronavirus HKU18-strain-HKU18-chu3-complete-genome	JQ065046-MrCoV-CN	China	2007
FJ376622-Munia-coronavirus-HKU13-3514-complete-genome	FJ376622-MuCoV-CN	China	2007
FJ376621-Thrush-coronavirus-HKU12-600-complete-genome	FJ376621-TCoV-CN	China	2007
MF431743-Porcine-deltacoronavirus-strain-SD-complete-genome	MF431743-PDCoV-CN	China	2014
FJ376620-Bulbul-coronavirus-HKU11-796-complete-genome	FJ376620-BCoV-CN	China	2007
JQ065047-Night-heron-coronavirus-HKU19-strain-HKU19-6918-complete genome	JQ065047-NhCoV-CN	China	2007
JQ065048-Wigeon-coronavirus-HKU20-strain-HKU20-9243-complete-genome	JQ065048-WCoV-CN	China	2008

The table points towards the molecular phylogenetic analysis by Maximum Likelihood method of DCoV. The first half of the table provides details to identify the sample from which the virus was isolated, protein that encodes the consensus sequences that are being analysed, the length of the consensus sequences thus providing the depth of the gene being analysed, minimum mapping

quality threshold used in IGV for the generation of the consensus sequences, the coverage of the consensus sequences from IGV, the year of collection of the sample and the NCBI accession number assigned to the particular consensus sequence. Corresponding short names have been used in the phylogenetic trees which contain the sample, virus, protein and the number of nucleotides. In case of the amino acid sequence analysis, the short names contain the “aa” after the protein. The second half of the table provides the details of the sequences that were used for the molecular phylogenetic analysis. They were found to be the most closely related sequences to the consensus sequences generated using BLASTN. Corresponding short names have been used in the phylogenetic trees which contain the NCBI accession number, virus and the country of collection. The collection date is given as retrieved from the corresponding NCBI nucleotide reference dataset which together with the country of collection can provide an insight into the possible evolution of the virus through the years.

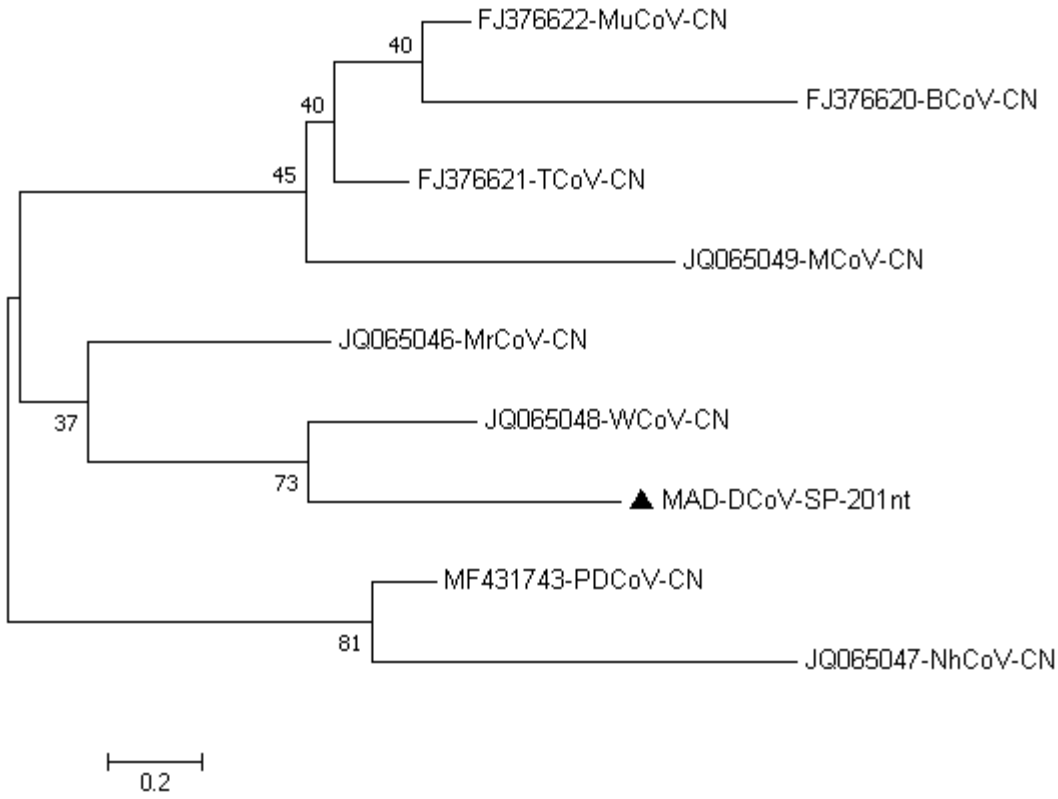


Figure S3.1: Molecular Phylogenetic analysis by Maximum Likelihood method of DCoV partial SP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model[4]. The tree with the highest log likelihood (-1292.92) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.6091)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 201 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S3.1: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 201 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

JQ065048-WCoV-CN								
JQ065049-MCoV-CN	49							
JQ065046-MrCoV-CN	52	59						
FJ376622-MuCoV-CN	48	48	54					
FJ376621-TCoV-CN	50	41	57	32				
MF431743-PDCoV-CN	57	58	55	57	56			
FJ376620-BCoV-CN	55	53	60	40	45	56		
JQ065047-NhCoV-CN	63	59	53	54	49	45	56	
MAD-DCoV-SP-201nt	37	52	53	58	58	59	58	61

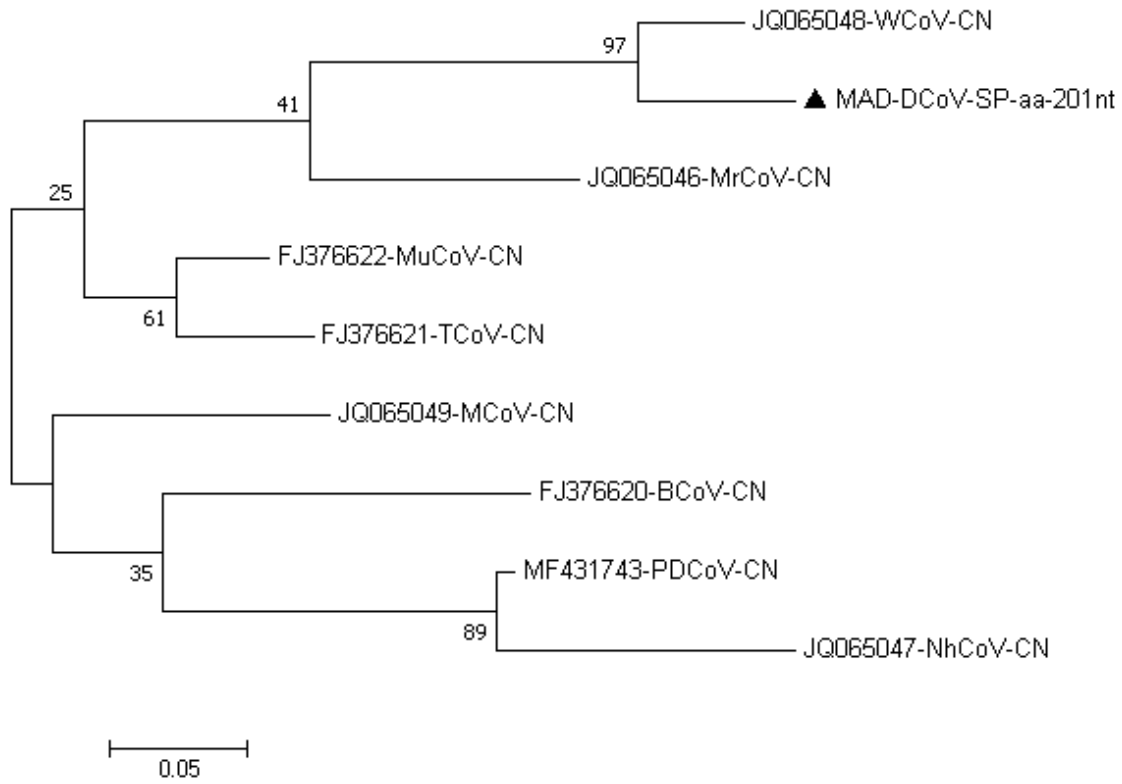


Figure S3.2. Molecular Phylogenetic analysis by Maximum Likelihood method for amino acid sequences of DCoV partial SP gene

The evolutionary history was inferred by using the Maximum Likelihood method based on the Le_Gascuel_2008 model[6]. The tree with the highest log likelihood (-501.04) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+*I*], 60.17% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 9 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 67 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S3.2. Estimates of Evolutionary Divergence between Sequences

The number of amino acid differences per sequence from between sequences are shown. The analysis involved 9 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 67 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

JQ065048-WCoV-CN								
JQ065049-MCoV-CN	11							
JQ065046-MrCoV-CN	12	15						
FJ376622-MuCoV-CN	13	11	9					
FJ376621-TCoV-CN	12	12	11	5				
MF431743-PDCoV-CN	17	11	13	11	12			
FJ376620-BCoV-CN	17	14	15	10	13	12		
JQ065047-NhCoV-CN	17	15	14	13	11	6	15	
MAD-DCoV-SP-aa-201nt	5	13	12	13	13	16	16	17

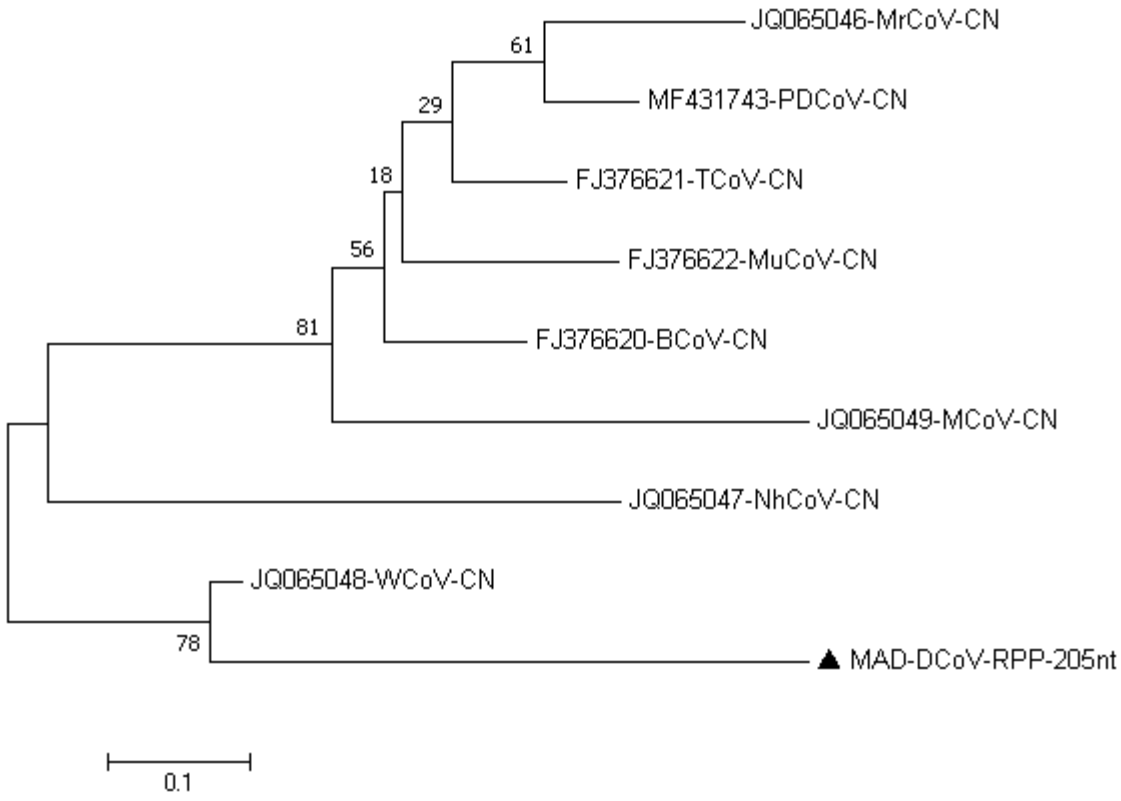


Figure S3.3: Molecular Phylogenetic analysis by Maximum Likelihood method of DCoV partial RPP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model[4]. The tree with the highest log likelihood (-1144.72) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.4466)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 205 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S3.3: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 205 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

JQ065049-MCoV-CN								
JQ065046-MrCoV-CN	48							
FJ376622-MuCoV-CN	43	35						
FJ376621-TCoV-CN	37	35	34					
MF431743-PDCoV-CN	47	29	33	29				
FJ376620-BCoV-CN	43	36	36	28	32			
JQ065047-NhCoV-CN	53	60	55	50	52	48		
JQ065048-WCoV-CN	48	58	46	46	53	43	45	
MAD-DCoV-RPP-205nt	46	59	50	51	58	53	52	39

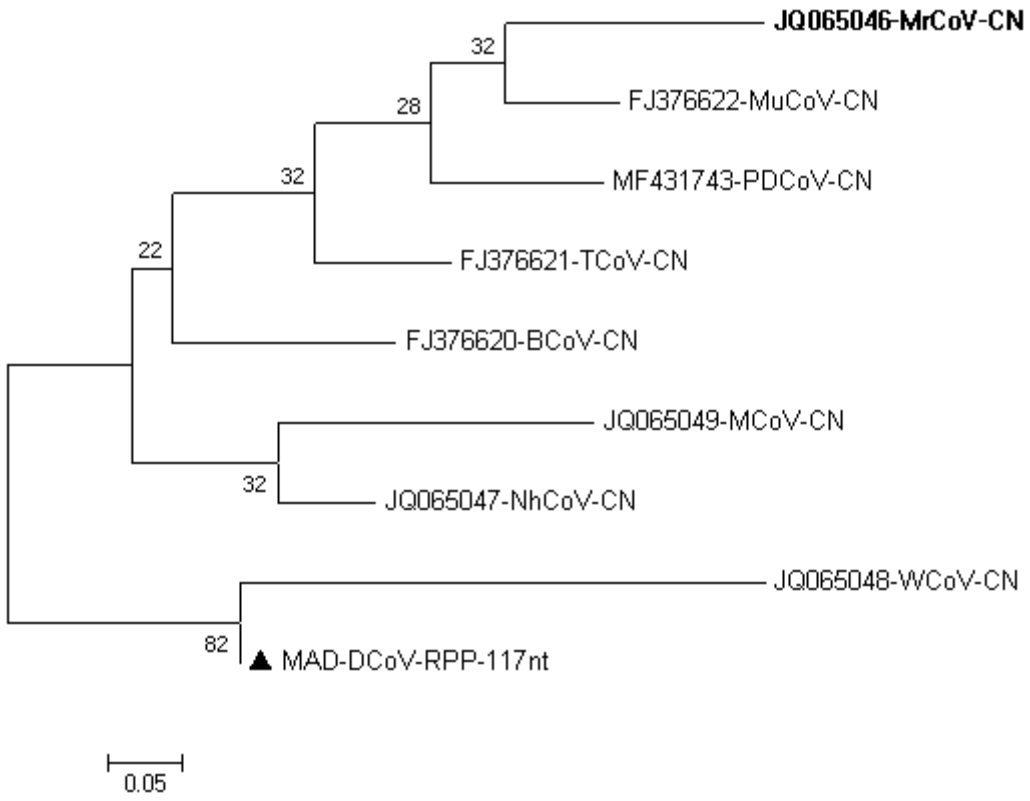


Figure S3.4: Molecular Phylogenetic analysis by Maximum Likelihood method of DCoV partial RPP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model[4]. The tree with the highest log likelihood (-653.38) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.4247)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 117 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S3.4: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 117 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

JQ065049-MCoV-CN								
JQ065046-MrCoV-CN	28							
FJ376622-MuCoV-CN	27	20						
FJ376621-TCoV-CN	21	22	21					
MF431743-PDCoV-CN	22	23	18	20				
FJ376620-BCoV-CN	27	23	22	22	27			
JQ065047-NhCoV-CN	20	28	24	26	21	22		
JQ065048-WCoV-CN	30	32	33	32	32	30	26	
MAD-DCoV-RPP-117nt	25	28	26	25	28	24	23	21

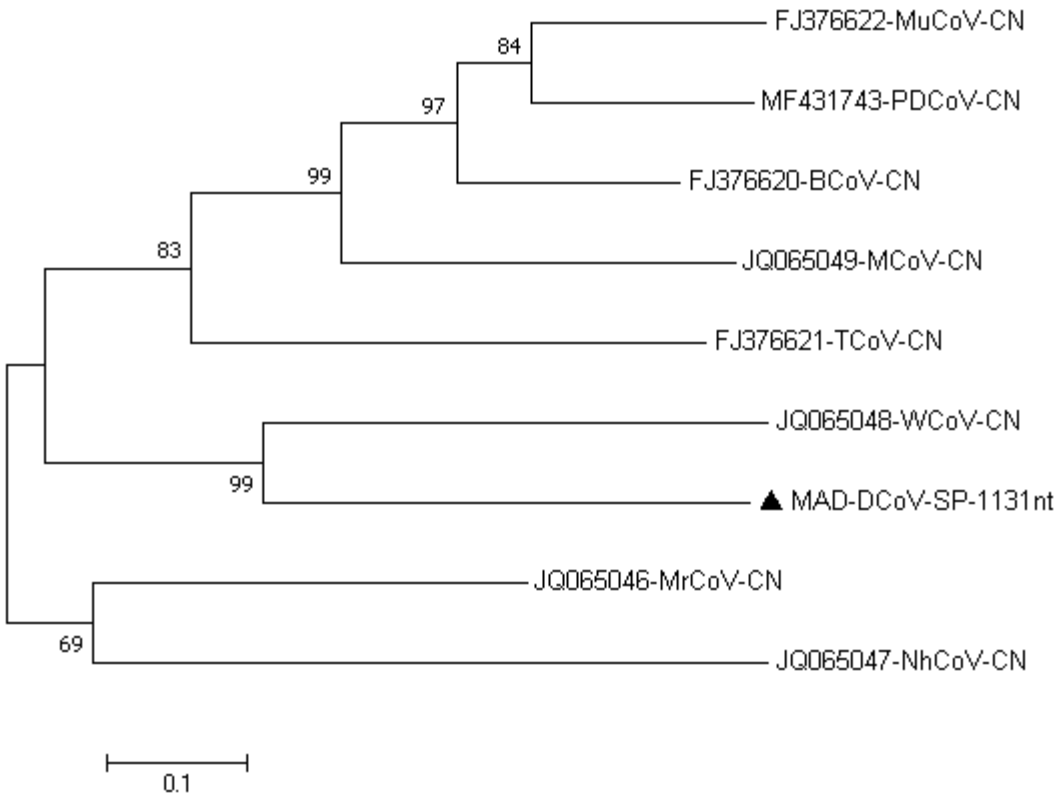


Figure S3.5: Molecular Phylogenetic analysis by Maximum Likelihood method of DCoV partial SP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model[4]. The tree with the highest log likelihood (-8892.42) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 1.0627)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 26.08% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 1125 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S3.5: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 1125 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

JQ065049-MCoV-CN								
JQ065046-MrCoV-CN	421							
FJ376622-MuCoV-CN	347	405						
FJ376621-TCoV-CN	389	413	388					
MF431743-PDCoV-CN	354	417	252	377				
FJ376620-BCoV-CN	308	423	274	390	261			
JQ065047-NhCoV-CN	436	408	443	416	421	423		
JQ065048-WCoV-CN	421	424	420	439	413	436	456	
MAD-DCoV-SP-1131nt	425	421	428	432	428	426	436	393

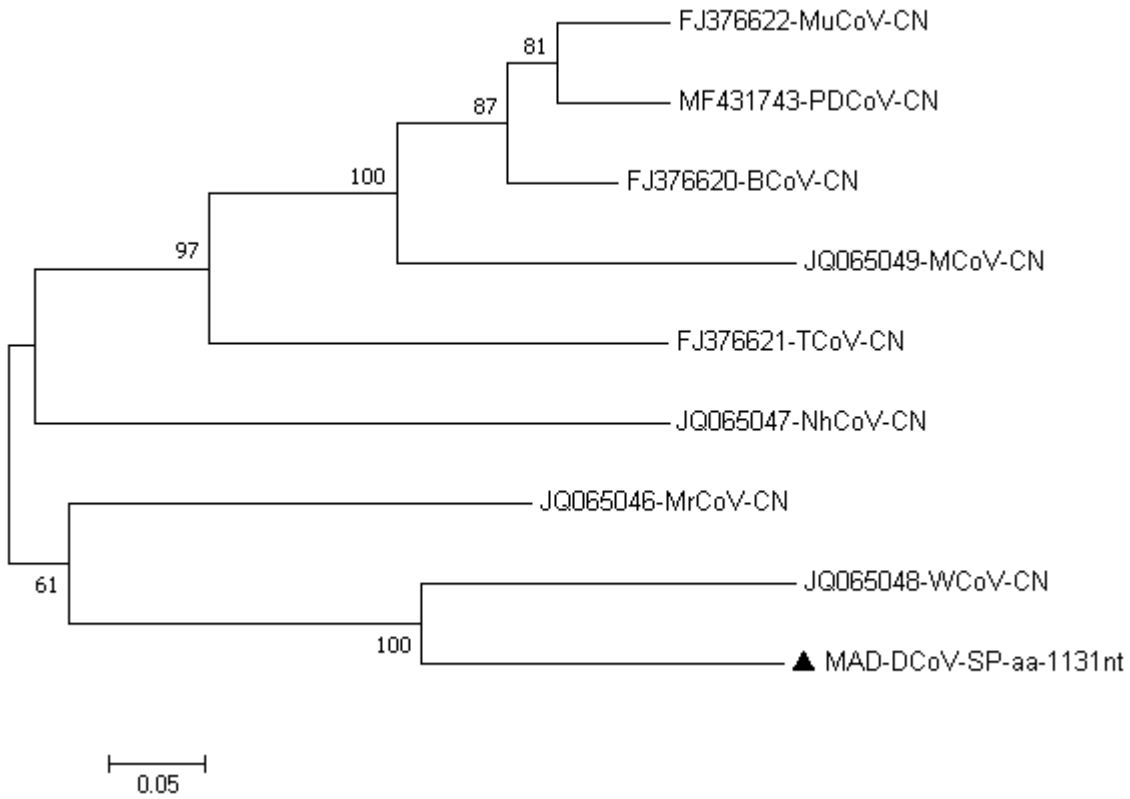


Figure S3.6: Molecular Phylogenetic analysis by Maximum Likelihood method for amino acid sequences of DCoV partial SP gene

The evolutionary history was inferred by using the Maximum Likelihood method based on the Le_Gascuel_2008 model[6]. The tree with the highest log likelihood (-3983.73) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.7265)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 9 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 375 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S3.6: Estimates of Evolutionary Divergence between Sequences

The number of amino acid differences per sequence from between sequences are shown. Standard error estimate(s) are shown above the diagonal. The analysis involved 9 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 375 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

JQ065049-MCoV-CN								
JQ065046-MrCoV-CN	133							
FJ376622-MuCoV-CN	89	129						
FJ376621-TCoV-CN	128	132	111					
MF431743-PDCoV-CN	96	132	43	114				
FJ376620-BCoV-CN	80	133	49	115	46			
JQ065047-NhCoV-CN	131	123	137	134	143	136		
JQ065048-WCoV-CN	149	130	141	147	135	140	144	
MAD-DCoV-SP-aa-1131nt	145	129	147	142	140	140	149	99

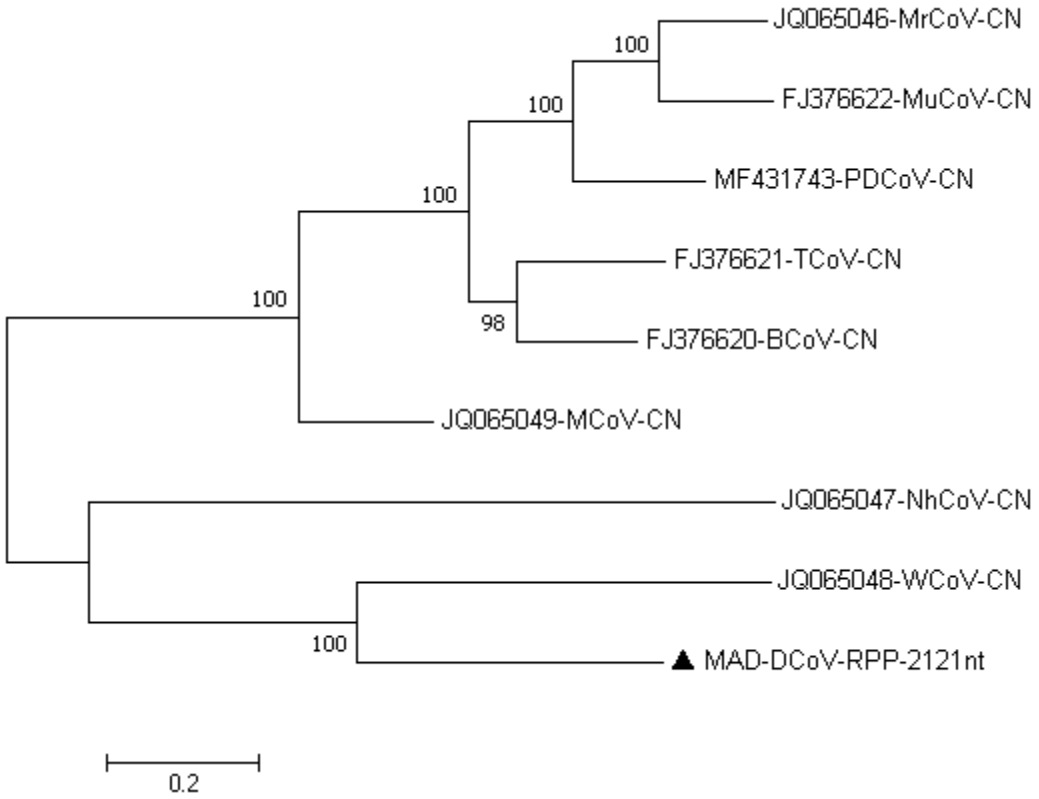


Figure S3.7: Molecular Phylogenetic analysis by Maximum Likelihood method of DCoV partial RPP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model[7]. The tree with the highest log likelihood (-15847.72) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 1.0085)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 9 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 2091 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S3.7: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 2091 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

JQ065048-WCoV-CN								
JQ065049-MCoV-CN	830							
JQ065046-MrCoV-CN	907	664						
FJ376622-MuCoV-CN	887	617	403					
FJ376621-TCoV-CN	872	569	614	557				
MF431743-PDCoV-CN	908	633	495	501	559			
FJ376620-BCoV-CN	866	577	576	566	433	534		
JQ065047-NhCoV-CN	878	835	954	912	891	928	887	
MAD-DCoV-RPP-2121nt	707	804	908	895	843	918	859	872

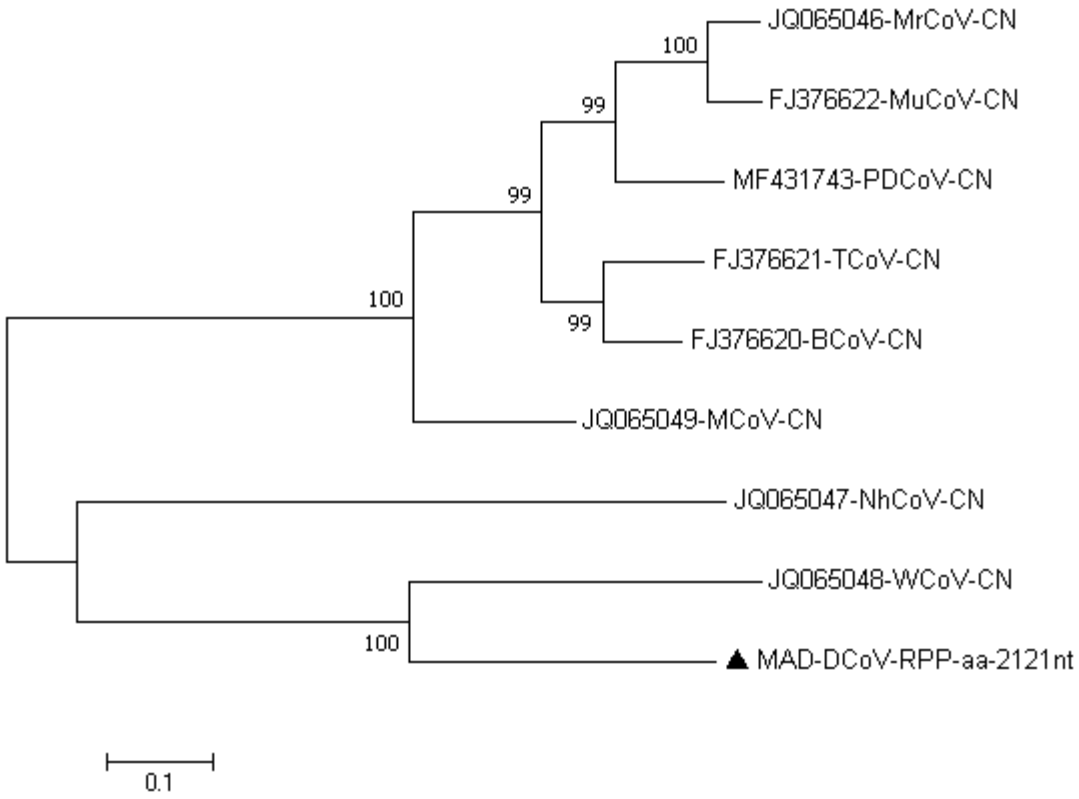


Figure S3.8: Molecular Phylogenetic analysis by Maximum Likelihood method for amino acid sequences of DCoV partial RPP gene

The evolutionary history was inferred by using the Maximum Likelihood method based on the Le_Gascuel_2008 model[6]. The tree with the highest log likelihood (-7469.23) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.6196)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 9 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 697 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S3.8: Estimates of Evolutionary Divergence between Sequences

The number of amino acid differences per sequence from between sequences are shown. The analysis involved 9 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 697 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

JQ065048-WCoV-CN								
JQ065049-MCoV-CN	316							
JQ065046-MrCoV-CN	328	181						
FJ376622-MuCoV-CN	323	189	60					
FJ376621-TCoV-CN	333	183	147	152				
MF431743-PDCoV-CN	327	183	117	116	141			
FJ376620-BCoV-CN	317	166	145	146	94	131		
JQ065047-NhCoV-CN	332	320	325	325	328	325	329	
MAD-DCoV-RPP-aa-2121nt	239	316	323	320	324	327	322	321

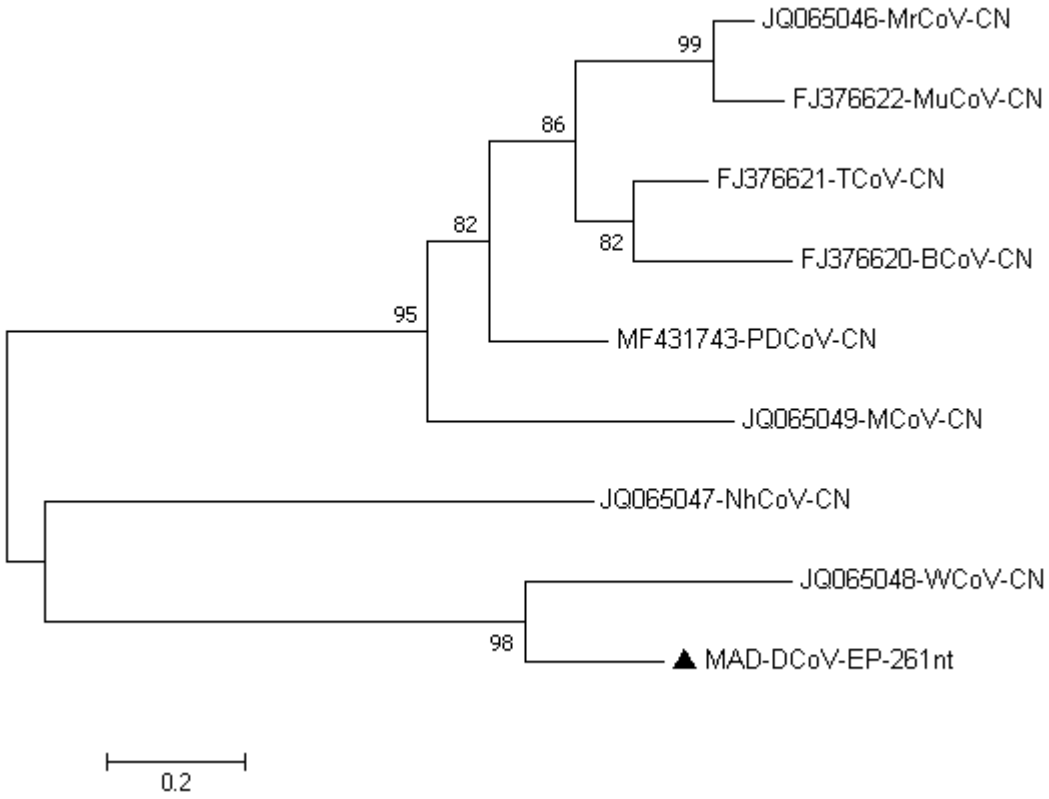


Figure S3.9: Molecular Phylogenetic analysis by Maximum Likelihood method of DCoV partial EP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model[4]. The tree with the highest log likelihood (-1858.26) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 1.3268)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 9 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 231 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S3.9: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 231 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

JQ065049-MCoV-CN								
JQ065046-MrCoV-CN	86							
FJ376622-MuCoV-CN	80	30						
FJ376621-TCoV-CN	84	58	60					
MF431743-PDCoV-CN	79	65	70	65				
FJ376620-BCoV-CN	85	69	67	50	68			
JQ065047-NhCoV-CN	114	117	114	115	107	117		
JQ065048-WCoV-CN	115	118	125	116	120	120	115	
MAD-DCoV-EP-261nt	111	112	111	121	110	117	111	74

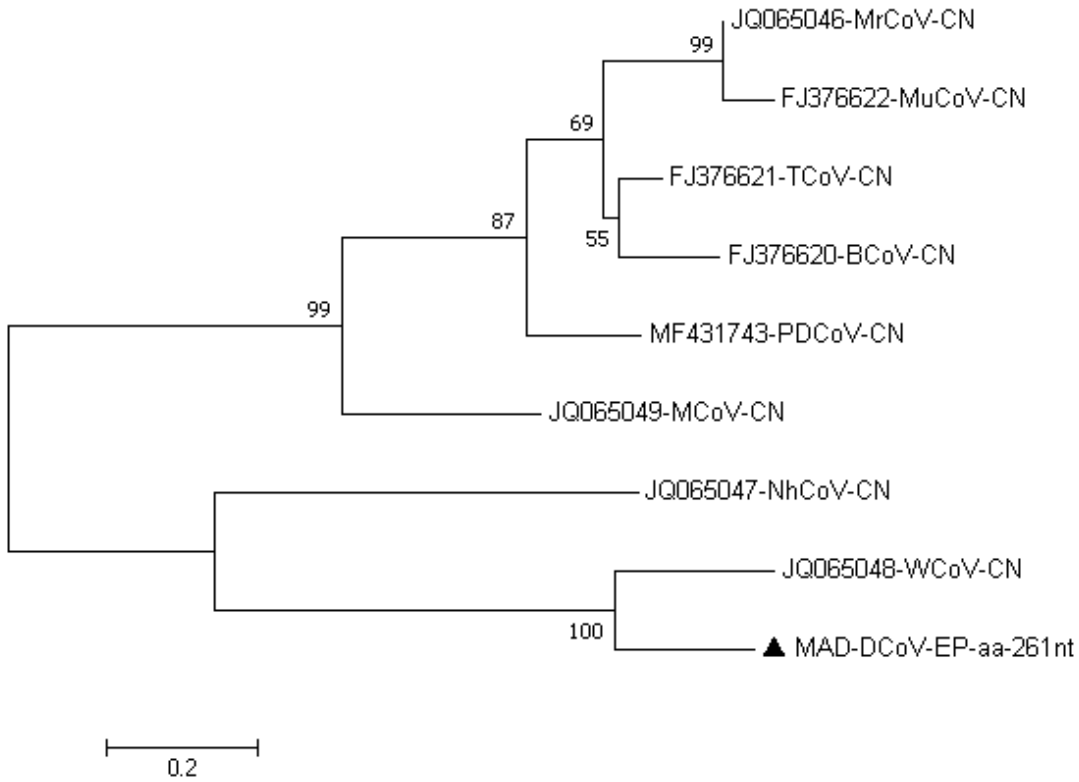


Figure S3.10: Molecular Phylogenetic analysis by Maximum Likelihood method for amino acid sequences of DCoV partial EP gene

The evolutionary history was inferred by using the Maximum Likelihood method based on the Le_Gascuel_2008 model[6]. The tree with the highest log likelihood (-962.16) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 1.9470)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 9 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 77 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S3.10: Estimates of Evolutionary Divergence between Sequences

The number of amino acid differences per sequence from between sequences are shown. The analysis involved 9 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 77 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

JQ065049-MCoV-CN								
JQ065046-MrCoV-CN	29							
FJ376622-MuCoV-CN	32	5						
FJ376621-TCoV-CN	27	14	17					
MF431743-PDCoV-CN	29	22	24	19				
FJ376620-BCoV-CN	31	18	19	12	21			
JQ065047-NhCoV-CN	49	47	47	46	47	49		
JQ065048-WCoV-CN	47	50	51	51	48	50	45	
MAD-DCoV-EP-aa-261nt	47	50	51	50	48	50	46	22

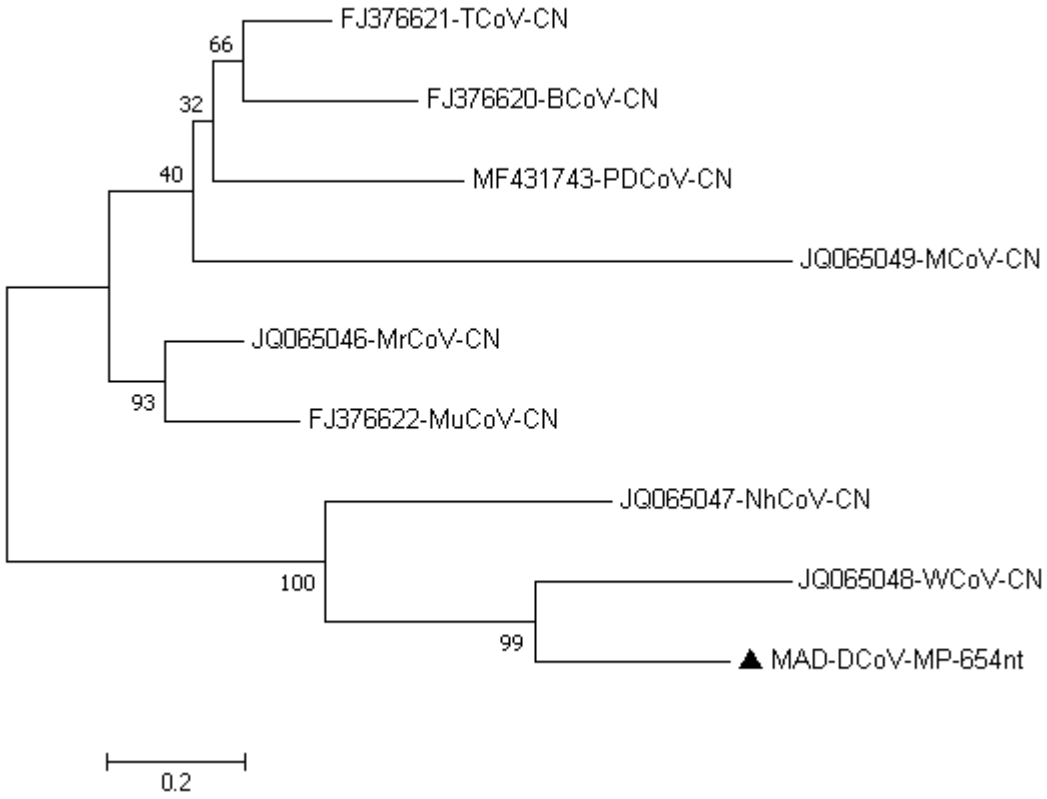


Figure S3.11. Molecular Phylogenetic analysis by Maximum Likelihood method of DCoV partial MP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model[4]. The tree with the highest log likelihood (-5074.04) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 1.0453)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 9 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 642 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S3.11: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 642 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

JQ065049-MCoV-CN								
JQ065046-MrCoV-CN	251							
FJ376622-MuCoV-CN	261	128						
FJ376621-TCoV-CN	223	173	184					
MF431743-PDCoV-CN	244	194	210	174				
FJ376620-BCoV-CN	232	192	193	145	193			
JQ065047-NhCoV-CN	290	262	246	258	268	252		
JQ065048-WCoV-CN	295	256	265	257	266	276	245	
MAD-DCoV-MP-654nt	285	273	252	265	281	271	231	195

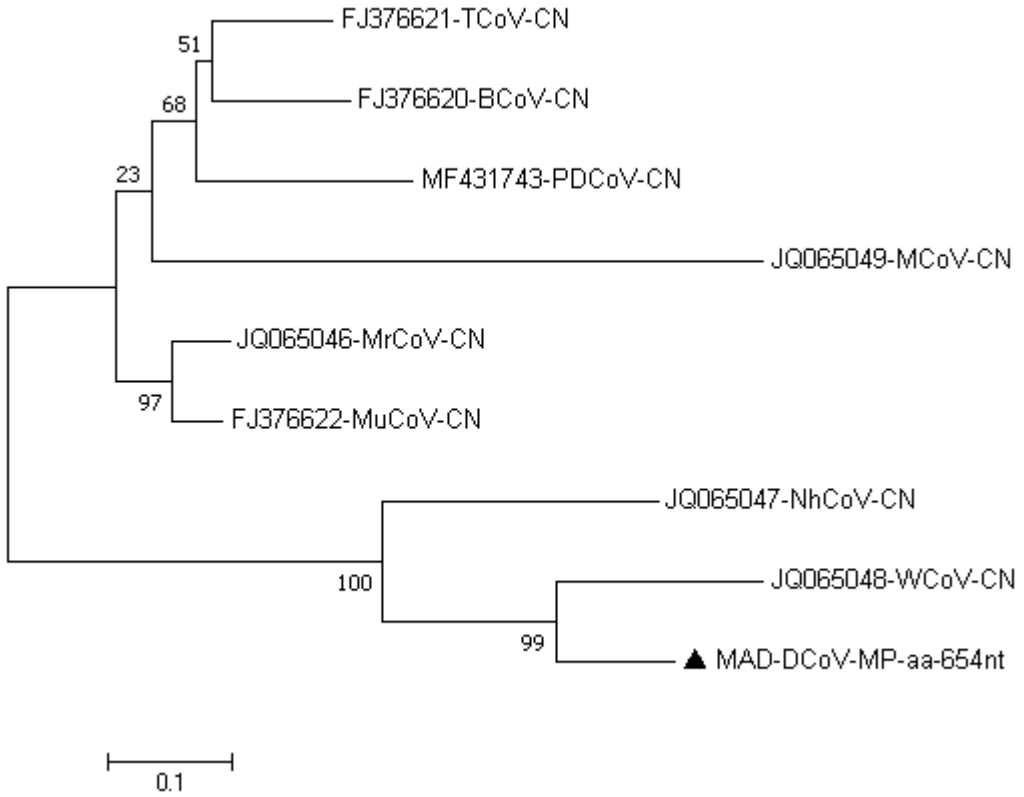


Figure S3.12: Molecular Phylogenetic analysis by Maximum Likelihood method for amino acid sequences of DCoV partial MP gene

The evolutionary history was inferred by using the Maximum Likelihood method based on the Le_Gascuel_2008 model[6]. The tree with the highest log likelihood (-2219.53) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.8155)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 9 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 214 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table 3.12: Estimates of Evolutionary Divergence between Sequences

The number of amino acid differences per sequence from between sequences are shown. The analysis involved 9 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 214 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

JQ065049-MCoV-CN								
JQ065046-MrCoV-CN	77							
FJ376622-MuCoV-CN	75	18						
FJ376621-TCoV-CN	73	43	40					
MF431743-PDCoV-CN	78	55	54	48				
FJ376620-BCoV-CN	76	49	47	36	48			
JQ065047-NhCoV-CN	107	85	83	82	95	90		
JQ065048-WCoV-CN	104	92	95	89	96	97	72	
MAD-DCoV-MP-aa-654nt	102	86	82	88	99	89	70	46

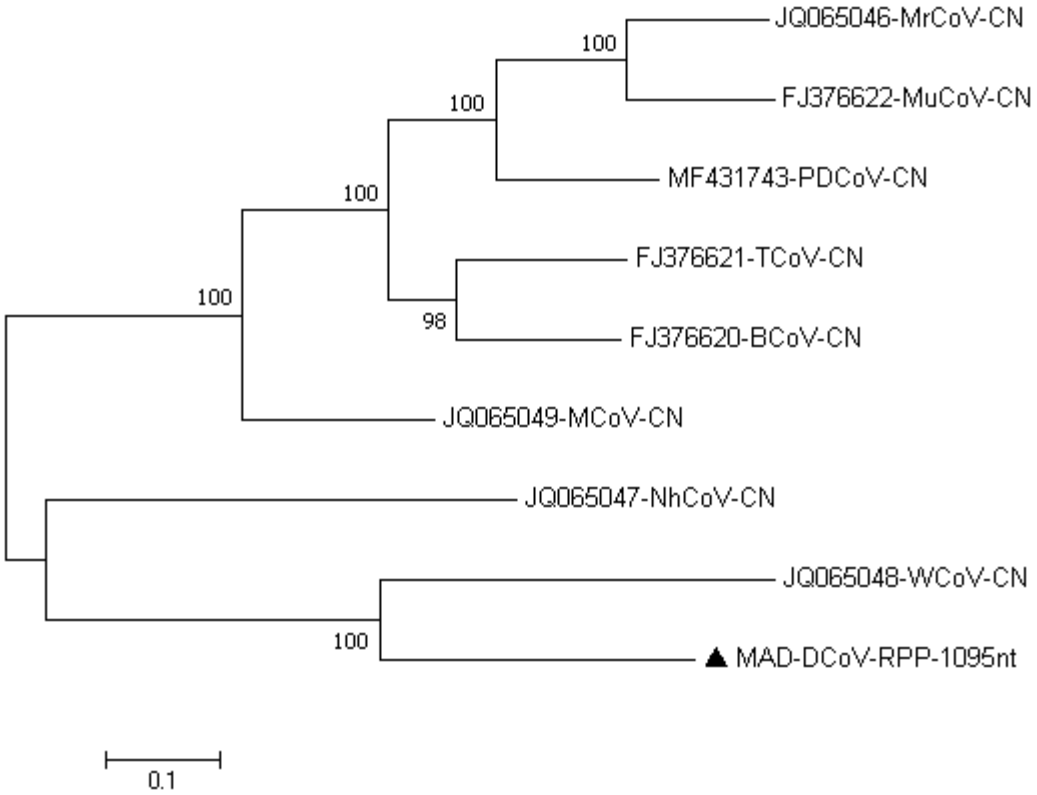


Figure S3.13: Molecular Phylogenetic analysis by Maximum Likelihood method of DCoV partial Orf1bStart gene

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model[4]. The tree with the highest log likelihood (-7597.55) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.7931)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 19.86% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 9 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 1095 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S3.13: Estimates of Evolutionary Divergence between Sequences

The number of base differences per sequence from between sequences are shown. The analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 1095 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

JQ065049-MCoV-CN								
JQ065046-MrCoV-CN	312							
FJ376622-MuCoV-CN	297	195						
FJ376621-TCoV-CN	267	280	279					
MF431743-PDCoV-CN	288	247	247	259				
FJ376620-BCoV-CN	270	281	260	203	264			
JQ065047-NhCoV-CN	350	392	392	376	392	375		
JQ065048-WCoV-CN	379	429	407	389	404	399	397	
MAD-DCoV-RPP-1095nt	373	433	419	395	423	402	378	317

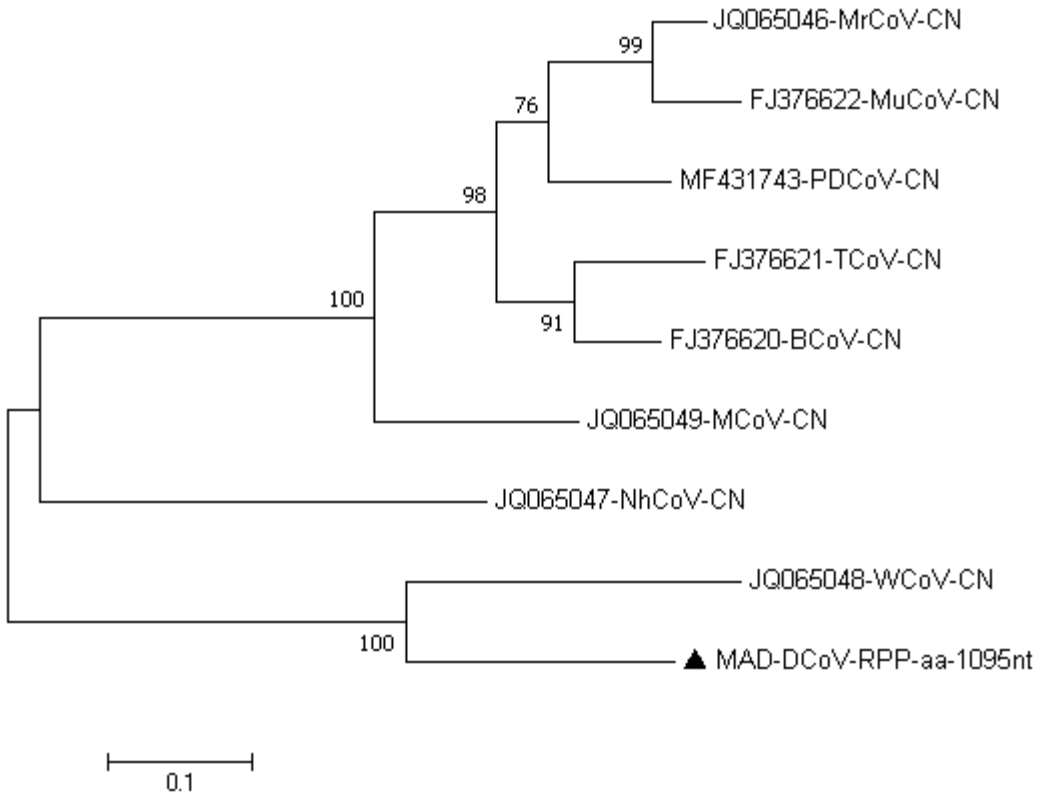


Figure S3.14: Molecular Phylogenetic analysis by Maximum Likelihood method for amino acid sequences of DCoV partial Orf1bStart gene

The evolutionary history was inferred by using the Maximum Likelihood method based on the Le_Gascuel_2008 model[6]. The tree with the highest log likelihood (-3466.48) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.5360)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 9 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 365 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S3.14: Estimates of Evolutionary Divergence between Sequences

The number of amino acid differences per sequence from between sequences are shown. The analysis involved 9 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 365 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

JQ065049-MCoV-CN								
JQ065046-MrCoV-CN	72							
FJ376622-MuCoV-CN	75	28						
FJ376621-TCoV-CN	75	68	66					
MF431743-PDCoV-CN	70	48	50	56				
FJ376620-BCoV-CN	74	54	52	42	56			
JQ065047-NhCoV-CN	121	127	126	124	124	118		
JQ065048-WCoV-CN	140	141	139	138	137	138	136	
MAD-DCoV-RPP-aa-1095nt	134	140	140	137	141	135	132	89

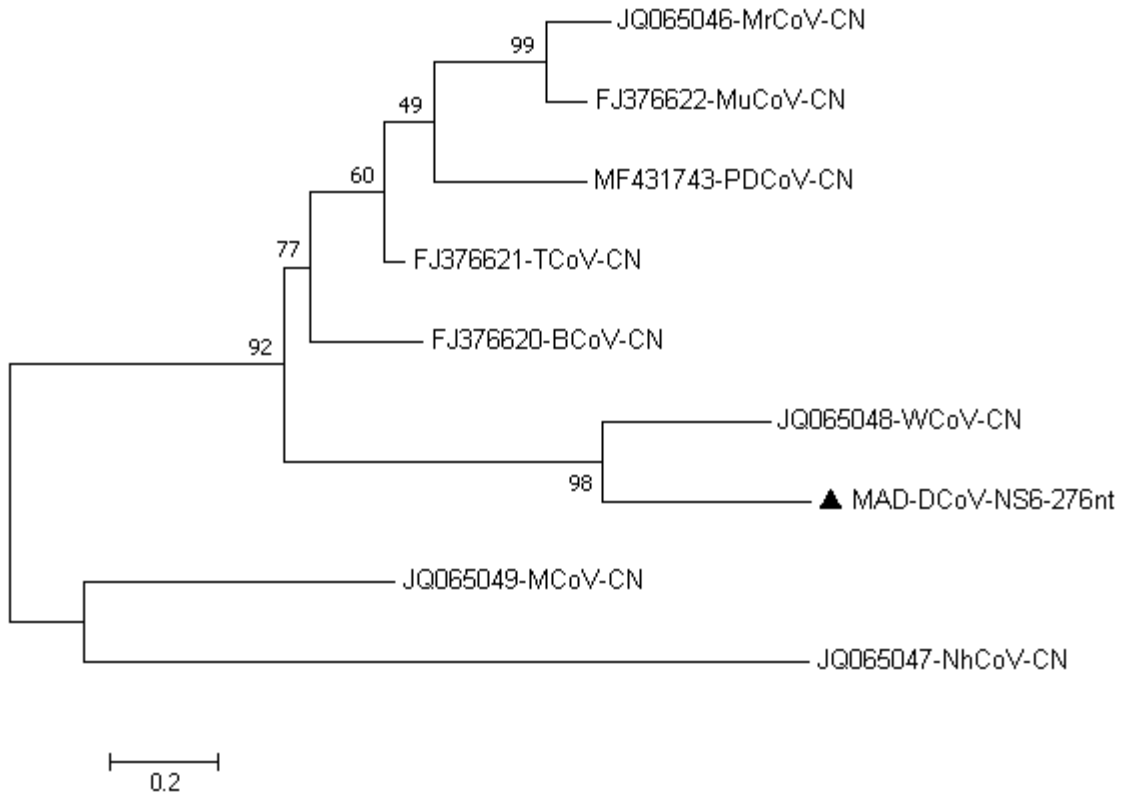


Figure S3.15: Molecular Phylogenetic analysis by Maximum Likelihood method of DCoV partial NS6 gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model[4]. The tree with the highest log likelihood (-2090.07) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 1.9020)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 9 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 240 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S3.15: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 240 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

JQ065049-MCoV-CN								
JQ065046-MrCoV-CN	125							
FJ376622-MuCoV-CN	121	38						
FJ376621-TCoV-CN	119	69	62					
MF431743-PDCoV-CN	118	79	78	64				
FJ376620-BCoV-CN	120	93	88	60	81			
JQ065047-NhCoV-CN	127	146	144	137	137	134		
JQ065048-WCoV-CN	131	119	123	99	113	109	136	
MAD-DCoV-NS6-276nt	123	122	126	112	120	113	140	84

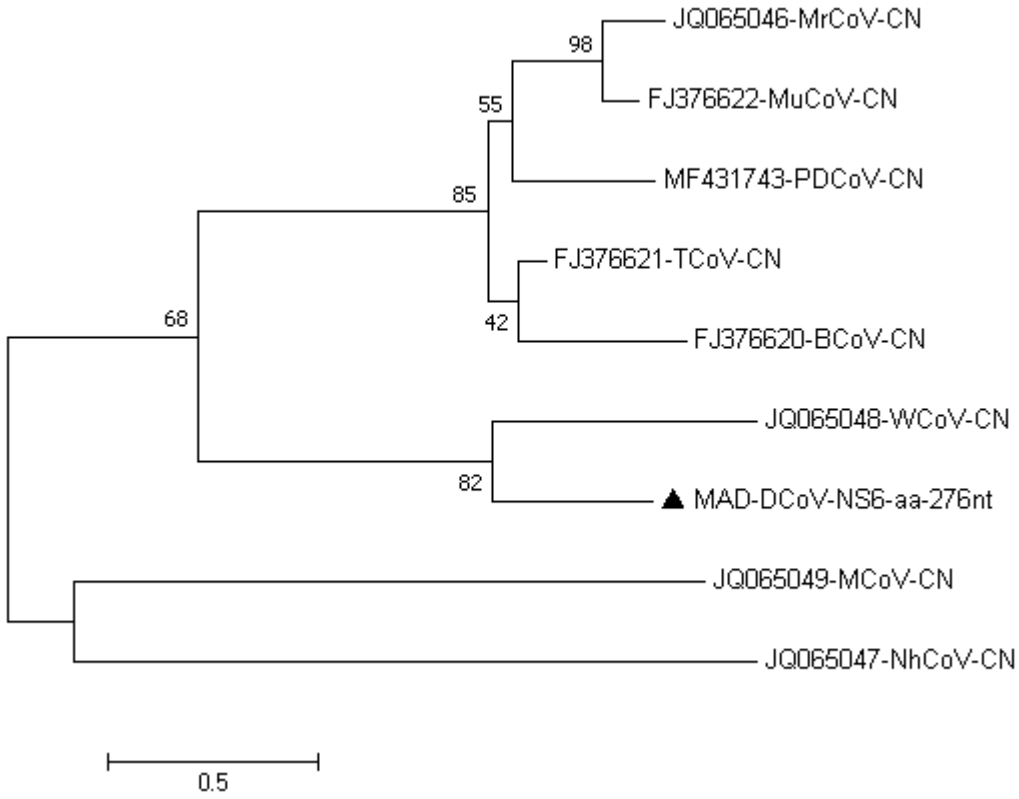


Figure S3.16: Molecular Phylogenetic analysis by Maximum Likelihood method for amino acid sequences of DCoV partial NS6 gene

The evolutionary history was inferred by using the Maximum Likelihood method based on the Le_Gascuel_2008 model[6]. The tree with the highest log likelihood (-1316.26) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 2.1381)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 9 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 80 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S3.16: Estimates of Evolutionary Divergence between Sequences

The number of amino acid differences per sequence from between sequences are shown. The analysis involved 9 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 80 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

JQ065049-MCoV-CN								
JQ065046-MrCoV-CN	57							
FJ376622-MuCoV-CN	59	15						
FJ376621-TCoV-CN	58	27	24					
MF431743-PDCoV-CN	57	27	28	25				
FJ376620-BCoV-CN	60	38	35	24	32			
JQ065047-NhCoV-CN	60	58	61	58	57	57		
JQ065048-WCoV-CN	59	55	54	48	50	54	60	
MAD-DCoV-NS6-aa-276nt	54	55	56	48	50	51	57	36

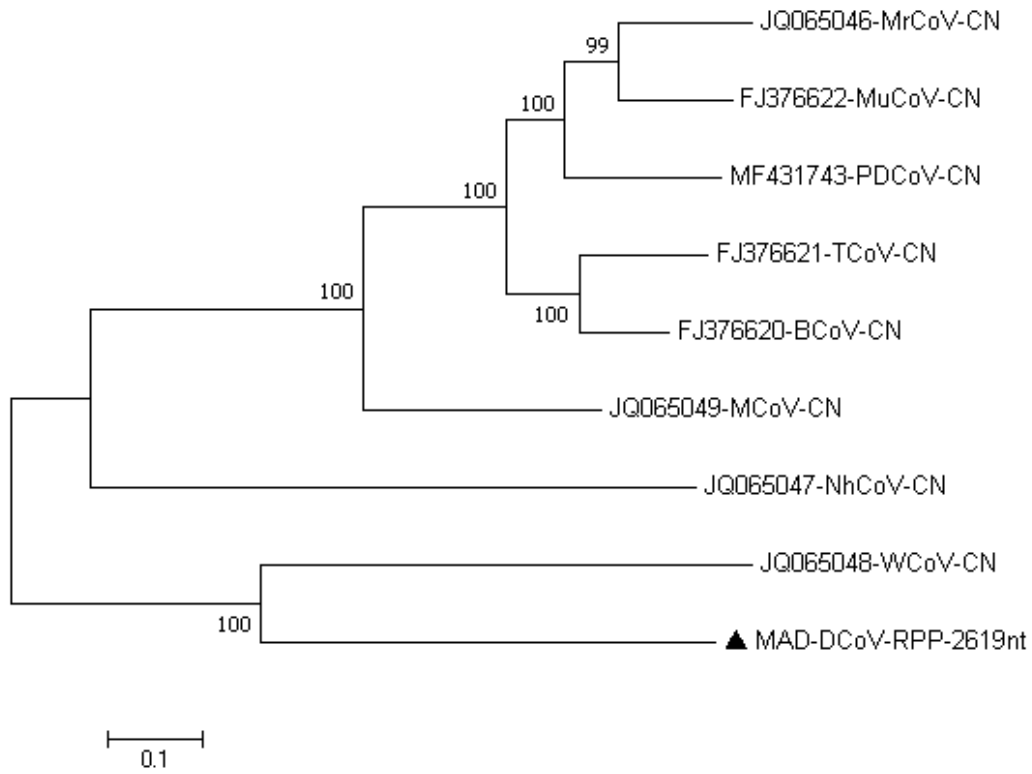


Figure 3.17: Molecular Phylogenetic analysis by Maximum Likelihood method of DCoV partial Orf1bNSP11-13 gene

The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model[7]. The tree with the highest log likelihood (-18412.00) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.8614)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 18.41% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 9 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 2607 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S3.17: Estimates of Evolutionary Divergence between Sequences

The number of base differences per sequence from between sequences are shown. The analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 2607 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

JQ065049-MCoV-CN								
JQ065046-MrCoV-CN	781							
FJ376622-MuCoV-CN	704	467						
FJ376621-TCoV-CN	698	604	609					
MF431743-PDCoV-CN	736	555	537	588				
FJ376620-BCoV-CN	681	596	552	406	585			
JQ065047-NhCoV-CN	956	971	949	970	969	958		
JQ065048-WCoV-CN	993	1061	1020	974	1051	982	1044	
MAD-DCoV-RPP-2619nt	946	1083	1040	1009	1051	985	1032	905

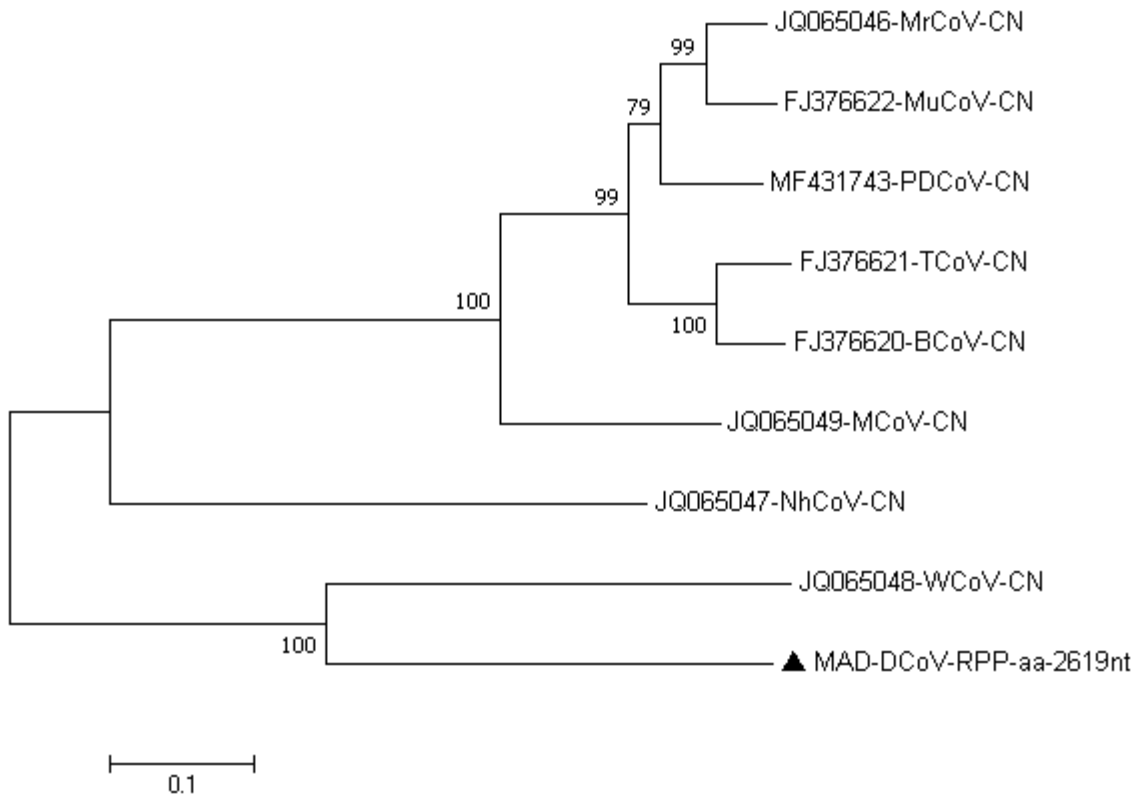


Figure S3.18: Molecular Phylogenetic analysis by Maximum Likelihood method for amino acid sequences of DCoV partial Orf1bNSP11-13 gene

The evolutionary history was inferred by using the Maximum Likelihood method based on the Le_Gascuel_2008 model[6]. The tree with the highest log likelihood (-8195.31) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.6597)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 9 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 869 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S3.18: Estimates of Evolutionary Divergence between Sequences

The number of amino acid differences per sequence from between sequences are shown. The analysis involved 9 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 869 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

JQ065049-MCoV-CN								
JQ065046-MrCoV-CN	174							
FJ376622-MuCoV-CN	181	67						
FJ376621-TCoV-CN	196	123	131					
MF431743-PDCoV-CN	183	99	101	121				
FJ376620-BCoV-CN	194	116	131	73	120			
JQ065047-NhCoV-CN	317	315	312	313	306	315		
JQ065048-WCoV-CN	351	346	349	340	346	335	347	
MAD-DCoV-RPP-aa-2619nt	351	356	365	358	353	351	336	283

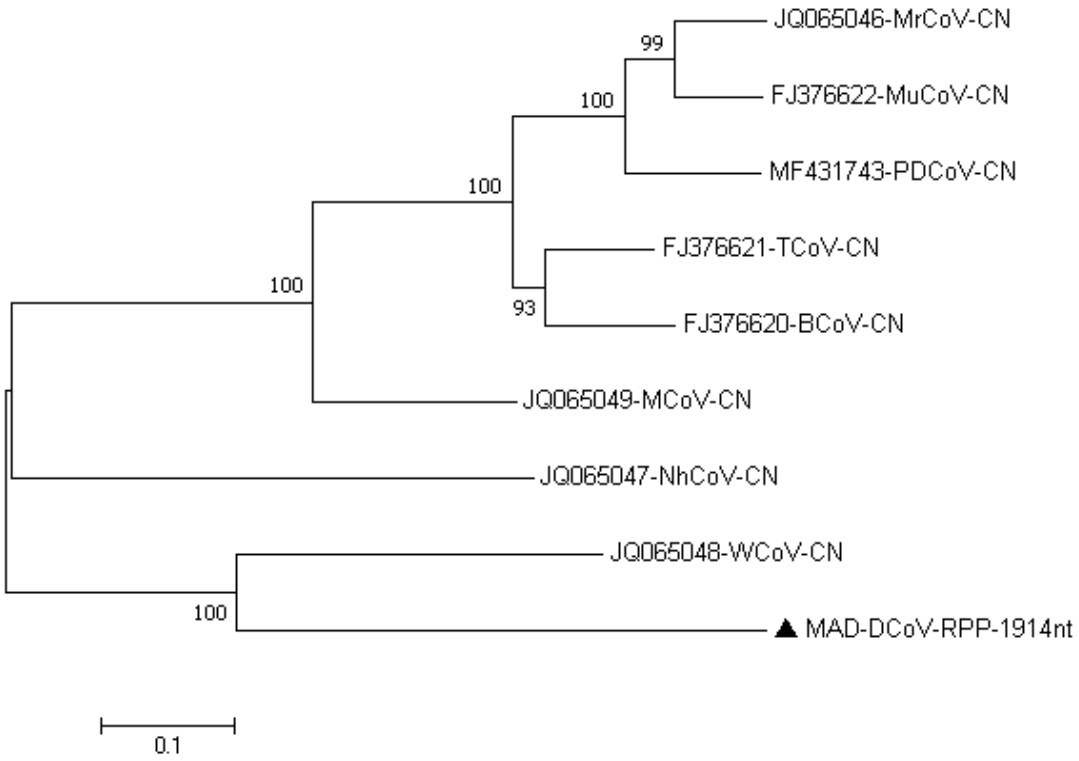


Figure S3.19: Molecular Phylogenetic analysis by Maximum Likelihood method of DCoV partial Orf1bNSP11 gene

The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model[7]. The tree with the highest log likelihood (-11913.38) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.7350)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 20.83% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 9 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 1851 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S3.19: Estimates of Evolutionary Divergence between Sequences

The number of base differences per sequence from between sequences are shown. The analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 1851 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

JQ065049-MCoV-CN								
JQ065046-MrCoV-CN	481							
FJ376622-MuCoV-CN	468	206						
FJ376621-TCoV-CN	392	338	328					
MF431743-PDCoV-CN	493	270	274	346				
FJ376620-BCoV-CN	426	353	344	253	351			
JQ065047-NhCoV-CN	603	661	625	640	623	617		
JQ065048-WCoV-CN	613	659	659	653	676	659	635	
MAD-DCoV-RPP-1914nt	634	680	675	665	701	658	659	559

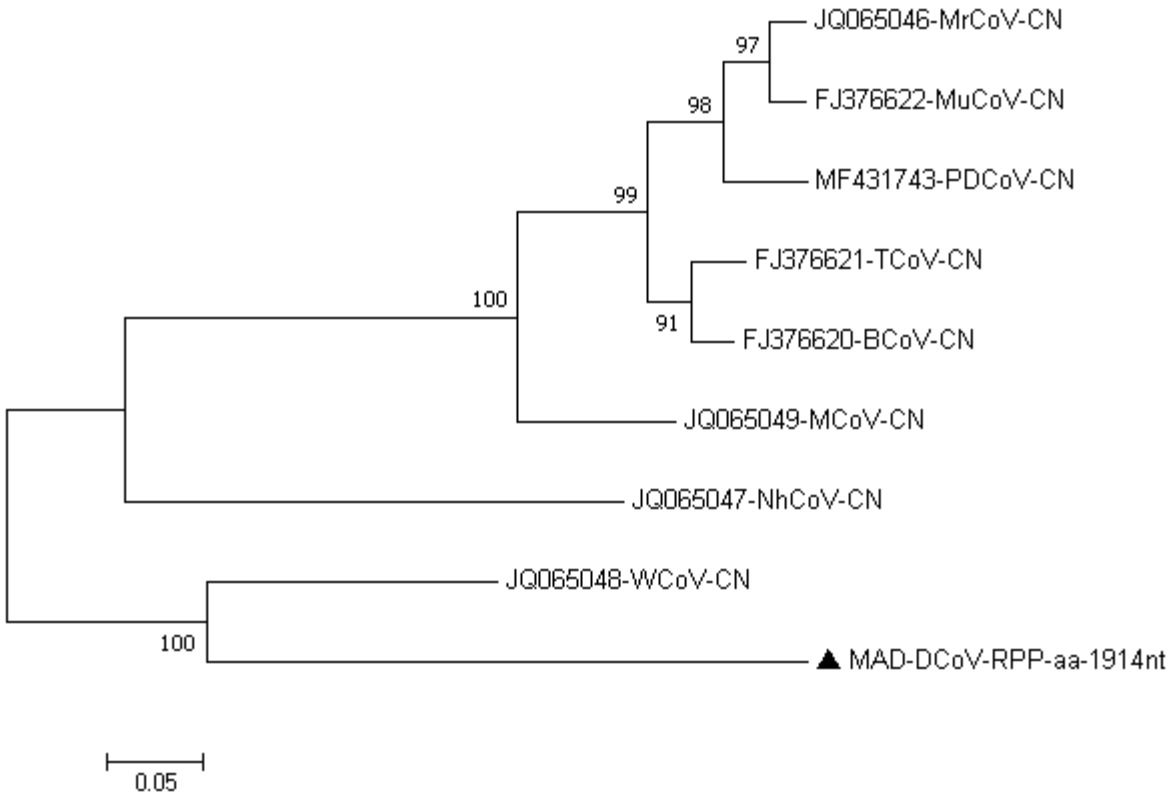


Figure S3.20. Molecular Phylogenetic analysis by Maximum Likelihood method for amino acid sequences of DCoV partial Orf1bNSP11 gene

The evolutionary history was inferred by using the Maximum Likelihood method based on the Le_Gascuel_2008 model[6]. The tree with the highest log likelihood (-5002.27) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.5922)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 9 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 617 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S3.20: Estimates of Evolutionary Divergence between Sequences

The number of amino acid differences per sequence from between sequences are shown. The analysis involved 9 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 617 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

JQ065049-MCoV-CN								
JQ065046-MrCoV-CN	100							
FJ376622-MuCoV-CN	99	22						
FJ376621-TCoV-CN	91	65	61					
MF431743-PDCoV-CN	103	46	48	57				
FJ376620-BCoV-CN	87	62	58	29	66			
JQ065047-NhCoV-CN	174	177	176	179	176	178		
JQ065048-WCoV-CN	181	191	186	187	193	178	188	
MAD-DCoV-RPP-aa-1914nt	211	216	210	216	219	210	203	162

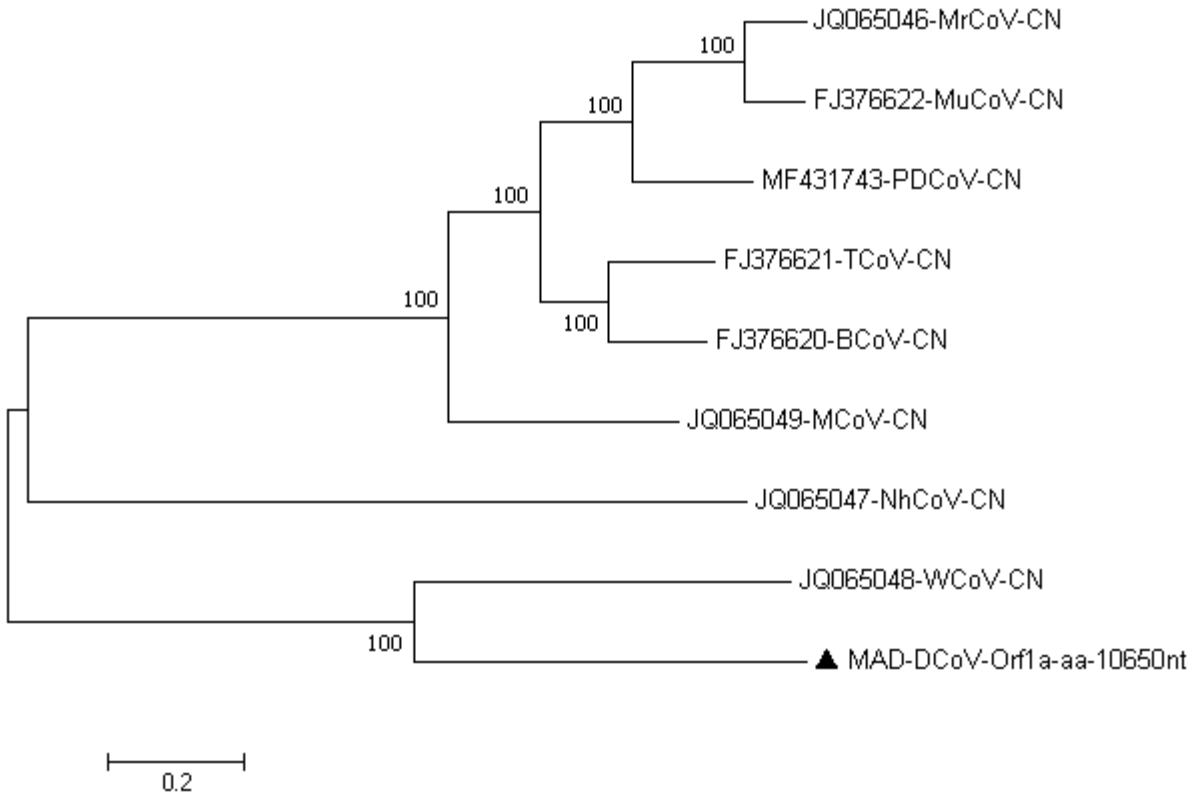


Figure S3.21: Molecular Phylogenetic analysis by Maximum Likelihood method for amino acid sequences of DCoV Orf1a gene

The evolutionary history was inferred by using the Maximum Likelihood method based on the Le_Gascuel_2008 model[6]. The tree with the highest log likelihood (-46030.60) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 1.2319)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 9 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 3379 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S3.21: Estimates of Evolutionary Divergence between Sequences

The number of amino acid differences per sequence from between sequences are shown. The analysis involved 9 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 3379 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

JQ065049-MCoV-CN								
JQ065046-MrCoV-CN	1311							
FJ376622-MuCoV-CN	1312	479						
FJ376621-TCoV-CN	1207	1120	1125					
MF431743-PDCoV-CN	1293	885	890	1063				
FJ376620-BCoV-CN	1193	1096	1084	733	1034			
JQ065047-NhCoV-CN	2000	1996	2004	1997	1989	1971		
JQ065048-WCoV-CN	2012	2037	2024	2043	2029	1993	2078	
MAD-DCoV-Orf1a-aa-10650nt	2012	2033	2031	2048	2045	2033	2086	1591

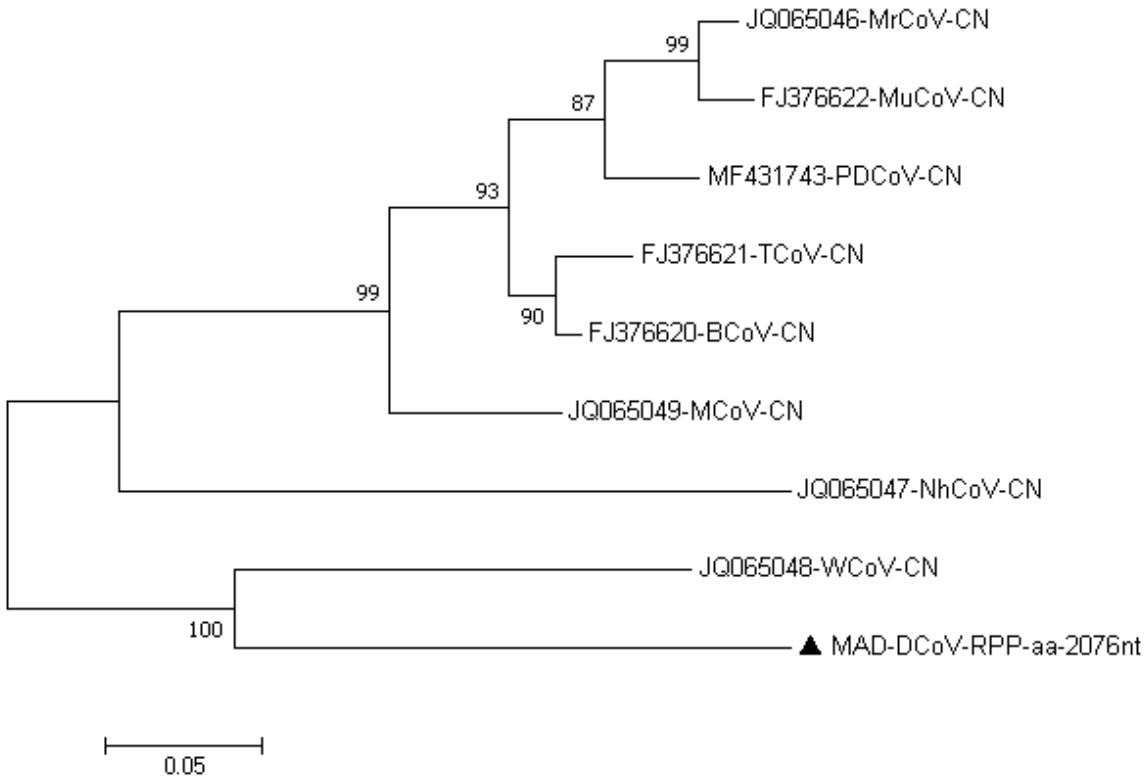


Figure S3.22: Molecular Phylogenetic analysis by Maximum Likelihood method for amino acid sequences of DCoV partial RPP gene

The evolutionary history was inferred by using the Maximum Likelihood method based on the Le_Gascuel_2008 model[6]. The tree with the highest log likelihood (-4534.10) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.3177)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 9 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 692 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S3.22: Estimates of Evolutionary Divergence between Sequences

The number of amino acid differences per sequence from between sequences are shown. The analysis involved 9 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 692 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

JQ065049-MCoV-CN								
JQ065046-MrCoV-CN	72							
FJ376622-MuCoV-CN	71	18						
FJ376621-TCoV-CN	69	52	54					
MF431743-PDCoV-CN	79	39	41	45				
FJ376620-BCoV-CN	61	50	55	21	46			
JQ065047-NhCoV-CN	142	134	131	139	132	136		
JQ065048-WCoV-CN	137	147	144	144	150	146	159	
MAD-DCoV-RPP-aa-2076nt	141	146	143	146	152	149	162	125

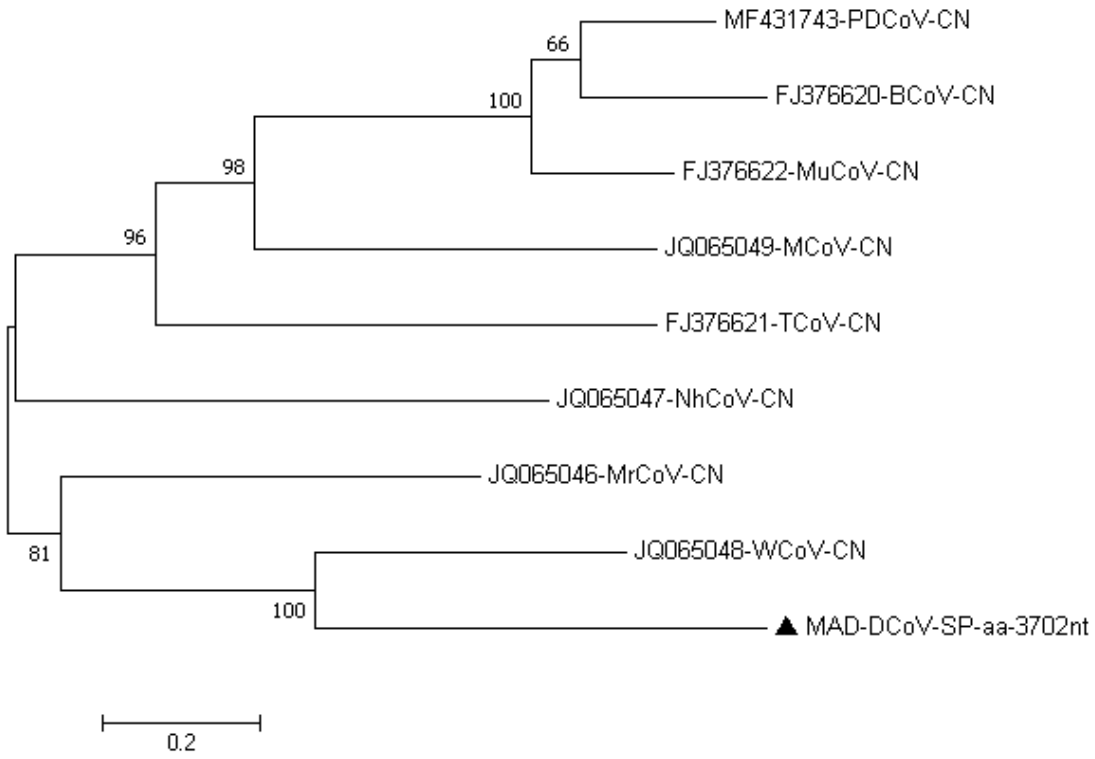


Figure S3.23: Molecular Phylogenetic analysis by Maximum Likelihood method for amino acid sequences of DCoV partial SP gene

The evolutionary history was inferred by using the Maximum Likelihood method based on the Whelan And Goldman + Freq. model[8]. The tree with the highest log likelihood (-16834.34) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 1.2876)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 12.14% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 9 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 1104 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S3.23: Estimates of Evolutionary Divergence between Sequences

The number of amino acid differences per sequence from between sequences are shown. The analysis involved 9 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 1104 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

JQ065049-MCoV-CN								
JQ065046-MrCoV-CN	568							
FJ376622-MuCoV-CN	509	576						
FJ376621-TCoV-CN	566	581	553					
MF431743-PDCoV-CN	526	583	302	554				
FJ376620-BCoV-CN	496	585	333	559	303			
JQ065047-NhCoV-CN	582	559	609	586	609	620		
JQ065048-WCoV-CN	590	555	585	618	589	599	605	
MAD-DCoV-SP-aa-3072nt	618	577	626	622	612	632	624	504

4. Virus related to goose adenovirus 4 (GoA4) and/or duck adenovirus 2 (DuA2) from MAD faecal sample

Figure and Table	Long Name (Format: Sample-virus-protein-length-quality-coverage-year)	Short name (Format: Sample-virus-protein-length-year)	Coverage	No. of nucleotides	Mapping quality threshold	NCBI accession number
DuA2						
S4.1	MAD-Adenovirus-52K-153nt-Q20-C-3-10-2016	MAD-AV-52K-153nt	3-10	153	20	MH028882
S4.2	MAD-Adenovirus-Hexon-179nt-Q20-C-4-25-2016	MAD-AV-Hexon-179nt	4-25	179	20	MH028883
S4.3	MAD-Adenovirus-Hexon-200nt-Q20-C-4-23-2016	MAD-AV-Hexon-200nt	4-23	200	20	MH028884
GoA4						
S4.4	MAD-Adenovirus-DNA-Polymerase-176nt-Q20-C-3-11-2016	MAD-AV-Pol-176nt	3-11	176	20	MH028875
S4.5	MAD-Adenovirus-DNA-Polymerase-144nt-Q20-C-8-180-2016	MAD-AV-Pol-144nt	8-180	144	20	MH028876
S4.6	MAD-Adenovirus-III-171nt-Q20-C-3-27-2016	MAD-AV-III-171nt	3-27	171	20	MH028877
S4.7	MAD-Adenovirus-Hexon-157nt-Q20-C-7-78-2016	MAD-AV-Hexon-157nt	7-78	157	20	MH028878
S4.8	MAD-Adenovirus-Hexon-164nt-Q20-C-3-15-2016	MAD-AV-Hexon-164nt	3-15	164	20	MH028879
S4.9	MAD-Adenovirus-Hexon-191nt-Q20-C-7-16-2016	MAD-AV-Hexon-191nt	7-16	191	20	MH028880
S4.10	MAD-Adenovirus-Hexon-228nt-Q20-C-4-39-2016	MAD-AV-Hexon-228nt	4-39	228	≤20	MH028881
NCBI sequences taken for phylogenetic analysis						
Long Name		Short Name (Format: NCBI accession number-virus-country/state)	Country of collection	Collection date		

KR135164-Duck-adenovirus-2-strain-CH-GD-12-2014-complete-genome	KR135164-DAd2-CN	China	2014
JF510462-Goose-adenovirus-4-strain-P29-complete-genome	JF510462-GAd4-HU	Hungary	-
FN824512-Pigeon-adenovirus-1-complete-genome-strain-IDA4	FN824512-PAd1-NL	Netherlands	1995
KC493646-Fowl-adenovirus-5-strain-340-complete-genome	KC493646-FAd5-IE	Ireland	1970

The table points towards the molecular phylogenetic analysis by Maximum Likelihood method of AV. The first half of the table provides details to identify the sample from which the virus was isolated, protein that encodes the consensus sequences that are being analysed, the length of the consensus sequences thus providing the depth of the gene being analysed, minimum mapping quality threshold used in IGV for the generation of the consensus sequences, the coverage of the consensus sequences from IGV, the year of collection of the sample and the NCBI accession number assigned to the particular consensus sequence. Corresponding short names have been used in the phylogenetic trees which contain the sample, virus, protein and the number of nucleotides. The second half of the table provides the details of the sequences that were used for the molecular phylogenetic analysis. They were found to be the most closely related sequences to the consensus sequences generated using BLASTN. Corresponding short names have been used in the phylogenetic trees which contain the NCBI accession number, virus and the country of collection. The collection date is given as retrieved from the corresponding NCBI nucleotide reference dataset which together with the country of collection can provide an insight into the possible evolution of the virus through the years.

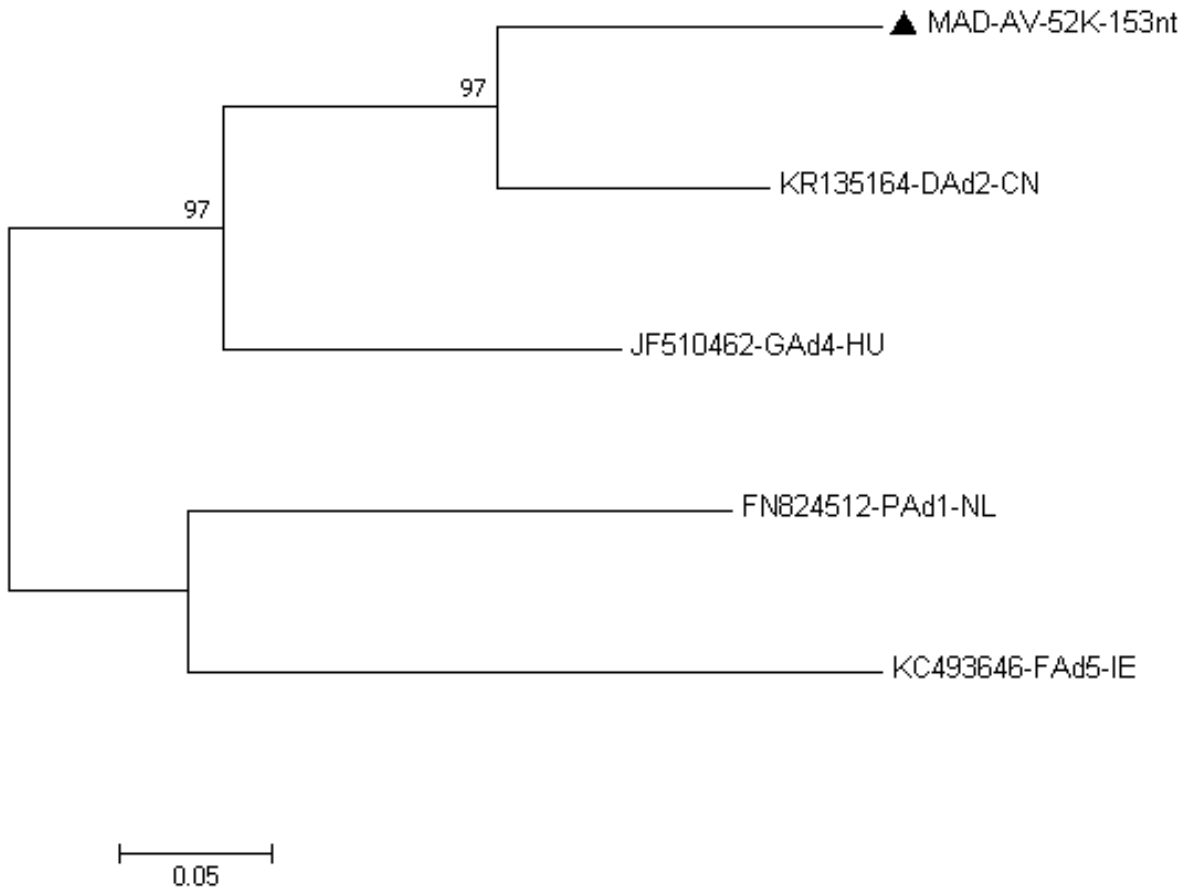


Figure S4.1: Molecular Phylogenetic analysis by Maximum Likelihood method of AV partial 52K gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Jukes-Cantor model[9]. The tree with the highest log likelihood (-674.58) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 24.51% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 5 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 153 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S4.1: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 153 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-AV-52K-153nt				
KR135164-DAd2-CN	26			
JF510462-GAd4-HU	37	35		
FN824512-PAd1-NL	50	48	43	
KC493646-FAd5-IE	51	50	51	43

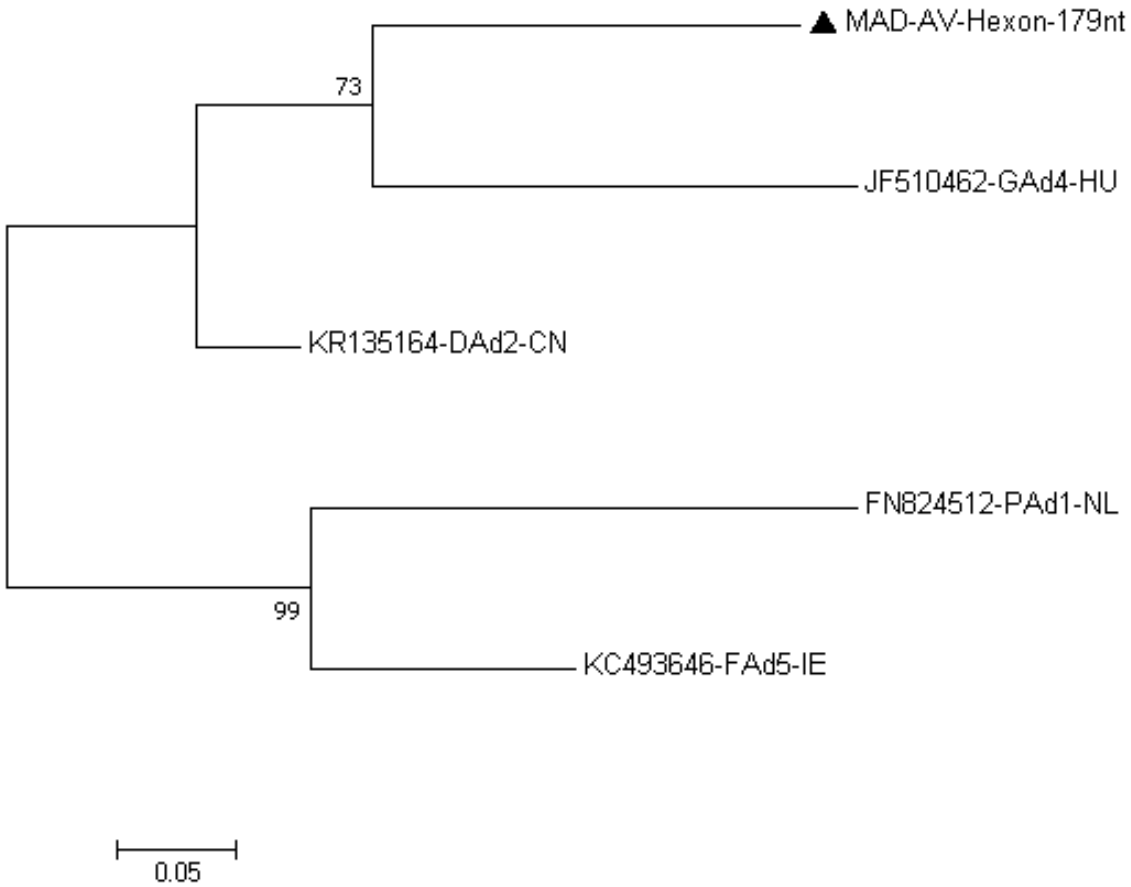


Figure S4.2: Molecular Phylogenetic analysis by Maximum Likelihood method of AV partial Hexon gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-693.56) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 30.17% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 5 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 179 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S4.2: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 179 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-AV-Hexon-179nt				
KR135164-DAd2-CN	34			
JF510462-GAd4-HU	36	38		
FN824512-PAd1-NL	57	43	50	
KC493646-FAd5-IE	49	37	44	34

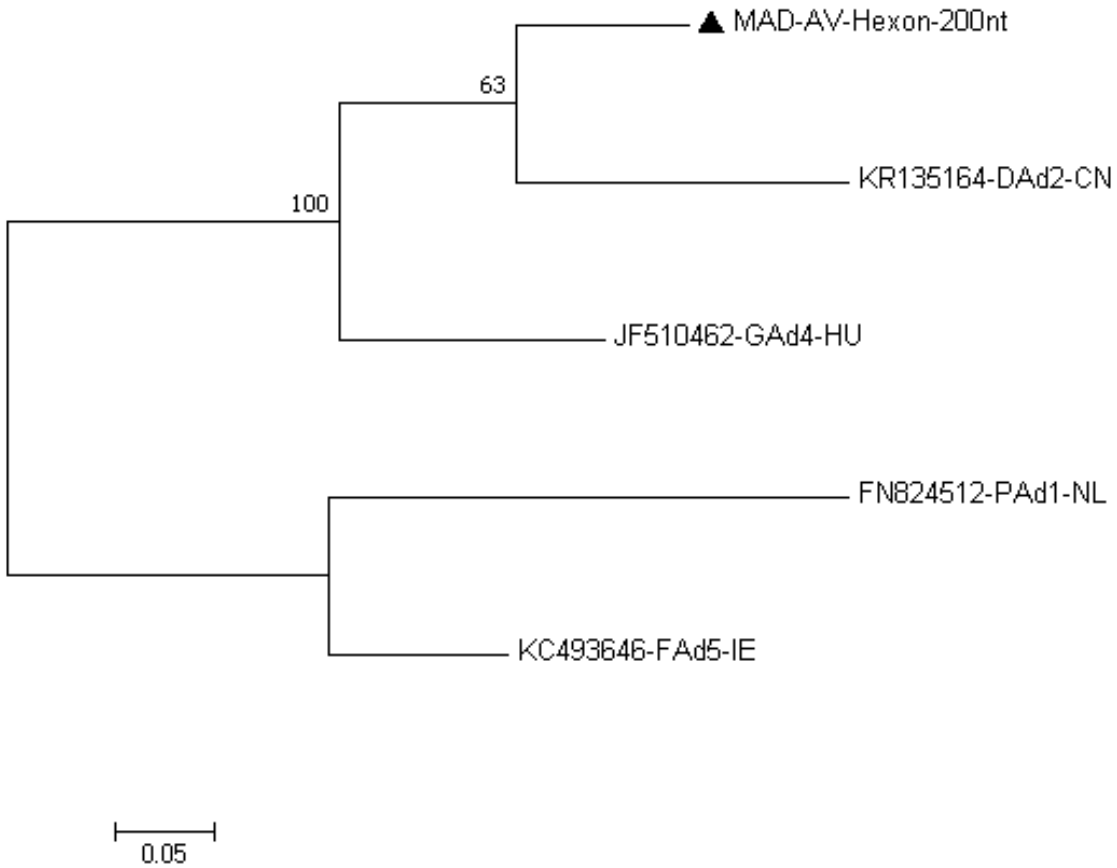


Figure S4.3: Molecular Phylogenetic analysis by Maximum Likelihood method of AV partial Hexon gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-845.82) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 27.25% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 5 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 200 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S4.3: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 200 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-AV-Hexon-200nt				
KR135164-DAd2-CN	37			
JF510462-GAd4-HU	41	49		
FN824512-PAd1-NL	68	64	71	
KC493646-FAd5-IE	61	63	58	44

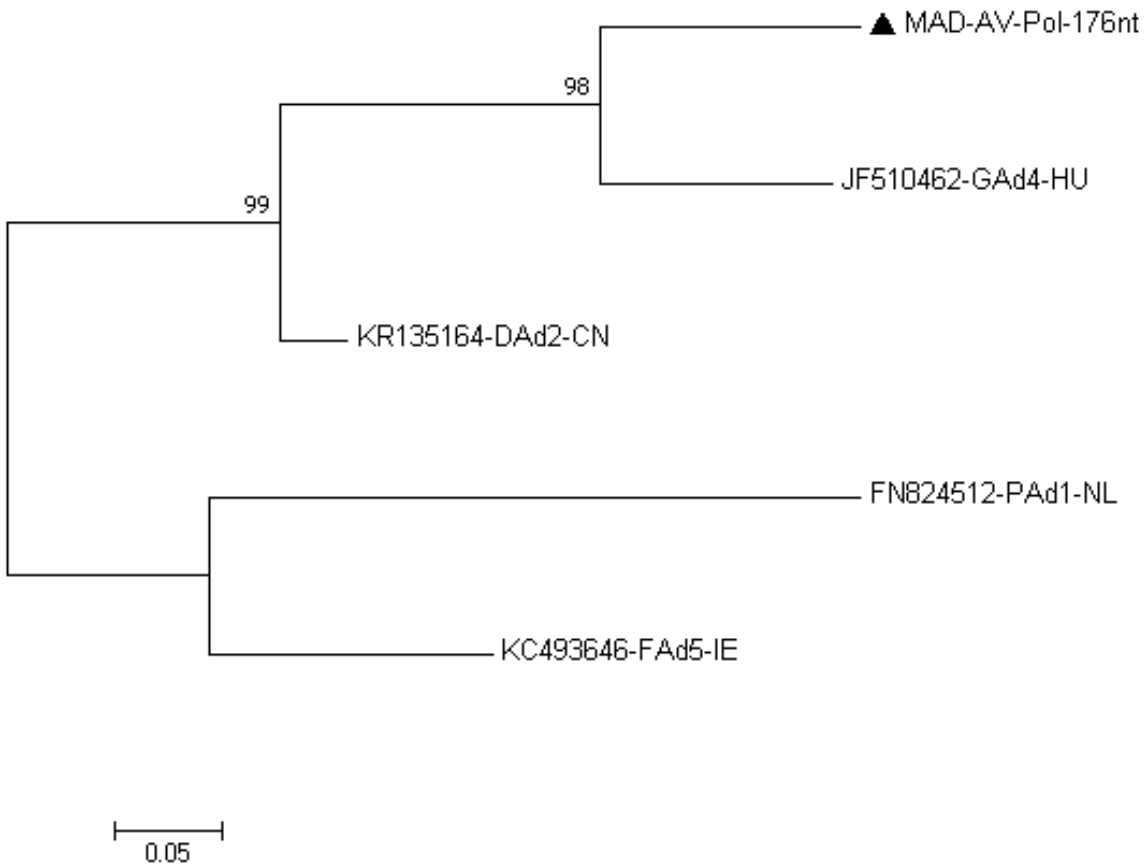


Figure S4.4: Molecular Phylogenetic analysis by Maximum Likelihood method of AV partial Pol gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-747.36) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 25.85% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 5 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 176 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S4.4: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 176 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-AV-Pol-176nt				
KR135164-DAd2-CN	38			
JF510462-GAd4-HU	31	37		
FN824512-PAd1-NL	65	56	59	
KC493646-FAd5-IE	57	44	56	47

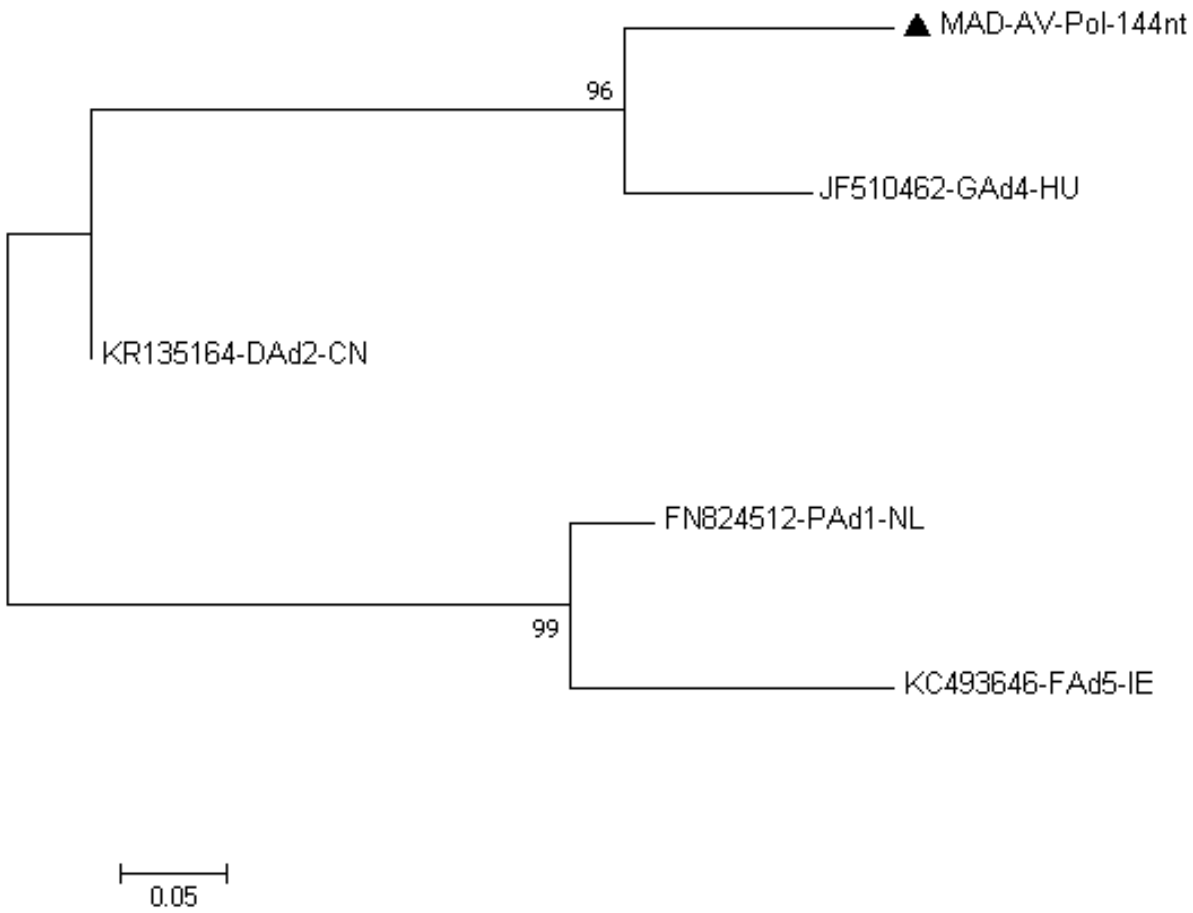


Figure S4.5: Molecular Phylogenetic analysis by Maximum Likelihood method of AV partial Pol gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Jukes-Cantor model[9]. The tree with the highest log likelihood (-566.00) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 30.90% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 5 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 144 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S4.5: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 144 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-AV-Pol-144nt				
KR135164-DAd2-CN	33			
JF510462-GAd4-HU	23	31		
FN824512-PAd1-NL	45	31	39	
KC493646-FAd5-IE	45	36	38	21

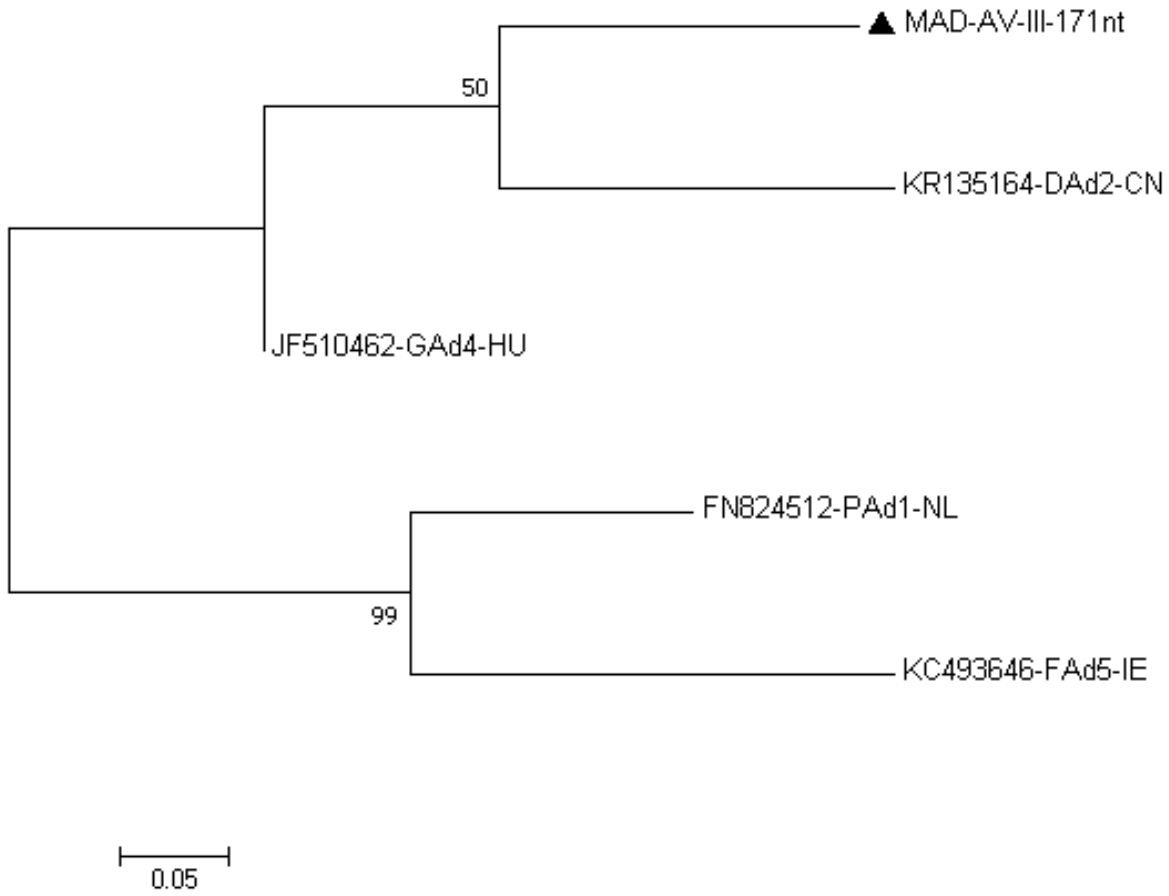


Figure S4.6: Molecular Phylogenetic analysis by Maximum Likelihood method of AV partial III gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-721.85) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 26.61% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 5 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 171 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S4.6: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 171 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-AV-III-171nt				
KR135164-DAd2-CN	40			
JF510462-GAd4-HU	33	37		
FN824512-PAd1-NL	61	48	45	
KC493646-FAd5-IE	60	61	48	39

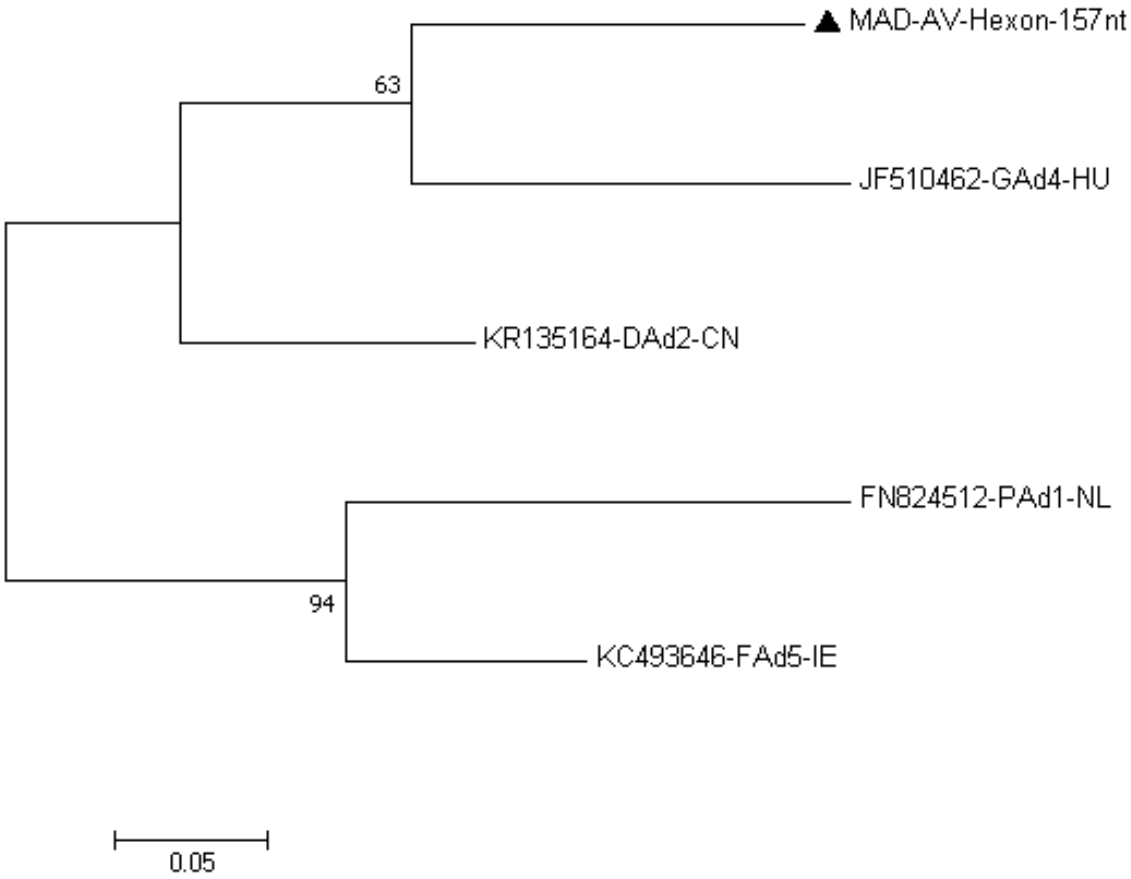


Figure S4.7: Molecular Phylogenetic analysis by Maximum Likelihood method of AV partial Hexon gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-605.36) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 31.21% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 5 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 157 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S4.7: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 157 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-AV-Hexon-157nt				
KR135164-DAd2-CN	30			
JF510462-GAd4-HU	30	33		
FN824512-PAd1-NL	42	38	41	
KC493646-FAd5-IE	42	31	33	27

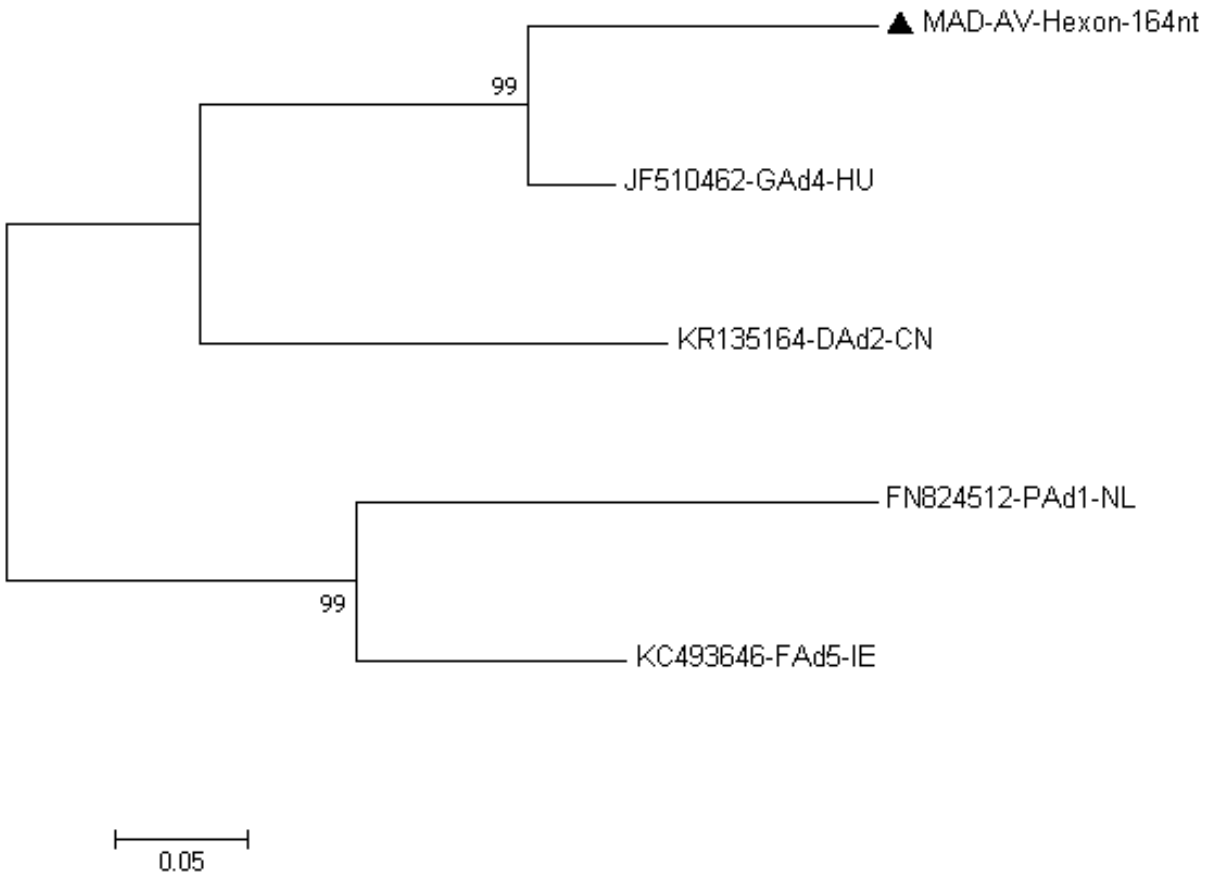


Figure S4.8: Molecular Phylogenetic analysis by Maximum Likelihood method of AV partial Hexon gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-618.97) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 30.79% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 5 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 164 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S4.8: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 164 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-AV-Hexon-164nt				
KR135164-DAd2-CN	35			
JF510462-GAd4-HU	21	31		
FN824512-PAd1-NL	49	42	45	
KC493646-FAd5-IE	46	39	39	30

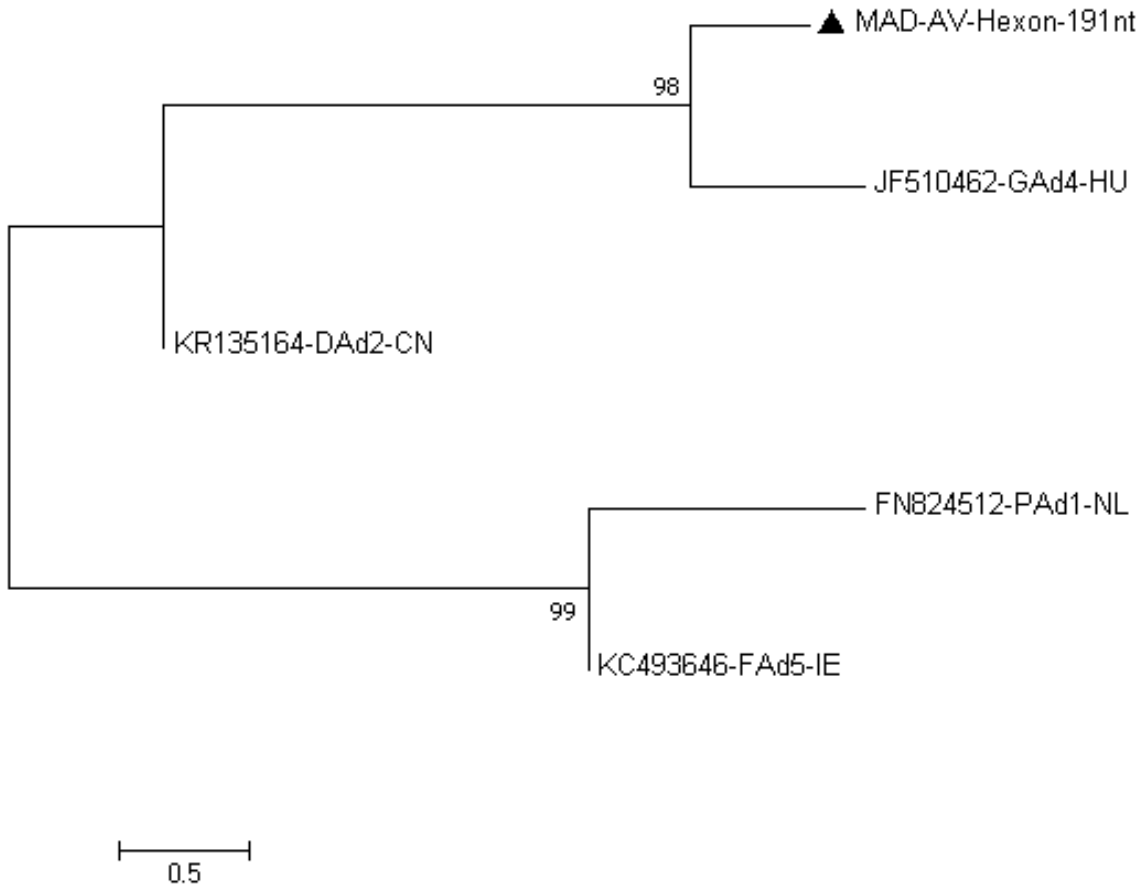


Figure S4.9: Molecular Phylogenetic analysis by Maximum Likelihood method of AV partial Hexon gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-778.30) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.6781)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 29.84% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 5 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 191 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S4.9: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 191 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-AV-Hexon-191nt				
KR135164-DAd2-CN	49			
JF510462-GAd4-HU	33	41		
FN824512-PAd1-NL	65	52	62	
KC493646-FAd5-IE	59	49	61	33

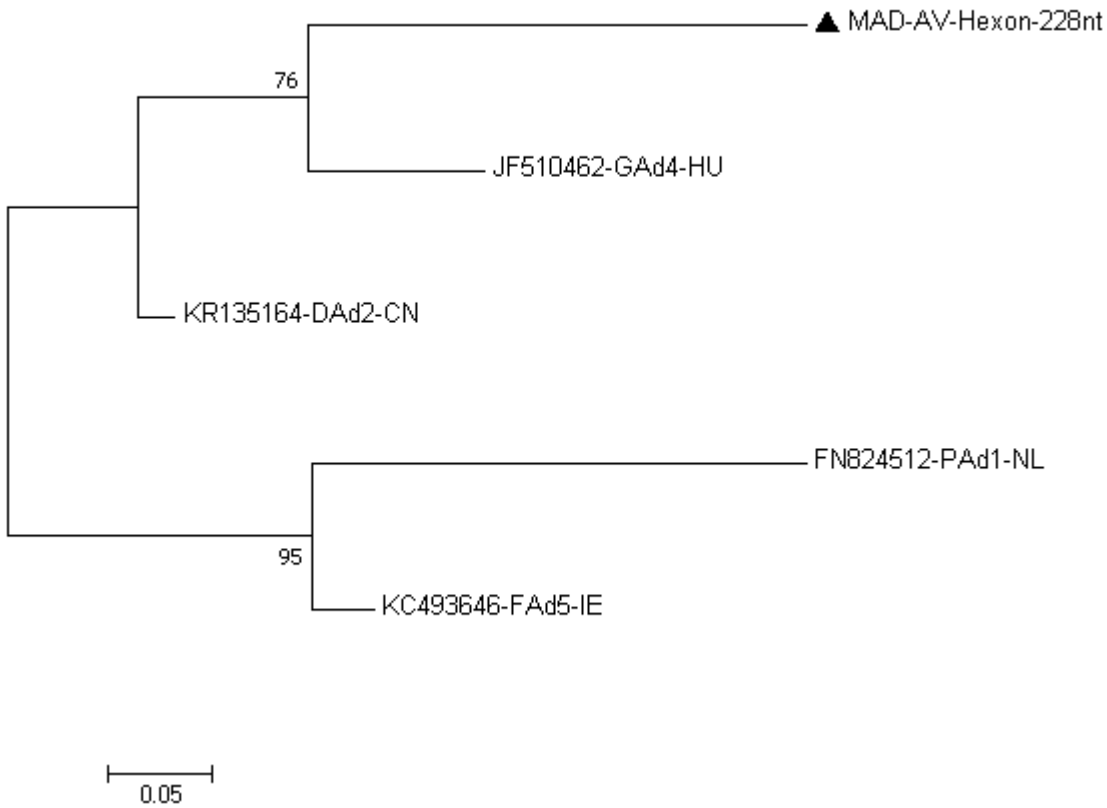


Figure S4.10: Molecular Phylogenetic analysis by Maximum Likelihood method of AV partial Hexon gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-781.58) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 33.99% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 5 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 228 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S4.10: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 228 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-AV-Hexon-228nt				
KR135164-DAd2-CN	43			
JF510462-GAd4-HU	40	28		
FN824512-PAd1-NL	61	41	42	
KC493646-FAd5-IE	54	36	41	36

5. Adeno-associated virus from MAD faecal sample

Figure and Table	Long Name (Format: Sample-virus-protein-length-quality-coverage-year)	Short name (Format: Sample-virus-protein-length)	Coverage	No. of nucleotides	Mapping quality threshold	NCBI accession number
S5.1	MAD-Adenoassociated-virus-rep-protein-94nt-Q20-C-4-5-2016	MAD-AAV-RP-94nt	4-5	94	20	MH107865
S5.2	MAD-Adenoassociated-virus-capsid-protein-279nt-Q32-C-7-27-2016	MAD-AAV-CP-279nt	7-27	279	32	MH107866
S5.3	MAD-Adenoassociated-virus-capsid-protein-142nt-Q32-C-3-4-2016	MAD-AAV-CP-142nt	3-4	142	32	MH107867
NCBI sequences taken for phylogenetic analysis						
Long Name		Short Name (Format: NCBI accession number-virus-country/state)	Country of collection	Collection date		
KX583629-Adenoassociated-virus-isolate-MHH-05-2015-complete-genome		KX583629-AAV-CN	China	2015		
KY475562-Goose-parvovirus-strain-RC16-complete-genome		KY475562-GPV-CN	China	2016		
KY679174-Duck-parvovirus-isolate-SC16-complete-genome		KY679174-DPV-CN	China	2016		
KY069274-Muscovy-duck-parvovirus-strain-LH-complete-genome		KY069274-MDPV-CN	China	2008		
KR265069-Goose-parvovirus-strain-WX2-capsid-protein-(VP)-gene-complete-cds		KR265069-GPV-CN	China	2014		

The table points towards the molecular phylogenetic analysis by Maximum Likelihood method of AAV. The first half of the table provides details to identify the sample from which the virus was isolated, protein that encodes the consensus sequences that are being analysed, the length of the consensus sequences thus providing the depth of the gene being analysed, minimum mapping quality threshold used in IGV for the generation of the consensus sequences, the coverage of the consensus sequences from IGV, the year of collection of the sample and the NCBI accession number assigned to the particular consensus sequence. Corresponding short names have been used in the phylogenetic trees which contain the sample, virus, protein and the number of nucleotides. The second half of the table provides the details of the sequences that were used for the molecular phylogenetic analysis. They were found to be the most closely related sequences to the consensus

sequences generated using BLASTN. Corresponding short names have been used in the phylogenetic trees which contain the NCBI accession number, virus and the country of collection. The collection date is given as retrieved from the corresponding NCBI nucleotide reference dataset which together with the country of collection can provide an insight into the possible evolution of the virus through the years.

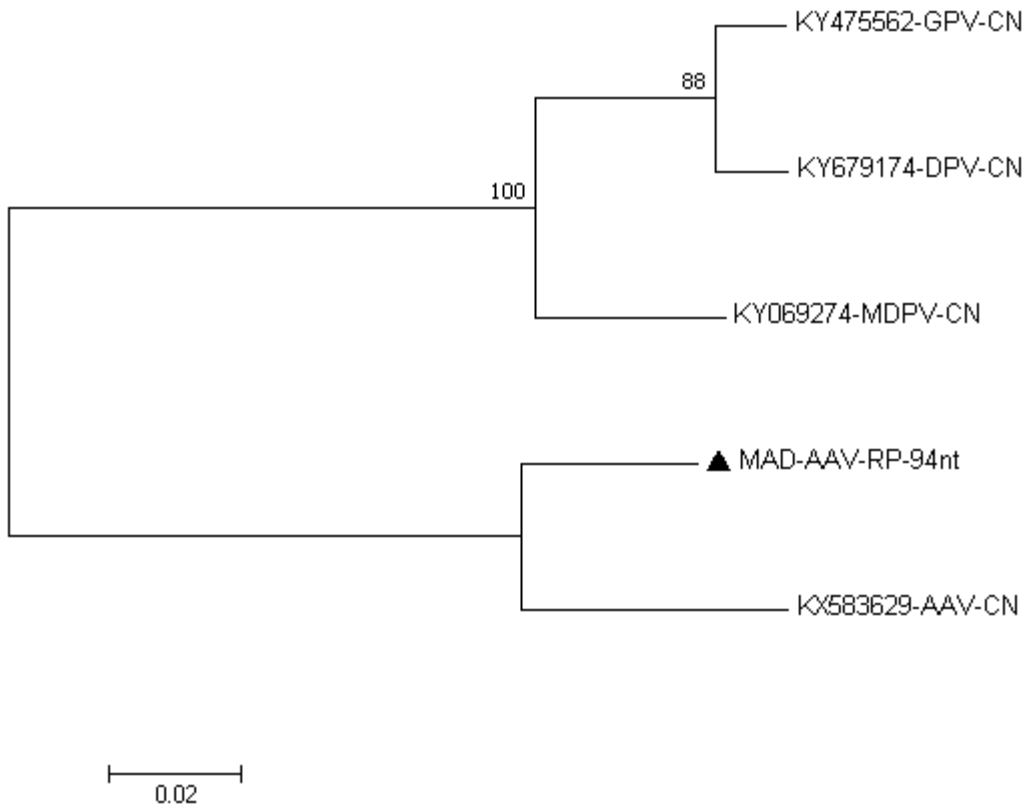


Figure S5.1: Molecular Phylogenetic analysis by Maximum Likelihood method of AAV partial RP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Jukes-Cantor model[9]. The tree with the highest log likelihood (-255.32) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 94 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S5.1: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 94 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-AAV-RP-94nt				
KX583629-AAV-CN	6			
KY475562-GPV-CN	16	18		
KY069274-MDPV-CN	17	18	6	
KY679174-DPV-CN	18	18	2	6

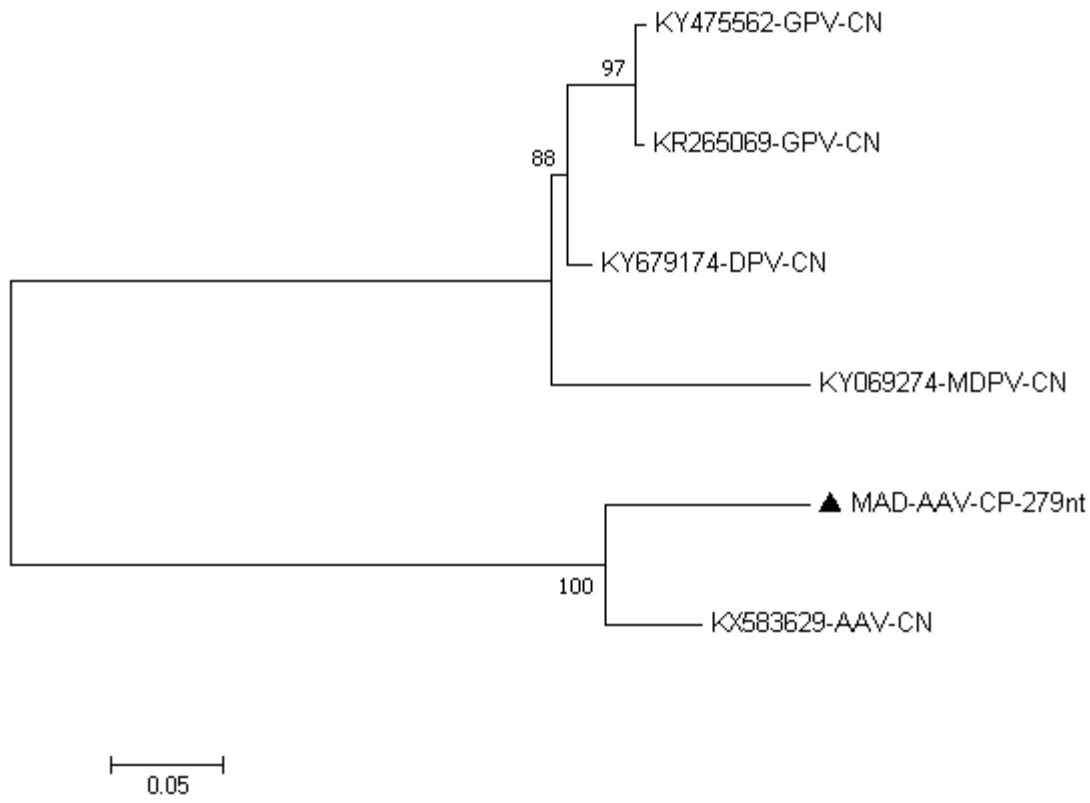


Figure S5.2: Molecular Phylogenetic analysis by Maximum Likelihood method of AAV partial CP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-889.81) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.5762)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 6 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 255 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [2].

Table S5.2: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 6 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 255 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-AAV-CP-279nt					
KX583629-AAV-CN	29				
KY475562-GPV-CN	87	82			
KY679174-DPV-CN	85	81	11		
KY069274-MDPV-CN	89	88	34	29	
KR265069-GPV-CN	85	81	2	11	32

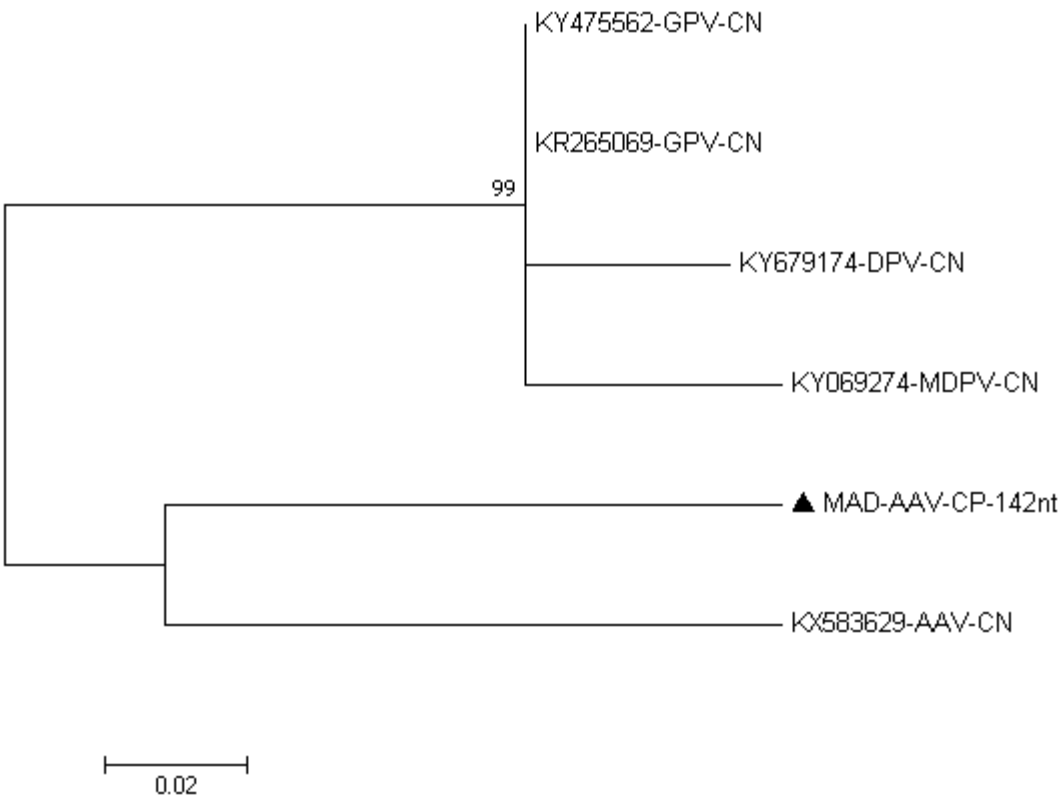


Figure S5.3: Molecular Phylogenetic analysis by Maximum Likelihood method of AAV partial CP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Jukes-Cantor model[9]. The tree with the highest log likelihood (-403.33) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 6 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 142 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S5.3: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 6 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 142 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-AAV-CP-142nt					
KX583629-AAV-CN	22				
KY475562-GPV-CN	23	23			
KY679174-DPV-CN	27	27	4		
KY069274-MDPV-CN	23	25	5	7	
KR265069-GPV-CN	23	23	0	4	5

6. Rotavirus G from MAD faecal sample

Figure and Table	Long Name (Format: Sample-virus-segment-protein-length-quality-coverage-year)	Short name (Format: Sample-virus-segment-protein-length)	Coverage	No. of nucleotides	Mapping quality threshold	NCBI accession number
S6.1	MAD-RotavirusG-seg1-VP1-230nt-Q10-C-3-14-2016	MAD-RG-s1-VP1-230nt	3-14	230	10	MH085089
S6.2	MAD-RotavirusG-seg3VP3-162nt-Q10-C-3-7-2016	MAD-RG-s3-VP3-162nt	3-7	162	10	MH085090
S6.3	MAD-RotavirusG-seg8-NSP2-149nt-Q10-C-3-6-2016	MAD-RG-s8-NSP2-149nt	3-6	149	10	MH085891
NCBI sequences taken for phylogenetic analysis						
Long Name		Short Name (Format: NCBI accession number-virus-segment-country/state)	Country of collection	Collection date		
Segment 1						
KC876010-RotavirusG-pigeon/HK18-strain-HK18-VP1-gene-complete-cds		KC876010-PRG-s1-CN	China	2011		
JN596592-RotavirusG-chicken/03V0567/DEU/2003-segment-1-complete-sequence		JN596592-CRG-s1-DE	Germany	2003		
KX362367-RotavirusB-strain-RVB/Pig-wt/VNM/12089-7/VP1-RNA-dependent-RNA-polymerase-(VP1)-gene-partial-cds		KX362367-PiRB-VN	Vietnam	2012		
Segment 3/4 (as per matched NCBI submitted reference sequence)						
JQ920005-RotavirusG-chicken/03V0567/DEU/2003-segment4-complete-sequence		JQ920005-CRG-s4-DE	Germany	2003		
KJ752086-RotavirusG-strain-RVG/chicken/ZAF/MRC-DPRU1679/2011/GXPX-segment3-RNA-capping-protein-VP3-(VP3)-gene-complete-cds		KJ752086-CRG-s3-ZA	South Africa	2011		
KC876012-RotavirusG-pigeon/HK18-strain-HK18-VP3-gene-complete-cds		KC876012-PRG-s4-CN	China	2011		
Segment 8						

KC876006-RotavirusG-pigeon/HK18-strain-HK18-NSP2-gene-complete-cds	KC876006-PRG-s8-CN	China	2011
JQ920009-RotavirusG-chicken/03V0567/DEU/2003-segment8-complete-sequence	JQ920009-CRG-s8-DE	Germany	2003
KJ752080-RotavirusG-strain-RVG/chicken/ZAF/MRC-DPRU1679/2011/GXPX-segment8-non-structural-protein-2-(NSP2)-gene-complete-cds	KJ752080-CRG-s8-ZA	South Africa	2011

The table points towards the molecular phylogenetic analysis by Maximum Likelihood method of RG. The first half of the table provides details to identify the sample from which the virus was isolated, protein that encodes the consensus sequences that are being analysed, the length of the consensus sequences thus providing the depth of the gene being analysed, minimum mapping quality threshold used in IGV for the generation of the consensus sequences, the coverage of the consensus sequences from IGV, the year of collection of the sample and the NCBI accession number assigned to the particular consensus sequence. Corresponding short names have been used in the phylogenetic trees which contain the sample, virus, protein and the number of nucleotides. The second half of the table provides the details of the sequences that were used for the molecular phylogenetic analysis. They were found to be the most closely related sequences to the consensus sequences generated using BLASTN. Corresponding short names have been used in the phylogenetic trees which contain the NCBI accession number, virus and the country of collection. The collection date is given as retrieved from the corresponding NCBI nucleotide reference dataset which together with the country of collection can provide an insight into the possible evolution of the virus through the years.

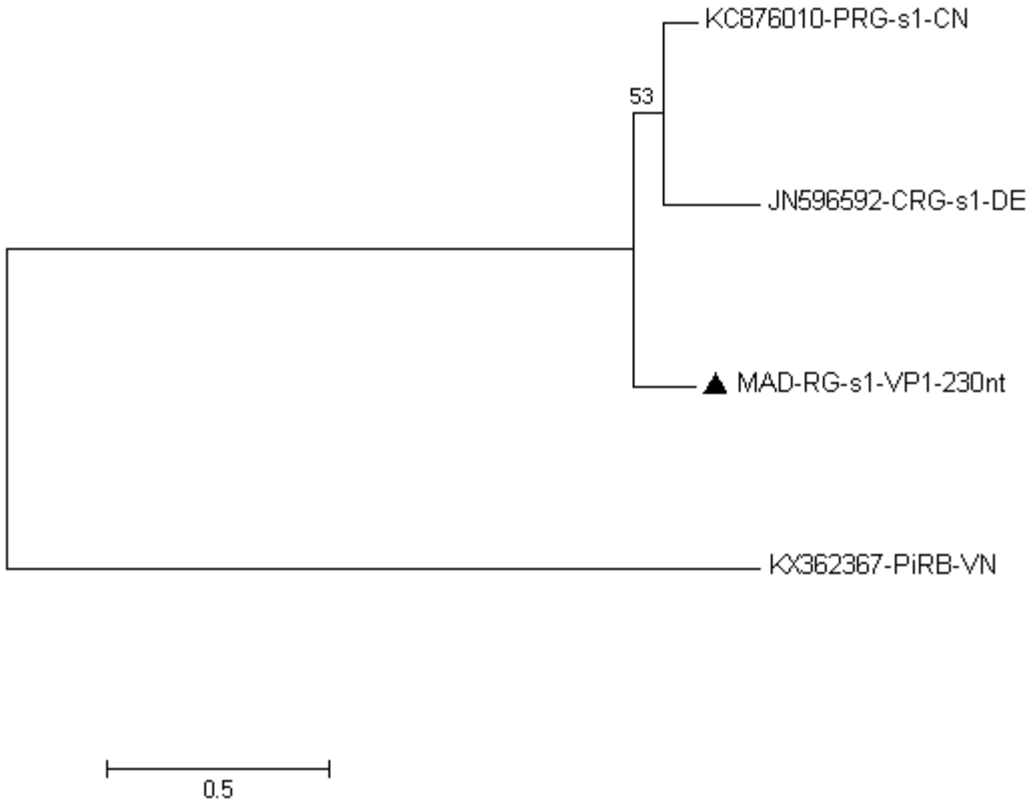


Figure S6.1: Molecular Phylogenetic analysis by Maximum Likelihood method of RG partial VP1 gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model[4]. The tree with the highest log likelihood (-805.99) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 36.76% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 4 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 227 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S6.1: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 4 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 227 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-RG-s1-VP1-230nt			
KC876010-PRG-s1-CN	44		
JN596592-CRG-s1-DE	57	38	
KX362367-PiRB-VN	92	88	87

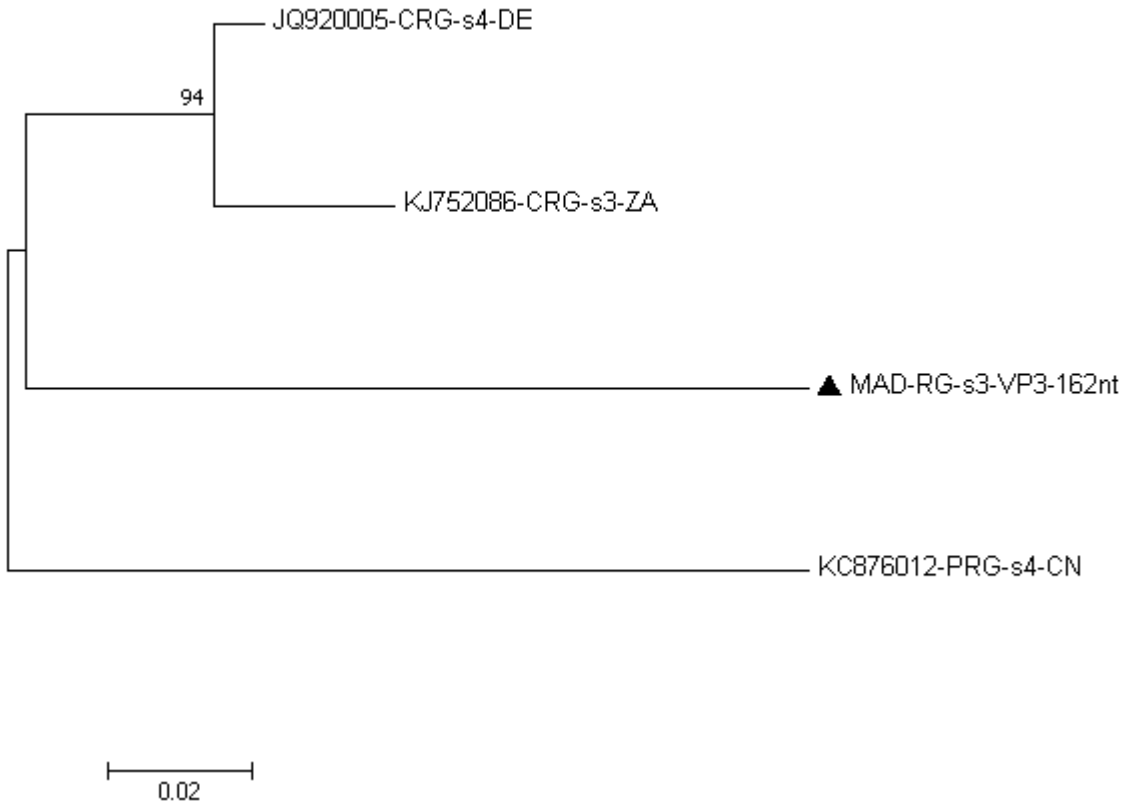


Figure S6.2: Molecular Phylogenetic analysis by Maximum Likelihood method of RG partial VP3 gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model[4]. The tree with the highest log likelihood (-388.80) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 4 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 162 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S6.2: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 4 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 162 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-RG-s3-VP3-162nt			
JQ920005-CRG-s4-DE	20		
KJ752086-CRG-s3-ZA	23	5	
KC876012-PRG-s4-CN	29	21	22

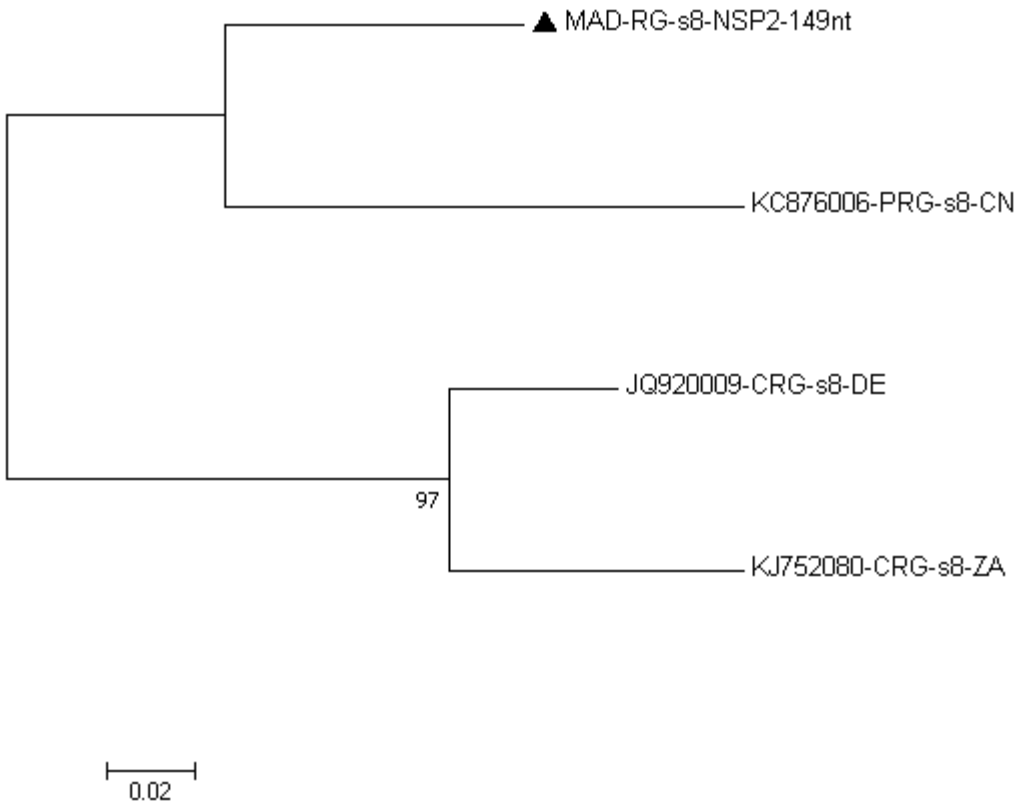


Figure S6.3: Molecular Phylogenetic analysis by Maximum Likelihood method of RG partial NSP2 gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model[4]. The tree with the highest log likelihood (-383.45) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.4597)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 4 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 149 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S6.3: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 4 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 149 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MAD-RG-s8-NSP2-149nt			
KC876006-PRG-s8-CN	19		
JQ920009-CRG-s8-DE	22	26	
KJ752080-CRG-s8-ZA	26	23	13

7. Hubei chryso-like virus 1 from MUD faecal sample

Figure and Table	Long Name (Format: Sample-virus-segment-protein-length-quality-coverage-year)	Short name (Format: Sample-virus-segment-protein-length)	Coverage	No. of nucleotides	Mapping quality threshold	NCBI accession number
S7.1	MUD-Hubei-chryso-like-virus-1-seg2-putative-protease-2175nt-Q32-C-4-71-2016	MUD-HCLV1-s2-PP-2175nt	4-71	2175	32	MH085093
S7.2	MUD-Hubei-chryso-like-virus-1-seg3-hypothetical-protein-536nt-Q32-C-9-42-2016	MUD-HCLV1-s3-HP-536nt	9-42	536	32	MH085094
S7.3	MUD-Hubei-chryso-like-virus-1-seg4-hypothetical-protein-1938nt-Q32-C-3-121-2016	MUD-HCLV1-s4-HP-1938nt	3-121	1938	32	MH085095
NCBI sequences taken for phylogenetic analysis						
Long Name		Short Name (Format: NCBI accession number-virus-segment-country/state)	Country or State of collection	Collection date		
Segment 2						
MF176389-Hubei-chryso-like-virus-1-strain-mosWSX51080-segment2-complete-sequence		MF176389-HCLV1-s2-WA	Western Australia	2015		
MF176262-Hubei-chryso-like-virus-1-strain-mos172gb42656-segment2-complete-sequence		MF176262-HCLV1-s2-WA	Western Australia	2015		
MF176310-Hubei-chryso-like-virus-1-strain-mos191gb77171-segment2-complete-sequence		MF176310-HCLV1-s2-WA	Western Australia	2015		
MF176281-Hubei-chryso-like-virus-1-strain-mos172X13576-segment2-complete-sequence		MF176281-HCLV1-s2-WA	Western Australia	2015		
MF176369-Hubei-chryso-like-virus-1-strain-mosWSgb49785-segment2-complete-sequence		MF176369-HCLV1-s2-WA	Western Australia	2015		
Segment 3						

MF176263-Hubei-chryso-like-virus-1-strain-mos172gb42656-segment3-complete-sequence	MF176263-HCLV1-s3-WA	Western Australia	2015
MF176370-Hubei-chryso-like-virus-1-strain-mosWSgb49785-segment3-complete-sequence	MF176370-HCLV1-s3-WA	Western Australia	2015
MF176390-Hubei-chryso-like-virus-1-strain-mosWSX51080-segment3-complete-sequence	MF176390-HCLV1-s3-WA	Western Australia	2015
MF176282-Hubei-chryso-like-virus-1-strain-mos172X13576-segment3-complete-sequence	MF176282-HCLV1-s3-WA	Western Australia	2015
Segment 4			
MF176391-Hubei-chryso-like-virus-1-strain-mosWSX51080-segment4-complete-sequence	MF176391-HCLV1-s4-WA	Western Australia	2015
MF176371-Hubei-chryso-like-virus-1-strain-mosWSgb49785-segment4-complete-sequence	MF176371-HCLV1-s4-WA	Western Australia	2015
MF176312-Hubei-chryso-like-virus-1-strain-mos191gb77171-segment4-complete-sequence	MF176312-HCLV1-s4-WA	Western Australia	2015
MF176283-Hubei-chryso-like-virus-1-strain-mos172X13576-segment4-complete-sequence	MF176283-HCLV1-s4-WA	Western Australia	2015
MF176264-Hubei-chryso-like-virus-1-strain-mos172gb42656-segment-4-complete-sequence	MF176264-HCLV1-s4-WA	Western Australia	2015

The table points towards the molecular phylogenetic analysis by Maximum Likelihood method of HCLV1. The first half of the table provides details to identify the sample from which the virus was isolated, protein that encodes the consensus sequences that are being analysed, the length of the consensus sequences thus providing the depth of the gene being analysed, minimum mapping quality threshold used in IGV for the generation of the consensus sequences, the coverage of the consensus sequences from IGV, the year of collection of the sample and the NCBI accession number assigned to the particular consensus sequence. Corresponding short names have been used in the phylogenetic trees which contain the sample, virus, protein and the number of nucleotides. The second half of the table provides the details of the sequences that were used for the molecular phylogenetic analysis. They were found to be the most closely related sequences to the consensus sequences generated using BLASTN. Corresponding short names have been used in the phylogenetic trees which contain the NCBI accession number, virus and the country of collection. The collection date is given as retrieved from the corresponding NCBI nucleotide reference dataset which together with the country of collection can provide an insight into the possible evolution of the virus through the years.

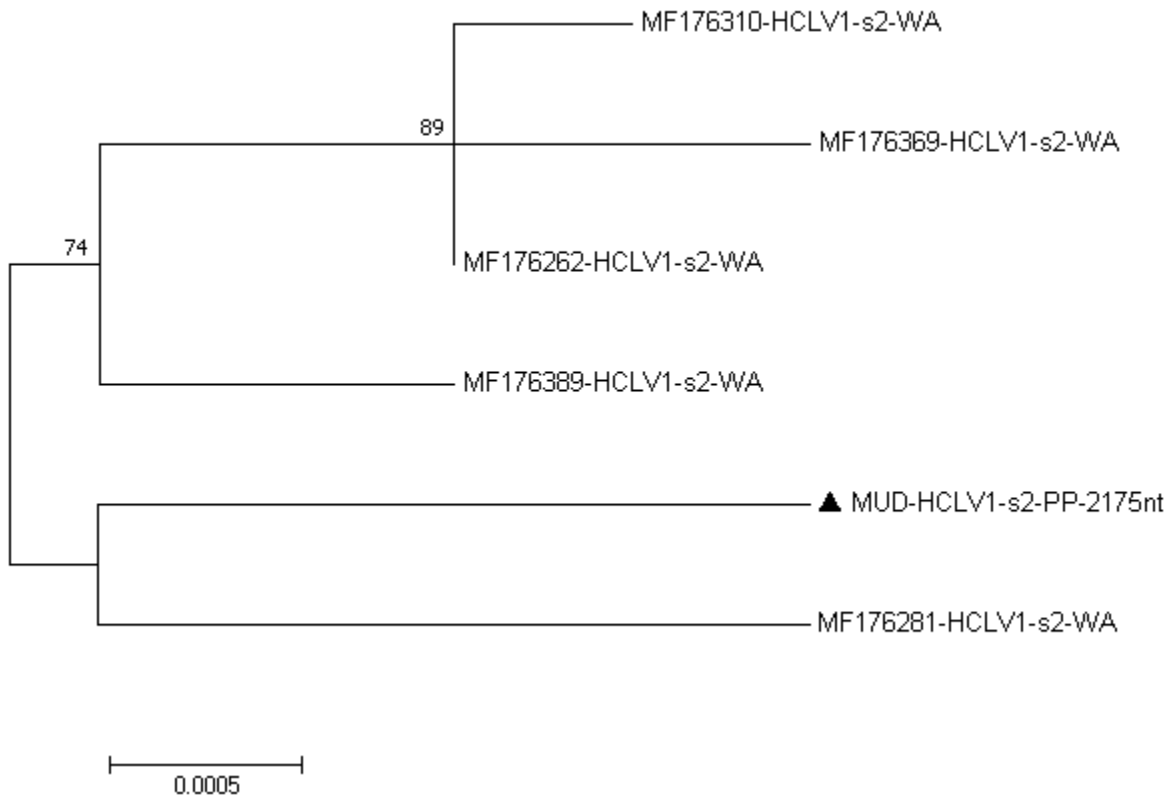


Figure S7.1: Molecular Phylogenetic analysis by Maximum Likelihood method of HCLV1 partial PP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model[5]. The tree with the highest log likelihood (-3129.64) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 6 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 2175 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S7.1: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 6 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 2175 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MUD-HCLV1-s2-PP-2175nt					
MF176389-HCLV1-s2-WA	7				
MF176262-HCLV1-s2-WA	7	4			
MF176310-HCLV1-s2-WA	8	5	1		
MF176281-HCLV1-s2-WA	8	7	7	8	
MF176369-HCLV1-s2-WA	9	6	2	3	9

Figure S7.2: Molecular Phylogenetic analysis by Maximum Likelihood method of HCLV1 partial HP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Jukes-Cantor model[9]. The tree with the highest log likelihood (-782.19) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 536 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S7.2: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 536 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MUD-HCLV1-s3-HP-536nt				
MF176390-HCLV1-s3-WA	2			
MF176263-HCLV1-s3-WA	2	0		
MF176370-HCLV1-s3-WA	3	1	1	
MF176282-HCLV1-s3-WA	4	2	2	3

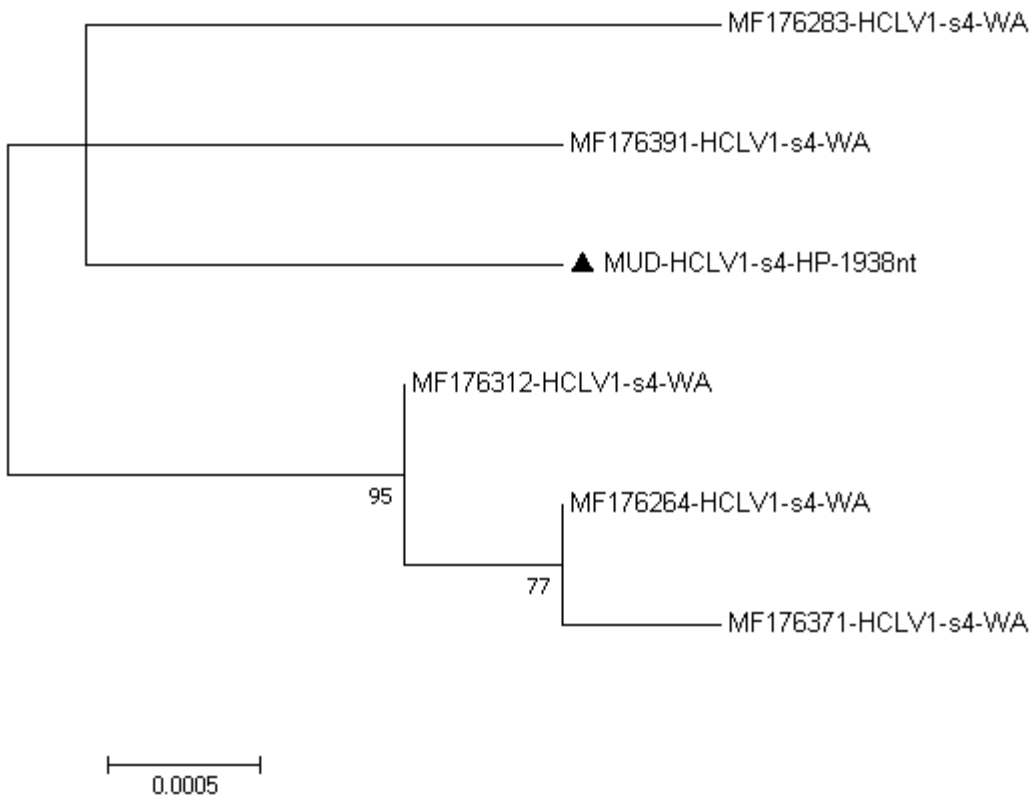


Figure S7.3: Molecular Phylogenetic analysis by Maximum Likelihood method of HCLV1 partial HP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model[5]. The tree with the highest log likelihood (-2775.63) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 6 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 1938 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S7.3: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 6 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 1938 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MUD-HCLV1-s4-HP-1938nt					
MF176283-HCLV1-s4-WA	7				
MF176391-HCLV1-s4-WA	6	7			
MF176312-HCLV1-s4-WA	6	7	6		
MF176264-HCLV1-s4-WA	7	8	7	1	
MF176371-HCLV1-s4-WA	8	9	8	2	1

8. Culex Negev like virus 3 of MUD faecal sample

Figure and Table	Long Name (Format: Sample-virus-protein-length-quality-coverage-year)	Short name (Format: Sample-virus-protein-length)	Coverage	No. of nucleotides	Mapping quality threshold	NCBI accession number
8.1	MUD-Culex-negev-like-virus3-Rp-531nt-Q32-C-3-22-2016	MUD-CNLV3-Rp-531nt	3-22	531	32	MH085096
8.2	MUD-Culex-negev-like-virus3-Rp-275nt-Q32-C-5-30-2016	MUD-CNLV3-Rp-275nt	5-30	275	32	MH085097
8.3	MUD-Culex-negev-like-virus3-putative-membrane-protein-456nt-Q32-C-3-19-2016	MUD-CNLV3-PMP-456nt	3-19	456	32	MH085098
NCBI sequences taken for phylogenetic analysis						
Long Name		Short Name (Format: NCBI accession number-virus-country/state)	Country or State of collection	Collection date		
MF176277-Culex-negev-like-virus3-strain-mos172X44875-complete-genome		MF176277-CNLV3-WA	Western Australia	2015		
MF281708-Biggievirus-Mos11-strain-258719/2008-ORF1-gene-partial-cds-and-ORF2-and-ORF3-genes-complete-cds		MF281708-BV-M11-IT	Italy	2008		
MF281709-Biggievirus-Mos11-strain-pool-11/2008-ORF1-gene-partial-cds-and-ORF2-and-ORF3-genes-complete-cds		MF281709-BV-M11-IT	Italy	2008		
KX924639-Biggievirus-Mos11-replicase-large-subunit-gene-partial-cds-and-hypothetical-protein-genes-complete-cds		KX924639-BV-M11-US	USA	2016		

The table points towards the molecular phylogenetic analysis by Maximum Likelihood method of CNLV3. The first half of the table provides details to identify the sample from which the virus was isolated, protein that encodes the consensus sequences that are being analysed, the length of the consensus sequences thus providing the depth of the gene being analysed, minimum mapping quality threshold used in IGV for the generation of the consensus sequences, the coverage of the consensus sequences from IGV, the year of collection of the sample and the NCBI accession number assigned to the particular consensus sequence. Corresponding short names have been used in the phylogenetic trees which contain the sample, virus, protein and the number of nucleotides. The second half of the table provides the details of the sequences that were used for the molecular

phylogenetic analysis. They were found to be the most closely related sequences to the consensus sequences generated using BLASTN. Corresponding short names have been used in the phylogenetic trees which contain the NCBI accession number, virus and the country of collection. The collection date is given as retrieved from the corresponding NCBI nucleotide reference dataset which together with the country of collection can provide an insight into the possible evolution of the virus through the years.

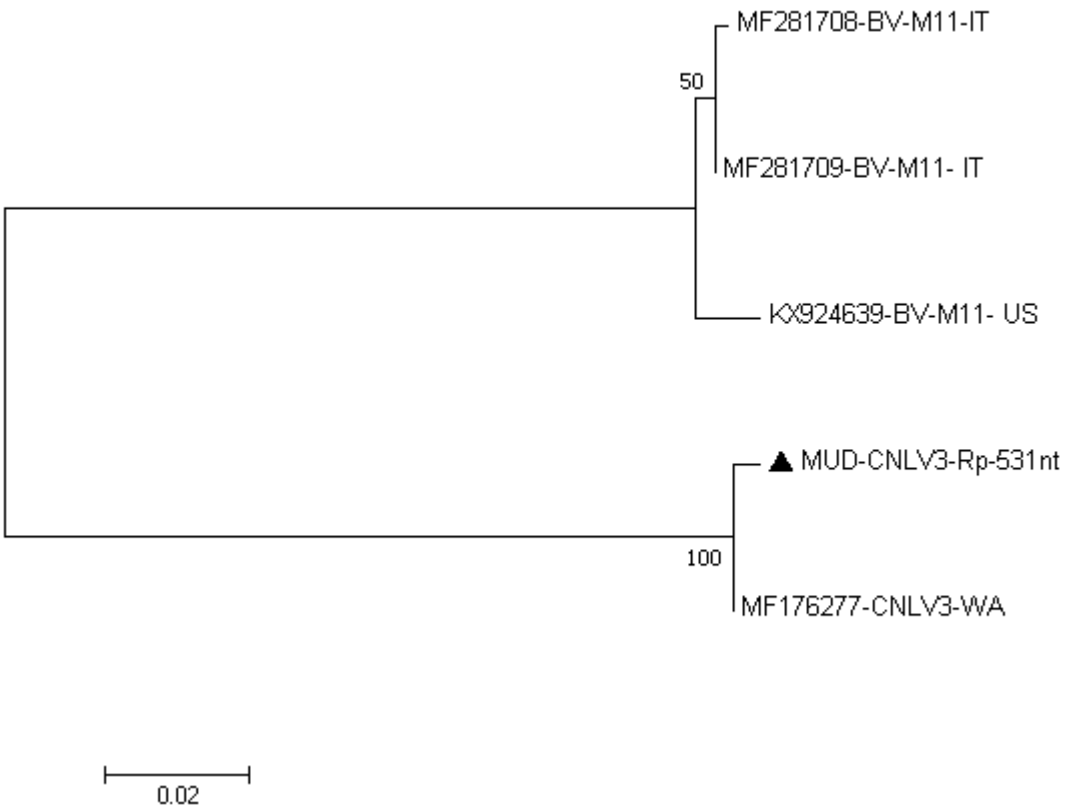


Figure S8.1: Molecular Phylogenetic analysis by Maximum Likelihood method of CNLV3 partial Rp gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model[4]. The tree with the highest log likelihood (-1086.99) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 531 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S8.1: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 531 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MUD-CNLV3-Rp-531nt				
MF176277-CNLV3-WA	2			
MF281708-BV-M11-IT	90	89		
MF281709-BV-M11-_IT	91	90	1	
KX924639-BV-M11-_US	93	92	7	6

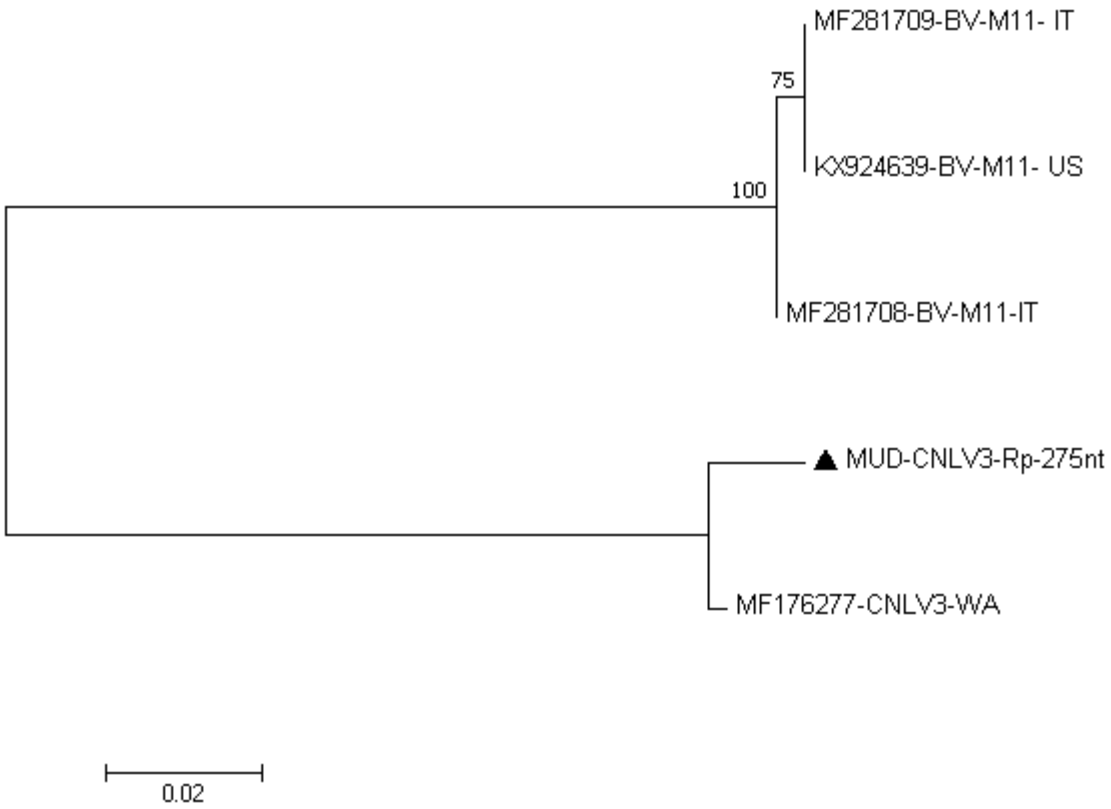


Figure S8.2: Molecular Phylogenetic analysis by Maximum Likelihood method of CNLV3 partial Rp gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model[4]. The tree with the highest log likelihood (-569.79) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 275 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S8.2: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 275 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MUD-CNLV3-Rp-275nt				
MF176277-CNLV3-WA	4			
MF281708-BV-M11-IT	48	46		
MF281709-BV-M11-_IT	49	47	1	
KX924639-BV-M11-_US	49	47	1	0

Figure S8.3: Molecular Phylogenetic analysis by Maximum Likelihood method of CNLV3 partial PMP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model[5]. The tree with the highest log likelihood (-893.63) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 456 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S8.3: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 456 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MUD-CNLV3-PMP-456nt				
MF176277-CNLV3-WA	5			
MF281708-BV-M11-IT	59	60		
MF281709-BV-M11-_IT	59	60	0	
KX924639-BV-M11-_US	62	61	3	3

9. **Hubei reo-like virus 7 like virus from MUD faecal sample**

Figure and Table	Long Name (Format: Sample-virus-protein-length-quality-coverage-year)	Short name (Format: Sample-virus-protein-length)	Coverage	No. of nucleotides	Mapping quality threshold	NCBI accession number
S9.1	MUD-Hubei-reo-like-virus7-Rp-240nt-Q20-C-3-11-2016	MUD-HRLV7L-Rp-240nt	3-11	240	20	MH085099
S9.2	MUD-Hubei-reo-like-virus7-Rp-184nt-Q32-C-3-6-2016	MUD-HRLV7L-Rp-184nt	3-6	184	32	MH085100
S9.3	MUD-Hubei-reo-like-virus7-Rp-117nt-Q32-C-4-5-2016	MUD-HRLV7L-Rp-117nt	4-5	117	32	MH085101
NCBI sequences taken for phylogenetic analysis						
Long Name		Short Name (Format: NCBI accession number-virus-country/state)	Country of collection	Collection date		
KX884635-Hubei-reo-like-virus7-strain-mosHB235771-Rp-gene-complete-cds		KX884635-HRLV7-CN	China	2013		
DQ087277-Aedes-pseudoscutellaris-reovirus-segment2-complete-sequence		DQ087277-APRV-FR	France	-		
KM978429-Fako-virus-strain-CSW87-segment2-RNA-dependent-RNA-polymerase-gene-complete-cds		KM978429-FV-CM	Cameroon	2010		
KX884633-Hubei-mosquito-virus5-strain-mosHB233040-RdRp-gene-partial-cds		KX884633-HMV5-CN	China	2013		

The table points towards the molecular phylogenetic analysis by Maximum Likelihood method of HRLV7L. The first half of the table provides details to identify the sample from which the virus was isolated, protein that encodes the consensus sequences that are being analysed, the length of the consensus sequences thus providing the depth of the gene being analysed, minimum mapping quality threshold used in IGV for the generation of the consensus sequences, the coverage of the consensus sequences from IGV, the year of collection of the sample and the NCBI accession number assigned to the particular consensus sequence. Corresponding short names have been used in the phylogenetic trees which contain the sample, virus, protein and the number of nucleotides. The second half of the table provides the details of the sequences that were used for the molecular phylogenetic analysis. They were found to be the most closely related sequences to the consensus sequences generated using BLASTN. Corresponding short names have been used in the phylogenetic trees which contain the NCBI accession number, virus and the country of collection.

The collection date is given as retrieved from the corresponding NCBI nucleotide reference dataset which together with the country of collection can provide an insight into the possible evolution of the virus through the years.

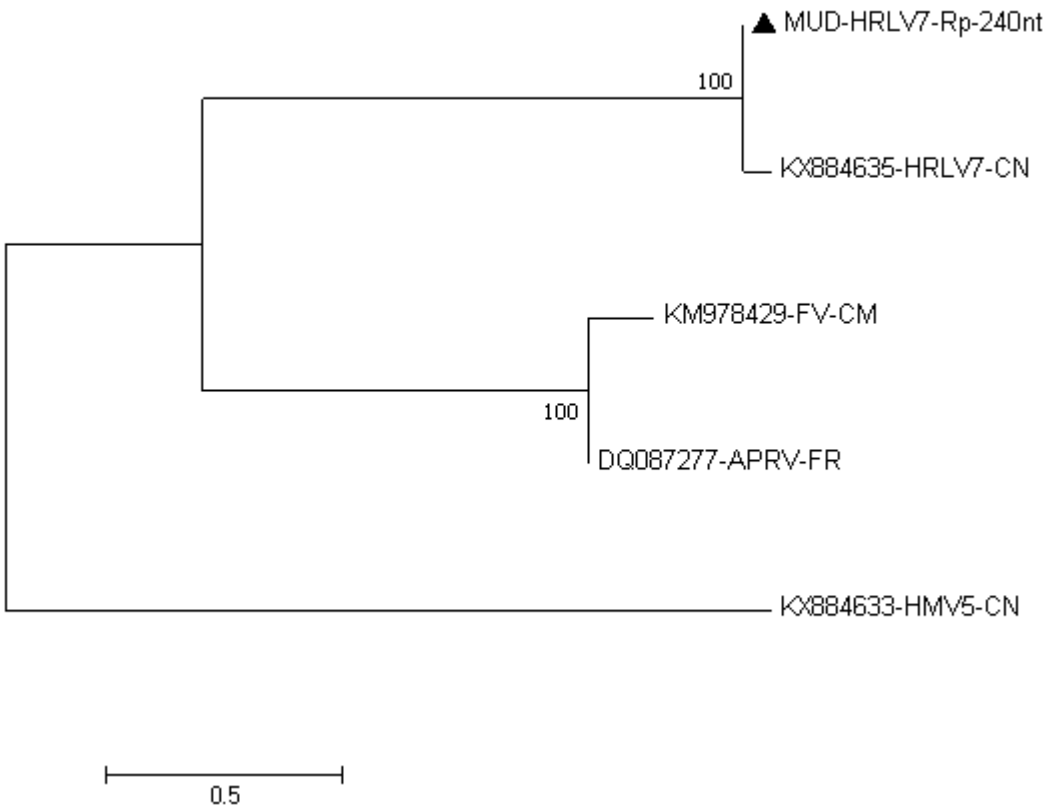


Figure S9.1: Molecular Phylogenetic analysis by Maximum Likelihood method of HRLV7L partial Rp gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model[4]. The tree with the highest log likelihood (-989.22) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 31.77% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 239 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S9.1: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 239 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MUD-HRLV7L-Rp-240nt				
KX884635-HRLV7-CN	13			
KX884633-HMV5-CN	112	111		
KM978429-FV-CM	97	97	97	
DQ087277-APRV-FR	90	91	93	27

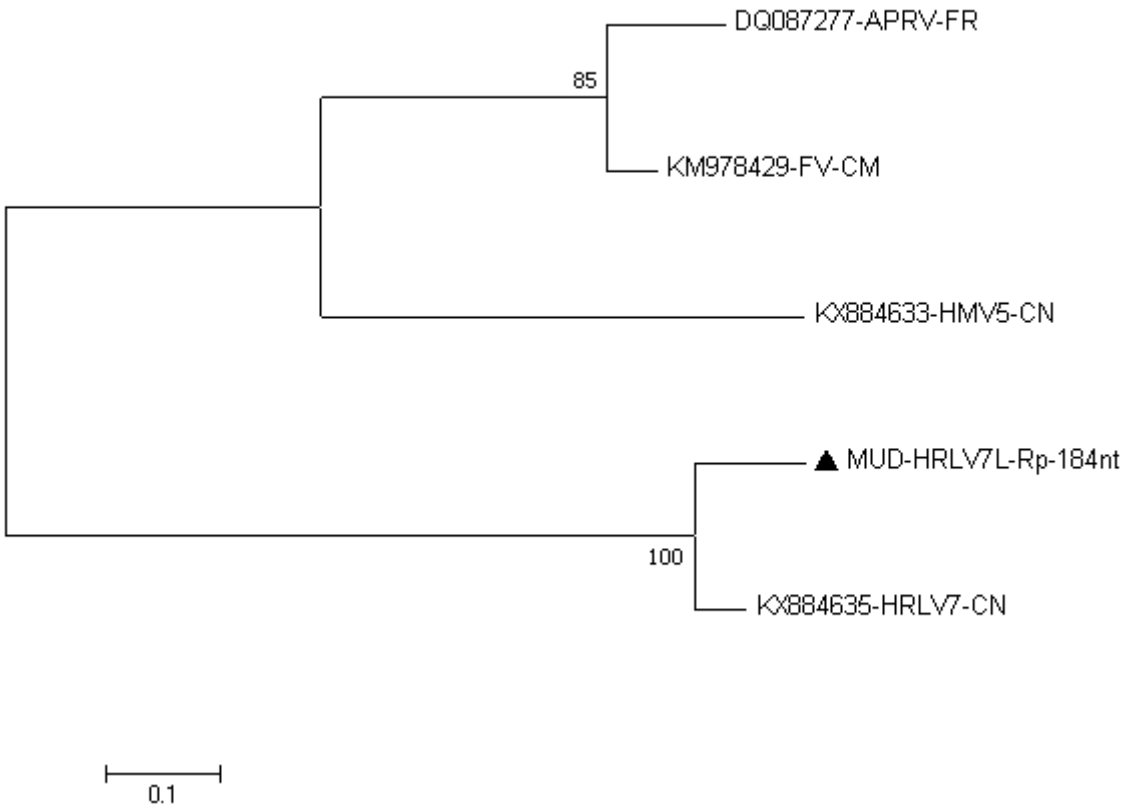


Figure S9.2: Molecular Phylogenetic analysis by Maximum Likelihood method of HRLV7L partial Rp gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model[4]. The tree with the highest log likelihood (-822.59) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 2.1378)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 184 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S9.2. Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 184 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MUD-HRLV7L-Rp-184nt				
KX884635-HRLV7-CN	23			
DQ087277-APRV-FR	95	85		
KM978429-FV-CM	87	87	23	
KX884633-HMV5-CN	98	95	72	70

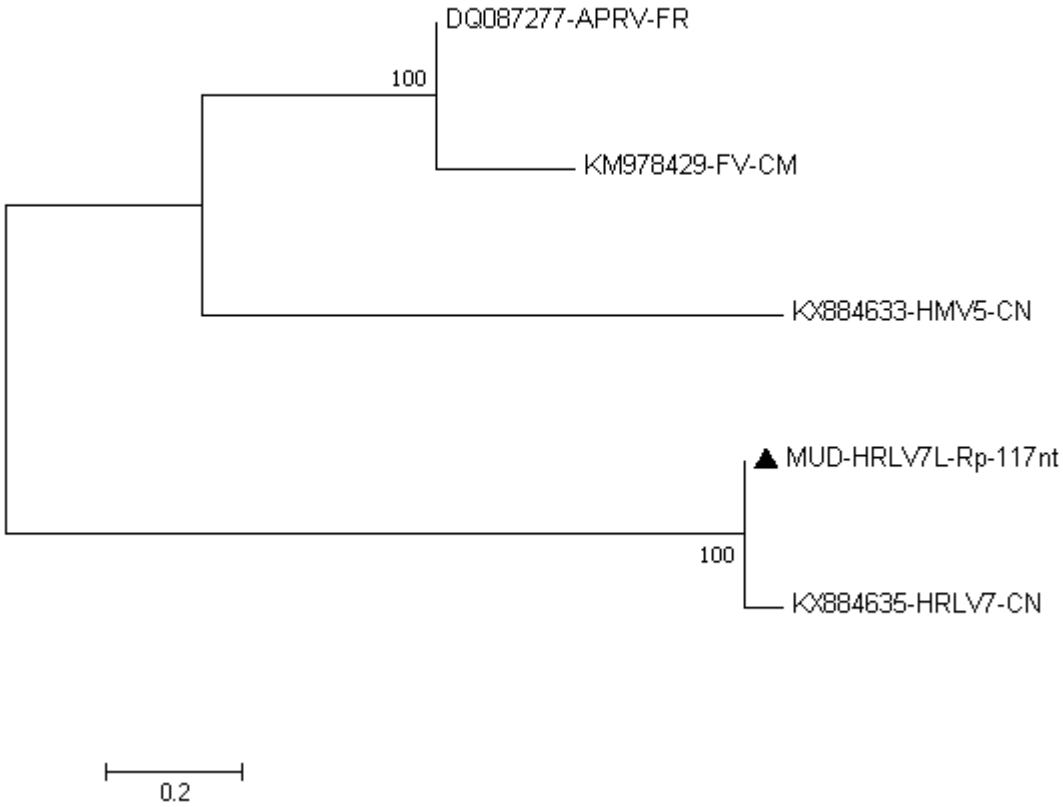


Figure S9.3: Molecular Phylogenetic analysis by Maximum Likelihood method of HRLV7L partial Rp gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model[4]. The tree with the highest log likelihood (-516.09) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 20.42% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 117 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S9.3: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 117 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MUD-HRLV7L-Rp-117nt				
KX884635-HRLV7-CN	6			
DQ087277-APRV-FR	56	58		
KM978429-FV-CM	53	56	19	
KX884633-HMV5-CN	63	63	53	58

10. **Enterobacteria phage phi92 from MUD faecal sample**

Figure and Table	Long Name (Format: Sample-virus-protein-length-quality-coverage-year)	Short name (Format: Sample-virus-protein-length)	Coverage	No. of nucleotides	Mapping quality threshold	NCBI accession number
S10.1	MUD-Enterobacteria-phage-phi92-Phi92-gp141-277nt-Q32-C-2-4-2016	MUD-EPP92-g141-277nt	2-4	277	32	MH188081
S10.2	MUD-Enterobacteria-phage-phi92-Phi92-gp141-aa-276nt-Q32-C-2-4-2016	MUD-EPP92-g141-aa-276nt	2-4	276	32	MH188081
NCBI sequences taken for phylogenetic analysis						
Long Name		Short Name (Format: NCBI accession number-virus-country/state)	Country of collection	Collection date		
FR775895-Enterobacteria-phage-phi92-complete-genome		FR775895-EPP92	-	-		
KU522583-Enterobacteria-phage-ECGD1-complete-genome		KU522583-EP	-	2015		
KX552041-Escherichia-phage-ESCO13-complete-genome		KX552041-EsP-FR	France	2014		
KX664695-Escherichia-phage-ESCO5-complete-genome		KX664695-EsP-FR	France	2014		
JX561091-Escherichia-phage-phAPEC8-complete-genome		JX561091-EsP-BE	Belgium	2010		
KR296694-Salmonella-phage-40-complete-genome		KR296694-SP40-IN	India	2002		

The table points towards the molecular phylogenetic analysis by Maximum Likelihood method of EPP92. The first half of the table provides details to identify the sample from which the virus was isolated, protein that encodes the consensus sequences that are being analysed, the length of the consensus sequences thus providing the depth of the gene being analysed, minimum mapping quality threshold used in IGV for the generation of the consensus sequences, the coverage of the consensus sequences from IGV, the year of collection of the sample and the NCBI accession number assigned to the particular consensus sequence. Corresponding short names have been used in the phylogenetic trees which contain the sample, virus, protein and the number of nucleotides. In case of the amino acid sequence analysis, the short names contain the “aa” after the protein. The second half of the table provides the details of the sequences that were used for the molecular phylogenetic analysis. They were found to be the most closely related sequences to the consensus

sequences generated using BLASTN. Corresponding short names have been used in the phylogenetic trees which contain the NCBI accession number, virus and the country of collection. The collection date is given as retrieved from the corresponding NCBI nucleotide reference dataset which together with the country of collection can provide an insight into the possible evolution of the virus through the years.

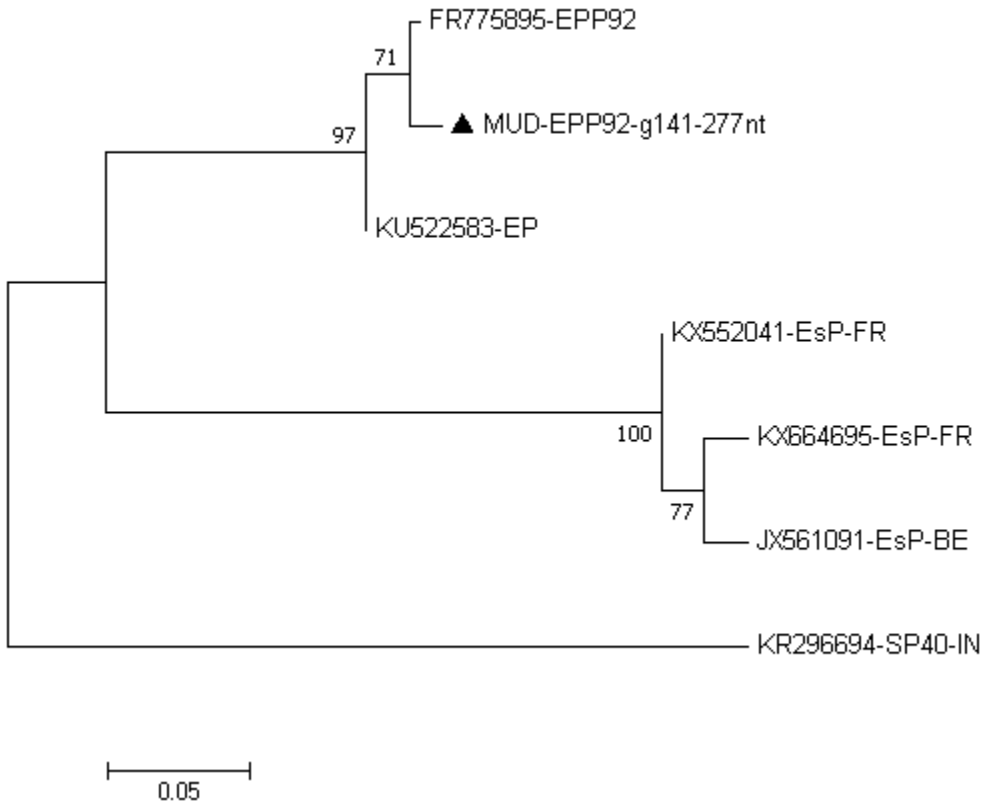


Figure S10.1: Molecular Phylogenetic analysis by Maximum Likelihood method of EPP92 partial g141 gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model[4]. The tree with the highest log likelihood (-899.41) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.4004)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 7 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 277 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S10.1: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 7 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 277 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

FR775895-EPP92						
MUD-EPP92-g141-277nt	4					
KU522583-EP	5	7				
KX552041-EsP-FR	54	54	52			
KX664695-EsP-FR	57	57	55	8		
JX561091-EsP-BE	61	61	59	8	8	
KR296694-SP40-IN	69	67	66	78	77	75

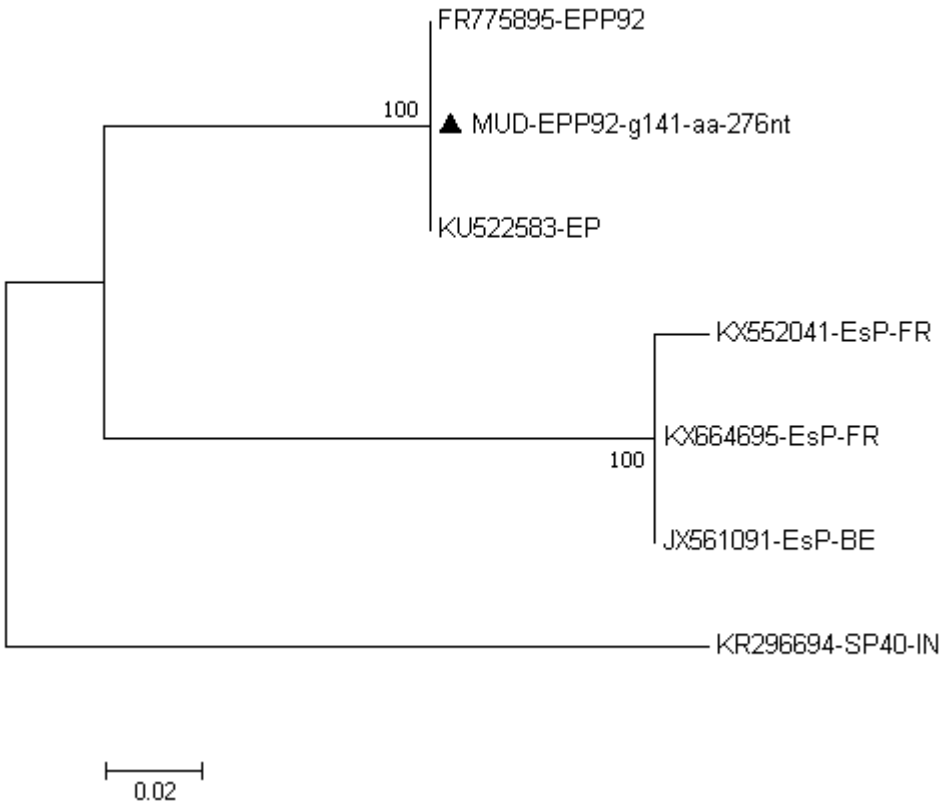


Figure S10.2: Molecular Phylogenetic analysis by Maximum Likelihood method for amino acid sequences of EPP92 partial g141 gene

The evolutionary history was inferred by using the Maximum Likelihood method based on the Le_Gascuel_2008 model[6]. The tree with the highest log likelihood (-431.10) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 7 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 92 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S10.2: Estimates of Evolutionary Divergence between Sequences

The number of amino acid differences per sequence from between sequences are shown. The analysis involved 7 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 92 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

FR775895-EPP92						
MUD-EPP92-g141-aa-276nt	0					
KU522583-EP	0	0				
KX552041-EsP-FR	15	15	15			
KX664695-EsP-FR	15	15	15	1		
JX561091-EsP-BE	15	15	15	1	0	
KR296694-SP40-IN	19	19	19	21	21	21

11. Human viruses from ST4, ST5 and ST6 faecal samples

Figure and Table	Long Name (Format: Sample-virus-protein-length-quality-coverage-year)	Short name (Format: Sample-virus-protein-length)	Coverage	No. of nucleotides	Mapping quality threshold	NCBI accession number
ssDNA viruses						
S11.1	ST6- Human-fecal-virus-Jorvi2-hypothetical-protein-208nt-Q46-C-1-2017	ST6-HFVJ2-HP-208nt	1	208	46	MH188072
S11.2	ST4-Torque-teno-mini-virus-5prime-untranslated-116nt-Q10-C-11-15-2017	ST4-TTMV-5UTR-116nt	11-15	116	10	MH188073
S11.2	ST5-Torque-teno-mini-virus-5prime-untranslated-116nt-Q10-C-10-13-2017	ST5-TTMV-5UTR-116nt	10-13	116	10	MH188073
Picornaviruses						
S11.3	ST4-Human-rhinovirus-C- genome-capsid-protein-VP2-163nt-Q8-C-5-6-2017	ST4-HRVC-VP2-163nt	5-6	163	8	MH188075
S11.4	ST6-Human-TMEV-like-cardiovirus-polyprotein-261nt-Q10-C-3-15-2017	ST6-HTMEVLCV-VP1-261nt	3-15	261	10	MH188076
S11.5	ST6-H Human-TMEV-like-cardiovirus-capsid protein-VP3-422nt-Q10-C-3-18-2017	ST6-HTMEVLCV-VP3-422nt	3-18	422	10	MH188077
S11.6	ST6-Saffold-virus-protein-2C-212nt-Q10-C-2-4-2017	ST6-SV-2C-212nt	2-4	212	10	MH188078
Contigs assembled						
S11.7	ST4-RotavirusA-VP4-909nt-Q20-C-3-52-2017	ST4-RVA-VP4-909nt	3-52	909	20	MH188079
S11.8	ST5-Norovirus-contig-7044nt-Q20-C-2-189-2017	ST5-NV-7044nt	2-189	7044	20	MH188080
NCBI sequences taken for phylogenetic analysis						
Long Name		Short Name (Format: NCBI accession number-virus-country/state)	Country of collection	Collection date		

MF118166-Human-fecal-virus-Tarto-complete-genome	MF118166-HFVT-EE	Estonia	-
MF118167-Human-fecal-virus-Jorvi2-complete-genome	MF118167-HFVJ2-FI	Finland	-
KT163899-Torque-teno-virus-isolate-P13-4-ORF1-gene-partial-cds	KT163899-TTV	-	-
KM259873-Torque-teno-mini-virus-ALA22-complete-genome	KM259873-TTMV-ES	Spain	2014
EF538883-TTV-like-mini-virus-isolate-LIL-y4-ORF2-and-ORF1-genes-complete-cds	EF538883-TTVLMV-FR	France	-
KX810063-TTV-like-mini-virus-isolate-Emory1-complete-genome	KX810063-TTVLMV-US	USA	1993
AB041962-Torque-teno-mini-virus5-DNA-complete-genome-isolate:TGP96	AB041962-TTMV5	-	-
KY983589-Human-rhinovirus-C11-strain-SC3107-complete-sequence	KY983589-HRVC-US	USA	2015
KM486097-Rhinovirus-C-strain-Mex14-Consensus-polyprotein-gene-complete-cds	KM486097-RVC-MX	Mexico	2014
JN815249-Human-rhinovirus-C-strain-HRV-C43-p1281-s6410-1999-polyprotein-gene-partial-cds	JN815249-HRVC-US	USA	1999
KY369878-Human-rhinovirus-C43-strain-SC174-complete-genome	KY369878-HRVC43-US	USA	2016
JX074056-Human-rhinovirus-C-strain-HRV-C43-p1154-sR1124-2009-polyprotein-gene-complete-cds	JX074056-HRVC43-US	USA	2009
LC004883-Rhinovirus-C-gene-for-polyprotein-VP2-VP3-VP1-region-partial-cds-strain:HRV-C/JPN/Yokohama63/2012	LC004883-RVC-JP	Japan	2012
AB747252-Saffold-virus-gene-for-polyprotein-partial-cds-isolate:Pak-3290	AB747252-SV-PK	Pakistan	2009
AB747248-Saffold-virus-gene-for-polyprotein-partial-cds-isolate:Pak-3097	AB747248-SV-PK	Pakistan	2009
FJ463616-Saffold-virus-isolate-Pak5152-polyprotein-(gp1)-gene-partial-cds-and-L*-protein-(L*)-gene-complete-cds	FJ463616-SV-PK	Pakistan	2009

AB747255-Saffold-virus-gene-for-polyprotein-partial-cds-isolate:Pak-3486	AB747255-SV-PK	Pakistan	2009
AB747250-Saffold-virus-gene-for-polyprotein-partial-cds-isolate:Pak-3641	AB747250-SV-PK	Pakistan	2009
EF165067-Saffold-virus-complete-genome	EF165067-SV-US	USA	-
GU595289-Human-TMEV-like-cardiovirus-isolate-HTCV-UC6-complete-genome	GU595289-HTMEVLCV-US	USA	2001
EU681179-Cardiovirus-D/VI2223/2004-polyprotein-gene-complete-cds	EU681179-CV-DE	Germany	-
EU376394-Human-TMEV-like-cardiovirus-complete-genome	EU376394-HTMEVLCV	-	-
EU681176-Cardiovirus-D/VI2229/2004-polyprotein-gene-complete-cds	EU681176-CV-DE	Germany	-
JN652233-Saffold-virus-strain-S19-polyprotein-gene-complete-cds	JN652233-SA-US	USA	2005
JF813004-Saffold-virus-strain-Can112051-06-adapted-complete-genome	JF813004-SV-CA	Canada	2007
GU565088-RotavirusA-strain-RVA/Vaccine/USA/RotaTeq-BrB-9/1996/G4P75-VP4-gene-complete-cds	GU565088-RVA-US	USA	1996
GU565077-RotavirusA-strain-RVA/Vaccine/USA/RotaTeq-WI78-8/1992/G3P75-VP4-gene-complete-cds	GU565077-RVA-US	USA	1992
D16351-Rotavirus-sp.-VP4-mRNA-encoding-VP5-and-VP8-complete-cds	D16351-RV	-	-
KC815661-Equine-rotavirusA-genotype-G6-P5-I2-R2-C2-M2-A13-N2-T6-E2-H3-outer-capsid-spike-protein-(VP4)-gene-complete-cds	KC815661-ERVA-JP	Japan	1982
L42980-Rotavirus-sp.-mRNA-fragment	L42980-RV	-	-
U53923-Bovine-rotavirus-spike-protein-VP4-(VP4)-mRNA-complete-cds	U53923-BRV-US	USA	-
MG002634-Norovirus-GII-strain-Hu/GII.P4-New-Orleans2009-	MG002634-NV-AU	Australia	2017

GII.4-Sydney2012/BNE5/2017/AU-polyprotein-major-capsid-protein-and-minor-structural-protein-genes-complete-cds			
MG002632-Norovirus-GII-strain-Hu/GII.P4-New-Orleans2009-GII.4-Sydney2012/BNE3/2017/AU-polyprotein-major-capsid-protein-and-minor-structural-protein-genes-complete-cds	MG002632-NV-AU	Australia	2017
KJ685411-Norovirus-Hu/GII/BG1C0398/2012/BGD-partial-genome	KJ685411-NV-BD	Bangladesh	2012
MF140641-Norovirus-GII-strain_Norovirus/GII/Hu/NL/2013/GII.P4-GII.4/Rotterdam/E7800009-p11-d0-ORF1-gene-partial-cds-and-ORF2-and-ORF3-genes-complete-cds	MF140641-NV-NL	Netherlands	2013
MF140642.1-Norovirus-GII-strain-Norovirus/GII/Hu/NL/2013/GII.P4-GII.4/Rotterdam/E1300306-p11-d83-ORF1-gene-partial-cds-ORF2-gene-complete-cds-and-ORF3-gene-partial-cds	MF140642-NV-NL	Netherlands	2013
JX459901-Norovirus-Hu/GII.4/Caringbah/NSW409G/2011/AU-complete-genome	JX459901-NV-AU	Australia	2011
JX459904-Norovirus-Hu/GII.4/Doonside/NSW536I/2011/AU-complete-genome	JX459904-NV-AU	Australia	2011

The table points towards the molecular phylogenetic analysis by Maximum Likelihood method of viruses isolated from human samples. The first half of the table provides details to identify the sample from which the virus was isolated, protein that encodes the consensus sequences that are being analysed, the length of the consensus sequences thus providing the depth of the gene being analysed, minimum mapping quality threshold used in IGV for the generation of the consensus sequences, the coverage of the consensus sequences from IGV, the year of collection of the sample and the NCBI accession number assigned to the particular consensus sequence. Corresponding short names have been used in the phylogenetic trees which contain the sample, virus, protein and the number of nucleotides. The second half of the table provides the details of the sequences that were used for the molecular phylogenetic analysis. They were found to be the most closely related sequences to the consensus sequences generated using BLASTN. Corresponding short names have been used in the phylogenetic trees which contain the NCBI accession number, virus and the

country of collection. The collection date is given as retrieved from the corresponding NCBI nucleotide reference dataset which together with the country of collection can provide an insight into the possible evolution of the virus through the years.

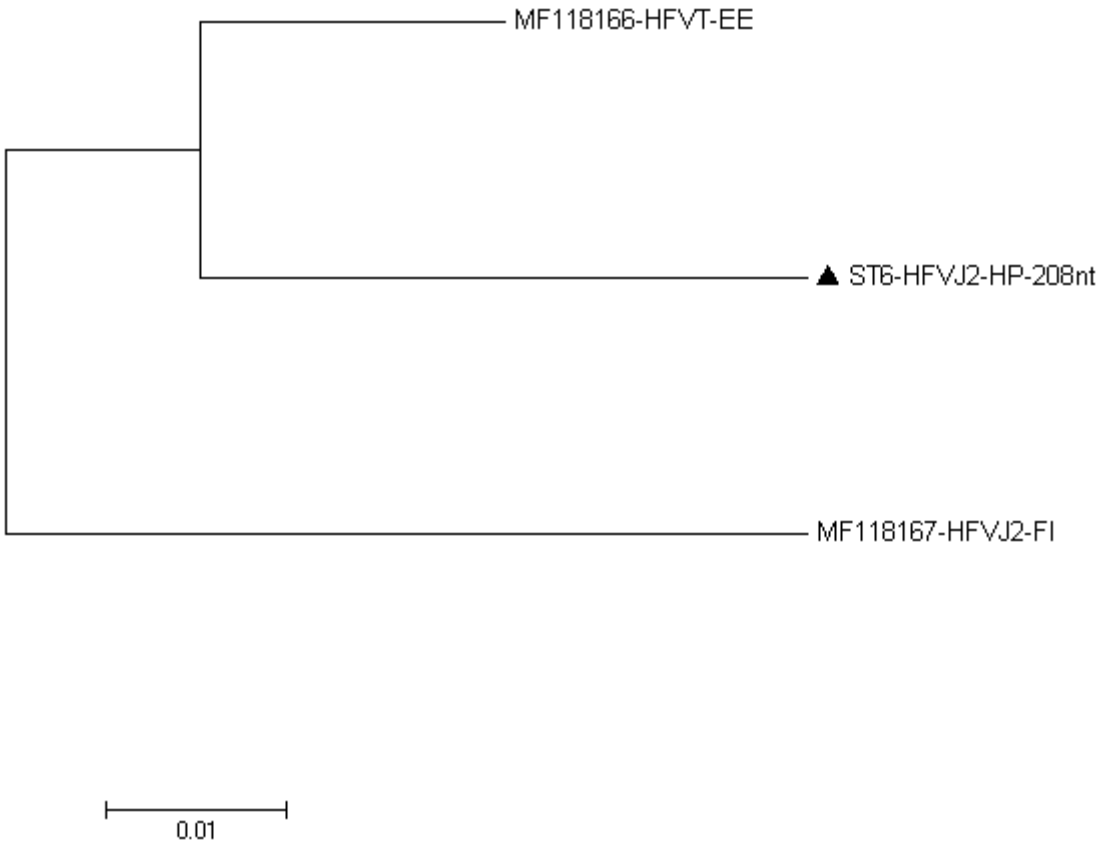


Figure S11.1: Molecular Phylogenetic analysis by Maximum Likelihood method of HFVJ2 partial HP gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model[4]. The tree with the highest log likelihood (-385.14) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 3 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 208 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S11.1: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 3 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 208 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

MF118166-HFVT-EE		
MF118167-HFVJ2-FI	14	
ST6-HFVJ2-HP-208nt	10	17

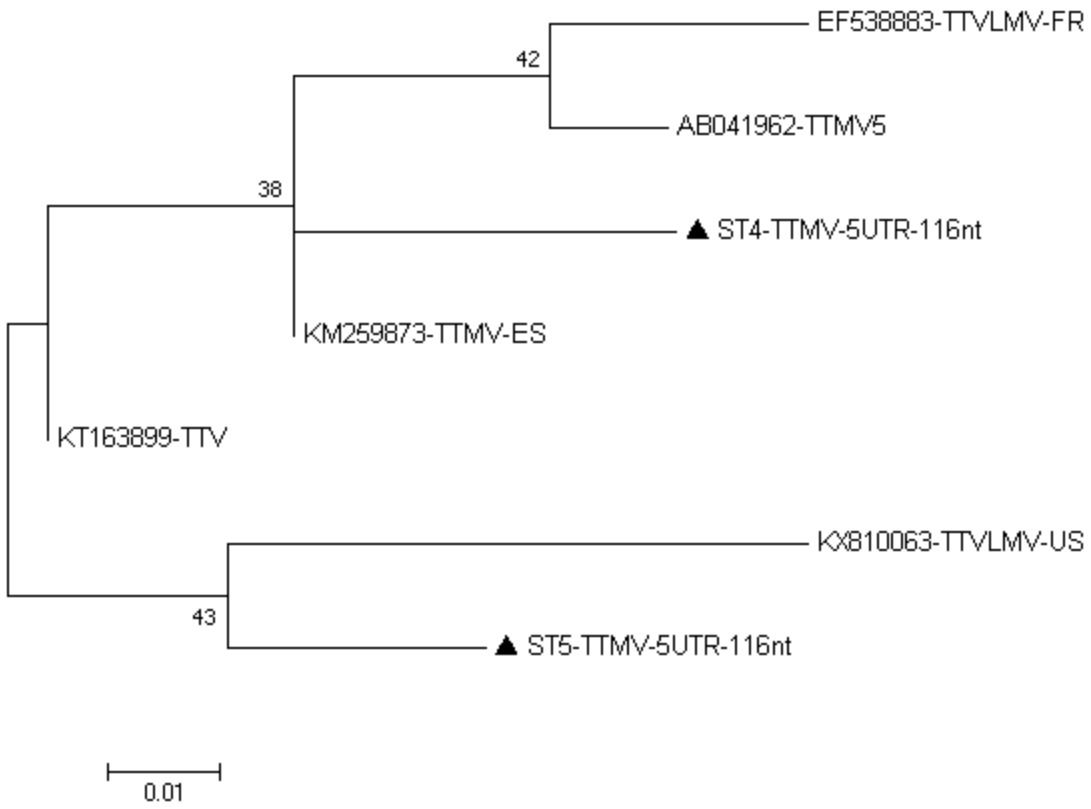


Figure S11.2: Molecular Phylogenetic analysis by Maximum Likelihood method of TTMV partial 5UTR gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Jukes-Cantor model[9]. The tree with the highest log likelihood (-260.34) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.1000)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 7 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 116 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S11.2: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 7 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 116 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

KT163899-TTV						
KM259873-TTMV-ES	2					
EF538883-TTVLMV-FR	6	4				
KX810063-TTVLMV-US	6	8	8			
AB041962-TTMV5	5	3	3	5		
ST5-TTMV-5UTR-116nt	4	6	6	6	7	
ST4-TTMV-5UTR-116nt	5	3	7	8	4	7

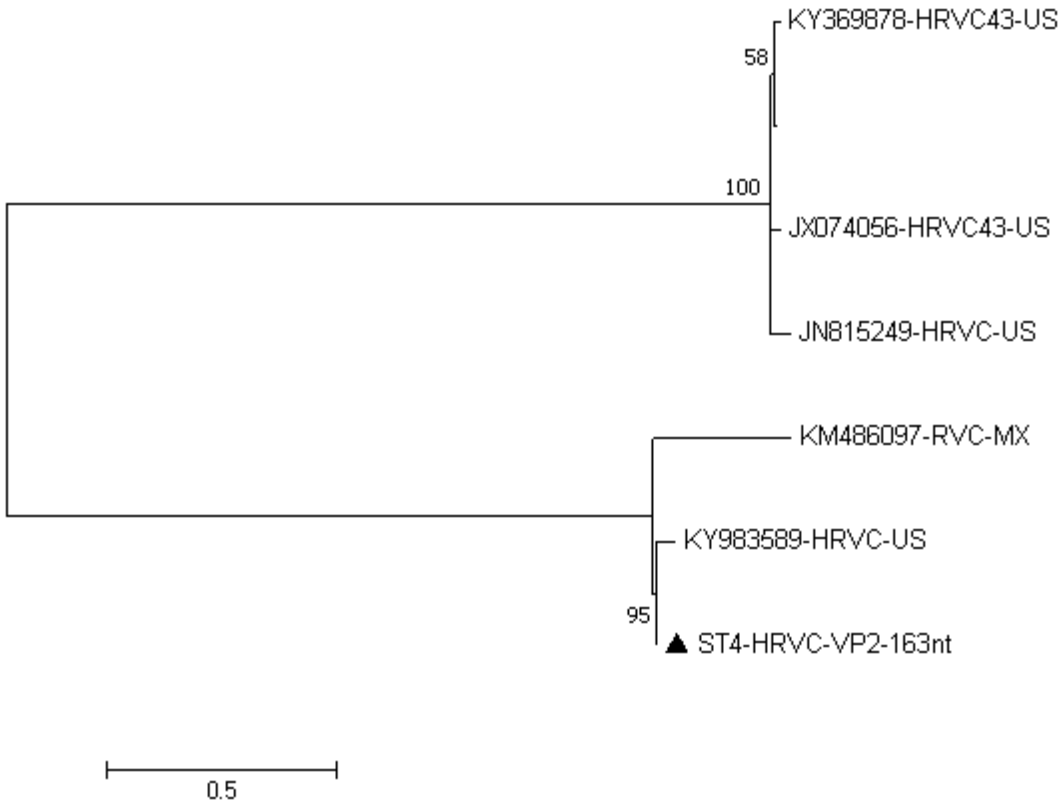


Figure S11.3: Molecular Phylogenetic analysis by Maximum Likelihood method of HRVC partial VP2 gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model[1]. The tree with the highest log likelihood (-537.01) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 66.13% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 7 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 163 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S11.3: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 7 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 163 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

KY983589-HRVC-US						
KM486097-RVC-MX	25					
JN815249-HRVC-US	34	39				
KY369878-HRVC43-US	29	40	9			
JX074056-HRVC43-US	33	43	9	6		
LC004883-RVC-JP	31	40	8	3	5	
ST4-HRVC-VP2-163nt	6	24	32	33	33	33

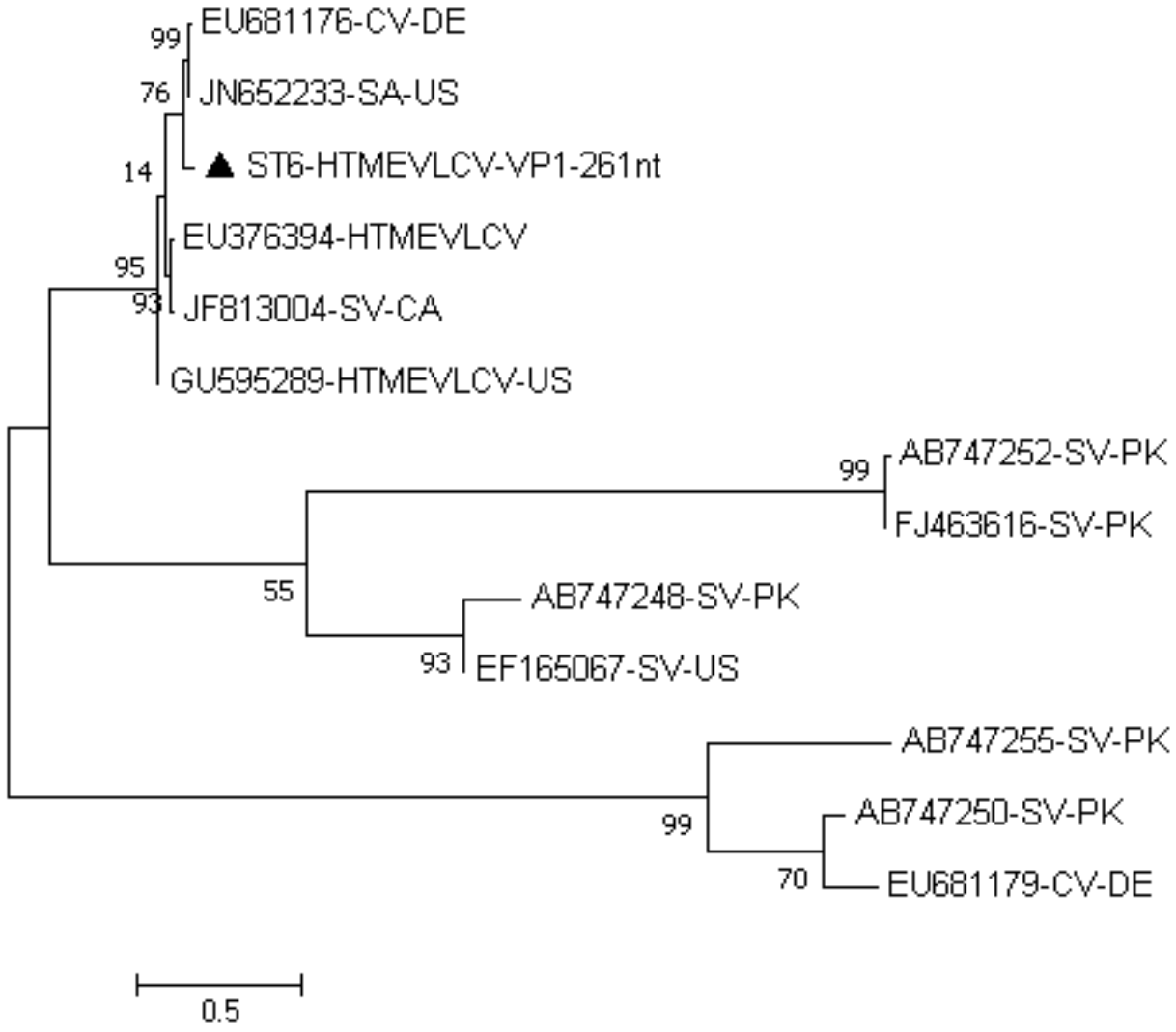


Figure S11.4: Molecular Phylogenetic analysis by Maximum Likelihood method of HTMEVLCV partial VP1 gene

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model[4]. The tree with the highest log likelihood (-1847.23) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 1.1715)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 13 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 255 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S11.4: Estimates of Evolutionary Divergence between Sequences

The number of base differences per sequence from between sequences are shown. The analysis involved 13 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 255 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

AB747252-SV-PK												
AB747248-SV-PK	107											
FJ463616-SV-PK	4	105										
AB747255-SV-PK	115	125	115									
AB747250-SV-PK	112	121	115	77								
EF165067-SV-US	103	34	101	127	121							
GU595289-HTMEVLCV-US	119	96	118	116	111	90						
EU681179-CV-DE	107	115	108	80	42	113	113					
EU376394-HTMEVLCV	116	95	115	115	112	95	11	111				
EU681176-CV-DE	114	99	113	115	114	93	22	114	21			
JN652233-SA-US	112	100	111	114	114	94	21	114	20	3		
JF813004-SV-CA	116	95	115	115	113	95	12	112	3	22	21	
ST6-HTMEVLCV-VP1-261nt	111	97	110	111	111	91	23	112	20	11	10	23

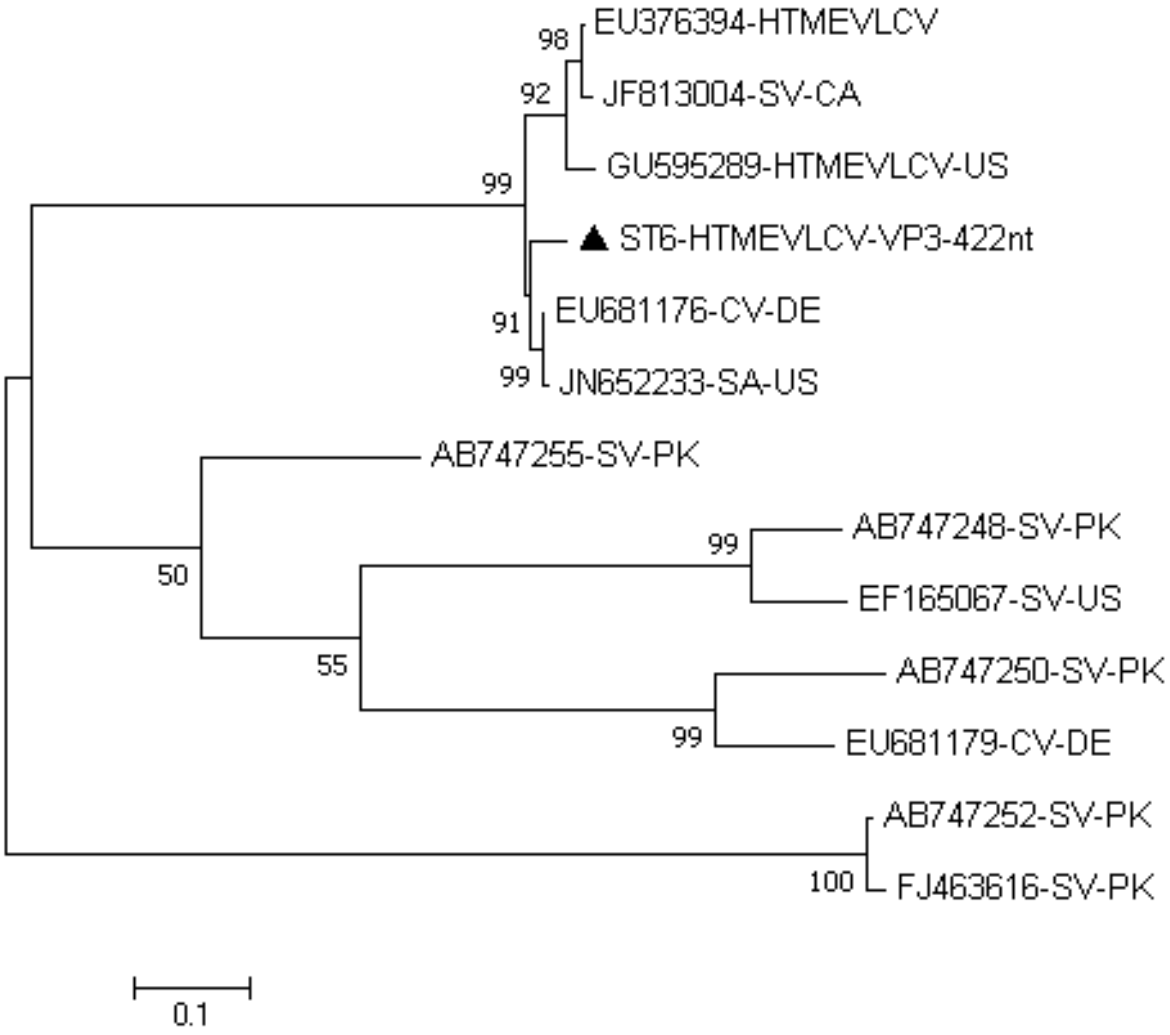


Figure S11.5: Molecular Phylogenetic analysis by Maximum Likelihood method of HTMEVLCV partial VP3 gene

The evolutionary history was inferred by using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model[5]. The tree with the highest log likelihood (-2386.84) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.5836)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 13 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 422 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S11.5: Estimates of Evolutionary Divergence between Sequences

The number of base differences per sequence from between sequences are shown. The analysis involved 13 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 422 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

AB747252-SV-PK												
AB747248-SV-PK	123											
FJ463616-SV-PK	9	124										
AB747255-SV-PK	108	95	111									
AB747250-SV-PK	124	97	127	93								
EF165067-SV-US	117	50	120	98	96							
GU595289-HTMEVLCV-US	123	110	127	100	114	110						
EU681179-CV-DE	128	102	128	97	67	100	110					
EU376394-HTMEVLCV	119	106	123	101	106	108	17	111				
EU681176-CV-DE	117	102	120	98	109	106	28	116	25			
JN652233-SA-US	118	101	121	97	111	104	30	118	27	2		
JF813004-SV-CA	120	104	124	103	106	109	20	115	5	28	30	
ST6-HTMEVLCV-VP3-422nt	126	106	127	102	106	108	35	115	32	17	19	35

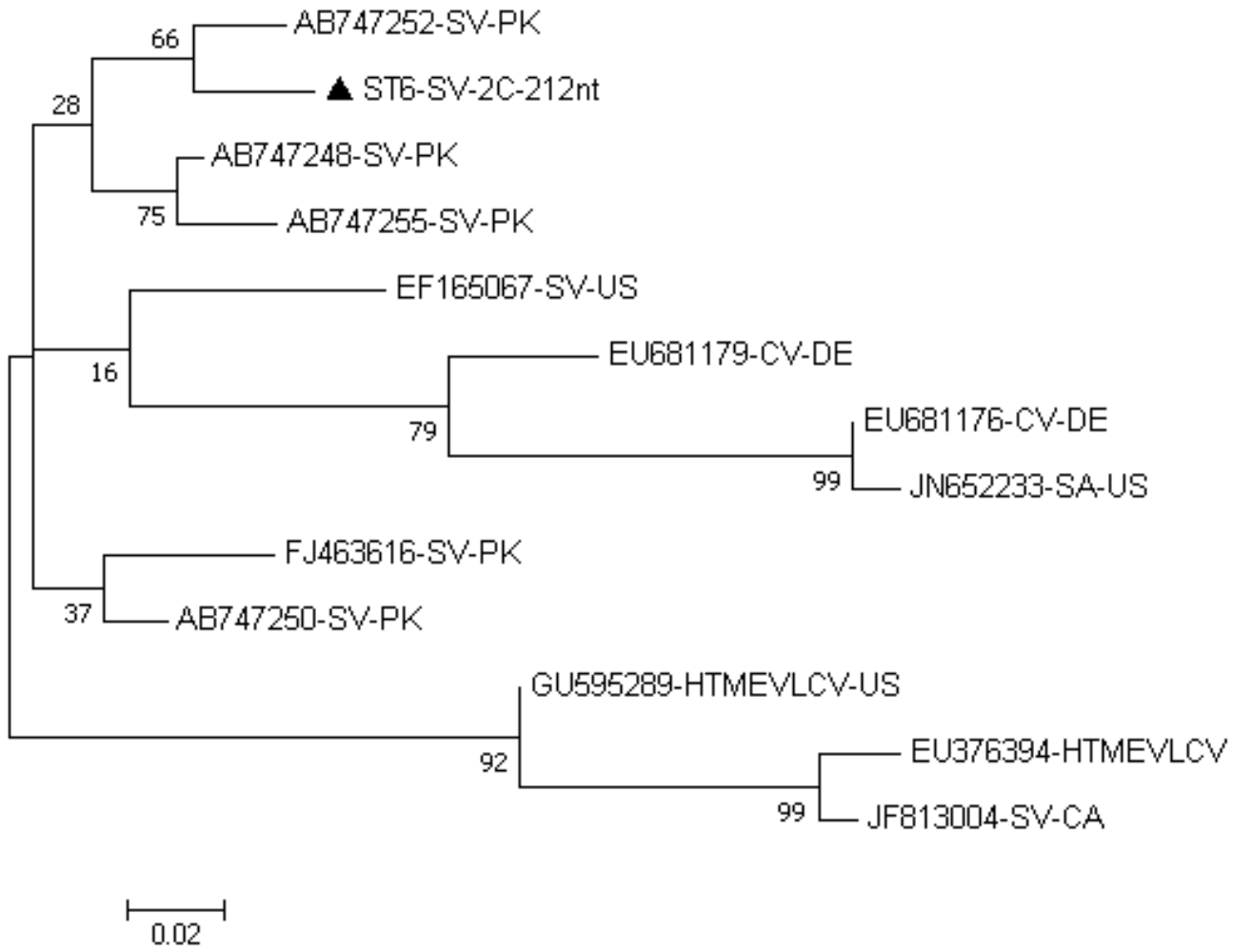


Figure S11.6: Molecular Phylogenetic analysis by Maximum Likelihood method of SV partial 2C gene

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model[4]. The tree with the highest log likelihood (-752.38) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.2798)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 13 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 212 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S11.6: Estimates of Evolutionary Divergence between Sequences

The number of base differences per sequence from between sequences are shown. The analysis involved 13 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 212 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

AB747252-SV-PK												
AB747248-SV-PK	11											
FJ463616-SV-PK	13	14										
AB747255-SV-PK	14	5	17									
AB747250-SV-PK	14	9	9	10								
EF165067-SV-US	14	16	17	16	16							
GU595289-HTMEVLCV-US	19	20	22	22	21	19						
EU681179-CV-DE	24	20	21	17	14	21	25					
EU376394-HTMEVLCV	26	27	29	27	28	26	13	29				
EU681176-CV-DE	27	25	26	22	21	24	23	17	24			
JN652233-SA-US	28	26	27	23	22	24	24	19	25	2		
JF813004-SV-CA	25	26	26	26	27	25	12	31	5	25	26	
ST6-SV-2C-212nt	8	11	13	14	16	18	21	26	30	27	28	29

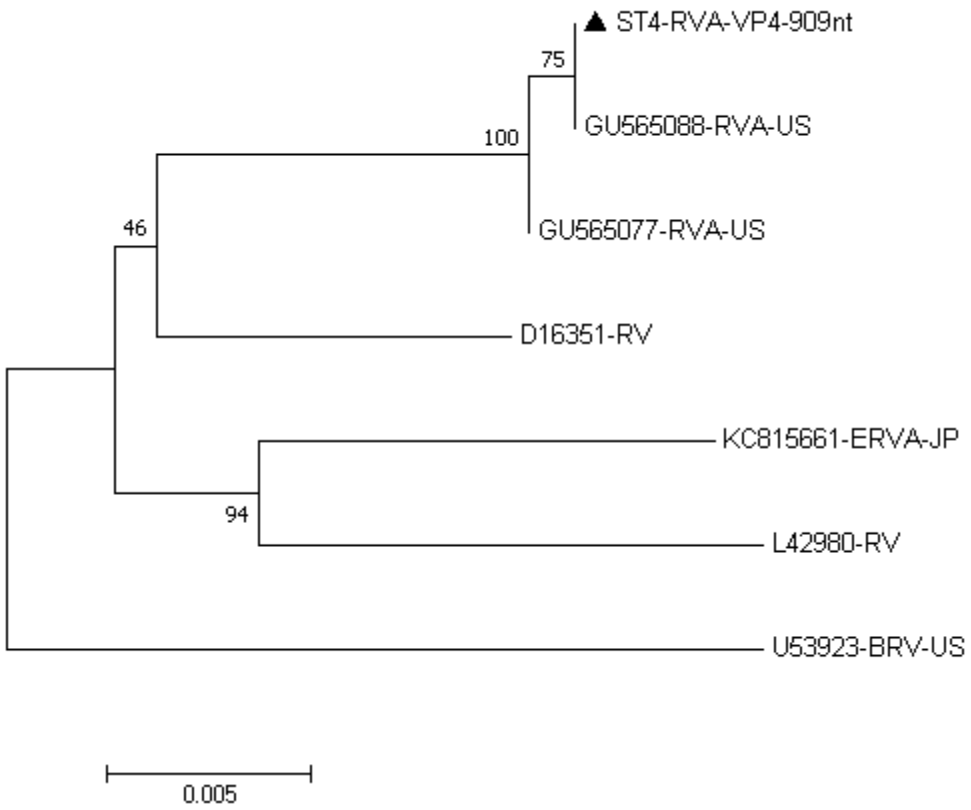


Figure S11.7: Molecular Phylogenetic analysis by Maximum Likelihood method of RVA partial VP4 gene
The evolutionary history was inferred by using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model[5]. The tree with the highest log likelihood (-1615.82) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 7 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 909 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S11.7: Estimates of Evolutionary Divergence between Sequences
The number of base differences per sequence from between sequences are shown. The analysis involved 7 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 909 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

ST4-RVA-VP4-909nt						
GU565088-RVA-US	0					
GU565077-RVA-US	1	1				
D16351-RV	17	17	16			
KC815661-ERVA-JP	22	22	21	22		
L42980-RV	23	23	22	23	21	
U53923-BRV-US	29	29	28	26	32	33

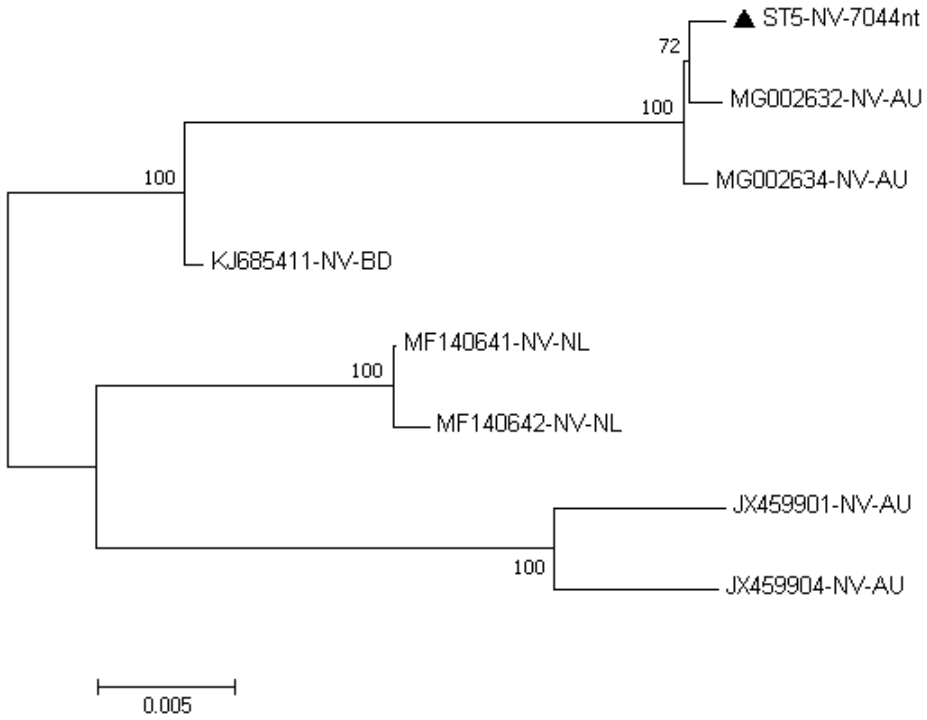


Figure S11.8: Molecular Phylogenetic analysis by Maximum Likelihood method of NV

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model[3]. The tree with the highest log likelihood (-12582.19) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.1000)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 7044 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[2].

Table S11.8: Estimates of Evolutionary Divergence between Sequences

The number of base differences per sequence from between sequences are shown. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 7044 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

ST5-NV-7044nt							
MG002634-NV-AU	17						
MG002632-NV-AU	17	16					
KJ685411-NV-BD	132	129	131				
MF140641-NV-NL	244	239	241	139			
MF140642-NV-NL	250	245	247	147	10		
JX459901-NV-AU	298	295	297	207	212	218	
JX459904-NV-AU	301	298	300	207	208	214	82

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Supplementary Material 3

Metagenomics detection and characterisation of viruses in faecal samples from Australian wild birds

Running Title of the Supplementary Material

Detailed optimised protocol for metagenomics of viruses and microbiome analysis.

Authors

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Materials required:

- 1X PBS
- 2mL microtubes with O-ring
- Microtubes, PCR tubes, 96 well plates
- pipettes and tips
- 0.8µm polyethersulfone (PES) spin filter (Sartorius, Catalogue number: VK01P042)
- Benzonase Nuclease, Purity > 90% (Millipore, Catalogue Number: 707463)
- Micrococcal nuclease (New England Biolabs, Catalogue Number: M0247S)
- 20X buffer {1M Tris pH8, 100mM calcium chloride (CaCl), 30mM magnesium chloride ($MgCl_2$)}
- 500mM ethylenediaminetetraacetic acid (EDTA; pH=8)
- QIAmp viral RNA mini kit (Qiagen; Catalogue number: 52904)
- 100% ethanol
- SeqPlex RNA amplification kit (Sigma, Catalogue number: SEQR)
- GenElute PCR Clean-up Kit (Sigma, Catalogue number: NA1020)
- Agilent High Sensitivity DNA Kit (Agilent, Catalogue number: 5067-4626)
- Ion Plus Fragment Library Kit (Thermo Fisher Scientific; Catalogue number: 4471252)
- Agencourt AMPure XP Kit (Beckman Coulter, Catalogue number: A63880)
- Magnet
- Low TE
- Ion Xpress Barcode Adapters 1-16 Kit (Thermo Fisher Scientific; Catalogue number: 4471250)
- Ion Library TaqMan™ Quantitation Kit (Thermo Fisher Scientific; Catalogue number: 4468802)
- Ion 520 & Ion 530 Kit-Chef (Thermo Fisher Scientific; Catalogue number: A27757)

Instruments required:

- small-scale analytical balance
- vortex
- TissueLyser II homogeniser (Qiagen) or equivalent homogeniser
- Microcentrifuge
- Beckman Coulter Airfuge Ultracentrifuge or equivalent ultracentrifuge
- Dry bath or water bath
- Nanodrop spectrophotometer
- Bioanalyzer
- Thermocycler
- QuantStudio™ 6 Flex Real-Time PCR System (Thermo Fisher Scientific; Catalogue number: 4485697) or equivalent real-time PCR instrument.
- Ion S5, Ion Chef Instrument (Thermo Fisher Scientific; Catalogue number: 4484177)
- Ion Torrent Sequencing and genetic analysis software (Thermo Fisher Scientific)

Step 1: Sample preparation

- Make 1:10 dilution of the faecal sample using sterile 1X PBS, i.e. 100mg:1ml

Step 2: Sample homogenization

- Transfer 1ml * 2 to two 2ml O ring tubes.
- Homogenize for 25Hz for 2min.
- Mix both tubes.
- Transfer 250µL to one tube. Now Tube A (1750µL) and Tube B (250µL).

Step 3: Centrifugation

- Centrifuge both tubes for 17000g for 3min at room temperature (RT).
- Collect supernatant.

Step 4: Filtration

- Filter both tubes using 0.8µm PES filter at 17000g for 1min or until all of the sample has passed through.
- Collect filtrate. Put Tube B on ice. Proceed Tube A for ultracentrifugation.

Step 5: Ultracentrifugation

- The sample was transferred into four 8*20mm ultra-clear (max of 450µL and min of 300µL) straight wall tubes.
- The tubes were placed in the A-95 rotor and balanced.
- The sample was then ultracentrifuged at 178,000g for 1 hour (30psi for 1hour) at RT.
- The supernatant was discarded. The pellet was suspended in a total of 130µL of sterile 1XPBS.

Step 6: Nuclease treatment

- To 130µL of the samples (both Tube A and B separately), 7µL of 20X buffer, 2µL of benzonase nuclease and 1µL of micrococcal nuclease was added.
- After mixing gently, the samples were incubated at 37 °C for 2 h.
- The nuclease reaction was stopped by adding 3µL of 500mM EDTA.

Step 7: Nucleic acid extraction

Kit used: QIAmp viral RNA mini kit

Note: No carrier RNA was added to the AVL buffer. If the kit is new, add 25ml of absolute ethanol to buffer AW1 and 30ml of absolute ethanol to buffer AW2. All steps performed at RT. All centrifugation performed at RT.

- Take 560µL of buffer AVL in the 1.5ml tube.
- Add 140µL of the sample to the buffer and mix by vortexing for 15s.
- Incubate at RT for 10min.
- Spin down.
- Add 560µL of absolute ethanol to sample and mix by vortexing for 15s. Spin down.
- Apply 630µL to QIAmp Minicolumn.
- Centrifuge 6000g or 8000rpm for 1min.
- Repeat the above two steps.
- Place column in clean collection tube.
- Add 500µL of buffer AW1.
- Centrifuge 6000g or 8000rpm for 1min. Place column in clean collection tube.

- Add 500µL of buffer AW2.
- Centrifuge 20000g or 14000rpm for 1min. Place column in clean collection tube.
- Centrifuge at full speed for 1min. Place column in the clean 1.5ml tube.
- Add 40µL of buffer AVE directly to spin column membrane without touching it. Close the cap and incubate for 1min at RT.
- Centrifuge 6000g or 8000rpm for 1min.
- Take the eluted buffer and place it on spin column membrane (double elution).
- Centrifuge 6000g or 8000rpm for 1min. The filtrate contains the virus nucleic acid.
- Store isolated nucleic acid at -20 ºC.

PAUSE POINT

Step 8: cDNA synthesis, amplification and purification

Kits used: SeqPlex RNA amplification kit and GenElute PCR Clean-up Kit

- Take 10µL of isolated nucleic acid and incubate for 95 ºC for 3min followed by snap cooling in -20 ºC cold ethanol.
- Add 2µL of Library Synthesis Solution and 1.2µL of nuclease free water to the nucleic acid making it a total volume of 13.2µL.
- Incubate in a thermocycler for 70 ºC for 5min, 18 ºC hold. Do not hold for more than 10 min. Remove reaction from thermocycler and place at RT or maintain at 18 ºC.
- Add 2µL of Library synthesis buffer, 3.2µL of nuclease free water and 1.6µL of Library synthesis enzyme to the above reaction mixture to make it a total volume of 20µL.
- Incubate in a thermocycler for 18 ºC for 10min, 25 ºC for 10min, 37 ºC for 30min, 42 ºC for 10min, 70 ºC for 20min and 4 ºC hold.
- Preferably proceed further or store at -20 ºC.
- To the above mixture add, 39.25µL of nuclease free water, 15µL of 5X amplification mix, 0.75µL of amplification enzyme making it a total volume of 75µL.
- Incubate in a thermocycler for 94 ºC for 2min, 25 cycles of 94 ºC for 30s and 70 ºC for 5min, 1 cycle of 70 ºC for 30min followed by 4 ºC hold.
- Purify the reaction mixture with GenElute PCR Clean-up Kit.
- After purification quantify the DNA.
- Proceed further or store at -20 ºC.

PAUSE POINT

Step 9: Primer removal and purification

Kits used: SeqPlex RNA amplification kit, GenElute PCR Clean-up Kit and Agilent High Sensitivity DNA Kit

- Take 2µg of purified DNA from the above and add 7.5µL of 10X primer removal buffer, 1.5µL of primer removal solution, 3.75µL of primer removal enzyme and make the total reaction volume to 75µL using nuclease free water.
- Incubate the reaction in 37 ºC for 60min, 65 ºC for 20min and 4 ºC hold.
- Purify the reaction mixture with GenElute PCR Clean-up Kit.
- After purification quantify the DNA using Agilent High Sensitivity DNA Kit in Bioanalyzer.
- Proceed further or store at -20 ºC.

Step 10: End repair and purification

Kits used: Ion Plus Fragment Library Kit and Agencourt AMPure XP Kit.

- Take 20µL of primer removed and purified sample DNA in a 1.5mL microtube and add nuclease free water to bring the volume to 79µL.
- Add 20µL of 5X end repair buffer and 1µL of end repair enzyme to make the total reaction mix to 100µL.
- Incubate the mix at RT for 20min.
- After the incubation purify the samples with Agencourt AMPure XP Kit with 180µL of AMPure XP Reagent. After the purification elute the sample in 25µL of Low TE buffer.
- Proceed further or store at -20 °C.

PAUSE POINT

Step 11: Adapter ligation and Nick repair

Kits used: Ion Plus Fragment Library Kit, Ion Xpress Barcode Adapters 1-16 Kit and Agencourt AMPure XP Kit.

- To the 25µL of eluted DNA samples add 10µL of 10X ligase buffer, 2µL of Ion P1 Adapter, 2µL of Ion Xpress Barcode X (barcode chosen), 2µL of dNTP mix, 49µL of nuclease free water, 2µL of DNA Ligase and 8µL of Nick Repair Polymerase.
- Incubate the reaction mix at 25 °C for 15min, 72 °C for 5min and 4 °C for up to 1h. Last stage is not a stopping point; continue directly to purification.
- Purify the samples using Agencourt AMPure XP Kit with 150µL of AMPure XP Reagent. After the purification elute the sample in 20µL of Low TE buffer.
- Store the DNA at -30 °C to -10 °C.

Step 12: Quantify the unamplified library

Kit used: Ion Library TaqMan™ Quantitation Kit

- Prepare 100-fold and 10000-fold dilution of the library using nuclease free water.
- Prepare 10-fold serial dilution of the *E.coli* DH10B Ion Control Library from 6.8pM to 0.0000068pM.
- To 9µL of the diluted library and control add 10µL of 2X TaqMan Master mix and 1µL of 20X Ion TaqMan Assay.
- Run RT-PCR in QuantStudio™ 6 Flex Real-Time PCR System using the following conditions in standard mode: 50 °C for 2min, 95 °C for 2min, 40 cycles of 95 °C for 15s and 60 °C for 1min followed by 10 °C hold.
- Calculate the concentration of undiluted library and determine the dilution that result in concentration of ~100pM.

PAUSE POINT

Step 13: Next generation sequencing

Kit used: Ion 520 & Ion 530 Kit-Chef

- Set up plan run in Ion Torrent Server as per manufacturer instructions.
- Prepare the Ion chip using Ion Chef as per manufacturer instructions.
- Run next generation sequencer Ion S5 as per manufacturer instructions.

Step 14: Next generation sequencing analysis

- BLASTN, BLASTX, MEGABLAST, Ion Torrent Server, TMAP, IGV for virus analysis
- Ion Reporter for microbiome analysis.

Step 15: Partial genome and evolutionary analysis of viruses

- IGV, BLASTN, BLASTX, MEGA for partial genome and evolutionary analysis.