POPULATION GENETICS OF THE BLUE CRAB (*Callinectes sapidus*) IN THE GULF OF MEXICO, CHESAPEAKE BAY, AND WESTERN SOUTH ATLANTIC

A Thesis

by

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ABSTRACT

The blue crab, *Callinectes sapidus*, is an important species in estuarine habitats, serving as both predator and prey to other species, and plays a pivotal ecological and economic role throughout its range. In recent years, however, its populations have been declining. Declining blue crab populations will negatively affect critically endangered organisms that depend on the blue crab, like Kemp's Ridley sea turtles and whooping cranes, as well as commercially important fish species, such as red drum, *Sciaenops ocellatus*. Despite its importance, little is known about its genetic population structure, which can be affected by population reductions. Previous research provides conflicting evidence of genetic variation in the blue crab across its range. Some studies have identified significant population structure in blue crabs in the Gulf of Mexico, attributed to seasonality, catastrophic events, and post-larval selection, while others have found genetic homogeneity in the Texas coast, possibly due to gene flow by larval dispersal. The results from previous studies are being used to implement management strategies, despite their limitations. In this study, population structure of the blue crab was assessed throughout the Gulf of Mexico, in the Chesapeake Bay, and southern Brazil using sixteen microsatellite markers. The results show high levels of gene flow for the blue crabs in the United States (G_{ST} = 0.005; D_{ST} = 0.015), with no genetic differentiation identified by any of the analyses. There is evidence of strong genetic differentiation between the U.S. and Brazil (G_{ST} = 0.067; D_{ST} = 0.056). No signs of a recent bottleneck were detected in any of the populations. Estimated N_E was very high for all populations.

This information will aid management decisions for the blue crab and help preserve this important species by improving stock delineations and providing a baseline of genetic diversity.

DEDICATION

This thesis is dedicated to my parents, for all of their sacrifices to ensure I could get the best education possible. This would not have been possible without your support and I will always be grateful.

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NOMENCLATURE

BR	Brazil
FCA	Factorial Correspondence Analysis
F_{ST}	Measure of genetic differentiation
G_{IS}	Measure of inbreeding in a population
GOM	Gulf of Mexico
H_E	Expected heterozygosity
H_O	Observed heterozygosity
HWE	Hardy-Weinberg Equilibrium
IBD	Isolation by distance
K	Number of cluster groups defined by structure
N_E	Effective population size
PCR	Polymerase chain reaction

SAMPLING LOCALITIES

LLM	(1) Lower Laguna Madre, TX
ROC	(2) Rockport, TX
POL	(3) Port Lavaca, TX
GAL	(4) Galveston, TX
AVI	(5) Avery Island, LA
SLI	(6) Slidell, LA

DIB	(7) D’Iberville, MS
APA	(8) Apalachicola, FL
CEK	(9) Cedar Key, FL
SERC	(10) Chesapeake Bay
LPA	(11) Lagoa dos Patos
ITA	(12) Itajaí
TRA	(13) Tramandaí
LAG	(14) Laguna

TABLE OF CONTENTS

LIST OF FIGURES

Page

LIST OF TABLES

Page

1. INTRODUCTION: LIFE HISTORY OF THE BLUE CRAB

The blue crab, *Callinectes sapidus*, has a complex life history, beginning life as pelagic larvae, which eventually settle into benthic habitats to metamorphose into sexually immature juvenile crabs that become adults through a series of molts. Each developmental stage is affected by a series of physical, chemical, and biological factors (Eckman, 1996). To clarify blue crab population structure, it is necessary to understand the effects various factors have on this organism. From larval dispersal to salinity, each component plays a role in determining gene flow and ultimately affects genetic diversity. Genetic diversity is key to a healthy population, particularly to those at risk of decline, like the blue crab (Reed & Frankham, 2003).

1.1 Mating

Unlike other estuarine species, mating and spawning of the blue crab takes place at different times. Blue crabs rely on chemical and visual cues to initiate courtship, which usually takes place after 18-20 molts. This allows them to be sexually receptive for about one week (Millikin & Williams, 1984). Males perform courtship displays as a response to chemical compounds released by pre-pubertal females. During displays, males rise up on their hind legs, with the chelae spread, and rotating its periopods (Bushmann, 1999). It is hypothesized that this behavior acts as a visual cue to females while also allowing males to transmit physical and chemical cues through the resulting increase in water flow (Kamio, Reidenbach, & Derby, 2008). Females respond by

rocking from side to side. Pair formations follow, though these may be resisted by the female and require multiple attempts (Bushmann, 1999).

Prior to initiating copulation, male crabs carry the females for a few days. At this stage, females are particularly vulnerable to predation, resulting in male guarding before and after mating (Jivoff, 1997). Males compete for females and Jivoff has found that large size is advantageous to males for two reasons. Larger males are able to guard females for longer periods than small males. Secondly, mating, for males, takes place during the intermolt stage. This phase in their lifecycle gets longer with size. Male competition is especially important because females use stored sperm for their lifetime production of eggs and produce broods of approximately 1.4×10^6 eggs (Hines et al., 2003; Jivoff, 2003; Millikin & Williams, 1984; Prager, McConaugha, Jones, & Geer, 1990; Van Engel, 1958; Wolcott, Hopkins, & Wolcott, 2005). While the female is still soft after molting, she turns on her back and opens her abdomen to expose the genital pores. For a day or two, copulation takes place while the female is carried by the male. Once the female's shell has hardened, she is no longer carried by the male (Churchill, 1919).

The fishing of large males poses a concern for blue crab mating. Though females are protected from this exploitation while gravid, continued fishing of large males could result in an excess of small males, and a skew in sex ratios (Abbe & Stagg, 1996; Uphoff, 1998). An overabundance of small males may lead to fewer matings due to their inability to properly guard females and their reduced sperm production. A skew in sex ratios also leads to less competition (Kingsolver et al., 2001). Jivoff (1997) found that, in

the presence of competitors, males will pass a larger percentage of their ejaculate to females. Carver et al (2004) found that in areas with heavy fishing pressure in the Chesapeake Bay, the average size of males were smaller and these males had less sperm. They also found that in some males, sperm levels were depleted pre-copulation, suggesting that these males are unable to replace seminal resources prior to mating.

Harvest and sexual selection can interact in complex ways, leading to profound impacts in populations (Allendorf & Hard, 2009; Hamon & Foote, 2005). Fishing, which generally targets sexually selected characters like large size and deeper body, can affect sexual selection and possibly result in evolutionary responses (Hanlon, 1998). This is particularly important as previous work suggests that human harvest results in a change in phenotypes much more quickly than other evolutionary forces (Allendorf & Hard, 2009; Darimont et al., 2009).

1.2 Spawning

A period of approximately 9 or 10 months takes place in between copulation and spawning (Churchill, 1919). Temperature and salinity play an important role for determining when spawning takes place. Though it generally occurs in the spring and summer, as eggs under warmer temperatures hatch faster than those in colder temperatures, it can occur later in the year during warmer winters (Sandoz, Mildred and Rogers, 1944).

Once the eggs are fertilized in the seminal receptacle, an egg mass commonly referred to as a sponge, berry, or pom-pom, is produced. Though this egg mass will be bright orange at first, it will darken with time, being completely black at hatching. Hines

et al. (2003) found that females have multiple spawning events and can spawn up to 18 times in their lifetime. Based on the massive number of offspring that the blue crab produced, Van Engel (1987) determined that the blue crab's life history is consistent with r-selection and suggested that the blue crab could be fished in high numbers, as the population should recover quickly if overfishing occurred.

After mating, males will remain in the lower salinity areas, while females move further out of the estuary to higher salinity regimes (Aguilar et al., 2005; Millikin & Williams, 1984; Van Engel, 1958). This behavior has been attributed to the development of salinity sensitive larvae and reduced predation (Dickinson *et al.* 2006). Tankersley *et al.* (1998) described the migratory process females undergo as occurring in two phases. They produce their brood during the first phase, as they move away from the mating location. In the second phase, the females move closer to coastal water to hatch their eggs, which are transported by ebb-tide, the period where water moves away from the shore. Afterwards, some will retreat to where they were for phase I to prepare another brood (Aguilar et al., 2005).

1.3 Larvae

The length of time before hatching is slightly shorter at higher water temperature, ranging from 12 to 17 days, and takes place in high salinity waters (Millikin & Williams, 1984).The events that take place after hatching are not well understood due to the pelagic life stages that follow hatching. The larvae migrate from estuaries to nearby shelf waters, where they undergo seven to eight molts as zoea over the course of four to five weeks, then metamorphose into the megalopal stage, which moves back into estuaries

and lasts 31 – 49 days (Andryszak, 1979; Costlow Jr. & Bookhout, 1959; Epifanio, 1995; Perry & Stuck, 1982; Zinski, 2006). It is important to note that the megalopae may not necessarily return to its parent estuaries, due to the movement of winds and currents (Epifanio, 1995; Epifanio, Valenti, & Pembroke, 1984). Both stages are primarily found in the upper 2 m of the water column and are controlled by near-surface circulation(Garvine, Epifanio, Epifanio, & Wong, 1997).

Megalopae, like the other life stages, rely on temperature and salinity to serve as cues for further development (Costlow Jr. & Bookhout, 1959). In general, the duration of the megalopal stage is longer with higher salinity. It has been suggested that once megalopae move into estuaries, the reduction in salinity enables them to develop past this stage (Millikin & Williams, 1984). In the summer, the newly recruited juveniles remain in low salinity waters, especially in areas with eelgrass (Heck & Orth, 1980). As winter approaches, the juvenile crabs move to deeper channels to hibernate (Van Engel, 1958).

Early juvenile blue crabs are also involved in pelagic emigration, typically through the use of nighttime flood tides (Etherington & Eggleston, 2000, 2003; Reyns & Eggleston, 2004). Juvenile crabs leave areas with high population density, though strong wind speeds may also lead to juvenile emigration. This form of secondary dispersal may impact distribution patterns (Blackmon & Eggleston, 2001; Reyns & Eggleston, 2004; Reyns, Eggleston, & Luettich, 2007).

Larval dispersal is considered a key mechanism for gene flow and is primarily determined by the major ocean currents. In the North Atlantic, blue crab larval dispersal is largely affected by several currents, which produce an anticyclonic gyre. The movement of the Gulf Stream, the North Atlantic Drift, the Canary Current, and the North Equatorial Current comprise the major currents in the North Atlantic (Scheltema, 1986). However, due to the difficulty in assessing larval dispersal, current understanding of its role in blue crab population structure is still limited. Previous work has indicated that hydrographic models are useful for understanding larval dispersal, though many other biophysical factors play a role in this (Cowen & Sponaugle, 2009; Gilg & Hilbish, 2003). Through the use of genetic markers, population structure can be determined and the extent of gene flow between populations can be established (Gilg & Hilbish, 2003).

1.4 Abiotic Factors

Blue crab are found from Nova Scotia to Argentina, spanning a wide variety in physical conditions, from the warm waters of the GOM to the cold waters in the Chesapeake Bay. Like other euryhaline crustaceans, blue crabs can survive in vastly different conditions. Although the varying conditions blue crab populations occur in are expected to generate genetic structure, previous work has been inconclusive (Darden, 2004; Kordos & Burton, 1993; McMillen-Jackson & Bert, 2004; Yednock & Neigel, 2014).

1.4.1 Salinity and Freshwater Inflow

The blue crab inhabits estuaries, which are areas where freshwater from rivers mix with salt water from the ocean (Montagna, Alber, Doering, & Connor, 2002).

Because of their influence on economically important fishery resources, Hildebrand & Gunter (1953) worked to determine the importance of freshwater inflows to Texas bays and estuaries. During a drought that lasted from 1948 to 1956, there was a sharp reduction in production of the bay oyster, white shrimp, and the black drum. This relationship between freshwater inflow and estuary productivity has been identified throughout the world and is now a key part of management strategies (Powell, Matsumoto, & Brock, 2002). For the blue crab, this is particularly important due to the role that salinity plays in its development.

The blue crab is highly adaptable to salinity, being capable of inhabiting environments from freshwater in Virginia to the hypersaline waters of Laguna Madre (Neufeld, Holliday, & Pritchard, 1980). This is surprising, since salinity affects a host of physiological responses in the blue crab. A correlation has been identified between salinity, blood pH, and osmoregulation (Mangum, Silverthorn, Harris, Towle, & Krall, n.d.). Finally, differential expression of key enzymes involved in active transport (Na-K ATPase, carbonic anhydrase) has been found under varying salinity concentrations (Genovese, Ortiz, Urcola, & Luquet, 2005; Gilles & Pequeux, 1985; Henry, 2006; Henry, Gehnrich, Weihrauch, & Towle, 2003; Luquet, Weihrauch, Senek, & Towle, 2005; Mantel & Farmer, 1983; Pequeux, 1995; Taylor & Taylor, 1992; David W. Towle & Weihrauch, 2001; D W Towle et al., 2001). Besides playing a role in physiology of adult crabs, salinity is closely linked to larval and juvenile development. As previously mentioned, each stage of the blue crab's life history is associated with a certain level of

salinity, possibly due to differential abilities for osmoregulation at each life stage (Tagatz, 1971).

Megalopae have reduced survival when there is a decrease in salinity and temperature (Costlow, 1967) showed that megalopae use salinity, combined with turbulence related to flood tide, as signals to determine when to move to the next stage of development and where to go in the water column. The megalopae continue to swim into the water column as they detect an increase in salinity and turbulence. They only descend after the flood tide has receded, when there is a resulting decrease in salinity and turbulence.

As a euryhaline species, the blue crab is dependent on the estuaries it inhabits. However, as urban development expands in coastal areas and dams continue to be built, there is reduced freshwater inflow to the estuaries (Montagna et al., 2002). Since salinity is closely linked to physiology, an increase in salinity because of reduced freshwater inflow could have a profound impact on this species.

1.4.2 Temperature

Another consequence of having a wide distribution is the variety in water temperature that the various populations of blue crab experience. Temperature, like salinity, has tremendous impact on the blue crab's development and physiology. In many species of lab-reared Brachyuran species, temperature and rate of larval development are related. This is likely the main reason for the variations in life history schedules observed throughout the blue crab's range (Smith, 1997).

Variations in both salinity and temperature may impact survival. Megalopae reared under different temperature regimes experience contrasting rates of survival. Costlow (1967) found that, although the megalopae reared at 20, 25, and 30°C experienced similar rates of survival, megalopae reared at 15°C had low survival rates, never higher than 50% survival. The physiological minimum temperature of the blue crab is close to 10°C. Below this temperature, the blue crab will no longer molt and will cease to grow (Brylawski & Miller, 2003). As temperature decreases, length of time needed for larval development increases (Costlow, 1967). For instance, a reduction in temperature from 30 to 20°C doubled the time needed to finish larval development. These lab-based experiments are supported by the differences in blue crab development found throughout its range.

The length of time it takes for a blue crab in the GOM to mature and reproduce is one year (O. P. Jensen, Seppelt, Miller, & Bauer, 2005). In the upper part of the blue crab's range, however, crabs go dormant between late November and late April as temperatures reach their physiological minimum temperature. This dormant phase extends their development time to 18 – 24 months. Low temperatures also lead to mortality of adult crabs. In the Chesapeake Bay, cold winters have negative impacts on crabs. Sharov *et al.* (2003) have found a correlation between the low water temperatures in January and the amount of dead crabs. Large crabs were impacted the most by low temperature.

Understanding the role that temperature plays in the development of the blue crab is important, especially because of the variation in environmental conditions

throughout the blue crab's range. As temperatures warm due to climate change, it is necessary to understand how this will impact the blue crab. Plankton, like the larval stages of the blue crab, are useful indicators of climate change (Beck et al., 2001; Edwards & Richardson, 2004; Hays, Richardson, & Robinson, 2005). Larval dispersal is credited as the reason for the genetic homogeneity previously found in the blue crab's range. Further testing with different markers could bring to light new information on the genetic variability of this species.

1.4.3 Pollutants

Marine life is increasingly threatened by pollutants in the ocean. These are introduced by a variety of mechanisms, from water runoff to oil leaks. Blue crabs are at a higher risk for exposure to toxicants because they inhabit estuaries, sheltered tidal flats, and salt marshes, which have high levels of pollutants and are susceptible to oil spills (Gundlach & Hayes, 1978; Sabourin, 1982). The blue crab's position in the food web, its broad distribution, feeding habits, and interaction with potentially contaminated sediment also makes it vulnerable to the effects of bioaccumulation(Duke, Lowe, & Wilson, 1970; Marcus & Mathews, 1987).

This exposure can sometimes lead to mass mortality events, behavioral impacts, and developmental abnormalities (Lee & Oshima, 1998; McCrea-Strub et al., 2011; Reichmuth, Roudez, Glover, & Weis, 2009). These effects have profound genetic consequences, ranging from the molecular level, like mutations, to the population level, like reduced genetic diversity (Bickham, Sandhu, Hebert, Chikhi, & Athwal, 2000).

A variety of pollutants occur in estuaries including polycyclic aromatic hydrocarbons (PAH), pesticides, crude oil, and metals. Wang & Stickle 1988 found that juvenile blue crabs experience diminished growth when exposed to crude oil. Conversely, the effects of PAHs on adult crab growth are less clear (Weis, Cristini, & Rao, 1992). These chemicals, as well as dioxins and dibenfozurans, also increase the time for the intermolt cycle significantly (Cantelmo et al., 1982). Other work has found that the exposure to both chlorinated hydrocarbons and PAHs can lead to modified behavior including reduced ability to capture prey items. The extent of these responses varies with concentration (Kinter & Pritchard, 1977). Chemicals used to clean up oil spills, like the surfactant sodium dodecyl sulfate (SDS), may also be harmful to the blue crab, as newly hatched larvae have been shown to be sensitive to them (Whiting, Cripe, & Lepo, 1996).

Other toxicants that lead to deleterious effects include pesticides and metals. Lee & Oshima (1998) found that exposure to pesticides like fenvalerate can lead to the inhibition of hatching. Other pesticides, like methoprene, a growth hormone used as an insecticide, stopped the production of chitin after molting (Horst & Walker, 1999). Abnormal eye spots may develop during exposure to metals, while mercuric chloride led to the development of embryos without a heartbeat (Lee & Oshima, 1998). The insecticide malathion has also been documented to increase blue crab mortality (Wendel & Smee, 2009)

As these details illustrate, the large range of pollutants that the blue crab may come in contact with can produce a variety of physiological and genetic effects. This is

of particular concern as human activity increases the amount of toxicants these animals are exposed to. Understanding the genetic diversity in the blue crab's range could help mitigate further loss of genetic diversity and protect this important species.

1.4.4 Extreme Weather Events

Hurricanes and tropical storms can wreak havoc in estuarine environments. Besides their cataclysmic power, they decrease salinity, while increasing turbidity, pollutants, nutrient loading, and dissolved organic carbon in their impact area (Tilmant et al., 2007). These changes in physical conditions of the environment can have dire impacts on the blue crab. Blue crab mortality was reported after hurricanes Andrew, Hugo, Frances, and Jeanne (Knott & Martore, 1992; Switzer, Winner, Dunham, Whittington, & Thomas, 2006; Tilmant et al., 2007). Storms can shape megalopal settlement distribution and influence nekton community structure by indirectly increasing population density (Eggleston, Reyns, Etherington, Plaia, & Xie, 2010; Etherington & Eggleston, 2000, 2003; Reyns, Eggleston, & Luettich, 2006; Reyns et al., 2007; Switzer et al., 2006).

The impact on blue crabs of rapidly reducing salinity following strong storms is complex. Decreased salinity is often listed as one of the causes for mass mortality of estuarine organisms following hurricanes (Knott & Martore, 1992; Switzer et al., 2006). Mobile benthic organisms like the blue crab, can avoid areas subject to these conditions. Hurricane Agnes in 1972 did not cause mass mortality of the blue crab, possibly due to the blue crab's mobility. Other hurricanes, however, have caused as much as a tenfold reduction in catches (Paerl et al., 2001).

A benefit to the blue crab that may arise from storms is larval transport. Eggleston et al., (2010) found that the nursery capacity of a North Carolina estuary was expanded due to tropical storms and that larval settlement distribution is also impacted by storms. This effect does vary with the magnitude and direction of the storm. Though storms can transport megalopae, a potential negative consequence of this is that depending on the strength of the storm, the megalopae may be transported to regions with high levels of freshwater, leading to mass-mortality.

Hypoxia is also attributed as one of the causes for organismal mortality after storms (Burkholder et al., 2004; M A Mallin et al., 2002; Michael A Mallin et al., 1999; Switzer et al., 2006; Tabb & Jones, 1962). Although salinity levels can rebound relatively quickly, hypoxic conditions take longer to return to normal (Tabb & Jones, 1962). Although blue crabs can sometimes leave regions with low dissolved oxygen, that is not always the case (Tatum, 1982).

As climate change is expected to increase the frequency and magnitude of storms, it is necessary to understand the potential impacts these storms may have on the blue crab. There is a high potential for increased reductions in blue crab stock. With knowledge of the genetic diversity of this organism, fisheries managers can prepare and address this after hurricanes occur.

1.4.5 Dissolved Oxygen

A frequent issue affecting estuarine organisms is the occurrence of hypoxia (>2 mg/L of oxygen) and anoxia (0 mg/L of oxygen). A consequence of eutrophication, hypoxia is becoming more frequent due to human activity and has been found to occur in

32 of 38 estuaries in the GOM (Brouwer et al., 2004). In addition to being caused by humans, hypoxia and anoxia can also take place due to the natural flow of tides. In shallow waters, hypoxia can sometimes occur as tides move hypoxic bottom waters up towards the surface. They can also occur cyclically while photosynthesis and respiration take place during the day and night, respectively (Brown-Peterson et al., 2005). Myriad responses are emitted by the blue crab as a response to hypoxia, ranging from behavioral to molecular responses.

The blue crab's behavioral response to hypoxia is varied. Bell *et al.* (2003) found that the blue crabs, despite being mobile, are ineffective at leaving hypoxic regions. They do sometimes move from regions experiencing hypoxia to oxygen-rich areas in Atlantic estuaries, though this behavior may impact population dynamics due to overcrowding (Das & Stickle, 1994; Lowery & Tate, 1986; Pihl, Baden, & Diaz, 1991). The blue crab is cannibalistic and with increased pressure from higher population density, it is possible that competition for resources could lead to an increase in cannibalization (Ferner, Smee, & Chang, 2005). This is of particular concern as shallow, oxygen rich areas are typically inhabited by smaller crabs that may be less capable of competing with the migrant, larger crabs (Aumann, Eby, & Fagan, 2006; Lenihan et al., 2001; Loesch, 1960).

In addition, hypoxic events can affect gene expression and protein activity in blue crabs. Specifically, differential expression of genes and activity of certain proteins has been found (Brouwer et al., 2004; Tanner, Burnett, & Burnett, 2006). For example, exposure to hypoxia may reduce the blue crab's ability to resist disease (Holman,

Burnett, & Burnett, 2004). Phenoloxidase, an enzyme involved in a crustacean mechanism for immune defense, had suppressed activity due to oxygen and pH during hypoxic events (Tanner et al., 2006). Gene expression for hemocyanin lowered significantly in lab-reared blue crabs exposed to five days of hypoxic conditions, possibly due to aerobic metabolic pathways ceasing activity (Brouwer et al., 2004). The increase in frequency of hypoxic events poses a threat to the blue crab because of their various impacts on the crab's behavior, physiology, and population dynamics. Management strategies should consider addressing hypoxic events to prevent collapse of the blue crab population.

1.5 Conclusion

Many biotic and abiotic factors can potentially influence the genetic structure of the blue crab. Fishing of large males may skew sex ratios. Blue crab distribution is impacted by larval and post-larval dispersal, which is in turn affected by wind, currents, tide, and storms. Pollutants, freshwater inflow, and hypoxic events all pose a risk to the blue crab habitat and may alter their physiology in negative ways. Although previous work has indicated that the blue crab exhibits little genetic differentiation, additional research with more markers can help determine whether this is truly the case. Determining the degree of genetic variation that occurs in the GOM can help fisheries managers better maintain a healthy population and prevent loss of diversity by delineating stock populations.

2. LITERATURE REVIEW

Understanding the genetic structure of a species is key to its conservation. The International Union for Conservation of Nature (IUCN) recognized the need to conserve genetic diversity because of its connection to heterozygosity and fitness (Reed & Frankham, 2003). Loss of genetic diversity can have devastating consequences to populations, such as inbreeding depression, demographic stochasticity, and amassment of deleterious mutations (Frankel & Soule, 1981; Frankham, 1995; Goodnight, 1987; Hedrick & Miller, 1992; Lande, 1988, 1994; Leberg, 1990; Luikart & Cornuet, 1998; Mills & Smouse, 1994; Nunney & Campbell, 1993). Of additional concern is the ability of a population to respond to environmental change, which is expected to be reduced with the loss of genetic variation (Amos & Balmford, 2001).

Molecular methods can help measure genetic diversity. In assessing the genetic health of a population, genetic markers can be used to calculate effective population size (N_E) and genetic diversity (e.g, allelic diversity and heterozygosity). N_E provides information on the number of individuals passing on genes to the next generation and establishes the rate of genetic drift, thus clarifying the amount of lost genetic variability, the level of increase in inbreeding, and the rate of frequency changes for alleles (Nunney & Campbell, 1993; Waples, 2013). Allelic diversity and heterozygosity provide complementary information on loss of genetic variation (F.W Allendorf, Luikhart, & Aitken, 2013)

To improve management and delineate stock populations, genetic markers have been increasingly applied to fisheries management. Information gathered assists with strategies to the continued, sustainable fishing of marine resources, and can help restore extirpated populations (Ward, 2000). Molecular methods can also be used to identify specimens, clarify fish behavior, and shed light on how selection shapes the population (Ward & Grewe, 1994). Although there are many applications for genetic markers in fisheries management, patterns of population structure for marine organisms vary widely.

There is conflicting evidence on the degree of population structure expected for marine organisms with a long-lived planktonic larval stage. A prevalent hypothesis for marine organisms with such characteristics was that long periods of larval duration resulted in increased long distance gene flow and genetic homogenization of populations (Hellberg, 2009; Selkoe et al., 2016; Ward, 2000). Therefore, lack of genetic population structure over large oceanic ranges, including entire basins or across basins, is expected for organisms with these life history characteristics.

This hypothesis was originally supported by studies using allozymes (Bohonak, 1999; Hedgecock, 1986). Since these studies, researchers found that genetic markers can produce varying results. For example, the use of three different markers, allozymes, nuclear loci, and mitochondrial DNA, to evaluate population structure in the American oyster have produced conflicting results, with the allozymes suggesting panmixia and the nuclear and mitochondrial loci identifying genetic breaks (Buroker, 1983; Karl & Avise, 1992). In contrast, the northern acorn barnacle exhibits high levels of gene flow

in both allozymes and microsatellites, disputing previous claims that this barnacle experienced a high degree of local adaptation (Brown, Kann, & Rand, 2001; Flight, O'Brien, Schmidt, & Rand, 2012). Riginos, Douglas, Jin, Shanahan, & Treml (2011) performed a review of marine population genetic studies in marine fishes and found that levels of genetic differentiation within the same species vary across markers. In general, they found that mitochondrial DNA sequences identified positive correlations between genetic structure and geographic distance. Similarly, in studies of the Atlantic cod, markers under selection identified significant differentiation, while neutral markers, like microsatellites, did not find genetic differentiation (Pampoulie et al., 2006). Although markers under selection appear to better reveal structure in marine organisms with very large population size, neutral markers can also reveal structure (Fred W Allendorf, Hohenlohe, & Luikart, 2010).

For example, in the Patagonian hoki, a reproductively isolated wintering population was identified with microsatellites (McKeown, Arkhipkin, & Shaw, 2015). In the eastern oyster, microsatellite studies supported other markers by identifying similar patterns of genetic differentiation (Anderson, Karel, Mace, Bartram, & Hare, 2014). Other studies have found population subdivision and localized recruitment in species that do have pelagic larvae, contradicting the prevailing hypothesis (Barber, Palumbi, Erdmann, & Moosa, 2002; Burton, 1986; Hellberg, Burton, Neigel, & Palumbi, 2002; Knowlton & Keller, 1986; Swearer et al., 2002). In a review of over 50 species in the Northeastern Pacific Coast., 41 of which had pelagic larvae, Kelly & Palumbi (2010) found no correlation between larval duration and population subdivision. Similarly, in

reviews by Riginos et al. (2011) and Weersing & Toonen (2009), they evaluated correlations between larval duration and genetic structure and found only slight correlations, across marine fishes and invertebrates.

The identified genetic structure could potentially be explained by constraints to larval dispersal from ocean currents or by patterns in larval life histories that encourage larvae to return to their natal location, such as gyres coinciding with spawning (Johannes, 1978; Shulman & Bermingham, 1995). Other explanations involve short duration of the pelagic stage, thereby limiting the distance traveled, swimming behavior of larvae, and larval mortality (Shulman & Bermingham, 1995). Based on the above-mentioned studies, though a pattern does exist between larval duration and levels of gene flow, there is evidence for exceptions to this rule, due to both biotic and abiotic factors (Palumbi, 1994). The lack of correlation between larval duration and population genetic structure caution against generalizations and each species needs to be assessed individually for effective management.

The variability in population structure across marine taxa with similar life histories complicates management efforts and emphasizes the need for further study (Barber et al., 2002; Hellberg et al., 2002; Knowlton & Keller, 1986; Selkoe et al., 2016; Swearer et al., 2002; Ward, 2000). Understanding marine connectivity is needed to improve fisheries management, especially when delineating stocks and marine reserves (Cowen, Lwiza, Sponaugle, Paris, & Olson, 2000). Expecting all marine organisms to have high levels of dispersal due to long larval duration leads to the assumption of intermixing between populations. This assumption could lead to incorrect management

decisions that would negatively impact a population, such as translocation of distinct populations causing outbreeding depression (Edmands, 1999).

In addition to maintaining genetic diversity and sustaining fisheries, proper fisheries management is needed for economic reasons. In 2014, the U.S. caught a total of 4.3 million metric tons of fishes and invertebrates, yielding over $5.5 billion in revenue (NOAA, 2014). The Gulf of Mexico (GOM) region alone was responsible for over a fifth of that revenue. Most of the harvests in the U.S. are associated with estuaries, which play key ecological functions (Gunter, 1967; Houde & Rutherford, 1993; Blaber et al., 2000). In addition to being home to a wide variety of fishes and invertebrates, they have high primary and secondary productivity (Beck et al., 2001). They also recycle nutrients and help protect shorelines from wave impacts (Costanza et al., 1997; Short & Wyllie-Echeverria, 1996). Despite their importance to coastal waters, estuaries are being lost at fast rates due to human activity (Edgar, Barrett, Graddon, & Last, 2000; Hinrichsen, D., Robey, B. & Upadhyay, 1998). This poses a threat to the survival of key estuarine species, like the blue crab.

The blue crab is an ecologically and economically important species. It helps maintain the health of the salt marshes it inhabits while also providing a significant source of revenue to the GOM and mid-Atlantic states of the United States (NOAA, 2014; Silliman & Bertness, 2002). A reported 40% of food consumed by wintering whooping cranes consists of the blue crab and its abundance can impact whooping crane mortality (Hunt & Slack, 1989; Pugesek, Baldwin, & Stehn, 2008). The Critically Endangered Kemp's Ridley sea turtle also relies on the blue crab as a food source, with

immature turtles stomach contents consisting of as much as 72% blue crab (Burke, Morreale, & Standora, 1994; Witzell & Schmid, 2005). This estuarine keystone species' natural range spans Nova Scotia to northern Argentina, though it has been introduced to Europe and Japan (Churchill, 1919; Millikin & Williams, 1984).

In 2014, 63,145.3 metric tons were caught in the Atlantic and Gulf states of the U.S., generating over $215.9 million in revenue (NOAA 2014). In southern Brazil, an average annual yield of 95 tons has been reported, with little data available for the rest of the country (Mendonça, Verani, & Nordi, 2010; Severino-Rodrigues, Musiello-Fernandes, Moura, Branco, & Canéo, 2013). In the U.S., since the mid-1980s, the fishery has been experiencing a severe decline, likely due to past overfishing, habitat degradation, and reduced freshwater flow into estuarine systems (Sutton, 2006). In the Chesapeake Bay, the largest blue crab fishery in the U.S., spawning stock declined by 81% from 1992-2000, while female size and mean size at maturity has decreased (Figure 1; Lipcius & Stockhausen, 2002). Larval abundance and post-larval recruitment decreased by 1 order of magnitude in the same time period (Lipcius & Stockhausen, 2002). The Gulf region has experienced a 13% decrease in blue crab landings while the mid-Atlantic has suffered a decrease of 36% (Figure 2; Pritzker, Sullivan, & Sobeck, 2014). The Texas fishery has also experienced a sharp reduction in the amount fished over the last 30 years (Figure 3). Similarly, in Brazil, the blue crab fishery has been reportedly in decline since 2004 due to environmental degradation and heavy exploitation (Mendonça et al., 2010).

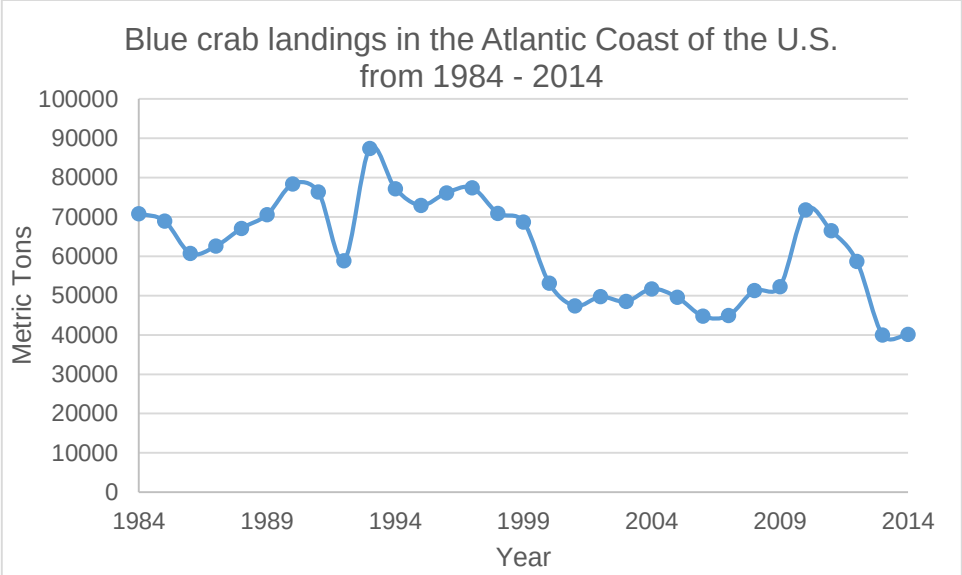

Figure 1. Blue crab landings, in metric tons, in the Atlantic Coast of the U.S. over thirty years (NOAA). The annual landings have generally varied between 400,000 metric tons and 900,000 metric tons, but faced a sharp decline from 2009 to 2014.

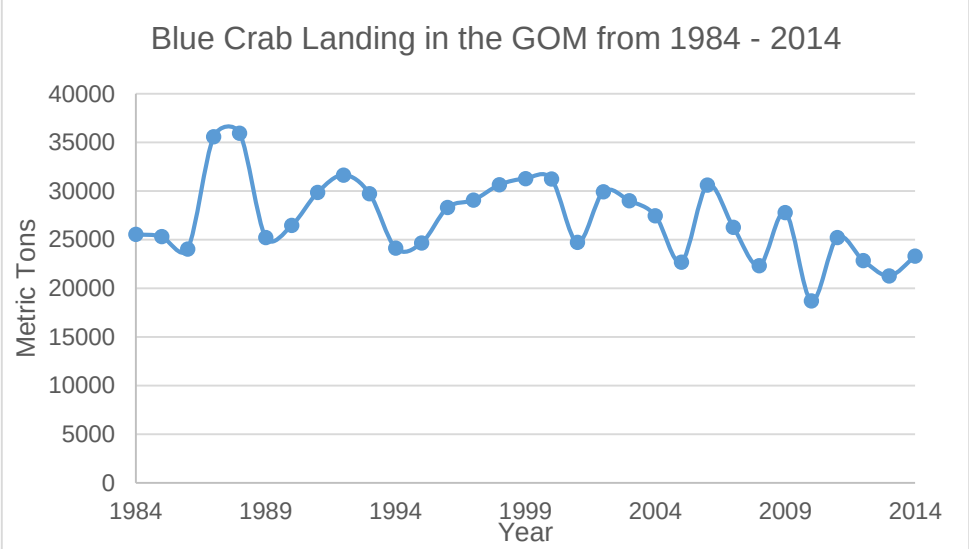

Figure 2. Blue crab landings, in metric tons, in the GOM over thirty years (NOAA). The GOM experiences more volatile trends in fishing quantities, but the amount of fishing has still declined in recent years.

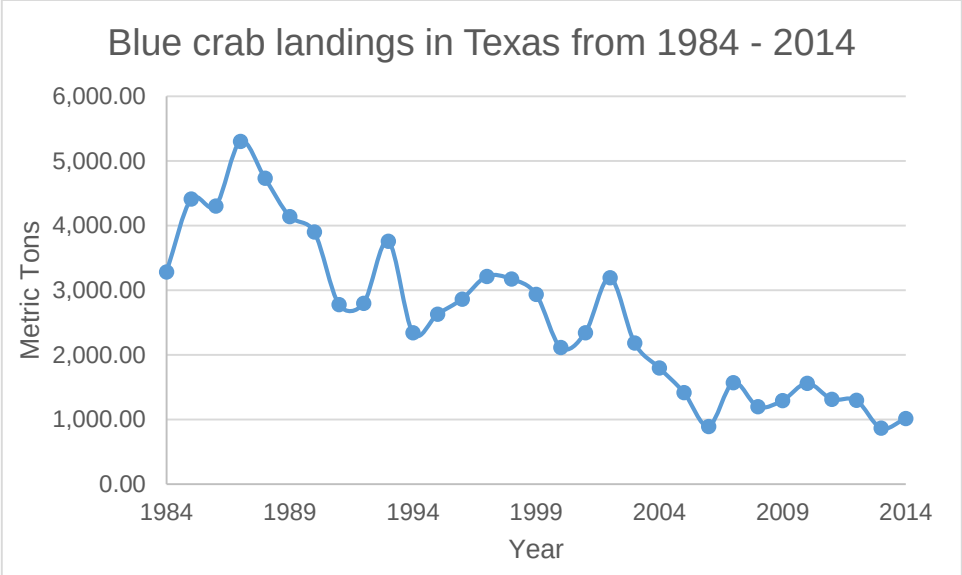

Figure 3. Blue crab landings, in metric tons, in Texas over thirty years (NOAA). Texas fisheries have sharply declined.

Despite its economic and ecological importance, current knowledge of the blue crab's molecular ecology is limited. Previous research provides conflicting evidence of genetic variation. For example, Kordos & Burton (1993) found high levels of spatial and temporal heterogeneity in their study of the Texas coast with three protein-coding genes. Similarly, (Darden, 2004) identified genetic structure in the western GOM and genetic homogeneity in the eastern GOM with the mitochondrial marker cytochrome oxidase I (COI). Though mitochondrial loci are useful markers, they have limitations and should not be the sole genetic marker used in a population genetic study (Hurst & Jiggins, 2005). The Gulf States Marine Fisheries Commission has proposed changing the current management populations to those seen in Figure 4, based on those results (VanderKooy, 2013b) . In contrast, another study conducted with mitochondrial loci throughout the

U.S. East and Gulf coasts did not find genetic structure, although they did find a pattern of decreasing genetic diversity along the East Coast and had previously identified some structure with allozymes (McMillen-Jackson, Bert, & Steele, 1994; McMillen-Jackson & Bert, 2004). A more recent study (2010 and 2011) in the GOM found an overall pattern of high gene flow and low genetic heterogeneity in the GOM, with significant differences between populations at a temporal scale (Yednock & Neigel, 2014). Samples at the GOM were compared to a locality in Venezuela, with significant differences being identified between the two regions. Unlike the work done by Kordos and Burton (1993) and Darden (2004), Yednock and Neigel (2014) did not find patterns of small-scale genetic structure. Due to the differences in results across genetic markers, additional research is necessary with other markers, such as microsatellites.

In the western south Atlantic, with samples from 2013 and 2014, high levels of gene flow were identified, using seven microsatellite markers (Lacerda et al., 2016). No population structure was found among localities that ranged in distance by 740 km. Due to the differences between samples from the GOM and Venezuela that were identified by Yednock and Neigel (2014), it is likely that samples from the GOM and U.S. Atlantic Coast will be significantly different from those in Brazil.

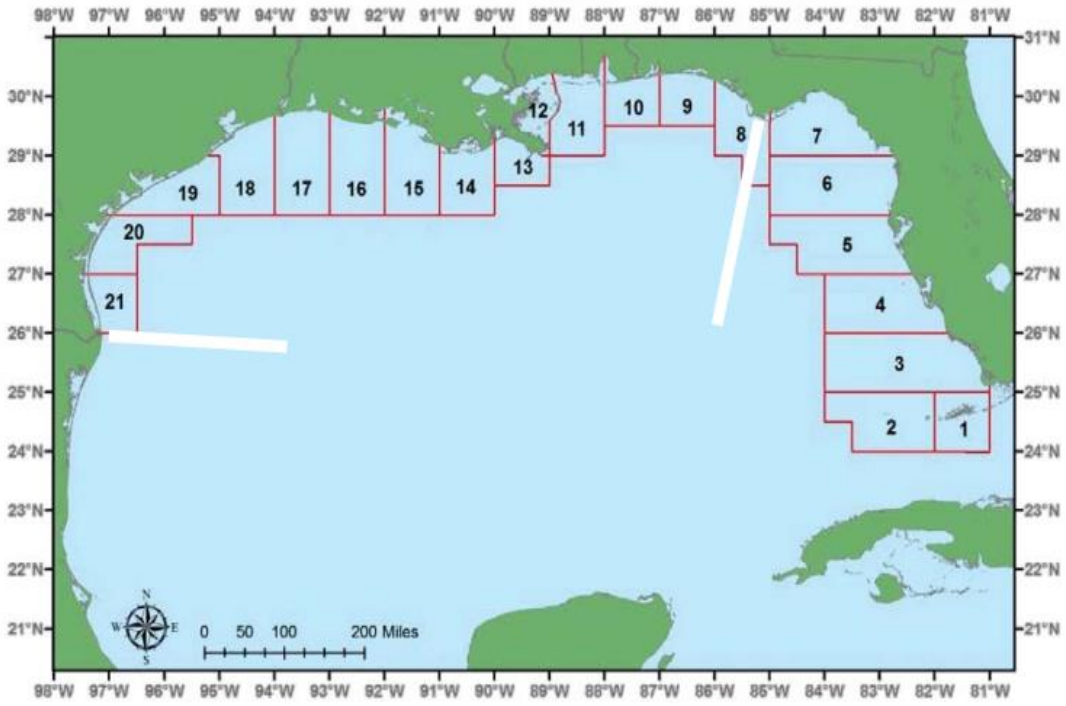

Figure 4. Proposed stock division of GOM blue crabs. The white lines define the break between eastern and western GOM blue crab stocks (VanderKooy 2013).

Given the importance of making informed management decisions and the limitations of previous studies, further analysis of the population structure of the blue crab is necessary. Additional loci will provide vital information on genetic diversity. This study will use microsatellites, short tandem repeated sequences located throughout the eukaryotic genome. They exhibit codominance, are generally selectively neutral, and can have as many as 50 alleles per locus (Jarne & Lagoda, 1996). The information collected from microsatellites will be used to identify genetic structure, to look for past population bottlenecks, and to measure the effective population size (N_E) of the

population. Despite their usefulness as a genetic marker, few microsatellites have been developed for the blue crab (Steven, Hill, Masters, & Place, 2005).

2.1 Objectives

In this study, I will use microsatellites to examine population structure and determine the genetic diversity of *C. sapidus* in the GOM, the Chesapeake Bay, and the western south Atlantic. It is expected that the blue crab populations will have a high degree of genetic diversity and that the populations in this study are panmictic due to larval dispersal.

3. METHODOLOGY

3.1 Sampling

Blue crabs were collected from nine localities across the GOM and one in the Chesapeake Bay (Figures 5-8; Table 1). In seven GOM localities they were sampled using double ring mesh nets with chicken as bait, and in the two remaining GOM localities, Rockport and D'Iberville, live crabs were purchased from local fishermen. Crabs from the Chesapeake Bay were sampled by a collaborator at the Smithsonian Environmental Research Center. Sampled crabs were stored in a cooler with dry ice, when available, or regular ice. A chela for each crab was dissected and stored in 100% ethanol for DNA preservation.

Table 1. Sampling locations and number of samples per location.

Sampling Locality	No. of samples 2013	No. of samples 2014	No. of samples 2015
Lower Laguna Madre (LLM)	-	-	24
Rockport (ROC)	-	24*	-
Port Lavaca (POL)	-	18	-
Galveston (GAL)	-	12	-
Avery Island (AVI)	-	20	-
Slidell (SLI)	-	11	-
D'Iberville (DIB)	-	24*	-
Apalachicola (APA)	-	21	-
Cedar Key (CEK)	-	13	-
Chesapeake Bay (SERC)	-	-	25
Lagoa dos Patos, Brazil (LPA)	30	28	-
Tramandai, Brazil (TRA)	34	-	-
Laguna, Brazil (LAG)	31	36	-
Itajai, Brazil (ITA)	21	33	-
Total:	116	240	49

* indicates localities where store-bought crabs were used. The first 10 localities in the table were sampled in this study; whereas the last four correspond to the samples obtained by Lacerda et al., 2016, for which genotypic data was used in this study.

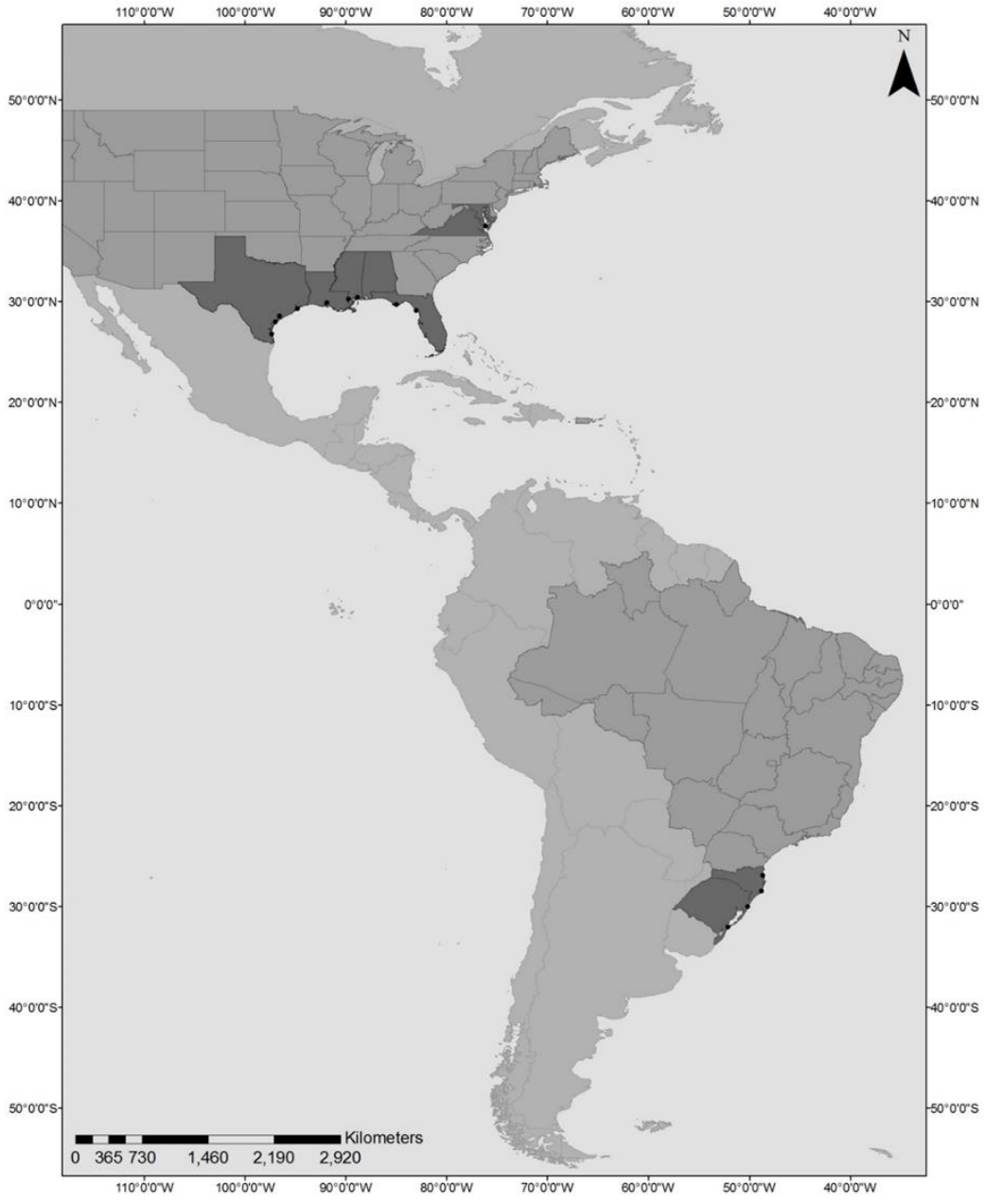

Figure 5. All sampling locations used in this study.

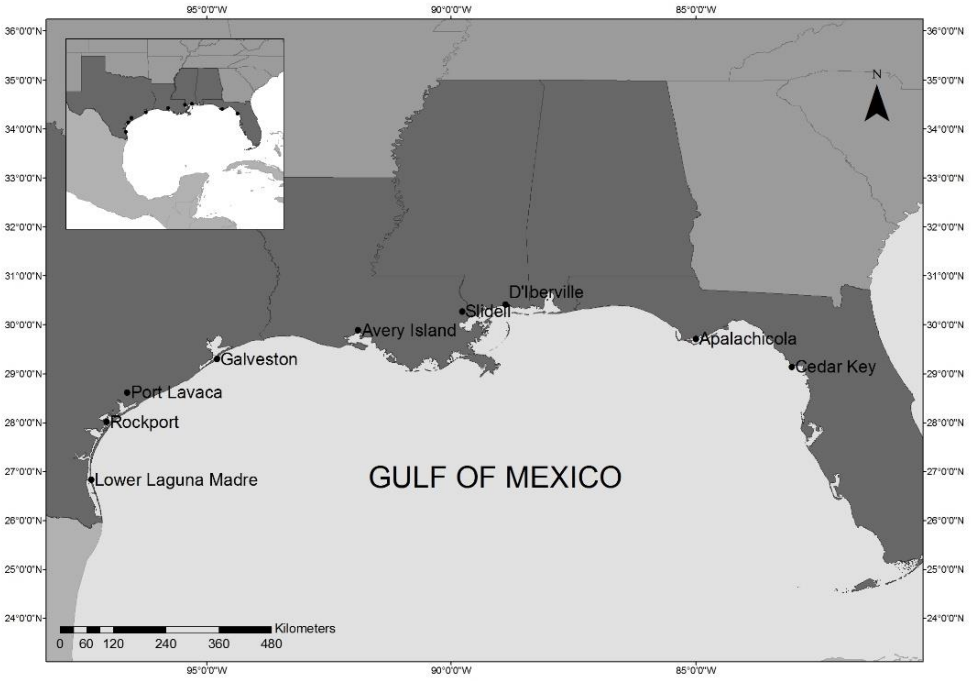

Figure 6. Sampling locations in the GOM.

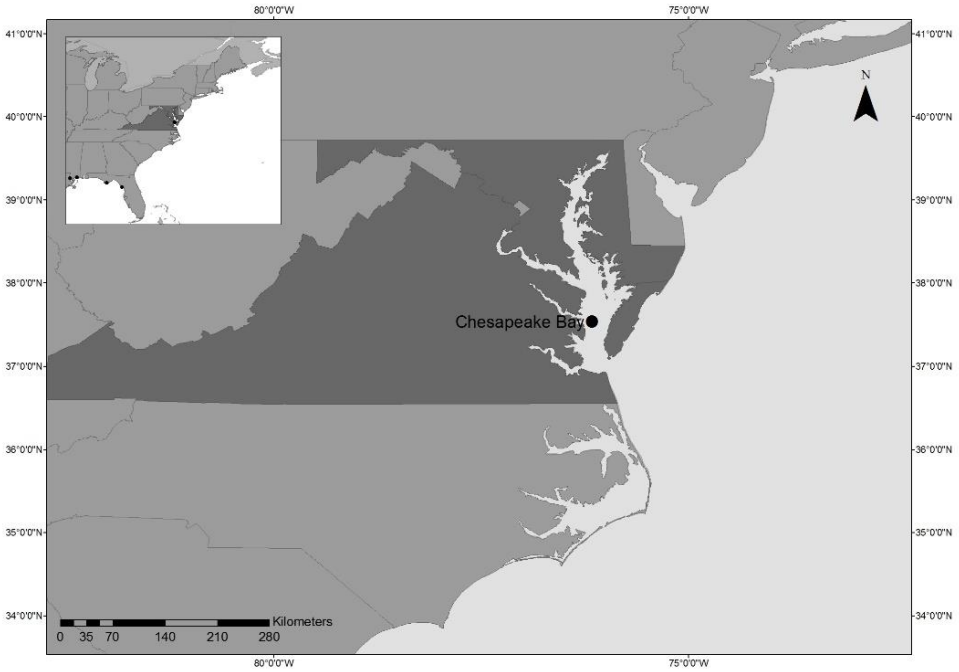

Figure 7. Samples from the Chesapeake Bay were collected by the Smithsonian Environmental Research Center.

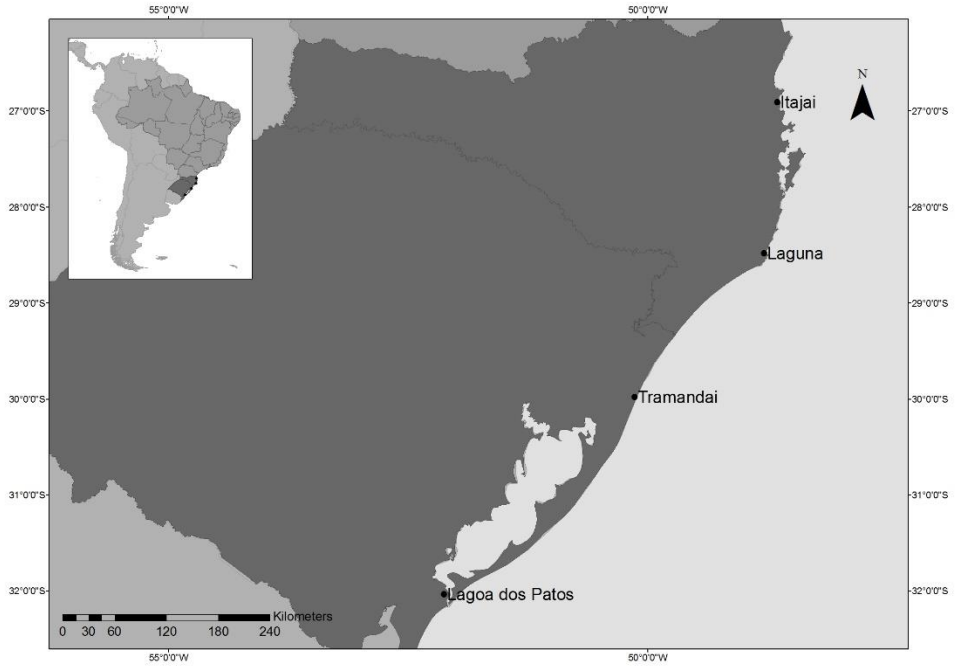

Figure 8. Genotyping data from Southern Brazil was collected and published in Lacerda et al. (2016).

3.2 Molecular Methods

DNA was extracted from muscle tissue dissected from the chela with Quick-gDNA™ MiniPrep kit (Zymo Research Corporation, Irvine, CA), according to manufacturer's 'Solid Tissue' instructions. DNA quality was checked visually in 2% agarose gel stained with 0.1X GelRed (Biotium, Inc., Hayward, CA).

The Hurtado lab has a dataset of 28, 607 microsatellites sequences for *C. sapidus*. Nine polymorphic microsatellites from this dataset were used (first nine in Table 2). Primers for these microsatellites were designed using Primer3 and WebSat (Martins, Lucas, Neves, & Bertioli, 2009; Untergasser et al., 2012). Seven additional loci, reported by Steven et al. (2005) and used by Lacerda et al. (2016), were also included (last seven in Table 2)

Table 2. Loci used in this study. The underlined portion of the forward sequence indicates the M13 tag and the underlined portion of the reverse primer indicates the added pigtail.

Locus ID	Primer Sequence (5'-3')	Repeat Motif	Label	T_A
Tet603	F: TGTAAAACGACGGCCAGTGGACGAACGAAAGCCAGATA R: GTGTCTTCCGAGAGGAAAATGAGAAAATG	$(ACAG)_7$	6-FAM™	50°C
Pen9028	F: TGTAAAACGACGGCCAGTTGTGTCCTATGTCCCAAGCA R: GTGTCTTAATGGCTGTTTGCCCTACAC	$(AAAGC)_5$	6-FAM™	58°C
Tet1057	F: TGTAAAACGACGGCCAGTTCGCTCTCTCTCCGTTTTTC R: GTGTCTTCGTAGGTGGGTAGATAAGTTTGC	$(ATCC)_5$	6-FAM™	50°C
Tet1329	F: TGTAAAACGACGGCCAGTCACAGCACATTACCCGTAGA R: GTGTCTTAAAAGGCTCGACGCCAGTAT	$(AGAT)_7$	6-FAM™	55°C
Pen23472	F: TGTAAAACGACGGCCAGTTCTCTCACCCGTCTTCATCA R: GTGTCTTAACTGAGGGACACACACAGC	$(AGGCG)_6$	6-FAM™	55°C
Di680	F: TGTAAAACGACGGCCAGTGGTCAAACAAAAAGTTCCACGA R: GTGTCTTGGGCGAACACACTTGAATAGA	$(AC)_{17}$	6-FAM™	55°C
Tri24376	F: TGTAAAACGACGGCCAGTGCGGCCTACGAGAAGACTAA R: GTGTCTTAAATGGAAAAAGACGCAACG	$(AAC)_9$	6-FAM™	58°C
Tet6290	F: TGTAAAACGACGGCCAGTTTGTCCGTGAAGTGTTCCTC R: GTGTCTTCCCTGATCCCTGCAAGACTA	$(AGAT)_{16}$	6-FAM™	60°C
Tet1886	F: TGTAAAACGACGGCCAGTTTCCCAGACTTGCATAGAGTCA R: GTGTCTTATGGCTCACAACACACCAACTA	$(TATC)_7$	6-FAM™	57°C
CSC-001	F: TGTAAAACGACGGCCAGTATTGGGTGGTTGCTTCAT R: ACGAGGAGAAAGTTGAGATTGC	$(CCTT)_{14}$	6-FAM™	55°C
CSC-004	F: TGTAAAACGACGGCCAGTACAACGGTAATTGTACGAGAA R: AGGCTAATGCCACCATCATC	$(TG)_{16}$	HEX™	58°C
CSC-007	F: TGTAAAACGACGGCCAGTGGGACAAACAACATGAAAGTGG R: GAAAACCTATTCCGGGAAGC	$(GA)_{35}$	6-FAM™	59°C
CSC-094	F: TGTAAAACGACGGCCAGTGTATCCACAACTGACTTTTCTCC R: GGAGAAACACCCTCAGAAAACC	$(TCTG)_6$	HEX™	55°C
CSA-035	F: TGTAAAACGACGGCCAGTGACTGGAGAAACGATAGGTG R: AACAAGGAGATTACACGGATTC	$(GT)_{29}$	NED™	46°C
CSA-073	F: TGTAAAACGACGGCCAGTGCCTATTTGCCTCGCTACCCC R: GTCACCAAAGTTGAGCAAGACTCTCT	$(GT)_{57}$	NED™	55°C
CSA-121	F: TGTAAAACGACGGCCAGTAATAAGAGAACAAACACACGGGG R: AACTGCTTGCCTTCCTTCCATC	$(AGAC)_9$	6-FAM™	55°C

Polymerase chain reactions (PCR) were performed following Schuelke (2000) method. An M13 universal tag sequence was added to the 5'-end of the forward primers (5'-TGTAAAACGACGGCCAGT-3') and a 7-bp pigtail was added to the 5'-end of the reverse primers (5'-GTGTCTT-3). The addition of the pigtail forces non-templated adenosine to be added to the 3' end, thus helping reduce genotyping error (Brownstein, Carpten, & Smith, 1996; Harker, 2001). The pigtail, however, was not added to the seven loci used by Lacerda et al. (2016) to prevent discrepancies in allele calling. To insert a fluorescent dye into each reaction, a third primer, M13 universal primer labeled with 6-FAM™, HEX™, or NED™, was added (Integrated DNA Technologies, Coralville, Iowa, USA; Applied Biosystems, Foster City, CA).

Due to the varying degree of amplification success for each marker, different PCR reaction mixes were utilized. The seven markers reported by Steven et al. (2005) and used by Lacerda et al. (2016) were amplified using the Type-It Microsatellite PCR Kit (QIAGEN, Valencia, California, USA). Each 5 μL reaction contained 1X Type-It Multiplex PCR Mastermix, 1X Q Solution, 1.25 μM of the forward primer, 5 μM each of the reverse and M13 primers, and 40-150 ng DNA. The reactions were performed on a BioRad MyCycler (Biorad, Hercules, California, USA). They began with a denaturing step at 95°C for 5 minutes, followed by 28 cycles of denaturation at 95°C for 30 seconds (s), annelation at 44 – 58°C for 90 s, and extension 72°C for 30s. An additional 10 cycles were used to embed the fluorescent dye, at 94°C for 30 s, 53°C for 45 s, and 72°C for 45 s. A final extension at 60°C for 30 min was used.

The nine microsatellite loci developed in our lab were amplified with PCR reactions containing 40-150 ng DNA; 1X PCR buffer; 1 U *Taq* DNA polymerase (New England BioLabs, Inc., Ipswich, MA); 1.25 μM forward primer; 5 μM of reverse and fluorescent M13 universal primer; 200 μM of each dNTP, for a final volume of 15 μL. The marker Pen23472 had 1.6 mM $MgCl_2$ added to each reaction. The thermocycler conditions differed as follows: a denaturation step at 95°C for 5 minutes, followed by 30 cycles of 95°C for 30 s, 53 – 63°C for 35 s, and extension at 72°C for 30 s. The same 10 cycles used above were included for incorporation of the fluorescent dye. A final extension at 72°C for 10 minutes was used.

Following PCR, samples were prepared for genotyping by diluting 1μL of PCR products into 8.7μL of formamide and 0.3 μL of MapMarker-ROX (BioVentures, Inc., Murfreesboro, TN) size standard. These were subsequently analyzed on an ABI 3130x1 Genetic Analyzer at the DNA Technologies Lab and Institute for Plant Genomics at Texas A&M University. GeneMarker v.1.6 (Softgenetics, State College, PA) was used for allele calling. Reproducibility was determined by randomly selecting 30% of the samples and repeating PCR and genotyping.

3.3 Statistical Methods

In addition to the microsatellite data we obtained, we also included genotypic data from Brazilian localities obtained by Lacerda et al., 2016. As mentioned earlier, this dataset only contained information of seven microsatellites. Therefore, analyses that included the Brazilian samples were based only on these microsatellites, whereas analyses for the samples from the US were based on the 16 microsatellites. The Brazilian

dataset was mainly used for analyses of genetic differentiation between the US and Brazil. However, we also conducted some basic analyses of genetic diversity for this dataset that were not presented by Lacerda et al. (2016).

PGDSpider v. 2.1.0.3 was used to convert data files between software packages (Lischer & Excoffier, 2012).To check for null alleles and scoring errors, MICRO-CHECKER (Van Oosterhout, Hutchinson, Wills, & P, 2004) was used. Linkage disequilibrium (LD) was tested in GENEPOP 4.2 (Raymond & Rousset, 1995).

3.3.1 Genetic Diversity

Expected and observed heterozygosity (H_E, H_O) and conformity to Hardy-Weinberg Equilibrium (HWE) were measured for each marker in GENEPOP 4.2 and GenAIEx (Peakall & Smouse, 2006, 2012). All GENEPOP analyses were performed with a dememorization number of 5,000, 500 batches, and 5,000 iterations per batch. Heterozygosity within populations (H_S), total heterozygosity (H_T), Nei's fixation index (D_{ST}), allelic richness (AR), number of alleles (N_A), and inbreeding coefficient (F_{IS}) with confidence intervals, for each locus and group were measured with FSTAT 2.9.3.2 (Goudet, 1995). Number of private alleles (N_P) per locus was determined in GenAlEx. To evaluate how sample size may affect allelic richness, a rarefaction analysis in Allelic Diversity Analyzer (ADZE; Szpiech *et al.* 2008) was performed. ADZE simulations were run for each group.

3.3.2 Population Structure

The statistical power of our dataset for detecting population structure was evaluated using POWSIM (Ryman & Palm, 2006). For each test, 1,000 simulations were

run at three levels of differentiation: F_{ST} = 0.01 (N_E = 500, t = 10); F_{ST} = 0.007 (N_E = 750, t = 10); and F_{ST} = 0.005 (N_E = 1000, t = 10).

Jost's differentiation (D_{ST}) and fixation index (G_{ST}) were calculated using FSTAT. Population differentiation was also tested using F-statistics calculated in GenAlEx, GENODIVE (Meirmans & Van Tienderen, 2004), and in FreeNA (Chapuis & Estoup, 2007). F_{ST} results from FreeNA were obtained with and without null allele corrections and GENODIVE was used to determine the p-values for the F_{ST} estimates. Isolation by distance (IBD) was tested using a Mantel test to compare a geographic distance and genetic distance matrix in Isolation By Distance, Web Service (IBDWS; Jensen *et al.* 2005). Genetic distances were obtained from GENODIVE and geographic distances were estimated in Google Earth (Google Developers, 2016). These were then compared in a linear regression to evaluate patterns of isolation by distance.
Analyses of molecular variance (AMOVA) was performed in GENODIVE. The data was grouped in multiple ways to examine variation across localities. We conducted different tests: (1) all localities separately; (2) followed by all localities in the U.S., then all localities in the GOM. Additionally, all localities in the eastern GOM were grouped for comparison against all localities in the western GOM. All localities in the GOM were compared against the Chesapeake Bay locality and all localities in the U.S. were compared against all localities in BR.

STRUCTURE 2.2.3 (Pritchard, Stephens, & Donnelly, 2000) was used to perform model-based clustering with a Bayesian approach to estimate genetic structure. In STRUCTURE, the algorithm estimates the percentage of the genome of an individual

derived from the presumed populations. K values from 2 to 10 were tested in three iterations, with 500,000 steps and a burn-in of 125,000 steps. The models tested in STRUCTURE were admixture with correlated allele frequencies, admixture with independent allele frequencies, no admixture with correlated allele frequencies, and no admixture with independent allele frequencies. All other settings were set to default. The most appropriate K value was determined in STRUCTURE HARVESTER with the Evanno method (Earl & vonHoldt, 2012; Evanno, Regnaut, & Goudet, 2005).

The spatial clustering program GENELAND (Guillot, Mortier, & Estoup, 2005) was used to infer the number of populations in each grouping. This program uses Bayesian analyses to determine the spatial extent of the estimated groups, with each group being homogenous. Ten independent runs were performed each time, with 50,000 iterations and a thinning value of 50. Geographic coordinates were converted into planar coordinates in ArcMap (Environmental Systems Research Institute, Redlands, California) and an uncertainty value of 100 m was selected. Each simulation was tested with two models, correlated and uncorrelated frequencies. The highest log likelihood value out of the ten runs was selected as the most appropriate cluster value.

GenAlEx was used to construct a genetic distance matrix, from which a principal coordinate analysis (PCA) was performed to identify population clusters. GENETIX (Belkhir, Borsa, Chikhi, Raufaste, & Bonhomme, 2004) was used to conduct a three-dimensional factorial correspondence analysis (FCA). This method seeks to identify correspondence between values in rows and columns, such as individuals and alleles.

For both sets of analyses, samples from the U.S., the GOM, and BR were compared against each other.

3.3.3 Effective Population Size and Recent Bottleneck Analyses

Effective population size (N_E) was estimated using two programs: NeEstimator (Do et al., 2014) and COLONY (Jones & Wang, 2009). NeEstimator calculates N_E based on linkage disequilibrium, the heterozygote excess method, and the molecular coancestry method. COLONY estimates N_E based on sibship assignments, which are calculated with a maximum likelihood method. Random mating was assumed in NeEstimator and COLONY reported both random and non-random mating results. In COLONY, female and male monogamy were assumed, as well as no inbreeding. MicroDrop (C. Wang, Schroeder, & Rosenberg, 2012) was used to obtain allelic dropout rates for each marker, one of the required inputs for COLONY. For genotyping errors per loci, the other requirement for COLONY, the recommended value of 0.05 was used (Rossi Lafferriere et al., 2016; J. Wang, 2004).

BOTTLENECK (Piry, Luikart, & Cornuet, 1999) was used to determine whether the blue crab population has recently undergone a bottleneck. BOTTLENECK calculates the distribution of H_E from the observed number of alleles for each sample and locus, under three possible mutation models. The mutation models, infinite allele model (IAM), stepwise mutation model (SMM), and the two-phase model (TPM) are used to calculate expected H_E, which is compared to H_O and subsequently used to determine whether a locus has heterozygote excess or deficit. For the TPM, the suggested model for microsatellites, Piry et al., 1999 recommended using 95% single-step mutations and 5%

multi-step mutations, as well as a variance of 12 among multiple steps. BOTTLENECK also examines the allele frequency distribution. Under mutation-drift equilibrium, an L-shaped distribution is expected, while a recent bottleneck is expected to cause a mode shift.

4. RESULTS

Reproducibility was 100% for all the samples that were repeated (30% of the total samples). Linkage disequilibrium was detected between two pairs of loci: *Tet1329* and *Tet1057* ($P = 0.025$) and *Tet1329* and *CSC094* ($P = 0.020$). Because *Tet1329* was present in both pairs, analyses were conducted excluding and including this locus to assess its impact on the results. Both sets of analyses are reported and present similar results. Analyses were also done without the loci with heavy stutter bands (CSC007, CSC001, CSC004, Pen23472, CSA035, CSA073, Di680) and are shown in Appendix A.

Table 3 shows genetic diversity estimations and inbreeding coefficients (F_{IS}) per locus for the US (16 loci) and Brazilian (7 loci) localities. No significant deviations of HWE after Bonferroni correction ($P < 0.003$) were observed in five microsatellites at any US location (Tet6290, Tet1329, Tet603, CSC007 and CSA121), whereas the others showed HWE departures in one to eight localities. Each locality have at least two loci departing HWE, with Chesapeake Bay being the US locality with the higher number of loci (9) showing departures of HWE. MICRO-CHECKER indicated the presence of potential null alleles in all but four loci (Table 3).

Tables 4 and 5 show genetic diversity estimations and percentage of missing data per locus for all localities. Percentage of missing data per locus ranged from 0.5% (*Tet1329*) to 16.5% (*Tet1886*), with an overall average of 5.6% of missing data. Number of alleles varied greatly between markers.

Table 3. Genetic diversity and inbreeding coefficient for 16 polymorphic loci for the ten localities in the U.S. and 7 polymorphic loci for the 4 localities in Brazil. Bolded values indicate significant departures from HWE ($P < 0.003$ for the 16 loci and $P < 0.007$ for the 7 loci. Italicized loci indicate presence of null alleles. N = number of samples genotyped; N_A/N_P = number of alleles and private alleles; AR = allelic richness, H_O; H_E = observed and expected heterozygosity, F_{IS} = inbreeding coefficient.

Apalachicola, Florida								Avery Island, Louisiana							
Primer	N	N_A/N_P	AR	H_O	H_E	P_{HW}	F_{IS}	Primer	N	N_A/N_P	AR	H_O	H_E	P_{HW}	F_{IS}
Tri24376	20	6/0	4.504	0.700	0.733	0.4529	0.0699	*Tri24376*	19	6/0	5.078	0.474	0.769	0.0037	0.4066
Tet6290	20	3/1	2.523	0.200	0.335	0.0164	0.4242	Tet6290	18	4/0	2.898	0.278	0.335	0.3450	0.1981
Tet1886	20	5/0	4.164	0.450	0.708	0.0116	0.3860	*Tet1886*	14	4/0	3.739	0.286	0.676	**0.0022**	0.6015
Tet1329	20	3/0	2.347	0.450	0.434	0.4665	-0.0118	Tet1329	20	3/0	2.350	0.500	0.516	1.0000	0.0571
Tet1057	18	4/0	3.407	0.222	0.532	**0.0010**	0.6012	*Tet1057*	17	4/0	3.691	0.235	0.590	**0.0001**	0.6202
Tet603	19	6/0	4.400	0.684	0.616	0.8158	-0.0833	Tet603	19	7/0	5.194	1.000	0.726	0.3830	-0.3545
Pen9028	20	7/0	4.836	0.500	0.606	0.2865	0.2000	*Pen9028*	19	7/0	4.816	0.421	0.601	0.0106	0.3239
Di680	19	14/0	8.591	0.684	0.881	0.0040	0.2488	Di680	18	13/0	8.815	0.833	0.897	0.1312	0.0989
Pen23472	20	6/1	4.247	0.450	0.703	0.0039	0.3816	Pen23472	16	5/0	4.218	0.625	0.666	0.0474	0.0937
CSC007	19	17/0	9.895	1.000	0.917	0.3357	-0.0638	CSC007	18	17/1	9.889	1.000	0.914	0.3192	-0.0662
CSC001	18	17/0	10.199	0.556	0.923	**0.0000**	0.4218	*CSC001*	15	11/0	8.408	0.267	0.884	**0.0000**	0.7157
CSA035	19	22/2	10.975	0.789	0.934	**0.0021**	0.1806	*CSA035*	18	18/1	10.380	0.722	0.926	0.0067	0.2470
CSC004	18	20/1	11.006	0.722	0.934	0.0032	0.2534	*CSC004*	18	17/0	9.625	0.722	0.895	0.0113	0.2205
CSC094	19	8/0	5.049	0.632	0.735	0.2438	0.1676	*CSC094*	17	9/0	6.618	0.529	0.817	0.0124	0.3780
CSA121	17	4/0	2.883	0.235	0.310	0.2692	0.2686	CSA121	18	5/0	3.289	0.278	0.341	0.1446	0.2130
CSA073	18	13/0	8.399	0.944	0.881	**0.0008**	-0.0433	CSA073	17	20/3	10.776	0.882	0.924	0.1175	0.0751
Cedar Key, Florida								D'Iberville, Mississippi							
Primer	N	N_A/N_P	AR	H_O	H_E	P_{HW}	F_{IS}	Primer	N	N_A/N_P	AR	H_O	H_E	P_{HW}	F_{IS}
Tri24376	11	5/1	4.384	0.273	0.599	0.0042	0.5775	*Tri24376*	24	6/0	4.367	0.333	0.681	**0.0006**	0.5258
Tet6290	12	3/0	2.837	0.333	0.542	0.0981	0.4211	Tet6290	24	4/0	2.721	0.417	0.350	1.0000	-0.1705
Tet1886	12	6/1	5.321	0.667	0.747	0.1796	0.1498	Tet1886	21	5/0	3.227	0.429	0.559	0.0363	0.2562
Tet1329	12	3/0	2.837	0.583	0.517	1.0000	-0.0845	Tet1329	24	5/1	2.873	0.500	0.510	0.3743	0.0417
Tet1057	12	4/0	3.968	0.250	0.712	**0.0001**	0.6733	*Tet1057*	20	4/0	3.829	0.450	0.661	0.0085	0.3423
Tet603	9	5/0	4.333	0.444	0.630	0.2621	0.3469	Tet603	24	8/1	5.071	0.750	0.663	0.6182	-0.1099
Pen9028	12	7/0	6.034	0.500	0.792	**0.0026**	0.4054	*Pen9028*	23	7/0	5.189	0.478	0.740	0.0371	0.3731
Di680	12	10/0	8.421	0.833	0.885	0.2161	0.1020	Di680	24	11/0	7.681	0.833	0.876	0.8556	0.0698
Pen23472	12	6/0	5.250	0.917	0.767	0.0662	-0.1524	Pen23472	24	6/0	4.546	0.583	0.703	0.4781	0.1910
CSC007	12	12/1	8.802	0.917	0.882	0.5527	0.0041	CSC007	24	21/2	10.606	0.958	0.935	0.0628	-0.0038
CSC001	11	11/0	8.920	0.545	0.884	**0.0014**	0.4231	*CSC001*	23	19/1	9.573	0.391	0.905	**0.0000**	0.5823
CSA035	12	15/1	10.519	0.500	0.913	**0.0002**	0.4864	*CSA035*	23	24/1	11.043	0.783	0.940	**0.0023**	0.1885
CSC004	12	15/0	10.883	0.417	0.924	**0.0000**	0.5785	CSC004	24	20/0	10.486	0.875	0.932	0.1945	0.0826
CSC094	12	7/1	5.547	0.583	0.722	0.2868	0.2338	*CSC094*	23	7/0	5.164	0.565	0.711	0.0405	0.2260
CSA121	11	4/1	2.909	0.273	0.248	1.0000	-0.0526	CSA121	23	3/0	2.453	0.304	0.269	1.0000	-0.1079
CSA073	12	15/2	10.583	0.917	0.917	0.1633	0.0435	CSA073	22	23/2	10.681	0.818	0.931	**0.0029**	0.1438

Table 3 (continued)

Galveston, Texas								Lower Laguna Madre, Texas							
Primer	N	N_A/N_P	AR	H_O	H_E	P_{HW}	F_{IS}	Primer	N	N_A/N_P	AR	H_O	H_E	P_{HW}	F_{IS}
Tri24376	12	4/0	3.817	0.250	0.681	0.0051	0.6580	*Tri24376*	19	6/1	4.315	0.263	0.701	**0.0000**	0.6407
Tet6290	12	2/0	1.837	0.167	0.153	1.0000	-0.0476	Tet6290	21	2/0	1.817	0.095	0.172	0.1448	0.4667
Tet1886	12	4/0	3.776	0.083	0.622	**0.0000**	0.8764	Tet1886	23	3/0	2.520	0.348	0.517	0.1305	0.3469
Tet1329	11	2/0	1.998	0.455	0.351	1.0000	-0.2500	Tet1329	24	4/1	2.579	0.542	0.453	0.8070	-0.1749
Tet1057	12	4/0	3.654	0.250	0.514	0.0158	0.5448	*Tet1057*	22	4/0	3.725	0.182	0.627	**0.0000**	0.7214
Tet603	12	10/2	7.218	0.833	0.778	0.9339	-0.0280	Tet603	24	9/0	4.396	0.625	0.615	0.2476	0.0043
Pen9028	12	7/0	6.119	0.500	0.785	0.0046	0.4000	*Pen9028*	23	8/0	5.479	0.565	0.718	0.0083	0.0009
Di680	12	10/1	7.511	0.833	0.823	0.0866	0.0308	Di680	16	14/0	9.513	0.938	0.910	0.5754	0.0090
Pen23472	12	5/0	4.464	0.667	0.708	0.0590	0.1020	*Pen23472*	24	7/1	4.661	0.667	0.734	**0.0022**	0.0004
CSC007	12	15/0	10.669	0.917	0.917	0.1462	0.0435	CSC007	19	22/1	11.125	1.000	0.929	1.0000	0.0000
CSC001	12	10/1	7.867	0.667	0.858	0.0130	0.2636	*CSC001*	20	16/0	9.256	0.450	0.901	**0.0000**	0.0000
CSA035	12	15/0	10.733	0.667	0.920	**0.0022**	0.3152	CSA035	22	22/0	11.051	0.955	0.941	0.7430	0.0108
CSC004	12	17/1	11.692	0.500	0.934	**0.0000**	0.4981	*CSC004*	24	20/0	9.514	0.583	0.905	**0.0003**	0.0003
CSC094	12	6/0	5.094	0.750	0.726	0.9416	0.0100	CSC094	24	8/0	5.069	0.708	0.694	0.5414	0.0063
CSA121	12	3/0	2.817	0.417	0.403	0.5598	0.0090	CSA121	24	5/0	3.393	0.292	0.418	0.0109	0.0007
CSA073	12	13/0	9.346	0.833	0.892	0.1170	0.1093	CSA073	23	19/2	10.255	0.739	0.929	**0.0000**	0.0000
Port Lavaca, Texas								Rockport, Texas							
Primer	N	N_A/N_P	AR	H_O	H_E	P_{HW}	F_{IS}	Primer	N	N_A/N_P	AR	H_O	H_E	P_{HW}	F_{IS}
Tri24376	18	6/1	4.632	0.389	0.727	**0.0008**	0.4871	*Tri24376*	21	4/0	3.594	0.286	0.612	0.0029	0.5506
Tet6290	18	2/0	1.997	0.222	0.401	0.0747	0.4688	Tet6290	22	2/0	1.967	0.273	0.298	0.5388	0.1064
Tet1886	17	6/0	5.089	0.353	0.744	**0.0019**	0.5472	*Tet1886*	17	7/1	5.057	0.294	0.715	**0.0006**	0.6078
Tet1329	18	4/0	2.778	0.444	0.539	0.3035	0.2023	Tet1329	22	3/0	2.539	0.591	0.492	0.5381	-0.1793
Tet1057	17	4/0	3.811	0.176	0.621	**0.0000**	0.7303	*Tet1057*	19	6/1	4.211	0.263	0.587	**0.0005**	0.5704
Tet603	18	7/0	5.063	0.778	0.684	0.1954	-0.1096	Tet603	22	6/0	4.512	0.682	0.630	0.5617	-0.0588
Pen9028	18	8/0	5.845	0.500	0.769	0.0192	0.3742	Pen9028	21	7/1	5.141	0.667	0.712	0.7482	0.0879
Di680	18	14/0	8.748	0.667	0.887	0.0238	0.2753	*Di680*	22	16/0	9.284	0.636	0.910	**0.0000**	0.3218
Pen23472	18	5/0	4.586	0.611	0.765	0.0041	0.2289	Pen23472	22	4/0	3.860	0.591	0.701	0.2596	0.1802
CSC007	18	20/0	11.069	0.944	0.937	0.6532	0.0203	CSC007	22	18/0	9.920	0.955	0.921	0.7960	-0.0126
CSC001	17	15/2	9.782	0.471	0.915	**0.0000**	0.5086	*CSC001*	20	13/1	8.191	0.200	0.879	**0.0000**	0.7825
CSA035	18	20/2	11.103	0.778	0.937	**0.0009**	0.1973	CSA035	22	25/1	11.711	0.864	0.950	**0.0022**	0.1143
CSC004	18	20/1	11.256	0.667	0.940	**0.0000**	0.3166	*CSC004*	21	17/1	9.726	0.524	0.917	**0.0000**	0.4486
CSC094	17	7/0	5.887	0.588	0.813	0.0273	0.3043	CSC094	21	8/0	5.784	0.762	0.743	0.3772	-0.0016
CSA121	18	5/0	3.042	0.389	0.336	1.0000	-0.1280	CSA121	22	3/0	2.785	0.500	0.406	0.7652	-0.2094
CSA073	18	17/0	10.542	0.889	0.931	0.1394	0.0733	CSA073	22	22/0	10.617	0.909	0.932	0.0206	0.0476

Table 3 (continued)

Chesapeake Bay								Slidell, Louisiana							
Primer	N	N_A/N_P	AR	H_O	H_E	P_{HW}	F_{IS}	Primer	N	N_A/N_P	AR	H_O	H_E	P_{HW}	F_{IS}
Tri24376	25	6/0	4.373	0.640	0.690	0.0407	0.0933	Tri24376	11	5/0	4.270	0.545	0.702	0.0267	0.2683
Tet6290	25	3/0	2.196	0.280	0.274	0.1841	-0.0000	Tet6290	10	2/0	1.921	0.200	0.180	1.0000	-0.0588
Tet1886	14	6/0	4.259	0.286	0.638	**0.0014**	0.5772	*Tet1886*	7	5/0	5.000	0.429	0.765	0.0305	0.5000
Tet1329	25	4/0	2.878	0.440	0.416	0.1340	-0.0373	*Tet1329*	11	3/0	2.627	0.182	0.368	0.0372	0.5402
Tet1057	23	6/1	4.640	0.348	0.711	**0.0000**	0.5269	Tet1057	11	4/0	3.152	0.273	0.318	0.2804	0.1892
Tet603	24	8/1	5.285	0.833	0.724	0.4200	-0.1302	Tet603	11	4/0	3.513	0.636	0.533	0.2368	-0.1475
Pen9028	24	8/0	6.176	0.458	0.804	**0.0002**	0.4470	*Pen9028*	11	5/0	4.384	0.364	0.599	0.0312	0.4326
Di680	17	12/0	8.703	0.647	0.898	**0.0002**	0.3071	Di680	11	11/0	8.418	0.636	0.843	0.0116	0.2893
Pen23472	25	7/1	5.297	0.520	0.790	**0.0009**	0.3600	Pen23472	11	4/0	3.918	0.545	0.632	0.0637	0.1837
CSC007	22	21/1	10.721	0.955	0.935	0.0812	0.0023	CSC007	10	12/0	9.849	1.000	0.900	1.0000	-0.0588
CSC001	25	14/1	9.168	0.240	0.912	**0.0000**	0.7460	*CSC001*	9	7/0	6.655	0.222	0.833	**0.0000**	0.7594
CSA035	23	22/1	10.887	0.609	0.940	**0.0000**	0.3714	CSA035	10	11/0	9.211	0.700	0.890	0.0487	0.2632
CSC004	24	21/1	10.721	0.583	0.938	**0.0000**	0.3959	*CSC004*	11	14/1	10.691	0.636	0.917	**0.0025**	0.3488
CSC094	25	8/0	5.507	0.520	0.754	**0.0008**	0.3283	CSC094	11	5/0	4.590	0.636	0.740	0.3199	0.1860
CSA121	24	6/1	3.479	0.417	0.418	0.8416	0.0234	CSA121	11	3/0	2.627	0.273	0.368	0.4373	0.3023
CSA073	24	21/1	10.432	0.875	0.931	0.0041	0.0809	CSA073	10	17/1	12.563	0.900	0.935	0.1406	0.0899
Lagoa dos Patos, Brazil								Tramandaí, Brazil							
Primer	N	N_A/N_P	AR	H_O	H_E	P_{HW}	F_{IS}	Primer	N	N_A/N_P	AR	H_O	H_E	P_{HW}	F_{IS}
CSC007	55	26/1	11.973	0.855	0.924	**0.0006**	0.0844	CSC007	28	23/0	12.359	0.929	0.926	0.0556	0.0154
CSC001	56	13/2	5.139	0.250	0.583	**0.0000**	0.5775	CSC001	24	7/1	5.919	0.208	0.510	**0.0002**	0.6055
CSA035	54	20/1	5.220	0.685	0.724	0.0628	0.0633	CSA035	33	11/0	6.340	0.485	0.517	0.2868	0.0775
CSC004	54	14/2	5.501	0.870	0.774	1.0000	-0.1158	CSC004	32	10/1	5.234	0.688	0.734	0.1091	0.0796
CSC094	57	5/0	2.204	0.263	0.240	**0.0012**	-0.086	CSC094	25	3/0	2.684	0.120	0.183	0.1977	0.3628
CSA121	57	3/1	1.730	0.070	0.101	0.0037	0.3139	CSA121	33	2/0	2.534	0.121	0.114	1.000	-0.0492
CSA073	58	26/0	5.689	0.828	0.795	0.0371	-0.0317	CSA073	34	12/1	12.563	0.794	0.710	**0.0000**	-0.1041
Itajaí, Brazil								Laguna, Brazil							
Primer	N	N_A/N_P	AR	H_O	H_E	P_{HW}	F_{IS}	Primer	N	N_A/N_P	AR	H_O	H_E	P_{HW}	F_{IS}
CSC007	40	26/1	12.866	0.950	0.938	0.0189	-0.0003	CSC007	67	33/4	12.579	0.925	0.949	**0.0001**	0.0322
CSC001	40	9/1	6.194	0.450	0.673	**0.0000**	0.3430	CSC001	67	16/2	9.714	0.358	0.600	**0.0000**	0.409
CSA035	52	17/0	6.636	0.635	0.604	0.7543	-0.0402	CSA035	65	22/3	10.707	0.646	0.669	0.258	0.0419
CSC004	43	10/3	6.135	0.767	0.729	**0.0012**	-0.0417	CSC004	66	19/2	10.427	0.773	0.747	0.0475	-0.0266
CSC094	45	4/0	2.680	0.333	0.400	0.0695	0.1776	CSC094	67	5/0	5.049	0.388	0.346	0.9077	-0.1132
CSA121	43	3/0	1.503	0.140	0.250	**0.0012**	0.4510	CSA121	67	3/0	2.680	0.060	0.058	1.000	-0.0154
CSA073	53	21/6	8.647	0.962	0.773	**0.0013**	-0.2352	CSA073	66	28/5	11.347	0.758	0.776	**0.0019**	0.0317

The seven markers used also in the Brazilian study (Lacerda et al. 2016) were highly polymorphic (N_A: 8 – 60) and consisted of mostly di-nucleotide repeat motifs. The markers developed for this study were not as polymorphic (N_A: 5 – 19), which is likely due to the use of primarily tetra- and penta-nucleotide repeat motifs.

Table 4. Genetic diversity, inbreeding coefficient, and percentage of missing data, per locus in U.S. localities. N_A = Mean number of alleles, H_O = observed heterozygosity; H_S = heterozygosity within populations; H_T = total heterozygosity, H_T' = corrected total heterozygosity.

Locus	N_A	H_O	H_S	H_T	H_T'	G_{IS}	*% Missing*
Tri24376	10	0.415	0.720	0.715	0.715	0.424	4.3%
Tet6290	5	0.246	0.316	0.321	0.322	0.219	3.2%
Tet1886	9	0.362	0.706	0.707	0.708	0.487	16.5%
Tet1329	6	0.469	0.474	0.486	0.487	0.011	0.5%
Tet1057	7	0.265	0.618	0.611	0.61	0.571	9%
Tet603	14	0.727	0.679	0.687	0.688	-0.07	3.2%
Pen9028	11	0.495	0.742	0.741	0.741	0.332	2.7%
Di680	19	0.754	0.915	0.920	0.92	0.176	10.1%
Pen23472	9	0.618	0.743	0.784	0.788	0.169	2.1%
CSC007	36	0.965	0.948	0.949	0.949	-0.017	6.4%
CSC001	30	0.401	0.937	0.936	0.936	0.572	9.6%
CSA035	43	0.737	0.965	0.963	0.963	0.237	4.8%
CSC004	36	0.623	0.962	0.960	0.96	0.353	3.2%
CSC094	11	0.627	0.773	0.77	0.769	0.188	3.7%
CSA121	8	0.338	0.363	0.360	0.36	0.071	4.3%
CSA073	46	0.871	0.952	0.957	0.958	0.085	5.3%

Table 5. Genetic diversity and inbreeding coefficient per locus in Brazil. N_A = Mean number of alleles, H_O = observed heterozygosity; H_S = heterozygosity within populations; H_T = total heterozygosity, H_T' = corrected total heterozygosity.

Locus	N_A	H_O	H_S	H_T	H_T'
CSC007	42	0.915	0.934	0.936	0.937
CSC001	24	0.317	0.582	0.588	0.591
CSA035	33	0.613	0.635	0.639	0.640
CSC004	31	0.775	0.747	0.747	0.747
CSC094	6	0.276	0.296	0.301	0.303
CSA121	3	0.074	0.082	0.082	0.082
CSA073	43	0.835	0.770	0.774	0.775

Table 6. Genetic diversity and inbreeding coefficient per locality and region. N_A/N_P = Mean number of alleles and private alleles, H_O = observed and; H_E = expected heterozygosity, and F_{IS} = inbreeding coefficient with confidence intervals per locality.

Locality	N_A/N_P	H_O	H_E	F_{IS}	F_{IS} 95% CI
APA	9.69/0.31	0.576	0.699	0.202	0.096 – 0.210
AVI	9.38/0.31	0.566	0.717	0.240	0.173 – 0.331
CEK	8.0/0.5	0.560	0.730	0.276	0.147 – 0.276
DIB	7.94/0.5	0.549	0.691	0.247	0.114 – 0.222
GAL	10.81/0.31	0.592	0.710	0.188	0.109 – 0.247
LLM	10.44/0.44	0.559	0.698	0.222	0.188 – 0.264
POL	10.0/0.38	0.554	0.747	0.285	0.202 – 0.285
ROC	10.06/0.38	0.562	0.713	0.235	0.160 – 0.263
SER	10.81/0.56	0.541	0.736	0.286	0.194 – 0.294
SLI	7.0/0.13	0.511	0.658	0.272	0.193 – 0.295
LPA	15.3/0.857	0.546	0.592	0.087	0.015 – 0.14
TRA	9.7/0.429	0.478	0.528	0.112	-0.004 – 0.193
ITA	12.9/1.571	0.605	0.624	0.041	-0.044 – 0.103
LAG	18/2.286	0.558	0.592	0.065	0.003 – 0.113
U.S. (16 loci)	18.750/--	0.561	0.739	0.233	0.183 – 0.283
U.S. (7 loci)	30/--	0.655	0.839	0.205	0.129 – 0.281
GOM	18.188/7.938	0.563	0.736	0.231	0.181 – 0.281
BR	26/--	0.119	0.553	0.596	0.031 – 0.179
U.S.& BR	28/11.9(U.S.)/7.9(BR)	0.604	0.718	0.155	0.102 – 0.208

Table 6 shows genetic diversity and inbreeding coefficient per locality.

Heterozygosity deficiencies were in general observed within localities and regions, as well as high values of F_{IS}. Differences between H_O and H_E, and of F_{IS} values, however,

were lower for Brazilian localities. Average number of alleles per locality did not vary much (N_A: 8 – 10) across the localities in the U.S, despite sample size. Slidell, Louisiana (n = 11), had the lowest average number of alleles (N_A = 7.0), whereas Galveston, Texas (n = 12) and the Chesapeake Bay (n = 25) both had the highest average number of alleles (10.81) in the U.S. Allelic diversity in Brazil was higher in localities with larger sample sizes. Laguna, Brazil (n = 67) had the highest overall average number of alleles (N_A = 18). Rarefaction analyses conducted in ADZE suggest the sample sizes used capture most of the allelic diversity present in the U.S. and Brazil (Figures 5 and 6).

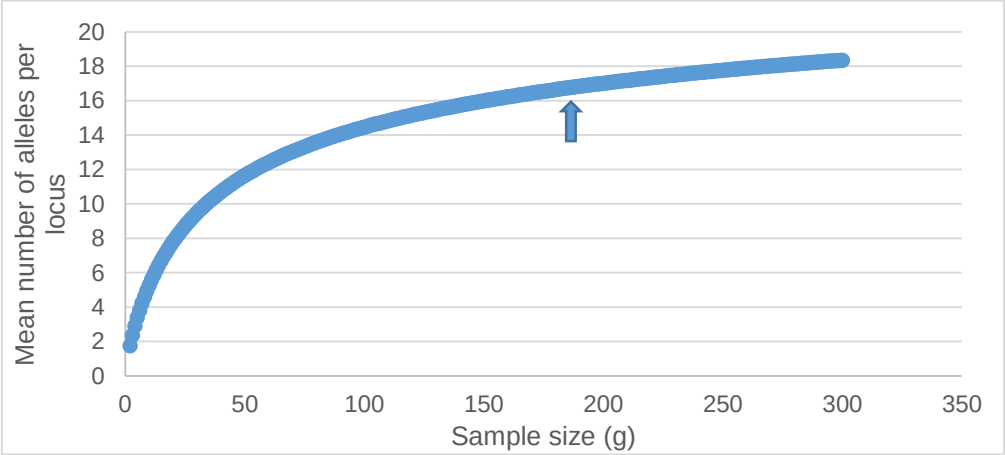

Figure 9. ADZE rarefaction analysis for the U.S., using sixteen loci. The arrow indicates where the used sample size falls along the curve.

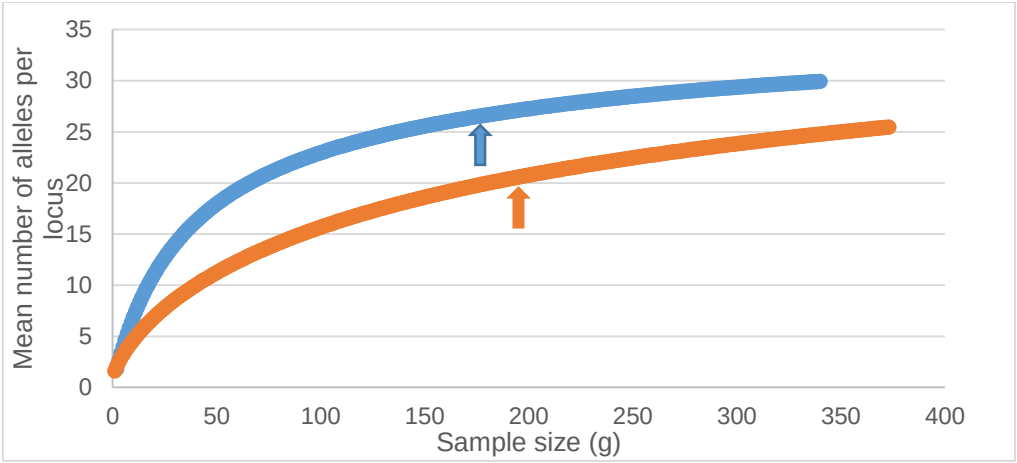

Figure 10. ADZE rarefaction analysis for the U.S. (blue line) and Brazil (orange line), using seven loci. The arrows indicate where the used sample size falls along the curve.

4.1 Analyses of Population Structure

POWSIM results indicate our dataset have a high probability (96.7–100%) of detecting population structure even at low F_{ST} values (0.005) (Table 7). Very low values of D_{ST} (Jost's differentiation) and G_{ST} (fixation index) were obtained for each locus within the US (Table 8). This was also observed within Brazil (Table 9). Combining information of all loci, low D_{ST} and G_{ST} values were still observed within the US (16 loci) and Brazil (7 loci), but their value substantially increased when information from the US and Brazil were combined (7 loci) (Table 10).

Table 7. Probability of detecting population structure, estimated in POWSIM.

	F_{ST} = 0.01	F_{ST} = 0.007	F_{ST} = 0.005
GOM	100%	100%	96.9%
U.S.	100%	99.7%	96.7%
U.S. & BR	100%	100%	100%

Table 8. Jost's differentiation, fixation index, and Nei's corrected fixation index (D_{ST}, D_{ST}', G_{ST}, G_{ST}') per locus in the U.S.

Locus	D_{ST}	D_{ST}'	G_{ST}	G_{ST}'
Tri24376	-0.005	-0.006	-0.007	-0.008
Tet6290	0.006	0.006	0.018	0.02
Tet1886	0.001	0.001	0.002	0.002
Tet1329	0.012	0.014	0.025	0.028
Tet1057	-0.006	-0.007	-0.01	-0.012
Tet603	0.008	0.009	0.012	0.013
Pen9028	-0.001	-0.001	-0.001	-0.001
Di680	0.005	0.005	0.005	0.006
Pen23472	0.041	0.045	0.052	0.057
CSC007	0.001	0.001	0.001	0.001
CSC001	-0.001	-0.001	-0.001	-0.001
CSA035	-0.002	-0.002	-0.002	-0.002
CSC004	-0.002	-0.003	-0.002	-0.003
CSC094	-0.003	-0.004	-0.004	-0.005
CSA121	-0.003	-0.003	-0.009	-0.009
CSA073	0.005	0.006	0.005	0.006

Table 9. Measures of population differentiation for all populations in Brazil. D_{ST} = Jost's differentiation, D_{ST}' = Jost's corrected differentiation, G_{ST} = fixation index, G_{ST}' = Nei's corrected fixation index, and G_{IS} = inbreeding coefficient for each locus.

Locus	D_{ST}	D_{ST}'	G_{ST}	G_{ST}'	G_{IS}
CSC007	0.003	0.004	0.003	0.004	0.020
CSC001	0.006	0.009	0.011	0.014	0.0456
CSA035	0.003	0.005	0.005	0.007	0.036
CSC004	0.000	0.000	0.000	0.001	0.037
CSC094	0.005	0.007	0.017	0.022	0.068
CSA121	0.000	0.000	0.004	0.005	0.096
CSA073	0.003	0.004	0.004	0.006	0.084

Table 10. Genetic diversity and structure per region. Mean observed heterozygosity, heterozygosity within populations, total heterozygosity, corrected total heterozygosity, Jost's differentiation, fixation index, Nei's corrected fixation index, and inbreeding coefficient for each group.

Group	H_O	H_S	H_T	H_T'	D_{ST}	D_{ST}'	G_{ST}	G_{ST}'	G_{IS}
GOM	0.559	0.736	0.741	0.741	0.019	0.019	0.006	0.007	0.241
U.S.	0.557	0.738	0.742	0.742	0.015	0.015	0.005	0.005	0.246
U.S. & BR	0.623	0.769	0.821	0.825	0.241	0.249	0.063	0.067	0.191
BR	0.550	0.591	0.594	0.595	0.009	0.009	0.005	0.006	0.069

F_{ST} pairwise tests found similar results. Pairwise comparisons between US localities conducted in FreeNA with and without null allele correction, and including/excluding locus *Tet1329* do not show significant differentiation (Tables 11 and 12). However, all FreeNA pairwise comparisons between US and Brazil, with and without correction of null alleles were significant (Table 13). Only one pairwise comparison within the US (Cedar Key and Galveston) was significant for tests conducted in Genodive including and excluding locus *Tet1329* (Table 14).

Table 11. F_{ST} with (lower) and without (upper) ENA for 16 loci for U.S. populations. Significance was tested at P = 0.05 and none were significant.

	APA	AVI	CEK	DIB	GAL	LLM	POL	ROC	SERC	SLI
APA	--	-0.00419	0.005372	-0.00458	0.004548	0.002501	-0.00232	0.000126	-0.00292	0.00005
AVI	0.008332	--	0.006273	0.004912	-0.0008	0.008251	-0.00231	0.004362	-0.0056	0.004087
CEK	0.013126	0.024061	--	-0.00798	-0.00552	-0.00304	-0.00266	0.002422	-0.0068	-0.00034
DIB	0.003991	0.015901	0.00861	--	0.002521	-0.00323	0.000314	-0.00096	-0.00462	0.001845
GAL	0.007746	0.011973	0.018743	0.009233	--	-0.00113	-0.00332	-0.00053	-0.00554	-0.00105
LLM	0.010953	0.007839	0.018407	0.002204	0.004784	--	0.002185	-0.00387	-0.00118	-0.0081
POL	0.008108	0.002195	0.001937	0.007483	0.012951	0.008974	--	0.003576	-0.00473	-0.00502
ROC	0.005834	0.006647	0.016769	0.007926	0.007098	0.002429	0.003808	--	-0.00088	-0.00679
SERC	0.003496	0.006503	0.007054	0.000306	-0.00321	-0.00037	0.004495	0.001269	--	0.000072
SLI	0.006511	0.022744	0.027957	0.009048	0.002012	0.006702	0.019189	0.015209	0.015133	--

Table 12. F_{ST} with ENA for 15 loci for U.S. populations. Significance was tested at P = 0.05 and none were significant.

	APA	AVI	CEK	DIB	GAL	LLM	POL	ROC	SERC	SLI
APA	--									
AVI	0.007703	--								
CEK	0.014763	0.025777	--							
DIB	0.004924	0.017079	0.010393	--						
GAL	0.008897	0.009808	0.019556	0.009640	--					
LLM	0.012383	0.007066	0.020144	0.002951	0.005589	--				
POL	0.001521	0.001744	-0.001978	0.003906	0.003938	0.002307	--			
ROC	0.006812	0.007198	0.018865	0.009285	0.007217	0.003140	-0.000355	--		
SERC	0.003984	0.003083	0.007281	-0.000121	-0.002655	-0.000199	-0.006204	0.000713	--	
SLI	0.007551	0.021236	0.029957	0.009836	0.002977	0.007594	0.010980	0.015844	0.017015	--

Table 13. F_{ST} values for 16 loci (lower) and 15 loci (upper) performed in GENODIVE without null allele correction. Bolded values indicate significance (P < 0.005).

	APA	AVI	CEK	DIB	GAL	LLM	POL	ROC	SER	SLI
APA	--	0.007	0.015	0.003	0.003	0.011	-0.001	0.009	0.001	0.002
AVI	0.008	--	0.021	0.018	0.005	0.007	-0.001	0.007	0	0.016
CEK	0.014	0.02	--	0.007	**0.01**	0.017	-0.006	0.013	-0.001	0.02
DIB	0.003	0.016	0.005	--	0.007	-0.001	0.001	0.008	-0.004	0.009
GAL	0.002	0.007	**0.01**	0.007	--	0	-0.004	0.002	-0.01	-0.002
LLM	0.01	0.007	0.016	-0.001	0	--	-0.001	-0.001	-0.004	0.003
POL	0.006	-0.001	-0.002	0.005	0.005	0.005	--	-0.004	-0.01	-0.001
ROC	0.008	0.007	0.011	0.007	0.002	-0.001	0	--	-0.002	0.009
SER	0	0.003	-0.001	-0.003	-0.01	-0.003	0	-0.001	--	0.008
SLI	0.001	0.018	0.019	0.009	-0.004	0.003	0.009	0.009	0.007	--

Table 14. F_{ST} values for the U.S. and Brazil with (lower) and without (upper) ENA for 7 loci. Bolded values indicate significance (P = 0.05).

	APA	AVI	CEK	DIB	GAL	LLM	POL	ROC	SERC	SLI	LPA	TRA	ITA	LAG
APA	--	0.00627	0.005372	-0.004576	0.004548	0.002501	-0.00232	0.000126	-0.002922	0.000055	0.176747	0.205076	0.156798	0.169967
AVI	-0.002431	--	0.00627	0.00491	-0.0008	0.00825	-0.0023	0.00436	-0.0056	0.00409	0.14783	0.1802	0.13517	0.14392
CEK	0.006781	0.01359	--	-0.007979	-0.005523	-0.003038	-0.002659	0.002422	-0.006804	-0.000344	0.154109	0.183627	0.133362	0.150437
DIB	-0.004524	0.00386	-0.004838	--	0.002521	-0.003227	0.000314	-0.000955	-0.004624	0.001845	0.160973	0.181741	0.140482	0.156023
GAL	0.005633	0.0045	0.000929	0.002062	--	-0.001133	-0.003319	-0.000525	-0.005537	-0.001049	0.12543	0.160641	0.115728	0.127553
LLM	0.002045	0.00903	0.000625	-0.001113	0.001399	--	0.002185	-0.003865	-0.001175	-0.008101	0.173807	0.197551	0.152749	0.172057
POL	-0.001718	0.00018	-0.002083	-0.002378	-0.001075	0.003591	--	0.003576	-0.004726	-0.00502	0.148855	0.175704	0.131031	0.145885
ROC	-0.001477	0.00364	0.005199	-0.002668	0.000925	-0.002965	0.003791	--	-0.000884	-0.006786	0.168551	0.194403	0.150065	0.166586
SERC	-0.001422	-0.002	0.000087	-0.003448	-0.000788	0.000072	-0.004935	-0.001529	--	0.000072	0.15215	0.173439	0.133221	0.151206
SLI	-0.002579	0.00313	0.000439	0.000122	-0.002642	-0.00699	-0.004422	-0.00526	0.000666	--	0.182802	0.214656	0.157577	0.178764
LPA	**0.17222**	**0.14705**	**0.157462**	**0.153346**	**0.124529**	**0.175419**	**0.144867**	**0.161135**	**0.151553**	**0.170796**	--	0.004079	0.010141	0.004201
TRA	**0.19275**	**0.17506**	**0.181193**	**0.168545**	**0.152116**	**0.19169**	**0.166634**	**0.182325**	**0.169853**	**0.196081**	0.005016	--	0.007433	0.008077
ITA	**0.1489**	**0.13204**	**0.135273**	**0.13384**	**0.112227**	**0.14959**	**0.127915**	**0.143427**	**0.13188**	**0.145**	0.012891	0.008491	--	0.005149
LAG	**0.166981**	**0.14447**	**0.154465**	**0.150426**	**0.126357**	**0.17392**	**0.143791**	**0.160462**	**0.151362**	**0.168259**	0.00349	0.007803	0.00846	--

An AMOVA for samples in the U.S. and the GOM indicated that there is between 28.6% and 31% (*P*-value = 0.001) variation among individuals within localities (Table 14). The variation among populations in both the U.S. and the GOM was low (0.2 – 0.5%; *P*-value > 0.05). When comparing the U.S. and Brazil, each geographic region in one group, the percent variation between the two groups was 14.4%, with a 42.1% (*P*-value = 0.001) variation among individuals from both populations. The variation for populations within each group was low, 0.2% (*P*- > 0.05). Comparisons between the GOM and Chesapeake Bay also indicate low variation for populations within each group, -0.6% (*P*-value > 0.05). The same value was calculated for variations for populations within the Eastern GOM and the Western GOM. The variation within populations for both comparisons was 30% (*P*-value = 0.001).

Table 15. AMOVA results for each grouping tested. Localities from the U.S (GOM and Chesapeake Bay) were compared to each other, as were localities in the GOM. Localities in the U.S. and BR were compared against each other, both individually and pooled as two separate groups. The localities from the GOM were pooled and compared against the Chesapeake Bay and the localities from the eastern GOM were pooled and compared against the pooled localities in the western GOM.

Group	Source of Variation	% Var.	F-stat	F-value	St. Dev.	CI 2.5%	CI 97.5%	P – value
U.S. (16 loci)	Within individuals	0.688	R_it	0.312	0.206	-0.048	0.608	--
	Among individuals within localities	0.31	R_is	0.311	0.209	-0.052	0.609	0.001
	Among populations within the U.S.	0.002	R_st	0.002	0.006	-0.007	0.14	0.372
GOM (16 loci)	Within individuals	0.709	R_it	0.291	0.226	-0.095	0.604	--
	Among individuals within localities	0.286	R_is	0.287	0.228	-0.098	0.605	0.001
	Among populations within the GOM	0.005	R_st	0.5	0.009	-0.007	0.025	0.225
U.S. & BR (7 loci)	Within individuals	0.477	R_it	0.523	0.045	0.421	0.588	--
	Among individuals within localities	0.424	R_is	0.471	0.071	0.327	0.567	0.001
	Among populations within the U.S. & BR	0.0099	R_st	0.099	0.046	0.04	0.174	0.001
GOM vs CB (16 loci)	Within individuals	0.704	R_it	0.296	0.199	-0.056	0.574	--
	Among individuals within populations	0.303	R_is	0.301	0.202	-0.058	0.581	0.001
	Among populations within each group	-0.006	R_sc	-0.006	0.007	-0.017	0.004	0.792
	Among populations within the U.S.	-0.001	R_ct	-0.001	0.007	-0.012	0.012	0.388
West vs East GOM (16 loci)	Within individuals	0.731	R_it	0.269	0.215	-0.111	0.570	--
	Among individuals within localities	0.278	R_is	0.276	0.215	-0.106	0.577	0.001
	Among populations within each group	0.001	R_sc	0.001	0.006	-0.008	0.013	0.394
	Among populations within the GOM	-0.010	R_ct	-0.010	0.002	-0.013	-0.005	1.000
U.S. vs B.R. (two groups)	Within individuals	0.444	R_it	0.556	0.041	0.454	0.609	--
	Among individuals within localities	0.405	R_is	0.477	0.073	0.339	0.575	0.001
	Among populations within each group	0.005	R_sc	0.006	0.004	0.001	0.011	0.552
	Among populations within the U.S. & BR	0.145	R_ct	0.145	0.068	0.049	0.250	0.001

No evidence for IBD was detected within the US or GOM localities (Table 15; Figures 11, 12). For the U.S., a negative slope (-6.186×10^{-6}) with $R^2 = 0.101$ was found. After removing the Chesapeake Bay locality, a positive slope (1.694×10^{-5}) was found, but the R^2 value was very low (0.0201). When comparing the U.S. and Brazil, the results are consistent with IBD with a large R^2 value (0.937) (Figure 13).

Table 16. IBDWS results for the U.S., GOM, and Brazil.

Group	Linear Model	Intercept	Slope	R^2
U.S.	Estimate	0.01610	-6.186×10^{-6}	0.101
	St. Error	0.00141	8.945×10^{-7}	
GOM	Estimate	-7.458×10^{-4}	1.694×10^{-5}	0.0201
	St. Error	2.166×10^{-6}	2.875×10^{-6}	
U.S. & BR	Estimate	-0.01442	1.598×10^{-5}	0.937
	St. Error	0.00304	4.244×10^{-7}	

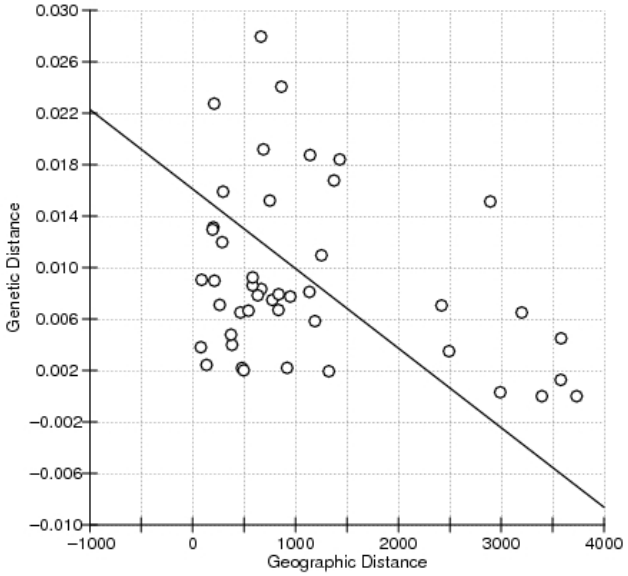

Figure 11. IBDWS linear regression of genetic and geographic distance of the U.S.

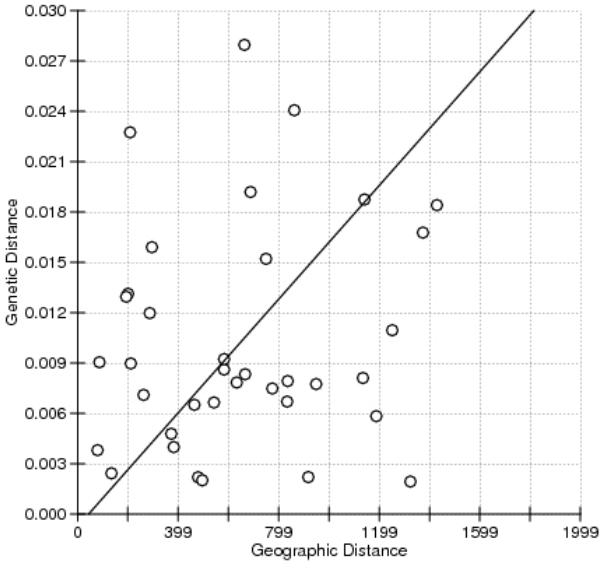

Figure 12. IBDWS linear regression of genetic and geographic distance of the GOM.

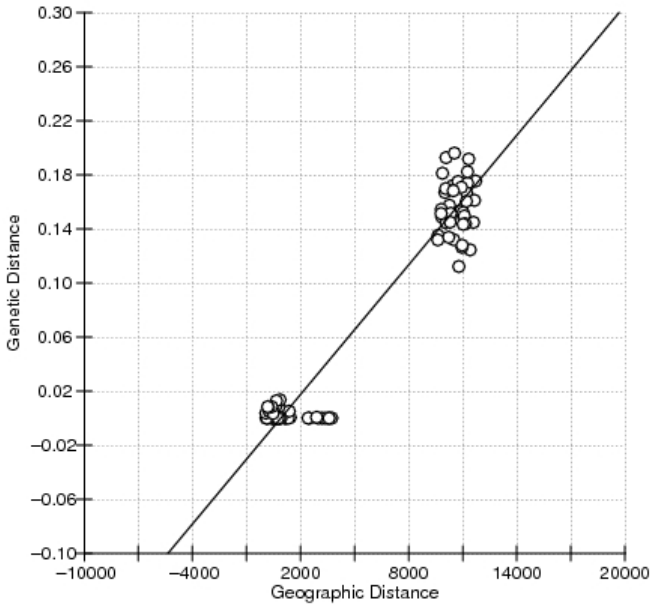

Figure 13. IBDWS linear regression of genetic and geographic distance of the U.S. and Brazil.

STRUCTURE also indicate not evident genetic differentiation within the US, but clear differentiation is observed between the US and Brazil (Figures 14 – 26). Although STRUCTURE HARVESTER selected K values ranged from 3 to 9, very little genetic structure was present among samples in the U.S and GOM. Results with and without the linked marker *Tet1329* produced different optimal K values, but both sets of results showed a lack of genetic structure within the U.S. and GOM. However, STRUCTURE results for the comparison of the U.S. and Brazil indicated almost complete genetic differentiation between the two regions. K values ranged from 3 to 4 but every model tested indicated genetic structure between the two locations. Results for K = 2 were also

reported for each simulation, as this value appeared to best represent the level of population differentiation found across geographic locations.

Spatial-based clustering (GENELAND) found one and two clusters, depending on the allele frequency model used. For uncorrelated allele frequencies, only one cluster was identified in the analysis of the U.S. and the analysis of the GOM. Two clusters were identified in the analysis of the U.S. and the analysis of the GOM when testing with correlated allele frequencies. Samples in the U.S. were all assigned to cluster 1 due to the higher posterior probability of their membership to the same cluster (50%). Posterior probability of population membership for cluster 2 was 49%. Samples in the GOM were all assigned to cluster 2 due to the higher posterior probability of their membership to this cluster (52% vs 48%). For the comparisons between the U.S. and Brazil, samples in the U.S. were assigned to cluster 1 (100%) and samples in Brazil were assigned to cluster 2 (100%), with both correlated and uncorrelated.

PCA does not reveal evident clustering patterns among localities in the U.S. (Figures 27 and 28) and GOM (Figures 29 and 30), and localities from the U.S. and Brazil clustered separately (Figure 31). The results from the FCA analyses (Figures 32 – 39) were consistent with the PCA results. The FCA graphs for the GOM and the U.S. did not identify separation of populations, although shows Cedar Key somehow more distant. When comparing the U.S. and Brazil, samples from Brazil clustered together and apart from samples from the U.S.

Figures 14-17. STRUCTURE Results for all populations in the GOM: (1) LLM, (2) ROC, (3) POL, (4) GAL, (5) AVI, (6) SLI, (7) DIB, (8) CEK, (9) APA. Figure 14 shows a model with admixture and correlated frequencies, Figure 15 shows admixture with independent frequencies, Figure 16 shows no admixture with correlated frequencies, and Figure 17 shows no admixture and independent frequencies.

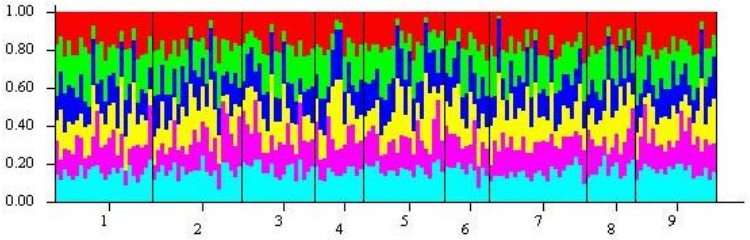

Figure 14. STRUCTURE results for the GOM (16 loci; K = 6)

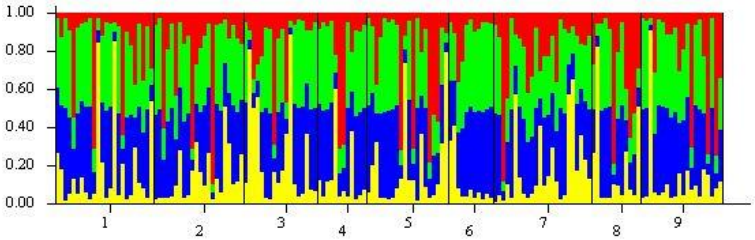

Figure 15. STRUCTURE results for the GOM (16 loci; K = 4)

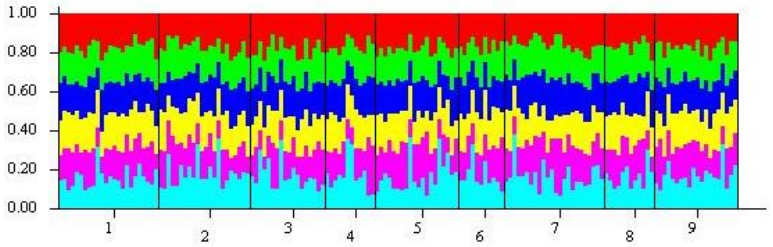

Figure 16. STRUCTURE results for the GOM (16 loci; K = 6)

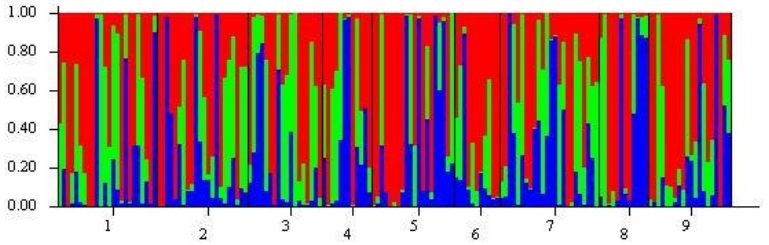

Figure 17. STRUCTURE results for the GOM (16 loci; K = 3)

Figures 18 – 21. STRUCTURE HARVESTER Results for all populations in the GOM (16 loci)

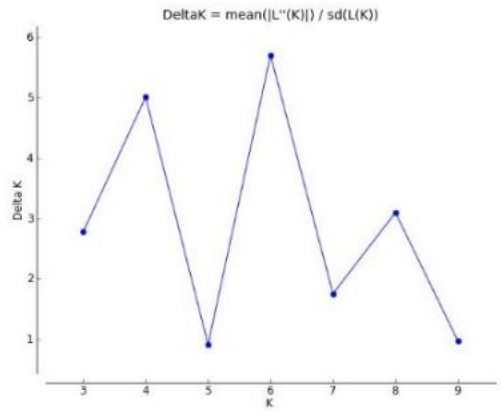

Figure 18. STRUCTURE HARVESTER Delta K for Figure 14

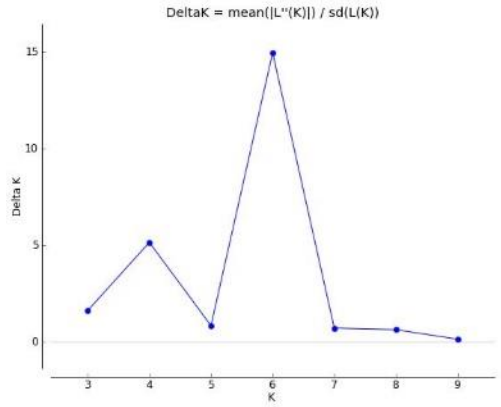

Figure 20. STRUCTURE HARVESTER Delta K for Figure 16

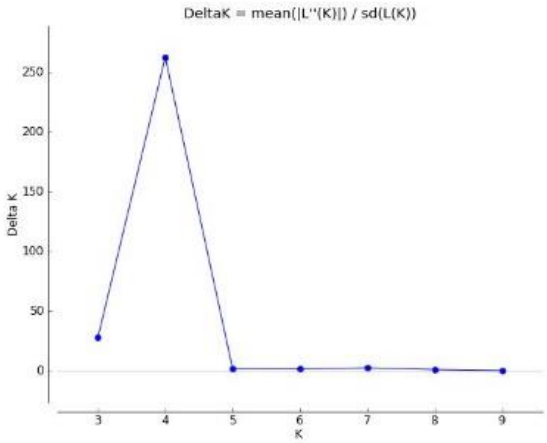

Figure 19. STRUCTURE HARVESTER Delta K for Figure 15

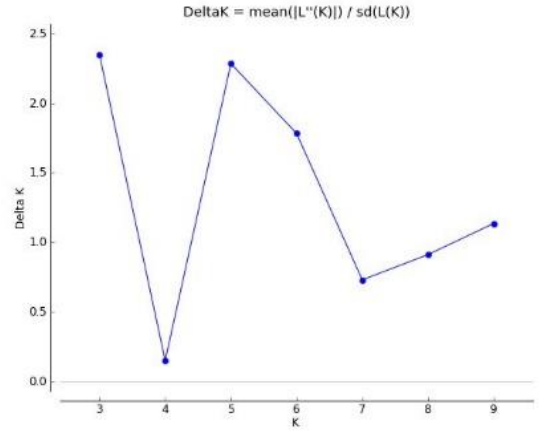

Figure 21. STRUCTURE HARVESTER Delta K for Figure 17

Figures 22 – 25: STRUCTURE Results for populations in the U.S. and BR – (1) LLM, (2) ROC, (3) POL, (4) GAL, (5) AVI, (6) SLI, (7) DIB, (8) CEK, (9) APA, (10) SER, (11) LPA, (12) TRA, (13) ITA, (14) LAG. Figure 22 shows a model with admixture and correlated frequencies, Figure 23 shows admixture with independent frequencies, Figure 24 shows no admixture with correlated frequencies, and Figure 25 shows no admixture and independent frequencies.

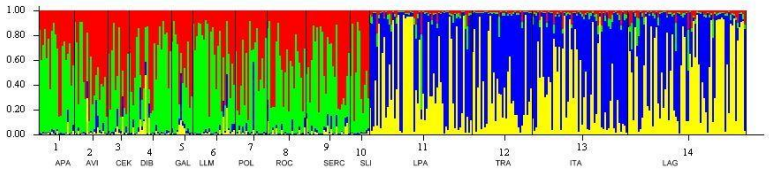

Figure 22. STRUCTURE results for the U.S. and BR (7 loci; K = 4)

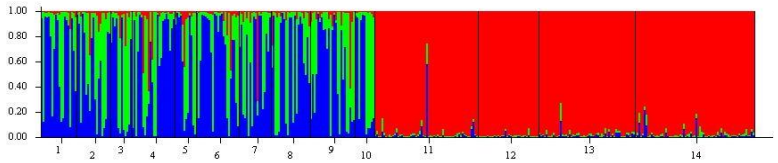

Figure 23. STRUCTURE results for the U.S. and BR (7 loci; K = 3)

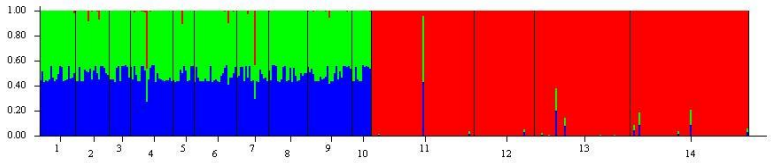

Figure 24. STRUCTURE results for the U.S. and BR (7 loci; K = 3)

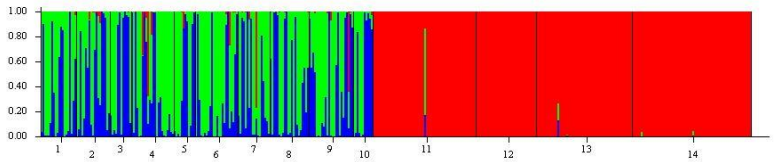

Figure 25. STRUCTURE results for the U.S. and BR (7 loci; K = 3)

Figures 26 – 29. STRUCTURE HARVESTER Results for all populations in the U.S. and BR (7 loci)

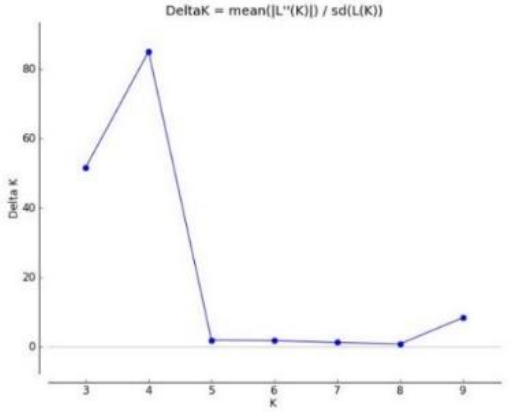

Figure 26. STRUCTURE HARVESTER Delta K for Figure 22

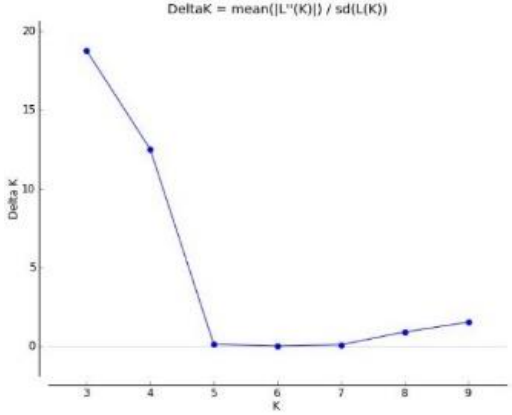

Figure 28. STRUCTURE HARVESTER Delta K for Figure 24

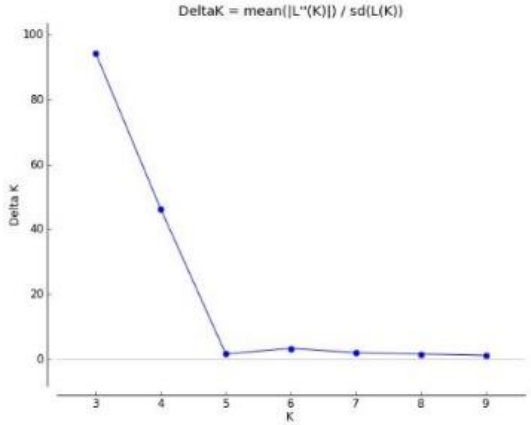

Figure 27. STRUCTURE HARVESTER Delta K for Figure 23

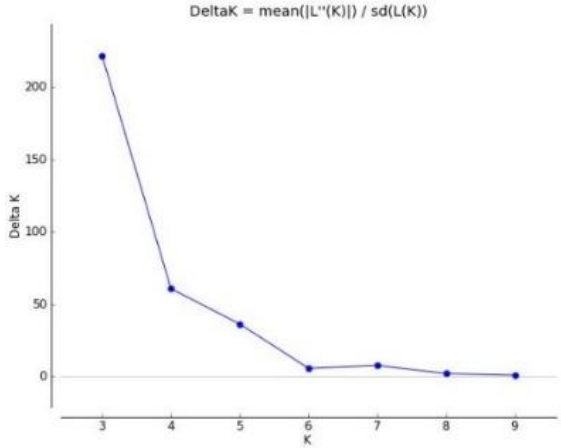

Figure 29. STRUCTURE HARVESTER Delta K for Figure 25

Figures 30 – 33: STRUCTURE Results for all populations in the U.S. - (1) APA, (2) AVI, (3) CEK, (4) DIB, (5) GAL, (6) LLM, (7) POL, (8) ROC, (9) SER, (10) SLI. Figure 30 shows a model with admixture and correlated frequencies, Figure 31 shows admixture with independent frequencies, Figure 32 shows no admixture with correlated frequencies, and Figure 33 shows no admixture and independent frequencies.

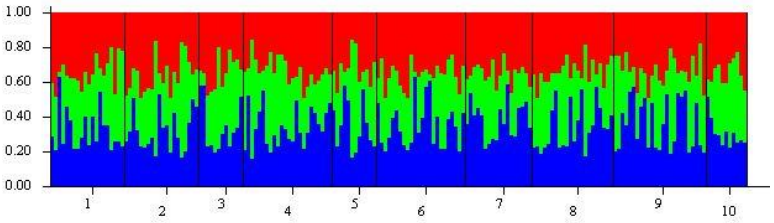

Figure 30. STRUCTURE results for all populations in the U.S. (16 loci; K = 3)

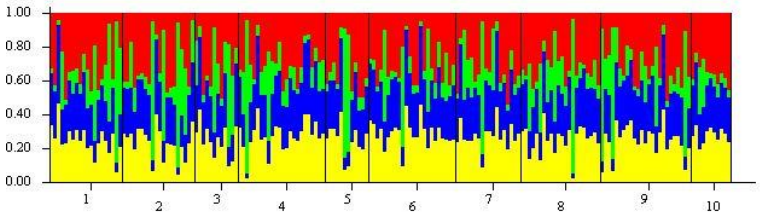

Figure 31. STRUCTURE results for all populations in the U.S. (16 loci; K = 4)

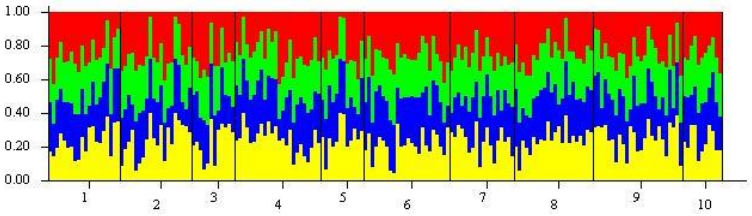

Figure 32. STRUCTURE results for all populations in the U.S. (16 loci; K = 4)

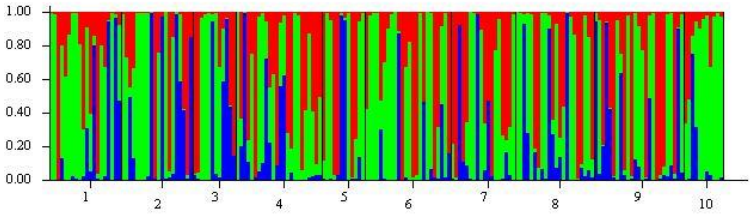

Figure 33. STRUCTURE results for all populations in the U.S. (16 loci; K = 3)

Figures 34 – 37. STRUCTURE HARVESTER Results for all populations in the U.S. (16 loci)

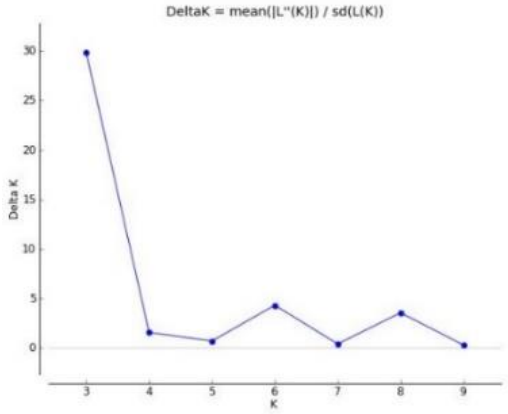

Figure 34. STRUCTURE HARVESTER Delta K for Figure 30

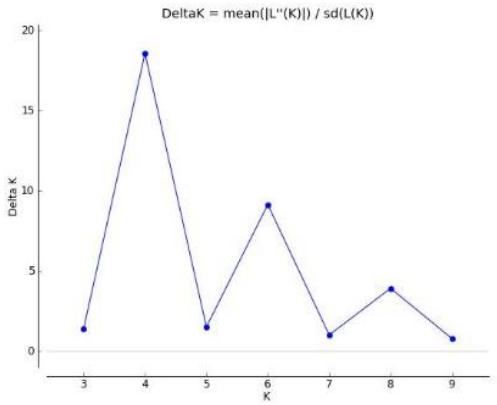

Figure 36. STRUCTURE HARVESTER Delta K for Figure 32

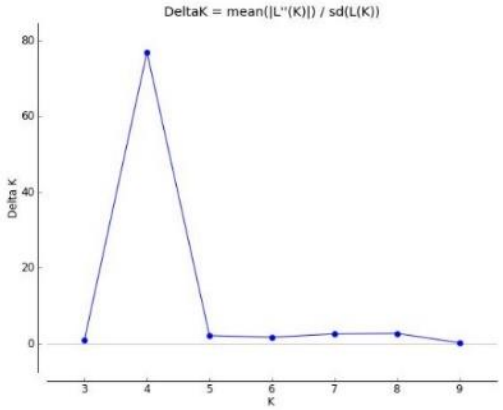

Figure 35. STRUCTURE HARVESTER Delta K for Figure 31

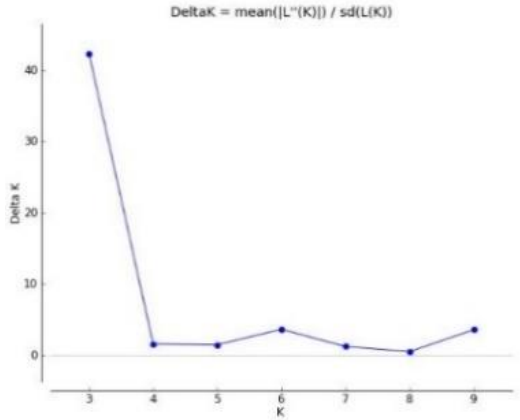

Figure 37. STRUCTURE HARVESTER Delta K for Figure 33

Figures 38 – 41 STRUCTURE Results for all populations in the U.S. (15 loci) - (1) APA, (2) AVI, (3) CEK, (4) DIB, (5) GAL, (6) LLM, (7) POL, (8) ROC, (9) SER, (10) SLI. Figure 38 shows a model with admixture and correlated frequencies, Figure 39 shows admixture with independent frequencies, Figure 40 shows no admixture with correlated frequencies, and Figure 41 shows no admixture and independent frequencies

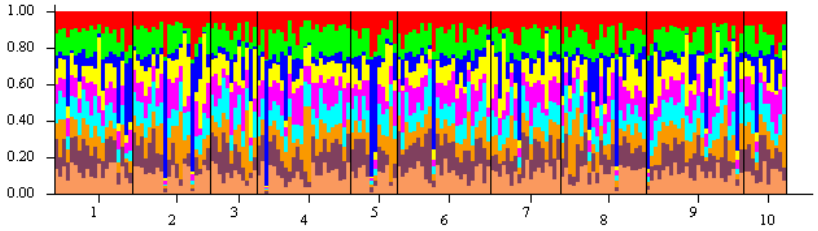

Figure 38. STRUCTURE results for all populations in the U.S. (15 loci; K = 9)

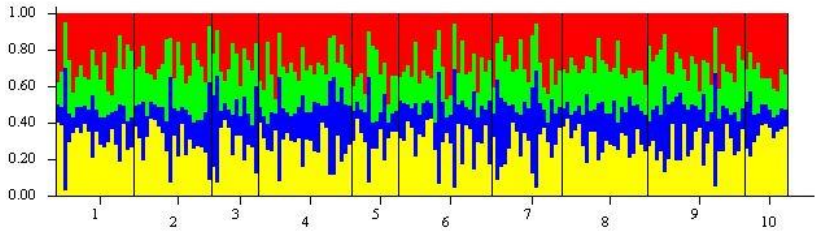

Figure 39. STRUCTURE results for all populations in the U.S. (15 loci; K = 4)

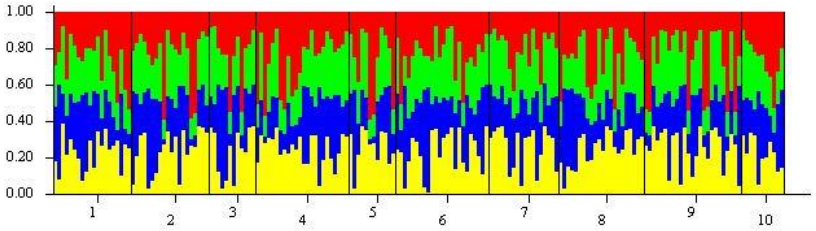

Figure 40. STRUCTURE results for all populations in the U.S. (15 loci; K = 4)

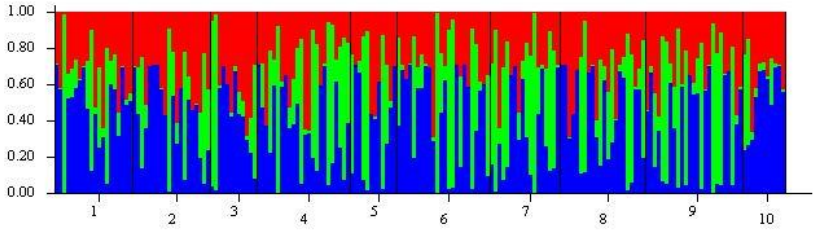

Figure 41. STRUCTURE results for all populations in the U.S. (15 loci; K = 3)

Figures 42 – 45. STRUCTURE HARVESTER Results for all populations in the U.S. (15 loci)

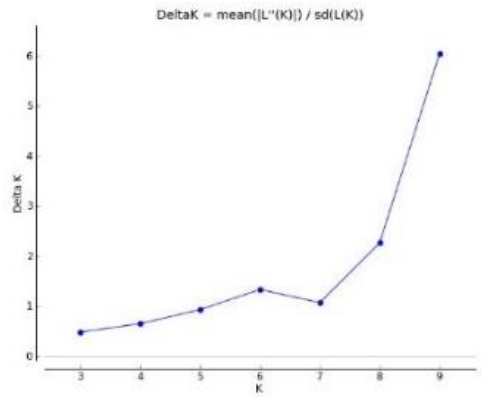

Figure 42. STRUCTURE HARVESTER Delta K for Figure 38

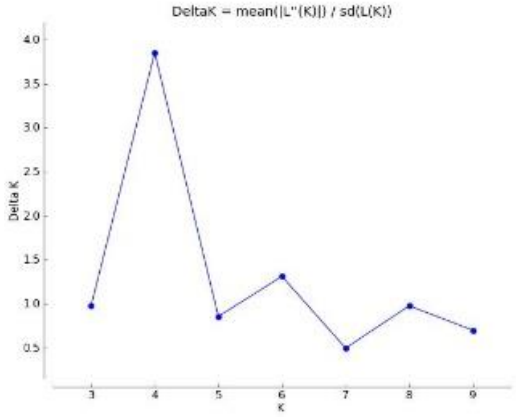

Figure 43. STRUCTURE HARVESTER Delta K for Figure 39

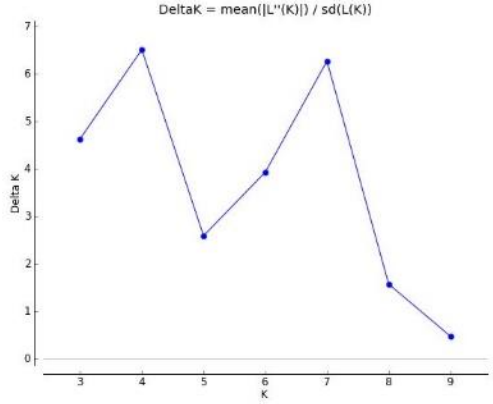

Figure 44. STRUCTURE HARVESTER Delta K for Figure 40

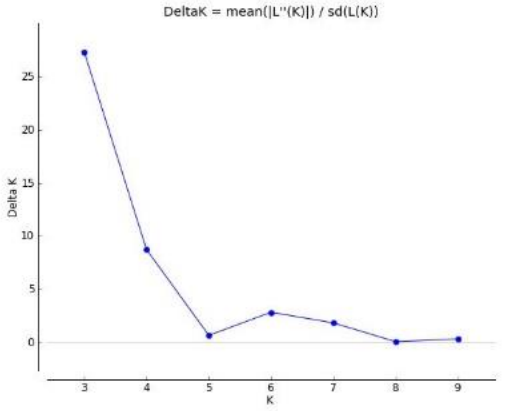

Figure 45. STRUCTURE HARVESTER Delta K for Figure 41

Figures 46 – 49: STRUCTURE and STRUCTURE HARVESTER Results for all populations in the GOM (15 loci) – (1) LLM, (2) ROC, (3) POL, (4) GAL, (5) AVI, (6) SLI, (7) DIB, (8) CEK, (9) APA. Figure 46 shows a model with admixture and correlated frequencies, Figure 47 shows admixture with independent frequencies, Figure 48 shows no admixture with correlated frequencies, and Figure 49 shows no admixture and independent frequencies

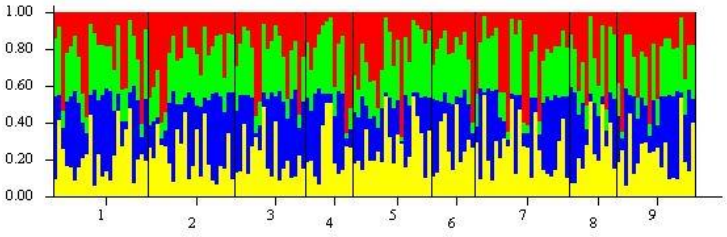

Figure 46. STRUCTURE results for all populations in the GOM (15 loci; K = 4)

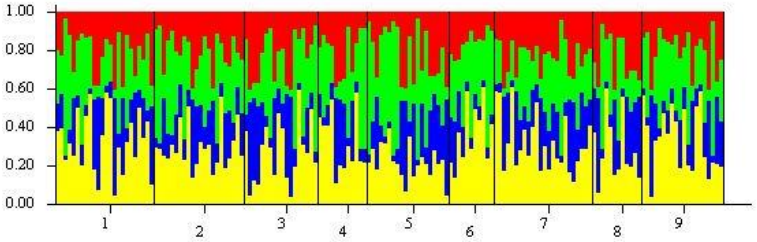

Figure 47. STRUCTURE results for all populations in the GOM (15 loci; K = 4)

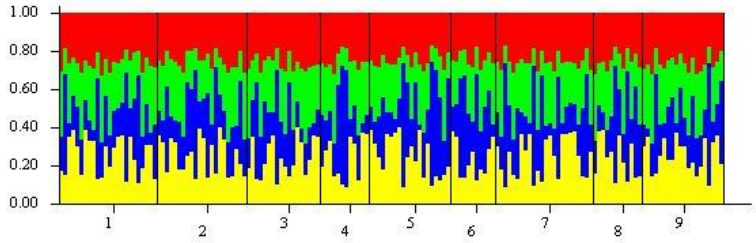

Figure 48. STRUCTURE results for all populations in the GOM (15 loci; K = 4)

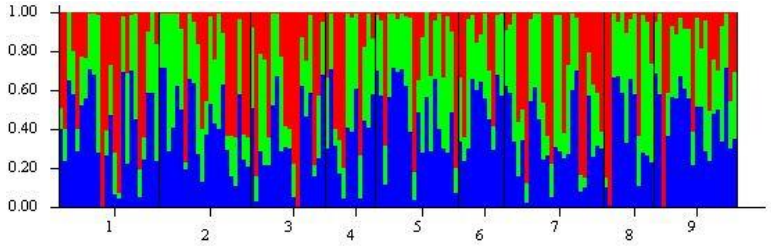

Figure 49. STRUCTURE results for all populations in the GOM (15 loci; K = 3)

Figures 50 – 53. STRUCTURE HARVESTER Results for all populations in the GOM. (15 loci)

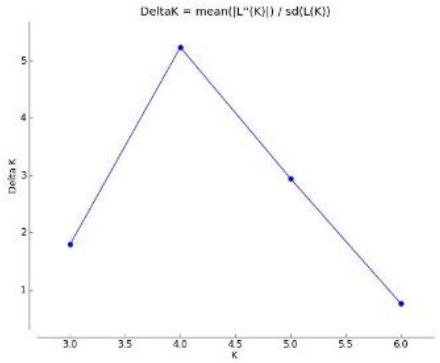

Figure 50. STRUCTURE HARVESTER Delta K for Figure 46

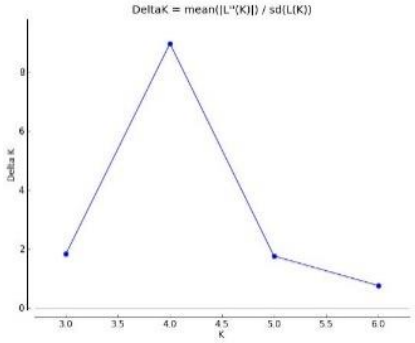

Figure 52. STRUCTURE HARVESTER Delta K for Figure 48

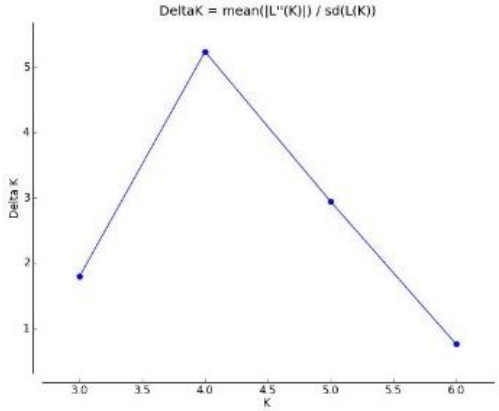

Figure 51. STRUCTURE HARVESTER Delta K for Figure 47

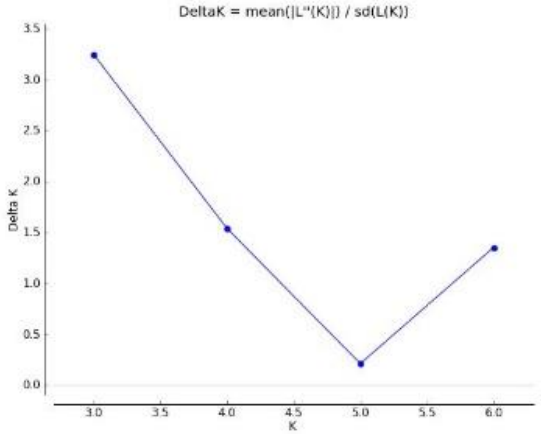

Figure 53. STRUCTURE HARVESTER Delta K for Figure 49

Figures 54 – 57: STRUCTURE Results K = 2 for the U.S.: (1) APA, (2) AVI, (3) CEK, (4) DIB, (5) GAL, (6) LLM, (7) POL, (8) ROC, (9) SER, (10) SLI. Figure 54 shows a model with admixture and correlated frequencies, Figure 55 shows admixture with independent frequencies, Figure 56 shows no admixture with correlated frequencies, and Figure 57 shows no admixture and independent frequencies

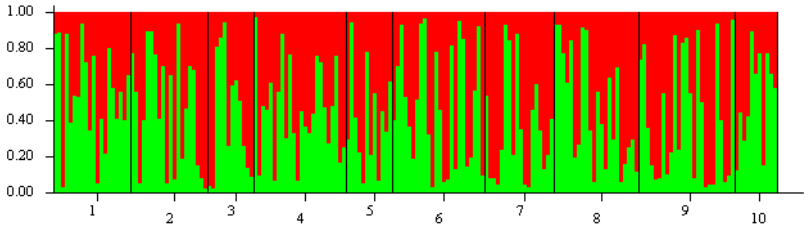

Figure 54. STRUCTURE results for all populations in the U.S. (K = 2)

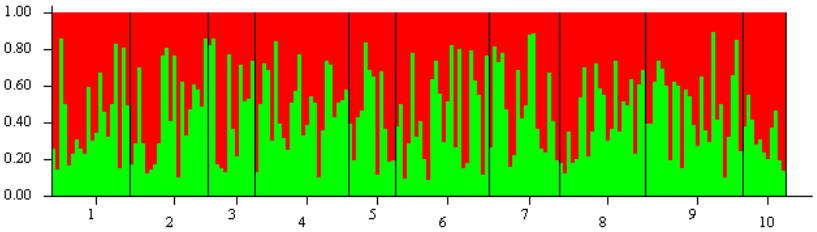

Figure 55. STRUCTURE results for all populations in the U.S. (K = 2)

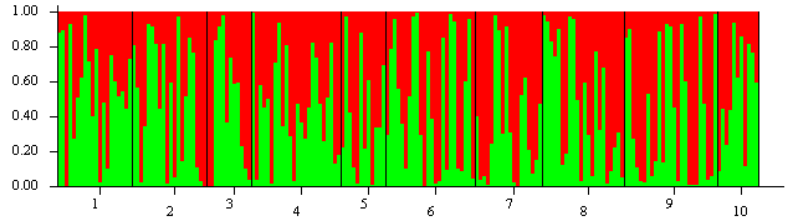

Figure 56. STRUCTURE results for all populations in the U.S. (K = 2)

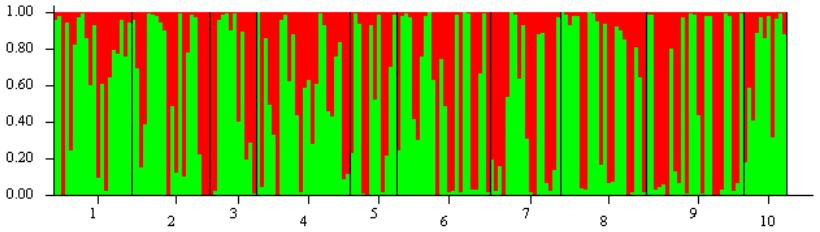

Figure 57. STRUCTURE results for all populations in the U.S. (K = 2)

Figures 58 – 62: STRUCTURE Results K = 2 for the GOM - (1) LLM, (2) ROC, (3) POL, (4) GAL, (5) AVI, (6) SLI, (7) DIB, (8) CEK, (9) APA. Figure 58 shows a model with admixture and correlated frequencies, Figure 59 shows admixture with independent frequencies, Figure 60 shows no admixture with correlated frequencies, and Figure 61 shows no admixture and independent frequencies

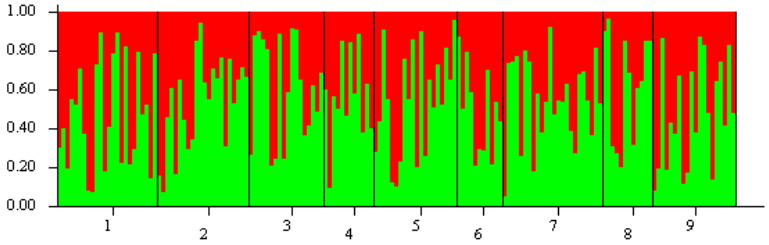

Figure 58. STRUCTURE results for all populations in the GOM (K = 2)

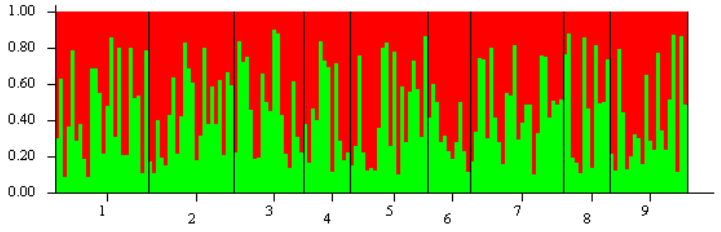

Figure 59. STRUCTURE results for all populations in the GOM (K = 2)

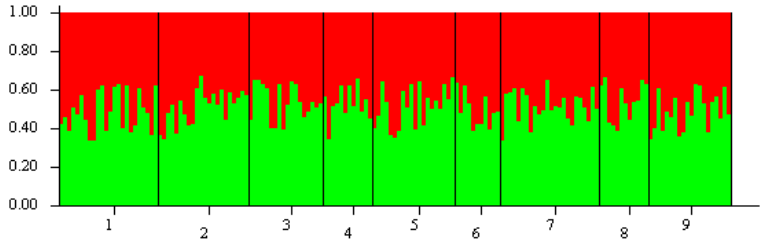

Figure 60. STRUCTURE results for all populations in the GOM (K = 2)

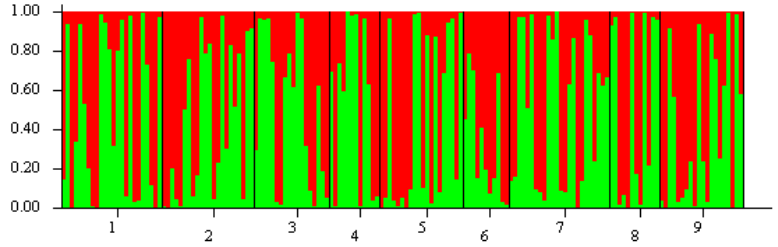

Figure 61. STRUCTURE results for all populations in the GOM (K = 2)

Figures 62 – 65: STRUCTURE Results for the U.S. and BR – (1) LLM, (2) ROC, (3) POL, (4) GAL, (5) AVI, (6) SLI, (7) DIB, (8) CEK, (9) APA, (10) SER, (11) LPA, (12) TRA, (13) ITA, (14) LAG. Figure 62 shows a model with admixture and correlated frequencies, Figure 63 shows admixture with independent frequencies, Figure 63 shows no admixture with correlated frequencies, and Figure 64 shows no admixture and independent frequencies

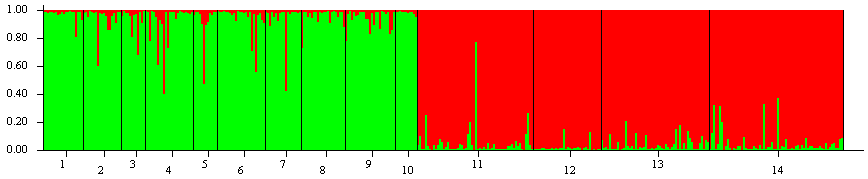

Figure 62. STRUCTURE results for all populations in the U.S. and BR (K = 2)

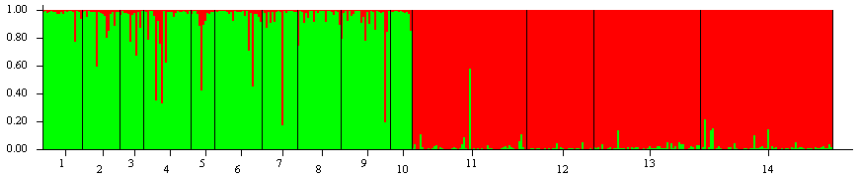

Figure 63. STRUCTURE results for all populations in the U.S. and BR (K = 2)

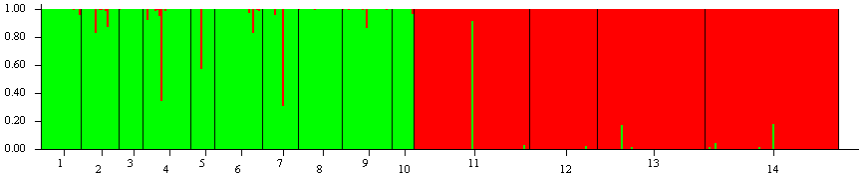

Figure 64. STRUCTURE results for all populations in the U.S. and BR (K = 2)

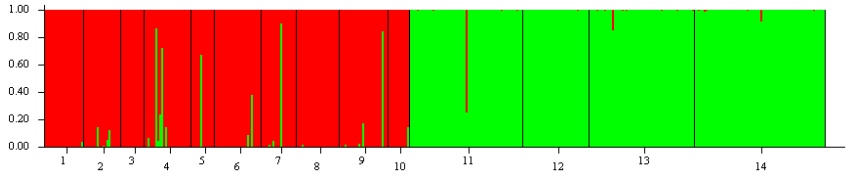

Figure 65. STRUCTURE results for all populations in the U.S. and BR (K = 2)

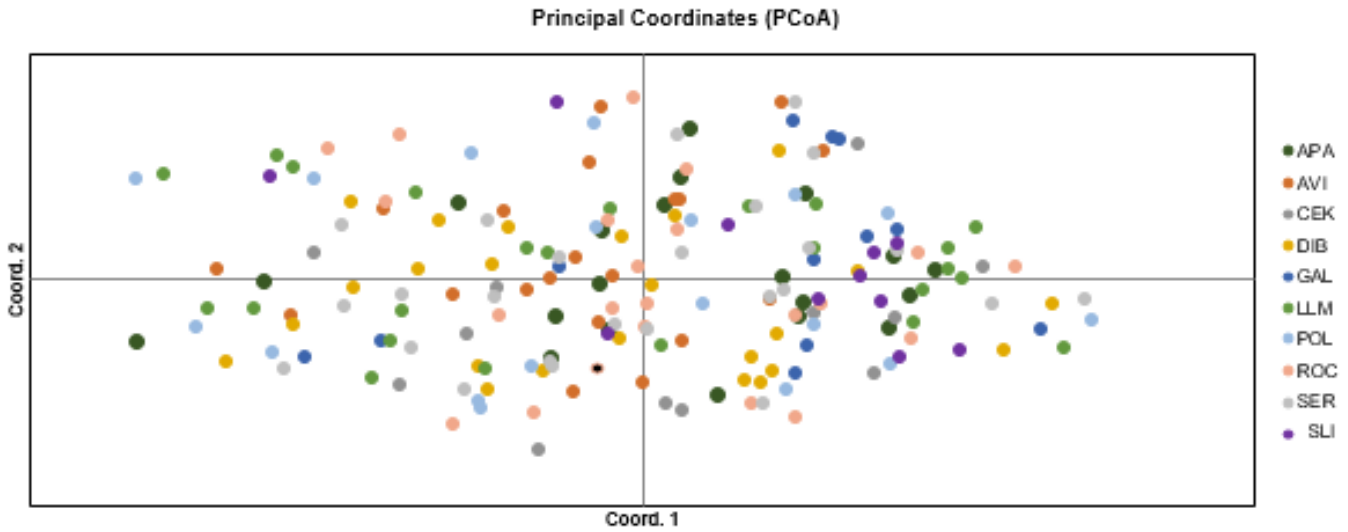

Figure 66. PCA for all samples in the U.S. with 16 loci

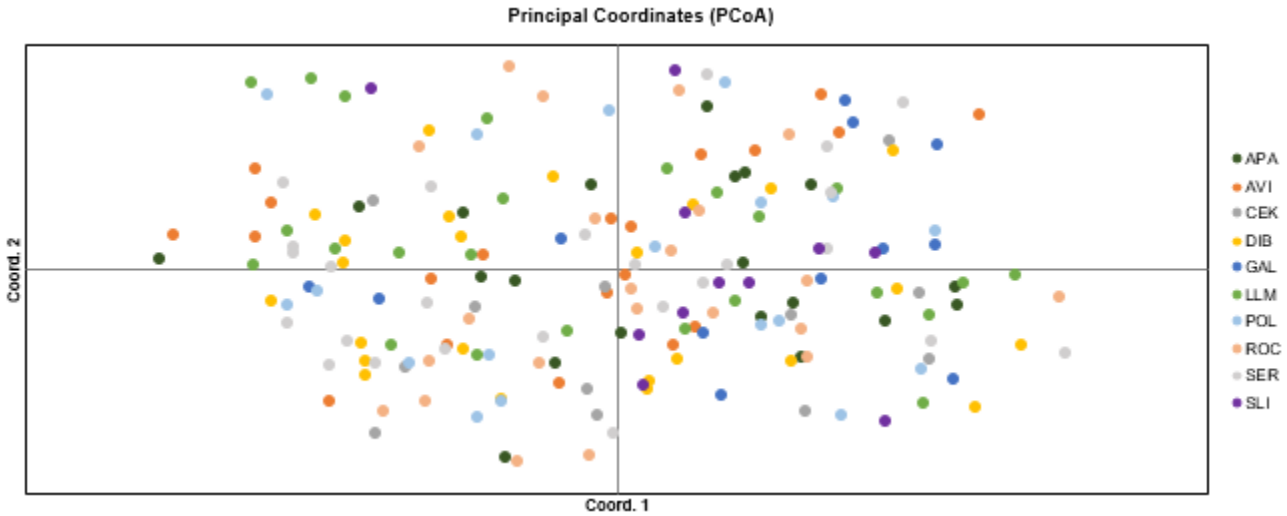

Figure 67. PCA for all samples in the U.S. with 15 loci

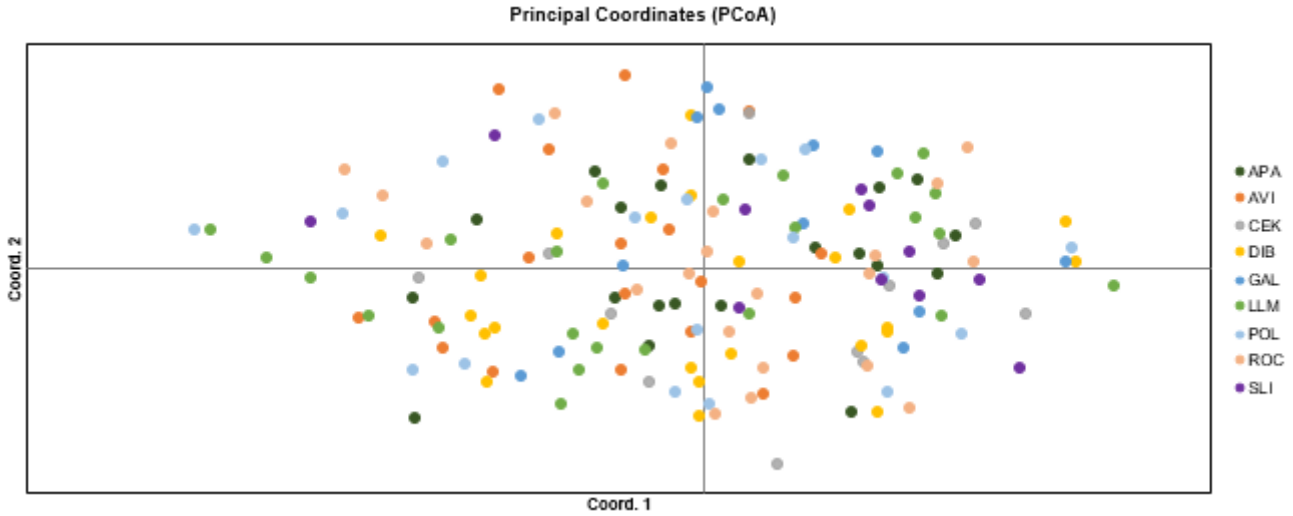

Figure 68. PCA for all samples in the GOM with 16 loci

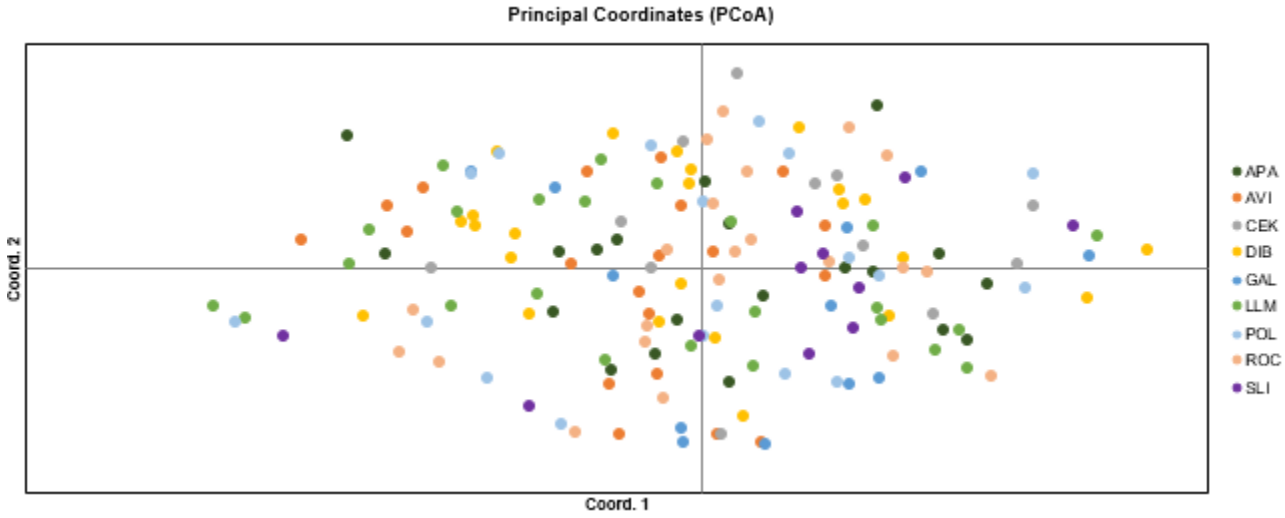

Figure 69. PCA for all samples in the GOM with 15 loci

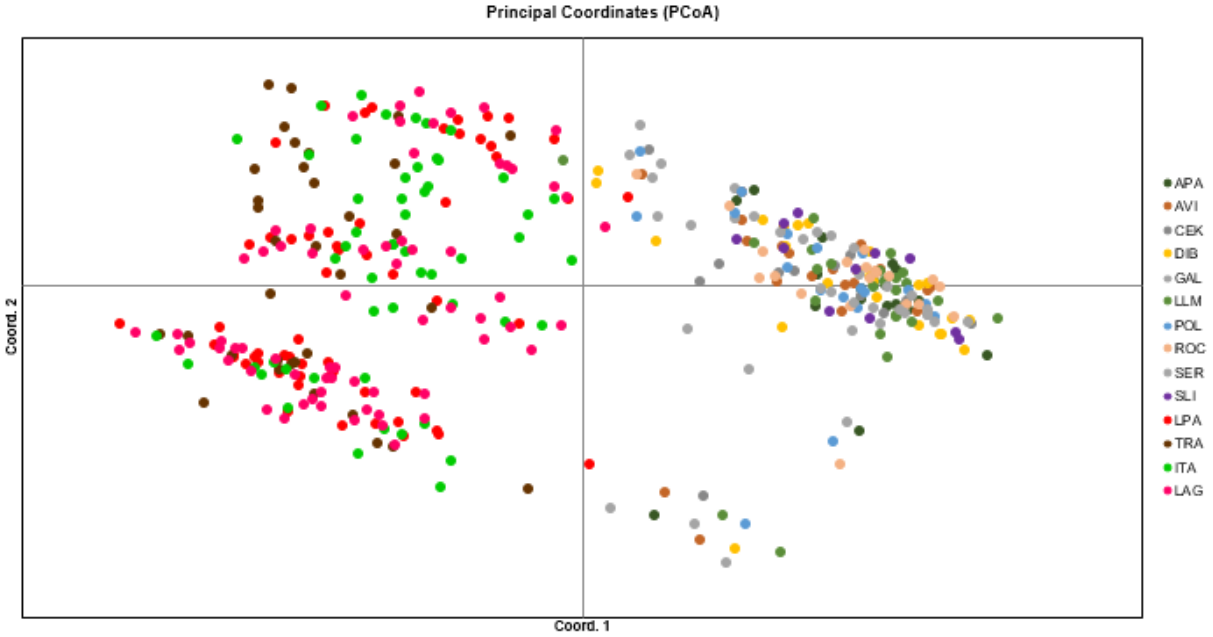

Figure 70. PCA for all samples in the U.S. and BR

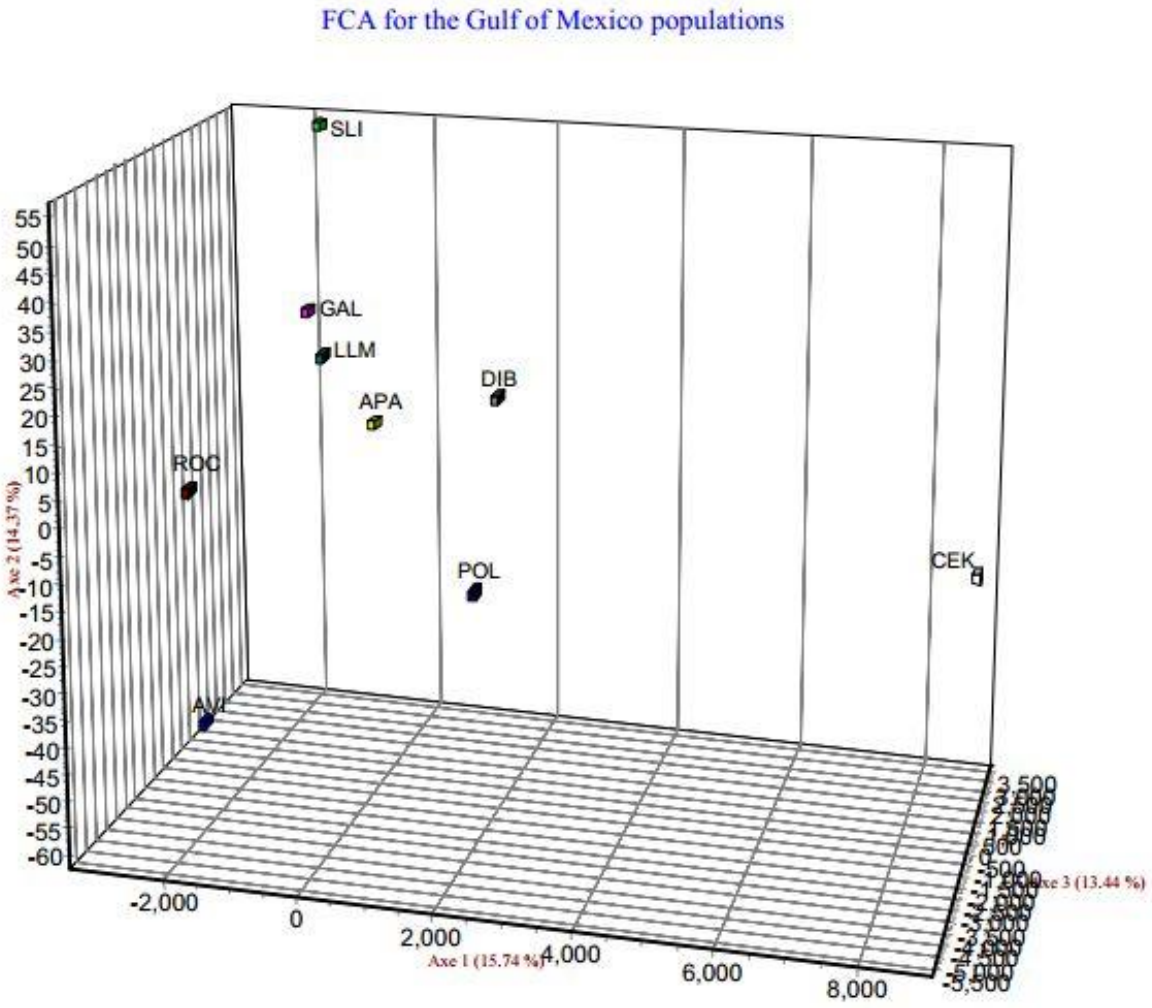

Figure 71. Mean FCA for the GOM with 16 loci

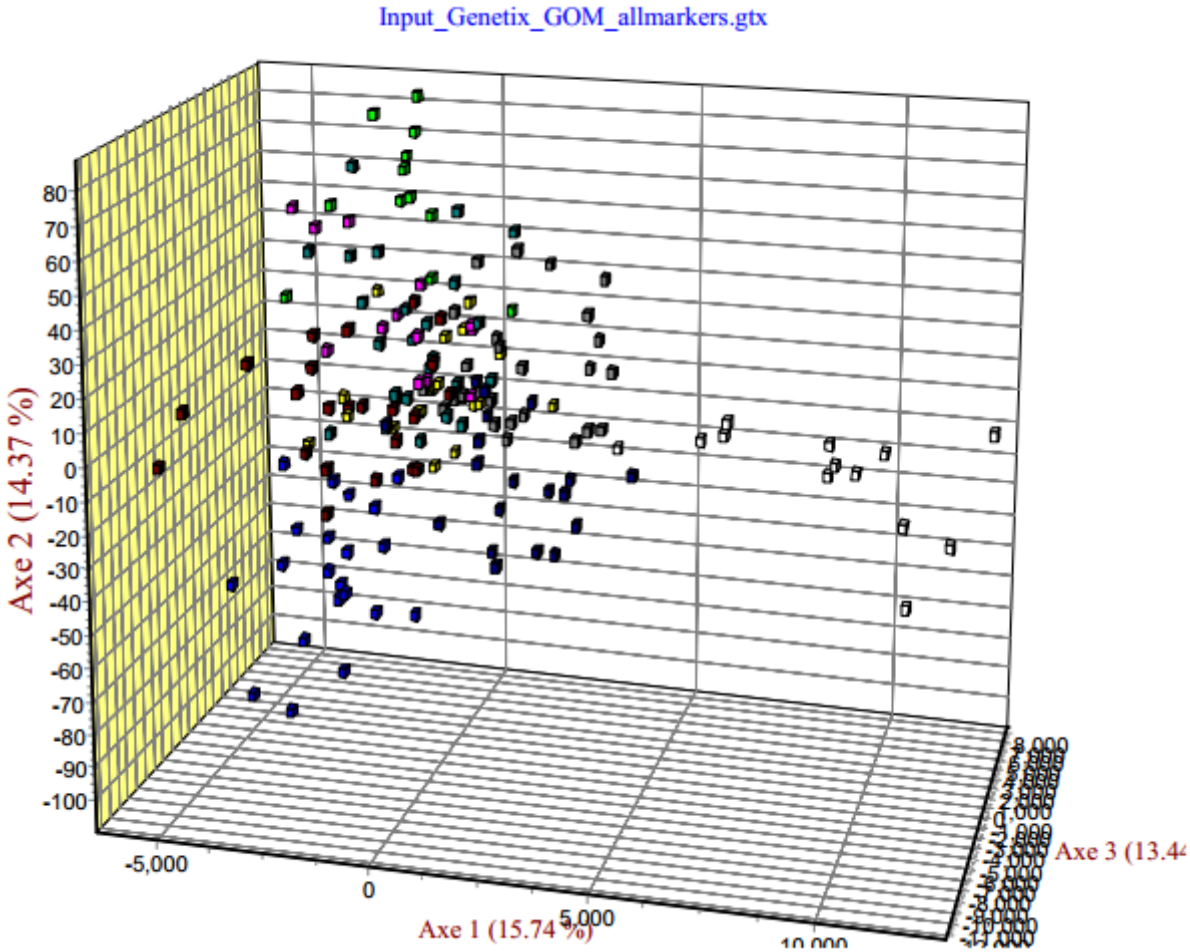

Figure 72. FCA for the GOM with 16 loci

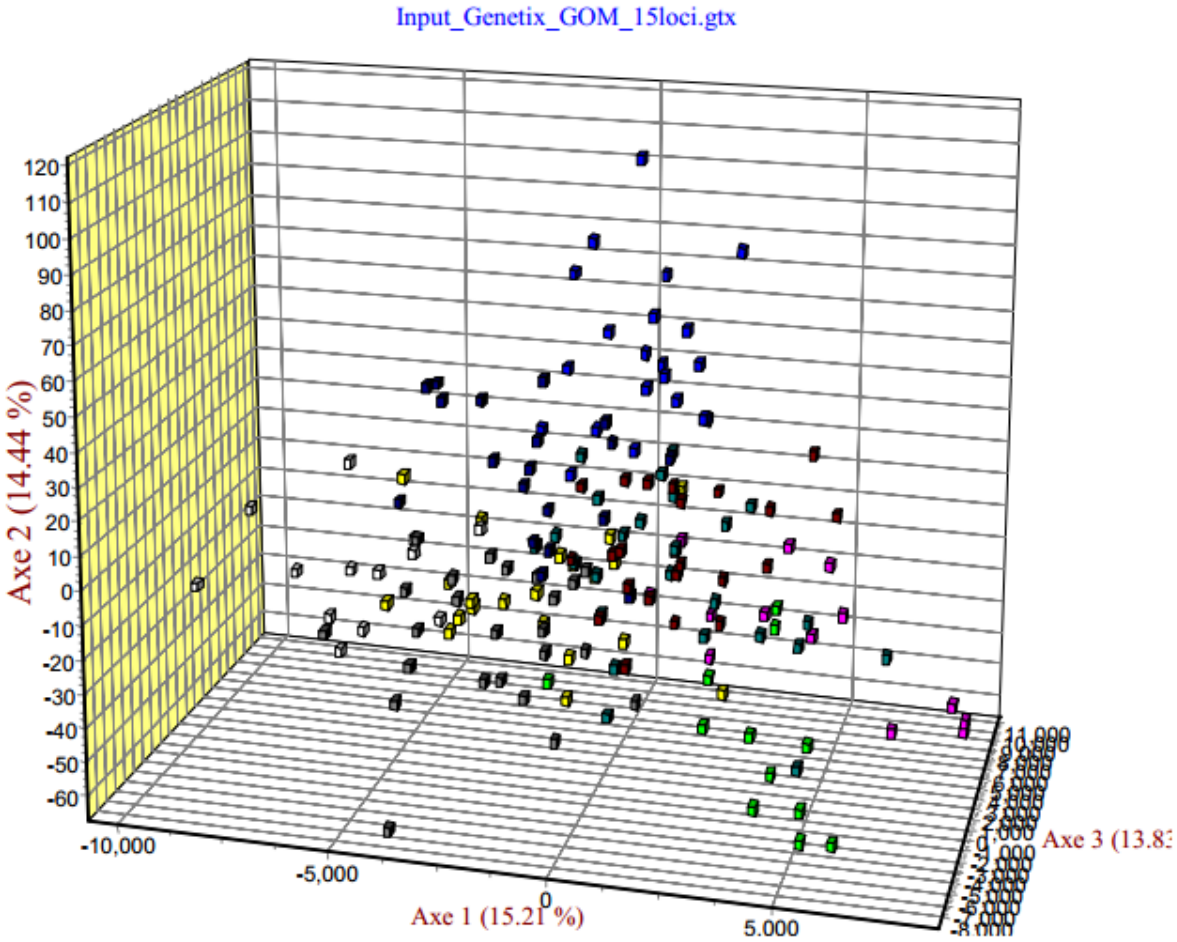

Figure 73. FCA for GOM with 15 loci

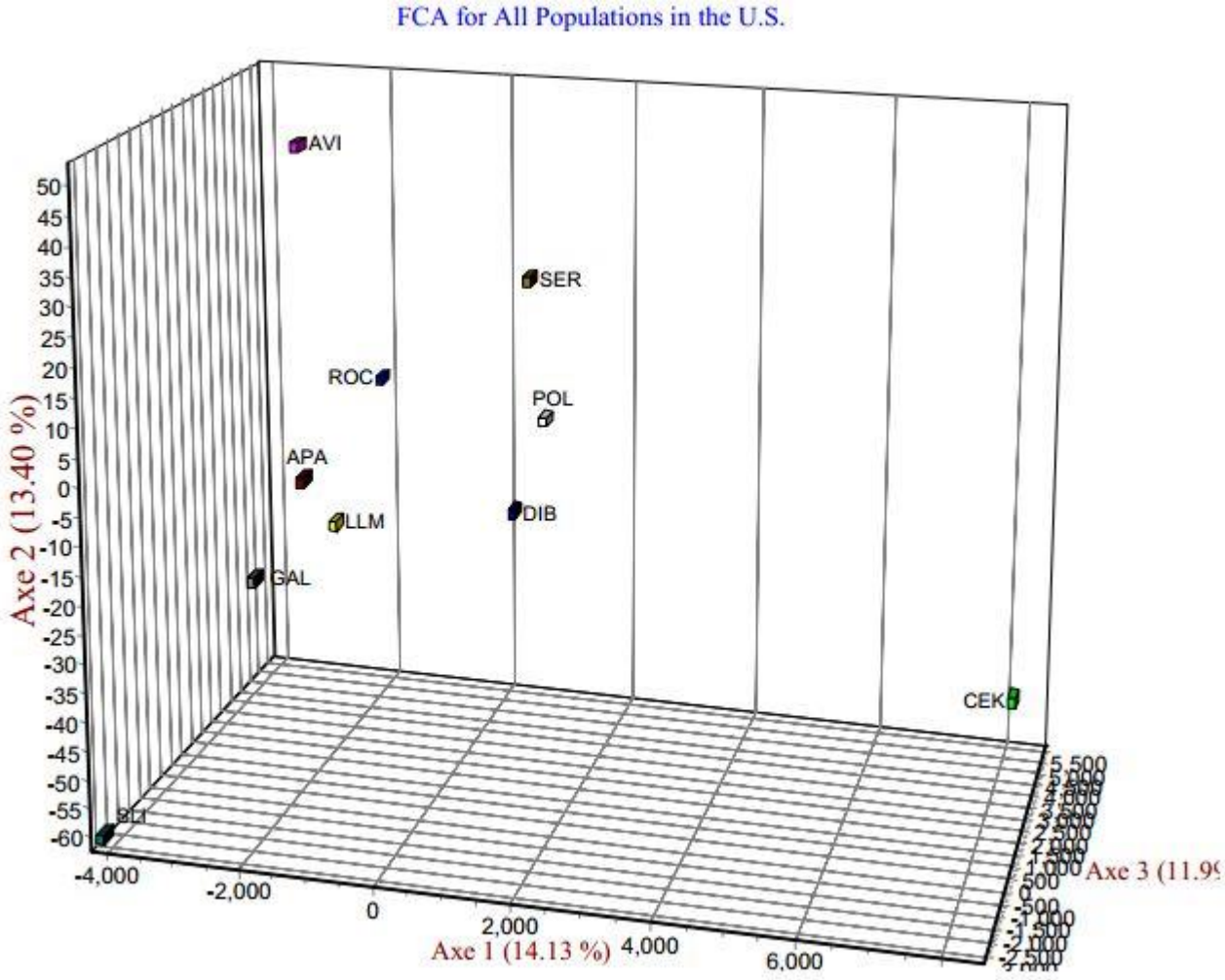

Figure 74. FCA for all populations in the U.S. with 16 loci

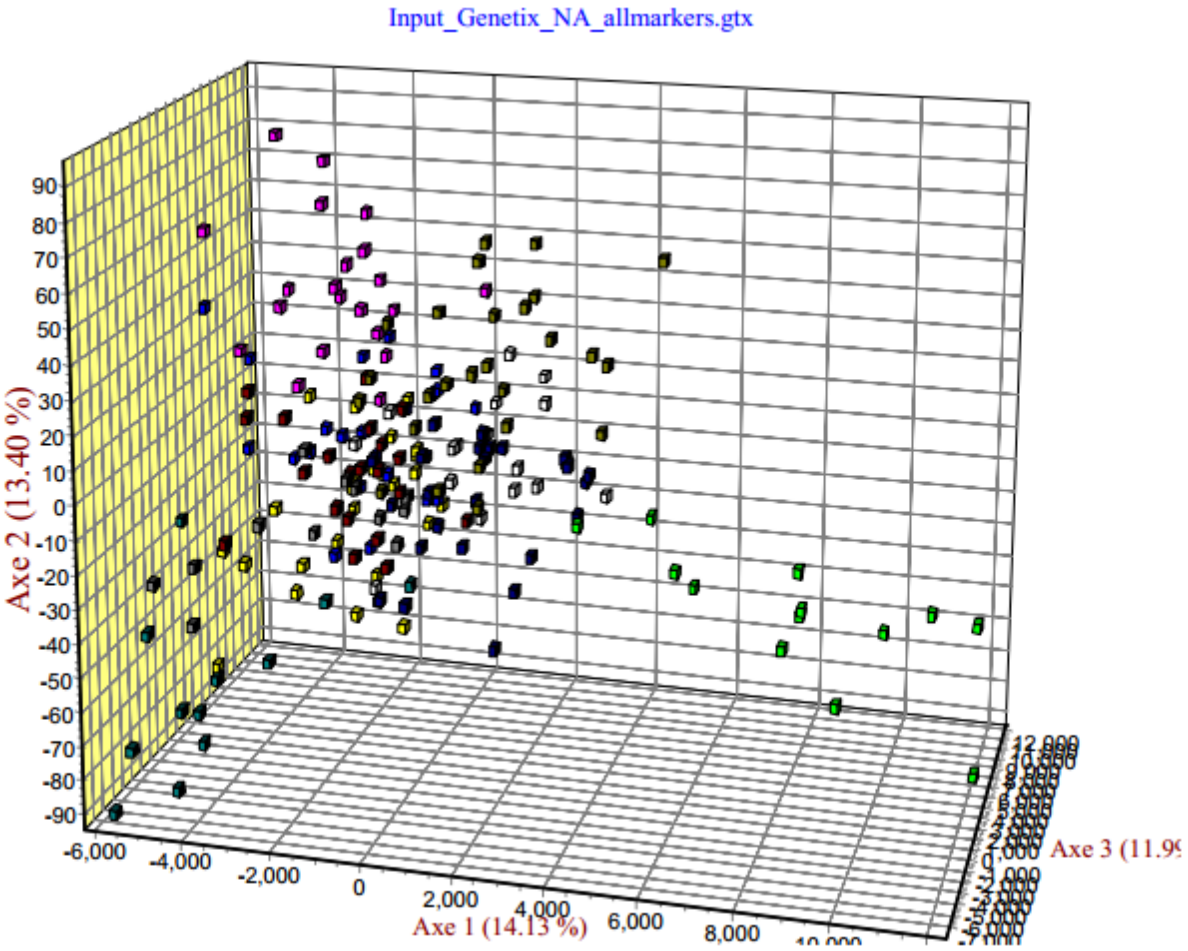

Figure 75. FCA for all populations in the U.S. with 16 loci

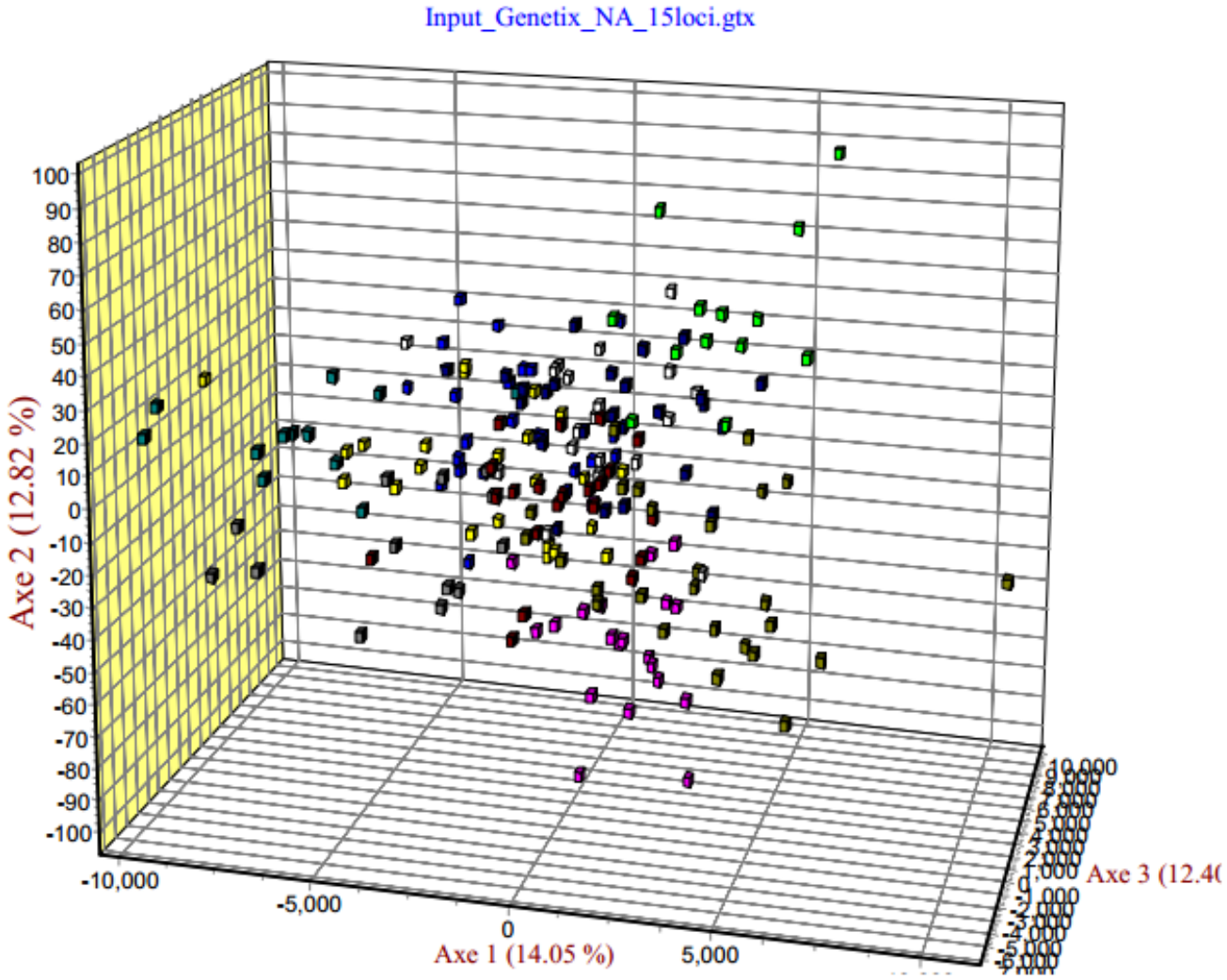

Figure 76. FCA for all populations in the U.S. with 15 loci

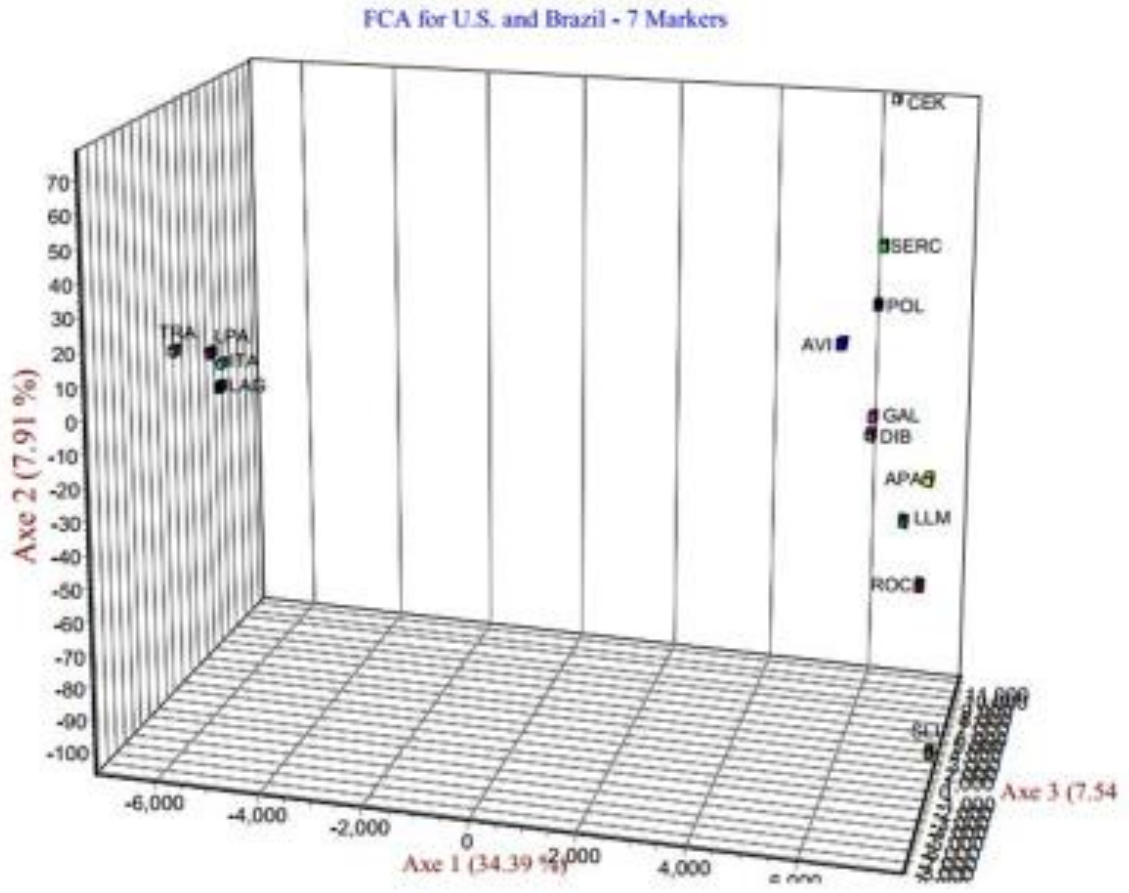

Figure 77. Mean FCA for all populations in the U.S. and in BR

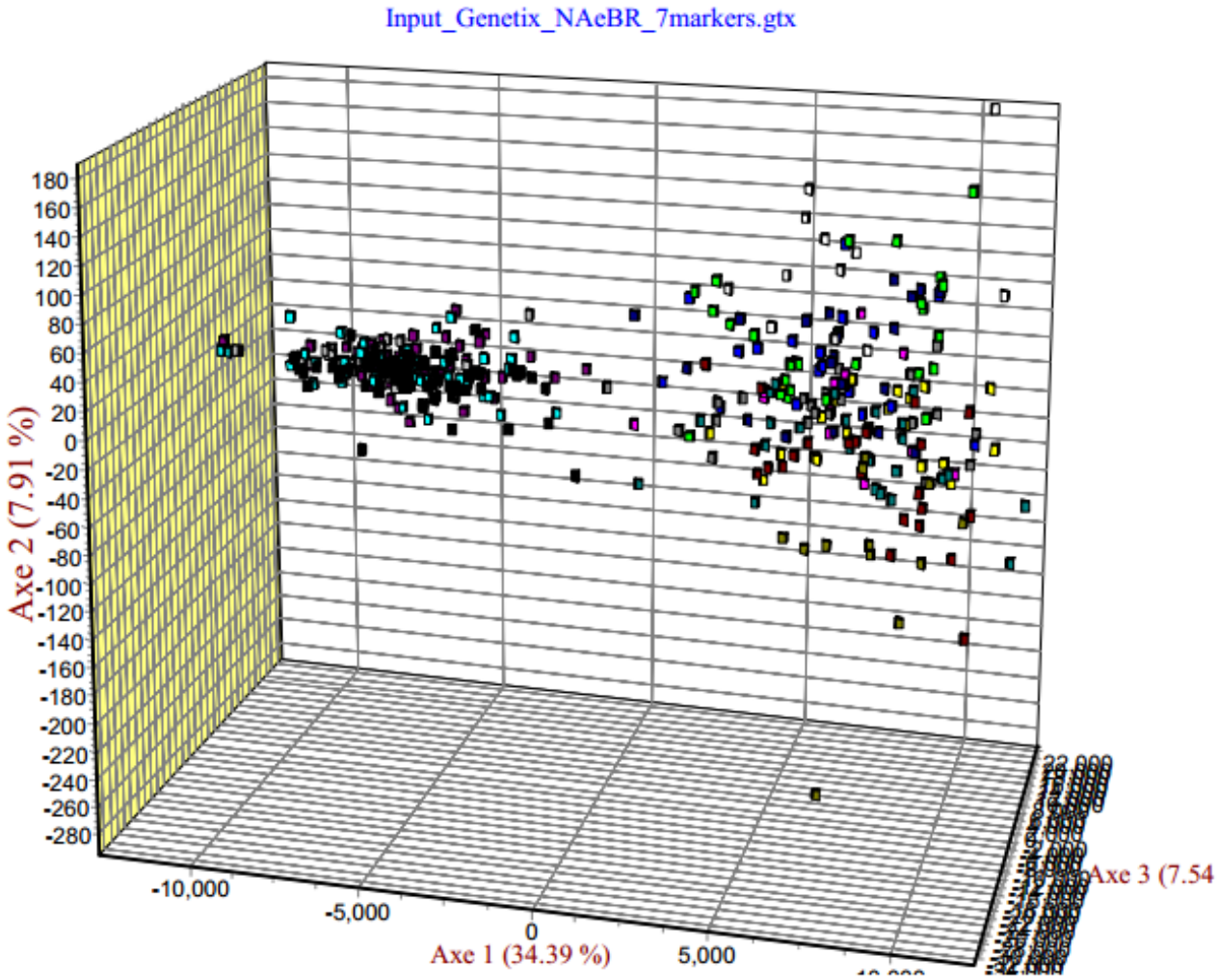

Figure 78. FCA for all populations in the U.S. and BR

4.2 Effective Population Size

N_E results varied greatly depending on the testing method used (Tables 16 - 18). The heterozygote excess method estimation values of Ne and 95% CI to be infinite. The LD method estimated in general negative N_E values and infinite upper limit for the 95% CI. These values using the LD method can be interpreted as indicative of a very large N_E (Waples and Do 2009). In contrast, the molecular coancestry (NeEstimator) and full-likelihood methods (COLONY) consistently produced low estimates relative to the other methods.

Table 17. N_E results for samples in the U.S.

Program	No. of loci	Method	Lowest Allele Frequency Used	N_E	CI 95
NeEstimator	16	Heterozygote Excess Method	0.05	Infinite	Infinite
		Heterozygote Excess Method	0.020	Infinite	Infinite
		Heterozygote Excess Method	0.010	Infinite	Infinite
		LD Method	0.05	-2141.9	1814.3 – Infinite
		LD Method	0.020	-6877.4	2925.1 – Infinite
		LD Method	0.010	-4060.3	7097.4 – Infinite
		Molecular Coancestry Method	–	77.3	1.9 – 285.1
	15	Heterozygote Excess Method	0.05	Infinite	Infinite
		Heterozygote Excess Method	0.020	Infinite	Infinite
		Heterozygote Excess Method	0.010	Infinite	Infinite
		LD Method	0.05	-3121.3	1346.8 – Infinite
		LD Method	0.020	-11335.5	2425.8 – Infinite
		LD Method	0.010	-3764.2	7629.8 – Infinite
		Molecular Coancestry Method	–	511.8	0.5 – 2569.2
COLONY	16	Full Likelihood – Random Mating	–	1212	960 – 1657
		Full Likelihood – Non-random Mating	–	705	536 – 976
	15	Full Likelihood – Random Mating	–	567	463 – 715
		Full Likelihood – Non-random Mating	–	313	254 – 402

Table 18. N_E results for the GOM.

Program	No. of loci	Method	Lowest Allele Frequency Used	N_E	CI 95
NeEstimator	16	Heterozygote Excess Method	0.05	Infinite	Infinite
		Heterozygote Excess Method	0.020	Infinite	Infinite
		Heterozygote Excess Method	0.010	Infinite	Infinite
		LD Method	0.05	-2446.1	1366.2 – Infinite
		LD Method	0.020	8004.1	1433.7 – Infinite
		LD Method	0.010	-3352.4	7973.3 – Infinite
		Molecular Coancestry Method	–	3922.5	3.9 – 19690.9
	15	Heterozygote Excess Method	0.05	Infinite	Infinite
		Heterozygote Excess Method	0.020	Infinite	Infinite
		Heterozygote Excess Method	0.010	Infinite	Infinite
		LD Method	0.05	-4085.0	1060.0 – Infinite
		LD Method	0.020	5996.1	1326.6 – Infinite
		LD Method	0.010	-3657.3	6310.3 – Infinite
		Molecular Coancestry Method	–	177.9	0.2 – 892.8
COLONY	16	Full Likelihood – Random Mating	–	550	437 – 715
		Full Likelihood – Non-random Mating	–	314	242 – 417
	15	Full Likelihood – Random Mating	–	508	407 – 652
		Full Likelihood – Non-random Mating	–	285	224 – 366

Table 19. N_E results for Brazil.

Program	No. of loci	Method	Lowest Allele Frequency Used	N_E	CI 95
NeEstimator	7	Heterozygote Excess Method	0.05	Infinite	Infinite
		Heterozygote Excess Method	0.020	Infinite	Infinite
		Heterozygote Excess Method	0.010	Infinite	Infinite
		LD Method	0.05	-275.0	-1340.4 – Infinite
		LD Method	0.020	-1473.3	944.5 – Infinite
		LD Method	0.010	-5027.4	1359.4 – Infinite
		Molecular Coancestry Method	–	42.7	0 – 214.3
COLONY	7	Full Likelihood – Random Mating	–	340	282 – 413
		Full Likelihood – Non-random Mating	–	250	204 – 309

No signatures of recent bottlenecks were suggested for samples in the U.S or Brazil with the Wilcoxon tests using the mutational models TPM and SMM or the Mode-Shift ADT test in the BOTTLENECK program (Table 19). The Wilcoxon test using the mutational model IAM suggested signatures of recent bottlenecks in five US and one Brazilian locality. Nonetheless, TPM is the most appropriate mutational model for microsatellites (Piry et al. 1999).

Table 20. BOTTLENECK results for the Wilcoxon one-tailed test for heterozygote excess and for the Mode-Shift test. Bolded values indicate significance. The TPM is the most appropriate model for microsatellites and L-shape distribution is indicative of no recent bottleneck.

Locality	Wilcoxon Test			A.D.T.
	IAM	TPM	SMM	L-shape
APA	**0.02884**	0.98930	0.9916	L-shape
AVI	0.07953	0.83875	0.92807	L-shape
CEK	**0.00775**	0.33427	0.44997	L-shape
DIB	0.05833	0.98930	0.99916	L-shape
GAL	**0.02533**	0.62822	0.21660	L-shape
LLM	0.09641	0.97116	0.98752	L-shape
POL	**0.00314**	0.21660	0.35285	L-shape
ROC	**0.00019**	0.82581	0.91232	L-shape
SERC	**0.01450**	0.82581	0.91232	L-shape
SLI	0.21660	0.76813	0.79813	L-shape
LPA	0.99219	1.00000	1.00000	L-shape
TRA	0.99219	1.00000	1.00000	L-shape
ITA	0.97266	1.00000	1.00000	L-shape
LAG	0.99219	1.00000	1.00000	L-shape

5. SUMMARY AND CONCLUSIONS

5.1 Null Alleles and Deviations from HWE

Null alleles were detected at almost every loci and almost every locality and are problematic because null alleles could lead to the overestimation of homozygosity, deviations from HWE, and increasing F_{ST} estimates by reducing genetic variability (Chapuis & Estoup, 2007; Nascimento de Sousa, Finkeldey, & Gailing, 2005).

There are many possible causes for the presence of null alleles in this study, though measures can be taken to understand the extent of their impact (Selkoe & Toonen, 2006). Some explanations are related to limitations in the laboratory. Mutations at flanking primer binding sites, low DNA quality, stuttering, and skewed allele amplification in heterozygotes could all lead to null alleles (Selkoe & Toonen, 2006). Two possibilities stand out in this study. Evidence of DNA degradation was found in many DNA samples, particularly those from samples collected in 2014. The likely culprit for DNA degradation was insufficient refrigeration, as the samples were stored in 100% ethanol. In the dinucleotide repeat loci, heavy patterns of stuttering were present, though this was mitigated by repeating PCR and scoring for 30% of individuals, with 100% of success. Other potential explanations involve biological reasons. Other marine invertebrates display similar levels of inbreeding and a possible explanation for this phenomenon involves higher mutation rates due to large numbers of cell cycles needed for high sperm production (Addison & Hart, 2005). The high values of F_{IS}, under this explanation, is consistent with an increased frequency in null alleles.

Despite the potential presence of null alleles, it does not appear that they influenced the F_{ST} estimations. These were performed with and without a null allele adjustment, with similar results in both cases. Furthermore, other studies on the blue crab with different markers have found low values of F_{ST}, suggesting the null alleles did not bias results (Yednock & Neigel, 2014). Carlsson (2008) has found that assignment tests are not impacted by null alleles and given how the results of the assignment tests presented here coincide with the estimated F_{ST} values, this serves as further evidence for the lack of influence of null alleles.

What remains to be explained is the deviations of HWE found in this study. If the assumption that null alleles did not affect results in this study is correct, it would then seem that the deviations in HWE are due to biological phenomena that violate the assumptions of HWE (Allendorf, Luikhart, & Aitken, 2013). This could be due to non-random mating, which seems likely because of the blue crab's courtship behavior, though this should be evident in all loci and it is not. Inbreeding could also explain the deviations from HWE, as could bottlenecks. In bottlenecks, heterozygosity will decrease and later increase, though this is a factor of the size of the bottleneck and the ability of the population to recover from it (Roderick & Navajas, 2003). Wahlund effects due to the sampling of genetically distinct populations is unlikely due to the very low levels of genetic subdivision in this study. Another form of the Wahlund effect, cohort Wahlund, could explain the deficit in heterozygotes due to the sweepstakes effect (Ruzzante, Taggart, & Cook, 1996; Underwood, Travers, & Gilmour, 2012). In species like the blue crab, with pelagic larvae, adults generally have differing reproductive success, resulting

in only some individuals contributing to the next generation. As such, genetic differences can occur within and between cohorts. If sweepstakes reproduction are playing a role in this system, this could explain the deviations from HWE (Underwood et al., 2012).

5.2 Gene Flow

Establishing the patterns of gene flow and population structure of the blue crab has confounded scientists for over a decade (Darden, 2004; Kordos & Burton, 1993; Lacerda et al., 2016; McMillen-Jackson & Bert, 2004; Yednock & Neigel, 2014). Although many studies have been performed to investigate genetic architecture of the blue crab, there is an abundance of conflicting evidence. This is further complicated by the unclear relationship between pelagic dispersal and gene flow. Although the blue crab has a relatively long pelagic larval duration (31 – 49 days), indicating high gene flow, there is evidence for local adaptation and population differentiation in marine organisms with similar life histories (Barber et al., 2002; Burton, 1986; Hellberg et al., 2002; Knowlton & Keller, 1986; Swearer et al., 2002). The differences in genetic markers, which ranged from allozymes to mitochondrial DNA to nuclear DNA, used in previous blue crab studies could potentially explain the variations in results, as was evidenced by a study on the American oyster (Buroker, 1983; Karl & Avise, 1992). However, microsatellites have found evidence of genetic subdivision in other organisms ((McKeown et al., 2015). Seasonality could also explain these differences, as temporal differences were identified in Yednock & Neigel (2014) and Kordos and Burton (1993). Post-recruitment selection could also explain these differences, though insufficient

evidence is currently available. The long planktonic larval phase of the blue crab seems to compensate for the distance between localities in establishing gene flow. It appears that, in the case of the blue crab, high dispersal equates to high gene flow. These results are consistent to studies with similar organisms, like the European lobster (Watson, McKeown, Coscia, Wootton, & Ironside, 2016).

In contrast, there is strong evidence for population subdivision between samples from the U.S. and BR. Previous work on the blue crab has identified genetic differences between populations in the GOM and in one locality in Venezuela (Yednock & Neigel, 2014). Venezuela could potentially be a genetic break between these two populations, though testing of samples from the Caribbean and the northern coast of South America could provide insight into the number of populations of the blue crab.

Table 21. Summary of previous studies on the population genetics of the blue crab.

Study	*Study Area*	*Marker*	*Genetic Structure*	*Highlights*
Kordos & Burton 1993	Texas Coast	Three enzyme-coding genes	Yes	Temporal heterogeneity identified as well
McMillen-Jackson, Bert, Steele 1994	New York to Texas	Allozymes	Yes*	*Genetic structure found in only some loci. Found genetic patchiness and clinal variation, despite high levels of gene flow
Berthelemy-Okazaki, Okazaki 1997	GOM	Allozymes	No	Found low levels of genetic variation
McMillen-Jackson & Bert 2004	U.S. Atlantic Coast, GOM, Yucatan, Mexico;	Restriction fragment length polymorphisms of mitochondrial genome	No	Found decreasing genetic diversity along geographic cline in the Atlantic Coast
Darden 2004	GOM	COI	Yes	Found patterns of restricted gene flow in the western GOM and no population structure in the eastern GOM
Yednock & Neigel 2014	Louisiana Coast; Lower Laguna Madre; Veracruz, Mexico; Zulia, Venezuela	Five nuclear protein-coding genes and 16S	No	Temporal heterogeneity identified in one locality (LUMCON Marine Lab). Near significant genetic heterogeneity was found in the GOM and significant differences were found between samples from the GOM and Venezuela.
Lacerda et al *2016*	Western South Atlantic	Seven microsatellites	No	Found high levels of gene flow in study area

5.2.1 Gulf of Mexico

The analyses reported here provide strong evidence for the lack of population structure across this region. Although many studies have found high significance in low values of F_{ST} in other marine organisms, only one pairwise F_{ST} comparison was significant here [F_{ST} = 0.01, P-value < 0.05 – CEK vs GAL] (Benestan et al., 2015; Knutsen, Jorde, Andŗe, & Stenseth, 2003; Shaw, Pierce, & Boyle, 1999).The power analysis suggests that the number of samples and the number of markers used were sufficient for capturing the low levels of population structure found here. In addition to very low F_{ST} values (F_{ST} = 0 – 0.0083) throughout the GOM, the AMOVA found very little variation between localities (0.2 – 0.5%; *P*-value > 0.05) and all clustering analyses suggested that the GOM was comprised of one cluster (Figures 15 – 39). No evidence of a division between the eastern and western GOM was found. Tests for population structure generally provided similar results. PCA results did not identify clusters, although FCA did place CEK, the easternmost locality, apart from other localities in the GOM. No evidence for isolation by distance was found in the GOM.

There is a deficit in heterozygosity (H_O = 0.559; H_T' = 0.741), particularly in comparison to reported mean heterozygosity values (H_O = 0.79) from other marine organisms (DeWoody & Avise, 2000). In Steven, Hill, Masters, and Place (2005), heterozygosity was higher, yet ranged from 0.26 to 0.97, with an overall pattern of heterozygote deficit. There were also fewer average number of alleles per locus (N_A = 9.3) than in other organisms (N_A = 19.9).

The relatively low N_A does suggest the potential loss of alleles due to bottleneck, particularly since this measure of diversity is more sensitive to bottlenecks than heterozygosity (Fred W. Allendorf, 1986). The values for G_{IS} in this study were, on average, lower than those in other genetic studies of the blue crab, which ranged from 0.545 to -0.010 (Yednock & Neigel, 2014).

5.2.2 Gulf of Mexico and Chesapeake Bay

Previous research with other marine organisms have found genetic heterogeneity between the two regions, and given the geographic distance, finding two distinct populations was expected (Boehm, Waldman, Robinson, & Hickerson, 2015; Bowen & Avise, 1990; Hollenbeck, Portnoy, Saillant, & Gold, 2015). Surprisingly, no evidence of population structure was identified between samples from the GOM and the Chesapeake Bay. The F_{ST} values were very low ($F_{ST} = 0 – 0.0083$), especially among pairwise comparison of GOM localities to the Chesapeake Bay locality (all $F_{ST} = 0$). The AMOVA identified low levels of variation between localities (0.2 – 0.5%; *P*-value > 0.05) and no separation between the two regions was identified in the clustering analyses. The lack of population structure indicates that the blue crab larvae are capable of dispersing through distances larger than 1,600 km.

Like in the GOM, there was a deficit in heterozygosity in samples from the U.S. ($H_O = 0.557$; $H_T' = 0.742$). The average N_A was slightly higher in the U.S ($N_A = 9.4$), relative to the GOM ($N_A = 9.25$). The inbreeding coefficient was lower, but not much, than the one calculated for the GOM ($G_{IS} = 0.191$). No evidence of a recent bottleneck was detected in this group of samples either and very high values of N_E (N_E = Infinite)

were identified. The high values of N_E were especially surprising given the decrease in blue crab populations of the Chesapeake Bay in recent years. Overfishing has been a problem and has led to record low levels in numbers of crabs (Pelton & Goldsborough, 2008). Dead zones in the ocean, where low-oxygen levels inhibit productivity, habitat loss due to sediment runoff and algal blooms, and infectious diseases have led to this decline in population (Pelton & Goldsborough, 2008; Shields & Overstreet, 2003). The lower than expected levels of heterozygosity and allelic diversity could be due to these events, though the lack of genetic evidence for a recent bottleneck seems to suggest otherwise. Null alleles could also explain the deficit in heterozygosity.

5.2.3 U.S. and Brazil

Significant differentiation was identified between the localities in the U.S. and the ones in BR. F_{ST} values (F_{ST} = 0.13 – 0.19) were several orders of magnitude higher than those for the U.S. The AMOVA found as much as 44.4% variation between the two countries, while all clustering analyses found clear separations between the two (Figures 14 – 39). Despite the high dispersal exhibited by larval blue crabs, it appears that the distances between the northern and southern West Atlantic are too large to be overcome by dispersal. Although the linear regression between genetic and geographic distances indicate the presence of IBD between the two populations, the lack of samples from the Caribbean and northern and central Brazil makes it difficult to conclusively point to IBD (Table 15; Figure 13).

The samples in BR were tested with fewer markers than the ones in the U.S, as they were originally part of Lacerda et al. (2016). Thus, it is uncertain whether the

differences in summary statistics between the two regions are due to the lower number of markers or actual biological differences. For instance, the BR samples had much lower H_O and H_T' (H_O = 0.550; H_T' = 0.595) than the samples in the U.S. On the other hand, they had higher average N_A (N_A = 13.9), though it is important to note that the seven markers used for the BR samples were highly polymorphic. It was interesting to find that the inbreeding coefficient for BR samples was much lower than those in the U.S. (G_{IS} = 0.069). The blue crab fishery in BR is not well regulated and evidence of overfishing, such as inbreeding, was expected in these samples.

5.3 Effective Population Size

All regional subsets analyzed here had varying results for N_E, though they suggest an overall large N_E (Tables 16 – 18). In reviews of the multiple methods for estimating N_E, J. Wang (2016) found that heterozygote excess and molecular coancestry methods were not as robust as the sibship frequency and LD methods. Though this does not fully explain the differences in estimates presented here, the sibship frequency method may not perform well with more than 10 microsatellite loci and if nonrandom sampling occurred (Waples, 2016). The same review by Waples (2016) found that the LD method improves in performance with the use of more loci. This would indicate that the blue crab populations have infinite N_E, according to the LD method.

5.4 Management

The blue crab fishery is an important source of income for countries throughout its range. Its economic importance places its key ecological role in jeopardy as anthropogenic activities have led to population decline. The results presented here are

important to ensuring better management of this critical species. Although no recent bottlenecks were detected with the markers used here, it is important to maintain the measures in place, such as female- and male-specific harvest regulations (e.g.: maintaining an abundance of 215 million female adults) and 10% harvest reductions, to keep this fishery on the path to recovery (NOAA, 2016).

The Chesapeake Bay has experienced the greatest levels of population decline and additional care should be taken to return the population size to previous levels (NOAA, 2014). One promising result of this study is that the very high levels of gene flow detected here suggest that populations from the GOM could help with recruitment for the Chesapeake Bay, without problems associated with translocation, like outbreeding depression. However, for blue crab larvae to proliferate in the Chesapeake Bay, remediation of their habitat is necessary. Additionally, the overall deficit in heterozygotes could hinder the populations' ability to adapt to environmental change (Gray, 1997)

The results presented here provide further evidence for genetic homogeneity in the U.S. Studies by Kordos & Burton (1993), McMillen-Jackson & Bert (2004), and Yednock & Neigel (2014) have indicated various levels of high gene flow in the blue crab's range in North America. Darden (2004) had found evidence of a genetic break between the east and west GOM, though this has not been supported in the other studies. Fisheries management in the GOM has relied on previous work that suggested this, despite the previous study being limited by its use of one mitochondrial marker (Darden, 2004; VanderKooy, 2013a). Although the differences in measured genetic structure

could be attributed to seasonality, the evidence presented here seems to overwhelmingly point towards no genetic structure in the GOM.

Current management of the GOM population is bounded by state lines, though this may still be necessary for bureaucratic purposes. The traditional method for stock delineation involve statistical comparisons between two possible stocks. Based on this method, there is one stock in the U.S. and one in Brazil. This method is conservative, though, and may not account for biological differences (Waples, 1998). To manage this fishery, though, additional information on population dynamics is needed.

The evidence of a genetic break between the U.S. and Brazil is important. Additional sampling is needed in the Caribbean and the northern portion of South America to pinpoint where the genetic break occurs. This will help managers throughout the blue crab's geographic range better understand the extent of its dispersal and improve management. In Brazil in particular, additional regulations and enforcement of these regulations are greatly needed. The lower levels of heterozygosity in those samples appear to point towards overfishing and mismanagement. Preserving the genetic diversity found in that region is important, especially in light of climate change.

5.5 Conclusions

This study provides supporting evidence to the hypothesis that pelagic larvae lead to high levels of dispersal and therefore high levels of gene flow. There is evidence that supports and contradicts this hypothesis, however, this study could provide insight into the patterns of gene flow for other crustaceans that have similar life history patterns. It also emphasizes the need for continued population genetic studies to explain the

reasons behind differences in patterns of genetic structure. The relationship between pelagic larvae and gene flow is far from clear across marine organisms, though in the blue crab, the two are positively correlated.

Unlike previous studies on population genetics of the blue crab in the U.S., this study did not find low, but statistically significant, levels of genetic structure. The results presented here for the U.S. are more consistent with what was identified by (Lacerda et al., 2016) in southern Brazil, where localities over 1,000 km apart from each other had high levels of gene flow. Notably, Yednock and Neigel (2014) found an overall F_{ST} of 0.09 in the GOM, an order of magnitude different from the overall FST presented here, $F_{ST} = 0.007$. This study also did not identify latitudinal gradients in genetic structure, as was suggested by one marker in (McMillen-Jackson & Bert, 2004). Although genetic structure in the Atlantic coast appears unlikely due to the high levels of gene flow across the blue crab's range, additional sampling could provide more information. Furthermore, despite extensive sampling in the coast of Texas, no patterns of genetic structure were identified there, unlike what had been previously reported (Kordos & Burton, 1993).

Microsatellite markers were used here due to their advantages over the previously used markers, such as high polymorphism, fast rate of evolution, reproducibility, and the improved capability of resolving genetic difference relative to other markers (Sunnucks, 2000). Based on the results presented here, it appears that other genetic markers overestimate the degree of population subdivision in this species, though it is important not to discount the role seasonality may be playing in causing

different results across time. To coalesce the contradicting results, genome-wide analysis with both neutral and markers under putative selection is necessary.

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Table 22. F_{ST} with (upper) and without (lower) ENA for 16 loci for U.S. populations. Significance was tested at P = 0.05 and none were significant.

	APA	AVI	CEK	DIB	GAL	LLM	POL	ROC	SERC	SLI
APA	--	-0.000721	0.024335	0.012048	0.000528	0.011112	0.014183	-0.003945	0.004643	0.006277
AVI	-0.006427	--	0.019052	-0.001322	0.003332	0.003466	-0.006155	-0.002821	0.005642	0.013518
CEK	0.024916	0.014805	--	0.018218	0.023013	0.033383	0.007987	0.016290	0.010057	0.056144
DIB	0.009891	-0.005695	0.012765	--	0.006077	-0.006982	0.008628	-0.010799	-0.003124	0.019835
GAL	-0.009164	-0.004618	0.011596	0.002066	--	0.007166	0.015368	-0.007561	-0.013082	0.000549
LLM	0.008804	-0.000067	0.030579	-0.013442	-0.002537	--	0.019900	-0.004052	-0.000442	0.015861
POL	0.007615	-0.012420	0.001643	0.003045	0.001145	0.012145	--	0.006098	0.014689	0.035703
ROC	-0.004039	-0.004668	0.008700	-0.015744	-0.014337	-0.010223	-0.001815	--	-0.002969	0.003132
SERC	0.002335	0.001807	0.002092	-0.006879	-0.021411	-0.006695	0.008462	-0.007159	--	0.022288
SLI	-0.006464	-0.000055	0.038782	0.015508	-0.013135	0.004204	0.020390	-0.003562	0.011972	--

Figure 79 shows a model with admixture and correlated frequencies, Figure 80 shows admixture with independent frequencies, Figure 81 shows no admixture with correlated frequencies, and Figure 82 shows no admixture and independent frequencies

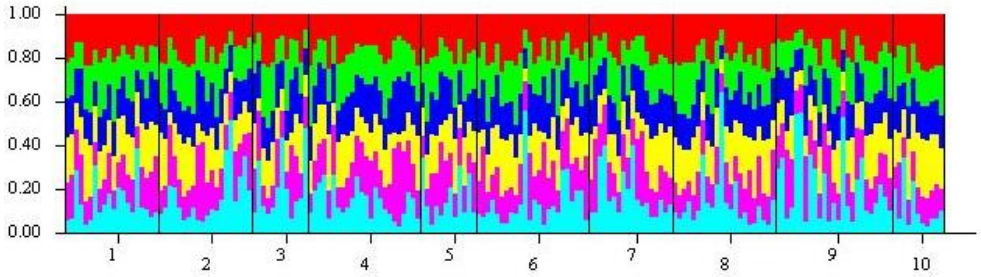

Figure 79. STRUCTURE results for all U.S. samples (9 loci; K = 6)

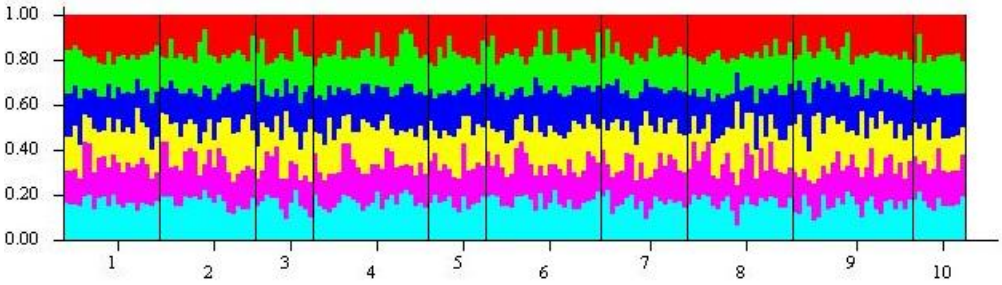

Figure 80. STRUCTURE results for all U.S. samples (9 loci; K = 6)

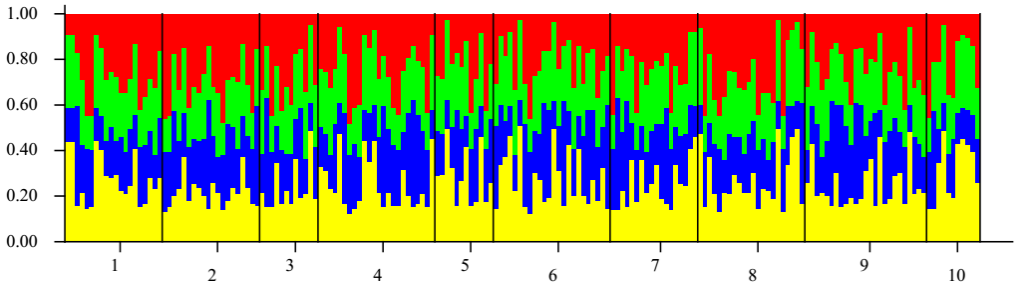

Figure 81. STRUCTURE results for all U.S. samples (9 loci; K = 4)

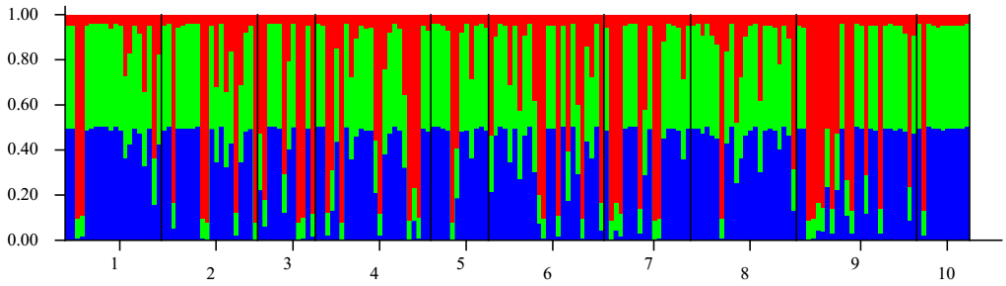

Figure 82. STRUCTURE results for all U.S. samples (9 loci; K = 3)

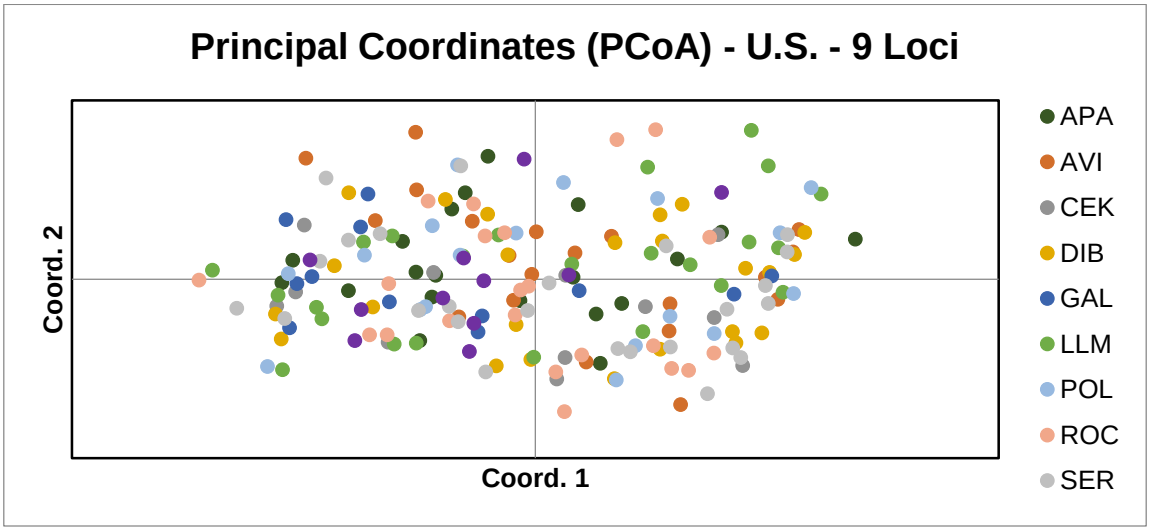

Figure 83. PCA for all samples in the U.S., using 9 loci

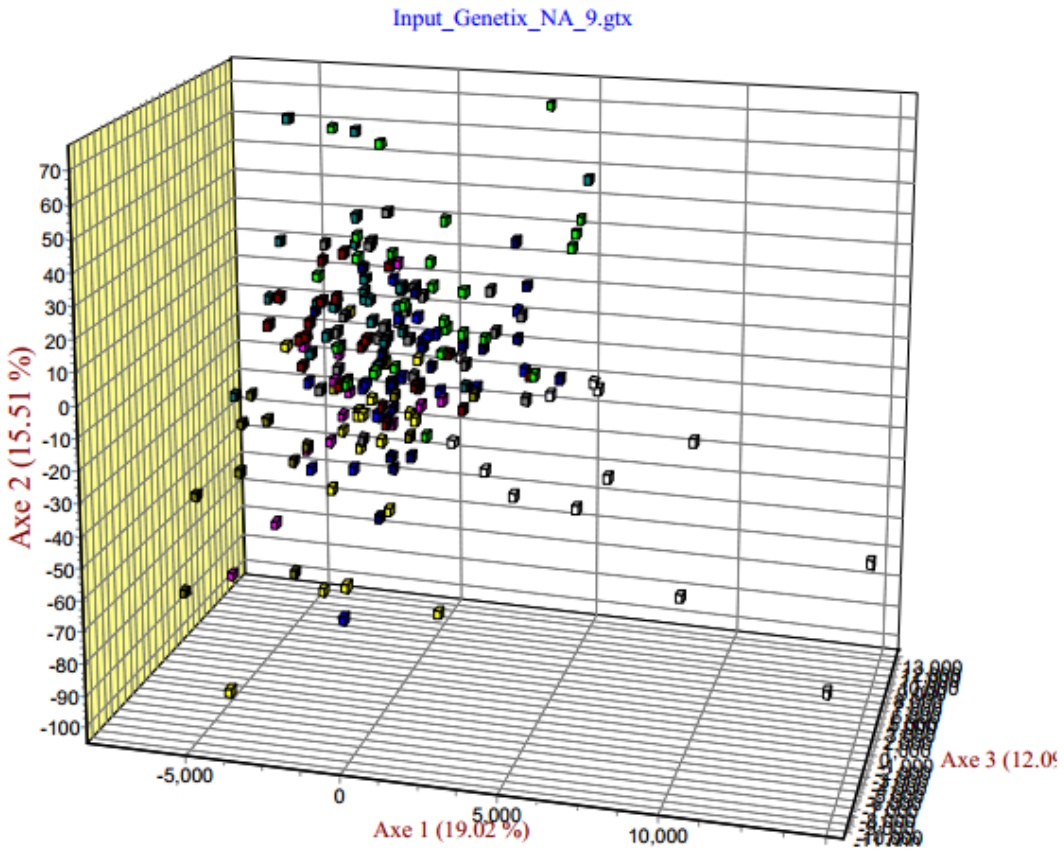

Figure 84. FCA for all samples in the U.S., using 9 loci

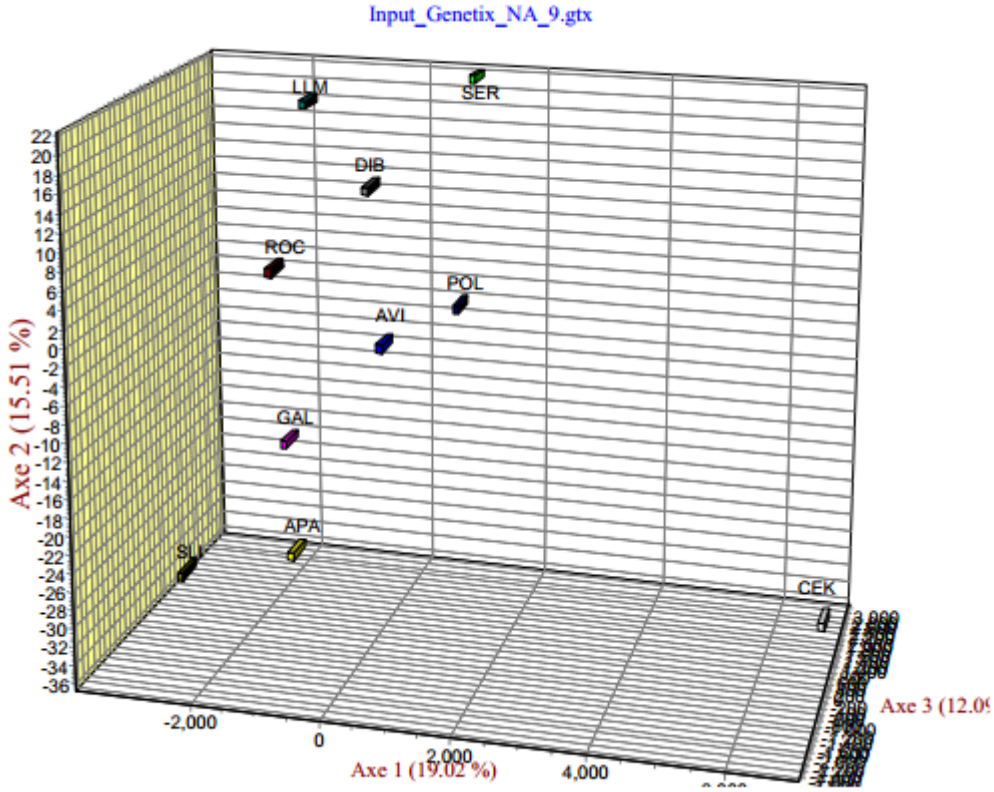

Figure 85. FCA means for each population in the U.S., using 9 loci

Table 23. BOTTLENECK analysis for all U.S. localities, using 9 loci. Bolded values indicate significance (P = 0.05)

Locality	Wilcoxon Test			A.D.T.
	IAM	TPM	SMM	L-shape
APA	0.500000	0.995117	0.997070	L-shape
AVI	0.410156	0.898438	0.935547	L-shape
CEK	0.212891	0.820313	0.875000	L-shape
DIB	0.455078	0.997970	0.999023	L-shape
GAL	0.367188	0.875000	0.935547	L-shape
LLM	0.714744	0.993164	0.997070	L-shape
POL	0.082031	0.714844	0.898438	L-shape
ROC	**0.024414**	0.990234	0.990234	L-shape
SERC	0.367188	0.998047	0.999023	L-shape
SLI	0.589844	0.981445	0.986328	L-shape

Table 24. N_E results for all samples in the U.S., using 9 loci

Program	No. of loci	Method	Lowest Allele Frequency Used	N_E	CI 95
NeEstimator	16	Heterozygote Excess Method	0.05	Infinite	Infinite
		Heterozygote Excess Method	0.020	Infinite	Infinite
		Heterozygote Excess Method	0.010	Infinite	Infinite
		LD Method	0.05	Infinite	49.9 – Infinite
		LD Method	0.020	Infinite	55.8 – Infinite
		LD Method	0.010	Infinite	55.8 - Infinite
		Molecular Coancestry Method	–	8.4	2.7 – 17.2
COLONY	16	Full Likelihood – Random Mating	–	169	133 – 213
		Full Likelihood – Non-random Mating	–	65	65 – 119