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A Worldwide Perspective on the Population Structure and Genetic Diversity of Bottlenose Dolphins *(Tursiops truncatus)* in New Zealand

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Abstract

Bottlenose dolphins (*Tursiops truncatus*) occupy a wide range of coastal and pelagic habitats throughout tropical and temperate waters worldwide. In some regions, "inshore" and "offshore" forms or ecotypes differ genetically and morphologically, despite no obvious boundaries to interchange. Around New Zealand, bottlenose dolphins inhabit 3 coastal regions: Northland, Marlborough Sounds, and Fiordland. Previous demographic studies showed no interchange of individuals among these populations. Here, we describe the genetic structure and diversity of these populations using skin samples collected with a remote biopsy dart. Analysis of the molecular variance from mitochondrial DNA (mtDNA) control region sequences ($n = 193$) showed considerable differentiation among populations ($F_{ST} = 0.17$, $\Phi_{ST} = 0.21$, $P < 0.001$) suggesting little or no female gene flow or interchange. All 3 populations showed higher mtDNA diversity than expected given their small population sizes and isolation. To explain the source of this variation, 22 control region haplotypes from New Zealand were compared with 108 haplotypes worldwide representing 586 individuals from 19 populations and including both inshore and offshore ecotypes as described in the Western North Atlantic. All haplotypes found in the Pacific, regardless of population habitat use (i.e., coastal or pelagic), are more divergent from populations described as inshore ecotype in the Western North Atlantic than from populations described as offshore ecotype. Analysis of gene flow indicated long-distance dispersal among coastal and pelagic populations worldwide (except for those haplotypes described as inshore ecotype in the Western North Atlantic), suggesting that these populations are interconnected on an evolutionary timescale. This finding suggests that habitat specialization has occurred independently in different ocean basins, perhaps with *Tursiops aduncus* filling the ecological niche of the inshore ecotype in some coastal regions of the Indian and Western Pacific Oceans.

All cetaceans including baleen whales, beaked whales, dolphins, and porpoises are highly mobile and many species undertake long-distance seasonal migrations (Baker et al. 1993; Rosel et al. 1999; Wells et al. 1999). This mobility has the potential to reduce the isolation and therefore the genetic differentiation in haplotype frequencies among regional populations. However, several studies have revealed demographic isolation (Würsig and Jefferson 1990;

Rossbach and Herzing 1999) or genetic differentiation at both the haplotype and nucleotide level among neighboring dolphin populations, despite no obvious physical barriers to interchange (e.g., Dowling and Brown 1993; Hoelzel 1998; Hoelzel et al. 1998; Pichler et al. 1998; Krützen et al. 2004; Oremus et al. 2007).

The bottlenose dolphin *(Tursiops truncatus)* occupies a wide range of coastal and pelagic habitats throughout tropical and temperate waters around the world (Leatherwood et al. 1983). At least one related species (currently *Tursiops aduncus*, although perhaps not a truly congener; refer to LeDuc et al. 1999; Wang et al. 1999; Natoli et al. 2004) is sympatric with *T. truncatus* along the coast of mainland China, in the Taiwan Strait (Wang et al. 1999), around Australia (Moller and Beheregaray 2001; Krützen et al. 2004) and off South Africa (Ross 1977; Ross and Cockcroft 1990; Natoli et al. 2004).

It appears that *T. truncatus* may have once or repeatedly, adapted to different environmental conditions resulting in several different forms or "ecotypes." In the North Atlantic, for example, Duffield et al. (1983) described 2 *T. truncatus* ecotypes based on hematology profiles and distribution: "inshore" and "offshore." Later studies confirmed this finding with independent lines of evidence from morphology, genetics, parasite load, and diet (Hersh and Duffield 1990; Mead and Potter 1990; Hoelzel et al. 1998; Natoli et al. 2004). In many regions of the world, however, there is insufficient evidence to distinguish between differential habitat use by individuals and true ecotype specialization of particular bottlenose dolphin genetic lineages. Distinct parapatric (adjacent) populations have been documented in the Western North Atlantic (Duffield et al. 1983; Hersh and Duffield 1990; Hoelzel et al. 1998; Torres et al. 2003; Kingston and Rosel 2004; Natoli et al. 2004) and to a lesser extent in the Eastern North Pacific (ENP), the Gulf of California (Lowther 2006; Segura et al. 2006), as well as along the western coast of South America (Van Waerebeek et al. 1990; Sanino et al. 2005).

Although it is generally assumed that the inshore ecotype inhabits coastal areas whereas the offshore ecotype inhabits pelagic waters, this assumption can be misleading: individuals described as the offshore ecotype have been reported close to shore in some areas (Wells et al. 1999), and individuals described as the inshore ecotype have been observed far from shore in regions where the continental shelf is broad (Kenney 1990). Moreover, around many islands in the Pacific Ocean, deep ocean habitats are found in close proximity to shallow coastal areas. Information on population structure and ecotype assignment of bottlenose dolphins from these islands has been limited to 1 or 2 populations with small sample sizes (Natoli et al. 2004). Further, there has been some confusion between the inshore ecotype of *T. truncatus* and the more coastal species of Indo-pacific bottlenose dolphin, *T. aduncus* (Reeves et al. 2004). For example, mitochondrial DNA (mtDNA) control region sequences of individuals from a coastal South African population previously reported to represent the inshore ecotype of *T. truncatus* (Goodwin et al. 1996; Smith-Goodwin 1997) were recently shown to match a sequence of the *T. aduncus* holotype (collected along the Ethiopian coast of the Red Sea; Perrin et al. 2007).

On a worldwide scale, pelagic *T. truncatus* seem to be characterized by high levels of genetic diversity, whereas coastal populations are characterized by low levels of genetic diversity (Natoli et al. 2004). Moreover, pelagic populations are likely to be the source of independent founder events that have generated somewhat discrete population segments in coastal areas perhaps as a result of resource specialization or philopatry (Hoelzel 1998; Natoli et al. 2004). Intensively studied populations in the Western North Atlantic (WNA) are commonly used as a model for comparison with other regions (Curry 1997; Curry and Smith 1997; Hoelzel et al. 1998; Natoli et al. 2004). However, considering the limited nature of studies conducted in the Central and Western Pacific (CWP) and the taxonomic uncertainty in some studies, it is unknown whether the pattern found in the WNA is representative of the worldwide population structure of the species or if it represents only an ocean basin, or even a region within an ocean. Although, testing of this hypothesis was initiated by Natoli et al. (2004), their sample size for the Pacific Ocean was limited to 18 samples from only 2 regions (1 from the ENP and 17 from China).

In New Zealand waters, bottlenose dolphins are found both in coastal and pelagic habitats (Constantine 2002); but as yet, there has been no independent evidence to classify individuals or populations as genetically more similar to inshore or offshore ecotypes found in other regions. In coastal waters, there are 3 discontinuous populations found in Northland, Marlborough Sounds, and Fiordland (Bräger and Schneider 1998; Schneider 1999; Constantine 2002, Figure 1). The Fiordland population appears to be further subdivided into 3 small communities inhabiting Milford, Doubtful, and Dusky Sounds (Boisseau 2003). Long-term studies using mark-recapture models have estimated abundance of around 446 adults for Northland (confidence interval [CI] = 418–487; Constantine 2002) and around 49 individuals for Doubtful Sound (coefficient of variation = 0.024; Gormley 2002). To date, there is no estimate for Marlborough Sounds; but a photo-identification catalog (Merriman et al. 2005) suggests a population of at least several hundreds. Comparison of individual identification photographs between Northland, Marlborough Sounds, and Fiordland suggests no exchange of individuals among populations (Bräger and Schneider 1998; Schneider 1999; Constantine 2002).

Here, we describe the population structure and genetic diversity of coastal bottlenose dolphins in New Zealand waters based on mtDNA control region sequences. We also describe the genetic relationship of New Zealand bottlenose dolphins to other *T. truncatus* from 18 regions worldwide, including the inshore and offshore ecotypes as described in the Western North Atlantic. With this more comprehensive dataset, we further test the hypothesis that the genetically distinct ecotypes reported in the Western North Atlantic are found worldwide, predicting that New Zealand coastal dolphins would group genetically with individuals representative of the inshore ecotype given their coastal habitat

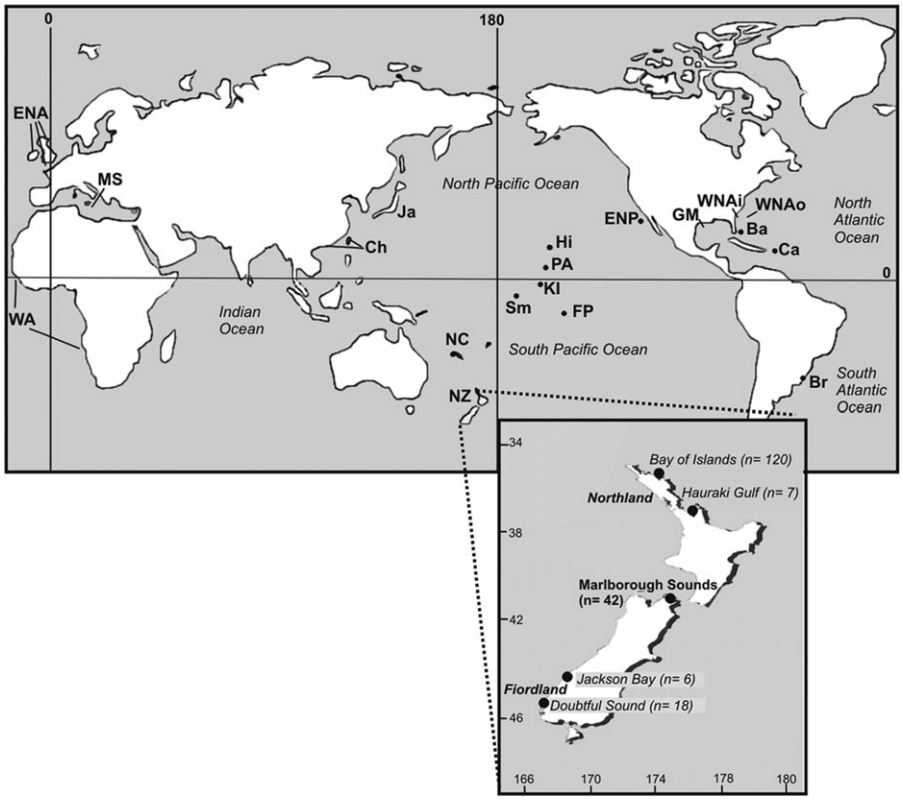

Figure 1. Locations represented by genetic samples of bottlenose dolphins (*Tursiops truncatus*) including New Zealand populations (insert). ENA = Eastern North Atlantic, MS = Mediterranean Sea, WA = West Africa, Ja = Japan, Ch = China–Taiwan, Hi = Hawai'i, PA = Palmyra Atoll, KI = Republic of Kiribati, Sm = Samoa, FP = French Polynesia, NC = New Caledonia, NZ = New Zealand, ENP = Eastern North Pacific, GM = Gulf of Mexico, WNAi = Western North Atlantic inshore, WNAo = Western North Atlantic offshore, Ba = Bahamas, Ca = Caribbean, and Br = Brazil.

preference or use. Alternatively, we considered that New Zealand dolphins have adopted a coastal habitat use independent of other coastal or insular populations, perhaps originating from a widespread, pelagic population, or complex of populations. The results provide new insights into the pattern of mtDNA diversity associated with habitat specialization and ecotype formation among *T. truncatus* worldwide.

Materials and Methods

New Zealand Dataset

A total of 193 samples were collected from bottlenose dolphins in coastal habitats around New Zealand (Figure 1) using a Paxarm biopsy sampling system (Krützen et al. 2002). Of these, 127 samples were from 2 locations in Northland (Bay of Islands 35°14′S, 174°06′E, $n = 120$ and Hauraki Gulf 36°40′S, 174°50′E, $n = 7$). Forty-two samples were collected from Marlborough Sounds (41°05′S, 174°15′E), 18 from Doubtful Sound in Fiordland (45°17′S, 167°168′E), and 6 from the neighboring Jackson Bay (44°S, 168°36′E). Analysis of individual identification photographs confirmed that some individuals photographed in Jackson Bay belonged to the Milford Sound community; therefore, samples collected in this area were assigned to the Fiordland population. Sixteen samples were obtained from strandings around New Zealand; these sequences were included in the worldwide analyses but not in the analyses of population structure for New Zealand as the assignment of individuals to populations was not possible.

Pacific Ocean Dataset

Excluding samples collected in New Zealand, a total of 218 samples representing 62 unique mtDNA control region sequences (i.e., haplotypes) were available from 8 populations from the CWP and 1 haplotype (represented by one sample) was available from the ENP. Haplotype sequences were obtained from published sequences, biopsy samples, "whale meat" products, and GenBank sources (Figure 1, Supplementary Appendix 1). From the CWP, 155 skin samples were collected using a biopsy sampling system; of those, 23 were collected from the Republic of Kiribati (Phoenix Archipelago, 2°49′S, 171°40′W), 117 from the main Hawaiian Islands (O'ahu, Hawai'i, Kaua'i, and Ni'ihau, 19°N–22°N, 156°W–160°W), 11 from the Palmyra Atoll (5° 52′N, 162° 06′W), 1 from Samoa (13°25′S, 172°36′W), 2 from French Polynesia (Tuamotu Archipelago 15°S,

148°W), and 1 from New Caledonia (22°51′S, 167°42′E; Figure 1, Supplementary Appendix 1). Previously unpublished sequences from 34 whale meat products identified as *T. truncatus* were obtained from commercial markets of Japan as part of the ongoing molecular monitoring of whale and dolphin products (Baker and Palumbi 1994; Baker et al. 2000; Endo et al. 2005). Most market products from dolphins were supplied by small-type coastal whaling (Endo et al. 2003) and therefore were assumed to originate from coastal areas around Japan.

Six mtDNA haplotype sequences of *T. truncatus* were obtained from GenBank (accession numbers: AF056231 and AF049101 from Wang et al. 1999; AF459508, AF459509, AF459523, and AF459522 from Ji GQ, Yang G, Liu S, Zhou KY, unpublished data). Additionally, 24 samples representing 19 unique haplotype sequences were reconstructed from 3 publications (Wang et al. 1999; Kakuda et al. 2002; Natoli et al. 2004) representing 3 regions (Japan, China-Taiwan, and ENP; Supplementary Appendix 1). Each publication included one reference sequence (published in GenBank or included in the publication) with a table of variable sites and haplotype frequencies. Haplotype sequences were reconstructed from these by inserting and aligning the reference sequence with the existing *T. truncatus* dataset using MacClade software Vs. 4.06 (Maddison WP and Maddison DR 2003).

Atlantic Ocean Dataset

A total of 158 samples representing 50 unique mtDNA haplotype sequences were available from 9 populations in the Atlantic Ocean from published sequences, strandings, and GenBank sources (Figure 1, Supplementary Appendix 1). For this study, 12 samples from Puerto Rico (17°N–18°N, 65°W–67°W) and 1 from the United States Virgin Islands (17°41.23′N, 64°49.32′W) were newly available from stranded individuals. Three haplotype sequences from the Bahamas were obtained from GenBank (accession numbers: AF155160, AF155161, and AF155162 from Parsons et al. [1999]). Additionally, 142 samples representing 37 haplotype sequences were reconstructed from 3 publications (Smith-Goodwin 1997; Parsons et al. 2002; Natoli et al. 2004) representing 8 regions and 2 ecotypes (Figure 1, Supplementary Appendix 1). Haplotype sequences were reconstructed following the procedure described above.

DNA Extraction, Polymerase Chain Reaction Amplification, and Sequencing

For tissue obtained from biopsy samples and stranded specimens, total genomic DNA was isolated from tissue samples using proteinase K digestion followed by standard phenol/chloroform methods (Sambrook et al. 1989), as modified for small tissue samples by Baker et al. (1994). Amplification of 800 bp of the mtDNA control region was performed using the primers light-strand tPro-whale Dlp-1.5 with the addition of an M13 tag to the 5′ end (Dalebout et al. 1998) and heavy-strand Dlp-8G (Pichler et al. 2001). Polymerase chain reaction (PCR) volume was 15 μl per reaction per sample. PCR conditions were as follows: 0.2 mM deoxynucleoside triphosphate, 2.5 mM MgCl, 1X PCR buffer, 0.4 μM of each primer, and 0.05 μl Platinum *Taq* (Invitrogen, Auckland, New Zealand). PCR cycling profile was 2 min at 94 °C, 35 cycles of 30 s at 94 °C, 40 s at 55 °C, and 40 s at 72 °C. PCR products were purified using ExoI and SAP (Werle et al. 1994) and sequenced with BigDye terminator chemistry using ABI 377 and ABI 3100 DNA sequencers (Applied Biosystem, Foster City, CA). Cycle sequencing used the primer tPro-whale Dlp-1.5. Variable sites of unique haplotypes were confirmed by sequencing the heavy strand using primer Dlp-8G.

For tissue obtained from Japanese whale meat markets, DNA extractions and initial PCR amplifications were conducted using "portable" PCR protocols (e.g., Baker and Palumbi 1994; Baker et al. 2006). In brief, tissue from each product was prepared for PCR amplification using Chelex resin (BioRad Laboratories, Hercules, CA) following Walsh et al. (1991). To comply with Convention on International Trade in Endangered Species (CITES) restrictions (Bowen and Avise 1994; Jones 1994), amplified products were isolated from "native" DNA by biotin labeling of one primer and binding to streptavidin-coated plates (Baker et al. 2006).

Taxonomy, Ecotype, and Habitat Classification

In order to avoid potential confusion with *T. aduncus*, sequences from biopsy samples, strandings specimens, and whale meat products were first compared with sequences from voucher specimens of *T. truncatus* available from the *Witness for the Whales* database (Vs. 4.3) within the Web-based program *DNA-surveillance* (Ross et al. 2003). Sequences used in the worldwide comparison were categorized into previously described ecotypes (i.e., inshore or offshore) by reviewing each published article for independent evidence from at least 2 sources (e.g., molecular or biochemical markers, diet, morphology). However, in some publications, the terms inshore or offshore were used with no evidence other than distribution. We considered that this evidence of classification by habitat (i.e., coastal or pelagic) was insufficient for classification of ecotype. All haplotype sequences from the Western North Atlantic inshore (WNAi), Bahamas, and Gulf of Mexico presented consistent diagnosis as the inshore ecotype, whereas haplotype sequences from the Western North Atlantic offshore (WNAo) presented evidence for diagnosis as the offshore ecotype. Haplotype sequences from all remaining populations were diagnosed as "unknown" in regards to ecotype. Regional populations were also grouped into 3 ocean basins: North Pacific (NP), South Pacific (SP), and Atlantic Ocean (AO; Table 1).

Sequences Analysis and Phylogenetic Reconstruction

Sequence alignments were performed using Sequencher (Vs. 4.1.2, Genes Codes Corp., Ann Arbor, MI) and edited manually. Unique haplotypes were identified using the software MacClade Vs. 4.06 (Maddison WP and Maddison

Table 1. Summary of mtDNA control region sequences available for *Tursiops truncatus* populations worldwide, showing the total number of samples (*n*), number of haplotypes, sequences length (No. of bp), published ecotype origin (when available), and genetic diversity values

Population	*n*	No. of haplotypes	No. of bp	Ecotype	Nucleotide diversity (π) %	Haplotypic diversity (*h*)	Source
South Pacific							
New Zealand (NZ)	209	22	391	U	2.2 ± 1.1	0.91 ± 0.007	BS, St
Republic of Kiribati (KI)	23	8	388	U	0.6 ± 0.3	0.83 ± 0.05	BS
New Caledonia (NC)	1	1	386	U	n/a	n/a	BS
Samoa (Sm)	1	1	391	U	n/a	n/a	BS
French Polynesia (FP)	2	2	391	U	n/a	n/a	BS
North Pacific							
Hawai'i (Hi)	117	19	385	U	2.2 ± 1.1	0.87 ± 0.016	BS
Palmyra Atoll (PA)	11	7	385	U	1.6 ± 0.9	0.93 ± 0.06	BS
China (Ch)	22	17	391	U	1.8 ± 1	0.95 ± 0.04	RS (1), GB
Japan (Ja)	41	19	387	U	1.3 ± 0.7	0.77 ± 0.07	RS (2), MP
Eastern North Pacific (ENP)	1	1	297	U	n/a	n/a	RS (3)
Atlantic							
Gulf of Mexico (GM)	10	6	297	I	0.7 ± 0.5	0.84 ± 0.1	RS (3)
Caribbean (Ca)	13	6	387	U	2.2 ± 1.2	0.82 ± 0.08	St
Bahamas (Ba)	7	5	297	I	0.5 ± 0.3	0.86 ± 0.14	RS (3), GB
Western North Atlantic inshore (WNAi)	29	6	297	I	0.7 ± 0.5	0.43 ± 0.11	RS (3)
Western North Atlantic offshore (WNAo)	25	11	297	O	2.2 ± 1.2	0.88 ± 0.05	RS (3, 5)
Eastern North Atlantic (ENA)	38	8	297	U	0.9 ± 0.5	0.73 ± 0.047	RS (3, 4)
Mediterranean Sea (MS)	18	11	294	U	2.1 ± 1.2	0.94 ± 0.03	RS (3)
West Africa (WA)	16	5	297	U	1.5 ± 0.9	0.72 ± 0.097	RS (3)
Brazil (Br)	2	1	297	U	n/a	n/a	RS (1)

bp, base pairs; I, inshore; O, offshore; U, unknown; and n/a, not available. Source: BS, biopsy samples; St, strandings; GB, GenBank sequences; MP, market products; and RS, reconstructed sequences. References: 1) Wang et al. 1999; 2) Kakuda et al. 2002; 3) Natoli et al. 2004; 4) Parsons et al. 2002; and 5) Smith-Goodwin 1997.

DR 2003). The neighbor-joining (NJ) algorithm, as implemented in the software PAUP* Vs. 4.0b10 (Swofford 2000), was used to reconstruct the phylogenetic relationships among New Zealand haplotypes. Bootstrap confidence estimates were based on 1000 replicates (Felsenstein 1985); the best fitting model of sequence evolution was found using Modeltest Vs. 3.7 (Posada and Crandall 1998). A maximum parsimony (MP) tree was also constructed using the branch and bound algorithm to search through numerous equally parsimonious trees. Because of the poorly resolved phylogeny within the subfamily *Delphininae* (LeDuc et al. 1999; Caballero, Jackson, et al. 2007), we chose a more distantly related species from the subfamily *Stenoninae*, the rough-toothed dolphin (*Steno bredanensis*; Oremus 2008), as an outgroup for all reconstructions (Caballero, Jackson, et al. 2007).

Population Structure and Genetic Diversity

Arlequin Vs. 2.001 (Schneider et al. 2000) was used to calculate F_{ST}, Φ_{ST}, *h* (haplotype diversity, Nei 1987), and π (nucleotide diversity, Tajima 1983) using Tamura-Nei distance correction (Tamura and Nei 1993). The significance of departure from a random distribution was evaluated using 10 000 permutations among individuals between populations (analysis of the molecular variance [AMOVA], Excoffier et al. 1992). An exact test of population differentiation based on haplotype frequencies (Raymond and Rousset 1995) was performed to test the null hypothesis of random distribution of individuals between pairs of populations. Populations with less than 5 samples were excluded from the test of differentiation. Sequential Bonferroni corrections were applied to pairwise comparisons where indicated (Rice 1989).

New Zealand Compared with Worldwide Populations

In order to compare New Zealand populations with the worldwide dataset, average gross (d_{xy}), and net (d_a) sequence divergence between populations and sequence diversity within populations (d_x, d_y) were estimated with Tamura–Nei distance correction, including calculation of standard errors using Mega 2.1. In order to better visualize the similarity of the New Zealand populations to the worldwide dataset, a mid-rooting dendrogram was built with Mega 2.1 (Kumar et al 2001) by NJ using net sequence divergence data (d_a) among populations.

Migration Rates among New Zealand Populations

Asymmetric female migration rates among populations were estimated using a Markov Chain Monte Carlo (MCMC) coalescent genealogy as implemented in the software Lamarc Vs. 2.0.1 (Kuhner 2006). Bayesian and maximum likelihood (ML) analyses were employed using 5 replicates per run over 5 different runs, implementing one initial and

final chain, a different random number seed, and 5 heating temperatures (1, 1.1, 1.2, 1.3, and 1.4) for each run. The burn-in option was used to allow the first 5% of each chain to be discarded and avoid unreasonable results as recommended in Kuhner et al. (2005). In order to estimate migration rates with accuracy in reasonable time, the sample size for Northland was reduced to $n = 70$ by random selection (Kuhner et al. 2005).

Worldwide Phylogeography

A network of the worldwide haplotype dataset was constructed using the statistical parsimony methodology described in Templeton et al. (1992), as implemented in the software TCS Vs. 1.13 (Clement et al. 2000). This method estimates an unrooted tree and provides a 95% plausible set for all sequence type linkages within the tree, with gaps considered as a fifth character state. To resolve any ambiguities (loops), we used the 3 criteria derived from the coalescent theory (Crandall and Templeton 1993; Templeton and Sing 1993; Crandall et al. 1994): 1) "frequency": high-frequency sequences are more likely to have been present in the population for a longer period of time; therefore, low-frequency sequences are more likely to be connected to sequences with high frequency; 2) "topology": sequences are more likely to be connected to interior sequences than to tip sequences; and 3) "geography": sequences are more likely to be connected to sequences from the same population or region, rather than to sequences occurring in distant populations.

Results

Phylogeography, Genetic Diversity, and Female Migration Rates among New Zealand Populations

Analysis of the 647-bp consensus fragment from the mtDNA control region sequences ($n = 193$; 16 samples from strandings were excluded from this analysis; refer to Materials and Methods) representing the 3 New Zealand populations revealed 24 unique maternal lineages (haplotypes; GenBank accession numbers: EU276389–EU276412), defined by 52 variable sites. Overall, there were 46 transition substitutions, 5 transversion substitutions (including one site with both a transition and transversion), and 2 single base insertion–deletions. The model of sequence evolution best fitting the dataset was HKY + I (Hasegawa et al. 1985). The estimated T_v/T_i ratio was 49.3, and estimated proportion of invariable sites (I) was 0.91.

Phylogenetic reconstructions (both NJ and MP) did not show a pattern of reciprocal monophyly or fixed nucleotide differences among populations, although strong frequency differences were observed. Most haplotypes were found in only one region: 15 unique to Northland, 7 to Marlborough Sounds, and 6 to Fiordland. Only one haplotype was shared among the 3 populations. Another haplotype was shared between Marlborough Sounds and Fiordland, and a third was shared between Northland and Marlborough Sounds (Figure 2).

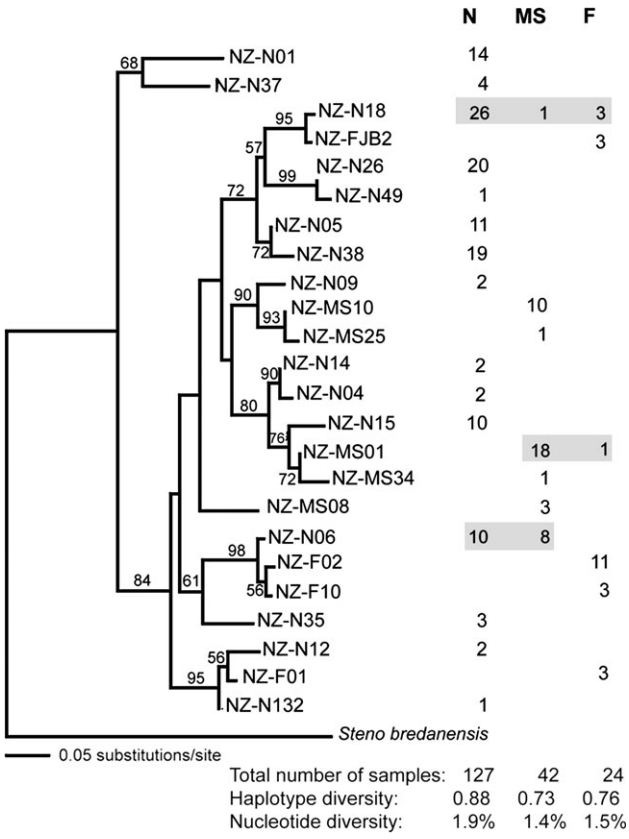

Figure 2. Phylogenetic reconstruction (NJ with HKY + I distance correction) of bottlenose dolphin mtDNA control region sequences, with bootstrap support (>50%) and rooted to the rough-toothed dolphin (*Steno bredanensis*). Shared haplotypes are shaded. N = Northland, MS = Marlborough Sounds, and F = Fiordland.

As expected from the strong frequency differences in haplotypes, the AMOVA results showed a high level of differentiation among the 3 regional populations ($F_{ST} = 0.171$, $P < 0.001$; $\Phi_{ST} = 0.206$, $P < 0.001$). Pairwise F_{ST} and Φ_{ST} comparisons showed that all 3 populations differed significantly from one other (Table 2). This was confirmed by an exact test of population differentiation based on haplotype frequencies. For such diverse populations, F_{ST} values are likely to be less informative regarding population divergence than Φ_{ST}, which incorporates both haplotype frequency and sequence divergence among haplotypes (Excoffier et al. 1992).

Northland had the highest estimates of haplotipic ($h = 0.88 \pm 0.01$) and nucleotide diversity ($\pi = 1.9\% \pm 1$). Fiordland was the next most diverse population ($h = 0.76 \pm 0.07$, $\pi = 1.5\% \pm 0.8$), and Marlborough Sounds was the least diverse population ($h = 0.73 \pm 0.04$; $\pi = 1.4\% \pm 0.7$; Figure 2).

The high level of differentiation indicated by the AMOVA was reflected in low levels of female migration estimated in Lamarc (Table 3). The ML coalescent results

Table 2. Pairwise F_{ST} (lower diagonal) and Φ_{ST} (upper diagonal) with their respective P values for the 3 New Zealand *Tursiops truncatus* populations

	Northland	Marlborough Sounds	Fiordland
	$n = 127$	$n = 42$	$n = 24$
N		0.194 ($P < 0.05$)	0.197 ($P < 0.05$)
MS	0.168 ($P < 0.001$)		0.298 ($P < 0.05$)
F	0.150 ($P < 0.001$)	0.239 ($P < 0.001$)	

N, Northland; MS, Marlborough Sounds; and F, Fiordland.

were discarded as migration rates and theta (θ) values did not stabilize over 5 runs, whereas both parameters stabilized when Bayesian searches were performed. Although CIs overlapped in all pairwise comparisons, some asymmetries in exchange rate was indicated: we found relatively low rates of female migration from both Marlborough Sounds and Fiordland to Northland; low rates of female migration between Marlborough Sounds and Fiordland; and rates of female migration from Northland to Marlborough Sounds and to Fiordland were estimated to be several fold higher than the reverse migration (Table 3).

Worldwide *T. truncatus* Genetic Diversity and Population Structure

To explore the phylogeographic relationship of New Zealand bottlenose dolphins to other populations worldwide, we analyzed a total of 586 samples representing 19 regional populations (Supplementary Appendix 1). The total length of sequences varied from 294 to 720 bp, allowing a consensus length of 391 bp for all analyses. For sequences shorter than 391 bp, haplotype identity was inferred from the available length. Although some potentially variable sites were not available for sequences less than 391 bp, visual inspection of the dataset showed that there was no ambiguity in defining unique haplotypes. However, 3 New Zealand haplotypes collapsed (NZ-FJB2 with NZ-N18, NZ-F10 with NZ-F02, and NZ-N38 with NZ-N05) when sequences were shortened to 391 bp. Examination of this 391-bp fragment revealed 89 variable sites defining 135 unique haplotypes. There were 82 transition substitutions, 9 transversion substitutions (including 2 sites showing both a transition and a transversion), and 4 single base insertion–deletions. For analyses of population structure and diversity, 5 regions represented by low sample numbers (a total of 7 samples of 5 haplotypes) were excluded, bringing the total number of samples analyzed to 579 and representing 130 unique haplotypes from 14 populations (including New Zealand, Table 1).

Haplotypic diversity of the 14 populations ranged from $h = 0.43 \pm 0.11$ for the WNAi to $h = 0.95 \pm 0.04$ for China (unknown), whereas nucleotide genetic diversity ranged from $\pi = 0.5\% \pm 0.30$ for Bahamas (inshore) to $\pi = 2.2\%$ for the Caribbean, Hawai'i, New Zealand (unknown), and WNAo (Table 1).

Overall, regional populations were highly differentiated ($F_{ST} = 0.16$ and $\Phi_{ST} = 0.34$; $P < 0.0001$). After applying sequential Bonferroni corrections, most pairwise comparisons remained significant for both F_{ST} (71 out of 91) and Φ_{ST} (83 out of 91; Table 4). The small sample size of some populations (i.e., Palmyra Atoll $n = 11$, Gulf of Mexico $n = 10$, and Bahamas $n = 7$; Table 1) may explain these nonsignificant results. There were few shared haplotypes among regional populations worldwide suggesting low levels of female migration (Supplementary Appendix 1). Among oceans, there was one shared haplotype between Japan (North Pacific) and New Zealand/Samoa (South Pacific), 4 between Hawai'i/Palmyra Atoll (North Pacific) and the Republic of Kiribati (South Pacific), and 1 between Palmyra Atoll (North Pacific) and French Polynesia (South Pacific; Supplementary Appendix 1). No haplotypes were shared between the Atlantic and Pacific Oceans.

Population Structure by Ecotype and Ocean Basin

We considered population differentiation for 2 higher order groupings: ecotype and ocean basin (Table 1). Unfortunately, a hierarchical analysis of these 2 groupings was not possible because of the imbalance of ecotype classification within oceans. Instead, we conducted 2 nonhierarchical AMOVA analyses including the entire dataset. Pairwise F_{ST} and Φ_{ST} comparisons by ecotype (inshore, offshore, and unknown) showed that all 3 were significantly different, irrespective of ocean origin (overall $F_{ST} = 0.110$; $\Phi_{ST} = 0.344$, $P < 0.0001$ for both; Table 5); F_{ST} and Φ_{ST} values showed far less difference between the offshore and unknown ecotypes than either of those to the inshore ecotype. This pattern was mirrored in the net and gross average sequence divergences (Table 6). Unknown and

Table 3. Most probable estimates of female migration rates per generation (N_{mf}) using Bayesian analysis between the 3 *Tursiops truncatus* populations in New Zealand

	To		
Migration from	Northland	Marlborough Sounds	Fiordland
N		3.99 (CI = 0.44–20.52)	4.89 (CI = 0.02–20.32)
MS	0.40 (CI = 0.03–2.36)		0.31 (CI = 0.00–3.12)
F	0.19 (CI = 0.00–1.70)	0.29 (CI = 0.00–2.01)	

N, Northland; MS, Marlborough Sounds; F, Fiordland; and CI, confidence interval.

Table 4. Pairwise F_{ST} (lower diagonal) and Φ_{ST} (upper diagonal) for 14 regional bottlenose dolphin populations worldwide (populations with <5 samples were excluded)

	NZ	KI	Ja	Ch	Hi	PA	GM	Ca	Ba	WNAi	WNAo	ENA	MS	WA
New Zealand		0.255	0.174	0.134	0.059	0.196	0.468	0.267	0.473	0.523	0.132	0.364	0.166	0.205
Kiribati	0.125		0.612	0.551	0.349	**0.149**	0.817	0.576	0.815	0.813	0.466	0.767	0.523	0.619
Japan	0.150	0.204		0.124	0.183	0.550	0.677	0.488	0.717	0.767	0.328	0.643	0.437	0.485
China	0.071	0.109	0.127		**0.100**	0.461	0.650	0.440	0.667	0.737	0.226	0.620	0.340	0.434
Hawai'i	0.111	0.141	0.161	0.093		0.290	0.517	0.315	0.515	0.585	0.159	0.435	0.205	0.273
Palmyra Atoll	**0.091**	**0.055**	0.173	**0.068**	**0.110**		0.706	0.379	0.675	0.721	0.323	0.646	0.308	0.428
Gulf of Mexico	0.117	0.164	0.201	**0.097**	0.142	0.123		0.470	0.669	0.769	0.581	0.771	0.597	0.707
Caribbean	0.127	0.174	0.210	0.110	0.152	**0.136**	**0.168**		0.438	0.631	**0.201**	0.439	**0.202**	**0.189**
Bahamas	**0.111**	**0.159**	0.199	**0.089**	**0.137**	**0.115**	**0.112**	**0.163**		0.711	0.532	0.753	0.569	0.683
WNAi	0.276	0.382	0.389	0.325	0.308	0.385	0.418	0.414	0.432		0.624	0.762	0.640	0.727
WNAo	0.105	0.146	0.181	0.086	0.129	0.109	0.138	0.149	**0.131**	0.356		0.380	**0.100**	**0.144**
ENA	0.169	0.224	0.251	0.166	0.194	0.195	0.224	0.232	**0.223**	0.413	0.194		0.222	0.369
MS	0.080	0.119	0.157	0.056	0.103	0.077	0.107	0.113	**0.099**	0.345	0.095	**0.102**		**0.089**
WA	0.165	0.220	0.251	0.157	0.191	0.189	0.220	0.229	**0.219**	0.447	0.194	0.235	**0.072**	

After Bonferroni correction ($P < 0.00055$), some pairwise comparisons were not significant (indicated in bold). NZ, New Zealand; KI, Republic of Kiribati; Ja, Japan; Ch, China–Taiwan; Hi, Hawai'i; PA, Palmyra Atoll; GM, Gulf of Mexico; Ca, Caribbean; Ba, Bahamas; WNAi, Western North Atlantic inshore; WNAo, Western North Atlantic offshore; ENA, Eastern North Atlantic; MS, Mediterranean Sea; and WA, West Africa.

offshore ecotypes presented higher values of genetic diversity than the inshore ecotype at both the haplotype and nucleotide level (unknown: $h = 0.97 \pm 0.002$, $\pi = 2.8\% \pm 1.4$; offshore: $h = 0.88 \pm 0.05$, $\pi = 2.2\% \pm 1.2$; and inshore: $h = 0.76 \pm 0.006$, $\pi = 1.9\% \pm 1.0$).

Pairwise F_{ST} and Φ_{ST} comparisons confirmed that the 3 ocean basins were significantly different, irrespective of ecotype classification (overall $F_{ST} = 0.067$; $\Phi_{ST} = 0.174$, $P < 0.0001$ for both; Table 7).

Overall, pairwise Φ_{ST} were higher than F_{ST} values as a result of high haplotypic diversity within populations and some extent of sequence divergence among populations (Supplementary Appendix 1).

Slightly higher values of genetic diversity at both the haplotype and nucleotide level were found in the AO ($h = 0.95 \pm 0.008$, $\pi = 2.8\% \pm 1.4$) compared with the NP ($h = 0.93 \pm 0.008$, $\pi = 2.2\% \pm 1.1$) or the SP ($h = 0.92 \pm 0.06$, $\pi = 2.6\% \pm 1.0$).

New Zealand Compared with Worldwide Populations

At a regional level, a dendrogram reconstruction based on sequence divergence (d_a) among worldwide populations suggested that New Zealand was more divergent from those populations found in the Atlantic Ocean than from those in the Pacific Ocean (Figure 3). In terms of ecotype, New Zealand and the CWP were more divergent from populations described as inshore (WNAi, Gulf of Mexico, and Bahamas) than from the offshore form (WNAo; Table 5), regardless of the habitat where samples were collected (coastal or pelagic) or ocean basin.

Table 5. Pairwise F_{ST} (lower diagonal) and Φ_{ST} (upper diagonal) of *Tursiops truncatus* ecotypes: inshore (I), offshore (O), and unknown (U)

	Inshore (I)	Offshore (O)	Unknown (U)
	$n = 46$	$n = 47$	$n = 493$
I		0.392	0.423
O	0.184		0.079
U	0.121	0.071	

For all comparisons, $P < 0.001$.

Worldwide Phylogeography

A statistical parsimony analysis revealed a very complex network of haplotypes with 31 closed loops, including 6 sequences connected to more than 7 other sequences each. There were 7 loops that could be resolved in more than one way potentially leading to different connections among haplotypes. There was no obvious pattern of monophyly of mtDNA lineages by ocean basin, regional population, or ecotype (Figure 4). However, samples described as inshore in the literature (WNAi, Gulf of Mexico, and Bahamas) clustered together whereas offshore or unknown ecotype origin haplotypes were scattered throughout the reminder of the network. Two haplotypes sampled in the Caribbean (Car-PR610 and Car-PR616) that were of unknown ecotype origin shared one fixed difference with the inshore group, suggesting that these samples belonged to the inshore ecotype.

Discussion

Our study presents one of the most comprehensive analyses of mtDNA structure and diversity of bottlenose dolphins to date in terms of sample size (586 individuals) and geographic sample coverage (19 populations) spanning 3 ocean basins. Our study includes and expands on the analysis of mtDNA by Natoli et al. (2004) by greatly increasing the sample size and geographic coverage for the

Table 6. Average net (d_a; lower diagonal), gross (d_{xy}; upper diagonal) sequence divergence between populations and within population diversity (d_x and d_y; diagonal) among New Zealand (NZ), published inshore, offshore, and unknown ecotypes including standard errors (SEs)

	New Zealand	Offshore	Inshore	Unknown
	$n = 209$	$n = 25$	$n = 46$	$n = 306$
NZ	2.5% (SE = 0.7)	2.8% (SE = 0.7)	4.6% (SE = 1.2)	2.8% (SE = 0.7)
Offshore	0.5% (SE = 0.2)	2.1% (SE = 0.6)	4.2% (SE = 1.1)	2.7% (SE = 0.7)
Inshore	2.4% (SE = 0.9)	2.1% (SE = 0.8)	1.7% (SE = 0.5)	4.4% (SE = 1.1)
Unknown	0.1% (SE = 0.1)	0.3% (SE = 0.1)	1.9% (SE = 0.6)	2.6% (SE = 0.7)

Pacific Ocean. The scope of the analyses allowed us to place the regional differences found among New Zealand coastal populations in a worldwide context.

Coastal New Zealand Populations Are Isolated but Retain Surprisingly High Diversity

Results from our study confirmed a high degree of isolation among New Zealand coastal populations. Significant population structure over relatively small geographic distances has been documented in several *T. truncatus* populations inhabiting coastal areas, including those along the coasts of the Gulf of Mexico (Duffield and Wells 1991; Sellas et al. 2005), the Bahamas (Parsons et al. 2006), and Western Australia, although the latter included individuals of uncertain taxonomy (Krützen et al. 2004). Parsons et al. (2006) suggested that the scale of population subdivision in this species reflects the genetic consequences of their social system and site fidelity. Bottlenose dolphins form stable, long-lasting associations, with individuals often showing strong site fidelity (Wells 1991), even in pelagic groups (Rossbach and Herzing 1999). However, in New Zealand, the only population that shows a high degree of local site fidelity is Doubtful Sound in Fiordland (Schneider 1999; Lusseau 2003). In the Bay of Islands, where the population has been studied intensively, there are no resident individuals, but rather a subset of regular users and infrequent visitors (Constantine 2002). A similar pattern seems to occur in Marlborough Sounds (Merriman et al. 2005).

Despite restricted female migration and significant population structure, all New Zealand populations showed relatively high genetic diversity (overall $h = 0.91$, $\pi = 2.2\%$) given their relatively small population sizes and degree of isolation. Natoli et al. (2004) reported haplotype diversity values ranging from $h = 0.43$ to 0.72 for coastal *T. truncatus* populations and from $h = 0.73$ to 0.94 for pelagic ones, concluding that coastal populations had comparatively lower genetic diversity. Krützen et al. (2004) analyzed 220 mtDNA control region sequences from coastal *Tursiops* sp. from Western Australia and identified only 8 unique haplotypes with a diversity of $h = 0.66$. In contrast, the analysis of 127 mtDNA control region sequences from Northland represented 15 unique haplotypes with a value of $h = 0.88$. The relatively high genetic diversity encountered in New Zealand, particularly Northland, is not explained by current population sizes or rates of female migration between local populations; the same pattern of high diversity was observed in most populations worldwide, except for those described as belonging to the inshore ecotype.

Bottlenose Dolphins Experience Long-distance Gene Flow

Results from a test of differentiation and the haplotype network suggested that restricted gene flow with long-distance dispersal events occurs between all populations except for those described as inshore ecotypes in the Western North Atlantic. The extent of movement by pelagic *T. truncatus* populations is poorly understood but it is thought to include at least occasional long-distance movements (Leatherwood and Reeves 1982; Wells et al. 1999). A recent study in the North Atlantic suggested that pelagic bottlenose dolphins are able to maintain high levels of gene flow over large distances (Quérouil et al. 2007). Additionally, Goodall et al. (2004) reported strandings of 6 bottlenose dolphins along the coast of Tierra del Fuego (55°S) and one live sighting in the Beagle Channel (Tierra del Fuego), suggesting that there is potential for ongoing interchange between the South Atlantic and South Pacific Oceans.

Habitat Specialization and Ecotypes Occur Independently between Oceans

As suggested by Natoli et al. (2004), the divergence of inshore WNA populations could have occurred for a variety of reasons including founder events from pelagic populations with subsequent philopatry. Without genetic input from other sources, small isolated populations are prone to

Table 7. Pairwise F_{ST} (lower diagonal) and Φ_{ST} (upper diagonal) of *Tursiops truncatus* by ocean basins

	North Pacific	South Pacific	Atlantic Ocean
	$n = 192$	$n = 236$	$n = 158$
NP		0.063	0.251
SP	0.070		0.216
AO	0.067	0.063	

AO, Atlantic Ocean; NP, North Pacific; and SP, South Pacific. For all comparisons, $P < 0.001$.

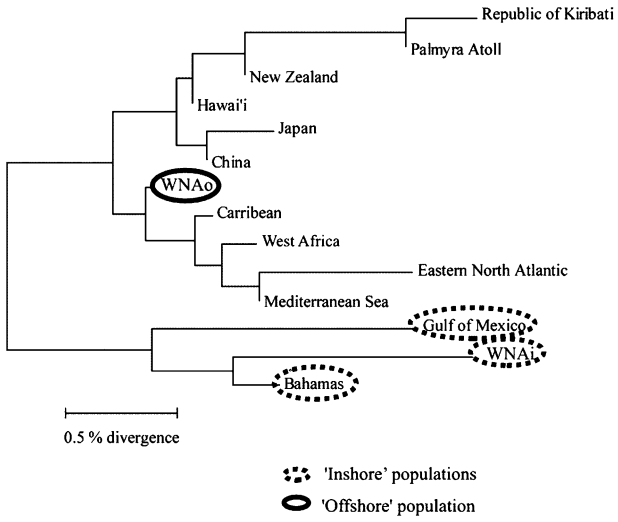

Figure 3. Dendrogram showing mtDNA control region sequence divergence (d_a) among worldwide regional populations of bottlenose dolphins based on a midpoint rooting NJ reconstruction. Inshore and offshore populations refer to the origins of sequences of known ecotype. WNAo = Western North Atlantic offshore and WNAi = Western North Atlantic inshore.

the effects of genetic drift diverging from the parental population and losing genetic diversity over time (Lacy 1987). On the other hand, differences in resource use could lead to assortative mating or ecological separation resulting in genetic differentiation (Hoelzel 1998).

Analyses conducted here showed that populations described as the inshore ecotype are highly differentiated from all other populations worldwide and restricted to the WNA, supporting previous suggestions that this ecotype could represent a different species or subspecies. Differences in ecology (distribution, foraging, and parasite load), morphology, and genetics led Mead and Potter (1990) to suggest that the WNA inshore and offshore ecotypes could be considered different species. Using nuclear markers amplified fragment length polymorphism (AFLP), Kingston and Rosel (2004) found that inshore and offshore ecotypes of *T. truncatus* in the WNA exhibited greater divergence than the 2 different species of common dolphin (*Delphinus delphis* and *Delphinus capensis*), also suggesting that the 2 ecotypes could represent different species. Interestingly, in the Indian Ocean and some (but not all) regions of the Pacific Ocean, populations thought to represent *T. aduncus* fill the ecological niche of this inshore *T. truncatus* ecotype.

Tursiops truncatus Offshore and Unknown Ecotypes Are Evolutionary Interconnected

Bottlenose dolphins found in coastal waters of New Zealand and CWP were genetically more divergent from those populations classified as inshore than from those classified as the offshore ecotype as described in the WNA. The WNA offshore ecotype seems to be genetically related to a number of worldwide haplotypes from populations found in coastal and pelagic habitats suggesting that, in contrast to the WNA inshore ecotype, its origins are not habitat specific. This supports the hypothesis that habitat use and ecotype have evolved independently in different oceans. If so, the pattern and evolutionary processes leading to highly differentiated ecotypes in the WNA are not entirely representative of *T. truncatus* worldwide. Moreover, *T. truncatus* populations described as offshore and unknown ecotypes present relatively high levels of genetic diversity and degree of isolation regardless of population habitat use; however, these populations seem to be interconnected through restricted gene flow. A similar pattern was observed in another worldwide distributed dolphin species such as spinner dolphins from French Polynesia (*Stenella longirostris longirostris*). Significant genetic differentiation and demographic isolation among neighboring communities indicated restricted gene flow; however, the high levels of genetic diversity found contrasted with this isolation suggesting instead a metapopulation structure (Oremus et al. 2007).

Alternatively, genetic diversity values observed in *T. truncatus* populations worldwide (except for those described as inshore ecotype) could reflect founder events due to recent colonization of coastal habitats. In this case, the observed values of genetic diversity would be a signal of the historical polymorphisms contained in large pelagic populations. However, such diversity values are unlikely to persist in small isolated populations without additional influx from other sources.

Conclusion

Our results suggest that the divergence of inshore populations and the formation of ecotypes in the Western North Atlantic do not necessarily reflect the worldwide pattern of *T. truncatus*; moreover, habitat specialization seems to have occurred independently in different ocean basins. Distinct inshore populations are highly differentiated and restricted to the WNA, potentially representing a different taxonomic unit. All other populations showed significant differentiation of mtDNA lineages among worldwide regions including relatively high mtDNA diversity; however, they were not phylogeographically distinct. These results suggest that offshore and unknown ecotypes are interconnected through long-distance gene flow and/or by interchange with pelagic populations. It is not clear what evolutionary processes have led to this pattern (e.g., foraging or reproductive strategies, environmental factors, social structure). Future research is needed to characterize potential pelagic populations of *T. truncatus* that might be linking coastal regions in the North and South Pacific Oceans. Independent lines of evidence (e.g., nuclear DNA markers and morphology; Caballero, Trujillo, et al. 2007) would aid in better describing different ecotypes or taxonomic units of this highly versatile species throughout its range.

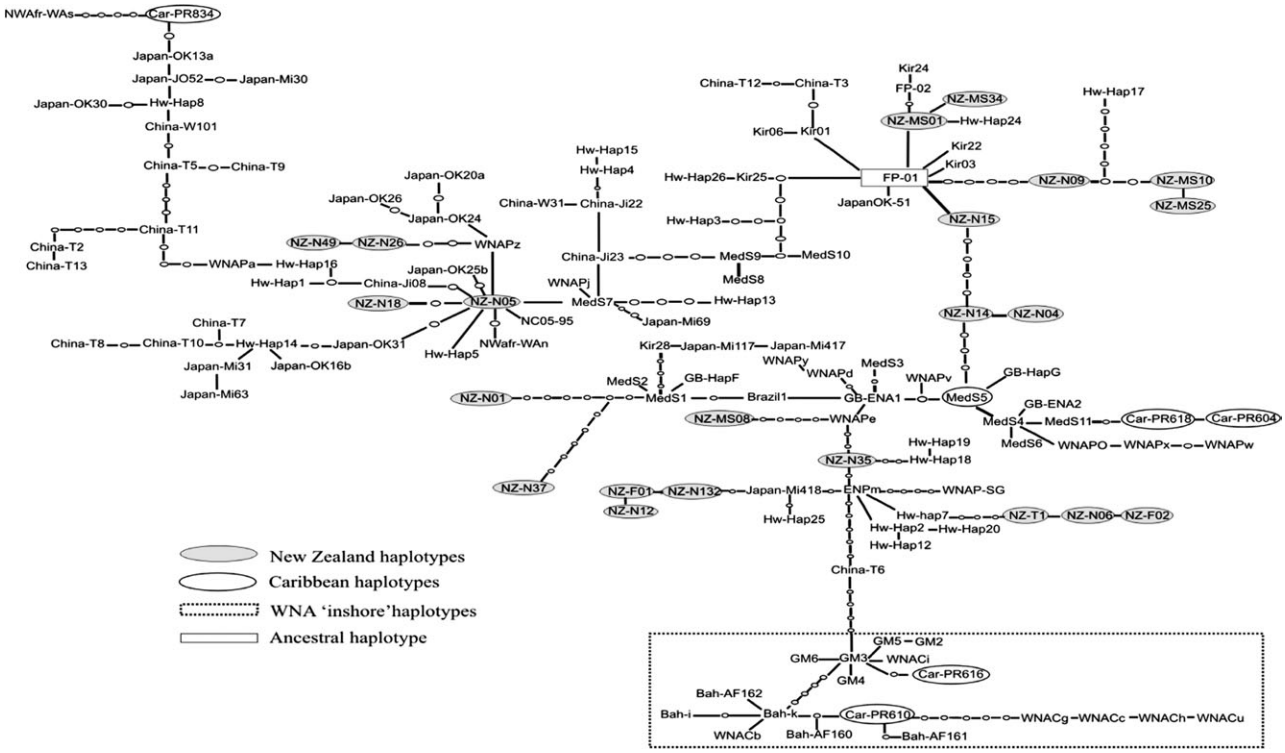

Figure 4. Worldwide parsimony network of *Tursiops truncatus* mtDNA control region haplotype sequences based on 19 regional populations of inshore, offshore, or unknown ecotype origin. Missing or unsampled intermediaries are shown by a small oval (O). Regional origins of haplotype sequences are indicated in the legend and in Supplementary Appendix 1.

Supplementary Material

Supplementary appendix table can be found at http://www.jhered.oxfordjournals.org/.

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