http://wwwnc.cdc.gov/EID/article/21/4/14-1545-Techapp1.pdf). The absence of correlation between the IgG statuses of mothers and children suggests that vertical transmission is probably not a major contributor to new infections, although it remains plausible that it may sometimes occur.

Data from Europe that suggest an association between PARV4 infection and progression to Centers for Disease Control and Prevention B-syndromes in HIV-positive persons are problematic because of confounding high rates of HCV infection and injection drug use in the PARV4-positive group (*8*). We sought evidence for this effect in our cohort, in which rates of HCV infection and injection drug use were likely to be negligible. We found no evidence of a PARV4 serostatus effect on HIV RNA load or CD4+ T cells in children ($p = 0.13$, $p = 0.68$, respectively; online Technical Appendix Table 1) or adults ($p = 0.15$, $p = 0.77$, respectively; online Technical Appendix Table 2).

We found an unexpected negative correlation between PARV4 IgG and HIV status in children ($p = 0.05$, Fisher exact test; online Technical Appendix Table 1). One possible explanation is that a detectable PARV4 IgG response is not mounted or maintained in the context of HIV infection; however, this theory is not supported by previous studies in which PARV4 IgG seems to be more prevalent in HIV-infected populations (*5*,*8*).

Our analysis was limited by small numbers tested and the retrospective approach to sample testing. Demographic data were not recorded for this cohort, so we are unable to explore further possible social or demographic risk factors that might correlate with PARV4 infection.

This study contributes to an evolving body of data suggesting that PARV4 is highly endemic to different settings across Africa. The unknown clinical effects and transmission routes of this virus remain pressing questions for future research.

P.C.M. is a National Institute for Health Research Clinical Lecturer and received funding from Oxford University Clinical Academic Graduate School and the John Fell Fund to cover the cost of this work. C.P.S., W.F.G., and P.S. were funded by the Biotechnology and Biological Sciences Research Council. P.J.R.G. is a Wellcome Trust Senior Investigator. P.K. is funded by the National Institute for Health Research Biomedical Research Centre, Oxford Martin Centre, and Wellcome Trust grant no. 091663.

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Co-infection with Avian (H7N9) and Pandemic (H1N1) 2009 Influenza Viruses, China

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DOI: http://dx.doi.org/10.3201/eid2104.141560

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To the Editor: Since the first case of avian-origin influenza (H7N9) virus infection was reported in March 2013 in Shanghai, China, this virus has caused ≈400 confirmed cases (http://www.who.int/influenza/human_animal_interface/influenza_h7n9/Data_Reports/en/). According to the Chinese Influenza Weekly Report (http://www.cnic.org.cn/eng/show.php?contentid=699), during the winter of 2013–14, multiple influenza viruses that infect humans co-circulated in southern China; these viruses were influenza A(H1N1) pdm09, seasonal influenza A(H3N2), and influenza B viruses, together with avian influenza (H7N9) virus,. Co-infection of humans with avian virus subtype H7N9 and human virus subtypes were reported in Jiangsu Province in June 2013 (*1*) and in Zhejiang Province in January 2014 (*2*), respectively. We report a fatal case of co-infection with influenza (H7N9) and A(H1N1)pdm09 viruses, which was further confirmed by virus isolation and neutralization antibody detection.

In a retrospective study, we identified co-infection with A(H1N1)pdm09 and novel avian-origin H7N9 virus in a 61-year-old female patient from the urban area of Shanghai, China. She had a 3-year history of thrombocythemia. In October 2013, she received an annual vaccine that included an A/California/7/2009(H1N1)pdm09–like virus, an A/Texas/50/2012(H3N2)–like virus, and an influenza B virus, as recommended by the World Health Organization (http://www.who.int/influenza/vaccines/virus/recommendations/2013_14_north/en/). On December 26, 2013, the patient experienced influenza-like symptoms; the next day, she was hospitalized. Despite oral administration of oseltamivir, a rapidly progressive pneumonia developed, and the patient died of multiple organ failure 9 days after admission. On December 31, 2013 (day 5), 1 serum and 2 throat swab samples were collected; both throat swab samples were positive for influenza A and A(H1N1)pdm09 virus, according to the universal and subtype-specific primers provided by the Chinese Center for Disease Control and Prevention (http://www.who.int/influenza/resources/documents/diagnostic_recommendations/en/). On January 3, 2014 (day 8), 1 bronchial secretion and 1 serum sample were also collected.

Recent DNA sequencing analysis of the stored bronchial secretion specimen, performed on a Roche 454 platform (*3*) (Roche, Basel, Switzerland) showed co-existence of H1, H7, N1, and N9 genomic segments, which were further confirmed by real-time reverse transcription PCR (RT-PCR). The cycle threshold values of H7 and H1 were 35.65 and 29.94, respectively.

We then determined antibody levels of the 2 serum samples collected on days 5 and 8 by using a microneutralization assay (*4*) with MDCK cells infected with 2 representative strains, A/Shanghai/4664T/2013(H7N9) and A/Shanghai/37T/2009(H1N1), respectively. The samples were serially diluted 2-fold (1:10 to 1:1,280) at a starting dilution of 1:10; titers were expressed as the reciprocal of the highest dilution of 50% neutralization. The microneutralization titers against novel subtype H7N9 virus were 160 and 320 for the samples collected on days 5 and 8, respectively. This finding indicated that the patient could have been infected by novel subtype H7N9 viruses (*5*); microneutralization titers against A/Shanghai/37T/2009(H1N1) were 320 for both samples.

To determine whether the reassortment had occurred between the 2 subtypes of influenza A viruses during the co-infection, we isolated the virus strains from the bronchial secretion on either MDCK cells (A[H1N1] pdm09) (http://www.who.int/influenza/gisrs_laboratory/manual_diagnosis_surveillance_influenza/en/) or embryonated eggs (H7N9) through several passages. The hemagglutination titers of the isolated H7N9 (A/Shanghai/Mix1/2014[H7N9]) and A(H1N1)pdm09 viruses (A/Shanghai/Mix1/2014[H1N1]) in allantoic fluid were 32 and 8, respectively. Cultures of the 2 isolates were subjected to RT-PCR for detection of 8 genomic segments. The RT-PCR products were sequenced on an ABI 3730XL Automatic DNA Analyzer by using an ABI Prism BigDye Terminator v3.1 Cycle Sequencing Kit (both from Applied Biosystems, Foster City, CA, USA). The full-genome sequences were submitted to the National Center for Biotechnology Information Influenza Virus Resource (GenBank accession nos. KJ946415, KJ946416 and KP058484–KP058489 for A/Shanghai/Mix1/201[H7N9] and KM607860, KM607861 and KP058490–KP058495 for A/Shanghai/Mix1/2014[H1N1]).

Phylogenetic trees were constructed by using the neighbor-joining method in MEGA 5.1 (http://www.megasoftware.net) to estimate relationships with selected influenza A virus reference sequences obtained from GenBank and the Global Initiative on Sharing Avian Influenza Data database. DNA sequence analysis showed that no genetic reassortment had occurred between the 2 subtypes because only H7N9 or A(H1N1)pdm09 genomic fragments were found in the culture of the respective isolate (Figure; online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/4/14-1560-Techapp1.pdf). Phylogenetic analyses of the hemagglutinin and neuraminidase genes revealed that the A/Shanghai/Mix1/2014(H7N9) and A/Shanghai/Mix1/2014(H1N1) viruses were clustered into the clade of A/Shanghai/01/2014(H7N9)-like viruses (*6*) (Figure, panels A and B) and A/Shanghai/6109/2014(H1N1)-like viruses (Figure, panels C and D), respectively. In addition, the phylogenetic trees of the 6 internal genes were close to those of H7N9 viruses or A(H1N1)pdm09-lineage viruses that had recently circulated in China (online Technical Appendix).

Co-infections with H7N9 and other influenza subtypes have been reported from Jiangsu (*1*) and Zhejiang (*2*) Provinces as well as in our study, indicating the potential risk for co-infection during the influenza season

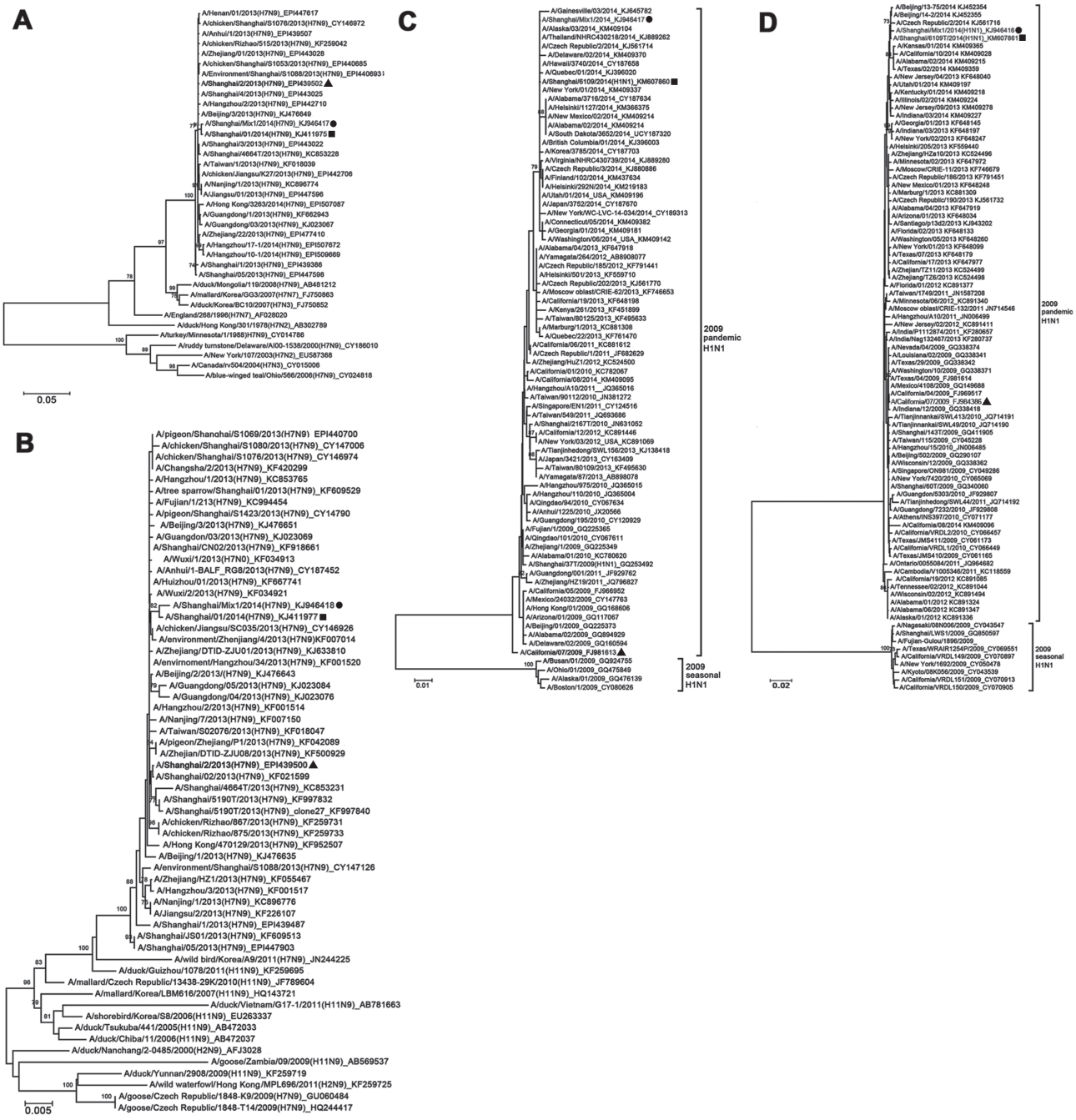

Figure. Phylogenetic analyses of A/Shanghai/Mix1/2014(H7N9) and A/Shanghai/Mix1/2014(H1N1) viruses from Shanghai, China, January 2014. A) Hemagglutinin and B) neuraminidase of A/Shanghai/Mix1/2014(H7N9); C) hemagglutinin and D) neuraminidase of A/Shanghai/Mix1/2014(H1N1). Multiple alignments were constructed by using the ClustalW (http://www.clustal.org/) algorithm based on the sequences of the hemagglutinin and neuraminidase genes. Genetic distances were calculated by using the Kimura 2-parameter method, and phylogenetic trees were constructed by using the neighbor-joining method with bootstrap analysis of 1,000 replicates in MEGA 5.1 (http://www.megasoftware.net). Horizontal distances are proportional to genetic distances. Numbers next to nodes indicate bootstrap value percentages (>70%). Circles indicate the A/Shanghai/Mix1/2014(H7N9) and A/Shanghai/Mix1/2014(H1N1) virus strains isolated from the co-infection patient from Shanghai, China. Triangles indicate the representative strains of the Shanghai area during the same period as the influenza (H7N9) outbreak in China. Squares indicate the representative strains of the Shanghai area during the same wave of the H7N9 outbreak in China. Scale bars indicate nucleotide substitutions per site. The Global Initiative on Sharing Avian Influenza Data database or GenBank accession numbers of the influenza viruses used in the construction of the phylogenetic analyses are displayed in the phylogenetic trees, which follow the taxon names.

in China. Although reassortment was not detected in this co-infection, a potential risk for emergence of a new pandemic strain by reassortment between these 2 viruses (with humans as mixing vessels) should not be ignored. To reduce the risk for emergence of new viral subtypes, the public health and scientific communities should enhance surveillance for co-infection with influenza (H7N9) virus and other influenza virus subtypes.

This research was supported by the National Megaprojects of China for Infectious Disease (nos. 2012ZX10004211 and 2014ZX10004002-003-004), National Natural Science Foundation of China (nos. 81341004, 81102283, and 81370131), Outstanding Academic Leader of Health System in Shanghai (no. XBR2013078), Ministry of Science and Technology (no. KJYJ-2013-01-01), Shanghai Municipal Health and Family Planning Commission (no. 2013QLG002), Key Discipline Construction Project of Pudong Health Bureau of Shanghai (no. PWZx2014-10), Academic Leader Training Project in Health System of Pudong Health Bureau of Shanghai (no. PWRd2010-01), and Key Medical Specialties of Shanghai (no. ZK2012A28).

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Nairobi Sheep Disease Virus RNA in Ixodid Ticks, China, 2013

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DOI: http://dx.doi.org/10.3201/eid2104.141602

To the Editor: Nairobi sheep disease virus (NSDV; genus *Nairovirus*, family *Bunyaviridae*) causes acute hemorrhagic gastroenteritis in sheep and goats (*1*,*2*). First identified in Nairobi, Kenya, in 1910, it is considered endemic in East Africa (*1*,*3*). Ganjam virus, a variant of NSDV, is found in India and Sri Lanka (*2*). NSDV has a limited effect on animals bred in areas to which the virus is endemic but can cause large losses of animals during introduction of new livestock or transport of animals through these areas (*4*). In humans, NSDV infection can cause febrile illness, headache, nausea, and vomiting (*5*).

Ticks are the main transmission vectors of NSDV and many other viral pathogens and therefore pose a major threat to public health (*6*,*7*). Here, we describe a newly discovered NSDV, named NSDV (China), identified by viral metagenomic analysis of ticks collected from the northeast region of the People's Republic of China (Liaoning, Jilin, and Heilongjiang provinces) during May–July, 2013, and divided into 9 groups according to tick species and sampling sites. Four tick species were morphologically identified: *Haemaphysalis longicornis* (84.8%); *Dermacentor silvarum* (7.2%); *D. nuttalli* (5.5%); and *Ixodes persulcatus* (2.5%) (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/21/4/14-1602-Techapp1.pdf).

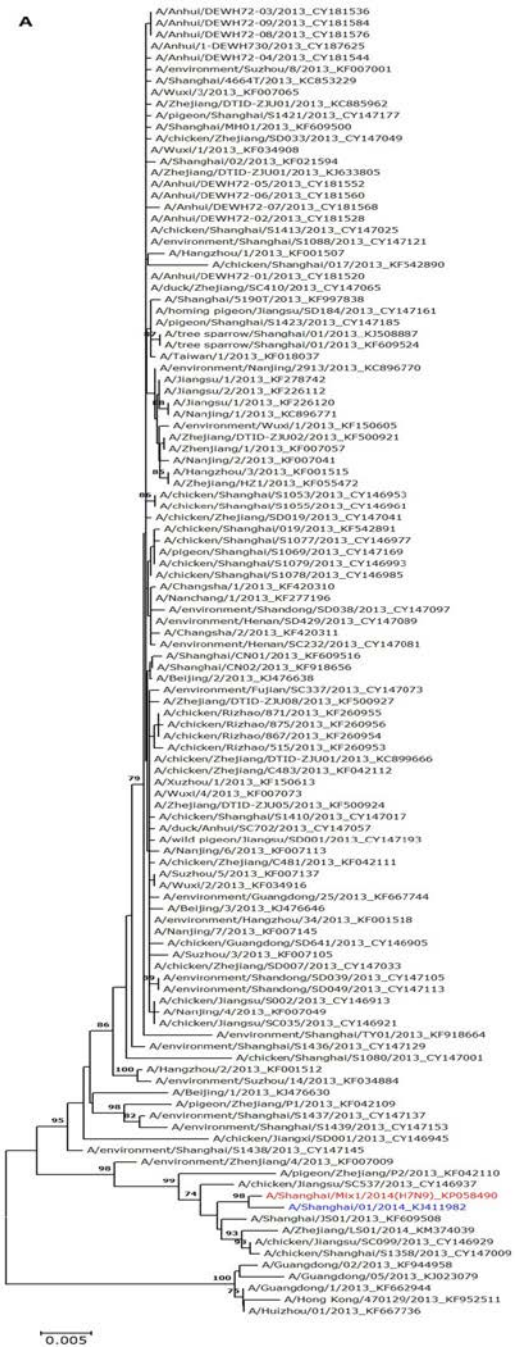
Of the 6,427 ticks collected, 3,410 were divided into 9 groups (average 379 ticks/group, range 163–512); each group was homogenized in SM buffer (50 mmol/L Tris, 10 mmol/L $MgSO_4$, 0.1 mol/L NaCl, pH 7.5). Viral RNA extraction, Solexa sequencing, and analysis are described in the online Technical Appendix. Among the sequences annotated to mammalian viruses, 65 contigs were found to target the small (n = 15), medium (n = 27), and large (n = 23) segments of the NSDV genome (online Technical Appendix Tables 2–4).

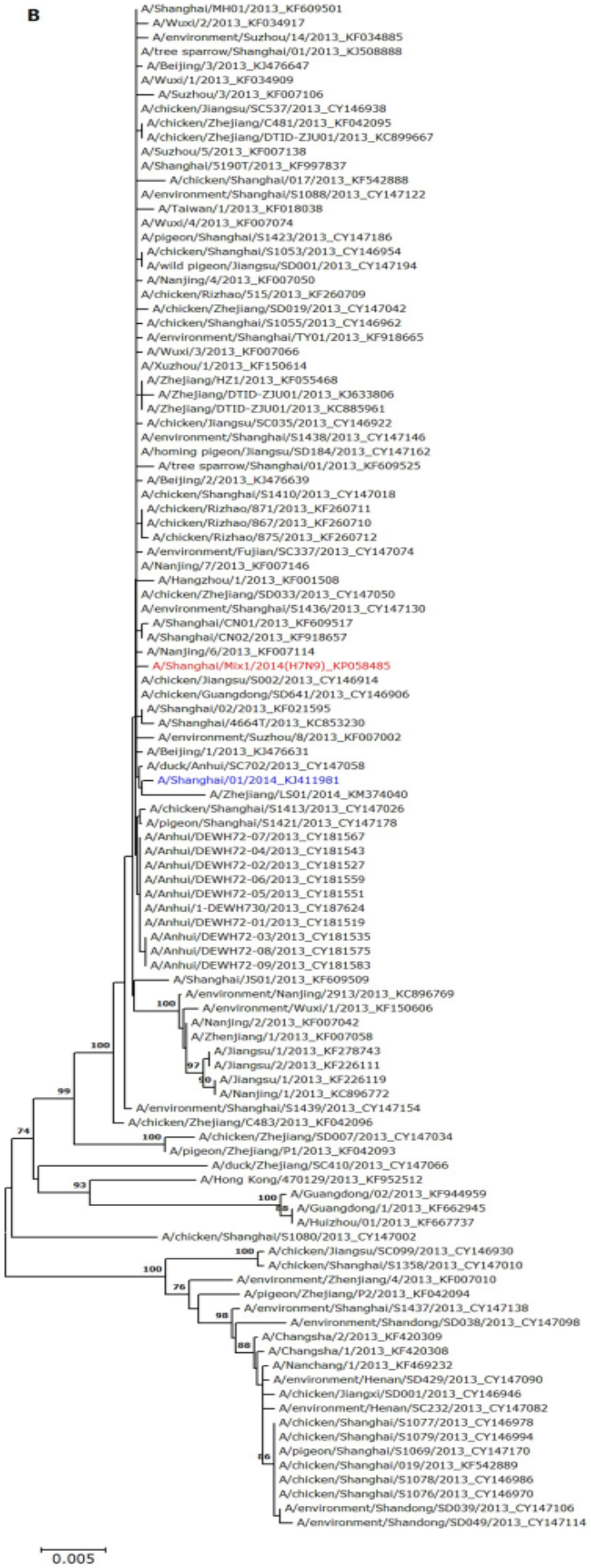
[1]These authors contributed equally to this article.

Article DOI: http://dx.doi.org/10.3201/eid2104.141560

Co-infection with Avian (H7N9) and Pandemic (H1N1) 2009 Influenza Viruses, China

Technical Appendix

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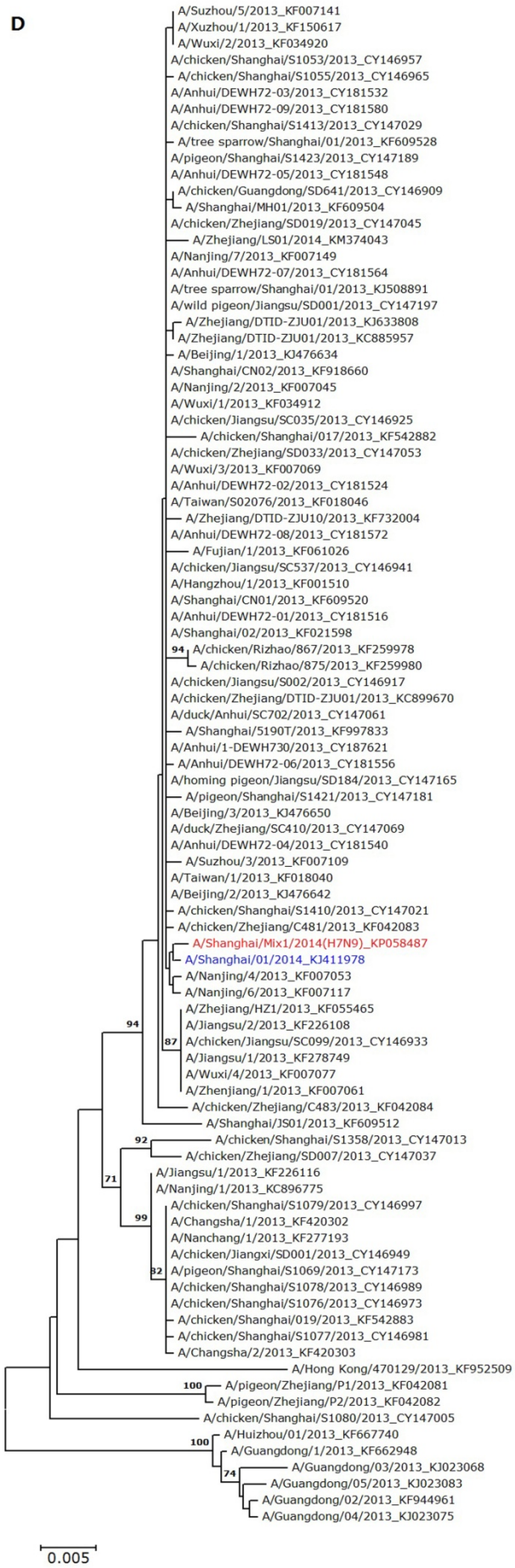
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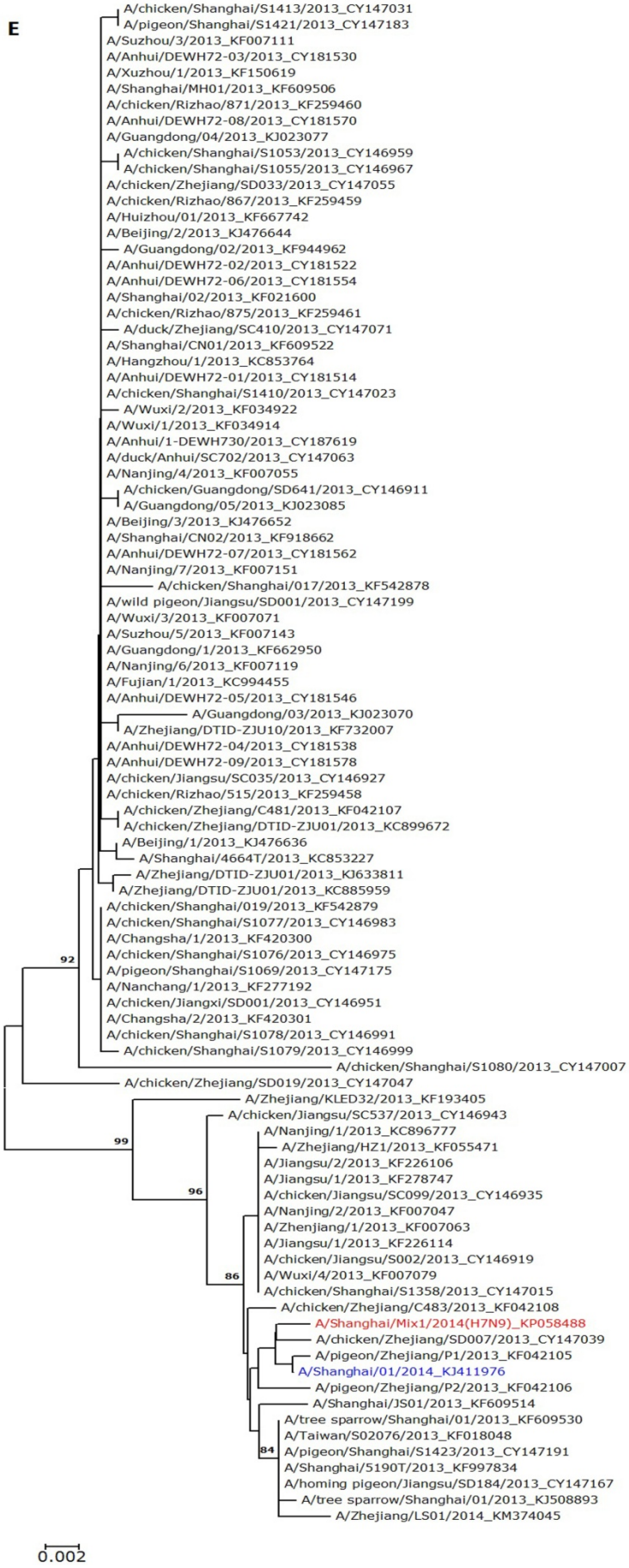
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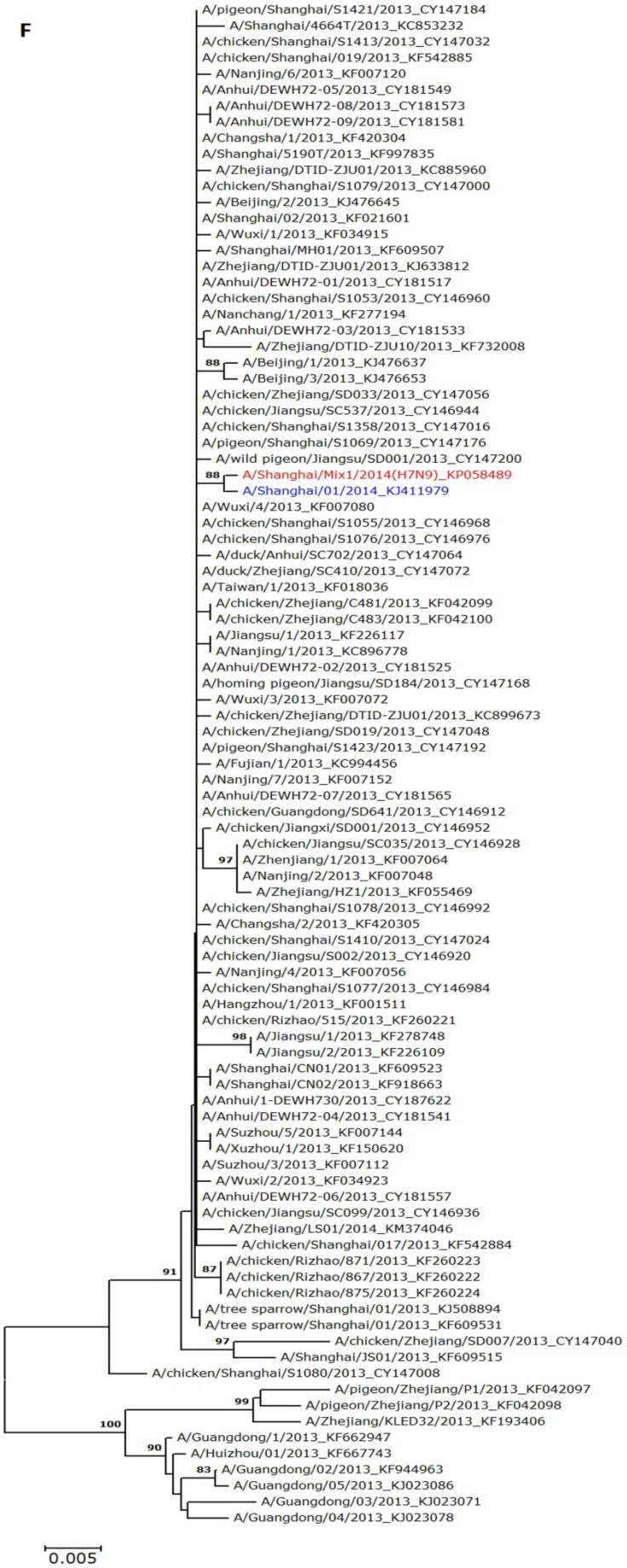
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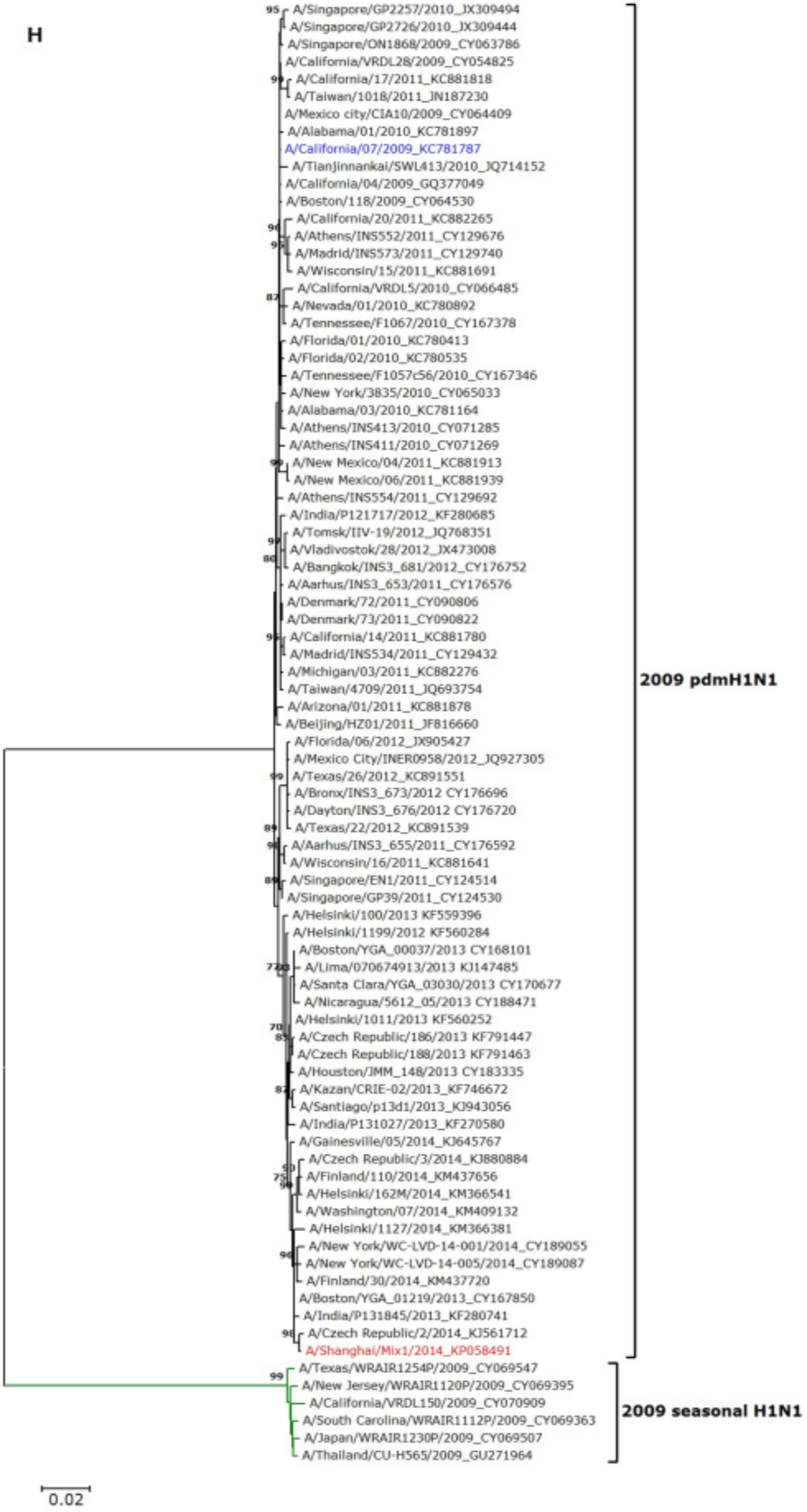
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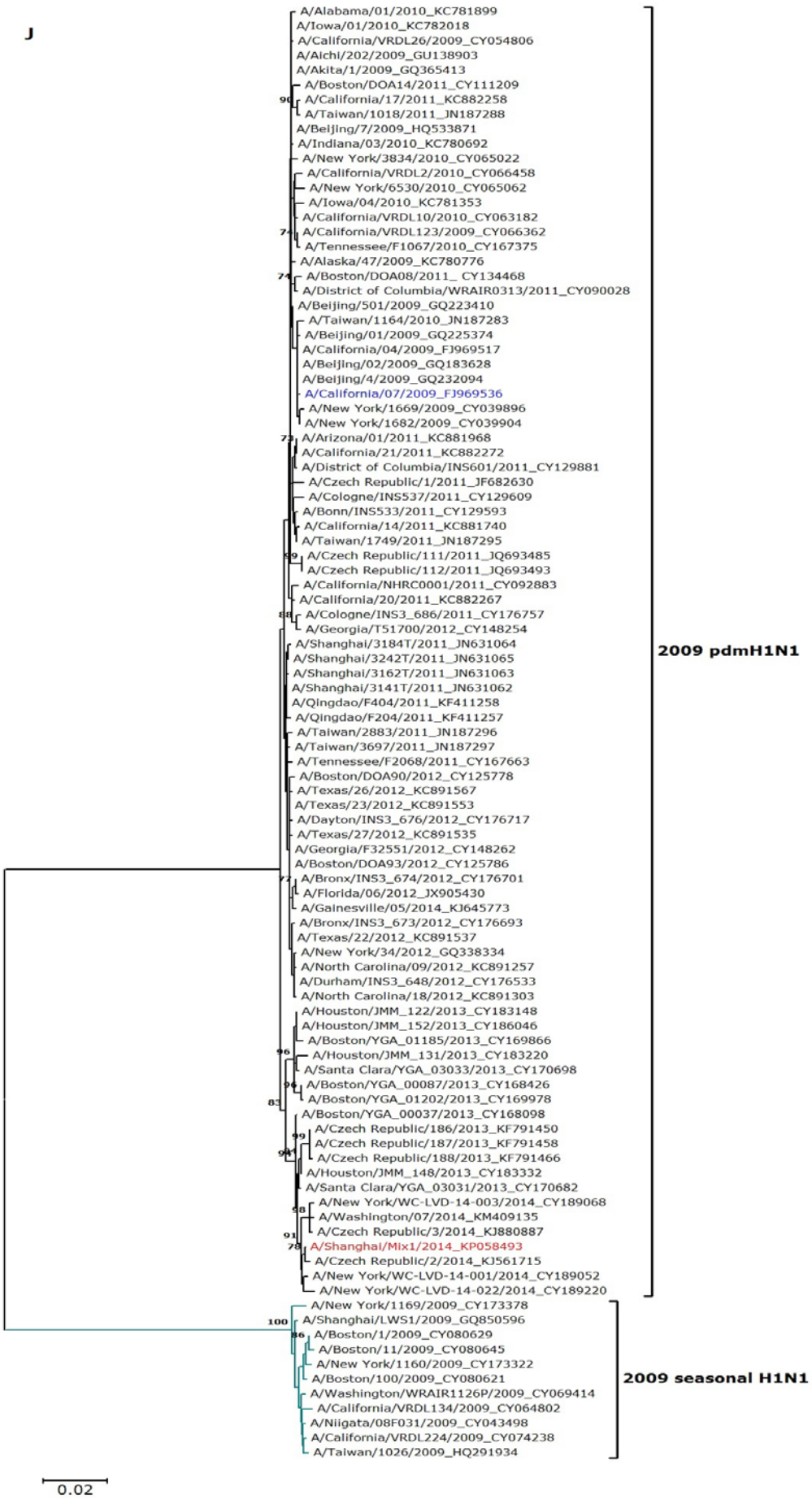
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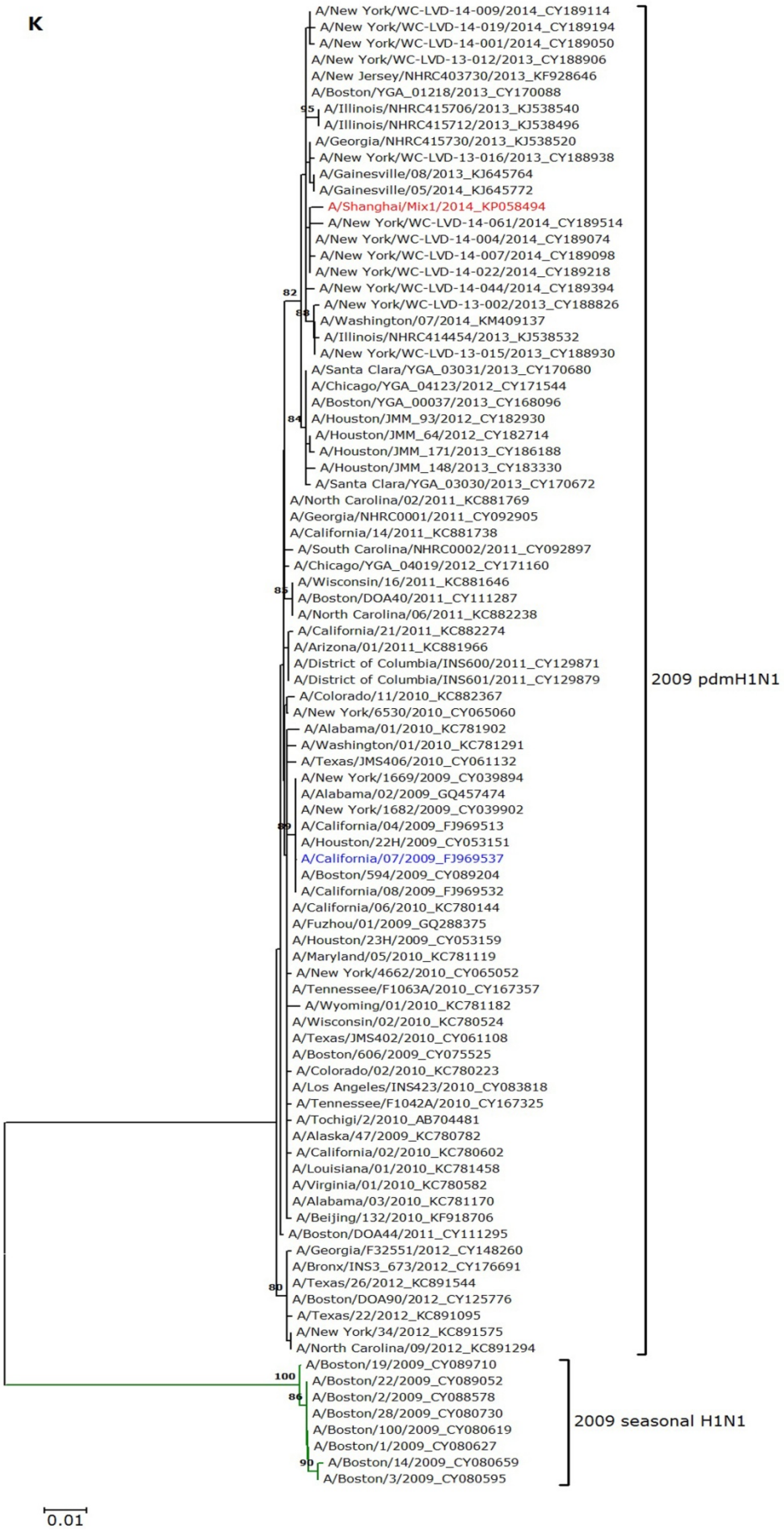
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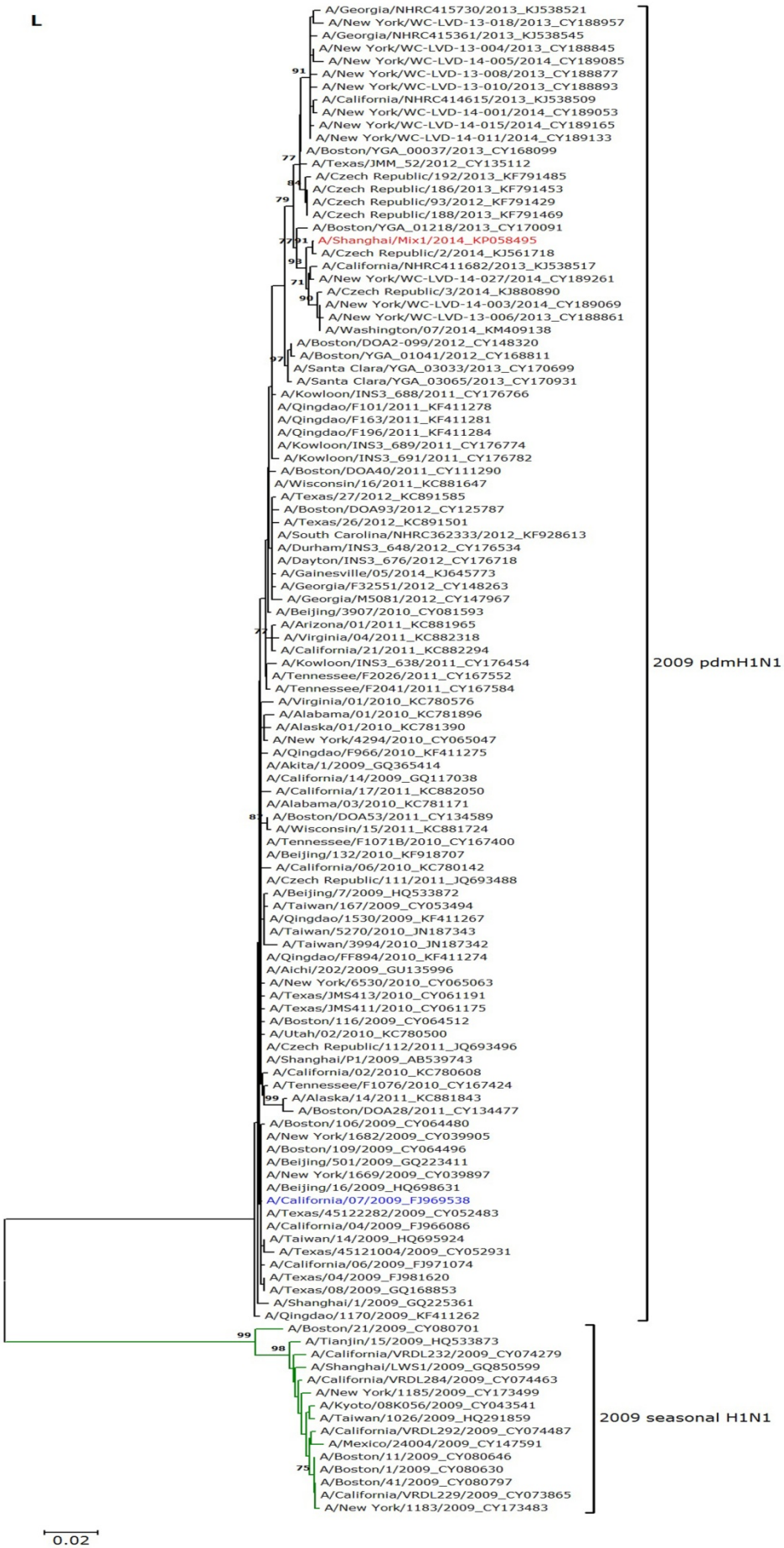
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A/Boston/41/2009_CY080797
A/California/VRDL229/2009_CY073865
A/New York/1183/2009_CY173483
2009 seasonal H1N1
0.02

Technical Appendix Figure. Phylogenetic analysis of 12 internal genes of the co-infection viruses in Shanghai, China. Phylogenetic trees were constructed by using the neighbor-joining method with bootstrap analysis of 1,000 replicates in MEGA 5.1 program. Numbers next to nodes indicate bootstrap value percentages (>70%). Panels A, B, C, D, E, F were the PB2, PB1, PA, NP, MP, NS phylogenetic trees of the A/Shanghai/Mix1/2014(H7N9) strain, respectively, and panels G, H, J, K, L, M were the PB2, PB1, PA, NP, MP, NS phylogenetic trees of the A/Shanghai/Mix1/2014(H1N1) strain, respectively. Red font indicates the A/Shanghai/Mix1/2014(H7N9) and A/Shanghai/Mix1/2014(H1N1) strains isolated from the co-infection patient from Shanghai, China. Blue font in the phylogenetic trees of the A/Shanghai/Mix1/2014(H7N9) strain indicates the representative strains of Shanghai area during the same wave of the H7N9 outbreak in China (*1*). Blue font in the phylogenetic trees of the A/Shanghai/Mix1/2014(H1N1) strain indicates the recommended influenza vaccine strain in the 2013–14 Northern Hemisphere influenza season (http://www.who.int/influenza/vaccines/virus/recommendations/2013_14_north/en/). Scale bars indicate nucleotide substitutions per site. The GISAID or GenBank accession numbers of the influenza viruses in the construction of the phylogenetic analyses are displayed in the phylogenetic trees, which followed the taxon names.