

Highly Pathogenic Avian Influenza Clade 2.3.4.4b in Antarctic Pinnipeds: first evidences of virus circulation in Weddell seals

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Abstract

The ongoing expansion of highly pathogenic avian influenza virus (HPAIV) H5N1 clade 2.3.4.4b has resulted in unprecedented outbreaks among wild birds and marine mammals, raising concerns about viral dissemination into Antarctica. Here, we investigated fecal samples from Weddell seals (*Leptonychotes weddellii*) and Southern Elephant seals (*Mirounga leonina*) collected in Antarctica using molecular screening and whole-genome sequencing. HPAIV H5N1 clade 2.3.4.4b was detected and genetically characterized, showing evidence of viral circulation in these Antarctic pinnipeds. Phylogenetic analyses placed the virus within the South American lineage currently circulating in wild birds and marine mammals. Our findings demonstrate the usefulness of non-invasive fecal sampling for pathogen surveillance and provide further evidence that Antarctic marine mammals are exposed to the ongoing H5N1 epizootic, emphasizing the need for continued monitoring under a One Health framework.

1. Introduction

The Antarctic Region, geographically defined by areas south of the Antarctic Polar Front and encompassing the Southern Ocean, ice shelves, the Antarctic Peninsula, and the South Shetland Islands, represents a critical habitat for marine wildlife. Among the local fauna, pinnipeds are particularly important components of Antarctic ecosystems, with several species relying on these habitats for foraging and breeding (1). Antarctica supports important populations of six pinniped species, including the Antarctic fur seal (*Arctocephalus gazella*) and five phocid species: the Ross seal (*Ommatophoca rossii*), crabeater seal (*Lobodon carcinophaga*), leopard seal (*Hydrurga leptonyx*), Weddell seal (*Leptonychotes weddellii*), and southern elephant seal (*Mirounga leonina*).

Until recently, reports of influenza A virus (IAV) infection in pinnipeds were sporadic. However, the emergence and global spread of highly pathogenic avian influenza A (H5N1) virus (HPAIV H5N1), particularly within clade 2.3.4.4b, has substantially increased the risk of infection in marine mammals. Between January and October 2023, HPAIV H5N1 clade 2.3.4.4b was associated with widespread transmission among pinnipeds along the South American coast, including South American fur seals (*Arctocephalus australis*), South American sea lions (*Otaria flavescens*), and southern elephant seals, resulting in extensive mortality events (2, 3, 4). In 2023, HPAIV H5N1 was subsequently detected in pinnipeds in the sub-Antarctic region, with positive carcasses of five southern elephant seals and one Antarctic fur seal reported at Jason Harbour, South Georgia. Some affected individuals exhibited respiratory clinical signs. Subsequent surveillance in the Antarctic Peninsula in 2024 documented additional H5N1-associated mortality events involving wild birds and pinnipeds (5, 6).

During the austral summer, the high density and sympatric coexistence of diverse wildlife species within coastal breeding and haul-out colonies may facilitate viral transmission and interspecific spillover. Indirect and direct close contact among individuals from different species may also increase opportunities for co-infection and, consequently, genetic reassortment, potentially contributing to the emergence of novel viral genotypes (Smeele et al., 2018). In addition, indirect contact with contaminated

material, like feces, also probably contributes to virus spread in these areas. These natural transmission dynamics may be further influenced by human-wildlife interactions associated with scientific activities, ecotourism, logistical operations, and fishing activities (7, 8). However, our knowledge on the epidemiology and pathogenesis of IAV in Antarctica is very limited, as this virus emerged in the region very recently. Thus, important data, like virus viability in water and in different types of sediments (under the extreme environmental conditions in Antarctica), and morbidity/mortality rates in different species of birds and marine mammals, are missing.

To further IAV circulation among marine pinnipeds, we report the detection of HPAIV H5N1 clade 2.3.4.4b in fresh fecal samples collected from Weddell seals and southern elephant seals in Antarctica between November and December 2024. To investigate the occurrence of HPAIV in Antarctic pinnipeds, non-invasive fecal samples were collected from both species at breeding and haul-out sites across the South Shetland Islands, Antarctic Peninsula, during the XLV Brazilian Antarctic Expedition.

2. Materials and Methods

2.1. Sampling sites

Non-invasive and opportunistic sampling was conducted at: Robert Point on Robert Island, President Head on Snow Island, and Harmony Point on Nelson Island (Fig. 1) in Antarctic Peninsula, between November and December 2024. Fieldwork and sample handling strictly followed international biosecurity protocols to prevent cross-contamination and ensure researcher safety. Fecal samples from 14 Weddell seals and from 41 southern elephant seals, apparently healthy individuals, were collected directly from the substrate using sterile disposable spatulas. Animals were visually identified to species level and fecal samples were collected immediately after defecation to ensure accurate species attribution. Samples were immediately transferred to sterile cryogenic tubes, flash-frozen, and stored at -80°C until molecular analyses.

2.2. Nucleic Acid Extraction and RT-qPCR for Influenza A Virus Detection

The feces samples were processed using 0,3 g of the total sample resuspended in 1 mL of PBS 1 X by vortexing. The mixture was clarified by centrifugation at 15,000 rpm x for 1 min. Total nucleic acid extraction was performed using the MagMAX™ CORE Kit on the KingFisher™ Flex System (Thermo Fisher Scientific), using 200 μL of supernatant of each sample.

The rt-qPCR were performed using the AgPath-ID™ One-Step Kit following the manufacture instructions performed in the QuantStudio™ 1 Real-Time PCR System. Those conditions aimed to detect the matrix (M) gene (101 bp) as a screening (9). Samples yielding positive results ($\text{ct} \leq 37$) were further evaluated for H5 hemagglutinin subtype via RT-qPCR, following the molecular detection protocols recommended by the World Health Organization (10).

2.3. Next-generation sequencing and phylogenetic analysis

When possible, amplification of all viral genome segments was performed using universal primers (11) with the SuperScript™ III One-Step RT-PCR System (Thermo Fisher Scientific). Next-generation sequencing (NGS) was performed on the Ion Torrent platform using an Ion 510 chip (Thermo Fisher Scientific). Sequencing libraries were prepared using the Ion Xpress™ Plus Fragment Library Kit (Thermo Fisher Scientific), according to the manufacturer's instructions. Reads were processed and assembled using the IRMA v.1.1.4 pipeline (12).

Phylogenetic analyses were conducted using HA and neuraminidase (NA) sequences retrieved from NCBI Virus NIH database. Sequences were downloaded on July 2026, applying the following filters: Alphainfluenzavirus (taxid:197911); genotype H5N* (H5 sequences) or H*N1 (N1 sequences); segment 4 (HA) or segment 6 (NA); Location and source: H5 geographic region: all sequences from Antarctic (n = 58), South America (n = 544) and Oceania (n = 57) were downloaded N1 geographic region: all sequences from Antarctic (n = 58) and a randomized subset of records (20) per category, stratified by collection year, from South America (n = 382 out of 2188) and Oceania (310 out of 1206) were downloaded. Additionally, HA and NA sequences were combined with the 50 most similar sequences identified using BLAST on the GISAID EpiFlu database. Identical nucleotide sequences were screened using an R script; duplicates from the same host species and sampling year were removed, whereas identical sequences originating from distinct hosts or sampling years were retained for downstream analyses.

The coding region of HA and NA segments were aligned using MAFFT v7.490 (13). Duplicate sequences and sequences with less than 80% nucleotide (nt) coverage relative to the longest sequence in the dataset were excluded (undetermined bases, Ns, were not counted in the total nucleotide count). Maximum-likelihood phylogenetic reconstructions were performed using IQ-TREE v3.1.1 (14). The ModelFinder Plus tool (-m MFP) was applied to determine the best-fitting nucleotide substitution model for each dataset (GTR + F+I+G4 for both datasets according to Bayesian Information Criterion). Branch support was assessed using the SH-like approximate likelihood ratio test (SH-aLRT) with 10,000 replicates and the number of computational threads was automatically determined (-nt AUTO). The resulting ML tree was subsequently visualized using FigTree (15) and edited using Inkscape.

3. Results

A total of seven out of 41 fecal samples from southern elephant seals tested positive for IAV, along the President Head (n = 4) and Robert Point (n = 3). Although none of these samples were positive for the H5 subtype via PCR, one sample from President Head was selected for whole-genome sequencing, yielding a partial H5 (660 bp) and N1 (sequences (536 bp). Among Weddell seals, four of the 14 fecal samples analyzed tested positive for the FLUAV M gene, while two of these samples were also H5 positive. One H5-positive sample was selected for whole-genome sequencing, yielding a partial H5 gene (1,299 bp) and a complete N1 gene (1,410 bp). Sequence analysis confirmed the virus as HPAI clade 2.3.4.4b. The H5 and N1 sequences from the Weddell seal have been deposited in GenBank under accession numbers

PZ441019 and PZ437028, respectively, while the partial H5 and N1 sequences from the southern elephant seal under accession numbers PZ700710 and PZ700711.

Four hundred eighty-eight HA sequences, ranging from 1,203 to 1,323 bp, were subjected to maximum likelihood phylogenetic analysis. Additionally, a shorter partial HA sequence (660 bp) from an elephant seal was included solely to ascertain its relative phylogenetic position. The sequence isolated from the Weddell seal (PZ441019_Leptonychotesweddellii_Antarctica_2024) clustered within the same clade (aLRT = 94.5%, Fig. 2A and Supplementary Fig. 1) as two other sequences isolated from the Antarctic Islands in 2024, derived from a Gentoo penguin (*Pygoscelis papua*) and a Southern giant petrel (*Macronectes giganteus*). This clade is nested among sequences from Antarctica, Argentina, Uruguay, and Brazil (aLRT = 86.7%), supporting the hypothesis of a viral introduction into Antarctica from South American lineages (Fig. 2B).

The partial HA sequence from the elephant seal (PZ700710_Miroungaleonina_Antarctica_2024) grouped within a clade (aLRT = 93.7%) composed of sequences isolated in Argentina, Chile, and Uruguay in 2026 (Fig. 2C). The long branch length and lack of statistical support at recent ancestral nodes likely stem from the limited length of this sequence. To better resolve its evolutionary relationships, we generated a secondary alignment by trimming all sequences to match the length of PZ700710_Miroungaleonina_Antarctica_2024 prior to phylogenetic reconstruction. This short-alignment tree reinforced the relationship between the elephant seal sample and isolates from Argentina, Chile, and Uruguay collected in 20025 and 2026 (aLRT = 99.8%, Supplementary Fig. 3A). Notably, in this reconstruction, the sample shared a direct common ancestor (aLRT = 99.4%) with a 2026 isolate from a Coscoroba swan (*Coscoroba coscoroba*) in Southern Brazil.

For the NA segment, 632 sequences (1,133 to 1,413 bp) were analyzed via phylogenetic reconstruction (Fig. 2D and Supplementary Fig. 2). As with HA, the partial 536 bp NA segment from the elephant seal (PZ700711_Miroungaleonina_SnowIsland_2024) was included to determine its placement. Consistent with the HA findings, the Weddell seal sequence (PZ437028_Leptonychotesweddellii_Antarctica_2024) grouped within a clade predominantly composed of Antarctic isolates (Fig. 2E). Furthermore, the NA sequence from the elephant seal mirrored the HA results, demonstrating a shared evolutionary history with samples from Argentina, Uruguay, and Brazil (Fig. 2F). Phylogenetic reconstruction based on the shortened NA alignment confirmed these topologies with robust support (Supplementary Fig. 3B).

4. Discussion

Since the onset of the spread of HPAIV H5N1 to the American Continent, concerns have arisen regarding the potential introduction of this virus into Antarctic ecosystems. While the virus spread in South America, substantial impacts on the Pacific coastal wildlife were documented and, additionally, the virus acquired genomic alterations which allowed its adaptation to mammals. Antarctic and sub-Antarctic regions are particularly vulnerable due to their dense, highly aggregated, and often isolated wildlife populations, and the introduction of HPAI could have severe ecological consequences (16).

This is the first record of H5N1 clade 2.3.4.4b detected in fecal material from a Weddell seal occurring in South America. This clade has been associated with widespread outbreaks in wild birds and sporadic spillover into mammalian hosts, underscoring its zoonotic potential.

The Weddell seal is widely distributed across the Antarctic continental shelf, with its range extending northward to South Georgia. Occasional vagrant individuals have been reported in South America, including Argentina, Uruguay, Brazil, Chile, and the Falkland Islands (17). Such movements may facilitate contact with other pinniped species in which H5N1 has previously been detected, including fur seals, sea lions, and elephant seals that disperse along South American coastlines. In addition, migratory bird species frequent overlapping habitats, increasing opportunities for interspecies transmission and environmental exposure (18). These ecological interactions may help explain the spillover of influenza virus into Weddell seals.

In 2026, the IUCN pre-assessment shifted the southern elephant seal (*M. leonina*) from Least Concern (LC) to Vulnerable (VU), estimating a 30% population reduction and signaling HPAI as the primary driver (19). This decline is illustrated by the 2023 breeding season in Argentina, where pup mortality reached at least 96%, with 17,000 dead elephant seals recorded alongside adult and subadult carcasses (20). Our findings demonstrate the active circulation of IAV within this species in apparently healthy individuals. Notably, the lineage circulating in Antarctica was subsequently detected in Southern Brazil in 2025, where it was identified in coscoroba swans (*Coscoroba coscoroba*) and caused outbreaks in a zoo in Sapucaia do Sul as well as in a commercial poultry farm in Montenegro, Rio Grande do Sul.

Our findings align with previous reports of H5N1 infection in other pinniped species and indicate that Weddell seals may be exposed to the virus through interspecies transmission or shared environmental pathways. Importantly, these results underscore the tangible risk of viral spillover within Antarctic ecosystems, where dense wildlife aggregations and limited immunological naïveté may amplify transmission dynamics. Occasional South American vagrant pinnipeds, already affected by H5N1, reach Antarctic coastlines and vice versa, represent a potential epidemiological bridge. In Antarctic, which is characterized by dense wildlife aggregations and limited immunological experience with avian influenza viruses, even rare long-distance movements by vagrant individuals could facilitate viral introduction and onward transmission. Continued surveillance of influenza A virus in Antarctic marine mammals is therefore essential to track viral evolution, assess ecological impacts, and inform conservation and biosecurity strategies. As wildlife species function both as reservoirs and sentinels of zoonotic pathogens, sustained monitoring efforts are crucial not only for safeguarding Antarctic biodiversity but also for anticipating and mitigating future pandemic threats.

Declarations

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Declaration of Interest Statement

The authors declared that they have no competing interests or conflicts of interest related to this research, authorship, or publication of this manuscript.

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CRediT author statement

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Figures

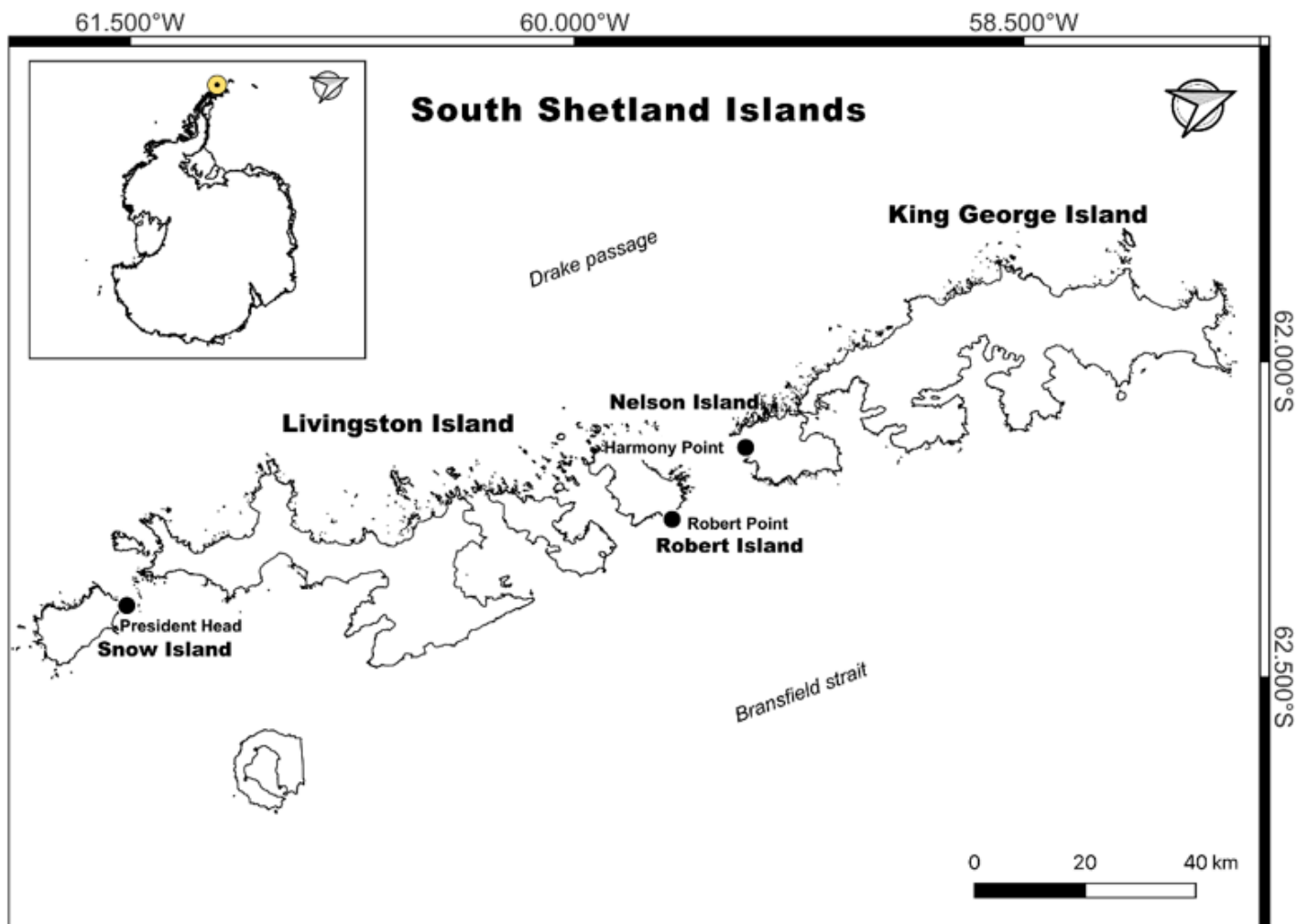


Figure 1

Sampling sites in the South Shetland Islands, Antarctic Peninsula, during the XLV Brazilian Antarctic Expedition (November–December 2024). Samples were collected at Robert Point (Robert Island), President Head (Snow Island), and Harmony Point (Nelson Island).

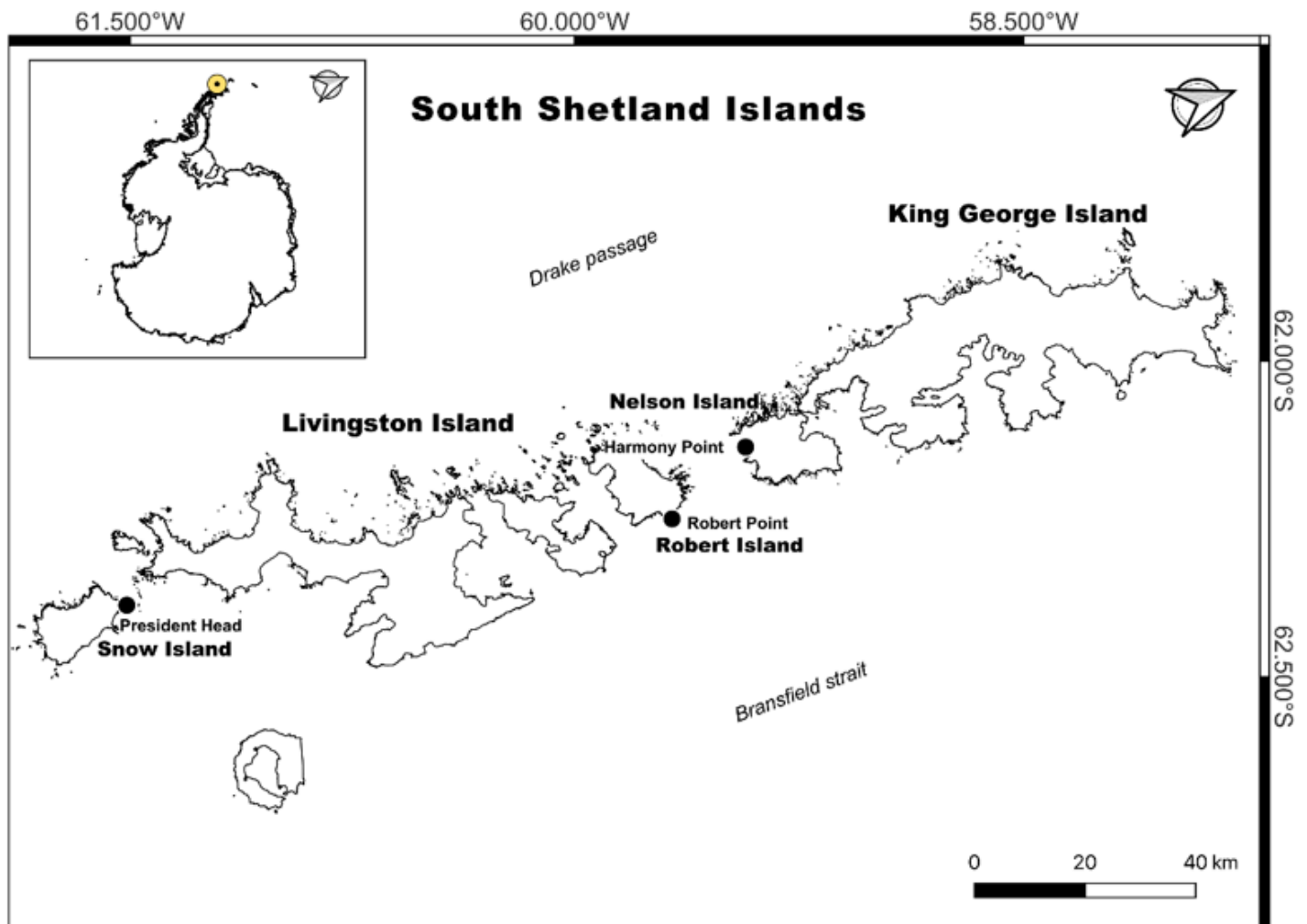


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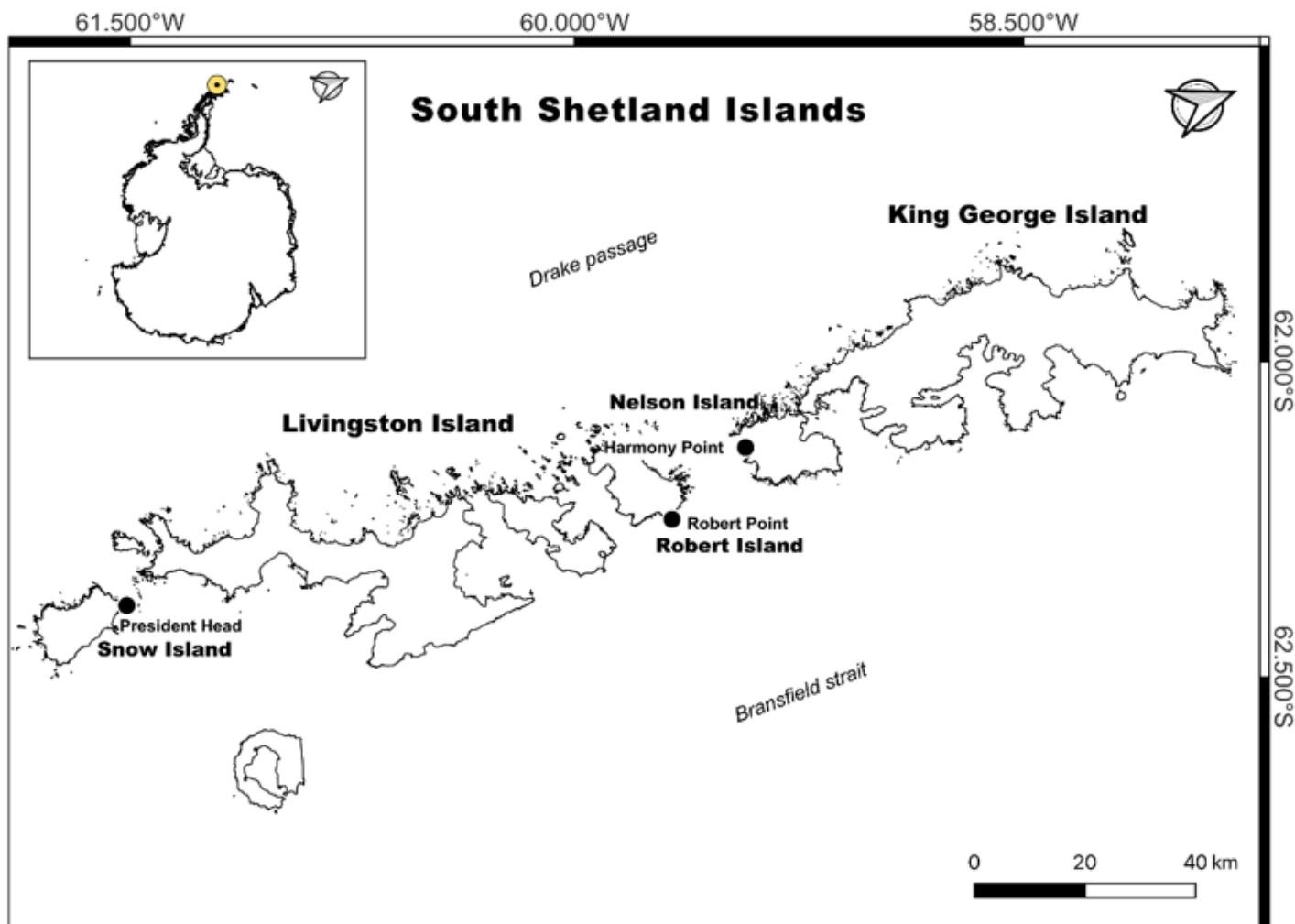


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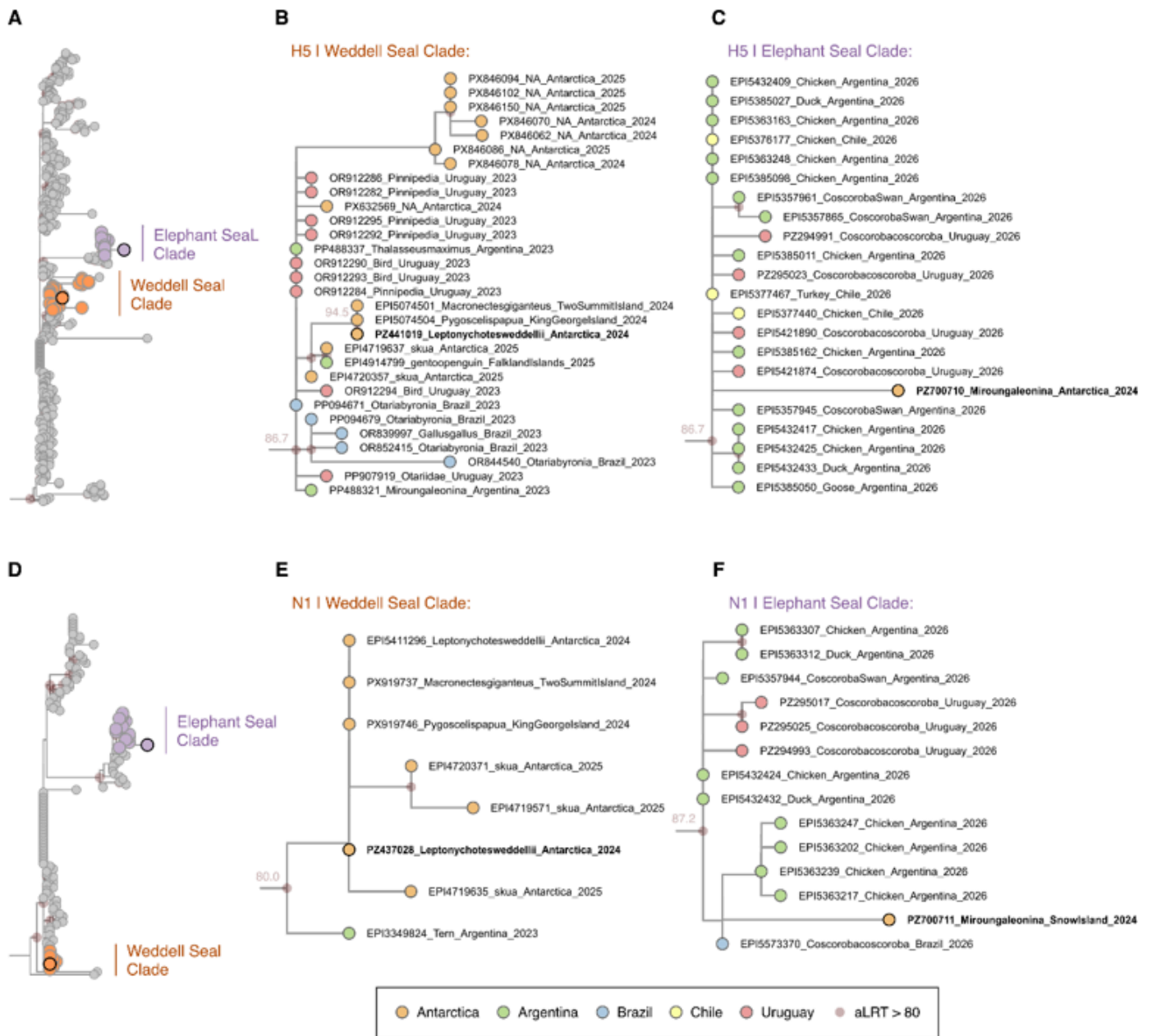


Figure 2

Maximum likelihood phylogenetic trees of H5 and N1 influenza segments highlighting seal-derived isolates. (A–C) Hemagglutinin (H5) segment phylogeny. (A) Overview tree showing the phylogenetic placement of the Weddell seal (*Leptonychotes weddellii*) and Southern elephant seal (*Mirounga leonina*) isolates within global and regional lineages (sample). (B) Detailed subtree of the H5 Weddell Seal Clade, highlighting co-clustering with avian isolates from the Antarctic Islands. (C) Detailed subtree of the H5 Elephant Seal Clade, indicating evolutionary relationships with lineages from South America. (D–F) Neuraminidase (N1) segment phylogeny. (D) Overview tree for the N1 segment including both seal isolates. (E) Subtree of the N1 Weddell Seal Clade, showing alignment with Antarctic isolates. (F) Subtree of the N1 Elephant Seal Clade, showing clade topology alongside South American isolates. Clades with

SH-aLRT ≥ 80 are indicated by red circles at the internal nodes and values were indicated for key ancestral nodes. Tip colors represent geographic origin/host species as detailed in the legend below.

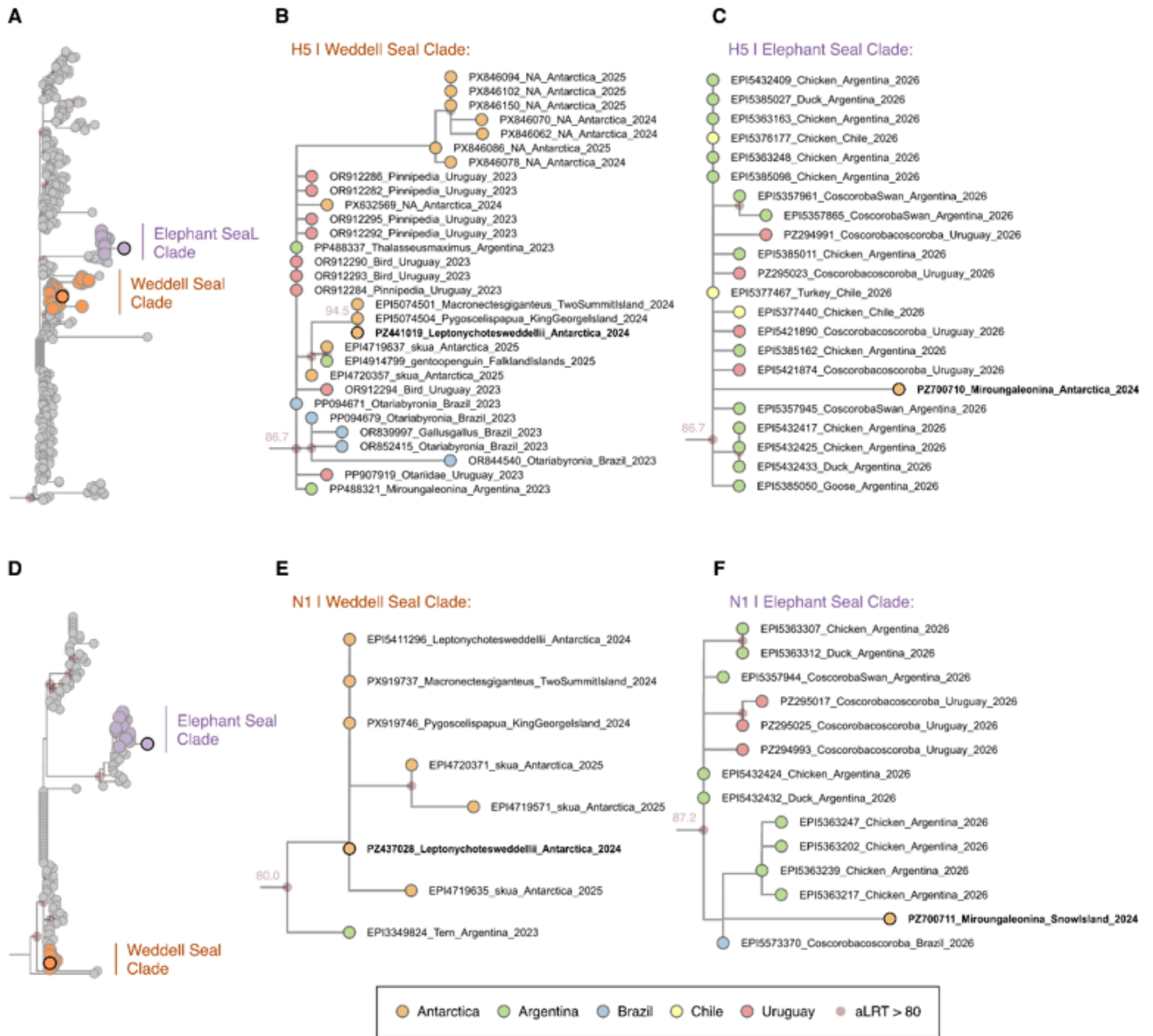


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