Novel Reassortant Highly Pathogenic Avian Influenza A(H5N2) Virus in Broiler Chickens, Egypt

Kareem E. Hassan, Jacqueline King, Magdy El-Kady, Manal Afifi, Hassanein H. Abozeid, Anne Pohlmann, Martin Beer, Timm Harder

We detected a novel reassortant highly pathogenic avian influenza A(H5N2) virus in 3 poultry farms in Egypt. The virus carried genome segments of a pigeon H9N2 influenza virus detected in 2014, a nucleoprotein segment of contemporary chicken H9N2 viruses from Egypt, and hemagglutinin derived from the 2.3.4.4b H5N8 virus clade.

Since 2006, Egypt's poultry industry has been plagued by endemic infections with highly pathogenic avian influenza (HPAI) virus, subtype H5N1, clade 2.2.1, of the goose/Guangdong (gs/GD) lineage (*1*). In addition, low pathogenicity avian influenza (LPAI) virus of subtype H9N2, G1 lineage, introduced in 2011 (*2*), and HPAI H5N8 (gs/GD clade 2.3.4.4b) introduced in 2016, have become entrenched in local poultry populations (*3*). Despite ongoing control measures, respiratory disease with increased mortality rates is endemic in poultry farms in Egypt.

The zoonotic nature of HPAI H5N1 2.2.1 viruses has caused in Egypt the highest number of human infection cases per country worldwide; a low level of sporadic benign human cases of H9N2 viral infection has also been reported from Egypt (*4*). Continued adaptation by point mutations, but not reassortment, to enhance replication in mammalian hosts has been repeatedly reported in avian influenza in Egypt (*5*). Here, we describe the detection of a new reassortant HPAI virus in commercial chicken holdings in Egypt. This virus carries the hemagglutinin (HA) gene of HPAI clade 2.3.4.4b H5N8 virus and 7 genome segments derived from Egyptian H9N2 viruses (*6*).

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DOI: https://doi.org/10.3201/eid2601.190570

The Study

During January–April 2019, we examined samples from 11 commercial broiler farms reporting respiratory clinical signs among chickens by using the Riems Influenza A Typing Assay (*7*). We detected co-presence of avian influenza viruses subtypes H5 and H9 with N2 (8 farms) as well as H5N2 only (3 farms).

We selected 8 samples representing H5N8, H9N2, and H5N1 from 2017–2018, plus 1 positive H5N2 sample from 2019, for full-genome sequencing (Table 1; Appendix 1 Table, https://wwwnc.cdc.gov/EID/article/26/1/19-0570-App1.pdf). Sanger- and next-generation sequencing results identified various reassortants new to Egypt (Figure 1). All H5 HA segments encoded a polybasic cleavage site, PLREKRRKR-GLF (H5 clade 2.3.4.4b) or PQGEKRRKKR-GLF (H5 clade 2.2.1.2), thus classifying those viruses as highly pathogenic. We identified the closest related sequences by BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) homology searches in the GISAID (http://platform.gisaid.org) and International Nucleotide Sequence Data Collaboration (http://www.insdc.org) databases. Phylogenetic analyses of each genome segment aided in clustering sequences (Appendix 1 Figure). We delineated the putative origin of each of the genome segments (Figure 1). The HA segment of the novel HPAI H5N2 reassortant virus was derived from clade 2.3.4.4b viruses with closest homology to viruses circulating in ducks in Egypt in 2017 (Figure 2), whereas ≥4 additional genome segments (polymerase basic 1, polymerase basic 2, polymerase, and nonstructural protein) originated from novel reassortant H9N2 viruses first detected in pigeons

Table. Characteristics of avian influenza viruses in samples from diseased poultry, Egypt*

Strain	Subtype	Collection date	Host species	Governorate	Flock size	Mortality rate, %	Genome sequence	Clade
A/duck/Egypt/AR518/2017	H5N8	2017 Mar 15	Duck	Giza	3,000	40	Full	2.3.4.4b
A/duck/Egypt/AR560/2018	H5N8	2018 May 20	Duck	Giza	5,000	35	Full	2.3.4.4b
A/turkey/Egypt/AR550/2018	H5N8	2018 Mar 2	Turkey	Beni-Suef	5,000	100	Full	2.3.4.4b
A/duck/Egypt/AR526/2017	H5N1	2017 Mar 20	Duck	Beni-Suef	3,000	15	Full	2.2.1.2
A/chicken/Egypt/AR528/2017	H5N1	2017 Mar 22	Chicken, layer	Beni-Suef	5,000	30	Full	2.2.1.2
A/chicken/Egypt/AR544/2018	H9N2	2018 Jan 20	Chicken, broiler	Giza	10,000	25	Full	G1.B
A/chicken/Egypt/AR545/2018	H9N2	2018 Mar 25	Chicken, broiler	Qualiobia	5,000	25	Full	G1.B
A/chicken/Egypt/AR546/2018	H9N2	2018 Mar 22	Chicken, broiler	El-Menia	8,000	10	Full	G1.B
A/chicken/Egypt/AI00994/2019	H5N2	2019 Jan 19	Chicken, broiler	Beheira	17,000	47	Full	2.3.4.4b
A/chicken/Egypt/AI00986/2019	H5N2	2019 Jan 5	Chicken, broiler	Fayoum	10,000	5	HA (partial)	2.3.4.4b
A/chicken/Egypt/AI00987/2019	H5, H9, N2	2019 Jan 9	Chicken, broiler	Beheira	7,000	15	HA (partial)	2.3.4.4b
A/chicken/Egypt/AI00988/2019	H5, H9, N2	2019 Jan 19	Chicken, broiler	Beheira	8,000	20	HA (partial)	2.3.4.4b
A/chicken/Egypt/AI00989/2019	H5, H9, N2	2019 Jan 27	Chicken, broiler	El-Menia	6,000	14	HA (partial)	2.3.4.4b
A/chicken/Egypt/AI00991/2019	H5, H9, N2	2019 Feb 16	Chicken, broiler	Beni-Suef	74,000	16	NA*	NA
A/chicken/Egypt/AI00992/2019	H5N2	2019 Mar 3	Chicken, broiler	Beheira	5,000	15	HA (partial)	2.3.4.4b
A/duck/Egypt/AI00993/2019	H5, N8, N2	2019 Jan 14	Chicken, broiler	Giza	4,000	15	NA	NA
A/chicken/Egypt/AI00995/2019	H5, H9, N2	2019 Jan 14	Chicken, broiler	Beheira	33,000	33.3	NA	NA
A/chicken/Egypt/AI00996/2019	H5, H9, N2	2019 Jan 15	Chicken, broiler	Beheira	10,000	60	NA	NA
A/chicken/Egypt/AI00997/2019	H5, N8, H9, N2	2019 Mar 9	Chicken, broiler	Dakhalia	40,000	7.5	NA	NA

*HA, hemagglutinin; NA, not available.

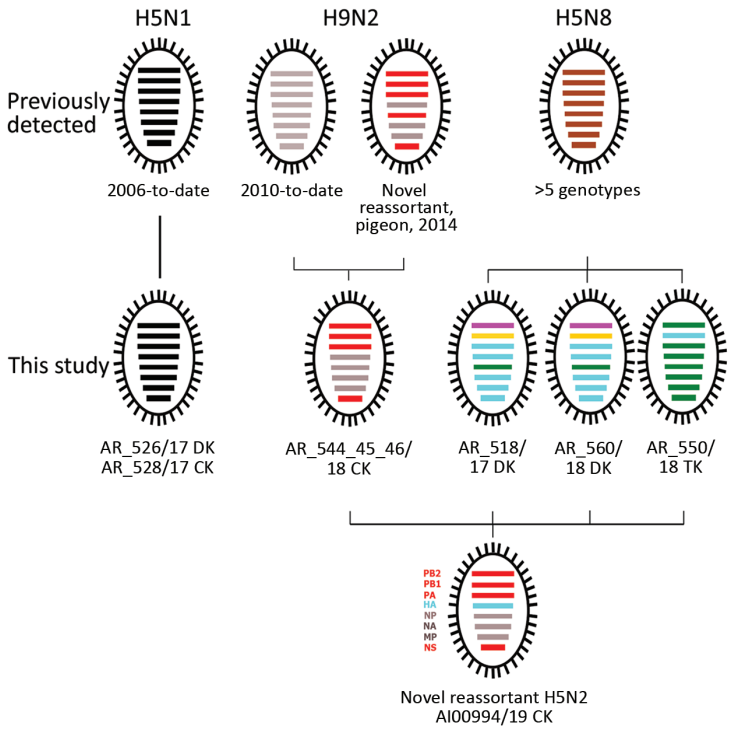

Figure 1. Genotype and reassortment analyses based on full-length genome sequences of avian influenza viruses in Egypt previously detected and those identified in this study. Colors indicate grouping of segment origin according to phylogenetic analyses (Appendix 1 Figure, https://wwwnc.cdc.gov/EID/article/26/1/19-0570-App1.pdf): highly pathogenic avian influenza (HPAI) H5N1 2.2.1.2 virus from Egypt (black); H9N2 subtype from Egypt circulating in chickens since 2010 (gray); H9N2 subtype from Egypt first detected in pigeons in 2014 (red); HPAI H5N8 viruses previously detected and circulating in Egypt (brown; different genotypes); Polymerase basic (PB) 2 segment most closely related to an H3N6 virus from Bangladesh (purple); PB1 segment most closely related to an H7N7 virus from Georgia (yellow); segments most closely related to H5N8 viruses from China (blue) or Russia (green).

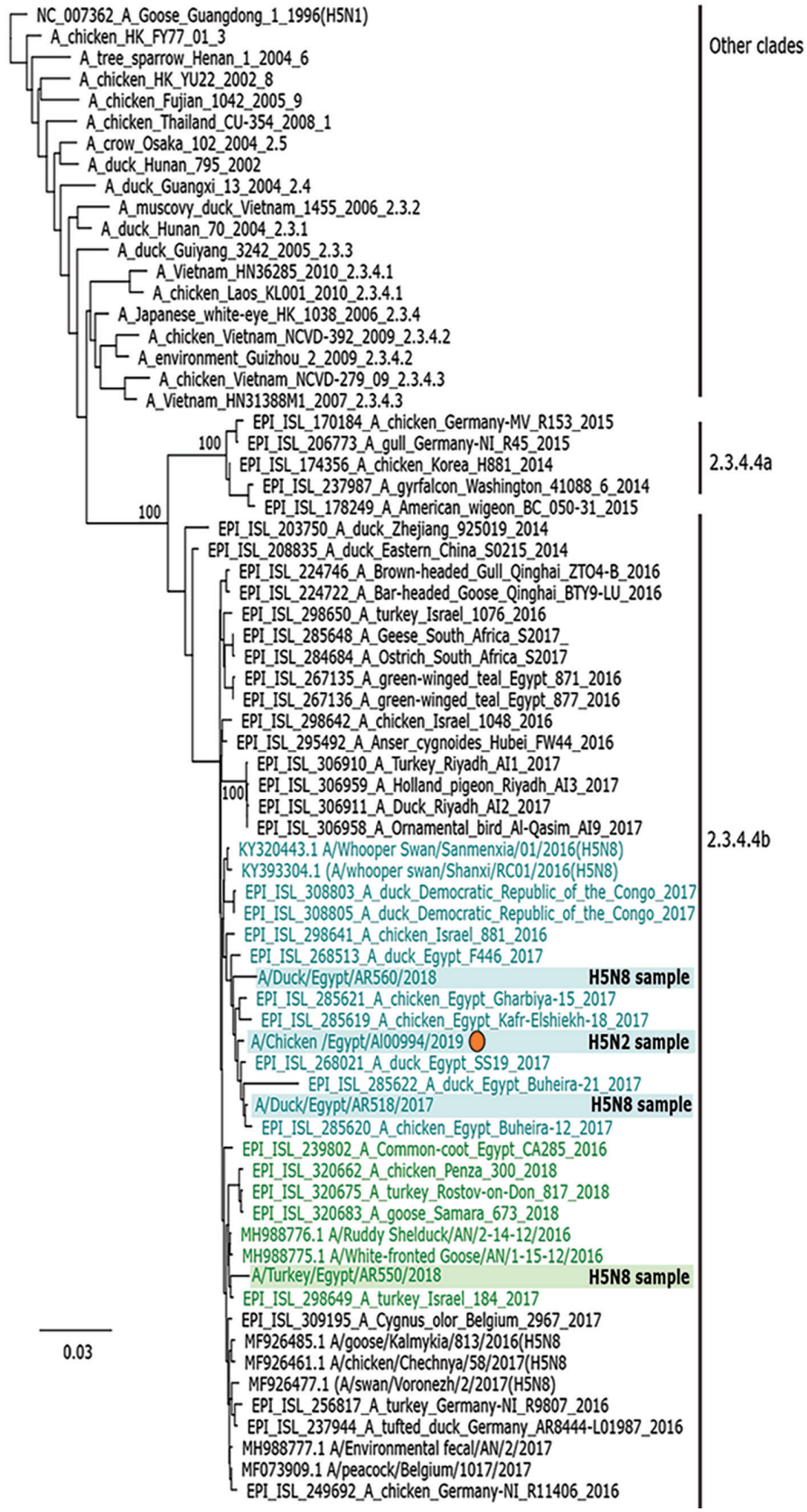

Figure 2. Phylogenetic analysis of the hemagglutinin segments of reassortant highly pathogenic avian influenza H5N2 and H5N8 viruses belonging to clade 2.3.3.4b from Egypt and reference viruses. Sequence analysis was based on alignment analyses by MAFFT version 7.450 embedded in the Geneious software suite, version 11.1.7 (https://www.geneious.com) with manual editing. We performed maximum-likelihood calculations using PhyML version 3.0 (http://www.atgc-montpellier.fr/phyml); we chose the best-fit model according to the Bayesian selection criterion using Model Finder embedded in Geneious. Colors indicate grouping of segment origin and match those shown in Figure 1: blue, most closely related to H5N8 viruses from China; green, most closely related to H5N8 viruses from Russia and Europe. GenBank or GISAID accession numbers (http://www.gisaid.org) are provided for reference sequences. Scale bar indicates nucleotide substitutions per site.

in Egypt during 2014 (*6*). The nucleoprotein segment and perhaps others were acquired from H9N2 viruses circulating in chickens in Egypt since 2010. Matrix and neuraminidase segments are identical in the pigeon and chicken H9N2 viruses. We identified no new mutations in the genome of reassortant H5N2 that would suggest increased adaptation to mammalian hosts. In addition, we observed 2 previously undescribed genotypes of HPAI H5N8 with distinct polymerase basic 1 and 2 segment origins (Figure 1). The composition of HPAI H5N1 viruses phylogenetically assigned to clade 2.2.1.2 (Figure 1; Appendix 1 Figure) was unaltered compared with other HPAI H5N1 viruses isolated since 2015.

Natural reassortants between H5 HPAI of the gs/GD lineage and H9N2 viruses, including subtype H5N2, have repeatedly emerged in Southeast Asia (*8*). So far, both the HPAI H5N1 2.2.1.2 and the co-circulating H9N2-G1 viruses appeared to be genotypically stable in poultry in Egypt. Successful forced reassortment of these viruses by co-cultivation and serum selection in embryonated chicken eggs ruled out a principal incompatibility between their genome segments (*5*); however, Naguib et al. did not rescue an H5N2 reassortant.

We and others have shown that HPAI H5 viruses of clade 2.3.4.4 have a high tendency to reassort with various influenza A viruses of wild birds or poultry (*9*). Thus, the incursion of clade 2.3.4.4b viruses into Egypt in 2016 not only added another antigenically distinct HPAI virus, but also signaled an increased reassortment risk. In fact, the 2.3.4.4b H5N8 virus proved to be a parent of the newly emerged H5N2 reassortant. Likewise, the second parental virus, an influenza A(H9N2) virus first detected in pigeons, was not described in Egypt before 2014. Genotypically, this H9N2 virus is distinct from the third parental virus, that is, the original H9N2 virus introduced to poultry in Egypt in 2010 (Figure 1). Infection of pigeons with clade 2.3.4.4b H5N8 HPAI virus has been described in Egypt, although pigeons are believed to be less susceptible to avian influenza infections (*10*). Although we cannot attribute the origin of the current HPAI H5N2 reassortant to a single host species, we cannot exclude pigeons as a possible host.

In March 2019, Egypt's Ministry of Agriculture announced the detection of a new influenza A(H5N2) virus from seemingly healthy ducks in the Dakahlia governorate (*11*); recently published information on this reassortant indicated the presence of a neuraminidase N2 segment of chicken H9N2 viruses in the background of an HPAI H5 clade 2.3.4.4b virus (*11*). Our data confirm the presence of a different H5N2 reassortant and its occurrence in chickens in different geographic regions of Egypt (Table 1). We detected the current reassortant HPAI H5N2 viruses in 2 different broiler farms in Beheira (January and March 2019) and 1 broiler farm in Fayoum (January 2019) governorates (Table 1). The HA amino acid sequence of these reassortants does not signal antigenic variation compared with parent HPAI H5 subtype of clade 2.3.4.4b. Antigenic and further phenotypic properties, such as host specificity, require investigation as soon as isolates are available. For the H5N2-positive samples, only FTA card material was available at Friedrich-Loeffler-Institut. However, H5N2 isolates were successfully generated at the Beni-Suef University, Egypt, but were currently not available for further antigenic and phenotypic analyses (M. El-Kady, unpub. data).

Intensified targeted surveillance in poultry and pigeons is urgently required and may lead to detection of additional reassortants. However, co-detection in a sample of H5N8 and H9N2 subtypes by reverse transcription quantitative PCR may blur the identification of H5N2 reassortants; plaque purification of such samples would aid in separating subtypes but cannot currently be used in routine diagnostics.

There is a risk for transboundary spread of HPAI A(H5N2) virus in northern Africa and the Middle East, and similar reassortment events are to be expected in regions where clade 2.3.4.4 HPAI and H9N2 viruses are co-circulating. Long-term solutions in combating avian influenza virus infections in poultry are sorely needed and would help to lower risks of human exposure to zoonotic avian influenza viruses such as the highly zoonotic H7N9 viruses in China that carry a full set of internal genes of an H9N2-G1–like avian influenza virus (*12*).

Acknowledgments

We acknowledge the originating and submitting laboratories that provided sequences available in the EpiFlu database (http://www.gisaid.org) (Appendix 2, https://wwwnc.cdc.gov/EID/26/1/19-0570-App2.xlsx).

K.E.H. received a grant from the Egyptian Ministry of Higher Education (Mission sector). This work was financed in part by the European Union Horizon 2020 program grant agreement DELTA-FLU (no. 727922).

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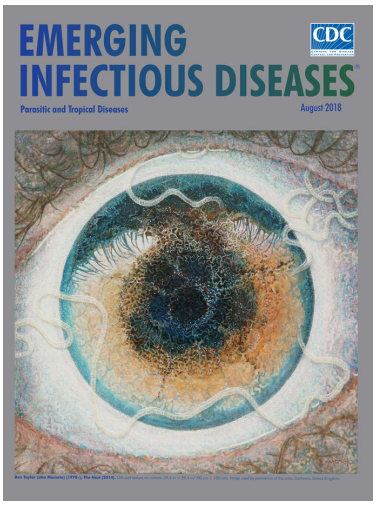
EID Podcast: A Worm's Eye View



Seeing a several-centimeters-long worm traversing the conjunctiva of an eye is often the moment when many people realize they are infected with *Loa loa*, commonly called the African eyeworm, a parasitic nematode that migrates throughout the subcutaneous and connective tissues of infected persons. Infection with this worm is called loiasis and is typically diagnosed either by the worm's appearance in the eye or by a history of localized Calabar swellings, named for the coastal Nigerian town where that symptom was initially observed among infected persons. Endemic to a large region of the western and central African rainforests, the *Loa loa* microfilariae are passed to humans primarily from bites by flies from two species of the genus *Chrysops*, *C. silacea* and *C. dimidiate*. The more than 29 million people who live in affected areas of Central and West Africa are potentially at risk of loiasis.

Ben Taylor, cover artist for the August 2018 issue of EID, discusses how his personal experience with the *Loa loa* parasite influenced this painting.

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Appendix 1

Appendix 1 Table. Avian influenza sequences included in study of highly pathogenic avian influenza virus A(H5N2), Egypt

AIV designation	AIV subtype	GISAID sequence accession no.							
		PB2	PB1	PA	HA	NP	NA	M	NS
A/Chicken/Egypt/AI00994/2019	H5N2	EPI1420452	EPI1420451	EPI1420416	EPI14204383	EPI1420434	EPI1420450	EPI1420384	EPI1420397
A/Duck/Egypt/AR518/2017	H5N8	EPI1420351	EPI1420350	EPI1420349	EPI1381391	EPI1420346	EPI1420345	EPI1420344	EPI1420347
A/Duck/Egypt/AR560/2018	H5N8	EPI1420334	EPI1420333	EPI1420332	EPI1381402	EPI1420330	EPI1420329	EPI1420328	EPI1420331
A/Turkey/Egypt/AR550/2018	H5N8	EPI1420343	EPI1420342	EPI1420341	EPI1381395	EPI1420339	EPI1420338	EPI1420336	EPI1420340
A/Duck/Egypt/AR526/2017	H5N1	EPI1420366	EPI1420364	EPI1420363	EPI1381393	EPI1420358	EPI1420357	EPI1420355	EPI1420362
A/Chicken/Egypt/AR528/2017	H5N1	EPI1420377	EPI1420376	EPI1420375	EPI1381432	EPI1420372	EPI14203571	EPI1420369	EPI1420373
A/Chicken/Egypt/AR544/2018	H9N2	EPI1420308	EPI1420307	EPI1420306	EPI1381412	EPI1420304	EPI1381411	EPI142035301	EPI1420305
A/Chicken/Egypt/AR545/2018	H9N2	EPI1420327	EPI1420326	EPI1420325	EPI131413	EPI1420323	EPI14203322	EPI1420321	EPI1420324
A/Chicken/Egypt/AR546/2018	H9N2	EPI1420318	EPI1420317	EPI1420316	EPI1381414	EPI1420314	EPI1420313	EPI1420312	EPI1420315
A/Chicken/Egypt/AI00986/2019	**H5**N2				EPI1542792				
A/Chicken/Egypt/AI00987/2019	**H5**, H9, N2				EPI1542793				
A/Chicken/Egypt/AI00988/2019	**H5**, H9, N2				EPI1542794				
A/Chicken/Egypt/AI00989/2019	**H5**, H9, N2				EPI1542795				
A/Chicken/Egypt/AI00990/2019	**H5**, H9, N2				EPI1542796				
A/Chicken/Egypt/AI00992/2019	**H5**N2				EPI1542798				

*Bold text indicates the hemagglutinin subtype sequenced in samples in which >1 HA subtype was detected. AIV, Avian influenza virus; HA, hemagglutinin, M, matrix; NA, neuraminidase; NP, nucleoprotein; NSP, nonstructural protein; PA, polymerase; PB, polymerase basic.

A
Influenza A virus (A/turkey/Wisconsin/1/1966(H9N2)
Influenza A virus (A/Chicken/Shanghai/F/98(H9N2)
Influenza A virus (A/Chicken/Beijing/1/94(H9N2)
Influenza A virus (A/duck/Hong Kong/Y439/1997(H9N2)
100
A/Duck/Egypt/AR526/2017
A/Chicken/Egypt/AR528/2017
H5N1 samples
Influenza A virus (A/duck/Giza/15292S/2015(H5N1)
Influenza A virus (A/chicken/Egypt/A10351A/2014(H5N1)
Influenza A virus (A/Egypt/MOH-NRC-8434/2014(H5N1)
Influenza A virus (A/chicken/Sharkeya/14209SS/2014(H5N1)
Influenza A virus (A/Egypt/MOH-NRC-7271/2014(H5N1)
Influenza A virus (A/Egypt/MOH-NRC-7305/2014(H5N1)
Influenza A virus (A/chicken/Italy/1875/2000(H7N1)
EPI_ISL_268511_A/Common-coot/Egypt/CA285/2016 (H5N8)
Influenza A virus (A/teal/Italy/3812/2005(H5N3)
Influenza A virus (A/spot-billed duck/Korea/KNU SYG06/2006(H5N3)
Influenza A virus (A/aquatic bird/Korea/w113/2006(H5N2)
Influenza A virus (A/chicken/Hubei/2856/2007(H5N1)
Influenza A virus (A/long-tailed shrike/Hong Kong/2762/2007(H5N1)
Influenza A virus (A/chicken/Hunan/3157/2006(H5N1)
Influenza A virus (A/bar-headed goose/Qinghai/F/2007(H5N1)
Influenza A virus (A/lesser kestrel/Hellongjiang/194/2007(H5N1)
Influenza A virus (A/duck/France/150233/2015(H5N2)
Influenza A virus (A/mallard duck/Georgia/11/2011(H1N1)
Influenza A virus (A/shelduck/Yasnopolyanske/4-08-02/2011(H1N1)
Influenza A virus (A/white-fronted goose/Netherlands/5/2011(H6N8)
Influenza A virus (A/turkey/Poland/08/2014(H9N2)
Influenza A virus (A/duck/France/150236/2015(H5N9)
Influenza A virus (A/Mallard/Netherlands/21/2013(H3N2)
Influenza A virus (A/chicken/France/150169a/2015(H5N1)
Influenza A virus (A/Mallard/Netherlands/33/2015(H4N6)
100
Influenza A virus (A/mallard/France/090360/2009(H9N2)
Influenza A virus (A/duck/Moscow/4652/2011(H4N6)
Influenza A virus (A/mallard/Republic of Georgia/13/2011(H6N2)
Influenza A virus (A/common Teal/Republic of Georgia/1/2011(H3N8)
Influenza A virus (A/black-headed gull/Netherlands/21/2014(H11N1)
Influenza A virus (A/mallard/Netherlands/29/2013(H3N8)
Influenza A virus (A/pigeon/Egypt/S10409A/2014(H9N2)
Influenza A virus (A/pigeon/Egypt/S10408B/2014(H9N2)
A/Chicken/Egypt/AR544/2018
A/Chicken /Egypt/AI00994/2019
H5N2 and H9N2 samples
A/Chicken/Egypt/AR546/2018
A/Chicken/Egypt/AR545/2018
Influenza A virus (A/chicken/Egypt/ABD1/2015(H9N2)
Influenza A virus (A/spot-billed duck/Shanghai/SH12/2014(H4N6)
Influenza A virus (A/duck/Zhejiang/6D10/2013(H2N8)
Influenza A virus (A/duck/Bangladesh/26980/2015(H7N9)
EPI_ISL_267135_A/green-winged_teal/Egypt/871/2016 (H5N8)
EPI_ISL_267136_A/green-winged_teal/Egypt/877/2016 (H5N8)
Influenza A virus (A/whooper swan/Shanxi/RC01/2016(H5N8)
Influenza A virus (A/duck/Democratic Republic of the Congo/17RS882-33/2017(H5N8)
Influenza A virus (A/Muscovy duck/Democratic Republic of the Congo/TCH4_PB2/2017(H5N8)
Influenza A virus (A/chicken/Uganda/17RS115-15/2017(H5N8)
Influenza A virus (A/white_winged_black_tern/Uganda/17RS115-3/2017(H5N8)
Influenza A virus (A/duck/Mongolia/521/2015(H3N8)
Influenza A virus (A/duck/Mongolia/30/2015(H3N8)
Influenza A virus (A/duck/Mongolia/482/2015(H2N3)
Influenza A virus (A/duck/Bangladesh/25891/2015(H4N6)
Influenza A virus (A/duck/Bangladesh/26918/2015(H3N6)
Influenza A virus (A/duck/Bangladesh/33137/2017(H3N2)
EPI_ISL_285622_A/duck/Egypt/Buheira-21/2017 (H5N8)
EPI_ISL_268021_A/duck/Egypt/SS19/2017 (H5N8)
EPI_ISL_285620_A/chicken/Egypt/Buheira-12/2017 (H5N8)
A/Duck/Egypt/AR518/2017
A/Duck/Egypt/AR560/2018
H5N8 samples
EPI_ISL_285619_A/chicken/Egypt/Kafr-Elshiekh-18/2017 (H5N8)
Influenza A virus (A/chicken/Belgium/807/2017(H5N8)
Influenza A virus (A/chicken/Chechnya/58/2017(H5N8)
Influenza A virus (A/swan/Krasnodar/44/2017(H5N8)
Influenza A virus (A/mute swan/Shimane/3211A001/2017(H5N6)
Influenza A virus (A/jungle crow/Hyogo/2803E011/2018(H5N6)
Influenza A virus (A/goose/Kalmykia/813/2016(H5N8)
Influenza A virus (A/Brahma chicken/Belgium/6153/2017(H5N8)
Influenza A virus (A/Guinea fowl/Belgium/6102/2017(H5N8)
Influenza A virus (A/swan/Voronezh/2/2017(H5N8)
Influenza A virus (A/Ruddy Shelduck/AN/2-14-12/2016(H5N8)
A/Turkey/Egypt/AR550/2018
H5N8 sample
Influenza A virus (A/White-fronted Goose/AN/1-15-12/2016(H5N8)
EPI_ISL_268513_A/duck/Egypt/F446/2017 (H5N8)
EPI_ISL_285621_A/chicken/Egypt/Gharbiya-15/2017 (H5N8)
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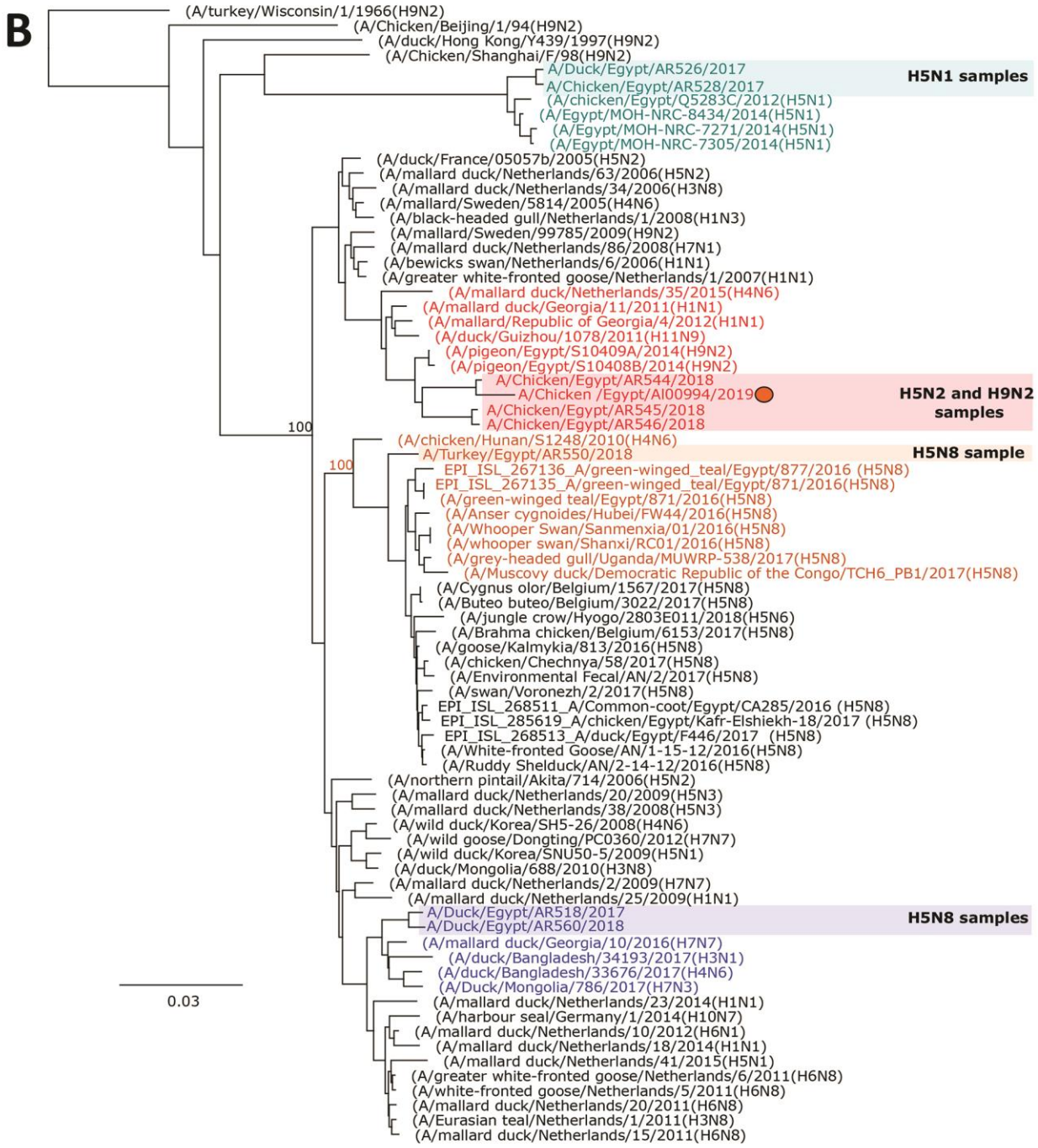
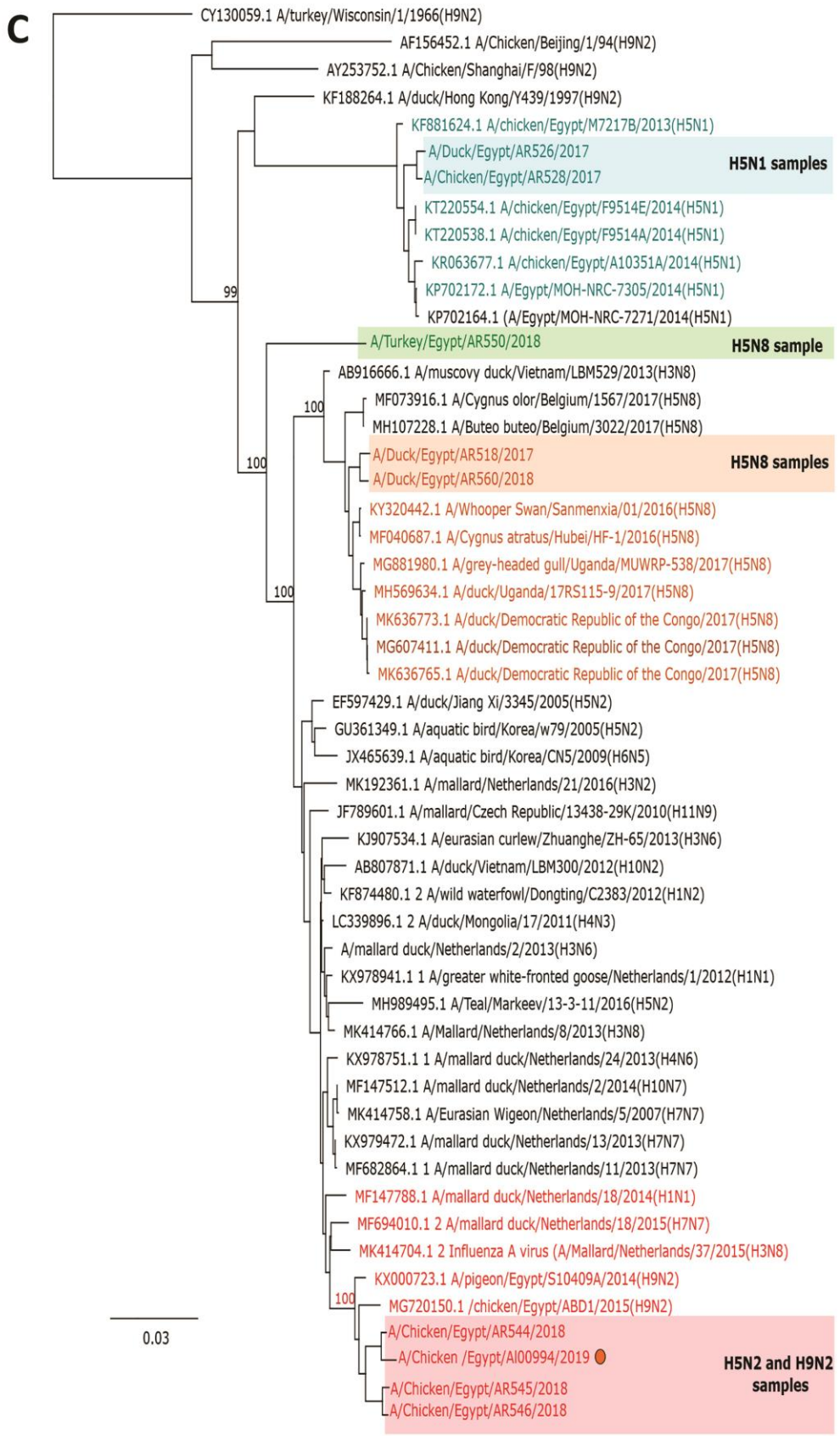

B
(A/turkey/Wisconsin/1/1966(H9N2)
(A/Chicken/Beijing/1/94(H9N2)
(A/duck/Hong Kong/Y439/1997(H9N2)
(A/Chicken/Shanghai/F/98(H9N2)
A/Duck/Egypt/AR526/2017
A/Chicken/Egypt/AR528/2017
(A/chicken/Egypt/Q5283C/2012(H5N1)
(A/Egypt/MOH-NRC-8434/2014(H5N1)
(A/Egypt/MOH-NRC-7271/2014(H5N1)
(A/Egypt/MOH-NRC-7305/2014(H5N1)
H5N1 samples
(A/duck/France/05057b/2005(H5N2)
(A/mallard duck/Netherlands/63/2006(H5N2)
(A/mallard duck/Netherlands/34/2006(H3N8)
(A/mallard/Sweden/5814/2005(H4N6)
(A/black-headed gull/Netherlands/1/2008(H1N3)
(A/mallard/Sweden/99785/2009(H9N2)
(A/mallard duck/Netherlands/86/2008(H7N1)
(A/bewicks swan/Netherlands/6/2006(H1N1)
(A/greater white-fronted goose/Netherlands/1/2007(H1N1)
(A/mallard duck/Netherlands/35/2015(H4N6)
(A/mallard duck/Georgia/11/2011(H1N1)
(A/mallard/Republic of Georgia/4/2012(H1N1)
(A/duck/Guizhou/1078/2011(H11N9)
(A/pigeon/Egypt/S10409A/2014(H9N2)
(A/pigeon/Egypt/S10408B/2014(H9N2)
A/Chicken/Egypt/AR544/2018
A/Chicken /Egypt/AI00994/2019
A/Chicken/Egypt/AR545/2018
A/Chicken/Egypt/AR546/2018
H5N2 and H9N2 samples
100
(A/chicken/Hunan/S1248/2010(H4N6)
A/Turkey/Egypt/AR550/2018
H5N8 sample
100
EPI_ISL_267136_A/green-winged_teal/Egypt/877/2016 (H5N8)
EPI_ISL_267135_A/green-winged_teal/Egypt/871/2016(H5N8)
(A/green-winged teal/Egypt/871/2016(H5N8)
(A/Anser cygnoides/Hubei/FW44/2016(H5N8)
(A/Whooper Swan/Sanmenxia/01/2016(H5N8)
(A/whooper swan/Shanxi/RC01/2016(H5N8)
(A/grey-headed gull/Uganda/MUWRP-538/2017(H5N8)
(A/Muscovy duck/Democratic Republic of the Congo/TCH6_PB1/2017(H5N8)
(A/Cygnus olor/Belgium/1567/2017(H5N8)
(A/Buteo buteo/Belgium/3022/2017(H5N8)
(A/jungle crow/Hyogo/2803E011/2018(H5N6)
(A/Brahma chicken/Belgium/6153/2017(H5N8)
(A/goose/Kalmykia/813/2016(H5N8)
(A/chicken/Chechnya/58/2017(H5N8)
(A/Environmental Fecal/AN/2/2017(H5N8)
(A/swan/Voronezh/2/2017(H5N8)
EPI_ISL_268511_A/Common-coot/Egypt/CA285/2016 (H5N8)
EPI_ISL_285619_A/chicken/Egypt/Kafr-Elshiekh-18/2017 (H5N8)
EPI_ISL_268513_A/duck/Egypt/F446/2017 (H5N8)
(A/White-fronted Goose/AN/1-15-12/2016(H5N8)
(A/Ruddy Shelduck/AN/2-14-12/2016(H5N8)
(A/northern pintail/Akita/714/2006(H5N2)
(A/mallard duck/Netherlands/20/2009(H5N3)
(A/mallard duck/Netherlands/38/2008(H5N3)
(A/wild duck/Korea/SH5-26/2008(H4N6)
(A/wild goose/Dongting/PC0360/2012(H7N7)
(A/wild duck/Korea/SNU50-5/2009(H5N1)
(A/duck/Mongolia/688/2010(H3N8)
(A/mallard duck/Netherlands/2/2009(H7N7)
(A/mallard duck/Netherlands/25/2009(H1N1)
A/Duck/Egypt/AR518/2017
A/Duck/Egypt/AR560/2018
H5N8 samples
(A/mallard duck/Georgia/10/2016(H7N7)
(A/duck/Bangladesh/34193/2017(H3N1)
(A/duck/Bangladesh/33676/2017(H4N6)
(A/Duck/Mongolia/786/2017(H7N3)
0.03
(A/mallard duck/Netherlands/23/2014(H1N1)
(A/harbour seal/Germany/1/2014(H10N7)
(A/mallard duck/Netherlands/10/2012(H6N1)
(A/mallard duck/Netherlands/18/2014(H1N1)
(A/mallard duck/Netherlands/41/2015(H5N1)
(A/greater white-fronted goose/Netherlands/6/2011(H6N8)
(A/white-fronted goose/Netherlands/5/2011(H6N8)
(A/mallard duck/Netherlands/20/2011(H6N8)
(A/Eurasian teal/Netherlands/1/2011(H3N8)
(A/mallard duck/Netherlands/15/2011(H6N8)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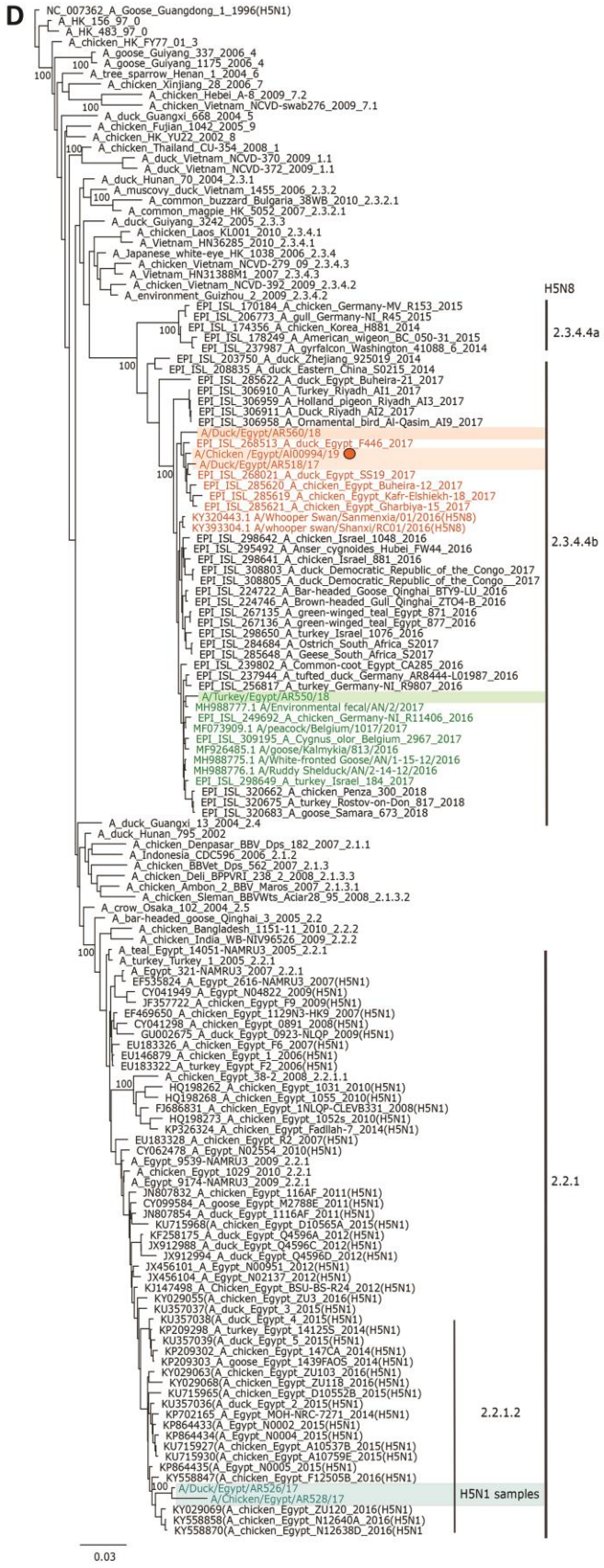
CY130059.1 A/turkey/Wisconsin/1/1966(H9N2)
AF156452.1 A/Chicken/Beijing/1/94(H9N2)
AY253752.1 A/Chicken/Shanghai/F/98(H9N2)
KF188264.1 A/duck/Hong Kong/Y439/1997(H9N2)
KF881624.1 A/chicken/Egypt/M7217B/2013(H5N1)
A/Duck/Egypt/AR526/2017
A/Chicken/Egypt/AR528/2017
H5N1 samples
KT220554.1 A/chicken/Egypt/F9514E/2014(H5N1)
KT220538.1 A/chicken/Egypt/F9514A/2014(H5N1)
KR063677.1 A/chicken/Egypt/A10351A/2014(H5N1)
KP702172.1 A/Egypt/MOH-NRC-7305/2014(H5N1)
KP702164.1 (A/Egypt/MOH-NRC-7271/2014(H5N1)
99
A/Turkey/Egypt/AR550/2018
H5N8 sample
AB916666.1 A/muscovy duck/Vietnam/LBM529/2013(H3N8)
100
MF073916.1 A/Cygnus olor/Belgium/1567/2017(H5N8)
MH107228.1 A/Buteo buteo/Belgium/3022/2017(H5N8)
100
A/Duck/Egypt/AR518/2017
A/Duck/Egypt/AR560/2018
H5N8 samples
KY320442.1 A/Whooper Swan/Sanmenxia/01/2016(H5N8)
MF040687.1 A/Cygnus atratus/Hubei/HF-1/2016(H5N8)
MG881980.1 A/grey-headed gull/Uganda/MUWRP-538/2017(H5N8)
MH569634.1 A/duck/Uganda/17RS115-9/2017(H5N8)
100
MK636773.1 A/duck/Democratic Republic of the Congo/2017(H5N8)
MG607411.1 A/duck/Democratic Republic of the Congo/2017(H5N8)
MK636765.1 A/duck/Democratic Republic of the Congo/2017(H5N8)
EF597429.1 A/duck/Jiang Xi/3345/2005(H5N2)
GU361349.1 A/aquatic bird/Korea/w79/2005(H5N2)
JX465639.1 A/aquatic bird/Korea/CN5/2009(H6N5)
MK192361.1 A/mallard/Netherlands/21/2016(H3N2)
JF789601.1 A/mallard/Czech Republic/13438-29K/2010(H11N9)
KJ907534.1 A/eurasian curlew/Zhuanghe/ZH-65/2013(H3N6)
AB807871.1 A/duck/Vietnam/LBM300/2012(H10N2)
KF874480.1 2 A/wild waterfowl/Dongting/C2383/2012(H1N2)
LC339896.1 2 A/duck/Mongolia/17/2011(H4N3)
A/mallard duck/Netherlands/2/2013(H3N6)
KX978941.1 1 A/greater white-fronted goose/Netherlands/1/2012(H1N1)
MH989495.1 A/Teal/Markeev/13-3-11/2016(H5N2)
MK414766.1 A/Mallard/Netherlands/8/2013(H3N8)
KX978751.1 1 A/mallard duck/Netherlands/24/2013(H4N6)
MF147512.1 A/mallard duck/Netherlands/2/2014(H10N7)
MK414758.1 A/Eurasian Wigeon/Netherlands/5/2007(H7N7)
KX979472.1 A/mallard duck/Netherlands/13/2013(H7N7)
MF682864.1 1 A/mallard duck/Netherlands/11/2013(H7N7)
MF147788.1 A/mallard duck/Netherlands/18/2014(H1N1)
MF694010.1 2 A/mallard duck/Netherlands/18/2015(H7N7)
MK414704.1 2 Influenza A virus (A/Mallard/Netherlands/37/2015(H3N8)
KX000723.1 A/pigeon/Egypt/S10409A/2014(H9N2)
100
MG720150.1 /chicken/Egypt/ABD1/2015(H9N2)
0.03
A/Chicken/Egypt/AR544/2018
A/Chicken /Egypt/AI00994/2019
A/Chicken/Egypt/AR545/2018
A/Chicken/Egypt/AR546/2018
H5N2 and H9N2 samples


D
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A_chicken_HK_FY77_01_3
A_goose_Guiyang_337_2006_4
A_goose_Guiyang_1175_2006_4
A_tree_sparrow_Henan_1_2004_6
A_chicken_Xinjiang_28_2006_7
A_chicken_Hebei_A-8_2009_7.2
A_chicken_Vietnam_NCVD-swab276_2009_7.1
A_duck_Guangxi_668_2004_5
A_chicken_Fujian_1042_2005_9
A_chicken_HK_YU22_2002_8
A_chicken_Thailand_CU-354_2008_1
A_duck_Vietnam_NCVD-370_2009_1.1
A_duck_Vietnam_NCVD-372_2009_1.1
A_duck_Hunan_70_2004_2.3.1
A_muscovy_duck_Vietnam_1455_2006_2.3.2
A_common_buzzard_Bulgaria_38WB_2010_2.3.2.1
A_common_magpie_HK_5052_2007_2.3.2.1
A_duck_Guiyang_3242_2005_2.3.3
A_chicken_Laos_KL001_2010_2.3.4.1
A_Vietnam_HN36285_2010_2.3.4.1
A_Japanese_white-eye_HK_1038_2006_2.3.4
A_chicken_Vietnam_NCVD-279_09_2.3.4.3
A_Vietnam_HN31388M1_2007_2.3.4.3
A_chicken_Vietnam_NCVD-392_2009_2.3.4.2
A_environment_Guizhou_2_2009_2.3.4.2
H5N8
EPI_ISL_170184_A_chicken_Germany-MV_R153_2015
EPI_ISL_206773_A_gull_Germany-NI_R45_2015
EPI_ISL_174356_A_chicken_Korea_H881_2014
EPI_ISL_178249_A_American_wigeon_BC_050-31_2015
EPI_ISL_237987_A_gyrfalcon_Washington_41088_6_2014
2.3.4.4a
EPI_ISL_203750_A_duck_Zhejiang_925019_2014
EPI_ISL_208835_A_duck_Eastern_China_S0215_2014
EPI_ISL_285622_A_duck_Egypt_Buheira-21_2017
EPI_ISL_306910_A_Turkey_Riyadh_AI1_2017
EPI_ISL_306959_A_Holland_pigeon_Riyadh_AI3_2017
EPI_ISL_306911_A_Duck_Riyadh_AI2_2017
EPI_ISL_306958_A_Ornamental_bird_Al-Qasim_AI9_2017
A/Duck/Egypt/AR560/18
EPI_ISL_268513_A_duck_Egypt_F446_2017
A/Chicken /Egypt/AI00994/19
A/Duck/Egypt/AR518/17
EPI_ISL_268021_A_duck_Egypt_SS19_2017
EPI_ISL_285620_A_chicken_Egypt_Buheira-12_2017
EPI_ISL_285619_A_chicken_Egypt_Kafr-Elshiekh-18_2017
EPI_ISL_285621_A_chicken_Egypt_Gharbiya-15_2017
KY320443.1 A/Whooper Swan/Sanmenxia/01/2016(H5N8)
KY393304.1 A/whooper swan/Shanxi/RC01/2016(H5N8)
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EPI_ISL_295492_A_Anser_cygnoides_Hubei_FW44_2016
EPI_ISL_298641_A_chicken_Israel_881_2016
EPI_ISL_308803_A_duck_Democratic_Republic_of_the_Congo_2017
EPI_ISL_308805_A_duck_Democratic_Republic_of_the_Congo__2017
EPI_ISL_224722_A_Bar-headed_Goose_Qinghai_BTY9-LU_2016
EPI_ISL_224746_A_Brown-headed_Gull_Qinghai_ZTO4-B_2016
EPI_ISL_267135_A_green-winged_teal_Egypt_871_2016
EPI_ISL_267136_A_green-winged_teal_Egypt_877_2016
EPI_ISL_298650_A_turkey_Israel_1076_2016
EPI_ISL_284684_A_Ostrich_South_Africa_S2017
EPI_ISL_285648_A_Geese_South_Africa_S2017
EPI_ISL_239802_A_Common-coot_Egypt_CA285_2016
EPI_ISL_237944_A_tufted_duck_Germany_AR8444-L01987_2016
EPI_ISL_256817_A_turkey_Germany-NI_R9807_2016
A/Turkey/Egypt/AR550/18
MH988777.1 A/Environmental fecal/AN/2/2017
EPI_ISL_249692_A_chicken_Germany-NI_R11406_2016
MF073909.1 A/peacock/Belgium/1017/2017
EPI_ISL_309195_A_Cygnus_olor_Belgium_2967_2017
MF926485.1 A/goose/Kalmykia/813/2016
MH988775.1 A/White-fronted Goose/AN/1-15-12/2016
MH988776.1 A/Ruddy Shelduck/AN/2-14-12/2016
EPI_ISL_298649_A_turkey_Israel_184_2017
EPI_ISL_320662_A_chicken_Penza_300_2018
EPI_ISL_320675_A_turkey_Rostov-on-Don_817_2018
EPI_ISL_320683_A_goose_Samara_673_2018
2.3.4.4b
A_duck_Guangxi_13_2004_2.4
A_duck_Hunan_795_2002
A_chicken_Denpasar_BBV_Dps_182_2007_2.1.1
A_Indonesia_CDC596_2006_2.1.2
A_chicken_BBVet_Dps_562_2007_2.1.3
A_chicken_Deli_BPPVRI_238_2_2008_2.1.3.3
A_chicken_Ambon_2_BBV_Maros_2007_2.1.3.1
A_chicken_Sleman_BBVWts_Aciar28_95_2008_2.1.3.2
A_crow_Osaka_102_2004_2.5
A_bar-headed_goose_Qinghai_3_2005_2.2
A_chicken_Bangladesh_1151-11_2010_2.2.2
A_chicken_India_WB-NIV96526_2009_2.2.2
A_teal_Egypt_14051-NAMRU3_2005_2.2.1
A_turkey_Turkey_1_2005_2.2.1
A_Egypt_321-NAMRU3_2007_2.2.1
EF535824_A_Egypt_2616-NAMRU3_2007(H5N1)
CY041949_A_Egypt_N04822_2009(H5N1)
JF357722_A_chicken_Egypt_F9_2009(H5N1)
EF469650_A_chicken_Egypt_1129N3-HK9_2007(H5N1)
CY041298_A_chicken_Egypt_0891_2008(H5N1)
GU002675_A_duck_Egypt_0923-NLQP_2009(H5N1)
EU183326_A_chicken_Egypt_F6_2007(H5N1)
EU146879_A_chicken_Egypt_1_2006(H5N1)
EU183322_A_turkey_Egypt_F2_2006(H5N1)
A_chicken_Egypt_38-2_2008_2.2.1.1
HQ198262_A_chicken_Egypt_1031_2010(H5N1)
HQ198268_A_chicken_Egypt_1055_2010(H5N1)
FJ686831_A_chicken_Egypt_1NLQP-CLEVB331_2008(H5N1)
HQ198273_A_chicken_Egypt_1052s_2010(H5N1)
KP326324_A_chicken_Egypt_Fadllah-7_2014(H5N1)
EU183328_A_chicken_Egypt_R2_2007(H5N1)
CY062478_A_Egypt_N02554_2010(H5N1)
A_Egypt_9539-NAMRU3_2009_2.2.1
A_chicken_Egypt_1029_2010_2.2.1
A_Egypt_9174-NAMRU3_2009_2.2.1
JN807832_A_chicken_Egypt_1116AF_2011(H5N1)
CY099584_A_goose_Egypt_M2788E_2011(H5N1)
JN807854_A_duck_Egypt_1116AF_2011(H5N1)
KU715968(A_chicken_Egypt_D10565A_2015(H5N1)
KF258175_A_duck_Egypt_Q4596A_2012(H5N1)
JX912988_A_duck_Egypt_Q4596C_2012(H5N1)
JX912994_A_duck_Egypt_Q4596D_2012(H5N1)
JX456101_A_Egypt_N00951_2012(H5N1)
JX456104_A_Egypt_N02137_2012(H5N1)
KJ147498_A_Chicken_Egypt_BSU-BS-R24_2012(H5N1)
KY029055(A_chicken_Egypt_ZU3_2016(H5N1)
KU357037(A_duck_Egypt_3_2015(H5N1)
2.2.1
KU357038(A_duck_Egypt_4_2015(H5N1)
KP209298_A_turkey_Egypt_14125S_2014(H5N1)
KU357039(A_duck_Egypt_5_2015(H5N1)
KP209302_A_chicken_Egypt_147CA_2014(H5N1)
KP209303_A_goose_Egypt_1439FAOS_2014(H5N1)
KY029063(A_chicken_Egypt_ZU103_2016(H5N1)
KY029068(A_chicken_Egypt_ZU118_2016(H5N1)
KU715965(A_chicken_Egypt_D10552B_2015(H5N1)
KU357036(A_duck_Egypt_2_2015(H5N1)
KP702165_A_Egypt_MOH-NRC-7271_2014(H5N1)
KP864433(A_Egypt_N0002_2015(H5N1)
KP864434(A_Egypt_N0004_2015(H5N1)
KU715927(A_chicken_Egypt_A10537B_2015(H5N1)
KU715930(A_chicken_Egypt_A10759E_2015(H5N1)
KP864435(A_Egypt_N0005_2015(H5N1)
KY558847(A_chicken_Egypt_F12505B_2016(H5N1)
2.2.1.2
A/Duck/Egypt/AR526/17
A/Chicken/Egypt/AR528/17
H5N1 samples
KY029069(A_chicken_Egypt_ZU120_2016(H5N1)
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KY558870(A_chicken_Egypt_N12638D_2016(H5N1
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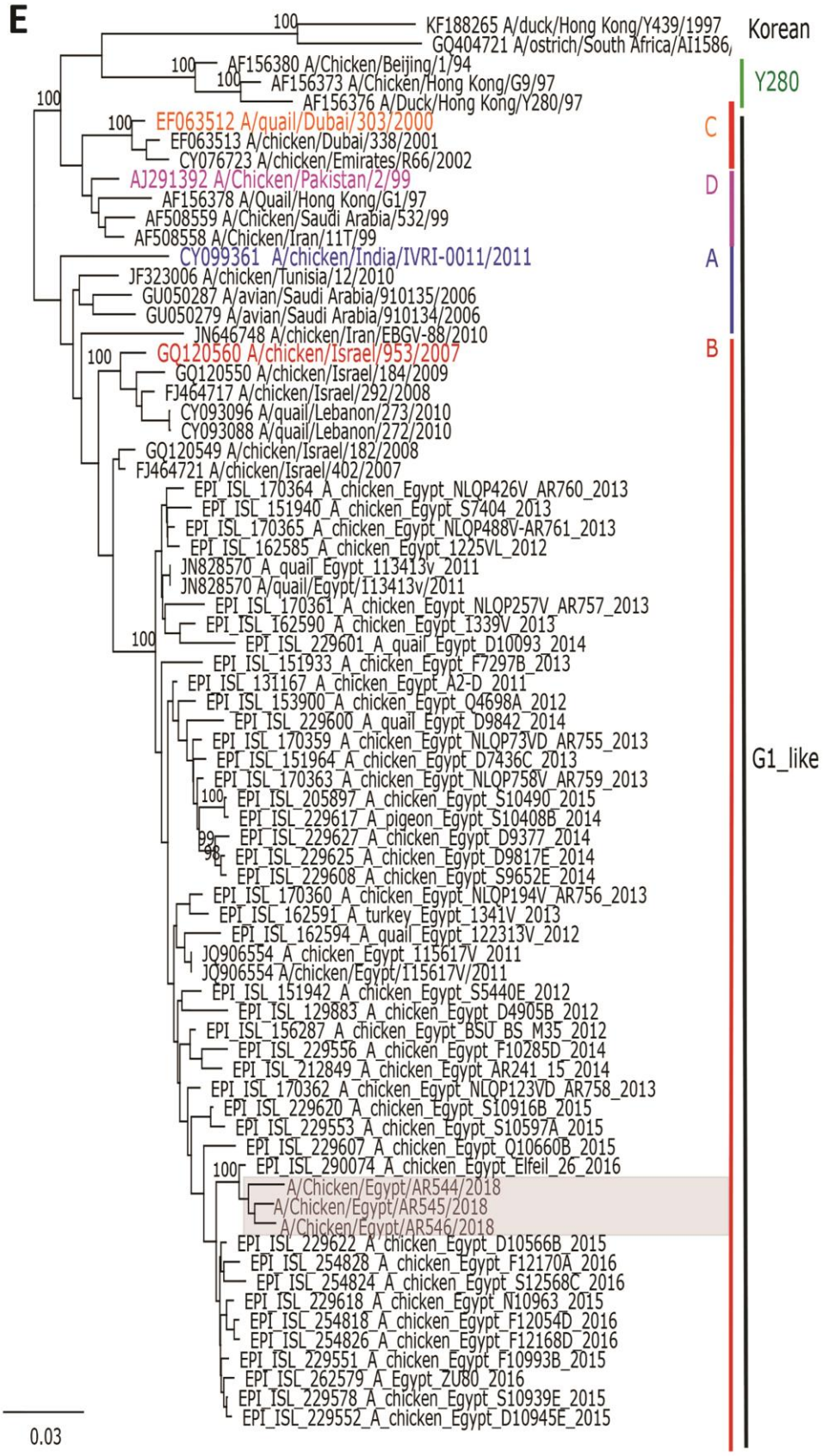
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Korean
AF156380 A/Chicken/Beijing/1/94
AF156373 A/Chicken/Hong Kong/G9/97
AF156376 A/Duck/Hong Kong/Y280/97
Y280
EF063512 A/quail/Dubai/303/2000
EF063513 A/chicken/Dubai/338/2001
CY076723 A/chicken/Emirates/R66/2002
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AJ291392 A/Chicken/Pakistan/2/99
AF156378 A/Quail/Hong Kong/G1/97
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AF508558 A/Chicken/Iran/11T/99
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GU050287 A/avian/Saudi Arabia/910135/2006
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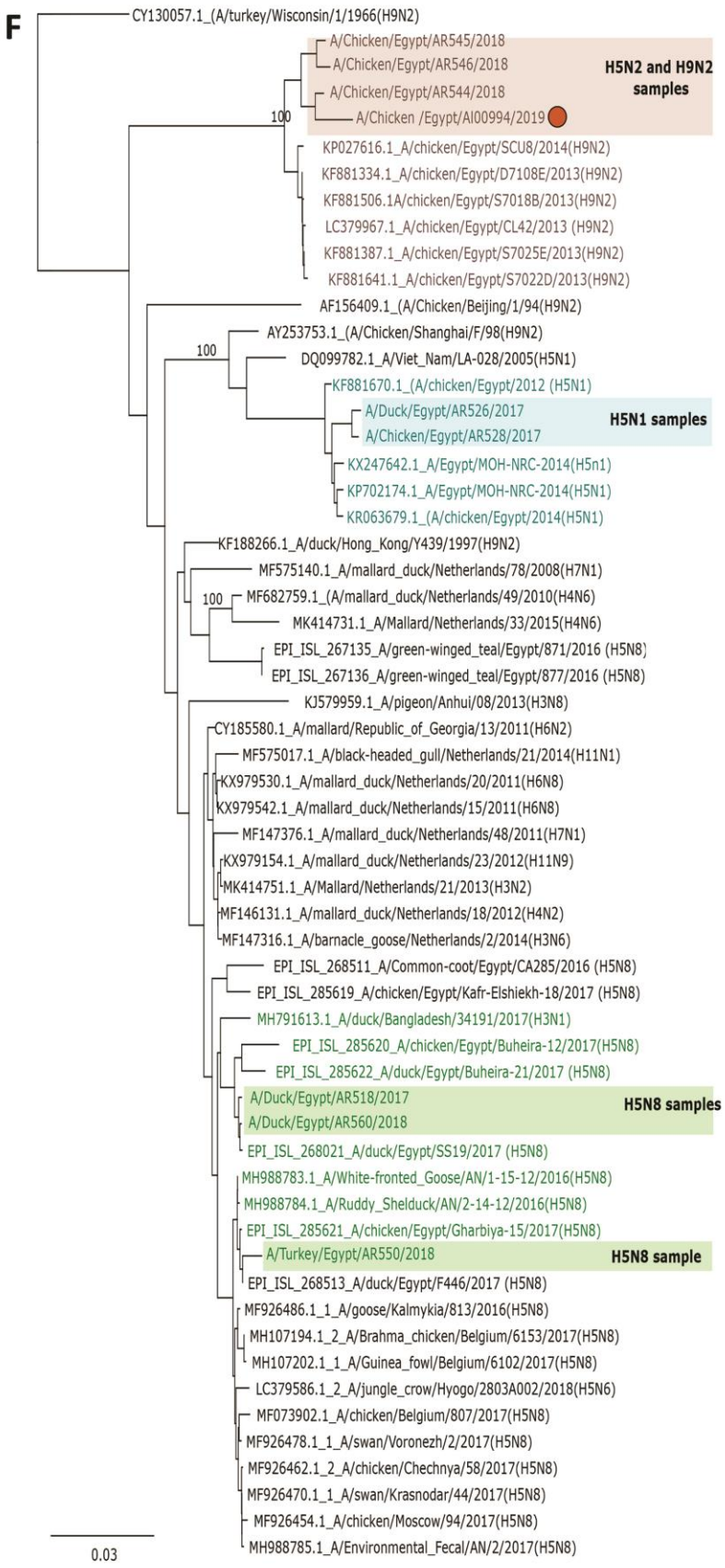
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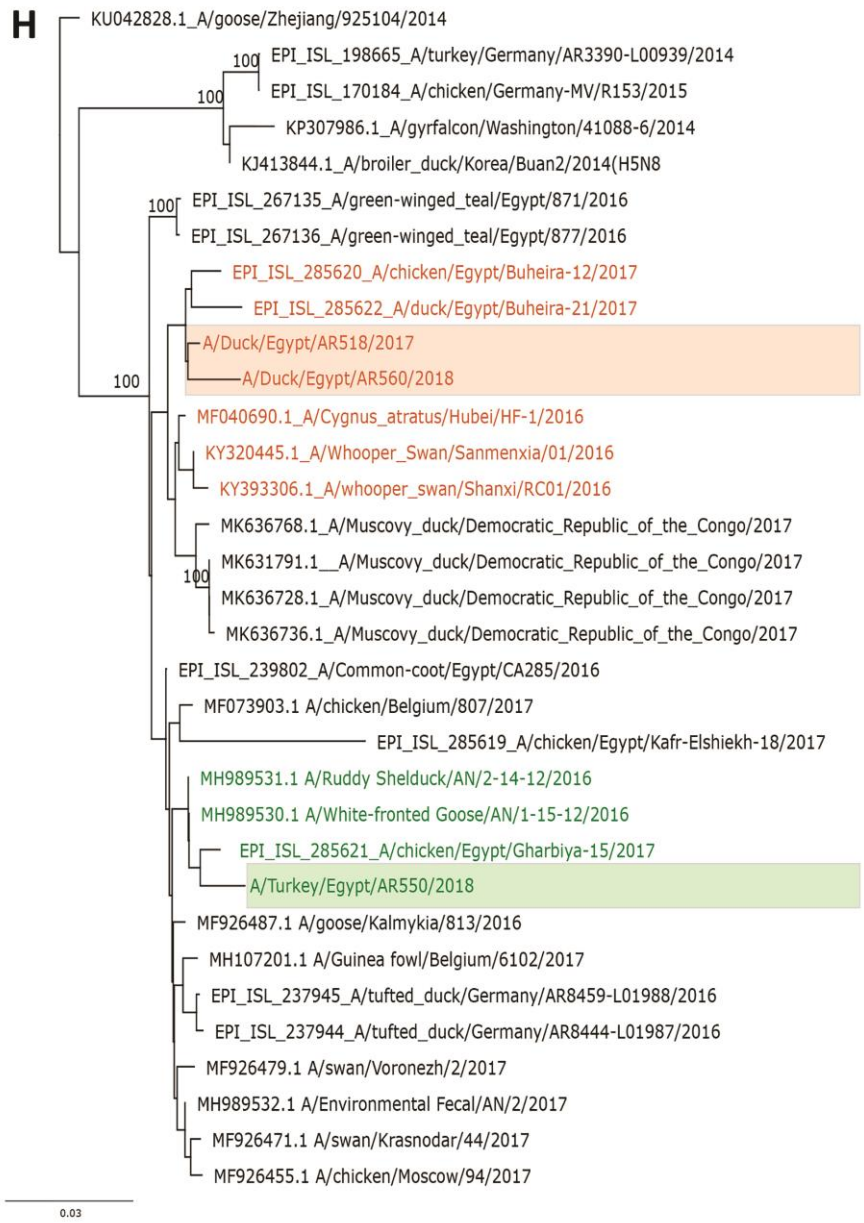
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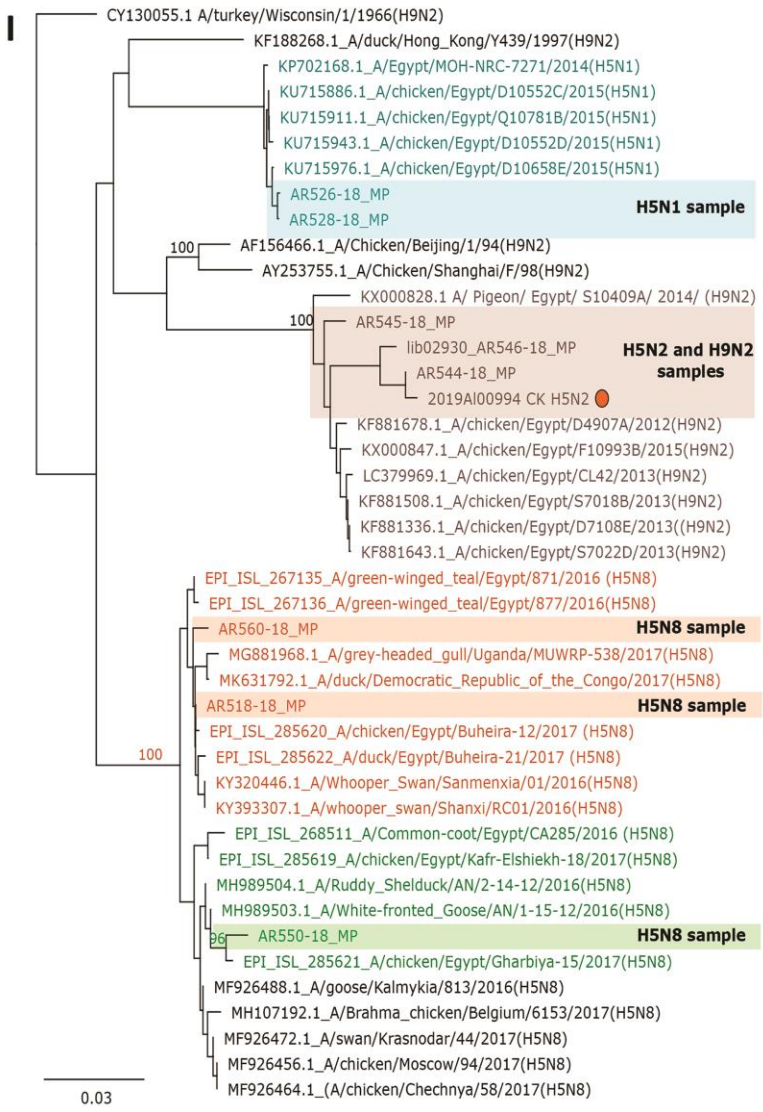
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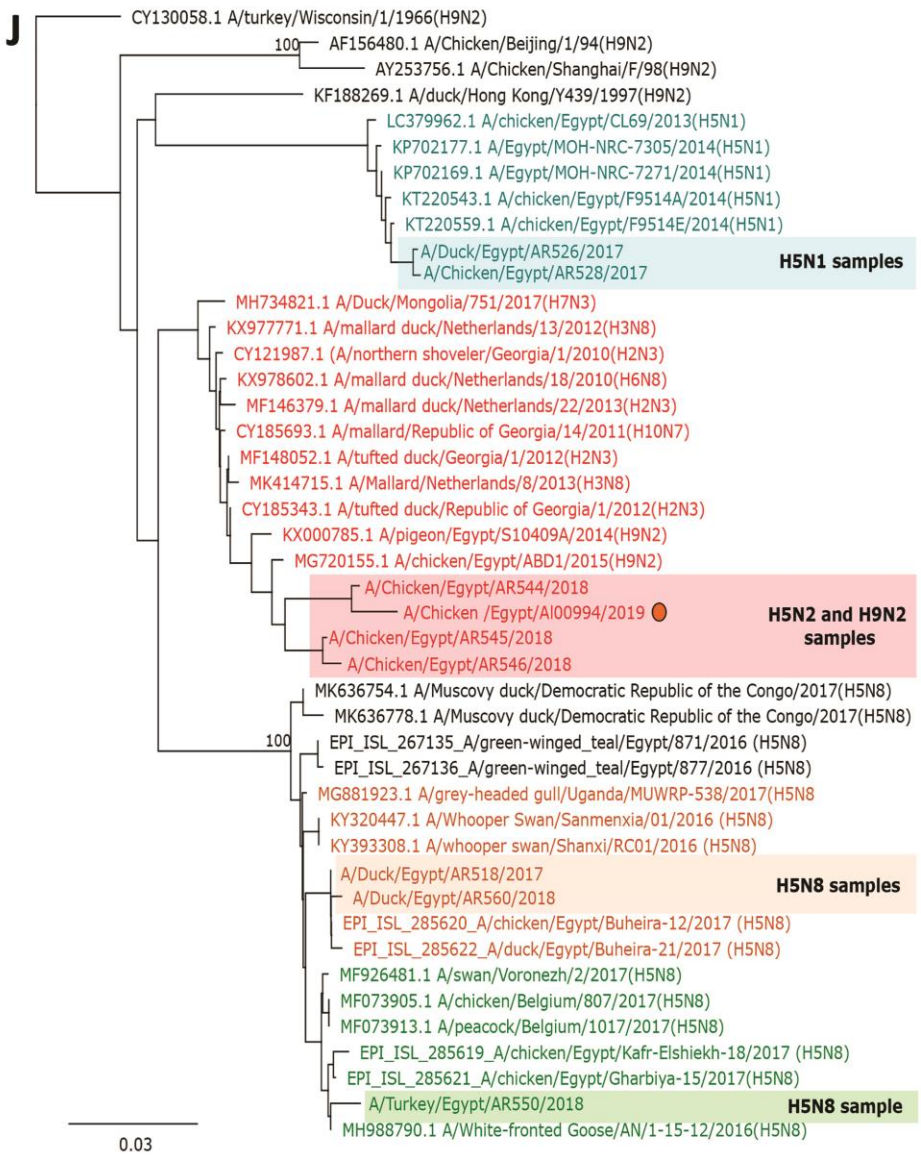

Appendix Figure. Phylogenetic analysis of segments of highly pathogenic avian influenza (HPAI) and low pathogenicity avian influenza (LPAI). Sequence analysis was based on alignment analyses by MAFFTv7.450 embedded in the Geneious software suite, version 11.1.7(https://www.geneious.com) with manual editing. We performed maximum likelihood calculations using PhyML version 3.0 (http://www.atgc-montpellier.fr/phyml) ,we chose the best-fit model according to the Bayesian selection criterion using Model Finder embedded in the Geneious software suite, version 11.1.7https://www.geneious.com). Colors indicate grouping of segment origin according to phylogenetic analyses: highly pathogenic avian influenza virus (HPAIV) H5N1 2.2.1.2 from Egypt (cyan); H9N2 subtype from Egypt circulating in chickens since 2010 (gray); H9N2 subtype from Egypt first detected in pigeons in 2014 (red); PB2 segment most closely related to an H3N6 virus from Bangladesh (purple); PB1 segment most closely related to an H7N7 virus from Georgia (blue); segments most closely related

to H5N8 viruses from China (orange) or Russia (green). Acknowledgments for sequences shared via EpiFlu are given in Appendix Table 2. A) Polymerase basic protein 2 (PB2) of HPAI H5N8, H5N1, and reassortant H5N2, and LPAI H9N2. B) Polymerase basic protein 1 (PB1) of HPAI H5N8, H5N1, and reassortant H5N2 and LPAI H9N2. C) Polymerase acidic protein (PA) of HPAI H5N8, H5N1, and reassortant H5N2, and LPAI H9N2. E) Hemagglutinin (HA _9) of LPAI H9N2. F) Nucleoprotein (NP) of HPAI H5N8, H5N1, and reassortant H5N2, and LPAI H9N2. G) Neuraminidase protein (NA_2) of HPAI reassortant H5N2 and LPAI H9N2. H) Neuraminidase protein (NA_8) of HPAI H5N8. I) Matrix protein (M) of HPAI H5N8, H5N1, and reassortant H5N2, and LPAI H9N2. J) Nonstructural protein (NS) of HPAI H5N8, H5N1, and reassortant H5N2, and LPAI H9N2.