Preliminary mitochondrial DNA analysis in eastern South Pacific bottlenose dolphins *Tursiops truncatus*

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ABSTRACT

Previous studies of eastern South Pacific bottlenose dolphins *Tursiops truncatus*, defined offshore and inshore ecotypes based on cranial and tooth morphology in Peru, documented the presence of a single resident coastal community ('pod-R') in central north Chile and confirmed the presence of offshore bottlenose dolphins off Chile. Morphological and behavioural differences between pod-R and the transient offshore animals suggested a reproductive isolation. To test this hypothesis and determine the pod-R probable origin, 331bp of mtDNA control region were analysed from the pod-R (n=8), Chilean offshore population (n=8), and inshore (n=3) and offshore (n=12) Peruvian ecotypes, as the first genetic analysis in *T. truncatus* of the eastern South Pacific. Levels of mtDNA diversity and phylogenetic relationships among haplotypes were determined. Three bottlenose dolphin specimens, morphologically identified as Peruvian inshore, grouped in an independent cluster supported by a bootstrap value of 100%. The net genetic distance between Peruvian inshore and Peruvian offshore was estimated at 2.9% and even higher when compared with the Chilean groups. The combination of morphological and mtDNA evidence conclusively argues for a reconsideration of the taxonomic status of the inshore ecotype. Further studies are required to determine boundaries of its distribution range and to estimate its population size and trend. Despite its inshore behavioural ecology, 'pod-R', which is the only resident bottlenose dolphin community recorded in Chile, presented a very high divergence from Peruvian inshore form (3.41% net interpopulational distance) and a relatively closer affinity with the Chilean offshore stock (0.87% net interpopulational distance). However, homogeneity tests showed significant genetic differences of pod-R with all other groups, including Chilean offshore. This, combined with a low nucleotide diversity of 0.0069 strongly suggests that 'pod-R' may be reproductively isolated and active protection measures and continuous monitoring of its status are recommended. Only one haplotype (from a total of 21) was shared by Peru and Chile offshore animals. The net genetic distance between them was estimated at 0.024% and no significant differences were found in haplotype frequencies, suggesting they form a single, wide-ranging offshore stock of unknown abundance.

KEYWORDS: GENETICS, BOTTLENOSE DOLPHIN, EASTERN SOUTH PACIFIC, TAXONOMY, CONSERVATION

INTRODUCTION

In the Southeast Pacific Ocean, bottlenose dolphins *Tursiops truncatus* are known to occur from the Galápagos archipelago, continental Ecuador, the entire coast of Peru, northern and central Chile south to about Concepción (37°S) and the offshore Chilean archipelagos of San Ambrosio y San Félix, Salas y Gómez islands and the Juan Fernández islands (e.g. Lévèque, 1963; Aguayo, 1975; Donovan, 1984; Van Waerebeek *et al.*, 1990; Félix, 1994; Sanino and Yáñez, 2001).

Van Waerebeek *et al.* (1990) found distinct cranial differences between offshore and inshore forms of bottlenose dolphins in Peru, as well as clear differences in diets and helminth parasite loads, suggesting reproductively isolated ecotypes. Otherwise, no studies on sub-specific morphological variation or population genetics of *T. truncatus* in the eastern South Pacific have been published, despite important and well-documented levels of by-catches and direct exploitation in Peru and Ecuador (e.g. Van Waerebeek *et al.*, 1990, 1997, 2002; Félix and Samaniego, 1994) and evidence of occasional harpooning and by-catch in Chile (Guerra *et al.*, 1987; Sanino and Yáñez, 2001).

Recently, management concerns, including direct takes (Sanino and Yáñez, 2001), about an inshore dwelling community of bottlenose dolphins, named pod-R, at Choros island (29°15'S, 71°26'W), north central Chile, and originally thought to form part of a putative coastal population of bottlenose dolphins in northern Chile, led to biopsy sampling of some of these animals.

Results of video-id studies showed very high site-fidelity of pod-R members, as well as morphological and behavioural differences with observed pods of offshore bottlenose dolphins. Pod-R has been closely watched in the area since 1981 (González *et al.,* 1989) and has allowed for the first attempt of commercial dolphin watching by local fishermen in Chile under a voluntary agreement ('Turismo Seguro' 1999-2000), between the Centre for Marine Mammals Research Leviathan (CMMR) and the local fishermen, which produced significant income for the local community (Sanino and Yáñez, 2000).

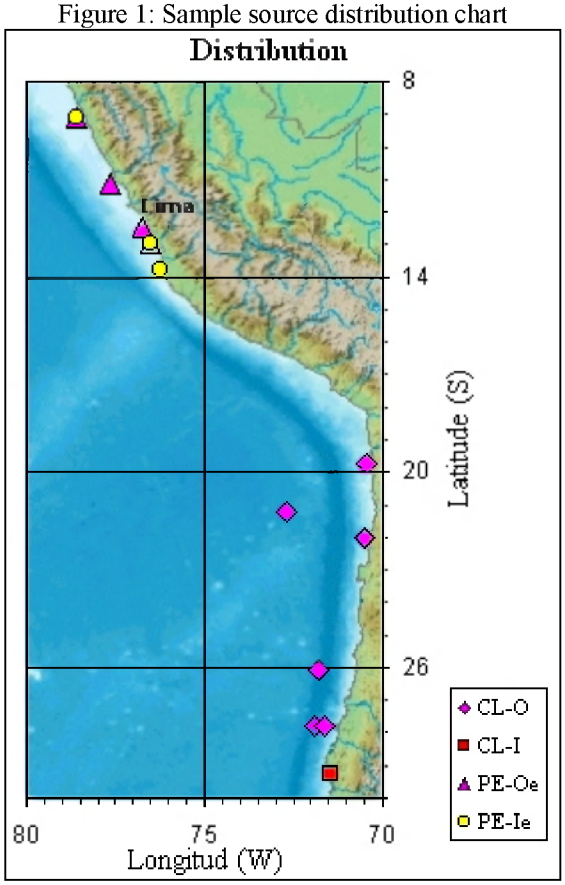
The area between the Chañaral island (29°2'S, 71°36'W) and the Choros islands is candidate for the development of the first Marine Protected Area (MPA) in Chile. As a biological resource, the issue was raised whether pod-R bottlenose dolphins are largely reproductively isolated or are part of, and mix with, other possible communities to form a putative wide-ranging coastal Chilean population. To test either hypothesis, and as a first evaluation of the overall mtDNA diversity and genealogy in *T. truncatus* populations of the Southeast Pacific, we here undertook to compare a mtDNA control region sequence of dolphins belonging to the pod-R with specimens from populations described as offshore Chile, inshore Peru and offshore Peru.

MATERIALS AND METHOD

Samples and localities

Sampling localities and sample sizes for the bottlenose dolphins are as follow (Fig. 1): Choros island in coastal Chile (CL-I, n=8), offshore Chile (CL-O, n=8), Peruvian inshore ecotype (PE-Ie, n=3) and a Peruvian sample including individuals of both confirmed offshore ecotype and indeterminate but probably-offshore specimens for which no skulls were collected (PE-Oe, n=12). Morphological characteristics used to distinguish ecotypes in Peru include tooth diameter and the morphology of pterygoids, palatine bones, antorbital process and the separation of occipital condyles according descriptions given by Van Waerebeek *et al.* (1990). Cues from accompanying fisheries data were also taken into account.

Figure 1: Sample source distribution chart



CL-I skin samples were collected using a Golden Bear bow with modified darts as described in IWC (1991), with a 6mm (diameter) tip. The samples were soaked in ethanol 70% during three weeks, after which the blubber hypodermis was eliminated and the epidermis/dermis was transferred to a DMSO saturated saline solution. Details of the samples are shown in Table 1A.

Table 1A: Chilean inshore *T. truncatus* samples, taken from pod-R in Choros Island, IV Region, Chile (TURSIOPS98/99)

BP Code	Tailstock side	Date dd/mm//yy	Identification
GPS-BP1	Right	08/02/1998	A1-0-19
GPS-BP2	Right	11/02/1998	A1-0-1
GPS-BP3	Right	11/02/1998	A1-2-11
GPS-BP4	Right	11/02/1998	A1-2-4
GPS-BP5	Left	15/02/1998	A1-0-17
GPS-BP6	Right	15/02/1998	A1-2-27
GPS-BP7	Right	16/02/1998	A1-2-9
GPS-BP8	Left	16/02/1998	A1-2-30

CL-O samples were collected using a Barnett crossbow as described in IWC (1991), during the third blue whale cruise of the IWC/SOWER program from the bow of the *Shonan Maru 2* (Findlay *et al.*, 1998). The samples were conserved in DMSO saline solution. Details of the samples are shown in Table 1B.

Table 1B: Chilean offshore *T. truncatus* samples, from SM2 (IWC SOWER97/98)

Form	Position	Date dd/mm/yy	N°biopsies
1004	19°42'51"S - 70°25'93"W	13/12/1997	3
1091	21°11'30"S - 72°40'57"W	18/12/1997	2
1108	22°0'10"S - 70°31'10"W	19/12/1997	1
1162	26°3'92"S - 71°45'93"W	23/12/1997	1
1195	27°47'54"S - 71°53'55"W	26/12/1997	1
1199	27°45'84"S - 71°36'91"W	26/12/1997	2

All tissue samples from Peru (PE-Ie and PE-Oe) were taken from either freshly landed specimens captured in a variety of fisheries or from body remains found on beaches near fishing towns. Most were stored in a DMSO solution, and some in 70% ethanol. Details of the samples are shown in Tables 1C and 1D, respectively for PE-Ie and PE-Oe bottlenose dolphins.

Table 1C: Peruvian inshore ecotype *T. truncatus* samples (CEPEC)

BP Code	Position	Site	Date dd/mm/yy	Ecotype support M=morphology,F=fisheries information
JAS 47	13°45'S - 76°13'W	San Andrés	18/03/1995	M
AGG 741	09°05'S - 78°36'W	Chimbote	18/03/1993	M
MFB 465	13°00'S - 76°30'W	Cerro Azul	12/13/1993	M, F

Table 1D: Peruvian offshore and indeterminate ecotype *T. truncatus* samples (CEPEC)

BP Code	Position	Site	Date dd/mm/yy	Ecotype (M=morphology,F=fisheries information)
MFB 185/187	13°00'S - 76°30'W	Cerro Azul	08/05/1993	Inferred offshore, is identical or was landed with offshore (M) MFB-185
MFB 186	13°00'S - 76°30'W	Cerro Azul	08/05/1993	Offshore (M), landed with offshore (M) MFB-185
MFB 441	13°00'S - 76°30'W	Cerro Azul	01/12/1993	Indeterminate
MFB 701	13°00'S - 76°30'W	Cerro Azul	10/07/1994	Inferred offshore, landed with MFB 702 (M)
MFB 702	13°00'S - 76°30'W	Cerro Azul	10/07/1994	Offshore (M)
KVW 2393	09°05'S - 78°36'W	Chimbote	15/02/1993	Indeterminate
KVW 2412	12°30'S - 76°45'W	Pucusana	18/02/1995	indeterminate, probably offshore (F)
KVW 2417	12°30'S - 76°45'W	Pucusana	29/03/1995	offshore (M)
KVW 2439	11°07'S - 77°37'W	Huacho	30/01/1997	Indeterminate
KVW 2440	11°07'S - 77°37'W	Huacho	01/30/1997	Indeterminate
RBC 54	09°05'S - 78°36'W	Chimbote	08/08/1996	Indeterminate
JAS 12	09°05'S - 78°36'W	Chimbote	12/02/1993	Indeterminate

Extraction of DNA

Total-cell DNA was extracted from samples of skin or other tissue. DNA extractions followed phenol/chloroform/isoamyl alcohol protocols as described in Sambrook *et al.* (1989). Extracted DNA were resuspended in 500ul of 0.1M Tris-HCl (pH 8.0), 0.05 rnM EDTA buffer.

Amplification of mtDNA control region

The 5' end of the mtDNA control region (331bp) was amplified using polymerase chain reaction (PCR). Sequences of the oligonucleotide primers for the PCR amplification were MT4-F (5'-CCTCCCTAAGACTCAAGGAAG-3', Arnason *et al.* 1993) and P2-R (5' GAAGAGGGATCCCTGCCAAGCGG-3', H. Hori, personal communication). F and R, respectively, denote a forward- or reverse-oriented primer with reference to the light strand.

Sequencing analysis

Cycle sequencing was performed with 100ng of PCR products using he PRISM™ Ready Reaction Dye Deoxy Terminator Kit of Applied Biosystems (ABI). Primers used for cycle sequencing were the same as indicated above. The reaction was performed through 25 cycles of 96°C for 10sec., 56°C for 20sec. and 60°C for 4min. The nucleotide sequence for each amplification was determined by electrophoresis through a 5% Long Ranger™ polyacrylamide matrix on an Applied Biosystems DNA Prism™ 377, following the protocols recommended by the manufacturer. For each sample both forward and reverse strands were sequenced. Sequences were aligned using DNA sequence comparison software 'Sequence Navigator' developed by ABI.

Levels of polymorphism

Genetic distances among different haplotypes were estimated using the Kimura's two parameters method based on genetic distance among haplotypes (Kimura, 1980). Nucleotide diversity was estimated following equation 10.5 of Nei (1987). The net genetic distance between populations was estimated by subtracting the average level of variation within each population, following equation 10.21 of Nei (1987).

MtDNA genealogy

Phylogenetic reconstruction of haplotypes was made using the neighbour-joining (NJ) method (Saitou and Nei, 1987). To evaluate the confidence limits on phylogenies, we conducted 1000 bootstrap simulations (Felsenstein, 1985). The phylogenies were rooted using the homologous sequence from a common dolphin *Delphinus* sp. (DNA Database access number: U02652).

Homogeneity test

Homogeneity test was conducted using the sequence (Kst*) and haplotype (Hst) statistics proposed by Hudson *et al.* (1992). The degree of divergence was inferred as being larger than zero, if an equal or more extreme value of the Kst* or Hst was observed in less than 5% of 10,000 Monte Carlo simulations.

RESULTS

Levels of polymorphism

For each sample sequence of the first 331 nucleotides were determined in the mtDNA control region. Amongst the total 31 samples we detected a total of 32 polymorphic sites, which defined 21 unique sequences (haplotypes). Nucleotide diversity for the total sample was estimated to be 0.02193 and the nucleotide diversity within a sample ranged from 0.00201 in the Peruvian inshore (PE-Ie) to 0.02007 in the Chile offshore group (CL-O) (Table 2).

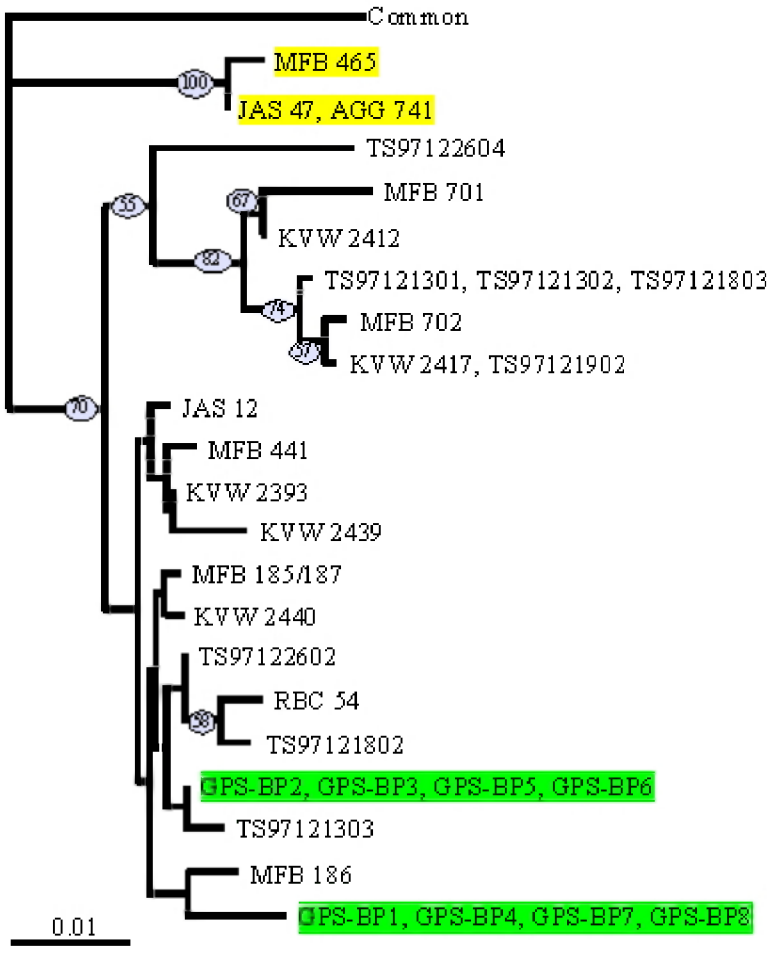
Table 2: Nucleotide diversity (diagonal in bold) and net interpopulational distances (upper right)

	PE-Ie (n=3)	PE-Oe (n=12)	CL-I (n=8)	CL-O (n=8)
PE-Ie (n=3)	**0.00201**	0.02900	0.03412	0.03349
PE-Oe (n=12)		**0.01794**	0.00564	0.00024
CL-I (n=8)			**0.00691**	0.00870
CL-O (n=8)				**0.02007**

Geographic distribution of haplotypes

The frequency of haplotypes in the bottlenose dolphin samples is shown in Fig. 2. Apart from haplotype '9', which was shared by CL-O and PE-Oe, no shared haplotype occurred among CL-I, CL-O, PE-Ie and PE-Oe. All the individuals in the Pe-Oe group showed a different haplotype. Six haplotypes were defined in eight CL-O individuals while only two haplotypes were defined in the eight CL-I animals. Two individuals previously defined as Peruvian inshore ecotype shared the same haplotype ('14').

Figure 2: Phylogenetic tree and frequencies of haplotypes (H) in each locality (CL-I: Inshore Chile, CL-O: offshore Chile, PE-Ie: inshore Peru, PE-Oe: offshore Peru). In circles the bootstrap values over 50% - in 1000 simulations.



Sampling Locality

H	CL-I	CL-O	PE-Ie	PE-Oe
4			1	
14			2	
21		1		
5				1
8				1
17		3		
6				1
9		1		1
13				1
3				1
7				1
10				1
1				1
11				1
20		1		
12				1
19		1		
16	4			
18		1		
2				1
15	4			

MtDNA haplotype genealogy

Fig. 2 shows a neighbour-joining-based phylogenetic tree of the haplotypes. The tree shows haplotypes '4' and '14' as highly divergent from the rest of the haplotypes. Haplotype '14' includes individuals identified previously as Peruvian inshore type. Haplotype '4' was represented by a single individual (MFB-465) of a working list of specimens as yet unassigned to ecotype (specimens were studied by KVW simultaneously with the DNA analysis). MFB-465 clustered very near to haplotype '14' so LAP purely on this result suggested that individual to be also of the inshore type. The independent morphological analysis by KVW of this individual's cranial characteristics confirmed this result. Apart from this highly divergent cluster, we identified two other clusters with a branch supported by a high bootstrap value (70%). However these clusters included individuals from different localities.

Net inter-populational distances

Table 2 shows the net inter-populational distances among localities. Individuals with haplotypes '4' or '14' were considered as of the Peruvian inshore type in this analysis, supported by morphological classification. All the pairwise comparisons involving this type (PE-Ie, n=3) showed large genetic distances ranging from 0.02900 to 0.03349. The other pairwise comparisons resulted in genetic distances between 0.00024 and 0.00870. The smallest genetic distance was found between CL-O and PE-Oe.

Homogeneity tests

All the pairwise comparisons among localities resulted in significant genetic differences. The exception was the comparison between CL-O and PE-Oe, which showed no significant differences (Hst=0.0223, P=0.05110; Kst=0.0068, P=0.27910).

DISCUSSION

The three bottlenose dolphin specimens from Peru identified as Peruvian Inshore (PE-Ie) through the evaluation of cranial non-metric characteristics, tooth diameters (following Van Waerebeek *et al.*, 1990), and fisheries data, grouped in a clade separate from all others with 100% bootstrap value. The three animals showed two haplotypes unique to PE-Ie. With a high, specific-level divergence of 2.9% (net inter-populational distance) between PE-Ie and PE-Oe, and even higher divergence values with the other localities. These data not only support conclusions of morphological differences between Peruvian inshore and offshore forms but considering their parapatric occurrence off Peru, it may be time to consider these forms as different subspecies, if not different species. Their relationship with other nominal species and in particular *Tursiops nuuanu* Andrews, 1911 of the eastern tropical Pacific, *T. truncatus gillii* Dall, 1873 of southern and Baja California and *T. gephyreus* Lahille, 1908 of Uruguay and Argentina needs to be established.

Surprisingly, Choros island bottlenose dolphins (pod-R), while inhabiting an inshore environment, forming a small pod and showing high site-fidelity, features that are commonly associated with inshore bottlenose dolphins, were highly divergent from the Peruvian inshore form (3.41% net interpopulational distance). In contrast, pod-R appeared much closer related (0.87% net interpopulational distance) to the Chilean offshore stock. Notably, the eight individuals showed only 2 haplotypes and a concomitant low nucleotide diversity of 0.0069. This agreed with video-ID results and intensive field research of pod-R which suggests that the group may be reproductively isolated (Sanino and Yáñez, 2001). A pairwise comparison with both the Chilean offshore group, as well as with the Peruvian offshore animals still showed significant genetic differences. These results can be called alarming for the survival of this community of some 30 individuals (Sanino and Yáñez, 2001), considering that there is no evidence for the existence of any widely distributed inshore bottlenose dolphin population in Chile at least south of Punta Coloso (23°43'S), near Antofagasta (see Aguayo, 1975; Sielfeld, 1980, 1983; Guerra *et al.,* 1987; Van Waerebeek *et al.,* 1990). North of Punta Coloso, a few specimens of undetermined ecotype are stored at the University of Antofagasta collection (Guerra *et al.* 1987) and unconfirmed reports of dolphins occurring in the surfzone off beaches require further investigation. Such reports are known from Peruvian waters despite the important hunting pressure on the Peruvian stock for at least decades (e.g. Read *et al.*, 1988; Van Waerebeek *et al.*, 1990), although the frequency of sightings and group sizes seem to have been declining. Indeed, CEPEC (unpublished data) holds many hundreds of sighting records of inshore bottlenose dolphins from tens of different locations in northern, central and south-central Peru, all opportunistic observations made by scientists just passing through coastal sites. In fact, there is hardly any other cetacean whose presence, or absence, is so visible from land that information gathered from locals through casual interviews reflects well data from scientific surveys. In Chile, apart from pod-R, documented records of inshore dwelling bottlenose dolphins are extremely limited. A large female was captured near Punta Coloso (23°43'S), Antofagasta on 11 June 1987 (Guerra *et al.*, 1987).

As expected, no significant differences were found between Peruvian and Chilean offshore bottlenose dolphins. Only one haplotype was shared between Peruvian and Chilean offshore animals (No. 9, Figure 2). Whenever observed, these powerful dolphins are often seen travelling with steady bearing at great speed, performing high leaps; whence they are thought to cover great distances with ease (personal observations by authors). Very few skulls of bottlenose dolphins are present in Chilean collections (none were listed by Sielfeld, 1980) and therefore have not yet been the subject of a detailed comparative study. Calvaria IRD-002 from Bahía Cisnes (27°16'S), northern Chile, donated by a local fisherman and deposited in the CMMR-Leviathan collection, shows the same cranial characteristics of the Peruvian offshore form (Van Waerebeek *et al.*, 1990).

Concluding from mtDNA results presented here, and from morphological research (Van Waerebeek *et al*, 1990), it is recommended that both Chilean and Peruvian inshore stocks of bottlenose dolphins be managed as separate reproductive units, both from each other and separate from the offshore bottlenose dolphin stock. Particular concern is expressed about the long-term perspectives of the Choros island pod-R bottlenose dolphins for demonstrating such low nucleotide diversity. The pod-R community requires active protection measures and continued monitoring of its status to safeguard its unique presence on the coast of Chile. Yañez (1997), in an assessment of conservation status of marine mammals in Chile, had earlier recognised this species as 'vulnerable'. Urgent attempts should be made also to define the northern and southern boundaries of the distribution range of the Peruvian inshore bottlenose dolphin, conclusively determine its taxonomic status and estimate its population size and trend.

Chilean and Peruvian offshore bottlenose dolphins probably form a single wide-ranging population, which we provisionally call 'Peru-Chile offshore stock'. Affinities with other bottlenose dolphins occurring in the offshore Southeast Pacific should be established, including with insular animals found around archipelagos. Regular, dedicated cetacean abundance surveys should be undertaken off western South America to monitor this unassessed population as well as other cetaceans of the region.

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REFERENCES

Aguayo, A. 1975. Progress report on small cetacean research in Chile. *J. Fish. Res. Board Canada* 32: 1123-1143.

Arnason, U, Gullberg, A. and Widegren, B. 1993. Cetacean mitochondrial DNA control region: sequences of all extant baleen whales and two sperm whale species. *Journal of Molecular Biology and Evolution* 10: 960-70.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using bootstrap. *Evolution* 44: 1097-1108.

Donovan, G.P. 1984. Small cetaceans seen during the IWC/IDCR research cruise in the eastern tropical Pacific, and in particular off Peru. *Rep. int. Whal. Commn.* 34: 561-567.

Félix, F. 1994. Ecology of the coastal bottlenose dolphin *Tursiops truncatus* in the Gulf of Guayaquil, Ecuador. *Investig. on Cetacea* 25: 235-256.

Félix, F. and Samaniego, J. 1994. Incidental catches of small cetaceans in the artisanal fisheries of Ecuador. *Rep. int. Whal. Commn.* (special issue 15): 475-480.

Findlay K., R. Pitman, R., Tsurui, T., Sakai, K., Ensor, P., Iwakami, H., Ljungblad, D., Shimada, H., Thiele, D., Van Waerebeek, K., Hucke, R. and Sanino, P. 1998. 1997/1998 IWC-Southern Ocean Whale and Ecosystem Research (IWC-SOWER) Blue Whale Cruise, Chile. *Final Report of the third IWC Blue Whale Cruise of the International Whaling Commission SOWER program*. 39pp.

González, J., Capella, J. and Gibbons, J. 1989. Delfines nariz de botella *Tursiops truncatus* (Montagu, 1821) habitando frente a isla Chañaral, Provincia de Huasco, Chile. *Estudios Oceanológicos* 8: 67-71. [In Spanish]

Guerra C., Van Waerebeek, K., Portflitt, G. and Luna, G. 1987. Presencia de cetáceos frente a la segunda región de Chile. *Estudios Oceanológicos* 6: 87-96. [In Spanish]

Hudson, R.R,, Boos, D.D, and Kaplan, N.L. 1992. A statistical test for detecting geographic subdivision. *Molecular Biology and Evolution* 9 (1), 38-151.

International Whaling Commission. 1991. Report of the Workshop on the Genetic Analysis of Cetacean Populations. *Rep. Int. Whal. Commn.* (special issue) 13: 3-21.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111-120.

Lévèque, R. 1963. Notes sur quatre cétacés de l'ocean Pacifique (Ecuador et Galapagos). *Mammalia* 27: 608-609. [In French]

Nei, M. 1987. *Molecular Evolutionary Genetics*. Columbia University Press, New York. x+5 12pp.

Read A.J.,Van Waerebeek, K., Reyes, J.C., McKinnon, J.S. and Lehman, L.C. 1988. The exploitation of small cetaceans in coastal Peru. *Biological Conservation* 46: 53-70.

Saitou, N. and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic tree. *Journal of Molecular Biology and Evolution* 4(4), 406-25.

Sambrook, J., Fritsch, E.F. and Maniatis, T. 1989. *Molecular cloning: A Laboratory Manual*. Second Edition. Cold Spring Harbor Laboratory, New York.

Sanino, G.P. and Yáñez, J.L.. 2000. Efectos del turismo de observación de cetáceos en punta de Choros, IV Región, Chile. *Revista Gestión Ambiental.* CL 6: 41-53. [In Spanish]

Sanino, G.P. and J.L. Yáñez. 2001. Nueva técnica de video identificación y estimación de tamaño poblacional en cetáceos, aplicada en delfines nariz de botella, *Tursiops truncatus*, de isla Choros, IV Región de Chile. *Boletín del Museo Nacional de Historia Natural,Chile*. 50: 37-63. [In Spanish]

Sielfeld, W. 1980. Mamíferos Marinos en colecciones y museos de Chile. *Anales del Instituto de la Patagonia.* 11: 273-280. [In Spanish]

Sielfeld, W. 1983. *Mamíferos Marinos de Chile*. Universidad de Chile, Santiago. 199pp. [In Spanish]

Van Waerebeek, K., Reyes, J.C., Read, A.J. and Mckinnon, J.S. 1990. Preliminary observations of bottlenose dolphins from the Pacific coast of South America. *In*: S. Leatherwood and R. Reeves (Eds.). *The Bottlenose Dolphin, Academic Press Inc.* San Diego: 143-157.

Van Waerebeek, K., Van Bressem, M.F., Félix, F., Alfaro, J., García-Godos, A., Chávez, L., Ontón, K., Montes, D. and Bello, R. 1997. Mortality of dolphins and porpoises in coastal fisheries off Peru and southern Ecuador in 1994. *Biological Conservation* 81: 43-49.

Van Waerebeek, K., Alfaro-Shigueto, J., Montes, D., Onton, K., Santillan, L. and Van Bressem, M.-F. 2002. Fisheries related mortality of small cetaceans in neritic waters of Peru in 1999-2001. Paper SC/54/SM10 submitted to the Scientific Committee of the International Whaling Commission, Shimonoseki, Japan, 26 April-10 May 2002.

Yáñez, J.L. 1997. Reunión de trabajo de especialistas en mamíferos acuáticos para categorización de especies según estado de conservación. *Noticiario Mensual del Museo Nacional de Historia Natural, Chile*. 330:8-16. [In Spanish]