Genetic Diversity and Structure From Antillean Manatee (Trichechus manatus manatus) in the Southern Gulf of México: Comparison Between Connected and Isolated Populations

Authors: Gómez-Carrasco, Guadalupe, Lesher-Gordillo, Julia María, Olivera-Gómez, León David, Bonde, Robert K, Arriaga-Weiss, Stefan, et al.

Source: Tropical Conservation Science, 11(1)

Published By: SAGE Publishing

URL: https://doi.org/10.1177/1940082918795560

Tropical Conservation Science
Volume 11: 1–10
© The Author(s) 2018
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/1940082918795560
journals.sagepub.com/home/trc
SAGE

Genetic Diversity and Structure From Antillean Manatee (*Trichechus manatus manatus*) in the Southern Gulf of México: Comparison Between Connected and Isolated Populations

Guadalupe Gómez-Carrasco[1], Julia María Lesher-Gordillo[1], León David Olivera-Gómez[1], Robert K Bonde[2], Stefan Arriaga-Weiss[1], Raymundo Hernández-Martínez[1], Guillermo Castañón-Nájera[1], Darwin Jiménez-Domínguez[1], Armando Romo-López[1], and Alberto Delgado-Estrella[3]

Abstract

Antillean manatees (*Trichechus manatus manatus*), a subspecies of the West Indian manatee, is listed as endangered species in the Red List of Threatened Species of the International Union for Conservation of Nature. The aims of this research were to survey on the possible regional genetic structure in the southern Gulf of Mexico and to compare genetic status of a landlocked population in Laguna de las Ilusiones (IL) with individuals from localities with no barriers to displacement and breed (open population [OP]). We analyzed 45 manatee skin samples collected from different locations in Tabasco ($n = 38$, including 19 from IL), Veracruz ($n = 3$), Campeche ($n = 2$), and Chiapas ($n = 2$). The genomic DNA was isolated and PCR amplifications were performed for each sample using 28 microsatellite loci, previously designed for West Indian manatees and described as polymorphic for this species. Two clusters ($k = 2$) were identified by STRUCTURE. The analysis of both a priori populations (IL and OP) indicate that the global values of F_{ST} and R_{ST} (F_{ST}=0.049, R_{ST}=0.077) were significant. The H_E for IL was 0.38 ± 0.03 and for OP was 0.49 ± 0.01. The average number of alleles N_A for IL was 2.21 ± 0.09 and for OP was 2.32 ± 0.09. The overall inbreeding coefficient was F_{IS}=−0.013 for analyzed populations. Genetic diversity was low. The IL population had slightly lower genetic diversity compared with OP, which could be explained by isolation of that small group, so conservation plans for IL should be considered as priority.

Keywords
Microsatellites, gene flow, bottleneck, inbreeding, Laguna de las Ilusiones

Introduction

Determining population structure in large mammals is important to facilitate the effective conservation management and help advance our understanding of the mechanisms that drive the evolution of populations (Hoelzer, Wallman, & Melnick, 1998). Microsatellites had been used to differentiate between stocks of manatees (Bonde, McGuire, & Hunter, 2012) and to achieve alternative estimates of genetic diversity. Antillean manatees (*Trichechus manatus manatus*), a subspecies

---

[1] Centro de Investigación para la Conservación y Aprovechamiento de los Recursos Tropicales (CICART) de la DACBiol; Universidad Juárez Autónoma de Tabasco, Villahermosa, Centro, Tabasco, México
[2] U.S. Geological Survey, Wetland and Aquatic Research Center, Gainesville, FL, USA
[3] Universidad Autónoma del Carmen, Ciudad del Carmen, Mexico

Received 11 May 2018; Revised 19 July 2018; Accepted 25 July 2018

Corresponding Author:
Julia María Lesher-Gordillo, Centro de Investigación para la Conservación y Aprovechamiento de los Recursos Tropicales (CICART) de la DACBiol; Universidad Juárez Autónoma de Tabasco, Carretera Villahermosa-Cárdenas km 0.5, entronque Bosques de Saloya; Villahermosa, Tabasco Código Postal: 86039. Villahermosa, Centro, Tabasco, México.
Email: lesher23@yahoo.com

of the West Indian manatee, is listed as endangered species in the Red List of Threatened Species of the International Union for Conservation of Nature (Self-Sullivan & Mignucci-Giannoni, 2008). It is suspected that habitat degradation and other anthropogenic threats have driven native manatee populations to be considered as endangered in Mexico (Secretaría de Medio Naturales Ambiente y Recursos [SEMARNAT], 2010); however, knowledge on manatee status and habitat requirements in Mexico is still scarce. Previous genetic studies have defined two populations in Mexico, the Gulf of Mexico and the southeastern Yucatan peninsula, and suggest that the Caribbean coasts may benefit from higher gene flow from neighboring populations in Belize and Florida, where the Gulf of Mexico is more isolated (Nourisson et al., 2011).

The southern Gulf of Mexico is a complex habitat for manatees, which inhabits mostly freshwater systems on extensive flood plains (Lefebvre, Marmontel, Reid, Rathbun, & Domning, 2001). Populations of manatees in this region are considered to be 1,000–1,500 individuals (United Nations Environment Programme, 2010), based on the best guessed estimation by specialists, but recent effort suggest that this is an overestimate (Puc-Carrasco, Morales-Vela, Olivera-Gomez, & González-Solis, 2017). The riverine environment is not homogeneous, and manatees seems to be more associated with lake systems connected to main rivers by secondary waterways, which involve seasonal movements in and out of these systems, following flood pulses (Puc-Carrasco, Olivera-Gómez, Arriaga-Hernandez, & Jimenez-Dominguez, 2016). This seasonal movement promotes discrete groups of manatees along the Usumacinta and Grijalva rivers, the largest regional river basins. Information on the population structure of manatees in Southern Gulf of Mexico by Nourisson et al. (2011) is not conclusive and suggests that more samples for this region are necessary to accurately identify the genetic structure of the population as a whole.

Natural and man-made modifications to the hydrology of particular areas have created isolated groups of manatees, as well as translocation efforts of individuals into closed lakes with touristic and waterway management intentions. The genetic health of these isolated populations is a matter of concern due to the small number of individuals and the time they have been isolated. One of these isolated populations is located in "Laguna de las Ilusiones" (IL), an area that was originally connected to the Carrizal River but access is no longer possible due to construction of a dam 40 years ago, preventing manatee movement in and out of the lake. After IL was landlocked, wild manatees from other areas were rescued and released into IL, as we know from "anecdotic" talks with people inhabited the lake borders, but they failed to provide exact dates and there are not any published record from environmental authorities. As far as we know, no new animals were translocated into the lake in the last 20 years. The extension of the 219 ha lake made this population of 18 to 39 manatees isolated and without proper management (Pablo-Rodríguez & Olivera-Gómez, 2012; Pérez-Garduza, 2013). Direct urban pressure on the IL population (Aragón-Martínez, Olivera-Gómez, & Jimenez-Dominguez, 2014) and concern about the health and fitness of the manatees provided an opportunity to conduct a survey of the genetic diversity of this population and compare the findings with opportunistic samples collected from wild manatees from other localities throughout the southern Gulf of Mexico.

The aims of this research were to survey the fine-scale regional genetic structure of manatees based on all the available samples and compare the genetic status of the wild OP in the southern Gulf of Mexico with the landlocked population of IL. This information will serve as a baseline for management strategies and to discuss potential threats.

Methods

Sampling

We analyzed 45 manatee samples of *T. m. manatus* collected from different locations in Tabasco ($n = 38$), Veracruz ($n = 3$), Campeche ($n = 2$), and Chiapas ($n = 2$; Figure 1). Skin samples, collected between 2006 and 2015, were used for DNA isolation. Small skin samples were taken from the tip of the caudal tail during health assessments of live manatees captured for radio tagging and from recently dead individuals. Samples were separated into two groups according to their origin. The first group corresponds to individuals from "IL" ($n = 19$), a landlocked lake located within the urbanized area of the city of Villahermosa (Figure 1(b)) where a small closed population (Pérez-Garduza, 2013) was isolated from the rest of the regional manatee population several decades ago. Animals in IL were most likely come from nearby areas in Tabasco, but their real origin is unknown. IL was not considered a priori as a genetically isolated population but spatially isolated. The second group of samples was taken from manatees in areas where there are no fixed barriers to displacement, so considered an OP ($n = 26$). We assumed that animals from this OP can move and breed freely along the study region.

Genomic DNA Extraction and Microsatellite Genotyping

The genomic DNA was isolated using QIAGEN'S DNeasy Blood and Tissue DNA Isolation kits

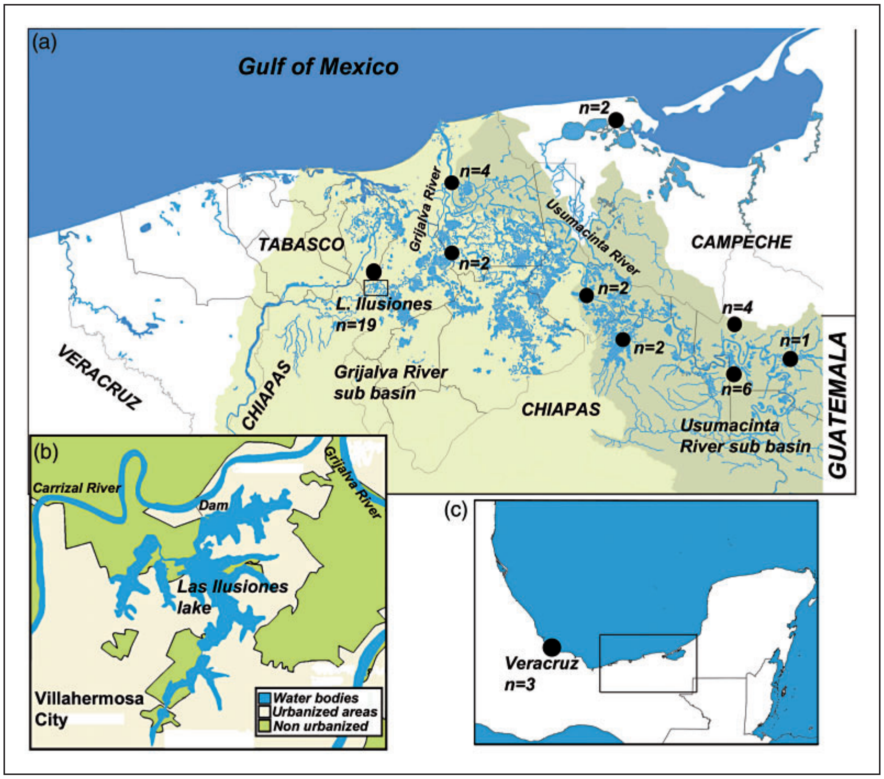

Figure 1. Study area in Southern Gulf of Mexico. The points represent the sampling areas. (a) sampling region in Tabasco, Chiapas, and Campeche, (b) lake of "Laguna de las Ilusiones," IL, which is a lake currently landlocked with a small manatee population, around 260 ha in extension. (c) The Gulf of Mexico region and location of samples in Veracruz, a small box shows extension of Figure 1(a).

(Valencia, CA, USA). Polymerase chain reaction (PCR) amplifications were performed for each sample using 28 microsatellite loci, previously designed for West Indian manatees and described as polymorphic: TmaH13 (Nourisson et al., 2011; Pause et al., 2007), TmaE14 (Nourisson et al., 2011; Pause et al., 2007), and TmaSC5 (Pause et al., 2007). The annealing temperature (*Tm*) was modified for the following microsatellites loci: TmaE02, TmaE26, TmaM79 (59°C; García-Rodríguez, Moraga-Amador, Farmerie, McGuire, & King, 2000; Nourisson et al., 2011); TmaE11 (57°C; García-Rodríguez et al., 2000; Nourisson et al., 2011); TmaE1 (54°C; Pause et al., 2007); TmaK01 (54°C; Nourisson et al., 2011; Pause et al., 2007); TmaE4 and TmaE07 (57°C; Pause et al., 2007); TmaJ02 (60°C; Nourisson et al., 2011; Pause et al., 2007); TmaKb60 (60°C; Pause et al., 2007); Tma-FWC01, Tma-FWC03, Tma-FWC04, Tma-FWC05, Tma-FWC07, Tma-FWC09, Tma-FWC10, Tma-FWC11, Tma-FWC12, Tma-FWC13, Tma-FWC15, Tma-FWC16, Tma-FWC17, Tma-FWC18 (58°C; Tringali et al., 2008); and Tma-FWC14 (57°C; Tringali et al., 2008).

Amplifications were performed in a T100 Thermal Cycler (Biorad) or a MyCycler thermocycler (Biorad) using the conditions as listed in the original publication for each primer, with the exception of the *Tm*, indicated earlier.

Amplifications were performed in a total volume of 22 µL, with 20 ng template DNA, 15 µL Platinum® PCR SuperMix (22 U/mL recombinant Taq DNA polymerase with Platinum® Taq Antibody, 22 mM Tris-HCl [pH 8.4], 55 mM KCl, 1.65 mM MgCl2, 220 µM dGTP, 220 µM dATP, 220 µM dTTP, 220 µM dCTP, and stabilizers; Invitrogen), and 200 nM per primer. To visualize the PCR products, 2% agarose gel electrophoresis, stained with ethidium bromide, was used (Barril & Nates, 2012; Porras Murillo, Bolaños Montero, & Barr, 2008). Gels were photo-documented using Molecular Imager® Gel Doc™ (Bio-Rad). The positive bands were analyzed using the automatic gel documentation and image analyzer UVband software (UVITEC, UK; Yazdian-Robati et al., 2015). In a random subsample, we verified number of alleles by automatic electrophoresis (Experion-Bio-Rad) and we found the same pattern than using conventional agarose gel electrophoresis. We compared our results with those of Nourisson et al. (2011), resulting in similar number of alleles. Noninformative loci were eliminated for posterior analysis.

Data Analysis

Genetic structure. The software STRUCTURE v2.3.1 (Pritchard, Stephens, & Donnelly, 2000) was used to identify the genetic subdivision of manatee populations in Tabasco. The Admixture model was applied; the number of populations (K) was set from 1 to 5 with a burn-in period of 100,000 reiterations followed by 1,000,000 Markow chain Monte Carlo (MCMC) iterations. The most probable number of populations, K, was determined using STRUCTURE HARVESTER (Earl & vonHoldt, 2011). The global values of genetic differentiation F_{ST}, as well pair wise F_{ST} and R_{ST} values, were calculated with GENALEX 6.502 (Peakall & Smouse, 2006) using an analysis of molecular variance. To identify possible bottleneck events, the null hypothesis was tested for excess of heterozygosity using BOTTLENECK Software 1.2.02 (Cornuet & Luikart, 1996; Piry, Luikart, & Cornuet, 1999), applying the Wilcoxon test for one tail under two models: stepwise mutation model (SMM) and the two-phase mutation model (TPM).

Genetic diversity. For genetic diversity, allelic richness (N_A), effective population size (N_E), observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficient (F_{IS}), the deviation from Hardy–Weinberg equilibrium, and linkage disequilibrium were estimated using GENALEX 6.502 (Peakall & Smouse, 2006) and GENEPOP 4.0.10 (Raymond & Rousset, 1995). The presence of null alleles and the index of the polymorphic content were estimated with CERVUS v. 3.0.7 (Kalinowski, Taper, & Marshall, 2007).

Results

All 45 samples from manatee skin tissue were used to isolate genomic DNA. Each sample was successfully amplified for 28 microsatellite loci and, from these, 67 alleles were obtained. Genetic diversity estimates obtained with software GENALEX 6.502 and GENEPOP 4.0.10 yield similar results. Therefore, we only present the results obtained from GENALEX 6.502 (Table 1), because of the extended use of this program in genetic diversity studies.

Genetic Structure

Two clusters ($k = 2$) were identified by the program STRUCTURE 2.3.4 as the appropriate number of clusters for assignment (Figure 2(a)). Figure 2(b) shows the samples reassigned by Q from each cluster. Analysis of a priori populations (IL and OP) indicate that the global values of F_{ST} and R_{ST} ($F_{ST} = 0.049$, $R_{ST} = 0.077$) were significant, as well as those which were analyzed by STRUCTURE clusters, F_{ST}=0.071, $R_{ST} = 0.111$, and both indicated moderate differentiation among groups (Frankham, Ballou, & Briscoe, 2004; Freeland, 2005). A significant heterozygote excess was found in IL (Wilcoxon signed rank test, $P_{\mathrm{TPM}} = 0.006$ and $P_{\mathrm{SMM}} = 0.031$) and OP (P_{TPM} *and* P_{SMM} <0.001) on the basis of mutational models. In IL, a strong normal L-shaped distribution was fitted.

Genetic Diversity

Values of average number of different alleles (N_A), average number of effective alleles (N_E), observed heterozygosity (H_O), and expected heterozygosity (H_E) estimated by GENALEX 6.502 for each group and for the entire region, are presented in Table 1. All microsatellite loci were not significant for Hardy–Weinberg equilibrium. No linkage disequilibrium was detected. The overall inbreeding coefficient F_{IS}=−0.013 was low but significant. Error proportion was estimated by the verification of 12% of samples using automatic electrophoresis and no error was found. Null alleles were not observed in any of the analyzed groups. Average polymorphism information content (PIC) was 0.372, with locus TmaFWCL15 (Tringali et al., 2008) which had the higher PIC (0.560) and locus TmaJ02 (Nourisson et al., 2011; Pause et al., 2007) which had the lowest PIC (0.192).

Discussion

Manatees were historically harvested in the southern Gulf of Mexico since pre-Hispanic times, but important hunting began in the colony and continued into 1920s.

Table 1. Average Allelic Frequency and Standard Deviation by Population and Total.

	n	N_A	N_E	H_O	H_E
A priori groups					
IL	19	2.21 ± 0.09	1.71 ± 0.08	0.50 ± 0.06	0.38 ± 0.03
OP	26	2.32 ± 0.09	1.99 ± 0.05	0.55 ± 0.04	0.49 ± 0.01
Global	45	2.27 ± 0.06	1.85 ± 0.05	0.52 ± 0.04	0.43 ± 0.02
STRUCTURE assigned groups					
Group red	19	2.29 ± 0.09	2.00 ± 0.04	0.61 ± 0.03	0.50 ± 0.01
Group green	26	2.29 ± 0.10	1.74 ± 0.07	0.46 ± 0.06	0.39 ± 0.03
Global	45	2.29 ± 0.07	1.87 ± 0.05	0.54 ± 0.04	0.44 ± 0.02

n= number of samples tested, N_A= average number of alleles, N_E= average number of effective alleles, H_O= observed heterozygosity and H_E= expected heterozygosity.

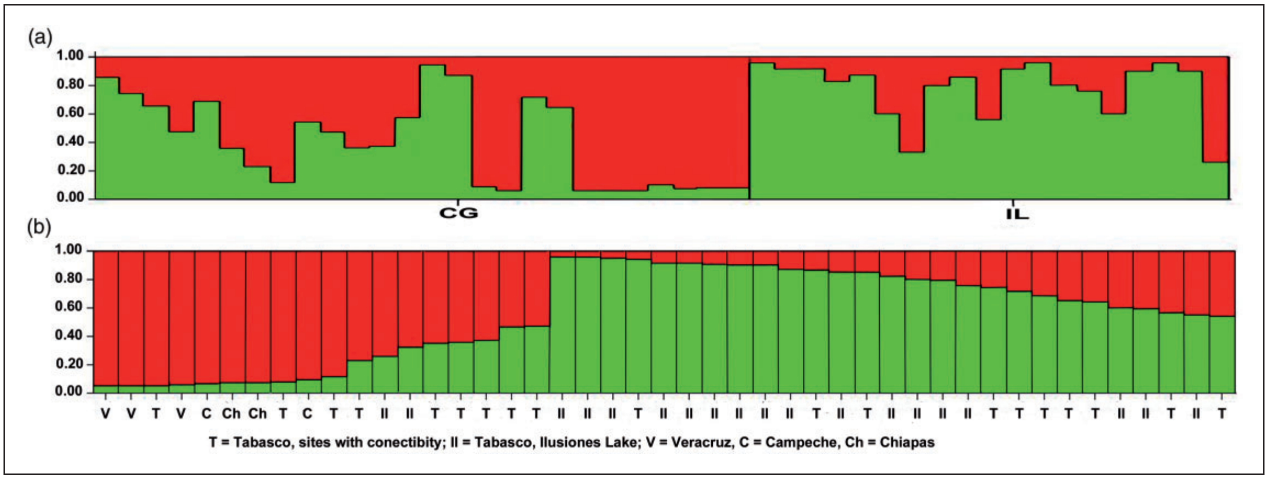

Figure 2. Genetic groups estimated from a Bayesian assessment of genetic partition applying STRUCTURE. (a) Genetic identity for each of the two populations: Cluster 1 (red) and Cluster 2 (green). (b) Graphical representation of $K = 2$ which assigned samples to each group; the values were assigned by Q.

Quantitative records of this hunting are not available in any documents, although the dimension of this hunting could be inferred because the first laws against commercial use of manatees in Mexico did not exist until the second decade of 20th century (Diario Oficial de la Federación, 1922). Current hunting is not significant but still occurs in some inaccessible regions in Mexico (L. D. Olivera-Gómez, 2008 personal communication). Rovirosa (1885) mentions that manatees were abundant in rivers and lakes of Tabasco. River runoff in this regional coastal plain represents about 30% for all of Mexico, thereby feeding a large and complex wetland area and hydrological system. Different from other Mexican states along the Gulf of Mexico (Veracruz, Tamaulipas, and Campeche), Tabasco remained without extensive agriculture or cattle farming until the middle of the 20th century (Sánchez-Munguía, 2005) which brought relative protection from hunting of manatees for food. However, Tabasco has focused in agriculture growth since the 1940s leading to current deforestation of 98% (Sánchez-Munguía, 2005) of the original vegetation. Tabasco was also important for oil and gas extraction since the 1940s, both along the shore and inland at areas associated with wetland productivity, which caused heavy changes in land use and in the hydrological drainage network.

Cold fronts north of Veracruz and Tamaulipas in Mexico and in Texas in the USA could act as a barrier of dispersion to and from northern Gulf of Mexico resulting in movements of manatees favoring eastward travel into the Yucatan Peninsula and along the Caribbean coast. Nourisson et al. (2011) estimated limited genetic mixing with individuals from the Mexican Caribbean population, resulting in the southern Gulf of Mexico as a possible source of individuals to prevent extensive inbreeding. Currently, the Antillean manatees from Tabasco, in the southern Gulf of Mexico coastal plain, is associated mainly with the Grijalva and Usumacinta river basins (Colmenero & Hoz-Zavala, 1986; Jiménez-Domínguez & Olivera-Gómez, 2014). This region is thought to host the largest manatee population in Mexico (Marsh, O'Shea, & Reynolds III, 2011). Unfortunately, genetic diversity in this population is lower when compared to that of the Caribbean coasts of Mexico (Nourisson et al., 2011).

Genetic diversity increases the chance of populations to adapt to environmental changes (Geffen, Luikart, & Waples, 2007). In this study, the genetic diversity, estimated on the heterozygosity and the number of alleles, was low (Table 1). Table 2 presents a comparison of this study with the results of previous works on West Indian manatees. The average expected heterozygosity was lower, although not significant, than that reported for manatees in Belize (Hunter et al., 2010), for all of Mexico (Nourisson, 2011), Florida (Pause Tucker et al., 2012), and Puerto Rico (Hunter et al., 2012). The expected heterozygosity in this study was similar to the number estimated by Nourisson et al. (2011) for the Gulf of Mexico, but lesser than what they estimated for the Mexican Caribbean, however, despite the fact that we used common loci, the total number of loci detected was different. In general, manatee heterozygosity had been lower than that from placental mammals (Garner, Rachlow, & Hicks, 2005) and from threatened mammals (DiBattista, 2008). The number of average alleles (N_A) observed was similar (Table 2), compared with studies on Florida and Antillean manatees from Belize and the Mexican Caribbean. In the Caribbean coasts, it increases the chance to account for more alleles shared with other Caribbean or Central America

Table 2. Comparison of the Total Allele Number, N_A, in Different Studies of the Genetic Diversity of West Indian Manatees Using Microsatellites.

Loci microsatellites	N_A average allele number (range)	Region	Author
28	2.27 ± 0.06 (2–4)	Gulf of Mexico	This article
16	3.1 (2–5)	Belize	Hunter et al. (2010)
13	2.62 ± 0.24	Gulf of Mexico	Nourisson et al. (2011)
	3.00 ± 0.32	Chetumal Bay	
	3.62 ± 0.48	Florida	
18	4.77 ± 0.51	Florida	Pause Tucker et al. (2012)
15	3.9 (2–6)	Puerto Rico	Hunter et al. (2012)

populations and even Florida. In the Gulf of Mexico, however, the average number of alleles was similar to that of the previous study (Nourisson et al., 2011). By specific loci, we have the same number of alleles at nine loci, and we have even more in some of the others. Furthermore, low diversity is also reported using mtDNA from manatees in Mexico (Castañeda & Morales-Vela, 2005; García-Rodríguez et al., 1998; Vianna et al., 2006).

Low genetic diversity in the Gulf of Mexico could be attributed to past bottleneck events even following historic hunting, habitat loss (Lefebvre et al., 2001), and low gene flow among populations (Nourisson et al., 2011). This could also be observed in the global values of F_{IS} which imply a low but significant level of inbreeding. The difference in H_E from OP and IL was important, approximately 0.1, considering that these are animals from the same geographic area and the time of isolation, which could be attributed to a founder effect, or it also could be derived by the process of isolation in the small population of IL. These isolated populations present a greater risk of loss of genetic diversity, as well as potential for extinction (Frankham, Bradshaw, & Brook, 2014). Therefore, the management of manatees in IL is critical and hence inbreeding is likely to be very different from the rest of the region.

Examining the samples from IL and the OP, we identified a bottleneck effect in both groups, suggesting that this effect in IL is derived from the founder individuals that were introduced from the OP population that was already under bottleneck effect as well. Nourisson et al. (2011) also detected recent bottleneck events in manatees from the Gulf of Mexico, and Hunter et al. (2012) discovered a possible bottleneck for manatees examined from Puerto Rico. Bottlenecks have been reported in other mammal populations, such as *Ursus arctos* (Xenikoudakis et al., 2015) and *Panthera onca* (Roques et al., 2016), attributed to recent anthropogenic influences upon natural distribution and isolation events. Contrastingly, González-Suárez, Aurioles-Gamboa, and Gerber (2010) found no evidence of a bottleneck in *Zalophus californianus* despite high hunting pressure from the past. And most notable is the case of the elephant seal where they recovered from a practically extinct population from Isla Guadalupe, Baja California, Mexico, to the current population of 225,000 animals, with little evidence of loss of fitness (Abadía-Cardoso, Freimer, Deiner, & Garza, 2017). However, they suggested that social structure, fitness, and population dynamics could have affected their findings. Although still under research, manatees in this region appear to be associated with specific river-lake systems adjacent to large rivers (Puc-Carrasco et al., 2017), where females have discovered localities that offered food and shelter to their calves and they continue to use those sites in a fashion of temporal movements in and out of the lake systems following flood pulses. This behavior is acting against large-scale dispersal of individuals and promoting regional bottlenecks that promote limited genetic flow.

The Grijalva and Usumacinta river basins are connected to each other nearly 12 km from the coast line forcing more interchange between individuals than they would have in other river basins along the coastal plain. In a healthy population, manatees need to travel along the high energy coast, with limited submerged vegetation because of plumes and siltation discharge of large rivers. We identified two genetic clusters in our samples (Figure 2) which indicate more structure in the region when compared with Nourisson et al. (2011).

The few samples we had from Veracruz and Campeche ($n = 5$) are from the red cluster, with ancestry levels in STRUCTURE higher than 0.9. This genetic cluster was also found in some samples from the most coastal sites of Tabasco, but high ancestry of this cluster is shown in individuals from Catazajá Lake in northern Chiapas and one individual from the upper Usumacinta River. In Las Ilusiones Lake, we found a mixed ancestry in the individuals, which suggest that the previous translocation of individuals occurred with individuals from several source populations.

There are just a few reports of sightings of manatees along the coast in the southern Gulf of Mexico and came from sparse opportunistic observations by local fishermen, park guards, or researchers working in other projects. A female manatee equipped with a satellite transmitter and tagged in 2015 at the confluence of Grijalva and Usumacinta river basins traveled about 50 km along the coast in a single night to other nearby freshwater systems (L. D. Olivera-Gómez, personal communication). In general, there are no geographical barriers to dispersal of animals within the region of southern Gulf of Mexico, but behavior could influence individuals to be more concentrated in particular areas within river basins (Puc-Carrasco et al., 2017). Hunter et al. (2010) identified two genetically distinct groups of manatees in Belize, where no visible barriers are present. In killer whales, from northeastern Pacific, genetic studies clearly differentiated between two ecotypes, transients and residents, which have been separated by diet and predatory behavior, even when they are currently sympatric (Hoelzel, Dahlheim, & Stern, 1998; Morin et al., 2010; Moura et al., 2015). In Florida, despite having evidences to winter separate between management units along the east and west coasts, genetic differentiation was weak (Pause Tucker et al., 2012).

Implications for Conservation

The origin of individuals within the IL is not clear; if they were occupying the lake when it was landlocked or if they were introduced from other sites, the original number of the population is unknown. The best population estimate in this lake today produced a range of 18 to 39 individuals (Pérez-Garduza, 2013). In IL, we identified slightly lower genetic diversity compared with OP, which could be explained by isolation. The amount of time of isolation in IL population is not known but estimated to be only three to four decades (Pablo-Rodríguez & Olivera-Gómez, 2012). In the Florida subspecies (*T. manatus latirostris*), the generation time is estimated in a range of 16 to 23 years (Haubold, Deutsch, & Fonnesbeck, 2006; Marmontel, O'Shea, Kochman, & Humphrey, 1996), where it would not likely be enough time to produce high levels of random genetic differentiation. However, as we include samples from animals from all ages, we have mixed generations in the study. Without alleles from new individuals, and if habitat quality decreased in this lake, genetic problems will be more evident in the future. Therefore, based on our findings, IL-specific management actions should be addressed to assure survival and fitness of this small isolated manatee population. The first step could be the decision of keeping this isolated population as it currently exists or translocate all the individuals to other areas. However, the younger individuals, with the red cluster genetic profile or with a higher number of alleles, could be introduced into the lake system. For OP, a travel corridor would help insure gene flow within river basins and among other basins to protect the species and promote increased genetic diversity. Access from the sea coast to large river basins could be restored with considerations to promote health genetic population management and involve local human communities in conservation actions.

Acknowledgments

The authors thank Dr Margaret Hunter of the USGS Wetland and Aquatic Research Center in Gainesville, FL, for providing us with manatee-specific primers used in this study. The authors also thank Dr Michelle Davis for her advice and help in the laboratory, as well as Dr Coralie Nourisson, for her guidance for applying for financial support from the SMM. Samples were collected under scientific collection permits SGPA/DGVS/d0/06; SGPA/DGVS–01103/07; SGPA/DGVS/00263/08; SGPA/DGVS/01754/09; SGPA/DGVS/04675/10; SGPA/DGVS/02901/11; SGPA/DGVS/03562/12; SGPA/DGVS/05846/13; SGPA/DGVS/11519/13; SGPA/DGVS/00808/14. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was funded by a grant from the Mexican Program for Faculty Improvement (Programa de Mejoramiento del Profesorado PROMEP). Project UJAT-CA-219. Financial support for training in Florida was provided by the Society for Marine Mammalogy (SMM) to first author. The first author wants to thank the Consejo Nacional para la Ciencia y la Tecnología (CONACYT; scholarship number: 399891) and to PISA (UJAT) for the financial support during her Master's degree studies.

ORCID iD

Julia María Lesher-Gordillo http://orcid.org/0000-0001-6973-2304

References

Abadía-Cardoso, A., Freimer, N. B., Deiner, K., & Garza, J. C. (2017). Molecular population genetics of the Northern Elephant Seal Mirounga angustirostris. *Journal of Heredity*, *108*(6), 618–627.

Aragón-Martínez, A., Olivera-Gómez, L. D., & Jimenez-Dominguez, D. (2014). Seasonal prevalence of antibodies to Leptospira interrogans in Antillean manatees from a landlocked lake in Tabasco, México. *Journal of Wildlife Diseases*, *50*(3), 505–511.

Barril, P., & Nates, S. (2012). Introduction to agarose and polyacrylamide gel electrophoresis matrices with respect to their detection sensitivities. S. Magdeldin (Ed.), *Gel electrophoresis principles and basic* (pp. 3–14). Croatia: InTech.

Bonde, R. K., McGuire, P. M., & Hunter, M. E. (2012). A review of the key genetic tools to assist imperiled species conservation: Analyzing West Indian manatee populations. *Journal of Marine Animals and Their Ecology*, *5*(1), 8–19.

Castañeda, A., & Morales-Vela, B. (2005). *Informe técnico sobre los análisis de DNA de las muestras de sangre de los manatíes de Quintana Roo, Tabasco y Campeche* [Technical report on the DNA analysis of the blood samples of the manatees of Quintana Roo, Tabasco and Campeche] (ECOSUR, Chetumal). Retrieved from http://www.conabio.gob.mx/institucion/proyectos/resultados/InfH164.pdf.pdf

Colmenero, L. C., & Hoz-Zavala, M. E. (1986). Distribución de los manatíes, situación y su conservación en México [Distribution of manatees, situation and their conservation in Mexico]. *Serie Zoología*, *56*, 955–1020.

Cornuet, J. M., & Luikart, G. (1996). Description and power analysis of two tests for inferring recent population bottlenecks from allele frequency data. *Genetics*, *144*, 2001–2014.

Diario Oficial de la Federación. (1922). *Diario Oficial de la Federación 19 de enero de 1922, Secretaría de Agricultura y Fomento: PREVENCIONES reglamentarias estableciendo veda absoluta para la pesca del "manatí", en aguas federales* [Official Gazette of the Federation January 19, 1922, Ministry of Agriculture and Development: REGULATORY PREVENTIONS establishing an absolute ban for the fishing of the "manatee" in federal waters] (p. 280). Ciudad de México, México.

DiBattista, J. D. (2008). Patterns of genetic variation in anthropogenically impacted populations. *Conservation Genetics*, *9*, 141–156.

Earl, D. A., & vonHoldt, B. M. (2011). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetic Resources*, *4*, 359–361.

Frankham, R., Ballou, J., & Briscoe, D. (2004). *Introduction to conservation genetics. Edn*. Cambridge, England: Cambridge University Press.

Frankham, R., Bradshaw, C. J. A., & Brook, B. W. (2014). Genetics in conservation management: Revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. *Biological Conservation*, *17*, 56–63.

Freeland, J. R. (2005). *Molecular ecology* (402 pp.). West Sussex, England: John Wiley & Sons Ltd.

García-Rodríguez, A. I., Bowen, B. W., Domning, D., Mignucci-Giannoni, A. A., Marmontel, M., Montoya-Ospina, R. A.,... McGuire, P. M. (1998). Phylogeography of the West Indian manatee (Trichechus manatus): How many populations and how many taxa? *Molecular Ecology*, *7*(9), 1137–1149.

García-Rodríguez, A. I., Moraga-Amador, D., Farmerie, W., McGuire, P., & King, T. L. (2000). Isolation and characterization of microsatellite DNA markers in the Florida manatee (*Trichechus manatus latirostris*) and their application in selected Sirenian species. *Molecular Ecology*, *9*, 2161–2163.

Garner, A., Rachlow, J. L., & Hicks, J. F. (2005). Patterns of genetic diversity and its loss in mammalian populations. *Conservation Biology*, *19*, 1215–1221.

Geffen, E., Luikart, G., & Waples, R. (2007). *Impacts of modern molecular genetic techniques on conservation biology*. Retrieved from https://digitalcommons.unl.edu/cgi/viewcontent.cgi?referer=https://scholar.google.com.mx/&httpsredir=1&article=1460&context=usdeptcommercepub

González-Suárez, M., Aurioles-Gamboa, D., & Gerber, L. R. (2010). Past exploitation of California sea lions did not lead to a genetic bottleneck in the Gulf of California. *Ciencias Marinas*, *36*(2), 199–211.

Haubold, E. M., Deutsch, C., & Fonnesbeck, C. (2006). Final biological status review of the Florida manatee (*Trichechus manatus latirostris*). Florida Fish and Wildlife Conservation Commission. *St. Petersburg, Florida*, *63*, 2–63.

Hoelzel, A. R., Dahlheim, M., & Stern, S. J. (1998). Low genetic variation among killer whales (*Orcinus orca*) in the eastern North Pacific and genetic differentiation between foraging specialists. *Journal of Heredity*, *89*(2), 121–128.

Hoelzer, G. A., Wallman, J., & Melnick, D. J. (1998). The effects of social structure, geographical structure, and population size on the evolution of mitochondrial DNA: II. Molecular clocks and the lineage sorting period. *Journal of Molecular Evolution*, *47*(1), 21–31.

Hunter, M. E., Auil-Gómez, N. E., Tucker, K. P., Bonde, R. K., Powell, J., & McGuire, P. M. (2010). Low genetic variation and evidence of limited dispersal in the regionally important Belize manatee. *Animal Conservation*, *13*, 592–602.

Hunter, M. E., Mignucci-Giannoni, A. A., Tucker, K. P., King, T. L., Bonde, R. K., Gray, B. A., & McGuire, P. M. (2012). Puerto Rico and Florida manatees represent genetically distinct groups. *Conservation Genetic*, *13*(6), 1623–1635.

Jiménez-Domínguez, D., & Olivera-Gómez, L. D. (2014). Características del hábitat del Manatí antillano (*Trichechus manatus manatus*) en sistemas fluviolagunares del sur del Golfo de México [Habitat characteristics of the Antillean manatee (*Trichechus manatus manatus*) in fluviolagunares systems of the southern Gulf of Mexico]. *THERYA*, *5*(2), 601–614.

Kalinowski, S. T., Taper, M. L., & Marshall, T. C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, *16*, 1099–1106.

Lefebvre, L. W., Marmontel, M., Reid, J. P., Rathbun, G. B., & Domning, D. P. (2001). *Distribution, status, and biogeography of the West Indian manatee*. In C. A. Woods & F. E. Sergile (Eds.), *Biogeography of the West Indies: Patterns and* *perspectives* (2nd ed., pp. 425–474). Boca Raton, FL: CRC Press.

Marmontel, M., O'Shea, T. J., Kochman, H. I., & Humphrey, S. R. (1996). Age determination in manatees using growth-layer-group counts in bone. *Marine Mammal Science*, *12*, 54–88.

Marsh, H., O'Shea, T. J., & Reynolds, J. E. III. (2011). *Ecology and conservation of the Sirenia: Dugongs and Manatees* (521 pp.). Cambridge, MA: Cambridge University Press.

Morin, P. A., Archer, F. I., Foote, A. D., Vilstrup, J., Allen, E. E., Wade, P., & Harkins, T. (2010). Complete mitochondrial genome phylogeographic analysis of killer whales (*Orcinus orca*) indicates multiple species. *Genome Research*, *20*(7), 908–916.

Moura, A. E., Kenny, J. G., Chaudhuri, R. R., Hughes, M. A., Reisinger, R. R., De Bruyn, P. J. N., & Hoelzel, A. R. (2015). Phylogenomics of the killer whale indicates ecotype divergence in sympatry. *Heredity*, *114*(1), 48–55.

Nourisson, C., Morales-Vela, B., Padilla-Saldivar, J., Pause-Tucker, K., Clark, A. M., Olivera-Gómez, L. D., … McGuire, P. (2011). Evidence of two genetic clusters of manatees with low genetic diversity in Mexico and implications for their conservation. *Genética*, *139*, 833–842.

Nourisson, C. (2011). *Estructura Genética de los manatíes en México* (Tesis de Doctorado, 124 pp.). El Colegio de Frontera Sur, Chetumal, Quintana Roo, México. Retrieved from https://ecosur.repositorioinstitucional.mx/jspui/bitstream/1017/660/1/0000507881_documento.pdf

Pablo-Rodríguez, N., & Olivera-Gómez, L. D. (2012). Situación de una población aislada de manatíes *Trichehus manatus* (Mammalia: Sirenia: Trichechidae) y conocimiento de la gente, en una Laguna Urbana, en Tabasco, México [Situation of an isolated population of manatees *Trichehus manatus* (Mammalia: Sirenia: Trichechidae) and knowledge of the people, in a Laguna Urbana, in Tabasco, Mexico]. *Universidad y Ciencia*, *28*(1), 15–26.

Pause, K. C., Nourisson, C., Clark, A., Kelloggs, M. E., Bonde, R. K., & McGuire, P. M. (2007). Polymorphic microsatellite DNA markers for the Florida manatee (*Trichechus manatus latirostris*). *Molecular Ecology Notes*, *7*, 1073–1076.

Pause Tucker, K., Hunter, M. E., Bonde, R. K., Austin, J. D., Clark, A. M., Beck, C. A., . . . Oli, M. K. (2012). Low genetic diversity and minimal population substructure in the endangered Florida manatee: Implications for conservation. *Journal of Mammalogy*, *93*(6), 1504–1511.

Peakall, R., & Smouse, P. E. (2006). GENALEX 6: Genetic analysis in excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, *6*, 288–295.

Pérez-Garduza, F. (2013). Evaluación de la eficiencia de cinco técnicas para determinar la abundancia de manatíes *Trichechus manatus* en ambientes poco visibles [Evaluation of the efficiency of five techniques to determine the abundance of manatees Trichechus manatus in inconspicuous environments] (MSc Thesis). Universidad Juárez Autónoma de Tabasco, México, 45 pp. Available with the author.

Piry, S., Luikart, G., & Cornuet, J. M. (1999). BOTTLENECK: A program for detecting recent effective population size reductions from allele data frequencies. *Journal of Heredity*, *90*, 502–503.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*(2), 945–959.

Porras Murillo, L. P., Bolaños Montero, J. R., & Barr, B. R. (2008). Variación genética y flujo de genes entre poblaciones de *Crocodylus acutus* (Crocodylia: Crocodylidae) en tres ríos del Pacífico Central Costa Rica. *Revista De Biología Tropical*, *56*(3), 1471–1480.

Puc-Carrasco, G., Olivera-Gómez, L. D., Arriaga-Hernandez, S., & Jimenez-Dominguez, D. (2016). Relative abundance of Antillean manatees in the Pantanos de Centla Biosphere Reserve in the coastal plain of Tabasco, Mexico. *Ciencias Marinas*, *42*(4), 261–270.

Puc-Carrasco, G., Morales-Vela, B., Olivera-Gómez, L. D., & González-Solis, D. (2017). First field-based estimate of Antillean manatee abundance in the San Pedro River system suggests large errors in current estimates for Mexico. *Ciencias Marinas*, *43*(4), 285–299.

Raymond, M., & Rousset, F. (1995). GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. *Journal of Heredity*, *86*, 248–249.

Roques, S., Sollman, R., Jácomo, A., Torres, N., Silveira, L., Chávez, C., . . . Palomares, F. (2016). Effects of habitat deterioration on the population genetics and conservation of the jaguar. *Conservation Genetics*, *17*, 125–139.

Rovirosa, J. N. (1885). Apuntes para la zoología de Tabasco. Vertebrados observados en el territorio de Macuspana [Notes for the zoology of Tabasco. Vertebrates observed in the territory of Macuspana]. *La Naturaleza*, *7*, 345–389.

Sánchez-Munguía, A. (2005). *Uso del suelo agropecuario y deforestación en Tabasco 1950-2000* (123 pp.). Villahermosa, Mexico: División Académica de Ciencias Biológicas. Universidad Juárez Autónoma de Tabasco.

Self-Sullivan, C., & Mignucci-Giannoni, A. (2008). *Trichechus manatus ssp. manatus. The IUCN Red List of Threatened Species 2008:*e.T22105A359161. Retrieved from http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T22105A9359161.en.

Secretaría de Medio Naturales Ambiente y Recursos. (2010). *Norma Oficial Mexicana 059, Protección ambiental Especies nativas de México de flora y fauna silvestres-Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-Lista de especies en riesgo* [Official Mexican Standard 059, Environmental protection Mexican native species of wild flora and fauna-Risk categories and specifications for inclusion, exclusion or change-List of species at risk]. Retrieved from http://www.profepa.gob.mx/innovaportal/file/435/1/NOM_059_SEMARNAT_2010.pdf

Tringali, M. D., Seyoum, S., Carney, S. L., Davis, M. C., Rodríguez-López, M. A., Reynolds, J. E., & Haubold, E. (2008). Eighteen new polymorphic microsatellite markers for the endangered Florida manatee, *Trichechus manatus latirostris*. *Molecular Ecology Resources*, *8*(2), 328–331.

United Nations Environment Programme. (2010). *Regional Management Plan for the West Indian Manatee (Trichechus manatus)* (United Nations Environment Program, CEP Technical Report 48). Kingston, Jamaica. Retrieved from https://www.fws.gov/caribbean/PDF/ManateeManagementPlan.pdf

Vianna, J. A., Bonde, R. K., Caballero, S., Giraldo, J. P., Lima, R. P., Clark, A., . . . Santos, F. R. (2006). Phylogeography, phylogeny and hybridization in trichechid sirenians: Implications for manatee conservation. *Molecular Ecology*, *15*(2), 433–447.

Xenikoudakis, G., Ersmark, E., Tison, J. L., Waits, L., Kindberg, J., Swenson, J. E., & Dalen, L. (2015). Consequences of a demographic bottleneck on genetic structure and variation in the Scandinavian brown bear. *Molecular Ecology*, *24*, 3441–3454.

Yazdian-Robati, R., Pourtaji, A., Rashedinia, M., Hosseinzadeh, H., Ghorbani, M., Razavi, B. M., & Abnous, K. (2015). Screening and identification of SUMP-proteins in sub-acute treatment with diazinon. *Iranian Journal of Basic Medical Sciences*, *18*(12), 1240–1244.