Exploring the Spread and Evolution of Avian Influenza through Bioinformatic Analyses

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Purpose

- Analyze the evolution and spread of influenza viruses by creating a time-calibrated maximum likelihood phylogenetic tree and geospatial map
- Apply new avian influenza functionality in Freyja to investigate ongoing transmission and spread

Introduction

- Zoonotic spillover: pathogen transfer from animals to humans, common through direct animal contact or fomites[1]
  - Ex. SARS-CoV-2 pandemic (wildlife trade), avian influenza: bird → human emergence[2]
  - Risks of reassortment and widespread infections in animal populations
- Analyzing pathogen spread and evolution through wastewater and environmental sampling[3]
  - Monitoring for outbreaks within communities before first case reported
  - Less expensive
  - More representative of the population
- Freyja
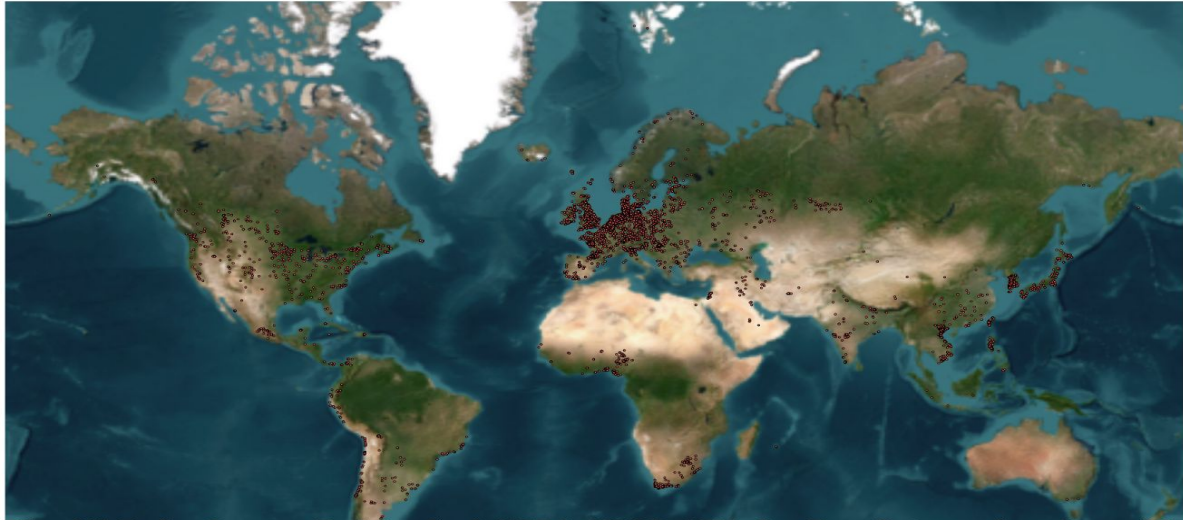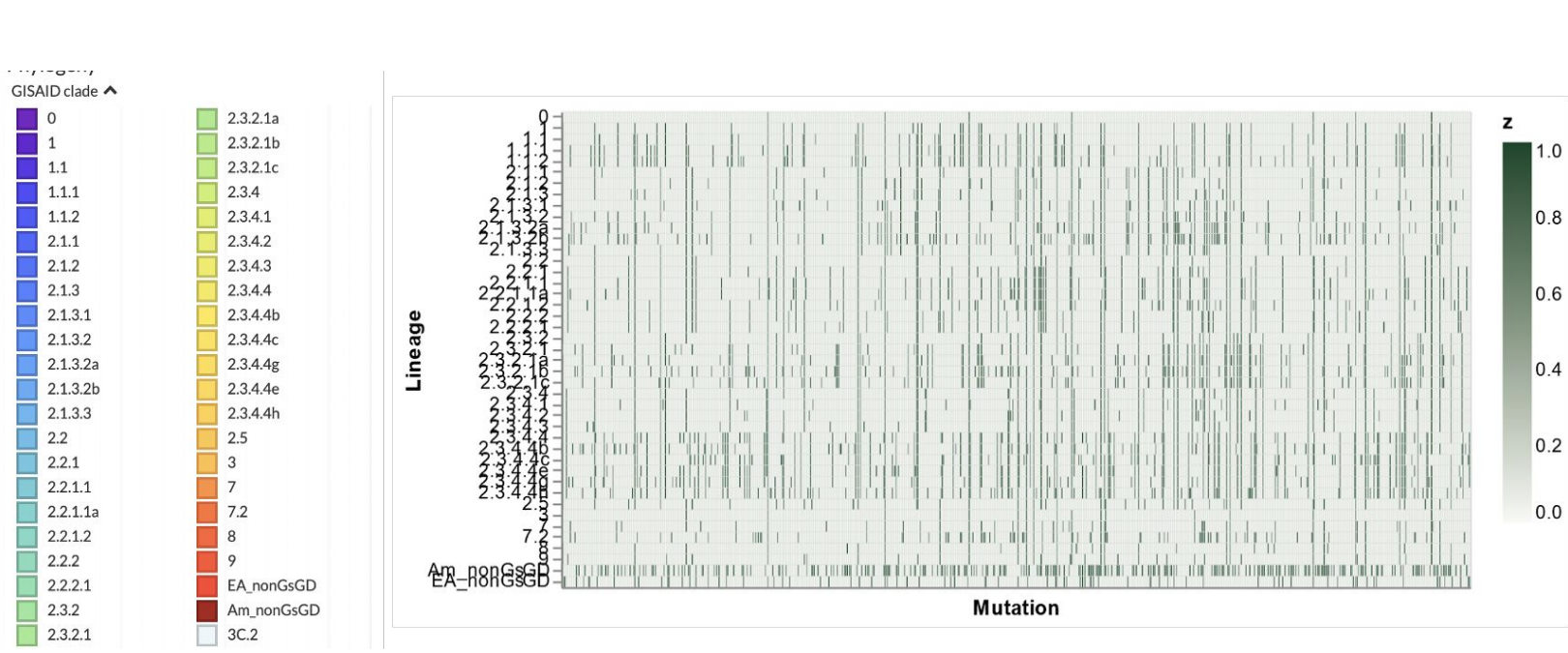 - Tool typically used to analyze virus lineages in mixed SARS-CoV-2 wastewater samples[4]

Figure 1. A geospatial map showing avian influenza case distribution between August 2018 to August 2023 was constructed with the Esri World Map basemap layer and plotted using Matplotlib[5]

Methods and Materials

Materials:

- Phylogenetic and Geospatial Analyses: GISAID data samples, EMPRES-i case data, Geopandas, Matplotlib, Cartopy, Contextily, MAFFT, TimeTree
- Variant Analyses: Freyja, iVAR

Methods:

- Obtained 1,689 influenza sequences for phylogeny creation from GISAID and geospatial data from EMPRES-i[7][8]
- Aligned flu sequences and processed temporal data to generate a time-calibrated maximum likelihood phylogenetic tree[6]
- Processed avian influenza data using various Python packages to create a geospatial map showing case distribution between August 2018-2023[5]
- Aligned avian influenza environmental samples to plot variant prevalence using Freyja[4]

Results

Figure 2. Barcodes used for Freyja analysis[4]

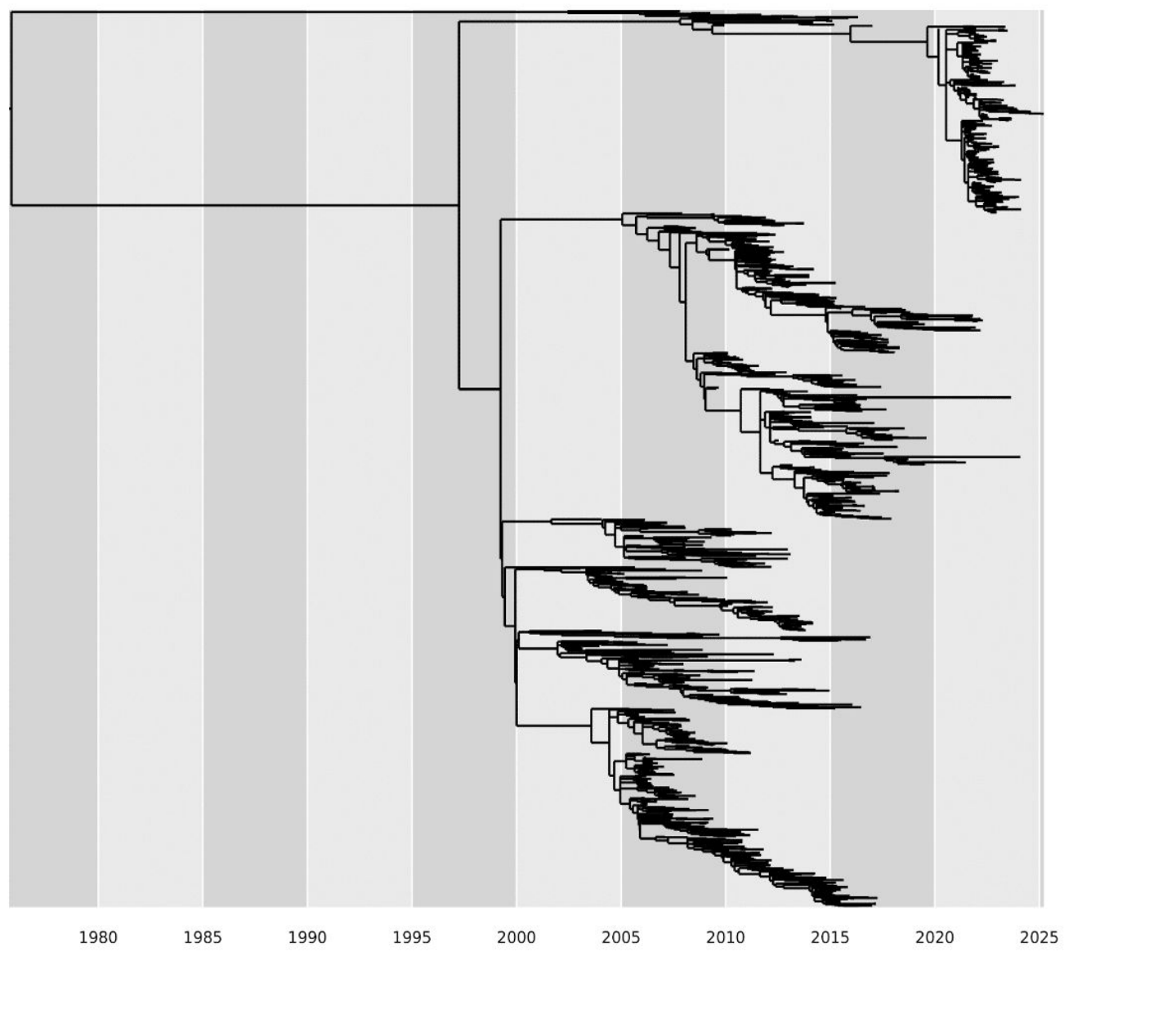Figure 3. Freyja analyses for a simulated set of flu viruses, showing lineage prevalence in each sample[4]

Figure 4. A time-calibrated maximum likelihood phylogenetic tree was constructed using TimeTree based on 1,689 influenza sequence samples[6]
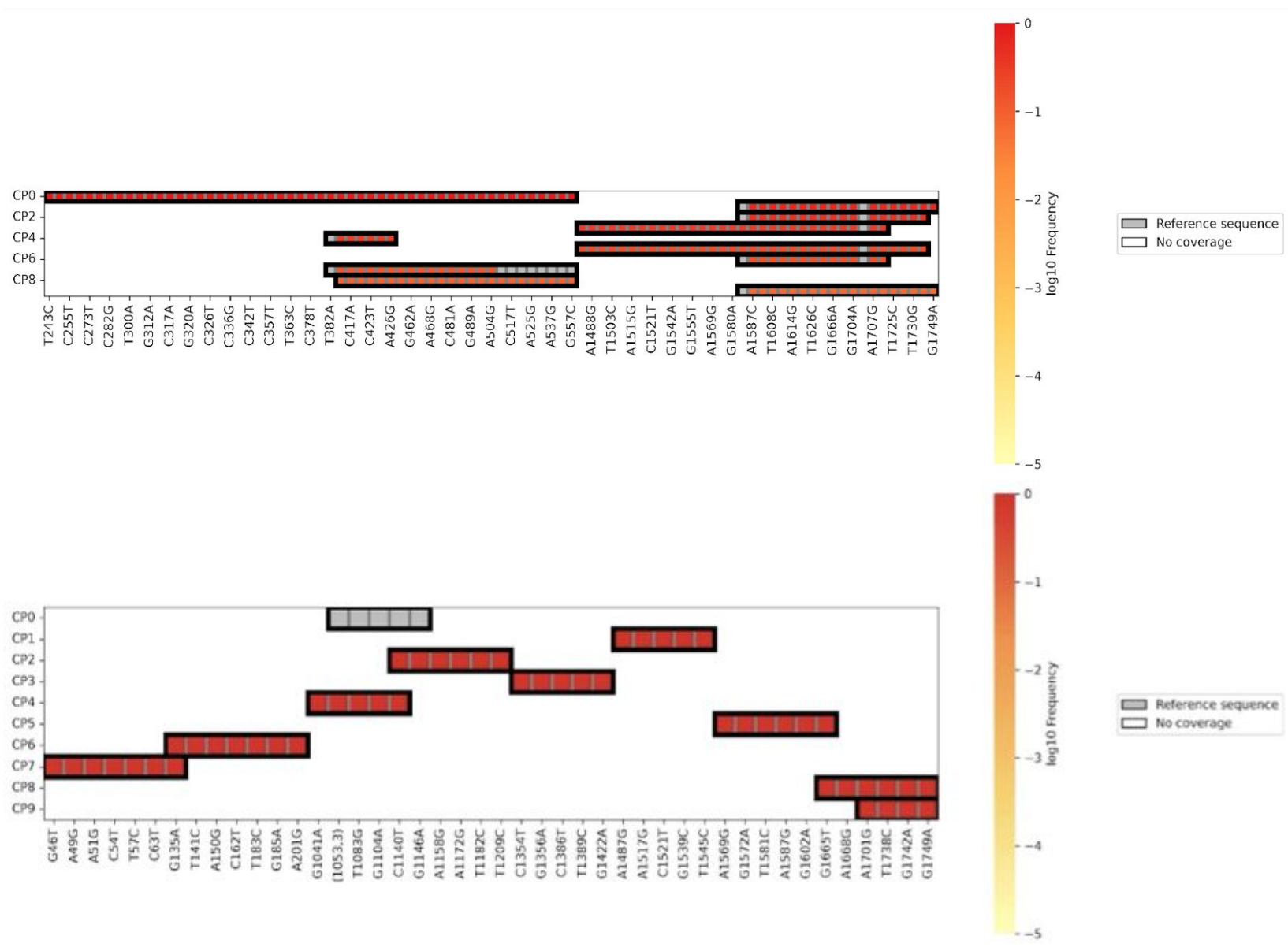
Figure 5. Covariant analysis for SRR7496088 and SRR23208411[4]

sample_outputs					
	summarized	lineages	abundances	resid	coverage
SRR7496088.tsv	[('Other', 0.9978474561560453)]	h52.3.4.4h h52.3.4.4	0.99572276 0.00212470	16.250032633310500	81.07954545454550
SRR7643082.tsv	[('Other', 0.9999999999999996)]	h5AmnonGsGD	1.00000000	0.9947018912430020	0.0
SRR23208411.tsv	[('Other', 0.9997940000021175)]	h5AmnonGsGD	0.99979400	17.24638512958310	34.65909090909090

Figure 6. Freyja output after demixing and aggregating 3 mixed environmental avian influenza samples for lineage prevalence. Bar chart shows plotting for lineage prevalence in a mixed environmental sample of avian influenza.[4]
Note: lineage prevalence was also plotted for samples SRR7643082 and SRR23208411 but didn't appear on the plot due to having mostly pure lineage.
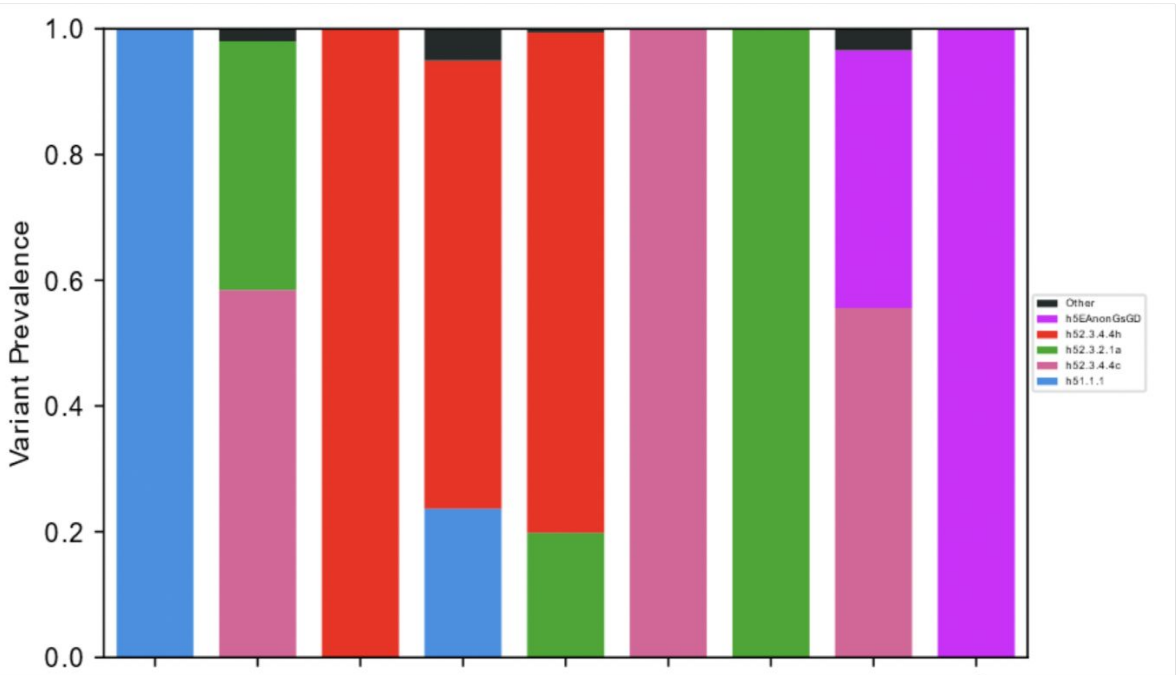
Results

- Freyja covariant and mixed lineage analyses show a high prevalence of mutated lineages within avian influenza environmental samples
- Signs of mixed lineages show risks of zoonotic spillover and genetic reassortment (Figure 6)
- Evolving lineages were detected in environmental samples of avian influenza, showing Freyja's adaptability to avian influenza viral strains
- Phylogenetic analyses of the influenza sequences suggest an evolutionary relationship
- Geospatial analyses using environmental data showed effects of migration on virus spread

Conclusion

- Phylogenetic analyses
  - Analyzes evolutionary relationships
  - Environmental samples can show virus mutation trends over time
- Geospatial mapping
  - Visualizes and tracks the geographic spread of known pathogens
  - Shows patterns and trends through mapping data
- Mixed sample analyses using Freyja
  - Ability to detect variants of influenza virus even at low concentrations
  - Surveillance benefits: tracing the evolution of the pathogen through new strains
- Implications:
  - Monitoring common migratory routes through geospatial mapping can pinpoint emerging regions
  - Sampling from frequent migration routes can prevent human transmission and improve public health

References

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