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Phylogeography, genetic diversity and population structure of common bottlenose dolphins in the Wider Caribbean inferred from analyses of mitochondrial DNA control region sequences and microsatellite loci: conservation and management implications

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Abstract

This study presents the first comprehensive genetic analyses of common bottlenose dolphin (*Tursiops truncatus*) based on mitochondrial DNA and microsatellite loci in the Wider Caribbean. Live captures of bottlenose dolphins have been occurring since the turn of the 20th century in Wider Caribbean waters where little is known about their population structure and genetic diversity. In this study, blood or tissue samples were obtained from stranded or captive dolphins from nine geographic regions. One hundred fifty-eight sequences of the mitochondrial DNA control region and nine microsatellite loci were analyzed and compared with previously published sequences. This study revealed the presence of 'inshore' ecotype and 'worldwide distributed form' haplotypes of bottlenose dolphins in Wider Caribbean waters. At the mitochondrial level, genetic differentiation between these two groups was significant ($F_{ST} = 0.805$, $P < 0.001$). Analyses of mitochondrial DNA sequences at a wider geographic level revealed three genetically differentiated ($F_{ST} = 0.254$, $\Phi_{ST} = 0.590$, $P < 0.001$) population units: Puerto Rico, Cuba/Colombia/Bahamas/Mexico, and Honduras. There was evidence of low female-mediated gene flow among these population units ($N_{mf} = 1.46$). Microsatellite analyses identified four somewhat different population units: Honduras/Colombia/Puerto Rico, Bahamas, Cuba and Mexico. The presence of 'worldwide distributed form' and 'inshore' ecotype haplotypes in particular population units, may be causing differences in the population structure pattern showed by each molecular marker. Decreased observed heterozygosity and three loci out of the Hardy–Weinberg equilibrium were found in the Honduras/Colombia/Puerto Rico population unit suggesting a Wahlund effect. The genetic differentiation and divergence between the two forms identified in this study must be taken into consideration for captive programs that aim to reproduce bottlenose dolphins from this region. Although genetic diversity at the mitochondrial and microsatellite level in these dolphins seems to be relatively high, additional demographic and abundance data must be obtained before more captures are allowed.

Introduction

The common bottlenose dolphin (*Tursiops truncatus*) is distributed worldwide in tropical and temperate waters. Despite being one of the most studied cetacean species (Reynolds, Wells & Eide, 2000) and the dolphin species most commonly displayed in captivity at aquariums and zoos, *T. truncatus* has been classified by the International Union for Conservation of Nature Red Data Book as 'insufficiently known'. It is therefore possible that some populations may be at risk but not enough data has been gathered and more information must be acquired (Wells & Scott, 1999). Particularly because most coastal populations face human pressure including, for example, habitat loss and degradation (Reeves *et al*., 2003), direct negative interactions with boats and fisheries (Wells *et al*., 2008), pollution, incidental catches and directed fisheries-related takes (Wells & Scott, 1999).

Similarly, its taxonomy has long been controversial (Hershkovitz, 1966). Today, *T. truncatus* and *T. aduncus* are currently accepted species (Perrin, Thewissen & Würsig, 2009) based on independent lines of evidence obtained from morphology, osteology and genetics (Wang, Chou & White, 1999, 2000a,b; Hale, Barreto & Ross, 2000; Möller & Beheregaray, 2001; Kakuda *et al*., 2002; Kemper, 2004; Kurihara & Oda, 2006, 2007). However, the taxonomic relationships within *Tursiops* are unclear at the global level, thus requiring local studies and examinations of type specimens. A new species, *Tursiops australis*, has been recently described in South Australia (Charlton-Robb *et al*., 2011) and cryptic subspecies have been found in the Black Sea and possibly the Indo Pacific Ocean (Perrin, Robertson, Van Bree *et al*., 2007; Möller *et al*., 2008; Viaud-Martínez *et al*., 2008). It appears that *T. truncatus* may have adapted to different environmental conditions resulting in several different forms or 'ecotypes'. In the Western North Atlantic (WNA) and Gulf of Mexico two ecotypes, 'inshore' and 'offshore ' were described based on morphology, parasite load, hematology profiles, genetics, diet and distribution (Duffield, Ridgway & Cornell, 1983; Hersh & Duffield, 1990; Hoelzel, Potter & Best, 1998, Kingston & Rosel, 2004, Mead & Potter, 1990; Natoli, Peddemors & Hoelzel, 2004; Sellas, Wells & Rosel, 2005). In many regions of the world, however, there is insufficient evidence to distinguish between differential habitat use by individuals (i.e. neritic vs. oceanic) and true ecotype specialization of particular bottlenose dolphin genetic lineages (Segura, Rocha-Olivares, Flóres-Ramírez *et al*., 2006). A recent study (Tezanos-Pinto *et al*., 2009) found that the ecotype previously described as 'offshore' based on mtDNA control region (CR) sequences (Hoelzel *et al*., 1998, Natoli *et al*., 2004), represents a worldwide distributed form than inhabits both neritic and oceanic habitats. Conversely, the 'inshore' ecotype found in the WNA is highly differentiated from all other populations worldwide, has lower values of genetic diversity and is restricted to the WNA, possibly representing a different taxonomic unit (Natoli *et al*., 2004).

Despite the potential for long-distance dispersal within *T. truncatus*, significant population structure over relatively small geographic distances have been detected among coastal regional populations such as those found along the coasts of the Gulf of Mexico, Florida, Bahamas, New Zealand, United Kingdom, Mediterranean and Black Seas (Wells, 1986; Hoelzel *et al*., 1998; Parsons *et al*., 2002; Torres *et al*., 2003; Natoli *et al*., 2004, 2005; Sellas *et al*., 2005; Parsons *et al*., 2006; Remington *et al*., 2007; Viaud-Martínez *et al*., 2008; Tezanos-Pinto *et al*., 2009; Urian *et al*., 2009). The only *T. truncatus* population studied to date, where no significant population structure was found is in the North Atlantic off the Azores and Madeira (Quérouil *et al*., 2007). In this region, long-distance movements provide opportunities for interbreeding between neighboring localities, resulting in lack of genetic differentiation.

In the Caribbean Sea and adjacent waters, there are only two formal studies on the genetic structure of *T. truncatus* published to date. Fine-scale population structure was found between three *Tursiops* populations in Northern Bahamas suggesting different units for conservation and management (Parsons *et al*., 2006). A worldwide comparison of *T. truncatus* mtDNA haplotypes (Tezanos-Pinto *et al*., 2009) that included 13 samples collected in the Caribbean suggested possible ancestral connectivity between Puerto Rico and the Mediterranean sea. This study also suggested the presence of the 'inshore' WNA ecotype in Puerto Rico.

Live-captures for this species exist since the turn of the 20th century. Until 1980, it was estimated that 1500 *Tursiops* were removed from the US, Mexico and the Bahamas for public display or research (Wells & Scott, 1999). When the US capture for captivity programs were eliminated (in the mid 1980s), other countries in the Wider Caribbean developed their own project-specific capture and display programs. In the late 1990s, facilities holding wild-caught bottlenose dolphins of Caribbean origin proliferated in this region and Europe (Fisher & Reeves, 2005; Van Warebeek *et al*., 2006). Today, such display facilities are found in Mexico, Cayman Islands, Cuba, Bahamas, Jamaica, Dominican Republic, British Virgin Islands, Antigua, Anguilla, Curaçao, Belize, Venezuela, Colombia and Honduras (Mignucci-Giannoni, 1998; Fisher & Reeves, 2005). New facilities are slated for Puerto Rico, St. Lucia, Aruba and Dominica. In Europe, at least 20 facilities include in their exhibition programs bottlenose dolphins captured in either Cuba or Mexico. Captures for public display also took place in the Dominican Republic (Parsons *et al*., 2010), Guyana and Haiti (Fisher & Reeves, 2005).

Despite the increasing demands of the captive industry for public-display dolphins, no study or population assessment has been carried out locally or regionally to evaluate the impacts of such takes. Furthermore, the genetic identity of many populations is still debatable, which may result in costly hybrid mistakes by captive breeding programs, including undesirable traits, introduction of foreign pathogens, outbreeding, or unplanned introductions outside the distribution range of the species or specific discrete populations (Frankham, 2003; Reeves & Brownell, 2009).

The aim of this study was to gain initial understanding of the phylogeography and population structure of bottlenose dolphins in the Wider Caribbean by analyzing mtDNA CR sequences and eleven microsatellite loci to answer three questions: (1) Are 'inshore' ecotype dolphins found in the Wider Caribbean?; (2) Should Caribbean *Tursiops* be treated as a regional stock or does each country have distinct stocks that should be managed accordingly in view of the increase capture and translocation of bottlenose dolphins in the Wider Caribbean for captivity?; and (3) What is the estimated genetic diversity for these groups and would they have enough resilience to continue supporting directed captures and the effects of stochastic environmental and/or demographic events?

Materials and methods

Sample collection

International collaboration was the main guiding methodology for this study, with over 21 colleagues, aquarists and veterinarians from different institutions providing or assisting with sample collection. Samples were obtained from stranded or captive dolphins (Table 1). Blood samples were obtained from captive dolphins in different aquariums in Europe and throughout the Wider Caribbean, following protocols approved by institutional animal care and use committees. Skin samples were obtained from dead stranded dolphins or specimens in museum collections. Skin samples were either preserved in 20% dimethyl sulfoxide (DMSO) saturated with sodium chloride or in 70% ethanol. Blood samples were stored in a lysis buffer solution. Samples were obtained from animals originating from a total of nine Caribbean geographic locations including Bahamas ($n = 15$), Colombia ($n = 4$), Cuba ($n = 65$), Honduras ($n = 6$), Jamaica ($n = 1$), Mexico (Gulf of Mexico and Quintana Roo, $n = 40$), Puerto Rico ($n = 26$), and the US Virgin Islands ($n = 1$) (Fig. 1). For additional phylogeographic comparisons and to find haplotypes shared between the Caribbean groups and other populations around the world, one sample from Japan and two samples from the Galápagos Islands were sequenced, and 306 previously published and available sequences from GenBank were used for comparisons. These included sequences from Gulf of Mexico (Natoli *et al*., 2004; Rosel, unpubl. data), Eastern North Pacific, WNA (coastal form), WNA (pelagic form), Mediterranean Sea, Eastern North Atlantic, West Atlantic, South Africa (Natoli *et al*., 2004), Bahamas (Natoli *et al*., 2004; Parsons *et al*., 2006), China (Wang *et al*., 1999), the Black Sea (Viaud-Martínez *et al*., 2008), Gulf of California (Segura *et al*., 2006), Azores, Madeira and mainland Portugal (Quérouil *et al*., 2007), New Caledonia, New Zealand, Kiribati Islands, Samoa, Japan and French Polynesia (Tezanos-Pinto *et al*., 2009), East Coast of the US (Rosel, unpubl. data), Brazil, Peru, Italy and Israel (Barreto, unpubl. data).

DNA extraction, polymerase chain reaction (PCR) amplification and mtDNA CR sequencing

DNA extraction from skin samples followed the protocol of Sambrook, Fritsch & Maniatis (1989) modified for small samples by Baker *et al*. (1994), and blood samples were extracted using the DNeasy kit (QIAGEN, Valencia, CA, USA). A portion of about 650 bp of the mitochondrial CR was amplified using the primers t-Pro-whale M13Dlp1.5 (5′-TGTAAAACGACAGCCAGTTCACCCAAAGCTG RARTTCTA-3′) and Dlp8 (5′-CCATCGWGATGTCTT ATTTAAGRGGAA-3′), following the amplification conditions from Baker *et al*. (1998). PCR products were cleaned using the PureLink PCR cleaning kit (INVITROGEN) and sequenced using the standard protocols of BigDye™ on an ABI 3100 Perkin-Elmer (Boston, MA, USA) automated capillary sequencer.

Microsatellite genotyping

One hundred twenty-three individuals from which we had mtDNA sequences, were genotyped with a panel of nine polymporphic loci: D08, D22 (Shinohara, Domingo-Roura & Takenaka, 1997), TexVet7, TexVet5 (Rooney, Merritt & Derr, 1999), MK6, MK8, MK9 (Krützen *et al*., 2001), EV1 (Valsecchi & Amos, 1996) and Tur48, Tur91, Tur117 (Nater, Kopps & Krützen, 2009). The loci were divided in two groups for amplification with a Multiplex PCR kit (QIAGEN), details of the groupings and the concentrations for each fluorescent dye are provided in the supplementary material (Supporting Information Table S1). PCR conditions were the same for both groups and consisted of 10–20 ng of genomic DNA, 5 μL of Multiplex Mix and 3 μL of primer mix in a 10 μL reaction. The PCR profile was as follows: 95°C for 15 min followed by 30 cycles of 94°C for 30 s, 60°C for 90 s and 71°C for 45 s, with a final extension of 72°C for 2 min. Both multiplexes were genotyped with the Beckman Coulterer system. All loci were run in Microchecker (Van Oosterhout *et al*., 2004) to check for null alleles, missed genotyping and stutter bands.

Data analyses

MtDNA CR sequence analyses

All sequences were manually edited and aligned using Sequencher 4.1 software (Gene Codes Corporation, Ann Arbor, MI, USA). Haplotypes were defined using MacClade (Maddison & Maddison, 2000) and for phylogeographic comparisons, two consensus regions of 293 and 386 bp were compiled, analyzed and compared with all sequences available from GenBank, in order to detect haplotypes shared among populations from around the world. The model of substitution was tested in Modeltest v3.06 (Posada & Crandall, 1998) and the settings for this model were used in the phylogenetic reconstructions using maximum parsimony, maximum likelihood and neighbor-

Table 1 Geographic region, sampling location, sampling sizes obtained, haplotypes and ecotype or form from *Tursiops truncatus* in the Wider Caribbean

Geographic region	Original sampling location	Sample size	Collection site/display facility	Haplotypes	Ecotype or form
Bahamas	Abaco Island	10	Dolphin Encounters, Bahamas	A (3), E (7)	Inshore Ecotype
	New Providence	1	Dolphin Experience (Bahamas, $n = 1$)	E	Inshore Ecotype
	Grand Bahama	1	Dolphin Experience (Bahamas, $n = 1$)	A	Inshore Ecotype
	Unknown	3	Dolphin Encounters, Bahamas	A (2), E (1)	Inshore Ecotype
Colombia	Ciénaga, Magdalena Province	1	Museo Universidad de los Andes, Colombia	MM	Worldwide Distributed Form
	Golfo de Morrosquillo, Córdoba Province	3	Oceanario Islas del Rosario, Colombia	C	Worldwide Distributed Form
Cuba	Bahía de Buenavista, Caibarien	65	Xel-ha (Mexico, $n = 6$), Xcaret (Mexico, $n = 5$), Dolphin Discovery (Mexico, $n = 38$), Aspro-Ocio (Spain $n = 5$), Dolphin Fantaseas (Anguilla, $n = 3$), Dolphin Fantaseas (Antigua, $n = 3$), Dolphin Discovery (Antigua, $n = 2$) and Dolphin Discovery (Anguilla, $n = 3$)	A (36), B (12), C (4), D (1), E (1), J (1), K (2), M (1), N (1), O (1), P (1), Q (1), R (1), S (1), L(1)	Inshore Ecotype (57),Worldwide Distributed Form (8)
Honduras	Between la Ceiba and Bahia de Trujillo	6	Dolphin Academy, Curaçao	C (4), G (2)	Worldwide Distributed Form
Jamaica	St. Ann's Bay	1	Dolphin Cove, Jamaica	T	Worldwide Distributed Form
Mexico	Holbox, Quintana Roo	9	Xcaret, Mexico	V (1), Y (3), Z (1), BB (2), QR01 (2)	Inshore Ecotype (4), Worldwide Distributed Form (5)
	Isla Mujeres, Quintana Roo	1	Dolphin Discovery, Mexico	W	Worldwide Distributed Form
	Paraiso, Tabasco, Gulf of Mexico	16	Escollera (Mexico, $n = 1$), Xcaret (Mexico, $n = 11$), Dolphin Discovery (Mexico, $n = 4$)	B (1), F (2), K (1), V (1), AA (5), BB (1), CC (1), DD (1), FF (1), TA02 (2)	Inshore Ecotype
	Celestún, Yucatán, Gulf of Mexico	1	IE Universidad Autónoma de México, México	FF	Inshore Ecotype
	Laguna de Alvarado, Veracruz, Gulf of Mexico	2	IE Universidad Autónoma de México (Mexico, $n = 1$), Dolphin Discovery (Mexico, $n = 1$)	K (1), U (1)	Inshore Ecotype
	Tampico, Tamaulipas, Gulf of Mexico	5	Dolphin Experience (Bahamas, $n = 5$)	D (4), X (1)	Inshore Ecotype
	Matamoros, Tamaulipas, Gulf of Mexico	4	Dolphin Cove, Jamaica	D	Inshore Ecotype
	Laguna de Términos, Campeche, Gulf of Mexico	2	Dolphin Cove, Jamaica	D (1), F (1)	Inshore Ecotype
Jamaica	St. Ann's Bay	1	Dolphin Cove, Jamaica	T	Worldwide Distributed Form
Puerto Rico	Ponce	3	Red Caribeña de Varamientos	C (1), I (2)	Worldwide Distributed Form
	Manati	1	Red Caribeña de Varamientos	C	Worldwide Distributed Form
	Peñuelas	1	Red Caribeña de Varamientos	GG	Worldwide Distributed Form
	San Juan	5	Red Caribeña de Varamientos	H (2), HH (1), JJ (1), LL (1)	Worldwide Distributed Form
	Naguabo	1	Red Caribeña de Varamientos	H	Worldwide Distributed Form
	Salinas	1	Red Caribeña de Varamientos	H	Worldwide Distributed Form
	Cataño	1	Red Caribeña de Varamientos	C	Worldwide Distributed Form
	Lajas	2	Red Caribeña de Varamientos	B (1), unknown	Inshore Ecotype
	Yauco	1	Red Caribeña de Varamientos	C	Worldwide Distributed Form
	Toa Baja	2	Red Caribeña de Varamientos	H (1), unknown	Worldwide Distributed Form
	Vega Baja	1	Red Caribeña de Varamientos	II	Worldwide Distributed Form
	Cabo Rojo	3	Red Caribeña de Varamientos	B (2), H (1)	Inshore Ecotype
	Aguadilla	1	Red Caribeña de Varamientos	C	Worldwide Distributed Form
	Barceloneta	1	Red Caribeña de Varamientos	H	Worldwide Distributed Form
	Humacao	1	Red Caribeña de Varamientos	H	Worldwide Distributed Form
	Isla de Vieques	2	Red Caribeña de Varamientos	H (1), KK (1)	Worldwide Distributed Form
US Virgin Islands	Long Point, St. Croix	1	Red Caribeña de Varamientos	B	Inshore Ecotype

Figure 1 Sampling sites and sizes for Wider Caribbean common bottlenose dolphins included in this study. Red and white circles indicate 'worldwide distributed form' and yellow and white circles indicate 'inshore' ecotype.

joining methods performed in Phylogenetic Analysis Using Parsimony *and other methods (PAUP) v4.0b1 (Sionauer Associates Inc., Sunderland, MA, USA) (Swofford, 2002). The rough-toothed dolphin *Steno bredanensis* was used as outgroup in these analyses.

To investigate the relationship between CR haplotypes found in the Wider Caribbean and to detect the presence of the ecotype previously defined as 'inshore' for the WNA, phylogenetic reconstructions by maximum parsimony, maximum likelihood (using the model HKY+I+G from Modeltest) and neighbor-joining were conducted. Wider Caribbean *T. truncatus* sequences were categorized into the 'inshore' ecotype or the 'worldwide distributed form' by reviewing each published paper for independent evidence from at least two sources (e.g. molecular or biochemical markers, diet, morphology). All haplotype sequences from the WNA coastal (WNAc), Bahamas, and Gulf of Mexico presented consistent diagnosis as the 'inshore' ecotype whereas the rest were classified as the 'worldwide distributed form' (Natoli *et al.*, 2004; Tezanos-Pinto *et al.*, 2009). This analyses also included sequences from two haplotypes from the Pacific (Galápagos Islands and Japan), six from Madeira (Quérouil *et al.*, 2007) and sequences described as WNA pelagic (WNAp) by (Natoli *et al.*, 2004). Analyses of haplotype and nucleotide diversity between the Caribbean sequences described as 'inshore' ecotype and 'worldwide distributed form' were calculated in the program Arlequin (Schneider, Roessli & Excoffier, 2000), and restricted to 386 bp of the CR.

In order to investigate genealogical relationships among Wider Caribbean *T. truncatus* CR haplotypes, Union of Maximum Parsimonious Trees (UMPT) (Cassens, Mardulyn & Milinkovitch, 2005) was used to calculate and construct a network of CR haplotypes. This method required two consecutive steps. First, a maximum parsimony analysis was performed for the CR haplotype data set and all most parsimonious trees were saved with their respective branch lengths. We used the tree bisection and reconnection branch-swapping (1000 replicates with random sequence addition) heuristic search option in PAUP* v.4b10. Second, all saved MP trees were combined into a single figure including all connections from MP trees into a single reticulated

graph, and merging branches, sampled or missing, that were identical among different trees (see Cassens, Mardulyn & Milinkovitch, 2005 for additional details on this analysis). The haplotype frequency was combined with the CR haplotype network, and the final network was drawn by hand.

Population structure analyses were performed in the program Arlequin (Excoffier, Smouse & Quattro, 1992) and restricted to 386 bp of the CR. To evaluate genetic boundaries between the sampling locations studied, we performed a spatial analysis of molecular variance (SAMOVA) (Dupanloup, Schneider & Excoffier, 2002). Genetic differences among the estimated population units detected in the SAMOVA analysis were then quantified by an analysis of molecular variance (AMOVA) as implemented in Arlequin (Excoffier *et al.*, 1992) based on conventional F_{ST} and Φ_{ST} statistics, using 10 000 random permutations. Genetic diversity reflected in haplotype and nucleotide diversity for each population unit were performed in the program Arlequin (Excoffier *et al.*, 1992) and restricted to 386 bp of the CR. The number of female migrants per generation (N_{mf}), as a measure of gene flow among localities, was estimated based on the F_{ST} value, using the equation $N_{mf} = 1/2(1/F_{ST}–1)$ (Takahata & Palumbi, 1985) assuming Wright's island model. Female migration rates per generation (N_{mf}) among each pair of population units were estimated using the Markov chain Monte Carlo (MCMC) coalescent approach in the program Migrate 3.0.3 (Beerli & Felsenstein, 2001; Beerli, 2003). The program was run with all the population units at the same time, using maximum likelihood. Multiple runs were performed to assess solution convergence with parameter estimates obtained using MCMC parameters as follow: ten short chains (500 used trees out of a sampled 10 000) by three long chains (5000 used trees out of a sampled 100 000) and a burn-in of 10 000.

Microsatellite analyses

The patterns of genetic structure were analyzed with Structure 2.3.1 (Pritchard, Stephens & Donnelly, 2000). The burn in period was set to 150 000 iterations and the probability estimates were determined using 5 000 000 Markov chain Monte Carlo (MCMC) iterations. Runs were conducted with K set from 1 to 9 with five runs for each value of K with the admixture model and correlated frequencies. To obtain the true value of K from the log probability of the data LnP(D), Evanno, Regnaut and Goudet (2005) developed an ad hoc statistic called ΔK that calculates the second order rate of change of Ln P(D) between the values of K. ΔK was calculated and the corresponding values for each K were plotted to determine the uppermost level of population structure for our dataset (Supporting Information Figure S1). The population units determined by structure were analyzed for the Hardy–Weinberg equilibrium (HW), genetic diversity, genetic differentiation and gender-biased dispersal. Deviation from HW equilibrium and genetic diversity were calculated as expected and observed heterozygosity (H_E and H_O) with the program Arlequin 2.0 (Schneider *et al.*, 2000). Allelic richness (AR) was calculated with FSTAT 2.9.3.2 (Goudet, 1995). Pairwise comparisons of genetic differentiation (F_{ST}) were conducted with the program GENEPOP and FSTAT was used to test the significance of the resulting estimates. Pairwise comparisons of genetic differentiation for R_{ST} values averaged over variance components and loci were calculated with RstCalc as recommended by Goodman (1997). As F_{ST} has proven to be restricted to show high levels of differentiation when loci show high values of heterozygosity, the index (D_{EST}) (Jost, 2008), was also obtained. D_{EST} was calculated with the program SMOGD (Crawford, 2010) and compared with both F_{ST} and R_{ST}. Linkage disequilibrium for each locus was calculated with GENEPOP. A sequential Bonferroni correction (Rice, 1989) was applied later to assess significance values. Gender-biased dispersal between the populations was tested with FSTAT 2.9.3.2 based on 100 randomizations and one-sided (Goudet, 1995).

Results

MtDNA CR phylogeography and ecotype classification

A total of 158 sequences were successfully obtained from the Wider Caribbean region. A total 386 bp of the CR were analyzed. Forty-one haplotypes were defined by 36 variable sites. Twenty-five haplotypes were defined in only one individual (Table 2). Haplotype sequences were submitted to GenBank as accession numbers JN596281–JN596321. Phylogenetic reconstructions by maximum parsimony, maximum likelihood (using the model HKY+I+G from Modeltest) and neighbor-joining were performed and combined with the haplotype frequency for each sampled region (Fig. 2). Two haplotypes were shared between Cuba and Bahamas (A and E), one haplotype was shared between Cuba, Mexico, Puerto Rico and the US Virgin Islands (B) and one haplotype was shared between Cuba, Honduras, Colombia and Puerto Rico (C). Haplotypes D and K were shared between Cuba and Mexico (Fig. 2). In wider phylogeographic comparisons using GenBank sequences, haplotype B was identified previously in the Bahamas (accession number AF155162) (Parsons *et al.*, 2006) and the Gulf of Mexico (Natoli *et al.*, 2004) and haplotype I, determined from two samples from Puerto Rico, was identified as haplotype MS.5 and TT009 previously found in the Mediterranean Sea and the Azores, respectively (Natoli *et al.*, 2004; Quérouil *et al.*, 2007; Tezanos-Pinto *et al.*, 2009).

Twenty-three haplotypes from the Wider Caribbean were grouped with haplotypes classified as 'inshore' WNA and 18 haplotypes were grouped in a node formed by the 'worldwide distributed form' (Fig. 3). Thirty-one out of 41 haplotypes detected in the Wider Caribbean were included in the UMPT analysis. Ten were excluded because they contained a high amount of missing data, as this is known to affect the performance of the algorithm used for combination of all most parsimonious trees into one network or haplotype genealogy. Twenty most parsimonious trees were obtained and these were combined in the haplotype genealogy

Table 2 Thirty-eight variable sites over 386 bp of the mitochondrial control region determining 41 Caribbean *Tursiops truncatus* haplotypes

Haplotypes (Variable sites)	5	10	42	53	83	88	89	94	97	103*	128	146	184*	224	233	244	253	255	257	258	259*	261	262	269	272	273	280	282	303	304	305	308	336	349	353	370	371	372
TtruCAR-A	T	T	G	-	A	T	A	C	A	T	C	T	T	C	A	C	T	T	T	T	C	T	C	A	T	C	T	A	G	A	T	C	C	T	C	C	T	C
TtruCAR-B	C	.	.	C	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	.	.	C	T	.	.	.	.	.	A	.	.	.	T	.	.	.	.	.
TtruCAR-C	C	.	.	–	G	.	G	.	.	–	.	.	C	T	.	A	-	.	C	.	T	C	.	.	.	.	.	G	A	.	.	.	T	.	.	.	C	T
TtruCAR-D	.	.	.	–	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.
TtruCAR-E	.	.	.	–	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	G	.	.	.	.	.	.	.	.
TtruCAR-F	.	.	.	–	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	A	.	.	.	.	.	.	.	.	.
TtruCAR-G	C	.	.	–	G	.	G	.	.	–	.	.	C	T	.	A	-	.	C	C	T	C	.	.	.	.	.	G	A	.	.	.	T	.	.	T	C	T
TtruCAR-H	C	.	.	–	G	.	G	.	.	–	.	.	C	.	.	A	-	.	C	.	T	C	.	.	.	T	.	G	A	.	.	.	T	.	.	.	C	T
TtruCAR-I	C	.	.	–	.	.	G	.	.	–	.	.	C	T	.	A	C	.	C	.	T	C	.	.	.	.	.	G	A	.	.	.	T	.	.	T	C	T
TtruCAR-J	C	.	.	–	G	.	G	.	.	–	.	.	C	T	.	A	-	.	C	.	T	C	.	.	.	.	.	G	A	.	.	.	T	.	T	?	?	?
TtruCAR-K	.	.	.	–	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	?	?	?
TtruCAR-L	.	.	.	–	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	?	?	?	?	?
TtruCAR-M	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	
TtruCAR-N	?	?	.	–	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.
TtruCAR-O	?	?	.	–	G	.	.	.	.	–	.	.	C	T	.	A	C	.	C	.	T	C	.	.	.	.	.	.	A	.	.	.	T	.	.	.	C	T
TtruCAR-P	?	?	.	–	G	.	G	.	.	–	.	.	C	T	.	A	-	.	C	.	T	C	.	.	.	.	.	G	A	.	.	.	T	.	.	.	C	T
TtruCAR-Q	C	.	A	–	.	.	.	T	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	G	.	.	C	G	A	.	C	.	.	.	.	.	.	.
TtruCAR-R	C	.	.	–	G	.	.	.	.	–	.	.	C	T	.	A	C	.	C	.	T	C	.	.	.	.	.	.	A	.	.	.	T	.	.	.	C	T
TtruCAR-S	.	.	.	–	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.
TtruCAR-T	C	.	.	–	.	.	G	.	.	–	.	.	C	T	.	A	C	.	C	.	T	.	.	.	.	.	.	G	A	.	.	.	T	.	.	T	C	T
TtruCAR-U	C	.	.	C	.	.	.	.	G	.	T	.	.	T	.	.	.	.	.	.	.	C	T	.	.	.	.	.	A	.	.	.	T	.	.	.	.	.
TtruCAR-V	C	.	.	C	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	A	.	.	.	T	.	.	.	C	T
TtruCAR-W	C	.	.	–	G	.	G	.	.	–	.	.	C	T	.	A	-	.	.	.	T	C	.	.	.	.	.	.	A	.	.	.	T	.	.	.	C	T
TtruCAR-X	.	.	.	–	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	C	.	.	.	.
TtruCAR-Y	C	.	.	–	G	.	G	.	.	–	.	.	C	T	.	A	C	.	C	.	T	C	.	.	.	.	.	.	A	.	.	.	T	.	.	?	?	?
TtruCAR-Z	C	.	.	C	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	.	.	C	T	.	.	.	.	.	A	.	.	.	T	.	?	?	?	?
TtruCAR-AA	.	.	.	–	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	A	.	.	.	.	?	?	?	?	?
TtruCAR-BB	C	.	.	C	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	A	.	.	.	T	.	.	?	?	?
TtruCAR-CC	C	.	.	C	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	A	.	.	.	T	?	?	?	?	?
TtruCAR-DD	C	.	.	C	.	.	.	.	G	.	T	C	.	.	.	.	.	.	.	.	.	C	T	.	.	.	.	.	A	.	.	.	T	?	?	?	?	?
TtruCAR-EE	C	.	.	C	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	.	.	C	T	.	C	.	.	.	A	.	.	.	?	?	?	?	?	?
TtruCAR-FF	C	.	.	C	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	.	.	C	T	.	C	.	.	.	A	.	.	.	T	.	.	.	.	.
TtruCAR-GG	C	.	.	–	G	.	G	.	.	–	.	.	C	T	.	A	-	.	C	.	T	C	.	.	.	.	.	G	A	.	.	.	?	?	?	?	?	?
TtruCAR-II	?	?	.	–	G	.	G	.	.	–	.	.	C	.	.	A	-	.	C	.	T	C	.	.	.	T	.	G	A	.	.	.	T	.	.	.	C	T
TtruCAR-HH	?	?	.	–	.	C	G	.	.	–	T	.	C	.	.	.	C	.	C	C	T	C	.	.	.	.	.	.	A	.	.	.	.	.	.	T	C	T
TtruCAR-JJ	.	.	.	–	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	A	G	.	.	.	.	.	.	.	.
TtruCAR-KK	?	?	.	–	G	.	G	.	.	–	.	.	C	.	.	A	-	.	C	.	T	C	.	.	.	T	.	G	A	.	.	.	T	.	.	.	C	T
TtruCAR-LL	C	C	.	–	.	C	G	.	.	–	T	.	C	T	.	T	C	.	C	.	T	C	.	.	.	.	.	.	A	.	.	.	.	.	.	T	C	T
TtruCAR-MM	C	.	.	–	G	.	G	.	.	–	.	.	C	T	.	A	-	C	C	.	T	C	.	.	.	.	.	G	A	.	.	.	T	.	.	.	C	T
TtruCARQR1	C	.	.	–	G	.	G	.	.	–	.	.	C	T	.	A	-	.	C	.	T	C	T	.	.	.	.	G	A	.	.	T	?	?	?	?	?	?
TtruCAR1TA02	.	.	.	–	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	?	?	?	?	?	?

(?) denotes missing information, (–) a gap in the allignement (insertions/deletions) and (*) a fixed-site between what appear to be 'inshore' ecotype and 'worldwide distributed form' haplotypes after comparison with previously published sequences (Natoli *et al*., 2004; Quérouil *et al*., 2007). Haplotype E = PR610 and haplotype JJ = PR616 as published in Tezanos-Pinto *et al*., 2009).

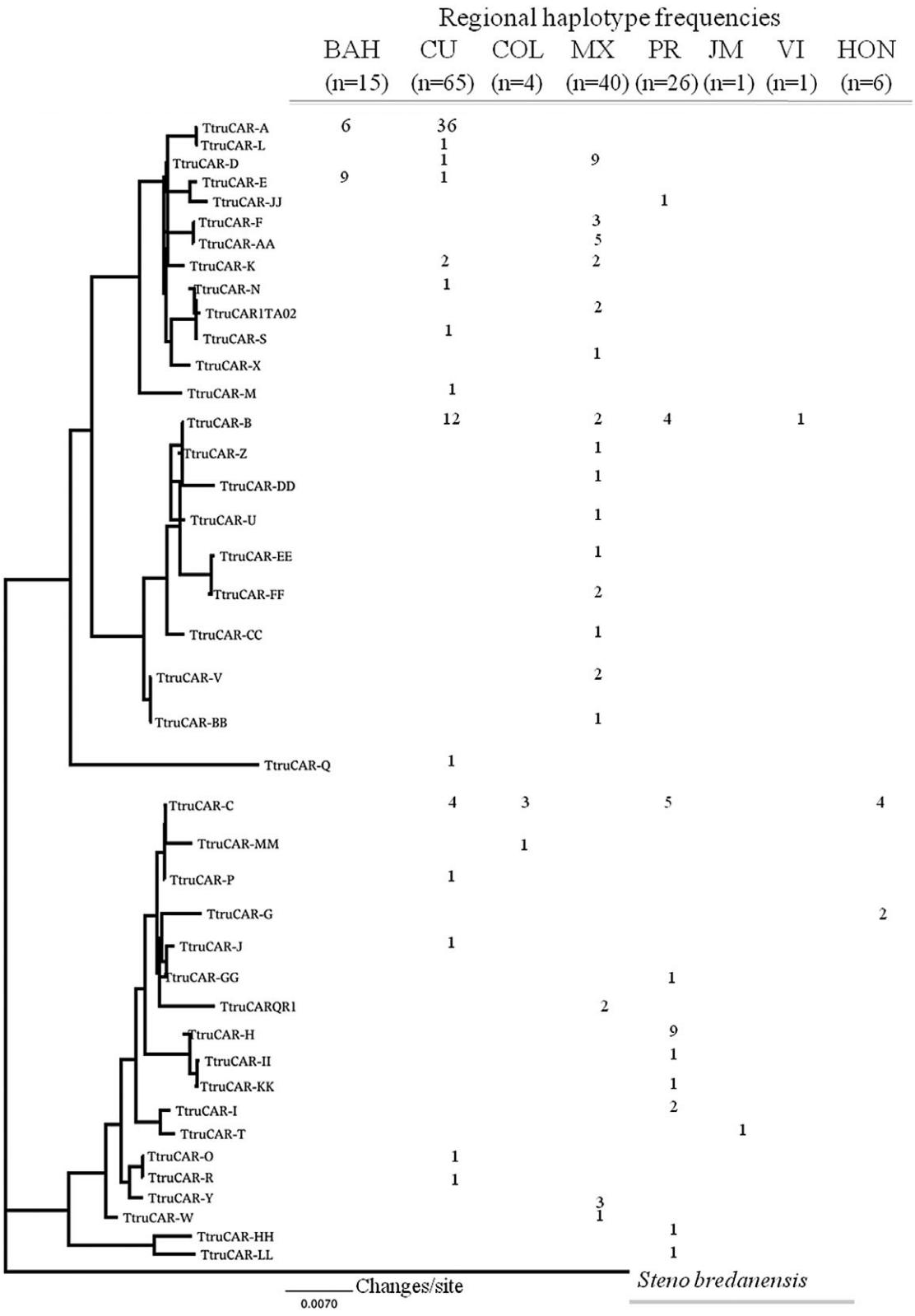

Figure 2 Maximum-likelihood phylogenetic reconstruction of Wider Caribbean control region haplotypes combined with the haplotype frequency found in each sampled region. Bootstrap support values higher than 50 are shown on branches.

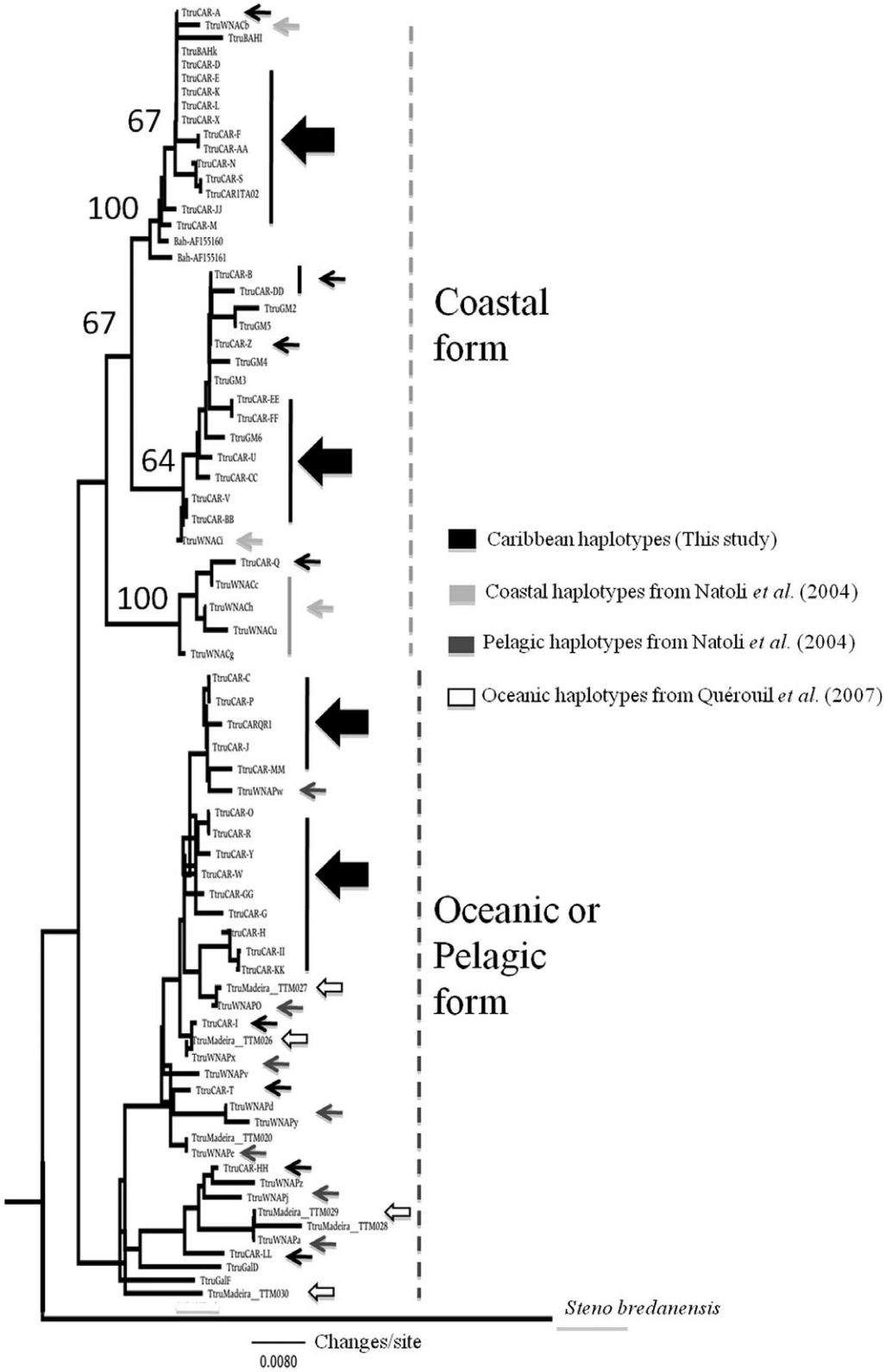

Figure 3 Maximum-likelihood phylogenetic reconstruction showing grouping of Wider Caribbean Control Region haplotypes with haplotypes previously defined as belonging to the 'inshore' ecotype and the 'worldwide distributed form' common bottlenose dolphins. Bootstrap support values higher than 50 are shown on branches.

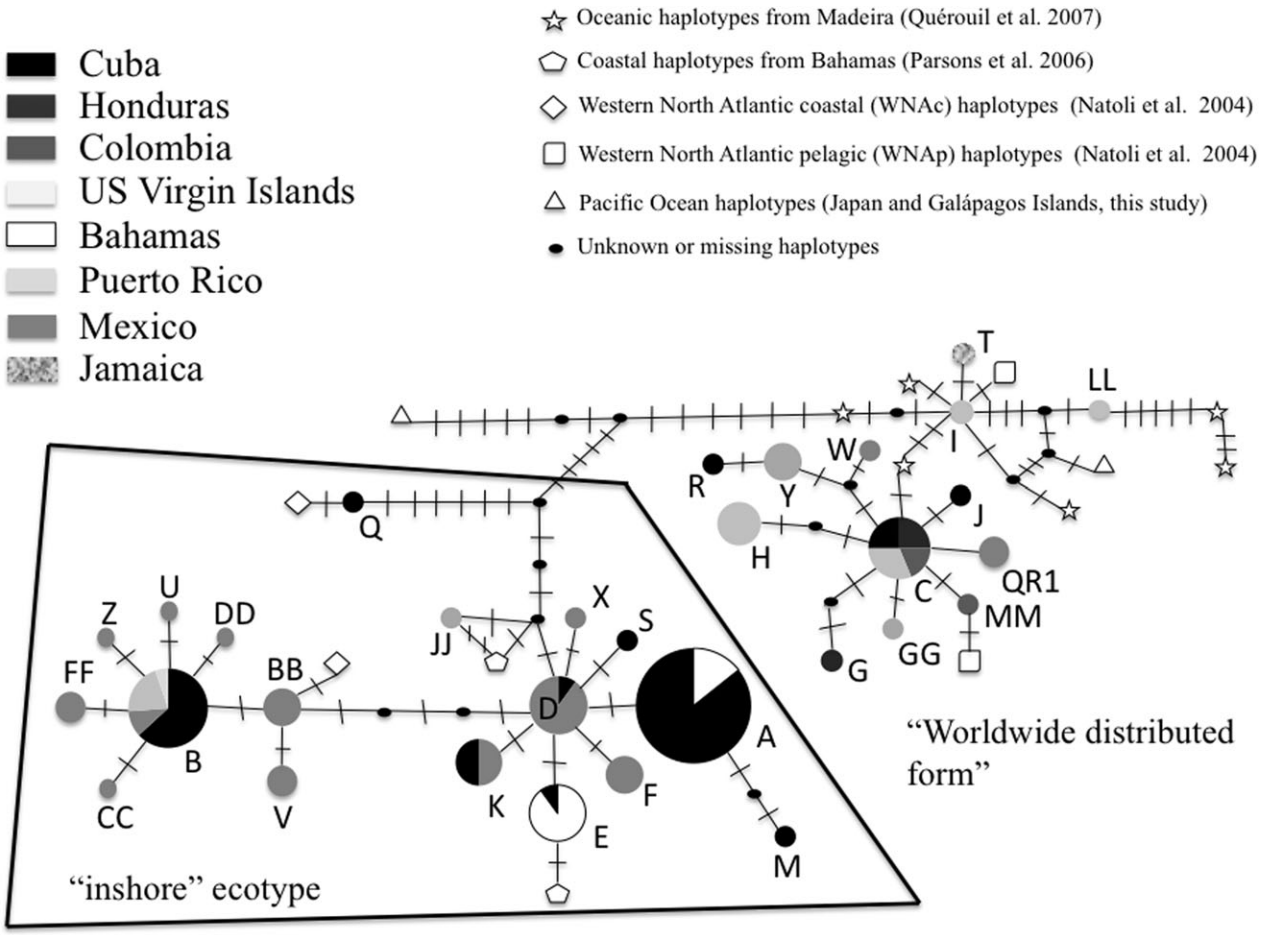

Figure 4 Haplotype genealogy obtained from the Union of Maximum Parsimonious Trees (UMPT) analysis. The size of the circles reflect frequency of a particular haplotype found in Cuba, Honduras, Colombia, US Virgin Islands, Bahamas, Puerto Rico, Mexico and Jamaica. Vertical bars represent substitutions between haplotypes.

(Fig. 4). The haplotypes B, C, D and I in the central position and connected with a high number of other haplotypes are probably the most ancestral. Haplotypes Q and BB were connected to WNA inshore haplotypes and I and MM connected to WNA offshore haplotypes. Haplotypes I and C were connected to haplotypes from Madeira classified as the 'worldwide distributed form'. Haplotypes E and JJ were connected with a haplotype previously classified as 'inshore' ecotype from Bahamas (BahAF155160 and BahAF155161, respectively. Haplotype E = PR610 Haplotype JJ = PR616 were published in Tezanos-Pinto *et al.* (2009). There were 15 unknown or missing haplotypes when conducting the UMPT analysis, which could be ancestral or haplotypes that were not sampled.

MtDNA CR population structure and genetic diversity

We performed all analysis considering sampling regions with $n \geq 2$. Thus, samples from the US Virgin Islands and Jamaica were excluded from all analysis ($n = 1$). Twelve sampling locations were included (see Table 1). We applied the SAMOVA algorithm searching for two to 11 potential population units. The largest mean F_{CT} index was found for three populations units ($F_{CT} = 0.613$) referred to as: (1) Puerto Rico; (2) Cuba/Colombia/Bahamas/Mexico (combining samples from Gulf of Mexico and Quintana Roo); and (3) Honduras. A non-hierarchical AMOVA analysis confirmed significant differences between the population units identified by the SAMOVA. The high degree of genetic differentiation among population units was reflected in the high F_{ST} and Φ_{ST} values obtained in the AMOVA ($F_{ST} = 0.254$, $\Phi_{ST} = 0.590$, $P < 0.001$, and values in Table 3).

For Wider Caribbean *T. truncatus* population units, overall $N_{mf} = 1.46$ females per generation (using $F_{ST} = 0.254$). Female migration rates per generation (N_{mf}) among each pair of populations suggest that the direction of female migration is from Puerto Rico to the Cuba/Colombia/Bahamas/Mexico population unit and from Honduras to the Cuba/Colombia/Bahamas/Mexico population unit (Table 4).

Table 3 Pairwise F_{ST} (below diagonal) and Φ_{ST} (above diagonal) values for control region among Wider Caribbean *Tursiops truncatus* population units

	Φ_{ST}		
F_{ST}	Puerto Rico	Cuba/Colombia/Bahamas/Mexico	Honduras
Puerto Rico	$h = 0.833 \pm 0.056$	0.552	0.683
	$\pi = 1.84 \pm 0.018$	*(<* ***0.0001****)*	*(0.071)*
Cuba/Colombia/Bahamas/Mexico	0.305	$h = 0.662 \pm 0.058$	0.591
	(< ***0.001****)*	$\pi = 1.5 \pm 0.008$	*(<* ***0.0001****)*
Honduras	0.586	0.229	$h = 0.800 \pm 0.122$
	(0.076)	*(<* ***0.0001****)*	$\pi = 0.28 \pm 0.002$

Probability values based on 10 000 permutations shown in italics. Significantly different values ($P < 0.05$) in bold. Haplotype (h) and nucleotide (π) % $\pm$ standard deviation (SD) are shown on the diagonal for each population unit.

Table 4 Most probable estimates of female migration rates per generation (N_{mf}) using maximum likelihood between the three Wider Caribbean *Tursiops truncatus* population units defined in this study (confidence interval at 95%)

	Migration to		
Migration from	Puerto Rico	Cuba/Colombia/Bahamas/Mexico	Honduras
Puerto Rico	–	1.51 (CI = 0.47 – 2.38)	7×10^{-16} (CI = 3.84×10^{-16} – 0.29)
Cuba/Colombia/ Bahamas/Mexico	5.13×10^{-13} (CI = 2.55×10^{-13} – 0.20)	–	5.11×10^{-13} (CI = 2.56×10^{-13} – 0.23)
Honduras	4.78×10^{-13} (CI = 2.39×10^{-13} – 0.60)	0.78 (CI = 0.0079 – 2.38)	–

Haplotype diversity values for Wider Caribbean haplotypes classified as 'inshore' ecotype ($n = 112$, $h = 0.578 \pm 0.049$, $\pi = 0.9\% \pm 0.5$) were lower than values estimated for those haplotypes assigned to the 'worldwide distributed form' ($n = 46$, $h = 0.71 \pm 0.056$, $\pi = 0.6\% \pm 0.4$), but nucleotide diversity was higher for haplotypes assigned to the 'inshore' ecotype. These two groups were significantly differentiated ($F_{ST} = 0.805$, $P < 0.001$).

We found relatively high haplotype and nucleotide diversity in most of the population units considered in this analysis, with the highest haplotype diversity found in the Puerto Rico population unit and the lowest nucleotide diversity found in the Honduras population unit (Puerto Rico $h = 0.85$, $\pi = 1.84\%$; Cuba/Colombia/Bahamas/Mexico $h = 0.66$, $\pi = 1.5\%$; Honduras $h = 0.80$, $\pi = 0.28\%$, Table 3).

Microsatellite genetic diversity, population structure and assignments

Because of the small sample size for Colombia and Honduras, and the irregular sample size in the rest of the countries sampled, a Bayesian clustering analysis was first performed in the structure to determine the number of population units observed in our data. Structure was performed under the admixture model with correlated frequencies as recommended by the structure when populations are likely to have a common ancestor. A clear peak can be observed at K = 4 (Supporting Information Figure S1) (Evanno *et al.*, 2005). To ensure the convergence of the run, fluctuations on the α parameter were observed; according to the Structure Manual, once the MCMC converged, α will stabilize around a value of 0.2 or less. The α parameter for K = 4, fluctuated from 0.05 to 0.25 in the beginning of the run and stabilized at 2.4[6] generations; The four population units detected by structure were: (1) Honduras/Colombia/Puerto Rico ($n = 29$); (2) Bahamas ($n = 11$); (3) Cuba ($n = 53$); and (4) Mexico (Quintana Roo and Gulf of Mexico) ($n = 29$) (Fig. 5). From this point onwards, Population Unit 1 will be referring to the cluster formed by Honduras, Colombia and Puerto Rico.

Genetic diversity values such as expected (H_E) and observed heterozygosity (H_O), number of alleles per population (n) and AR were obtained for nine loci in the four population units analyzed along with deviations from HW equilibrium (Table 5). Heterozigosity values were very similar for Cuba and Mexico while H_E was highest in Bahamas and lowest in Population Unit 1. After Bonferroni correction (*P-value* = 0.001562, Table 5), Population Unit 1 (Honduras–Colombia–Puerto Rico) showed three loci out of equilibrium and the largest difference between H_E and H_O. Cuba and Mexico showed only one microsatellite significantly out of HW equilibrium and no loci was out of HW equilibrium for the Bahamas population unit.

Pairwise population differentiation indices F_{ST}, R_{ST} and D_{EST} were calculated for all sampling locations (Table 6). R_{ST} values were higher than D_{EST} and F_{ST} values, suggesting a deeper ancestral differentiation between sampling locations with some degree of recent gene flow. This could be the case especially between Bahamas and Population Unit 1, showing the smallest F_{ST} value (0.045) and a relatively high R_{ST} value (0.132). This could be related to the fact that all Population Unit 1 individuals were represented by 'worlwide distributed form' haplotypes and all individuals from the Bahamas population unit were represented by 'inshore' ecotype haplotypes. All the Mexico pairwise comparisons had the highest values for all the indices, suggesting certain degree of isolation of this population from the Caribbean. Intermediate differentiation was found between Cuba

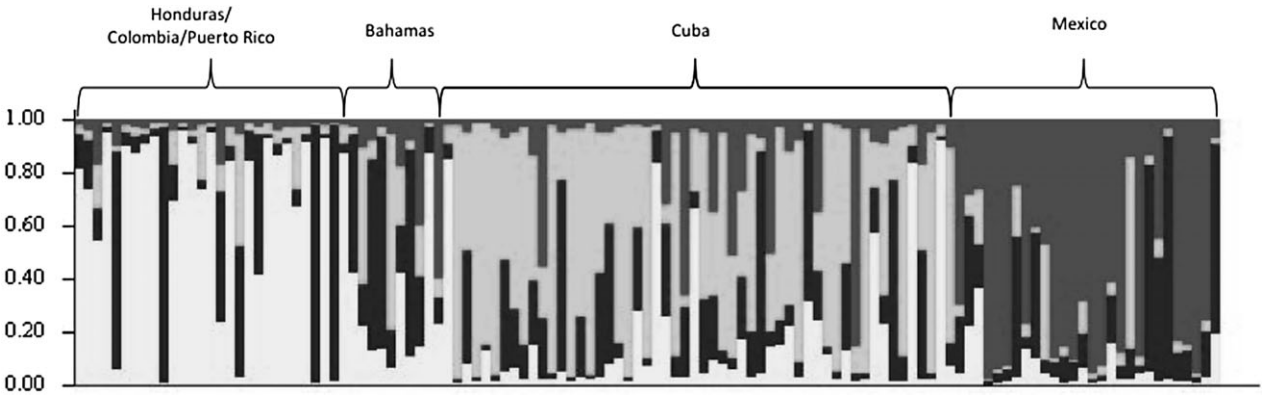

Figure 5 Barplot of the likelihood (*Y*-axis) of each individual's (*X*-axis) assignment to a particular population units for K = 4.

and Bahamas ($F_{ST} = 0.0643$) as well as between Cuba and Population Unit 1 ($F_{ST} = 0.0709$). D_{EST} and F_{ST} values do not show strong differences in our populations, probably because of the intermediate to low levels of genetic diversity found. The gender-biased dispersal test performed by FSTAT was not significant with a *P-value* = 1.000 for the assignment *T*-test and *P-value* = 0.9100 for F_{ST} test between males and females.

Discussion

This study presents the first comprehensive analyses of common bottlenose dolphin mitochondrial DNA and microsatellite markers in the Wider Caribbean and provides key information to scientist, managers and governmental agencies regarding management of these dolphins as an important resource for the captive industry in European and Latin American countries.

Ecotypes and divergence in the Wider Caribbean region

Our analyses demonstrate the presence of at least two genetically differentiated forms of common bottlenose dolphins in the Wider Caribbean, the 'inshore' ecotype and the 'worldwide distributed form'. Specifically, the 'inshore' ecotype commonly found in the WNA, Bahamas and Mexico is also present in many of the Caribbean regions analyzed here. Particularly, the Cuba/Colombia/Bahamas/Mexico mtDNA population unit presented a considerable number of individuals that were assigned to the 'inshore' ecotype. However, it is possible that the 'inshore' ecotype is also present in Honduras but given the small sample size of this population unit in our study ($n = 6$), it was undetected. The distribution of the 'inshore' ecotype and 'worldwide distributed form' overlap in several regions sampled in this study, for example in the Yucatán Península (Quintana Roo), Mexico. Therefore, we suggest that these forms are found in parapatry or maybe even in sympatry in these regions (Islas-Villanueva, 2005); however, future studies investigating distribution and habitat use are needed to clarify this. Some haplotypes described as belonging to the 'worldwide distributed form' were shared between the Caribbean and the Azores as well as with the Mediterranean Sea. This result seems to suggest past or present gene flow among these areas (Silva *et al.*, 2008), supporting the hypothesis of evolutionary interconnection between common bottlenose populations worldwide with founder events and colonization of island and coastal habitats by particular groups as previously suggested (Natoli *et al.*, 2004; Tezanos-Pinto *et al.*, 2009).

Similarly to results obtained in the WNA (Hoelzel *et al.*, 1998; Natoli *et al.*, 2004; Tezanos-Pinto *et al.*, 2009), for the Wider Caribbean, sequences assigned to the 'inshore' ecotype were highly differentiated from those representing the 'worldwide distributed form' ($F_{ST} = 0.805$, $P < 0.001$). Our data further suggest that the 'inshore' ecotype should be recognized as a distinct lineage within *Tursiops truncatus*. Mitochondrial data suggests little, if any, maternal gene flow at present. Specific adaptations to a neritic environment include an inshore distribution, differences in ecology, foraging, parasite load, morphology and genetics (Mead & Potter, 1990; Kingston & Rosel, 2004). Previous studies suggested that the WNA 'inshore' ecotype could be considered a different taxonomic unit (Natoli *et al.*, 2004). Whether this ecotype represents a true species/subspecies grants further investigation; however, it is clear that the 'inshore' ecotype is found in the Wider Caribbean and seems to be following an independent evolutionary trajectory. Additional studies on common bottlenose dolphins in the Wider Caribbean investigating historical demography are needed in order to clarify possible divergence dates between the 'inshore' ecotype and the 'worldwide distributed form' as well as present migration rates between ecotypes and population units.

Population structure and genetic diversity

At a phylogeographic level, significant population structure was found here within three population units detected using mitochondrial DNA CR data: Puerto Rico, Cuba/Colombia/Bahamas/Mexico and Honduras. Each of

Table 5 Genetic diversity for nine nuclear microsatellites in six populations analyzed

Locus	Honduras/Colombia/ Puerto Rico/ N = 29	Bahamas N = 11	Cuba N = 53	Mexico N = 29
D08	n = 5 AR = 3.835	n = 2 AR = 2.000	n = 5 AR = 2.799	n = 7 AR=4.592
n = 8	H_O = 0.44444	H_O = 0.63636	H_O = 0.169	H_O = 0.60000
	H_E = 0.54437	H_E = 0.45455	H_E = 0.257	H_E = 0.65424
	P = 0.01792	P = 0.47976	P = 0.00460	P = 0.66241
D22	n = 8 AR = 4.433	n = 5 AR = 4.634	n = 9 AR = 4.761	n = 8 AR = 6.139
0.08594	H_O = 0.41379	H_O = 0.81818	H_O = 0.62	H_O = 0.70000
n = 12	H_E = 0.52208	H_E = 0.62338	H_E = 0.670	H_E = 0.81808
	P = 0.00491	P = 0.88384	P = 0.56903	P = 0.12477
TV5	n = 4 AR =3.898	n = 3 AR = 3.00	n = 5 AR = 4.6274	n = 5 AR = 4.119
n = 7	H_O = 0.51724	H_O = 0.81818	H_O = 0.68	H_O = 0.43333
	H_E = 0.70599	H_E = 0.67100	H_E = 0.726	H_E = 0.59492
	P = 0.02154	**P =** 0.75678	P = 0.29071	P = 0.06164
MK6	**n = 6 AR = 4.837**	n = 7 AR = 6.403	n = 6 AR = 5.789	n = 6 AR = 5.196
n = 10	**H_O = 0.36000**	H_O = 0.81818	H_O = 0.788	H_O = 0.73333
	H_E = 0.71673	H_E = 0.75325	H_E = 0.835	H_E = 0.78079
	P = 0.00001	P = 0.75678	P = 0.33938	P = 0.34257
MK8	n = 7 AR = 5.046	n = 5 AR = 5.00	n = 7 AR = 5.559	**n = 6 AR = 5.145**
n = 10	H_O = 0.60714	H_O = 0.55556	H_O = 0.711	**H_O = 0.48148**
	H_E = 0.72857	H_E = 0.81046	H_E = 0.777	**H_E = 0.76101**
	P = 0.02742	P = 0.08594	P = 0.20957	**P = 0.00035**
MK9	**n = 6 AR = 4.886**	n = 4 AR=4.00	**n = 7 AR = 5.174**	n = 7 AR = 4.976
n = 9	**H_O = 0.25926**	H_O = 0.77778	**H_O = 0.509**	H_O = 0.65517
	H_E = 0.72607	H_E = 0.69935	**H_E = 0.694**	H_E = 0.71204
	P = 0.00000	P = 0.73810	**P =** 0.00024	P = 0.32541
Tur117	n = 5 AR = 2.895	n = 2 AR = 2.00	n = 5 AR = 3.594	n = 5 AR = 4.064
n = 8	H_O = 0.13793	H_O = 0.11111	H_O = 0.510	H_O = 0.48276
	H_E = 0.22686	H_E = 0.11111	H_E = 0.49	H_E = 0.62795
	P = 0.00585	P = 1.00000	P = 0.02377	P = 0.04864
Tur91	**n = 4 AR = 3.864**	n = 2 AR=2.00	n = 4 AR = 3.738	n = 5 AR = 3.587
n = 6	**H_O = 0.12500**	H_O = 0.33333	H_O = 0.458	H_O = 0.53571
	H_E = 0.62677	H_E = 0.29412	H_E = 0.624	H_E = 0.58442
	P = 0.00000	P = 1.0000	P = 0.01296	P = 0.48871
Tur48	n = 4 AR = 3.542	n = 4 AR = 4.00	n = 4 AR = 2.553	n = 2 AR = 1.881
n = 6	H_O = 0.56000	H_O = 0.55556	H_O = 0.28	H_O = 0.11111
	H_E = 0.52816	H_E = 0.54248	H_E = 0.281	H_E = 0.17121
	P = 0.16995	P = 0.27816	P = 0.76118	P = 0.18363
Observed and expected heterozygosity	H_O = 0.38053	H_O = 0.60269	H_O = 0.52535	H_O = 0.52588
	H_E = 0.59173	H_E = 0.55108	H_E = 0.59612	H_E = 0.63385

N = dolphin sample size; for each locus: n = total number of alleles, H_O = observed heterozygosity, H_E = expected heterozygosity and AR = allelic richness. Loci out of equilibrium after Bonferroni correction (0.001562) are shown in bold.

Table 6 Population differentiation between pairwise populations with nine microsatellites

	Honduras/Colombia/Puerto Rico/Bahamas	Cuba	Mexico
Honduras/Colombia/Puerto Rico/Bahamas	–	**0.0583*****	**0.1094*****
Cuba	**0.0597**** (0.0659)	–	**0.0694*****
Mexico	**0.1363**** (0.1767)	**0.1056**** (0.1546)	–

Significant scores are in bold and the *P*-value is shown below them. Below diagonal: F_{st} values (*P-values* were obtained after 3000 permutations) along with the harmonic mean of Jost's (2008) D_{EST} across loci shown in (). Above diagonal: R_{ST} values. Degrees of significance: ** 0.001 and *** 0.0001.

these population units has relatively high haplotype and nucleotide diversity, similar to the values reported for other common bottlenose populations studied elsewhere around the world (Natoli *et al*., 2004; Quérouil *et al*., 2007; Tezanos-Pinto *et al*., 2009). These seem to be discrete units, with very low female migration among them (< 1 female per generation). Nucleotide diversity was low for the Honduras population unit, probably because of the small sample size used in this analysis ($n = 6$).

It has been suggested that the rejection of panmixia given by significant values of F_{ST} is not enough to determine population structure and to assign management units (Taylor & Dizon, 1999; Palsboll, Berube & Allendorf 2007). In this study, we used the results from the Bayesian clustering analysis (Structure 2.3.3) to determine these units. The Evanno method applied to the Structure results, detected the value of K for the uppermost level of population structure for the populations tested, identifying K = 4 as the number of subgroups: (1) Honduras/Colombia/Puerto Rico; (2) Bahamas; (3) Cuba; and (4) Mexico. This structure pattern is somewhat different from the results obtained from the mitochondrial DNA CR analyses. Results from the microsatellite analyses may be reflecting present levels of gene flow mediated by both males and females, different to possibly ancestral gene flow evidenced in the mitochondrial DNA CR analyses. Also, the presence of 'worldwide distributed form' and 'inshore' ecotype haplotypes in particular population units, may be causing differences in the population structure pattern showed by each molecular marker.

Microsatellite expected heterozygosity (H_E) values are very similar among the four populations but observed ones (H_O) are considerably lower in Population Unit 1 (Honduras/Colombia/Puerto Rico) that is entirely constituted by 'worldwide distributed form' dolphins, while Bahamas shows the highest value and a population entirely constituted by 'inshore' ecotype. In our study, populations with a high number of individuals with 'worldwide distributed form' haplotypes showed the highest levels of mitochondrial genetic diversity and the lowest levels of expected heterozygosity with microsatellites (Population Unit 1) (Table 5). This is in disagreement with findings from other studies where populations composed by 'worldwide distributed form' individuals, showed higher values of both mitochondrial and nuclear genetic diversity (Natoli *et al*., 2004; Quérouil *et al*., 2007; Tezanos-Pinto *et al*., 2009).

Population Unit 1 is mostly composed by 'worldwide distributed form' individuals and it also has the highest amount of loci out of HW equilibrium (three out of nine). Cuba and Mexico have only one locus out of equilibrium and a small proportion of 'worlwide distributed form' individuals while Bahamas has no 'worldwide distributed form' individuals and all loci in HW equilibrium. The entire set of samples from Puerto Rico came from stranded animals and therefore their origin is not entirely clear. This fact plus the unknown migratory dynamics of animals around islands could be a confounding effect that may be observed in these results. Another possible explanation could be that the decreased heterozygosity in Population Unit 1 could be due to a substructure within the population, better known as the Wahlund-effect, because of the admixture of 'inshore' ecotype and 'worlwide distributed form' individuals or the admixture of 'worldwide distributed form' individuals from different populations. The Wahlund effect explains decreased heterozygosity and HW disequilibrium in fragmented populations when they are treated as a single unit (Hartl & Clark, 1997). In this case, we hypothesize that this Wahlund effect could possibly result from local females (possibly belonging to the 'inshore' ecotype) mating with transient males belonging to the 'worldwide distributed form', as has been observed in groups of other mammals (Goossens *et al*., 2001), even though the sex-biased dispersal test was not significant for our present sampling (Prugnolle & de Meeus, 2002).

High population differentiation was detected for all microsatellite indices (F_{ST}, R_{ST} and D_{EST}). Population differentiation was stronger between Mexico and all the other populations, suggesting a certain degree of isolation of this population. The F_{ST} value between Population Unit 1 and Bahamas was the smallest, while the R_{ST} was considerably higher. This could suggest that the differences between these two populations are ancestral and are driven by a very different origin, as indicated by their divergent haplotypes, but with more recent gene flow reflected in the smaller F_{ST}. Differences between F_{ST} and D_{EST} were not pronounced. This could be due to the fact that F_{ST} values are constrained toward higher levels of genetic diversity according to Jost (2008), but population units in this study showed intermediate to low levels of heterozygosity. The largest differences lie between Population Unit 1 and Mexico and between Cuba and Mexico. However, D_{EST} estimates are particularly affected when migration is included in the model (Ryman & Leimar, 2009), two very important factors in natural populations. The fact that we are comparing populations that have very different mitochondrial lineages and that seem to be mixing more in some populations than in others makes for a difficult assessment to which of these indices is better in determining population structure in such a complex species like *T. truncatus*. Another complication for determining management units arises from the fact that our sample has a mix of captive-wild individuals and strandings. A recent study showed that estimating population structure based only on carcasses can fail to detect population differentiation and lead to an erroneous decision-making process about management units (Bilgmann *et al*., 2011). This 'carcass' effect could be one of the reasons why we failed to observe sex-biased dispersal in our sample. Another obvious reason for these results can also be the irregular sampling of the regions and very small sample sizes for Honduras and Colombia.

Management and conservation implications

Managers of threatened and protected populations face the challenge of balancing conservation with responsible use of the resource. This can be achieved by using a multitude of

tools, such as the species biology, zoogeography and genetics. The shifts in demographic rates that drive population decline usually have nongenetic origins, such as habitat degradation or human-induced mortality (Lande, 1988). However, genetic factors may hasten the extinction process once a population is small. A reduction in genetic diversity affects the long-term adaptability of the population to environmental changes. In the short term, it reduces reproduction and survival (i.e. inbreeding depression) and leads to increased risk of threat or even extinction (Westemeier *et al*., 1998; Frankham, Ballou & Briscoe, 2002).

Common bottlenose dolphins in the Wider Caribbean seems to represent a genetically 'healthy' population in terms of their mitochondrial and microsatellite genetic diversity, but may also represent a challenge for management purposes (Torres *et al*., 2003; Sellas *et al*., 2005). It seems that at least two independent evolutionary lineages are found the in the Wider Caribbean, the 'inshore' ecotype and the 'worldwide distributed form'. The genetic differentiation and divergence between these forms should be taken into consideration for captive programs that aim to reproduce bottlenose dolphins from this region. Similarly, releases or reintroductions into natural habitats should carefully evaluate the site for such releases, taking into consideration not only the genetic makeup of each individual but also the social structure of each local population and the genetic differentiation between the population units detected in this study for the Wider Caribbean. Live-captures not only affect the demography of a population but they can potentially impact the reproductive success of the remaining animals in the wild through disruption of social associations. This may be of special concern for Cuban animals, as this population seems to be distinct and discrete (from microsatellite analyses) and represented mostly by 'inshore' ecotype animals. This population has been heavily exploited in recent years (Van Warebeek *et al*., 2006). In Sarasota Bay, USA, the social structure of bottlenose dolphins has been described in detail (Wells, 1986) In this region, dolphins exhibit complex patterns characterized by long-term associations and a high degree of site fidelity. Furthermore, reproductive success in this region is related to the size of each nursery group. Females raising young in smaller groups (as might be the case following the capture of females) have significantly lower reproductive success than females of similar age raising their young in larger, more stable groups (Wells, 1986; Wells *et al*., 2008).

Increased human-related mortalities and/or catastrophic events such as a severe harmful algal bloom, morbillivirus outbreak or oil spills could lead to a population decline. Such a possibility is not unrealistic. In 2006, nearly 2% of the resident population of bottlenose dolphins in Sarasota Bay died from ingestion of recreational fishing gear following a severe red tide (Fire *et al*., 2008). The biological effects of the Deepwater Horizon oil spill in the Gulf of Mexico on bottlenose dolphins have yet to be determined.

Local studies aiming to investigate vital rates, social structure, abundance, demography and stock structure of local populations should be undertaken before captures of animals occur. This is necessary to provide a framework to manage these populations sustainably in the long term; particularly, knowledge of the population size of each local unit is needed to understand what level of live-capture they can sustain.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Graphic representation of Evanno *et al.*, 2005 ad hoc statistic ΔK, a clear peak in the modal value of this distribution is observed in K = 4.

Table S1 Nine polymporphic microsatellites were multiplexed in two locus groups (LG1 and LG2) with the same PCR conditions described in Methods. For each LG: name of microsatellite, type of dye and concentration in ρM of the fluorescent marker.

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