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ORIGINAL ARTICLE

# Panmixia in the American eel extends to its tropical range of distribution: Biological implications and policymaking challenges

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## Abstract

The American eel (*Anguilla rostrata*) has long been regarded as a panmictic fish and has been confirmed as such in the northern part of its range. In this paper, we tested for the first time whether panmixia extends to the tropical range of the species. To do so, we first assembled a reference genome (975 Mbp, 19 chromosomes) combining long (PacBio and Nanopore and short (Illumina paired-end) reads technologies to support both this study and future research. To test for population structure, we estimated genotype likelihoods from low-coverage whole-genome sequencing of 460 American eels, collected at 21 sampling sites (in seven geographic regions) ranging from Canada to Trinidad and Tobago. We estimated genetic distance between regions, performed ADMIXTURE-like clustering analysis and multivariate analysis, and found no evidence of population structure, thus confirming that panmixia extends to the tropical range of the species. In addition, two genomic regions with putative inversions were observed, both geographically widespread and present at similar frequencies in all regions. We discuss the implications of lack of genetic population structure for the species. Our results are key for the future genomic research in the American eel and the implementation of conservation measures throughout its geographic range. Additionally, our results can be applied to fisheries management and aquaculture of the species.

KEYWORDS
American Eel, Anguilla, panmixia, population structure

Thomas J. Kwak authorship is posthumous.

For Affiliation refer page on 1884

## 1 | INTRODUCTION

Despite their apparent openness, marine environments can present multiple barriers to gene flow, such as those caused by oceanic fronts, currents (Sotka et al., 2004), and habitat discontinuities (Catarino et al., 2015). Such barriers, as well as life history characteristics like the presence or absence of pelagic larvae or tolerance to environmental variables, could affect species dispersal potential and genetic homogeneity. Therefore, marine species with wide distributions present various patterns of genetic structure, from heterogenic patterns such as chaotic genetic patchiness (e.g., Vendrami et al., 2021) to true panmixia. An example of the latter is the European eel (*Anguilla anguilla*; Als et al., 2011; Dannewitz et al., 2005; Enbody et al., 2021; Palm et al., 2009), a sister species of the American eel (*Anguilla rostrata*; LeSueur, 1817), with a similar life cycle. These two species, and other freshwater eels, are often cited as the poster child for catadromy, even when this life history pattern is facultative, as is the case for the American eel (Thibault et al., 2007). This is to say that spawning, egg fertilization, and egg hatching occur in the open ocean, but most adult life stages are spent in brackish or freshwater environments, sometimes moving between both feeding grounds (Figure 1). Immature adult American eels in their growing phase (yellow stage) inhabit geographically distant and environmentally dissimilar habitats, from tropical Caribbean watercourses to Greenland (Benchetrit & McCleave, 2016; Cairns et al., 2022). Upon reaching sexual maturity and while developing all the traits characteristic of the mature adult (silver stage), such as a paddle-like caudal region and enlarged eyes, that are required for the open ocean migration, individuals return to their breeding ground. The current scientific consensus is that this semelparous fish mates in the southwest of the Sargasso Sea, in a single area delimited by a front and partially overlapping with the breeding grounds of the European eel, with which infrequent hybridization occurs (Pujolar et al., 2014; Wielgoss et al., 2014). This area appears to be the destination of sexually mature adults' (silver stage) breeding migrations (Béguer-Pon et al., 2015, 2017; Wright et al., 2022) and is where the youngest larvae have been observed (McCleave, 1993; McCleave et al., 1987; Miller et al., 2015; Munk et al., 2010; Rypina et al., 2014).

There is little doubt of panmixia for American Eel specimens in the northern and eastern portion of the species' range, which extends from Greenland to the northeast of Florida, USA (Avise et al., 1986; Côté et al., 2013). Additionally, Bonvechio et al. (2018) did not find evidence for genetic differentiation between American eel from the Atlantic and Gulf of Mexico coasts of Florida. However, leptocephali dispersal to this area is expected to follow different routes and be aided by different current patterns than those used for dispersion to southern estuaries and rivers (Kleckner & McCleave, 1982; Miller et al., 2015; Munk et al., 2010). Moreover, most information about landings, historical abundance, and other data pertaining to the species´ stocks is only available for Atlantic North American drainages (Cairns et al., 2022), where the decline of the species is already a source of concern, due to both anthropogenic and natural threats as well as susceptibility to climate change (Drouineau et al., 2018; Jacoby et al., 2015; Miller et al., 2009). In Canada, the American eel is considered Threatened by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC, 2012), while in the United States, the Atlantic States Marine Fisheries Commission classified American Eel stocks as Depleted (ASMFC, 2017). It is also listed as Endangered on the International Union for Conservation of Nature (IUCN) Red List (Jacoby et al., 2018).

Information about the American Eel in the southern portion of its range is extremely limited, yet this vast area encompasses around

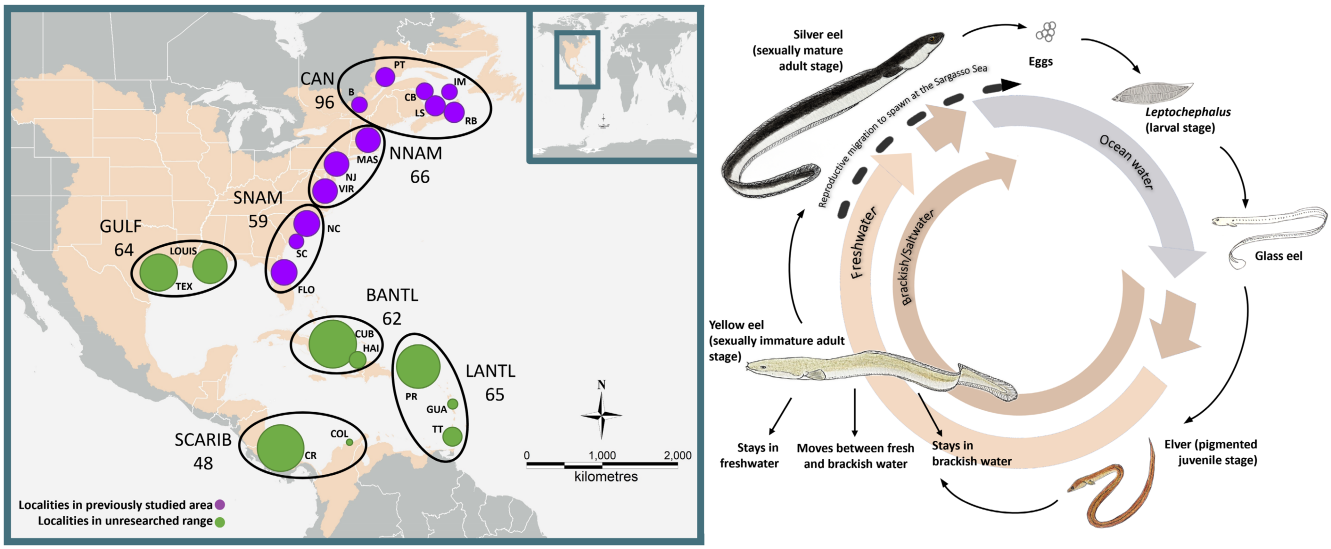


FIGURE 1 Left: Sampling localities and geographical groups. Circles are proportional to sample size, plausible range is shown in beige. Map background and plausible range visualization courtesy of David Cairns. For more information, see Table 1. Right: Diagram shows the life cycle of the American eel.

half of the geographic area inhabited by the species (Benchetrit & McCleave, 2016; Cairns et al., 2022) and harbors both commercial and subsistence fisheries (Cairns, 2020). This knowledge gap hinders effective management and the implementation of conservation policies for a resource that, due to the particularities of its geographically varied life cycle, is indeed shared among multiple countries. Effectively managing a panmictic species through such a vast geographical area would necessitate cooperation between many provinces, states, and countries. Additionally, while spatially varying selection (single-generation local selection; Levene, 1953) has previously been documented (Babin et al., 2017; Gagnaire et al., 2012; Pavey et al., 2015), lack of population structure, and reshuffling of allele frequencies in each generation points to nongenetic mechanisms of adaptation (e.g., phenotypic plasticity from the epigenetic to the behavioral level) as key for the species.

Besides excluding Caribbean drainages and lacking spatial coverage of the Gulf of Mexico, previous population structure research of the species only partially covered the genome (i.e., Avise et al. (1986) with mitochondrial DNA restriction–fragment–length–polymorphism; Côté et al. (2013) and Bonvechio et al. (2018) with microsatellites). These limitations, accompanied by the lack of a high-quality reference genome, hinder future research development and the translation of research into policy-making. Therefore, to fill these knowledge gaps, we developed a new American eel reference genome and used low-coverage whole-genome sequencing to document the genetic population structure of the American eel across most of its range, including for the first time tropical localities.

## 2 | MATERIALS AND METHODS

### 2.1 | Reference genome

We aligned the American eel sequences to a new and improved reference genome (GenBank accession number GCA_018555375.2) developed for this study. This reference genome was obtained by using the MagAttract HMW DNA Kit from Qiagen (Qiagen, Toronto, ON) to extract high-quality genomic DNA from the liver of an American eel in the yellow stage, captured in the St. Lawrence River near the locality of Bécancour (latitude 46.3506° N, longitude 72.4351° W), Québec, Canada. Twenty-nine SMRT cells from the Pacific Biosystems Sequel system at the Centre d'expertise et de services Génome Québec were used to sequence sheared large insert libraries from the extracted DNA, yielding 70X of raw coverage. Additional sequencing was performed at the McGill Genome Center Advanced Genomics Technologies laboratory where DNA was extracted from muscle tissue using the Circulomics Nanobind Tissue Kit (Pacific Biosciences). Nanopore libraries were constructed using both the ligation kit (SQK-LSK110) and the Rapid Barcoding Kit (SQK-RBK114.24) (Oxford Nanopore Technologies). The Nanopore sequencing was performed using the PromethION platform with flowcell model FLO-PRO002 using pore chemistry R9.4.1. We ran two flowcells for 10X depth in raw coverage. Assembly was done with wtdbg2 v.2.5 (Ruan & Li, 2020) and Pilon v1.23 (–diploid–fix all–mindepth 5) (Walker et al., 2014) was used to polish it using 24X of Illumina paired-end reads. Finally, we used RagTag v1.1.0 with default parameters (Alonge et al., 2022) to super-scaffold the American eel genome using the European eel genome. Synteny against the current reference European eel genome (GenBank accession number GCA_013347855.1) was evaluated using SyMAP v4.2 (Soderlund et al., 2006, 2011). We did the synteny alignment with MUMmer v4.0.0.1 (Marçais et al., 2018) and visualized the synteny result as described in Symap's "Running MUMmer from the command line" document. The quality of the assembly and gene content statistics were obtained with Benchmarking Universal Single-Copy Orthologs (BUSCO) v3.0.1 (–m geno) (Simão et al., 2015).

### 2.2 | DNA extraction and library preparation

Six to 50 samples (fin clips or tip of the tail if glass eels or elvers) from 21 locations (Table 1) from throughout the entire known distribution of the species (with the exception of Greenland, from the Gulf of St. Lawrence to Trinidad and Tobago (Figure 1) were obtained opportunistically or reused from previous studies. Samples from the Atlantic coast of North America were previously collected by Côté et al. (2013) and Pavey et al. (2015).

DNA was extracted using a salt-based extraction protocol by Aljanabi and Martinez (1997), modified to add an RNase A treatment (Qiagen). Following DNA extraction, DNA integrity was first verified with agarose gel electrophoresis. Then, we checked contamination levels using NanoDrop (ThermoFisher Scientific) and finally quantified the DNA by using AccuClear® Ultra High Sensitivity dsDNA Quantitation Kit (Biotium). For the next steps, we retained extractions containing only high-quality DNA that was further purified using magnetic beads followed by checks of integrity and quality, as mentioned above. To normalize DNA concentration, we diluted each sample to 2 ng/μL through two dilution steps. Low coverage whole-genome libraries for each individual were performed in house following the protocol described in Therkildsen and Palumbi (2017) and Mérot et al. (2021) with slight modifications. Briefly, input DNA concentration was increased to 2 ng/μL, and size selection Axygen magnetic beads ratio in the first size selection step was modified to 0:45:1. We quantified the DNA concentration in resulting preparations using AccuClear® Ultra High-Sensitivity dsDNA Quantitation Kit and used Agilent Bioanalyzer's High-Sensitivity DNA Chip to determine the average size of the DNA fragments. High-quality libraries were pooled in equimolar concentrations and sent for 150 bp paired-end sequencing in a NovaSeq6000 sequencer with flow cell S4 at the Centre d'expertise et de services Génome Québec (Montréal, Canada). Each pool was sequenced using one separate lane and targeted mean coverage was 4X. Some tissue samples (e.g., some Haiti samples) producing very low-quality DNA after three extraction attempts were excluded from the study.

**TABLE 1** Sampling site information. Coordinates are approximate in some localities.

| Sampled region | #indv | Groups | # indv | Sampling year | Life stage | Latitude and longitude |
|---|---|---|---|---|---|---|
| Beauharnois (B) | 14 | CAN | 96 | 2011 | Adult eel | 45.31°N −73.9° W |
| Cascumpeque Bay (CB) | 18 | | | 2011 | Adult eel | 46.77° N −64.07° W |
| Ile de la Madeleine (IM) | 14 | | | 2011 | Adult eel | 47.53° N −61.69° W |
| Lake of Shining (LS) | 15 | | | 2011 | Adult eel | 46.5° N −63.39° W |
| Petite Trinité (PT) | 17 | | | 2008 | Adult eel | 49.52° N −67.28° W |
| Riviere Bourgeois (RB) | 18 | | | 2011 | Adult eel | 45.63° N −60.96° W |
| Massachusetts (MAS) | 22 | NNAM | 66 | 2008/2009 | Glass/elver | 41.68° N −70.92° W |
| New Jersey (NJ) | 22 | | | 2008 | Glass/elver | 39.56° N −74.58° W |
| Virginia (VIR) | 22 | | | 2008 | Glass/elver | 37.22° N −76.49° W |
| Florida (FLO) | 23 | SNAM | 59 | 2008 | Glass/elver | 30.02° N −81.33° W |
| North Carolina (NC) | 23 | | | 2008 | Glass/elver | 34.77° N −76.81° W |
| South Carolina (SC) | 13 | | | 2008 | Glass/elver | 32.93° N −80.01° W |
| Louisiana (LOUIS) | 31 | GULF | 64 | 2015/2017/2018 | Adult eel | 29.51° N −90.41° W; 29.90° N −91.15° W; 29.58° N −90.56° W; 29.64° N −90.58° W; 29.83° N −91.13° W; 29.88° N −91.16° W; 30.61° N −91.42° W; 30.61° N −91.44° W; 30.64° N −91.48° W; 30.61° N −91.44° W; 29.75° N −91.14° W; 29.88° N −91.16° W; 29.75° N −90.72° W; 29.94° N −91.18° W; 29.56° N −90.79° W; 29.63° N −90.55° W; 29.75° N −91.14° W |
| Texas (TEX) | 33 | | | 2016/2018/2019 | Adult eel | 28.73° N −97.16° W; 28.62° N −96.62° W; 31.19° N −93.56° W; 28.04° N −97.86° W; 30.32° N −96.52° W |
| Cuba (CUB) | 47 | BANTL | 62 | 2017 | Glass/elver | 20.39° N −74.54° W; 21.10° N −76.10° W |
| Haiti (HAI) | 15 | | | 2018 | Glass/elver | 19.73° N −72.11° W; 19.70° N −72.02° W;19.70° N −71.75° W |
| Guadelopue (GUA) | 9 | LANTL | 65 | 2015/2016 | Adult eel | 16.22° N −61.78° W; 16.08° N −61.76° W |
| Puerto Rico (PR) | 39 | | | 2018 | Adult eel | 18.36° N −65.71° W; 18.41° N −66.09° W; 18.36° N −65.71° W; 18.36° N −65.76° W; 18.37° N −65.76° W; 18.35° N −65.72° W; 18.36° N −66.06° W |
| Trinidad and Tobago (TT) | 17 | | | 2019 | Adult eel | 10.70° N −61.62° W; 11.26° N −60.70° W |
| Colombie (COL) | 5 | SCARIB | 48 | 2013 | Adult eel | 11.23S −73.80° W |
| Costa Rica (CR) | 43 | | | 2017/2018 | Adult eel | 9.66° N −82.83° W; 9.69° N −82.86° W; 9.68° N −82.84° W; 9.67° N −82.83° W; 9.62° N −82.68° W |

Due to very unbalanced (and in some localities, small) sample sizes, we followed Lou et al. (2021) suggestions to increase the statistical robustness of population structure analysis. Thus, we developed a block design, grouping localities by geographic area (Figure 1; Table 1).

## 2.3 | Preparation pipeline

Raw low-coverage sequences were processed with the whole genome sequencing pipeline designed by E. Normandeau (https://github.com/enormandeau/wgs_sample_preparation) which is used for trimming, sequence quality assessment, reference genome alignment, indel correction, and PCR duplicates deletion. Trimming and quality control were done with fastp (Chen et al., 2018). Trimmed sequences were aligned to the reference genome using BWA-MEM (Li, 2013). Duplicates were removed and bam indexes were created with MarkDuplicates (Picard v1.119). We realigned target sequences around the indels with GATK 3.8 IndelRealigner (McKenna et al., 2010). Overlapping ends of paired reads were soft clipped with BamUtil 1.0.14 function clipOverlap

(Jun et al., 2015). Mean coverage per chromosome was calculated using mosdepth (Pedersen & Quinlan, 2018) to estimate maximum depth filters in the next step.

Processed sequences were analyzed with ANGSD v0.937 (Korneliussen et al., 2014) following the angsd_pipeline developed by Claire Mérot (https://github.com/clairemerot/angsd_pipeline) to estimate allele frequency considering the most frequent allele as major allele (-doMAF 1 -doMajorMinor 1) on the whole data set as well as depth and genotype likelihoods (GL-2, GATK method). Positions were kept if between 2X and 5520X (-setMinDepthInd 2, -setMaxDepth 5520, which is determined by multiplying three times the mean coverage per chromosome obtained in the previous section by number of individuals: 12*460), present in a minimum of 80% of individuals (-minInd 0.8), with minor allele frequency (MAF) above of 5% (–minMaf 0.05) and *p*-value of the likelihood ratio test for SNP variability threshold of 0.000001 (-SNP_pval). Additionally, input sequences were filtered to keep mapping and base quality above 30 and 20, respectively (–minMapQ 30 -minQ 20), and the analysis was performed excluding scaffolds that account for only 1.76% of the genome. Individual depth was calculated as the average site's depth for each individual. Depth after filtration in each variant position was obtained by using the functions -doCounts 1 -doDepth 1 -maxDepth 1000 -dumpCounts 2. Sites with heterozygote excess, likely to be paralogs, genotyping errors, coverage-related issues, or duplications due to copy number variants, were estimated as in Pečnerová et al. (2021), by using per-site inbreeding coefficients (F) estimated in PCAngsd v0.97 (Meisner & Albrechtsen, 2018). Sites with $F < -0.95$ were considered in heterozygote excess and eliminated from our main data set.

A principal components analysis (PCA) was used to check for outliers, positive control duplicates, and sequences with low coverage. The PCA was conducted in PCAngsd v0.97, and an eigenvalue correction (scaling two transformation; Legendre & Legendre, 2012) was added in R v3.6.3 (R Core Team, 2020) before visualizing with plotly (Plotly Technologies Inc, 2015) and ggplot2 (Wickham, 2016). Additionally, we inferred "true" genotypes using the function -bcf in ANGSD (-postCutoff 0.80). We used the custom script available at https://gitlab.com/YDorant/Toolbox/-/blob/master/00-VCF_Reshaper.sh to transform vcf files in StAMPP (Pembleton et al., 2013) format.

## 2.4 | Spatial population structure analysis

### 2.4.1 | PCA and PCA-based analysis

We performed a second whole-genome PCA, to visualize clustering and geographical patterns due to shared genetic variation with PCAngsd v1.11 (Meisner & Albrechtsen, 2018). This PCA was visualized as previously described. We followed this same approach in each chromosome to identify putative structural variations and their locations. Once identified (see results), we performed a third PCA without putative chromosomal rearrangements underlying the whole-genome PCA pattern.

We verified if the haplogroups created by such putative structural variants deviated from Hardy–Weinberg equilibrium (HWE) using a Chi-square test with Yates correction (X2-Y), Fisher's exact test (F), and log-likelihood test with continuity correction (G), all implemented in the HW_TEST software (Santos et al., 2020). Additionally, we tested whether haplogroup proportions were distributed evenly between geographical regions with the chi-square test of independence with Yates' correction using the chisq.test function from package stats v3.6.3. We also estimated Fisher's exact test *p*-values by Monte Carlo simulation with $2^8$ replicates using the fisher.test function from package stats v3.6.3. Finally, we ran pairwise comparisons of haplogroups' frequencies between regions with post-hoc pairwise chi-square, Fisher's exact test, and G test estimated with the function pairwiseNominalIndependence from the package rcompanion (Mangiafico, 2015) controlling for false discovery with the Benjamini and Hochberg correction (Benjamini & Hochberg, 1995). All statistical analyses with package stats and rcompanion were done in R v3.6.3.

### 2.4.2 | PC-based population/habitat-blind outliers

To identify highly linked regions characteristic of inversions, we performed PC-based genome-wide selection scans using the extended selection models implemented in PCAngsd (Meisner et al., 2021). Such models are based on FastPCA (Galinsky et al., 2016), hereby named PCAngsd-S1 following Meisner et al. (2021) and R package pcadapt (Luu et al., 2017; Privé et al., 2020), named PCAngsd-S2. We chose alpha = 0.05 as the threshold for outlier detection after adjusting for the false discovery rate with the Bonferroni correction as in Meisner et al. (2021). Manhattan plots of SNP-based *p*-values were plotted with package qqman (Turner, 2018) in R 4.10.

### 2.4.3 | Discriminant analysis of principal components (DAPC)

As described by Jombart et al. (2010) and using the script available at https://github.com/therkildsen-lab/genomic-data-analysis/blob/master/scripts, we performed a linear DAPC of the PCA covariance matrices obtained with PCAngsd with the lda function implemented in the R package MASS. Following the "$k-1$ criterion" proposed by Thia (2023), we retained 6 PC axes for the DAPC.

### 2.4.4 | Admixture-like clustering analysis with NGSadmix

Bayesian clustering analysis with NGSadmix (Skotte et al., 2013) was done after pruning the data set for linkage disequilibrium (LD) using plink function indep with VIF = 2 and $N = 5$, window size 10 kb (following estimations of LD decay described for the European Eel in Hemmer-Hansen et al. (2014)) and removing

chromosomes 11 and 13 since these chromosomes present putative structural variants that could bias such analysis (see Results). We performed clustering analysis using sites present in ~90% of individuals (414 samples) and ran each K (from 2 to 8) twice. Sample ancestry assignations were visualized with CLUMPAK (Kopelman et al., 2015).

#### 2.4.5 | Pairwise genetic distance (Fst)

The average pairwise genetic distance (Fst) between geographical regions was estimated from both the genotype likelihoods using ANGSD's realSFS and the estimated "true" genotypes using StAMPP. Site allele frequencies were obtained for each geographic region (-doSaf 1) using the reference genome as ancestral (–anc) to polarize the spectrum and restricting loci to those previously obtained when estimating genotype likelihoods (–sites –doMajorMinor 3). To avoid the effect of uneven sample sizes, geographic regions were randomly subsampled to the smallest group size. The joint 2D site frequency spectra (SFS, also called allele frequency spectrum) and the weighted pairwise Fst values were estimated using realSFS (–fold 1, considering 500,000 sites). We estimated Hudson's Fst (Hudson et al., 1992) as described by Bhatia et al. (2013, equation 10) and implemented in realSFS (-whichFst 1). Hudson's Fst (1992) estimator has been suggested to be less biased for small sample sizes and therefore more relevant for our data set. Pairwise Weir and Cockerham (1984) Fst values were calculated using the package StAMPP (1000 boostraps across loci) in a randomly selected subset of 100,000 sites. This was repeated 10 times, and we reported the average pairwise Fst values.

## 3 | RESULTS

### 3.1 | Reference genome

We obtained a genome assembly of approximately 975Mbp with high continuity (scaffold N50 = 56.6 Mb, contig N50 = 5 Mb). The 19 chromosome-like assembled scaffolds contained 98.2% of the total assembled genome length. The completeness of the core gene content was 85.9% against the database actinopterygii_odb10, with 80.0% complete and single copy and 5.9% duplicated. High synteny against the sister species genome (GCA_013347855.1) was observed in the synteny alignment (Figure S1).

### 3.2 | Spatial population structure analysis

The average sequencing depth estimated by mosdepth was 4.06X, going from 3.95X in chr 16 and chr 18 to 4.4X in chr 19 (Table S1). After calculating genotype likelihoods with ANGSD, 8,593,829 variant sites (4.15X average depth) were found. We excluded 17,021 sites due to heterozygosity excess, representing less than 0.2% of the entire data set, therefore, a total of 460 individuals and 8,576,808 sites were retained for downstream analyses. In the LD-pruned data set, we retained 5,661,242 variants.

### 3.3 | Principal components analysis (PCA) and PCA-based analysis

To infer population structure through individual allele frequency estimation, we performed two PCAs to visualize genotype likelihood relationships. All nine discrete groups observed in the whole-genome PCA (Figure 2a) are likely caused by differential segregation due to polymorphic chromosomal rearrangements. Here, groups along PC1 are correlated with a large structural variant found in chromosome 11, while differences along PC2 correspond with another structural variant found in chromosome 13 (PCA of chromosomes 11 and 13 in Figure S2). In each of these two chromosomes, the rearrangement appears to be a polymorphic inversion, with samples divided into three haplogroups (homozygotes for each state and heterozygotes as an intermediate group) as described by Huang et al. (2020). PCA and PC-based methods have been previously used in the detection of inversions (Nowling et al., 2020, 2022), with PCA clusters due to putative inversions observed in other fish species (e.g., Atlantic Cod *Gadus morhua*; Berg et al., 2017). A similar PCA pattern to the one observed here, with nine clusters due to two inversions, was observed in the fly *Coelopa frigida* by Mérot et al. (2021). Neither PCA (whole genome or without chromosomes 11 and 13) show groups that correspond with geographical origin (Figure 2a,b; PC 3 and PC 4 plotted in Figure S3) and there is little variance explained by the first two principal components (PC1 = 0.46% and 0.41%, respectively, PC2 = 0.38% and 0.36%, respectively). To further explore this signal and verify the putative inversions' localization, we produced Manhattan plots of PC-based population blind selection scans (Figure 3). Several outliers (q-value after Bonferroni correction <0.05) were found, 49,616 of them with PCAngsd-S2. With the PCAngsd-S1 method, we detected 19,213 outliers in PC1 and 11,130 in PC2. All outliers found with PCAngsd-S1 were also found with PCAngsd-S2 but not vice versa, thus we considered the outliers found with PCAngsd-S1 to be true positives for each PC. It is also noteworthy that no outlier was shared between PC1 and PC2. Most outliers were localized in a block in chromosome 11 or 13 for PC1 and PC2, respectively (see Figure 3), and could represent regions of low recombination (high LD) that are characteristic of inversions or other chromosomal arrangements. As observed by Nowling et al. (2020), when an SNP's PC association values are plotted against an SNP's position, "inversion regions stand out due to the presence of a step-function like pattern, with a large number of associated SNPs in the inverted region and few outside of the region." In addition, there were 120 SNPs that appeared as possible outliers (108 in PC1, 11 in PC2). Some of these appear to be the result of high LD. We believe this because in the LD-pruned data set, we observed

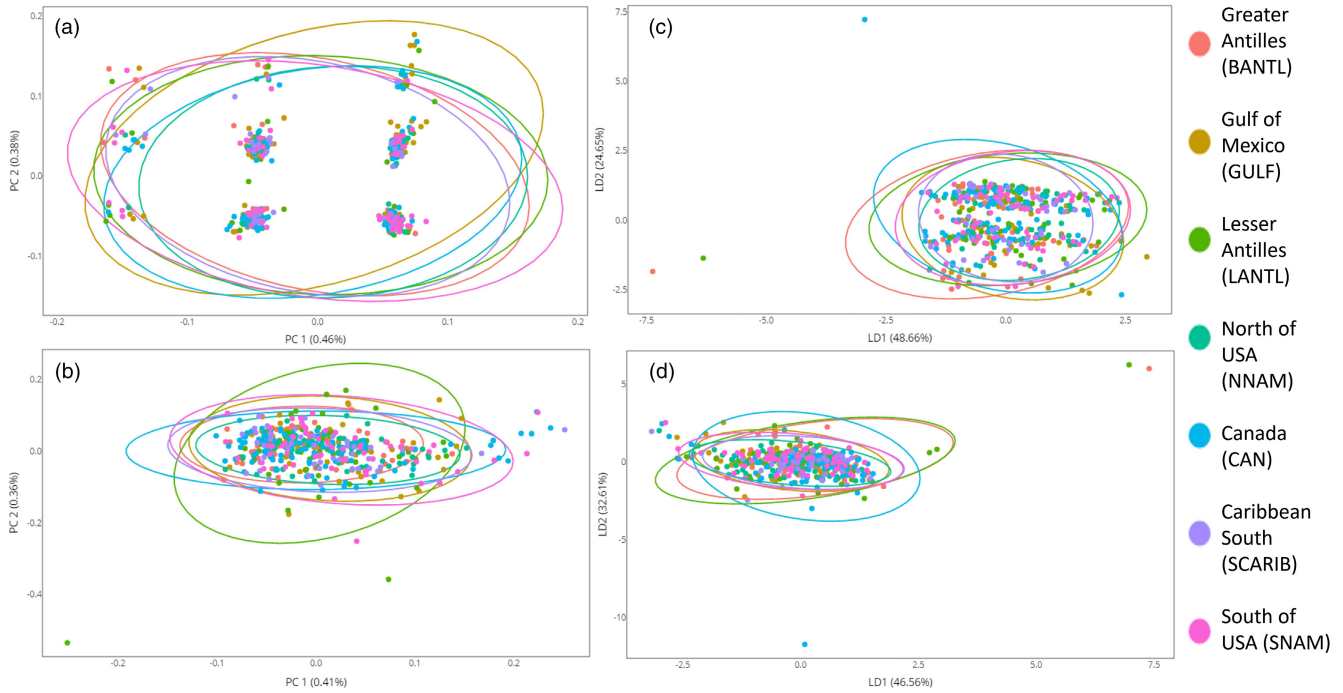


**FIGURE 2** (a) Whole-genome principal components analysis (PCA). (b) PCA without chromosomes 11 and 13. (c) Whole-genome discriminant analysis of principal components (DAPC). (d) DAPC without chromosomes 11 and 13.

only 67 outliers (instead of 108, in addition to the putative chr 11 inversion) in PC1. LD pruning at 10 kb eliminated the signal of the putative inversion in chr 13, and only one outlier (10 less than in the non-pruned data set) was found in PC2 in the pruned data set.

We found no evidence of spatial structure in these two putative inversions. In chromosome 11, approximately 49.3%, 43.5%, and 7.2% of individuals sampled belong to each haplogroup, respectively. In chromosome 13, individuals' frequency in each haplogroup was 50.2%, 40.9%, and 8.9% respectively. All seven geographic regions are represented in each haplogroup. Both putative inversions are in Hardy–Weinberg equilibrium (chr 11 $p$-values >0.2, chr 13 $p$-values >0.8) for the whole data set and for each geographic region (Table S2). The haplogroups are distributed in similar proportions in all regions (chr 11 Chi-square $p$-value = 0.73, Fisher's exact test $p$-value = 0.73; chr 13 11 Chi-square $p$-value = 0.53, Fisher's exact test $p$-value = 0.61) and none of the pairwise comparisons were significant after adjusting for multiple corrections (Fisher's adjusted $p$-value <0.5; Table S3). Therefore, we conclude that putative inversions' frequencies behave neutrally across the species' range.

We retained 6 PCs following suggestions by Thia (2023) to avoid under or overfitting of the DAPC. Whole-genome DAPC results are presented in Figure 2c, and DAPC results for the whole-genome minus chromosomes 11 and 13 are presented in Figure 2d. The whole-genome DAPC shows three clusters that correspond to the individuals in each haplogroup created by the putative inversion in chr 11, but neither DAPC shows any geographically linked clusters.

### 3.4 | Admixture-like clustering analysis with NGSadmix

We used NGSadmix to infer admixture proportions from genotype likelihoods in a data set pruned for LD and without chromosomes 11 and 13. In the admixture analysis (Figure 4), we did not identify any cluster at any of the K modeled, with most individual ancestries splitting similarly among all groups.

### 3.5 | Pairwise genetic distance (Fst)

Weighted pairwise Hudson's *Fst* values estimated with realSFS are all under 0 (Table 2). Negative values sometimes have no biological sense and are interpreted as being 0 (Smaragdov et al., 2018; Willing et al., 2012). Therefore, these pairwise *Fst* values indicate no genetic differences between geographic regions. Negative values are usually believed to arise due to uneven sample size. Here, sample sizes were normalized for the *Fst* calculations so they could be discarded as causing any effect. In multiallelic markers such as microsatellites, it has been suggested that negative values could be the effect of small sample size/number of alleles ratio (Gerlach et al., 2010), but here we only considered bi-allelic SNP markers. Negative values can also appear when within-population alleles are more different than are between-populations (Wilkinson et al., 2011) and usually appear when comparing a population to itself or to one where less than 50% of individuals are genetically different (Smaragdov et al., 2018). Therefore, negative Hudson's *Fst* values could, in this case, be a product of the variance within regions being higher than

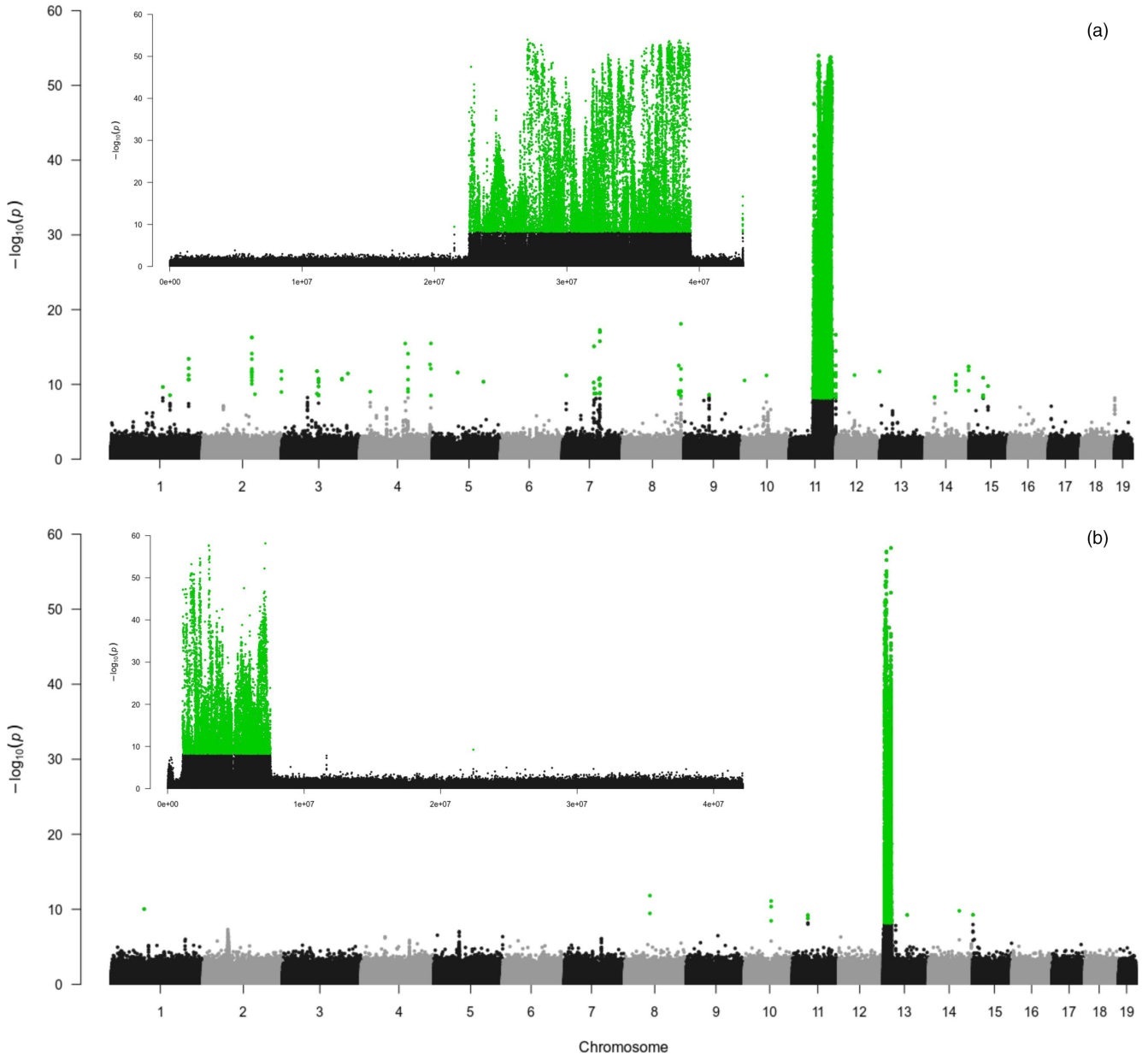


FIGURE 3 Manhattan plots of population/habitat blind selection scans based on principal components. Outliers (q-value after Bonferroni correction <0.05) are highlighted in green. (a) Principal component 1 (PC1), zoom on chromosome 11. (b) Principal component 2 (PC2) zoom in chromosome 13.

that between regions and a reflection of the lack of genetic differences between them. Pairwise Weir and Cockerham (1984) mean *F*st estimates obtained from "hard" or true genotypes are shown in Table 2. In all 10 estimations, average *F*st values are low (≤0.00018) and no *p*-value was consistently under 0.01 (Table S4). It is thus likely that *F*st values are equivalent to zero, confirming that genetic differentiation is nonexistent and supporting the hypothesis of panmixia throughout the entire range of American eel, including the tropics.

## 4 | DISCUSSION

The goal of this study was to revisit the panmixia paradigm for the American eel by expanding sampling coverage to the species' tropical range and using low-coverage sequence analysis of the entire genome. Panmixia in eastern North America and Greenland has already been shown (Côté et al., 2013) but here, we confirm the absence of geographical genetic structure throughout the species' range by including American Eel sampled throughout the southern portion of the range including the Caribbean, South America, and the Gulf of Mexico. Our results also reveal novel insights on genomic structure, that is low-coverage whole-genome sequencing revealed two putative chromosomal rearrangements. Despite a body of research associating genomics inversions to local adaptation (e.g., Schaal et al., 2022; Thorstensen et al., 2022) neither of these two putative inversions showed evidence of being differently represented in different sampling locations and thus did not contribute to population structure. Altogether, our results reveal for the first time

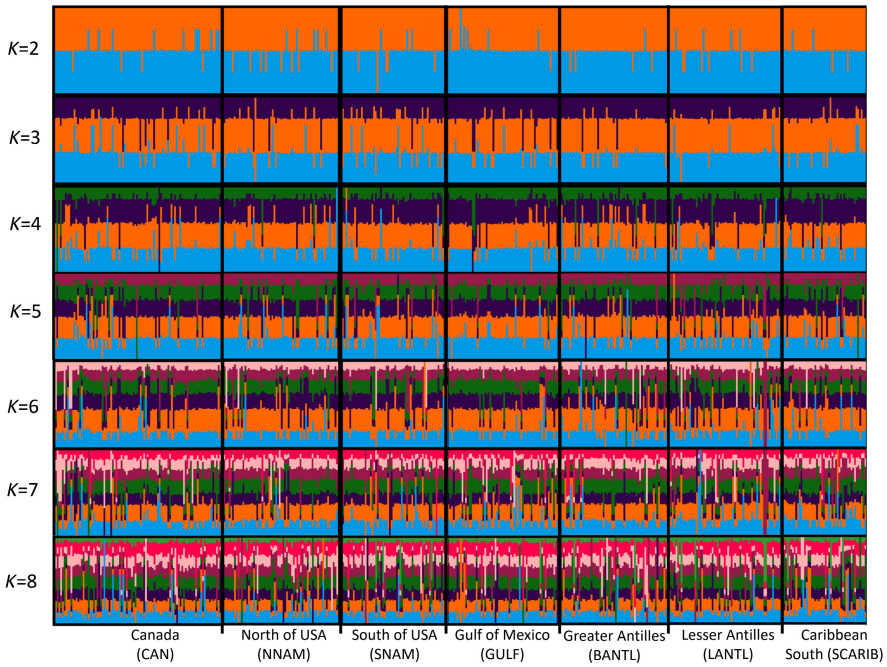


**FIGURE 4** Admixture proportions were obtained using NGSadmix. From top to bottom is the analysis assuming from 2 to 8 ancesral populations ($K = 2–8$). For each K, each column represents one individual, colors represent estimated ancestries, and black vertical lines delimit geographic regions.

**TABLE 2** Pairwise FST distances.

| | CAN | NNAM | SNAM | GULF | BANTL | LANTL | SCARIB |
|---|---|---|---|---|---|---|---|
| CAN | | −0.00061 | −0.0007 | −0.00076 | −0.0007 | −0.00073 | −0.00065 |
| NNAM | 0.00005 | | −0.00084 | −0.00084 | −0.00072 | −0.00081 | −0.00071 |
| SNAM | 0.00005 | 0.00001 | | −0.00092 | −0.00077 | −0.00083 | −0.00078 |
| GULF | 0.00010 | 0.00010 | 0.00005 | | −0.00087 | −0.00094 | −0.00082 |
| BANTL | 0.00005 | 0.00004 | 0.00002 | 0.00010 | | −0.00079 | −0.00074 |
| LANTL | 0.00009 | 0.00002 | 0.00008 | 0.00010 | 0.00005 | | −0.0008 |
| SCARIB | 0.00003 | 0.00009 | 0.00008 | 0.00018 | 0.00008 | 0.00012 | |

*Note*: Regions are ordered southward. Above the diagonal are weighted Hudson's Fst values (1992) as expressed in Bathia (2013; formule 10). Below the diagonal are the Weir and Cockerham (1984) Fst mean values obtained from inferred "hard genotypes."

that panmixia extends to the tropical range of American Eel. Here we discuss our finding implications for juvenile migration as well as for the development of fishery management policies and conservation strategies. Moreover, we discuss how our results shed light on the species' occurrence throughout a highly heterogenous environment despite the apparent absence of local adaptation.

## 4.1 | American eel reference genome

Previous draft genomes for the species using only short sequence reads (Pavey et al., 2017) estimated the haploid genome size at 1.41 Gb, which is in the range of the genome sizes estimated for the species using bulk fluorometric assays and Feulgen image analysis densitometry (C values between 1.01 and 1.66; in www.genomesize.com, accessed January 9, 2023). However, Jansen et al. (2017) estimated the species' haploid genome size to be between 799.0 and 813.0 Mb based on *k*-mer profiles of Illumina-sequencing data. Our final assembly lies in between C-value estimations and those based on *k*-mer profiles and is similar in size to haploid genomes of the closely related European Eel (Table 3). Our improved reference genome has 19 chromosomes, a number consistent with the number of chromosomes reported for European eel (GCA_013347855.1) and Japanese eel (*Anguilla japonica*, GCA_025169545.1), the two phylogenetically closest species (Aoyama et al., 2001). BUSCO completeness was 85.9%, which is in the range (between 50% and 95%) of what has been reported for other nonmodel species by Seppey et al. (2019), while model species completeness is often above 95%. Further improvements and new genome iterations should increase this metric, but in its current state, the genome is a valuable resource for freshwater eel genomics.

## 4.2 | Panmixia implications for glass eel migration

Historically, the breeding area, migratory routes, and navigation cues of North Atlantic eels have all been the subject of much speculation. Some researchers have proposed more than one or an alternative

**TABLE 3** North Atlantic eel's published genomes.

| Common name | American eel (this paper) | American eel (previous) | European eel | European eel | European eel (linked assembly) | | European eel |
|---|---|---|---|---|---|---|---|
| Scientific name | *Anguilla rostrata* | *Anguilla rostrata* | *Anguilla anguilla* | *Anguilla anguilla* | *Anguilla anguilla* | | *Anguilla anguilla* |
| Total sequence length | 975.29 Mb | 1.41 Gb | 893.6 Mbp | 1.01 Gb | 979.04 Mb principal pseudohaplotype at chromosome level | 965.24 Mb alternate-pseudohaplotype at contig level | 1.03Gb |
| # of chromosomes | 19 | 0 | 0 | 0 | 19 | 0 | 19 |
| # of contigs | 2074 | 3,07,315 | 2356 | 8,65,467 | 693 | 2338 | 1799 |
| Contig N50 | 5.02 Mb | 7.35 kb | 1.19 Mb | 2.5 kb | 5.11 Mb | 723.28 kb | 13.16 Mb |
| Contig L50 | 50 | 47,985 | NA | 84,717 | 54 | 378 | 26 |
| # of scaffolds | 1093 | 79,208 | NA | 5,01,148 | 54 | NA | 1466 |
| Scaffolds N50 | 56.61 Mb | 86.64 kb | NA | 59.65 kb | 57.2 Mb | NA | 55.98 Mb |
| Scaffolds L50 | 8 | 4171 | NA | 2979 | 7 | NA | 8 |
| Complete BUSCO (%) | 85.90% | NA | 79.80% | NA | 98.40% | NA | NA |
| Repeat elements (%) | 5.90% | NA | NA | NA | 9.20% | NA | NA |
| Reference | This paper | Pavey et al., 2017 | Jansen et al., 2017 | NA | NA | NA | NA |
| Database accession | GCA_018555375.2 | GCA_001606085.1 | PRJEB20018 | GCA_000695075.1 | GCF_013347855.1 | GCA_013347865.1 | GCA_018320845.1 |
| Year published in database | 2021 | 2016 | 2017 | 2014 | 2020 | 2020 | 2021 |
| Technology | PacBio Sequel; Oxford Nanopore PromethION | Illumina HiSeq 169X | Oxford Nanopore MinION | Illumina GAIIx 20X | PacBio Sequel I CLR; Illumina NovaSeq; Arima Genomics Hi-C; Bionano Genomics DLS 51.23X | PacBio Sequel I CLR; Illumina NovaSeq; Arima Genomics Hi-C; Bionano Genomics DLS 51.23X | Oxford Nanopore PromethION |

breeding area (Chang et al., 2020, 2022; Kleckner & McCleave, 1980, 1982; Westerberg et al., 2018). For instance, a smaller breeding area in the western Caribbean Sea was proposed by Kleckner and McCleave (1980, 1982) and again discussed by Miller et al. (2015). In all cases, speculation was triggered by the collection of small larvae close to the Yucatan peninsula. However, after Schmidt's initial findings (Schmidt, 1923), most surveys focused exclusively on the Sargasso Sea, thus a different breeding area can not be confirmed or totally denied, even if its existence remains unlikely (Miller et al., 2015). Chang et al. (2020) proposed an alternative spawning area for both Atlantic eel species in the Mid-Atlantic Ridge. This hypothesis was challenged by Hanel et al. (2022), who pointed to several deficiencies, particularly the misinterpretation of otolith chemistry data from Martin et al. (2010). Chang et al. (2022) recognize the mistake, but authors conclude that while their reasoning does not preclude spawning in the Sargasso Sea, it does "suggest that spawning occurs in multiple places in the Sargasso Sea and that spawning over the Mid-Atlantic Ridge is possible, if not likely." However, whatever the number and/or extent of the spawning area, our results support the current scientific consensus through the confirmation of random mating in the entire species, which would most likely happen only if all individuals spawned synchronously in a single location. Up to now field data seem to confirm that the spawning area is in the western part of the Sargasso Sea where the newly hatched larvae have been found (reviewed in Miller et al., 2015, see also Figure 15 in it; additional collections for the European Eel in Miller et al., 2019).

Furthermore, our results raise several questions pertaining to the American Eel larval dispersal. Despite larvae's possible active swimming capabilities (Rypina et al., 2014), it is unlikely that the species' eggs or larvae are capable of moving countercurrent to cross from the Florida Strait to the Gulf of Mexico. Larvae collected in the Caribbean Sea and the Gulf of Mexico (EEL database https://www.ices.dk/data/data-portals/Pages/Eggs-and-larvae.aspx; Miller et al., 2015) are thought to reach this area through the Greater Antilles passages, namely the Windward and Mona Passages (Kleckner & McCleave, 1982; Miller et al., 2015; Munk et al., 2010). Active elver and glass eel fisheries in Cuba and La Hispaniola (Cairns, 2020) suggest that this area receives a high influx of eel at early life stages. The southernmost Caribbean records of larvae include one observation southeast of Puerto Rico, three observations north of St. Croix, and a single observation northeast of Panama (EEL database https://www.ices.dk/data/data-portals/Pages/Eggs-and-larvae.aspx). Except for these, no larvae have been detected near South America and the Lesser Antilles, thus migratory pathways leading to those growing grounds where yellow eels are known to occur (Benchetrit & McCleave, 2016; Cairns et al., 2022) remain unknown.

### 4.3 | Panmixia precludes local adaptation to environmental variables

The absence of genetic structure makes classic inheritable local adaptation impossible, which is striking given the broad range of environments American eel is known to inhabit. Phenotype, life history, and behavior of American eel changes with both latitude and habitat (Cairns et al., 2008; Jessop, 2010; Laflamme et al., 2012). Confirmation of panmixia across the entire species' range suggests that within-generation habituation to local conditions is driven by spatially varying selection (polygenic selection within each generation; Babin et al., 2017; Gagnaire et al., 2012; Laporte et al., 2016; Pavey et al., 2015; reviewed in Pujolar et al., 2022), epigenetic adaptation (Liu et al., 2022), and/or habitat choice (Mensinger et al., 2021), alone or in synergy. In the absence of a genetic basis for these local differences, it can be expected that for genotypically determined traits, American eel individuals will react similarly to environmental cues range-wide. Spatially varying selection associated with salinity, temperature, and pollution has been previously described from individuals inhabiting North Atlantic drainages and the St. Lawrence River (Babin et al., 2017; Gagnaire et al., 2012; Laporte et al., 2016; Pavey et al., 2015). Environmental factors behind spatially varying selection and/or influencing phenotypic plasticity (e.g., Côté et al., 2014), in the Saint Lawrence River system and North Atlantic drainages, would also be expected to have a similar effect in southern, less-studied parts of the species' range. Admittedly, however, there are no studies pertaining to behavioral or phenotypic differences comparing eels inhabiting Atlantic drainages in North America to those in the Gulf of Mexico or continental Caribbean. Like most North America East Coast drainages and the St. Lawrence R. system, eel density in Puerto Rico rivers decreases upstream, where individuals tend to be larger, and larger individuals are more frequently female (Kwak et al., 2019; Torres-Molinari et al., 2023). Wang and Tzeng (1998, 2000) found that elvers in all pigmentation stages recruiting in North Carolina, Florida and La Hispaniola are, on average, smaller than those recruiting further in the northern part of the range. Later, Jessop (2010) and Laflamme et al. (2012) corroborated with North American samples (from Florida to the St. Lawrence River System) that elvers recruiting at higher latitudes were longer, on average. Moreover, by performing a meta-analysis, Jessop (2010) found positive correlations in females between both latitude and migration distance with both length and age at metamorphosis, but, in males, only with age at metamorphosis.

The faster growth rate of southern-bound recruits has been attributed to warmer waters by Wang and Tzeng (1998), and a positive effect of higher rearing temperatures on change in mass in glass eels has been empirically observed (Blakeslee et al., 2018). Additionally, RNA/DNA ratios (a metabolic proxy that is influenced by temperature, among other variables) were found to vary with latitude between North American localities (Laflamme et al., 2012). Higher ratios are associated with higher protein synthesis and higher recent growth in marine fish larvae; however, the same growth rate can be achieved under higher temperatures with less RNA, and therefore, a smaller RNA/DNA ratio (Chícharo & Chícharo, 2008). However, American eel recruits have the compounded effect of time spent in oceanic waters in addition to the water temperature effect. Recruitment in temperate regions extends from December in Florida to June–July in Newfoundland–Labrador (Helfman et al., 1987 in Côté et al., 2013).

It peaks from January to March midway in North Carolina and New Jersey (Powles & Warlen, 2002) and from late April to late June in Nova Scotia (Jessop, 1998 in COSEWIC, 2012), while in the tropics, (Cuba) the peak occurs from October to November (Fernández & Vázquez, 1978). Thus, American eel larvae recruiting to the Gulf of Mexico and the Caribbean could present smaller RNA/DNA ratios than those recruiting to higher latitudes in North America, while presenting similar or higher growth rates and lengths. Unfortunately, there is not enough information about the species in the Caribbean to discuss more about life history or demographic trends.

In the context of spatially varying selection, selective pressures in one geographic area could be rapidly reflected in the genomic makeup of American eel in another area. That is to say that changes in allele frequency due to either temporally or spatially varying selection in one region could be rapidly observed in another region where the selective pressure is not present. Besides selective pressure influencing which individuals survive to adulthood and can complete the reproductive migration to the Sargasso Sea, it is important to also consider that there may be discrepancies in the number of individuals that will make the spawning migration from each region, ultimately leading to regional discrepancies in contributions to the mating pool. This has important implications for the conservation of the species. For instance, overfishing or habitat loss (Jacoby et al., 2015, 2018; Miller et al., 2009) could regionally decrease the number of mature silver stage adults, thereby compromising the reproductive migration back to the Sargasso Sea. This would effectively decrease the gene pool and the numbers of individuals of the next generation across the entire range of distribution of the species (this consequence of panmixia and the possible "rescue effect" of one region have been discussed in COSEWIC, 2006).

Despite our results supporting range-wide panmixia, there is still much to be done to understand phenotypic and behavioral variation across the species' range, including for both recruitment and habitat preference, that have been documented across the northern range of the species. As previously discussed, genetic variants under spatially varying selection can be associated with environmental variables (Babin et al., 2017; Gagnaire et al., 2012; Laporte et al., 2016; Pavey et al., 2015). Here we did not pursue such analysis because we lack reliable environmental and/or life history data for most sampling locations from the southern portion of the range and the opportunistic nature of our sampling efforts resulted in samples being from multiple cohorts and ontogenetic stages. Further studies are needed to test if spatially varying selection in the southern portion of the American Eel range generates within-generation genetic differences, as has been documented in Northeastern USA and Canada.

### 4.4 | American eel management and conservation

The main management and conservation implication of this study is that the entire species of American eel is a single, panmictic population and fishery stock. Hence, interjurisdictional international cooperation is necessary for its effective assessment and management, as is range-wide cooperation and consistency in conservation policies. Currently, there are active American eel commercial fisheries in several countries, each with distinct regulations and quotas (Gollock et al., 2018: Shiraishi, 2020; Cairns, 2020; Cornic et al., 2021). However, our knowledge about stock trends and landings is fragmented at best. Eel research is scarce in Latin America and the Caribbean (Benchetrit & McCleave, 2016; Kwak et al., 2019; Roghair et al., 2014; Torres-Molinari et al., 2023; Wang & Tzeng, 1998) and data throughout that region are sometimes unreliable. Similarly, data are sparse, fairly recent, and inconsistent across all Gulf of Mexico rivers, and even the North American Atlantic data are uneven depending on locations (Cairns, 2020; Cairns et al., 2022; Cornic et al., 2021). Moreover, growth habitats, river continuum, and contamination by organic and elemental pollutants should be carefully taken into account to manage the species, as suggested in recent studies (e.g., Bourillon et al., 2020, 2022; Miller et al., 2016; Righton et al., 2021). Stock assessments, as well as demographic, abundance, contamination, and life history data sets are almost all from the Western Atlantic (Cairns, 2020, Cairns et al., 2022, Cornic et al., 2021). Only recently have efforts been made to coordinate conservation action. Notably two workshops, in 2018 and 2021, were organized by the Sargasso Sea Commission. These were the result of Decisions 17.186 and 17.187 on *Anguilla* spp., which were adopted in the 17th Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) (CoP17, Johannesburg, 2016) and called for, among other things, the compilation and sharing of data between eel range states and other parties involved in conservation and trade of the genus. Additionally, the International Council for the Exploration of the Sea (ICES) has also recently begun an effort to compile range-wide data on the species (ICES, 2023). The nonexistence of a population/locality genetic fingerprint also means there is no way to infer fishery origin using genetic data, making it easy to conceal illegal traffic of live eels. International trade of the species has exploded in recent decades, fueled by a complete ban on exports of European eel, as well as growth in demand from Asian eel-rearing aquaculture facilities (Gollock et al., 2018; Shiraishi, 2020).

However, the lack of both genetic structure and local adaptation does not mean that individual American eels living in different localities are interchangeable, as translocation experiments have demonstrated. Previous controlled experiment studies and translocation experiments in American eel have shown that growth rates, size at maturation, and migration timing characteristics from their recruitment locations can be retained throughout their lives once translocated (Béguer-Pon et al., 2017; Côté et al., 2015; Pratt & Threader, 2011; Stahl et al., 2023; Verreault et al., 2010), possibly hindering the success of breeding migrations of translocated individuals. Durif et al. (2022) hypothesized that the route back to the Sargasso Sea gets imprinted during migration to coastal waters, therefore, translocation events could affect orientation during the migration, although evidence indicates that translocated eels are capable of finding their way to the Sargasso Sea in at least the initial part of the journey (Béguer-Pon et al., 2017; Stahl et al., 2023). Translocation could also modify local sex ratios (Côté et al., 2015;

Pratt & Threader, 2011). Thus, translocation for conservation or commercial purposes is not advised without further research.

Besides multiple threats to the survival of the species, climate change is an added source of concern (Drouineau et al., 2018; Jacoby et al., 2015; Miller et al., 2009). As weather patterns change, resilience capacity and adaptive potential in the form of standing genetic variation become more critical. As discussed in the previous section, a demographic decline of eels in one region not only would affect the number of reproductive migrants (COSEWIC, 2006) but could also alter the species' genetic pool. Thus, the maintenance of genetic diversity through the species range is paramount. Global climate change effects in panmictic *Anguilla* spp. were considered in detail by Drouineau et al. (2018); nonetheless, we would like to highlight that due to panmixia being linked to the existence of only one reproduction area, the species survival is also highly dependent on the maintenance of favorable conditions in this area and the ability of individual eels to migrate to and from there. It has been reported that the Sargasso Sea's physiochemical characteristics have changed in the last decades (Bates & Johnson, 2020) but it is unclear how much this would affect freshwater anguillids.

## 5 | CONCLUSIONS

Our work extends the American eel's panmixia paradigm to the previously understudied regions of the Caribbean and Gulf of Mexico, where population dynamics are mostly unknown. The findings of our study are of particular importance from a management and conservation standpoint because it highlights the need for cooperative management of the species as a fishery resource. In addition, we developed a high-quality reference American eel genome which will serve as an important tool for genomic research in the genus *Anguilla*. Finally, our results will benefit future conservation efforts for the species under climate change pressure and manmade threats.

### AUTHOR CONTRIBUTIONS

L.B. conceived the research idea. G.U.D. and L.B. coordinated new data acquisition, designed the research, and wrote the initial paper draft. J.B. provided contacts and helped to acquire samples. A.E., W.O.M., C.A.L.A., D.H., E.B., E.F., F.L.P.L., J.W., R.M., R.S.M., and T.J.K. contributed new data. G.U.D., B.B., S.H.C., S.J.R., and C.B. did wet lab work. G.U.D. and E.N. did bioinformatic analysis or sequences preparation. E.N., H.H.V.D., and J.R. worked in the genome assembly. A.E., W.O.M., C.A.L.A., D.H., E.B., E.F., R.M., R.S.M., C.B., S.H.C., H.H.V.D., J.R., and E.N. contributed with multiple revisions, editions, and additions. All authors but T.J.K (because his premature death) revised the text. J.B. declares that (1) the research was not undertaken under the auspices of Natural Resources Canada as part of his employment responsibilities, (2) the work was conducted during the author's previous affiliation with Fisheries and Oceans Canada, and (3) any expressed policy positions therein are not those of Natural Resources Canada, International Affairs and Trade Division Natural Resources Canada.


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[Correction added on 21 December 2023, after first online publication: the institution name for co-author William O. McLarney has been updated in this version.]


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## CONFLICT OF INTEREST STATEMENT

The authors report no conflict of interest.

## DATA AVAILABILITY STATEMENT

Raw sequences and associated metadata will be publicly available in the SRA database from GenBank (BioProject PRJNA964587) upon publication.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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