Circulation Patterns of Human Seasonal Influenza a Virus in Chile Before H1N1pdm09 Pandemic

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Circulation patterns of human seasonal Influenza A virus in Chile before H1N1pdm09 pandemic

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Abstract

Understanding the diversity and circulation dynamics of seasonal influenza viruses is key to public health decision-making. The limited genetic information of pre-pandemic seasonal IAVs in Chile has made it difficult to accurately reconstruct the phylogenetic relationships of these viruses within the country. The objective of this study was to determine the genetic diversity and estimate the antigenic evolution of pre-pandemic human seasonal IAVs in Chile. We sequenced the complete genome of 42 historic IAV obtained between 1996 to 2007. The phylogeny was determined using HA sequences and complemented using other segments. Time-scale phylogenetic analyses revealed that diversity of pre-pandemic human seasonal IAVs in Chile have been influenced by continuous introductions of new A/H1N1 and A/H3N2 lineages and constant viral exchange between Chile and the rest of the countries every year. Besides, wide genetic and antigenic diversity was observed co-circulating in some years and introduced lineages every year become extinct at the end of each season. These results provide important knowledge about genetic diversity and evolutionary patterns of pre-pandemic human seasonal IAVs in Chile, which can help to design optimal surveillance systems and prevention strategies in the country, however, future studies with current sequences should be conducted.

Keywords: ***Seasonal*** *Influenza A, Phylogeny, H1N1, H1N2, H3N2***,** pre-pandemic

Introduction

Influenza A virus (IAV) is an important concern in public health causing epidemics of respiratory disease and between 290,000-650,000 deceases worldwide annually[1]. The last influenza pandemic was caused by a novel lineage of Influenza A/H1N1

(A/H1N1pdm09) causing more than 123,000 global deaths from March to December 2009[2]. This strain displaced the previous human seasonal IAV A/H1N1 subtype that was circulating before the pandemic[3]. Nowadays, A/H1N1pdm09 is co-circulating seasonally with A/H3N2 and influenza B viruses[3], and its viral dynamic is well known due to the surveillance efforts and novel sequencing platforms[4,5]. Before the A/H1N1pdm09 pandemic, the information about IAV genetic diversity was scarce, and only HA gene was commonly sequenced. The viral dynamic of IAV circulating before the 2009 pandemic is still unknown in most of the world, especially in developing and least-developed countries.

The viral circulation is important to maintain seasonal IAV strains. Seasonal IAV is driven by the introduction of new lineages from other countries rather than a local persistence of lineages that remains circulating from previous epidemics[6–9]. For example, studies suggest that A/H3N2 viruses originate from an ecological source located in East and Southeast Asia, and from there spread to other regions of the world[9,10]. On the contrary, Asian regions play a limited role in the dissemination of new lineages of A/H1N1 viruses[3,10]. Recently, it has also been suggested a more complex metapopulation model of the spatial spread proposing several geographic areas as potential sources of new variants[3,6,7]. In this way, the annual seasonal pattern is characterized by an increase in activity during the winter season in temperate regions[11], and during the rainy season in the tropics[12].

In South America and in general in the southern hemisphere, the epidemiological and evolutionary dynamics of circulating IAVs have only been partially explored due to the lack of IAV sequences. Some studies indicate that in South America IAV strains do not persist locally between seasons, and genetic diversity is driven by the northern regions of the continent, mainly influenced by North America[13–15]. In Chile, the information about

IAV before the A/H1N1pdm09 pandemic is scarce, only include 43 HA sequences are publicly available and there are no studies on the epidemiology and/or evolutionary dynamics of human IAVs. Therefore, this study aimed to determine the diversity of pre-pandemic human seasonal IAVs in Chile filling the information gap in the region.

Results

Genetic evolution of pre-pandemic human seasonal IAVs in Chile. Human IAV isolates were genetically characterized to evaluate the diversity and genetic evolution of pre-pandemic human seasonal IAVs in Chile. Forty-two out of 57 IAV isolates were successfully whole genome sequenced (GenBank accession numbers MN054079-MN055475). These IAVs were obtained in 1996, 2000, 2001, 2003, 2004, 2005, and 2007, and are the first whole genome IAVs sequenced previous 2009, excepting to isolates from 2000 and 2001. All Chilean pre-pandemic influenza sequences were incorporated in a table summarizing overall results (Table 1). Based on subtypes, 54 strains were classified as H3N2, 30 as H1N1, and 1 H1N2.

Time-scale phylogenetic analyzes of the HA1 region were performed to study the H1 and H3 subtypes independently. The phylogeny showed that the H1 Chilean sequences are distributed in 15 different clades, which are related to viruses from different locations, especially South and North America (Figure 1, Supplementary Table 1), evidence an extensive global exchange of viruses between different geographic regions (continents). At least three lineages were observed co-circulating in Chile during the same year, especially in 2000, 2006, and 2008 (Figure 1, Supplementary Table 1). The evolutionary analysis shows that the Chilean HA sequences are the last to appear in their respective clades, suggesting that Chile is one of the regions with the latest IAV arrival. An inter-seasonal

extinction of Chilean H1 lineages was observed, as it is also observed in other geographical regions; however, some viruses were transmitted to other countries after their arrival in Chile in 2000 and 2008. (clades B and N) (Figure 1). On the other hand, the H1N2 isolate (clade F) was grouped with viruses of the same subtype that were circulating globally between 2001-2003. The mean evolutionary rate for H1 subtype was 3.3×10^{-3} substitutions/site/year (95% HPD: $2.9\text{-}3.7 \times 10^{-3}$ substitutions/site/year).

According to the time-scale phylogenetic analysis of the H3 subtype (Figure 2), the mean evolutionary rate for the HA1 region was 4×10^{-3} substitutions/site/year (HPD 95%: $3.4\text{-}4.8 \times 10^{-3}$ substitutions/site/year). As in the results obtained for the H1 subtype, an extensive global exchange of viruses between different geographic regions was identified. An inter-seasonal extinction of the Chilean H3 lineages was also evidenced. Phylogeny revealed that Chilean H3 sequences are distributed in 22 different clades (A-U), being related to viruses from different geographic regions. Unlike the H1 subtype, these sequences are commonly related not only to sequences from South America and North America but also from Europe and Asia (Figure 2). A co-circulation of two to five lineages in the same year was observed, specifically in 1996, 2000, 2003, 2004, 2005, 2006, and 2007. Viral interchange from Chile to other geographical regions was evidenced in at least 7 opportunities, in 2001, 2003, 2004, 2006, and 2007 (clades F, G, I, M, Q, S, and U) (Figure 2).

The time to the most recent common ancestor (TMRCA) and related sequences (continent of origin) of Chilean H1 and H3 sequences are summarized in Supplementary Table 1.

The phylogenetic relationships of the rest of the genes NA, PB2, PB1, PA, NP, M and NS were in concordance with the results observed for the HA trees (Supplementary figures 1 and 2).

Antigenic evolution of human seasonal IAVs in Chile. Genetic analyzes based on HA1 amino acid sequences were performed to determine the genetic clusters and estimate the antigenic evolution (predicted from the genetic analysis) of worldwide human seasonal IAV sequences (1990-2008). These analyzes show that these IAVs were grouped into four genetic clusters for H1 and three genetic clusters for H3 (Figures 3 and 4, respectively). For each subtype, clusters were named according to the year of emergence, being cluster 1 the first to emerge. All clusters evidence circulation in all geographic regions, including South America and Chile. In general, Asia is the geographic region where IAV strains from each genetic cluster were isolated for the first time, while South America, including Chile, is the region with the latest IAV arrival, according to publicly available IAV sequences. Co-circulation of different IAV clusters of the same subtype in Chile was also observed in 2000, 2006, and 2008 for subtype H1, while for subtype H3 in 2003 (Supplementary tables 2 and 3).

Discussion

We evaluated the genetic diversity and evolution of the seasonal human IAVs in Chile between 1994 and 2008. IAV subtypes A/H1N1, A/H1N2, and A/H3N2 co-circulated in the Chilean population before the 2009 pandemic. The A/H3N2 subtype was the most commonly detected and sequenced in that period. These results are consistent with the global circulation patterns described for seasonal IAVs[3,10,16]. In general, A/H3N2 has been

143 the dominant subtype since it first emerged in 1968[17], despite the re-emergence of the
144 H1N1 subtype in 1977[18]. The subtype A/H1N2 identified in this study in 2003 correspond
145 to a reassortant virus that circulated around the world between 2001 and 2003[19].
146 The average evolution rate estimated for the HA1 region of the H3 subtype was
147 higher (4 x 10-3; HPD 95%: 3.4-4.8 x 10^{-3} substitutions/site/year) than that estimated for
148 the H1 subtype (3.3 x 10-3; HPD 95%: 2.9-3.7 x 10^{-3} substitutions/site/year), which is
149 consistent with previous studies[3,10]. As expected, phylogenetic analyzes for H1 and H3
150 subtypes showed an extensive viral exchange between Chile and the other regions of the
151 world, evidencing a continuous genetic flow inside and outside Chile, beyond the existence
152 of a closed evolutionary system in the country. Pre-pandemic Chilean IAVs are mainly
153 related to sequences from South and North America. This is consistent with previous
154 studies, which reported that viruses arriving in South America originate mainly in North
155 America, and that there is a continuous viral exchange between South American
156 countries[8,14,15]. However, A/H3N2 virus introductions would also come from Asia and
157 Europe, indicating that the epidemic outbreaks that occur in Chile every year are influenced
158 by viruses from different geographical regions, which may differ antigenically among
159 them.
160 In general, previous studies have described that A/H3N2 lineages do not persist
161 locally between epidemics, while A/H1N1 lineages can persist for several seasons and
162 show more complex global dynamics[3]. In this study, a circulation of multiple A/H1N1 and
163 A/H3N2 lineages was evidenced during the same season, which came from different
164 geographic regions and generally disappeared at the end of each outbreak in Chile. This
165 shows a wide genetic diversity in each flu season in Chile, which are produced by the

166 introduction of new A/H1N1 and A/H3N2 lineages from other countries rather than the
167 local persistence of lineages from the previous season.

168 All IAV genetic clusters (based on amino acid sequences) determined in this study
169 have circulated worldwide, showing the globally distribution of this virus. In general, Asia
170 is the geographic region where IAV strains from each genetic cluster were isolated for the
171 first time. A similar situation occurs in Oceania, Europe, and North America. While Africa
172 and South America, including Chile, are the regions with the latest IAV arrival. However,
173 few sequences from Africa and South America have been published compared to the rest of
174 the continents and therefore there could be information bias. Although the surveillance was
175 improved after the IAV pandemic in 2009, it is still insufficient in some countries, such as
176 Chile. Previously, it has been shown that Asia plays an important role in the transmission of
177 seasonal human IAVs, showing that most lineages eventually originated from this
178 geographic region[3,6,9,20,21]. We also detected the co-circulation of different IAV clusters of
179 the same subtype in some years in Chile, suggesting that the efficacy of the influenza
180 vaccine may have been insufficient during those years. The seasonal influenza vaccine is
181 designed to protect against strains that research shows are most likely to spread and cause
182 illness among people during the next flu season. However, influenza vaccines only contain
183 one A/H1N1 strain and one A/H3N2 strain[22], showing a reduction in the risk of disease of
184 between 40 and 60%[23].

185 Notably, the genetic analysis, based on amino acid sequences, carried out in this
186 study is highly correlated with previously published antigenic analyzes, based on
187 hemagglutination inhibition assay, specifically for the H3 subtype[21,24]. This confirms that
188 this is a good tool to predict IAV antigenic evolution. However, we did not differentiate
189 between some previously described antigenic clusters[24], because there are only a few amino

190 acid substitutions in HA1 domain between strains from these clusters. Only 1 amino acid
191 substitution in HA1 domain can cause a high antigenic impact[25].
192 In conclusion, the results obtained in this study indicate that pre-pandemic human
193 seasonal IAVs in Chile are influenced by continuous introductions of viral variants from
194 other geographic regions and that there is a continuous viral exchange between different
195 countries. Moreover, a wide genetic diversity was observed co-circulating in the same
196 season in Chile. These IAV lineages become extinct at the end of each season, being
197 replaced for new lineages introduced from other countries each year. This is the first study
198 on human IAV epidemiology in Chile, providing an important knowledge about genetic
199 diversity and evolutionary patterns of human seasonal IAVs in Chile, which can help to
200 design optimal surveillance systems and prevention strategies in the country. A limitation
201 of this study was the small number of IAV sequences (data) published so far in South
202 American countries, especially Chile. Greater IAV surveillance, sequencing and
203 phylogeographic analyzes are necessary to support these results, including post-pandemic
204 IAVs that are currently circulating.
205
206 **Materials and methods**
207 **Viruses**
208 Fifty-seven human IAVs pre-pandemic isolates were provided by the Virology
209 Laboratory, Faculty of Medicine, University of Chile, Santiago, Chile. These isolates were
210 collected in Santiago, Chile, from suspected patients. To obtain these strains, the sample
211 collection was performed using Vacuum-Assisted Nasopharyngeal Aspirates (NPAs) and
212 the viral isolation attempted in Madin-Darby Canine Kidney (MDCK) cells. The isolates
213 were obtained from samples collected in 1996 (19 isolates), 2000 (11), 2001 (11), 2003 (3),

2004 (9), 2005 (3), and 2007 (1). The isolated were preserved at -80 ºC until sequencing. The samples collected to obtain the isolates were approved by the Ethics Committee of the Faculty of Medicine at Universidad de Chile and Roberto del Río Hospital to perform the exams and research (project Nº 194 0527 Apr 1994 – Mar 1997; Nº 194 0527 Apr 1994 – Mar 1997; Nº 198 0892 Apr 1998 – Mar 2007).

Virus confirmation and propagation

First the IAV isolates were tested by a real time RT-PCR (rRT-PCR) to reconfirm the IAV presence. After, isolates were propagated in MDCK cells to yield enough virus to attempt the whole genome sequencing. Briefly, RNA was extracted by TRIzol™ LS Reagent (Invitrogen™, Carlsbad, CA, USA) and tested by rRT-PCR amplifying a conserved region of the matrix gene[26]. rRT-PCR positive isolates were propagated in MDCK cells, which were previously cultured using minimum essential medium (MEM) supplemented with 10% fetal calf serum (FCS) and 1% antibiotic-antimycotic solution[27]. Confluent MDCK monolayers were washed twice with PBS containing 1 µg/mL of trypsin treated with N-tosyl-L-phenylalanyl chloromethyl ketone (TPCK) (Sigma-Aldrich, St. Louis, Mo, USA), inoculated with each IAV isolate, and incubated for virus absorption for 1h at 37 ºC. Subsequently, cells were rinsed with PBS to eliminate the unbound virus, and IAV growth medium (MEM supplemented with 1 µg/mL of TPCK-treated trypsin, 0.3% bovine serum albumin, and 1% antibiotic-antimycotic solution) was added. The monolayers were incubated at 37 °C and observed for cytopathic effect (CPE) daily for 5 days. The supernatants of cultures without CPE were re-inoculated in MDCK cells and observed for another 5 days[28]. Isolated were tested by rRT-PCR and Hemagglutination assay to confirm

the presence of IAV[28,29]. RNA was submitted to the Molecular Virology Laboratory, Pontificia Universidad Católica de Chile for further steps.

Whole genome sequencing

Whole IAV genome was amplified by a multisegment RT-PCR (mRT-PCR)[30], and sequenced by Illumina. The mRT-PCR was performed at Molecular Virology Laboratory, Pontificia Universidad Católica de Chile. Briefly, RNA was subjected to reverse transcription and PCR amplification with the SuperScript III high-fidelity RT-PCR kit (Invitrogen, Carlsbad, CA, USA), using the primers Opti1-F1 (5-GT TA CGC GCC AGC AAA AGC AGG), Opti1-F2 (5-GTT ACG CGC CAG CGA AAG CAG G), y Opti1-R1 (5′-GTT ACG CGC CAG TAG AAA CAA GG). A 50 µL total RT-PCR volume containing 25 µL of buffer, 0.35 µL of Opti1-F1, 0,65 of Opti1-F2 and 1 µL of Opti1-R1, 1 µL of Enzyme Mix, 17 µL of water, and 5 µL of RNA was performed. The thermal cycler program consisted in five cycle of 55°C for 2 min, 42°C for 60 sec and 94°C for 2 min; 26 cycles of 94°C for 30 sec, 57°C for 30 sec and 68°C for 3,5 min, and one cycle of 68ºC for 10 min. PCR products were purified using Agencourt AMPure XP 5-ml kit (Beckman Coulter) and those with ≥25 ng/µL of DNA concentration were submitted for sequencing. The purified PCR products were submitted to the Center for Research on Influenza Pathogenesis (CRIP), Icahn School of Medicine at Mount Sinai (New York City, NY, USA) for sequencing on an Illumina HiSeq 2000 sequencer.

Phylogenetic analysis

Phylogenetic analyzes for all IAV segments were performed. Sequence alignments were separately constructed for HA (H1 and H3 subtypes), NA (N1 and N2 subtypes) and each internal gene segment (PB2, PB1, PA, NP, M, and NS), using MUSCLE v3.8.3[31]. Reference sequences available in Global Initiative on Sharing All Influenza Data (GISAID) EpiFlu Database[32] were incorporated including all Chilean sequences available. The CD-HIT program[33] was used to cluster the sequences according to the genetic diversity found in each continent per year, and thus select some representative sequences of each cluster. This allowed to reduce the data sets for the construction of phylogenetic trees. The phylogenetic trees were constructed by the maximum likelihood method using IQ-TREE with substitution model selection (ModelFinder implememted in IQ-TREE) option and 1,000 bootstraps[34].

Additionally, for the HA segment, the encoding the HA1 domain was analyzed using time-scaled Bayesian analyses. HA1 domain is the most variable region of the virus[35–38]; therefore, is selected for time scaled tree[14]. Phylogenetic relationships of the HA from subtypes H1 (510) and H3 (735) were inferred for each data set separately using the time-scaled Bayesian approach using Markov chain Monte Carlo (MCMC) methods available via the BEAST v1.10.4 package[39]. A relaxed uncorrelated lognormal (UCLN) molecular clock was used, with a general-time reversible (GTR) model of nucleotide substitution with a gamma-distributed rate variation among sites. For the H1 subtype was used an expansion growth demographic model, while for the H3 subtype was used a constant population size model. The MCMC was run for each data set for at least 200 million iterations, with sub-sampling every 10,000 iterations. The BEAGLE library was used to improve computational performance[40]. All parameters reached convergence, as assessed visually using Tracer v1.6 (http://tree.bio.ed.ac.uk/software/tracer/), with

statistical uncertainty reflected by values of the 95% highest posterior density (HDP). The initial 10% of the chain was removed as burn-in, and maximum clade credibility (MCC) trees were summarized using TreeAnnotator v1.8.0[41].

Antigenic analysis

We analyzed amino acid sequences, deduced from nucleotide sequences, encoding the HA1 domain (327 amino acids for subtype H1 and 329 amino acids for subtype H3). The sequence alignment was done with MUSCLE v3.8.3[31]. We computed pairwise distances between amino acid sequences to construct a dissimilarity matrix, using the method p-distance in MEGA software (version 7.0.26)[42]. Genetic clusters were defined by Ward's method based on the Euclidean distances among strains, and a 3-dimensional (3D) genetic map was constructed with Multidimensional Scaling (MDS) method, using the XLSTAT software (version 2020.1.2)[25]. Worldwide human seasonal IAV sequences, between 1990 and 2008, were included in the analyses. Reference sequences were obtained from GISAID EpiFlu Database[32].

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Author Contributions

JM and VN designed the study. LA and GB obtained the isolates. JM and VN performed the propagation and preparation viruses for sequencing. RAM and PPC performed the sequencing. JM and CV performed the phylogenetic analyzes. RT performed the antigenic analyzes. JM and RT made the figures. JM, RT and VN wrote the manuscript and all the authors contributed to revise. All authors have read the final version of the manuscript and accept responsibility for the veracity and originality of the work.

Additional Information

Competing Interests: The authors declare no competing interests.

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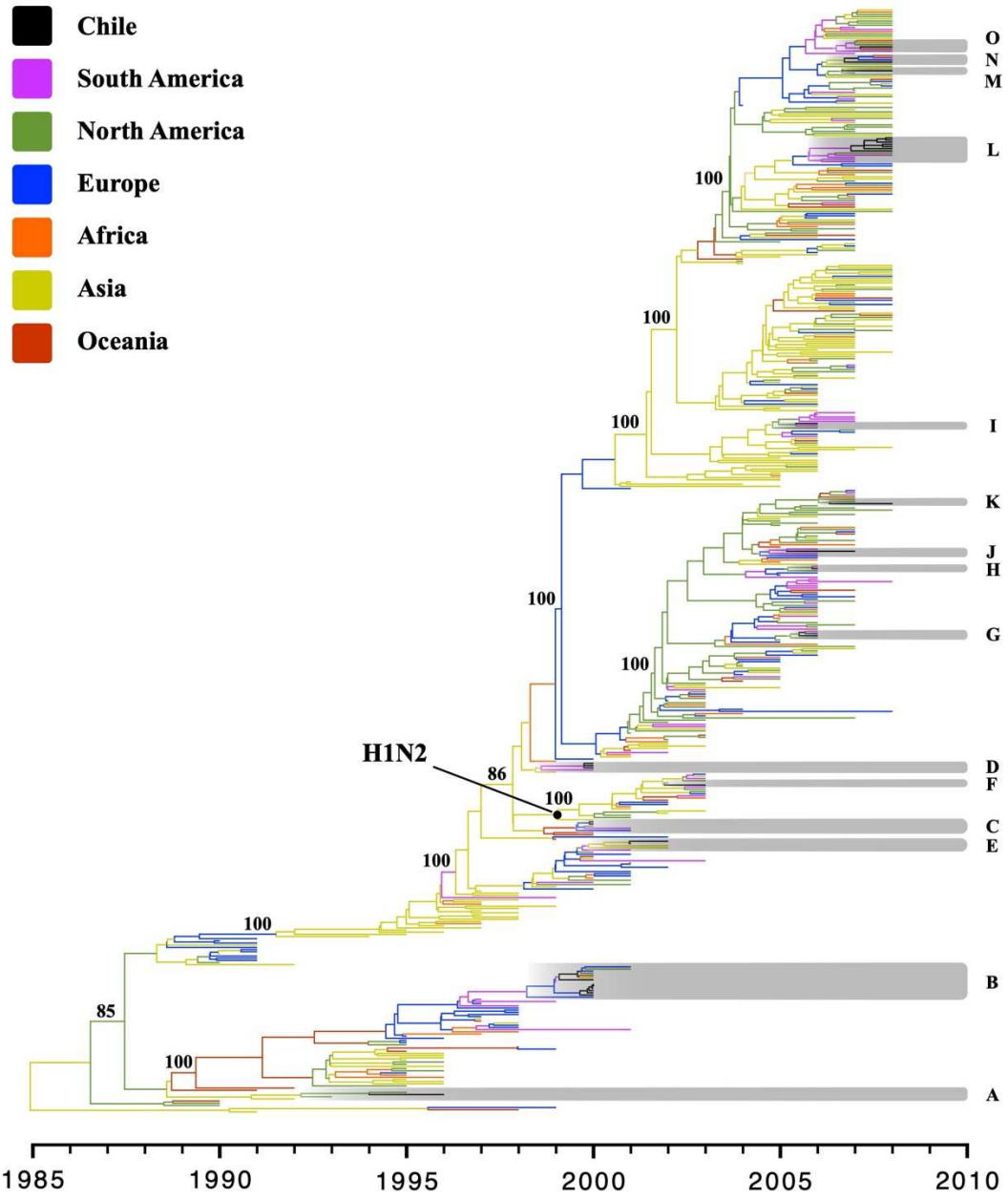


418

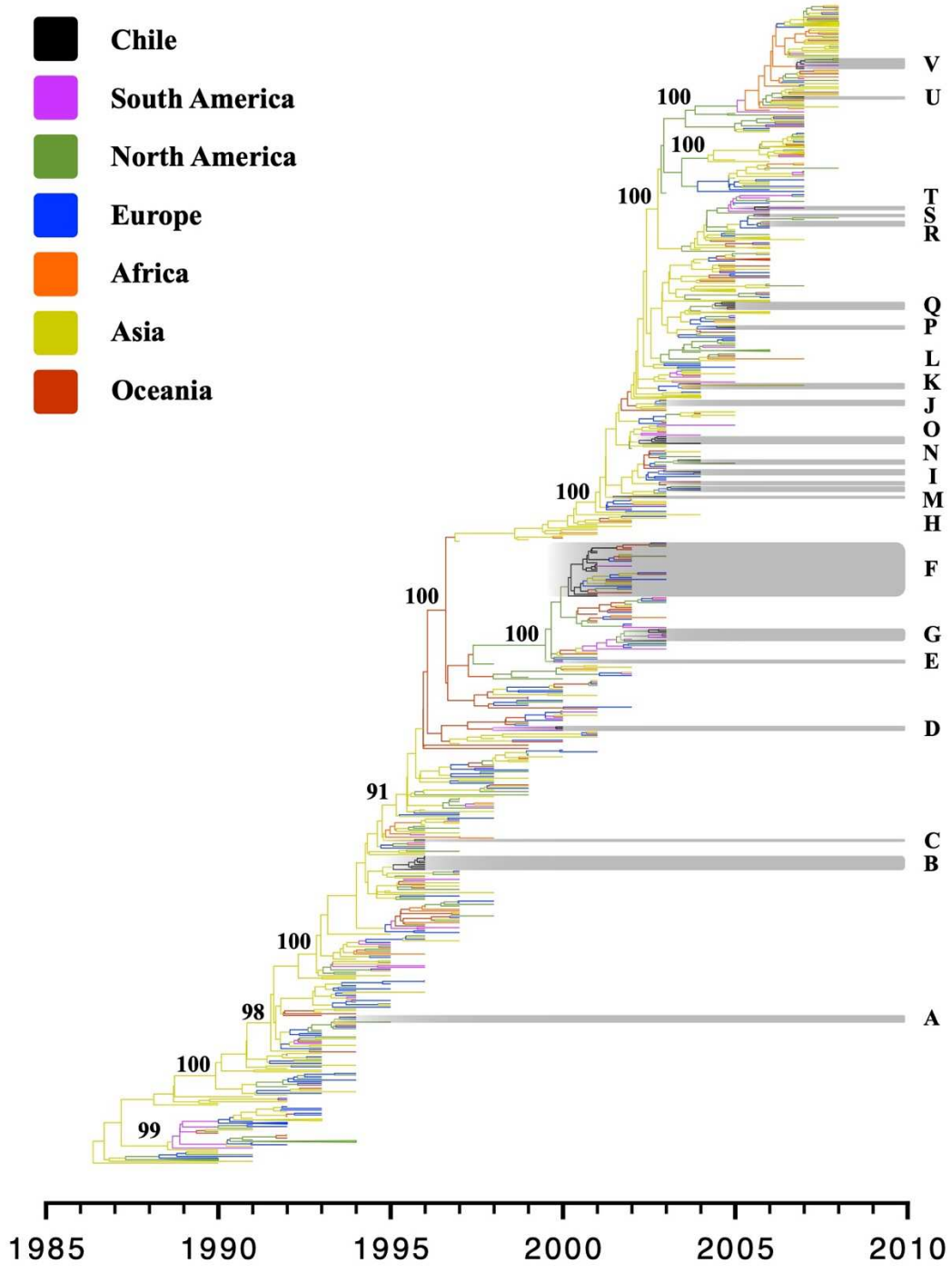
419 **Figure 1**. **Time-scale Bayesian MCC tree of the HA1 portion of IAVs subtype H1**

420 **isolated around the world in the period 1990-2008**. Branches are shaded by continent of

421 origin. The clades that Chilean sequences group are highlighted and identified with the

422 letters A-O. The A/H1N2 clade is identified. The posterior probabilities are included for

423 key nodes.



424

425 **Figure 2**. **Time-scale Bayesian MCC tree of the HA1 portion of IAVs subtype H3**

426 **isolated around the world in the period 1990-2008**. Branches are shaded by continent of

427 origin. The clades that Chilean sequences group are highlighted and identified with the

428 letters A-V. The posterior probabilities are included for key nodes.

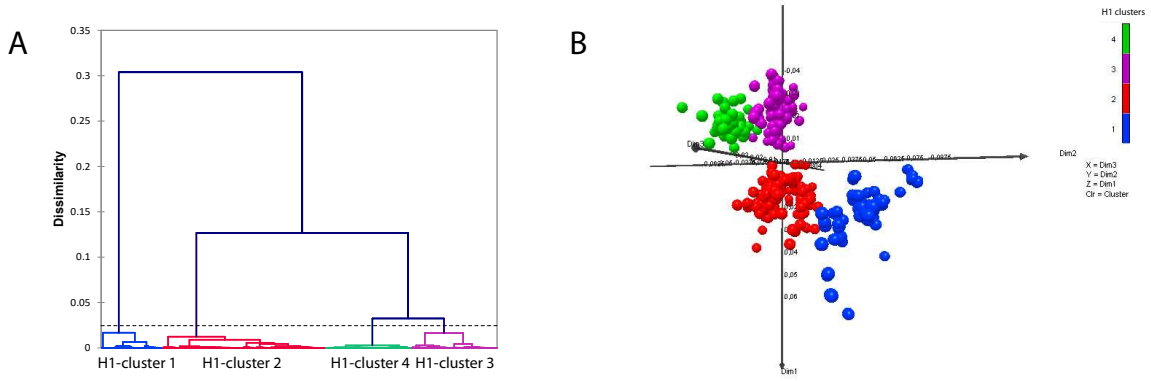


Figure 3. Genetic analysis of H1 influenza A viruses (IAVs) based on HA1 amino acid sequences. (A) Genetic clusters were defined by Ward's method based on the Euclidean distances among the strains. (B) A 3-dimensional (3D) genetic map was constructed by Multidimensional Scaling (MDS) method. All axes represent amino acid distance (percent of distance) and the orientation of the map within these axes is free. Circles represent IAV strains used in this study. Color represents the genetic clusters: H1-cluster 1 is blue, H1-cluster 2 is red, H1-cluster 3 is purple, and H1-cluster 4 is green.

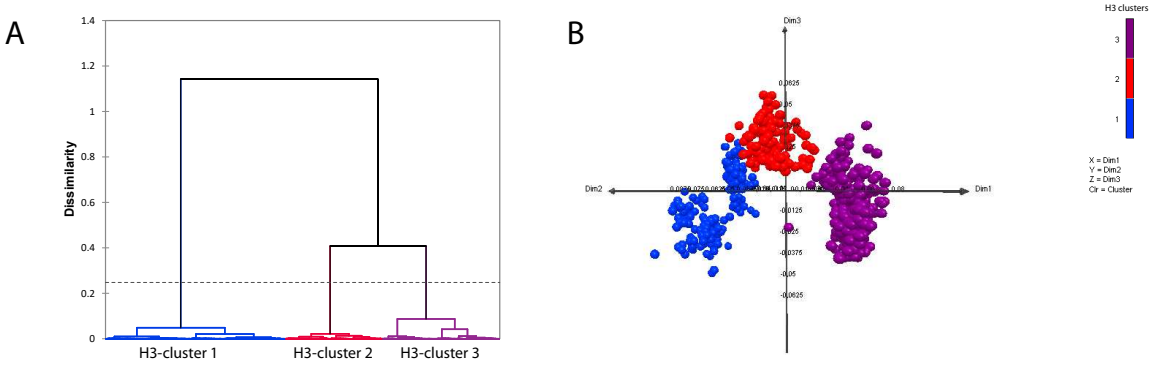


Figure 4. Genetic analysis of H3 influenza A viruses (IAVs) based on HA1 amino acid sequences. (A) Genetic clusters were defined by Ward's method based on the Euclidean distances among the strains. (B) A 3-dimensional (3D) genetic map was constructed by Multidimensional Scaling (MDS) method. All axes represent amino acid distance (percent

443 of distance) and the orientation of the map within these axes is free. Circles represent IAV
444 strains used in this study. Color represents the genetic clusters: H3-cluster 1 is blue, H3-
445 cluster 2 is red, and H3-cluster 3 is purple.
446
447 **Table 1**. Chilean human-origin IAV sequences obtained between 1994 and 2008. Forty-two
448 genomes of the subtype H1N1(12) and H3N2 (30), were obtained in this study. Only two
449 complete genomes of the H1N1 subtype were previously published and were isolated in
450 2000 and 2001.
451

Year isolation	H1N1 subtype							
	HA	**NA**	**PB2**	**PB1**	**PA**	**NP**	**M**	**NS**
1996	1	1	1	1	1	1	1	1
2000	12	12	12	12	12	12	12	12
2001	1	1	1	1	1	1	1	1
2002	1	-	-	-	-	-	-	-
2006	3	-	-	-	-	-	1	-
2007	1	1	-	-	-	-	-	-
2008	11	11	-	-	-	-	7	-
All	**30**	**26**	**14**	**14**	**14**	**14**	**22**	**14**

Year isolation	H3N2 subtype							
	HA	**NA**	**PB2**	**PB1**	**PA**	**NP**	**M**	**NS**
1994	1	-	-	-	-	-	-	-

1996	12	11	11	11	11	11	11	11
1997	-	1	-	-	-	-	-	-
2000	3	-	-	-	-	-	-	-
2001	11	8	8	8	8	8	9	8
2003	10	2	2	2	2	2	2	2
2004	5	5	5	5	5	5	5	5
2005	5	3	3	3	3	3	3	3
2006	3	2	-	-	-	-	-	-
2007	4	3	1	1	1	1	5	1
All	**54**		**30**	**30**	**30**	**30**	**35**	**30**

452

453

Figures

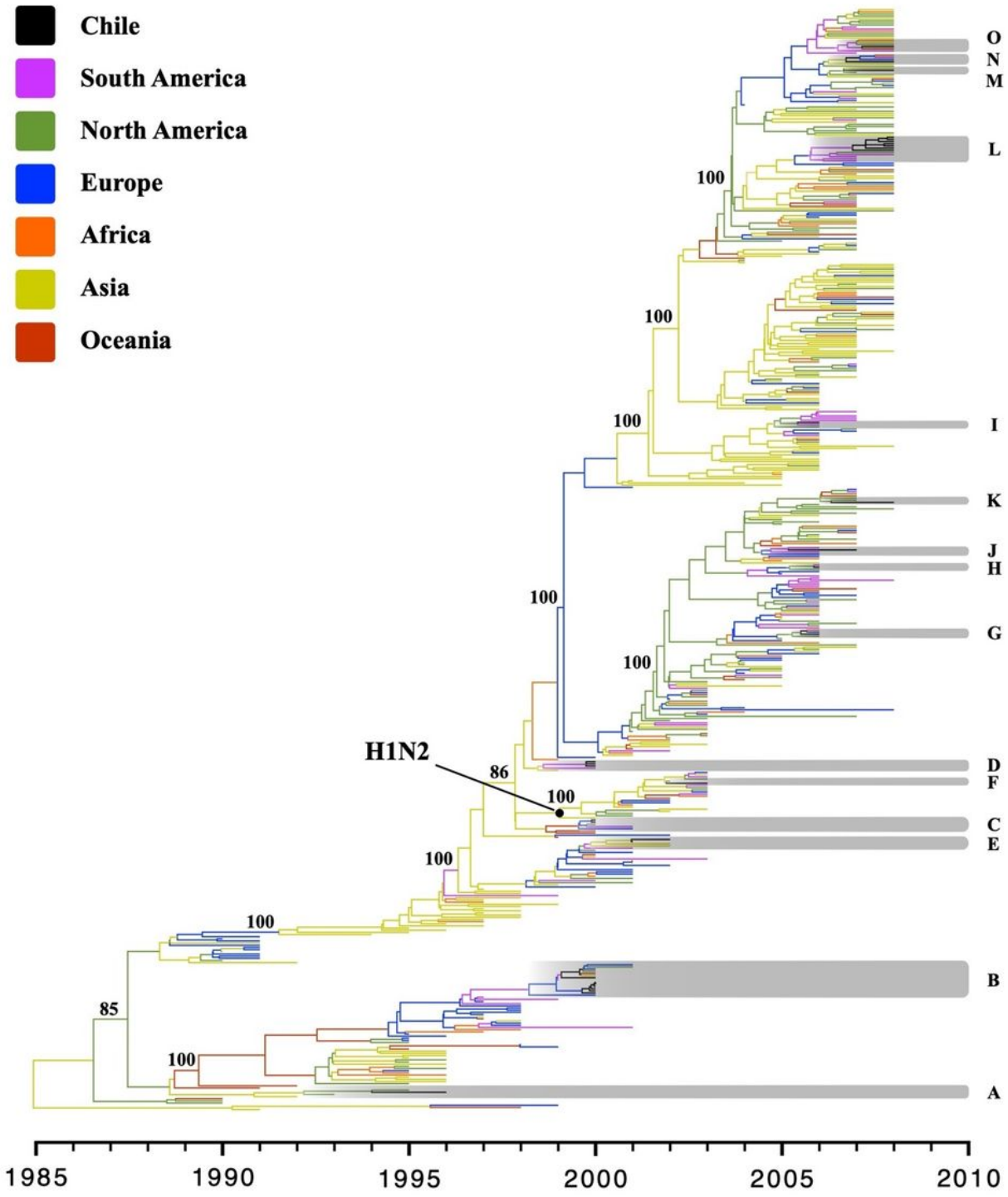


Figure 1

Time-scale Bayesian MCC tree of the HA1 portion of IAVs subtype H1 isolated around the world in the period 1990-2008. Branches are shaded by continent of origin. The clades that Chilean sequences group

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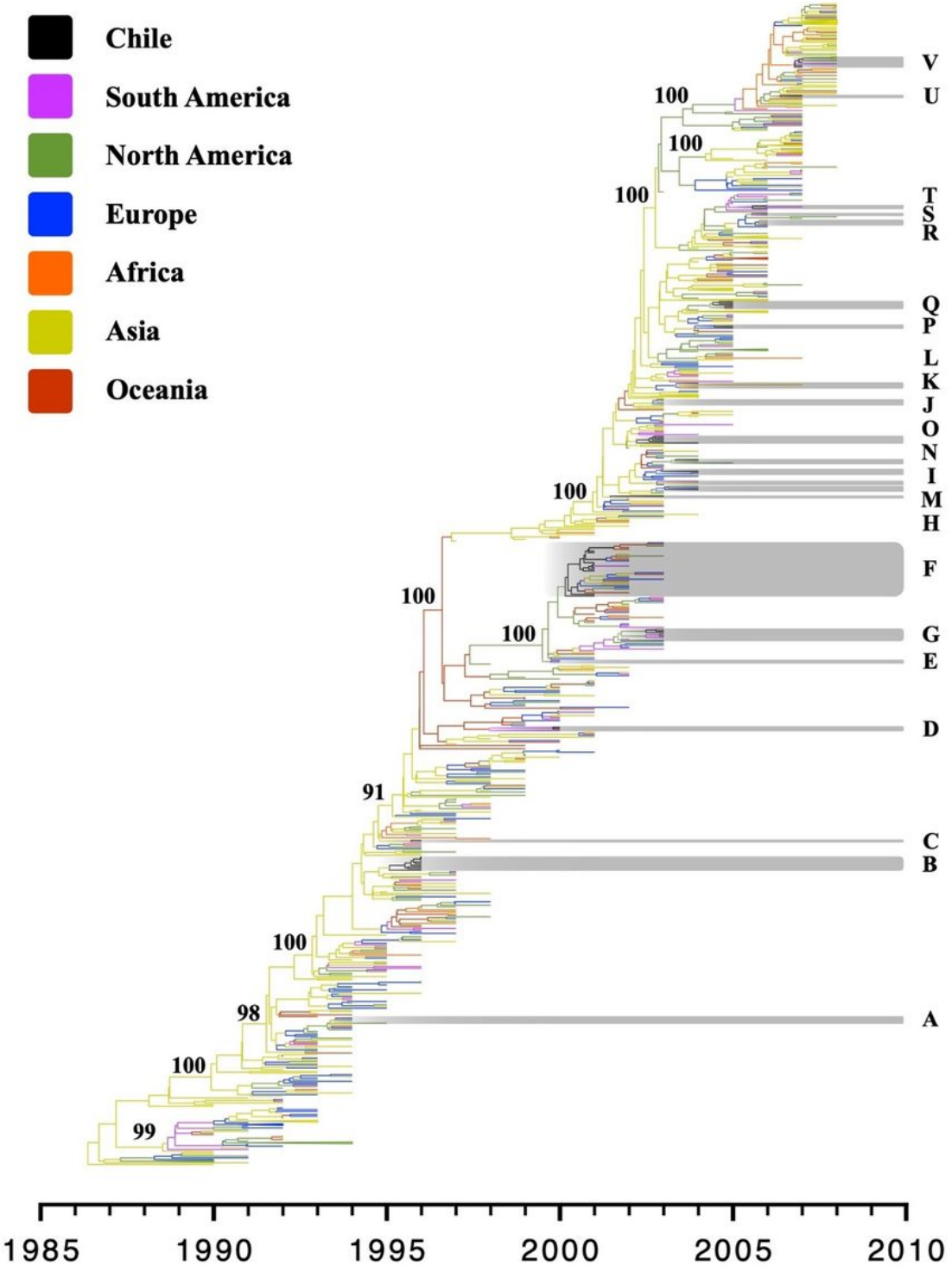

Figure 2

Time-scale Bayesian MCC tree of the HA1 portion of IAVs subtype H3 isolated around the world in the period 1990-2008. Branches are shaded by continent of origin. The clades that Chilean sequences group are highlighted and identified with the letters A-V. The posterior probabilities are included for key nodes.

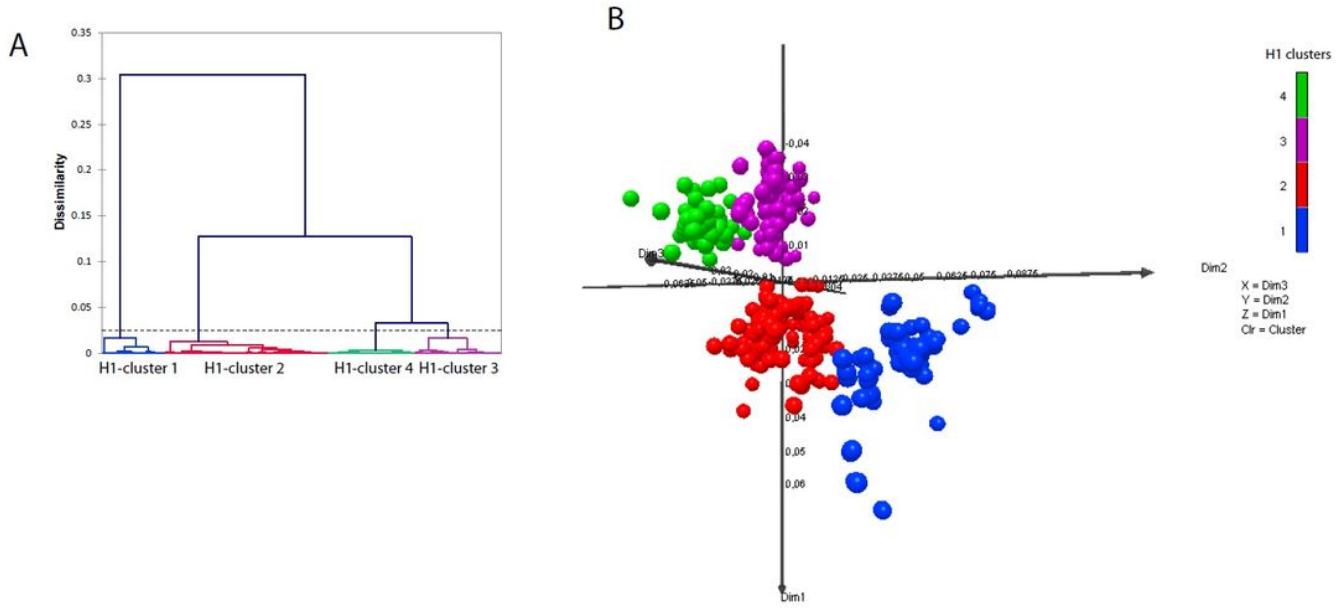

Figure 3

Genetic analysis of H1 influenza A viruses (IAVs) based on HA1 amino acid sequences. (A) Genetic clusters were defined by Ward's method based on the Euclidean distances among the strains. (B) A 3-dimensional (3D) genetic map was constructed by Multidimensional Scaling (MDS) method. All axes represent amino acid distance (percent of distance) and the orientation of the map within these axes is free. Circles represent IAV strains used in this study. Color represents the genetic clusters: H1-cluster 1 is blue, H1- cluster 2 is red, H1-cluster 3 is purple, and H1-cluster 4 is green.

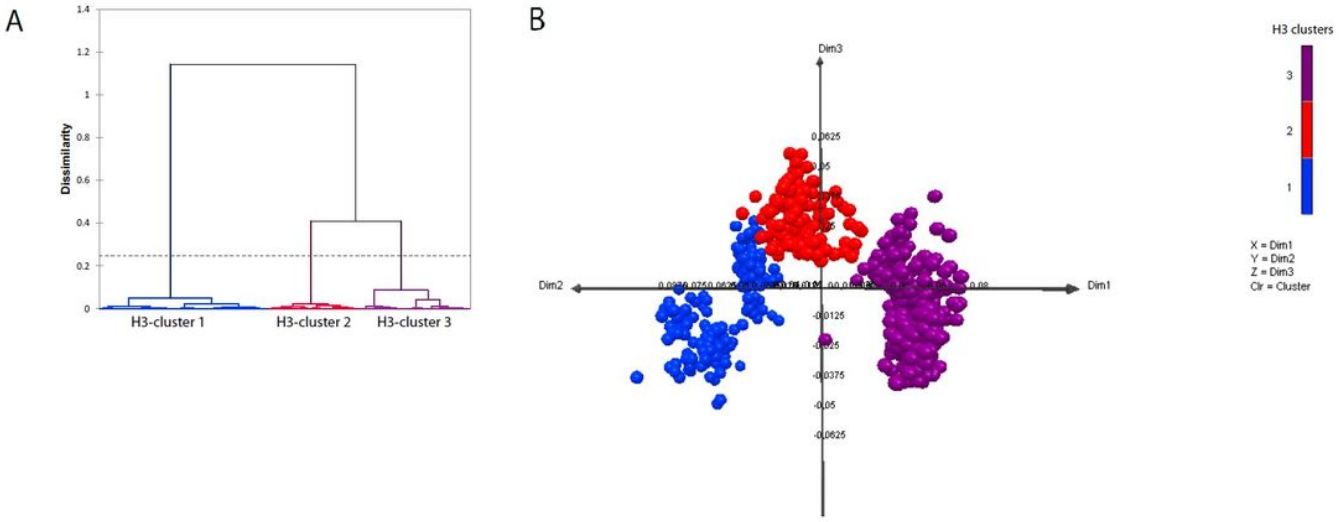

Figure 4

Genetic analysis of H3 influenza A viruses (IAVs) based on HA1 amino acid sequences. (A) Genetic clusters were defined by Ward's method based on the Euclidean distances among the strains. (B) A 3-dimensional (3D) genetic map was constructed by Multidimensional Scaling (MDS) method. All axes represent amino acid distance (percent of distance) and the orientation of the map within these axes is 443 free. Circles represent IAV strains used in this study. Color represents the genetic clusters: H3-cluster 1 is blue, H3- cluster 2 is red, and H3-cluster 3 is purple.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterial.pdf