How do influenza viruses mutate?

2 main molecular mechanisms

Reassortment
(antigenic shift)

Mutations
(antigenic drift)

Consequences of the changes:
- Host range, ability to spread
- Drug susceptibility change
- Vaccine escape

Sebastian Maurer-Stroh

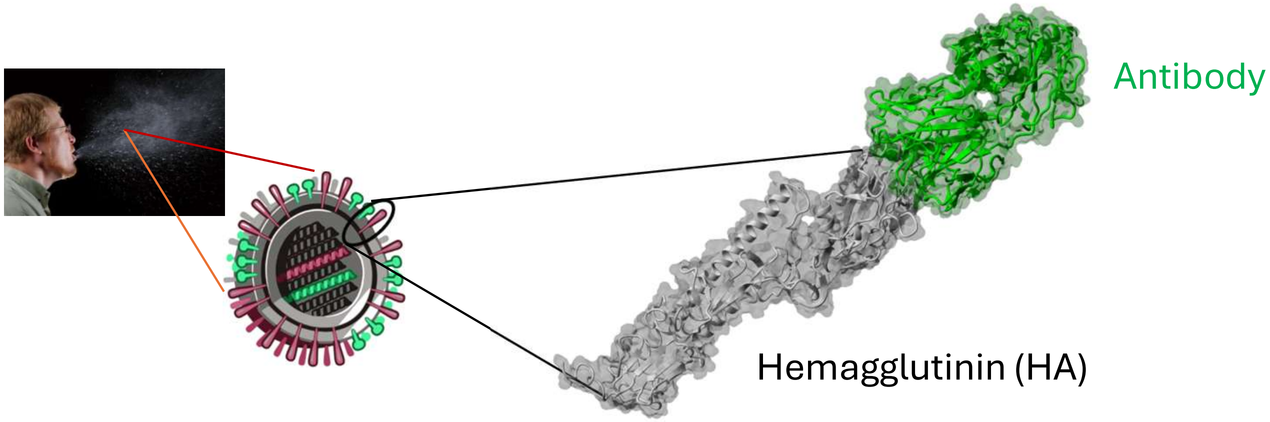
How do influenza viruses mutate?

2 main molecular mechanisms

Reassortment
(antigenic shift)

Mutations
(antigenic drift)

Antigenic Drift (Mutations)



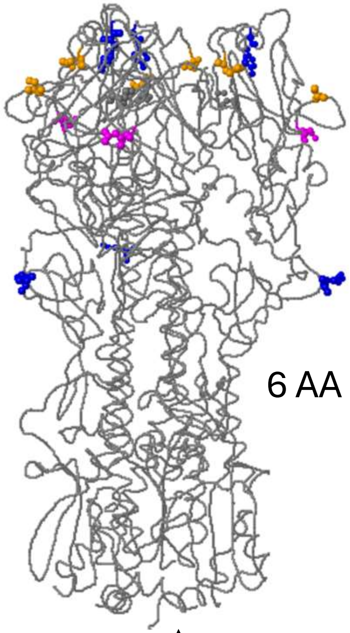
Hemagglutinin (the H in H1N1, H3N2, **H5N1** etc.) is the major surface protein of influenza viruses and is recognized by our immune system through antibodies which prevent the virus from infecting our cells.

Seasonal H3N2 vaccine strain differences

WHO Global Influenza Programme

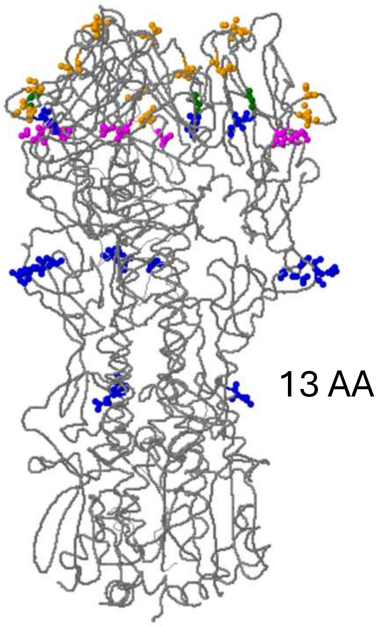
Croatia/2023 vs.

Thailand/2022



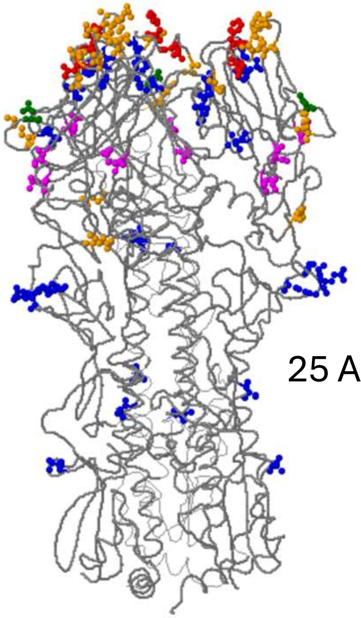
6 AA diff

Darwin/2021



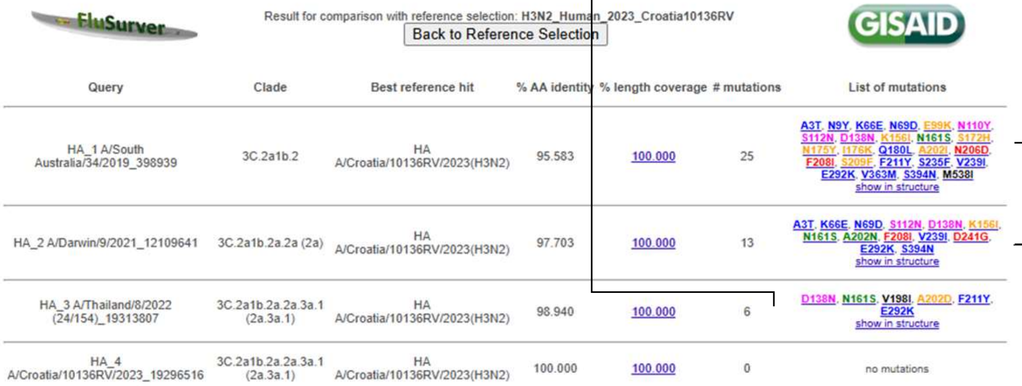
13 AA diff

South Australia/2019



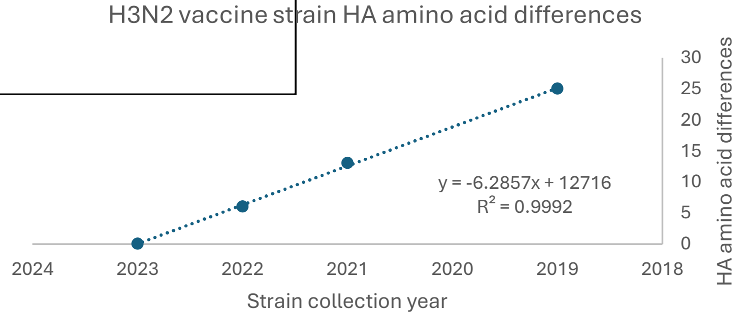
25 AA diff

Seasonal H3 mutation rate is ~6 HA amino acids per year



Result for comparison with reference selection: H3N2_Human_2023_Croatia10136RV

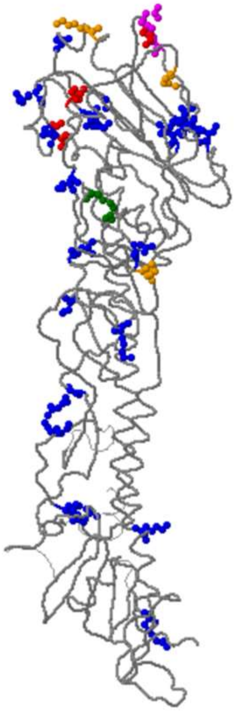
Query	Clade	Best reference hit	% AA identity	% length coverage	# mutations	List of mutations
HA_1 A/South Australia/34/2019_398939	3C.2a1b.2	HA A/Croatia/10136RV/2023(H3N2)	95.583	100.000	25	A3T, N9Y, K66E, N69D, E99K, N110Y, S112N, D138N, K156I, N161S, S172H, N175Y, I176K, Q180L, A202I, N206D, F208I, S209F, F211Y, S235F, V239I, E292K, V363M, S394N, M538I show in structure
HA_2 A/Darwin/9/2021_12109641	3C.2a1b.2a.2a (2a)	HA A/Croatia/10136RV/2023(H3N2)	97.703	100.000	13	A3T, K66E, N69D, S112N, D138N, K156I, N161S, A202N, F208I, V239I, D241G, E292K, S394N show in structure
HA_3 A/Thailand/8/2022 (24/154)_19313807	3C.2a1b.2a.2a.3a.1 (2a.3a.1)	HA A/Croatia/10136RV/2023(H3N2)	98.940	100.000	6	D138N, N161S, V198I, A202D, F211Y, E292K show in structure
HA_4 A/Croatia/10136RV/2023_19296516	3C.2a1b.2a.2a.3a.1 (2a.3a.1)	HA A/Croatia/10136RV/2023(H3N2)	100.000	100.000	0	no mutations



GISRS
GLOBAL INFLUENZA SURVEILLANCE & RESPONSE SYSTEM
Uniting the world in the fight against influenza

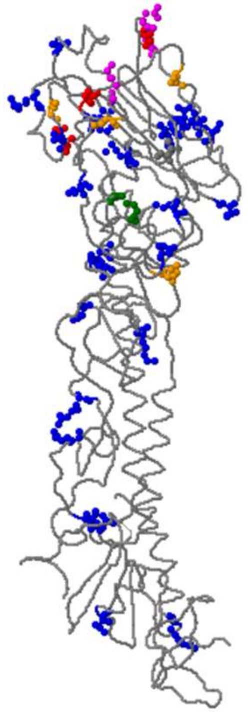
Clade 1
(A/VietNam/1203/2004)

Clade 2.3.2.1a
(A/Hubei/1/2010)



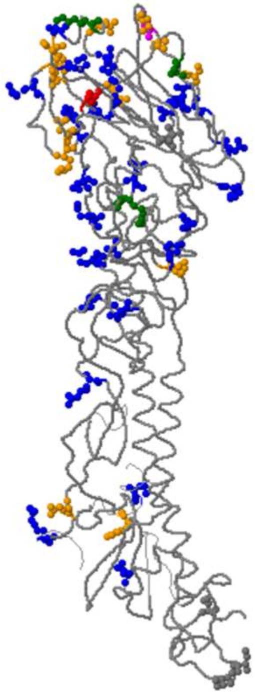
33 AA diff

Clade 2.3.2.1c
(A/Alberta/1/2014)



40 AA diff

Clade 2.3.4.4b
(A/Astrakhan/3212/
2020)

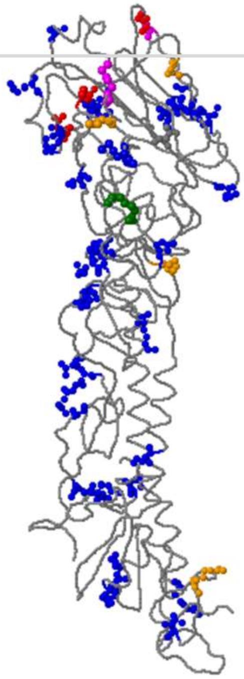


44 AA diff

Clade 2.3.2.1a
(A/Victoria/149/2024)

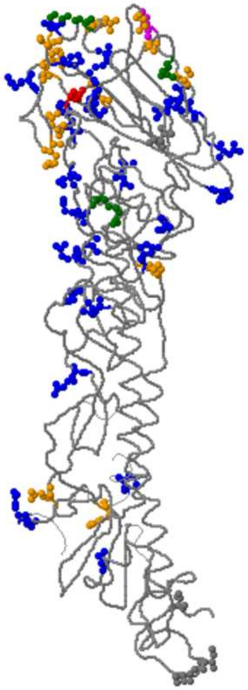
43 AA diff

Clade 2.3.2.1c
(A/Cambodia/SVH24
0441/2024)

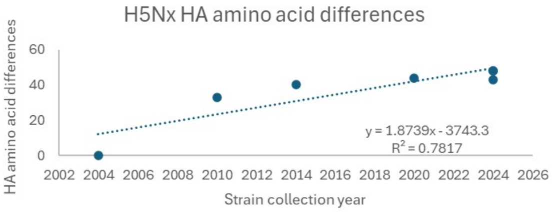


48 AA diff

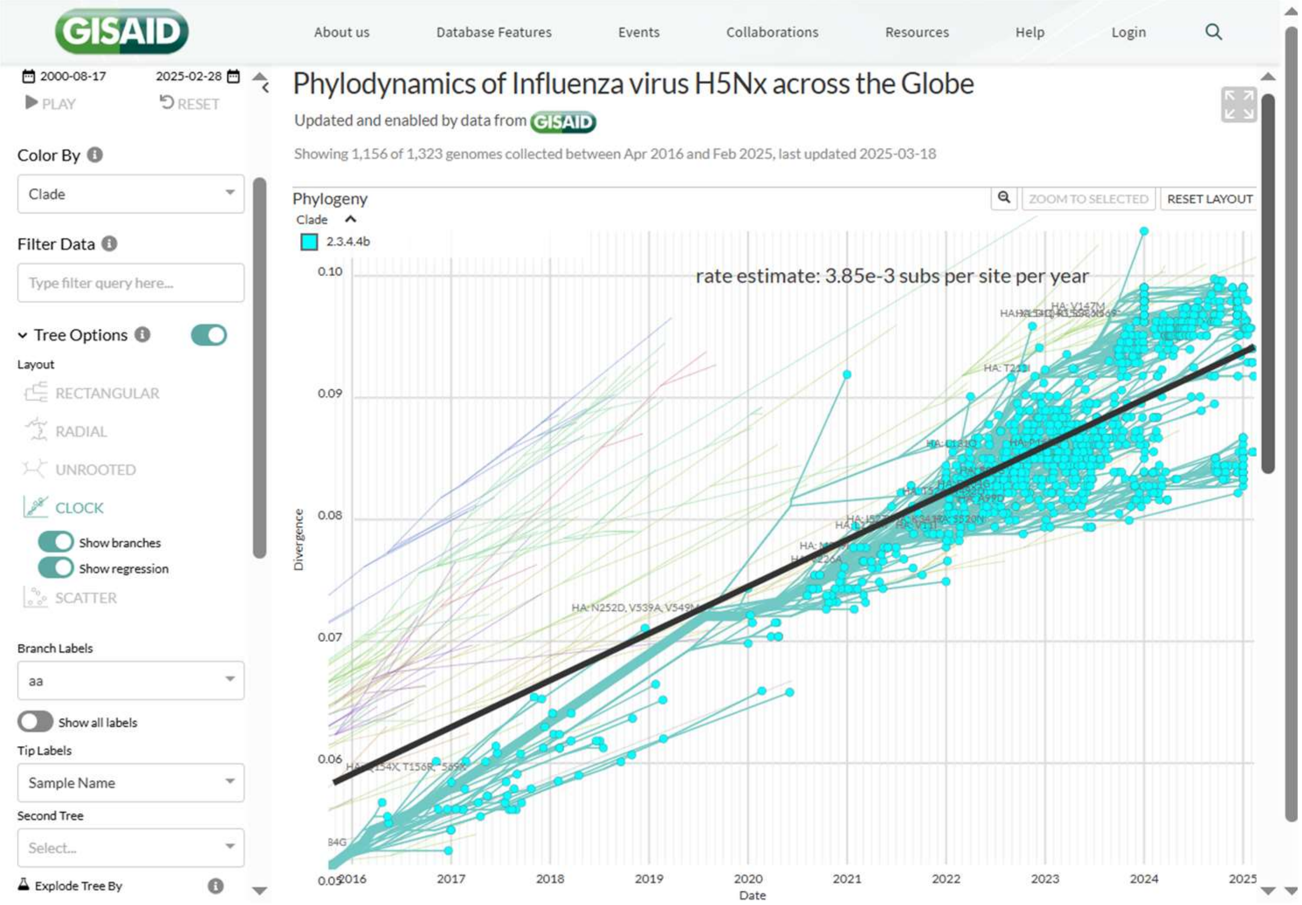
Clade 2.3.4.4b
A/Texas/37/2024



48 AA diff



Avian H5 mutation rate is ~2 HA amino acids per year



Phylodynamics of Influenza virus H5Nx across the Globe

Updated and enabled by data from GISAID

Showing 1,156 of 1,323 genomes collected between Apr 2016 and Feb 2025, last updated 2025-03-18

Color By: Clade

Layout: CLOCK

Show branches

Show regression

Branch Labels: aa

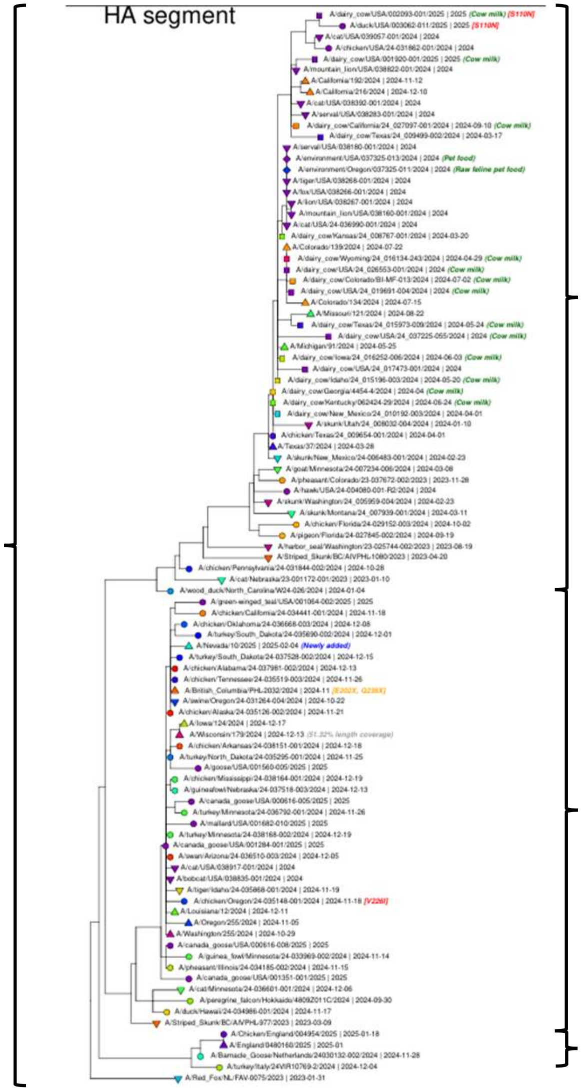
Tip Labels: Sample Name

rate estimate: 3.85e-3 subs per site per year

HA nucleotide substitutions per site per year

Subtype		Clade	
H3N2	4.70E-03		
H1N1	3.63E-03		
B Vic	1.92E-03		
H5Nx	3.89E-03	***2.3.2.1a***	4.17E-03
		2.3.2.1c/e/g	4.82E-03
		2.3.4.4b	3.85E-03

Genotypes in 2.3.4.4b outbreak



North America Cow-related

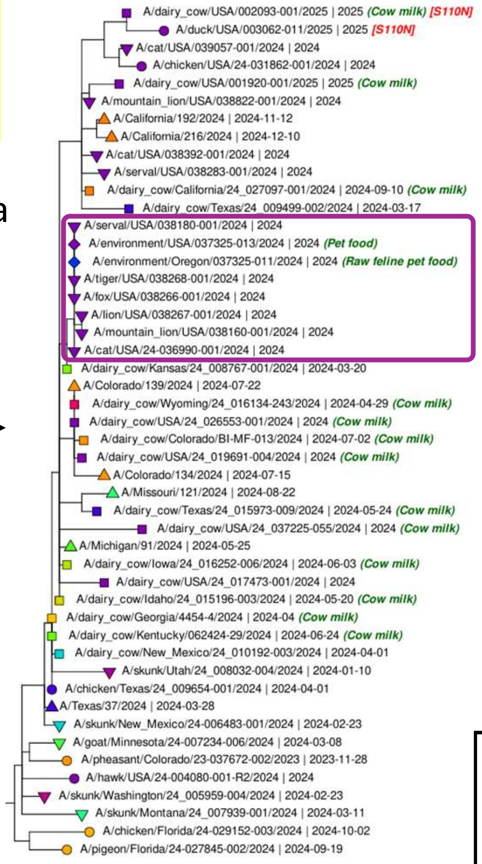
B3.13

North America Bird-related

D1.1

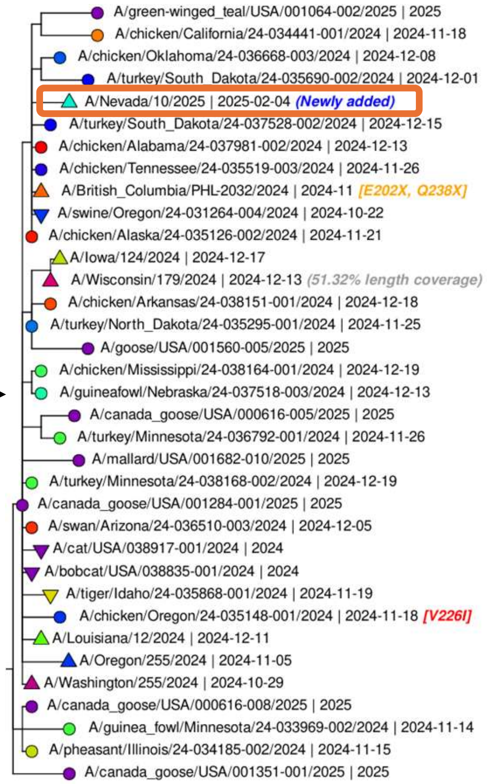
DI.2

UK Bird-related



Pet food/zoo cluster

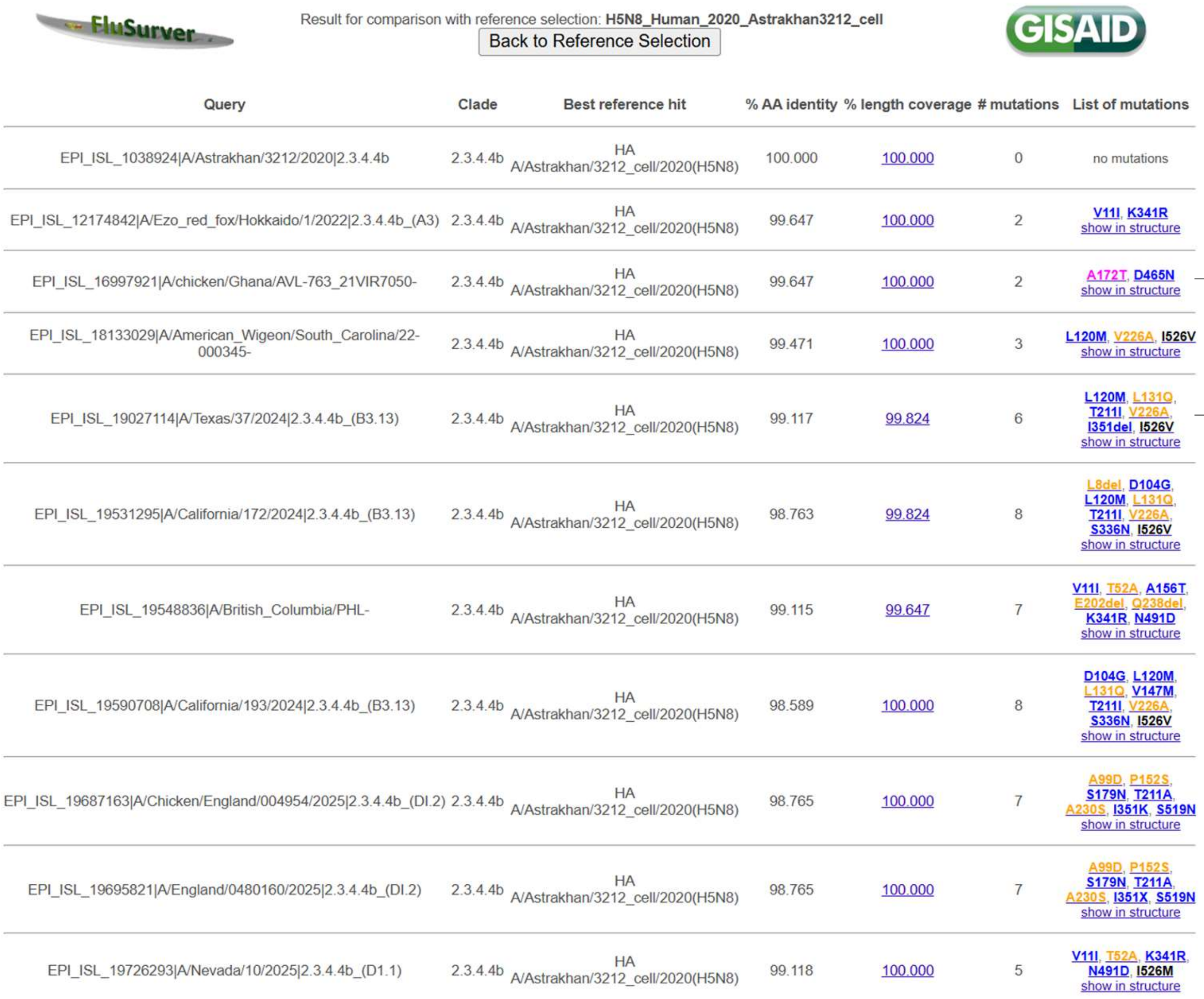
Cow-related



We gratefully acknowledge the Authors from Originating and Submitting laboratories of sequence and metadata on which this analysis is based.

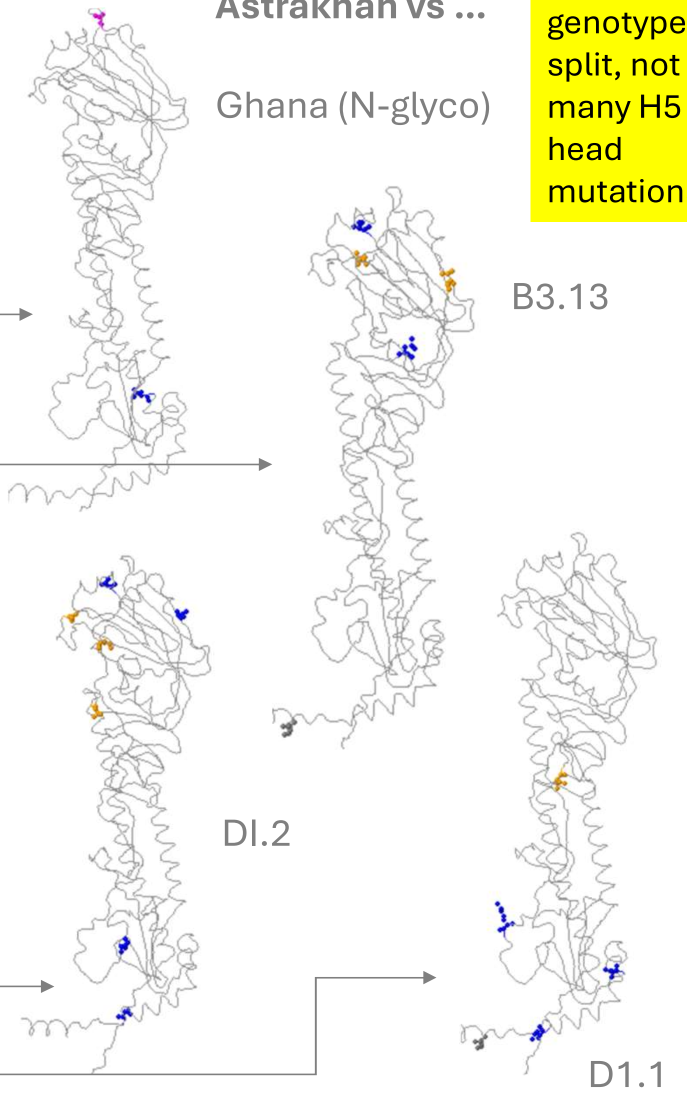
Astrakhan vs …

Despite genotype split, not many H5 head mutations



Result for comparison with reference selection: **H5N8_Human_2020_Astrakhan3212_cell**

Query	Clade	Best reference hit	% AA identity	% length coverage	# mutations	List of mutations
EPI_ISL_1038924|A/Astrakhan/3212/2020|2.3.4.4b	2.3.4.4b	HA A/Astrakhan/3212_cell/2020(H5N8)	100.000	100.000	0	no mutations
EPI_ISL_12174842|A/Ezo_red_fox/Hokkaido/1/2022|2.3.4.4b_(A3)	2.3.4.4b	HA A/Astrakhan/3212_cell/2020(H5N8)	99.647	100.000	2	V11I, K341R show in structure
EPI_ISL_16997921|A/chicken/Ghana/AVL-763_21VIR7050-	2.3.4.4b	HA A/Astrakhan/3212_cell/2020(H5N8)	99.647	100.000	2	A172T, D465N show in structure
EPI_ISL_18133029|A/American_Wigeon/South_Carolina/22-000345-	2.3.4.4b	HA A/Astrakhan/3212_cell/2020(H5N8)	99.471	100.000	3	L120M, V226A, I526V show in structure
EPI_ISL_19027114|A/Texas/37/2024|2.3.4.4b_(B3.13)	2.3.4.4b	HA A/Astrakhan/3212_cell/2020(H5N8)	99.117	99.824	6	L120M, L131Q, T211I, V226A, I351del, I526V show in structure
EPI_ISL_19531295|A/California/172/2024|2.3.4.4b_(B3.13)	2.3.4.4b	HA A/Astrakhan/3212_cell/2020(H5N8)	98.763	99.824	8	L8del, D104G, L120M, L131Q, T211I, V226A, S336N, I526V show in structure
EPI_ISL_19548836|A/British_Columbia/PHL-	2.3.4.4b	HA A/Astrakhan/3212_cell/2020(H5N8)	99.115	99.647	7	V11I, T52A, A156T, E202del, Q238del, K341R, N491D show in structure
EPI_ISL_19590708|A/California/193/2024|2.3.4.4b_(B3.13)	2.3.4.4b	HA A/Astrakhan/3212_cell/2020(H5N8)	98.589	100.000	8	D104G, L120M, L131Q, V147M, T211I, V226A, S336N, I526V show in structure
EPI_ISL_19687163|A/Chicken/England/004954/2025|2.3.4.4b_(DI.2)	2.3.4.4b	HA A/Astrakhan/3212_cell/2020(H5N8)	98.765	100.000	7	A99D, P152S, S179N, T211A, A230S, I351K, S519N show in structure
EPI_ISL_19695821|A/England/0480160/2025|2.3.4.4b_(DI.2)	2.3.4.4b	HA A/Astrakhan/3212_cell/2020(H5N8)	98.765	100.000	7	A99D, P152S, S179N, T211A, A230S, I351X, S519N show in structure
EPI_ISL_19726293|A/Nevada/10/2025|2.3.4.4b_(D1.1)	2.3.4.4b	HA A/Astrakhan/3212_cell/2020(H5N8)	99.118	100.000	5	V11I, T52A, K341R, N491D, I526M show in structure



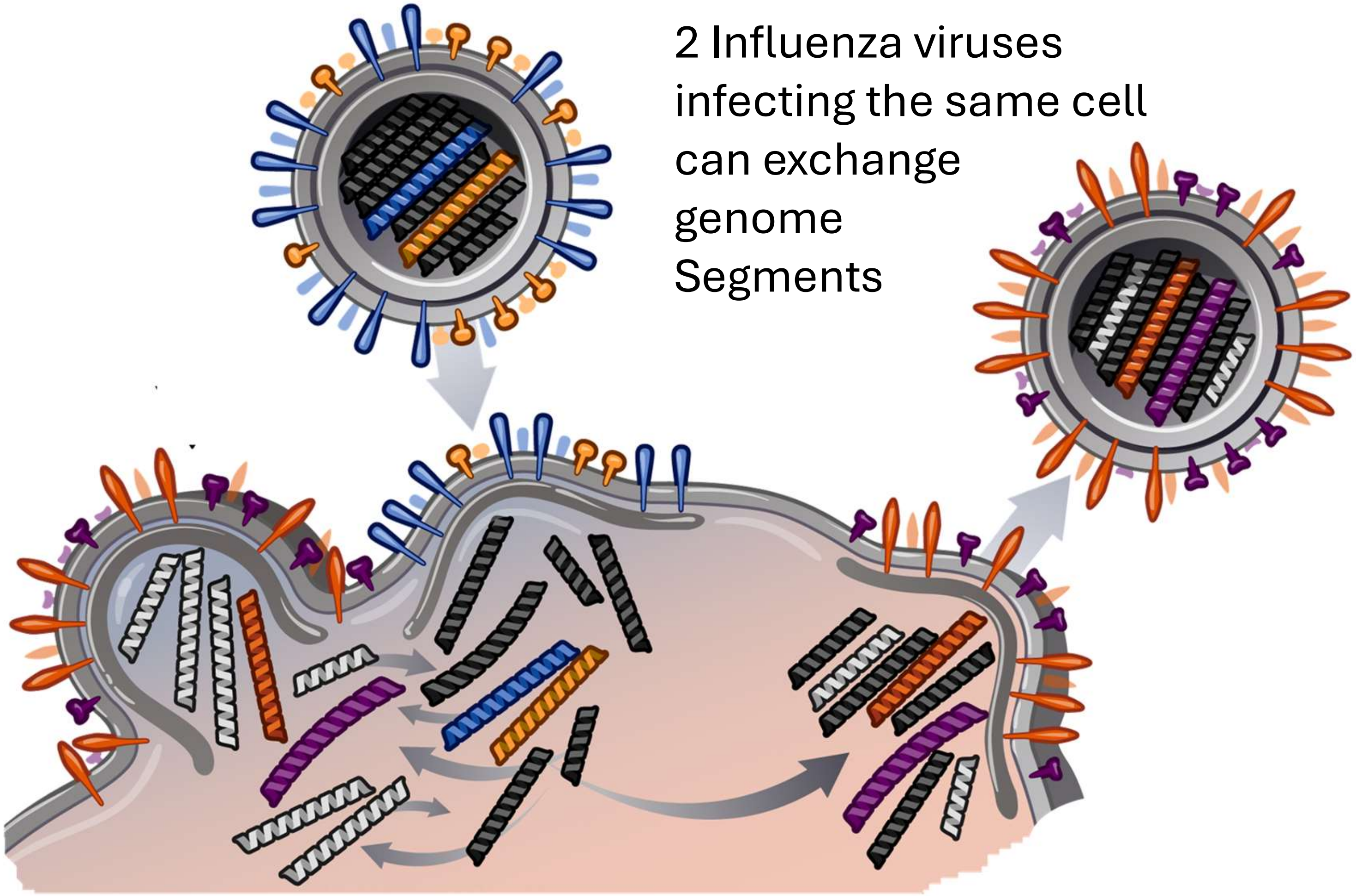
How do influenza viruses mutate?

2 main molecular mechanisms

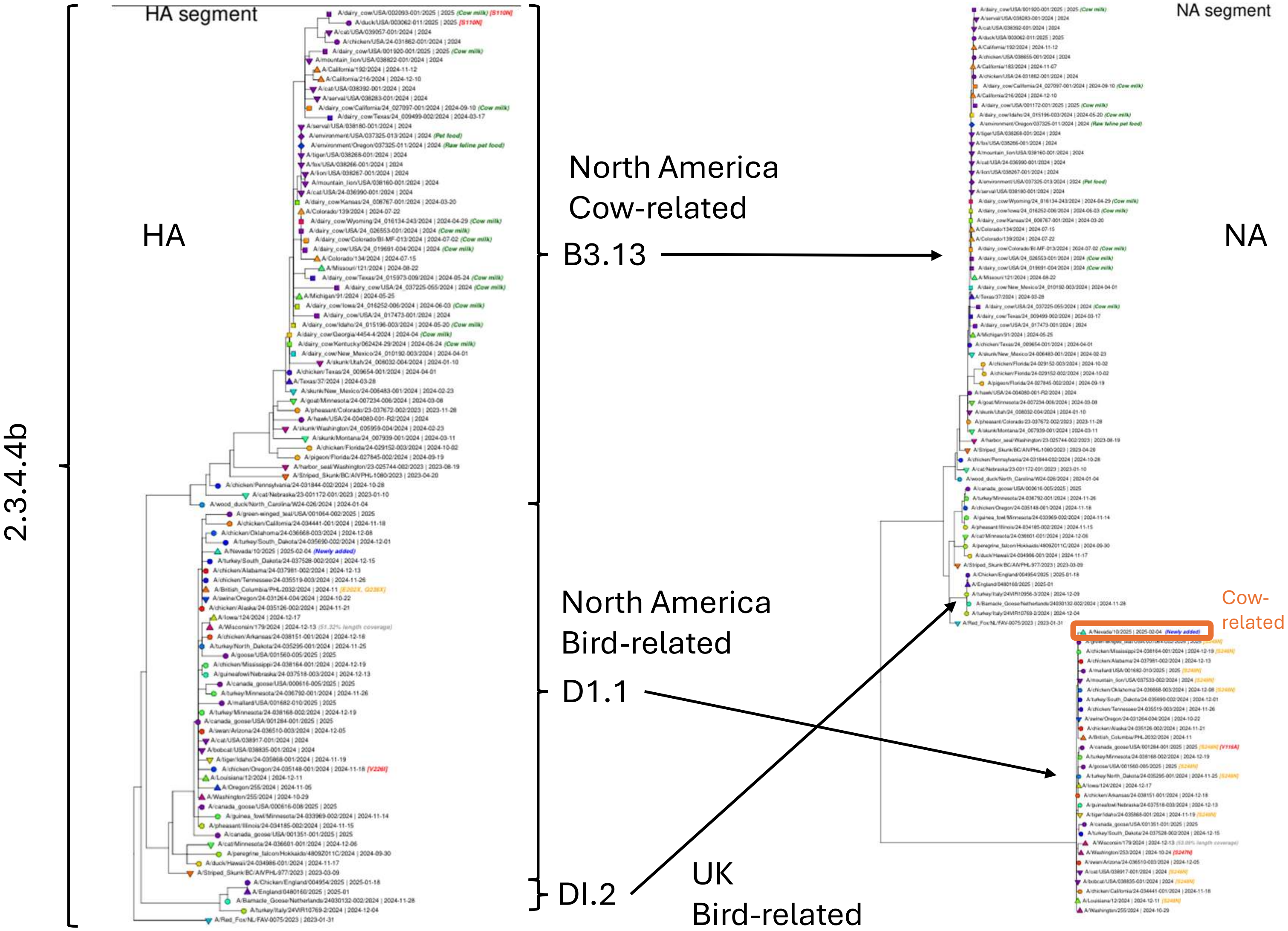
Reassortment
(antigenic shift)

Mutations
(antigenic drift)

Reassortment = Genome Mixing



2 Influenza viruses infecting the same cell can exchange genome Segments



2.3.4.4b

HA

North America
Cow-related

B3.13

NA

North America
Bird-related

D1.1

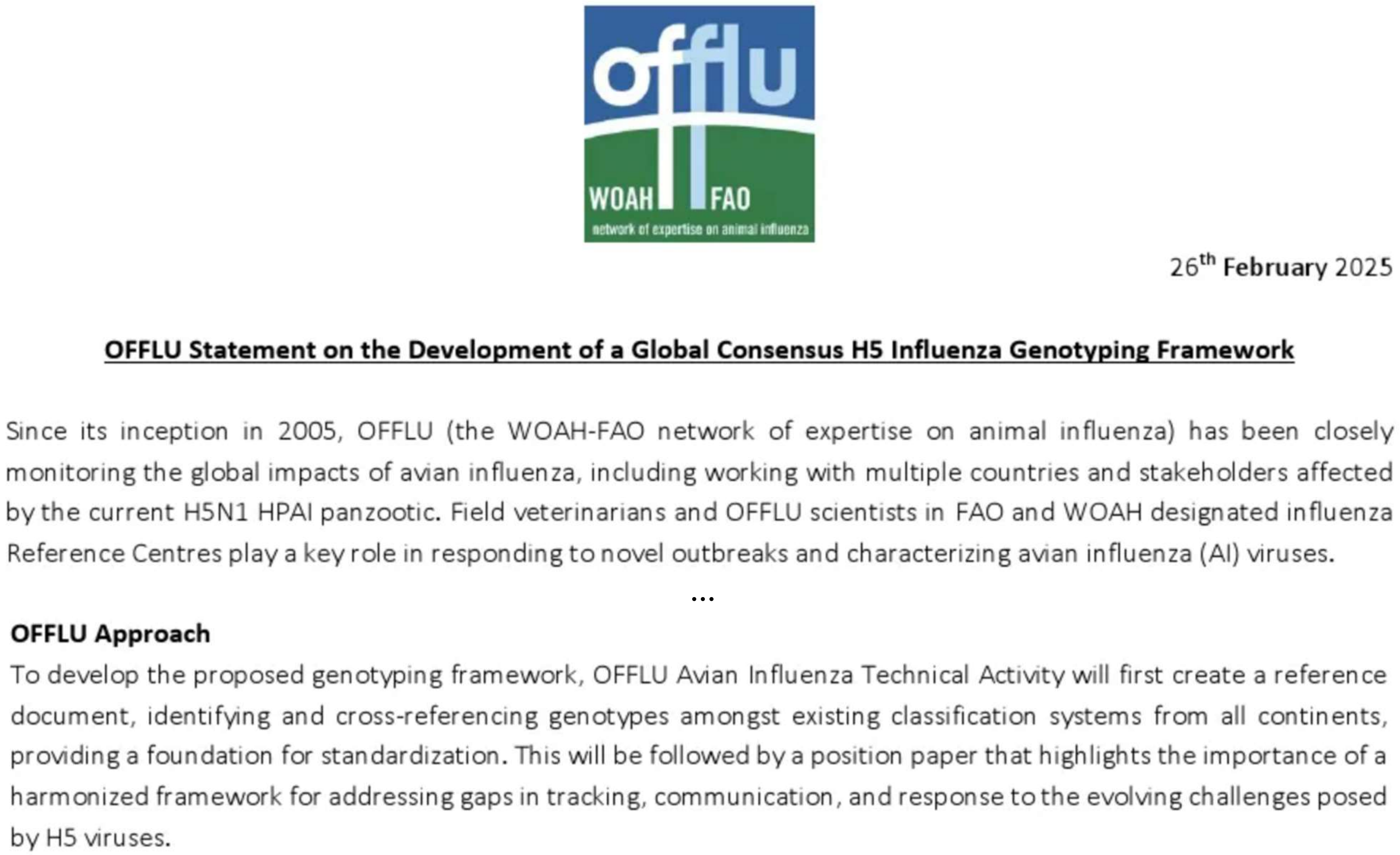
DI.2

UK
Bird-related

Cow-
related

e.g. NA reassorted

Genotypes differentiate reassortment patterns across all 8 segments



26th February 2025

OFFLU Statement on the Development of a Global Consensus H5 Influenza Genotyping Framework

Since its inception in 2005, OFFLU (the WOAH-FAO network of expertise on animal influenza) has been closely monitoring the global impacts of avian influenza, including working with multiple countries and stakeholders affected by the current H5N1 HPAI panzootic. Field veterinarians and OFFLU scientists in FAO and WOAH designated influenza Reference Centres play a key role in responding to novel outbreaks and characterizing avian influenza (AI) viruses.

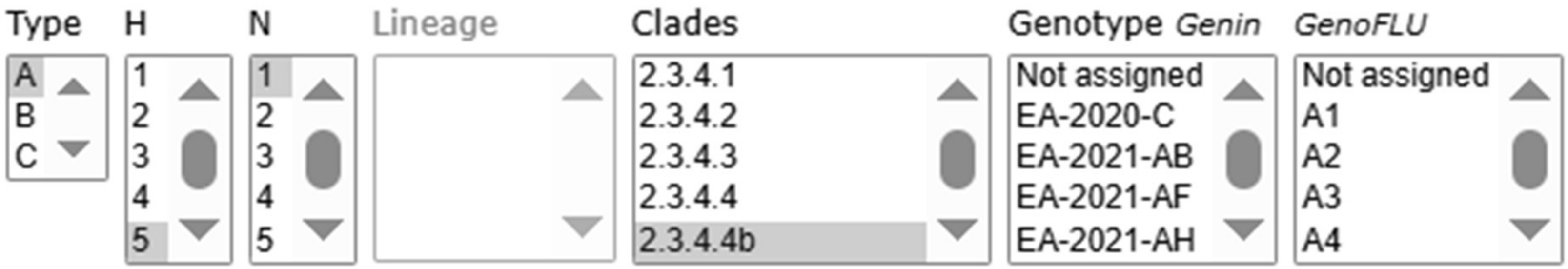
…

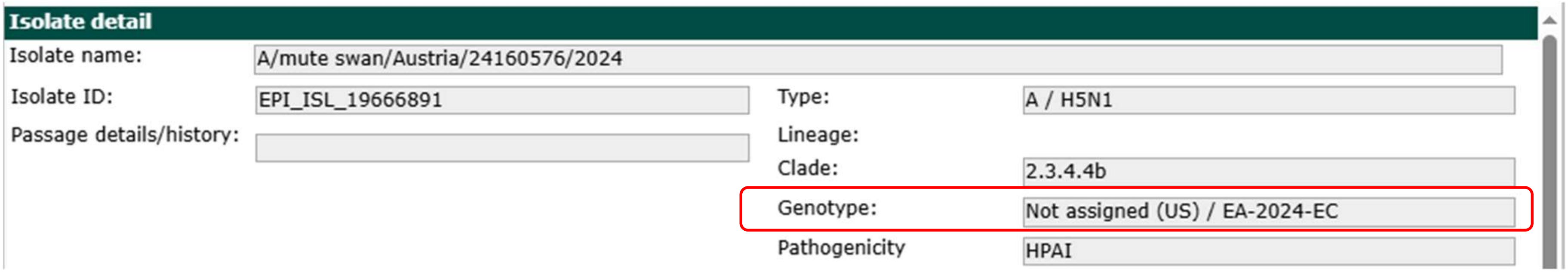
OFFLU Approach

To develop the proposed genotyping framework, OFFLU Avian Influenza Technical Activity will first create a reference document, identifying and cross-referencing genotypes amongst existing classification systems from all continents, providing a foundation for standardization. This will be followed by a position paper that highlights the importance of a harmonized framework for addressing gaps in tracking, communication, and response to the evolving challenges posed by H5 viruses.

https://www.offlu.org/wp-content/uploads/2025/02/OFFLU-Genotyping-Holding-Statement-February-2025-Final.pdf

EpiFlu filters by genotype for H5 Clade 2.3.4.4b (a Rosetta Stone for regional tool nomenclature)





Isolate detail

Isolate name: A/mute swan/Austria/24160576/2024

Isolate ID: EPI_ISL_19666891

Passage details/history:

Type: A / H5N1

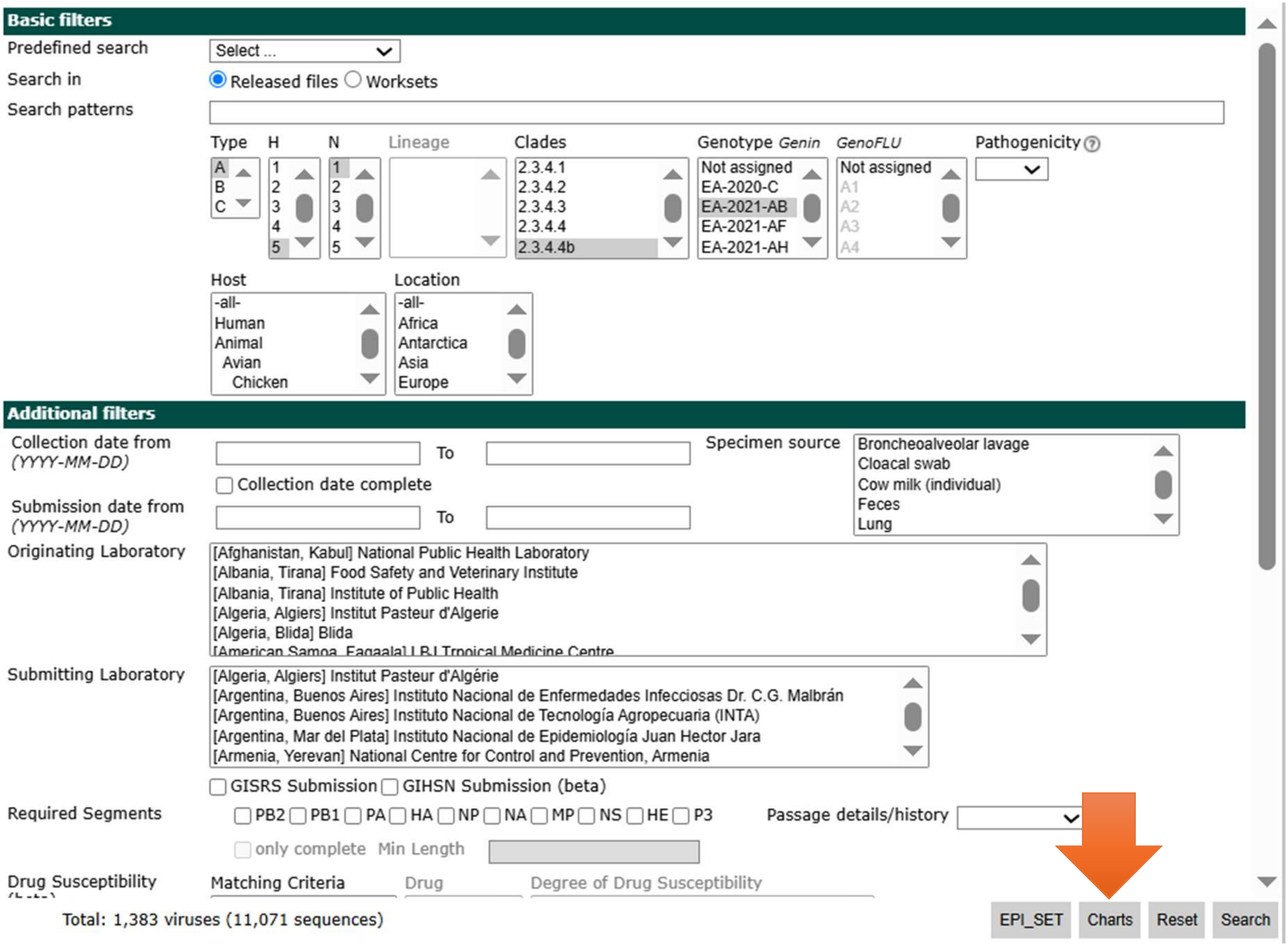
Lineage:

Clade: 2.3.4.4b

Genotype: Not assigned (US) / EA-2024-EC

Pathogenicity HPAI

We gratefully acknowledge the Authors from Originating and Submitting laboratories of sequence and meta data on which the analysis is based.



Basic filters

Predefined search: Select ...

Search in: ● Released files ○ Worksets

Search patterns:

Type: A (selected), B, C

H: 1, 2, 3, 4, 5 (selected)

N: 1 (selected), 2, 3, 4, 5

Lineage

Clades: 2.3.4.1, 2.3.4.2, 2.3.4.3, 2.3.4.4, 2.3.4.4b (selected)

Genotype *Genin*: Not assigned, EA-2020-C, EA-2021-AB (selected), EA-2021-AF, EA-2021-AH

GenoFLU: Not assigned, A1, A2, A3, A4

Pathogenicity

Host: -all-, Human, Animal, Avian, Chicken

Location: -all-, Africa, Antarctica, Asia, Europe

Additional filters

Collection date from *(YYYY-MM-DD)* To

☐ Collection date complete

Submission date from *(YYYY-MM-DD)* To

Specimen source: Broncheoalveolar lavage, Cloacal swab, Cow milk (individual), Feces, Lung

Originating Laboratory:
[Afghanistan, Kabul] National Public Health Laboratory
[Albania, Tirana] Food Safety and Veterinary Institute
[Albania, Tirana] Institute of Public Health
[Algeria, Algiers] Institut Pasteur d'Algerie
[Algeria, Blida] Blida
[American Samoa, Fagaala] LBJ Tropical Medicine Centre

Submitting Laboratory:
[Algeria, Algiers] Institut Pasteur d'Algérie
[Argentina, Buenos Aires] Instituto Nacional de Enfermedades Infecciosas Dr. C.G. Malbrán
[Argentina, Buenos Aires] Instituto Nacional de Tecnología Agropecuaria (INTA)
[Argentina, Mar del Plata] Instituto Nacional de Epidemiología Juan Hector Jara
[Armenia, Yerevan] National Centre for Control and Prevention, Armenia

☐ GISRS Submission ☐ GIHSN Submission (beta)

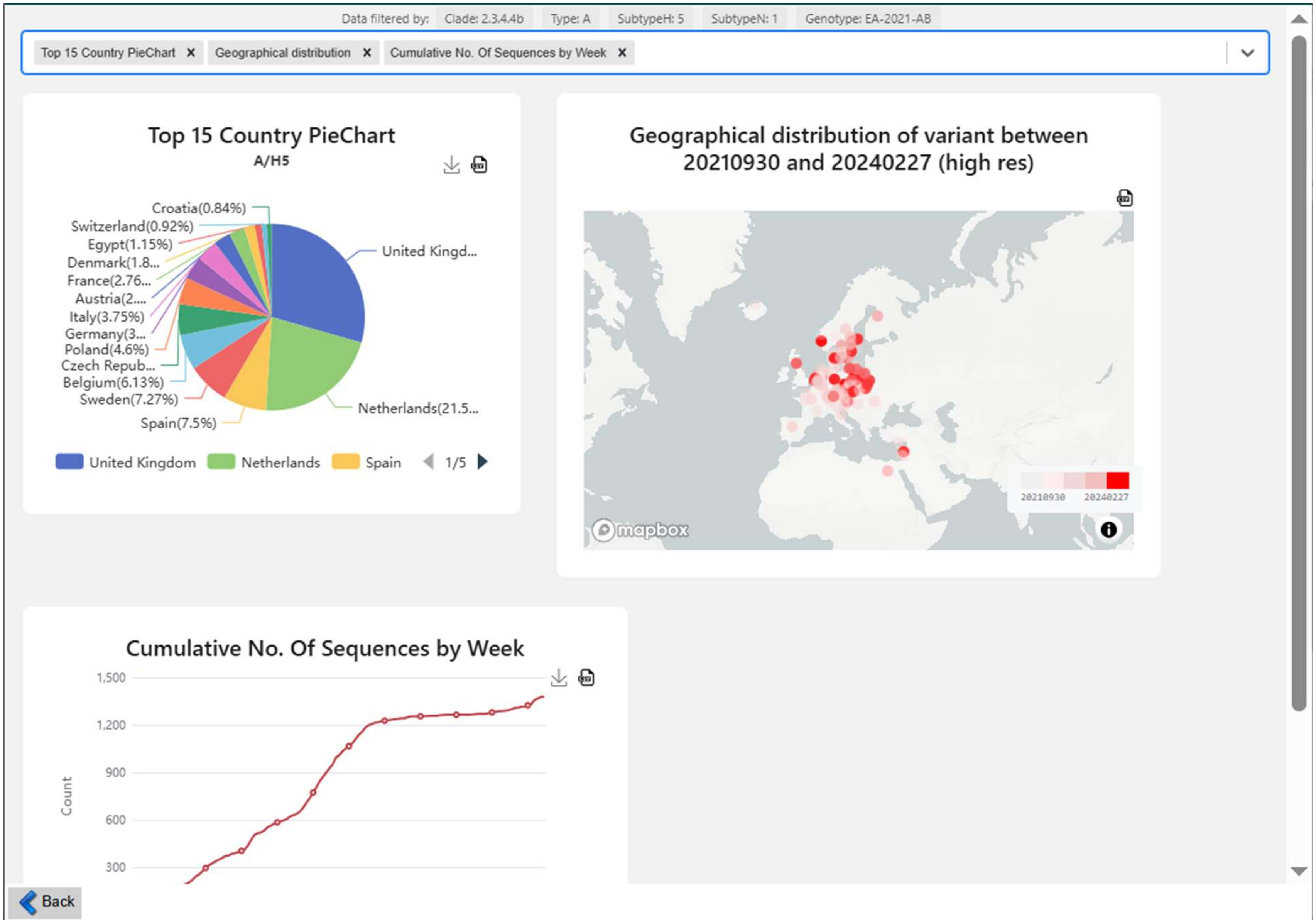
Required Segments: ☐ PB2 ☐ PB1 ☐ PA ☐ HA ☐ NP ☐ NA ☐ MP ☐ NS ☐ HE ☐ P3

Passage details/history

☐ only complete Min Length

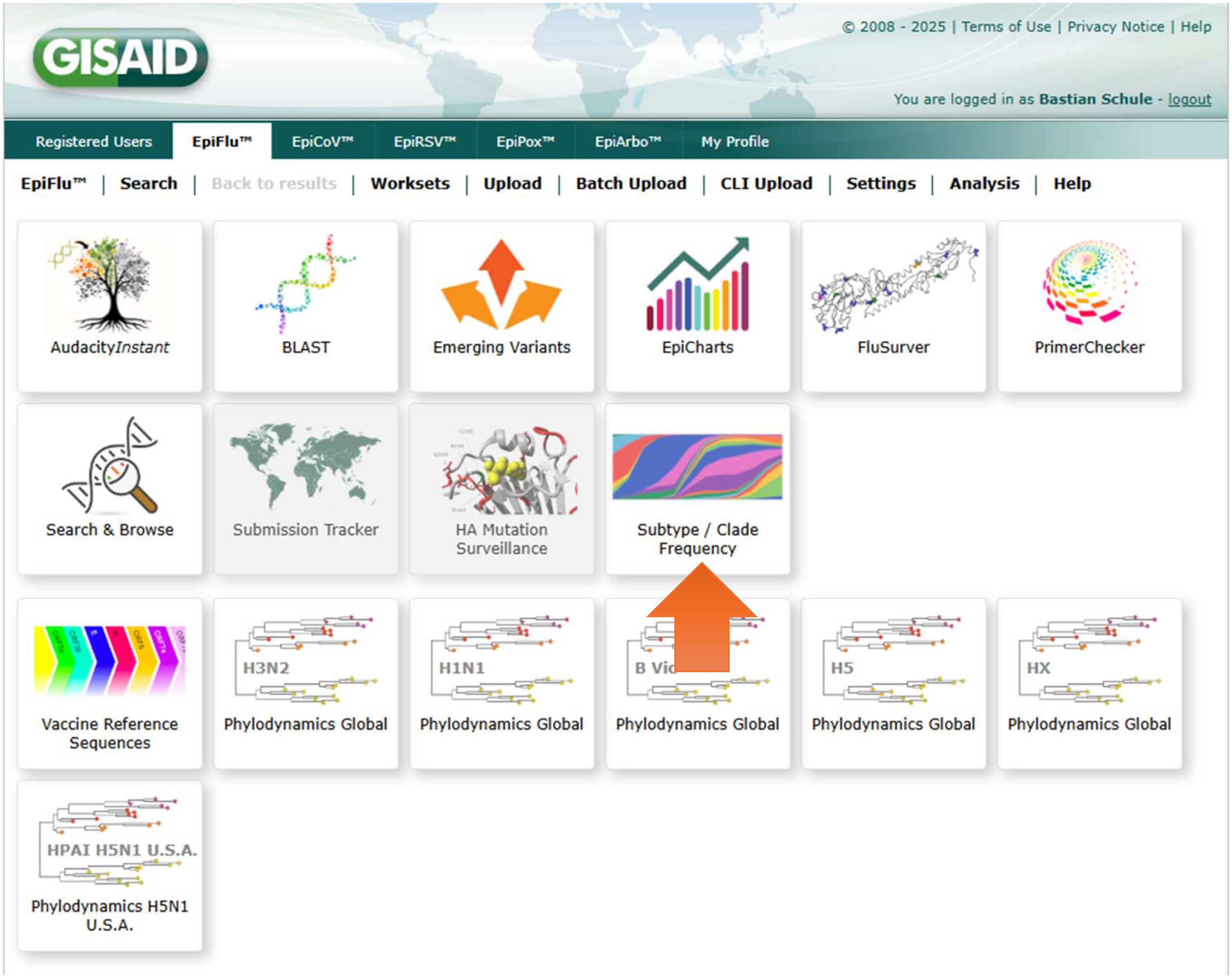
Drug Susceptibility (beta): Matching Criteria, Drug, Degree of Drug Susceptibility

Total: 1,383 viruses (11,071 sequences)



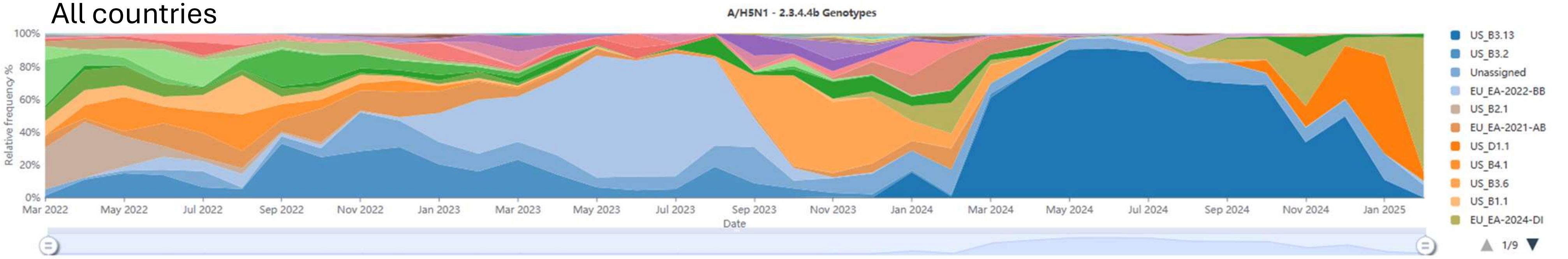
Data filtered by: Clade: 2.3.4.4b Type: A SubtypeH: 5 SubtypeN: 1 Genotype: EA-2021-AB

Top 15 Country PieChart ✕ Geographical distribution ✕ Cumulative No. Of Sequences by Week ✕

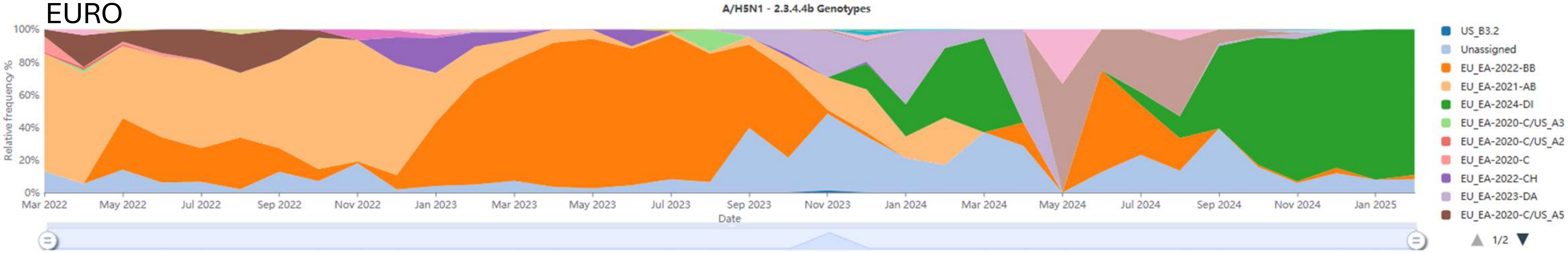


You are logged in as **Bastian Schule** - logout

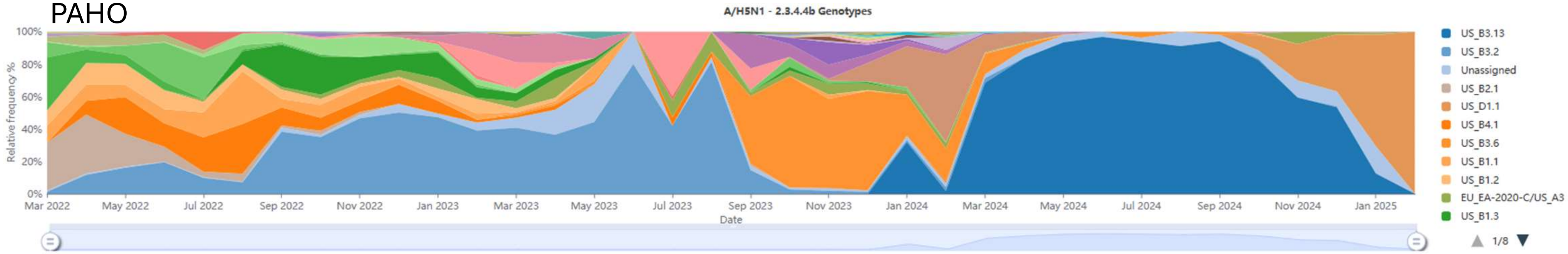
All countries

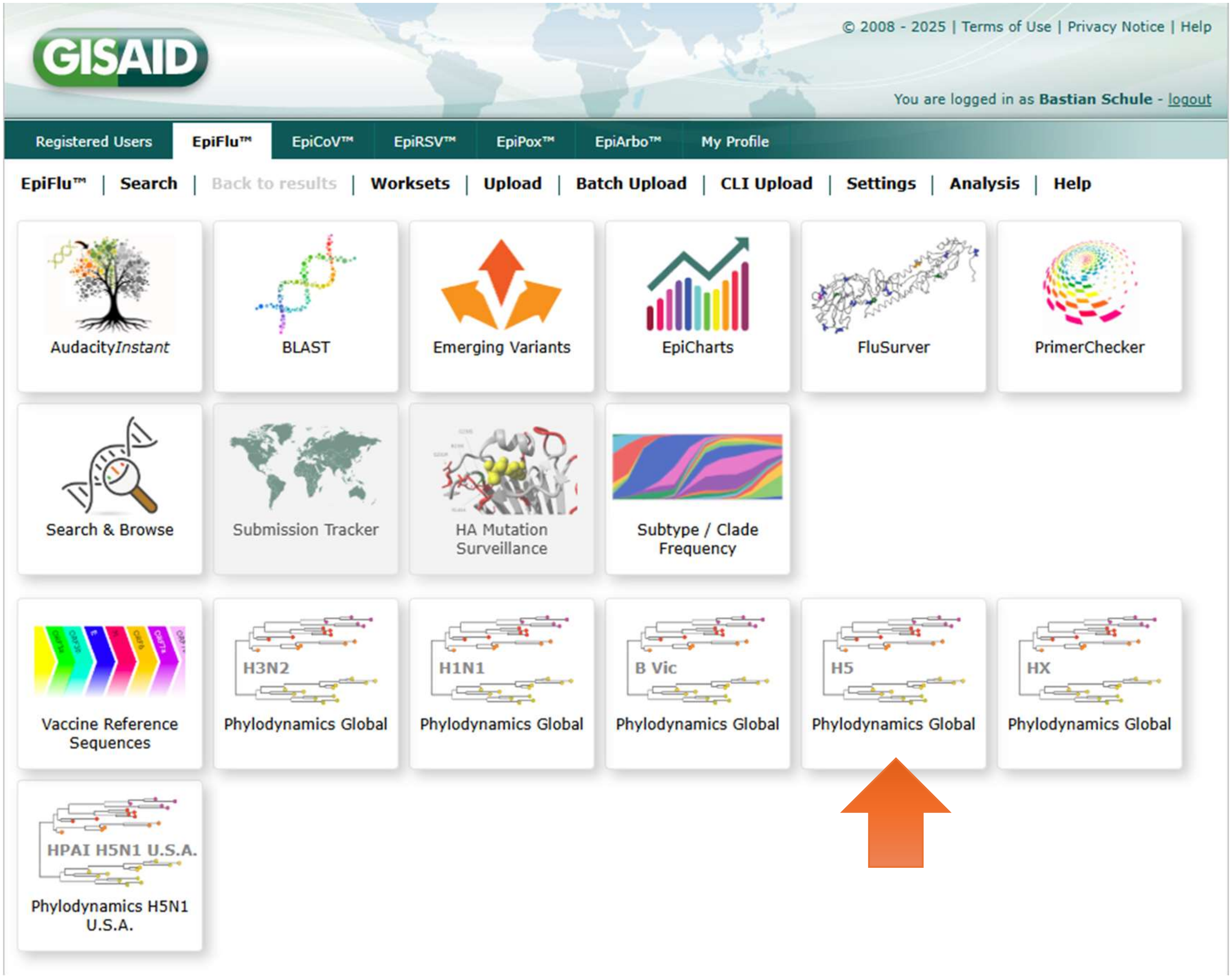


EURO

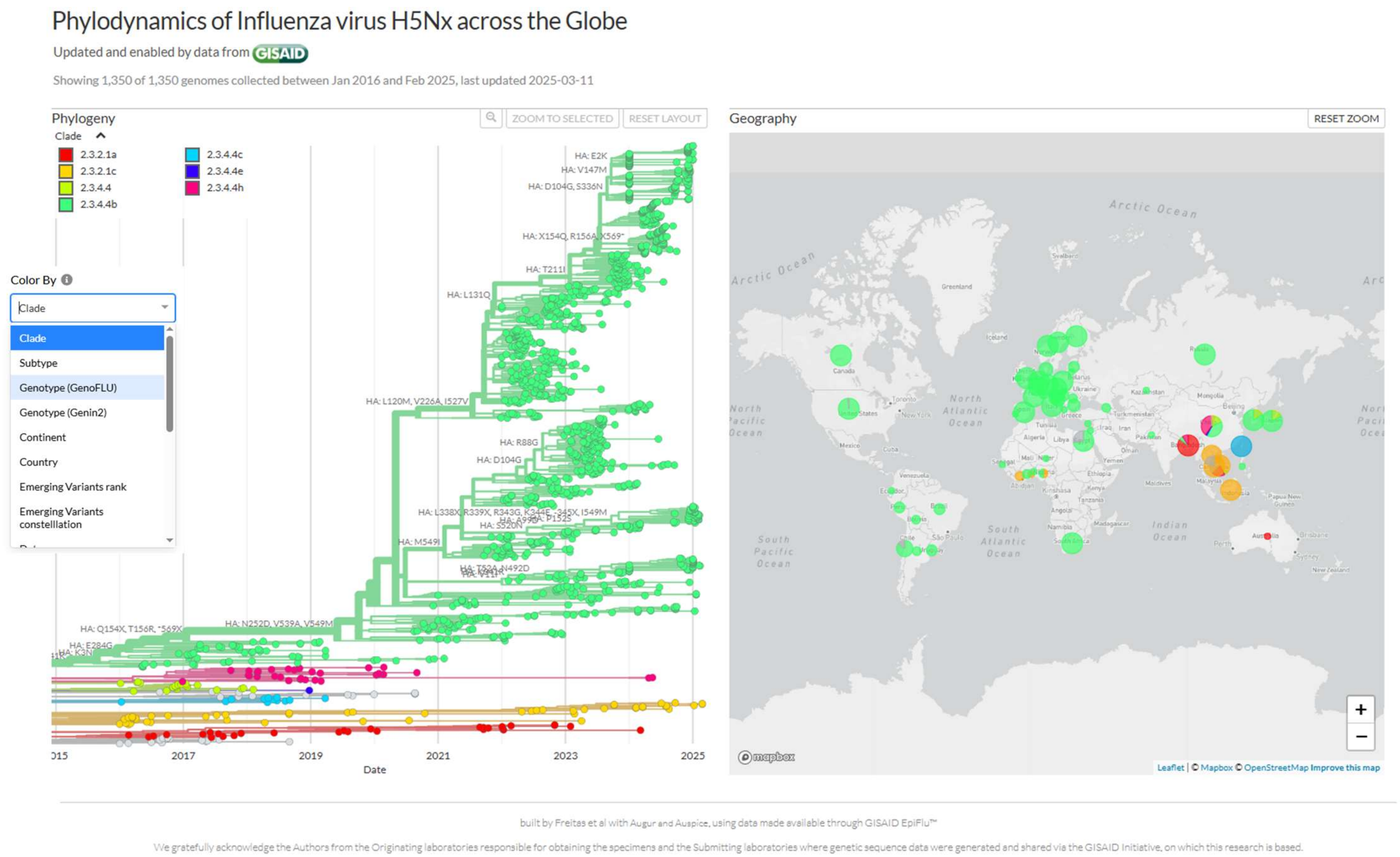


PAHO





You are logged in as **Bastian Schule** - logout



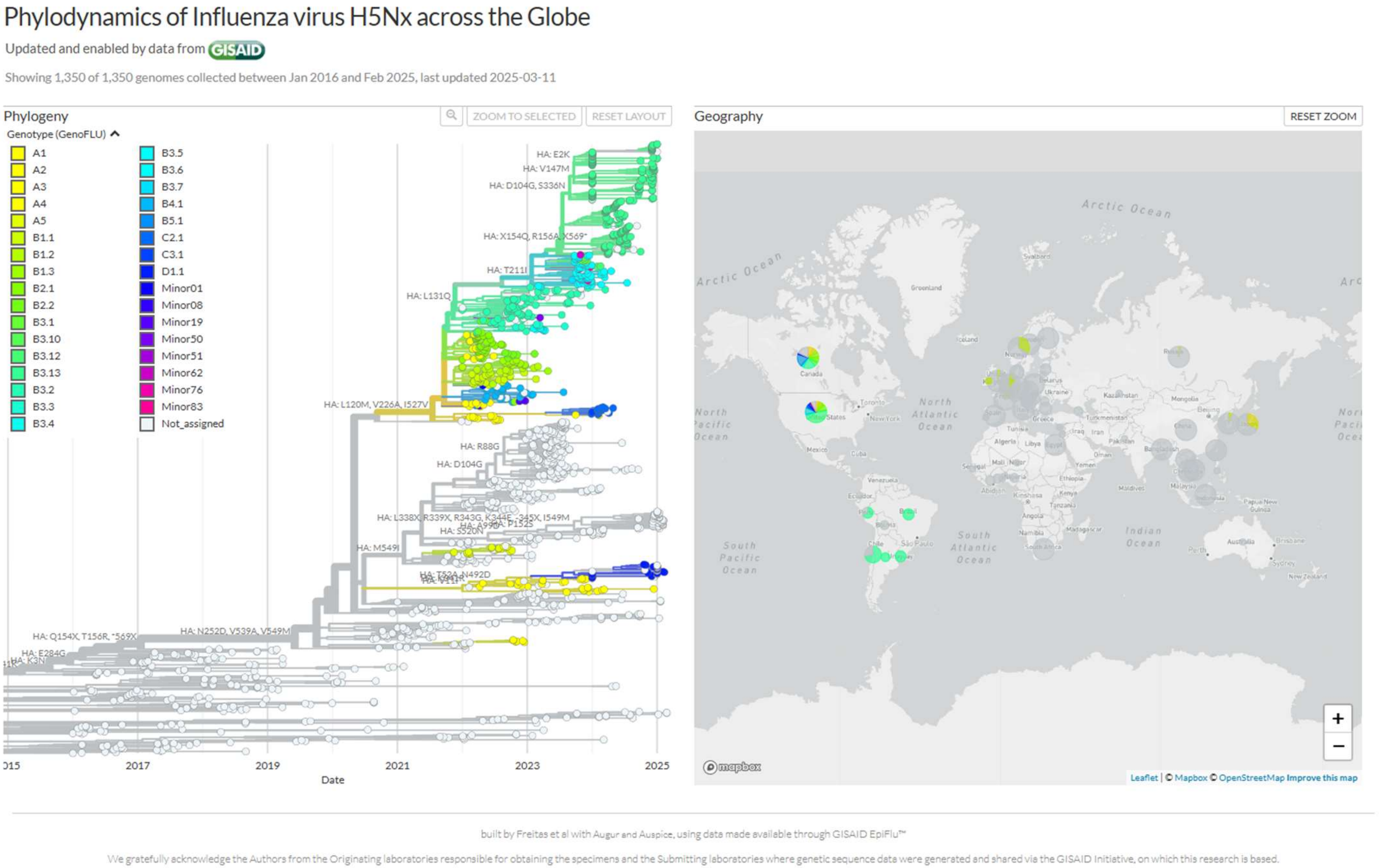
Phylodynamics of Influenza virus H5Nx across the Globe

Updated and enabled by data from GISAID

Showing 1,350 of 1,350 genomes collected between Jan 2016 and Feb 2025, last updated 2025-03-11

built by Freitas et al with Augur and Auspice, using data made available through GISAID EpiFlu™

We gratefully acknowledge the Authors from the Originating laboratories responsible for obtaining the specimens and the Submitting laboratories where genetic sequence data were generated and shared via the GISAID Initiative, on which this research is based.



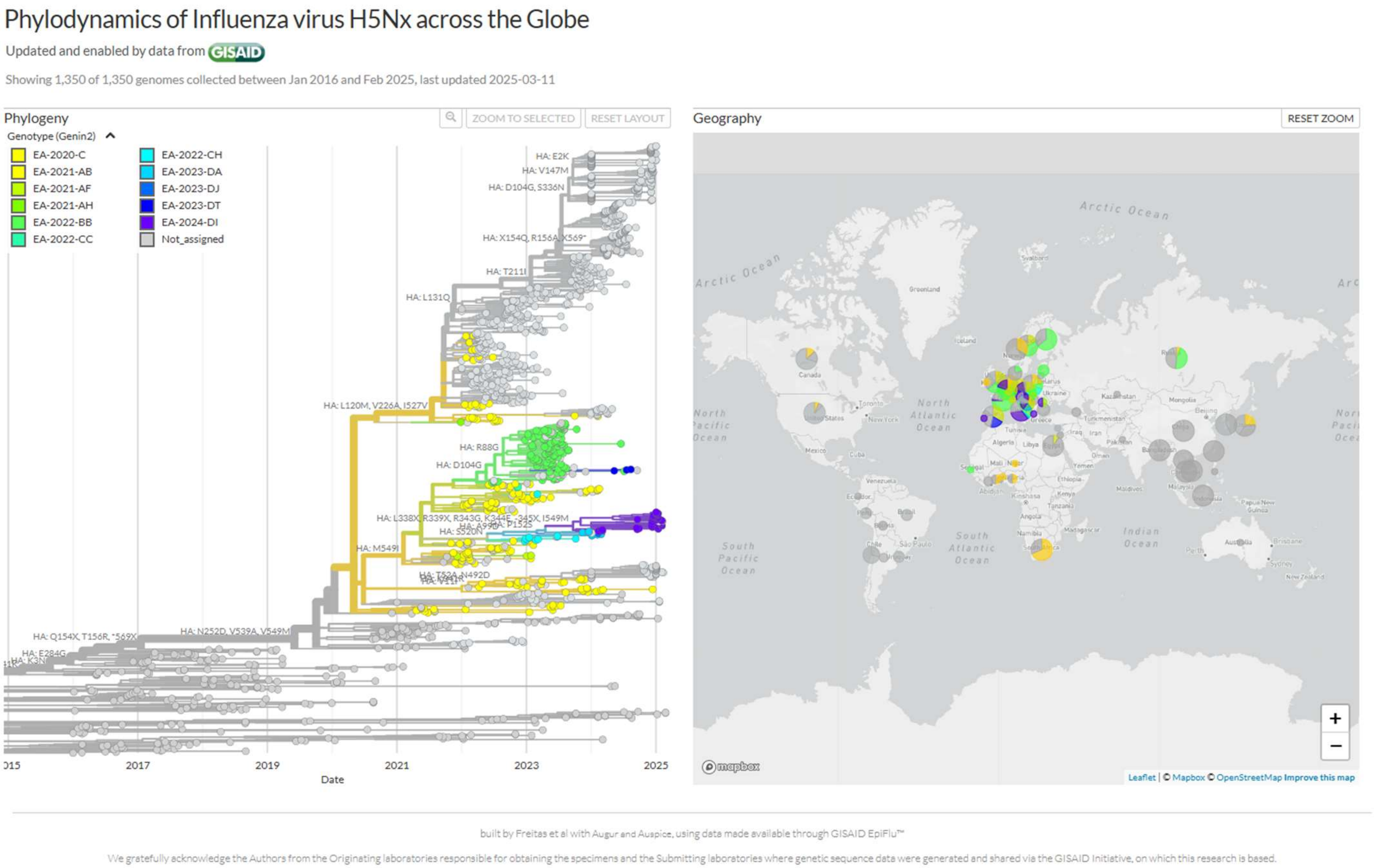
Phylodynamics of Influenza virus H5Nx across the Globe

Updated and enabled by data from GISAID

Showing 1,350 of 1,350 genomes collected between Jan 2016 and Feb 2025, last updated 2025-03-11

built by Freitas et al with Augur and Auspice, using data made available through GISAID EpiFlu™

We gratefully acknowledge the Authors from the Originating laboratories responsible for obtaining the specimens and the Submitting laboratories where genetic sequence data were generated and shared via the GISAID Initiative, on which this research is based.



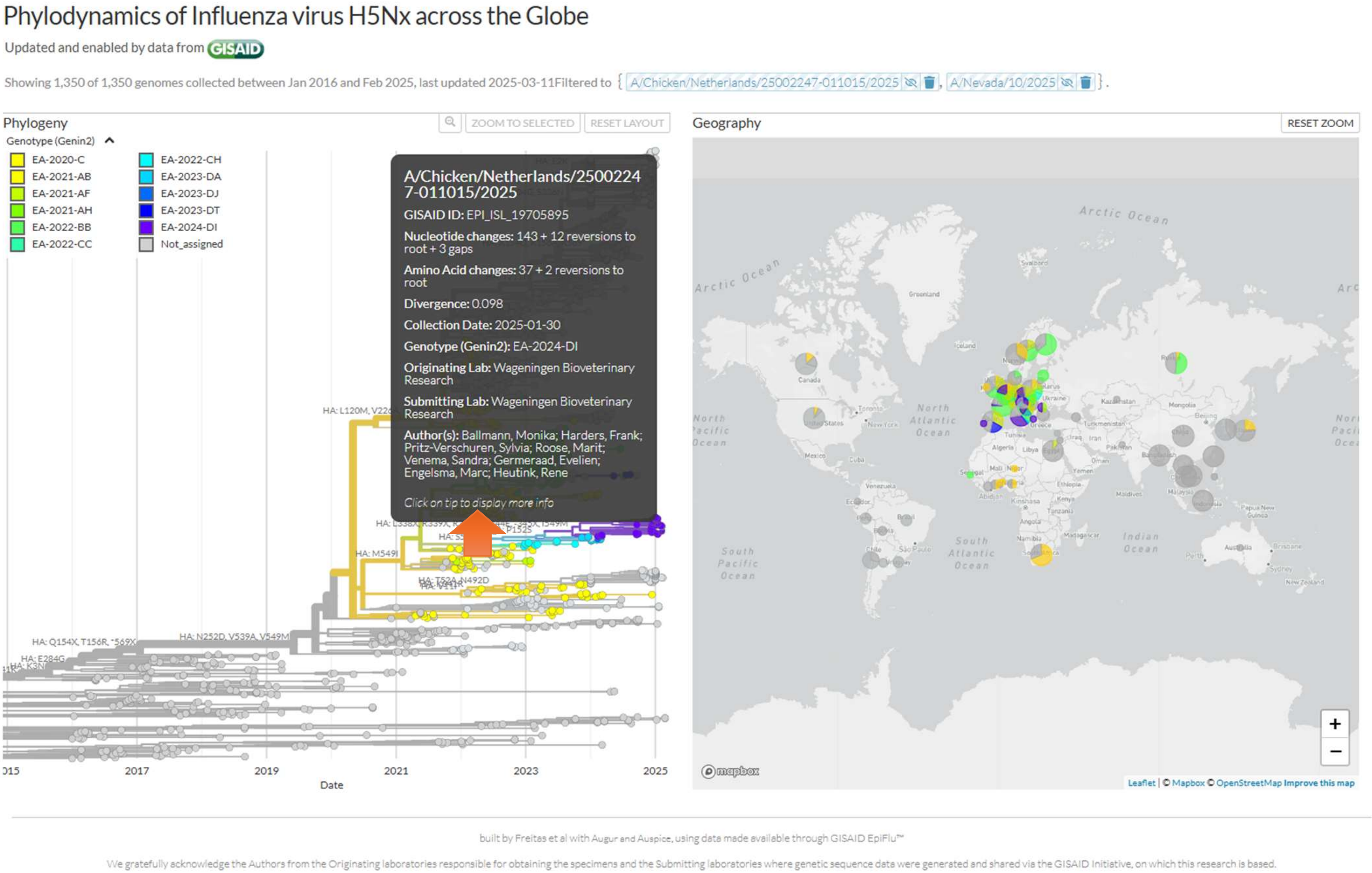
Phylodynamics of Influenza virus H5Nx across the Globe

Updated and enabled by data from GISAID

Showing 1,350 of 1,350 genomes collected between Jan 2016 and Feb 2025, last updated 2025-03-11

built by Freitas et al with Augur and Auspice, using data made available through GISAID EpiFlu™

We gratefully acknowledge the Authors from the Originating laboratories responsible for obtaining the specimens and the Submitting laboratories where genetic sequence data were generated and shared via the GISAID Initiative, on which this research is based.



Phylodynamics of Influenza virus H5Nx across the Globe

Updated and enabled by data from GISAID

Showing 1,350 of 1,350 genomes collected between Jan 2016 and Feb 2025, last updated 2025-03-11Filtered to { A/Chicken/Netherlands/25002247-011015/2025, A/Nevada/10/2025 }.

A/Chicken/Netherlands/25002247-011015/2025

GISAID ID: EPI_ISL_19705895

Nucleotide changes: 143 + 12 reversions to root + 3 gaps

Amino Acid changes: 37 + 2 reversions to root

Divergence: 0.098

Collection Date: 2025-01-30

Genotype (Genin2): EA-2024-DI

Originating Lab: Wageningen Bioveterinary Research

Submitting Lab: Wageningen Bioveterinary Research

Author(s): Ballmann, Monika; Harders, Frank; Pritz-Verschuren, Sylvia; Roose, Marit; Venema, Sandra; Germeraad, Evelien; Engelsma, Marc; Heutink, Rene

Click on tip to display more info

built by Freitas et al with Augur and Auspice, using data made available through GISAID EpiFlu™

We gratefully acknowledge the Authors from the Originating laboratories responsible for obtaining the specimens and the Submitting laboratories where genetic sequence data were generated and shared via the GISAID Initiative, on which this research is based.

A/Chicken/Netherlands/25002247-011015/2025

Collection date	2025-01-30
Originating laboratory	Wageningen Bioveterinary Research
GISAID ID	EPI_ISL_19705895
Analyze with	Audacity*Instant* - FluSurver
Continent	Europe
Genotype (GenoFLU)	Not_assigned
Country	Netherlands
clade	2.3.4.4b
Host	Chicken
Submitting laboratory	Wageningen Bioveterinary Research
Genotype (Genin2)	EA-2024-DI
Emerging Variants rank	2.3.4.4b/+HA_S157P,+HA_N252D
Clade	2.3.4.4b
Subtype	H5N1
Emerging Variants constelllation	+HA_S157P,+HA_N252D
Authors	Ballmann, Monika; Harders, Frank; Pritz-Verschuren, Sylvia; Roose, Marit; Venema, Sandra; Germeraad, Evelien; Engelsma, Marc; Heutink, Rene

Isolate detail

Isolate name: A/Chicken/Netherlands/25002247-011015/2025
Isolate ID: EPI_ISL_19705895
Passage details/history: Original
Type: A / H5N1
Lineage:
Clade: 2.3.4.4b
Genotype: Not assigned (US) / EA-2024-DI
Pathogenicity: HPAI

Sample information

Collection date: 2025-01-30
Host: Gallus gallus
Additional host information:
Domestic status:
Is vaccinated:
Sampling strategy:
Location: Netherlands / Provincia Friesland
Additional location information:
Health status: Sick
Specimen source:
Strain or commercial product name used for vaccination:
Sequencing strategy:
Sequencing technology:
Assembly method:
Coverage:

Institute information

Originating lab: Wageningen Bioveterinary Research
Address: Wageningen Bioveterinary Research, Houtribweg 39, 8222 RA Lelystad, Netherlands
Sample ID given by the originating lab:
Submitting lab: Wageningen Bioveterinary Research
Address: Wageningen Bioveterinary Research, Houtribweg 39, 8222 RA Lelystad, Netherlands
Sample ID given by the submitting laboratory: 25002247-011015
Authors: Ballmann, Monika; Harders, Frank; Pritz-Verschuren, Sylvia; Roose, Marit; Venema, Sandra; Germeraad, Evelien; Engelsma, Marc; Heutink, Rene

Publications

Publication

In vivo antiviral resistance

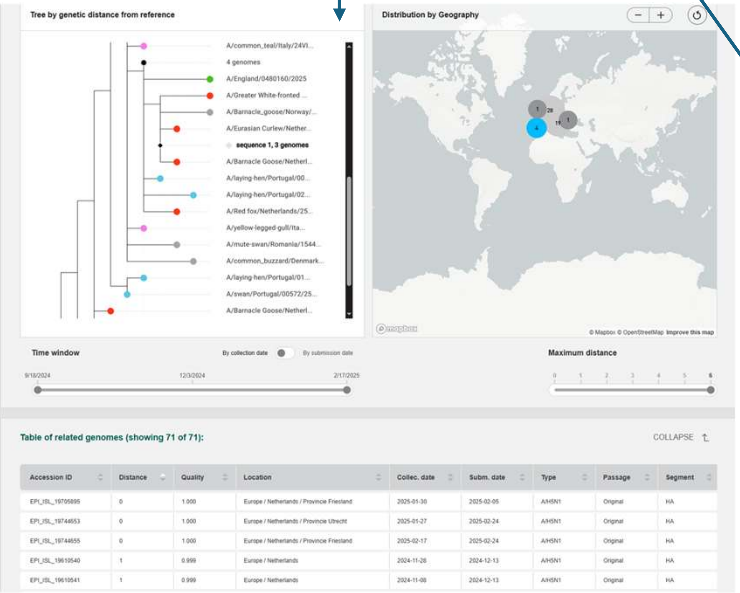
Antiviral resistance annotated by GISAID

	Oseltamivir	Zanamivir	Peramivir	Laninamivir	Baloxavir
pos + subtype + aa	NI	NI	NI	-	-
pos + subtype	-	-	-	-	-
pos	-	-	-	-	-

Additional information

Antigenic characterization:
Note:

Sequence



Tree by genetic distance from reference

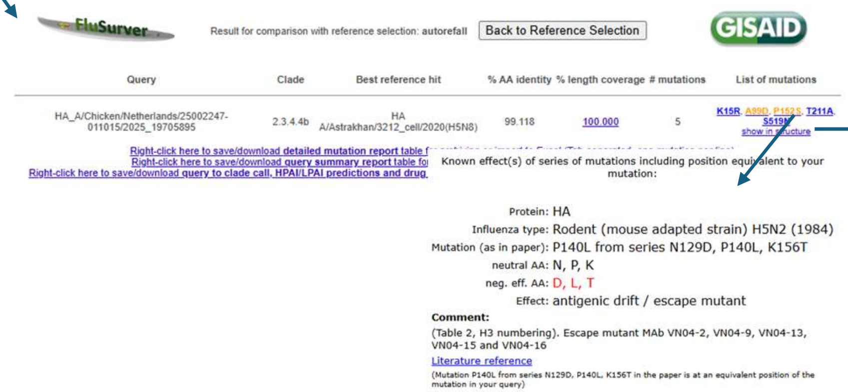
Distribution by Geography

Time window

Maximum distance

Table of related genomes (showing 71 of 71):

Accession ID	Distance	Quality	Location	Collec. date	Subm. date	Type	Passage	Segment
EPI_ISL_19705895	0	1.000	Europe / Netherlands / Provincie Friesland	2025-01-30	2025-02-05	A/H5N1	Original	HA
EPI_ISL_19744653	0	1.000	Europe / Netherlands / Provincie Utrecht	2025-01-27	2025-02-24	A/H5N1	Original	HA
EPI_ISL_19744655	0	1.000	Europe / Netherlands / Provincie Friesland	2025-02-17	2025-02-24	A/H5N1	Original	HA
EPI_ISL_19610540	1	0.999	Europe / Netherlands	2024-11-28	2024-12-13	A/H5N1	Original	HA
EPI_ISL_19610541	1	0.999	Europe / Netherlands	2024-11-08	2024-12-13	A/H5N1	Original	HA



Result for comparison with reference selection: autorefall

Query	Clade	Best reference hit	% AA identity	% length coverage	# mutations	List of mutations
HA_A/Chicken/Netherlands/25002247-011015/2025_19705895	2.3.4.4b	HA A/Astrakhan/3212_cell/2020(H5N8)	99.118	100.000	5	K15R, A99D, P152S, T211A, S519N show in structure

Right-click here to save/download detailed mutation report table f
Right-click here to save/download query summary report table fo
Right-click here to save/download query to clade call, HPAI/LPAI predictions and drug

Known effect(s) of series of mutations including position equivalent to your mutation:

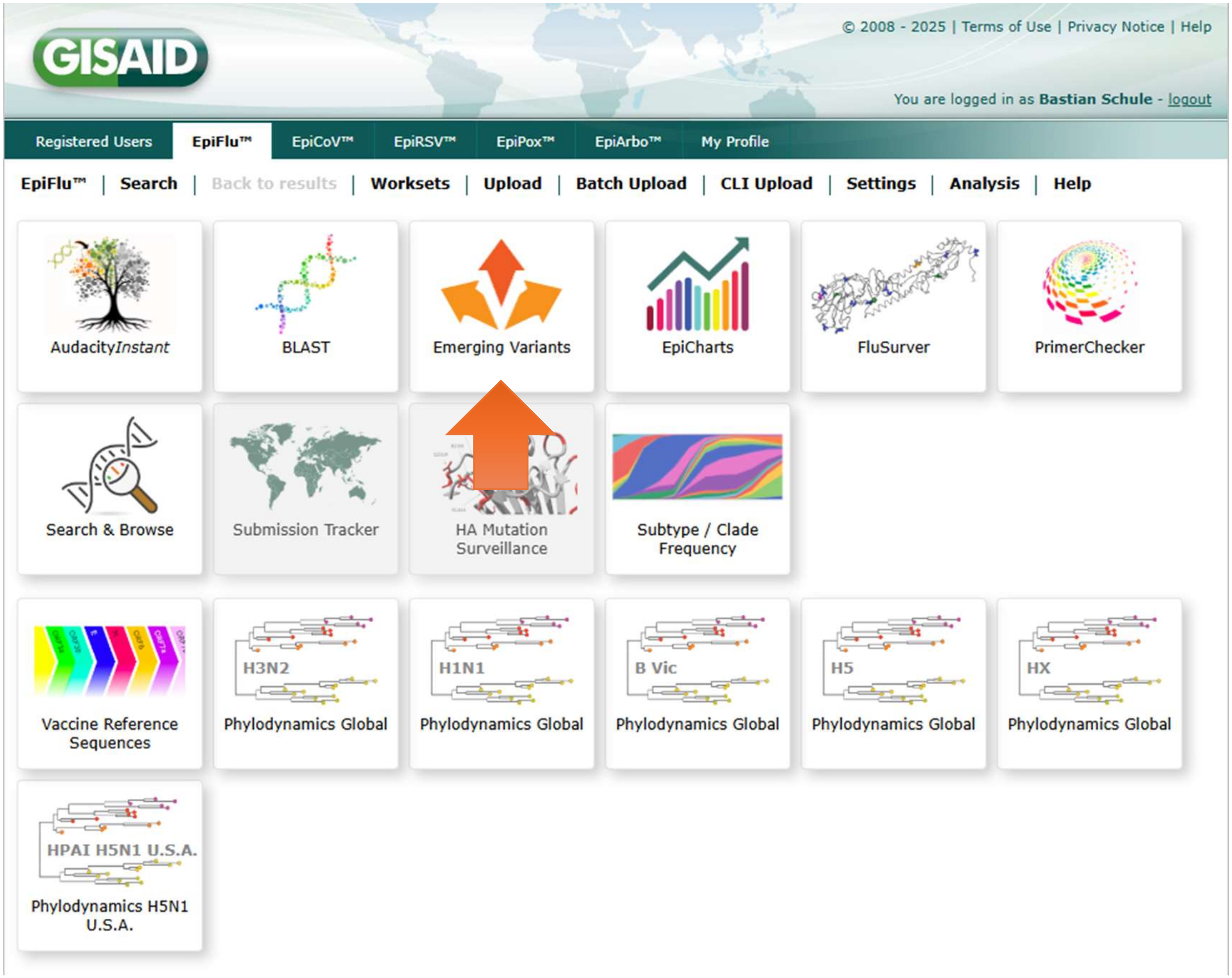
Protein: HA
Influenza type: Rodent (mouse adapted strain) H5N2 (1984)
Mutation (as in paper): P140L from series N129D, P140L, K156T
neutral AA: N, P, K
neg. eff. AA: D, L, T
Effect: antigenic drift / escape mutant

Comment:

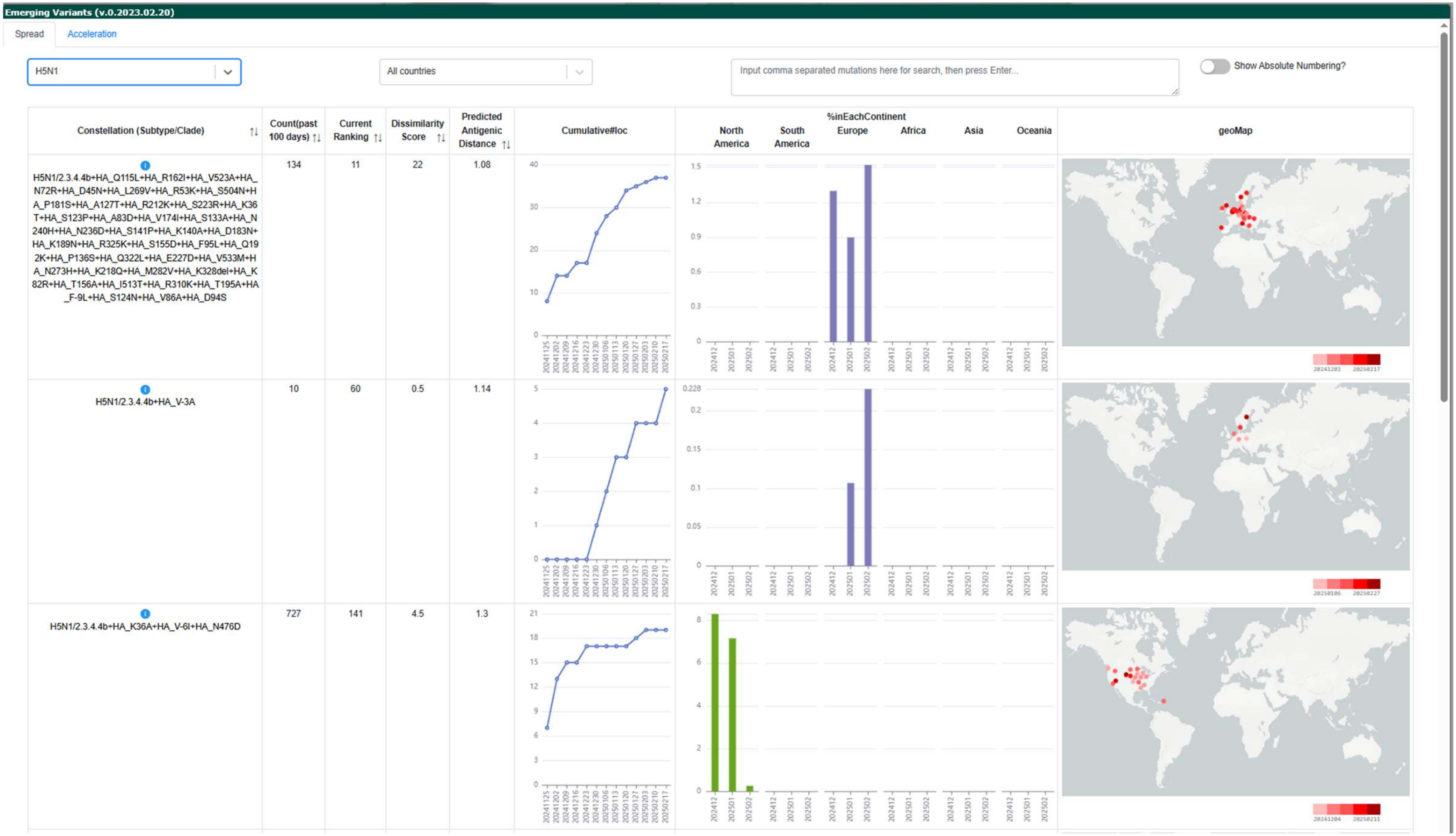
(Table 2, H3 numbering). Escape mutant MAb VN04-2, VN04-9, VN04-13, VN04-15 and VN04-16

Literature reference

(Mutation P140L from series N129D, P140L, K156T in the paper is at an equivalent position of the mutation in your query)



You are logged in as **Bastian Schule** - logout



Emerging Variants (v.0.2023.02.20)

H5N1

All countries

Input comma separated mutations here for search, then press Enter...

Constellation (Subtype/Clade) ↑↓	Count(past 100 days) ↑↓	Current Ranking ↑↓	Dissimilarity Score ↑↓	Predicted Antigenic Distance ↑↓	Cumulative#loc	%inEachContinent North America	South America	Europe	Africa	Asia	Oceania	geoMap
H5N1/2.3.4.4b+HA_Q115L+HA_R162I+HA_V523A+HA_N72R+HA_D45N+HA_L269V+HA_R53K+HA_S504N+HA_P181S+HA_A127T+HA_R212K+HA_S223R+HA_K36T+HA_S123P+HA_A83D+HA_V174I+HA_S133A+HA_N240H+HA_N236D+HA_S141P+HA_K140A+HA_D183N+HA_K189N+HA_R325K+HA_S155D+HA_F95L+HA_Q192K+HA_P136S+HA_Q322L+HA_E227D+HA_V533M+HA_N273H+HA_K218Q+HA_M282V+HA_K328del+HA_K82R+HA_T156A+HA_I513T+HA_R310K+HA_T195A+HA_F-9L+HA_S124N+HA_V86A+HA_D94S	134	11	22	1.08								
H5N1/2.3.4.4b+HA_V-3A	10	60	0.5	1.14								
H5N1/2.3.4.4b+HA_K36A+HA_V-6I+HA_N476D	727	141	4.5	1.3								

A Big Thanks to our Colleagues @

Global Influenza Programme at WHO

Global Influenza Surveillance and Response System

GISAID Data Science Initiative

OFFLU Network of WOAH und FAO