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Short Communication

First mitochondrial DNA analysis of the spectacled porpoise (*Phocoena dioptrica*) from Tierra del Fuego, Argentina

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ABSTRACT

The spectacled porpoise (*Phocoena dioptrica*, Lahille, 1912) is one of the cetacean species about which least is known. Most information on the biology and ecology of this species has been obtained from stranded specimens and sightings at sea. In this study, analysis of 380 bp fragment of mitochondrial DNA (mtDNA) control region sequences ($N = 50$) was performed to provide a preliminary assessment of the genetic variation in spectacled porpoise specimens found stranded or by caught on the coast of Tierra del Fuego, Argentina. Results showed high levels of mtDNA diversity, as expected in large size and stable populations, and similar to other species of porpoises. The star-like shape phylogeny of haplotypes indicates a recent population expansion. This is the first report on the genetic variation of this species. Other lines of evidence (microsatellite loci, single-nucleotide polymorphism (SNPs)) are needed to improve our knowledge on the molecular biology of the spectacled porpoise.

The amount of genetic variation in a population can provide important clues to the evolutionary trends in population size and stability. As predicted by population genetic theory, due to genetic drift and/or inbreeding, small populations should show lower levels of genetic variability than large populations (Hartl and Clark 2007). The analysis of the processes involved in the population structure of cetaceans is difficult to attain due to their high mobility and the few physical barriers of the environment in which they live. Studies of intraspecific variation using molecular markers such as mitochondrial DNA (mtDNA) have been useful in the analysis of the population structure of these species (Pichler et al. 1998; Rosel and Rojas-Bracho 1999; Avise 2004; Pimper et al. 2010; Taguchi et al. 2010; Jayasankar et al. 2011).

The spectacled porpoise (*Phocoena dioptrica*, Lahille, 1912) is a small odontocete distributed in temperate to cold circumpolar waters in the southern hemisphere (Fig. 1, adapted from Sekiguchi et al. 2006; Goodall 2009; Van Waerebeek et al. 2010). It is one of the six extant species of porpoises (Phocoenidae) and one of the least known. Its limited biological data are based mainly on opportunistic records of stranded specimens, while the understanding of its distribution is based on only a few sightings at sea (Read 1999; Sekiguchi et al. 2006). The species has been found between 40° and 60°S off South America, Australia, New Zealand as well as Sub-Antarctic Islands of Falkland (Malvinas) Islands, South Georgia, Auckland Islands and Heard Island (Fraser 1968; Fordyce et al. 1984; Brownell et al. 1989; Evans et al. 2001).

Most of the strandings are found on the east coast of Tierra del Fuego and the coast of southern Argentina (Goodall and Schiavini 1995). With more than 300 specimens, it is the second most numerous species of cetacean collected in Tierra del Fuego and stored in the Goodall collection (RNP) held in the Museo Acatushún de Aves y Mamíferos Marinos Australes at Estancia Harberton, Tierra del Fuego. There are two possible explanations for these findings: specimens got trapped and stranded on the gently sloping beaches with macro-tidal regimen, or were bycaught in artisanal shallow water gillnetting fisheries (Goodall et al. 1994; Goodall 2009).

Nothing is known about migration patterns and separation of stocks around its range of distribution. Stable isotope analysis of Tierra del Fuego specimens indicates offshore foraging in oceanic waters near to the Antarctic Convergence (Riccialdelli et al. 2010).

The 2011 IUCN Red List of Threatened Species listed it as 'data deficient'. In Appendix II of CITES it is considered as a species not necessarily threatened, but that may become so if not closely monitored. At this time, there is not enough information (such as effective population size, stocks, movement patterns, population structure, levels of bycatch) for management and conservation of

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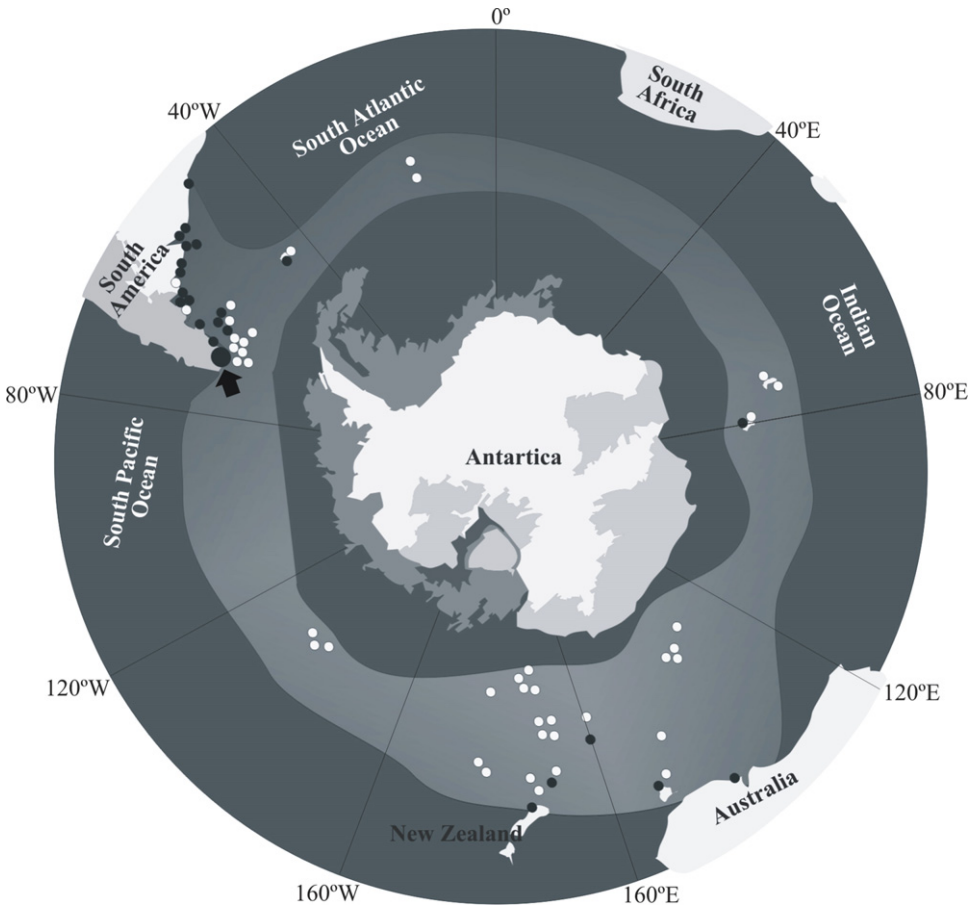

Fig. 1. Distribution of spectacled porpoise in the Southern Ocean (light gray). Black circles indicate strandings and white circles indicate sightings, according to Sekiguchi et al. (2006), Goodall (2009) and Van Waerebeek et al. (2010). Circle size is proportional to the number of specimens stranded or sighted at each site. The arrow shows origin of samples (Tierra del Fuego, Argentina) used in the present work.

the species. Our objectives was to analyze the sequence variation of the mtDNA control region of the spectacled porpoise found ashore in Tierra del Fuego, to provide a preliminary assessment of the genetic diversity in this species.

Samples of dry skin, teeth and bones of 156 specimens of spectacled porpoise were provided by the Goodall collection, found along the coasts of Tierra del Fuego, Argentina during the years 1974–2011. Total DNA was isolated from dry skin samples following Pimper et al. (2010), and from small fragments of bone and teeth following Pimper et al. (2009). The first 380 base pairs of the mtDNA control region were amplified using primers L15824 and H16265 (Rosel et al. 1999b) with temperature profiles following Taguchi et al. (2010). Amplicons were quantified by 1.6% agarose gel electrophoresis staining in ethidium bromide and UV visualization with DNA low-mass ladder. PCR products were sequenced in both directions on an ABI PrismTM Sequencer 3130×*l* Genetic Analyzer (Applied Biosystems, Inc.) from the service of the Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires.

Sequences were aligned and edited using Sequencher 4.9 (Gene Codes Corporation). Variable sites and unique haplotypes were identified using MacClade 4.08a (Maddison and Maddison 1992). Arlequin 3.11 (Excoffier et al. 2005) was used for computing estimates of nucleotide diversity (π) and haplotype diversity (h) according to Nei (1987), and to test neutrality with Tajima's D (Tajima 1989) and Fu's Fs (Fu 1997). The significance of these tests of neutrality was assessed using 1000 iterations. Phylogenetic relationships were reconstructed among haplotypes using a median-joining network with the program Network 4.5.0.0 (Bandelt et al. 1999).

DNA was extracted successfully from 84 samples (54%). A fragment of 380 bp of the mtDNA control region was successfully amplified and sequenced in 50 samples. Only a small amount of samples were successfully amplified and sequenced due to highly degraded and low concentrated DNA in dry skin, teeth and bone samples.

We identified a total of 28 haplotypes defined by 32 variable sites (Genbank Accession Numbers JQ599383–JQ599410). All variable sites were transition substitutions except two transversions. No heteroplasmy, either in the nucleotide sequence or in the length of the amplified fragments was detected. The overall haplotype diversity (h) (0.966 ± 0.012) and the nucleotide diversity (%π) (0.99 ± 0.56) demonstrated high levels of genetic diversity. Values for Tajima's D (-1.58, $p = 0.03$), and Fu's Fs (-20.06, $p = 0.0$) were negative and statistically significant, indicating an excess of rare alleles as expected under a recent expansion model. The network of haplotypes showed a star-like shape, suggesting a relatively recent expansion in size in the area studied (Fig. 2).

Genetic data presented here allow a first insight into the genetic variation of the spectacled porpoise. Samples from Tierra del Fuego, Argentina, show substantially high levels of mtDNA diversity due to the existence of a very high proportion of unique haplotypes that differ by only a small number of bases. These values are similar to that of other species of porpoises and small delphinid species ($h = 0.84$–0.97, %$\pi = 0.99$–1.26 in harbour's porpoise *Phocoena phocoena* Rosel et al., 1999a; $h = 0.968$, %$\pi = 1.06$ in Dall's porpoise *Phocoenoides dalli* Hayano et al., 2003; $h = 0.949$–0.968, %$\pi = 1.8$ in short-beaked common dolphin *Delphinus delphis* Luca et al., 2009),

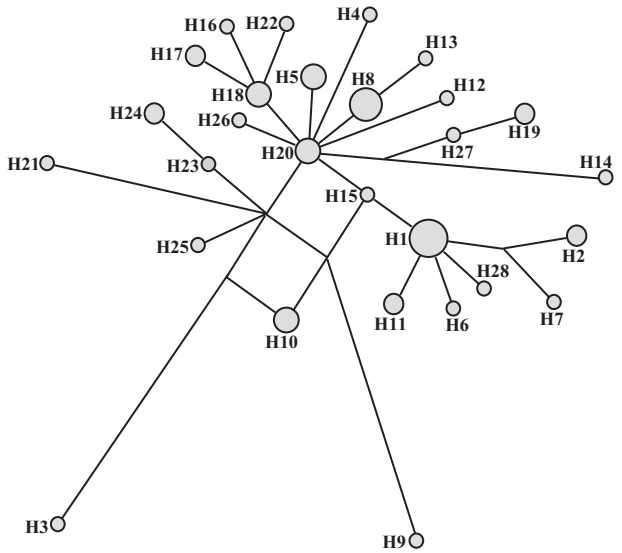

Fig. 2. Inferred genealogical relationship among the 28 mtDNA haplotypes in spectacled porpoise based on the median-joining algorithm. The diameter of each circle is proportional to the number of individuals found for the haplotype. Lines connecting haplotypes indicate single mutational differences.

and as those expected in wide distributions and/or large size populations (Grant and Bowen 1998).

These results, together with neutrality tests and phylogenetic relationship analysis, indicated that spectacled porpoises from Tierra del Fuego might be a large or expanding aggregation, with probable immigration from other areas of their distribution range.

It is yet unclear why so many spectacled porpoises have been found stranded on the coasts of Tierra del Fuego, while a preference of this species for deep waters has been shown by sightings at sea and in analysis of diet (Riccialdelli et al. 2010). There are many factors that can explain and shape the distribution of marine pelagic species. Some of them are water currents, sea floor topography, water temperature and prey distribution (Baird and Whitehead 2000; Cotté et al. 2009; Möller et al. 2011).

Further analysis of spectacled porpoise samples from throughout the range of the species' distribution, as well as the analysis of other molecular markers, would provide information about their genetic structure and migration patterns. In addition, monitoring any changes in genetic diversity within and between areas over time will assess the effects of anthropogenic pressures (such as bycatch) and climatic change in the species.

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