Article DOI: https://doi.org/10.3201/eid2404.172124

Novel Highly Pathogenic Avian Influenza A(H5N6) Virus in the Netherlands, December 2017

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Technical Appendix

Technical Appendix Table 1. Calculated time to the most recent common ancestor for selected segments of avian influenza (H5N6) virus with 95% credible interval and posterior value*

Segment	Node†	tMRCA	95% HPD		Posterior value
			Earliest date	Latest date	
NA	1	2013 Sep 07	2012 Jun 04	2014 Oct 01	0.9965
	2	2015 Nov 17	2014 Dec 10	2016 Jul 14	0.9989
	3	2016 May 24	2015 Aug 16	2016 Nov 23	0.9868
	4	2017 Oct 16	2017 Jul 07	2017 Dec 06	0.9988
HA	1	2016 Jun 01	2016 Jan 15	2016 Sep 24	0.9994
	2	2016 Jun 22	2016 Feb 20	2016 Oct 07	0.2298
	3	2016 Oct 08	2016 Jul 19	2016 Nov 22	0.9997
	4	2017 Jul 26	2017 Feb 26	2017 Oct 31	0.9950
	5	2017 Sep 14	2017 Jun 04	2017 Nov 20	0.9994

*HPD, highest posterior density interval; tMRCA, time to most recent common ancestor (median).
†Nodes of the time-scaled phylogenetic tree.

Technical Appendix Table 2. Analysis of sequence features in influenza viruses associated with increased human risk*

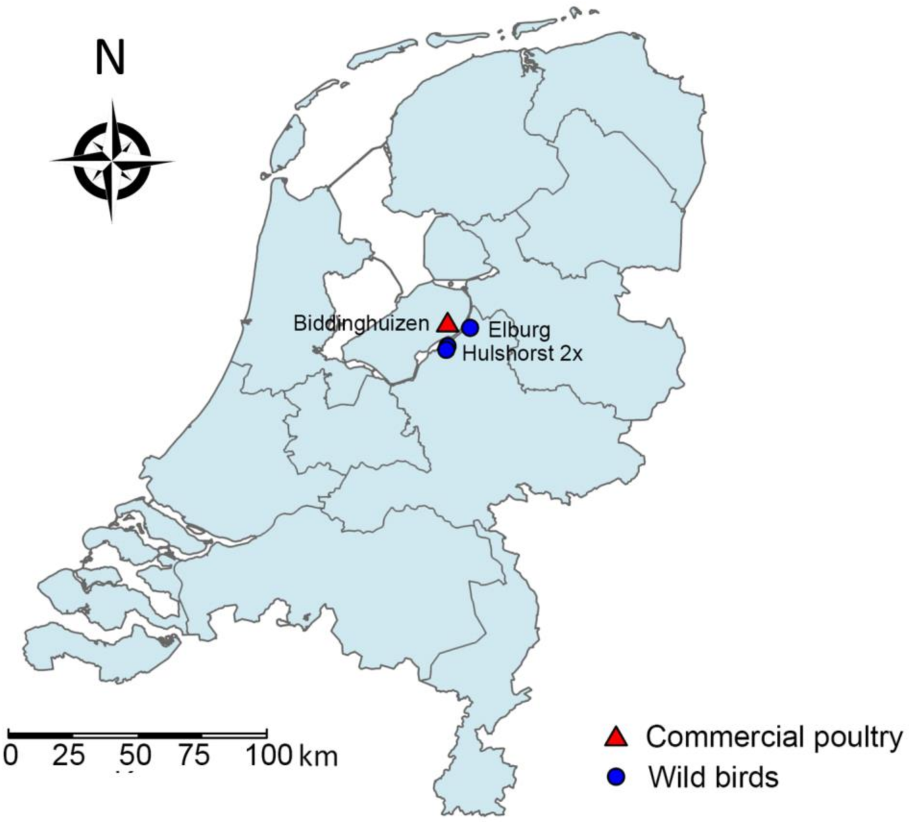
Protein	Position	Present	Increased
PB2	D256G	No	Replication
	Q591K	No	Replication
	E627K	No	Virulence
	D701N	No	Replication
	S714R	No	Replication
PB1†	V3A	No	Virulence
	H99Y	No	Airborne transmission
	K207R	No	Virulence
	N328K	No	Virulence
	I368V	No	Airborne transmission
	N375S	No	Virulence
PA	T85I	No	Replication
	T97I	No	Virulence
	G186M	No	Replication
	L336S	No	Replication
	S409N	No	Human transmission
	K615N	No	Replication
	G631S	No	Virulence
H5	Various residues	No	Binding to α2–6
	119Y, 172A, 238L, 240S	No	Airborne transmission
	450K, 459K, 403I	No	Acid stability
NP	R99K	No	Airborne transmission
	N319K	No	Replication
	S345N	No	Airborne transmission
	Q357K	No	Virulence
N6	V116A	No	Resistance to oseltamivir, zanamivir
	E119V	No	Resistance to oseltamivir, zanamivir
	Q136K	No	Resistance to zanamivir
	D199N	Yes	Resistance to oseltamivir

Protein	Position	Present	Increased
	H275Y	No	Resistance to oseltamivir, zanamivir
M1	N30D	Yes‡	Virulence
	T139A	No	Virulence
	Y215A	Yes‡	Virulence
NS1	P42S	Yes‡	Virulence
	D92E	No	Virulence
	L103F	Yes‡	Virulence
	I106M	Yes‡	Virulence
	80–84	No	Virulence

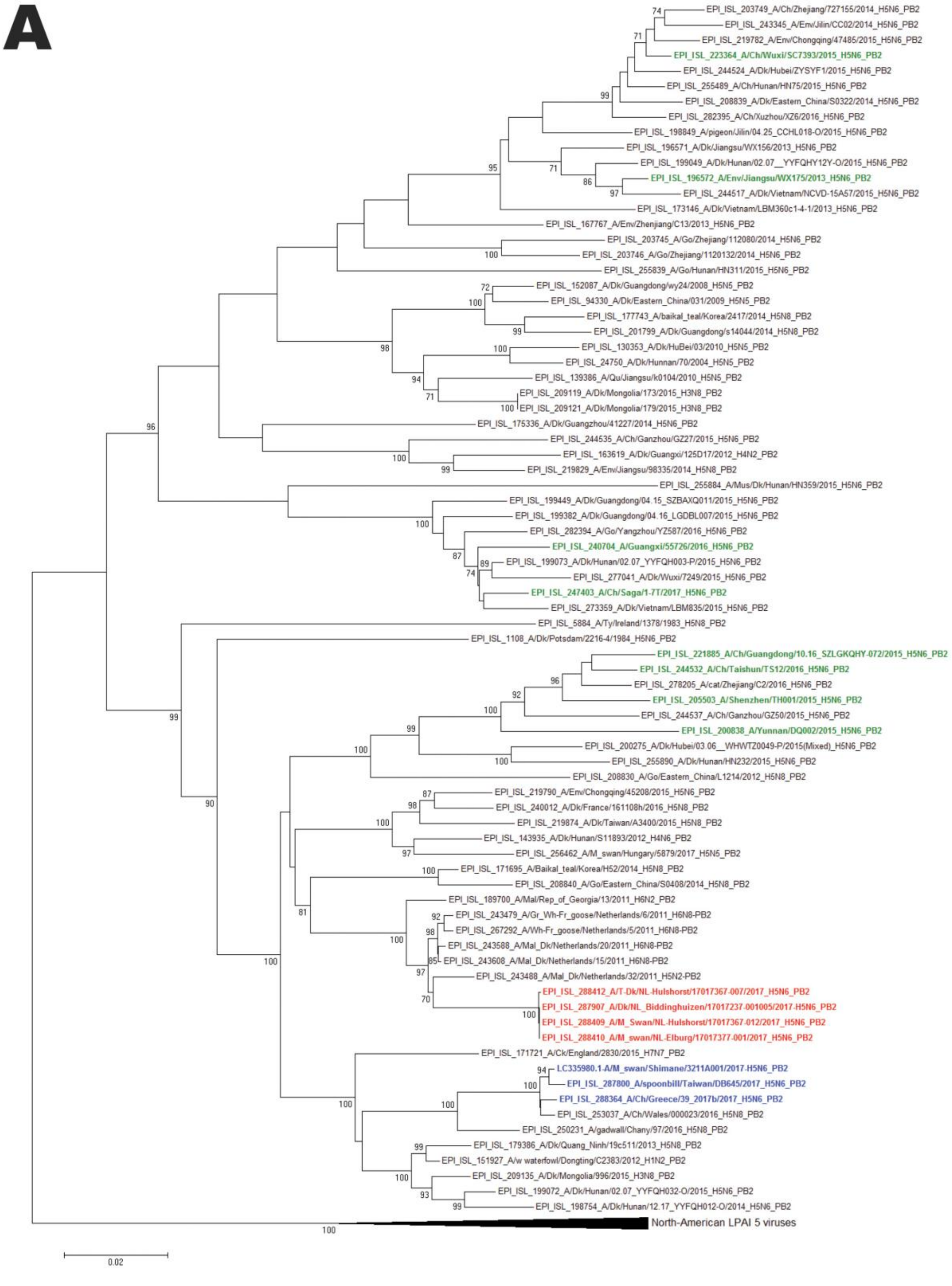
*PB, polymerase basic; PA, polymerase; HA, hemagglutinin; NA, neuraminidase; M, matrix; NP, nucleoprotein, NS, nonstructural protein.

†PB1-F2 truncation amino acids 12–90

‡Sequence features of H5 Clade 2.3.4.4 viruses



Technical Appendix Figure 1. Map of the Netherlands, showing locations of the duck farm (red) and the dead wild birds infected with HPAI H5N6 (blue).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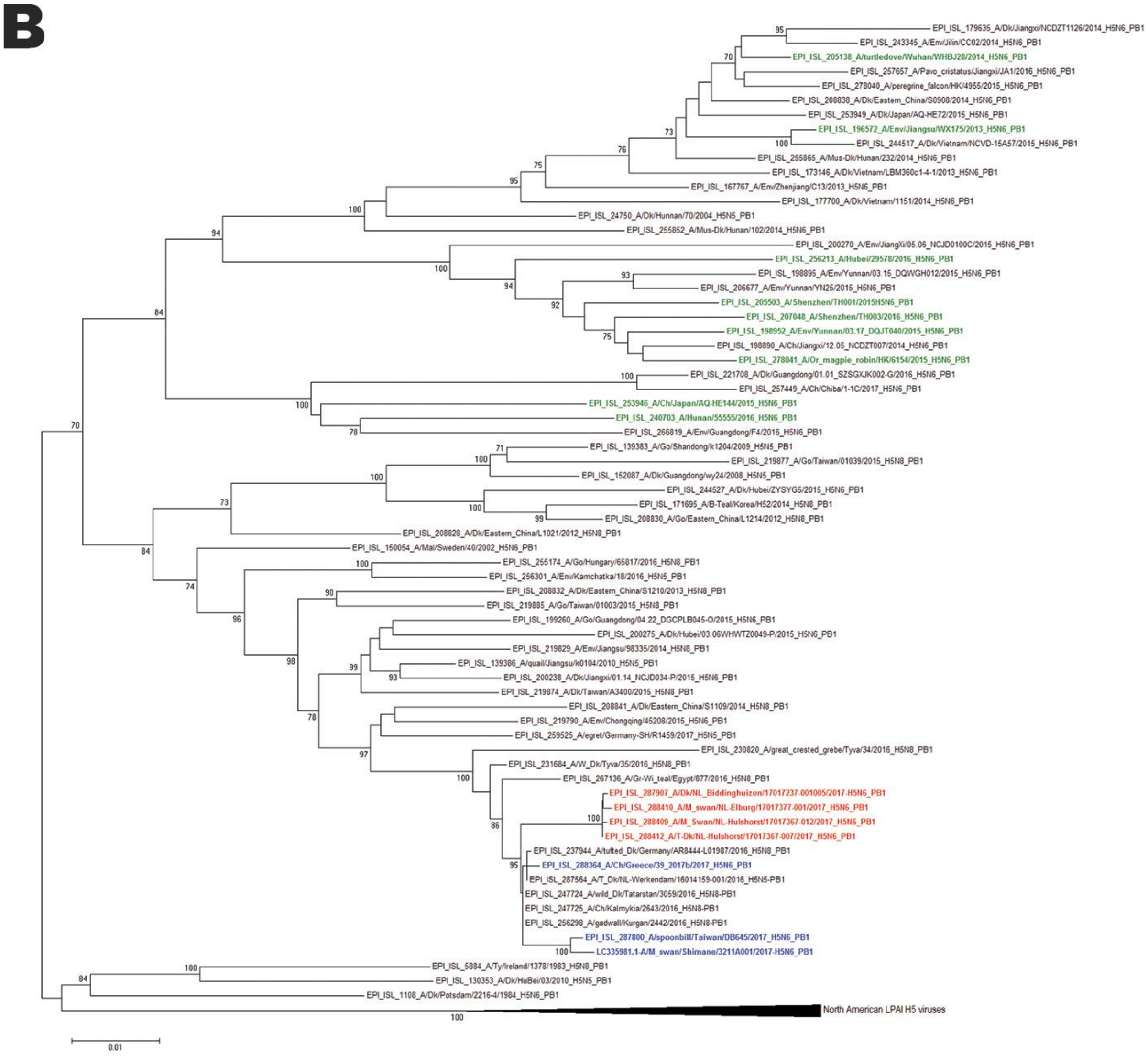
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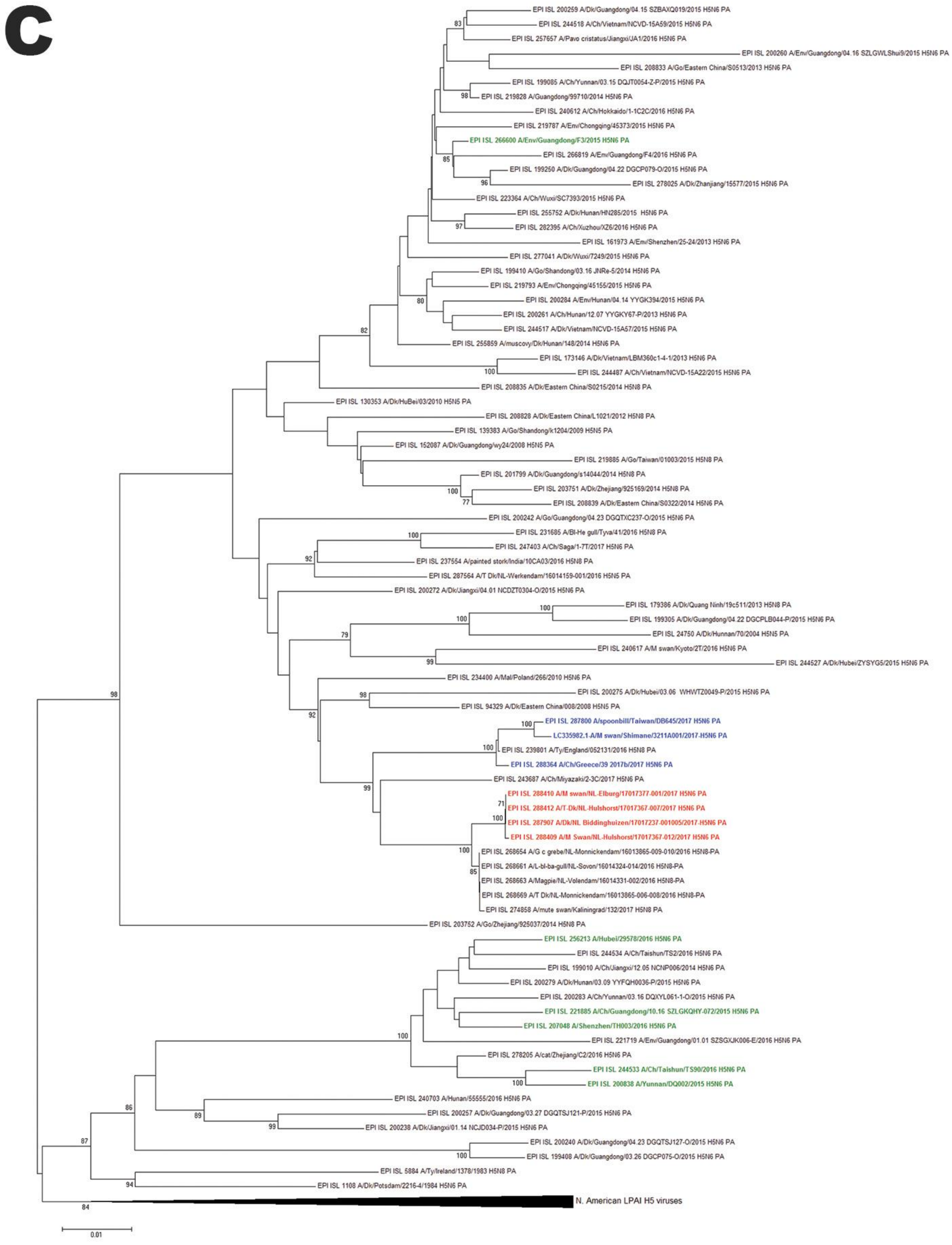
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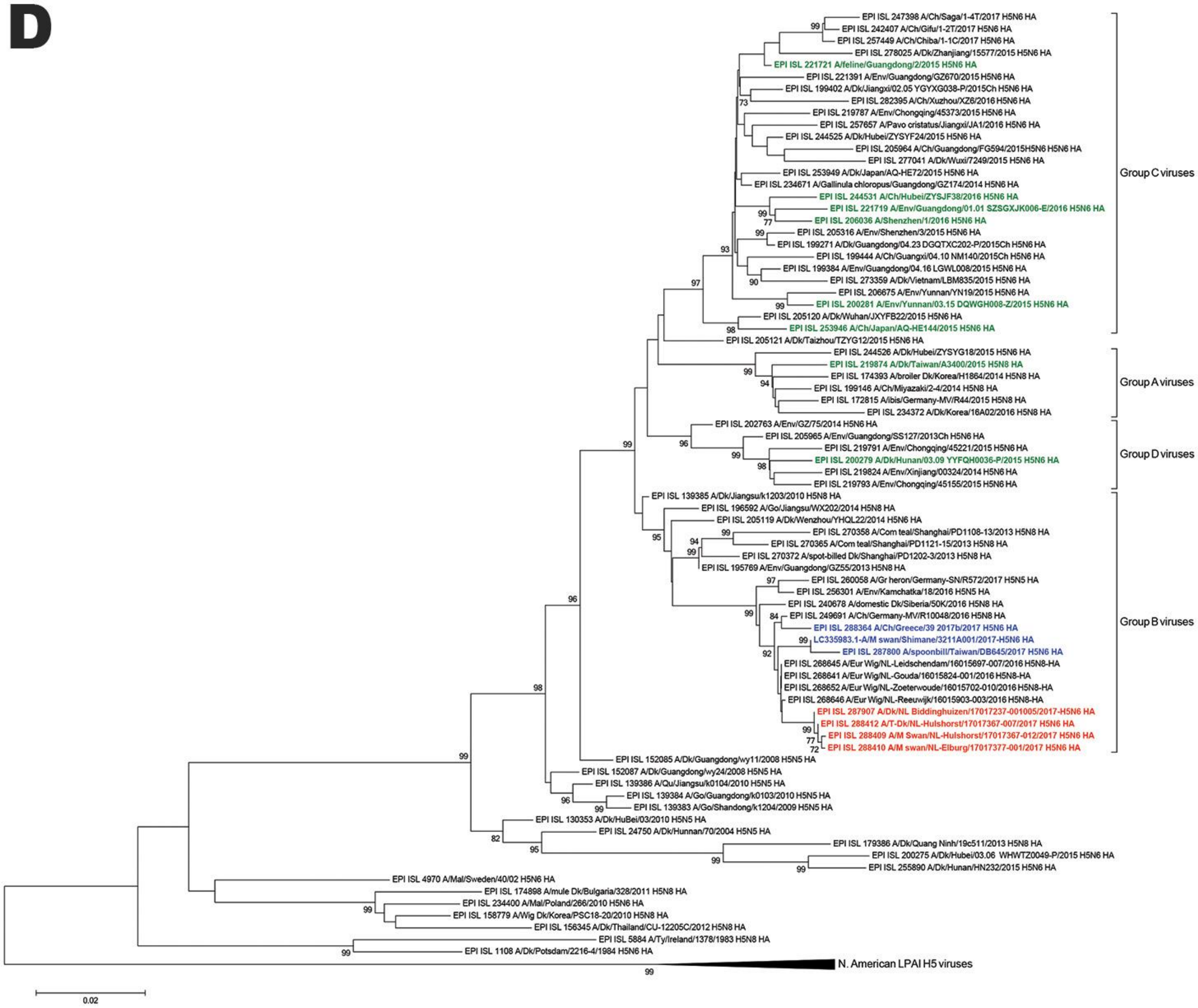
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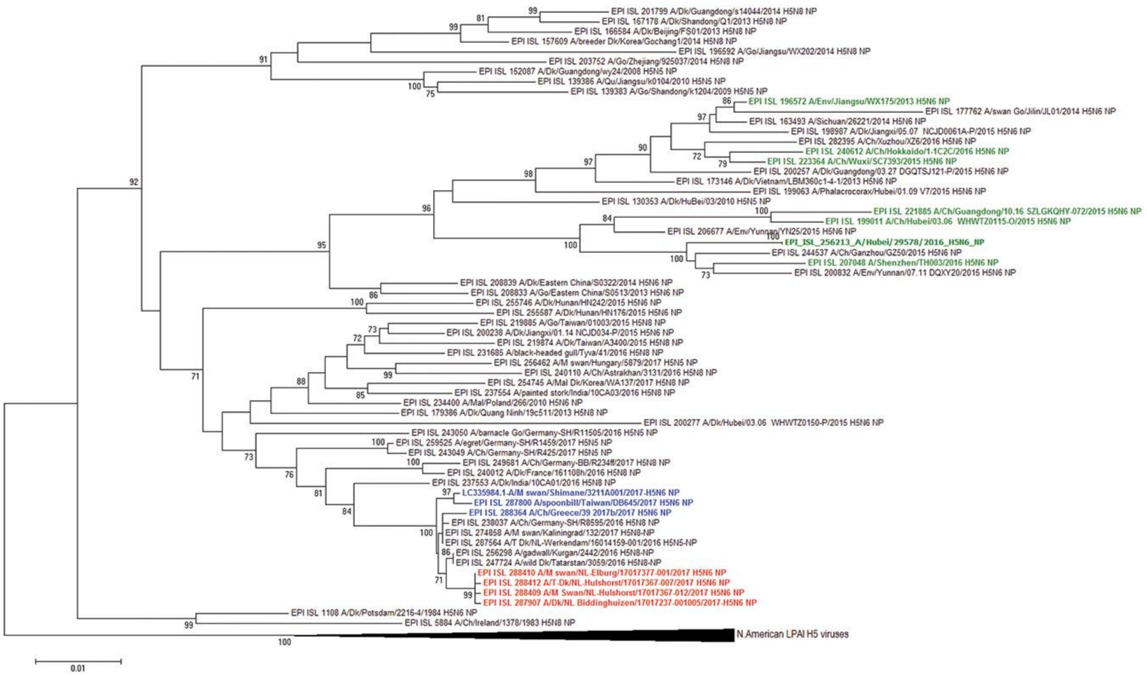
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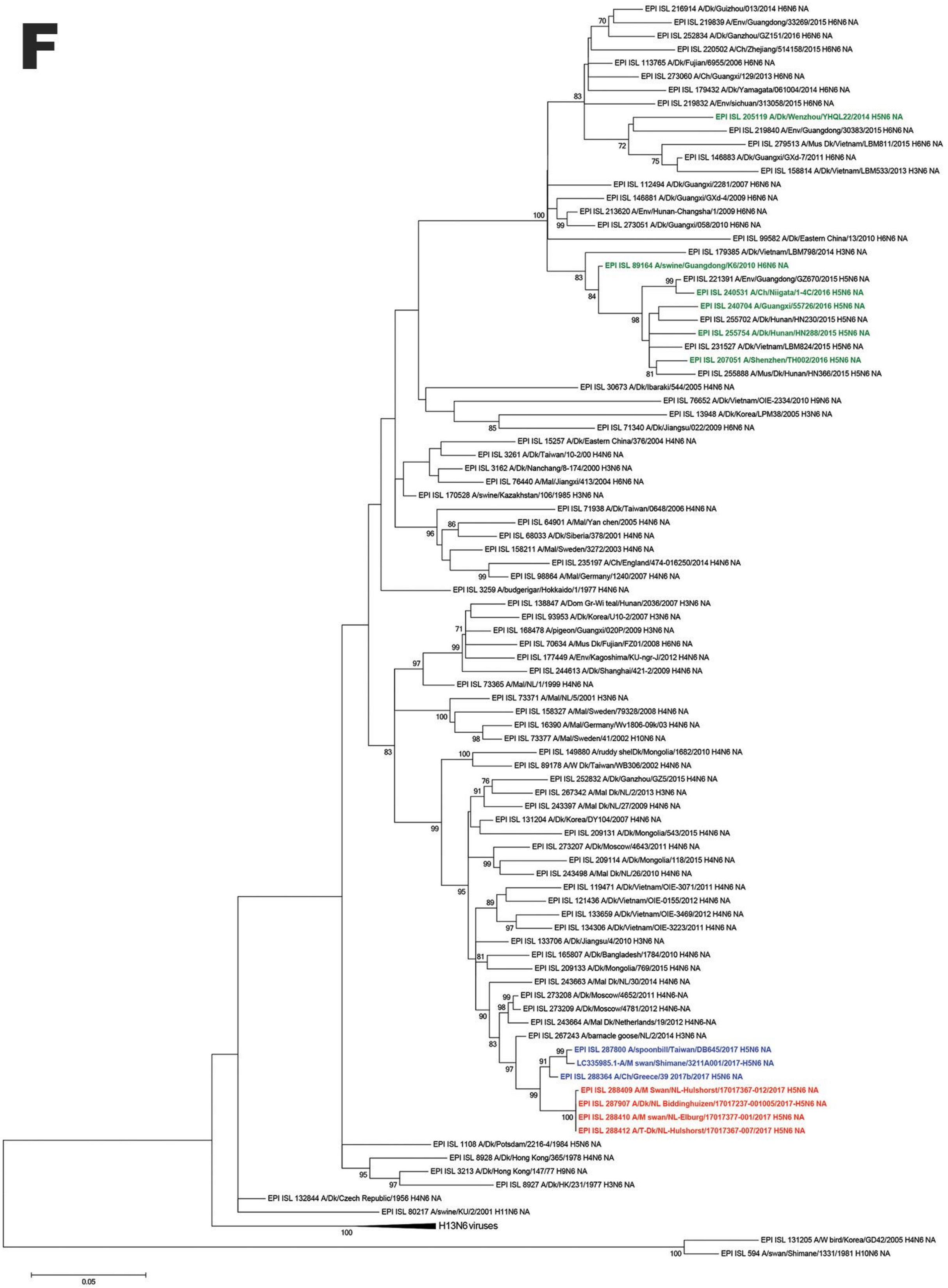
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89
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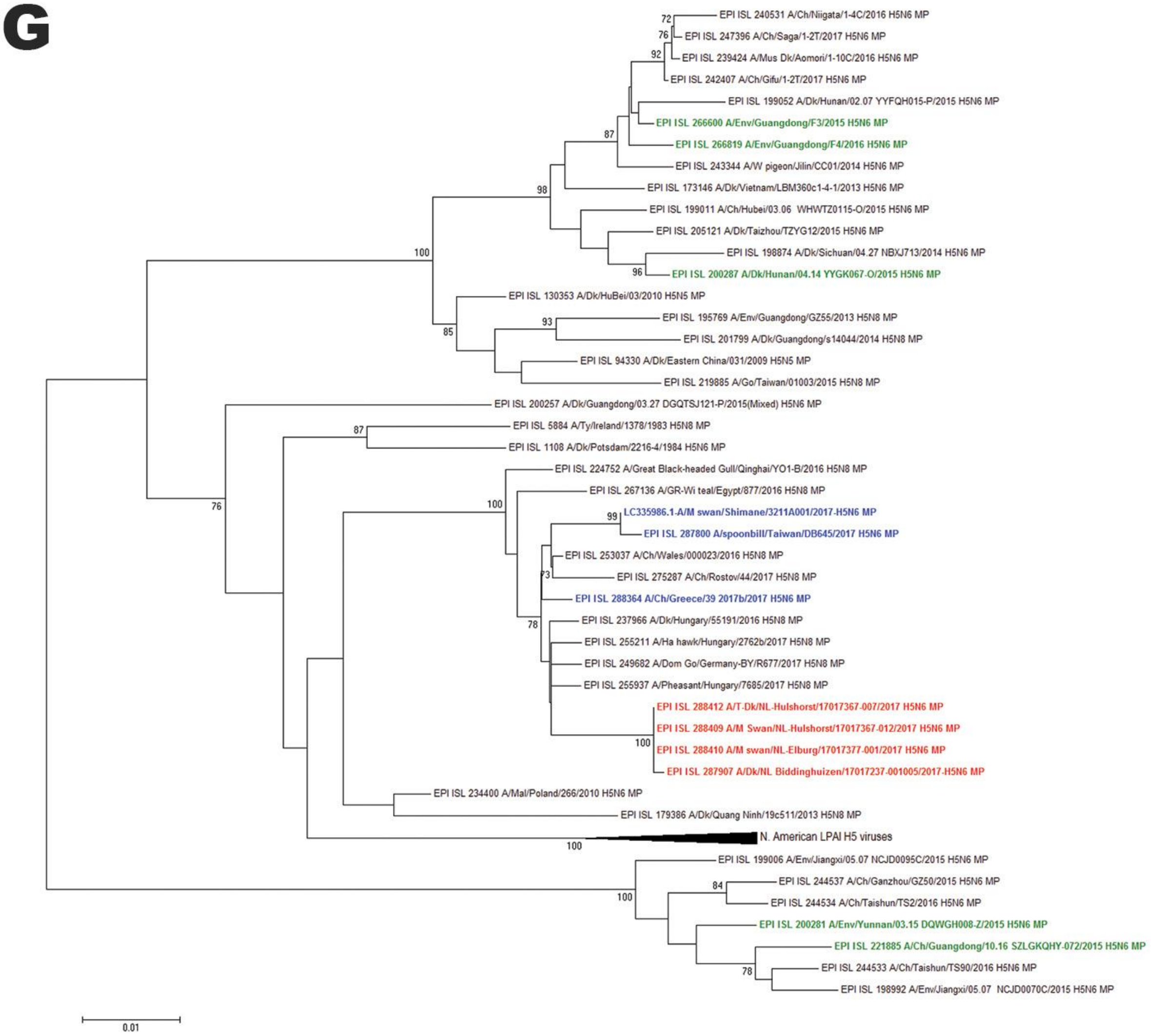
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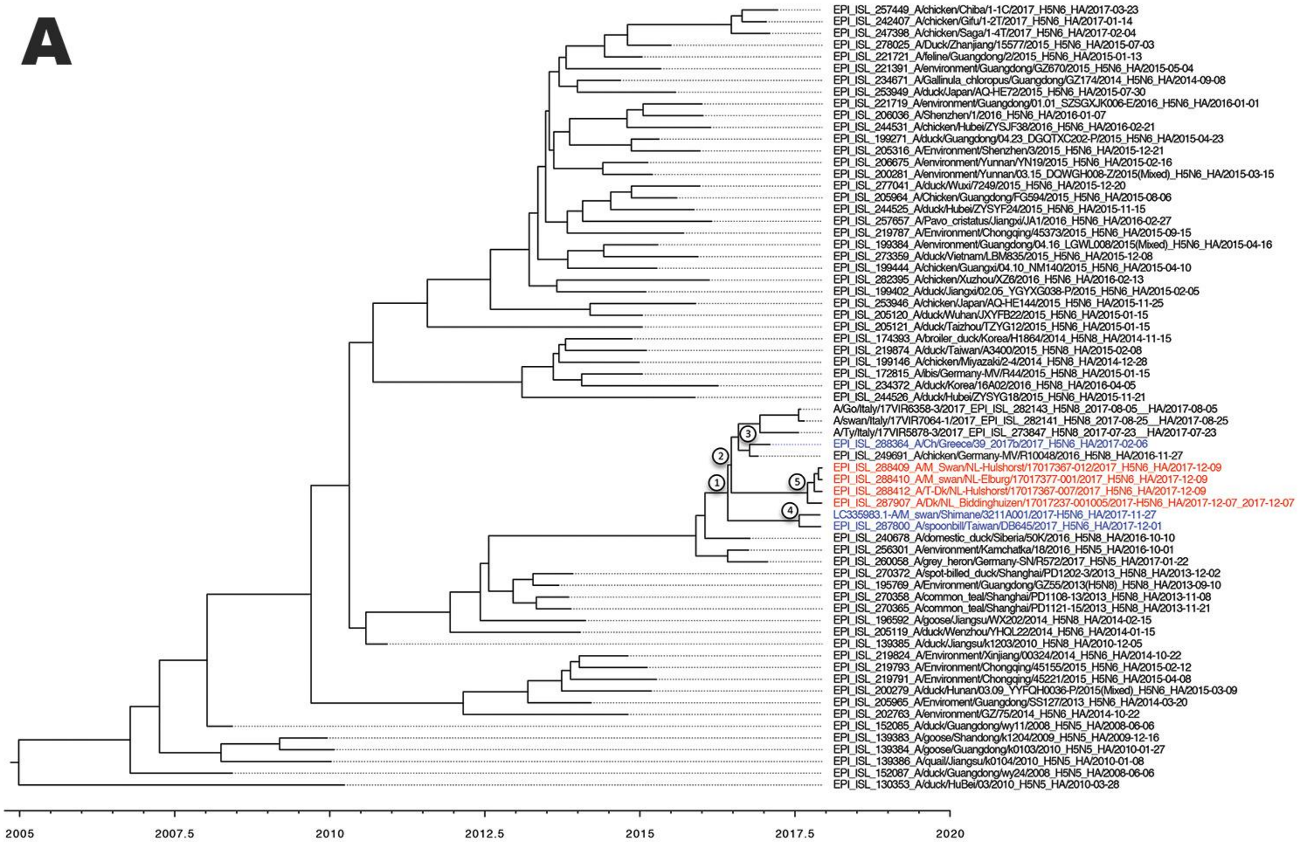
Technical Appendix Figure 2 (following pages). Phylogenetic analysis was performed for each gene segment. Related sequences in the GISAID database (http://platform.gisaid.org) were clustered using the CD-HIT algorithm, using identity setting of 0.97–0.985. Phylogenetic trees were generated using the neighbor-joining method with 1,000 bootstrap replicates within the MEGA6 software package. The maximum composite likelihood model was used with a gamma distribution (shape parameter = 1) for rate variation. The optimal phylogenetic trees are shown, and are drawn to scale. The GISAID accession numbers of the viruses are shown in the trees, as are the novel viruses isolated from the commercial duck farm (EPI ISL 287907), the tufted (EPI ISL 288412), and the 2 mute swans (EPI ISL 288409 and EPI ISL 288410). Groups A–D of clade 2.3.4.4 are indicated. The H5N6 viruses isolated in the Netherlands are in red, the H5N6 viruses isolated in Greece, Japan, and Taiwan are in blue, and the taxons comprising H5N6 viruses isolated from humans are in green. Segments shown are A) polymerase basic (PB2); B) polymerase basic (PB1); C) polymerase (PA); D) hemagglutinin (HA); E) nucleoprotein (NP); F) neuraminidase (NA); G) matrix protein (MP); H) nonstructural protein (NS).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①
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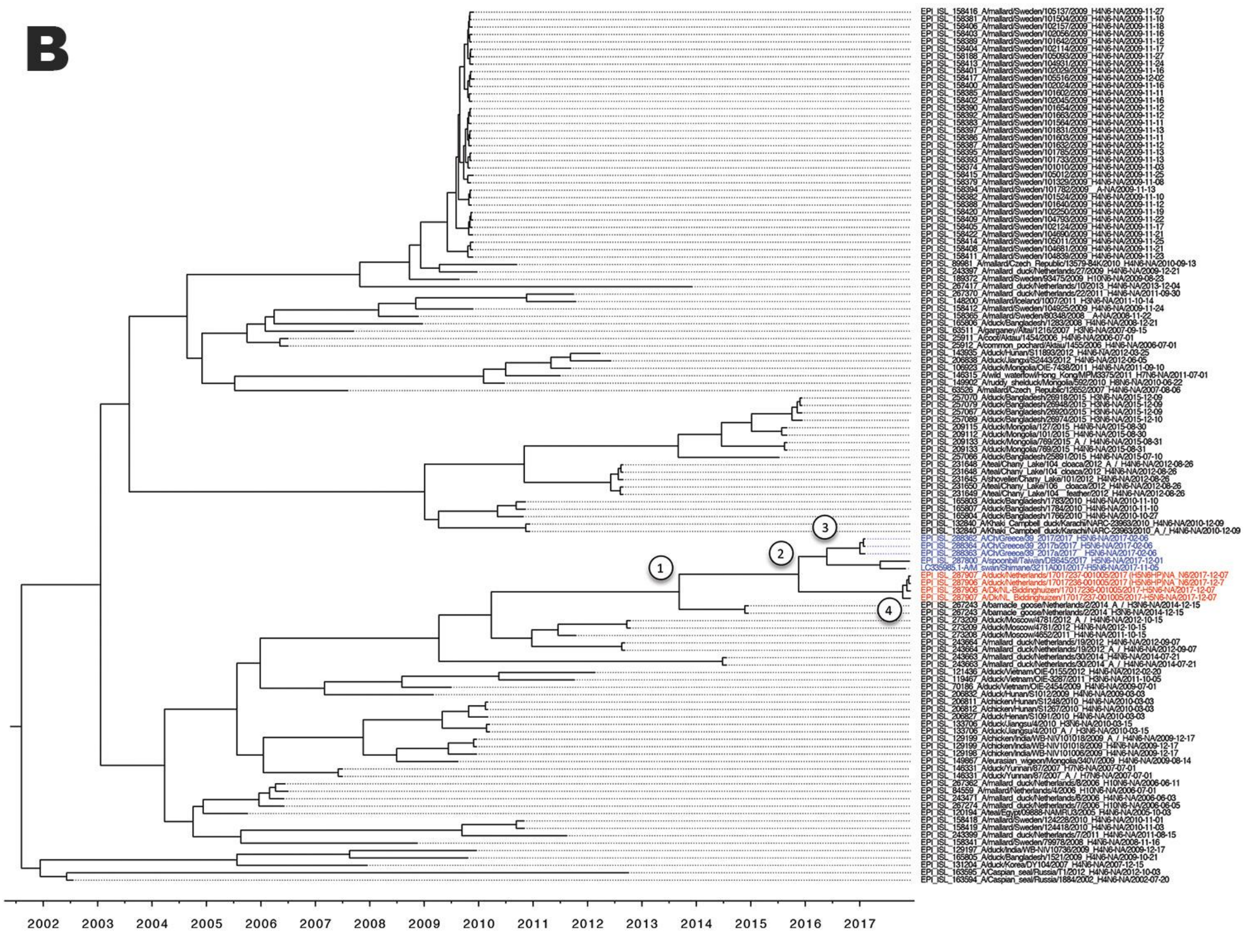

Technical Appendix Figure 3 (following pages). Molecular dating was performed for the (A) hemagglutinin (HA) and (B) neuraminidase (NA) gene segments. We estimated the tMRCA of 86 HA and 118 NA gene segment sequences; the GISAID accession numbers of the viruses are shown in the trees. Time scaled phylogenies (dates shown on the horizontal axis) were reconstructed using a Bayesian Markov chain Monte Carlo (MCMC) framework implemented in the BEAST software package (v 1.8.4) (http://beast.community/beast) with the SRD06 nt substitution model, the Bayesian Skyline coalescent model, and an uncorrelated lognormal relaxed molecular clock. Three MCMC runs of 50 × 106 states sampling each 5,000 steps were combined to obtain an effective sample size of >200. Maximum clade credibility (MCC) trees were reconstructed with 10% burn-in and the posterior distribution of relevant parameters were assessed in Fig Tree v 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree). The H5N6 viruses isolated in the Netherlands are in red and those isolated in Greece, Japan, and Taiwan are in blue.