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Influenza between August 2002 and August 2003

Influenza A and B viruses continued to cause outbreaks of disease throughout the world and the two types of viruses co-circulated in many countries. Overall, influenza activity was mild to moderate. Influenza A H3N2 viruses predominated in some countries, such as Japan and Italy, while B viruses predominated in others e.g Finland and Israel. Although AH1N1 and AH1N2 viruses circulated at low level in most parts of the world, they were prevalent in North America where H1N2 viruses were the principal cause of outbreaks in Canada. Of particular note during 2003 were 1) two cases of AH5N1 infection in Hong Kong during February; a 33 year old man who died and his 9 year old son; 2) more than 80 cases of H7N7 infection (mainly conjunctivitis), including one fatality, associated with oubreaks of highly pathogenic H7N7 in poultry in the Netherlands during March to May.

Characteristics of viruses

During the past year, the Influenza Centre characterized approximately 1300 viruses from 36 countries. Sixty five per cent were type A, of which 78% were H3N2, 17% were H1N1 and 5% were H1N2, and 35% were influenza B (Table 1). Influenza B were more prominent among viruses received during the latter part of 2002 (Table 2). They predominanted in Madagascar from September to November (in contrast to the AH3N2 viruses which caused a severe outbreak during July and August) and were isolated almost exclusively in Spain and Portugal during November and December, and predominated in Finland and Israel during December to Feburary. AH3N2 viruses predominated in north African countries, Senegal during August to November and Egypt during December and January and accounted for the majority of viruses isolated in many countries during the first half of 2003, European countries such as Austria, Italy and Switzerland, Hong Kong SAR of China and Japan, and more recently for outbreaks (June-July) in Australia, Israel, Madagascar, New Zealand, South Africa and S. American countries.

AH1N1 and H1N2 viruses

In haemagglutination inhibition (HI) tests using post-infection ferret antisera, the HAs of most of the H1N1 and H1N2 viruses were shown to be indistinguishable from each other and to be closely related to that of the current vaccine strain, A/New Caledonia/20/99 (H1N1) and A/Egypt/96/02 (H1N2) (Table 3). Few gave reduced HI titres of 4-fold or more with antisera to A/New Caledonia/20/99-like reference viruses.

HA and NA sequences of these viruses were in general similar to those of viruses isolated during the previous year. Of the H1N1 viruses most fell within phylogenetic group 1 (Figure 1), differing by two common amino acid changes from HA1 of A/New Caledonia/20/99, valine 166 to alanine (V166A) and tryptophan 252 to arginine (W252R) (Figure 2, Table 4). Their NA sequences were also similar to that of A/New Caledonia/20/99, differing by two amino acids, glutamic acid 332 to lysine (E332K) and asparagine 450 to aspartic acid (N450D) (Figures 3 and 4, Table 4). The HA sequences of H1N2 viruses had 4 changes relative to that of A/New Caledonia/20/99, valine 166 to alanine (V166A), valine 175 to isoleucine (V175I), alanine 190 to threonine (A190T) and alanine 215 to threonine (A215T); many recent isolates had an additional change, threonine 89 to alanine (T89A), causing the loss of a glycosylation site at asparagine 87. Their NA sequences were similar to those of H1N2 viruses circulating during early 2002; they were close to those of A/Moscow/10/99 (H3N2)-like viruses such as A/Singapore/15/01 (Figure 7) but were distinguished by 3 amino acid changes,

methionine 24 to threonine (M24T), glutamic acid 199 to lysine (E199K) and lysine 431 to asparagine (K431N) (Figure 8, Table 4).

AH3N2 viruses

Most of the H3N2 viruses isolated during August to November 2002 were closely related antigenically to the vaccine strain A/Panama/2007/99 and the more recent A/Moscow/10/99-like reference viruses, A/New York/55/01, A/Hong Kong/1550/02 and A/Egypt/130/02 (Table 5). An increasing proportion of more recent isolates were distinguishable from A/Panama/2007/99 and were more closely related to A/Fujian/411/02 and other A/Fujian/411/02-like reference viruses, such as A/Finland/170/03, A/Wyoming/3/03 or A/UK/1861/03 (Table 6). However, a number of isolates gave low HI titres with all the recent reference post-infection ferret antisera; HA sequences showed that many of such recent isolates were closely related to A/Fujian/411/02.

The HA and NA sequences of most of the viruses isolated between August 2002 and July 2003 fell into one of two phylogenetically distinguishable lineages, one represented by A/New York/55/01 (or Egypt 130/02; previously designated 1c), the other represented by A/Fujian/411/02 (previously represented by A/Hong Kong/1143/02 and designated 1b) (Figure 5). HAs within the former group (A/New York/55/01) of viruses, isolated during the latter half of 2002, were similar in sequence to the HAs of viruses prevalent during the early part of the year. Variants more divergent in sequence, which emerged during January to April 2003 were distinguished by additional amino acid changes, relative to the HA sequence of A/Panama/2007/99 of 1) glycine 49 to serine (G49S), lysine 158 to arginine (K158R), tyrosine 233 to histidine (Y233H) and asparagine 312 to lysine (N312K), represented by e.g. A/Latvia/1506/03; or 2) tyrosine 159 to asparagine (Y159N), tryptophan 222 to arginine (W222R), glycine 225 to aspartic acid (G225D) and possessed isoleucine at 226, represented by e.g. A/Hannover/154/03 [additional changes included glycine 49 to aspartic acid (G49D) and glutamic acid 62 to glycine (E62G), or valine 202 to isoleucine (V202I) and several had reverted to asparagine 144] (Figure 5 and 6, Table 7). The NA sequences which fell within the A/New York/55/01 phylogenetic group were similar to those of viruses prevalent during the previous year (Figures 7 and 8).

The HAs of A/Fujian/411/02-like viruses have also diverged to a significant extent from the sequence of A/Panama/2007/99 HA (Figure 5) and have accumulated 11 characteristic changes, leucine 25 to isoleucine (L25I), arginine 50 to glycine (R50G), histidine 75 to glutamine (H75Q), glutamic acid 83 to lysine (E83K),alanine 131 to threonine (A131T), histidine 155 to threonine (H155T), glutamine 156 to histidine (Q156H; in most recent isolates), serine 186 to glycine (S186G), valine 202 to isoleucine (V202I), tryptophan 222 to arginine (W222R) and glycine 225 to asparatic acid (G225D) (Figures 5 and 6, Table 7). Although these common differences do not alter the glycosylation of the HA, several viruses posssessed other changes which would alter glycosylation, e.g. threonine 128 to alanine (T128A) removes a glycosylation site at asparagine 126, and may contribute to variability in the patterns of reactivity observed in HI tests. The sequences of the NAs of these viruses exhibited 9 amino acid differences from the NA of A/Moscow/10/99: alanine 18 to serine (A18S), leucine 23 to phenylalanine (L23F), valine 30 to isoleucine (V30I), cysteine 42 to phenylalanine (C42F), glycine 143 to valine (G143V), glycine 216 to valine (G216V), proline 267 to

threonine (P267T), valine 307 to isoleucine (V307I) and lysine 385 to asparagine (K385N) (Figures 7 and 8, Table 7).

Influenza B viruses

The HAs of the majority of B viruses were antigenically closely related to the prototype vaccine strain B/Hong Kong/330/01 and the vaccine strain B/Shandong/7/97, as well as more recent reference viruses, B/Hong Kong/335/01, B/Tehran/80/02 and B/Brisbane/32/02 (B/Victoria/2/87 lineage) (Table 8). Few (3%) were B/Sichuan/379/99-like (B/Yamagata/16/88 lineage); these viruses were isolated infrequently, but were more prevalent in some Asian countries.

The HA sequences of the majority of the Victoria-lineage viruses isolated during 2003 were close to that of B/Shandong/7/97, the only common difference being isoleucine 121 to threonine (I121T) (Figures 9 and 10, Table 9), and therefore show little change over the past 6 years. Changes in residues 197 and 199 affect glycosylation of asparagine 197 of many isolates. The NAs of these viruses were similar to that of B/Sichuan/379/99 (Figure 11); amino acid changes included glutamic acid 148 to glycine (E148G), serine 198 to asparagine (S198N) and threonine 389 to alanine (T389A), and in several recent isolates arginine 65 to histidine (R65H) and/or asparagine 199 to aspartic acid (N199D) (Figure 12, Table 9). The majority of B isolates were, therefore, reassortants, possessing a Shandong-like HA and a Sichuan-like NA, and similar to viruses prevalent during 2002, represented by B/Tehran/80/02. Relatively few viruses (mainly from Asia) were similar to B/Hong Kong/330/01, with a HA characterized by the amino acid changes, histidine 116 to arginine (H116R), isoleucine 121 to asparagine (I121N) and aspartic acid 164 to glutamic acid (D164E) (Figures 9 and 10, Table 9).

The HAs of most of the B/Sichuan/379/99-like viruses fell within the B/Harbin/7/94 sublineage; many, e.g. B/Hong Kong/273/02, were characterized by the changes lysine 129 to asparagine (K129N), aspartic acid 233 to alanine (D233A) and glycine 256 to arginine (G256R), in addition to the histidine 40 to tyrosine (H40Y) change typical of Harbin-lineage viruses circulating during the previous year.

Swine Influenza Viruses

Of 78 viruses characterized (11 isolated in France, 65 in Italy and 2 in Switzerland), 33 were of the H1N1 subtype, 16 were H1N2 and 29 were H3N2. Most H1N1 isolates were antigenically closely related, in HI and NI tests, to the reference viruses A/swine/Ille et Vilaine/1455/99 (Sw/IV/1455/99) or A/swine/Côtes d'Armor/1482/99 (Sw/CA/1482/99) (Table 10). Most of the H1 sequences obtained for selected viruses confirmed the close relationship of isolates from France, Italy and Switzerland with Sw/IV/1455/99. The H1N2 viruses, isolated in France and Italy, were antigenically most closely related to the reference swine virus Sw/CA/790/97 (Table 10). The HA sequences of H1N2 viruses isolated during 2001 and 2002 fell into one of two phylogenetic groups, one represented by Sw/CA/790/97, the other by Sw/CA/604/99. The H3N2 viruses, all isolated in Italy, were antigenically closely related to the recent reference strain Sw/Italy/1523/98 (Table 11), although most of the HA sequences of recent isolates were distinguished phylogenetically from that of Sw/Italy/1523/98.

Reagents

Influenza viruses and antisera sent to National Influenza Centres and other Collaborating Centres are listed in Tables 12 and 13.

Acknowledgements

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Table 1: Human influenza isolates characterized August 2002 - August 2003

Country	H1N1 A/New Caledonia 20/99	H1N2 A/Egypt 96/02	H3N2 A/Panama/2007/99 or A/Fujian/411/02	B B/Sichuan 379/99	B B/HK 335/01
Albania			1		2
Australia			23		7
Austria			14	1	
Bulgaria			7		
Congo	2				
Croatia	1			1	
Czech Republic	3		4		4
Denmark		3	53		23
Egypt			33		6
Finland	4	4	3	2	8
France	14	5	15	2	31
Germany	1	1	66		11
Greece			5		10
Hong Kong SAR China		1	40	3	5
Hungary	2		1		4
Iceland	6		3		11
Indonesia			2	1	1
Ireland			2		1
Israel	1		17		28
Italy	1	2	78		5
Japan			6		2
Latvia	3		18		2
Madagascar			9		13
Malaysia				1	
Netherlands	1		8		1
New Caledonia			1		
New Zealand	2		9	1	
Norway	3	5	10		7
Portugal	3				44
Romania	16		1		16
Russia	9		11		5
Senegal	10	1	33		14
Serbia and Montenegro		1	4		6
Singapore			2		
Slovakia		3	1		1
South Africa		4	7		2
Spain	37		16		73
Sweden	2		21		2
Switzerland	1	3	35		9
Taiwan	1				
Thailand			2	1	1
Turkey					1
United Kingdom			3		6
Total = 1118	**123**	**33**	**574**	**13**	**375**
	(11%)	**(3%)**	**(51%)**	**388 (35%)**	

Table 2. Human influenza viruses isolated August 2002 - August 2003

	H1N1	H1N2	H3N2	B	
Month of isolation	A/New Caledonia 20/99	A/Egypt 96/02	A/Panama/2007/99 or A/Fujian/411/02	B/Sichuan 379/99	B/HK 335/01
2002					
August		1	4		2
September			6		8
October	1		14	2	4
November	4		12	1	31
December	3	2	36		90
2003					
January	10	4	106	2	71
February	38	9	121	2	67
March	45	10	142	1	56
April	13	2	26		10
May	3		7	1	
June	3	1	42		
July	1		14	2	
August			1		
Total = 1031	121 (12%)	29 (3%)	531 (51%)	11	339
				350 (34%)	

Table 3. Antigenic analyses of influenza A H1N1 and H1N2 viruses

Viruses	Isolation Date	Haemagglutination inhibition titre* Post infection ferret sera A/Bay 7/95	A/Beij 262/95	A/NC 20/99	A/Mad 57794/00	A/Eg 96/02
A/Bayern/7/95		**1280**	320	80	40	40
A/Beijing/262/95		80	**2560**	320	320	320
A/New Caledonia/20/99		40	640	**640**	320	640
A/Madagascar/57794/00		40	640	640	**640**	640
A/Egypt/96/02		<	320	640	320	**1280**
H1N1						
A/Dakar/51/02	25.10.02	<	160	320	320	1280
A/Congo/6/02	Dec-02	<	80	320	640	1280
A/Navarra/RR1252/02	Dec-02	80	80	320	640	1280
A/Lyon/1541/02	2.12.02	<	80	160	160	640
A/Paris/0655/03	Jan-03	40	160	320	320	640
A/Prague/8/03	26.1.03	<	40	320	320	640
A/Netherlands/02/03	29.1.03	<	80	80	160	640
A/Latvia/1381/03	3.2.03	40	160	640	320	1280
A/Segovia/17/03	17.2.03	<	160	320	320	640
A/St. Petersburg/49/03	17.2.03	40	640	320	640	640
A/Israel/2/03	18.2.03	<	320	640	640	1280
A/Hungary/7/03	20.2.03	80	640	640	320	1280
A/Sachsen/252/03	3.3.03	40	160	320	160	640
A/Lisbon/2/03	11.3.03	<	80	320	320	320
A/Moscow/6/03	12.3.03	<	640	640	640	640
A/Geneva/4099/03	14.3.03	<	320	320	320	640
A/Bucharest/796/03	19.3.03	40	320	320	160	320
A/Finland/299/03	27.3.03	<	160	320	160	640
A/Barcelona/69830/03	10.4.03	<	320	320	320	320
A/Zagreb/4889/03	9.5.03	<	80	640	320	640
A/Iceland/46/03	10.6.03	<	160	160	160	320
A/Dakar/13/03	11.6.03	40	160	320	160	320
H1N2						
A/Hong Kong/1670/02	30.8.02	40	80	640	640	1280
A/Lyon/1723/02	18.12.02	<	80	160	160	640
A/Slovakia/258/03	6.2.03	<	80	160	160	640
A/Belgrade/1062/03	21.2.03	<	160	80	160	640
A/Rheinland-Pfalz/34/03	28.2.03	<	320	640	320	1280
A/Parma/24/03	3.3.03	40	160	160	160	640
A/Geneva/3762/03	6.3.03	<	160	320	320	640
A/Denmark/56/03	13.3.03	<	320	320	—	320
A/Finland/294/03	22.3.03	<	160	320	160	640
A/Oslo/3389/03	20.4.03	<	160	160	160	640

1 . <,<40

Table 4. Amino acid differences characteristic of HA and NA sequences of H1N1 and H1N2 viruses

Variant group	Representative strain	Amino acid changes[1]	
		HA	NA
H1N1	A/Israel/2/03	V166A W252R	E332K N450D (V48I)
H1N2	A/Denmark/56/03	T89A V166A V175I A190T A215T	M24T[2] E199K K431N

1. Relative to A/New Caledonia/20/99 (H1N1)
2. Relative to A/Singapore/15/01 (H3N2)

Table 5. Antigenic analyses of influenza A H3N2 viruses (1)

Viruses	Isolation Date	Haemagglutination inhibition titre[1]						
		Post infection ferret sera						
		A/Pan 2007/99	A/NY 55/01	A/HK 1550/02	A/Egypt 130/02	A/Fuj 411/02	A/Send 4952/02	A/Fin 170/03
A/Panama/2007/99		**5120**	1280	5120	2560	80	160	80
A/New York/55/01		5120	**2560**	2560	5120	160	320	160
A/Hong Kong/1550/02		5120	2560	**5120**	2560	160	320	80
A/Egypt/130/02		1280	640	1280	**2560**	160	80	160
A/Fujian/411/02		80	40	80	80	**640**	640	320
A/Sendai/4952/02		320	320	640	320	320	**640**	320
A/Finland/170/03		160	80	160	160	640	640	**320**
A/Netherlands/369/02	30.12.02	2560	1280	2560	—	160	320	—
A/Austria/79807/03	—	2560	1280	1280	2560	80	—	80
A/Rome/3/03	Feb-03	1280	2560	1280	—	<	40	—
A/Prague/34/03	2.2.03	640	640	640	—	<	<	—
A/Slovakia/252/03	5.2.03	320	2560	320	2560	160	—	—
A/Latvia/1506/03	10.2.03	1280	2560	1280	2560	160	40	—
A/Perugia/7/03	10.2.03	1280	2560	1280	5120	40	—	<
A/Hannover/94/03	17.2.03	5120	5120	2560	—	40	80	—
A/Belgrade/1061/03	21.2.03	640	1280	—	2560	160	80	80
A/Geneva/3340/03	25.2.03	1280	2560	2560	2560	80	80	<
A/Greece/109/03	21.3.03	320	1280	320	640	40	40	—
A/Oslo/3391/03	1.5.03	1280	640	320	2560	160	—	40
A/Dakar/11/02	29.5.02	1280	640	—	2560	<	160	<
A/Lyon/476/03	5.2.03	320	320	160	—	160	160	—
A/Netherlands/17/03	16.2.03	320	320	640	320	640	—	640
A/Firenze/10/03	24.2.03	320	160	320	—	640	320	—
A/Berlin/35/03	27.2.03	320	320	320	—	640	1280	—
A/Parma/18/03	3.3.03	320	640	640	320	1280	—	640
A/Moscow/48/03	3.3.03	80	80	160	160	640	—	160
A/Geneva/3767/03	6.3.03	160	160	320	160	640	—	320
A/Dakar/10/03	10.3.03	160	80	—	160	320	640	160
A/St. Petersburg/122/03	11.3.03	160	320	320	320	320	—	320
A/Denmark/44/03	13.3.03	320	640	640	320	640	320	320
A/Stockholm/10/03	17.3.03	320	80	320	320	1280	—	640
A/Iceland/22/03	19.3.03	160	320	160	320	320	—	320
A/Finland/300/03	28.3.03	160	320	320	320	640	—	320
A/Barcelona/364/03	23.4.03	160	80	160	160	1280	—	320
A/Madagascar/69863/03	19.5.03	40	80	80	80	160	—	80
A/Israel/3650/03	3.6.03	160	320	160	640	640	—	320
A/Johannesburg/28/03	4.6.03	80	80	160	160	640	—	160
A/Hong Kong/1227/03	5.7.03	320	160	—	160	320	640	320

1 . <,<40

Table 6. Antigenic analyses of influenza A H3N2 viruses (2)

Viruses	Isolation Date	Haemagglutination inhibition titre[1]						
		Post infection ferret sera						
		A/Pan 2007/99	A/NY 55/01	A/Eg 130/02	A/Fuj 411/02	A/Fin 170/03	A/Wy 3/03	A/UK 1861/03
A/Panama/2007/99		**5120**	1280	2560	80	80	640	160
A/New York/55/01		5120	**2560**	5120	160	160	640	160
A/Egypt/130/02		1280	640	**2560**	160	160	320	80
A/Fujian/411/02		80	40	80	**640**	320	1280	320
A/Finland/170/03		160	80	160	640	**320**	1280	320
A/Wyoming/3/03		1280	320	320	2560	1280	**5120**	1280
A/UK/1861/03		320	80	160	160	80	640	**640**
A/Austria/77956/03	—	1280	2560	5120	320	80	320	80
A/Belgrade/1285/03	24.2.03	640	1280	2560	80	80	320	80
A/Goteborg/1/03	5.3.03	2560	1280	2560	80	80	320	80
A/Hannover/154/03	7.3.03	1280	320	1280	40	160	40	—
A/Albania/20/03	14.3.03	640	640	2560	40	40	40	40
A/Montpellier/1279/03	27.3.03	2560	2560	5120	160	160	640	320
A/Geneva/4841/03	2.4.03	320	160	1280	<	40	<	—
A/Lyon/1331/03	5.5.03	1280	2560	2560	160	320	320	—
A/Netherlands/222/03	—	320	80	80	1280	640	5120	640
A/Shanghai/369/03	—	320	80	80	160	320	1280	320
A/Oslo/2221/03	Feb-03	640	320	640	1280	640	2560	640
A/Singapore/55/03	10.2.03	1280	320	320	160	160	1280	320
A/Stockholm/9/03	17.3.03	320	160	320	640	320	2560	320
A/Indonesia/22/03	25.3.03	160	80	80	160	160	640	320
A/Geneva/4620/03	26.3.03	320	160	320	320	320	640	320
A/Lyon/1158/03	31.3.03	320	160	160	320	320	1280	640
A/Auckland/6/03	16.5.03	160	80	80	320	640	1280	320
A/Poitiers/1375/03	22.5.03	320	160	160	320	320	1280	640
A/Johannesburg/36/03	June-03	320	160	320	1280	640	2560	640
A/Madagascar/70074/03	13.6.03	160	160	320	320	320	1280	320
A/Israel/3'/03	19.6.03	320	320	640	640	640	2560	320
A/Hong Kong/1205/03	28.6.03	320	160	160	320	320	1280	640
A/Dakar/19/03	30.6.03	320	80	160	640	160	2560	320

1. <,<40

Table 7. Amino acid changes characteristic of H3N2 sequence variants

Variant group[1]	Representative strain	Amino acid changes	
		HA[2]	NA[3]
1b	A/Fujian/411/02	L25I R50G H75Q E83K A131T H155T Q156H S186G V202I W222R G225D	A18S L23F V30I C42F G143V G216V P267T V307I K385N
1c	A/New York/55/01	A106V N144D S186G	K172R T265I P267T S332F D399E Q432E L437W
	A/Latvia/1506/03	G49S[4] K158R Y233H N312K	
	A/Hannover/154/03	Y159N[4] W222R G225D V226I (G49D) (E62G) (V202I)	

1. Designation based on previously distinguished groups (Annual Report, 2001 and 2002); 2. Relative to A/Panama/2007/99; 3. Relative to A/Moscow/10/99; 4. Relative to A/New York/55/01.

Table 8. Antigenic analyses of influenza B viruses

Viruses	Isolation date	Haemagglutination inhibition titre[1]					
			Post infection ferret sera				
		B/Shan[2] 7/97	B/Shan 7/97	B/HK 335/01	B/Te 80/02	B/Bris 32/02	B/Sich 379/99
B/Shandong/7/97		**2560**	**640**	640	160	320	<
B/Hong Kong/335/01		2560	160	**640**	80	320	<
B/Tehran/80/02		2560	320	320	**320**	320	<
B/Brisbane/32/02		2560	320	640	160	**320**	<
B/Sichuan/379/99		<	<	<	<	<	**160**
B/Dakar/31/02	11.9.02	2560	640	640	320	—	<
B/Shizuoka/C1/02	Nov-02	2560	640	640	320	320	<
B/Madagascar/67860/02	12.11.02	1280	640	640	160	320	<
B/Lisbon/3/03	13.11.02	2560	320	640	160	—	<
B/Lyon/1436/02	20.11.02	1280	320	640	80	—	<
B/Paris/242/02	Dec-02	1280	160	320	40	—	<
B/Morocco/66/02	Dec-02	2560	320	320	320	320	<
B/Hong Kong/315/02	16.12.02	2560	320	320	320	320	<
B/England/450/02	20.12.02	2560	320	160	160	160	<
B/Madrid/G1334/02	26.12.02	2560	320	160	160	160	<
B/Egypt/213/03	5.1.03	2560	320	640	320	320	<
B/Ireland/363/03	10.1.03	5120	160	160	80	160	<
B/Indonesia/19/03	22.1.03	5120	640	640	320	320	<
B/Prague/7/03	27.1.03	640	320	320	40	160	<
B/Netherlands/1/03	28.1.03	2560	640	320	160	320	40
B/Voronezh/236/03	28.1.03	640	320	640	80	160	<
B/Trieste/1/03	Feb-03	5120	160	160	80	160	<
B/Rome/3/03	Feb-03	2560	640	640	320	160	<
B/Slovakia/238/03	4.2.03	5120	160	160	80	160	<
B/Belgrade/622/03	5.2.03	2560	640	320	40	80	<
B/Barcelona/215/03	6.2.03	5120	640	320	160	320	<
B/Valladolid/11/03	7.2.03	5120	320	160	80	320	<
B/Moscow/8/03	17.2.03	2560	320	640	80	320	<
B/Hungary/4/03	21.2.03	1280	320	640	160	160	<
B/Denmark/26/03	3.3.03	2560	640	640	160	320	<
B/Albania/6/03	6.3.03	1280	320	160	160	160	<
B/Turkey/15/03	17.3.03	2560	640	160	160	80	<
B/Lipetsk/17/03	18.3.03	2560	640	640	160	320	<
B/Latvia/3248/03	14.3.03	2560	160	320	80	320	<
B/Greece/116/03	26.3.03	1280	320	640	80	320	<
B/Oslo/3377/03	26.3.03	2560	640	320	80	80	<
B/Hannover/52/03	27.3.03	2560	640	640	320	320	<
B/Geneva/5079/03	Apr-03	2560	320	320	80	160	<
B/Israel/102/03	3.4.03	5120	640	320	40	—	<
B/Iceland/36/03	10.4.03	2560	320	320	80	160	<
B/Stockholm/2/03	10.4.03	320	320	80	160	80	<
B/Hong Kong/273/02	23.10.02	<	<	<	<	<	160
B/Austria/81287/03	—	40	<	<	<	<	160
B/Finland/231/03	29.1.03	<	<	<	<	<	80
B/Zagreb/4117/03	20.2.03	40	40	<	<	<	160
B/Bucharest/795/03	19.3.03	<	<	<	<	<	160
B/Malaysia/522/03	23.5.03	<	<	<	<	<	160
B/Townsville/1/03	3.7.03	40	<	<	<	<	160
B/Chanthaburi/218/03	27.7.03	<	<	<	<	<	160

1. <,<40; 2. hyperimmune sheep serum

Table 9. Amino acid changes characteristic of B sequence variants

Lineage	Representative strain	Amino acid changes	
		HA	**NA**
Sichuan[1]	B/Lyon/125/02	L58F/S N126D	E148G
Harbin[2]	B/Hong Kong/273/02	H40Y K129N D233A G256R	D329N A358E
Victoria[3]	B/Tehran/80/02	I121T	E148G[1] S198N T389A (R65H) (N199D)
	B/Hong Kong/330/01	H116R I121N D164E (K129E)	M50T C54S V60F E69K N345S R360K K436T (M369I)

1. Relative to B/Sichuan/379/99; 2. Relative to B/Hong Kong/557/00; 3. Relative to B/Shandong/7/97

Table 10. Antigenic analyses of swine H1N1 and H1N2 viruses

Viruses	Haemagglutination inhibition titre[1]									
			post infection ferret sera							
	A/Braz[2] 11/78	sw/Fin[2] 2899/82	A/Braz 11/78	Sw/Fin 2899/82	Sw/It 1513-1/98	Sw/CA 1482/99	Sw/IV 1455/99	Sw/Scot 410440/94	Sw/CA 790/97	Sw/CA 604/99
A/Brazil/11/78	**5120**	320	640	<	<	80	<	160	<	40
Sw/Finistere/2899/82	<	**5120**	<	**1280**	**160**	2560	160	<	<	<
Sw/Italy/1513-1/98	80	**5120**	<	**1280**	**640**	2560	320	<	<	<
Sw/CA/1482/99	160	5120	<	1280	640	**5120**	640	<	<	<
Sw/IV/1455/99	<	1280	160	160	<	320	**640**	<	<	<
Sw/Scotland/410440/94	5120	80	160	<	<	<	<	**5120**	640	160
Sw/CA/790/97	5120	160	320	<	<	<	<	320	**5120**	320
Sw/CA/604/99	5120	160	<	<	<	<	<	320	2560	**2560**
Sw/CA/1205/02	40	5120	<	640	160	2560	640	<	<	<
Sw/CA/1214/02	40	5120	<	1280	160	2560	1280	<	<	<
Sw/CA/1624/02	40	5120	<	1280	320	2560	1280	<	<	<
Sw/Switzerland/2258/02	<	5120	<	1280	160	2560	1280	<	<	<
Sw/Switzerland/A5301/03	<	5120	<	1280	320	2560	1280	<	40	<
Sw/Italy/267424/02	40	5120	<	640	160	1280	1280	<	<	<
Sw/Italy/4230/02	<	5120	<	320	160	1280	640	<	<	<
Sw/Italy/73226/03	<	5120	<	640	160	2560	1280	<	<	<
Sw/Italy/289171/03	<	1280	<	160	80	320	640	<	<	<
Sw/CA/1203/02	5120	40	40	40	<	<	<	160	640	160
Sw/CA/1428/02	5120	80	80	80	<	<	<	320	1280	320
Sw/CA/1623/02	5120	80	80	80	<	<	<	320	1280	160
Sw/Italy/110093/02	5120	<	160	<	<	<	<	160	640	80
Sw/Italy/4675/03	5120	40	160	<	<	<	<	160	640	160
Sw/Italy/11271/03	5120	80	160	<	<	<	<	320	1280	160

1. <,<40; 2. hyperimmune rabbit serum

Table 11. Antigenic analyses of swine H3N2 viruses

Viruses	Haemagglutination inhibition titre[1]				
	Post infection ferret sera				
	Sw/Fin 3633/84	Sw/It 1407-2/95	Sw/It 1477/96	Sw/Eire 471/96	Sw/Italy 1523/98
Sw/Finistere/3633/84	**2560**	<	160	640	160
Sw/Italy/1407-2/95	<	**1280**	<	<	<
Sw/Italy/1477/96	160	<	**5120**	80	320
Sw/Eire/471/96	640	<	160	**1280**	160
Sw/Italy/1523/98	320	<	1280	80	**1280**
Sw/Italy/172368/01	160	<	640	80	640
Sw/Italy/8304/02	160	<	640	80	640
Sw/Italy/275734/02	320	<	2560	160	1280
Sw/Italy/274838/02	320	<	1280	160	1280
Sw/Italy/245950-2/02	320	<	2560	160	1280
Sw/Italy/250793/02	320	<	1280	160	1280
Sw/Italy/8291/02	160	<	640	80	640
Sw/Italy/65838-1/03	160	<	640	80	640

1. <,<40

Table 12. Viruses despatched August 2002 - July 2003

Country	A	B	Swine	Equine	Avian
Australia	7	4			
Austria	5	3			
Czech Republic	4	3			
Denmark	3	2			
Finland	2				2
France	1	3			
Germany	1				
Hungary		1			
Ireland	5	5			
Italy			5	1	
Japan	7	4			2
Netherlands	3				
Poland		1			
Portugal					1
Russia	2	2			
Switzerland	5	2	6		
United Kingdom	12	11	1		2
United States	9	4			
Total = 131	**66**	**45**	**12**	**1**	**7**

Table 13. Antisera despatched August 2002 - July 2003

Country	A	B	Swine	Avian
Australia	2			
Austria	5	3		
Czech Republic	4	3		
Denmark	3	2		
France		3		
Germany	1			
Italy			7	
Japan	2			1
Netherlands	3			
Portugal				1
Switzerland	7	2	7	
United Kingdom	5	6		1
United States	5			
Total = 73	**37**	**19**	**14**	**3**

Figure 1. Phylogenetic comparison of nucleotide sequences encoding H1 haemagglutinins

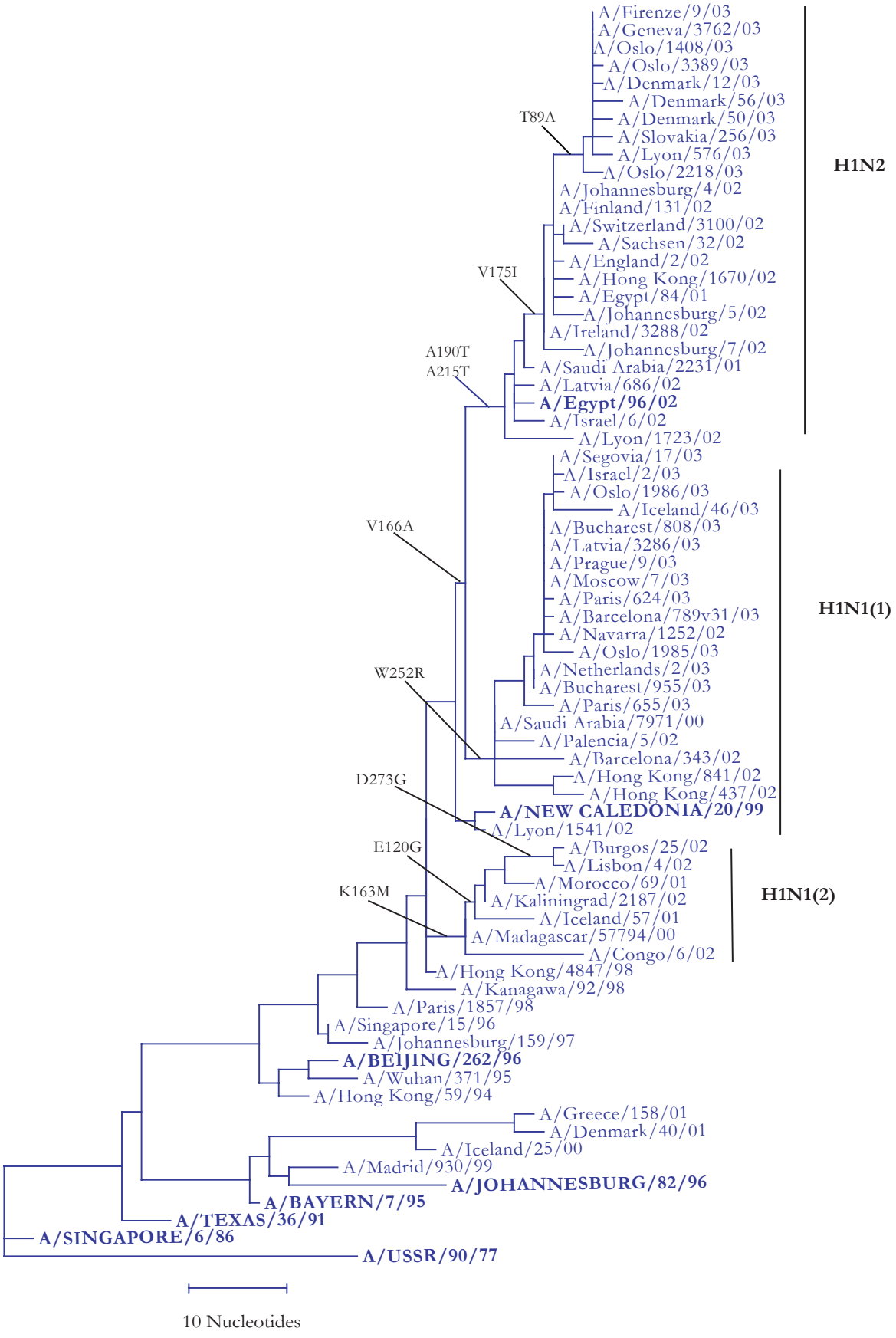


Figure 2. HA sequences (amino acids 1-300) of H1N1 and H2N2 viruses

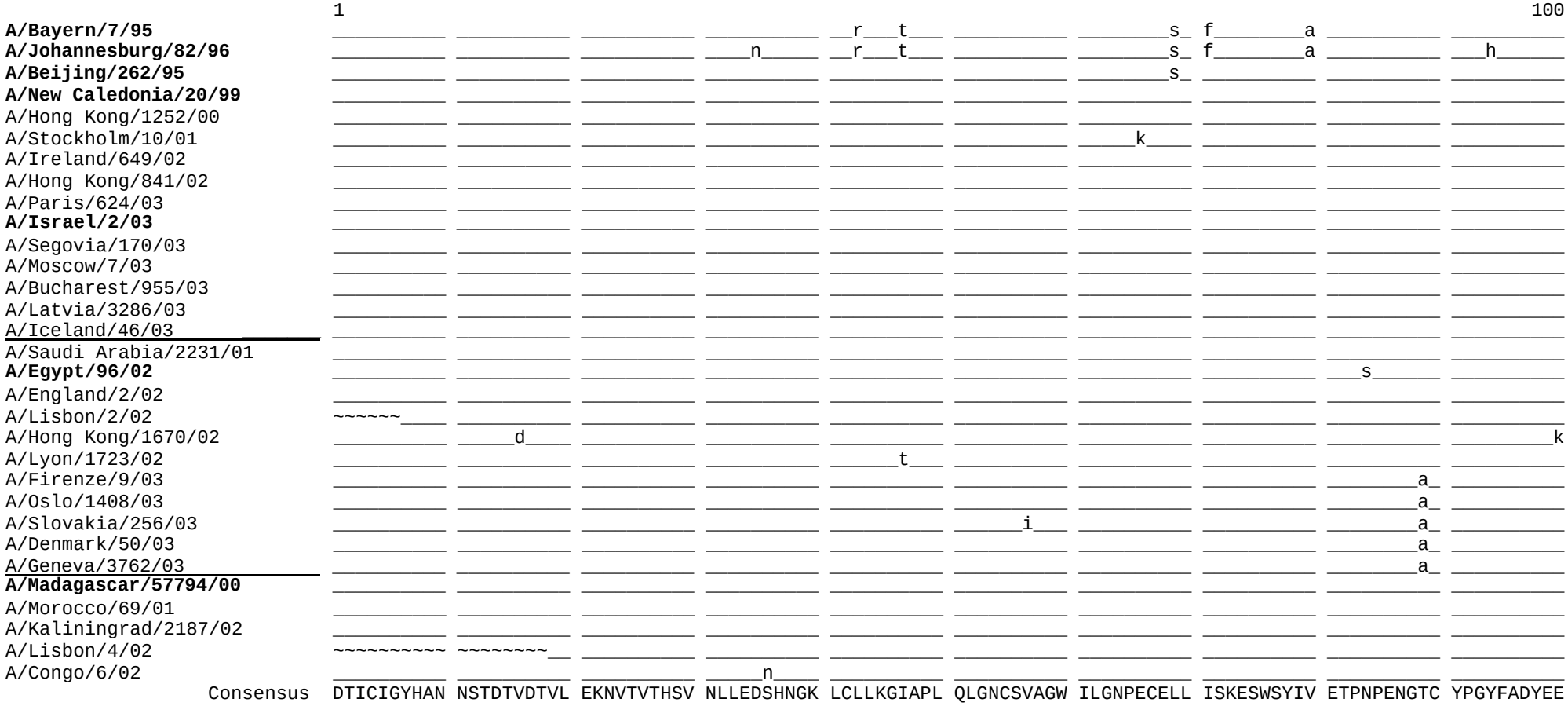


Figure 2 (continued)

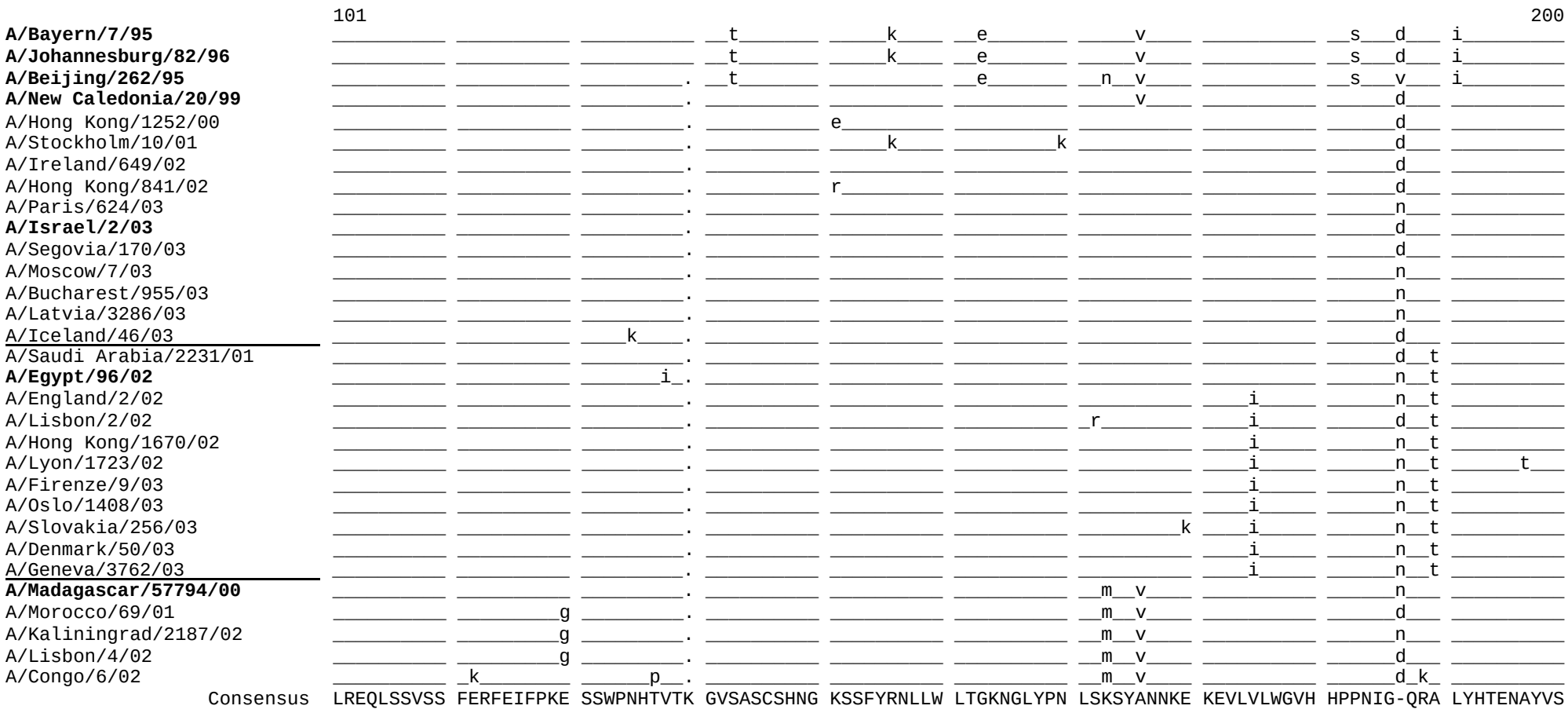


Figure 2 (continued)

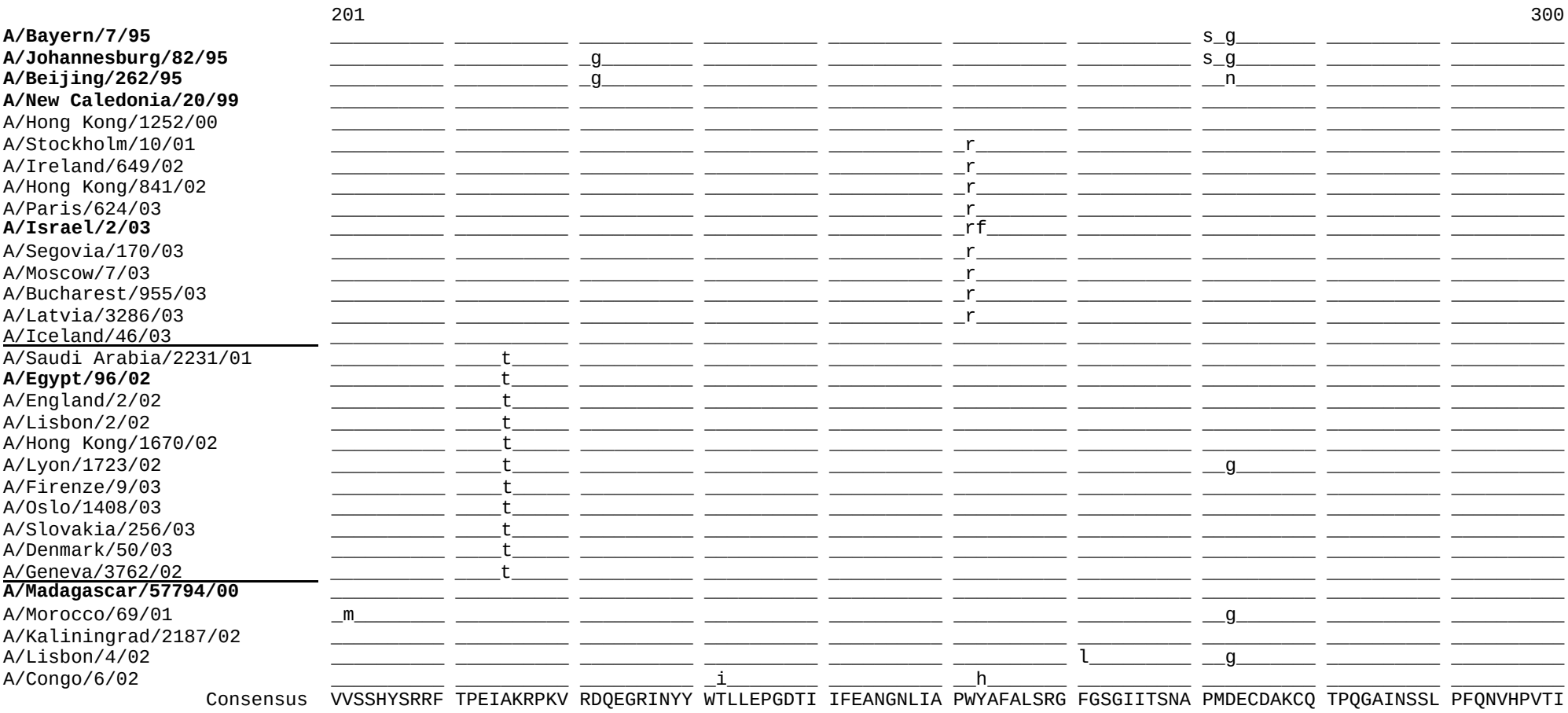


Figure 3. Phylogenetic comparison of nucleotide sequences encoding N1 neuraminidases

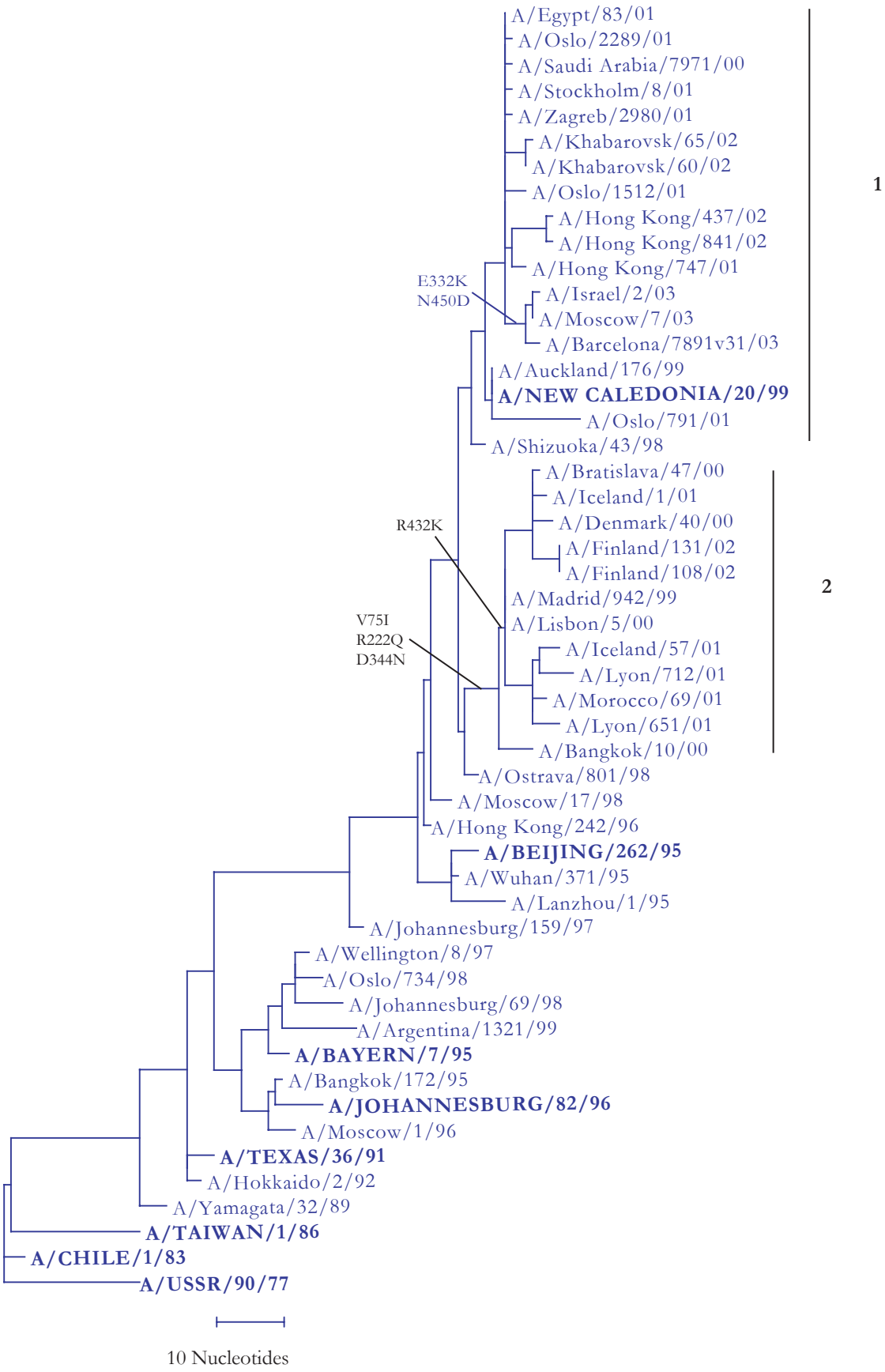


Figure 4. NA sequences of H1N1 viruses

Figure 4 (Continued)

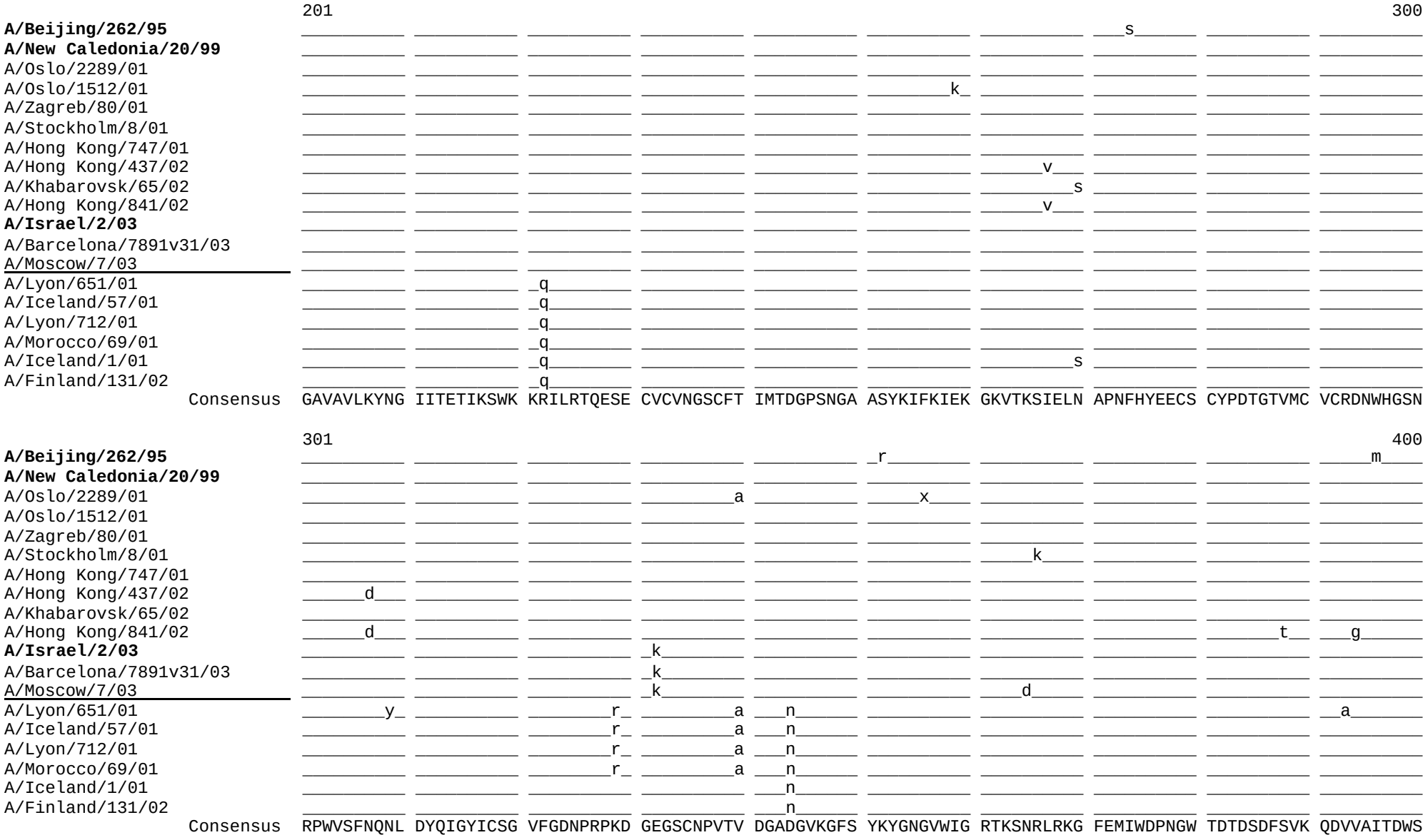


Figure 4 (Continued)

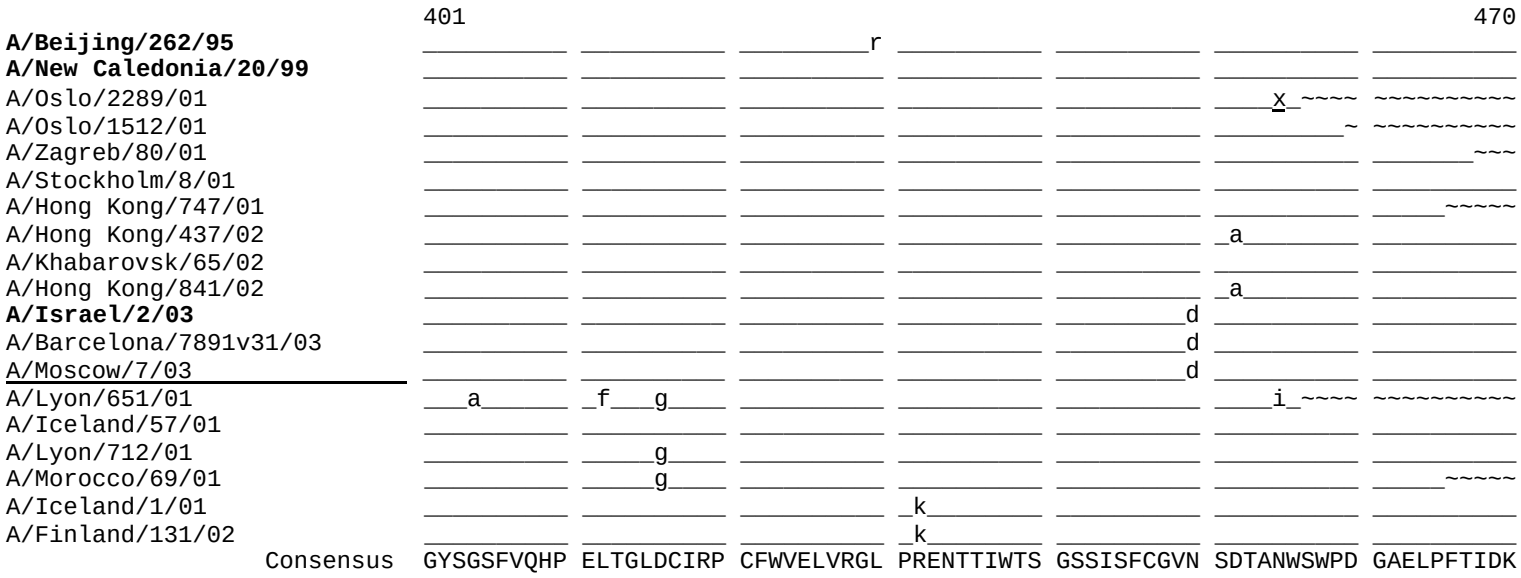


Figure 5. Phylogenetic comparison of nucleotide sequences encoding H3 haemagglutinins

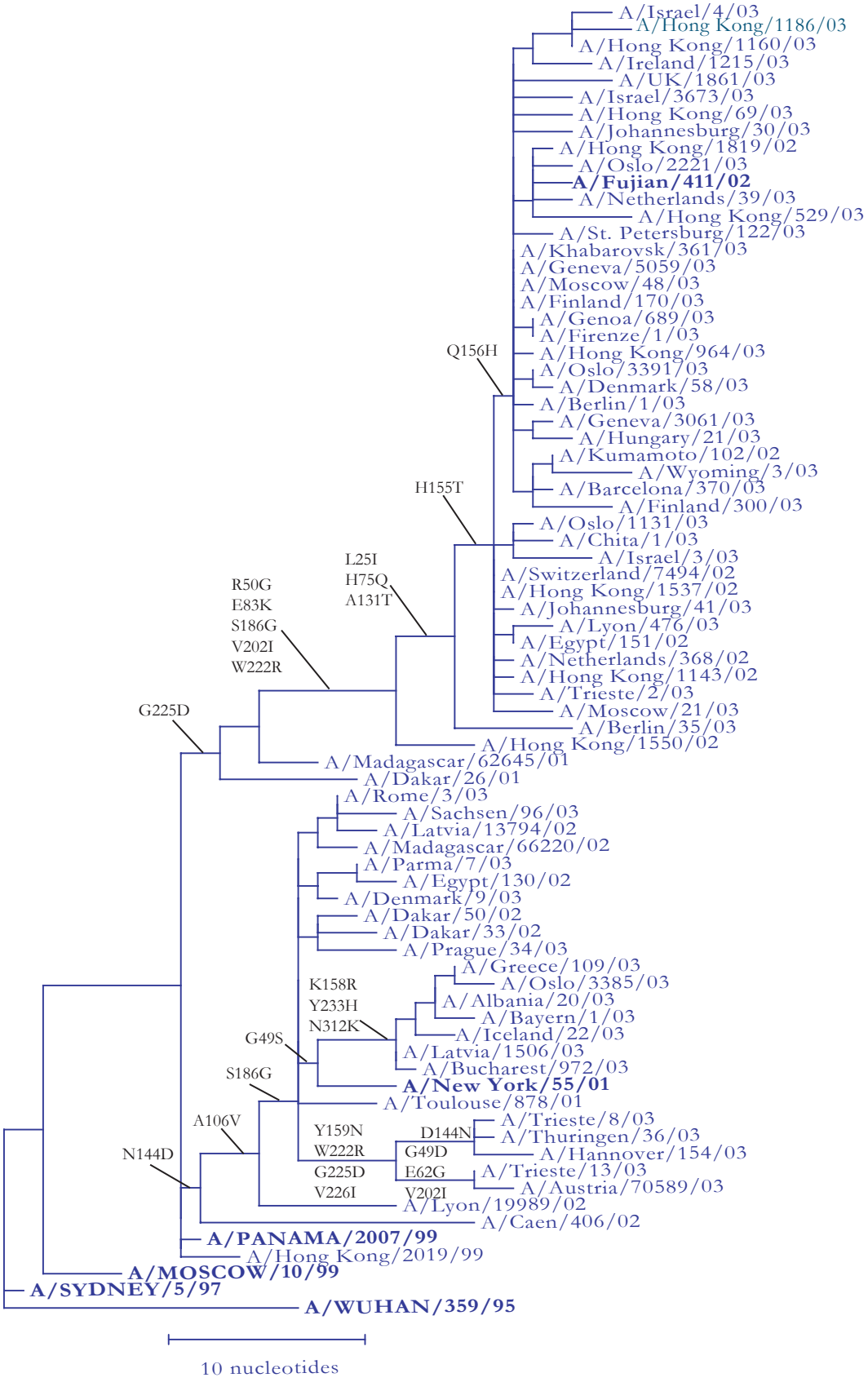


Figure 6. HA sequences (amino acids 1-300) of H3N2 viruses

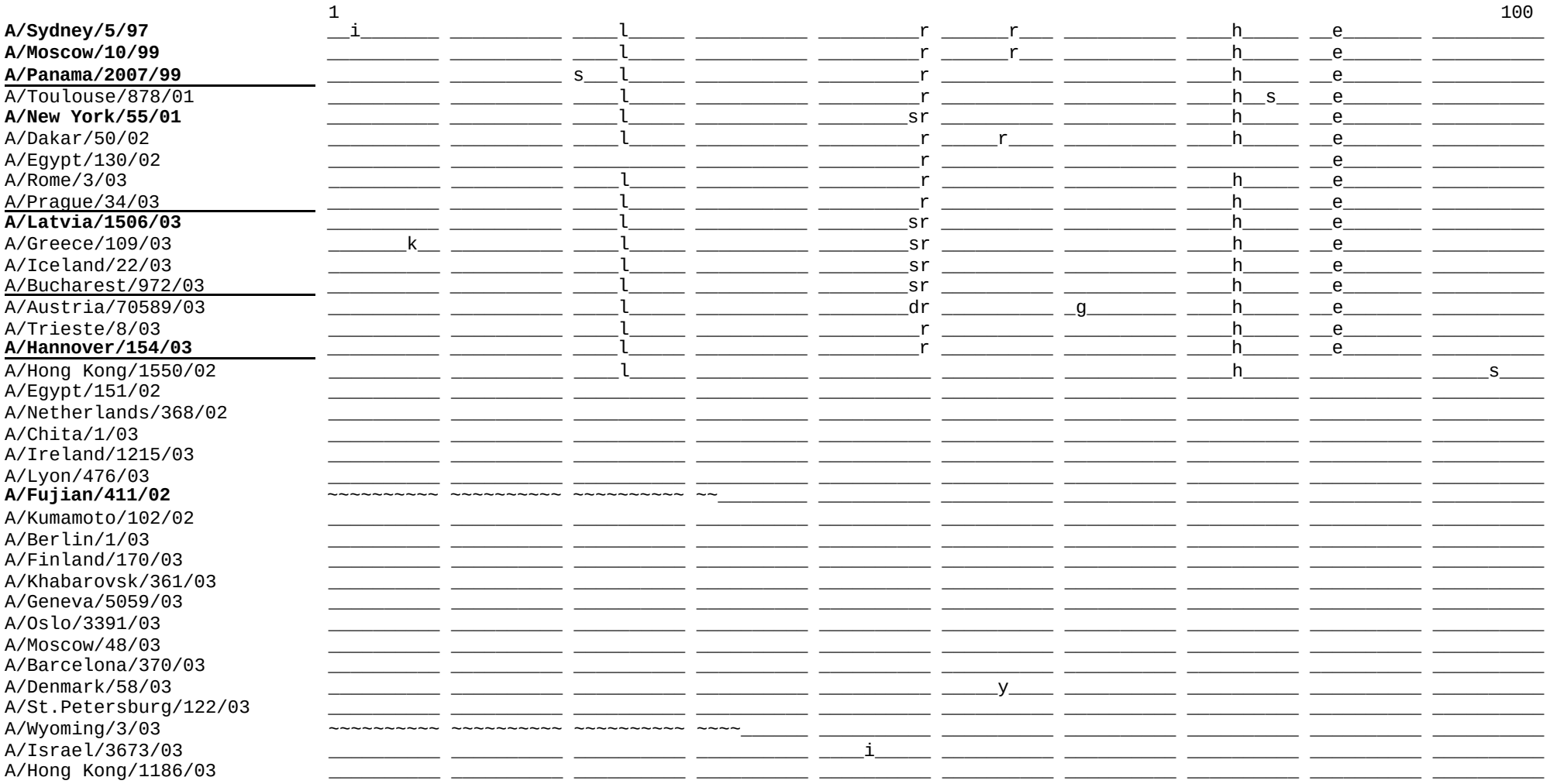


Figure 6 (continued)

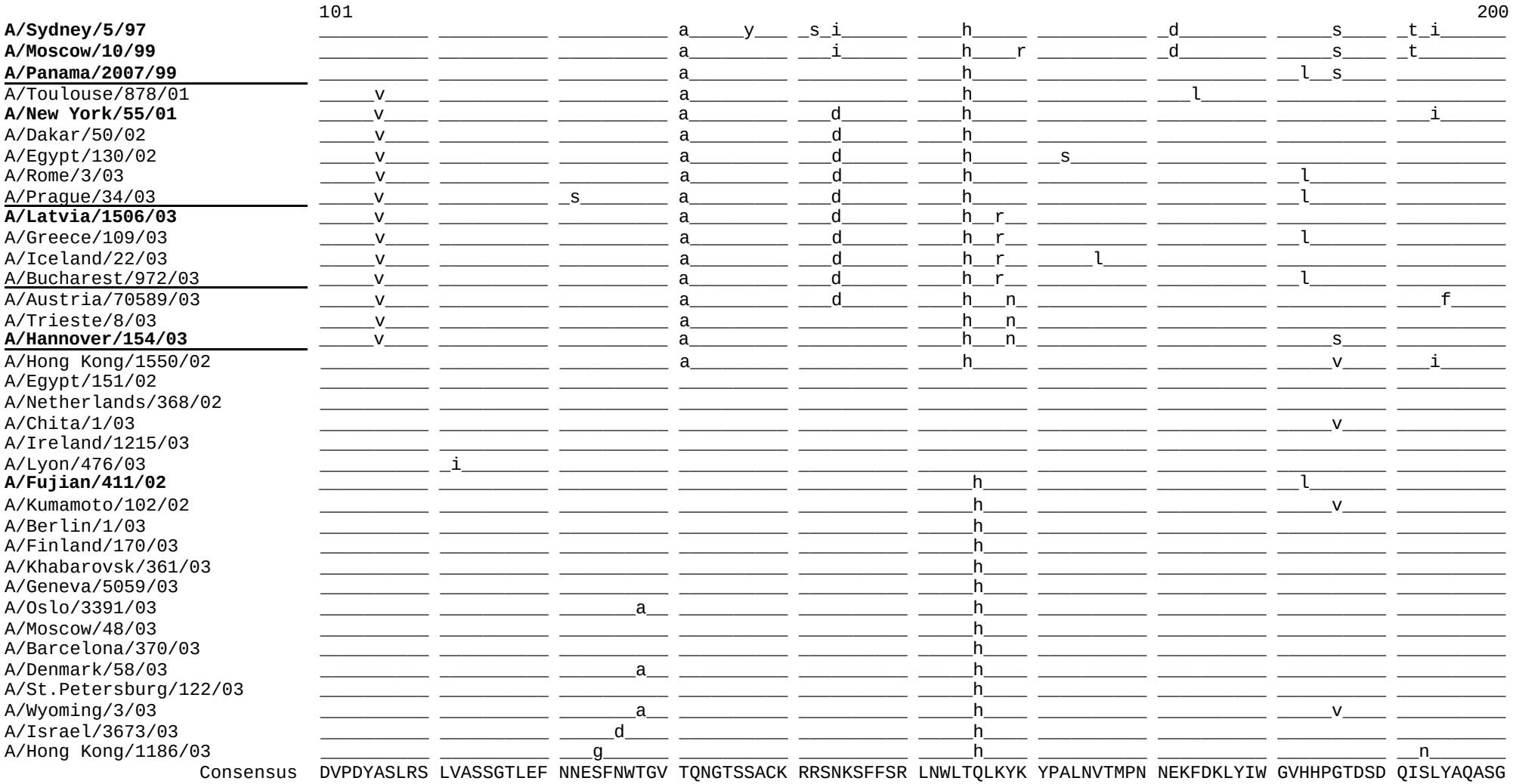


Figure 6 (continued)

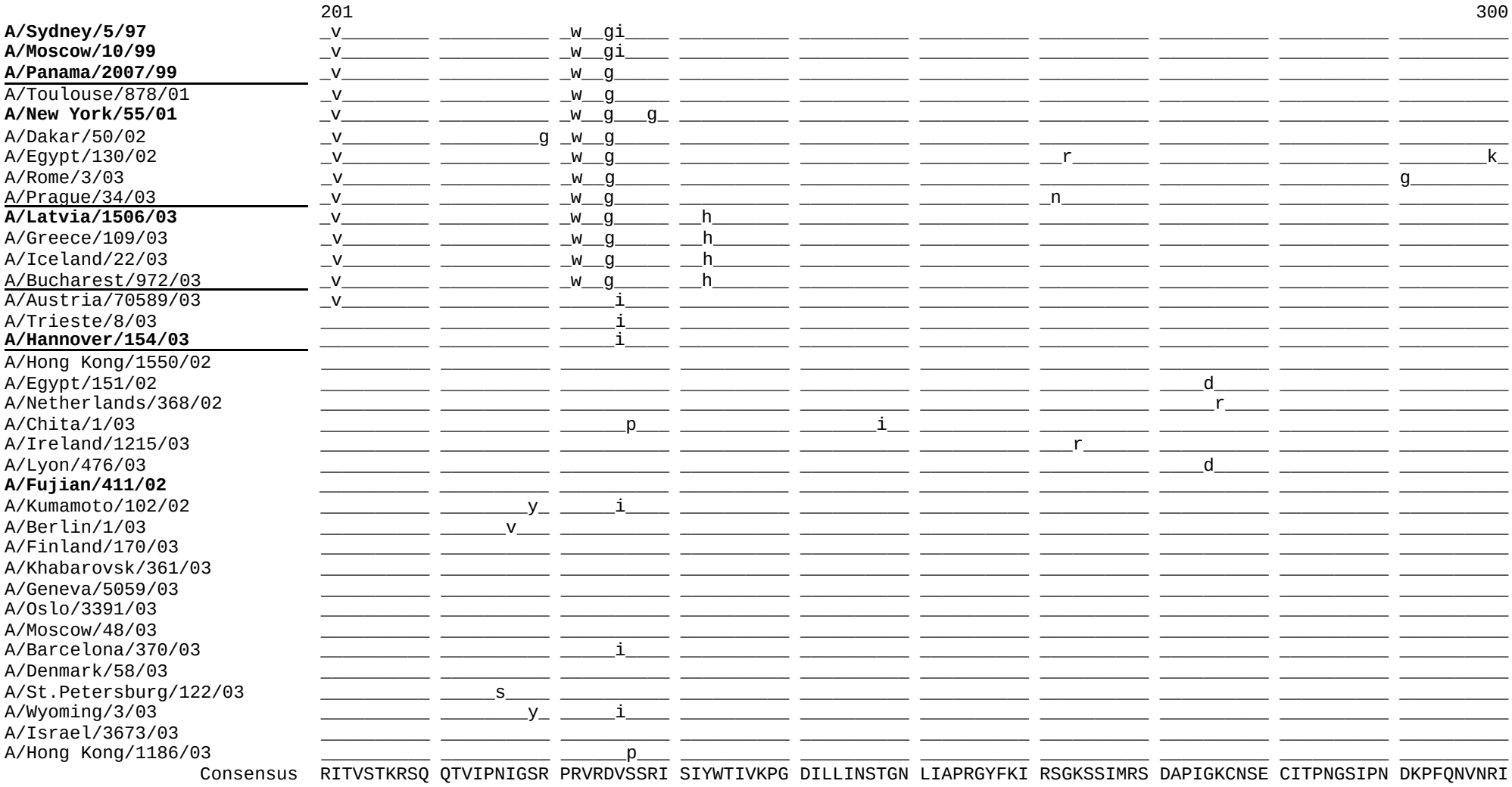


Figure 7. Phylogenetic comparison of nucleotide sequences encoding N2 neuraminidases

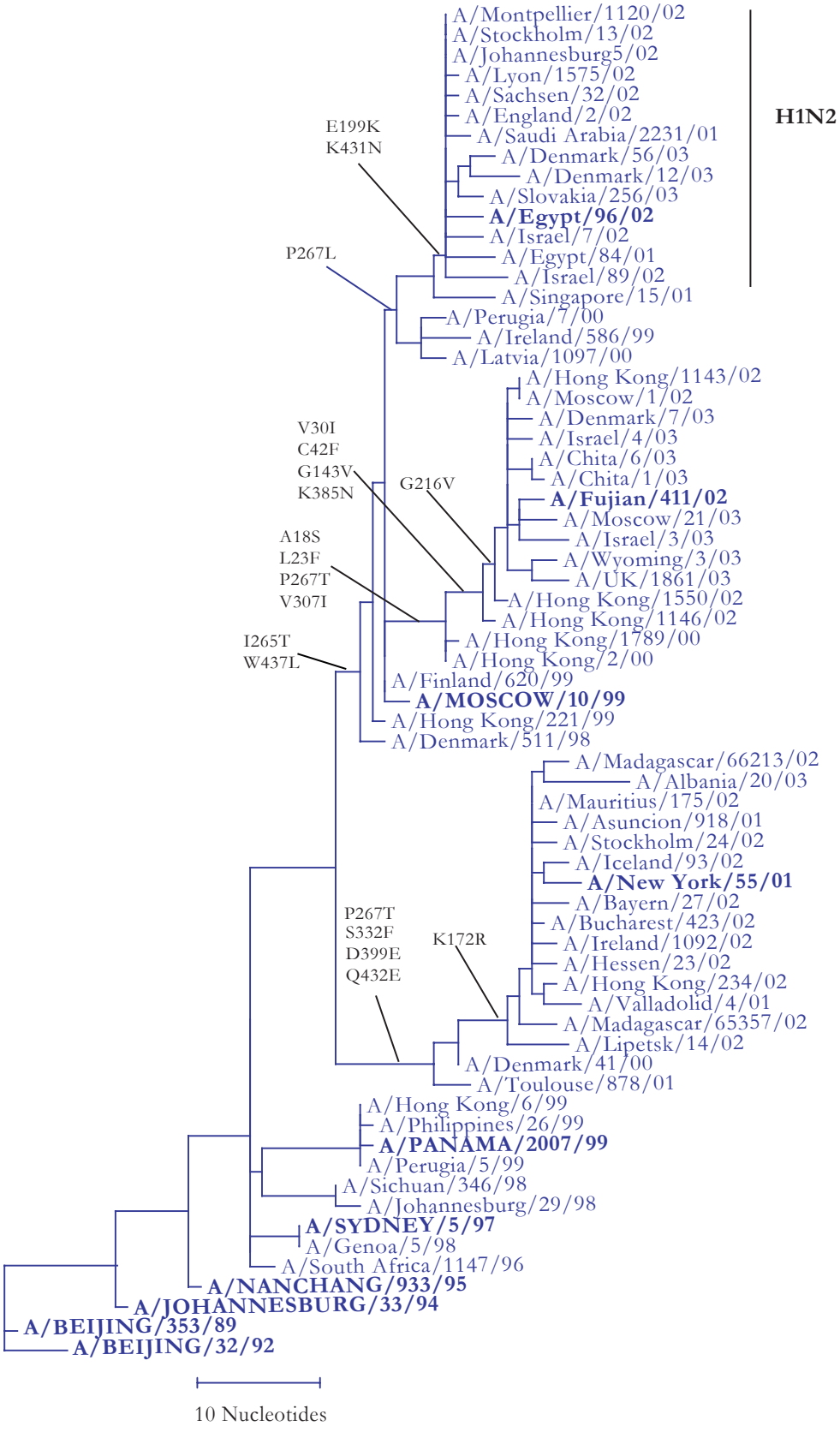


Figure 8. NA sequences of H3N2 and H1N2 viruses

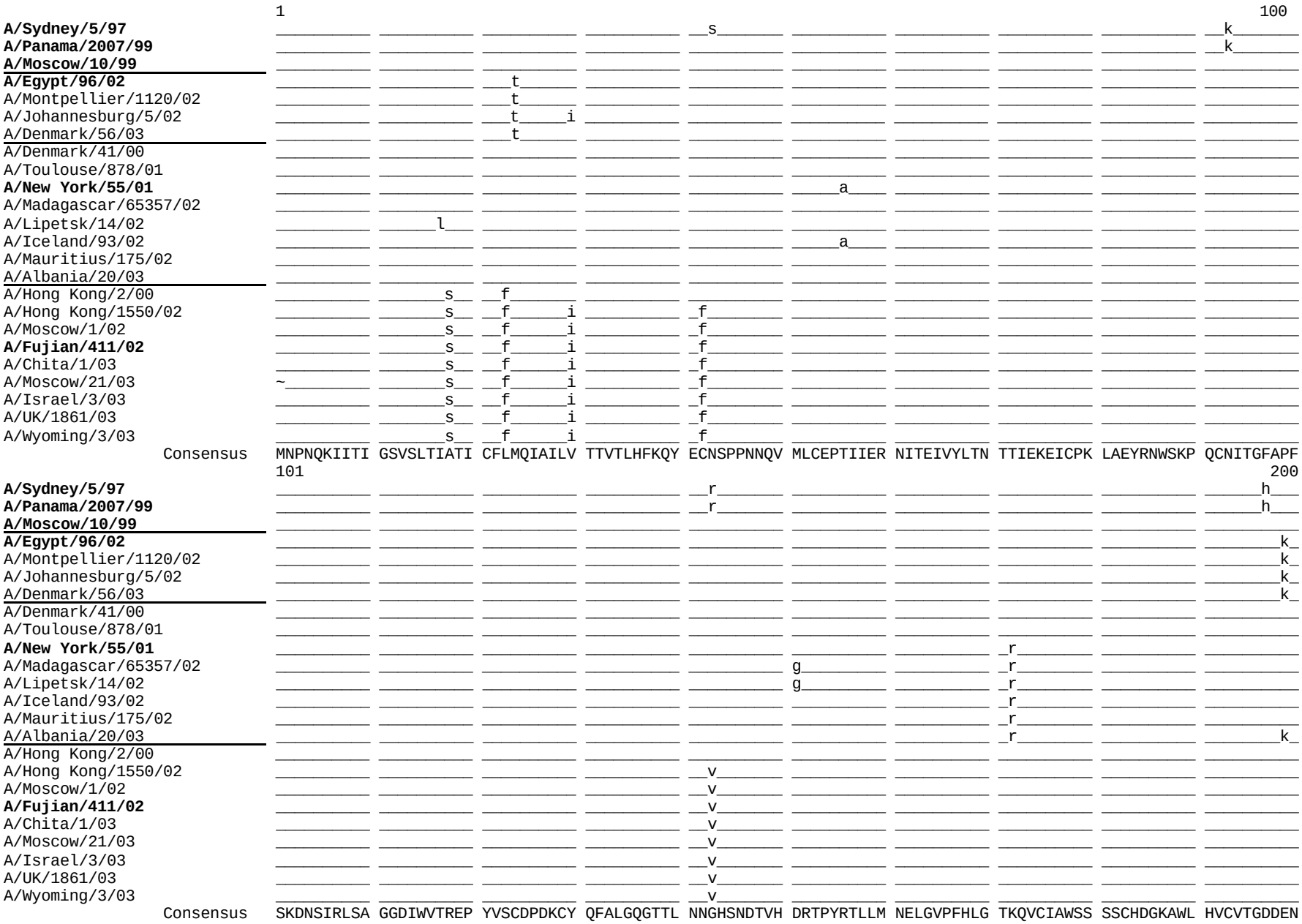


Figure 8 (Continued)

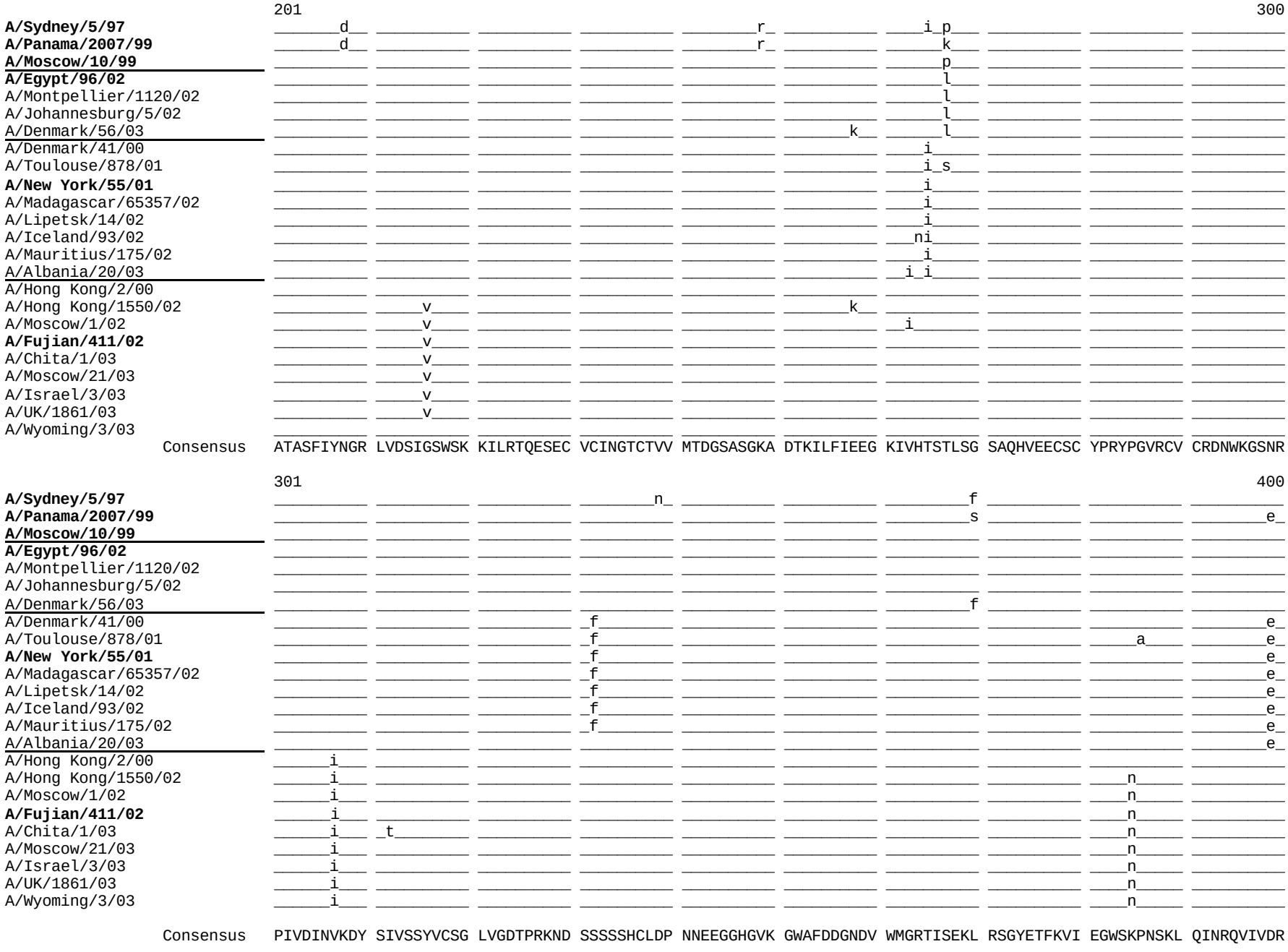


Figure 8 (Continued)

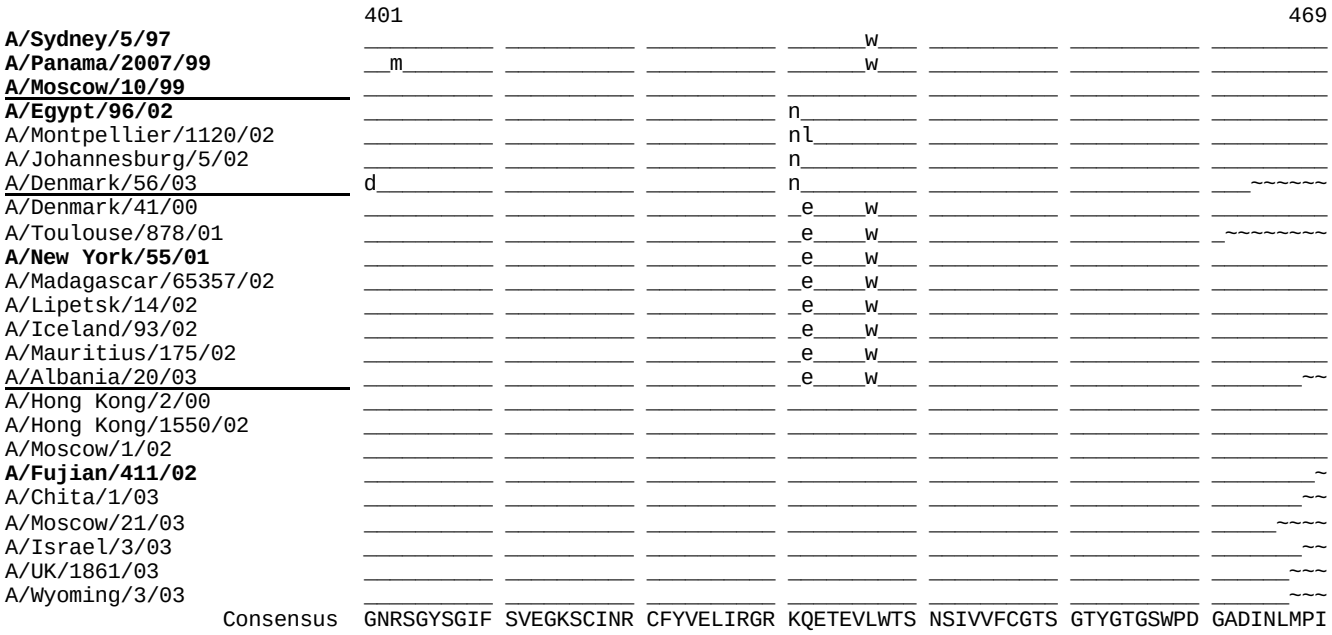


Figure 9. Phylogenetic comparison of nucleotide sequences encoding B haemagglutinins

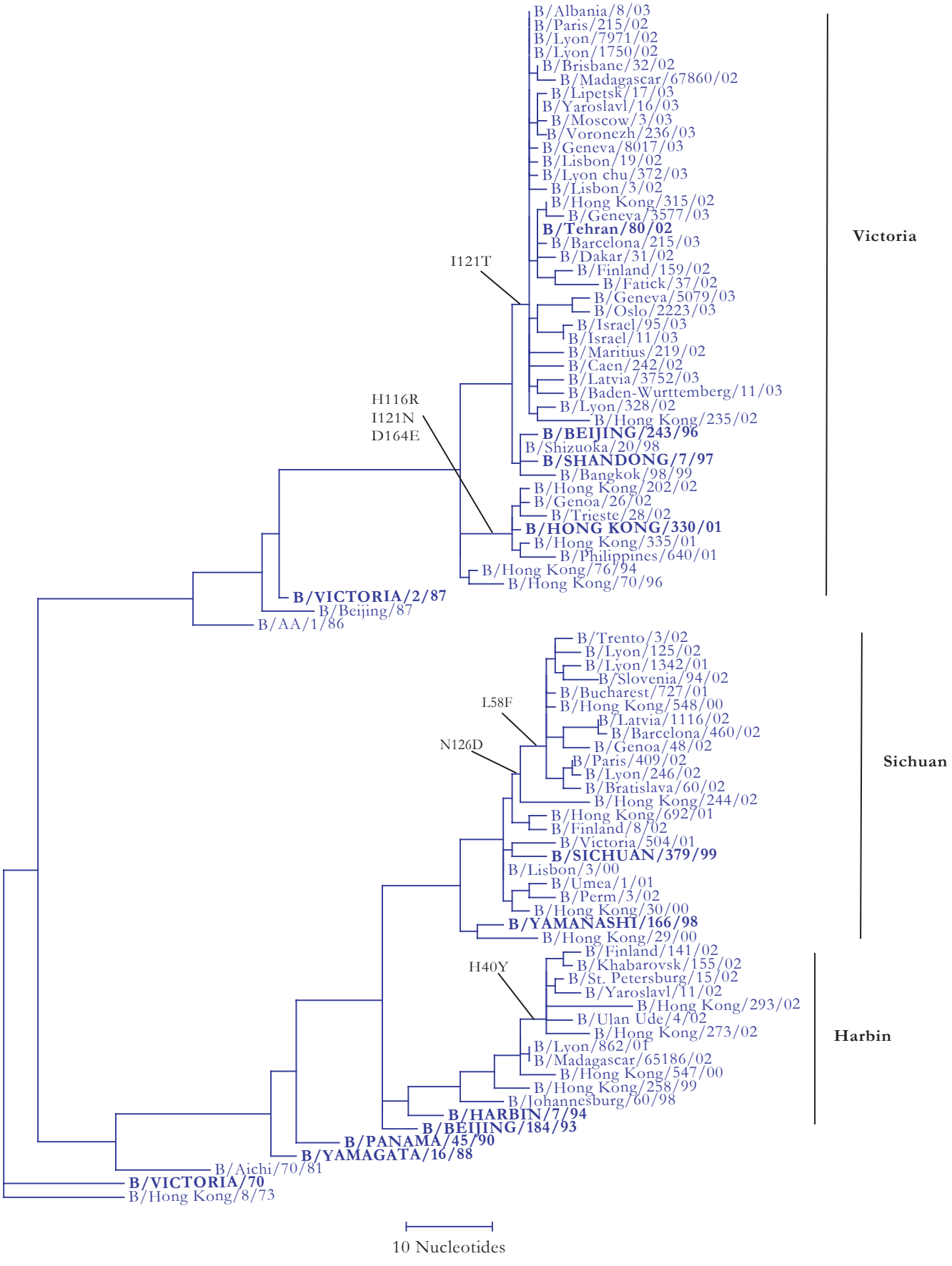


Figure 10. HA sequences (amino acids 1-300) of B viruses

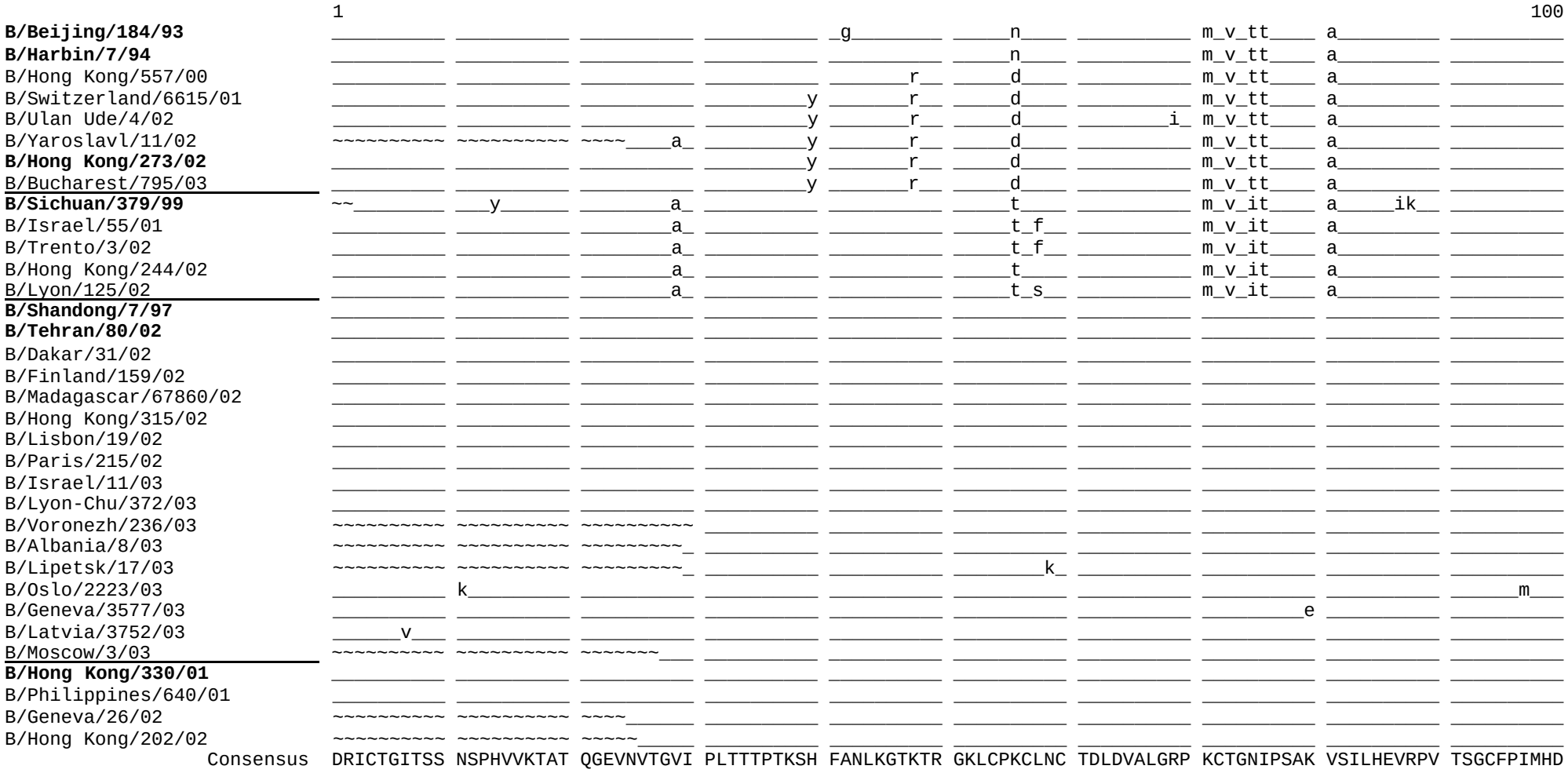


Figure 10 (Continued)

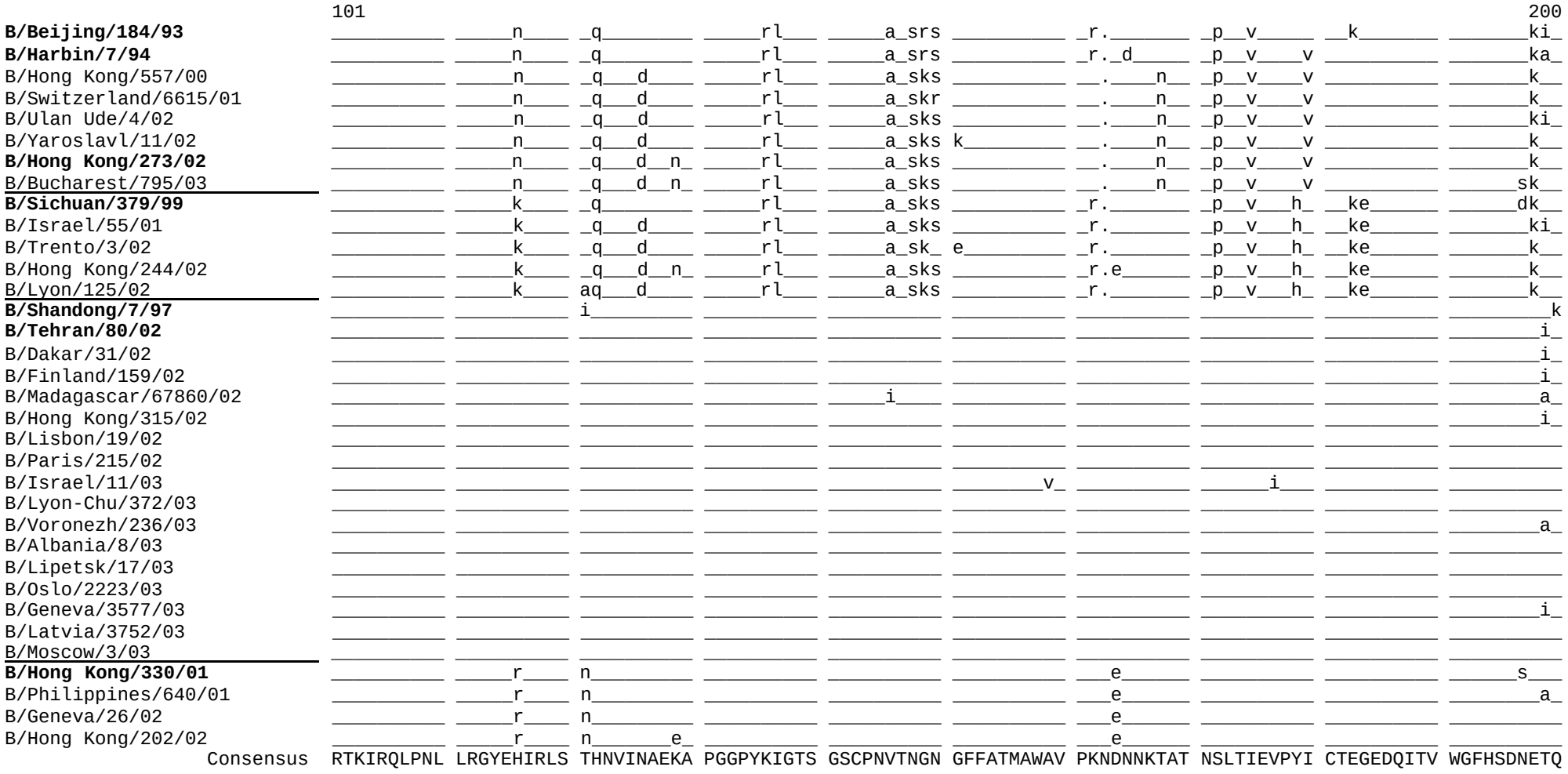


Figure 10 (Continued)

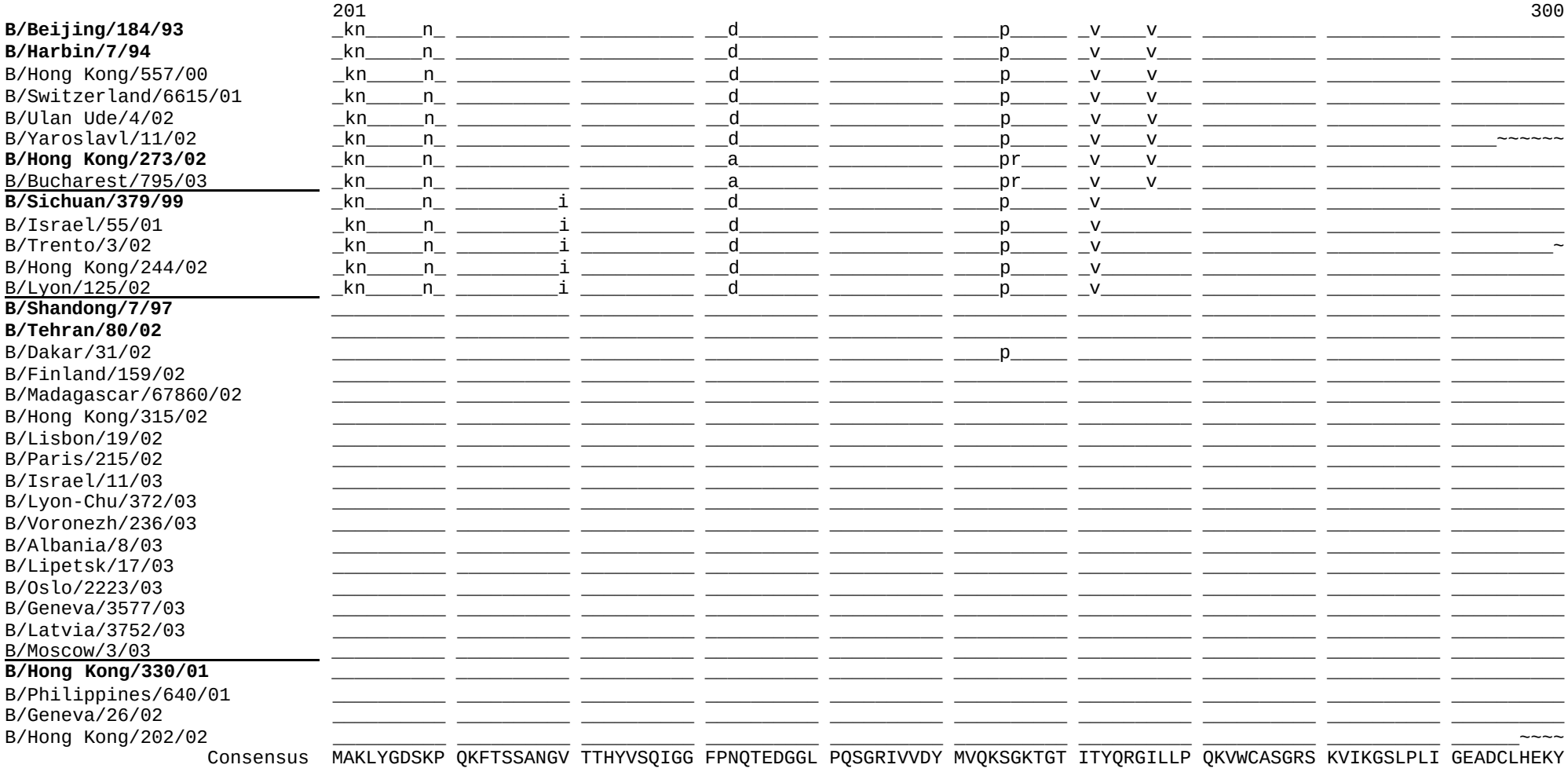
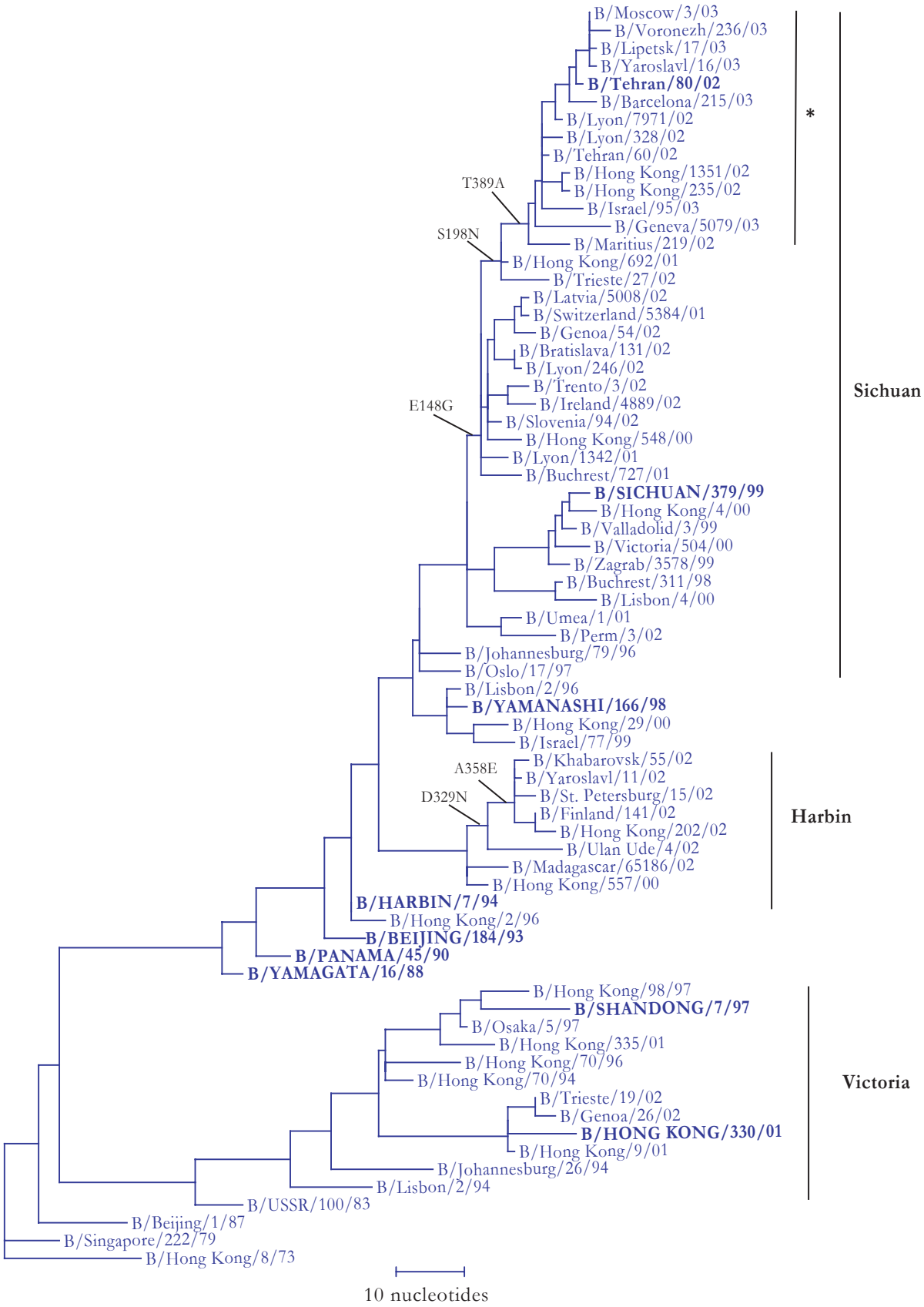
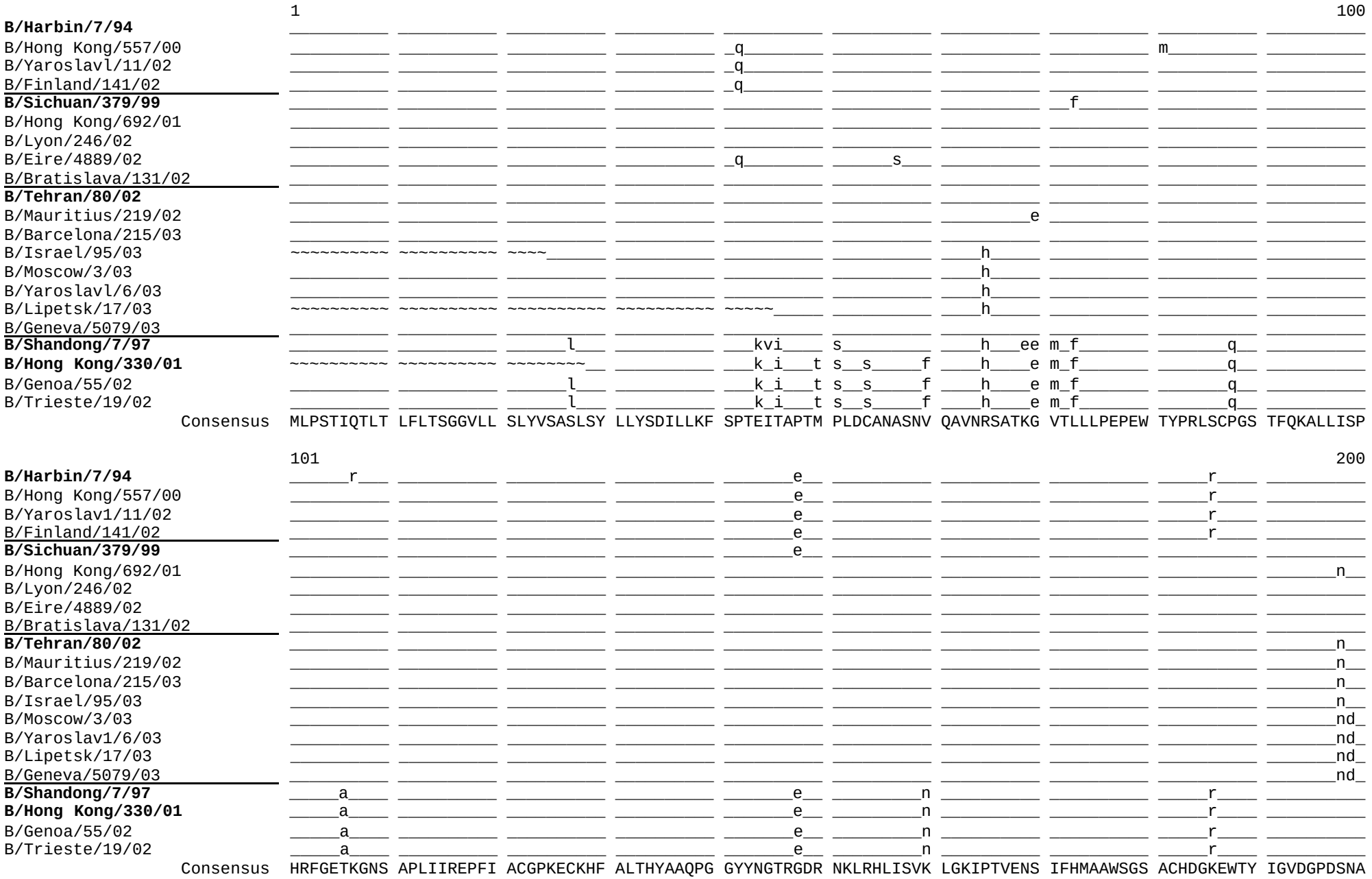


Figure 11. Phylogenetic comparison of nucleotide sequences encoding B neuraminidases



*** Victoria/Sichuan reassortants**

Figure 12. NA sequences of B viruses



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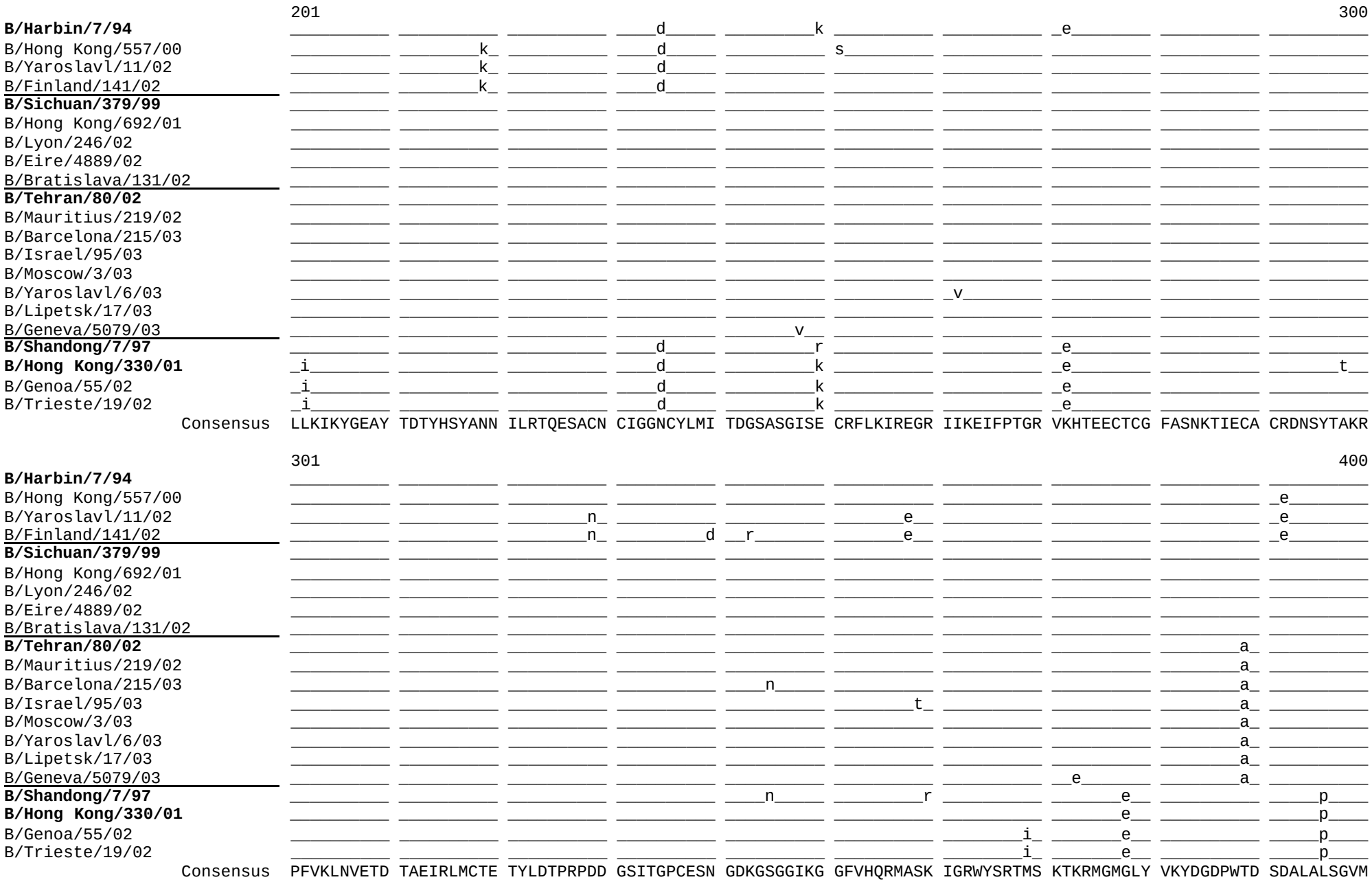

Figure 12 (continued)