Conservation Genetics (2026) 27:55
https://doi.org/10.1007/s10592-026-01775-w

RESEARCH

Rangewide population genetic analysis of the Wood Turtle (*Glyptemys insculpta*)

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Received: 19 September 2025 / Accepted: 2 March 2026

Abstract

Wood Turtle (*Glyptemys insculpta*) populations are declining in many portions of the species' native range due to multiple factors that might influence functional connectivity and population genetic structure. We used 13 microsatellite markers to examine patterns of genetic structure in the Wood Turtle across its native range in Eastern and Midwestern North America. For $n=45$ collections with 15 or more individuals (total $N=1{,}258$), multiple clustering approaches revealed two major genetic groups corresponding to the midwestern and eastern collections. Interestingly, a sample from lower Michigan clustered with the Eastern group while a sample from the Upper Peninsula of Michigan clustered with the Midwestern group. Evidence of gene flow between these two major groups arose from the most proximate sites near the edges of each group. These results suggest that Lake Superior and Lake Michigan were historically substantial (but perhaps not complete) barriers to gene flow. Our results suggest that Evolutionarily Significant Unit (ESU) status is warranted for Midwestern and Eastern Wood Turtles in North America. Within the eastern group, we observed a strong pattern of clinal allele frequency variation, with evidence of incipient genetic differentiation between multiple collections from the Potomac and Monongahela Rivers from collections in river basins further to the north. Estimation of full-sibling families indicated a range of distance between close family members of 16.8–301 km, suggesting the possibility of extremely long-distance (though rare) dispersal. Mean expected heterozygosity ranged from 0.553 to 0.722 and allelic richness ranged from 4.1 to 6.8. For a species with such a long generation interval (approximately 40 years (yrs)), isolated populations on the low end of this range of both measures of genetic variation might suffer from negative fitness effects of inbreeding and warrant further monitoring efforts. Our results support the management of this species at, or within, the Hydrologic Unit Code-4 (HUC4) subregion scale.

Keywords Wood Turtle · Conservation units · ESU · Genetic structure · Hierarchical · Isolation-by-distance

Introduction

The Wood Turtle (*Glyptemys insculpta*) is distributed primarily in fluvial and riparian habitats from Nova Scotia, Canada in the eastern limit of the range, to Minnesota and Iowa (USA) at the western range limit, and Virginia (USA) at the southern range limit (Fig. 1) (Ernst and Lovich 2009; Jones and Willey 2020). This species is listed as Endangered by the International Union for the Conservation of Nature (IUCN) Red List (van Dijk and Harding 2011), is listed in Appendix II of the Conservation on International Trade in Endangered Species of Wild Fauna and Flora (CITES), and is currently under consideration for listing under the US Endangered Species Act (ESA) (Jones et al. 2018). Wood turtles are also listed as threatened under the Species at Risk Act (SARA) in Canada (COSEWIC 2018) and considered a regional species of greatest conservation need (RSGCN) in the northeastern United States (Jones et al. 2018; NEFWDTC 2024). Populations are declining in most areas of their native range (van Dijk and Harding 2011; Willey et al. 2022) with threats including loss and degradation of

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Published online: 22 April 2026

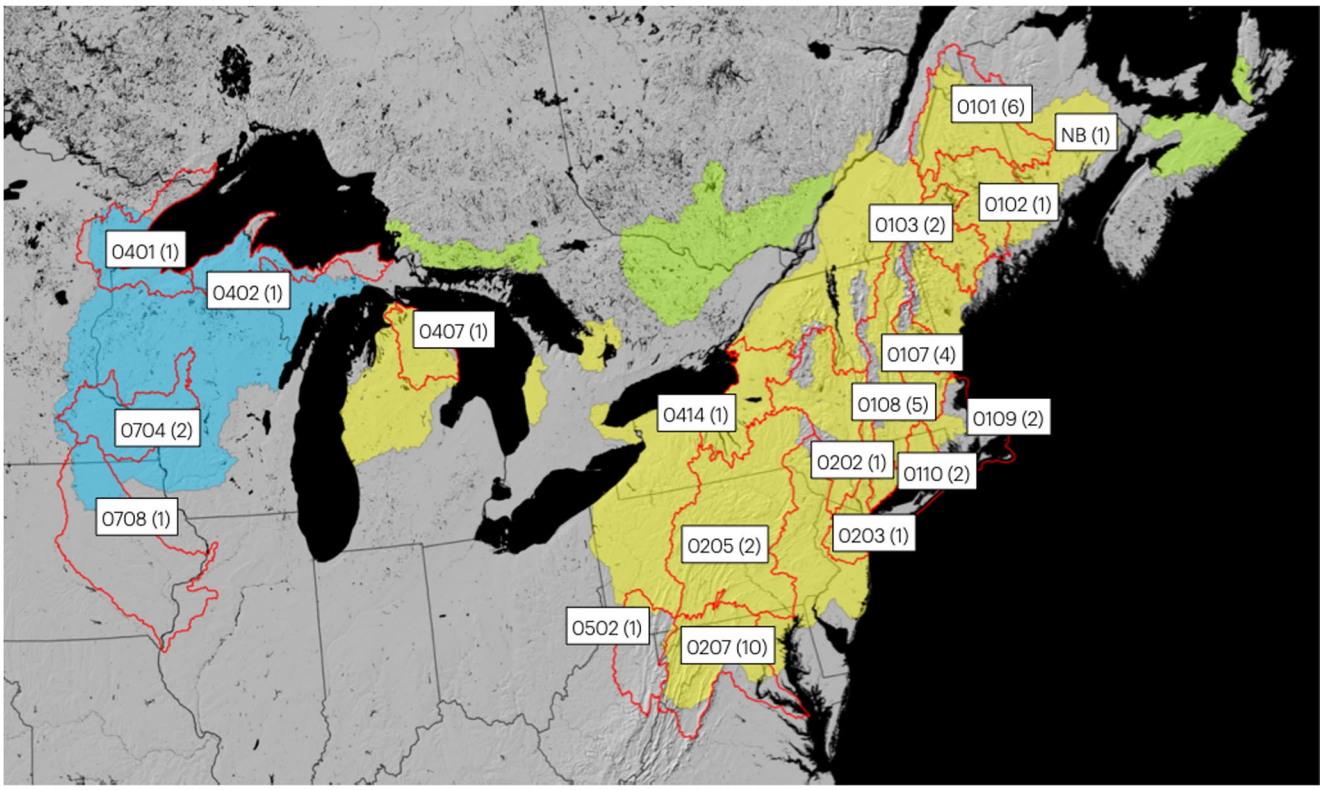

Fig. 1 Range map and Wood Turtle collection localities by occupied HUC4. Collection localities with = 15 individuals sampled ($n = 45$) are shown as the total number of sites within each HUC4 subregion. The total number of sites in a HUC4 is shown in parentheses. The blue shading represents the Midwestern genetic group defined in this paper. The yellow shading represents the Eastern genetic group defined in this paper. The green shading represents unsampled Canadian portions of the species range. Red outlines show occupied 4-digit hydrologic unit code boundaries (HUC4). The map is centered on the Great Lakes region of the Canada-USA border. Map prepared in ArcGIS Pro using North America Albers Equal Area Conic (projected coordinate system for North America). Shapefile distribution maps for the Wood Turtle were provided by TTWG (2025) and TTWG (2025) and are used with permission. Source attribution for basemap data: Airbus, USGS, NGA, NASA, CGIAR, NCEAS, NLS, OS, NMA, Geodatastyrelsen, GSA, GSI, and the GIS User Community. The map is shown at the same scale as Fig. 2.

riparian habitats by development and agriculture, population fragmentation by roads and development, depredation by human-subsidized meso-predators, mortality from roads and other anthropogenic disturbances (such as farming and logging), nest destruction and adult mortality from flooding, and collection and removal for the pet trade (Ernst and Lovich 2009; Spradling et al. 2010; COSEWIC 2018; Jones and Willey 2021; Tamplin 2024). Further, the species appears to also be threatened by a shifting and shrinking climatic envelope (Mothes et al. 2020).

To date, there is little range-wide population genetic information available for the Wood Turtle. Amato et al. (2008) examined variation in the mitochondrial control region across the species range and found support for a dominant influence of range expansion on genetic structure. Several other studies have used nuclear microsatellites to examine patterns of population genetic structure at smaller geographic scales (within or across adjacent river basins; (Tessier et al. 2005; Castellano et al. 2009; Spradling et al. 2010; Fridgen et al. 2013; Willoughby et al. 2013; Bouchard et al. 2019). These studies provide useful information about the genetic structure of the Wood Turtle for specific subregions, but the limited geographic scope precludes the identification of species-wide genetic structure. This semi-aquatic turtle tends to move along streams, generally within 300 m of waterways (Harding and Bloomer 1979; Arvisais et al. 2002; Jones and Willey 2020; Chatfield et al. 2022). Capture-recapture and radiotelemetry studies reveal short dispersal distances, usually less than 10 to 20 km (Daigle 1997; Behler and Castellano 2005; Walde et al. 2007; Jones and Willey 2020; Chatfield et al. 2022). The combination of previous genetic studies conducted at smaller spatial scales and tagging results leads to the expectation that we will observe historic effects of expansion post-glaciation on genetic structure, genetic differentiation at the subregion scale (HUC4), and a pattern of isolation-by-distance (IBD), whereby geographically proximate populations are less genetically divergent than those more distant (Bouchard et al. 2019).

In this study, we use microsatellite markers to examine the most spatially comprehensive collection of Wood Turtle

population samples available to date. We examined collections from Eastern North America ranging from Virginia, USA to New Brunswick, Canada along with four Midwestern US states. Our objectives were to: (1) describe patterns of genetic structure; (2) test for an IBD and hierarchical pattern in allele frequency divergence; (3) describe within-population genetic variation (heterozygosity and allelic richness); and (4) evaluate spatial patterns in the distribution of putative siblings and parent/offspring groups. Our results reveal a range-wide hierarchical population genetic structure that will assist with conservation planning at various spatial scales.

Methods

Sampling

Tissues were collected from the following US states and Canadian Province: Maine, New Hampshire, Massachusetts, Connecticut, New Jersey, Pennsylvania, Maryland, Virginia West Virginia, Vermont, Delaware, New York, Rhode Island, Wisconsin, Minnesota, Iowa, Michigan, and New Brunswick. We targeted 20 individuals per collection site. Adults were prioritized and when hatchlings were sampled, we usually sampled no more than one hatchling per nest. An exception occurred in one site (MAAlBr) where we specifically targeted multiple hatchlings per nest to test genetic sibship reconstruction approaches (see *Full-sib-based estimates of dispersal* below). In most cases, a study site was represented by collections from a single kilometer of stream, consistent with a regional population assessment protocol (Jones et al. 2018; Jones and Willey 2021), but far smaller than the potential lifetime movement distance or dispersal capability of an adult Wood Turtle (Jones and Willey 2020; Chatfield et al. 2022). In fewer cases, multiple 1-km sampling areas along the same named stream were combined into a single site locality. Even in extreme cases, the longest section of stream along which samples were pooled to form a sample locality was approximately 17 km, a distance comparable to long-distance movements observed repeatedly in the wild (Jones and Willey 2020; Chatfield et al. 2022).

We collected blood, tail tissue, toenail, and shell tissues. Other soft body parts (such as toes or foot) were occasionally collected from recent mortalities. Blood was preserved in 95% ethanol, lysis buffer (e.g., Queens lysis) and Phosphate-Bufferred Saline (PBS), or Flinders Technology Associates (FTA) cards depending on the collector. Other tissue types were preserved in 95% ethanol. Other than the PBS or FTA cards, samples were stored at -20˚C until processing.

Laboratory methods

DNA was primarily extracted from blood, tail, nail, and shell tissues using a Qiagen DNeasy Blood and Tissue Kit™ (Qiagen, Inc., Germantown, MD) according to manufacturer's protocols. Blood and tail and other soft tissue were incubated overnight at 55˚C, and nail and shell samples were incubated for 2 days on a shaking incubator (Henry Troemner LLC, Thorofare, NJ). DNA concentration was measured using a Nanodrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE). Samples with DNA concentrations greater than 40 ng/ul were diluted with DNA/RNA-free water to 20–25 ng/ul.

Seventeen microsatellite markers were selected based on performance in other published studies of Wood Turtles. Most markers were described for the congeneric Bog Turtle (*Glyptemys muhlenbergii*), but were tested on Wood Turtles (King and Julian 2004). Ten additional markers were included that were described for Blanding's Turtle (*Emydoidea blandingii*) and Painted Turtle (*Chrysemys picta*). Four multiplexes were performed with 3–5 markers each for a total of 17 loci. *GmuD51* was isolated for Polymerase Chain Reaction (PCR) and then added to a multiplex prior to electrophoresis due to better performance.

We conducted 10 µl PCRs in a 96-well plate using a thermal cycler (MJ Research, PTC-200). Each reaction consisted of 5 µl of Qiagen Multiplex PCR Master Mix, 1 µl template DNA, 1 µl of primer (6-FAM primers were 0.15 µm, PET and VIC primers were 0.2 µM and NED primers were 0.25 µM), and 3 µl of PCR grade water. PCR on nails included 1 µl BSA and 2 µl of PCR grade water. Forward primers were fluorescently labeled and acquired from Applied Biosystems (colors NED and PET, Foster City, CA) and Integrated DNA Technologies (colors 6-FAM and VIC, Coralville, IA). Thermocycling conditions included an initial 15 min at 95˚C followed by 35 cycles of 94˚C for 30 s, 57˚C for 90 s (or 51˚C for *GmuD51*), 72˚C for 90 s and a final cycle of 72˚C for 10 min. One negative control was included on each plate. PCR products were diluted to 1:50 with PCR grade water. The PCR products were run on an Applied Biosystems 3130xl Genetic Analyzer with a LIZ600 ladder for size standard. Peaks were scored using Geneious version 9 (Biomatters Ltd, Auckland, New Zealand).

Population genetic analyses

Of the seventeen loci amplified, four were excluded at the scoring stage. Three loci (*GmuD51*, *GmuD55*, and *GmuB21*) were excluded because they amplified inconsistently and had substantial missingness (>30%) across individuals. Locus *Eb19* was monomorphic in the entire dataset and excluded. Individuals that did not genotype successfully at

nine or more loci were excluded due to missingness across loci. After this filtering step, the final data set consisted of $N = 1{,}258$ individuals from 45 wild collections of size $n \geq 15$ individuals per collection. Summaries of Wood Turtle individuals sampled by collection and state (Table S1) and HUC (Table S2) are provided in supplemental materials.

Exact tests for deviation from Hardy Weinberg (HW) proportions and for gametic (linkage) disequilibrium (LD) were performed with R version 4.4.0 (R Core Team 2025) and the package *genepop* (Rousset et al. 2020). We used 20,000 dememorization steps, 100 batches, and 20,000 iterations. Expected and observed heterozygosity, F_{IS} (a measure of deviation of observed and expected heterozygosity), and rarified estimates of allelic richness were calculated using the R package *hierfstat* (Goudet et al. 2015).

We examined population clustering with two approaches. We used Principal Components Analysis (PCA) based on collection-level allele frequencies. We calculated allele frequencies with the *tab* function applied to a *genpop* data object with the R package *adegenet* (Jombart 2008; Jombart and Ahmed 2011). We removed the largest allele (in base pairs) from each locus to address lack of independence among allele frequencies. We used the *pca* function in the R package *vegan* (Oksanen et al. 2013) to perform PCA with centered allele frequencies.

Our second approach was to use Discriminant Analysis of Principal Components (DAPC) to further examine patterns of genetic structure. DAPC does not have an underlying evolutionary model and therefore is not prone to defining discrete genetic groups when the underling genetic structure is continuous in nature (i.e., IBD) (Jombart et al. 2010). We performed *k*-means clustering with the function *find.clusters* in the R package *adegenet* and used a Bayesian Information Criteria (BIC) approach to determine the range of *K* values to examine with the function *dapc*. We retained 140 principal components (PCs) for the *k*-means clustering step. We used 41 PCs for the *dapc* function based on results from the *a*-score optimization procedure obtained with function *optim.a.score*.

An IBD pattern is expected to arise from a stepping-stone model of gene flow (Kimura and Weiss 1964). We would also expect allele frequency variation to occur in a cline across the region demonstrating IBD. We tested for clinal allele frequency variation indirectly through testing for a correlation between either principal component (PC) or discriminant axis (DA) scores and collection latitude. We used latitudes derived from the centroid of each collection location. For PCA, we used PC-scores along the second principal component axis for each collection obtained from the PCA on collection-level allele frequency estimates. For DAPC, we calculated the centroid of individual-level DA-scores along the second discriminant axis. Pearson's product moment correlation (r) was calculated with the *cor.test* function from the *stats* package in R, including 95% CI and *P*-value. We also tested for IBD with a Mantel test implemented with the function *mantel* in the R package *vegan* for eastern genetic group collections. We calculated pairwise geographic distance with function *distm* in the R package geosphere based on the WGS84 datum. This function calculates the shortest distance in meters between two points on an ellipsoid based on their latitude and longitude. We used pairwise F_{ST} (Weir and Cockerham 1984) as the genetic distance. We performed 10,000 permutations to test for statistical significance of the Mantel test.

We further examined genetic differentiation with *F*-statistics. Based on previous work for Wood Turtle and evidence of a tendency for spatially limited dispersal, we predicted that genetic structure would be hierarchically influenced by watershed structure. Our sampling design allowed us to test a four-level hierarchy. The innermost or smallest spatial scale in the hierarchy was the collection. The next tier was the HUC8 subbasin. HUC8 subbasins occur hierarchically within HUC4 subregions (Fig. 1). The outermost or largest spatial scale in the hierarchy corresponded to regions (Midwestern or Eastern) nested within the total range, where assignment to either of these regions was based on results of the genetic analyses described above. Our primary interest was to estimate the effect of each level independently of the effect of lower levels in the hierarchy. We thus estimated the effect of collections within HUC8 subbasins ($F_{C/SB}$; where the '/' represents 'within'), the effect of HUC8 subbasins within HUC4 subregions ($F_{SB/SR}$), the effect of HUC4 subregions within regions ($F_{SR/R}$), and the effect of regions within the total ($F_{R/T}$). We also estimated the effect of collections within the total ($F_{C/T}$), which corresponds to the standard overall or global F_{ST}. We calculated these hierarchical *F*-statistics with the function *varcomp.glob* from the R package *hierfstat.* We performed 10,000 permutations to test for statistical significance of each level of the hierarchy with the *hierfstat* functions *test.within*, *test.between*, and *test.between.within* following de Meeûs and Goudet (2007). We also calculated pairwise F_{ST} among each collection with Weir and Cockerham's θ (Weir and Cockerham 1984) with the R package *hierfstat* and Hedrick's G'_{ST} (Hedrick 2005) with the R package *mmod.* G'_{ST} is well-suited for highly variable microsatellite markers (Hedrick 2005).

We further tested for hierarchical structuring at the HUC8 (subbasin) scale within the two HUC4s (subregions) for which we had the most collections, including at least two HUC8s with at least two collections each. Subbasin 0101 (northern Maine) had six collections representing a total of four HUC8s, two of which had two collections each. Subbasin 0207 (Maryland) had ten collections representing a

total of four HUC8s. One of these HUC8s had four collections, one had three collections, and another had two collections. Hierarchical structuring according to HUC8s should lead to a higher proportion of non-significant tests for allele frequency divergence and lower pairwise F_{ST}'s for within HUC8 comparisons relative to among HUC8 comparisons. We used chi-square genic tests for allele frequency differentiation with the R package *strataG* (Archer et al. 2017) with *P*-values based on 10,000 permutations. We compared the number of significant tests for pairwise comparisons within and among HUC8s without correcting for multiple tests. We also compared mean pairwise F_{ST}'s within and among HUC8s with each of these two HUC4s.

Full-sib-based estimates of dispersal

We used reconstructed full-sibling families within each major genetic group to test for putative dispersal events. To test this approach for our marker set and species, we first examined offspring at the hatchling life stage sampled from known nests with known mothers from one site (MAAlBr). We genotyped 25 offspring with known nest/mother combinations. We used *rcolony* to estimate full sibship with the software *Colony* ver. 2 (Jones and Wang 2010). We assumed polygamy for both males and females, assumed no inbreeding, we scaled full-sibship sizes, and used no sibship prior. We performed long, high precision runs with the full likelihood model. We report probability of inclusion (Pr (Inc.) and the probability of exclusion (Pr(Exc.). A lower probability of inclusion indicates support for splitting an estimated full-sib family. The probability of exclusion is the probability that individuals of a given family represent the best configuration of full-sibs. A low probability of exclusion suggests a full-sib family could be incomplete (i.e. perhaps a singleton individual belongs to another family). In addition to some insight into the mating structure of this species at one site, we used these results to infer the degree to which we accurately obtained estimates of membership to full-sib family of size two or larger if we based that inference on a probability of inclusion ≥ 0.90 or ≥ 0.95.

Closely related individuals were identified among collections to test for dispersal. Under this approach, inferred full-sibs from different collection sites represented a putative dispersal event, where one of the individuals dispersed prior to the sampling event. Sibship-based approaches can have high accuracy to infer dispersal events at the scale of a collection (Whiteley et al. 2014). We again used *Colony* ver. 2.0 and *rcolony* (Jones and Wang 2010) under the same settings as above. Since we combined individuals of unknown ages within each collection, the estimated full-sibs could be full-sib pairs or parent-offspring pairs. We restricted inference to full-sib families with Pr (Inc.) ≥ 0.90 and we examined the Midwestern and Eastern genetic groups separately. We performed an additional analysis with all individuals combined to test for putative between genetic group dispersal.

Results

Tests for Hardy-Weinberg and linkage disequilibrium

The final dataset included 1,258 individuals from 45 collections. Out of 570 total tests for HW proportions, 56 tests were significant ($P < 0.05$) where 30 were expected by chance at $\alpha = 0.05$. Bonferroni correction for the approximately 45 HW tests conducted per locus (adjusted $\alpha' = 0.00114$) led to six tests remaining significant after adjustment with no locus remaining out of HW proportions in more than one collection. Bonferroni correction for the approximately 13 HW tests conducted per locus (adjusted $\alpha' = 0.00417$) led to ten tests remaining significant after adjustment. Two of these tests were from the IA collection (*GmuD70* $F_{IS} = 0.498$ and *Cp2* $F_{IS} = 0.211$), two from MAAlBr (*GmuA19* $F_{IS} = 0.105$, *GmuD28* $F_{IS} = 0.01$), and two from VADiv (*GmuD28* $F_{IS} = -0.037$, *Eb17* $F_{IS} = -0.248$). Thus, this latter approach indicated that one locus (*GmuD28*) was out of HW proportions twice after correction by the 13 locus tests within each population, but F_{IS} was relatively small and occurred in different directions. We chose not to remove any loci based on these HW results.

Out of 3,421 total tests for linkage disequilibrium (LD), 335 tests were significant ($P < 0.05$) where 171 were expected by chance at $\alpha = 0.05$. Following table-wide Bonferroni correction (adjusted $\alpha = 0.000015$), 17 tests for LD remained significant, including two tests in VABox and ten tests in MI_CR. Following less stringent Bonferroni correction for the at most 78 locus-pair tests per collection (adjusted $\alpha = 0.00064$), 27 tests remained significant. This included four tests in MAAlBr, two tests in MACro, 12 tests in MI_CR, two tests in VABox, and two tests in VADiv. We chose not to remove any loci based on these LD results due to the collection-specific nature of the observed patterns.

Patterns of genetic structure

PCA based on collection allele frequencies provided strong evidence for two major genetic groups, one in the Midwest that included collections from Minnesota, Iowa, Wisconsin and Upper Peninsula Michigan, and the other ranging from Virginia to New Brunswick in the eastern part of the species range, including a collection from lower Michigan. These groups were well-separated along the first PC axis, which explained 24.4% of the variation (Fig. 2). The remaining

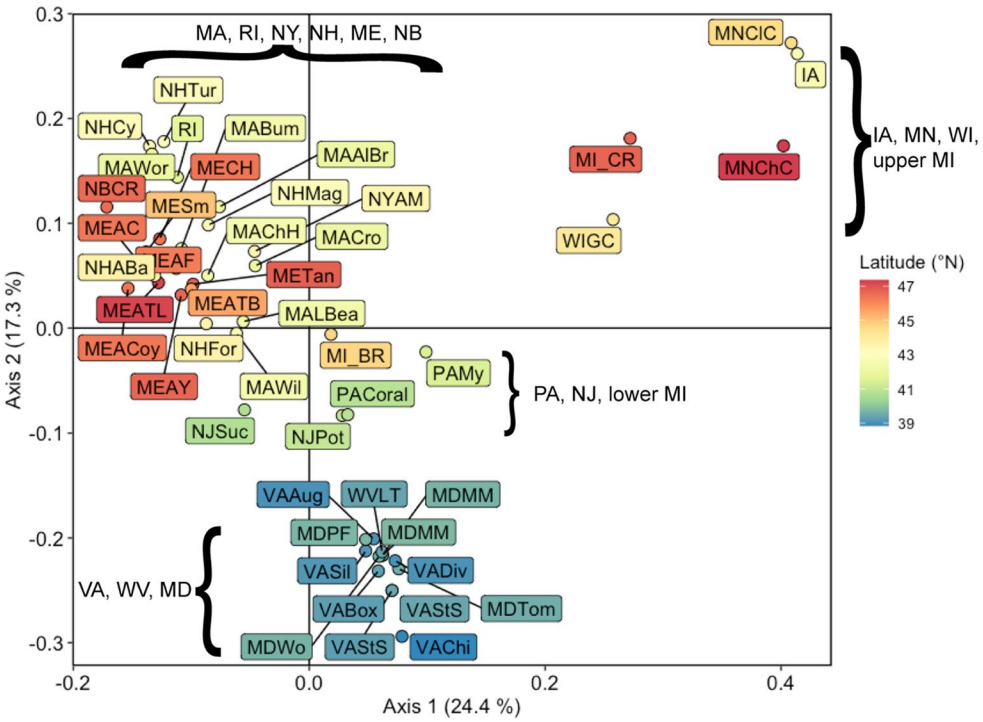

Fig. 2 PCA based on allele frequencies for 45 Wood Turtle collections. Collection (data points) and their estimated allele frequencies were the unit of analysis. The US state or Canadian province of origin is shown as the first two letters of each collection abbreviation. Proportion of variance explained by each PC axis is shown in parentheses. Each data point was given a repelled label corresponding to collection pseudocode. Color of each label and data point corresponds to the collection latitude in degrees North. Curley brackets and associated state (or province) abbreviations are shown to clarify the geographic pattern that occurs along both axes. 'lower MI' is lower Michigan, 'upper MI' is the Upper Peninsula Michigan

collections (including MI_BR from lower Michigan) were primarily separated along PC2 (17.3% of the variation, Fig. 2). Along PC2, southern collections from the Potomac and Monongahela Rivers had the lowest scores, northern collections from the Kennebec, St. John, Connecticut, Merrimack Rivers, coastal New England, and New Brunswick had the highest PC2 scores, and collections from the Susquehanna and Hudson Rivers were more intermediate.

DAPC results were highly congruent with those from the PCA, including strong evidence for two major groups corresponding to the Midwest and East and a continuous isolation-by-distance pattern in the east. BIC values associated with clusters (K) had a minimum at approximately 20 (Figure S1). However, biological interpretation changed little with increasing values of K beyond 3, likely due to strong isolation-by-distance (Figure S2). With $K=3$ k-means-defined genetic clusters, the Midwestern collections occurred in one group and all the rest of the collections from the eastern portion of the range, including those from MI_BR, were divided among two continuously distributed groups (Fig. 3). At $K=3$, proportional assignment of individuals to DAPC clusters provided evidence of mixed ancestry within the eastern collections. Twenty-one of 40 eastern collections had non-zero mean DAPC assignments for each of the two eastern clusters (Table S3). Several individuals were intermediately located between the Midwestern and Eastern groups (Fig. 3). These might be offspring of recent migrants and therefore may provide evidence of admixture between the major genetic groups (Table S3). The two individuals providing evidence for Eastern-to-Midwest admixture were

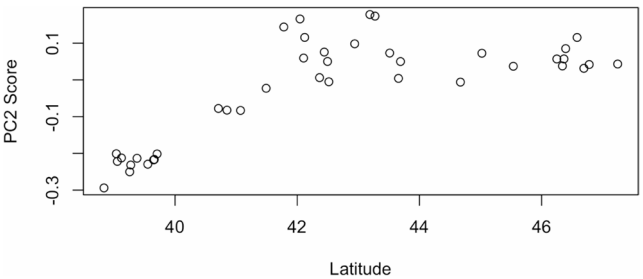

Fig. 3 Discriminant Analysis of Principal Components (DAPC) assuming $K=3$ for 45 Wood Turtle collections. Individuals (points and colored lines connected to points) are colored by the population to which they were assigned by the discriminant analysis. Putatively admixed individuals are labeled with the color that corresponds to their population assignment but are connected with colored lines to the population from which they were sampled. The location of collection labels (or the black line segment issuing from the collection label) represents the centroid of that collection's scores along each axis. The percentage of variation explained by each discriminant axis is shown in parentheses

from PAMy (PAMy_929) and MI_BR (MI_BR_35). There were five individuals providing evidence for Midwest-to-East admixture; WIGC _14, WIGC_21, and WIGC_22 were assigned to the more southern green eastern cluster. MI_CR _7 and MI_CR_14 were assigned to the more northern pink eastern cluster (Fig. 3.

We observed variation consistent with clinal allele frequency variation and IBD within the eastern genetic group (Figs. 2 and 4, and 3). PC2 scores for eastern-group collections (excluding the five collections that belong to the Midwest major genetic group) were positively correlated with latitude (Pearson's $r=0.74$, 95% CI 0.56–0.85, $P=4.9e^{-8}$)

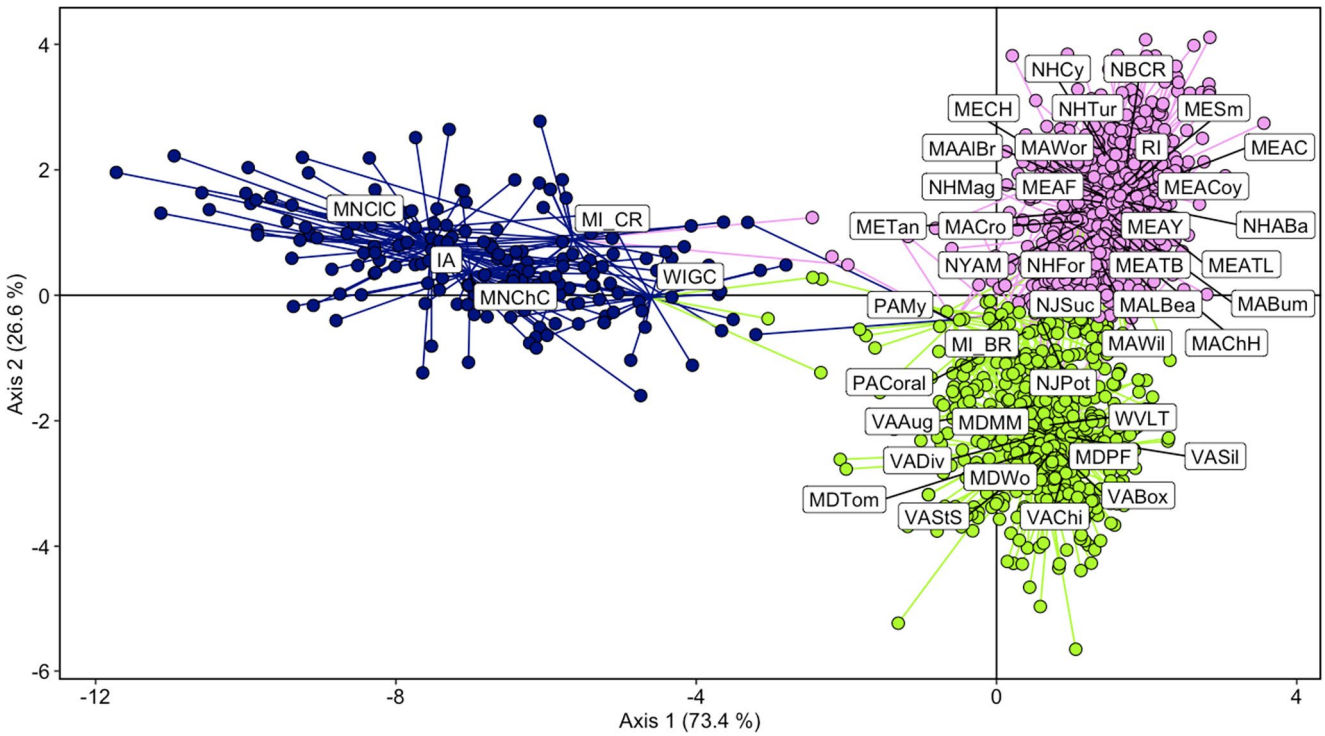

Fig. 4 Test for geographically continuous cline in population subdivision in the eastern portion of the Wood Turtle range for all collections with N ≥ 15. PC2 score per collection is on the y-axis. Latitude of each collection is on the x-axis. Midwestern (except east assigning MI_BR) collections were excluded

(Fig. 4). Centroids of individual scores along DA 2 were also highly correlated with latitude (Pearson's $r=0.81$, 95% CI 0.66–0.89, $P=2.7e^{-10}$). Finally, a Mantel test based on pairwise F_{ST} and geographic pairwise distances within the eastern genetic group was highly significant ($r=0.60$, $P=0.001$).

Hierarchical F-statistics confirmed pronounced genetic differentiation between regions, with less but statistically significant differentiation at lower levels in the hierarchy. Differentiation between regions within the total range ($F_{R/T}$) was 0.107 ($P<0.003$). Differentiation among HUC4 subregions within regions ($F_{SR/R}$) was 0.055 ($P<0.001$). Differentiation among HUC8 subbasins within HUC4 subregions was 0.015 ($P<0.001$). Differentiation among collections within HUC8 subbasins was 0.017 ($P<0.001$). Global F_{ST}, or differentiation among collections within the total ($F_{C/T}$,) was 0.182 ($P<0.001$). The geographic pattern of greater genetic similarity of more geographically proximate populations was similar between two measures of pairwise genetic differentiation. Pairwise F_{ST} (Weir and Cockerham's θ) ranged from −0.001 to 0.271 (Fig. 5) and pairwise Hedrick's G'_{ST} ranged from −0.001 to 0.685 (Figure S3) with largest values occurring between collections from the Midwestern and Eastern groups.

Further analysis of the two most densely sampled HUC4 subregions suggested that hierarchical structure varied among subregions. There was little evidence for HUC8 subbasin-level genetic structure within the northern Maine HUC4 0101 subregion. Pairwise tests of genic differentiation were all highly significant ($P<0.001$). Mean pairwise $\theta(F_{ST})$ within HUC8 subbasins was 0.031. Mean pairwise θ for comparisons among HUC8 subbasins was 0.037. In contrast, there was evidence for HUC8 subbasin structuring within the southern HUC4 0207 subregion. Pairwise tests of genic differentiation were not significant for six out of 45 total tests in this HUC4 ($P>0.05$). Three of 10 (30%) within HUC8 subbasin tests were not significant, whereas three of 35 (8.6%) among HUC8 subbasin tests were not significant. Mean pairwise θ within HUC8 subbasins was 0.017. Mean pairwise θ for comparisons among HUC8 subbasins was 0.022.

Genetic variation within collections

Mean estimates of genetic variation were moderately variable among collections (Table 1). Mean expected heterozygosity within collections (H_S) ranged from 0.552 (MNChC) to 0.722 (MALBea). The collections with the five lowest

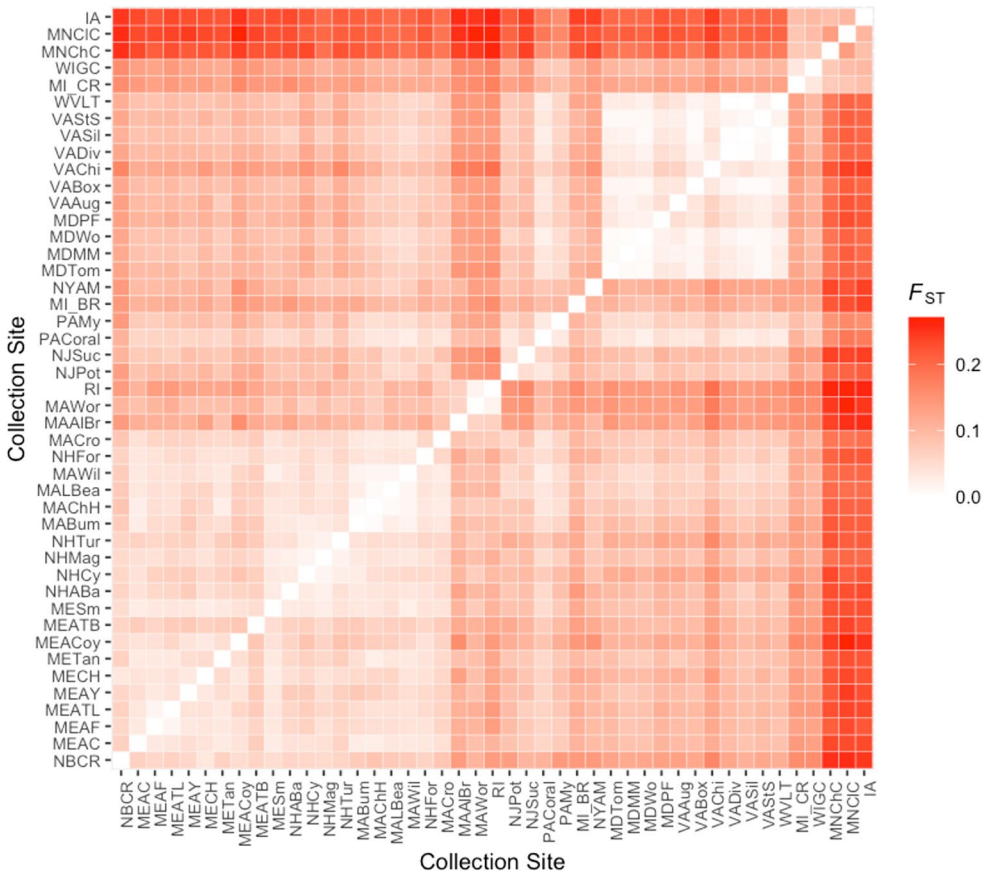

Fig. 5 Pairwise F_{ST} (Weir and Cockerham 1984) for 45 Wood Turtle collection sites. Collection names are pseudocodes and were arranged so that geographically proximate collections appear close together. Diagonal values were left blank. Identical values occur above and below the diagonal for visualization purposes

values of H_S were MNChC (H_S = 0.565), RI (H_S = 0.574), MEACoy (H_S = 0.584), MAWor (0.584), and MAAlBr (0.591; Table 1). Estimates of allelic variation rarified to the smallest sample size (allelic richness, *AR; N*=15) ranged from 4.1 (MNChC) to 6.8 (PACoral; Table 1). The collections with the five lowest values of *AR* were MNChC (*AR*=4.1), MAAlBr (*AR*=4.4), RI (*AR*=4.6), MEACoy (*AR*=4.8), and MEATB (*AR*=4.9; Table 1). Note that the MAAlBr collection was from nests and was included to test sibship clustering approaches with this marker set, thus this estimate should not be used as an estimate of genetic variation present in the entire population. Mean F_{IS} ranged from −0.101 (NJPot) to 0.080 (IA; Table 1). The five collections with greatest positive mean F_{IS} were MABum (F_{IS} = 0.052), MI_CR (F_{IS} = 0.062), PAMy (F_{IS} = 0.063), and METan (F_{IS} = 0.069) and IA (F_{IS} = 0.080).

Full-sib based estimates of dispersal

For the MAAlBr hatchling sample test case, we obtained an estimate of 11 full-sib families ranging in size from 1 to 7 full-siblings (Table 2). Five of the full-sib families included two or more individuals. All five of these families had concordance between genetic assignments and the known mother, providing some degree of validation of this approach. Two of these families (families 2 and 9) were potentially maternal half-sibships (Probability=0.85). The largest full-sib family (7 sibs) was from known female 3016. Two other singleton full-sib families were assigned to female 3016 and are either true full-sibs that were incorrectly assigned or maternal half-sibs, in which case there would be an estimated three fathers for this family. The probability of this half-sib cluster was low (*P*=0.02) and therefore we would not have been able to resolve relationships without the nest and mother information. Female 50 was assigned four small families. These could have been one incorrectly reconstructed full-sibship, or half sib structure was present, with the potential for more than two fathers (possibly four). Again, we could not have accurately reconstructed these relationships without nest/mother information (the overall probability of this cluster was 0.02). If we used a strict cutoff of Pr (Inc.)≥0.95, we would retain 40% (2/5) of the full-sib families of size two or larger. If we used a less strict cutoff of Pr (Inc.)≥0.90, we would retain 80% (4/5) of the full-sib families of size two or larger. Only two of these full-sib families would be complete, but only errors of omission

Table 1 Summary of genetic variation within 45 wild-captured Wood Turtle collections for which sample size was greater than or equal to 15. Collection names were given pseudocodes to obscure collection locations. Collections are arranged by HUC4 subregion. *N* is sample size. HUC4 subregion is the four-digit USGS Hydrologic Unit Code. Values for all genetic metrics are means across loci within collections. H_O is observed heterozygosity. H_S is mean expected heterozygosity. F_{IS} is a measure of departure from HW proportions. *AR* is allelic richness, a measure of allelic variation rarefied to the smallest collection sample size ($N=15$)

Collection	*N*	HUC4 Subregion	H_O	H_S	F_{IS}	AR
NBCR	18	NB	0.650	0.650	-0.021	5.6
MEAY	23	0101	0.643	0.630	-0.019	5.5
MECH	19	0101	0.663	0.637	-0.045	5.8
MEAC	24	0101	0.663	0.645	0.036	5.3
MEAF	46	0101	0.603	0.637	0.034	5.8
MEATL	36	0101	0.619	0.638	0.03	5.5
METan	17	0101	0.597	0.641	0.069	5.4
MEACoy	16	0102	0.568	0.584	-0.006	4.8
MEATB	69	0103	0.66	0.651	-0.015	4.9
MESm	23	0103	0.63	0.651	0.025	5.7
NHABa	15	0107	0.665	0.670	-0.026	5.5
NHTur	21	0107	0.744	0.691	-0.08	5.8
NHCy	15	0107	0.653	0.698	0.048	6.5
NHMag	25	0107	0.716	0.699	-0.031	6.2
NHFor	26	0108	0.649	0.659	0.021	5.6
MABum	26	0108	0.645	0.669	0.052	5.7
MAChH	16	0108	0.704	0.682	-0.03	6.2
MALBea	16	0108	0.723	0.722	0.014	6.0
MAWil	24	0108	0.698	0.674	-0.048	6.1
MAAlBr	26	0109	0.587	0.591	0.023	4.4
MAWor	22	0109	0.581	0.584	-0.001	5.0
MACro	28	0110	0.697	0.669	-0.054	5.6
RI	17	0110	0.611	0.574	-0.065	4.6
NJPot	16	0202	0.722	0.660	-0.101	6.4
NJSuc	16	0203	0.694	0.662	-0.077	5.6
PAMy	19	0205	0.611	0.660	0.063	6.2
PACoral	38	0205	0.681	0.684	-0.02	6.8
MDMM	42	0207	0.685	0.666	-0.047	6.2
MDTom	30	0207	0.678	0.681	0.015	6.2
MDWo	21	0207	0.696	0.683	-0.051	6.5
WVLT	24	0207	0.689	0.659	-0.034	5.7
VABox	69	0207	0.705	0.668	-0.067	6.0
VAStS	63	0207	0.667	0.664	-0.016	6.1
VAChi	16	0207	0.638	0.600	-0.056	5.3
VADiv	66	0207	0.684	0.663	-0.035	5.7
VASil	21	0207	0.677	0.675	0.016	6.1
VAAug	18	0207	0.655	0.646	-0.035	5.7
MNChC	47	0401	0.565	0.552	-0.023	4.1
MI_CR	26	0402	0.654	0.688	0.062	5.9
MI_BR	22	0407	0.636	0.659	0.024	6.0
NYAM	19	0414	0.689	0.660	-0.045	5.3
MDPF	15	0502	0.662	0.632	-0.044	5.9
MNClC	17	0704	0.625	0.606	-0.032	5.2
WIGC	26	0704	0.752	0.709	-0.062	6.8
IA	59	0708	0.546	0.600	0.08	5.2

would occur. We therefore chose to use a Pr (Inc.)≥0.90 cutoff for analysis of the larger data set. We are likely to obtain estimates of full-sib families that are accurate, with family size possibly biased low (if the uncertainty above is due to incorrectly splitting larger full sib families in the cases of families 2, 3, 4, 7, and 9) and therefore we consider this a conservative estimate of dispersal at an appropriate cutoff of Pr (Inc.).

Table 2 Colony results for the MAAlBr test sample of hatchlings collected from known nests with known mothers. Each family was given an arbitrary number as an ID. The number of full-sibs assigned to each of the 11 families is shown in the second column. Probabilities of inclusion (Pr (Inc.)) and exclusion (Pr (Exc.)) are shown. Individuals assigning to each estimated full-sib family appear in the fifth column. One half-sib family assignment had relatively high confidence (probability = 0.85; shown in bold) where these two full-sib families were estimated to have the same mother but different fathers. Other half-sib estimates had low confidence (probability < 0.05) and could not be reconciled with this full-sib arrangement (not shown). The Mother ID based on field identified association with a nest is shown in the last column

Full-sib Family Number	Full-sib Family size	Pr (Inc.)	Pr (Exc.)	Estimated Full-sibs	Field-based Mother ID
1	1	1.00	1.00	MAAlBr3046	Unknown
2	3	0.98	0.92	**MAAlBr3052**,**MAAlBr3054**,**MAAlBr3098**	3030
3	7	0.90	0.90	MAAlBr3056,MAAlBr3075,MAAlBr3090,MAAlBr3091,MAAlBr3092,MAAlBr3093,MAAlBr3099	3016
4	1	1.00	0.30	MAAlBr3076	3016
5	2	0.16	0.16	MAAlBr3077,MAAlBr3073	50
6	1	1.00	0.03	MAAlBr3079	50
7	1	1.00	0.14	MAAlBr3080	3016
8	1	1.00	0.53	MAAlBr3081	50
9	2	0.92	0.43	**MAAlBr3096**,**MAAlBr3053**	3030
10	1	1.00	0.39	MAAlBr3071	50
11	6	0.95	0.95	MAAlBr3066,MAAlBr3067,MAAlBr3061,MAAlBr3063,MAAlBr3064,MAAlBr3065	3022

Full-sib reconstruction identified three putative dispersing individuals in the eastern portion of the range and none in the midwestern region. Thirty full-sib families were of size two or larger at a Pr (Inc.) ≥ 0.90 within the eastern major genetic group (Table 3). 93% of these families contained two individuals, the remaining two families contained three (Table 3). Full-sib families larger than size three were recovered, but none had Pr (Inc) > 0.003. Three full-sib families contained estimated sibs from different collection sites. These included sibs (with estimated straight-line distances in parentheses) from MEATB and NHMag (301 km), MECH and MEMon (202 km), and MDMM and MDTom (21.4 km). Within the Midwest genetic group, 7 estimated full-sibling families had Pr(Inc) ≥ 0.90. Each of these families were of size two. No individuals from different geographic locations were assigned to the same full-sib family (Table 3). Full-sib reconstruction of all individuals combined did not reveal any putative dispersers between the Eastern and Midwestern genetic groups.

Discussion

We conducted the most extensive population genetic analysis of the Wood Turtle to date. Our results demonstrate a clear genetic break between Midwestern and Eastern populations of this species in North America. This provides initial guidance towards the designation of conservation units at a range-wide spatial scale for the Wood Turtle. We observed an IBD pattern at the scale of the entire eastern portion of the distribution along with evidence of hierarchical genetic structure. This result is consistent with dispersal among adjacent watersheds. Examination of within-population genetic variation revealed that some populations were relatively genetically depauperate if we assume relatively small differences in genetic variation are biologically meaningful for such a long-lived species. Although we cannot discount anthropogenic dispersal, we observed putative natural long-range dispersal (up to 300 km) based on sampling locations of estimated full-sibs, which is longer dispersal than has been observed previously for this species.

Large-scale genetic groups

At the largest (range-wide) geographic scale examined here, expectations for patterns of genetic structure include consideration of glacial refugia during the last glacial maximum (~12,000 yrs ago). The northern portion of the Wood Turtle species range was completely ice-covered during the last glacial period (Amato et al. 2008). Remains of Wood Turtles from Mississippi (Phillips 2006), Georgia (Holman 1967, 1985), and Tennessee (Parmalee and Klippel 1981)

Table 3 Colony results for all individuals belonging to the eastern major genetic group. The probability of inclusion (Pr (Inc.)) is shown in the first column, values are sorted from larger to smaller. The second column contains individual IDs (a combination of site pseudocode and individual number) of the assigned full-sibs in each full-sib family (all families retained were of size 2 or 3). If individuals were collected in different sites we consider this a putative dispersal event (Y). Note that full-sib and parent-offspring relationship could not be distinguished with this approach because we sampled individuals of varying and unknown ages

Pr (Inc.)	Estimated Full-sibs	Putative dispersal event?
Eastern Genetic Group		
1.00	METan487, METan494	N
1.00	NHTur531, NHTur533	N
1.00	MACro52, MACro4092	N
1.00	MEAY348, MEAY355	N
1.00	MEACoy237, MEACoy245	N
1.00	CT1, CT9	N
1.00	NHACu593, NHACu594	N
1.00	NYAML2R1, NYAML8R1	N
1.00	NHYo655, NHYo664	N
1.00	MEATB423, NHMag672, NHMag679	Y
1.00	MEAF256, MEAF261	N
1.00	MALBea132, MALBea133	N
0.98	METan489, METan490	N
0.98	MEATB40, MEATB437	N
0.98	MEAY354, MEAY356	N
0.98	MAWil113, MAWil4078	N
0.98	MECH439, MEMon391	Y
0.98	MEAY346, MEAY358	N
0.97	MESm470, MESm472	N
0.96	MDMM507, MDTom214	Y
0.96	VAStSWV16, VAStSWV17	N
0.95	MDMM200, MDMM7276	N
0.95	MACro51, MACro4086	N
0.95	VABox616, VABox820	N
0.93	NHTur526, NHTur530	N
0.92	VADiv1146, VADiv212, VADiv4	N
0.91	MIBR45, MIBR48	N
0.92	MDBaCh2, MDBaCh24	N
0.90	MEMon392, MEMon395	N
0.90	MDMM7305, MDMM7306	N
Midwest Genetic Group		
1.00	MICR005, MICR026	N
1.00	WIGC11, WIGC4	N
0.99	IA088, IA109	N
0.99	MNChC024, MNChC037	N
0.98	MICR003, MICR019	N
0.96	MICR018, MICR025	N
0.90	MNChC020, MNChC022	N

reveal that populations resided in one or multiple southern refugia. In addition, dated remains from southwestern Iowa suggest the species range included regions farther to the west approximately 10,000 yrs ago (Hill 2022). Wood turtles are proposed to have recolonized previously ice-covered areas from one or multiple refugia (Amato et al. 2008).

Previous phylogeographic work based on the control region of the mitochondrial genome is consistent with these expectations (Amato et al. 2008). Amato et al. (2008) describe low diversity in the control region of mtDNA across the Wood Turtle range (maximum pairwise difference of 2% between haplotypes) and a lack of genetic divergence in mitochondrial haplotypes between the Midwestern and Eastern portions of the species range. The authors discuss two explanations for their results: (1) dispersal from a southern Appalachian refugium to the Midwest and the Northeast based on low genetic diversity of mtDNA haplotypes across the range; or (2) a bottleneck or selective sweep within the southern refugium. Amato et al. (2008) also speculate that their data reveal a more northern east-to-west long-range dispersal route, north of Lakes Ontario, Erie, and Huron. However, this single-gene mitochondrial analysis had limited resolution.

The strong genetic divergence we observed with microsatellites between Wood Turtles from the Midwest and East provides a new perspective on range-wide genetic patterns. This regional genetic divergence could have occurred following glacial recolonization over this approximately 10,000-year period. For example, similar patterns have been observed for other herpetofauna in this region (e.g. Painted Turtle, *Chrysemys picta*; (Starkey et al. 2003). Consistency in the two analyses would require that Amato et al. (2008) lacked the ability to resolve divergence of the Midwestern populations following recolonization. A bottleneck in a refugium (or refugia) would influence mtDNA more than the nuclear genome due to smaller effective population size of the mitochondrial genome (Ovenden and White 1990; James et al. 2017; Allendorf et al. 2022). The relatively high within-population genetic variation across Wood Turtle populations observed here seems inconsistent with major bottlenecks. It is also possible that a selective sweep could have influenced the mitochondrial genome, and obscured divergence owing to limited gene flow and genetic drift that remains apparent within the nuclear genome. Ultimately, it is challenging to explain extant patterns of genetic structure given multiple historical scenarios that could lead to similar current genetic patterns, especially since expectations of the influence of evolutionary processes differ for the nuclear and mitochondrial genomes. Further, microsatellites have relatively high mutation rates for nuclear portions of the genome (Whittaker et al. 2003), making it difficult to tease apart historic effects of gene flow, genetic drift, and mutation. However, we suggest dispersal from at least one southern refugium is consistent with both data sets and further tests of this hypothesis would likely require a more detailed sampling of both mitochondrial and nuclear genomes.

Our analysis and sampling design provides information about the location of the genetic break between the Midwestern and Eastern genetic groups. The lower Michigan collection clearly assigned to the eastern genetic group, while the Upper Peninsula Michigan collection clearly belonged to the Midwestern group. The cohesiveness of the lower Michigan collection and those to the east could be explained by gene flow from populations further to the east via Lake Erie, overland through the Port Huron/Detroit region, or south of Lake Erie. Lakes Michigan and Superior strongly appear to have provided more historic resistance to gene flow, though our analysis suggests occasional gene flow has occurred. We sampled at both a larger geographic scale and at a higher density of collection sites than has been available for this species to date, however, the spatial resolution of this major genetic break remains somewhat coarse since we had one sample from each of the Michigan Upper Peninsula and lower Michigan. Thus, while we obtained a set of collections with few spatial gaps throughout the species distribution, some marginal portions of the species range contain low density populations that could not provide enough individuals for population-level analyses. We also had very limited representation within the Canadian portion of the range and had limited ability to resolve the potential for east-to-west gene flow north of the Great Lakes. However, extensive gene flow via that route would be expected to lead to lower than observed genetic differentiation between the Eastern and Midwestern genetic groups and therefore appears unlikely.

The strong clinal allele frequency pattern we observed from north to south along the eastern portion of the species range extended from Virginia to New Brunswick. The pattern we observed is consistent with the stepping-stone model of migration, which includes short-range and occasional long-range gene flow (Kimura and Weiss 1964). This pattern is also consistent with an IBD model where gene flow decreases with increasing distance between populations under equilibrium conditions (Wright 1943). An IBD pattern is expected from postglacial recolonization (Nadeau et al. 2016) and is consistent with phylogeographic expectations (south-to-north recolonization) and the dispersal behavior of Wood Turtles. Individuals tend to move along streams and rarely are located more than 300 m from waterways (Harding and Bloomer 1979; Arvisais et al. 2002; Behler and Castellano 2005). Jones and Sievert (2009) documented Wood Turtles in Massachusetts dispersing up to 16.8 km after flood events. Turtles tracked by Jones and Sievert (2009) confirms that Wood Turtles can move overland or in the stream corridor and observed distances suggest movement should primarily occur among neighboring populations. The strength of observed clinal allele frequency variation suggests that this is the result of long-term patterns of gene flow. An IBD pattern should be more pronounced at equilibrium between genetic drift and gene flow (Slatkin 1993) and is expected to be stronger in long-established compared to recently established populations (Mimura and Aitken 2007).

Incipient genetic divergence might be occurring within the eastern portion of the Wood Turtle range examined here. There was some indication of a Northeastern group (Maine, New Hampshire, Rhode Island, New York, Massachusetts, New Brunswick) and a Southeastern group (Virginia, Maryland, West Virginia) with several intermediate collections in Pennsylvania and New Jersey. A mechanism for this pattern could be the development of IBD at a post-glacial time scale with more recent disruption to gene flow in central regions due to more recent fragmentation and population decline. An alternative explanation would need to involve two more distinct genetic groups (Northeast and Southeast) that are connected by a central zone of admixture. In either case, we did not find evidence for two currently disjunct genetic groups in Eastern North America. Further genetic structuring within the eastern portion of the Wood Turtle range might be revealed by sample collection in portions of the range not included here. For example, we lacked samples from potentially genetically isolated Wood Turtle populations in Nova Scotia (Graef et al. 2003) and the St. Lawrence River was found to limit gene flow in Quebec (Tessier et al. 2005).

We found support that neutral genetic variation is hierarchically arranged in the Wood Turtle. Differentiation among regions (Midwestern vs. Eastern) was greatest, followed by differentiation among HUC4 subregions within the two regions. Genetic differentiation among HUC8 subbasins within HUC4 subregions and among collections within HUC8 subbasins were of similar magnitude. We lacked replication within many HUC8 subbasins, therefore some caution is warranted in our interpretations of the effects of lowest levels of the hierarchy. Where we had the densest sampling within HUC4 subregions, we also observed more evidence of hierarchical structure at the HUC8 subbasin scale in one HUC4 but not another, suggesting that the degree of hierarchical structuring varies spatially. Our analysis is consistent with and extends previous genetic studies that have occurred at smaller spatial scales for this species (Castellano et al. 2009; Fridgen et al. 2013; Tessier et al. 2005; Bouchard et al. 2019). For example, Bouchard et al. (2019) found evidence for river drainage and river distance explaining a substantial portion of genetic differentiation among Wood Turtle populations in Quebec and Ontario. Collectively, Wood Turtle population genetic analyses suggest metapopulations (defined as a set of spatially divided subpopulations connected by gene flow and each with some probability of extinction; Allendorf et al. 2022) occur at the spatial scale of HUC4 subregions, with apparent variation among HUC4 subregions in the degree of finer-scale hierarchical structuring.

Little is known about long-distance dispersal of Wood Turtles. Individual turtles moving among sites have been documented based on individual identification, and movements up to 50 km are known to occur (T. S. B. Akre, unpublished data) The three close relatives (either putatively full sibs or parent-offspring pairs) that provided evidence for large distance movements (up to ~300 km) in our analysis could be due to rare long-distance dispersal. It is possible that past tagging studies have missed a long but narrow tail to the dispersal distribution. Another non-mutually exclusive possibility is that anthropogenic displacement is responsible for our results, as has been suggested elsewhere (Amato et al. 2008). Wood turtles are subjected to illegal trade (COSEWIC 2018) and we cannot rule out human-caused movements.

Within-population genetic variation

At the finest spatial scale, our collection protocol focused on 'local populations' that were, at most, pooled individuals sampled less than 17 km apart. Collections at this spatial scale generally conformed to Hardy-Weinberg expectations. The vast majority of closely related individuals (likely full-sibs or parent offspring) were also detected within our predefined collections. Thus, we appear to have sampled at the appropriate spatial scale to compare estimates of genetic variation and to potentially identify populations that might have experienced demographic events (including bottlenecks) that have led to low standing genetic variation.

The lowest estimated within-collection genetic variation occurred in the northern Minnesota collection, at the northwest periphery of the species range. Greater genetic variation tended to occur in the Southeast, closest to the proposed glacial refugia, but differences in genetic variation along the eastern portion of the range were slight. Collections with multiple HW (IA) or LD (MI_CR) violations also tended to have lower genetic variation. Inbreeding and high mean coancestry (i.e. pronounced family structure) could be the cause of these HW and LD signals. For example, the IA collection had two significant loci with positive F_{IS}, which could be due to inbreeding. It seems feasible that small populations with limited recruitment could contain a high fraction of closely related individuals. However, we cannot rule out other causes of positive F_{IS}, such as undetected population substructure or population-specific null alleles. The pronounced family structure we observed in MAAlBr, where we targeted hatchlings from a limited number of nests, reveals that cohorts can contain full and likely half siblings and this was probably the cause of HW violations observed in that collection. Similar to HW violations, a signal of LD can also be caused by low effective population size, inbreeding, and family structure (Waples 2025). This could be responsible for the large signal of LD from MI_CR. However, LD can also be caused by a 'mixture LD effect' (Waples 2025), which is essentially a two-locus Wahlund effect due to unaccounted for population subdivision. The hypothesis that inbreeding is the cause of these positive F_{IS} values and the HW and LD signal in several populations could be investigated with genomic approaches that reliably estimate individual inbreeding coefficients (Kardos et al. 2015).

The observed range of heterozygosity (0.522–0.722) and allelic richness (4.1–6.8) at first appears to suggest that none of the populations is substantially more genetically depauperate than others. However, the life history of Wood Turtles suggests that some caution is warranted in this interpretation. Longevity exceeds 50 yrs (Lovich et al. 1990) and generation time has been estimated as 36–47 yrs (van Dijk and Harding 2011; Jones and Willey 2021) for this species. With such a long generation interval, genetic variation will be lost very slowly on an annual time scale. This creates a time lag between anthropogenic factors such as fragmentation or habitat degradation and their genetic consequences (Anderson et al. 2010; Bouchard et al. 2019). On one hand, in a completely isolated population of relatively small effective population size (e.g., $N_e = 50$), standing variation will be maintained on the time scale of decades, allowing more time for conservation planning (Marsack and Swanson 2009). For example, for an N_e of 50 over 100 yrs, an isolated population will lose between approximately 2.1 and 2.7% of its heterozygosity for the estimated generation interval range above. On the other hand, within-population genetic variation may tell us little about genetic connectivity, or lack thereof, for species such as these. The same hypothetical isolated population of $N_e = 50$ might suffer from fitness effects of inbreeding and lack of gene flow, but this might go undiagnosed for species like the Wood Turtle because of small observed absolute magnitude of differences in heterozygosity. Based on this logic, we recommend further investigation of the lower percentile H_e and AR populations identified here to see if they might be demographically isolated and if inbreeding depression might be a concern.

Conservation recommendations

Our results suggest that it may be appropriate to designate the two larger major genetic groups (Midwestern and Eastern) as ESUs. ESUs generally exhibit long-term reproductive isolation (approximately 100's of generations) and ecological or adaptive distinctiveness (Allendorf et al. 2022). Our data suggest that the long-term reproductive isolation criterion has been met, where the groups are expected to be the outcome of unique past evolutionary events that are unlikely to re-evolve on ecological time scales (10's of generations). We found some evidence of limited gene

flow between the two major genetic groups. However, the amount of genetic differentiation suggests that gene flow, under the assumptions of the island model of migration (Wright 1931), is approximately two turtles every generation (approximately 40 yrs) and is therefore unlikely to overcome the evolutionary consequences of long-term separation. Ecological conditions differ between the two regions, and thus adaptive divergence could occur at this spatial scale. Additional information on adaptive variation would be helpful for ESU determination. Even absent that information, an important component of the evolutionary legacy and evolutionary potential of the Wood Turtle would be lost if either the Midwestern or the Eastern genetic group went extinct. Future work would also benefit from inclusion of collections from the Canadian range that we did not sample, where divergence across ecological gradients has been observed in some cases (Tessier et al. 2005).

At a finer spatial scale, maintenance of metapopulation structure and genetic connectivity is a conservation target that emerges from our study. Metapopulation structure should be maintained at the HUC4 subregion and HUC8 subbasin scales. If assisted gene flow (i.e., genetic rescue; Whiteley et al. 2015) is considered in the future, translocations at this scale are the least likely to lead to outbreeding depression, defined as when outcrossed individuals from genetically divergent populations have lower fitness than either parental form. The pattern of IBD along the eastern portion of the range suggests that translocations might be performed at distances further than adjacent HUC4 watersheds without risk of outbreeding depression because alleles should have spread throughout the entire set of interacting subpopulations (Slatkin 1993). However, local adaptation can occur under a stepping-stone pattern of gene flow (Irwin 2012), so greater geographic (and therefore genetic) distance along with more pronounced environmental differences would lead to a greater risk of outbreeding depression. Our considerations for the geographic scale of translocations is also relevant for potential repatriation efforts for seized animals. In combination with assignment tests, repatriation to assigned natal HUC4 subregion could be a target. We predict limited fitness costs if assignment tests incorrectly assign individuals to adjacent HUC4 subregions. Finally, continued monitoring of relatively genetically depauperate populations is warranted because loss of genetic variation will be slow to respond to population decline for species with such long generation interval. As such, genetic rescue might be warranted at lower thresholds of loss of genetic variation in species such as the Wood Turtle compared to short generation interval species.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10592-026-01775-w.

Acknowledgments Collectors who contributed more than 30 samples (and not listed above) were: V. Young, N. Gerard, L. Johnson, and N. Sherwood. Numerous other collectors and volunteers contributed samples to this project. E. Winter, S. Painter, and A. Lodmell performed lab work. All turtles were collected and handled according to designated scientific collection permits and followed ethical treatment and care under policies for each agency or institution. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government. The findings and conclusions in this publication are those of the authors and should not be construed to represent any official U.S. Department of Agriculture or U.S. Government determination or policy.

Author contributions A.R.W., D.W.-S., and M.T.J. wrote the main manuscript text. A.W. and D.W.-S. performed population genetic analyses. M.T.J. prepared figure 1. A.R.W. prepared other figures. T.S.B.A., D.B., D.M.D., C.E., L.E., K.D.G., E.L., M.N.M., T.M., D.M., J.M., J.M., A.R., H.P.R., R.R., S.M.S., J.T., E.T., D.T.Y., and L.L.W. provided critial samples and helped with sampling design. All authors reviewed the manuscript.

Funding For this study was provided by two US Fish and Wildlife Service-sponsored Competitive State Wildlife Grants (CSWGs) to the Massachusetts Division of Fisheries and Wildlife and its partners, and by a Regional Conservation Needs Grant. Key participants in the CSWG process include P. Sievert, M. M. Winters, P. deMaynadier, N. Sherwood, G. Johnson, and B. Zarate.

Data availability All genetic data with population names obscured as pseudocodes are available at DRYAD (upon publication available at: https://doi.org/10.5061/dryad.jm63xsjrc, currently located at: http://datadryad.org/share/LINK_NOT_FOR_PUBLICATION/uYCtjyDsw9roMKqfPd52VTP81vMQ8yxYnwlXNGv8y4c).

Declarations

Competing interests The authors declare no competing interests.

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Conservation Genetics (2026) 27:71
https://doi.org/10.1007/s10592-026-01795-6

CORRECTION

Correction: Rangewide population genetic analysis of the Wood Turtle (Glyptemys insculpta)

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Published online: 2 June 2026

Correction to: Conservation Genetics (2026) 27:55
https://doi.org/10.1007/s10592-026-01775-w

In this article, Figure captions 3 and 4 are exchanged; they were ordered incorrectly. The correct order is displayed below.

Incorrect version:

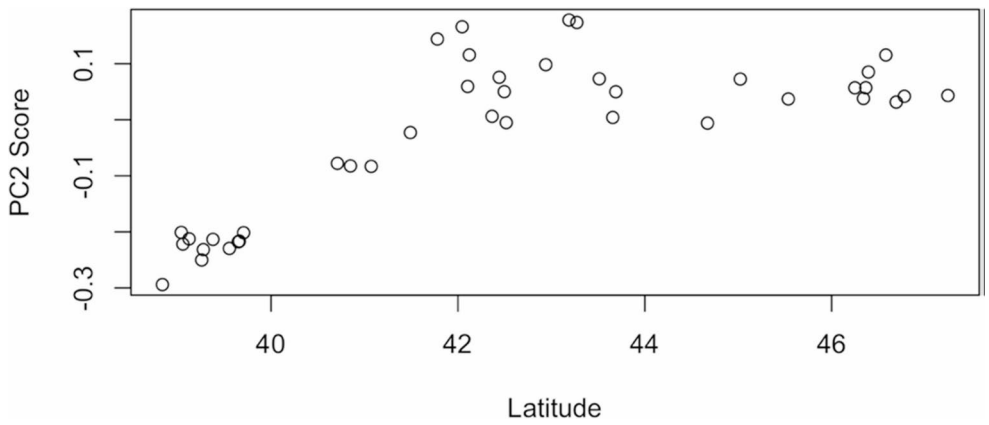


Fig.3 Discriminant Analysis of Principal Components (DAPC) assuming K = 3 for 45 Wood Turtle collections. Individuals (points and colored lines connected to points) are colored by the population to which they were assigned by the discriminant analysis. Putatively admixed individuals are labeled with the color that corresponds to their population assignment but are connected with colored lines to the population from which they were sampled. The location of collection labels (or the black line segment issuing from the collection label) represents the centroid of that collection's scores along each axis. The percentage of variation explained by each discriminant axis is shown in parentheses

The original article can be found online at https://doi.org/10.1007/s10592-026-01775-w.

Extended author information available on the last page of the article

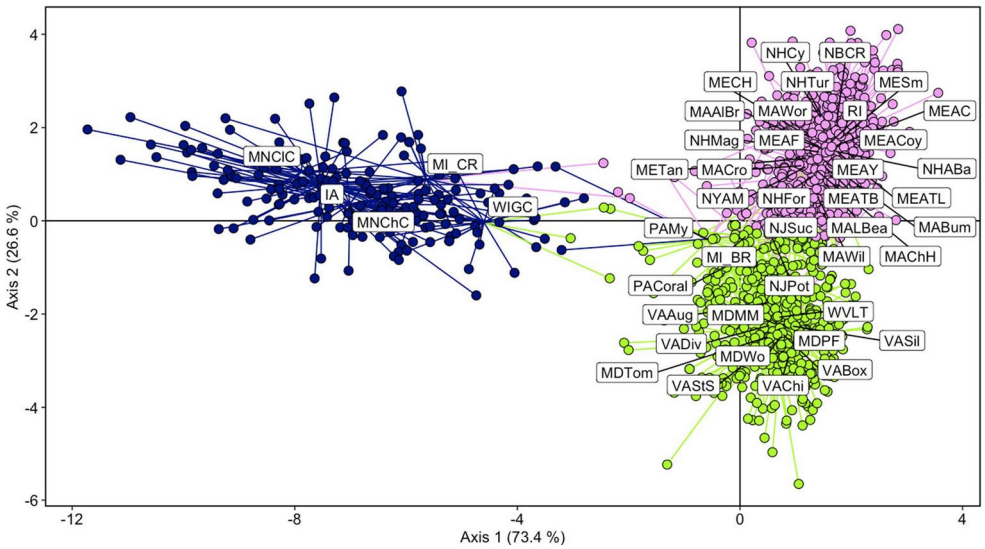
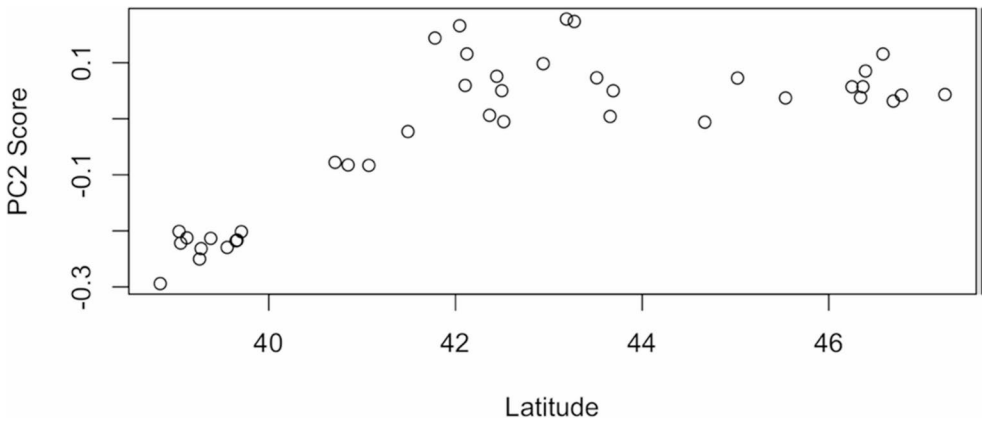


Fig. 4 Test for geographically continuous cline in population subdivision in the eastern portion of the Wood Turtle range for all collectionswith N ≥ 15. PC2 score per collection is on the y-axis. Latitude of each collection is on the x-axis. Midwestern (except east assigning MI_BR) collections were excluded

Corrected version:



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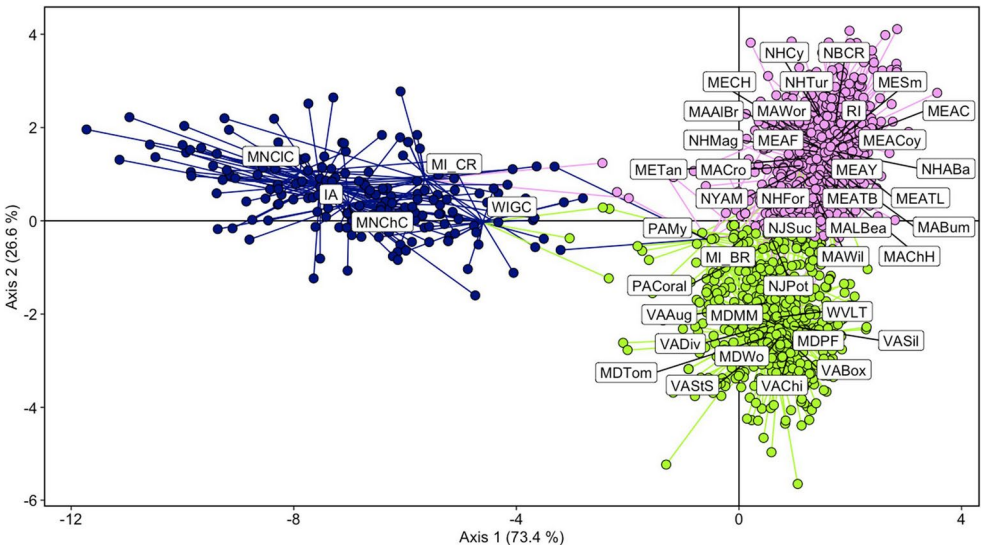

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The original article has been corrected.

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