

Molecular Characterization of Closely Related H6N2 Avian Influenza Viruses Isolated from Turkey, Egypt, and Uganda

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Citation: Lastname, F.; Lastname, F.; Lastname, F. Title. *Viruses* **2021**, *13*, x. <https://doi.org/10.3390/xxxxx>

Academic Editor: Firstname Lastname

Academic Editor: Firstname Lastname

Received: 27 February 2021

Accepted: 30 March 2021

Published: date

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Table S1. Pairwise nucleotide similarities of virus isolates.

Gene		GD/18	UG/731	EG/1566C	EG/1556OP
PB2	GD/18	1			
	UG/731	0.91506	1		
	EG/1566C	0.9853	0.91082	1	
	EG/1556OP	0.9978	0.91564	0.98484	1
PB1	GD/18	1			
	UG/731	0.961213	1		
	EG/1566C	0.97354	0.96076	1	
	EG/1556OP	0.999824	0.960759	0.99912	1
PA	GD/18	1			
	UG/731	0.951806	1		
	EG/1566C	0.997671	0.950274	1	
	EG/1556OP	0.998138	0.950787	0.99535	1
HA	GD/18	1			
	UG/731	0.966591	1		
	EG/1566C	0.982039	0.966632	1	
	EG/1556OP	0.981418	0.965985	0.999411	1
NP	GD/18	1			
	UG/731	0.949747	1		
	EG/1566C	0.953384	0.975428	1	
	EG/1556OP	0.953384	0.975428	1	1
NA	GD/18	1			
	UG/731	0.947689	1		
	EG/1566C	0.998772	0.948232	1	
	EG/1556OP	0.998772	0.948232	1	1
M	GD/18	1			
	UG/731	0.975291	1		
	EG/1566C	0.9967	0.976016	1	
	EG/1556OP	0.9967	0.9760161	1	1
NS	GD/18	1			
	UG/731	0.984703	1		
	EG/1566C	0.979922	0.971398	1	
	EG/1556OP	0.979922	0.971398	1	1

Table S2. Pairwise amino acid similarities of virus isolates.

Protein		GD/18	UG/731	EG/1566C	EG/1556OP
PB2	GD/18	1			
	UG/731	0.994785	1		
	EG/1566C	0.994785	0.989542	1	
	EG/1556OP	1	0.994785	0.994785	1
PB1	GD/18	1			
	UG/731	0.996029	1		
	EG/1566C	0.998678	0.994702	1	
	EG/1556OP	1	0.996029	0.998678	1
PB1-F2	GD/18	1			
	UG/731	0.966098	1		
	EG/1566C	0.988827	0.977527	1	
	EG/1556OP	0.988827	0.977527	1	1
PA	GD/18	1			
	UG/731	0.992991	1		
	EG/1566C	0.998602	0.994398	1	
	EG/1556OP	0.998602	0.994398	1	1
PA-X	GD/18	1			
	UG/731	0.984	1		
	EG/1566C	0.996024	0.979959	1	
	EG/1556OP	0.996024	0.979959	1	1
HA	GD/18	1			
	UG/731	0.97314	1		
	EG/1566C	0.99113	0.98217	1	
	EG/1556OP	0.98934	0.98037	0.99823	1
NP	GD/18	1			
	UG/731	0.989991	1		
	EG/1566C	0.987879	0.993958	1	
	EG/1556OP	0.987879	0.993958	1	1
NA	GD/18	1			
	UG/731	0.95227	1		
	EG/1566C	0.99575	0.95707	1	
	EG/1556OP	0.99575	0.95707	0.99165	1
M1	GD/18	1			
	UG/731	1	1		
	EG/1566C	1	1	1	
	EG/1556OP	1	1	1	1
M2	GD/18	1			
	UG/731	0.989637	1		
	EG/1566C	1	0.989637	1	
	EG/1556OP	1	0.989637	1	1
NS1	GD/18	1			
	UG/731	0.968955	1		
	EG/1566C	0.995624	0.964598	1	
	EG/1556OP	0.995624	0.964598	1	1
NEP	GD/18	1			
	UG/731	0.991701	1		
	EG/1566C	0.991701	0.983333	1	
	EG/1556OP	0.991701	0.983333	1	1

Table S3. Amino acid markers reported in the literature to be associated with host tropism, increased virulence, and escape from the host immune system.

Protein	Amino Acid Marker	Phenotype	Viruses with the marker	Reference(s)^c
PB2	I63 (with PB1 T677)	Pathogenic in mice	All	[27]
	V89 (with, D309, K339, G477, V495, T676)	Enhanced polymerase activity, Increased virulence in mice	All	[25]
	S590/R591	Increased polymerase activity, Human host adaptation	EG/1566C	[26]
PB1	V3 (with N328 and N375)	Increased polymerase activity, Increased virulence in ferrets and mice	All	[28, 31]
	P13	Increased polymerase activity, Adaptation to mice	All	[30]
	Y436	Increased polymerase activity and virulence in mallards, ferrets and mice	All	[29]
	V473	Increased polymerase activity and replication efficiency in mammalian cells	All	[32]
	T677 (with PB2 I63)	Pathogenic in mice	All	[27]
PB1-F2	S66	Increased virulence in mammals	All	[34, 35]
	L82	Increased pathogenicity in mice	GD/18	[36]
PA	A37	Significantly increased viral growth and polymerase activity in mammalian cells	All	[38]
	S277 (with Q278 and P653)	Adaptation to mammalian hosts	All	[37]
PA-X	P28	Increased host shutdown in mammalian cells	All	[39]
	S65	Increased host shutdown in mammalian cells	All	[39]
HA^a	A156	Increased virus binding to α 2–6 sialic acid receptor, Transmissibility in Guinea pigs	All	[40, 41]
	T189	Increased virus binding to α 2–6 SA receptor	UG/731, EG/1566C, EG/1556OP	[42]
NP	V105	Adaptation of duck AIVs to chickens	GD/18, EG/1566C, EG/1556OP	[43]
NA^b	T117	Reduced susceptibility to oseltamivir and zanamivir	All	[44]
M1	D30	Increased virulence in mice	All	[45]
	A215	Increased virulence in mice	All	[45]

NS1	S42	Increased virulence in mice, Antagonism of IFN induction	All	[47]
	F138 (with ESEV in PDZ binding do- main)	Increased replication in mam- malian cells, Decreased inter- feron response	All	[46]
	A149	Increased virulence and de- creased interferon response in chickens	All	[48]

No amino acid substitution was detected from the literature for M2 nor NEP (NS2) proteins.

^aAccording to H5 numbering

^bAccording to N2 numbering

^cReference numbers were given in accordance with the references in the main text

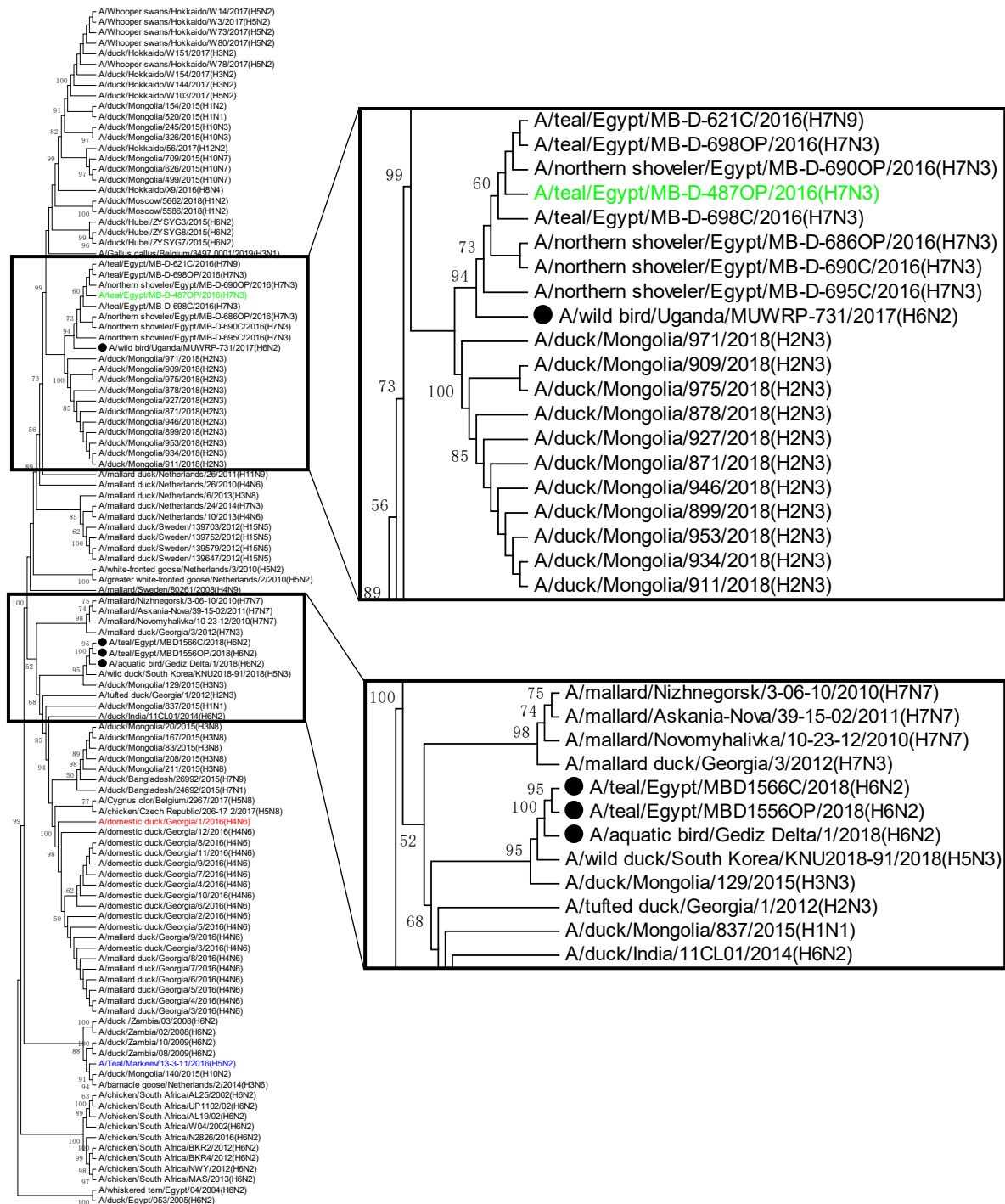


Figure S1. Phylogenetic relationships between GD/18, EG/1566C, EG/1556OP and UG/731 based on PB1 genes. All four viruses were indicated with black circles. Phylogenetic trees were constructed using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model. PB1 genes of GD/18, EG/1566C and EG/1556OP clustered with Korean and Mongolian sequences. PB1 gene of UG/731 mainly located in the same cluster with Egypt and Mongolia PB1 sequences. Reference viruses (most closely related strains for at least one gene segment) are color-coded: A/domestic duck/Georgia/1/2016 (H4N6) in red, A/Teal/Markseev/13-3-11/2016 (H5N2) in blue, and A/teal/Egypt/MB-D-487OP/2016 (H7N3) in green.

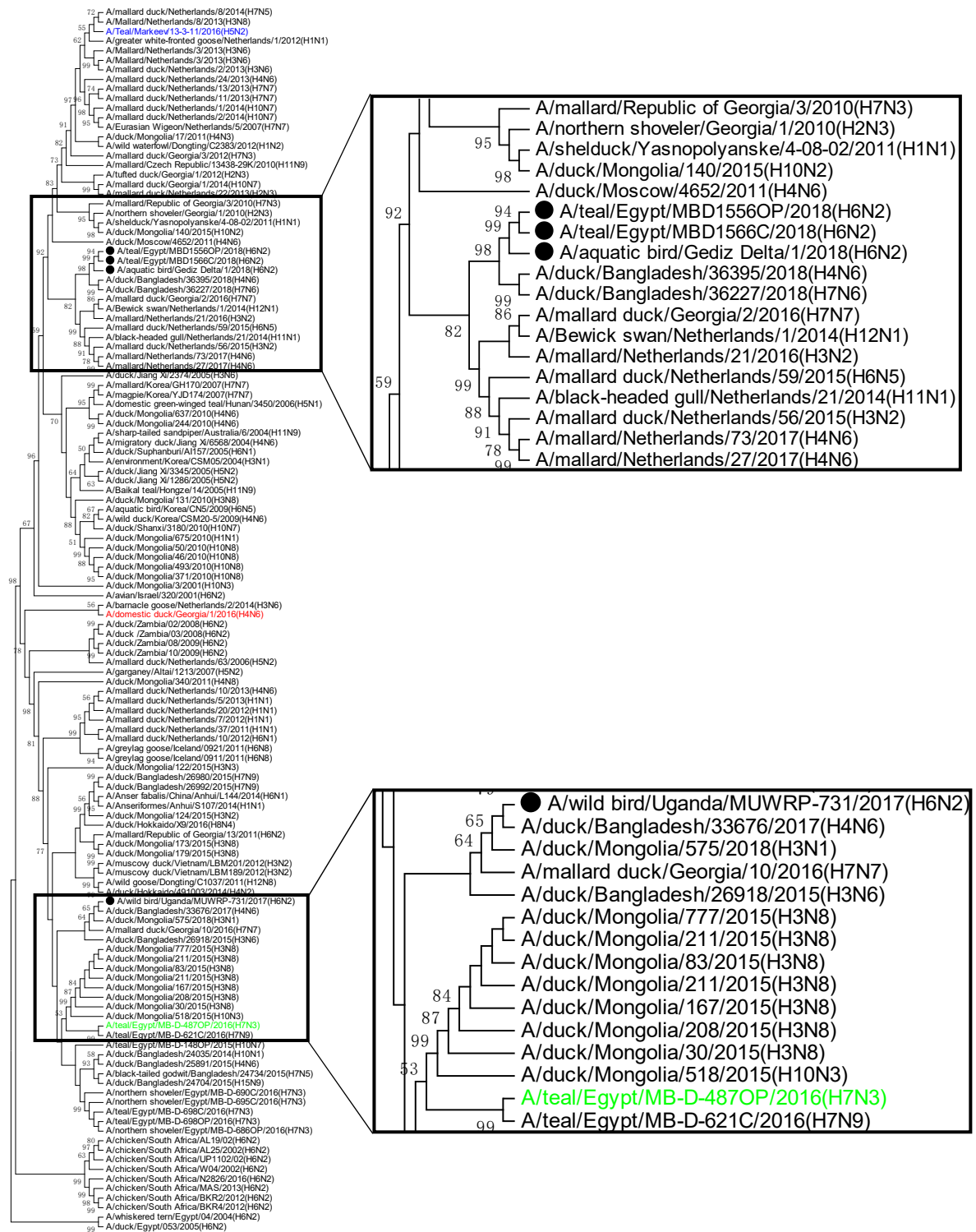


Figure S2. Phylogenetic relationships between GD/18, EG/1566C, EG/1556OP and UG/731 based on PA genes. All four viruses were indicated with black circles. Phylogenetic trees were constructed using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model. PA genes of GD/18, EG/1566C and EG/1556OP clustered together with Bangladesh and Netherlands sequences. PA of UG/731 clustered with Mongolia, Egypt, and Bangladesh sequences. Reference viruses (most closely related strains for at least one gene segment) are color-coded: A/domestic duck/Georgia/1/2016 (H4N6) in red, A/Teal/Markeev/13-3-11/2016 (H5N2) in blue, and A/teal/Egypt/MB-D-487OP/2016 (H7N3) in green.

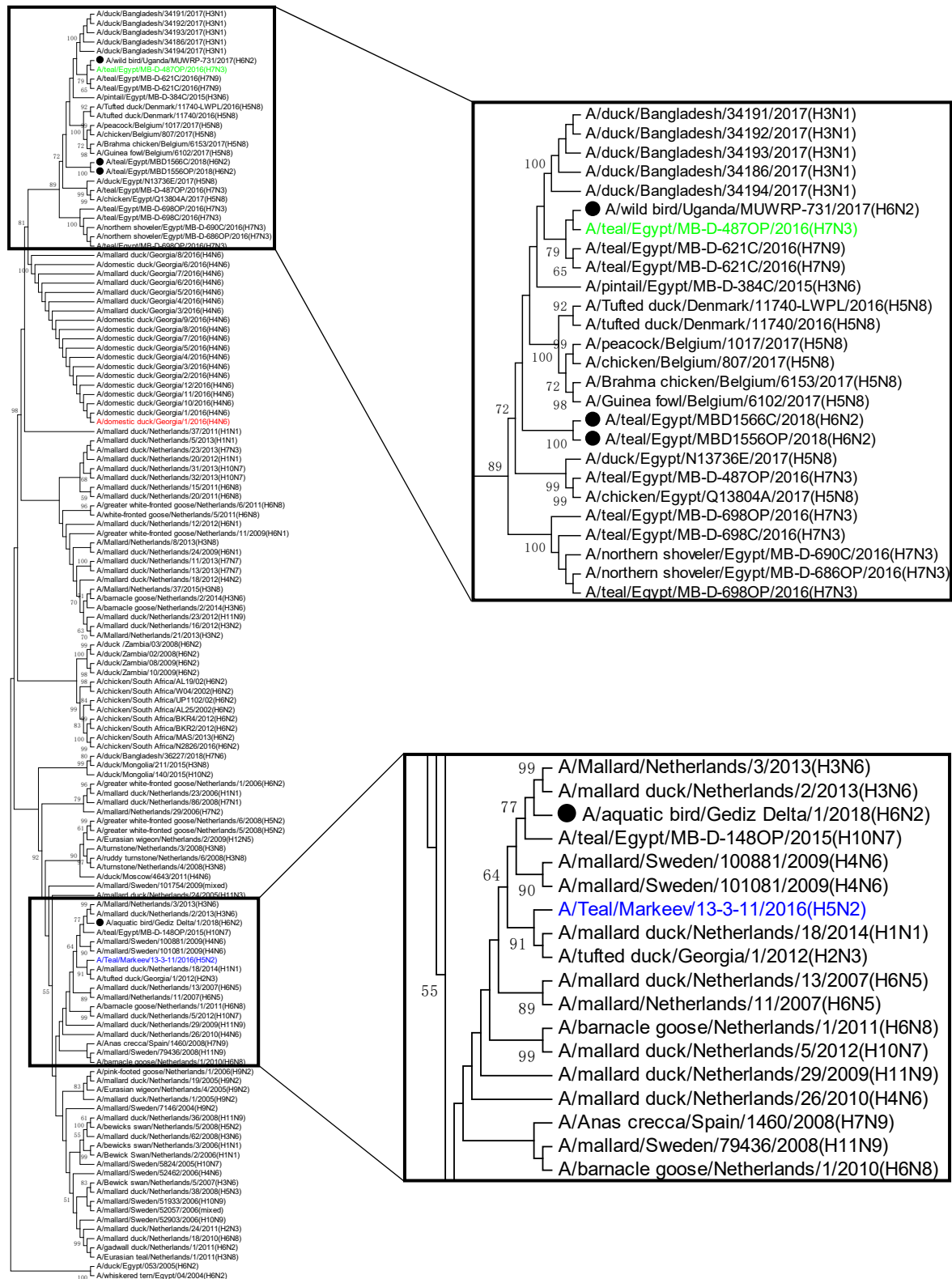


Figure S3. Phylogenetic relationships between GD/18, EG/1566C, EG/1556OP and UG/731 based on NP genes. All four viruses were indicated with black circles. Phylogenetic trees were constructed using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model. NP of UG/731, EG/1566C and EG/1556OP clustered with Bangladesh, Egypt, and some European sequences. NP of GD/18 clustered with European sequences. Reference viruses (most closely related strains for at least one gene segment) are color-coded: A/domestic duck/Georgia/1/2016 (H4N6) in red, A/Teal/Markeev/13-3-11/2016 (H5N2) in blue, and A/teal/Egypt/MB-D-487OP/2016 (H7N3) in green.

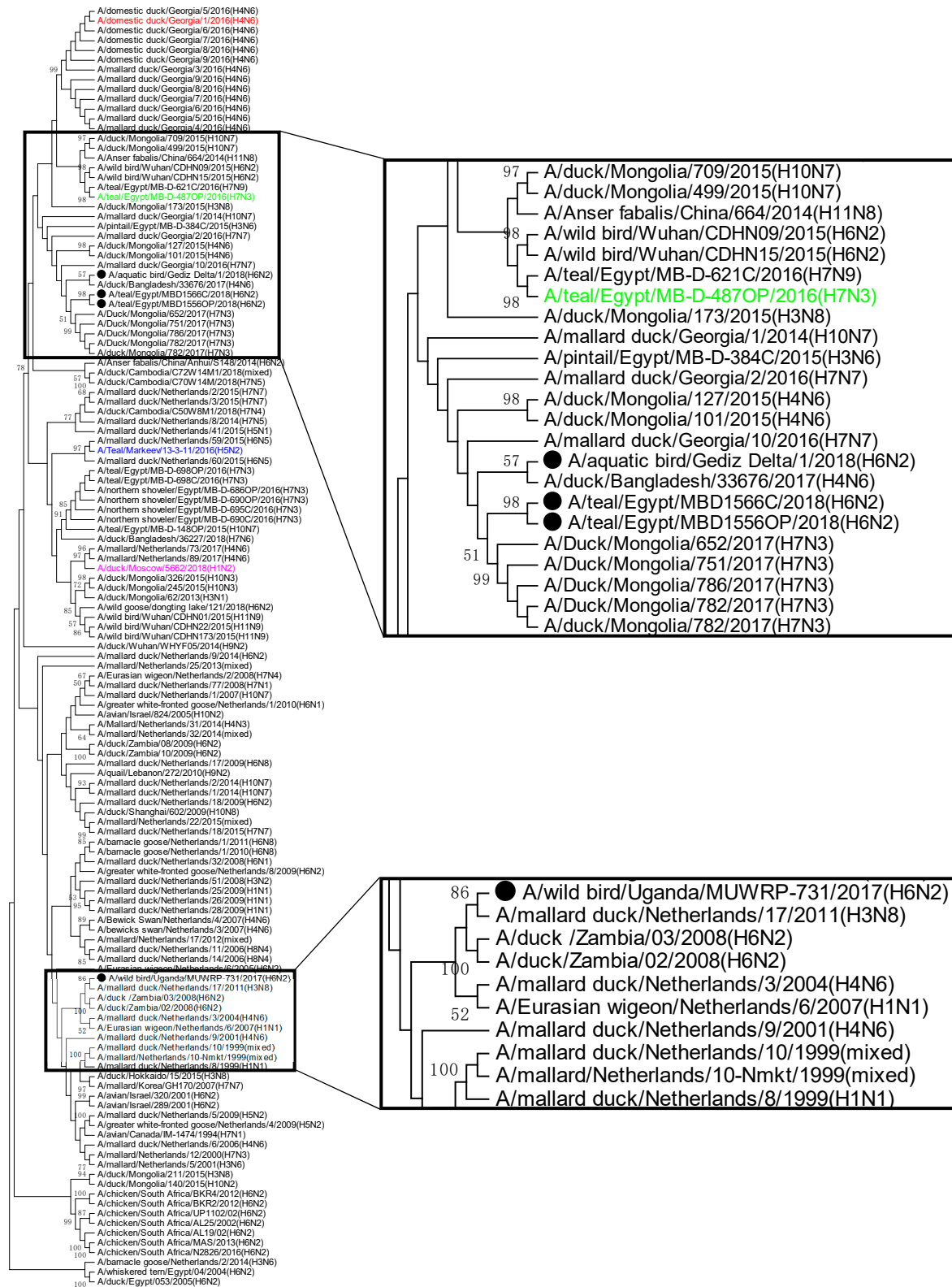


Figure S4. Phylogenetic relationships between GD/18, EG/1566C, EG/1556OP and UG/731 based on M genes. All four viruses were indicated with black circles. Phylogenetic trees were constructed using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model. M gene sequences of GD/18, EG/1566C and EG/1556OP clustered together with Bangladesh, Mongolia, and Georgia sequences. M gene of UG/731 clustered with Netherlands sequences. Reference viruses (most closely related strains for at least one gene segment) are color-coded: A/domestic duck/Georgia/1/2016 (H4N6) in red, A/Teal/Markeev/13-3-11/2016 (H5N2) in blue, A/teal/Egypt/MB-D-487OP/2016 (H7N3) in green, and A/duck/Moscow/5662/2018 (H1N2) in purple.

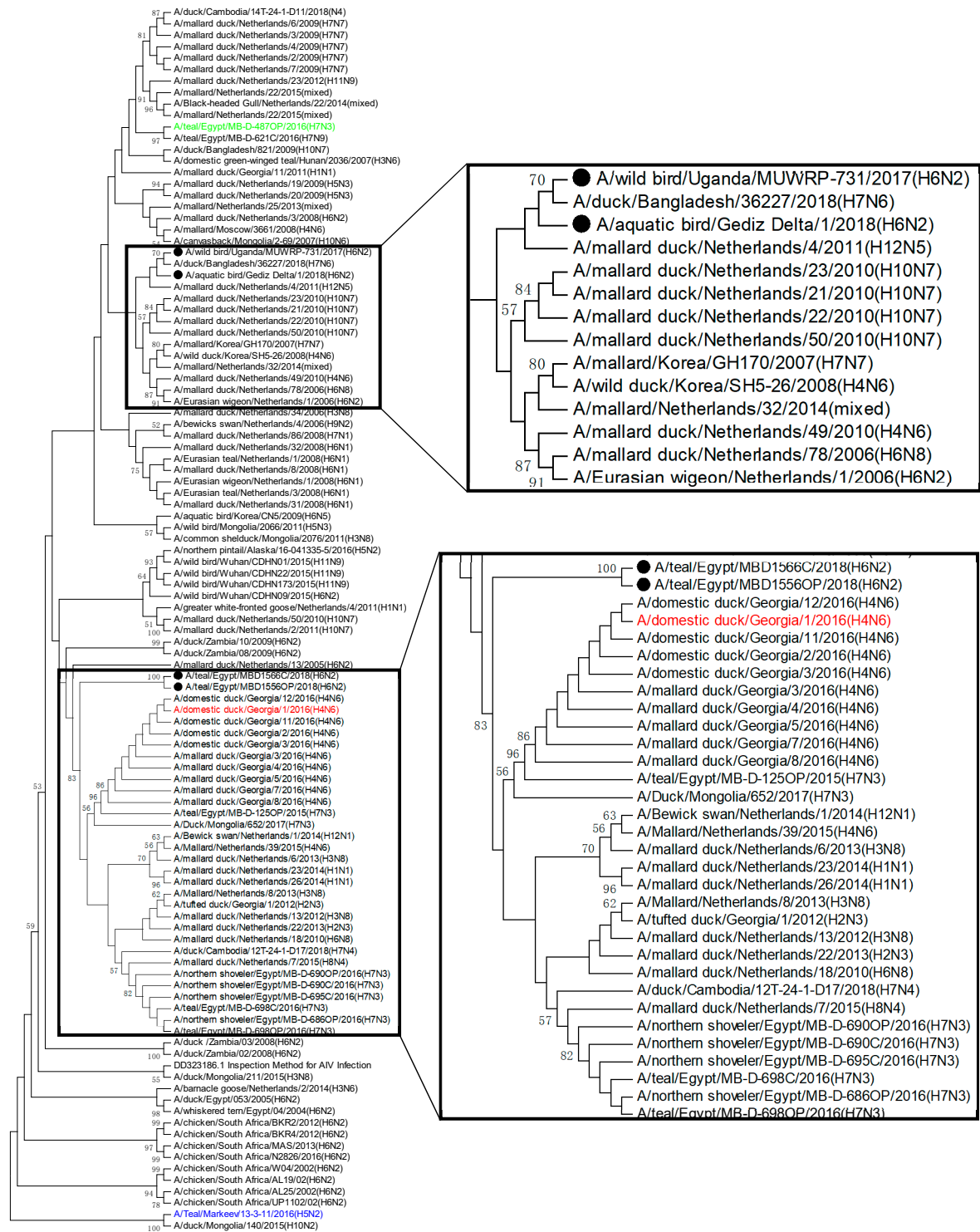


Figure S5. Phylogenetic relationship between GD/18, EG/1566C, EG/1556OP and UG/731 based on NS gene. All four viruses were indicated with black circles. Phylogenetic tree was constructed using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model. NS gene of EG/1566C and EG/1556OP clustered together Georgia, Netherlands, and Egypt sequences. NS of UG/731 and GD/18 clustered with Bangladesh, Korea, and Netherlands sequences. Reference viruses (most closely related strains for at least one gene segment) are color-coded: A/domestic duck/Georgia/1/2016 (H4N6) in red, A/Teal/Markeev/13-3-11/2016 (H5N2) in blue, and A/Teal/Egypt/MB-D-487OP/2016 (H7N3) in green.