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ARTICLE

Population genetic structure of the South American Bryde's whale

Estructura genética poblacional de la ballena de Bryde en América del Sur

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Resumen.- Un análisis genético basado en secuencias de la región control del ADN mitocondrial fue realizado para investigar tanto la identidad de la especie como la estructura genética poblacional de la ballenas de Bryde Sudamericanas. El análisis genético se basó en muestras históricas, biopsias y varamientos de Chile (n= 10) y Brasil (n= 8). Para fines de comparación, secuencias publicadas de ballenas de Bryde de diferentes localidades de los océanos Índico y Pacífico (incluyendo Perú, n= 24) fueron incorporadas en los análisis. El análisis filogenético identificó las ballenas de Bryde de América del Sur como *Balaenoptera brydei*. Ninguna diferenciación genética estadísticamente significativa fue encontrada entre ballenas de Bryde de Chile y Perú. Sin embargo, fuertes diferencias genéticas se encontraron entre animales del Atlántico Sur occidental (Brasil) y Pacífico Sur oriental (Perú y Chile). También fuertes diferencias genéticas fueron encontradas entre todas las localidades de América del Sur y aquellos del Pacífico Norte occidental, Fiji y Java. Estos resultados sugieren movimiento de *B. brydei* en el Pacifico Sur oriental en el rango latitudinal correspondientes a Chile y Perú. Estos resultados también sugieren ningún o muy limitado movimiento de ballenas entre los océanos del Pacífico Sur y Atlántico Sur. Esto es consistente con la idea de que *B. brydei* no se distribuye más al sur de los 40ºS en ambos lados de América del Sur.

Palabras clave: *Balaenoptera brydei*, ADN mitocondrial, Pacífico Sur oriental, Atlántico Sur occidental

Abstract.- A genetic analysis based on mitochondrial DNA control region sequences was conducted to investigate both species identity and populations genetic structure of South American Bryde's whales. The genetic analysis was based on historical, biopsy and stranding samples from Chile (n= 10) and Brazil (n= 8). For comparative purposes published sequences of the Bryde's whales from different localities of the Indian and Pacific Oceans (including Peru, n= 24) were incorporated into the analysis. Results of the phylogenetic analysis identified the Bryde's whales of South America as *Balaenoptera brydei*. No statistically significant genetic differentiation was found between Chilean and Peruvian Bryde's whales. However, striking differences were found between western South Atlantic (Brazil) and eastern South Pacific (Peru and Chile) animals. In addition, striking genetic differences were found between all South American localities and those from the western North Pacific, Fiji and Java. These results suggest movement of *B. brydei* in the eastern South Pacific in the latitudinal range corresponding to Chile and Peru. These results also suggest no or very limited movement of whales between the South Pacific and the South Atlantic Oceans. This is consistent with the notion that *B. brydei* is not distributed further south of approximately 40ºS on both sides of South America.

Key words: *Balaenoptera brydei*, mitochondrial DNA, eastern South Pacific, western South Atlantic

INTRODUCTION

Bryde's whales have been difficult to study in several regions of the world due to its similarity with the sei whale *Balaenoptera borealis* (Lesson, 1828). Whaling stations in the eastern South Pacific continued to confuse both species, in Peru until 1973 and in Chile until the end of commercial whaling in 1983 (Valdivia *et al.* 1981, Gallardo *et al.* 1983). In Brazil, this confusion remained until 1967 at some stations, and until the end of the commercial

whaling period (1988) in others (Zerbini *et al*. 1997). In the North Pacific, Bryde's and sei whale catches were reported separately at a much earlier date, in the mid-1950s.

In the eastern South Pacific, analyses based on sighting and catch distribution suggested that Bryde's whales are distributed in Peruvian waters almost throughout the year with abundance changing seasonally (Pastene & Ohsumi 1998)[1]. The first record of Bryde's whale in Chile was reported by Clarke & Aguayo (1965) on the basis of an individual caught off Iquique (20°S). Subsequently Gallardo *et al.* (1983) reported the occurrence of Bryde's whales based on sighting data between 32°-36°S approximately. Pastene *et al.* (1983)[2] confirmed the occurrence of Bryde's whales in central Chile through the examination of three animals caught in the latitudinal range of 33º-35ºS. Aguayo *et al.* (1998) reported a single sighting of a Bryde's whale in the adjacent waters of San Felix and San Ambrosio Islands (26º59'S; 86º39'W) on 3 September 1994. Currently, the distribution of Bryde's whales in the eastern South Pacific off Chile based on animals confirmed as Bryde's whales and 'like' Bryde's whales (Fig. 1) show a continuity in geographical distribution between the latitudinal range of 18º43'S and 37º58'S and in the longitudinal range between the Chilean coast and 76º16'W. Moreover, seasonal pattern of occurrence of Bryde's whales in the eastern South Pacific was consistent with the hypothesis of a north (Peru)-south (to latitude 38ºS in Chile) movement of whales in the eastern South Pacific in spring and summer (Pastene 1982).

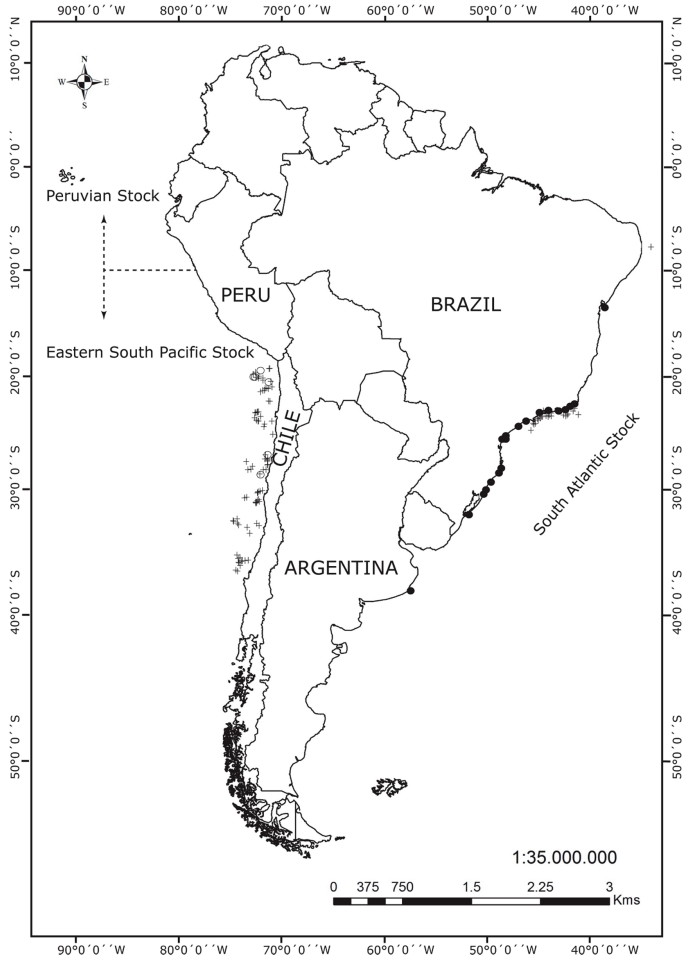

Figure 1. Distribution of sightings (+), like Bryde's (o) and strandings (•) of Bryde's whale in Chilean and Brazilian waters. Sources: Clarke & Aguayo (1965), Gallardo *et al*. (1983), Pastene *et al.* (1983), Zerbini *et al*. (1997), Aguayo *et al*. (1998), Findlay *et al*. (1998), Siciliano *et al*. (2004), Andriolo *et al*. (2010), Figuereido *et al*. (2014) and unpublished data / Distribución de los avistamientos (+), probable Bryde (o) y varamientos (•) de ballenas de Bryde en aguas de Chile y Brazil. Fuentes: Clarke & Aguayo (1965), Gallardo *et al*. (1983), Pastene *et al.* (1983), Zerbini *et al.* (1997), Aguayo *et al*. (1998), Findlay *et al*. (1998), Siciliano *et al*. (2004), Andriolo *et al.* (2010), Figueiredo *et al*. (2014) y datos no publicados

[1]Pastene LA & S Ohsumi. 1998. A brief review of the information on distribution and stock identity of Bryde's whales (Balaenoptera edeni) in the eastern South Pacific. Paper SC/50/CAWS6 presented to the IWC Scientific Committee, June 1998 (unpublished), 18 pp.
[2]Pastene LA, M Acevedo & VA Gallardo. 1983. A note on Chilean Bryde's whales. Paper SC/35/Ba4 presented to the IWC Scientific Committee June 1983 (unpublished), 9 pp.

In the western South Atlantic, analysis of sighting and stranding data suggested that Bryde's whales occur regularly in coastal areas mainly in southeastern and southern Brazil (Fig. 1). Sighting of this species were recorded mainly in summer and autumn while strandings have been recorded through the year (Zerbini *et al.* 1997, Siciliano *et al.* 2004). Bastida & Rodriguez (2009) reported that in the Atlantic coastal waters of South America, the southernmost records corresponded to a couple of strandings recorded on the northern coasts of Argentina (38°06'S; 57°34'W, Fig. 1). Little is known about the relationship between western South Atlantic and eastern South Pacific Bryde's whales.

The taxonomy of Bryde's whales is still unresolved. Some authors (*e.g.*, Wada *et al.* 2003) recognize two species, the smaller one *B. edeni* Anderson, 1879 (Eden's whale) and a larger one *B. brydei* Olsen, 1913 (Bryde's whale), while others (*e.g.*, Kershaw *et al.* 2013) assign these species a sub-specific status: *B. edeni edeni* and *B. edeni brydei*, respectively. The smaller one inhabits primarily coastal and continental shelf waters of the Northern Indian Ocean and the western Pacific Ocean while the larger one inhabits tropical and warm temperature waters worldwide (Rice 1998). Wada *et al.* (2003) provided genetics, external morphology and osteology evidences to separate *B. brydei* and *B. edeni* into two distinct species. The present study follows the taxonomic classification of Wada *et al.* (2003).

Peru, Chile and Brazil were involved in commercial whaling of Bryde's whales until approximately the mid 1980's. The International Whaling Commission's Scientific Committee (IWC-SC) defined the geographic boundaries of stocks for management purposes: a 'Peruvian stock' from 10ºN-10ºS and from the South American coast to 110ºW, including the Galápagos Archipelago; and an 'Eastern South Pacific stock' from the South American coast and 150ºW, excluding the Peruvian stock area (Donovan 1991). Whales caught in Brazil were from the 'South Atlantic stock' (Donovan 1991). There is, however, limited biological evidence to support those geographic delineations.

The objective of this study were, firstly to examine the species identity and secondly to elucidate the population genetic structure of the species around South America, through the genetic analyses of samples collected on both sides of South America. Given the known geographical distribution of *B. edeni* and *B. brydei* (Rice 1998), our expectation was that the species distributed in South American waters correspond to the latter one. Within the species, our expectation was that the genetic composition of whales from Peru and Chile is similar based on the hypothesised seasonal movement in the eastern South Pacific (Pastene 1982), and that whales from the eastern South Pacific and western South Atlantic are genetically distinct given that their distribution is limited to regions north of 40ºS and therefore no or only limited interchange of whales occurs between those ocean basins due to the landmass barrier (southern South America) that extends south to 53ºS.

MATERIALS AND METHODS

SAMPLES

Biopsy samples from the Chilean Bryde's whales were obtained by the IWC Southern Ocean Whale and Ecosystem Research Program (IWC-SOWER) survey conducted in December 1997 (Findlay *et al.* 1998)[3] (n= 8). Biopsy skin samples were collected using the Paxarm system (Krützen *et al.* 2002) and crossbows. In addition baleen plates were available from whales caught in Chile in April 1983 (n= 2). Muscle samples from the Brazilian Bryde's whales were obtained from stranded animals along the southern and southeastern coast of Brazil in January, February, March, August, September and October between 2004 and 2010 (n= 8). Skin/muscle samples were stored in ethanol 100% until their use. Sequences from the Peruvian Bryde's whales published in Kanda *et al.* (2007) were obtained from muscle samples collected during a former Japanese coastal whaling operation in Peru in January-March 1983. Samples were stored at -20°C until their use. Figure 2 shows the geographic distribution of the samples used in the present study and Table 1 shows ancillary information of the sampling in Chile and Brazil.

LABORATORY ANALYSIS

Genomic DNA was extracted from approximately 0.05 g of the outer epidermal layer of the skin or muscle using the protocol of Sambrook & Russell (2001). In the case of baleen plates, DNA was extracted using the Bio 101 'Genclean Kit for Ancient DNA'. Extracted DNAs were stored in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

[3]Findlay K, R Pitman, T Tsurui, K Sakai, P Ensor, H Iwakami, D Ljungblad, H Shimada, D Thiele, K Van Waerebeek, R Hucke-Gaete & GP Sanino-Vatier. 1998. 1997/1998 IWC-Southern Ocean Whale and Ecosystem Research (IWC-SOWER) Blue Whale Cruise, Chile. Paper SC/SO/Rep.2 presented to the IWC Scientific Committee (unpublished), 40 pp.

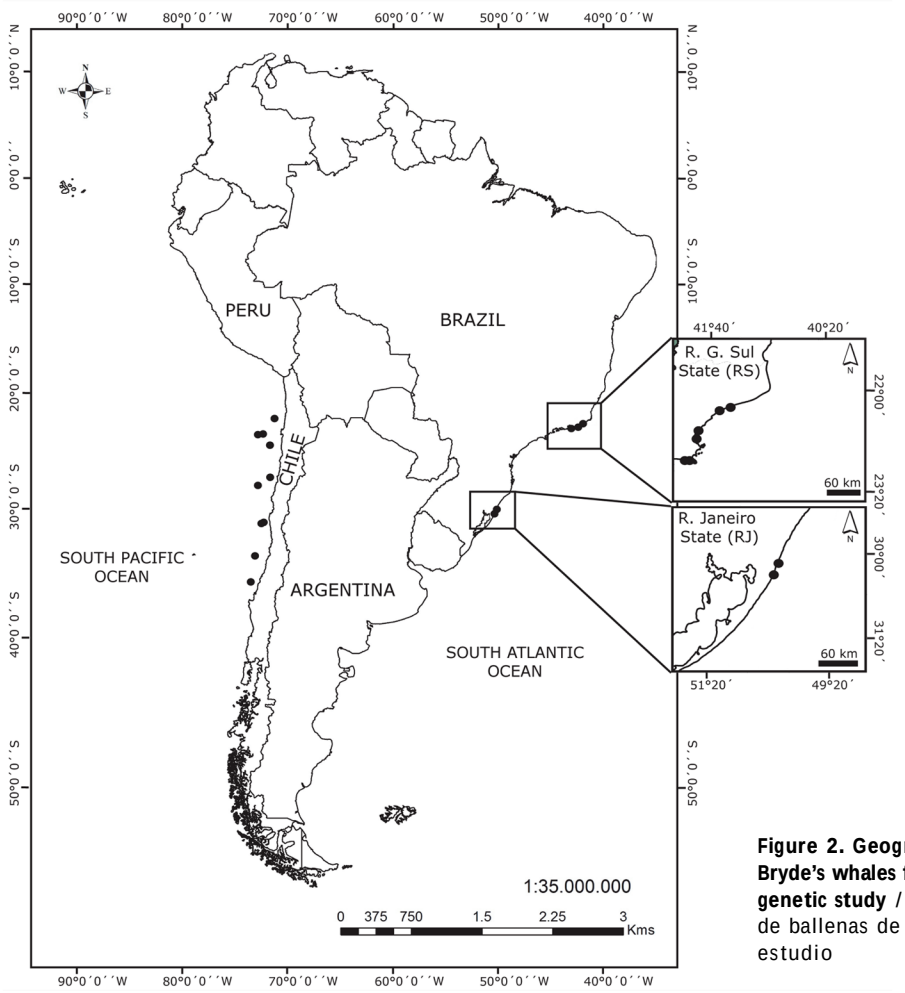

Figure 2. Geographical distribution of the samples of Bryde's whales from Chile and Brazil used in the present genetic study / Distribución geográfica de las muestras de ballenas de Bryde de Chile y Brasil usadas en este estudio

Sequencing analyses of the 299 base pairs (bp) control region of mitochondrial DNA (mtDNA) was conducted using the primers MT4 (Arnason *et al.* 1993) and P2 (Kanda *et al.* 2007). 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 (TaKaRa Ex Taq). After an initial denaturation step at 95°C for 5 min, a PCR amplification cycle of 30 s at 94°C, followed by 30 s at 50°C and 30 s at 72°C was repeated 30 times. The amplification was completed with a final extension step of 10 min at 72°C. Subsequent cycle sequencing reactions were performed with 100 ng of products generated in the above PCR amplifications using the Prism™ dRhodamine Terminator Cycle Sequencing Kit (Applied Biosystems, Inc.). The oligo-nucleotides used to prime the cycle sequencing reaction were the same as those used in the initial PCR amplification. A total of 25 cycles with 10 s at 96°C, 20 s at 56°C and 4 min at 60°C were performed. Purification of cycle sequencing reactions was made using AutoSeq G-50 Dye Terminator Removal Kit. The nucleotide sequence of each cycle sequencing reaction was determined by electrophoresis through a 5% Long Ranger™ (FMC, Inc.) denaturing polyacrylamide matrix on a DNA Prism™ 377 DNA Sequencer (Applied Biosystems, Inc.) under standard conditions. Both DNA strand were sequenced for each sample. A consensus sequence was created for each sample after resolving potential disagreements in aligned sequences.

Table 1. Samples of the Bryde's whales from Chilean and Brazilian waters used in the present analyses / Muestras de ballenas de Bryde de Chile y Brazil utilizadas en el análisis

Location	Sampling date	Source	Latitude (S)	Longitude (W)	Reference
Chilean waters	10 Apr 1983	Catch	33º55'	72º46'	Pastene (1983)
Chilean waters	12 Apr 1983	Catch	35º19'	73º09'	Pastene (1983)
Chilean waters	14 Dec 1997	Biopsy	24º08'	71º45'	Findlay *et al*. (1998)
Chilean waters	19 Dec 1997	Biopsy	21º46'	71º15'	Findlay *et al*. (1998)
Chilean waters	21 Dec 1997	Biopsy	22º47'	72º25'	Findlay *et al*. (1998)
Chilean waters	21 Dec 1997	Biopsy	22º50'	72º39'	Findlay *et al*. (1998)
Chilean waters	24 Dec 1997	Biopsy	27º02'	71º38'	Findlay *et al*. (1998)
Chilean waters	26 Dec 1997	Biopsy	27º41'	72º54'	Findlay *et al*. (1998)
Chilean waters	30 Dec 1997	Biopsy	30º53'	72º32'	Findlay *et al*. (1998)
Chilean waters	30 Dec 1997	Biopsy	30º49'	72º28'	Findlay *et al*. (1998)
Brazilian waters	26 Feb 2004	Stranding	22º14'	41º32'	This study
Brazilian waters	23 Jan 2005	Stranding	22º33'	41º58'	This study
Brazilian waters	28 Sep 2006	Stranding	22º40'	41º59'	This study
Brazilian waters	18 Aug 2007	Stranding	22º58'	42º02'	This study
Brazilian waters	11 Oct 2008	Stranding	22º57'	42º05'	This study
Brazilian waters	08 Mar 2010	Stranding	22º17'	41º40'	This study
Brazilian waters	20 Jan 2005	Stranding	30º13'	50º13'	This study
Brazilian waters	21 Jan 2005	Stranding	30º20'	50º16'	This study

Analysis of genetic data

Phylogenetic analysis

Sequences were aligned by eye using Sequence Navigator (Applied Biosystem, Inc.). Variable sites and unique sequences (haplotypes) were identified using the program MacClade (Maddison & Maddison 2000).

A phylogenetic tree of haplotypes was generated using the Neighbor-Joining method (Saitou & Nei 1987) as implemented in the program PHYLIP (Felsenstein 1993). Genetic distances among haplotypes were estimated using the program DNADIST of PHYLIP, based on Kimura's 2-parameter model (Kimura 1980). A transition-transversion ratio of 5:1 was used. For comparative purposes, mtDNA control region sequences of *B. edeni* published by Yoshida & Kato (1999) and Wada *et al*. (2003) and of *B. brydei* published by Kanda *et al*. (2007), were included in the estimation. The genealogy was rooted using the homologous sequence from *B. omurai* and *B. borealis* published in Wada *et al*. (2003). To estimate support for each node a total of 1,000 bootstrap simulations were conducted and the majority-rule consensus genealogy estimated. The genealogy of mtDNA haplotypes was also inferred using the maximum likelihood (ML) method as implemented in the program MEGA6 (Tamura *et al*. 2013). The Tamura-Nei's three-parameter substitution model with Gamma distribution (Tamura & Nei 1993) was set as substitution model to reconstruct the ML tree based on the results of the ModelTest. *B. omurai* and *B. borealis* were used as outgroups. Support for the groupings was estimated with 1,000 bootstrap replications.

Genetic diversity and population differentiation

The degree of mtDNA diversity within each area was estimated using the nucleotide diversity (Nei & Li 1979). The level of differentiation of mtDNA between populations was estimated using net interpopulation genetic distance, dA (Nei 1987). Heterogeneity tests among whales from different 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*

Table 2. Frequencies of haplotypes in *B. brydei* by geographical locality (WNP= western North Pacific). Haplotypes were numbered following Kanda *et al*. (2007). Haplotypes 52-55 are new haplotypes with regard those in Kanda *et al*. (2007) / Frecuencias de haplotipos en *B. brydei* por localidad geográfica. (WNP= Pacífico Noroccidental). Haplotipos fueron numeradas siguiendo a Kanda *et al*. (2007). Haplotipos 52-55 son nuevos en relación a aquellos de Kanda *et al*. (2007)

Haplotype	WNP	FIJI	PERU	Chile	Brazil	JAVA	Total
1	50	15	0	0	0	0	65
2	2	0	0	0	0	0	2
3	35	0	1	0	0	0	36
4	3	0	0	0	0	0	3
5	156	3	1	0	0	0	160
6	8	0	0	0	0	0	8
7	14	0	0	0	0	0	14
8	14	0	1	0	0	0	15
9	3	0	0	0	0	0	3
10	6	0	0	0	0	0	6
11	5	0	0	0	0	0	5
12	4	0	0	0	0	0	4
13	5	0	0	0	0	0	5
14	1	0	0	0	0	0	1
15	8	0	0	0	0	0	8
16	6	0	0	0	0	0	6
17	2	0	1	0	0	0	3
18	14	0	0	0	0	0	14
19	8	0	0	0	0	0	8
20	4	0	0	0	0	0	4
21	3	0	0	0	0	0	3
22	5	0	0	0	0	0	5
23	2	0	0	0	0	0	2
24	2	0	0	0	0	0	2
25	7	0	0	0	0	0	7
26	7	0	0	0	0	0	7
27	1	0	0	0	0	0	1
28	6	0	0	0	0	0	6
29	8	0	0	0	0	0	8
30	2	0	0	0	0	0	2
31	1	0	0	0	0	0	1
32	1	0	0	0	0	0	1
33	3	0	0	0	0	0	3
34	2	0	0	0	0	0	2
35	1	0	0	0	0	0	1
36	2	3	1	1	7	0	14
37	0	2	0	0	0	0	2
38	0	1	0	0	0	0	1
39	0	0	1	0	0	0	1
40	0	0	1	0	0	0	1
41	0	0	1	0	0	0	1
42	0	0	5	3	0	0	8
43	0	0	1	0	0	0	1
44	0	0	1	0	0	0	1
45	0	0	3	2	0	0	5
46	0	0	5	1	0	0	6
47	0	0	1	0	0	0	1
48	0	0	0	0	0	19	19
49	0	0	0	0	0	2	2
50	0	0	0	0	0	1	1
51	0	0	0	0	0	1	1
52	0	0	0	1	0	0	1
53	0	0	0	1	0	0	1
54	0	0	0	1	0	0	1
55	0	0	0	0	1	0	1
Total	401	24	24	10	8	23	490

or *Kst** was observed. For the analyses of mtDNA diversity and population differentiation, sequences from *B. brydei* from the eastern South Pacific, western North Pacific, western South Pacific and eastern Indian Ocean published by Kanda *et al*. (2007) were used for comparative purposes.

RESULTS

DNA was successfully extracted and sequenced from the ten and eight samples from Chilean and Brazilian waters, respectively. The final data set included the first 299 nucleotides of the mtDNA control region from a total of 490 specimens (including the 18 from Chile and Brazil and those examined by Kanda *et al*. 2007). There were 38 segregating sites discriminating a total of 55 haplotypes in the total sample (Fig. 3 and Table 2). Haplotypes were numbered following Kanda *et al*. (2007) (Br01, Br02, etc.). All mutations were transitions. The additional 18 samples from Chile and Brazil discriminated four new haplotypes in relation to the data set used by Kanda *et al*. (2007): haplotypes '52', '53', '54' and '55' (Fig. 3 and Table 2). The sequences of these four haplotypes have been deposited in GenBank under Accession numbers KT191131, KT191132, KT191133 and KT191134, respectively.

Figure 3 includes three haplotypes of *B. edeni*, one from *B. omurai* and one from *B. borealis* (Yoshida & Kato 1999, Wada *et al*. 2003). Fixed differences in the nucleotide sequence between the 55 haplotypes (n= 490) and *B. edeni* were observed at eleven nucleotide positions (positions 28, 45, 46, 58, 77, 82, 84, 149, 190, 194, and 237, see Fig. 3).

PHYLOGENETIC ANALYSIS

The haplotypes of South American whales clustered together Kanda *et al*. (2007)'s *B. brydei* haplotypes. The 55 'Br' haplotypes were clearly separated from *B. edeni*, *B. omurai* and *B. borealis* (Fig. 4), a pattern supported by high bootstrap values. These haplotypes were associated with Wada *et al*. (2003)'s *B. brydei*. *B. edeni* and *B. omurai* are closer related with *B. borealis* than with *B. brydei*, and this pattern was also supported by high bootstrap values. Within *B. brydei*, three main clades were observed but none was supported by high bootstrap values, and none was geographic-specific. A similar phylogenetic pattern was found by the ML method (Fig. 5).

	10	20	30	40	50	60	70	
	1111222	2333333444	4455555566	7778888899	11111111 9923334445	1111222222 6999000001	2222222222 1122223333	22222222 33455578
	1790179018	9014789035	6734568903	3791234934	6784890590	3046356783	4536890234	78002333
Br01	CTATCTACTT	TTGCGTATAC	-TTAACT--T	TTAGGGGAAT	--TTAGCACT	TTATAAGCAT	CATCCAGCGA	ACCTTTAT
Br02	..G.....	....	....	....	TA.....	....	..CTT...A.	......G.
Br03	..G.....	....	....	....	TA.....	.....G.	....TG..A.	.....CG.
Br04	..G.....	....	....	....	....	C.....G.	....TG.TA.	....
Br05	..G.....	....	....	....	....	C.....G.	....TG.TAG	....
Br06	..G.....	....	....	....	....	C.....G.	....TG.TA.	.T.....C
Br07	....	....	....	....	....	....	....	.....C..
Br08	..G.....	....	....	....	....	C.....G.	....TG.TAG	...C....
Br09	..G.....	....	....	....	....	C.....G.	....TG..A.	.T......
Br10	..G.....	....	....	....	....	C.....G.	....TG.TA.	.T......
Br11	..G.....	....	....	....	....	C.....G.	...TTG.TA.	.T.....C
Br12	..G.....	....	....	....	....	.....TG.	....TG..A.	....
Br13	..G.....	....	....	....	....	C.....A.G.	....TG.TA.	....
Br14	..G.....	....	....	....	.....G..	C.....G.	....TG.TA.	....
Br15	..G.....	....	....	....	....	.....TG.	....TG....	....
Br16	..G.T....	....	....	....	....	C.....G.	....TG.TA.	.T......
Br17	..G.....	....	....	....	....	.....G.	....TG..A.	.T......
Br18	..G.....	....	....	....	TA.....	....	...TT...A.	......G.
Br19	..G.....	....	....	....	....G.....	C.....G.	....TG.TA.	....
Br20	..G.....	....	....	....	TA.....	.....G.	....TG..A.	......G.
Br21	..GC.....	....	....	....	....	C.....G.	....TG.TA.	...C....
Br22	..G.....	....	....	....	....	C.....	....TG.TA.	.T......
Br23	..G.....	....	....	....	....	...C.....	....TG..A.	.TT.....
Br24	..G....T..	.........T	C	.....G.	TA.....G..	.....G.T..	....TG..A.	....
Br25	..G.....	....	.C........	....	....	C.....G.	....TG.TA.	....
Br26	..G.....	....	....	....	....	C.....	....TG.TA.	...C....
Br27	..G.....	C.........	....	....	....G.....	C.....G.	....TG.TA.	....
Br28	..G.....	....	....	....	....	C.....G.	....TG.TAG	.T......
Br29	..G.....	....	....	....	....	C.....G.	....TG.TA.	...C....
Br30	..G....T..	....	....	....	TA.C...G..	.....G.	T...TG..A.	....
Br31	..G.....	....	....	....	....	C.....G.	...TTG..A.	.T......
Br32	..G.....	....	....	....	....	C.....G.	....T..TA.	.T......
Br33	..G.....	....	....	....	....	C.....G.	T...TG.TAG	...C....
Br34	..G....T..	.........T	C	.....G.	TA.....G..	....	....TG..A.	....C...
Br35	..G.....	....	....	....	....	C.....G.	....TG..A.	.T.....C
Br36	..G.....	....	....	....	TA.....	.....G.	...TTG..A.	..T.....
Br37	..G.....	....	....	....	TA.....	.....G.	....TG..A.	..T.....
Br38	..G.....	....	.....T....	....	....	C.....G.	....TG.TA.	.T.....C
Br39	..G.....	....	....	....	....	.....TG.	....TG..A.	.T......
Br40	..G.....	....	....	....	TA.....	...C....G.	T...TG..A.	....
Br41	..G....T..	.........T	C	.....G.	TA.....G..	....	....TG..A.	....
Br42	..G.....	....	....	....	TA.....	.....G.	....TG..A.	....
Br43	..G....T..	....	....	....	TA.C...G..	....	...TTG..A.	....
Br44	..G....T..	....	....	....	TA.C...G..	.....G.	....TG..A.	....
Br45	..G.....	....	....	....	....	.....G.	....TG..A.	....
Br46	..G....T..	....	....	.....A....	TA.C...G..	.....G.	....TG..A.	..T.....
Br47	....	....	....	....	....	....	....TG....	..T.....
Br48	..G.....	....	....	....	....	....	...TTG....	.T......
Br49	..G....T..	.........T	....G....C	....	.....G..	....	....TG..A.	C
Br50	..G.....	....	....	....	....	....	....TG....	.T......
Br51	..G.....	....	....	....	TA.....	.....G.	...TTG..A.	......G.
BR52	..G.....	....	....	....	TA.....	...C....G.	....TG..A.	....
BR53	..G.....	....	....	....	....	.....G.	....TG..A.	.....C..
BR54	....	....	....	....	....	....	....TG....	....
BR55	..G.....	....	....	....	TA.....C	.....G.	...TTG..A.	..T.....
Edeni1	.....T.C	.........-	CC....C...	.C..A.T.G.	TA.C...GTC	.CG.....	TG..TG..A.	TT.....
Edeni2	.....T.C	.........-	CC....C...	.C..A.T.G.	TA.C...GTC	.CG.....	T...TG..A.	TT..C...
Edeni3	.....T.C	.........-	CC....C...	.C..A.T.G.	TA.C...GTC	.CG.....	TG..TGA.A.	TT.....
Omurai	T..CT.G.C.	.C.T.CTCGA	T..GG.CAT.	CC.AATTG..	..C.GTA..C	.....TGC	TC..TG..A.	TTT..G..
Sei	.C...C.T..	..C.A....-	.CC.....	.CGA..T..A	TA.C...G..	C...G...G.	T...TG..A.	C.T....C

Figure 3. Variable sites defining 55 mtDNA haplotype in *B. brydei*. The column on the left are haplotype ID. The numbers above are the nucleotide positions of the polymorphic sites starting from the 5' end of the mtDNA control region. Haplotypes '2' through '55' are listed with reference to haplotype '1'. A dot indicates an identical nucleotide at the position relative to haplotype '1'. Haplotypes of *B. edeni*, *B. omurai* and *B. borealis* published in Yoshida & Kato (1999) and Wada *et al*. (2003) are included for comparative purposes / Sitios variables definiendo los 55 haplotipos de ADNmt in *B. brydei*. La columna sobre la izquierda son los ID de los haplotipos. Los números de arriba son las posiciones de nucleótidos de los sitios polimórficos a partir del extremo 5' de la región de control del ADNmt. Los haplotipos '2' a través de '55' se listan en referencia al haplotipo '1'. Un punto indica un nucleótido idéntico en la posición relativa del haplotipo '1'. Los haplotipos de *B. edeni*, *B. omurai* y *B. borealis* publicados en Yoshida & Kato (1999) y Wada *et al*. (2003) se incluyen con fines comparativos

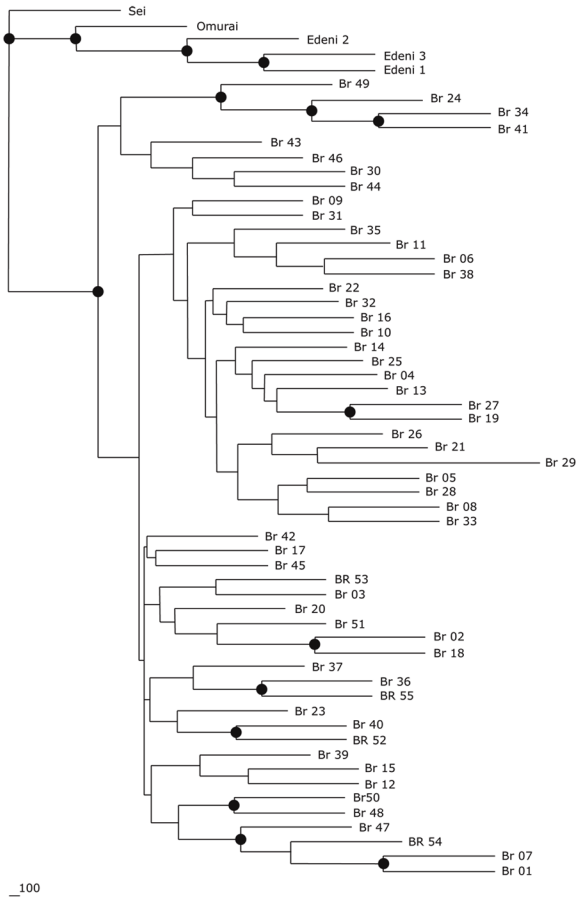

Figure 4. Neighbor-Joining-based tree of Bryde's whale mtDNA haplotypes. Haplotypes were numbered following Kanda *et al*. (2007) (Br01, Br02, etc.) (see Table 2 and Fig. 3). Clades supported by over 50% in 1,000 bootstrap simulations are indicated by a dot. Note that haplotype 'Br36' corresponds to Wada *et al*. (2003)'s *B. brydei* haplotype / Árbol filogenético de los haplotipos de ADN mitochondrial generado por el método Neighbor-Joining. Haplotipos fueron numerados siguiendo a Kanda *et* al. (2007) (Br01, Br02, etc.) (ver Tabla 2 y Fig. 3). Clados apoyados por más de 50% en 1.000 simulaciones son indicados por un punto. Se debe notar que el haplotipo 'BR36' corresponde al haplotipo de *B. brydei* en Wada *et al*. (2003)

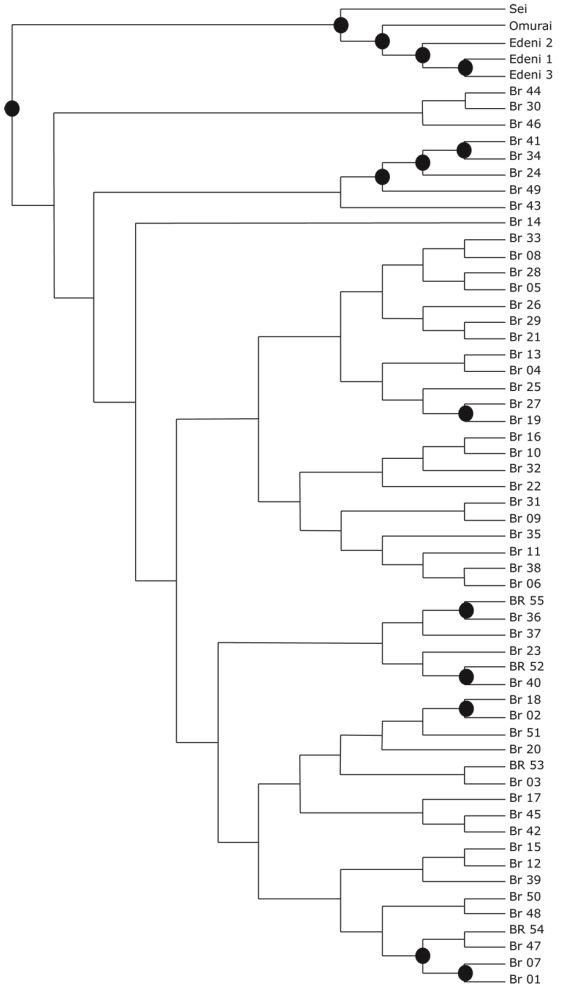

Figure 5. ML-based tree of Bryde's whale mtDNA haplotypes. Haplotypes were numbered following Kanda *et al*. (2007) (Br01, Br02, etc.) (see Table 2 and Fig. 3). Clades supported by over 50% in 1,000 bootstrap simulations are indicated by a dot / Árbol filogenético de los haplotipos de ADN mitochondrial generado por el método de ML. Haplotipos fueron numerados siguiendo a Kanda *et al.* (2007) (Br01, Br02, etc.) (ver Tabla 2 y Fig. 3). Clados apoyados por más de 50% en 1.000 simulaciones son indicados por un punto

GEOGRAPHICAL DISTRIBUTION OF *B. BRYDEI* HAPLOTYPES

Among the 55 *B. brydei* haplotypes there was a total of 7 haplotypes in the Chilean sample, with 3 being specific to that locality. Four haplotypes (representing 70% of the total samples) were shared with the Peruvian sample. Only 2 haplotypes were discriminated in the Brazilian sample, one specific to that locality (one individual) and the other shared with all other localities except Java (7 individuals) (Table 2).

GENETIC DIVERSITY

Nucleotide diversity of *B. brydei* was similar among localities except in the case of Brazil where it was significantly lower (Table 3).

POPULATION DIFFERENTIATION

The comparison of dA between *B. brydei* from Peru and Chile yielded a negative value suggesting a close genetic relationship. However, the dA of *B. brydei* from Peru and Chile in relation to Brazilian whales was larger (Table 3).

There were not significant genetic differences between Chilean and Peruvian *B. brydei*. However both Chilean and Peruvian whales differed significantly from Brazilian whales. South American *B. brydei* differed significantly from all other populations of the Indian and Pacific Oceans (Table 4).

Table 3. Nucleotide diversity (on the diagonal) and Kimura's net-interpopulation genetic distance (dA) among populations of *B. brydei*. In parenthesis is the SE (WNP= western North Pacific) / Diversidad nucleotídica (sobre la diagonal) y distancia neta interpoblacional de Kimura (dA) entre poblaciones de *B. brydei*. En paréntesis se denota la desviación estándar (WNP= Pacífico Noroccidental)

	WNP n= 401	Fiji n= 24	Peru n= 24	Chile n= 10	Brazil n= 8	Java n= 23
WNP	0.0101 (0.0006)	0.0012	0.0048	0.0058	0.0121	0.0115
Fiji		0.0072 (0.0018)	0.0069	0.0080	0.0126	0.0130
Peru			0.0104 (0.0018)	-0.0004	0.0060	0.0088
Chile				0.0083 (0.0019)	0.0057	0.0090
Brazil					0.0008 (0.0006)	0.0117
Java						0.0063 (0.0029)

Table 4. Results of the statistical test for genetic heterogeneity of *B. brydei* from South America (WNP= western North Pacific) / Resultados de las pruebas estadísticas de heterogeneidad genética de *B. brydei* de Sudamérica (WNP= Pacífico Noroccidental)

	Hst	Kst*	Chi-square
WNP-Peru	0.0140 (P = 0.0001)	0.0377 (P = 0.0001)	P = 0.0001
WNP-Chile	0.0077 (P = 0.0001)	0.0216 (P = 0.0001)	P = 0.0001
WNP-Brazil	0.0214 (P = 0.0001)	0.0379 (P = 0.0001)	P = 0.0001
Fiji-Peru	0.1366 (P = 0.0001)	0.2347 (P = 0.0001)	P = 0.0001
Fiji-Chile	0.1275 (P = 0.0001)	0.2522 (P = 0.0001)	P = 0.0001
Fiji-Brazil	0.2630 (P = 0.0001)	0.3964 (P = 0.0001)	P = 0.0001
Peru-Chile	-0.0125 (P = 0.8535)	-0.0163 (P = 0.9601)	P = 0.8939
Peru-Brazil	0.1640 (P = 0.0001)	0.2236 (P = 0.0001)	P = 0.0024
Chile-Brazil	0.2195 (P = 0.0014)	0.3323 (P = 0.0001)	P = 0.0039
Java-Peru	0.2377 (P = 0.0001)	0.3336 (P = 0.0001)	P = 0.0001
Java-Chile	0.2511 (P = 0.0001)	0.3514 (P = 0.0001)	P = 0.0001
Java-Brazil	0.4836 (P = 0.0001)	0.5292 (P = 0.0001)	P = 0.0001

DISCUSSION

This paper presents the results of the first genetic analysis of South American Bryde's whales. Results suggested that whales from Peru, Chile and Brazil belong to *B. brydei* according to the taxonomic classification suggested by Wada *et al.* (2003).

The level of nucleotide diversity in the Brazilian *B. brydei* was significantly lower. To confirm this result, the analysis of additional samples from Brazil will be necessary. The current data set is limited to just 8 stranded animals, which could not be representative of the diversity of the population. Further analysis of additional samples, particularly from offshore areas, will be necessary to confirm the low level of genetic diversity in the Brazilian whales.

As expected, substantial genetic differentiation was found between eastern South Pacific and western South Atlantic *B. brydei*. This is consistent with the notion that *B. brydei* is distributed mainly north of approximately 40ºS on both sides of South America, and that migration between the two ocean basins do not occur or that it is very limited. To evaluate the relative effects of divergence and migration between both sides of South America, the approach of Nielsen & Wakeley (2001) should be used in future.

Populations separated by landmass have been reported for other species. For example common minke whale *B. acutorostrata* from both sides of the Japanese archipelago (Sea of Japan and western North Pacific) are genetically differentiated (Pastene *et al*. 2007). Genetic differentiation between animals in the west and east coast of South America has also been reported for other species such as the South American sea lion *Otaria flavescens* (Artico *et al*. 2010), humpback whale *Megaptera novaeangliae* (Engel *et al*. 2008) and dusky dolphins *Lagenorhynchus obscurus* (Cassens *et al*. 2003), which reveals a common phylogeographic pattern.

In contrast, *B. brydei* from Chile and Peru were closely related as suggested by a negative value of the net inter-populational distance and no statistically significant mtDNA differences between whales in these 2 localities. This result suggests that *B. brydei* from Chile and Peru belong to a same population. It should be recognized that the sample sizes used in the comparative genetic analysis between Peru and Chile's whales, were small, 24 and 10 samples, respectively. The analysis of a larger sample size is recommended to confirm this conclusion. However, if the effect size between the putative Peruvian and Chilean populations is at the same level as that between Chile and Brazil, then the small sample size used in this study should have been able to detect the difference, which it did not.

The sighting distribution data is consistent with the hypothesis of a north (Peru)-south (Chile) movement of *B. brydei* in the eastern South Pacific in spring and summer, which is consistent with our genetic results. Sightings of *B. brydei* have been related to a coastal upwelling ecosystem in central Chile in spring and summer (Gallardo *et al*. 1983). As the stomach contents of whales examined in central Chile were composed of pelagic fishes (unpublished information), the putative north-south migration in spring and summer could be related to food availability. Clearly further genetic studies based on samples from a broader longitudinal and latitudinal range are necessary to investigate additional structure within the western South Atlantic population.

Results of the genetic analyses provide no support for a differentiation of *B. brydei* in the eastern South Pacific as suggested by the IWC when it delineated a 'Peruvian stock' and an 'Eastern South Pacific stock' in the 80's (Donovan 1991), although the small number of samples in the present genetic analysis should be emphasized again. Genetic results presented here are consistent with the IWC delineation of a separated 'South Atlantic stock' (Donovan 1991), but it should be noted that the samples used in the present analysis covered only a small portion of the South Atlantic (southern and south eastern coast of Brazil) and the analyses of additional samples from broader geographical areas is necessary to investigate additional structure in this population as mentioned above.

In summary, no significant genetic differences were found between *B. brydei* of Chile and Peru. *B. brydei* from Peru and Chile were genetically different from whales from Brazil. *B. brydei* from all South American localities were genetically different from whales from other localities of the Pacific Ocean and Indian Ocean confirming that this species is highly structured within and between ocean basins. The genetic differences among populations were smaller within an oceanic basin than between oceanic basins. Furthermore the level of genetic differentiation, especially at the inter-equatorial level, was smaller than that of common minke whale (Pastene *et al*. 2007, 2010) and humpback whale (Baker *et al*. 1994), which could be due to their more recent separation compared to other baleen whale species (Kanda *et al*. 2007).

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