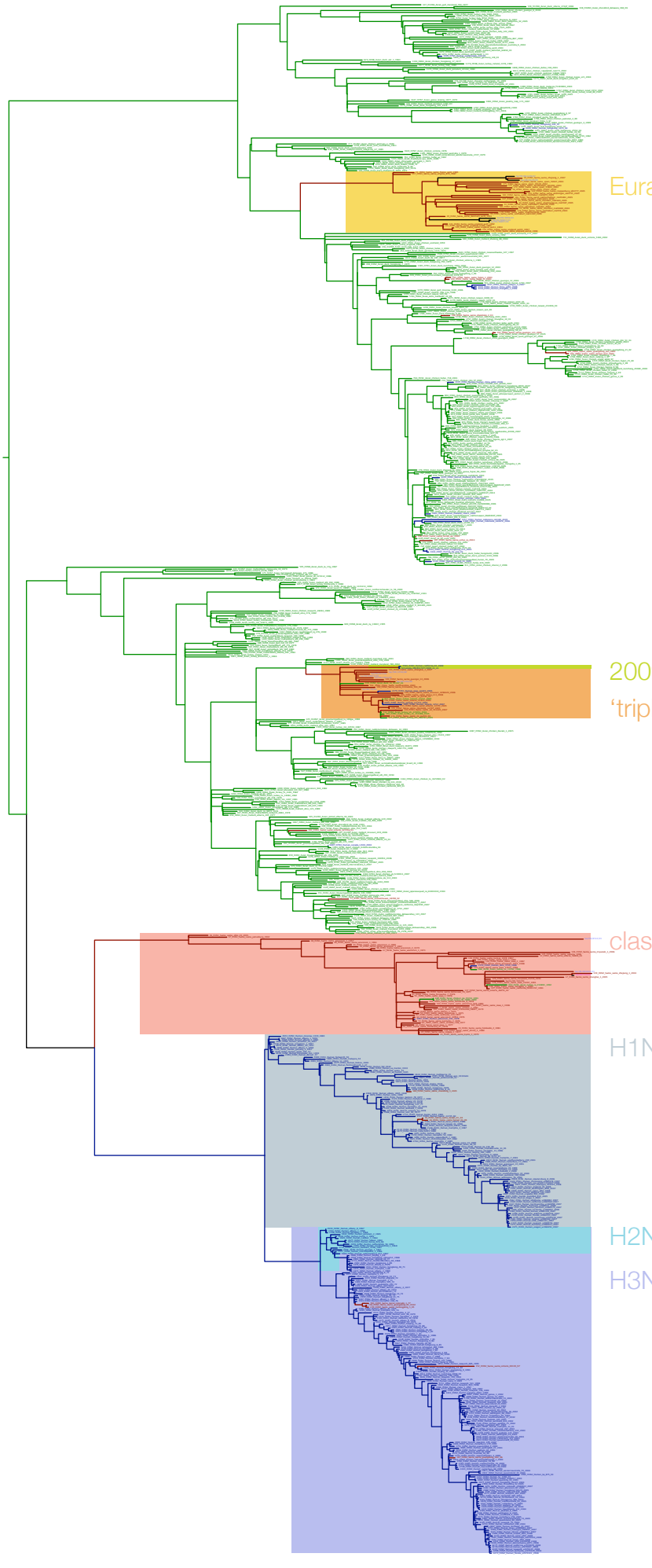
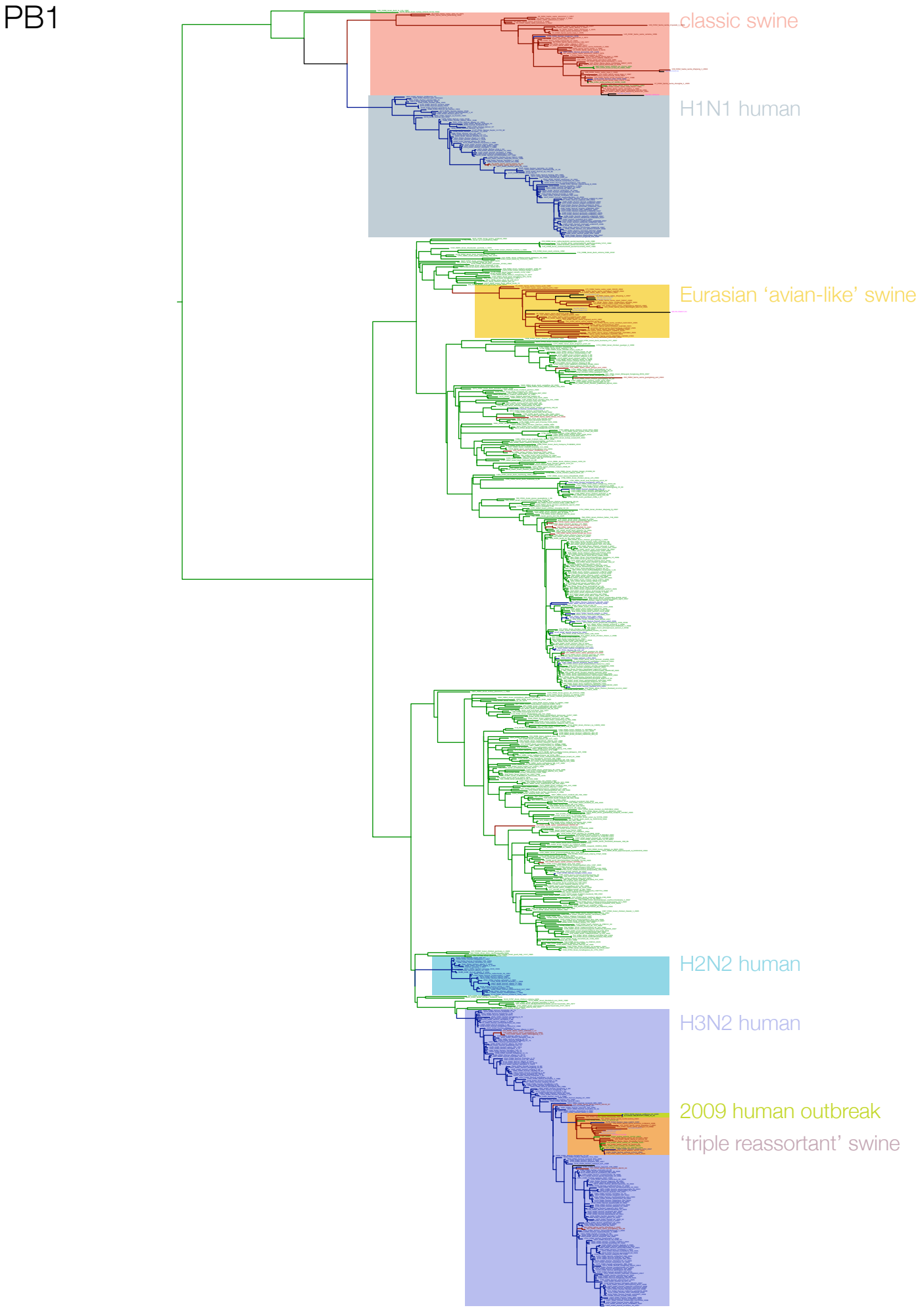
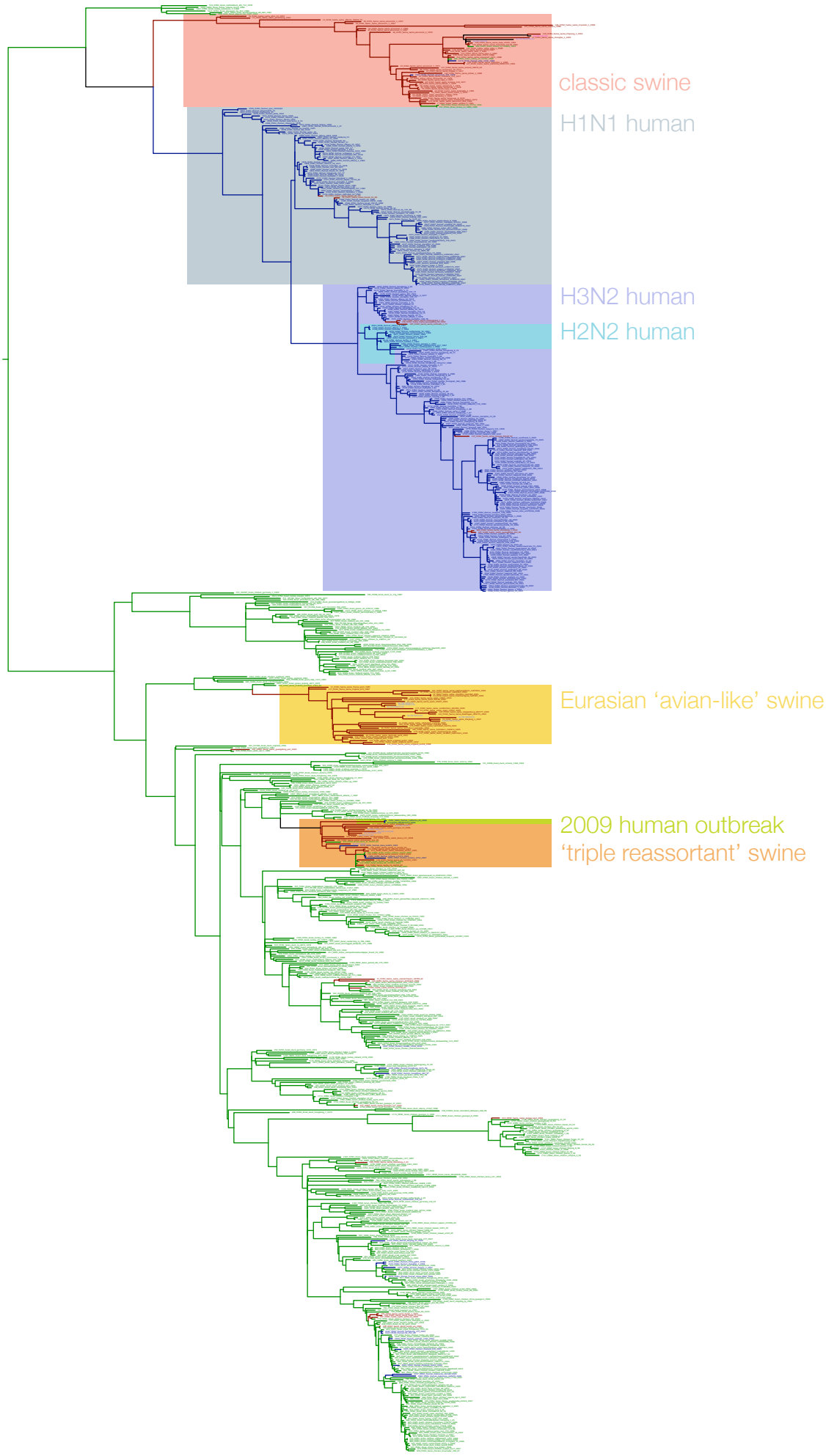
**Supplementary Figure 1.** Phylogenetic relationships of each gene segment (PB2, PB1, PA, HA, NP, NA, M & NS) of swine influenza A viruses indicating genetic components of the swine-origin influenza A (H1N1) virus. Clade labels indicate major swine lineages. Human viruses are coloured blue, swine viruses in red and avian viruses in green.

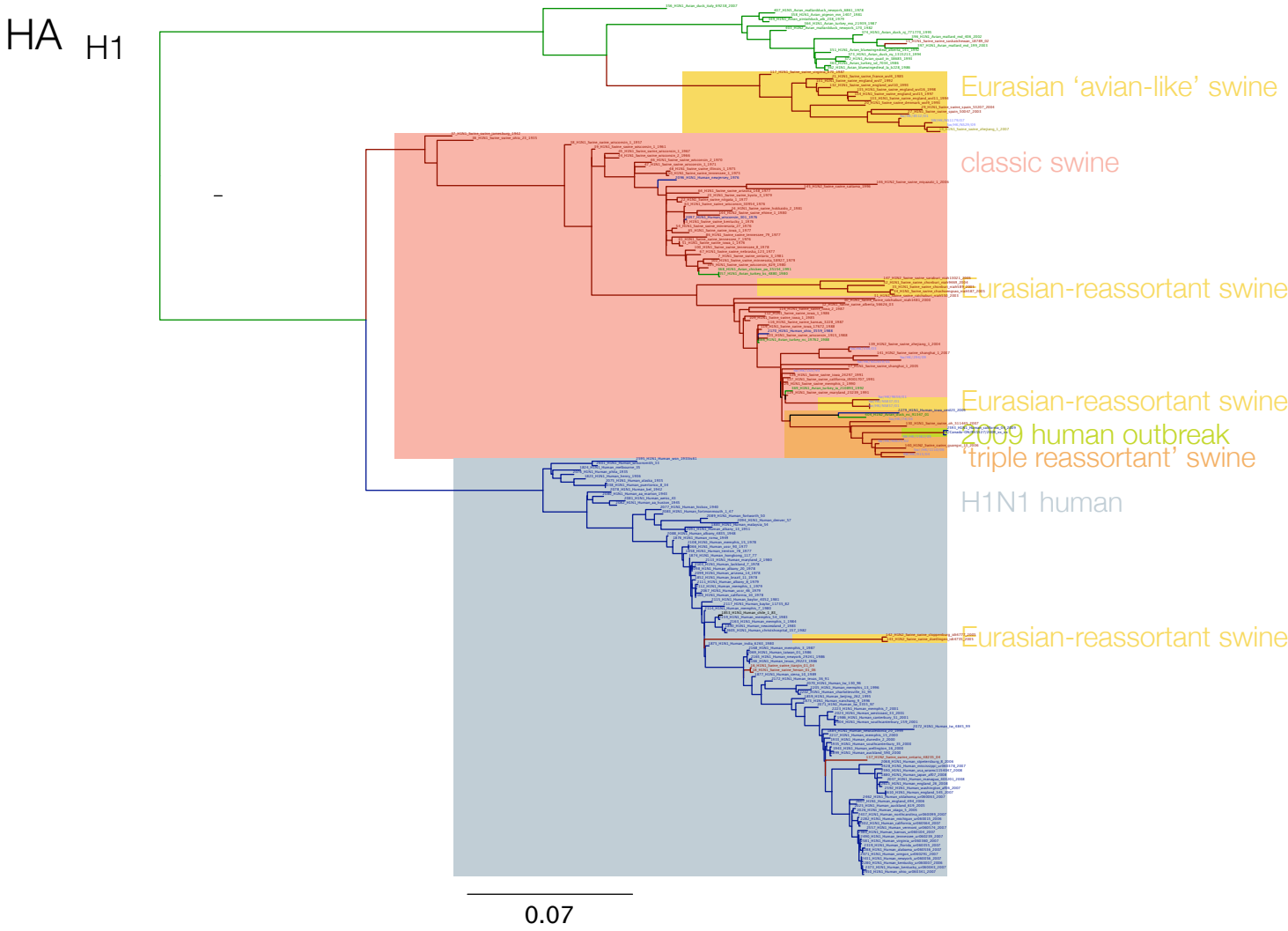
PB2

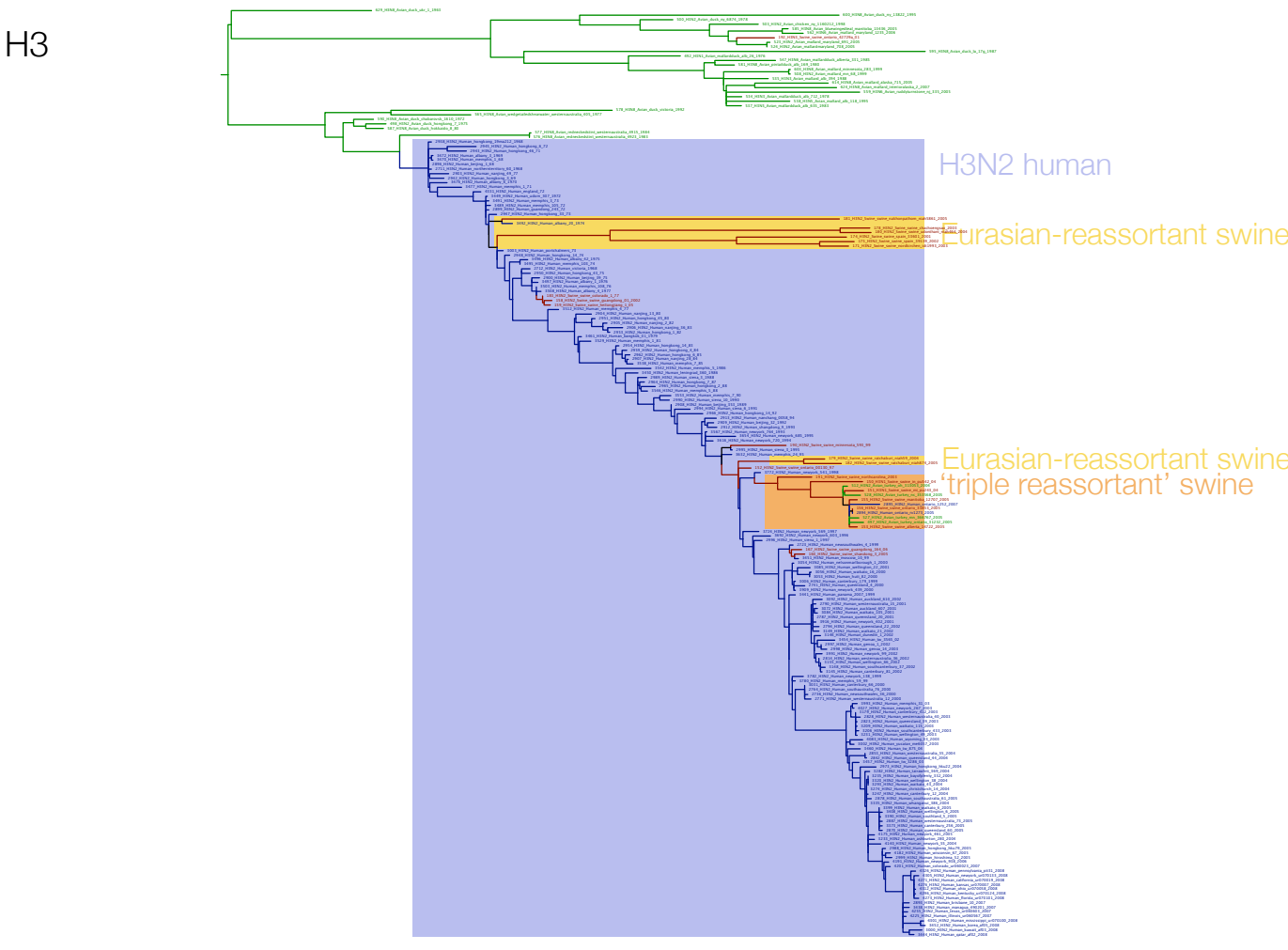

Eurasian ‘avian-like’ swine
2009 human outbreak
‘triple reassortant’ swine
classic swine
H1N1 human
H2N2 human
H3N2 human
0.03


PB1
classic swine
H1N1 human
Eurasian ‘avian-like’ swine
H2N2 human
H3N2 human
2009 human outbreak
‘triple reassortant’ swine
0.05

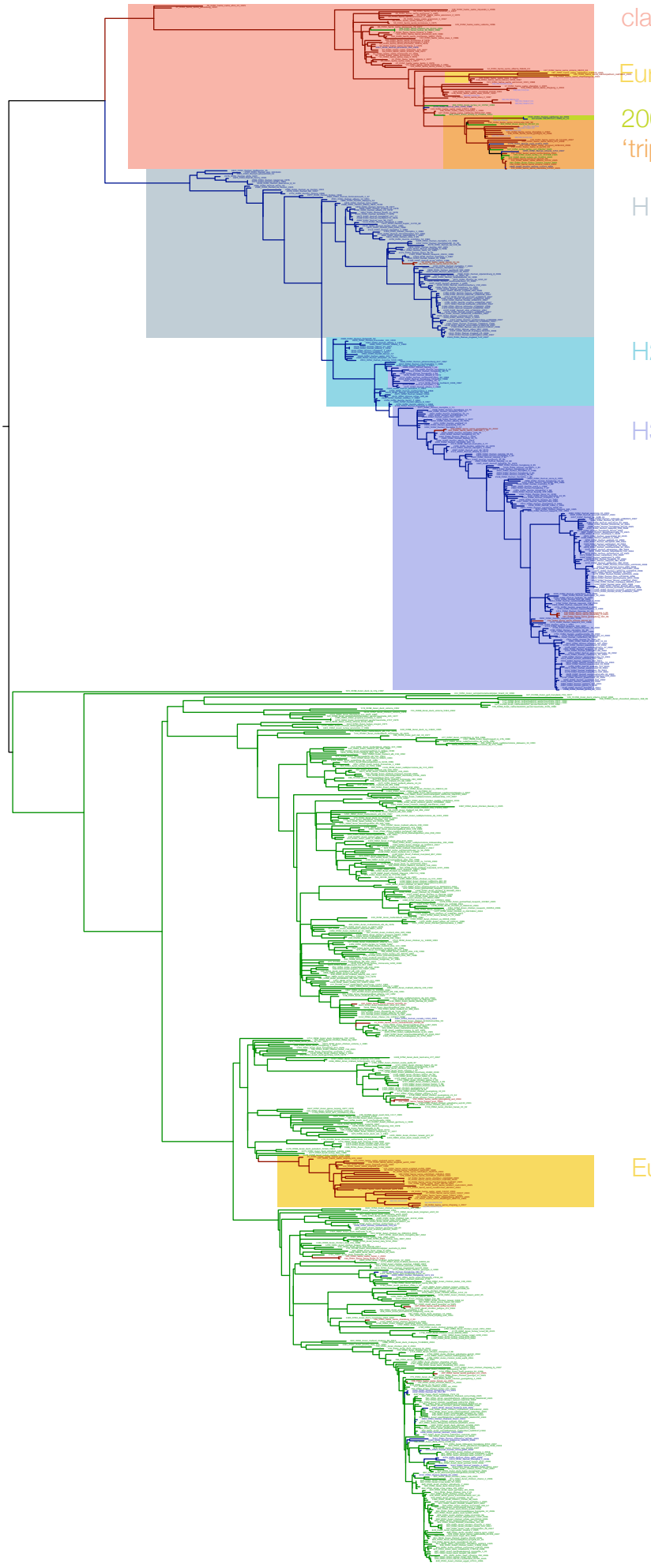
PA

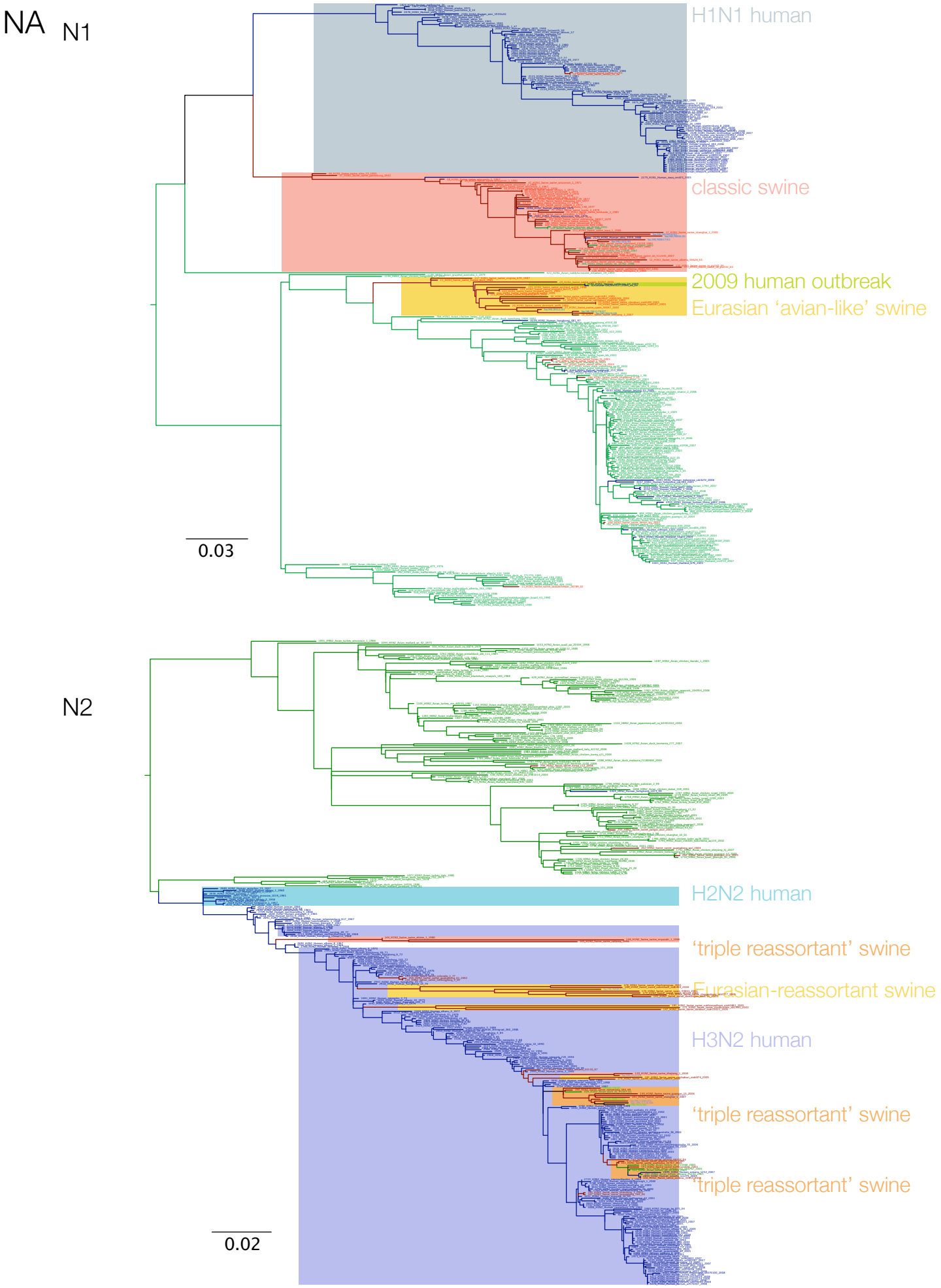

classic swine
H1N1 human
H3N2 human
H2N2 human
Eurasian ‘avian-like’ swine
2009 human outbreak
‘triple reassortant’ swine
0.05


HA
H1
Eurasian 'avian-like' swine
classic swine
Eurasian-reassortant swine
Eurasian-reassortant swine
2009 human outbreak
'triple reassortant' swine
H1N1 human
Eurasian-reassortant swine
0.07

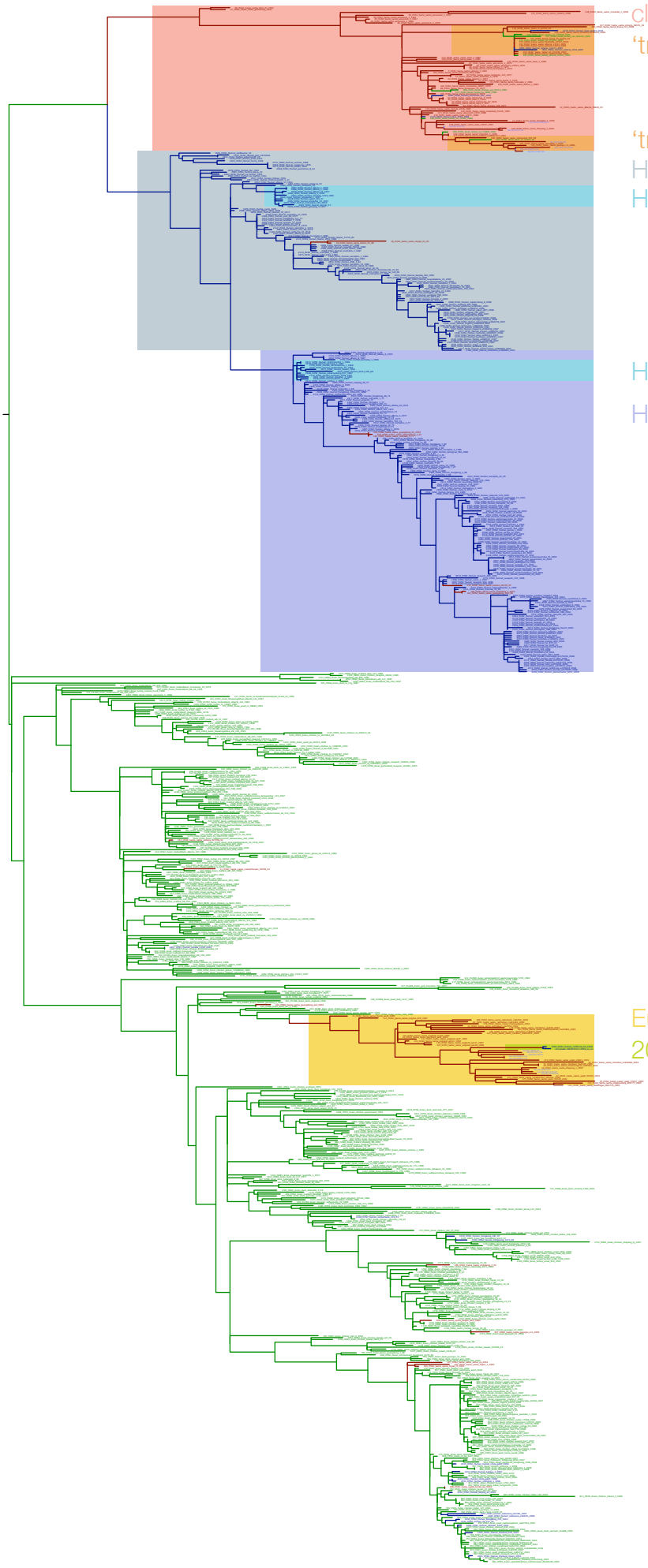

H3
H3N2 human
Eurasian-reassortant swine
Eurasian-reassortant swine
'triple reassortant' swine
0.05

NP

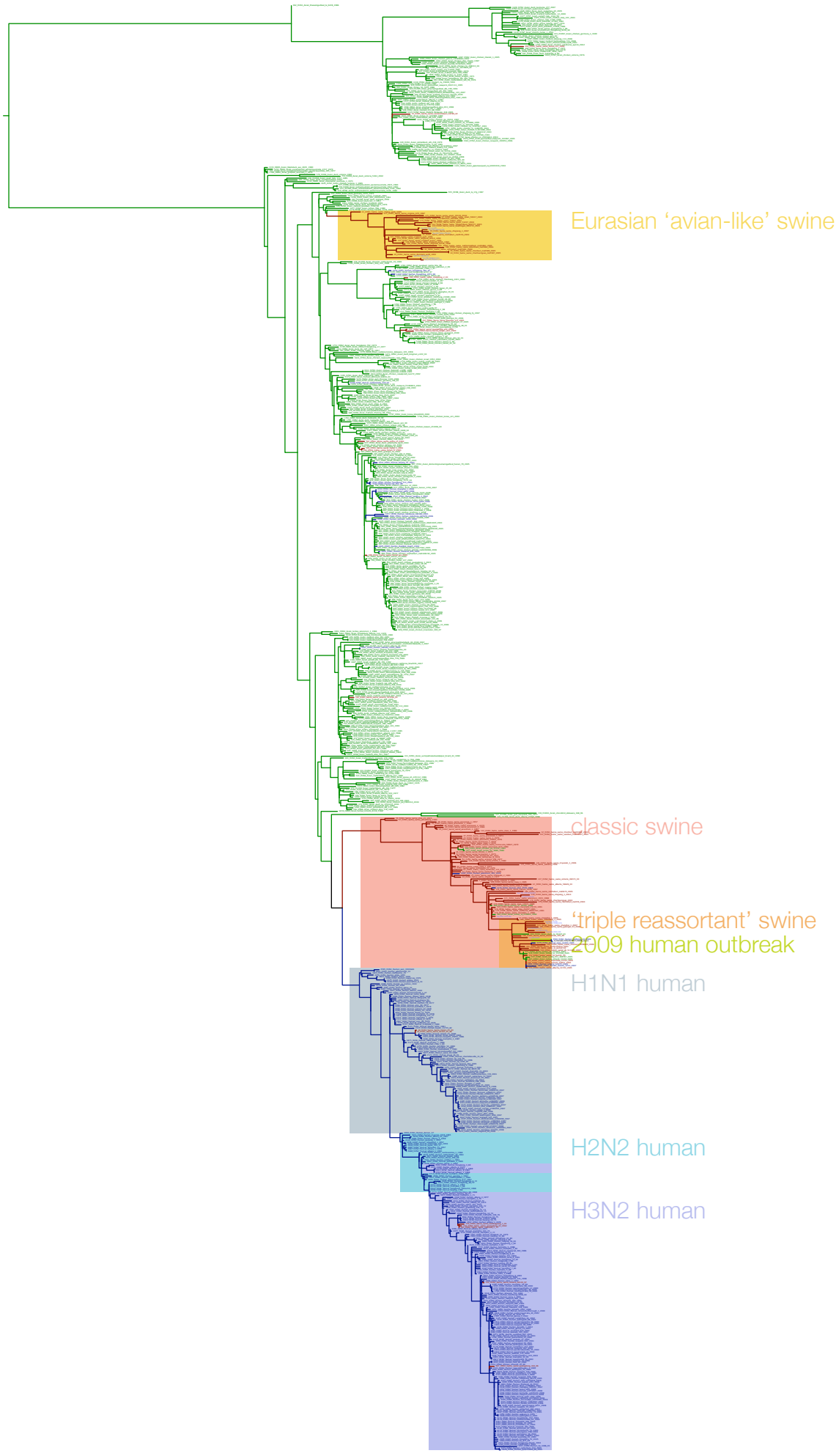

classic swine
Eurasian-reassortant swine
2009 human outbreak
'triple reassortant' swine
H1N1 human
H2N2 human
H3N2 human
Eurasian 'avian-like' swine
0.05


NA
N1
H1N1 human
classic swine
2009 human outbreak
Eurasian 'avian-like' swine
0.03
N2
H2N2 human
'triple reassortant' swine
Eurasian-reassortant swine
H3N2 human
'triple reassortant' swine
'triple reassortant' swine
0.02

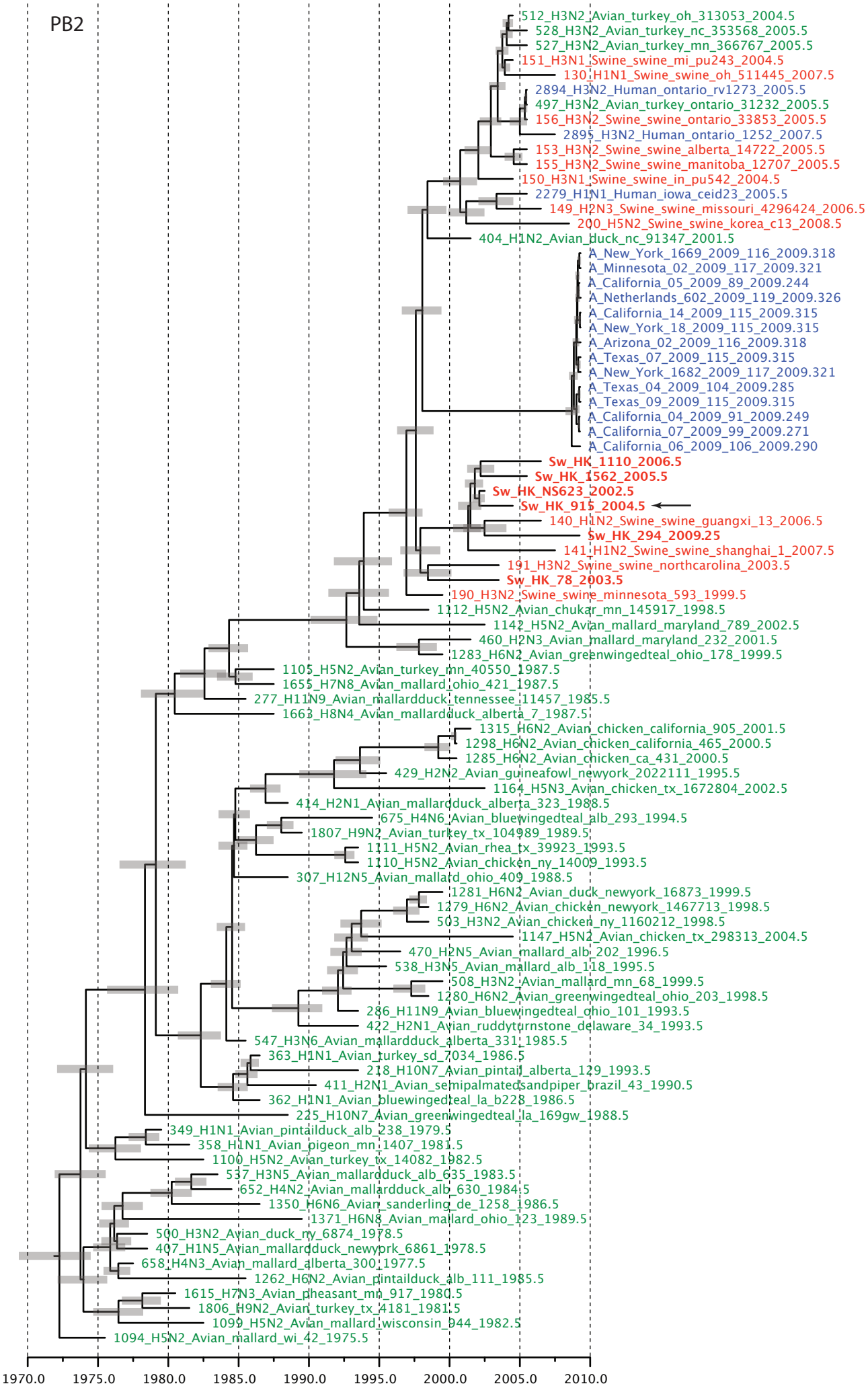
M

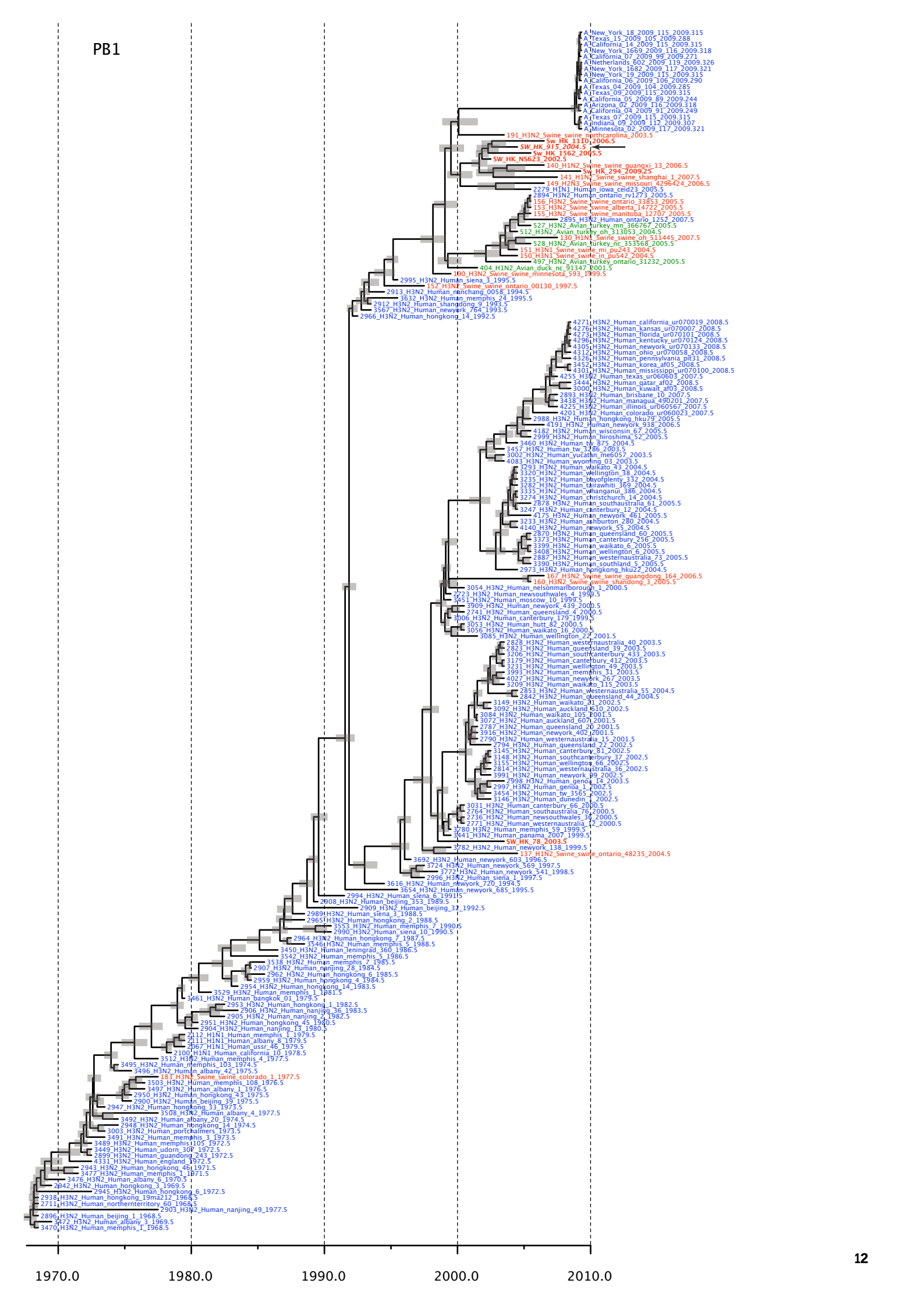

classic swine
'triple reassortant' swine
'triple reassortant' swine
H1N1 human
H2N2 human
H2N2 human
H3N2 human
Eurasian 'avian-like' swine
2009 human outbreak
0.03

NS


Eurasian ‘avian-like’ swine
classic swine
‘triple reassortant’ swine
2009 human outbreak
H1N1 human
H2N2 human
H3N2 human
0.1

**Supplementary Figure 2.** Phylogenetic relationships scaled to time for each gene segment (PB2, PB1, PA, HA, NP, NA, M & NS) of the swine-origin influenza A (H1N1) virus as represented in Figure 2 of the main text but with full virus names and GenBank accession numbers. Internal nodes are reconstructed common ancestors with 95% credible intervals on their date given by the grey bars. Human viruses are coloured blue, swine viruses in red and avian viruses in green. Newly described Hong Kong sequences have bold labels. Sw/HK/915/04 (H1N2) is highlighted with an arrow when present (see main text). Timeline is in years.


PB2
512_H3N2_Avian_turkey_oh_313053_2004.5
528_H3N2_Avian_turkey_nc_353568_2005.5
527_H3N2_Avian_turkey_mn_366767_2005.5
151_H3N1_Swine_swine_mi_pu243_2004.5
130_H1N1_Swine_swine_oh_511445_2007.5
2894_H3N2_Human_ontario_rv1273_2005.5
497_H3N2_Avian_turkey_ontario_31232_2005.5
156_H3N2_Swine_swine_ontario_33853_2005.5
2895_H3N2_Human_ontario_1252_2007.5
153_H3N2_Swine_swine_alberta_14722_2005.5
155_H3N2_Swine_swine_manitoba_12707_2005.5
150_H3N1_Swine_swine_in_pu542_2004.5
2279_H1N1_Human_iowa_ceid23_2005.5
149_H2N3_Swine_swine_missouri_4296424_2006.5
200_H5N2_Swine_swine_korea_c13_2008.5
404_H1N2_Avian_duck_nc_91347_2001.5
A_New_York_1669_2009_116_2009.318
A_Minnesota_02_2009_117_2009.321
A_California_05_2009_89_2009.244
A_Netherlands_602_2009_119_2009.326
A_California_14_2009_115_2009.315
A_New_York_18_2009_115_2009.315
A_Arizona_02_2009_116_2009.318
A_Texas_07_2009_115_2009.315
A_New_York_1682_2009_117_2009.321
A_Texas_04_2009_104_2009.285
A_Texas_09_2009_115_2009.315
A_California_04_2009_91_2009.249
A_California_07_2009_99_2009.271
A_California_06_2009_106_2009.290
Sw_HK_1110_2006.5
Sw_HK_1562_2005.5
Sw_HK_NS623_2002.5
Sw_HK_915_2004.5
140_H1N2_Swine_swine_guangxi_13_2006.5
Sw_HK_294_2009.25
141_H1N2_Swine_swine_shanghai_1_2007.5
191_H3N2_Swine_swine_northcarolina_2003.5
Sw_HK_78_2003.5
190_H3N2_Swine_swine_minnesota_593_1999.5
1112_H5N2_Avian_chukar_mn_145917_1998.5
1142_H5N2_Avian_mallard_maryland_789_2002.5
460_H2N3_Avian_mallard_maryland_232_2001.5
1283_H6N2_Avian_greenwingedteal_ohio_178_1999.5
1105_H5N2_Avian_turkey_mn_40550_1987.5
1655_H7N8_Avian_mallard_ohio_421_1987.5
277_H11N9_Avian_mallardduck_tennessee_11457_1985.5
1663_H8N4_Avian_mallardduck_alberta_7_1987.5
1315_H6N2_Avian_chicken_california_905_2001.5
1298_H6N2_Avian_chicken_california_465_2000.5
1285_H6N2_Avian_chicken_ca_431_2000.5
429_H2N2_Avian_guineafowl_newyork_2022111_1995.5
1164_H5N3_Avian_chicken_tx_1672804_2002.5
414_H2N1_Avian_mallardduck_alberta_323_1988.5
675_H4N6_Avian_bluewingedteal_alb_293_1994.5
1807_H9N2_Avian_turkey_tx_104989_1989.5
1111_H5N2_Avian_rhea_tx_39923_1993.5
1110_H5N2_Avian_chicken_ny_14009_1993.5
307_H12N5_Avian_mallard_ohio_409_1988.5
1281_H6N2_Avian_duck_newyork_16873_1999.5
1279_H6N2_Avian_chicken_newyork_1467713_1998.5
503_H3N2_Avian_chicken_ny_1160212_1998.5
1147_H5N2_Avian_chicken_tx_298313_2004.5
470_H2N5_Avian_mallard_alb_202_1996.5
538_H3N5_Avian_mallard_alb_118_1995.5
508_H3N2_Avian_mallard_mn_68_1999.5
1280_H6N2_Avian_greenwingedteal_ohio_203_1998.5
286_H11N9_Avian_bluewingedteal_ohio_101_1993.5
422_H2N1_Avian_ruddyturnstone_delaware_34_1993.5
547_H3N6_Avian_mallardduck_alberta_331_1985.5
363_H1N1_Avian_turkey_sd_7034_1986.5
218_H10N7_Avian_pintail_alberta_129_1993.5
411_H2N1_Avian_semipalmatedsandpiper_brazil_43_1990.5
362_H1N1_Avian_bluewingedteal_la_b228_1986.5
225_H10N7_Avian_greenwingedteal_la_169gw_1988.5
349_H1N1_Avian_pintailduck_alb_238_1979.5
358_H1N1_Avian_pigeon_mn_1407_1981.5
1100_H5N2_Avian_turkey_tx_14082_1982.5
537_H3N5_Avian_mallardduck_alb_635_1983.5
652_H4N2_Avian_mallardduck_alb_630_1984.5
1350_H6N6_Avian_sanderling_de_1258_1986.5
1371_H6N8_Avian_mallard_ohio_123_1989.5
500_H3N2_Avian_duck_ny_6874_1978.5
407_H1N5_Avian_mallardduck_newyork_6861_1978.5
658_H4N3_Avian_mallard_alberta_300_1977.5
1262_H6N2_Avian_pintailduck_alb_111_1985.5
1615_H7N3_Avian_pheasant_mn_917_1980.5
1806_H9N2_Avian_turkey_tx_4181_1981.5
1099_H5N2_Avian_mallard_wisconsin_944_1982.5
1094_H5N2_Avian_mallard_wi_42_1975.5
1970.0
1975.0
1980.0
1985.0
1990.0
1995.0
2000.0
2005.0
2010.0


PB1
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A/Texas_15_2009_105_2009.288
A/California_14_2009_115_2009.315
A/New_York_1669_2009_116_2009.318
A/California_07_2009_99_2009.271
A/Netherlands_602_2009_119_2009.326
A/New_York_1682_2009_117_2009.321
A/New_York_19_2009_115_2009.315
A/California_06_2009_106_2009.290
A/Texas_04_2009_104_2009.285
A/Texas_09_2009_115_2009.315
A/California_05_2009_89_2009.244
A/Arizona_02_2009_116_2009.318
A/California_04_2009_91_2009.249
A/Texas_07_2009_115_2009.315
A/Indiana_09_2009_112_2009.307
A/Minnesota_02_2009_117_2009.321
191_H3N2_Swine_swine_northcarolina_2003.5
Sw_HK_1110_2006.5
SW_HK_915_2004.5
Sw_HK_1562_2005.5
SW_HK_NS623_2002.5
140_H1N2_Swine_swine_guangxi_13_2006.5
Sw_HK_294_2009.25
141_H1N2_Swine_swine_shanghai_1_2007.5
149_H2N3_Swine_swine_missouri_4296424_2006.5
2279_H1N1_Human_iowa_ceid23_2005.5
2894_H3N2_Human_ontario_rv1273_2005.5
156_H3N2_Swine_swine_ontario_33853_2005.5
153_H3N2_Swine_swine_alberta_14722_2005.5
155_H3N2_Swine_swine_manitoba_12707_2005.5
2895_H3N2_Human_ontario_1252_2007.5
527_H3N2_Avian_turkey_mn_366767_2005.5
512_H3N2_Avian_turkey_oh_313053_2004.5
130_H1N1_Swine_swine_oh_511445_2007.5
528_H3N2_Avian_turkey_nc_353568_2005.5
151_H3N1_Swine_swine_mi_pu243_2004.5
150_H3N1_Swine_swine_in_pu542_2004.5
497_H3N2_Avian_turkey_ontario_31232_2005.5
404_H1N2_Avian_duck_nc_91347_2001.5
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2995_H3N2_Human_siena_3_1995.5
152_H3N2_Swine_swine_ontario_00130_1997.5
2913_H3N2_Human_nanchang_0058_1994.5
3632_H3N2_Human_memphis_24_1995.5
2912_H3N2_Human_shandong_9_1993.5
3567_H3N2_Human_newyork_764_1993.5
2966_H3N2_Human_hongkong_14_1992.5
4271_H3N2_Human_california_ur070019_2008.5
4276_H3N2_Human_kansas_ur070007_2008.5
4273_H3N2_Human_florida_ur070101_2008.5
4296_H3N2_Human_kentucky_ur070124_2008.5
4305_H3N2_Human_newyork_ur070133_2008.5
4312_H3N2_Human_ohio_ur070058_2008.5
4326_H3N2_Human_pennsylvania_pit31_2008.5
3452_H3N2_Human_korea_af05_2008.5
4301_H3N2_Human_mississippi_ur070100_2008.5
4255_H3N2_Human_texas_ur060603_2007.5
3444_H3N2_Human_qatar_af02_2008.5
3000_H3N2_Human_kuwait_af03_2008.5
2893_H3N2_Human_brisbane_10_2007.5
3438_H3N2_Human_managua_490201_2007.5
4225_H3N2_Human_illinois_ur060567_2007.5
4201_H3N2_Human_colorado_ur060023_2007.5
2988_H3N2_Human_hongkong_hku79_2005.5
4191_H3N2_Human_newyork_938_2006.5
4182_H3N2_Human_wisconsin_67_2005.5
2999_H3N2_Human_hiroshima_52_2005.5
3460_H3N2_Human_tw_875_2004.5
3457_H3N2_Human_tw_3286_2003.5
3002_H3N2_Human_yucatan_me6057_2003.5
4083_H3N2_Human_wyoming_03_2003.5
3293_H3N2_Human_waikato_43_2004.5
3320_H3N2_Human_wellington_38_2004.5
3235_H3N2_Human_bayofplenty_332_2004.5
3282_H3N2_Human_tairawhiti_369_2004.5
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3274_H3N2_Human_christchurch_14_2004.5
2878_H3N2_Human_southaustralia_61_2005.5
3247_H3N2_Human_canterbury_12_2004.5
4175_H3N2_Human_newyork_461_2005.5
3233_H3N2_Human_auburton_280_2004.5
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3399_H3N2_Human_waikato_6_2005.5
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2973_H3N2_Human_hongkong_hku22_2004.5
167_H3N2_Swine_swine_guangdong_164_2006.5
160_H3N2_Swine_swine_shandong_3_2005.5
3054_H3N2_Human_nelsonmarlborough_1_2000.5
2723_H3N2_Human_newsouthwales_4_1999.5
3451_H3N2_Human_moscow_10_1999.5
3909_H3N2_Human_newyork_439_2000.5
2741_H3N2_Human_queensland_4_2000.5
3006_H3N2_Human_canterbury_179_1999.5
3053_H3N2_Human_hutt_82_2000.5
3056_H3N2_Human_waikato_16_2000.5
3085_H3N2_Human_wellington_22_2001.5
2828_H3N2_Human_westernaustralia_40_2003.5
2823_H3N2_Human_queensland_39_2003.5
3206_H3N2_Human_southcanterbury_433_2003.5
3179_H3N2_Human_canterbury_412_2003.5
3231_H3N2_Human_wellington_49_2003.5
3993_H3N2_Human_memphis_31_2003.5
4027_H3N2_Human_newyork_267_2003.5
3209_H3N2_Human_waikato_115_2003.5
2853_H3N2_Human_westernaustralia_55_2004.5
2842_H3N2_Human_queensland_44_2004.5
3149_H3N2_Human_waikato_21_2002.5
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3916_H3N2_Human_newyork_402_2001.5
2790_H3N2_Human_westernaustralia_15_2001.5
2794_H3N2_Human_queensland_22_2002.5
3145_H3N2_Human_canterbury_81_2002.5
3148_H3N2_Human_southcanterbury_37_2002.5
3155_H3N2_Human_wellington_66_2002.5
2814_H3N2_Human_westernaustralia_36_2002.5
3991_H3N2_Human_newyork_99_2002.5
2998_H3N2_Human_genoa_14_2003.5
2997_H3N2_Human_genoa_1_2002.5
3454_H3N2_Human_tw_3565_2002.5
3146_H3N2_Human_dunedin_1_2002.5
3031_H3N2_Human_canterbury_66_2000.5
2764_H3N2_Human_southaustralia_76_2000.5
2736_H3N2_Human_newsouthwales_36_2000.5
2771_H3N2_Human_westernaustralia_12_2000.5
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3441_H3N2_Human_panama_2007_1999.5
SW_HK_78_2003.5
3782_H3N2_Human_newyork_138_1999.5
137_H1N2_Swine_swine_ontario_48235_2004.5
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3727_H3N2_Human_newyork_541_1998.5
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3616_H3N2_Human_newyork_720_1994.5
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2994_H3N2_Human_siena_6_19911[illegible]
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2909_H3N2_Human_beijing_32_1992.5
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2965_H3N2_Human_hongkong_2_1988.5
3553_H3N2_Human_memphis_7_1990.5
2990_H3N2_Human_siena_10_1990.5
2964_H3N2_Human_hongkong_7_1987.5
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3450_H3N2_Human_leningrad_360_1986.5
3542_H3N2_Human_memphis_5_1986.5
3538_H3N2_Human_memphis_7_1985.5
2907_H3N2_Human_nanjing_28_1984.5
2962_H3N2_Human_hongkong_6_1985.5
2959_H3N2_Human_hongkong_4_1984.5
2954_H3N2_Human_hongkong_14_1983.5
3529_H3N2_Human_memphis_1_1981.5
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2953_H3N2_Human_hongkong_1_1982.5
2906_H3N2_Human_nanjing_36_1983.5
2905_H3N2_Human_nanjing_2_1982.5
2951_H3N2_Human_hongkong_45_1980.5
2904_H3N2_Human_nanjing_13_1980.5
2112_H1N1_Human_memphis_1_1979.5
2111_H1N1_Human_albany_8_1979.5
2067_H1N1_Human_ussr_46_1979.5
2100_H1N1_Human_california_10_1978.5
3512_H3N2_Human_memphis_4_1977.5
3495_H3N2_Human_memphis_103_1974.5
3496_H3N2_Human_albany_42_1975.5
183_H3N3_Swine_swine_colorado_1_1977.5
3503_H3N2_Human_memphis_108_1976.5
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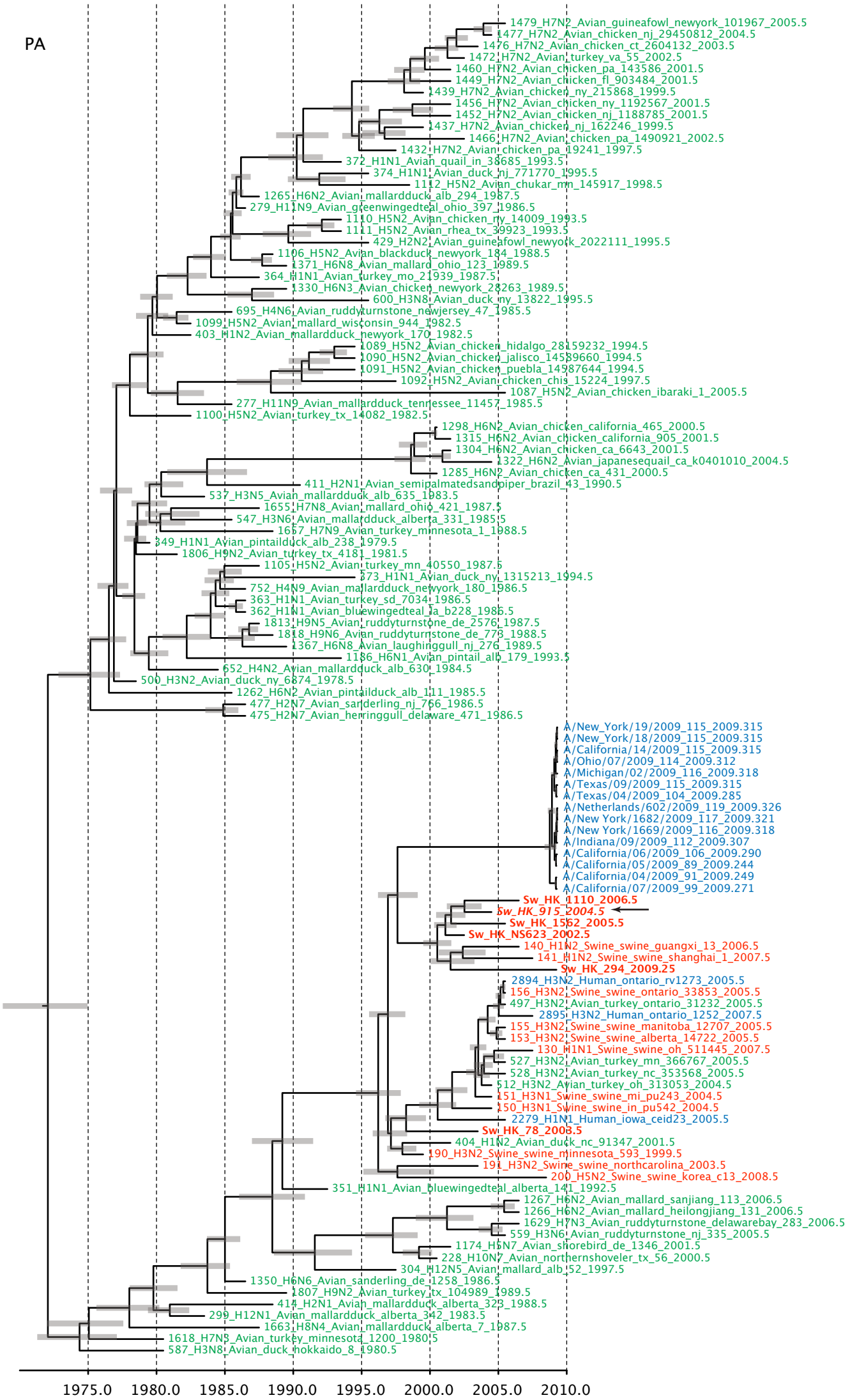
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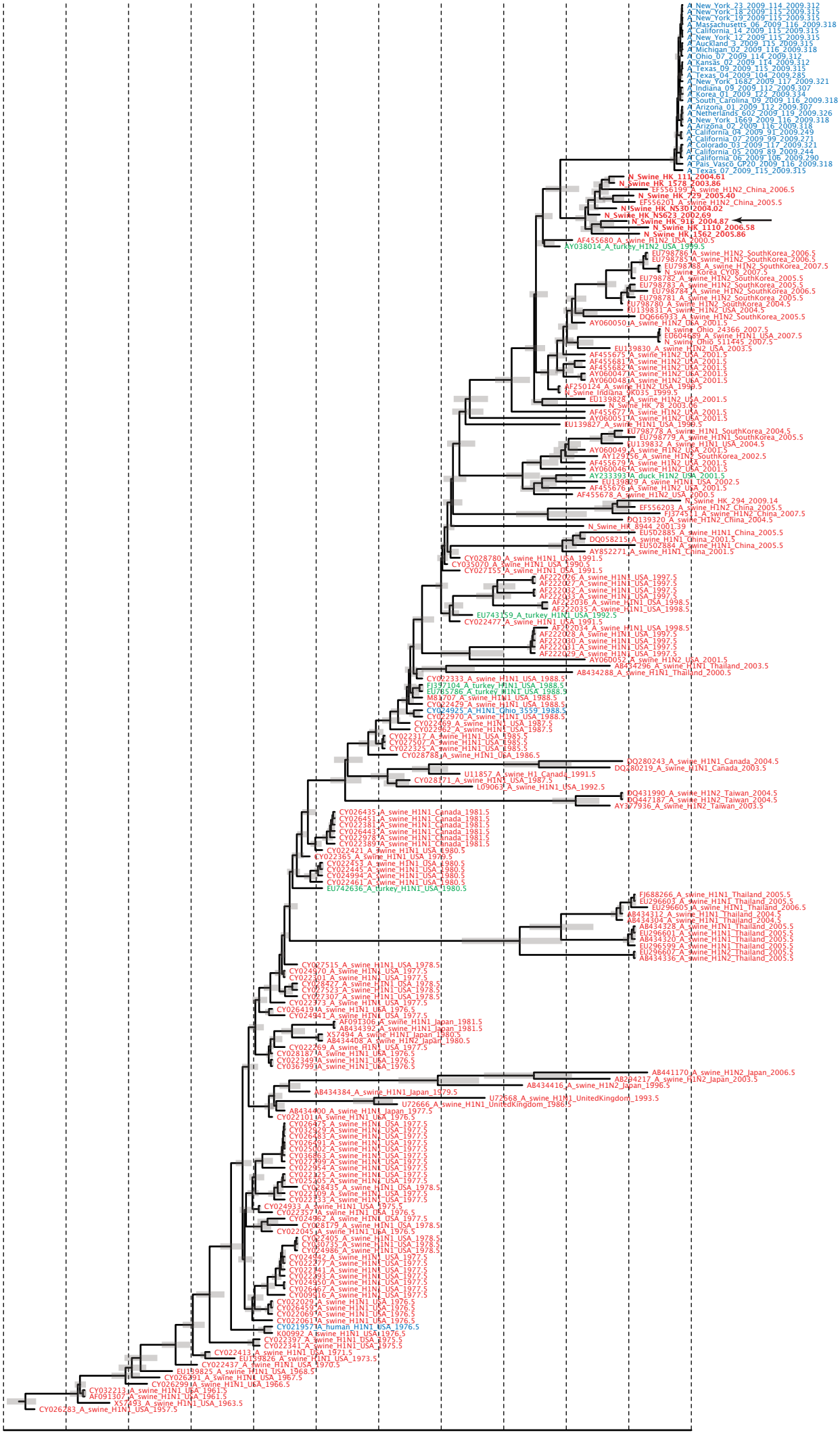
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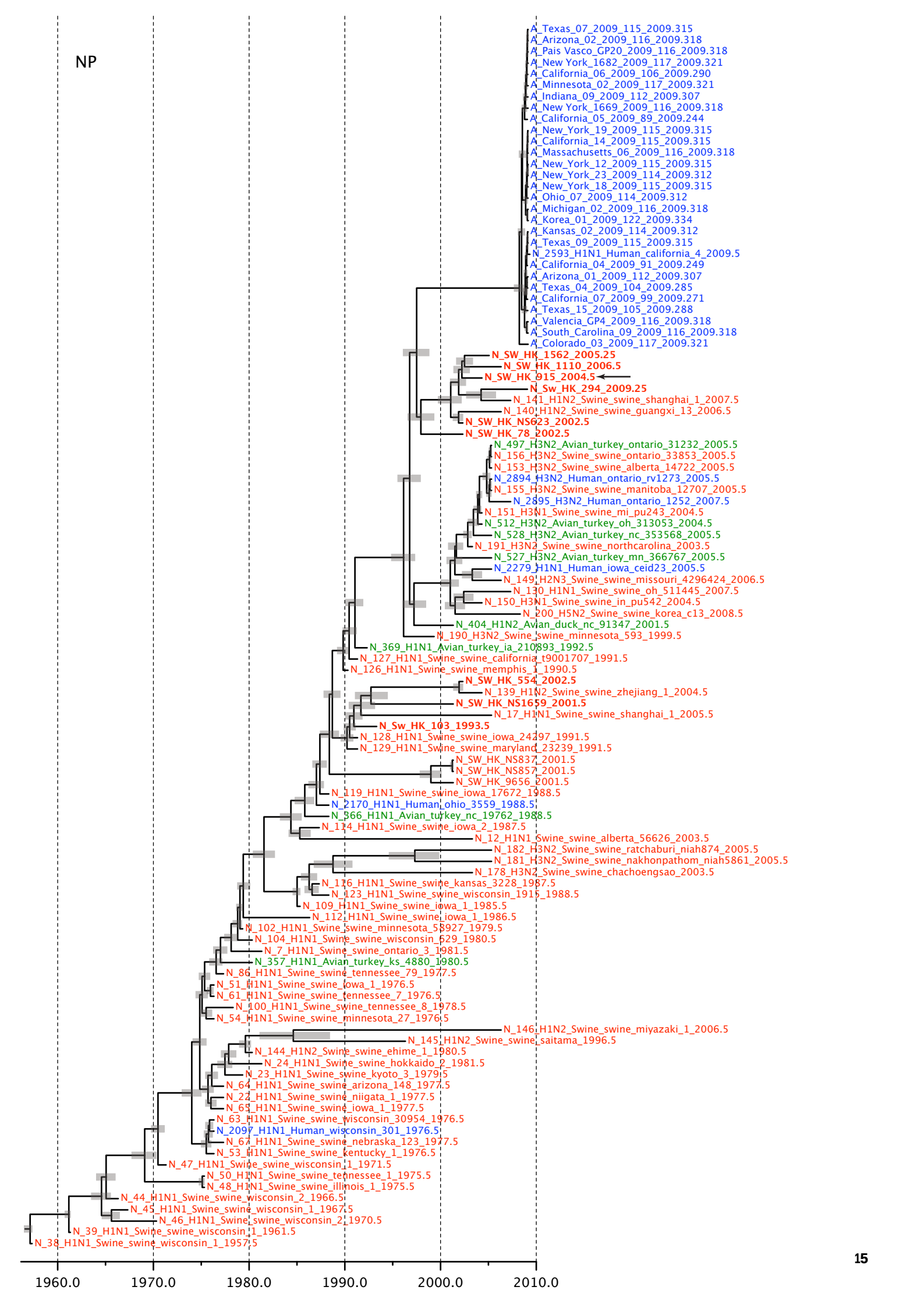
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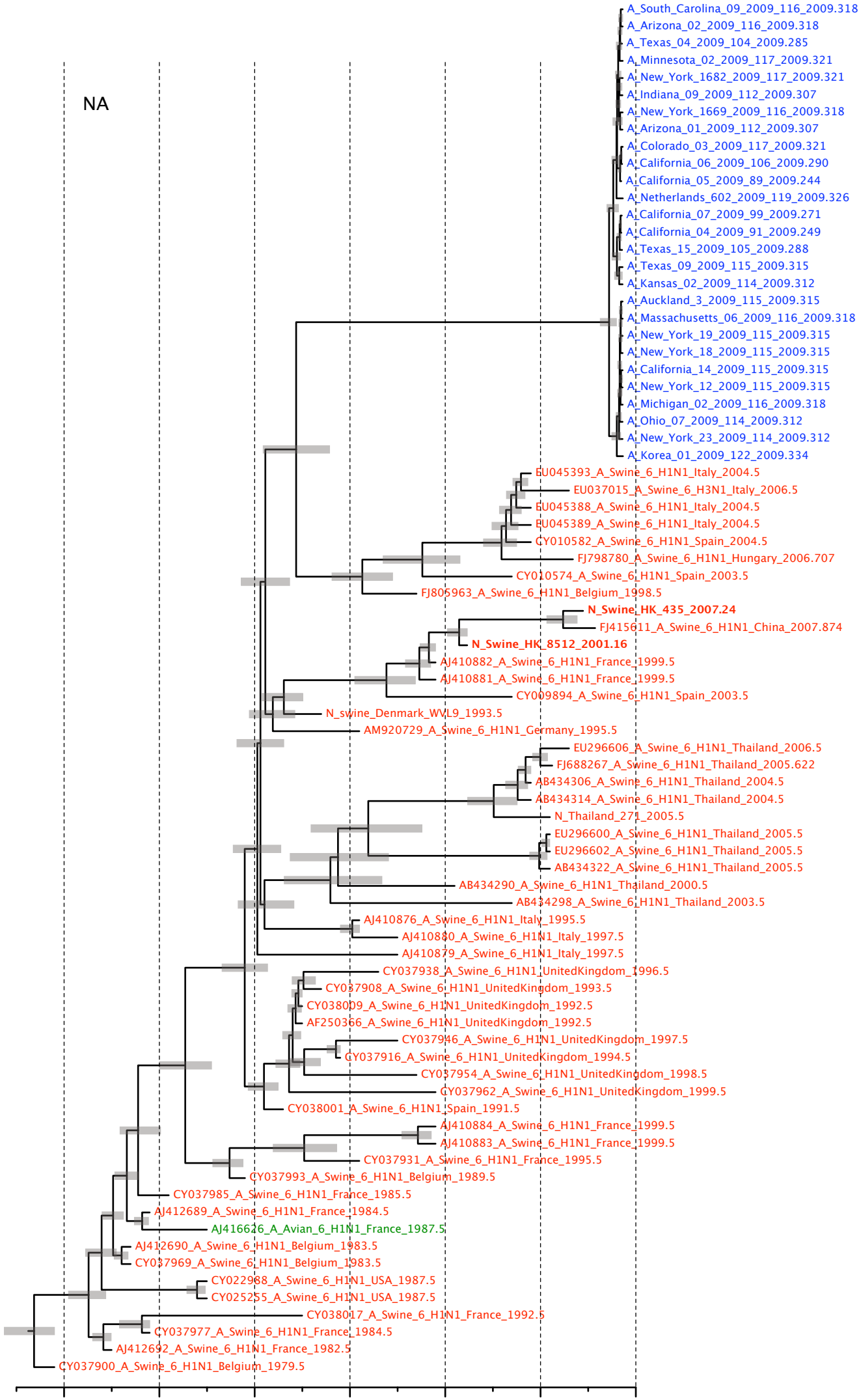
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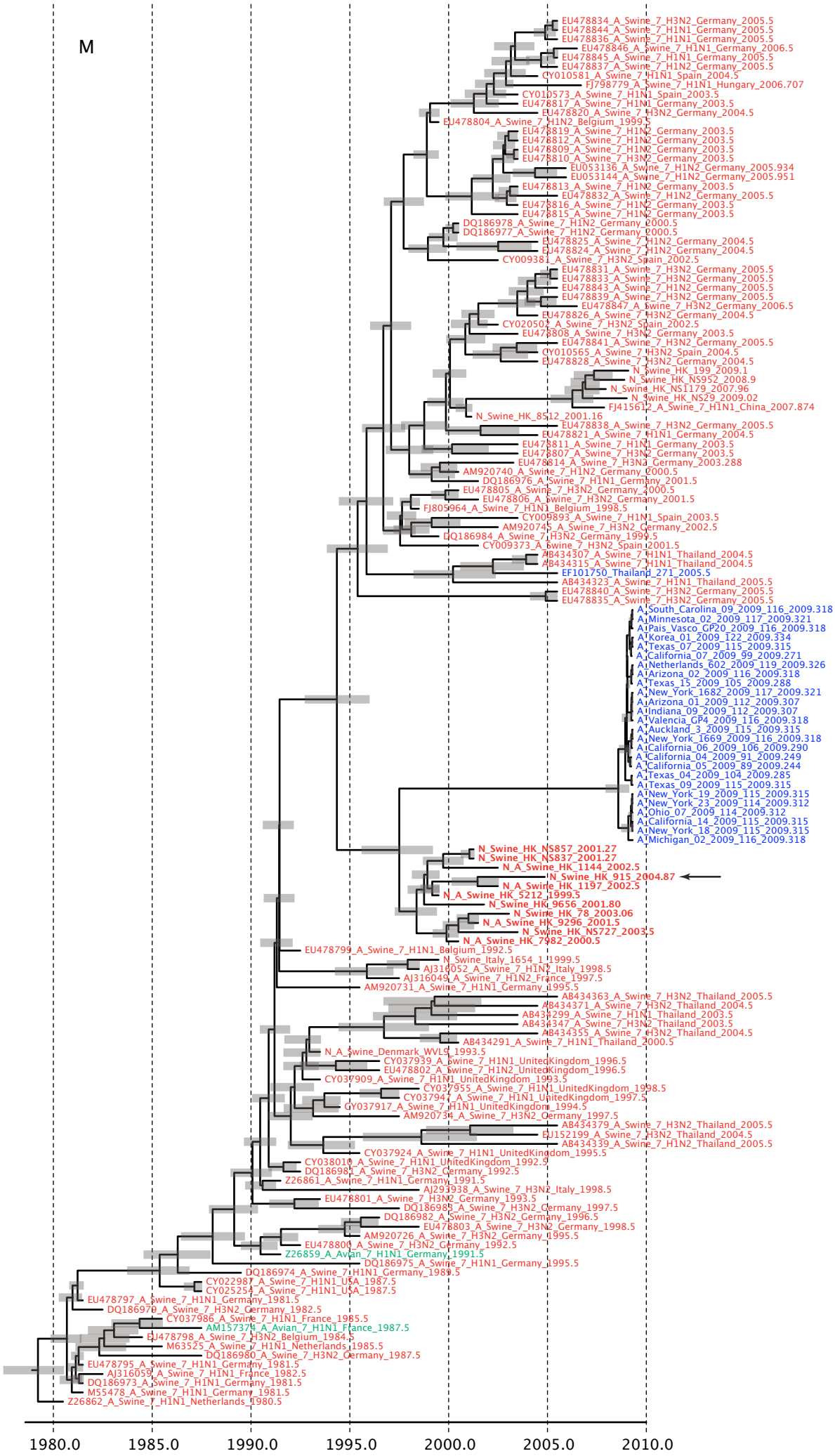
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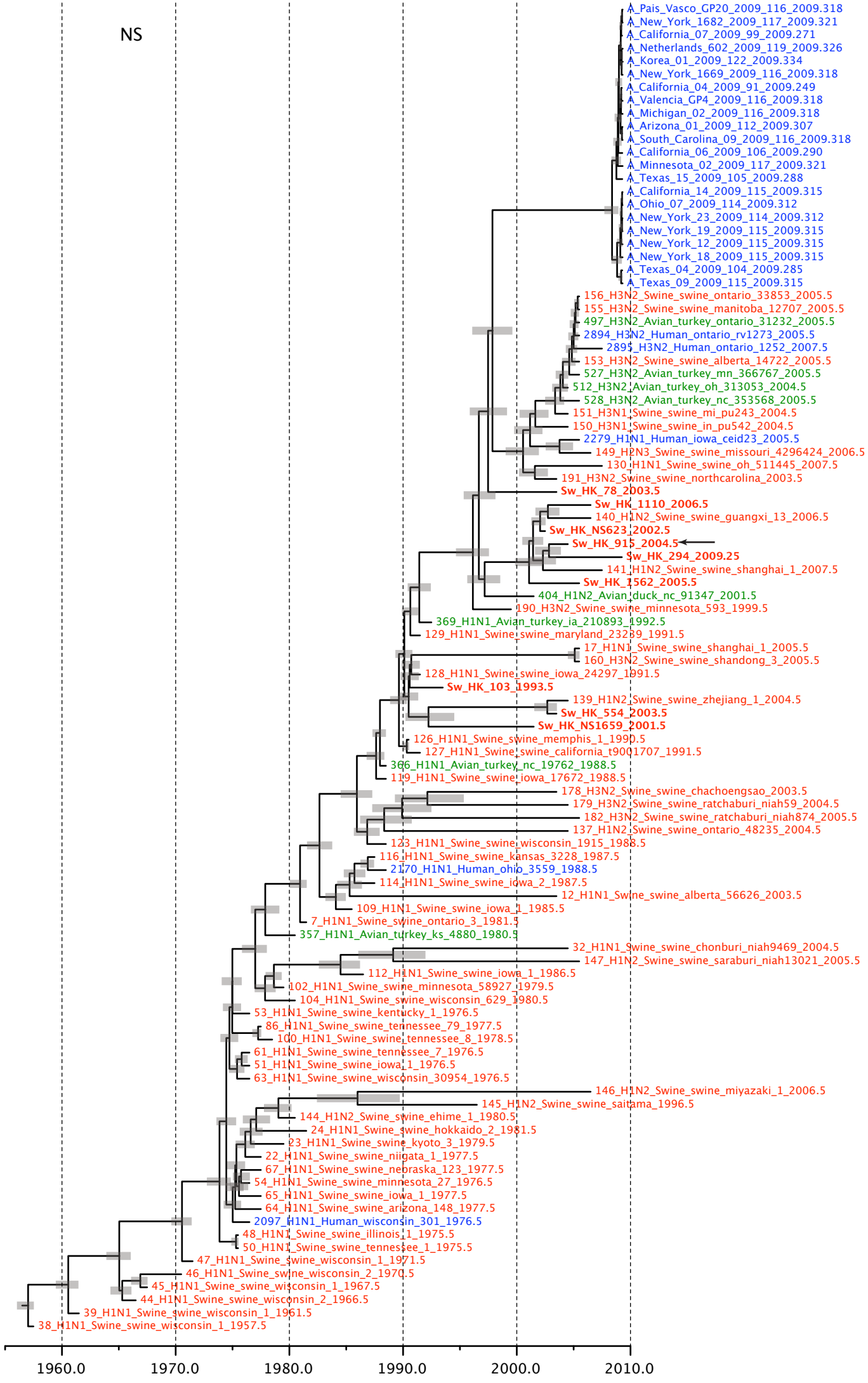
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A_Arizona_01_2009_112_2009.307
A_South_Carolina_09_2009_116_2009.318
A_California_06_2009_106_2009.290
A_Minnesota_02_2009_117_2009.321
A_Texas_15_2009_105_2009.288
A_California_14_2009_115_2009.315
A_Ohio_07_2009_114_2009.312
A_New_York_23_2009_114_2009.312
A_New_York_19_2009_115_2009.315
A_New_York_12_2009_115_2009.315
A_New_York_18_2009_115_2009.315
A_Texas_04_2009_104_2009.285
A_Texas_09_2009_115_2009.315
156_H3N2_Swine_swine_ontario_33853_2005.5
155_H3N2_Swine_swine_manitoba_12707_2005.5
497_H3N2_Avian_turkey_ontario_31232_2005.5
2894_H3N2_Human_ontario_rv1273_2005.5
2895_H3N2_Human_ontario_1252_2007.5
153_H3N2_Swine_swine_alberta_14722_2005.5
527_H3N2_Avian_turkey_mn_366767_2005.5
512_H3N2_Avian_turkey_oh_313053_2004.5
528_H3N2_Avian_turkey_nc_353568_2005.5
151_H3N1_Swine_swine_mi_pu243_2004.5
150_H3N1_Swine_swine_in_pu542_2004.5
2279_H1N1_Human_iowa_ceid23_2005.5
149_H2N3_Swine_swine_missouri_4296424_2006.5
130_H1N1_Swine_swine_oh_511445_2007.5
191_H3N2_Swine_swine_northcarolina_2003.5
Sw_HK_78_2003.5
Sw_HK_1110_2006.5
140_H1N2_Swine_swine_guangxi_13_2006.5
Sw_HK_NS623_2002.5
Sw_HK_915_2004.5
Sw_HK_294_2009.25
141_H1N2_Swine_swine_shanghai_1_2007.5
Sw_HK_1562_2005.5
404_H1N2_Avian_duck_nc_91347_2001.5
190_H3N2_Swine_swine_minnesota_593_1999.5
369_H1N1_Avian_turkey_ia_210893_1992.5
129_H1N1_Swine_swine_maryland_23239_1991.5
17_H1N1_Swine_swine_shanghai_1_2005.5
160_H3N2_Swine_swine_shandong_3_2005.5
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Sw_HK_103_1993.5
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Sw_HK_NS1659_2001.5
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119_H1N1_Swine_swine_iowa_17672_1988.5
178_H3N2_Swine_swine_chachoengsao_2003.5
179_H3N2_Swine_swine_ratchaburi_niah59_2004.5
182_H3N2_Swine_swine_ratchaburi_niah874_2005.5
137_H1N2_Swine_swine_ontario_48235_2004.5
123_H1N1_Swine_swine_wisconsin_1915_1988.5
116_H1N1_Swine_swine_kansas_3228_1987.5
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86_H1N1_Swine_swine_tennessee_79_1977.5
100_H1N1_Swine_swine_tennessee_8_1978.5
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51_H1N1_Swine_swine_iowa_1_1976.5
63_H1N1_Swine_swine_wisconsin_30954_1976.5
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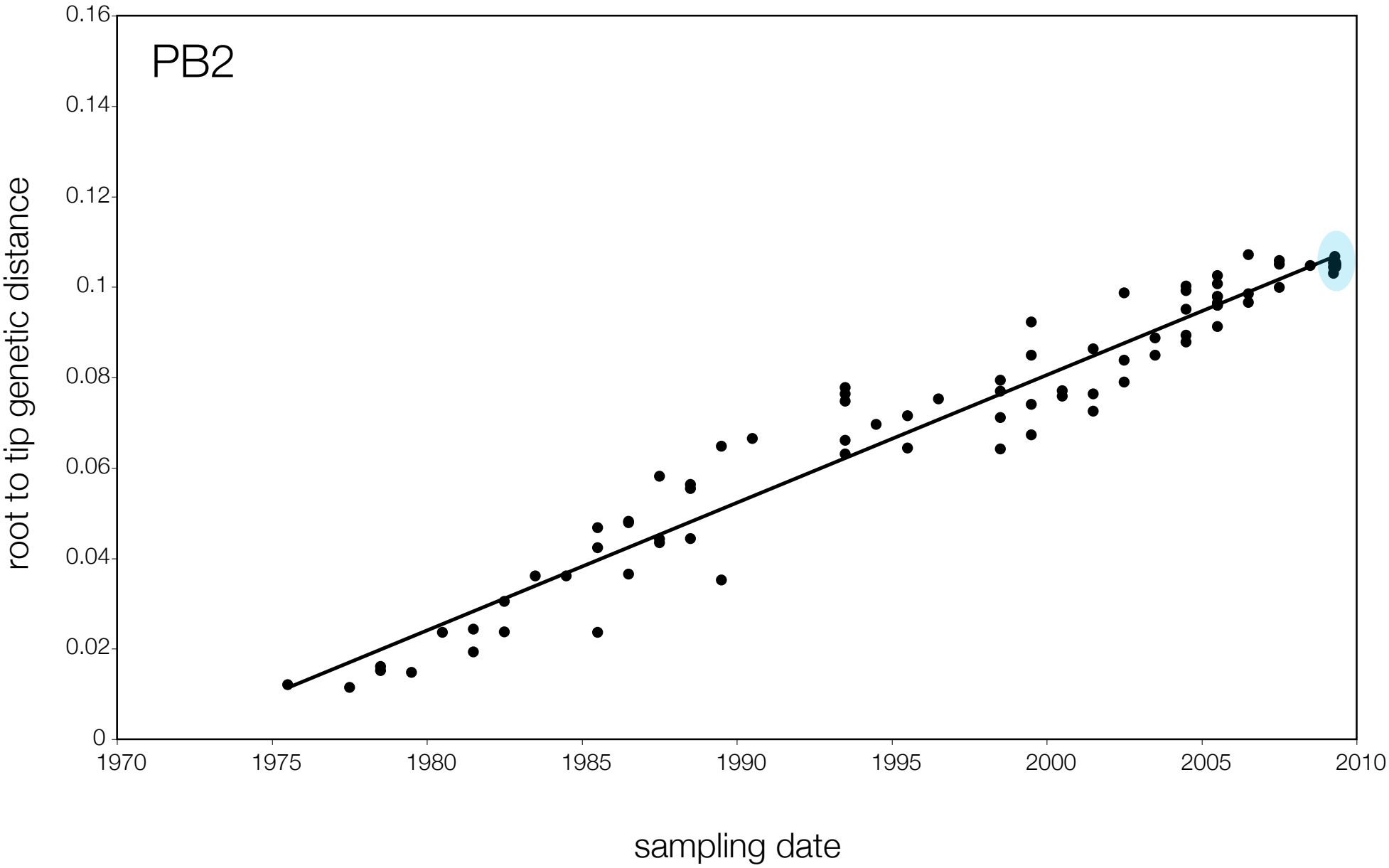
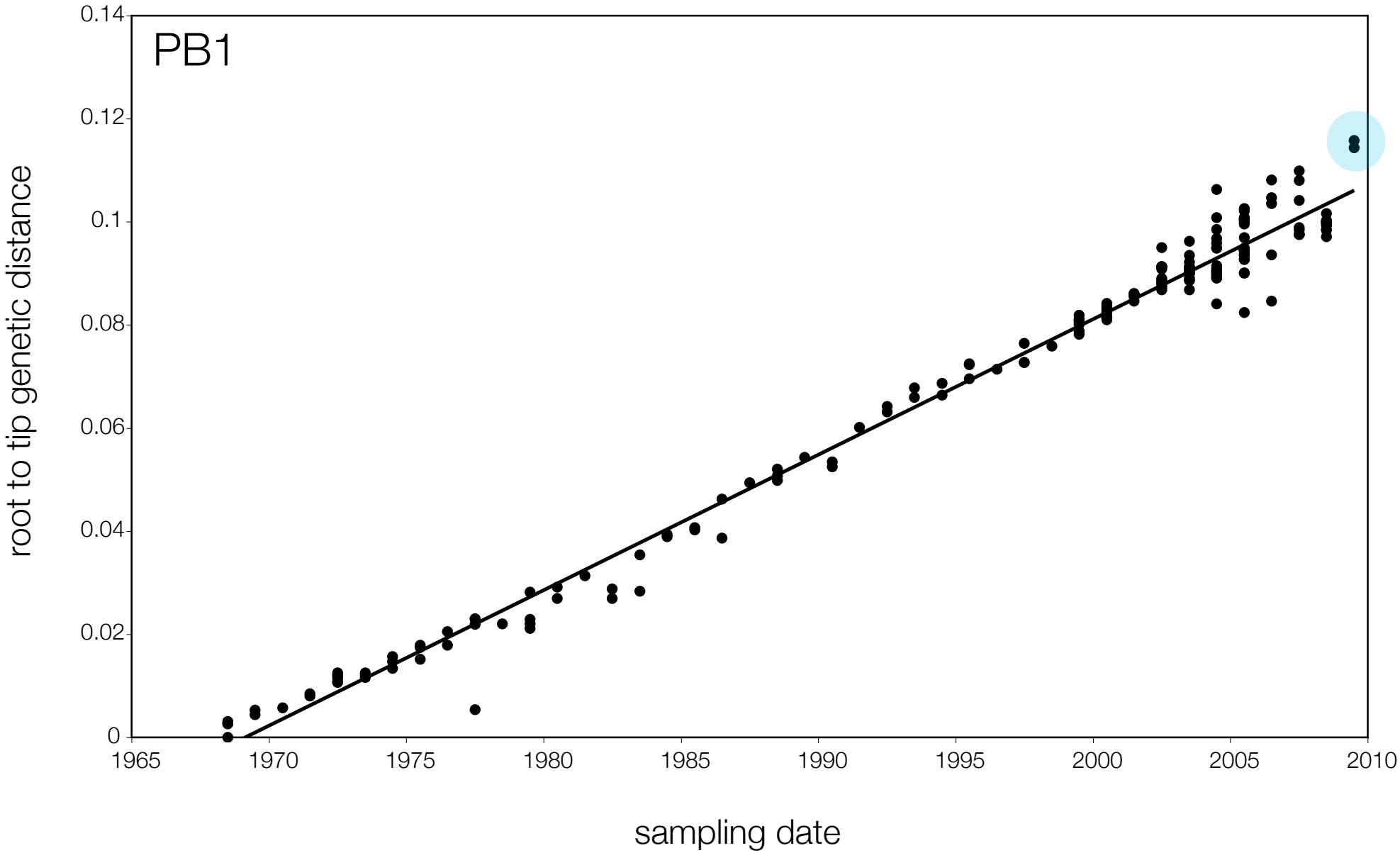
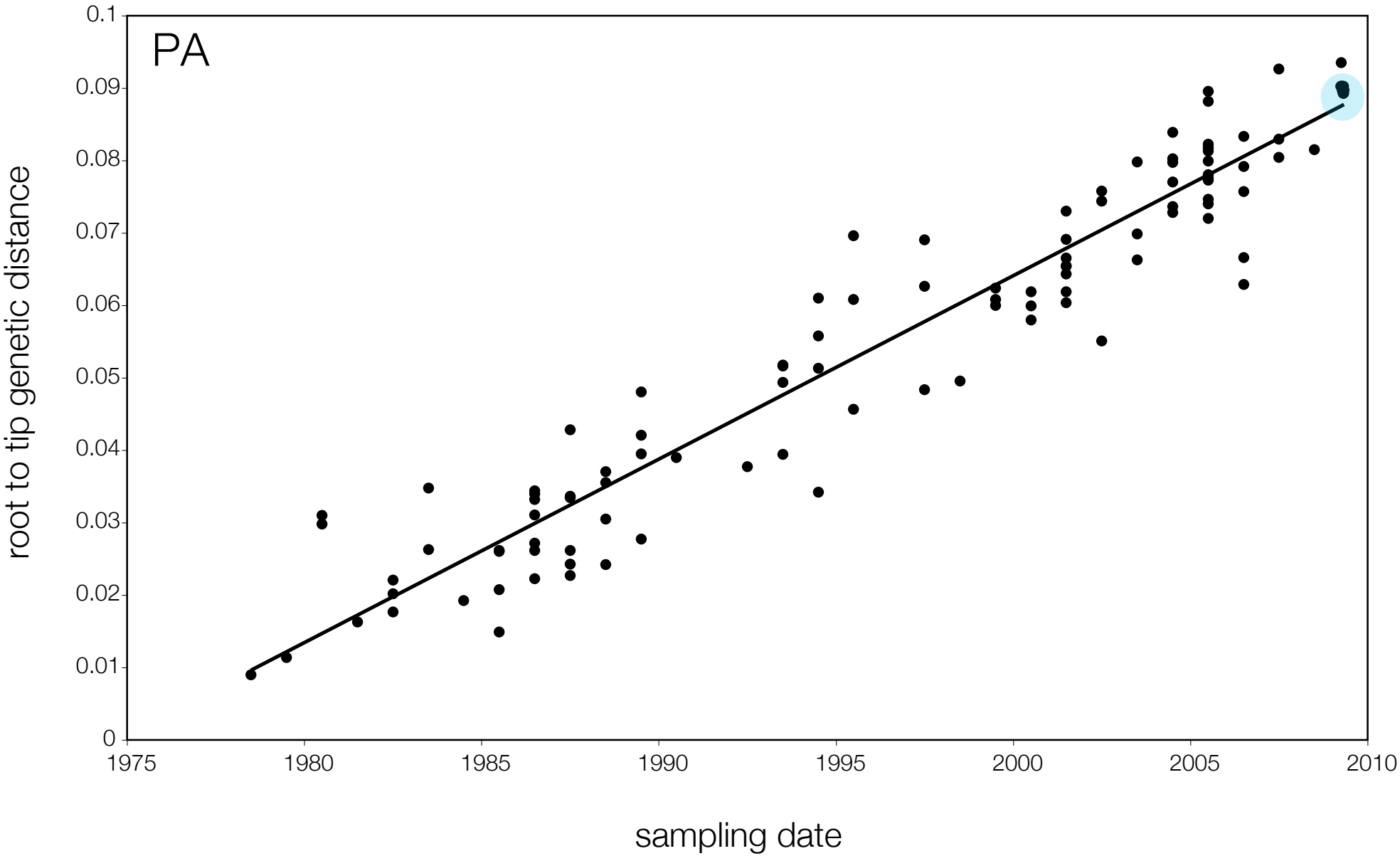
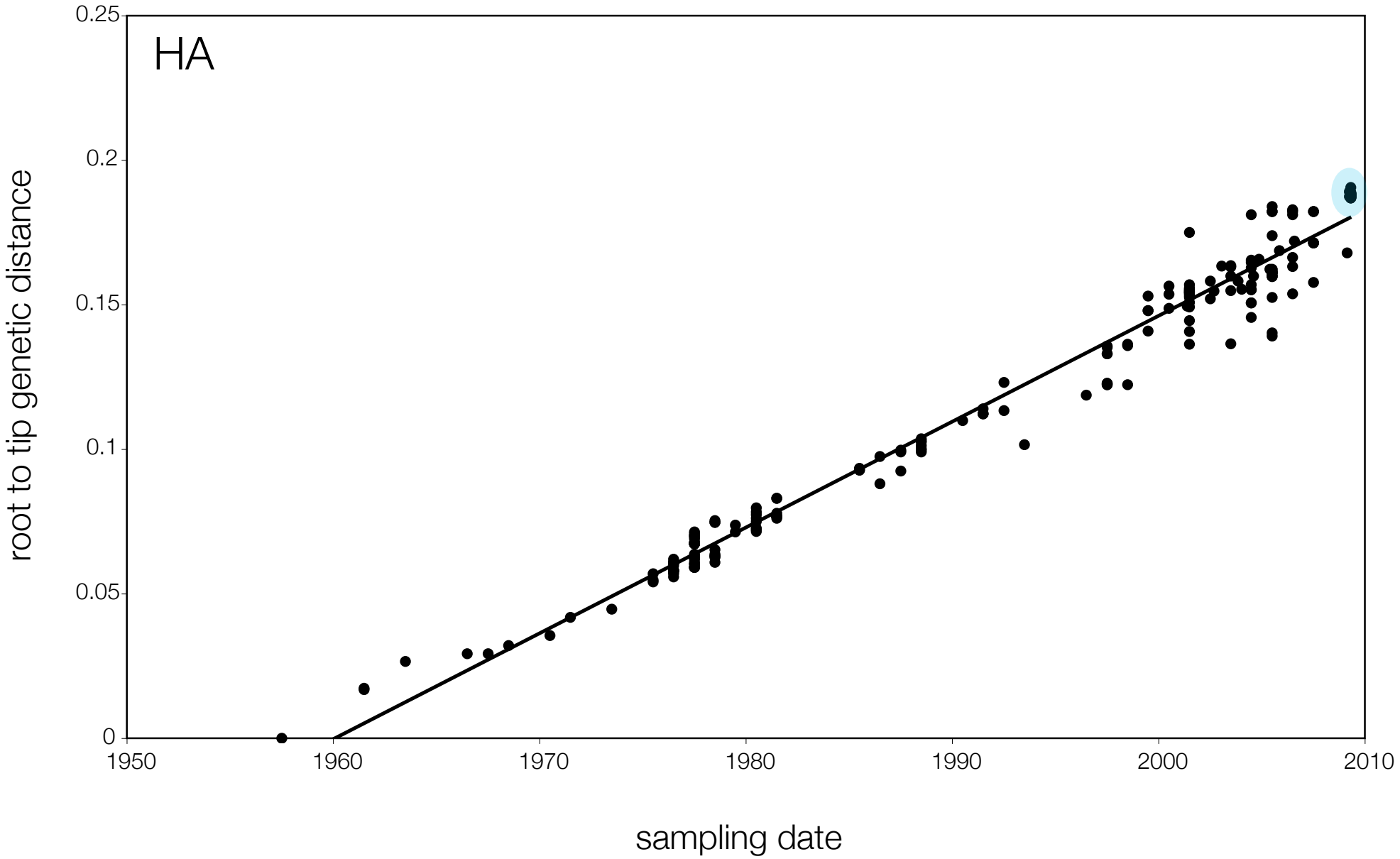
24_H1N1_Swine_swine_hokkaido_2_1981.5
23_H1N1_Swine_swine_kyoto_3_1979.5
22_H1N1_Swine_swine_niigata_1_1977.5
67_H1N1_Swine_swine_nebraska_123_1977.5
54_H1N1_Swine_swine_minnesota_27_1976.5
65_H1N1_Swine_swine_iowa_1_1977.5
64_H1N1_Swine_swine_arizona_148_1977.5
2097_H1N1_Human_wisconsin_301_1976.5
48_H1N1_Swine_swine_illinois_1_1975.5
50_H1N1_Swine_swine_tennessee_1_1975.5
47_H1N1_Swine_swine_wisconsin_1_1971.5
46_H1N1_Swine_swine_wisconsin_2_1970.5
45_H1N1_Swine_swine_wisconsin_1_1967.5
44_H1N1_Swine_swine_wisconsin_2_1966.5
39_H1N1_Swine_swine_wisconsin_1_1961.5
38_H1N1_Swine_swine_wisconsin_1_1957.5
1960.0
1970.0
1980.0
1990.0
2000.0
2010.0

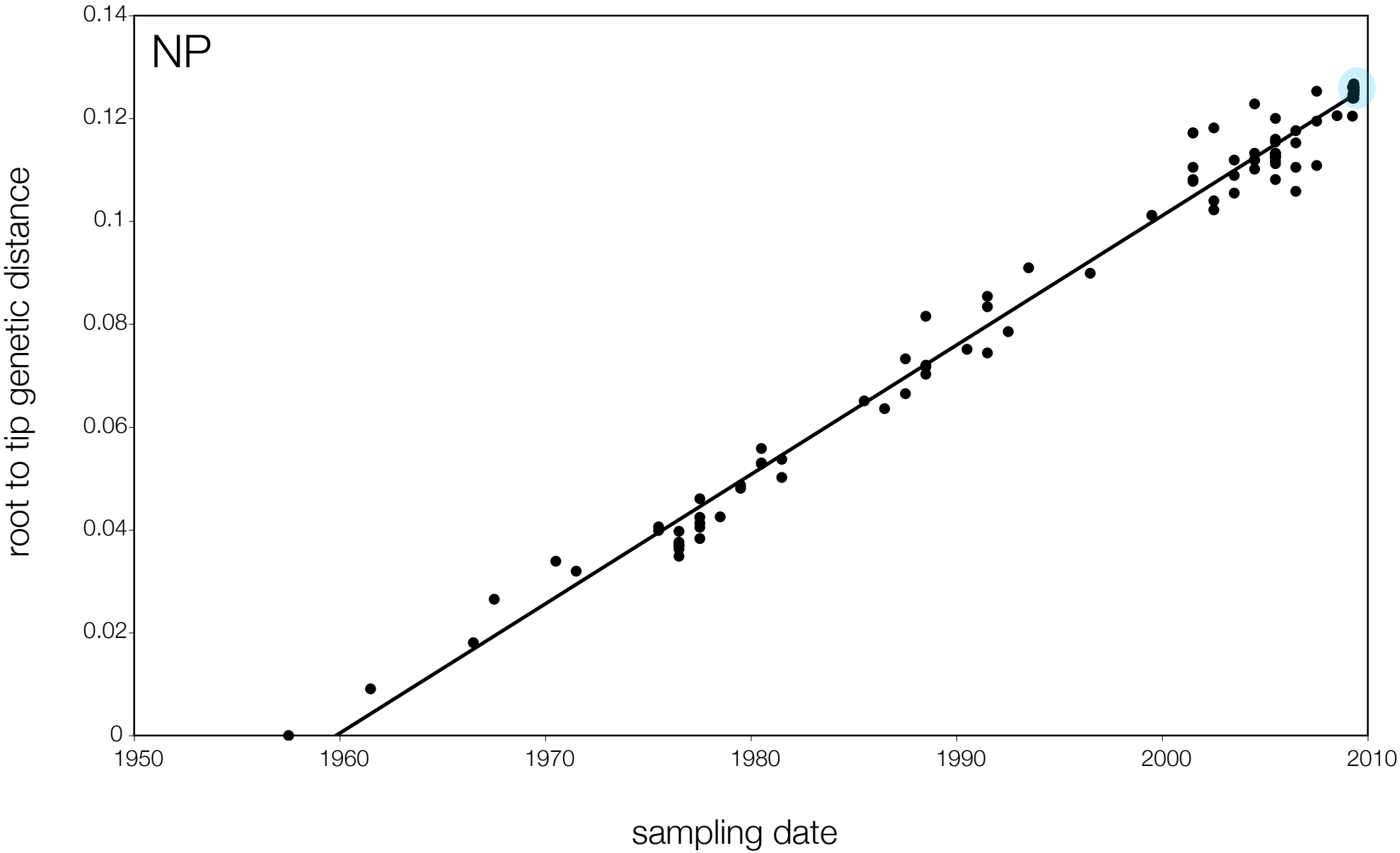
**Supplementary Figure 3.** For each gene segment (PB2, PB1, PA, HA, NP, NA, M & NS), we plot the isolation date of each influenza sequence against the genetic distance from that sequence to the root of the phylogeny. The linear regression gradient is therefore an estimate of the rate of sequence evolution and the x-intercept is an estimate of the TMRCA of the whole phylogeny. Phylogenies were estimated using neighbour-joining, with rooting chosen to maximise the regression fit. The chosen root was typically very close to the earliest sampled sequence. Residual analysis was performed to identify and remove significant outliers, which most likely result from isolation data annotation errors in the sequence database. For each gene, the degree of scatter about the linear regression reflects evolutionary rate heterogeneity among lineages, such that a "strict clock" corresponds to all the points falling exactly on the regression line. The 2009 outbreak sequences (highlighted in light blue) are entirely typically of the long termtrends in divergence, hence there is no evidence that the branch leading to the outbreak has evolved unusually rapidly or slowly. For further discussion of this methodology, see Drummond AJ, Pybus OG, Rambaut A. 2003. Inference of viral evolutionary rates from molecular sequences. *Advances in Parasitology* **54**:331-358.

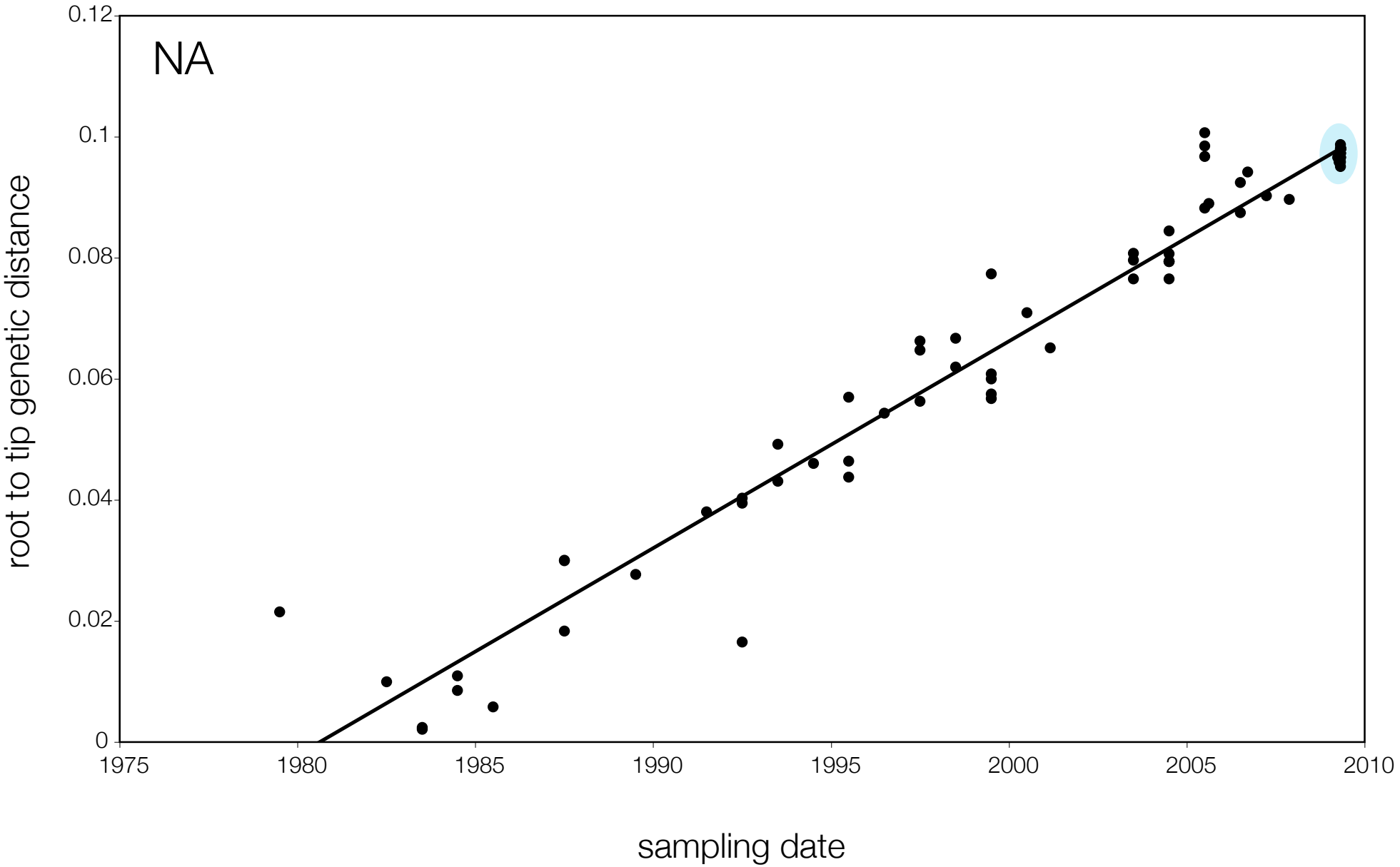

PB2
root to tip genetic distance
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
1970
1975
1980
1985
1990
1995
2000
2005
2010
sampling date

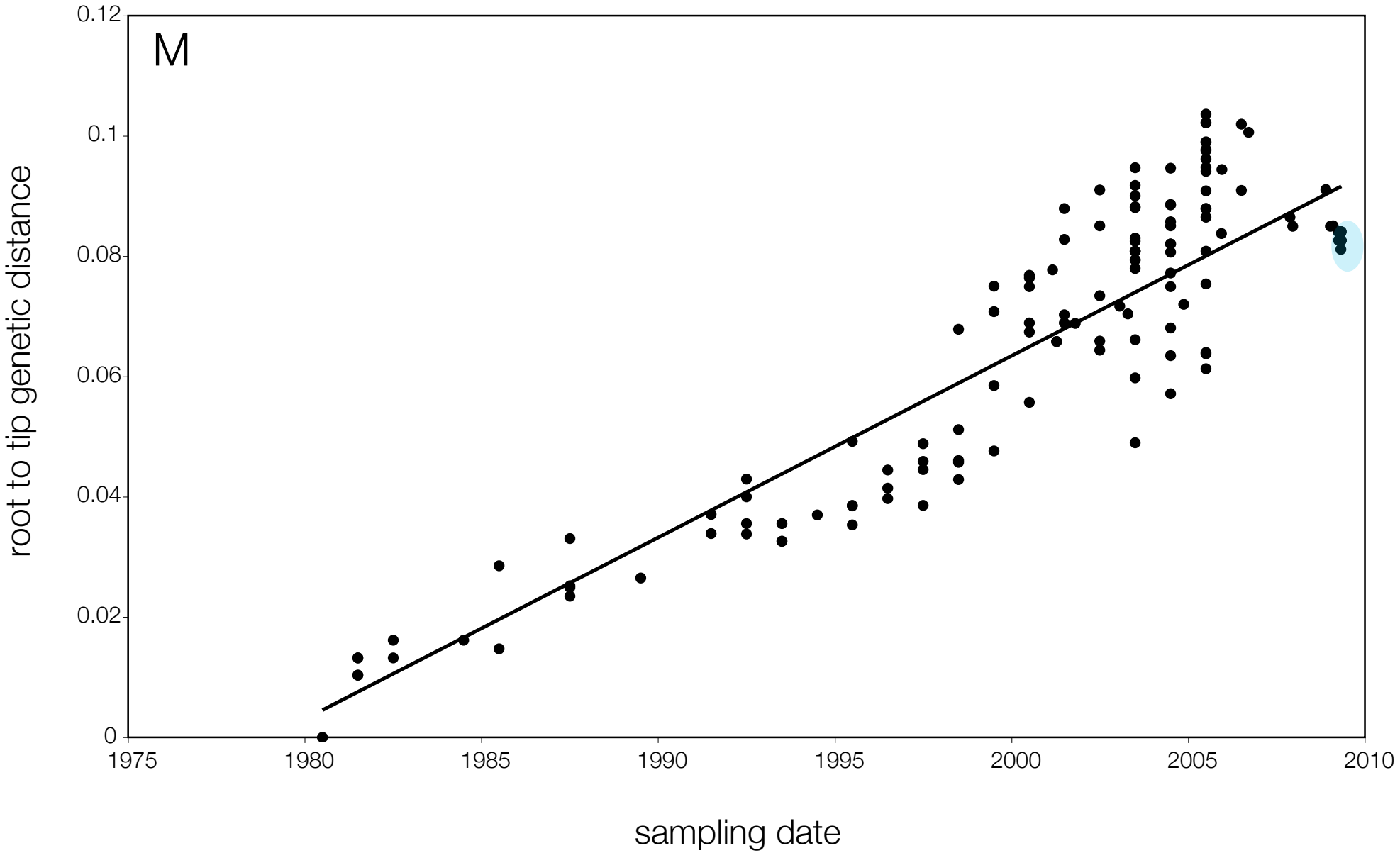

PB1
root to tip genetic distance
sampling date
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
1965
1970
1975
1980
1985
1990
1995
2000
2005
2010

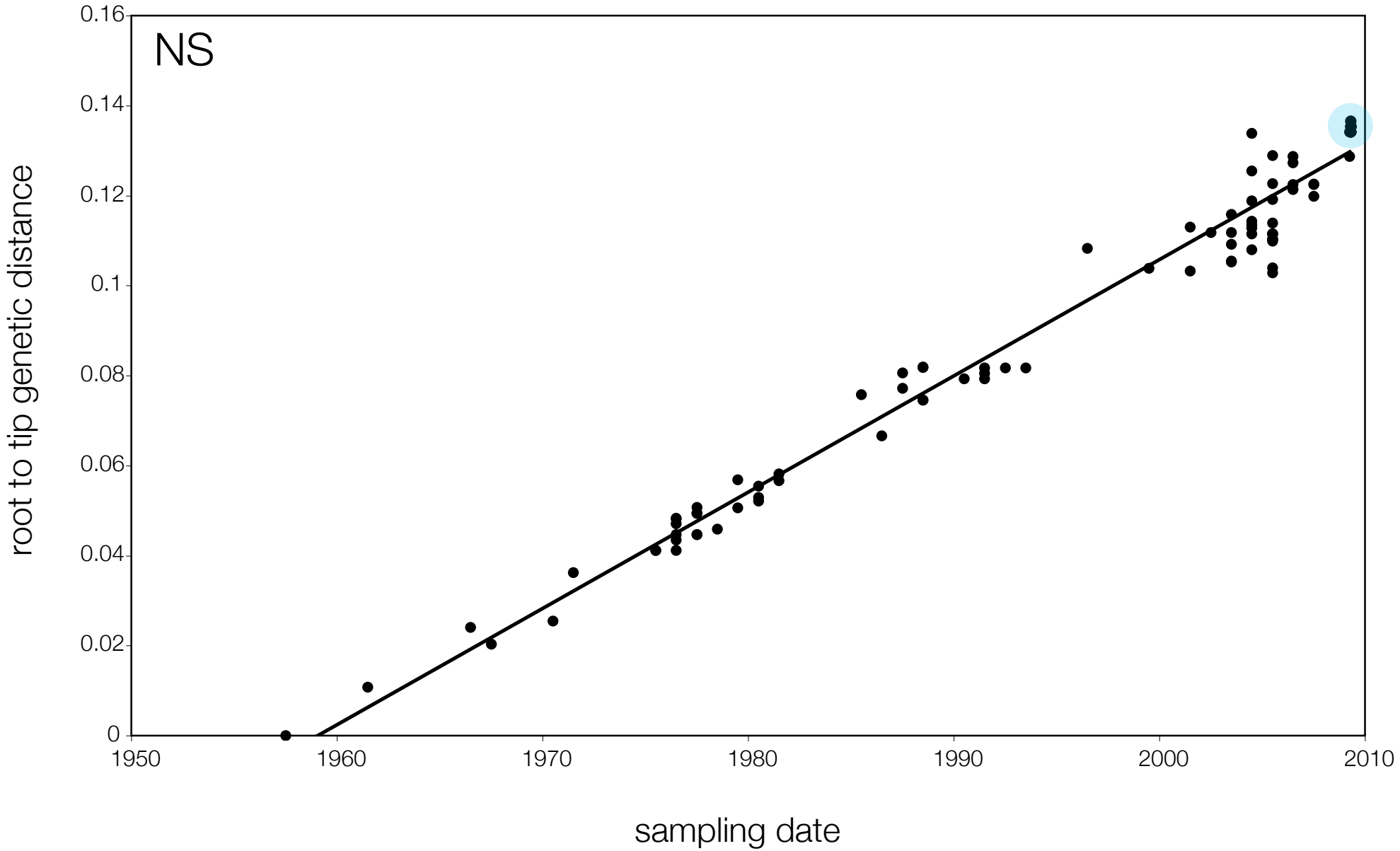

PA
root to tip genetic distance
sampling date
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
1975
1980
1985
1990
1995
2000
2005
2010


HA
root to tip genetic distance
0.25
0.2
0.15
0.1
0.05
0
1950
1960
1970
1980
1990
2000
2010
sampling date


NP
root to tip genetic distance
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
1950
1960
1970
1980
1990
2000
2010
sampling date


NA
root to tip genetic distance
0.12
0.1
0.08
0.06
0.04
0.02
0
1975
1980
1985
1990
1995
2000
2005
2010
sampling date


M
root to tip genetic distance
0.12
0.1
0.08
0.06
0.04
0.02
0
1975
1980
1985
1990
1995
2000
2005
2010
sampling date


NS
root to tip genetic distance
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
1950
1960
1970
1980
1990
2000
2010
sampling date

## Table S1 SLAC Results

| gene | outbreak dN/dS[a] | reference dN/dS | estimated relative rate[b] | mean TMRCA | mean TMRCA (adjusted) |
|---|---|---|---|---|---|
| HA | 0.32 | 0.21 | 1.22 | 28-Aug-2008 | 11-Oct-2008 |
| NA | 0.26 | 0.18 | 1.16 | 8-Aug-2008 | 12-Sep-2008 |
| MP | 0.19 | 0.05 | 1.43 | 3-Aug-2008 | 26-Oct-2008 |
| NP | 0.18 | 0.05 | 1.39 | 27-Mar-2008 | 19-Jul-2008 |
| NS | 2.15 | 0.23 | 3.85 | 21-May-2008 | 6-Feb-2009 |
| PA | 0.11 | 0.06 | 1.15 | 7-Oct-2008 | 6-Nov-2008 |
| PB1 | 0.212 | 0.06 | 1.45 | 24-Oct-2008 | 22-Dec-2008 |
| PB2 | 0.15 | 0.11 | 1.11 | 9-Sep-2008 | 4-Oct-2008 |

[a]Calculated using SLAC (S. Kosakovsky Pond, available at http://datamonkey.org)

[b]substitution rate in outbreak clade relative to non-outbreak sequences based on excess non-synonymous mutations inferred from the higher dN/dS ratio.

## Table S2 SNAP Results

| gene | outbreak dN/dS[a] | reference dN/dS | estimated relative rate[b] | mean TMRCA | mean TMRCA (adjusted) |
|---|---|---|---|---|---|
| HA | 0.24 | 0.09 | 1.41 | 28-Aug-2008 | 9-Nov-2008 |
| NA | 0.32 | 0.1 | 1.59 | 8-Aug-2008 | 17-Nov-2008 |
| MP | 0.3 | 0.03 | 1.82 | 3-Aug-2008 | 5-Dec-2008 |
| NP | 0.14 | 0.02 | 1.39 | 27-Mar-2008 | 19-Jul-2008 |
| NS | 0.31 | 0.13 | 1.43 | 21-May-2008 | 5-Sep-2008 |
| PA | 0.18 | 0.05 | 1.43 | 7-Oct-2008 | 10-Dec-2008 |
| PB1 | 0.16 | 0.03 | 1.45 | 24-Oct-2008 | 22-Dec-2008 |
| PB2 | 0.13 | 0.07 | 1.15 | 9-Sep-2008 | 10-Oct-2008 |

[a]Calculated using SNAP (B. Korber, available at http://www.hiv.lanl.gov/content/sequence/SNAP/SNAP.html)

[b]substitution rate in outbreak clade relative to non-outbreak sequences based on excess non-synonymous mutations inferred from the higher dN/dS ratio.

## Table S3 Hong Kong swine genetic origins

Lineage of segments from Sw/HK sequences based on Phylogenetic analysis

| All Seqs | PB2 | PB1 | PA | HA (H1) | NP | NA | M | NS |
|---|---|---|---|---|---|---|---|---|
| Swine/HK/103/93 | C | C | C | C | C | N1 | C | C |
| Sw/HK/NS1659/01 | C | C | C | C | C | N1 | C | C |
| Sw/HK/8512/01 | C | E | E | E | E | N1 | E | E |
| Sw/HK/NS837/01 | E | E | E | E | C | N1 | E | E |
| Sw/HK/9656/01 | E | E | E | E | C | N1 | E | E |
| Sw/HK/NS1179/07 | E | E | E | E | E | N1 | E | E |
| Sw/HK/NS29/09 | E | E | E | E | E | N1 | E | E |
| Sw/HK/554/03 | C | C | C | C | C | N2 | C | C |
| Sw/HK/NS857/01 | E | E | E | E | C | N2 | E | E |
| Sw/HK/NS623/02 | T | T | T | T | T | N2 | T | T |
| Sw/HK/78/03 | T | H | T | T | T | N2 | E | T |
| Sw/HK/1110/06 | T | T | T | T | T | N2 | T | T |
| Sw/HK/915/04 | T | T | T | T | T | N2 | E | T |
| Sw/HK/1562/05 | T | T | T | T | T | N2 | T | T |
| Sw/HK/294/09 | T | T | T | C | T | N2 | T | T |
| A/California/04/2009 | T | T | T | T | T | N1 | E | T |

| | |
|---|---|
| Classical Swine | C |
| Eurasian Swine | E |
| Triple Reassortant | T |
| Human | H |

## Table S4 S-OIV sequences available on NCBI Influenza virus database at time of analysis.

| | Days[1] | PB2[2] | PB1 | PA | HA | NP | NA | MP | NS |
|---|---|---|---|---|---|---|---|---|---|
| A/Arizona/01/2009 | 112 | | | | GQ117067 | GQ117063 | GQ117064 | GQ117066 | GQ117065 |
| A/Arizona/02/2009 | 116 | GQ117076 | GQ117075 | | GQ117079 | GQ117074 | GQ117077 | GQ117078 | |
| A/Auckland/3/2009 | 115 | | | | | | FJ973552 | FJ973553 | |
| A/California/04/2009 | 91 | FJ966079 | FJ966080 | FJ966081 | FJ966082 | FJ966083 | FJ966084 | FJ966085 | FJ966086 |
| A/California/05/2009 | 89 | FJ966955 | FJ966958 | FJ966957 | FJ966952 | FJ966953 | FJ966956 | FJ966954 | |
| A/California/06/2009 | 106 | FJ966963 | FJ966965 | FJ966964 | FJ966960 | FJ966961 | FJ971075 | FJ966962 | FJ971074 |
| A/California/07/2009 | 99 | FJ984387 | FJ969531 | FJ969529 | FJ981613 | | FJ984386 | FJ966975 | |
| A/California/14/2009 | 115 | GQ117035 | GQ117034 | GQ117037 | GQ117040 | GQ117033 | GQ117036 | GQ117039 | GQ117038 |
| A/Colorado/03/2009 | 117 | | | | GQ117119 | GQ117117 | GQ117118 | | |
| A/Indiana/09/2009 | 112 | | GQ117093 | GQ117095 | GQ117097 | GQ117092 | GQ117094 | GQ117096 | |
| A/Kansas/02/2009 | 114 | | | | GQ117059 | GQ117057 | GQ117058 | | |
| A/Korea/01/2009 | 122 | | | | GQ131023 | GQ131024 | GQ132185 | GQ131025 | GQ131026 |
| A/Massachusetts/06/2009 | 116 | | | | GQ117043 | GQ117041 | GQ117042 | | |
| A/Michigan/02/2009 | 116 | | | GQ117109 | GQ117112 | GQ117107 | GQ117108 | GQ117111 | GQ117110 |
| A/Minnesota/02/2009 | 117 | GQ117070 | GQ117069 | | | GQ117068 | GQ117071 | GQ117073 | GQ117072 |
| A/Netherlands/602/2009 | 119 | | | | CY039527 | | CY039528 | | |
| A/New York/1669/2009 | 116 | CY039900 | CY039899 | CY039898 | CY039893 | CY039896 | CY039895 | CY039894 | CY039897 |
| A/New York/1682/2009 | 117 | CY039908 | CY039907 | CY039906 | CY039901 | CY039904 | CY039903 | CY039902 | CY039905 |
| A/New York/12/2009 | 115 | | | | FJ984337 | FJ984336 | FJ984335 | | FJ984334 |
| A/New York/18/2009 | 115 | FJ984351 | FJ984353 | FJ984354 | FJ984355 | FJ984352 | FJ984350 | FJ984348 | FJ984349 |
| A/New York/19/2009 | 115 | | FJ984392 | FJ984393 | FJ984394 | FJ984391 | FJ984390 | FJ984388 | FJ984389 |
| A/New York/23/2009 | 114 | | | | FJ984364 | FJ984363 | FJ984362 | | FJ984361 |
| A/Ohio/07/2009 | 114 | | | FJ984401 | GQ117100 | GQ117098 | GQ117099 | | |
| A/Pais Vasco/GP20/2009 | 116 | | | | FJ985763 | FJ985761 | FJ985764 | FJ985760 | FJ985762 |
| A/South Carolina/09/2009 | 116 | | | | GQ117056 | GQ117052 | GQ117053 | GQ117055 | GQ117054 |
| A/Texas/04/2009 | 104 | FJ969525 | FJ981615 | FJ981618 | FJ981614 | FJ981617 | FJ981620 | FJ966980 | |
| A/Texas/07/2009 | 115 | GQ117089 | GQ117088 | | GQ117091 | GQ117087 | | GQ117090 | |
| A/Texas/09/2009 | 115 | GQ117027 | GQ117026 | GQ117029 | GQ117032 | GQ117025 | GQ117028 | GQ117031 | GQ117030 |
| A/Texas/15/2009 | 105 | | GQ122094 | | GQ122097 | GQ122092 | GQ122096 | GQ122095 | GQ122093 |
| A/Valencia/GP4/2009 | 116 | | | | FJ985758 | FJ985756 | FJ985759 | FJ985755 | FJ985757 |

1. The sampling date as number of days since 1-Jan-2009.

2. Accession numbers for each available genomic segment

**Supplementary notes:**

**Adaptation and purifying selection**

We suggest in the main text that the increased presence of amino-acid polymorphisms may be present in the outbreak sequences relative to the swine influenza reference sequences due to the rapid growth in number of infections and the small window of time over which they were sampled. The fairly uniform distribution of non-synonymous changes across the outbreak genomes (data not shown) and the relatively consistent dN/dS ratios across genes (Supplementary Tables S1 & S2) support this view. We predict that many of these mutations will be mildly deleterious and, given time, will be removed by purifying selection along an emergent 'trunk' lineage, and that the estimated evolutionary rate and gene-specific dN/dS pattern will approach that observed in the swine influenza data sets sampled over a time period of decades (see below). An interesting alternative is that the elevated rate in the outbreak sequences (and higher dN/dS ratio) is due to a burst of adaptive evolution in a new host, rather than a relaxation of purifying selection and that many of the 'extra' non-synonymous mutations will become fixed in the population. If the current outbreak persists in the human population, it will be possible to distinguish these hypotheses.

**Detailed molecular characterization**

The presence of residue Asn 31 in the M2 protein invariably confers resistance to the adamantanes, a group of antiviral drugs used for treatment of human influenza[1]. Sequence analysis revealed that Asn 31 was present in all human isolates of the current outbreak, this mutation was also present in swine H1N1 and H1N2 viruses that were most closely related in the M gene. The widespread occurrence of the Asn 31 mutation in closely related viruses indicate that it may have descended from the

swine lineage of amantadine-resistant M2 genes rather than independently acquired. It should be noted that majority of the seasonal H1N1 and H3N2 viruses are resistant to amantadine, except Brisbane-like H1N1 viruses[2]. No mutations were observed in the NA gene that confers Oseltamivir resistance.

The molecular determinants of interspecies transmission of the A/California/04/2009-like virus to humans are unclear. In these viruses the amino acid residue at the receptor binding pocket of HA1– position Gln 226 and Gly 228 retain configurations (2,3-NeuAcGal linkages) predicted to have affinity for mammalian cell-surface receptors[3–4]. Amino acid residues relevant to receptor binding were identical to those of classical swine H1N1 and North American H1N1 and recent H1N1 viruses in both the 130-loop and 190-helix. However, the amino acids at positions 133 and 135 are different from the current seasonal vaccine strain A/Brisbane/59/2007(H1N1) used in both the northern and southern hemispheres, indicating that these viruses may show less cross-reaction to the current vaccine strain[5–6].

Mutations Glu627Lys and Asp701Asn of the PB2 gene that are thought to be associated with adaptation to mammals and increased virulence of influenza viruses in mice were not present[7–12]. The C terminal of the NS1 gene is truncated in the A/California/04/2009-like viruses and four residues of the PDZ ligand domain are not present[11].

**Supplementary Methods**

Viral RNA was directly extracted from infected allantoic fluid or cell culture using QIAamp viral RNA minikit (Qiagen, Inc., Valencia, Calif.). cDNA were synthesized by reverse transcription reaction and gene amplification by PCR were performed

using specific primers for each gene segments. PCR products were purified with the QIAquick PCR purification kit (Qiagen Inc.) and sequenced by synthetic oligonucleotides. Reactions were performed using Big Dye-Terminator v3.1 Cycle Sequencing Reaction Kit on an ABI PRISM 3700 DNA Analyzer (Applied Biosystems) following the manufacturer's instructions. All sequences were assembled and edited with Lasergene version 6.1 (DNASTAR, Madison, WI). Full genome sequences of these viruses are available for download at GISAID under the accession numbers (EPI177540 to EPI77658 and EPI177947).

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