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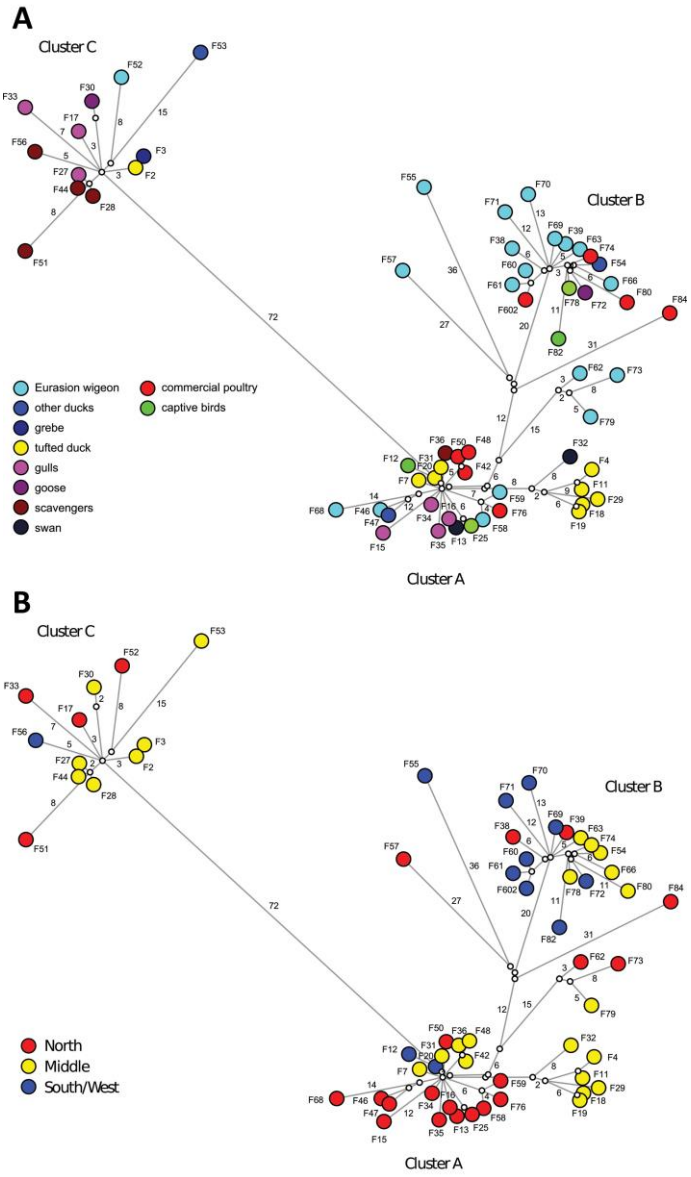
Multiple Reassorted Viruses as Cause of Highly Pathogenic Avian Influenza A(H5N8) Virus Epidemic, the Netherlands, 2016

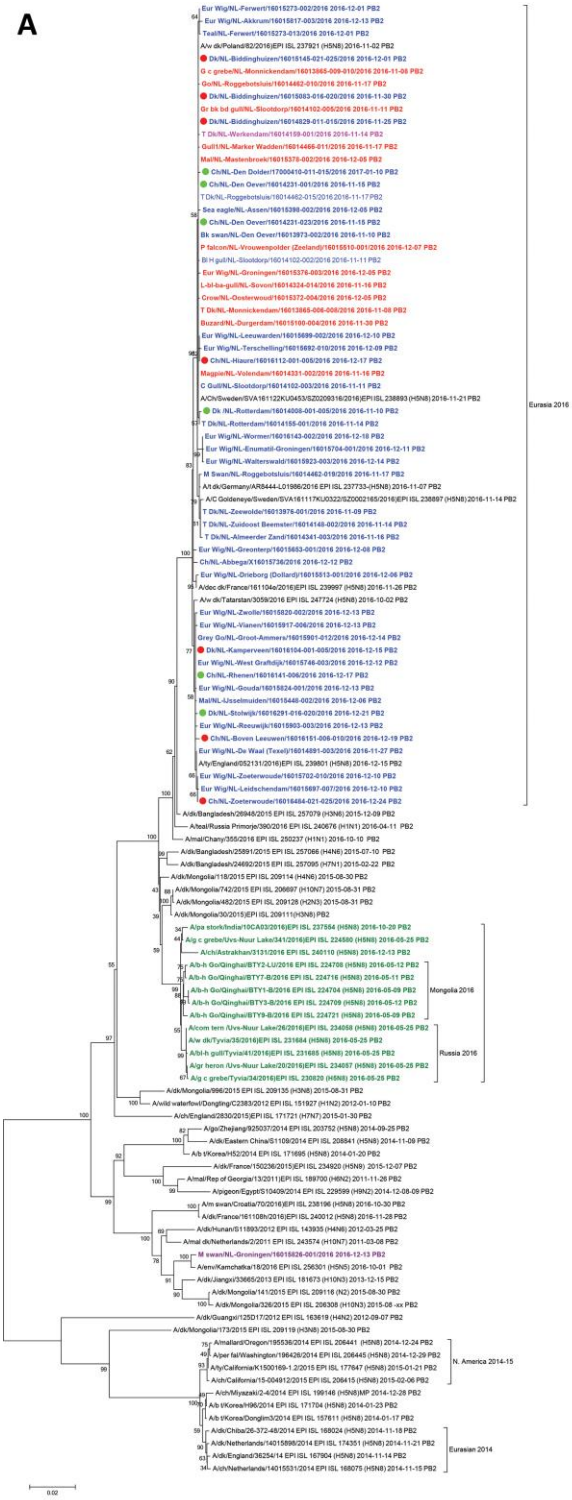
Technical Appendix 2



Technical Appendix 2 Figure 1. Network analysis: Median joining network of concatenated 8 gene segments. The network included all the most parsimonious trees linking the sequences. The virus isolates

are represented by circles, and their numbering is listed (Technical Appendix 1 Table 3). The smaller red circles are the intermediate vectors. Numbers (red) refer to the number of mutations between genotypes and/or median vectors. The GISAID accession numbers of the virus isolates (F-numbers) are listed in online Technical Appendix 1 Table 1.

Technical Appendix 2 Figure 2. Phylogenetic trees: The evolutionary history was inferred using the Neighbor-Joining method (*1*). The optimal trees for each gene segment are shown. One sequence for every virus isolated from a wild bird, poultry or captive bird is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Tamura-Nei method (*2*) and are in the units of the number of base substitutions per site. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). Evolutionary analyses were conducted in MEGA6 (*3*). The GISAID accession numbers of the viruses used in this study are listed in online Technical Appendix 1 Tables 1 and 2. The H5N8 viruses isolated in Russia-Mongolia are marked in green, the H5N8 viruses isolated in the Netherlands in 2016 and harboring the PA I gene segment are marked in bleu, those having the PA II gene segment in red. The H5N5 virus isolated from a tufted duck near Werkendam is marked in pink, that isolated from a mute swan near Groningen in purple.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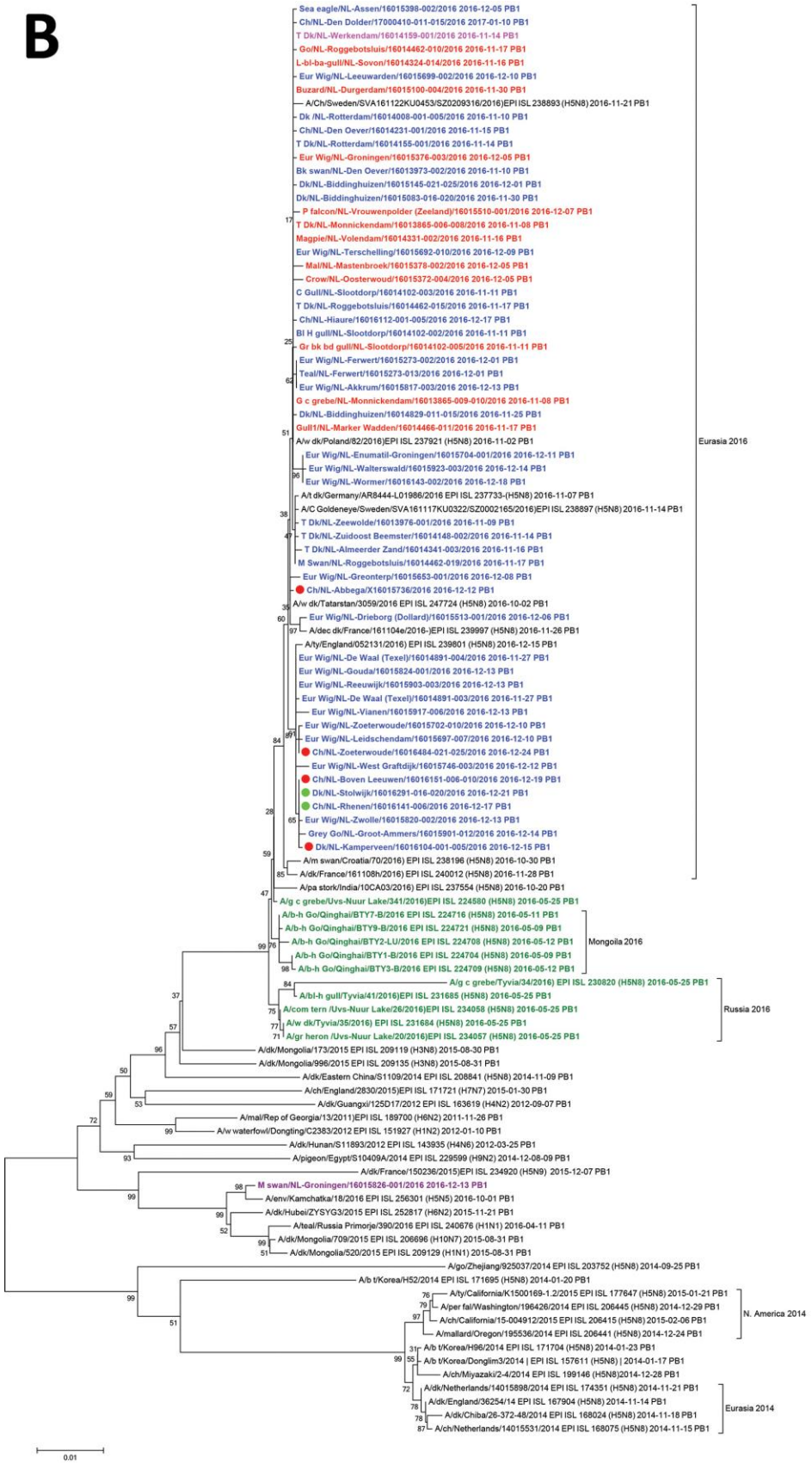
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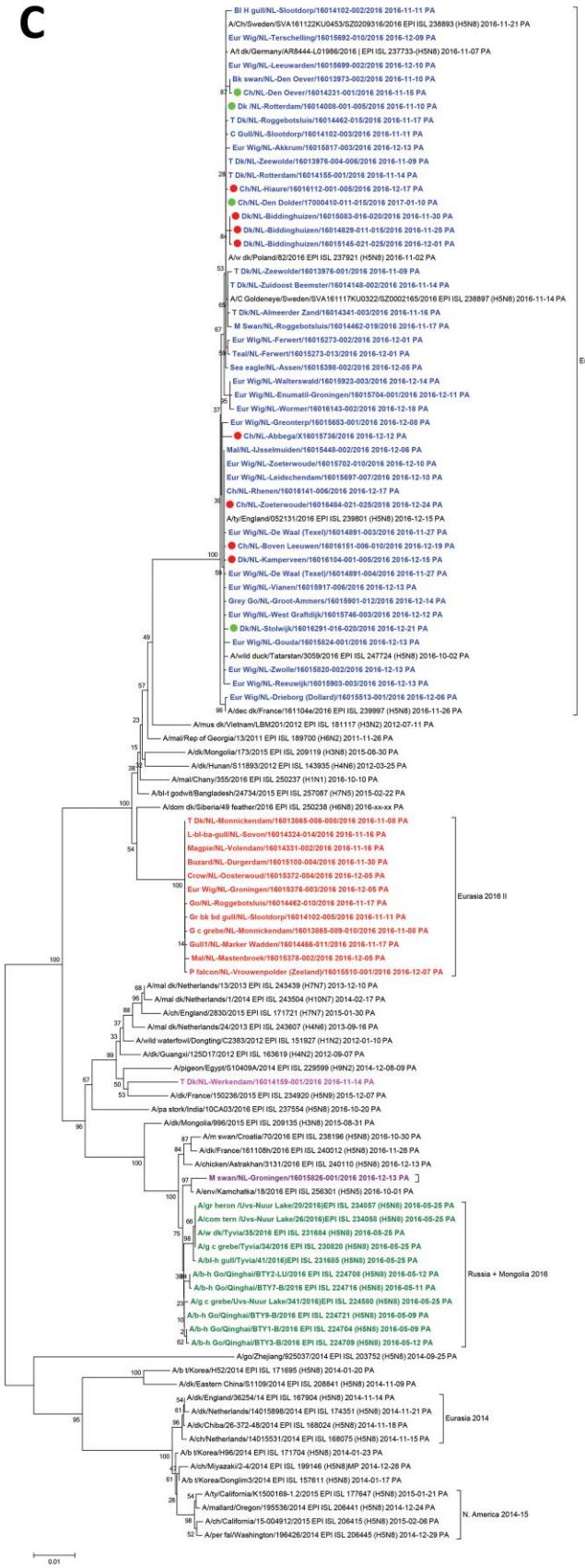
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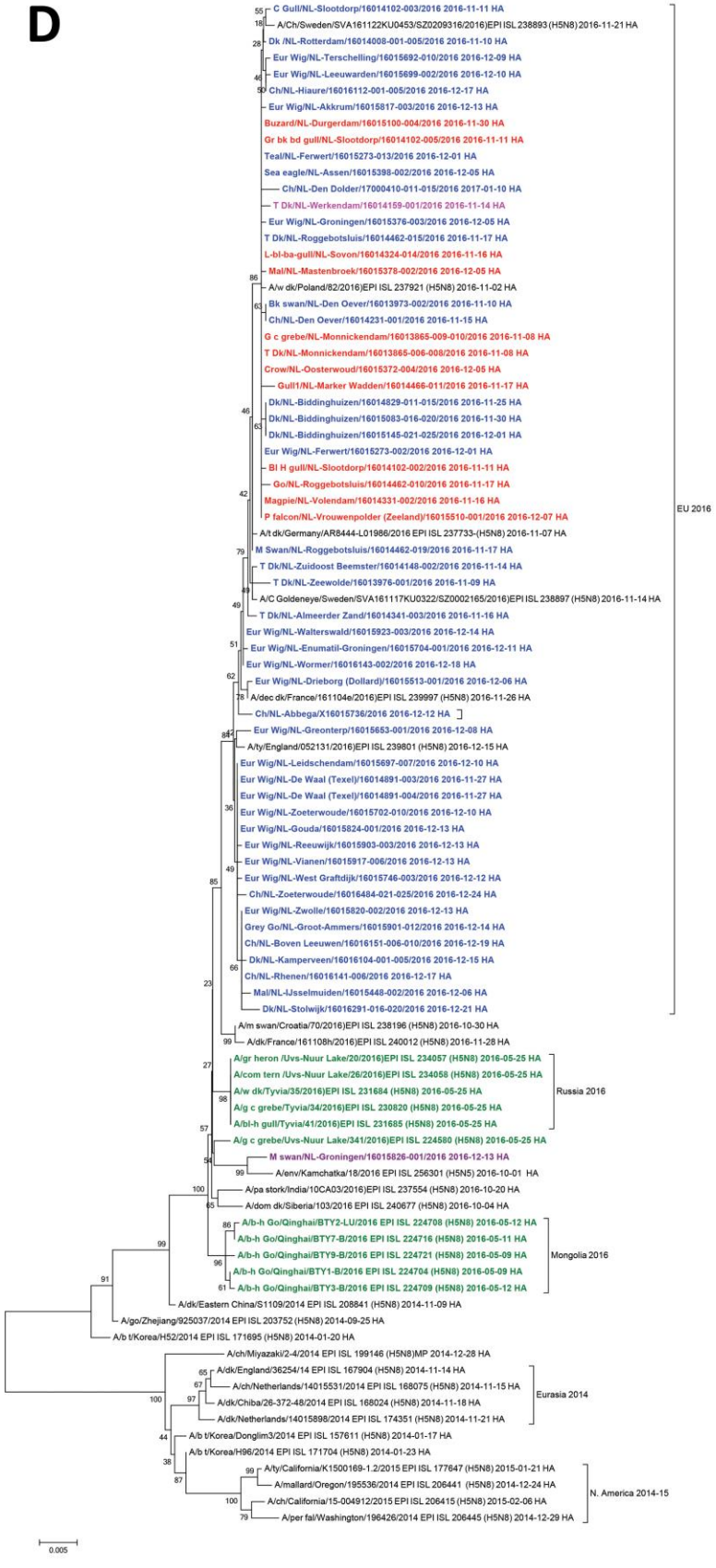
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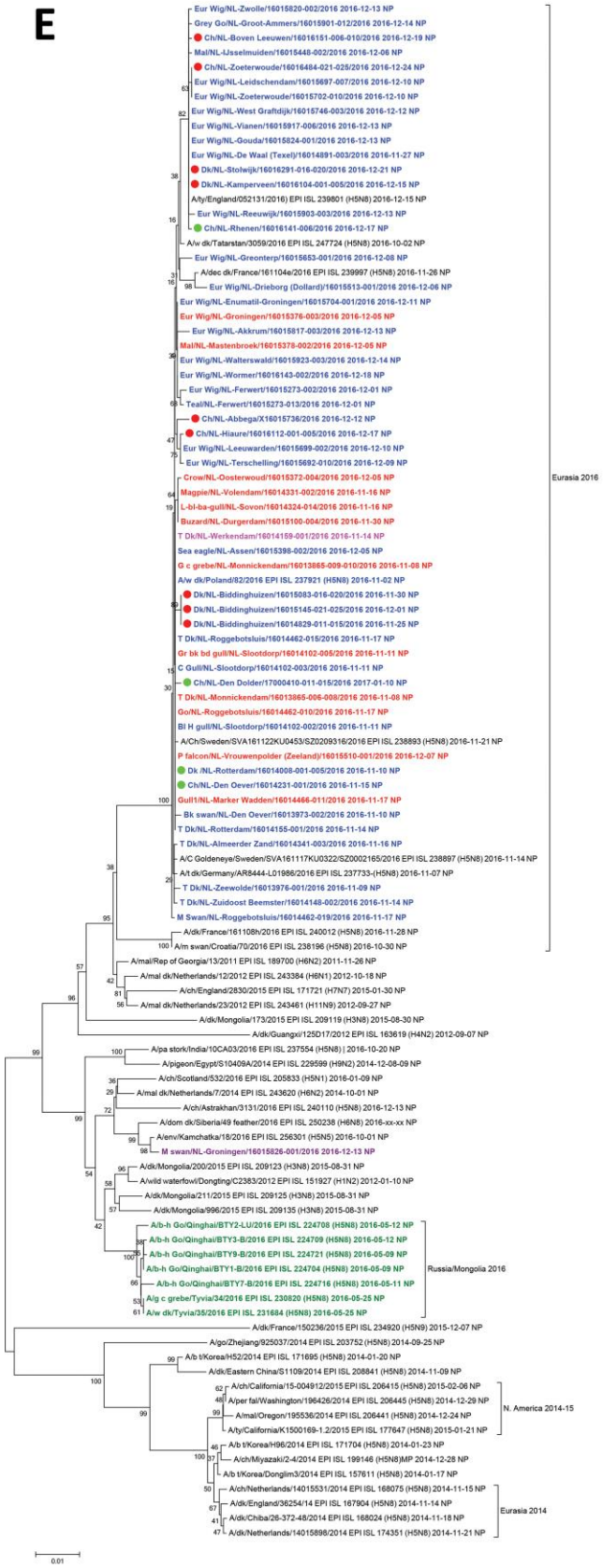
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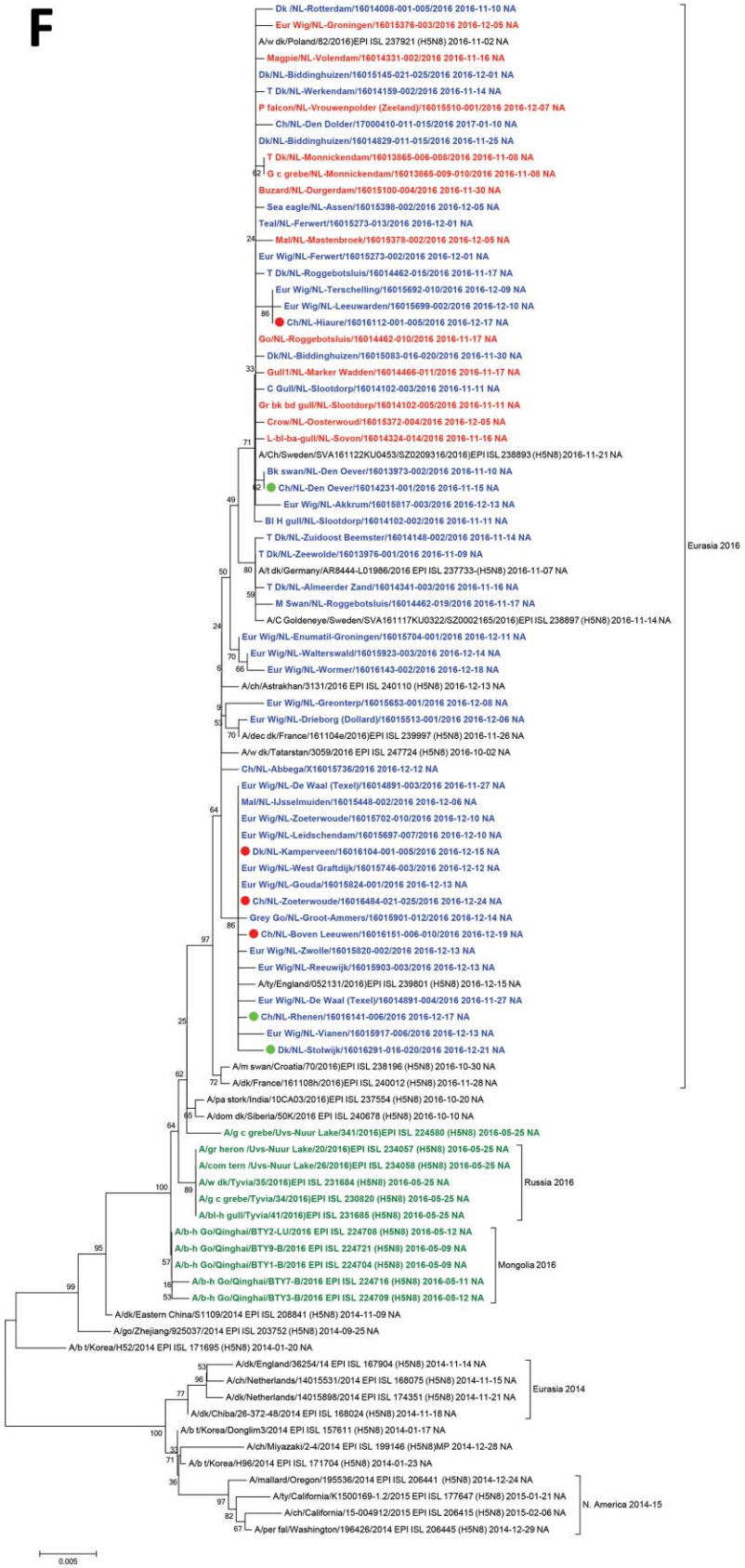
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Eurasia 2016
Russia 2016
Mongolia 2016
Eurasia 2014
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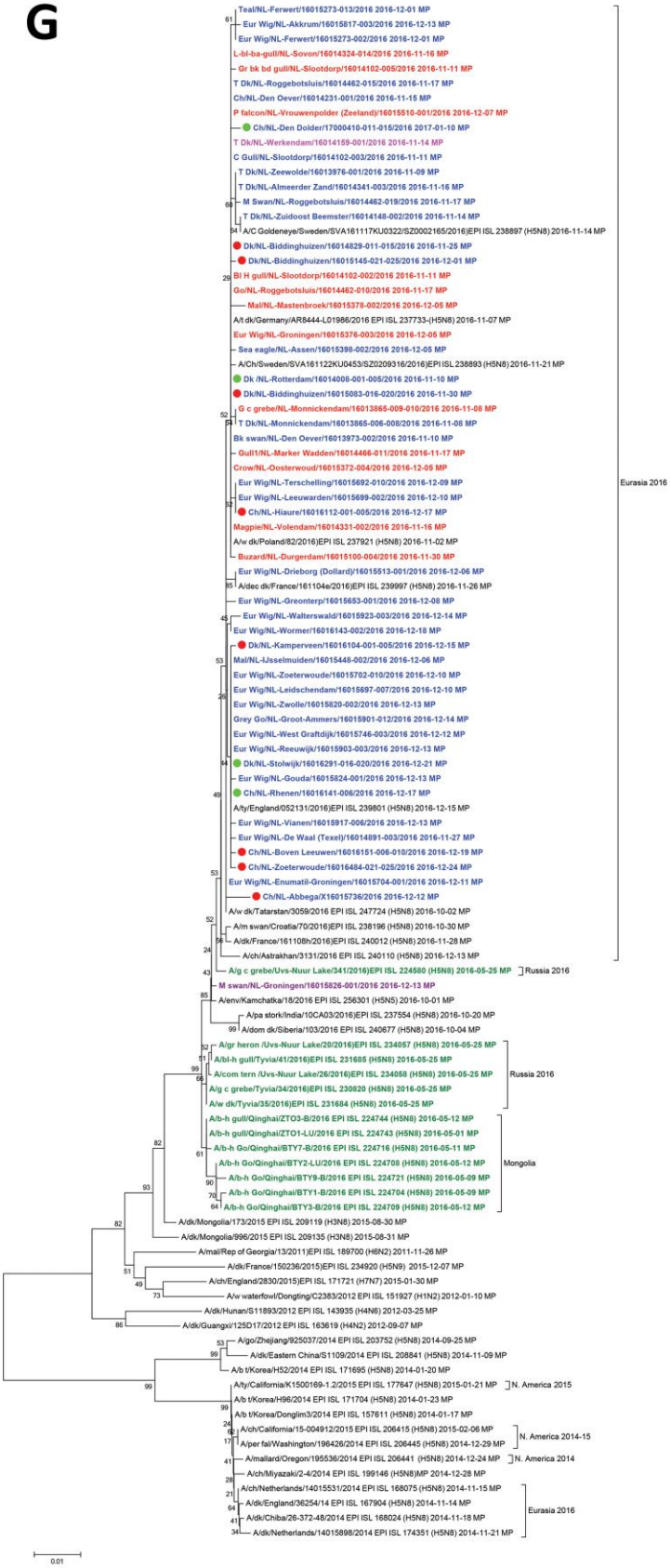
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Ch/NL-Den Oever/16014231-001/2016 2016-11-15 MP
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Eur Wig/NL-Wormer/16016143-002/2016 2016-12-18 MP
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Dk/NL-Stolwijk/16016291-016-020/2016 2016-12-21 MP
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A/ch/Astrakhan/3131/2016 EPI ISL 240110 (H5N8) 2016-12-13 MP
Eurasia 2016
A/g c grebe/Uvs-Nuur Lake/341/2016)EPI ISL 224580 (H5N8) 2016-05-25 MP
Russia 2016
M swan/NL-Groningen/16015826-001/2016 2016-12-13 MP
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A/com tern /Uvs-Nuur Lake/26/2016)EPI ISL 234058 (H5N8) 2016-05-25 MP
A/g c grebe/Tyvia/34/2016)EPI ISL 230820 (H5N8) 2016-05-25 MP
A/w dk/Tyvia/35/2016)EPI ISL 231684 (H5N8) 2016-05-25 MP
Russia 2016
A/b-h gull/Qinghai/ZTO3-B/2016 EPI ISL 224744 (H5N8) 2016-05-12 MP
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Mongolia
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N. America 2015
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Eurasia 2016
0.01

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Teal/NL-Ferwert/16015273-013/2016 2016-12-01 NS
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Mongolia 2016
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Russia 2016
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Eurasia 2014
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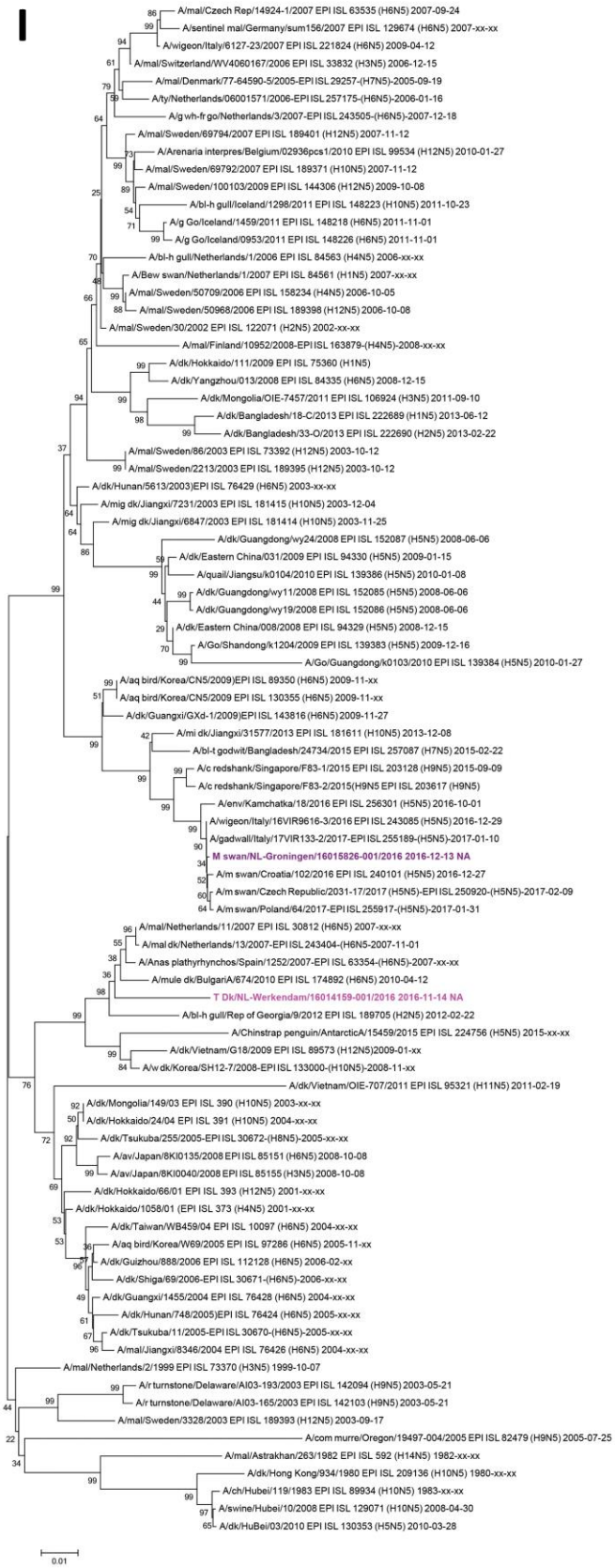
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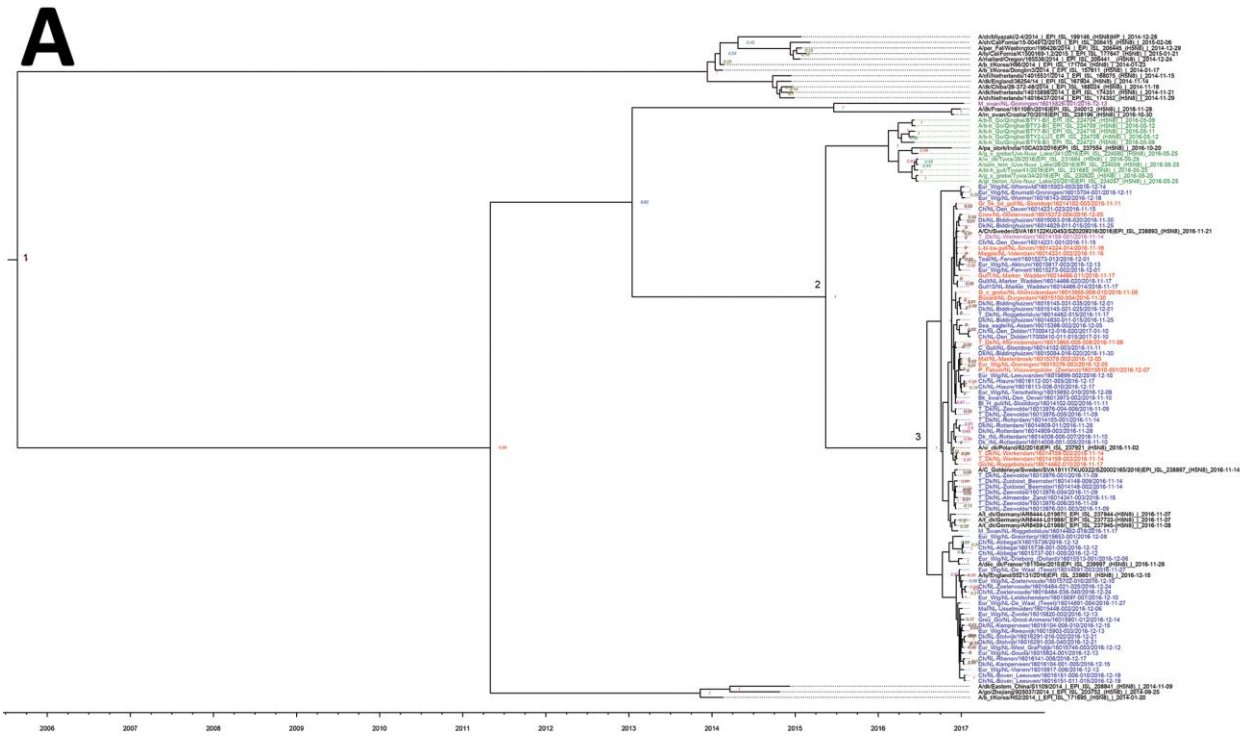
Technical Appendix 2 Figure 3. Molecular clock: Time-scaled phylogenies (dates shown on the horizontal axis) were inferred using relaxed-clock Bayesian Markov Chain Monte Carlo analysis for each gene segment separately (PB2, PB1, PA, HA, NP, N8, MP, NS) and also for N5. In this analysis, all sequences (mostly two, originating from the tracheal and cloacal swabs) obtained for the viruses isolated from wild birds, poultry or captive birds were included. The calculated time of most recent common ancestor (tMRCA) for the numbered nodes is listed in Table 1 in the main text), as are the highest posterior density interval and posterior value. The Russian-Mongolian H5N8 viruses are marked in green, the 2016 H5N8 viruses in the Netherlands are marked in blue (PA cluster I) and red (PA cluster II), and the H5N5 sequences isolated from a tufted duck near Werkendam (pink) and a mute swan near Groningen are also shown. The GISAID accession numbers of the viruses used in this study are listed in online Technical Appendix 1 Tables 1 and 2.



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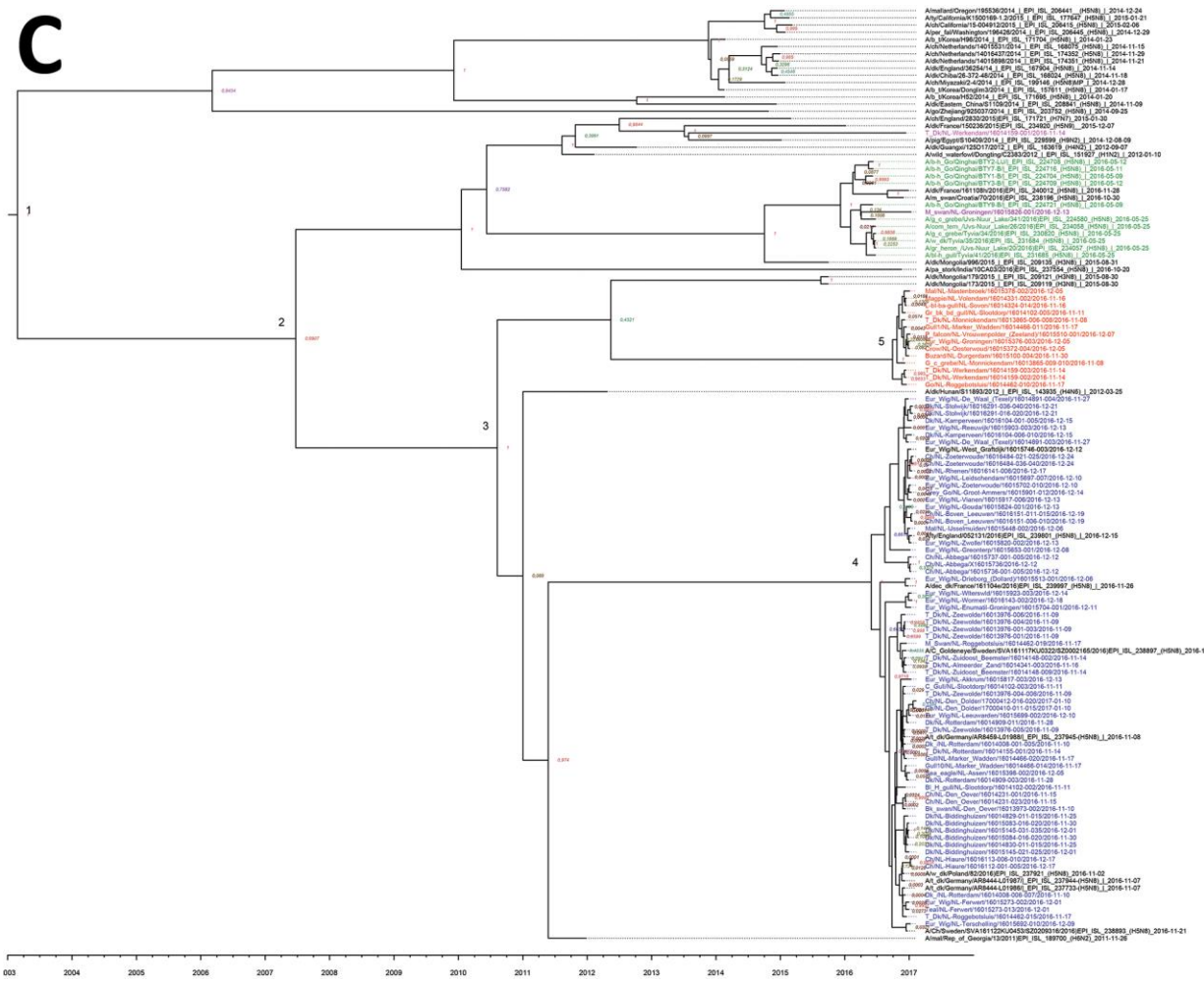
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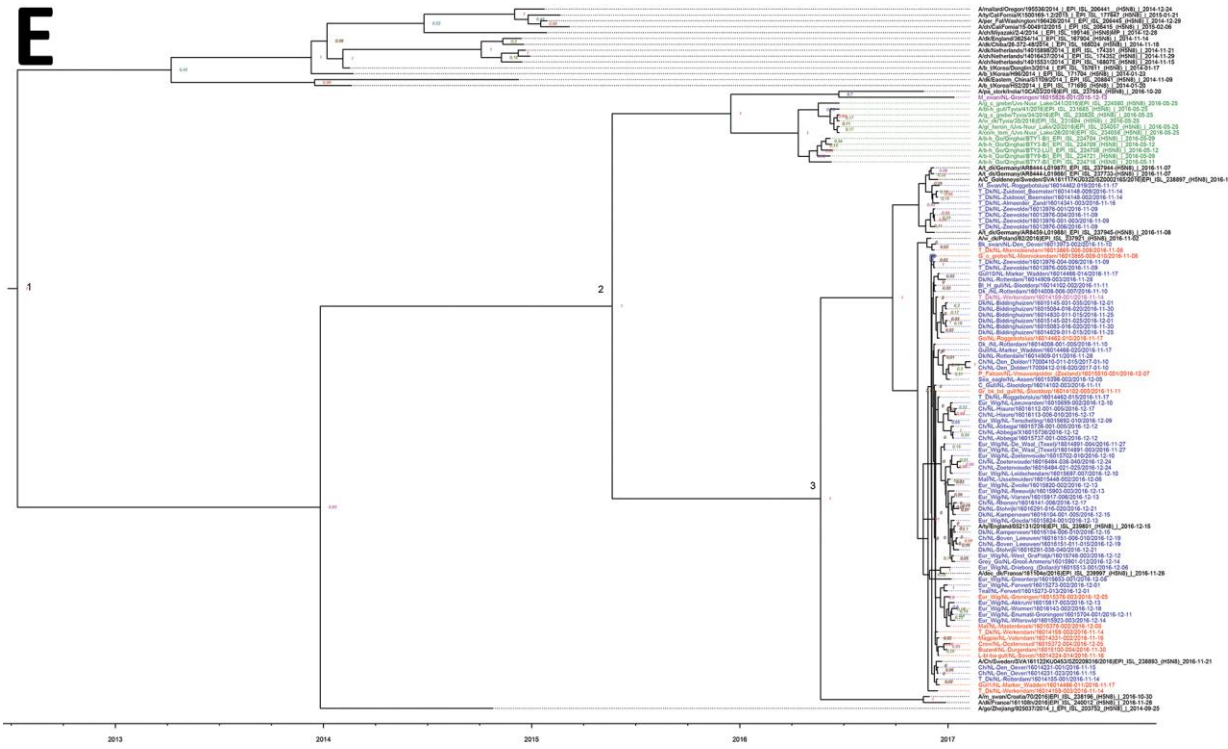
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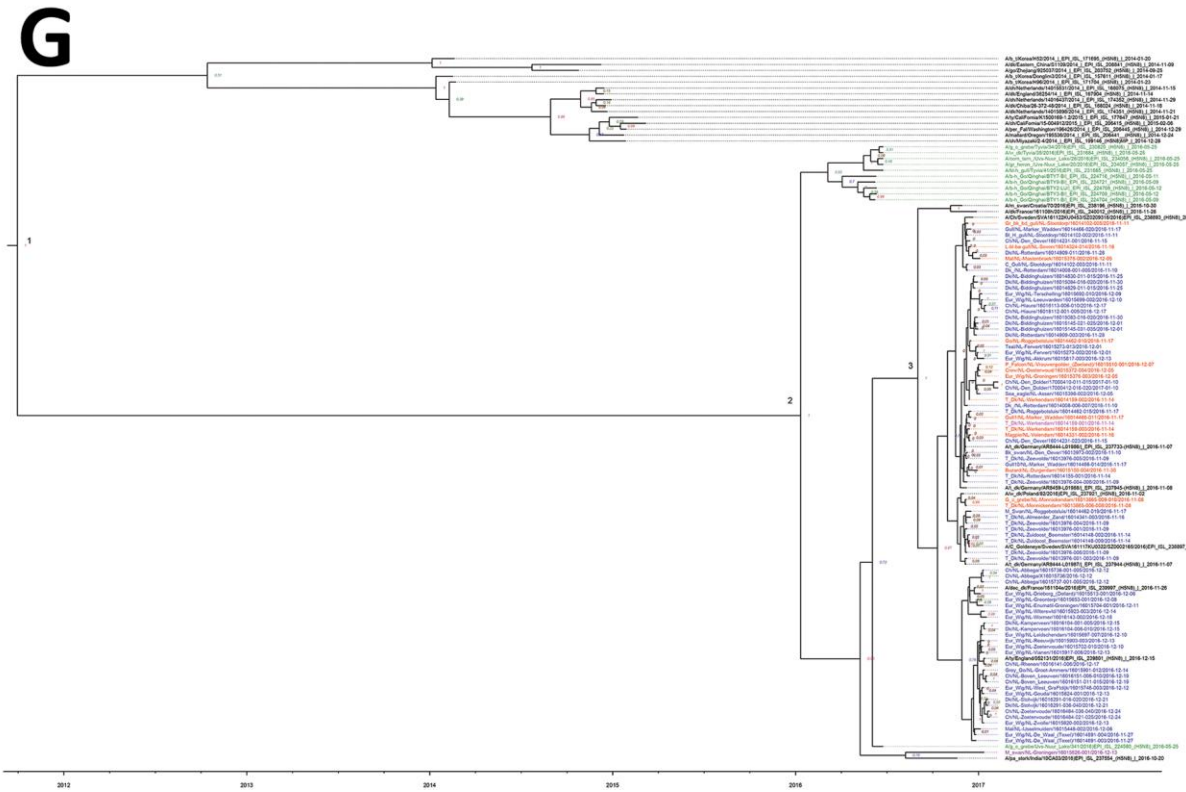
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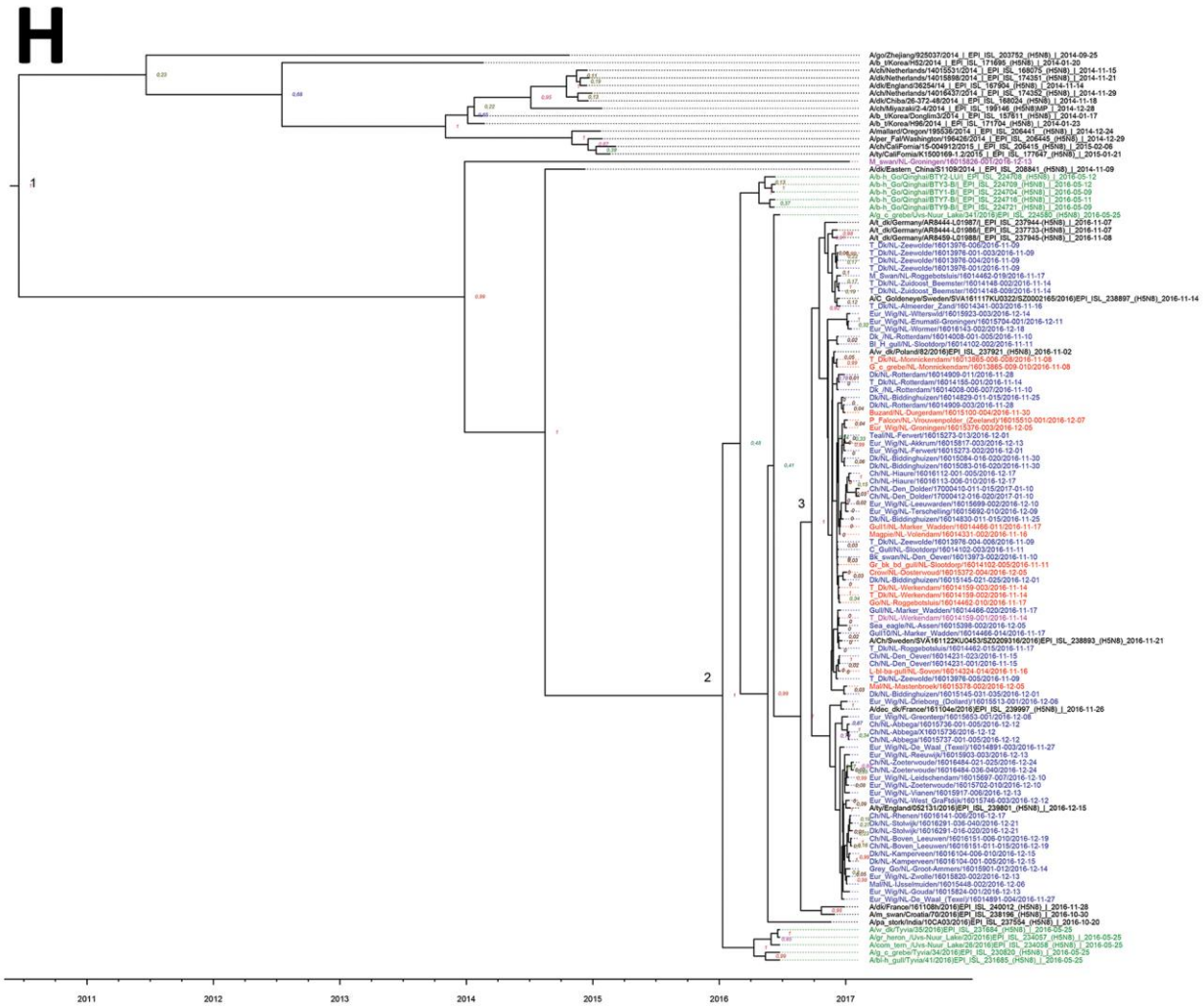
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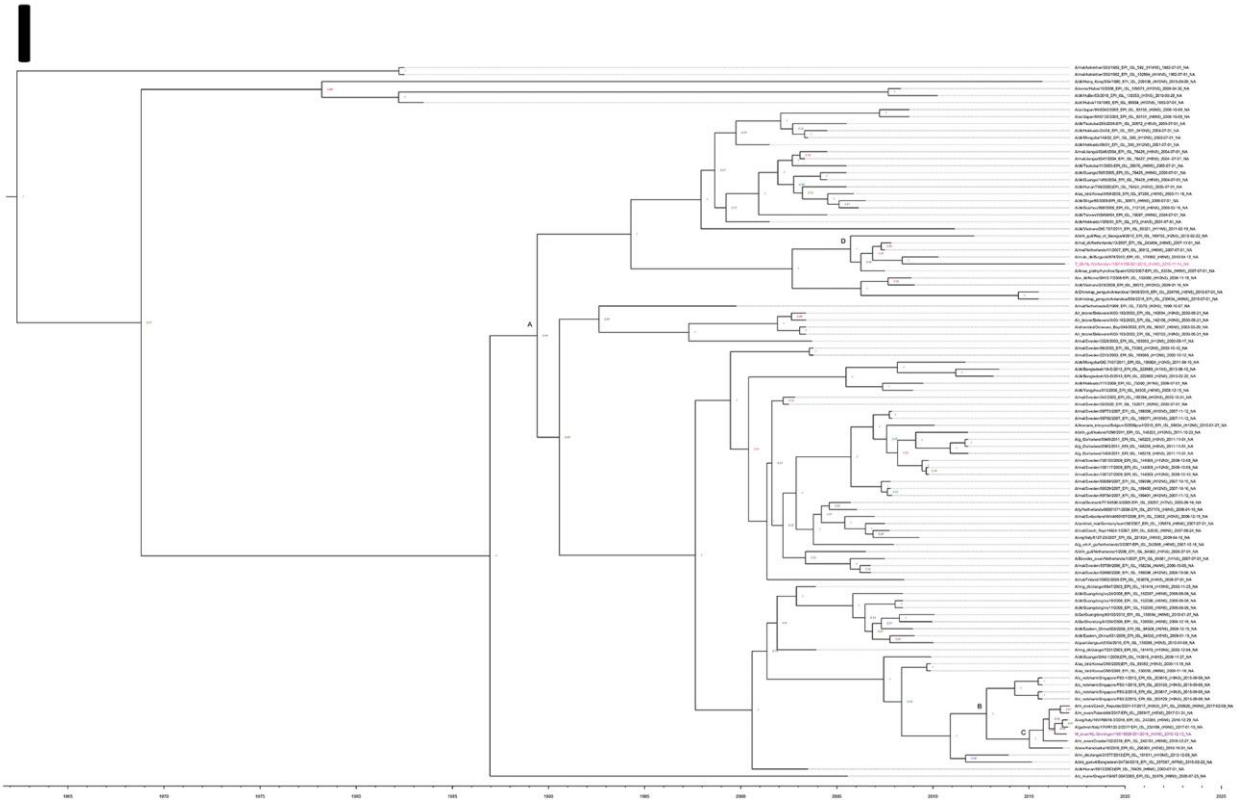

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