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Genetic variation and populations structure among larval *Lethenteron* spp. within the Yukon River drainage, Alaska

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ABSTRACT

The absence of information on genetic variation and population structure of brook lampreys *Lethenteron* spp. in the eastern part of their distribution limits our understanding of the migration ecology and spatial population genetic structure of the species. We examined genetic variation within and among three aggregations of *Lethenteron* spp. larvae in the Yukon River drainage, Alaska, using microsatellite genotypes. A total of 120 larval lampreys were genotyped at eight microsatellite loci. Global F_{ST} was 0.053 (95% CI 0.021–0.086), while pairwise F_{ST} values ranged from 0.048–0.057). Model-based Bayesian clustering analyses with sample locality priors (LOCPRIOR) identified three distinct, but admixed, genetic clusters that corresponded with the three aggregations. Estimates of contemporary gene flow indicate substantial reciprocal migration among sites consistent with no or low-fidelity natal homing. These results are largely

in agreement with previous reports of historic and contemporary gene flow among *Lethenteron* spp. in other parts of their geographic distribution.

KEYWORDS

Genetics, lampreys, microsatellite genotyping, population structure, Yukon River drainage

1 | INTRODUCTION

The population genetic structure of anadromous lamprey species suggests high dispersal and gene flow among populations spanning broad geographic regions (Bryan *et al*. 2005; Almada *et al*. 2008; Waldman *et al*. 2008; Spice *et al*. 2012; Yamazaki *et al*. 2014; Artamonova *et al*. 2015). The lack of population structure within anadromous lamprey species is widely attributed to a lack of natal homing and long-distance migration ability (Bryan *et al*. 2005; Waldman *et al*. 2008; Spice *et al*. 2012; Yamazaki *et al*. 2014). Previous studies largely focused on the population genetic structure of anadromous lampreys throughout their geographic range, yet genetic structuring of lamprey populations within a single river basin has only been recently examined (Hess *et al*. 2014; Clemens *et al*. 2017). These studies reported moderate levels of temporal genetic subdivision and identified signatures of local adaptation, despite low genetic

differentiation at neutral markers (Hess *et al*. 2014; Clemens *et al*. 2017). Documenting both range-wide and localized population genetic structure of lampreys is important for conservation and management, since many species have been experiencing drastic declines due to increased harvest pressures, anthropogenic disturbance of habitats and changing environmental conditions (Renaud 1997; Close *et al*. 2002; Masters *et al*. 2006).

Five nominal lamprey species assigned to three distinct genera are known in Alaskan freshwaters (Arctic lamprey *Lethenteron camtschaticum* (Tilesius 1811); western river lamprey *Lampetra ayresii* (Günther 1870); Pacific lamprey *Entosphenus tridentatus* (Richardson 1836); Alaskan brook lamprey *Lethenteron alaskense* Vladykov & Kott 1978; and western brook lamprey *Lampetra richardsoni* Vladykov & Follett 1965), but species of *Lethenteron* Creaser & Hubbs 1922 are found farther north than any other lampreys (Mecklenburg *et al*. 2002; Renaud 2011). Arctic lamprey *Lethenteron camtschaticum* are a jawless, semelparous fish widely distributed at mid-to high latitudes in northwestern North America and eastern Eurasia (Mecklenburg *et al*. 2002; Renaud *et al*. 2011). Anadromous *L. camtschaticum* are the most common lamprey species in Alaska, with known spawning aggregations throughout the Yukon, Kuskokwim and Susitna River drainages (Morrow 1980; Mecklenburg *et al*. 2002). In addition, anadromous *L. camtschaticum* are harvested from the lower Yukon River for both subsistence and commercial purposes (Brown *et al*. 2005; Renaud 2011). Subsistence harvests of anadromous *L. camtschaticum* is an important traditional resource to native Alaskan communities within the lower Yukon River drainage with initial documentation of subsistence

harvests dating back to the late 1800s (Renaud 2011). A test commercial fishery targeting *L. camtschaticum* was started in 2003 and currently remains in operation with annual harvest limits set at 20,000 kg (Estensen *et al*. 2017). Despite annual commercial and subsistence harvest of this species, information regarding the population genetic structure among *Lethenteron* spp. within Alaska rivers is non-existent, highlighting the need for genetic information in this region (ADF&G 2006; Mesa & Copeland 2009; Thorsteinson & Love 2016).

Previous studies using mitochondrial and nuclear microsatellite markers demonstrated highly connected populations of *Lethenteron* spp. in the western part of their distribution (Yamazaki *et al*. 2011, 2014; Artamonova *et al*. 2011, 2015). Although genetic data using cytochrome oxidase subunit I (*coI*) for *Lethenteron* spp. are largely restricted in geographical scope to eastern Eurasia, there is no evidence of genetic divergence in sequenced regions of the mitochondrial genome (Artamonova *et al*. 2011, 2015). Larval and adult *L. camtschaticum* examined using nuclear microsatellite markers indicated varying levels of genetic divergence in eastern Eurasia (Yamazaki *et al*. 2014). Genetic variability revealed two ancestral clusters that exhibited a latitudinal pattern, with northern-most individuals having higher probabilities of being assigned to the same genetic cluster (Yamazaki *et al*. 2014). This regional variation contrasts with the range-wide genetic homogeneity documented among populations of anadromous sea lamprey *Petromyzon marinus* L. 1758, but it is similar to low measures of genetic differentiation among Pacific lamprey *E. tridentatus* stocks (Bryan *et al*. 2005; Goodman *et al*. 2008; Spice *et al*. 2012). To date, population genetic studies of *Lethenteron* spp. have only

evaluated the western portion of the species range in Eurasia, but it is not known whether similar genetic patterns exist among *Lethenteron* spp. in the eastern portion of its range in Alaska.

We investigated the genetic structure of the eastern portion of the species range of *Lethenteron* spp. using microsatellite genotyping of larvae collected among three sites within the Yukon River drainage. For this study, we use the term *Lethenteron* spp. because we know at least two nominal species of the genus *Lethenteron*, *L. camtschaticum* and *L. alaskense,* are known to occur in the Chena River, a tributary of the Yukon River basin (Sutton 2017). Furthermore, morphological characteristics and molecular markers could not distinguish between parasitic (*L. camtschaticum*) and non-parasitic (*L. alaskense*) species (April *et al*. 2011; Balakirev *et al*. 2014; Sutton 2017). In fact, minimal genetic divergence between these putative species is consistent with no reproductive isolation and high rates of gene flow in the absence of physical barriers (Yamazaki *et al*. 2011).

Although morphologically similar in their larval form, parasitic and non-parasitic lampreys diverge radically in feeding behavior and body size through successive life-history stages (Zanandrea 1959; Vladykov & Kott 1979). Anadromous *L. camtschaticum* feeds on the blood and flesh of host fishes in marine environments before returning to freshwater tributaries to spawn, while *L. alaskense* is a fluvial, non-parasitic species that reside and filter feed in freshwater tributaries for the duration of their life cycle and do not feed as adults (Mecklenburg *et al*. 2002; Renaud *et al*. 2009, 2011). Current lamprey taxonomies recognize life-history variants as distinct species (Mecklenburg *et al*. 2002; Renaud 2011; Potter *et al*. 2015). Although

the distinctiveness, relationship and validity of *L. alaskense* relative to *L. camtschaticum* are not yet well established, the ongoing debate surrounding the nature and degree of separation between parasitic and non-parasitic forms is beyond the scope of this study. For this study, we tested the null hypothesis of no population structure among *Lethenteron* spp. collected from its eastern range at three sites in the Yukon River drainage. Furthermore, we tested whether genetic variation observed in this study using a set of nuclear microsatellite markers was equivalent with the levels of diversity previously described from the western portion of the species range.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Larval *Lethenteron* spp. were collected from three tributaries along the Yukon River drainage, Alaska (Figure 1 and Supporting Information Table S1). Larvae from the East Fork of the Andreafsky and Gisasa Rivers were opportunistically collected in 2014 within 3.5 river kilometres (rkm) of annual Pacific salmon *Oncorhynchus* Sukley 1861 species enumeration weirs using a modified bottom sampler (Lasne *et al*. 2010). A total of 40 tissue samples from individuals of varying size classes were collected at sites (n = 10) along the East Fork of the Andreafsky River over a 1 month period to minimize sampling the same cohort. To further reduce the probability of sampling from the same cohort, a maximum of six tissue samples were

collected at each site where *Lethenteron* spp. were present. A total of 40 individual tissue samples from the Gisasa River were collected from one site (n = 1) within a 24 h period regardless of size due to time constraints. All larval *Lethenteron* spp. sampled within the East Fork of the Andreafsky and Gisasa Rivers were released live near their collection site.

Larvae from the Chena River were collected from a 149 rkm reach in 2011 using a Model ABP-2 backpack electrofishing unit (ETS Electrofishing LLC; www.etselectrofishing.com) in an effort to characterize the distribution and ecology of *Lethenteron* spp. in interior Alaska rivers (Sutton 2017). A maximum of 10 individuals were collected at each site where *Lethenteron* spp. were present. These larvae were euthanized using MS-222 and frozen for subsequent molecular analyses. For this study, a total of 40 tissue samples from larvae of varying size classes were selected from sites (n = 29) that encompassed the full extent of sampling efforts. No more than two tissue samples from the same collection site were selected to reduce the probability of sampling the same cohort. All tissue samples used in this study were preserved in 96% molecular biology-grade ethanol in the field and placed in cold storage (–20^{o}C) for long-term preservation.

We predicted this study design would result in a pattern of relatedness between individuals collected at varying spatial scales within each site. Relatedness between individuals was expected to be highest at the Gisasa River, since all 40 individuals were collected from a single site within a 24-hour period. In contrast, relatedness between individuals was expected to be lowest in the Chena River where samples were collected over a 2 week period from 29 sites that stretched over 149 rkm. Finally, we predicted relatedness between individuals in the East

Fork of the Andreafsky River would be intermediate because samples were collected over a 1 month period from 10 sites spanning 3.5 rkm.

2.2 | Microsatellite genotyping

Total genomic DNA from larval tissue samples (n = 120) were isolated through tissue lysis followed by salt-and-alcohol preferential precipitation of proteins and nucleic acids, respectively using the Gentra Puregene Tissue Kit (Qiagen; www.qiagen.com) following the manufacturer's protocol. Eight microsatellite loci previously identified and developed from brook lamprey *Lethenteron* spp. N from Japan were targeted for genotyping (Takeshima *et al*. 2005). To generate allele amplicons for the targeted loci, a three-primer PCR approach was implemented (Schuelke 2000). The primer combination included: a locus specific primer modified to include a standard M13 sequence 52tail; a locus specific complementary primer; a fluorescently labelled oligonucleotide corresponding to the M13 segment of the locus specific primer. The standard M13 sequence 52tail was incorporated on the locus specific forward primer for all loci except Lspn-088 (Supporting Information Table S2).

PCRs were performed in 25 μl reactions with 0.5 μl variable concentration genomic DNA template, 1x *GoTaq* buffer (Promega; www.promega.com), 0.05 mM locus specific primer with M13 tail, 0.25 mM locus specific complementary primer, 0.25 mM fluorescently labelled M13 primer (FAM or HEX), 0.6 mM deoxynucleotide triphosphate (dNTP), 25 μg/ml bovine

serum albumin (BSA), 1.5–2 μM Mg^{2+} and 0.025 U μl^{-1} of GoTaq polymerase. The Mg^{2+} concentrations in the reaction mix varied by locus (Supporting Information Table S2). Thermal cycler conditions were identical to those reported in Takeshima *et al*. (2005). The PCR products were run on 2.5% agarose gels with a negative control to confirm amplification length and lack of template contamination. These products were analysed using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems; www.appliedbiosystems.com) and allele sizes were determined using GeneMapper 3.7 (Applied Biosystems).

Potential genotyping errors resulting from null alleles or large allelic drop out were evaluated with the software program Micro-Checker (van Oosterhout *et al*. 2004). Micro-Checker indicated the occurrence of null alleles at two loci (see results); therefore, the program FREENA (Chapuis & Estoup 2007) was used to estimate the frequency of null alleles for each locus and calculate F_{ST} estimates adjusted for presence of putative null alleles.

Because all tissue samples collected for this study were from larval *Lethenteron* spp., we investigated the likelihood that collected samples were from a small number of families. The probability of sampled full-sibling pairs was estimated using the full-pedigree likelihood (FL) approach in COLONY 2.0.6.2 (Jones & Wang 2010) under a female and male polygamy mating system, known allele frequencies, no sibship prior and a long run length with five independent runs. One randomly selected individual from each full-sibling pair collected from the same tributary was removed from the dataset for downstream analyses to avoid relatedness bias.

Deviations from genotype frequencies expected under Hardy-Weinberg equilibrium (HWE) and tests for linkage disequilibrium (LD) between pairs of loci in each population were evaluated with exact probability tests implemented in GENEPOP 4.0.10 (10^4 dememorization, 10^3 batches, 10^4 iterations per batch; Raymond & Rousset 1995). To confirm that the final dataset (*i.e.*, sample sizes, number of loci and observed allele frequencies) had the statistical power to detect genetic differentiation, the program POWSIM (Ryman & Palm 2006) was employed to estimate power and type I error (α) for χ^2-test and Fisher's exact test. Simulations at varying degrees of genetic differentiation were repeated 1000 times; the proportion of significant ($P < 0.05$) simulations yielded an estimate of power (α error when $F_{ST} = 0$).

2.3 | Microsatellite genetic diversity

Statistics summarizing allele frequencies, effective number of alleles (N_e), expected heterozygosity (H_E) and observed heterozygosity (H_O) were calculated with the program GENALEX 6.5 (Peakall & Smouse 2012). Estimates of allelic richness (A_r) and inbreeding coefficients (F_{IS}) were calculated with the program FSTAT 2.9.3.2 (Goudet 2001).

2.4 | Genetic differentiation

To assess the degree of genetic differentiation between populations, an estimate of Weir and Cockerham's (1984) F_{ST} was implemented in the program GENEPOP. Fisher's exact probability tests for genetic differentiation for each population pair across all loci was conducted in GENEPOP to evaluate the statistical significance of the observed F_{ST} values. Global F_{ST} and 95% CI were estimated in FSTAT with 20,000 permutations.

2.5 | Genetic structure

The model-based Bayesian clustering analysis within Structure 2.3.4 (Pritchard *et al*. 2000) was used to identify genetic structure and estimate individual membership probabilities (*q*) to genetic clusters (*k*). Initial analyses of genetic structure were performed among all individuals without prior information on the locality of samples. An additional set of analyses included prior information on the locality of samples (LOCPRIOR) that has been recommended to improve the performance of Structure models when working with a limited number of individuals and loci (Hubisz *et al*. 2009). To further maximize clustering performance, Kalinowski (2011) recommended testing realistic values of *k*. Tested values ranged from 1 to 4 (*i.e*., the number of sampled tributaries + 1), with 40 independent replicates performed for each *k* value. In each replicate analysis, Markov Chain Monte Carlo (MCMC) simulations consisted of 750,000 iterations discarding samples from the first 250,000 iterations as burn-in.

Multiple models (n = 16) were run with different parameter combinations (*i.e*., $\alpha = 1/k$ and (un)correlated model selection; Falush *et al*. 2003) following the recommendations of Wang (2017). The default ancestry prior yields more accurate individual assignments to source populations when sample sizes are balanced (Wang 2017). Because the sample size ratios between each combination of source populations was close to 1 ($n_1n_2^{-1} = 1.03$, $n_1n_3^{-1} = 0.94$, $n_2n_3^{-1} = 0.91$), the default ancestry prior was used for each run. For all Structure models, the top 10 replicates (25%) for each k-value were identified based on log likelihood lnPr(X|K) output. Only the top 10 replicates were submitted to Structure Harvester (Earl & vonHoldt 2012) to reduce the probability of spurious results influencing our inference of k and to maximize the probability of identifying optimal alignment (*H*2) using the software program CLUMPP 1.2 (Jakobsson & Rosenberg 2007). Optimal values of k were identified by comparing the highest estimated log likelihood lnPr(X|K) reported by Structure and the ” K method (Evanno *et al*. 2005) reported by Structure Harvester. The software program CLUMPP was used to identify the optimal alignment of estimated cluster membership matrices over the top 10 runs for the chosen k. Plots were visualized using DISTRUCT v 1.1 (Rosenberg 2004).

To evaluate between-population variation with no model-based assumptions and compare membership probability assignments to those of a model-based approach, a discriminant analysis of principal components (DAPC) was run using the R-package (www.r-project.org) adegenet 2.0.1 (Jombart *et al*. 2010). The initial DAPC clustering was run using the full number of principal components (PC) and discriminant functions. To avoid over-fitting the DAPC, the

optimal number of PCs were selected using the cross-validation function xvalDapc using an 80% training set and a 20% validation set. The cross-validation was replicated 1,000 times to identify number of PCs achieving highest mean success and lowest mean-squared error (MSE). An additional DAPC was run using the optimal number of PCs, but only one discriminant function was retained to visualize between-population variation at $k = 2$.

Contemporary migration rates (m) were estimated using a Bayesian assignment method in the program BAYESASS 3.0.1 (Wilson & Rannala 2003) employing a MCMC procedure that does not assume HWE. Reported values of m were used to estimate proportions of non-migrant individuals within each population and evaluate directionality of migration among population pairs within the last two generations. A total of 21^6 MCMC iterations and 5^6 burn-in were used to estimate m and produce convergent trace outputs for five independent runs with varied seed numbers. Delta values for migration rates (m), inbreeding coefficient (f) and allele frequencies (a) were adjusted to attain acceptance rates between 20 and 60% of the total iterations ($m = 0.30$, $f = 0.83$ and $a = 0.54$, respectively). The convergence of the MCMC algorithm was assessed using the software TRACER 1.6 (Rambaut *et al*. 2014) by visually plotting posterior parameter estimates.

3 | RESULTS

3.1 | Microsatellite genetic diversity

A total of 120 individuals were successfully genotyped at eight loci. One locus (Lspn-002-2) was monomorphic in all genotyped individuals and excluded from downstream analyses. Micro-Checker indicated the occurrence of a null allele at locus Lspn-019c in samples collected in the Chena River and at locus Lspn-050 in samples collected at all three sites (Supporting Information Table S3). However, when F_{ST} estimates were adjusted for the presence of putative null alleles, unbiased estimates of F_{ST} were highly similar to original values and the statistical test of population differentiation remained unchanged (Supporting Information Table S4). Global F_{ST} with and without using ENA correction was 0.053 (95% CI 0.021–0.086) and 0.053 (95% CI 0.020–0.089), respectively. Because the putative null alleles appear to have a limited effect on data analyses and subsequent interpretation of results, the original genotypic data was used for downstream analyses.

COLONY identified a total of 26 full-sibling pairs with probabilities ranging from 0.01–0.91. The fewest full-sibling pairs (seven) were identified in the Chena River, while the highest (15) were identified in the Gisasa River. The number of family clusters (*i.e.*, number of male and female parents) was found to be highest in the Chena River and lowest in the Gisasa River (Supporting Information Table S5). These results supported the prediction that relatedness among individuals within each of the three sample sites decreased as the number and distance between sample sites increased. The estimated effective population size (N_e) assuming random mating was 35 individuals (95% CI 22–55). After removing one randomly selected individual

from each full-sibling pair collected from the same tributary, the final dataset contained 94 individuals with 5.17% missing data. A total of 31, 30 and 33 individuals were retained from the East Fork of the Andreafsky, Gisasa and Chena rivers, respectively, with 5.07%, 6.67% and 3.9% missing data, respectively.

Significant deviations from HWE were detected at locus Lspn-019c and Lspn-050 after Bonferroni correction (critical value $P < 0.01$). No linkage disequilibrium (LD) was observed between pairs of loci. Using allele frequencies from 94 individuals genotyped at seven loci, the proportion of significant simulations in POWSIM were above 90% at F_{ST} values e 0.02 (Supporting Information Figure S2), indicating that the final genotypic dataset had the power to identify genetic differentiation when $F_{ST} = 0$, the α error was 0.037 (Supporting Information Table S6).

The number of observed alleles per locus varied from 2 to 5 (Supporting Information Table S7). Overall genetic variation (*e.g.*, A_r, N_e, H_E and H_O) was highest in the Chena River (Table 1). Estimates of allelic richness per population were based on a minimum sample size of 21 individuals and ranged from 2.696 to 2.958. Average values of F_{IS} ranged from 0.079 in the East Fork of the Andreafsky River to 0.195 in the Gisasa River, respectively.

3.2 | Genetic differentiation

Measures of F_{ST} for all population pairs ranged from 0.048 to 0.057 (Table 2), with a global F_{ST} of 0.052 (95% CI 0.022–0.086). Tests of population differentiation remained highly significant among all pairs of populations after Bonferroni correction (critical value $P < 0.05$).

3.3 | Genetic structure

Results for the Structure models without prior information on the locality of samples reported the highest log-likelihood at $k = 1$ for all combinations of model parameters (Supporting Information Table S8). The top model selected by the ” *K* method (α = 0.50, uncorrelated frequency model) identified $k = 2$. Average individual membership coefficients for this model ranged from 0.497 to 0.503, suggesting almost equal proportions of global ancestry between genetic clusters (Supporting Information Figure S1).

The top performing Structure model with the LOCPRIOR parameter (α = 0.50, correlated allele frequency model) identified $k = 3$ as the optimal number of genetic clusters (Figure 2a). This inference was supported by both the highest log-likelihood and ” *K* method (Supporting Information Table S9). The second-best performing model (α = 0.25, correlated frequency model) identified $k = 2$ as the optimal number of clusters and was also supported by both inference methods. The admixture proportions from this model were visualized to investigate the uppermost level of genetic structure among samples (Figure 2b).

After cross-validation, a total of 12 PCs and 2 discriminate functions were retained for the DAPC. The majority of individuals had mixed membership proportions between or among clusters (Figure 3a). The maximum membership probability was > 0.95 in all three clusters, while the proportion of samples successfully assigned to their original clusters ranged from 0.667 in the Chena River to 0.833 in the Gisasa River (Figure 3a). The overall successful proportion of sample assignments was 0.755. When only one discriminate function was retained, the proportion of samples successfully assigned to their original clusters ranged from 0.333 in the Gisasa River to 0.758 in the Chena River. The overall proportion of successful assignments was 0.574 with maximum membership probabilities ranging from 0.489 in the East Fork of the Andreafsky River to 0.998 in the Chena River. Densities of individuals on a given discriminant function were not well differentiated between the Gisasa and the East Fork of the Andreafsky rivers (Figure 3b).

Recent migration rates (m) among population pairs were estimated using BAYESASS. The proportion of non-migrant individuals ranged from 0.880 in the East Fork of the Andreafsky River to 0.850 in the Chena River (Figure 4). The lowest proportion of migrants (m = 0.03) was detected from the East Fork of the Andreafsky River into the Chena River. Estimated migration among populations was symmetrical, with the highest estimated migration rates occurring from upstream to downstream among all three localities.

4 | DISCUSSION

This study is the first examination of *Lethenteron* spp. genetic diversity and population genetic structure in the eastern part of their geographic distribution. Results are largely in agreement with observations of gene flow among *Lethenteron* spp. populations throughout their western range (Yamazaki *et al*. 2011, 2014; Artamonova *et al*. 2011, 2015). The values of F_{ST} (0.048–0.052) reported in this study were indicative of restricted, but continuous gene flow among sampled tributaries and fell within the range of pairwise F_{ST} values reported for anadromous *L. camtschaticum* in eastern Eurasia estimated using nuclear microsatellite markers (–0.049 to 0.171; Yamazaki *et al*. 2011, 2014). The uppermost level of population genetic structure ($k = 2$) identified by Structure indicated historic gene flow between two ancestral lineages with a latitudinal orientation. Despite spanning different spatial scales, the uppermost level of population genetic structure observed in this study was similar the pattern observed in eastern Eurasia; samples across a broad geographic range exhibited admixed ancestry from two ancestral lineages that largely clustered on a latitudinal gradient (Yamazaki *et al*. 2014). According to these authors, both homing behaviour (*i.e*., lack of natal philopatry) and extensive migration capabilities facilitate contemporary and historic gene flow among anadromous populations of *L. camtschaticum*. Although the subsequent level of population genetic structure ($k = 3$) identified in this study exhibited geographic structure (samples within tributaries largely clustered together despite admixture), the admixed ancestry of individuals at multiple levels of population genetic substructure support previous hypothesizes on homing behaviour in lampreys.

Genetic homogenization of widely dispersed anadromous lamprey populations has been attributed to a lack of natal homing (Bryan *et al*. 2005; Waldman *et al*. 2008; Spice *et al*. 2012; Yamazaki *et al*. 2014). Previous research indicates that sea lamprey *P. marinus* and Pacific lamprey *E. tridentatus* do not exhibit natal homing during spawning migrations, possibly due to extensive migrations for marine feeding and involuntary movements while parasitically attached to marine hosts (Bergstedt & Seelye 1995; Bryan *et al*. 2005; Waldman *et al*. 2008; Hatch & Whiteaker 2009; Spice *et al*. 2012). In addition, stream-resident larvae release bile acids that act as a migratory pheromone to guide sexually maturing, adult lampreys to suitable spawning habitat independent of natal provenance (Moore & Schleen 1980; Bjerselius *et al*. 2000; Sorensen *et al*. 2003; Vrieze *et al*. 2011; Yun *et al*. 2011). No studies have verified the role of larval pheromones on migratory behaviour in *L. camtschaticum*, but it is suspected to be similar among lamprey genera (Moser *et al*. 2015). If larval migratory pheromones contribute to a lack of natal homing in *Lethenteron* spp., an increased portion of migrant individuals would be expected to occur within sampled tributaries. This perception is consistent with our estimates of contemporary migration. Reciprocal migration occurred among sampled sites. Interestingly, although migration was symmetric, fewer individuals were estimated to have migrated from the southernmost (East Fork of the Andreafsky River) into the northernmost (Chena River) tributaries. This migration pattern was also observed in Eurasian populations where the two northernmost populations had a reduced number of migrants when compared to the remaining southern populations (Yamazaki *et al*. 2014). The reduced number of upstream migrants in this

study appears to reflect slightly elevated measures of genetic differentiation between the two most distant tributaries. If this migratory pattern occurred over historic periods, it may contribute to the geographic genetic substructure reported in this study.

Because the microsatellites used in this study were isolated and optimized from a resident, nonparasitic brook lamprey *Lethenteron* sp. N from Japan, there is a potential for ascertainment bias in this study. Microsatellite loci have been reported to be longer and more variable in the species from which they are developed, despite successful cross-species amplification (Ellegren *et al*. 1995; Vowles & Amos 2006). The loci used in this study successfully amplified in both *Lethenteron* sp. N and anadromous adult and larval *L. camtschaticum* collected across a broad region of eastern Eurasia (Takeshima *et al*. 2005; Yamazaki *et al*. 2011, 2014). Furthermore, the numbers of alleles in *L. camtschaticum* and measures of genetic diversity were greater or equal to those reported for *Lethenteron* sp. N. (Takeshima *et al*. 2005). Although this is an indication that ascertainment bias did not affect the results of previous studies, the number of alleles observed in this study were consistently less than or equal to the number observed among anadromous *L. camtschaticum* from eastern Eurasia (Yamazaki *et al*. 2014). This may reflect a historical event that has reduced overall measures of genetic diversity among *Lethenteron* spp. in the eastern part of their geographic distribution. It could also indicate ascertainment bias or limited sampling efforts, which could bias comparisons of F_{ST} between western and eastern portions of the species range. However, measures of allelic richness and reported values of F_{ST} within tributaries were within or slightly below the ranges

observed by Yamazaki *et al*. (2014), suggesting that ascertainment bias had limited effects on the interpretation of results. Interestingly, the allele sizes reported in both studies for the same microsatellite loci did not overlap. Although there are standardization issues and we are unable to directly compare the two datasets, this brings into question the connectivity of *Lethenteron* spp. throughout their geographic distribution. It is possible that the Bering Sea acts as a barrier to gene flow between eastern and western populations of *L. camtschaticum*. Similarly, measures of genetic differentiation among populations of *P. marinus* along the coast of North America and Portugal indicate that *P. marinus* separated by the Atlantic Ocean exhibit reduced gene flow (Rodriguez-Munoz *et al*. 2004; Bryan *et al*. 2005). This highlights the need for an unbiased set of standardized markers to characterize patterns of genetic diversity and population genetic structure among *L. camtschaticum* throughout their geographic range.

The small spatial scale of sampling efforts within the East Fork of the Andreafsky and Gisasa rivers (3.5 and 0 rkm), compared with efforts in the Chena River (149 rkm), may have affected our interpretation of results. The larger geographic distribution among sites within the Chena River may explain why measures of genetic diversity were highest among samples from the northernmost tributary, approximately 950 rkm from the mouth of the Yukon River drainage. This contrasts patterns generally observed within upstream reaches of branched tributaries, in which measures of genetic diversity and allelic richness are reduced when compared with lower reaches (Morrissey & Kerchkov 2009). Higher genetic diversity would be expected in tributaries lower in the drainage that have higher numbers of migrants from greater numbers of sources as

compared with more isolated upper tributaries. With a lower number of collection sites spanning a limited area in the East Fork of the Andreafsky and Gisasa rivers, the genetic diversity among individuals within these two tributaries may not have been represented as well as had been for the Chena River. An effect of sampling effort may also be indicated by the relatedness we observed among individuals. The highest number of families (*i.e.*, mothers and fathers) and lowest number of estimated full siblings were identified within the Chena River. In contrast, the highest number of estimated full siblings were identified within the Gisasa River where all individuals were collected from one site within a 24 h period. This result demonstrated a correlation between spatial-scale sampling and relatedness. To reduce the probability of sampling the same families, we recommend samples should be collected throughout the reach of a tributary, since a smaller spatial scale of sampling efforts (≤ 3.5 rkm) correlated to a greater number of estimated full siblings and smaller number of families. Given our methodology to reduce relatedness bias, our study effectively minimized this sampling bias and likely provided evidence of population genetic structure that emerged among sites. An alternative hypothesis to explain the population genetic structure observed in this study is that resident, non-parasitic life-history variants contributed to the values of F_{ST} reported in this study.

Significant measures of genetic differentiation have been reported among nonparasitic resident lamprey populations of the same species, despite high levels of gene flow between parasitic and non-parasitic forms (Espanhol *et al*. 2007; Blank *et al*. 2008; Mateus *et al*. 2011; Boguski *et al*. 2012; Docker *et al*. 2012; Bracken *et al*. 2015; Rougemont *et al*. 2015). Although

the scope of this study did not include an explicit examination of genetic differentiation between *L. camtschaticum* and *L. alaskense* within the Yukon River drainage, adult parasitic and non-parasitic forms are known to occur sympatrically in the Chena River (Sutton 2017). Studies have repeatedly shown that diagnostic morphological characteristics cannot distinguish *L. camtschaticum* and *L. alaskense* during their larval stage (Kucheryavyi *et al*. 2007; Artamonova *et al*. 2011; Nazarov *et al*. 2011; Renaud 2011; Sutton 2017). However, it has been suggested that larval body length may be an indicator of life history, since larvae ≥180 mm have only been reported for resident, non-parasitic forms (Mundahl & Sagan, 2005; Docker 2009; Sutton 2017). A portion of samples collected from the Chena River and used in this study were > 180 mm. If body length is an indicator of life history, then it is likely that both *L. camtschaticum* and *L. alaskense* were sampled within the Chena River. This life-history variation may explain the reduced number of migrants into the Chena River from other tributaries because brook lampreys are believed to be more sedentary when compared with anadromous counterparts. The occurrence of *L. alaskense* within the East Fork of the Andreafsky and Gisasa rivers is unknown, but all larvae from the East Fork of the Andreafsky River were < 150 mm, increasing the probability that only parasitic forms were sampled. Nonparasitic adults have not been observed in these tributaries and the precise timing and location of spawning grounds are currently unknown. With these data, we recommend that adult *Lethenteron* spp. are sampled to address questions of divergence between species pairs within Alaska tributaries and compare that to patterns of lampreys worldwide.

Sampled *Lethenteron* spp. within the Yukon River drainage exhibit reduced, but ongoing, levels of gene flow and hierarchical genetic structure. The use of microsatellites in this study, which are putatively neutral markers, precludes inference of adaptation among sampled *Lethenteron* spp. within the Yukon River drainage. However, adaptive variation has been related to adult body size, run timing and geography in populations of *E. tridentatus*, despite largely homogenized variation at neutral markers (Goodman *et al*. 2008; Spice *et al*. 2012; Hess *et al*. 2013, 2014). A diagnostic single nucleotide polymorphism associated with run timing and migration distance was reported for *E. tridentatus* in the Columbia River basin, suggesting adaptive variation is an underlying mechanism driving migration distance in individuals (Hess *et al*. 2014). If reductions in upstream migrants occurred historically within the Yukon River drainage, processes of genetic drift and selection could have given rise to local adaptation and increased measures of genetic differentiation in the absence of physical barriers (*i.e*., dams). Because anadromous *L. camtschaticum* are harvested for both subsistence and commercial purposes in the lower Yukon River drainage, identifying mechanisms underlying genetic differentiation is of interest to fisheries managers. Previous research efforts clearly highlight the necessity of utilizing both neutral and adaptive markers to further understand patterns of divergence and identify genetic drivers of population genetic structure among *E. tridentatus* within the Columbia River basin (Spice *et al*. 2012; Hess *et al*. 2013, 2014). More effort is required to resolve the relative importance of the factors (*e.g*., spatial-scale, parasitism, adaptive

traits, *etc*.) driving the patterns of genetic variation observed in the eastern portion of the range of the species complex, *Lethenteron* spp.

This study tested the null hypothesis of no population structure among *Lethenteron* spp. aggregations found at three sites in the Yukon River drainage. Results indicated reduced, but continuous gene flow among sampled tributaries facilitated by reciprocal migration among sampled sites. Measures of genetic differentiation and population genetic structure were similar to those observed among *L. camtschaticum* in the western portion of their distribution. These results are inconsistent with high-fidelity natal homing and are in agreement with expectations based on other species of lamprey. In addition, this study demonstrated that sampling efforts over a small spatial scale correlated to a greater number of estimated full siblings and smaller number of families suggesting sampling efforts should occur throughout the reach of a tributary to minimize relatedness bias. Future collection efforts should include additional major Alaskan river drainages to provide a comprehensive evaluation of population genetic structure and further characterize diversity of *Lethenteron* spp. throughout the state with the goal of improving our understanding of the broad-range population genetic structure of *Lethenteron* spp. complex throughout its geographic distribution.

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SUPPORTING INFORMATION

Supporting information can be found in the online version of this paper.

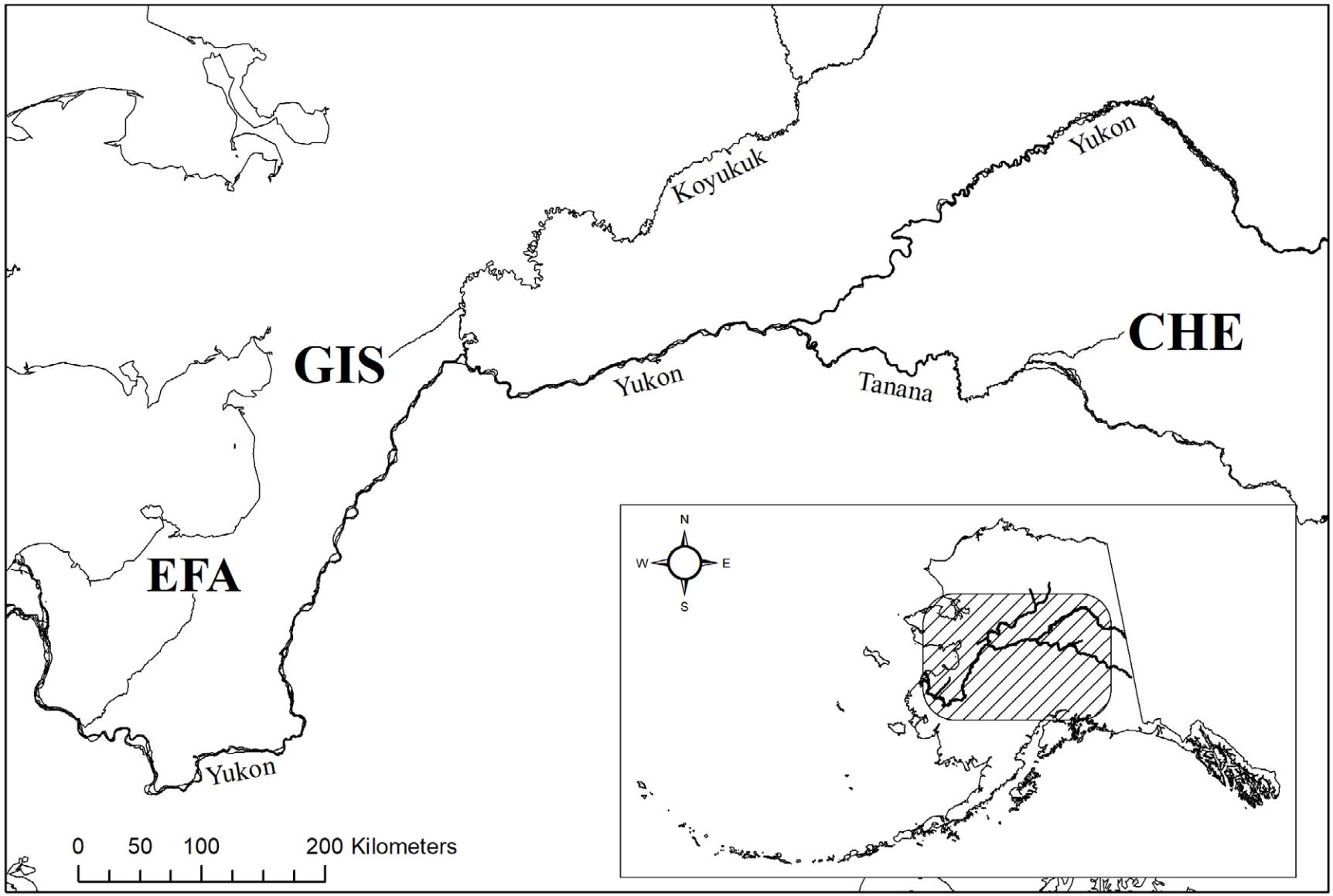

FIG. 1

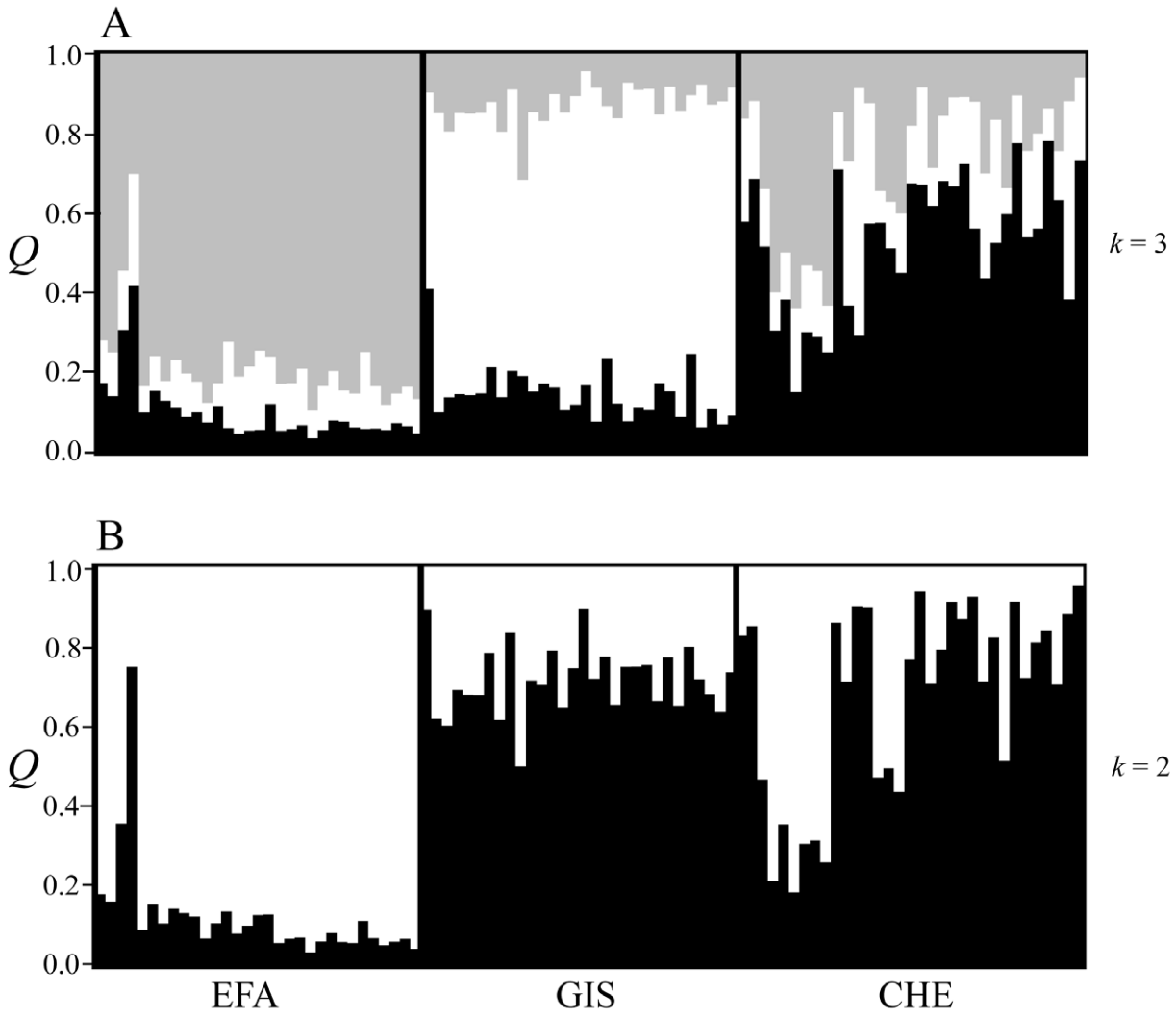

FIG. 2

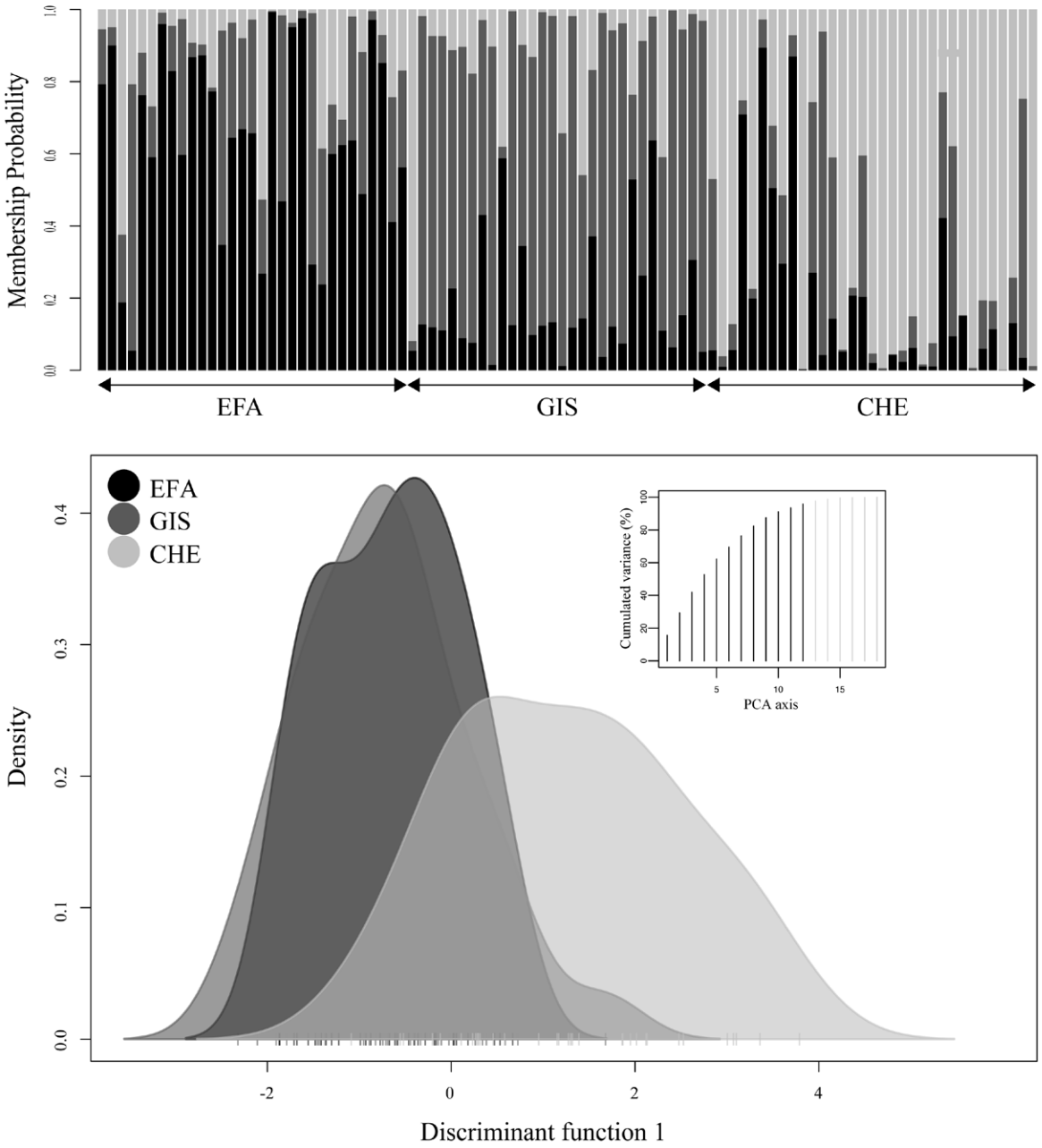

FIG. 3

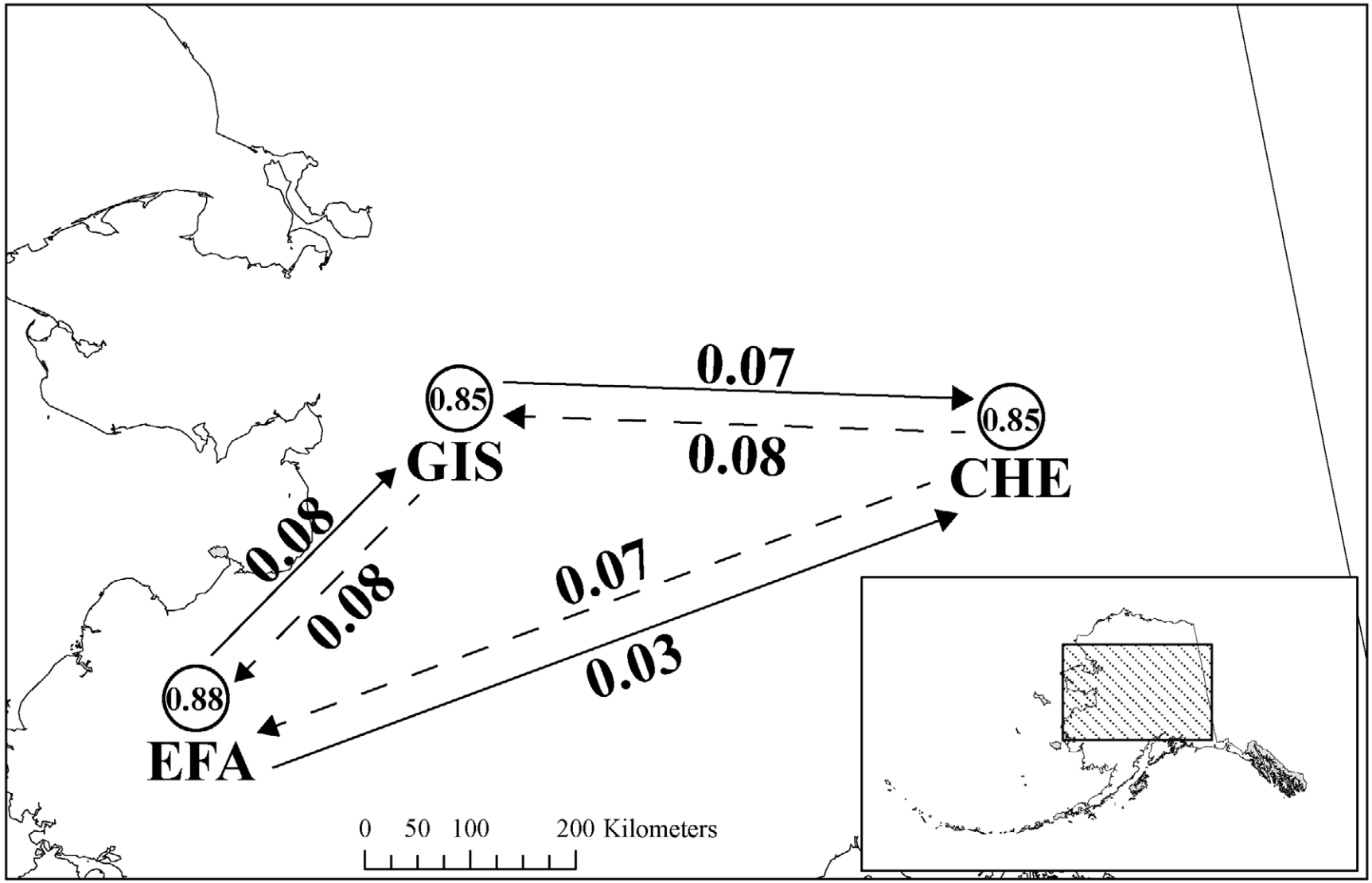

FIG. 3

Figure Captions

Figure 1. Map of rivers sampled for *Lethenteron* spp. within Yukon River drainage, Alaska. EFA, East Fork of the Andreafsky River; GIS, Gisasa River; CHE, Chena River.

Typesetter

1 Change Kilometers to km.

2 Replace ornate compass rose with simple latin cross surmounted by N.

Figure 2. Structure bar plots generated from *Lethenteron* spp. microsatellite data. (a) Global analysis of population structure with prior information on the locality of samples (LOCPRIOR; $K = 3$); (b) Global analysis of population structure with prior information on the locality of samples (LOCPRIOR; $K = 2$). EFA, East Fork of the Andreafsky River; GIS, Gisasa River; CHE, Chena River.

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1 Change A, B to (a), (b).

Figure 3. Results of discriminate analysis of principal components (DAPC). (a) Barplot of individual *Lethenteron* spp. along the two first discriminant functions ($K = 3$). (b) Density of individual *Lethenteron* spp. utilizing a single linear function ($K = 2$). EFA, East Fork of the Andreafsky River; GIS, Gisasa River; CHE, Chena River.

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1 Label top panel (a) and lower panel (b).

Figure 4. Contemporary migration rates (*m*) among *Lethenteron* spp. populations estimated using BAYESASS. Numbers within circles denote the proportion of non-immigrants within populations. Arrows indicate the direction of gene flow and the corresponding *m* values.

TABLE 1 Summary statistics for *Lethenteron* spp. collected from three Alaskan rivers.

	East Fork of the Andreafsky River	**Gisasa River**	**Chena River**
n	31	30	33
A_r	2.696	2.768	2.958
N_e	2.714	2.857	3.143
H_E	0.364	0.385	0.515
H_O	0.342	0.316	0.436
F_{IS}	0.079	0.195	0.170

n, Number of individuals genotyped at all seven loci; A_r, allelic richness; N_e, effective number of alleles; H_E, expected heterozygosity; H_O, observed heterozygosity; F_{IS}, inbreeding coefficient.

TABLE 2 Pairwise F_{ST} values for sampled *Lethenteron* spp. population pairs. Bold F_{ST} values are significant tests of population differentiation after Bonferroni correction (P, 0.0166).

	East Fork of the Andreafsky River	**Gisasa River**	**Chena River**
East Fork of the Andreafsky River	–		
Gisasa River	**0.050**	–	
Chena River	**0.056**	**0.052**	–