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Population structure of nuclear and mitochondrial DNA variation among South American Burmeister's porpoises (*Phocoena spinipinnis*)

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Abstract

Little is known about the biology of Burmeister's porpoises (*Phocoena spinipinnis*), a small cetacean species endemic to South American waters. Information on stock structure, however, is urgently needed, as the species suffers from considerable mortality due to local fishery activities throughout its distribution range. Using mitochondrial control region sequences and 11 species-specific microsatellite loci, we assessed the genetic differentiation among 118 stranded, incidentally or directly-caught Burmeister's porpoises from different localities in Peruvian, Chilean, and Argentine waters. F-statistics and Bayesian clustering analyses indicate a major population differentiation along the South American Pacific coast, separating Peruvian from both Chilean and Argentine individuals. Interestingly, this population boundary is consistent with the population structure found in another sympatrically-occurring cetacean species: the dusky dolphin (*Lagenorhynchus obscurus*). Given that vulnerability to local depletion for South American coastal porpoises and dolphins is probably highest in the Peruvian population (due to high exploitation levels and recurrent El Niño events), the genetic data reported here considerably strengthen the need for conservation efforts focused on regulation of catches in local waters. Moreover, we discuss possible genetic differentiation among Burmeister's porpoises (i) from the Atlantic and Pacific Ocean and (ii) from different Peruvian harbors. Finally, cross-species amplifications suggest that our newly-developed microsatellite markers will be useful in population genetic studies in the five other extant porpoise species.

Introduction

Some cetacean populations throughout the world are under threats from human activities such as direct catches, incidental mortality due to fishery activities, and modification of inshore habitat. Small cetacean species with restricted distribution range and habitat use are especially vulnerable to human-induced perturbations (Pichler and Baker 2000; Dawson et al. 2001). Knowledge of population structure is fundamental for assessing population changes, interpreting catch statistics, and achieving sustainable management. In many cetaceans, however, stock boundaries are not well understood, precluding any development of effective management schemes.

The Burmeister's porpoise, *Phocoena spinipinnis* (Burmeister 1865), is a small cetacean species

endemic to South American waters. Along the Atlantic coast, it is commonly found from Santa Catarina, Southern Brazil (28°48′ S) to the San José Gulf, Argentina (42°23′ S), while along the Pacific coast, it occurs from Bahia de Paita, Peru (05°01′ S) to Chiloe Island, Chile (42°30′ S) (Rice 1998; Van Waerebeek et al. 2002b). Although Burmeister's porpoises are described to preferentially inhabit coastal waters (Brownell and Praderi 1984), their direct observation is extremely difficult due to their small size, unobtrusive swimming habits, and to the sometimes inaccessible coastline. A recent review showed that, to that date, only 28 confirmed sightings exist for the Southeast Pacific (Van Waerebeek et al. 2002b). Knowledge about the species biology, and more specifically its stock structure and dispersal patterns, is therefore particularly scarce. For example, although sightings and strandings suggest that Burmeister's porpoises may be common in portions of the Magellan Strait and the Beagle Canal (Goodall 1978; Goodall et al. 1995b), it is still unclear whether the species' distribution is continuous between the Atlantic and Pacific Ocean (Corcuera et al. 1995; Goodall et al. 1995a). On the contrary, it has been suggested that Pacific and Atlantic Burmeister's porpoises comprise two different stocks (Brownell and Praderi 1984), a hypothesis which is consistent with size differences observed between individuals from Northern Argentina and Peru/Chile (Corcuera et al. 1995). However, potential population boundaries have never been investigated using molecular data.

More accurate stock designations are urgently needed given that Burmeister's porpoises are subjected to high human-induced mortality levels throughout their range. While porpoises are incidentally caught in gill-net fisheries off Argentina and Uruguay, the situation is more severe in Peruvian and Northern Chilean waters where direct catches of small cetaceans, used for human consumption and increasingly as bait in shark fisheries, have been observed since the direct collapse of the anchovy fishery in the 1970's (Read et al. 1988; Van Waerebeek and Reyes 1990a, b). The annual cetacean catch off Peru has been estimated to be 15–20 thousands individuals per year for the period 1991–1993 (Van Waerebeek and Reyes 1994b). Despite a ban on small cetacean exploitation in 1990, further reinforced in 1994, captures of several species, including dusky dolphins (*Lagenorhynchus obscurus*), long-beaked common dolphins (*Delphinus capensis*), and Burmeister's porpoises, still occur in Peruvian fisheries (Van Waerebeek et al. 1997; Van Waerebeek et al. 1999; Van Waerebeek et al. 2002a). Indeed, between 1995 and 1999, Van Waerebeek et al. (1999) reported on small cetacean exploitation in 83.5% of the visited harbors and found the remains of at least 452 captured cetaceans, 43.5% of which were *P. spinipinnis*. Given that hunting of small cetaceans is prohibited, fishermen increasingly try to commercialize their catches hidden from public view. As evidenced by beach-cast carcasses found in the proximity of harbors, cetacean specimens are increasingly cut up on board and parts of no value are discarded before entering the ports (Van Waerebeek et al. 2002a). Despite these difficulties in establishing accurate catch records, it is likely the number of Burmeister's porpoises taken annually in Peruvian waters reaches at least the high hundreds (Van Waerebeek, unpublished data).

In addition, porpoises in the Peruvian upwelling system might be particularly vulnerable to local depletion as they inhabit a very rich, but unstable environment: Recurrent El Niño events cause high mortality rates among animals and plants, with the most severe oscillations of the last century having occurred in 1982–1983 and 1997–1998 (Berta and Sumich 1999).

Here, we report on nuclear microsatellite and mitochondrial DNA sequence variation among Burmeister's porpoises from different locations along the South American coasts. Molecular analyses allow us to address the following issues: The genetic population structure of the species is assessed and putative stock boundaries are identified. Considering that the species suffers considerable human-induced mortality and the vulnerability to local depletion is probably highest in the Peruvian upwelling system, we particularly assess the genetic variability and test for a recent bottleneck in Peruvian Burmeister's porpoises and investigate their genetic divergence from other populations. The important sampling in Peruvian waters allows us to additionally examine whether genetic differentiation on a small geographic scale can be detected. Porpoises sampled in, or close to, different harbors might originate from distinct fishing grounds (Van Waerebeek, unpublished data). Given that sampling locality (harbor) of a

carcass is probably a good approximation of locality of the individual's take, the identification of fixed genetic differences among local groups could provide valuable information to catch statistics studies.

Finally, we provide information on the utility of our newly isolated *P. spinipinnis* microsatellite markers for population genetic studies in the other five extant porpoise species.

Materials and methods

Sample collection and DNA extraction

Skin, bone, and tooth samples were collected from stranded, incidentally- or directly-caught Burmeister's porpoise specimens (*Phocoena spinipinnis*) from Peru (n = 95), Chile (n = 16), and Argentina (n = 7) (Figure 1a). The bone (n = 2) and tooth (n = 3) samples came from different localities along the coast of Tierra del Fuego, Argentina. While only a few Peruvian samples (n = 13) were sampled less than 12 h *post mortem* (sampled in 1989/1990 in different harbors before the onset of the ban), most samples (n = 82) were opportunistically collected during the last 10 years from discarded, partly decayed, carcasses (cf. Introduction). Eighty-six of the 95 Peruvian samples can be assigned to precise localities (harbors and their nearby beaches) along the coast (Figure 1b; from North to South): Talara (04°60′ S, n = 1), San José (06°46′ S, n = 7), Pacasmayo (06°80′ S, n = 4), Pimentel (06°85′ S, n = 1), Salaverry (08°14′ S, n = 25), Chimbote (09°05′ S, n = 10), Los Chimus (09°5′ S, n = 3), Ancon (11°46′ S, n = 2), Chancay (11°56′ S, n = 9), Pucusana (12°30′ S, n = 17), Cerro Azul (13°10′ S, n = 6), and Tambo de Mora (13°27′ S, n = 1). Skin samples were preserved in a saturated salt solution (NaCl) containing 20% dimethyl sulfoxide (DMSO).

Standard proteinase K digestion and phenol–chloroform extraction procedures (Hillis et al. 1996) were used for all skin samples. Total genomic DNA from bone and tooth samples was extracted using the Qiagen DNeasy Tissue Kit, with a slightly

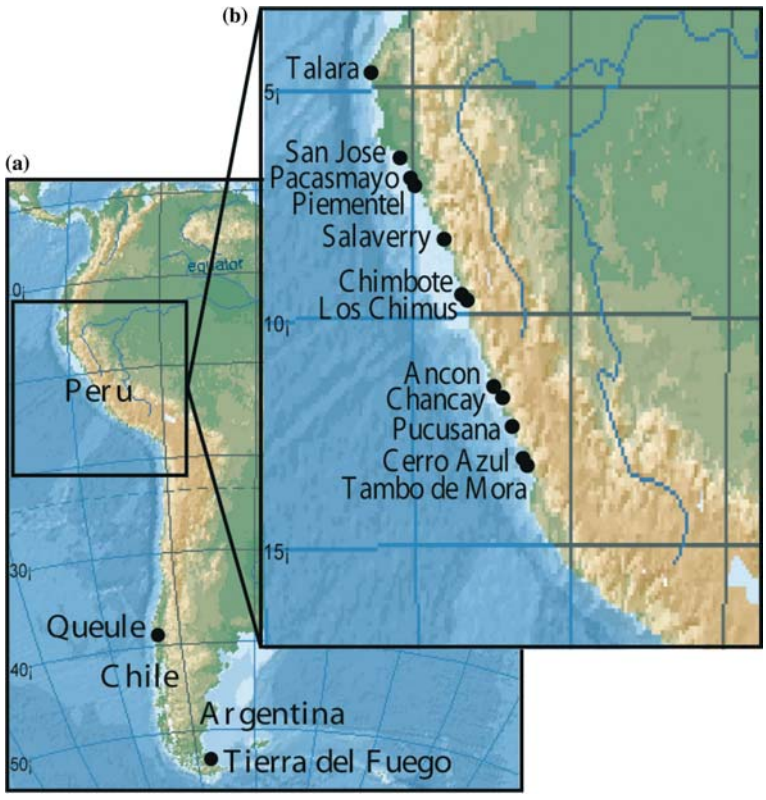

Figure 1. Geographical locations of Burmeister's porpoise sampling sites in (a) South American and (b) Peruvian waters.

modified protocol as described in Cassens et al. (2005). An examination of DNA quality by electrophoresis on 1% agarose gels (Biozym) revealed partially or highly degraded DNA for many of the extractions, especially from skin, tooth, and bone samples of beach-cast carcasses.

Microsatellite isolation and genotyping

Nuclear DNA variation was assayed using species-specific microsatellite loci that were isolated (as described in Cassens et al. 2005) from a genomic library of a Peruvian *Phocoena spinipinnis* individual. Primers flanking the repeated sequence were designed using the program OligoFaktory (unpublished, Laboratory of Evolutionary Genetics, Free University of Brussels). We screened 12 individuals for variation at 35 microsatellite loci and selected 11 loci (Table 1) on the basis of variability and unambiguousness of amplification pattern interpretation. Sequences of the cloned alleles are available from GenBank (DQ02239). The 5′-end of the reverse primer from each selected locus was fluorescently-labeled. Forward primers were designed with a GTTTCTT 5′-tail to reduce variability in adenylation of amplification products and thereby improve allele binning (Brownstein et al. 1996). Each PCR was performed in a final volume of 25 μl containing 0.75 U of *Fast Start Taq* DNA polymerase (Roche Molecular Biochemicals), 0.25 mM of each dNTP, 2 mM $MgCl_2$, 1× PCR buffer (50 mM Tris/HCl, pH 8.3), 0.6 pmol/μl of each primer, and 1 μl (~10–500 ng) of genomic DNA. For difficult DNA extractions (i.e., from bone, teeth, and decayed skin samples), 400 μg/μl BSA (Sigma) was added to the PCR reaction (Hillis et al. 1996). The PCR reaction profile was as follows: 95 °C for 4 min 30 s for activation of the polymerase, 35 cycles of 30 s at 95 °C – 30 s at the optimized annealing temperature according to the OligoFaktory program (52.5, 56, or 58 °C) – 30 s at 72 °C, then followed by a final elongation at 72 °C for 1 h. PCR products were separated electrophoretically using an Applied Biosystems 3100 or 3730 automated sequencer. Allelic sizes were scored against the size standard

Table 1. Characterization of the 11 microsatellite loci used in this study

Locus	Primer sequences (5′–3′)	Repeat	T_a (°C)	Size (bp)
PS1	F: GTTTCTTGAGTTTGGCAAATAACCTACC R: CCAATAAGAAACACTTACAGTTGAA	$(AC)^{17}$	56	113–134
PS2	F: GTTTCTTACGTGCCTATTTTAGGATAAA R: CTAATTTCCTCTGTGCTGC	$(GT)^{23}$	56	85–117
PS3	F: GTTTCTTATCTTCTCAGGCTGTTCTCTACA R: CAGATGGTGGAAAGAAAAAAGAA	$(TG)^{19}$	56	99–112
PS4	F: GTTTCTTCAGGCTGCTAATAAAGTTATTTC R: TCACTCATCAACTCCATGCAA	$(AC)^{16}$	56	99–107
PS5	F: GTTTCTTGTTTTCTAATGTGTTACTTTAAGGT R: ACAAAGTTATATGAAAGCATGTGTA	$(GT)^{19}$	56	89–101
PS6	F: GTTTCTTCACACGCACATATACCTGC R: GGAAAAGGATAAAGCAGATAAGA	$(AC)^{17}$	52.5	98–114
PS7	F: GTTTCTTAAAATAAAGAAGTGAAAAGGATAGG R: AAGCCTGCTACCAACACA	$(GT)^{13}$	52.5	81–89
PS8	F: GTTTCTTCTCTATTTTTGACTGCTTT R: ATTAGTTACCCATTTTATCATAA	$(AC)^{17}$	52.5	87–95
PS9	F: GTTTCTTTATATGTAGACCTATAGCTATATTT R: TTCAGGTGGAAATCTCTGT	$(AC)^{23}$	52.5	94–108
PS10	F: GTTTCTTTCAGTGTTTGCTGTATACATTCTTG R: GATGCAGTCTCCTTAGATACTATG	$(GT)^{17}$	56	93–103
PS11	F: GTTTCTTTAGGAATGAGTTTCTCTCTAAT R: TTTTTTTAGCTTCATCAACA	$(GT)^{19}$	52.5	97–103

For each locus, the forward primer (F) includes a GTTTCTT tail (underlined) at its 5′-end to force A+ alleles and, hence, improve binning of alleles; the reverse primer (R) was fluorescently labeled. The "repeat" column shows the structure of the repeat region in the cloned allele. T_a, annealing temperature; size, range of allele sizes found.

GS500 LIZ (Applied Biosystems) and analyzed using the GENESCAN 3.7 and GENOTYPER 3.7 software (Applied Biosystems).

For 2 of the 11 loci, an obvious heterozygote deficiency was observed with the initial primer pairs used, suggesting the existence of null alleles. The design of new primers (listed in Table 1) that were shifted by a few base pairs revealed a second allele for many of the formerly ''homozygous'' individuals.

Cross-species amplification of the 11 microsatellite loci in representatives of each of the 5 other species of true porpoises (family *Phocoenidae*) was performed using the 11 *P. spinipinnis* pairs of primers. While amplification success in a single individual was tested for the vaquita (*Phocoena sinus*), spectacled porpoise (*P. dioptrica*), Dall's porpoise (*Phocoenoides dalli*), and finless porpoise (*Neophocoena phocaenoides*), we additionally examined intraspecific variability (number of alleles, observed and expected heterozygosities) in 13 harbor porpoise individuals (*Phocoena phocoena*) from Northeast Atlantic waters. PCR conditions were the same as described above.

Microsatellite analysis

If not otherwise stated, we assigned the 118 surveyed Burmeister's porpoises to three geographical 'sampling units': Peru, Chile, and Argentina. We tested for significant heterozygote deficiency per locus and per 'sampling unit' using a Hardy–Weinberg exact test (Guo and Thompson 1992) based on 100,000 Markov chain iterations as implemented in ARLEQUIN, version 2.000 (Schneider et al. 2000). Using FSTAT, version 2.9.3.2 (Goudet 1995, 2001), the assumption of independence within each pair of loci was assessed with a likelihood-ratio statistic, whose null distribution was obtained by 2000 permutations. Critical significance levels for multiple testing were corrected following the sequential Bonferroni procedure (Rice 1989).

The following statistics of nuclear genetic diversity within 'sampling units' were calculated per locus as well as averaged over the 11 microsatellite loci using the software ARLEQUIN, version 2.000 (Schneider et al. 2000): number of alleles per locus (A), allele frequencies, observed heterozygosity (H_O), and expected heterozygosity (H_E), computed according to (Nei 1987). The program FSTAT, version 2.9.3.2. (Goudet 1995; 2001) was used to estimate the allelic richness (AR), a standardized measure of the number of alleles per locus that takes into account unequal sample sizes (El Mousadik and Petit 1996; Petit et al. 1998). Allelic richness was calculated for the smallest number of individuals typed for a locus in a sample, i.e., for the seven individuals assigned to the Argentine sampling unit. To investigate whether populations have experienced a recent reduction of their effective population size, we applied two different methods to our microsatellite data set, both implemented in the software BOTTLENECK, version 1.2.02 (Piry et al. 1999): (1) The distribution of allele frequencies was examined for a so-called 'mode-shift' that discriminates recently-bottlenecked from stable populations (Luikart et al. 1998), and (2) we analyzed whether a significant number of loci exhibit a heterozygosity excess, given that allelic diversity reduces faster than heterozygosity in bottlenecked populations (Cornuet and Luikart 1996). Heterozygosity excess was estimated based on 50,000 replications (under the two-phase model, Di Rienzo et al. 1994, with 5% and 10% multi-step mutations) and tested for significance with a one-tailed Wilcoxon signed rank test. Given that significance levels can depend on the mutation model used (Storz et al. 2002), first we checked whether the approximate proportion of multi-step mutations per locus was estimated to be less than 10%, using the program MISAT, version 1.1 (Nielsen 1997).

Because of the uncertainty that the geographical assignment of individuals to 'sampling units' would represent biologically significant entities, a Bayesian method implemented in the program STRUCTURE, version 2.0 (Pritchard et al. 2000) was used to determine whether our *a priori* group definition (i.e., Peru, Chile, and Argentina) was consistent with genetic information. STRUCTURE uses a model-based clustering approach to assign individuals to groups while minimizing Hardy–Weinberg disequilibrium and gametic phase disequilibrium among loci within groups. The number of groups (K) most compatible with the observed data can be obtained by maximizing the estimated log-likelihood of the data for different values of $K(\ln \Pr(X/K))$. We performed a series of independent runs for K from 1 to 6 populations,

assuming correlated allele frequencies and an admixture model, with a burn-in of 5000 iterations and a data collection period of 200,000 iterations. Three runs for each value of K were performed to check for convergence. The program can also be used to report, for each individual, on its so-called 'membership coefficient' in each of the inferred populations and on the probability that the individual or one of its ancestors came from a population other than the one where it has been sampled. This information allowed us to identify migrants (or descendents of migrants) and their possible origin. To investigate whether genetic differentiation can be detected on smaller geographic scales, we performed additional analyses which included either only (i) Peruvian or (ii) Chilean and Argentine individuals (burn-in of 500,000 iterations, data collection period of 0.5×10^7 iterations, K from 1 to 6, and 1 to 4, respectively).

Nuclear population structuring was further estimated in terms of classical F-statistics. We first used an allele size permutation procedure to test whether microsatellite allele sizes are informative with respect to genetic differentiation (Hardy et al. 2003) as implemented in SPAGeDi, version 1.1 (Hardy and Vekemans 2002). Non-rejection (based on 15,000 permutations) of the null hypothesis ($F_{ST} = R_{ST}$) led to the calculation of genetic differentiation using F_{ST} values (Weir and Cockerham 1984; Michalakis and Excoffier 1996) as F_{ST} is more appropriate than R_{ST} when differentiation is caused mainly by drift. F_{ST} values were calculated among the three pre-defined geographic sampling localities (Peru, Chile, Argentina). F-statistics was also used to examine fine-scale genetic structure within Peruvian waters, comparing Burmeister's porpoises from San José (n = 7), Salaverry (n = 25), Chimbote (n = 10), Chancay (n = 9), Pucusana (n = 17), and Cerro Azul (n = 6) (cf. Figure 1b). All F-statistics estimations were performed using the Arlequin software (Schneider et al. 2000) and tested for statistical significance with 100,000 permutations.

Mitochondrial sequence analysis

Nuclear copies of mitochondrial sequences are frequently co-amplified in Phocoenid species, hampering considerably the analysis of mitochondrial gene fragments (Cassens and Milinkovitch, in preparation). Targeting very long mitochondrial sequences, amplification increases mitochondrial specificity through the exclusion of, mostly shorter, nuclear copies. We therefore first amplified a 9 kb fragment that encompasses more than half of the mitochondrial genome, using the Expand^{TM} Long Template PCR system (Roche Molecular Biochemicals) and the following primers: L10534/NII (5′-tttgcagcctgcgaagcagctatcggact-3′) and H2825/NIV (5′-ggattgcgctgttatccct agggtaacttgttccg-3′). Long PCRs were successful when performed on fresh samples (13 Peruvian and 16 Chilean individuals), i.e., yielding high molecular-weight DNA (cf. Introduction). Each PCR was performed in a final volume of 50 μl containing 3.75 U of *Taq* Long Expand polymerase, 0.35 mM of each dNTP, 3.75 mM $MgCl_2$, 1× PCR buffer type 1, 0.6 pmol/μl of each primer, and 1 μl (up to 500 ng) of genomic DNA. The PCR profile was carried out as follows: 94 °C for 2 min, then 10 cycles of 94 °C for 10 s – 62 °C for 30 s – 68 °C for 6 min, followed by 25 cycles of 94 °C for 10 s – 62 °C for 30 s – 68 °C for 6 min (increased by 20 s at each cycle), and a final elongation step of 15 min at 68 °C. Long PCR products included the complete cytochrome *b* gene and control region, from which a portion was directly sequenced on both strands (BigDye^{TM} Terminator Cycle Sequencing Kit, Applied Biosystems) and analyzed on an ABI 3100 sequencer using several internal sequencing primers (Table 2). All mitochondrial haplotypes have been deposited in Genbank under the accession numbers (DQ022940-DQ022949).

Sequences were aligned using Clustal X (Thompson et al. 1997) and variable positions were determined manually. Genetic variability within populations was estimated in terms of haplotypic (H) and nucleotide (π) diversity, as implemented in the program Arlequin, version 2.000 (Schneider et al. 2000). To estimate phylogeographic structure, we inferred a median-joining graph (Bandelt et al. 1999) using the program Network, version 2.0 (available at http://www.fluxus-engineering.com/sharenet.htm). Pairwise F-statistics (conventional F_{ST} from haplotype frequencies as well as Φ_{ST} calculated from absolute number of differences) were calculated to estimate mitochondrial genetic differentiation among the pre-defined populations, using Arlequin, version 2.000 (Schneider et al. 2000).

Table 2. Internal primers used for the sequence analysis of Burmeister's porpoise control region (cf. Material and methods for details) and their approximate locations on the mitochondrial DNA molecule (location). L, forward primer; H, reverse primer

Primer	Location	Sequence	Reference
Sequ1_L	Cytochrome b (3′-end)	5′-TCATTGTGGGCCAATTAGCA-3′	This study
Sequ2_L	Control region (5′-end)	5′-GCTATGTATTATTGTGCATTC-3′	This study
Sequ3_L	Control region (5′-end)	5′-CCATACGACTATGTTAAAGT-3′	This study
Sequ4_L	Control region	5′-ATCACGAGCTTAACCACCAT-3′	This study
Sequ5_H	Control region	5′-ATGGTGGTTAAGCTCGTGAT-3′	This study
Sequ6_H	Control region (3′-end)	5′-GTCATTAGTCCATCGAGATG-3′	This study
Sequ7_H	Control region (3′-end)	5′-GTTATGTGTGAGCATGGGCT-3′	This study
Sequ8_H	tRNA-phe	5′-TCTCGAGATTTTCAGTGTCTTGCTTT-3′	Hoelzel et al. (1991)

Results

Microsatellite variability

After adjusting the significance levels for multiple comparisons, none of the three sampling units significantly deviates from Hardy–Weinberg and linkage disequilibria. Nuclear genetic diversity is moderate, with a mean allele number of 7.2 and mean expected heterozygosity of 0.72 (all 118 individuals across the 11 microsatellites studied; Table 3). Highest mean variability is found in Peruvian Burmeister's porpoises. However, genetic diversity levels are highly variable across loci: expected heterozygosity for example is about equal in Peruvian and Chilean "populations" for the loci PS2 and PS4 and highest in Argentine waters for the locus PS6 (Table 3). An analysis of allele frequency distributions reveals that a large number of alleles are not shared among populations: 34, 3, and 1 private alleles are found in the Peruvian, Chilean, and Argentine populations, respectively (data not shown). None of the performed bottleneck tests (mode-shift and heterozygosity excess analyses) detects a recent reduction in population size for any of the three "populations".

Nuclear genetic differentiation

A clear population structuring could be detected using the Bayesian clustering approach: the STRUCTURE program suggests that the sampled Burmeister's porpoises most likely only represent two different groups ($P = 1$ with $K = 2$). Moreover, the assignment of individuals to these two groups clearly differentiates Peruvian waters from all other sampling localities (Table 4). While most of the 118 porpoises could undoubtedly be assigned to one of the two clusters (with a membership coefficient higher than 0.93), four individuals exhibit genotypes that might originate outside from the cluster where they were sampled, i.e., suggesting some degree of admixture between the two clusters (Table 5 (a)). Our analyses suggest that migration events in past generations account for these observation, with an increased probability that a parent (N88) or a grandparent (N35, N67, 5B9) of the sampled individual immigrated from the other population (Table 5 (b)).

Nuclear genetic differentiation is very low on smaller geographic scales. The Bayesian clustering analyses including either only Peruvian or only Chilean + Argentine individuals estimated, on average, the highest likelihood and smallest variance for $K = 1$. For higher values of K, estimates of $\ln \Pr(X|K)$ tend to be variable across runs. Furthermore, mean alpha values (proportion of admixed individuals) are large (>4 for Chile/

Table 3. Summary of genetic variability statistics for the nuclear microsatellite loci

n:	All 118	Peru 95	Chile 16	Argentina 7
A (mean)	7.2	6.8	3.6	2.7
PS1	10	10	6	3
PS2	7	7	5	4
PS3	6	5	4	1
PS4	9	8	5	2
PS5	4	4	1	1
PS6	8	7	4	5
PS7	8	8	4	3
PS8	10	10	2	3
PS9	7	6	5	3
PS10	6	6	2	2
PS11	4	4	2	3

Table 3. Continued

n:	All 118	Peru 95	Chile 16	Argentina 7
AR (mean)	4.4	4.1	2.9	2.6
PS1	5.7	5.1	4.5	3.0
PS2	4.7	4.4	4.2	4.0
PS3	3.3	3.3	2.6	1.0
PS4	4.9	4.4	4.3	2.0
PS5	3.1	3.3	1.0	1.0
PS6	4.1	3.9	3.2	4.8
PS7	5.0	4.5	3.0	2.7
PS8	5.4	5.6	2.0	2.8
PS9	4.7	3.9	3.1	2.9
PS10	4.0	3.9	1.9	2.0
PS11	3.4	3.4	1.9	2.7
H_O (mean)	0.65	0.71	0.48	0.33
PS1	0.77	0.80	0.69	0.57
PS2	0.73	0.73	0.75	0.67
PS3	0.59	0.68	0.37	Monom.
PS4	0.74	0.75	0.87	0.43
PS5	0.44	0.54	Monom.	Monom.
PS6	0.68	0.70	0.62	0.57
PS7	0.69	0.77	0.44	0.29
PS8	0.73	0.81	0.44	0.43
PS9	0.69	0.71	0.75	0.43
PS10	0.58	0.67	0.25	0.14
PS11	0.53	0.63	0.12	0.14
H_E (mean)	0.72	0.71	0.48	0.46
PS1	0.83	0.78	0.71	0.65
PS2	0.74	0.71	0.74	0.74
PS3	0.64	0.66	0.38	Monom.
PS4	0.78	0.74	0.74	0.36
PS5	0.49	0.57	Monom.	Monom.
PS6	0.73	0.70	0.58	0.83
PS7	0.79	0.74	0.54	0.39
PS8	0.80	0.83	0.52	0.49
PS9	0.75	0.71	0.56	0.66
PS10	0.72	0.68	0.23	0.49
PS11	0.68	0.64	0.29	0.40

n, number of porpoises analyzed; A, number of alleles per locus (PS1–PS11) and mean number of alleles across the 11 loci (mean); AR, allelic richness expected for seven individuals (i.e., the sample size of the sampling unit 'Argentina'); H_O, observed heterozygosity; H_E, expected heterozygosity. *Monom*, monomorphic.

Argentina and >5 within Peruvian waters), suggesting that no well-defined population structure can be detected at this geographical scale, using the STRUCTURE program (Pritchard et al. 2000).

Consistent with the STRUCTURE analysis, we observe highly significant F_{ST} values (ranging from 0.239 to 0.274) in all pairwise comparisons including the Peruvian population (Table 6 (a)), whereas a low ($F_{ST} = 0.079$) but significant differentiation is detected between the Argentine and Chilean 'stocks'. Likewise, comparisons among Peruvian harbors in terms of pairwise F-statistics revealed low but significant differences only between Burmeister's porpoises from Salaverry vs. Chimbote and Chancay *vs*. Pucusana (Table 6 (b)) while all other pairwise comparisons yield non-significant values.

Table 4. Proportion of individuals from each sampling unit assigned to each of the two clusters inferred from the 'Structure' analysis (see text for details), n, sample size

Sampling units	n	Inferred population clusters	
		1	2
Peru	95	0.978	0.022
Chile	16	0.007	0.993
Argentina	7	0.019	0.981

Cross-species amplifications

All 11 *P. spinipinnis* microsatellite markers were successfully amplified and analyzed in the other five extant Phocoenid species. Despite the very low number of individuals tested, heterozygosity was observed at 2 (*P. sinus*; 1 individual), 6 (*N. phocoenoides*; 1 individual), 9 (*A. dioptrica*; 1 individual), 10 (*P. dalli*; 1 individual), and 11 (*P. phocoena*; 13 individuals) loci. The level of intraspecific variability in harbor porpoises (*Phocoena phocoena*) was surprisingly high: over all loci, the mean number of alleles, observed, and expected heterozygosities, are 7, 0.81, and 0.79, respectively.

Mitochondrial sequence diversity and population structure

A mitochondrial DNA fragment, encompassing ten bases of the cytochrome *b* gene, the complete tRNA-thr and tRNA-pro genes, and approximately 94% of the control region were compared among 29 Burmeister's porpoises from Peru and Chile. A TA-repeat region, found at the 5′-end of the control region (Rosel et al. 1995) was removed from the final alignment. Of a total of 975 nucleotides analyzed, 19 sites are polymorphic and define 10 distinct haplotypes. Genetic variability in

Table 5. Identification and analysis of the four Burmeister's porpoise individuals that showed highest probability of contributing to admixture

Individual	Sampling locality	(a)		(b)			
		1	2	A	B_0	B_{+1}	B_{+2}
N35	Peru	0.851	0.149	0.585	0.000	0.019	0.396
N67	Peru	0.889	0.111	0.594	0.000	0.002	0.404
N88	Peru	0.331	0.669	0.000	0.003	0.920	0.077
5B9	Chile	0.127	0.873	0.743	0.000	0.000	0.257

(a) Estimated probabilities of membership in clusters 1 and 2 when population structure was inferred without any prior information on sampling localities, (b) posterior probability that the individual is correctly assigned to its given sampling locality (A), and that the individual comes from the second population (B_0), or has a parent (B_{+1}) or grandparent (B_{+2}) coming from the second population.

Table 6. Nuclear genetic differentiation in terms of pairwise F-statistics among (a) the three Burmeister's porpoise sampling localities along the South-American coast (Peru, Chile, and Argentina) and (b) harbors within Peruvian waters

(a)	Peru	Chile			
Chile	0.239***				
Argentina	0.274***	0.079***			
(b)	San José	Salaverry	Chimbote	Chancay	Pucusana
Salaverry	0.001 ns				
Chimbote	0.005 ns	0.013*			
Chancay	0.018 ns	0.009 ns	0.014 ns		
Pucusana	−0.011 ns	0.007 ns	0.011 ns	0.023*	
Cerro Azul	0.015 ns	−0.010 ns	0.015 ns	0.002 ns	0.005 ns

terms of haplotypic and nucleotide diversity is highest in the Peruvian population (Table 7). Genetic differentiation at the mitochondrial locus is in agreement with nuclear markers, with both F_{ST} and ϕ_{ST} statistics confirming the significant differentiation of the Peruvian population (Table 7). The median-joining haplotype network reveals that haplotypes from the north (Peru) and south (Chile) of the South-American Pacific coast fall into two different haplogroups separated by one fixed mutation (located in the tRNA-thr gene) (Figure 2).

Table 7. Mitochondrial sequence variability within the three Burmeister's porpoise sampling units in terms of haplotype (H, diagonal/left side) and nucleotide (π, diagonal/right side) diversities

	Peru		Chile	
Peru	0.83 (±0.07)	0.15 (±0.10)	0.293***	
Chile	0.547***		0.59 (±0.12)	0.10 (±0.07)

Standard deviations are given between parentheses. Mitochondrial genetic differentiation among sampling units is given in terms of pairwise F_{ST} (above diagonal) and Φ_{ST} (below diagonal) values. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; ns, $P > 0.05$.

Discussion

The Burmeister's porpoise is a small cetacean species that is restricted to South-American coastal waters. Burmeister's porpoises are only exceptionally sighted, due to their shy and elusive behaviour. Information about the species' biology is scarce and predominantly based on remains of stranded, incidentally- or directly-captured individuals.

Nuclear and mitochondrial population structure

Our molecular genetic data allow for a first insight into the population structure of Burmeister's porpoises. A major population differentiation is observed along the South-American Pacific coast, separating Peruvian from both Chilean and Argentine individuals. Genetic evidence for the

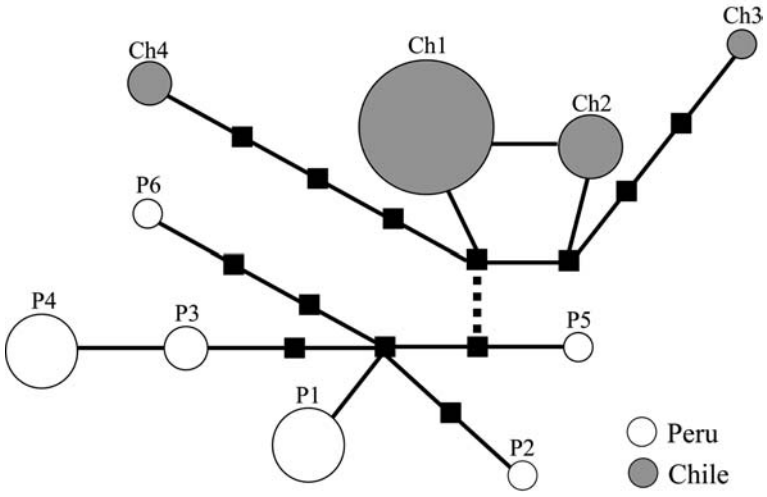

Figure 2. Median-joining graph depicting the genealogical relationships among mitochondrial Burmeister's porpoise haplotypes. Circle size is proportional to the number of individuals exhibiting the corresponding haplotype. Small unlabeled black squares represent missing, non-sampled haplotypes. Each branch connecting two haplotypes corresponds to a single mutational step with the dashed line representing the fixed substitution in the tRNA-thr gene that separates Peruvian and Chilean haplogroups.

existence of these two Burmeister's porpoise stocks come from our analyses of both mitochondrial sequence and nuclear microsatellite data. Mitochondrial control region haplotypes of individuals from Peruvian and Chilean waters cluster into two distinct haplogroups separated by a fixed substitution (Figure 2), leading to significant pairwise F_{ST} and ϕ_{ST} values (Table 7). In agreement with mitochondrial DNA data, our analyses of the geographical distribution of microsatellite variability at 11 nuclear loci further corroborate the hypothesis of an isolated Peruvian Burmeister's porpoise stock. Almost all, and only, Peruvian individuals are assigned with highest likelihood to one of the two inferred populations in the STRUCTURE analysis (Table 4). Likewise, F-statistics indicate a highly-significant nuclear differentiation between porpoises from the North (Peru) and the South (Chile and Argentina) (Table 6 (a)). Given that Burmeister's porpoises are described (Goodall et al. 1995a, b) to occur from Paita (05°01′S), Peru, to Valdivia (39°50′S), Chile, the existence of a genetically differentiated Peruvian Burmeister's porpoise stock is surprising and would not have been detectable by using the distribution data available to date. Most significantly, there is congruence between the population structure inferred here for Burmeister's porpoises and that described (Cassens et al. 2003, 2005) for dusky dolphins (*Lagenorhynchus obscurus*), another small cetacean species that occurs sympatrically in South American waters. It is striking that Peruvian individuals are significantly differentiated in both species (especially with respect to maternal lineages), suggesting that non-species-specific factors might have considerably influenced the distribution of genetic variation in South-Eastern Pacific coastal small cetaceans. For example, given that the Peruvian upwelling system is an unstable environment (due to recurrent El Niño events), genetic drift – caused by historical fluctuating population sizes – might have accelerated genetic differentiation of Peruvian stocks in many coastal species. It has been suggested that Burmeister's porpoises may be significantly impacted by El Niño phenomena through the resulting changes in food composition and availability. It may be that porpoises do not undertake long-range movements in the search of food (Reyes and Van Waerebeek 1995). This is consistent with findings of emaciated porpoises that were landed at a Peruvian port and several specimens that were found stranded on beaches during a severe El Niño oscillation in 1982–1983 (Reyes and Van Waerebeek 1995).

Our data suggest that recent gene flow among Burmeister's porpoise stocks is limited along the South American Pacific coast: A handful of sampled individuals (Table 5) indicate most probably

rare migration events that occurred one (N88) or two (N35, N67, 5B9) generations ago. There is however no clear evidence for different migration rates in male and female porpoises (i.e., we found significant population structuring both at mitochondrial and nuclear loci). This warrants further investigation, given that male-biased dispersal has been inferred in many other cetacean species, including baleen whales (Baker et al. 1998), sperm whales (Lyrholm et al. 1999), other porpoise species (Walton 1997; Rosel et al. 1999a; Escorza-Trevino and Dizon 2000), as well as the sympatric dusky dolphin (Cassens et al. 2005).

Especially in southern Chile and Argentina (i.e., in Tierra del Fuego and Patagonia channels), only few shore surveys have been carried out and the exact distribution of Burmeister's porpoises still needs to be established (Goodall et al. 1995a, b). Based on a presumed gap in austral distribution and differences in body size, a population boundary has been proposed between Atlantic and Pacific individuals (Brownell and Praderi 1982; Corcuera et al. 1995). Interestingly, nuclear differentiation among Burmeister's porpoises from Argentina and Chile is much weaker than the well-defined population differentiation along the South American Pacific coast: While the STRUCTURE analysis (with Peruvian individuals excluded) assigned with highest likelihood all Atlantic and Pacific porpoises to a single population cluster without any evidence for substructuring, conventional F-statistics yielded low but significant F_{ST} values for the population pair Chile *vs*. Argentina (Table 6 (a)). However, it is likely that differences between the two oceans become more pronounced (and perhaps detectable in a Bayesian clustering approach) with larger sampling sizes.

Conservation and management perspectives

Throughout their distribution range, Burmeister's porpoises are under threat from human activities (Crespo et al. 1994; Van Waerebeek and Reyes 1994a; Van Waerebeek et al. 1997). An assessment of the demographic impact of high removal rates, however, has not been possible yet as key information on stock boundaries and dispersal behavior was not available to date (the species is listed as "data deficient" by the IUCN). The molecular genetic data presented here is the first objective set of evidence indicating the existence of two management units, with a pronounced population boundary along the Pacific coast of South America. Given that non-natural mortality levels due to incidental and direct takes are reported to be highest in Peruvian fisheries (Van Waerebeek and Reyes 1990a, 1994a, b; Van Waerebeek et al. 1997), the evidence for a genetically isolated stock off Peru strengthens the value of conservation efforts that focus on an effective regulation of small cetacean catches in local waters. As shown in New Zealand Hector's dolphins (*Cephalorhynchus hectori*), high human-induced mortality can lead to a severe depletion of an isolated population in which large amounts of genetic variability have been lost (Pichler et al. 1998; Pichler and Baker 2000; Slooten et al. 2000; Dawson et al. 2001). The exploitation of small cetaceans in Peruvian waters has been observed for three decades (representing most probably less than 10 Burmeister's porpoise generations) and the most severe El Niño oscillations of the century occurred in 1982–1983 and 1997–1998 (Berta and Sumich 1999). However, neither a significant reduction of nuclear and mitochondrial variability (Tables 3 and 7) nor evidence for a recent bottleneck (heterozygosity excess and mode-shift test) was observed in the Peruvian Burmeister's porpoise population. It is possible that these human-induced and natural events are too recent and/or too moderate for their impact to be detected with our sampling (over the last 15 years) and the available molecular data (e.g., see Cornuet and Luikart (1996) for power analysis of bottleneck tests). Note however that the lack of a detectable signature associated with possible recent bottlenecks does not mean that these demographic events will not have a significant impact on the levels of genetic variability, hence, on the long-term survival of Peruvian Burmeister's porpoises.

For management purposes, Peruvian Burmeister's porpoises should be considered a single, isolated stock. In addition, the detection of a weak, but significant nuclear differentiation among some of the Peruvian harbors (Table 6 (b)) warrants further investigation, in particular because the differentiated harbors Salaverry *vs*. Chimbote and Pucusana *vs*. Chancay are neighbor localities along the coastline separated by less than 200 km. These local genetic differentiation levels are comparable to those between Chilean and Argentine Burmeister's

porpoises from sampling locations that are separated by more than 1500 km. Based on the analysis of eight nuclear microsatellite loci, even lower F_{ST} values (0.0018) were observed among Northwest Atlantic populations of harbor porpoises (*Phocoena phocoena*) that are 1000 km apart (Rosel et al. 1999b). Still, no population structure was detected in the STRUCTURE analysis that included only Peruvian individuals, and probably a more thorough sampling is needed to identify a potential fine-scale structure within Peruvian waters. If confirmed, differences among harbors would add evidence to the hypothesis that cetacean catches landed in different harbors have been captured in distinct fishing grounds; information which has important implications for catch statistics and management policies.

Cross-species amplification

The successful amplification of all 11 Burmeister's porpoise microsatellite loci in all the extant species of phocoenids as well as the observed levels of polymorphism suggest these markers will prove highly informative in population genetic studies in these species.

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References

Baker CS, Medrano-Gonzalez L, Calambokidis J, et al. (1998) Population structure of nuclear and mitochondrial DNA variation among humpback whales in the North Pacific. *Mol. Ecol.*, **7**, 695–707.

Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.*, **16**, 37–48.

Berta A, Sumich JL (1999) *Marine Mammals Evolutionary Biology*, Academic Press, San Diego, CA.

Brownell RL, Praderi R (1982) Status of Burmeister's porpoise, *Phocoena spinipinnis*, in southern South American waters. *FAO Fisheries Series (5) [Mammals in the Sea]*, **4**, 91–96.

Brownell RL, Praderi R (1984) Phocoena spinipinnis. *Mammalian Species*, **217**, 1–4.

Brownstein MJ, Carpten D, Smith JR (1996) Modulation of non-templated nucleotide addition by Taq DNA polymerase: primer modifications that facilitate genotyping. *BioTechniques*, **20**, 1004–1010.

Cassens I, Van Waerebeek K, Best PB, et al. (2003) The phylogeography of dusky dolphins (*Lagenorhynchus obscurus*): A critical examination of network methods and rooting procedures. *Mol. Ecol.*, **12**, 1781–1792.

Cassens I, Van Waerebeek K, Best PB, et al. (2005) Evidence for male dispersal along the coasts but no migration in pelagic waters in dusky dolphins (*Lagenorhynchus obscurus*). *Mol. Ecol.*, **14**, 107–121.

Corcuera JF, Monzon F, Aguilar A, Borrell A, Raga JA (1995) Life history data, organochlorine pollutants and parasites from eight Burmeister's porpoises, *Phocoena spinipinnis*, caught in northern Argentine waters. *Rep. Int. Whaling Comm. (Special Issue)*, **16**, 365–372.

Cornuet J-M, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, **144**, 2001–2014.

Crespo EA, Corcuera JF, Lopez Cazorla A (1994) Interactions between marine mammals and fisheries in some coastal fishing areas of Argentina. *Rep. Int. Whaling Comm. (Special Issue)*, **15**, 269–281.

Dawson S, Pichler FB, Slooten E, Baker CS (2001) The North Island Hector's dolphin is vulnerable to extinction. *Marine Mammal Sci.*, **17**, 366–371.

Di Rienzo A, Peterson AC, Garza JC, et al. (1994) Mutational processes of simple-sequence repeat loci in human populations. *Proc. Natl. Acad. Sci., USA*, **91**, 3166–3170.

El Mousadik A, Petit RJ (1996) High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* (L.) Skeels] endemic to Morocco. *Theoret. Appl. Genet.*, **92**, 832–839.

Escorza-Trevino S, Dizon AE (2000) Phylogeography, intraspecific structure and sex-biased dispersal of Dall's porpoise, *Phocoenoides dalli*, revealed by mitochondrial and microsatellite DNA analyses. *Mol. Ecol.*, **9**, 1049–1060.

Goodall RNP (1978) Report on the small cetaceans stranded on the coasts of Tierra del Fuego *Sci. Rep. Whales Res. Inst., Tokyo*, **30**, 197–230.

Goodall RNP, Norris KS, Harris G, Oporto JA, Castello HP (1995a) Notes on the biology of the Burmeister's porpoise, *Phocoena spinipinnis*, off southern South America. *Rep. Int. Whaling Comm. (Special Issue)*, **16**, 317–347.

Goodall RNP, Würsig B, Würsig M, Harris G, Norris KS (1995b) Sightings of Burmeister's porpoise, *Phocoena spinipinnis*, off southern South America. *Rep. Int. Whaling Comm. (Special Issue)*, **16**, 297–316.

Goudet J (1995) FSTAT (Version 1.2): a computer program to calculate F-statistics. *J. Heredity* **86**, 485–486.

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3.). Available from http://www.unil.ch/izea/softwares/fstat.html.

Guo SW, Thompson EA (1992) Permorming the exact test for Hardy–Weinberg proportions for multiple alleles. *Biometrics*, **48**, 361–372.

Hardy O, Charbonnel N, Fréville H, Heuertz M (2003) Microsatellite allele sizes: A simple test to assess their significance on genetic differentiation. *Genetics*, **163**, 1467–1482.

Hardy O, Vekemans X (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Mol. Ecol. Notes*, **2**, 618–620.

Hillis DM, Moritz C, Mable BK (1996) *Molecular Systematics*, Sinauer Associates, Inc., Sunderland, MA, 655.

Hoelzel AR, Hancock JM, Dover GA (1991) Evolution of the cetacean mitochondrial D-loop region. *Mol. Biol. Evol.*, **8**, 475–493.

Luikart G, Allendorf FW, Sherwin B, Cornuet J-M (1998) Distortion of allele frequency distributions provides a test for recent population bottlenecks. *J. Heredity*, **12**, 238–247.

Lyrholm T, Leimar O, Johanneson B, Gyllensten U (1999) Sex-biased dispersal in sperm whales: Contrasting mitochondrial and nuclear genetic structure of global populations. *Proc. Roy. Soc., London*, **266**, 347–354.

Michalakis Y, Excoffier L (1996) A generic estimation of population subdivision using distances between alleles with special reference to microsatellite loci. *Genetics*, **142**, 1061–1064.

Nei M (1987) *Molecular Evolutionary Genetics*, Columbia University Press, New York, USA

Nielsen R (1997) A likelihood approach to populations samples of microsatellite alleles *Genetics*, **146**, 711–716.

Petit RJ, El Mousadik A, Pons O (1998) Identifying populations for conservation on the basis of genetic markers. *Conserv. Biol.*, **12**, 844–855.

Pichler FB, Baker CS (2000) Loss of genetic diversity in the endemic Hector's dolphin due to fisheries-related mortality. *Proc. Roy. Soc., London B*, **267**, 97–102.

Pichler FB, Dawson SM, Slooten E, Baker CS (1998) Geographic isolation of Hector's dolphin populations described by mitochondrial DNA sequences. *Conserv. Biol.*, **12**, 676–682.

Piry S, Luikart G, Cornuet J-M (1999) BOTTLENECK: A computer program for detecting recent reductions in the effective population size using allele frequency data. *J. Heredity*, **90**, 502–503.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Read AJ, Waerebeek KVan, Reyes JC, McKinnon JS, Lehman LC (1988) The exploitation of small cetaceans in coastal Peru. *Biol. Conserv.*, **46**, 53–70.

Reyes J, Van Waerebeek K (1995) Aspects of the biology of Burmeister's porpoise from Peru. *Rep. Int. Whaling Comm. (Special Issue)*.

Rice DW (1998) *Marine Mammals of the World Systematics and Distribution*, Allen Press, Inc, Lawrence, KS.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Rosel PE, France SC, Wang JY, Kocher TD (1999a) Genetic structure of harbour porpoise *Phocoena phocoena* populations in the northwest Atlantic based on mitochondrial and nuclear markers. *Mol. Ecol.*, **8**, 41–54.

Rosel PE, France SC, Wangs JY, Kocher TD (1999b) Genetic structure of harbour porpoise *Phocoena phocoena* populations in the northwest Atlantic based on mitochondrial and nuclear markers. *Mol. Ecol.*, **8**, S41–S54.

Rosel PE, Haygood MG, Perrin WF (1995) Phylogenetic relationships among true porpoises. *Mol. Phylogenet. Evol.*, **4**, 463–474.

Schneider S, Roessli D, Excoffier L (2000) *Arlequin ver. 2.000: a software for population genetics data analysis*, Genetics and Biometry Laboratory, University of Geneva, Geneva, Switzerland.

Slooten E, Fletcher D, Taylor BL (2000) Accounting for uncertainty in risk assessment: Case study of Hector's dolphin mortality due to gillnet entanglement. *Conserv. Biol.*, **14**, 1264–1270.

Storz JF, Ramakrishnan U, Alberts SC (2002) Genetic effective size of a wild primate population: Influence of current and historical demography. *Evolution*, **56**, 817–829.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The Clustal X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucl. Acids Res.*, **24**, 4876–4882.

Van Waerebeek K, Alfaro-Shigueto J, Montes D, et al. (2002a) *Fisheries related Mortality of Small cetaceans in neritic Waters of Peru in 1999–2001*, Scientific Committee, IWC, Shimonoseki, Japan10.

Van Waerebeek K, Reyes JC (1990a) Catch of Small Cetaceans at Pucusana Port, Central Peru, during 1987. *Biol. Conserv.*, **51**, 15–22.

Van Waerebeek K, Reyes JC (1990b) Incidental catch and sightings of Burmeister's porpoise in Peru, 1988–1989. IWC Scientific Committee, 13 pp.

Van Waerebeek K, Reyes JC (1994a) Interactions between small cetaceans and Peruvian fisheries in 1988–1989 and analysis of trends. *Rep. Int. Whaling Comm. (Special Issue)*, **15**, 495–502.

Van Waerebeek K, Reyes JC (1994b) Post-ban small cetacean takes off Peru: a review. *Rep. Int. Whaling Comm. (Special Issue)*, **15**, 503–520.

Van Waerebeek K, Santillan L, Reyes JC (2002b) An unusually large aggregation of Burmeister's porpoise *Phocoena spinipinnis* off Peru, with a review of sightings from the eastern South Pacific. *Noticiario Mensual*, **350**, 12–17.

Van Waerebeek K, Van Bressem M-F, Alfaro-Shigueto J, et al. (1999) A preliminary analysis of recent captures of small cetaceans in Peru and Chile. *Paper presented to the 51st Annual Meeting of the International Whaling Commission Scientific Committee*, 9 pp.

Van Waerebeek K, Bressem M-FVan, Felix F, et al. (1997) Mortality of dolphins and porpoises in coastal fisheries off Peru and southern Ecuador in 1994. *Biol. Conserv.*, **81**, 43–49.

Walton MJ (1997) Population structure of harbour porpoises *Phocoena phocoena* in the seas around the UK and adjacent waters *Proc. Roy. Soc., London*, **264**, 89–94.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.