

### Background

H5 highly pathogenic avian influenza viruses (AIV) of the A/Goose/Guangdong/1/96-like lineage continue to evolve and circulate among avian species causing outbreaks in domestic and wild birds globally. Global spread via migratory birds to all continents except Oceania have resulted in large-scale outbreaks in domestic poultry, mass death of wild birds, and spillover infections to mammalian species. The expanded global spread is accompanied by increased genetic diversity of the H5Nx viruses through genetic reassortment of local AIV.

### Objectives

This study aims to analyze the genetic composition of 22 H5Nx isolates derived from poultry markets in Guangdong, China from 2020 to 2022 and to assess the impact of AIV gene segments introduced via migratory birds on the local AIV gene pool.

### Results

#### H5 is detected more frequently at the retail market than at wholesale market

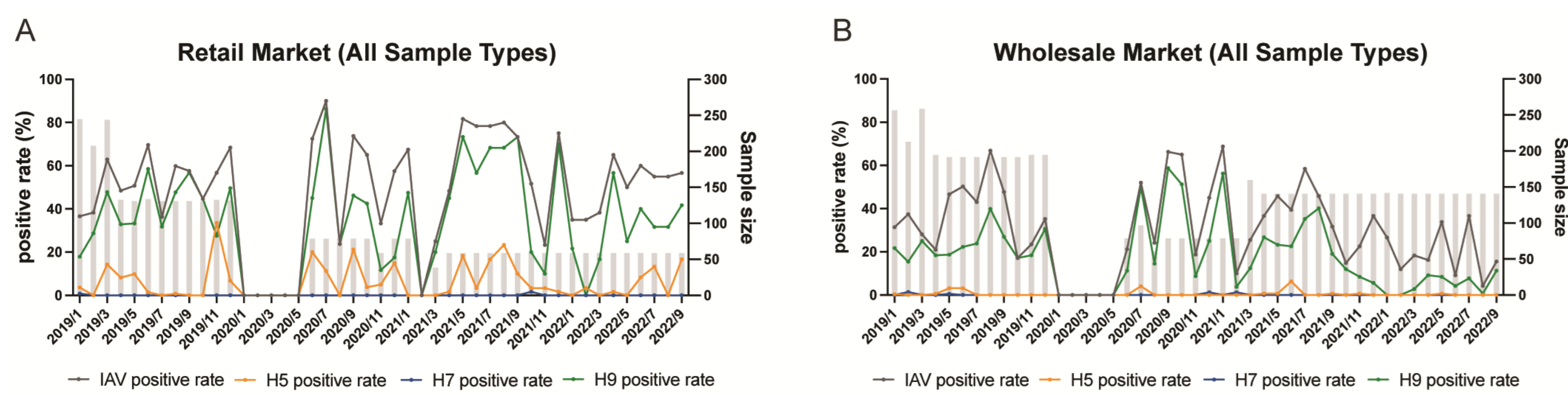


FIG : Positive rates of H5, H7 & H9 avian influenza virus from all sample types including oropharyngeal, cloacal, cavity and environmental swabs. Samples were tested for influenza(A) and subtyped via RT-PCR.

#### H5 isolates that clustered into clade 2.3.4.4b & clade 2.3.4.4h were re-emerging & continuously circulating in China, respectively

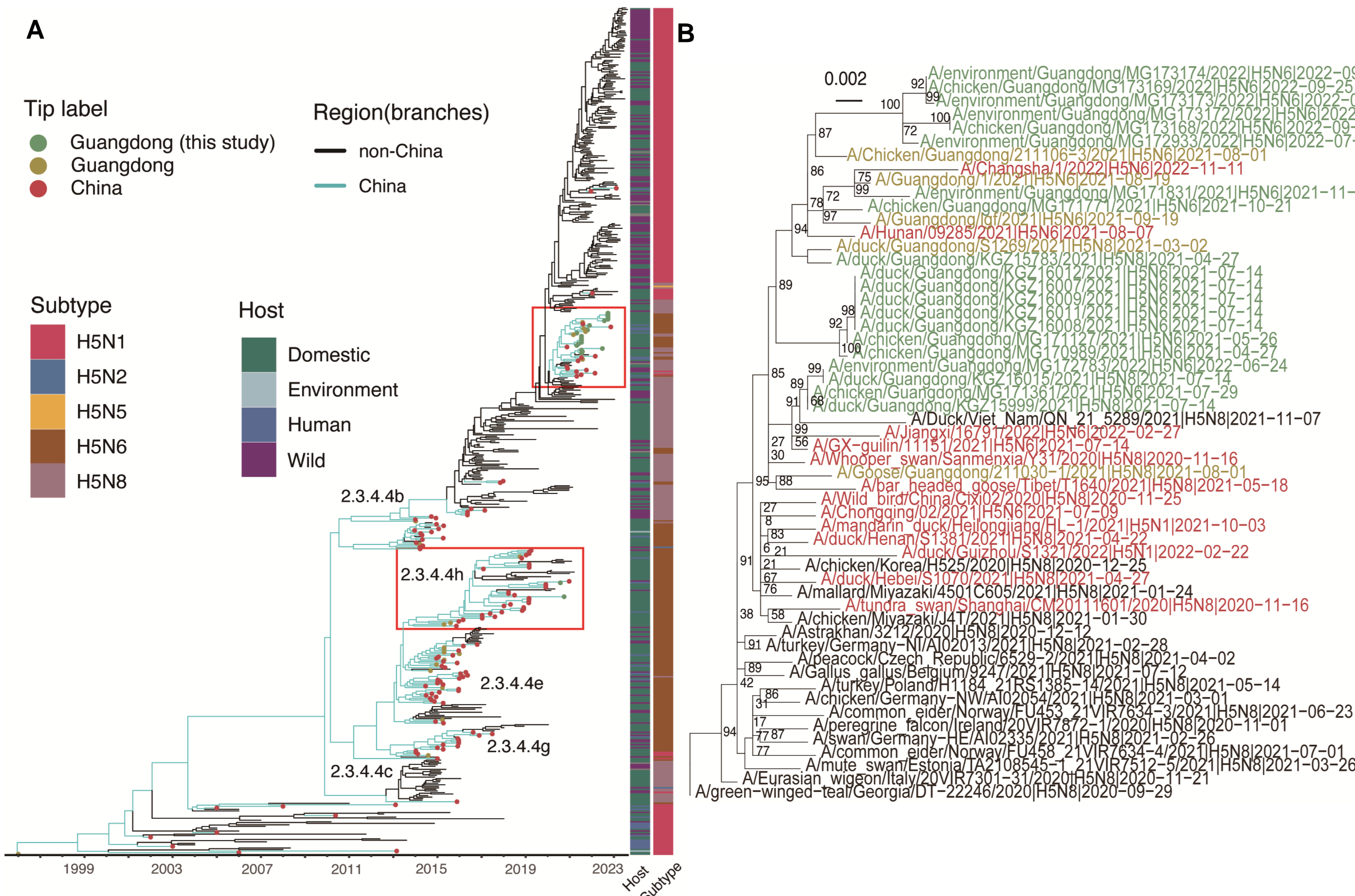


FIG : H5 hemagglutinin phylogenetic tree (A) with zoomed-in images of clade 2.3.4.4b (n=20) (B) and clade 2.3.4.4h (n=2) (C). Study isolates, Guangdong non-study isolates, China isolates are coloured in red, yellow and green.

22 H5 isolates were identified by selecting samples with CT < 35 for NGS sequencing by Illumina MiSeq and assembled via De-Novo assembly (CLCGenomics). HPAI H5 virus sequences from avian and human hosts between 2014-01-01 and 2023-06-30 were downloaded from GISAID, aligned via MAFFT, and maximum-likelihood phylogenetic tree construction via IQ-Tree LSD.

### Acknowledgements

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#### N6 NA gene circulates within Asia while N8 NA gene pool suggests introduction from outside of Asia

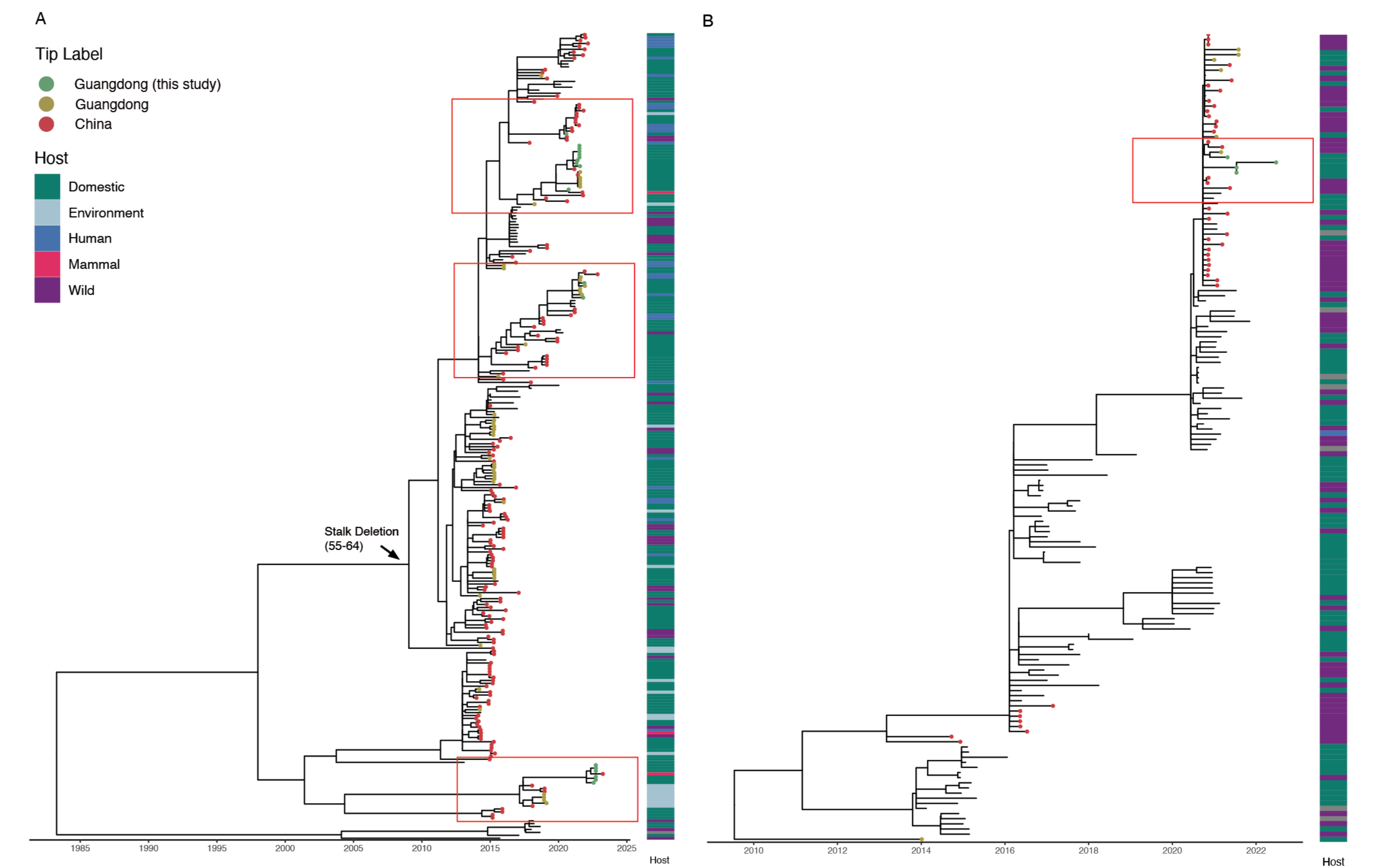
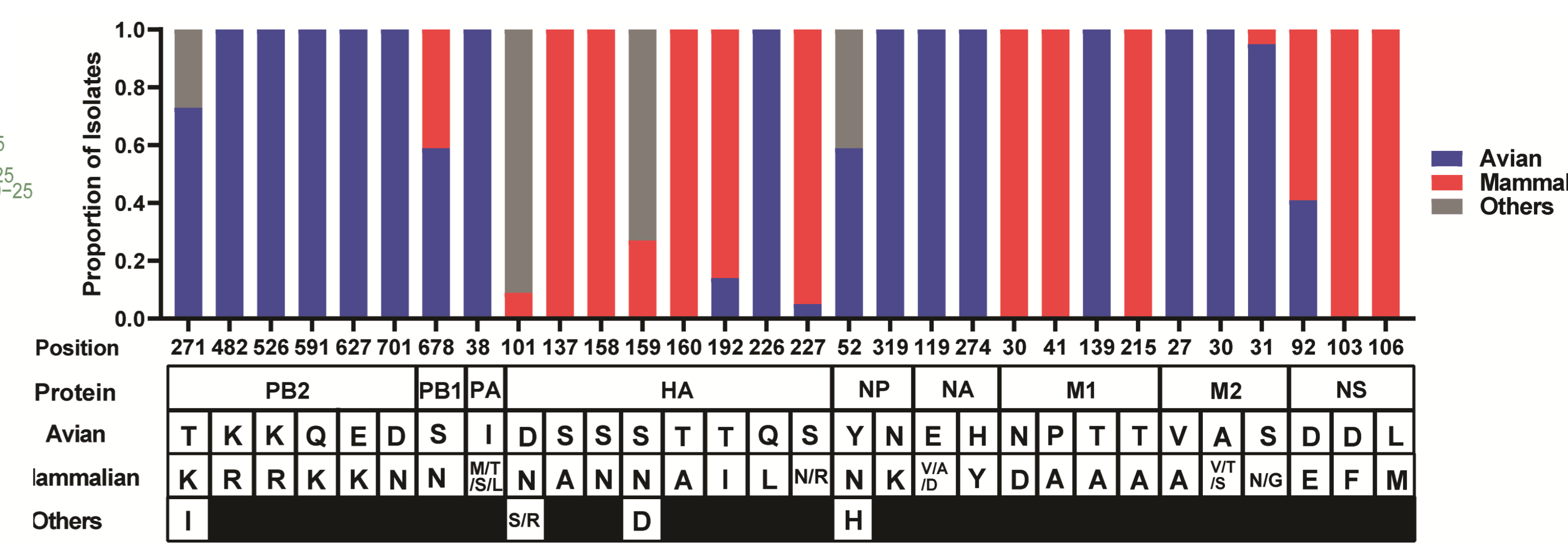


FIG : H5 neuraminidase phylogenetic tree (A) H5N6 (n=18) and (B) H5N8 (n=4). (A) Three N6 clusters belonging to clades circulating in Asia since 2014. The most bottom cluster show BLAST similarity with H6N6 NA detected from domestic waterfowl in China from 2018 – 2020 (B) H5N8 viruses belonging to a cluster of N8 NAs of 2.3.4.4b viruses detected in both wild birds and domestic poultry across Eurasia from late-2020 to mid-2021.

#### Adaptation markers found in H5 viruses



#### Transient genotypes were identified among the isolates

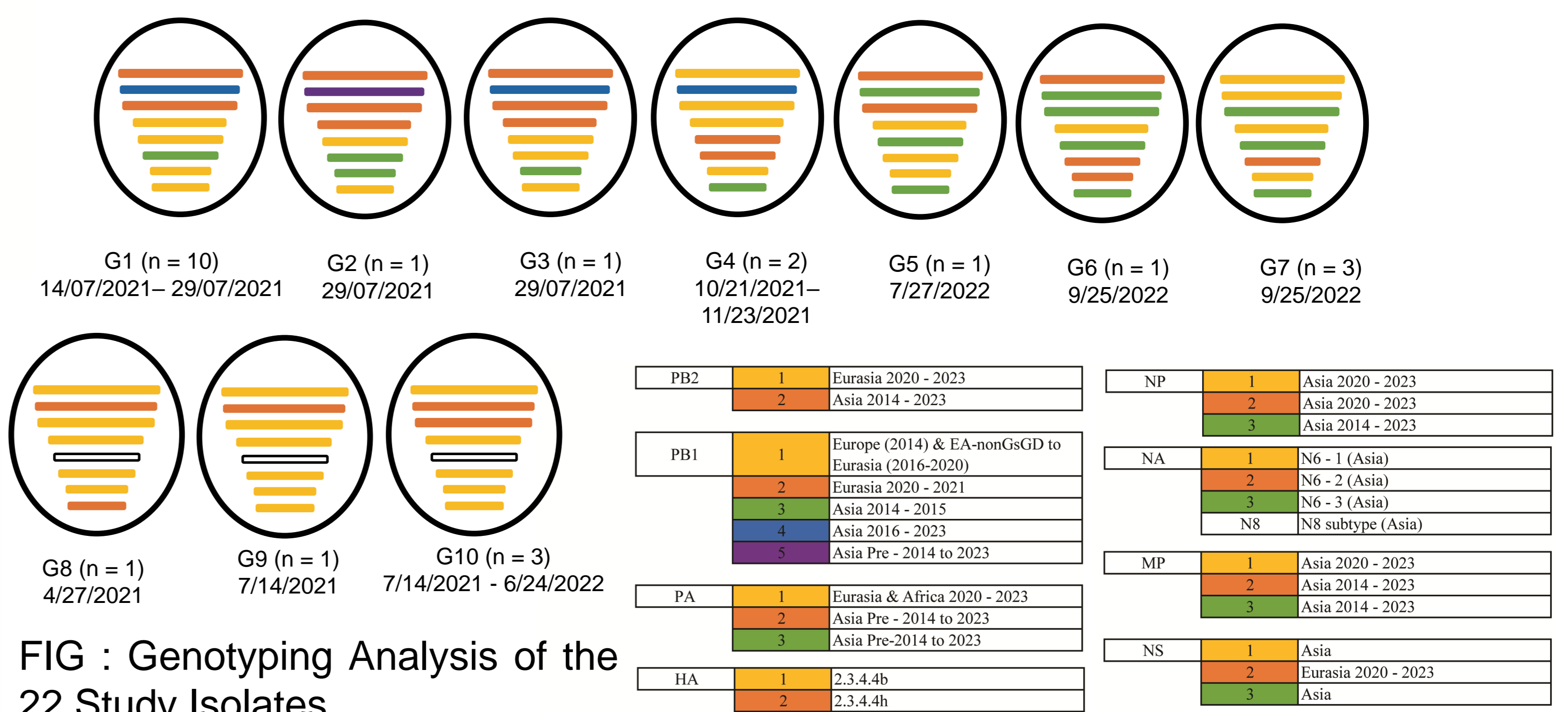


FIG : Genotyping Analysis of the 22 Study Isolates

### Conclusion

- We identified 20 isolates of H5N6 & H5N8 subtypes that clustered in the re-emerged clade 2.3.4.4.b with the global H5Nx isolates, and 2 isolates of H5N6 subtype that clustered in the clade 2.3.4.4.h persistent in China.
- The N6 NA originates from long term circulation in Asia since 2014, with one cluster showing reassortment with H6N6. Meanwhile, the N8 NA gene pool contains more recent isolates from Asia, Europe and Africa since 2020.
- We detected 10 genotypes from the 22 H5 isolates, suggesting high genetic diversity of H5Nx that circulated in China in 2020-2022. Several gene segments (PB2, PB1, PA & NS) were found to circulate with strains outside Asia and with wild birds.
- Most of the genotypes were transiently detected during one surveillance event.