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Influenza A(H5N1) Virus Clade 2.3.2.1a in Traveler Returning to Australia from India, 2024

Appendix

Methods

The time-structured phylogenetic tree was estimated in BEAST v.1.10.4 (Appendix Figure 2) (1). The ML tree was used to generate a regression of root-to-tip genetic distances in TempEst (2), resulting high correlation coefficient of 0.83 and R^2 value of 0.69, indicating a strong temporal signal. BEAST analysis was performed using an SRD06 codon model, an uncorrelated lognormal relaxed molecular clock model, and a Bayesian Skyline tree prior. Three independent runs of 100 million generations were performed. Convergence was assessed using Tracer v1.6. Tree files were combined using LogCombiner v1.8 following removal of a 10% burn-in and Maximum clade credibility (MCC) trees were generated using TreeAnnotator v1.8. Trees were visualized with ggtree 3.10.1.

Results

The mean date of when A/Victoria/149/2024 diverged from the most closely related 2.3.2.1a HA sequences in GISAID was June 2020 (95% highest posterior density [HPD]: December 2019 - January 2021). That the most recent common ancestor (MRCA) was almost 4 years before the human case reported here underscores substantial missing or unsampled data, highlighting the need for more sequence data from the region.

References

1. Drummond AJ, Suchard MA, Xie D, Rambaut A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol Biol Evol.* 2012;29:1969–73. [PubMed https://doi.org/10.1093/molbev/mss075](https://doi.org/10.1093/molbev/mss075)
2. Rambaut A, Lam TT, Max Carvalho L, Pybus OG. Exploring the temporal structure of heterochronous sequences using TempEst (formerly Path-O-Gen). *Virus Evol.* 2016;2:vew007. [PubMed https://doi.org/10.1093/ve/vew007](https://doi.org/10.1093/ve/vew007)

Appendix Table. Top matches to each segment using BLAST in GISAID and Genbank (BLAST analysis performed on 15 July 2024)*

Segment	Database	Closest match	Clade	% identity
PB2	GISAID EpiFlu	EPI2752307 A/duck/Wakayama/22A6T/2022(H5N1)	2.3.4.4b	99%
	NCBI nt	LC774734 A/peregrine falcon/Fukui/NIES212/2022(H5N1)	2.3.4.4b	99.34%
PB1	GISAID EpiFlu	EPI2789452 A/environment/Kagoshima/KU-H1/2022 (H5N1)	2.3.4.4b	99%
	NCBI nt	OR950546 A/duck/Yunlin/20WB0265–1/2020(H1N6)	LPAI	98.33%
PA	GISAID EpiFlu	EPI2742639 A/white-fronted goose/Korea/22WC099/2022 (H5N1)	2.3.4.4b	99%
	NCBI nt	OR864262 A/duck/People's Republic of China/JX02PA/2023(H4N6)	LPAI	98.75%
HA	GISAID EpiFlu	EPI2147679 A/duck/Bangladesh/46162/2020(H5N1)	2.3.2.1a	98%
	NCBI nt	OM938222 A/duck/Bangladesh/46157/2020(H5N1)	2.3.2.1a	98.53%
NP	GISAID EpiFlu	EPI2162602 A/chicken/Bangladesh/50592/2021(H5N1)	2.3.2.1a	99%
	NCBI nt	OP023558 A/chicken/Bangladesh/50585/2021(H5N1)	2.3.2.1a	99.13%
NA	GISAID EpiFlu	EPI3116264 A/chicken/Bangladesh/18-B-569/2022(H5N1)	2.3.2.1a	98%
	NCBI nt	PP680409 A/duck/Bangladesh/58667/2023(H5N1)	2.3.2.1a	98.52%
M	GISAID EpiFlu	EPI2062119 A/duck/Bangladesh/19D1817/2021(H5N1)	2.3.4.4b	99%
	NCBI nt	LC765296 A/crow/Fukuoka/TU48–37/2022(H5N1))	2.3.4.4b	99.59%
NS	GISAID EpiFlu	EPI3360023 A/duck/Bangladesh/18-D-2109/2023(H?N?)	2.3.2.1a	99%
	NCBI nt	OP023561 A/chicken/Bangladesh/50585/2021(H5N1)	2.3.2.1a	98.93%

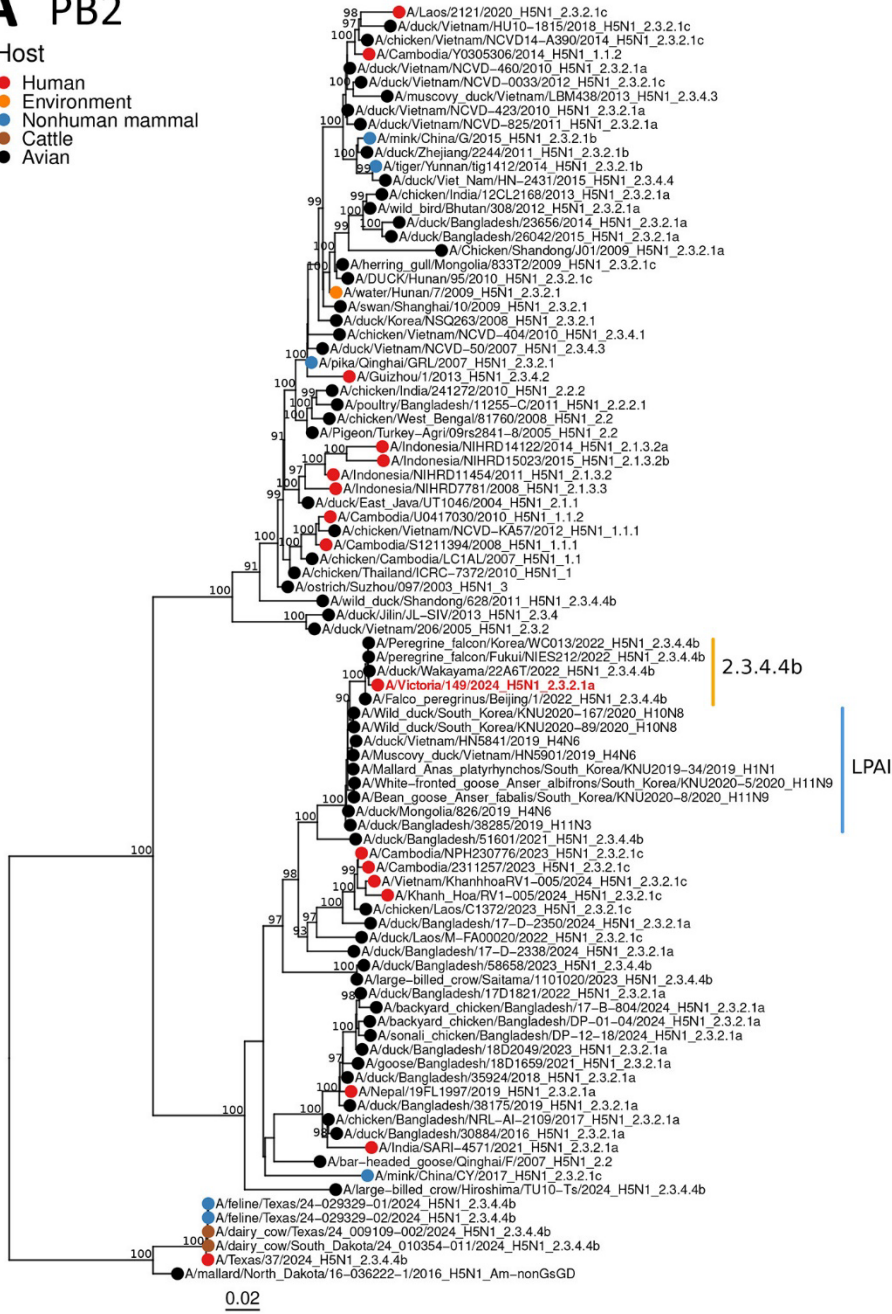
*HA, hemagglutinin; M, matrix; NA, neuraminidase; NP, nucleoprotein; NS, nonstructural; PA, polymerase acidic; PB1, polymerase basic 1; PB2, polymerase basic 2.

Appendix Figure 1 (following pages). Detailed phylogenetic analysis of genes of A/Victoria/149/2024. Maximum-likelihood nucleotide trees are shown for A) PB2, B) PB1, C) PA, D) HA, E) NP, F) NA, G) MP, H) NS genes with a sample of BLAST-matched nucleotide sequences available (up to 21 May 2024) from GISAID and GenBank. Bootstrap values >90% for key nodes are shown. Scale bars indicate the number of nucleotide substitutions per site for each gene. HA, hemagglutinin; MP, matrix protein; NA, neuraminidase; NP, nucleoprotein; NS, nonstructural; PA, polymerase acidic; PB1, polymerase basic 1; PB2, polymerase basic 2.

A PB2

Host

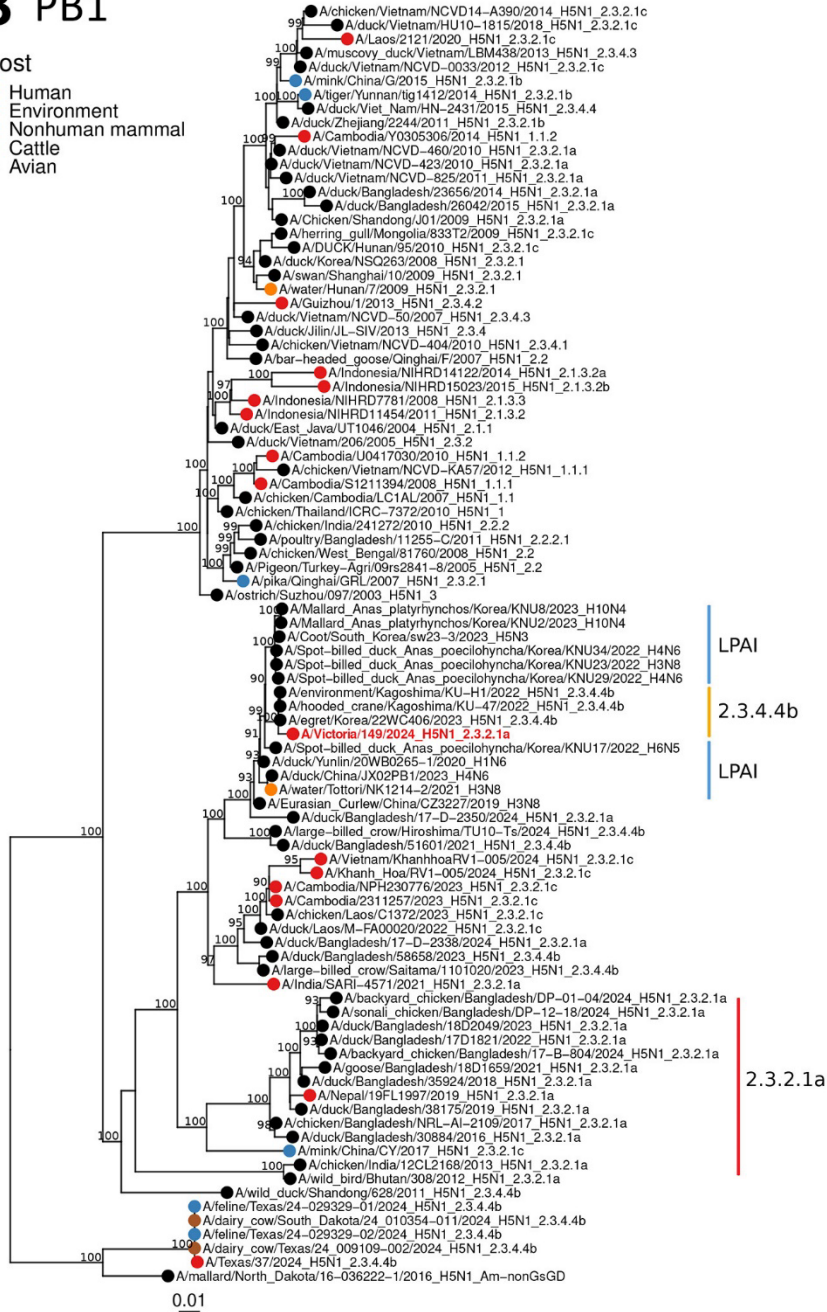
- Human
- Environment
- Nonhuman mammal
- Cattle
- Avian



B PB1

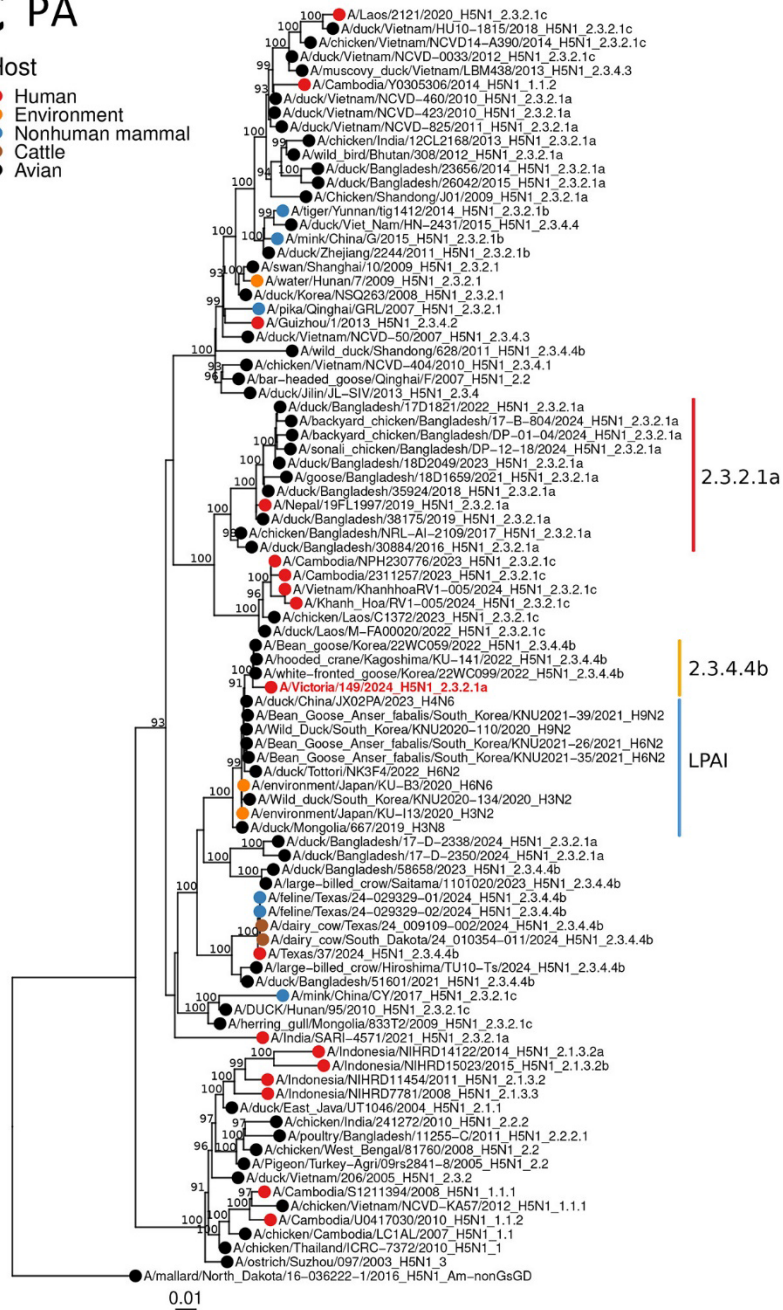
Host

- Human
- Environment
- Nonhuman mammal
- Cattle
- Avian



C PA

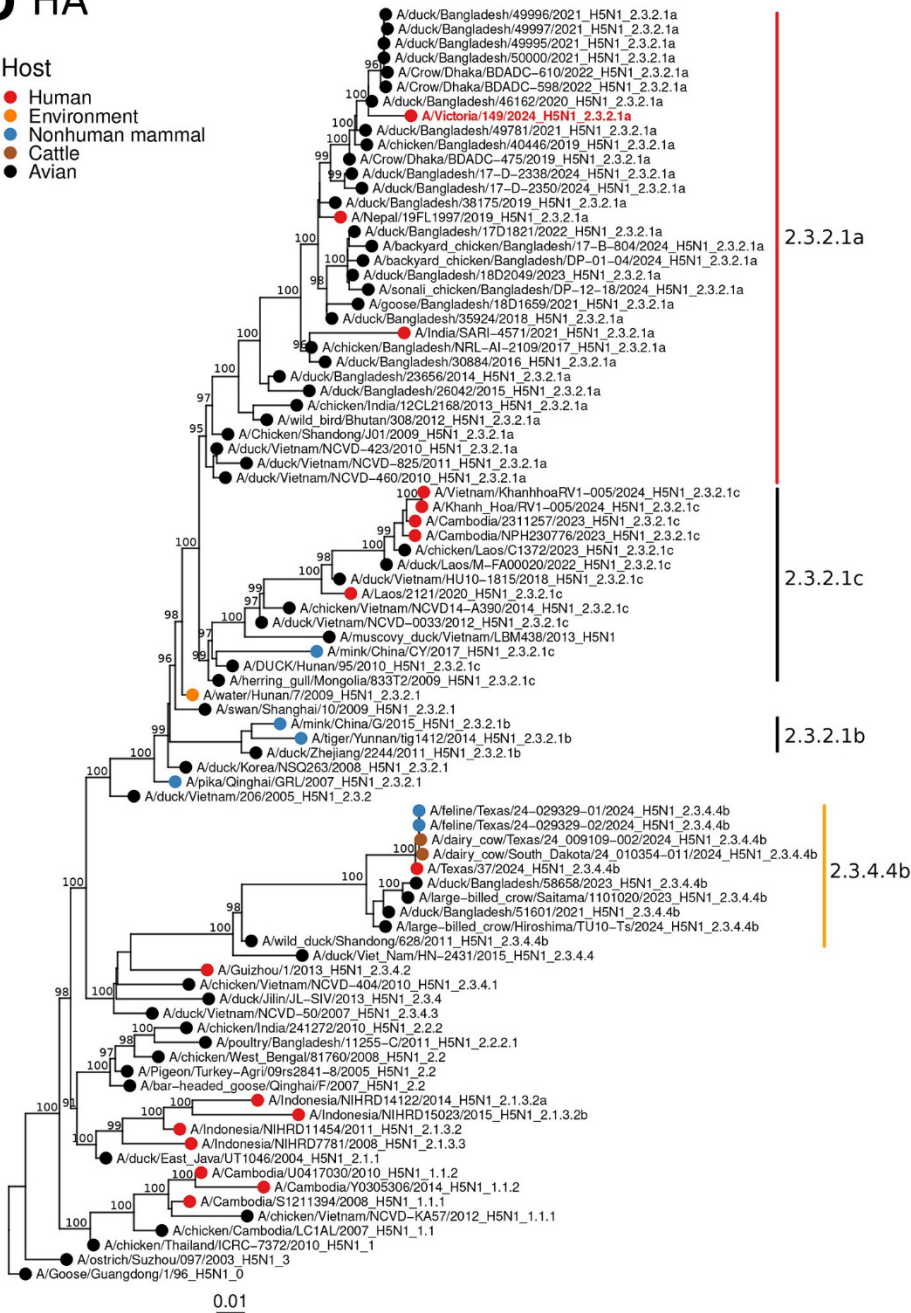
- Host
- Human
 - Environment
 - Nonhuman mammal
 - Cattle
 - Avian



D HA

Host

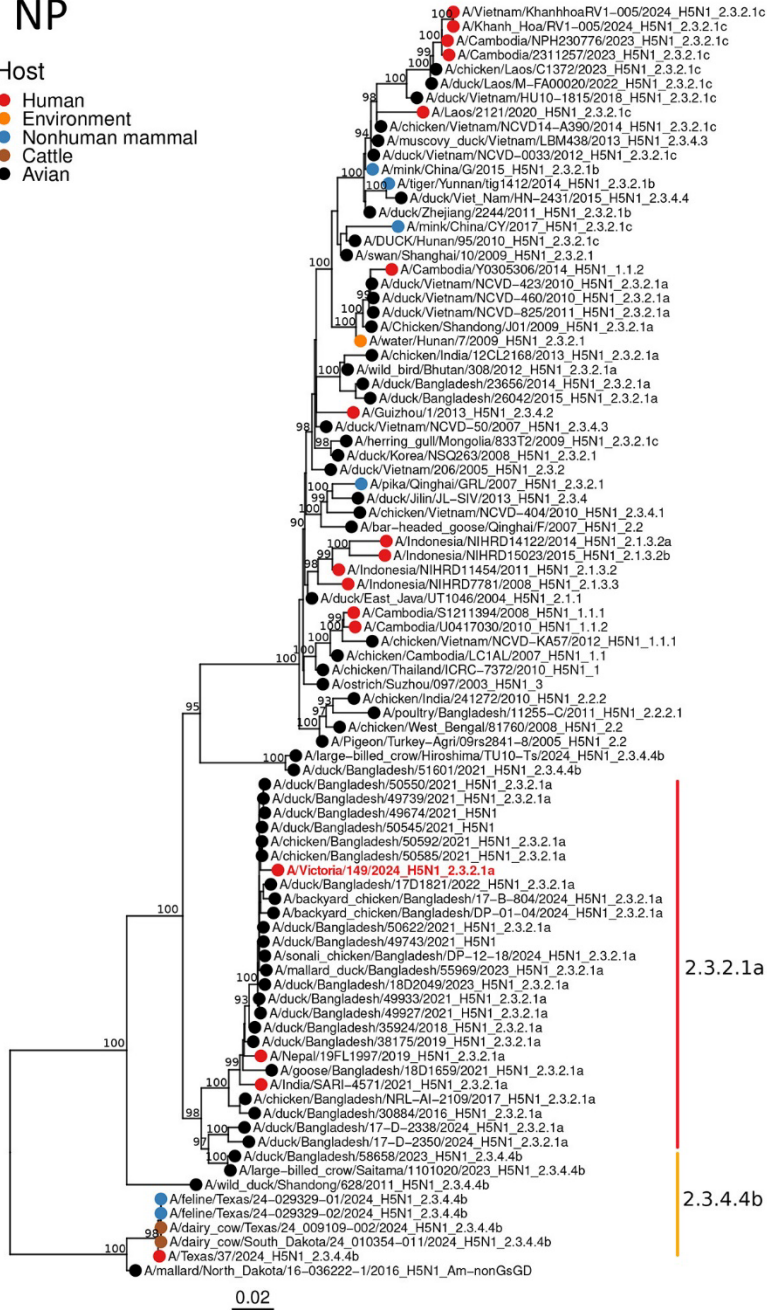
- Human
- Environment
- Nonhuman mammal
- Cattle
- Avian



E NP

Host

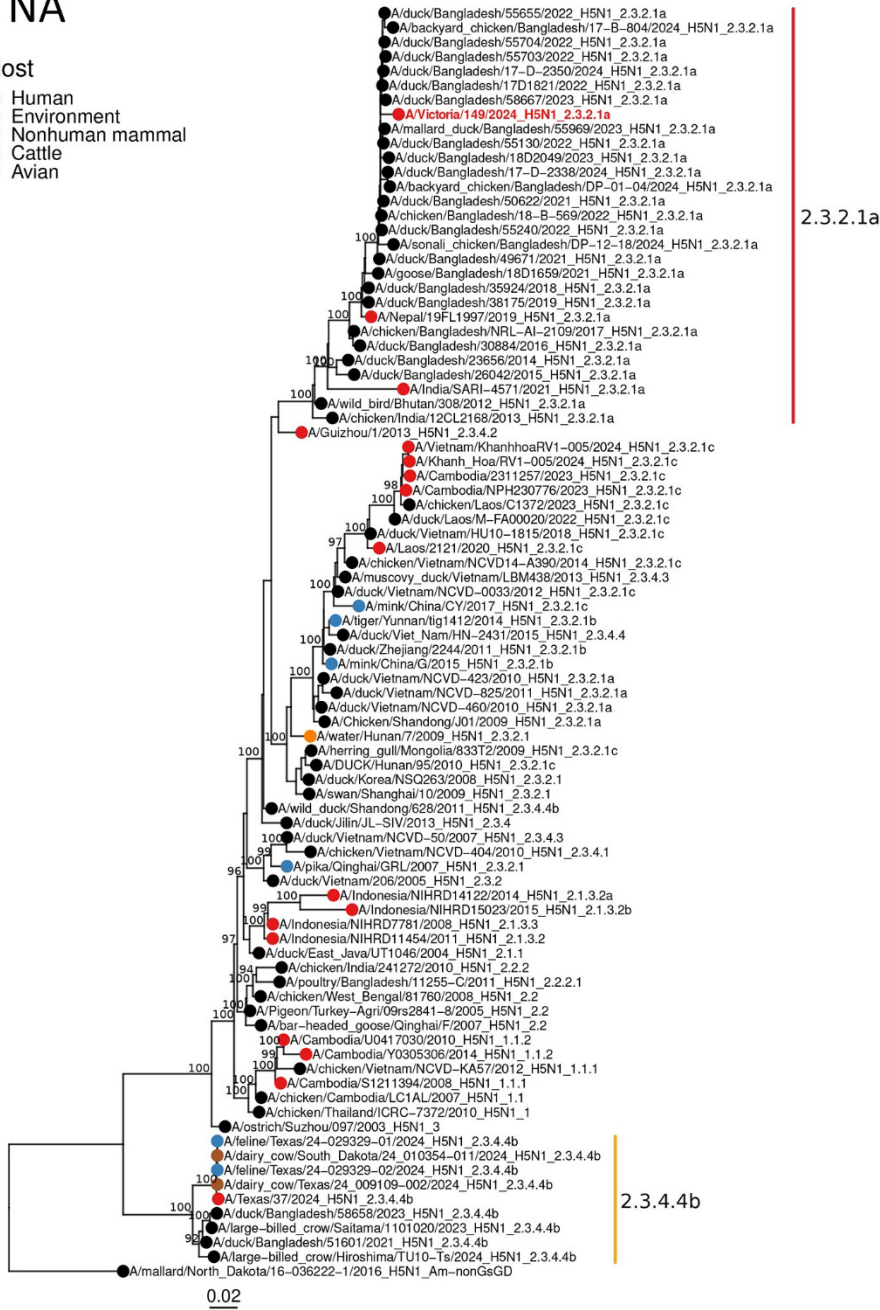
- Human
- Environment
- Nonhuman mammal
- Cattle
- Avian



F NA

Host

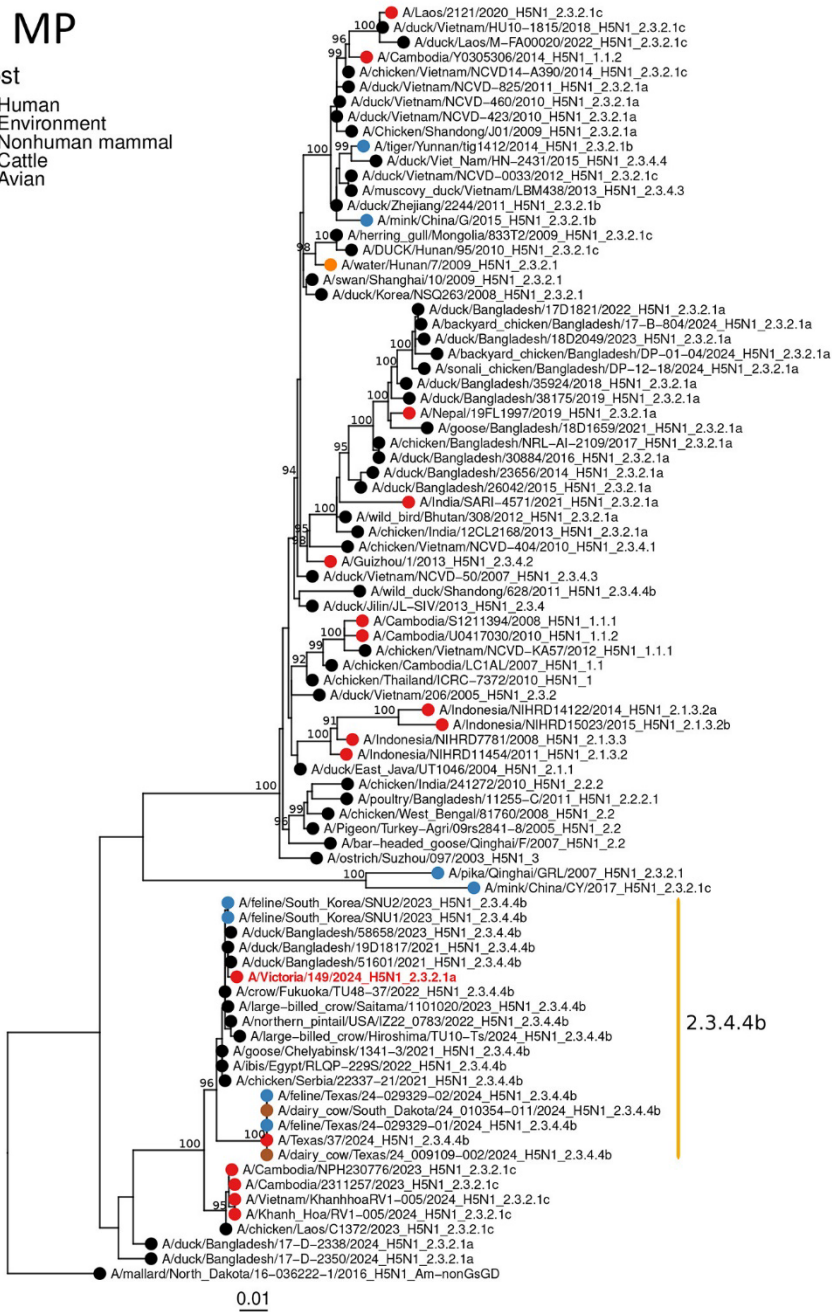
- Human
- Environment
- Nonhuman mammal
- Cattle
- Avian



G MP

Host

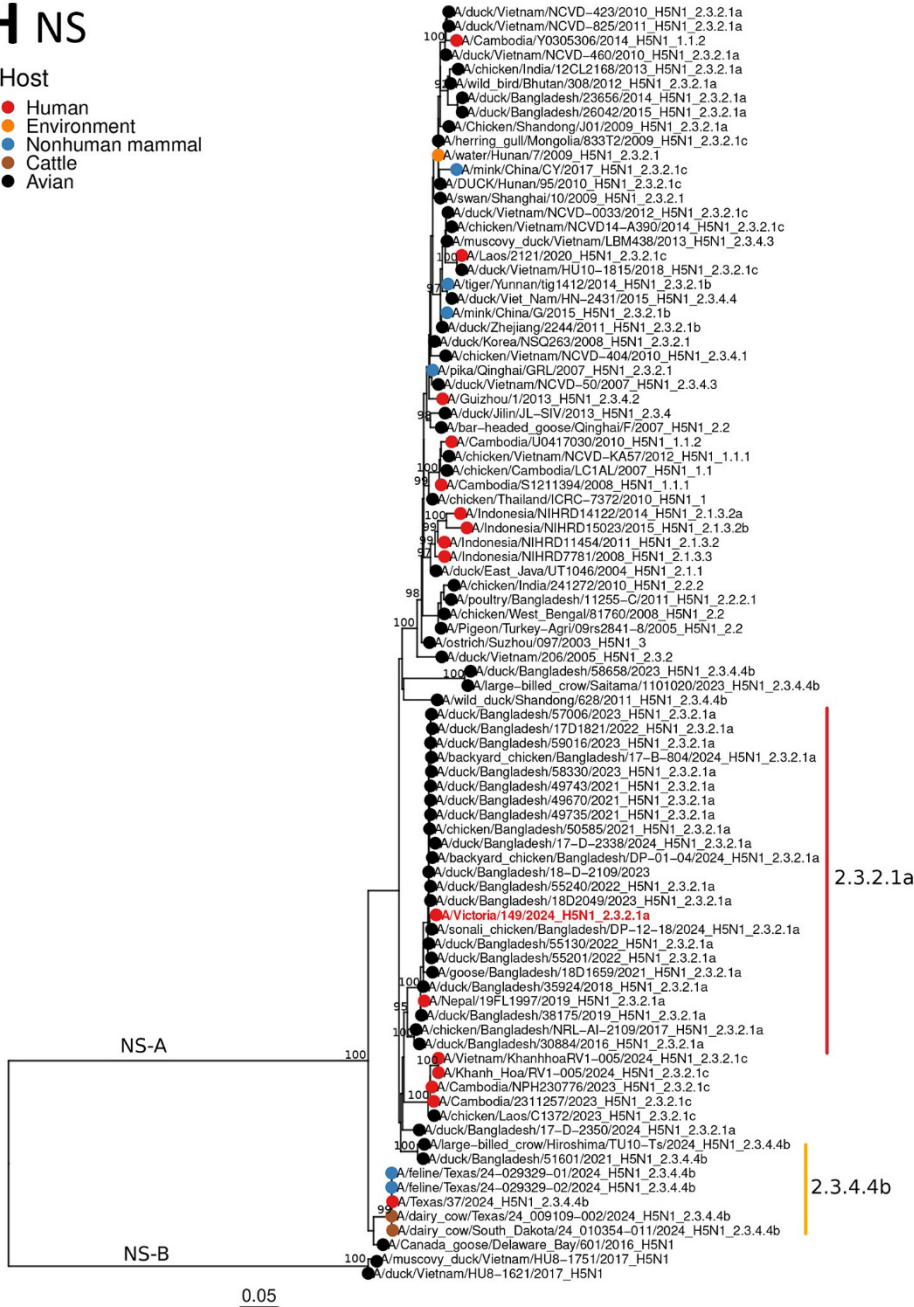
- Human
- Environment
- Nonhuman mammal
- Cattle
- Avian



H NS

Host

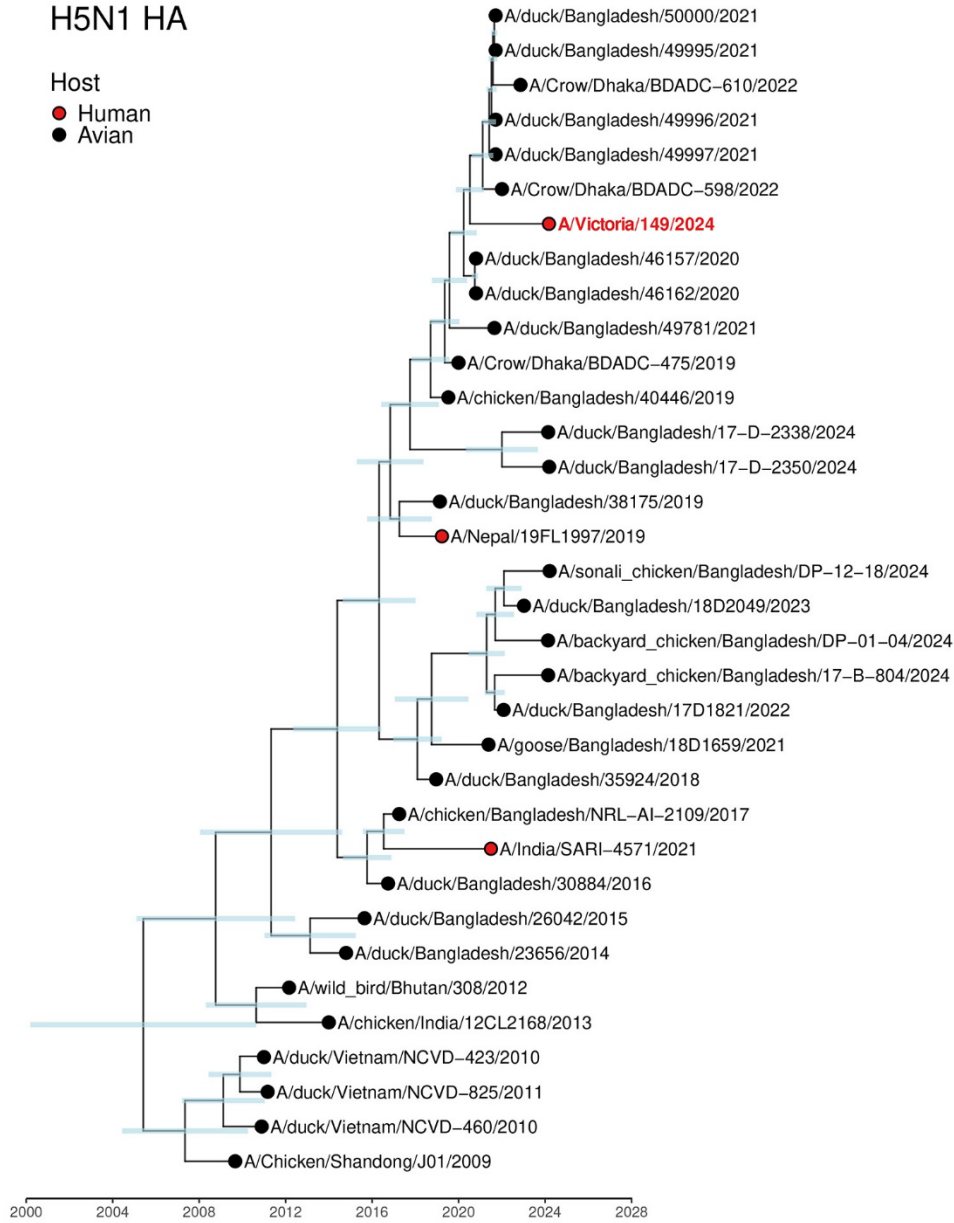
- Human
- Environment
- Nonhuman mammal
- Cattle
- Avian



H5N1 HA

Host

- Human
- Avian



Appendix Figure 2. A time structured phylogenetic analysis of the H5N1 clade 2.3.2.1a viruses shown in Figure 1 (main article). See methods described previously in Appendix.