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Supporting Online Material for

Reassortment of Pandemic H1N1/2009 Influenza A Virus in Swine

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Supporting Online Material for

Transmission and reassortment of pandemic H1N1/2009 influenza A virus in swine

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Materials and Methods

Tracheal or nasal swabs were taken fortnightly from slaughtered swine at an abattoir in Hong Kong. Swab materials were inoculated into 9-to-10-day old chicken embryonated eggs and MDCK cells, and virus isolates identified and subtyped by hemagglutination inhibition (HI) assays as previously described (*S1*).

Post-H1N1/2009 vaccination (n=3), acute and convalescent post-H1N1/2009 infection human sera (n=2) and a ferret antiserum to A/California/4/09 were tested by HI assay for

reactivity to the viral antigens A/Brisbane 59/2007 (seasonal influenza H1N1), A/California/4/2009 (H1N1/2009) and reassortant virus A/swine/Hong Kong/201/2010 (*S1*).

Viral RNA extraction, cDNA synthesis, PCR, and sequencing were carried out as described (*S2*,*S3*). The genetic composition of A/swine/Hong Kong/201/2010 was confirmed by direct PCR detection and re-sequencing of the 8 gene segments from the original swab specimen. Furthermore, the original swab specimen and virus isolate were screened with H1N1/2009 specific primers which confirmed the absence of H1N1/2009 HA and internal genes.

We compared 32 newly sequenced Hong Kong swine influenza genomes with all genomes available in GenBank representing influenza A diversity from human, swine and avian hosts. Phylogenetic trees were constructed for each genomic segment independently. A best-fit nucleotide substitution model (*S4*) was calculated and maximum likelihood phylogenies reconstructed using the program Garli 0.96 (*S5*). Phylogenetic support was calculated with 1000 neighbor-joining bootstrap replicates in PAUP* 4.0 (*S6*).

To assess the infectivity and transmission of A/swine/Hong Kong/201/2010, five 4 to 6-week-old seronegative piglets were infected intranasally with 10^6 plaque forming units (PFU) of the virus in 0.6ml of Eagle's minimal essential Medium (MEM) and co-housed in the same cage with two naïve piglets. Two other piglets were mock infected with an equivalent volume of MEM. All animals were moved to the BSL-3 lab 4 days prior to infection and baseline body weights were established 3 days prior to infection. Body weights were recorded daily at approximately the same time (9:30-10:30 am) for each individual. Nasal and rectal swabs were collected daily from each piglet and placed into 0.6 ml of cold sterile phosphate buffered saline and stored at −80 °C. Viral RNA was extracted from the clarified swab supernatant using MagMAX™ 96 Viral RNA Isolation Kit (Applied Biosystems) on the MagMAX™ Express

(Applied Biosystems). cDNA synthesis and determination of influenza M gene copy number were carried out as described previously (*S2, S7*). A virus preparation was titrated with standard plaque assay to construct a ten-fold dilution series (10^1-10^6 PFU/ml) that was used to determine the relative equivalent units (REU) for viral infectivity based on the cycle threshold (Ct) values obtained (*S8, S9*).

Blood was collected via venipuncture of the anterior vena cava from pigs. Specific antibodies against A/swine/Hong Kong/201/2010 were tested by HI.

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Table S1. Summary of isolation of influenza A viruses (H1N1 and H1N2) from swine, June 2009 to February 2010, Hong Kong

Sampling occasion	Sample number	Influenza A positive	
		H1N1/2009	H1N1 / H1N2
11 Jun 2009	252	0	1
25 Jun 2009	252	0	1
9 Jul 2009	252	0	0
23 Jul 2009	252	0	3
13 Aug 2009	252	0	7
27 Aug 2009	252	0	0
10 Sep 2009	252	0	0
24 Sep 2009	252	0	0
8 Oct 2009	252	0	0
22 Oct 2009	252	2	1
5 Nov 2009	252	0	2
19 Nov 2009	252	0	0
3 Dec 2009	247	2	3
17 Dec 2009	244	5	3
7 Jan 2010	206	1	1
21 Jan 2010	180	0	0
4 Feb 2010	200	0	0
TOTAL	4101	10	22

Table S2. Antigenic analysis of influenza A viruses (H1N1/H1N2) by hemagglutination inhibition test

Serum	Antibody titers* to viral antigen				
	A/Brisbane/59/2007 (Seasonal H1N1)	A/California/4/2009 (Pandemic H1N1)	A/Sw/HK/201/2010 (Novel reassortant H1N1)	A/Sw/HK/NS29/2009 (EA linage H1N1)	A/Sw/HK/4167/1999 (CS lineage H1N1)
Human post H1N1/2009 vaccine (Patient A)	<10	160	<10	–[#]	–
Human post H1N1/2009 vaccine (Patient B)	10	320	320	–	–
Human post H1N1/2009 vaccine (Patient C)	320	80	<10	–	–
Human post H1N1/2009 infection (Patient D) acute	<10	<10	<10	–	–
Human post H1N1/2009 infection (Patient D) convalescent	<10	160	40	–	–
Human post H1N1/2009 infection (Patient E) acute	<10	<10	<10	–	–
Human post H1N1/2009 infection (Patient E) convalescent	20	160	10	–	–
Ferret anti-A/California/4/2009 serum	<10	1280	<10	80	2560
Ferret anti-A/swine/Hong Kong/NS29/2009 serum	<10	160	10240	5120	1280
Ferret anti-A/swine/Hong Kong/4167/1999 serum	<20	5120	160	640	20480

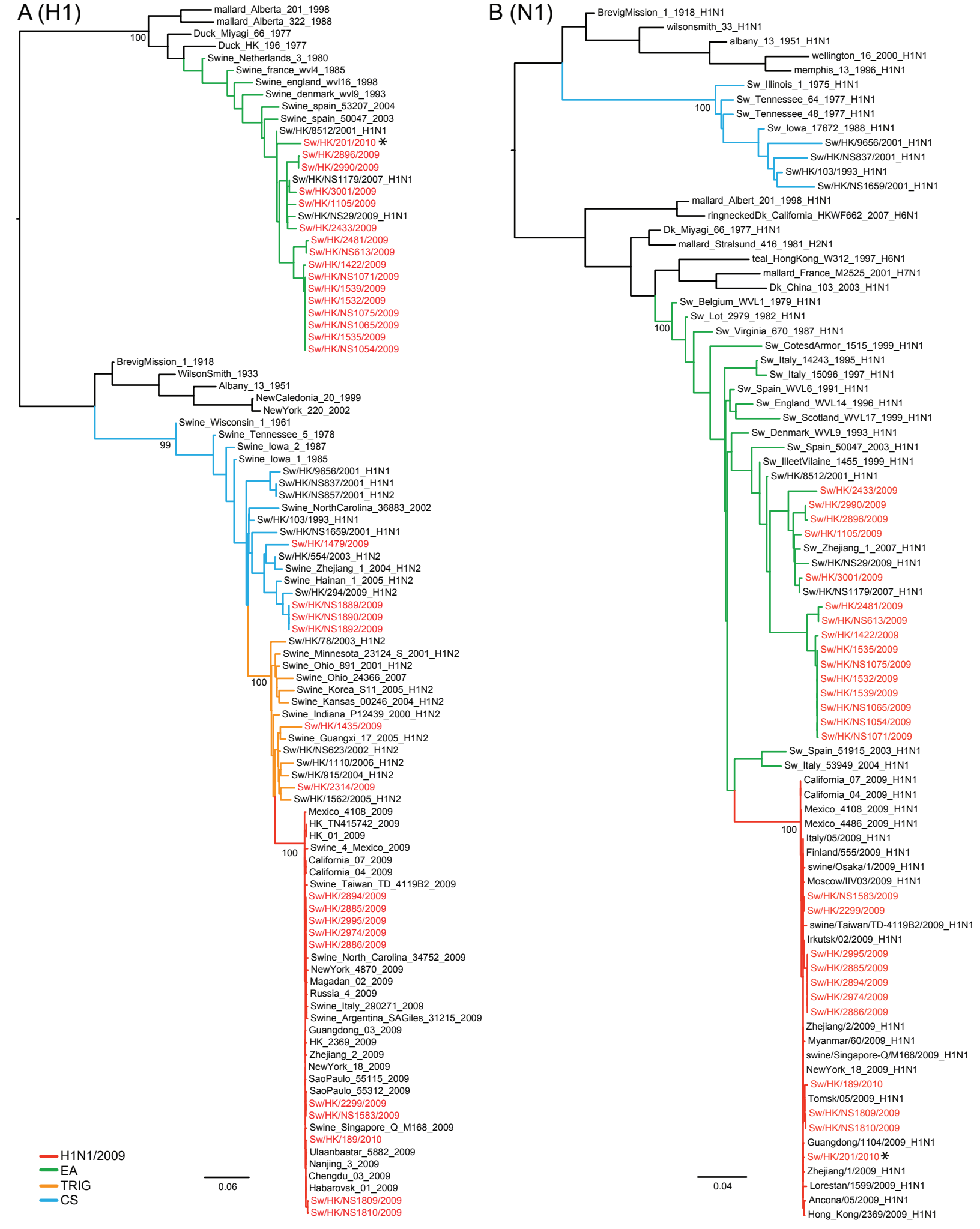
*Reciprocal antibody titers are presented. [#]– indicates titer not tested. Underline text indicates homologous titers. Abbreviations: CS, classical swine; EA, European avian-like; HK, Hong Kong; Sw, swine.

Fig. S1. Maximum likelihood phylogenies of the **(A–H)** H1-HA, N1-NA, PB2, PB1, PA, NP, M and NS genes, respectively, of representative influenza A viruses. * denotes phylogenetic position of the novel reassortant virus A/swine/Hong Kong/201/2010. Only bootstrap supports for major swine H1N1/H1N2 and H1N1/2009 lineages (>70%) are shown. Bar represents nucleotide substitutions per site.

Fig. S2. Mean body weight changes of swine challenged or exposed to A/swine/Hong Kong/201/2010.

Fig. S3. Virus shedding of A/swine/Hong Kong/201/2010 in challenged or contact exposed swine. Virus shedding was not detected either in mock infected pigs or rectal swabs from any individual collected at any time point throughout the study. All infected and contact animals seroconverted to A/swine/Hong Kong/201/2010 (data not shown).

Figure S1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Swine_france_wvl4_1985
Swine_england_wvl16_1998
Swine_denmark_wvl9_1993
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Sw/HK/1105/2009
Sw/HK/NS29/2009_H1N1
Sw/HK/2433/2009
Sw/HK/2481/2009
Sw/HK/NS613/2009
Sw/HK/1422/2009
Sw/HK/NS1071/2009
Sw/HK/1539/2009
Sw/HK/1532/2009
Sw/HK/NS1075/2009
Sw/HK/NS1065/2009
Sw/HK/1535/2009
Sw/HK/NS1054/2009
BrevigMission_1_1918
WilsonSmith_1933
Albany_13_1951
NewCaledonia_20_1999
NewYork_220_2002
Swine_Wisconsin_1_1961
Swine_Tennessee_5_1978
99
Swine_Iowa_2_1987
Swine_Iowa_1_1985
Sw/HK/9656/2001_H1N1
Sw/HK/NS837/2001_H1N1
Sw/HK/NS857/2001_H1N2
Swine_NorthCarolina_36883_2002
Sw/HK/103/1993_H1N1
Sw/HK/NS1659/2001_H1N1
Sw/HK/1479/2009
Sw/HK/554/2003_H1N2
Swine_Zhejiang_1_2004_H1N2
Swine_Hainan_1_2005_H1N2
Sw/HK/294/2009_H1N2
Sw/HK/NS1889/2009
Sw/HK/NS1890/2009
Sw/HK/NS1892/2009
Sw/HK/78/2003_H1N2
Swine_Minnesota_23124_S_2001_H1N2
Swine_Ohio_891_2001_H1N2
100
Swine_Ohio_24366_2007
Swine_Korea_S11_2005_H1N2
Swine_Kansas_00246_2004_H1N2
Swine_Indiana_P12439_2000_H1N2
Sw/HK/1435/2009
Swine_Guangxi_17_2005_H1N2
Sw/HK/NS623/2002_H1N2
Sw/HK/1110/2006_H1N2
Sw/HK/915/2004_H1N2
Sw/HK/2314/2009
Sw/HK/1562/2005_H1N2
Mexico_4108_2009
HK_TN415742_2009
HK_01_2009
100
Swine_4_Mexico_2009
California_07_2009
California_04_2009
Swine_Taiwan_TD_4119B2_2009
Sw/HK/2894/2009
Sw/HK/2885/2009
Sw/HK/2995/2009
Sw/HK/2974/2009
Sw/HK/2886/2009
Swine_North_Carolina_34752_2009
NewYork_4870_2009
Magadan_02_2009
Russia_4_2009
Swine_Italy_290271_2009
Swine_Argentina_SAGiles_31215_2009
Guangdong_03_2009
HK_2369_2009
Zhejiang_2_2009
NewYork_18_2009
SaoPaulo_55115_2009
SaoPaulo_55312_2009
Sw/HK/2299/2009
Sw/HK/NS1583/2009
Swine_Singapore_Q_M168_2009
Sw/HK/189/2010
Ulaanbaatar_5882_2009
Nanjing_3_2009
Chengdu_03_2009
Habarovsk_01_2009
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Sw/HK/NS1810/2009
H1N1/2009
EA
TRIG
CS
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albany_13_1951_H1N1
wellington_16_2000_H1N1
memphis_13_1996_H1N1
Sw_Illinois_1_1975_H1N1
Sw_Tennessee_64_1977_H1N1
100
Sw_Tennessee_48_1977_H1N1
Sw_Iowa_17672_1988_H1N1
Sw/HK/9656/2001_H1N1
Sw/HK/NS837/2001_H1N1
Sw/HK/103/1993_H1N1
Sw/HK/NS1659/2001_H1N1
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ringneckedDk_California_HKWF662_2007_H6N1
Dk_Miyagi_66_1977_H1N1
mallard_Stralsund_416_1981_H2N1
teal_HongKong_W312_1997_H6N1
mallard_France_M2525_2001_H7N1
Dk_China_103_2003_H1N1
Sw_Belgium_WVL1_1979_H1N1
Sw_Lot_2979_1982_H1N1
100
Sw_Virginia_670_1987_H1N1
Sw_CotesdArmor_1515_1999_H1N1
Sw_Italy_14243_1995_H1N1
Sw_Italy_15096_1997_H1N1
Sw_Spain_WVL6_1991_H1N1
Sw_England_WVL14_1996_H1N1
Sw_Scotland_WVL17_1999_H1N1
Sw_Denmark_WVL9_1993_H1N1
Sw_Spain_50047_2003_H1N1
Sw_IlleetVilaine_1455_1999_H1N1
Sw/HK/8512/2001_H1N1
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Sw/HK/NS1179/2007_H1N1
Sw/HK/2481/2009
Sw/HK/NS613/2009
Sw/HK/1422/2009
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Sw/HK/1539/2009
Sw/HK/NS1065/2009
Sw/HK/NS1054/2009
Sw/HK/NS1071/2009
Sw_Spain_51915_2003_H1N1
Sw_Italy_53949_2004_H1N1
California_07_2009_H1N1
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Mexico_4486_2009_H1N1
100
Italy/05/2009_H1N1
Finland/555/2009_H1N1
swine/Osaka/1/2009_H1N1
Moscow/IIV03/2009_H1N1
Sw/HK/NS1583/2009
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swine/Taiwan/TD-4119B2/2009_H1N1
Irkutsk/02/2009_H1N1
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Sw/HK/2885/2009
Sw/HK/2894/2009
Sw/HK/2974/2009
Sw/HK/2886/2009
Zhejiang/2/2009_H1N1
Myanmar/60/2009_H1N1
swine/Singapore-Q/M168/2009_H1N1
NewYork_18_2009_H1N1
Sw/HK/189/2010
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Ancona/05/2009_H1N1
Hong_Kong/2369/2009_H1N1
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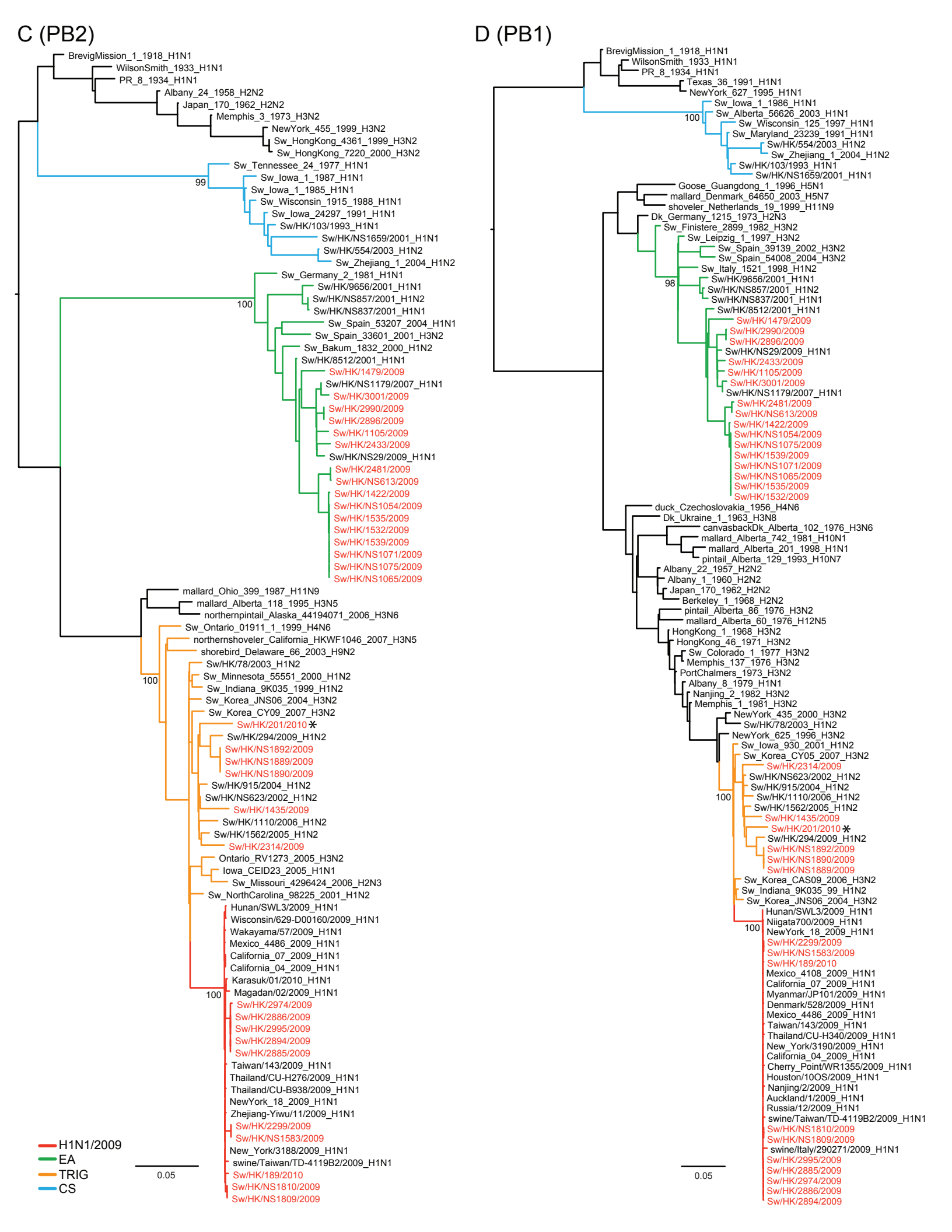
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Sw_HongKong_4361_1999_H3N2
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99
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Sw_Iowa_1_1985_H1N1
Sw_Wisconsin_1915_1988_H1N1
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Sw/HK/103/1993_H1N1
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Sw_Zhejiang_1_2004_H1N2
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100
Sw/HK/9656/2001_H1N1
Sw/HK/NS857/2001_H1N2
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northernpintail_Alaska_44194071_2006_H3N6
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California_04_2009_H1N1
Karasuk/01/2010_H1N1
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Magadan/02/2009_H1N1
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WilsonSmith_1933_H1N1
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Sw_Iowa_1_1986_H1N1
100
Sw_Alberta_56626_2003_H1N1
Sw_Wisconsin_125_1997_H1N1
Sw_Maryland_23239_1991_H1N1
Sw/HK/554/2003_H1N2
Sw_Zhejiang_1_2004_H1N2
Sw/HK/103/1993_H1N1
Sw/HK/NS1659/2001_H1N1
Goose_Guangdong_1_1996_H5N1
mallard_Denmark_64650_2003_H5N7
shoveler_Netherlands_19_1999_H11N9
Dk_Germany_1215_1973_H2N3
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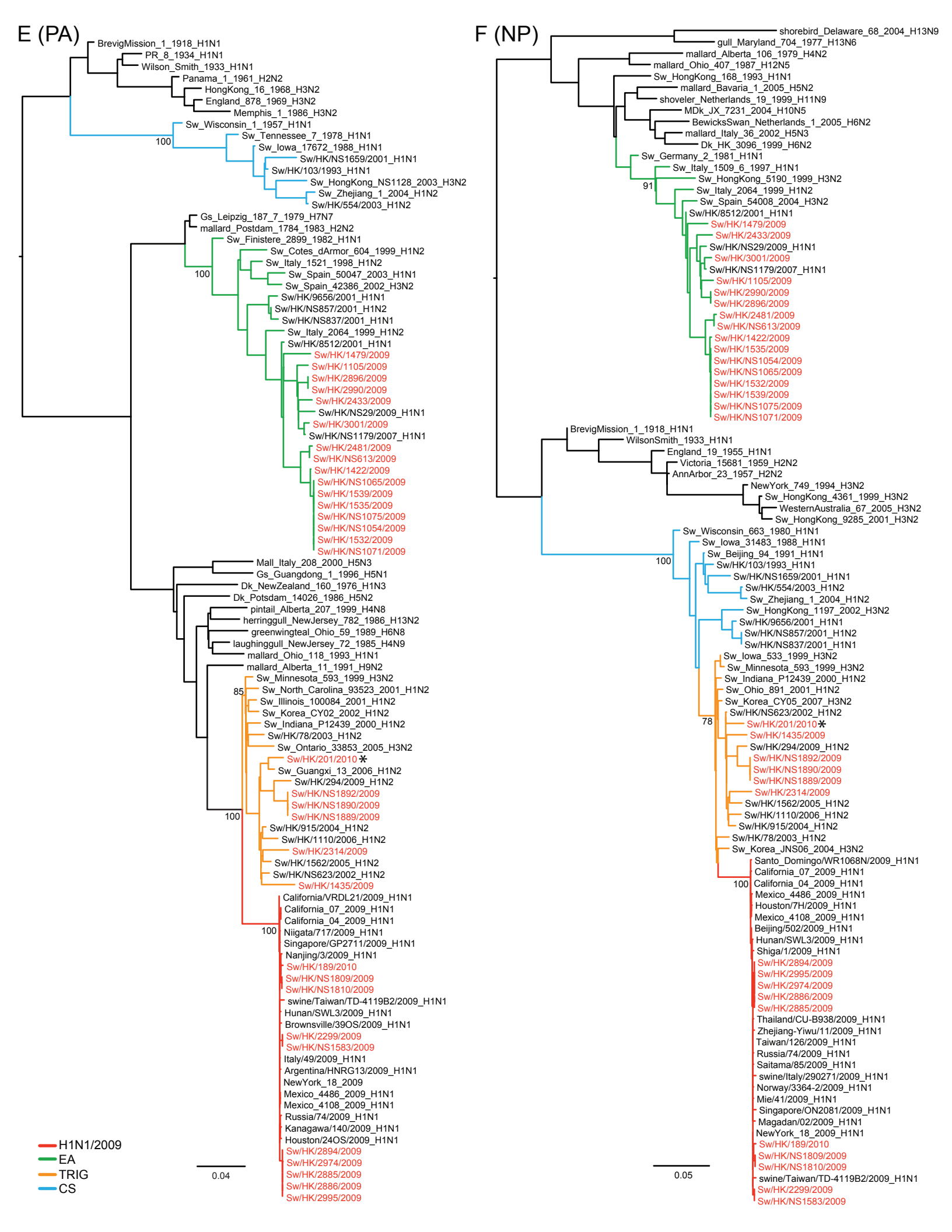
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greenwingteal_Ohio_59_1989_H6N8
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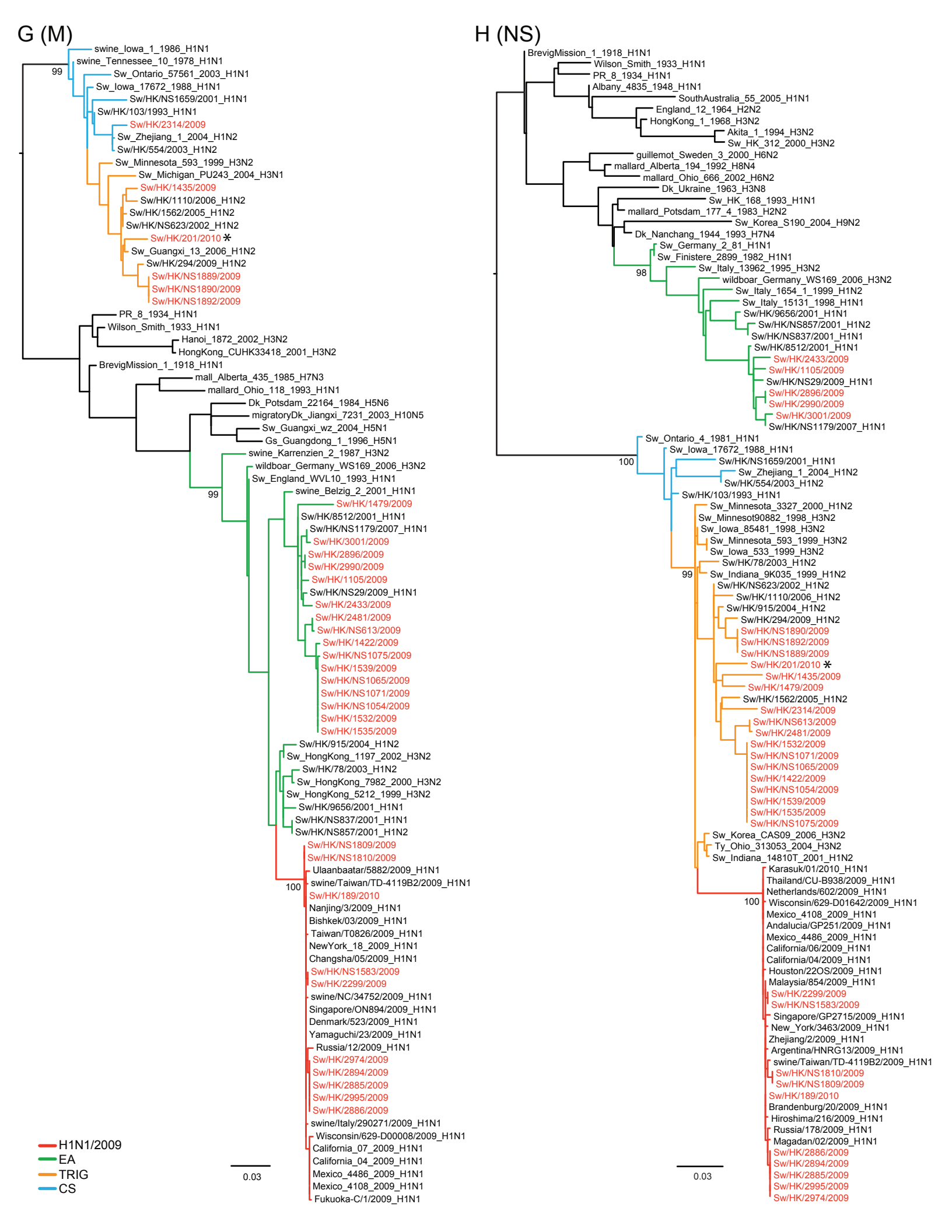
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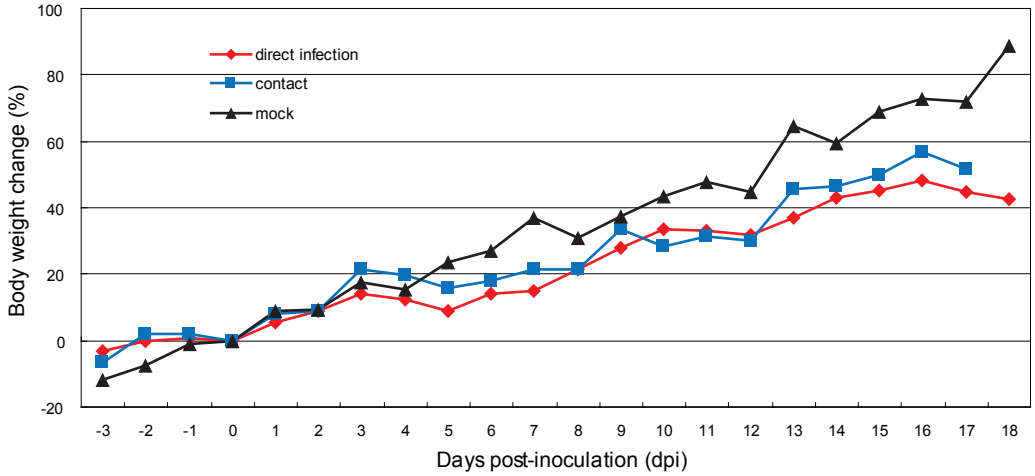

Figure S3