# A Population Genetic Assessment of the Extant Subspecies of Seaside Sparrow (*Ammospiza maritima*) on the Atlantic Coast

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To the College of Graduate Studies:

We are submitting a thesis written by Mackenzie Roeder entitled "A Population Genetic Assessment of the Extant Subspecies of Seaside Sparrow (*Ammospiza maritima*) on the Atlantic Coast." We have examined the final copy of this thesis for form and content. We recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science in biology.

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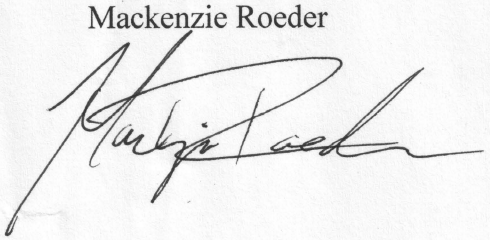

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## Abstract

Seaside Sparrows (*Ammospiza maritima*) are tidal salt marsh endemic passerines found along the Atlantic and Gulf Coasts. At present there has not been a modern genetic assessment of the Atlantic Coast clade, which consists of two extant subspecies: the Northern Seaside Sparrow, *A. m. maritima* (Wilson 1811), and MacGillivray's Seaside Sparrow, *A. m. macgillivraii* (Audubon 1870). The currently described ranges of these subspecies are from Massachusetts to North Carolina (Northern) and North Carolina to Florida (MacGillivray's). We analyzed genetic (microsatellite and mitochondria) data from 400 Seaside Sparrows from Connecticut to Florida (2000 – 2017). Sampling efforts were focused (1) near the currently defined geographic boundary between the subspecies (Dare County, NC), and (2) the type locality for MacGillivray's Seaside Sparrow (Charleston, SC). Bayesian cluster analysis (program STRUCTURE) indicates three genetically distinct population segments, which were recovered regardless of how the data were subsampled. The population in Charleston, SC was the most strongly differentiated population, and this population also harbored a unique mitochondrial (mtDNA) "signature," likely reflecting long-standing isolation. These results indicate discordance with the currently described ranges of the subspecies of Seaside Sparrow, and provide grounds for the consideration of separate management plans for the three populations.

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## CHAPTER 1: Background Information

The concept of identifiable geographical variance below species level in birds was first referred to as "subspecies" in 1886 by the American Ornithologists Union (AOU 1886). The concept, however, had been discussed multiple times beforehand as traditional Linnaean taxonomy could not account for sub-specific variance so19th century biologists used phrases such as forms, varieties, or races to convey this concept. The emergence of the term "subspecies" was accompanied by its own taxonomic addition, the trinomial (Winker 2010). The term has a longstanding history of controversy though much of it stems from a lack of consensus on the definition of a subspecies and what standards to expect for their diagnosability (Mayr 1943, 1982; Winker 2010). Some of this confusion, no doubt, was a product of misunderstanding the species concept and an increasing reliance on morphological differences as the boundary between species. Ernst Mayr's criterion (Mayr 1943, 1996) for classifying species, a focus on interbreeding versus reproductive isolation, helps to clarify the issue. Under this definition of species, morphological differences are helpful but not of utmost importance. Thus populations that are reproductively isolated from other populations are considered species. Any populations within these species showing diagnosable geographical variance that are not reproductively isolated may be considered subspecies. Mayr's definition of species even addresses the issue of hybrids. Rather than reproductive isolation being a rigid barrier, isolating mechanisms are more broadly considered properties of individuals within the species that prevent the interbreeding of populations, not individuals (Mayr 1943, 1996).

More recently, however, additional species concepts have been proposed (biological, phylogenetic, ecological, etc.), continuing the confusion among biologists of

varying disciplines (de Queiroz 2005). The problem, ultimately, is that different taxa can be identified (or not) depending on the concept used and thus the continued confusion of the definition of species naturally leads to contention over the definition of subspecies. The most important, though seemingly obvious, criterion shared among these concepts is that the species is the central biological criteria for taxonomic classification and the fundamental category for classifying biological populations (Mayr 1943, de Queiroz 2005).

Subspecies are groups, or local populations, that show diagnosable variation across the range of the species as a whole. Mayr (1943) describes three main questions to be addressed when identifying subspecies: is the population reproductively isolated, are geographically varied populations the only type of subspecies, and how are subspecies distinguished from one another? The first question is addressed directly by the biological species concept: if the two populations (not just individuals) successfully breed when they come in contact they are not separate species. Take, for example, the Northern Flicker (*Colaptes auratus*), which is currently treated as a single species despite their clear morphological differences. The ranges of the two subspecies, the Yellow-shafted Flicker (*C.a. auratus*) and the Red-shafted Flicker (*C. a. cafer*), overlap in an area that covers the length of North America. Populations within this overlap interbreed without any reproductive consequences confirming that they are not separate species, per the biological species concept (Mayr 1943, Tyler Flockhart and Wiebe 2009). Until recently even molecular methods were unable to distinguish the two subspecies from each other. The first evidence of genomic differentiation between these two taxa was found using

next generation sequencing, though the levels of differentiation were low and no fixed SNPs were identified (Aguillon et al. 2018).

The second question addresses the potential causal mechanisms for observed variation between populations that have served as the criteria for defining taxa. In birds differences between populations are most often classified geographically though ecological races are recognized in other taxa, particularly in plants, but also in several avian taxa including Crossbills (*Loxia*) and Darwin's finches (*Geospiza*) (Mallet 2008). Mayr argues that in most cases there is no benefit in differentiating between the two as many ecological differences between groups are the product of an initial geographical isolation (Mayr 1943). The third question is the one most often argued in debates on the classification of subspecies: how are they diagnosed in continuous populations? As subspecies are not reproductively isolated from one another populations often show gradients of differentiation making border delineations seem arbitrary when no distributional gap is evident (Mayr 1943). Often times the 75% rule is applied, wherein 75 percent of the individuals from one population must be able to be distinguished from 99% of individuals of another population (Amadon 1949). The obvious issue with this rule is that the percentage of diagnosability will likely change with access to more specimens for comparison or if certain standards for specimen comparison are not set; e.g., specimens in breeding versus non-breeding condition (Mayr 1943, Cicero and Johnson 2006). Ultimately Mayr's response to the third question are the following guidelines for identifying subspecies (i) members share a unique geographic range within that of the species, (ii) they share similar phenotypic characteristics, and (iii) have a unique natural history compared to those of other populations within the species (Brien

and Mayr 1991). What, then, is the value of subspecies and research on infraspecific variation? Subspecies are a useful tool for understanding populations: their potential niche differences, behavioral differences, and local adaptations. The study of subspecies considered to be biological units (multiple concepts agree that there is diagnosability between populations) allows insight into the varying processes influencing species across their entire geographic range and can help identify potential conservation concerns (Mayr 1982, Winker 2010).

There are several methods currently used to delineate subspecies. Traditionally, avian taxonomists relied heavily on the analysis of morphological characters and linked them to geographical patterns (Paxton et al. 2010). This practice, largely due to the vast collections of avian museum specimens, generated most of the initial taxonomic rankings of birds. Plumage variation, in particular, has been one of the most cited subjects of criticism against the delineation of subspecies due to its inherent subjectivity. Over the past two decades the field of taxonomy has become dominated by genetic methods; e.g., the analysis of microsatellite data, mitochondrial DNA, Next-Generation sequencing. These methods can address a variety of issues including population relatedness, potential hybrid zones, bottlenecks, population sizes, migration rates, dispersal distances, and population dynamics (Selkoe and Toonen 2006). These new tools have been invaluable to the field of population genetics, as well as countless others. The past 10 – 15 years have seen rapid advancements in genetic technology, a vast expansion in its applications, and of the methods of statistical interpretations of the resulting data. Genetic population assessments analyze patterns of genetic diversity within and among populations (Ball and Avise 1992, Chan and Arcese 2002, Friis et al. 2016). From a conservation standpoint,

genetic diversity is an important part of maintaining a species adaptive potential (Holderegger et al. 2006). Genetic diversity can be broken down into two types: neutral and adaptive. Adaptive genetic markers are genes that have an effect on the fitness of the organism; they are affected by natural selection. Neutral genetic markers are genes, or loci, that have no effect on fitness. They are often used in population genetics because (in the case of microsatellites) comparisons of their frequencies within and among groups provide insight into dispersal, gene flow, and genetic drift (Holderegger et al. 2006). Due to variations in mutation rates between neutral genetic markers (e.g. microsatellite loci versus mitochondrial genes) insight into population level genetic changes can be gained on both contemporary and more evolutionarily distant time scales (Ball and Avise 1992, Chan and Arcese 2002, Wayne and Morin 2004).

Microsatellites are often used to understand subspecific genetic variation in avian systems as they can be used to infer rates of gene flow and genetic drift as well as to infer contractions and expansions of past populations (Selkoe and Toonen 2006). These analyses are useful in the planning of conservation efforts as they provide insight into the level of genetic diversity among populations; which can be predictive of their ability to respond to stressors or conservation challenges (Greenberg et al. 2016). Microsatellite data was used to examine differentiation within and between five subspecies of Song Sparrows, *Melospiza melodia*, in the San Francisco Bay Region; three of these subspecies are found in tidal salt marsh (*M. m. samuelis*, *M. m. maxillaris*, and *M. m. pusillula*) one in upland habitats (*M. m. gouldii*) and one in riparian habitats (*M. m. heermanni*) (Chan and Arcese 2002). Previous studies (Hare and Shields 1992, Zink and Dittmann 1993, Fry and Zink 1998) had failed to show any genetic differentiation among the morphologically

varied subspecies using mitochondrial DNA. Microsatellite analyses confirmed low levels of genetic differentiation within and among populations. Despite these estimates of divergence being low they were still statistically significant. One of the tidal marsh endemic subspecies (*M. m. pusillula*) showed the greatest levels of differentiation when compared to all other subspecies thus indicating that the prioritization of conservation efforts be focused on *M. m. pusilulla* based on both statistically significant (highest levels of differentiation among the subspecies) and biologically significant (degree of threat of habitat loss and fragmentation) factors (Chan and Arcese 2002).

Analysis of seven microsatellite loci from isolated colonies of Reddish Egrets, *Egretta rufescens,* showed genetic differentiation throughout the range that clustered in three main regions; the Baja California peninsula, Mexico, and Great Inagua, Bahamas (Hill et al. 2012). Minimal evidence of gene flow between these populations led to the recommendation that conservation efforts be focused on each of the three distinct population segments (Hill et al. 2012). Analyses of seven microsatellite loci were also used to measure levels of genetic differentiation between two subspecies of Swamp Sparrow; *Melospiza georgiana.* The Coastal Plain Swamp Sparrow (*M. g. nigrescens*) is found in brackish tidal marsh while the Southern Swamp Sparrow (*M. g. georgiana*) is found in freshwater wetlands. These analyses showed discordance with previous mitochondrial analyses indicating no levels of genetic differentiation (Greenberg et al. 2016). Microsatellite analyses suggested two distinct genetic clusters as well as indication of a possible intergrade zone in an area of known morphological intergrades. These findings provide support for the subspecific status of these taxa and coupled with the knowledge of dwindling salt marsh habitat subspecific delineation for these two

populations would allow for the implementation of population specific management plans (Greenberg et al. 2016).

The Seaside Sparrow (*Ammospiza maritima*), first described in 1811 (Wilson 1811) is a polytypic species comprised of nine subspecies whose ranges extends along the thin strip of coastal marshes from Massachusetts down along the Atlantic coast, around Florida, and along the Gulf coast to the south east border of Texas (Austin 1983, Nelson et al. 2000). They are one of few tidal salt marsh endemic birds, and they are considered vulnerable due to the loss and fragmentation of saltmarsh habitat (Greenberg et al. 2006, Malamud-Roam et al. 2006, Crain et al. 2009, Correll et al. 2017). In this thesis I use a population genetic approach, using 15 microsatellite loci, to better understand the genetic characteristics of the extant populations of Seaside Sparrows along the Atlantic Coast.

# Chapter 2

## A population genetic assessment of the Atlantic Coast Seaside Sparrows*

*The following chapter is being formatted for submission to The Auk or The Condor with the following coauthors: S. Woltmann, C. Hill, C. Elphick, M. Conway, and A. Kocek

### 2.1 Introduction

Conservation efforts depend upon a clear understanding of the biological characteristics of the organism, including its distribution and geographic range (Chan and Arcese 2002, Greenberg et al. 2016). This is sometimes challenging for species with large, contiguous ranges that cover heterogeneous landscapes, or experience different degrees of anthropogenic impact. To describe variation (or perceived variation) within species, early ornithologists relied on the use of the subspecies concept. The concept of the subspecies has a longstanding history in avian ecology even though its definition remains a topic of debate (Haig et al. 2006, Phillimore and Owens 2006, Draheim et al. 2010, Haig and D'Elia 2010, Patten 2015). The ranges of subspecies remain poorly understood in many avian taxa, because classification by plumage can be difficult and modern genetic assessments vary in agreement with morphological classifications (Chan and Arcese 2002, Johnson et al. 2003, García et al. 2016). Furthermore, genetic variation between populations is often unknown, as many named subspecies have yet to be evaluated with genetic approaches (Remsen 2010, Woltmann et al. 2014). A better understanding of the geographic ranges of subspecies, and the degree of genetic variation within these ranges, could improve conservation efforts at both the population and species level. In practice, the study of genetic variation within and among populations has been useful for understanding drivers of divergence, piecing together evolutionary

histories, and implementing conservation and management plans (Feulner et al. 2004, Friis et al. 2016, Walsh et al. 2017).

Microsatellite data have been used to describe intraspecific variation in many avian taxa. An analysis of the genetic diversity of Reddish Egrets (*Egretta rufescens*), a patchily distributed species found from the northern Pacific coast of Mexico to the Caribbean, using microsatellite data indicated dramatic genetic differentiation throughout the range. Of the three currently described subspecies of Reddish Egret (*E. r. rufescens,* found in northeastern Mexico, the Bahamas, and on the Gulf coast of the United States, *E.r. dickeyi,* found in the Baja California peninsula, *and E. r. colorata,* found in the Yucatan peninsula) genetic structure showed support for *E. r. dickeyi* as well as an additional population within the currently described range of *E. r. rufescens* in Great Iguana, Bahamas. No significant genetic differences were noted between dark and light color morphs within the same area (Hill et al. 2012). A population genetic assessment of the five putative subspecies of Song Sparrow, *Melospiza melodia,* in the San Francisco Bay region (*M. m. samuelis, M. m. maxillaris, M. m. pusillula, M. m. gouldii,* and *M. m. heermanni*) showed low estimates of divergence despite marked phenotypic variation between subspecies (Chan and Arcese 2002). Microsatellite data were used to assess the genetic structure of Least Tern (*Sternula antilarrum*) populations in the United States. The genetic structure found did not align with the currently described ranges of the subspecies (the California Least Tern (*S. a. browni*), the Interior Least Tern (*S. a. athalassos*), and the Eastern Least Tern (*S. a. antillarum*) which suggests a taxonomic reevaluation may be warranted (Draheim et al. 2010).

The Seaside Sparrow is a tidal marsh endemic member of the sparrow family Passerellidae. They are found in tidal salt marshes along the Atlantic and Gulf coasts of the United States and have a complex taxonomic history (Austin 1983, McDonald 1988). Up to three species have been recognized – *A. maritimus, A. nigrescens,* and *A. mirabilis,* though they are now considered subspecies *(A .m. maritima, A. m. nigrescens, and A. m. mirabilis)* (Chapman 1899, Robbins 1983, McDonald 1988). The Seaside Sparrow was first described in 1811 by Alexander Wilson as *Fringilla maritima* from the tidal salt marshes of southern New Jersey. The first subspecies of Seaside Sparrow (*A. m. macgillivraii*) was discovered in Charleston, South Carolina in 1834 by John Bachman and described by John James Audubon (Austin 1983). The taxonomic confusion for the subspecies begins here as Audubon's description is vague - describing MacGillivray's Seaside Sparrow as being intermediate in appearance between the Seaside and Saltmarsh Sparrow but without listing any diagnosable characteristics (Audubon 1870, McDonald 1988). Mysteriously the type specimen Audubon chose (located in the U.S. National Museum of Natural History) is in juvenile plumage, and juvenile plumages in Seaside Sparrows are not considered diagnosable to subspecies (Griscom 1944). Two other subspecies (*A. m. waynei* and *A. m. pelonotus*) on the southeastern Atlantic coast were later considered synonymous with *macgillivraii* (Kale 1983, McDonald 1988). By 1919, nine subspecies had been described with slight plumage variations (see Table 1). Seaside Sparrows molt annually, in the early fall, and most of the original descriptions of their plumage were based on specimens collected in late spring when the plumage is fairly worn; exacerbating the taxonomic confusion (Austin 1983, McDonald 1988). Two main taxonomic assessments of Seaside Sparrows were Griscom and Nichols (1920) and

Griscom (1944). These revisions noted within-population variation in Seaside Sparrows and posited the presence of light and dark morphs within each of the subspecies, though later Griscom acknowledged that the variation was a gradient (McDonald 1988). Since the 1980s two of these subspecies (*A. m. pelonotus* and *A. m. nigrescens*) have become extinct, and one is currently listed as federally endangered (*A. m. mirabilis)* (Kale 1983, Robbins 1983, Avise and Nelson 1989, Woltmann et al. 2014).

The extant Atlantic Coast Seaside Sparrows are the Northern Seaside Sparrow (*A. m. maritima*) and MacGillivray's Seaside Sparrow (*A. m. macgillivraii*); the Federally endangered Cape Sable Seaside Sparrow (*A. m. mirabilis*) in the Florida Everglades is also part of the "Atlantic" mtDNA group (Nelson et al. 2000). The Northern Seaside Sparrow has a breeding range from Massachusetts southward to North Carolina (the putative beginning of MacGillivray's range). In the winter it migrates south into MacGillivray's range though some birds have been found in the Chesapeake Bay area and occasionally as far north as Massachusetts during the winter months; individuals will apparently remain sedentary as far north as New York when winters are mild (Wilson 1811, Austin 1983, Funderburg 1983, Robbins 1983). Northern Seaside Sparrows are described as a pale gray above and pale below with dark malar striping, yellow lores and forewing, with a bill longer than most sparrows (Wilson 1811, Funderburg 1983). MacGillivray's Seaside Sparrow has a putative breeding range from Dare County, North Carolina to the northeastern tidal salt marshes of Florida. Audubon's original description of MacGillvray's Seaside Sparrow describes the plumage as darker than the Northern Seaside Sparrow but was otherwise vague in diagnostic characters, as many early descriptions were (Austin 1983, Funderburg 1983).

Two studies have described two phylogenetically distinct groups within the Seaside Sparrow: the Gulf coast Seaside Sparrows and the Atlantic Coast Seaside Sparrows; though, interestingly the Cape Sable Seaside Sparrow (*A. m. mirabilis*) was found to belong to the Atlantic group rather than the geographically closer Gulf group (Avise and Nelson 1989, Nelson et al. 2000). A taxonomic review (McDonald 1988), however, found that plumage variation among the subspecies did not fit neatly into these two phylogenetic groups. Plumage characteristics of 1318 museum specimens were compared to determine if diagnosable plumage differences existed between the subspecies. McDonald found variation from light to dark within subspecies but found no evidence to support Griscom's light and dark morphs. The Dusky and Cape Sable Seaside Sparrows (*A. m. nigrescens* and *A. m. mirabilis,* respectively) were the only readily distinguishable subspecies, and the rest of the specimens (both Gulf and Atlantic) could more or less be placed in a continuum of light to dark, but in no geographically relevant pattern. No consistently diagnosable plumage characteristics were noted among the Atlantic coast subspecies. This assessment brings to light the many shortcomings of the original Seaside Sparrow subspecies classifications. No morphological evidence was found to support the classification of several of the currently accepted taxa and demonstrates a clear need for a reevaluation of the subspecies, particularly on the Atlantic coast (McDonald 1988).

Recently, microsatellite analyses showed discordance between the currently accepted taxonomic delineations (which were originally described based on morphological characteristics) and the population genetic characteristics of Seaside Sparrows along the Gulf of Mexico. The two Gulf Coast Florida taxa (*peninsulae* and

*juncicola*) were not genetically distinguishable, but are distinct from the two remaining western subspecies (*fisheri* and *sennetti).* The southern Texas population (*sennetti*) is genetically distinct from all other subspecies and there is evidence of an admixture zone between *sennetti* and *fisheri* (Woltmann et al. 2014). A petition for the federal listing of MacGillivray's Seaside Sparrow was filed in 2010, necessitating a Species Status Assessment (Center for Biological Diversity 2010). At present at least one subspecies (*A. m. nigrescens*) has gone extinct and confusion remains on morphologically diagnosing these subspecies; a modern genetic assessment will provide insight into the structure of genetic variation and potential conservation risks. Here we used 15 microsatellite loci to understand range-wide population genetic characteristics of Seaside Sparrows along the Atlantic coast. The goals of this study are to (1) describe the distribution of genetic structure within and among populations of Seaside Sparrows on the Atlantic coast, (2) ask whether this structure is in concordance with current sub-specific taxonomy, and (3) describe evidence of potential conservation concerns.

## 2.2 METHODS

### 2.2.1 Methods

Permits: Birds were banded and blood-sampled under Federal Banding Permits 23828 and 22990 and state permits 55903 (Virginia), 17-SC01133 (North Carolina), R17-04 (USFWS Special Use Permit: Cedar Island NWR), BB-17-06 (SC), and 29-WJH-16-230 #29 (Georgia). All samples were collected in accordance with the guidelines in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All protocols were approved by the Institutional Animal Care and Use Committee of Austin

Peay State University (Permit number: 17-008).

### 2.2.2 Study Area

Mist nets were used to capture 400 birds from 12 localities ranging from Florida north to Connecticut (Table 2, Figure 1) between 2000 and 2017. Blood samples were collected and stored in Queen's lysis buffer (Seutin et al. 1991). Sampling localities were chosen to evenly sample throughout the entire range of Seaside Sparrows on the Atlantic coast with particular attention paid to the currently defined boundary (Dare County, NC) between the two extant subspecies (*A. m. maritima* and *A. m. macgillivraii*), as well as the type locality for MacGillivray's Seaside Sparrow (Charleston, SC). Samples from the southern portion of the range were collected as follows: Mathews and Gloucester Counties, VA (May 2017), Dare County, NC (July 2016 and May 2017), Carteret County, NC (September 2003, July 2005, and May 2017), Georgetown County, SC (July 2000), Charleston County, SC (February - May 2000 and May 2017), Glynn County, GA (July 2016 and May 2017), and Nassau and Duval Counties, FL (July 2016 and June 2017) (see Table 2). As there are no reliable diagnostic plumage criteria to distinguish the two subspecies these ranges and sampling dates were chosen to assure that all migratory birds potentially wintering in these areas had moved north. Further, all birds sampled from NC south were adults in breeding condition (based on the presence of either a cloacal protuberance or brood patch) or hatch years indicating that they were locally breeding birds.

### 2.2.3 Laboratory Methods

#### 2.2.3.1 Microsatellites

DNA was extracted using Qiagen DNeasy or ThermoScientific Tissue and Blood kits, quantified using a Nanodrop 2000 (ThemoScientific, Delaware, USA). We amplified 15 microsatellite loci: Aca01, Aca11 (Hill et al. 2008), Am02, Am12, Am14, Am18, Am20, Am32 (Lehmicke et al. 2012), Sosp13, Sosp14 (Sardell et al. 2010), ZoleC06, ZoleC11, ZoleE11, ZoleF11, and ZoleG03 (Poesel et al. 2009). All primers were modified with a 19 bp M13 tag (Boutin-Ganache et al. 2001, Woltmann et al. 2014). Polymerase chain reactions (PCR) were run in 10µl reactions with 3.00 mM $MgCl_2$, 0.16 mM dNTPs, 1X buffer, 0.06 µM forward primer, 0.36 µM reverse primer, 0.60 µM dye-labeled m13 primer, 0.10 units Taq polymerase, and 10-20 ng DNA. Amplification of some loci was improved by the addition of Dimethyl sulfoxide (DMSO; 0.30 µL): Am32, Am20, Aca01, Am18, Sosp13, Sosp14, ZoleH02, ZoleC11, ZoleC06, ZoleE11, ZoleF11. Additionally, Betaine (1.00 µL) was used to improve amplification of some loci: : Aca01, Am12, Am14, Am18, Am20, Am32, Sosp13, Sosp14, ZoleC06, ZoleC11, ZoleE11. Thermocycler protocols were as follows: 94˚C for 1 minute, 33-35 cycles of 94 ˚C for 30 seconds, 55-60 ˚C for 30 seconds, 72 ˚C for 30 seconds, and a final extension 72 ˚C for 5 minutes. Reference individuals from the Gulf Coast study (Woltmann et al. 2014) were included to insure consistent genotyping between labs and studies. PCR products were pooled (post-PCR) into four panels (dyes: PET, VIC, NED, and 6-FAM) and run on an Applied Biosystems 3730 capillary sequencer. All genotyping was done by MR and SW using GeneMarker (v. 2.7.0; SoftGenetics, LLC.; State College, Pennsylvania).

#### 2.2.3.2 mtDNA

We sequenced the mitochondrial nicotinamide adenine dinucleotide dehydrogenase subunit 2 (ND2; 1042bp) from 9-20 samples from each site (Table 2). PCRs were run in 25 μL volumes with 1.5mM $MgCl_2$, 0.8 mM dNTPs, 1.25 μM forward primer (L5215, (Hackett 1996)), 1.25 μM reverse primer (H6313, (Johnson and Sorenson 1998)), 1X buffer, 2.5 units of *Taq* polymerase, and 10 ng DNA. The thermocycling protocol was as follows: 94˚C for 30 s, 34 cycles of 94 ˚C for 30 s, 52˚C for 30 s, and 72˚C for 1 min, with a final extension of 72˚C for 7 minutes. SimpleSeq Sanger DNA sequencing was done by Eurofins Genomics (Louisville, KY, USA) and sequences were aligned using Sequencher (v. 5.4.6, Genecodes Corporation, Ann Arbor, Michigan).

### 2.2.4 Data Analysis

#### 2.2.4.1 Summary Statistics

MICROCHECKER (Van Oosterhout et al. 2004) found no evidence of scoring errors or null alleles in the microsatellite data. All microsatellite data conformed to Hardy-Weinberg Equilibrium (HWE) and there was no evidence of linkage-disequilibrium (LD) (GenePop v. 4.2 (Rousset 2008). Population genetic characteristics (mean number of alleles, allelic richness, and observed and expected heterozygosity) were generated with GenAlEx v 6.503 (Peakall and Smouse 2012). Effective genetic population size ($N_e$) was calculated using the sibship assignment method in COLONY (v 2.0.6.3; (Jones and Wang 2010)) using the full-likelihood option. COLONY uses estimated levels of relatedness (probabilities of chosen individuals being full-siblings, half-siblings, or completely

unrelated) to estimate $N_e$, if the calculated $N_e$ is small then there is a high likelihood that the offspring in the population are related (Ackerman et al. 2017). Initial replicate runs starting at different random number seeds indicated that estimates of $N_e$ were consistent between runs, so we used a single run for each sampling locality. We chose this method for its increased accuracy over the linkage disequilibrium and heterozygote excess methods and its flexibility on assumptions regarding samples (Jones and Wang 2010, Hughes et al. 2012). To compare estimates of $N_e$ between the Atlantic and Gulf Coast (data from Woltmann 2014) we used the linkage disequilibrium method (with a random mating model, 0.5 lowest allele frequency, and a jackknife approach to estimating 95% confidence intervals) using NeEstimator (Do et al. 2014). Temporal estimates of $N_e$ (using the Pollak method) were done for all populations with samples collected in separate years (locations 3a, 4a, and 10). Mitochondrial ND2 sequences were analyzed in DnaSP (v 6,(Rozas et al. 2003)) and haplotype frequency networks were generated using TCS (v 1.21; (Clement et al. 2000)). Isolation by Distance (IBD) Desktop (Bohonak 2002) was used to estimate the degree of IBD across all sampling localities using 5,000 permutations. The program BOTTLENECK (V 1.2.02) was used to detect recent bottlenecks in each sampling locality (Luikart and Cornuet 1999).

#### 2.2.4.2 Genetic Structure

We used a Bayesian clustering method in the program STRUCTURE (v 2.3.4) to explore the distribution of genetic structure throughout our sampling range (Pritchard et al. 2000). Admixture models with correlated allele frequencies were run using sampling locations (*loc prior*) to test for genetic clusters (*K*) (Stephens et al. 2000). Run lengths

ranged between 5 x $10^5$ – 1 x $10^6$ MCMC iterations after a burnin of 5 x $10^4$ – 1 x $10^5$ iterations. We explored several variations of the model parameters, including *loc prior* vs no *loc prior*, and correlated vs non-correlated allele frequencies. Initial testing explored up to a range of 16 possible K that was later reduced based upon the preliminary runs. We used a hierarchical approach to our analyses; the same parameters were run as follows: (i) the entire dataset (N =400), (ii) the data set with a reduced number of individuals in several sampling localities to ensure a balanced number (~ 30 individuals per site) of samples from each site, (iii) a run for each of the currently described subspecific ranges; one including just the data from north of Dare County, NC and one including just the data south of Dare County, (iv) smaller runs within areas of genetic structure to check for substructure, (v) a run separating out samples collected in different years within areas of genetic structure to check for possible temporal structure; none was found so those samples were treated as contemporaneous. STRUCTURE HARVESTER (Earl and vonHoldt 2012) was used to process STRUCTURE outputs, which were then processed with CLUMPP (Jakobsson and Rosenberg 2007) and DISTRUCT (Rosenberg 2004).

### 2.3 Results

#### 2.3.1 Summary Statistics

The average number of alleles per locus ($N_a$) ranged from 7.2 – 9.9 (Table 3) and was lowest in New Jersey, though this may be due to the small sample size from that locality. Allelic richness ($A_r$) ranged from 6.6 – 7.5. Point estimates of effective genetic population size ($N_e$) when using COLONY ranged from 57 – 156 (Figure 2). Estimates of mean pairwise relatedness (calculated in GenAlEx v 6.503 (Peakall and Smouse 2012))

confirmed elevated levels of relatedness in several of our sampling localities but not all (see Figure 7). Estimates of $N_e$ using the LD method were generally higher (point estimates ranging from 32 to 989) but most of the confidence intervals included infinity. This was consistent with the estimates of $N_e$ using the LD method for populations of Seaside Sparrows along the Gulf Coast (Woltmann et al. 2014), and could indicate that populations are indeed quite large, or that these estimates are being affected by factors such as migration (Table 6). The likelihood-based method using temporal allele frequency changes is another way to estimate effective genetic population size for multi-year data that has been shown to produce more accurate estimates with narrower confidence intervals (Berthier et al. 2002). We used this approach where multi-year data was available to compare with the other two methods and our results were consistent with the lower estimates of $N_e$ indicated by COLONY (Wang 2005) (see Table 6).

More than half (62%) of the pairwise $F_{ST}$ values were statistically significant ($P<0.001$) (Table 4). Both locations 3a and 9 (Charleston, SC and Queens, NY) showed statistically significant pairwise $F_{ST}$ values when compared to all sites except site 8 (New Jersey), though it is likely that estimates involving site 8 were affected by low sample size (N=11). There was a significant pattern of Isolation by Distance (IBD) across our sampling sites (slope = 0.01646, Z = 3.1303, $r = 0.4212$, $P = 0.001$) but with several outliers that showed higher genetic distance than would be expected solely based upon geographic distance alone (Figure 3).

### 2.3.3. Bayesian Inference of *K*

The analysis of the entire Atlantic coast data set using STRUCTURE showed the most support for a best *K* of 3, based upon the most conservative interpretation of the log likelihoods and the "Evanno" method (Evanno et al. 2005) (see Figure 4). A best K of 4 only differed in treating location 9 (Queens County and Richmond County, New York) as a separate population. Hierarchical analyses of the dataset confirmed a best K of 3 (figure 5). We present here the results from runs employing the correlated alleles model with the *loc prior* option. Bar plots show three clearly defined clusters in FL-GA (locations 1 and 2), Charleston, SC (location 3a), and the remainder of the range to CT (locations 3b – 11) (see Figure 5). Samples from Charleston, SC were also run as two separate groups based on collection year (2000 and 2017) to check for evidence of temporal structure or recent bottlenecks but none were found (see Figure 5). The Wilcoxon 2-tailed tests for each population, run in the program BOTTLENECK, were not significant providing no support for bottlenecks in any of the sampled populations (see Table 7).

### 2.3.5 Mitochondrial Analysis

We recovered 17 ND2 haplotypes from our subset of 116 individuals. One common haplotype was shared by all sampling localities and two haplotypes were shared by a small subset of the 116 individuals (Table 5). The first of these two haplotypes was shared exclusively by individuals from location 3a; the second was shared by two individuals from location 3a, one from location 1 (Florida) and one from location 8 (New Jersey) (see Figure 8). This confirms the genetic differentiation of the population in

Charleston, SC (location 3) and indicates a higher degree of isolation than the other two genetic clusters (sampling localities 1-2, and 3b-10) indicated by the microsatellite data.

### 2.3.4 $N_e$ and Relatedness within populations

Estimates of $N_e$ were also calculated for the three genetic clusters mentioned above using COLONY (see figure 6). The general trend shows lower estimates of $N_e$ for the southern two clusters in comparison to the larger, northern, cluster (Figure 6). Lower values of $N_e$, when using the Sib-ship method indicates higher levels of relatedness within the samples. Mean estimates of within-population relatedness confirm this, showing evidence of relatives in the samples for both of the southern clusters (FL-GA, and Charleston, SC), New York, and Connecticut (Figure 7).

## 2.4 Discussion

Analyses of both microsatellite and mitochondrial data indicate the presence of genetic structure within the Atlantic Coast clade of Seaside Sparrows. Our microsatellite analyses consistently indicated three genetically distinct population segments: one ranging from Florida to Georgia (locations 1 and 2), one entirely restricted to Charleston, SC (location 3a), and the other spanning the remainder of the coast to Massachusetts (locations 3b – 11; Figure 4). Our mitochondrial ND2 data indicate range-wide panmixia with the exception of site 3a (Charleston, South Carolina). Based upon these findings, neither microsatellite nor mitochondrial data indicate that genetically differentiated populations of Seaside Sparrows along the Atlantic coast are concordant with putative subspecific ranges. Our microsatellite data are not consistent with the recognition of two

populations (*A. m. maritima* and *A. m. macgillivraii)* along the Atlantic Coast and no evidence was found to corroborate a genetic differentiation at the presumed contact zone between the subspecies ( Dare Co. North Carolina) (AOU 1957). Instead, we detected three genetically distinct clusters: one spanning Florida and Georgia, one restricted to Charleston, South Carolina, and one continuing the rest of the range to Connecticut). Our mitochondrial ND2 data did not corroborate the three genetic clusters identified by our microsatellite data but this is not surprising as mitochondrial data are typically used to identify long-term isolation and microsatellite data more recent divergence (Sunnucks 2000, Greenberg et al. 2016). Our analyses did, however, indicate a geographically unique haplotype shared among birds in the marshes sampled in Charleston, South Carolina. A clearer understanding of this long term isolation is beyond the scope of this paper, but possibilities include isolation due to habitat fragmentation coinciding with limited dispersal rates, possible indication of divergence based upon a migratory divide, or could simply be evidence of ancestral polymorphisms (Bulgin et al. 2003, Walsh et al. 2012, Greenberg et al. 2016). This genetic and taxonomic discordance corroborates the lack of diagnosability of subspecies on the Atlantic coast demonstrated by McDonald's museum specimen category test (McDonald 1988).

There was evidence of IBD across the entire sampling range indicating that levels of gene flow are sufficient among most local populations to prevent isolation but gene flow still decreases with increasing geographic distance (Walsh et al. 2012). These findings are atypical for Passerelidae generally (Lee et al. 2001, Walsh et al. 2012), but are consistent with patterns of isolation by distance found in other tidal salt marsh

obligates: the Saltmarsh Sparrow (*A. caudacutus*) (Walsh et al. 2012) and Gulf Coast Seaside Sparrows (Woltmann et al. 2014).

Our estimates of $N_e$ were fairly consistent across all sampling localities (57 – 156) but estimates of mean pairwise relatedness were not (elevated in certain localities but not others); this indicates we are likely estimating $N_n$ (neighborhood size) rather than $N_e$ (effective genetic population size). The difference is that $N_e$ is the effective number of individuals in the entire population while $N_n$ is the effective number of individuals within an area of the population (Nunney 2016). $N_e$ assumes a completely panmictic population but does not consider the isolating effects of Euclidean distance on mating probabilities when members of the population are distributed across a landscape. In such instances the population as a whole becomes broken down into overlapping neighborhoods ($N_n$) of breeding individuals (Shirk and Cushman 2014). We would expect $N_e$ to vary across populations because it relies strongly on habitat availability, which should, theoretically, vary across populations. $N_n$, on the other hand, is related to the dispersal behavior of the species, which should be fairly consistent across populations (Nunney 2016). Thus, based upon the relatively low point estimates (compared to those calculated with the LD method) and consistency of values among localities it seems likely that our estimates of $N_e$ are more so estimates of $N_n$. These estimates indicate elevated levels of within-population relatedness and show evidence of limited dispersal in Florida, Georgia, Charleston, SC, which is consistent with our current knowledge of Seaside Sparrow dispersal rates in non-migratory populations (Post and Greenlaw 2009). In residential populations (e.g. South Carolina) dispersal from natal sites is limited for both sexes (males: 55-602 meters, females: 20-294 meters) and distance between natal site and first

year breeding site was similarly close (Post and Greenlaw 2009). The lack of evidence of elevated levels of within-population relatedness at our sites further north is likely due to the increased dispersal rates of migratory populations. Though studies have demonstrated site fidelity of migratory Seaside Sparrows on both the breeding and wintering grounds (Post 1974, Winder et al. 2012) levels of dispersal should still be higher as their return rates are within the typical range of migratory passerines (44.7%, average between the sexes) (Post 1974). One exception, showing higher levels of within-population relatedness in the northern range, was found in our New York sites (Figure 7). Seaside Sparrows at these sites (Idlewild Park, Queens NY and Sawmill Creek, Staten Island NY) showed similar levels of within-population relatedness to those of the resident populations in South Carolina, Florida and Georgia. One possible explanation for this finding is that limited and fragmented habitat (Hartig et al. 2002) restrict dispersal ability; wherein migratory Seaside Sparrows returning for the breeding season may show a stronger site fidelity as nesting habitat is limited in the region.

One possible isolating mechanism that could be driving the observed genetic structure, low Ne, and signs of limited dispersal seen in these Atlantic Seaside Sparrow populations is a migratory divide. Migratory divides occur between sympatric populations that vary in their seasonal migratory timing or routes (Battey et al. 2017). During the non-breeding season northern and southern populations of Seaside Sparrows on the Atlantic coast coexist then separate during the breeding season as migratory birds move north; aside from some populations in North Carolina which likely includes both resident and migratory individuals (Post and Greenlaw 2009, Winder et al. 2012). Geographical variation in migration times has been documented in Seaside Sparrows

(Robbins 1983) and could act as a barrier to gene flow (Battey et al. 2017). Ultimately, we suggest a taxonomic reassessment of the Atlantic coast Seaside Sparrows is necessary as neither phenotypic (McDonald 1988) nor genetic data (this paper) agree with the current taxonomy. The combination of low effective genetic population size, evidence of limited dispersal, and the evidence of genetic isolation in the Charleston, SC population raises conservation concerns.

### 2.5 Conclusions

Our findings indicate three genetic clusters of Seaside Sparrows along the Atlantic coast between Connecticut and northern Florida that do not support the currently accepted sub-specific ranges: one extending from at least Connecticut, south to northern South Carolina, one in Charleston, South Carolina, and one encompassing Georgia and northeast Florida. The southern two clusters show evidence of limited dispersal and lower $N_e$ than the northern cluster. Our findings indicate the need for a taxonomic reassessment of Seaside Sparrows along the Atlantic coast, including morphological analyses of museum specimens, to more accurately reflect genetic and morphological variation. Further ecological and demographic studies are needed to understand the ranges of birds that exhibit migratory versus resident behavior as well as to better our understanding of their dispersal rates, breeding site fidelity, and wintering ground fidelity. These data could lead to a more accurate taxonomic classification of Seaside Sparrows on the Atlantic coast as well as provide grounds for effective conservation management where necessary. At the very least more data is needed to understand the levels of isolation of the population in Charleston, South Carolina; particularly from nearby populations.

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**Table 1: A description of the range, year of classification, and distinguishing features of Seaside Sparrow subspecies**

| Species | Range | Described by (Name, Location, Year) | Description |
|---|---|---|---|
| *Ammospiza maritima* | Tidal Salt marshes – Atlantic and Gulf Coasts, North America[1] | Alexander Wilson, Southern New Jersey, 1811 | Originally *Fringilla maritima* – Pale gray above and dusky-blue below with a white chin. Dark malar striping, bill longer than most sparrows<br>Lores and forewing yellow |
| **Subspecies** | | | |
| *A. m. maritima* | Massachusetts southward to southern Virginia/northern North Carolina[1] | See above | See Above, migratory subspecies – Breeding grounds range from Massachusetts southward to the putative beginning of *macgillivraii* (Dare County, North Carolina). Migrates into range of *macgillivraii* during winter months with some birds staying in the Chesapeake bay area and occasionally as far north as Massachusetts.[1] |
| *A. m. macgillivraii* | South from Dare County, North Carolina, to southeastern (Camden County) Georgia[1] | John Bachman & John James Audubon, Charleston, South Carolina, 1834 | Originally *Fringilla macgillivraii*<br>Color phases vary from light to dark, overall grayish-white with dark olive streaking<br>Some buff on underpants<br>Lores and forewing yellow |
| *A. m. pelonotus* | Duval County, Florida<br>Status - Extinct[1] | Harry C. Oberholser, New Smyrna Beach, Florida, 1931 | Grayish-white with a tinge of buff on under parts<br>Back grayish-brown with heavy black streaking<br>Lores and forewing yellow[1] |
| *A. m. nigrescens* | Orange and Brevard Counties – central eastern Florida<br>Status - Extinct[1] | Robert Ridgway, Indian River, Florida, 1873 | Under parts white with heavy, defined, black streaking - no buff<br>Very dark overall, back black<br>Lores and forewing yellow |
| *A. m. mirabilis* | Cape Sable, Florida<br>Restricted to inland marshes on the southwestern tip of Florida, particularly everglades<br>Status - endangered[1] | H. Howell, Southern tip of Florida,<br>1919 | Originally described as separate species *Ammodramus mirabilis*<br>Under parts white with slight ashy-tinge, streaks are dark-olive, thin, and sharply defined<br>Lores and forewing yellow |
| *A. m. peninsulae* | Dixie County, Florida south to Pasco County, Florida – mid-northwestern edge of Florida.[1] | Joel Asaph Allen, Tarpon Springs, Florida, 1888 | White/brownish-gray underneath with think black streaks<br>Overall dark olive with some reddish-brown<br>Lores and forewing yellow |
| *A. m. juncicola* | Wakulla, Florida southeast to Taylor County, Florida – far western edge of Florida[1] | Ludlow Griscom & John T. Nichols, Wakulla, Florida,1920 | Grey under parts with a buff tinge with diffuse dark streaking<br>Dark overall, back brownish with broad black streaks<br>Lores and forewing yellow |
| *A. m. fisheri* | Pensacola, Florida to San Antonio Bay, Texas[1] | Frank M. Chapman, Grand Isle, Louisiana, 1899 | Varies from darker overall to lighter across range, grayish under parts with an ochre tinge. Streaks range from heavy dark streaking to very diffuse streaking. Head and nape dark rusty brown, back brown overall.<br>Lores and forewing yellow |
| *A. m. sennetti* | Southern Texas, Nueces and Copano Bays[1] | Joel Asaph Allen, Corpus Christie, Texas, 1888 | Much lighter and more gray than *fisheri*, light grayish-white under parts with very diffuse gray streaking. Back gray overall<br>Lores and forewing yellow |

References for table:
(Wilson 1811, Austin 1983, Funderburg 1983)

**Table** 2**.** Sampling localities, subspecies (presumed based on location and sampling date), sample sizes, and contributors for Seaside Sparrows used in the population genetic assessment along the Atlantic coast.

| Site | Subspecies | Locality | Year | Representative coordinates | N | Contributor |
|---|---|---|---|---|---|---|
| **1** | *macgillivraii* | FL (Nassau and Duval Counties) | 2016, 2017 | 30.54213, -81.52391 | 30 | C. Enloe |
| **2** | *macgillivraii* | GA (Glynn County) | 2016, 2017 | 31.15943, -81.44789 | 25 | C. Hill/this study |
| **3a** | *macgillivraii* | SC-south (Charleston County) | 2000, 2017 | 32.75555, -80.01852 | 60 | C. Hill/this study |
| **3b** | *macgillivraii* | SC-north(Georgetown County) | 2000 | 33.22937, -79.21650 | 26 | C. Hill |
| **4a** | *macgillivraii* | NC-south (Carteret County) | 2003, 2005, 2017 | 34.93591, -76.35296 | 39 | C. Hill/this study |
| **4b** | *macgillivraii* | NC-north (Dare County) | 2016, 2017 | 35.71831, -75.50426 | 27 | C. Hill/this study |
| **5** | *maritima* | VA (Gloucester County) | 2017 | 37.28131, -76.39025 | 35 | C. Hill/this study |
| **6** | *maritima* | MD (Worcester, Somerset, and Dorchester Counties) | 2016 | 38.04432, -75.26238 | 34 | M. Conway |
| **7** | *maritima* | DE (Kent and Sussex Counties) | 2016 | 39.08808, -75.46445 | 23 | M. Conway |
| **8** | *maritima* | NJ (Atlantic and Ocean Counties) | 2016 | 39.34712, -74.71769 | 11 | M. Conway |
| **9** | *maritima* | NY (Richmond and Queens Counties) | 2016 | 40.60883, -74.19333 | 33 | A. Kocek |
| **10** | *maritima* | CT (New Haven and Middlesex Counties) | 2003, 2017 | 41.26165, -72.54961 | 57 | C.Elphick/ C. Hill |

**Table 3: Population genetic characteristics for each of the 12 Seaside Sparrow sampling localities along the Atlantic Coast. Site numbers refer to Fig. 1.**

| Site | $N_a$ | $A_r$ | $H_o$ | $H_e$ | $N_e$ | $N_e$ 95% CI |
|---|---|---|---|---|---|---|
| 1 | 8.000 | 6.622 | 0.773 | 0.787 | 73 | 45 – 136 |
| 2 | 8.000 | 6.687 | 0.792 | 0.792 | 100 | 56 – 251 |
| 3a | 8.667 | 6.720 | 0.807 | 0.812 | 64 | 39 – 125 |
| 3b | 8.933 | 7.294 | 0.826 | 0.818 | 72 | 43 – 151 |
| 4a | 9.867 | 7.425 | 0.786 | 0.825 | 97 | 60 – 195 |
| 4b | 8.667 | 7.060 | 0.790 | 0.808 | 156 | 86 – 507 |
| 5 | 9.600 | 7.305 | 0.798 | 0.812 | 113 | 71 – 209 |
| 6 | 9.067 | 7.472 | 0.822 | 0.820 | 98 | 63 – 180 |
| 7 | 9.467 | 7.386 | 0.842 | 0.822 | 72 | 41 – 189 |
| 8 | 7.200 | 7.200 | 0.800 | 0.791 | - | - |
| 9 | 8.533 | 6.784 | 0.806 | 0.788 | 57 | 35 – 99 |
| 10 | 9.733 | 7.137 | 0.810 | 0.815 | 91 | 42 – 1080 |

Na = Mean number of alleles across loci, Ar = Allelic richness, Ho = observed heterozygosity, He = expected heterozygosity, $N_e$ = effective genetic population size, based on analyses in the program Colony. Estimates of $N_e$ not available for NJ; sample size too low.

**Table 4: Pairwise $F_{ST}$ for all 12 sampling localities for Seaside Sparrows along the Atlantic Coast based on micro-satellite data. Values shown in bold are statistically significant (P<0.001), those shown in parentheses were not statistically significant (P>0.001) based on 5,000 permutations of the data.**

| | 1 | 2 | 3a | 3b | 4a | 4b | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | - | (0.0073) | **0.0350** | **0.0277** | **0.0336** | **0.0281** | **0.0240** | **0.0178** | **0.0288** | **0.0188** | **0.0491** | **0.0280** |
| 2 | | - | **0.0317** | **0.0175** | **0.0255** | **0.0227** | **0.0206** | **0.0142** | **0.0267** | (0.0088) | **0.0452** | **0.0245** |
| 3a | | | - | **0.0185** | **0.0188** | **0.0250** | **0.0238** | **0.0171** | **0.0274** | **0.0164** | **0.0352** | **0.0246** |
| 3b | | | | - | (0.0061) | (0.0053) | **0.0116** | (0.0037) | (0.0113) | (0.0057) | **0.0253** | (0.0102) |
| 4a | | | | | - | (0.0026) | **0.0067** | (0.0031) | (0.0091) | (0.0023) | **0.0212** | (0.0071) |
| 4b | | | | | | - | **0.0067** | (0.0032) | (0.0120) | (0.0065) | **0.0179** | (0.0047) |
| 5 | | | | | | | - | (-0.0013) | (0.0018) | (0.0022) | **0.0184** | **0.0103** |
| 6 | | | | | | | | - | (0.0027) | (0.0030) | **0.0182** | (0.0068) |
| 7 | | | | | | | | | - | (0.0073) | **0.0273** | **0.0116** |
| 8 | | | | | | | | | | - | (0.0114) | (0.0091) |
| 9 | | | | | | | | | | | - | **0.0226** |
| 10 | | | | | | | | | | | | - |

**Table 5:** ND2 haplotype frequencies for each sampling locality. Site numbers are from Fig. 1; the number of individuals sequenced per population is in parentheses.

| | **Haplotype** | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site # (N) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
| 1 (9) | 0.78 | - | 0.11 | - | - | 0.11 | - | - | - | - | - | - | - | - | - | - | - |
| 2 (10) | 0.80 | - | - | 0.10 | - | - | 0.10 | - | - | - | - | - | - | - | - | - | - |
| 3a(20) | 0.40 | 0.35 | 0.25 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3b (9) | 1.00 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 (10) | 0.80 | - | - | - | 0.10 | - | - | 0.10 | - | - | - | - | - | - | - | - | - |
| 5 (11) | 0.82 | - | - | - | - | - | - | - | 0.09 | - | - | - | - | - | - | - | 0.09 |
| 6 (10) | 0.80 | - | - | - | - | - | - | - | - | 0.10 | - | - | - | - | - | 0.10 | - |
| 7 (9) | 0.89 | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.11 | - | - |
| 8 (9) | 0.67 | - | 0.11 | - | - | - | - | - | - | - | 0.11 | - | - | 0.11 | - | - | - |
| 9 (9) | 1.00 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 10 (10) | 0.60 | - | - | - | 0.10 | - | - | - | - | - | - | 0.10 | 0.10 | 0.10 | - | - | - |

**Table 6:** Comparison of effective genetic population size ($N_e$) point estimates and 95% confidence intervals between Atlantic Coast Seaside Sparrows (this study) and Gulf Coast Seaside Sparrows (Woltmann et al. 2014) using the Linkage Disequilibrium method and the Pollak Temporal method (where temporal samples were available).

| Sampling locality | Coast | Ne (LD method) | Ne 95% Confidence interval | Temporal Estimate of Ne | 95% CI |
|---|---|---|---|---|---|
| 1 | Atlantic | 184 | 88 - ∞ | - | - |
| 2 | Atlantic | 222 | 81 - ∞ | - | - |
| 3a | Atlantic | 555 | 231 - ∞ | 58 | 31-168 |
| 3b | Atlantic | 268 | 94 - ∞ | - | - |
| 4a | Atlantic | 160 | 95 - 427 | 52 | 21.8 - ∞ |
| 4b | Atlantic | ∞ | 1791 - ∞ | - | - |
| 5 | Atlantic | 989 | 182 - ∞ | - | - |
| 6 | Atlantic | 925 | 178 - ∞ | - | - |
| 7 | Atlantic | 262 | 170 - ∞ | - | - |
| 8 | Atlantic | ∞ | ∞ | - | - |
| 9 | Atlantic | 32 | 26 - 41.6 | - | - |
| 10 | Atlantic | 95 | 73 - 133 | 74 | 31 - 12009 |
| 1 | Gulf | 556 | 71 - ∞ | - | - |
| 2 | Gulf | 283 | 82 - ∞ | - | - |
| 3 | Gulf | 2047 | 159 - ∞ | - | - |
| 4 | Gulf | ∞ | 451 - ∞ | - | - |
| 5 | Gulf | ∞ | 457 - ∞ | - | - |
| 6 | Gulf | 341 | 149 - ∞ | - | - |
| 7 | Gulf | 84 | 54 - 164 | - | - |
| 8 | Gulf | 176 | 86 - 5378 | - | - |
| 9 | Gulf | 857 | 150 - ∞ | - | - |

**Table 7:** Tests for evidence of a bottleneck in each sampled population using the program BOTTLENECK using the stepwise mutation model

| Population | Mean Expected Heterozygosity | Wilcoxon 2-tailed test |
|---|---|---|
| 1 | 0.80072 | 0.977966 |
| 2 | 0.80805 | 0.678772 |
| 3a | 0.81842 | 0.083252 |
| 3b | 0.83434 | 0.761536 |
| 4a | 0.83536 | 0.890381 |
| 4b | 0.82278 | 0.561401 |
| 5 | 0.82349 | 0.488708 |
| 6 | 0.83835 | 0.599487 |
| 7 | 0.83047 | 0.803955 |
| 8 | 0.82915 | 0.135376 |
| 9 | 0.80028 | 0.977966 |
| 10 | 0.82195 | 0.803955 |

Figures

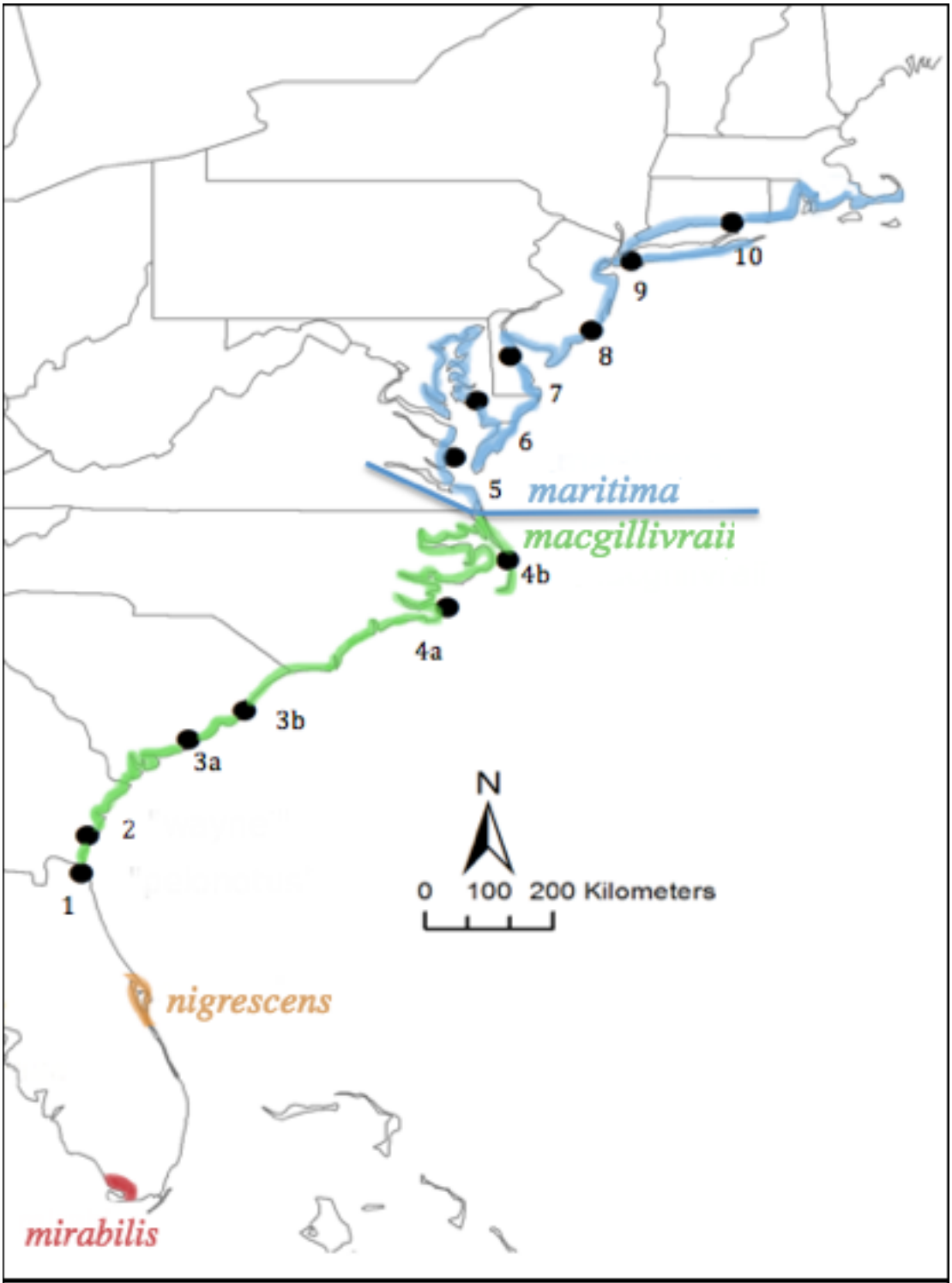


**Figure 1.** Representative sampling localities and the putative breeding ranges of Seaside Sparrow subspecies along the Atlantic Coast.

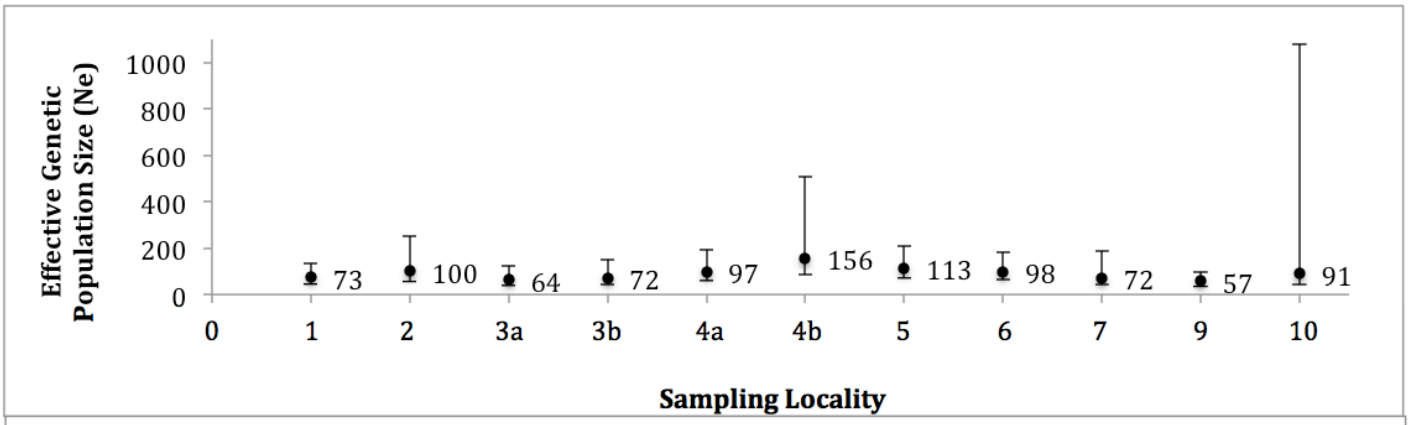


**Figure 2.** Estimates of effective genetic population size ($N_e$) for each sampling locality using the sibship estimate method in COLONY, New Jersey (8) has been left out as a low sample size (N=11) contributed to upper bounds of ∞

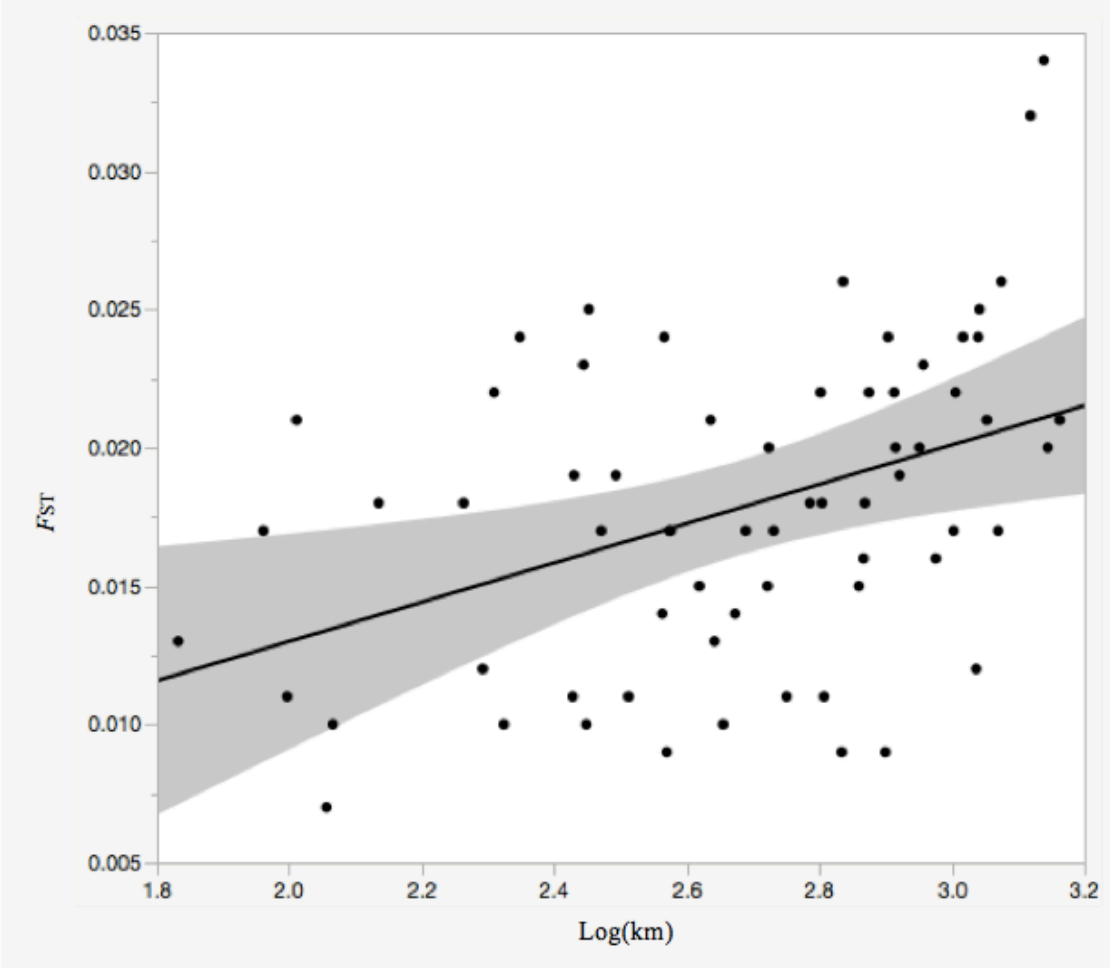


**Figure 3.** Isolation by Distance across all sampling localities. Plot shows genetic distance ($F_{ST}$) vs. log(geographic distance) calculated in IBD (Bohonak 2002) and visualized in JMP Pro 12.

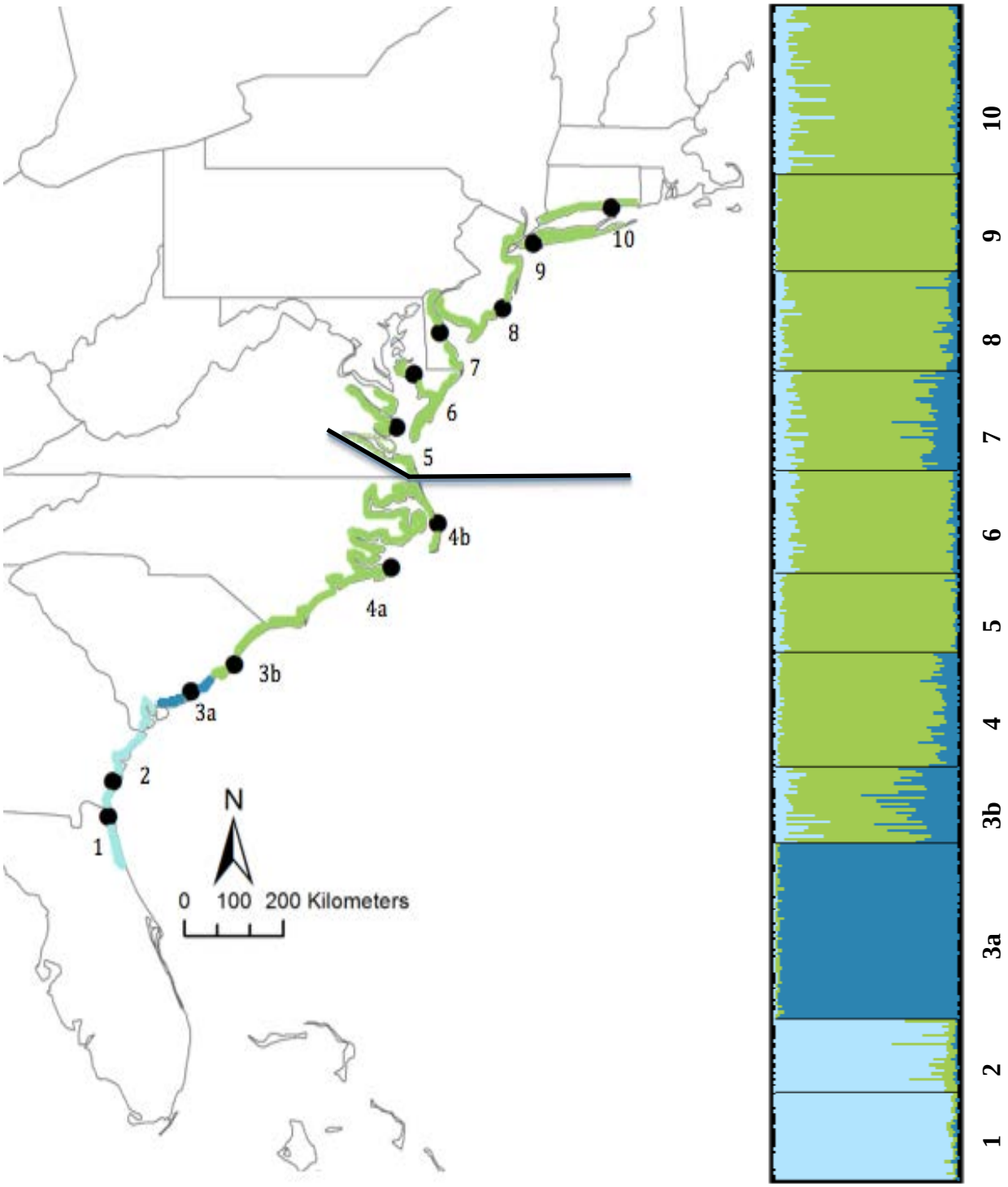


**Figure 4.** STRUCTURE bar plots showing the geographic distribution of detected genetic structure along the Atlantic coast, best K of 3. Samples from Charleston, SC (3a) were divided into groups based on collection year (2000 and 2017).

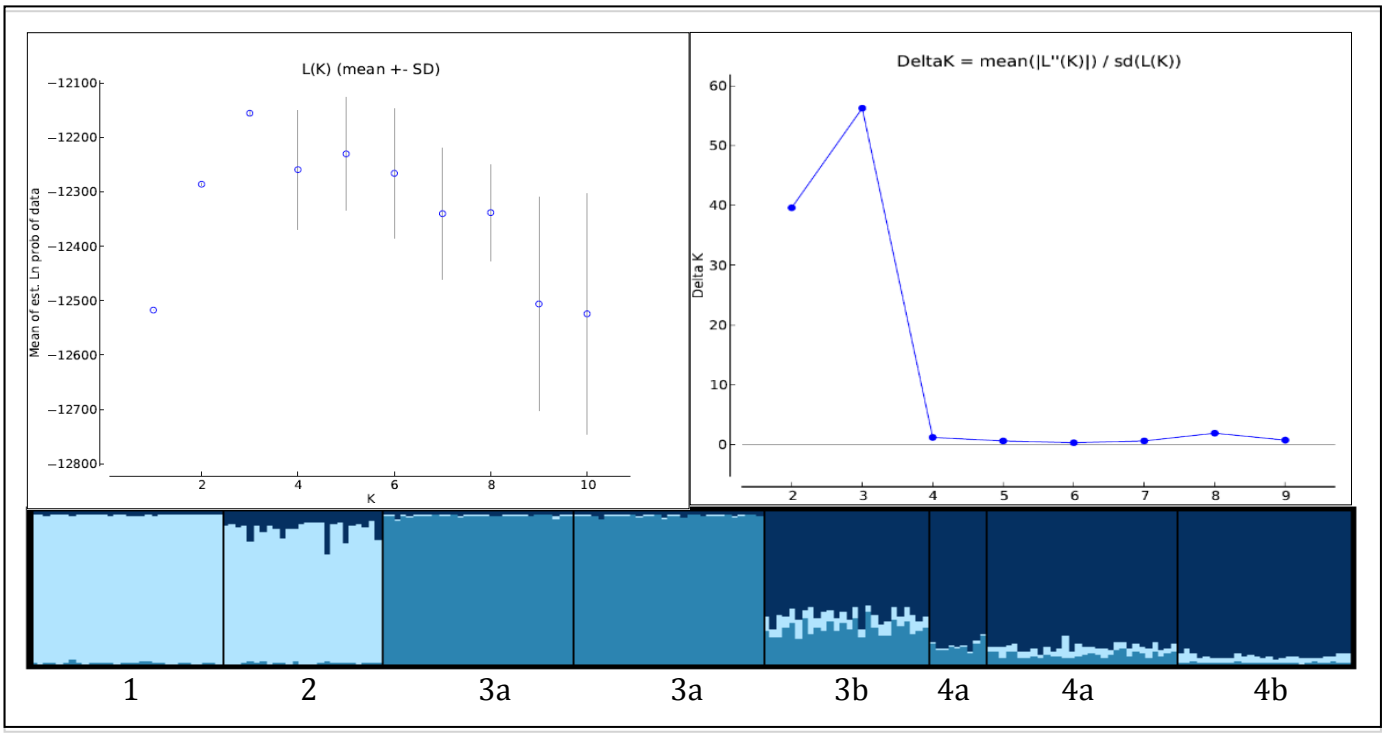


**Figure 5.** Maximum likelihood (mean ln(k)) and Evanno (K) plots for determining best K for the putative range of MacGillivray's Seaside Sparrow with a hypothesis of K=10 from STRUCTURE and STRUCTURE HARVESTER. STRUCTURE bar plot showing the geographic distribution of detected genetic structure (best K of 3). Samples from Charleston, SC (3a) and Carteret County North Carolina (4a) were divided into groups based on collection year (2000 and 2017, and 2003/2005 and 2017 respectively).

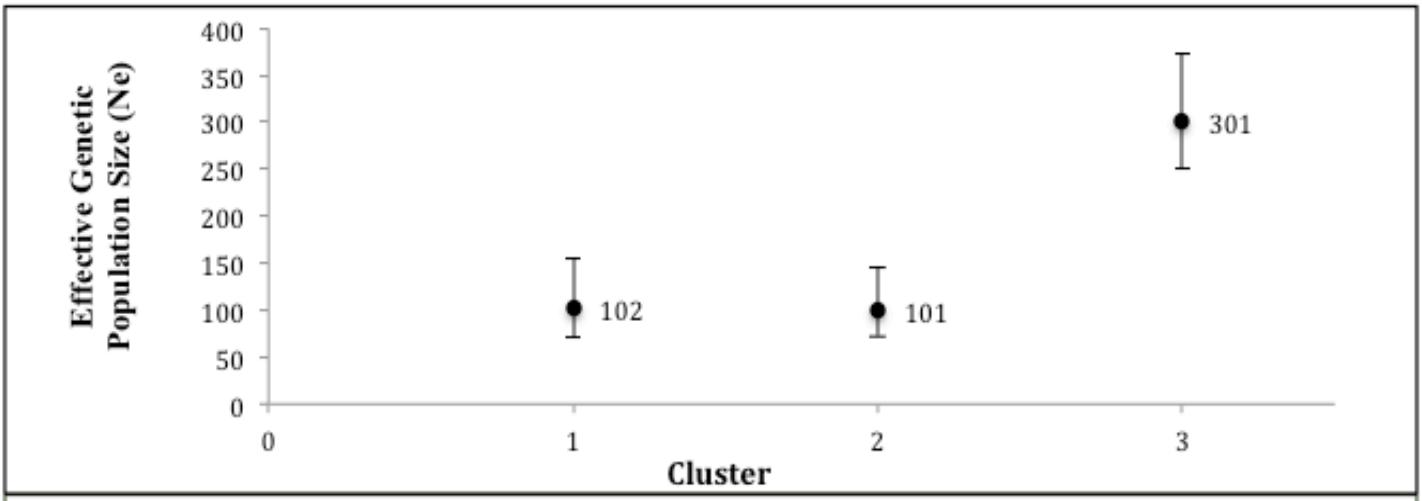


**Figure 6.** Effective genetic population size ($N_e$) for the 3 genetically distinct population segments calculated with the sib-ship assignment method in COLONY. Cluster one includes samples from Florida and Georgia, Cluster two includes Charleston, South Carolina, and cluster 4 covers the remaining localities north of Charleston to Connecticut. N = 30 each to minimize effects of sample size.

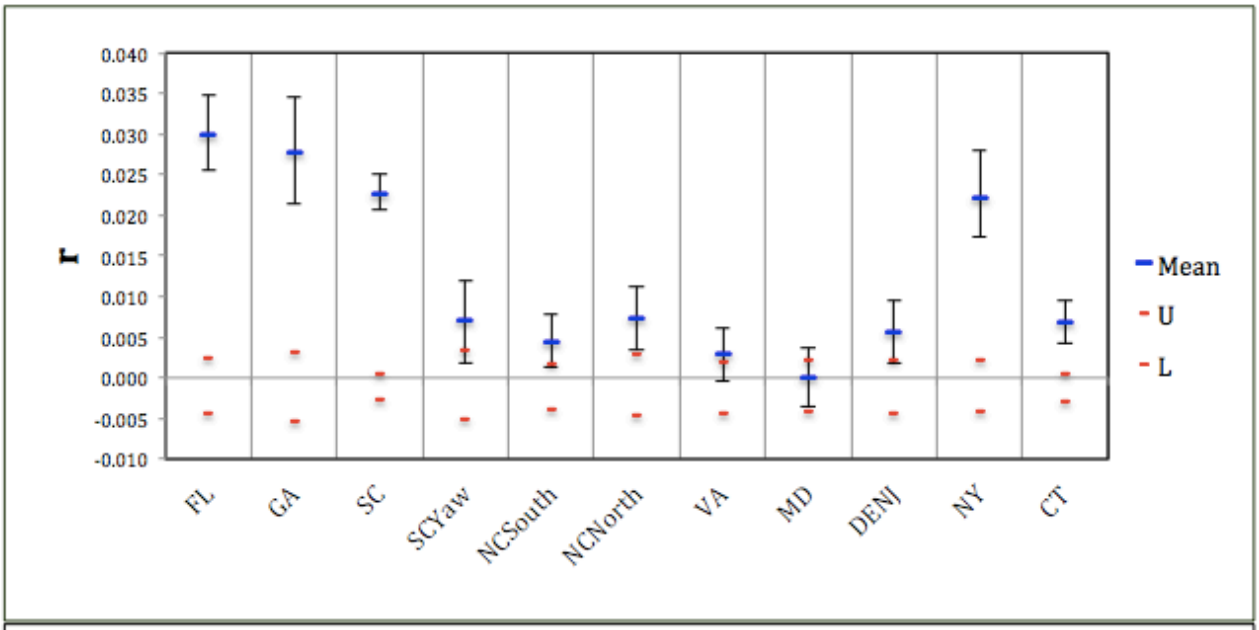


**Figure 7.** Estimates of mean pairwise relatedness within populations, calculated in GenAlEx. NJ samples were included into the DE population due to small sample size. The null line (0.000) indicates no relatedness in the sample; the red bars are the confidence intervals of the null.

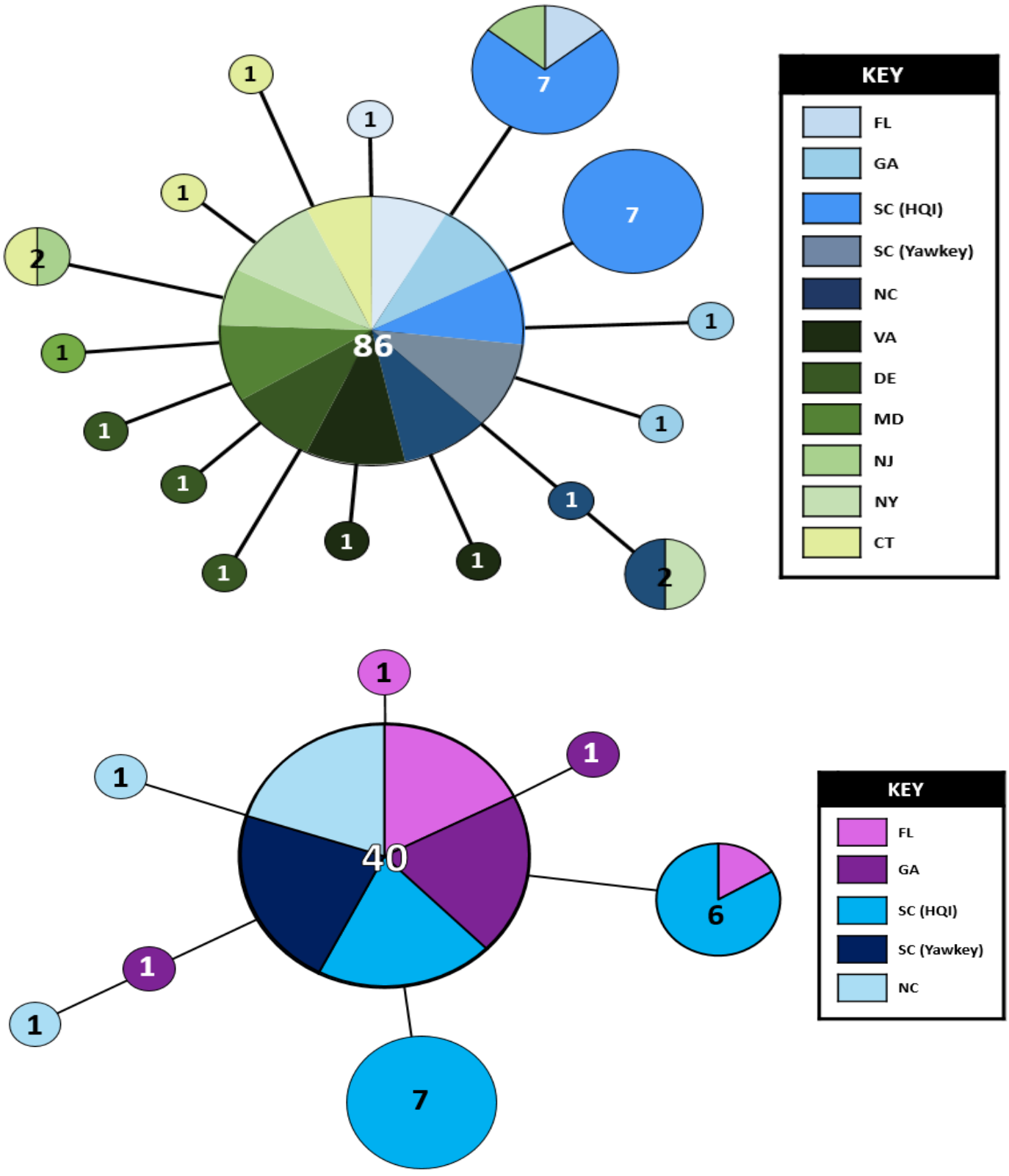


**Figure 8.** Haplotype frequency networks for mitochondrial ND2. The top network is for the entire set of sequenced samples along the Atlantic coast, the bottom network is for the described range of MacGillivray's Seaside Sparrow (FL – NC).