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Genetic Characterization of Highly Pathogenic Avian Influenza A(H5N1) Clade 2.3.4.4b, Antarctica, 2024

Appendix 1

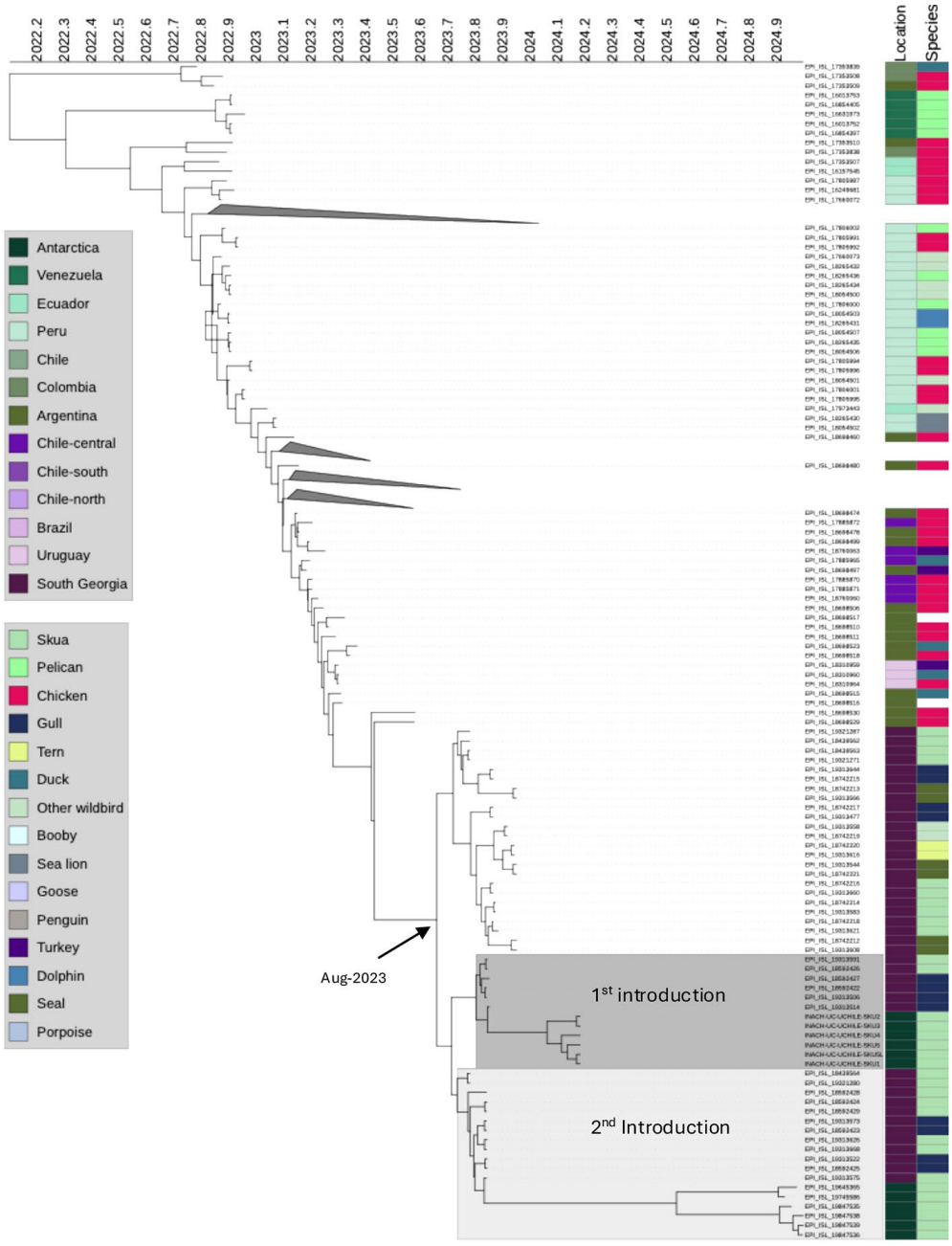
Additional Methods

The samples were reextracted according to the protocol previously described (*3*, main text). Briefly, extraction was performed by using TRIzol lysis (15596018; Invitrogen, https://www.thermofisher.com) plus E.Z.N.A Viral RNA Kit (R6874; Omega Biotek, https://www.omegabiotek.com). Then, the whole genome was amplified, and PCR products were purified by using SPRISelect Beads (B2338; Beckman Coulter, https://beckman.com). High-quality samples were sequenced by using the nanopore technology (ONT) platform with the Native Barcoding Kit (SQK-NBD114.96; Oxford Nanopore Technologies, https://www.nanoporetech.com) according to the manufacturer's instructions for R.10 flow cells. The genomes were assembled by referencing the genome segments of A/*Falco_rusticolus*/EdoMex/CPA-19638–22/2022(H5N1) (GenBank accession nos. OP691321–8).

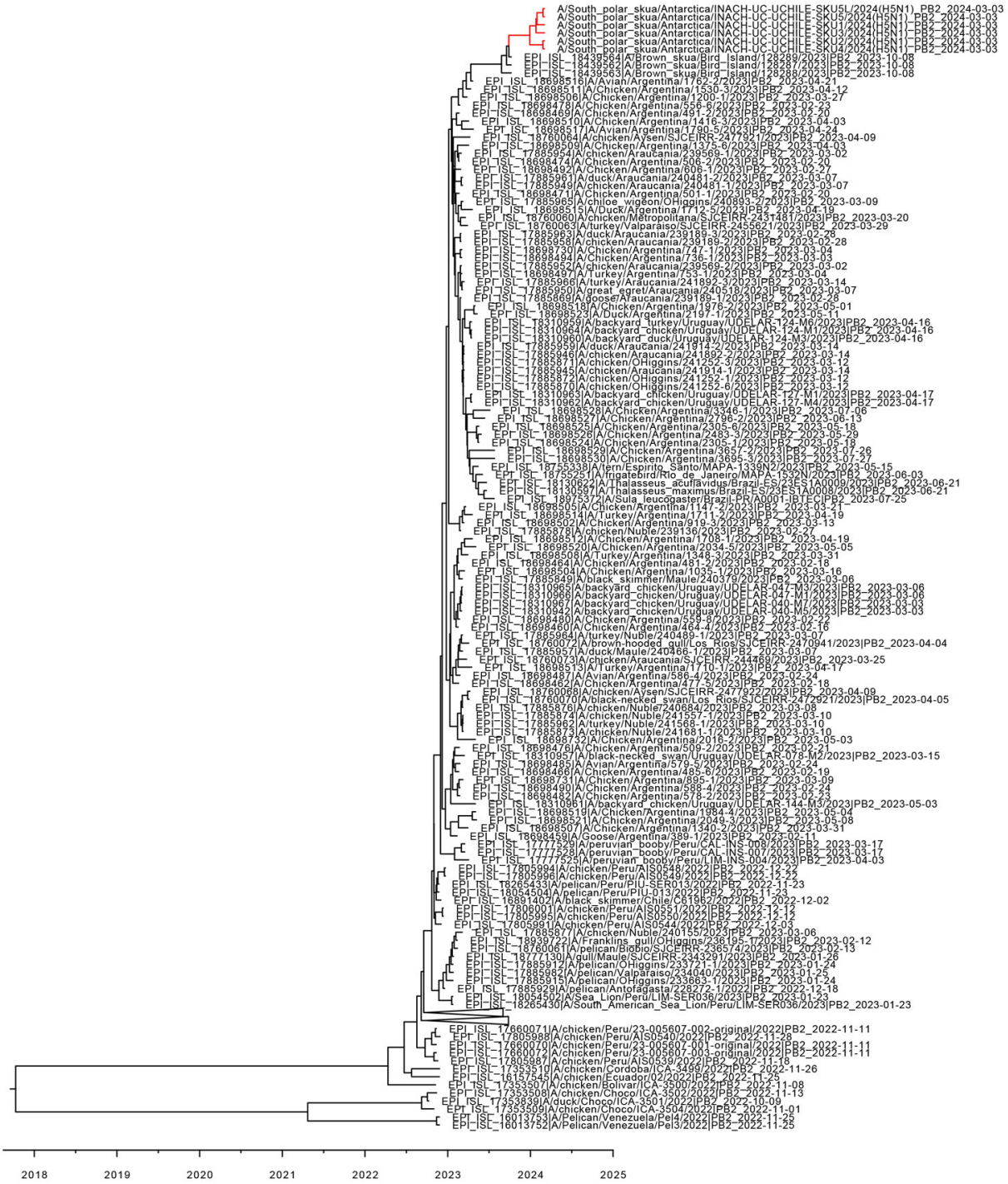
Appendix 1 Table. GenBank accession numbers*

Date	Sample identification no.	Subtype	Coverage†	Influenza virus gene segments							
				PB2	PB1	PA	HA	NP	NA	M	NS
03–03–24	INACH-UC-UCHILE-SKU1	H5N1	24.077	PQ304442	PQ304438	PQ304437	PQ304440	PQ304443	PQ318382	PQ304441	PQ304439
03–03–24	INACH-UC- UCHILE -SKU2	H5N1	18.192	PQ304431	PQ304434	PQ304436	PQ304435	PQ304433	PQ318403	PQ304430	PQ304432
03–03–24	INACH-UC- UCHILE -SKU3	H5N1	8.834	PQ304423	PQ304426	PQ304428	PQ304425	PQ304429	PQ318398	PQ304424	PQ304427
03–03–24	INACH-UC- UCHILE -SKU4	H5N1	18.477	PQ304582	PQ304586	PQ304587	PQ304584	PQ304583	PQ318371	PQ304588	PQ304585
03–03–24	INACH-UC- UCHILE -SKU5	H5N1	16.184	PQ304483	PQ304486	PQ304487	PQ304488	PQ304484	PQ318388	PQ304482	PQ304485
03–03–24	INACH-UC- UCHILE -SKU5L	H5N1	17.681	PQ304563	PQ304565	PQ304564	PQ304566	PQ304567	PQ318393	PQ304568	PQ304569

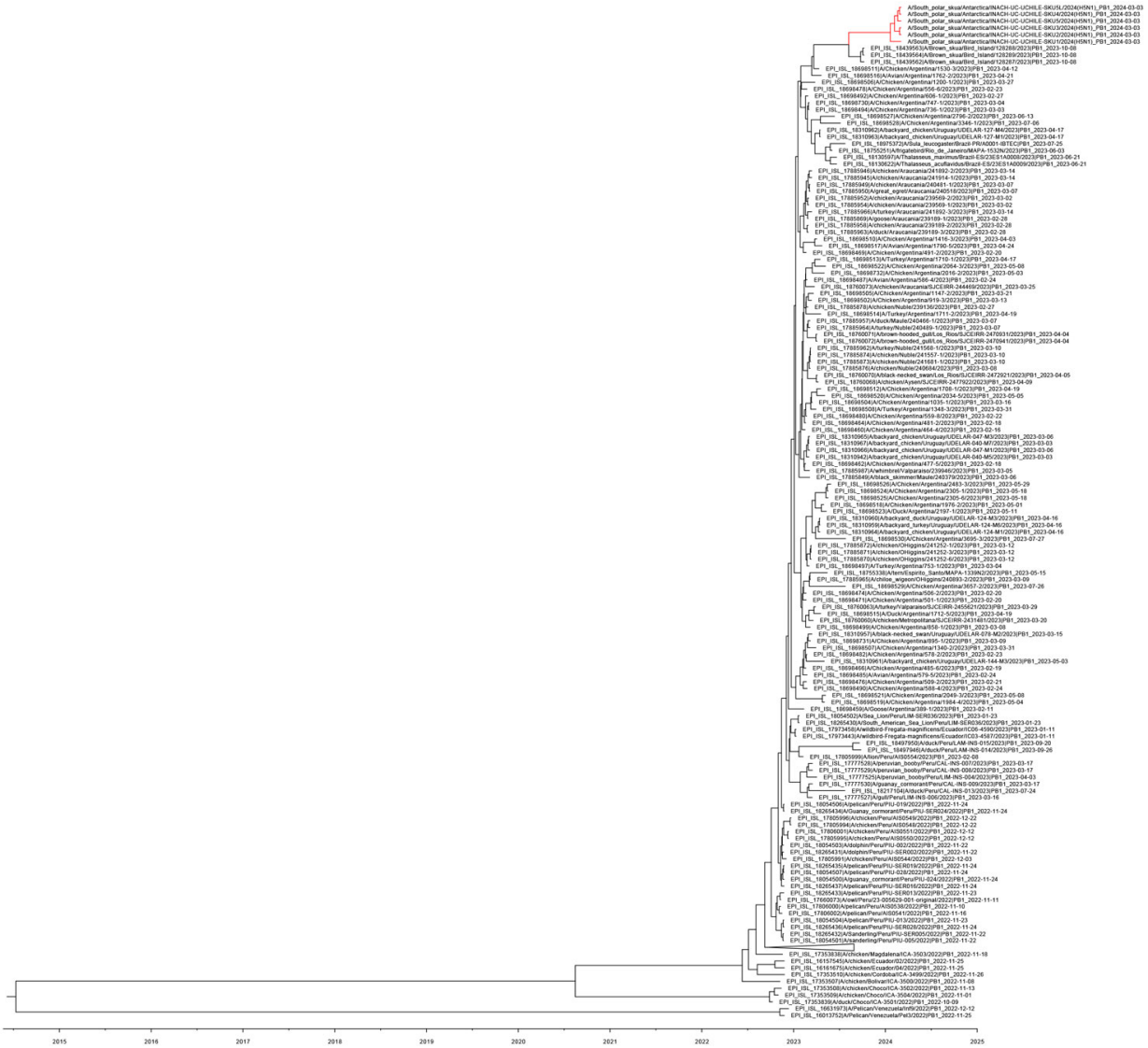
*HA, hemagglutinin; M, matrix; NA, neuraminidase; NP, nucleoprotein; NS, nonstructural; PA, polymerase acidic; PB1, polymerase basic 1; PB2, polymerase basic 2.
†Sequencing depth or coverage, number of reads obtained.



Appendix 1 Figure 1. Phylogenetic analysis of H5 clade 2.3.4.4b influenza viruses identified in Antarctica, 2024. Maximum clade credibility tree depicting time to most recent common ancestor estimates, generated by using a log-normal distribution and exponential growth models. Tree was reconstructed by using H5 gene sequences of strains sequenced from South America, South Georgia Islands, and Antarctica. The 6 sequences from this study were grouped into a monophyletic cluster during the first introduction. Another subcluster contained sequences from King George Island (Antarctica), detected on December 25, 2024 during the second introduction (at bottom of tree; EPI_ISL_19847536, EPI_ISL_19847539, EPI_ISL_19847538, EPI_ISL_19847535, EPI_ISL_19745586, EPI_ISL_19645365). Scale bar indicates nucleotide substitutions per site.

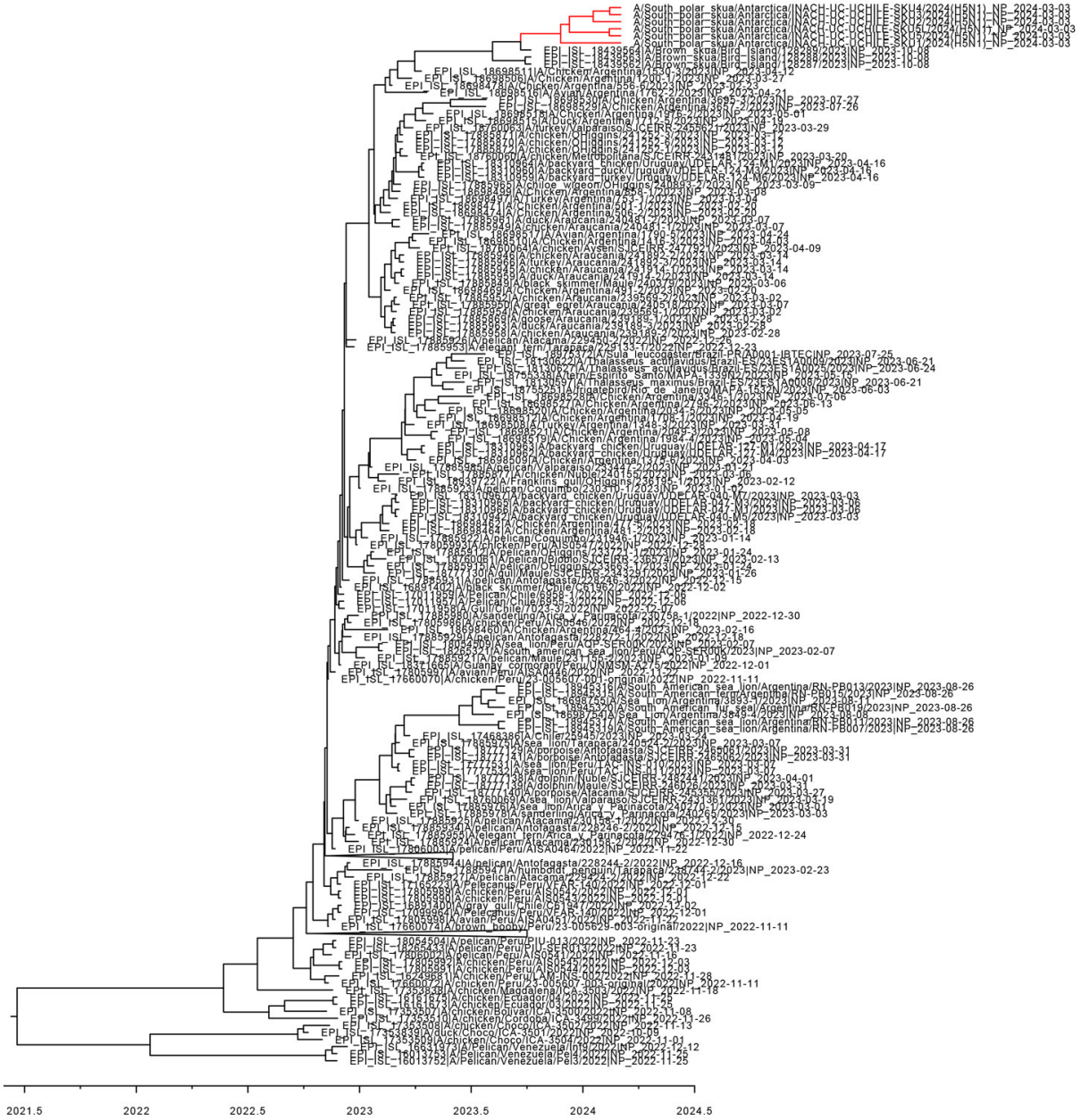


Appendix 1 Figure 2. Time-scaled maximum clade credibility tree for polymerase basic 2 genes of avian influenza virus. Red branches indicate sequences from this study.

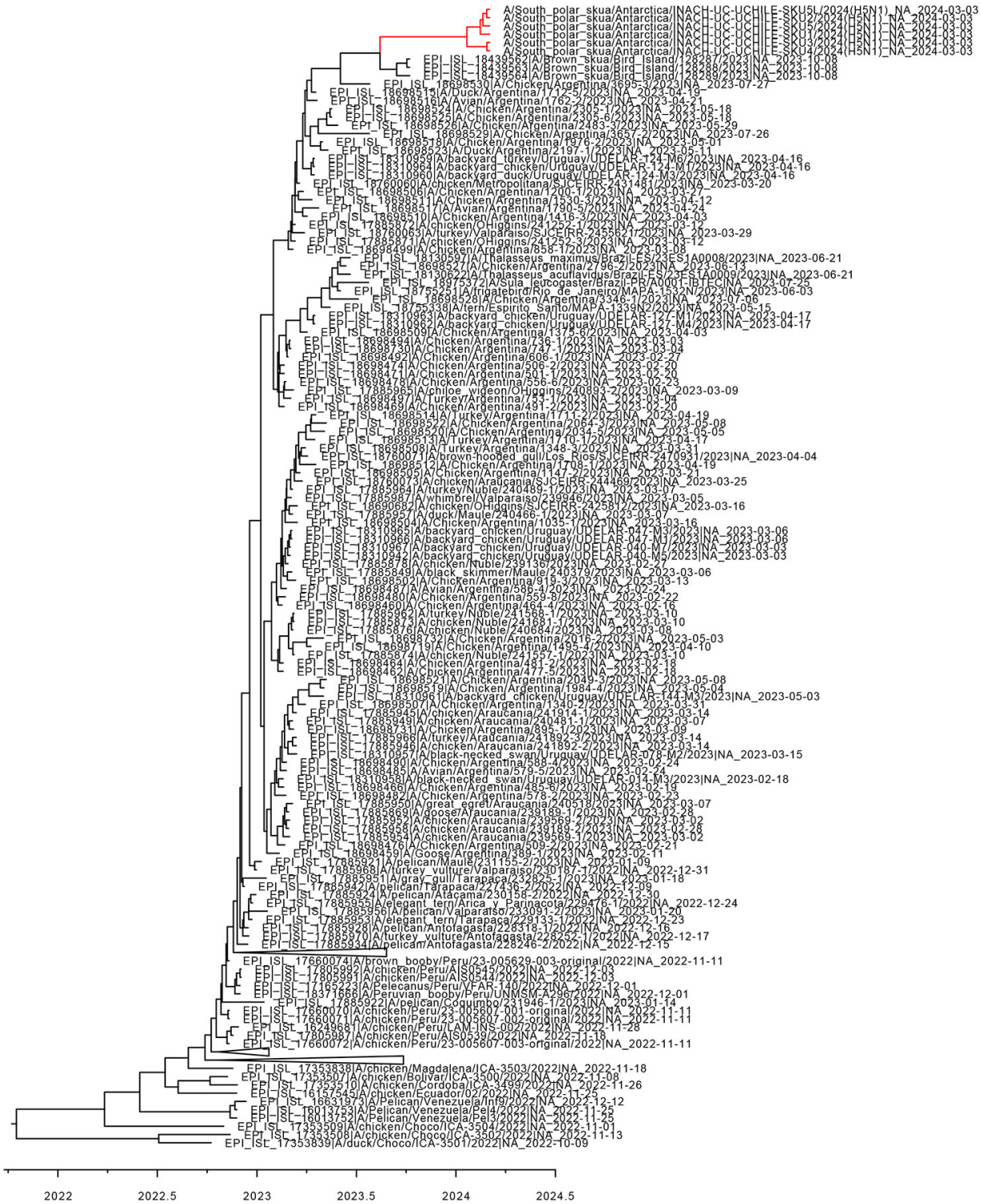


Appendix 1 Figure 3. Time-scaled maximum clade credibility tree for polymerase basic 1 genes of avian influenza virus. Red branches indicate sequences from this study.

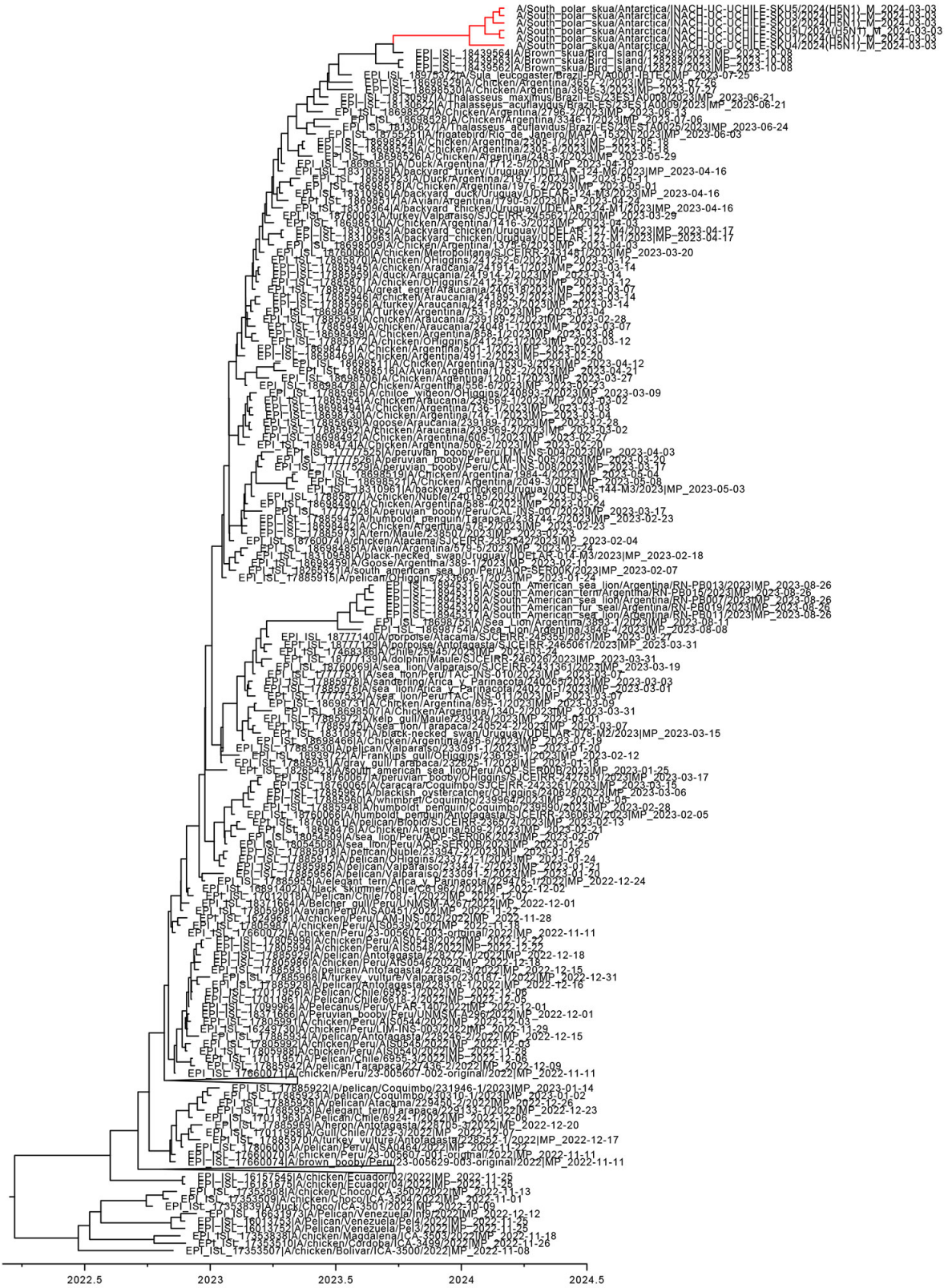
Appendix 1 Figure 4. Time-scaled maximum clade credibility tree for polymerase acidic genes of avian influenza virus. Red branches indicate sequences from this study.



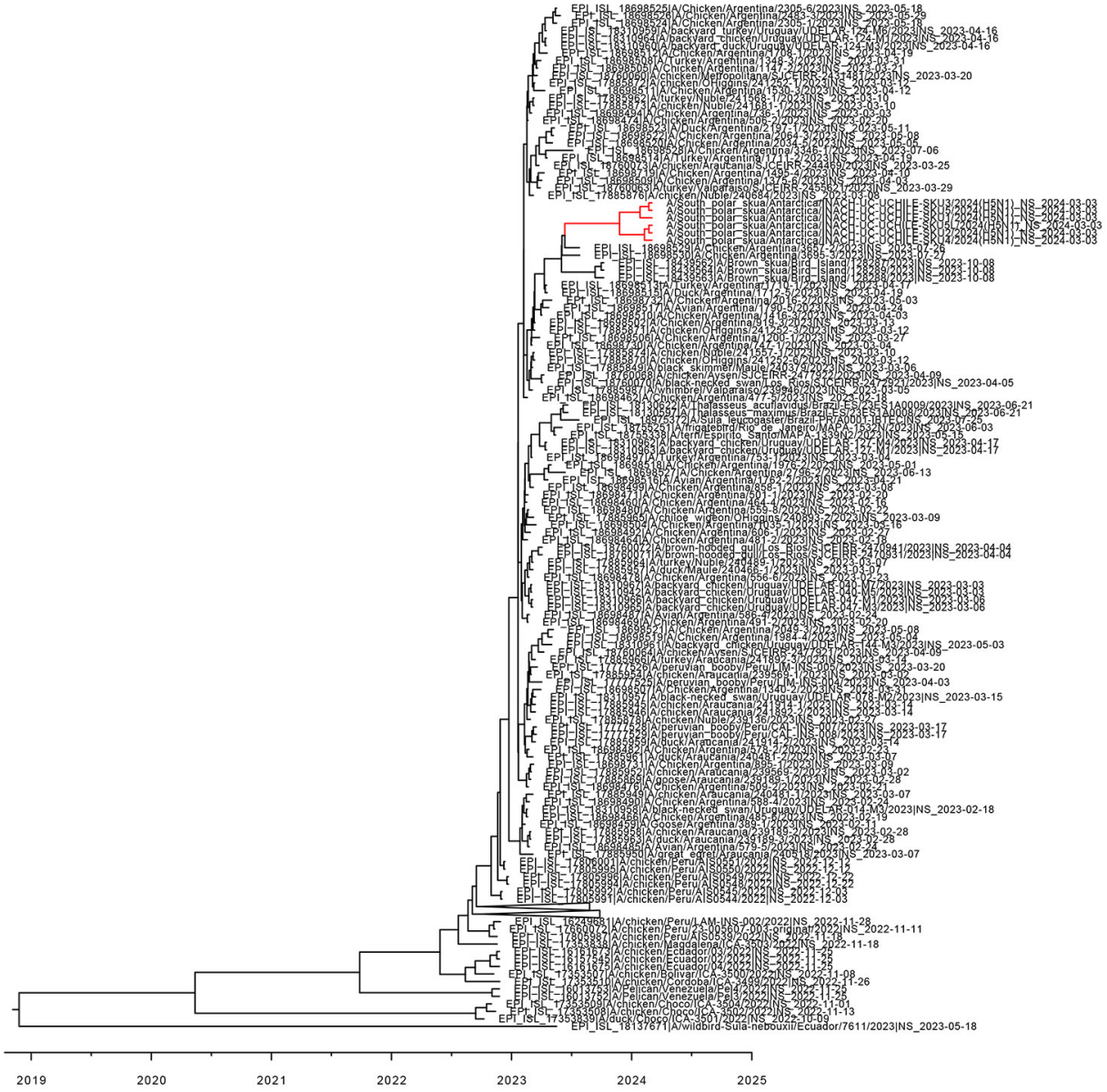
Appendix 1 Figure 5. Time-scaled maximum clade credibility tree for nucleoprotein genes of avian influenza virus. Red branches indicate sequences from this study.



Appendix 1 Figure 6. Time-scaled maximum clade credibility tree for neuraminidase genes of avian influenza virus. Red branches indicate sequences from this study.



Appendix 1 Figure 7. Time-scaled maximum clade credibility tree for matrix genes of avian influenza virus. Red branches indicate sequences from this study.



Appendix 1 Figure 8. Time-scaled maximum clade credibility tree for nonstructural genes of avian influenza virus. Red branches indicate sequences from this study.