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

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)
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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
A/Chicken/Egypt/AR546/2018
A/Chicken/Egypt/AR545/2018
H5N2 and H9N2 samples
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)
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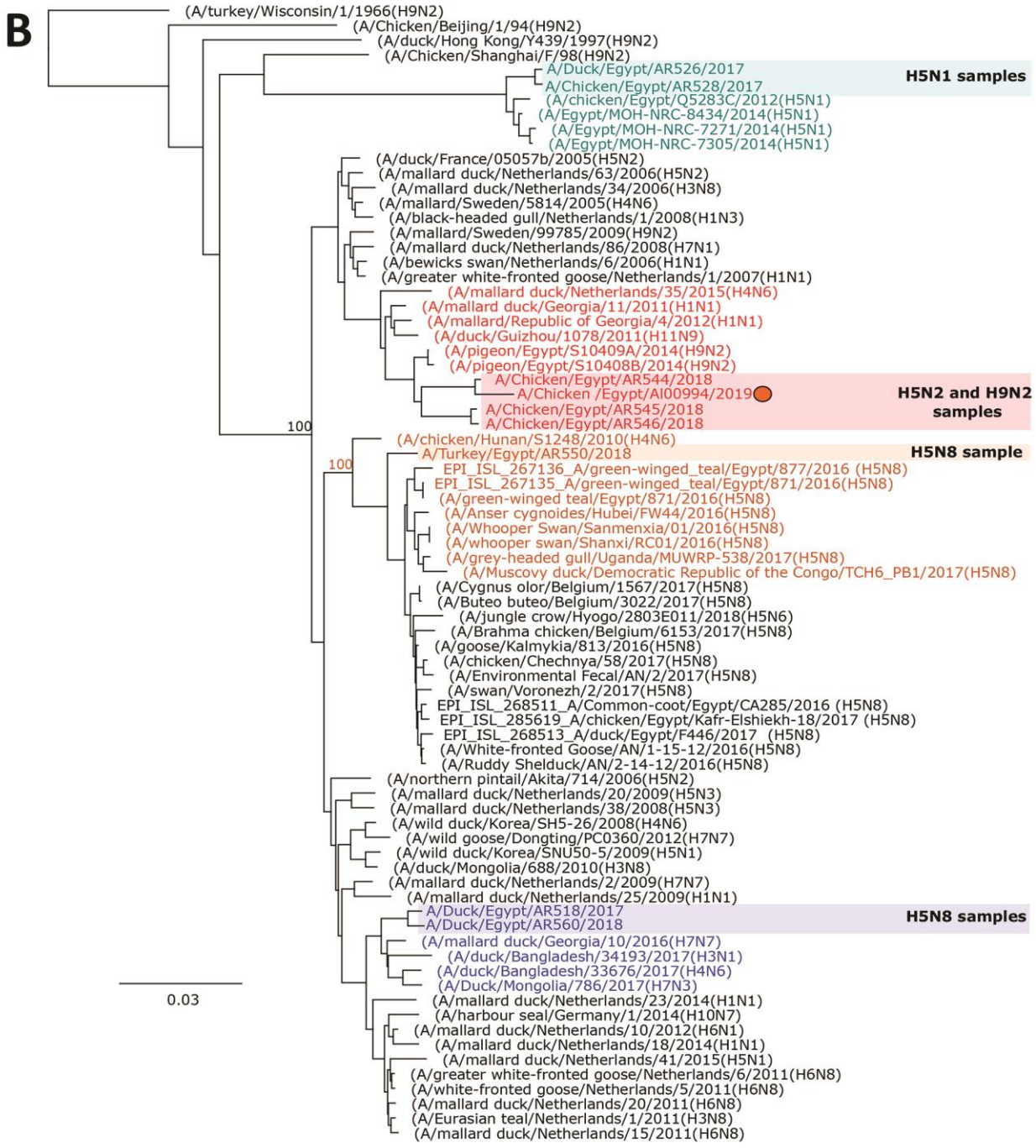
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(A/duck/Hong Kong/Y439/1997(H9N2)
(A/Chicken/Shanghai/F/98(H9N2)
A/Duck/Egypt/AR526/2017
A/Chicken/Egypt/AR528/2017
H5N1 samples
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(A/Egypt/MOH-NRC-8434/2014(H5N1)
(A/Egypt/MOH-NRC-7271/2014(H5N1)
(A/Egypt/MOH-NRC-7305/2014(H5N1)
(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
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(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)
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(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)
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(A/duck/Bangladesh/33676/2017(H4N6)
(A/Duck/Mongolia/786/2017(H7N3)
(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)
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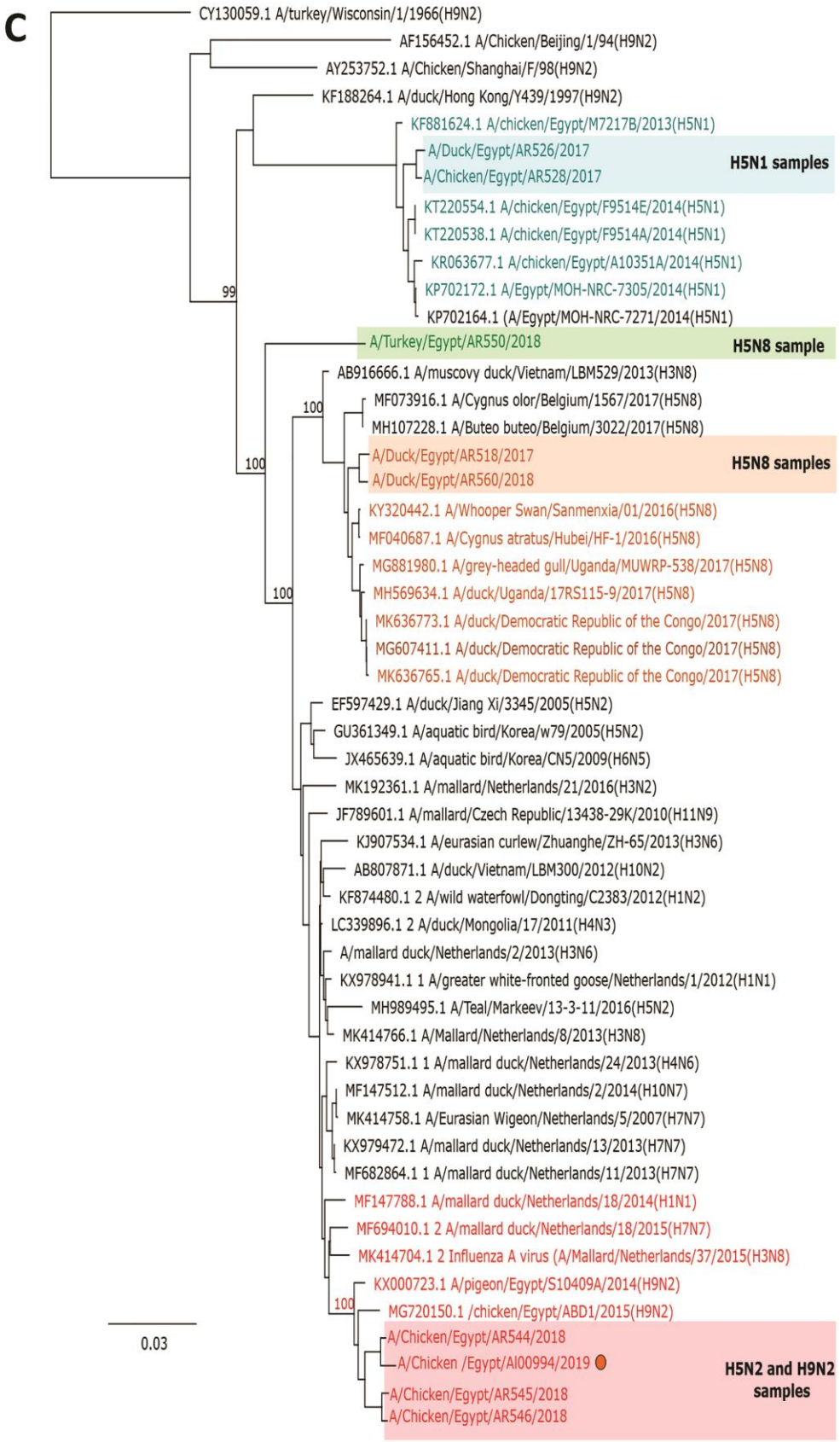
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KF188264.1 A/duck/Hong Kong/Y439/1997(H9N2)
KF881624.1 A/chicken/Egypt/M7217B/2013(H5N1)
A/Duck/Egypt/AR526/2017
A/Chicken/Egypt/AR528/2017
H5N1 samples
KT220554.1 A/chicken/Egypt/F9514E/2014(H5N1)
KT220538.1 A/chicken/Egypt/F9514A/2014(H5N1)
KR063677.1 A/chicken/Egypt/A10351A/2014(H5N1)
KP702172.1 A/Egypt/MOH-NRC-7305/2014(H5N1)
KP702164.1 (A/Egypt/MOH-NRC-7271/2014(H5N1)
A/Turkey/Egypt/AR550/2018
H5N8 sample
AB916666.1 A/muscovy duck/Vietnam/LBM529/2013(H3N8)
MF073916.1 A/Cygnus olor/Belgium/1567/2017(H5N8)
MH107228.1 A/Buteo buteo/Belgium/3022/2017(H5N8)
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)
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)
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MG720150.1 /chicken/Egypt/ABD1/2015(H9N2)
A/Chicken/Egypt/AR544/2018
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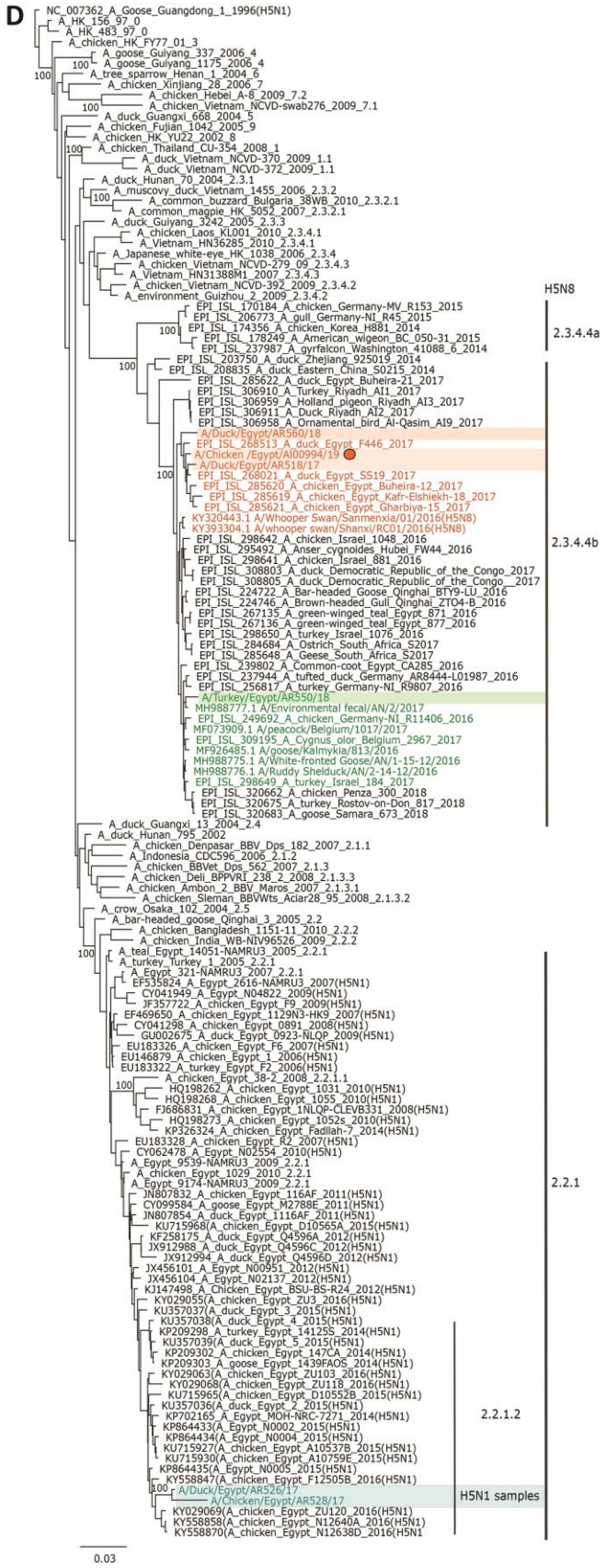
A/Chicken/Egypt/AR546/2018
H5N2 and H9N2 samples
99
100
100
100
100
0.03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A_common_magpie_HK_5052_2007_2.3.2.1
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A_chicken_Laos_KL001_2010_2.3.4.1
A_Vietnam_HN36285_2010_2.3.4.1
A_Japanese_white-eye_HK_1038_2006_2.3.4
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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_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
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A_Egypt_9174-NAMRU3_2009_2.2.1
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KY029069(A_chicken_Egypt_ZU120_2016(H5N1)
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KY558870(A_chicken_Egypt_N12638D_2016(H5N1)
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2.3.4.4b
2.2.1
2.2.1.2
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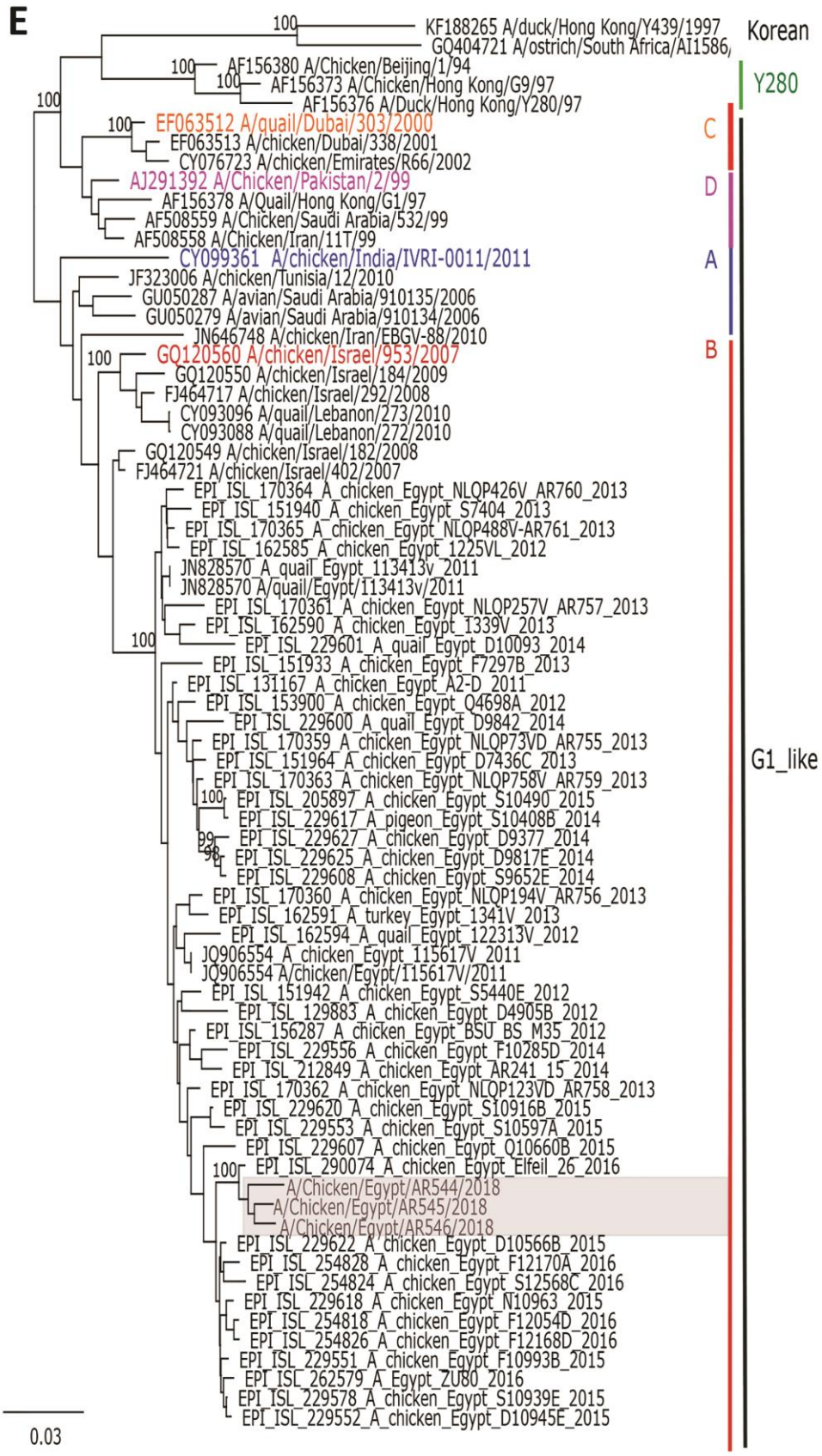
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Y280
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EF063513 A/chicken/Dubai/338/2001
CY076723 A/chicken/Emirates/R66/2002
C
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AF508559 A/Chicken/Saudi Arabia/532/99
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D
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JF323006 A/chicken/Tunisia/12/2010
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JN646748 A/chicken/Iran/EBGV-88/2010
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CY093096 A/quail/Lebanon/273/2010
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GQ120549 A/chicken/Israel/182/2008
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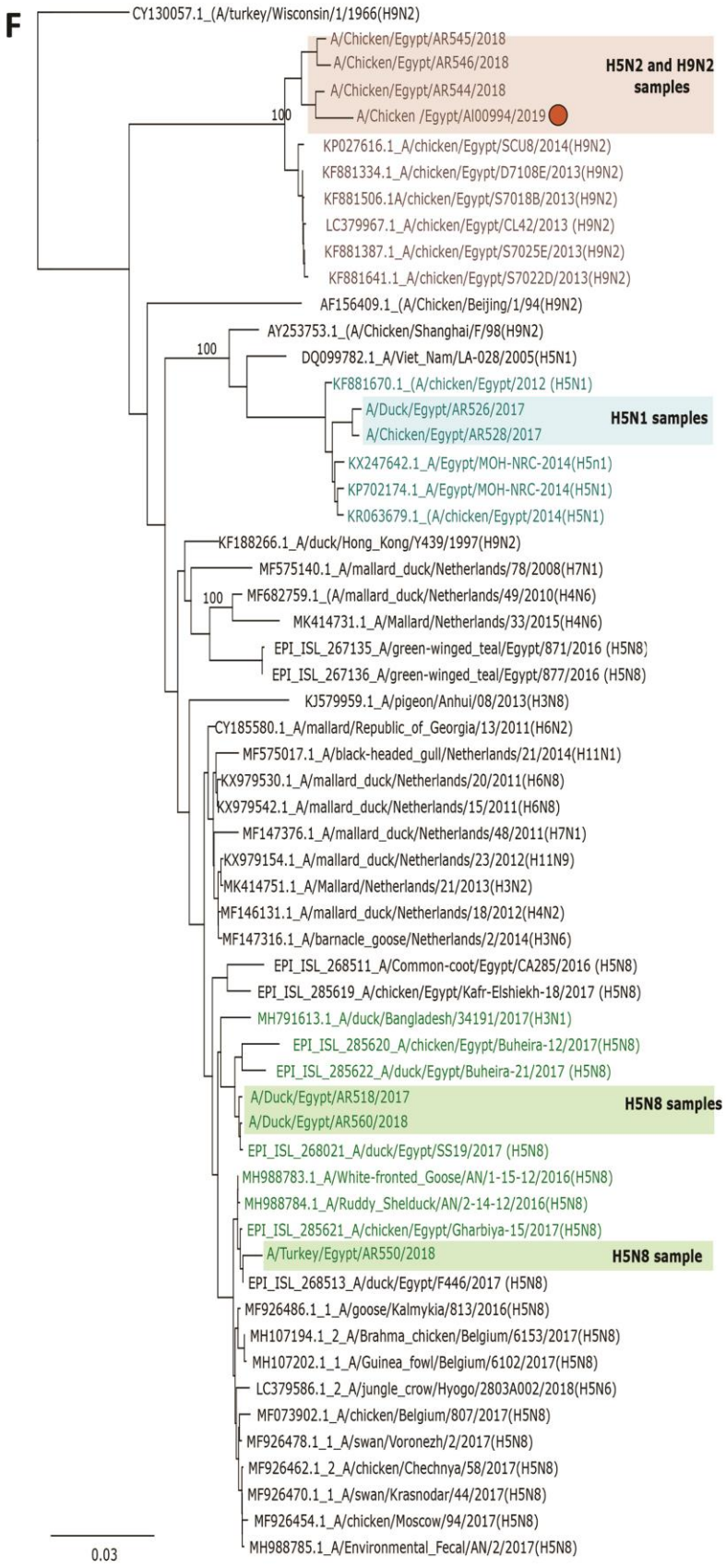
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KF881387.1_A/chicken/Egypt/S7025E/2013(H9N2)
KF881641.1_A/chicken/Egypt/S7022D/2013(H9N2)
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AY253753.1_(A/Chicken/Shanghai/F/98(H9N2)
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KX979542.1_A/mallard_duck/Netherlands/15/2011(H6N8)
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KX979154.1_A/mallard_duck/Netherlands/23/2012(H11N9)
MK414751.1_A/Mallard/Netherlands/21/2013(H3N2)
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MF147316.1_A/barnacle_goose/Netherlands/2/2014(H3N6)
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LC379586.1_2_A/jungle_crow/Hyogo/2803A002/2018(H5N6)
MF073902.1_A/chicken/Belgium/807/2017(H5N8)
MF926478.1_1_A/swan/Voronezh/2/2017(H5N8)
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MF926470.1_1_A/swan/Krasnodar/44/2017(H5N8)
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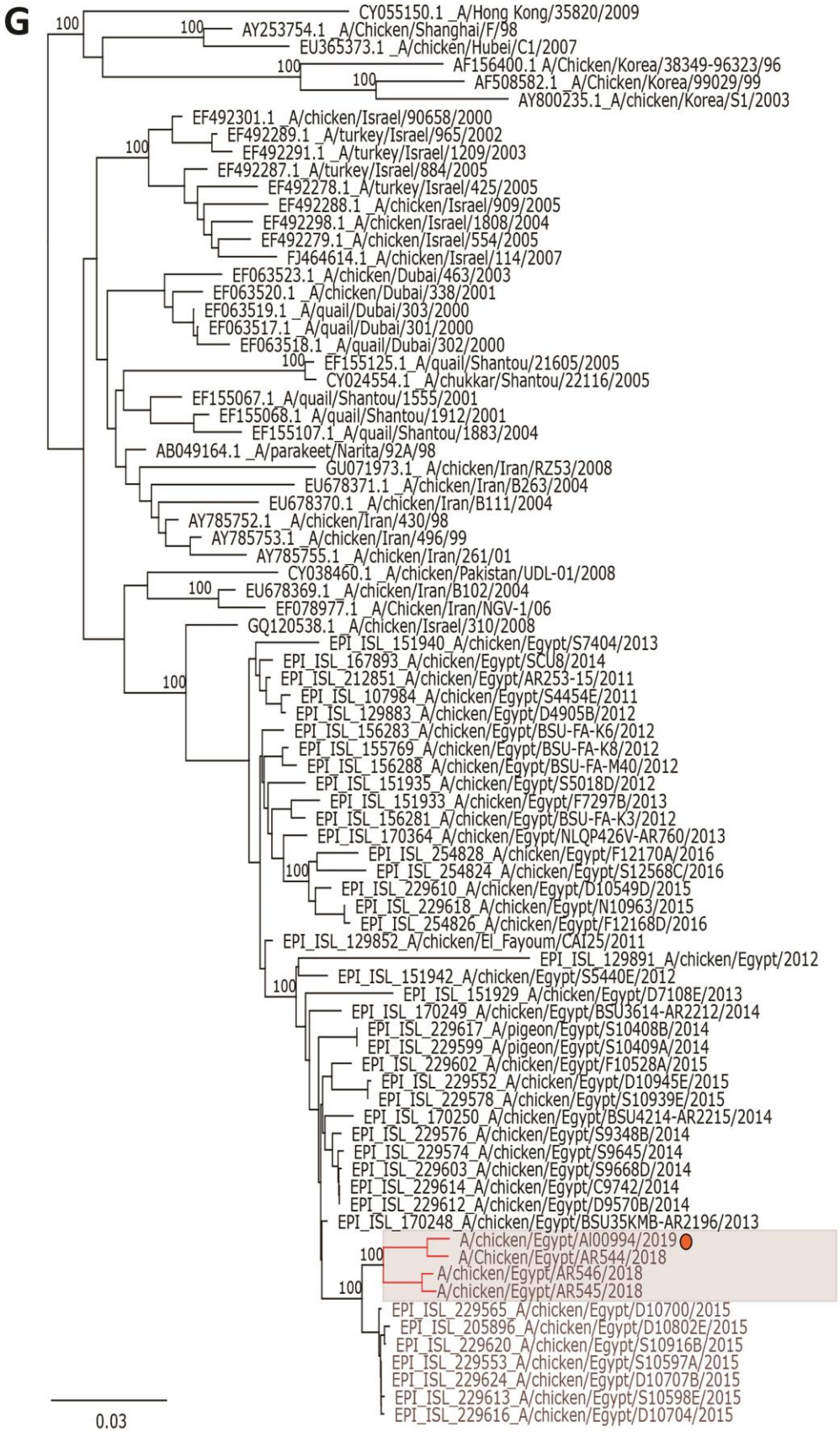
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AB049164.1 _A/parakeet/Narita/92A/98
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EU678371.1 _A/chicken/Iran/B263/2004
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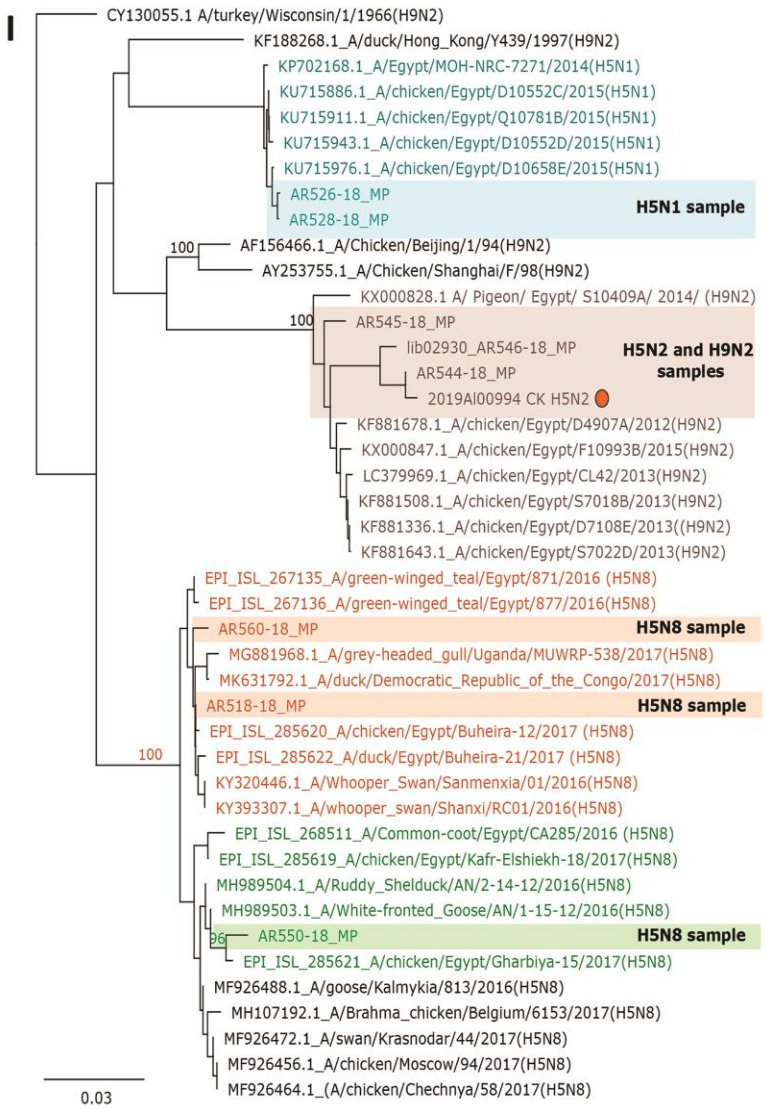
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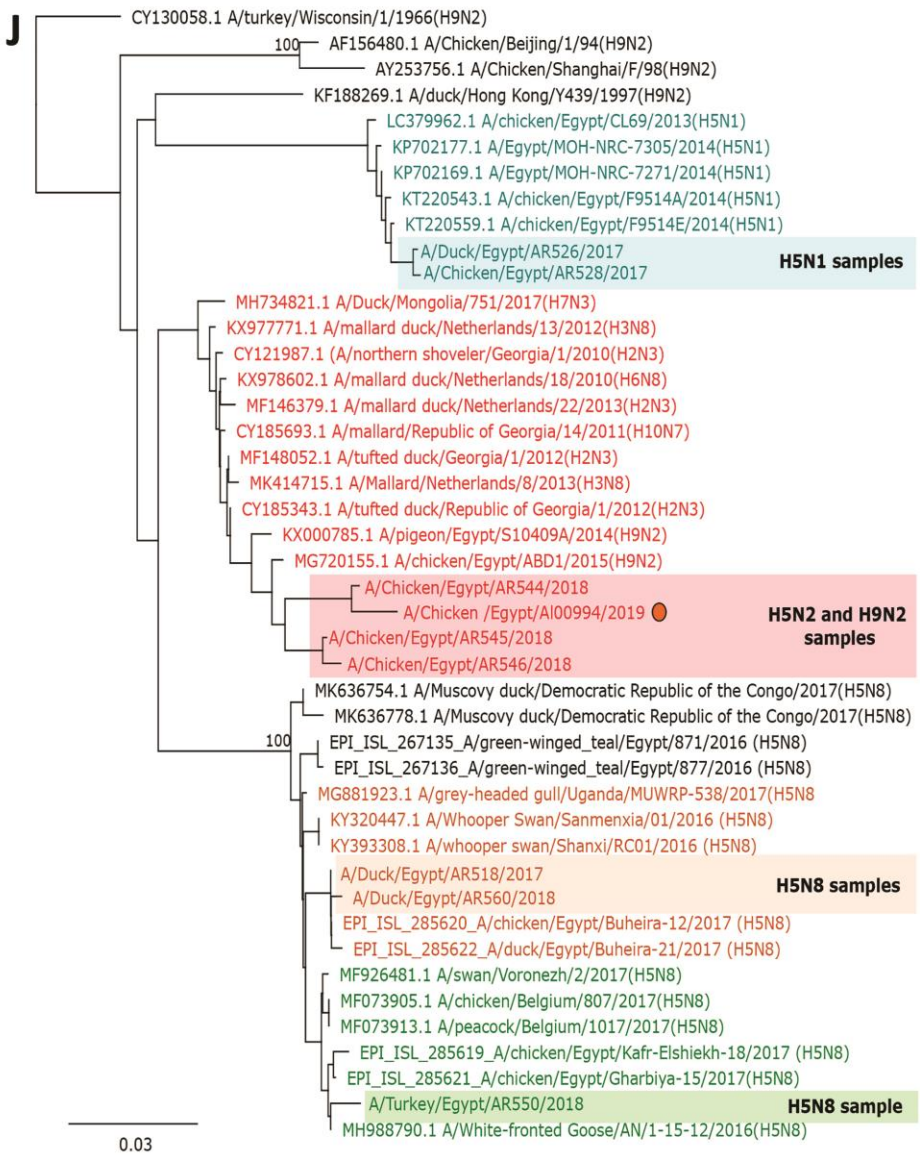

Appendix Figure. Phylogenetic analysis of segments of highly pathogenic avian influenza (HPAI) and low pathogenicity avian influenza (LPAI). Sequence analysis was based on alignment analyses by MAFFTv7.450 embedded in the Geneious software suite, version 11.1.7(https://www.geneious.com) with manual editing. 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.