Detection and spread of high pathogenicity avian influenza virus H5N1 in the Antarctic Region

Running title: HPAIV in the Antarctic

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Abstract

Until recent events, the Antarctic was the only major geographical region in which high pathogenicity avian influenza virus (HPAIV) had never previously been detected. The current panzootic of H5N1 HPAIV has decimated wild bird populations across Europe, North America, and South America. Here we report on the detection of clade 2.3.4.4b H5N1 HPAIV in the Antarctic and sub-Antarctic regions of South Georgia and the Falkland Islands, respectively. We initially detected H5N1 HPAIV in samples collected from brown skuas at Bird Island, South Georgia on 8th October 2023. Since this detection, mortalities were observed in brown skuas, kelp gulls, South Georgia shag, Antarctic tern, elephant seals and fur seals at multiple sites across South Georgia. Subsequent testing confirmed H5N1 HPAIV across several sampling locations in multiple avian species and two seal species. Simultaneously, we also confirmed H5N1 HPAIV in southern fulmar and black-browed albatross in the Falkland Islands. Genetic assessment of the virus indicates spread from South America, likely through movement of migratory birds. Critically, genetic assessment of sequences from mammalian species demonstrates no increased risk to human populations. Here we describe the detection, species impact and genetic composition of the virus and propose both introductory routes and potential long-term impact on avian and mammalian species across the Antarctic region. We also speculate on the threat to specific populations following recent reports in the area.

42 # Introduction

43 Following the emergence and global expansion of A/goose/Guangdong/1/96 (GsGd)-lineage H5 high
44 pathogenicity avian influenza viruses (HPAIV) there have been repeat epizootics in wild birds and
45 poultry populations globally. This lineage spread to other Asian countries, evolving into diverse
46 clades classified according to the H5 haemagglutinin (HA) gene phylogeny [1]. In the autumn of 2021,
47 the situation escalated considerably with the detection of a clade 2.3.4.4b HPAIV subtype H5N1 in
48 Europe[2]. Subsequently, two unprecedented epizootic waves with this lineage in 2021/22 and 2022/23
49 were associated with mass mortality events in wild birds together with unprecedented numbers of
50 incursions into poultry premises[2, 3, 4, 5, 6]. High levels of viral adaptation to wild bird species[7], and
51 increased fitness advantage through continued genetic reassortment[8] likely underpin the broad impact
52 infection has had across many avian species[3]. This wide host range has facilitated the transmission of
53 the lineage across a large geographic area, including from Europe to North America[9, 10], where it has
54 since rapidly expanded its range southward into South America via migratory avian species. Incursion
55 into South American countries, starting in November of 2022, represented the first recorded instances
56 of GsGd-lineage H5 HPAIV in the region[11, 12, 13]. Mass mortality events in the region have been
57 particularly severe and reported across several different bird species in addition to marine mammals[3,
58 11, 14, 15], highlighting the extensive ecological impact of HPAIV and the ongoing threat it presents to
59 naïve hosts.

60 The Antarctic region includes the ice shelves, waters, and all the island territories in the
61 Southern Ocean situated inside of the 'Antarctic Convergence' or 'Antarctic Polar Front', a marine
62 belt encircling Antarctica where Antarctic waters meet those of the warmer sub-Antarctic[16]. Several
63 islands are located inside the Antarctic region, including South Georgia, while the Falkland Islands
64 among others are located outside the Antarctic Convergence in the sub-Antarctic zone (Figure 1A).
65 There have been no previous reports of HPAIV inside the Antarctic region[17, 18]. Antarctica and the
66 sub-Antarctic islands possess unique ecosystems which support the population strongholds of several
67 avian and marine mammal species. The relative isolation of these islands from human populations has
68 provided species across the Antarctic with only limited protection from anthropogenic environmental

change[19]. Indeed, wildlife populations in the Antarctic face a broad range of challenges from introduced species[20], to longline fisheries[21, 22], and rapid climate change[23, 24, 25]. Several native bird species including wandering albatross (*Diomedea exulans*), macaroni penguins (*Eudyptes chrysolophus*), grey-headed albatross (*Thalassarche chrysostoma*), and white-chinned petrel (*Procellaria aequinoctialis*), are listed as either vulnerable or endangered[26]. Iconic long-lived species with late maturity, such as albatross, exhibit low resilience to rapid increases in population mortality[27]. High mortality disease outbreaks therefore represent a substantial threat to already vulnerable seabird populations[28, 29].

While geographically isolated, several Antarctic seabird species routinely range between the South Atlantic and Southern Ocean, visiting the South American coast to either forage or overwinter[30]. Brown skuas (*Stercorarius antarcticus*), kelp gulls (*Larus dominicanus*), southern giant petrel (*Macronectes giganteus*), and snowy sheathbills (*Chionis albus*) have previously been identified as potential vectors of infectious pathogens into this vulnerable ecosystem due to their migratory traits, scavenging behaviour, and previously identified roles as carriers of low pathogenicity avian influenza viruses (LPAIV)[31, 32, 33, 34, 35, 36, 37, 38]. Evidence of low pathogenicity avian influenza virus (LPAIV) in the Antarctic region was first identified from serological studies in the 1980s[39, 40, 41]. A range of subtypes have since been reported (H1[41], H3[32], H4[36], H5[42], H6[33], H7[40], H9[32], and H11[34]) including genetic analysis of the influenza virus subtypes H4N7[36], H5N5[34, 39, 40, 41, 42], H6N8[33] and H11N2[34, 43, 44]. In contrast to the more prevalent H11N2 influenza viruses, H4-H6 subtypes were found to share high sequence similarity with viruses from South America, indicating more recent introduction events[33, 34, 36]. Evidence of LPAIV transmission to the continent from the Americas demonstrates the elevated risk of clade 2.3.4.4b H5 HPAIV being introduced to the Antarctic, encouraging researchers in 2022 to employ additional biosecurity measures while maintaining surveillance activities[31, 45]. During the austral summer of 2022/23, sampling and surveillance was conducted at several sites in the Antarctic region, and as of March 2023, HPAIV had not been detected[17, 18].

Here we describe the observation of morbidity and mortality events across different species as well as the positive detection of H5N1 HPAIV in a variety of species in South Georgia, inside the

Antarctic region and the sub-Antarctic Falkland Islands. We detail the suspicion, diagnostic evaluation, and clinical presentations of HPAIV in the region. Genetic analysis is used to characterise potential introduction routes and the consequences of HPAIV circulation in this region are considered.

Results

Case description and virus detection

Provision for diagnostic investigation of avian influenza is limited in the Antarctic and sub-Antarctic regions. For some samples in the description below, local RT-PCR testing at the KEMH Pathology and Food, Water & Environmental Laboratory on the Falkland Islands was used to screen for the presence of avian influenza virus H5N1 viral RNA (vRNA). This was initially undertaken for a Southern Fulmar as described below and this detection, alongside the increase clinical disease and mortalities observed on South Georgia triggered shipment of samples to the World Organisation for Animal Health (WOAH) and Food and Agriculture Organisation (FAO) International Reference Laboratory for avian influenza, swine influenza and Newcastle disease virus at the Animal and Plant Health Agency (APHA), Weybridge, UK for confirmatory and further diagnostic evaluation. An overview of both observations and testing is detailed below.

On the 17th September, researchers of the British Antarctic Survey (BAS) on Bird Island, South Georgia (an island approximately 0.5 km Northwest of the main island of South Georgia) (Figure 1B) discovered a single southern giant petrel showing behaviours indicative of loss of coordination, neurological twitching, and lethargy. This individual was observed being predated and scavenged upon by brown skuas and other southern giant petrels (*Macronectes giganteus*). On 8th October, brown skuas were observed in the same locality showing lethargy, neck spasms, twitching, and an inability to fly, and by 10th October, bird mortality was seen on Bird Island, with the highest number of mortalities occurring at the roosting site of non-breeding birds. Swab samples were collected from the three brown skua (*Stercorarius antarcticus*) on 8th October 2023 and a further brown skua on the 11th October which were all later found dead on Bird Island. These initial sampling

events all yielded PCR positivity for HPAIV H5N1. The series of sampling events and species testing positive from this point onwards are detailed in Table 1 and Supplementary Table S1.

Briefly, both brown skuas and kelp gulls then tested positive at two different locations on South Georgia (Hound Bay and St. Andrews Bay) on the 30th October with further gulls and skuas (Moltke Harbour) swabbed a day later (31st October) also testing positive. Further positive samples were taken on the 3rd (Harpon Bay) and 8th (Penguin River) November from both brown skuas and kelp gulls. Further escalation in mortality occurred by 17th November 2023, when 57 skuas were observed to have died at Bird Island although samples could not be retrieved. On the 27th of November a South Georgia shag tested positive from King Edward Cove alongside an Antarctic tern sampled dead on the 6th of December.

Alongside avian species, in early December clinical disease consistent with mammalian infection with HPAIV was observed in colonies of southern elephant seal (*Mirounga leonine*) and Antarctic fur seal (*Arctocephalus gazella*) at Jason Harbour on South Georgia (Supplementary table S2). Clinical presentation included difficulty breathing, with coughing and short sharp breath intake. Individuals also showed accumulation of viscous fluid around the nasal passage. Five southern elephant seals and 1 Antarctic fur seal from Jason Harbour collected on the 9th December tested positive for the virus (Supplementary table S2).

Overall, between the initial detection of HPAIV on the 8th of October and 9th December, from South Georgia a total of 33 avian carcasses and 17 mammalian carcasses were sampled including representatives of five different avian species and two mammalian species across eight different locations. Of these, 66% (n=28/33 avian carcasses; n=5/17 mammalian carcasses) tested positive for HPAIV H5N1.

Concurrent to the events occurring on South Georgia, on 30th October, a southern fulmar (*Fulmarus glacialoides*) was reported dead on Stanley, Falkland Islands and tested positive for the virus (Figure 1C). Numerous other swabs were taken from different avian species across the Falkland Islands (Supplementary Table 1) but only three black-browed albatross (*Thalassarche melanophris*) tested positive from Saunders Island (n=1) and Steeple Jason (n=2) being collected on the 19th and the 26th of November, respectively. In total between the 30th October and 10th December samples

150 were taken and tested from a total of 13 carcasses from nine different bird species with 31% testing
151 positive for HPAIV H5N1 (Table 1 and Supplementary Table S1).
152
153 **Virus isolation**

154 Wherever samples tested positive for HPAIV H5N1, attempts to isolate viable virus were undertaken.
155 From the samples collected from South Georgia, virus isolation was successful from two brown skuas
156 and a kelp gull taken from Hound Bay on the 30th October, a brown skua and kelp gull collected from
157 Moltke harbour on the 31st October, a kelp gull from Harpon Bay on the 3rd November and both a
158 South Georgia shag and an Antarctic tern from King Edward Cove on the 27th November and the 6th
159 December, respectively.
160 Virus isolation was also successful from swabs taken from carcasses found dead on the
161 Falklands Islands, including from samples from two southern fulmar collected on the 30th October and
162 the 13rd November from the Falklands Islands, and from black-browed albatross from Saunders Island
163 and Steeple Jason on the 19th November and the 26th November, respectively.
164 From a mammalian perspective, no viable virus could be recovered from any of the samples
165 testing positive by the generic AIV, HPAIV H5-specific assay and N1-specific rRT-PCR assays[46, 47, 48]
166 (Supplementary Table 2).
167
168 **Genomic and phylogenetic analysis**

169 Whilst successful virus isolation gave material for full virus genomic sequencing and analysis,
170 wherever possible, samples for which only rRT-PCR detection was possible were also assessed for
171 full genome generation and sequence analysis. A total of 20 full genomes were generated from avian
172 samples and a further three full genomes were generated from samples collected from seals. These
173 included ten full virus genomes from brown skuas (from Bird Island, Hound Bay, Moltke Harbour,
174 and Penguin River); six full virus genomes from kelp gulls (from Hound Bay, Moltke Harbour,
175 Harpon Bay, and Penguin River); and both a South Georgia shag and an Antarctic tern (both from

176 King Edward Cove). A further avian full virus genome was generated from two Southern Fulmars,
177 both from Stanley Island on the Falklands Islands (Supplementary table S1).

178 From a mammalian perspective we generated two full virus genomes from southern elephant
179 seals (both from Jason Harbour); and one full virus genome from an Antarctic fur seal (from Jason
180 Harbour) were generated (Supplementary table S2).

181 The sequences generated were then analysed with representative global H5N1 clade 2.3.4.4b
182 full-genome sequences to assess genetic ancestry by inferring maximum-likelihood phylogenetic trees
183 (Supplementary Figure 1). The sequences from South Georgia and the Falkland Islands clustered with
184 H5N1 viral sequences obtained from South America between October 2022 and October 2023, in
185 particular Argentina, Uruguay, Peru, and Chile across all gene segments. All sequences from South
186 Georgia and the Falkland Islands were genotyped according to the United States of America H5N1
187 schema, given the spread of these viruses from North to South America in early 2022[11], and found to
188 be part of the B3.2 genotype[49]. Comparison of the full data set revealed that they shared 99.86-100%
189 nucleotide identity across all eight influenza gene segments (Supplementary table S3).

190 To further investigate the introduction of H5N1 HPAIV into South Georgia and the Falkland
191 Islands, representative H5N1 clade 2.3.4.4b HA sequences from North and South America were used
192 to perform time-resolved phylogenetic analysis (Figure 2A). This analysis provided evidence for
193 distinct, separate introductions of H5N1 into South Georgia and the Falkland Islands, with both sets of
194 sequences sharing a common ancestor with sequences from South America dating back to July 2023
195 (Falkland Islands, range: December 2022 to November 2023) and August 2023 (South Georgia,
196 range: February November 2023). However, both sets of sequences produced long branch lengths
197 compared to South American sequences, potentially due to unsampled ancestry. The sequences from
198 South Georgia formed a single monophyletic group suggestive of a single introduction, whilst those
199 from the Falkland Islands formed a paraphyletic group, with sequences from Argentina and Brazil. To
200 investigate the source of these introductions, a Bayesian stochastic search variable selection (BSSVS)
201 analysis was performed using the country or location from which the sequences originated as a
202 discrete trait and then the support for these transmissions was quantified using Bayes factors (BFs).

203 This analysis demonstrated that Argentina was the likely source of H5N1 HPAIV for both South
204 Georgia (moderate support with a BF of 3-10) and the Falkland Islands (very strong support with a BF
205 of 30-100) (Figure 2B). The spread of H5N1 HPAIV within South Georgia was then also investigated
206 using the same approach (Figure 2C). This analysis further supported that the initial introduction into
207 South Georgia occurred on Bird Island, before onward spread to Penguin River (definitive support
208 with a BF >100), Hound Bay (strong support with a BF of 10-30) and Moltke Harbour (moderate
209 support with a BF of 3-10). Interestingly, both Harpon Bay and Jason Harbour did not demonstrate a
210 statistically relevant connection to Bird Island based on the BSSVS analysis, but also there was no
211 connection between these sampling locations and any outside of South Georgia either. Nevertheless,
212 this demonstrates that after introduction into South Georgia, H5N1 HPAIV was then spread onward to
213 multiple locations across the islands, whereby it infected multiple avian and mammalian species.

214 Alongside interrogation of phylogenetic ancestry, the sequences derived from all species were
215 assessed for the presence or absence of adaptive mutations that may indicate adaptation to replication
216 in mammals. From the mammalian samples, only a single amino acid change of interest (PB2 E627K
217 in one southern elephant seal sequence from Jason Harbour) was observed. None of the sequences
218 contained any of the other PB2 mutations of particular interest (i.e., T271A, K526R, Q591K or
219 D701N) or any other mutations associated with adaptation to replication in mammals. Interestingly, a
220 kelp gull sequence from Harpon Bay displayed a single amino acid change of interest, PB2 D701N,
221 but no others. Both southern fulmar sequences obtained from the Falkland Islands contained Q591K
222 and D701N mutations. There were also no mutations in either mammalian or avian species that would
223 affect the susceptibility of this virus to antivirals (Supplementary Table S4).

224 Discussion

225 Since the emergence and global expansion of Gs/Gd-lineage H5Nx HPAIV in 1996,
226 Antarctica and Oceania were the only two continents in which it has not been detected. Moreover,
227 until now, the Antarctic region remained the only major geographical region in which HPAIV had
228 never been detected. The island of South Georgia lies in the Southern Ocean inside the Antarctic

229 convergence, a marine belt encircling Antarctica which defines the Antarctic Region. The island is an
230 area of high biodiversity and high conservation priority with multiple species being defined as
231 vulnerable to the incursion of infectious diseases[50, 51, 52]. The Falkland Islands constitute a remote
232 cluster of islands in the South Atlantic Ocean situated approximately 1500 km to the west of South
233 Georgia. The Falkland Islands are situated outside of the Antarctic convergence, in the sub-Antarctic
234 region. Both the Falkland Islands archipelago and South Georgia represent key areas that are host to
235 significant avian biodiversity and the presence of HPAIV on these islands represents a significant risk
236 to the susceptible bird populations. South Georgia is home to approximately 29 avian species which
237 breed on the islands and is recognised as an 'Important Bird Area' by Birdlife International[53].
238 Therefore, any colony or population that comes under threat from an HPAIV outbreak on South
239 Georgia may have direct impact upon the wider population of seabirds. Despite seabird colonies
240 showing space partitioning between colonies[54], there is often a high degree of species overlap within
241 colonies. Often this is due to the movement of nonbreeders or juvenile birds[55]. It is therefore, not
242 unreasonable to suspect that birds on South Georgia may freely interact, which may aid the spread of
243 disease, as has been documented previously[37, 56]. Indeed, in the northern hemisphere it has been found
244 that northern gannets (*Morus bassanus*) increased their interactions due to high levels of colony
245 prospecting from surviving birds[56].
246 The interlinkages between avian and mammalian species testing positive for H5N1, in closely
247 linked ecosystems across the Antarctic region, means that there is potential for the virus to spread
248 further and there have been reports of the virus being detected on mainland Antarctica[57, 58] as well as
249 causing low level mortality events in penguins (APHA, unpublished results)[58, 59]. Circumpolar and
250 trans-Pacific migrants such as grey-headed albatross[60], white-chinned petrel[61], southern giant petrels,
251 and northern giant petrels (*Macronectes halli*) may facilitate this spread. Indeed, phylogeographic
252 analysis has suggested a dynamic geneflow between southern Atlantic populations and Macquarie
253 Island (an island in the southwestern Pacific Ocean, about halfway between New Zealand and
254 Antarctica)[62], and as such the threat of transmission to New Zealand and Australasia must be
255 considered.

256 From a mammalian infection standpoint there have been several reports globally of wild
257 aquatic mammals, including seals, being infected with H5Nx HPAIV since 2020, where infection has
258 been attributed to the predation of sick or dead infected birds[11, 15, 63, 64]. Information to date suggests
259 that HPAIV infection in seals often leads to a neurological presentation with infrequent detection of
260 viral material being detected through standard swab sampling activities[63]. This may explain the initial
261 lack of influenza vRNA detection in elephant seal swab samples taken in this study, despite the
262 consistency of clinical presentation seen in elephant seals with that reported elsewhere. However,
263 follow up invasive sampling clearly demonstrated that swab samples from elephant seals were
264 positive for vRNA. Whilst weather conditions prevented a thorough sampling of species from across
265 the different islands/regions experiencing mortality events in seals, those achieved did include
266 samples testing positive. Whilst the impact of the virus on avian populations appears to be reducing
267 (as of March 1st 2024), infection of both fur seals and elephant seals resulting in mortality events
268 continues to be noted. Importantly, where mortality events continue to occur in cetacean species, the
269 opportunity for infection of scavenging birds exists which may then continue the cycle of risk through
270 the potential for disease events in birds and further shedding of virus into new environments.
271 However, carcass removal, disposal and environmental clean-up is not an option. The Antarctic
272 region is one of the most remote environments on earth and is the location of enormous breeding
273 colonies of various avian species that may be susceptible, and succumb, to infection with HPAIV.
274 Further, the potential for virus survival in this cold environment is increased and it may be that
275 infectious virus remains for longer periods in carcasses preserved by the local climate. Local ecology
276 of species could also influence the scale of impact throughout Antarctica. Although all species remain
277 vulnerable to large scale infection events, it is possible that the density of animals may preclude some
278 species from succumbing to viral spread[65]. For example, wandering albatross nest at low density
279 (approximately 0.0022 nests per m^2)[66], which could limit spread between breeding individuals.
280 However, non-breeding birds congregate in groups to display and dance[67] which may provide
281 opportunities for disease spread. Similar ecological considerations must be made when considering
282 burrow nesting species (such as white-chinned petrel, diving petrel [*Pelecanoides spp.*], and prion
283 species [small petrels in the genera *Pachyptila* and *Halobaena*]), which nest in separated burrow

284 systems and may limit spread. Penguins are also susceptible to HPAIV, and mortality has been
285 observed following infection[68]. Penguin species nest in high densities (dependent upon species
286 ranging between 0.25 - 1.7 nests per m^2)[69, 70], and although limited impact has been seen to date, if
287 HPAIV does start to impact more significantly on penguin colonies, it could show rapid infection and
288 spread. However, the current situation does not suggest that significant mortality events will be seen
289 in penguin species. The report of an outbreak in Gentoo penguin (*Pygoscelis papua*) chicks has not
290 resulted in dissemination and mortality events in adult birds[59]. Further, reports of Adelie penguin
291 (*Pygoscelis adeliae*) testing positive for vRNA on swab material in the absence of clinical disease
292 suggests further that either resistance to infection, or prior exposure to an antigenically related H5
293 AIV giving some level of protection[58]. Importantly, sampling of penguin populations is ongoing and
294 although a very small number of king penguin (*Aptenodytes patagonicus*) have tested positive for the
295 virus (APHA, unpublished data)[59], extensive mortality events have not been seen suggesting that the
296 impact may not be as severe on these populations as initially feared. Activities within the region are
297 ongoing to track mortality events and autonomous authorities are on high alert to signal the potential
298 for incursions across the broader area although as suggested previously the proximity of affected
299 species, both avian and mammalian, suggests that penguins may be less susceptible to this genotype
300 of the virus.

301 Genomic analysis of the avian sequences obtained from South Georgia and the Falkland
302 Islands suggested separate, distinct introductions of the B3.2 HPAIV genotype into the two locations.
303 The B3.2 genotype emerged in early 2022 in the midwestern United States of America as a reassortant
304 formed following coinfection with the original H5N1 that was transmitted from Europe to North
305 America in late 2021[9] with a North American virus from which the novel genotype emerged,
306 containing the PB2, PB1, NP and NS gene segments of the North American AIVs[49]. This genotype
307 has been demonstrated to have been introduced into South America four times between October 2022
308 and March 2023[11]. Analysis of all available full-genome sequences from South America demonstrated
309 that 95% (184 of 193) of H5N1 HPAIV sequences corresponded to this genotype. Given the close
310 geographical proximity of South Georgia and the Falkland Islands to South America, and that wild
311 bird species are known to migrate between the mainland and these islands, it is not surprising that the

312 B3.2 was detected as the cause of the disease events. The phylogenetic analyses undertaken
313 demonstrated that the viruses detected in South Georgia and the Falkland Islands shared common
314 ancestors with those detected in mainland South America from mid-2023 and further analyses
315 demonstrated that Argentina was the likely source of HPAIV. However, the long branch lengths
316 observed across all gene segments suggests unsampled evolutionary ancestry. Further to this, analysis
317 of the sequences obtained from the sampling sites on South Georgia suggests that after the initial
318 introduction, there was spread of HPAIV to multiple locations which is consistent with eye-witness
319 accounts from the island.

320 The observation that all sequences generated from positive samples across these sampling
321 activities conducted on South Georgia cluster closely within a monophyletic group, along with the
322 BSSVS analysis demonstrates a singular introduction and that they do not constitute a divergent
323 lineage. Further, the mammalian sequences cluster amongst the avian sequences demonstrating that
324 they are not forming a separate evolutionary lineage. Contrary to this, the two sequences from the
325 Falkland Islands do not form a monophyletic group and cluster with sequences from Argentina and
326 Brazil. However, assessment of whether this is the result of multiple, separate introductions is limited
327 due to the small number of sequences available at the time of writing. Further sampling activities
328 within the Falkland Islands should be considered to further investigate the introduction of H5N1.

329 Critically, assessment of sequences derived from mammalian species did not indicate any
330 adaptive mutations or increased risk to human populations. A single sequence from a single Southern
331 elephant Seal sequence from Jason Harbour) contained the PB2 E627K mutation that is associated
332 with adaptation to replication in mammalian species and a Kelp Gull sequence from Harpon Bay
333 displayed PB2 D701N. No other mammalian or avian sequences investigated from South Georgia
334 contained any of the other PB2 mutations of particular interest (i.e., T271A, K526R, Q591K or
335 D701N). This contrasts with reports coming from sequences derived from mortality events in
336 mammals across South America[11, 71, 72, 73]. However, the two southern fulmar sequences obtained
337 from the Falkland Islands contained Q591K and D701N mutations which match the mammal
338 sequences from South America, potentially indicating different sources for introduction into the

339 Antarctic region. The data generated here indicates that human populations on these islands are not at
340 any increased risk from infection from these viruses. Further, should this situation change, there were
341 no mutations that would affect the susceptibility to antivirals observed in the data generated in this
342 study. Still, continual monitoring of the virus, especially where it appears to be the causative agents of
343 mortality events in seals (and potentially further avian species where scavenging occurs on dead seals
344 occurs), is critical to maintain an understanding of whether adaptive events may occur. Clearly, with
345 continual infection events occurring in mammals globally it is of great importance to understand
346 where mutations may represent a genuine zoonotic risk as well as understand where adaptations that
347 have occurred in mammals become tolerated in avian species.

348 In conclusion, whilst the H5N1 B3.2 HPAIV has been translocated to the fragile ecosystem in
349 the sub-Antarctic region, the current impact appears to be relatively minimal for avian species with
350 minimal impact upon penguin populations being reported to date. Further whilst infection and
351 significant mortality events have been observed in both fur and elephant seals, there remains no
352 evidence of viral adaptation for enhanced infection of mammals and, consequently no increased risk
353 to the human populations on the islands is predicted. Whilst this data supports a low impact on
354 penguin species and little to no risk to humans, the global release of data restricts a fulsome
355 interpretation here. It is of high interest to understand what impact repeat introduction events may be
356 having in the region. Currently, there are only a limited number of sequences deposited in public
357 databases from H5N1 HPAIV detections in South America during summer 2023, which limits
358 interpretation. This factor, as with countless other studies highlights the importance of real-time
359 global data sharing as a key tool in understanding the emergence and spread of these viruses. The
360 current lack of publicly available data precludes a conclusive assessment of potential incursion routes.
361 Multiple disciplines globally continue to monitor the situation in Antarctica to see whether fears of
362 ecological disaster in the region will be realised.

363 # Methods

364 **Virological detection**

365 On the Falkland Islands, initial diagnostic assessment of samples was undertaken at the KEMH
366 Pathology and Food, Water & Environmental Laboratory utilising the QIAamp Viral RNA Mini Kit
367 (Qiagen) and the Oasig OneStep RT-qPCR kit for H5N1 (Genesig). A preliminary diagnosis was made
368 of avian influenza H5N1 infection. Following reports of increasing mortalities and the observation of
369 disease consistent with HPAIV infection in avian and mammalian species in South Georgia,
370 oropharyngeal (OP) and cloacal (C) swabs collected from birds were submitted to the Animal and Plant
371 Health Agency (APHA)-Weybridge for laboratory virological investigation. Total nucleic acid was
372 extracted from all samples[47] for testing by a suite of three AIV real-time reverse transcription
373 polymerase chain reaction (rRT-PCR) assays consisting of the Matrix (M)-gene assay for generic
374 influenza A virus detection[46]; an assay for specific detection of HPAIV H5 clade 2.3.4.4b[47] and an N1-
375 specific rRT-PCR to confirm the neuraminidase type[48]. A positive result was denoted in each case by a
376 Cq value ≤36.0. The samples were also screened for avian paramyxovirus type 1 (APMV-1) by an rRT–
377 PCR assay targeting the large polymerase (L) gene[74] where a positive result was denoted by a Cq value
378 ≤37.0. All amplifications were carried out in an AriaMx qPCR System (Agilent, United Kingdom). The
379 OP swabs, C swabs or brain material were used for virus isolation in 9- to 11-day-old specific pathogen-
380 free (SPF) embryonated fowls' eggs (EFEs) according to the internationally recognised methods[75].

381 **Whole-Genome Sequencing and Phylogenetic Analysis**

382 Wherever possible, sequence generation was undertaken on original clinical material. However,
383 material derived following successful virus isolation in eggs was also utilised for sequence generation.
384 For whole-genome sequence analysis, the extracted vRNA was converted to double-stranded cDNA
385 and amplified using a one-step RT-PCR using SuperScript III One-Step RT-PCR kit (Thermo Fisher
386 Scientific). The primers used were as follows: Optil-F1 5'-TTACGCGCCAGCAAAAGCAG-3',
387 Optil-F2 5'-GTTACGCGCCAGCGAAAGCAGG-3' and Optil-R1 5'-
388 GTTACGCGCCAGTAGAAACAAG-3' that have been previously described[76, 77]. PCR products were
389 purified with Agencourt AMPure XP beads (Beckman Coultrer) prior to sequencing library
390 preparation using the Native Barcoding Kit (Oxford Nanopore Technologies) and sequenced using a
391 GridION Mk1 (Oxford Nanopore Technologies) according to manufacturer's instructions. Assembly

392 of the influenza A viral genomes was performed using a custom in-house pipeline as described
393 previously[8] but adapted for nanopore sequence reads. All influenza sequences generated and used in
394 this study are available through the GISAID EpiFlu Database (https://www.gisaid.org). All H5N1
395 HPAIV clade 2.3.4.4b sequences available in the EpiFlu database between 1st September 2020 and
396 22nd January 2024 were downloaded to create a sequence dataset. As North America and Europe were
397 over-represented in this dataset, these were sub-sampled to maintain representative sequences using
398 PARNAS[78]. The remaining dataset was separated by segment and aligned using Mafft v7.520[79], and
399 manually trimmed to the open-reading frame using Aliview version 1.26[80] The trimmed alignments
400 were then used to a infer maximum-likelihood phylogenetic tree using IQ-Tree version 2.2.3[81] along
401 with ModelFinder were downloaded to create a sequence dataset. As North America and Europe were
402 over-represented in this dataset, these were sub-sampled to maintain representative sequences using
403 PARNAS[78]. The remaining dataset was separated by segment and aligned using Mafft v7.520[79], and
404 manually trimmed to the open-reading frame using Aliview version 1.26[80] The trimmed alignments
405 were then used to a infer maximum-likelihood phylogenetic tree using IQ-Tree version 2.2.3[81] along
406 with ModelFinder[8] and 1,000 ultrafast bootstraps[82]. For the time-resolved phylogenetic analysis, all
407 HA sequences available from South America, and representatives from North America were
408 combined with the sequences from South Georgia and the Falkland Islands and used to infer
409 phylogeny using BEAST version 1.10.4[83] with the BEAGLE library[84]. The Shapiro-Rambaut-
410 Drummond-2006 (SRD06)[85] nucleotide substitution model was implemented with a four-category
411 gamma distribution model of site-specific rate variation and separate partitions for[83] with the
412 BEAGLE library[83, 84]. The Shapiro-Rambaut-Drummond-2006 (SRD06)[85] nucleotide substitution
413 model was implemented with a four-category gamma distribution model of site-specific rate variation
414 and separate partitions for codon positions 1 plus 2 versus position 3 with the Hasegawa-Kishino-
415 Yano (HKY) HKY substitution models on each with a strict clock and a coalescent GMRF Bayesian
416 skyride tree prior. Three independent Markov Chain Monte Carlo (MCMC) runs were performed and
417 combined using the Log Combiner tool in the BEAST package. Each chain consisted of 200,000,000
418 steps and was sampled every 20,000 steps, and the first 10% of samples were discarded as the burn-in.
419 Discrete geographical transition events were reconstructed using a symmetric continuous-time

Markov Chain model with an incorporated Bayesian stochastic search variable selection (BSSVS) to determine which transition rates sufficiently summarize connectivity[86]. SpreaD3 was used to determine the rates of transmission using a Bayes factor (BF) test. The BF represents the ratio of two competing statistical models, represented by their marginal likelihood, and, in this case, was used to determine the likelihood of transmission between geographical locations. The support of the BF for transmission was interpreted as described previously[87]). BF and representative transitions related to South Georgia and the Falkland Islands were visualised on maps in R (v4.3.2) using the packages GGPlot2[88], rnaturalearth[89], rnaturalearth data and sf[90]. Nucleotide identity between sequences was determined as described previously[82]. Sequences were genotyped according to the USDA schema, using the GenoFLU tool (https://github.com/USDA-VS/GenoFLU)[49].

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Author contributions

Conceptualisation: ACB, JJ, AB, EMF, ZF; formal analysis: AB, JJ, SMR, KF, ACB, AMPB; investigation: SMR, JLJ, DdS, FB, MB, RH, AMPB, JPD, BM; resources: ACB, JJ, IHB, ZF, EMF;

445 writing—original draft, ACB, JJ, AMPB, SR; writing—review and editing: ACB, JJ, APMB, AB, ZF,
446 EMF, SMR, IHB. All authors have read and agreed to the final version of the manuscript.

447 Competing interests

448 The authors declare no competing interests.

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459 Additional information

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461 Figure Legends

462 **Figure 1. Geographical distribution of H5N1 HPAIV detections from South Georgia and The**
463 **Falkland Islands. (A)** Map showing the locations of South America, the Falkland Islands, South
464 Georgia, the Antarctic peninsula, and other major islands in the Antarctic region. The location of the
465 Antarctic Convergence (Antarctic Polar Front) is indicated by a blue hashed line. **(B)** Map showing
466 the location, species, and date of influenza positive samples collected from South Georgia. Photo
467 credits: Brown skua, Viktorialvanets/Shutterstock.com; Kelp gull, Holmes, P./Pixabay.com; Antarctic
468 tern, Stubblefield Photography/Shutterstock.com; South Georgia shag, Lindie R./Shutterstock.com;

Antarctic fur seal, Carroll, P./Unsplash.com; Southern elephant seal, Osborn, D./Shutterstock.com. **(C)** Map showing the location, species, and date of influenza positive samples collected from the Falkland Islands. Locations with mortalities but no positive samples are also labelled. Photo credits: Black-browed albatross, jmarti20/Pixaby.com; Southern fulmar, juan68/Shutterstock.com.

Figure 2. H5N1 HPAIV transmission from the South American continent to Falkland Islands and South Georgia. (A) Maximum clade credibility (MCC) phylogeny of the HA gene by BEAST analysis. The phylogeny is scaled by collection date of samples. Tip points are coloured by country of origin. **(B)** Analysis of South American, Falklands Islands and South Georgia H5N1 sequences suggests transmission from the mainland to the islands. BSSVS analysis is shown to describe potential transmission routes. Arrows are coloured according to relative strength, inferred using a bayes factor (BF), by which transmission events are supported **(C)** Analysis of South Georgia H5N1 sequences suggests transmission between geographically related HPAIV detections. BSSVS analysis is shown to describe potential transmission routes across the island. Arrows are coloured according to relative strength, inferred using a bayes factor (BF), by which transmission events are supported.

Supplementary Figure 1. Representative global H5N1 HPAIV sequences collected since 1st September 2020 were combined with the sequences generated from samples collected from South Georgia and the Falkland Islands and used to infer maximum likelihood phylogenetic trees. Tips are coloured according to region of origin, and the sequences from South Georgia and the Falkland Islands are indicated.

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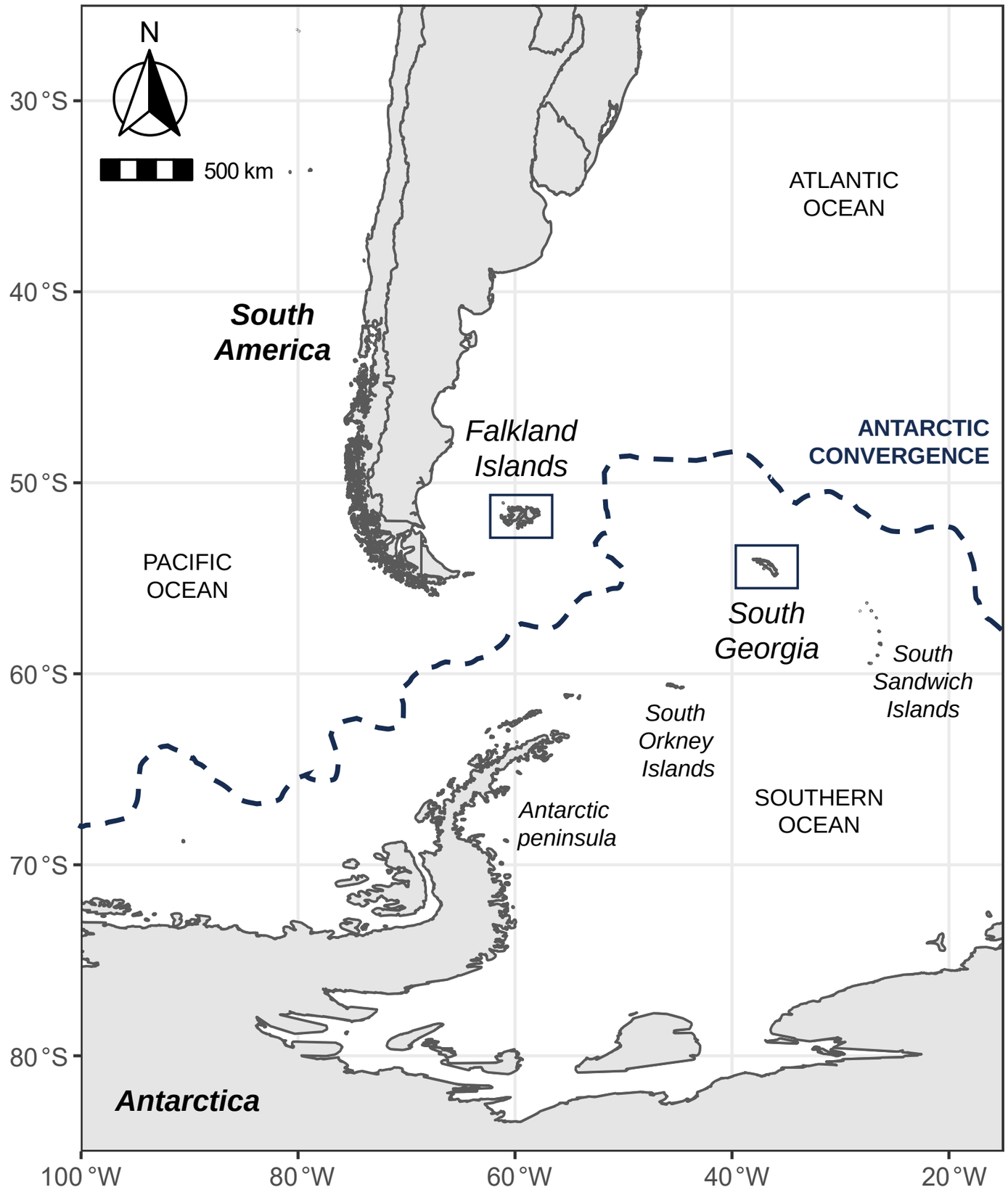
Figure 1A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America
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CONVERGENCE
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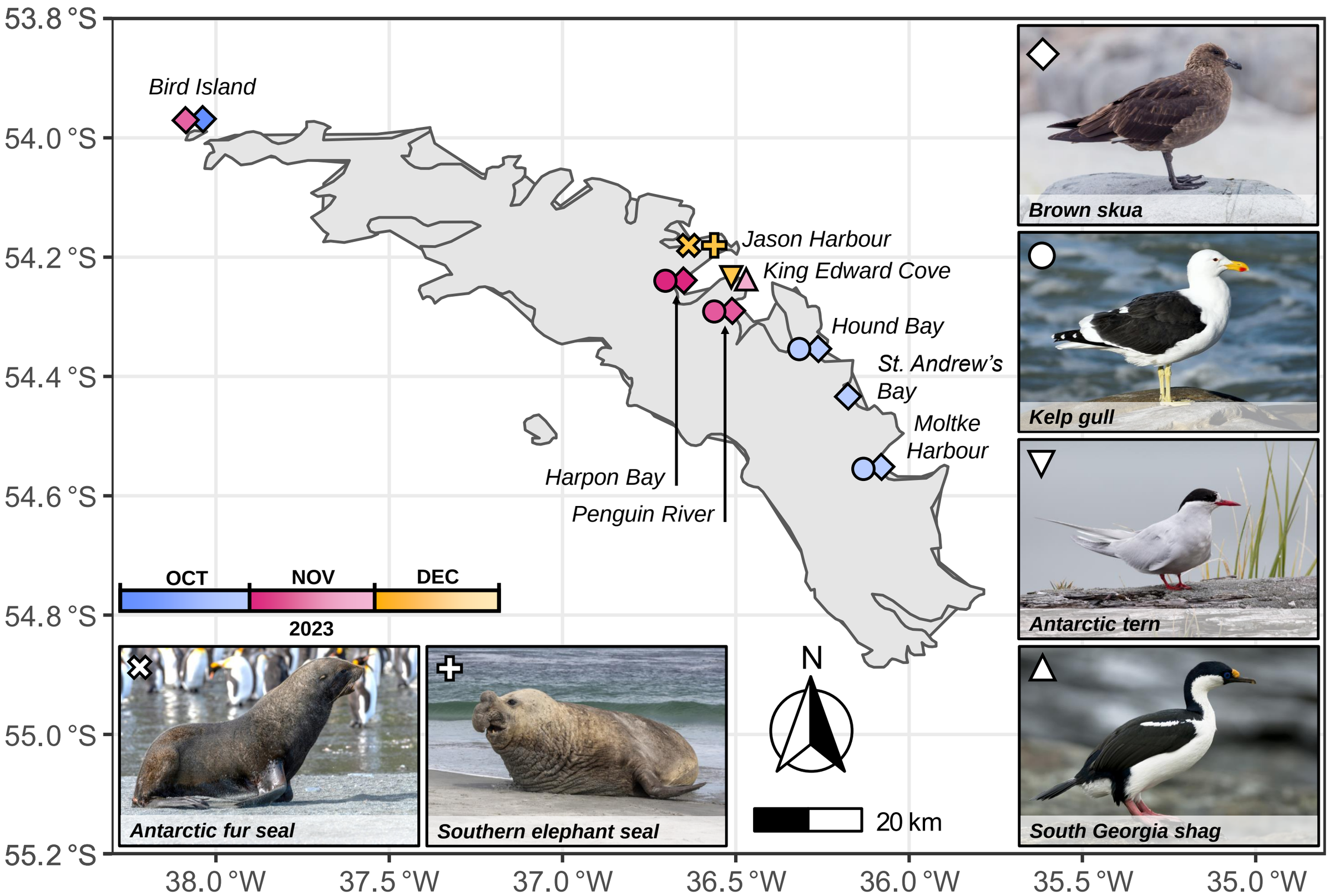
Figure 1B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Bird Island
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King Edward Cove
Hound Bay
St. Andrew's Bay
Moltke Harbour
Harpon Bay
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Brown skua
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Antarctic tern
South Georgia shag
Antarctic fur seal
Southern elephant seal

Figure 1C

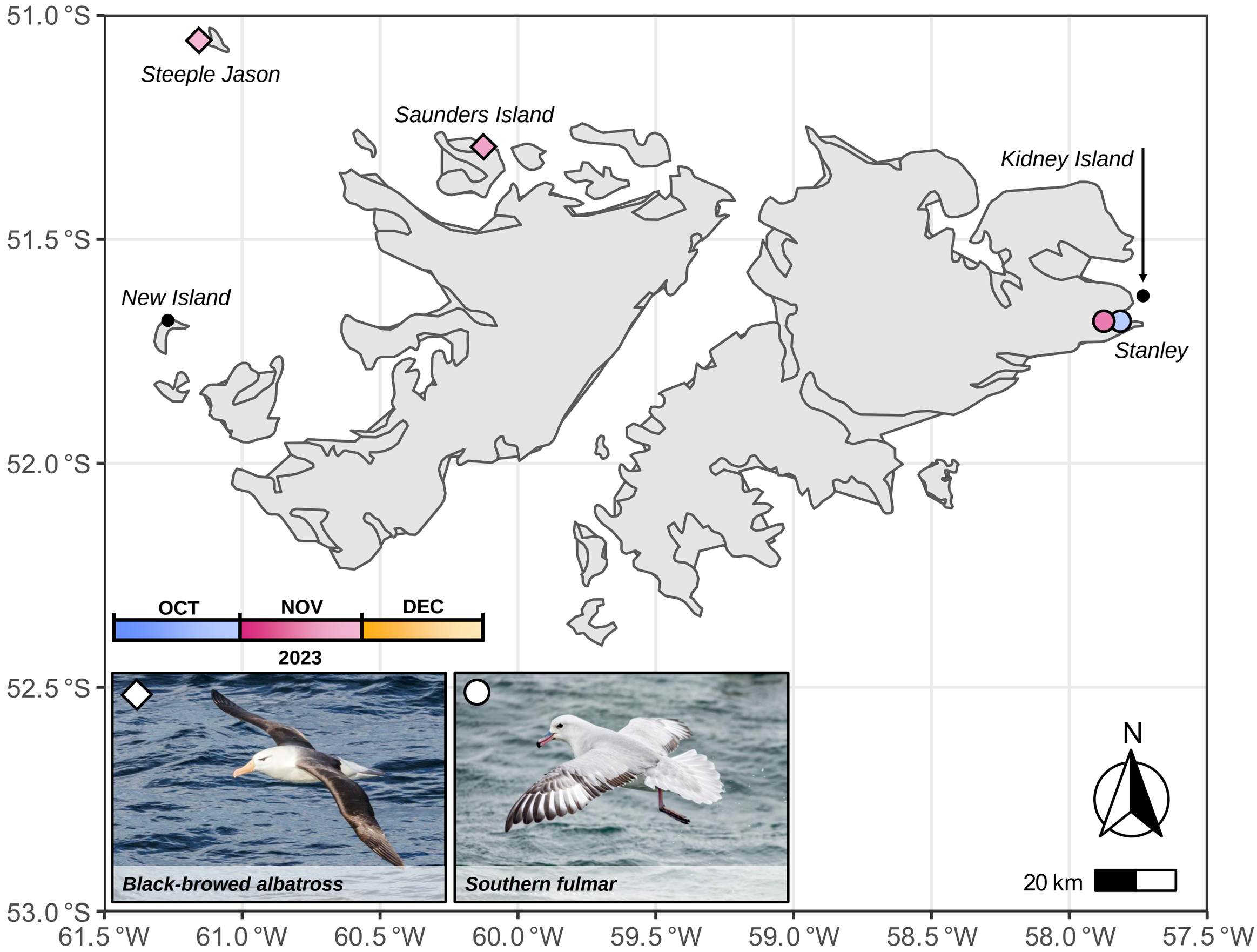
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Figure 2A

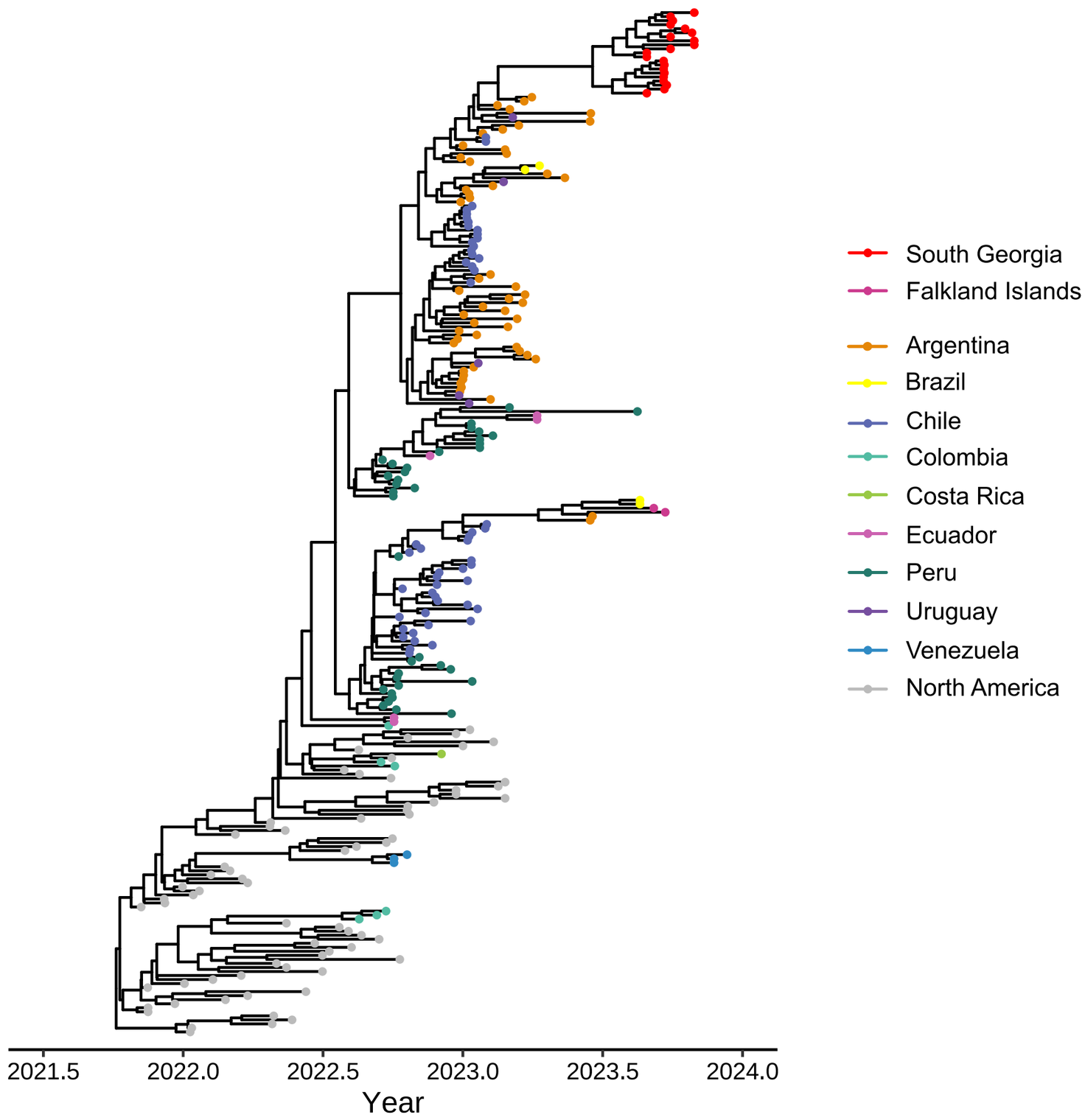

South Georgia
Falkland Islands
Argentina
Brazil
Chile
Colombia
Costa Rica
Ecuador
Peru
Uruguay
Venezuela
North America
2021.5
2022.0
2022.5
2023.0
2023.5
2024.0
Year

Figure 2B

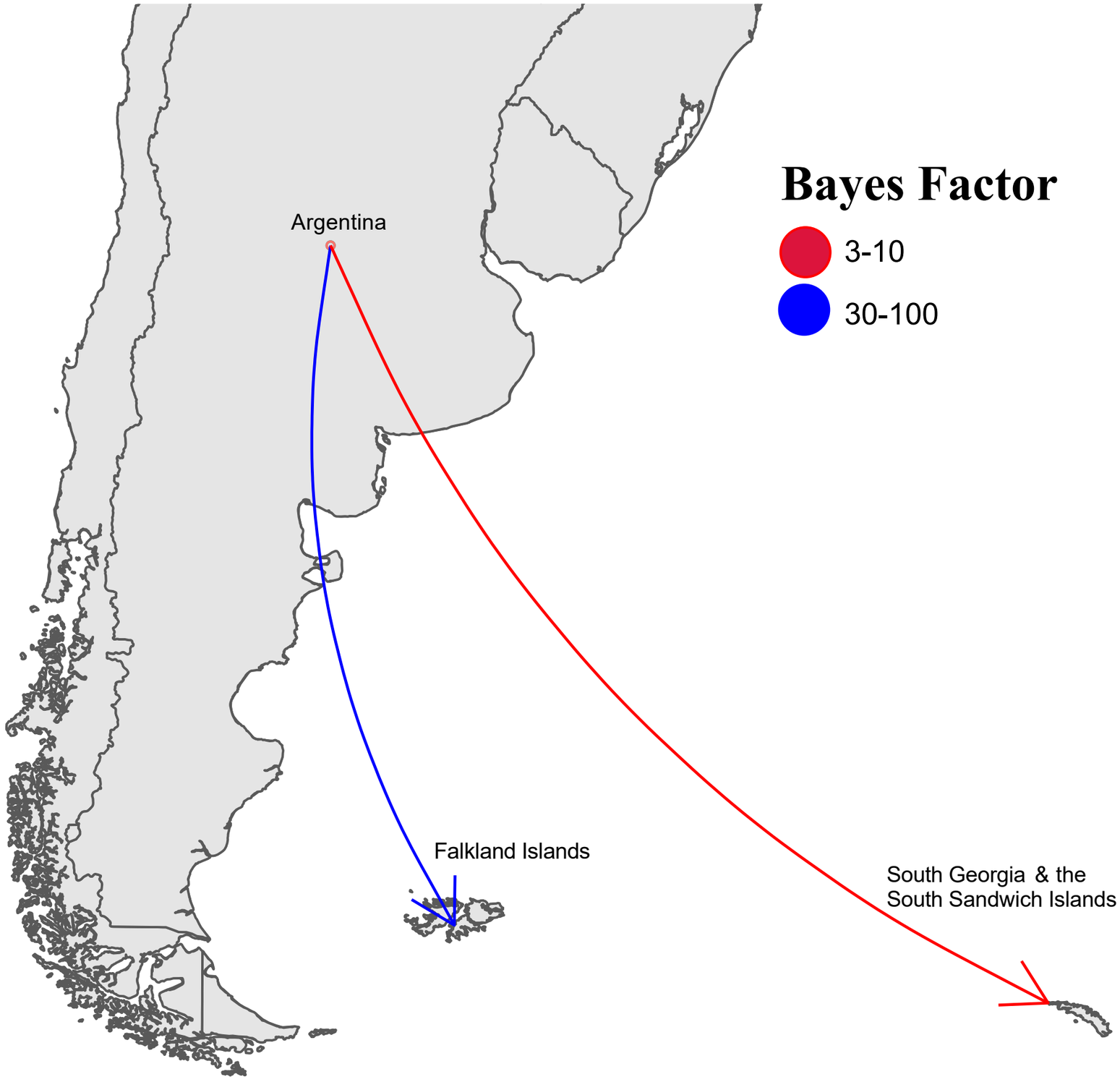

Argentina
Bayes Factor
3-10
30-100
Falkland Islands
South Georgia & the
South Sandwich Islands

Figure 2C

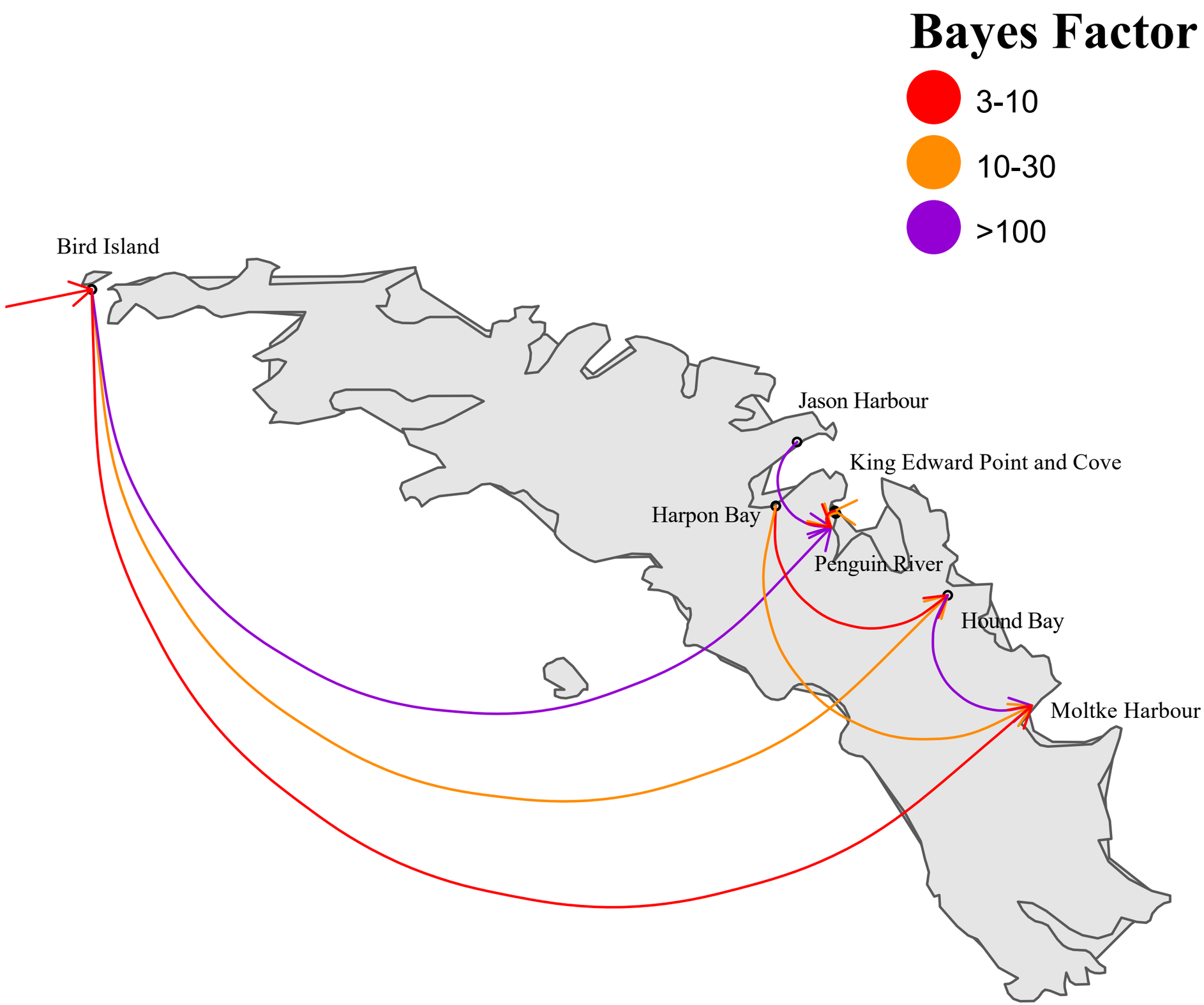

Bayes Factor
3-10
10-30
>100
Bird Island
Jason Harbour
King Edward Point and Cove
Harpon Bay
Penguin River
Hound Bay
Moltke Harbour

Table 1: Summary of H5 HPAIV testing results from avian and mammalian species from South Georgia and the Falkland Islands.

Location	Common name	Scientific name	H5 HPAIV +ve animals (total tested)	Sequences obtained
South Georgia	Brown skua	*Stercorarius antarcticus*	15 (15)	9
	Kelp gull	*Larus dominicanus*	11 (11)	6
	Antarctic tern	*Sterna vittata*	1 (1)	1
	South Georgia shag	*Leucocarbo georgianus*	1 (5)	1
	King penguin	*Aptenodytes patagonicus*	0 (1)	0
	Southern elephant seal	*Mirounga leonina*	5 (13)	2
	Antarctic Fur Seal	*Arctocephalus gazella*	1 (4)	1
Falkland islands	Black browed albatross	*Thalassarche melanophris*	2 (4)	0
	Southern fulmar	*Fulmarus glacialoides*	2 (2)	2
	Austral thrush	*Turdus falcklandii*	0 (1)	0
	Chicken	*Gallus gallus domesticus*	0 (1)	0
	Falkland steamer duck	*Tachyeres brachypterus*	0 (1)	0
	Grey-backed storm petrel	*Garrodia nereis*	0 (1)	0
	Imperial shag	*Leucocarbo atriceps*	0 (1)	0
	Rock shag	*phalacrocorax magellanicus*	0 (1)	0
	Southern rockhopper penguin	*Eudyptes chrysocome*	0 (1)	0

Figure S1

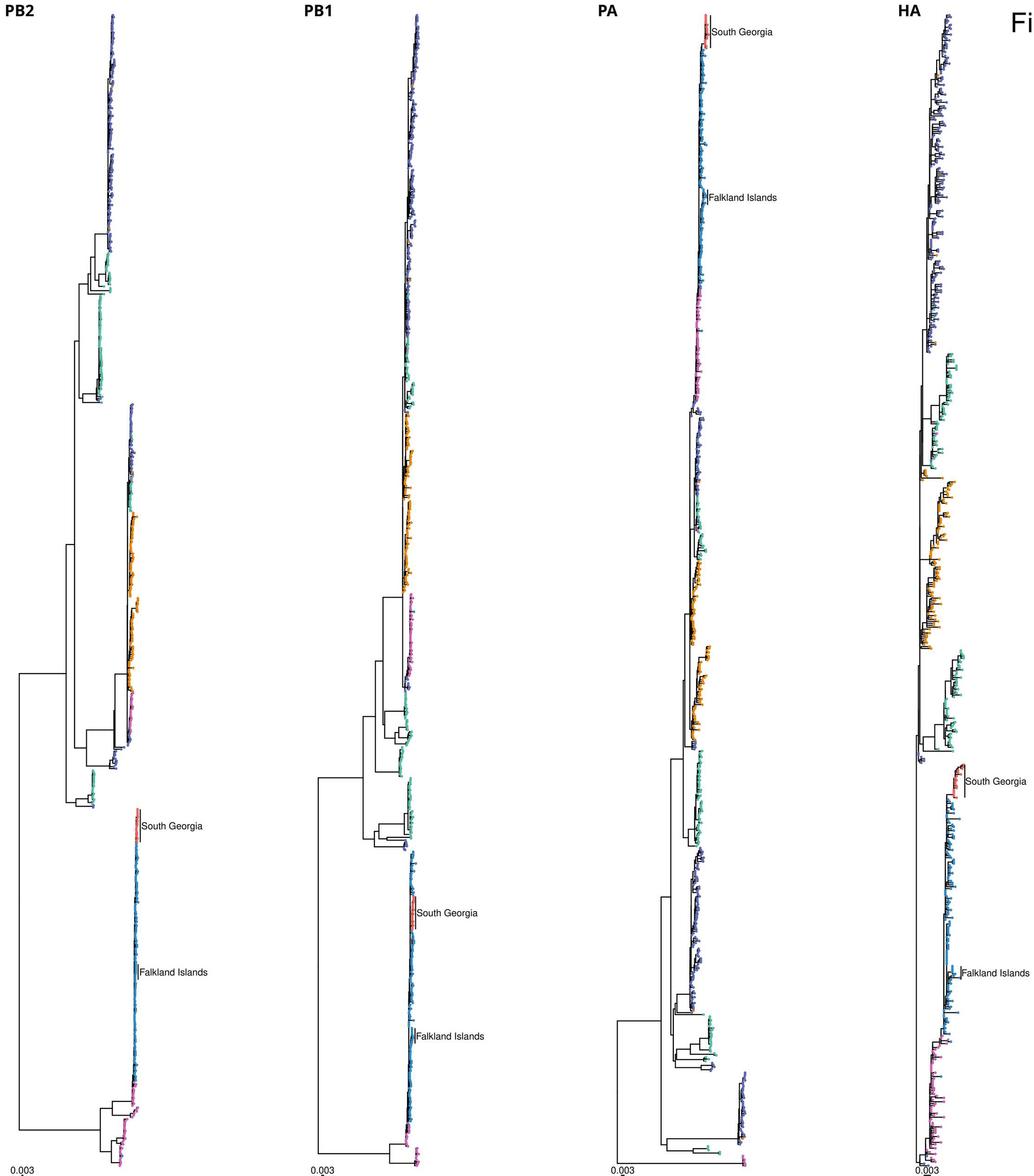
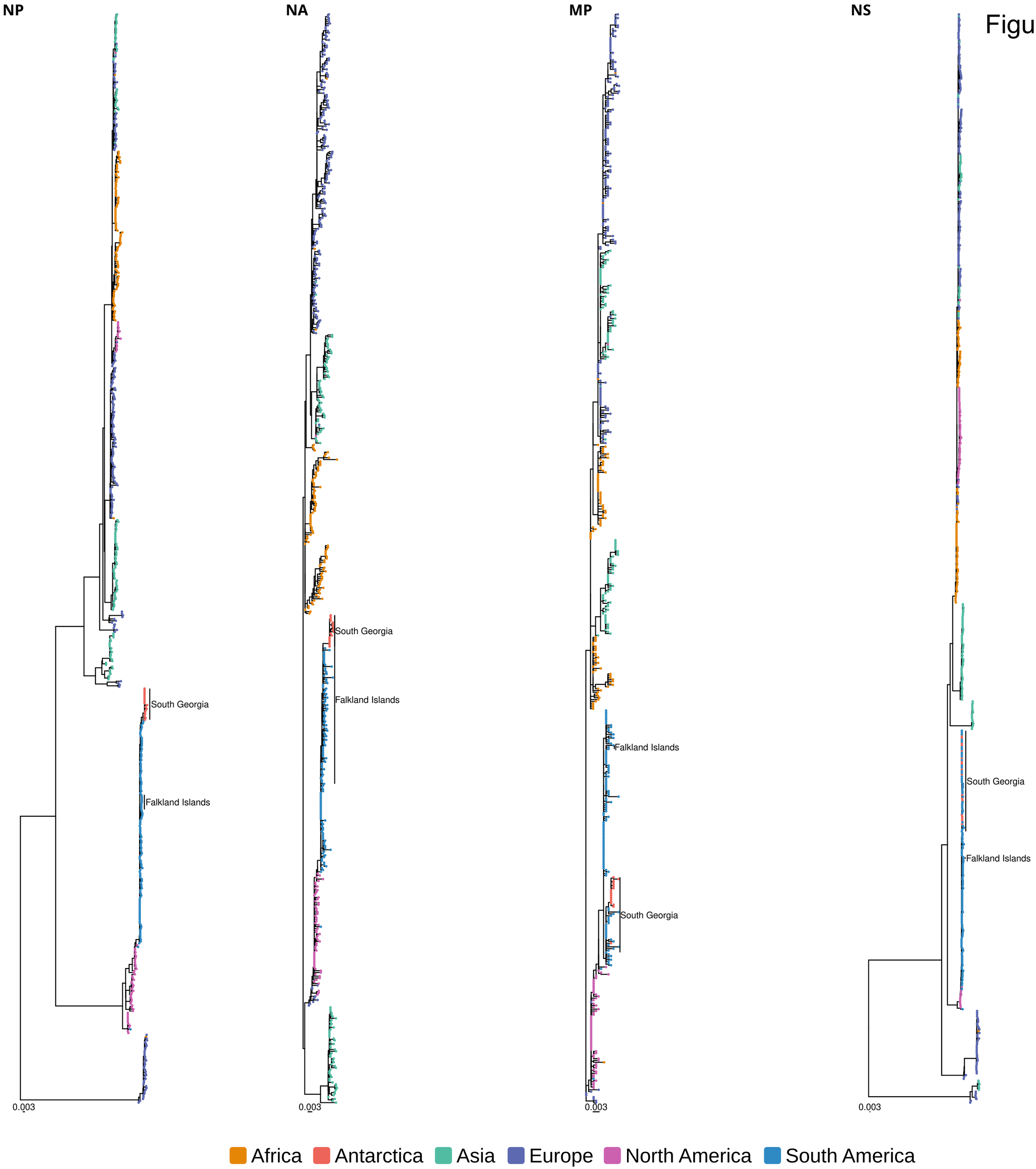

PB2
PB1
PA
HA
South Georgia
Falkland Islands
0.003
Africa
Antarctica
Asia
Europe
North America
South America

Figure S2


NP
NA
MP
NS
South Georgia
Falkland Islands
South Georgia
Falkland Islands
Falkland Islands
South Georgia
South Georgia
Falkland Islands
0.003
0.003
0.003
0.003
Africa
Antarctica
Asia
Europe
North America
South America

Supplementary Table 1: Summary of date, location and species of avian samples collected, and testing result for H5N1 HPAIV from South Georgia and the Falklands Islands.

Location	Collection date	Common name	Scientific name	Swab type	RRT-PCR assay			RRT-PCR Interpretation[a]	Virus isolation[b]	Virus strain name	GISAID accession number
					M-gene	H5-HP	N1				
Bird Island, South Georgia	08/10/2023	Brown skua	*Stercorarius antarcticus*	OP[c]	28.46	27.75	29.82	**H5N1 HPAIV**	Negative	A/brown_skua/Bird_Island/128287/2023	EPI_ISL_18439562
				C[d]	27.1	26.2	28.3	**H5N1 HPAIV**	Negative	N/P[e]	N/A[f]
		Brown skua	*Stercorarius antarcticus*	OP	28.09	26.25	28.99	**H5N1 HPAIV**	Negative	A/brown_skua/Bird_Island/128288/2023	EPI_ISL_18439563
				C	35.01	33.65	35.69	**H5N1 HPAIV**	Negative	N/P	N/A
		Brown skua	*Stercorarius antarcticus*	OP	31.07	30.16	32.36	**H5N1 HPAIV**	Negative	A/brown_skua/Bird_Island/128289/2023	EPI_ISL_18439564
				C	32.63	31.85	33.87	**H5N1 HPAIV**	Negative	N/P	N/A
	11/11/2023	Brown skua	*Stercorarius antarcticus*	OP	28.91	27.91	28.37	**H5N1 HPAIV**	Negative	A/Brown_Skua/Bird_Island/141232/2023	EPI_ISL_18742214
				C	-	-	38.04	Negative	Negative	N/P	N/A
Hound Bay, South Georgia	30/10/2023	Kelp gull	*Larus dominicanus*	C	-	-	-	Negative	N/P	N/P	N/A
				OP	27.27	25.24	27.96	**H5N1 HPAIV**	Negative	A/Kelp_Gull/Hound_Bay/133744/2023	EPI_ISL_18592422
		Brown skua	*Stercorarius antarcticus*	C	32.36	29.52	32.75	**H5N1 HPAIV**	**Positive**	A/Brown_Skua/Hound_Bay/133947/2023	EPI_ISL_18592428
				OP	32.31	29.87	32.09	**H5N1 HPAIV**	N/P	N/P	N/A
		Kelp gull	*Larus dominicanus*	C	-	-	-	Negative	N/P	N/P	N/A
				OP	35.89	34.71	35.8	**H5N1 HPAIV**	Negative	N/P	N/A
		Kelp gull	*Larus dominicanus*	C	-	-	37.81	Negative	N/P	N/P	N/A
				OP	28.18	26.27	29.22	**H5N1 HPAIV**	**Positive**	A/Kelp_Gull/Hound_Bay/133747/2023	EPI_ISL_18592423
		Brown skua	*Stercorarius antarcticus*	C	28.86	26.87	29.88	**H5N1 HPAIV**	Negative	N/P	N/A
				OP	29.61	27.84	30.53	**H5N1 HPAIV**	N/P	N/P	N/A
		Brown skua	*Stercorarius antarcticus*	C	26.92	24.65	27.28	**H5N1 HPAIV**	**Positive**	A/Brown_Skua/Hound_Bay/133949/2023	EPI_ISL_18592429
				OP	27.61	27.39	29.47	**H5N1 HPAIV**	N/P	N/P	N/A
St Andrew's Bay, South Georgia		Brown skua	*Stercorarius antarcticus*	C	36.31	35.76	36.67	**H5N1 HPAIV**	N/P	N/P	N/A
				OP	29.34	26.5	28.64	**H5N1 HPAIV**	Negative	N/P	N/A
		Brown skua	*Stercorarius antarcticus*	C	32.43	30.89	33.86	**H5N1 HPAIV**	Negative	N/P	N/A
				OP	34.98	31.47	34.54	**H5N1 HPAIV**	N/P	N/P	N/A
Moltke Harbour, South Georgia	31/10/2023	Brown skua	*Stercorarius antarcticus*	C	-	-	-	Negative	N/P	N/P	N/A
				OP	31.43	28.42	31.77	**H5N1 HPAIV**	Negative	A/Brown_Skua/Moltke_Harbour/133752/2023	EPI_ISL_18592424
		Kelp gull	*Larus dominicanus*	C	-	-	-	Negative	N/P	N/P	N/A
				OP	36.29	33.65	35.55	**H5N1 HPAIV**	Negative	N/P	N/A
		Kelp gull	*Larus dominicanus*	C	38.84	-	39.19	Negative	N/P	N/P	N/A
				OP	29.21	27.54	29.78	**H5N1 HPAIV**	**Positive**	A/Kelp_Gull/Moltke_Harbour/133754/2023	EPI_ISL_18592425
		Brown skua	*Stercorarius antarcticus*	C	30.65	28.43	31.7	**H5N1 HPAIV**	N/P	N/P	N/A
				OP	27.25	25.51	28.42	**H5N1 HPAIV**	**Positive**	A/Brown_Skua/Moltke_Harbour/133755/2023	EPI_ISL_18592426
		Brown skua	*Stercorarius antarcticus*	C	32.66	30.37	33.76	**H5N1 HPAIV**	N/P	N/P	N/A
				OP	30.87	29.32	31.65	**H5N1 HPAIV**	Negative	N/P	N/A
		Kelp gull	*Larus dominicanus*	C	-	-	-	Negative	N/P	N/P	N/A
				OP	29.97	28.92	31.5	**H5N1 HPAIV**	Negative	N/P	N/A
Harpon Bay, South Georgia	03/11/2023	Kelp gull	*Larus dominicanus*	C	32.77	31.46	33.9	**H5N1 HPAIV**	N/P	N/P	N/A
				OP	30.67	28.51	31.04	**H5N1 HPAIV**	**Positive**	A/Kelp_Gull/Harpon_Bay/133943/2023	EPI_ISL_18592427
		Brown skua	*Stercorarius antarcticus*	C	30.09	29.26	31.53	**H5N1 HPAIV**	N/P	N/P	N/A
				OP	27.4	24.62	27.54	**H5N1 HPAIV**	Negative	N/P	N/A
		Kelp gull	*Larus dominicanus*	C	38.93	-	37.63	Negative	N/P	N/P	N/A
				OP	31.93	29.55	32.32	**H5N1 HPAIV**	Negative	N/P	N/A
		Kelp gull	*Larus dominicanus*	C	-	-	-	Negative	N/P	N/P	N/A
				OP	36.53	33.81	35.96	**H5N1 HPAIV**	Negative	N/P	N/A
Penguin River, South Georgia	08/11/2023	Kelp gull	*Larus dominicanus*	OP	27.88	26.95	27.32	**H5N1 HPAIV**	Negative	A/Kelp_Gull/Penguin_River/141234/2023	EPI_ISL_18742215
				C	-	-	-	Negative	N/P	N/P	N/A
		Brown skua	*Stercorarius antarcticus*	OP	27.96	26.77	28.27	**H5N1 HPAIV**	Negative	A/Brown_Skua/Penguin_River/141236/2023	EPI_ISL_18742216
				C	35.95	35.41	35.53	**H5N1 HPAIV**	N/P	N/P	N/A
		Kelp gull	*Larus dominicanus*	OP	34.41	33.4	34.16	**H5N1 HPAIV**	N/P	N/P	N/A
				C	30.31	29.69	29.92	**H5N1 HPAIV**	Negative	A/Kelp_Gull/Penguin_River/141239/2023	EPI_ISL_18742217
		Brown skua	*Stercorarius antarcticus*	OP	31.95	31.25	37.48	**H5N1 HPAIV**	Negative	A/Brown_Skua/Penguin_River/141240/2023	EPI_ISL_18742218
				C	36.65	28.5	29.27	**H5N1 HPAIV**	Negative	N/P	N/A
King Edward Cove, South Georgia	25/11/2023	South Georgia shag	*Leucocarbo georgianus*	OP	-	-	-	Negative	N/P	N/P	N/A
				C	-	-	-	Negative	N/P	N/P	N/A
	27/11/2023	South Georgia shag	*Leucocarbo georgianus*	OP	34.7	27.59	31.3	**H5N1 HPAIV**	N/P	N/P	N/A
				C	26.66	19.9	23.62	**H5N1 HPAIV**	**Positive**	A/South_Georgia_Shag/King_Edward_Cove/141245/2023	EPI_ISL_18742219
	06/12/2023	South Georgia shag	*Leucocarbo georgianus*	OP	-	-	-	Negative	N/P	N/P	N/A
				C	-	-	-	Negative	N/P	N/P	N/A
		South Georgia shag	*Leucocarbo georgianus*	Brain	-	-	-	Negative	N/P	N/P	N/A
				OP	-	-	-	Negative	N/P	N/P	N/A
		South Georgia shag	*Leucocarbo georgianus*	C	-	-	-	Negative	N/P	N/P	N/A
				Brain	-	-	-	Negative	N/P	N/P	N/A
		King penguin	*Aptenodytes patagonicus*	OP	-	-	-	Negative	N/P	N/P	N/A
				C	-	-	-	Negative	N/P	N/P	N/A
				Brain	-	-	-	Negative	N/P	N/P	N/A
		Antarctic tern	*Sterna vittata*	OP	25.88	20.58	24.38	**H5N1 HPAIV**	Negative	N/P	N/A
				Brain	19.81	13.29	17.07	**H5N1 HPAIV**	Negative	A/Antarctic_tern/King_Edward_Point/141271/2023	EPI_ISL_18742220
Stanley, Falkland Islands	30/10/2023	Southern fulmar	*Fulmarus glacialoides*	OP	20.5	18.65	20.71	**H5N1 HPAIV**	Negative	A/Southern_fulmar/Falkland_Island/133789/2023	EPI_ISL_18522961
				C	18.85	17.57	19.62	**H5N1 HPAIV**	Negative	N/P	N/A
				Brain	21.51	20.49	22.41	**H5N1 HPAIV**	**Positive**	N/P	N/A
	06/11/2023	Grey-backed storm petrel	*Garrodia nereis*	OP	39.83	-	-	Negative	N/P	N/P	N/A
				C	-	-	-	Negative	N/P	N/P	N/A
		Falkland steamer duck	*Tachyeres brachypterus*	OP	39.37	-	-	Negative	N/P	N/P	N/A
				C	-	-	-	Negative	N/P	N/P	N/A
	13/11/2023	Southern fulmar	*Fulmarus glacialoides*	OP	28.81	25.58	26.85	**H5N1 HPAIV**	**Positive**	A/Southern_Fulmar/Falkland_Island/138870/2023[g]	EPI_ISL_18918640
				C	32.89	29.45	30.98	**H5N1 HPAIV**	Negative	N/P	N/A
	24/11/2023	Austral thrush	*Turdus falcklandii*	OP	-	-	-	Negative	N/P	N/P	N/A
				C	-	-	-	Negative	N/P	N/P	N/A
	25/11/2023	Rock shag	*phalacrocorax magellanicus*	OP	-	-	-	Negative	N/P	N/P	N/A
				C	-	-	-	Negative	N/P	N/P	N/A
	27/11/2023	Chicken	*Gallus gallus domesticus*	OP	-	-	-	Negative	N/P	N/P	N/A
				C	-	-	-	Negative	N/P	N/P	N/A
Saunders Island, Falkland Islands	19/11/2023	Black browed albatross	*Thalassarche melanophris*	OP	36.51	33.92	35.84	**H5N1 HPAIV**	Negative	N/P	N/A
				C	31.61	29.46	30.81	**H5N1 HPAIV**	Negative	N/D[h]	N/A
Steeple Jason, Falkland Islands	26/11/2023	Black browed albatross	*Thalassarche melanophris*	OP	-	-	-	Negative	N/P	N/P	N/A
				C	-	-	-	Negative	N/P	N/P	N/A
		Black browed albatross	*Thalassarche melanophris*	OP	34.75	34.6	32.87	**H5N1 HPAIV**	Negative	N/P	N/A
				C	30.47	30.94	29.16	**H5N1 HPAIV**	Negative	N/D	N/A
New Island, Falkland Islands	01/12/2023	Imperial shag	*Leucocarbo atriceps*	OP	-	-	-	Negative	N/P	N/P	N/A
				C	-	-	-	Negative	N/P	N/P	N/A
	04/12/2023	Black browed albatross	*Thalassarche melanophris*	C	-	-	-	Negative	N/P	N/P	N/A
				OP	-	-	-	Negative	N/P	N/P	N/A
Kidney Island, Falkland Islands	10/12/2023	Southern rockhopper penguin	*Eudyptes chrysocome*	C	-	-	-	Negative	N/P	N/P	N/A
				OP	-	-	-	Negative	N/P	N/P	N/A

a, RRT-PCR interpretation from M-gene, H5-HP and N1; **b**, virus isolation in embryonated fowl eggs;**c**, oropharyngeal (OP); **d**, cloacal (C); **e**, not performed (N/P); **f**, not applicable (N/A); **g**, sequence derived from egg amplified material;**h**, performed but could not be determined (ND); -, No Cq

Supplementary Table 2: Summary of date, location and species of mamalian samples collected and testing result for H5 HPAIV from South Georgia.

Location	Collection date	Age	Common name	Scientific name	Swab type	RRT-PCR assay			RRT-PCR Interpretation[a]	Virus isolation[b]	Virus strain name	GISAID accession number
						M-gene	H5-HP	N1				
Moltke Harbour, South Georgia	31/10/2023	Pup	Southern elephant seal	*Mirounga leonina*	Nasal	37.75	-	39.5	Negative	Negative	N/P[c]	N/A[d]
					Anal	-	-	-	Negative	N/P	N/P	N/A
			Southern elephant seal	*Mirounga leonina*	Nasal	-	-	-	Negative	N/P	N/P	N/A
					Anal	-	-	-	Negative	N/P	N/P	N/A
			Southern elephant seal	*Mirounga leonina*	Nasal	38.8	-	-	Negative	Negative	N/P	N/A
					Anal	-	-	-	Negative	N/P	N/P	N/A
King Edward Cove, South Georgia	06/12/2023	Pup (newborn)	Antarctic fur seal	*Arctocephalus gazella*	Oral	-	-	-	Negative	N/P	N/P	N/A
					Nasal	-	-	-	Negative	N/P	N/P	N/A
					Anal	-	-	-	Negative	N/P	N/P	N/A
					Brain	-	-	-	Negative	N/P	N/P	N/A
Jason Harbour, South Georgia	09/12/2023	Weaner (molting)	Southern elephant seal	*Mirounga leonina*	Oral	37.21	34.44	36.58	**H5N1 HPAIV**	Negative	A/Southern_Elephant_Seal/Jason_Harbour/141027/2023	EPI_ISL_18742221
					Anal	-	-	-	Negative	N/P	N/P	N/A
					Nasal	-	-	39.93	Negative	N/P	N/P	N/A
					Brain	-	-	-	Negative	N/P	N/P	N/A
			Southern elephant seal	*Mirounga leonina*	Oral	-	-	-	Negative	N/P	N/P	N/A
					Anal	-	-	-	Negative	N/P	N/P	N/A
					Nasal	-	-	-	Negative	N/P	N/P	N/A
					Brain	-	-	-	Negative	N/P	N/P	N/A
			Southern elephant seal	*Mirounga leonina*	Brain	-	-	-	Negative	N/P	N/P	N/A
			Southern elephant seal	*Mirounga leonina*	Oral	-	-	-	Negative	N/P	N/P	N/A
					Anal	-	-	-	Negative	N/P	N/P	N/A
					Nasal	-	-	-	Negative	N/P	N/P	N/A
					Brain	-	-	-	Negative	N/P	N/P	N/A
			Southern elephant seal	*Mirounga leonina*	Oral	-	-	-	Negative	N/P	N/P	N/A
					Anal	-	-	-	Negative	N/P	N/P	N/A
					Nasal	-	-	37.97	Negative	N/P	N/P	N/A
					Brain	-	-	-	Negative	N/P	N/P	N/A
			Southern elephant seal	*Mirounga leonina*	Oral	-	-	-	Negative	N/P	N/P	N/A
					Anal	32.32	30.47	32.09	**H5N1 HPAIV**	Negative	N/D[f]	N/A
					Nasal	38.27	-	39.31	Negative	N/P	N/P	N/A
					Brain	-	-	-	Negative	N/P	N/P	N/A
			Southern elephant seal	*Mirounga leonina*	Oral	37.84	-	38.38	Negative	N/P	N/P	N/A
					Anal	35.51	34.25	34.21	**H5N1 HPAIV**	Negative	N/D	N/A
					Nasal	-	-	37.88	Negative	N/P	N/P	N/A
					Brain	33.35	32.84	33.2	**H5N1 HPAIV**	Negative	N/D	N/A
			Southern elephant seal	*Mirounga leonina*	Oral	-	-	-	Negative	N/P	N/P	N/A
					Anal	-	-	-	Negative	N/P	N/P	N/A
					Nasal	-	-	-	Negative	N/P	N/P	N/A
					Brain	-	-	-	Negative	N/P	N/P	N/A
			Southern elephant seal	*Mirounga leonina*	Oral	-	-	39.86	Negative	N/P	N/P	N/A
					Anal	-	-	-	Negative	N/P	N/P	N/A
					Nasal	-	-	-	Negative	N/P	N/P	N/A
					Brain	32.78	31.02	32.63	**H5N1 HPAIV**	Negative	N/D	N/A
			Southern elephant seal	*Mirounga leonina*	Oral	-	-	39.64	Negative	N/P	N/P	N/A
					Anal	37.98	-	36.14	Negative	N/P	N/P	N/A
					Nasal	36.05	35.1	36.23	**H5N1 HPAIV**	Negative	N/D	N/A
					Brain	37.04	-	39.35	Negative	N/P	N/P	N/A
					Liver	-	-	-	Negative	N/P	N/P	N/A
					Lung	32.03	31.41	32.24	**H5N1 HPAIV**	Negative	A/Southern_Elephant_Seal/Jason_Harbour/141078/2023	EPI_ISL_18742213
					Blood	-	-	-	Negative	N/P	N/P	N/A
		Adult male	Antarctic fur seal	*Arctocephalus gazella*	Oral	-	-	-	Negative	N/P	N/P	N/A
					Anal	-	-	-	Negative	N/P	N/P	N/A
					Nasal	36.9	-	-	Negative	N/P	N/P	N/A
					Brain	-	-	-	Negative	N/P	N/P	N/A
			Antarctic fur seal	*Arctocephalus gazella*	Oral	30.66	29.87	31.18	**H5N1 HPAIV**	Negative	A/Antarctic_Fur_Seal/Jason_Harbour/141037/2023	EPI_ISL_18742212
					Anal	-	-	-	Negative	N/P	N/P	N/A
					Nasal	32.41	33.81	35.49	**H5N1 HPAIV**	Negative	N/P	N/A
					Brain	31.61	33.71	32.59	**H5N1 HPAIV**	Negative	N/P	N/A
			Antarctic fur seal	*Arctocephalus gazella*	Oral	-	-	-	Negative	N/P	N/P	N/A
					Anal	-	-	-	Negative	N/P	N/P	N/A
					Nasal	-	-	-	Negative	N/P	N/P	N/A
					Brain	-	-	-	Negative	N/P	N/P	N/A

a, RRT-PCR interpretation from M-gene, H5-HP and N1; **b**, virus isolation in embryonated fowl eggs;**c**, not performed (N/P); **d**, not applicable (N/A); **f**, performed but could not be determined (ND); -, No Cq

Table S3: Percentage nucleotide (nt) identity across H5N1 HPAIV sequences generated from South Georgia and The Falkland Islands.

		A/Kelp Gull/Hound Bay/133744/2023	A/Kelp Gull/Hound Bay/133747/2023	A/Brown Skua/Moltke Harbour/133752/2023	A/Kelp Gull/Moltke Harbour/133754/2023	A/Brown Skua/Moltke Harbour/133755/2023	A/Kelp Gull/Harpon Bay/133943/2023	A/Brown Skua/Hound Bay/133947/2023	A/Brown skua/Hound Bay/133949/2023	A/Brown skua/Bird Island/128289/2023	A/Brown skua/Bird Island/128288/2023	A/Brown skua/Bird Island/128287/2023	A/Kelp Gull/Penguin River/141239/2023	A/Brown Skua/Penguin River/141236/2023	A/Kelp Gull/Penguin River/141234/2023	A/Brown Skua/Bird Island/141232/2023	A/Southern Elephant Seal/Jason Harbour/141078/2023	A/Antarctic Fur Seal/Jason Harbour/141037/2023	A/Southern Elephant Seal/Jason Harbour/141027/2023	A/Southern Fulmar/Falkland Islands/138870/2023	A/Southern fulmar/Falkland Islands/133789/2023
PB2	A/Kelp Gull/Hound Bay/133744/2023	100	99.86	99.82	99.82	99.87	99.96	99.91	99.78	99.96	99.91	99.96	99.78	99.78	99.82	99.78	99.96	99.78	99.82	99.52	99.43
	A/Kelp Gull/Hound Bay/133747/2023	99.86	100	99.77	99.77	99.73	99.82	99.95	99.73	99.91	99.86	99.91	99.73	99.73	99.77	99.73	99.82	99.73	99.68	99.46	99.41
	A/Brown Skua/Moltke Harbour/133752/2023	99.82	99.77	100	99.82	99.69	99.78	99.82	99.96	99.87	99.82	99.87	99.69	99.69	99.74	99.69	99.78	99.69	99.65	99.43	99.34
	A/Kelp Gull/Moltke Harbour/133754/2023	99.82	99.77	99.82	100	99.69	99.78	99.82	99.78	99.87	99.82	99.87	99.69	99.69	99.74	99.69	99.78	99.69	99.65	99.43	99.34
	A/Brown Skua/Moltke Harbour/133755/2023	99.87	99.73	99.69	99.69	100	99.82	99.78	99.65	99.82	99.78	99.82	99.65	99.65	99.69	99.65	99.82	99.65	99.69	99.39	99.3
	A/Kelp Gull/Harpon Bay/133943/2023	99.96	99.82	99.78	99.78	99.82	100	99.87	99.74	99.91	99.87	99.91	99.74	99.74	99.78	99.74	99.91	99.74	99.78	99.56	99.47
	A/Brown Skua/Hound Bay/133947/2023	99.91	99.95	99.82	99.82	99.78	99.87	100	99.78	99.96	99.91	99.96	99.78	99.78	99.82	99.78	99.87	99.78	99.74	99.52	99.43
	A/Brown Skua/Hound Bay/133949/2023	99.78	99.73	99.96	99.78	99.65	99.74	99.78	100	99.82	99.78	99.82	99.65	99.65	99.69	99.65	99.74	99.65	99.61	99.39	99.3
	A/Brown skua/Bird Island/128289/2023	99.96	99.91	99.87	99.87	99.82	99.91	99.96	99.82	100	99.96	100	99.82	99.82	99.87	99.82	99.91	99.82	99.78	99.56	99.47
	A/Brown skua/Bird Island/128288/2023	99.91	99.86	99.82	99.82	99.78	99.87	99.91	99.78	99.96	100	99.96	99.78	99.78	99.82	99.78	99.87	99.78	99.74	99.52	99.43
	A/Brown skua/Bird Island/128287/2023	99.96	99.91	99.87	99.87	99.82	99.91	99.96	99.82	100	99.96	100	99.82	99.82	99.87	99.82	99.91	99.82	99.78	99.56	99.47
	A/Kelp Gull/Penguin River/141239/2023	99.78	99.73	99.69	99.69	99.65	99.74	99.78	99.65	99.82	99.78	99.82	100	100	99.96	100	99.74	100	99.96	99.39	99.3
	A/Brown Skua/Penguin River/141236/2023	99.78	99.73	99.69	99.69	99.65	99.74	99.78	99.65	99.82	99.78	99.82	100	100	99.96	100	99.74	100	99.96	99.39	99.3
	A/Kelp Gull/Penguin River/141234/2023	99.82	99.77	99.74	99.74	99.69	99.78	99.82	99.69	99.87	99.82	99.87	99.96	99.96	100	99.96	99.78	99.96	99.91	99.43	99.34
	A/Brown Skua/Bird Island/141232/2023	99.78	99.73	99.69	99.69	99.65	99.74	99.78	99.65	99.82	99.78	99.82	100	100	99.96	100	99.74	100	99.96	99.39	99.3
	A/Southern Elephant Seal/Jason Harbour/141078/2023	99.96	99.82	99.78	99.78	99.82	99.91	99.87	99.74	99.91	99.87	99.91	99.74	99.74	99.78	99.74	100	99.74	99.78	99.47	99.39
	A/Antarctic Fur Seal/Jason Harbour/141037/2023	99.78	99.73	99.69	99.69	99.65	99.74	99.78	99.65	99.82	99.78	99.82	100	100	99.96	100	99.74	100	99.96	99.39	99.3
	A/Southern Elephant Seal/Jason Harbour/141027/2023	99.82	99.68	99.65	99.65	99.69	99.78	99.74	99.61	99.78	99.74	99.78	99.96	99.96	99.91	99.96	99.78	99.96	100	99.34	99.25
	A/Southern Fulmar/Falkland Islands/138870/2023	99.52	99.46	99.43	99.43	99.39	99.56	99.52	99.39	99.56	99.52	99.56	99.39	99.39	99.43	99.39	99.47	99.39	99.34	100	99.91
	A/Southern fulmar/Falkland Islands/133789/2023	99.43	99.41	99.34	99.34	99.3	99.47	99.43	99.3	99.47	99.43	99.47	99.3	99.3	99.34	99.3	99.39	99.3	99.25	99.91	100
PB1	A/Kelp Gull/Hound Bay/133744/2023	100	99.82	99.91	99.82	99.82	99.91	99.91	99.91	99.78	99.87	99.87	99.78	99.78	99.87	99.78	99.91	99.78	99.91	99.34	99.43
	A/Kelp Gull/Hound Bay/133747/2023	99.82	100	99.86	99.77	99.72	99.82	99.91	99.82	99.68	99.77	99.77	99.68	99.68	99.77	99.68	99.82	99.68	99.82	99.36	99.45
	A/Brown Skua/Moltke Harbour/133752/2023	99.91	99.86	100	99.91	99.82	99.91	99.91	100	99.86	99.95	99.95	99.86	99.86	99.95	99.86	99.91	99.86	99.91	99.37	99.46
	A/Kelp Gull/Moltke Harbour/133754/2023	99.82	99.77	99.91	100	99.73	99.82	99.82	99.91	99.77	99.86	99.86	99.77	99.77	99.86	99.77	99.82	99.77	99.82	99.28	99.37
	A/Brown Skua/Moltke Harbour/133755/2023	99.82	99.72	99.82	99.73	100	99.82	99.82	99.82	99.69	99.78	99.78	99.69	99.69	99.78	99.69	99.82	99.69	99.82	99.29	99.38
	A/Kelp Gull/Harpon Bay/133943/2023	99.91	99.82	99.91	99.82	99.82	100	99.91	99.91	99.78	99.87	99.87	99.78	99.78	99.87	99.78	99.91	99.78	99.91	99.34	99.43
	A/Brown Skua/Hound Bay/133947/2023	99.91	99.91	99.91	99.82	99.82	99.91	100	99.91	99.78	99.87	99.87	99.78	99.78	99.87	99.78	99.91	99.78	99.91	99.34	99.43
	A/Brown Skua/Hound Bay/133949/2023	99.91	99.82	100	99.91	99.82	99.91	99.91	100	99.87	99.96	99.96	99.87	99.87	99.96	99.87	99.87	99.87	99.91	99.34	99.43
	A/Brown skua/Bird Island/128289/2023	99.78	99.68	99.86	99.77	99.69	99.78	99.78	99.87	100	99.91	99.91	99.74	99.74	99.82	99.74	99.78	99.74	99.78	99.21	99.3
	A/Brown skua/Bird Island/128288/2023	99.87	99.77	99.95	99.86	99.78	99.87	99.87	99.96	99.91	100	100	99.82	99.82	99.91	99.82	99.87	99.82	99.87	99.3	99.38
	A/Brown skua/Bird Island/128287/2023	99.87	99.77	99.95	99.86	99.78	99.87	99.87	99.96	99.91	100	100	99.82	99.82	99.91	99.82	99.87	99.82	99.87	99.3	99.38
	A/Kelp Gull/Penguin River/141239/2023	99.78	99.68	99.86	99.77	99.69	99.78	99.78	99.87	99.74	99.82	99.82	100	100	99.91	100	99.78	100	99.87	99.21	99.3
	A/Brown Skua/Penguin River/141236/2023	99.78	99.68	99.86	99.77	99.69	99.78	99.78	99.87	99.74	99.82	99.82	100	100	99.91	100	99.78	100	99.87	99.21	99.3
	A/Kelp Gull/Penguin River/141234/2023	99.87	99.77	99.95	99.86	99.78	99.87	99.87	99.96	99.82	99.91	99.91	99.91	99.91	100	99.91	99.87	99.91	99.87	99.3	99.38
	A/Brown Skua/Bird Island/141232/2023	99.78	99.68	99.86	99.77	99.69	99.78	99.78	99.87	99.74	99.82	99.82	100	100	99.91	100	99.78	100	99.87	99.21	99.3
	A/Southern Elephant Seal/Jason Harbour/141078/2023	99.91	99.82	99.91	99.82	99.82	99.91	99.91	99.91	99.78	99.87	99.87	99.78	99.78	99.87	99.78	100	99.78	99.91	99.34	99.43
	A/Antarctic Fur Seal/Jason Harbour/141037/2023	99.78	99.68	99.86	99.77	99.69	99.78	99.78	99.87	99.74	99.82	99.82	100	100	99.91	100	99.78	100	99.87	99.21	99.3
	A/Southern Elephant Seal/Jason Harbour/141027/2023	99.91	99.82	99.91	99.82	99.82	99.91	99.91	99.91	99.78	99.87	99.87	99.87	99.87	99.87	99.87	99.91	99.87	100	99.34	99.43
	A/Southern Fulmar/Falkland Islands/138870/2023	99.34	99.36	99.37	99.28	99.29	99.34	99.34	99.34	99.21	99.3	99.3	99.21	99.21	99.3	99.21	99.34	99.21	99.34	100	99.82
	A/Southern fulmar/Falkland Islands/133789/2023	99.43	99.45	99.46	99.37	99.38	99.43	99.43	99.43	99.3	99.38	99.38	99.3	99.3	99.38	99.3	99.43	99.3	99.43	99.82	100
PA	A/Kelp Gull/Hound Bay/133744/2023	100	99.77	99.72	99.72	99.77	100	99.77	99.77	99.86	99.86	99.86	99.81	99.81	99.81	99.81	99.81	99.81	99.67	98.98	98.84
	A/Kelp Gull/Hound Bay/133747/2023	99.77	100	99.77	99.77	99.63	99.77	99.81	99.81	99.91	99.91	99.91	99.86	99.86	99.86	99.86	99.77	99.86	99.72	99.02	98.88
	A/Brown Skua/Moltke Harbour/133752/2023	99.72	99.77	100	99.72	99.58	99.72	99.77	99.86	99.86	99.86	99.86	99.81	99.81	99.81	99.81	99.72	99.81	99.67	98.98	98.84
	A/Kelp Gull/Moltke Harbour/133754/2023	99.72	99.77	99.72	100	99.63	99.72	99.77	99.77	99.86	99.86	99.86	99.81	99.81	99.81	99.81	99.72	99.81	99.67	98.97	98.83
	A/Brown Skua/Moltke Harbour/133755/2023	99.77	99.63	99.58	99.63	100	99.77	99.63	99.63	99.72	99.67	99.67	99.67	99.67	99.67	99.67	99.67	99.67	99.53	98.84	98.7
	A/Kelp Gull/Harpon Bay/133943/2023	100	99.77	99.72	99.72	99.77	100	99.77	99.77	99.86	99.86	99.86	99.81	99.81	99.81	99.81	99.81	99.81	99.67	98.98	98.84
	A/Brown Skua/Hound Bay/133947/2023	99.77	99.81	99.77	99.77	99.63	99.77	100	99.81	99.91	99.91	99.91	99.86	99.86	99.86	99.86	99.77	99.86	99.72	99.02	98.88
	A/Brown Skua/Hound Bay/133949/2023	99.77	99.81	99.86	99.77	99.63	99.77	99.81	100	99.91	99.91	99.91	99.86	99.86	99.86	99.86	99.77	99.86	99.72	99.02	98.88
	A/Brown skua/Bird Island/128289/2023	99.86	99.91	99.86	99.86	99.72	99.86	99.91	99.91	100	100	100	99.95	99.95	99.95	99.95	99.86	99.95	99.81	99.12	98.98
	A/Brown skua/Bird Island/128288/2023	99.86	99.91	99.86	99.86	99.72	99.86	99.91	99.91	100	100	100	99.95	99.95	99.95	99.95	99.86	99.95	99.81	99.12	98.98
	A/Brown skua/Bird Island/128287/2023	99.86	99.91	99.86	99.86	99.72	99.86	99.91	99.91	100	100	100	99.95	99.95	99.95	99.95	99.86	99.95	99.81	99.12	98.98
	A/Kelp Gull/Penguin River/141239/2023	99.81	99.86	99.81	99.81	99.67	99.81	99.86	99.86	99.95	99.95	99.95	100	100	100	100	99.81	100	99.77	99.07	98.93
	A/Brown Skua/Penguin River/141236/2023	99.81	99.86	99.81	99.81	99.67	99.81	99.86	99.86	99.95	99.95	99.95	100	100	100	100	99.81	100	99.77	99.07	98.93
	A/Kelp Gull/Penguin River/141234/2023	99.81	99.86	99.81	99.81	99.67	99.81	99.86	99.86	99.95	99.95	99.95	100	100	100	100	99.81	100	99.77	99.07	98.93
	A/Brown Skua/Bird Island/141232/2023	99.81	99.86	99.81	99.81	99.67	99.81	99.86	99.86	99.95	99.95	99.95	100	100	100	100	99.81	100	99.77	99.07	98.93
	A/Southern Elephant Seal/Jason Harbour/141078/2023	99.81	99.77	99.72	99.72	99.67	99.81	99.77	99.77	99.86	99.86	99.86	99.81	99.81	99.81	99.81	100	99.81	99.77	98.98	98.84
	A/Antarctic Fur Seal/Jason Harbour/141037/2023	99.81	99.86	99.81	99.81	99.67	99.81	99.86	99.86	99.95	99.95	99.95	100	100	100	100	99.81	100	99.77	99.07	98.93
	A/Southern Elephant Seal/Jason Harbour/141027/2023	99.67	99.72	99.67	99.67	99.53	99.67	99.72	99.72	99.81	99.81	99.81	99.77	99.77	99.77	99.77	99.77	99.77	100	98.93	98.79
	A/Southern Fulmar/Falkland Islands/138870/2023	98.98	99.02	98.98	98.97	98.84	98.98	99.02	99.02	99.12	99.12	99.12	99.07	99.07	99.07	99.07	98.98	99.07	98.93	100	99.77
	A/Southern fulmar/Falkland Islands/133789/2023	98.84	98.88	98.84	98.83	98.7	98.84	98.88	98.88	98.98	98.98	98.98	98.93	98.93	98.93	98.93	98.84	98.93	98.79	99.77	100
HA	A/Kelp Gull/Hound Bay/133744/2023	100	99.77	99.88	99.77	99.94	99.94	99.88	99.88	99.88	99.77	99.82	99.71	99.53	99.77	99.47	99.71	99.47	99.71	99	99.06
	A/Kelp Gull/Hound Bay/133747/2023	99.77	100	99.88	99.77	99.82	99.82	99.88	99.88	99.88	99.77	99.82	99.71	99.53	99.77	99.47	99.59	99.47	99.71	99	99.06
	A/Brown Skua/Moltke Harbour/133752/2023	99.88	99.88	100	99.88	99.94	99.94	100	100	100	99.88	99.94	99.88	99.71	99.88	99.65	99.71	99.65	99.88	99.12	99.18
	A/Kelp Gull/Moltke Harbour/133754/2023	99.77	99.77	99.88	100	99.82	99.82	99.88	99.88	99.88	99.77	99.82	99.71	99.53	99.77	99.47	99.59	99.47	99.71	99	99.06
	A/Brown Skua/Moltke Harbour/133755/2023	99.94	99.82	99.94	99.82	100	100	99.94	99.94	99.94	99.82	99.88	99.77	99.59	99.82	99.53	99.77	99.53	99.76	99.06	99.12
	A/Kelp Gull/Harpon Bay/133943/2023	99.94	99.82	99.94	99.82	100	100	99.94	99.94	99.94	99.82	99.88	99.77	99.59	99.82	99.53	99.77	99.53	99.77	99.06	99.12
	A/Brown Skua/Hound Bay/133947/2023	99.88	99.88	100	99.88	99.94	99.94	100	100	100	99.88	99.94	99.82	99.65	99.88	99.59	99.71	99.59	99.82	99.12	99.18
	A/Brown Skua/Hound Bay/133949/2023	99.88	99.88	100	99.88	99.94	99.94	100	100	99.91	100	99.88	99.94	99.82	99.65	99.88	99.59	99.71	99.59	99.12	99.18
	A/Brown skua/Bird Island/128289/2023	99.88	99.88	100	99.88	99.94	99.94	100	100	100	99.88	99.94	99.82	99.65	99.88	99.59	99.71	99.59	99.82	99.12	99.18
	A/Brown skua/Bird Island/128288/2023	99.77	99.77	99.88	99.77	99.82	99.82	99.88	99.88	99.88	100	99.94	99.82	99.65	99.88	99.59	99.71	99.59	99.88	99	99.18
	A/Brown skua/Bird Island/128287/2023	99.82	99.82	99.94	99.82	99.88	99.88	99.94	99.94	99.94	99.94	100	99.88	99.71	99.94	99.65	99.77	99.65	99.88	99.06	99.24
	A/Kelp Gull/Penguin River/141239/2023	99.71	99.71	99.88	99.71	99.77	99.77	99.82	99.82	99.82	99.82	99.88	100	99.82	99.59	99.65	99.65	99.65	99.88	99.94	99.12
	A/Brown Skua/Penguin River/141236/2023	99.53	99.53	99.71	99.53	99.59	99.59	99.65	99.65	99.65	99.65	99.71	99.82	100	99.65	99.82	99.47	99.82	99.82	98.77	98.94
	A/Kelp Gull/Penguin River/141234/2023	99.77	99.77	99.88	99.77	99.82	99.82	99.88	99.88	99.88	99.88	99.94	99.82	99.65	100	99.59	99.71	99.71	99.82	99.12	99.3
	A/Brown Skua/Bird Island/141232/2023	99.47	99.47	99.65	99.47	99.53	99.53	99.59	99.59	99.59	99.59	99.65	99.65	99.82	99.59	100	99.41	99.88	99.77	98.71	98.88
	A/Southern Elephant Seal/Jason Harbour/141078/2023	99.71	99.59	99.71	99.59	99.77	99.77	99.71	99.71	99.71	99.71	99.77	99.65	99.47	99.71	99.41	100	99.41	99.65	98.83	99
	A/Antarctic Fur Seal/Jason Harbour/141037/2023	99.47	99.47	99.65	99.47	99.53	99.53	99.59	99.59	99.59	99.59	99.65	99.65	99.82	99.71	99.88	99.41	100	99.77	98.83	99
	A/Southern Elephant Seal/Jason Harbour/141027/2023	99.71	99.71	99.88	99.71	99.76	99.77	99.82	99.82	99.82	99.88	99.88	99.88	99.82	99.82	99.77	99.65	99.77	100	98.94	99.12
	A/Southern Fulmar/Falkland Islands/138870/2023	99	99	99.12	99	99.06	99.06	99.12	99.12	99.12	99	99.06	98.94	98.77	99.12	98.71	98.83	98.83	98.94	100	99.59
	A/Southern fulmar/Falkland Islands/133789/2023	99.06	99.06	99.18	99.06	99.12	99.12	99.18	99.18	99.18	99.18	99.24	99.12	98.94	99.1	98.88	99	99	99.12	99.59	100
NP	A/Kelp Gull/Hound Bay/133744/2023	100	99.8	99.67	99.86	99.93	99.93	99.6	99.93	99.93	99.93	99.93	99.93	99.93	99.93	99.93	99.87	99.93	99.8	99.06	99.13
	A/Kelp Gull/Hound Bay/133747/2023	99.8	100	99.6	99.8	99.87	99.87	99.67	99.87	99.87	99.87	99.87	99.87	99.87	99.87	99.87	99.8	99.87	99.73	99	99.06
	A/Brown Skua/Moltke Harbour/133752/2023	99.67	99.6	100	99.8	99.73	99.6	99.4	99.73	99.73	99.73	99.73	99.73	99.73	99.73	99.73	99.67	99.73	99.6	98.86	98.93
	A/Kelp Gull/Moltke Harbour/133754/2023	99.86	99.8	99.8	100	99.93	99.8	99.59	99.93	99.93	99.93	99.93	99.93	99.93	99.93	99.93	99.86	99.93	99.8	99.05	99.12
	A/Brown Skua/Moltke Harbour/133755/2023	99.93	99.87	99.73	99.93	100	99.87	99.67	100	100	100	100	100	100	100	100	99.93	100	99.87	99.13	99.2
	A/Kelp Gull/Harpon Bay/133943/2023	99.93	99.87	99.6	99.8	99.87	100	99.67	99.87	99.87	99.87	99.87	99.87	99.87	99.87	99.87	99.8	99.87	99.73	99	99.06
	A/Brown Skua/Hound Bay/133947/2023	99.6	99.67	99.4	99.59	99.67	99.67	100	99.67	99.67	99.67	99.67	99.67	99.67	99.67	99.67	99.6	99.67	99.53	98.8	98.86
	A/Brown Skua/Hound Bay/133949/2023	99.93	99.87	99.73	99.93	100	99.87	99.67	100	100	100	100	100	100	100	100	99.93	100	99.87	99.13	99.2
	A/Brown skua/Bird Island/128289/2023	99.93	99.87	99.73	99.93	100	99.87	99.67	100	100	100	100	100	100	100	100	99.93	100	99.87	99.13	99.2
	A/Brown skua/Bird Island/128288/2023	99.93	99.87	99.73	99.93	100	99.87	99.67	100	100	100	100	100	100	100	100	99.93	100	99.87	99.13	99.2
	A/Brown skua/Bird Island/128287/2023	99.93	99.87	99.73	99.93	100	99.87	99.67	100	100	100	100	100	100	100	100	99.93	100	99.87	99.13	99.2
	A/Kelp Gull/Penguin River/141239/2023	99.93	99.87	99.73	99.93	100	99.87	99.67	100	100	100	100	100	100	100	100	99.93	100	99.87	99.13	99.2
	A/Brown Skua/Penguin River/141236/2023	99.93	99.87	99.73	99.93	100	99.87	99.67	100	100	100	100	100	100	100	100	99.93	100	99.87	99.13	99.2
	A/Kelp Gull/Penguin River/141234/2023	99.93	99.87	99.73	99.93	100	99.87	99.67	100	100	100	100	100	100	100	100	99.93	100	99.87	99.13	99.2
	A/Brown Skua/Bird Island/141232/2023	99.93	99.87	99.73	99.93	100	99.87	99.67	100	100	100	100	100	100	100	100	99.93	100	99.87	99.13	99.2
	A/Southern Elephant Seal/Jason Harbour/141078/2023	99.87	99.8	99.67	99.86	99.93	99.8	99.6	99.93	99.93	99.93	99.93	99.93	99.93	99.93	99.93	100	99.93	99.93	99.06	99.13
	A/Antarctic Fur Seal/Jason Harbour/141037/2023	99.93	99.87	99.73	99.93	100	99.87	99.67	100	100	100	100	100	100	100	100	99.93	100	99.87	99.13	99.2
	A/Southern Elephant Seal/Jason Harbour/141027/2023	99.8	99.73	99.6	99.8	99.87	99.73	99.53	99.87	99.87	99.87	99.87	99.87	99.87	99.87	99.87	99.8	99.87	100	99	99.06
	A/Southern Fulmar/Falkland Islands/138870/2023	99.06	99	98.86	99.05	99.13	99	98.8	99.13	99.13	99.13	99.13	99.13	99.13	99.13	99.13	99.06	99.13	99	100	99.93
	A/Southern fulmar/Falkland Islands/133789/2023	99.13	99.06	98.93	99.12	99.2	99.06	98.86	99.2	99.2	99.2	99.2	99.2	99.2	99.2	99.2	99.13	99.2	99.06	99.93	100
NA	A/Kelp Gull/Hound Bay/133744/2023	100	99.79	99.72	99.79	99.65	99.79	99.79	99.72	99.57	99.57	99.57	99.72	99.72	99.79	99.72	99.79	99.72	99.79	99.08	99.01
	A/Kelp Gull/Hound Bay/133747/2023	99.79	100	99.93	100	99.72	99.72	100	99.93	99.79	99.79	99.79	99.93	99.93	100	99.93	100	99.93	100	99.29	99.22
	A/Brown Skua/Moltke Harbour/133752/2023	99.72	99.93	100	99.93	99.57	99.57	99.86	99.93	99.65	99.65	99.65	99.79	99.79	99.86	99.79	99.86	99.79	99.86	99.15	99.08
	A/Kelp Gull/Moltke Harbour/133754/2023	99.79	100	99.93	100	99.72	99.72	100	99.93	99.79	99.79	99.79	99.93	99.93	100	99.93	100	99.93	100	99.29	99.22
	A/Brown Skua/Moltke Harbour/133755/2023	99.65	99.72	99.57	99.72	100	99.57	99.72	99.65	99.5	99.5	99.5	99.65	99.65	99.72	99.65	99.72	99.65	99.72	99.01	98.94
	A/Kelp Gull/Harpon Bay/133943/2023	99.79	99.72	99.57	99.72	99.57	100	99.72	99.65	99.5	99.5	99.5	99.65	99.65	99.72	99.65	99.72	99.65	99.72	99.01	98.94
	A/Brown Skua/Hound Bay/133947/2023	99.79	100	99.86	100	99.72	99.72	100	99.93	99.79	99.79	99.79	99.93	99.93	100	99.93	100	99.93	100	99.29	99.22
	A/Brown Skua/Hound Bay/133949/2023	99.72	99.93	99.93	99.93	99.65	99.65	99.93	100	99.72	99.72	99.72	99.86	99.86	99.93	99.86	99.93	99.86	99.93	99.29	99.15
	A/Brown skua/Bird Island/128289/2023	99.57	99.79	99.65	99.79	99.5	99.5	99.79	99.72	100	99.86	99.86	99.72

Table S4: amino acid polymorphisms in PB2 at positions associated with mammalian adaptation (271, 591, 627 and 701) in H5N1 HPAIV sequences derived from mammalian samples collected in South Georgia.

Sequence name	**Amino acid mamalian adapation polymorphism in PB2**			
	T271A	**Q591K**	**E627K**	**D701N**
A/Southern Elephant Seal/Jason Harbour/141078/2023	T	Q	K	D
A/Antarctic Fur Seal/Jason Harbour/141037/2023	T	Q	E	D
A/Southern Elephant Seal/Jason Harbour/141027/2023	T	Q	E	D