Population differentiation in the franciscana (*Pontoporia blainvillei*) from two geographic locations in Brazil as determined from mitochondrial DNA control region sequences

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Abstract: The franciscana, *Pontoporia blainvillei*, is a dolphin that experiences extensive incidental mortality in fisheries throughout its restricted distribution and is perhaps the most exploited species along the Atlantic coast of South America. However, the basic information required for effective conservation of this species is lacking. To understand the population structure of this platanistoid dolphin, we sequenced 418 base pairs (bp) of the control region and 68 bp of the adjacent pro-tRNA gene of the mtDNA from 20 franciscana that were captured incidentally by gill-net fisheries of Rio Grande do Sul and Rio de Janeiro, Brazil. Of 11 haplotypes found, 5 were exclusive to franciscana from Rio Grande do Sul and 6 were found only in franciscana from Rio de Janeiro and no haplotypes were shared between locations. Reconstruction of the phylogenetic relationships among the haplotypes through a maximum-likelihood analysis of sequences revealed two distinct lineages that were consistent with the geographic sampling locations. Analysis of molecular variance also showed the population structure ($\phi_{ST} = 0.403$, $p < 0.0001$). Furthermore, the estimate of nucleotide diversity for the northern population (0.38 ± 0.13%) was significantly lower than for the southern population (1.01 ± 0.30%). The genetic evidence indicated that at least two populations of franciscana exist.

Résumé : Le Dauphin de la Plata, *Pontoporia blainvillei*, est un animal fréquemment victime de blessures mortelles encourues lors d'opérations de pêche dans sa répartition restreinte et il représente peut-être l'espèce la plus exploitée le long de la côte atlantique sud-américaine. Cependant, l'information de base qui serait nécessaire à des tentatives efficaces de conservation de l'espèce fait défaut. Pour comprendre la structure démographique de ce platanistoïde, nous avons procédé au séquençage de 418 paires de base de la région de contrôle et de 68 paires de base du gène adjacent de l'ARNt-pro dans l'ADNmt de 20 dauphins capturés accidentellement dans des filets maillants à Rio Grande do Sul et à Rio de Janeiro au Brésil. Des 11 haplotypes identifiés, 5 étaient exlusifs aux dauphins Rio Grande do Sul et 6 étaient exclusifs aux dauphins Rio de Janeiro et aucun haplotype n'était commun aux dauphins des deux endroits. La reconstitution des relations phylogénétiques entre les haplotypes par analyse de la vraisemblance maximale des séquences a révélé l'existence de deux lignées correspondant aux positions géographiques des sites d'échantillonnage. L'analyse de la variance moléculaire a également mis en relief la structure des populations ($\phi_{ST} = 0{,}403$, $p < 0{,}0001$). De plus, la divergence entre les nucléotides des populations (0,83%) était plus élevée que la diversité des nucléotides au sein de chacune des populations du nord (0,38%) et du sud (1,01%). Les analyses génétiques indiquent qu'il existe au moins deux populations de Dauphins de la Plata.

[Traduit par la Rédaction]

Introduction

The franciscana, *Pontoporia blainvillei*, is the smallest and the only marine platanistoid dolphin. It is endemic to the coastal Atlantic waters of South America and occurs from the central coast of Brazil to the central coast of Argentina the scarcity of information available. The low reproductive capacity (Kasuya and Brownell 1979), restricted distribution, and high incidental mortality rate caused by coastal fisheries make this species especially susceptible to depletion.

Since threats to this species occur at different levels along its distribution, owing to heterogeneity in local and seasonal fishing effort, knowledge of population structure is critical to management decisions on a regional basis. Based on osteological differences, Pinedo (1991) was able to distinguish two types of franciscana, a northern form and a southern form, with a boundary between them in the state of Santa Catarina, Brazil. Given that taxonomy has a strong influence on priorities for the conservation of endangered species, it is prudent to base such taxonomic decisions on multiple lines of evidence, including molecular analysis, which complements more traditional studies (Avise 1989).

Many population genetics studies have included an analysis of variations in mitochondrial DNA (mtDNA), a molecule that has characteristics which make it especially suitable for determining population structure, particularly in mammals (Brown et al. 1979; Brown 1983; Wilson et al. 1985; Hoelzel and Dover 1989). The control region of mtDNA is a portion that evolves rapidly and therefore allows fine-scale resolution of population structure and microevolutionary partitions (e.g., Rosel et al. 1995; Wang et al. 1996; Brennin et al. 1997; Wang and Berggren 1997). In the present study, we analysed mtDNA control region sequences from the two forms of franciscana to test the morphological classification suggested by Pinedo (1991).

Materials and methods

Sample collection and DNA extraction

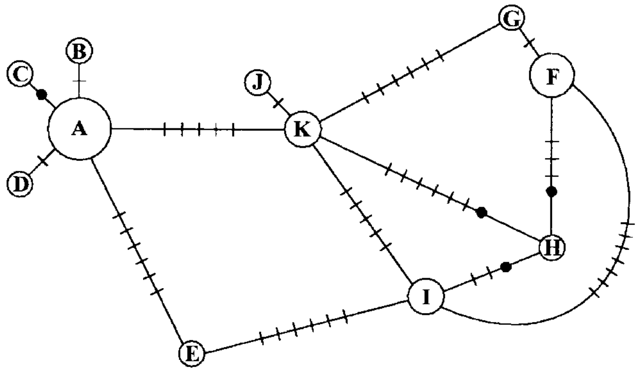
Fresh muscle samples were collected from 20 franciscana that were killed incidentally by gill-net fisheries operating in the coastal waters of Brazil between September 1993 and November 1994 (all were captured in different nets and on different days). Samples were collected from the waters of the Brazilian states of Rio Grande do Sul (10) and Rio de Janeiro (10) (see Fig. 1). The tissues were preserved in a solution of 0.25 M disodium EDTA and 20% DMSO and saturated with NaCl (Seutin et al. 1991). Approximately 1 g of tissue was ground with a mortar and pestle cooled in liquid nitrogen. The resultant powder was added to 3.5 mL of lysis buffer (4 M urea, 0.2 M NaCl, 0.1 M Tris-HCl, pH 8.0, 0.5% *n*-lauroylsarcosine, 10 mM 1,2-cyclohexanediaminetetraacetic acid) and incubated at 37°C for 2 days. Sixty-five units of proteinase K were added to the sample and this solution was incubated at 37°C for another day. Total DNA was extracted twice with a mixture of 70% phenol and 30% chloroform and once with chloroform before precipitation with 0.1 M sodium acetate and two volumes of 95% ethanol. Samples with visible

Table 1. Mitochondrial DNA haplotypes of the franciscana, *Pontoporia blainvillei*, and the 19 variable sites that characterise each haplotype. The frequency of occurrence and the sampling locations are also shown for each haplotype.

Haplotype	16	25	76	103	144	168	**202**	305	341	347	354	355	**356**	359	397	429	462	463	464	Frequency	Sampling location
A	T	T	A	A	C	T	**G**	A	A	A	C	T	**C**	A	G	C	A	A	C	6	N
B	.	.	.	.	.	.	**.**	.	.	.	.	.	**.**	.	.	.	.	.	.	1	N
C	.	.	.	.	.	.	**.**	.	.	.	.	.	**.**	.	.	.	C	.	.	1	N
D	.	.	.	.	.	.	**.**	.	G	.	.	.	**.**	.	.	.	.	.	.	1	N
E	.	.	.	.	.	.	**.**	G	.	G	T	.	**.**	G	A	T	.	.	.	1	N
F	.	.	.	G	T	.	**A**	G	.	.	T	C	**T**	.	.	.	.	.	T	3	S
G	.	.	.	G	T	.	**A**	G	.	.	T	.	**T**	.	.	.	.	.	T	1	S
H	.	G	G	.	T	.	**A**	G	.	.	N	C	**T**	.	N	T	.	.	N	1	S
I	.	.	.	.	.	.	**A**	G	.	.	.	C	**T**	.	A	T	.	.	.	2	S
J	C	.	.	.	.	C	**A**	G	.	.	.	.	**T**	.	.	.	.	G	.	1	S
K	C	.	.	.	.	C	**A**	G	.	.	.	.	**T**	.	.	.	.	.	.	2	S

Note: Diagnostic sites 202 and 356 are indicated in boldface type. The sampling locations are north (N) and south (S) of Santa Catarina, Paraná, and São Paulo states. Dots represent sequence identity to haplotype A. Representative sequences of a northern (B) and a southern (K) haplotype were deposited in GenBank (accession numbers AF037593 and AF037594, respectively).

Fig. 2. The minimum-spanning network for mitochondrial DNA haplotypes of franciscana. Dashes represent transitions and dots represent transversions. The sizes of the circles approximate the frequency of occurrence of each haplotype in the sample analysed (20). The lengths of the line joining the haplotypes approximate the number of substitutions separating the haplotypes.



haplotypes, used in the above equations, were estimated under the Jukes–Cantor model, using the computer program MEGA (Kumar et al. 1993). Determination of the phylogenetic associations among the haplotypes employed the maximum-likelihood algorithm of the Phylogenetic Inference Package (PHYLIP) version 3.5c (Felsenstein 1995). To test the hypothesis that at least two genetically distinct (northern and southern) populations of franciscana exist in the coastal waters of Brazil, the distance values between haplotypes, generated by the above analysis, were subjected to the analysis of molecular variance (AMOVA) of Excoffier et al. (1992) with 10 000 permutations. Also, to demonstrate the relationships among the haplotypes, a minimum-spanning network of the nucleotide changes was constructed.

Results

A total of 486 base pairs (bp) of mtDNA (68 bp of proline tRNA and 418 bp control region) was sequenced from 20 franciscana. Nineteen variable sites resolved 11 unique sequences (or haplotypes) of which 5 were found exclusively in animals sampled from the Rio de Janeiro region (northern group) and the other 6 were exclusive to animals from Rio Grande do Sul (southern group), i.e., no haplotypes were shared among animals from these two sampling regions (examination of specimen information revealed no apparent sex bias in the distribution of haplotypes). Diagnostic characters were found for distinguishing northern and southern groups (i.e., each group was fixed for different bases at sites 202 and 356) (Table 1). Three haplotypes of the northern group were closely related to the most commonly occurring haplotype (A), differing by only one substitution: B and D differed by one transition and C differed by a transversion. In the southern group, K–J and G–F differed by one substitution. Other relationships were not as simple (Fig. 2). The relationship of haplotype E to the other haplotypes is unclear in the network. The closest haplotype to E differs by six transitions (both A and I). Haplotype A occurred most frequently (six) followed by F (three), I (two), and K (two). The remaining seven haplotypes all had single representatives.

Table 2. Genetic diversity indices for two populations of franciscana, *Pontoporia blainvillei*.

	Northern population	Southern population	Estimate of haplotypic diversity
Northern population	0.38±0.13	0.83±0.36	0.67
Southern population	—	1.01±0.30	0.89

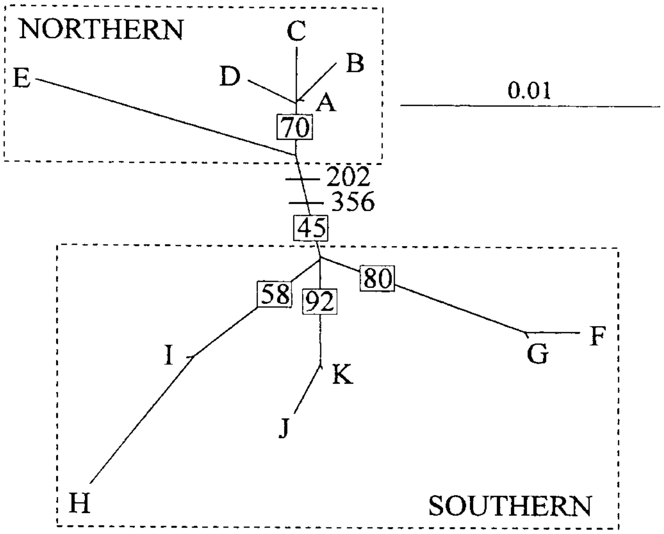
Note: The estimates of nucleotide diversity (× 100; mean ± standard error) are shown along the diagonal and the nucleotide divergence estimate (× 100; mean ± standard error) is shown above the diagonal.

Nucleotide diversity of the northern population was significantly lower (~2.7 times) than in the southern population ($t = 1.915$, $p < 0.05$ (one-tailed test), $\mathrm{df} = \infty$; see Kumar et al. 1993; note: a one-tailed test was chosen because there was evidence that the northern population was smaller (see later)). Sequence divergence between the populations was higher than within the northern population but lower than within the southern population. Estimates of haplotypic diversity were lower for the northern group than the southern group as well (see Table 2).

The phylogenetic tree produced by the maximum-likelihood approach showed two distinct groupings of haplotypes that corresponded to the geographical sampling locations (Fig. 3). Haplotypes A–E constituted the northern lineage, while haplotypes F–K made up the southern lineage (Fig. 3). Haplotype E appears to be a divergent haplotype whose relationship to the rest of the northern haplotypes is supported by diagnostic differences at sites 202 and 356 and the results of the neighbour-joining approach (results not shown). Rosel et al. (1995) also reported similar low bootstrap values for lineages of harbour porpoise haplotypes that were clearly separated by network interrelationships and geography.

Further support for the partition of the northern and southern forms was obtained from the AMOVA. The variance among populations ($\phi_{ST} = 0.403$) was highly significant ($p < 0.0001$).

Fig. 3. Relationship of mitochondrial DNA haplotypes of franciscana, based on the maximum-likelihood method of phylogeny reconstruction. Numbers in the boxes placed on the major lineages show bootstrap values. Diagnostic sites 202 and 356 are also indicated on the tree.



Discussion

The results of the present study provided direct genetic evidence for at least two populations of franciscana in Brazilian waters: one to the south and one to the north of Santa Catarina, Paraná, and São Paulo states. The phylogeny of the haplotypes, two diagnostic substitutional differences (sites 202 and 356), network of haplotype interrelationships, and AMOVA show that the franciscana samples separated into two lineages. With the small sample size of the present study, we were not able to examine other aspects or details of population structure of the franciscana (e.g., subpopulations within these populations, differences in philopatric behaviour between the sexes (see Wang et al. 1996), zoogeography, etc.). Nevertheless, the results of this study provide direct genetic support for population distinctions that are critical for conservation. Management-decision makers must recognise at least two (and potentially more) genetically distinct populations of franciscana. The crude separation of franciscana into a northern and a southern population may not be sufficient for effective conservation of this species. Analysis of many more samples throughout its distribution is necessary to refine our understanding of franciscana populations. Once a distribution-wide genetic survey is complete, information on differences in population demographics, incidental mortality, and reproductive seasonality may be integrated and considered when conservation strategies are designed.

The lower genetic diversity found in the northern population (relative to the southern population) is consistent with the belief that this is a smaller (Secchi et al.)[2] or declining population. The network of interrelationships among haplotypes also supports this hypothesis; the southern population contained matrilines that shared a more ancient ancestry than the northern lineage, while all northern haplotypes (except E) were closely related and most of the samples belonged to one haplotype (A). However, these findings also suggest that the northern population was established more recently by a founder lineage from the south or, alternatively, that an ancient lineage has survived in tropical waters even though most haplotypes dispersed south into more temperate regions. Although the divergent haplotype E may indicate that a second lineage also colonised (or survived in) the northern regions, we believe that the shared diagnostic sites (202 and 356) indicate a single colonising (or surviving) lineage. These competing hypotheses regarding the population size or direction and pattern of franciscana dispersal require further study. To test the contribution of heavy exploitation to the low diversity estimates for the northern population, comparisons of the present diversity estimates with pre-exploitation levels or with estimates of the popula-

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[2] E.R. Secchi, M.C.O. Santos, S. Siciliano, and P.H. Ott. Is the abundance of coastal cetaceans determined by co-existence with sympatric species?: A study case in Brazil. In preparation.

tion in the future are required. Additional genetic, morphological, and palaeontological studies are required to evaluate these hypotheses. Nevertheless, the low diversity indicates that the smaller northern population may be particularly vulnerable to decline and may be in need of special conservation attention.

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