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RESOURCE ARTICLE

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A new genomic resource to enable standardized surveys of SNPs across the native range of brook trout (*Salvelinus fontinalis*)

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Abstract

Understanding how genetic diversity is distributed across spatiotemporal scales in species of conservation or management concern is critical for identifying large-scale mechanisms affecting local conservation status and implementing large-scale biodiversity monitoring programmes. However, cross-scale surveys of genetic diversity are often impractical within single studies, and combining datasets to increase spatiotemporal coverage is frequently impeded by using different sets of molecular markers. Recently developed molecular tools make surveys based on standardized single-nucleotide polymorphism (SNP) panels more feasible than ever, but require existing genomic information. Here, we conduct the first survey of genome-wide SNPs across the native range of brook trout (*Salvelinus fontinalis*), a cold-adapted species that has been the focus of considerable conservation and management effort across eastern North America. Our dataset can be leveraged to easily design SNP panels that allow datasets to be combined for large-scale analyses. We performed restriction site-associated DNA sequencing for wild brook trout from 82 locations spanning much of the native range and domestic brook trout from 24 hatchery strains used in stocking efforts. We identified over 24,000 SNPs distributed throughout the brook trout genome. We explored the ability of these SNPs to resolve relationships across spatial scales, including population structure and hatchery admixture. Our dataset captures a wide spectrum of genetic diversity in native brook trout, offering a valuable resource for developing SNP panels. We highlight potential applications of this resource with the goal of increasing the integration of genomic information into decision-making for brook trout and other species of conservation or management concern.

KEYWORDS
conservation genomics, fisheries management, native brook trout, SNP panels

1 | INTRODUCTION

The distribution of genetic diversity at local to broad spatial scales and at past and present points in time is rarely understood, despite the recognized importance of genetic diversity to species conservation (Blanchet et al., 2017; Habel et al., 2014; Schwartz et al., 2007). Genetic studies to inform species conservation and management frequently comprise singular surveys of local populations using unique sets of molecular markers, limiting the degree to which spatially or temporally discrete populations can be directly compared. This approach precludes inferences about evolutionary processes shaping conservation status across spatiotemporal scales (Dodds et al., 2021; Heffernan et al., 2014; Schmidt et al., 2023) and impedes large-scale monitoring of genetic biodiversity to achieve global conservation targets (Hoban et al., 2014, 2021). In particular, knowledge of how broad-scale evolutionary processes respond to processes occurring at local scales, and vice versa, is critical for a mechanistic understanding of factors shaping biodiversity and predicting biodiversity responses to ecological and environmental change. Such relationships are indiscernible from studies of local populations at single points in time, limiting the spatial and temporal scope of conservation and management decision-making.

Recently developed genomic tools that apply panels of single-nucleotide polymorphisms (SNPs) to perform cost-effective, high-throughput genotyping offer practical solutions for surveying genetic diversity across spatiotemporal scales by making standardized surveys among independent studies more feasible than ever (Meek & Larson, 2019). For example, amplicon sequencing (e.g. GTseq; Campbell et al., 2015) and sequence capture (e.g. RAD capture; Ali et al., 2016) can be used to efficiently genotype previously identified regions of the genome, are easily transferable across research efforts and do not require specialized equipment once developed. Additionally, because these methods are typically used to target SNPs, genotyping error resulting from inconsistent allele calls among studies is low relative to other marker types (e.g. microsatellites; Moran et al., 2006). However, as with all surveys of allele frequencies, the degree of ascertainment bias associated with evaluating previously identified loci in additional individuals representing spatially or temporally discrete populations must be carefully considered. Efforts to develop genomic resources that comprise high-resolution surveys of genome-wide diversity across spatial or temporal scales are now necessary for enabling rapid and cost-effective development of SNP panels to inform conservation and management. Such resources help to reduce barriers to integrating genomic methods into widespread practice and facilitate data integration to produce greater spatiotemporal coverage than is often otherwise practical.

Here, we develop a genomic resource that can be leveraged by users to easily design SNP panels for investigating questions of conservation and management concern across the native range of an imperilled species. We also illustrate the potential for this resource to resolve relationships across spatial scales. We develop this resource for native populations of brook trout (*Salvelinus fontinalis*), the only charr native to much of eastern North America, from northern Quebec (Canada) to Georgia (United States; U.S.) and the Laurentian Great Lakes. Brook trout prefer pristine cold-water habitat, therefore this species is often considered a sentinel of environmental quality and ecosystem health (Power, 1980). In recent decades, declines in the abundance of native brook trout have resulted in the reduction of self-sustaining populations in nearly one third of sub-watersheds along the U.S. east coast and across the U.S. portion of the Lake Superior basin (EBTJV, 2006; Hudy et al., 2008; USFWS, 2016). Among other factors, contemporary populations of brook trout are increasingly impacted by degraded environmental conditions, such as habitat fragmentation (Letcher et al., 2007; Whiteley et al., 2013) and rising stream temperatures (Letcher et al., 2015; Meisner, 1990a, 1990b), including due to a changing global climate (Andrew et al., 2022; EBTJV, 2006; Hudy et al., 2008; Merriam et al., 2019).

Substantial resources have been invested across the native range of brook trout to generate information to guide conservation and management decision-making. These efforts include numerous genetic studies to assess diverse relationships in native populations, including characterizing the spatial distribution of genetic diversity in local waterways (Hargrove et al., 2022; Morgan et al., 2021; Nathan et al., 2020; Stott et al., 2010), quantifying introgression of domestic alleles as a result of stocking hatchery-reared fish (Erdman et al., 2022; Lehnert et al., 2020; Létourneau et al., 2018; White et al., 2018) and identifying landscape variables correlated with genetic diversity (Castric et al., 2001; Torterotot et al., 2014; Whiteley et al., 2013; Wood et al., 2014). So far, these studies primarily comprise surveys of neutral genetic diversity in local populations or meta-populations representing a limited portion of the native species range. Exceptions include a survey of microsatellites in brook trout across much of the native range by Kazyak et al. (2022), though only a few Canadian populations were sampled and resulting insights were limited to neutral relationships. Ferchaud et al. (2020) surveyed SNPs representing neutral and adaptive regions of the genome in brook trout across the Canadian extent of the native range, but analyses were not extended to U.S. populations. These studies supply natural resource managers with valuable information for conservation and management. However, information on both neutral and adaptive relationships in populations across the native range remains scarce, and the ability to directly integrate existing datasets is constrained by studies that largely comprise surveys of unique sets of molecular markers.

We use native populations of brook trout to accomplish the following objectives: (1) identify genome-wide SNPs in wild brook trout across much of the native species range and domestic brook trout from major hatchery strains used in rehabilitation efforts, (2) explore the potential for identified SNPs to resolve relationships among wild populations and between wild and domestic populations at local to continental spatial scales, (3) direct users on how to design SNP panels from the putatively neutral and adaptive SNPs identified here, and (4) equip decision makers with guidance on the application of genomic information to address key conservation and management questions. Previous genetic studies of native brook trout report extensive and hierarchical population structure (e.g. Danzmann et al., 1998; Ferchaud et al., 2020; Kazyak et al., 2022; Mamoozadeh et al., 2023). Therefore, we expected the SNPs identified in this study to be capable of resolving evolutionary lineages reflecting phylogeographic history at a broad spatial scale and populations corresponding with tributaries at a local spatial scale. Additionally, a growing number of genetic studies report limited introgression of domestic alleles into wild populations despite supplementation with domestically raised individuals (e.g. Annett et al., 2012; Lehnert et al., 2020; White et al., 2018). Therefore, we expected the SNPs identified here to largely be capable of distinguishing wild and domestic individuals. By accomplishing our objectives, we establish a new resource for native brook trout that enables standardized surveys of genomic variation across spatial scales anywhere across the native species range. Developing this resource for native brook trout also offers an illustrative example for creating analogous resources in additional species.

2 | MATERIALS AND METHODS

2.1 | Sample collection

We analysed wild brook trout across much of the native species range, including locations across Canada, the U.S. and the Great Lakes, that were either analysed in previous genetic studies or newly analysed in this study (Table S1). We acquired samples through a collaborative effort by several academic institutions and natural resource agencies. Samples primarily consisted of fin clips collected from brook trout in lakes, rivers and streams captured using electrofishing, hook-and-line angling or gillnets. Detailed sampling methods for most wild-caught individuals are described in Aunins et al. (2015), Ferchaud et al. (2020), Fraser and Bernatchez (2005), Hoxmeier et al. (2015), Kazyak et al. (2018), Kazyak et al. (2022), Morgan et al. (2021), Nathan et al. (2020), USFWS (2016) and Wood et al. (2014). Wild individuals from the southernmost portion of the native species range (Georgia, North Carolina and Tennessee) were preferentially sampled from populations known to exhibit limited introgression from stocked domestic individuals. Wild individuals from elsewhere across the native range were sampled from populations without prior knowledge of domestic introgression. Sampling efforts for wild-caught brook trout occurred between the years 2000 and 2019.

We also acquired samples for many of the domestic strains of brook trout historically or currently used in wild rehabilitation efforts. Domestic brook trout have been stocked in locations across the native species range, primarily to increase recreational fishing opportunities amid declining wild populations. The earliest stocking efforts began in the 1800s and used domestic strains derived from wild populations in the northeastern U.S. Although some contemporary stocking efforts still use domestic strains derived from nonlocal sources of broodstock, locally derived strains are increasingly used for wild rehabilitation efforts. We collected samples of domestic individuals from state and federal fish hatcheries located across the native species range for analysis in this study (Table S2).

2.2 | RAD library preparation and sequencing

We extracted total genomic DNA for most tissue samples using the magnetic bead-based protocol described by Ali et al. (2016). DNA for remaining samples was isolated using a Gentra Puregene Tissue Kit (Qiagen), DNEasy 96 Blood and Tissue Kit (Qiagen), E-Z 96 Tissue DNA Kit (Omega Bio-Tek) or a modified version of the salt extraction protocol described by Aljanabi and Martinez (1997). We quantified DNA isolations using a BioTek FLx800 microplate reader (BioTek Instruments) and Quant-iT PicoGreen assays (Thermo Fisher Scientific). We selected DNA isolations that exhibited high quality and quantity for two to eight individuals from each wild population or domestic strain to include in restriction site-associated DNA sequencing (RADseq) libraries. This experimental approach allowed us to survey the range of genetic diversity represented by a larger number of populations across the native species range. RADseq libraries were prepared according to Ali et al. (2016), but with the following modifications: we used 100 ng of DNA per individual for restriction enzyme digestion, and ligation reactions were incubated for a period of 12 h. We sequenced six libraries on two lanes of an Illumina HiSeq 4000 next-generation sequencing platform at the Michigan State University Research Technology Support Facility. DNA from individuals with low read counts was prepared as a new RADseq library and sequenced on a single lane as described earlier. All libraries underwent PE150 sequencing.

2.3 | SNP discovery and quality filtering

We de-multiplexed the FASTQ files that resulted from sequencing using the *process_radtags* module of *Stacks* v1.4 or v2.4 (Catchen et al., 2011, 2013; Rochette et al., 2019). We included options for a barcode mismatch threshold of one base pair and to remove reads with uncalled bases or low-quality scores. We mapped demultiplexed reads to the chromosome-level *Salvelinus* sp. reference genome assembly produced by Christensen et al. (2018, but see Christensen et al., 2021). Reads were mapped to the *Salvelinus* sp. reference genome using the BWA-MEM alignment algorithm implemented in *BWA* v0.7.17 (Li, 2013). We used *SAMtools* v1.9 (Li et al., 2009) to exclude secondary and supplementary alignments and alignments with quality scores <30. Quality filtered alignments were then analysed in the *gstacks* module of *Stacks* v2.4 to call SNPs using default settings. We exported genotypes as a VCF file using the *populations* module of *Stacks*.

We performed quality filtering of the dataset exported from *Stacks* using *VCFtools* v0.1.15 (Danecek et al., 2011). Loci and individuals missing an excessive proportion of genotypes were removed from the dataset by excluding SNPs missing $\geq 75\%$ of genotypes followed by individuals missing ≥90% of genotypes. We then removed genotypes with quality scores ≤30 and read depths <6. To reduce the probability of falsely heterozygous genotypes, we used *jvarkit* to remove genotypes with an allele balance >0.80 or <0.20. We then excluded SNPs with a minor allele count <3. Finally, SNPs and individuals exhibiting large proportions of missing genotypes were iteratively removed to produce a dataset with individuals genotyped at >70% of loci and SNPs genotyped at >80% of individuals.

We performed additional filtering of our dataset by removing paralogous loci and loci rendered monomorphic by the filtering process. Paralogs were identified by assessing the proportion of heterozygotes and deviations in read ratios within heterozygotes using *HDplot* (McKinney et al., 2017). We excluded SNPs corresponding with >50% heterozygotes or read ratio deviations >|4|. Monomorphic SNPs were removed using the *dartR* v1.1.6 (Gruber et al., 2018) package in *R* (R Core Team, 2021). Finally, to reduce the probability of linkage disequilibrium among loci, we used a custom *R* script to retain only the first SNP on each RAD locus. The resulting dataset comprised brook trout sampled from wild populations and domestic strains and is hereafter referred to as the 'full dataset'. This dataset can be used to easily design SNP panels for surveying a standardized set of SNPs in focal populations. We demonstrate the utility of this dataset by exploring its ability to resolve relationships among brook trout at local to continental spatial scales.

2.4 | Relationships of wild and domestic populations across spatial scales

We used principal component analysis (PCA) and discriminant analysis of principal components (DAPC) to assess the ability of the SNPs identified here to resolve major genetic groups of brook trout apparent at a continental scale that spanned eastern North America. These multivariate methods efficiently summarize complex genetic information without strong assumptions about an underlying population genetic model (Jombart et al., 2009, 2010). We used PCA to summarize overall variability among individuals. PCA was performed using *adegenet* v2.1.1 (Jombart, 2008) with centred and non-scaled allele frequencies. We used DAPC to assess relationships between groups of individuals and hierarchical structure among groups. Groups were defined prior to DAPC using sequential *K*-means clustering of principal components (PCs). The optimal number of PCs to include in DAPC was determined by evaluating cluster reassignment probabilities. We used Bayesian information criterion (BIC) calculated for each value of *K* to infer the most likely range of *K* in results from DAPC analyses. Both *K*-means clustering and DAPC were performed in *adegenet*. We performed PCA and DAPC analyses twice, once using the full dataset and a second time using a dataset limited to brook trout sampled from wild populations.

We used results from multivariate analyses performed at a continental scale to delineate major genetic groups of brook trout. We then used these groups to explore relationships among wild populations and between wild and domestic populations at regional to local scales. We performed PCA and DAPC within each major genetic group as described earlier. These analyses were performed twice for each group, once using datasets that included both wild and domestic individuals and a second time using datasets limited to wild individuals.

2.5 | Private genetic variation

To further understand the degree of genetic distinctiveness among the brook trout analysed here, we quantified private variation within major genetic groups of brook trout identified from multivariate analyses. In regions where brook trout exhibit larger levels of private genetic variation, potentially higher degrees of ascertainment bias may be possible when using SNP panels derived from our dataset. We used *dartR* to determine the number of private alleles within each genetic group compared to all other genetic groups. We also calculated the number of loci within each genetic group that exhibit fixed allelic differences compared to all other genetic groups.

2.6 | Outlier loci

We conducted exploratory analyses to determine whether the SNPs identified in this study include loci that appear as outliers relative to neutral population structure and thus may be candidates to include in SNP panels aimed at more rigorous explorations of adaptive genetic relationships. We performed outlier detection analyses using *pcadapt* v4.1.0 (Luu et al., 2017) and *BayeScan* v2.1 (Foll & Gaggiotti, 2008). *Pcadapt* assumes candidate loci are outliers with respect to population structure as ascertained by PCA. We determined the number of PCs to retain for *pcadapt* analyses by visualizing scree plots of PCA eigenvalues. *BayeScan* uses differences in allele frequencies among populations to identify candidate outlier loci. We performed *BayeScan* analyses using prior odds for the neutral model of 2:1, 20 pilot runs, a burn-in of 25,000 iterations followed by 25,000 additional iterations and a thinning interval of 10. Outlier detection analyses were performed using a dataset comprising wild individuals from the full dataset, where individuals were organized by major genetic group for the *BayeScan* analysis. We also performed outlier detection analyses for wild individuals within each major genetic group, where for *BayeScan* analyses we organized individuals by sampling site since results from multivariate analyses indicated that population structure generally corresponded with sampling location. In results from both *pcadapt* and *BayeScan*, we retained loci with expected false discovery rates (FDR) <0.05, then generated a final list of candidate outliers that comprised loci identified using both methods. A minor allele frequency threshold of 0.05 was implemented prior to outlier detection analyses to minimize bias from SNPs with minor allele frequencies altered by analysing a subset of the full dataset.

2.7 | Example SNP panel design

Our dataset can be used to design SNP panels for various applications. For example, users interested in exploring relationships among brook trout populations from a particular region within the native species range may design a panel that comprises loci most effective at differentiating populations within that region. To demonstrate this application, we tested SNP panel designs for the Northern and Southern major genetic groups identified in this study. We used *HIERFSTAT* (Goudet, 2005) to rank SNPs by F_{ST}, then selected the 500 and 5000 SNPs that exhibited the largest F_{ST} values. This ranking and locus selection were conducted separately for the Northern and Southern groups. We tested panels comprising 500 and 5000 SNPs within each group because these numbers of loci are practical for GTseq and RAD capture, respectively, though both GTseq and RAD capture can be used to target more or fewer loci (see guidelines in Meek & Larson, 2019). We performed PCA as described earlier to test the ability of the SNP panels we identified based on ranked F_{ST} to resolve the major relationships apparent within each group based on the full dataset. We also used PCA to test the ability of the SNPs identified within the Northern group to resolve relationships within the Southern group, and vice versa. The purpose of this particular analysis was to assess the ability of a SNP panel designed for populations in one geographic region to resolve relationships among populations in another geographic region. Finally, we used PCA to compare the ability of SNPs randomly selected within the Northern and Southern groups (e.g. without taking F_{ST} into account) to resolve major relationships within each group.

2.8 | Comparison of SNPs versus microsatellites

We used the SNPs identified in this study and microsatellites surveyed by Kazyak et al. (2022) to compare the ability of SNPs versus microsatellites to resolve relationships among wild populations of brook trout. We analysed data subsets comprising individuals from locations sampled in both studies. We also compared individuals sampled from nearby locations on the same tributary. Although some individuals were identical between the SNP and microsatellite datasets, many were not, and were also sampled in different years. We evaluated relationships at a continental scale by comparing brook trout from all available sampling sites ($n=20$ sites), which spanned a broad extent of the native species range. We also evaluated regional- and local-scale relationships by comparing brook trout from sampling sites in two geographic regions: (1) a southern Appalachian region encompassing four sites and (2) a mid-Atlantic region encompassing seven sites. We performed DAPC as described earlier and compared results between SNPs and microsatellites. For analyses based on microsatellites, we performed DAPC using all available individuals from Kazyak et al. (2022), and a subset of randomly selected individuals from each site to produce sample sizes matching the SNP dataset.

3 | RESULTS

3.1 | SNP dataset

We sequenced a total of 402 wild and domestic brook trout in this study. Quality filtering of the dataset exported from *Stacks* resulted in a dataset comprising 267 individuals and 24,337 SNPs (Table S3). This dataset is hereafter referred to as the 'full dataset' and consisted of 201 wild-caught brook trout from 82 sites [1–6 individuals per site (mean = 2); Table S1; Figure 1a] and 66 domestic brook trout representing 24 hatchery strains [1–6 individuals per strain (mean = 3); Table S2]. Individuals in the full dataset were missing an average of 8.10% (SD = 7.88%) of genotypes. Mean coverage per SNP per individual was 30.96 (SD = 12.42).

The *Salvelinus* sp. genome assembly provided an effective reference for the brook trout analysed here. An average of 97.34% (SD = 2.95) of reads mapped to the reference genome; 70.12% (SD = 5.06) of these reads remained after quality filtering read alignments. SNPs comprising the full dataset were distributed throughout the *Salvelinus* sp. reference genome (Figure S1). A total of 20,055 SNPs (82.4%) were located on assembled chromosomes, which contained an average of 514 SNPs (range = 56–1194 SNPs) per chromosome. The distance between neighbouring SNPs on chromosomes was 75,185 bp on average. Remaining SNPs (4282 SNPs; 17.6%) were located on unplaced scaffolds, which included an average of two SNPs (range = 1–49 SNPs) per scaffold. Given structural variation between the *Salvelinus* sp. reference genome and reference genomes available for other salmonid species (Christensen et al., 2018), we expect some degree of uncertainty in the location of SNPs reported here relative to their actual position within the brook trout genome.

3.2 | Relationships of wild and domestic populations across spatial scales

Results from multivariate analyses performed at a continental scale reflected a large degree of genetic heterogeneity among wild brook trout from distinct geographic regions. BIC calculated for each of the K values we assessed with DAPC indicated that the most likely K for both the full dataset and a dataset limited to wild individuals ranged from 5 to 9. At these values for K, DAPC resolved groups exhibiting limited degrees of admixture that presumably reflect deep evolutionary relationships shaped by phylogeographic history (Figure 1b). We used the groups resolved at $K=5$ to define major genetic groups apparent at a continental scale (Figure 1a,b). These groups primarily corresponded with southern ('Southern group'), upper interior ('Upper Interior group'), mid-Atlantic ('Mid-Atlantic group'), northern ('Northern group') and northeastern ('Northeastern group') regions of the native species range. Although these regional identifiers do not reflect the geographic location of a small number of sampling sites within each group, we use them here for convenience. The major genetic groups resolved at $K=5$ were largely consistent between the full dataset and a dataset limited to wild individuals. The

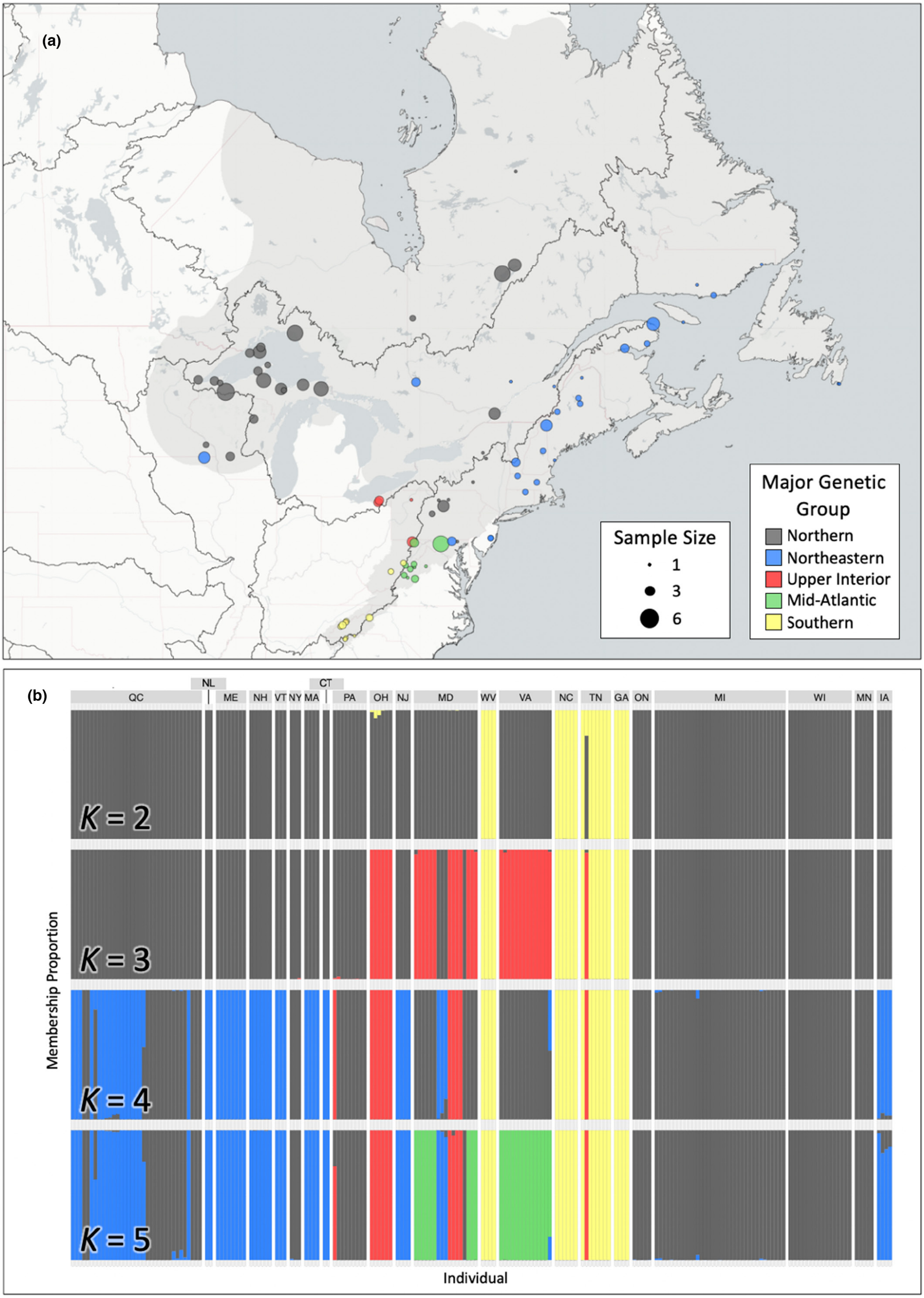
(a)
Sample Size
1
3
6
Major Genetic Group
Northern
Northeastern
Upper Interior
Mid-Atlantic
Southern
(b)
NL
CT
QC
ME
NH
VT
NY
MA
PA
OH
NJ
MD
WV
VA
NC
TN
GA
ON
MI
WI
MN
IA
K = 2
K = 3
K = 4
K = 5
Membership Proportion
Individual

FIGURE 1 (a) Map depicting the sampling sites of the brook trout analysed in this study. Sites are colour coded by assignment to the major genetic groups delimited by DAPC at $K=5$. Points are scaled by the number of individuals genotyped from each site. The full extent of the native species range (grey shading) and level two sub-watershed boundaries (black lines) are also shown. (b) Results from DAPC performed using a dataset limited to wild individuals and $K=2$–5. Vertical bars correspond with individuals and are colour coded to reflect the proportion of membership to a particular DAPC group. Individuals are arranged by sampling site within each state or province. A $K=5$ was used to delimit major genetic groups.

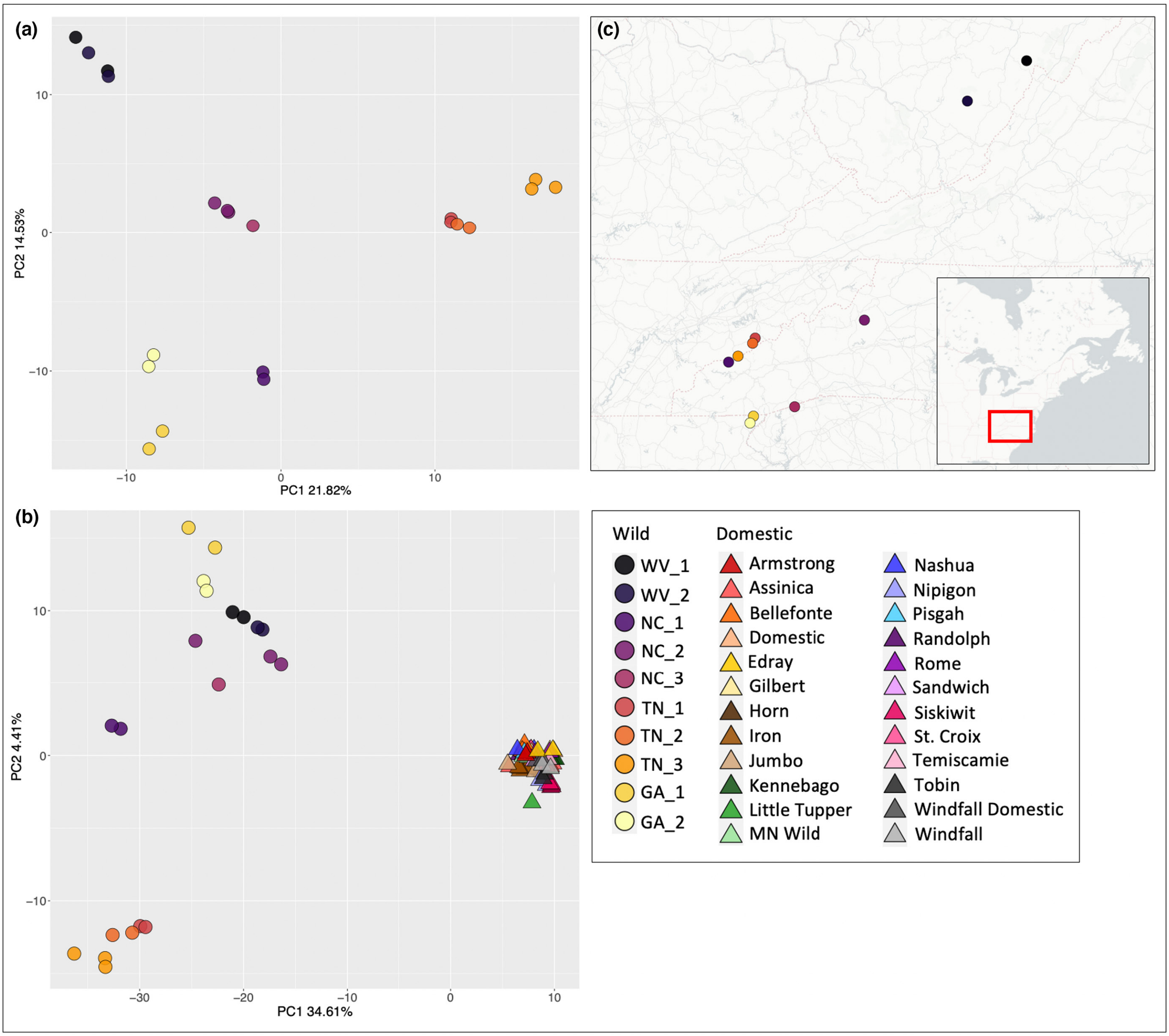

FIGURE 2 Results from PCA of the Southern group. Results are shown for PC axes 1 and 2 using datasets limited to wild individuals (circles; a) or wild and domestic individuals (circles and triangles respectively; b). Axis labels portray the proportion of variation explained by each PC. Colour coding by sampling site for wild individuals and by strain for domestic individuals is shown. (c) Map depicting sampling sites for wild individuals. Points are colour coded by site as in a and b.

Mid-Atlantic, Upper Interior and Southern groups were also apparent in results from PCA (Figure S2).

Multivariate analyses performed separately for each of the five major genetic groups revealed large degrees of regional and local population structure (Figures 2 and 3; Figures S3–S5). For example, in the Southern group, PCA performed using a dataset limited to wild individuals revealed distant relationships among sampling sites (Figure 2), including sites separated by less than 11 km river distance. In comparison, wild brook trout in the Northern group (Figure 3) exhibited comparatively close genetic relationships in

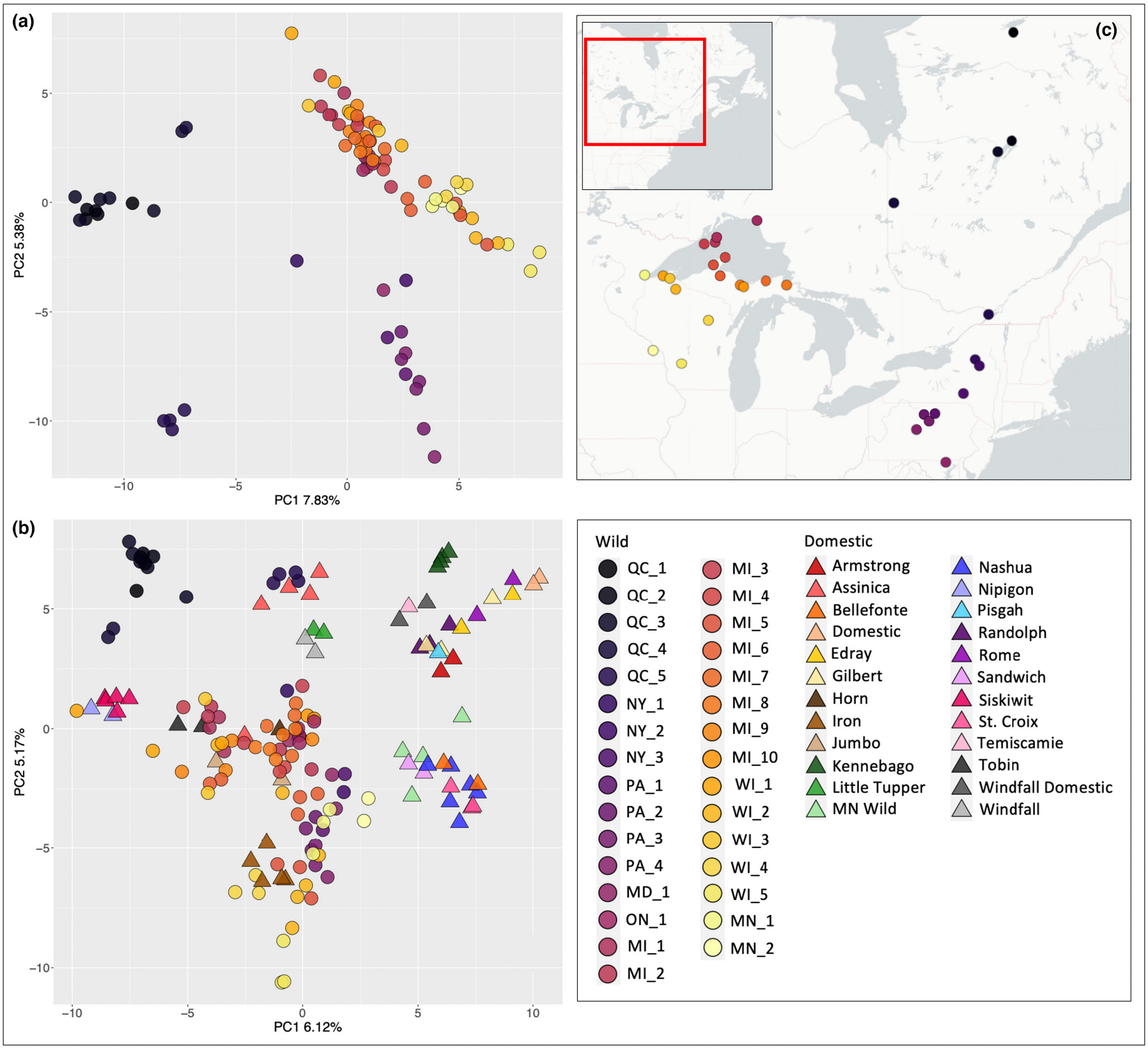

FIGURE 3 Results from PCA of the Northern group. Results are shown for PC axes 1 and 2 using datasets limited to wild individuals (circles; a) or wild and domestic individuals (circles and triangles respectively; b). Axis labels portray the proportion of variation explained by each PC. Colour coding by sampling site for wild individuals and by strain for domestic individuals is shown. (c) Map depicting sampling sites for wild individuals. Points are colour coded by site as in a and b.

results from PCA, especially among individuals from the Great Lakes region. DAPC results for both the Southern and Northern groups revealed hierarchical structure that frequently corresponded with broad geographic regions at low values for K and nearby sampling sites at high values for K (Figure S6), as well as low levels of individual admixture.

Multivariate analyses performed within each major genetic group revealed variable relationships between wild and domestic individuals. For example, PCA and DAPC revealed a distant relationship between wild individuals and domestic strains for the Southern group (Figure 2); this result at least partially reflects the sampling strategy implemented in southern areas of the native range where populations with a known history of stocking were avoided. In comparison, wild individuals from the Northern group displayed a close relationship with a subset of domestic strains (Figure 3).

3.3 | Private genetic variation

Analyses to detect private variation within major genetic groups revealed large numbers of private alleles within each group, highlighting potentially unique genetic variation characteristic of distinct geographic regions. The number of private alleles ranged from 1086 to 3938 alleles (mean = 2228 alleles) across genetic groups (Figure 4),

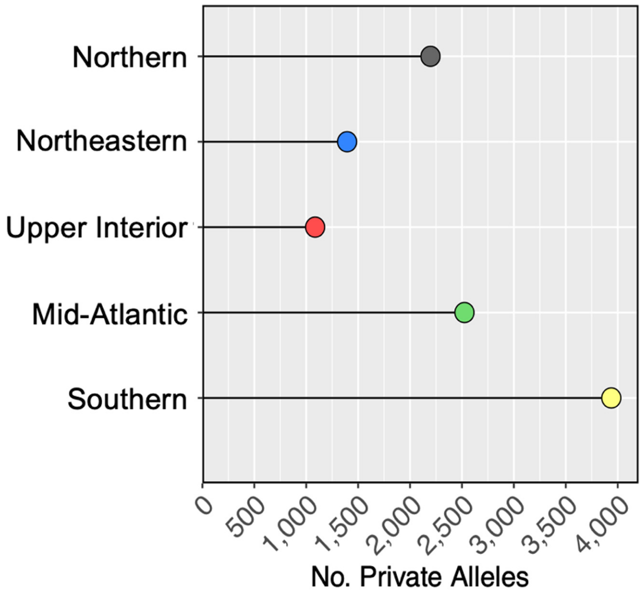

FIGURE 4 Private alleles identified within major genetic groups of brook trout. Private variation was evaluated within each group relative to all other groups.

with the Southern group exhibiting the largest number of private alleles. We also detected private alleles exhibiting fixed allelic differences between the Southern group (n=11 alleles) and remaining groups (results not shown); private variation corresponding with fixed allele frequencies was not observed in any other group.

3.4 | Outlier loci

Results from analyses to explore the presence of SNPs that appear as outliers with respect to neutral population structure included the detection of outliers at varying spatial scales. For analyses performed using wild individuals from the full dataset, 26 loci were identified as outliers by both *pcadapt* and *BayeScan* (FDR <0.05; Figure S7). We also identified outlier loci within each major genetic group, ranging from 10 loci in the Upper Interior group to 63 loci in the Mid-Atlantic group (FDR <0.05; Figure S7). Many of the outliers detected by *BayeScan* were weak outliers identified by *pcadapt* (Figure S8); this result and the low number of loci identifed as outliers by both outlier detection methods may stem from the relative sensitivity of each method. Additionally, results from outlier detection analyses may be influenced by the hierarchical structure characteristic of many brook trout populations, including those analysed here. The outlier loci identified in this study were distributed throughout the brook trout genome (Figure S8). These loci represent 0.44%–1.32% of the SNPs within each data subset that exhibited minor allele frequencies >0.05. We expect that for some of the loci that failed to meet this requirement, analyses based on larger numbers of individuals per sampling site would produce minor allele frequencies larger than those inferred here. Our results thus likely offer a conservative estimate of outlier loci in the SNP dataset presented here, and collectively indicate that SNP panels derived from this dataset may facilitate more rigorous explorations of adaptive relationships across spatial scales.

3.5 | Example SNP panel design

The panels of 500 and 5000 SNPs we identified within the Northern and Southern genetic groups based on ranked F_{ST} were capable of resolving the relationships apparent in the full dataset, but the larger SNP panel was needed to accomplish this task in the Northern group. The SNP sets we identified based on ranked F_{ST} largely differed between groups (Figure S9). In the Southern group, we found that relationships apparent in PCA results based on the full dataset were recovered with both the 500 and 5000 SNP panels (Figure 5). However, in the Northern group, relationships apparent in the full dataset were only recovered using the 5000 SNP panel. For panels applied to different geographic regions, we found that the Northern panel with 5000 SNPs was able to resolve the deepest relationships within the Southern group (Figure 5), but these relationships were not resolved using the Northern panel with 500 SNPs (results not shown). We observed a similar pattern for the Southern panels applied to the Northern genetic group (Figure 5; results for 500 SNP panel not shown). Finally, we found that panels based on SNPs that were randomly selected within each group performed nearly as well as panels based on F_{ST} ranked within each group (Figure 5), though the overall levels of genetic variation explained by PCA were slightly lower. For randomly selected SNPs, relationships within the Northern group were again only recovered with the 5000 SNP panel (results for 500 SNP panel not shown). Improved resolution of relationships within the Northern group based on 500 SNPs may be possible if SNPs exhibiting the largest F_{ST} between each population pair are used to ensure the most informative SNPs for distinguishing each population are included in the panel.

3.6 | Comparison of SNPs versus microsatellites

Continental-scale comparisons of the SNP dataset produced here and the microsatellite dataset from Kazyak et al. (2022) revealed patterns that differed substantially between marker types. Results also varied within marker type between analyses performed using all available wild individuals and subsets of wild individuals. We compared brook trout from 20 sites across the native species range (Figure S10). We evaluated DAPC results for K=5 because this value of K was used to delineate major genetic groups from the full SNP dataset. Results based on SNPs (Figure S11A) revealed patterns similar to those derived from the full set of wild individuals (Figure 1b); except the Northeastern major genetic group was not resolved as a separate group. Additionally, individuals from two sites that were originally assigned to the Mid-Atlantic major genetic group were assigned to different groups here. Results based on microsatellites (Figure S11B) revealed groups that were largely non-overlapping with those resolved using SNPs. For example, brook trout sampled from the southernmost

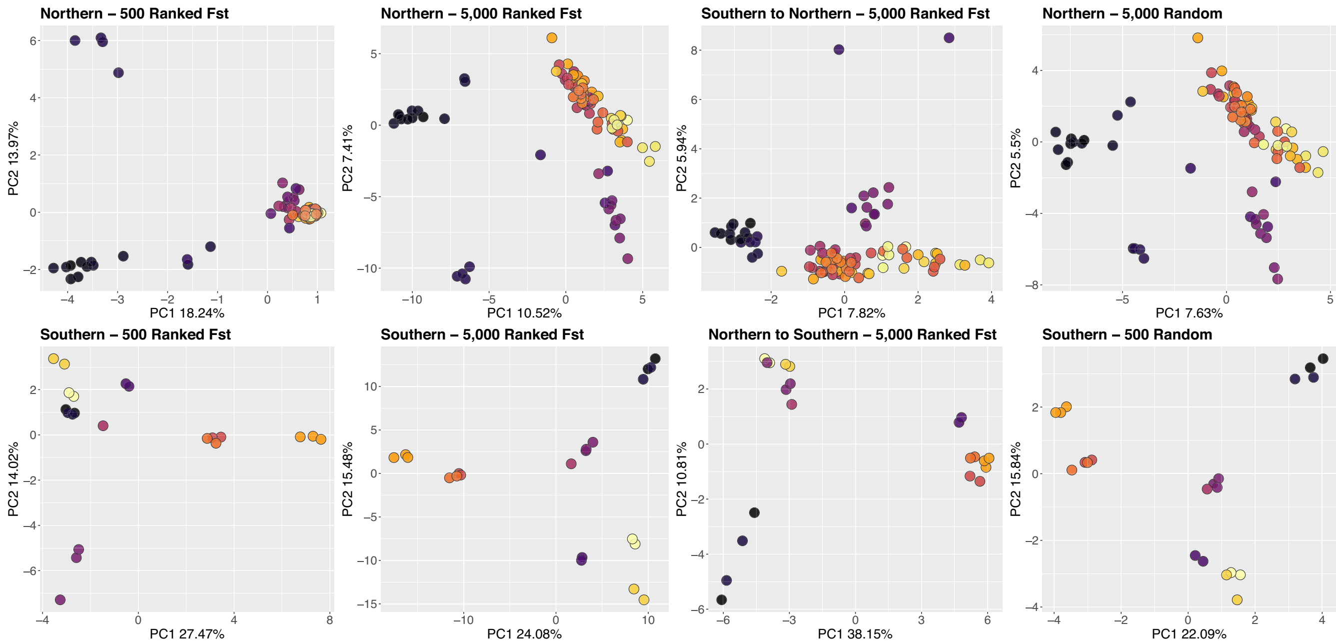

FIGURE 5 Results from PCA performed using SNP panels created for the Northern (*top row*) and Southern (*bottom row*) genetic groups. Results from SNP panels created within the Southern group but applied to the Northern group, and vice versa, are also shown. Panels comprising 500 or 5000 SNPs selected using ranked F_{ST} values or randomly selected SNPs were compared. Results are shown for PC axes 1 and 2 using datasets limited to wild individuals. Axis labels portray the proportion of variation explained by each PC. Colour coding by sampling site is identical to Figures 2 and 3.

extent of the native species range assigned to two groups in analyses based on microsatellites. Individuals from these sites assigned to a single group reflective of the Southern major genetic group in analyses based on SNPs. For analyses based on microsatellites, group assignments for eight of the sites compared between marker types (20% of sites) differed between analyses performed using all available individuals and the dataset with reduced sample sizes.

For regional- to local-scale comparisons, DAPC revealed similar patterns between results based on SNPs and microsatellites, though the relative performance of each marker type varied by geographic region. For microsatellites, results also varied by the number of individuals analysed. In the southern Appalachian region, BIC from DAPC performed using microsatellites and all available individuals indicated that the most likely K was ≥4. We compared results for $K=4$ because this K corresponded with the number of sampling sites analysed in this region (Figure 6). At this K, the full microsatellite dataset resolved a distinct group per sampling site. Similar results were observed for the SNP dataset and the microsatellite dataset with reduced sample sizes. However, the microsatellite dataset with reduced sample sizes also exhibited two groups with large degrees of admixture, making them difficult to distinguish from each other. In the Mid-Atlantic region, BIC from the full microsatellite dataset indicated that the most likely values for K were 6–9. We compared results for $K=7$ because this K corresponded with the number of sampling sites analysed in this region. DAPC resolved a distinct group per sampling site based on the full microsatellite dataset (Figure S12). These groups were also largely apparent in the microsatellite dataset with reduced sample sizes, though groups exhibited elevated levels of admixture. In results based on the SNP dataset, individuals from three sites assigned to the same group so that only a subset of groups were apparent.

4 | DISCUSSION

The primary goal of this study was to develop a resource that paves the way for standardized surveys of genome-wide SNPs in brook trout across the native species range. We accomplished this goal by surveying wild brook trout from 82 sites across eastern North America and domestic brook trout from 24 hatchery strains. We produced a dataset comprising over 24,000 neutral and outlier SNPs distributed throughout the brook trout genome. We illustrated the potential for this dataset to resolve relationships among wild populations and between wild and domestic populations at local to continental spatial scales. The SNP dataset presented here provides a new genomic resource that reduces barriers to implementing genomic studies of native brook trout and integrating genomic information into conservation and management decision-making for this species. More broadly, this study offers a blueprint for developing highly useful genomic resources for additional species.

4.1 | Relationships of wild and domestic populations across spatial scales

We found large degrees of population structure in wild brook trout at local to continental scales, revealing patterns consistent with

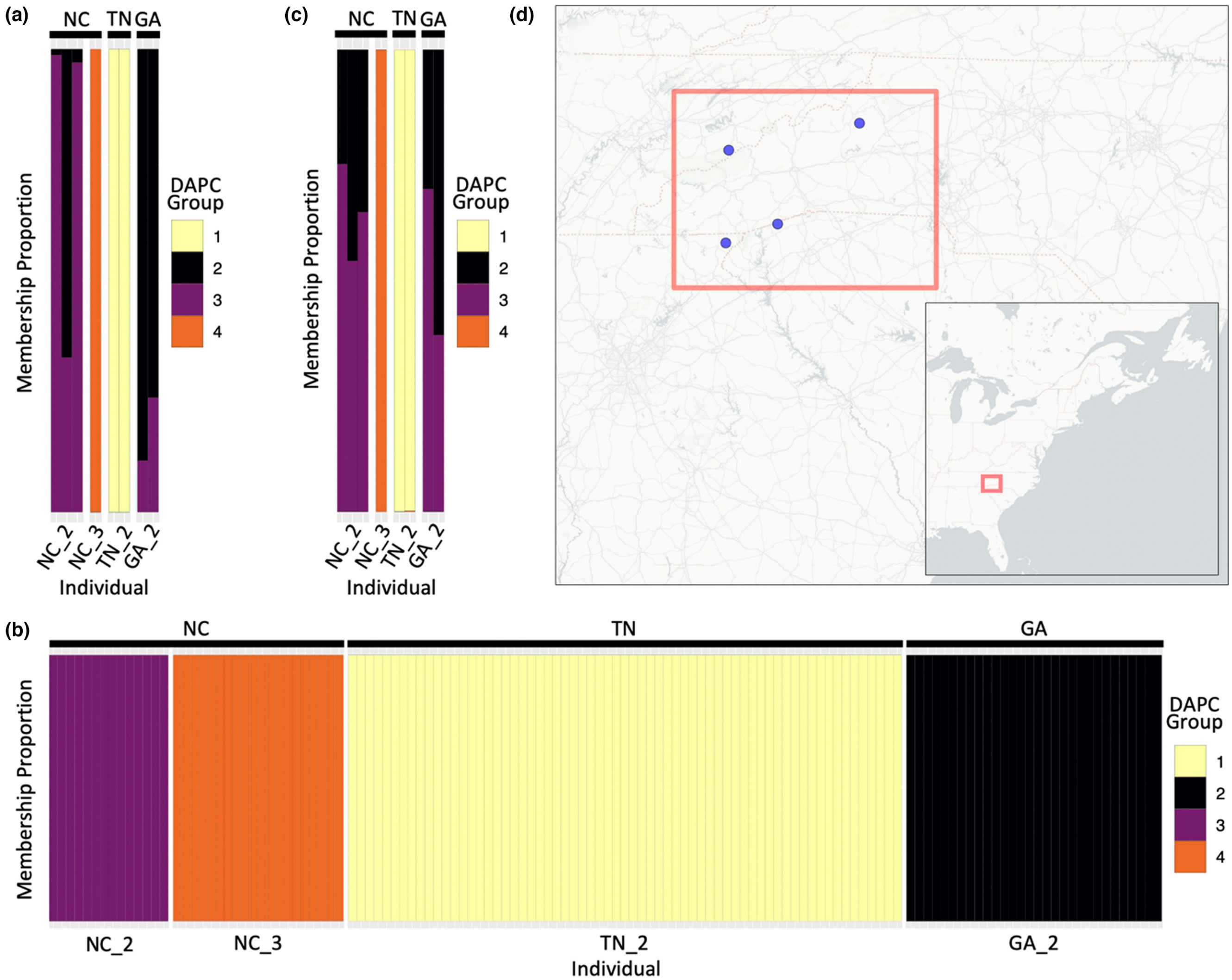

FIGURE 6 Results from DAPC performed using SNPs or microsatellites in four populations from the southern extent of the brook trout native range that were sampled in this study and in Kazyak et al. (2022). Results for $K=4$ are shown for all datasets. Vertical bars correspond with individuals and are coloured to reflect the proportion of membership to a particular DAPC group. Individuals are arranged by site within each state. Results based on (a) SNPs, (b) microsatellites and (c) microsatellites with sample sizes matching the SNP dataset. (d) Map depicting sampling sites (blue points) for the individuals analysed here.

those reported in previous genetic studies. The five major genetic groups (Northern, Northeastern, Upper Interior, Mid-Atlantic and Southern) apparent at a continental scale in this study are similar to groups resolved using mitochondrial DNA (Danzmann et al., 1998) and microsatellites (Kazyak et al., 2022). These groups presumably reflect postglacial dispersal and recolonization patterns in eastern North America, as well as historical and contemporary selection and drift. Similarly, the presence of highly structured populations at regional and local scales in this study has been reported in numerous genetic studies (Beer et al., 2019; Kanno et al., 2011; Kazyak et al., 2021; Mamoozadeh et al., 2023; Morgan et al., 2021; Weathers et al., 2019), including studies spanning large regions of the native species range (Ferchaud et al., 2020; Kazyak et al., 2022). Results from these studies collectively indicate that brook trout is a highly structured species, with large levels of genetic differentiation possible across small geographic distances. Collectively, these results highlight the utility of our dataset for resolving relationships among wild populations of brook trout across spatial scales.

We observed relationships between wild and domestic populations of brook trout that varied by geographic region but that were consistent across spatial scales. In general, wild populations from northern regions of the native range exhibited comparatively close relationships with domestic populations. This result may reflect the northern origin of most hatchery strains used to supplement wild populations, populations at higher latitudes that frequently exhibit lower levels of differentiation than populations at lower latitudes and/or a history of introgression due to past hatchery stocking. In comparison, wild populations from elsewhere across the native range exhibited distant relationships with domestic populations. This result was expected for the southernmost portion of the native range given our experimental design, where populations with known stocking history were not sampled.

Our ability to distinguish wild and domestic individuals indicates that introgression from hatchery strains is not prevalent in many areas. This finding is consistent with microsatellite-based studies of brook trout across the native range (Annett et al., 2012; Kazyak et al., 2018; Kazyak et al., 2021; Lehnert et al., 2020; Morgan et al., 2021; White et al., 2018; but see Hargrove et al., 2022), which report little to no domestic introgression in wild populations. The SNPs identified in this study offer a new resource for exploring patterns of domestic introgression in future studies that may improve upon the resolution afforded by microsatellites.

4.2 | Molecular markers for studies of native brook trout

Our comparisons between SNPs and microsatellites revealed similar relationships among individuals at local and regional scales, but different relationships at a continental scale. These differences were apparent even when the number of individuals per sampling site in the microsatellite dataset was normalized to match the SNP dataset. Inconsistent results between marker types as observed here potentially stem from comparing markers discovered using different source populations and/or markers reflecting differing information content (e.g. neutral-only vs. both neutral and adaptive regions of the genome). The relative importance of these factors may also vary by spatial scale. Comparisons of SNPs and microsatellites for resolving fine-scale population structure in other salmonids have indicated that, when comparing the most informative SNPs, a SNP-to-microsatellite ratio of 200:1 can provide equivalent accuracy (Hess et al., 2011). We analysed two orders of magnitude more SNPs in this study. Further, the continental-scale relationships resolved by Kazyak et al. (2022) and Kazyak et al. (2021) based on their full microsatellite dataset are similar to those based on the full SNP dataset in this study. Given these factors, we believe the relationships resolved in this study offer an accurate representation of native brook trout.

By exploring the ability of our dataset to resolve relationships across spatial scales, and comparing results between marker types, we offer important insights into experimental designs possible with surveys of genome-wide SNPs. We inferred relationships among wild populations and between wild and domestic populations by analysing over 24,000 SNPs in one to six individuals per sampling site or domestic strain. Although sampling designs ideal for a particular study are expected to vary by focal population, relationships targeted for inference and the number and composition of genetic markers selected for analysis (Flesch et al., 2018; Lotterhos & Whitlock, 2015; Nazareno et al., 2017; Rellstab et al., 2015), our results demonstrate the potential for the SNP dataset presented here to facilitate inferences about neutral relationships that are at least equivalent to those based on microsatellites.

We view the dataset developed in this study as providing a practical tool for users to more efficiently conduct standardized genomic studies of brook trout across the native range. However, even though RADseq methods such as those employed here allow much more of the genome to be surveyed than non-genomic methods, these methods only facilitate analyses of SNPs associated with restriction enzyme recognition sites. In comparison, whole genome sequencing methods allow SNPs found anywhere in the genome to be analysed, and these methods are becoming increasingly affordable for non-model applications (see review by Lou et al., 2021). We thus expect RADseq, and increasingly, whole genome sequencing, datasets to play an important role in SNP panel development and data standardization now and into the future.

4.3 | Genomic resources to address key conservation and management questions for native brook trout

Genomic resources, including the genome-wide SNPs identified here, enable increased opportunities to inform the conservation and management of brook trout across the native species range (Table 1). Microsatellites previously developed to assess range-wide genetic relationships in brook trout (Kazyak et al., 2022; King et al., 2012) have facilitated important insights into neutral demographic relationships. Our SNP dataset builds upon these efforts by establishing an additional resource for evaluating range-wide demographic relationships. Additionally, the genomic dataset presented here includes loci that appeared as outliers with respect to neutral population structure at both continental and regional spatial scales. Such loci may enable assessment of adaptive relationships, a key benefit over microsatellites, that remain largely unexplored for brook trout across the native range (but see Elias et al., 2018; Ferchaud et al., 2020; Fraser et al., 2014). Analyses of adaptive variation can be used to identify management units corresponding with adaptive groups that reflect adaptation to local conditions, detect regions of the genome linked with specific environmental variables and identify genetic variation underlying key adaptive traits, among other applications relevant to conservation and management (see additional examples and references in Table 1).

Our SNP dataset also enables standardized surveys of genomic variation where data generated among independent studies can be directly integrated, highlighting another major benefit over microsatellites, which are difficult to standardize across studies. Standardized surveys are critical for developing datasets with greater spatial or temporal coverage than is often practical within the scope of a single study. Surveys of genomic variation in temporally spaced sample collections or sample collections spanning broad geographic regions have been used to identify climate and harvest variables underlying changes in population biomass for Atlantic cod (*Gadus morhua*; Bonanomi et al., 2015), characterize trends in effective population size and identify associated environmental and anthropogenic drivers for Atlantic salmon (*Salmo salar*; Lehnert et al., 2019) and determine the spatial distribution, historical prevalence and replacement potential of genetic variation underlying important adaptive traits in Chinook

TABLE 1 Key questions central to the management of native brook trout that can be addressed using genome-wide SNPs and SNP panels derived from these datasets.

Application	Key questions	References
(1) Identifying management units	(a) Which individuals comprise populations?* (b) Which populations comprise adaptive groups?* (c) On what spatial and temporal scales do populations and adaptive groups occur?*	*Reviews*: Funk et al. (2012), Hohenlohe et al. (2021) *Examples*: Barbosa et al. (2018), Johansen et al. (2020), Meek et al. (2020), Vaux et al. (2021)
(2) Conservation prioritization	(a) Which adaptive groups exhibit the greatest mismatch with predicted future conditions?* (b) Which adaptive groups exhibit the greatest adaptive potential?* (c) Which populations are most at risk of extirpation?* (d) Are there populations or adaptive groups that harbour unique genetic variation warranting special protection, including variation associated with ecologically important traits?	*Reviews*: Capblancq et al. (2020), Funk et al. (2018) *Examples*: Bay et al. (2018), Lehnert et al. (2019), Prince et al. (2017), Shryock et al. (2020)
(3) Targeted supplementation	(a) Have populations and adaptive groups been impacted by introgression from domestically raised individuals?* (b) Are there populations and adaptive groups that offer a suitable source of individuals for translocation?* (c) Are there populations and adaptive groups that offer a suitable source of individuals for genetic rescue?	*Reviews*: Bell et al. (2019), Fitzpatrick et al. (2023), Laikre et al. (2010), Seddon et al. (2014), White et al. (2023) *Examples*: Dresser et al. (2017), Fitzpatrick et al. (2020), Lamaze et al. (2012)
(4) Managing landscape features	(a) How is genetic connectivity among populations and adaptive groups affected by physical barriers to movement?* (b) What ecological and environmental variables most strongly affect the genetic diversity of populations and adaptive groups?*	*Reviews*: Forester et al. (2018), Scribner et al. (2016) *Examples*: Gehri et al. (2021), Jackson et al. (2018), Micheletti et al. (2018)
(5) Evaluating restoration efforts	(a) Has neutral and adaptive genetic diversity changed following targeted translocation, genetic rescue or landscape modification?* (b) Have alleles associated with distinct adaptive traits been restored following targeted translocation, genetic rescue or landscape modification?	*Reviews*: Flanagan et al. (2018), Seaborn et al. (2021) *Examples*: Campbell et al. (2017), Flesch et al. (2020), Fraik et al. (2021)
(6) Monitoring	(a) Are key genetic metrics that measure neutral and adaptive diversity, effective size or inbreeding changing over time?* (b) Are inferences from genetic monitoring consistent at local to broad spatiotemporal scales?*	*Reviews*: Flanagan et al. (2018), Leroy et al. (2018), Schwartz et al. (2007) *Examples*: Bi et al. (2019), Chen et al. (2016), Gugger et al. (2021), Osborne et al. (2022)

Note: Table adapted from Kazyak et al. (2022) and updated here to reflect additional applications possible with analyses of adaptive variation. References that provide subject reviews and empirical examples are also provided. Questions able to be addressed using SNP panels derived from the dataset presented in this study are identified with an asterisk, though evaluation of additional questions may be possible. In the context of this table, populations are defined as demographically independent management units and adaptive groups are defined as adaptively similar groups of populations.

salmon (*Oncorhynchus tshawytscha*; Prince et al., 2017; Thompson et al., 2019). Analogous approaches in terrestrial systems have been used to monitor temporal changes in the frequency of alleles underlying infectious disease (Bröniche-Olsen et al., 2016), identify genomic variation associated with distinct climate variables and infer vulnerability to changing climatic conditions (Bay et al., 2018) and characterize fitness consequences of reduced population sizes and immigration rates (Chen et al., 2016). Evaluation of these and other relationships in native populations of brook trout is now more attainable with the SNP dataset developed in this study.

4.4 | Next steps for users

The SNP dataset presented here enables rapid and cost-effective development of SNP panels designed to meet specific user needs. SNP panel development, including for GTseq and RAD capture, typically requires an initial discovery step that consists of sampling a representative collection of individuals, preparing libraries for sequencing [e.g. RADseq or low coverage whole genome sequencing (Therkildsen & Palumbi, 2017) libraries] and performing bioinformatic analyses of sequence data to identify SNPs and produce genotypes. Resulting SNP data are then used to design SNP panels comprising an informative subset of loci to be genotyped in additional individuals. The initial discovery step that occurs prior to SNP panel design is time-consuming, costly and requires extensive genomic expertise. Our SNP dataset eliminates the need for an initial discovery step, allowing users to begin directly with SNP panel design. For example, users interested in developing a RAD capture panel from our dataset can begin by selecting a focal set of SNPs then design custom capture probes (e.g. Arbor BioSciences myBaits® target capture kit) to target these loci in the panel. These tasks can be accomplished using the data resources provided with this study.

Ideally, the subset of SNPs included in a SNP panel is selected based on the ability of loci to address questions central to a particular study. A SNP panel designed for one application may therefore be less effective at addressing the goals of additional applications (but see May et al., 2020 for a multi-purpose panel design example). Similarly, the number of loci included in a SNP panel depends on the degree of statistical power needed to resolve focal relationships and the method selected for genotyping additional individuals (Bootsma et al., 2020; McKinney et al., 2020; Storer et al., 2012; see Meek & Larson, 2019 for guidelines comparing RAD capture vs. GTseq). Given these factors, the dataset presented here was not used to design a discrete SNP panel. Instead, this dataset sets the stage for users to design SNP panels tailored to specific user needs. For instance, our SNP panel design example demonstrates the ability of users to design panels to identify genetically distinct populations of brook trout within focal geographic regions. This example illustrates that the number of SNPs needed to resolve focal relationships varies by geographic region and that panels capable of resolving genetically distinct populations in one region may be less capable of resolving these relationships when applied to another region. Although we captured high levels of genetic variation within each region by taking F_{ST} into account during SNP panel design, additional power for resolving these relationships may be possible by considering F_{ST} between population pairs within each region. This gain in power may become increasingly important at finer spatial scales, particularly given the large number of private alleles observed within major genetic groups. However, we found that randomly selected SNPs performed similarly well in resolving relationships among populations and may increase the applicability of panels to multiple regions.

Our dataset can also be used to design SNP panels for additional purposes. To design a panel that maximizes statistical power for identifying instances of domestic introgression, users can identify SNPs that exhibit large levels of genetic differentiation (e.g. F_{ST}) between focal hatchery strains and wild populations (Anderson, 2010; Nugent et al., 2023; Wringe et al., 2019). Additionally, our dataset may be useful for kinship analyses to resolve relationships among individuals, where users design a panel comprising SNPs with the highest minor allele frequencies in focal populations (Anderson & Garza, 2006; Holman et al., 2017; Lew et al., 2015; Liu et al., 2016; May et al., 2020). Finally, it is also possible that SNP panels derived from the dataset presented here will be informative of relationships for brook trout outside of the native species range and enable comparisons between native and invasive populations. Such applications may include identifying diagnostic loci to determine the native origin of invasive populations. Datasets developed for such targeted purposes can ultimately be combined to address additional questions relevant to conservation and management if derived from the baseline data presented here.

This study is the first to survey genome-wide SNPs across the native range of brook trout and offers a valuable characterization of genomic diversity across the native range. However, genotyping individuals from additional geographic locations is certain to reveal genetic variation not represented in the SNPs identified here, particularly because brook trout frequently exhibit substantial population subdivision over small spatial scales. This may also be the case given the large number of private alleles detected in this study. Careful consideration must therefore be given to ascertainment of the SNPs identified here in additional populations.

The degree to which ascertainment bias affects inferences for additional populations will depend on the genetic distinctiveness of newly analysed populations relative to the populations analysed here and the relationships targeted for inference (e.g. Bradbury et al., 2011; McTavish & Hillis, 2015; Paz-Vinas et al., 2021; Rosenblum & Novembre, 2007). For example, northern populations of brook trout frequently exhibit lower levels of genetic diversity compared to more southerly populations (Hall et al., 2002; Kazyak et al., 2022; this study), at least partially due to historical patterns of glaciation and post-glacial recolonization (Danzmann et al., 1998). Therefore, we expect ascertainment bias to be a lesser concern in northern populations, where sampling in this study was most sparse, compared to more southerly populations, where sampling was more extensive. This is also likely given the larger number of private alleles we detected within the Southern genetic group compared to remaining (more northerly) groups. However, the heightened risk for ascertainment bias in more southerly populations is likely offset in our dataset by greater sampling effort in this region. Regardless, given fine-scale sampling across a broad spatial extent, we expect that the overarching patterns of genomic diversity represented by brook trout across the native species range have been captured by our efforts. Furthermore, given the large number of SNPs presented here, it is likely that users will be able to overcome ascertainment bias and identify loci effective at resolving target relationships, for example by selecting loci that exhibit high minor allele frequencies in a focal geographic region. The SNPs presented here thus offer a valuable resource for enabling surveys of genome-wide diversity, including in studies for which such surveys may not otherwise be practical.

5 | CONCLUDING REMARKS

Standardized surveys of genomic variation that employ SNP panels generated using recently developed GTseq and RAD capture protocols (a natural extension of the SNPs presented here) make the generation of large-scale, high-resolution genomic datasets more feasible than ever. These methods offer enhanced capabilities for developing standardized datasets that have played important roles in conservation and management decision-making for some species (Charlier et al., 2012; Van Doornik et al., 2013; Waples et al., 1993) and are becoming increasingly available for fishes of conservation and management concern [bull trout (*S. confluentus*), Bohling et al., 2021; walleye (*Sander vitreus*), Bootsma et al., 2020; Euclide et al., 2022; chum salmon (*O. keta*), McKinney et al., 2022; sockeye salmon (*O. nerka*), Chang et al., 2022]. Such datasets also enable a new horizon of opportunities to understand and predict patterns of neutral and adaptive diversity across spatial and temporal scales (Blanchet et al., 2017; Capblancq et al., 2020; Lasky et al., 2020; Waldvogel et al., 2020). Large-scale, high-resolution genomic datasets ultimately help to equip natural resource managers with information to manage for the long-term resiliency of populations and species. These datasets also provide managers with a practical mechanism for monitoring genetic biodiversity at national scales. In the context of native brook trout conservation, we expect enhanced capabilities for conducting genome-wide surveys will facilitate novel explorations of neutral and adaptive relationships across spatiotemporal scales. Inference of such relationships is critical for supporting the long-term persistence of this culturally, ecologically and economically important species.

AUTHOR CONTRIBUTIONS

Mariah H. Meek, Andrew R. Whiteley, Benjamin H. Letcher and David C. Kazyak conceived of and designed the study, Charlene Tarsa performed the laboratory work and Nadya R. Mamoozadeh analysed the data and wrote the manuscript. All authors contributed substantially to the development of ideas throughout the course of the study and to revisions of the original manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to report for this work.

DATA AVAILABILITY STATEMENT

Raw sequencing reads have been made publicly available through the NCBI Sequence Read Archive (PRJNA874748). Data files including genotypes for the full dataset, contigs for SNPs contained in the full dataset and meta-data for individuals comprising the full dataset are available from the Dryad digital repository (doi: https://doi.org/10.5061/dryad.2fqz612sb). A list of SNPs comprising the full dataset that were identified as significant in outlier detection analyses is also available from Dryad.

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