EVIDENCE FOR HIERARCHICAL STRUCTURING AND LARGE-SCALE CONNECTIVITY IN EASTERN PACIFIC OLIVE RIDLEY SEA TURTLES (LEPIDOCHELYS OLIVACEA)

by

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ABSTRACT

Author: Silver-Gorges, Ian, M. MS
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Title: Evidence for Hierarchical Structuring and Large-Scale Connectivity in Eastern Pacific Olive Ridley Sea Turtles (Lepidochelys Olivacea)
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Inferring genetic population structure in endangered, highly migratory species such as sea turtles is a necessary but difficult task in order to design conservation and management plans. Genetically discrete populations are not obvious in highly migratory species, yet require unique conservation planning due to unique spatial and behavioral life-history characteristics. Population structure may be inferred using slowly evolving mitochondrial DNA (mtDNA), but some populations may have diverged recently and are difficult to detect using mtDNA. In these cases, rapidly evolving nuclear microsatellites may better elucidate population structuring. Bayesian inference and ordination may be useful for assigning individuals to inferred populations when populations are unknown. It is important to carefully examine population inference results to detect hierarchical population structuring, and to use multiple, mathematically diverse methods when inferring and describing population structure from genetic data.

Here I use Bayesian inference, ordination, and multiple genetic analyses to investigate population structure in Olive ridley sea turtles (ORs; *Lepidochelys olivacea*) nesting in northwestern Costa Rica (NWCR) and across the entire Eastern Tropical Pacific (ETP). Mitochondrial DNA did not show structure within NWCR, and existing data from prior studies are not appropriately published to compare NWCR to Mexican ORs. In NWCR, Bayesian inference suggested one population, but ordination suggested four moderately structured populations with high internal relatedness, and moderate to high levels of connectivity. In the ETP, Bayesian inference suggested a Mexican and Central American population, but hierarchical analysis revealed a third subpopulation within Mexico. Ordination revealed nine cryptic clusters across the ETP that primarily corresponded to Mexican and Central American populations but contained individuals from both populations, some from other, distant nesting sites. The subpopulation within Mexico was well-defined after ordination, and all clusters displayed high

internal relatedness and moderate genetic differentiation. Bottlenecks were detected in both putative populations, at seven Mexican and two Central American nesting beaches, and in six out of nine inferred clusters, including three out of four Mexican clusters. Bottleneck events may have played some role in cluster differentiation. Migration was significant from Mexico to Central America at multiple levels, but did not necessarily agree with potential migrants elucidated by ordination. Migration was generally lower between ordination-inferred clusters than between nesting sites or Bayesian-inferred clusters. Phylogenetic trees generally supported structuring by ordination, rather than by Bayesian inference. Structuring in ordination not tied to bottleneck events could be due to mating behaviors or patterns of nesting beach colonization dictated by environmental features.

In this study, ordination provided a more practical and nuanced framework for defining MUs and DIPs in ETP ORs than did STRUCTURE. This may be due to hierarchical structuring within ETP ORs that may be present in other sea turtle populations and species. In the case of ETP ORs, hierarchical structure may be an artefact of recent population bottlenecks and subsequent recolonization of nesting beaches, or due to mating at foraging grounds or along migratory routes. Bayesian inference may not be the best method for population inference in highly migratory species such as sea turtles, which have a high potential for broad scale genetic connectivity, and therefore may display hierarchical population structuring not easily related to nesting sites. Future studies, and perhaps published studies, should incorporate Bayesian inference and ordination, as well as other measures of population divergence and descriptive statistics, when searching for population structure in highly migratory species such as sea turtles.

INTRODUCTION

Sea Turtle Population Biology

Understanding the population genetics of endangered species is critical to identifying where and how many distinguishable populations there may be in a region, and can aid in developing conservation plans for those populations. For sea turtle conservation this is often done by designating Management Units (MUs), which are genetically discrete groupings of nesting assemblages (Komoroske et al. 2017). Nesting assemblages are obvious choices for defining turtle populations, as females are easily accessible for sampling as they come ashore to nest, and typically display natal homing (Lohmann et al. 2008, Lohmann et al. 2017). Defining MUs is a critical step towards effective sea turtle conservation and is continually highlighted as a priority for global sea turtle research (Hamann et al. 2010, Rees et al. 2016).

Six out of the seven species of sea turtles are listed as threatened or endangered by the International Union for the Conservation of Nature (IUCN; IUCN 2018). These species are distributed globally, and have faced intense pressure from habitat loss due to anthropogenic development and abiotic alteration as a result of climate change. Populations are also impacted by indirect and direct take from fisheries operations, as well as by legal and illegal poaching of eggs and adult turtles for consumption and decorative products (Valverde et al. 2012, Foran and Ray 2016). In addition, sea turtles undertake extensive developmental and seasonal migrations between breeding and foraging grounds (Plotkin 2003, Broderick et al. 2007, Shillinger et al. 2008) that expose them to additional risk factors. For example, these migrations may put turtles at risk of higher annual mortality due to spatial overlap with anthropogenic activities (Hart et al. 2014, Vander Zanden et al. 2016). Increased exposure to risk factors complicates efforts to define MUs, as genetically related turtles may be found at opposite ends of an ocean basin (Bowen et al. 1997). Sea turtles in different locations require unique management plans, but genetically related, regionally-defined populations (such as ontogenetically discrete assemblages, related breeding or foraging assemblages, as well as distant but genetically similar nesting assemblages) necessitate management plans to account for this genetic connectivity.

Sea Turtle Population Genetics

Mitochondrial DNA

Genetic analyses of mitochondrial DNA (mtDNA) and highly polymorphic regions in nuclear DNA (nDNA) have allowed researchers to designate more informative MUs that capture much of the genetic variation within a species regionally and globally (Bowen et al. 2007, Komoroske et al. 2017). Research focusing on the D-loop of mtDNA has been integral towards this end (Bowen et al. 2007). Sequences (haplotypes) from the D-loop of mtDNA diverge on a scale of tens of thousands to millions of years in Testudines (Avise et al. 1992). Sea turtles typically share mtDNA D-loop haplotypes within regions (such as isolated islands or the northern and southern areas of an ocean basin; Bowen et al. 2007). Previously, studies utilized ~400 basepair (bp) mtDNA D-loop sequences to characterize sea turtle populations until 2006, when oligonucleotide primers were reported that allowed for the amplification of ~800bp sequences (Abreu-Grobois et al. 2006). While the ~400bp sequences capture many of the variable sites within the D-loop, the ~800bp sequences overlap the ~400bp sequences and help to further refine the relationships between populations. Haplotype sequences are made publicly available on Genbank (www.ncbi.nlm.nih.gov/genbank/) at the time of publication, allowing researchers to compare haplotypes between populations to determine population structure. mtDNA D-loop haplotypes (based on both the ~400bp and ~800bp sequences) and haplotype frequencies have been used extensively to delineate MUs in most sea turtle species (Bowen et al. 2007).

Nuclear DNA

Codominant, repetitive, hypervariable markers in nDNA termed microsatellites, have also been used to characterize population structure in sea turtles. Microsatellites are more likely to vary between individuals than are D-loop sequences, but certain alleles may be conserved within reproductively isolated MUs (Komorske et al. 2017). Additionally, microsatellite alleles are passed down by both males and females to offspring in diploid species. Thus, an informative panel of microsatellites may capture divergences that are too recent to be represented by D-loop sequences or that are occurring in near-panmictic populations (i.e. Rodriguez-Zarate et al. 2018) amd illustrate male-mediated gene-flow (i.e. FitzSimmons et al. 1997, Roberts et al. 2004).

nDNA can also used to conduct bottleneck, migration, and kinship analyses to better understand the factors influencing the formation of MUs (see Blouin et al. 2003, Putman and Carbone 2014 for reviews of these analyses). However, microsatellite allele lengths (in bps; the metric measured to genotype individuals) are sensitive to polymerase chain reaction (PCR) temperatures and variation in electrophoresis conditions, making it difficult to directly compare among studies.

Genetic Analyses in Population Biology

F and F-like Statistics and Analysis of Molecular Variance

mtDNA sequences and nDNA genotypes may be subjected to mathematical equations and models to characterize and understand population structure. These analyses ultimately can be used to define MUs. F-statistics and F-like statistics (*F*st and *F*is, **Θ**st, D; Wright 1949, Weir and Cockerham 1984, Jost 2008, respectively) and Analysis of Molecular Variance (AMOVA; Excoffier et al. 1992) are commonly used to identify MUs with significantly different haplotype and allele frequencies. These metrics can also provide a quantitative measure of the strength of population structuring (*F*st) and even inbreeding (*F*is).

Bottleneck Analysis

It is also possible to investigate evolutionary processes that form MUs. For example, population bottlenecks may occur when populations are reduced dramatically in size. Globally, many sea turtle populations have experienced bottlenecks after reaching historic minima in the mid-20th century (see Jensen et al. 2013A and references therein). Populations now recovering from bottleneck events are expected to have relatively low genetic diversity (Bouzat 2010 and references therein), which may have consequences for population resilience (Keller et al. 1994, Frankham et al. 1999, Eldridge et al. 1999). The genetic signatures left by bottleneck events may be identified using metrics and likelihood functions that analyze mtDNA (D; Tajima 1989) and nDNA (M-ratio: Garza and Williamson 2001; BOTTLENECK: Piry et al. 1999, Cornuet and Luikart, 1996). However, methods for detecting bottlenecks using nDNA data have come under criticism for their sensitivity to the fit of the data to specific mutational models and for their sensitivity to specific input parameters (Cornuet and Luikart 1996, Leblois et al. 2006, Peery et

al. 2012). Despite this, such metrics may be useful for understanding population structure if properly calculated and interpreted (Putman and Carbone 2014). Geographically discrete bottleneck signatures, such as those present at only some nesting sites within an ocean basin, may identify recent and ongoing take of turtles (i.e. fisheries bycatch, poaching of eggs and adults on beaches) that can be addressed in policy and management plans.

Relatedness Analysis

Relatedness (r) may also be informative towards population structure (Blouin 2003). Relatedness is measured on a scale from 0 to 1 for any two individuals based on how much of their genome is shared (0 being none, 1 being all). In a diploid family, meiosis occurs between each "generation link" (L), and the probability of a copy of a gene being passed on from one individual to another is 0.5 (Davies et al. 2012). The relatedness coefficient between any two individuals is then the sum of the probability of a gene being passed down for all of the links between the two individuals, or

$$r=\sum_p(0.5)^L$$

where p is the number of paths between two individuals and L is each link between two individuals within one path. For example, a parent has a 50% chance of passing a copy of any gene to its offspring. r for parent-offspring (po) relationships is:

$$r_{po}=0.5^1= 0.5$$

There is only one path between the two individuals, and there is only one link. If that offspring has a full sibling, there are two links between the siblings (offspring to parent, parent to sibling), and two paths connecting the siblings (one through each parent). Therefore:

$$r_{sib}=0.5^2+0.5^2= 0.5$$

In practice, estimating relatedness coefficients from genotypic data is not based on categorical relationships between individuals, but on the percentage of the genome (based on shared alleles) that is identical by descent. Lynch and Ritland (1999) and Queller and Goodnight (1989) developed algorithms to estimate relatedness between individuals based on genotypes and allele frequencies. While these estimators are sensitive to the number of loci used and the number of sampled related individuals, and are not always in complete agreement (Wang 2002, Blouin 2003), on a population scale they may provide a sense of structuring and can complement F-statistics (Queller and Goodnight 1989).

Sea turtles are known to display natal homing during breeding seasons (Lohmann et al. 2017) and therefore turtles at one nesting beach, or within a certain nesting area, may be more related to each other than to turtles that nest further away. However, relatedness patterns may also be influenced by mating behavior at nesting aggregations (such as arribadas) or by mating away from nesting beaches (i.e. at foraging grounds). Related groups of turtles are expected within nesting assemblages, but related groups that do not share nesting beaches may suggest that other areas are used for mating, such as foraging grounds and migratory routes. These areas would gain importance as areas to protect to maintain the genetic diversity within and between MUs. Related groups that do not correspond to nesting regions may also suggest that other environmental or endogenous factors are affecting dispersal from and recruitment to nesting assemblages.

Migration Analysis

Migration, or the transition of individuals from one population to another geographically separate population, may also shape and affect population structure (Putman and Carbone 2014, Sundqvist et al. 2016 and references therein). Migration may erode population structure if individuals consistently move between populations, and may even lead to misleading estimates of population differentiation (Dias 1996). This is particularly true for asymmetric migration, in which individuals move more frequently from some populations to other populations, rather than evenly between populations. In some cases, asymmetric migration may produce misleading signatures of population differentiation (i.e. Whitlock and Mcauley, 1996) that can lead to misdirected management efforts if not properly identified (Dias 1996, Pringle et al. 2011).

Software and metrics that estimate migration based on genetic data are often subject to internal assumptions of loci being in linkage equilibrium or low levels of migration between populations with high levels of differentiation (i.e. BayesAss; Wilson and Rannala 2003, Faubet et al. 2007). Some analyses only measure migration within one generation (i.e. GeneClass; Piry et al. 2004), and/or assume symmetric migration (i.e. Gst: Nei 1973; G'st: Hedrick 2005, and D: Jost 2008). Such analyses may not be appropriate for species such as sea turtles, which are already highly migratory (in a spatial sense of the term) and long lived (and therefore are likely to have overlapping generations). Further, asymmetric migration is known in marine species with environmentally driven dispersal of neonates and juveniles (Siegel et al. 2003, Cowen and

Sponaugle 2009). For example, sea turtles display current driven dispersal as hatchlings (Hays et al. 2010), and adult movements may even be constrained or influenced by environmental features (i.e. Rodriguez-Zarate et al. 2018). Analyzing asymmetric migration (i.e. in divMigrate; Sundqvist et al. 2016) is not a well-tested practice. While this analysis may be inaccurate with high levels of migration (Sundqvist et al. 2016), it should prove useful to understanding sea turtle population structure and designating effective MUs.

Genetic Distance Analysis

Identifying the factors that lead to the formation of MUs may further validate putative MUs, and provide valuable information towards conservation of MUs. However, MUs must be identified before population structure may be characterized and investigated. Identifying MUs ad-hoc is not always straight-forward. For sea turtles, one MU may span multiple nesting beaches and show connectivity to distant foraging grounds. Differences between nesting turtles from different ocean basins may be empirically hypothesized and tested, but it is not so simple to identify genetic boundaries when looking within a region (such as an ocean basin). Constructing exploratory phylogenetic trees of individuals or sampling sites may provide a basis for MU designation, but are sensitive to the tree-building method used and require prior designation of sub-populations (Putman and Carbone 2014).

Population Inference

Bayesian Inference

STRUCTURE (Pritchard et al. 2000) is a canonical program for investigating population structure. STRUCTURE uses Bayesian inference to identify the most likely partitions in genetic data sets (K), but is processing-heavy and subject to assumptions of Hardy-Weinberg equilibrium that may reduce the informative power of a data set (Putman and Carbone 2014). Hierarchical analyses are often necessary to elucidate the true population structure, as sub-structuring may be obscured by broader structuring (Kalinowksi 2011, Putman and Carbone 2014). STRUCTURE has been shown to incorrectly identify K based on its internal ad-hoc likelihood estimator (Pritchard et al. 2000, Evanno et al. 2005), and is subject to sampling bias when populations are not evenly samples (Peuchmaille 2016). STRUCTURE may also be inherently biased towards

K=2 (Janes et al. 2017). These limitations can be ameliorated to an extent using post-processing algorithms such as those included in Structure Harvester (Earl 2012), but these programs still rely on the STRUCTURE output to determine the most likely number of genetic groupings.

Ordination

Thus, it is important to compare results from multiple different clustering algorithms. Ordination of genetic data, in which individuals are plotted as points on a 2-dimensional plane, does not rely upon the same assumptions as Bayesian algorithms nor does it require as much processing power. Common ordination methods include Principal Component Analysis (PCA; in which axes are constructed to maximize variance among data) and Principal Coordinate Analysis (PCoA; in which the data are projected onto a coordinate plane to maximize dissimilarities between points). However, PCA assumes that variance among points will be homogenous, which is not often true for genetic data sets, and PCoA requires the correct specification of a mutational model to accurately infer true differences between points, which is difficult with datasets that do not conform nicely to any common mutational models (Jombart et al. 2009, Odong et al. 2013, Putman and Carbone 2014).

The R package *adegenet* (Jombart 2008, Jombart and Ahmed 2011) implements a method termed Discriminant Analysis of Principal Components (DAPC, Jombart et al. 2010) which plots individuals in a coordinate plane based on genotypic data, uses a k-means algorithm to identify the most likely *K*, and then calculates axes to maximize distance between clusters and minimize variance within clusters. DAPC may outperform STRUCTURE when hierarchical structuring is present in a dataset (Jombart et al. 2010), however the limitations of DAPC and ordination in general are not well known (Putman and Carbone 2014). Selecting the number of axes retained to plot individuals and the most likely *K* are left up to the user and may therefore introduce bias into an analysis (Putman and Carbone 2014). Despite the disadvantages and differences of these exploratory methods, comparing their results on a single dataset can increase confidence in identifying true population structure present within a dataset, and can ultimately lead to more effective MU designations.

Olive Ridley Sea Turtles

Global Population Biology

MUs are not well defined for Olive ridley (OR) sea turtles (*Lepidochelys olivacea*). ORs are the most abundant sea turtle and are not listed as endangered (Abreu-Grobois & Plotkin 2012). However they are subject to illegal and legal take of eggs (Valverde et al. 2012), and constitute a large proportion of fishers bycatch (Moore et al. 2009). Researchers have documented decades-long declines in the number of individuals participating in mass nesting events termed "arribadas" (Fonseca et al. 2009). Despite this, OR population structure has not been comprehensively studied and MUs remain largely undefined.

ORs may be divided into broad, genetically distinct regional populations based on mtDNA in the Atlantic Ocean (ATL; Bowen et al. 1997, Plot et al. 2012), the Indo-Pacific region (IP; Bowen et al. 1997, Jensen et al. 2013B, Bahri et al. 2018), India and Sri Lanka (IND; Bowen et al. 1997, Shanker et al. 2004B), and the Eastern Tropical Pacific Ocean (ETP; Bowen et al. 1997, Lopez-Castro & Rocha-Olivares 2005, Cortes et al. 2015). The ATL population of Olive ridleys is the smallest (Bowen et al. 1997, Plot et al. 2012). Only turtles nesting at Guinea Bissau have been systematically sampled and had mtDNA haplotypes sequenced at ~800bps (Plot et al. 2012). The IP population does not host any arribada beaches but is widespread across northern Australia, the southeast Asian island, and the Asian and continent (Bowen et al. 1997, Jensen et al. 2013B, Bahri et al. 2018). Several ~800bp mtDNA sequences are available from Indonesia, Papua New Guinea, and Australia, but only Australian Olive ridleys have been studied with an intent towards designating MUs (Jensen et al. 2013B). The IND population is large and has faced intense anthropogenic pressure (Shanker et al. 2004A). Haplotypes from this region share a 7bp deletion with Kemps ridley sea turtles (*Lepidochelys kempii*) and are thought to be basal for the species (Bowen et al. 1997, Shanker et al. 2004B). However, mtDNA sequences are only available at ~400bps from this population.

Eastern Pacific Population Biology

The ETP population is robust, with multiple arribada nesting sites and high-density solitary nesting sites, but seemingly minimal structuring among continental sites (Lopez-Castro and Rocha-Olivares 2005, Valverde et al. 2012). In accordance with this, phylogeographic

studies of ORs have suggested that the EP population is no more than 250,000-300,000 years old (Bowen et al. 1997, Jensen et al. 2013B). Analyses of mtDNA and nDNA data suggest that Olive ridleys nesting on the Baja Peninsula (Mexico) may comprise a discrete MU (Lopez-Castro & Rocha-Olivares 2005, Rodriguez-Zarate et al. 2013). nDNA has shown that arribada and non-arribada ORs in Costa Rica do not comprise discrete populations (Jensen et al. 2006, Koval 2015). Koval (2015) analyzed microsatellite data from solitary and arribada ORs nesting at three sites in Northwestern Costa Rica (NWCR; Playa Grande, Playa Ostional, and Playa Nancite) using STRUCTURE and DAPC. STRUCTURE suggested all nesting sites and behaviors comprised one population (in accordance with Jensen et al. 2006), but DAPC identified 4 clusters comprising individuals from all nesting sites and both behaviors with moderate overall structure (Fst=0.103; Koval 2015). There was no evidence of a bottleneck event overall, within sampling sites, or within DAPC clusters (Koval 2015).

While mtDNA and nDNA data do not show obvious population structure among continental ETP nesting sites at fine scales, nDNA suggest a north-south population split across the entire ETP when analyzed in STRUCTURE (Rodriguez-Zarate et al. 2018). However, it is difficult to test for this split using mtDNA as haplotypes are not well-reported from Central America. Further, while Rodriguez-Zarate et al. (2018) found that environmental variables (namely oceanographic features) play a role in this structuring, the authors did not report hierarchical or comparative analyses for population structuring. They suggest two panmictic populations of ETP ORs, one comprising Mexican turtles and the other comprising Central American turtles.

Study Questions

In light of the recently uncovered north-south population split in ETP Olive ridleys (Rodriguez-Zarate et al. 2018), as well as the lack of a robust mtDNA data set from Central America, I sought to answer four questions in this study:

1) Would analysis of mtDNA sequence data support or refine population structure in NWCR or ETP ORs?

2) Would analysis of ETP OR nDNA elucidate substructuring within Mexican and Central American populations?

3) Would ordination (DAPC, as implemented in *adegenet*) produce cryptic clusters in both NWCR and ETP ORs?

4) What processes might be contributing to the formation of inferred populations, and which inferred populations were best supported by multiple analyses (genetic distances trees, bottleneck analysis, relatedness analysis, and migration analysis)?

METHODS

NWCR mtDNA Analysis

Blood samples and skin samples from 118 Olive ridley turtles collected in 1999 (Playa Nancite, n=7; Clusella-Trullas et al. 2006), and in 2011-2012 and 2013-2014 (Playa Ostional n=78; Playa Grande, n=33) were processed at Purdue University Fort Wayne in 2014 (Koval 2015). DNA was extracted from samples using QIAGEN DNeasy Blood and Tissue Kits following the manufacturer's protocol and frozen at -20 ºC. Samples were diluted to 25 ng μl^{-1} before PCR.

~800 bp sequences from the D-loop of the mtDNA control region were amplified for 60 turtles from Playas Nancite, Ostional, and Grande using primers LTEi9 (5'-AGCGAATAATCAAAAGAGAAGG-3') and H950 (5'-GTCTCGGATTTAGGGGTTTA-3'; Abreu-Grobois et al. 2006, Jensen et al. 2013B). PCR reactions were conducted using QIAGEN Taq PCR Master Mix Kits following the manufacturer's protocol. I used an incubation profile previously described by Jensen et al. (2013B): denaturing at 94°C for 5 minutes, then 35 cycles of 45 seconds at 94°C, 45 seconds at 52°C, and 45 seconds at 72°C, and final extension at 72°C for 5 minutes. PCR products were run on agarose gels to ascertain quality and product size.

PCR products measured to ~800 bps were purified using ExoSAP-It (Thermo) and sent to Genewiz (New Jersey, USA) for Sanger sequencing. Forward and reverse sequences were trimmed to ~400bps and ~800bps, assembled, and aligned in Geneious v.11 (Kearse et al. 2012) using the CLUSTALW algorithm (Thompson et al. 2003). ~400bp and ~800bp Olive ridley mtDNA sequences from prior studies (Bowen et al. 1997, Shanker et al. 2004B, Lopez-Castro & Rocha-Olivares 2005, Plot et al. 2012, Jensen et al. 2013B, Revuelta et al. 2015,Beltran et al. 2016, Bahri et al. 2018) were procured from Genbank and aligned with sequences from this study. New and existing haplotypes from NWCR were determined using DnaSP 6 (Rozas et al. 2017). All haplotypes were named as per National Marine Fisheries Services protocols (Peter Dutton, personal communication). New sequences (n=4) were re-sequenced for confirmation and deposited in Genbank (Accession #s MK749418, MK749419, MK749420, MK749421). Haplotype networks were generated for sequences from this study in TCS (Clement et al. 2000) and modified for publication using tcsBU (Murias dos Santos et al. 2015). Genetic diversity at all

sites was quantified by calculating mean Haplotype Diversity (*H*) and mean Nucleotide Diversity (π) in Arlequin v.3.5 (Excoffier & Lischer 2010).

Microsatellite Amplification

Amplification and characterization of microsatellite loci from ORs in NWCR (Koval 2015) and the ETP; Rodriguez-Zarate et al. 2018) are described in the original studies, respectively. NWCR data were obtained from the author (Julianne Koval, personal communication), and ETP OR data were obtained from https://doi.org/10.5061/dryad.nj344m5 (Rodriguez-Zarate et al. 2018). Data from Koval (2015) will be referred to as NWCR, and data from Rodriguez-Zarate et al. (2018) will be referred to as ETP. Abbreviations of ETP sampling sites used here may be found in the supplemental data for Rodriguez-Zarate et al. (2018).

Analyses

F and F-like Statistics and Analysis of Molecular Variance

Pairwise and overall *F*st (Wright 1949) and Φst (an *F*st estimator for mtDNA), single site *F*is (Wright 1949), pairwise θst (Weir & Cockerham 1984), Jost's D (Jost 2008), and Analysis of Molecular Variance (AMOVA; Excoffier et al. 1992) were quantified in Arlequin v. 3.5 (Excoffier and Lischer 2010) and GenAlex (Peakall and Smouse 2006) for mtDNA and nDNA (when applicable) from NWCR ORs. Alpha levels for pairwise *F*st and D were adjusted using a Bonferroni correction for multiple tests. *F*st, or Wright's Fixation Index, compares haplotype or allele frequencies between putative populations relative to the overall sample and produces a measurement of population differentiation between 0 and 1 (0 being no difference between populations; 1 being total difference between populations). *F*is (Wright 1949) is similar to *F*st, and measures the amount of genetic variation contained within individuals within subpopulations. Θst, or Weir and Cockerham's (1984) *F*st, is an estimator of Wright's *F*st for mtDNA data that takes the number of haplotypes and sample size into account. D (Jost 2008) measures the fraction of allelic variation among populations and is thought to be more appropriate for analyzing microsatellite data than *F*st (Putman and Carbone 2014). AMOVA is similar to conventional ANOVAs, but uses the sum of square molecular distances between haplotypes or alleles within and between populations to assess population differentiation.

Population Inference

STRUCTURE (Pritchard et al. 2000) and *adegenet* (Jombart et al. 2008) were used to assign individuals to putative MUs (*K*) based on microsatellite genotypes. *K* represents the number of inferred genetically discrete populations; whether each inferred population represents an MU is explored in the discussion. Initial STRUCTURE parameters and results are reported in Koval (2015; NWCR) and Rodriguez-Zarate et al. (2018; ETP). I analyzed Mexican and Central American population identified by Rodriguez-Zarate et al. (2018) for internal structuring. STRUCTURE was run 10 times for each *K*=1-15, with a burn-in of 50,000 generations and an MCMC of 100,000 generations for Mexican and Central American populations. Each run assumed correlated allele frequencies (Falush *et al.* 2003) and historical admixture between populations (Pritchard *et al.* 2000). Runs were repeated with and without sampling location as a prior (LOCPRIOR; Hubisz et al. 2009). STRUCTURE was run hierarchically until the most likely *K*=1 (see below).

STRUCTURE output files were analyzed in STRUCTURE HARVESTER v.0.6.92 (Earl 2012) to determine the true number of *K* using multiple metrics. The estimated log probability of data pr(X|*K*) given a particular value of *K* allows the estimation of the most likely number of clusters (Pritchard *et al.* 2000). The ad hoc delta-*K* method (Evanno *et al.* 2005) reports the second-order rate of change of the log probability of data regarding the number of clusters, which typically peaks at the appropriate value of *K*. The admixture model calculates the fractional probability (*Q*) of individuals belonging to each population.

A Discriminant Analysis of Principal Components (DAPC; Jombart et al. 2010) was run for both data sets using *adegenet* (Jombart et al. 2008) as implemented in R. Genotype data were transformed into a coordinate format for Principal Coordinate Analysis (PCA). The most likely number of clusters was determined using k-means clustering and a Bayesian Information Criterion (BIC), and all principal components (PCs) were retained. A Discriminant Analysis (DA) was run on the PCA to maximize separation between groups. As suggested by Jombart et al. (2010), 100% of the initial PCs were retained when identifying *K*, ~80% of PCs were retained during discriminant analysis, and the first n≤10 axes of the discriminant analysis were retained. DAPC was also run by sampling site for both data sets to examine geographic population structuring. Each DAPC was cross-validated and re-run with suggested PCs to minimize error (Jombart et al. 2010).

STRUCTURE and *adegenet K* assignments were tested for population structure (*F*st and *F*is within AMOVA in Arlequin; Pairwise *F*st and D in GenAlex), genetic distances in Neighbor-Joining trees (Saitou and Nei 1987), bottleneck signatures (BOTTLENECK; Cornuet and Luikart 1996, Piry et al. 1999), relatedness (LRM: Lynch and Ritland 1999; and QGM: Queller and Goodnight 1986, in GenAlex), and differential migration (divMigrate, Sundqvist et al. 2016). *F*st, AMOVA, and D were calculated over 10,000 bootstrap replicates.

Genetic Distance Analysis

Neighbor-joining (NJ; Saitou and Nei 1987) distance trees were constructed for all individuals, sampling sites, and DAPC clusters using Nei et al.'s (1983) Da with 1000 bootstrap replicates as implemented in the program *Populations 1.2.30* (http://bioinformatics.org/populations/; Olivier Langella, 1999). NJ trees are constructed by pairing the least genetically distant individuals or units at the tips of the tree, and working backwards to build the branches and base of the tree (Saitou and Nei 1983). *Populations* offers many options for calculating distances between individuals and populations, each of which vary slightly (see http://bioinformatics.org/populations/). I chose Da (Nei et al. 1983) as a distance measure in part because of a potential bug in *Populations* that prevented the completion of trees based on other distance measures, and in part because Da is thought to produce more accurate topologies than other distance measures (see Nei et al. 1983). NJ trees are not as robust as maximum likelihood or Bayesian methods for inferring ancestral (i.e. population of origin) relationships between individuals and populations (Putman and Carbone, 2014), but may prove useful as an exploratory method for further assessing and comparing population structuring inferred by other methods.

Bottleneck Analysis

Bottleneck analysis was conducted for sampling sites and all inferred populations (via STRUCTURE and DAPC) in BOTTLENECK (Piry et al. 1999). BOTTLENECK was run for 10,000 iterations of the two-phase mutation model (TPM; DiRienzo et al. 1994) as suggested for microsatellite data by Piry (1999; 95% Stepwise Mutation Model [SMM; Ohta and Kimura 1973] in the TPM with a variance of 12). BOTTLENECK was also run with TPM settings suggested by Piry et al. in web documentation for the program

(http://www1.montpellier.inra.fr/CBGP/software/Bottleneck/pub.html; 0% SMM in the TPM and 36 variance). In the SMM, microsatellites are only modeled to mutate by one repeat length per mutational event, while in the TPM they may mutate by one or more repeats per mutational event. The TPM is thought to be more representative of actual processes of mutation and evolution than the SMM (DiRienzo et al. 1994, Piry et al. 1999). The TPM is generally more conservative than the SMM in inferring bottleneck events, as alleles that differ by more than one repeat still have a probability of coming from one mutational event, rather than multiple mutational events (Sainudiin et al. 2004).

Relatedness Analysis

Pairwise relatedness values between individuals were calculated over 10000 iterations using estimators designed by Ritland and Lynch (1999) and Queller and Goodnight (1989) as implemented in GenAlex (Peakall and Smouse 2006). Mean pairwise relatedness was then examined relative to overall relatedness for sampling sites and all inferred populations (via STRUCTURE and DAPC). Queller and Goodnight's (QGM; 1989) estimator is a coefficient based only on estimated identity by descent (IBD; Grafen 1985). Ritland and Lynch's (LRM; 1999) estimator uses a regression calculation to determine relatedness coefficients for any pair of individuals based on shared IBD alleles, but can perform poorly if few related individuals are sampled, or if loci are too highly polymorphic (Blouin 2003). Both estimators may also have high variances when few loci ($n<20$) are used, but, as mentioned above, can provide a good estimation of relatedness between groups of individuals (Queller and Goodnight 1989, Blouin 2003).

Migration Analysis

divMigrate (Sundqvist et al. 2016) was used to calculate differential migration between all sampling sites, STRUCTURE clusters, and DAPC clusters from both data sets. divMigrate estimates directional migration based on one of three population metrics specified by the user: Nm (Alcala et al. 2014), Gst (Hedrick and Goodnight 2005) and D (Jost 2008). In idealized asymmetric migration from one population (A) to another (B), population B is expected to share alleles with population A in the same proportions (albeit lower frequencies) as they exist in population A, and may also contain alleles not found in population A.

divMigrate uses the genetic distance measures mentioned above and allele frequencies to calculate scaled, relative migration between two populations on a 0 to 1 scale based on this theory of the genetic signature of migration. divMigrate is known to incorrectly identify migration at high levels of migration and low (n<~50) sample sizes (Sundqvist et al. 2016), and each estimator performs differently. Gst is thought to perform best at accurately identifying differential migration relative to Nm and D (Sundqvist et al. 2016), but I chose to run divMigrate with all three parameters for 1000 bootstrap replicates each to avoid biases from any individual estimator.

RESULTS

Mitochondrial DNA Analysis

I observed ten ~800bp haplotypes from 60 turtles nesting at Playa Nancite (n=7), Playa Grande (n=17), and Playa Ostional (n=36; Table 1; Figures 1&2). Haplotypes are named sequentially as per National Marine Fisheries Service conventions (Peter Dutton, personal communication). Overall haplotype diversity (*H*=0.5657±0.0783 SD) and nucleotide diversity (π=0.0014±0.0012 SD) were comparable to those reported in other studies of Pacific Olive ridleys (i.e. Jensen et al. 2013B, Lopez-Castro & Rocha-Olivares 2005) and did not vary between sites (as suggested by overlapping standard deviations; Table 1).

There was no evidence that any of the three sites were genetically distinct from each other as determined by pairwise Φst (Φst= -0.05 ± 0.00021, p = 0.37 - 0.79), pairwise θst (θst = -0.03 ± 0.02, p = 0.61 - 0.79), and AMOVA (*F*st=-0.02, p=0.65). I attempted to compare haplotype frequencies from this study to those reported from previous studies on EP Olive ridley mtDNA sequences (i.e. Lopez-Castro & Rocha-Olivares 2005) but were unable to accomplish this analysis due to the lack of a consistent, systematic nomenclature for Olive ridley haplotypes (Peter Dutton, personal communication). However, Lo46 comprised 68% of the haplotypes I found, which is consistent with (albeit lower than) Lopez-Castro and Rocha-Olivares' (2005) findings from Mexican Olive ridleys (~90%) and suggests a lack of population differentiation between Mexican nesting assemblages and these Costa Rican nesting assemblages.

Summary of Nuclear DNA Results from Previous Studies

Summary and descriptive statistics for microsatellite data from NWCR and the ETP, as well as initial STRUCTURE and DAPC results, are reported in the original studies: Koval (2015) and Rodriguez-Zarate et al. (2018). Briefly, Koval (2015) found that *K*=1 in NWCR ORs, and Rodriguez-Zarate et al. (2018) found that *K*=2 in ETP ORs, with a division between Mexican and Central American ORs (AMOVA; *F*ct=0.028). Koval (2015) also conducted DAPC and found 4 clusters with moderate differentiation (*F*st=0.103) that each contained arribada and solitary nesting individuals from all three sampling sites. Koval (2015) did not find evidence for

bottleneck events. Pairwise *F*st, pairwise D, and AMOVA are reported for ETP ORs in Rodriguez-Zarate et al. (2018).

Population Inference in ETP ORs

Hierarchical analysis in STRUCTURE with location as a prior (locprior) weakly suggested a putative subpopulation in Mexico consisting primarily of turtles nesting at PAR (Figure 3A). AMOVA and pairwise *F*st confirmed significant, yet moderate structuring between PAR and other Mexican sites (AMOVA: *F*st=0.067, p<0.001; Pairwise *F*st: *F*st=0.066, p<0.001). Hierarchical analysis of STRUCTURE in Mexico without locprior, as well as in Central America with and without locprior were unable to discern obvious structure (Figure 3A, B). DAPC by nesting site confirmed STRUCTURE results: Mexican and Central American nesting beaches split along the first axis, PAR separated from other Mexican nesting beaches, and the Central American nesting beaches showed admixture (Figure 4). However, assignment proportions were not high (mean=65.3±0.002SE; Figure 4).

DAPC by inferred clusters produced similar results to the same analysis run on NWCR ORs. DAPC elucidated 9 discrete clusters with high assignment proportions (mean=0.99+-0.003SE; Figure 4). Clusters largely aligned with Mexican and Central American populations, but contained individuals from multiple nesting sites, some as distant as ~3500km as the crow flies (i.e. BCS and PMA) within both putative populations (Figure 4). AMOVA showed that DAPC clusters were moderately differentiated from each other (Variance Explained=10.37%, Fst=0.103, p<0.001; Table 2) and pairwise *F*st (0.037-0.091) and D (0.111-0.507) confirmed that all clusters were significantly different from one another (Table 3). *F*is indicated significant, moderate inbreeding in all primarily Mexican clusters (1,5,8,9) and one primarily Central American cluster (6; Table 4).

I removed individuals assigned to one highly differentiated cluster (#8 in Figure 4) and re-ran DAPC on the remaining individuals, which highlighted separation between the remaining inferred clusters (Figure 5). Hierarchical discriminate analysis of Mexican and Central American subpopulations further highlighted discrete clusters within each subpopulation that largely corresponded to clusters in the ETP-wide DAPC (Figure 6, Table 5).

Genetic Distance Analysis

The NJ trees confirmed elements of STRUCTURE and DAPC analyses. I compared Nei et al.'s (1983) Da to Latter's (1972) Fst in a NJ tree of NWCR individuals (Figure 10), to determine if different distance calculations would affect general tree topology. Trees constructed with Da (Nei et al. 1983) and *F*st (Latter 1972) agreed on general structuring (Figure 10) and here I discuss trees constructed with Da. I caution that these trees are not intended to be their own analyses of population structuring, but rather should be used to supplement analyses in STRUCTURE and *adegenet*. Trees were left unrooted, as I did not intend to determine evolutionary relationships between populations.

The NJ tree of NWCR individuals indicated weak overall structuring as indicated by near 0 bootstrap values at the base of the tree, although individuals from the same DAPC clusters, rather than from sites or behaviors, tended to be grouped together with higher confidence towards the tips of the tree (Figure 10). The NJ tree of CR sites indicated weak but persistent separation between sites and behaviors as indicated by branch lengths and bootstrap values (Figure 11). Playa Nancite was more distant from other sites and behaviors, but this should be interpreted cautiously for reasons mentioned above. The NJ tree of DAPC clusters showed more persistent separation than the NJ tree of sites, as indicated by higher bootstrap values at nodes (Figure 11).

The NJ tree of ETP individuals showed weak ETP-wide structuring, but individuals grouped into clades largely consistent with DAPC clusters with slightly higher bootstrap values than at the base of the tree (Figure 12). The NJ tree constructed with nesting sites as populations showed a Mexican and Central American split, and highlighted structure among Mexican sites and the lack of structure among Central American sites (Figure 13). The NJ tree of DAPC clusters highlighted the Mexican-Central American split, but had higher bootstrap values than the NJ tree of nesting sites (Figure 14).

Bottleneck Analysis

BOTTLENECK results varied depending on the proportion of SMM in the TPM, and on the test used to validate the significance of results. In general, 95% SMM inferred more population expansion after bottleneck events than 0% SMM, which only inferred one instance of

heterozygosity excess (Table 5). The two-tailed Wilcoxon test conferred significance (alpha=0.05) on heterozygosity deficiencies slightly more often than the sign test, specifically in Central American DAPC clusters. However, both tests largely agreed on bottleneck significance, or the lack thereof.

With no SMM in the TPM, there were no inferred bottleneck events. This may be due to the constraints and limitations of the mutation models used in BOTTLENECK (Luikart et al. 1998, Piry et al. 1999, Putman and Carbone 2014). With 95% SMM in the TPM, both Mexico and Central America had significant heterozygote deficiency. In Mexico, bottlenecks were detected at 6 sites, while bottlenecks were only detected at 2 sites in Central America (Table 5). The Wilcoxon test detected bottlenecks in 3 out of 4 Mexican DAPC clusters, and 3 out of 5 Central American DAPC clusters. However, the sign test only detected a bottleneck in cluster 8.

Relatedness Analysis

LRM and QGM showed agreement in general patterns of relatedness, but differed in exact values of relatedness within nesting sites and putative populations. In general, LRM was more conservative than QGM. In NWCR, relatedness was negligible overall and within nesting sites and behaviors (Table 6). Both measures suggested that Playa Nancite displayed significantly high relatedness relative to the entire sample, but this should be interpreted cautiously as the sample size was low (n=7) and individuals were missing data. Relatedness was significantly high within clusters, and ranged from 0.31-0.57 (LRM) and 0.053-0.235 (QGM).

Relatedness was higher in Mexico and Central American nesting sites, and in the two ETP populations overall, than in NWCR sites/behaviors and overall (Tables 5 and 6). Relatedness ranged from 0.009-0.072(LRM)/0.014-0.226(QGM) in Mexico and 0.003-0.031(LRM)/0.022-0.097(QGM) in Central America. Relatedness was not significantly high at PLA (QGM) and NS (both LRM and QGM). Relatedness in clusters ranged from 0.018-0.054(LRM)/0.001-0.311(QGM). Relatedness within DAPC clusters was significantly high and comparable to nesting sites and subpopulations in all clusters, save for #9 (QGM). Relatedness was on average higher at nesting beaches within Mexico than across all Mexican individuals, and vice-a-verse in Central America.

Migration Analysis

All three measures of migration (Nm, Gst, D) agreed on general trends in migration, although D was the most conservative metric across all analyses. Nm and Gst in particular agreed on specific levels of migration, whereas D produced different values from either of those measures.

Differential migration between NWCR nesting behaviors and sites was equitable, although Playa Nancite was indicated as a potential source population (Figure 7a-c). However, this should be interpreted cautiously as these individuals were missing genotypic data. Migration between DAPC clusters was equitable and generally lower than migration between nesting behaviors and sites. Cluster 3 was an exception, and was indicated as a source for other clusters (Figure 7d-e).

Differential migration between ETP sites was higher within than between Mexican and Central American populations, but there was evidence for significant differential migration from Mexico to Central America (Tables 7 and 8). MIS was indicated as a significant source population for both Mexican and Central American sites by all three measures, while PAR was indicated as a significant recipient for Mexican and Central American migrants by Nm and Gst (Tables 7 and 8, Figure 8). DivMigrate indicated low levels of migration between and to Mexican clusters, and significant levels of migration between and to Central American clusters (Table 9, Figure 9). Clusters 3 and 6, primarily comprised of Central American individuals, were indicated as source populations for almost all clusters by all three metrics.

Table 1. Olive ridley mtDNA control region haplotype frequencies from three nesting beaches in Costa Rica (PN: Playa Nancite; PG: Playa Grande, PO: Playa Ostional) and overall (OV) for this study. Also shown are the number of individuals from each site (n), mean (±SD) haplotype diversity (*H*), and mean (±SD) nucleotide diversity (π).

Site	**n**	***H* (±SD)**	**π (±SD)**	**Lo46**	**Lo73**	**Lo27**	**Lo54**	**Lo31**	**Lo52**	**Lo60**	**Lo96**	**Lo62**	**LoN11**
PN	7	0.5238 (±0.2086)	0.001128 (±0.001028)	5	0	1	0	0	0	1	0	0	0
PG	17	0.5074 (±0.1403)	0.001316 (±0.001042)	12	1	0	0	0	2	1	1	0	0
PO	36	0.6151 (±0.954)	0.001583 (±0.001155)	24	0	1	3	2	2	0	0	1	3
OV	60	0.5657 (±0.0783)	0.001434 (±0.01061)	41	1	2	3	2	4	2	1	1	3

Table 2. Hierarchical AMOVA of ETP ORs with DAPC clusters as populations calculated after 10,000 permutations. Population structure to explain variance in the data is examined between DAPC clusters, within DAPC clusters, and within all individuals in the dataset.

Source of Variation	**Percentage of Variation**	**F statistic**	**P Value**
Between Clusters	10.37	*F*st=0.104	<0.001
Within Clusters	3.34	*F*is=0.037	<0.001
Within Individuals	86.29	*F*it=0.137	<0.001

Table 3. Pairwise population differentiation indices (Fst, CITE; D, Jost 2008) for ETP DAPC clusters calculated after 10,000 permutations. Pairwise Fst values are above the diagonal (top right), and pairwise D values are below the diagonal (bottom left). All values were significant (alpha=0.00139)

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1		0.055	0.049	0.061	0.052	0.059	0.054	0.062	0.037
2	0.298		0.041	0.053	0.058	0.074	0.051	0.066	0.041
3	0.271	0.237		0.045	0.050	0.029	0.044	0.061	0.040
4	0.328	0.303	0.257		0.065	0.089	0.052	0.073	0.039
5	0.241	0.290	0.252	0.323		0.054	0.065	0.067	0.049
6	0.231	0.311	0.111	0.376	0.191		0.084	0.082	0.091
7	0.318	0.324	0.280	0.324	0.359	0.388		0.074	0.037
8	0.300	0.344	0.319	0.377	0.303	0.310	0.422		0.056
9	0.250	0.309	0.309	0.283	0.318	0.507	0.301	0.373	

Table 4. Inbreeding coefficients (*F*is) for ETP DAPC clusters calculated after 10,000 permutations. Bold typeface indicates inbreeding (alpha=0.0056).

Cluster	Majority Location	*F*is
1	Mexico	**0.075**
2	Central America	-0.012
3	Central America	0.019
4	Central America	-0.059
5	Mexico	**0.099**
6	Central America	**0.107**
7	Central America	0.059
8	Mexico	**0.094**
9	Mexico	**0.109**

Table 5. Bottleneck results (TPM, Sign Test and Wilcoxon Test) and relatedness measures (LRM, Lynch and Ritland 1999; QGM, Queller and Goodnight 1989) for ETP ORs after 10,000 bootstrap replicates by putative populations (Mexico and Central America), nesting sites, and DAPC clusters. "0" indicates 0% SMM in the TPM, "95" indicates 95% SMM in the TPM. Bold typeface indicates significant values (alpha=0.05) for heterozygote deficiency or excess. (E) indicates significant heterozygote excess detected by BOTTLENECK. DAPC clusters are ordered by Mexican (M) and Central American (C) populations.

Site	TPM Sign 0	TPM Wilcoxon 0	TPM Sign 95	TPM Wilcoxon 95	LRM	QGM
Mexico	0.374	0.921	**0.000**	**0.000**	**0.013**	**0.022**
BCS	0.628	0.431	**0.015**	**0.019**	**0.009**	**0.026**
EVE	0.355	0.160	0.165	0.193	**0.016**	**0.086**
PLA	0.176	0.232	0.172	0.105	**0.012**	0.014
NVA	0.154	0.695	**0.014**	**0.007**	**0.025**	**0.064**
PVG	0.157	0.492	**0.013**	**0.032**	**0.022**	**0.079**
MIS	0.575	0.275	0.069	0.131	**0.033**	**0.226**
PTI	0.608	0.845	0.055	0.105	**0.028**	**0.112**
CPA	0.615	0.625	**0.013**	**0.032**	**0.033**	**0.115**
TCO	0.354	0.921	0.059	0.130	**0.020**	**0.055**
SJC	0.360	0.846	0.062	0.105	**0.017**	**0.060**
BCR	0.363	0.922	**0.013**	**0.032**	**0.023**	**0.110**
ESC	0.167	0.232	0.062	**0.032**	**0.008**	**0.022**
PAR	0.156	0.232	**0.002**	**0.003**	**0.072**	**0.222**
Central America	0.622	0.492	**0.016**	**0.014**	**0.024**	**0.044**
GH	0.359	0.769	0.061	0.105	**0.011**	**0.039**
SPD	0.607	0.492	**0.002**	**0.014**	**0.018**	**0.053**
SJG	0.357	0.625	0.360	0.275	**0.031**	**0.081**
SB	0.631	0.275	0.387	0.432	**0.013**	**0.036**
NC	0.617	0.557	0.058	0.131	**0.011**	**0.031**
NF	0.162	0.232	**0.002**	**0.003**	**0.017**	**0.097**
NV	0.608	1.0	0.061	0.105	**0.011**	**0.078**
NS	0.6	0.625	0.182	0.275	0.003	0.022
PMA	0.168	0.16	0.390	0.275	**0.011**	**0.034**
Cluster 1 (M)	0.166	0.845	0.065	**0.024**	**0.025**	**0.154**
Cluster 5 (M)	0.369	0.275	0.061	**0.024**	**0.026**	**0.194**
Cluster 8 (M)	0.168	0.3227	**0.002**	**0.003**	**0.054**	**0.213**
Cluster 9 (M)	**0.047 (E)**	**0.0419 (E)**	0.599	0.846	**0.018**	0.001
Cluster 2 (C)	0.354	0.769	0.063	**0.014**	**0.027**	**0.134**
Cluster 3 (C)	0.352	0.375	0.062	**0.014**	**0.019**	**0.095**
Cluster 4 (C)	0.365	0.492	0.065	0.130	**0.035**	**0.166**
Cluster 6 (C)	0.607	0.556	0.065	**0.014**	**0.030**	**0.311**
Cluster 7 (C)	0.617	0.556	0.396	0.432	**0.044**	**0.117**

Table 6. Relatedness measures (LRM, Lynch and Ritland 1999; QGM, Queller and Goodnight 1989) for NWCR ORs after 10,000 bootstrap replicates for the entire dataset, by nesting sites and behaviors, and by DAPC clusters. Bold typeface indicates significant values (alpha=0.0125).

Site	**LRM**	**QGM**
Costa Rica	-0.008	-0.006
Playa Grande	-0.012	-0.014
Playa Ostional Arribada	-0.010	-0.010
Playa Ostional Solitary	-0.023	-0.050
Playa Nancite	**0.040**	**0.125**
Cluster 1	**0.057**	**0.235**
Cluster 2	**0.036**	**0.145**
Cluster 3	**0.031**	**0.225**
Cluster 4	**0.056**	**0.053**

Table 7. Relative migration calculated by divMigrate (Sundqvist et al. 2016) between ETP sites inferred using Nm (A; Alcala et al. 2014), Gst (B: Hedrick and Goodnight 2005), and D (C; Jost 2008) over 1000 bootstrap replicates. Values represent migration from sites on the y-axis (left) to sites on the x-axis (top) on a scale from 0 (low) to 1 (high). Dashed lines indicate the interface between Mexican and Central American sites (PAR and GH, respectively). Bold values indicate significant differential migration (alpha=0.05).

A	BCS	EVE	PLA	NVA	PVG	MIS	PTI	CPA	TCO	SJC	BCR	ESC	PAR	GH	SPD	SJG	SB	NC	NF	NV	NS	PMA
BCS		0.178	0.319	0.214	0.179	0.063	0.115	0.208	0.156	0.42	0.153	0.464	0.093	0.115	0.126	0.077	0.136	0.172	0.08	0.103	0.118	0.114
EVE	0.267		0.184	0.135	0.159	0.086	0.07	0.133	0.121	0.182	0.157	0.243	0.059	0.097	0.082	0.068	0.108	0.113	0.057	0.101	0.095	**0.081**
PLA	0.383	0.11		0.134	0.176	0.052	**0.122**	0.107	0.168	0.19	0.13	0.184	**0.104**	0.09	0.068	0.054	0.082	0.089	0.055	0.077	0.072	0.078
NVA	0.221	0.092	0.14		0.105	0.05	0.099	0.135	0.089	0.147	0.116	0.24	0.056	0.073	0.069	0.05	0.079	0.09	0.06	0.066	0.066	0.077
PVG	0.267	0.09	0.216	0.111		0.058	0.083	0.142	0.285	0.132	0.118	0.156	0.073	0.077	0.069	0.048	0.08	0.079	0.05	0.063	0.061	0.077
MIS	**0.219**	0.203	**0.167**	0.088	**0.224**		**0.122**	**0.098**	**0.171**	0.133	**0.178**	**0.181**	**0.086**	**0.133**	**0.127**	**0.084**	**0.127**	**0.133**	**0.075**	**0.139**	**0.116**	**0.102**
PTI	0.115	0.062	0.047	0.096	0.12	0.025		0.105	0.109	0.075	0.111	0.116	0.085	0.045	0.043	0.022	0.041	0.061	0.026	0.028	0.027	0.043
CPA	0.158	0.05	0.105	0.067	0.17	0.037	0.066		0.122	0.076	0.081	0.082	0.088	0.04	0.041	0.029	0.043	0.048	0.035	0.037	0.038	0.039
TCO	0.191	0.073	0.169	0.08	0.225	0.049	0.076	0.12		0.108	0.114	0.126	0.07	0.068	0.061	0.042	0.07	0.078	0.048	0.058	0.057	0.066
SJC	0.627	0.136	0.22	0.174	0.15	0.065	0.094	0.192	0.122		0.141	0.437	**0.106**	0.084	0.088	0.056	0.099	0.111	0.063	0.077	0.083	0.078
BCR	0.277	0.168	0.141	0.119	0.217	0.062	0.087	0.098	0.205	0.158		0.265	0.068	0.059	0.055	0.038	0.059	0.1	0.039	0.051	0.048	0.051
ESC	0.476	0.176	0.275	0.211	0.227	0.075	0.112	0.194	0.174	0.442	0.167		0.095	0.11	0.114	0.064	0.125	0.149	0.08	0.107	0.113	0.101
PAR	0.074	0.052	0.029	0.04	0.128	0.02	0.12	0.068	0.116	0.042	0.086	0.052		0.04	0.042	0.021	0.036	0.042	0.019	0.022	0.023	0.044
GH	0.088	0.048	0.06	0.068	0.064	0.032	0.054	0.063	0.061	0.077	0.058	0.077	0.053		0.52	0.137	0.606	0.869	0.216	0.516	0.666	0.646
SPD	0.087	0.045	0.055	0.062	0.067	0.031	0.052	0.061	0.055	0.071	0.053	0.078	0.051	0.531		0.184	0.466	0.57	0.184	0.328	0.439	0.475
SJG	0.067	0.035	0.043	0.037	0.053	0.024	0.043	0.045	0.046	0.051	0.041	0.052	**0.044**	0.217	0.248		0.272	0.222	0.111	0.192	0.288	0.213
SB	0.106	0.056	0.076	0.075	0.09	0.034	0.068	0.072	0.078	0.096	0.061	0.079	0.061	0.629	0.498	0.17		0.862	0.202	0.424	0.697	0.599
NC	0.106	0.054	0.068	0.076	0.074	0.033	0.067	0.072	0.077	0.082	0.06	0.082	0.056	0.888	0.451	0.143	0.84		0.244	0.531	0.487	0.671
NF	0.071	0.04	0.044	0.048	0.062	0.031	0.055	0.055	0.066	0.054	0.049	0.07	**0.048**	0.359	0.244	0.113	0.244	0.33		0.337	0.312	0.274
NV	0.089	0.052	0.062	0.064	0.063	0.034	0.058	0.061	0.061	0.077	0.05	0.068	**0.062**	1	0.423	0.192	0.668	0.851	0.262		0.622	0.498
NS	0.099	0.053	0.072	0.063	0.072	0.034	0.064	0.063	0.068	0.081	0.057	0.08	**0.061**	0.708	0.417	0.175	0.7	0.549	0.202	0.496		0.49
PMA	0.077	0.036	0.053	0.053	0.056	0.025	0.047	0.05	0.058	0.062	0.043	0.065	0.045	0.475	0.336	0.139	0.467	0.474	0.186	0.34	0.371	

B	BCS	EVE	PLA	NVA	PVG	MIS	PTI	CPA	TCO	SJC	BCR	ESC	PAR	GH	SPD	SJG	SB	NC	NF	NV	NS	PMA
BCS		0.181	0.323	0.218	0.183	0.064	0.119	0.212	0.159	0.423	0.157	0.466	0.095	0.117	0.128	0.08	0.138	0.174	0.083	0.107	0.121	0.117
EVE	0.268		0.187	0.138	0.161	0.087	0.072	0.135	0.122	0.183	0.16	0.244	0.06	0.098	0.084	0.07	0.109	0.114	0.059	0.102	0.097	**0.082**
PLA	0.386	0.114		0.138	0.179	0.054	**0.125**	0.11	0.172	0.193	0.134	0.186	**0.108**	0.091	0.07	0.056	0.083	0.09	0.058	0.079	0.074	0.08
NVA	0.224	0.095	0.142		0.107	0.052	0.101	0.138	0.091	0.149	0.12	0.243	0.058	0.075	0.071	0.052	0.082	0.092	0.063	0.068	0.069	0.08
PVG	0.269	0.092	0.218	0.113		0.06	0.085	0.145	0.288	0.133	0.121	0.158	0.074	0.078	0.071	0.051	0.081	0.081	0.053	0.065	0.063	0.079
MIS	**0.22**	0.205	**0.17**	0.089	**0.226**		**0.124**	**0.099**	**0.173**	0.135	**0.18**	**0.183**	**0.087**	**0.135**	**0.129**	**0.086**	**0.128**	**0.134**	**0.077**	**0.14**	**0.117**	**0.103**
PTI	0.116	0.066	0.047	0.098	0.123	0.026		0.108	0.111	0.076	0.114	0.117	0.087	0.047	0.045	0.024	0.043	0.064	0.027	0.03	0.028	0.045
CPA	0.159	0.051	0.108	0.068	0.173	0.039	0.069		0.125	0.076	0.084	0.083	0.09	0.042	0.044	0.032	0.045	0.051	0.038	0.039	0.04	0.041
TCO	0.192	0.075	0.172	0.081	0.227	0.05	0.078	0.122		0.109	0.116	0.127	0.072	0.07	0.063	0.045	0.072	0.08	0.051	0.061	0.059	0.068
SJC	0.627	0.138	0.222	0.176	0.152	0.067	0.097	0.194	0.124		0.144	0.44	**0.108**	0.086	0.091	0.059	0.102	0.113	0.066	0.08	0.086	0.08
BCR	0.279	0.17	0.144	0.121	0.219	0.063	0.089	0.1	0.206	0.161		0.267	0.069	0.059	0.056	0.04	0.059	0.101	0.04	0.051	0.049	0.051
ESC	0.478	0.179	0.277	0.213	0.23	0.077	0.115	0.197	0.176	0.444	0.17		0.097	0.112	0.117	0.067	0.127	0.151	0.082	0.109	0.115	0.103
PAR	0.075	0.053	0.029	0.041	0.129	0.02	0.122	0.068	0.118	0.043	0.087	0.053		0.041	0.043	0.021	0.037	0.044	0.02	0.023	0.023	0.045
GH	0.089	0.049	0.062	0.069	0.066	0.033	0.056	0.065	0.062	0.079	0.059	0.078	0.054		0.521	0.14	0.607	0.869	0.219	0.518	0.667	0.647
SPD	0.088	0.046	0.057	0.064	0.07	0.032	0.054	0.063	0.057	0.073	0.055	0.079	0.053	0.533		0.187	0.467	0.571	0.187	0.33	0.441	0.476
SJG	0.069	0.036	0.045	0.039	0.054	0.026	0.045	0.048	0.048	0.053	0.043	0.053	**0.046**	0.22	0.25		0.274	0.224	0.113	0.194	0.29	0.215
SB	0.107	0.058	0.078	0.078	0.092	0.035	0.07	0.075	0.08	0.098	0.063	0.08	0.064	0.631	0.5	0.173		0.863	0.205	0.427	0.698	0.601
NC	0.107	0.056	0.07	0.078	0.076	0.034	0.07	0.074	0.079	0.084	0.061	0.083	0.058	0.888	0.452	0.146	0.842		0.247	0.533	0.489	0.672
NF	0.073	0.041	0.045	0.05	0.065	0.033	0.057	0.057	0.068	0.055	0.05	0.071	**0.05**	0.36	0.246	0.115	0.246	0.332		0.339	0.314	0.275
NV	0.09	0.054	0.063	0.065	0.065	0.035	0.06	0.063	0.062	0.078	0.051	0.069	**0.063**	1	0.425	0.195	0.669	0.852	0.263		0.623	0.5
NS	0.1	0.055	0.074	0.066	0.073	0.035	0.067	0.065	0.07	0.082	0.058	0.081	**0.063**	0.709	0.419	0.178	0.701	0.551	0.205	0.498		0.492
PMA	0.078	0.038	0.055	0.056	0.058	0.027	0.049	0.053	0.06	0.064	0.044	0.066	0.046	0.478	0.34	0.143	0.47	0.476	0.189	0.342	0.374	

C	BCS	EVE	PLA	NVA	PVG	MIS	PTI	CPA	TCO	SJC	BCR	ESC	PAR	GH	SPD	SJG	SB	NC	NF	NV	NS	PMA
BCS		0.178	0.273	0.18	0.153	0.129	0.111	0.194	0.164	0.359	0.117	0.435	0.146	0.141	0.159	0.07	0.181	0.197	0.08	0.098	0.132	0.15
EVE	0.448		0.204	0.178	0.26	0.173	0.103	0.178	0.223	0.28	0.192	0.517	0.152	0.197	0.137	0.094	0.213	0.227	0.126	0.194	0.177	**0.16**
PLA	0.298	0.095		0.115	0.179	0.103	0.144	0.11	0.135	0.18	0.118	0.249	0.1	0.193	0.115	0.07	0.145	0.198	0.07	0.137	0.119	0.114
NVA	0.2	0.112	0.187		0.127	0.097	0.149	0.154	0.128	0.155	0.113	0.217	0.082	0.101	0.102	0.068	0.101	0.128	0.078	0.089	0.085	0.103
PVG	0.331	0.122	0.23	0.146		0.108	0.113	0.156	0.319	0.229	0.124	0.252	0.135	0.155	0.115	0.059	0.158	0.154	0.069	0.088	0.102	0.152
MIS	**0.478**	0.29	0.199	0.231	**0.279**		0.23	0.27	0.253	0.218	0.279	0.304	0.151	0.24	**0.245**	0.135	**0.261**	**0.256**	0.107	0.219	0.242	**0.26**
PTI	0.215	0.065	0.227	0.127	0.12	0.103		0.133	0.168	0.163	0.145	0.262	0.131	0.08	0.067	0.042	0.077	0.086	0.061	0.071	0.068	0.08
CPA	0.528	0.091	0.134	0.192	0.193	0.093	0.084		0.158	0.313	0.098	0.249	0.184	0.062	0.06	0.038	0.064	0.074	0.044	0.05	0.049	0.058
TCO	0.392	0.135	0.172	0.16	0.234	0.121	0.114	0.152		0.279	0.165	0.355	0.156	0.104	0.095	0.053	0.114	0.111	0.061	0.082	0.075	0.1
SJC	0.741	0.18	0.321	0.223	0.182	0.118	0.121	0.217	0.186		0.139	0.426	0.144	0.133	0.113	0.062	0.142	0.139	0.081	0.085	0.094	0.097
BCR	**0.375**	0.255	0.171	0.177	0.275	0.141	0.201	0.153	0.328	0.206		0.338	0.175	0.325	0.235	0.084	0.296	0.264	0.12	0.209	0.186	0.209
ESC	0.467	0.168	0.27	0.208	0.238	0.15	0.124	0.21	0.213	0.399	0.164		0.145	0.187	0.16	0.066	0.186	0.213	0.122	0.145	0.175	0.164
PAR	0.242	0.219	0.151	0.158	0.308	0.166	0.183	0.334	0.264	0.149	0.144	0.174		0.097	0.097	0.086	0.095	0.105	0.064	0.087	0.107	0.111
GH	0.209	0.114	0.098	0.121	0.129	0.111	0.088	0.111	0.117	0.137	0.171	0.222	0.11		0.604	0.141	0.59	1	0.243	0.493	0.718	0.668
SPD	0.16	0.09	0.085	0.094	0.085	0.088	0.067	0.102	0.072	0.105	0.103	0.144	0.08	0.497		0.206	0.502	0.616	0.209	0.339	0.408	0.528
SJG	0.121	0.068	0.078	0.047	0.103	0.072	0.077	0.071	0.079	0.09	0.074	0.132	0.082	0.26	0.297		0.351	0.312	0.184	0.253	0.327	0.247
SB	0.199	0.1	0.128	0.094	0.147	0.084	0.102	0.105	0.115	0.118	0.128	0.152	0.099	0.584	0.429	0.169		0.679	0.197	0.36	0.636	0.47
NC	0.214	0.098	0.109	0.113	0.115	0.099	0.091	0.108	0.129	0.11	0.122	0.171	0.107	0.82	0.466	0.137	0.6		0.271	0.535	0.464	0.588
NF	0.112	0.122	0.085	0.079	0.093	0.08	0.084	0.098	0.117	0.121	0.095	0.165	0.083	0.483	0.394	0.179	0.329	0.455		0.428	0.467	0.377
NV	**0.27**	0.127	0.13	0.113	0.128	0.112	0.101	**0.125**	0.143	0.173	0.127	0.197	0.136	0.911	0.416	0.208	0.572	0.753	0.38		0.689	0.433
NS	0.256	0.113	0.124	0.084	0.153	0.103	0.092	0.102	0.125	0.147	0.155	0.211	0.107	0.666	0.415	0.214	0.708	0.501	0.218	0.511		0.557
PMA	0.198	0.063	0.086	0.071	0.099	0.067	0.096	0.078	0.124	0.117	0.099	0.171	0.093	0.39	0.272	0.12	0.321	0.383	0.191	0.332	0.369	

Table 8. Relative migration calculated by divMigrate (Sundqvist et al. 2016) between Mexico (M) and Central America (C) using Nm (Alcala et al. 2014), Gst (Hedrick and Goodnight 2005), and D (Jost 2008) over 1000 bootstrap replicates. Values represent migration from sites on the y-axis (left) to sites on the x-axis (top) on a scale from 0 (low) to 1 (high). Dashed lines indicate the interface between inference with Nm, Gst, and D. Bold values indicate significant differential migration (alpha=0.05).

	Nm		Gst		D	
	M	C	M	C	M	C
M		**1**		**1**		0.816
C	0.662		0.66		1	

Table 9. Relative migration calculated by divMigrate (Sundqvist et al. 2016) between ETP DAPC clusters using Nm (A; Alcala et al. 2014), Gst (B: Hedrick and Goodnight 2005), and D (C; Jost 2008) over 1000 bootstrap replicates. Values represent migration from sites on the y-axis (left) to sites on the x-axis (top) on a scale from 0 (low) to 1 (high). (M) indicates clusters comprised of primarily Mexican individuals. (C) indicates clusters comprised of primarily Central American individuals. Bold values indicate significant differential migration (alpha=0.05).

A	1(M)	2(C)	3(C)	4(C)	5(M)	6(C)	7(C)	8(M)	9(M)
1(M)		**0.155**	0.095	0.119	0.102	0.069	**0.165**	0.044	0.177
2(C)	0.086		0.044	0.089	0.064	0.026	0.137	0.068	0.164
3(C)	0.085	**0.274**		**0.674**	0.065	0.073	**0.579**	0.066	0.145
4(C)	0.08	0.13	0.048		0.059	0.032	0.12	0.079	0.186
5(M)	0.189	**0.218**	**0.154**	**0.158**		0.108	**0.191**	0.126	0.135
6(C)	**0.108**	**0.85**	**1**	**0.286**	0.08		**0.546**	0.075	**0.123**
7(C)	0.087	0.089	0.055	0.115	0.06	0.027		0.054	0.136
8(M)	**0.243**	0.109	0.075	0.075	0.086	0.054	**0.122**		0.148
9(M)	0.18	0.142	0.145	0.147	0.124	0.041	**0.235**	0.084	

B	1(M)	2(C)	3(C)	4(C)	5(M)	6(C)	7(C)	8(M)	9(M)
1(M)		**0.158**	0.099	0.123	0.103	0.071	**0.169**	0.044	0.179
2(C)	0.089		0.045	0.091	0.066	0.026	0.139	0.069	0.168
3(C)	0.086	**0.276**		**0.676**	0.065	0.074	**0.582**	0.067	0.15
4(C)	0.082	0.132	0.05		0.061	0.033	0.121	0.081	0.191
5(M)	0.189	**0.221**	**0.156**	**0.161**		0.109	**0.194**	0.127	0.137
6(C)	**0.109**	**0.851**	**1**	**0.287**	0.081		**0.547**	0.076	**0.125**
7(C)	0.091	0.091	0.056	0.117	0.062	0.027		0.057	0.139
8(M)	**0.244**	0.112	0.078	0.077	0.087	0.056	**0.125**		0.15
9(M)	0.184	0.147	0.153	0.152	0.127	0.042	**0.241**	0.086	

C	1(M)	2(C)	3(C)	4(C)	5(M)	6(C)	7(C)	8(M)	9(M)
1(M)		0.139	0.089	0.122	0.616	0.114	0.136	0.439	0.245
2(C)	0.098		0.12	0.192	0.112	0.134	0.228	0.145	0.13
3(C)	**0.179**	**0.289**		**0.584**	0.231	0.34	**0.459**	**0.193**	0.096
4(C)	0.124	0.225	0.145		0.116	0.146	0.239	0.145	0.124
5(M)	0.79	**0.198**	0.194	0.166		0.198	**0.205**	0.549	0.228
6(C)	**0.272**	**0.722**	**1**	**0.512**	**0.372**		**0.882**	**0.273**	**0.157**
7(C)	0.09	0.152	0.123	0.195	0.08	0.122		0.072	0.121
8(M)	0.47	0.135	0.089	0.092	0.462	0.118	0.125		0.221
9(M)	0.145	0.096	0.066	0.09	0.13	0.076	0.127	0.15	

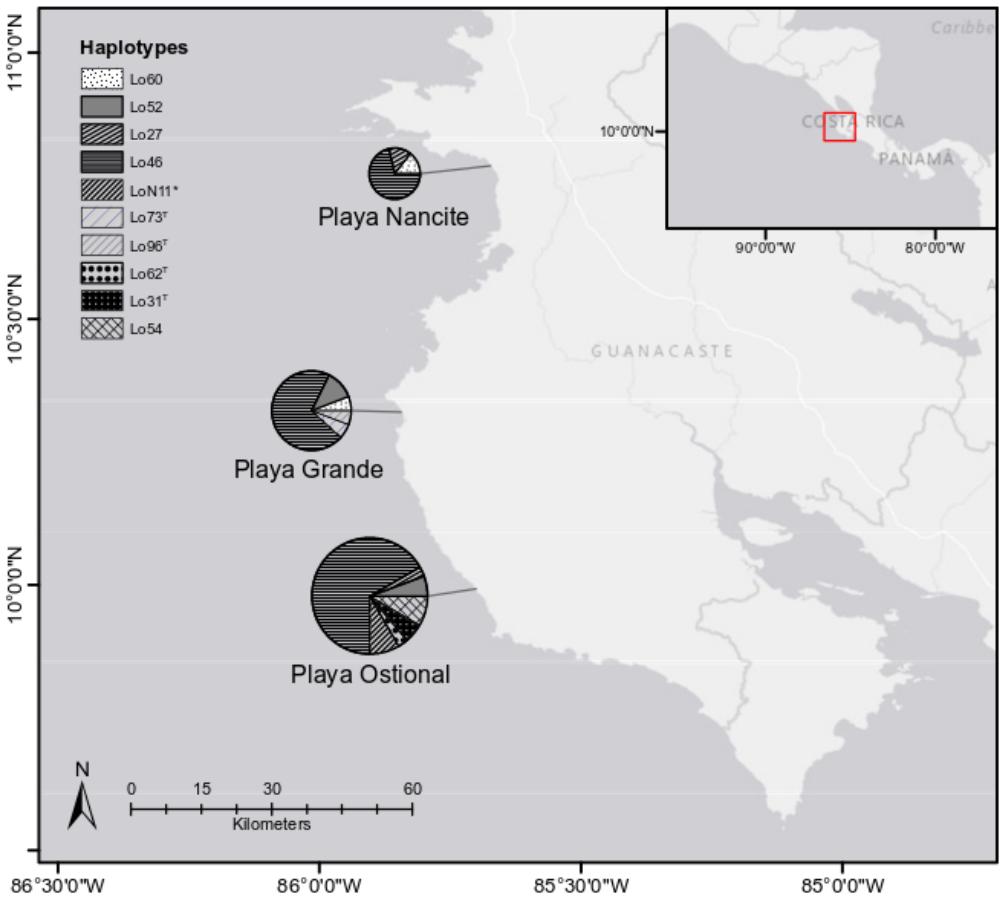


Figure 1. Map of NWCR sampling sites and corresponding Olive ridley haplotypes. Site names are listed next to each node, and node size corresponds to sample size. "*" indicates haplotypes present in the NMFS database that are unnamed. Superscript "T" indicates haplotypes first reported from nesting beaches in this study.

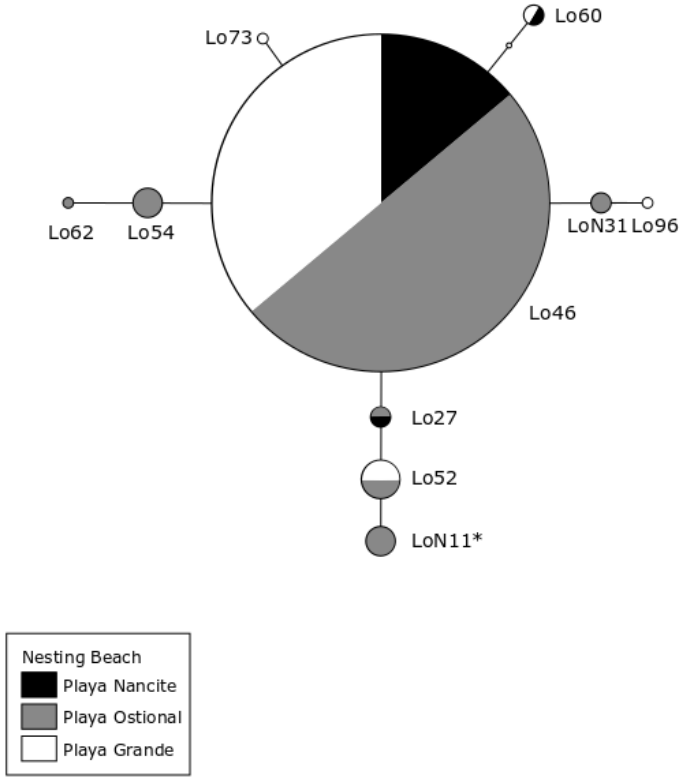


Figure 2. Network of Olive ridley mtDNA control region haplotypes found at three sites in NWCR. Node size corresponds to haplotype frequency. Bars in between nodes represent one mutational step. Nodes without names are mutational steps not reported from this study.

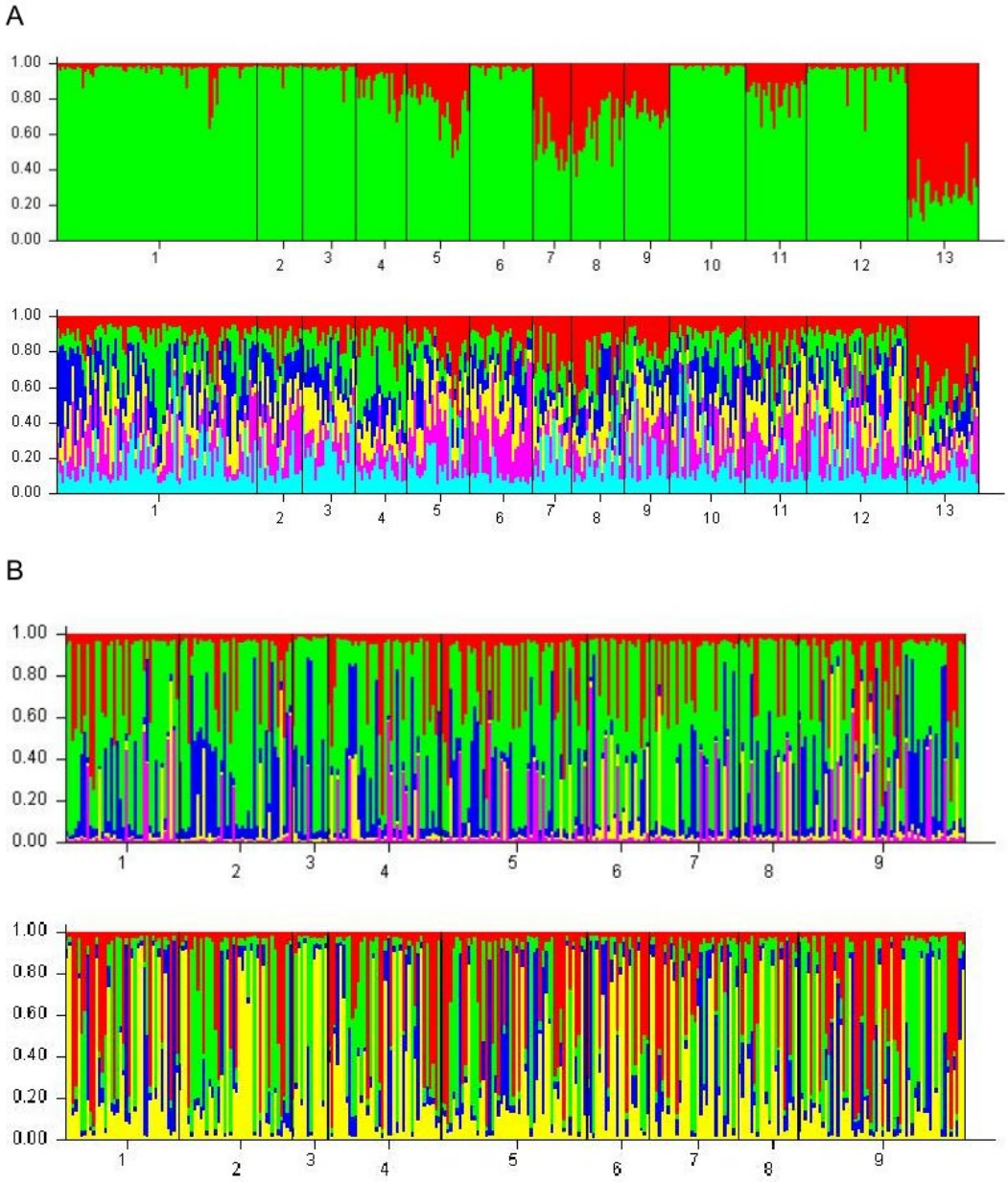


Figure 3.STRUCTURE barplots from Bayesian inference of populations in Mexican (A, *K*=2) and Central American (B, *K*=5) ORs. Colors correspond to different populations, and bar heights correspond to the probability of an individual being assigned to each population. STRUCTURE was run with (top) and without (bottom) location as a prior for both populations. Numbers along the x-axes correspond to sampling sites in order from North to South in Mexico and Central America as published in Rodriguez-Zarate et al. (2018)

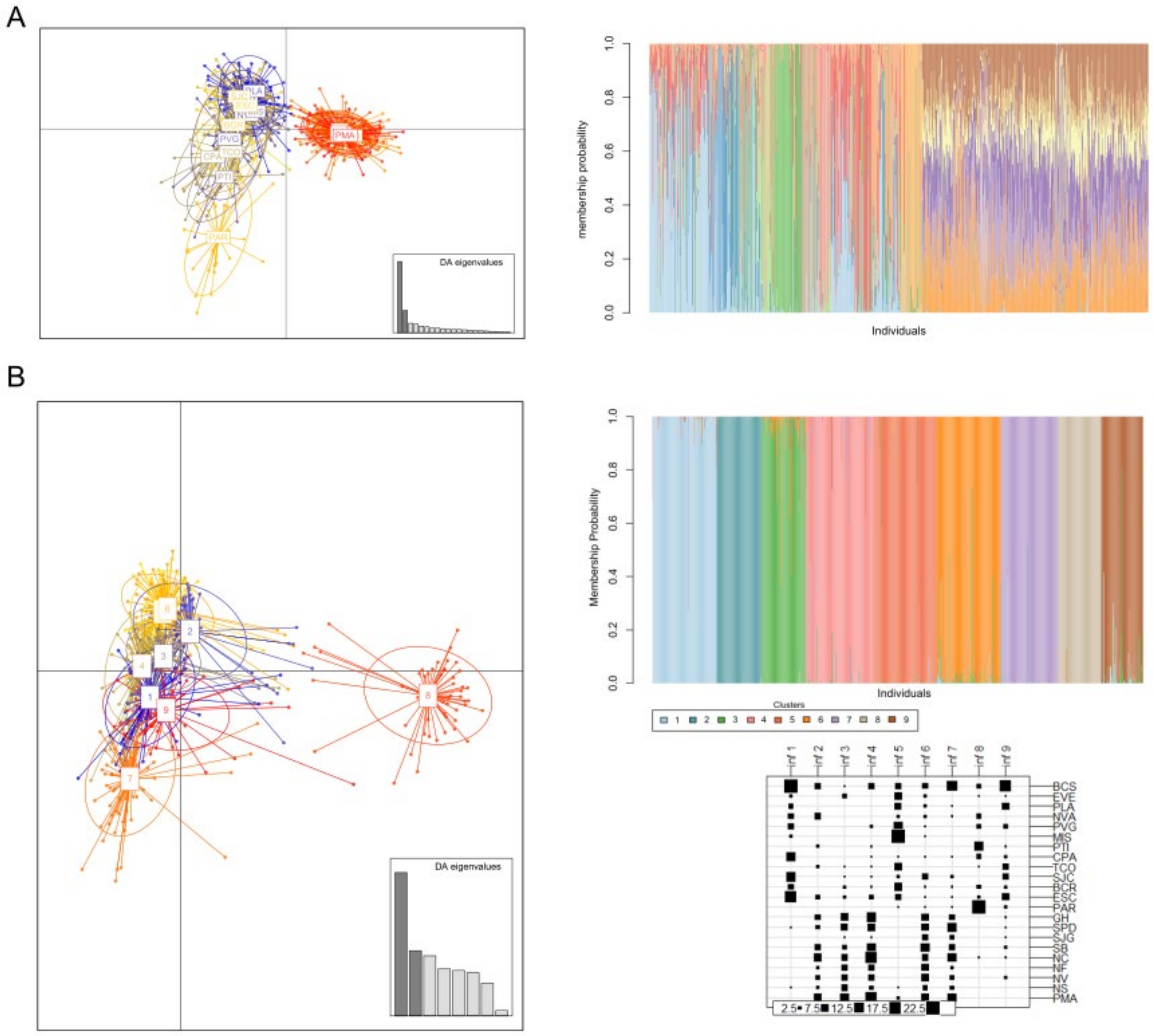


Figure 4. DAPC scatter plots (left), barplots (top and center right), and box plot (bottom right) from ETP ORs analyzed by nesting sites (A) and by inferred clusters (B). Scatter plot inertia ellipses summarize dispersion from the centroids of nesting sites (A) and inferred clusters (B), which are labelled by nesting site or cluster number. Bar heights correspond to the probability of an individual being assigned to each population. Individuals are unlabeled. In barplot A individuals are grouped by sites moving from North (left) to South (right). In barplot B individuals are grouped by inferred clusters. The box plot shows the number of individuals (represented by box sizes) from each site (along the y-axis) that are assigned to each of the 9 inferred clusters (listed along the top x-axis).

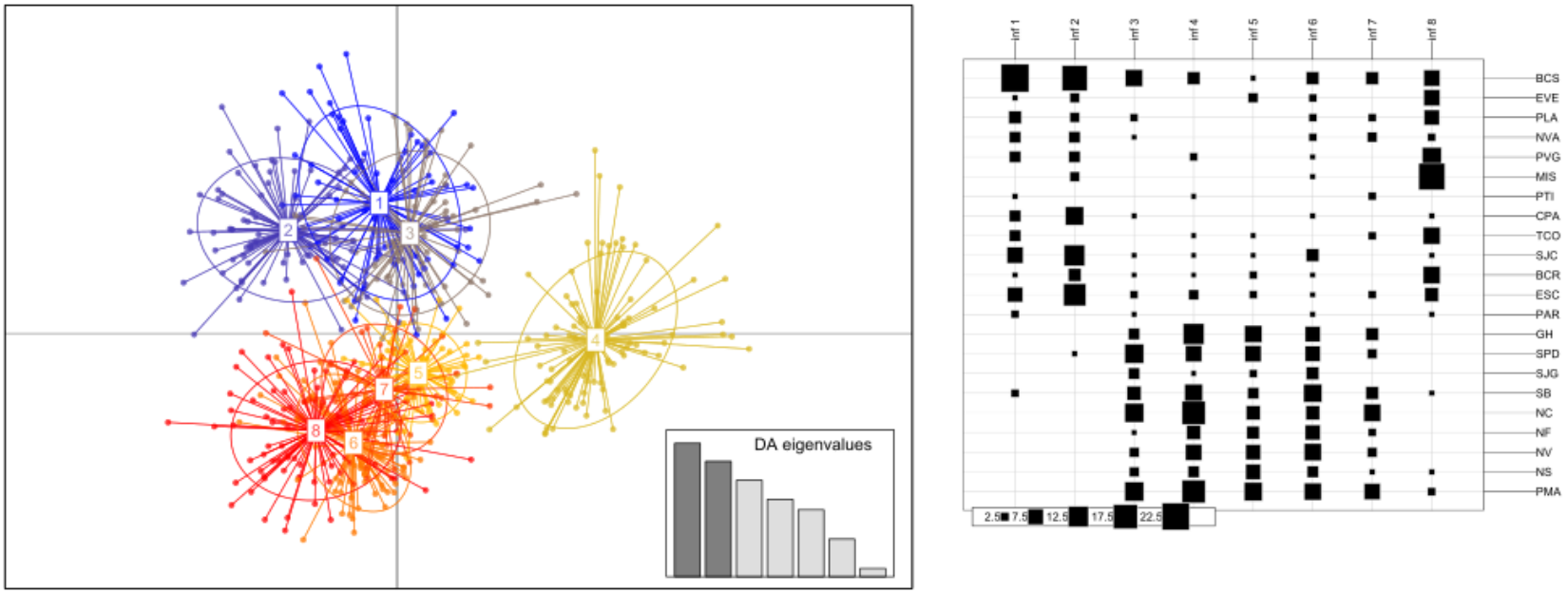


Figure 5. DAPC scatter plot (left) and box plot (right) from ETP ORs analyzed by inferred clusters after removal of individuals assigned to a unique cluster (#8, Figure 4). Scatter plot inertia ellipses summarize dispersion from the labelled centroids of inferred clusters. The box plot shows the number of individuals (represented by box sizes) from each site (along the y-axis) that are assigned to each of the 8 inferred clusters (along the top x-axis).

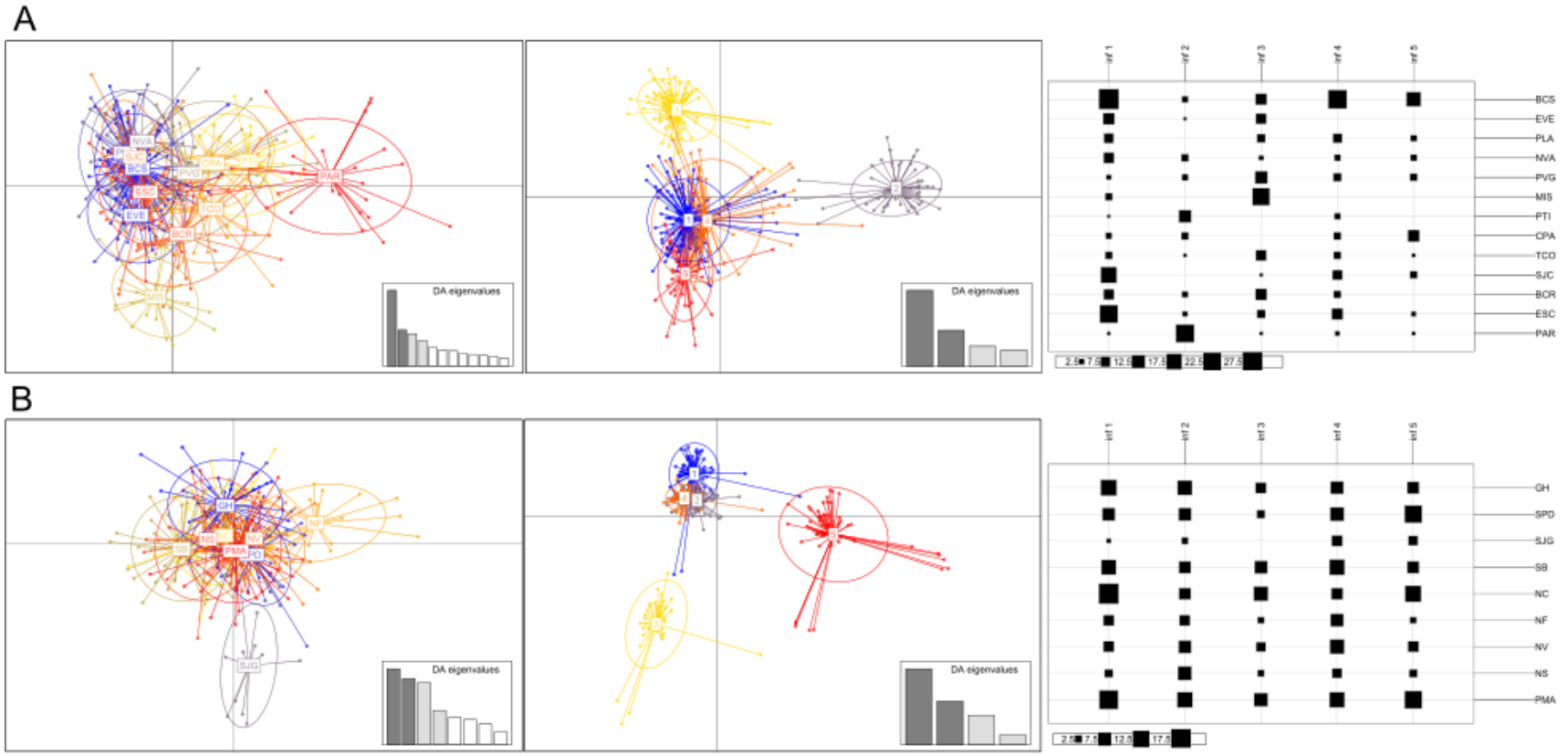


Figure 6. DAPC scatter plots by sites (left) and inferred clusters (center), and box plots (right) from Mexican (A) and Central American (B) ORs. Scatter plot inertia ellipses summarize dispersion from the centroids of nesting sites and inferred clusters, which are labelled by nesting site or cluster number. The box plots show the number of individuals (represented by box sizes) from each site (along the y-axis) that are assigned to each of the 9 inferred clusters (along the top x-axis).

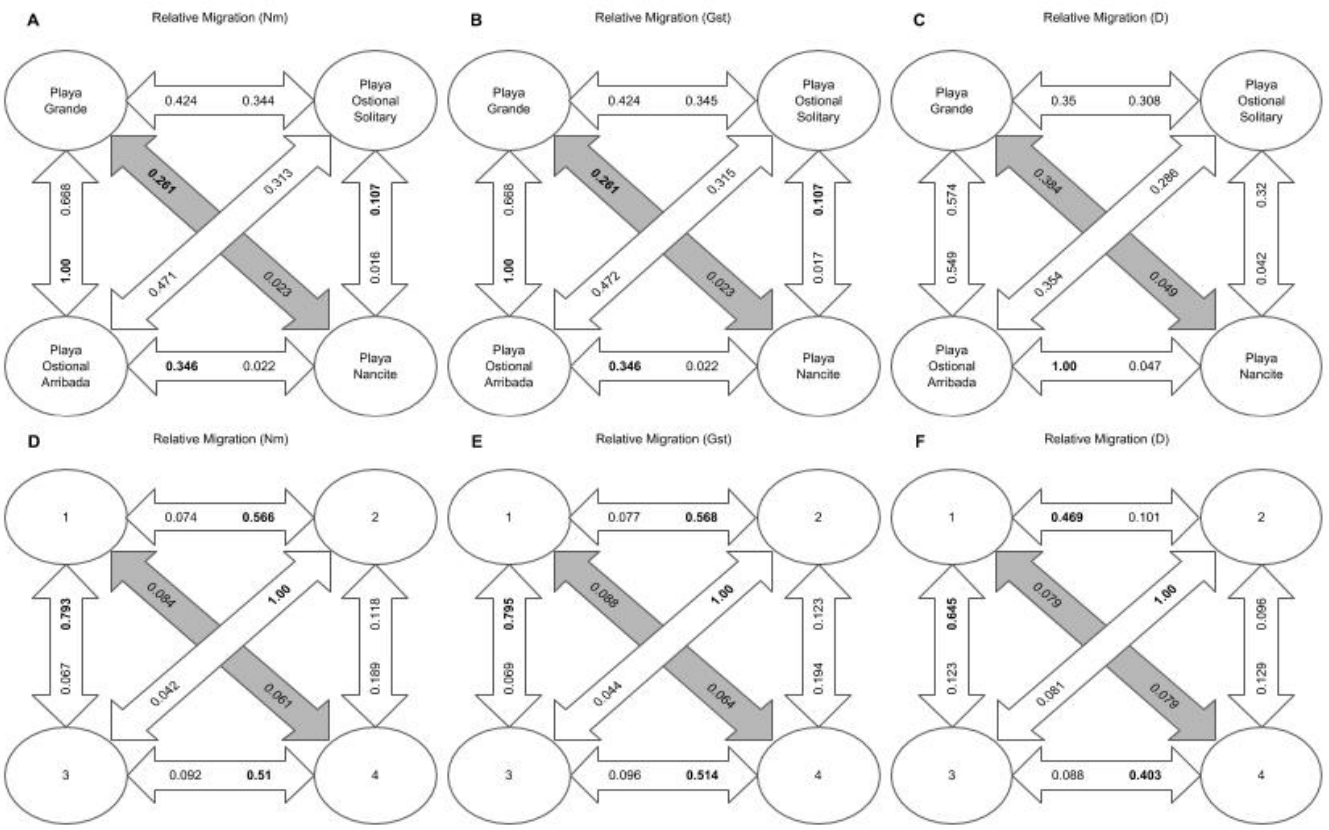


Figure 7. Diagrams of relative migration calculated by divMigrate (Sundqvist et al. 2016) between NWCR sites and behaviors (A-C) and DAPC clusters (D-F). Migration was inferred using Nm (A, D; Alcala et al. 2014), Gst (B, E; Hedrick and Goodnight 2005), and D (C, F; Jost 2008) over 1000 bootstrap replicates. Arrows represent migration between sites, and numbers at each arrowhead indicate the potential for migration (on a 0 to 1 scale) to the site nearest the arrowhead from the site at the other end of that arrow. Bold values indicate significantly asymmetric migration between sites (alpha=0.05). Arrow coloring is intended only as a visual aid.

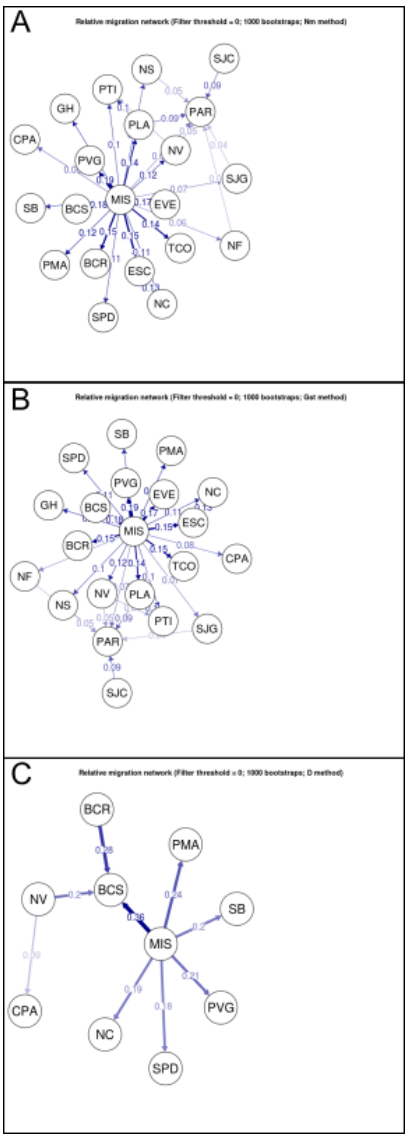


Figure 8. Diagrams of relative migration calculated by divMigrate (Sundqvist et al. 2016) between ETP sites, shown as circles. Migration was inferred using Nm (A; Alcala et al. 2014), Gst (B: Hedrick and Goodnight 2005), and D (C; Jost 2008) over 1000 bootstrap replicates. Arrows represent migration between sites, and numbers indicate the potential for asymmetric migration (on a 0 to 1 scale) between sites. The color of each arrow is indicative of the level of migration between sites, from low (light blue) to high (dark blue). The proximity of the circles to one another is dictated by the level of migration between circles, from low (distant) to high (near). Sites are only shown with significant asymmetric migration.

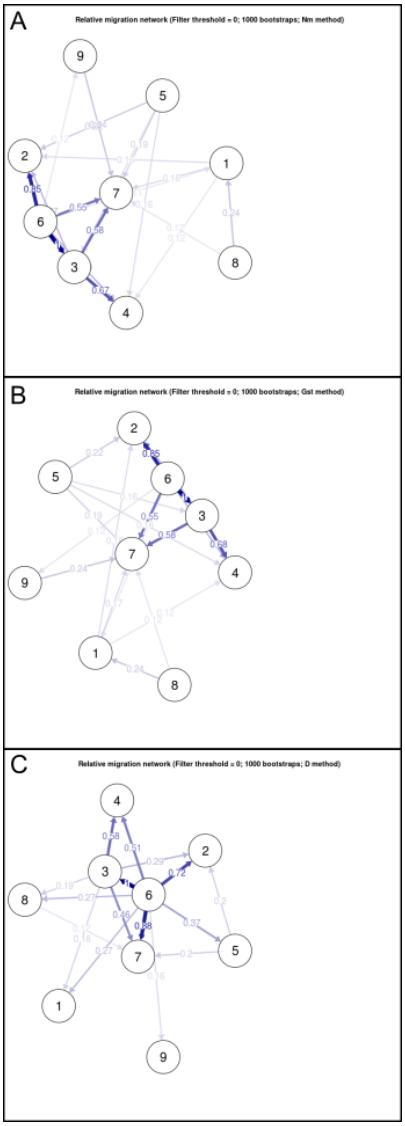


Figure 9. Diagram of relative migration calculated by divMigrate (Sundqvist et al. 2016) between ETP clusters, shown as circles. Migration was inferred using Nm (A; Alcala et al. 2014), Gst (B: Hedrick and Goodnight 2005), and D (C; Jost 2008) over 1000 bootstrap replicates. Arrows represent migration between sites, and numbers indicate the potential for asymmetric migration (on a 0 to 1 scale) between sites. The color of each arrow is indicative of the level of migration between sites, from low (light blue) to high (dark blue). The proximity of the circles to one another is dictated by the level of migration between circles, from low (distant) to high (near). Sites are only shown with significant asymmetric migration.

A)

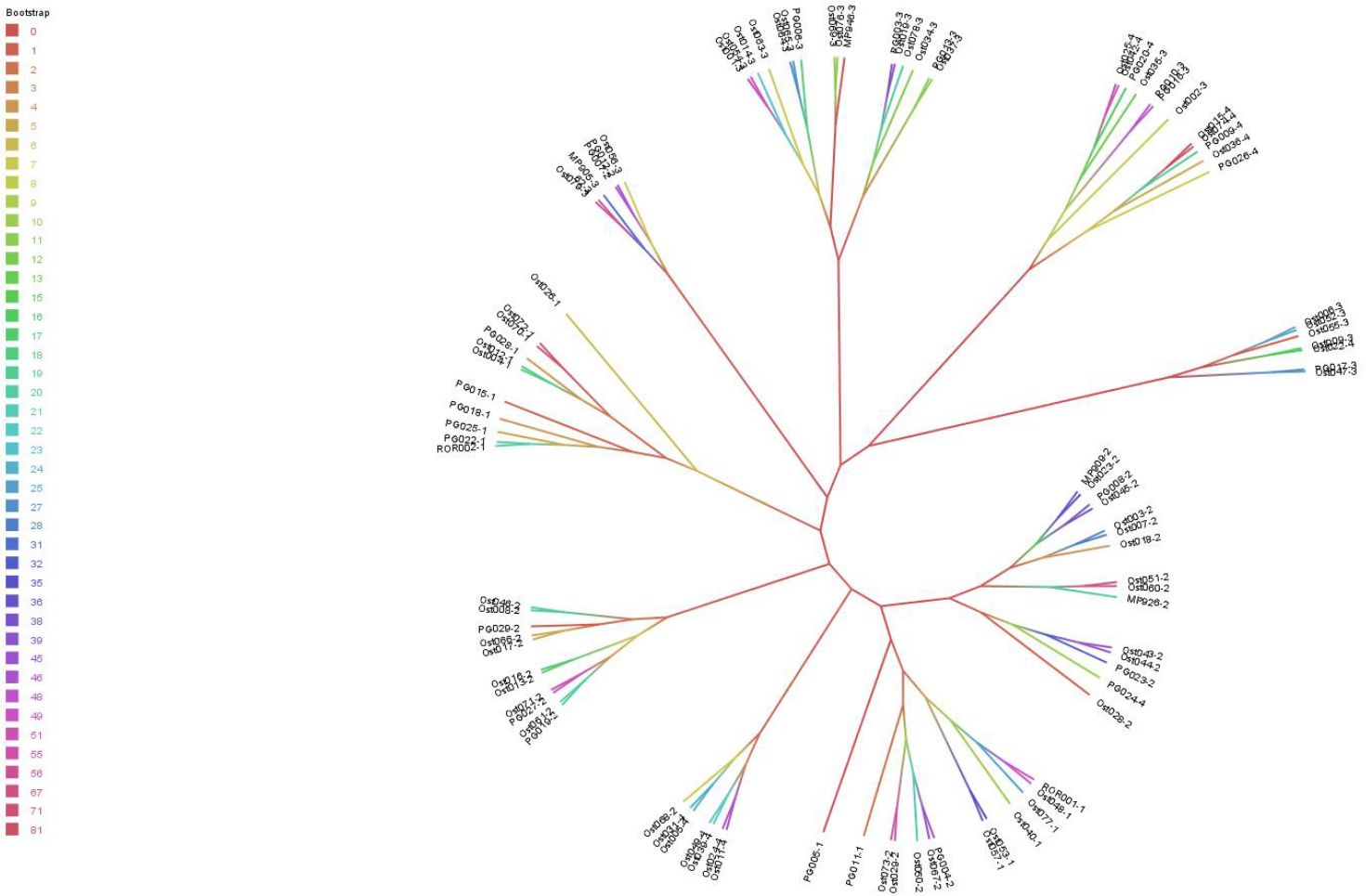
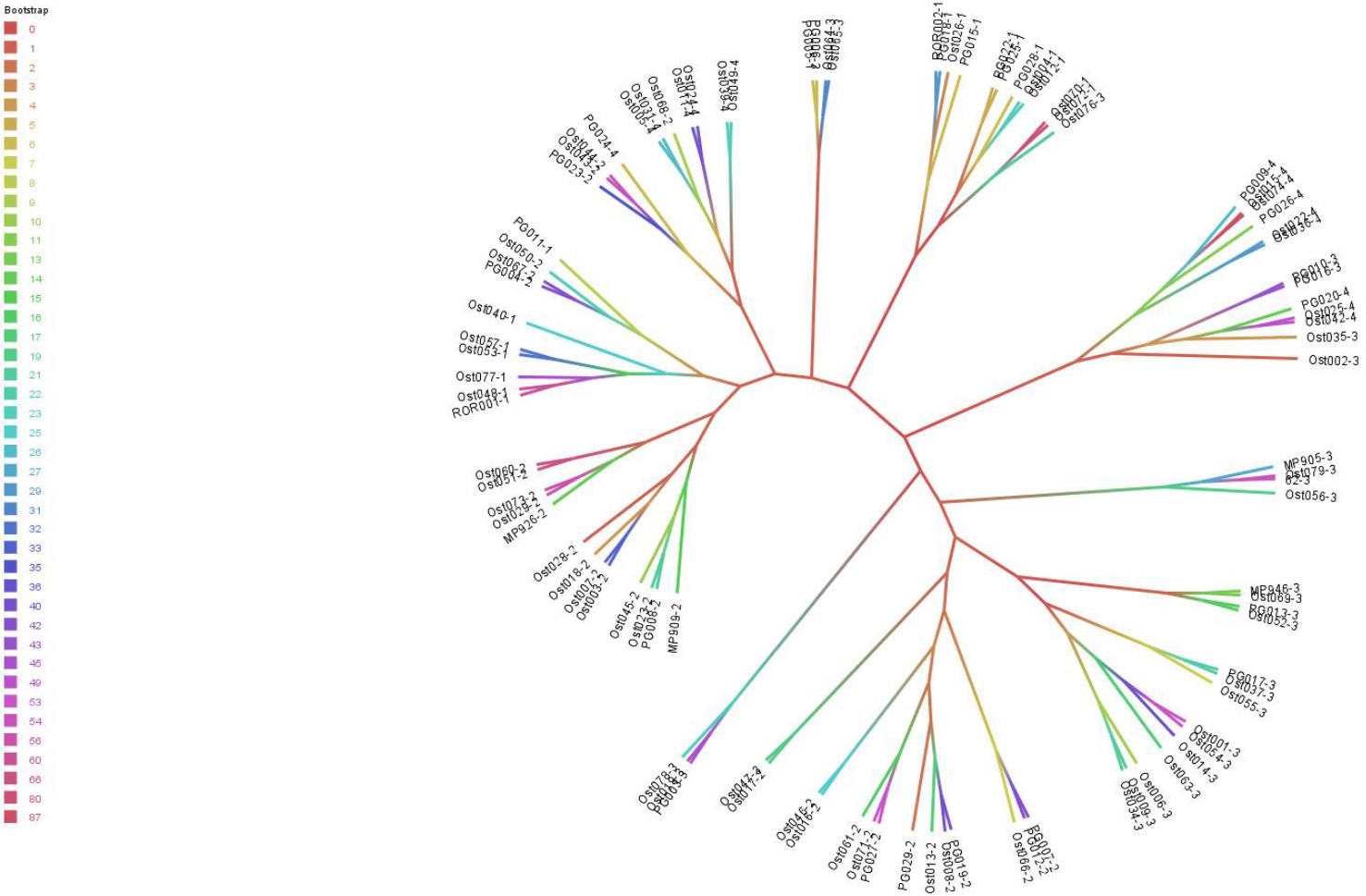


Figure 10. Unrooted neighbor-joining trees of NWCR individuals constructed using Da (A; Nei et al. 1983) and Fst (Latter 1972) over 1000 bootstrap replicates. Bootstrap values are represented by branch color. Tips are labelled with each individual, which nesting site they were sampled at, and to which DAPC they correspond.

Figure 10 Continued

B)



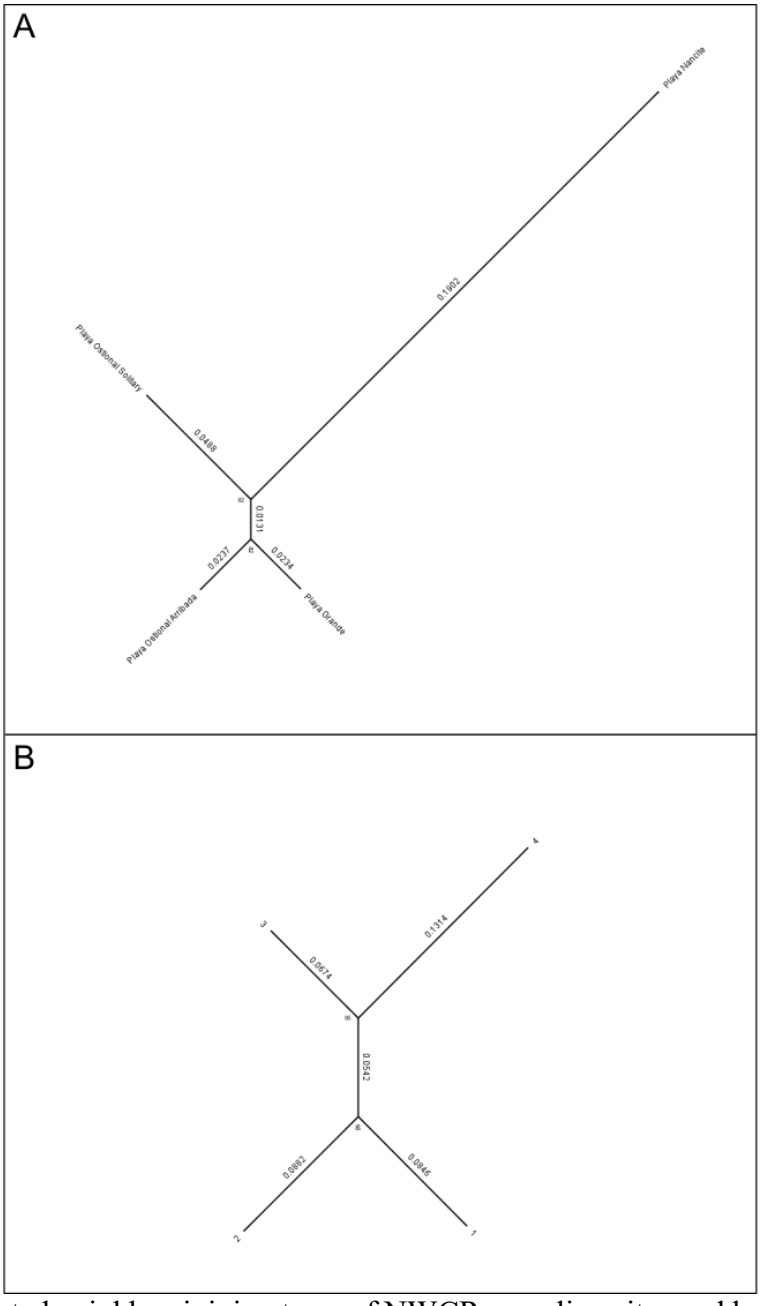


Figure 11. Unrooted neighbor-joining trees of NWCR sampling sites and behaviors (A) and DAPC clusters (B) constructed using Da (A; Nei et al. 1983) over 1000 bootstrap replicates. Nodes are labelled with bootstrap values, branches are labelled with genetic distances (Da) between sites/behaviors and nodes, and tips are labelled with nesting site and behavior (if applicable).

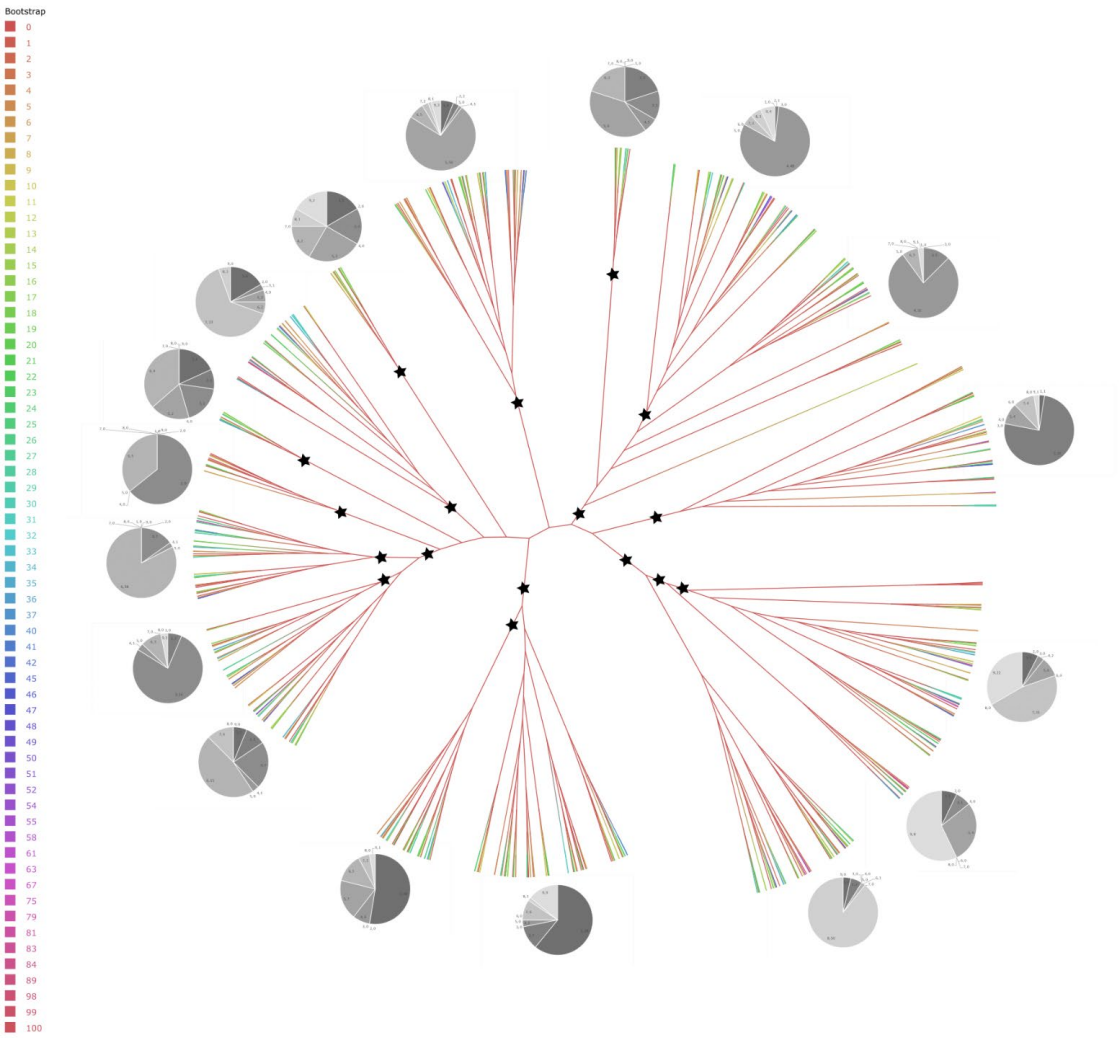


Figure 12. Unrooted neighbor-joining tree of ETP individuals constructed using Da (A; Nei et al 1983) over 1000 bootstrap replicates. Bootstrap values are represented by branch color. Tips represent individuals, but are unlabeled. Pie charts represent the proportion of individuals present from each DAPC cluster (1-9) in *ad hoc* clades, denoted on the tree by black stars. Sub-clades (i.e. stars following another star) have individual pie charts. Pie charts are located adjacent to the clades they represent.

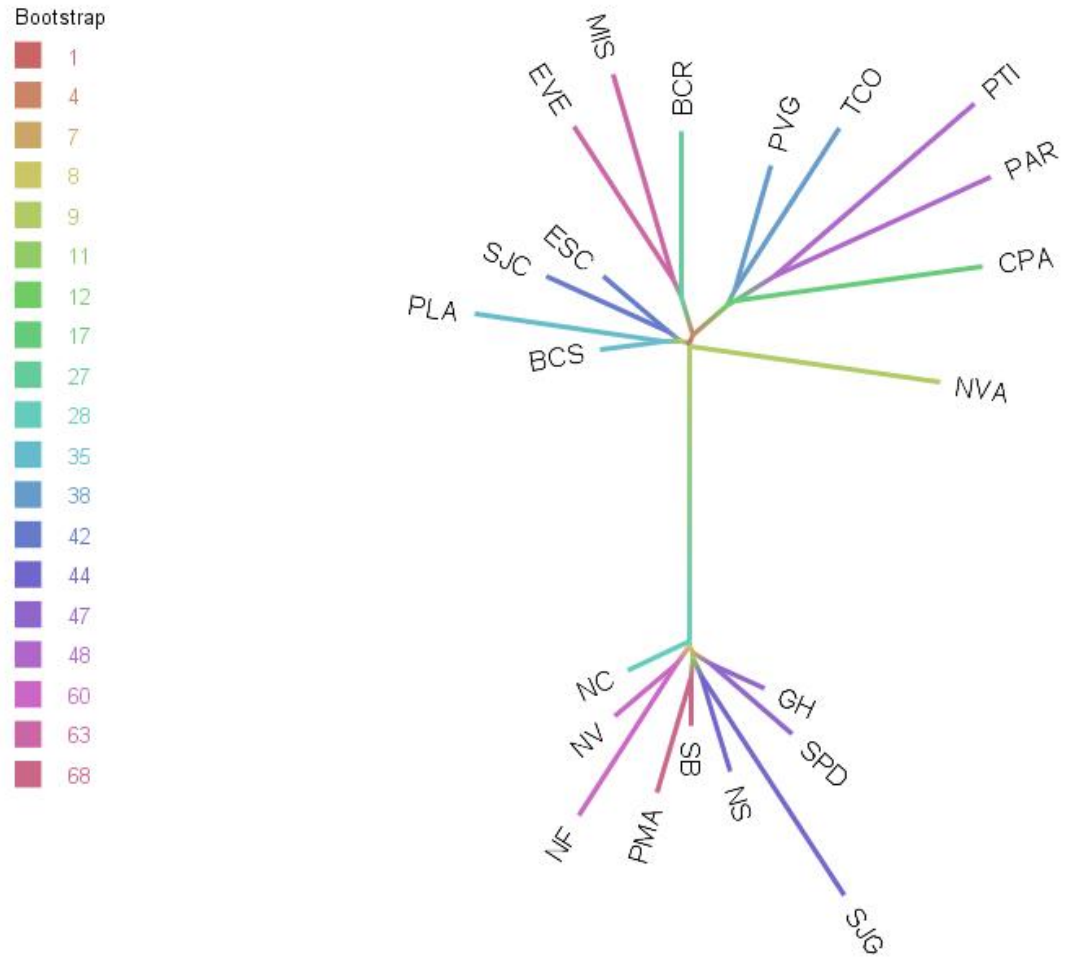


Figure 13. Unrooted neighbor-joining tree of ETP sampling sites constructed using Da (A; Nei et al. 1983) over 1000 bootstrap replicates. Bootstrap values are represented by branch color. Tips are labelled with sampling sites.

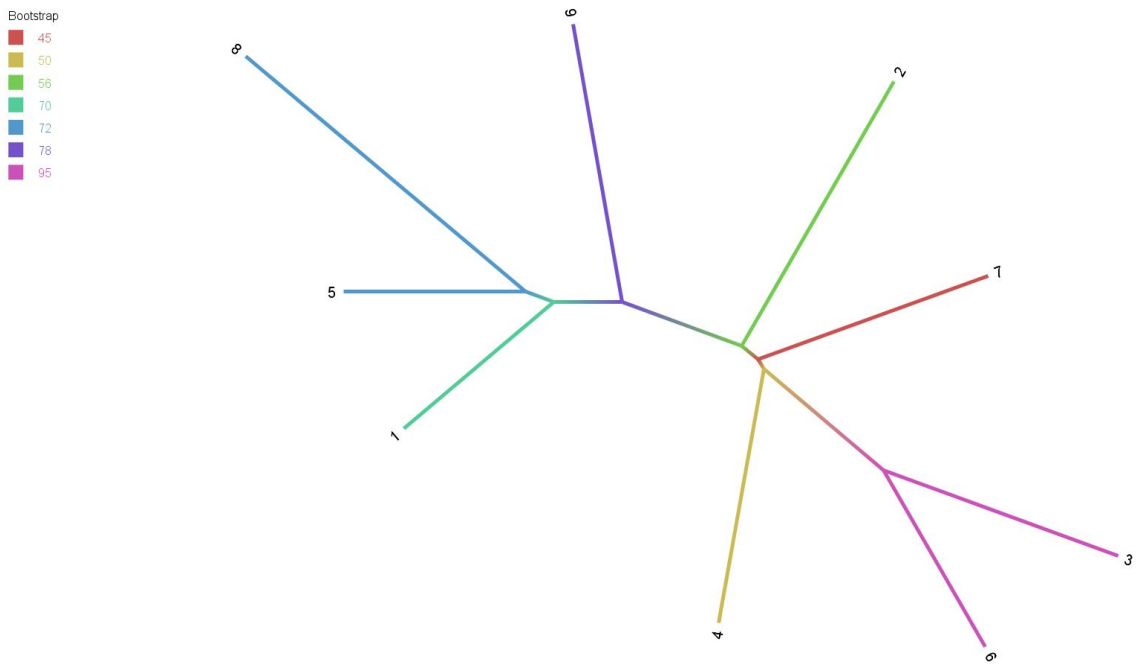


Figure 14. Unrooted neighbor-joining tree of ETP DAPC clusters constructed using Da (A; Nei et al. 1983) over 1000 bootstrap replicates. Bootstrap values are represented by branch color. Tips are labelled with DAPC cluster numbers.

DISCUSSION

Overview

This study was designed to answer 4 questions regarding NWCR and ETP OR population structure, and regarding the utility of population inference methods in general.

Question 1: Would mtDNA analysis support or refine population structure in NWCR and ETP ORs?

Analyses of mtDNA did not support or refine population structure in NWCR or ETP ORs. mtDNA did not suggest structuring between or within NWCR sites or differing behaviors, which confirmed Koval's (2015) findings of NWCR ORs belonging to one larger population. I was unable to compare our data set with a data set from Mexican ORs (Lopez-Castro and Rocha-Olivares 2005) due to issues detailed below, but it is possible that divergences within NWCR ORs and among ETP ORs are too recent to be detected in mtDNA haplotype data.

Question 2: Would analysis of ETP OR nDNA elucidate substructuring within Mexican and/or Central American populations?

Analysis of ETP OR nDNA elucidated substructuring within Mexican and Central American OR populations. In the ETP, hierarchical Bayesian analysis suggested weak structure within Mexican ORs, and weak, cryptic structure in Central American ORs (Figure 3). In Mexico, there is evidence that PAR may comprise a unique subpopulation (Fst=0.067), while in Central America population structure may not be easily related to nesting locations. STRUCTURE analysis of the entire ETP and of Mexican ORs did not strongly suggest this partitioning, which was highlighted by DAPC.

Ordination confirmed and possibly refined hierarchical STRUCTURE results. DAPC of ETP ORs by nesting site highlighted the singular nature of PAR ORs and refined that putative population (Figure 4A). DAPC by most likely groupings found nine clusters which largely corresponded to Mexican and Central American populations, but contained individuals from both populations (Figure 4B). Individuals nesting primarily at PAR and PTI comprised a unique

cluster separated from all other ETP ORs. This, as well as the near-even distribution of Central American ORs into 5 clusters, follow results of hierarchical Bayesian inference.

Question 3: Would DAPC produce cryptic clusters in both NWCR and ETP ORs?

DAPC produced cryptic clusters in both NWCR and ETP ORs. DAPC clusters from the ETP spanned multiple nesting beaches and were particularly admixed between sites in Central America (Figure 4B). It is impossible to determine if NWCR ORs would fit into the ETP DAPC clusters as the two data sets cannot be merged without extensive allele size calibration. I discuss possible explanations for this cryptic clustering in discussions of Ecological and Conservation Significance below.

Question 4: What processes might be contributing to the formation of inferred populations, and which inferred populations were best supported by multiple analyses?

Bottleneck events, related groups of individuals, and differential migration all contributed to the formation of inferred populations. Populations inferred in NWCR and the ETP using ordination were most consistently and strongly supported by all analyses, while populations inferred by Bayesian inference were less consistently and more weakly supported by all analyses.

Genetic Distance Analysis

The NJ tree of NWCR individuals showed that individuals grouped primarily by DAPC cluster, and indicated more support for DAPC groupings than for nesting sites/behaviors. As with NWCR ORs, ETP individuals in the NJ tree were grouped broadly by DAPC clusters, and there was higher bootstrap support for DAPC clusters than for nesting sites in those NJ trees, respectively. NJ trees do not appear to have the same power as Bayesian inference or ordination in inferring broad population structuring, as suggested by low bootstrap values at the base of most NJ trees presented here.

Bottleneck Analysis

Inferred structuring may have been due in part to bottleneck events. Bottlenecks were detected overall in both Mexico and Central America (Table 5), which supports inference for those two populations. This confounds Rodriguez-Zarate et al.'s (2018) findings of "isolation by

environment," as bottlenecked populations may have unique genetic signatures (Piry et al. 1999, Putman and Carbone 2014). In ETP ORs, isolation by environment may play a role in population structuring by facilitating or limiting gene flow and migration between sites and inferred populations. However, bottleneck events (particularly in Mexico) have likely contributed the formation of the Mexican population, and to the formation of subpopulation within Mexico.

Bottlenecks were detected at 9 continental nesting sites (7 in Mexico; 2 in Central America; Table 5). Bottlenecks detected at continental sites may be due to historically intense fishing pressure, harverst, and nest-site poaching, particularly in Mexico (i.e. Marquez et al. 1996). The bottleneck at PAR may have been severe enough to cause differentiation of that site from all other ETP sites. However, asymmetric migration may confound some bottleneck estimates (see Sundqvist et al. 2016 and references therein). PAR was identified as a possible population sink, which may confound bottleneck testing and might have caused PAR to display a unique signature in population structuring inference. Bottlenecks detected at BCS confirm findings of the Baja Peninsula as a unique population that may warrant designation as a unique MU (i.e. Lopez-Castro and Rocha-Olivares 2005, Rodriguez-Zarate et al. 2013).

Bottleneck events were not detected in NWCR or at all ETP sites. This may be due to a lack of bottlenecking at these sites or population recovery past the point of detecting bottlenecks at these sites. This may also be due to the limits of bottleneck metrics and software, specifically in their sensitivity to parameter inputs as seen in this study (Table 6; Putman and Carbone 2014). Migration was found to be significant from Mexico to Central America (Table 8), and it is possible that Mexican ORs are contributing to genetic rescues at Central American sites where I did not detect bottleneck events (Ingvarrson 2001).

Bottlenecks were detected by the two-tailed Wilcoxon test in 6 out of 9 ETP DAPC clusters (3 in Mexico, 3 in Central America. Table 5). It is difficult to determine exactly what might be leading to genetic bottleneck signatures in the DAPC clusters since they contain individuals from distant nesting beaches. However, many of the clusters with bottleneck signatures also had significantly high *F*is (Table 4). Low recruitment to clusters and mating between related individuals might produce bottleneck-like signatures, and this paradigm may have even been engendered by bottleneck events. I might also expect these results (bottleneck signatures and inbreeding) if the clusters reflect mating behaviors (see discussion of Ecological Significance below), and if ETP ORs display mating site fidelity.

Relatedness Analysis

Structuring, especially cryptic clustering in DAPC, may be explained by mating patterns, which could lead to high relatedness in clusters. In NWCR, relatedness was low across sites and behaviors but high within DAPC clusters. It is possible that relatedness was low at sites due to low nesting site fidelity in NWCR ORs. Sampling effort may also be suspect: if the NWCR OR population is very large relative to other OR populations, and NWCR ORs are no more or less fecund than ORs elsewhere, then the probability of sampling related individuals in NWCR will be lower than it is elsewhere. Arribadas number in the tens of thousands, and sampling efforts may have to be larger at arribada beaches to account for increased assemblage size.

Relatedness was higher at ETP nesting sites than at NWCR nesting sites and behaviors. This may be an artefact of sampling and genotyping (see Blouin 2003), but could be due to smaller populations outside of NWCR or higher natal site fidelity outside of NWCR. The latter would be a difficult phenomenon to explain if it were true, but could be due to: a) the abundance and proximity of nesting beaches, particularly arribada beaches, and a possible lack of evolutionary pressure to home to natal beaches in NWCR; b) pressure to avoid competition for suitable nesting habitat by nesting outside of NWCR; and/or c) low magnetic declination near NWCR leading to navigational miscalculations by nesting turtles (i.e. Brothers and Lohmann 2018).

Relatedness may serve as an indicator of nesting-site fidelity. Relatedness was low at sites and overall in NWCR. Relatedness in Central American ORs overall was higher than relatedness at Central American nesting sites on average, while relatedness in Mexican ORs overall was lower than relatedness at Mexican nesting sites on average. Nesting beaches in Mexico may partition related individuals better than the overall sample, which may be explained by natal site-fidelity. In Central America, individuals may display lower site fidelity, and thus the relatedness signature at nesting beaches is lower than for the overall population. This lowered site fidelity could explain the negligible relatedness signatures at NWCR nesting sites. Further, relatedness was higher in Central America overall than in Mexico overall. Central American ORs may generally display lower site fidelity than Mexican ORs, but be more-recently established, hence higher overall relatedness within the Central American population.

Relatedness was comparable between DAPC clusters and nesting sites in the ETP, as well as Mexican and Central American populations. In many cases, DAPC relatedness was higher

than relatedness in putative populations or at nesting sites. While it is possible that the DAPC clusters result from related groups of individuals, if this were the case then DAPC might have suggested at least some individual nesting sites (such as PAR) as unique clusters. This, as well as moderate-strong signatures of divergence mentioned above (i.e. pairwise *F*st and D, and AMOVA) suggest that the DAPC clusters are not artefacts of analysis but true groupings of individuals as a result of some evolutionary process(es). It may also lend support to the DAPC clusters as more effective population groupings for ETP ORs than STRUCTURE clusters (see discussion of Bayesian inference and ordination below).

Migration Analysis

Migration was comparable between NWCR sites/behaviors, but uneven between DAPC clusters. Migration indicated significant asymmetric gene flow between Mexican and Central American ORs, and highlighted specific source (MIS) and sink (PAR) populations. Migration was present between sites throughout the ETP, not solely within Mexico and Central America. Migration was limited between Mexican DAPC clusters, but significant between Central American clusters and from Mexican clusters to Central American clusters. This information is important for effective management of ETP ORs, and was not evident from Bayesian inference alone. DAPC grouped individuals from Mexico in primarily Central American clusters and vice-a-versa; these individuals may in fact represent recent migrants between the two populations. However, individuals from MIS (the primary source population in the ETP) were not often clustered with Central American individuals after DAPC.

NWCR OR mtDNA

Chelonian mitochondrial DNA has been shown to accrue mutations on a scale of tens of thousands to hundreds of thousands of years. Past phylogeographic studies of ORs have suggested that the EP population is no more than 250,000-300,000 years old (Bowen et al. 1997, Jensen et al. 2013B). Thus, while at least 14 control region haplotypes are reliably documented from EP ORs (Bowen et al. 1997, Lopez-Castro and Rocha-Olivares 2005, Jensen et al. 2013B), it is likely none have established themselves at a level similar to the basal haplotype (Lo46; Bowen et al. 1997, Lopez-Castro and Rocha-Olivares 2005, this study) despite evidence for population structuring (Rodriguez-Zarate et al. 2018). However, I found Lo46 at a slightly lower

rate in NWCR ORs (~70%) than has been reported for Mexican ORs (~90%; Lopez-Castro and Rocha-Olivares 2005). Lopez-Castro and Rocha-Olivares (2005) posited that the Baja Peninsula nesting population is basal to EP ORs given the high presence of Lo46. It is possible then that the lower rate of Lo46 found here in NWCR ORs may be characteristic of environmental partitioning between Mexican and non-Mexican ORs (Rodriguez-Zarate et al. 2018).

This hypothesis cannot be tested without an organized and complete global database of OR haplotypes and accurate and complete reporting of haplotype datasets. Haplotype frequencies at different sites may be compared using pairwise F-like statistics (i.e. Φst and **Θ**st; Wright 1949, Weir and Cockerham 1984) and AMOVAs (Excoffier et al. 1992) given complete datasets and corresponding sequences. Sequences from published studies should be retrievable from Genbank, and reported haplotype frequencies may then be reconstructed to test population differentiation hypotheses. When I attempted to do so for ETP ORs, I found serious discrepancies, ambiguities, and author-oversight in naming and reporting of haplotypes not just for ETP ORs but for ORs globally.

There are two databases that contain OR haplotypes: Genbank, and a database maintained by the National Marine Fisheries Service (NMFS; Peter Dutton, personal communication). Haplotypes on Genbank (n=45 as of this study) are publicly available but are not organized between studies. Many studies use the alphanumeric nomenclature first established by Bowen et al. (1997), but some use unique names for their sequences (see below). Haplotypes in the NMFS database (n=96 as of this study) are named sequentially from when they were provided to NMFS (i.e. Lo1, Lo2; see below), and are not publicly available, but some haplotypes named using the NMFS system are listed on Genbank (see Jensen et al. 2013B). Haplotypes presented in this study are named using the NMFS system, since the NMFS database is more complete than Genbank.

There are further issues with reported haplotypes: some reported haplotypes are not publicly available; some papers use multiple nomenclatures to describe single haplotypes; and some publicly available haplotypes are listed under different names than were reported in their original publication, sometimes sharing the same name with a different haplotype. For example, haplotypes Q-W are reported from ETP Olive ridleys (Lopez-Castro & Rocha-Olivares 2005) but are unavailable from Genbank and there is no polymorphic-site data to allow for manual

reconstruction of these haplotypes. This prevented us from using our mtDNA data to look for evidence for population structuring between Mexican and non-Mexican EP ORs.

Haplotype N (NMFS Lo46; Accession #'s AF051776 & AF514311; Bowen et al. 1997, Shanker et al. 2004B) is the most abundant haplotype known for EP Olive ridley turtles and was first reported at ~400bps (Bowen et al. 1997, Lopez-Castro & Rocha-Olivares 2005). However, on Genbank the ~800bp haplotype that matches the ~400bp haplotype "N" is labelled "K" (Accession # AY920519), identical to a haplotype "K" that is known only from the Indian population (Bowen et al. 1997, Shanker et al. 2004B). Further, the ~800bp haplotype labelled "N" (Accession # AY920521) matches the ~400bp haplotype "O" as described by Bowen et al. (1997).

Nearly every publication reporting OR haplotypes utilizes a unique naming system, in addition to the original haplotype names assigned by Bowen et al. (1997; Table 2). Shanker et al. (2004B) reported five haplotypes stemming from haplotype "K" (Bowen et al. 1997) in India. These haplotypes were named K-1 thru K-5. Jensen et al. (2013B) reported over 20 new haplotypes from IP turtles using NMFS conventions and explicitly stated which ~800bp haplotypes corresponded with ~400bp haplotypes reported by Bowen et al. (1997). Bahri et al. (2018) used the NMFS formula (seemingly without consulting NMFS) for naming sequences deposited in Genbank from Indonesian ORs. While Jensen et al. (2013B) used "Lo2" to name the ~800bp sequence that overlaps with the ~400bp sequence named "G" by Bowen et al. (1997), the ~800bp "Lo2" reported by Bahri et al. (2018) is entirely unique.

The lack of consistent, systematic haplotype naming is a preventable obstacle, yet there seems to be no inertia to push studies to systematically name haplotypes or to deposit unique or named haplotypes in Genbank (i.e. Plot et al. 2012, Bahri et al. 2018). mtDNA D-loop haplotype nomenclatures exist for Loggerhead (*Caretta caretta*) and Green (*Chelonia mydas*) sea turtles (i.e. http://accstr.ufl.edu/resources/mtdna-sequences/), Leatherback sea turtles (*Dermochelys coriacea*; Dutton et al. 1999, Dutton et al. 2013), and Hawksbill (*Eretmochelys imbricata*; Leroux et al. 2012) sea turtles. Researchers and managers are increasingly focusing on Olive ridley population structure, and such studies will benefit from a consensus, global nomenclature for Olive ridley control region haplotypes. It has been 20+ years since the last global phylogeography of ridley turtles (genus *Lepidochelys*) was published (Bowen et al. 1997), and a

new effort to study ridley phylogeography will necessitate better organization of mtDNA haplotypes.

Bayesian Inference vs. Ordination

Bayesian inference (BI), as implemented in STRUCTURE (Pritchard et al. 2000) proved useful at highlighting population structure on the scale of the entire ETP. However, I was unable to detect population structuring at the same resolution using BI as with ordination, as implemented in *adegenet* (Jombart et al. 2008). I was specifically unable to detect differentiation between PAR and other sites (which was confirmed by F-statistics, AMOVA, and ordination) without hierarchical analysis in BI. While it is possible that PAR comports a false signature of differentiation as a putative population sink, analysis using BI alone did not identify this possibility and would have perhaps lead to incorrect conservation efforts at PAR (see Conservation Significance below).

Population inference using STRUCTURE may have been limited by assumptions of Hardy-Weinberg equilibrium (HWE) and an inability to account for high levels of admixture between sites, particularly in the southern ETP. The presence of three linked loci in the CR dataset lowered resolution from eight loci to six loci when analyzing population structure using BI (Koval 2015). It is possible that including more than six loci would have allowed me to detect more than one population in NWCR ORs using STRUCTURE. Even with more loci, as in the EP-wide dataset, hierarchical analysis of STRUCTURE results did not elucidate discrete populations in the southern ETP, and only slightly resolved structure in Mexican ORs by highlighting the unique genetic signature of turtles nesting at PAR.

STRUCTURE and DAPC allowed me to detect broad divisions between Mexican and Central American ETP ORs, but inference using DAPC suggested finer-scale population structuring that inference in STRUCTURE did not suggest. Inference in STRUCTURE should in theory suggest these groups, as it performs well to identify populations with $F\text{st} \geq 0.02$ (Latch et al. 2006). Inference in STRUCTURE may have been unable to elucidate within-subpopulation structuring due to the clustering method and assumptions programmed into STRUCTURE. STRUCTURE is based on the theory that alleles within established natural populations will be in HWE. This presumption, briefly stated, means that relative allele and genotype frequencies at each locus should not deviate from expected frequencies. STRUCTURE uses a Bayesian model

and Gibbs sampling of a Monte-Carlo Markov Chain of simulated populations (*K*) to look for groups of individuals with loci in HWE (Pritchard et al. 2000). In this context, the probability of seeing my data (X) given groups of individuals (Z) with unique genetic signatures (P; P(X|Z,P)) may then be used (via ad hoc methods) to estimate the best *K*. Thus, for *K*=2, individuals fit into 1 of 2 ancestral populations (excluding admixed individuals) with different allele frequencies.

The assumption of populations with different allele frequencies that are in HWE may not be appropriate for all data (Pritchard et al. 2000, see examples in Kalinowksi 2011 and Putman and Carbone 2014). HWE arises when gene-flow between populations is minimal. STRUCTURE implements algorithms to account for admixture and migrant individuals (Pritchard et al. 2000), and populations with correlated allele frequencies (Falush et al. 2003), but only when both occur at relatively low levels (i.e. probability of migration = 0.001-0.1; Pritchard et al. 2000). In both data sets analyzed here, clusters identified by DAPC did not have allele frequencies that differed notably from parent populations (i.e. overall in Koval 2015, and Mexican and Central American in Rodriguez-Zarate et al. 2018). This could be due to gene flow between clusters in both data sets, particularly in Central American ORs. STRUCTURE may also require many loci (i.e. 200-300) and a certain minimum number of non-migrant or non-admixed individuals to recognize numerous migrant and admixed individuals (Pritchard et al. 2000). Low-enough levels of admixture for STRUCTURE to define *K* may only occur at a broad scale (i.e. Mexican vs non-Mexican sites) in ETP ORs. High migration between Central American clusters may have lowered the likelihood cost of STRUCTURE grouping all Central American sites together, rather than clearly identifying DAPC-like clusters (i.e. Kalinowski 2011; see below).

While inference in STRUCTURE distinguishes sea turtle populations on a broad scale (i.e. Rodriguez-Zarate et al. 2018), sea turtles may exhibit levels of intra-population gene flow and migration that exceeds STRUCTURE's capabilities on finer scales. All sea turtles display some level of natal breeding philopatry (Lohmann et al. 2017), in which they return to the region that they originated from to breed. Female turtles may even return to the exact beach from which they hatched to lay their own nests. Male sea turtles, not limited by the need for a nesting beach, are known to show limited natal breeding philopatry relative to female sea turtles. Studies that examine both mtDNA and nDNA from the same individual nesting females may find more evidence for structuring with mtDNA than with nDNA (FitzSimmons et al. 1997, Roberts et al.

2004), which suggests males may breed with multiple females from relatively distant nesting beaches. This could confound clustering in STRUCTURE, as any sampled female may have been fathered by an unsampled male that might have originated from a different population (or nesting area). Further, female natal beach site-fidelity is thought to be limited in ETP ORs (Kalb 1999), and any female at one nesting beach may have originated from a more distant nesting beach. This seems to be the case in NWCR ORs and non-Mexican ORs especially, as relatedness values within sampling sites were negligible (NWCR) and less than relatedness within Mexican nesting sites (ETP: Central America). Multiple sources of admixture between potential subpopulations such as DAPC clusters (i.e. admixed heredity and low site fidelity) could have led analysis of STRUCTURE results to suggest the most likely K=2.

As hinted at above, the lack of male genetic information in both data sets is problematic. Due to natal breeding fidelity, we assume that sea turtle populations should be structured around nesting beaches. The samples here consist of female nesting turtles with putatively limited natal breeding fidelity, who are offspring of females with putatively limited natal breeding fidelity and males with perhaps even more limited natal breeding fidelity. Further, since female ORs are thought to reach sexual maturity at 10-15 years of age (Zug et al. 1998) and display varied reproductive phenology (breeding remigration of 1-2 years; Kalb 1999, Dornfeld et al. 2015), the females sampled here are likely offspring from different reproductive seasons, each comprised by different individuals with different genetic makeups. Perhaps a more appropriate sampling regime for future population studies would be to sample hatchlings from nests from the geographic range of the study. This would provide more information towards identifying contemporary admixture and structure based on all breeding individuals, and would not be biased towards males or females (as hatchling sex may not be identified based on external morphology).

In determining the most likely K, STRUCTURE may settle on local likelihood maxima and choose mathematically cost-effective structuring schemes in favor of real, yet less-cost-effective schemes (Pritchard et al. 2000, Kalinowski 2011). Kalinowski (2011) showed this with simulated hierarchical populations. In ETP ORs, STRUCTURE may be correctly identifying Mexican and non-Mexican populations, but ignore finer-scale structuring. In analysis of the Mexican population alone, analysis of STRUCTURE results identifies PAR as a unique population, but groups all other sites together. DAPC results also identify individuals from PAR, and PTI, as members of a unique cluster. A neighbor-joining tree of all ETP sites confirms the

Mexican/non-Mexican split, but also provides evidence for hierarchical structure within the Mexican population. PAR and PTI, the two sites that comprise DAPC cluster #8, are most-closely related to a clade that includes PVG, CPA, and TCO. The NJ tree also groups EVE, MIS, and BCR (DAPC cluster #5) and BCS, PLA, SJC, and ESC (DAPC clusters #1 & 9) together. The PAR-PTI clade may be preventing STRUCTURE from identifying other relationships between nesting sites, as in simulated populations in Kalinowksi (2011).

ETP ORs might be thought of in the same framework as simulated populations in Kalinowski (2011). STRUCTURE has two options: 1) separate PAR from Mexican and Central American ORs such that K=3; or 2) include PAR in the Mexican population such that K=2. STRUCTURE confers a likelihood penalty for increasing K to 3 for PAR, but also confers a likelihood gain for resolving population structuring. On the other hand, STRUCTURE confers a likelihood gain for inferring only 2 populations, but might confer slightly less of a gain for including PAR in the Mexican population even though PAR has a unique genetic signature. When all is summed, it may be more "cost-effective" for STRUCTURE produce results that infer K=2 rather than 3.

Analyzing data hierarchically within STRUCTURE (i.e. the entire ETP, then just Mexico, then just Central America) can elucidate such population structuring, but with each level of analysis comes decreased analytical power through the loss of individual genotypes and potentially some alleles. Additionally, it is difficult to know at which level it is appropriate to cease such an analysis. After analyzing Mexican sites separately, is it appropriate to remove PAR and repeat the analysis? It would not seem so based on STRUCTURE bar plots, since individuals were still not completely assigned to the PAR group, and individuals at other sites were also admixed. F-statistics and AMOVA would suggest removal of PAR and re-analysis, but they do not operate within the STRUCTURE framework and it is not necessarily appropriate to force population inference in STRUCTURE based on results from external analyses.

Population inference in DAPC detected fine-scale, cryptic structuring in both NWCR and ETP ORs. In DAPC, genotypes are decomposed into coordinate data via PCA. DAPC then uses a k-means clustering algorithm to delineate clusters, the most likely of which is determined using a Bayesian Information Criterion (BIC). As implemented in DAPC, k-means and BIC identify the most likely K as having the least within-group variance while penalizing higher values of K. The assumptions, implications, and limits of DAPC are not well known (Putman and Carbone 2014).

Jombart et al. (2010) concede that k-means may not detect the correct clusters in complex situations, and that some clusters may be artefacts of the algorithm. Another issue is the ad-hoc implementation of DAPC, in which the user chooses how much % variance and how many principal axes to retain. Here, I follow Jombart et al.'s (2010) suggestions for retaining % variance and principal axes, and perform cross validation (within adegenet) to minimize error when retaining principal axes. However, there remains subjectivity in balancing discriminatory power and overfitting clusters (i.e. through retaining more principal axes; Jombart et al. 2010).

DAPC has been shown to outperform STRUCTURE in inferring clusters in populations with complicated structuring, such as with hierarchical-island and hierarchical-stepping stone models (Jombart et al. 2010). In the first model, random-mating subpopulations are confined to "islands". There is gene flow within and between islands, but the latter is of less magnitude than the former. In the second model, random-mating subpopulations are confined to distinct zones. There is gene flow between adjacent subpopulations within each zone, and limited gene flow between zones, typically at a point of contact. In simulated populations with these models (K=6 and 12, respectively), STRUCTURE predicted K= 2 and 3, respectively. DAPC predicted K=6 and 11 respectively.

Hierarchical structuring may be occurring in ETP ORs, thus explaining branching in the NJ trees and the clusters detected by analysis of DAPC results and not STRUCTURE results. Sea turtle populations may not fit standard island or stepping stone hierarchical models. A hierarchical stepping stone model might be appropriate for sea turtles assemblages with high breeding site fidelity, in which females nest very close to their natal beach, and where females and males only mate near their natal beach. Then, along a stretch of coast, there should be genetic variation along a gradient and some gene flow between adjacent nesting assemblages. This is the case for loggerhead turtles (*Caretta caretta*) nesting in the western Atlantic ocean, which display mitochondrial variation along a gradient from Virginia, USA to south Florida, USA, and then around the Florida panhandle into the Gulf of Mexico (Shamblin 2012) In the ETP, such a pattern might be disrupted by environmental factors near the interface between Mexican and Central American nesting beaches (Rodriguez-Zarate et al. 2018). However, while relatedness values suggest some level of fidelity to nesting sites across the ETP, migration appears to be minimal between relatively adjacent beaches in Mexican and Central American ORs (Table 7).

The hierarchical island model, in which multiple subpopulations comprise broad, relatively genetically isolated populations, may be better suited for ETP ORs, but perhaps not purely by nesting site. Migration is present (sometimes at comparable levels) between all sites, not only between adjacent sites within Mexican and Central American populations, and is significant from some Mexican sites to Central American sites (Table 7). Migration is also lower between Mexican clusters and between Mexican and Central American clusters than it is between Central American clusters (Table 9). In a true hierarchical island population, migration is expected be highest between subpopulations within broad populations, but not between broad populations. This suggests that DAPC may have elucidated randomly mating subpopulations with minimal gene flow in Mexico, at least. However, migration is high between non-Mexican clusters, which may be due to relative panmixia or recent divergence in non-Mexican ORs, even when separated by site or cluster. This is not to shed doubt on the non-Mexican clusters, but rather suggests that they are not as discrete as Mexican clusters, which were even weakly detected by STRUCTURE (ie PAR, cluster #8).

Thus, ETP ORs may comprise as many as 9 discrete genetic units. These units are generally split into Mexican and Central American groups, but do not strictly adhere to Mexican and Central American populations. There is evidence for significant gene flow between nesting sites and all 9 groups in the ETP, particularly within Central America and from Mexico to Central America. The Mexican clusters (1, 5, 8, 9) may comprise discrete populations in a hierarchical island model, while the Central American clusters (2, 3, 4, 6, 7) may comprise subpopulations of a broader Central American population in the same model.

STRUCTURE assumes that populations are in a state of relative isolation and equilibrium, especially across broad distances, and does not perform well in identifying hierarchical structuring. ETP ORs, and many other sea turtle populations in general, may not fit STRUCTURE's assumptions well-enough for analysis of STRUCTURE results to accurately suggest populations. DAPC may be better suited for analyzing population structure in sea turtles, which are highly migratory, have enormous potential for broad migration and genetic connectivity, and may in many cases be recovering from anthropogenic bottleneck events. This is supported with the weight of bottleneck, relatedness, migration, and phylogenetic analyses.

Ecological Significance

Population inference in DAPC elucidated genetically discrete clusters in ETP ORs that may have formed from bottleneck events, family lineages, and/or cryptic mating behaviors. Sea turtle populations declined globally in the 19th and 20th centuries due to fisheries impacts (Lewison and Crowder 2007), as well as legal and illegal take of turtles for consumptive use (Valverde et al. 2012, Foran and Ray 2016). This is true for the ETP, where legal take of OR eggs is still allowed at Playa Ostional, and sporadic poaching of females and eggs still occurs range-wide. ETP OR populations have begun to recover since the mid-20th century (Abreu and Plotkin 2012), although some assemblages are thought to be in decline (Fonseca et al. 2009), and in some cases entire arribada nesting assemblages have been lost (see Marquez et al. 1996).

Such bottleneck events may leave detectable genetic signatures, as was the case in some ETP sites, putative populations, and DAPC clusters (discussed in Conservation Significance below). However, not all sites and clusters displayed bottleneck signatures. At nesting sites structuring and relatedness may be due to site fidelity (see above). In DAPC clusters without bottleneck signatures, it is possible that family lineages contributed to contemporary structuring. If certain limited lineages were reproductively successful during the peak of sea turtle take in the ETP, descendants of these lineages could comprise contemporary ETP ORs. As those descendants reached sexual maturity, they could have nested with limited natal site fidelity over generations, perhaps constrained by oceanographic features (i.e. Rodriguez-Zarate et al. 2018, see below). DAPC may have detected such lineages, which could explain the relatedness of turtles within clusters.

Since oceanographic features are known to play a role in genetic structuring at the scale of the entire ETP (Rodriguez-Zarate et al. 2018), perhaps they also play a role within Mexican and Central American populations. If minor oceanographic features are more prominent offshore of Mexico than offshore of Central American countries, descendants of bottlenecked lineages may have been structured, and may continue to be structured, more so in the Mexican population than in the Central American population, hence the low levels of migration detected between Mexican clusters. In Central American ORs, oceanographic currents may not prevent migration and may even facilitate it, thus explaining the connectivity between clusters and relatively low levels of relatedness at nesting sites. Connectivity between Mexican and Central American ORs may be dictated by broad scale currents such as the California Current and Equatorial Currents.

Baja California del Sur, MX (BCS) ORs are found in every Central American DAPC cluster, and individuals from Mismaloya, MX (MIS) may migrate as far south as Panama (PMA). It is possible that some of these putative migrants may have followed the California Current south from Mexico, and then been directed to Central America via the Equatorial Counter Current and/or Costa Rica Coastal Current (see Figure 1a in Rodriguez-Zarate et al. 2018).

The clusters could be due to contemporary genetic structuring through mating at discrete locations. Little is known about the specific locations where sea turtles mate (Rees et al. 2016). Although some mating occurs offshore of nesting beaches, it may occur elsewhere, such as on migratory routes between foraging and breeding areas, and perhaps even at foraging areas. Sea turtles tend to display multi-year fidelity to foraging grounds (Broderick et al. 2007, Schofield et al. 2010). If turtles utilize discrete, even if broad, foraging grounds and mating occurs at or en route to foraging or breeding grounds, I may expect to observe structuring that corresponds to foraging regions. ETP ORs are thought to be nomadic foragers with broad foraging ranges (Eguchi et al. 2007, Plotkin 2010), but comprehensive data on foraging ETP ORs, such as home range estimates from telemetry data and stable isotope values, do not exist. If ETP ORs display some level of fidelity to broad foraging regions and mating occurs at foraging grounds or between foraging and nesting grounds, then it may contribute to the structuring detected by DAPC. However, more data are needed to investigate correlations between genetic structuring and trophic and spatial ecology of ETP ORs.

There was not strong evidence to suggest that ordination captured specific migration patterns between sites and putative populations. Although clusters contained individuals from both populations, they did not always contain individuals from a significant source population in Mexico (MIS). DAPC may have unintentionally identified demographically discrete populations with few immigrants in Mexico, but did not do so in Central America. DAPC clusters may reflect deeper, or different, population structuring than can be captured in estimates of migration.

Conservation Significance

Conserving highly migratory, long-lived species such as sea turtles is a difficult endeavor. Sea turtles occupy diverse habitats and shift habitats ontogenetically, and resources for conservation efforts are limited. Time-series abundance data from nesting beaches and foraging grounds may identify high-use areas, and conservation plans may focus on areas with the highest

density of turtles or areas experiencing declines in abundance. However, such plans do not account for population connectivity and gene-flow that can only be elucidated by genetic studies. For example, one foraging ground may contain turtles from three genetically discrete populations. Although abundance data may indicate stability, genetic data may show that individuals from one population are declining at that site. Such data allow researchers and conservationists to investigate those declines and design plans to aid in population recovery.

Designating genetically discrete populations is therefore critical to effectively conserving sea turtles, but it must be done so correctly and practically. Here, I show that population inference in STRUCTURE only elucidates broad populations of ETP ORs, while DAPC uncovers fine-scale structuring. ETP ORs are certainly split at the Mexican-Central American interface (Rodriguez-Zarate et al. 2018), but there is important sub-structuring within these populations and connectivity between these populations. Although the clusters are difficult to explain, they merit investigation and conservation planning. The clusters may comprise MUs, but MUs are typically spatially continuous groups of nesting assemblages. It may be more appropriate to designate the clusters as Demographically Independent Populations (DIPs; Komoroske et al. 2017). DIPs exhibit low levels of differentiation but comport conservation importance (see Taylor et al. 2010, Dutton et al. 2013). DAPC cluster 8 provides one obvious example of a putative DIP, although PAR alone might comprise an MU worthy of prompt conservation planning. It is possible that each DAPC cluster may be a unique DIP, and should be incorporated into management plans as such, especially after further investigation. This is a challenging notion, but if the DAPC clusters could be related to mating at discrete foraging grounds, protecting those areas could ensure the persistence of the DAPC clusters. Satellite telemetry of post-nesting Olive ridleys will be crucial to determining the formation of the DAPC clusters. Finding discrete high use areas that correspond with the DAPC clusters would add a tangible, spatial element to population structure in ETP ORs that managers and conservationists can protect by designating Marine Protected Areas, for example. Further, DAPC highlighted connectivity between Mexican and Central American ORs (see Figure 4) that was confirmed by migration estimates (Tables 7 and 9, Figures 8 and 9). This connectivity spans ~3,500 km as the crow flies (between BCS and PMA), and can be affected if not interrupted by unregulated fisheries and shipping industries. Management and conservation plans must account for this broad connectivity. Knowledge of migratory routes and strategies used by ORs, gained via

satellite telemetry, will be important if there is to be effective conservation of ORs away from nesting beaches.

Some of the putative DIPs showed evidence for inbreeding (Table 4) and bottleneck events (Table 5). It should be of concern to managers and conservationists that these process might be going on for genetically discrete populations of unknown origins across multiple, distant nesting beaches. If recent or ongoing take is not occurring at nesting beaches, it may be occurring elsewhere, such as at foraging grounds or along migratory routes. Although I did not test for decreases in population size here, ORs are globally in decline (IUCN 2018). By focusing conservation efforts on nesting beaches, managers and conservationists may be missing an unidentified population outflow for ETP ORs. Concern should be highest in the Mexican population of ETP ORs, where 3 out of 4 putative DIPs showed evidence of bottleneck events, and all putative DIPs showed significant inbreeding.

Population inference using ordination, and not Bayesian inference, efficiently produced well-supported groupings in ETP ORs that should be incorporated into conservation and management plans, rather than results of Bayesian inference alone. Designing conservation plans based solely on panmictic Mexican and Central American populations would be incorrect and impractical. Monitoring even a majority of the nesting habitat in Mexico would be impossible, and such a plan might suggest monitoring only beaches with the highest abundance of nesting turtles. Monitoring away from nesting beaches (i.e. at foraging areas) is relatively expensive and might seem wasteful in this scenario, since the turtles at any given beach all come from the same genetic population and it should be possible to examine population trends for all Mexican ORs by monitoring nesting beaches.

In Central America, a management plan for a panmictic population would require cooperation from conservation entities from as many as 6 nations. When determining priority areas for monitoring and protection, conservation efforts and funding in a regional management framework might be biased towards nations with the highest abundance of nesting ORs, namely Costa Rica and Panama (due to the presence of arribada beaches). Alternatively, each nation might enact individual plans to monitor their own nesting beaches, eschewing standardization or a regional conservation mindset. These scenarios may already be playing out, and the lack of cooperation, standardization, and urgency in conserving ORs could be contributing to their global decline (IUCN 2018).

Ordination, as implemented in DAPC, provides us with a higher-resolution picture of population structure in ETP ORs than does STRUCTURE, and could allow for more practical and effective management plans. Ordination highlights the presence of Mexican and Central American populations, but highlights connectivity between both populations and of connectivity and internal structure within both populations. Conservation plans based on ordination could account for that connectivity and internal structuring, and could examine possible causes of bottleneck events and inbreeding not strictly related to nesting sites. The PTI-PAR DIP is a clear example of this. A conservation plan premised upon panmixia might not suggest monitoring at PTI or PAR if other sites were more frequently used. Ordination highlights that turtles at those sites are genetically unique and provides impetus for monitoring at those beaches. Further, ordination provides impetus to investigate connectivity between PTI and PAR, as well as the processes that form these putative DIPs in general.

Conservation at nesting beaches alone may not protect every genetically discrete group of ETP ORs. Conservation at foraging grounds might be an important facet of a regional conservation plan, if it would work towards preserving the PTI-PAR cluster and other putative DIPs. Such a plan would also necessitate spatial and temporal investigations of mating behaviors to better understand the DAPC clusters. If clusters do correspond to mating at specific sites or regions, it may be possible to highlight those areas in conservation planning. These areas may overlap with fishing grounds or shipping lanes, and policies could be implemented to mitigate impacts from these industries on discrete clusters. Identifying these hotspots in waters belonging to Central American nations might provide a useful framework for a regional conservation plan for otherwise panmictic ORs. For example, if one of the clusters corresponds to turtles that are found at a foraging ground off of Nicaragua, the country could enact policies to protect that foraging ground. In doing so, they would likely protect turtles that nest along the entire Central American coast and directly contribute to the conservation of the species as well as to preserving a vast eco-tourism industry based on sea turtles (Delgado and Nichols 2005, Senko et al. 2011). The clusters provide a more tangible path towards regional conservation than do panmictic Mexican and Central American populations.

Conclusion

Sea turtles are difficult species to conserve due to their long migrations and broad distributions. Defining MUs and other discrete population units such as DIPs around which to base conservation plans must be done correctly to effectively conserve genetically discrete populations of sea turtles. In this study, ordination provided a more practical and nuanced framework for defining MUs and DIPs in ETP ORs than did STRUCTURE. This may be due to hierarchical structuring within ETP ORs that may be present in other sea turtle populations and species. In the case of ETP ORs, hierarchical structure may be an artefact of recent population bottlenecks and subsequent recolonization of nesting beaches, or due to mating at foraging grounds or along migratory routes.

It appears that population inference based on BI, such as is implemented in STRUCTURE, may not be best suited for defining MUs and DIPs for sea turtles with hierarchical structuring. Gene flow between distant breeding areas may lead to the violation of critical assumptions made by programs using BI, namely that the most suitable discrete populations display different allelic signatures, are in Hardy-Weinberg equilibrium, and are not highly admixed. The "best" genetically discrete groupings of sea turtles may not always conform to these constraints. Barriers that prevent gene flow may be relatively limited in the ETP and marine environments generally (Hellberg et al. 2002), or may only be present at broad spatial scales (i.e. Rodriguez-Zarate et al. 2018). Migration between genetically discrete groupings may be common in sea turtles given their migratory nature, and perhaps even evolutionarily advantageous (i.e. through genetic rescue; Ingvarsson 2001). Ordination makes no assumptions about the characteristics of genetically discrete populations, and simply maximizes variance between groups of the most genetically similar individuals.

Results from exploratory ordination must be carefully examined in the context of the study system, and can be supplemented with analyses to understand how genetically discrete groupings may have formed. Here I showed that ordination validated broad-scale population structure, recognized gene flow between broad populations, and grouped related individuals together within populations in what may represent DIPs not tied to nesting beaches. This connectivity and lack of terrestrial structuring is feasible for sea turtles, was supported by bottleneck, relatedness, and migration analyses, and merits investigation, both as an ecological phenomenon and as basis for a management framework. Microsatellite data from previous

studies that relied only on BI of genetically discrete sea turtle populations should be re-analyzed with ordination, and results should be compared between both methods and investigated to better understand how these discrete groupings formed and interact with one another. Future studies should always compare multiple exploratory (Bayesian inference, ordination, phylogenies) and explanatory (bottleneck, relatedness, migration) methods for population inference. The outcome may lead to at least subtle, but important, changes in the designation of MUs, DIPs, and management plans for sea turtles globally, and could lead to a better understanding of sea turtle biology and life history.

REFERENCES

Abreu-Grobois FA, Horrocks J, Formia A, Dutton PH, LeRoux R, Velez-Zuazo X, Soares L, Meylan P (2006) New mtDNA Dloop primers which work for a variety of marine turtle species may increase the resolution of mixed stock analyses Proceedings of the 26th Annual Symposium on Sea Turtle Biology Isle of Crete, Greece, p 179

Abreu-Grobois A, Plotkin P (2012) Lepidochelys olivacea The IUCN Red List of Threatened Species

Alcala N, Goudet J, Vuilleumier S (2014) On the transition of genetic differentiation from isolation to panmixia: what we can learn from GST and D. Theor Popul Biol 93:75-84

Avise JC, Bowen BW, Lamb T, Meylan AB, Bermingham E (1992) Mitochondrial DNA evolution at a turtle's pace: evidence for low genetic variability and reduced microevolutionary rate in the Testudines. Mol Biol Evol 9:457-473

Bahri S, Atmadipoera AS, Madduppa HH (2018) Genetic Diversity of Olive Ridley Lepidochelys Olivacea Associated with Current Pattern in Cendrawasih Bay, Papua. Jurnal Ilmu dan Teknologi Kelautan Tropis 9:747-760

Balazs GH, Chaloupka M (2004) Thirty-year recovery trend in the once depleted Hawaiian green sea turtle stock. Biol Conserv 117:491-498

Beltran JA, Campista S, Peinado LI, Cornejo I, Castillo H, Martin del Campo JR, Sanchez JG (2016) Genetic diversity of the Olive ridley turtle (Lepidochelys olivacea) in northwestern Mexico: limit distribution. Universidad Autonoma de Sinaloa

Bernardo J, Plotkin PT (2007) An evolutionary perspective on the arribada phenomenon and reproductive behavioral polymorphism of olive ridley sea turtles (Lepidochelys olivacea). In: Biology and Conservation of Ridley Sea Turtles, p 59-87

Blouin MS (2003) DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. Trends Ecol Evol 18:503-511

Bouzat JL (2010) Conservation genetics of population bottlenecks: the role of chance, selection, and history. Conserv Genet 11:463-478

Bowen BW, Clark AM, Abreu-Grobois FA, Chaves A, Reichart HA, Ferl RJ (1997) Global phylogeography of the ridley sea turtles (Lepidochelys spp.) as inferred from mitochondrial DNA sequences. Genetica 101:179-189

Bowen BW, Karl SA (2007) Population genetics and phylogeography of sea turtles. Mol Ecol 16:4886-4907

Broderick AC, Coyne MS, Fuller WJ, Glen F, Godley BJ (2007) Fidelity and over-wintering of sea turtles. Proc R Soc B 274:1533-1538

Brothers JR, Lohmann KJ (2018) Evidence that magnetic navigation and geomagnetic imprinting shape spatial genetic variation in sea turtles. Curr Biol 28:1325-1329. e1322

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Mol Ecol 9:1657-1659

Clukey KE, Lepczyk CA, Balazs GH, Work TM, Lynch JM (2017) Investigation of plastic debris ingestion by four species of sea turtles collected as bycatch in pelagic Pacific longline fisheries. Mar Pollut Bull 120:117-125

Clusella Trullas S, Spotila JR, Paladino FV (2006) Energetics during hatchling dispersal of the olive ridley turtle Lepidochelys olivacea using doubly labeled water. Physiol Biochem Zool 79:389-399

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001-2014

Cortes APR, Suárez SS, Noria OC (2015) Evaluación de la diversidad genética de Lepidochelys olivacea en la colonia de anidación Ixtapilla, Michoacán. Biológicas Revista de la DES Ciencias Biológico Agropecuarias Universidad Michoacana de San Nicolás de Hidalgo 16:31-35

Cowen RK, Sponaugle S (2009) Larval dispersal and marine population connectivity. Annual Review of Marine Science 1:443-466

Davies NB, Krebs JR, West SA (2012) An introduction to behavioural ecology, Vol. John Wiley & Sons

Delgado S, Nichols W (2005) Saving sea turtles from the ground up: awakening sea turtle conservation in northwestern Mexico. Marit Stud 4:89-104

Di Rienzo A, Peterson A, Garza J, Valdes A, Slatkin M, Freimer N (1994) Mutational processes of simple-sequence repeat loci in human populations. Proceedings of the National Academy of Sciences 91:3166-3170

Dias PC (1996) Sources and sinks in population biology. Trends Ecol Evol 11:326-330

Dornfeld TC, Robinson NJ, Tomillo PS, Paladino FV (2015) Ecology of solitary nesting olive ridley sea turtles at Playa Grande, Costa Rica. Mar Biol 162:123-139

Dutton PH, Bowen BW, Owens DW, Barragan A, Davis SK (1999) Global phylogeography of the leatherback turtle (Dermochelys coriacea). J Zool 248:397-409

Dutton PH, Roden SE, Stewart KR, LaCasella E, Tiwari M, Formia A, Thome JC, Livingstone SR, Eckert S, Chacon-Chaverri D (2013) Population stock structure of leatherback turtles (Dermochelys coriacea) in the Atlantic revealed using mtDNA and microsatellite markers. Conserv Genet 14:625-636

Earl DA (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Cons Gen Res 4:359-361

Eguchi T, Gerrodette T, Pitman RL, Seminoff JA, Dutton PH (2007) At-sea density and abundance estimates of the olive ridley turtle Lepidochelys olivacea in the eastern tropical Pacific. Endang Species Res 3:191-203

Eldridge MD, King JM, Loupis AK, Spencer PB, Taylor AC, Pope LC, Hall GP (1999) Unprecedented low levels of genetic variation and inbreeding depression in an island population of the black-footed rock-wallaby. Conserv Biol 13:531-541

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611-2620

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479-491

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10:564-567

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164:1567-1587

Faubet P, Waples RS, Gaggiotti OE (2007) Evaluating the performance of a multilocus Bayesian method for the estimation of migration rates. Mol Ecol 16:1149-1166

Fonseca LG, Murillo GA, Guadamúz L, Spínola RM, Valverde RA (2009) Downward but stable trend in the abundance of arribada olive ridley sea turtles (Lepidochelys olivacea) at Nancite Beach, Costa Rica (1971–2007). Chelonian Conserv Biol 8:19-27

Foran DR, Ray RL (2016) Mitochondrial DNA Profiling of Illegal Tortoiseshell Products Derived from Hawksbill Sea Turtles. J Forensic Sci 61:1062-1066

Frankham R, Lees K, Montgomery ME, England PR, Lowe EH, Briscoe DA (1999) Do population size bottlenecks reduce evolutionary potential? Animal Conservation Forum. Cambridge University Press, p 255-260

Frantz A, Cellina S, Krier A, Schley L, Burke T (2009) Using spatial Bayesian methods to determine the genetic structure of a continuously distributed population: clusters or isolation by distance? J Appl Ecol 46:493-505

Garza J, Williamson E (2001) Detection of reduction in population size using data from microsatellite loci. Mol Ecol 10:305-318

Hamann M, Godfrey MH, Seminoff JA, Arthur K, Barata PCR, Bjorndal KA, Bolten AB, Broderick AC, Campbell LM, Carreras C, Casale P, Chaloupka M, Chan SKF, Coyne MS, Crowder LB, Diez CE, Dutton PH, Epperly SP, FitzSimmons NN, Formia A, Girondot M, Hays GC, Cheng IJ, Kaska Y, Lewison R, Mortimer JA, Nichols WJ, Reina RD, Shanker K, SPotila JR, Tomas J, Wallace BP, Work TM, Zbinden J, Godley BJ (2010) Global research priorities for sea turtles: informing management and conservation in the 21st century. Endang Species Res 11:245-269

Hart KM, Lamont MM, Sartain AR, Fujisaki I (2014) Migration, foraging, and residency patterns for Northern Gulf loggerheads: implications of local threats and international movements. PLoS One 9:e103453

Hays GC, Fossette S, Katselidis KA, Mariani P, Schofield G (2010) Ontogenetic development of migration: Lagrangian drift trajectories suggest a new paradigm for sea turtles. J Royal Soc Interface 7:1319-1327

Hedrick PW (2005) A standardized genetic differentiation measure. Evolution 59:1633-1638

Hellberg ME, Burton RS, Neigel JE, Palumbi SR (2002) Genetic assessment of connectivity among marine populations. Bull Mar Sci 70:273-290

Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the assistance of sample group information. Mol Ecol Resour 9:1322-1332

Ingvarsson PK (2001) Restoration of genetic variation lost–the genetic rescue hypothesis. Trends Ecol Evol 16:62-63

Janes JK, Miller JM, Dupuis JR, Malenfant RM, Gorrell JC, Cullingham CI, Andrew RL (2017) The K= 2 conundrum. Mol Ecol 26:3594-3602

Jensen M, Abreu-Grobois FA, Frydenberg J, Loeschcke V (2006) Microsatellites provide insight into contrasting mating patterns in arribada vs. non-arribada olive ridley sea turtle rookeries. Mol Ecol 15:2567-2575

Jensen MP, FitzSimmons NN, Dutton PH (2013A) Molecular genetics of sea turtles. In: The Biology of Sea Turtles, Vol 3, p 135-161

Jensen MP, Limpus CJ, Whiting SD, Guinea M, Prince RIT, Dethmers KEM, Adnyana KEMD, Kennett R, FitzSimmons NN (2013B) Defining olive ridley turtle Lepidochelys olivacea management units in Australia and assessing the potential impact of mortality in ghost nets. Endang Species Res 21:241-253

Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403-1405

Jombart T, Pontier D, Dufour A (2009) Genetic markers in the playground of multivariate analysis. Heredity 102:330

Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet 11:94

Jombart T, Ahmed I (2011) adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. Bioinformatics 27:3070-3071

Jost LOU (2008) GST and its relatives do not measure differentiation. Mol Ecol 17:4015-4026

Kalb HJ (1999) Behavior and physiology of solitary and arribada nesting olive ridley sea turtles (Lepidochelys olivacea) during the internesting period. Texas A & M University

Kalinowski ST (2011) The computer program STRUCTURE does not reliably identify the main genetic clusters within species: simulations and implications for human population structure. Heredity 106:625

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Thierer CDT, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647-1649

Keller LF, Arcese P, Smith JN, Hochachka WM, Stearns SC (1994) Selection against inbred song sparrows during a natural population bottleneck. Nature 372:356

Komoroske LM, Jensen MP, Stewart KR, Shamblin BM, Dutton PH (2017) Advances in the application of genetics in marine turtle biology and conservation. Frontiers in Marine Science 4:156

Koval J (2015) Use of microsatellites to compare solitary vs arribada nesting olive ridley turtles (Lepidochelys olivacea) along the Eastern Pacific coast of Costa Rica. Purdue University

Latter B (1972) Selection in finite populations with multiple alleles. III. Genetic divergence with centripetal selection and mutation. Genetics 70:475-490

Leroux RA, Dutton PH, Abreu-Grobois FA, Lagueux CJ, Campbell CL, Delcroix E, Chevalier J, Horrocks JA, Hillis-Starr Z, Troeng S, Harrison E, Stapleton S (2012) Re-examination of population structure and phylogeography of hawksbill turtles in the wider Caribbean using longer mtDNA sequences. J Hered 103:806-820

Lewison RL, Crowder LB (2007) Putting longline bycatch of sea turtles into perspective. Conserv Biol 21:79-86

Lohmann KJ, Putman NF, Lohmann CM (2008) Geomagnetic imprinting: a unifying hypothesis of long-distance natal homing in salmon and sea turtles. Proceedings of the National Academy of Sciences 105:19096-19101

Lohmann KJ, Witherington BE, Lohmann CM, Salmon M (2017) Orientation, navigation, and natal beach homing in sea turtles. In: The Biology of Sea Turtles, Volume I. CRC Press, p 121-150

Lopez-Castro MC, Rocha-Olivares A (2005) The panmixia paradigm of eastern Pacific olive ridley turtles revised: consequences for their conservation and evolutionary biology. Mol Ecol 14:3325-3334

Luikart G, Allendorf F, Cornuet J, Sherwin W (1998) Distortion of allele frequency distributions provides a test for recent population bottlenecks. J Hered 89:238-247

Lynch M, Ritland K (1999) Estimation of pairwise relatedness with molecular markers. Genetics 152:1753-1766

Márquez R, Peñaflores C, Vasconcelos J (1996) Olive ridley turtles (Lepidochelys olivacea) show signs of recovery at La Escobilla, Oaxaca. Mar Turt Newsl 73:5-7

Moore JE, Wallace BP, Lewison RL, Žydelis R, Cox TM, Crowder LB (2009) A review of marine mammal, sea turtle and seabird bycatch in USA fisheries and the role of policy in shaping management. Mar Policy 33:435-451

Múrias dos Santos A, Cabezas MP, Tavares AI, Xavier R, Branco M (2015) tcsBU: a tool to extend TCS network layout and visualization. Bioinformatics 32:627-628

Nei M (1973) Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences 70:3321-3323

Nei M, Tajima F, Tateno Y (1983) Accuracy of estimated phylogenetic trees from molecular data. J Mol Evol 19:153-170

Odong T, Van Heerwaarden J, van Hintum T, van Eeuwijk F, Jansen J (2013) Improving hierarchical clustering of genotypic data via principal component analysis. Crop Sci 53:1546-1554

Ohta T, Kimura M (1973) A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. Genetics Research 22:201-204

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288-295

Piry S, Luikart G, Cornuet J (1999) BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. J Hered 90:502-503

Piry S, Alapetite A, Cornuet J-M, Paetkau D, Baudouin L, Estoup A (2004) GENECLASS2: a software for genetic assignment and first-generation migrant detection. J Hered 95:536-539

Plot V, De Thoisy B, Blanc S, Kelle L, Lavergne A, Roger-Berubet H, Tremblay Y, Fossette S, Georges J-Y (2012) Reproductive synchrony in a recovering bottlenecked sea turtle population. J Anim Ecol 81:341-351

Plotkin PT (2003) Adult migrations and habitat use. In: The Biology of Sea Turtles, Vol 2, p 225-241

Plotkin PT (2010) Nomadic behaviour of the highly migratory olive ridley sea turtle Lepidochelys olivacea in the eastern tropical Pacific Ocean. Endang Species Res 13:33-40

Pringle JM, Blakeslee AM, Byers JE, Roman J (2011) Asymmetric dispersal allows an upstream region to control population structure throughout a species' range. Proceedings of the National Academy of Sciences 108:15288-15293

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945-959

Puechmaille SJ (2016) The program STRUCTURE does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. Mol Ecol Resour 16:608-627

Putman AI, Carbone I (2014) Challenges in analysis and interpretation of microsatellite data for population genetic studies. Ecology and Evolution 4:4399-4428

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evolution 43:258-275

Rees AF, Alfaro-Shigueto J, Barata PCR, Bjorndal KA, Bolten AB, Bourjea J, Broderick AC, Campbell LM, Cardona L, Carreras C, Casale P, Ceriani SA, Dutton PH, Eguchi T, Formia A, Fuentes MMPB, Fuller WJ, Girondot M, Godfrey MH, Hamann M, Hart KM, GC Hays, Hochscheid S, Kaska Y, Jensen MP, Mangel JC, Mortimer JA, Naro-Maciel E, Ng CKY, Nichols WJ, Phillott AD, Reina RD, Revuelta O, Schofield G, Seminoff JA, Shanker K, Tomas J, van de Merwe JP, Van Houtan KS, Vander Zanden HB, Wallace BP, Wedemeyer-Strombel KR, Work TM, Godley BJ (2016) Are we working towards global research priorities for management and conservation of sea turtles? Endang Species Res 31:337-382

Revuelta O, Carreras C, Domènech F, Gozalbes P, Tomás J (2015) First report of an olive ridley (Lepidochelys olivacea) inside the Mediterranean Sea. Mediterr Mar Sci 16:346-351

Rodríguez-Zárate CJ, Rocha-Olivares A, Beheregaray LB (2013) Genetic signature of a recent metapopulation bottleneck in the olive ridley turtle (Lepidochelys olivacea) after intensive commercial exploitation in Mexico. Biol Conserv 168:10-18

Rodríguez-Zárate CJ, Sandoval-Castillo J, van Sebille E, Keane RG, Rocha-Olivares A, Urteaga J, Beheregaray LB (2018) Isolation by environment in the highly mobile olive ridley turtle (Lepidochelys olivacea) in the eastern Pacific. Proc R Soc B 285:20180264

Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A (2017) DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol Biol Evol 34:3299-3302

Sainudiin R, Durrett RT, Aquadro CF, Nielsen R (2004) Microsatellite mutation models: insights from a comparison of humans and chimpanzees. Genetics 168:383-395

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406-425

Schofield G, Hobson VJ, Fossette S, Lilley MK, Katselidis KA, Hays GC (2010) Biodiversity Research: fidelity to foraging sites, consistency of migration routes and habitat modulation of home range by sea turtles. Diversity Distributions 16:840-853

Scribner KT, Congdon JD, Chesser RK, Smith MH (1993) Annual differences in female reproductive success affect spatial and cohort-specific genotypic heterogeneity in painted turtles. Evolution 47:1360-1373

Senko J, Schneller AJ, Solis J, Ollervides F, Nichols WJ (2011) People helping turtles, turtles helping people: understanding resident attitudes towards sea turtle conservation and opportunities for enhanced community participation in Bahia Magdalena, Mexico. Ocean Coast Manage 54:148-157

Shamblin BM, Bolten AB, Bjorndal KA, Dutton PH, Nielsen JT, Alberto Abreus-Grobois F, Reich KJ, Witherington BE, Bagley DA, Ehrhart LM, Tucker AD, Addison DS, Arenas A, Johnson C, Carthy RR, Lamont MM, Dodd MG, Gaines MS, LaCasella E, Nairn CJ (2012) Expanded mitochondrial control region sequences increase resolution of stock structure among North Atlantic loggerhead turtle rookeries. Mar Ecol Prog Ser 469:145-160

Shanker K, Pandav B, Choudhury BC (2004A) An assessment of the olive ridley turtle (Lepidochelys olivacea) nesting population in Orissa, India. Biol Conserv 115:149-160

Shanker K, Ramadevi J, Choudhury BC, Singh L, Aggarwal RK (2004B) Phylogeography of olive ridley turtles (Lepidochelys olivacea) on the east coast of India: implications for conservation theory. Mol Ecol 13:1899-1909

Shillinger GL, Palacios DM, Bailey H, Bograd SJ, Swithenbank AM, Gaspar P, Wallace BP, Spotila JR, Paladino FV, Piedra R, Eckert SA, Block BA (2008) Persistent leatherback turtle migrations present opportunities for conservation. PLoS Biol 6:e171

Siegel D, Kinlan B, Gaylord B, Gaines S (2003) Lagrangian descriptions of marine larval dispersion. Mar Ecol Prog Ser 260:83-96

Sundqvist L, Keenan K, Zackrisson M, Prodöhl P, Kleinhans D (2016) Directional genetic differentiation and relative migration. Ecology and Evolution 6:3461-3475

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585-595

Taylor BL, Martien K, Morin P (2010) Identifying units to conserve using genetic data. In: Marine mammal ecology and conservation—a handbook of techniques. Oxford University Press, p 306-344

Thompson JD, Gibson TJ, Higgins DG (2003) Multiple sequence alignment using ClustalW and ClustalX. Current Protocols in Bioinformatics:2-3

Valverde RA, Orrego CM, Tordoir MT, Gómez FM, Solis DS, Hernandez RA, Gomez GB, Brenes LS, Baltodano JP, Fonseca LG, Spotila JR (2012) Olive ridley mass nesting ecology and egg harvest at Ostional Beach, Costa Rica. Chelonian Conserv Biol 11:1-11

Vander Zanden HB, Bolten AB, Tucker AD, Hart KM, Lamont MM, Fujisaki I, Reich KJ, Addison DS, Mansfield KL, Phillips KF, Pajuelo M, Bjorndal KA (2016) Biomarkers reveal sea turtles remained in oiled areas following the Deepwater Horizon oil spill. Ecol Appl 26:2145-2155

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358-1370

Whitlock MC, Mccauley DE (1999) Indirect measures of gene flow and migration: FST≠ 1/(4Nm+ 1). Heredity 82:117-125

Wilson GA, Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163:1177-1191

Wright S (1949) The genetical structure of populations. Ann Hum Genet 15:323-354

Zug GR, Wilson RV, Ernst CH (1998) Lepidochelys olivacea. In: Catalogue of American Amphibians and Reptiles (CAAR)