Aquatic Mammals 2017, *43*(4), 378-386, DOI 10.1578/AM.43.4.2017.378

Genetic Composition and Connectivity of the Antillean Manatee (*Trichechus manatus manatus*) in Panama

Edgardo Díaz-Ferguson,[1] Margaret Hunter,[2] and Héctor M. Guzmán[3]

[1]*Institute of Scientific Research and High Technology Services (INDICASAT-AIP), Building-219, City of Knowledge, Clayton, Panamá*
E-mail: diazedgardo7213@gmail.com
[2]*U.S. Geological Survey, Wetland and Aquatic Research Center, 7920 NW 71st Street, Gainesville, FL 32653, USA*
[3]*Smithsonian Tropical Research Institute, PO Box 0843-03092, Balboa, Panamá*

Abstract

Genetic diversity and haplotype composition of the West Indian manatee (*Trichechus manatus*) population from the San San Pond Sak (SSPS) wetland in Bocas del Toro, Panama, was studied using a segment of the mitochondrial DNA (control region). No genetic information to date has been published for manatee populations in Panama. Due to the secretive behavior and small population size of the species in the area, DNA extraction was conducted from opportunistically collected fecal (N = 20), carcass tissue (N = 4), and bone (N = 4) samples. However, after DNA processing, only 10 samples provided enough quality DNA for sequencing—three fecal, four tissue, and three bone samples. We identified three haplotypes in total: J01 was previously published (N = 3), while the other two haplotypes, J02 (N = 3) and J03 (N = 4), are reported for the first time. The genetic diversity was similar to previous analyses conducted in the Caribbean with moderate values of nucleotide (π = 0.00152) and haplotypic (Hd = 0.57) diversity. Connectivity assessment between the SSPS population with published range-wide manatee haplotypes was based on sequence similarity, genetic distance, and genetic differentiation. The identified J01 haplotype is also found in populations to the north along the Central American and Gulf of Mexico coasts indicating reduced differentiation (Fst = 0.0094). In contrast, comparisons between SSPS sequences and South American populations (not including Colombia), the West Indies, and Florida showed fewer similarities (Fst = 0.049 and 0.058, respectively). These results corroborate previous phylogeographic patterns already established for manatee populations and situate the manatee population in Panama within the Belize/Mexico cluster. In addition, these findings provide a baseline for comparative studies of manatees in other areas of Panama and Central America. These results can assist with management decisions regarding conservation of genetic diversity, future introductions, connectivity, and effective population size of manatee populations along the Central American corridor.

Key Words: *Trichechus manatus*, conservation genetics, Antillean manatee, West Indian manatee, Caribbean connectivity, Belize, San San Pond Sak, Panama, mitochondrial DNA, phylogeography

Introduction

Understanding genetic diversity is key to assessing effective population size, health, and fitness of natural populations. In addition, unraveling genetic composition allows us to infer connectivity patterns and origins of populations, which is essential for accurate management and conservation strategies (Frankham et al., 2002). The West Indian manatee (*Trichechus manatus*) inhabits the Caribbean Antilles, including Central and South America, as far south as Bahia in Brazil and Florida in the United States. *Trichechus manatus* is imperiled throughout its distribution and is categorized as vulnerable by the International Union for Conservation of Nature (Lefebvre et al., 2001; IUCN, 2007). In most of Central America, the size of manatee populations has been reduced considerably by overexploitation from the 17th to 19th centuries (Lefebvre et al., 2001). The current total size of Central American populations is estimated at less than 2,000 individuals (Quintana-Rizzo & Reynolds, 2010; Castelblanco-Martínez et al., 2012) and is expected to decline at a rate of at least 10% over the next 20 y (see Deutsch et al., 2008). Manatee populations are currently impacted by threats related to habitat degradation, coastal development, pollution, collisions with watercraft and net entanglement, and continued hunting (O'Shea et al., 1995; Hunter et al., 2010, 2012; Castelblanco-Martínez et al., 2012).

Regional phylogeographic studies for the West Indian manatee conducted by García-Rodríguez et al. (1998) and Vianna et al. (2006) resulted in three genetic clusters based on sequence divergence: (1) Florida and Central America, Antilles, and the Caribbean coast of South America; (2) Mexico, Central America, and the Caribbean coast of South America; and (3) the northeastern coast of South America (Brazil and Guyana). The genetic structure of manatee populations has been assessed in detail in Brazil, Colombia, Belize, Puerto Rico, Mexico, and Florida (Hunter et al., 2010, 2012; Nourisson et al., 2011; Luna et al., 2012; Satizabal et al., 2012; Tucker et al., 2012). However, in Central America, no manatee sequences have been published from Guatemala, Honduras, Nicaragua, Costa Rica, or Panama (Quintana-Rizzo & Reynolds, 2007, as cited in Hunter et al., 2010). It has been suggested that the Central American populations are supplemented by historical expansion and colonization events from the Belize and Mexico populations, which are considered the largest population centers for the region (Mou-Sue et al., 1990; Vianna et al., 2006).

In Panama, there is evidence of two resident population centers for manatees. A population was artificially introduced in Lake Gatun of the Panama Canal watershed (Mou-Sue et at., 1990; Lefebvre et al., 2001; Muschett, 2008; Muschett & Vianna, 2015). This population was established in 1964 with nine West Indian manatees and one Amazonian manatee (MacLaren, 1967). In 1980, it was estimated to have grown to 25 manatees (Schad et al., 1981). The second resident population inhabits ~120 km of coast and rivers in the Bocas del Toro Province and the indigenous territory, Comarca Ngabe-Bugle, on the eastern side of the country.

The manatee habitat on the east coast contains extensive coastal ecosystems dominated by coral reefs, seagrass beds, and mangroves and includes at least four major river systems that manatees utilize (Mou-Sue et al., 1990). Within the Bocas del Toro Providence, three of the rivers—(1) San San, (2) Changuinola, and (3) Cañaveral—are located inside two internationally protected wetland areas: (1) the San San Pond Sak (SSPS) and (2) the Damani-Guariviara wetlands. Based on replicate aerial surveys in the late 1980s, manatees were commonly observed in the three rivers (Mou-Sue et al., 1990). The population in the SSPS has been the only area evaluated in detail (Guzmán & Condit, in press).

In the entire Bocas del Toro riverine system, the manatee population is estimated at approximately 30 manatees (Guzmán & Condit, in press). However, little is known about this population, and more information on population size, sex ratio, number of effective breeders, genetic composition, genetic diversity, and historical connectivity patterns is needed for addressing conservation measures (Guzmán & Condit, in press). The main objectives of this study were to characterize the mitochondrial DNA (mtDNA) diversity and genetic composition of the manatee population from the SSPS protected area of Panama and to understand the historical connectivity with respect to other Caribbean regions and other previously studied populations.

Methods

Study Area

The SSPS was designated as a Ramsar site (a wetland of international importance protected by an international treaty for conservation and sustainable use) in 1993 and is located in the District of Changuinola in Bocas del Toro Province (9° 31.679' N – 82° 30.889' W) along the northwestern Caribbean coast of Panama. The wetland encompasses an area of 164.14 km^2 flooded by three main rivers: (1) Negro, (2) San San, and (3) Changuinola. This study evaluated manatees only in the San San riverine system (12.8 km) and its tributary, the Rio Negro (5.7 km), covering *ca*. 2.9 km^2 of riverbed (Figure 1). The Changuinola River is larger and formed by several interconnected artificial channels providing suitable habitat with abundant and diverse food for manatees. However, this river is not connected to the San San River, and their mouths are separated by *ca*. 10 km. A detailed description of the study area can be found in Guzmán & Condit (in press).

Sampling and DNA Extraction from Tissues, Bones, and Feces

DNA extraction was conducted using fecal (N = 20), carcass tissue (N = 4), and bone (N = 4) samples collected from manatees. Tissue and bone samples were collected from dead individuals found floating in the San San River or buried at unknown dates and sampling events between 2010-2012 and 2016. No necropsy examination was conducted on the carcasses. Tissue samples collected from the carcasses were processed by local people without recording additional data (e.g., size or sex). In addition, 20 fresh fecal samples were collected from a feeding habitat in the Rio Negro in 2013 on the surface along the river margins and close to vegetation following the collection protocol detailed by Muschett et al. (2009). Fecal samples were collected during different sampling events conducted over several weeks. All samples were collected using sterile latex gloves and stored in vials containing 95% ethanol.

Tissue samples were extracted using a DNeasy kit (QIAGEN, INC., Valencia, California, USA). DNA was extracted from bones using the porous regions of vertebrae and ribs (Burdin et al., 2012). Approximately 50 to 100 mg of bone debris and cells were collected using a forensic drill. These cells were lysed overnight. Clear lysate was transferred to a new sample tube and processed using a PrepFiler® Express BTA Forensic DNA extracted kit (Applied Biosystems, Inc., Pembroke Pines, Florida, USA) through an Automate Express DNA Extraction System (Applied Biosystems by Life Technologies, Carlsbad, California, USA). Samples were suspended in 50 µl of elution butter.

Fecal samples were centrifuged, and the ethanol preservative was decanted. The fecal pellet containing manatee cells was suspended using 1 mL of PCR distilled water. This volume of water containing fecal matter was filtered using a 25-µm mesh size filter. Genetic material was extracted from the filter using a water DNA extraction kit (Power Water, MO BIO, Carlsbad, California, USA). DNA concentration and quality of the nucleic acids for all samples were assessed using a Nanodrop spectrophotometer.

PCR and Sequencing

DNA extracted from tissue and feces was concentrated and cleaned using an ethanol precipitation protocol. DNA samples were amplified using control region primers CR5 (5'-TACCCATCAACACCCAAAGC-3') and CR4 (5'-AGATGTCTTATTTAAGAGGAA-3'), designed by García-Rodríguez et al. (1998). The PCR reactions were adjusted to laboratory procedures described by Hunter et al. (2012) using an annealing temperature of 55° C. Positive amplifications of PCR products were confirmed using a 1.5% agarose gel and cleaned using a PCR Clean Up Kit (QIAGEN Inc., Carlsbad, California, USA). Cleaned products were submitted in a BigDye 3.1 cycle sequence reaction and then cleaned using

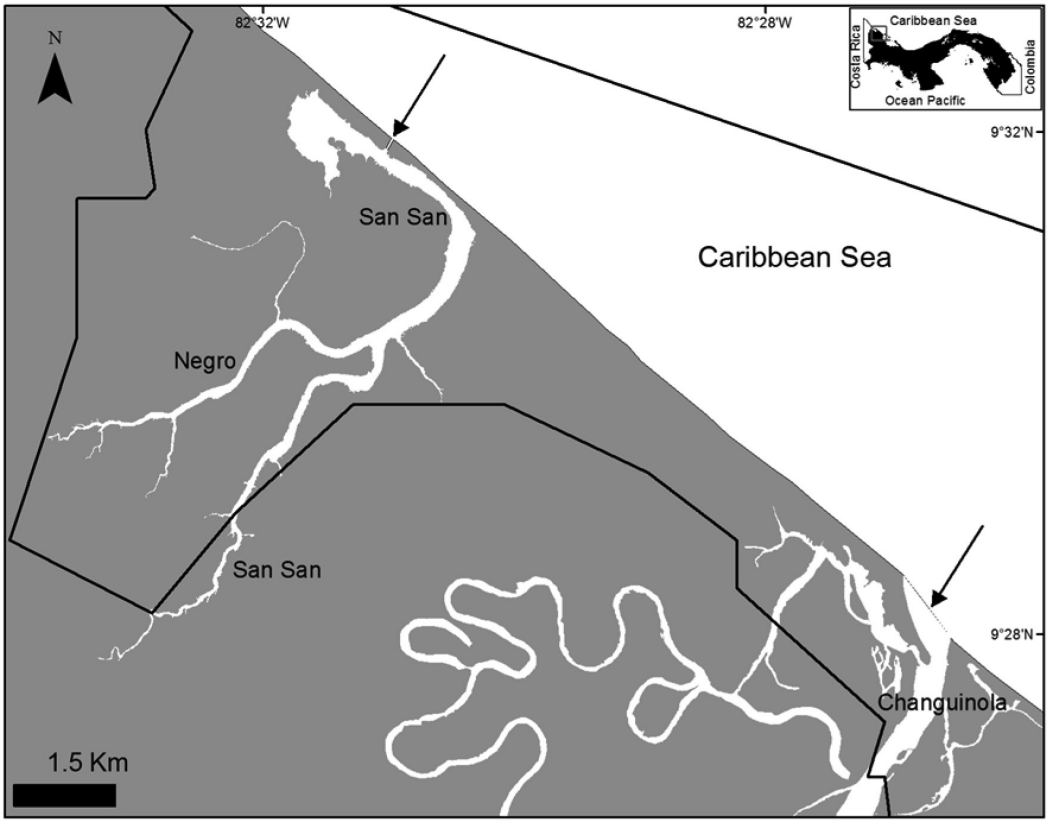

Figure 1. Map of Panama's San San Pond Sak (SSPS) wetland where samples were collected. The black line represents the boundary of the protected area. The arrows indicate the mouths of the rivers. Tissues from dead manatees were collected in San San River and San San Lagoon (top arrow), while feces were only collected in the Rio Negro area.

the "BigDye XTerminator" (Applied Biosystems, Inc.). Once cleaned, all sequences were run using an electronic ABI 3130 sequencer. The quality of each sequence was verified using the software *Sequencing Analysis*, Version 5.2 (Applied Biosystems, Inc.). Sequences were exported as Fasta files to *Geneious*, Version 10.0.7 (Kearse et al., 2012) where they were aligned, trimmed, and edited. Sequences were then deposited in the GenBank database where accessions numbers were assigned.

Genetic Composition, Genetic Diversity, and Connectivity

Genetic diversity was measured as the total number of haplotypes (H), nucleotide diversity (π), and haplotype diversity (Hd) of the analyzed sequences using *DNAsp*, Version 5.1 (Librado & Rozas, 2009). Assessment of historical connectivity was conducted by comparing the Panama sequences with those deposited in GenBank from other Caribbean regions (García-Rodríguez et al., 1998; Vianna et al., 2006; Hunter et al., 2010, 2012; Luna et al., 2012; Satizabal et al., 2012). Genetic connectivity was indirectly assessed with genetic differentiation (pairwise Fst) and genetic distance (Da) values from the three identified clusters (using 15 sequences total; Vianna et al., 2006). Also, a pairwise Fst test comparing SSPS sequences with sequences from different Caribbean regions was calculated (permutation test using 1,000 replicates). Estimates of sequence divergence were determined using the HKY two-parameter genetic distance model, and a UPGMA tree was generated to show differences and similarities between samples from Panama with other regions of the Caribbean using *Geneious*, Version 10.0.7. Genetic distances between haplotypes were then calculated using the Kimura 2-parameter algorithm using *DNAsp*, Version 5.1.

Results

DNA Extraction and Sequencing Among Different Manatee Tissues

DNA was extracted from three different sources—(1) fecal matter, (2) soft tissue, and (3) bones—with concentrations varying among samples (7 to 20 ng/µl). DNA extracted from tissues had a low initial concentration between 7.8 to 12.1 ng/µl but improved to 14.1 to 22.1 ng/µl (OD ratios > 1.5) after ethanol precipitation. All tissue samples led to high-quality DNA and sequences. Alternatively, seven out of 20 fecal samples yielded DNA with reduced DNA concentrations between 5 and

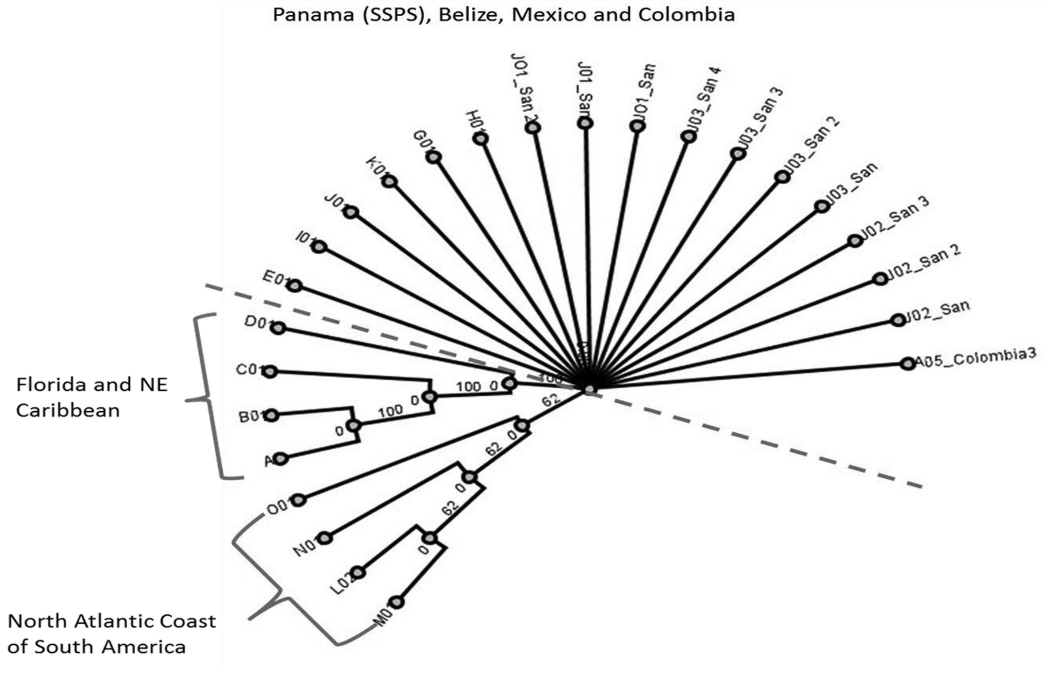

Figure 2. Genetic distance tree based on select mitochondrial DNA control region sequences (HKY-UPGMA 100 replicates bootstrap) from West Indian manatees (*Trichechus manatus manatus*). Previously reported haplotypes for the Caribbean basin are included in the analysis (Vianna et al., 2006). Panama's SSPS wetland samples are closer in distance to the Central American and Gulf of Mexico coasts.

10 ng/µl, even after ethanol precipitation (see Muschett et al., 2009). High-quality sequences were obtained from only three of those samples. Extracted bone samples (N = 4) also resulted in low DNA concentrations (2 to 5 ng/µl). The three sequences obtained from bone had good quality scores despite the initial low DNA concentration.

Genetic Composition, Genetic Diversity, and Historical Connectivity

From the ten samples that were successfully amplified, three haplotypes (J01, J02, and J03) were found. Genetic distances among haplotypes can be visualized in Figure 2. Genetic diversity was assessed by calculation of nucleotide (π = 0.00152) and haplotype (Hd = 0.57) diversities. Sequences from Panama had one new *Trichechus* polymorphic site leading to two new haplotypes: J02 and J03. These new sequences were submitted to the GenBank database with accession numbers (KR218318 to KR218324) (Table 1). All sequences obtained from bones corresponded to the J01 haplotype, which was previously reported in other Caribbean areas (i.e., Mexico and Belize) and Colombia (Table 1). Thus, when the Panama sequences were compared to the three West Indian manatee clusters, the reduced differentiation and a smaller genetic distance was apparent between these samples and the Central America group (Cluster II), thereby linking the populations (Table 2).

Discussion

For the first time, manatee populations in Panama have been described in terms of their haplotype composition, thereby providing the first sequences from Panama into GenBank and establishing linkages with other manatee populations within the

Table 1. DNA concentration and summary of sequence information of West Indian manatee (*Trichechus manatus manatus*) samples from the San San Pond Sak (SSPS) area of Panama

Sample #	Sample source	[DNA ng/µl]	GenBank accession #	Haplotype code	Geographic location
TM1PTYSS	Tissue	7.8	KR218318	J02	San San River
TM2PTYSS	Tissue	9.0	KR218319	J02	San San River
TM3PTYSS	Tissue	10.5	KR218320	J02	San San River
TM4PTYSS	Tissue	12.1	KR218321	J03	Rio Negro
Fecal1PTYSS	Feces	5.0	KR218322	J03	Rio Negro
Fecal2PTYSS	Feces	7.0	KR218323	J03	Rio Negro
Fecal 3PTYSS	Feces	6.5	KR218324	J03	Rio Negro
Bone1PTYSS	Bone	2.0	MF346774	J01	Rio Negro
Bone2PTYSS	Bone	2.5	MF346775	J01	Rio Negro
Bone3PTYSS	Bone	5.0	MF346776	J01	Rio Negro

Table 2. Indirect assessment of genetic differentiation based on Fst-pairwise permutation test (1,000 replicates, upper matrix values) and genetic distances (Da, lower matrix values) between Panama's San San Pond Sak samples and the Caribbean lineages established by García et al. (1998) and Vianna et al. (2006); West Indies and Florida (Cluster I–Haplotypes A, B, C & D), Central America & Gulf of Mexico (Cluster II–Haplotypes H, I, J & K), and Atlantic Coast of South America (Cluster III–Haplotypes L, M, N & O). Bold values indicate significance at $p \leq 0.005$.

Caribbean region/site	San San Pond Sak, Panama	West Indies & Florida	Central America & Gulf of Mexico	Atlantic coast of South America
San San Pond Sak, Panama	--	0.058	**0.0094**	0.049
West Indies & Florida	0.92	--	0.049	0.038
Central America & Gulf of Mexico	**0.74**	0.88	--	0.040
Atlantic coast of South America	0.92	0.84	0.87	0.000

Caribbean region. This connectivity and genetic population health information may help contribute to manatee conservation in the future. In Panama, a previous mtDNA genetic analysis was conducted using fecal samples (Muschett et al., 2009) and by utilizing a different set of primers (Kocher et al., 1989); however, no haplotypes were assigned to these sequences or reported to GenBank. In addition, parameters such as genetic diversity, connectivity, and differentiation were not presented.

Most methods used to collect tissue for genetic analysis in marine mammals involve invasive procedures (e.g., blood draw and muscular tissue biopsy) or bones from carcasses. Optimization of a noninvasive method for recovering genetic material from fecal matter suspended in water (Taberlet & Luikart, 1999; Taberlet et al., 1999) is useful in cryptic or elusive species with reduced population sizes such as manatees and dugongs (*Dugong dugon*) (Tikel et al., 1996; Muschett et al., 2009; Bonde et al., 2012).

Methodologically, our study contributes an alternative method for DNA extraction from fecal material (Muschett et al., 2009) using a Power Water kit (MO BIO Laboratories, Inc. [now owned by Qiagen]) traditionally used for environmental DNA (eDNA) extraction. However, samples collected in ecological studies are subjected to harsh environmental conditions, and DNA yield from fecal samples is reduced despite improved isolation methods. The ability to build reference databases based on opportunistic samples is limited by poor quality sequences and unknown numbers of unique individuals (Scott-Mills et al., 2000). Indeed, we were only able to produce reliable sequences in three out of 20 processed fecal samples (15% success rate from fecal samples). In general, manatee samples showed a low concentration of DNA (Table 1); however, bone samples had the lowest concentration among all of the samples, and muscle tissue had the highest concentration (Table 1).

Genetic Composition, Genetic Diversity, and Historical Connectivity

The two new Antillean manatee haplotypes, J02 and J03, identified from Panama are closely related to others previously reported in GenBank from manatees in Belize and Mexico (J01), and in Colombia (E01, G01, H01, and I01). Although few samples were analyzed, this genetic information links the SSPS populations with Central America and the Gulf of Mexico (i.e., Belize and Mexico), and to a lesser degree with its South American neighbor, Colombia (see Gilpin & Soule, 1986; Vianna et al., 2006; Satizabal et al., 2012). In fact, this genetic connectivity may be the product of historical colonization events originating from South American populations and/or recent migrations from the Central American populations—for example, Belize. However, the existence of unique haplotypes (J02 and J03) in a limited number of SSPS samples may suggest restricted contemporary migration and a significant haplotype frequency shift among locations. Restricted gene flow was also identified by García-Rodríguez et al. (1998). Comparisons using nuclear data analyses with additional samples from within and outside the country are needed to address the current connectivity and to better define population boundaries.

The phylogenetic separation between Panama and Colombia may be due to the gap in suitable habitat along the southeast coast of Panama, which has more open water, high wave action, and unsuitable coastal habitats for manatees, resulting in a barrier to gene flow and colonization events (García-Rodríguez et al., 1998). This separation leading to isolation has also been observed in other marine Caribbean taxa (Shulman & Bermingham, 1995; Díaz-Ferguson et al., 2010, 2011)

The genetic divergence between the Central American and Caribbean populations (i.e., Puerto Rico, Florida, and Dominican Republic) supports previous evidence of limited connectivity (Hunter et al., 2010, 2012; Castelblanco-Martínez et al., 2012) and reduced migration of females described by Hunter et al. (2012) and Satizabal et al. (2012). Dispersal patterns in manatees may be reduced by philopatric female behavior, small or isolated populations, weather, and dependence on freshwater sources. Nonetheless, some manatees have been reported to travel long distances (e.g., from Florida to Cuba; Alvarez-Alemán et al., 2010). The immigration of genetically distinct individuals can substantially reduce the effects of inbreeding and could also benefit small and isolated manatee populations (Frankham et al., 2002).

Mitochondrial genetic diversity has been studied in other Caribbean and Central American manatee populations and resulted in π and Hd values ranging from 0.000 to 0.044 and 0.00 to 1.00, respectively (García-Rodríguez et al., 1998; Vianna et al., 2006). Our study produced nucleotide and haplotype diversity values within that range ($\pi = 0.00152$ and Hd = 0.570). These values are similar to other Caribbean populations with approximately the same sample size (*N*) and total haplotype number (HT) (i.e., Dominican Republic, $N = 6$, HT = 2, Hd = 0.53, and $\pi = 0.001$; Mexico, $N = 14$, HT = 3, Hd = 0.61, and $\pi = 0.040$) (García-Rodríguez et al., 1998, 2000; Vianna et al., 2006). Thus, the reduced diversity observed in this study seems to be in agreement with previous studies and coherent with the reduced population size of the SSPS population. In general, obtained values of genetic diversity are

lower in manatees than in many other endangered or bottleneck mammal populations (Gebremedhin et al., 2009; Bushell, 2013). We did identify private haplotypes in the San San River and the Rio Negro; however, this is likely due to sampling artifacts. These two rivers are connected, and manatees most likely travel between them. Additional samples and individual identification using microsatellite markers may begin to reveal distinct maternal lineages and/or individual site fidelity that could contribute to this phenomenon.

Historical and modeling data suggest that the SSPS population in Panama experienced past exploitation, and the current population estimate suggests limited recovery (*ca*. 30 animals) (Guzmán & Condit, in press). Values of genetic diversity found in this study corroborated the existence of a small population size with a possible historical connection with the Central American populations (e.g., Belize and Mexico) to the north. This low diversity can negatively affect fitness, decrease population viability, and may increase susceptibility to disease (Frankham et al., 2002; Hunter et al., 2012).

Final Considerations and Conservation Implications

Results from this research could aid managers and local scientists in decisions regarding manatee conservation of genetic diversity, management of effective population size (i.e., introduction of animals from other areas), and re-establishment of regional connectivity patterns and migration corridors. Finally, these results can serve as a baseline for assessing and monitoring the genetic health of Panamanian manatee populations and for future comparison with other areas in Panama (i.e., Lake Gatun in the Panama Canal water basin) and other Central American populations that have not yet been examined (i.e., Guatemala, Honduras, Nicaragua, and Costa Rica).

Acknowledgments

We thank Mr. Sixto Herrera for helping during the collection of fecal samples and Mario Rivera for general assistance in the field. We also thank all the personnel from AAMVECONA for facilitating our work in the area and providing field support. The project was partially sponsored by the Inter-American Developing Bank and the Smithsonian Tropical Research Institute. This research was conducted under permits issued by the Environmental Authority of Panama (ANAM) for the collecting of tissue and fecal samples, and provisions stipulated by the Smithsonian Animal Care and Use Committee. We also want to thank Diomedes Trejos and Any Rojas from the Legal Medicine Laboratory of the Republic of Panama for their help while conducting the DNA extraction protocol from bone samples. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Literature Cited

Alvarez-Alemán, A., Beck, C., & Powell, J. (2010). First report of a Florida manatee (*Trichechus manatus latirostris*) in Cuba. *Aquatic Mammals*, *36*(2), 148-153. https://doi.org/10.1578/AM.36.2.2010.148

Bonde, R. K., McGuire, P. M., & Hunter, M. E. (2012). Genetic tools to assist imperiled species conservation: Analyzing the West Indian manatee population. *Journal of Marine Animals and their Ecology*, *5*, 8-19.

Burdin, A., Potgieter, B., & O'Corry-Crowe, G. (2012). *Climate change: A view through the prism of Steller's sea cow extinction*. Boca Raton: Florida Atlantic University. 17 pp.

Bushell, J. (2013). *The genetic diversity and population structure of dugongs (*Dugong dugon*) of Thailand* (Master of Science thesis). San Jose State University, San Jose, CA. 50 pp.

Castelblanco-Martínez, D., Nourisson, C., Quintana-Rizzo, E., Padilla-Saldivar, J., & Schmitter-Soto, J. (2012). Potential effects of human pressure and habitat fragmentation on population viability of the Antillean manatee, *Trichechus manatus manatus*: A predictive model. *Endangered Species Research*, *18*, 129-145. https://doi.org/10.3354/esr00439

Deutsch, C. J., Self-Sullivan, C., & Mignucci-Giannoni, A. A. (2008). *Trichechus manatus*. In International Union for Conservation of Nature (Ed.), *The IUCN red list of threatened species, Version 2014.2*. Retrieved from wwwiucnredlist.org

Díaz-Ferguson, E., Haney, R., Wares, J., & Silliman, B. (2010). Population genetics of a trochid gastropod broadens picture of Caribbean Sea connectivity. *PLOS ONE*, *5*(9), e12675.

Díaz-Ferguson, E., Haney, R., Wares, J., & Silliman, B. (2011). Genetic structure and connectivity patterns of two Caribbean rocky intertidal gastropods. *Journal of Molluscan Studies*, *78*, 112-118. https://doi.org/10.1093/mollus/eyr050

Frankham, R., Ballou, J. R., & Briscoe, D. A. (2002). *Introduction to conservation genetics*. Cambridge, UK: Cambridge University Press. https://doi.org/10.1017/cbo9780511808999

García-Rodríguez, A. I., Moraga-Amador, D., Farmerie, W., McGuire, P. M., & 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. https://doi.org/10.1046/j.1365-294X.2000.10534.x

García-Rodríguez, A. I., Bowen, B. W., Domning, D. P., 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*, 1137-1149. https://doi.org/10.1046/j.1365-294x.1998.00430.x

Gebremedhin, B., Ficetola, G. F., Naderi, S., Rezaei, H. R., Maudet, C., Rioux, D., . . . Taberlet, P. (2009). Combining genetic and ecological data to assess the conservation status of the endangered Ethiopian walia ibex. *Animal Conservation*, *12*, 89-100. https://doi.org/10.1111/j.1469-1795.2009.00238.x

Gilpin, M. E., & Soule, M. E. (1986). Minimum viable populations: Processes of species extinction. In M. E. Soule (Ed.), *Conservation biology: The science of scarcity and diversity* (pp. 19-34). Sunderland, MA: Sinauer Associates.

Guzmán, H. M., & Condit, R. (In press). Abundance of manatees (*Trichechus manatus*) in a Panama wetland estimated from side-scan sonar. *Wildlife Society Bulletin*.

Hunter, M. E., Auil-Gomez, 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. https://doi.org/10.1111/j.1469-1795.2010.00383.x

Hunter, M. E., Mignucci-Giannoni, A. A., Pause Tucker, K., King, T. L., Bonde, R. K., Gray, B., & McGuire, P. M. (2012). Puerto Rico and Florida manatees represent genetically distinct groups. *Conservation Genetics*, *13*, 1623-1635. https://doi.org/10.1007/s10592-012-0414-2

International Union for Conservation of Nature (IUCN). (2007). *The IUCN red list of threatened species*. Retrieved from www.iucnredlist.org

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., . . . Drummond, A. (2012). *Geneious Basic*: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, *28*, 1647-1649. https://doi.org/10.1093/bioinformatics/bts199

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., Villablanca, F. X., & Wilson, A. C. (1989). Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences*, *86*, 6196-6200.

Lefebvre, L. W., Marmontel, M., Reid, J. P., Rathbun, G. B., & Domning, D. P. (2001). Status and biogeography of the West Indian manatee. In C. Woods & F. Sergile (Eds.), *Biogeography of the West Indies: Patterns and perspectives* (2nd ed., pp. 425-474). Boca Raton, FL: CRC Press. https://doi.org/10.1201/9781420039481.ch22

Librado, P., & Rozas, J. (2009). *DnaSP* v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, *25*, 1451-1452. https://doi.org/10.1093/bioinformatics/btp187

Luna, F., Bonde, R. K., Attademo, F., Saunders, J., Meigs Friend, G., Passavante, J., & Hunter, M. (2012). Phylogeographic implications for release of critically endangered manatee calves rescue in northeast Brazil. *Aquatic Conservation*, *22*, 665-672. https://doi.org/10.1002/aqc.2260

MacLaren, J. P. (1967). Manatees as a naturalistic biological mosquito control method. *Mosquito News*, *27*, 387-393.

Mou-Sue, L., Chen, D., Bonde, R. K., & O'Shea, T. J. (1990). Distribution and status of manatees in Panama. *Marine Mammal Science*, *6*, 234-241. https://doi.org/10.1111/j.1748-7692.1990.tb00247.x

Muschett, G. (2008). *Distribución y estudios genéticos del manatí (*Trichechus manatus*) en la Cuenca hidrográfica del Canal de Panamá* [Distribution and genetic studies of the manatee (*Trichechus manatus*) in the drainage basin of the Panama Canal] (Master of Science thesis). Pontificia Universidad Católica de Chile, Santiago, Chile.

Muschett, G., & Vianna, J. A. (2015). Distribution and abundance of the West Indian manatee (*Trichechus manatus*) in the Panama Canal. *bio$R_x$iv*. https://doi.org/10.1101/026724

Muschett, G., Bonacic, C., & Vianna, J. (2009). A non-invasive sampling method for genetic analysis of the West Indian manatee (*Trichechus manatus*). *Marine Mammal Science*, *25*, 955-963. https://doi.org/10.1111/j.1748-7692.2009.00310.x

Nourisson, C., Morales-Vela, B., Padilla-Saldivar, J., Tucker, K. P., Clark, A., Olivera-Gomez, L., . . . McGuire, P. M. (2011). Evidence of two genetic clusters of manatees with low genetic diversity in Mexico and implications for their conservation. *Genetica*, *139*, 833-842. https://doi.org/10.1007/s10709-011-9583

O'Shea, T. J., Ackerman, B. B., & Percival, H. F. (1995). *Population biology of the Florida manatee* (National Biological Service Information and Technical Report 1). Washington, DC: U.S. Department of the Interior.

Quintana-Rizzo, E., & Reynolds III, J. E. (2010). Regional management plan for the West Indian manatee (*Trichechus manatus*) (CEP Technical Report 48). Kingston, Jamaica: Caribbean Environment Programme, United Nations Environment Programme.

Satizabal, P., Mignucci-Giannoni, A. A., Duchene, S., Caicedo-Herrera, D., Perea-Sicchar, C., Garcia-Davila, C., . . . Caballero, S. (2012). Phylogeography and sex biased dispersal across riverine manatee populations (*Trichechus inunguis* and *Trichechus manatus*) in South America. *PLOS ONE*, *7*, e52468. https://doi.org/10.1371/journal.pone.0052468

Schad, R. C., Montgomery, G., & Chancellor, D. (1981). La distribución y frecuencia del manatí en el lago Gatún y en el Canal de Panamá [The distribution and frequency of the manatee in Gatun Lake and the Panama Canal]. *ConCiencia*, *8*, 1-4.

Scott-Mills, L., Ciatta, J., Lair., K., Schwartz, M. K., & Tallmon, D. A. (2000). Estimating animal abundance

using non-invasive DNA sampling: Promise and pitfalls. *Ecological Applications*, *10*, 283-294. https://doi.org/10.1890/1051-0761(2000)010[0283:EAAUND]2.0.CO;2

Shulman, M. J., & Bermingham, E. (1995). Early life histories, ocean currents and the population genetics of Caribbean reef fishes. *Evolution*, *49*, 897-910. https://doi.org/10.2307/2410412

Taberlet, P., & Luikart, G. (1999). Non-invasive genetic sampling and individual identification. *Biological Journal of the Linnean Society*, *68*, 41-55. https://doi.org/10.1111/j.1095-8312.1999.tb01157.x

Taberlet, P., Waits, L., & Luikart, G. (1999). Noninvasive genetic sampling: Look before you leap. *Trends in Ecology Evolution*, *14*, 323-327. https://doi.org/10.1016/S0169-5347(99)01637-7

Tikel, D., Blair, D., & Marsh, H. D. (1996). Marine mammal faeces as a source of DNA. *Molecular Ecology*, *5*, 456-457. https://doi.org/10.1111/j.1365-294X.1996.tb00337.x

Tucker, K. P., Hunter, M. E., Bonde, R. K., Austin, J. D., Clark, A. M., Beck, C. A., . . . Oli, M. K. (2012). Genetic diversity and minimal population substructure in the endangered Florida manatee: Implications for conservation. *Journal of Mammalogy*, *93*, 1504-1511. https://doi.org/10.1644/12-MAMM-A-048.1

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*, 433-447. https://doi.org/10.1111/j.1365-294X.2005.02771.x