# Museum specimens of a landlocked pinniped reveal recent loss of genetic diversity and unexpected population connections

Matti T. Heino[1,15*], Tommi Nyman[2], Jukka U. Palo[3,4], Jenni Harmoinen[1,16], Mia Valtonen[5,6,16], Małgorzata Pilot[7,17,18], Sanni Översti[8,9], Elina Salmela[10,11], Mervi Kunnasranta[12,13], A. Rus Hoelzel[14], Minna Ruokonen[1], Jouni Aspi[1]

[1]Ecology and Genetics Research Unit, University of Oulu, Oulu, Finland

[2]Department of Ecosystems in the Barents Region, Svanhovd Research Station, Norwegian Institute of Bioeconomy Research, Svanvik, Norway

[3]Department of Forensic Medicine, University of Helsinki, Helsinki, Finland

[4]Forensic Chemistry Unit/Forensic Genetics, Finnish Institute for Health and Welfare, Helsinki, Finland

[5]Department of Environmental and Biological Sciences, University of Eastern Finland, Joensuu, Finland

[6]Institute of Biotechnology, University of Helsinki, Helsinki, Finland

[7]School of Biological and Biomedical Sciences, Durham University, Durham, UK

[8]Transmission, Infection, Diversification and Evolution Group, Max-Planck Institute for the Science of Human History, Jena, Germany

[9]Organismal and Evolutionary Biology Research Programme, Faculty of Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland

[10]Faculty of Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland

[11]Department of Biology, Faculty of Science, University of Turku, Turku, Finland

[12]University of Eastern Finland, Joensuu, Finland

[13]Natural Resources Institute Finland, Joensuu, Finland

[14]Department of Biosciences, Durham University, Durham, UK

[15]Current affiliation: Department of Forensic Medicine, University of Helsinki, Helsinki, Finland

[16]Current affiliation: Wildlife Ecology Group, Natural Resources Institute Finland, Helsinki, Finland

[17]Museum and Institute of Zoology, Polish Academy of Sciences, Gdańsk, Poland

[18]Faculty of Biology, University of Gdańsk, Gdańsk, Poland

*Corresponding author. Email: matti.heino@helsinki.fi

## Abstract:

**Aim:** The Saimaa ringed seal (*Pusa hispida saimensis*) is endemic to Lake Saimaa in Finland. The subspecies is thought to have originated when parts of the ringed seal population of the Baltic region were trapped in lakes emerging due to post-glacial bedrock rebound around 9,000 years ago. During the 20th century, the population experienced a drastic human-induced bottleneck. Today encompassing a little over 400 seals with extremely low genetic diversity, it is classified as endangered. Our main aim was to evaluate the role of the 20th century bottleneck in the erosion of genetic diversity in the Saimaa seal population. We also evaluated connections with other ringed seals from the Baltic Sea, Lake Ladoga, North America, Svalbard and the White Sea.

**Location:** Lake Saimaa, Finland, together with the Baltic Sea and the Arctic Ocean.

**Methods:** We sequenced sections of the mitochondrial control region from 60 up to 125 years old museum specimens of the Saimaa ringed seal. The generated dataset was combined with publicly available sequences. We studied how genetic variation has changed through time in this subspecies, and how it is phylogenetically related to other ringed seal populations.

**Results:** We observed temporal fluctuations in haplotype frequencies and loss of haplotypes accompanied by a recent reduction in female effective population size. In apparent contrast with the traditionally held view of the Baltic origin of the population, the Saimaa ringed seal mtDNA variation shows also affinities to North American ringed seals.

**Main conclusions:** Our results suggest that the Saimaa ringed seal has experienced recent genetic drift associated with small population size. The results further suggest that extant Baltic ringed seals do not represent well the ancestral population of the Saimaa ringed seal, which calls for re-evaluation of the deep history of this subspecies.

# 1 INTRODUCTION

Modern DNA sequencing technologies and analytical methods, especially simulation-based approaches, enable detailed molecular inferences on population-level processes and demographic history in human and wildlife populations (Foote et al., 2021; Hoban et al., 2012; Taylor et al., 2021; Vilaça et al., 2021). However, there are limits as to what can be deduced from present-day genetic data. This is largely since the contemporary genetic composition of any population has been shaped by the actions of all evolutionary forces (drift, mutation, migration and selection) throughout the whole history of the population. Understanding the population dynamics at a certain period would require disentangling the effects of these forces, which is often impossible. The confounding signals arising from the actions of the different forces at different times can be to some extent minimized, for example, by marker choice (ignoring selection, assuming steady mutation rates), but drift and gene flow are harder to partition.

These limitations of contemporary variation can nevertheless be circumvented by directly obtaining data on past diversity. Temporal sampling of varied genetic data, *e.g.*, from subfossil, archaeological or museum specimens, can reveal the developments leading to the palimpsest of current genetic diversity and provide a substantially more accurate view of past evolutionary history. In this context, zoological museum collections have proven an invaluable source of direct knowledge on temporal changes in DNA diversity and, ultimately, on the demography of various wildlife species (Hofreiter et al., 2015; Huynen et al., 2012; Nakahama, 2021; Rizzi et al., 2012).

The Saimaa ringed seal (*Pusa hispida saimensis*) offers an excellent model population for exploring the effects of small size and isolation on genetic diversity. Endemic to Lake Saimaa in Finland (Fig. 1), its origin and timing of isolation are deemed as well established. It is considered a subspecies of the Holarctic ringed seal, which, numbering in millions is the most abundant Arctic pinniped (Reeves, 1998). Based on the understanding of the geological history of the Baltic Sea region, the population originates from marine ringed seals that colonized the Baltic basin during the deglaciation of the Scandinavian Ice Sheet, c. 10,000 years ago (Ukkonen, 2002; Ukkonen et al., 2014). In consequence of post-glacial bedrock rebound, parts of the Baltic ringed seal population (currently *P. h. botnica*) were trapped in emerging freshwater lakes, including lakes Saimaa and Ladoga (subspecies *P. h. ladogensis*). Hence, the Saimaa ringed seal has lived in complete isolation for c. 860 generations (Palo et al., 2003), during which it has evolved into a morphologically, behaviorally and genetically distinct subspecies (Berta & Churchill, 2012; Hyvärinen & Nieminen, 1990; Kunnasranta, 2001). Today, the Saimaa ringed seal population encompasses c. 420–430 seals (Metsähallitus, 2020) and is classified as endangered, both nationally (Liukko et al., 2016) and internationally (Kunnasranta et al., 2021). During the 20th century, the population experienced a drastic human-induced bottleneck from hunting and later due to environmental pollutants.

Due to its limited lacustrine habitat, the population has probably never been large; based on the size and resources of the lake, the maximum number of seals has been estimated as being few thousands (Hyvärinen et al., 1999). In the late 1800s, there were possibly as many as 1,000 seals remaining in Lake Saimaa, as suggested by bounty statistics (Kokko et al., 1999) and local knowledge (Hyvärinen et al., 2004). The dwindling of the population started in the early 1900s and continued through most of the century despite protection of the subspecies in 1955. In the 1980s, the population reached its ultimate low of fewer than 150 seals (Sipilä et al., 1990). Since then, intensified conservation efforts

(aiming at $N > 400$) set the population to a slow increase in the 1990s, and the positive trend has now continued for more than 20 years (Kunnasranta et al., 2021).
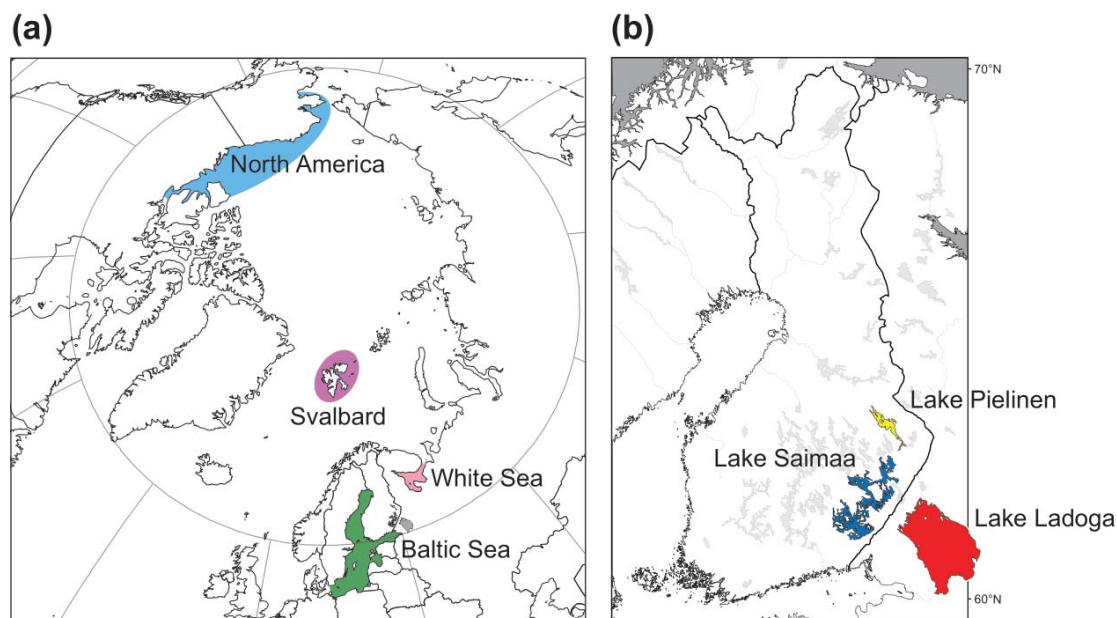


**Figure 1.** Maps showing the areas from which (a) marine, and (a) freshwater ringed seals were sampled for this study. (Note that a permanent population is not present in Lake Pielinen).

In comparison with other ringed seal subspecies – and almost any mammal population –, genetic diversity of the Saimaa ringed seal is extremely low (Martinez-Bakker et al., 2013; Palo et al., 2003; Valtonen et al., 2012, 2014). Roughly 55% of the autosomal heterozygosity and 90% of the mitochondrial nucleotide diversity present today in the marine population has been lost during the founder event and isolation. The diversity is also substantially lower than in Ladoga ringed seals (Nyman et al., 2014; Valtonen et al., 2012, 2014). Although the Saimaa ringed seal is known to have suffered a substantial reduction in numbers during the 20th century, it is not known how much of the total reduction of genetic diversity can be attributed to these recent events. Previous genetic studies have estimated a population trajectory spanning back to the isolation timepoint (Nyman et al., 2014; Palo et al., 2003; Valtonen et al., 2012), but have suggested a minor role for the 20th century bottleneck in the total diversity loss. Also, Nyman et al. (2014), exploring the plausible demographic histories leading to the current diversity using Approximate Bayesian Computation (ABC) analyses, could not find conclusive support for models including the recent bottleneck, over models including only an ancient bottleneck associated with the colonization of Lake Saimaa.

Using mitochondrial DNA (mtDNA) control-region sequences obtained from 60 up to 125 years old museum specimens, as well as previously published contemporary data, we explored changes in Saimaa ringed seal mtDNA diversity. Our main aim was to evaluate whether the loss of genetic variation of the Saimaa ringed seal can be primarily attributed to slow erosion during its isolation

after a founder event, as previously suggested (Nyman et al., 2014; Palo et al., 2003; Valtonen et al., 2012), or to the 20th-century bottleneck (Peart et al., 2020; Stoffel et al., 2018). We also evaluate the history of the Saimaa population by positioning the diversity in Saimaa on the cladistics canvas of mtDNA variation found in ringed seals from the Baltic Sea, Lake Ladoga, North America (Chukchi and Beaufort Sea), Svalbard and the White Sea.

# 2 METHODS

## 2.1 DNA extraction, amplification and sequencing

We obtained tissue samples (bones, claws or skin) of 63 historical Saimaa ringed seals from the collections of the Finnish Museum of Natural History (Supplementary Table S1). DNA extractions, amplifications and sequencing were performed either at the University of Oulu, Finland, or at Durham University, U.K.

At the University of Oulu, DNA was extracted in the ancient-DNA laboratory of the Centre for Material Analysis, following the protocol by Rohland et al. (2010). For bones and claws, a small portion of the surface of each sample was removed, and circa 250 mg of powder was obtained by drilling into the sample; the powder was then subjected to DNA extraction. For hides, a circa 250 mg piece was cut into small pieces with a sterile scalpel and then subjected to extraction. We targeted a circa 704-bp section of the mitochondrial control region (with some length variation observed among the samples). The whole region was amplified and sequenced in short variable-sized overlapping fragments depending on sample quality (for primers, see Supplementary Table S2). We first attempted to amplify each sample using primer pairs H16305/R328, F322/R471 and F425/L224. If this failed, amplification of shorter fragments was performed using primer pairs H16305/R109, F108/R328, F322/R471, F425/R605 and F603/L224. Each PCR included 1X AmpliTaq Gold® 360 Buffer, 2 mM of $MgCl_2$, 1 mg/ml of BSA, 200 μM of each dNTP, 0.4 μM of each primer and 0.6 U of AmpliTaq Gold® 360 DNA Polymerase. The PCR profile consisted of initial denaturation in 95 °C for 10 min, then 50 cycles of 95 °C for 30 s, 50 °C for 30 s and 72 °C for 30 s, and finally 72 °C for 7 min. To identify possible mis-incorporated bases caused by post-mortem changes, we PCR amplified and sequenced every region at least twice. As a control, some samples were also extracted twice. Negative controls were included in each extraction and PCR batch. PCR products were purified using Exonuclease I (Thermo Fisher Scientific) and Shrimp Alkaline Phosphatase (Thermo Fisher Scientific). Sequencing reactions were then done using BigDye Terminator v1.1 Cycle Sequencing Kit (Thermo Fisher Scientific), and the products were run on ABI 3730 DNA Analyzer (Applied Biosystems). DNA sequences were edited and assembled into contigs using CodonCode Aligner v4.0.4 (CodonCode Corporation). We also included previously unpublished sequence data from additional modern samples from Lake Saimaa ($N = 5$) and the Baltic Sea ($N = 2$) (for methods, see Valtonen et al. (2012)).

At the Durham University, DNA extraction was carried out following the method described in de Bruyn et al. (2009). The mtDNA control region was amplified in three overlapping fragments of 185-210 bp, from which 490 bp sequence was reconstructed (for primers, see Supplementary Table 2). PCR reactions were performed in a volume of 16 μl containing 1X Qiagen Multiplex PCR Master Mix, 0.2 μM of each primer, and 5 μl of the 5-times diluted extract. The thermal cycling profile was as

follows: 95 °C for 15 min, 45 cycles of 94 °C for 30 s, 50 °C for 90 s, and 72 °C for 60 s, followed by a final extension at 72 °C for 10 min. Negative controls were used for all PCR runs. PCR products were purified using QIAquick PCR Purification Kits (Qiagen) according to the manufacturer's instructions. Purified PCR products were sequenced at the sequencing facility of Durham University. As in Oulu, each sample was sequenced in both directions, and the chromatograms of each sequence were checked by eye.

We obtained the full target region of circa 704 bp from 56 out of 63 museum specimens, 49 of which were fully replicated. A partial target region was obtained from four additional specimens, three of which were fully replicated. We included samples that had unreplicated sequence areas in the analyses, after replacing any unique SNPs in their un-replicated parts with IUPAC ambiguity codes. For example, if the un-replicated part had a thymine (T) in a position in which all other Saimaa ringed seals had a cytosine (C), the T was changed to Y. We additionally included in the analyses five previously unreported modern sequences from Saimaa and two from the Baltic Sea (Supplementary Table 1). All newly reported sequences have been deposited in GenBank under accession numbers XXXXXX-XXXXXX.

## 2.2 Haplotype network

A haplotype network for seals from the Baltic area was constructed based on a combined dataset including sequences of museum and modern samples. For the museum samples, only the 56 samples from which the full target region was obtained were included. The modern data included sequences from 208 Saimaa, 21 Baltic and 16 Ladoga ringed seals sampled during the years 1980–2008, 2006–2009 and 1991–2000, respectively (Valtonen et al., 2012), as well as 57 sequences obtained from Saimaa ringed seal placentas collected from breeding sites during the years 2009–2011 (Valtonen et al., 2015). Sequences were aligned using the FFT-NS-i method within the online version of MAFFT v7 (https://mafft.cbrc.jp/alignment/server/; (Katoh et al., 2002, 2019; Katoh & Standley, 2013)). A total of 24 bases from the beginning and 20 bases from the end of the alignment were excluded to accommodate the seven previously unpublished modern sequences. The final alignment included 663 bp from 358 samples. We first converted the Fasta-formatted alignment file into a TCS input file using FaBox (Villesen, 2007), and then built a haplotype network using TCS (Clement et al., 2000), treating gaps as 5$^{th}$ state. The network was visualized with tcsBU (Múrias Dos Santos et al., 2016).

## 2.3 Temporal genetic variation

To study temporal genetic variation in the Saimaa ringed seal population, the full-length sequences from Saimaa ($N$ = 321) were divided into five temporal groups (TG) based on the history of the population outlined in Kunnasranta et al. (2021): TG1 pre–bottleneck 1894–1939 ($N$ = 23), TG2 bottleneck 1960–1979 ($N$ = 33), TG3 bottleneck 1980–1989 ($N$ = 81), TG4 recovery 1990–1999 ($N$ = 56), and TG5 increase 2000–2011 ($N$ = 128).

Temporal changes in the haplotype frequencies in the Saimaa ringed seal population were visualized as a haplotype network divided into the aforementioned five temporal groups. The figure was drawn manually by editing a network built with TempNet (Prost & Anderson, 2011). We tested for the existence of differences in the spatial distribution of samples across the different temporal groups using chi-square tests. For this, we first excluded the individual from Lake Pielinen, currently uninhabited by seals, as well as nine historical specimens for which the collection site within Lake

Saimaa was unknown. To improve concordance to the test's assumptions, we pooled time periods TG1 and TG2, and combined Kolovesi and Haukivesi into a single area, resulting in a contingency table of 4 time periods by 4 areas. As over 20% of the expected frequencies were still below 5, we conducted a second test using a 5 time periods by 2 areas dataset, in which sampling locations were coded as 'northern' (Northern Saimaa, Kolovesi, and Haukivesi basins) or 'southern' (Pihlajavesi and Southern Saimaa basins).

As the sample sizes per temporal group are relatively small, the significance of the haplotype frequency differences between the contemporary population (TG5) and earlier temporal groups (TG1–TG4) was tested using a resampling approach. For each haplotype in temporal groups TG1–TG4, $N$ observations were drawn with a probability of success equal to the frequency of that particular haplotype in the reference population (TG5) and $N$ equal to the sample size in the temporal group in question. For the six haplotypes absent from the reference population TG5, resampling was performed using a frequency of 0.023132 instead of zero, as a haplotype of that frequency would have a 5% chance of not showing up in a sample of $N = 128$. The resampling was repeated 100,000 times per haplotype and temporal group, and the resultant empirical distribution of the expected number of occurrences of each haplotype was recorded and compared with the observed number. The underlying idea is that a nonsignificant haplotype frequency difference may be due to sampling variation, whereas a significant difference could be caused by other factors, such as genetic drift or natural selection. The resampling was performed with an in-house script written for R v3.6.0. by ES (Översti et al., 2019).

## 2.4 Past effective female population size

We studied changes in the past effective female population size of the Saimaa ringed seal by constructing Bayesian skyline plots (Drummond et al., 2005) in BEAST v1.10.4 (Suchard et al., 2018) based on the 321 full-length sequences. The best-fitting substitution model was inferred using model averaging with bModelTest (Bouckaert & Drummond, 2017) in BEAST v2.6.3 (Bouckaert et al., 2019) and analysing the results with Tracer v.1.7.1 (Rambaut et al., 2018). The resulting optimal substitution model was TN93 (Tamura & Nei, 1993) accounting for proportion of invariant sites and rate variation across sites modelled with four gamma categories (Yang, 1994). The data was fitted into two molecular clock models: strict and relaxed uncorrelated lognormal clock. The most suitable clock model was determined with Akaike's information criteria through MCMC (AICM) (Baele et al., 2012) implemented in Tracer v1.6 (Rambaut et al., 2018). A strict-clock model was preferred over a relaxed clock (AICM = 11.86 with 100 bootstrap replicates) and was used in the subsequent analyses. The sampling years of the sequences were used as a source for external calibration of the molecular clock (i.e. 'tip-calibration'). We performed three parallel analyses, each with 500 million steps. Every 50,000[th] step was sampled, and the first ten percent of the steps were discarded as a burn-in. Consistency between runs was evaluated in Tracer, and chains were combined with LogCombiner included in the BEAST software package. Tracer v1.7.2 (Rambaut et al., 2018) was used to confirm that an adequate effective sample size (ESS>200) had been reached for each parameter and for visualization of the Bayesian skyline plot.

Furthermore, although it has been shown that tip-calibration yields more consistent results than internal node calibration (Rieux et al., 2014), on an evolutionary scale, samples in this study can be considered recent. Thus, to assess if the temporal structure of the data used is sufficient to calibrate

the molecular clock, we conducted a date-randomization test. We performed twenty random shufflings of tip dates with the R package TIPDATINGBEAST v1.1-0 (Rieux & Khatchikian, 2017) and ran the data-randomized datasets with BEAST v1.10.4 as described above.

## 2.5 Phylogenetic relationships between ringed seal populations

Finally, we studied broader phylogenetic connections among ringed seals from Lake Saimaa and other representative subspecies and populations in the Arctic and Baltic regions. As an addition to the data described above, sequences from Palo (2003) and Martinez-Bakker et al. (2013) were included in this analysis. As for the Baltic region dataset mentioned above, sequences were aligned using the FFT-NS-i method in MAFFT. We used only the sequence region that overlapped in all the datasets, which resulted in an alignment of 348 bp. Next, sequences that had any ambiguous bases in polymorphic sites were removed, bringing the number of sequences down to 551. In order to make the data more manageable for the analysis, sequences were collapsed into 211 unique haplotypes using the DNA to haplotype collapser and converter in FaBox (Villesen, 2007). A Bayesian phylogenetic analysis of the sequence dataset was run in MrBayes v3.2.7a (Ronquist et al., 2012) on the CIPRES Server (Miller et al., 2010). To retain information included in alignment gaps, gaps were recoded to binary presence/absence characters following the method of Simmons & Ochoterena (2000) as implemented in FastGap v1.2 (Borchsenius, 2009). In the run, we used a mixed substitution model with gamma-categorized rate variation for nucleotide sites, while the recoded gaps were treated as a separate partition of variable, gamma-corrected 'restriction' sites. Ratepr, statefreqpr, shape, revmat, and tratio were left as default and unlinked across partitions, but the branch length prior was set to unconstrained:Exp(100.0). Two runs with four incrementally heated chains each were run for 10 million generations, while sampling trees every 1,000 generations. Run convergence was assessed in Tracer and, after removing the first 10% of the sampled trees as a burn-in, a midpoint-rooted summary tree showing all compatible groupings was calculated in MrBayes.

# 3 RESULTS

## 3.1 Haplotype network

Our haplotype network for the Baltic area ringed seal mtDNA variation (Fig. 2) shows similar haplotype assemblage as in Valtonen et al. (2012, 2015), but the museum samples carried four additional Saimaa-specific haplotypes (H9, H10, H12 and H14) while lacking four previously observed haplotypes (H4, H6, H7 and H8). In total, the 'Saimaa clade' of the network consists of fourteen distinct haplotypes (H1–H14), with one haplotype from the Baltic Sea and the single sequence from Lake Pielinen (H11) being placed in the center of this assemblage. We emphasize that Lake Pielinen has not harbored a permanent ringed seal population in historical times, so the sample from this lake clearly represents a migrant individual that has strayed to Lake Pielinen from Lake Saimaa via the 67 km long River Pielisjoki. As in the results of Valtonen et al. (2012), the network shows all the Lake Saimaa haplotypes clustering relatively close to each other, with two haplotypes (H1 and H3) dominating through time. Haplotype H14 encountered in Lake Saimaa has a 64-bp deletion but also additional SNPs, which explain its outlier position in the network.
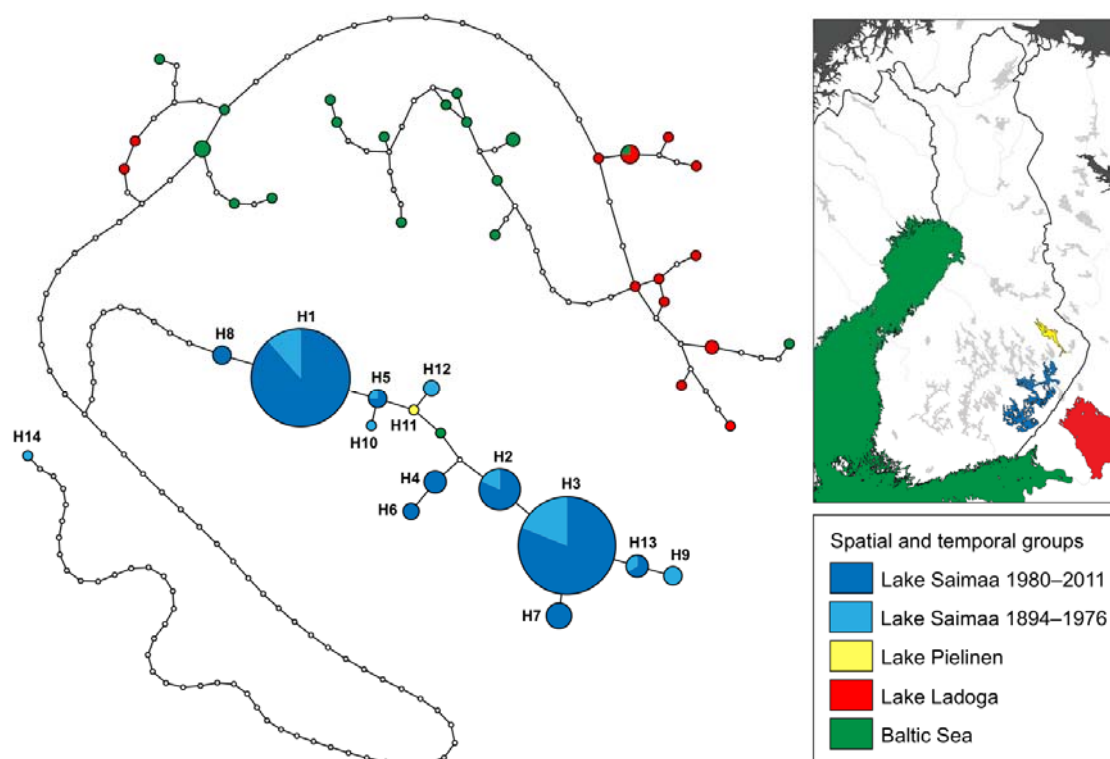
**Figure 2.** Haplotype network of ringed seals in the Baltic Sea and lakes Saimaa, Pielinen, and Ladoga. The sizes of the circles correspond to the number of sampled individuals with the haplotype, section colors denote sampling site and collection period within Lake Saimaa (see map and legend in inset). Small white circles denote unobserved haplotypes. Note that haplotype H14 is present in a single individual that has a 64-bp deletion (each bp in the deletion is counted as one change) as well as unique substitutions.

## 3.2 Temporal genetic variation

Division of the samples into five temporal groups (TG1–TG5) allowed exploring changes through time in the Lake Saimaa mtDNA haplotype pool. The temporal haplotype network (Fig. 3) and the haplotype table (Table 1) show substantial fluctuation in haplotype frequencies through time. The most common haplotype today, H3, seems to have been dominant also during the two first periods (TG1: 1894–1939 and TG2: 1960–1979). The currently second most common haplotype, H1, however, seems to have become common only after TG1: 1894–1939, and it was even more common than H3 during TG3: 1980–1989 and TG4: 1990–1999. The third most common haplotype (H2) is observed only from period TG2: 1960–1979 onwards, with a slight increase in frequency towards the Present.
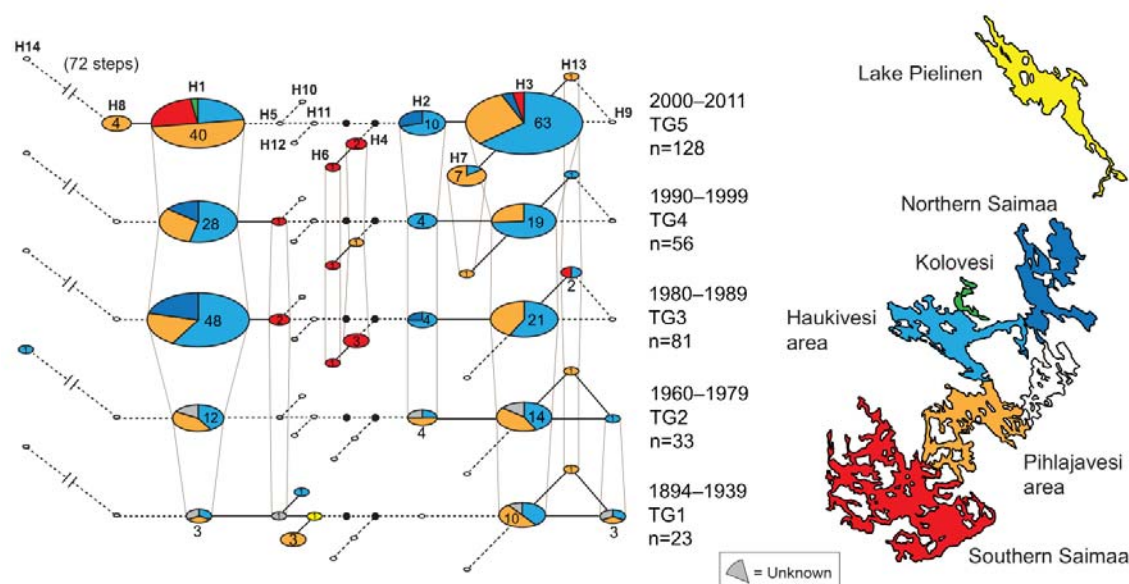
**Figure 3.** Haplotype network of Saimaa ringed seals during five different time intervals spanning periods from 1894 to the Present. In each network, circle size is proportional to haplotype frequency, and sector colors denote sampling areas as shown in the map to the right of the networks.

**Table 1. Number of each haplotype observed in each temporal period.**

| Period | TG1 | TG2 | TG3 | TG4 | TG5 |
|---|---|---|---|---|---|
| Haplotype | | | | | |
| H1 | 3 | 12 | 48 | 28 | 40 |
| H2 | 0 | 4 | 4 | 4 | 10 |
| H3 | 10 | 14 | 21 | 19 | 63 |
| H4 | 0 | 0 | 3 | 1 | 2 |
| H5 | 1 | 0 | 2 | 1 | 0 |
| H6 | 0 | 0 | 1 | 1 | 1 |
| H7 | 0 | 0 | 0 | 1 | 7 |
| H8 | 0 | 0 | 0 | 0 | 4 |
| H9 | 3 | 1 | 0 | 0 | 0 |
| H10 | 1 | 0 | 0 | 0 | 0 |
| H11 | 1 | 0 | 0 | 0 | 0 |
| H12 | 3 | 0 | 0 | 0 | 0 |
| H13 | 1 | 1 | 2 | 1 | 1 |
| H14 | 0 | 1 | 0 | 0 | 0 |
| Total | 23 | 33 | 81 | 56 | 128 |

Despite the relatively small temporal group-specific sample sizes, our resampling tests (Fig. 4) showed that many of the observed differences in haplotype frequencies are unlikely to be caused by mere sampling variation: five haplotypes in TG1–TG4 showed a nominally significant difference from TG5 within at least one time period (seven significant differences in all). While this result is not corrected for multiple testing (*i.e.*, for the 4 x 14 = 56 haplotype-wise tests), only 2.8 significant results would be expected by chance at a significance level of $\alpha = 0.05$ in 56 independent tests, and the probability of obtaining seven or more significant results by chance would be 0.021.
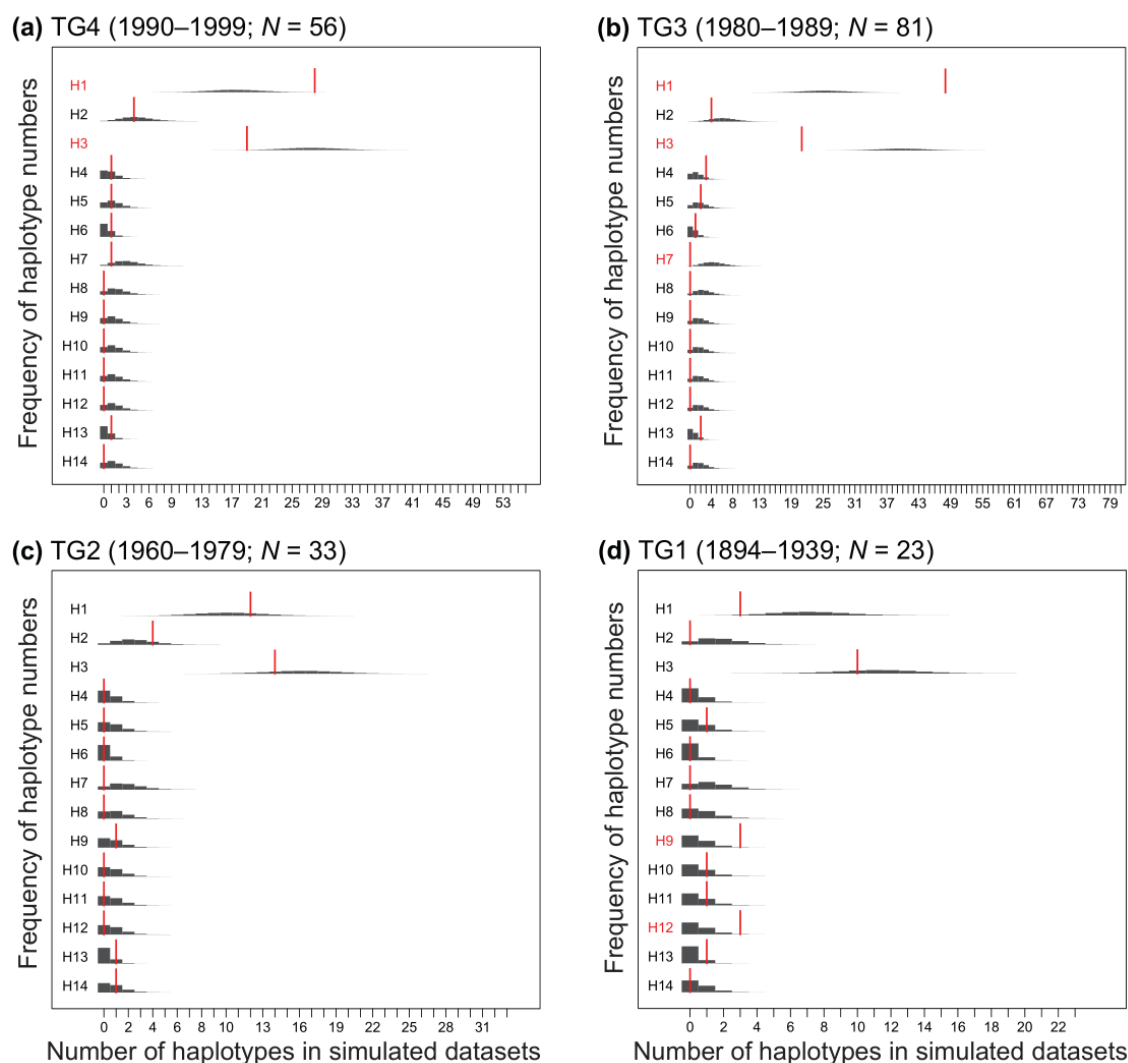


**Figure 4.** Haplotype frequency differences between the modern population and earlier temporal groups (TG1–TG4). Dark grey bars depict the expected distribution of the number of observations per haplotype in each temporal group based on 100,000 rounds of resampling from the modern distribution (TG5, *N* = 128). Red bars indicate the observed numbers within each temporal group, and red font denotes haplotypes with a nominally significant frequency difference as compared the modern population (*i.e.*, with an observed number of occurrences outside 95% of the simulated probability mass).

Taking into account also the Lake Pielinen specimen, eight different haplotypes were observed in the earliest time interval TG1 (1894–1939). Three of these (H10, H11 and H12) were not observed in later time. The resampling analysis suggested that the overrepresentation of H9 and H12 in this time interval cannot be explained by small sample size alone (Fig. 4). H12 is only observed in TG1, with a frequency of ≈ 13%. The fact that three unique haplotypes were observed only in the smallest sample of the oldest time period shows that some variation has been permanently lost and also suggests that some of the past variation remains unsampled. In TG2 (1960–1979), only six haplotypes were observed despite a larger sample size than in TG1, suggesting substantial loss of haplotype diversity between 1894 and 1960. TG3 (1980–1989) demonstrates a step towards the modern haplotype distribution, with H1 and H3 dominating. Nevertheless, compared to modern times, H1 is nominally significantly overrepresented and H3 underrepresented both in the 1980s and 1990s samples (TG3 and TG4). Furthermore, H7, which has a frequency of ≈ 5% in the modern TG5 (2000–2011), appears for the first time in TG4 (1990–1999). Haplotype H8 is only observed in TG5 (2000–2011) despite the larger summed sample size of the earlier time periods ($N$ = 128 vs. $N$ = 193).

Our tests for differential sampling across time periods indicated differences in the 4 temporal groups by 4 areas dataset ($\chi2$ = 12.7, df = 9, $P$ = 0.0005), but in this case the assumptions of the test are slightly violated (over 20% of the expected frequencies were below 5). The test assumptions were, however, met by the 5 temporal groups by 2 areas dataset, which also resulted in statistically significant differences in sampling ($\chi2$ = 12.744, df = 4, $P$ = 0.013).

## 3.3 Past effective female population size

The Bayesian skyline plot depicting the past effective population size of Saimaa ringed seal females (Fig. 5) suggests that the population has slowly decreased in size at least since the beginning of the $12^{th}$ century, but with an abrupt escalation of the population decline from the middle of the $19^{th}$ century onwards. In our date-randomization test, the 95 % highest posterior densities for the Clock.rate and TreeModel.rootHeight parameters overlapped somewhat between the actual and randomized datasets (Supplementary Fig. S1).

## 3.4 Phylogenetic relationships among ringed seal populations

In our ringed seal mtDNA phylogeny (Fig. 6), the Saimaa ringed seal clade is unexpectedly nested within the Arctic, and more specifically North American, ringed seal diversity rather than the Baltic ringed seal diversity. The ringed seals from the White Sea, which are geographically the closest Arctic population to Saimaa, are however not closely related to Saimaa. This is also the case for the geographically adjacent Ladoga ringed seal population, as individuals from Lake Ladoga are widely scattered throughout the phylogeny. Interestingly, the aforementioned Saimaa ringed seal individual 5688 (haplotype H14) with the 64-bp deletion is grouped with 1.0 posterior support together with two North American individuals that share the same deletion as well as SNPs. Five Baltic ringed seal haplotypes are nested inside the Saimaa ringed seal clade.
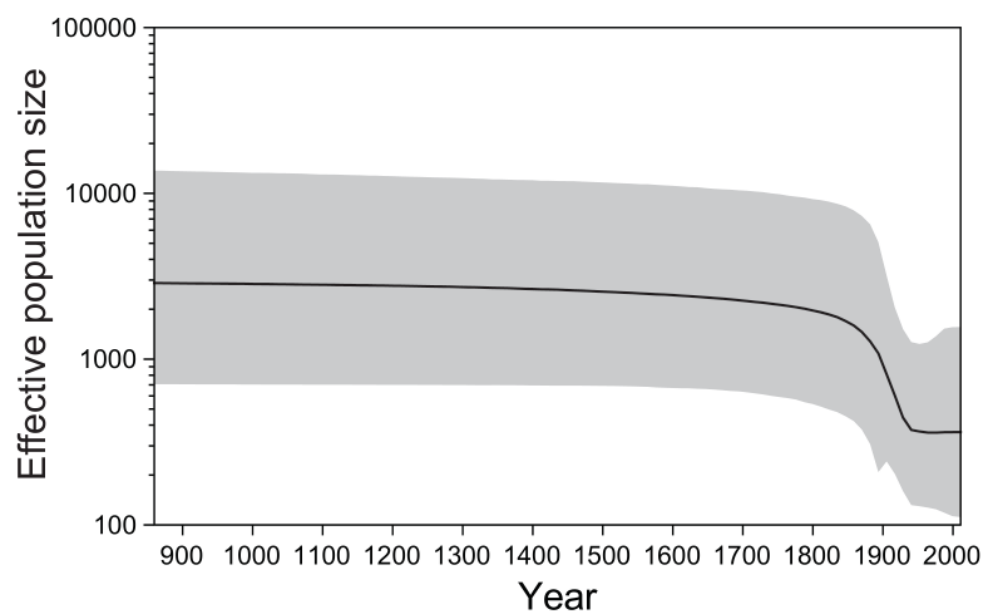
**Figure 5.** Bayesian skyline plot depicting the estimated historical effective population size of Saimaa ringed seal females on a logarithmic scale. Calendar years are shown on the X-axis. The thick line shows the median and the shaded area represents the 95% highest posterior density interval.
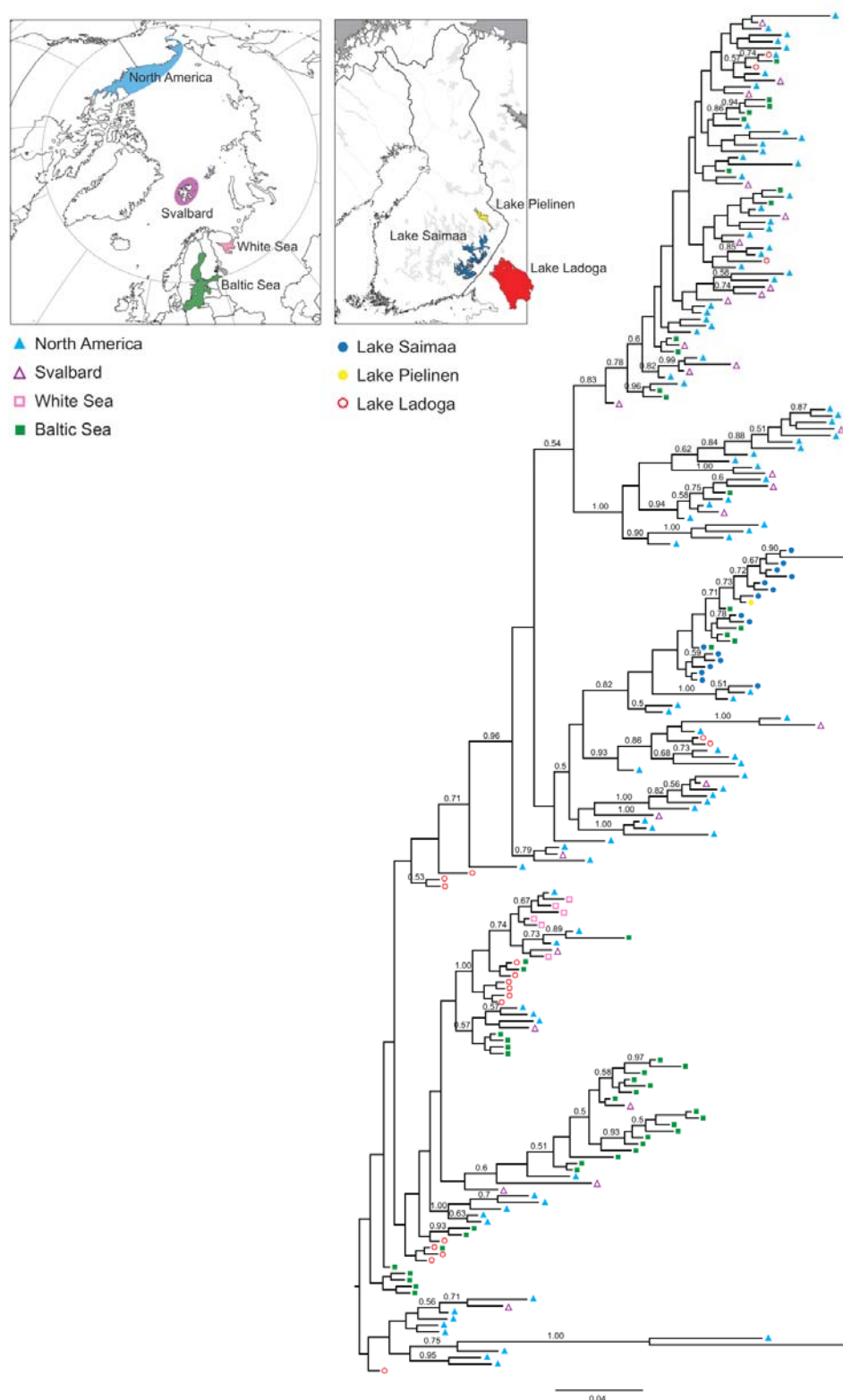
**Figure 6.** Bayesian phylogenetic tree of 211 unique mitochondrial control-region haplotypes found in freshwater, Baltic and Arctic ringed seal populations. Symbols and colors at the tips of the tree indicate the areas where each haplotype is present (see inset map and legend). Numbers above branches are posterior probabilities, only values over 0.50 are shown.

# 4 DISCUSSION

Improvements in molecular-genetic methods have increasingly made museum collections an invaluable source for gaining insights into the past genetic composition of animal and plant species (Burrell et al., 2015; Nakahama, 2021; Raxworthy & Smith, 2021). Museum material allow inferences of long-term changes in the frequencies of genetic variants (Bi et al., 2019), and can be used to improve estimates of demographic trajectories (Hung et al., 2014). Especially for endangered species, genotyping museum samples can allow direct demonstration of loss of genetic diversity over time due to diminished population size (Dussex et al., 2021; van der Valk et al., 2019; von Seth et al., 2021). Here, we sequenced mitochondrial control-region sequences from 60 museum-preserved samples of the endangered Saimaa ringed seal, and then combined these data with previously published contemporary sequences from Lake Saimaa. We used this combined dataset spanning 117 years to infer the genetic impacts of the 20th-century population bottleneck, caused mainly by relentless persecution of Saimaa ringed seals during the first half of the century. We also combined our Saimaa dataset with existing data from other Holarctic ringed seal subspecies, in order to investigate the origin of the lake-endemic Saimaa ringed seal population.

## 4.1 The genetic signature of the 20th-century bottleneck

The Saimaa ringed seal ranks among the genetically least diverse animal subspecies on the Earth, both in terms of mitochondrial (Martinez-Bakker et al., 2013; Nyman et al., 2014; Palo, 2003; Valtonen et al., 2012, 2014) and nuclear (Martinez-Bakker et al., 2013; Nyman et al., 2014; Palo, 2003; Palo et al., 2003; Stoffel et al., 2018; Valtonen et al., 2014, 2015) genetic diversity. The genetic uniformity of the subspecies is even more striking when considering the fact that ringed seals in general tend to be highly diverse (Martinez-Bakker et al., 2013; O'Corry-Crowe, 2008; Olsen et al., 2011; Palo et al., 2001). As estimated by Nyman et al. (2014), Palo et al. (2003) and Valtonen et al. (2012), the Saimaa ringed seal has lost 33.9% and 89.4% of its mitochondrial haplotype and nucleotide diversity respectively, as well as 55-69% of its overall heterozygosity in relation to its relatives in the Arctic Ocean and the Baltic Sea.

As such, the anthropogenic population collapse of the Saimaa ringed seal in the 20th century is well known and thoroughly documented (Hyvärinen et al., 1998; Kokko et al., 1998; Ranta et al., 1996; Sipilä et al., 1990). However, whether this bottleneck fully explains the extremely low genetic diversity of the population is less evident. The Saimaa ringed seal is long-lived and has an estimated generation time of 11 years (Palo et al., 2001). Hence, Nyman et al. (2014), Palo et al. (2003) and Valtonen et al. (2012) inferred that the main portion of diversity loss occurred well prior to the recent bottleneck, *i.e.*, during the long post-glacial isolation of the Saimaa ringed seal. Nyman et al. (2014) used Approximate Bayesian Computation inference based on combined nuclear and mitochondrial data and postulated that incorporating the recent bottleneck does not improve model fit in relation to a demographic model that includes only a bottleneck during colonization of the Lake Saimaa system nearly 10,000 years ago.

However, it should be emphasized that abrupt or slow post-glacial genetic erosion does not preclude the possibility that the recent bottleneck would have led to further marked loss of diversity. Indeed, our Bayesian skyline plot (Fig. 5) showed a sharp reduction in female effective population size towards the Recent. The commencement of the dip in the estimated trajectory closely parallels the

recent population collapse, which started in the early 19th century and intensified during the first half of the 20th century, largely as a result of a growing human population and increased access to modern firearms in the region. In this respect, our results are also consistent with the recent results of Stoffel et al. (2018) and Peart et al. (2020), who found statistically significant signatures of the recent bottleneck based on nuclear microsatellites and ddRAD-based SNP's, respectively. The effects of the still-continuing population recovery, which started in the 1980s, were not manifested in our most recent temporal samples of 2011.

The patterns we observed in the Bayesian skyline plot need to be interpreted with caution, as the 95% posterior density interval of the trajectory is wide, and population substructure and unbalanced sampling may confound skyline analyses (Heller et al., 2013). Moreover, in our date-randomization test, the 95% highest posterior densities for the clockRate and TreeHeight parameters overlapped somewhat between the actual and randomized datasets (Supplementary Fig. S1). This implicates that the temporal timeframe of the sampled sequences might not be wide enough to produce entirely reliable estimates of evolutionary rate and time. Future analyses should aim at strengthening the temporal signal by including older samples, longer sequences, and/or internal node calibrations, as suggested by Rieux & Khatchikian (2017).

## 4.2. Fluctuations in haplotype frequencies and recent loss of diversity

We observed substantial fluctuation in mtDNA haplotype frequencies through time in the Saimaa ringed seal population. While Valtonen et al. (2012, 2014) demonstrated significant differentiation in haplotype frequencies between the years 1980 and 2008, our analyses incorporating museum material extend the pattern nearly a hundred years back in time. As shown by our temporal networks (Fig. 3) and resampling analyses (Fig. 4), several haplotypes have probably been completely lost, while some haplotypes that are dominant today have only recently increased in frequency. The observed differences in haplotype frequencies in the different temporal groups are likely real and not caused by sampling bias. The observation of three unique haplotypes in the sample of 23 individuals that lived between 1894–1939, but not found among the nearly 300 individuals from later times, provides concrete evidence for diversity loss. Given the small sample sizes in the oldest temporal groups, it is also likely that much of the past variation remains unsampled. The observed haplotype loss and drastic frequency changes suggest that, during the last 125 years, the reduction in effective female population size in the Saimaa ringed seal has been dramatic. In this, the fate of the Saimaa ringed seal resembles another severely persecuted mammal, the grey wolf (*Canis lupus*). The wolves in the past have harbored high mtDNA diversity that has collapsed in the turn of the 20th century especially in Western Europe (Dufresnes et al., 2018).

When interpreting the results above, a potential complication arises because of the previously documented genetic substructure within Lake Saimaa (Valtonen et al., 2014), which could bias inferences if the main regions of the lake are differentially represented in the temporal samples. Statistically significant differential sampling indeed seemed to be present in the dataset. The fact that samples from Southern Saimaa are only present in the three most recent temporal groups (TG3, TG4 and TG5) might explain the absence of some Southern Saimaa-specific haplotypes in the earlier time intervals and would at the same time bias estimated diversity of the most recent time periods upwards. However, regionally biased sampling does not seem to explain the loss of haplotypes H9,

H10, H12 and H14 in the most recent time intervals, as these haplotypes were observed in lake regions that are well represented in temporal groups TG3–TG5.

## 4.3 Origin of the Saimaa ringed seal

According to the commonly held view, the Saimaa ringed seal population originated from Baltic ringed seals trapped in an inland lake system that gradually emerged due to post-glacial land uplift around 9,000 years ago (Hyvärinen & Nieminen, 1990; Nyman et al., 2014). However, the mtDNA phylogeny presented here (Fig. 6) suggests that the modern Baltic ringed seal population is a poor proxy for the ancestral Saimaa population. All the Saimaa haplotypes form a cluster that is not nested within the Baltic haplotypes. In fact, none of the geographically adjacent populations in the Baltic Sea, Lake Ladoga, and the White Sea are close to the Saimaa ringed seal cluster. Rather, the Saimaa ringed seal clade is nested within the Arctic, especially North American, ringed seal diversity, which suggests that the history of the Saimaa ringed seal population is more complex than previously thought. The fact that some Baltic ringed seal haplotypes are nested inside the Saimaa ringed seal mtDNA clade (Figs. 2 and 6) might conceivably suggest returning gene flow from Lake Saimaa into the Baltic population. However, the current outlet of Lake Saimaa is River Vuoksi, which runs into Lake Ladoga, from which the route to the Baltic Sea continues via River Neva. The absence of Saimaa-clade haplotypes from Lake Ladoga therefore suggests that their presence in the Baltic Sea reflects incomplete lineage sorting.

As already noted by Palo (2003) and Valtonen et al. (2012), particularly problematic for the post-glacial 'Out of Baltic' scenario for the Saimaa ringed seal is the presence of multiple closely related, but still diverse, haplotypes in Lake Saimaa. It is unlikely that a random sampling (through lineage sorting either during the initial colonization phase or later) from the Baltic haplotype pool would retain such a closely related set of haplotypes. The presence of a single mtDNA clade within Lake Saimaa could theoretically be explained by an extended colonization bottleneck (Nyman et al., 2014) and differentiation of a few founding lineages during the isolation. The current diversity within Lake Saimaa is, however, too high to have emerged post-glacially. As pointed out by Palo (2003), the accumulation of the diversity observed in the Saimaa clade would require at least 95,000 years (c. 8,600 generations), or Saimaa-specific mutation rates that are roughly 10x faster than normally estimated for mammalian mtDNA.

The observed pattern could have emerged if the Baltic Sea area has experienced multiple colonization waves of ringed seals after the last glacial period (Schmölcke, 2008), so that the Saimaa population represents survivors of an early wave, and the extant Baltic and Ladoga populations derive from a later wave that replaced the earlier Baltic population. Based on ancient specimens, Bro-Jørgensen (2021) inferred that such a replacement has happened in the Baltic Sea grey seal (*Halichoerus grypus* (2014). However, according to Ukkonen et al. (2014), there is no paleontological evidence suggesting that the Baltic ringed seal population would have experienced extinctions and recolonizations after the initial immigration into the basin after the last glacial period, though additional immigration from the Arctic may have occurred.

On the other hand, the pattern could be explained if Saimaa ringed seals originated from an ancestral population that inhabited proglacial lake systems situated on the edge of the Fennoscandian Ice Sheet. Current models support the existence of such refugia other aquatic species, such as the

Atlantic salmon (*Salmo salar* L.) (Asplund et al., 2004; Nilsson et al., 2001; Tonteri et al., 2005, 2007), grayling (*Thymallus thymallus*) (Koskinen et al., 2000), and European perch (*Perca fluviatilis*) (Nesbø et al., 1999).

## 4.4 Conclusions

The use of museum specimens allowed us to directly investigate genetic patterns in the endangered Saimaa ringed seal population through more than a hundred years. Although erosion of the initial genetic diversity has continued throughout the isolation, we observed 20th century loss of haplotypes and relatively drastic fluctuations in haplotype frequencies, demonstrating a genetic effect of the human-induced population collapse. Combining newly generated and already published data from multiple ringed seal populations additionally allowed us to investigate broad phylogeographic patterns in ringed seals. In apparent contrast with the traditionally held view of the Baltic origin of the population, the Saimaa ringed seal mtDNA variation shows enigmatic affinities to North American ringed seals. These results add to the growing body of evidence which calls for a re-evaluation of the deep history of the Saimaa ringed seal population. Future data on still-unsampled populations, for example in the Arctic Ocean, as well as genomic data and ancient DNA could provide keys to understanding the origin and demographic history of the Saimaa ringed seal. Lake Saimaa may harbor a ringed seal population that is even more unique than previously thought.

## Acknowledgements

**CONFLICT OF INTEREST**

The authors declare no conflicts of interest.

**DATA AVAILABILITY STATEMENT**

The sequences reported in this study have been deposited in GenBank under accession numbers X. The script used for haplotype resampling is deposited in X.

# References

Asplund, T., Veselov, A., Primmer, C. R., Bakhmet, I., Potutkin, A., Titov, S., … Lumme, J. (2004). Geographical structure and postglacial history of mtDNA haplotype variation in Atlantic salmon (*Salmo salar* L.) among rivers of the White and Barents Sea basins. *Annales Zoologici Fennici*, *41*(3), 465–475.

Baele, G., Lemey, P., Bedford, T., Rambaut, A., Suchard, M. A., & Alekseyenko, A. v. (2012). Improving the accuracy of demographic and molecular clock model comparison while accommodating phylogenetic uncertainty. *Molecular Biology and Evolution*, *29*(9), 2157–2167. https://doi.org/10.1093/molbev/mss084

Berta, A., & Churchill, M. (2012). Pinniped taxonomy: review of currently recognized species and subspecies, and evidence used for their description. *Mammal Review*, *42*(3), 207–234. https://doi.org/10.1111/j.1365-2907.2011.00193.x

Bi, K., Linderoth, T., Singhal, S., Vanderpool, D., Patton, J. L., Nielsen, R., … Good, J. M. (2019). Temporal genomic contrasts reveal rapid evolutionary responses in an alpine mammal during recent climate change. *PLOS Genetics*, *15*(5), e1008119. https://doi.org/10.1371/journal.pgen.1008119

Borchsenius, F. (2009). *FastGap 1.2.* http://www.aubot.dk/FastGap_home.htm

Bouckaert, R. R., & Drummond, A. J. (2017). bModelTest: Bayesian phylogenetic site model averaging and model comparison. *BMC Evolutionary Biology*, *17*(1), 1–11. https://doi.org/10.1186/s12862-017-0890-6

Bouckaert, R., Vaughan, T. G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., … Drummond, A. J. (2019). BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLOS Computational Biology*, *15*(4), e1006650. https://doi.org/10.1371/journal.pcbi.1006650

Bro-Jørgensen, M. H. (2021). Ancient genomics of Baltic seals⬚: Insights on the past Baltic grey seal and harp seal populations (PhD dissertation, Stockholm University, Department of Archaeology and Classical Studies). Retrieved from http://urn.kb.se/resolve?urn=urn:nbn:se:su:diva-189293

Burrell, A. S., Disotell, T. R., & Bergey, C. M. (2015). The use of museum specimens with high-throughput DNA sequencers. *Journal of Human Evolution*, *79*, 35–44. https://doi.org/10.1016/j.jhevol.2014.10.015

Clement, M., Posada, D., & Crandall, K. A. (2000). TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, *9*(10), 1657–1659. https://doi.org/10.1046/j.1365-294x.2000.01020.x

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods 2012 9:8*, *9*(8), 772–772. https://doi.org/10.1038/nmeth.2109

de Bruyn, M., Hall, B. L., Chauke, L. F., Baroni, C., Koch, P. L., & Hoelzel, A. R. (2009). Rapid Response of a Marine Mammal Species to Holocene Climate and Habitat Change. *PLOS Genetics*, *5*(7), e1000554. https://doi.org/10.1371/journal.pgen.1000554

Drummond, A. J., Rambaut, A., Shapiro, B., & Pybus, O. G. (2005). Bayesian Coalescent Inference of Past Population Dynamics from Molecular Sequences. *Molecular Biology and Evolution*, *22*(5), 1185–1192. https://doi.org/10.1093/molbev/msi103

Dufresnes, C., Miquel, C., Remollino, N., Biollaz, F., Salamin, N., Taberlet, P., & Fumagalli, L. (2018). Howling from the past: historical phylogeography and diversity losses in European grey wolves. *Proceedings of the Royal Society B*, *285*(1884). https://doi.org/10.1098/RSPB.2018.1148

Dussex, N., van der Valk, T., Morales, H. E., Wheat, C. W., Díez-del-Molino, D., von Seth, J., … Dalén, L. (2021). Population genomics of the critically endangered kākāpō. *Cell Genomics*, *1*(1), 100002. https://doi.org/10.1016/j.xgen.2021.100002

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, *10*(3), 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Foote, A. D., Hooper, R., Alexander, A., Baird, R. W., Baker, C. S., Ballance, L., … Morin, P. A. (2021). Runs of homozygosity in killer whale genomes provide a global record of demographic histories. *Molecular Ecology*, *30*(23), 6162–6177. https://doi.org/10.1111/mec.16137

Hasegawa, M., Kishino, H., & Yano, T. aki. (1985). Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution 1985 22:2*, *22*(2), 160–174. https://doi.org/10.1007/bf02101694

Heller, R., Chikhi, L., & Siegismund, H. R. (2013). The Confounding Effect of Population Structure on Bayesian Skyline Plot Inferences of Demographic History. *PLOS ONE*, *8*(5), e62992. https://doi.org/10.1371/journal.pone.0062992

Ho, S. Y. W., Lanfear, R., Bromham, L., Phillips, M. J., Soubrier, J., Rodrigo, A. G., & Cooper, A. (2011). Time-dependent rates of molecular evolution. *Molecular Ecology*, *20*(15), 3087–3101. https://doi.org/10.1111/j.1365-294x.2011.05178.x

Ho, S. Y. W., Shapiro, B., Phillips, M. J., Cooper, A., & Drummond, A. J. (2007). Evidence for Time Dependency of Molecular Rate Estimates. *Systematic Biology*, *56*(3), 515–522. https://doi.org/10.1080/10635150701435401

Hoban, S., Bertorelle, G., & Gaggiotti, O. E. (2012). Computer simulations: tools for population and evolutionary genetics. *Nature Reviews. Genetics*, *13*(2), 110–122. https://doi.org/10.1038/nrg3130

Hofreiter, M., Paijmans, J. L. A., Goodchild, H., Speller, C. F., Barlow, A., Fortes, G. G., … Collins, M. J. (2015). The future of ancient DNA: Technical advances and conceptual shifts. *BioEssays : News and Reviews in Molecular, Cellular and Developmental Biology*, *37*(3), 284–293. https://doi.org/10.1002/bies.201400160

Hung, C. M., Shaner, P. J. L., Zink, R. M., Liu, W. C., Chu, T. C., Huang, W. S., & Li, S. H. (2014). Drastic population fluctuations explain the rapid extinction of the passenger pigeon. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(29), 10636–10641. https://doi.org/10.1073/pnas.1401526111

Huynen, L., Millar, C. D., & Lambert, D. M. (2012). Resurrecting ancient animal genomes: the extinct moa and more. *BioEssays : News and Reviews in Molecular, Cellular and Developmental Biology*, *34*(8), 661–669. https://doi.org/10.1002/bies.201200040

Hyvärinen, H., Kunnasranta, M., Nieminen, P., & Taskinen, J. (2004). *Hyle : Saimaan oma norppa*. Helsinki: Tammi.

Hyvärinen, H., & Nieminen, M. (1990). Differentiation of the ringed seal in the Baltic Sea, Lake Ladoga and Lake Saimaa. *Finnish Game Research*, *47*, 21–27.

Hyvärinen, H., Sipilä, T., Koskela, J., & Kunnasranta, M. (1999). The Saimaa ringed seal. In E. Kuusisto (Ed.), *Saimaa, a Living Lake* (pp. 126–136). Helsinki: Tammi.

Hyvärinen, H., Sipila, T., Kunnasranta, M., & Koskela, J. T. (1998). Mercury pollution and the Saimaa ringed seal (*Phoca hispida saimensis*). *Marine Pollution Bulletin*, *36*(1), 76–81. https://doi.org/10.1016/s0025-326x(98)90037-6

Katoh, K., Misawa, K., Kuma, K. I., & Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, *30*(14), 3059–3066. https://doi.org/10.1093/nar/gkf436

Katoh, K., Rozewicki, J., & Yamada, K. D. (2019). MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics*, *20*(4), 1160–1166. https://doi.org/10.1093/bib/bbx108

Katoh, K., & Standley, D. M. (2013). MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Molecular Biology and Evolution*, *30*(4), 772–780. https://doi.org/10.1093/molbev/mst010

Kokko, H., Helle, E., Lindström, J., Ranta, E., Sipilä, T., & Courchamp, F. (1999). Backcasting population sizes of ringed and grey seals in the Baltic and Lake Saimaa during the 20th century. *Annales Zoologici Fennici*, *36*(2), 65–73.

Kokko, H., Lindström, J., Ranta, E., Sipilä, T., & Koskela, J. (1998). Estimating the demographic effective population size of the Saimaa ringed seal (*Phoca hispida saimensis* Nordq.). *Animal Conservation Forum*, *1*(1), 47–54. https://doi.org/10.1111/j.1469-1795.1998.tb00225.x

Koskinen, M. T., Ranta, E., Piironen, J., Veselov, A., Titov, S., Haugen, T. O., … Primmer, C. R. (2000). Genetic lineages and postglacial colonization of grayling (*Thymallus thymallus*, Salmonidae) in Europe, as revealed by mitochondrial DNA analyses. *Molecular Ecology*, *9*(10), 1609–1624. https://doi.org/10.1046/j.1365-294x.2000.01065.x

Kunnasranta, M. (2001). Behavioural biology of two ringed seal (*Phoca hispida*) subspecies in the large European lakes Saimaa and Ladoga (PhD dissertation, University of Eastern Finland, Department of Biology).

Kunnasranta, Mervi, Niemi, M., Auttila, M., Valtonen, M., Kammonen, J., & Nyman, T. (2021). Sealed in a lake — Biology and conservation of the endangered Saimaa ringed seal: A review. *Biological Conservation*, *253*, 108908. https://doi.org/10.1016/j.biocon.2020.108908

Liukko, U.-M., Henttonen, H., Hanski, I. K., Kauhala, K., Kojola, I., Kyheröinen, E.-M., & Pitkänen, J. (2016). *The 2015 Red List of Finnish Mammal Species*. Ympäristöministeriö and Suomen ympäristökeskus.

Martinez-Bakker, M. E., Sell, S. K., Swanson, B. J., Kelly, B. P., & Tallmon, D. A. (2013). Combined Genetic and Telemetry Data Reveal High Rates of Gene Flow, Migration, and Long-Distance Dispersal Potential in Arctic Ringed Seals (*Pusa hispida*). *PLOS ONE*, *8*(10), e77125. https://doi.org/10.1371/journal.pone.0077125

Metsähallitus. (2020). *Saimaannorppa*. https://www.metsa.fi/luonto-ja-kulttuuriperinto/lajien-suojelu/saimaannorppa/

Miller, M. A., Pfeiffer, W., & Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *2010 Gateway Computing Environments Workshop, GCE 2010*. https://doi.org/10.1109/gce.2010.5676129

Múrias Dos Santos, A., Cabezas, M. P., Tavares, A. I., Xavier, R., & Branco, M. (2016). tcsBU: a tool to extend TCS network layout and visualization. *Bioinformatics*, *32*(4), 627–628. https://doi.org/10.1093/bioinformatics/btv636

Nakahama, N. (2021). Museum specimens: An overlooked and valuable material for conservation genetics. *Ecological Research*, *36*(1), 13–23. https://doi.org/10.1111/1440-1703.12181

Nesbø, C. L., Fossheim, T., Vøllestad, L. A., & Jakobsen, K. S. (1999). Genetic divergence and phylogeographic relationships among european perch (*Perca fluviatilis*) populations reflect glacial refugia and postglacial colonization. *Molecular Ecology*, *8*(9), 1387–1404. https://doi.org/10.1046/j.1365-294x.1999.00699.x

Nilsson, J., Gross, R., Asplund, T., Dove, O., Jansson, H., Kelloniemi, J., … Lumme, J. (2001). Matrilinear phylogeography of Atlantic salmon (*Salmo salar* L.) in Europe and postglacial colonization of the Baltic Sea area. *Molecular Ecology*, *10*(1), 89–102. https://doi.org/10.1046/j.1365-294x.2001.01168.x

Nyman, T., Valtonen, M., Aspi, J., Ruokonen, M., Kunnasranta, M., & Palo, J. U. (2014). Demographic histories and genetic diversities of Fennoscandian marine and landlocked ringed seal subspecies. *Ecology and Evolution*, *4*(17), 3420–3434. https://doi.org/10.1002/ece3.1193

O'Corry-Crowe, G. (2008). Climate change and the molecular ecology of Arctic marine mammals. *Ecological Applications : A Publication of the Ecological Society of America*, *18*. https://doi.org/10.1890/06-0795.1

Olsen, M. T., Volny, V. H., Bérubé, M., Dietz, R., Lydersen, C., Kovacs, K. M., … Palsbøll, per J. (2011). A simple route to single-nucleotide polymorphisms in a nonmodel species: identification and characterization of SNPs in the Artic ringed seal (*Pusa hispida hispida*). *Molecular Ecology Resources*, *11*, 9–19. https://doi.org/10.1111/j.1755-0998.2010.02941.x

Översti, S., Majander, K., Salmela, E., Salo, K., Arppe, L., Belskiy, S., … Onkamo, P. (2019). Human mitochondrial DNA lineages in Iron-Age Fennoscandia suggest incipient admixture and eastern introduction of farming-related maternal ancestry. *Scientific Reports*, *9*(1), 1–14. https://doi.org/10.1038/s41598-019-51045-8

Palo, J. (2003). Genetic diversity and phylogeography of landlocked seals (PhD dissertation, University of Helsinki, Department of Ecology and Systematics).

Palo, J. U., Hyvärinen, H., Helle, E., Mäkinen, H. S., & Väinölä, R. (2003). Postglacial loss of microsatellite variation in the landlocked Lake Saimaa ringed seal. *Conservation Genetics*, *4*(2), 117–128. https://doi.org/10.1023/a:1023303109701

Palo, J. U., Mäkinen, H. S., Helle, E., Stenman, O., & Väinölä, R. (2001). Microsatellite variation in ringed seals (*Phoca hispida*): genetic structure and history of the Baltic Sea population. *Heredity*, *86*(5), 609–617. https://doi.org/10.1046/j.1365-2540.2001.00859.x

Peart, C. R., Tusso, S., Pophaly, S. D., Botero-Castro, F., Wu, C. C., Aurioles-Gamboa, D., … Wolf, J. B. W. (2020). Determinants of genetic variation across eco-evolutionary scales in pinnipeds. *Nature Ecology & Evolution*, *4*(8), 1095–1104. https://doi.org/10.1038/s41559-020-1215-5

Pesole, G., Gissi, C., de Chirico, A., & Saccone, C. (1999). Nucleotide substitution rate of mammalian mitochondrial genomes. *Journal of Molecular Evolution*, *48*(4), 427–434. https://doi.org/10.1007/pl00006487

Prost, S., & Anderson, C. N. K. (2011). TempNet: a method to display statistical parsimony networks for heterochronous DNA sequence data. *Methods in Ecology and Evolution*, *2*(6), 663–667. https://doi.org/10.1111/j.2041-210x.2011.00129.x

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. *Systematic Biology*, *67*(5), 901–904. https://doi.org/10.1093/sysbio/syy032

Ranta, E., Lindström, J., & Kokko, H. (1996). Ecological Risk Analysis: The Case of the Saimaa Ringed Seal. *Ambio*, *25*(5), 363–365.

Raxworthy, C. J., & Smith, B. T. (2021). Mining museums for historical DNA: advances and challenges in museomics. *Trends in Ecology & Evolution*, *36*(11), 1049–1060. https://doi.org/10.1016/j.tree.2021.07.009

Reeves, R. R. (1998). Distribution, abundance and biology of ringed seals (*Phoca hispida*): an overview. *NAMMCO Scientific Publications*, *1*, 9–45. https://doi.org/10.7557/3.2979

Rieux, A., Eriksson, A., Li, M., Sobkowiak, B., Weinert, L. A., Warmuth, V., … Balloux, F. (2014). Improved calibration of the human mitochondrial clock using ancient genomes. *Molecular Biology and Evolution*, *31*(10), 2780–2792. https://doi.org/10.1093/molbev/msu222

Rieux, A., & Khatchikian, C. E. (2017). tipdatingbeast: an r package to assist the implementation of phylogenetic tip-dating tests using beast. *Molecular Ecology Resources*, *17*(4), 608–613. https://doi.org/10.1111/1755-0998.12603

Rizzi, E., Lari, M., Gigli, E., de Bellis, G., & Caramelli, D. (2012). Ancient DNA studies: New perspectives on old samples. *Genetics Selection Evolution*, *44*(1), 1–19. https://doi.org/10.1186/1297-9686-44-21

Rohland, N., Siedel, H., & Hofreiter, M. (2010). A rapid column-based ancient DNA extraction method for increased sample throughput. *Molecular Ecology Resources*, *10*(4), 677–683. https://doi.org/10.1111/j.1755-0998.2009.02824.x

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., … Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Systematic Biology*, *61*(3), 539–542. https://doi.org/10.1093/sysbio/sys029

Schmölcke, U. (2008). Holocene environmental changes and the seal (Phocidae) fauna of the Baltic Sea: coming, going and staying. *Mammal Review*, *38*(4), 231–246. https://doi.org/10.1111/j.1365-2907.2008.00131.x

Schwarz, G. (1978). Estimating the Dimension of a Model. *The Annals of Statistics*, *6*(2), 461–464.

Simmons, M. P., & Ochoterena, H. (2000). Gaps as Characters in Sequence-Based Phylogenetic Analyses. *Systematic Biology*, *49*(2), 369–381. https://doi.org/10.1093/sysbio/49.2.369

Sipilä, T., Helle, E., & Hyvärinen, H. (1990). Distribution, population size and reproductivity of the Saimaa ringed seal (*Phoca hispida saimensis* Nordq.) in Finland, 1980–1984. *Finnish Game Research*, *47*, 3–10.

Stoffel, M. A., Humble, E., Paijmans, A. J., Acevedo-Whitehouse, K., Chilvers, B. L., Dickerson, B., … Hoffman, J. I. (2018). Demographic histories and genetic diversity across pinnipeds are shaped by human exploitation, ecology and life-history. *Nature Communications*, *9*(1), 1–12. https://doi.org/10.1038/s41467-018-06695-z

Suchard, M. A., Lemey, P., Baele, G., Ayres, D. L., Drummond, A. J., & Rambaut, A. (2018). Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evolution*, *4*(1). https://doi.org/10.1093/ve/vey016

Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, *10*(3), 512–526. https://doi.org/10.1093/oxfordjournals.molbev.a040023

Taylor, R. S., Jensen, E. L., Coltman, D. W., Foote, A. D., & Lamichhaney, S. (2021). Seeing the whole picture: What molecular ecology is gaining from whole genomes. *Molecular Ecology*, *30*(23), 5917–5922. https://doi.org/10.1111/mec.16282

Tonteri, A., Veselov, A. J., Titov, S., Lumme, J., & Primmer, C. R. (2007). The effect of migratory behaviour on genetic diversity and population divergence: a comparison of anadromous and freshwater Atlantic salmon *Salmo salar*. *Journal of Fish Biology*, *70*, 381–398. https://doi.org/10.1111/j.1095-8649.2007.01519.x

Tonteri, Anni, Titov, S., Veselov, A., Zubchenko, A., Koskinen, M. T., Lesbarrères, D., … Primmer, C. R. (2005). Phylogeography of anadromous and non-anadromous Atlantic salmon (*Salmo salar*) from northern Europe. *Annales Zoologici Fennici*, *42*(1), 1–22.

Ukkonen, P. (2002). The early history of seals in the northern Baltic. *Annales Zoologici Fennici*, *39*, 187–207.

Ukkonen, P., Aaris-Sørensen, K., Arppe, L., Daugnora, L., Halkka, A., Lõugas, L., … Storå, J. (2014). An Arctic seal in temperate waters: History of the ringed seal (*Pusa hispida*) in the Baltic Sea and its adaptation to the changing environment. *The Holocene, 24*(12), 1694–1706. https://doi.org/10.1177/0959683614551226

Valtonen, M., Heino, M., Aspi, J., Buuri, H., Kokkonen, T., Kunnasranta, M., … Nyman, T. (2015). Genetic Monitoring of a Critically-Endangered Seal Population Based on Field-Collected Placentas. *Annales Zoologici Fennici*, *52*(1–2), 51–65. https://doi.org/10.5735/086.052.0205

Valtonen, M., Palo, J. U., Aspi, J., Ruokonen, M., Kunnasranta, M., & Nyman, T. (2014). Causes and consequences of fine-scale population structure in a critically endangered freshwater seal. *BMC Ecology*, *14*(1), 1–15. https://doi.org/10.1186/1472-6785-14-22

Valtonen, M., Palo, J. U., Ruokonen, M., Kunnasranta, M., & Nyman, T. (2012). Spatial and temporal variation in genetic diversity of an endangered freshwater seal. *Conservation Genetics*, *13*(5), 1231–1245. https://doi.org/10.1007/s10592-012-0367-5

van der Valk, T., Díez-del-Molino, D., Marques-Bonet, T., Guschanski, K., & Dalén, L. (2019). Historical Genomes Reveal the Genomic Consequences of Recent Population Decline in Eastern Gorillas. *Current Biology*, *29*(1), 165-170.e6. https://doi.org/10.1016/j.cub.2018.11.055

Vilaça, S. T., Piccinno, R., Rota-Stabelli, O., Gabrielli, M., Benazzo, A., Matschiner, M., … Bertorelle, G. (2021). Divergence and hybridization in sea turtles: Inferences from genome data show evidence of ancient gene flow between species. *Molecular Ecology*, *30*(23), 6178–6192. https://doi.org/10.1111/mec.16113

Villesen, P. (2007). FaBox: an online toolbox for fasta sequences. *Molecular Ecology Notes*, *7*(6), 965–968. https://doi.org/10.1111/j.1471-8286.2007.01821.x

von Seth, J., Dussex, N., Díez-del-Molino, D., van der Valk, T., Kutschera, V. E., Kierczak, M., … Dalén, L. (2021). Genomic insights into the conservation status of the world's last remaining Sumatran rhinoceros populations. *Nature Communications*, *12*(1), 1–11. https://doi.org/10.1038/s41467-021-22386-8

Yang, Z. (1994). Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: Approximate methods. *Journal of Molecular Evolution*, *39*(3), 306–314. https://doi.org/10.1007/bf00160154

# Supplementary Material

**Supplementary Table S1.** Studied ringed seal specimens sorted by known/approximate year of death. The table shows sequence ID, catalog ID, subspecies status, whether the full target sequence was obtained, year of death, location, sex, source collection and type of sample/tissue.

**Supplementary Table S2.** Primers used to amplify the mtDNA control region in ringed seals.

**Supplementary Figure S1.** Results from date-randomization test for the (a) Clock.rate and (b) TreeModel.rootHeight parameters. For both parameters, 95% highest posterior densities (HPD) for the true data (red) and for twenty date-randomized datasets (black) are presented on a logarithmic scale.