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More pieces for the puzzle: novel information on the genetic diversity and population structure of *Steno bredanensis* (Artiodactyla: Delphinidae) in Central America and the Caribbean Sea

Dalia C. Barragán-Barrera*[1, 2, 3]; https://orcid.org/0000-0003-4023-9908
Camilo A. Correa-Cárdenas[3, 4]; https://orcid.org/0000-0001-5009-6213
María Alejandra Duarte-Fajardo[3, 5]; https://orcid.org/0000-0002-6494-6941
Lissette Trejos Lasso[6, 7]; https://orcid.org/0000-0002-2495-0452
Betzi Pérez-Ortega[6, 8, 9]; https://orcid.org/0000-0001-5414-6329
Shakira G. Quiñones-Lebrón[6]; https://orcid.org/0000-0003-1822-4443
Antonio A. Mignucci-Giannoni[10, 11]; https://orcid.org/0000-0003-1443-4873
José Julio Casas[7, 12, 13]; https://orcid.org/0000-0001-9951-0542
Roberto Santamaria Valverde[12]; https://orcid.org/0000-0001-7371-8273
Nohelia Farías-Curtidor[14]; https://orcid.org/0000-0002-2617-8988
Susana Caballero[3]; https://orcid.org/0000-0002-9285-3873

1. Instituto Javeriano del Agua, Pontificia Universidad Javeriana, Carrera 7ª No. 40-62, Bogotá, Colombia; daliac.barraganbarrera@gmail.com (*Correspondence)
2. R&E Ocean Community Conservation Foundation, Oakville, Canada; daliac.barraganbarrera@gmail.com
3. Laboratorio de Ecología Molecular de Vertebrados Acuáticos-LEMVA, Departamento de Ciencias Biológicas, Universidad de los Andes, Carrera 1 No. 18A-10, Bogotá, Colombia; daliac.barraganbarrera@gmail.com, camilocc510@gmail.com, aduarte108@gmail.com, sj.caballero26@uniandes.edu.co
4. Grupo de Investigación en Enfermedades Tropicales del Ejército (GINETEJ), Laboratorio de Referencia e Investigación, Dirección de Sanidad, Ejército Nacional de Colombia, Bogotá, Colombia; camilocc510@gmail.com
5. Fundación Malpelo y otros Ecosistemas Marinos, Bogotá, Colombia; aduarte108@gmail.com
6. Fundación Panacetacea Panamá, Ciudad de Panamá, Panamá; ltrejos@miambiente.gob.pa, betziperez@yahoo.com, shakiguani@gmail.com
7. Ministerio de Ambiente, Avenida Ascanio Villalaz, edificio 500, Ancón, Panamá; ltrejos@miambiente.gob.pa, jcasas@miambiente.gob.pa
8. Biology Department and Redpath Museum – McGill University, Montreal, Canada; betziperez@yahoo.com
9. Instituto Smithsonian de Investigaciones Tropicales. Ciudad de Panamá, Panamá; betziperez@yahoo.com
10. Centro de Conservación de Manatíes del Caribe, Universidad Interamericana de Puerto Rico, 500 Carr. Dr. John Will Harris, Bayamón, Puerto Rico 00957; mignucci@manatipr.org
11. Center for Conservation Medicine and Ecosystem Health, Ross University School of Veterinary Medicine, PO Box 334 Basseterre, St. Kitts West Indies; mignucci@manatipr.org
12. Facultad de Ciencias del Mar, Universidad Marítima Internacional de Panamá – UMIP, Ciudad de Panamá, Panamá; jcasas@miambiente.gob.pa, santamariaroberto43@gmail.com
13. Estación Científica Coiba AIP, Ciudad de Panamá, Panamá; jcasas@miambiente.gob.pa
14. Fundación Macuáticos Colombia, Calle 27 Nº 79-167, Medellín, Colombia; nohefa@gmail.com

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ABSTRACT

Introduction: The rough-toothed dolphin (*Steno bredanensis*) inhabits oceanic waters of tropical latitudes and exhibits philopatry in some oceanic islands. However, the species has been observed in shallow coastal waters in a few areas. Particularly in Central America, the rough-toothed dolphin has been reported by occasional records and strandings. For instance, the first confirmed record of this species in the Panama's Caribbean was on July 17, 2012, in a coastal region of the Chiriquí Lagoon during a bottlenose dolphin monitoring survey. Similarly, the first rough-toothed dolphin mass stranding reported for the Pacific of Panama was on April 20, 2016, at the Ostional Beach, where 60 dolphins stranded and ten died. These sightings and events offered a valuable opportunity to obtain samples to conduct genetic studies, which are scarce in the region.

Objective: In this study, we present the first assessment of genetic diversity for rough-toothed dolphins based on mitochondrial DNA Control Region (mtDNA-*CR*) in the Panamanian Pacific and the Wider Caribbean.

Methods: Samples were collected in Colombia (N=5), Panama (N-Caribbean=1, N-Pacific=9), and Puerto Rico (N=3) from free-ranging and stranded individuals. DNA was extracted from each sample, and a mtDNA segment of around 534 to 748 bp was amplified through the PCR reaction. The obtained sequences were compared with rough-toothed dolphin haplotypes previously published in NCBI (N=70), from the Atlantic, Indian, and the Pacific Oceans.

Results: Our findings showed significant population structure among ocean basins (strong differentiation with Φ_{ST} data), and high genetic diversity within each phylogroup. Only the Atlantic Ocean showed high genetic differentiation within the basin, detecting three phylogroups: the Caribbean, northern, and southern Atlantic.

Conclusions: These findings support previous genetic studies that indicate high levels of population structure among ocean basins, although this species seems to be widely dispersed. However, samples from Panama and the Caribbean appear to show connectivity between highly differentiated Atlantic and Pacific Oceans. Therefore, our results highlight the need for more research to assess the rough-toothed dolphin genetic and population status in Central America, as the piece of the puzzle needed to clarify its taxonomy and genetic differentiation worldwide. This information is needed due to the rough-toothed dolphin IUCN categorization as "Least Concern" and its classification into appendix II according to CITES. While individuals are potentially threatened by incidental fishing, no management units are currently used to conserve this species despite its high genetic differentiation.

Key words: Delphinids; cetaceans; Control Region; mtDNA; Caribbean; Pacific Ocean; conservation.

RESUMEN

Más piezas del rompecabezas: información preliminar sobre la diversidad genética y estructura poblacional de *Steno bredanensis* (Artiodactyla: Delphinidae) en Centroamérica y el Mar Caribe

Introducción: El delfín de dientes rugosos (*Steno bredanensis*) habita aguas oceánicas de latitudes tropicales y muestra filopatría en algunas islas oceánicas. Sin embargo, la especie ha sido observada en algunas áreas costeras de aguas poco profundas. Particularmente en Centroamérica, los delfines de dientes rugosos han sido reportados por registros ocasionales y varamientos. Por ejemplo, el primer registro confirmado de la especie en el Caribe Panameño ocurrió el 17 de julio de 2012 en una región costera de la Laguna de Chiriquí, durante un monitoreo de delfín nariz de botella. De manera similar, el primer reporte de un varamiento masivo de delfines de dientes rugosos en el Pacífico Panameño ocurrió el 20 de abril de 2016, en la Playa Ostional, donde 60 delfines vararon y diez murieron. Estos avistamientos y eventos ofrecen una valiosa oportunidad para obtener muestras con el fin de realizar estudios genéticos, los cuales son escasos en la región.

Objetivo: En este estudio, presentamos la primera evaluación de la diversidad genética de los delfines de dientes rugosos basado en la Región Control de ADN mitocondrial (*CR*-ADNmt) en el Pacífico Panameño y la región Caribe.

Métodos: Las muestras fueron colectadas en Colombia (N=5), Panamá (N-Caribe=1, N-Pacífico=9), y Puerto Rico (N=3) de individuos vivos y varados. El ADN fue extraído para cada muestra, y un segmento de ADNmt de aproximadamente 534 a 748 pb fue amplificado mediante la reacción en cadena de la polimerasa PCR. Las secuencias obtenidas fueron comparadas con haplotipos de delfines de dientes rugosos de los Océanos Atlántico, Índico y Pacífico, publicados previamente en NCBI (N=70).

Resultados: Nuestros resultados mostraron una estructura poblacional significativa entre las cuencas oceánicas (una alta diferenciación con base en datos de Φ_{ST}), y una alta diversidad genética dentro de cada filogrupo. Solo el Océano Atlántico mostró una alta diferenciación dentro de la cuenca, detectando tres filogrupos: el Caribe, Atlántico norte y sur.

Conclusiones: Estos resultados soportan los estudios genéticos previos que indican altos niveles de estructura poblacional entre las cuencas oceánicas, aunque esta especie parece estar ampliamente distribuida. Sin embargo, las muestras de Panamá y el Caribe parecen mostrar conectividad entre las cuencas altamente diferenciadas del Océano Atlántico y Pacífico. Por lo tanto, nuestros resultados destacan la necesidad de realizar más investigación para determinar el estado genético y poblacional de los delfines de dientes rugosos en Centroamérica, como la pieza del rompecabezas que falta para esclarecer su taxonomía y diferenciación genética a nivel mundial. Esta información es necesaria debido a que el delfín de dientes rugosos está categorizado ante la UICN como “Preocupación Menor” y está clasificado en el apéndice II de CITES. Aunque los individuos pueden estar potencialmente amenazados por captura incidental, no existen actualmente unidades de manejo para conservar esta especie a pesar de su alta diferenciación genética.

Palabras clave: Delfínidos; cetáceos; Región Control; ADNmt; Caribe; Océano Pacífico; conservación.

Nomenclature: SMT1: Supplementary material Table 1; SMF1: Supplementary material Figure 1.

INTRODUCTION

The rough-toothed dolphin, *Steno bredanensis,* (G. Cuvier in Lesson, 1828) is distributed worldwide in tropical, sub-tropical, and warm-temperate latitudes (Jefferson, 2018). In general, the species has oceanic habits, but shows some preference for volcanic islands where deep waters are close to the coast, such as the Canary Islands, French Polynesia, and Hawaii (Kerem et al., 2016). Particularly in these Indo-Pacific and Pacific islands, where the species has been extensively studied, the rough toothed-dolphins show some degree of philopatry (Baird, 2016; Oremus et al., 2012). In the Eastern Mediterranean, the rough-toothed dolphin is reported as an oceanic species with a seasonal migration pattern between neritic and oceanic habitats (Kerem et al., 2016). Consequently, the species may occupy both habitats, despite the apparent tendency to be distributed in oceanic areas. Indeed, in countries along the Western Atlantic and the Caribbean including Brazil, Colombia, Honduras, and recently in Panama, rough toothed dolphins have been reported mainly in neritic zones (Barragán-Barrera et al., 2015; Farías-Curtidor & Ayala, 2015; Farías-Curtidor & Barragán-Barrera, 2017, Farías-Curtidor & Barragán-Barrera, 2019; Kuczaj & Yeater, 2017; Ott & Danilewicz, 1996; Santos et al., 2019).

It is clear the need to study the rough-toothed dolphins worldwide, in order to understand their distributional patterns as a first step to assess adequately its conservation threats, since marine mammal coastal populations are more exposed to threats than the oceanic ones (Avila et al., 2018). Central America deserves special attention as a big gap of information about rough-toothed dolphins, despite that the entire region may potentially be occupied by this species (Kiszka et al., 2019). For instance, only in 2012 (July 17 at 9:50 a.m.), the rough-toothed dolphin was reported and confirmed genetically for the first time in the Caribbean of Panama, when a group of about six adults was observed jumping and traveling close to shore within the Chiriquí Lagoon during a common bottlenose dolphin (*Tursiops truncatus*) (Montagu, 1821) survey (Barragán-Barrera et al., 2015). Similarly, in 2016, a rare mass-stranding event of 60 rough-toothed dolphins, of which ten died on the beach despite local efforts to rescue them, was reported for the first time at the Ostional Beach, on the Pacific coast of Panama (May-Collado et al., 2017). In general, the few sightings in Central American Caribbean have taken place in coastal areas (May-Collado et al., 2017), and even some individuals appear to show residency patterns like off the coast of Utila in Honduras (Kuczaj & Yeater, 2017).

Definition of neritic and/or oceanic habits of rough-toothed dolphins, as well their population status, is needed to assess adequate management plans. The main threat reported for the species is bycatch in oceanic waters of Brazil (Donato et al., 2019; Monteiro-Neto et al., 2000). However, neritic individuals also may be affected by contamination and fishery interactions, particularly in the Atlantic coast of the USA and in Brazil, where mass strandings have been reported (Baptista et al., 2016;

Donato et al., 2019; Ewing et al., 2020; Lailson-Brito et al., 2012; Lemos et al., 2013; Meirelles & Barros, 2007; Struntz et al., 2004). In light of this, genetic studies based on samples collected opportunistically from stranding events in areas where non-monitoring programs are established, for example in Central America, could be useful to provide an initial status of rough-toothed dolphins' population structure. A recent work aimed to assess this was conducted using samples collected worldwide, and showed clear genetic differentiation among ocean basins based on both nuclear and mitochondrial markers (Albertson et al., 2022). Particularly, a strong distinction was detected between Atlantic and Pacific oceans, which suggested a potential incipient speciation to at least subspecies level (Albertson et al., 2022; da Silva et al., 2015). However, the authors recognized the need for including more samples that represent a larger area in the Pacific and especially in the Indian Ocean to confirm this assumption (Albertson et al., 2022). Additionally, this work only included six samples from the Caribbean and one from Brazil, which could imply no divergence detection between these two areas, despite a strong differentiation previously described based on mitochondrial DNA (mtDNA) data (da Silva et al., 2015).

Following the Albertson et al. (2022) recommendation, herein we provide new insights into genetic diversity and population structure of rough-toothed dolphins based on mtDNA Control Region (mtDNA-*CR*) using new samples collected from the Central American Pacific coast, specifically from Panama, as well as new samples from the Caribbean. Additionally, this study aimed to corroborate or not the population differentiation reported by Albertson et al. (2022), emphasizing on dolphins from Central America, which potentially may provide the resolution needed to clarify the potential subspeciation process among ocean basins. This study provides relevant baseline data about the genetic status of the rough-toothed dolphin in the region, as a first step to understand its population status, and thus propose future adequate management plans for this enigmatic species in Central America.

METHODS

Study area: The study area in Central America, where rough-toothed dolphin samples were collected, encompasses the Azuero Peninsula on the Pacific coast of Panama and the Chiriquí Lagoon on the Caribbean coast of Panama (Fig. 1). The Azuero Peninsula, where the Ostional beach is situated, is located at the central portion of Panama, whose coast is dominated by small portions of mangrove and beach vegetation (Friedman & Grandmont, 2019). In the Ostional beach, a rare event of a mass stranding was reported on April 19th, 2016 (Fig. 2A). The Chiriquí Lagoon, where one sample of one individual from a group of six free-ranging adult dolphins was collected on July 17th, 2012 (Fig. 2B), is a semi-enclosed lagoon located in the Bocas del Toro Province at Western Caribbean of Panama, an area highly influenced by precipitation (Guzmán & Guevara, 1998).

Additionally, samples from the Caribbean basin, coming from Colombia and Puerto Rico, were included in this study (Fig. 1). Samples from Colombia were collected from two locations: 1) in waters of Dibulla, located in La Guajira Peninsula on the northern portion of Colombia, where four samples of free-ranging adult individuals from a group of around 15 dolphins were collected on May 19th, 2015 (Fig. 2C) (Farías-Curtidor & Ayala, 2015; Farías-Curtidor & Barragán-Barrera, 2017, Farías-Curtidor & Barragán-Barrera, 2019), and 2) in Gaira, located in the Magdalena department, where one sample from a stranded individual was collected. These two areas are in the Eastern Colombian Caribbean and are influenced by upwelling events (Arévalo-Martínez & Franco-Herrera, 2008; Fajardo, 1979; Gutiérrez et al., 2015), so cetaceans have been usually reported there (e.g., Barragán-Barrera, do Amaral, et al., 2019, Barragán-Barrera, Luna-Acosta, et al., 2019; Farías-Curtidor et al., 2017; Fraija et al., 2009; Pardo & Palacios, 2006). Regarding

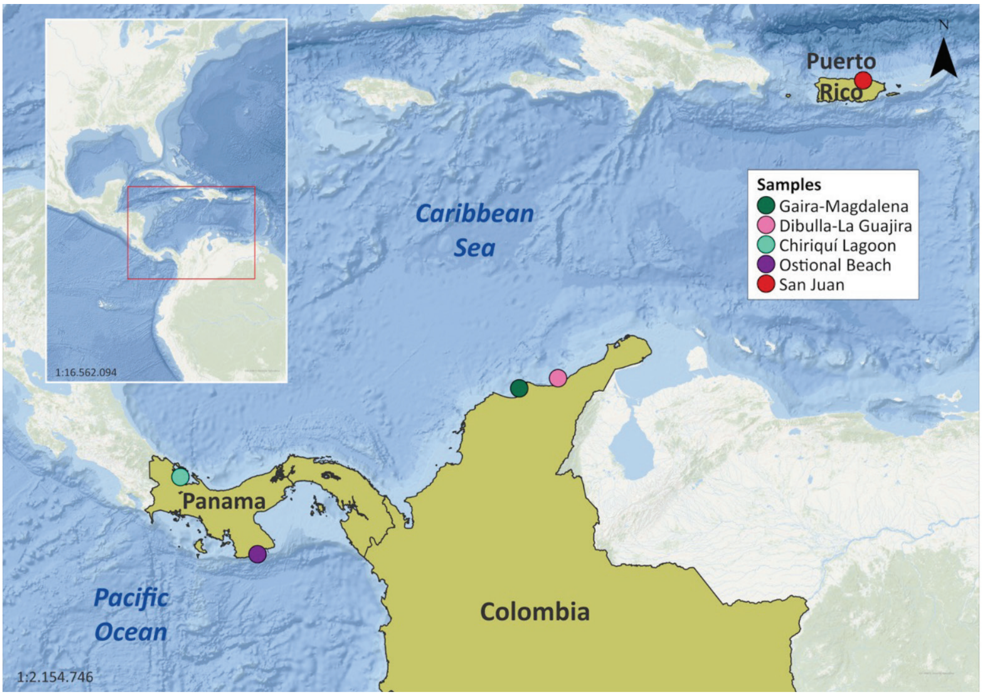

Fig. 1. Location of the samples of Rough-toothed dolphins (*Steno bredanensis*) in the Caribbean coast of Colombia (Gaira-Magdalena and Dibulla-La Guajira), Panama (Chiriquí Lagoon), and Puerto Rico (San Juan), as well as the Pacific coast of Panama (Ostional Beach).

Puerto Rico, it is a Caribbean archipelago that is part of the Greater Antilles (Fig. 1), and due its localization between the Caribbean Sea and the Atlantic Ocean, holds a high marine mammals' diversity in the Wider Caribbean (Mignucci-Giannoni, 1989).

Sample collection: Following Barragán-Barrera et al. (2017), rough-toothed dolphins tissue samples in the Chiriquí Lagoon (N = 1) and Dibulla-La Guajira Peninsula (N = 4) were collected from free-ranging animals using the PAXARMS system (Krützen et al., 2002). Samples from the Azuero Peninsula (N = 9), Gaira-Magdalena (N = 1), and Puerto Rico (N = 3) were collected from stranded animals. Samples were preserved in 70 % ethanol and at −20 °C (Amos & Hoelzel, 1991) for subsequent laboratory analysis.

DNA extraction, PCR, sequencing, and sexing: DNA was extracted from tissue samples using the DNeasy kit (QIAGEN) following the manufacturer's instructions. A portion of mtDNA-*CR* of around 534 to 748 bp was amplified by the polymerase chain reaction (PCR), using the primers Dlp5G (5'- GGAGTACTATGTCCTGTAACCA-3') or Dlp8G (5'-CCATCGWAGATGTCTTATTTAAGRARTTCTA-3') and Dlp1.5 (5'-TCACCCAAAGCTGRARTTCTA-3'), following the protocol described in Baker et al. (1998). PCR products were purified following a Polietilenglicol protocol (PEG 20 %), and DNA was sequenced using the Sanger sequencing method (Sanger & Coulson, 1975). Following Gilson et al. (1998), the sex of each individual sampled was identified using the male-specific SRY gene and ZFY/ZFX genes of males and females.

Data analysis: The software Geneious v. 4.8.5 (Drummond et al., 2009) was used to edit manually all obtained sequences. These haplotypes were compared to 70 GenBank

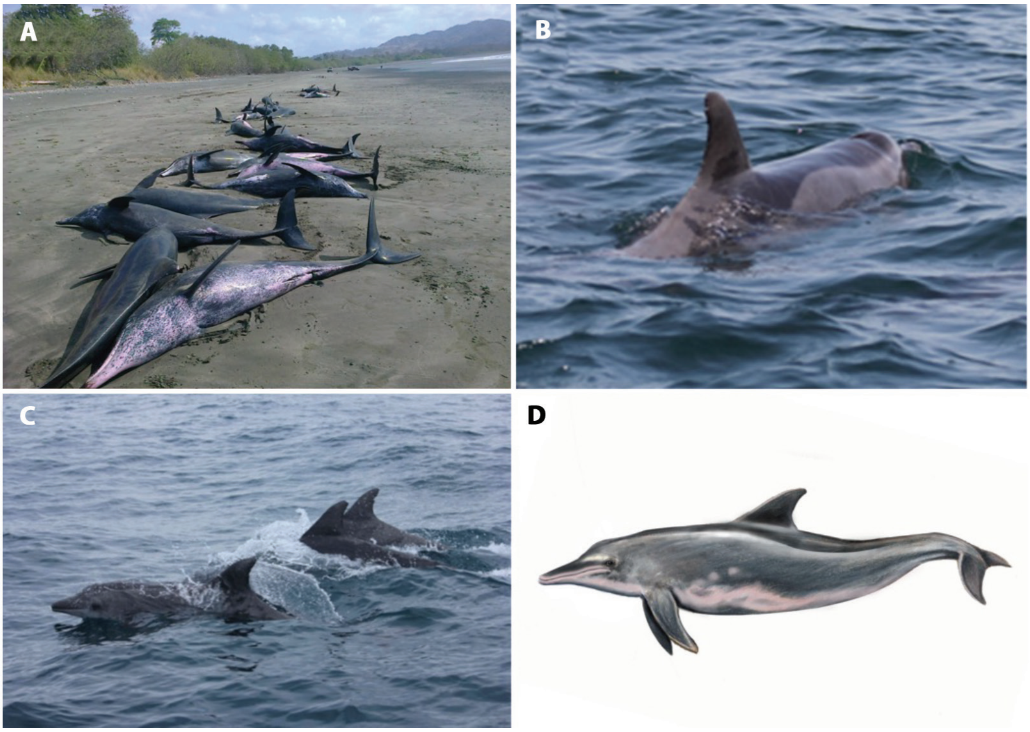

Fig. 2. Sightings of rough-toothed dolphins (*Steno bredanensis*) in which tissue samples for this study were collected. A) A rare mass-stranding occurred in April 2016 at Ostional Beach in the Azuero Peninsula, Pacific coast of Panama; photo courtesy Lissette Trejos. B) The first confirmed sighting of rough-toothed dolphins in the Caribbean coast of Panama, at Chiriquí Lagoon, reported in July 2012; photo by Mónica Acosta. C) A group recorded in May 2015 consisting of 14 adults and one calf in La Guajira Peninsula, Caribbean coast of Colombia; photo by Nohelia Farías-Curtidor. D) A species description illustrated by Emmanuel Laverde © www.arteyconservacion.com

published sequences from Brazil, China, Eastern Pacific coast of Central and North America, India, Indonesia, Japan, Mediterranean Sea, New Zealand, Puerto Rico, Samoa Island, Society Islands, South Africa, South Korea, Spain, Taiwan, Thailand, and USA (Western Atlantic coast and Hawaii) (SMT1). The R script RemoveRedundantTaxa was used to characterize the haplotypes. All sequences were aligned obtaining an alignment of 383 bp. In order to understand the relationships among all sequences, the CIPRES Science Gateway V. 3.3 software was used (Miller et al., 2010) to build a maximum likelihood phylogenetic tree using the evolutionary model of Generalized-Time-Reversible γ + Invariant (GTR γ + I) substitution and 1 000 bootstrap replicates.

Because no information about haplotype frequencies from Indonesia and Thailand was available, these were not included in the subsequent analyses. To determine the number of phylogrups for all haplotypes obtained worldwide, the software Arlequin v. 3.5 (Excoffier & Lischer, 2010) was used to assess the best F_{CT} value by running several possible combinations of geographic areas (two to eight groups) based on ocean basin and/or previous studies (Albertson et al., 2017, Albertson et al., 2022; da Silva et al., 2015; Oremus et al., 2012). The same software was used to assess the fixation indices (F_{IS} and F_{IT}). Once the number of phylogrups was identified, the PopART software (Clement et al., 2000) was used with the TCS method to build a haplotype network to visualize to what population the haplotypes used in this study

Table 1

Estimates of mitochondrial differentiation among the five phylogrups identified for rough-toothed dolphins (*Steno bredanensis*) in the Atlantic, Indian, and Pacific Oceans. F_{ST} value is shown above diagonal and Φ_{ST} below diagonal. P-value is indicated under each value in parentheses (significant values are considered as P < 0.05). Haplotype (*h*) and nucleotide diversity (π) are shown on the diagonal for each phylogrup.

F_{ST} Φ_{ST}	Brazil-South Atlantic Ocean	Caribbean	North Atlantic Ocean	Indian Ocean	Pacific Ocean
Brazil- South Atlantic Ocean	$h = 0.628$ $\pi = 0.121$	0.233 (P = 0.009)	0.268 (P < 0.001)	0.262 (P < 0.001)	0.189 (P < 0.001)
Caribbean	0.644 (P < 0.001)	$h = 0.978$ $\pi = 0.109$	0.100 (P < 0.001)	0.046 (P < 0.001)	0.043 (P < 0.001)
North Atlantic Ocean	0.746 (P < 0.001)	0.904 (P < 0.001)	$h = 0.840$ $\pi = 0.060$	0.124 (P = 0.009)	0.104 (P = 0.009)
Indian Ocean	0.562 (P < 0.001)	0.768 (P < 0.001)	0.340 (P < 0.001)	$h = 0.929$ $\pi = 0.167$	0.057 (P = 0.027)
Pacific Ocean	0.490 (P < 0.001)	0.780 (P < 0.001)	0.570 (P < 0.001)	0.269 (P < 0.001)	$h = 0.942$ $\pi = 0.083$

belonged and their frequency. To assess the genetic subdivision among phylogrups identified, the software Arlequin v. 3.5. (Excoffier & Lischer, 2010) was used to conduct an analysis of molecular variance (AMOVA) (Excoffier et al., 1992), so the pairwise comparison of population differentiation indices F_{ST} and Φ_{ST} between all phylogrups were assessed. The same software was used to assess diversity indices as nucleotide (π) and haplotype diversity (*h*), as well as the neutrality defining *Tajima's D* index for each phylogrup.

RESULTS

A total of 18 samples were collected, and 17 were successfully amplified (94,44 %) except one from the Ostional Beach. In total, the 17 sequences obtained represent 13 new haplotypes ranging between 534 and 748 bp. For Panama, one haplotype was identified in the Caribbean while five were identified for the Pacific. Among these five haplotypes, sequences SbEPP1 (748 bp) and SbEPP1.1 (691 bp), which were identical, were reported as different haplotypes due the first one was longer, and we do not have any evidence of polymorphism present in the non-amplified region. For Colombia (Caribbean basin), three haplotypes were identified in Dibulla-La Guajira and one in Gaira-Magdalena. For Puerto Rico, three haplotypes were identified (SMT1). All these new haplotypes were deposited and are available in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) under accession numbers OR436925-OR436936.

Comparison among these new haplotypes with previously 70 GenBank published sequences from Atlantic, Indian, and Pacific Oceans, showed strong differentiation among ocean basins. Despite of this, four Caribbean haplotypes, including the ones from Colombia (Dibulla-La Guajira), Panama, and Puerto Rico (SbGCC2, SbGCC3, SbPC1, and SBPRC3) nested within the Pacific Ocean clade (the maximum likelihood phylogenetic tree illustrates in green color the Caribbean haplotypes; Fig. 3). Consequently, reciprocal monophyly was not detected between the Atlantic and Pacific Oceans, and this last one appears to be a paraphyletic group (boostrap support > 95 %; Fig. 3). The remaining four Caribbean haplotypes (SbMCC1, SbPRC1, SBPRC2, and SbGCC1) exhibited connectivity to the entire Atlantic Ocean. The SbMCC1 haplotype from Gaira-Magdalena (Colombian Caribbean) nested with haplotypes from the Atlantic coast of the USA, as well as with the haplotypes SbPRC1 and SBPRC2 from Puerto Rico. The Colombian haplotype SbGCC1 from Dibulla-La Guajira

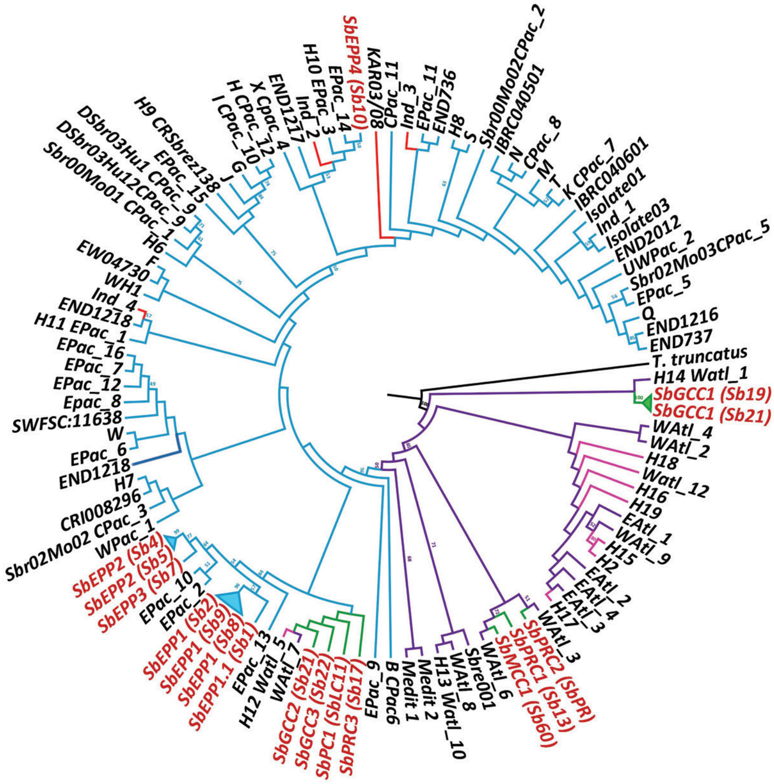

Fig. 3. Phylogenetic reconstruction by maximum likelihood of rough-toothed dolphins (*Steno bredanensis*) Control Region (752 bp) haplotypes in the Atlantic (purple and pink clades), Indian (red), and Pacific (blue) Oceans. Caribbean clades are colored green. Phylogeny in a circular polar form shows bootstrap support in branches with percentages of 100 % or > 50 %. Red letters represent the samples used for this study. Outgroup: Common bottlenose dolphin, *Tursiops truncatus*.

showed a higher connectivity to the Atlantic basin since it nested with haplotypes from the Atlantic coast of the USA and Canary Islands (Spain) (Fig. 3). Despite this, high population structure was detected between the Caribbean and the North Atlantic phylogrups. Regarding the haplotypes from the Ostional Beach in the Panamanian Pacific (SbEPP1, SbEPP1.1, SbEPP2, SbEPP3, and SbEPP4), these exhibited connectivity with haplotypes from the entire Pacific basin, since they nested with haplotypes from Central Pacific (mainly Hawaii and Society Island), Indo-Pacific (India), Eastern Pacific (Pacific coast of America), and Thailand (Fig. 3).

In the analysis of population structure, we identified five phylogrups based on the best F_{CT} value ($F_{CT} = 0.47$, P = 0.005): 1) Brazil-South Atlantic Ocean, 2) Caribbean, 3) North Atlantic Ocean, 4) Indian Ocean, and 5) Pacific Ocean. The Caribbean phylogrup was formed by haplotypes from the Caribbean coast of Colombia, Panama, and Puerto Rico. The North Atlantic Ocean phylogrup consisted of haplotypes from the Eastern Mediterranean Sea, Canary Islands (Spain), and the Atlantic coast of the USA. The Indian Ocean phylogrup was formed by haplotypes from India and South Africa. The Pacific Ocean phylogrup included haplotypes from the Central Pacific, China, Eastern Pacific, Hawaii, Japan, Pacific waters of Panama, Samoa Islands, Society Island, South Korea, Taiwan, and Western Pacific. Pairwise comparisons showed strong population structure among phylogrups,

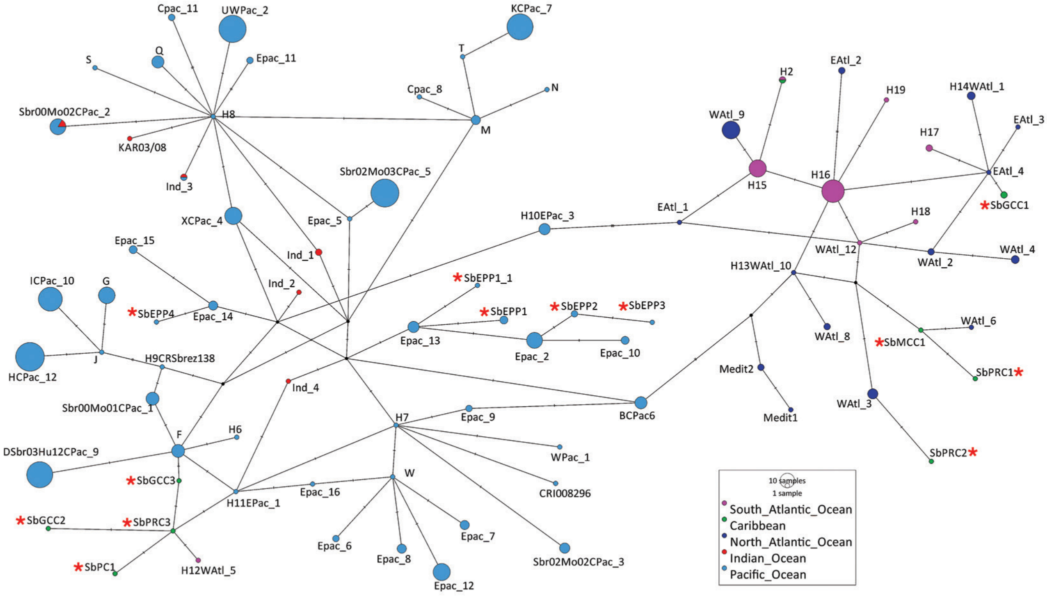

Fig. 4. Haplotype network reconstruction for rough-toothed dolphins (*Steno bredanensis*) in the Caribbean, North Atlantic Ocean, South Atlantic Ocean (Brazil), Indian Ocean, and Pacific Ocean, under parsimony criteria with the TCS algorithm (N = 83, 383 bp). The circle's size indicates the frequency of each haplotype. Black dot indicates the hypothetical ancestral haplotype, and the perpendicular lines between the haplotypes refer to the number of nucleotide substitutions between them. New haplotypes reported in this study are indicated with a red asterisk (*).

mainly at nucleotide level (Φ_{ST}) (Table 1). The haplotype network (Fig. 4) showed the relationship among haplotypes and their frequencies from the five phylogrups.

The genetic diversity indexes showed high haplotype and nucleotide diversity for each phylogrup, with the highest haplotype diversity in the Caribbean, followed by the Pacific Ocean and the Indian Ocean. Brazil-South Atlantic Ocean exhibited the lowest haplotype diversity (Table 1). Regarding *Tajima's D* index, it was significant only for the Brazil-South Atlantic Ocean phylogrup and showed a negative value (*Tajima's D* = -1.792, P = 0.013). In general, the fixation indices for all phylogrups showed a significant value only for the endogamy index (F_{IS} = 0.501, P < 0.001).

DISCUSSION

This is the first study on the genetic population structure of rough-toothed dolphins using samples from Central America (specifically from Panama) based on mtDNA-*CR* data. Although we included individuals from new geographic regions that had not been sampled before (Panama and the Caribbean), our data supports strong population structure reported previously for the species among the Atlantic, Indian, and Pacific Oceans (Albertson et al., 2022; da Silva et al., 2015), and provide new insights about genetic differentiation within the Atlantic basin (da Silva et al., 2015; Donato et al., 2019). In general, our findings highlight that individuals in Central America may contain crucial genetic information to elucidate population status of rough-toothed dolphins in both Atlantic and Pacific Oceans, where a process of incipient subspeciation has been suggested (Albertson et al., 2022).

Genetic structure of Panamanian and Caribbean dolphins in the Atlantic Ocean

Our findings suggest the Atlantic Ocean is represented by three phylogrups: one in the Caribbean, a second one in the North

Atlantic Ocean, and a third one in Brazil-South Atlantic Ocean. Although two samples (haplotype SbGCC1) from Dibulla-La Guajira (Colombian Caribbean) nested with haplotypes from the Atlantic coast of the USA and Canary Islands (Spain), as well the Chiriquí Lagoon sample (SbPC1, Panamanian Caribbean) nested with haplotypes from the Atlantic coast of the USA (Fig. 3), a high population structure exists between the Caribbean and North Atlantic areas (Table 1). These findings may be explained by the oligotrophic condition of Caribbean waters (Aguirre, 2014; Corredor, 1979; Müller-Karger & Aparicio, 1994), which promote high dolphin dispersion toward productive Caribbean areas located mainly in coastal areas (Barragán-Barrera, do Amaral, et al., 2019). Consequently, despite oceanic habits of species such as pantropical spotted dolphins (*Stenella attenuata*) (Gray, 1846) and rough-toothed dolphins, individuals have been registered in coastal areas along the Caribbean Sea (Barragán-Barrera et al., 2015, Barragán-Barrera, do Amaral, et al., 2019; Farías-Curtidor & Ayala, 2015; Farías-Curtidor & Barragán-Barrera, 2017, Farías-Curtidor & Barragán-Barrera, 2019; Kuczaj & Yeater, 2017). For instance, rough-toothed dolphins in Dibulla-La Guajira have been observed in waters between 2 to 6.8 nautical miles from shore (Farías-Curtidor & Ayala, 2015; Farías-Curtidor & Barragán-Barrera, 2017, Farías-Curtidor & Barragán-Barrera, 2019). Additionally, this species has been reported as philopatric in coastal Caribbean waters of Honduras (Kuczaj & Yeater, 2017). These philopatric habits towards coastal areas in Central America, likely because of lack of resources in oceanic waters, may result in the genetic differentiation observed here. However, the high haplotype and nucleotide diversity also suggests a high connectivity among individuals within the Caribbean phylogrup. Therefore, more research focused on distribution, habitat use, local genetic patterns, and satellite tagging is needed to confirm if rough-toothed dolphins exhibit philopatric habits along specific Central American Caribbean areas, or if dolphins migrate between coastal and oceanic areas as has been reported in the Mediterranean Sea (Kerem et al., 2016).

The genetic differentiation observed between rough-toothed dolphins from the Caribbean and North Atlantic Ocean with Brazil-South Atlantic Ocean had been previously described (Donato et al., 2019; Silva et al., 2015), and agrees with other genetic studies with delphinids such as the Atlantic spotted dolphin *Stenella frontalis* (Cuvier, 1829) (do Amaral et al., 2021), bottlenose dolphin (Fruet et al., 2014), clymene dolphin *Stenella clymene* (Gray, 1850) (Nara et al., 2017), and common dolphin (Amaral et al., 2012), which reported a similar isolation pattern. Oceanographical features like the Amazon River mouth and the North Brazilian Current appear to act as a barrier that is segregating fauna from the northern and southern Brazil (Costa et al., 2017). Consequently, and due the isolation pattern for delphinid species in southern Brazil, some populations may be at risk (Fruet et al., 2014). Particularly rough-toothed dolphins from Brazil are threatened due to bycatch (Donato et al., 2019; Monteiro-Neto et al., 2000), which may imply a reduction of this population. Indeed, the significant and negative *D'Tajima* value found for this phylogrup suggests it is in expansion after a historical bottleneck (Weber et al., 2004). The population reduction resulted in unique haplotypes emerging, so the expansion observed here. Therefore, although we don't have information about the causes of historical bottleneck, our findings support the da Silva et al. (2015) recommendation in considering the Brazil-South Atlantic Ocean phylogrup as a distinct management unit due to the restricted gene flow.

Genetic diversity of Panamanian dolphins in the Pacific Ocean

New samples from the Pacific coast of Panama exhibited a genetic connectivity across the Pacific Ocean. These findings are not in agreement with the Albertson et al. (2022) previous study, in which they reported population structure between the Central, Eastern, and Western

Pacific. Particularly in Central Pacific, where oceanic islands are present including Hawaii, Samoa, and Society Islands, rough-toothed dolphins show genetic structure and high philopatry, with restricted movements even between closer islands (Albertson et al., 2017, Albertson et al., 2022; Baird, 2016; Oremus et al., 2012). However, this kind of residency pattern has not been reported in the Pacific basin of Central America. The few rough-toothed dolphin records in this region have been done in oceanic waters (May-Collado et al., 2017). Consequently, it is possible this species reflects oceanic habits, and conducts long migrations along the Pacific Ocean (Learmonth et al., 2007), as is reflected by several shared haplotypes among individuals, as well as by their high haplotype diversity.

Long and multiple migrations patterns across Pacific islands have been reported in green turtle, *Chelonia mydas* (Linnaeus, 1758) (e.g., Dutton et al., 2014; Jensen et al., 2016), which are able to travel distances larger than 2 000 km influenced mainly by sea currents (Read et al., 2014). Additionally, oceanographic events like El Niño Southern Oscillation (ENSO), may change the ocean current direction in relation to the warm (North-Equatorial Current) and cold currents as Humboldt Current (Richmond, 1990). As a result, temporary migration routes have been reported for many species, like fishes (Victor et al., 2004), green turtles (Seminoff et al., 2008), and marine mammals (Learmonth et al., 2007), which allows genetic connectivity along the Pacific Ocean.

One rough-toothed dolphin haplotype from Panama (SbEPP4) was nested with two haplotypes from India and Thailand. Despite this, significant population structure was detected between Indian and Pacific Oceans although F_{ST} and Φ_{ST} values were lower (Table 1). Similarly, pairwise comparisons between the Pacific and Caribbean basins showed lower F_{ST} and Φ_{ST} values (Table 1). These findings may be the result of some shared ancestral haplotypes between these three oceans basins (Fig. 3; SMT1), which may imply potential highly conserved haplotypes or genetic connectivity. Indeed, Albertson et al. (2022) found few shared haplotypes between the Atlantic and Pacific Oceans, as well as did not detect population structure between the Indian and Pacific Ocean based on nuclear marker data. Likely, individuals from Central America may be maintaining genetic connectivity among the three ocean basins, but until no more samples will be obtained, or satellite tagging research is conducted, this assumption can not be tested.

Conservation and research implications for dolphins in Central America

Although this is the first genetic assessment of rough-toothed dolphins in Central America, this study highlights the need for conducting monitoring and sample collection as well as more genetic assessments along the region. Particularly individuals from Central America are very useful to understand whether genetic connectivity between Atlantic and Pacific Oceans exist or are ancestral. Furthermore, the role of Panama Isthmus closure in the divergence of these phylogrups has not been completely understood (Albertson et al., 2022). Despite this, a potential subspeciation has been reported between these two oceans basin (Albertson et al., 2022), and our findings based on new Central America and Caribbean samples support the population structure despite their grouping with Pacific haplotypes.

The high and significant endogamy index value detected here was intriguing since it suggests the species has a low intrinsic genetic diversity due to considerable inbreeding. (Weber et al., 2004). Likely, variable sites within the species are not significant in relation to DNA analyzed here, despite mtDNA-*CR* having a high mutational rate because it is not under natural selection. Consequently, if the rough-toothed dolphin is an endogamic species, its status in the IUCN Red List as "Least Concern" (Kiszka et al., 2019) should be changed to some threatened level. However, our findings must be considered with caution, as we only provide information from a short portion of the maternal lineage. Analyzing bi-parental genes, and specifically microsatellite data, are needed to confirm this endogamy hypothesis. However, previous research with rough-toothed dolphins from French Polynesia using 14 microsatellite loci did not detect endogamy (Oremus et al., 2012). Consequently, samples from Centro America could be useful to assess if endogamy exists for this species.

Understanding the population or sub-speciation status of rough-toothed dolphins will be essential for their conservation, and sample collection in Central America is critical to achieving this goal. The Brazil-South Atlantic Ocean phylogrup is an example of effectiveness of large sampling to assess its conservation status, since the increasing number of samples collected has allowed the genetic isolation detection (da Silva et al., 2015; Donato et al., 2019).

The Central American rough-toothed dolphins, which are distributed in both Atlantic and Pacific Oceans, may be exposed to different threats, but no information about risks has been obtained yet in this region. The closest risk case for this species has been reported in the Colombian Caribbean, where few isolated bycatch cases were registered (Avila et al., 2018; Avila & Giraldo, 2022). However, both Caribbean and Pacific waters along the Central American region, mainly in Costa Rica, present high bycatch risk for oceanic delphinids as the pantropical spotted dolphin (Pino, 2021). Considering this, rough-toothed dolphins may be at risk in Central America, but this information is virtually unknown. We hope this study provides the baseline data needed to assess the rough-toothed dolphin genetic and population status in Central America, which will be essential for its management in the region. Herein, we invite to Marine Mammalogists working in Central America from Belize, Costa Rica, El Salvador, Honduras, Guatemala, and Nicaragua, to create transnational conservation efforts to increase sampling of rough-toothed dolphins in both the Eastern Pacific and Caribbean Sea in order to clarify the intraspecific genetic dynamics of this species. Furthermore, Panama must promote continuous genetic surveillance of its populations.

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See supplementary material
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