SC/64/SH7

Population genetic structure of the South American Bryde's whale

Luis A. Pastene[1], Jorge Acevedo[2], Salvatore Siciliano[3], Thais G.C. Sholl[3], Jailson F. de Moura[3], Paulo Henrique Ott[4] and Anelio Aguayo-Lobo[5]

[1]*Institute of Cetacean Research, Toyomi 4-5, Chuo-ku, Tokyo 104-0055, Japan*
[2]*Centro Regional de Estudios del Cuaternario Fuego-Patagonia y Antártica (CEQUA), 21 de Mayo 1690, Punta Arenas, Chile*
[3]*Grupo de Estudos de Mamíferos Marinhos da Região dos Lagos (GEMM-Lagos), Escola Nacional de Saúde Pública/FIOCRUZ, Rua Leopoldo Bulhões, 1480 - 6o. andar, sala 620 Manguinhos, Rio de Janeiro, RJ 21041-210, Brazil*
[4]*Universidade Estadual do Rio Grande do Sul (UERGS) and Grupo de Estudos de Mamíferos Aquáticos do Rio Grande do Sul (GEMARS), Av. Tramandai, 976, Imbé, Rio Grande do Sul, RS 95625-000, Brazil*
[5]*Instituto Antártico Chileno, Plaza Muñoz Gamero 1055, Punta Arenas, Chile*

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) were incorporated into the analysis. Results of the phylogenetic analysis identified the Bryde's whales of South America as Wada *et al*. (2003)'s Bryde's whale (*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. Also striking genetic differences were found between all South American localities and those from the western North Pacific, Fiji and Java. These results suggest that at least part of the Peruvian Bryde's whales moves into Chilean waters in some period of the year. These results also suggest no or very limited movement of whales between eastern South Pacific and western South Atlantic Oceans. This is consistent with the notion that Bryde's whales do not distribute further south of approximately 40^{0}S on both side of South America.

INTRODUCTION

Bryde's whale (*Balaenoptera edeni*) has been a species difficult to study in several regions of the world due to its similarity with the sei whale (*B. borealis*). 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. 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 in a much earlier date, in the mid 1950s.

Wada *et al*. (2003) described a new species of baleen whale *B. omurai* (Omura's whale) based on the morphological and mtDNA analyses of nine animals caught around the Solomon Sea and the eastern Indian Ocean near the Cocos Islands (identified at the time of the catches as 'Bryde's whales'). The description of this new species was confirmed later through the analysis of nuclear markers based on SINE and mtDNA (Sasaki *et al*., 2006). Furthermore Wada *et al*. (2003) provided 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's 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 *B. edeni*.

In the eastern South Pacific, analyses based on sighting and catch distribution suggested that Bryde's whales distribute in Peruvian waters almost around the year with abundance changing seasonally (Pastene and Ohsumi, 1998). The first record of Bryde's whale in Chile was reported by Clarke and Aguayo (1965) on the basis of an individual caught off Iquique (20°S). Subsequently Gallardo *et al*. (1983) reported the occurrence of Bryde's whale based on sighting data between 32°-36°S approximately. Pastene *et al*. (1983) confirmed the occurrence of Bryde's whales in central Chile through the examination of three animals caught in the latitudinal range 33°-35°S. Aguayo *et al*. (1998) reported a single sighting of Bryde's whale in the adjacent waters of San Felix and San Ambrosio Islands (26°59'S; 86°39'W) on 3 September 1994. Little is known about the distribution, pattern of movement of Bryde's whales distributed in Chilean waters and about their relationship with the Peruvian whales.

Similarly little is known about the distribution and movement pattern of Bryde's whales 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. Sighting of this species were recorded mainly in summer and autumn while stranding were recorded through the year (Zerbini *et al*., 1997; Siciliano *et al*., 2004). Little is known about the relationship between western South Atlantic and eastern South Pacific Bryde's whales.

In both eastern South Pacific and western South Atlantic no record of Bryde's whales has been made south of 40°S, which suggest no or limited interchange of whales between oceans through the southern tip of the South American continent.

Peru, Chile and Brazil were involved in commercial whaling of Bryde's whales until approximately the mid 1980's, and the IWC SC had 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 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 are, however, limited biological evidences to support those geographic delineations.

The objective of this study was to examine both the species identity (based on the classification proposed by Wada *et al*., 2003), and the population genetic structure of Bryde's whales off South America through the genetic analyses of samples collected in Peru, Chile and Brazil.

MATERIALS AND METHODS

Samples

Biopsy samples from the Chilean Bryde's whales were obtained by the SOWER survey conducted in December 1997 (Findlay *et al.*, 1998) (n=8). Biopsy samples were collected using Paxarm system and crossbows. In addition baleen plates were available from whales caught in Chile in April 1983 (n=2). Genetic samples from the Brazilian Bryde's whales were obtained from stranded animals along the southern and south eastern coast of Brazil in the months of January, February, March, August, September and October between the years 2004 and 2010 (n=8). Genetic samples from the Peruvian Bryde's whales were obtained during a former Japanese coastal whaling operation in Peru in 1983 (n=24) (Kanda *et al*., 2007).

Table 1 shows ancillary information of the sampling in Chile and Brazil. Figure 1 shows details of the geographical distribution of the Brazilian samples.

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

Laboratory 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™ 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™ (FMC, Inc.) denaturing polyacrylamide matrix on a DNA Prism™ 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 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* or *Kst** was observed.

The effective number of migrants (*Nem*) was estimated assuming Wright's island model using equation $Hst = 1/(1 + 2Nem)$ (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 sei whale as well the Omura and Eden whales (Wada *et al*., 2003).

RESULTS

Genetic diversity

In the total sample of 490 animals (including the samples examined by Kanda *et al*., 2007) there were 38 segregating sites discriminating a total of 55 haplotypes (Table 2). All mutations were transitions. Then the additional 18 samples from Chile and Brazil discriminated four new haplotypes in relation to the data set used by Kanda *et al*. (2007).

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

Population differentiation

Table 2 shows the geographical distribution of haplotypes. There was a total of seven haplotypes in the Chilean sample, with three being specific to that locality. Four haplotypes (representing 70% of the total samples) were shared with the Peruvian sample. Only two haplotypes were discriminated in the Brazilian sample, one specific to that locality (one individual) and the other shared with all other localities except Java (seven individuals).

Table 3 shows the net-interpopulational distances among populations. The comparison between Bryde's whales from Peru and Chile yielded a negative value suggesting a close genetic relationship. However the genetic distances of Bryde's whales from Peru and Chile in relation to Brazilian whales are larger.

Table 4 shows the results of the statistical test for population differentiation for several statistics. Chilean Bryde's whale differed significantly from all other populations of Bryde's whales, except that from Peru. Both Chilean and Peruvian Bryde's whales differed significantly from Brazilian whales.

Migrants per generation
Assuming the island model of migration, the values of *Hst* translated into two female migrants per generation between Chile and Brazil Bryde's whales.

Phylogenetic analysis
Figure 2 shows the neighbor-joining based phylogenetic tree of Bryde's whale haplotypes. Bryde's whales from the eastern South Pacific and western South Atlantic 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 genetic analysis of South American Bryde's whales. Results suggested that whales from Peru, Chile and Brazil belong to *B. brydei* according to the classification suggested by Wada *et al*. (2003).

The level of nucleotide diversity in the Brazilian Bryde's whales 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 eight 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 Bryde's whales, which suggest limited movement of whales between these two ocean basins. In fact the results suggested that only two females migrate between these two ocean basins per generation. This is consistent with the notion that Bryde's whales do not distribute further south of approximately 40°S on both sides of South America.

In contrast Bryde's whales 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 two localities. This result suggests that Bryde's whales from Chile and Peru belong to a same stock. It should be recognized that the sample sizes used in the comparative genetic analysis between Peru and Chile's Bryde's whales, were small, 24 ad 10 samples, respectively. The analysis of a larger sample size is recommended to confirm this conclusion. However, if the differences between the putative Peruvian and Chilean populations are 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 was not the case.

There are other non-genetic evidences relating eastern South Pacific Bryde's whales from Peru and Chile. Figure 3 shows the distribution of sighting and catches of confirmed and 'like' Bryde's whales in the eastern South Pacific off Chile. The figure is based on a total of 135 animals confirmed as Bryde's whales thirteen of them correspond to catches and 122 to sightings. Whales were distributed in the latitudinal range 18°43'S and 37°58'S and in the longitudinal range between the Chilean coast and 76°16'W. Figure 4 shows the distribution of sightings and catches of Bryde's whales, by season. Records were made approximately between 29°S and 38°S in summer (December-February) and between approximately 18°S and 33°S in spring (September-November). Records in fall and winter were scarce. Seasonal differences in the occurrence of whales could be related to latitudinal movement through the Chilean coast and therefore the available sighting distribution and the genetic data presented in this study are consistent with the hypothesis of a north (Peru)-south (till latitude 38°S in Chile) movement of Bryde's whales in the eastern South Pacific in spring summer. This hypothesis should be further examined through the analysis of former 'sei' whale catches/effort in Chile (which include an unknown proportion of Bryde's whales), and larger number of genetic samples.

Sightings of Bryde's whales were related to a coastal upwelling ecosystem in central Chile in spring summer (Gallardo *et al*., 1983). As the stomach contents of whales examined in central Chile were composed of pelagic fishes (unpublished information), the north-south migration in spring/ summer could be related to food availability. As suggested by Gallardo *et al*. (1983), Bryde's whale appears to show more correlation with the presence of food of the right kind and in the adequate quantities, than with a given range of temperature.

In the western South Atlantic there is some information suggesting seasonal movement (latitudinal and longitudinal) of whales related to food availability. Siciliano *et al.* (2004) reported on feeding events of Bryde's whales in south eastern Brazil (~22-24°S) during austral summer and autumn, which agrees with data provided by Zerbini *et al.* (1997). During these seasons, sardines approach the coast to spawn in shallower waters (Saccardo & Rossi Wongtschowski, 1991), and it is likely that Bryde's whales move inshore while following sardine schools (Zerbini *et al.*, 1997). Bryde's whale predation on schooling fish, like Brazilian sardines, generally agrees with the reported habits of the species from a few areas such as South Africa (Best, 1977; Best & Rickett, 1984) and Gulf of California, Mexico (Tershy *et al*., 1993).

Two stranded Bryde's whales (GEMM 052 and 078) had plenty of small shrimps (*Acetes americanus*) in their stomachs. It is not clear if this was an opportunistic feeding or represents a regular prey item for this stock while using shallow waters off SE Brazil.

Moura & Siciliano (2011) recently reviewed the stranding records of Bryde's whales along south eastern Brazil (~22-25°S). A total of 47 stranding were reported from 1972 to 2010. No significant differences were found in stranding rates between seasons, supporting the idea that Bryde's whales are commons in south eastern Brazilian coast along the year (Siciliano *et al.*, 2004).

In southern Brazil, however, only nine strandings of Bryde´s whales were recorded south of 30°S, between 1989 and 2011 (Zerbini *et al*., 1997; GEMARS, unpublished information). Most of these records (n = 6; 66.7%) occurred between December and January, suggesting some seasonality in movements and perhaps reflecting a latitudinal migration along the Brazilian coast. Alternatively, Bryde´s whales in southern Brazil could approximate the shore during the summer, thus increasing the probability of stranding.

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 our analyses provide no support for a differentiation of Bryde's whales in the eastern South Pacific as suggested by the IWC when delineated a 'Peruvian stock' and an 'Eastern South Pacific stock' in the 80's (Donovan, 1991), although it should be emphasized again the small number of samples in the present genetic analysis. 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.

Bryde's whales from Peru, Chile and Brazil were significant different from Bryde's whales from other localities of the Pacific Ocean and Indian Ocean confirming that this species is highly structured within and between ocean basins.

ACKNOWLEDGEMENTS

We thank M. Goto, Institute of Cetacean Research, for his assistance in the analysis of data. We also thank the researchers from GEMARS for collaborating in the collection and necropsies of the specimens along the Brazilian coast.

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Table 1. Samples of the Bryde's whales from Chilean and Brazilian waters used in the present analyses.

Species	**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 GEMM052	26 Feb 2004	Stranding	22º14'	41º32'	This study
Brazilian waters GEMM078	23 Jan 2005	Stranding	22º33'	41º58'	This study
Brazilian waters GEMM112	28 Sep 2006	Stranding	22º40'	41º59'	This study
Brazilian waters GEMM130	18 Aug 2007	Stranding	22º58'	42º02'	This study
Brazilian waters GEMM157	11 Oct 2008	Stranding	22º57'	42º05'	This study
Brazilian waters GEMM183	08 Mar 2010	Stranding	22º17'	41º40'	This study
Brazilian waters GEMARS1223	20 Jan 2005	Stranding	30º13'	50º13'	This study
Brazilian waters GEMARS1224	21 Jan 2005	Stranding	30º20'	50º16'	This study

Table 2. Frequencies of haplotypes in the Bryde's whale by geographical locality (WNP=western North Pacific).

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**
	401	**24**	**24**	**10**	**8**	**23**	**490**

Table 3. Nucleotide diversity (on the diagonal) and net-interpopulational distances among populations of Bryde's whale. In parenthesis is the SE (WNP= western North Pacific)

	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 Bryde's whales from South America (WNP= western North Pacific).

	Hst	Kst*	Chi-square
WNP-Peru	0.0140 (P=0.0000)	0.0377 (P=0.0000)	P=0.0000
WNP-Chile	0.0077 (P=0.0000)	0.0216 (P=0.0000)	P=0.0000
WNP-Brazil	0.0214 (P=0.0000)	0.0379 (P=0.0000)	P=0.0000
Fiji-Peru	0.1366 (P=0.0000)	0.2347 (P=0.0000)	P=0.0000
Fiji-Chile	0.1275 (P=0.0000)	0.2522 (P=0.0000)	P=0.0000
Fiji-Brazil	0.2630 (P=0.0000)	0.3964 (P=0.0000)	P=0.0001
Peru-Chile	**-0.0125 (P=0.8535)**	**-0.0163 (P=0.9601)**	**P=0.8939**
Peru-Brazil	**0.1640 (P=0.0000)**	**0.2236 (P=0.0000)**	**P=0.0024**
Chile-Brazil	**0.2195 (P=0.0014)**	**0.3323 (P=0.0000)**	**P=0.0039**
Java-Peru	0.2377 (P=0.0000)	0.3336 (P=0.0000)	P=0.0000
Java-Chile	0.2511 (P=0.0000)	0.3514 (P=0.0000)	P=0.0000
Java-Brazil	0.4836 (P=0.0000)	0.5292 (P=0.0000)	P=0.0000

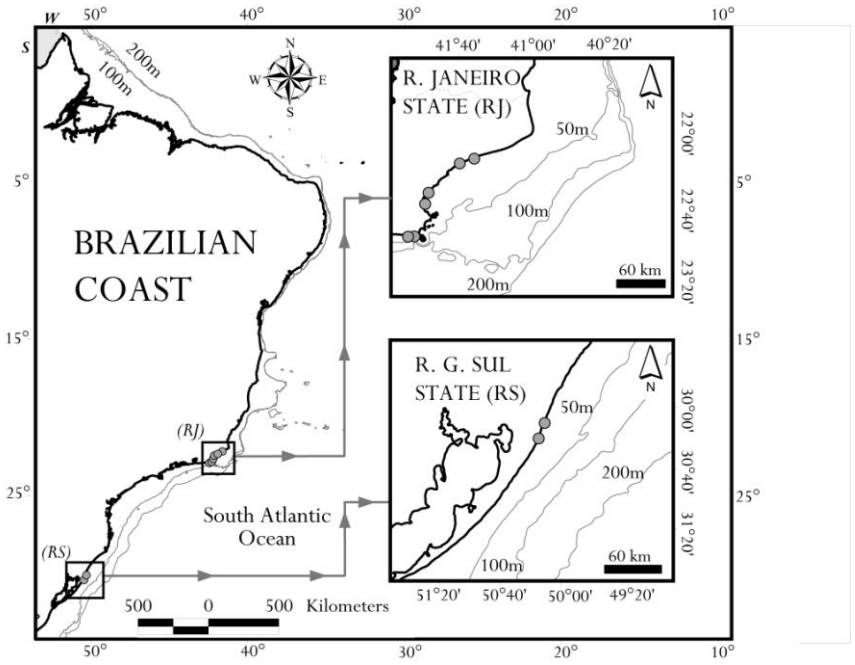

Figure 1. Geographic distribution of the samples of Bryde's whales from Brazil used in the present genetic study.

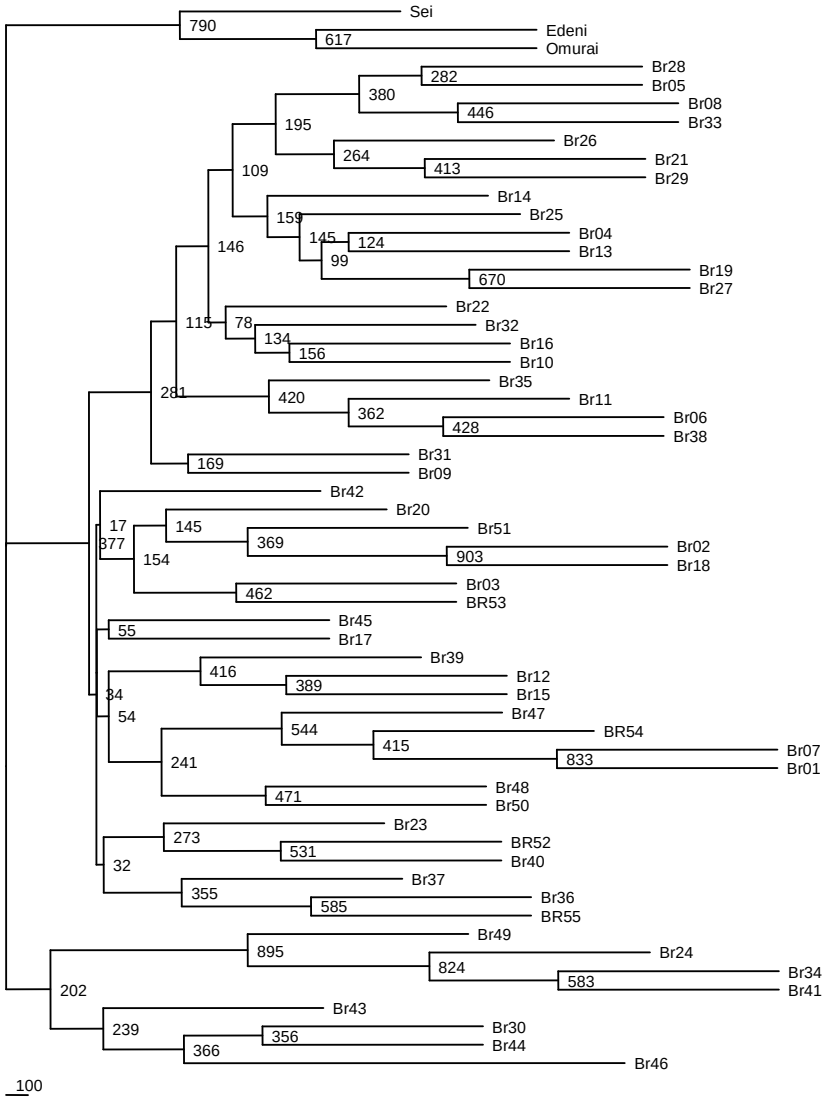

Figure 2. Neighbor-joining based tree of Bryde's whale mtDNA haplotypes. Figures in the nodes indicate high bootstrap values (in 1,000 simulations).

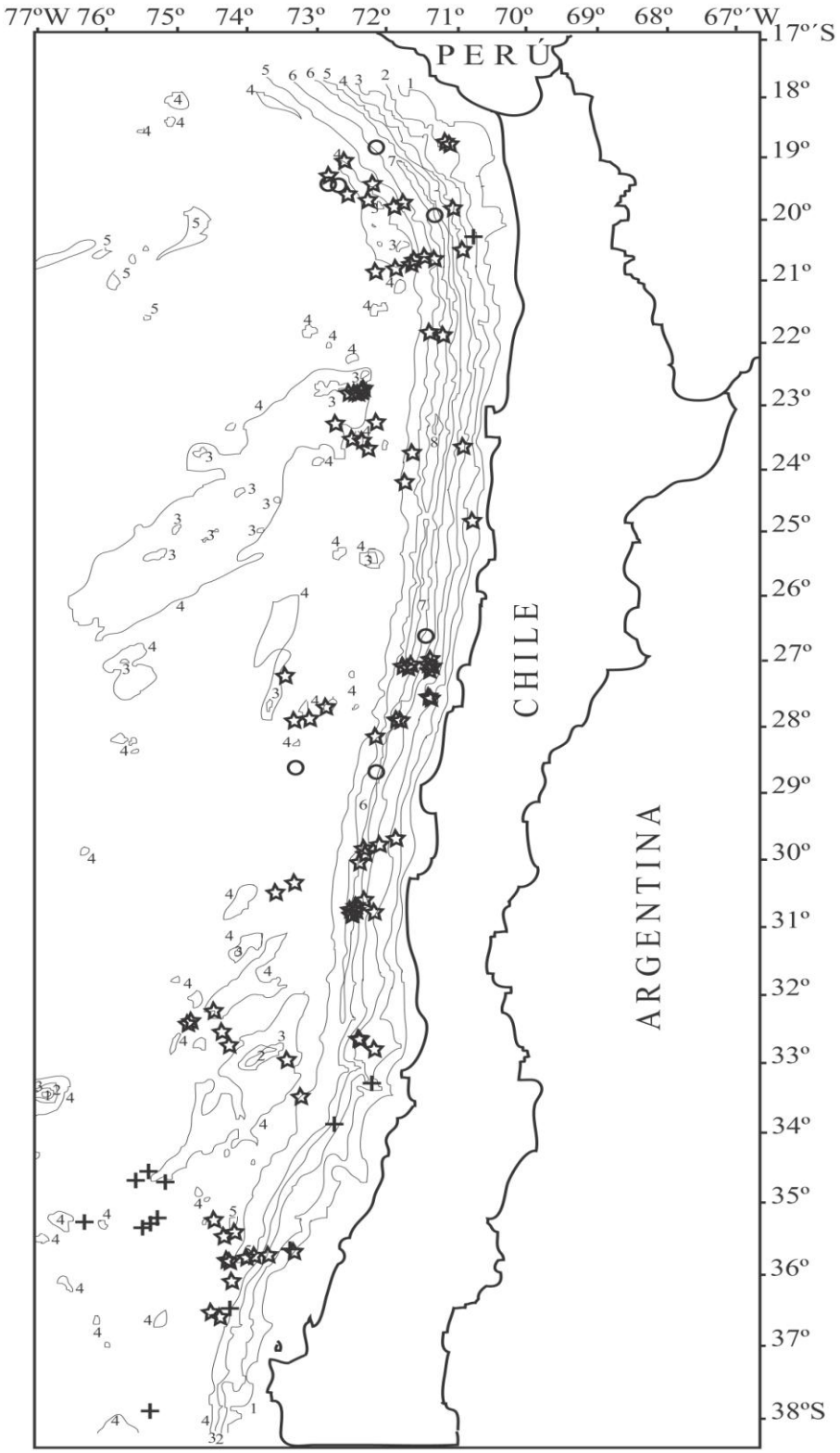

Figure 3. Distribution of sightings and catches of Bryde's whale in Chilean waters (sightings in stars, catches in cross, like Bryde's whales in circle) (source: Gallardo *et al.*, 1983; Findlay *et al.*, 1998; Clarke and Aguayo, 1965; unpublished information). The numbers in each line denote depth x 1000m.

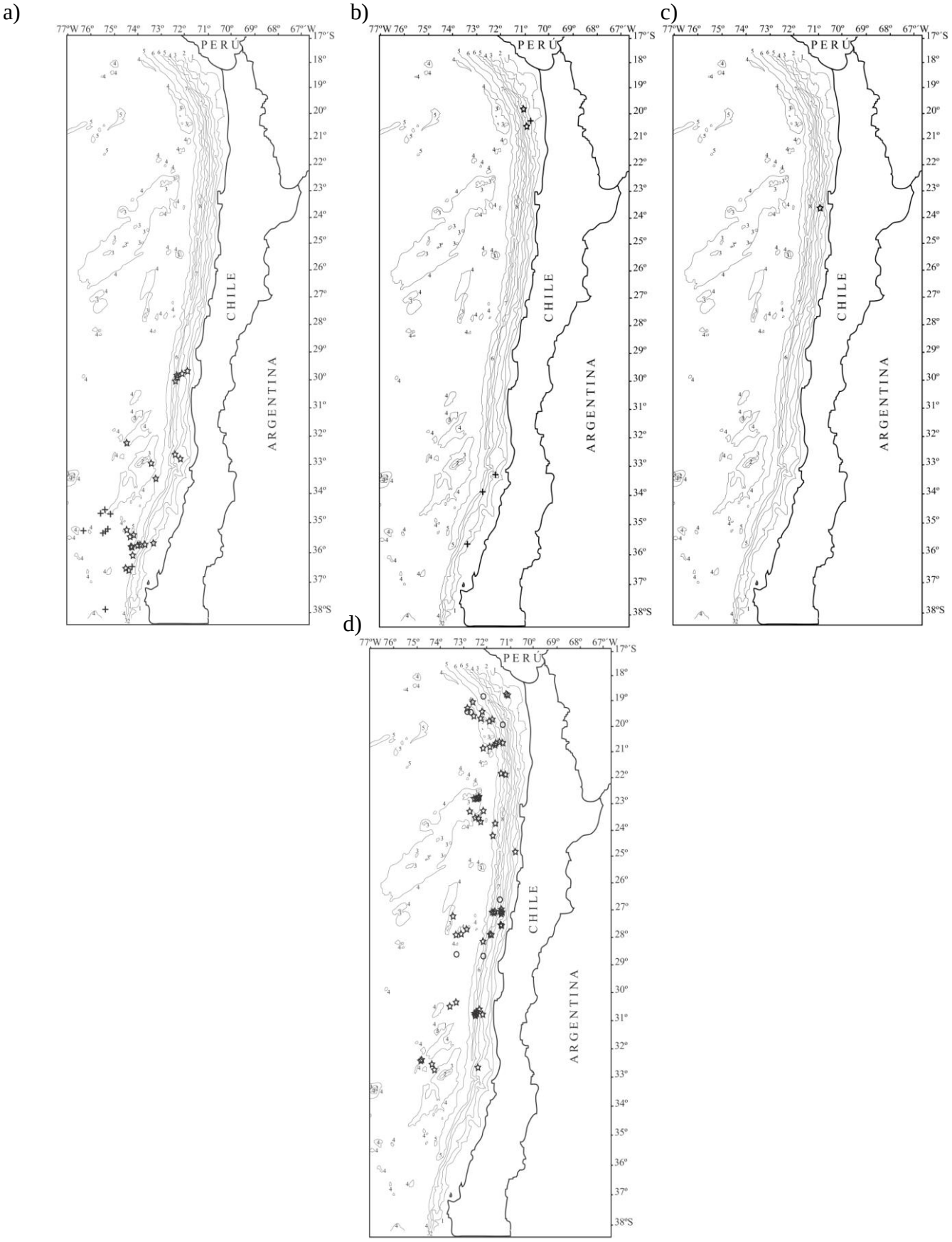

Figure 4. Distribution of sightings and catches of Bryde's whales in Chilean waters, by season: summer (a), fall (b), winter (c) and spring (d) (sightings in stars, catches in cross and like Bryde's whales in circle) (source: Gallardo *et al*., 1983; Findlay *et al*., 1998; Clarke and Aguayo, 1965; unpublished information).