

## Supplementary information

### Molecular characterization of a novel avian influenza A (H2N9) strain isolated from wild duck in Korea in 2018

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**Fig. S1. Information of bird species identification**

**Table S1. Initial input of barcoding**

**Table S2. Detailed NGS analysis**

**Table S3. Amino acid residues of the NA, PB2, PB1, PA, NP, NS1 and M1 gene of A/wild duck/ Korea/K102/2018 (H2N9)**

### Reference

**All phylogenetic trees.(HA, M, NA, NP, NS, PA, PB1, PB2)**

# BOLD TaxonID Taxonomy Report

Order	Family	Species
Anseriformes[99 individuals][4 species]		
	Anatidae[99 individuals][4 species]	
		Anas platyrhynchos[81 individuals]
		Anas platyrhynchos x A. crecca[1 individuals]
		Anas poecilorhyncha[16 individuals]
		Tadorna tadorna[1 individuals]

**Fig. S1. Information of bird species identification**

**Table S1. Initial input of barcoding**

AACGTGATCGTCACCGCTCACGCCTTCGTAATAATCTTCTTCATGGTAATGCCATCATAATTGGAGGGTTCGGCAACTGATTGGTCCCCCTGATAATCGGTGCCCCGACATAGCATTCCCACGAATAAACAACATAAGCTTCT  
GACTCCTCCCACCATATTCCTCCTTCTACTCGCCTCATCCACTGTAGAAGCTGGCGCTGGTACGGGTTGAACCGTATACCCACCTTAGCAGGCAACCTAGCCCACGCCGAGCCTCAGAGGACCTGGCTATCTTCTCACTT  
ACCTGGCTGGTGTCTCCTCCATCCTCGGAGCCATTAACCTTACACACAGCCATCAACATAAAACCCCCGCACTCTCACAATACCAAACCCCACTTTTCGTCTGATCAGTCCTAATTACCGCCATCCTGCTCCTCCTACTACT  
CCCCGTCTCGCCGCCGCATCACAAATGCTACTAACCGACCGAAACCTAAACACCACATTCTTTGATCCTGCCGGAGGGCGAGACACAATCCTGTACCAACACCT

**Table S2. Detailed NGS analysis**

Sample	Reference						Sequenced Sample							R_ORF			S_ORF			method	R_ORF-S_ORF length	Contig Count	
	Segment#	gene name	desc.	Accession_ID	desc. all	ref bp	# of Pre-processed reads	# of Influenza Virus extracted reads	# of non-Influenza Virus reads	Virus reads %	#M_Reads	Unique Matches	S_Con_bp	%Cov.(S/R)	Length	S_position	E_position	Length	S_position				E_position
K102	1	PB2	A/duck/Guangdong/707/2000/H2N9	KF260787	gi 523793430 gb KF260787 Influenza A virus (A/duck/Guangdong/707/2000(H2N9)) segment 1 polymerase PB2 (PB2) gene, complete cds	2280	23263654	2186688	21076966	0.103748	17148	0.029	2292	1	2280	1	2280	2274	19	2292	standard :segment 8	6	1
	2	PB1, PB1-F2		KF260543	gi 523792777 gb KF260543 Influenza A virus (A/duck/Guangdong/707/2000(H2N9)) segment 2 polymerase PB1 (PB1) and PB1-F2 protein (PB1-F2) genes, complete cds	2277					17999	0.03	2280	1	2274	1	2274	2274	3	2276		0	1
	3	PA, PA-X		KF260299	gi 523792051 gb KF260299 Influenza A virus (A/duck/Guangdong/707/2000(H2N9)) segment 3 polymerase PA (PA) and PA-X protein (PA-X) genes, complete cds	2151					16372	0.027	2151	1	2151	1	2151	2151	1	2151		0	1
	4	HA		KF258945	gi 523788781 gb KF258945 Influenza A virus (A/duck/Guangdong/707/2000(H2N9)) segment 4 hemagglutinin (HA) gene, complete cds	1689					53303	0.089	1688	1	1689	1	1689	1689	1	1689		0	1
	5	NP		KF259811	gi 523790757 gb KF259811 Influenza A virus (A/duck/Guangdong/707/2000(H2N9)) segment 5 nucleocapsid protein (NP) gene, complete cds	1497					97019	0.162	1505	0.99	1497	1	1497	1506	1	1506		-9	1
	6	NA		KF259722	gi 523790579 gb KF259722 Influenza A virus (A/duck/Guangdong/707/2000(H2N9)) segment 6 neuraminidase (NA) gene, complete cds	1413					4713	0.008	1419	0.9795	1413	1	1413	1395	5	1399		18	1
	7	M2, M1		KF259292	gi 523789549 gb KF259292 Influenza A virus (A/duck/Guangdong/707/2000(H2N9)) segment 7 matrix protein 2 (M2) and matrix protein 1 (M1) genes, complete cds	982					333564	0.556	980	0.999	759	1	759	759	1	759		0	1
	8	NEP, NS1		KF260055	gi 523791319 gb KF260055 Influenza A virus (A/duck/Guangdong/707/2000(H2N9)) segment 8 nuclear export protein (NEP) and nonstructural protein 1 (NS1) genes, complete cds	845					35329	0.059	822	0.9728	693	1	693	690	2	691		3	1

**Table S3.** Amino acid residues of the NA, PB2, PB1, PA, NP, NS1 and M1 gene of A/wild duck/ Korea/K102/2018 (H2N9)

Gene segments	Amino acid	K/2018 <sup>a</sup>	S/2013 <sup>b</sup>	A/2013 <sup>c</sup>	H/MPL133 <sup>d</sup>	H/MPL961 <sup>e</sup>	Comments	Reference
NA	M26I	I	I	I	I	I	M26I: Increased virulence in mice	[1]
	I107V	V	V	V	V	V	I107V: Increased virulence in mice	[2]
	R144K	K	K	K	K	K	R144K: Increased virulence in mice and mammals	[3]
	N146S	S	S	S	S	S	N147S: Increased virulence in mammals	[1]
	T224I	I	I	I	I	I	T224I: Increased virulence in mammals	[4-5]
PB2	L89V	V	V	V	V	V	L89V: Enhanced polymerase activity. Increased virulence in mice	[6-7]
	K251R	R	R	R	R	R	K251R: Increased virulence in mice	[8]
	G309D	D	D	D	D	D	G309D: Enhanced polymerase activity. Increased virulence in mice	[7]
	H447Q	Q	Q	Q	Q	Q	H447Q: Increased polymerase activity. Increased virulence in mammals.	[9-10]
PB1	D/A3V	V	V	V	V	V	D/A3V: Increased polymerase activity. Increased virulence in mammals.	[9-10]
	V13P	P	P	P	P	P	V13P: Increased polymerase activity. Increased virulence in mammals. Mammalian host marker	[11-12]
	R207K	K	K	K	K	K	R207K: Increased polymerase activity in mammalian cells	[13]
	S375N/T	N	N	N	N	N	S375N/T: Increased polymerase activity. Increased virulence in mammals. Human host marker	[9-10, 14]
PA	H266R	R	R	R	R	R	H266R: Increased polymerase activity. Increased virulence in mammals and birds	[15]
	F277S	S	S	S	S	S	F277S: contributed to the virulence and mammalian adaptation	[16]
	S515T	T	T	T	T	T	S515T: Increased polymerase activity. Increased virulence in mammals and birds	[13, 15]

	V41I	I	I	I	I	I	V41I: Contribute to viral transmissibility	[17]
NP	A184K	K	K	K	K	K	A184K: Increased the replication and pathogenicity of H5N1	[18-19]
	D210E	E	E	E	E	E	D210E: Contribute to viral transmissibility	[17]
NS1	P42S	S	S	S	S	S	P42S: Increased virulence in mice	[20-21]
M1	N30D	D	D	D	D	D	N30D: Increased virulence in mice	[22-23]
	T215A	A	A	A	A	A	T215A: Increased virulence in mice	[22-23]

<sup>a</sup>K/2018, A/wild duck/Korea/K102/2018 (H2N9);

<sup>b</sup>S/2013, A/Shanghai/1/2013 (H7N9-human isolate)

<sup>c</sup>A/2013, A/Anhui/1/2013 (H7N9-human isolate)

<sup>d</sup>H/MPL133, A/northern shoveler/Hong Kong/MPL133/2010 (H2N9);

<sup>e</sup>H/MPL961, A/northern shoveler/Hong Kong/MPL961/2011(H2N9);

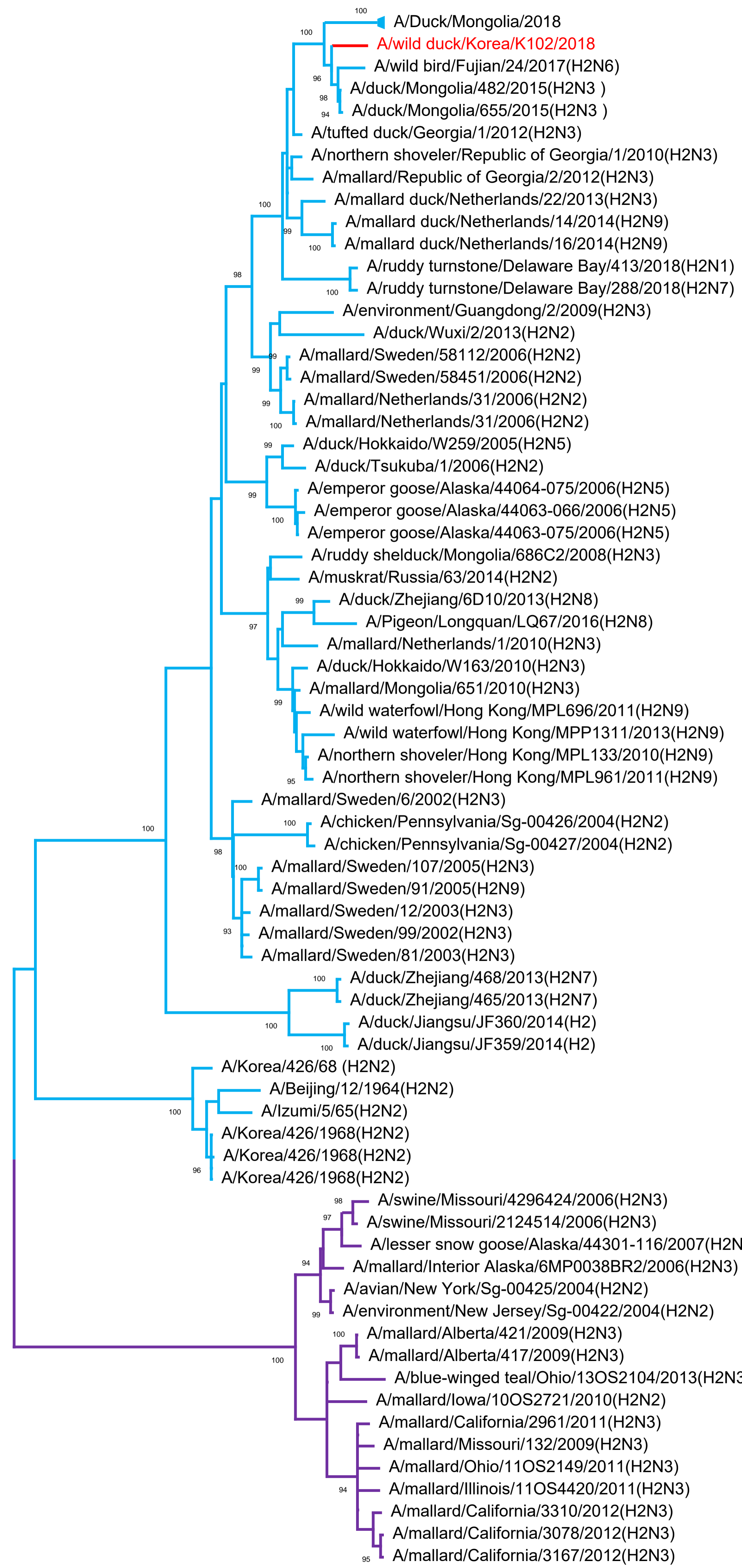
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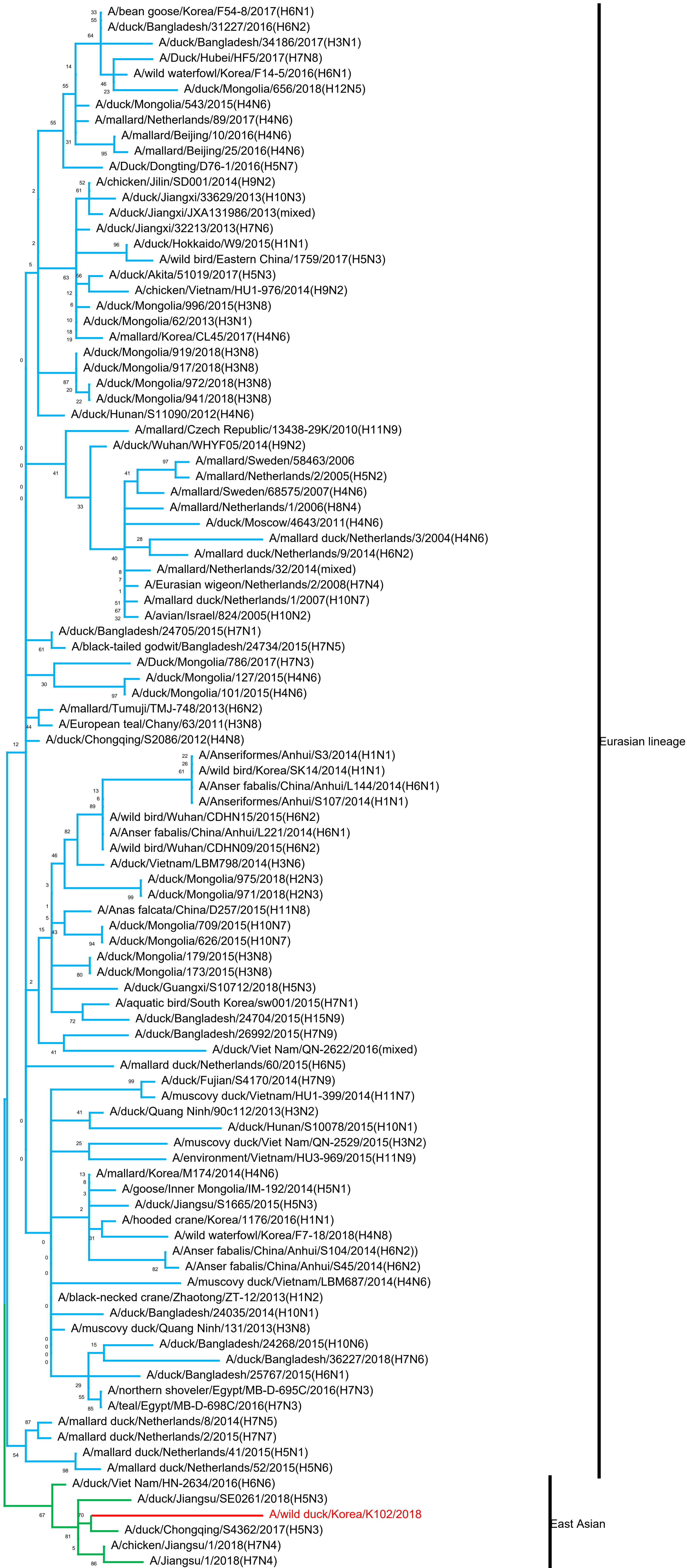
HA



Eurasian lineage

North American lineage

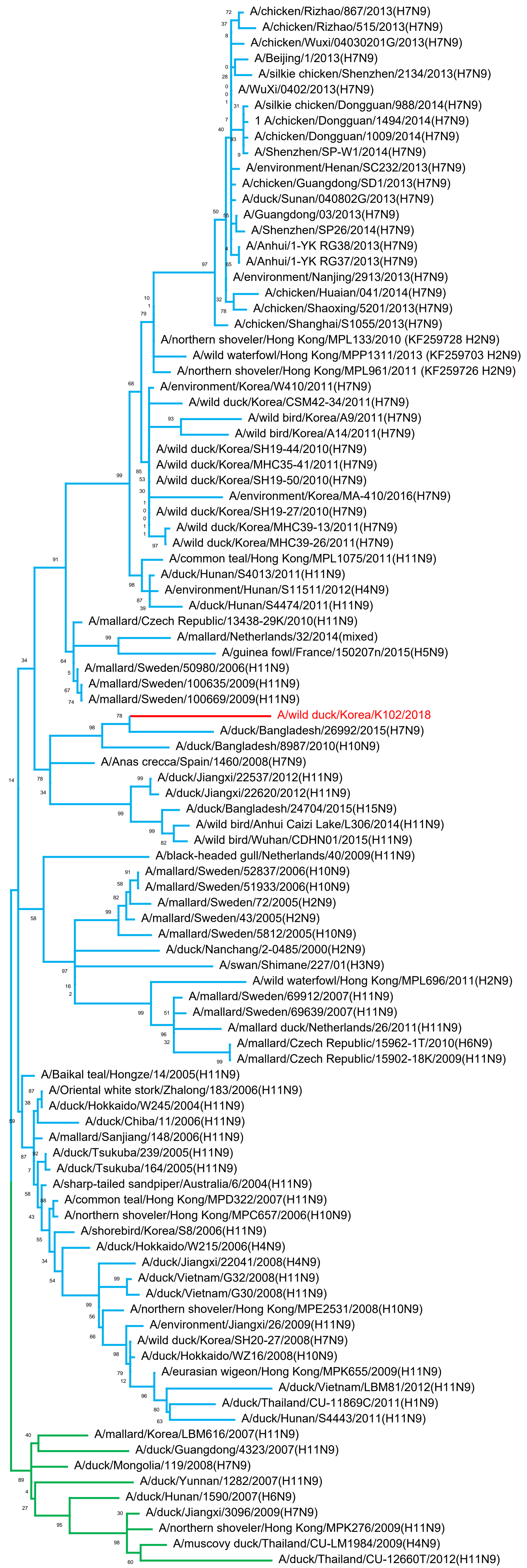
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Eurasian lineage

East Asian

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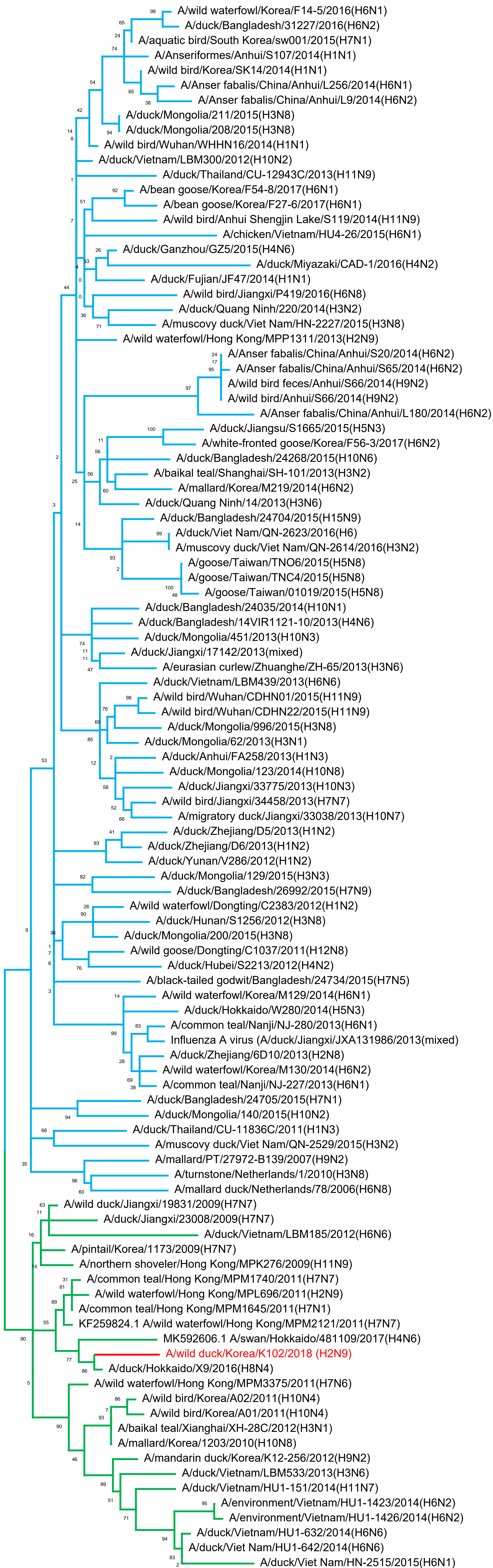


NA

Eurasian lineage

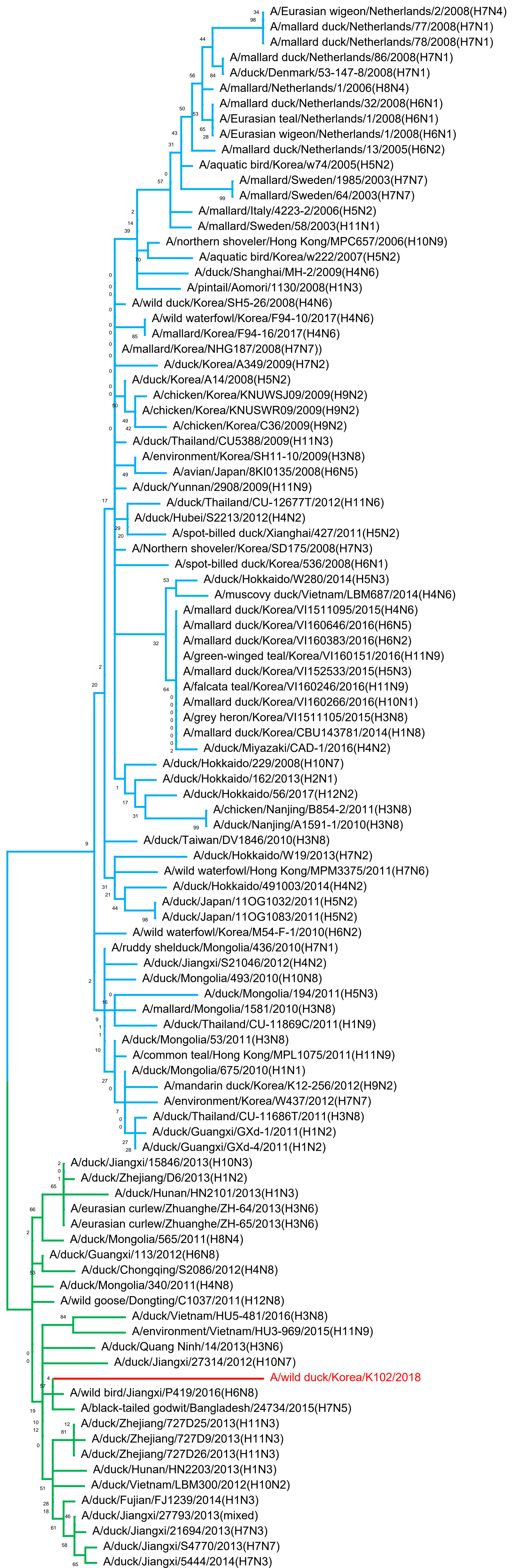
East Asia

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Eurasian lineage

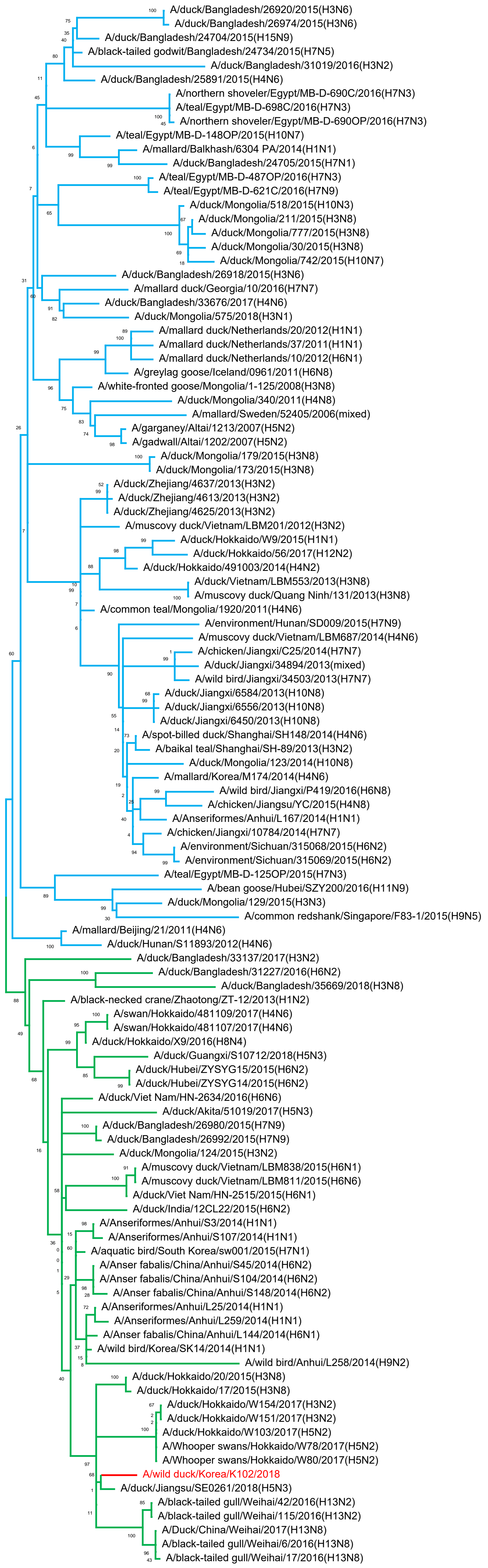
East Asia



NS

Eurasian lineage

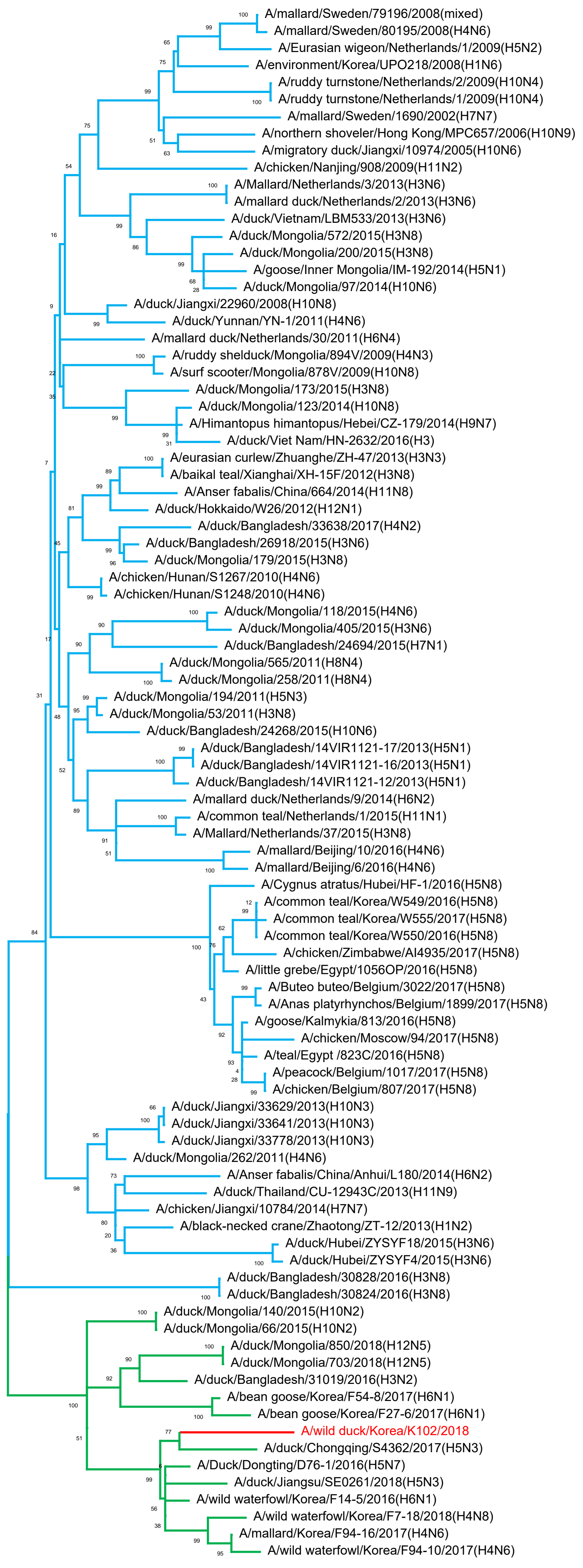
East Asia



Eurasian lineage

East Asia

PB1



Eurasian lineage

East Asia

0.0050



0.0050