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Highly Pathogenic Avian Influenza A(H5N8) Virus from Waterfowl, South Korea, 2014

To the Editor: To date, 18 hemagglutinin (HA) subtypes and 11 neuraminidase (NA) subtypes have been identified in influenza A viruses (*1*–*4*). Influenza A viruses containing HA subtypes 1–16 circulate in aquatic birds (*1*,*2*), whereas those harboring HA subtypes 17 and 18 are found in bats (*3*,*4*).

On January 18, 2014, the government of South Korea reported an outbreak of highly pathogenic avian influenza A(H5N8) virus in breeding ducks in the southern part of Jeollabuk-Do Province (*5*). More than 12 million poultry have since been culled, but the spread of the virus continues in duck and chicken farms. We report the genetic characterization of this virus.

On February 15, 2014, a total of 200 fecal samples were collected from waterfowl in the Pungse River in Chungnam Province, which is geographically close to Jeollabuk-Do Province. All samples were inoculated into hens' eggs, and influenza A viruses were confirmed by PCR by using influenza A–specific nucleoprotein (NP) primers. We obtained 1 isolate, A/waterfowl/Korea/S005/2014 (H5N8), and sequenced the full regions of all 8 genes as described (*6*). These sequences were deposited into GenBank under accession nos. KJ511809–KJ511816.

We conducted a BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi, http://platform.gisaid.org/epi3/frontend#4ead5c) to identify the closest gene sequences to those of A/waterfowl/Korea/S005/2014 (H5N8) (Table). Sequences for polymerase basic (PB) 2 (99% homology), HA (97% homology), and NP (99% homology) genes were closely related to those of A/wild duck/Shandong/628/2011 (H5N1). Sequences for PB1 (99% homology), polymerase acidic subunit (PA) (98% homology), matrix (M) (99% homology), and nonstructural (NS) (99% homology) genes were closely related to those of A/duck/Jiangsu/1-15/2011 (H4N2). Sequences for the NA (98% homology) gene were closely related to that of A/duck/Jiangsu/k1203/2010 (H5N8). Phylogenic analysis showed that all 8 genes of A/waterfowl/Korea/S005/2014 (H5N8) belonged to the Eurasian lineage, and that the HA gene clustered with clade 2.3.4 (online Technical Appendix Figure 1, http://wwwnc.cdc.gov/EID/article/20/9/14-0390-Techapp1.pdf).

We further analyzed the amino acid sequences of the virus isolate (online Technical Appendix Table 1). Positions 138 and 160 of the HA protein (H3 numbering) contained an alanine (A) residue, which was previously found to be related to enhanced binding to the human influenza receptor (*7*). The connecting peptide of HA contained an insertion of 4 basic amino acids (arginine-arginine-arginine-lysine), which is the same as in the HA of A/duck/Korea/Buan2/2014 (H5N8), an isolate from a duck farm in South Korea (GenBank accession no. KJ413839.1–KJ413846.1). Aspartic acid was found in M1 at position 30 and alanine at position 215; this combination has been connected with increased virulence in mice (*8*). The NS1 sequence contained serine at position 42, which is related to the enhanced pathogenicity in mice, but a truncation of the amino acids at positions 218–230 that has been linked with reduced pathogenicity in mice (*9*) was not identified. Asparagine was identified at position 31 of M2, which is the same in M2 of A/duck/Korea/Buan2/2014 (H5N8) and confers resistance to amantadine and rimantadine (*10*).

Because all 8 genes of A/waterfowl/Korea/S005/2014 (H5N8) are closely related to those of the A/duck/

Table. Nucleotide homology of genes of influenza virus strain A/waterfowl/Korea/S005/2014 (H5N8) to the closest related influenza virus strains*

Gene	Closest related virus strain	Nucleotide identity, %
PB2	A/wild duck/Shandong/628/2011 (H5N1)	99
PB1	A/duck/Jiangsu/1-15/2011 (H4N2)	99
PA	A/duck/Jiangsu/1-15/2011 (H4N2)	98
HA	A/wild duck/Shandong/628/2011 (H5N1)	97
NP	A/wild duck/Shandong/1/2011 (H5N1)	99
NA	A/duck/Jiangsu/k1203/2010 (H5N8)	98
M	A/duck/Jiangsu/1-15/2011 (H4N2)	99
NS	A/duck/Jiangsu/1-15/2011 (H4N2)	99

*PB, polymerase basic subunit; PA, polymerase acidic subunit; HA, hemagglutinin; NP, nucleoprotein; NA, neuraminidase; M, matrix; NS, nonstructural.

Korea/Buan2/2014 (H5N8) isolate that was obtained from a duck farm, it is likely that A/waterfowl/Korea/S005/2014 (H5N8) originated from infected waterfowl that had visited poultry on an infected farm (online Technical Appendix Figure 1). Our laboratory has studied the feces of wild birds in Chungnam Province since 2009, surveying >20,000 fecal samples from wild birds in this area each year, but we had not previously isolated avian influenza A(H5N8) virus from any samples.

The genetic analysis of the A/waterfowl/Korea/S005/2014 (H5N8) isolate indicates that this novel strain may have been created by the reassortment of PB2, HA, and NP segments from H5N1-like avian influenza virus; PB1, PA, M, and NS segments from H4N2-like avian influenza virus; and NA segments from H5N8-like avian influenza virus (online Technical Appendix Figure 2). Most genes of the virus we isolated are related to those of avian influenza viruses isolated in China, but the HA gene of A/waterfowl/Korea/S005/2014 (H5N8) showed only 97% homology to the closest HA gene in GenBank, which indicates that this gene may have been created in poultry in South Korea. To our knowledge, no outbreak of this virus in poultry farms in China has been reported, and we found no previous reports in the literature that migratory birds could carry the virus. Taken together, our data suggest that A/waterfowl/Korea/S005/2014 (H5N8) may have been reassorted in a duck farm in South Korea.

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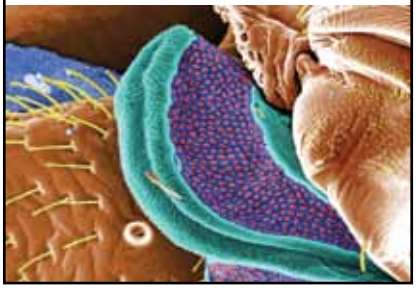

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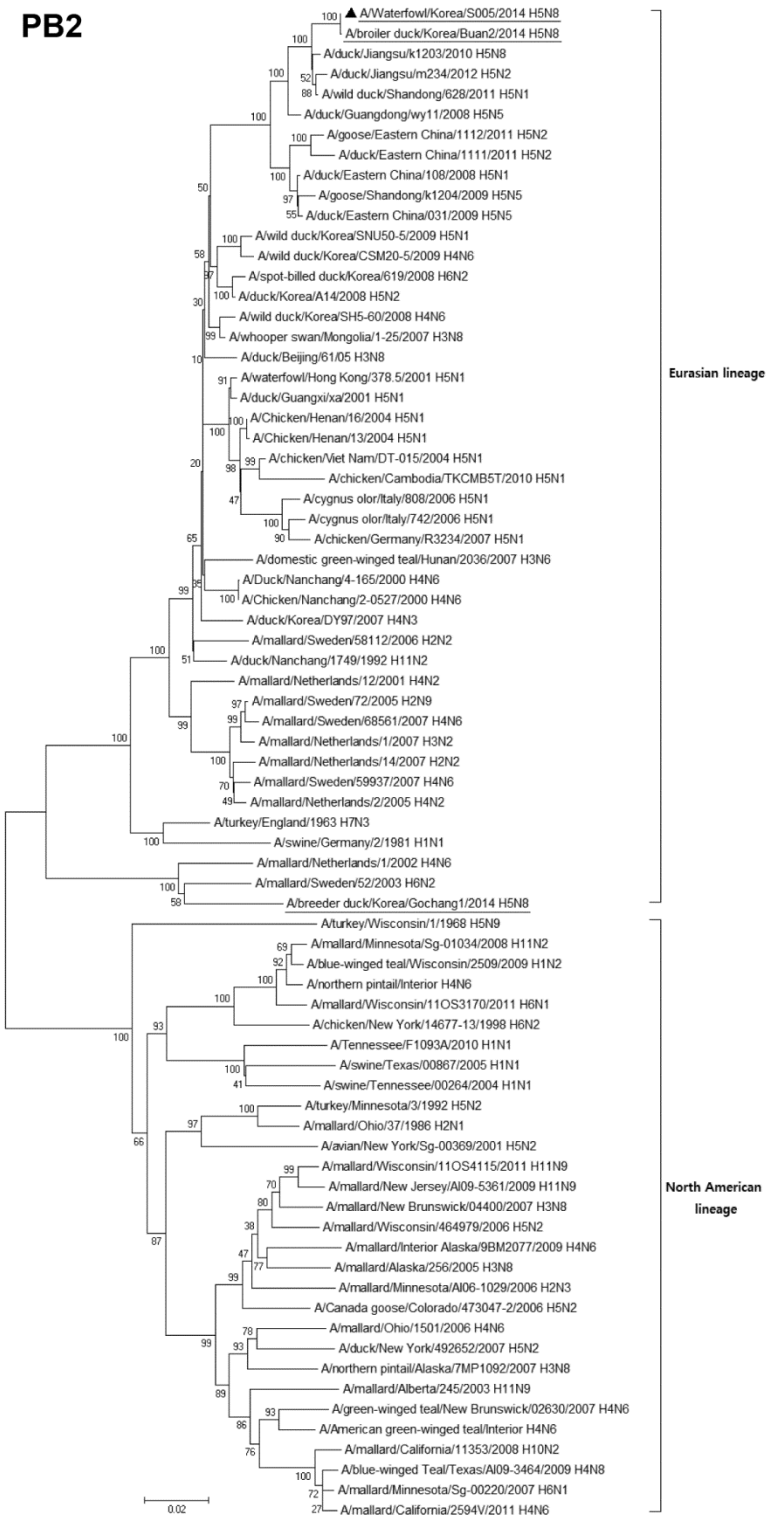
Highly Pathogenic Avian Influenza A(H5N8) Virus from Waterfowl, South Korea, 2014

Technical Appendix

Technical Appendix Figure 1 (following pages). Phylogenetic analysis of PB2, PB1, PA, HA, NP, NA, M, and NS genes of influenza virus strain A/waterfowl/Korea/S005/2014 (H5N8) (indicated by triangles). The trees were constructed using the neighbor-joining method in MEGA5 (http://www.megasoftware.net) with 1,000 bootstrap replicates. Scale bars indicate nucleotide substitutions per site. The HA was rooted to A/Goose/Guangdong/1/1996. The clade of HA gene was determined by BLAST search (http://www.fludb.org/brc/h5n1Classifier.spg?method=ShowCleanInputPage&decorator=influenza). Underlines indicate recent H5N8 isolates. PB, polymerase basic subunit; PA, polymerase acidic subunit; HA, hemagglutinin; NP, nucleoprotein; NA, neuraminidase; M, matrix; NS, nonstructural.


PB2
A/Waterfowl/Korea/S005/2014 H5N8
A/broiler duck/Korea/Buan2/2014 H5N8
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A/duck/Jiangsu/m234/2012 H5N2
A/wild duck/Shandong/628/2011 H5N1
A/duck/Guangdong/wy11/2008 H5N5
A/goose/Eastern China/1112/2011 H5N2
A/duck/Eastern China/1111/2011 H5N2
A/duck/Eastern China/108/2008 H5N1
A/goose/Shandong/k1204/2009 H5N5
A/duck/Eastern China/031/2009 H5N5
A/wild duck/Korea/SNU50-5/2009 H5N1
A/wild duck/Korea/CSM20-5/2009 H4N6
A/spot-billed duck/Korea/619/2008 H6N2
A/duck/Korea/A14/2008 H5N2
A/wild duck/Korea/SH5-60/2008 H4N6
A/whooper swan/Mongolia/1-25/2007 H3N8
A/duck/Beijing/61/05 H3N8
A/waterfowl/Hong Kong/378.5/2001 H5N1
A/duck/Guangxi/xa/2001 H5N1
A/Chicken/Henan/16/2004 H5N1
A/Chicken/Henan/13/2004 H5N1
A/chicken/Viet Nam/DT-015/2004 H5N1
A/chicken/Cambodia/TKCMB5T/2010 H5N1
A/cygnus olor/Italy/808/2006 H5N1
A/cygnus olor/Italy/742/2006 H5N1
A/chicken/Germany/R3234/2007 H5N1
A/domestic green-winged teal/Hunan/2036/2007 H3N6
A/Duck/Nanchang/4-165/2000 H4N6
A/Chicken/Nanchang/2-0527/2000 H4N6
A/duck/Korea/DY97/2007 H4N3
A/mallard/Sweden/58112/2006 H2N2
A/duck/Nanchang/1749/1992 H11N2
A/mallard/Netherlands/12/2001 H4N2
A/mallard/Sweden/72/2005 H2N9
A/mallard/Sweden/68561/2007 H4N6
A/mallard/Netherlands/1/2007 H3N2
A/mallard/Netherlands/14/2007 H2N2
A/mallard/Sweden/59937/2007 H4N6
A/mallard/Netherlands/2/2005 H4N2
A/turkey/England/1963 H7N3
A/swine/Germany/2/1981 H1N1
A/mallard/Netherlands/1/2002 H4N6
A/mallard/Sweden/52/2003 H6N2
A/breeder duck/Korea/Gochang1/2014 H5N8
Eurasian lineage
A/turkey/Wisconsin/1/1968 H5N9
A/mallard/Minnesota/Sg-01034/2008 H11N2
A/blue-winged teal/Wisconsin/2509/2009 H1N2
A/northern pintail/Interior H4N6
A/mallard/Wisconsin/11OS3170/2011 H6N1
A/chicken/New York/14677-13/1998 H6N2
A/Tennessee/F1093A/2010 H1N1
A/swine/Texas/00867/2005 H1N1
A/swine/Tennessee/00264/2004 H1N1
A/turkey/Minnesota/3/1992 H5N2
A/mallard/Ohio/37/1986 H2N1
A/avian/New York/Sg-00369/2001 H5N2
A/mallard/Wisconsin/11OS4115/2011 H11N9
A/mallard/New Jersey/AI09-5361/2009 H11N9
A/mallard/New Brunswick/04400/2007 H3N8
A/mallard/Wisconsin/464979/2006 H5N2
A/mallard/Interior Alaska/9BM2077/2009 H4N6
A/mallard/Alaska/256/2005 H3N8
A/mallard/Minnesota/AI06-1029/2006 H2N3
A/Canada goose/Colorado/473047-2/2006 H5N2
A/mallard/Ohio/1501/2006 H4N6
A/duck/New York/492652/2007 H5N2
A/northern pintail/Alaska/7MP1092/2007 H3N8
A/mallard/Alberta/245/2003 H11N9
A/green-winged teal/New Brunswick/02630/2007 H4N6
A/American green-winged teal/Interior H4N6
A/mallard/California/11353/2008 H10N2
A/blue-winged Teal/Texas/AI09-3464/2009 H4N8
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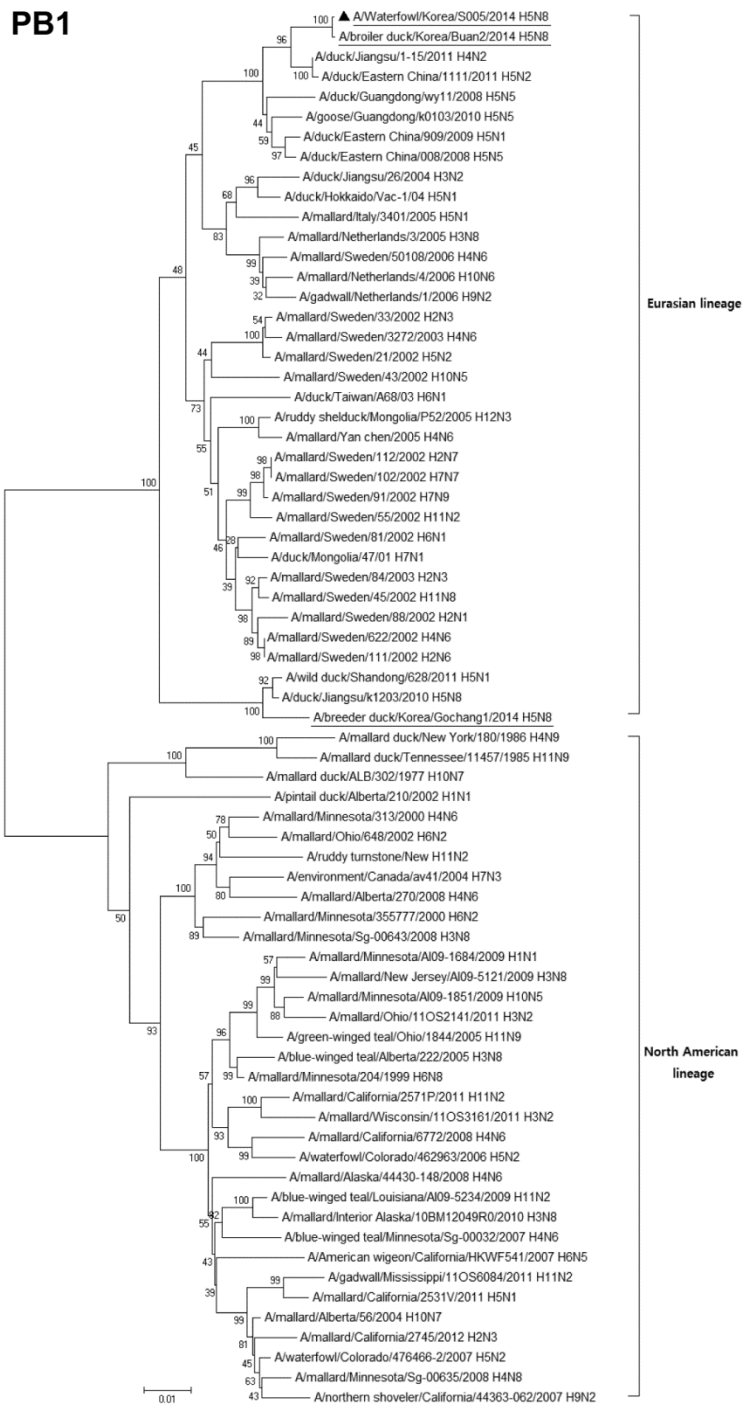
A/mallard/California/2594V/2011 H4N6
North American lineage
0.02


PB1
▲ A/Waterfowl/Korea/S005/2014 H5N8
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A/duck/Eastern China/1111/2011 H5N2
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A/duck/Eastern China/909/2009 H5N1
A/duck/Eastern China/008/2008 H5N5
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A/mallard/Sweden/3272/2003 H4N6
A/mallard/Sweden/21/2002 H5N2
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A/mallard/Sweden/81/2002 H6N1
A/duck/Mongolia/47/01 H7N1
A/mallard/Sweden/84/2003 H2N3
A/mallard/Sweden/45/2002 H11N8
A/mallard/Sweden/88/2002 H2N1
A/mallard/Sweden/622/2002 H4N6
A/mallard/Sweden/111/2002 H2N6
A/wild duck/Shandong/628/2011 H5N1
A/duck/Jiangsu/k1203/2010 H5N8
A/breeder duck/Korea/Gochang1/2014 H5N8
Eurasian lineage
A/mallard duck/New York/180/1986 H4N9
A/mallard duck/Tennessee/11457/1985 H11N9
A/mallard duck/ALB/302/1977 H10N7
A/pintail duck/Alberta/210/2002 H1N1
A/mallard/Minnesota/313/2000 H4N6
A/mallard/Ohio/648/2002 H6N2
A/ruddy turnstone/New H11N2
A/environment/Canada/av41/2004 H7N3
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A/mallard/Minnesota/AI09-1851/2009 H10N5
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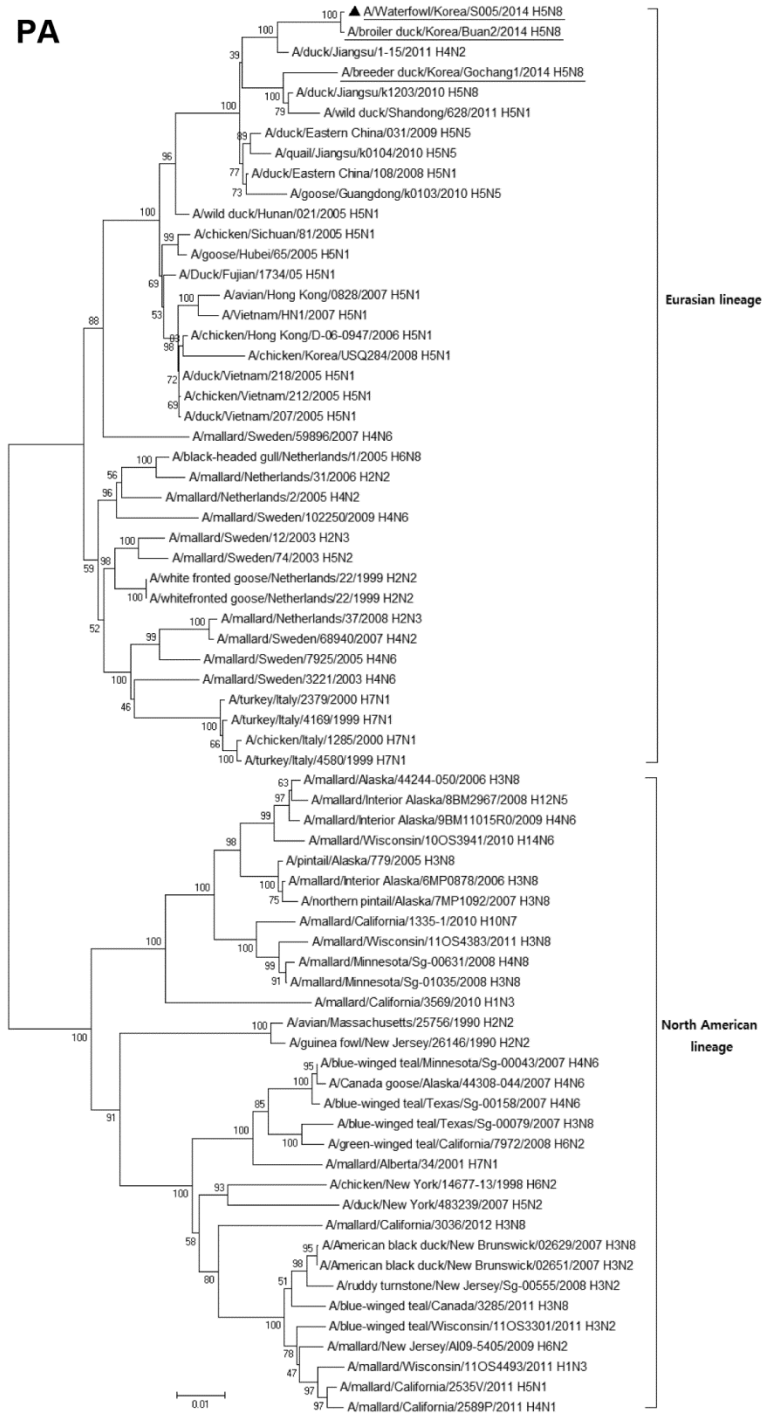
0.01


PA
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A/wild duck/Hunan/021/2005 H5N1
A/chicken/Sichuan/81/2005 H5N1
A/goose/Hubei/65/2005 H5N1
A/Duck/Fujian/1734/05 H5N1
A/avian/Hong Kong/0828/2007 H5N1
A/Vietnam/HN1/2007 H5N1
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A/whitefronted goose/Netherlands/22/1999 H2N2
A/mallard/Netherlands/37/2008 H2N3
A/mallard/Sweden/68940/2007 H4N2
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A/turkey/Italy/2379/2000 H7N1
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A/turkey/Italy/4580/1999 H7N1
Eurasian lineage
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A/duck/New York/483239/2007 H5N2
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A/American black duck/New Brunswick/02629/2007 H3N8
A/American black duck/New Brunswick/02651/2007 H3N2
A/ruddy turnstone/New Jersey/Sg-00555/2008 H3N2
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North American lineage
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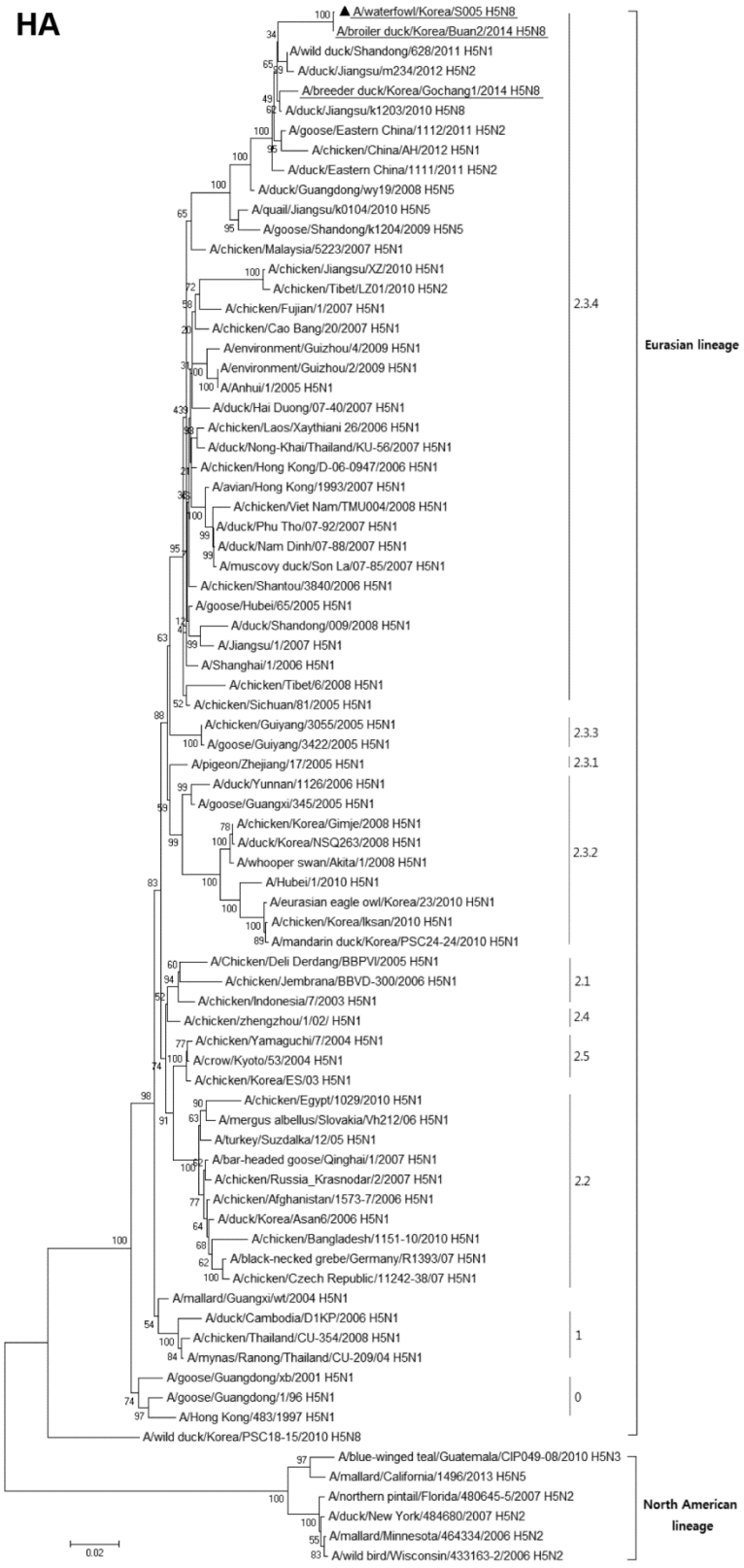
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A/duck/Jiangsu/k1203/2010 H5N8
A/goose/Eastern China/1112/2011 H5N2
A/chicken/China/AH/2012 H5N1
A/duck/Eastern China/1111/2011 H5N2
A/duck/Guangdong/wy19/2008 H5N5
A/quail/Jiangsu/k0104/2010 H5N5
A/goose/Shandong/k1204/2009 H5N5
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A/Anhui/1/2005 H5N1
A/duck/Hai Duong/07-40/2007 H5N1
A/chicken/Laos/Xaythiani 26/2006 H5N1
A/duck/Nong-Khai/Thailand/KU-56/2007 H5N1
A/chicken/Hong Kong/D-06-0947/2006 H5N1
A/avian/Hong Kong/1993/2007 H5N1
A/chicken/Viet Nam/TMU004/2008 H5N1
A/duck/Phu Tho/07-92/2007 H5N1
A/duck/Nam Dinh/07-88/2007 H5N1
A/muscovy duck/Son La/07-85/2007 H5N1
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A/duck/Shandong/009/2008 H5N1
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A/Shanghai/1/2006 H5N1
A/chicken/Tibet/6/2008 H5N1
A/chicken/Sichuan/81/2005 H5N1
A/chicken/Guiyang/3055/2005 H5N1
A/goose/Guiyang/3422/2005 H5N1
A/pigeon/Zhejiang/17/2005 H5N1
A/duck/Yunnan/1126/2006 H5N1
A/goose/Guangxi/345/2005 H5N1
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A/duck/Korea/NSQ263/2008 H5N1
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A/Hubei/1/2010 H5N1
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A/chicken/Korea/Iksan/2010 H5N1
A/mandarin duck/Korea/PSC24-24/2010 H5N1
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A/chicken/Jembrana/BBVD-300/2006 H5N1
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A/chicken/zhengzhou/1/02/ H5N1
A/chicken/Yamaguchi/7/2004 H5N1
A/crow/Kyoto/53/2004 H5N1
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A/mallard/California/1496/2013 H5N5
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A/wild bird/Wisconsin/433163-2/2006 H5N2
2.3.4
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2.5
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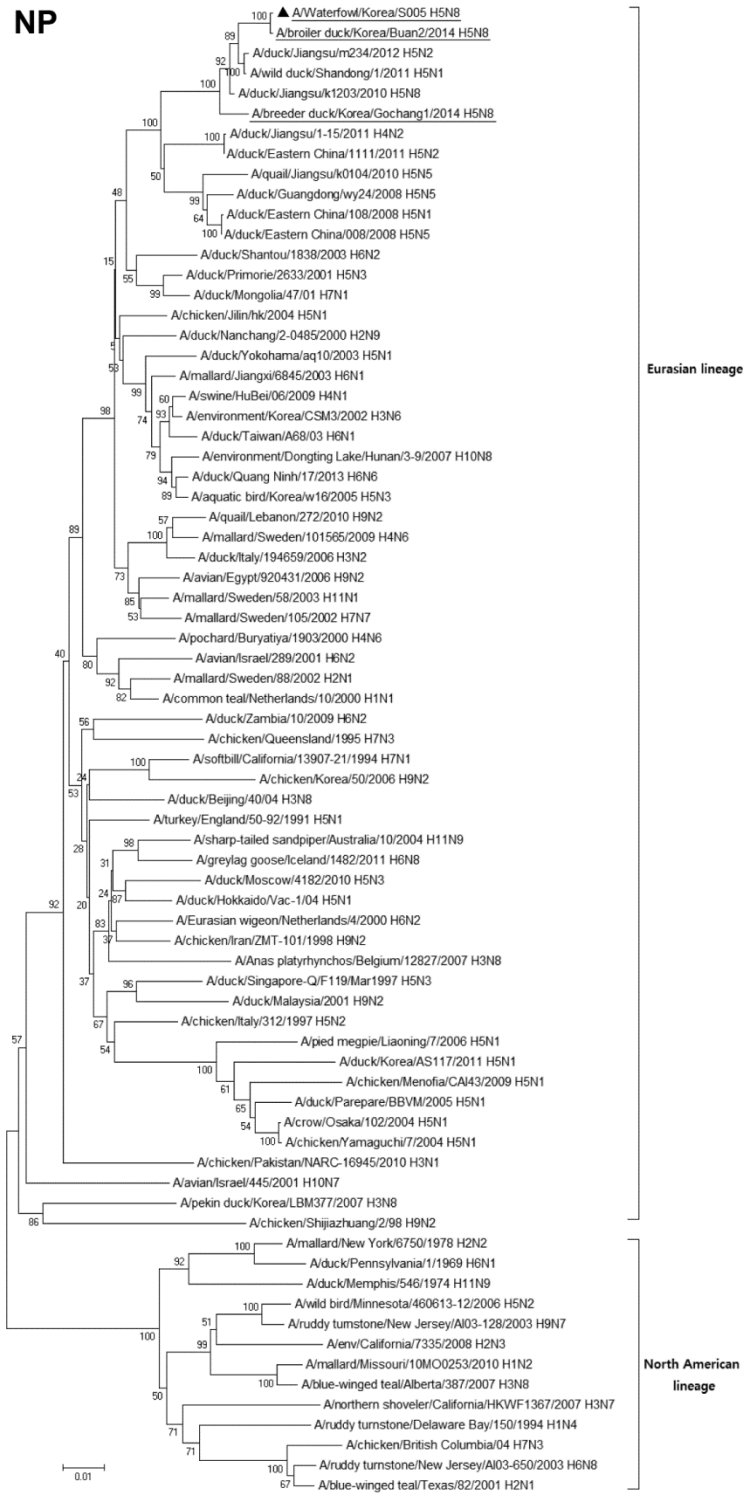
Eurasian lineage
North American lineage
0.02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A/duck/Jiangsu/k1203/2010 H5N8
A/breeder duck/Korea/Gochang1/2014 H5N8
A/duck/Jiangsu/1-15/2011 H4N2
A/duck/Eastern China/1111/2011 H5N2
A/quail/Jiangsu/k0104/2010 H5N5
A/duck/Guangdong/wy24/2008 H5N5
A/duck/Eastern China/108/2008 H5N1
A/duck/Eastern China/008/2008 H5N5
A/duck/Shantou/1838/2003 H6N2
A/duck/Primorie/2633/2001 H5N3
A/duck/Mongolia/47/01 H7N1
A/chicken/Jilin/hk/2004 H5N1
A/duck/Nanchang/2-0485/2000 H2N9
A/duck/Yokohama/aq10/2003 H5N1
A/mallard/Jiangxi/6845/2003 H6N1
A/swine/HuBei/06/2009 H4N1
A/environment/Korea/CSM3/2002 H3N6
A/duck/Taiwan/A68/03 H6N1
A/environment/Dongting Lake/Hunan/3-9/2007 H10N8
A/duck/Quang Ninh/17/2013 H6N6
A/aquatic bird/Korea/w16/2005 H5N3
A/quail/Lebanon/272/2010 H9N2
A/mallard/Sweden/101565/2009 H4N6
A/duck/Italy/194659/2006 H3N2
A/avian/Egypt/920431/2006 H9N2
A/mallard/Sweden/58/2003 H11N1
A/mallard/Sweden/105/2002 H7N7
A/pochard/Buryatiya/1903/2000 H4N6
A/avian/Israel/289/2001 H6N2
A/mallard/Sweden/88/2002 H2N1
A/common teal/Netherlands/10/2000 H1N1
A/duck/Zambia/10/2009 H6N2
A/chicken/Queensland/1995 H7N3
A/softbill/California/13907-21/1994 H7N1
A/chicken/Korea/50/2006 H9N2
A/duck/Beijing/40/04 H3N8
A/turkey/England/50-92/1991 H5N1
A/sharp-tailed sandpiper/Australia/10/2004 H11N9
A/greylag goose/Iceland/1482/2011 H6N8
A/duck/Moscow/4182/2010 H5N3
A/duck/Hokkaido/Vac-1/04 H5N1
A/Eurasian wigeon/Netherlands/4/2000 H6N2
A/chicken/Iran/ZMT-101/1998 H9N2
A/Anas platyrhynchos/Belgium/12827/2007 H3N8
A/duck/Singapore-Q/F119/Mar1997 H5N3
A/duck/Malaysia/2001 H9N2
A/chicken/Italy/312/1997 H5N2
A/pied megpie/Liaoning/7/2006 H5N1
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A/chicken/Menofia/CAI43/2009 H5N1
A/duck/Parepare/BBVM/2005 H5N1
A/crow/Osaka/102/2004 H5N1
A/chicken/Yamaguchi/7/2004 H5N1
A/chicken/Pakistan/NARC-16945/2010 H3N1
A/avian/Israel/445/2001 H10N7
A/pekin duck/Korea/LBM377/2007 H3N8
A/chicken/Shijiazhuang/2/98 H9N2
A/mallard/New York/6750/1978 H2N2
A/duck/Pennsylvania/1/1969 H6N1
A/duck/Memphis/546/1974 H11N9
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A/blue-winged teal/Alberta/387/2007 H3N8
A/northern shoveler/California/HKWF1367/2007 H3N7
A/ruddy turnstone/Delaware Bay/150/1994 H1N4
A/chicken/British Columbia/04 H7N3
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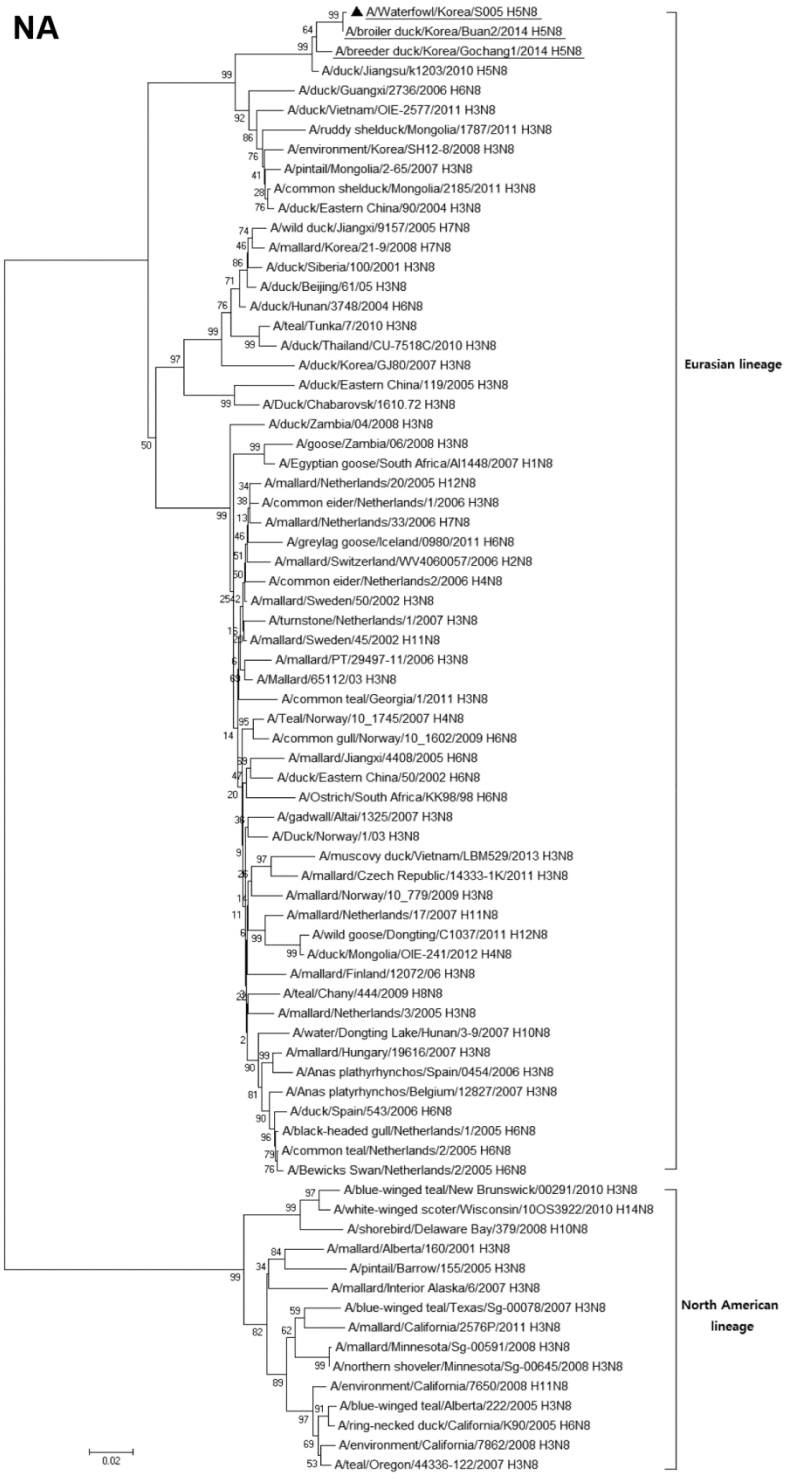
Eurasian lineage
North American lineage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A/duck/Guangxi/2736/2006 H6N8
A/duck/Vietnam/OIE-2577/2011 H3N8
A/ruddy shelduck/Mongolia/1787/2011 H3N8
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A/wild duck/Jiangxi/9157/2005 H7N8
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A/duck/Siberia/100/2001 H3N8
A/duck/Beijing/61/05 H3N8
A/duck/Hunan/3748/2004 H6N8
A/teal/Tunka/7/2010 H3N8
A/duck/Thailand/CU-7518C/2010 H3N8
A/duck/Korea/GJ80/2007 H3N8
A/duck/Eastern China/119/2005 H3N8
A/Duck/Chabarovsk/1610.72 H3N8
A/duck/Zambia/04/2008 H3N8
A/goose/Zambia/06/2008 H3N8
A/Egyptian goose/South Africa/AI1448/2007 H1N8
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A/common eider/Netherlands/1/2006 H3N8
A/mallard/Netherlands/33/2006 H7N8
A/greylag goose/Iceland/0980/2011 H6N8
A/mallard/Switzerland/WV4060057/2006 H2N8
A/common eider/Netherlands2/2006 H4N8
A/mallard/Sweden/50/2002 H3N8
A/turnstone/Netherlands/1/2007 H3N8
A/mallard/Sweden/45/2002 H11N8
A/mallard/PT/29497-11/2006 H3N8
A/Mallard/65112/03 H3N8
A/common teal/Georgia/1/2011 H3N8
A/Teal/Norway/10_1745/2007 H4N8
A/common gull/Norway/10_1602/2009 H6N8
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A/mallard/Netherlands/17/2007 H11N8
A/wild goose/Dongting/C1037/2011 H12N8
A/duck/Mongolia/OIE-241/2012 H4N8
A/mallard/Finland/12072/06 H3N8
A/teal/Chany/444/2009 H8N8
A/mallard/Netherlands/3/2005 H3N8
A/water/Dongting Lake/Hunan/3-9/2007 H10N8
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A/Anas platyrhynchos/Belgium/12827/2007 H3N8
A/duck/Spain/543/2006 H6N8
A/black-headed gull/Netherlands/1/2005 H6N8
A/common teal/Netherlands/2/2005 H6N8
A/Bewicks Swan/Netherlands/2/2005 H6N8
Eurasian lineage
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A/white-winged scoter/Wisconsin/10OS3922/2010 H14N8
A/shorebird/Delaware Bay/379/2008 H10N8
A/mallard/Alberta/160/2001 H3N8
A/pintail/Barrow/155/2005 H3N8
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A/ring-necked duck/California/K90/2005 H6N8
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North American lineage
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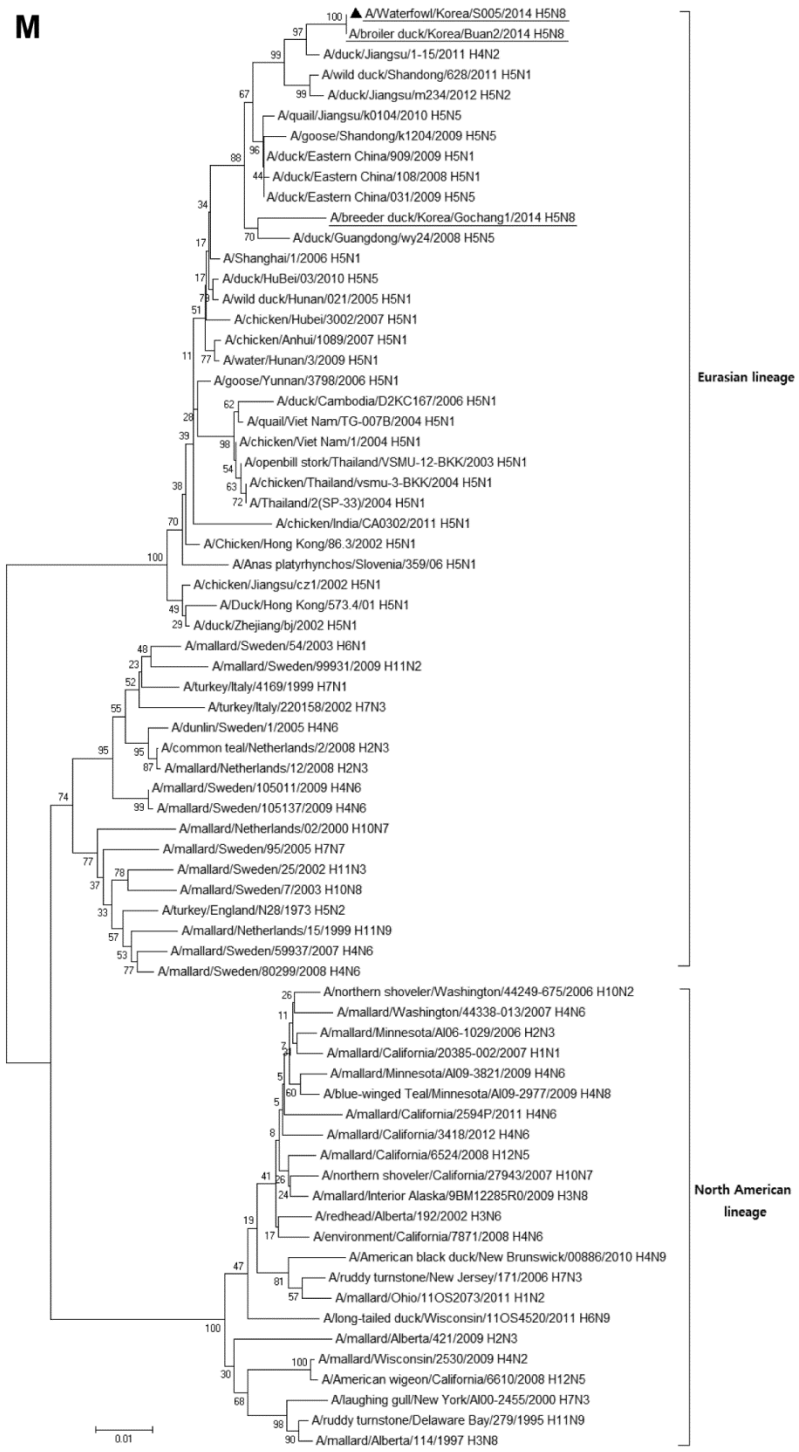
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A/wild duck/Shandong/628/2011 H5N1
A/duck/Jiangsu/m234/2012 H5N2
A/quail/Jiangsu/k0104/2010 H5N5
A/goose/Shandong/k1204/2009 H5N5
A/duck/Eastern China/909/2009 H5N1
A/duck/Eastern China/108/2008 H5N1
A/duck/Eastern China/031/2009 H5N5
A/breeder duck/Korea/Gochang1/2014 H5N8
A/duck/Guangdong/wy24/2008 H5N5
A/Shanghai/1/2006 H5N1
A/duck/HuBei/03/2010 H5N5
A/wild duck/Hunan/021/2005 H5N1
A/chicken/Hubei/3002/2007 H5N1
A/chicken/Anhui/1089/2007 H5N1
A/water/Hunan/3/2009 H5N1
A/goose/Yunnan/3798/2006 H5N1
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A/chicken/Viet Nam/1/2004 H5N1
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A/Thailand/2(SP-33)/2004 H5N1
A/chicken/India/CA0302/2011 H5N1
A/Chicken/Hong Kong/86.3/2002 H5N1
A/Anas platyrhynchos/Slovenia/359/06 H5N1
A/chicken/Jiangsu/cz1/2002 H5N1
A/Duck/Hong Kong/573.4/01 H5N1
A/duck/Zhejiang/bj/2002 H5N1
A/mallard/Sweden/54/2003 H6N1
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A/dunlin/Sweden/1/2005 H4N6
A/common teal/Netherlands/2/2008 H2N3
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A/mallard/Sweden/25/2002 H11N3
A/mallard/Sweden/7/2003 H10N8
A/turkey/England/N28/1973 H5N2
A/mallard/Netherlands/15/1999 H11N9
A/mallard/Sweden/59937/2007 H4N6
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Eurasian lineage
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A/mallard/Minnesota/AI09-3821/2009 H4N6
A/blue-winged Teal/Minnesota/AI09-2977/2009 H4N8
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A/mallard/California/3418/2012 H4N6
A/mallard/California/6524/2008 H12N5
A/northern shoveler/California/27943/2007 H10N7
A/mallard/Interior Alaska/9BM12285R0/2009 H3N8
A/redhead/Alberta/192/2002 H3N6
A/environment/California/7871/2008 H4N6
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A/ruddy turnstone/New Jersey/171/2006 H7N3
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A/long-tailed duck/Wisconsin/11OS4520/2011 H6N9
A/mallard/Alberta/421/2009 H2N3
A/mallard/Wisconsin/2530/2009 H4N2
A/American wigeon/California/6610/2008 H12N5
A/laughing gull/New York/AI00-2455/2000 H7N3
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North American lineage
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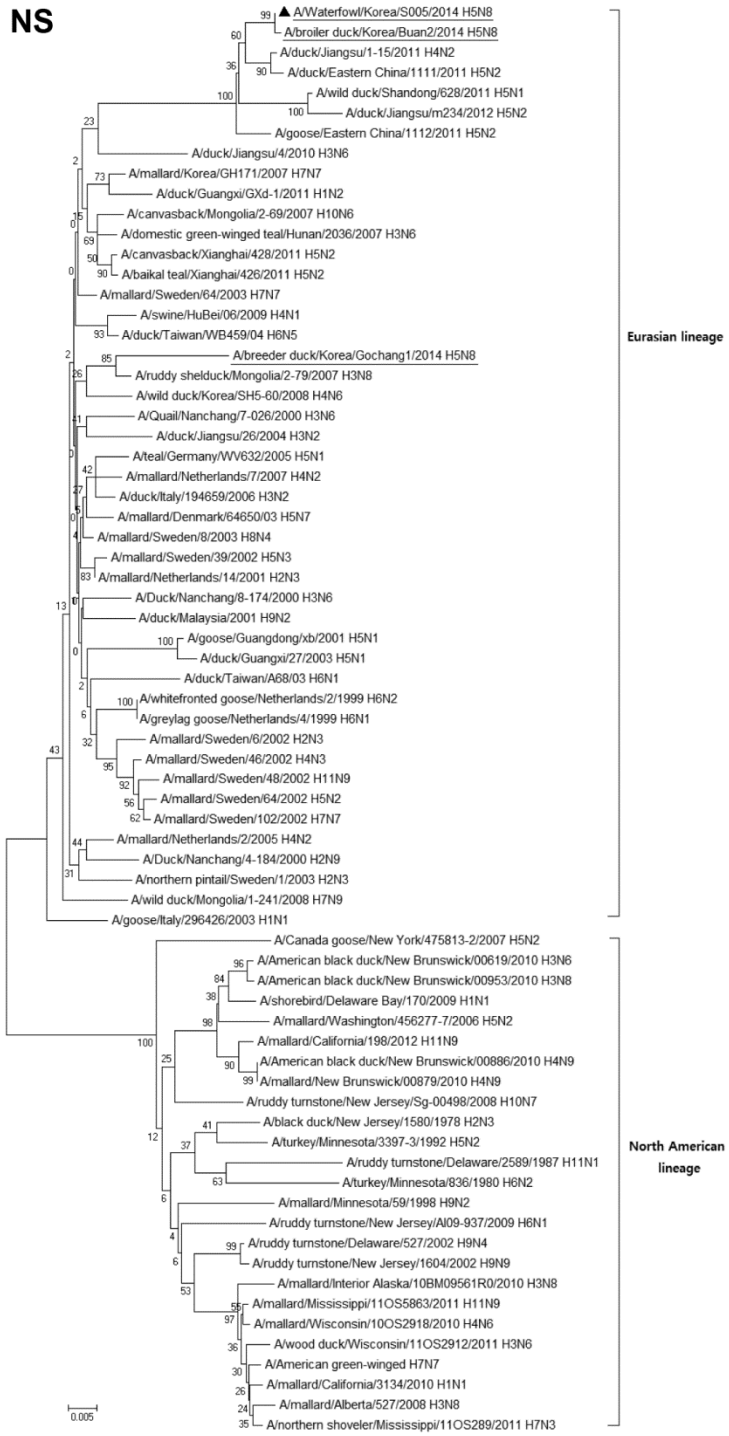
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A/wild duck/Shandong/628/2011 H5N1
A/duck/Jiangsu/m234/2012 H5N2
A/goose/Eastern China/1112/2011 H5N2
A/duck/Jiangsu/4/2010 H3N6
A/mallard/Korea/GH171/2007 H7N7
A/duck/Guangxi/GXd-1/2011 H1N2
A/canvasback/Mongolia/2-69/2007 H10N6
A/domestic green-winged teal/Hunan/2036/2007 H3N6
A/canvasback/Xianghai/428/2011 H5N2
A/baikal teal/Xianghai/426/2011 H5N2
A/mallard/Sweden/64/2003 H7N7
A/swine/HuBei/06/2009 H4N1
A/duck/Taiwan/WB459/04 H6N5
A/breeder duck/Korea/Gochang1/2014 H5N8
A/ruddy shelduck/Mongolia/2-79/2007 H3N8
A/wild duck/Korea/SH5-60/2008 H4N6
A/Quail/Nanchang/7-026/2000 H3N6
A/duck/Jiangsu/26/2004 H3N2
A/teal/Germany/WV632/2005 H5N1
A/mallard/Netherlands/7/2007 H4N2
A/duck/Italy/194659/2006 H3N2
A/mallard/Denmark/64650/03 H5N7
A/mallard/Sweden/8/2003 H8N4
A/mallard/Sweden/39/2002 H5N3
A/mallard/Netherlands/14/2001 H2N3
A/Duck/Nanchang/8-174/2000 H3N6
A/duck/Malaysia/2001 H9N2
A/goose/Guangdong/xb/2001 H5N1
A/duck/Guangxi/27/2003 H5N1
A/duck/Taiwan/A68/03 H6N1
A/whitefronted goose/Netherlands/2/1999 H6N2
A/greylag goose/Netherlands/4/1999 H6N1
A/mallard/Sweden/6/2002 H2N3
A/mallard/Sweden/46/2002 H4N3
A/mallard/Sweden/48/2002 H11N9
A/mallard/Sweden/64/2002 H5N2
A/mallard/Sweden/102/2002 H7N7
A/mallard/Netherlands/2/2005 H4N2
A/Duck/Nanchang/4-184/2000 H2N9
A/northern pintail/Sweden/1/2003 H2N3
A/wild duck/Mongolia/1-241/2008 H7N9
A/goose/Italy/296426/2003 H1N1
Eurasian lineage
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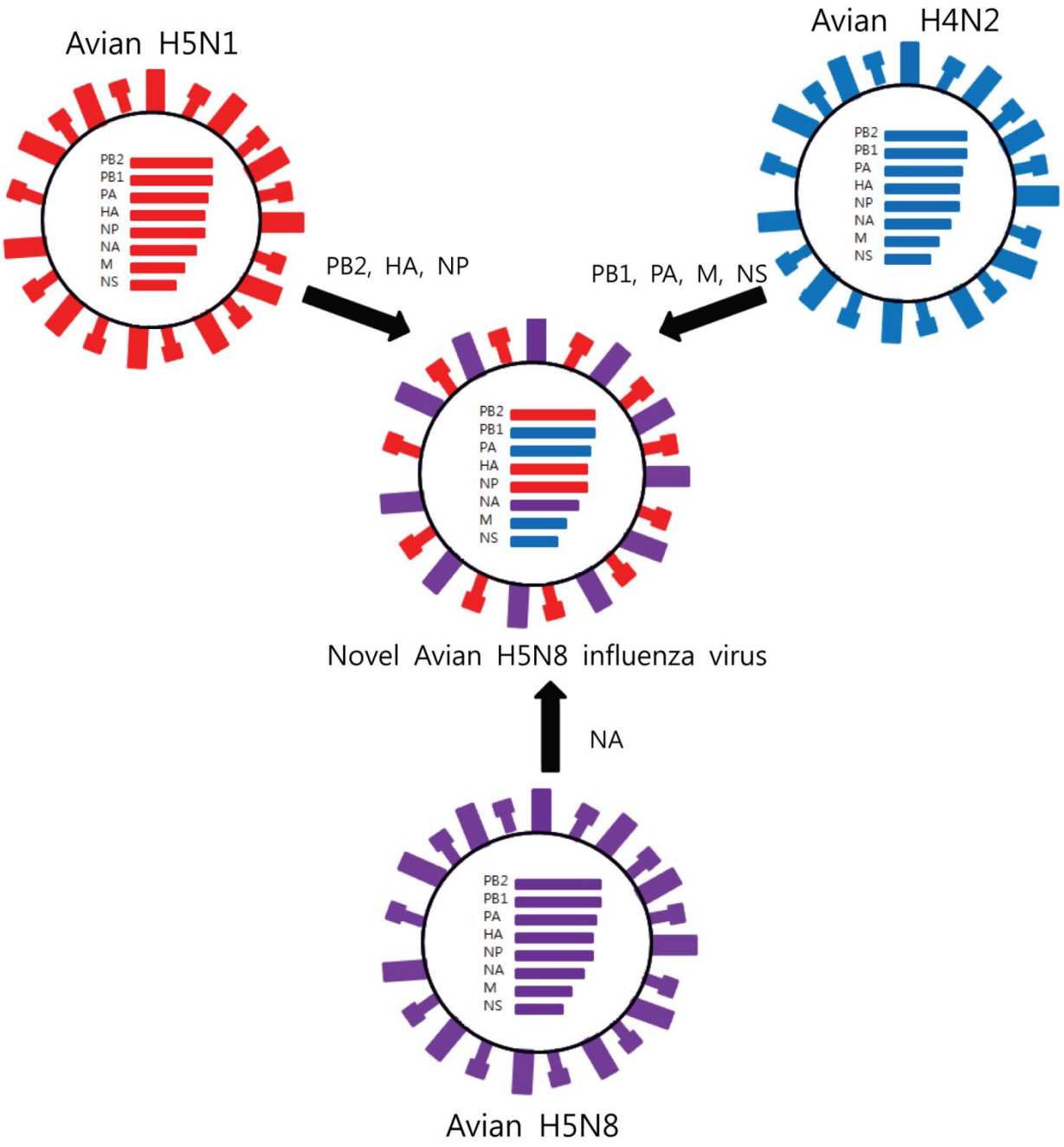
A/American black duck/New Brunswick/00619/2010 H3N6
A/American black duck/New Brunswick/00953/2010 H3N8
A/shorebird/Delaware Bay/170/2009 H1N1
A/mallard/Washington/456277-7/2006 H5N2
A/mallard/California/198/2012 H11N9
A/American black duck/New Brunswick/00886/2010 H4N9
A/mallard/New Brunswick/00879/2010 H4N9
A/ruddy turnstone/New Jersey/Sg-00498/2008 H10N7
A/black duck/New Jersey/1580/1978 H2N3
A/turkey/Minnesota/3397-3/1992 H5N2
A/ruddy turnstone/Delaware/2589/1987 H11N1
A/turkey/Minnesota/836/1980 H6N2
A/mallard/Minnesota/59/1998 H9N2
A/ruddy turnstone/New Jersey/AI09-937/2009 H6N1
A/ruddy turnstone/Delaware/527/2002 H9N4
A/ruddy turnstone/New Jersey/1604/2002 H9N9
A/mallard/Interior Alaska/10BM09561R0/2010 H3N8
A/mallard/Mississippi/11OS5863/2011 H11N9
A/mallard/Wisconsin/10OS2918/2010 H4N6
A/wood duck/Wisconsin/11OS2912/2011 H3N6
A/American green-winged H7N7
A/mallard/California/3134/2010 H1N1
A/mallard/Alberta/527/2008 H3N8
A/northern shoveler/Mississippi/11OS289/2011 H7N3
North American lineage
0.005



Technical Appendix Figure 2. Schematic diagram of influenza virus strain A/waterfowl/Korea/S005/2014 (H5N8). Novel highly pathogenic avian influenza virus is likely to be created by genes from 3 avian influenza viruses. PB, polymerase basic subunit; PA, polymerase acidic subunit; HA, hemagglutinin; NP, nucleoprotein; NA, neuraminidase; M, matrix; NS, nonstructural.

Technical Appendix Table. Identification of amino acids of influenza virus strain A/waterfowl/Korea/S005/2014 (H5N8) involved in binding to human-type influenza receptor, enhancing antiviral drugs, and causing pathogenesis in poultry and mammals

Viral protein*	Amino acid position	A/waterfowl/Korea/S005/2014 (H5N8)†	Comments
PB2	627	E	E627K: adaptation to mammalian host
HA	138 (H3 numbering)	A	S138A: Increased binding to human-type influenza receptor
	160 (H3 numbering)	A	T160A: N-glycosylation loss and increased binding to human-type influenza receptor
	226 (H3 numbering)	Q	Q226L: Increased binding to human-type influenza receptor
	228 (H3 numbering)	G	G228S: Increased binding to human-type influenza receptor
	339-348	RE RRRK R/GLF	Polybasic amino acid insertion: high pathogenesis in poultry and mammals
NA	69-72 (N9 numbering)	No deletion	Deletion of amino acids 69-73:Increased pathogenesis in mice
	292 (N2 numbering)	R	R292K: Resistance to oseltamivir and zanamivir
M1	30	D	N30D: Increased pathogenesis in mice
	215	A	T215A: Increased pathogenesis in mice
M2	31	N	S31N: Resistance to amantadine and rimantadine
NS1	42	S	P42S: Increased pathogenesis in mice
	218-230	No truncation	Lack of PDZ domain binding motif: reduced pathogenesis in mice

*PB, polymerase basic subunit; PA, polymerase acidic subunit; HA, hemagglutinin; NP, nucleoprotein; NA, neuraminidase; M, matrix; NS, nonstructural.

†A, alanine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; K, lysine; L, leucine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine.