New Reassortant Clade 2.3.4.4b Avian Influenza A(H5N6) Virus in Wild Birds, South Korea, 2017–2018

Technical Appendix

Methods

Virus Isolation

Fecal samples were tested for influenza A virus by egg inoculation using 9–1-day-old specific-pathogen-free embryonating chicken eggs. After a 72-h incubation period, allantoic fluids were harvested from an axenic medium, and undiluted allantoic fluids were tested for hemagglutinin (HA) activity. For the definitive diagnosis of influenza A virus, RNA was extracted from HA-positive allantoic fluid using an RNeasy Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. The extracted RNA was tested for the influenza A virus matrix gene by real-time reverse transcription PCR (RT-PCR) as described previously (1). The hosts of the positive fecal sample were identified as Mandarin ducks (*Aix galericulata*) and mallards (*Anas platyrhynchos*) using DNA barcoding techniques as previously described (2).

Full-genome Sequencing

Full-length genome sequencing was performed for 19 of 40 isolates (5 from Cheongmi-cheon River collected on 22 December 2017, 13 from Gokgyo-cheon River collected on 23 December 2017, and 1 from Gokgyo-cheon River collected on 18 January 2018). For molecular analysis, RNA was extracted using the RNeasy kit (QIAGEN) according to the manufacturer's instructions. The 8 genes of each virus were amplified using 1-step RT-PCR. The RT-PCR amplicons (2 µg) of all 8 gene segments was used to prepare Ion Fragment sequencing libraries (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. Briefly, amplicons were loaded onto beads, and emulsion PCR was conducted before sequencing with an Ion 318 chip on an Ion Torrent Personal Genome Machine. De novo and directed assembly of genome sequences were performed using Geneious R9 software (http://www.geneious.com). We

provided the genome sequences in the EpiFlu database of Global Initiative on Sharing All Influenza Data (GISAID; https://www.gisaid.org), under accession nos. EPI1190336–40, EPI1190342–61, EPI1190381, EPI1190386, EPI1190392, EPI1190410, EPI1190418, EPI1190426, EPI1190426, EPI1190433, EPI1190446, EPI1190498, EPI1190503, EPI1190508, EPI1190514–19, EPI1200474–509, EPI1200511–87.

Phylogenetic Analysis

For phylogenetic analysis, complete coding regions were aligned using MAFFT (https://mafft.cbrc.jp/alignment/software/), and manual editing and tree reconstruction were conducted using Geneious 8 software (https://www.geneious.com/). The sequence of clade 2.3.4.4 H5 highly pathogenic avian influenza viruses representing each subgroup and low pathogenic avian influenza virus strains related to new reassortment of H5N6 highly pathogenic avian influenza viruses in 2017–18 were selected from GISAID Epiflu and GenBank (https://www.ncbi.nlm.nih.gov/genomes/FLU) database as a reference for phylogenetic tree analysis. A maximum-likelihood tree was estimated by the MEGA7 software using the Hasegawa-Kishino-Yano model (*3*) of nucleotide substitution with gamma-distributed rate variation among sites with 4 rate categories. Statistical analysis of the phylogenetic tree was performed by bootstrap analysis on 1,000 replicates. The percentages of replicate trees (>70%) in which the associated taxa clustered together in the bootstrap test are shown next to the branches.

To better visualize the genetic relatedness of viruses, HA gene was analyzed using the median joining method implemented by NETWORK ver. 5.0 with epsilon set to 0. Nucleotide sequences of complete protein coding sequence region of subgroup B H5N6 and H5N8 identified from Asia and Europe in 2016 and 2017 (n = 250) were retrieved from GISAID Epiflu database on February 13, 2018. The sequences were pruned by using the online software cd-hit (*4*) at 97% homology to filter-out 66 representative sequences. The nucleotide sequences of HA segment obtained in this study (n = 19) were added to the dataset. A total of 85 nt sequences were aligned using MAFFT, and alignments were manually edited in Geneious 8 software (https://www.geneious.com/).

For estimation of the time to the most recent common ancestor, Bayesian analysis was performed for all 8 gene segments using BEAST version 1.8.4 (http://beast.bio.ed.ac.uk). We used an Hasegawa-Kishino-Yano substitution model with 4 gamma categories and specified an

uncorrelated lognormal relaxed clock and GMRF Bayesian skyride tree prior for each segment. A Markov chain Monte Carlo method was used with 50 million chain lengths to draw inference under this model. A maximum clade credibility tree with common ancestor height was generated for each dataset using TreeAnnotator version 1.8.4 after 10% burn-in. The maximum clade credibility trees were visualized using the program FigTree v1.4.2.

References

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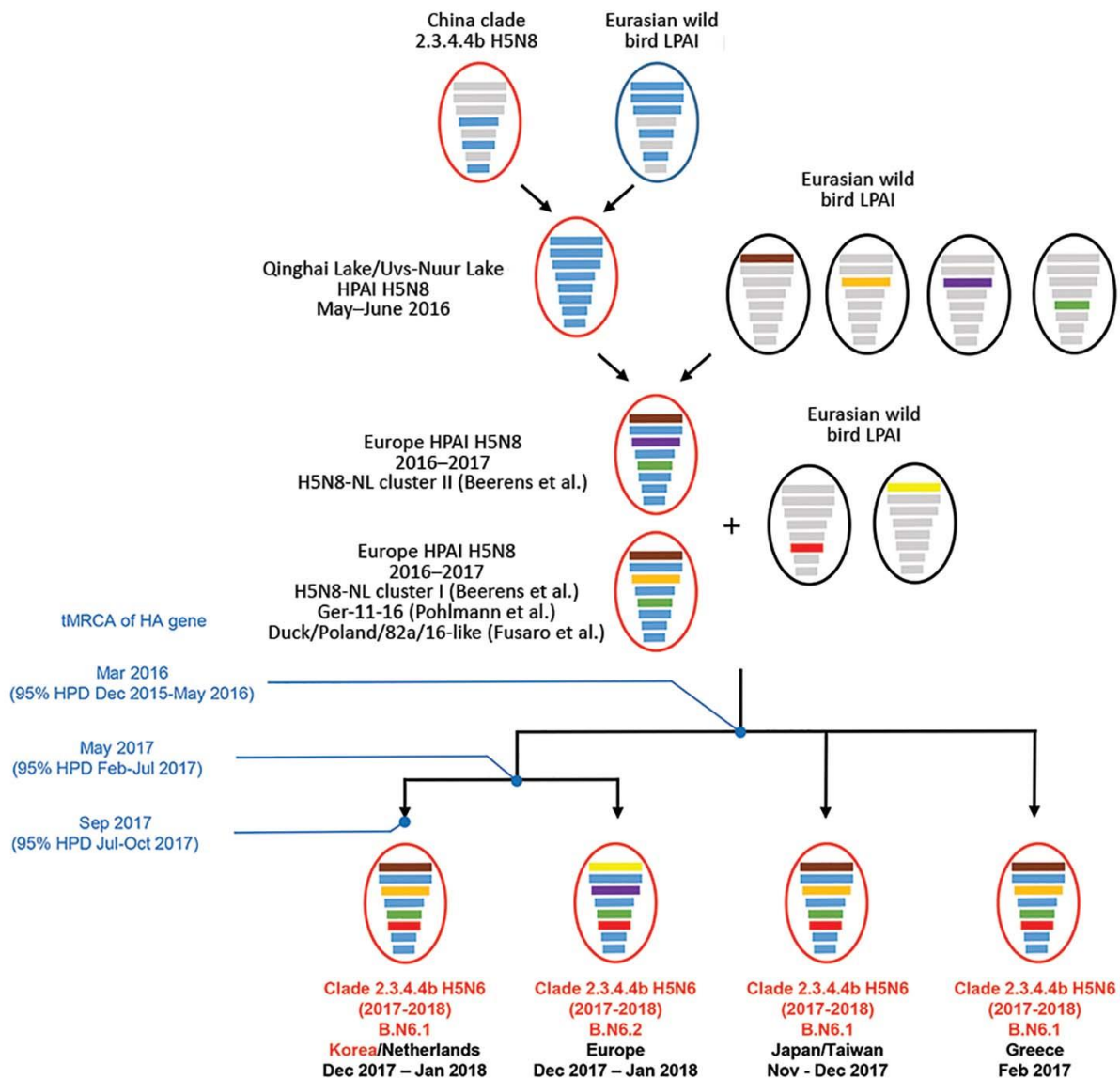
Technical Appendix 1 Figure 1. Proposed reassortment events leading to the new subgroup B influenza A(H5N6) viruses. Eight gene segments in each of the schematic virus particles are arranged from top to bottom to represent the PB2, PB1, PA, HA, NP, NA, M, and NS genes. The circled HPAI viruses are colored in red, and unknown LPAI viruses are colored in black. For each gene segment, different phylogenetic groupings are in different colors. The mean time to the most recent common ancestor of HA gene are shown at the node. HA, hemagglutinin; HPAI, highly pathogenic avian influenza; HPD, highest posterior density; LPAI, low pathogen avian influenza; M, matrix; NA, neuraminidase; NP, nucleoprotein; NS, nonstructural; PA, polymerase acidic; PB, polymerase basic; tMCRA, time to the most recent common ancestor.

A

A/Mandarin duck/Korea/K17-1817/2017|2017-12-22|H5N6
A/Mandarin duck/Korea/K17-1828/2017|2017-12-22|H5N6
A/Mandarin duck/Korea/K17-1815/2017|2017-12-22|H5N6
A/Mandarin duck/Korea/K17-1826/2017|2017-12-22|H5N6
A/Mallard/Korea/K17-1825/2017|2017-12-22|H5N6
A/Mandarin duck/Korea/K17-1894/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1873/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K18-3/2018|2018-01-18|H5N6
A/Mandarin duck/Korea/K17-1891/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1885/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1879/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1887/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1881/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1869/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1862/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1866/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1893/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1896/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1889/2017|2017-12-23|H5N6

Korea H5N6 2017-18 (B.N6.1)

A/Great Black-backed Gull/Netherlands/1/2017|2017-12-18|H5N6

Netherlands H5N6 2017 (B.N6.1)

A/turkey/England/052131/2016|2016-12-15|H5N8
A/eurasianwigeon/Germany-NI/AR249-L02143/2017|2017-01-04|H5N8
A/turkey/Rostov/11/2017|2017-01-01|H5N8
A/chicken/Greece/39.2017b/2017|2017-02-06|H5N6

Greece H5N6 2017 (B.N6.1)

A/tufted duck/Germany/AR8459-L01988/2016|2016-11-08|H5N8
A/Tufted Duck/Switzerland/V237/2016|2016-11-13|H5N8
A/wild duck/Poland/82A/2016|2016-11-02|H5N8
A/domesticduck/Germany-MV/R9869/2016|2016-11-23|H5N8
A/long-eared owl/Voronezh/15/2017|2017-01-06|H5N8
A/Goose/Hungary/1030/2017|2017-01-11|H5N8
A/mute swan/Shimane/3211A001/2017|2017-11-05|H5N6
A/spoonbill/Taiwan/DB645/2017|2017-12-01|H5N6

Japan/Taiwan H5N6 2017 (B.N6.1)

A/wigeon/Italy/17VIR57-3/2017|2017-01-03|H5N8
A/mute swan/Krasnodar/25/2017|2017-01-06|H5N8
A/swan/Italy/17VIR7064-1/2017|2017-08-25|H5N8
A/Bar-headed Goose/Qinghai/BTY1-B/2016|2016-05-09|H5N8
A/great crested grebe/Tyva/34/2016|2016-05-25|H5N8
A/chicken/Astrakhan/3131/2016|2016-12-13|H5N8
A/duck/India/10CA01/2016|2016-10-17|H5N8
A/painted stork/India/10CA03/2016|2016-10-20|H5N8
A/gadwall/Chany/97/2016|2016-09-10|H5N8
A/grey heron/Korea/W779/2017|2017-01-27|H5N8
A/chicken/Korea/H903/2017|2017-02-08|H5N8

Qinghai/Uvs-like

A/duck/Mongolia/30/2015|2015-08-29|H3N8
A/duck/Hunan/12.17.YYFQH012-O/2014|2014-12-17|H5N6
A/chicken/Croatia/104/2017|2017-03-07|H5N5
A/M Swan/NL-Groningen/16015826-001/2016|2016-12-13|H5N5
A/environment/Kamchatka/18/2016|2016-10-01|H5N5

Ger-12-16-N5

A/chicken/Taiwan/x37/2016|2016-12-21|H5N8
A/duck/Taiwan/A3400/2015|2015-02-08|H5N8
A/shoveler/Chany/82K/2014|2014-09-10|H3N8
A/Environment/Chongqing/45208/2015|2015-03-18|H5N6
A/duck/France/161108h/2016|2016-11-28|H5N8
A/black swan/Germany-BW/R1364/2017|2017-02-12|H5N8
A/mute swan/Croatia/70/2016|2016-10-30|H5N8
A/Mute swan/Hungary/3139/2017|2017-01-26|H5N8
A/turkey/Germany-NI/R10523/2016|2016-12-13|H5N8
A/greylag goose/Germany-NI/AR1395-L02144/2017|2017-02-02|H5N8
A/chicken/Czech Republic/206-17 2/2017|2017-01-06|H5N8
A/Mute swan/Hungary/6092/2017|2017-02-15|H5N8

Ger-12-16

A/chicken/Netherlands/16007311-037041/2016|2016-06-08|H7N9
A/mallard duck/Netherlands/15/2011|2011-09-14|H6N8
A/Eurasian teal/Netherlands/1/2011|2011-10-15|H3N8
A/Italy/3/2013|2013-09-07|H7N7
A/mallard duck/Netherlands/16/2012|2012-08-17|H3N2
A/mallard duck/Netherlands/30/2014|2014-07-21|H4N6
A/Mute Swan/Netherlands/17017367-012/2017|2017-12-09|H5N6
A/pochard duck/England/AVP 18003254/2018|2018-01-10|H5N6
A/canada goose/England/AV58 18OPpoolEP1/2018|2018-01-05|H5N6
A/Tufted Duck/Netherlands/17017367-007/2017|2017-12-09|H5N6
A/Duck/Netherlands/17017236-001-005/2017|2017-12-07|H5N6
A/Duck/Netherlands/17017237-001-005/2017|2017-12-07|H5N6
A/Mute Swan/Netherlands/17017377-001/2017|2017-12-09|H5N6
A/mute swan/England/AVP 18 001986/2017|2017-12-31|H5N6
A/common pochard/Germany-BY/AR09-L02421/2017|2017-12-28|H5N6
A/Great-backedGull/Netherlands/2/2017|2017-12-18|H5N6

Eurasia LPAI

Europe H5N6 2017-18 (B.N6.2)

A/duck/Wenzhou/YHQL22/2014|2014-01|H5N6
A/duck/Eastern China/S1210/2013|2013-12-10|H5N8
A/breeder duck/Korea/Gochang1/2014|2014-01-16|H5N8
A/mallard duck/Shanghai/SH-9/2013|2013-11-18|H5N8
A/goose/Zhejiang/925037/2014|2014-09-25|H5N8

clade 2.3.4.4b (2013-2014)

A/pigeon/Jilin/04.25 CCHL018-O/2015|2015-04-25|H5N6
A/duck/Hunan/12.07 YYGK111-P/2013|2013-12-07|H5N6
A/chicken/Guangxi/04.10 NM140/2015|2015-04-10|H5N6

clade 2.3.4.4c

A/duck/Jiangsu/WX 156/2013|2013-07-22|H5N6
A/environment/Zhenjiang/C13/2013|2013-12-20|H5N6

clade 2.3.4.4d

A/goose/Zhejiang/112080/2014|2014-11-20|H5N6
A/duck/Jiangsu/k1203/2010|2010-12-05|H5N8
A/duck/Eastern China/L1021/2012|2012-10-21|H5N8
A/duck/Beijing/FS01/2014|2014-01-20|H5N8
A/duck/Chiba/26-372-48/2014|2014-11-18|H5N8
A/goose/Taiwan/01019/2015|2015-01-11|H5N8
A/duck/Hubei/ZYSYG18/2015|2015-11-21|H5N6
A/baikal teal/Korea/1437/2014|2014-01-20|H5N8
A/crane/Kagoshima/KU21/2014|2014-12-17|H5N8

clade 2.3.4.4a

Ger-11-16

Eurasia clade 2.3.4.4b

B

A/Mandarin duck/Korea/K17-1889/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1893/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1869/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1866/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1862/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1896/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1891/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K18-3/2018|2018-01-18|H5N6
A/Mandarin duck/Korea/K17-1881/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1894/2017|2017-12-23|H5N6

Korea H5N6 2017-18 (B.N6.1)

A/Mandarin duck/Korea/K17-1885/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1873/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1879/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1887/2017|2017-12-23|H5N6
A/Mallard/Korea/K17-1825/2017|2017-12-22|H5N6
A/Mandarin duck/Korea/K17-1815/2017|2017-12-22|H5N6
A/Mandarin duck/Korea/K17-1828/2017|2017-12-22|H5N6
A/Mandarin duck/Korea/K17-1826/2017|2017-12-22|H5N6
A/Mandarin duck/Korea/K17-1817/2017|2017-12-22|H5N6

A/Great Black-backed Gull/Netherlands/1/2017|2017-12-18|H5N6
A/common pochard/Germany-BY/AR09-L02421/2017|2017-12-28|H5N6
A/Duck/Netherlands/17017236-001-005/2017|2017-12-07|H5N6
A/Tufted Duck/Netherlands/17017367-007/2017|2017-12-09|H5N6
A/pochard duck/England/AVP 18 0003254/2018|2018-01-10|H5N6
A/Great Black-backed Gull/Netherlands/2/2017|2017-12-18|H5N6
A/Mute Swan/Netherlands/17017367-012/2017|2017-12-09|H5N6
A/Duck/Netherlands/17017237-001-005/2017|2017-12-07|H5N6
A/Mute Swan/Netherlands/17017377-001/2017|2017-12-09|H5N6
A/mute swan/England/AVP 18 001986/2017|2017-12-31|H5N6
A/canada goose/England/AV58 18OPpoolEP1/2018|2018-01-05|H5N6

Europe H5N6 2017-18 (B.N6.1, B.N6.2)

A/turkey/England/052131/2016|2016-12-15|H5N8
A/ChNL-Zoeterwoude/16016484-021-025/2016|2016-12-24|H5N6
A/tufted duck/Germany/AR8444-L01987/2016|2016-11-07|H5N8
A/Magpie/NL-Volendam/16014331-002/2016|2016-11-16|IA /H5N8
A/chicken/Greece/39 2017b/2017|2017-02-06|H5N6 **Greece H5N6 2017 (B.N6.1)**
A/T Dk/NL-Werkendam/16014159-001/2016|2016-11-14|H5N5
A/wild duck/Poland/82A/2016|2016-11-02|H5N8
A/long-eared owl/Voronezh/15/2017|2017-01-06|H5N8
A/mute swan/Shimane/3211A001/2017|2017-11-05|H5N6
A/spoonbill/Taiwan/DB645/2017|2017-12-01|H5N6 **Japan/Taiwan H5N6 2017 (B.N6.1)**
A/mute swan/Krasnodar/25/2017|2017-01-06|H5N8
A/turkey/Italy/17VIR5878-3/2017|2017-07-23|H5N8
A/chicken/Italy/17VIR3078/2017|2017-04-07|H5N8
A/shelduck/Italy/17VIR1572-24/2017|2017-01-03|H5N8
A/mute swan/Croatia/70/2016|2016-10-30|H5N8
A/Cygnus olor/Belgium/1567/2017|2017-02-21|H5N8
A/Turkey/Hungary/2030/2017|2017-01-18|H5N8
A/duck/Hungary/55191/2016|2016-11-11|H5N8
A/Duck/Hungary/1588/2017|2017-01-16|H5N8
A/chicken/Italy/17VIR1751-3/2017|2017-02-27|H5N8
A/duck/India/10CA01/2016|2016-10-17|H5N8
A/wild duck/Tyva/35/2016|2016-05-25|H5N8
A/great crested grebe/Tyva/34/2016|2016-05-25|H5N8
A/black-headed gull/Tyva/41/2016|2016-05-25|H5N8
A/gadwall/Chany/97/2016|2016-09-10|H5N8
A/painted stork/India/10CA03/2016|2016-10-20|H5N8
A/grey heron/W779/2017|2017-01-27|H5N8
A/common teal/Korea/W547/2016|2016-12-15|H5N8
A/chicken/Korea/H903/2017|2017-02-08|H5N8
A/mallard duck/Korea/WA137/2017|2017-01-24|H5N8
A/Bar-headed Goose/Qinghai/BTY1-B/2016|2016-05-09|H5N8

Qinghai/Uvs-like clade 2.3.4.4b

A/duck/Mongolia/179/2015|2015-08-30|H3N8
A/goose/Zhejiang/112080/2014|2014-11-20|H5N6
A/cormorant/Germany-SH/R896/2017|2017-01-30|H5N5
A/duck/Eastern China/S1109/2014|2014-11-09|H5N6
A/Phalacrocorax/Hubei/01.09 V7/2015|2015-01-09|H5N6
A/duck/Guangdong/04.22 DGCPLB018-O/2015|2015-04-22|H5N6
A/Environment/Jiangsu/98335/2014|2014-10-28|H5N6
A/Environment/Kamchatka/18/2016|2016-10-01|H5N5
A/M Swan/NL-Groningen/16015826-001/2016|2016-12-13|H5N5 **Ger-12-16-N5.1**
A/Goose/Hungary/65817/2016|2016-12-18|H5N8
A/greylag goose/Germany-NI/AR1395-L02144/2017|2017-02-02|H5N8
A/greylag goose/Germany-NI/AR703-L02138/2017|2017-01-25|H5N8 **Ger-12-16.2**
A/duck/Eastern China/L1021/2012|2012-10-21|H5N8
A/goose/Zhejiang/925037/2014|2014-09-25|H5N8
A/duck/Chiba/26-372-48/2014|2014-11-18|H5N8
A/scarlet ibis/Germany/AR44-L01279/2015|2015-01|H5N8
A/baikal teal/Korea/1437/2014|2014-01-20|H5N8
A/duck/Beijing/FS01/2014|2014-01-20|H5N8
A/common teal/Korea/KU-12/2015|2015-01-22|H5N8
A/tundra swan/Tottori/C6nk/2014|2014-11-18|H5N8

clade 2.3.4.4a

A/duck/Hubei/ZYSYG18/2015|2015-11-21|H5N6
A/duck/Jiangsu/k1203/2010|2010-12-05|H5N8
A/goose/Guangdong/s13124/2013|2013-12-12|H5N8
A/duck/Taizhou/TZYG12/2015|2015-01|H5N6
A/Baikal teal/Korea/H52/2014|2014-01-20|H5N8
A/duck/Eastern China/S0215/2014|2014-02-15|H5N8
A/duck/Zhejiang/925169/2014|2014-09-25|H5N8
A/duck/Guangdong/03.26 DGCP077-O/2015|2015-03-26|H5N6

clade 2.3.4.4c

A/environment/Guangdong/GZ893/2015|2015-11-18|H5N6
A/Shenzhen/TH001/2015|2015-12-29|H5N6
A/chicken/Hubei/ZYSJF11/2016|2016-02-21|H5N6
A/Hubei/29578/2016|2016-04-15|H5N6
A/environment/JiangXi/05.06 NCJD0100C/2015|2015-05-06|H5N6

clade 2.3.4.4c (H9N2-like)

A/muscovy/duck/Hunan/102/2014|2014-11-11|H5N6
A/duck/Hunan/02.26 YYFQH297-P/2014|2014-02-26|H5N6
A/environment/Guangdong/04.16 LGWL008/2015|2015-04-16|H5N6
A/turtledove/Wuhan/WHBJ12/2014|2014-12|H5N6
A/environment/Zhenjiang/C13/2013|2013-12-20|H5N6
A/duck/Vietnam/NCVD-15A57/2015|2015-08-01|H5N6

clade 2.3.4.4d

C

A/Mandarin duck/Korea/K17-1887/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1894/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1885/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1881/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1879/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1817/2017|2017-12-22|H5N6
A/Mandarin duck/Korea/K17-1815/2017|2017-12-22|H5N6
A/Mallard/Korea/K17-1825/2017|2017-12-22|H5N6
A/Mandarin duck/Korea/K17-1873/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1828/2017|2017-12-22|H5N6   Korea H5N6 2017-18 (B.N6.1)
A/Mandarin duck/Korea/K17-1893/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1896/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1891/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K18-3/2018|2018-01-18|H5N6
A/Mandarin duck/Korea/K17-1882/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1866/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1869/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1889/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1826/2017|2017-12-22|H5N6

A/Great Black-backed Gull/Netherlands/1/2017|2017-12-18|H5N6   Netherlands H5N6 2017 (B.N6.1)
A/turkey/England/052131/2016|2016-12-15|H5N8
A/eurasian wigeon/Germany-NI/AR249-L02143/2017|2017-01-04|H5N8
A/Ch/NL-Zoeterwoude/16016484-021-025/2016|2016-12-24|H5N8
A/chicken/Greece/39 2017b/2017|2017-02-08|H5N6   Greece H5N6 2017 (B.N6.1)
A/spoonbill/Taiwan/DB845/2017|2017-12-01|H5N6   Japan/Taiwan H5N6 2017 (B.N6.1)
A/mute swan/Shimane/3211A001/2017|2017-11-05|H5N6
A/turkey/Italy/17VIR5878-3/2017|2017-07-23|H5N8
A/domestic duck/Germany-MV/R9869/2016|2016-11-23|H5N8
A/Bl H gull/NL-Slootdorp/16014102-002/2016|2016-11-11|H5N8
A/wild duck/Poland/82A/2016|2016-11-02|H5N8
A/Dk/NL-Rotterdam/16014008-001-005/2016|2016-11-10|H5N8
A/domestic duck/Germany-MV/R9784/2016|2016-11-21|H5N8
A/Ch/NL-Abbega/X16015736/2016|2016-12-12|H5N8
A/mallard/Republic of Georgia/13/2011|2011-11-26|H6N2

A/Gull1/NL-Marker Wadden/16014466-011/2016|2016-11-17|H5N8
A/P falcon/NL-Vrouwenpolder (Zeeland)/16015510-001/2016|2016-12-07|H5N8
A/Eur Wig/NL-Groningen/16015376-003/2016|2016-12-05|H5N8
A/Magpie/NL-Volendam/16014331-002/2016|2016-11-16|H5N8
A/mute swan/Kaliningrad/132/2017|2017-02-13|H5N8
A/Duck/Netherlands/17017237-001-005/2017|2017-12-07|H5N6
A/Mute Swan/Netherlands/17017377-001/2017|2017-12-09|H5N6
A/Tufted Duck/Netherlands/17017367-007/2017|2017-12-09|H5N6
A/pochard duck/England/AVP 18 003254/2018|2018-01-10|H5N6
A/Duck/Netherlands/17017236-001-005/2017|2017-12-07|H5N6
A/canada goose/England/AV5818OPpoolEP1/2018|2018-01-05|H5N6   Europe H5N6 2017-18 (B.N6.2)
A/Mute Swan/Netherlands/17017367-012/2017|2017-12-09|H5N6
A/common pochard/Germany-BY/AR09-L02421/2017|2017-12-28|H5N6
A/mute swan/England/AVP 18 001986/2017|2017-12-31|H5N6
A/Great Black-backed Gull/Netherlands/2/2017|2017-12-18|H5N6

A/chicken/Italy/17VIR1684-2/2017|2017-02-27|H5N8
A/Goose/Hungary/982/2017|2017-01-11|H5N8
A/Goose/Hungary/64909/2016|2016-12-14|H5N8
A/mute swan/Croatia/70/2016|2016-10-30|H5N8
A/chicken/Republic of Macedonia/AR1167-L02131/2017|2017-01-21|H5N8
A/duck/France/161108n/2016|2016-11-28|H5N8
A/Goose/Hungary/65817/2016|2016-12-18|H5N8
A/turkey/Germany-NI/R10523/2016|2016-12-13|H5N8   Qinghai/Uvs-like 2.3.4.4b
A/greylag goose/Germany-NI/AR1395-L02144/2017|2017-02-02|H5N8
A/chicken/Astrakhan/3131/2016|2016-12-13|H5N8
A/Brown-headed Gull/Qinghai/ZTO4-B/2016|2016-05-11|H5N8
A/great crested grebe/Tyva/34/2016|2016-05-25|H5N8
A/chicken/Croatia/104/2017|2017-03-07|H5N5
A/environment/Kamchatka/18/2016|2016-10-01|H5N5
A/duck/India/10CA01/2016|2016-10-17|H5N8
A/duck/Mongolia/996/2015|2015-08-31|H3N8
A/duck/Mongolia/66/2015|2015-08-30|H10N2
A/duck/Mongolia/520/2015|2015-08-31|H1N1
A/chicken/Kumamoto/1-7T/2016|2016-12-27|H5N6
A/duck/Korea/ES2/2016|2016-11-16|H5N6
A/northern shoveler/Georgia/1/2010|2010|H2N3
A/T Dk/NL-Werkendam/16014159-001/2016|2016-11-14|H5N5
A/greater white-fronted goose/Netherlands/1/2012|2012-11-20|H1N1
A/gadwall/Chany/97/2016|2016-09-10|H5N8
A/painted stork/India/10CA03/2016|2016-10-20|H5N8
A/turkey/Italy/17VIR538-1/2017|2017-01-20|H5N8
A/chicken/Korea/H903/2017|2017-02-08|H5N8   Chany/97-like 2.3.4.4b
A/mallard duck/Korea/WA137/2017|2017-01-24|H5N8
A/greyheron/W779/2017|2017-01-27|H5N8   Korea H5N8 2017
A/common teal/Korea/W547/2016|2016-12-15|H5N8
A/goose/Guangdong/04.23 DGQTXC237-O/2015|2015-04-23|H5N6
A/muscovy duck/Aomori/1-2T/2016|2016-11-28|H5N6
A/Mandarin duck/Korea/K16-187-3/2016|2016-10-28|H5N6   clade 2.3.4.4c
A/duck/Guangdong/01.01 SZSGXJK002-G/2016|2016-01-01|H5N6
A/environment/Guangdong/F3/2015|2015-12-29|H5N6
A/duck/Hunan/12.17 YYGK0056/2014|2014-12-17|H5N6
A/duck/Eastern China/L1021/2012|2012-10-21|H5N8
A/duck/Eastern China/S0215/2014|2014-02-15|H5N8
A/duck/Taiwan/A3400/2015|2015-02-08|H5N8
A/crane/Kagoshima/KU1/2014|2014-11-23|H5N8
A/tundra swan/Tottori/C6nk/2014|2014-11-18|H5N8   clade 2.3.4.4a
A/common teal/Korea/KU-12/2015|2015-01-22|H5N8
A/duck/Chiba/26-372-48/2014|2014-11-18|H5N8
A/goose/Eastern China/L1214/2012|2012-12-14|H5N8
A/goose/Guangdong/s13124/2013|2013-12-12|H5N8
A/duck/Wenzhou/YHQL22/2014|2014-01|H5N6
A/mallard duck/Shanghai/SH-9/2013|2013-11-18|H5N8   clade 2.3.4.4b (2013-2014)
A/duck/Eastern China/S1109/2014|2014-11-09|H5N8
A/breeder duck/Korea/Gochang1/2014|2014-01-16|H5N8
A/goose/Guangdong/04.22 DGCP120-P/2015|2015-04-22|H5N6
A/mute swan/Kyoto/8T/2016|2016-12-22|H5N6
A/duck/Hubei/ZYSYG18/2015|2015-11-21|H5N6
A/Hunan/55555/2016|2016-11-18|H5N6
A/duck/Guangdong/03.27 DGQTSJ121-P/2015|2015-03-27|H5N6
A/Shenzhen/TH001/2015|2015-12-29|H5N6
A/chicken/Taishun/TS2/2016|2016-02-19|H5N6

NL-cluster I 2.3.4.4b

NL-cluster II 2.3.4.4b

D

A/Mandarin duck/Korea/K17-1873/2017/2017-12-23/H5N6
A/Mandarin duck/Korea/K17-1879/2017/2017-12-23/H5N6
A/Mandarin duck/Korea/K17-1887/2017/2017-12-23/H5N6
A/Mandarin duck/Korea/K17-1881/2017/2017-12-23/H5N6
A/Mandarin duck/Korea/K17-1885/2017/2017-12-23/H5N6
A/Mandarin duck/Korea/K17-1894/2017/2017-12-23/H5N6
A/Mandarin duck/Korea/K17-1886/2017/2017-12-23/H5N6
A/Mandarin duck/Korea/K18-3/2018/2018-01-18/H5N6
A/Mandarin duck/Korea/K17-1815/2017/2017-12-22/H5N6
A/Mandarin duck/Korea/K17-1893/2017/2017-12-23/H5N6
A/Mandarin duck/Korea/K17-1828/2017/2017-12-22/H5N6
A/Mandarin duck/Korea/K17-1826/2017/2017-12-23/H5N6
A/Mandarin duck/Korea/K17-1889/2017/2017-12-23/H5N6
A/Mandarin duck/Korea/K17-1817/2017/2017-12-22/H5N6
A/Mallard/Korea/K17-1825/2017/2017-12-22/H5N6
A/Mandarin duck/Korea/K17-1882/2017/2017-12-23/H5N6
A/Mandarin duck/Korea/K17-1888/2017/2017-12-23/H5N6
A/Mandarin duck/Korea/K17-1891/2017/2017-12-23/H5N6
A/Mandarin duck/Korea/K17-1896/2017/2017-12-23/H5N6

Korea H5N6 2017-18 (B.N6.1)

A/common pochard/Germany-BY/AR09-L02421/2017/2017-12-28/H5N6
A/Great Black-backed Gull/Netherlands/1/2017/2017-12-18/H5N6
A/canada goose/England/AV58 18O Ppool EP1/2018/2018-01-05/H5N6
A/pochard duck/England/AVP 18 003254/2018/2018-01-10/H5N6
A/mute swan/England/AVP 18 001986/2017/2017-12-31/H5N6
A/Tufted Duck/Netherlands/17017367-007/2017/2017-12-09/H5N6
A/Duck/Netherlands/17017237-001-005/2017/2017-12-07/H5N6
A/Duck/Netherlands/17017236-001-005/2017/2017-12-07/H5N6
A/Great Black-backed Gull/Netherlands/2/2017/2017-12-18/H5N6
A/Mute Swan/Netherlands/17017367-012/2017/2017-12-08/H5N6
A/Mute Swan/Netherlands/17017377-001/2017/2017-12-09/H5N6

Europe H5N6 2017-18 (B.N6.1, B.N6.2)

A/eurasian wigeon/Germany-NI/AR249-L02143/2017/2017-01-04/H5N8
A/Eur Wig/NL-De Waal (Texel)/16014891-004/2016/2016-11-27/H5N8
A/Eur Wig/NL-Vianen/16015917-009/2016/2016-12-13/H5N8
A/Eur Wig/NL-Zwolle/16015820-002/2016/2016-12-13/H5N8
A/Eur Wig/NL-Zoeterwoude/16015702-010/2016/2016-12-10/H5N8
A/turkey/England/052131/2016/2016-12-15/H5N8
A/mute swan/Shimane/3211A001/2017/2017-11-05/H5N6
A/spoonbill/Taiwan/DB645/2017/2017-12-01/H5N6

Japan/Taiwan H5N6 2017 (B.N6.1)

A/chicken/SergiyevPosad/38/2017/2017-03-02/H5N8
A/swan/Italy/17VIR7084-1/2017/2017-08-25/H5N8
A/turkey/Italy/17VIR5878-3/2017/2017-07-23/H5N8
A/chicken/Italy/17VIR3078/2017/2017-04-07/H5N8
A/turkey/Rostov/1/2017/2017-01-01/H5N8
A/chicken/Astrakhan/3131/2016/2016-12-13/H5N8
A/chicken/Germany-NI/R11406/2016/2016-12-24/H5N8
A/Eur Wig/NL-Walterswald/16015923-003/2016/2016-12-14/H5N8
A/T Dk/NL-Zeewolde/16013676-001/2016/2016-11-09/H5N8
A/chicken/Greece/392017b/2017/2017-02-08/H5N6

Greece H5N6 2017 (B.N6.1)

A/chicken/Germany-MV/R10048/2016/2016-11-27/H5N8
A/Mal/NL-Mastenbroek/16015378-002/2016/2016-12-05/H5N8
A/C Gull/NL-Slootdorp/16014102-003/2016/2016-11-11/H5N8
A/mute swan/Germany-NI/AR1529-L02145/2017/2017-02-14/H5N8
A/turkey/Germany-NI/R9807/2016/2016-11-22/H5N8
A/Eur Wig/NL-Akkrum/16015817-003/2016/2016-12-13/H5N8
A/Eur Wig/NL-Groningen/16015376-003/2016/2016-12-05/H5N8
A/BIH gull/NL-Slootdorp/16014102-002/2016/2016-11-11/H5N8
A/Ch/NL-Abbega/X16015736/2016/2016-12-12/H5N8
A/Peregrine falcon/Hungary/4882/2017/2017-02-07/H5N8
A/Goose/Hungary/1030/2017/2017-01-11/H5N8
A/wild duck/Tatarstan/3059/2016/2016-10-02/H5N8
A/mute swan/Hungary/6092/2017/2017-02-15/H5N8
A/turkey/Germany-BB/R234ff/2017/2017-01-09/H5N8
A/mute swan/Hungary/5316/2017/2017-02-08/H5N8
A/Whitefronted goose/Hungary/801/2017/2017-01-09/H5N8
A/mute swan/Hungary/51049/2016/2016-10-19/H5N8
A/Mute swan/Hungary/3513/2017/2017-01-27/H5N8
A/Cormorant/Hungary/6102/2017/2017-02-15/H5N8
A/Cygnus olor/Belgium/1567/2017/2017-02-21/H5N8
A/Mute swan/Hungary/5879/2017/2017-02-14/H5N5
A/common buzzard/Germany-SN/R1117/2017/2017-02-08/H5N5
A/turkey/Germany-SH/R425/2017/2017-01-22/H5N5
A/grey heron/Germany-SN/R572/2017/2017-01-22/H5N5
A/environment/Kamchatka/18/2016/2016-10-01/H5N5
A/great crested grebe/Tyva/34/2016/2016-05-25/H5N8
A/Bar-headed Goose/Qinghai/BTY14-LU/2016/2016-05-14/H5N8
A/painted stork/India/10CA03/2016/2016-10-20/H5N8
A/turkey/Italy/17VIR538-1/2017/2017-01-20/H5N8
A/domestic duck/Siberia/103/2016/2016-10-04/H5N8
A/chicken/Korea/H903/2017/2017-02-08/H5N8
A/common teal/Korea/W547/2016/2016-12-15/H5N8
A/grey heron/W779/2017/2017-01-27/H5N8
A/mallard duck/Korea/WA 137/2017/2017-01-24/H5N8

Korea H5N8 2016-2017

A/duck/India/10CA01/2016/2016-10-17/H5N8
A/duck/Eastern China/S1109/2014/2014-11-09/H5N8
A/duck/Zhejiang/W24/2013/2013-11-14/H5N8
A/breeder duck/Korea/Gochang1/2014/2014-01-16/H5N8
A/Environment/Jiangsu/98335/2014/2014-10-28/H5N8
A/duck/Wenzhou/YHQL22/2014/2014-01/H5N6
A/duck/Jiangsu/k1203/2010/2010-12-05/H5N8
A/duck/Shandong/Q1/2013/2013-04-25/H5N8
A/goose/Guangdong/s13124/2013/2013-12-12/H5N8

A/Mandarin duck/Korea/K16-187-3/2016/2016-10-28/H5N6
A/duck/Korea/ES2/2016/2016-11-16/H5N6
A/muscovy duck/Aomori/1-7T/2016/2016-11-28/H5N6
A/duck/Guangdong/01.01 SZSGXJK002-G/2016/2016-01-01/H5N6
A/swine/Guangdong/1/2014/2014-05-18/H5N6
A/duck/Guangdong/GD01/2014/2014-03/H5N6
A/goose/Eastern China/L1214/2012/2012-12-14/H5N6

clade 2.3.4.4c

A/crane/Kagoshima/KU41/2014/2014-12-24/H5N8
A/goose/Taiwan/01019/2015/2015-01-11/H5N8
A/turkey/Iowa/14318-1/2015/2015-04-30/H5N2
A/baikal teal/Korea/1437/2014/2014-01-20/H5N8
A/domestic duck/Germany-NI/R3468/2014/2014-12-18/H5N8
A/duck/Chiba/26-372-48/2014/2014-11-18/H5N8
A/environment/Kagoshima/KU-ngr-H/2014/2014-12-01/H5N8

clade 2.3.4.4a

A/Sichuan/2621/2014/2014-04-21/H5N6
A/environment/Zhenjiang/C13/2013/2013-12-20/H5N6

clade 2.3.4.4d

A/Anhui/1/2005/2005/H5N1

Qinghai/Uvs-like

clade 2.3.4.4b

E

A/Mandarin duck/Korea/K17-1885/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1887/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1881/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1879/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1899/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1893/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1891/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1873/2017|2017-12-23|H5N6
93 ─ A/Mandarin duck/Korea/K17-1866/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1862/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1899/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1889/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1894/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K18-3/2018|2018-01-18|H5N6
86 ─ A/Mandarin duck/Korea/K17-1815/2017|2017-12-22|H5N6
A/Mandarin duck/Korea/K17-1817/2017|2017-12-22|H5N6
A/Mandarin duck/Korea/K17-1828/2017|2017-12-22|H5N6
A/Mandarin duck/Korea/K17-1826/2017|2017-12-22|H5N6
A/Mallard/Korea/K17-1825/2017|2017-12-22|H5N6

Korea H5N6 2017-18 (B.N6.1)

A/Great Black-backed Gull/Netherlands/1/2017|2017-12-18|H5N6
A/common pochard/Germany-BY/AR09-L02421/2017|2017-12-28|H5N6
A/pochard duck/England/AVP 003254/2018|2018-01-10|H5N6
A/mute swan/England/AVP 18 001986/2017|2017-12-31|H5N6
A/Great Black-backed Gull/Netherlands/2/2017|2017-12-18|H5N6
88 ─ A/Mute Swan/Netherlands/17017377-001/2017|2017-12-09|H5N6
A/canada goose/England/AV58 18OPpoolEP1/2018|2018-01-05|H5N6
A/Mute Swan/Netherlands/17017367-012/2017|2017-12-09|H5N6
A/Tufted Duck/Netherlands/17017367-007/2017|2017-12-09|H5N6
A/Duck/Netherlands/17017236-001-005/2017|2017-12-07|H5N6
A/Duck/Netherlands/17017237-001-005/2017|2017-12-07|H5N6

Europe H5N6 2017-18 (B.N6.1, B.N6.2)

Ger-11-16 2.3.4.4b

A/chicken/Wales/000023/2016|2016-12-31|H5N8
A/turkey/Rostov/11/2017|2017-01-04|H5N8
A/turkey/Italy/17VIR5878-3/2017|2017-07-23|H5N8
100 ─ A/mute swan/Krasnodar/25/2017|2017-01-06|H5N8
A/chicken/Tatarstan/88/2017|2017-05-08|H5N8
A/Ch/NL-Abbega/X16015736/2016|2016-12-12|H5N8
A/chicken/Belgium/807/2017|2017-02-01|H5N8
A/Eur Wig/NL-Drieborg (Dollard)/16015513-001/2016|2016-12-08|H5N8
A/Eur Wig/NL-Greonterp/16015663-001/2016|2016-12-08|H5N8
97 ─ A/mute swan/Shimane/3211A001/2017|2017-11-05|H5N6
A/spoonbill/Taiwan/DB845/2017|2017-12-01|H5N6

Japan/Taiwan H5N6 2017 (B.N6.1)

A/chicken/Greece/39.2017b/2017|2017-02-08|H5N6

Greece H5N6 2017 (B.N6.1)

A/Eur Wig/NL-Groningen/16015376-003/2016|2016-12-05|H5N8
A/wild duck/Poland/82A/2016|2016-11-02|H5N8
A/Gull/NL-Marker Wadden/16014466-011/2016|2016-11-17|H5N8
73 ─ A/tufted duck/Germany/AR8459-L01988/2016|2016-11-08|H5N8
A/turkey/Germany-SH/R8595/2016|2016-11-09|H5N8
A/Bl H gull/NL-Slootdorp/16014102-002/2016|2016-11-11|H5N8
A/Magpie/NL-Volendam/16014331-002/2016|2016-11-16|H5N8
A/duck/India/10CA01/2016|2016-10-17|H5N8
99 ─ A/mallard/Chany/185/2016|2016-09-10|H3N8
A/pintail/Chany/198/2016|2016-09-10|H3N8
91 ─ A/domestic duck/Germany-BB/R6811f/2017|2017-01-26|H5N8
100 ─ A/turkey/Germany-BB/R377ff/2017|2017-01-17|H5N8
A/Goose/Hungary/65817/2016|2016-12-18|H5N8
93 ─ A/mute swan/Croatia/70/2016|2016-10-30|H5N8
A/GuineaFowl/Hungary/596/2017|2017-01-06|H5N8
A/duck/France/161108n/2016|2016-11-28|H5N8

Ger-12-16 2.3.4.4b

A/mallard duck/Netherlands/32/2013|2013-08-05|H10N7
A/mallard duck/Netherlands/23/2012|2012-09-27|H11N9
A/turkey/Germany-SH/R425/2017|2017-01-22|H5N5
99 ─ A/cormorant/Germany-SH/R896/2017|2017-01-30|H5N5

Ger-12-16-N5 2.3.4.4b

87 ─ A/barnacle goose/Germany-SH/R11505/2018|2016-12-22|H5N5
100 ─ A/swan/Germany-SN/R10645/2016|2016-12-13|H5N5
83 ─ A/M Swan/NL-Groningen/16015826-001/2016|2016-12-13|H5N5

Ger-12-16-N5.1 2.3.4.4b

A/chicken/Astrakhan/3131/2016|2016-12-13|H5N8
100 ─ A/chicken/Taiwan/x37/2016|2016-12-21|H5N8
A/duck/Taiwan/A3400/2015|2015-02-08|H5N8
88 ─ A/goose/Taiwan/01019/2015|2015-01-11|H5N8
71 ─ A/duck/Jiangxi/01.14 NCJD034-P/2015|2015-01-14|H5N6
A/duck/Mongolia/208/2015|2015-08-31|H3N8
89 ─ A/duck/Mongolia/167/2015|2015-08-30|H3N8
100 ─ A/great crested grebe/Tyva/34/2016|2016-05-25|H5N8
A/Bar-headed Goose/Qinghai/BTY1-B/2016|2016-05-09|H5N8

Qinghai/Uvs-like 2.3.4.4b

99 ─ A/gadwall/Chany/97/2016|2016-09-10|H5N8
72 ─ A/painted stork/India/10CA03/2016|2016-10-20|H5N8

Chany/97-like 2.3.4.4b

A/Environment/Jiangxi/08488/2015|2015-11-19|H12N2
A/Anas platyrhynchos/Belgium/17330dis42/2013|2013-09-26|H9N2
96 ─ A/grey heron/W779/2017|2017-01-27|H5N8
A/chicken/Korea/H903/2017|2017-02-08|H5N8
100 ─ A/mallard duck/Korea/WA137/2017|2017-01-24|H5N8
98 ─ A/commonteal/Korea/W547/2016|2016-12-15|H5N8

Korea H5N8 2017 2.3.4.4b

A/goose/Zhejiang/925037/2014|2014-09-25|H5N8
70 ─ A/goose/Zhejiang/925106/2014|2014-09-25|H5N6
70 ─ A/duck/Zhejiang/925019/2014|2014-09-25|H5N6
70 ─ A/mallard duck/Shanghai/SH-9/2013|2013-11-18|H5N8
A/breeder duck/Korea/Gochang1/2014|2014-01-16|H5N8

2.3.4.4b (2013-2014)

74 ─ A/goose/Guangdong/s13124/2013|2013-12-12|H5N8
A/duck/Eastern China/L0405/2010|2010-04-05|H5N8
91 ─ A/duck/Beijing/FS01/2014|2014-01-20|H5N8
75 ─ A/goose/Hunan/HN325/2015|2015-12-18|H5N6
100 ─ A/chicken/Miyazaki/7/2014|2014-12-16|H5N8
70 ─ A/duck/Chiba/26-372-48/2014|2014-11-18|H5N8
99 ─ A/scarlet ibis/Germany/AR44-L01279/2015|2015-01|H5N8

2.3.4.4a

A/goose/Eastern China/S0513/2013|2013-05-13|H5N6
100 ─ A/Hubei/29578/2016|2016-04-15|H5N6
A/Shenzhen/TH001/2015|2015-12-29|H5N6
83 ─ A/feline/Guangdong/1/2014|2015-01-04|H5N6
A/swan goose/Jilin/JL01/2014|2014-06-01|H5N6
93 ─ A/duck/Jiangxi/NCDZT1123/2014|2014-06-29|H5N6
100 ─ A/chicken/Xuzhou/XZ9/2016|2016-02-13|H5N6
64 ─ A/duck/Guangdong/04.16 SZLGDBL003/2015|2015-04-16|H5N6
A/goose/Yunnan/07.14 DQWCK119/2015|2015-07-14|H5N6
A/duck/Jiangxi/04.01 NCDZT0312-O/2015|2015-04-01|H5N6
A/duck/Guangdong/04.22 DGCPLB018-O/2015|2015-04-22|H5N6
A/Pavo cristatus/Jiangxi/JA1/2016|2016-02-27|H5N6
A/Hunan/55555/2016|2016-11-18|H5N6
92 ─ A/Mandarin duck/Korea/K16-187-3/2016|2016-10-28|H5N6
A/chicken/Niigata/2-7T/2016|2016-11-30|H5N6
99 ─ A/duck/Korea/ES2/2016|2016-11-16|H5N6

Asia H5N6

F

A/Mandarin duck/Korea/K17-1891/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K18-3/2018|2018-01-18|H5N6
A/Mandarin duck/Korea/K17-1869/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1866/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1881/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1894/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1873/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1893/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1896/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1889/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1887/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1879/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1885/2017|2017-12-23|H5N6
A/Mandarin duck/Korea/K17-1815/2017|2017-12-22|H5N6
A/Mandarin duck/Korea/K17-1826/2017|2017-12-22|H5N6
A/Mallard/Korea/K17-1825/2017|2017-12-22|H5N6
A/Mandarin duck/Korea/K17-1817/2017|2017-12-22|H5N6
A/Mandarin duck/Korea/K17-1828/2017|2017-12-22|H5N6
A/Mandarin duck/Korea/K17-1862/2017|2017-12-23|H5N6

Korea H5N6 2017-18 (B.N6.1)

A/common pochard/Germany-BY/AR09-L02421/2017|2017-12-28|H5N6
A/Great Black-backed Gull/Netherlands/1/2017|2017-12-18|H5N6
A/Duck/Netherlands/17017236-001-005/2017|2017-12-07|H5N6
A/Duck/Netherlands/17017237-001-005/2017|2017-12-07|H5N6
A/Great Black-backed Gull/Netherlands/2/2017|2017-12-18|H5N6
A/canada goose/England/AV5818OPpoolEP1/2018|2018-01-05|H5N6
A/Tufted Duck/Netherlands/17017367-007/2017|2017-12-09|H5N6
A/mute swan/England/AVP 18 001986/2017|2017-12-31|H5N6
A/pochard duck/England/AVP18 003254/2018|2018-01-10|H5N6
A/Mute Swan/Netherlands/17017367-012/2017|2017-12-09|H5N6
A/Mute Swan/Netherlands/17017377-001/2017|2017-12-09|H5N6

Europe H5N6 2017-18 (B.N6.1, B.N6.2)

A/chicken/Greece/39 2017b/2017|2017-02-06|H5N6 **Greece H5N6 2017 (B.N6.1)**

A/mute swan/Shimane/3211A001/2017|2017-11-05|H5N6
A/spoonbill/Taiwan/DB645/2017|2017-12-01|H5N6 **Japan/Taiwan H5N6 2017 (B.N6.1)**

clade 2.3.4.4b H5N6

A/barnacle goose/Netherlands/2/2014|2014-12-15|H3N6
A/duck/Moscow/4652/2011|2011-10|H4N6
A/mallard duck/Netherlands/19/2012|2012-09-07|H4N6
A/mallard duck/Netherlands/30/2014|2014-07-21|H4N6
A/duck/Mongolia/262/2011|2011-09-01|H4N6
A/teal/Chany Lake/106 cloaca/2012|2012-08-26|H4N6
A/duck/Yunnan/87/2007|2007|H7N6
A/eurasian wigeon/Mongolia/340V/2009|2009-08-14|H4N6
A/duck/Jiangsu/4/2010|2010-03|H3N6
A/duck/Henan/S1091/2010|2010-03-03|H4N6
A/chicken/Hunan/S1267/2010|2010-03-03|H4N6

A/duck/Vietnam/LBM360c1-4-1/2013|2013-02-06|H5N6
A/duck/Wenzhou/YHQL22/2014|2014-01|H5N6
A/duck/Hubei/03.06WHWTZ0049-P/2015|2015-03-06|H5N6 **Clade 2.3.4.4d**

A/environment/Guangdong/GZ693/2015|2015-11-18|H5N6
A/chicken/Niigata/1-4C/2016|2016-11-28|H5N6
A/feline/Guangdong/1/2014(H5N6)|2015-01-04|H5N6
A/muscovy/duck/Hunan/HN353/2015|2015-12-19|H5N6
A/environment/Jilin/CC02/2014|2014-06-19|H5N6
A/Guangdong/SZ872/2015|2015-12-28|H5N6 **clade 2.3.4.4c**

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Korea H5N6 2017-18 (B.N6.1)
- A/Mandarin duck/Korea/K17-1891/2017|2017-12-23|H5N6
- A/Mandarin duck/Korea/K17-1894/2017|2017-12-23|H5N6
- A/Mandarin duck/Korea/K17-1869/2017|2017-12-23|H5N6
- A/Mandarin duck/Korea/K18-3/2018|2018-01-18|H5N6
- A/Mandarin duck/Korea/K17-1896/2017|2017-12-23|H5N6
- A/Mandarin duck/Korea/K17-1893/2017|2017-12-23|H5N6
- A/Mandarin duck/Korea/K17-1889/2017|2017-12-23|H5N6
- A/Mandarin duck/Korea/K17-1887/2017|2017-12-23|H5N6
- A/Mandarin duck/Korea/K17-1885/2017|2017-12-23|H5N6
- A/Mandarin duck/Korea/K17-1881/2017|2017-12-23|H5N6
- A/Mandarin duck/Korea/K17-1879/2017|2017-12-23|H5N6
- A/Mandarin duck/Korea/K17-1873/2017|2017-12-23|H5N6
- A/Mandarin duck/Korea/K17-1815/2017|2017-12-22|H5N6
- A/Mandarin duck/Korea/K17-1817/2017|2017-12-22|H5N6
- A/Mallard/Korea/K17-1825/2017|2017-12-22|H5N6
- A/Mandarin duck/Korea/K17-1826/2017|2017-12-22|H5N6
- A/Mandarin duck/Korea/K17-1828/2017|2017-12-22|H5N6
- A/Mandarin duck/Korea/K17-1862/2017|2017-12-23|H5N6
- A/Mandarin duck/Korea/K17-1866/2017|2017-12-23|H5N6

98

Europe H5N6 2017-18 (B.N6.1, B.N6.2)
- A/Great Black-backed Gull/Netherlands/1/2017|2017-12-18|H5N6
- A/Duck/Netherlands/17017237-001-005/2017|2017-12-07|H5N6
- A/Great Black-backed Gull/Netherlands/2/2017|2017-12-18|H5N6
- A/mute swan/England/AVP 18 001986/2017|2017-12-31|H5N6
- A/pochard duck/England/AVP 18 003254/2018|2018-01-10|H5N6
- A/canada goose/England/AV5818OPpoolEP1/2018|2018-01-05|H5N6
- A/Duck/Netherlands/17017236-001-005/2017|2017-12-07|H5N6
- A/Mute Swan/Netherlands/17017367-012/2017|2017-12-09|H5N6
- A/Mute Swan/Netherlands/17017377-001/2017|2017-12-09|H5N6
- A/Tufted Duck/Netherlands/17017367-007/2017|2017-12-09|H5N6
- A/common pochard/Germany-BY/AR09-L02421/2017|2017-12-28|H5N6

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85

- A/duck/Hungary/55191/2016|2016-11-11|H5N8
- A/Pheasant/Hungary/6553/2017|2017-02-16|H5N8
- A/shelduck/Italy/17VIR1572-24/2017|2017-01-03|H5N8
- A/domestic goose/Germany-BY/R677/2017|2017-01-25|H5N8
- A/Mute swan/Hungary/5316/2017|2017-02-09|H5N8
- A/greylag goose/Germany-NI/AR1395-L02144/2017|2017-02-02|H5N8
- A/chicken/Czech Republic/206-17 2/2017|2017-01-06|H5N8
- A/mute swan/Czech Republic/1058-17/2017|2017-01-18|H5N8

Japan/Taiwan H5N6 2017 (B.N6.1)
- 99 A/mute swan/Shimane/3211A001/2017|2017-11-05|H5N6
- A/spoonbill/Taiwan/DB645/2017|2017-12-01|H5N6

- A/Ch/NL-Abbega/X16015736/2016|2016-12-12|H5N8
- A/Greylag goose/Hungary/320/2017|2017-01-04|H5N8
- A/swan/Italy/17VIR537-2/2017|2017-01-19|H5N8

Greece H5N6 2017 (B.N6.1)
- A/chicken/Greece/39 2017b/2017|2017-02-06|H5N6

- A/tufted duck/Germany-SH/R8444/2016|2016-11-07|H5N8
- A/long-eared owl/Voronezh/15/2017|2017-01-06|H5N8
- A/chicken/Rostov/44/2017|2017-04-18|H5N8
- A/chicken/Belgium/807/2017|2017-02-01|H5N8
- A/chicken/Sergiyev Posad/38/2017|2017-03-02|H5N8
- A/swan/Italy/17VIR7064-1/2017|2017-08-25|H5N8
- A/chicken/Astrakhan/3131/2016|2016-12-13|H5N8
- 72 A/great crested grebe/Uvs-Nuur Lake/341/2016|2016-05-25|H5N8
- A/duck/India/10CA01/2016|2016-10-17|H5N8
- A/chicken/Korea/H903/2017|2017-02-08|H5N8
- 65 A/painted stork/India/10CA03/2016|2016-10-20|H5N8
- 92 94 A/gadwall/Chany/97/2016|2016-09-10|H5N8
- A/grey heron/W779/2017|2017-01-27|H5N8
- 99 A/cormorant/Germany-SH/R896/2017|2017-01-30|H5N5
- A/great crested grebe/Tyva/34/2016|2016-05-25|H5N8
- A/Bar-headed Goose/Qinghai/BTY1-B/2016|2016-05-09|H5N8

Qinghai/Uvs-like 2.3.4.4b

- A/duck/Mongolia/179/2015|2015-08-30|H3N8

2.3.4.4c
- 98 A/chicken/Niigata/1-4C/2016|2016-11-28|H5N6
- A/muscovy duck/Aomori/1-1T/2016|2016-11-28|H5N6
- A/duck/Hunan/02.07 YYFQH015-P/2015|2015-02-07|H5N6
- A/wild pigeon/Jilin/CC01/2014|2014-06-19|H5N6
- A/duck/Guangdong/03.27 DGQTSJ131-O/2015|2015-03-27|H5N6
- 99 A/Gallinula chloropus/Guangdong/GZ174/2014|2014-09-08|H5N6

2.3.4.4d
- A/environment/Zhenjiang/C13/2013|2013-12-20|H5N6
- 99 A/goose/SiChuan/15/2015|2015-04-07|H5N6

- A/chicken/Guangdong/GD1602/2016|2016-03-22|H5N6

2.3.4.4a
- 99 A/crane/Kagoshima/KU21/2014|2014-12-17|H5N8
- 94 A/mallard/Korea/H2102/2015|2015-01-14|H5N8
- 100 A/duck/Chiba/26-372-48/2014|2014-11-18|H5N8
- A/duck/Beijing/FS01/2014|2014-01-20|H5N8

2.3.4.4b East Asia 2013-14
- 86 A/duck/Eastern China/L0405/2010|2010-04-05|H5N8
- A/goose/Guangdong/s13124/2013|2013-12-12|H5N8
- A/mallard duck/Shanghai/SH-9/2013|2013-11-18|H5N8
- 91 A/breeder duck/Korea/Gochang1/2014|2014-01-16|H5N8
- 98 A/goose/Zhejiang/925106/2014|2014-09-25|H5N6
- 77 A/duck/Eastern China/S1109/2014|2014-11-09|H5N8

- A/environment/Guangdong/04.16SZLGWLShui9/2015|2015-04-16|H5N6

China H9N2
- A/chicken/Sichuan/SIC36/2014|2014-12|H9N2
- 100 A/pigeon/Zhejiang/77037/2014|2014-07-07|H9N2
- A/Phalacrocorax/Hubei/01.09 V5/2015|2015-01-09|H5N6
- A/environment/Jiangxi/05.07 NCJD0070C/2015|2015-05-07|H5N6
- 83 A/Environment/Guangdong/40113/2015|2015-05-11|H5N6
- A/Shenzhen/TH001/2015|2015-12-29|H5N6

Technical Appendix 1 Figure 2. Maximum-likelihood phylogenetic tree of the PB2, PB1, PA, HA, NP, NA, M, and NS genes. The new subgroup B H5N6 isolates are colored according to the isolated location (South Korea, red; Europe, blue; Japan/Taiwan, green; Greece, purple). The percentages of replicate trees (>70%) in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches. Each cluster is labeled according to classification of Pohlmann et al. (*5*) and Beerens et al. (*6*).HA, hemagglutinin; M, matrix; NA, neuraminidase; NP, nucleoprotein; NS, nonstructural; PA, polymerase acidic; PB, polymerase basic. Scale bars indicate nucleotide substitutions per site.
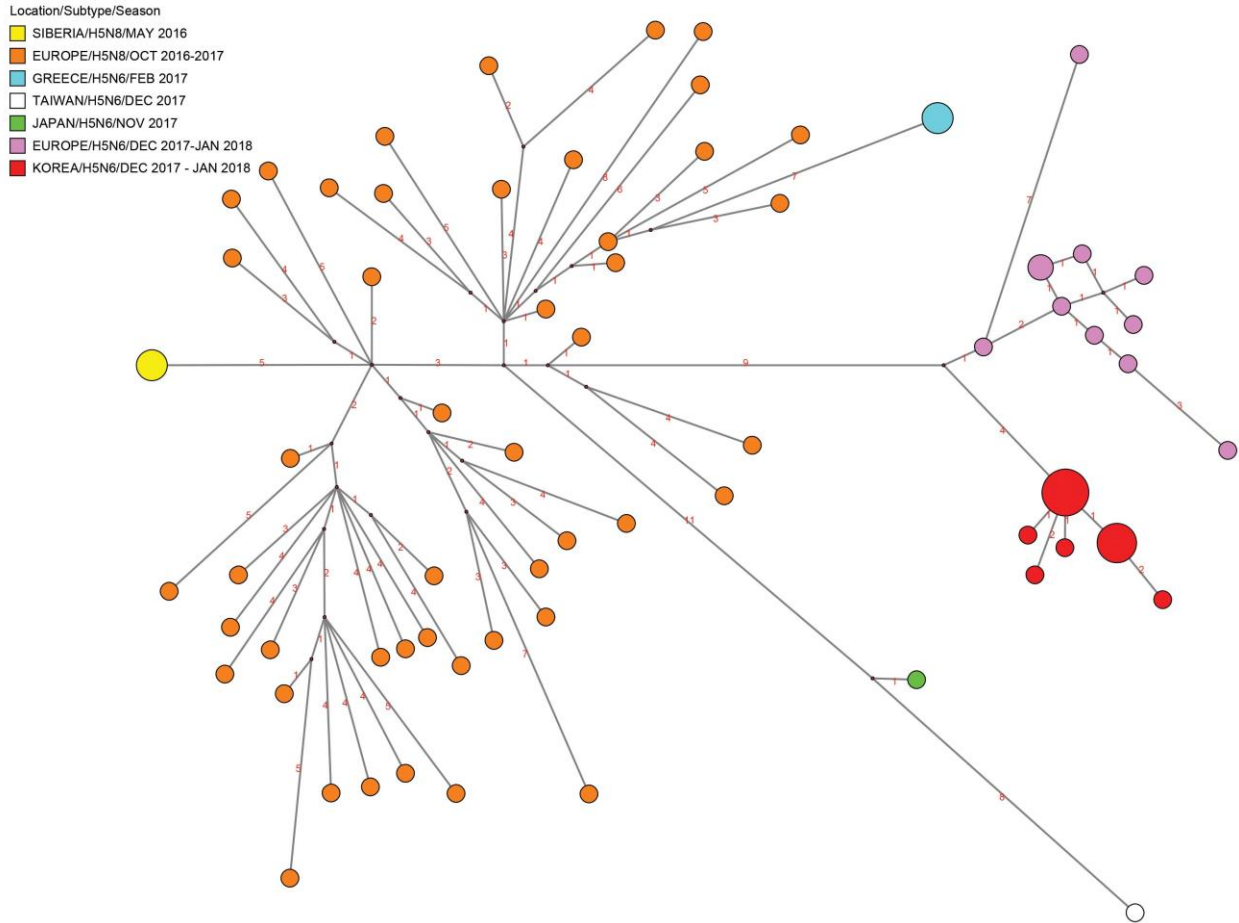
Technical Appendix 1 Figure 3. Median-joining phylogenetic network of subgroup B H5 highly pathogenic avian influenza viruses. The median-joining network was constructed from the HA gene. It includes all the most parsimonious trees linking the sequences. Each unique sequence is represented by a circle sized relative to its frequency in the dataset. Branch length is proportional to the number of mutations. Isolates are colored according to the location. HA, hemagglutinin.
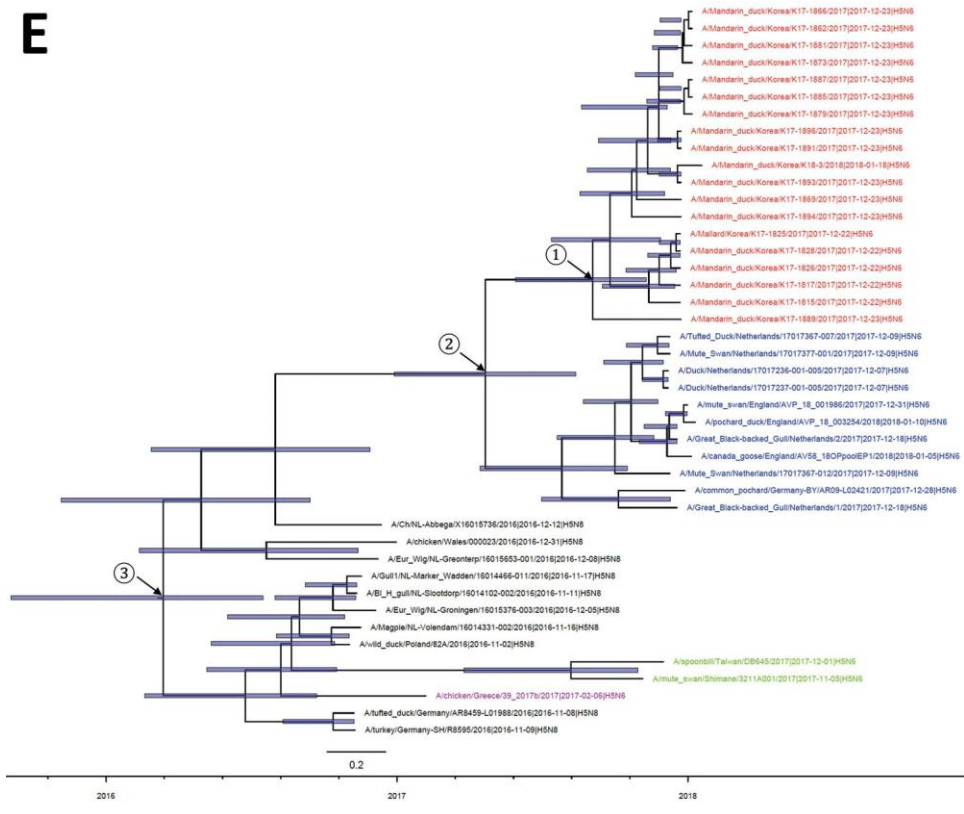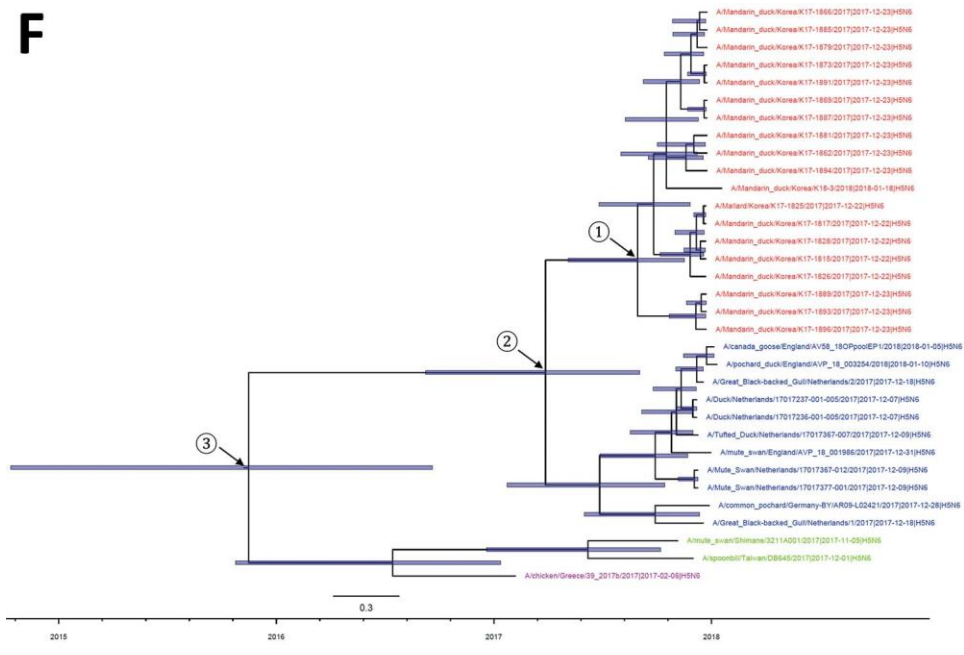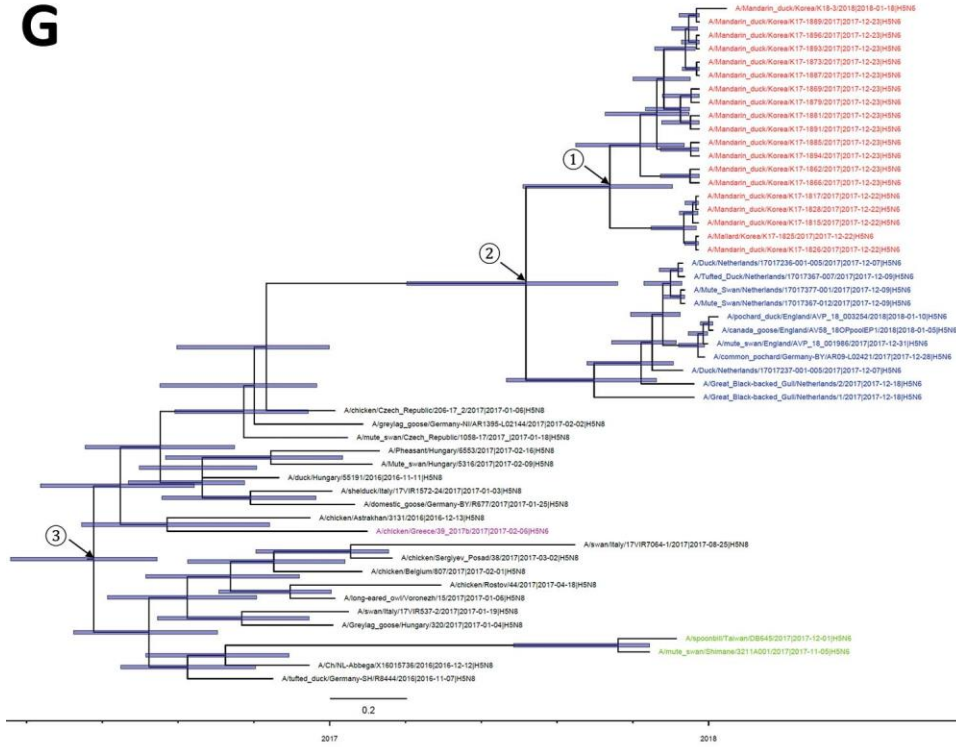
C

A/Mandarin_duck/Korea/K17-1826/2017|2017-12-22|H5N6
A/Mandarin_duck/Korea/K17-1887/2017|2017-12-23|H5N6
A/Mandarin_duck/Korea/K17-1828/2017|2017-12-22|H5N6
A/Mandarin_duck/Korea/K17-1873/2017|2017-12-23|H5N6
A/Mandarin_duck/Korea/K17-1881/2017|2017-12-23|H5N6
A/Mallard/Korea/K17-1825/2017|2017-12-22|H5N6
A/Mandarin_duck/Korea/K17-1817/2017|2017-12-22|H5N6
A/Mandarin_duck/Korea/K17-1885/2017|2017-12-23|H5N6
A/Mandarin_duck/Korea/K17-1879/2017|2017-12-23|H5N6
A/Mandarin_duck/Korea/K17-1815/2017|2017-12-22|H5N6
A/Mandarin_duck/Korea/K17-1894/2017|2017-12-23|H5N6
A/Mandarin_duck/Korea/K17-1862/2017|2017-12-23|H5N6
A/Mandarin_duck/Korea/K17-1866/2017|2017-12-23|H5N6
A/Mandarin_duck/Korea/K17-1869/2017|2017-12-23|H5N6
A/Mandarin_duck/Korea/K17-1889/2017|2017-12-23|H5N6
A/Mandarin_duck/Korea/K17-1891/2017|2017-12-23|H5N6
A/Mandarin_duck/Korea/K18-3/2018|2018-01-18|H5N6
A/Mandarin_duck/Korea/K17-1893/2017|2017-12-23|H5N6
A/Mandarin_duck/Korea/K17-1896/2017|2017-12-23|H5N6
A/Great-backed_Gull/Netherlands/1/2017|2017-12-18|H5N6
A/mute_swan/Shimane/3211A001/2017|2017-11-05|H5N6
A/spoonbill/Taiwan/DB645/2017|2017-12-01|H5N6
A/turkey/Italy/17VIR5878-3/2017|2017-07-23|H5N8
A/chicken/Greece/39_2017b/2017|2017-02-06|H5N6
A/Ch/NL-Zoeterwoude/16016484-021-025/2016|2016-12-24|H5N8
A/eurasian_wigeon/Germany-NI/AR249-L02143/2017|2017-01-04|H5N8
A/turkey/England/052131/2016|2016-12-15|H5N8
A/Ch/NL-Abbega/X16015736/2016|2016-12-12|H5N8

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A/Mandarin_duck/Korea/K17-1826/2017|2017-12-22|H5N6
A/Mandarin_duck/Korea/K17-1817/2017|2017-12-22|H5N6
A/Mandarin_duck/Korea/K17-1891/2017|2017-12-23|H5N6
A/Mandarin_duck/Korea/K17-1869/2017|2017-12-23|H5N6
A/Mandarin_duck/Korea/K17-1893/2017|2017-12-23|H5N6
A/Mandarin_duck/Korea/K17-1889/2017|2017-12-23|H5N6
A/Mandarin_duck/Korea/K17-1862/2017|2017-12-23|H5N6
A/Mallard/Korea/K17-1825/2017|2017-12-22|H5N6
A/Mandarin_duck/Korea/K17-1828/2017|2017-12-22|H5N6
A/Mandarin_duck/Korea/K17-1896/2017|2017-12-23|H5N6
A/Mandarin_duck/Korea/K17-1866/2017|2017-12-23|H5N6
A/Mandarin_duck/Korea/K17-1815/2017|2017-12-22|H5N6
A/Mandarin_duck/Korea/K18-3/2018|2018-01-18|H5N6
A/Mandarin_duck/Korea/K17-1879/2017|2017-12-23|H5N6
A/Mandarin_duck/Korea/K17-1894/2017|2017-12-23|H5N6
A/Mandarin_duck/Korea/K17-1885/2017|2017-12-23|H5N6
A/Mandarin_duck/Korea/K17-1887/2017|2017-12-23|H5N6
A/Mandarin_duck/Korea/K17-1873/2017|2017-12-23|H5N6
A/Mandarin_duck/Korea/K17-1881/2017|2017-12-23|H5N6
A/pochard_duck/England/AVP_18_003254/2018|2018-01-10|H5N6
A/canada_goose/England/AV58_18OPpoolEP1/2018|2018-01-05|H5N6
A/mute_swan/England/AVP_18_001986/2017|2017-12-31|H5N6
A/Duck/Netherlands/17017237-001-005/2017|2017-12-07|H5N6
A/Duck/Netherlands/17017236-001-005/2017|2017-12-07|H5N6
A/Tufted_Duck/Netherlands/17017367-007/2017|2017-12-09|H5N6
A/Mute_Swan/Netherlands/17017377-001/2017|2017-12-09|H5N6
A/Mute_Swan/Netherlands/17017367-012/2017|2017-12-09|H5N6
A/Great-backed_Gull/Netherlands/2/2017|2017-12-18|H5N6
A/Great-backed_Gull/Netherlands/1/2017|2017-12-18|H5N6
A/common_pochards/Germany-BY/AR09-L02421/2017|2017-12-28|H5N6
A/eurasian_wigeon/Germany-NI/AR249-L02143/2017|2017-01-04|H5N8
A/Eur_Wig/NL-Vlanen/16015917-006/2016|2016-12-13|H5N8
A/Eur_Wig/NL-De_Waal_(Texel)/16014891-004/2016|2016-11-27|H5N8
A/Eur_Wig/NL-Zwolle/16015820-002/2016|2016-12-13|H5N8
A/turkey/England/052131/2016|2016-12-15|H5N8
A/Eur_Wig/NL-Zoeterwoude/16015702-010/2016|2016-12-10|H5N8
A/mute_swan/Shimane/3211A001/2017|2017-11-05|H5N6
A/spoonbill/Taiwan/DB645/2017|2017-12-01|H5N6
A/Peregrine_falcon/Hungary/4882/2017|2017-02-07|H5N8
A/Goose/Hungary/1030/2017|2017-01-11|H5N8
A/Eur_Wig/NL-Groningen/16015376-003/2016|2016-12-05|H5N8
A/Eur_Wig/NL-Akkrum/16015817-003/2016|2016-12-13|H5N8
A/chicken/Germany-MV/R10048/2016|2016-11-27|H5N8
A/turkey/Germany-NI/R9807/2016|2016-11-22|H5N8
A/C_Gull/NL-Slootdorp/16014102-003/2016|2016-11-11|H5N8
A/mute_swan/Germany-NI/AR1529-L02145/2017|2017-02-14|H5N8
A/Mall/NL-Mastenbroek/16015378-002/2016|2016-12-05|H5N8
A/Bl_H_gull/NL-Slootdorp/16014102-002/2016|2016-11-11|H5N8
A/T_Dk/NL-Zeewolde/16013976-001/2016|2016-11-09|H5N8
A/chicken/Greece/39_2017b/2017|2017-02-06|H5N6
A/chicken/Italy/17VIR3078/2017|2017-04-07|H5N8
A/turkey/Italy/17VIR5878-3/2017|2017-07-23|H5N8
A/swan/Italy/17VIR7064-1/2017|2017-08-25|H5N8
A/chicken/Sergjyev_Posad/38/2017|2017-03-02|H5N8
A/chicken/Germany-NI/R11406/2016|2016-12-24|H5N8
A/chicken/Astrakhan/3131/2016|2016-12-13|H5N8
A/turkey/Rostov/1/2017|2017-01-01|H5N8
A/Eur_Wig/NL-Walterswald/16015923-003/2016|2016-12-14|H5N8
A/Ch/NL-Abbega/X16015736/2016|2016-12-12|H5N8

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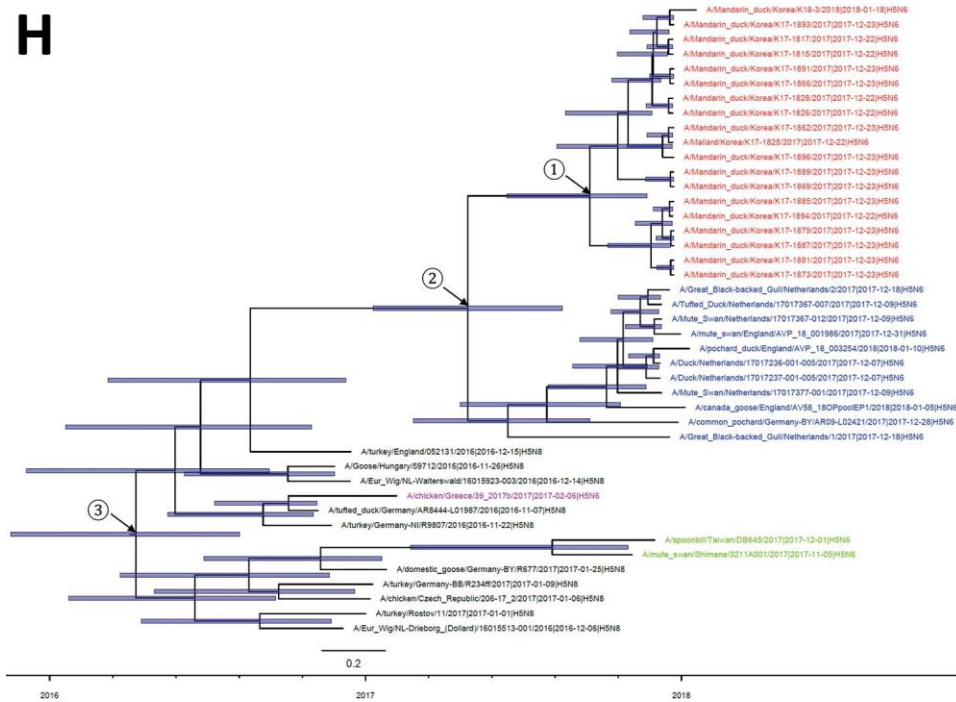
Technical Appendix 1 Figure 4. Temporally structured maximum clade credibility phylogenetic tree (years on the horizontal axis) of the PB2, PB1, PA, HA, NP, NA, M, and NS genes of subgroup B HPAIV. The new subgroup B H5N6 Isolates are colored according to the isolated location (South Korea, red;

Europe, blue; Japan/Taiwan, green; and Greece, purple). The horizontal bars indicate the 95% highest posterior density (HPD) intervals of the most recent common ancestor. HA, hemagglutinin; HPD, highest posterior density; M, matrix; NA, neuraminidase; NP, nucleoprotein; NS, nonstructural; PA, polymerase acidic; PB, polymerase basic. Scale bars indicate nucleotide substitutions per site.