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Phylogenetic Analysis of M2 Proteins from Avian and Swine Influenza A Viruses

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ABSTRACT

M2 is transmembrane matrix protein that plays a key role in the replication cycle of influenza virus A. Four identical M2 protein chains form a M2 proton channel, which transports protons inside the host cell. Although the M2 proteins are highly conservative, some positions are characterized by a considerable degree of variability. In this paper, data of 359 M2 sequences were collected for the study. The sequences were separated into three groups sequences that belong to the avian influenza viruses; sequences that belong to viruses whose hosts are swine; and sequences that belong to viruses whose hosts are birds, swine and other hosts or an undefined host. In this work, a phylogenetic analysis was done for each of the above groups.

Key Words: M2 protein, influenza A virus, phylogenetic analysis, virus hosts, swine influenza, avian (bird) influenza

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INTRODUCTION

M2 protein is an important element in the replication cycle of influenza virus A. It plays a key role in this process by acidifying a virion and the interior of host cells (Holsinger et al., 1994; Shimbo et al., 1996; Forrest et al., 2000; Wu and Voth, 2003; Le and Leluk, 2011). The M2 proteins are characterized by low variability; however, various strains of influenza virus A in this work are different.In this publication, an attempt is made to explain the evolutionary relationships between various swine and avian strains of influenza virus A, with regard to M2 protein.

MATERIALS AND METHOD

M2 protein amino acid sequences

M2 protein sequences were found by using BLAST search from the UniProt Knowledgebase (Altschul et al., 1990; Madden et al., 1996; Altschul et al., 1997; UniProtKB, 2014). This has resulted in 359 M2 protein sequences.

All of the collected sequences were divided into four groups: 1. sequence groups belonging to viruses whose hosts are birds; 2. sequence groups belonging to viruses whose hosts are swine; 3. sequence groups belonging to viruses whose hosts are birds (first partial sequences of this group) and swine (last partial sequences of this group); and 4. sequence groups belonging to viruses whose hosts are birds, swine and others, or with an unidentified host.

Bioinformatics software used in the search

PHYLIP is a software program for inferring phylogenies (Felsenstein, 1989). Phylogenetic trees were created by using two different algorithms from the PHYLIP software (maximum parsimony and maximum likelihood).

ClustalX – software used in this publication to generate phylogenetic trees (Larkin et al., 2007). ClustalX used the NJ (neighbor joining) algorithm—the method of the nearest neighborhood—to create the trees.

SSSSg – a program for creating phylogenetic trees. It uses an algorithm based on the following criteria: the percentage of identity, the absolute length of the compared sequences, and the distribution of identical positions along the comparing sequences (Gajewska and Leluk, 2005).

ConSurf – an online program for identifying functional regions in proteins and was used to create the phylogenetic trees (Glaser et al., 2003).

RESULTS

This publication presents 20 phylogenetic trees. The main manuscript only shows four trees, one for each of the groups. The other phylogenetic trees were placed in the annex.

Figures description

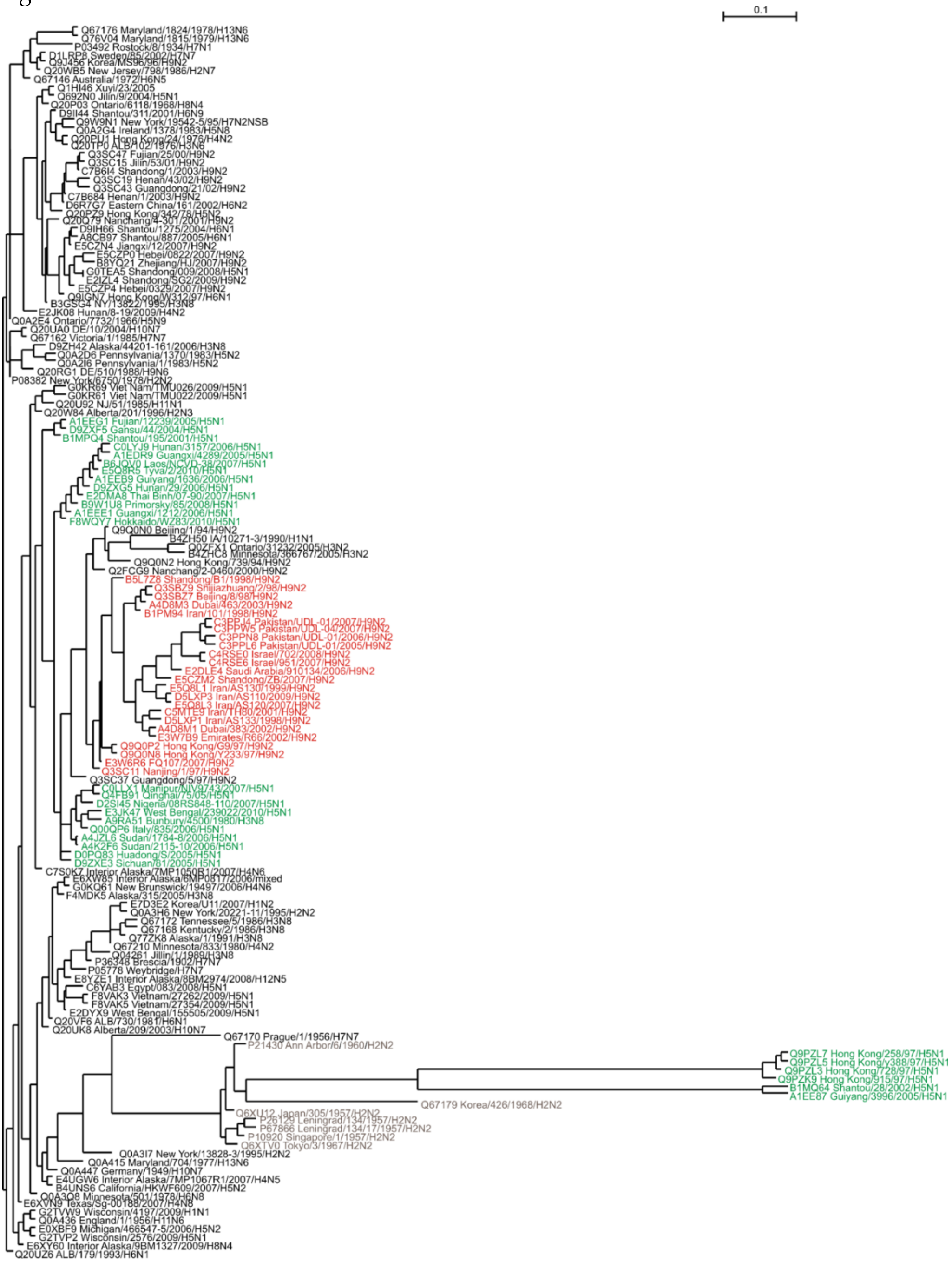
Figure 1: Phylogenetic tree constructed using SSSSg from a sequence group belonging to viruses whose hosts are birds.

Figure 2: Phylogenetic tree constructed using PHYLIP (maximum likelihood algorithm) from a sequence group belonging to viruses whose hosts are swine.

Figure 3: Phylogenetic tree constructed using PHYLIP (maximum likelihood algorithm) from selected sequences in both the group belonging to viruses whose hosts are birds and the group belonging to viruses whose hosts are swine.

Figure 4: Phylogenetic tree constructed using PHYLIP (maximum likelihood algorithm) from a sequence group belong to viruses whose hosts are birds and swine, or another or unidentified host.

Figure 1: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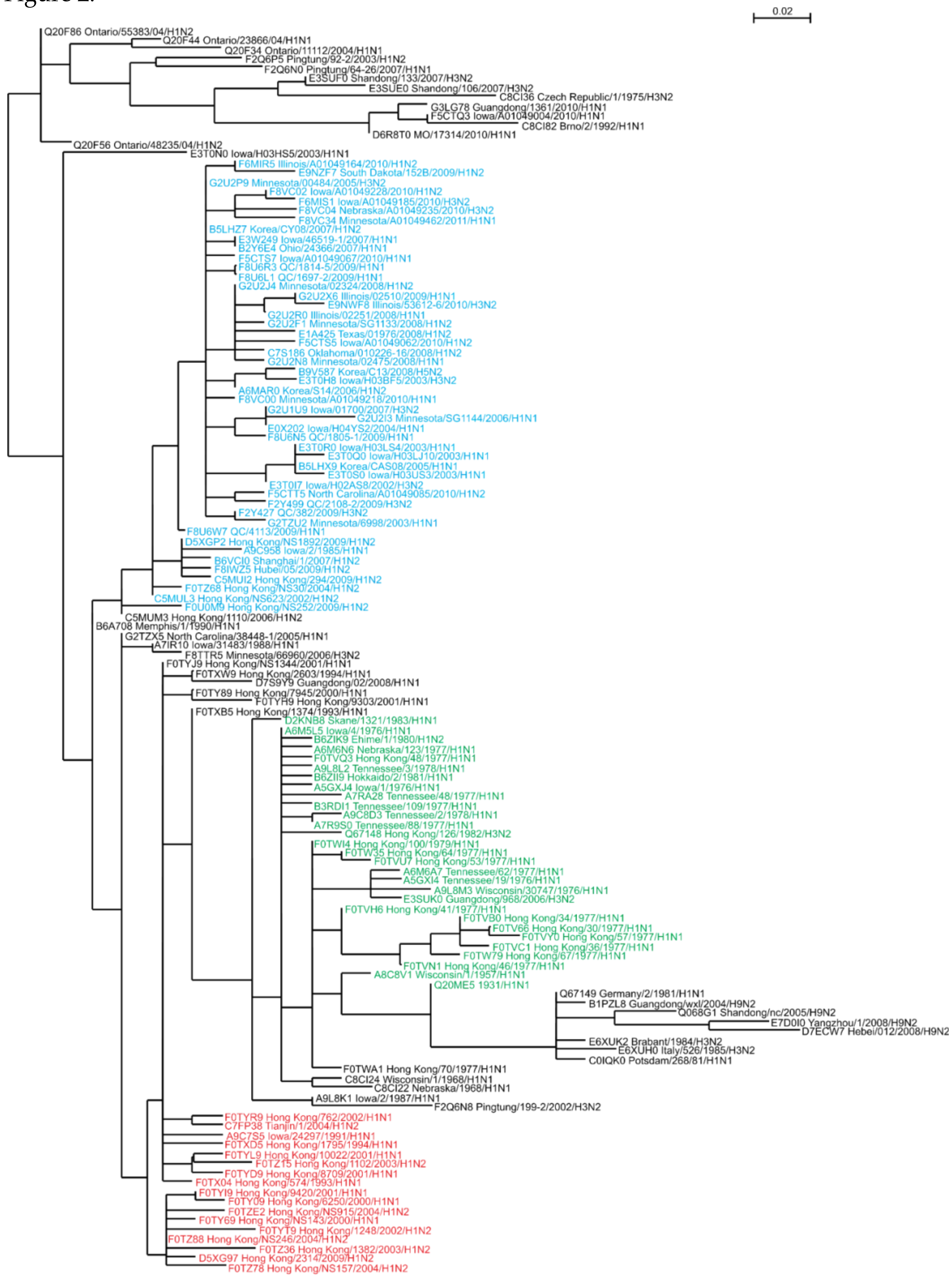
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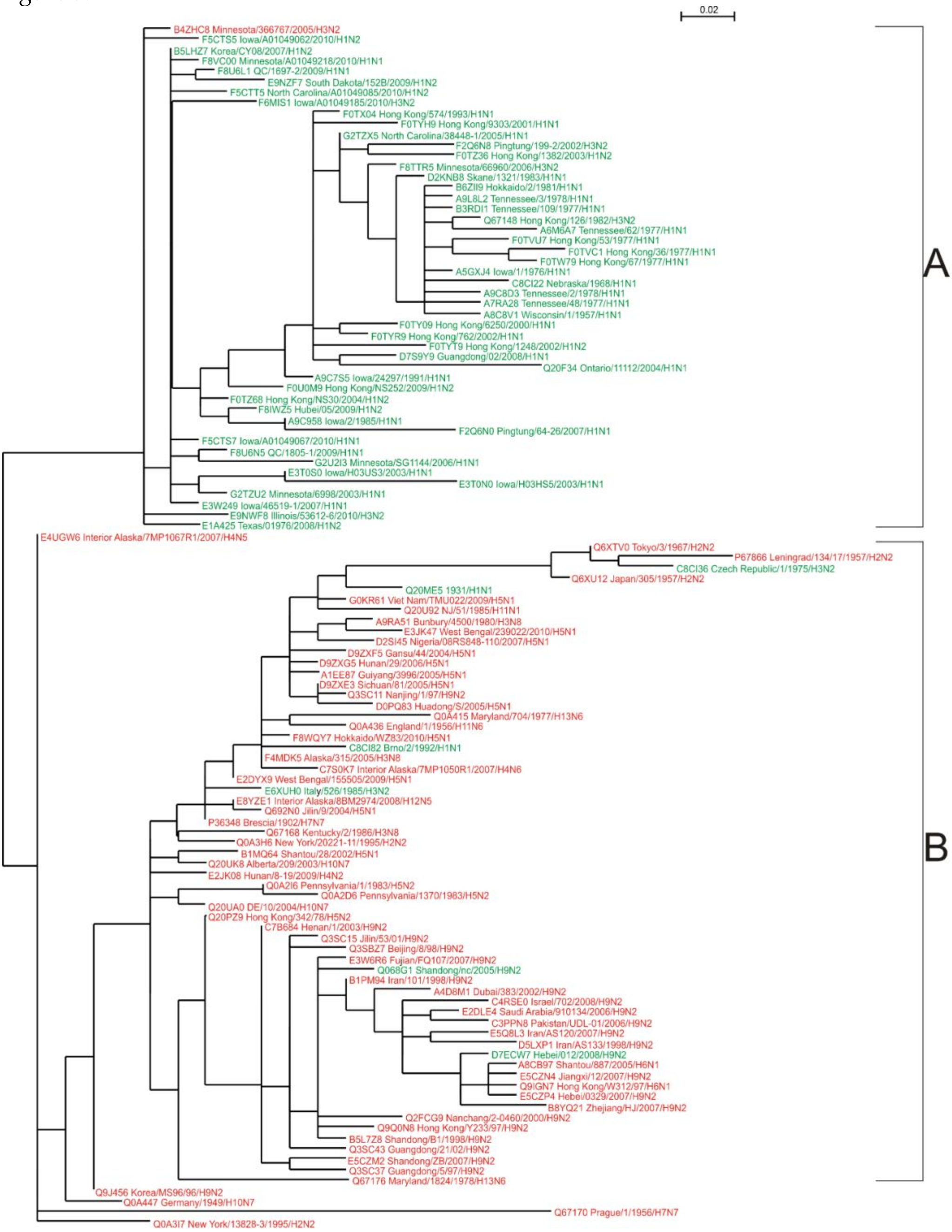
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Q20PZ9 Hong Kong/342/78/H5N2
C7B684 Henan/1/2003/H9N2
Q3SC15 Jilin/53/01/H9N2
Q3SBZ7 Beijing/8/98/H9N2
E3W6R6 Fujian/FQ107/2007/H9N2
Q068G1 Shandong/nc/2005/H9N2
B1PM94 Iran/101/1998/H9N2
A4D8M1 Dubai/383/2002/H9N2
C4RSE0 Israel/702/2008/H9N2
E2DLE4 Saudi Arabia/910134/2006/H9N2
C3PPN8 Pakistan/UDL-01/2006/H9N2
E5Q8L3 Iran/AS120/2007/H9N2
D5LXP1 Iran/AS133/1998/H9N2
D7ECW7 Hebei/012/2008/H9N2
A8CB97 Shantou/887/2005/H6N1
E5CZN4 Jiangxi/12/2007/H9N2
Q9IGN7 Hong Kong/W312/97/H6N1
E5CZP4 Hebei/0329/2007/H9N2
B8YQ21 Zhejiang/HJ/2007/H9N2
Q2FCG9 Nanchang/2-0460/2000/H9N2
Q9Q0N8 Hong Kong/Y233/97/H9N2
B5L7Z8 Shandong/B1/1998/H9N2
Q3SC43 Guangdong/21/02/H9N2
E5CZM2 Shandong/ZB/2007/H9N2
Q3SC37 Guangdong/5/97/H9N2
Q67176 Maryland/1824/1978/H13N6
Q9J456 Korea/MS96/96/H9N2
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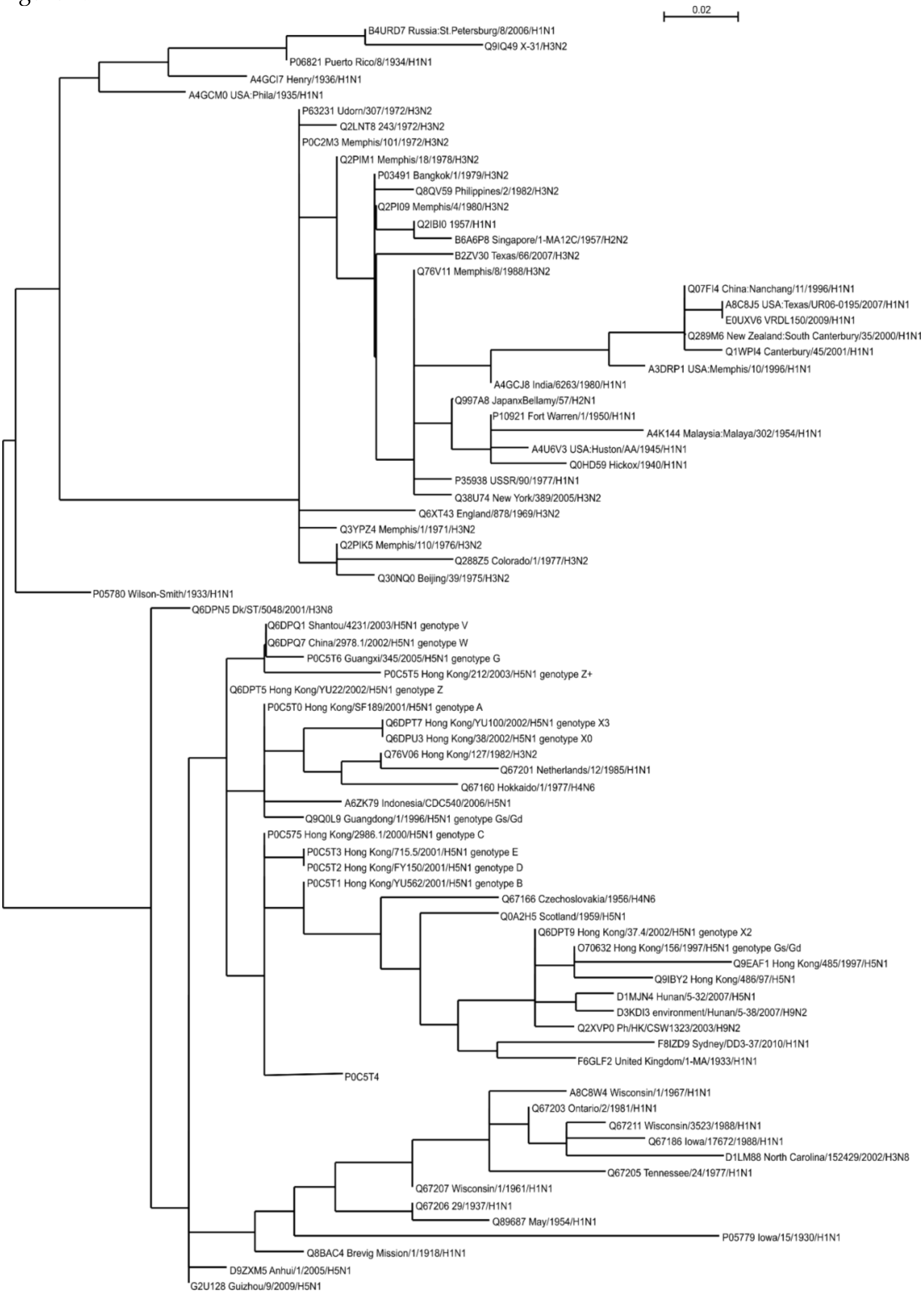
Q67170 Prague/1/1956/H7N7
Q0A3I7 New York/13828-3/1995/H2N2

Figure 4: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A4GCM0 USA:Phila/1935/H1N1
P63231 Udorn/307/1972/H3N2
Q2LNT8 243/1972/H3N2
P0C2M3 Memphis/101/1972/H3N2
Q2PIM1 Memphis/18/1978/H3N2
P03491 Bangkok/1/1979/H3N2
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Q2PI09 Memphis/4/1980/H3N2
Q2IBI0 1957/H1N1
B6A6P8 Singapore/1-MA12C/1957/H2N2
B2ZV30 Texas/66/2007/H3N2
Q76V11 Memphis/8/1988/H3N2
Q07FI4 China:Nanchang/11/1996/H1N1
A8C8J5 USA:Texas/UR06-0195/2007/H1N1
E0UXV6 VRDL150/2009/H1N1
Q289M6 New Zealand:South Canterbury/35/2000/H1N1
Q1WPI4 Canterbury/45/2001/H1N1
A3DRP1 USA:Memphis/10/1996/H1N1
A4GCJ8 India/6263/1980/H1N1
Q997A8 JapanxBellamy/57/H2N1
P10921 Fort Warren/1/1950/H1N1
A4K144 Malaysia:Malaya/302/1954/H1N1
A4U6V3 USA:Huston/AA/1945/H1N1
Q0HD59 Hickox/1940/H1N1
P35938 USSR/90/1977/H1N1
Q38U74 New York/389/2005/H3N2
Q6XT43 England/878/1969/H3N2
Q3YPZ4 Memphis/1/1971/H3N2
Q2PIK5 Memphis/110/1976/H3N2
Q288Z5 Colorado/1/1977/H3N2
Q30NQ0 Beijing/39/1975/H3N2
P05780 Wilson-Smith/1933/H1N1
Q6DPN5 Dk/ST/5048/2001/H3N8
Q6DPQ1 Shantou/4231/2003/H5N1 genotype V
Q6DPQ7 China/2978.1/2002/H5N1 genotype W
P0C5T6 Guangxi/345/2005/H5N1 genotype G
P0C5T5 Hong Kong/212/2003/H5N1 genotype Z+
Q6DPT5 Hong Kong/YU22/2002/H5N1 genotype Z
P0C5T0 Hong Kong/SF189/2001/H5N1 genotype A
Q6DPT7 Hong Kong/YU100/2002/H5N1 genotype X3
Q6DPU3 Hong Kong/38/2002/H5N1 genotype X0
Q76V06 Hong Kong/127/1982/H3N2
Q67201 Netherlands/12/1985/H1N1
Q67160 Hokkaido/1/1977/H4N6
A6ZK79 Indonesia/CDC540/2006/H5N1
Q9Q0L9 Guangdong/1/1996/H5N1 genotype Gs/Gd
P0C575 Hong Kong/2986.1/2000/H5N1 genotype C
P0C5T3 Hong Kong/715.5/2001/H5N1 genotype E
P0C5T2 Hong Kong/FY150/2001/H5N1 genotype D
P0C5T1 Hong Kong/YU562/2001/H5N1 genotype B
Q67166 Czechoslovakia/1956/H4N6
Q0A2H5 Scotland/1959/H5N1
Q6DPT9 Hong Kong/37.4/2002/H5N1 genotype X2
O70632 Hong Kong/156/1997/H5N1 genotype Gs/Gd
Q9EAF1 Hong Kong/485/1997/H5N1
Q9IBY2 Hong Kong/486/97/H5N1
D1MJN4 Hunan/5-32/2007/H5N1
D3KDI3 environment/Hunan/5-38/2007/H9N2
Q2XVP0 Ph/HK/CSW1323/2003/H9N2
F8IZD9 Sydney/DD3-37/2010/H1N1
F6GLF2 United Kingdom/1-MA/1933/H1N1
P0C5T4
A8C8W4 Wisconsin/1/1967/H1N1
Q67203 Ontario/2/1981/H1N1
Q67211 Wisconsin/3523/1988/H1N1
Q67186 Iowa/17672/1988/H1N1
D1LM88 North Carolina/152429/2002/H3N8
Q67205 Tennessee/24/1977/H1N1
Q67207 Wisconsin/1/1961/H1N1
Q67206 29/1937/H1N1
Q89687 May/1954/H1N1
P05779 Iowa/15/1930/H1N1
Q8BAC4 Brevig Mission/1/1918/H1N1
D9ZXM5 Anhui/1/2005/H5N1
G2U128 Guizhou/9/2009/H5N1

DISCUSSION

Group 1 (avian)

The analysis of birds' phylogenetic trees shows common features. Sequences accumulate in some specific groups. Green color indicates sequences that mainly belong to the H5N1 strain. Red color indicates sequences that mainly belong to the H9N2 strain. Brown (gray) color indicates sequences that mainly belong to the H2N2 strain. Pink and black indicate sequences that belong to various strains.

Based on the phylograms (ClustalX, ConSurf, SSSSg), it can be concluded that strain H5N1 is closely related to strain H9N2 (in terms of protein M2), but the PHYLIP tree (maximum likelihood) is different. The M2 protein group belonging to strain H2N2 is more diverse in relation to the other strains. The H2N2 strain forms a separate group for each tree. For the SSSSg and PHYLIP (maximum likelihood) trees, H2N2 is somewhat correlated with the H5N1 strain.

Viruses marked with black and pink colors also make some groups (they show a tree in PHYLIP (maximum likelihood)); however, they are no longer homogeneous in strains of the virus. Contrary, these form a mosaic of different strains, but the M2 proteins are similar.

Individual strains of influenza virus are identified based on structural differences (more specifically, the antigenicity) in hemagglutinin and neuraminidase. Thus, the close relationship of virus proteins belonging to a single strain on the phylograms was not expected. M2 proteins have evolved separately; moreover, M2 is very conservative, compared to other components of the virus.

For the M2 protein sequences of strains H5N1, H9N2 and H2N2 used in this publication, a relationship can be observed between hemagglutinin and neuraminidase volatility and the M2 proteins. Trees contain distinct clusters of M2 protein sequences belong to the H5N1, H9N2, and H2N2 strains. This may prove a conjunction between adaptive hemagglutinin and neuraminidase with M2 protein during the evolution of these strains.

Group 2 (swine)

The dominant strain in a sequence group whose hosts are swine is H1N1. The analysis of M2 proteins of this strain shows a large variation of this one strain, in terms of M2 protein.

Proteins that belong to the H1N1 strain are not one large group in the phylograms; rather, they are divided and grouped into several smaller groups. This is best seen on the tree created using the SSSSg program, which shows a relatively large variation within the analyzed proteins of one H1N1 strain only. Some dependencies can be seen based on when the strains appeared. Each tree can distinguish a coherent set of virus sequences.

The green color represents a group of sequences derived from the 1970s (strain H1N1). The blue color clusters are the most recent sequences, derived mostly from the years 2005 to 2010. A large part of this group represents the H1N2 and H3N2 strains. The red sequences are from around the year 2000 (clearly visible as separate clades on ClustalX and ConSurf trees). Moreover, the sequences in this group are derived from one place: Hong Kong. The pink and black colors mark the sequences that form some groups; however, they are not homogeneous, in terms of the criteria adopted above.

The amino acid sequences of the M2 protein derived from swine viruses are less diverse, in terms of the number of virus strains from which they derive. However, they show a similar diversity, in terms of the construction of the M2 protein.

Group 3 (avian and swine only)

The red color sequences are from birds, while the green color sequences are from swine. For the trees consisting of the selected sequences, the expected result for the group of viruses whose hosts are birds and of viruses whose hosts are swine was a division of the tree into two distinct groups (a group of bird sequences and a group of pig sequences). We saw a clear division into two groups when analyzing the formed trees, in most cases. This division was seen in the trees created by thee ClustalX and PHYLIP (maximum likelihood) programs and using the ConSurf. Such a relationship can also be traced for the other two trees; however, it is no longer so distinct. The ClustalX program created a tree that could be divided into two distinct groups. Group A consists primarily of bird sequences (except for five sequences that belong to swine viruses: C8CI82, D7ECW7, E6XUH0, Q068G1, and Q20ME5). In contrast, Group B included swine sequences (except for the bird sequence B4ZHC8).

The ConSurf program generated a tree that also splits into two distinct groups. Group A is a group of bird sequences (except for sequences C8CI82, D7ECW7, E6XUH0, Q068G1, and Q20ME5, which belong to swine viruses). Group B of the phylogram consists of swine sequences, except for one bird sequence: B4ZHC8.

The PHYLIP program (maximum parsimony algorithm) created a tree in which swine sequences were grouped together in one clear clade. The bird sequences were divided into two groups; however, these groups can be traced back to their relationship.

The phylogram created using the PHYLIP program (maximum likelihood algorithm) could be separated into two distinct groups. Group A in this case contains swine sequences except for one bird sequence: B4ZHC8. Group B consists mainly of bird sequences, except for six swine sequences: C8CI36, C8CI82, D7ECW7, E6XUH0, Q068G1, and Q20ME5.

The tree created using the SSSSg program can be divided into several groups. Group A contains bird sequences, except for two swine sequences: C8CI82 and E6XUH0. Group B is a group of swine sequences, except for six sequences of birds: B4ZHC8, Q0A3I7, Q0A415, Q0A436, Q0A447, and Q2FCG9. Group C contains bird sequences, except for one swine sequence: D7ECW7. Group D is also a group of bird sequences, except for three swine sequences: C8CI36, Q068G1, and Q20ME5. In the case of B group's bird sequences: Q0A436, Q0A447, and Q2FCG9 are located at the base of the clade, so they have nothing to be classified into.

ClustalX, PHYLIP (maximum likelihood), and the ConSurf server had similar results, in terms of separation sequences into two distinct groups for swine and birds. Although the tree topology is different, there were some similarities. The SSSSg program provided slightly different results. E phylogram created using this program can be divided into four groups, which are relatively homogeneous regarding the host of the virus. In this tree, it can be seen that group A is linked to group C (bird sequences). However, extensive portions of group B (swine) are more closely linked to group C, although this does not mean that the results are invalid—it may be the subject of future discussion. Group D (bird sequences) appears to be distant from the groups consisting of bird sequences (group A and C).

The results show that avian viruses differ from swine viruses not regarding surface glycoproteins, but also in terms of the M2 protein. This means that the viruses adapt to their hosts in parallel, in terms of the M2 protein, hemagglutinin and neuraminidase, although M2 protein only acts as a proton pump, through which it is possible to start replication. There may be an unknown compound between these that has made the evolution of these two elements related.

Group 4 (other or unidentified host)

The last group contains the smallest number of sequences, but they quite diverse within the group. The trees are divided into two separate clades. These results were provided by the ClustalX, ConSurf, and PHYLIP (maximum likelihood) programs. In contrast, the SSSSg program provided different result, in which several characteristic groups could be observed.

These groups are relatively uniform in terms of the virus strains, even though the strains came from different years and different places on Earth. This is another condition that might indicate a functional connection between the M2 proteins of surface virus glycoproteins.

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ANNEX

Avian
Figures: 5, 6, 7, 8
Swine
Figures: 9, 10, 11, 12
Avian and swine
Figures: 13, 14, 15, 16
Other
Figures: 17, 18, 19, 20

Content of annex

The annex contains a description of all of the M2 records used in this publication.
Sequence M2 records belonging to viruses whose hosts are birds.
Sequence M2 records belonging to viruses whose hosts are swine.
Sequence M2 records belonging to viruses whose hosts are both birds and swine, or an unidentified host.

Figure descriptions (annex)

Avian

Figure 5

Figure 5: Phylogenetic tree constructed using PHYLIP (maximum likelihood algorithm) from sequence group belonging to viruses whose hosts are birds.

Figure 6

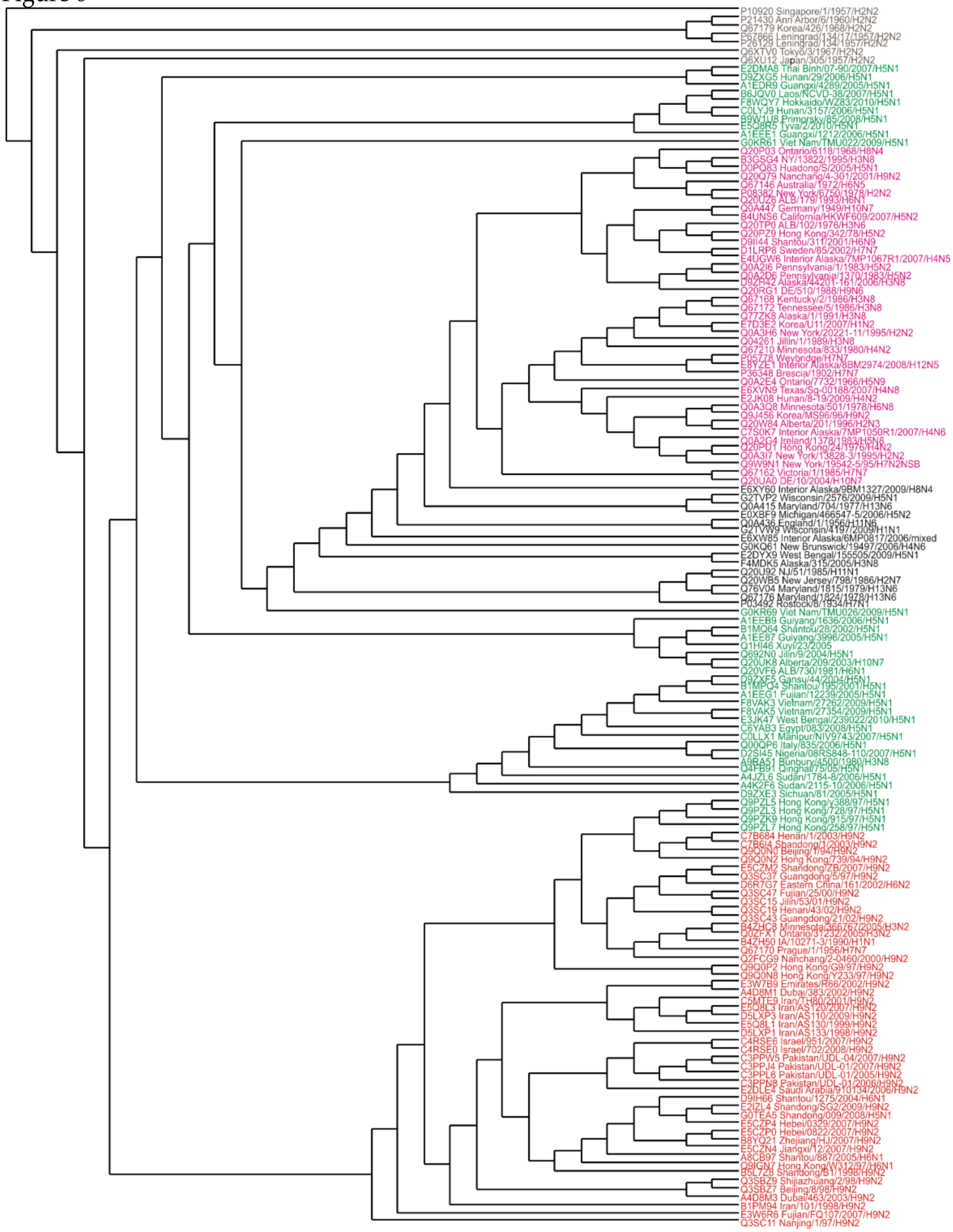

Figure 6: Phylogenetic tree constructed using PHYLIP (maximum parsimony algorithm) from sequence group belonging to viruses whose hosts are birds.

Figure 7

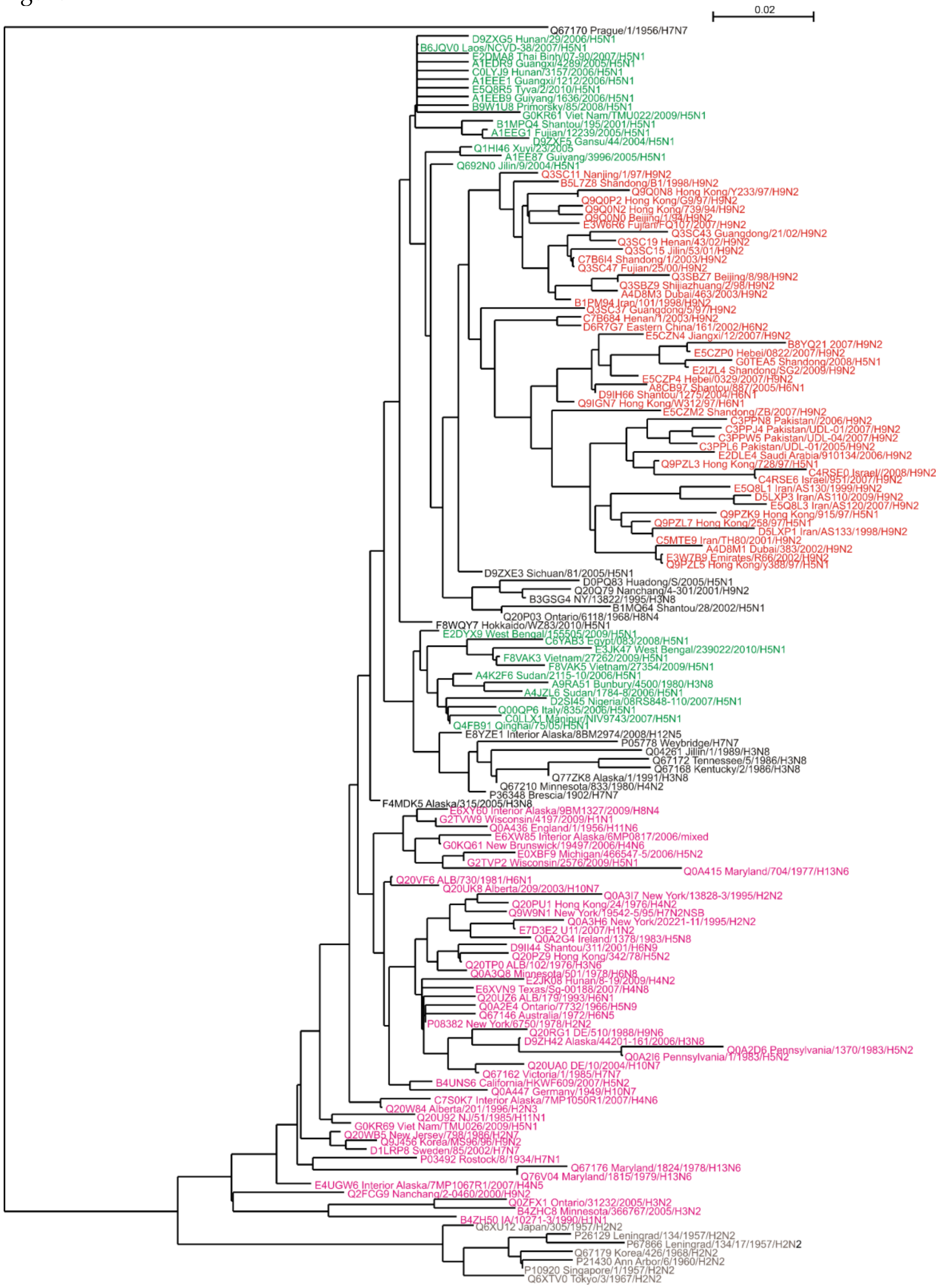

Figure 7: Phylogenetic tree constructed using ClustalX from sequence group belonging to viruses whose hosts are birds.

Figure 8

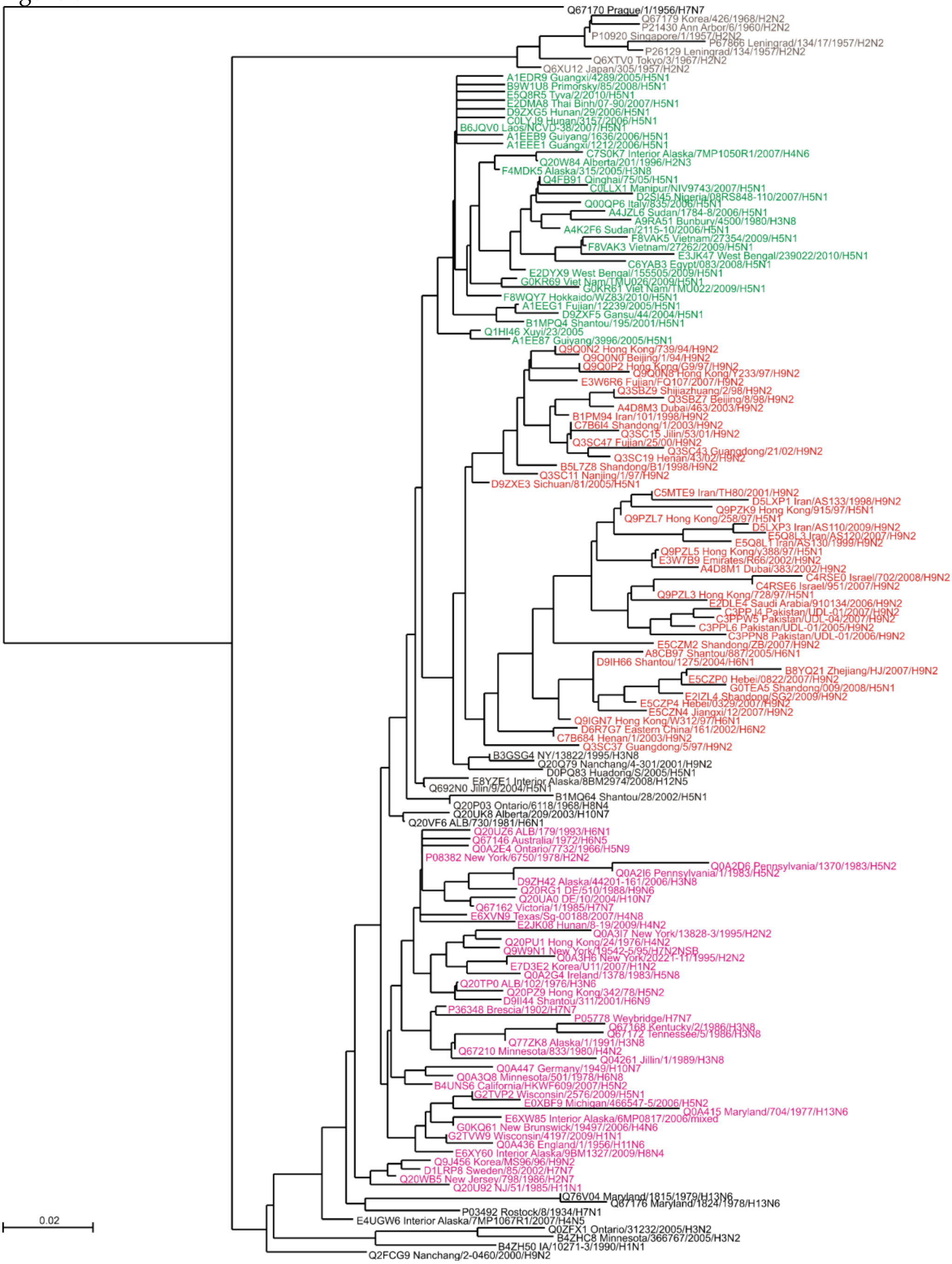

Figure 8: Phylogenetic tree constructed using Consurf from sequence group belonging to viruses whose hosts are birds.

Swine

Figure 9

Figure 9: Phylogenetic tree constructed using SSSSg from sequence group belonging to viruses whose hosts are swine.

Figure 10

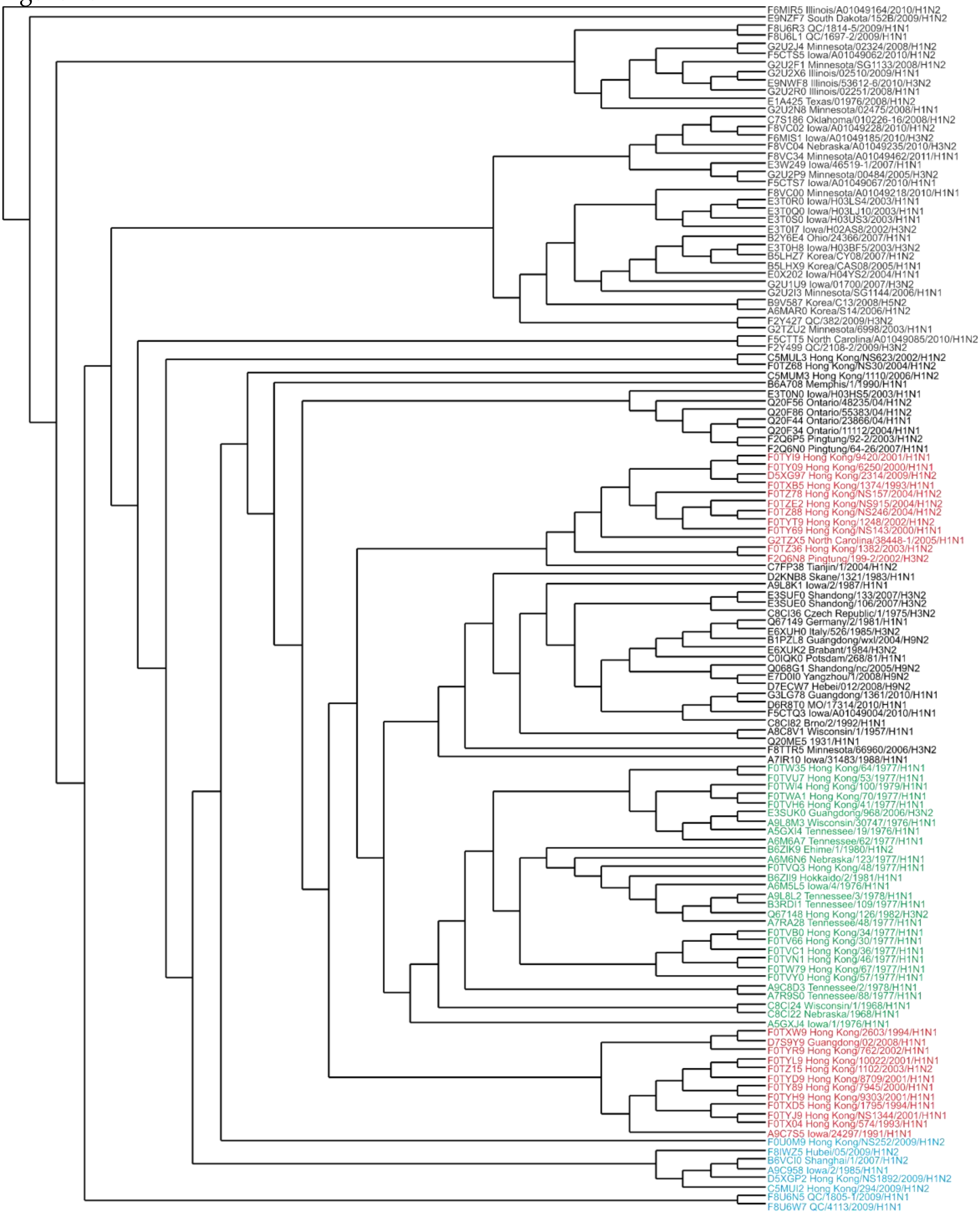

Figure 10: Phylogenetic tree constructed using PHYLIP (maximum parsimony algorithm) from sequence group belonging to viruses whose hosts are swine.

Figure 11

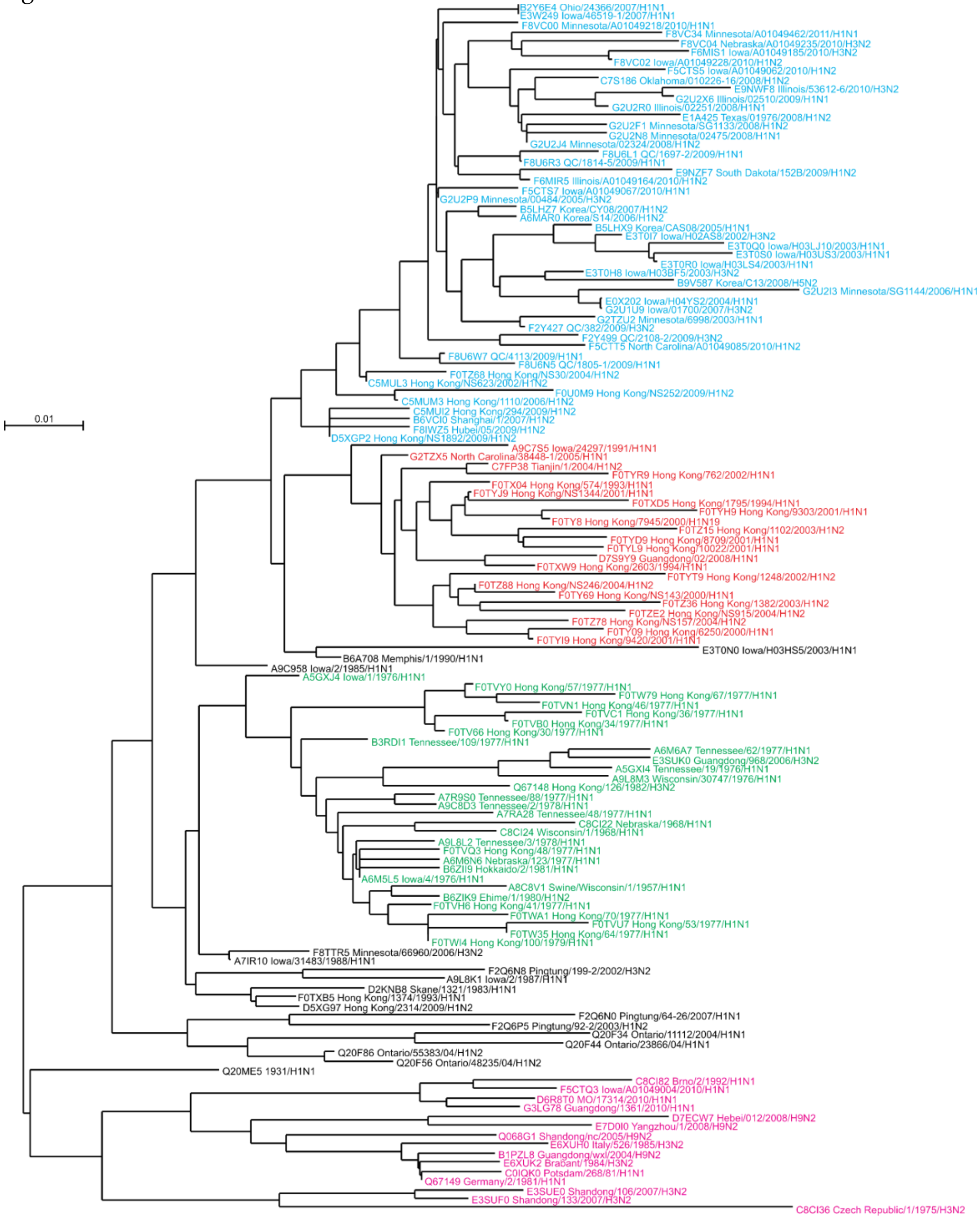

Figure 11: Phylogenetic tree constructed using ClustalX from sequence group belonging to viruses whose hosts are swine.

Figure 12

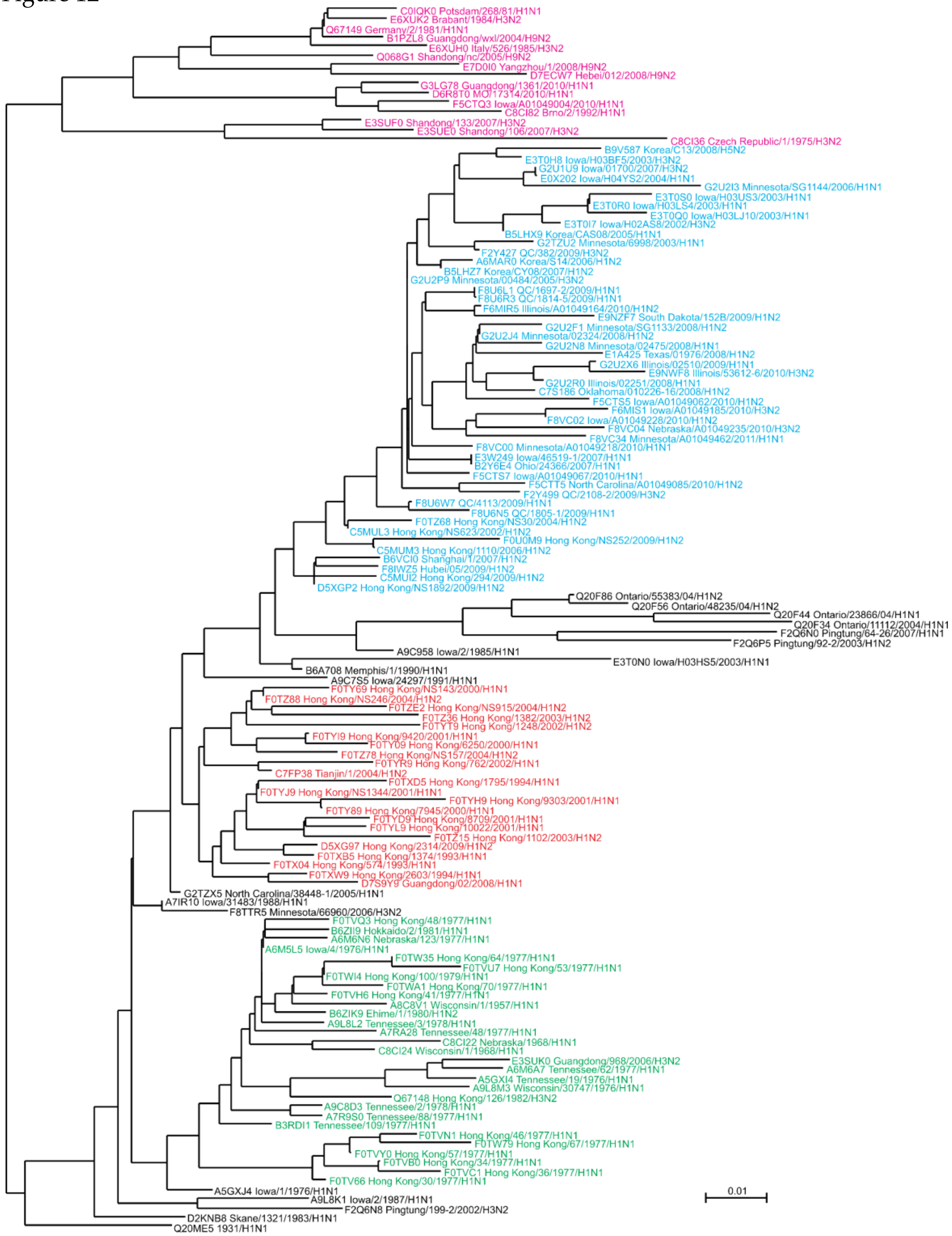

Figure 12: Phylogenetic tree constructed using Consurf from sequence group belonging to viruses whose hosts are swine.

Avian and swine

Figure 13

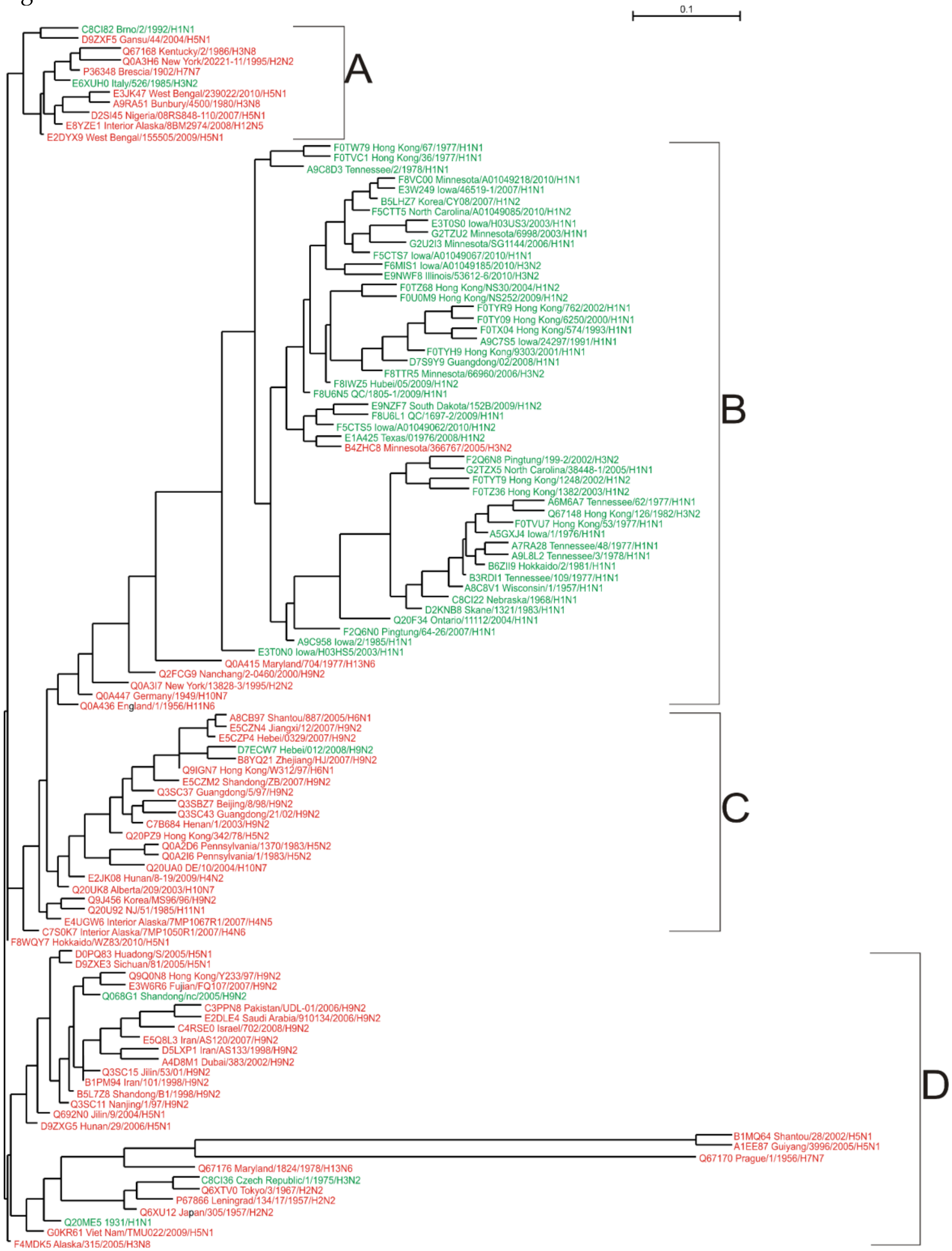

Figure 13: Phylogenetic tree constructed using SSSSg from selected sequences in both the group belonging to viruses whose hosts are birds and group belonging to viruses whose hosts are swine.

Figure 14

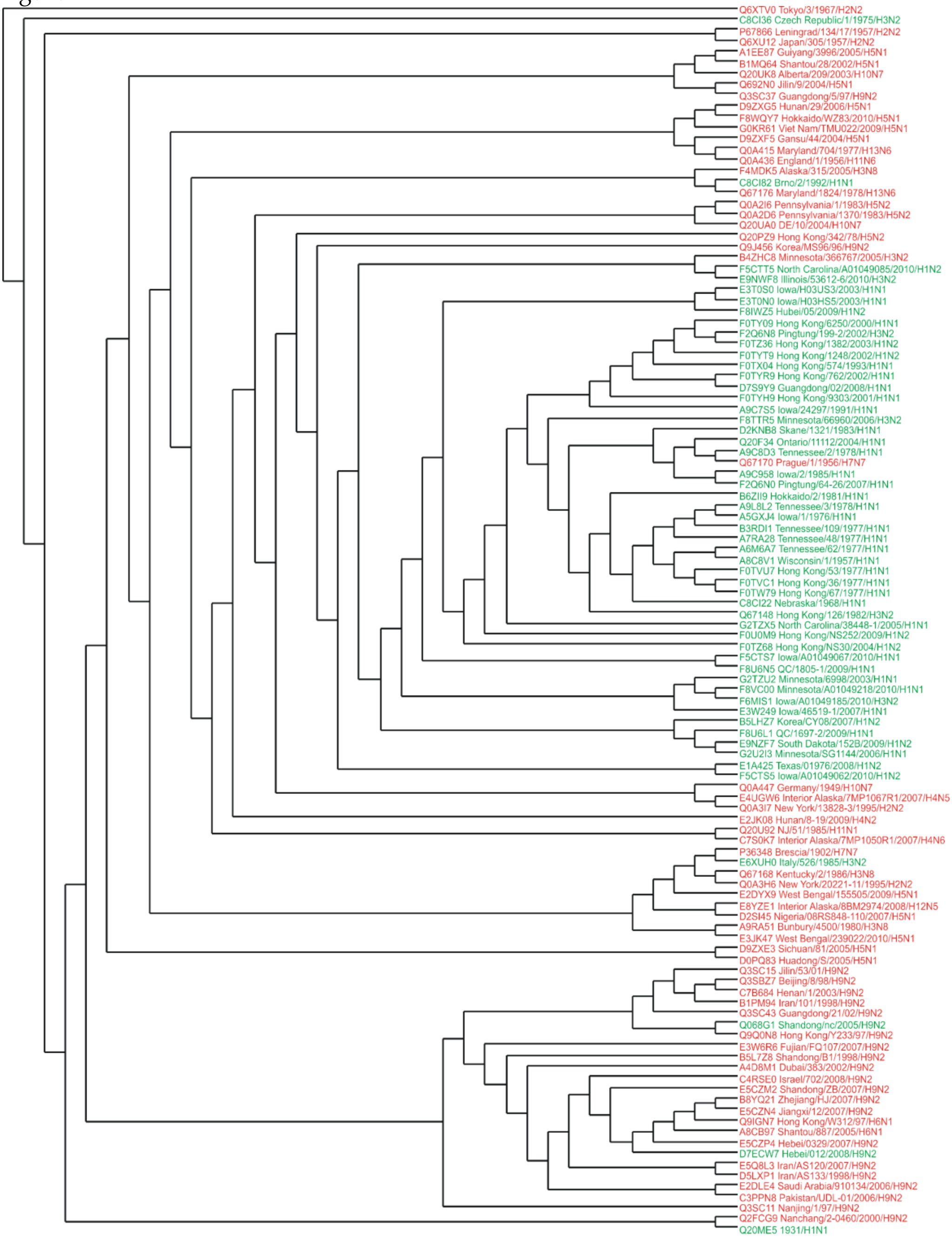

Figure 14: Phylogenetic tree constructed using PHYLIP (maximum parsimony algorithm) from selected sequences in both the group belonging to viruses whose hosts are birds and the group belonging to viruses whose hosts are swine.

Figure 15

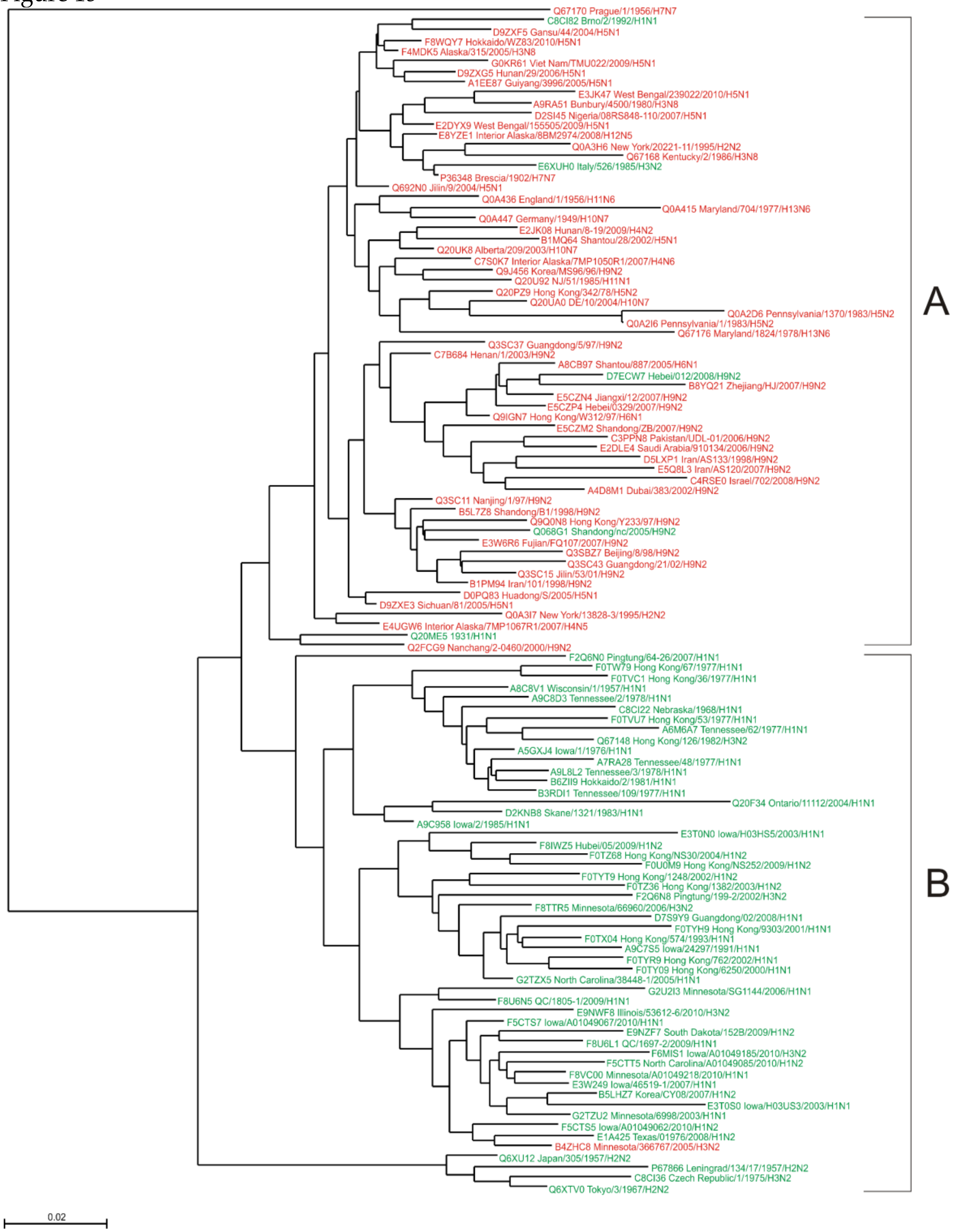

Figure 15: Phylogenetic tree constructed using ClustalX from selected sequences in both the group belonging to viruses whose hosts are birds and the group belonging to viruses whose hosts are swine.

Figure 16

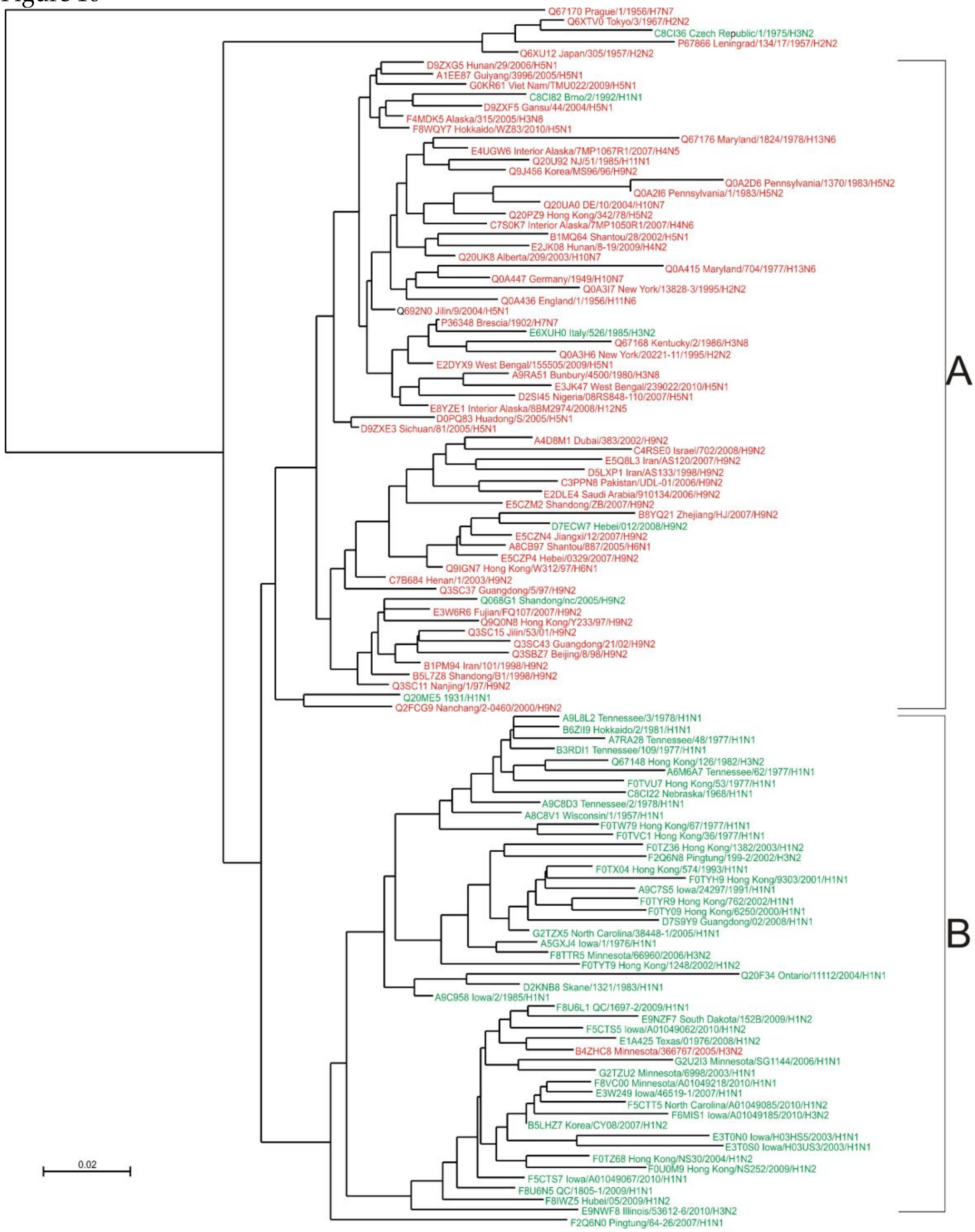

Figure 16: Phylogenetic tree constructed using Consurf from selected sequences in both the group belonging to viruses whose hosts are birds and the group belonging to viruses whose hosts are swine.

Other

Figure 17

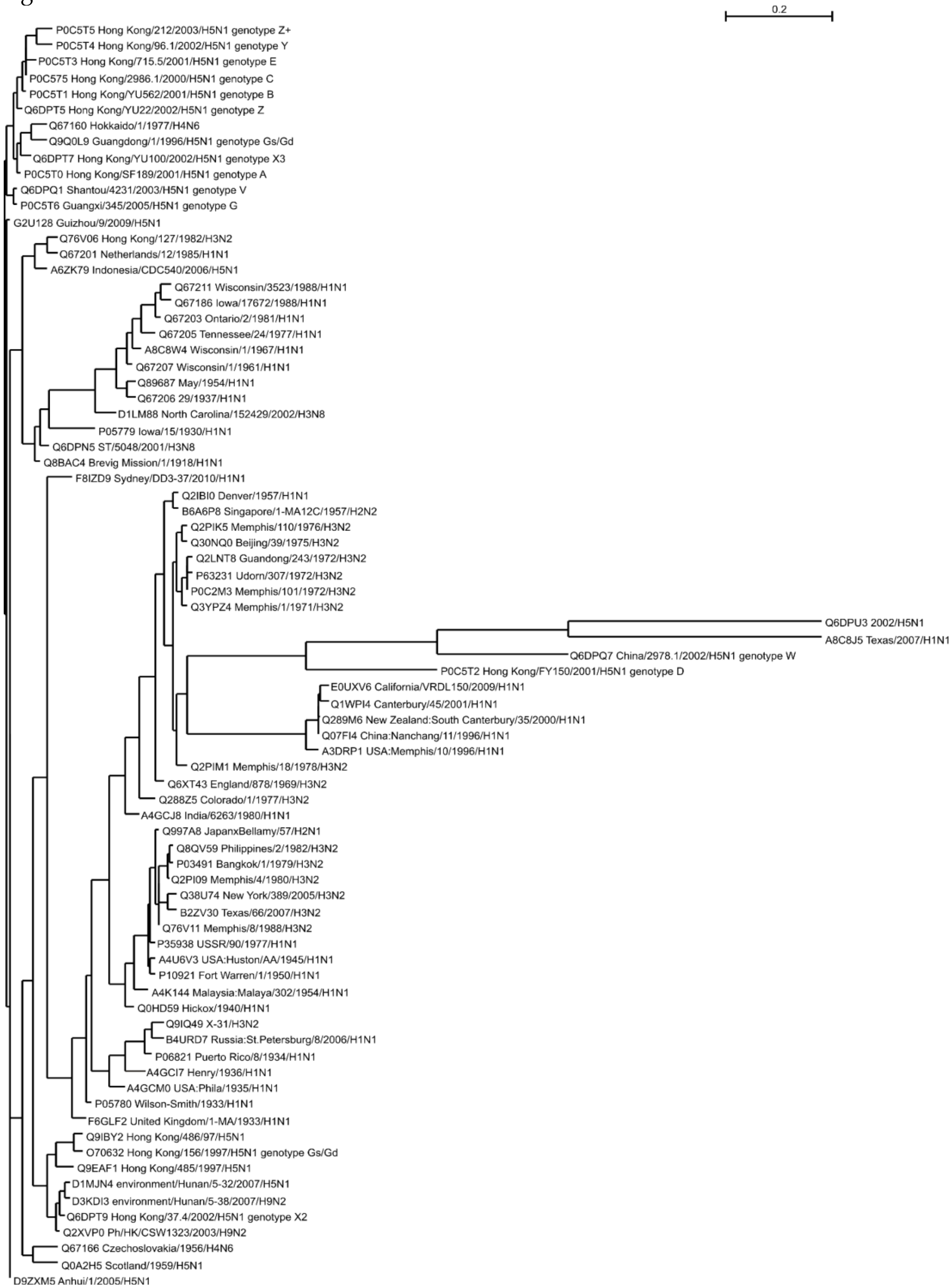

Figure 17: Phylogenetic tree constructed using SSSSg from sequence group belonging to viruses whose hosts are birds, swine, or others, or an unidentified host.

Figure 18

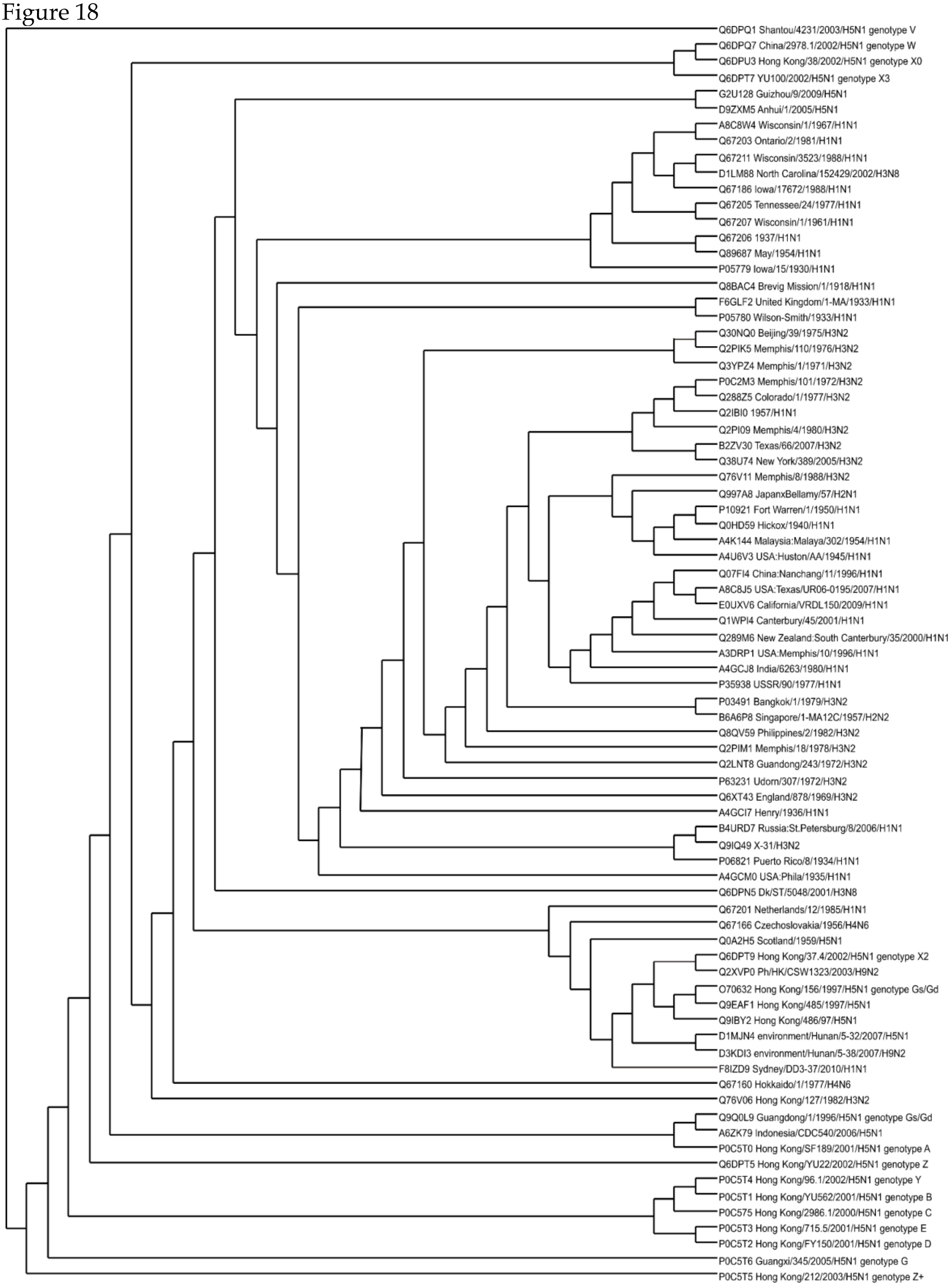

Figure 18: Phylogenetic tree constructed using PHYLIP (maximum parsimony algorithm) from the sequence group belonging to viruses whose hosts are birds, swine, and others, or an unidentified host.

Figure 19

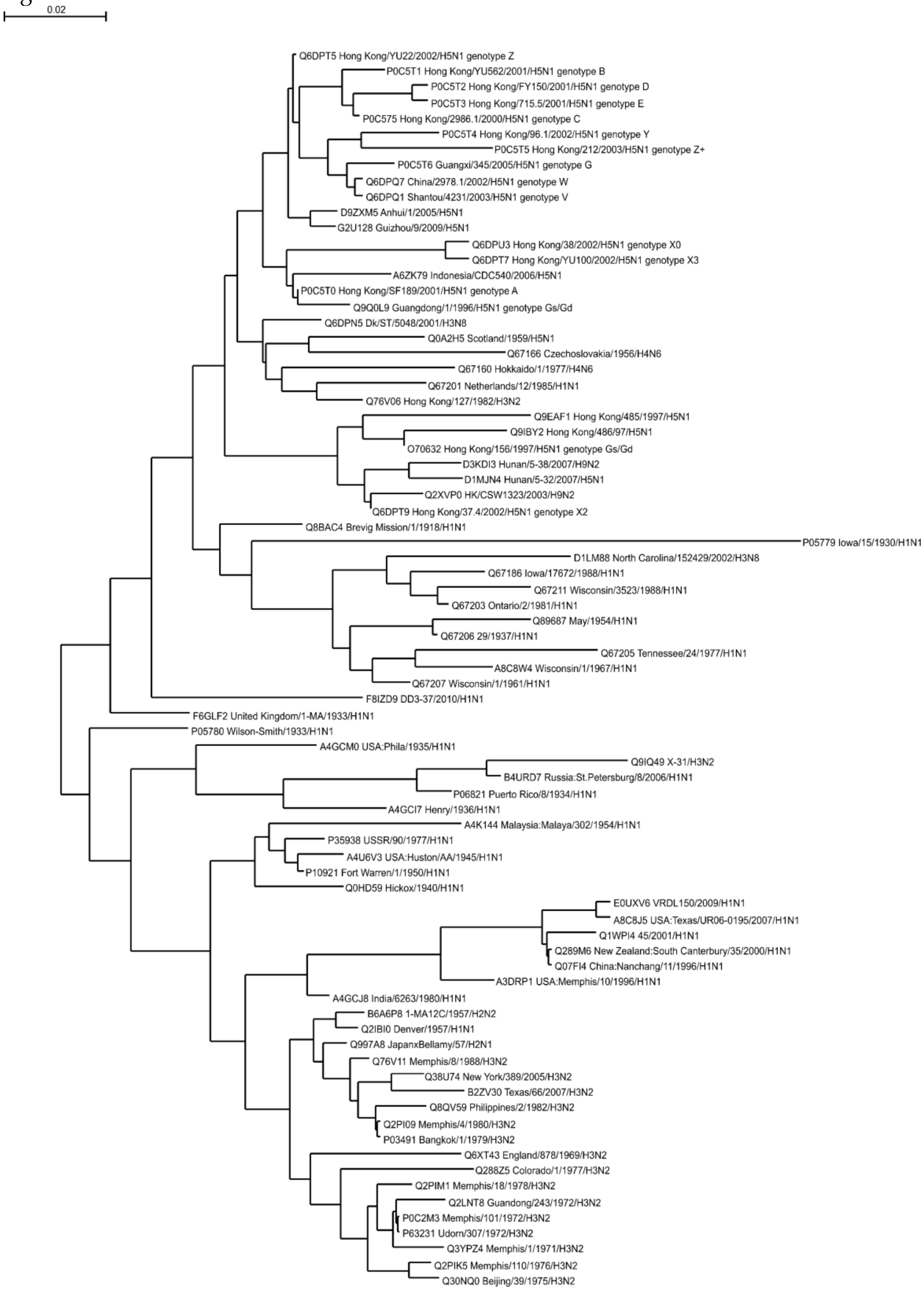

Figure 19: Phylogenetic tree constructed using ClustalX from the sequence group belonging to viruses whose hosts are birds, swine, and others, or an unidentified host.

Figure 20

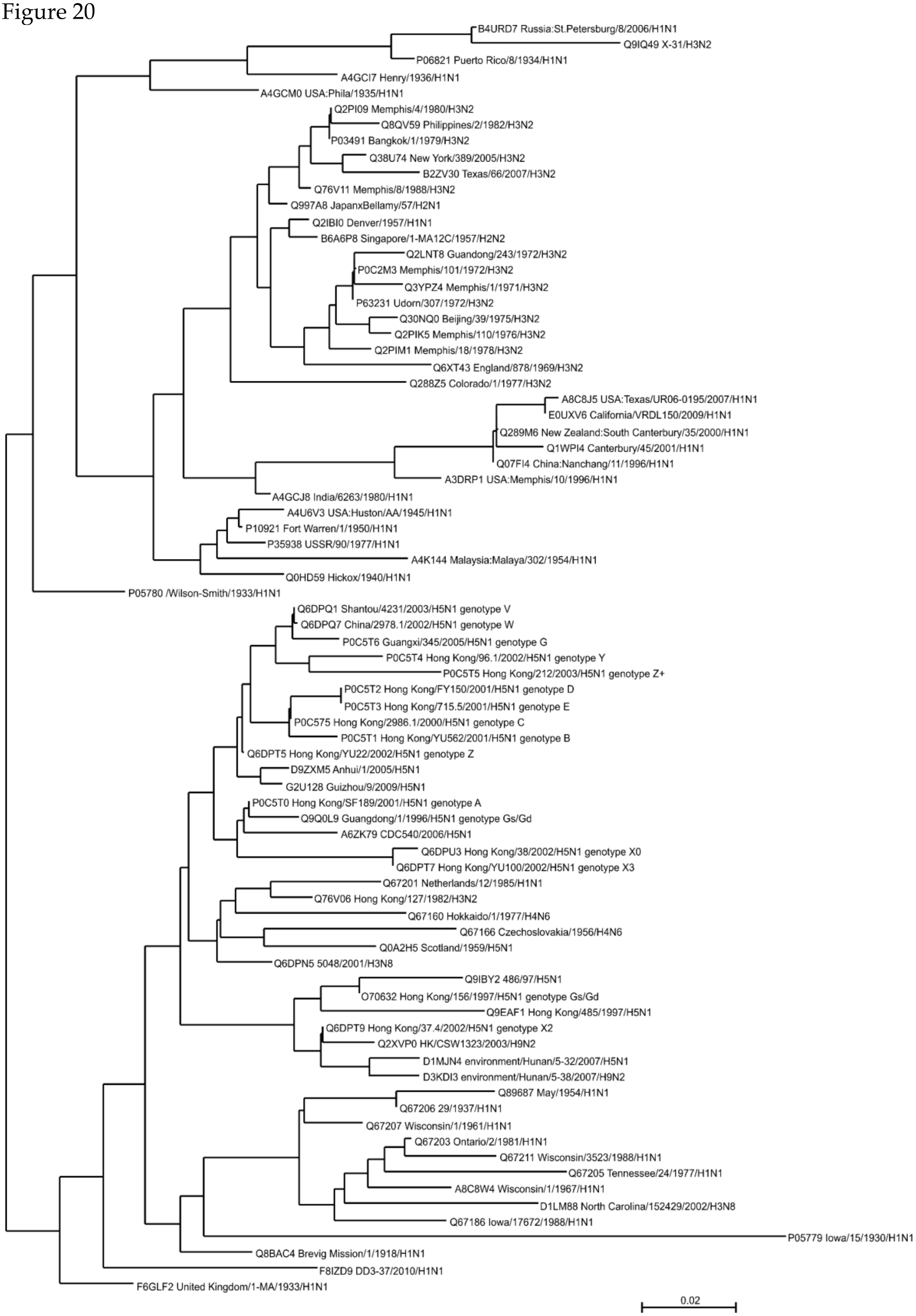

Figure 20: Phylogenetic tree constructed using Consurf program from the sequence group belonging to viruses whose hosts are birds, swine, and others, or an unidentified host.

The annex contains description of all M2 records used in this publication.

Sequence M2 records belonging to viruses whose hosts are birds.

A1EDR9 - A/goose/Guangxi/4289/2005/H5N1
A1EE87 - A/duck/Guiyang/3996/2005/H5N1
A1EEB9 - A/goose/Guiyang/1636/2006/H5N1
A1EEE1 - A/chicken/Guangxi/1212/2006/H5N1
A1EEG1 - A/chicken/Fujian/12239/2005/H5N1
A4D8M1 - A/chicken/Dubai/383/2002/H9N2
A4D8M3 - A/chicken/Dubai/463/2003/H9N2
A4JZL6 - A/chicken/Sudan/1784-8/2006/H5N1
A4K2F6 - A/chicken/Sudan/2115-10/2006/H5N1
A8CB97 - A/quail/Shantou/887/2005/H6N1
A9RA51 - A/red-necked stint/Bunbury/4500/1980/H3N8
B1MPQ4 - A/duck/Shantou/195/2001/H5N1
B1MQ64 - A/chicken/Shantou/28/2002/H5N1
B1PM94 - A/chicken/Iran/101/1998/H9N2
B3GSG4 - A/duck/NY/13822/1995/H3N8
B4UNS6 - A/American green-winged teal/California/HKWF609/2007/H5N2
B4ZH50 - A/turkey/IA/10271-3/1990/H1N1
B4ZHC8 - A/turkey/Minnesota/366767/2005/H3N2
B5L7Z8 - A/chicken/Shandong/B1/1998/H9N2
B6JQV0 - A/chicken/Laos/NCVD-38/2007/H5N1
B8YQ21 - A/chicken/Zhejiang/HJ/2007/H9N2
B9W1U8 - A/chicken/Primorsky/85/2008/H5N1
C0LLX1 - A/chicken/Manipur/NIV9743/2007/H5N1
C0LYJ9 - A/chicken/Hunan/3157/2006/H5N1
C3PPJ4 - A/chicken/Pakistan/UDL-01/2007/H9N2
C3PPL6 - A/chicken/Pakistan/UDL-01/2005/H9N2
C3PPN8 - A/chicken/Pakistan/UDL-01/2006/H9N2
C3PPW5 - A/chicken/Pakistan/UDL-04/2007/H9N2
C4RSE0 - A/chicken/Israel/702/2008/H9N2
C4RSE6 - A/chicken/Israel/951/2007/H9N2
C5MTE9 - A/chicken/Iran/TH80/2001/H9N2
C6YAB3 - A/chicken/Egypt/083/2008/H5N1
C7B684 - A/chicken/Henan/1/2003/H9N2
C7B6I4 - A/chicken/Shandong/1/2003/H9N2
C7S0K7 - A/mallard/Interior Alaska/7MP1050R1/2007/H4N6
D0PQ83 - A/mallard/Huadong/S/2005/H5N1
D1LRP8 - A/mallard/Sweden/85/2002/H7N7
D2SI45 - A/chicken/Nigeria/08RS848-110/2007/H5N1
D5LXP1 - A/chicken/Iran/AS133/1998/H9N2
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D6R7G7 - A/duck/Eastern China/161/2002/H6N2
D9IH66 - A/duck/Shantou/1275/2004/H6N1
D9II44 - A/wild duck/Shantou/311/2001/H6N9
D9ZH42 - A/glaucous gull/Alaska/44201-161/2006/H3N8
D9ZXE3 - A/chicken/Sichuan/81/2005/H5N1
D9ZXF5 - A/chicken/Gansu/44/2004/H5N1
D9ZXG5 - A/duck/Hunan/29/2006/H5N1
E0XBF9 - A/mallard/Michigan/466547-5/2006/H5N2
E2DLE4 - A/avian/Saudi Arabia/910134/2006/H9N2
E2DMA8 - A/muscovy duck/Thai Binh/07-90/2007/H5N1
E2DYX9 - A/chicken/West Bengal/155505/2009/H5N1

E2IZL4 - A/chicken/Shandong/SG2/2009/H9N2
E2JK08 - A/duck/Hunan/8-19/2009/H4N2
E3JK47 - A/chicken/West Bengal/239022/2010/H5N1
E3W6R6 - A/duck/Fujian/FQ107/2007/H9N2
E3W7B9 - A/chicken/Emirates/R66/2002/H9N2
E4UGW6 - A/northern pintail/Interior Alaska/7MP1067R1/2007/H4N5
E5CZM2 - A/chicken/Shandong/ZB/2007/H9N2
E5CZN4 - A/chicken/Jiangxi/12/2007/H9N2
E5CZP0 - A/chicken/Hebei/0822/2007/H9N2
E5CZP4 - A/chicken/Hebei/0329/2007/H9N2
E5Q8L1 - A/chicken/Iran/AS130/1999/H9N2
E5Q8L3 - A/chicken/Iran/AS120/2007/H9N2
E5Q8R5 - A/grebe/Tyva/2/2010/H5N1
E6XVN9 - A/blue-winged teal/Texas/Sg-00188/2007/H4N8
E6XW85 - A/northern pintail/Interior Alaska/6MP0817/2006/mixed
E6XY60 - A/mallard/Interior Alaska/9BM1327/2009/H8N4
E7D3E2 - A/duck/Korea/U11/2007/H1N2
E8YZE1 - A/northern pintail/Interior Alaska/8BM2974/2008/H12N5
F4MDK5 - A/pintail/Alaska/315/2005/H3N8
F8VAK3 - A/chicken/Vietnam/27262/2009/H5N1
F8VAK5 - A/duck/Vietnam/27354/2009/H5N1
F8WQY7 - A/duck/Hokkaido/WZ83/2010/H5N1
G0KQ61 - A/American black duck/New Brunswick/19497/2006/H4N6
G0KR61 - A/duck/Viet Nam/TMU022/2009/H5N1
G0KR69 - A/duck/Viet Nam/TMU026/2009/H5N1
G0TEA5 - A/duck/Shandong/009/2008/H5N1
G2TVP2 - A/mallard/Wisconsin/2576/2009/H5N1
G2TVW9 - A/mallard/Wisconsin/4197/2009/H1N1
P03492 - A/aves/Rostock/8/1934/H7N1
P05778 - A/Chicken/Weybridge/H7N7
P08382 - A/Mallard/New York/6750/1978/H2N2
P10920 - A/aves/Singapore/1/1957/H2N2
P21430 - A/aves/Ann Arbor/6/1960/H2N2
P26129 - A/aves/Leningrad/134/1957/H2N2
P36348 - A/Chicken/Brescia/1902/H7N7
P67866 - A/aves/Leningrad/134/17/1957/H2N2
Q00QP6 - A/mallard/Italy/835/2006/H5N1
Q04261 - A/aves/Jillin/1/1989/H3N8
Q0A2D6 - A/Chicken/Pennsylvania/1370/1983/H5N2
Q0A2E4 - A/Turkey/Ontario/7732/1966/H5N9
Q0A2G4 - A/Turkey/Ireland/1378/1983/H5N8
Q0A2I6 - A/Chicken/Pennsylvania/1/1983/H5N2
Q0A3H6 - A/guinea fowl/New York/20221-11/1995/H2N2
Q0A3I7 - A/chicken/New York/13828-3/1995/H2N2
Q0A3Q8 - A/Turkey/Minnesota/501/1978/H6N8
Q0A415 - A/Gull/Maryland/704/1977/H13N6
Q0A436 - A/Duck/England/1/1956/H11N6
Q0A447 - A/Duck/Germany/1949/H10N7
Q0ZFX1 - A/turkey/Ontario/31232/2005/H3N2
Q1HI46 - A/spotbill duck/Xuyi/23/2005
Q20P03 - A/Turkey/Ontario/6118/1968/H8N4
Q20PU1 - A/duck/Hong Kong/24/1976/H4N2
Q20PZ9 - A/duck/Hong Kong/342/78/H5N2
Q20Q79 - A/chicken/Nanchang/4-301/2001/H9N2
Q20RG1 - A/ruddy turnstone/DE/510/1988/H9N6

Q20TP0 - A/canvasback duck/ALB/102/1976/H3N6
Q20U92 - A/ruddy turnstone/NJ/51/1985/H11N1
Q20UA0 - A/shorebird/DE/10/2004/H10N7
Q20UK8 - A/mallard/Alberta/209/2003/H10N7
Q20UZ6 - A/pintail/ALB/179/1993/H6N1
Q20VF6 - A/mallard duck/ALB/730/1981/H6N1
Q20W84 - A/mallard/Alberta/201/1996/H2N3
Q20WB5 - A/laughing gull/New Jersey/798/1986/H2N7
Q2FCG9 - A/quail/Nanchang/2-0460/2000/H9N2
Q3SBZ7 - A/chicken/Beijing/8/98/H9N2
Q3SBZ9 - A/chicken/Shijiazhuang/2/98/H9N2
Q3SC11 - A/duck/Nanjing/1/97/H9N2
Q3SC15 - A/chicken/Jilin/53/01/H9N2
Q3SC19 - A/chicken/Henan/43/02/H9N2
Q3SC37 - A/chicken/Guangdong/5/97/H9N2
Q3SC43 - A/chicken/Guangdong/21/02/H9N2
Q3SC47 - A/chicken/Fujian/25/00/H9N2
Q4FB91 - A/Bar-headed Goose/Qinghai/75/05/H5N1
Q67146 - A/Shearwater/Australia/1972/H6N5
Q67162 - A/Chicken/Victoria/1/1985/H7N7
Q67168 - A/aves/Kentucky/2/1986/H3N8
Q67170 - A/aves/Prague/1/1956/H7N7
Q67172 - A/aves/Tennessee/5/1986/H3N8
Q67176 - A/Gull/Maryland/1824/1978/H13N6
Q67179 - A/aves/Korea/426/1968/H2N2
Q67210 - A/Turkey/Minnesota/833/1980/H4N2
Q692N0 - A/chicken/Jilin/9/2004/H5N1
Q6XTV0 - A/aves/Tokyo/3/1967/H2N2
Q6XU12 - A/aves/Japan/305/1957/H2N2
Q76V04 - A/Gull/Maryland/1815/1979/H13N6
Q77ZK8 - A/aves/Alaska/1/1991/H3N8
Q9IGN7 - A/Teal/Hong Kong/W312/97/H6N1
Q9J456 - A/Chicken/Korea/MS96/96/H9N2
Q9PZK9 - A/chicken/Hong Kong/915/97/H5N1
Q9PZL3 - A/Chicken/Hong Kong/728/97/H5N1
Q9PZL5 - A/Chicken/Hong Kong/y388/97/H5N1
Q9PZL7 - A/Chicken/Hong Kong/258/97/H5N1
Q9Q0N0 - A/Chicken/Beijing/1/94/H9N2
Q9Q0N2 - A/Chicken/Hong Kong/739/94/H9N2
Q9Q0N8 - A/Pigeon/Hong Kong/Y233/97/H9N2
Q9Q0P2 - A/Chicken/Hong Kong/G9/97/H9N2
Q9W9N1 - A/Chicken/New York/19542-5/95/H7N2NSB

Sequence M2 records belonging to viruses whose hosts are swine.

A5GXI4 - A/swine/Tennessee/19/1976/H1N1
A5GXJ4 - A/swine/Iowa/1/1976/H1N1
A6M5L5 - A/swine/Iowa/4/1976/H1N1
A6M6A7 - A/swine/Tennessee/62/1977/H1N1
A6M6N6 - A/swine/Nebraska/123/1977/H1N1
A6MAR0 - A/swine/Korea/S14/2006/H1N2
A7IR10 - A/swine/Iowa/31483/1988/H1N1
A7R9S0 - A/swine/Tennessee/88/1977/H1N1
A7RA28 - A/swine/Tennessee/48/1977/H1N1
A8C8V1 - strain A/Swine/Wisconsin/1/1957/H1N1

A9C7S5 - A/swine/Iowa/24297/1991/H1N1
A9C8D3 - A/swine/Tennessee/2/1978/H1N1
A9C958 - A/swine/Iowa/2/1985/H1N1
A9L8K1 - A/swine/Iowa/2/1987/H1N1
A9L8L2 - A/swine/Tennessee/3/1978/H1N1
A9L8M3 - A/swine/Wisconsin/30747/1976/H1N1
B1PZL8 - A/swine/Guangdong/wxl/2004/H9N2
B2Y6E4 - A/swine/Ohio/24366/2007/H1N1
B3RDI1 - A/swine/Tennessee/109/1977/H1N1
B5LHX9 - A/swine/Korea/CAS08/2005/H1N1
B5LHZ7 - A/swine/Korea/CY08/2007/H1N2
B6A708 - A/swine/Memphis/1/1990/H1N1
B6VCI0 - A/swine/Shanghai/1/2007/H1N2
B6ZII9 - A/swine/Hokkaido/2/1981/H1N1
B6ZIK9 - A/swine/Ehime/1/1980/H1N2
B9V587 - A/swine/Korea/C13/2008/H5N2
C0IQK0 - A/swine/Potsdam/268/81/H1N1
C5MUI2 - A/swine/Hong Kong/294/2009/H1N2
C5MUL3 - A/swine/Hong Kong/NS623/2002/H1N2
C5MUM3 - A/swine/Hong Kong/1110/2006/H1N2
C7FP38 - A/swine/Tianjin/1/2004/H1N2
C7S186 - A/swine/Oklahoma/010226-16/2008/H1N2
C8CI22 - A/swine/Nebraska/1968/H1N1
C8CI24 - A/swine/Wisconsin/1/1968/H1N1
C8CI36 - A/swine/Czech Republic/1/1975/H3N2
C8CI82 - A/swine/Brno/2/1992/H1N1
D2KNB8 - A/swine/Skane/1321/1983/H1N1
D5XG97 - A/swine/Hong Kong/2314/2009/H1N2
D5XGP2 - A/swine/Hong Kong/NS1892/2009/H1N2
D6R8T0 - A/swine/MO/17314/2010/H1N1
D7ECW7 - A/swine/Hebei/012/2008/H9N2
D7S9Y9 - A/swine/Guangdong/02/2008/H1N1
E0X202 - A/swine/Iowa/H04YS2/2004/H1N1
E1A425 - A/swine/Texas/01976/2008/H1N2
E3SUE0 - A/swine/Shandong/106/2007/H3N2
E3SUF0 - A/swine/Shandong/133/2007/H3N2
E3SUK0 - A/swine/Guangdong/968/2006/H3N2
E3T0H8 - A/swine/Iowa/H03BF5/2003/H3N2
E3T0I7 - A/swine/Iowa/H02AS8/2002/H3N2
E3T0N0 - A/swine/Iowa/H03HS5/2003/H1N1
E3T0Q0 - A/swine/Iowa/H03LJ10/2003/H1N1
E3T0R0 - A/swine/Iowa/H03LS4/2003/H1N1
E3T0S0 - A/swine/Iowa/H03US3/2003/H1N1
E3W249 - A/swine/Iowa/46519-1/2007/H1N1
E6XUH0 - A/swine/Italy/526/1985/H3N2
E6XUK2 - A/swine/Brabant/1984/H3N2
E7D0I0 - A/swine/Yangzhou/1/2008/H9N2
E9NWF8 - A/swine/Illinois/53612-6/2010/H3N2
E9NZF7 - A/swine/South Dakota/152B/2009/H1N2
F0TV66 - A/swine/Hong Kong/30/1977/H1N1
F0TVB0 - A/swine/Hong Kong/34/1977/H1N1
F0TVC1 - A/swine/Hong Kong/36/1977/H1N1
F0TVH6 - A/swine/Hong Kong/41/1977/H1N1
F0TVN1 - A/swine/Hong Kong/46/1977/H1N1
F0TVQ3 - A/swine/Hong Kong/48/1977/H1N1

F0TVU7 - A/swine/Hong Kong/53/1977/H1N1
F0TVY0 - A/swine/Hong Kong/57/1977/H1N1
F0TW35 - A/swine/Hong Kong/64/1977/H1N1
F0TW79 - A/swine/Hong Kong/67/1977/H1N1
F0TWA1 - A/swine/Hong Kong/70/1977/H1N1
F0TWI4 - A/swine/Hong Kong/100/1979/H1N1
F0TX04 - A/swine/Hong Kong/574/1993/H1N1
F0TXB5 - A/swine/Hong Kong/1374/1993/H1N1
F0TXD5 - A/swine/Hong Kong/1795/1994/H1N1
F0TXW9 - A/swine/Hong Kong/2603/1994/H1N1
F0TY09 - A/swine/Hong Kong/6250/2000/H1N1
F0TY69 - A/swine/Hong Kong/NS143/2000/H1N1
F0TY89 - A/swine/Hong Kong/7945/2000/H1N1
F0TYD9 - A/swine/Hong Kong/8709/2001/H1N1
F0TYH9 - A/swine/Hong Kong/9303/2001/H1N1
F0TYI9 - A/swine/Hong Kong/9420/2001/H1N1
F0TYJ9 - A/swine/Hong Kong/NS1344/2001/H1N1
F0TYL9 - A/swine/Hong Kong/10022/2001/H1N1
F0TYR9 - A/swine/Hong Kong/762/2002/H1N1
F0TYT9 - A/swine/Hong Kong/1248/2002/H1N2
F0TZ15 - A/swine/Hong Kong/1102/2003/H1N2
F0TZ36 - A/swine/Hong Kong/1382/2003/H1N2
F0TZ68 - A/swine/Hong Kong/NS30/2004/H1N2
F0TZ78 - A/swine/Hong Kong/NS157/2004/H1N2
F0TZ88 - A/swine/Hong Kong/NS246/2004/H1N2
F0TZE2 - A/swine/Hong Kong/NS915/2004/H1N2
F0U0M9 - A/swine/Hong Kong/NS252/2009/H1N2
F2Q6N0 - A/swine/Pingtung/64-26/2007/H1N1
F2Q6N8 - A/swine/Pingtung/199-2/2002/H3N2
F2Q6P5 - A/swine/Pingtung/92-2/2003/H1N2
F2Y427 - A/swine/QC/382/2009/H3N2
F2Y499 - A/swine/QC/2108-2/2009/H3N2
F5CTQ3 - A/swine/Iowa/A01049004/2010/H1N1
F5CTS5 - A/swine/Iowa/A01049062/2010/H1N2
F5CTS7 - A/swine/Iowa/A01049067/2010/H1N1
F5CTT5 - A/swine/North Carolina/A01049085/2010/H1N2
F6MIR5 - A/swine/Illinois/A01049164/2010/H1N2
F6MIS1 - A/swine/Iowa/A01049185/2010/H3N2
F8IWZ5 - A/swine/Hubei/05/2009/H1N2
F8TTR5 - A/swine/Minnesota/66960/2006/H3N2
F8U6L1 - A/swine/QC/1697-2/2009/H1N1
F8U6N5 - A/swine/QC/1805-1/2009/H1N1
F8U6R3 - A/swine/QC/1814-5/2009/H1N1
F8U6W7 - A/swine/QC/4113/2009/H1N1
F8VC00 - A/swine/Minnesota/A01049218/2010/H1N1
F8VC02 - A/swine/Iowa/A01049228/2010/H1N2
F8VC04 - A/swine/Nebraska/A01049235/2010/H3N2
F8VC34 - A/swine/Minnesota/A01049462/2011/H1N1
G2TZU2 - A/swine/Minnesota/6998/2003/H1N1
G2TZX5 - A/swine/North Carolina/38448-1/2005/H1N1
G2U1U9 - A/swine/Iowa/01700/2007/H3N2
G2U2F1 - A/swine/Minnesota/SG1133/2008/H1N2
G2U2I3 - A/swine/Minnesota/SG1144/2006/H1N1
G2U2J4 - A/swine/Minnesota/02324/2008/H1N2
G2U2N8 - A/swine/Minnesota/02475/2008/H1N1

G2U2P9 - A/swine/Minnesota/00484/2005/H3N2
G2U2R0 - A/swine/Illinois/02251/2008/H1N1
G2U2X6 - A/swine/Illinois/02510/2009/H1N1
G3LG78 - A/swine/Guangdong/1361/2010/H1N1
Q068G1 - A/swine/Shandong/nc/2005/H9N2
Q20F34 - A/swine/Ontario/11112/2004/H1N1
Q20F44 - A/swine/Ontario/23866/04/H1N1
Q20F56 - A/swine/Ontario/48235/04/H1N2
Q20F86 - A/swine/Ontario/55383/04/H1N2
Q20ME5 - A/swine/1931/H1N1
Q67148 - A/swine/Hong Kong/126/1982/H3N2
Q67149 - A/swine/Germany/2/1981/H1N1

Sequence M2 records belonging to viruses whose hosts are both birds and swine, or an unidentified host

A3DRP1 - A/aves,swine/USA:Memphis/10/1996/H1N1
A4GCI7 - A/aves,swine/Henry/1936/H1N1
A4GCJ8 - A/aves,swine/India/6263/1980/H1N1
A4GCM0 - A/aves,swine/USA:Phila/1935/H1N1
A4K144 - A/aves,swine/Malaysia:Malaya/302/1954/H1N1
A4U6V3 - A/aves,swine/USA:Huston/AA/1945/H1N1
A6ZK79 - A/Indonesia/CDC540/2006/H5N1
A8C8J5 - A/aves,swine/USA:Texas/UR06-0195/2007/H1N1
A8C8W4 - A/aves,swine/Wisconsin/1/1967/H1N1
B2ZV30 - A/Texas/66/2007/H3N2
B4URD7 - A/aves,swine/Russia:St.Petersburg/8/2006/H1N1
B6A6P8 - A/Singapore/1-MA12C/1957/H2N2
D1LM88 - A/equine/North Carolina/152429/2002/H3N8
D1MJN4 - A/environment/Hunan/5-32/2007/H5N1
D3KDI3 - A/environment/Hunan/5-38/2007/H9N2
D9ZXM5 - A/aves,swine/Anhui/1/2005/H5N1
E0UXV6 - A/California/VRDL150/2009/H1N1
F6GLF2 - A/United Kingdom/1-MA/1933/H1N1
F8IZD9 - A/Sydney/DD3-37/2010/H1N1
G2U128 - A/aves,swine/Guizhou/9/2009/H5N1
O70632 - A/aves,swine/Hong Kong/156/1997/H5N1 genotype Gs/Gd
P03491 - A/aves,swine/Bangkok/1/1979/H3N2
P05779 - A/aves,swine/Iowa/15/1930/H1N1
P05780 - A/aves,swine/Wilson-Smith/1933/H1N1
P06821 - A/aves,swine/Puerto Rico/8/1934/H1N1
P0C2M3 - A/aves,swine/Memphis/101/1972/H3N2
P0C575 - A/aves,swine/Hong Kong/2986.1/2000/H5N1 genotype C
P0C5T0 - A/aves,swine/Hong Kong/SF189/2001/H5N1 genotype A
P0C5T1 - A/aves,swine/Hong Kong/YU562/2001/H5N1 genotype B
P0C5T2 - A/aves,swine/Hong Kong/FY150/2001/H5N1 genotype D
P0C5T3 - A/aves,swine/Hong Kong/715.5/2001/H5N1 genotype E
P0C5T4 - A/aves,swine/Hong Kong/96.1/2002/H5N1 genotype Y
P0C5T5 - A/aves,swine/Hong Kong/212/2003/H5N1 genotype Z+
P0C5T6 - A/aves,swine/Guangxi/345/2005/H5N1 genotype G
P10921 - A/aves,swine/Fort Warren/1/1950/H1N1
P35938 - A/aves,swine/USSR/90/1977/H1N1
P63231 - A/aves,swine/Udorn/307/1972/H3N2
Q07FI4 - A/aves,swine/China:Nanchang/11/1996/H1N1
Q0A2H5 - A/aves,swine/Scotland/1959/H5N1
Q0HD59 - A/aves,swine/Hickox/1940/H1N1

Q1WPI4 - A/Canterbury/45/2001/H1N1
Q288Z5 - A/aves,swine/Colorado/1/1977/H3N2
Q289M6 - A/aves,swine/New Zealand:South Canterbury/35/2000/H1N1
Q2IBI0 - A/Denver/1957/H1N1
Q2LNT8 - A/Guandong/243/1972/H3N2
Q2PI09 - A/aves,swine/Memphis/4/1980/H3N2
Q2PIK5 - A/aves,swine/Memphis/110/1976/H3N2
Q2PIM1 - A/aves,swine/Memphis/18/1978/H3N2
Q2XVP0 - A/Ph/HK/CSW1323/2003/H9N2
Q30NQ0 - A/aves,swine/Beijing/39/1975/H3N2
Q38U74 - A/New York/389/2005/H3N2
Q3YPZ4 - A/aves,swine/Memphis/1/1971/H3N2
Q67160 - A/aves,swine/Hokkaido/1/1977/H4N6
Q67166 - A/aves,swine/Czechoslovakia/1956/H4N6
Q67186 - A/aves,swine/Iowa/17672/1988/H1N1
Q67201 - A/aves,swine/Netherlands/12/1985/H1N1
Q67203 - A/aves,swine/Ontario/2/1981/H1N1
Q67205 - A/aves,swine/Tennessee/24/1977/H1N1
Q67206 - A/aves,swine/29/1937/H1N1
Q67207 - A/aves,swine/Wisconsin/1/1961/H1N1
Q67211 - A/aves,swine/Wisconsin/3523/1988/H1N1
Q6DPN5 - A/Dk/ST/5048/2001/H3N8
Q6DPQ1 - A/aves,swine/Shantou/4231/2003/H5N1 genotype V
Q6DPQ7 - A/aves,swine/China/2978.1/2002/H5N1 genotype W
Q6DPT5 - A/aves,swine/Hong Kong/YU22/2002/H5N1 genotype Z
Q6DPT7 - A/aves,swine/Hong Kong/YU100/2002/H5N1 genotype X3
Q6DPT9 - A/aves,swine/Hong Kong/37.4/2002/H5N1 genotype X2
Q6DPU3 - A/aves,swine/Hong Kong/38/2002/H5N1 genotype X0
Q6XT43 - A/aves,swine/England/878/1969/H3N2
Q76V06 - A/aves,swine/Hong Kong/127/1982/H3N2
Q76V11 - A/aves,swine/Memphis/8/1988/H3N2
Q89687 - A/aves,swine/May/1954/H1N1
Q8BAC4 - A/aves,swine/Brevig Mission/1/1918/H1N1
Q8QV59 - A/aves,swine/Philippines/2/1982/H3N2
Q997A8 - A/JapanxBellamy/57/H2N1
Q9EAF1 - A/Hong Kong/485/1997/H5N1
Q9IBY2 - A/Hong Kong/486/97/H5N1
Q9IQ49 - A/aves,swine/X-31/H3N2
Q9Q0L9 - A/aves,swine/Guangdong/1/1996/H5N1 genotype Gs/Gd

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