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Mitochondrial DNA supports the low genetic diversity of *Tursiops truncatus* (Artiodactyla: Delphinidae) in Bocas del Toro, Panama and exhibits new Caribbean haplotypes

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ABSTRACT

Introduction: The common bottlenose dolphin (*Tursiops truncatus*) is one of the most studied cetaceans worldwide; however, information about the genetic structure of wild populations is scarce in some regions like Central America and the Caribbean. There are two known genetic forms identified in the Caribbean based on mitochondrial DNA Control Region (mtDNA-*CR*) data: the 'inshore (or coastal) form' and the 'Worldwide distributed form'. In general, the inshore form refers to coastal and highly philopatric populations that show low genetic diversity. Worldwide distributed form refers to highly mobile populations with coastal and oceanic individuals that do not show philopatry and usually display high genetic diversity.

Objective: To determine the preliminary genetic status of common bottlenose dolphins in La Guajira, Colombian Caribbean, using a hypervariable portion of mtDNA-*CR*. The obtained haplotypes were compared with samples collected in Panama (likely 'inshore form') and with haplotypes previously found in other areas of the Caribbean.

Methods: In 2016, a total of 26 skin samples were obtained by remote biopsy system (PAXARMS) in two locations, La Guajira (Colombia, N=7) and Bocas del Toro (Panama, N=19). DNA was extracted, samples sexed, and a segment of mtDNA-*CR* (~550-750 bp) was amplified by PCR. The successfully amplified DNA sequences were manually reviewed and cleaned, and subsequently compared with 44 haplotypes previously reported for the Caribbean.

Results: The mtDNA-*CR* sequences from Bocas del Toro shared the same unique inshore haplotype previously reported for this population, while the samples from La Guajira represented six novel haplotypes, five belonging to the Worldwide distributed form and one to the 'inshore form.' Population structure analysis revealed two

phylogroups for the Caribbean (F_{ST}=0.1353, Φ_{ST}=0.3330) with high haplotype diversity: Panama(Bocas del Toro)-Bahamas-Cuba-Mexico (h=0.8489, π=4.2536 %) and Colombia-Costa Rica-Honduras-Puerto Rico (h=0.8837, π=4.2423 %).

Conclusions: These findings support the results previously reported for common bottlenose dolphins in Bocas del Toro-Panama and reinforce the need to protect this vulnerable 'inshore' population by treating it as a unique population management unit. Mitochondrial DNA analysis of samples collected from La Guajira dolphins provide the first insight into the genetic diversity of common bottlenose dolphins in this region, indicating the presence of both inshore and Worldwide distributed genetic forms. The potential connectivity of this last form among La Guajira-Colombia, Costa Rica, and Honduras in Central America highlights the need for more genetic and ecological studies to determine the appropriate management units for this species in Central America and the Caribbean.

Key words: bottlenose dolphin; cetaceans; mtDNA; genetic diversity; La Guajira, Colombia, Central America.

RESUMEN

ADN mitocondrial soporta la baja diversidad genética de *Tursiops truncatus (*Artiodactyla: Delphinidae) en Bocas del Toro, Panamá y detecta nuevos haplotipos en el Caribe

Introducción: El delfín nariz de botella común (*Tursiops truncatus*) es uno de los cetáceos más estudiados a nivel mundial. Sin embargo, la información sobre la estructura genética de sus poblaciones silvestres es escasa en algunas regiones, como Centroamérica y el Caribe. Con base en datos de la Región de Control del ADN mitocondrial (ADNmt-*CR*), dos formas genéticas han sido identificadas en el Caribe: la 'forma *inshore* (o costera)' y la 'forma mundialmente distribuida'. En general, la forma costera se refiere a poblaciones costeras y altamente filopátricas que muestran baja diversidad genética. La forma mundialmente distribuida se refiere a poblaciones altamente móviles con individuos costeros y oceánicos que no muestran filopatría y generalmente muestran alta diversidad genética.

Objetivo: Para determinar el estado genético preliminar de los delfines nariz de botella comunes en La Guajira, Caribe colombiano, se realizó un análisis genético utilizando una porción hipervariable de ADNmt-*CR*. Los haplotipos obtenidos se compararon con muestras recolectadas en Panamá (probablemente la forma costera) y con haplotipos encontrados previamente en otras áreas del Caribe.

Métodos: En 2016 se obtuvo un total de 26 muestras de piel colectadas con el sistema de biopsia remota (PAXARMS) en dos localidades, La Guajira (Colombia, N=7) y Bocas del Toro (Panamá, N=19). Se extrajo el ADN, se sexaron las muestras, y un segmento de ADNmt-*CR* (~550-750 pb) se amplificó mediante PCR. Las secuencias de ADN amplificadas con éxito se revisaron y limpiaron manualmente; posteriormente, se compararon con 44 haplotipos reportados previamente en el Caribe.

Resultados: Las secuencias de ADNmt-*CR* de Bocas del Toro compartieron el mismo haplotipo costero único reportado previamente para esta población, mientras que las muestras de La Guajira representaron seis haplotipos nuevos, cinco pertenecientes a la forma mundialmente distribuida y uno a la forma costera. El análisis de la estructura de la población reveló dos filogrupos para el Caribe (F_{ST}=0.1353, Φ_{ST}=0.3330) con alta diversidad haplotípica: Panamá(Bocas del Toro)-Bahamas-Cuba-México (h=0.8489, π=4.2536 %) y Colombia-Costa Rica-Honduras-Puerto Rico (h=0.8837, π=4.2423 %).

Conclusiones: Estos hallazgos respaldan los resultados previamente reportados para los delfines nariz de botella comunes en Bocas del Toro-Panamá y refuerzan la necesidad de proteger a esta vulnerable población costera tratándola como una unidad de manejo poblacional única. Para La Guajira, estos resultados de ADNmt resultan en el primer esfuerzo por determinar la diversidad y estructura genética del delfín nariz de botella común en esta región, los cuales sugieren que ambas formas genéticas están presentes en el área, siendo la forma mundialmente distribuida la predominante. La conectividad potencial de esta última forma entre La Guajira-Colombia, Costa Rica y Honduras en Centroamérica destaca la necesidad de realizar más estudios genéticos y ecológicos para determinar las unidades de manejo apropiadas para esta especie en Centroamérica y el Caribe.

Palabras clave: delfín nariz de botella; cetáceos; ADNmt; diversidad genética; La Guajira, Colombia, Centroamérica.

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

INTRODUCTION

The common bottlenose dolphin, *Tursiops truncatus* (Montagu, 1821) is a cosmopolitan species inhabiting a variety of nearshore and oceanic environments in tropical and temperate waters. It is currently listed as Least Concern by the International Union for the Conservation of Nature (IUCN) (Wells et al., 2019). This broadly distributed species is one of the most studied cetaceans worldwide (Reynolds et al., 2000). However, genetic information is still scarce for populations in some regions, particularly in coastal areas of Central America and the Caribbean, where few studies have been conducted. The lack of information regarding population structure may result in poor management practices, since coastal populations show a high degree of genetic differentiation and face more anthropogenic threats due to the proximity to human populations (e.g., Barragán-Barrera et al., 2017; Caballero et al., 2012; Fruet et al., 2014; Natoli et al., 2004; Parsons et al., 2006; Tezanos-Pinto et al., 2009). Therefore, targeted studies like this one, address critical data gaps for geographic regions of interest and are key for ensuring effective conservation and management programs.

Common bottlenose dolphins are highly adaptable to different environmental conditions, where habitat selection and resource specialisation likely shape patterns of movement, gene flow, and population structure (Hoelzel et al., 1998; Wiszniewski et al., 2010). For example, 'inshore' and 'offshore' ecotypes have been described in the Gulf of Mexico and the Northwest Atlantic based on a variety of factors such as genetics, haemoglobin profile, and morphometry (Duffield et al., 1983; Hersh & Duffield, 1990; Hoelzel et al., 1998; Natoli et al., 2004; Sellas et al., 2005). However, Tezanos-Pinto et al. (2009) provided a worldwide phylogeographic perspective highlighting that the 'offshore' of the Northwest Atlantic is genetically interconnected to several mitochondrial DNA (mtDNA) haplotypes distributed worldwide regardless of the habitat where samples were collected (coastal or pelagic) or ocean basin. Therefore, although the 'inshore form' is present in the Wider Caribbean, only the populations located in Florida-western North Atlantic, the Bahamas (Caballero et al., 2012; Natoli et al., 2004; Tezanos-Pinto et al., 2009), and Bocas del Toro Archipelago, Panama (Barragán-Barrera et al., 2017) have been successfully classified as entire 'inshore form'.

The inshore populations found in the western North Atlantic, which are highly structured among bays (e.g., Sellas et al., 2005), have recently been proposed as a new species based on an integrative approach comparing mitochondrial and nuclear DNA with morphometrics (Costa et al., 2022). These findings indicate that inshore bottlenose dolphins are likely under-characterized and deserve additional attention. Given this, it is imperative to conduct studies on dolphin populations in the Caribbean and Central America regions. This research should take into consideration that the inshore form is primarily found in the Wider Caribbean, specifically in Bocas del Toro (Barragán-Barrera et al., 2017; Caballero et al., 2012; Tezanos-Pinto et al., 2009).

The 'inshore' population found in Bocas del Toro exhibits a strong population structure and low genetic diversity (all individuals possess a single mitochondrial DNA Control Region (mtDNA-*CR*) haplotype) compared to other populations in the Caribbean (Barragán-Barrera et al., 2017). The population is small, shows high site fidelity, and restricted coastal feeding habits; all characteristics usually attributed to the inshore ecotype (Barragán-Barrera, Luna-Acosta et al., 2019; May-Collado et al., 2015, May-Collado et al., 2017). However, recent observations in the field suggest that oceanic individuals may be entering within the north-west region, which may result in potential genetic flow (B. Pérez, personal communication, 2022). Additional analyses are needed to better understand the genetic isolation and the 'inshore form' genetic connectivity between the Bocas del Toro dolphins and other dolphin populations in the Caribbean.

Little is known about the population found in La Guajira (Colombian Caribbean). To date,

most of the studies have focused on habitat use and occurrence (Combatt & González, 2007; Palacios et al., 2013). In the absence of data on genetic diversity and population structure, it is unknown if bottlenose dolphins in La Guajira belong to the inshore form or 'Worldwide distributed form', or if both forms coexist. This information gap prevents adequate management plans for this population, despite bottlenose dolphins in Colombia are facing threats related to bycatch, interactions with fishing gear, direct capture, vessel traffic/transit, pollution, and pathogens (Avila & Giraldo, 2022).

Here, we evaluate the genetic diversity and population structure of the common bottlenose dolphin in Bocas del Toro and La Guajira by comparing the mtDNA-*CR* marker to 44 haplotypes previously published from various locations in the Caribbean Sea (Barragán-Barrera et al., 2017; Caballero et al., 2012). Results from this work will contribute to the understanding of the genetic structure of bottlenose dolphins in the Central American Caribbean

and represent a critical first step to local conservation efforts.

METHODS

Study area: The Bocas del Toro Archipelago is located on the western Caribbean coast of Panama (Fig. 1), and has great marine biodiversity associated with coral reefs, seagrasses, and mangroves (Coates et al., 2005; Guzmán et al., 2005). La Guajira, in the western coast of Colombia, is the northernmost area of South America (Fig. 1). It is one of the most biodiverse regions in Colombia; it holds several coastal and marine ecosystems such as mangroves, coral reefs, coastal wetland, sandy beaches, and seagrasses (Corporación Autónoma Regional de La Guajira & Instituto de Investigaciones Marinas y Costeras, 2012).

Sample collection: Tissue samples were collected from wild common bottlenose dolphins using remote biopsy darts fired from a
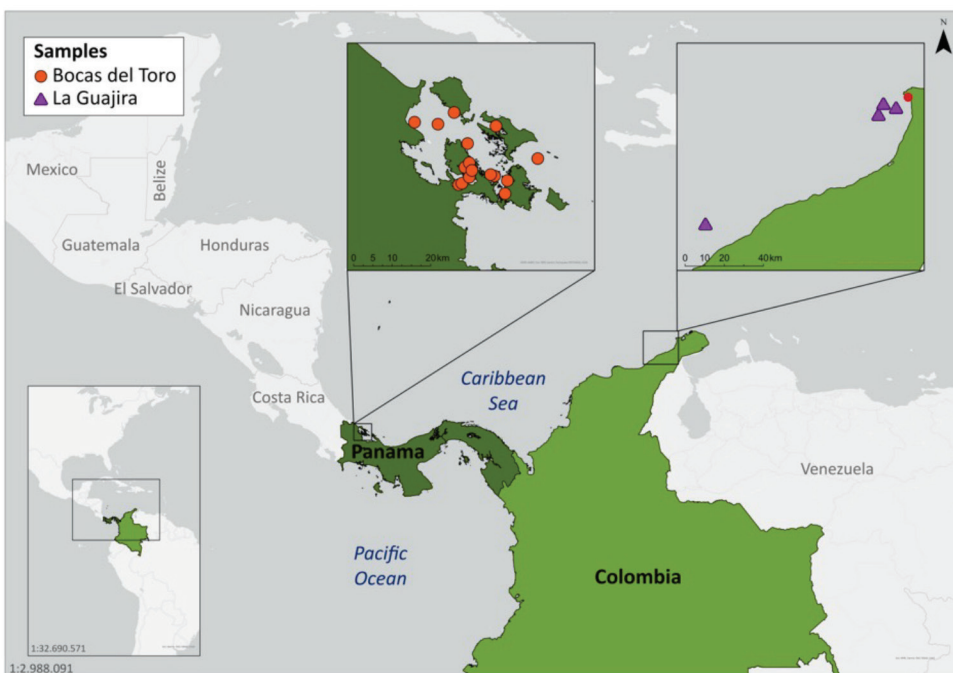

Fig. 1. Sample locations in Bocas del Toro Archipelago, Panama (orange dots) and La Guajira, Colombia (purple triangles) in the Caribbean Sea. Red dot indicates the location of Cabo de la Vela in La Guajira.

modified rifle (PAXARMS) (Krützen et al., 2002). This is a standardised methodology for collecting small skin biopsies from small cetaceans (Tezanos-Pinto & Baker, 2012). Samples were collected in February, March, and October of 2016 in Bocas del Toro, and in May and June of 2016 in La Guajira. GPS data and photographs of the dolphin biopsied were also collected. The latter allowed individual identification avoiding re-sampling (Fruet et al., 2014; Krützen et al., 2002). Samples were preserved in 70 % ethanol and stored at −20 °C (Amos & Hoelzel, 1991) for further laboratory analysis.

DNA extraction, PCR, sequencing, and sexing: DNA was extracted from skin samples using the DNeasy kit (QIAGEN). Fragments of approximately 535 - 684 bp of mtDNA-*CR* were amplified through the polymerase chain reaction (PCR), using the primers Dlp8G (5'-CCATCGWAGATGTCT-TATTTAAGRARTTCTA-3') or Dlp5G (5'-GGAGTACTATGTCCTGTAACCA-3') and Dlp1.5 (5'-TCACCCAAAGCTGRARTTC-TA-3') (Baker et al., 1998). Amplification followed this protocol: an initial pre-denaturation step of 94 ºC as denaturation for 2 min, with 34 cycles of 2–4 repeat times of 30 s at 94 ºC, followed by 45 s at 55 ºC as annealing and an extension at 72 ºC, with a final elongation after the last cycle of 10 min at 72 ºC (Baker et al., 1998). PCR products were visualized on agarose gel before sequencing. DNA was cleaned and sequenced in both directions with the Sanger sequencing method (Sanger & Coulson, 1975) on an ABI 3500 DNA automated sequencer using the Big Dye^{TM} Terminator v.3.1 Cycle Sequencing kit. In order to identify the sex of the samples collected, we used male-specific SRY gene and ZFY/ZFX genes of males and females (Gilson et al., 1998). Fragments were amplified by PCR using the protocol proposed by Gilson et al. (1998) and visualised on agarose gel to determine sex.

Data analysis: All mitochondrial sequences were checked, cleaned manually and aligned using the software Geneious v. 2022.1.1 (Drummond et al., 2009). To confirm that the samples collected were from common bottlenose dolphins and not from other delphinid species of the delphinid complex *Delphinus-Stenella-Tursiops*, we used BLAST in NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to compare our sequences to publicly available reference sequences and confirm species of origin. Unique haplotypes were assigned using the R script RemoveRedundantTaxa in RStudio (v. 2022.02.3+492). Haplotypes obtained were compared to 44 previously published sequences on NCBI GenBank from the Bahamas, Colombia, Costa Rica, Cuba, Honduras, Jamaica, Mexico, Puerto Rico, and Virgins Islands (Accession numbers: JN596281-JN596321) (Caballero et al., 2012), Costa Rica (Accession numbers: KY817220-KY817221), and Panama (Bocas del Toro) (Accession number: KX833116) (Barragán-Barrera et al., 2017). The complete alignment was 386 bp.

To identify which of the two genetic forms of common bottlenose dolphins the samples analysed belonged to, a Maximum Likelihood phylogenetic tree was constructed with the evolutionary model of Generalised-Time-Reversible γ + Invariant (general reversible time substitution model GTR γ + I) substitution and 1 000 bootstrap replicates using RAxML-HPC BlackBox tool in CIPRES Science Gateway V. 3.3 (Miller et al., 2010). As outgroup we defined a sample from an Atlantic spotted dolphin, *Stenella frontalis* (Cuvier, 1829) collected in La Guajira in 2016, since bootstrap value indicates 100 support segregating *T. truncatus* from *S. frontalis,* and this species has been used before as outgroup for mtDNA analysis on bottlenose dolphins (e.g., Wang et al., 1999). Also, a haplotype network was constructed using the TCS method in PopART software (Clement et al., 2000) to visualise to what population the haplotypes used in this study belonged and their frequency. Finally, we defined the number of phylogroups for the Caribbean by calculating F_{CT} using Arlequin v. 3.5 (Excoffier & Lischer, 2010). We used the same software to assess estimates of genetic structure (F_{ST} and Φ_{ST}), nucleotide (π), and haplotype diversity (h).

Here, samples from Jamaica and the US Virgin Islands were excluded due to the low number of samples (N = 1).

RESULTS

A total of 26 tissue samples from common bottlenose dolphins were collected; seven in La Guajira, Colombia, and 19 in Bocas del Toro, Panama. All samples from Panama were successfully amplified, sequenced and included in genetic structure and diversity analyses. Among these samples, only a single haplotype was found. Six out of the seven samples from La Guajira were successfully amplified, each representing a unique haplotype. Overall, ten males and nine females were identified in Bocas del Toro samples, and four males and three females were identified in La Guajira samples.

When the sequences obtained from this study were compared with 44 previously Caribbean haplotypes (Barragán-Barrera et al., 2017; Caballero et al., 2012; SMT1), all samples from La Guajira resulted in new haplotypes not previously reported for the Caribbean, while the only haplotype found in the samples from Bocas del Toro was identified as the same unique inshore haplotype reported previously (Barragán-Barrera et al., 2017). New *T. truncatus* haplotypes were submitted to GenBank (https://www.ncbi.nlm.nih.gov/genbank/) as accession numbers OR090913-OR090918 and the new *S. frontalis* (outgroup) haplotype was submitted to GenBank as accession number OR090919.

Further comparisons conducted through phylogenetic analyses, showed samples from Bocas del Toro nested with the previously reported haplotype BOC (Accession number: KX833116), which had been reported by Barragán-Barrera et al. (2017) as 'inshore.' Five out of six samples from La Guajira nested with the Worldwide distributed form, and only one sample nested with the inshore form, grouping with the inshore Q haplotype from Cuba (Fig. 2). The haplotype network (Fig. 3) shows the relationships among haplotypes, frequency, and locations.

In the genetic structure analysis using the new sequences found here with the previous ones reported in the Caribbean, we evaluated from two to five phylogroups. Based on the most probable phylogroups, we defined two (F_{CT} = 0.2655, P = 0.0352). One phylogroup formed by Bocas del Toro (Panama)-Bahamas-Cuba-Mexico (mostly formed by inshore haplotypes) and the other one by Colombia-Costa Rica-Honduras-Puerto Rico (mostly formed by Worldwide distributed form haplotypes). We reported similar values of haplotype and nucleotide diversity between both phylogroups (Table 1), but values of F_{ST} and Φ_{ST} showed strong population structure between them, especially at the nucleotide level (F_{ST} = 0.1353, Φ_{ST} = 0.3330, P < 0.0001, Table 1). We found relatively high haplotype and nucleotide diversity in both phylogroups considered in this analysis, with the highest haplotype diversity found in the Colombia-Costa Rica-Honduras-Puerto Rico phylogroup (Table 1).

Table 1

Estimates of mitochondrial differentiation among the two forms ('inshore form' and 'Worldwide distributed form') of common bottlenose dolphin (*Tursiops truncatus*) located in the Caribbean Sea. F_{ST} value is shown above diagonal and Φ_{ST} below diagonal. P-value is indicated under each value. Haplotype (*h*) and nucleotide diversity (*π*) are shown on the diagonal for each phylogroup.

F_{ST} / Φ_{ST}	Bocas del Toro (Panama)-Bahamas-Cuba-México	Colombia-Costa Rica-Honduras-Puerto Rico
Bocas del Toro (Panama)-Bahamas-Cuba-México	*h* = 0.8489 +/- 0.0179 *π* = 4.2536% +/- 0.021	0.1353*
Colombia-Costa Rica-Honduras-Puerto Rico	0.3330*	*h* = 0.8837 +/- 0.0337 *π* = 4.2423% +/- 0.0214

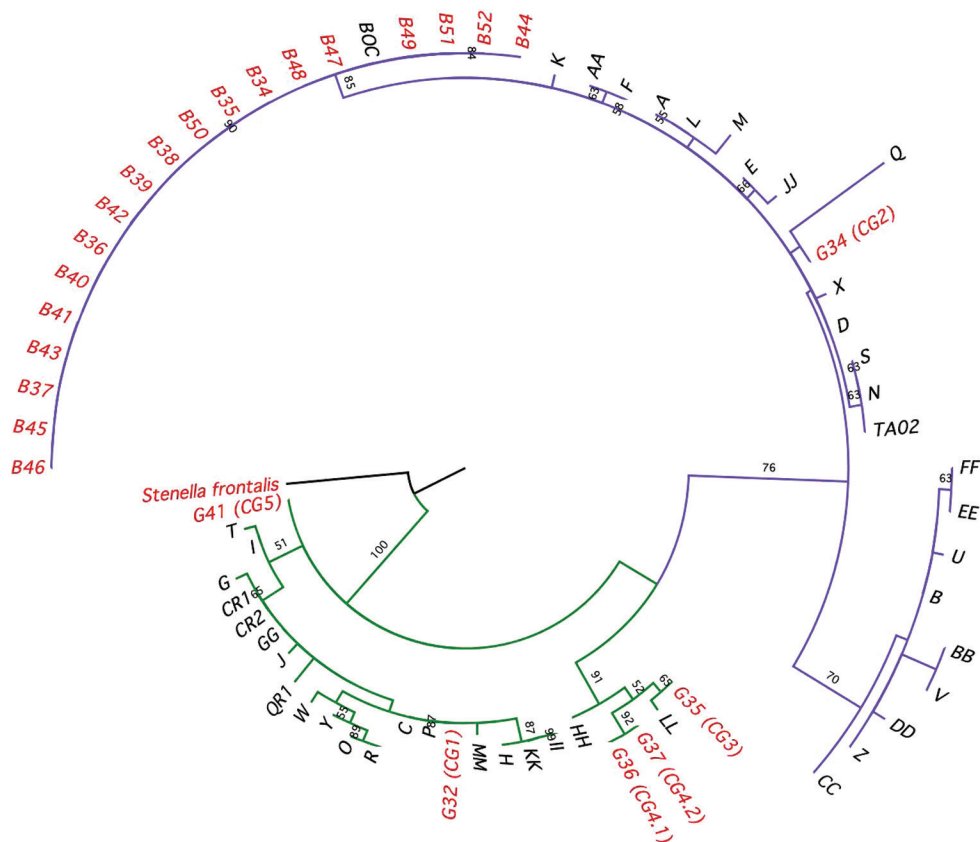Significant values (p < 0.05) are indicated with an asterisk (*).

Fig. 2. Phylogenetic reconstruction by Maximum Likelihood of Control Region haplotypes from common bottlenose dolphins (*Tursiops truncatus*) in the Caribbean Sea (N = 70, 386 bp). Phylogeny in a circular polar form shows bootstrap support in branches with percentages > 50 %. Purple line groups the 'inshore form' and the green line groups the 'Worldwide distributed form'. Red letters represent the samples used for this study and the braces indicate the new haplotypes. Outgroup: Atlantic spotted dolphin (*Stenella frontalis*).

DISCUSSION

This study provides new insights into the population structure and genetic diversity of common bottlenose dolphins from La Guajira (Colombia) and Bocas del Toro (Panama) in the western Caribbean using mtDNA-*CR* data. The results confirm that a single unique haplotype is present among the individuals sampled in Bocas del Toro, and it is unique to the Caribbean Sea (Barragán-Barrera et al., 2017). This population has low mitochondrial diversity and both sexes appear to be highly philopatric (Barragán-Barrera et al., 2017). Previous photo-ID data supports high site fidelity

(e.g., May-Collado et al. 2012, 2015, 2019), and genetic data shows a strong population structure based on nuclear data (nine microsatellite loci), as well as that both sexes share the same unique inshore haplotype not reported in other Caribbean area (Barragán-Barrera et al., 2017). This site fidelity may be explained by the prey availability for dolphins within the Archipelago (Barragán-Barrera, Luna-Acosta et al., 2019), as well as for the shallow areas suitable as nursery (May-Collado et al., 2019). Even though most studies have demonstrated male-biased dispersal (Baker et al. 1993; Escorza-Trevino & Dizon, 2000; Möller et al. 2004; Rosel et al. 1999), philopatry in both sexes has been documented
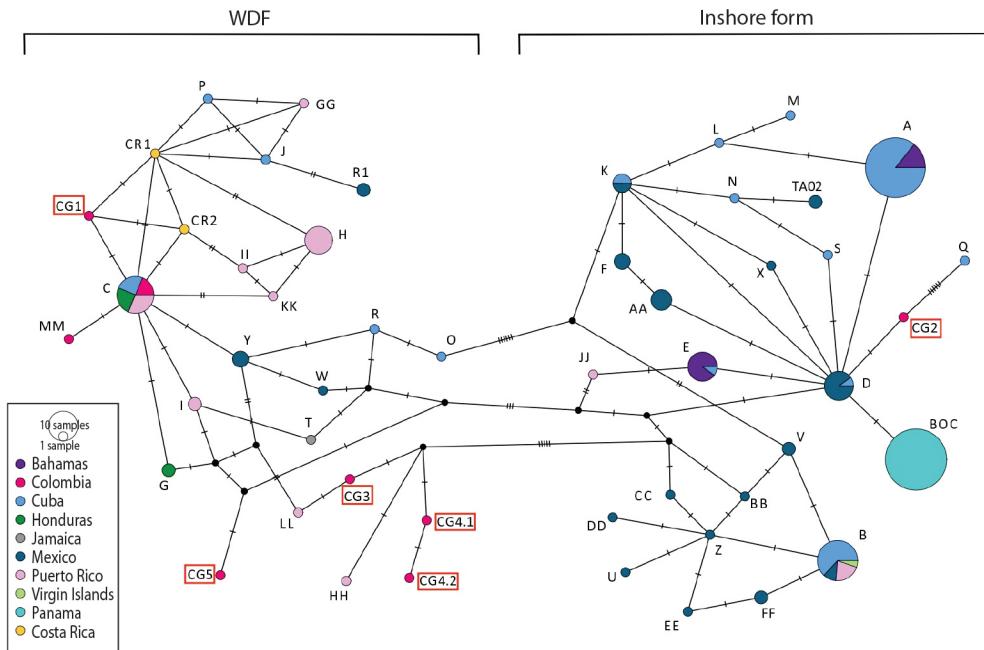
Fig. 3. Haplotype network reconstruction for common bottlenose dolphins (*Tursiops truncatus*) in the Caribbean Sea under parsimony criteria with the TCS algorithm (N = 50, 386 bp). The size of the circles indicates the frequency of each haplotype. Black dots indicate the hypothetical ancestral haplotype, and the perpendicular lines between the haplotypes refer to the number of nucleotide substitutions between them. New haplotypes are highlighted within a red square.

before in odontocetes. For example, in resident killer whales located in British Columbia and Washington State (Bigg et al., 1987) and pilot whales located in the Faeroe Islands (Amos et al., 1993). In some bottlenose dolphin populations, males are more mobile than females even in resident populations (Möller & Beheregaray, 2004).

Conversely, the genetic pattern of the population of La Guajira is very different. Here, we infer that dolphins are mainly of the Worldwide distributed form, in addition to a few inshore dolphins. A previous study (Combatt & González, 2007) suggested that dolphins in La Guajira probably belonged to the inshore form (based on observations and occurrence data). However, in general, La Guajira Peninsula does not have closed and protected bays as does the Bocas del Toro Archipelago. This geography and oceanic foraging may promote admixture among different forms. Nonetheless,

the area is influenced by upwelling conditions, mainly in the low (south) and high (north) Guajira, offering nutrients to dolphin prey species (Barragán-Barrera, Luna-Acosta et al., 2019; Gutiérrez Leones et al., 2015; Paramo et al., 2011). Although this upwelling is variable throughout the year and between years (Andrade & Barton, 2005), it has been related to presence of dolphins in La Guajira (Farías-Curtidor et al., 2017). The area appears to be important for transit, and consequently, a very mobile common bottlenose dolphin population may visit La Guajira when oceanographic conditions are favourable. Nevertheless, the individual nested with 'inshore form' haplotypes could suggest the potential presence of inshore populations in the high Guajira (where the sample was collected), or admixture between forms. The sampled inshore individual was a male in a group of 15 individuals that were feeding close to a fishing boat in the Cabo de la

Vela (12.113333º N & -72.294533º W) (Fig. 1). In this area, mainly in the north, some enclosed bays may offer adequate habitats for dolphins, but sufficient monitoring data to confirm this assumption does not exist.

La Guajira appears to be an important transit area for common bottlenose dolphins in the Wider Caribbean, where the presence of the Worldwide distributed form individuals formed by dolphins with both coastal and oceanic habits, may allow for genetic flow among inshore populations. In the Wider Caribbean, two differentiated phylogroups were detected based on the high F_{ST} and Φ_{ST} values: the phylogroup formed by Bocas del Toro (Panama)-Bahamas-Cuba-Mexico that is mostly represented by the inshore form, and the one formed by Colombia-Costa Rica-Honduras-Puerto Rico which is mostly represented by Worldwide distributed form individuals. Despite this differentiation, similar haplotype and nucleotide values were found between the two phylogroups, likely because some countries such as Colombia (La Guajira), Cuba, Mexico, and Puerto Rico shelter both genetic forms (Caballero et al., 2012). Furthermore, three haplotypes (CG3, CG4.1, and CG4.2) from three samples obtained in La Guajira seem to be intermediate haplotypes between inshore and Worldwide distributed forms (Fig. 2 and Fig. 3). Bootstrap support values segregating both forms are high (Fig. 2), but more extensive sampling for genetic and genomic analyses are needed to elucidate the origin of these intermediate haplotypes. Here, we hypothesize these haplotypes could be either conserved haplotypes or the result of the genetic exchange between inshore and Worldwide distributed forms. It is interesting to note that the HH and LL haplotypes form Puerto Rico also nest in this intermediate zone. A previous study using mitochondrial DNA suggested possible ancestral connectivity between Puerto Rico and the Mediterranean Sea (Tezanos-Pinto et al., 2009); therefore, this may support our second hypothesis.

The common bottlenose dolphin is likely at risk in Colombia and worldwide (Avila et al., 2018; Avila & Giraldo, 2022), particularly coastal populations that are more vulnerable to human activities (Avila et al., 2018). In the Caribbean, a recent review documented at least 68 threats for common bottlenose dolphins: in Aruba (2 threats), Bahamas (2), Bermuda (1), Colombia (2), Cuba (42), Dominican Republic (5), Guyana (3), Haiti (2), Honduras (1), Mexico (1), Puerto Rico (2), and Venezuela (3) (Avila et al., 2018). These threats are related to anthropogenic activities that include fisheries (reported as interaction with fishing activities in six cases), hunting (25), tourism (23), scientific research (7), unreported direct human activities (1), urban development (1), and unidentified sources (5) (Avila et al., 2018). However, common bottlenose dolphins in the Caribbean could be exposed to more threats in other countries that were not included in the review. These threats include contamination by mercury exposure, reported in Bocas del Toro, Panama (Barragán-Barrera, Luna-Acosta et al., 2019), and in Colombia and Belize for this species and other small delphinids (Barragán-Barrera, Farías-Curtidor et al., 2019; González-Velásquez et al., 2020), as well as boat traffic, reported extensively in Bocas del Toro-Panama (e.g., Kassamali-Fox et al., 2020; May-Collado et al., 2012, 2017; May-Collado & Quiñones-Lebrón, 2014; Pérez-Ortega et al., 2021). The general lack of monitoring in the Central American Caribbean may further threaten inshore populations of common bottlenose dolphins.

Considering the threats that bottlenose dolphins face in the Wider Caribbean, identifying genetically distinct dolphin populations is critical to designing and implementing management plans. The case of common bottlenose dolphins in Bocas del Toro exemplifies this situation, where a small (seemingly isolated) population that is vulnerable to contaminant exposure and boat traffic from dolphin-watching activities (Barragán-Barrera, Luna-Acosta et al., 2019; May-Collado et al., 2017), has been proposed to be categorised as endangered at the local level based on ecological and genetic studies (Barragán-Barrera et al., 2017,

Barragán-Barrera, Luna-Acosta et al. 2019; May-Collado et al., 2017).

The definition of population structure is required for effective conservation planning. Considering the threatened level for the common bottlenose dolphins in the Central American Caribbean, further genetic studies are needed. The 'inshore form,' previously reported in the Caribbean (Barragán-Barrera et al., 2017; Caballero et al., 2012; Tezanos-Pinto et al., 2009), needs to be studied in detail (particularly in Central America) to detect the inshore form level of isolation from the western North Atlantic (Costa et al., 2022). The results of population structure studies would provide critical information for local management plans. Conversely, in the case of Worldwide distributed form dolphins, a regional plan at the Caribbean level is urgent to maintain the genetic flow between populations. At the national level, Colombia and Panama must strengthen research and develop effective management plans to accomplish better boat and fishing practices, and reduce anthropogenic stressors for the inshore populations. La Guajira deserves special attention as a potentially important transit area for both common bottlenose dolphins' forms in the Caribbean Sea.

Here, we provide further evidence that dolphins in Bocas del Toro belonged to the 'inshore form,' and for the first-time provide genetic evidence that the dolphins sampled in La Guajira include both genetic forms, mainly the Worldwide distributed form. The single inshore haplotype in La Guajira may indicate that the northern portion of the Colombian Caribbean may be occupied by 'inshore' individuals. However, there is insufficient data to confirm this assumption or establish the common bottlenose dolphin conservation status, unlike in Bocas del Toro.

This study constitutes the first step to decreasing the knowledge gaps related to the genetic status of common bottlenose dolphins in the western Caribbean and highlights the urgent need of conducting more studies in Colombia and in the Central American Caribbean. Confirming the real conservation status

of common bottlenose dolphins and the genetic connectivity between populations is key to determine at which scale management must be implemented. Under this context, further research is critical for conservation management of inshore populations that could be isolated and at high risk, but their status is currently unknown.

See supplementary material
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