A Phylogeographic Contact Zone for Arctic Grayling (Thymallus arcticus) in Alberta, Canada

Jessica R. Reilly, Joshua M. Miller

A phylogeographic contact zone for Arctic Grayling *Thymallus arcticus* in Alberta, Canada

Jessica R Reilly[1], Joshua M Miller[2]

[1]Alberta Environment and Parks, Government of Alberta, Cochrane, AB, Canada

[2]Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada

Running Title: Phylogeography of Arctic Grayling in Alberta

Abstract

Arctic Grayling *Thymallus arcticus* are a salmonid with a Holarctic distribution, extending from north-eastern Eurasia through north-western North America. Throughout their range, Arctic Grayling face a number of threats including angling mortality, habitat fragmentation and loss and climate change. Thus, there is a need to protect the species through targeted management actions. Genetic information can assist in determining the appropriate scale for these actions through description of Designatable Units (DUs). Here we use newly collected mitochondrial DNA sequence data to assess the phylogeographic structure of Arctic Grayling in Alberta, Canada and link these with previously collected mitochondrial and microsatellite data to determine how many DUs may exist across Canada. Our assessment of 831 base pairs of sequence data in 96 individuals found two deeply divergent lineages in Alberta. When combined with 22 previously collected sequences our results highlight that Alberta is a contact zone for the observed lineages of Arctic Grayling in North America. Reassessment of nine microsatellites genotyped in 1,116 individuals further highlighted inter-basin divergence, likely the result of historical processes. Given the divergence and geographic distribution of the genetic diversity, Arctic Grayling in Canada merit consideration for separate DUs in future species status assessments and management plans. Continuing research should aim to expand sampling geographically (e.g. regions east of Great Slave Lake and along the Arctic coastline) to clarify possible colonization routes, and add to or synthesize work on Arctic Grayling behaviour, morphology, and life-history to address the limited understanding of local adaptions within this species.

Introduction

There is broad agreement on the need to preserve intraspecific variation and protect evolutionary potential in the face of changing environments (Sgro et al. 2011; Eizaguirre and Baltazar-Soares 2014; Funk et al. 2019). Since the inception of the evolutionarily significant unit concept (Ryder 1986), conservation-based decisions and legislation are now often structured around groups below the species level (e.g., U.S. Endangered Species Act (U.S. Fish and Wildlife Service 2020), Canada Species at Risk Act (SARA; Government of Canada 2016). Within Canada,

provisions for subspecies, populations, or population components can be made if they are deemed discrete and evolutionarily significant (COSEWIC 2018). Such Designatable Units (DUs) allow a pragmatic approach to describing and protecting biodiversity, and enable monitoring and conservation actions at a smaller more manageable scale, rather than across an entire species range.

Mee and colleagues (2015) proposed a process to identify DUs in Canada to avoid potential subjectivity or inconsistency. Their decision tree consisted of four hierarchical criteria: named taxonomic entity or reproductive isolation, phylogenetic distinctness, local adaptation, and presence in different eco-geographic regions. Given that phylogenetic data is a key consideration in the delineation (i.e., number and spatial extent) of DUs there is a need for genetic information on species at risk in Canada.

Here we use newly collected mitochondrial DNA (mtDNA) sequence data to assess for the presence of phylogeographic structure of a fish species of conservation concern, the Arctic Grayling *Thymallus arcticus*. In North America, Arctic Grayling occur in drainages from Hudson Bay to Alaska, including the Northwest Territories, Yukon, and Nunavut, as well as northern areas of British Columbia, Alberta, Saskatchewan and Manitoba. In addition, a disjunct population is present in Montana, USA, and the species was once found in Michigan, USA (extirpated in 1936; Scott and Crossman 1998). Arctic Grayling face a number of threats including angling mortality, habitat fragmentation and loss due to land use changes, as well as climate change (Federal Registrar 2014; Cahill 2015). Throughout their range, many populations are considered secure, while others have collapsed or been extirpated (Northcote 1995; Behnke 2002). For example, there has been a marked decline in Arctic Grayling abundance in Alberta, with a recent estimate of up to 70% reduction since the 1960s (Cahill 2015).

Despite concerns around localized population losses, Arctic Grayling do not currently have an at-risk status under SARA. However, in 2019, the Freshwater Fishes Sub-committee of the Committee on the Status of Endangered Wildlife in Canada considered the populations in the Western Arctic eco-geographic region, including those in Alberta, as having the highest priority for a full status assessment due to the risk of extinction (COSEWIC 2020; Accessed March 2020). Status assessments of Arctic Grayling outside of the Western Arctic region are not

currently a priority (COSEWIC 2020; Accessed Mar 2020). The Western Arctic region is over 1.8 million km^2 (i.e., 20% of Canada (Rosenberg International Forum on Water Policy 2013)) and includes the Mackenzie River basin and other drainages into the Arctic Ocean, excluding the Arctic archipelagos. This large area encompasses declining Arctic Grayling populations at southern latitudes (e.g., Lashmar and Ptolemy 2002; Cahill 2015) and secure populations in the north (e.g., Working Group on General Status of NWT Species 2016). Different status designations at the federal level could be applied that are more reflective of these local conditions if there was adequate support (e.g. Mee et al. 2015) to divide the Western Arctic region into smaller DUs. Smaller management units within DUs can also be established by other management agencies, such as provincial and territorial governments, although they would lack the protections enabled under SARA.

There have been recent assessments of mtDNA variation of Arctic Grayling in North America that could help inform DU designations, but information gaps still exist. Redenbach and Taylor (1999) assessed sequence data and restriction fragment length polymorphisms (RFLP) and identified two potential refugia for Arctic Grayling during the Wisconsinan Ice Age: the Beringia Refugium north of the ice sheets and a southern refugium located in the upper Missouri River system or southern Alberta. These authors also postulated that Arctic Grayling may have survived in the Brooks Range Refugium, Nahanni River Refugium, and Mississippi Refugium based on the contemporary species range (Crossman and McAllister 1986), fossil evidence (Miller et al. 1993), and/or morphological differences (McCart and Pepper 1971), but results were inconclusive and additional sampling was recommended (Redenbach and Taylor 1999). Further analysis of mtDNA diversity was conducted by Stamford and Taylor (2004) at 31 localities distributed throughout the western portion of the species' range. These authors described three major lineages originating from three areas: a Nahanni lineage from the Nahanni River Refugium (Group A), a North Beringia lineage from the Brooks Range Refugium (Group B), and a South Beringia lineage from the Yukon River Valley Refugium (Group C). Their findings also suggest that a disjunct population belonging to the Group B lineage may have survived the last glaciation in a refugium located in the upper Missouri River system or southern Alberta, which eventually gave rise to current-day populations in Montana and

Saskatchewan. Notably, these previous studies did not sample a large north-eastern portion of the range (including Nunavut and parts of the Northwest Territories), nor the watersheds of Alberta that represent the southernmost extent of extant Arctic Grayling (omitting the disjunct population in Montana). Thus, new genetic data from Alberta Arctic Grayling would address an information gap for this recreationally and culturally important species and specifically inform DU delineation within the Western Arctic region. In addition, genetic assessment of populations in the province could provide insights on post-glacial colonization routes of freshwater fish species because Alberta is a possible contact zone for all extant North American lineages of Arctic Grayling.

The intention of this study is to compare and expand on the previous genetic assessments, fill in missing information on Arctic Grayling populations in Alberta and review the delineation of DUs across Canada. Specifically, our first objective is to determine the number and spatial extent of genetic lineages of Arctic Grayling in the province using new mtDNA sequence information. Second, we leverage previously collected data on mtDNA and microsatellite variation to better understand possible post-glacial colonization routes across North America. In terms of microsatellite variation, range expansion after glaciation is expected to leave predicable signals in the genome (Hewitt 1999, 2000), with variation highest closer to refugia, and then decreasing with distance along the expansion front due to successive founder effects. Finally, we place these results in the context of possible DU designations following the decision tree proposed by Mee and colleagues (2015), with a special focus on the Western Arctic region.

Methods

Sample collection

The study area was located in northern Alberta, Canada. Arctic Grayling were collected at 15 sites, including four sites in the Athabasca River basin, five sites in the Peace River basin and five sites in the Hay River basin (Table 1) by Reilly et al. (2014) or the provincial government of Alberta using angling or backpack electrofishing from May to September, 2007 – 2012. There are no known movement barriers between sites. We assumed that Arctic Grayling in this study

represented both sexes and different age classes based on the range in length (64mm to 402mm). Tissue samples consisted of dried pelvic fin clips and scales stored at room temperature, or of adipose and pelvic fin clips stored in 95% ethanol at -20°C.

DNA extraction, PCR, alignment, and quality trimming

Total genomic DNA was extracted using Qiagen DNeasy Blood and Tissue Kits following the manufacturer's protocol. A fragment of the mtDNA Dloop and cytochrome B gene was amplified via PCR using either the primers CLeu3-F (5'-GGAACCAAAAACTCTTGGTGCAACTC-3'; (Bernatchez and Osinov 1995)) with CGlu-R (5'-GACTTGAAGAACCACCGTTG-3'; (Park et al. 1993)), or DloopCytB-IntF (5'-TTTGTAGCCATGCTTCTAGGC-3') with DloopCytB-IntR (5'-TGCGACTCTCGATACTTGTCC-3'). PCR products were cleaned with Exo/Sap, prepared for sequencing using BigDye version 3.1, and then sequenced in both the forward and reverse direction on an ABI 3730 DNA Analyzer at the Molecular Biology Services Unit at the University of Alberta. Sequence chromatograms were aligned in Geneious (version 11.1.5) using the MAFFT algorithm (version 7.450; (Katoh and Standley 2013)). Alignments were then inspected by eye and per individual consensus sequences generated. Finally, a global alignment across all individuals was generated using the MAFFT algorithm and exported for analyses.

Sequence analyses

We first constructed a rooted phylogenetic tree using IQ-tree (version 1.6.10; Nguyen et al. 2014) implemented on the Cipres Science Gateway (Miller et al. 2010). IQ-tree uses a maximum likelihood algorithm for tree construction, and we employed 1000 bootstrap replicates to assess robustness of the inferred relationships. In this analysis a sequence from *Thymallus baicalolenensis* is the outgroup (GenBank Accession KY078221.1) as this species was recently shown to be the closest sister taxa to *T. arcticus* (Weiss et al. 2021). We then assessed relationships among the newly generated sequences using a median-joining haplotype network generated in PopArt (Leigh and Bryant 2015) using TCS algorithm (Clement et al. 2000) with default settings.

We calculated sequence diversity statistics (number of haplotypes, haplotype diversity, and nucleotide diversity) using DnaSP (version 5.10.01; Librado and Rozas 2009) and average divergence between the two main genetic lineages discovered (see Results) with MEGA X (Kumar et al. 2018; Stecher et al. 2020). For the latter, test significance was assessed with 500 bootstrap replicates. We conducted analyses of molecular variance (AMOVA) using Arlequin (version 3.5.2.1; Excoffier and Lischer 2010) to see how variation was partitioned among groups of individuals. For both the diversity statistics and AMOVA analyses we considered two groupings of our individuals: 1) sampling sites nested within basins, and 2) sampling sites nested within the two main genetic lineages discovered (see Results).

We integrated our new sequences with previously collected mtDNA sequences for North American Arctic Grayling. This included those collected by Stamford and Taylor (2004) (N = 14; GenBank accession numbers AY528426 - AY528439), three sequences from Koskinen et al. (2002) (accession numbers AY168400 - AY168402), and five unpublished sequences for Arctic Grayling collected in Alaska (accession numbers KT630692- KT630696), for a combined dataset of N = 118 sequences. Following the methods outlined above, we aligned the new sequences to ours in Geneious then created an additional haplotype network with this expanded dataset.

Microsatellite diversity

Using data from Reilly et al. (2014) we examined geographic patterns of microsatellite diversity. In their work, Reilly et al. (2014) genotyped 1,116 Arctic Grayling captured from 40 sites in the Hay, Peace, and Athabasca River basins at nine microsatellite loci to examine fine-scale population structure within basins. Using this dataset we first assessed broad-scale genetic groups among the basins using the program STRUCTURE version 2.3.4 (Pritchard et al. 2000; Falush et al. 2003). STRUCTURE implements Bayesian clustering analysis, which assumes a model with K unknown clusters representing genetic populations, and then assigns individual ancestry among clusters based on allele frequencies. We ran ten repetitions for K = 1 – 13, with a burn-in of 100,000 iterations and MCMC length of 200,000 iterations. These runs used the admixture model, correlated allele frequencies among populations, and did not assume prior population information. All other parameters were left at default values. Results were post-

processed using CLUMPAK (Kopelman et al. 2015) and the ΔK statistic (Evanno et al. 2005) was used to infer the best K. We then focused on the 29 sites from the Athabasca River basin (N = 880 individuals) and examined how allelic richness of each sample site varied with latitude and longitude. Allelic richness values were taken from Reilly et al. (2014) where values had been standardized by sample size using HP-Rare version 1.0 (Kalinowski 2005). Specifically, using the R statistical suite (version 3.6.0; R Core Team 2019) we fit two weighted linear regression models with per-site allelic richness as the dependant variable, and either latitude or longitude as the independent variable. In both models, weights corresponding to the number of samples at each site. We did not consider a single model which incorporated both latitude and longitude as the two are highly correlated due to the locations of the sample sites (Pearson's product-moment correlation = 0.674, t = 4.7352, DF = 27, p-value = 6.206×10^{-5}).

Results

After alignment and quality trimming we were able to obtain 831 base pairs (bp) of sequence data for 96 individuals. These sequences have been deposited on GenBank with accession numbers MT212424-MT212519 [embargoed until publication]. The maximum likelihood phylogenetic tree showed two divergent clades within Arctic Grayling (Figure 1A). Within one of these clades, two subclades can be seen, however the bootstrap support separating them was low (66%). The two divergent clades were also recovered in the haplotype network, separated by 18 mutations (Figure 1B). One clade, which we will call the Nahanni lineage, was represented by a single haplotype. The other clade, which we will call the Beringia lineage, contained five haplotypes separated by up to four mutations, but with two haplotypes representing the majority of samples (Table 2). Average pairwise divergence between the two lineages was 2.2% ± 0.64% (mean ± SD).

Samples from the same location may have both Nahanni and Beringia haplotypes (Figure 2, Table S.1). However, the Nahanni haplotype was restricted to locations in the Hay River and Peace River basins. When grouped by sampling sites within basins, the AMOVA showed that the majority of variation was within sampling sites followed by among basins and then among sampling sites within basins. However, when sampling sites were grouped within

lineages, nearly all variation was attributed to the lineages not among or within sampling sites (Table 3). We note though that differences in sample sizes among sites, as well as the presence of only a single haplotype within the Nahanni lineage, influences the variance of these diversity statistics.

The 22 previously collected mtDNA sequences overlapped at 438 bp with our samples from Alberta. Despite the reduced sequence length, we still resolved two divergent genetic lineages (Figure S.1). In this dataset, Arctic Grayling from Alberta contained four haplotypes, of which three were shared with those previously found. One lineage was represented by a single haplotype shared by Alberta Arctic Grayling and a sequence from Stamford and Taylor (2004). The second lineage contained nine haplotypes separated by at most four base-pair changes. Here, three haplotypes were found in our newly sequenced individuals with one rare haplotype unique to Alberta Arctic Grayling and the two most common haplotypes shared with those previously found. Three main patterns were observed after examining the geographic distribution of the haplotypes from this combined analysis (Figure 3): 1) concordance of the Nahanni lineage (i.e., Group A haplotypes) from Stamford and Taylor (2004), 2) the majority of Beringia haplotypes we observed in the Hay River and Peace River basins clustered with those from 'Group C' of Stamford and Taylor (2004), and 3) haplotypes found in the Athabasca River basin clustered with those of 'Group B' from Stamford and Taylor (2004) (Table S.1).

Initial assessment of inter-basin population structure using the microsatellite data from Reilly et al. (2014) strongly supported K = 2 (Table S.2), which separated the Athabasca River basin from the Hay River and Peace River basins (Figure 4). However, it is known that the program STRUCTURE can be biased towards selection of K = 2 even when there is additional genetic structure within the dataset (Janes et al. 2017; Cullingham et al. 2020). Therefore, we looked for evidence of hierarchical genetic structure within the Peace River and Hay River basins by analyzing those basins separately for K = 1 - 4, and keeping all run parameters the same as for the complete dataset. Again, K = 2 was heavily supported (Table S.3), with both sites in the Hay River basin clustering together with two sites in the lower Peace River basin. However, at K = 3 the two sites in the Hay River basin differentiate from the two genetic clusters in the Peace River basin (Figure 4). Within the Athabasca River basin, microsatellite

allelic richness was strongly correlated to latitude and longitude, being higher in more northeastern locations (Figure S.2; Pearson's product-moment correlation to latitude = 0.667, t = 4.6513, DF = 27, p-value = $7.774e^{-05}$, Pearson's product-moment correlation to longitude = 0.408, t = 2.3197, DF = 27, p-value = 0.0282).

Discussion

Major phylogeographic lineages of Arctic Grayling

In this study we used mitochondrial DNA sequence variation to examine divergence and biogeographic patterns of Arctic Grayling in Alberta, Canada. Our first objective was to enumerate and describe the distribution of distinct lineages of Arctic Grayling in the province. We observed two deeply divergent lineages we called Nahanni and Beringia (Figure 1) to reflect their putative refugial origins during the Wisconsin Ice Age. Fish belonging to the Nahanni lineage were found throughout the Hay River basin and at one sampling locality in the Peace River basin (Figure 2). Beringia lineage fish were primarily distributed throughout the Athabasca River and Peace River basins, with a few located in the Hay River basin.

The observation of two major lineages matches a previous phylogenetic assessment (Weiss et al. 2006), but differs from the interpretation of Stamford and Taylor (2004). These authors identified three major lineages: one that is concordant with the Nahanni lineage we describe, and two lineages, rather than one, which originated within the Beringia Refugium. This additional split was only weakly supported across the analyses considered (Stamford and Taylor 2004) and while our phylogenetic tree also showed two subclades within the Beringia lineage, the distinction was attributed to a small number of base pair changes among haplotypes (Figure 1) that may indicate a more recent isolation event. Considering the current evidence, we propose that two lineages best characterize the significant phylogeographic patterns of Arctic Grayling in North America. However, consideration of a larger portion of the mitochondrial genome or use of nuclear markers may better resolve the question of one or two major Beringian lineages. Additional lineages may also be identified upon assessment of individuals from the currently un-sampled north-eastern portion of the species range.

Our results highlight that Alberta is a contact zone for the Nahanni and Beringia lineages of Arctic Grayling. At this point we are unable to conclusively say whether this contact is reflective of historical processes, contemporary ones, or both. There has been limited stocking of Arctic Grayling within the province, most of which has occurred outside of the species range or was within isolated water bodies that are not hydrologically connected to surrounding river systems. For example, there are no known cases of Arctic Grayling being stocked into the Hay River basin (Government of Alberta 2021). Additionally, it is highly unlikely that the two original donor populations used for Arctic Grayling stocking in the province contained individuals belonging to the Nahanni lineage because they are situated in the Athabasca River basin (i.e., Freeman Lake) and the upper Peace River basin (i.e., Beaverlodge River) where Nahanni lineage haplotypes have not been detected. Lastly, the results from the microsatellite-based Bayesian clustering analysis lend weight to contact being the result of historical processes. Specifically, we found the highest level of differentiation distinguished fish from the Athabasca River basin relative to those from the Peace River and Hay River basins. When searching for genetic substructure within the Peace River and Hay River basins, the main signal at K = 2 distinguished locations known or likely to have individuals from the Nahanni lineage. Differentiation between watersheds became apparent only when examining K = 3. These results are similar to the fine-scale assessment of population genetic structure of Arctic Grayling in Alberta conducted by Reilly et al. (2014). Here the authors found distinct populations in Hardy-Weinberg equilibrium within basins.

Post-glacial colonization routes across North America

The broad-scale patterns of mtDNA and microsatellite variation can be used to develop hypotheses of post-glacial dispersal routes (Hewitt 1999, 2000). The Nahanni lineage we observed may have survived glaciation in the Nahanni River valley to later disperse into the Liard, Nahanni, Hay and Peace rivers (Stamford and Taylor 2004). Although we did not find evidence of two deeply divergent lineages originating from Beringia, the geographic distribution of the haplotypes within the Beringia lineage may reflect post-glacial colonization patterns. The majority of Beringia haplotypes we observed in the Hay River and Peace River basins clustered

with the 'Group C' haplotypes of Stamford and Taylor (2004) (Figure 3). This may indicate that Arctic Grayling in these regions are mostly decedents of fish who dispersed through the Yukon River southward into the Peace River via the route described in Stamford (1996). In contrast, the haplotypes found in the Athabasca River basin clustered with the 'Group B' haplotypes that Stamford and Taylor (2004) predominately sampled in Montana, northern Saskatchewan and northern Alaska. It remains unclear if the Athabasca River basin was colonized by fish that dispersed through glacial lakes (e.g., Lake Agassiz) from a Missouri River refugium (Redenbach and Taylor 1999), by Arctic Grayling travelling along the Arctic coastline and then into inland waters moving east and then south from northern Beringia, or by a mix of these two scenarios. Previous genetic studies on Burbot *Lota lota* (Van Houdt et al. 2005), Lake Trout *Salvelinus namaycush* (Wilson and Hebert 1998), and Northern Pike *Esox lucius* (Skog et al. 2014) provide evidence that both routes were possible. Regardless of a northern origin (i.e., Beringia Refugium) or southern origin (i.e., upper Missouri River/southern Alberta Refugium), our work suggests that colonization into the Athabasca River basin proceeded in a southern direction given that microsatellite diversity declined with decreasing latitude (Figure S.2).

We do not believe that contemporary stocking would influence these hypotheses around post-glacial colonization routes. In the mid-1980s there were three stocking events of Arctic Grayling from the Athabasca River basin into the Peace River basin (Schroeder 1987) that could have resulted in fish with 'Group B' haplotypes occurring in the Peace River basin, specially within Wapiti River and Burnt River (Figure 3; Redenbach and Taylor 1999; Stamford and Taylor 2004). However, we deem it highly improbable that stocking resulted in contemporary admixture between the Beringian groups due to substantial distances (i.e., 150 to over 1000 river kilometers) between the stocked waterbodies and the study locations, the low numbers of stocked fish (i.e., 165 to 1400 fingerlings), and the lack of evidence that stocking resulted in self-sustaining populations (Government of Alberta 2021).

Designatable Units of Arctic Grayling in Canada

Mee et al. (2015) considered four factors in hierarchical sequence when determining the number and spatial extent of DUs. Specifically, they assigned DU status under any of the

following scenarios: 1) a group comprises a taxonomic entity or is reproductively isolated in sympatry from other groups, 2) the group has a unique phylogeographic history, 3) the group has a trait resulting from an independent case of local adaptation, or 4) the group inhabits a different eco-geographic region.

Given the divergence and geographic distribution of the genetic diversity we observed, Arctic Grayling in Canada meet the criteria for separate DU designations as proposed by Mee and colleagues (2015). While Arctic Grayling in North America are part of a single named species, we argue that the strong spatial structure and deep divergence between the Nahanni and Beringia lineages fulfill the criteria of phylogenetic distinctness. The local adaptation criterion is more difficult to assess because we are not aware of research comprehensively investigating regional differences in behaviour, morphology, or life history of Arctic Grayling in Canada. However, the presence of distinct genetic clusters at small spatial scales (Stamford and Taylor 2005; Peterson and Ardren 2009; Reilly et al. 2014) suggest that local adaptations could have developed (Savolainen et al. 2013; Blanquart et al. 2013; Hoban et al. 2016) and previous studies provide clues of which aspects of Arctic Grayling biology may be the targets of such adaptations. For example, there is evidence of two morphological phenotypes (i.e., large-scale and small-scale forms) from Alaska (McCart and Pepper 1971; Reed and Mccann 1973) and there may be physiological and behavioural adaptations in terms of salinity tolerance (Blair et al. 2016; Heim et al. 2016), thermal tolerance (LaPerriere and Carlson 1973; Lohr et al. 1996; Blair et al. 2016) and inter-annual site fidelity to summer feeding areas (Buzby and Deegan 2000). Lastly, when considering the final criterion, the species is found in four eco-geographic regions: the Pacific, Yukon, Western Hudson Bay, and Western Arctic. Phylogenetic history of fish from the Western Hudson Bay region is currently unknown. Considering the available information on Arctic Grayling and following the process proposed by Mee et al. (2015), there is support for five DUs: DU1 (Pacific Region), DU2 (Yukon Region), DU3 (Western Hudson Bay Region), DU4 (Western Arctic Region – Beringia lineage) and DU5 (Western Arctic Region – Nahanni lineage).

Additional DUs may be appropriate should future research reveal evidence of finer scale structure or local adaptations. In Canada, DU assignment during species assessments is

ultimately the decision of the Committee on the Status of Endangered Wildlife in Canada, which may consider other information and guidelines outside of the Mee et al. (2015) process. In addition, given that both the Nahanni and Beringia lineages of Arctic Grayling are present at several locations within and outside of Alberta it is possible that the lineages may interbreed with one another. For example, a few locations in the Peace River and Hay River basins show both lineages are present, though we would argue that it is not widespread. However, such contemporary hybridization presents potentially difficult management scenarios as the consequences of hybridization can range from adaptive introgression and hybrid vigor (e.g. Pardo-Diaz et al. 2012; Oziolor et al. 2019), to extinction of parental lineages through hybrid swarm formation (Todesco et al. 2016). Currently there is no consensus on how to treat hybrids in conservation and management including via legislation (Haig and Allendorf 2006; Hamilton and Miller 2016; Kovach et al. 2016; Lind-Riehl et al. 2016; Chan et al. 2019). Therefore, each case will need continued monitoring and consideration on how to best address hybrid individuals.

Conclusion

In the face of the diverse threats impacting Arctic Grayling (Federal Registrar 2014; Cahill 2015) partitioning the species into multiple DUs could help to better understand local population status and focus conservation actions where the species is most at risk. Going forward, additional samples, both in terms of regions of the genome as well as physical locations would help provide more evidence for colonization patterns and delineation of DUs (Funk et al. 2012; Coates et al. 2018; Barbosa et al. 2018). Specifically, samples from the Canadian territories (i.e., east of Great Slave Lake and along the Arctic coastline) would clarify possible colonization routes for the Beringia and Nahanni lineages and help to delineate the spatial extent of the Nahanni lineage. We also recommend targeted research and a synthesis of published works on regional differences in Arctic Grayling behaviour, morphology, and life history characteristics, as well as further investigation of the genomic basis of such traits, to address the limited understanding of local adaptions within this species. This would improve

387 the current weight of evidence approach when evaluating the discreteness and evolutionary
388 significance of Arctic Grayling populations, particularly those within the Western Arctic region.
389
390 **Acknowledgements**
391 Financial support for this project was generously provided by Trout Unlimited Canada -
392 Northern Lights Fly Fishers Chapter and a Circumpolar/Boreal Alberta Research grant to JRR.
393 We thank Alberta Environment and Parks for providing samples, Sophie Dang and Dr. Tara
394 Fulton for completing the mtDNA laboratory component, and Dr. David Coltman for providing
395 logistical support for data analyses. We also thank Dr. Catherine Cullingham, Chris Cahill, Dr.
396 Andrew Paul and several anonymous reviewers for providing helpful comments on early drafts
397 of the manuscript. Fish collection was conducted in accordance with guidelines approved by the
398 University of Alberta's Animal Care and Use Committee (Protocol Number 758/09/13) and was
399 permitted under provincial fish research licenses (License Numbers 12-2003 FRL, 12-0437 FRL,
400 and 12-1201 FRL).
401

Literature Cited

Barbosa, S., F. Mestre, T. A. White, J. Paupério, P. C. Alves, and J. B. Searle. 2018. Integrative approaches to guide conservation decisions: Using genomics to define conservation units and functional corridors. Molecular Ecology 27(17):3452–3465. Wiley/Blackwell (10.1111).

Behnke, R. J. 2002. Trout and salmon of North America. The Free Press, New York.

Bernatchez, L., and A. Osinov. 1995. Genetic diversity of trout (genus Salmo) from its most eastern native range based on mitochondrial DNA and nuclear gene variation. Molecular Ecology 4(3):285–298. John Wiley & Sons, Ltd.

Blair, S. D., D. Matheson, Y. He, and G. G. Goss. 2016. Reduced salinity tolerance in the Arctic grayling (Thymallus arcticus) is associated with rapid development of a gill interlamellar cell mass: implications of high-saline spills on native freshwater salmonids. Conservation Physiology 4(1).

Blanquart, F., O. Kaltz, S. L. Nuismer, and S. Gandon. 2013. A practical guide to measuring local adaptation. Ecology Letters 16(9):1195–1205. John Wiley & Sons, Ltd.

Buzby, K. M., and L. A. Deegan. 2000. Inter-annual fidelity to summer feeding sites in Arctic grayling. Environmental Biology of Fishes 59(3):319–327.

Cahill, C. L. 2015. Status of the Arctic grayling (Thymallus arcticus) in Alberta: update 2015. Edmonton.

Chan, W. Y., A. A. Hoffmann, and M. J. H. van Oppen. 2019. Hybridization as a conservation management tool. Conservation Letters 12(5):e12652. John Wiley & Sons, Ltd (10.1111).

Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9(10):1657–1659. Blackwell Science Ltd.

Coates, D. J., M. Byrne, and C. Moritz. 2018. Genetic Diversity and Conservation Units: Dealing With the Species-Population Continuum in the Age of Genomics .

COSEWIC. 2018. COSEWIC guidelines for recognizing designatable units [Approved November 2018]. http://cosewic.ca/index.php/en-ca/reports/preparing-status-reports/guidelines-recognizing-designatable-units.

COSEWIC. 2020. COSEWIC candidate wildlife species; Updated March 6, 2020. http://cosewic.ca/index.php/en-ca/reports/candidate-wildlife-species.

Crossman, E. J., and D. E. McAllister. 1986. Zoogeography of freshwater fishes of the Hudson Bay drainage, Ungava Bay and the Arctic Archipelago. Pages 53–104 *in* C. H. Hocutt and E. O. Wiley, editors. Zoogeography of North American Fishes. John Wiley & Sons.

Cullingham, C. I., J. M. Miller, R. M. Peery, J. R. Dupuis, R. M. Malenfant, J. C. Gorrell, and J. K. Janes. 2020. Confidently identifying the correct K value using the ΔK method: when does K = 2? Molecular Ecology 29:862–869.

Eizaguirre, C., and M. Baltazar-Soares. 2014. Evolutionary Conservation - Evaluating the adaptive potential of species. Evolutionary Applications 7(9):963–967.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. Molecular Ecology 14(8):2611–2620. Department of Ecology and Evolution, Biology building, University of Lausanne, CH 1015 Lausanne, Switzerland.

Excoffier, L., and H. E. L. Lischer. 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. Blackwell Publishing Ltd.

Falush, D., M. Stephens, and J. K. Pritchard. 2003. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. Genetics 164(4):1567–1587.

Federal Registrar. 2014. Endangered and Threatened Wildlife and Plants; Revised 12-Month Finding on a Petition To List the Upper Missouri River Distinct Population Segment of Arctic Grayling as an Endangered or Threatened Species.

Funk, W. C., B. R. Forester, S. J. Converse, C. Darst, and S. Morey. 2019. Improving conservation policy with genomics: a guide to integrating adaptive potential into U.S. Endangered Species Act decisions for conservation practitioners and geneticists. Conservation Genetics 20(1):115–134.

Funk, W. C., J. K. McKay, P. A. Hohenlohe, and F. W. Allendorf. 2012. Harnessing genomics for delineating conservation units. TREE 27(9):489–496.

Government of Alberta. 2021. Fisheries and Wildlife Information Management System. https://www.alberta.ca/access-fwmis-data.aspx.

460 Government of Canada. 2016. About the Species at Risk Act.
461 https://www.canada.ca/en/environment-climate-change/services/environmental-
462 enforcement/acts-regulations/about-species-at-risk-act.html.
463 Haig, S. M., and F. W. Allendorf. 2006. Hybrids and Policy. Pages 150–163 *in* J. M. Scott, D. D.
464 Goble, and F. W. Davis, editors. The Endangered Species Act at Thirty: Vol. 2 : Conserving
465 Biodiversity in Human-Dominated Landscapes. Island Press, Washington.
466 Hamilton, J. A., and J. M. Miller. 2016. Adaptive introgression as a resource for management
467 and genetic conservation in a changing climate. Conservation Biology 30(1):33–41.
468 Blackwell Publishing Inc.
469 Heim, K. C., M. S. Whitman, and L. L. Moulton. 2016. Arctic grayling (Thymallus arcticus) in
470 saltwater: a response to Blair et al. (2016). Conservation Physiology 4(1).
471 Hewitt, G. M. 1999. Post-glacial re-colonization of European biota. Biological Journal of the
472 Linnean Society 68(1–2):87–112.
473 Hewitt, G. M. 2000. The genetic legacy of the Quaternary ice ages. Nature 405(6789):907–913.
474 Hoban, S., J. L. Kelley, K. E. Lotterhos, M. F. Antolin, G. Bradburd, D. B. Lowry, M. L. Poss, L. K.
475 Reed, A. Storfer, and M. C. Whitlock. 2016. Finding the Genomic Basis of Local Adaptation:
476 Pitfalls, Practical Solutions, and Future Directions. The American Naturalist 188(4):379–
477 397. The University of Chicago Press.
478 Van Houdt, J. K. J., L. De Cleyn, A. Perretti, and F. A. M. Volckaert. 2005. A mitogenic view on the
479 evolutionary history of the Holarctic freshwater gadoid, burbot (Lota lota). Molecular
480 Ecology 14(8):2445–2457.
481 Janes, J. K., J. M. Miller, J. R. Dupuis, R. M. Malenfant, J. C. Gorrell, C. I. Cullingham, and R. L.
482 Andrew. 2017. The K = 2 conundrum. Molecular Ecology 26(14):3594–3602.
483 Kalinowski, S. T. 2005. hp-rare 1.0: a computer program for performing rarefaction on measures
484 of allelic richness. Molecular Ecology Notes 5(1):187–189. John Wiley & Sons, Ltd.
485 Katoh, K., and D. M. Standley. 2013. MAFFT Multiple Sequence Alignment Software Version 7:
486 Improvements in Performance and Usability. Molecular Biology and Evolution 30(4):772–
487 780.
488 Kopelman, N. M., J. Mayzel, M. Jakobsson, N. A. Rosenberg, and I. Mayrose. 2015. Clumpak: a

program for identifying clustering modes and packaging population structure inferences across K. Molecular Ecology Resources 15(5):1179–1191.

Koskinen, M. T., I. Knizhin, C. R. Primmer, C. Schlötterer, and S. Weiss. 2002. Mitochondrial and nuclear DNA phylogeography of Thymallus spp. (grayling) provides evidence of ice-age mediated environmental perturbations in the world's oldest body of fresh water, Lake Baikal. Molecular Ecology 11(12):2599–2611. John Wiley & Sons, Ltd.

Kovach, R. P., G. Luikart, W. H. Lowe, M. C. Boyer, and C. C. Muhlfeld. 2016. Risk and efficacy of human-enabled interspecific hybridization for climate-change adaptation: response to Hamilton and Miller (2016). Conservation Biology 30(2):428–430. John Wiley & Sons, Ltd.

Kumar, S., G. Stecher, M. Li, C. Knyaz, and K. Tamura. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Molecular Biology and Evolution 35(6):1547–1549.

LaPerriere, J. D., and R. F. Carlson. 1973. Thermal tolerances of interior Alaskan Arctic grayling (Thymallus arcticus).

Lashmar, M., and J. Ptolemy. 2002. Williston Watershed Arctic Grayling.

Leigh, J. W., and D. Bryant. 2015. popart: full-feature software for haplotype network construction. Methods in Ecology and Evolution 6(9):1110–1116.

Librado, P., and J. Rozas. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25(11):1451–1452.

Lind-Riehl, J. F., A. L. Mayer, A. M. Wellstead, and O. Gailing. 2016. Hybridization, agency discretion, and implementation of the U.S. Endangered Species Act. Conservation Biology 30(6):1288–1296.

Lohr, S. C., P. A. Byorth, C. M. Kaya, and W. P. Dwyer. 1996. High-Temperature Tolerances of Fluvial Arctic Grayling and Comparisons with Summer River Temperatures of the Big Hole River, Montana. Transactions of the American Fisheries Society 125(6):933–939.

McCart, P., and V. A. Pepper. 1971a. Geographic Variation in the Lateral Line Scale Counts of the Arctic Grayling, Thymallus arcticus . Journal of the Fisheries Research Board of Canada 28(5):749–754.

Mee, J. A., L. Bernatchez, J. D. Reist, S. M. Rogers, and E. B. Taylor. 2015. Identifying

designatable units for intraspecific conservation prioritization: a hierarchical approach applied to the Lake Whitefish species complex (Coregonus spp.). Evolutionary Applications 8(5):423–441.

Miller, B. B., D. F. Palmer, W. D. McCoy, A. J. Smith, and M. L. Colburn. 1993. A Pre-Illinoian Pleistocene Fossil Assemblage from Near Connersville, Southeastern Indiana. Quaternary Research 40(2):254–261.

Miller, M. A., W. Pfeiffer, and T. Schwartz. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Pages 1–8 2010 Gateway Computing Environments Workshop (GCE).

Nguyen, L.-T., H. A. Schmidt, A. von Haeseler, and B. Q. Minh. 2014. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. Molecular Biology and Evolution 32(1):268–274.

Northcote, T. G. 1995. Comparative biology and management of Arctic and European grayling (Salmonidae, Thymallus). Reviews in Fish Biology and Fisheries 5(2):141–194.

Oziolor, E. M., N. M. Reid, S. Yair, K. M. Lee, S. Guberman VerPloeg, P. C. Bruns, J. R. Shaw, A. Whitehead, and C. W. Matson. 2019. Adaptive introgression enables evolutionary rescue from extreme environmental pollution. Science 364(6439):455–457.

Pardo-Diaz, C., C. Salazar, S. W. Baxter, C. Merot, W. Figueiredo-Ready, M. Joron, W. O. McMillan, and C. D. Jiggins. 2012. Adaptive Introgression across Species Boundaries in Heliconius Butterflies. Plos Genetics 8(6). Pardo-Diaz, C (reprint author), Univ Cambridge, Dept Zool, Cambridge, England Univ Cambridge, Dept Zool, Cambridge, England Smithsonian Trop Res Inst, Panama City, Panama Museum Natl Hist Nat, CNRS, UMR 7205, CP50, Paris, France Fed Univ Para, Inst Estudo.

Park, L., M. Brainard, D. Dightman, and G. Winans. 1993. Low levels of intraspecific variation in the mitochondrial DNA of chum salmon (Oncorhynchus keta). Molecular Marine Biology and Biotechnology 2(6):362–370.

Peterson, D. P., and W. R. Ardren. 2009. Ancestry, population structure, and conservation genetics of Arctic grayling (Thymallus arcticus) in the upper Missouri River, USA. Canadian Journal of Fisheries and Aquatic Sciences 66(10):1758–1774. NRC Research Press.

547 Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using
548 multilocus genotype data. Genetics 155(2):945–959.
549 R Core Team. 2019. R: A language and environment for statistical computing, reference index
550 version 3.2.2. R Foundation for Statistical Computing, Vienna, Austria.
551 Redenbach, Z., and E. B. Taylor. 1999. Zoogeographical implications of variation in
552 mitochondrial DNA of Arctic grayling (Thymallus arcticus). Molecular Ecology 8(1):23–35.
553 John Wiley & Sons, Ltd.
554 Reed, R. J., and J. A. Mccann. 1973. Analyses of Some Meristic and Morphometric Data from the
555 Arctic Grayling, Thymallus arcticus, in Alaska (4):819–822.
556 Reilly, J. R., C. A. Paszkowski, and D. W. Coltman. 2014. Population Genetics of Arctic Grayling
557 Distributed Across Large, Unobstructed River Systems. Transactions of the American
558 Fisheries Society 143(3):802–816. John Wiley & Sons, Ltd.
559 Rosenberg International Forum on Water Policy. 2013. Rosenberg International Forum: The
560 Mackenzie Basin.
561 Ryder, O. A. 1986. Species conservation and systematics: the dilemma of subspecies. Trends in
562 Ecology & Evolution 1(1):9–10.
563 Savolainen, O., M. Lascoux, and J. Merilä. 2013. Ecological genomics of local adaptation. Nature
564 Reviews Genetics 14(11):807–820.
565 Schroeder, D. 1987. Seine netting of M.D. of Peace Pond #2 and Relocation of Arctic grayling to
566 Long Lake (34:62-6-W6). Edmonton.
567 Scott, W. B., and E. J. Crossman. 1998. Freshwater fishes of Canada. Galt House Pub, Oakville.
568 Sgro, C. M., A. J. Lowe, and A. A. Hoffmann. 2011. Building evolutionary resilience for
569 conserving biodiversity under climate change. Evolutionary Applications 4(2):326–337.
570 Sgro, CM (reprint author), Monash Univ, CESAR, Clayton, Vic 3800, Australia Monash Univ,
571 CESAR, Clayton, Vic 3800, Australia Monash Univ, Australian Ctr Biodivers, Sch Biol Sci,
572 Clayton, Vic 3800, Australia Univ Adelaide, ACEBB, Sch Earth & Environm Sci, .
573 Skog, A., L. A. Vøllestad, N. C. Stenseth, A. Kasumyan, and K. S. Jakobsen. 2014. Circumpolar
574 phylogeography of the northern pike (Esox lucius) and its relationship to the Amur pike (E.
575 reichertii). Frontiers in Zoology 11(1):1–8.

576 Stamford, M. D. 2002. Mitochondrial and microsatellite DNA diversity throughout the range of a
577 cold adapted freshwater salmonid : phylogeography, local population structure and
578 conservation genetics of Arctic grayling (Thymallus arcticus) in North America. University
579 of British Columbia.
580 Stamford, M. D., and E. B. Taylor. 2004. Phylogeographical lineages of Arctic grayling (Thymallus
581 arcticus) in North America: divergence, origins and affinities with Eurasian Thymallus.
582 Molecular Ecology 13(6):1533–1549. John Wiley & Sons, Ltd (10.1111).
583 Stamford, M. D., and E. B. Taylor. 2005. Population subdivision and genetic signatures of
584 demographic changes in Arctic grayling (Thymallus arcticus) from an impounded
585 watershed. Canadian Journal of Fisheries and Aquatic Sciences 62(11):2548–2559.
586 Stecher, G., K. Tamura, and S. Kumar. 2020. Molecular Evolutionary Genetics Analysis (MEGA)
587 for macOS. Molecular Biology and Evolution 37(4):1237–1239.
588 Todesco, M., M. A. Pascual, G. L. Owens, K. L. Ostevik, B. T. Moyers, S. Hübner, S. M. Heredia,
589 M. A. Hahn, C. Caseys, D. G. Bock, and L. H. Rieseberg. 2016. Hybridization and extinction.
590 Evolutionary Applications 9(7):892–908.
591 U.S. Fish and Wildlife Service. 2020. Endangered Species Act: Overview.
592 https://www.fws.gov/endangered/laws-policies/.
593 Weiss, S. J., D. V Gonçalves, G. Secci-Petretto, G. K. Englmaier, A. Gomes-Dos-Santos, G. P. J.
594 Denys, H. Persat, A. Antonov, C. Hahn, E. B. Taylor, and E. Froufe. 2021. Global systematic
595 diversity, range distributions, conservation and taxonomic assessments of graylings
596 (Teleostei: Salmonidae; Thymallus spp.). Organisms Diversity & Evolution 21:25–42.
597 Wilson, C. C., and P. D. N. Hebert. 1998. Phylogeography and postglacial dispersal of lake trout
598 (Salvelinus namaycush) in North America. Canadian Journal of Fisheries and Aquatic
599 Sciences 55(4):1010–1024. NRC Research Press.
600 Working Group on General Status of NWT Species. 2016. NWT Species 2016-2020 – General
601 Status Ranks of Wild Species in the Northwest Territories. Yellowknife NT.
602
603

Table 1. Site locations of Arctic Grayling captured in the Hay River, Peace River, and Athabasca River basins in Alberta, Canada. Shown are the site codes, sampling location, and total numbers of individuals genotyped (*N*).

Watercourse	Site code	Latitude (dec. deg.)	Longitude (dec. deg)	*N*
Hay River basin				
Dizzy Creek	DiC	59.222	-116.135	4
James Creek	Ja	59.364	-116.297	10
Slavey Creek	SlC	59.009	-116.934	6
Whitesand River	WhR	59.625	-115.695	2
Yates River	Ya	59.791	-116.468	3
Peace River basin				
Caribou River	Ca	58.775	-115.889	12
Carl Creek	CaC	58.771	-115.803	4
Kemp River	KeR	57.549	-117.628	1
Wapiti River	Wa	54.737	-120.000	6
Wentzel River	WeR	58.720	-114.665	3
Athabasca River basin				
Athabasca River	AtN	53.980	-116.934	12
Dismal Creek	Di	53.108	-115.684	12
Driftpile River	Dr	55.078	-115.735	11
House River	Ho	55.642	-112.150	10

Table 2: Diversity statistics for 831bp of mtDNA assessed in 96 Arctic Grayling from Alberta, Canada. Statistics were calculated when samples were grouped either per basin or based on lineage.

Sample grouping	N samples	N haplotypes	Haplotype diversity	Haplotype diversity SD	Nucleotide diversity (PI)	Nucleotide diversity SD
Athabasca	45	4	0.245	0.081	0.00031	0.00011
Hay	28	3	0.463	0.098	0.00929	0.00172
Peace	19	3	0.573	0.061	0.01104	0.00105
Beringia	68	5	0.526	0.051	0.00167	0.00017
Nahanni	28	1	0.000	0.000	0.00000	0.00000

Table 3: Analysis of molecular variance for 831bp of mtDNA assessed in 96 Arctic Grayling from Alberta, Canada. Statistics were calculated when samples were grouped either per basin or based on lineage

	Basin				Lineage			
Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among groups	2	174.844	2.213	41.88	1	358.969	8.975	94.23
Among populations within groups	14	221.246	2.957	55.94	15	37.121	0.434	4.56
Within populations	79	9.129	0.116	2.19	79	9.129	0.116	1.21

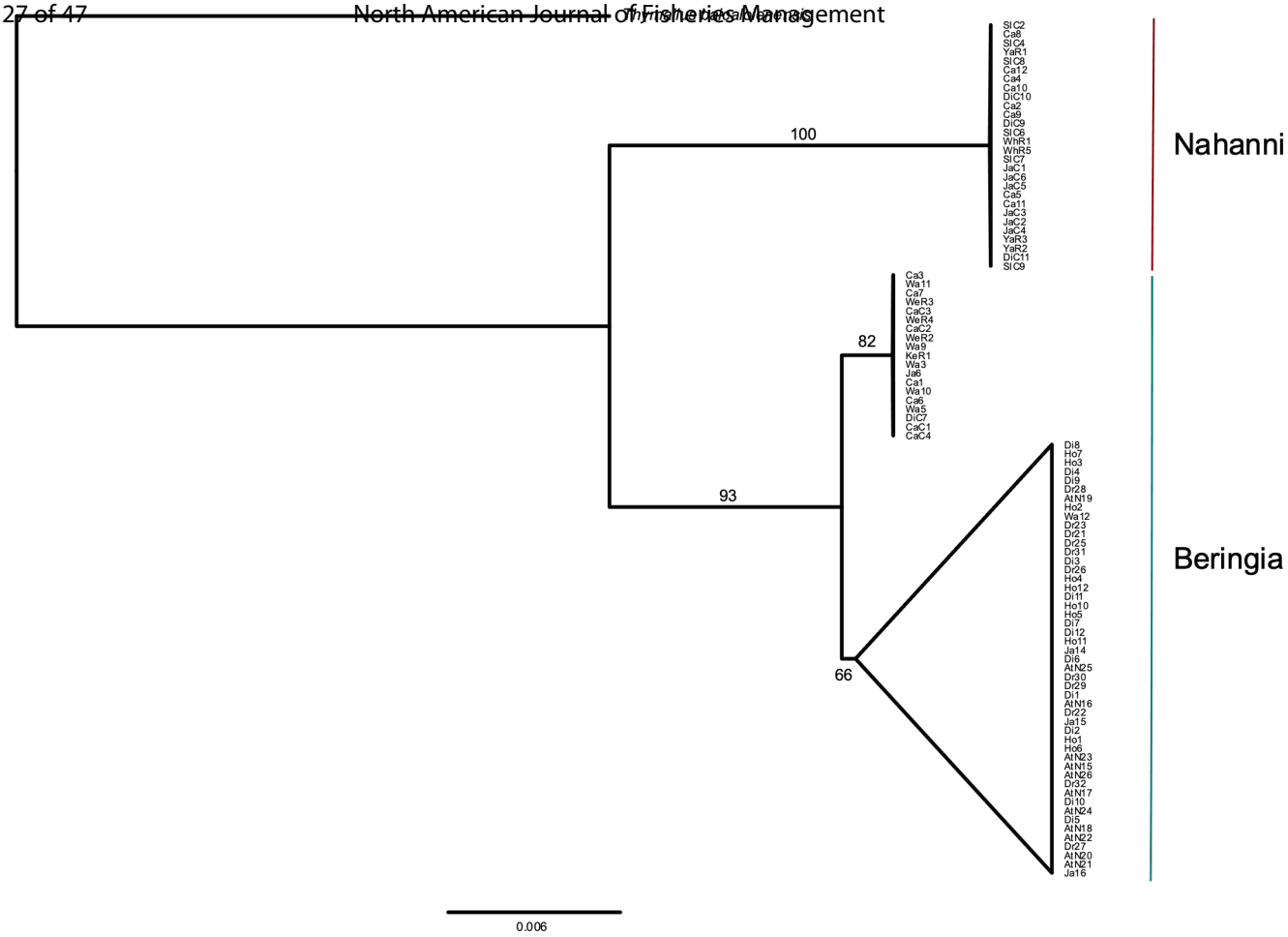
Figure 1: **(A)** Maximum-likelihood tree of 831bp of mtDNA in 96 Arctic Grayling samples from Alberta, Canada rooted with *T. baicalolenensis*. Sample prefixes correspond to locations as in Table 1. Numbers next to branches represent percent bootstrap support. The triangle is a cartoon representation for diversity among several haplotypes. Scale bar represents expected number of substitutions per site. **(B)** Median-joining haplotype network of 831bp mtDNA in 96 Arctic Grayling samples from Alberta, Canada. Each circle represents a single haplotype and hashes across branches represent the number of DNA mutations between haplotypes. The size of the circle proportional to the number of samples with that haplotype, and colors represent the basin where a sample was collected. The white node represents an unsampled, hypothetical haplotype.

Figure 2: Maps showing the distribution of haplotypes based on 831bp mtDNA in 96 Arctic Grayling from Alberta, Canada. Each circle represents a sampling location with the size proportional to the number of individuals sequenced at that location and colours representing either the genetic lineage (left panel) or individual haplotypes observed (right panel).

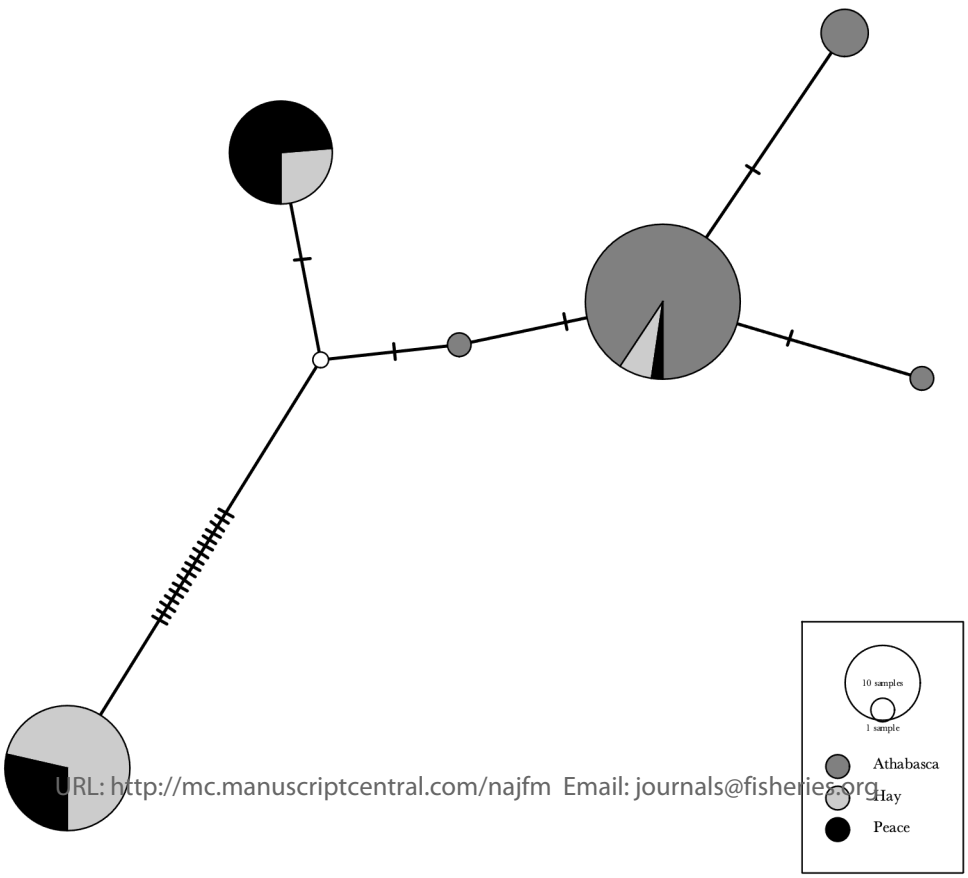
Figure 3: Map showing the distribution of haplotype groupings across North America based on the combined dataset of 438bp of mtDNA. Each circle represents a sampling location with the size proportional to the number of individuals considered at that location and colours representing Group A, B, and C haplotypes described by Stamford and Taylor (2004).

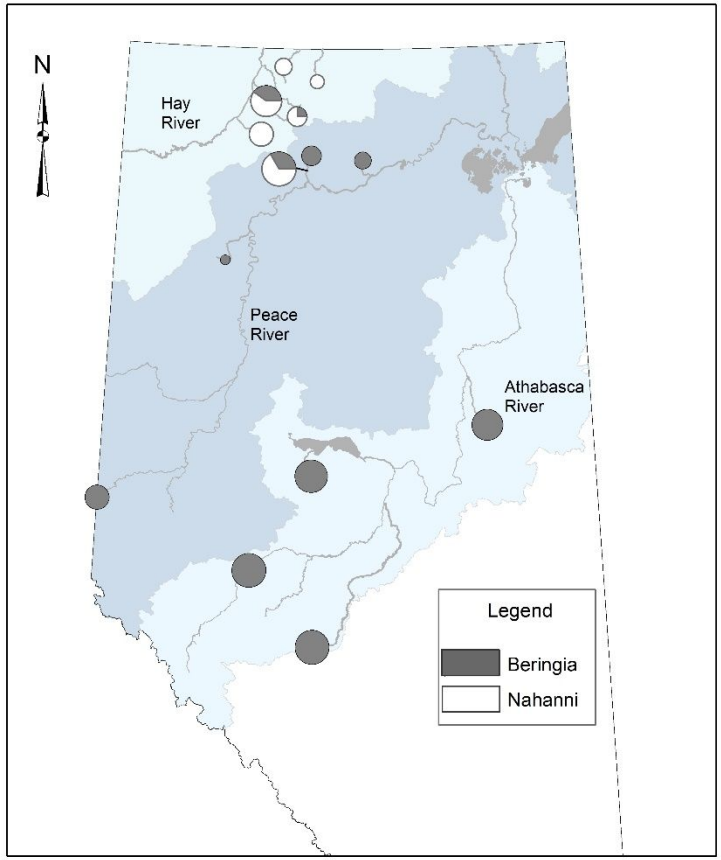
Figure 4: Ancestry barplot based on nine microsatellite loci for 1,116 Arctic Grayling from Alberta, Canada. The number of genetic clusters (K) is shown next to each plot, and each lineage is identified by a different color. Individuals are represented as a vertical bar, with the proportion of color in a bar equal to the percent ancestry to that cluster. Basins are indicated below each barplot.

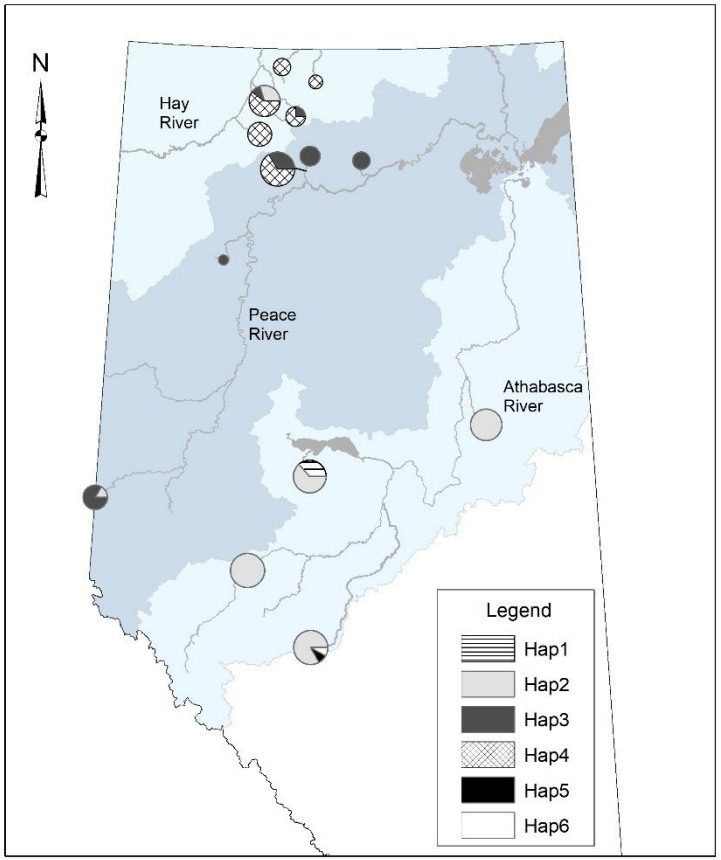
A)

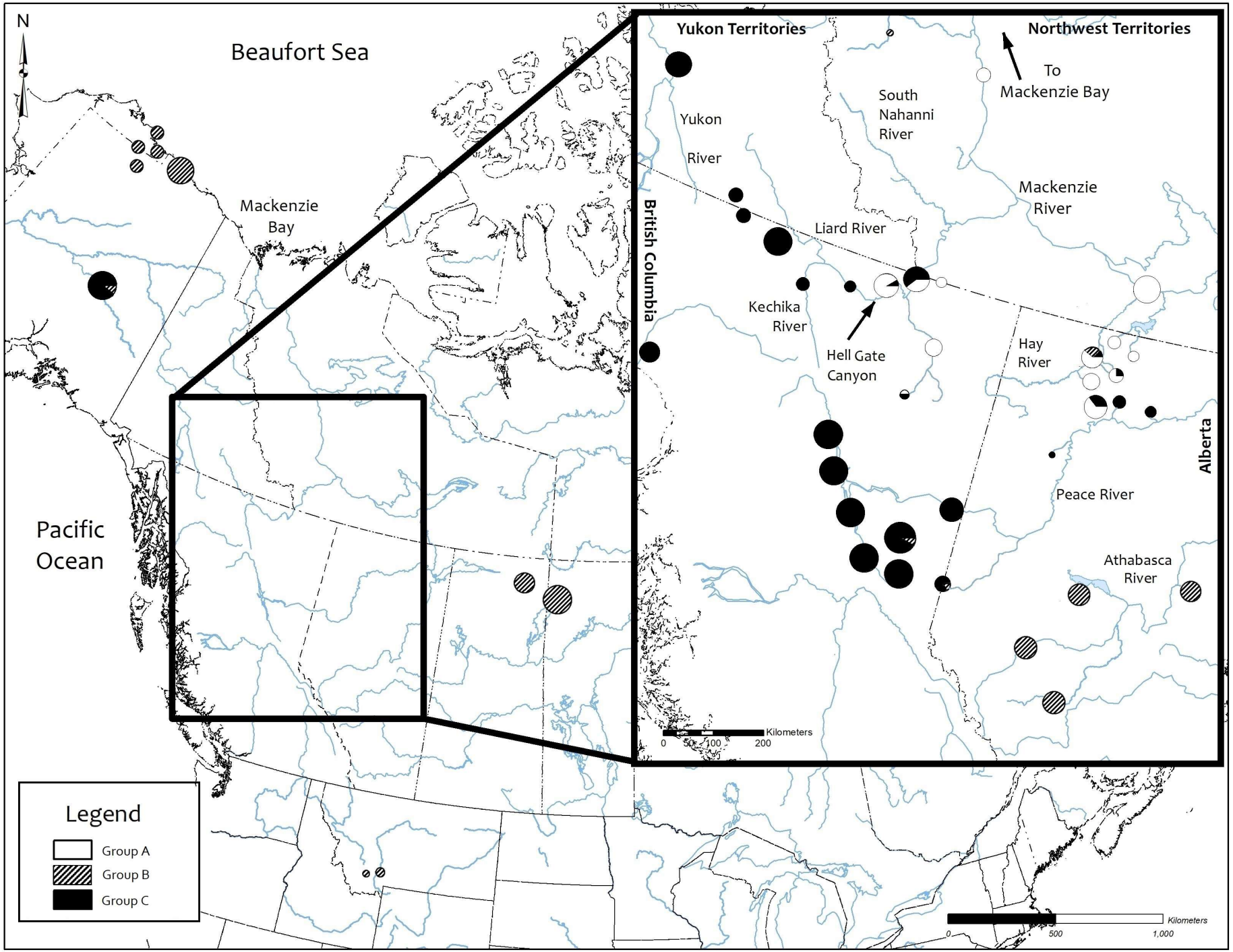

SIC2
Ca8
SIC4
YaR1
SIC8
Ca12
Ca4
Ca10
DiC10
Ca2
Ca9
DiC9
SIC6
WhR1
WhR5
SIC7
JaC1
JaC6
JaC5
Ca5
Ca11
JaC3
JaC2
JaC4
YaR3
YaR2
DiC11
SIC9
100
Nahanni
Ca3
Wa11
Ca7
WeR3
CaC3
WeR4
CaC2
WeR2
Wa9
KeR1
Wa3
Ja6
Ca1
Wa10
Ca6
Wa5
DiC7
CaC1
CaC4
82
93
Di8
Ho7
Ho3
Di4
Di9
Dr28
AtN19
Ho2
Wa12
Dr23
Dr21
Dr25
Dr31
Di3
Dr26
Ho4
Ho12
Di11
Ho10
Ho5
Di7
Di12
Ho11
Ja14
Di6
AtN25
Dr30
Dr29
Di1
AtN16
Dr22
Ja15
Di2
Ho1
Ho6
AtN23
AtN15
AtN26
Dr32
AtN17
Di10
AtN24
Di5
AtN18
AtN22
Dr27
AtN20
AtN21
Ja16
66
Beringia
0.006

B)


10 samples
1 sample
Athabasca
Hay
Peace






N
Beaufort Sea
Mackenzie Bay
Pacific Ocean
Yukon Territories
Northwest Territories
To Mackenzie Bay
South Nahanni River
Yukon River
Mackenzie River
British Columbia
Liard River
Kechika River
Hell Gate Canyon
Hay River
Alberta
Peace River
Athabasca River
Kilometers
0 50 100 200
Legend
Group A
Group B
Group C
Kilometers
0 500 1,000

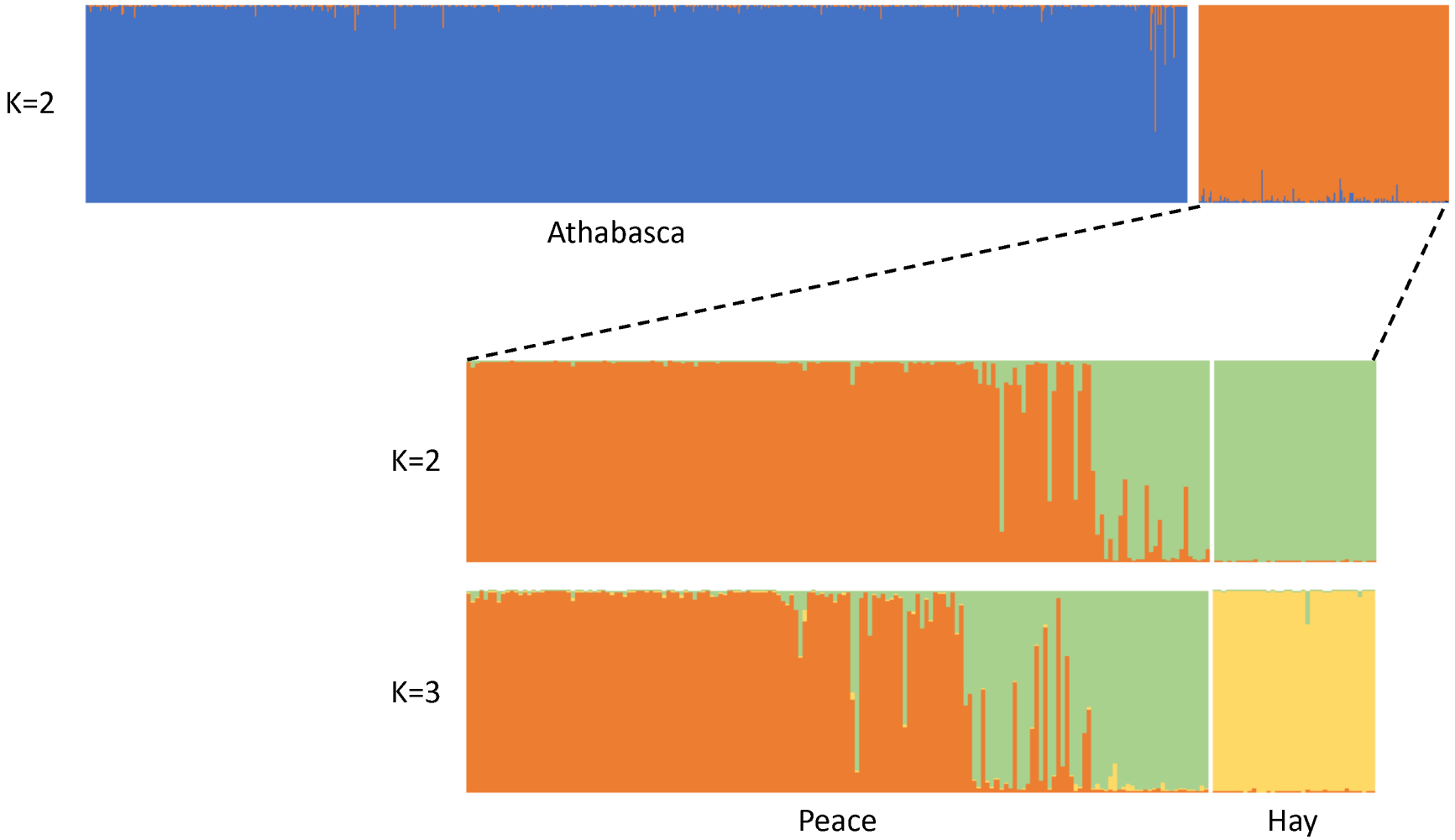

K=2
Athabasca
K=2
K=3
Peace
Hay

Table S.1: Comparison of observed haplotypes, and the number of individuals assigned to the Beringia and Nahanni lineages defined by the current study based on the 831bp dataset, or the Group A, B and C haplotypes described by Stamford and Taylor (2004) based on the combined dataset of 438bp.

Watercourse	Site code	Current study								Stamford and Taylor (2004)			
		Hap1	Hap2	Hap3	Hap4	Hap5	Hap6	Beringia	Nahanni	Group A	Group B	Group C	N.A.[1]
Hay River basin													
Dizzy Creek	DiC	0	0	1	3	0	0	1	3	3	0	1	0
James Creek	Ja	0	3	1	6	0	0	4	6	6	3	1	0
Slavey Creek	SlC	0	0	0	6	0	0	0	6	6	0	0	0
Whitesand River	WhR	0	0	0	2	0	0	0	2	2	0	0	0
Yates River	Ya	0	0	0	3	0	0	0	3	3	0	0	0
Peace River basin													
Caribou River	Ca	0	0	4	8	0	0	4	8	8	0	4	0
Carl Creek	CaC	0	0	4	0	0	0	4	0	0	0	4	0
Kemp River	KeR	0	0	1	0	0	0	1	0	0	0	1	0
Wapiti River	Wa	0	1	5	0	0	0	6	0	0	1	5	0
Wentzel River	WeR	0	0	3	0	0	0	3	0	0	0	3	0
Athabasca River basin													
Athabasca River	AtN	0	12	0	0	0	0	12	0	0	12	0	0
Dismal Creek	Di	0	10	0	0	1	1	12	0	0	11	0	1
Driftpile River	Dr	4	7	0	0	0	0	11	0	0	11	0	0
House River	Ho	0	10	0	0	0	0	10	0	0	10	0	0

[1]No assignment

Table S.2: Mean likelihood value, standard deviation (SD), and Delta K values from STRUCTURE analyses of 1,116 Arctic Grayling from the Athabasca River, Peace River, and Hay River basins genotyped at nine microsatellite loci.

K	Mean ln(P|K)	SD	Delta K
1	-42079.33	0.11	
2	-38300.79	0.82	3254.48
3	-37190.35	2.58	187.92
4	-36564.41	18.60	1.27
5	-35914.92	56.54	2.22
6	-35390.82	249.80	1.01
7	-35117.83	303.99	0.88
8	-35113.17	448.22	0.73
9	-34779.78	322.69	0.74
10	-34684.07	384.43	0.14
11	-34641.24	383.50	0.68
12	-34857.48	253.44	0.63
13	-34913.91	25.74	

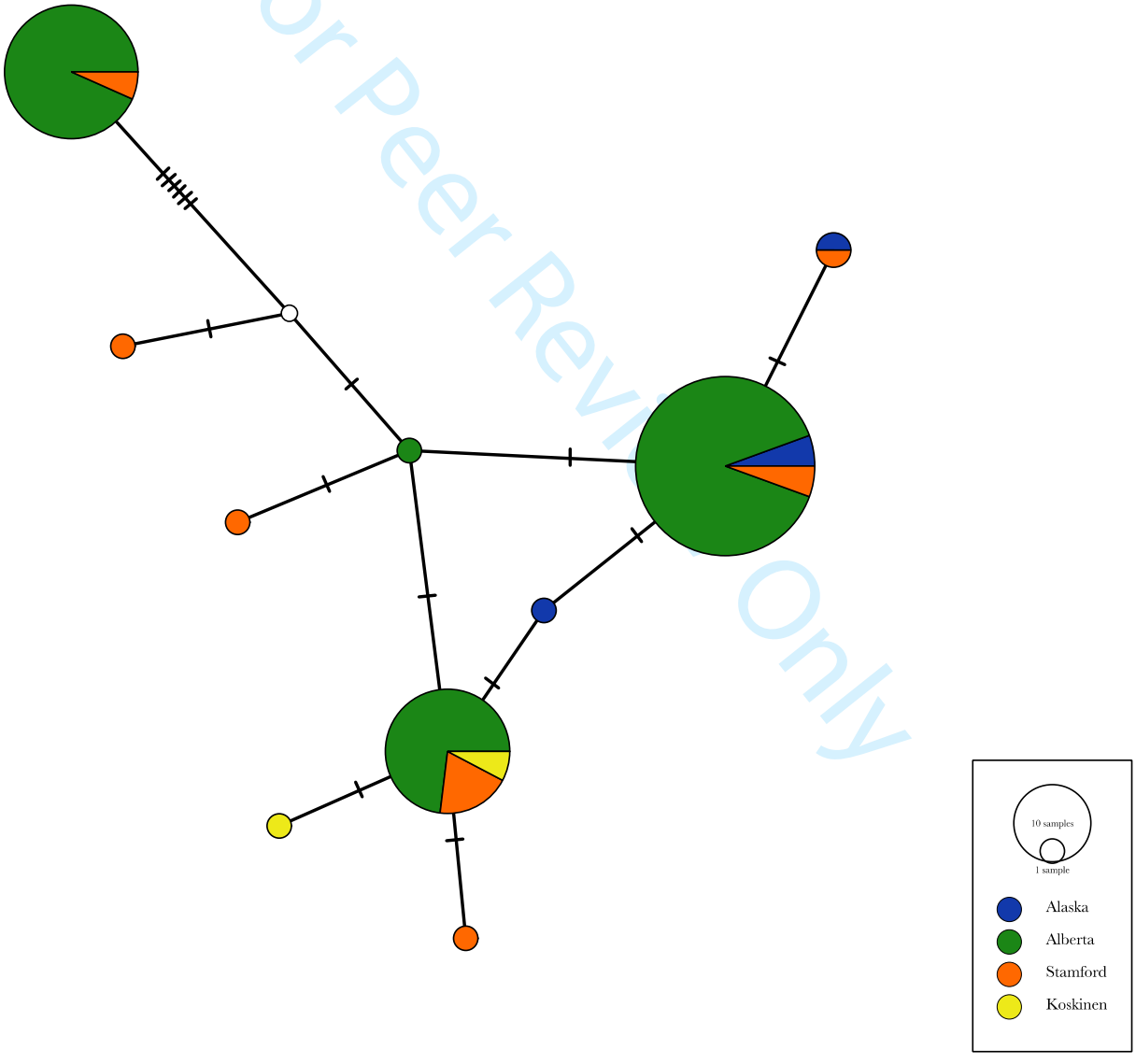
Table S.3: Mean likelihood value, standard deviation (SD), and Delta K values from STRUCTURE analyses of 207 Arctic Grayling from the Athabasca River, Peace River, and Hay River basins genotyped at nine microsatellite loci.

K	Mean ln(P|K)	SD	Delta K
1	-8434.56	0.46	
2	-7640.70	1.50	313.65
3	-7317.67	1.55	99.26
4	-7148.42	1.88	

Figure S.1: Median-joining haplotype network of 432bp mtDNA in 118 Arctic Grayling samples. Each circle represents a single haplotype and hashes across branches represent the number of DNA mutations between haplotypes. The size of the circle proportional to the number of samples with that haplotype, and colors represent the study or location in which that haplotype was observed. The open circle represents an unsampled, hypothetical haplotype. In the legend Stamford refers to Stamford and Taylor (2004) and Koskinen to Koskinen et al. (2002).

Figure S.2: Scatter plot showing relationship between geographic location and microsatellite genetic diversity of Arctic Grayling within the Athabasca River basin. Each point represents a single sample site and points are coloured based on average allelic richness (Ar) measured at nine microsatellite loci using data from Reilly et al. (2014).

Supplementary Figure 1: Median-joining haplotype network of 432bp mtDNA in 118 Arctic Grayling samples. Each circle represents a single haplotype and hashes across branches represent the number of DNA mutations between haplotypes. The size of the circle proportional to the number of samples with that haplotype, and colors represent the study or location in which that haplotype was observed. The open circle represents an unsampled, hypothetical haplotype. In the legend Stamford refers to Stamford and Taylor (2004) and Koskinen to Koskinen et al. (2002).



Supplementary Figure 2: Scatter plot showing relationship between geographic location and microsatellite genetic diversity of Arctic Grayling within the Athabasca watershed. Each point represents a single sample site and points are coloured based on average allelic richness (Ar) measured at 9 microsatellite loci.