ORIGINAL ARTICLE

# A melting pot in the Arctic: Analysis of mitogenome variation in Arctic char (*Salvelinus alpinus*) reveals a 1000-km contact zone between highly divergent lineages

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## Abstract

Analysing the geographical distribution of evolutionary linages can reveal the potential locations of past refugia and colonisation routes and thus can improve understanding of current patterns of genetic variation and adaptive potential. We analysed 94 full mitogenome sequences to assess phylogeographic relationships amongst ten Arctic char (*Salvelinus alpinus*) populations, from western Greenland, eastern Greenland, Iceland and Norway. In addition, we excised D-loop sequences, which were combined with previously published data in order to provide a circumpolar phylogeographical overview. In western Greenland, a secondary contact zone between Arctic and Atlantic evolutionary lineages was identified, spanning >1000 km, which geographically parallels a similar contact zone in Labrador, Canada. In eastern Greenland, Iceland and Norway, the Atlantic lineage was exclusively observed, whereas the northern-most western Greenland populations belonged to the Arctic lineage. The Arctic and Atlantic lineages were estimated to have diverged ca. 400,000 years BP, corresponding to the onset of the Saale glaciation, whereas the time of the most recent common ancestor (TMRCA) of the Arctic lineage was ca. 15,000 years BP. The Atlantic lineage comprised two subclades, with an estimated TMRCA of 60,000 BP, suggesting a complex history involving cryptic refugia or multiple recolonisations. Codon-based tests revealed no evidence for positive selection within the 13 coding genes, indicating that there are no mitochondrial genetic adaptations within or between lineages. Higher genetic diversity observed within the contact zone likely correlates with higher standing genetic variation that could contribute to adaptive responses and morphological diversification, which Arctic char is renowned.

**KEYWORDS**
D-loop, mitogenome, phylogeography, *Salvelinus alpinus*, secondary contact

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# 1 | INTRODUCTION

Pleistocene ice ages have had a pronounced impact on present-day species and intraspecies evolutionary lineages both in the northern and the southern hemisphere (Bernatchez & Wilson, 1998; Hewitt, 2000, 2004; Ruzzante et al., 2008, 2020; Shafer et al., 2010; Waters et al., 2001). In glaciated regions, populations survived in isolated refugia, which sometimes led to genetic divergence between lineages. During warmer interglacial periods, de-glaciated landscapes were recolonised, which sometimes led to secondary contact between previously isolated lineages (Hewitt, 2000, 2004). Whereas studies of the ecological and evolutionary consequences of glaciation history initially focussed on temperate regions, a growing number of studies have shown that Arctic biota also can harbour distinctly divergent lineages and complex phylogeographic histories (Alsos et al., 2005; Brunner et al., 2001; Van Houdt et al., 2005; Liu et al., 2016; Mathiesen et al., 2017; Moore et al., 2015). In fact, Arctic (and Antarctic) regions can presently be considered interglacial refugia for cold-adapted species (Lanier et al., 2015). This is of relevance in the context of ongoing anthropogenic climate change, which is expected to be particularly severe in Arctic regions (Leduc et al., 2016). Here, cold adapted Arctic species will have limited possibilities for migrating to cooler environments and may experience increased competition and predation from boreal species (e.g., Andrews et al., 2019; Renaud et al., 2012). Consideration of phylogeographical patterns in Arctic species is therefore important for demarcating major units for conservation and for identifying possible secondary contact between divergent lineages that could increase standing genetic variation, and thus potential for adapting to altered temperature regimes.

The present study examines Arctic char (*Salvelinus alpinus*) with specific focus on Greenland. This cold adapted Salmonid species is the most northerly distributed freshwater fish and can only tolerate a narrow range of temperatures (Knudsen et al., 2016). It is distributed throughout the Arctic and sub-Arctic regions of the Holarctic, as well as cold mountainous regions of boreal and temperate regions (Brunner et al., 2001; Klemetsen et al., 2003). Although all populations spawn in freshwater in rivers or lakes, many Arctic populations are anadromous and undertake marine feeding migrations. Hence, recolonisation after the Last Glacial Maximum (LGM – ca. 21,000 years ago (Hughes & Gibbard, 2015)) may have occurred through inland routes via proglacial lakes and isostatic rebound, as well as marine routes (Brunner et al., 2001; Klemetsen et al., 2003; Moore et al., 2015; Wilson et al., 1996). Given these attributes, Arctic char is a suitable species for investigating the impacts of past glacial cycles on Arctic freshwater species, and at the same time, it can shed light on how cold-adapted species are vulnerable to ongoing climate change (Hein et al., 2012; Layton et al., 2021).

The effect of the Pleistocene glaciations on present phylogeographical patterns in Arctic char has been investigated in several studies (Alekseyev et al., 2009; Brunner et al., 2001; Moore et al., 2015; Salisbury et al., 2019; Taylor et al., 2008; Wilson et al., 1996). Brunner et al. (2001) conducted the first extensive Holarctic study by sequencing the mitochondrial DNA (mtDNA) D-loop (control

region). Later, Moore et al. (2015) analysed D-loop variation in additional populations and combined the data with previously published sequences (Alekseyev et al., 2009; Brunner et al., 2001; Power et al., 2009; Taylor et al., 2008). Both Brunner et al. (2001) and Moore et al. (2015) found evidence for five phylogeographic lineages: the Arctic, Atlantic, Beringian, Siberian and Acadian lineages. The Arctic lineage has been observed throughout the Arctic Archipelago and the North American Arctic Coast, as well as in populations of the Chukotka Peninsula (Russia) and western Greenland. The Beringian lineage has been found in large regions of in eastern Russia and western North America; the Siberian lineage has been observed in large regions of Russia; the Acadian haplotype has been detected in Newfoundland and Maine; and the Atlantic lineage is mainly in Europe but interestingly also in southern Greenland and coastal eastern Canada. Moore et al. (2015) observed only a few instances where different phylogeographical lineages were detected in the same populations. However, a recent study by Salisbury et al. (2019), based on sequencing ca. 500 BP of the mitochondrial D-loop, documented co-occurrence of Acadian and Atlantic lineages in Newfoundland and Arctic and Atlantic lineages in Labrador, Canada.

A common limitation of previous studies has been the low representation of populations from Greenland. Thus far, only three populations have been included in prior studies (Brunner et al., 2001; Moore et al., 2015). Given its geographical location between North America and Europe, Greenland has likely acted as a stepping stone for the Atlantic lineage to reach North America and could potentially represent a zone of secondary contact between Arctic and Atlantic lineages. Whereas the Arctic lineage has been observed both in the northwest and southwest of Greenland, the Atlantic lineage has so far only been found in the southwest (Brunner et al., 2001; Moore et al., 2015). Whether this denotes a true pattern or is due to limited sampling is presently unknown. A denser sampling is thus warranted to close this gap in our knowledge of the circumpolar phylogeography of Arctic char.

Phylogeographic patterns have historically been investigated using the maternally inherited mitochondrial DNA (mtDNA), which is still widely used (Avise, 2009; Galtier et al., 2009), although with the important reservation that the evolutionary history of mtDNA may not necessarily reflect that of entire species and populations (Ballard & Whitlock, 2004). Most studies have relied on short sequences (<1000 BP) of fast-evolving regions, such as the D-loop. However, with an increasing use of next-generation sequencing, there has been a shift towards analysing the complete mitochondrial genome (mitogenome) (e.g., Andersen et al., 2017; Carr & Marshall, 2008; Jacobsen et al., 2012; Morin et al., 2010; Pavlova et al., 2017; Winkelmann et al., 2013). This has several advantages as longer sequences in general resolve phylogenies better than shorter ones (DeFilippis & Moore, 2000; Rokas & Carroll, 2005; Jacobsen et al., 2012; Morin et al., 2010). Moreover, by analysing the whole mitogenome, it is possible to test for positive selection acting within the molecule. Although historically it has been thought that mtDNA exhibits neutral evolution, several recent studies have

**TABLE 1** Overview of sequenced mitogenome samples with information about sample site, life history and sample size, as well as number of haplotypes and estimated nucleotide diversity with standard deviation

| Area | Abbreviation | Locality | Coordinates | | Sampling site | Life history | Sample size | Number of haplotypes | Nucleotide diversity (π) |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | | Latitude | Longitude | | | | | |
| Qaanaaq | QAAN–1 | Sea | 77.47 | −69.23 | sea | A | 10 | 4 | 0.00010 (±0.00004) |
| Uummannaq | UUMM–2 | Sermeelat Kangerluat river | 70.54 | −50.77 | river/lake | A | 10 | 4 | 0.00090 (±0.00002) |
| Disko Bay | DISK–1 | Sea | 69.25 | −53.52 | sea | A | 9 | 9 | 0.00522 (±0.00130) |
| Sisimiut | SISI–1 | Ulkebugten | 66.94 | −53.60 | river | A[a] | 10 | 5 | 0.00602 (±0.00077) |
| Manitsoq | MANI–1 | Kangerdluarssuk | 65.57 | −52.38 | river | A | 10 | 3 | 0.00365 (±0.00161) |
| Nuuk | NUUK–1 | Kapisilit river | 64.42 | −50.20 | river | A | 10 | 5 | 0.00018 (±0.00003) |
| Qaqortoq | QAQO–2 | Eqaluit river | 60.76 | −45.54 | river | A | 9 | 6 | 0.00428 (±0.00172) |
| Scoresby Sound | SCOR–1 | Sea next to river | 70.35 | −28.14 | sea | A | 8 | 4 | 0.00053 (±0.00010) |
| Iceland | ICEL–1 | Vatnshlidarvatn | 65.52 | −19.64 | lake | L | 9 | 7 | 0.00046 (±0.00009) |
| Norway | NORW–2 | Luktvatn | 66.04 | 13.58 | lake | L | 9 | 4 | 0.00007 (±0.00003) |

Anadromous (A) and Landlocked (L) populations are denoted.

[a]Above a waterfall that Arctic char may pass at high water levels.

detected evidence of positive selection acting on the coding genes, with many examples from fish (Belanger-Deschenes et al., 2013; Bernatchez & Dodson, 1985; Dalziel et al., 2006; Gagnaire et al., 2012b; Garvin et al., 2011; Jacobsen et al., 2014; Jacobsen et al., 2015). As positive selection would violate the neutrality assumed by most phylogenetic software, it may lead to errors in data analysis and interpretation (Galtier et al., 2009). Moreover, the finding of positive selection would further emphasise the need for separate management of evolutionary lineages or populations of interest (Pavlova et al., 2017).

In this study, we generated and analysed 94 mitogenome sequences from Arctic char to investigate phylogeographic patterns and assess the colonisation history of Greenland. The sample locations included ten different Arctic char populations representing seven populations in western Greenland and one population from each of eastern Greenland, Iceland and Norway. The overall objective was to assess if both the Atlantic and Arctic lineages and possibly other lineages are distributed across western Greenland. To this end, we excised D-loop sequences from the mitogenome sequences and combined them with previously published sequences representing all five phylogeographic lineages, allowing us to directly identify the lineages present at our study locations. We also mapped the lineages found in our study region along with lineages observed in previous studies to provide a circumpolar overview of the phylogeography of Arctic char. Second, we made use of the high information content of full mitogenome sequences to estimate divergence time between lineages. Prior studies have raised the possibility of cryptic refugia (Moore et al., 2015; Wilson et al., 1996), and we investigated if the same could be the case for Greenland by searching for the presence of unique sub-clades or lineages within the Greenlandic sample location and by estimating time since divergence. We also used divergence time estimates between lineages to evaluate the possible biological significance of secondary contact such as the possibility of increased levels of standing variation, which could contribute to adaptive potential. Third and finally, we investigated the possibility of positive selection having acted on the Arctic char mitogenome. Our approach offered new insights into the distribution of phylogeographic lineages in Arctic char, offering further perspective on the lasting impact of past glaciation on present-day biodiversity and adaptive potential.

## 2 | MATERIALS AND METHODS

### 2.1 | Sampling and populations

A total of 94 mitogenomes were sequenced from Arctic char representing 10 sampling sites across western Greenland (seven locations), eastern Greenland, Iceland and Norway, each represented by one sampling location (see Table 1 for further information about the sampled populations and Figure 1 for geographical locations). The Greenlandic samples were collected in 2012–2015, whereas the Norwegian and Icelandic samples were collected in 2011 and
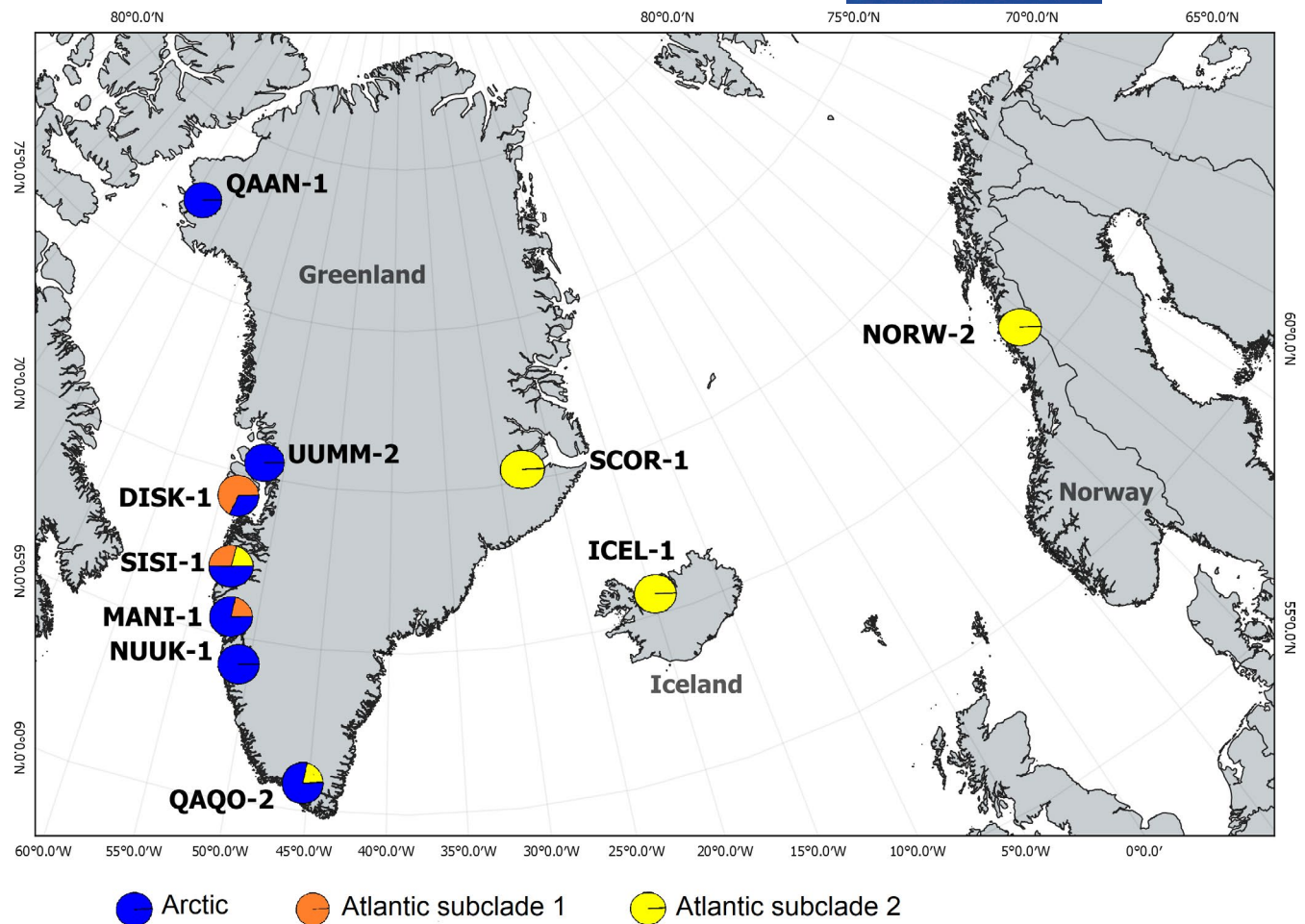
**FIGURE 1** Map showing the locations of the sample sites in Greenland, Iceland and Norway. The proportions of phylogeographic lineages (Arctic and Atlantic, including the two identified Atlantic sub-lineages) in the populations are shown by pie charts

2016, respectively. All samples were anadromous fish, except from Iceland (ICEL-1) and Norway (NORW-2) that represented land-locked populations. The samples SCOR-1, DISK-1 and QAAN-1 were caught in the sea but likely represent populations in nearby rivers, whereas the other samples were taken in rivers or lakes. Sampling was conducted by angling or gillnet fishing. Adipose fin clips were taken, stored in 96% ethanol and kept frozen at −20℃ until DNA extraction. Sampling in Greenland took place according to survey licences G14-034 and G15-013 from the government of Greenland.

Our analyses also included 56 unique D-loop haplotype sequences described in Moore et al. (2015) (see Table S2 in that paper, and Note S1 in supporting information). These data represented all five described evolutionary linages of Arctic char and were combined with D-loop sequences excised from the mitogenomes sequenced in the present study, which allowed us to identify the phylogeographic lineages represented in our study. Finally, we combined data on the geographical distribution of phylogeographic lineages from previous studies (Alekseyev et al., 2009; Brunner et al., 2001; Esin et al., 2017; Moore et al., 2015; Salisbury et al., 2019; Taylor et al., 2008) and the present study in order to map the circumpolar distribution of phylogeographic lineages.

## 2.2 | Mitogenome sequence capture and next-generation sequencing

DNA was extracted from 8 to 10 individuals per sample location using the E.Z.N.A.® Tissue DNA Kit (OMEGA, Bio-tek, CA, USA) following the manufacturer's recommendations. For each individual sample, DNA concentration was measured on a Qubit® 2 fluorometer (ThermoFisher Scientific, Waltham, MA, USA), and a total of 0.5–1.2 µg of DNA was diluted in a final volume of 130 µl TE buffer. Fragmentation of the DNA was performed by sonication, using an S220 focussed ultrasonicator (Covaris Inc., Woburn, MA, USA). Following sonication, HiBind®spin DNA extraction columns (E.Z.N.A.® Gel extraction kit—enzymatic reaction protocol) were used to concentrate the fragmented DNA and remove smaller fragments (<100 BP) before library preparation. Elution of the purified DNA was conducted in 50 µl of elution buffer, which was the recommended starting volume for building DNA libraries.

DNA libraries were prepared for Illumina sequencing using the NEBNext® Ultra™ II—DNA Library Prep Kit for Illumina® (New England BioLabs, Ipswich, MA, USA) according to the manufacturer's recommendations for an initial DNA amount >50 ng. Size selection was performed using Agencourt® AMPure® XP beads (Beckman

Coulter Life Sciences, Indianapolis, IN, USA) following the recommended conditions for an insert size of 250 BP and a total library size (insert +adaptor + primers) of 420 BP. After size selection, DNA was eluted in a volume of 18 μl 0.1×TE buffer and incubated for 5 minutes to increase final DNA yield. Subsequently, index-tagged libraries were made for all 94 individuals through index PCR amplification, using the NEBNext® Multiplex Oligos for Illumina® (New England BioLabs, Ipswich, MA, USA), which consists of dual barcoded primers for a total of 96 unique combinations. The number of PCR cycles varied between 4 and 7 and was determined by the concentration of DNA after sonication. The final purification step of the indexed libraries was performed using Agencourt® AMPure® XP beads in 25 μl 0.1×TE. At the end of library preparation, concentrations of all libraries were measured on a Qubit® 2.0 Fluorometer, and all individuals from each population were pooled (8–10 individuals per pool). As initial tests showed high similarity between the average library fragment sizes of the individual libraries, we pooled individuals based on their DNA concentration.

For each pool, we conducted separate DNA sequence capture of the full mitochondrial genome using MYbaits® (MYcroarray, Ann Arbor, MI, USA). Capture probes were designed using a published Arctic char mitogenome sequence as reference (Genbank accession number NC_000861 (Doiron et al., 2002)). We followed the manufacturer's recommendations (http://www.mycroarray.com/mybaits/manuals.html) and used a hybridisation step of >18 h. The KAPA Hifi Hotstart Ready Mix PCR kit (Kapa Biosystem Ltd, Cape Town, South Africa) was used for final PCR amplification. Molarity of the pooled capture libraries was measured using a Bioanalyzer (Agilent Technologies Denmark Aps), and the individual pools were pooled in equimolar concentration for a final library including all 94 indexed individuals. Final mitogenome sequencing was outsourced to Beijing Genomic Institute, BGI (Hongkong, China), and was conducted on an Illumina MiSeq platform using 150-BP paired end chemistry.

## 2.3 | Filtering, mitogenome assembly and data sets

After sequencing, individuals were sorted by barcodes at BGI and subsequently quality-filtered using the FASTQ quality filter programme implemented in the FASTX-Toolkit (available from: http://hannonlab.cshl.edu/fastx_toolkit/). Subsequently all identical reads were collapsed and then imported into Geneious v9.1.6 (http://www.geneious.com, (Kearse et al., 2012)), where they were aligned to a published Arctic char mitogenome sequence (GenBank accession no. NC_00086 (Doiron et al., 2002)), with individual sequences identified according to a majority rule criterion (>50% of reads for any single insertion or deletion) (for details, see Note S2).

We constructed two data sets for further downstream analyses. The first consisted of a 500-BP fragment from the D-loop, excised from the mitogenome sequences and combined with previously published D-loop sequences from Moore et al. (2015). The D-loop data set totaled 150 sequences representing all known phylogeographic lineages in Arctic char: the Siberian, Atlantic, Acadian, Beringian and Arctic lineages. Following Moore et al. (2015) we included two homologous sequences from brook trout (*Salvelinus fontinalis*) and lake trout (*Salvelinus namaycush*) as outgroups. This data set was used primarily for identifying the phylogeographic lineages in our sample locations as defined in previous studies. The second data set, used for most subsequent analyses, consisted of the generated mitogenome sequences along with the published mitogenome sequence (GenBank accession no. NC_00086) that represents the Acadian phylogeographic lineage.

## 2.4 | Assessment of D-loop phylogeny

The number of haplotypes was calculated using DnaSP v5.1 (Librado & Rozas, 2009). The best fit substitution model (TrN+G+I) was identified via the corrected Akaike information criterion (AICc). A maximum-likelihood phylogenetic tree was constructed using this substitution model, with a total of 1000 replicates generated for calculation of branch support. MEGA v7 (Kumar et al., 2016) was used for these analyses.

## 2.5 | Mitogenome phylogeny, estimation of divergence time and genetic differentiation

The number of haplotypes was calculated using DnaSP v5.1 (Librado & Rozas, 2009). Phylogenetic relationships and time of most recent common ancestor (TMRCA) were analysed in BEAST v1.8.2 (Drummond & Rambaut, 2007) using the entire mitogenome sequence. The best-fit substitution model (TrN+G) was identified via the corrected Akaike information criterion (AICc >5 to the next best model) using MEGA v7 (Kumar et al., 2016). An initial rate heterogeneity test was performed in TRACER v1.6 (Rambaut et al., 2014), using an uncorrelated log-normal clock. Given that the standard deviation of the uncorrelated lognormal relaxed clock (ucld.stdev) was abutting against zero (95%HPD of ucld.stdev $=4.25 \times 10^{-5}$ – 0.23) a strict molecular clock could not be rejected (Drummond et al., 2007). Hence, subsequent analyses were conducted using a strict clock. As different demographic scenarios between lineages may affect divergence time estimates (Ho et al., 2008), final analyses were conducted using *BEAST (Heled & Drummond, 2010). Here, different demographic scenarios were allowed between each of the three major evolutionary lineages found within the data set (the Arctic, Acadian and Atlantic lineages), using either the piecewise linear and the piecewise linear constant root models. Both the Yule or the birth-death speciation priors were tested as inter-lineage tree priors and the best combination was chosen based on Bayes factor values as calculated in TRACER (Rambaut et al., 2014).

To estimate divergence time (TMRCA), we used a full mitogenome substitution rate of $1.537 \times 10^{-8}$ sub/site/year estimated for whitefish (*Coregonus* spp.) (Jacobsen et al., 2012). The final Markov chain Monte Carlo sample obtained was based on a run for 50,000,000 generations, with genealogies sampled every

5000 generations. Convergence was assessed by effective sample size and additional runs. A maximum clade credibility tree with mean heights for branches was estimated in TREEANNOTATOR (Drummond & Rambaut, 2007) using an initial burn-in of 10%, and the final phylogenetic tree was visualised using FIGTREE v1.3.1 (Andre Rambaut, University of Edinburgh, http://tree.bio.ed.ac.uk/software/figtree/). Finally, to test the robustness of the Bayesian phylogeny a maximum likelihood approach was furthermore conducted using MEGA v7 (Kumar et al., 2016). The best-fit TrN+G substitution model with 8 gamma categories was used and a total of 100 bootstraps were generated for calculation of branch support (bootstrap values).

To investigate if climatic change during the past ice ages correlated with divergence time of the major evolutionary lineages of Arctic char, published Deuterium data from the past 420,000 years from the Vostok ice-core, Antarctica (Petit et al., 1999), was incorporated in the mitogenome phylogeny to visualise how temperature has fluctuated back to the TMRCA. Deuterium is the heavy isotope of hydrogen. It does not undergo radioactive decay and is therefore stable. The isotopic content in ice-cores can be used as a proxy for past temperature because of fractionation processes. In ice cores, a smaller amount of deuterium is found in colder periods, because it takes more energy to evaporate the heavier isotopes from the surface of the ocean (Petit et al., 1999). Furthermore, heavier molecules tend to be lost more rapidly by precipitation as the moist air moves towards the poles and cools down.

## 2.6 | Analysis of population structure using the mitogenome data set

Nucleotide diversity ($\pi$) and the associated standard deviation for individual populations was analysed using DnaSP v5.1 (Librado & Rozas, 2009). Analysis of differentiation ($\Phi_{ST}$) between pairs of populations and across all populations was performed using ARLEQUIN 3.5.2.2 (Excoffier & Lischer, 2010). The TrN substitution model was applied and 99,999 permutations of individuals amongst populations were used for testing significance of differentiation.

## 2.7 | Tests for selection

Several tests were applied to investigate the possibility of positive or relaxed purifying selection acting on the mitogenomes. All mitogenome sequences were aligned to the annotated reference (Genbank accession no. NC_000861) in GENEIOUS v9.1.6 (Kearse et al., 2012). Subsequently, all 13 coding genes were extracted separately and terminal stop codons were removed. NADH6 (ND6) was reverse translated, as it is located on the minus strand. Alignments of either the single genes or all concatenated genes were subsequently analysed for selection. A total of four different analyses were performed. First the direction of all nonsynonymous changes within the data set was reconstructed following a parsimonious principle with the closely

related *Salvelinus namaycush* as outgroup (Genbank accession numbers: MF621737-39). Quantification of non-conservative amino acid substitutions, i.e., replacement of an amino acid by one belonging to a different amino acid class, was performed following the approach from Doiron et al. (2002).

To test for positive selection amongst the Arctic and the Atlantic lineages McDonald-Kreitman tests (Mcdonald & Kreitman, 1991) were conducted in DnaSP v5.1 (Librado & Rozas, 2009). Analyses were conducted on the single gene data sets, as well as for the concatenated data set Subsequently, using the concatenated coding genes, CODEML in PAML (Yang, 1997) was used to test for significant differences in *dN/dS* amongst a predefined set of branches of the phylogenetic tree. A total of three different models were analysed and the best model chosen based on likelihood ratio tests. The different models included one with a single *dN/dS* across the entire phylogenetic tree (*OneRate*), one where the within lineage *dN/dS* was different from the between lineage (background) *dN/dS* (*TwoRates*) and one that allowed different *dN/dS* for each of the three lineages, as well as for the background (*FourRates*).

Finally, to test for codon-level signatures of selection, the FUBAR (Fast Unbiased Bayesian AppRoximation (Murrell et al., 2013)) and MEME (Mixed Effect Model of Evolution) tests were applied (Murrell et al., 2012). FUBAR tests for positive (diversifying) and negative (purifying) selection across the individual codon positions and is less likely to produce false positives owing to assignment of codons experiencing different selection pressure in the same categories, as compared to more classical random effects likelihood tests (Murrell et al., 2013). MEME tests for episodic selection within a phylogeny and hence for the possibility for specific evolutionary lineages to show evidence for positive selection (Murrell et al., 2012). Both tests were conducted for all 13 mitochondrial coding genes using the entire mitogenome data set with the HYPHY package (Pond & Frost, 2005) on the DataMonkey server (http://www.datamonkey.org/dataupload.php). Significance level was determined as posterior probability >.9 for FUBAR and $p < .05$ for MEME tests (for more details on selection tests, see Note S3).

## 3 | RESULTS

### 3.1 | Mitogenome sequencing and assembly

Alignment of individual reads to the Canadian reference mitogenome led to an average of 33,824 (between 20,423 and 54,678) reads per individual and an average sequencing depth of 305 (ranging from 184 to 493) (for details on read counts and filtering, see Note S2). Individual base positions were highly supported. The few regions showing some uncertainties with respect to base calling included polymer regions where Illumina sequencing is known to have problems in calling the true number of bases. However, even within these regions the consensus base call normally represented more than 75% of all individual reads, thus exhibiting high support for the generated mitogenome sequences.

## 3.2 | Analysis of D-loop phylogeny

We observed a total of 63 haplotypes based on the D-loop sequences excised from the mitogenome sequences alongside previously published data. The 94 mitogenomes included seven new haplotypes. Of these, four were from Disk-1, whilst the latter three were found in Norw-2, QAQO-2 and UUMM-2. As expected, the Maximum Likelihood tree retrieved all five different phylogeographic lineages within Arctic char (Figure 2), and it showed that all sequences generated in the present study belonged to either the Atlantic or Arctic lineage. These two lineages are not direct sister-clades and show clear differentiation from each other (Figure 2). Five new haplotypes were observed in the Atlantic lineage and two new haplotypes in the Arctic lineage, whereas those remaining corresponded to haplotypes previously observed by Brunner et al. (2001) and Moore et al. (2015). This also includes the haplotype ARC19, which made up 95.3% of all Arctic lineage haplotypes in Moore et al. (2015) and 96% of the 53 Arctic haplotypes in the present study. It should be noted that ARC19 and other haplotypes were divided into several separate haplotypes in the mitogenome data set.

## 3.3 | Mitogenome phylogeny and estimation of divergence time

Forty-eight haplotypes were observed amongst the 95 mitogenome sequences. The majority of shared haplotypes were distributed between individuals within the same populations, although there were also haplotypes shared across different populations. These constituted Arctic haplotype Arc2 shared between the populations QAAN-, DISK-1, SISI-1, and MANI-1 spanning almost 1500 km of distance and an Atlantic haplotype ATL18 shared between the populations QAQO-2 on the south-west coast and SCOR-1 on the mid-east coast of Greenland (Table S1).

Model comparisons conducted in TRACER (Rambaut et al., 2014) showed no major support for any specific model combination (Bayes Factors <2) and all four analyses recovered nearly identical results of TMRCA. However, a combination including a birth-death interlineage prior and a piecewise linear intra-lineage prior was slightly better supported and thus used for phylogenetic inferences and estimation of divergence times (Table S2).

Both the Bayesian and Maximum Likelihood approaches supported the same mitogenome phylogeny and showed, as expected, three distinct clades representing the Arctic, Acadian and Atlantic lineages defined by Moore et al. (2015) and Brunner et al. (2001). Assignment to phylogeographical lineage was the same for the mitogenome data set as for the D-loop data set. The Bayesian tree is shown in Figure 3 and the distribution of phylogeographic lineages across populations is shown in Figure 1. The Arctic lineage was only present in Arctic char from Western Greenland and comprised all sequenced individuals from QAAN-1, UUMM-2 and NUUK-1 and was furthermore present in the populations DISK-1, SISI-1, MANI-1

and QAQO-2. The Atlantic lineage comprised all fish from SCOR-1 (Eastern Greenland), ICEL-1, NORW-2 and was furthermore observed in the Western Greenland populations DISK-1, SISI-1, MANI-1 and QAQO-2. Hence, both the Arctic and Atlantic lineages are present in Western Greenland, and in several cases both lineages were found in the same sampled locations (Figure 1). The distribution of lineages suggests a broad zone of secondary contact extending ≥1000 km from the southern tip of Greenland northward to Disko Island. The circumpolar overview of the distribution of lineages (Figure 4) further illustrates the secondary contact zone in Greenland and shows that this is roughly geographically parallel to the secondary contact between Arctic and Atlantic lineages in Labrador in Eastern North America (Salisbury et al., 2019).
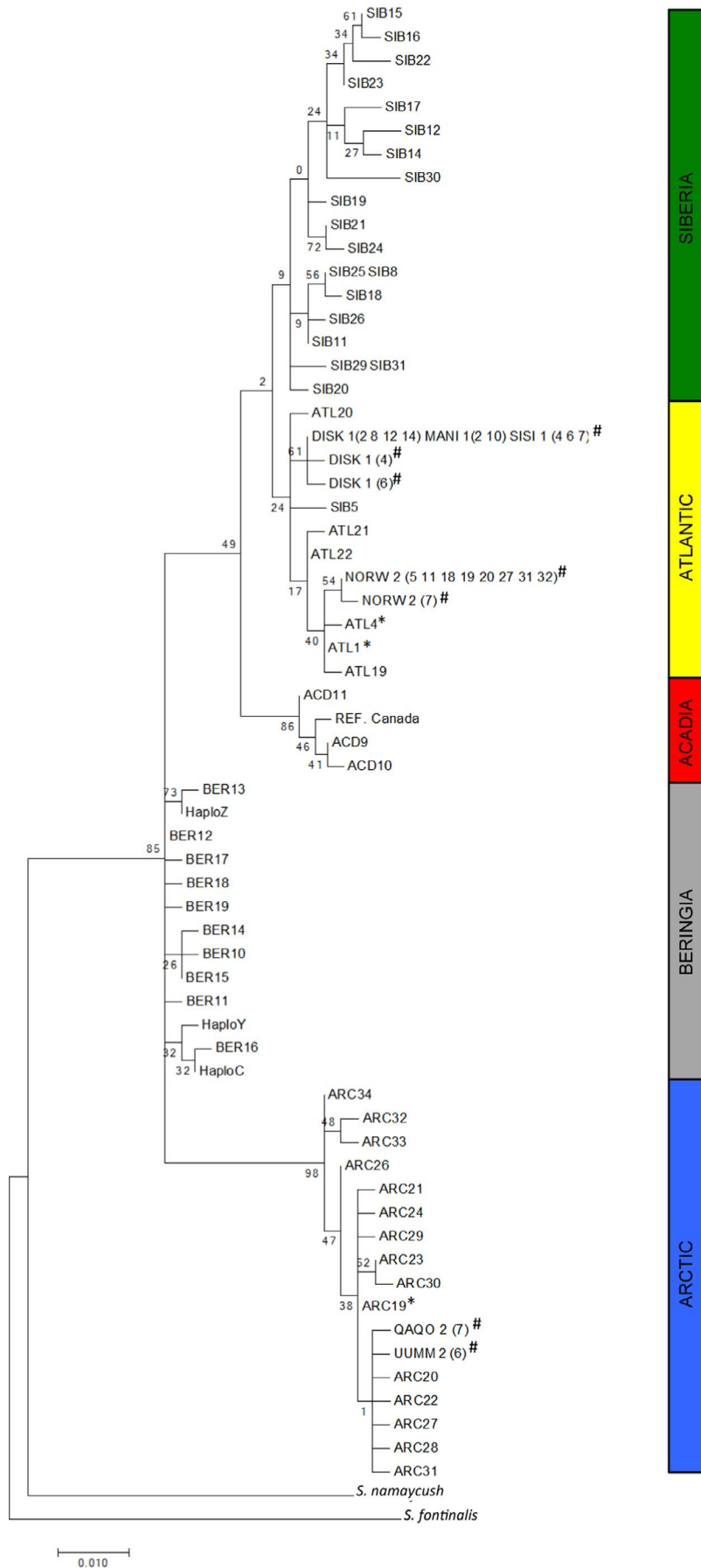
Interestingly, the Atlantic clade appears to be further divided into two sub-clades (Figure 3). The first (Sub-clade 1) was observed in DISK-1, SISI-1 and MANI-1 in Western Greenland, whereas the second (Sub-clade 2) was found in all individuals from NORW-1, ICEL-1 and SCOR-1 and was furthermore observed in the Western Greenland SISI-1 and QAQO-2 sample location. The two sub-clades of the Atlantic lineage observed in the mitogenome data set were also evident, albeit not as distinctly in the D-loop data set (Figure 2).

Divergence times (TMRCA) between the major linages generally corresponded to known ice ages and overlapped with periods showing smaller amounts of detected deuterium in the Vostok data. TMRCA between Arctic and Atlantic lineages was estimated to 391,788 BP (95% HPD 334,191–454,938 BP). The TMRCA of the Arctic clade was estimated to 15,275 BP (95% HPD 8631–22,472 BP). These estimates overlap with the Saale glacial period (128,000–386,000 BP) and the beginning of the Holocene (0–12,000 BP), respectively. TMRCA between the Acadian and Atlantic lineage was estimated to 106,195 BP (95% HPD 80,736–131,033), which aligns with the onset of the Weichselian glaciation (12,000–115,000 BP). Finally, TMRCA of Sub-clades 1 and 2 was estimated to 58,779 BP (95%HPD 40,561–75 211), that is, during the last (Weichselian) glaciation. The divergence time within Sub-clade 2 between the Western Greenlandic haplotypes and those observed in SCOR-1, ICEL-1 and NORW-1 was approximately 9000 years (Figure 4).
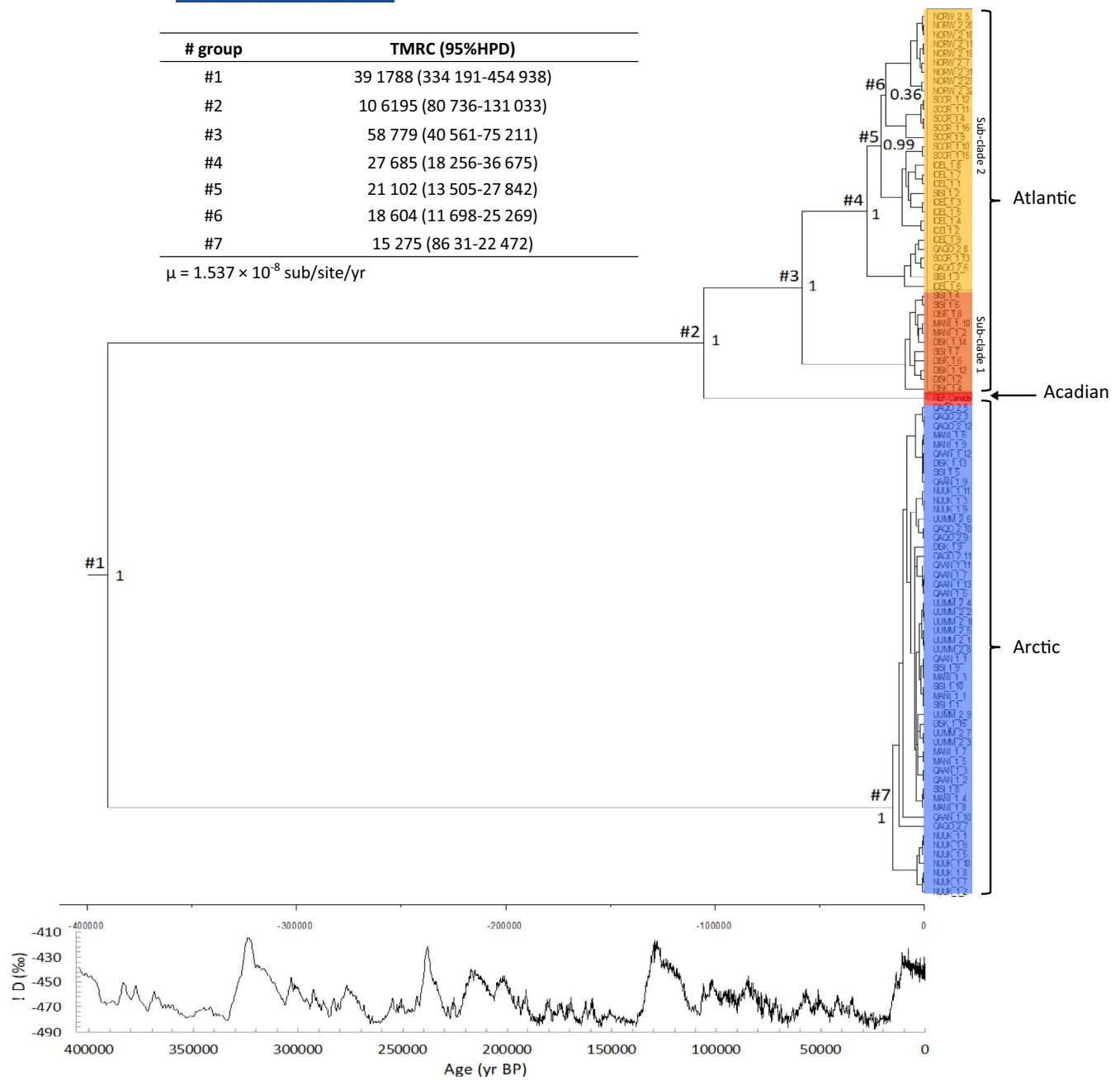
## 3.4 | Analysis of population structure

Nucleotide diversity ($\pi$) within sample locations ranged from 0.00007 (NORW-1) to 0.00602 (SISI-1) (Table 1). As expected, sample locations harbouring both phylogeographical lineages showed higher $\pi$ values (from 0.00365 to 0.00602) than sites where only a single lineage was observed (from 0.00007 to 0.00090). Significant differentiation was observed across all populations ($\Phi_{ST} = 0.77$, $p < .001$), and significant pairwise differentiation was found between most sample sites, with $\Phi_{ST}$ ranging from 0 to 0.99. The only nonsignificant comparisons were between DISK-1 and SISI-1, SISI-1 and MANI-1, SISI-1 and QAQO-2 and finally MANI-1 and QAQO-2 (Table 2).

**FIGURE 2** Maximum-likelihood tree showing the genetic relationships between phylogeographic lineages and haplotypes based on D-loop sequences. The data include seven haplotype sequences from the present study along with 56 sequences from Moore et al. (2015) representing the Siberian, Atlantic, Acadian, Beringian and Arctic lineages found in Arctic char. The hash symbol "#" denotes the new haplotypes found in the present study. Asterisks "*" denote haplotypes shared between the current study and Moore et al. (2015). Brook trout (*Salvelinus fontinalis*) and lake trout (*Salvelinus namaycush*) are included as outgroups

| # group | TMRC (95%HPD) |
|---------|---------------|
| #1 | 39 1788 (334 191-454 938) |
| #2 | 10 6195 (80 736-131 033) |
| #3 | 58 779 (40 561-75 211) |
| #4 | 27 685 (18 256-36 675) |
| #5 | 21 102 (13 505-27 842) |
| #6 | 18 604 (11 698-25 269) |
| #7 | 15 275 (86 31-22 472) |

$\mu = 1.537 \times 10^{-8}$ sub/site/yr



**FIGURE 3** Bayesian tree showing the genetic relationships between the analysed mitogenome sequences, along with the populations they were found in. The time of the most recent common ancestor (TMRCA) for the observed clades is also shown. In addition, deuterium data from the past 420,000 years from the Vostok ice-core Antarctica are shown, where low deuterium content marks cold time periods (Petit et al., 1999). TMRCA between the Atlantic and Arctic lineages, and the Atlantic and Acadian linages overlap with the onset the Saale and Weichselian glaciations

## 3.5 | Tests for selection

Analysis of the direction of a nonsynonymous change in the phylogeny showed an almost equal number of nonsynonymous changes along the branches, leading to the combined Atlantic–Acadian clade, and the Arctic clade, respectively (Figure 5; see also Table S3). Four amino acid changes occurred along the branch, leading to the Acadian lineage. Given that this lineage is only represented by one individual,

it is uncertain whether these differences represent mutations fixed within the Acadian clade or specific to the sequenced individual. Thirty-one of the total 40 observed nonsynonymous changes were found in the terminal branches with two codon positions (ND2 221 and ND1 188) showing the occurrence of multiple, but evolutionary independent, nonsynonymous changes within the same position. Only three replacements showed a negative matrix score, which indicates a nonconservative replacement for transmembrane proteins

**FIGURE 4** Circumpolar distribution of the five major phylogeographical lineages observed in Arctic char, based on data from the present study and previously published data (Alekseyev et al., 2009; Brunner et al., 2001; Esin et al., 2017; Moore et al., 2015; Salisbury et al., 2019; Taylor et al., 2008). The lineages are represented by different colours, as shown in the legend to the right. The exact populations types within the contact zone in Labrador, Canada, is also shown

**TABLE 2** $F_{ST}$ between Arctic char (*Salvelinus alpinus*) populations

| | QAAN–1 | UUMM–2 | DISK–1 | SISI–1 | MANI–1 | NUUK–1 | QAQO–2 | SCOR–1 | ICEL–1 | NORW–2 |
|---|---|---|---|---|---|---|---|---|---|---|
| QAAN–1 | - | - | - | - | - | - | - | - | - | - |
| UUMM–2 | **0.24339** | - | - | - | - | - | - | - | - | - |
| DISK–1 | **0.62973** | **0.63099** | - | - | - | - | - | - | - | - |
| SISI–1 | **0.41895** | **0.42075** | −0.04053 | - | - | - | - | - | - | - |
| MANI–1 | **0.11368** | **0.11897** | **0.28532** | 0.08173 | - | - | - | - | - | - |
| NUUK–1 | **0.42223** | **0.46072** | **0.63062** | **0.42504** | **0.14432** | - | - | - | - | - |
| QAQO–2 | **0.14029** | **0.14495** | **0.25859** | 0.04973 | −0.08994 | **0.16603** | - | - | - | - |
| SCOR–1 | **0.97333** | **0.97397** | **0.36546** | **0.42175** | **0.74662** | **0.96982** | **0.70875** | - | - | - |
| ICEL–1 | **0.97512** | **0.97571** | **0.38961** | **0.44123** | **0.76033** | **0.97182** | **0.72412** | **0.29939** | - | - |
| NORW–2 | **0.99180** | **0.99240** | **0.40097** | **0.45720** | **0.77598** | **0.98826** | **0.74211** | **0.50770** | **0.48399** | - |

*Note.:* Bold values are significant ($p < .05$).

and hence the possibility for structural change at the protein level. All occurred in the terminal part of the tree and was not shared amongst different haplotypes (Table S3).

McDonald–Kreitman tests found no evidence for positive selection across the Arctic and Atlantic lineages. However, a significantly higher *dN/dS* was observed within the lineages than interlineage

**FIGURE 5** Maximum-likelihood tree showing the genetic relationships between all mitogenome haplotype sequences and mapping all nonsynonymous substitutions in the phylogenetic tree. Direction of change is inferred by using brook trout as an outgroup and visualised along the respective branches by coloured bars. Each bar represents a mutation with the colour representing a gene and the adjacent number denoting the codon position. The total number of nonsynonymous changes found in each gene either residing in the terminal part of the phylogeny or fixed between the Atlantic–Acadian and Arctic Clades are shown in the table in the lower right corner. Hash (#) denotes the existence of mutations that have occurred independently multiple times in the tree. Sample names are shown for each haplotype

*dN/dS* (Table S4), supporting relaxed purifying selection. The same pattern was observed for the CODEML tests conducted in PAML. Here, the models with two or four different rates of dN/dS outperformed the model with only one rate. The four rate model allowing different *dN/dS* between all three lineages showed the best fit (Table S5). In this model, *dN/dS* was higher in all three lineages (0.1033–0.5808) than the between lineage rate (0.0370).

Finally, the codon-specific analyses showed that signatures of possible selection within each of the 13 coding genes were dominated by purifying selection (Table 3). Only one site was found to be under potential diversifying (positive) selection by the FUBAR test. This site was detected in base position 564 (codon 188) of the ND1 gene within a predicted transmembrane domain (Table S6). This mutation leads to the replacement of the amino acid alanine with threonine, two amino acids that have highly similar physiochemical

properties. The mutation was one of the two mutations that had independently occurred within the same position (Figure 5; Table 3).

## 4 | DISCUSSION

This study represents the first investigation of phylogeography in Arctic char based on mitogenome sequencing that closes an important gap in our knowledge of the circumpolar phylogeographic structure of the species by including several sample locations from Greenland. We found a broad contact zone in western Greenland involving Arctic and Atlantic phylogeographical lineages, which roughly is geographically parallel to a contact zone involving the same lineages observed in Labrador (Salisbury et al., 2019). We furthermore identified two subclades within the Atlantic lineage,

**TABLE 3** Results of the three analyses of selection within each of the 13 coding genes

| Gene | Number of haplotypes | Length in codons | FUBAR | | MEME |
| | | | Diversifying (Positive) selection | Purifying (Negative) selection | Episodic (positive) selection |
| --- | --- | --- | --- | --- | --- |
| ATP6 | 6 | 227 | 0 | 7 | No |
| ATP8 | 2 | 55 | NA | NA | NA |
| COX1 | 8 | 516 | 0 | 10 | No |
| COX2 | 7 | 230 | 0 | 6 | No |
| COX3 | 6 | 261 | 0 | 10 | No |
| CYTB | 8 | 380 | 0 | 15 | No |
| ND1 | 9 | 324 | 1[a] | 6 | No |
| ND2 | 12 | 349 | 0 | 9 | No |
| ND3 | 5 | 116 | 0 | 1 | No |
| ND4 | 11 | 461 | 0 | 12 | No |
| ND4L | 4 | 98 | 0 | 0 | No |
| ND5 | 15 | 612 | 0 | 22 | No |
| ND6 | 8 | 173 | 0 | 7 | No |

*Notes.*: NA =not available as the alignment of all ATP8 sequences showed too little variation for analysis of selection (<3 haplotypes).
[a]Codon 188.

suggesting more complex dynamics than a simple dual postglacial recolonisation scenario of Arctic/Atlantic lineages. Moreover, estimated divergence time between the Arctic and Atlantic lineages was approximately 400,000 years, suggesting that important evolutionary divergence could also have built up across the nuclear genome over this time scale. Finally, we found little evidence for positive selection acting on the mitogenome of Arctic char. We therefore assume that observed mitogenome variation reflects neutral processes, but admixture (i.e., interbreeding) between divergent lineages, as indicated by their mitogenomes, could be an important factor influencing nuclear genomic variation and the populations' adaptability in the face of anthropogenic climate change.

## 4.1 | Distribution of phylogeographic lineages

A previous study of Arctic char documented the presence of both Arctic and Atlantic lineages in western Greenland, although this was based on the analysis of only one landlocked and one anadromous population (Brunner et al., 2001). All haplotypes in the anadromous population were from the Arctic, and all haplotypes in the landlocked population were from the Atlantic lineage. The results of our study show that this is not a general pattern. Although no landlocked populations were included from western Greenland, four of the seven anadromous populations exhibited both Atlantic and Arctic haplotypes, whereas the remaining three exhibited exclusively Arctic haplotypes. When considering our results along with the circumpolar distribution of phylogeographic lineages, a picture emerges of northwestern Greenland populations being dominated by or fixed for the Arctic lineage, whereas central and

southwestern Greenland populations represent a zone of secondary contact between lineages that extends for at least 1,000 kilometres. Whether or not this contact zone also encompasses parts of eastern Greenland cannot be resolved by our study. However, at least the northeastern SCOR-1 population and the populations from Iceland (ICEL-1) and Norway (NORW-1) were fixed for Atlantic haplotypes, and in general, only Atlantic haplotypes have been reported from Europe (Figure 4).

A parsimonious interpretation of the results would suggest expansion after the Last Glacial Maximum of the Arctic lineage from Atlantic Canada via northwestern Greenland towards the south, and expansion of the Atlantic lineage from Europe, possibly via Iceland to eastern Greenland and subsequently from southwestern Greenland towards the north. The latter expansion could also have led to the introduction of the Atlantic lineage in Labrador and Newfoundland. However, the two subclades found within the Atlantic lineage have a divergence time of ca. 60,000 years, which suggests that the Atlantic lineage has a more complex phylogeographical history than the aforementioned scenario of expansion and secondary contact. Nearly all Atlantic haplotypes in midwestern Greenland, that is, DISK-1, SISI-1 and MANI-1, belonged to Sub-clade 1, which was conspicuously absent from QAQO-2, SCOR-1, ICEL-1 and NORW-1. Atlantic haplotypes in the latter populations all belonged to Sub-clade 2. It is possible that the two sub-clades represent two separate colonisation events of western Greenland by the major Atlantic lineage. In this case, Sub-clade 1 could represent an earlier recolonisation by Arctic char, which persisted and diverged in a cryptic refugium. Geological evidence does not suggest a large ice-free area in this part of Greenland during the last glaciation (Funder et al., 2011), but the presence of cryptic refugia along the eastern coast of Canada

has been suggested by Moore et al. (2015). Simultaneous recolonisation from two different refugial areas in Europe represents an alternative possibility. Indeed, the haplotype ATL20, which is most closely related to Sub-clade 1 based on D-loop sequences, is derived from Loch Rannoch, Scotland (Moore et al., 2015), and was furthermore not observed in eastern Canada (Salisbury et al., 2019), thus providing some support for the possibility that Sub-clade 1 diverged in Europe, rather than Greenland or eastern Canada.

The populations analysed in western Greenland were all anadromous and therefore in principle subject to gene flow. Yet, despite the fact that most of the populations in the secondary contact zone harbour both Arctic and Atlantic haplotypes, one population (NUUK-1) appears to be fixed for Arctic lineage haplotypes. This raises the general question whether co-occurrence of lineages within most populations also reflects admixture at the genomic level, or if the finding of a single lineage within NUUK-1 could indicate a mosaic of populations representing different lineages. Demonstration of admixture (or not) cannot be resolved by mtDNA data alone. However, results based on 53 nuclear SNPs from 16 populations of anadromous Arctic char from western Greenland, including NUUK-1, showed pronounced isolation by distance amongst populations, and spatial autocorrelation analysis suggested connectivity amongst populations on a geographical scale encompassing hundreds of kilometres (Madsen et al., 2020). The observed heterogeneity at the nuclear level does not suggest co-occurrence of the two lineages, but instead suggests that admixture has taken place. Moreover, another study analysing contemporary and historical SNP variation (the latter based on DNA from otoliths collected in the 1950s) showed lower effective population size ($N_e$) in the NUUK-1 population (point estimate of $N_e$ 188) than other populations ($N_e$ from 538 to 610) (Christensen et al., 2018). As random genetic drift is stronger at mtDNA than nuclear loci (Ballard & Whitlock, 2004), then genetic drift alone may explain the reduced variation and fixation of the Arctic lineage in NUUK-1.

As a whole, the finding of divergent phylogeographic lineages of Arctic char in western Greenland adds to the number of reports of secondary contact in aquatic organisms within the sub-Arctic and Arctic regions. Other examples include threespine stickleback (*Gasterosteus aculeatus*) (Liu et al., 2016), blue mussel (*Mytilus* spp.) (Mathiesen et al., 2017) and kelp (*Saccharina latissima*) (Neiva et al., 2018). This region, likely also encompassing Labrador in eastern Canada, may therefore constitute a general suture zone (Hewitt, 2000; Swenson & Howard, 2005) with multiple phylogeographic breaks and hybrid zones across different organisms.

## 4.2 | Secondary contact between the Atlantic and Arctic lineages

Divergence time estimates between lineages were obtained using a mitogenome substitution rate of $1.537 \times 10^{-8}$ sub/site/year, which was estimated in European whitefish (*Coregonus* sp.) based on evidence of population expansion 13,000 years BP (Jacobsen et al.,

2012). This rate is somewhat higher than other estimated substitution rates for salmonid mtDNA based on older divergence events (e.g., $1 \times 10^{-8}$ sub/site/year; Smith (1992)) and may indicate time dependency (Ho et al., 2005, 2011). The use of this rate was supported in European whitefish, based on the finding of relaxed purifying selection in the terminal branches (Jacobsen et al., 2012). In the present study, evidence of relaxed purifying selection was also observed as *dN/dS* was significantly higher within lineages than the between lineage rate. Nonetheless, the estimates of deeper evolutionary events may be underestimated and hence should be considered with caution.

Using a mutation rate estimate of 5%–10% divergence per million years, Moore et al. (2015) estimated that the Arctic lineage diverged from the Beringian lineage between 716,000 and 1,432,000 years BP. Our estimate of divergence time between the Arctic and Atlantic lineages is more recent, likely coinciding with the onset of the Saale slaciation 391,788 BP (95%HPD 334,191–454,938 BP). The divergence time between the Acadian and the Atlantic lineage was estimated to be 106,195 BP (95% HPD 80,736–131,033) within the onset of the last glacial cycle. Both estimates overlap with known glacial periods with expected low amounts of detected deuterium in the Vostok data. This suggests an allopatric origin of evolution of the lineages and indicates that the glacial cycles had a key role in shaping the observed phylogenetic pattern in Arctic char. What are the consequences, if any, of secondary contact between the highly divergent Arctic and Atlantic lineages? Not surprisingly, nucleotide diversity was higher for populations harbouring mitogenomes from both lineages than populations exhibiting only Arctic or Atlantic mitogenomes. It is doubtful, however, if this has any direct adaptive consequences. In fact, of the 40 nonsynonymous substitutions, only one (codon 188 in ND1) evolved under possible diversifying selection. The mutation led to the replacement of the amino acid alanine with threonine in two independent places within the Acadian and Arctic evolutionary lineages (Figure 5). Although it cannot be excluded that the mutation is fixed in the Acadian lineage as it was only represented by one sequence, the mutation was located in the terminal part of the phylogeny for the Arctic linage pointing to a recent event. Further, the amino acid replacements have highly similar physiochemical properties compared with the ancestral one, and the mutation was observed within a transmembrane domain, which is in general believed to be of less overall importance for protein function (da Fonseca et al., 2008; Jacobsen et al., 2016). In addition, the three replacements that exhibited a negative matrix score occurred in the terminal part of the tree. Hence, although these mutations may be under positive selection, we find it more likely to be the outcome of relaxed purifying selection, leading to amino acid replacements of a weak deleterious (or neutral) effect (Ho et al., 2005, 2011; Jacobsen et al., 2016). Although some studies have identified positive selection acting on the mitogenome in fish, this has usually been attributed to metabolic efficiency, which can be associated with differences in migratory behaviour (Consuegra et al., 2015; Gagnaire et al., 2012a; Garvin et al., 2011; Jacobsen et al., 2015). Geographical differences in seasonal cycles of sea surface temperature have been

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documented in western Greenland, which translate into differences of time windows available for feeding migrations in the sea. This may pose important differences in selection regimes for anadromous char populations (Madsen et al., 2020), but mitogenome variation as analysed here seemingly does not play a role in adaptation to differences in sea surface temperature.

Indirectly however, the ancient divergence time of the two lineages implies that significant differences must have accumulated at the nuclear genomic level. Following secondary contact, this would be expected to lead to high nuclear genomic variation as compared with populations descending exclusively from a single lineage. This is supported for Greenland Arctic char as analysis of 53 nuclear SNPs shows considerable genetic differentiation between northwest Greenland (QAAN-1) and east Greenland, Norway and Iceland (Madsen et al., 2020), which are fixed for either Arctic or Atlantic mitogenome haplotypes. High $F_{ST}$ values could also reflect very long geographical distances (> 4000 km waterway distance), effectively isolating the populations. Moreover, heterozygosity is higher for southwestern- and midwestern Greenland populations than for northwest Greenland (QAAN-1) and east Greenland, which only harbour one evolutionary lineage. There is growing appreciation that evolutionary adaptive responses largely rely on standing genetic variation, rather than *de novo* mutations (Barrett & Schluter, 2008; Jones et al., 2012; Lai et al., 2019). In principle, therefore, populations resulting from secondary contact could have more opportunities for responding adaptively to rapid environmental change, such as contemporary climate change. It would consequently be of considerable interest to analyse and compare genome-wide nuclear variation in Arctic char populations within and secondary contact zones in Greenland and Labrador and Newfoundland (Salisbury et al., 2019).

Admixture of highly diverged nuclear genomes could also introduce genomic incompatibilities that could interact with natural selection at other loci and contribute to the establishment of hybrid zones and sympatric divergent morphs (Bierne et al., 2011). For example, in lake whitefish (*Coregonus clupeaformis*) from North America, secondary contact between phylogeographic lineages plays an important role in the evolution of sympatric morphologically divergent morphs (Rougeux et al., 2017). Arctic char is renowned for its morphological variation, with several divergent morphs often observed within the same lakes (Jonsson & Jonsson, 2001; Kapralova et al., 2011; Knudsen et al., 2006; Salisbury et al., 2018). However, although the study by Salisbury et al. (2018) included morphologically distinct sympatric populations in a secondary contact zone between the Arctic and Atlantic lineages, there was no association between morphs and mitochondrial lineages. Also, genome-wide analysis of pairs of sympatric morphs from some of these systems did not suggest parallelism at the genomic level (Salisbury et al., 2020). Other renowned cases of sympatric morphs in Lake Thingvallavatn, Iceland, and Lake Fjellfroskvatn, Norway (Kapralova et al., 2011; Knudsen et al., 2006), are found in regions where only the Atlantic phylogeographic lineage has been observed (see Figure 4). Morphologically divergent morphs

of Arctic char have been observed in several lakes in west and east Greenland (Riget et al., 2000). The secondary contact zone in western Greenland therefore poses interesting possibilities for future analysis of sympatric morphs, and comparison to systems in east Greenland (where we assume only the Atlantic lineage is present) could provide a direct assessment of the possible role of mixture of phylogeographic lineages in morphological evolution.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Genbank at https://www.ncbi.nlm.nih.gov/genbank/ (reference number MT880631-MT880724).

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.