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Exploration of the emergence of the Victoria lineage of influenza B virus

Brief Report

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Summary. The Victoria lineage represented by B/Victoria/2/87 is one of the two major distinctive haemagglutinin (HA) lineages of influenza B virus, and its recent re-emergence has aroused great concerns. However, it remains unknown when, where, and how this HA lineage emerged in the world. In this study, the HA1 domain of the HA gene of fourteen influenza B viruses isolated in China in 1972–1984 was sequenced. The sequences were phylogenetically analyzed with the HA1 sequences of 41 other important influenza B isolates. The results unveiled some earlier footprints of the Victoria lineage in China, and the epidemic history of the Victoria lineage could be traced back from the year 1985 to 1975. Moreover, phylogenetic analysis, the history of China, and the epidemiology of influenza B virus indicated that the Victoria lineage possibly emerged in China in the 1970s through gradual evolution from a minor lineage.

*

The Victoria lineage represented by B/Victoria/2/87 is one of the two major distinctive haemagglutinin (HA) lineages of influenza B virus, the other being the Yamagata lineage represented by B/Yamagata/16/88 [8, 11]. The Victoria lineage circulated widely in the world in the 1980s and re-appeared globally in recent years [6, 8–15], and this has aroused great concerns. Consequently, a strain of the Victoria lineage, B/Malaysia/2506/2004, was recommended to be included in influenza vaccine for the year 2006–2007 (www.flu.lanl.gov/vaccine/).

It seemed that the Victoria lineage emerged suddenly and widely in the world, as the earliest strains of the Victoria lineage reported previously, B/Canada/3/85,

B/Finland/24/85, B/Ibaraki/2/85 and B/Victoria/3/85, were all isolated in 1985 from North America, Europe, Asia, and Australia, respectively [6, 8–15, 17]. These four strains were all distinct from their contemporaries of the Yamagata lineage [8, 13]. Therefore, it is still unknown when, where, and how the Victoria lineage emerged in the world, which is significant for understanding the evolution of influenza B viruses.

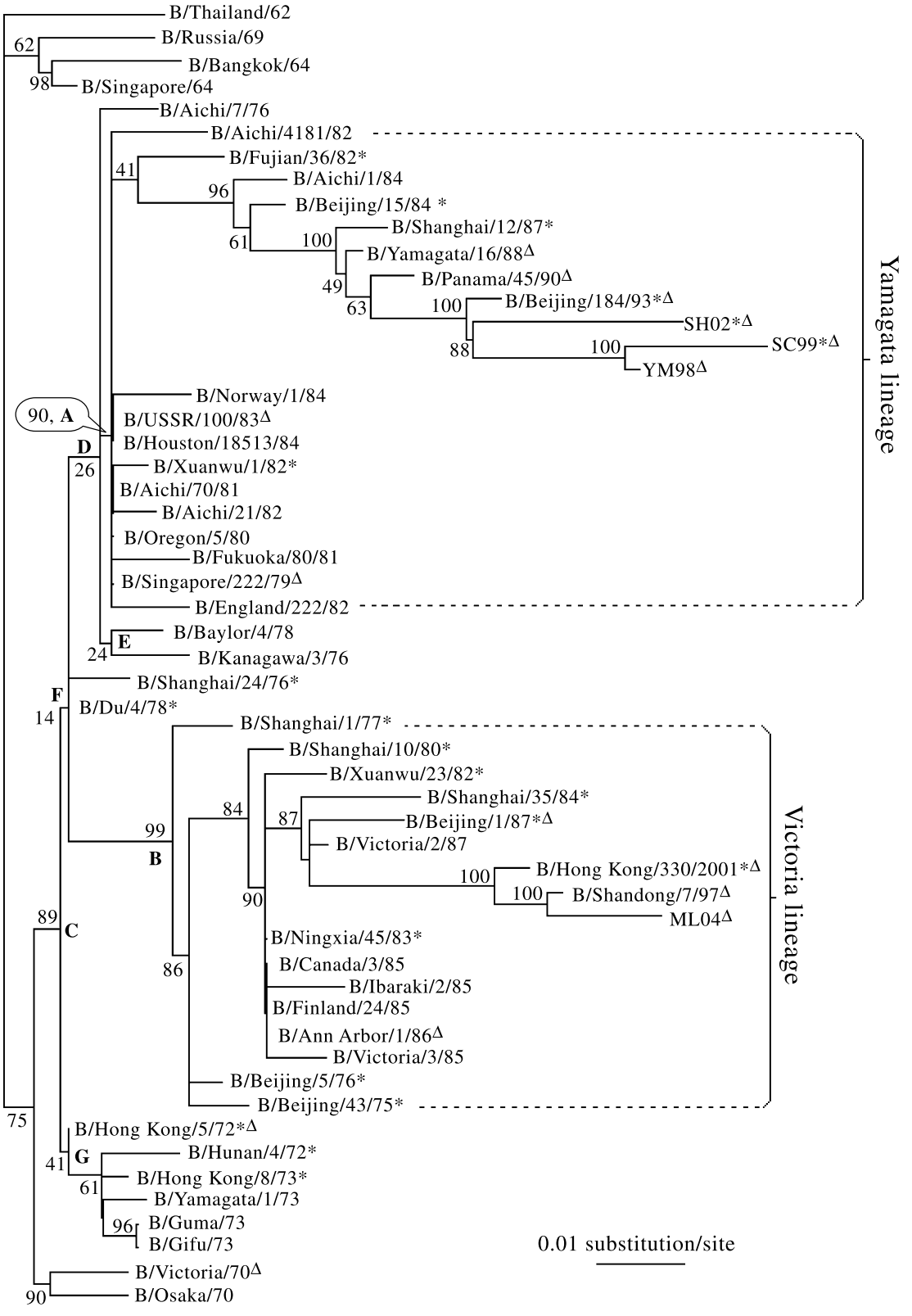
The molecular basis of the divergence of the two HA lineage is the evolution of the HA1 domain in the viral HA gene [6, 8–15, 17]. The HA1 domain, 344–347 aa long and highly variable, encodes protective antigenic epitopes [18]. Through the linear regression of the divergence time and the number of observed substitutions in HA1 sequences between the two HA lineages, the Victoria lineage possibly emerged in 1969 [8]. However, since multiple substitutions could occur at the same position, the observed substitution number is in curvilinear rather than linear relation to the divergence time [7], and therefore the Victoria lineage possibly began its emergence after the year 1969.

The circulation of the Victoria lineage was likely confined to China for nearly 10 years in the 1990s [1, 15]. This indicates that its roots in China were probably deeper than in other regions. Therefore, we suspected that the Victoria lineage possibly emerged in 1970s in China. However, little was known about the real circulation of influenza B viruses in China before the year 1985.

To address these issues, we sequenced the HA1 domain of the following fourteen influenza B strains isolated in 1972–1984 in China: B/Hong Kong/5/72, B/Hunan/4/72, B/Beijing/43/75, B/Beijing/5/76, B/Shanghai/24/76, B/Shanghai/1/77, B/Du/4/78, B/Shanghai/10/80, B/Fujian/36/82, B/Xuanwu/1/82, B/Xuanwu/23/82, B/Ningxia/45/83, B/Beijing/15/84 and B/Shanghai/35/84 according to the method reported previously [1, 11]. The GenBank accession numbers of their HA1 domain sequences are AF299369–AF299382.

For phylogenetic analysis in this study, HA1 sequences of 41 other influenza B strains were also selected from the Influenza Sequence Database (www.flu.lanl.gov). Their accession numbers in the database are AB027386–AB027394, AB027407, AB027495, AF050061, AF101066–AF101071, AF299384, AF319590, AY504610, ISDN38226, ISDN126672, ISDNYAM98, K02713, L19646, M10298, M18384, M21874, M22943, M22944, M22946, M36105–M36107, M58418, M58420, M65171, U70384, X00897, X13552 and X13553. They included all of the 38 strains isolated from 1960 to 1985 with HA1 sequences reported to Influenza Sequence Database and all fifteen global representative

Fig. 1. The consensus phylogenetic tree of 55 influenza B strains, based on their HA1 nucleotide sequences, constructed with software of PHYLIP 3.62 using the maximum likelihood method. B/Thailand/62 was selected as the outgroup. "*" and "Δ" indicate Chinese strains and the global representative strains, respectively. Numbers near some nodes indicate percent bootstrap support based on 500 replicates. B/Yamagata/166/98, B/Sichuan/379/99, B/Shanghai/361/2002 and B/Malaysia/2506/2004 are abbreviated as YM98, SC99, SH02 and ML04, respectively. A distance bar is shown in the lower right corner


B/Thailand/62
B/Russia/69
B/Bangkok/64
B/Singapore/64
B/Aichi/7/76
B/Aichi/4181/82
B/Fujian/36/82*
B/Aichi/1/84
B/Beijing/15/84 *
B/Shanghai/12/87*
B/Yamagata/16/88Δ
B/Panama/45/90Δ
B/Beijing/184/93*Δ
SH02*Δ
SC99*Δ
YM98Δ
Yamagata lineage
90, A
B/Norway/1/84
B/USSR/100/83Δ
B/Houston/18513/84
D
B/Xuanwu/1/82*
B/Aichi/70/81
B/Aichi/21/82
B/Oregon/5/80
B/Fukuoka/80/81
B/Singapore/222/79Δ
B/England/222/82
B/Baylor/4/78
E
B/Kanagawa/3/76
B/Shanghai/24/76*
F
B/Du/4/78*
B/Shanghai/1/77*
B/Shanghai/10/80*
B/Xuanwu/23/82*
B/Shanghai/35/84*
B/Beijing/1/87*Δ
B/Victoria/2/87
B/Hong Kong/330/2001*Δ
B/Shandong/7/97Δ
ML04Δ
Victoria lineage
B
C
B/Ningxia/45/83*
B/Canada/3/85
B/Ibaraki/2/85
B/Finland/24/85
B/Ann Arbor/1/86Δ
B/Victoria/3/85
B/Beijing/5/76*
B/Beijing/43/75*
B/Hong Kong/5/72*Δ
G
B/Hunan/4/72*
B/Hong Kong/8/73*
B/Yamagata/1/73
B/Guma/73
B/Gifu/73
B/Victoria/70Δ
B/Osaka/70
0.01 substitution/site

strains selected for influenza vaccine production from 1970 to 2006 by the global influenza surveillance network of the World Health Organization (www.who.int/entity/csr/resources/publications/surveillance/Influenza.pdf; www.flu.lanl.gov/vaccine/). The selection was based on antigenic analyses by hemagglutination inhibition tests, which directly pertain to the evolution of the HA1 domain.

The total 55 HA1 sequences were aligned with CLUSTAL W [16], and the consensus phylogenetic tree (Fig. 1) was constructed using the maximum likelihood method with software of PHYLIP 3.62 [3, 4]. Substitution rates among sites were assumed to be in Gamma distribution because the substitution rates were found to differ obviously among the sites [18]. The transition/transversion ratio was set as 10 because the transitional substitutions were obviously more frequent than the transversional substitutions through online alignments of nearly 1000 HA1 sequences of influenza B viruses reported to the Influenza Sequence Database (data not shown). The topology and bootstrap values of the phylogenetic tree (Fig. 1) changed little if the tree was constructed by the neighbor-joining method or the maximum parsimony method [7] (data not shown).

High bootstrap values in percentage at the node of A and B (both $\geq$90%) in Fig. 1 suggested with confidence that the strains in parentheses respectively belonged to the corresponding major HA lineages [2]. The earliest isolates of the Victoria lineage in the figure was B/Beijing/43/75, while its earliest strains reported previously were isolated in 1985 [6, 8–15, 17]. Therefore, the epidemic history of the Victoria lineage could be traced back from the year 1985 to 1975 in this study.

Figure 1 demonstrates that, among the seven global representative strains for the periods of 1970–1990, only B/Ann Arbor/1/86 and B/Beijing/1/87 belonged to the Victoria lineage. The two strains were distinct from their contemporaries of the Yamagata lineage. Therefore, the Victoria lineage possibly emerged from a minor lineage.

A high bootstrap value at node C (89%) in Fig. 1 suggested with confidence that both B/Osaka/70 and B/Victoria/70 could be the common ancestors of all of the stains isolated after 1970 in the tree, and so the two major HA lineages possibly began divergence in the early 1970s.

Low bootstrap values (all $<$42%) and the topology at the nodes of D–G in Fig. 1 suggested that B/Hong Kong/5/72 (the world representative strain for 1972–1979), B/Aichi/7/76, B/Kanagawa/3/76, B/Shanghai/24/76, B/Du/4/78 and B/Baylor/4/78 were similar to each other, and they were intermediate between the Victoria lineage and the strain of B/Victoria/70, although some of them were more similar to the Yamagata lineage. This further suggested that the Victoria lineage possibly emerged in 1970s.

All of the early strains of the Victoria lineage isolated before the year 1985 in Fig. 1, including B/Beijing/43/75, B/Beijing/5/76, B/Shanghai/1/77, B/Shanghai/10/80, B/Xuanwu/23/82, B/Ningxia/45/83 and B/Shanghai/35/84, were isolated in China, while none of the strains isolated in the rest of the world from 1960 to 1984 belonged to the Victoria lineage. This indicated that the Victoria lineage possibly emerged from a minor lineage in the 1970s in China. Since a minor lineage of a

pathogen in its early emergence usually circulates in a limited region, it is unlikely that the Victoria lineage simultaneously originated in another region. In the 1970s, China was in the social convulsion of the Cultural Revolution and insulated from many countries [18], which could hinder the spread of the lineage from China. In 1980s, China gradually opened up to the world, which would facilitate the global spread of the Victoria lineage from China in the 1980s. Thus, Chinese history of this period coincided with the "sudden" emergence of the Victoria lineage in many places in the world in 1985, as stated in the beginning.

Table 1. The accumulation (shown with shadow) of the amino acid residues specific for the Yamagata or Victoria lineage before 1990

Strains	Positions numbered according to the HA1 sequence of B/Victoria/2/87																					
	56	71	88	116	122	129	136	137	148	149	162	163	164	165	183	198	202	203	109	255	262	267
Reference strains																						
B/Victoria/70	N	N	K	N	R	A	I	V	N	G	K	-	-	N	K	K	V	T	K	P	V	V
B/Hong Kong/5/72	N	K	K	N	R	T	I	V	N	G	K	-	-	-	K	E	V	K	K	P	A	V
B/Aichi/7/76	N	K	K	N	R	T	I	V	N	G	K	-	-	N	K	E	V	K	K	P	V	V
B/Kanagawa/3/76	K	K	K	N	R	T	I	I	N	G	K	-	-	N	K	E	V	K	K	P	A	V
B/Shanghai/24/76	N	K	K	N	Y	T	I	V	N	G	K	-	-	N	K	E	V	K	K	P	V	V
B/Baylor/4/78	N	K	K	N	R	M	I	I	N	G	K	-	-	N	K	E	V	K	N	P	A	V
B/Du/4/78	N	K	K	N	R	T	I	V	N	G	K	N	N	N	K	E	V	K	K	P	V	V
Yamagata lineage																						
B/Singapore/222/79	N	K	K	N	R	R	I	I	N	G	K	-	-	N	K	E	V	K	K	P	V	V
B/Aichi/70/81	N	K	K	N	S	R	I	I	N	G	K	-	-	D	K	K	V	K	K	P	V	V
B/Aichi/1/84	N	K	K	N	R	R	R	L	N	G	R	-	-	D	K	K	E	K	N	P	V	V
B/Shanghai/12/87	N	K	R	N	H	R	R	L	S	R	R	-	-	D	K	K	K	K	N	P	V	V
B/Yamagata/16/88	N	M	R	N	H	R	R	L	S	R	R	-	-	D	K	K	K	N	N	P	V	V
Victoria lineage																						
B/Beijing/43/75	K	K	K	N	H	T	I	V	N	G	K	-	-	N	K	E	V	K	K	S	T	V
B/Shanghai/1/77	K	K	K	N	H	T	I	V	N	G	K	-	-	N	K	E	A	K	K	S	T	V
B/Shanghai/10/80	K	K	K	N	H	T	I	V	N	G	K	-	N	N	K	E	V	K	K	S	T	I
B/Xuanwu/23/82	K	K	K	N	H	T	I	V	N	G	K	N	N	N	E	E	V	K	K	S	T	I
B/Beijing/1/87	K	K	K	H	H	T	K	V	N	G	K	N	D	N	E	E	V	K	K	S	T	I

Although 21 of the 55 strains in the phylogenetic tree (Fig. 1) were isolated in China, the phylogenetic tree was possibly not China-biased because all of the global representative strains from 1970 to 2005 were included, and each of them should be able to represent a large group of isolates. In addition, China is a large and populous country which could provide many niches for the virus circulation.

Among the fourteen strains isolated before the year 1985 in China, five isolated from 1972 to 1978 belonged to the B/Hong Kong/5/72-like viruses, six isolated from 1975 to 1984 belonged to the Victoria lineage, and the remaining three isolated from 1982 to 1984 belonged to the Yamagata lineage (Fig. 1). Interestingly, two isolates, B/Xuanwu/23/82 and B/Xuanwu/1/82, were both isolated in the Xuanwu District in Beijing in 1982, but they belonged to the Victoria lineage and the Yamagata lineage, respectively (Fig. 1). This demonstrates that the two HA lineages could co-circulate at the same time in the same place.

The amino acid residues specific for either of the two major HA lineages before the year 1990 were identified by sequence alignment (Table 1). The amino acid residues specific for a major HA lineage were shared by most strains of the major HA lineage isolated in at least three continuous years in the world and not shared by most strains of the other major HA lineage isolated in the same period in the world. These specific amino acid residues were assumed to be the basic features of the major HA lineages. Consistent with the phylogenetic tree (Fig. 1), Table 1 suggested that B/Beijing/43/75 was an early isolate of the Victoria lineage, as it had accumulated four amino acid residues specific to the Victoria lineage, while B/Singapore/222/79, an early isolate of the Yamagata lineage, had only one amino acid residue specific to the Yamagata lineage. The Victoria lineage had gradually accumulated nine specific amino acid residues plus two insertions by the year 1987, while the Yamagata lineage had gradually accumulated thirteen specific amino acid residues by the year 1988 without insertions or deletions. Therefore, the Yamagata lineage possibly began its emergence in 1979, and the Victoria lineage possibly began its emergence shortly before the year 1975.

According to the numbers of residues that differ between the strains in Table 1, B/Aichi/7/76, B/Kanagawa/3/76 and B/Shanghai/24/76 were intermediate not only between B/Singapore/222/79 and B/Beijing/43/75, but also between B/Singapore/222/79 or B/Beijing/43/75 and B/Victoria/70. Therefore, B/Aichi/7/76, B/Kanagawa/3/76 and B/Shanghai/24/76 were possibly the ancestors of the two major HA lineages. Using a similar method, B/Baylor/4/78 was found to be more similar to the Yamagata lineage, and B/Du/4/78 was a little more divergent from both of the two major HA lineages due to its two insertions at positions 163 and 164, which were ignored by the PHYLIP software in the construction of the phylogenetic tree.

In summary, some early footprints of the Victoria lineage of influenza B viruses in China were found, and the epidemic history of the Victoria lineage could be traced back from the year 1985 to 1975, and our data indicated that the Victoria lineage possibly emerged in 1970s in China through gradual evolution from a minor lineage. The circulation of influenza B viruses before the year 1985 in China was also partially unveiled by phylogenetic analyses.

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References

1. Chen J, Guo Y, Guo J, Dong J (2002) Characterization of HA1 gene of influenza B virus circulated in 1990 through 2000 in China. Chin J Exper Clin Virol 16: 278–280
2. Efron B, Halloran E, Holmes S (1996) Bootstrap confidence levels for phylogenetic trees. Proc Natl Acad Sci USA 93: 7085–7090
3. Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17: 368–376
4. Hasegawa M, Kishino H, Saitou N (1991) On the maximum likelihood method in molecular phylogenetics. J Mol Evol 32: 443–445
5. Hsü ICY (1999) The rise of modern China, 6th edn. Oxford University Press, New York
6. Kanegae Y, Sugita S, Endo A, Ishida M, Senya S, Osako K, Nerome K, Oya A (1990) Evolutionary pattern of the haemagglutinin gene of influenza B viruses isolated in Japan: cocirculating lineage in the same epidemic season. J Virol 64: 2860–2865
7. Li WH (1997) Molecular evolution. Sinauer Associates, Sunderland, MA
8. Luo C, Morishita T, Satou K, Tateno Y, Nakajima K, Nobusawa E (1999) Evolutionary pattern of influenza B viruses based on the HA and NS genes during 1940–1999: origin of the NS genes after 1997. Arch Virol 144: 1881–1891
9. Matsuzaki Y, Sugawara K, Takashita E, Muraki Y, Hongo S, Katsushima N, Mizuta K, Nishimura H (2004) Genetic diversity of influenza B virus: the frequent reassortment and cocirculation of the genetically distinct reassortant viruses in a community. J Med Virol 74: 132–140
10. McCullers JA, Saito T, Iverson AR (2004) Multiple genotypes of influenza B virus circulated between 1979 and 2003. J Virol 78: 12817–12828
11. Nerome R, Hiromoto Y, Sugita S, Tanabe N, Ishida M, Matsumoto M, Lindstrom SE, Takahashi T, Nerome K (1998) Evolutionary characteristics of influenza B virus since its first isolation in 1940: dynamic circulation of deletion and insertion mechanism. Arch Virol 143: 1569–1583
12. Pyhala R, Kleemola M, Kumpulainen V, Vartiainen E, Lappi S, Ponka A, Cantell K (1992) Immune response to inactivated influenza virus vaccine: antibody reactivity with epidemic influenza B viruses of two highly distinct evolutionary lineages. Vaccine 10: 631–636
13. Rota PA, Wallir TR, Harmon MW, Rota JS, Kendal AP, Nerome K (1990) Cocirculation of two distinct evolutionary lineages of influenza type B virus since 1983. Virology 175: 59–68
14. Rota PA, Hemphill ML, Whistler T, Regnery HL, Kendal AP (1992) Antigenic and genetic characterization of the haemagglutinins of recent cocirculating strains of influenza B virus. J Gen Virol 73: 2737–2742
15. Shaw MW, Xu X, Li Y, Normand S, Ueki RT, Kunimoto GY, Hall H, Klimov A, Cox NJ, Subbarao K (2002) Reappearance and global spread of variants of influenza B/Victoria/2/87 lineage viruses in the 2000–2001 and 2001–2002 seasons. Virology 303: 1–8
16. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res 22: 4673–4680

17. Yamashita M, Krystal M, Fitch WM, Palese P (1988) Influenza B virus evolution: cocirculating lineages and comparison of evolutionary pattern with those of influenza A and C viruses. Virology 163: 112–122
18. Zou S, Prud'homme I, Weber JM (1997) Evolution of the haemagglutinin gene of influenza B virus was driven by both positive and negative selection pressures. Virus Genes 14: 181–185

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