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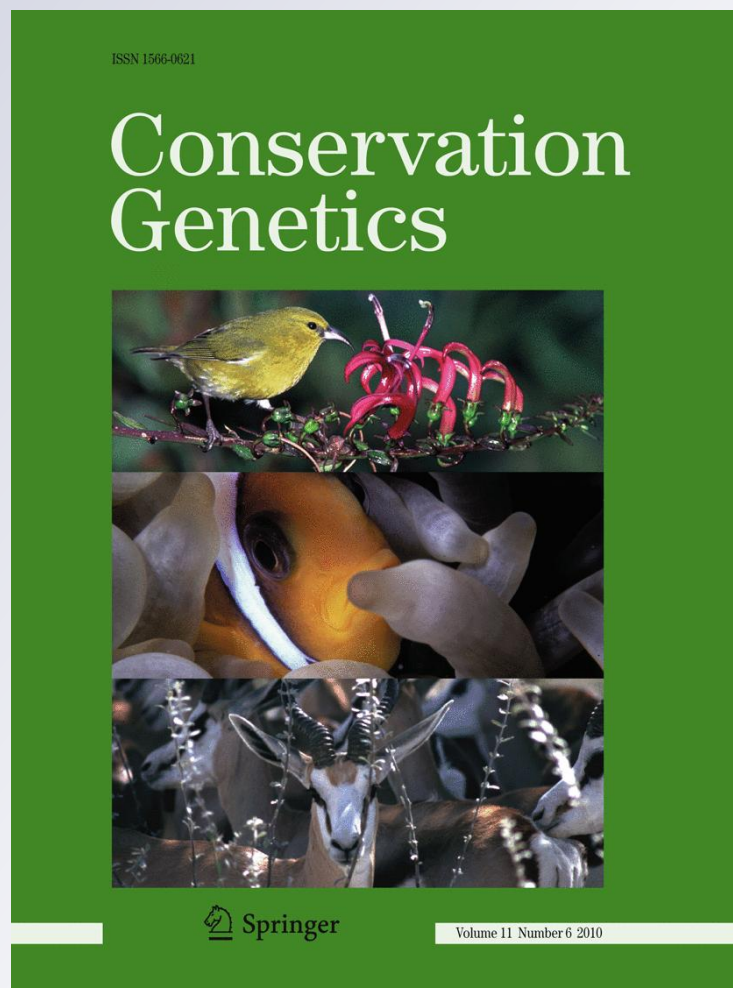
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## Mitochondrial DNA variation and population structure of Commerson's dolphins (*Cephalorhynchus commersonii*) in their southernmost distribution

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**Abstract** The Commerson's dolphin, *Cephalorhynchus commersonii*, is found in shallow waters of the continental shelf off the eastern coast of South America between 40°S and 56°S. This species is taken incidentally in artisanal gillnet fisheries, especially along the shallow coastline of northern Tierra del Fuego and southern Patagonia. The biological importance of by-catch is likely to be

underestimated if the boundaries of subpopulations are not properly defined. Here, we report on the sequence variation of the mitochondrial DNA control region of the Commerson's dolphin from five areas defined in Tierra del Fuego, Argentina and Chile, to provide a preliminary assessment of population structure where conservation efforts are most needed. A 466 bp fragment of the mitochondrial DNA control region was sequenced from 196 samples of skin, teeth and bone, defining 20 haplotypes from 17 polymorphic sites. Nucleotide ( $\pi = 0.40\%$ ) and haplotype ( $h = 0.807$ ) diversity were low compared to some other odontocete populations, but similar to that of other species of this genus. Genetic differentiation evaluated through analyses of molecular variance (AMOVA) showed significant overall differences among areas within Tierra del Fuego ( $\Phi_{ST} = 0.059$ ,  $P < 0.01$ ). An analysis of sex-specific population structure suggested that the dispersal rates of both females and males are low, indicative of females displaying greater site fidelity. The results from mtDNA control region sequences alone revealed significant differentiation among studied areas, which should be considered as independent management units. We recommend that the impact of localized gillnet mortalities should be managed on a local scale in these areas of Tierra del Fuego.

**Electronic supplementary material** The online version of this article (doi:10.1007/s10592-010-0102-z) contains supplementary material, which is available to authorized users.

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**Keywords** Commerson's dolphin · mtDNA variation · Population structure · Conservation genetics

### Introduction

The genus *Cephalorhynchus* includes four species of small dolphins, each endemic to a different region of cold-temperate to subantarctic waters of the Southern Hemisphere (New Zealand, South Africa, Chile, Argentina and

Kerguelen Islands) (Brownell Jr. and Donovan 1988). Of the four, Commerson's dolphin (*Cephalorhynchus commersonii* Lacépède 1804) has the largest area of distribution. Subspecies *C. c. commersonii* is found in shallow waters of the continental shelf along the southeastern coast of South America between about 40°S and 56°S, including the central and eastern Strait of Magellan and waters around the Falkland (Malvinas) Islands (Goodall et al. 1988a; Goodall 1994; White et al. 1999). An isolated population occurring about 8,000 km to the east of the South American populations at the Kerguelen Islands in the Indian Ocean (Robineau 1984) which differs in morphology, pigmentation and genetic data, is considered the subspecies *C. c. kerguelensis* (Robineau et al. 2007).

Across some of its range, Commerson's dolphins have historically been subject to directed exploitations for use as bait in the local crab (*Lithodes antarctica*) fishery, as well as for oil, sport, live capture for exhibition, scientific specimens and even for human consumption (Goodall and Cameron 1980; Goodall et al. 1988a, 1994). Although this directed exploitation no longer occurs, the species is still being caught incidentally in artisanal shore-based gillnet fisheries, especially along the shallow coastline of northern Tierra del Fuego and southern Patagonia, Argentina (Goodall et al. 1994; Iñíguez et al. 2003). It is the most abundant dolphin near shore and the most affected by incidental catch off Tierra del Fuego (Goodall et al. 1994). Other threats to this species could include pollution, habitat degradation in coastal waters, oil drilling and production, and commercial shipping traffic throughout its range. The 2009 IUCN Red List of Threatened Species listed the Commerson's dolphin 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.

All *Cephalorhynchus* species are characterized by relatively low abundance ( $<10^4$ ) and all are subjected to some degree of incidental or directed mortality in fishing nets (Dawson 1991; Goodall et al. 1988a, b, 1994; Pichler et al. 2001; Best 2007). Species of the genus *Cephalorhynchus* also have relatively low reproductive rates (Lande 1991; Slooten 1991), and appear to be depth-limited coastal species with small home ranges (Bräger et al. 2002). Thus the biological impact of by-catch or any other threat is likely to be underestimated if the existence of distinct population units is not properly recognized. This has previously been shown to be the case for Hector's dolphin where relatively high levels of incidental by-catch resulted in the decline of some local populations, due in part to the lack of dispersal between populations (Pichler and Baker 2000). However, the local divisions of Commerson's dolphins are less well described and there is little or no information on the mating and social systems, in relationship to dispersal and gene flow.

Here, we report on the sequence variation of the mitochondrial DNA control region of Commerson's dolphin in Tierra del Fuego, both Argentina and Chile. These regions form the southerly extreme of the Commerson's range. Other main concentrations along the Argentine coast include: Rawson to Camarones, Comodoro Rivadavia, Puerto Deseado, San Julián, Puerto Santa Cruz, and Rio Gallegos (Fig. 1). Our objectives were to provide a preliminary assessment of the genetic structure of local populations and to identify geographic areas where conservation efforts are most needed.

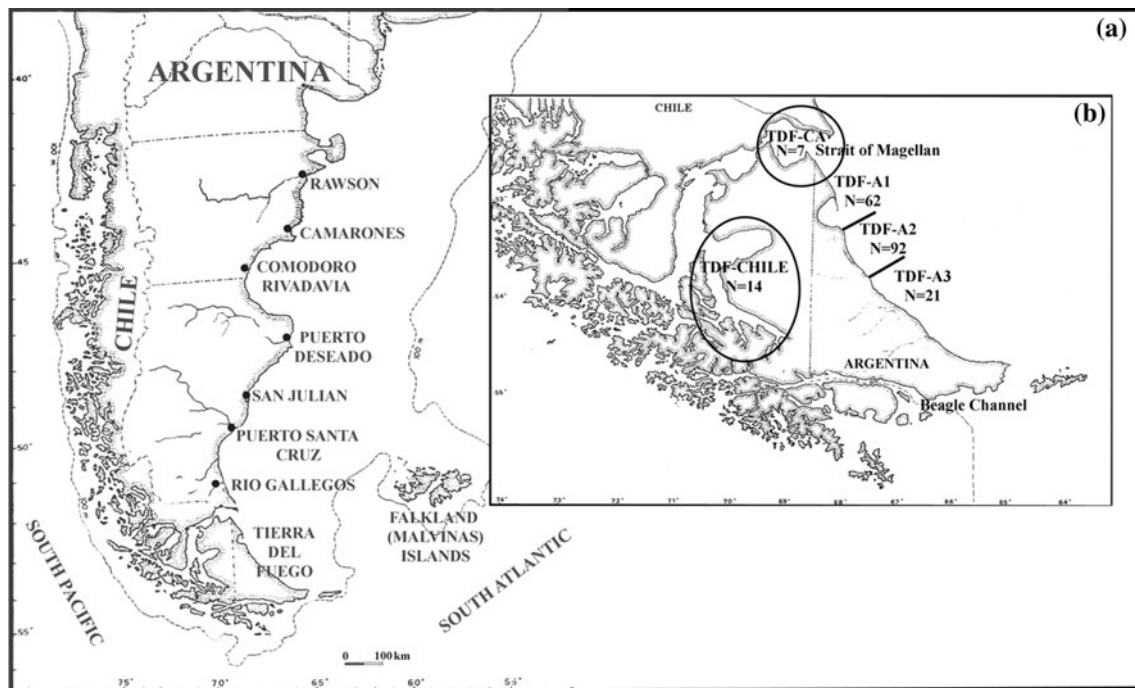
## Materials and methods

### Samples

A total of 312 samples of Commerson's dolphins from Argentine and Chilean Tierra del Fuego were collected from dry skin ( $n = 87$ ), teeth ( $n = 178$ ) and bones ( $n = 34$ ) of different specimens provided by the Goodall collection (RNP) at the Museo Acatushún de Aves y Mamíferos Marinos Australes at Estancia Harberton, Ushuaia, Tierra del Fuego. These were obtained from the extensive by-catch and occasional beachcast specimens collected opportunistically along the coasts of Tierra del Fuego, both Argentina and Chile, during the years 1974–2008 (Goodall 1978; Goodall et al. 1994). A small number of skin biopsy samples ( $n = 13$ ) from Tierra del Fuego, Chile, were collected with a lightweight biopsy dart fired from a modified veterinary rifle, Paxarm (Krützen et al. 2002).

The Archipelago of Tierra del Fuego (54°S, 70°W) is made up of one large island, along with many medium and smaller islands. The Strait of Magellan separates southern Patagonia (the southern South American mainland) from Tierra del Fuego. The Isla Grande of Tierra del Fuego is divided politically by Chile and Argentina. For our analyses, five coastal areas were defined along the coast of the Isla Grande de Tierra del Fuego (TDF): one on the west coast called TDF-Chile, one along the northeast coast called TDF-Chile-Argentina (TDF-CA), and three on the east coast of TDF-Argentina: TDF-A1, TDF-A2 and TDF-A3 (Fig. 1). These areas were chosen according to ecological and topographical characteristics of the coast following Goodall et al. (1994). The Atlantic Tierra del Fuego coast has an extension of 330 km with a northwest-southeast orientation and a macrotidal regime. TDF-CA has a maximum depth of 70 m, a high tidal range and strong currents. TDF-A1 and TDF-A2 coasts have sand or clay beaches, with mud flats, bays and river mouths with gently sloping shores. The southeastern area (TDF-A3) has rocky irregular coasts, gravel beaches in bays and high





**Fig. 1** **a** Map showing known distribution of Commerson's dolphins along the coast of South America, with locations referred to in text. **b** Five areas defined in Tierra del Fuego, Argentina, and Tierra del Fuego, Chile for Commerson's dolphin samples

cliffs (Bujalesky 2002). The area in TDF-Chile is characterized by a wide bay, inland passages and a large fiord influenced by subantarctic adjacent waters, precipitation, continental run-off and melting ice (Antezana 1999). The choice of area divisions was further verified by implementing the program Barrier v2.2 (Manni et al. 2004). This program uses a computational geometry algorithm for identifying genetic discontinuities within a geographical coordinate system, i.e. for finding locations where genetic distances show an abrupt change (Manni and Guérard 2004).

#### DNA extraction

Total cellular DNA was isolated from dry skin and biopsy samples by digestion with Proteinase K followed by a standard phenol:chloroform extraction method (Sambrook et al. 1989) as modified for small samples by Baker et al. (1994). Total DNA was extracted from small fragments of bone and single teeth following Pimper et al. (2009).

#### Mitochondrial DNA sequencing, haplotype and sex identification

Amplification of the mtDNA control region used a series of nested primers to amplify fragments of different length depending on quality of DNA. The first attempt was to amplify an 800-bp fragment bounded by the primers M13dlp1.5 (5'-TGTA AACGACAGCCAGTTCACCCAAAGCTGRARTT

CTA-3'; Dalebout et al. 1998) and dlp8G (5'-GGAGTAC TATGTCCTGTAACCA-3'; Lento et al. 1998). We then tried 550 bp with M13dlp1.5-dlp5 (5'-CCATCGWGATGT CTTATTTAAGRGGAA-3'; Dalebout et al. 1998), 400 bp with M13dlp1.5-dlp4 (5'-GCGGGWTRYTGRTTTCAC G-3'; Baker et al. 1998) and finally a 206-bp fragment with dlpFBP (5'-GTACATGCTATGTATTATTGTGC-3'; Pichler and Baker 2000) and dlp4.

Amplification reactions of dry skin and biopsy samples were carried out in a total volume of 10  $\mu$ l with 1 $\times$  PCR buffer, 2.5 mM MgCl<sub>2</sub>, 0.4  $\mu$ M of each primer, 0.2 mM deoxynucleoside triphosphate (dNTP) and 0.125 U of Platinum<sup>®</sup> Taq DNA Polymerase (Invitrogen Life Technologies). For teeth and bone samples, reactions were performed in a total volume of 50  $\mu$ l with 1 $\times$  PCR buffer, 5 mM MgCl<sub>2</sub>, 0.4  $\mu$ M of each primer, 0.2 mM deoxynucleoside triphosphate (dNTP), 0.5 U of Platinum<sup>®</sup> Taq DNA Polymerase (Invitrogen Life Technologies) and 10 mg/mL bovine serum albumin (BSA) that was added to overcome inhibition of polymerase chain reaction (PCR). The PCR temperature profile was as follows: a preliminary denaturing period of 3 min at 94°C followed by 30 cycles of denaturation for 30 s at 94°C, primer annealing for 45 s at 55°C and polymerase extension for 60 s at 72°C. Amplicons were quantified by 1.6% agarose gel electrophoresis staining in ethidium bromide and UV visualization with DNA low-mass ladder. PCR products were purified for sequencing with SAPEX (Amersham), cleaned

with Agencourt® CleanSEQ® Kit (Beckman Coulter) and sequenced with BigDye™ terminator chemistry v.3.1 (Applied Biosystems, Inc.) on an ABI Prism™ Sequencer 3130×1 Genetic Analyzer (Applied Biosystems, Inc.) from the service of the Centre for Genomics & Proteomics, University of Auckland, School of Biological Sciences.

The sex of specimens was identified in the field from genital examination, external colour patterns (when possible) and/or the shape of pelvic bones, and in the laboratory via molecular sexing. The protocol consisted of a multiplex amplification of a fragment of the male-specific sry gene (ca. 240 bp) and fragments of the ZFY/ZFX genes (ca. 450 bp) as positive control (Gilson et al. 1998). Male and female positive controls, and a negative PCR control were added to the reaction.

### Data analysis

Sequences were aligned and edited using Sequencher 4.6 (Gene Codes Corporation), BioEdit 7.0.5.3 (Hall 1999) and ClustalX v.1.8 (Higgins and Sharp 1988). ClustalX analysis was performed with the default parameters, namely an opening gap penalty = 10 and gap extension penalty = 0.2. Variable sites and unique haplotypes were identified using MacClade version 4.0 (Maddison and Maddison 1992), Microsoft Excel and GenAlEx6.1 (Peakall and Smouse 2006). Haplotypes were confirmed by re-sequencing of different individuals, by quality control scores (ABI equivalent to PHRED > 30) and visual inspection in Sequencher 4.6 (Gene Codes Corporation). Sequences with PHRED scores less than 30 were repeated.

Arlequin v3.11 (Excoffier et al. 2005) was used for computing estimates of nucleotide diversity ( $\pi$ ) and haplotype diversity ( $h$ ) according to Nei (1987), and to test neutrality with Tajima's  $D$  (Tajima 1989) and Fu's  $F_s$  (Fu 1997). The significance of these tests of neutrality was assessed using 1,000 iterations. Analysis of molecular variance (AMOVA) (Excoffier et al. 1992) based on  $F_{ST}$  (using haplotypes frequencies) and  $\Phi_{ST}$  (using genetic distances with Kimura algorithm) were conducted to investigate differentiation among areas and sexes. The statistical significance of these values was tested by 5,000 permutations of the data.

To test for a bias in dispersal by males or females, mtDNA was analyzed by coding individuals as homozygotes according to Oremus et al. (2007), using the 'biased dispersal' option implemented in FSTAT (Goudet 2001). This approach is a traditional analysis of population differentiation and therefore the  $F_{ST}$  value for the philopatric sex is expected to be higher than that of the dispersing sex (Prugnolle and de Meeus 2002). We note, however, that given the maternal inheritance of mtDNA, this test is indicative only of male-biased dispersal, not

male-mediated gene flow. To test for significance we used a randomization procedure with 10,000 permutations.

Asymmetric female migration rates between pairwise local populations were estimated using a Markov Chain Monte Carlo (MCMC) coalescent genealogy as implemented in the software Lamarc v2.1.3 (Kuhner 2006). Bayesian analysis was employed using F84 mutation model, 5 replicates per run over 5 different runs (with different random number seed), implementing one initial and final chain, 125000 steps with an initial burn-in of 5000 steps and 5 heating temperatures (1, 1.1, 1.2, 1.3, and 1.4) for each run. Convergence was assessed by using the program TRACER v1.5 (Rambaut and Drummond 2009) by computing the effective sample size (ESS). Rambaut and Drummond suggest an ESS of 100 or 200 implies convergence has been attained. Our runs consistently achieved ESS values in the thousands or better. We therefore conclude that we ran LAMARC long enough to obtain stable, reproducible, reliable results.

The phylogenetic relationship of individual haplotypes was reconstructed by a maximum parsimony tree (heuristic search with tree bisection reconnection branch swapping, 1000 replicates, stepwise addition starting tree and a random sequence addition) with the computer program PAUP (Swofford 1998). This reconstruction also included the haplotypes Ccom-K and Ccom-L detected previously in Kerguelen Island animals (GenBank Accession numbers AF393541 and AF393542) (Pichler et al. 2001). As outgroups, we included sequences from *Cephalorhynchus eutropia* (Chilean dolphin), *C. heavisidii* (Heaviside's dolphin) and *C. hectori* (Hector's dolphin) (GenBank Accession numbers EU121123, AF390997, AF057997, respectively) (Caballero et al. 2008; Jansen van Vuuren et al. 2002; Pichler et al. 1998). To take into account the potential coexistence of both ancestral and derived haplotypes in the population sample (Posada and Crandall 2001), we also reconstructed phylogenetic relationships among haplotypes using a median-joining network using the program Network v.4.5.0.0 (Bandelt et al. 1999).

## Results

### Genetic diversity and haplotype identity

DNA was extracted successfully from a total of 275 samples. Sex-specific markers were amplified successfully from 96% of these samples (177 males/88 females), giving a ratio of 2:1, males to females ( $\chi^2 = 29.9$ ;  $P < 10^{-4}$ , for departure from 1:1). The fragment of the 5' end of the mtDNA control region was successfully amplified from 273 samples. The total length of sequence obtained varied among samples ranging from ca. 150 to 650 bp, with

sequences from bone and teeth samples being the shortest ones. For the analyses presented here, 466 bp sequence was chosen because it was successfully amplified in 196 samples and resolved most [or all] of the haplotypes. From this fragment, a total of 20 haplotypes defined by 17 variable sites were detected (Table 1). Haplotypes differed by 1–7 bp with a mean of 3.2 bp. All variable sites were transition substitutions. No heteroplasmy, either in the nucleotide sequence or in the length of the amplified fragments was detected. Five of the 20 haplotypes had been identified previously by Pichler et al. (2001) and the 15 new haplotypes were submitted to GenBank (Accession numbers HM368538–HM368552). One (*C.com-M*) of the three haplotypes found previously by Pichler et al. (2001) only from the Kerguelen Islands was represented by a single individual in the larger sample from South America presented here (Figs. 2, 3). A small number of sequence errors were detected in the previous haplotypes of Pichler et al. (2001). The original electropherograms of these were reviewed and the submitted sequences have now been corrected (Supplementary Material). In total, 22 haplotypes have now been described in Commerson's dolphins, 2 of which have been found only in those from the Kerguelen Island. For the overall South American sample, the most common haplotype (*C.com-A*) was shared by 31.1% of the samples, with a second common haplotype (*C.com-C*) shared by 22.4%.

The overall haplotype diversity ( $h$ ) (0.807) and the nucleotide diversity ( $\pi$ ) (0.40) demonstrated moderate levels of genetic variability. The  $h$  values by area ranged from 0.476 to 0.805, and  $\pi$  values ranged from 0.20 to 0.41% (Table 2). TDF-A1, TDF-A2 and TDF-A3 showed 11, 16 and 6 lineages respectively and relative high levels of genetic diversity, while TDF-CA displayed 2 lineages and low values of genetic diversity (Fig. 2 and Table 2).

#### Phylogenetic relationships

The phylogenetic reconstruction of the 22 known mtDNA haplotypes of Commerson's dolphins resulted in a single most parsimonious tree 68 steps long, consistency index (CI) excluding uninformative characters = 0.6071 and retention index (RI) = 0.71 (Fig. 3). The differences among sampled areas of South America were mainly related to differences in haplotype frequencies and the distribution of private haplotypes (Fig. 3). Some clades show a certain concordance with the geographical origin of the samples. One clade (haplotypes *C.com-K-L-M*) with 64% bootstrap support clustered haplotypes found in Kerguelen Island animals and was distinguished from the other haplotypes by three nucleotide substitutions. Another clade (haplotypes *C.com-G-D-E*) with 60% bootstrap support was found mainly in TDF-Argentina.

The network showed two common haplotypes (*C.com-A* and *C.com-C*) from which emerged a very frequent haplotype (*C.com-D*) and several closely related haplotypes comprising six private haplotypes from TDF-A2, three singly occurring haplotypes unique to TDF-A1 and one singly occurring haplotype unique to TDF-Chile (Fig. 2).

#### Population differentiation

The results from the landscape genetics analyses provide support for a partitioning of the samples into distinct groups based on mtDNA data. Four areas were identified: TDF-Chile, TDF-CA, TDF-A1, TDF-A2 + TDF-A3. The overall AMOVA showed significant differentiation among the five areas from Tierra del Fuego, for both  $\Phi_{ST}$  and  $F_{ST}$  (Table 3). Pairwise comparisons of  $F_{ST}$ , after sequential Bonferroni correction, revealed that genetic differentiation was driven largely by differentiation within TDF-Argentina and between TDF-Chile and TDF-A1 areas (Table 4). Pairwise comparisons of  $\Phi_{ST}$ , after sequential Bonferroni correction, revealed significant genetic differentiation only between TDF-A1 and TDF-A3. The AMOVA using only Argentine data rendered significant genetic differentiation among locations ( $\Phi_{ST} = 0.055$ ,  $P < 0.01$ ,  $F_{ST} = 0.072$ ,  $P < 10^{-4}$ ). The relative high sample size of TDF-A1 and TDF-A2 supports the detected genetic differentiation between these studied areas. Genetic differentiation between the remained areas studied should be considered with some caution because of limited sample size.

The results of AMOVA revealed significant  $F_{ST}$  and  $\Phi_{ST}$  values for females and males, rejecting the null hypothesis of panmixia for both sexes (Table 3). However, the comparison of  $F_{ST}$  values between males and females showed significantly greater population differentiation for females ( $P < 0.05$ ), suggesting greater male dispersal.

Values for Tajima's  $D$  were not statistically significant, whereas Fu's  $F_s$  values were negative and statistically significant for the data sets of TDF-Chile and TDF-A2 ( $-2.877$ ,  $P = 0.013$ ,  $-6.255$  and  $P = 0.013$ , respectively). The negative values suggest probable population expansion.

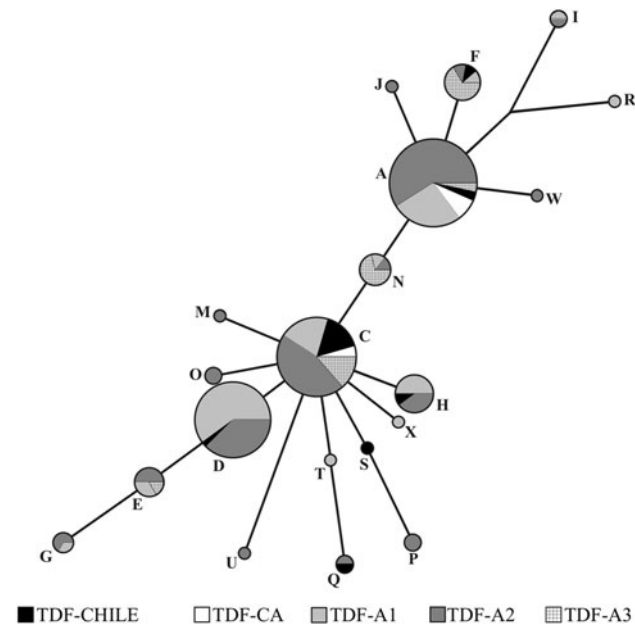
Estimates of effective female  $N_e m$  per generation based on coalescence methods are shown in Table 5. For the analysis TDF-CA was grouped with TDF-A1 due to the small sample size of the first one. The estimated values are similar to those obtained by estimates derived from  $F_{ST}$ , although some differences were found in values between TDF-A3 and TDF-Chile (Table 5). While CIs overlapped in all pairwise comparisons, some general tendencies were evident: (i) relatively high rates of female migration between TDF-CA + TDF-A1 and TDF-A2; (ii) relatively low female migration rates between most distant areas within TDF-Argentina, TDF-CA + TDF-A1 and TDF-A3;

**Table 1** Positions of the 17 variable sites within the 466 bp consensus fragment of the mtDNA control region that define the 20 haplotypes in Commerson's dolphin from Tierra del Fuego in Argentina and Chile

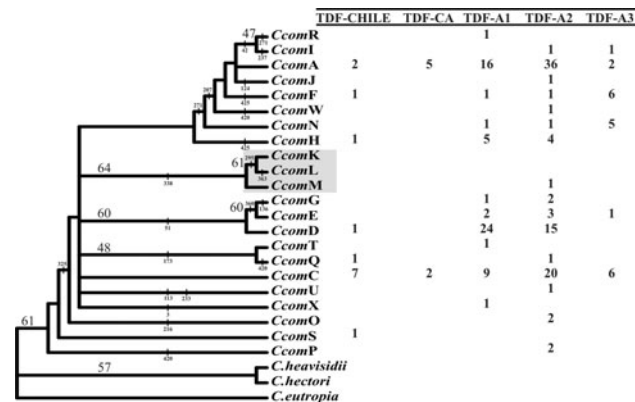
	3	42	51	113	124	136	173	207	216	233	237	271	325	338	360	420	425	GenBank Accession number
<i>C.com-A</i>	C	G	T	C	G	T	C	G	A	A	C	G	C	T	T	C	T	AF393536
<i>C.com-B</i>	.	.	.	.	A	.	.	A	.	.	.	A	.	.	.	.	.	AF393538
<i>C.com-D</i>	.	.	C	.	A	.	.	A	.	.	.	A	.	.	.	.	.	AF393539
<i>C.com-E</i>	.	.	C	.	A	.	.	A	.	.	.	A	.	.	C	.	.	AF393540
<i>C.com-F</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	HM368538
<i>C.com-H</i>	.	.	.	.	A	.	.	A	.	.	.	A	.	.	.	.	C	HM368540
<i>C.com-I</i>	.	A	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	HM368541
<i>C.com-J</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	HM368542
<i>C.com-O</i>	.	.	.	.	A	.	.	A	G	.	.	A	.	.	.	.	.	HM368544
<i>C.com-T</i>	.	.	.	.	.	.	.	A	.	.	.	A	.	.	.	.	.	HM368549
<i>C.com-X</i>	T	.	.	.	.	.	T	A	.	.	.	A	.	.	.	.	.	HM368552
<i>C.com-N</i>	.	.	.	.	.	.	.	A	.	.	.	A	.	.	.	.	.	HM368543
<i>C.com-W</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	HM368551
<i>C.com-R</i>	.	A	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	HM368547
<i>C.com-M</i>	.	.	.	.	.	.	.	A	.	.	.	A	.	C	.	.	.	AF393543
<i>C.com-U</i>	.	.	.	T	.	.	.	A	.	G	.	A	.	.	.	.	.	HM368550
<i>C.com-P</i>	.	.	.	.	.	.	.	A	.	.	.	A	T	.	.	T	.	HM368545
<i>C.com-Q</i>	.	.	.	.	.	.	T	A	.	.	.	A	.	.	.	T	.	HM368546
<i>C.com-G</i>	.	.	C	.	.	C	.	A	.	.	.	A	.	.	C	.	.	HM368539
<i>C.com-S</i>	.	.	.	.	.	.	.	A	.	.	.	A	T	.	.	.	.	HM368548

Dots indicate match to reference haplotype, *C.com-A*





**Fig. 2** Inferred genealogical relationship among the 20 mtDNA haplotypes in Commerson's dolphins 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



**Fig. 3** Phylogenetic relationships among the 20 haplotypes defined in Commerson's dolphins by the 466 bp fragment of the mtDNA control region based on Maximum Parsimony analysis. Branch termini represent haplotypes. Haplotype frequencies by area are shown on the right. *Cephalorhynchus heavisidii*, *C. hectori* and *C. eutropia* were used as outgroups. The gray box indicates the Kerguelen Islands haplotypes (Pichler et al. 2001)

(iii) some asymmetries in female migration rates were evident from northern to southern areas. We also used IM Program to generate probability distributions for ongoing gene flow between populations. Unfortunately, unimodal posterior distributions of parameter estimates could not be achieved.

**Table 2** Comparative haplotype (*h*) and percent nucleotide diversity ( $\pi$ ) among Commerson's dolphins in the five areas studied around Tierra del Fuego in Argentina and Chile

Location	<i>n</i>	<i>h</i> ( $\pm$ SD)	% $\pi$ ( $\pm$ SD)	Sex		
				M	F	?
TDF-Chile	14	0.758 $\pm$ 0.116	0.335 $\pm$ 0.234	8	6	0
TDF-CA	7	0.476 $\pm$ 0.171	0.204 $\pm$ 0.180	5	2	0
TDF-A1	62	0.766 $\pm$ 0.035	0.373 $\pm$ 0.244	40	20	2
TDF-A2	92	0.776 $\pm$ 0.031	0.411 $\pm$ 0.261	58	32	2
TDF-A3	21	0.805 $\pm$ 0.045	0.384 $\pm$ 0.258	12	8	1
Total	196	0.807 $\pm$ 0.016	0.40 $\pm$ 0.25	123	68	5

Sex information is included for comparison (? = sex unknown)

**Table 3**  $F_{ST}$  and  $\Phi_{ST}$  values for males (M) and females (F), and corresponding *P*-values calculated from 10,000 random permutation tests, for five areas around Tierra del Fuego in Argentina and Chile

	<i>n</i>	$F_{ST}$	$\Phi_{ST}$
F + M + ?	196	0.076 ( $P < 10^{-4}$ )	0.059 ( $P = 0.0004$ )
F	68	0.154 ( $P = 0.002$ )	0.091 ( $P = 0.0025$ )
M	123	0.049 ( $P = 0.006$ )	0.050 ( $P = 0.026$ )

? = sex unknown

**Table 4** Genetic differentiation among areas based on pairwise  $F_{ST}$  (values shown in the upper matrix) and  $\Phi_{ST}$  (values in the lower matrix) in Commerson's dolphin from Tierra del Fuego in Argentina and Chile

	TDF-Chile	TDF-CA	TDF-A1	TDF-A2	TDF-A3
TDF-Chile		0.160	<b>0.109*</b>	0.062	0.050
TDF-CA	0.224		0.154	0.016	0.210
TDF-A1	0.049	0.205		<b>0.035*</b>	<b>0.152**</b>
TDF-A2	0.034	0.034	0.038		<b>0.116**</b>
TDF-A3	0.102	0.013	<b>0.145*</b>	0.042	

Statistically significant results calculated from 5,000 random permutations tests are shown in bold type

\*  $P < 0.05$ ; \*\*  $P < 0.01$

## Discussion

### Mitochondrial DNA diversity and phylogenetic haplotype relationships

The populations of Commerson's dolphin in the southernmost part of their South Atlantic distribution show substantially lower levels of mtDNA diversity than those reported in most odontocete populations, but similar to that of other species of this genus (Pichler and Baker 2000). Four out of five analyzed areas exhibited similar estimates of haplotype diversities. The analysis of haplotype and nucleotide diversities demonstrated that TDF-CA exhibited

**Table 5** Most probable estimates of migrants per generation ( $N_e m_{ij}$ ) for Commerson's dolphin using Bayesian analysis between four local areas around Tierra del Fuego in Argentina and Chile

	$N_e m_{12}$	$N_e m_{21}$	95% CI	$F_{ST}$	$Nm$
(1) TDF-CA + TDF-A1	10.53		4.66–13.45	0.018	27.28
(2) TDF-A2		7.02	4.21–7.26		
	$N_e m_{13}$	$N_e m_{31}$			
(1) TDF-CA + TDF-A1	1.72		0.000072–3.50	0.146	2.92
(3) TDF-A3		1.069	0.00012–3.66		
	$N_e m_{23}$	$N_e m_{32}$			
(2) TDF-A2	3.29		0.39–3.69	0.116	3.81
(3) TDF-A3		3.11	0.39–6.97		
	$N_e m_{14}$	$N_e m_{41}$			
(1) TDF-CA + TDF-A1	4.05		0.0017–5.57	0.099	4.55
(4) TDF-Chile		1.68	0.0013–3.31		
	$N_e m_{24}$	$N_e m_{42}$			
(2) TDF-A2	5.29		1.09–5.75	0.062	7.56
(4) TDF-Chile		3.08	0.61–6.31		
	$N_e m_{34}$	$N_e m_{43}$			
(3) TDF-A3	1.41		0.00012–4.39	0.050	9.50
(4) TDF-Chile		1.12	0.000045–1.96		

$Nm$  column refer to the values estimated according  $F_{ST} = 1/(2N_e m + 1)$ . For the analysis TDF-CA was grouped with TDF-A1  
*CI* confidence interval

lowest values of genetic diversity. However, this result was obtained on a small sample and should be considered preliminary. The analysis of 175 individuals from TDF-Argentina revealed 13 haplotypes (out of 20 for all sampled areas) unique in the studied area and suggested genetic differentiation among different main geographic areas (TDF-Chile and TDF-Argentina).

Except for some clades, which show a certain concordance with the geographical origin of the samples, the phylogenetic tree does not exhibit an evident geographical structure. The haplotype network clearly indicated that *C.com-A* and *C.com-C* haplotypes might be proposed as ancestral haplotypes by their internal position in the network, by the number of lineages that arise from them, and by their frequencies. The network showed a rather star-like shape suggesting a relatively recent expansion in size. TDF-Chile and TDF-A2 showed negative significant values of Fu's  $F_s$  suggesting an excess of low frequency haplotypes arising from population growth (Fu 1997; Ramos-Onsins and Rozas 2002). These results might be explained by the recolonization of southern waters (Pichler et al. 2001) after the definitive ice retreat, 10 Ka ago (Rabassa et al. 2000). During such an event TDF-A2 and TDF-Chile would have undergone population expansion.

Within the South American coastal sample, *C.com-M* (unique to TDF-A2) was the only haplotype shared between the Kerguelen Island and the South American samples. Haplotype *C.com-M*, previously thought to be restricted to the Kerguelen subspecies (Pichler et al. 2001; Robineau et al. 2007), was detected in only one female of the South American subspecies, found dead on the beach due to by-catch. This single specimen could be explained

by a 'return' dispersal from Kerguelen Islands or from other unsampled regions where the haplotype is shared, including the Falkland (Malvinas) Islands. Alternatively, the haplotype could be an ancestral lineage that has been retained in the South American subspecies following the colonization of the Kerguelen Islands (Pichler et al. 2001).

#### Geographic differentiation within Tierra del Fuego

We detected significant population structuring over relatively small geographical distances. Overall analyses based on  $F_{ST}$  and  $\Phi_{ST}$  values indicated significant partitioning of genetic variability among the five areas. No obvious geographic boundaries separate these regional populations in TDF-Argentina. Instead, as Dawson and Slooten (1993) suggest for the Hector's dolphin, based on behavioural observations and movements of individuals, isolation among local populations could be a result of ecological preference and strong philopatry. More striking differences across short distances have been found in the Hector's dolphin (Pichler et al. 1998). Currently, there are no available estimates of home range or movements along-shore for Commerson's dolphin in the region, although isotope studies have shown that these animals feed over the continental shelf and occasionally the slope, moving offshore for this reason about 180 km (Ricciardelli et al. 2008). For other members of the genus *Cephalorhynchus* ranges have been estimated. Hector's dolphins at Banks Peninsula in New Zealand show high site fidelity and have typical alongshore ranges of less than 60 km, with a maximum distance of 106 km (Bräger et al. 2002; Rayment et al. 2009). A study at Isla Chiloé, Chile, found that Chilean dolphins also exhibit a

high degree of site fidelity between years, with a maximum displacement distance of 45 km (Heinrich 2006). Heavyside's dolphin off South Africa moved on average 60 km alongshore and up to 22 km offshore over a two-month period of study (Elwen et al. 2006).

Pairwise comparisons based on haplotype frequencies ( $F_{ST}$ ) exhibited significant heterogeneity in TDF-Argentina. They showed that there are significant genetic differences among the three TDF-Argentina neighbour areas (TDF-A1, TDF-A2 and TDF-A3). Short-range population fragmentation may be due to natal fidelity (Pichler and Baker 2000) resulting in population differentiation even along a contiguous coastline. The absence of genetic differentiation in any pairwise comparisons between the TDF-CA area and the others may be explained by a small sample size. TDF-Chile showed significant genetic differentiation with respect to TDF-A1 but exhibited genetic homogeneity when compared with the two areas located at the south edge of TDF-Argentina (TDF-A2 and TDF-A3).

The magnitudes of estimates of gene flow based on coalescence are similar to those obtained by estimates derived from conventional  $F_{ST}$ . The estimates of gene flow between local areas were low, on the order of one to ten individuals per generation with the exception of the two neighbour areas of TDF-A1 and TDF-A2. These estimates provide only relative information about female movement because the methods rely on some assumptions related to population history and migration rates.

Taking into account the absence of genetic differentiation and the relative high gene flow based on  $F_{ST}$  between TDF-A3 and TDF-Chile, one is tempted to propose a southern connection between Chilean and southern Argentine areas. However, in relation to the low level of gene flow detected through coalescence methods, this hypothesis should be considered with some caution. Further studies with higher population sizes as well as other molecular markers can provide additional information to analyse this hypothesis. Mitochondrial DNA data also suggested some directional bias in gene flow indicating movements of females towards southern areas, perhaps following prey movements and/or climatic change.

During these last years, change in range distribution of marine mammals due to global warming have been documented and analyzed with theoretical and empirical studies (Harlin-Cognato et al. 2007; Azzellino et al. 2008; Kovacs and Lydersen 2008). It has been proposed that global warming can carry changes in marine isotherms and consequently expansions or contractions in the species distribution (Clarke et al. 2007; MacLeod 2009; Salvadeo et al. 2010). In response to these increases in water temperature, cold-temperate water species such as *Cephalorhynchus* may change their range by shifting poleward or contracting based on their current distribution (MacLeod 2009).

### Signals of greater female philopatry

A bias toward males was found among all the samples. This was also reported in Hector's dolphins with beachcast and by-catch samples (Pichler 2001). Also more young, immature males are captured in nets (Goodall et al. 2008). One possible explanation would be that male dolphins might rove between groups to encounter receptive females, with young males being less experienced and eager to explore, and are thus more likely to encounter nets and become entangled (Slooten et al. 1993).

In mammals, females are usually philopatric while males tend to disperse (Greenwood 1980). In cetaceans, some molecular studies support this hypothesis (Escorza-Trevino and Dizon 2000; Möller and Beheregaray 2004), while behavioural and/or genetic evidence for other odontocetes indicate that both females and males remain in their natal group (Amos et al. 1993). Also, cetaceans may be more prone to engage in temporary dispersal, so genetic differentiation may reflect philopatry and gene flow. The sex-biased population structure analysis suggests that the dispersal rates of females and males are low, but greater in males. Analysis of larger sample sizes and male-specific or biparentally inherited markers will be needed to confirm this apparent lack of strong male biased dispersal in Commerson's dolphin.

### Management implications

The distribution of Commerson's dolphins is likely to be closely related to the distribution of their prey, near river mouths and shallow coasts (Coscarella 2005). Coastal fronts affect the density and abundance of food resources and determine the distribution pattern of the species. Reports of incidental capture of Commerson's dolphins in gillnets, trammel nets and mid-water trawls (Crespo et al. 1997) in Argentina give us information about the distribution and relative abundance of this species by region (Goodall and Cameron 1980; Goodall et al. 1988c, 1994). On the east coast of TDF-Argentina (TDF-A1–A2–A3) incidental take of small cetaceans using artisanal shore-based nets were documented each year (Goodall et al. 1994). In TDF-A1 and TDF-A2 the incidental captures were homogenous in space across each area. The coast in TDF-A3 has more cliffs and rocky shores than the other areas (TDF-A1, TDF-A2) (Bujalesky 2002), so the incidental capture was mainly located near river mouths.

The apparently high levels of inshore gillnetting by artisanal fishermen during austral summer (November to March) overlapped in time and space with reported Commerson's dolphin inshore movement (Leatherwood et al. 1988), following the inshore movement of young fish, Patagonian blenny or *robalo* (*Eleginops maclovinus*) and

smelt or *pejerrey* (*Austrotherina nigrans*) (Goodall et al. 1994). Incidental catches may be frequent enough to reduce or eliminate a local population, as is the case of the harbour porpoise (*Phocoena phocoena*) in several areas, including the Gulf of Maine off the US northeast coast, the English Channel and the Baltic Sea (Read 1996; Hutchinson 1996; Northridge 2002).

There is no current official information on the level of fishing effort and the occurrence and extent of by-catch in artisanal inshore fisheries in Tierra del Fuego. The first census for artisanal fishermen took place in Argentina during the first semester of this year promoted by the *Unión Argentina de Pescadores Artesanales* (UAPA). In 2007, the *Cámara Fueguina de Pescadores Artesanales* was founded in Tierra del Fuego. The recent creation of such organizations and the future availability of new statistics about this activity will be an improvement towards effective mitigation management measures.

In the present analysis of mtDNA we provide new insights into the genetic structure of *C. commersonii* populations using a large collection of samples of skin, teeth and bones. Our results over a small area suggest low dispersal rate in both sexes, although greater female philopatry was evident. This generates significant heterogeneity among areas of Commerson's dolphin, as is seen in the other species of this genus. Thus, the areas analyzed here represent important components in the evolutionary legacy of this species and should be considered as independent management units. We recommend that the impact of localized gillnet mortalities should be managed on a local scale in these areas of Tierra del Fuego. There is a need to continue and increase our systematic surveys of cetacean by-catch levels in gillnets all along the coast of Tierra del Fuego and to work with the organizations mentioned above in seeking alternative fishing techniques that replace passive nets.

Although the analysis of mtDNA variation and distribution was highly informative for examining female gene flow over a small geographic area, other lines of evidence (microsatellite loci, SNPs or morphometric/morphological traits) are needed to improve our knowledge of evolutionary significant units for Commerson's dolphins.

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