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A note on the genetic differentiation of the Bryde's whales from the Gulf of California

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ABSTRACT

Preliminary results of the comparison of mitochondrial DNA (mtDNA) control region sequences of Bryde's whales from the Gulf of California (GCA) and published sequences of whales from the Pacific and Indian Oceans are presented. The analyses were conducted to confirm the species identity of whales in the GCA and to elucidate the genetic relationship of this population with populations from other oceanic regions. The analyses included 503 samples, 401 from the western North Pacific, 24 from the western South Pacific (Fiji), 24 from the eastern South Pacific (Peru), 23 from the eastern Indian Ocean (Java) and 31 from the GCA. Phylogenetic analysis associated the Bryde's whales from the GCA with the "*brydei*" form. A total of 54 haplotypes was identified in the total sample, of these five were found in the GCA with three being specific to this locality (involving 71% of the samples) and two shared with the Peruvian population. The GCA whales exhibited the highest value of nucleotide diversity (0.0116 SE 0.0009), but it was not significantly different from other localities. The smallest genetic distance resulted from the comparison between the GCA and Peruvian populations. The statistical analysis of heterogeneity based on Chi-square, *Kst* and *Hst* showed significant differences between the GCA and each of the other regions. It is concluded preliminarily that the Bryde's whales of the GCA belong to the "*brydei*" form, and that this population is genetically differentiated from other populations in the Pacific and Indian Oceans.

INTRODUCTION

Bryde's whales distribute along all the Pacific coast of Mexico (Leatherwood *et al.*, 1982), being one of the most common baleen whale species in the Gulf of California (GCA) (Leatherwood *et al*., 1982; Urban and Flores, 1996), where is observed through the year.

Little is known about the genetic population structure of the Bryde's whale of the GCA. Dizon *et al.* (1996) examined the mitochondrial DNA diversity of Bryde's whales from the GCA but the analyses were focused mainly on taxonomy and were based on a limited number of samples. Previous studies showed seasonal fluctuations in the abundance of animals in the south of the Gulf suggesting seasonal movement of whales to and from the GCA (Salvadeo *et al*., 2011). On the other hand photo identification studies suggested that whales in the north of the Gulf could be resident (Breese and Tershy, 1987).

Wada *et al*. (2003) provided genetic evidence to separate *B. brydei* (Bryde's whale) and *B. edeni* (Eden's whale) into two distinct species. The Eden's whale has been recorded for coastal areas in Japan and Philippines. The Bryde' whale has a pelagic distribution in all oceans of the world but limited to the regions comprised approximately between 40°N and 40°S. The taxonomic status of Bryde's whales is still unresolved and the International Whaling Commission's Scientific Committee (IWC SC) is still naming the Bryde's whale 'complex' as *Balaenoptera edeni*.

This paper presents preliminary results of a genetic study based on mitochondrial DNA (mtDNA) of Bryde's whales of the GCA. The study was conducted with two main objectives, first to determine the species identity of whales in this locality according to Wada *et al.* (2003)'s classification, and second elucidate the genetic relationship of the GCA whales with populations from the Pacific and Indian Oceans investigated by Kanda *et al.* (2007).

MATERIALS AND METHODS

Samples

Genetic samples from the GCA Bryde's whales were obtained by skin biopsy sampling during the months January and November in the period between 2004 and 2012 (n=31). Biopsy samples were collected using Paxarm system and crossbows. Figure 1 shows the geographical distribution of the Bryde's whale samples from GCA used in this study.

Mitochondrial DNA sequences of Bryde's whale from the western North Pacific, western South Pacific (Fiji), eastern South Pacific (Peru) and eastern Indian Ocean (Java) published by Kanda *et al.* (2007) were used for comparative purposes.

Molecular genetic analysis

Genomic DNA was extracted from approximately 0.05g of the outer epidermal layer of the skin, muscle or baleen tissue using the protocol of Sambrook *et al*. (1989). Extracted DNAs were stored in TE buffer (10mM Tris-HCl, 1mM EDTA, ph8.0).

Sequencing analyses of the 299bp control region of mitochondrial DNA (mtDNA) was conducted using the primers MT4 (Arnason *et al*. 1993) and P2 (5'-GAA GAG GGA TCC CTG CCA AGC GG-3'). Reactions were carried out in 50 uL volumes containing 100 mM KCl, 20 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT; 0.5% Tween 20, 0.5% Nonidet P-40, 200 uM dNTPs, 2.5 pM of each oligo-nucleotide and one unit of Taq DNA polymerase. After an initial denaturation step at 95° C for 5 minutes, a PCR amplification cycle of 30 seconds at 94°C, followed by 30 seconds at 50°C and 30 seconds at 72°C was repeated 30 times. The amplification was completed with a final extension step of 10 minutes at 72°C. Subsequent cycle sequencing reactions were performed with 100ng of products generated in the above PCR amplifications using the Prism^{TM} dRhodamine Terminator Cycle Sequencing Kit (Applied Biosystems, Inc.). The oligo-nucleotides used to prime the cycle sequencing reaction were the same as employed in the initial PCR amplification listed above. A total of 25 cycles with 10 seconds at 96°C, 20 seconds at 56°C and four minutes at 60°C were performed. The nucleotide sequence of each cycle sequencing reaction was determined by electrophoresis through a 5% Long Ranger^{TM} (FMC, Inc.) denaturing polyacrylamide matrix on a DNA Prism^{TM} 377 DNA Sequencer (Applied Biosystems, Inc.) under standard conditions. Both strand samples were sequenced in their entirety for all samples.

Analysis of genetic data

Genetic distances among unique sequences (haplotypes) were estimated using the Kimura's two parameters method (Kimura, 1980). The degree of mtDNA diversity within each Area was estimated using the nucleotide diversity (Nei and Li, 1979). The net-interpopulational distances among populations were estimated using equation 10.20 of Nei (1987).

Heterogeneity tests among oceanic regions were conducted as described in Hudson *et al*. (1992), using the chi-square, the *Hst* and the *Kst** statistics. The level of statistical significance was estimated from 10,000 Monte Carlo simulations as the proportion of simulations in which a similar or more extreme value of chi-square, *Hst* or *Kst** was observed.

The effective number of migrants (*Nem*) was estimated assuming Wright's island model using equation of Hst = 1/(1 + 2*Nem*) (Takahata and Palumbi, 1985).

A phylogenetic tree of haplotypes was generated using the neighbor-joining method (Saitou and Nei, 1987). To estimate confidence intervals, 1,000 bootstrap simulations were conducted. The phylogeny was rooted using the homologous sequence from the Omura and Eden whales (Wada *et al*., 2003).

RESULTS

Genetic diversity

In the total sample of 503 animals (including the samples examined by Kanda *et al*., 2007) there were 39 segregating sites (all transitions) discriminating a total of 54 haplotypes (Table 1). Then the additional 31 samples from GCA discriminated three new haplotypes in relation the data set used by Kanda *et al*. (2007) (haplotypes 52, 53 and 54 in Table 1).

Nucleotide diversity by geographic locality is shown in Table 2. The diversity in the GCA Bryde's whale was the highest, but it was not significantly different from other localities.

Population differentiation

Table 1 shows the geographical distribution of haplotypes by geographical locality. There was a total of five haplotypes in the GCA whales, two of them (41 and 45 represented by nine samples) was shared with the Peruvian population, and three (52, 53 and 54 representing 22 samples) were specific for the GCA. Table 2 shows the net-interpopulational distances among populations. The comparison between Bryde's whales from Peru and GCA yielded the smallest values among all pairwise comparisons.

Table 3 shows the results of the statistical test for population differentiation for several statistics. GCA Bryde's whale differed significantly from all other populations of Bryde's whales included the Peruvian population.

Assuming the island model of migration, the values of *Hst* translated into 18 female migrants per generation between western North Pacific and GCA; nine between Peru and GCA; three between Fiji and GCA and two between Java and GCA.

Phylogenetic analysis

Figure 2 shows the neighbor-joining based phylogenetic tree of Bryde's whale haplotypes. Bryde's whales in the GCA clustered with Wada *et al*. (2003)'s Bryde's whale (*B. brydei*). Within the brydei, no geographic specific clades were found.

DISCUSSION

This paper presented the results of the first population-level genetic analysis on Brydes' whale from Baja California. For studying the population genetic structure of Bryde's whales from the GCA we took advantage of published sequences of Bryde's whales from several oceanic regions. First the phylogenetic analyses suggested that whales from the GCA belong to the 'Brydei' form according the terminology used by Wada y Numachi (1991) and Dizon *et al.* (1996; 1997) or to th especies *B. brydei* according to the classification of Wada *et. al*. (2003).

Second the heterogeneity test based on haplotype and sequence statistics showed that Bryde's whales from the GCA are genetically differentiated from brydei-type whales from other regions in the Pacific and Indian Oceans. Regarding the North Pacific these results support the population division proposed by the IWC (1996).

Our results showed a good concordance between the level of genetic differentiation and geographical distances. Whales from the GCA were genetically closer to Peruvian and western North Pacific animals than to the geographical distant populations of Fiji and Java. This was evident by the results of the net inter-populational genetic distances, Hst/Kst* and the number of migrant females by generation.

The question now is whether or not there is additional structure within the GCA. As mentioned earlier, previous studies suggested the possibility of two populations, one being resident and the other transient with movement inside/outside the Gulf (Urbán y Flores, 1996, Dizon *et al*., 1996, y IWC 1996). Our data are consistent with this hypothesis, for example the resident population could be associated with the specific haplotypes to the GCA (52, 53 and 54) while the transient population could be associated with the shared haplotypes with other populations in the eastern Tropical Pacific (41 and 45).

The alternative explanation is of a single population in the GCA with specific and also shared haplotypes with adjacent populations. The time of divergence between the GCA and Peruvian populations is estimated from 0.19Mya to 0.37Mya (for substitution rates of 1% and 0.5% per million years, respectively). Then the time of divergence could not be long enough for a complete sorting of haplotypes between the CGA and adjacent populations.

To elucidate between both explanations the genetic analysis of samples obtained in the Pacific Ocean outside the GCA as well the analyses of the samples within the GCA on a temporal basis, will be necessary.

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Table 1. Frequencies of haplotypes in the Bryde's whale by geographical locality (WNP=western North Pacific; GCA=Gulf of California).

HAPLOTYPES	WNP	FIJI	PERU	GCA	JAVA	SUM
1	50	15	1	0	0	65
2	2	0	0	0	0	2
3	35	0	1	0	0	36
4	3	0	0	0	0	3
5	156	3	1	0	0	160
6	8	0	0	0	0	8
7	14	0	0	0	0	14
8	14	0	1	0	0	15
9	3	0	0	0	0	3
10	6	0	0	0	0	6
11	5	0	0	0	0	5
12	4	0	0	0	0	4
13	5	0	0	0	0	5
14	1	0	0	0	0	1
15	8	0	0	0	0	8
16	6	0	0	0	0	6
17	2	0	1	0	0	3
18	14	0	0	0	0	14
19	8	0	0	0	0	8
20	4	0	0	0	0	4
21	3	0	0	0	0	3
22	5	0	0	0	0	5
23	2	0	0	0	0	2
24	2	0	0	0	0	2
25	7	0	0	0	0	7
26	7	0	0	0	0	7
27	1	0	0	0	0	1
28	6	0	0	0	0	6
29	8	0	0	0	0	8
30	2	0	0	0	0	2
31	1	0	0	0	0	1
32	1	0	0	0	0	1
33	3	0	0	0	0	3
34	2	0	0	0	0	2
35	1	0	0	0	0	1
36	2	3	1	0	0	6
37	0	2	0	0	0	2
38	0	1	0	0	0	1
39	0	0	1	0	0	1
40	0	0	1	0	0	1
41	0	0	1	**2**	0	3
42	0	0	5	0	0	5
43	0	0	1	0	0	1
44	0	0	1	0	0	1
45	0	0	3	**7**	0	10
46	0	0	5	0	0	5
47	0	0	1	0	0	1
48	0	0	0	0	19	19
49	0	0	0	0	2	2
50	0	0	0	0	1	1
51	0	0	0	0	1	1
52	0	0	0	**12**	0	12
53	0	0	0	**7**	0	7
54	0	0	0	**3**	0	3
Total	**401**	**24**	**24**	**31**	**23**	**503**

Table 2. Nucleotide diversity in the Bryde's whale by locality (in the diagonal with standard errors in parenthesis), and net-interpopulational distances (WNP=western North Pacific; GCA=Gulf of California).

	WNP n=401	Fiji n=24	Peru n=24	**GCA n=31**	Java n=23
WNP	0.01012 (0.00058)	0.00122	0.00477	**0.01160**	0.01145
Fiji		0.00720 (0.00175)	0.00685	**0.01497**	0.01304
Peru			0.01042 (0.00181)	**0.00369**	0.00888
GCA				**0.01164 (0.00091)**	**0.01185**
Java					0.00629 (0.00292)

Table 3. Results of the heterogeneity test among localities (WNP=western North Pacific; GCA=Gulf of California).

	Hst	*Kst**	Chi-square
WNP-Fiji	0.0215 (P=0.0000)	0.0166 (P=0.0000)	P=0.0000
WNP-Peru	0.0140 (P=0.0000)	0.0377 (P=0.0000)	P=0.0000
WNP-GCA	**0.0273 (P=0.0000)**	**0.0867 (P=0.0000)**	**P=0.0000**
WNP-Java	0.0528 (P=0.0000)	0.0885 (P=0.0000)	P=0.0000
Fiji-Peru	0.1366 (P=0.0000)	0.2347 (P=0.0000)	P=0.0000
Fiji-GCA	**0.1640 (P=0.0000)**	**0.3299 (P=0.0000)**	**P=0.0000**
Fiji-Java	0.3760 (P=0.0000)	0.4722 (P=0.0000)	P=0.0000
Peru-GCA	**0.0511 (P=0.0000)**	**0.1296 (P= 0.0000)**	**P= 0.0000**
Peru-Java	0.2377 (P=0.0000)	0.3336 (P=0.0000)	P=0.0000
GCA-Java	**0.2550 (P=0.0000)**	**0.3268 (P=0.0000)**	**P=0.0000**

Figure 1. Geographical distribution of the samples of Bryde's whales in the GCA used in the present genetic study.

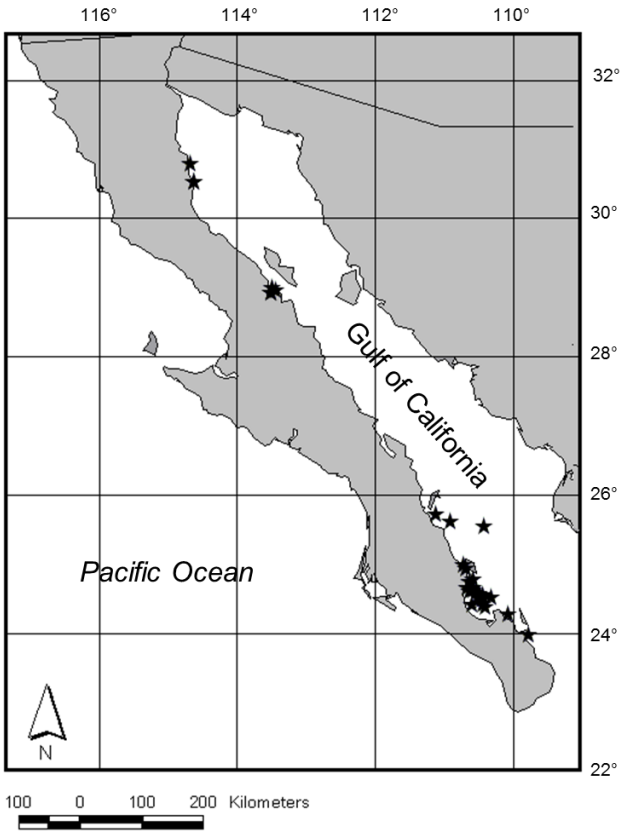

Figure 2. Phylogenetic relationship among 54 Bryde's whale haplotypes. In square are the haplotypes specific for the GCA locality. Closed circles indicate high bootstrap values (over 50% in 1,000 simulations).