

Supplementary data

Genetic characterization of a novel North America-Origin avian Influenza A (H6N5) virus isolated from Bean geese in South Korea in 2018

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Figure S1. Host information by COI gene sequence. Initial input of Barcoding COI gene (A) and information of bird species identification (B)

Table S1. Information of strains with more than 99% genetic homology

Figure S2. Phylogenetic tree of whole genome of the K6 avian influenza virus (AIV) compared to influenza sequences available in NCBI and GISAID.

Figure S3. Maximum likelihood phylogenetic trees showing inferred relationships among nucleotide sequences for the complete coding regions of K6 and K17 gene segments.

Figure S4. Raw ELISA data to conduct TCID₅₀ assay.

Figure S5. Raw ELISA data to conduct TCID₅₀ assay to measure virus K6-H6N5, H1N1, H7N1 titer in lung at day 3 (A); day 6 (B); day 14 (C) post infected.

Figure S6. Lungs from (A) uninfected control; (B) K6-H6N5; (C) H7N1; (D) H7N1 - infected mouse at 6 dpi.

Figure S7. Location map of mandarin duck marked with satellite transmitters in Korea in October in 2017.

Figure S8. Spatial distribution patterns of Bean geese during Jan 2017- Nov 2017.

(A)

CTCTAGGCGACGACCAAATTTACATGTAATCGTTACCGCTCACGCCTTTGTAATA
ATCTTCTTTATAGTCATAACCCATCATGATCGGAGGATTCCGGCAACTGATTAGTCC
CCCTCATAATCGGTGCCCGGACATAGCATTCCCGCGAATAAACAACATAAGCT
TTTGAATCCTCCCCCATCATTCTCCTGCTACTAGCTTCATCCACTGTAGAAGCT
GGCGCCGGCACAGGCTGAACTGTCTACCCTCCCCTAGCAGGTAACCTCGCCCAC
GCCGGAGCTTCAGTAGACCTGGCTATCTTCTCACTCCACTTAGCCGGTATCTCCT
CCATTCTTGGAGCCATCAACTTTATCACCACAGCTATCAACATAAAACCCCCCG
CACTCTCACAATACCAAACCCCACTATTTGTCTGATCCGTGCTAATTACCGCCAT
CCTACTCCTTCTATCACTCCCCGTAATCGCCGCGGTATTACAATACTACTAACT
GATCGAAACCTAAACACCACATTCTTCGATCCCGCTGGAGGAGGAGACCCAAT
CCTGTACCAACACCTATTCTGATTTTTC

(B)

BOLD TaxonID Taxonomy Report

Order	Family	Species
Anseriformes[99 individuals][11 species]		
	Anatidae[99 individuals][11 species]	
		Anser albifrons[23 individuals]
		Anser anser[19 individuals]
		Anser brachyrhynchus[5 individuals]
		Anser caerulescens[11 individuals]
		Anser cygnoides[10 individuals]
		Anser erythropus[4 individuals]
		Anser fabalis[11 individuals]
		Anser indicus[6 individuals]
		Anser rossii[2 individuals]
		Anser sp.[1 individuals]
		Branta canadensis[7 individuals]

Figure S1. Host information by COI gene sequence. Initial input of Barcoding COI gene (A) and information of bird species identification (B)

Table S1. Information of strains with more than 99% genetic homology

Gene	K6		K17	
	Strain	% genetic homology	Strain	% genetic homology
PB2	A/Mallard/Alaska/AH0029066S.1.A/2016(H12N5) (MN254630.1)	99.34	A/Mallard/Alaska/AH0029066S.1.A/2016(H12N5) (MN254630.1)	99.62
	A/Aix galericulata/South Korea/K17-1638-5/2017(H6N5) (MK830104.1)	99.21	A/Mallard/California/D1713638/2017(H12N5) (MK995808.1)	99.15
	A/glaucous-winged gull/Southcentral Alaska/16MB03648/2016(H5N2) (CY239407.1)	99.17	A/Mallard/Utah/D1801802/2018(H5N2) (MK995816.1)	99.1
			A/glaucous-winged gull/Southcentral Alaska/16MB03648/2016(H5N2) (CY239407.1)	99.44
PB1	A/Mallard/Alaska/AH0029066S.2.A/2016(H12N5) (MN254483.1)	99.56	A/American black duck/Maryland/16OS2661/2017(H7N3) (MK236794.1)	99.27
	A/glaucous-winged gull/Southcentral Alaska/16MB03648/2016(H5N2) (CY239406.1)	99.38		
	A/northern pintail/Alaska/16-041335-6/2016(H5N2) (MH546889.1)	99.21		
	A/northern pintail/Alaska/16-041335-18/2016(H5N2) (MH546873.1)	99.21		
	A/northern pintail/Alaska/16-041335-16/2016(H5N2) (MH546857.1)	99.16		
	A/mallard duck/California/UCD738/2016(H4N5) (KY990805.1)	99.03		
PA	A/Aix galericulata/South Korea/K17-1638-5/2017(H6N5) (MK830102.1)	99.49	A/Mallard/California/D1713634/2017(H9N2) (MK995798.1)	99.33
	A/emperor goose/Alaska/UGAI15-6737/2015(H3N8) (KX949459.1)	99.26	A/Northern Shoveler/California/D1715961/2017(H8N4) (MK995838.1)	99.28
	A/emperor goose/Alaska/UGAI15-6758/2015(H3N8) (KX949467.1)	99.26	A/Green-Winged Teal/California/D1800952/2018(H9N2) (MK995782.1)	99.28
	A/emperor goose/Alaska/UGAI15-6759/2015(H3N8) (KX949475.1)	99.26	A/Green-Winged Teal/California/D1800177/2018(mixed) (MK995772.1)	99.28
	A/emperor goose/Alaska/UGAI15-6734/2015(H3N8) (KY131296.1)	99.21	A/mallard/Alberta/71/2017(H3N8) (MH411975.1)	99.28
	A/Mallard/California/D1713634/2017(H9N2) (MK995798.1)	99.16	A/mallard/Alberta/40/2017(H3N8) (MH411894.1)	99.33
	A/mallard/Alberta/71/2017(H3N8) (MH411975.1)	99.16	A/mallard/Alberta/362/2017(H3N8) (MH637046.1)	99.24
	A/mallard/Alberta/40/2017(H3N8) (MH411894.1)	99.16	A/mallard/Alberta/81/2017(H3N8) (MH412146.1)	99.28

A/mallard/Southcentral Alaska/12ML01469/2014(mixed) (CY194170.1)	99.16	A/lesser snow goose/Oregon/16-006224- 1/2016(H5N3) (MH546530.1)	99.19
A/Northern Shoveler/California/D1715961/2017 (H8N4) (MK995838.1)	99.12	A/blue-winged teal/Alberta/98/2017(H3N8) (MH412308.1)	99.28
A/Green-Winged Teal/California/D1800952/2018(H9N2) (MK995782.1)	99.12	A/northern shoveler/California/AH0076719/2016(H7 N3) (KY550793.1)	99.1
A/Green-Winged Teal/California/D1800177/2018(mixed) (MK995772.1)	99.12	A/mallard/Alberta/182/2017(H3N8) (MH637074.1)	99.15
A/mallard/Alberta/273/2017(H4N6) (MH981933.1)	99.12	A/Green-Winged Teal/Nevada/D1612806/2016(H5N2) (MK928161.1)	99.06
A/mallard/Alberta/498/2017(H4N6) (MH932484.1)	99.12	A/blue-winged teal/Alberta/205/2017(H4N6) (MH932335.1)	99.28
A/blue-winged teal/Alberta/210/2017(H4N6) (MH932445.1)	99.12	A/mallard/Alberta/249/2017(H4N6) (MH932343.1)	99.28
A/blue-winged teal/Alberta/209/2017(H4N6) (MH932407.1)	99.12	A/mallard/Alberta/243/2017(H4N6) (MH932273.1)	99.28
A/mallard/Alberta/231/2017(H4N6) (MH932391.1)	99.12	A/mallard/Alberta/245/2017(H4N6) (MH932128.1)	99.28
A/mallard/Alberta/228/2017(H4N6) (MH932383.1)	99.12	A/American green-winged teal/Illinois/17OS2545/2017(H4N6) (MK237839.1)	99.15
A/blue-winged teal/Alberta/207/2017(H4N6) (MH932311.1)	99.12	A/mallard/Alberta/251/2017(H4N6) (MH932224.1)	99.28
A/mallard/Alberta/129/2017(mixed) (MH932359.1)	99.12	A/blue-winged teal/Illinois/17OS2553/2017(H6N1) (MK237200.1)	99.01
A/blue-winged teal/Alberta/205/2017(H4N6) (MH932335.1)	99.12	A/mallard/Southcentral Alaska/12ML01469/2014(mixed) (CY194170.1)	99.32
A/blue-winged teal/Alberta/211/2017(H4N6) (MH932303.1)	99.12	A/bufflehead/Alaska/512/2014(H4N8) (KT338435.1)	99.23
A/mallard/Alberta/249/2017(H4N6) (MH932343.1)	99.12	A/mallard/Washington/16-027073- 5/2016(H5N2) (MH546802.1)	99.28
A/mallard/Alberta/253/2017(H4N6) (MH932287.1)	99.12	A/mallard/Washington/16-027073- 48/2016(H5N2) (MH546794.1)	99.28
A/mallard/Alberta/243/2017(H4N6) (MH932273.1)	99.12	A/mallard/Washington/16-027073- 46/2016(H5N2) (MH546786.1)	99.28
A/mallard/Alberta/241/2017(H4N6) (MH932257.1)	99.12	A/mallard/Washington/16-027073- 22/2016(H5N2) (MH546770.1)	99.28
A/blue-winged teal/Alberta/206/2017(H4N6) (MH932265.1)	99.12	A/mallard/Washington/16-027073- 21/2016(H5N2) (MH546762.1)	99.28

A/mallard/Alberta/255/2017(H4N6) (MH932241.1)	99.12	A/mallard/Alberta/498/2017(H4N6) (MH932484.1)	99.28
A/mallard/Alberta/234/2017(H4N6) (MH932249.1)	99.12	A/mallard/Alberta/231/2017(H4N6) (MH932391.1)	99.28
A/mallard/Alberta/251/2017(H4N6) (MH932224.1)	99.12	A/emperor goose/Alaska/UGAI15- 6737/2015(H3N8) (KX949459.1)	99.41
A/mallard/Alberta/233/2017(H4N6) (MH932184.1)	99.12	A/emperor goose/Alaska/UGAI15- 6758/2015(H3N8) (KX949467.1)	99.41
A/mallard/Alberta/123/2017(H4N6) (MH932144.1)	99.12	A/emperor goose/Alaska/UGAI15- 6759/2015(H3N8) (KX949475.1)	99.41
A/mallard/Alberta/245/2017(H4N6) (MH932128.1)	99.12	A/blue-winged teal/Alberta/210/2017(H4N6) (MH932445.1)	99.27
A/mallard/Alberta/232/2017(H4N6) (MH932112.1)	99.12	A/blue-winged teal/Alberta/209/2017(H4N6) (MH932407.1)	99.27
A/mallard/Alberta/201/2017(H4N6) (MH932120.1)	99.12	A/mallard/Alberta/228/2017(H4N6) (MH932383.1)	99.27
A/mallard/Alberta/362/2017(H3N8) (MH637046.1)	99.12	A/blue-winged teal/Alberta/207/2017(H4N6) (MH932311.1)	99.27
A/blue-winged teal/Alberta/98/2017(H3N8) (MH412308.1)	99.12	A/mallard/Alberta/129/2017(mixed) (MH932359.1)	99.27
A/mallard/Alberta/81/2017(H3N8) (MH412146.1)	99.12	A/mallard/Alberta/253/2017(H4N6) (MH932287.1)	99.27
A/mallard/Washington/16-027073- 5/2016(H5N2) (MH546802.1)	99.12	A/mallard/Alberta/241/2017(H4N6) (MH932257.1)	99.27
A/mallard/Washington/16-027073- 48/2016(H5N2) (MH546794.1)	99.12	A/blue-winged teal/Alberta/206/2017(H4N6) (MH932265.1)	99.27
A/mallard/Washington/16-027073- 46/2016(H5N2) (MH546786.1)	99.12	A/mallard/Alberta/123/2017(H4N6) (MH932144.1)	99.27
A/mallard/Washington/16-027073- 22/2016(H5N2) (MH546770.1)	99.12	A/mallard/Alberta/232/2017(H4N6) (MH932112.1)	99.27
A/mallard/Washington/16-027073- 21/2016(H5N2) (MH546762.1)	99.12	A/mallard/Alberta/273/2017(H4N6) (MH981933.1)	99.27
A/mallard/California/16-038989- 4/2016(H5N2) (MH546570.1)	99.12	A/blue-winged teal/Alberta/211/2017(H4N6) (MH932303.1)	99.27
A/mallard/Southcentral Alaska/12ML00991_AAF1/2014(H5N2) (CY194194.1)	99.12	A/mallard/Alberta/255/2017(H4N6) (MH932241.1)	99.27
A/bufflehead/Alaska/512/2014(H4N8) (KT338435.1)	99.07	A/mallard/Alberta/201/2017(H4N6) (MH932120.1)	99.27
A/mallard/Washington/16-028147- 4/2016(H5N2) (MH546810.1)	99.07	A/mallard/Washington/16-028147- 4/2016(H5N2) (MH546810.1)	99.23
A/mallard/Washington/16-027073- 45/2016(H5N2) (MH546778.1)	99.07	A/emperor goose/Alaska/UGAI15- 6734/2015(H3N8) (KY131296.1)	99.36

A/lesser snow goose/Oregon/16-006224-1/2016(H5N3) (MH546530.1)	99.07	A/mallard/Southcentral Alaska/12ML00991_AAF1/2014(H5N2) (CY194194.1)	99.27
A/mallard/Alberta/53/2017(H3N8) (MH932351.1)	99.02	A/mallard/Alberta/233/2017(H4N6) (MH932184.1)	99.27
A/mallard/Alberta/30/2017(H3N8) (MH932327.1)	99.02	A/mallard/Alberta/234/2017(H4N6) (MH932249.1)	99.27
A/mallard/Alberta/230/2017(H3N8) (MH932295.1)	99.02	A/mallard/Washington/16-027073-45/2016(H5N2) (MH546778.1)	99.23
A/mallard/Alberta/246/2017(H3N8) (MH932176.1)	99.02	A/mallard/California/16-038989-4/2016(H5N2) (MH546570.1)	99.18
A/mallard/Alberta/292/2017(H3N8) (MH932160.1)	99.02	A/mallard/Nevada/16-035274-2/2016(H5N2) (MH546674.1)	99.14
A/blue-winged teal/Alberta/4/2017(H3N8) (MH932152.1)	99.02	A/mallard/Oregon/16-028808-2/2016(H5N2) (MH546714.1)	99.09
A/mallard/Alberta/270/2017(mixed) (MH932136.1)	99.02	A/mallard/Southcentral Alaska/12ML01175/2014(mixed) (CY206803.1)	99.14
A/blue-winged teal/Alberta/3/2017(H3N8) (MH411905.1)	99.02	A/northern pintail/Alaska/UGAI15-6407/2015(H3N8) (KX949451.1)	99.14
		A/green-winged teal/Alaska/UGAI15-7287/2015(H3N8) (KX949491.1)	99.14
		A/northern pintail/Alaska/UGAI15-7291/2015(H3N8) (KX949507.1)	99.14
		A/northern pintail/Alaska/UGAI15-7300/2015(H6N1) (KY131359.1)	99.14
		A/green-winged teal/Alaska/UGAI15-6067/2015(H8N4) (KY131249.1)	99.14
		A/American green-winged teal/Nevada/16-033700-1/2016(H5N2) (MH546202.1)	99.05
		A/northern pintail/Alaska/UGAI15-7288/2015(H3N8) (KX949499.1)	99.09
		A/green-winged teal/Alaska/UGAI15-7286/2015(H3N8) (KX949483.1)	99.09
		A/mallard/Alberta/53/2017(H3N8) (MH932351.1)	99.18
		A/mallard/Alberta/30/2017(H3N8) (MH932327.1)	99.18
		A/mallard/Alberta/230/2017(H3N8) (MH932295.1)	99.18
		A/mallard/Alberta/246/2017(H3N8) (MH932176.1)	99.18
		A/mallard/Alberta/292/2017(H3N8) (MH932160.1)	99.18
		A/blue-winged teal/Alberta/4/2017(H3N8) (MH932152.1)	99.18
		A/mallard/Alberta/270/2017(mixed) (MH932136.1)	99.18

			A/blue-winged teal/Alberta/3/2017(H3N8) (MH411905.1)	99.18
			A/northern pintail/Alaska/UGAI15-7399/2015(H3N6) (KY131391.1)	99.04
			A/northern pintail/Alaska/UGAI15-6994/2015(H6N1) (KY131328.1)	99.04
			A/mallard/Alaska/UGAI15-7301/2015(mixed) (KX949547.1)	99
HA	A/Aix galericulata/South Korea/K17-1638-5/2017(H6N5) (MK830100.1)	99.3		
NP	A/lesser scaup/Wisconsin/17OS5811/2017(H6N1) (MK236919.1)	99.67	A/American green-winged teal/Missouri/17OS3212/2017(H3N8) (MK236738.1)	99.55
	A/Northern Pintail/Arizona/D1800538/2018(H12N2) (MK995828.1)	99.6	A/blue-winged teal/Illinois/17OS2636/2017(H4N6) (MK237055.1)	99.42
	A/American wigeon/California/LDC523/2016(H6N1) (MH251193.1)	99.6	A/mallard/Ohio/16OS0620/2016(H10N7) (KY561279.1)	99.23
	A/northern shoveler/Missouri/17OS4821/2017(H6N1) (MK237918.1)	99.6	A/Blue-winged Teal/North Carolina/AH0114567S.5.A/2017(H4N6) (MN254118.1)	99.11
	A/American wigeon/Missouri/17OS4780/2017(H6N1) (MK237159.1)	99.53	A/Northern Shoveler/Nevada/D1615770/2016(H10N9) (MK928247.1)	99.04
	A/American green-winged teal/Tennessee/18OS0447/2018(H5N2) (MK237929.1)	99.47	A/mallard/Idaho/16-034834-1/2016(H5N2) (MH546580.1)	99.1
	A/Gadwall/Ohio/18OS2668/2018(H6N1) (MN431215.1)	99.06	A/mallard duck/Illinois/17OS1431/2017(H10N7) (MG280472.1)	99.04
	A/American Wigeon/Ohio/18OS1967/2018(H6N1) (MN431212.1)	99	A/green-winged teal/Missouri/15OS6706/2015(H6N1) (KY563727.1)	99.03
	A/American Green-winged Teal/Ohio/18OS2656/2018(H6N1) (MN430901.1)	99	A/mallard/Idaho/UGAI16-1929/2016(H3N8) (CY235197.1)	99.03
	A/mallard/Arkansas/AI09-5761/2009 (H2N3) (CY141020.1)	99		
NA	A/Aix galericulata/South Korea/K17-1638-5/2017(H6N5) (MK830105.1)	99.16		
MP	A/mallard/Kentucky/AH00729344/2016 (H7N2) (KY550970.1)	99.59	A/Northern Pintail/Arizona/D1800538/2018(H12N2) (MK995826.1)	99.22
	A/mallard duck/Ohio/15OS7510/2015(H11N3) (KY561112.1)	99.49	A/Blue-winged Teal/South Dakota/AH0037508S.7.A/2015(N8) (MN253741.1)	99.12
	A/Northern shoveler/Mississippi/16OS5984/2016 (H6N1) (MG280067.1)	99.39	A/Mexican duck/EstadodeMexico; Lerma/M_UIFMVZ377/2016(H5N2) (MK828141.1)	99.03

A/Mallard Duck/Ohio/16OS0899/2016(H2N3) (MN552532.1)	99.29	A/mallard/Ohio/17OS5967/2017(H3N1) (MK236986.1)	99
A/mallard/Idaho/UGAI16-1941/2016(mixed) (CY235246.1)	99.29	A/mallard/Pennsylvania/UGAI15-4892/2015(H1N1) (MH341857.1)	99
A/blue-winged teal/Louisiana/UGAI15-2510/2015(H7N3) (KY013813.1)	99.29	A/mallard duck/Ohio/16OS0859/2016(H3N8) (MG280174.1)	99
A/blue-winged teal/Texas/UGAI14-3653/2014(mixed) (CY205500.1)	99.29	A/mallard duck/Ohio/16OS0862/2016(H3N8) (MG279891.1)	99
A/northern pintail/Alaska/819/2014(H4N6) (KT338548.1)	99.29	A/mallard duck/Ohio/16OS0846/2016(H3N8) (MG280490.1)	99
A/American green-winged teal/Alaska/306/2014(H3N3) (KT338352.1)	99.29	A/mallard duck/Ohio/16OS0843/2016(H3N8) (MG280070.1)	99
A/Mallard/Pennsylvania/AH0119501S .7.A/2017(Mixed) (MN253842.1)	99.19	A/American wigeon/Maryland/16OS5139/2016(H6N8) (MG280072.1)	99
A/Mallard/Illinois/17OS2751/2017(H5N2) (MN431105.1)	99.19	A/blue-winged teal/Texas/UGAI15-6811/2015(H4N8) (KY014007.1)	99
A/mallard/Washington/16-027073-5/2016(H5N2) (MH546806.1)	99.19	A/blue-winged teal/Texas/UGAI15-6848/2015(H4N8) (KY014015.1)	99
A/mallard/Washington/16-027073-48/2016(H5N2) (MH546798.1)	99.19	A/mallard/Utah/AH0020690/2015(H7N3) (KY551350.1)	99
A/mallard/Washington/16-027073-46/2016(H5N2) (MH546790.1)	99.19	A/mallard/Idaho/AH0011522/2015(H7N7) (KY551319.1)	99
A/mallard/Washington/16-027073-45/2016(H5N2) (MH546782.1)	99.19		
A/mallard/Washington/16-027073-22/2016(H5N2) (MH546774.1)	99.19		
A/mallard/Washington/16-027073-21/2016(H5N2) (MH546766.1)	99.19		
A/Northern shoveler/Ohio/16OS5078/2016(H10N7) (MG280008.1)	99.19		
A/mallard/Idaho/UGAI16-1957/2016(mixed) (CY235299.1)	99.19		
A/cinnamon teal/Idaho/UGAI16-1910/2016(mixed) (CY235050.1)	99.19		
A/American wigeon/California/HS001/2015(H6N2) (KY983210.1)	99.19		
A/mallard/Louisiana/UGAI15-0711/2015(H7N3) (KY013743.1)	99.19		
A/blue-winged teal/Texas/UGAI15-2283/2015(H7N3) (KY013783.1)	99.19		
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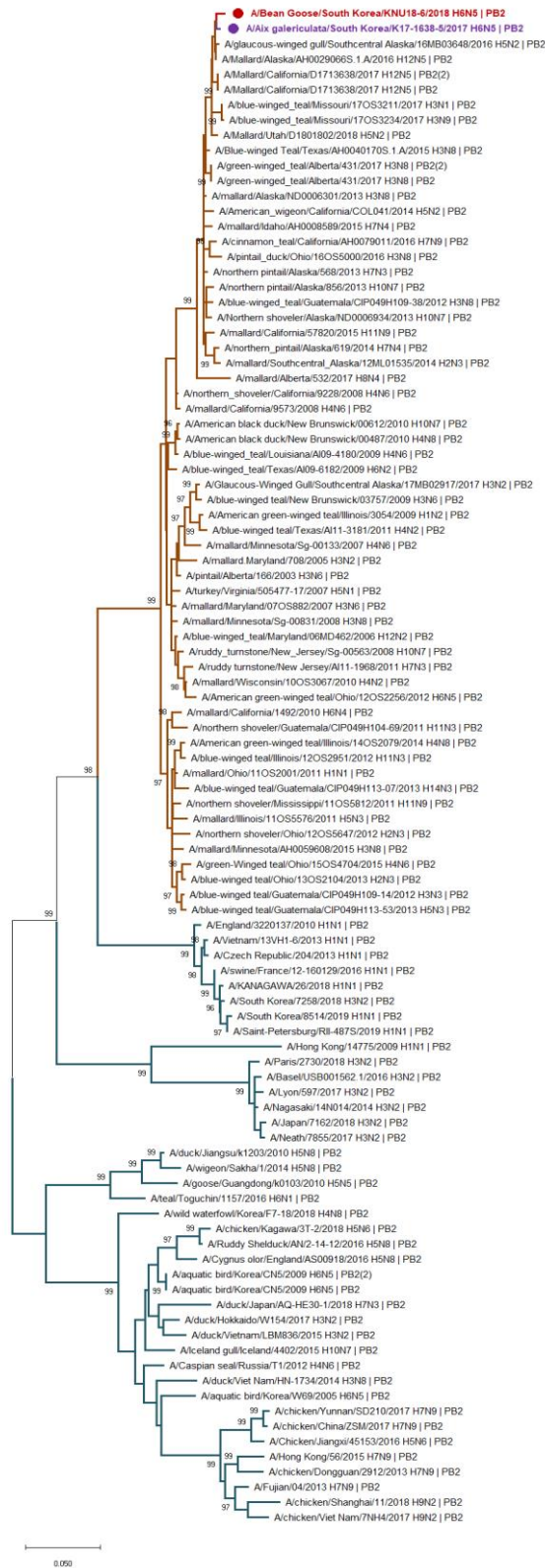
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	A/Aix galericulata/South Korea/K17-1638-5/2017(H6N5) (MK830107.1)	99.4	A/northern pintail/Alaska/16-041335-16/2016(H5N2) (MH546863.1)	99.44

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A/northern pintail/Alaska/16-041335-16/2016(H5N2) (MH546863.1)	99.4	A/mallard/Alberta/414/2017(H11N9) (MH637219.1)	99.44
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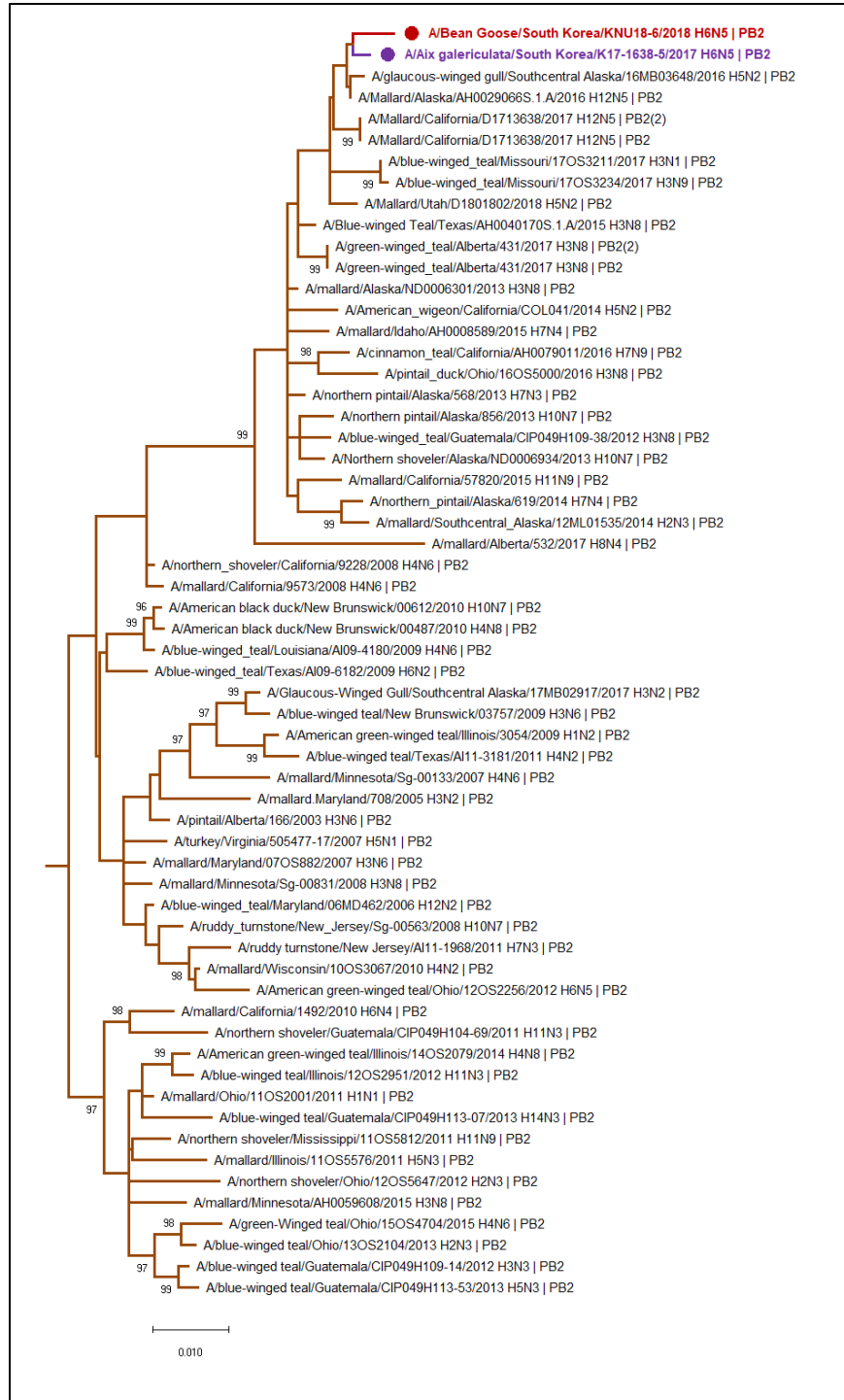
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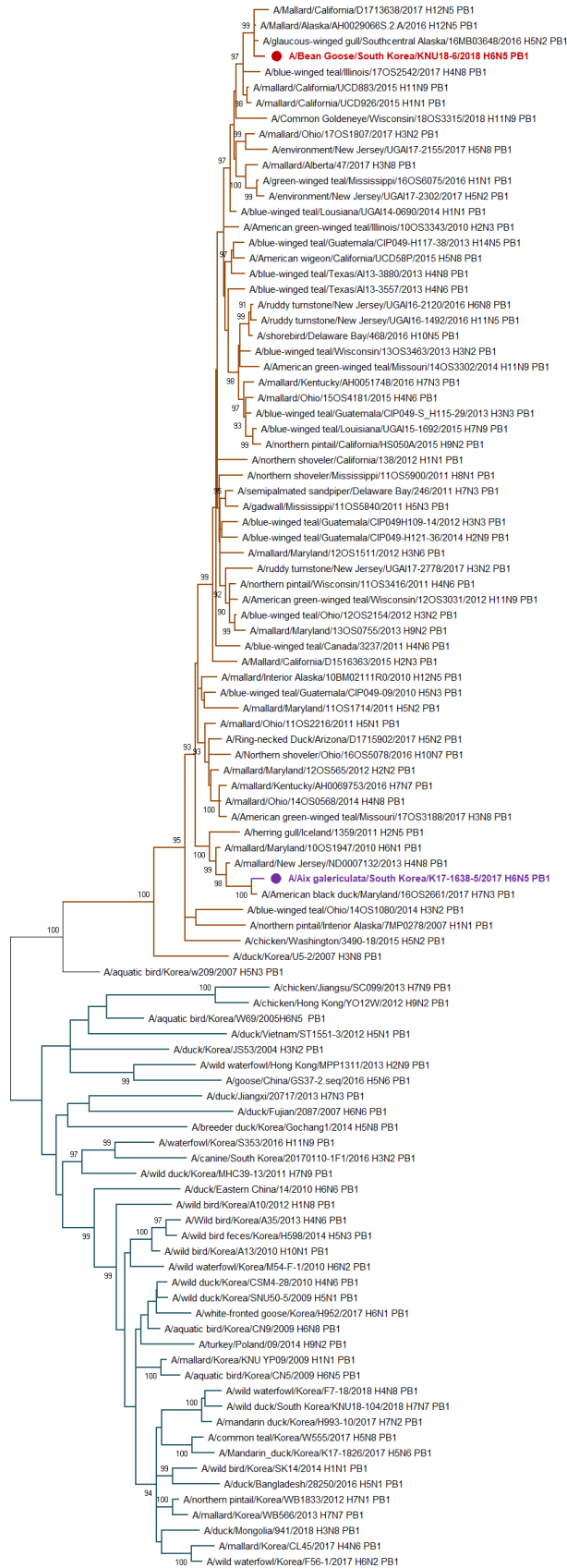
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EA

a. PB2
NAM



B. PB1

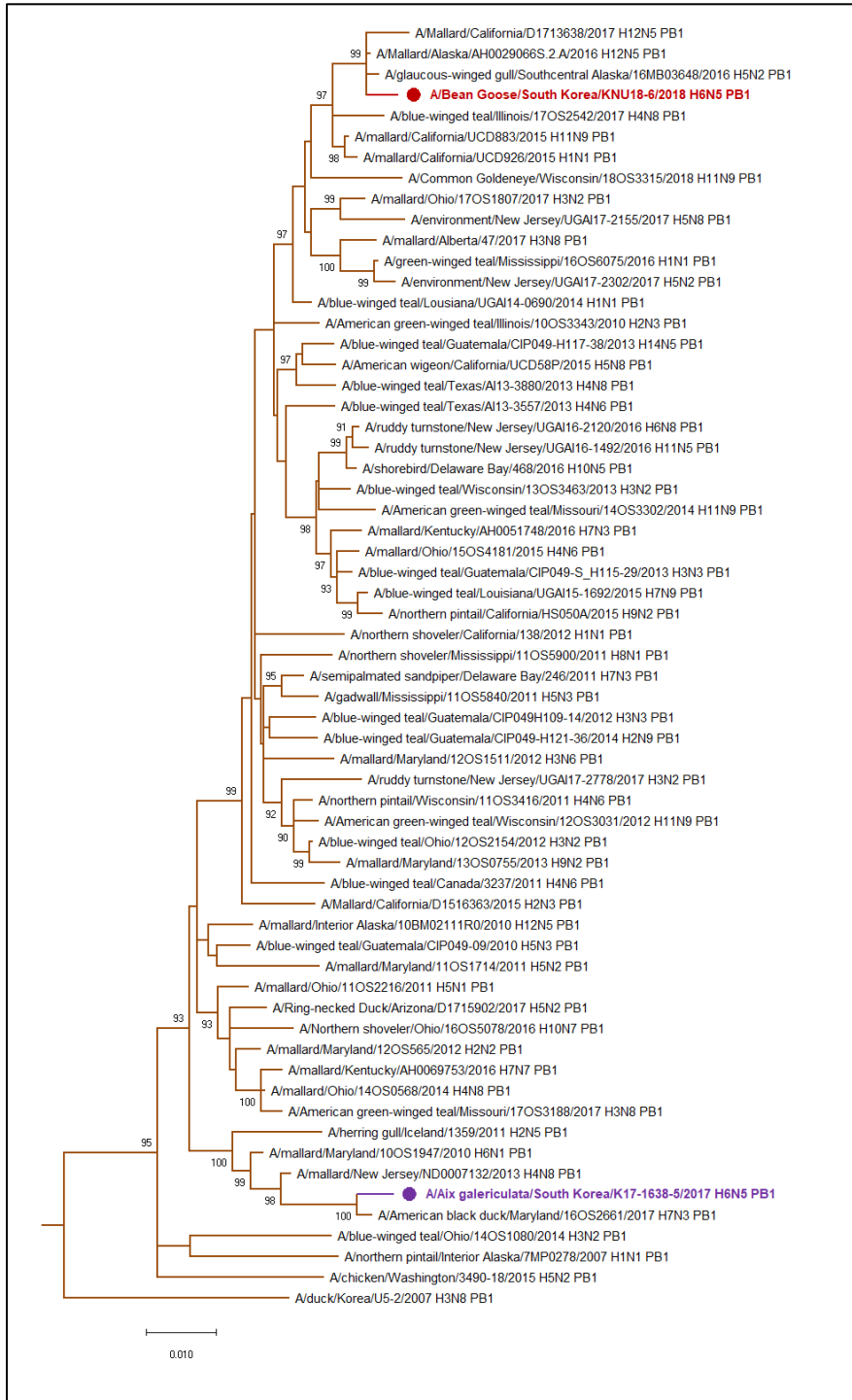


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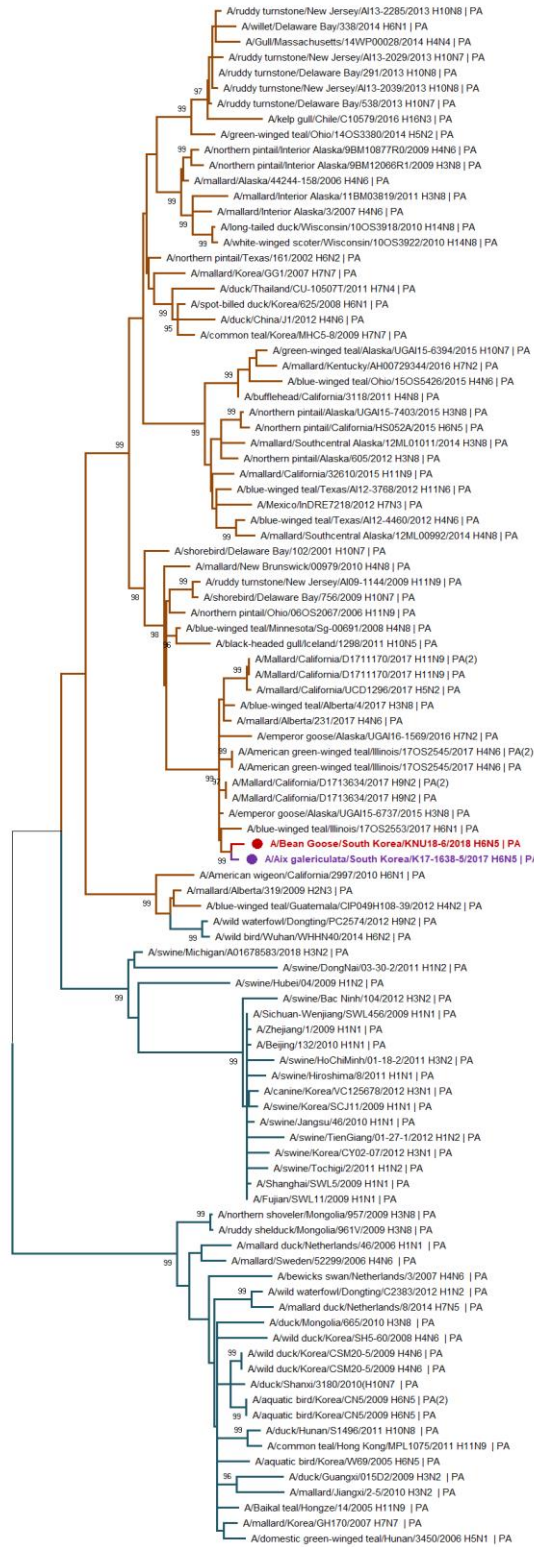
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b. PB1
NA_m



C. PA

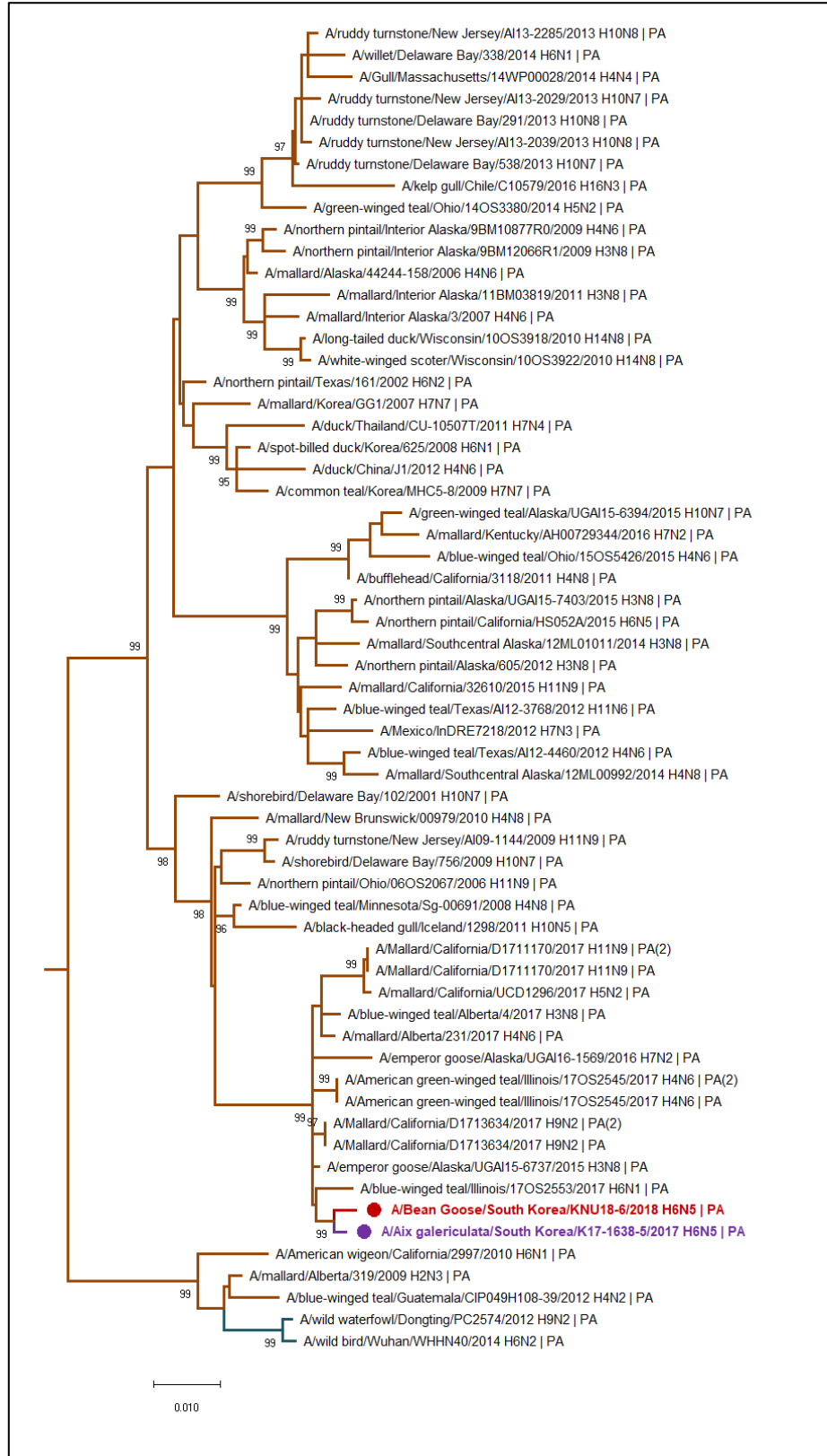


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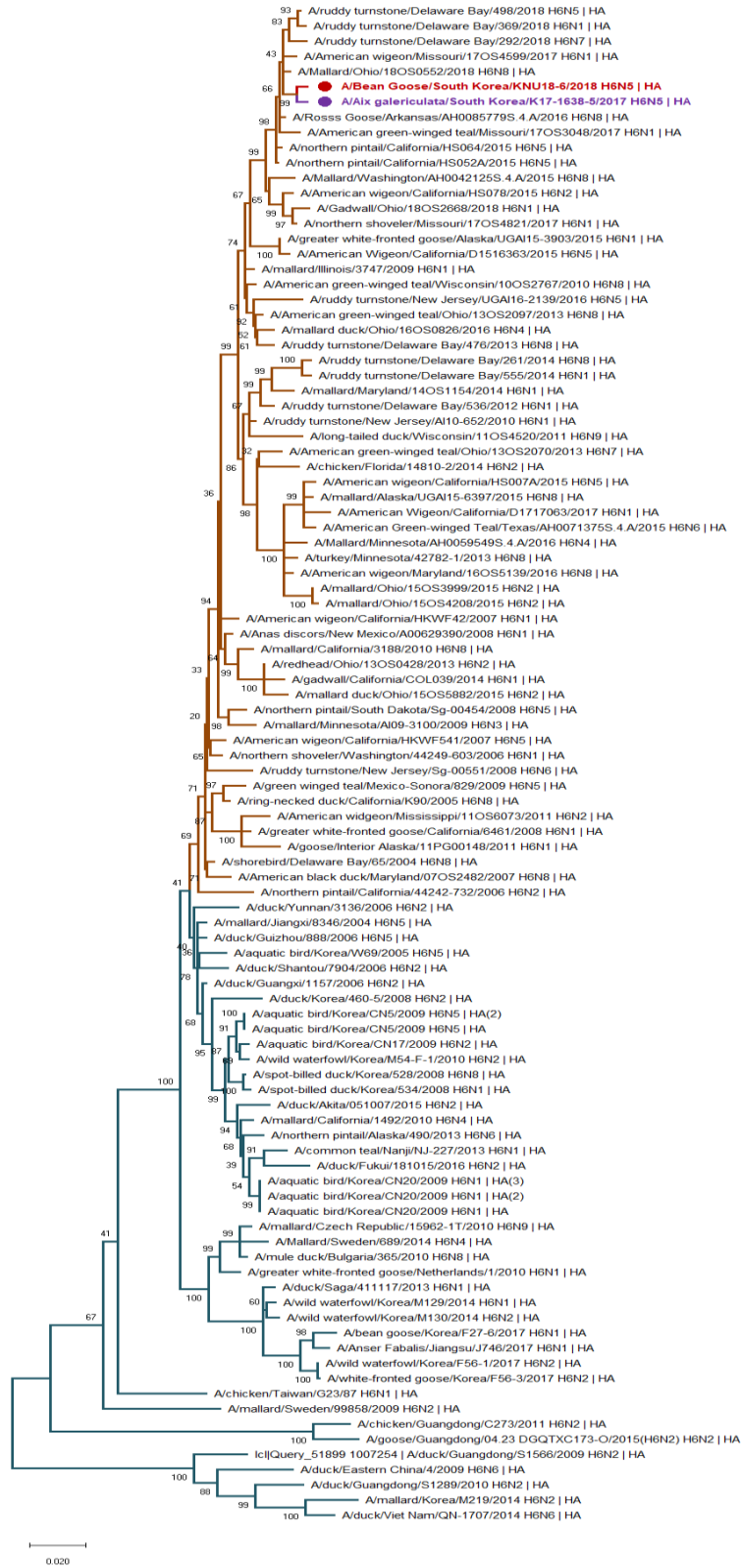
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c. PA
NAm



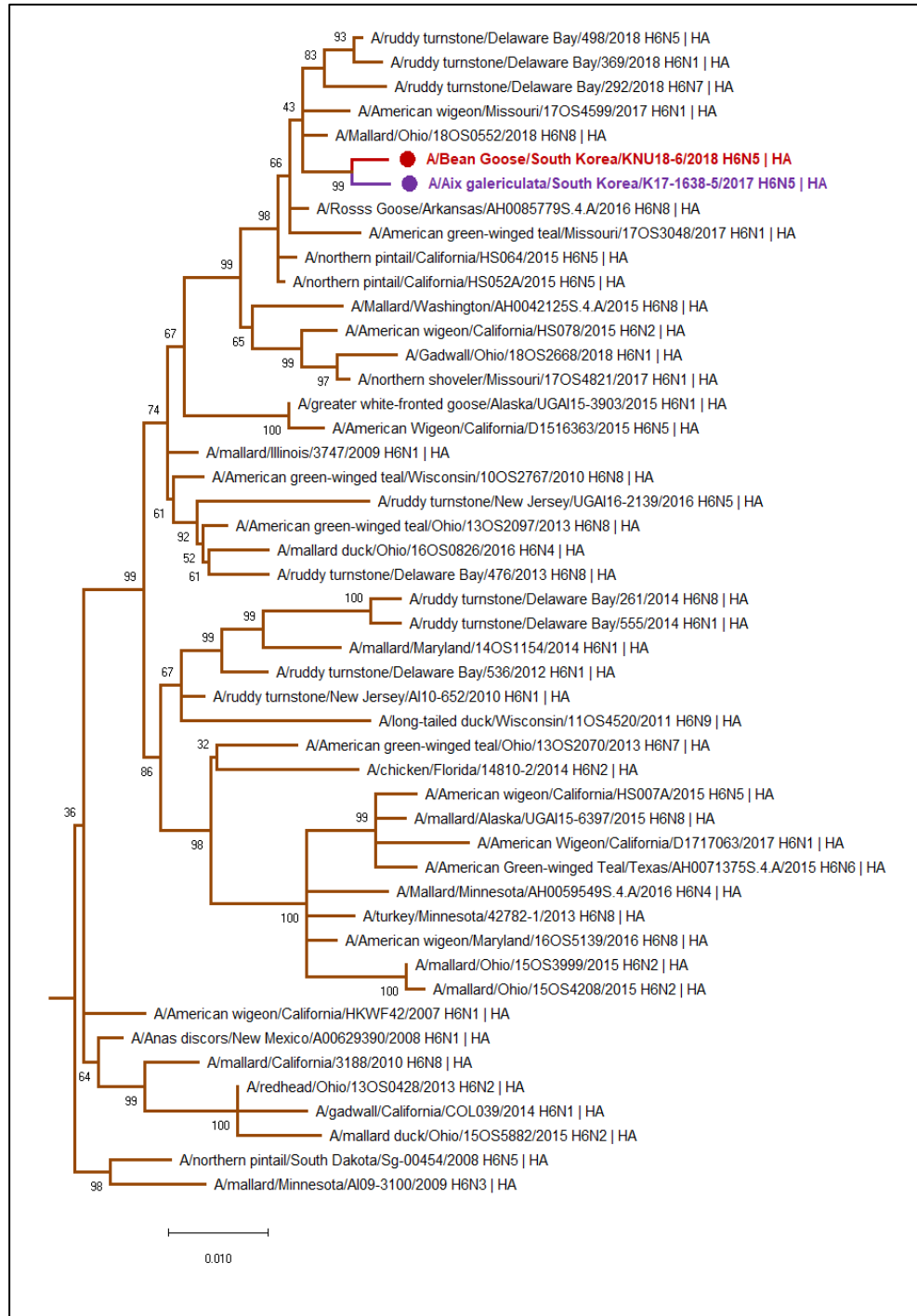
D. HA



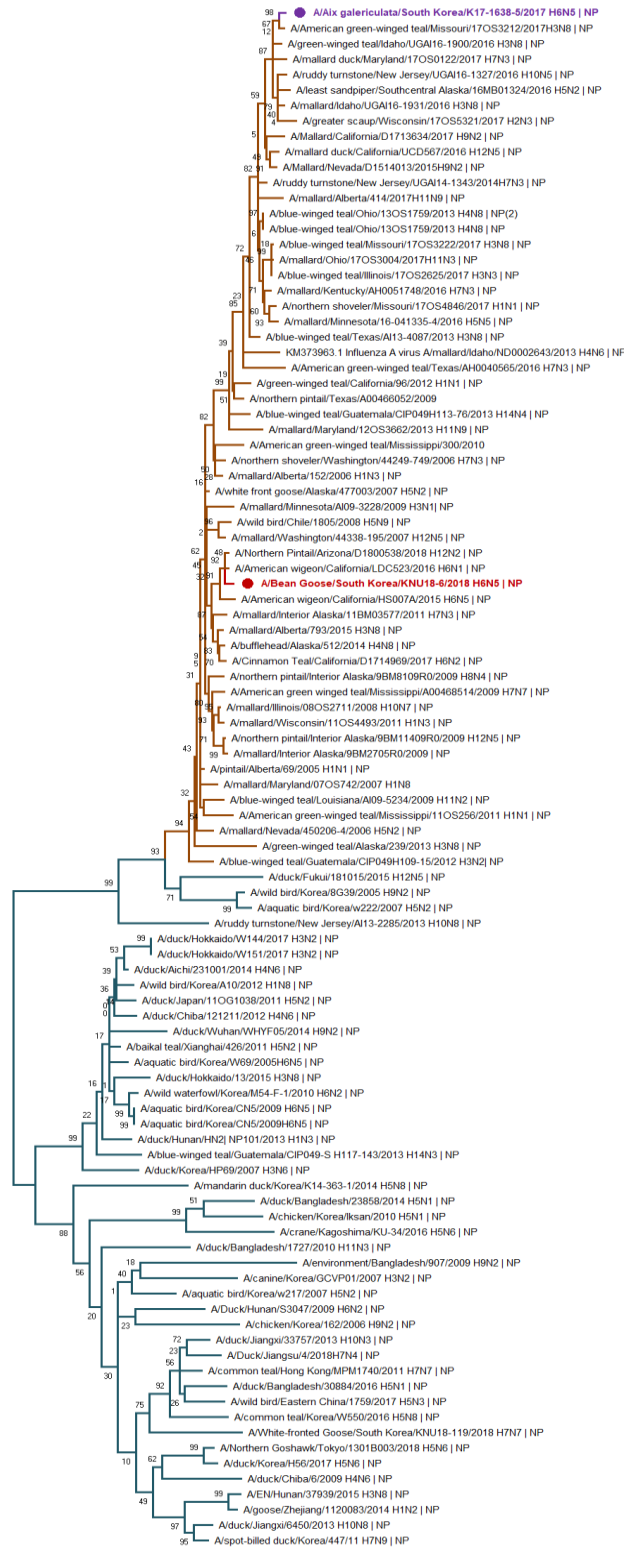
NA m

EA

d. HA
NAm



E. NP

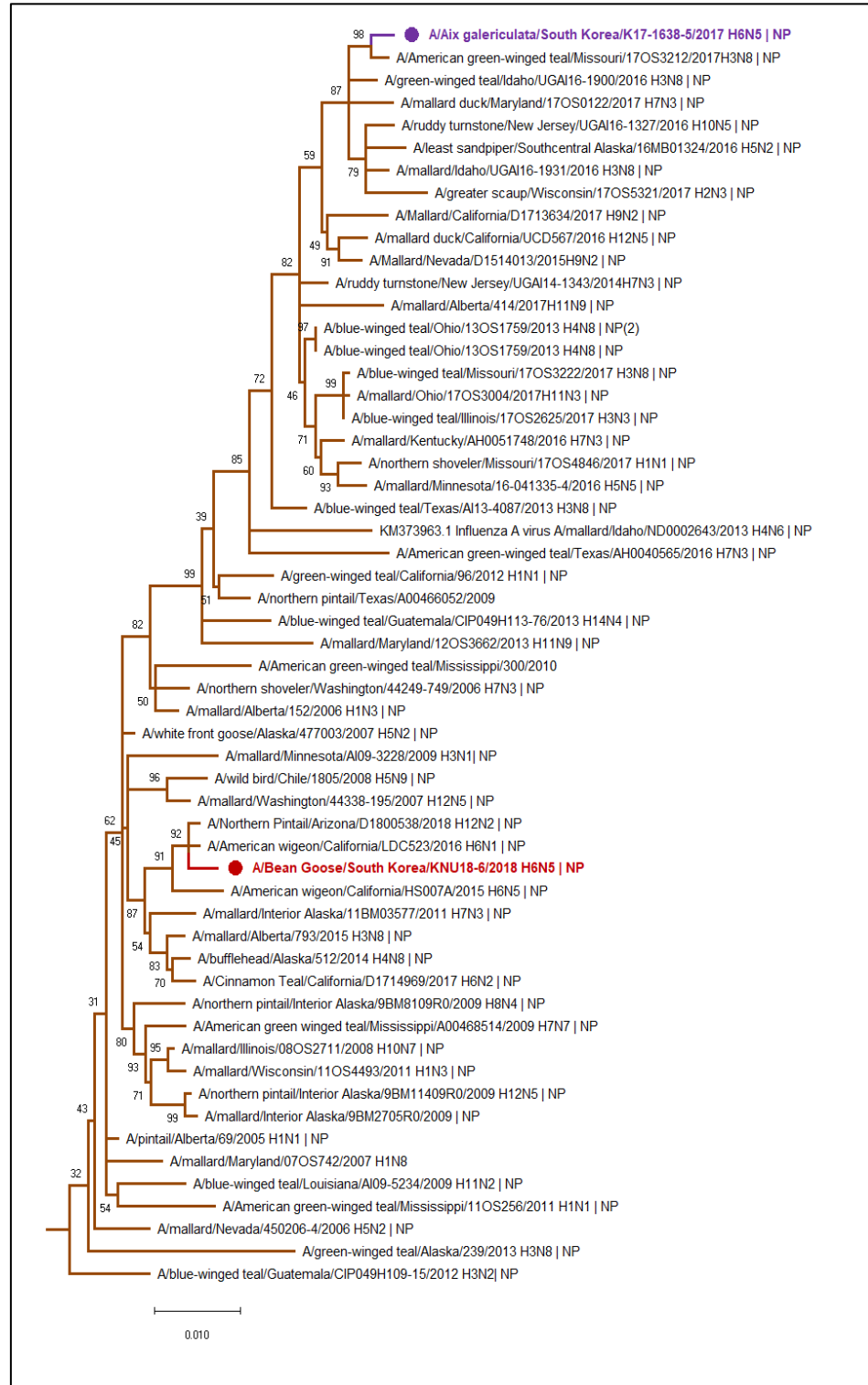


NAm

EA

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e. NP
NA_m



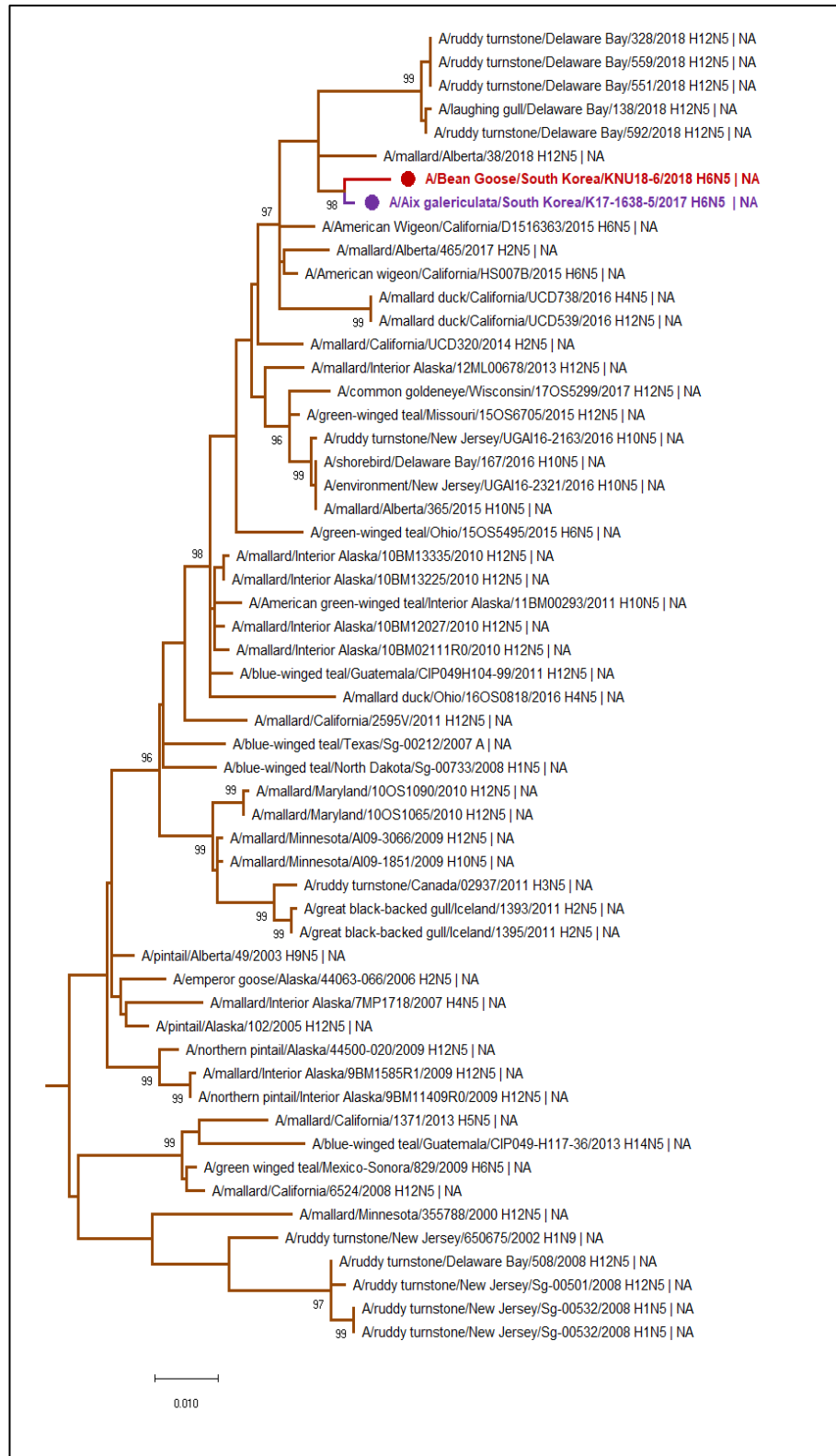
F. NA



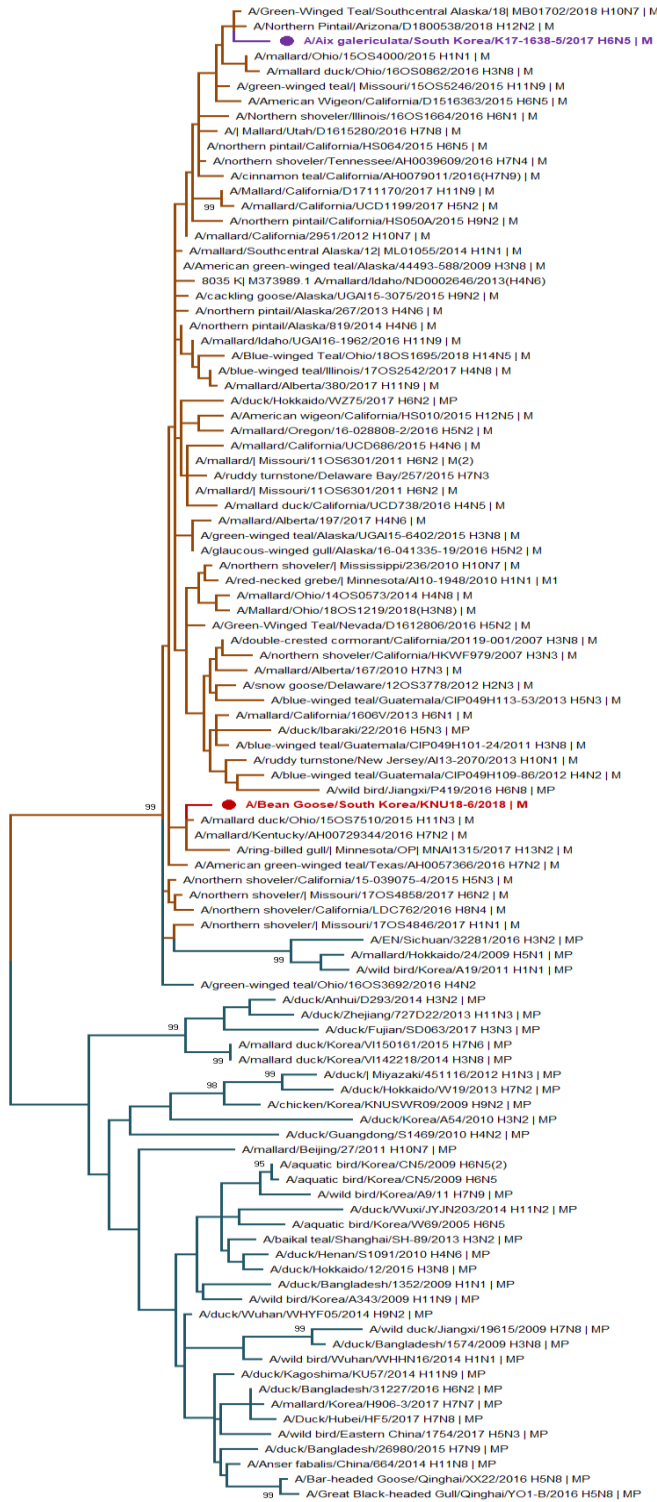
NA

EA

f. NA
NA_m



G. MP

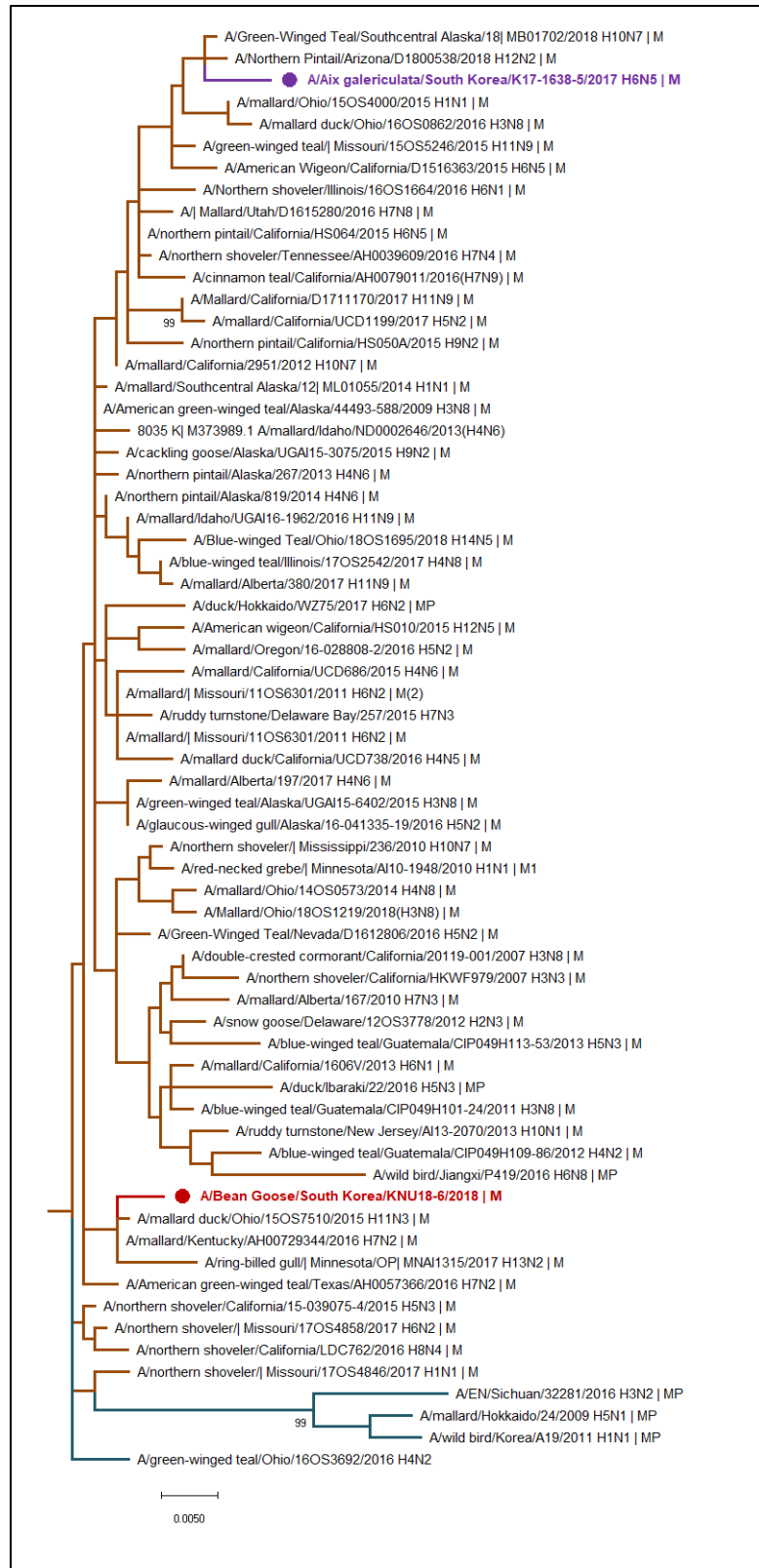


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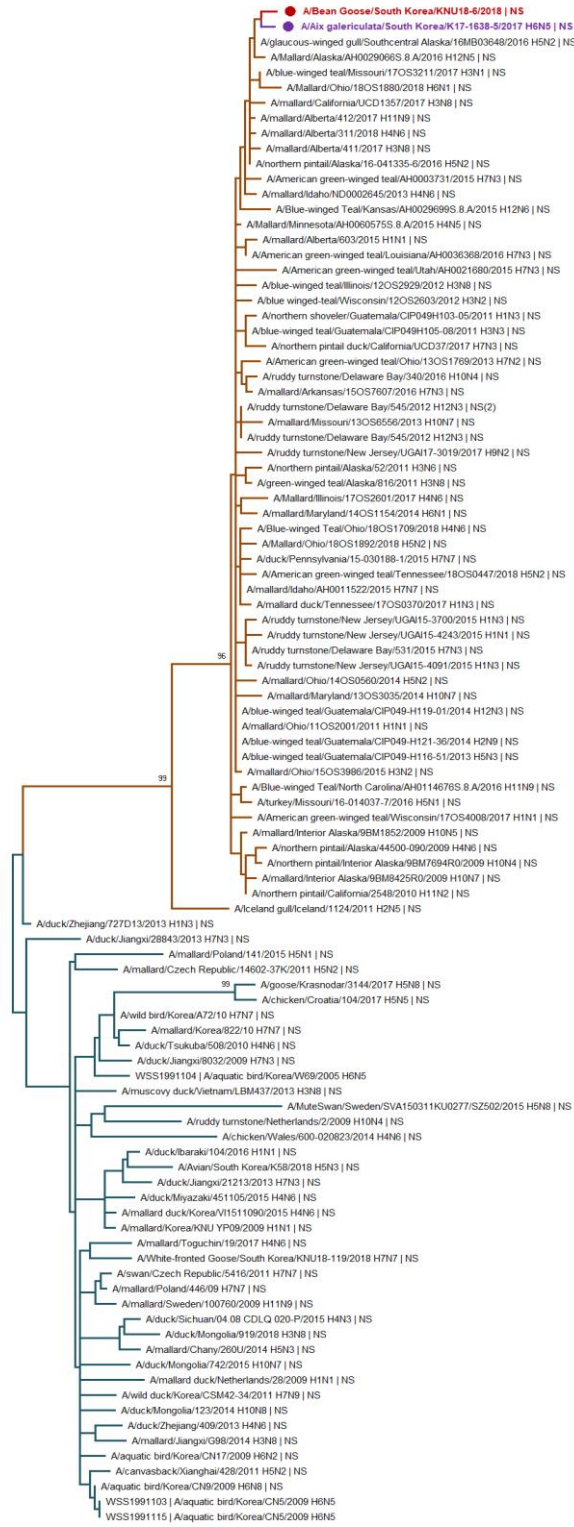
EA

0.010

g. MP
NA_m



H. NS



NAm

EA

0.010

h. NS
NAm

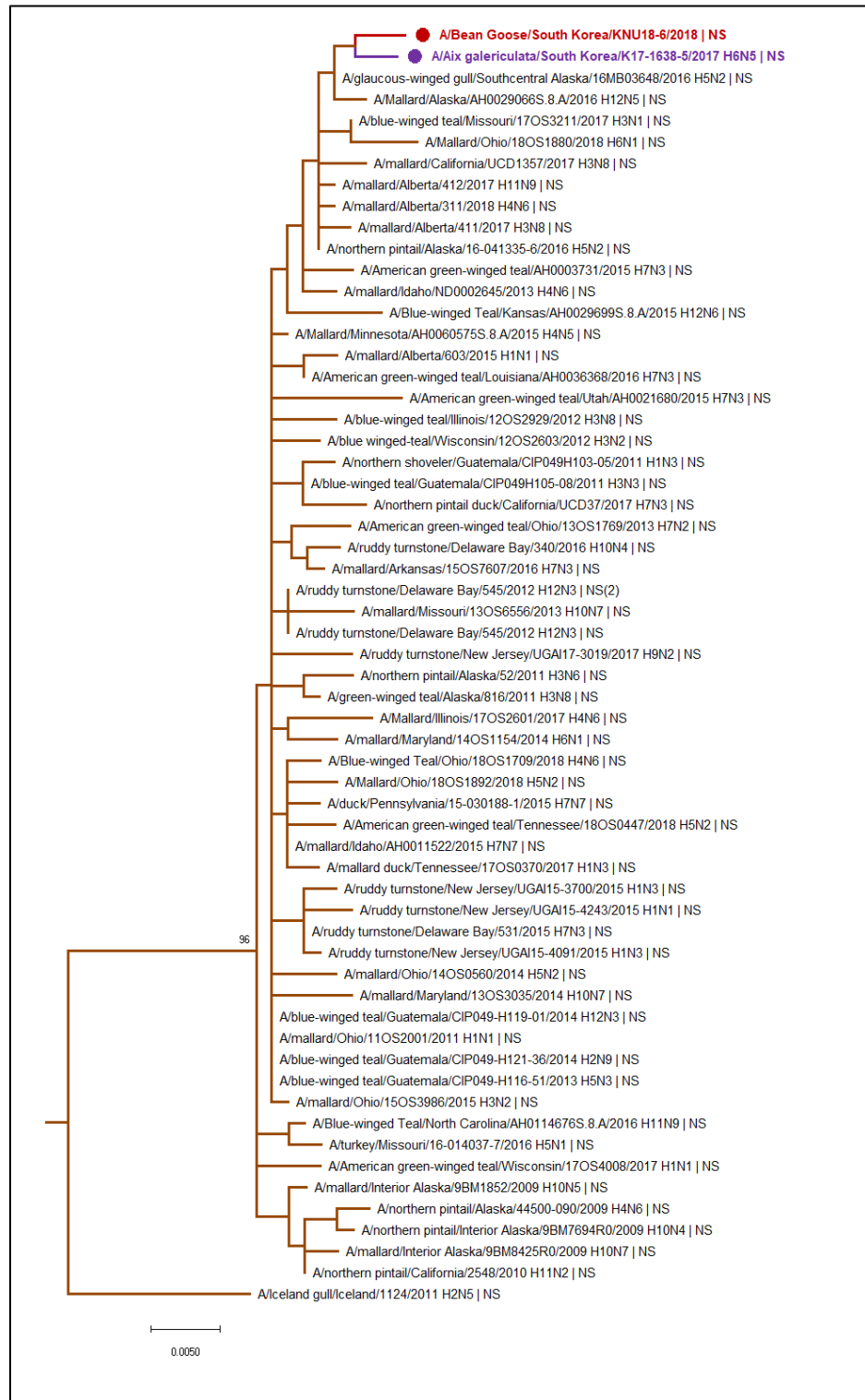
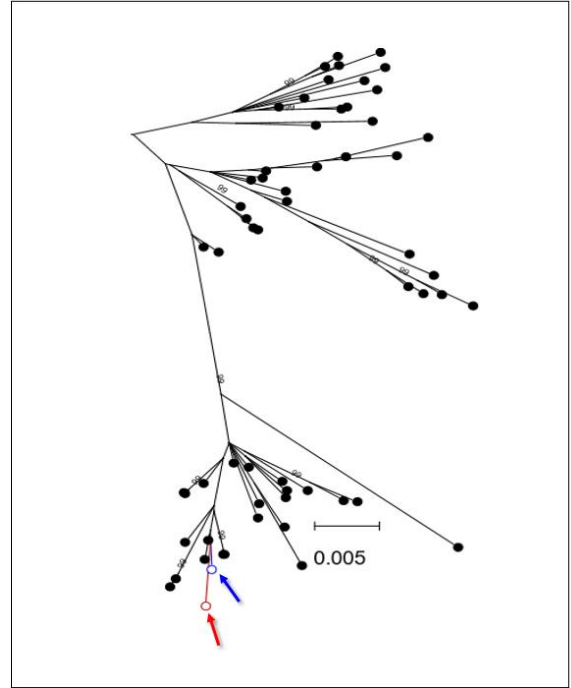
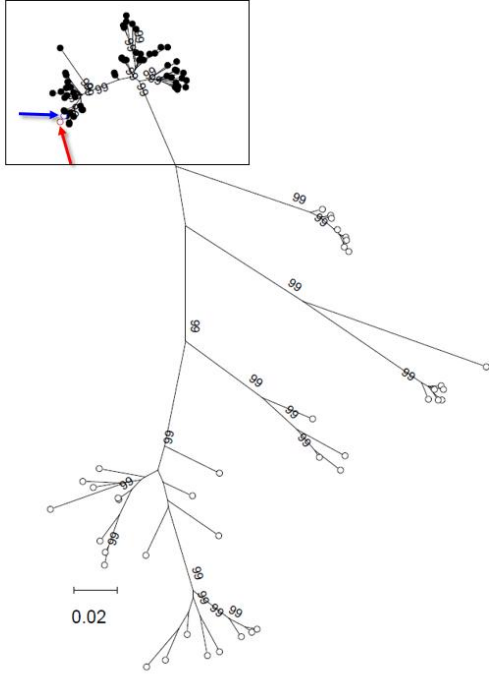
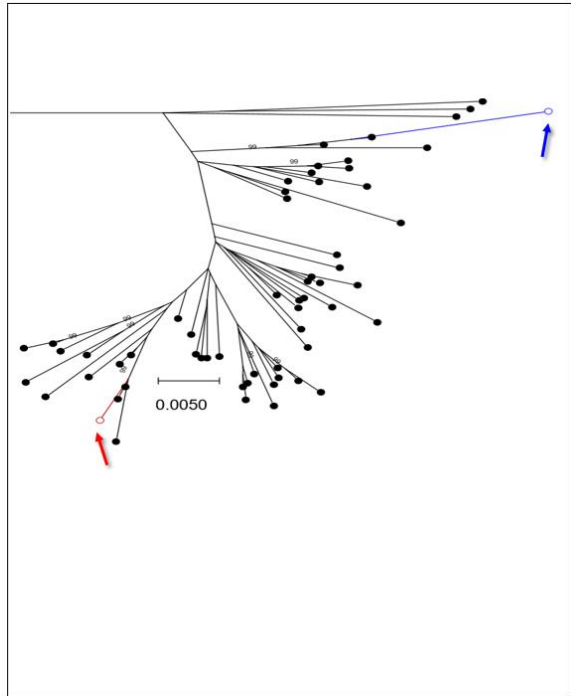
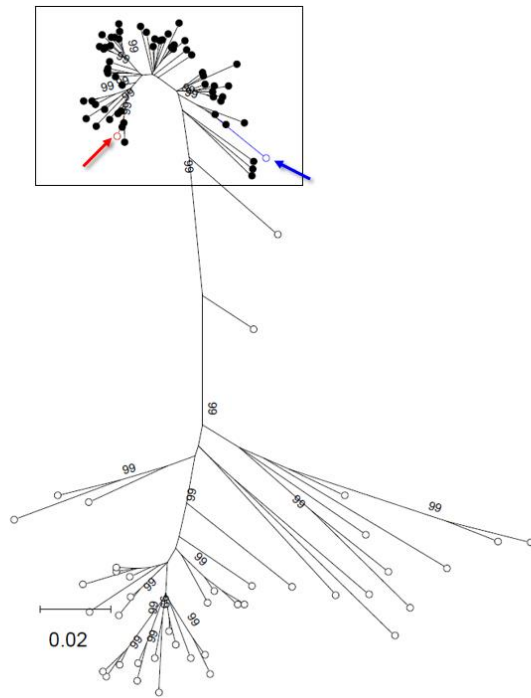


Figure S2. Phylogenetic tree of whole genome of the K6 avian influenza virus (AIV) compared to influenza sequences available in NCBI and GISAID. K6 was marked in red color and K17 was marked in purple color. Nam, North America lineage; EA, Eurasian lineage.

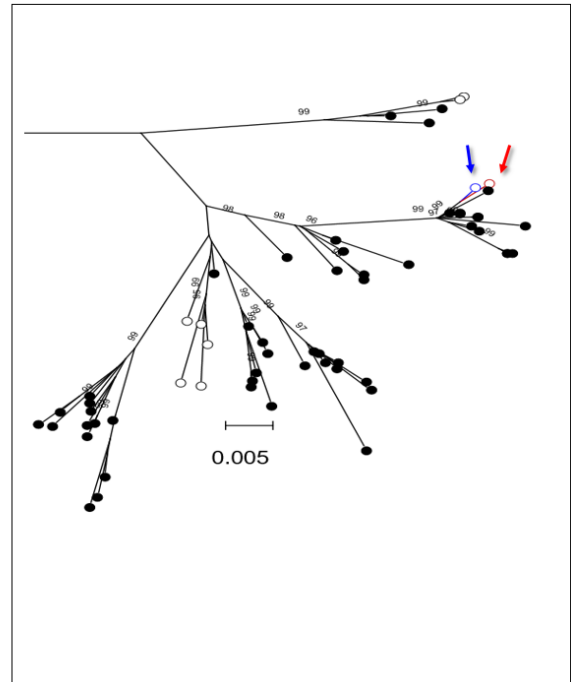
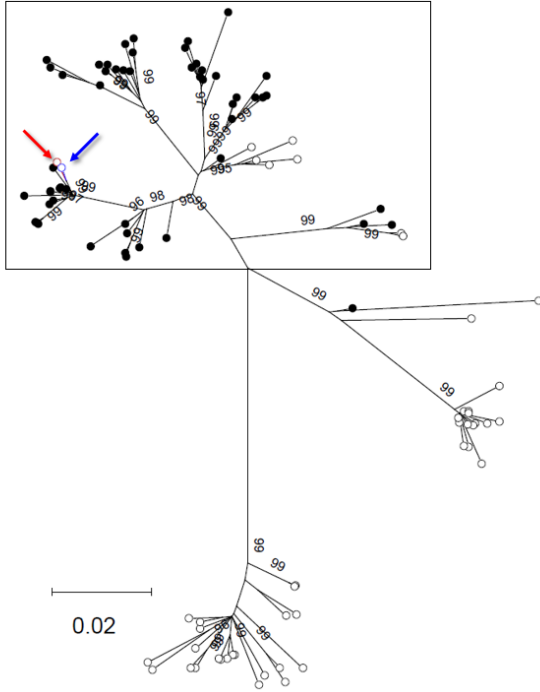
PB2



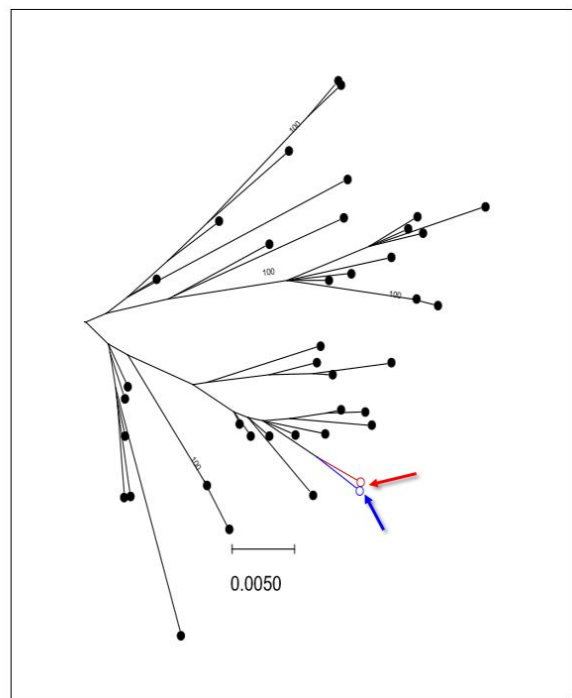
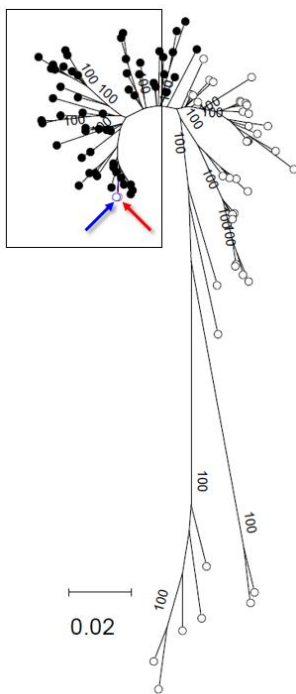
PB1



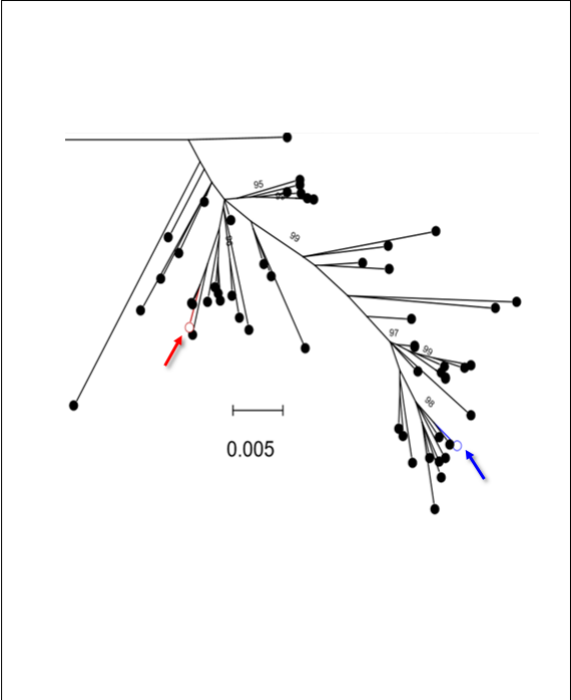
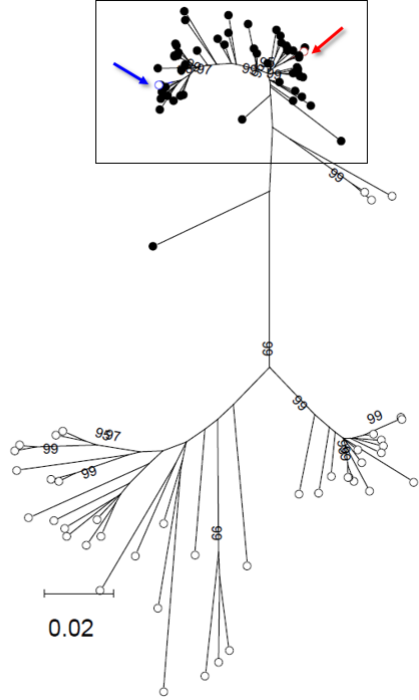
PA



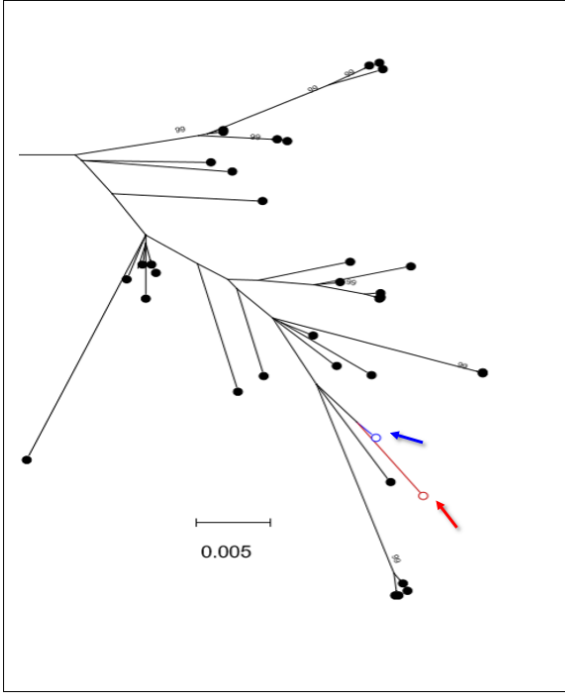
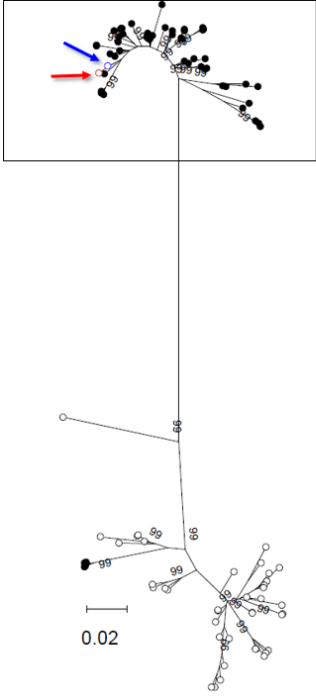
HA



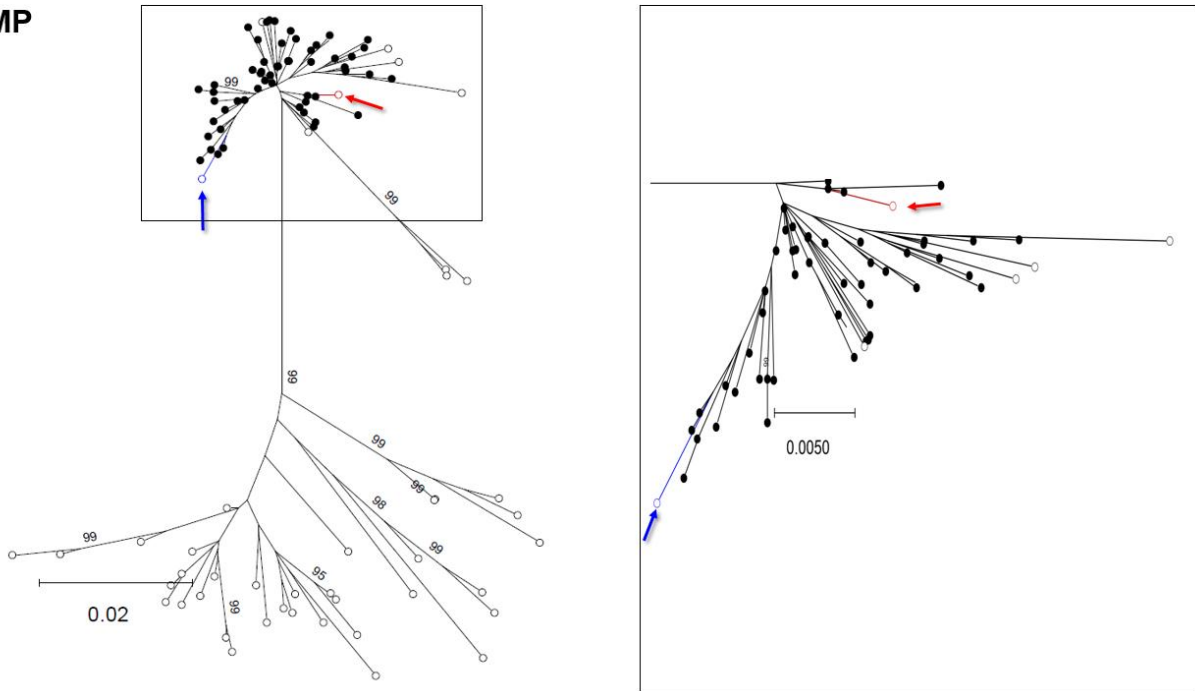
NP



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MP



NS

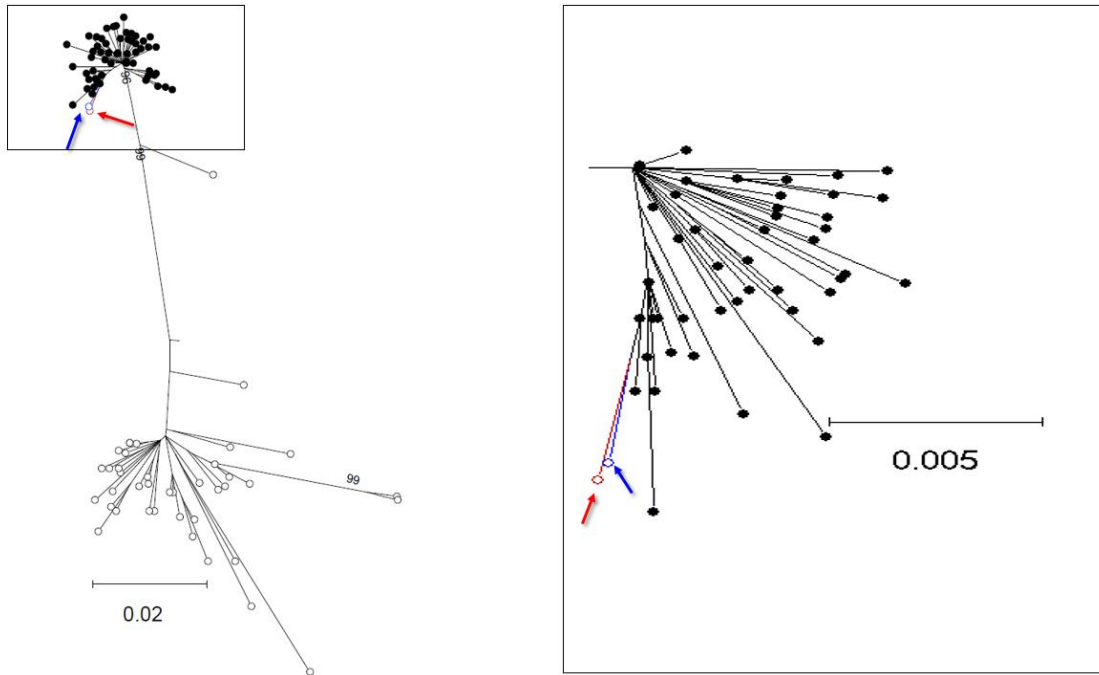
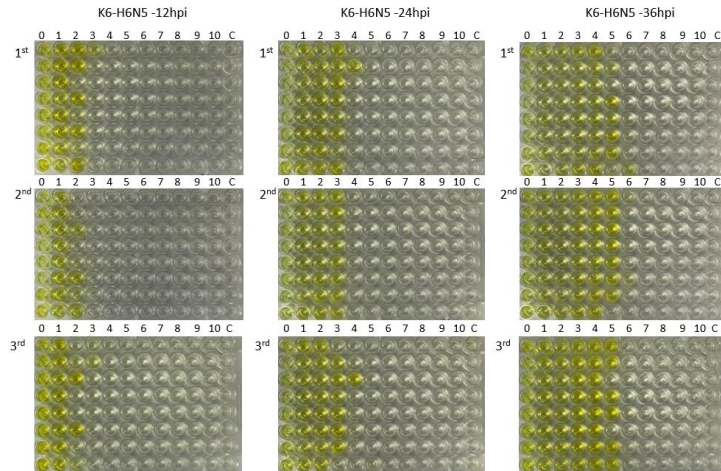


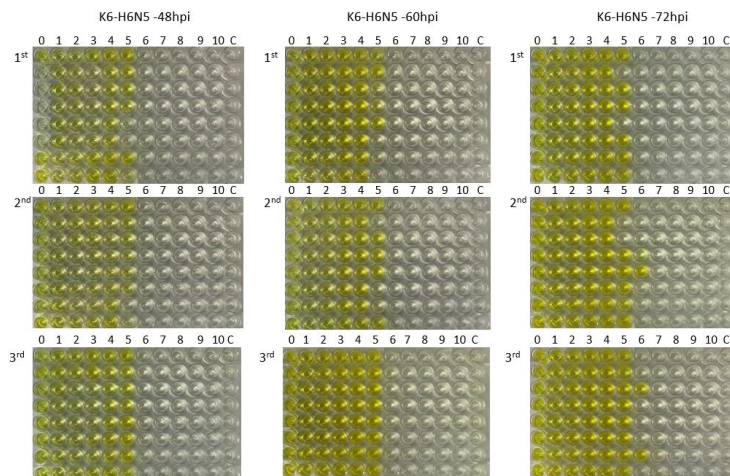
Figure S3. Maximum likelihood phylogenetic trees showing inferred relationships among nucleotide sequences for the complete coding regions of K6 and K17 gene segments. Red open circle indicates K6-H6N5 and blue open circle indicates K17. These

are highlighted with red and blue arrows used for K6 and K17, respectively. Closed and open circles indicate lineages of North America and Eurasia, respectively.

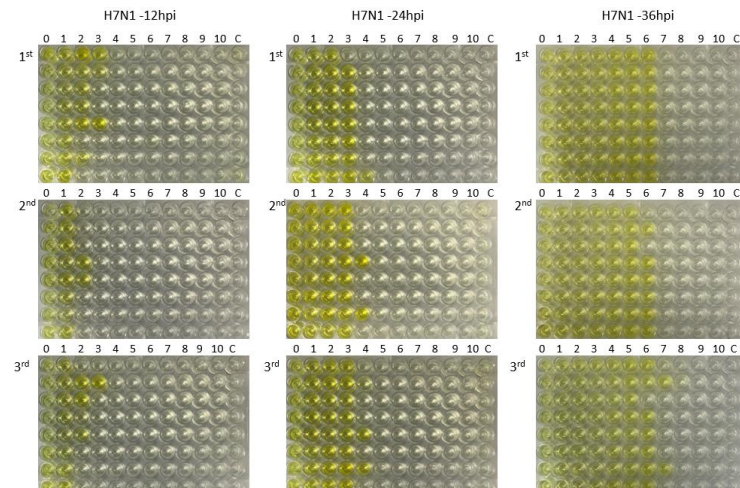
A.



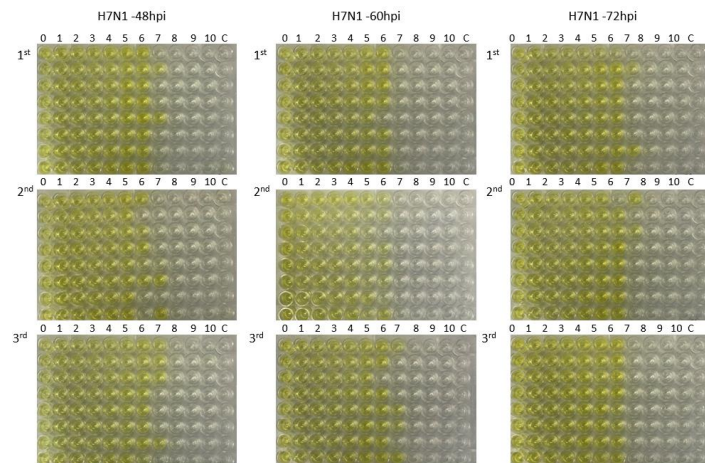
B.



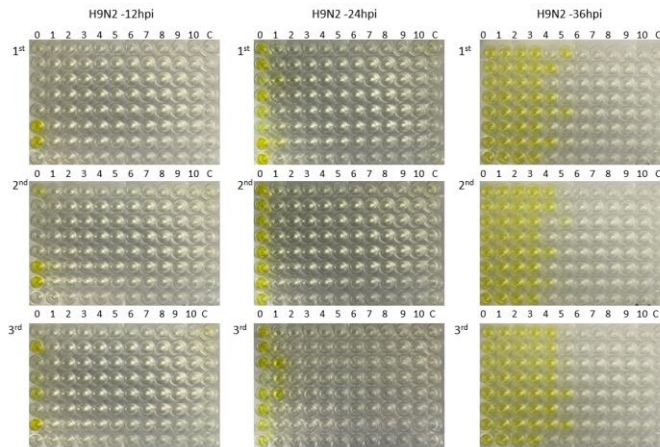
C.



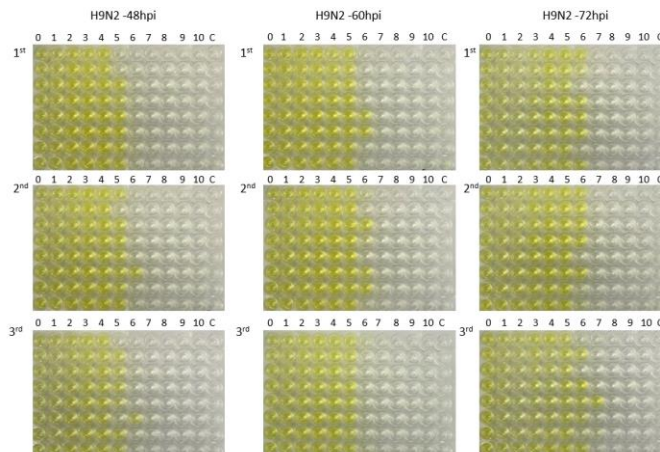
D.



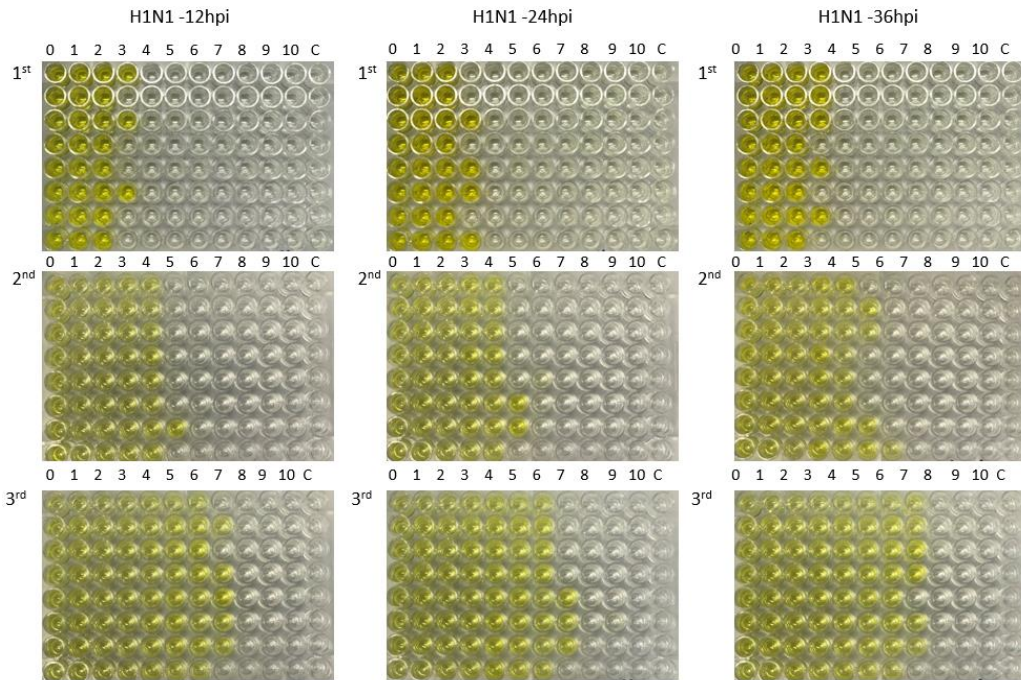
E.



F.



G.



H.

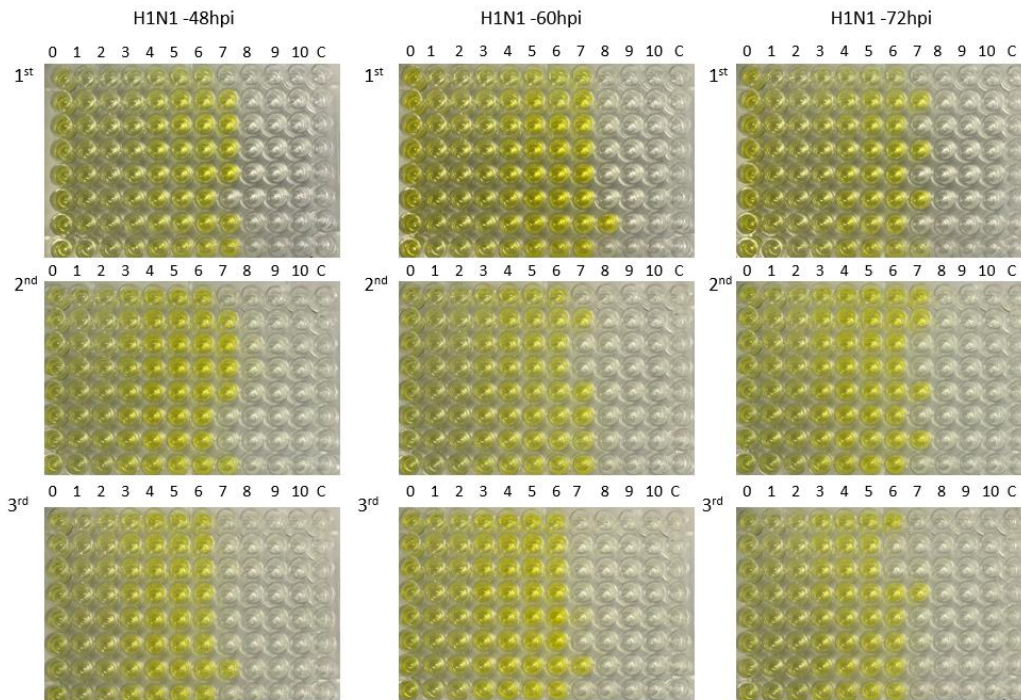
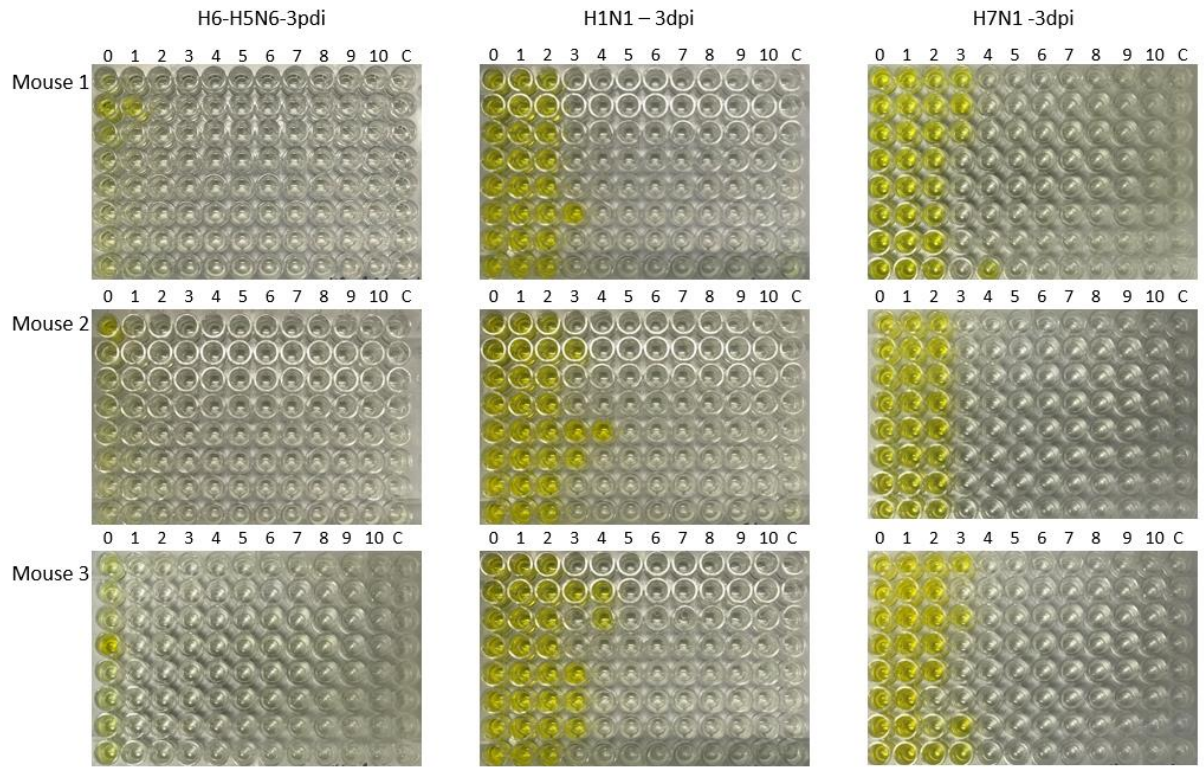


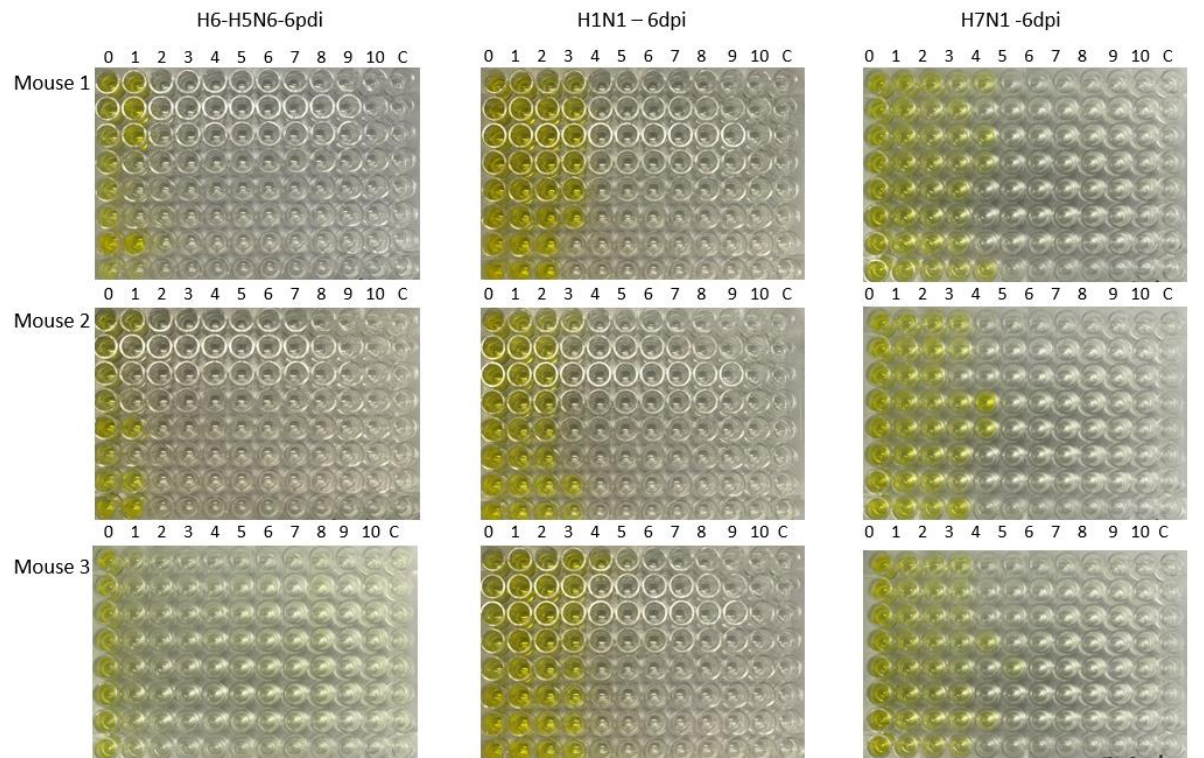
Figure S4. Raw ELISA data to conduct TCID₅₀ assay. Viruses were inoculated into MDCK monolayers at a multiplicity of infection (MOI) of 0.01. The supernatant were collected at different time point: 12, 24, 36, 48, 60 and 72 hours post of infection (hpi), respectively. The viral supernatant of different viruses (A,B - K6 (H6N5); C,D - H7N1; E,F - H9N2; G,H - H1N1) was serial 10-fold diluted and added to MDCK cell. After 3

days inoculation, medium was removed and cells was washed with PBS then fixed with 80% acetone followed by blocking with 5% non-fat milk. Cells was continuously washed with PBS-T (0.01 Tween 20) and reacted with 0.1 $\mu\text{g}/\text{well}$ of anti-influenza nucleoprotein (Medix Biochemica, Finland). Subsequently, secondary mAb in the form of horseradish peroxidase (HRP)-conjugated rabbit anti-mouse IgG (Abcam, Cambridge, UK) was added to each well according to the manufacture's protocol. Washing with PBS-T was performed five times to remove nonspecific binding and 3,3',5,5'-tetra methyl benzidine (Sigma-Aldrich) substrate solution was added. The reaction was stop by 0.18M H_2SO_4 and the OD value at 450nm was observed. TCID₅₀ titer was calculated. 0-10: 10^0 - 10^{10} folds dilution of stock, C- mock-infection.

A.



B.



C.

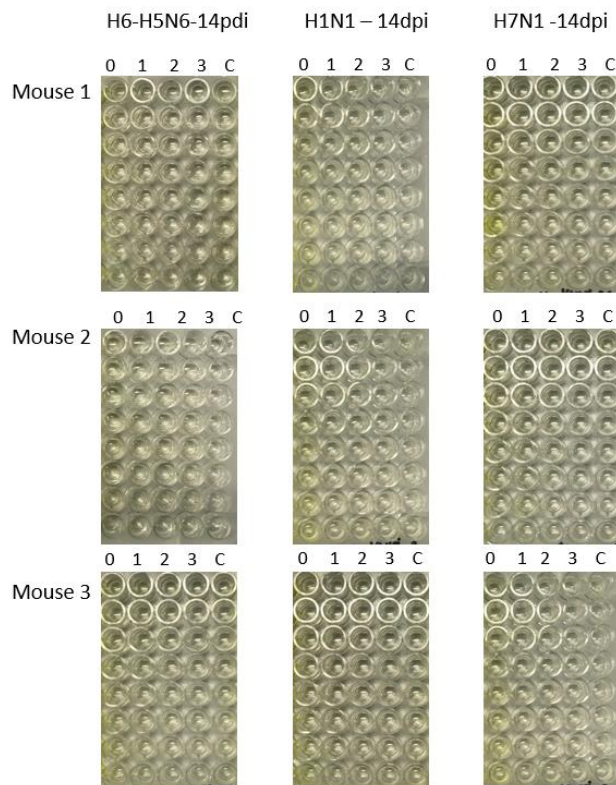
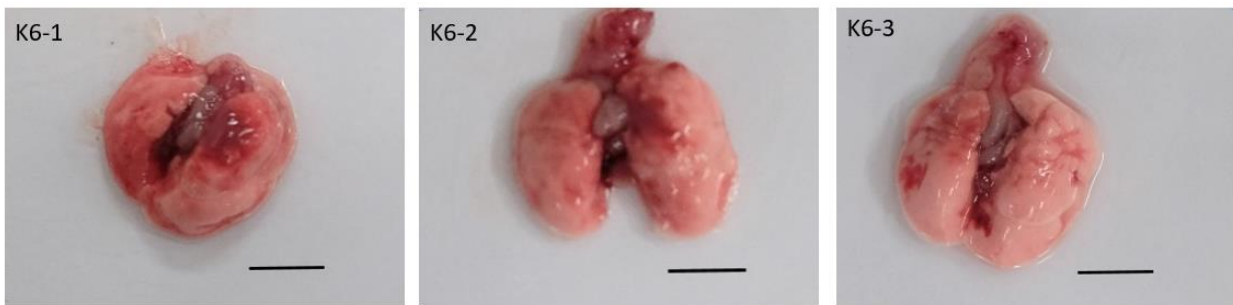


Figure S5. Raw ELISA data to conduct TCID₅₀ assay to measure virus K6-H6N5, H1N1, H7N1 titer in lung at day 3 (A); day 6 (B); day 14 (C) post infected. 0, 10⁰- fold dilution of stock; 1, 10¹- fold dilution of stock; 2, 10²- fold dilution of stock; 3, 10³- fold dilution of stock; 4, 10⁴- fold dilution of stock; 5, 10⁵- fold dilution of stock; 6, 10⁶- fold dilution of stock; 7, 10⁷- fold dilution of stock; 8, 10⁸- fold dilution of stock; 9, 10⁹- fold dilution of stock; 10, 10¹⁰- fold dilution of stock; C, mock-infection;

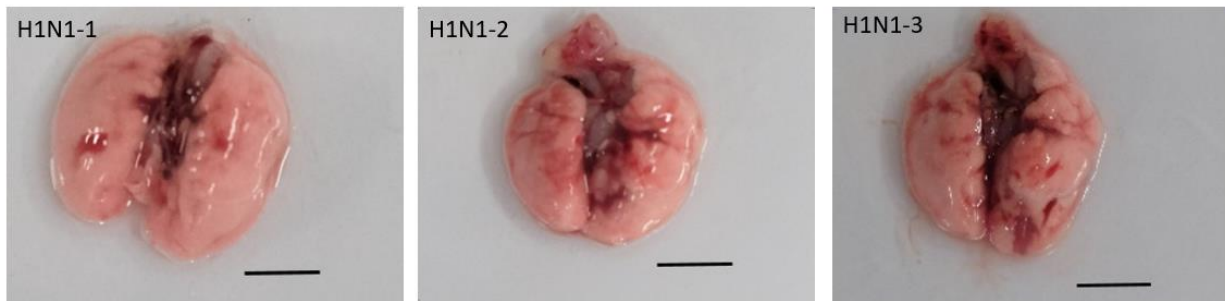
A



B



C



D

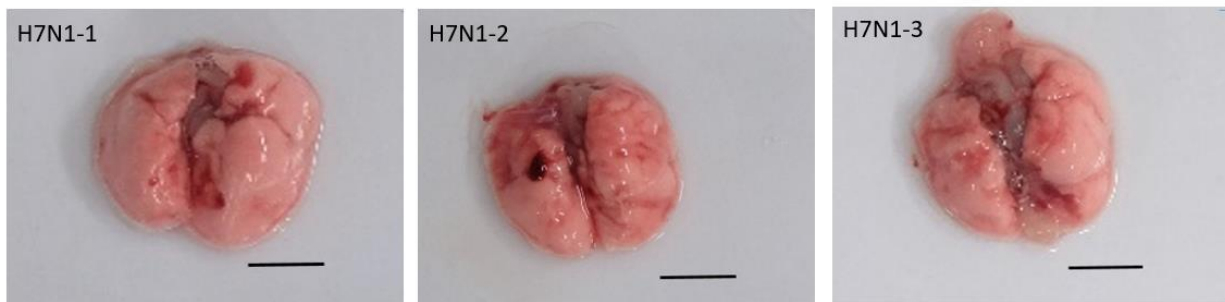


Figure S6. Lungs from (A) uninfected control; (B) K6-H6N5; (C) H7N1; (D) H7N1 -infected mouse at 6 dpi. Scale bar: 0.5cm

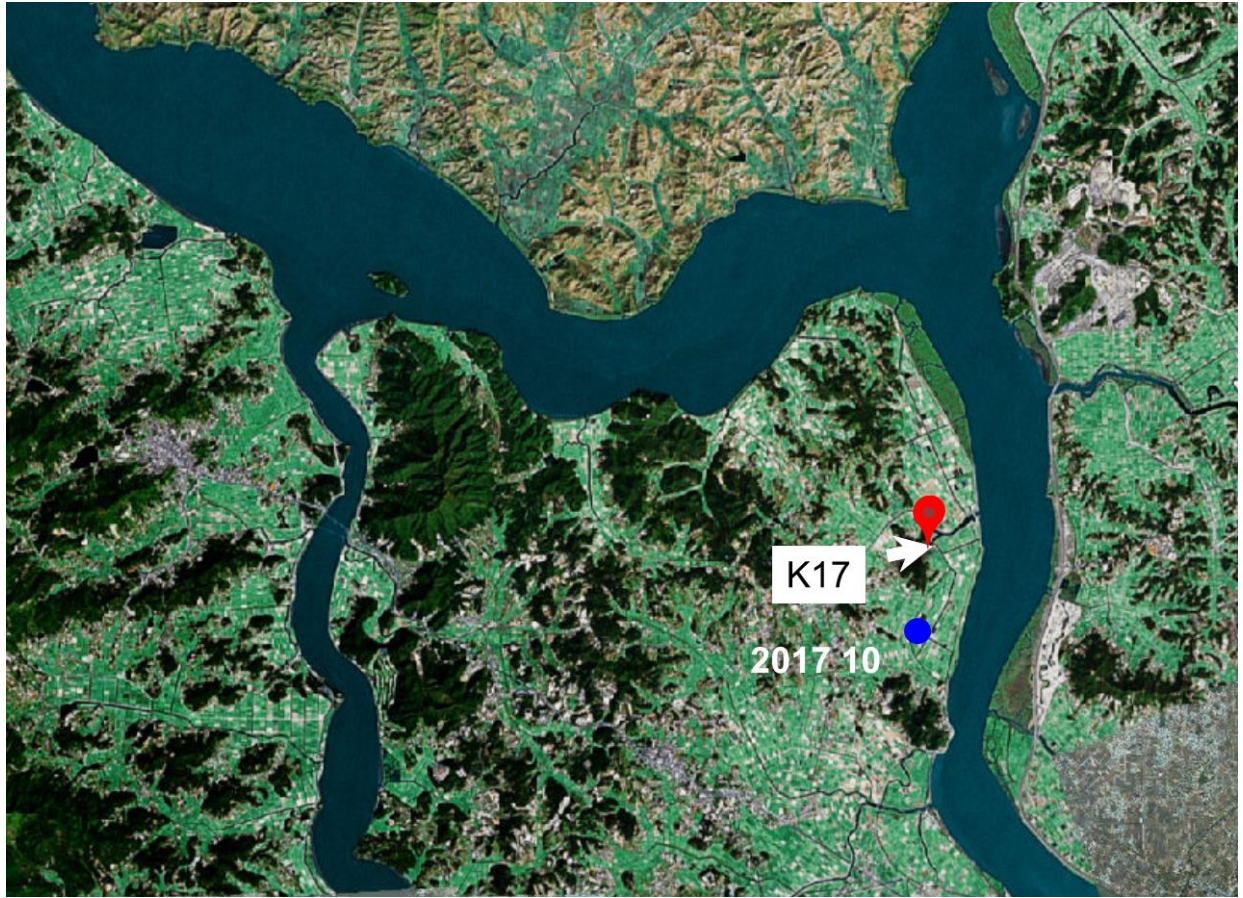


Figure S7. Location map of mandarin duck marked with satellite transmitters in Korea in October in 2017. White arrow indicates the isolation place of *A/Aix galericulata*/South Korea/K17-1638-5/2017(H6N5) and blue dot indicates the position of mandarin duck in October 2017. Map was retrieved from National Institute of Biological Resources, Korea.

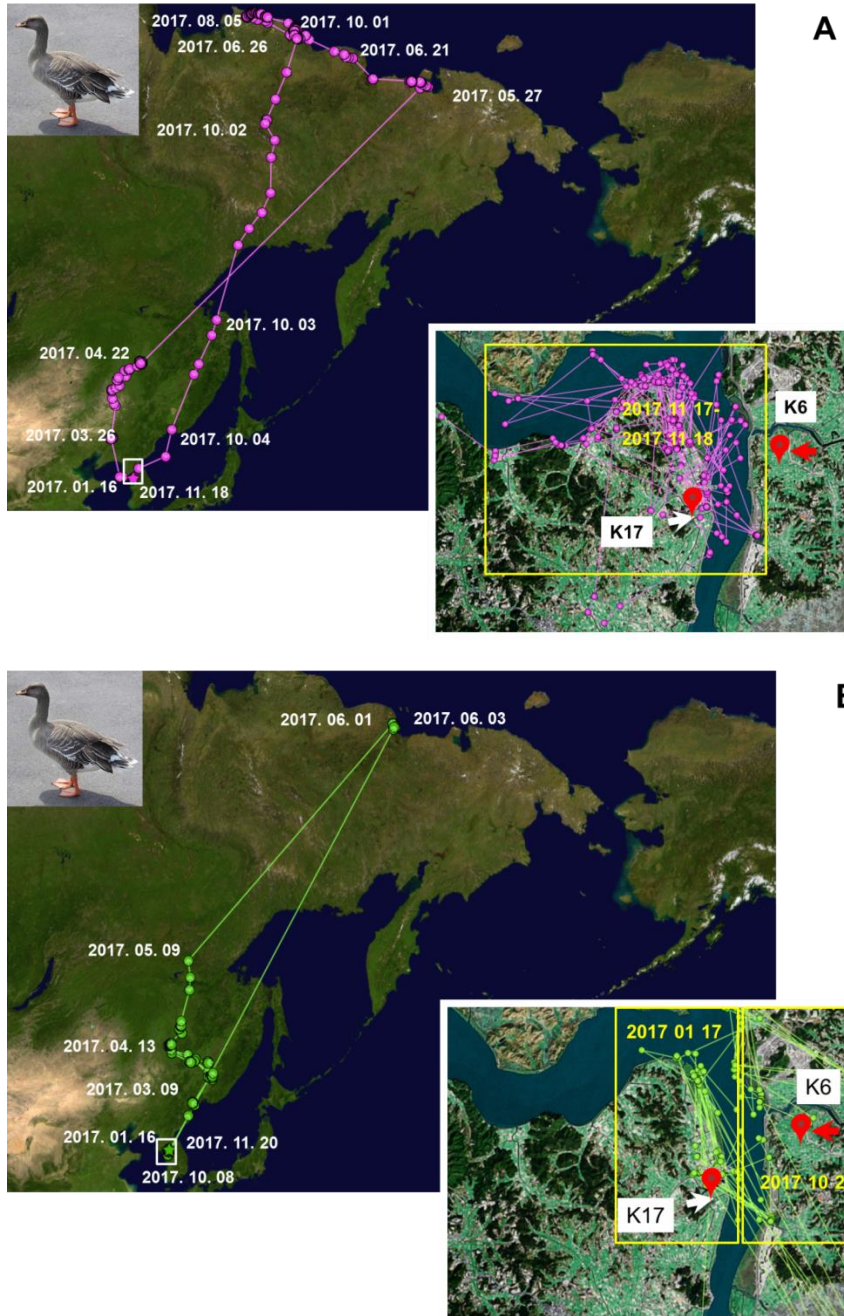


Figure S8. Spatial distribution patterns of Bean geese during Jan 2017- Nov 2017. Two satellite tracking devices (A, **br1691**; B, **br1692**) are shown. Red and white arrows indicate the isolated places of K6 and K17, respectively. Maps were retrieved from National Institute of Biological Resources, Korea. Date of movement (yellow color) is marked in yellow lined box in insert.

