



SURVEILLANCE REPORT

Influenza virus characterisation

Summary Europe, December 2013

Summary

The 2013–14 influenza season has been slow to start in EU/EEA countries resulting in a low number (46) of influenza-positive specimens, with collection dates since 1 September 2013, being received by WHO CC, London.

- Type A and type B viruses have been received in the ratio 9:1.
- A(H3N2) and A(H1N1)pdm09 viruses have been received in the ratio 2:1.
- Compared to the 2012–13 influenza season where genetic subgroup 6C dominated among A(H1N1)pdm09 viruses, and based on the current global situation, genetic subgroup 6B viruses have been detected in increasing frequency. Subgroup 6B viruses have been antigenically similar to the vaccine virus, A/California/07/2009.
- Recently circulating A(H3N2) viruses have fallen within genetic subgroup 3C represented by the recommended vaccine virus for the 2013–14 season, A/Texas/50/2012; some new genetic clusters defined by specific HA amino acid substitutions have been observed for which antigenic characterisation is pending.
- No B/Victoria-lineage viruses have been received to date, but phylogenetic analysis of recently circulating viruses reveals that all are in genetic clade 1A with no significant HA amino acid substitutions, suggestive of antigenic similarity to cell-propagated reference viruses of the B/Brisbane/60/2008 genetic clade.
- Two genetic clades of B/Yamagata-lineage viruses continue to circulate: clade 3 represented by B/Wisconsin/1/2010 and clade 2 represented by B/Massachusetts/2/2012 (the recommended vaccine component for the 2013–14 influenza season). The great majority of recently circulating viruses fall within clade 2.

Influenza-positive specimens with collection dates since 1 September 2013 (with week 40, the start of weekly monitoring of influenza activity for the 2013–14 influenza season, commencing on 30 September 2013) were received from six countries in the EU/EEA region at the MRC National Institute for Medical Research, WHO Collaborating Centre for Reference and Research on Influenza. A summary of specimens received is shown in Table 1, with characterisation of all specimens being in process.

While numbers received (46) are low, indicative of a late start to the 2013–14 influenza season, influenza type A viruses (91%) are dominating over type B (9%) reflecting what is often seen at the beginning of Northern Hemisphere influenza seasons. For type A, H3N2 viruses have been received in greater numbers than H1N1pdm09 viruses (ratio 2:1). Among influenza B virus receipts, all fall in the B/Yamagata lineage. Antigenic characterisation, based on propagated viruses, has yet to be performed.

Table 1. Summary of clinical samples and isolates received from ECDC-affiliated countries, with collection dates since 1 September 2013

MONTH	TOTAL RECEIVED	A	H1N1pdm09		H3N2		B	B Victoria lineage		B Yamagata lineage	
Country			Number received	Number propagated ¹	Number received	Number propagated ²		Number received	Number propagated ¹	Number received	Number propagated ¹
SEPTEMBER											
France	1				1	in process					
United Kingdom	2		1	in process	1	in process					
OCTOBER											
France	3		1	in process	1	in process				1	in process
Norway	6				6	in process					
Portugal	1									1	in process
Spain	2		2	in process							
United Kingdom	2				1	in process				1	in process
NOVEMBER											
France	5		2	in process	3	in process					
Germany	3		1	in process	2	in process					
Norway	3		1	in process	1	in process				1	in process
Portugal	2				2	in process					
Spain	7		3	in process	4	in process					
United Kingdom	2		2	in process							
DECEMBER											
France	3		1	in process	2	in process					
Spain	4	2			2	in process					
	46	2	14	0	26	0	0	0	0	4	0
			30.4%		56.5%			0.0%		8.7%	
6 Countries			91.3%				8.7%				

1. Propagated to sufficient titre to perform HI assay (the totalled number does not include any from batches that are in process)

2. Propagated to sufficient titre to perform HI assay in presence of 20nM oseltamivir (the totalled number does not include any from batches that are in process)

Influenza A(H1N1)pdm09 virus analyses

Figure 1A shows a phylogenetic tree for the HA genes of representative H1N1 viruses, with collection dates up to June 2013, characterised at the London WHO CC. Over the last four years, HA genes have been observed to fall into eight designated genetic groups, with a ninth 'outlier' group largely restricted to countries of west Africa. However, viruses collected after 31 January 2013 fell into genetic groups 6 and 7 with group 6 viruses clustering in three subgroups, 6A–C (Figure 1A). Both groups carry the substitutions **S185T** and **S203T** in **HA1** and **E47K** and **S124N** in **HA2** compared to A/California/7/2009.

The two groups and three subgroups are further defined by the following amino acid substitutions in **HA1/HA2**:

Group 6 viruses:

D97N, with the subgroups defined by the substitutions:

6A: **H138R** and **V249L**, e.g. A/Hong Kong/5659/2012.

6B: **K163Q**, **A256T**, **K283E** and **E172K**, e.g. A/Norway/2417/2013.

6C: **V234I**, **K283E** and **E172K** e.g. A/Estonia/77389/2013.

Group 7 viruses:

S143G and **A197T**, often with **S84G**, **K163I** and **V193A**, e.g. A/Norway/1675/2013.

The majority of H1N1 viruses from EU/EEA countries collected during the 2012–13 season clustered within genetic groups 6 and 7, with viruses belonging to subgroup 6C predominating, notably so for those with collection dates since 1 March 2013 ([see September report](#)) [1].

A recent search of the [GISAID database](#) [2] for HA sequences of worldwide H1N1pdm09 specimens with collection dates after 31 July 2013 yielded 75 'released' sequences, 69 of which fell within genetic subgroup 6B being detected in Bangladesh, Brazil, Hong Kong, India, Japan, Puerto Rico, Sweden and USA, the remaining six being in subgroup 6C (Costa Rica, Japan, USA: Figure 1B). Previously circulating subgroup 6B viruses have reacted well with antisera raised against the vaccine virus, A/California/7/2009.

Figure 1A. Phylogenetic comparison of influenza A(H1N1)pdm09 HA genes

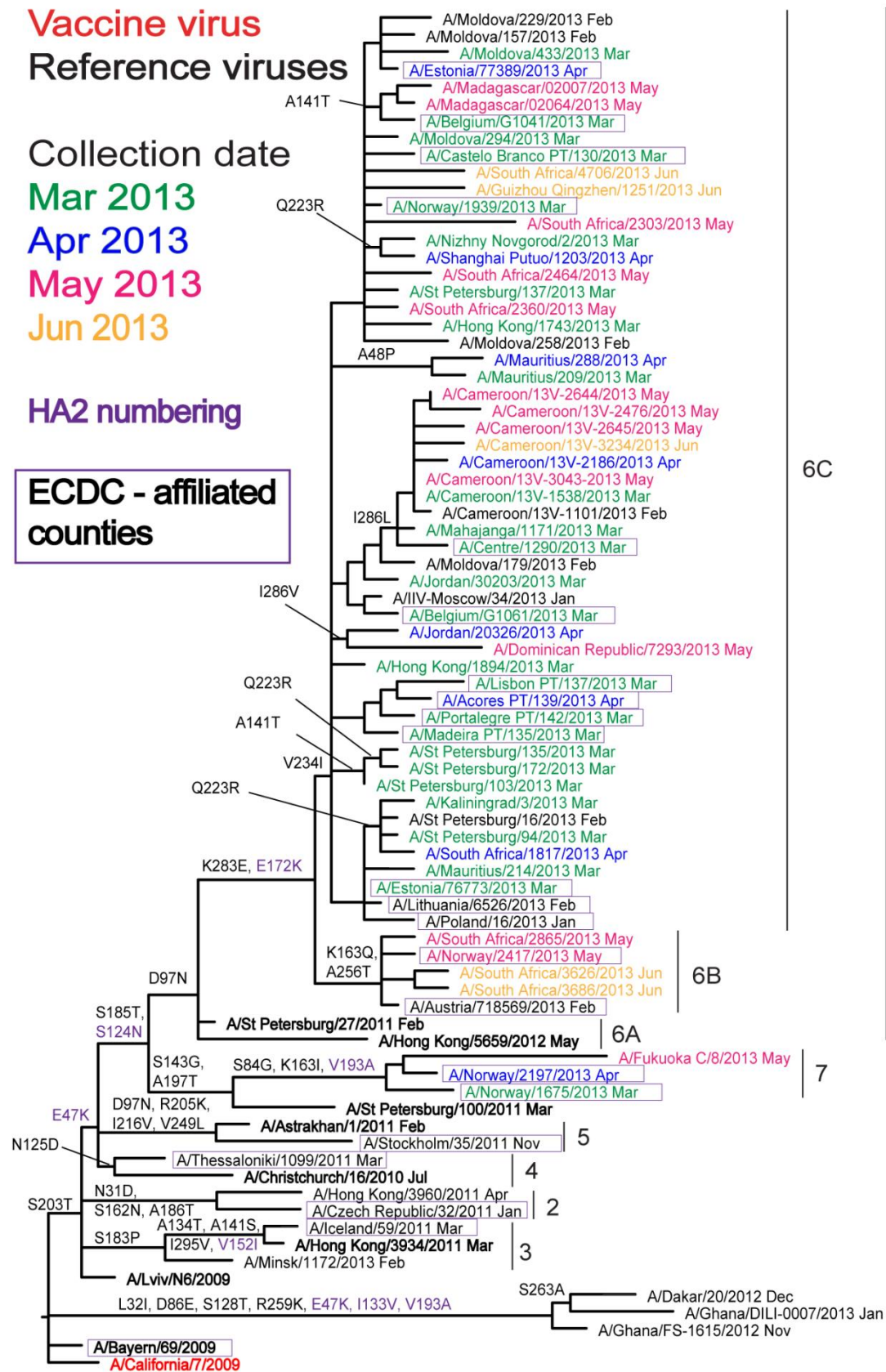
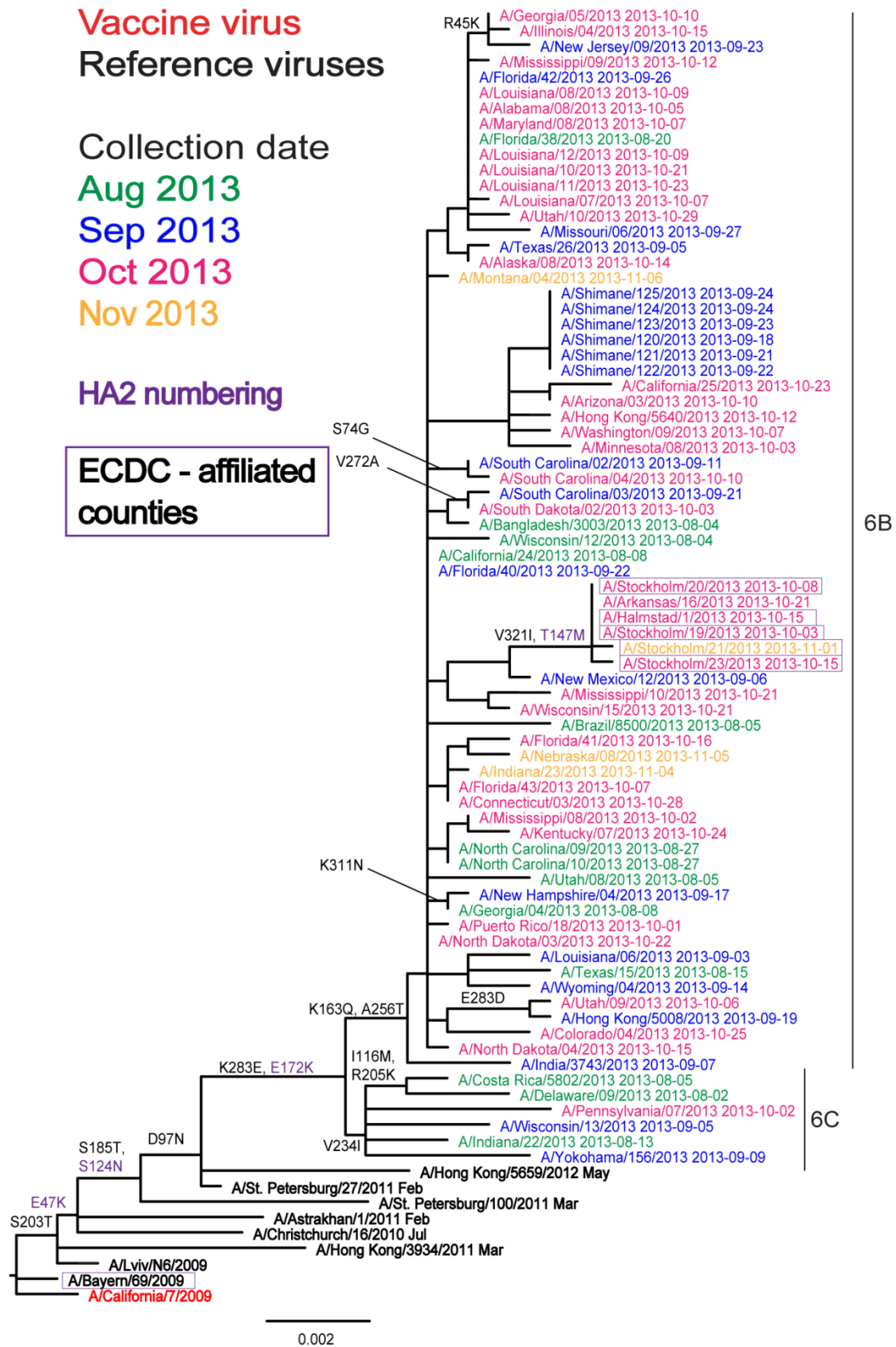


Figure 1B. Phylogeny of influenza A(H1N1)pdm09 HA genes: recently collected specimens



Influenza A(H3N2) virus analyses

Figure 2A shows a phylogenetic tree for the HA genes of representative H3N2 viruses, with collection dates up to August 2013, characterised at the London WHO CC. Viruses from EU/EEA countries collected since 1 January 2013 have HA genes that fall predominantly within genetic group 3C, mainly within subgroup 3C.3 with some in subgroup 3C.2.

The amino acid substitutions in **HA1/HA2** associated with the grouping of recently collected viruses are:

Group 3 viruses:

N145S and **V223I**, with the subgroups defined by the substitutions:

3A: **N144D** (resulting in the loss of a potential glycosylation site) and **D158N**, e.g. A/Stockholm/18/2011;

3B: **A198S**, **N312S** and **D158N**, e.g. A/Athens/112/2012;

3C: **S45N** (resulting in gain of a potential glycosylation site), **T48I**, **A198S** and **N312S**, e.g. the prototype vaccine virus A/Victoria/361/2011. The 3C subgroup can be sub-divided into three subsets:

3C.1 **Q33R**, **S145N** and **N278K**, e.g. A/Berlin/93/2011, A/Texas/50/2012 and A/Hawaii/22/2012;

3C.2 As 3C.1 plus **N145S**, e.g. A/Ireland/M28390/2013;

3C.3 As 3C.2 plus **T128A** (resulting in the loss of a potential glycosylation site) and **R142G**, e.g. A/Samara/73/2013

A recent search of the [GISAID database](#) [2] for HA sequences of H3N2 specimens with collection dates after 7 July 2013 yielded 65 'released' sequences, including 12 from Norway and Sweden: 24 (four from the EU/EEA) of these fell within genetic subgroup 3C.2 and 41 (eight from the EU/EEA) in subgroup 3C.3 (Figure 2B). While there is no evidence for antigenic change associated with any of the assigned genetic groups, subgroups and subsets, including the emerging 3C.3 subset that carries substitutions in HA1 at amino acid residues 128 and 142, a number of new virus clusters among recently circulating H3N2 viruses, defined by specific amino acid substitutions, have emerged. Antigenic characterisation of the viruses forming these newly emerged genetic clusters is pending.

Note: in Figures 2A/B the G186V substitutions in HA1 occur during adaptation of H3N2 viruses to passage in hens' eggs and are not group specific.

Influenza B virus analyses

B/Victoria-lineage viruses

Phylogenetic analysis of the HA genes of representative B/Victoria lineage viruses is shown in Figure 3. The HA genes of all recently circulating viruses, including the nine with collection dates in August/September available in GISAID at the time of compilation of this report, fall within the B/Brisbane/60 genetic clade (1A), with only a small number of amino acid substitutions compared with the HA of the previously used trivalent and recommended quadrivalent vaccine virus, B/Brisbane/60/2008.

B/Yamagata-lineage viruses

Figure 4 shows a phylogenetic analysis of the HA genes of representative B/Yamagata-lineage viruses. The HA genes of recent viruses continue to fall into two genetic clades: clade 3 (represented by a previous vaccine virus B/Wisconsin/1/2010 and reference viruses B/Stockholm/12/2011 and B/Novosibirsk/1/2012) and clade 2 (represented by the reference viruses B/Brisbane/3/2007, B/Estonia/55669/2011, B/Hong Kong/3577/2012 and the 2013–14 vaccine virus B/Massachusetts/02/2012). The two clades are differentiated by substitutions at HA1 positions 48, 108, 150, 165, 181 and 229. The HA genes of viruses of clade 2 encode **K48**, **A108**, **S150**, **N165**, **A181** and **G229**; the HA genes of viruses in clade 3 encode **R48**, **P108**, **I150**, **Y165**, **T181** and **D229**. As observed previously, the large majority of recently circulating B/Yamagata lineage viruses fall in genetic clade 2.

Figure 2A. Phylogenetic comparison of influenza A(H3N2) HA genes

Vaccine virus
Reference viruses

Collection date
May 2013
Jun 2013
Jul 2013
Aug 2013

HA2 numbering

ECDC - affiliated counties

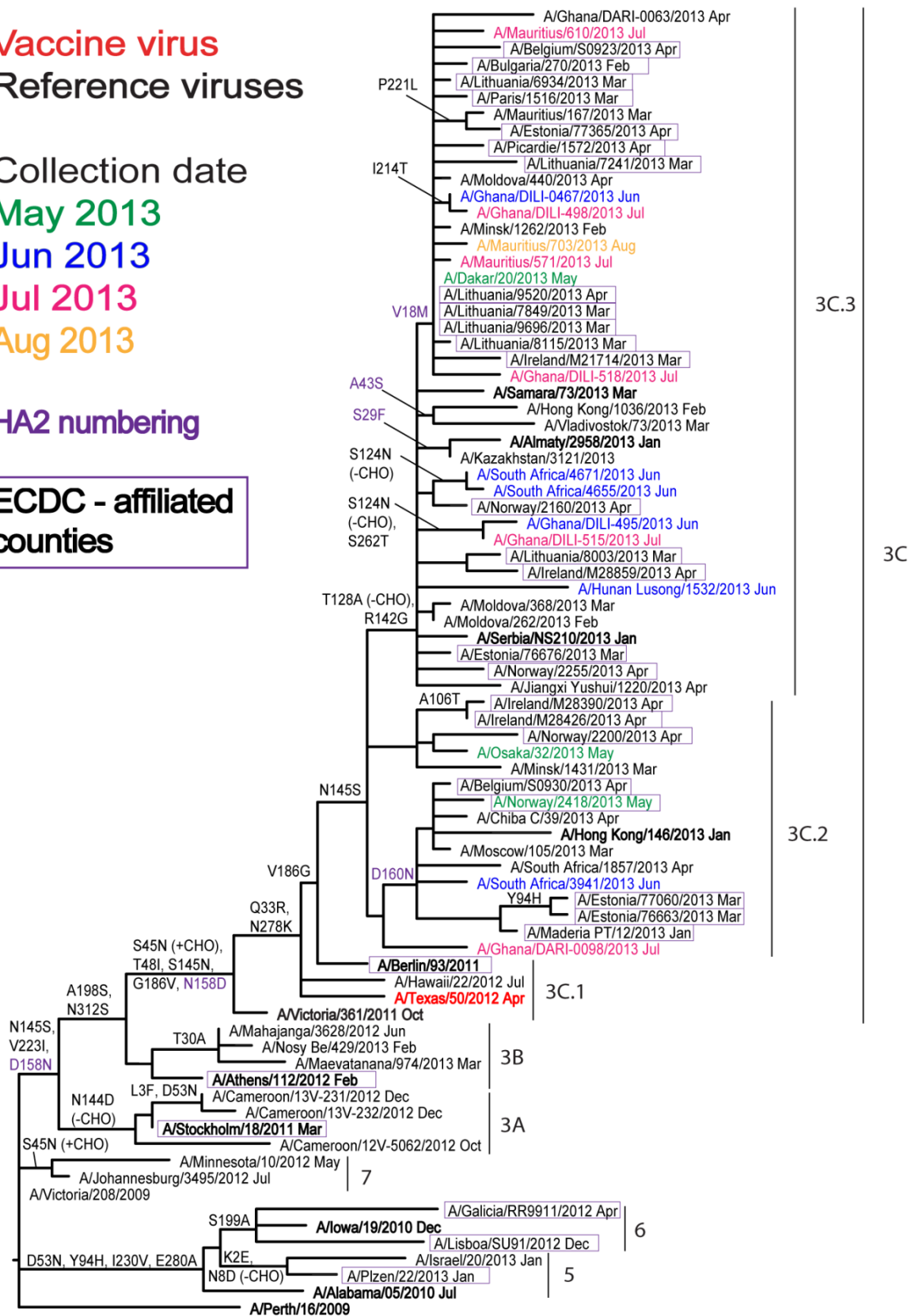


Figure 2B. Phylogeny of influenza A(H3N2) HA genes: recently collected specimens

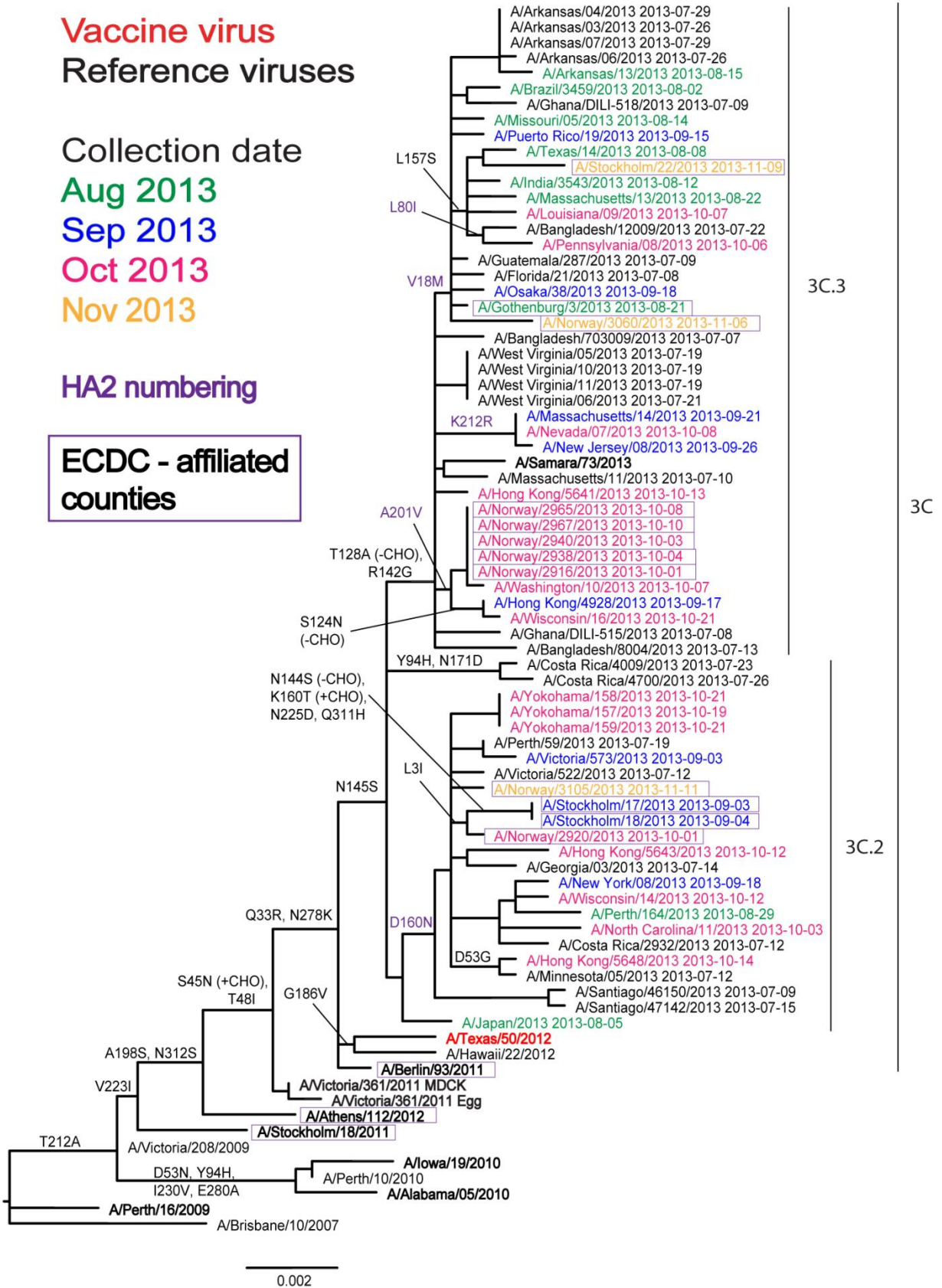


Figure 3. Phylogenetic comparison of influenza B/Victoria-lineage HA genes

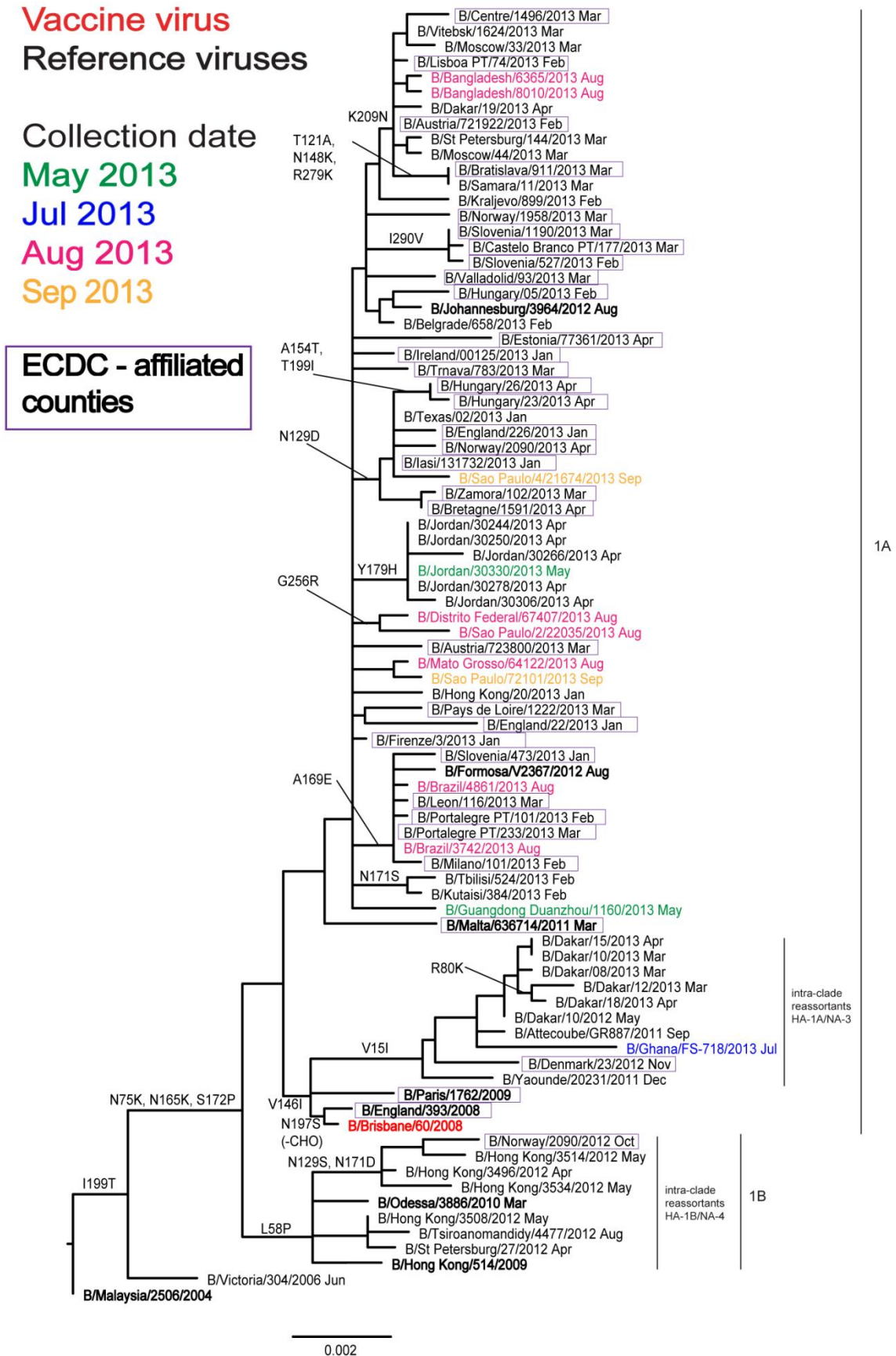
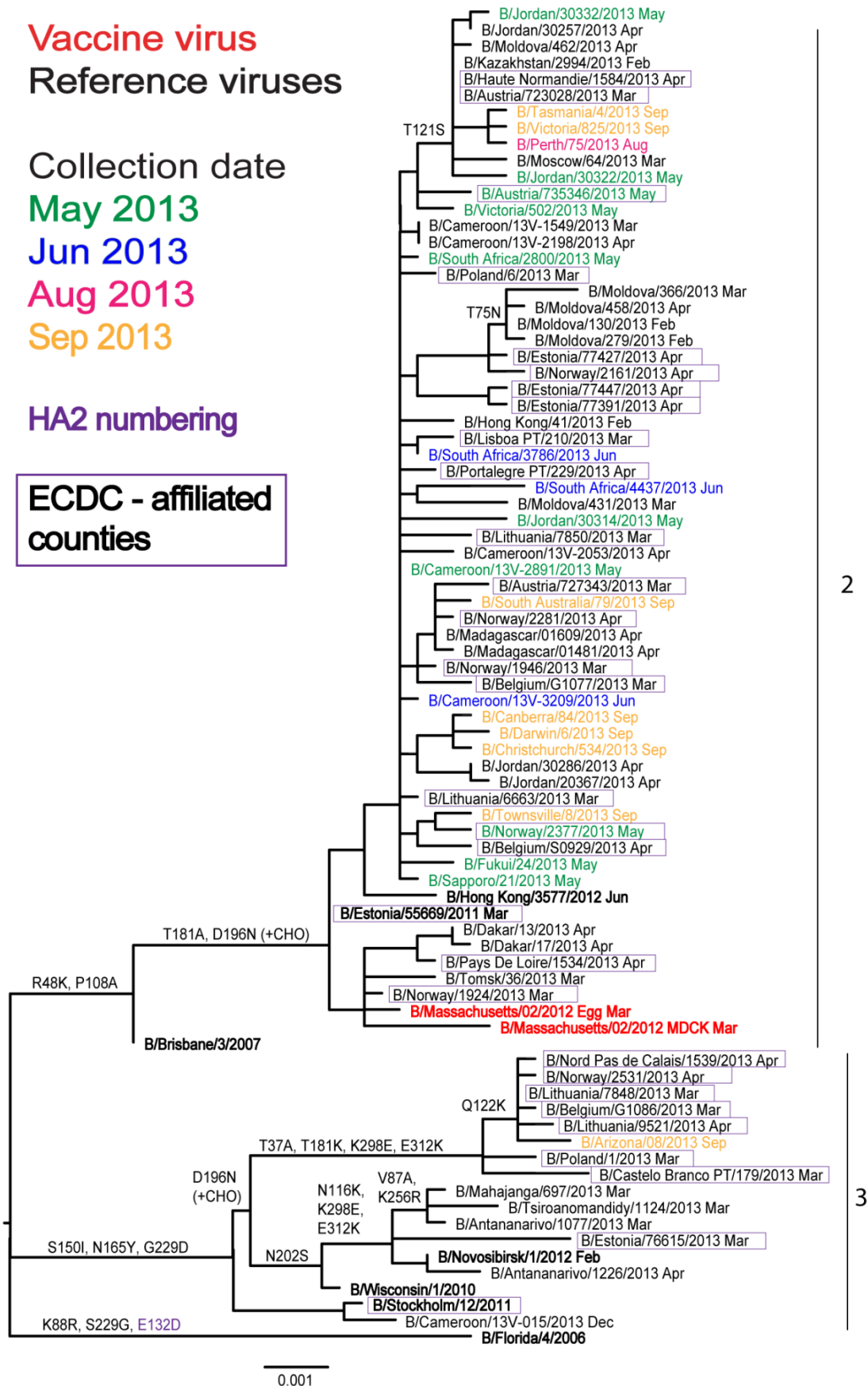


Figure 4. Phylogenetic comparison of influenza B/Yamagata-lineage HA genes



Influenza A(H7N9) virus

On 1 April 2013, the [WHO Global Alert and Response](#) [3] reported that the China Health and Family Planning Commission notified the World Health Organization (WHO) of three cases of human infection with influenza A(H7N9). The cases were confirmed by laboratory testing on 29 March by the Chinese CDC. A description of the characteristics of H7N9 viruses can be found on the [WHO website](#) [4]. WHO is updating information on the outbreak [regularly](#) [4] and ECDC is posting [epidemiological updates](#) [5]. A [Rapid Risk Assessment](#) [6] for these A(H7N9) viruses was carried out and posted on 3 April 2013, and an updated risk assessment was [posted by WHO](#) [7]. As of 6 November 2013, 139 laboratory-confirmed cases and 45 associated fatalities (case-fatality ratio of 32%) had been reported to WHO with a further six laboratory-confirmed cases being reported on [10 and 17 December](#) [8, 9], inclusive of two in Hong Kong SAR. A second case imported from China has been reported in [Taiwan](#) [10].

A description of results generated by the WHO Collaborating Centre for Reference and Research on Influenza at the MRC National Institute for Medical Research in London, and evaluated at the WHO Vaccine Composition Meetings held at WHO Geneva on 18–20 February 2013 and 23–25 September 2013, can be found at: http://www.nimr.mrc.ac.uk/documents/about/Interim_Report_February_2013.pdf

<http://www.nimr.mrc.ac.uk/documents/about/NIMR-report-Sep2013final.pdf>

Note on the figures

The phylogenetic trees were constructed using RAxML and drawn using FigTree. The bars indicate the proportion of nucleotide changes between sequences. Reference strains are viruses to which post-infection ferret antisera have been raised. The colours indicate the month of sample collection. Isolates from WHO NICs in ECDC countries are highlighted within boxes. Sequences for many viruses from non-EU/EEA countries were recovered from GISAID. We gratefully acknowledge the authors, originating and submitting laboratories of the sequences from GISAID's EpiFlu Database which were downloaded for use in the preparation of this report (all submitters of data may be contacted directly via the GISAID website [2]), along with all laboratories who submitted sequences directly to the London WHO Collaborating Centre.

References

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