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Geographical variation in franciscana (*Pontoporia blainvillei*) external morphology

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Four distinct Franciscana Management Areas (FMAs) have been proposed based on several lines of evidence including genotype, phenotype, population response and distribution. To determine if differences in external morphology fit this division, a canonical variate analysis was carried out for males and/or females from FMAs I to IV using up to 14 characters. A total of 78 adult specimens were analysed. More than 90% of the differences between groups were summarized by three canonical variates. Females were larger than males in all areas. Females from FMA IV were of intermediate length between those from FMA I and FMA III and individuals from FMA II were smaller than those from all other areas. Position of dorsal fin and morphology of the anterior body region, differentiate individuals from FMA I and FMA III. Morphological differences found in this study give additional support for the proposed FMAs. Since habitat characteristics and franciscana feeding ecology vary regionally, it is possible that observed morphological differences are due to ecological divergence for niche occupation. The indication of a discontinuous distribution, consistency between genetic and morphological evidence, and a short time genetic divergence, might indicate that franciscanas inhabiting FMA I represent a distinct subspecies.

Keywords: *Pontoporia blainvillei*, stock identity, external morphology, multiple-group principal component analysis, canonical variate analysis, south-western Atlantic

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INTRODUCTION

Phenotypic data such as osteological and morphological characters as well as coloration patterns, along with other proxies such as molecular biology, reproduction, contaminant loads, parasite and feeding ecology, can offer valuable insights to identify demographically discrete units for management and conservation of threatened or exploited cetacean populations (e.g. Kasuya *et al.*, 1988; Christensen *et al.*, 1990; Perrin *et al.*, 1991; Heyning & Perrin, 1994; Andrade *et al.*, 1997; Wang *et al.*, 1999, 2000b; Rodriguez *et al.*, 2002; Perrin *et al.*, 2003; Secchi *et al.*, 2003; Jefferson & Van Waerebeek, 2004). However, for cetaceans, because it is difficult to assemble large sample sizes of fresh specimens, there are only a few studies with measurements of external morphology (e.g. Christensen *et al.*, 1990; Perrin *et al.*, 1991; Gao *et al.*, 1995; Wang *et al.*, 2000b). Franciscana, *Pontoporia blainvillei* (Gervais & d'Orbigny, 1844), is a small cetacean with distribution restricted to coastal waters of the western South Atlantic, where it is seriously threatened by incidental catches in gillnets. Several studies on genetics and osteology have shown the existence of distinct populations along the coast (Pinedo, 1991; Secchi *et al.*, 1998; Higa *et al.*, 2002; Ramos *et al.*, 2002; Lázaro *et al.*, 2004). Four Franciscana Management Areas (FMAs) were proposed using the phylogeographical concept applied to genotypical, phenotypical, population response and distributional data. FMA I: the coastal waters of Espírito Santo and Rio de Janeiro States; FMA II: the coastal waters of São Paulo, Paraná and Santa Catarina States; FMA III: the coastal waters of Rio Grande do Sul and Uruguay; FMA IV: the coastal waters of northern Argentina (Secchi *et al.*, 2003).

There is strong evidence that the populations from FMAs I, II and III represent distinct demographic entities with limited gene flow, especially between the former and any other FMA. However, evidence for splitting FMA III from IV is weak. The genetic results from microsatellite analysis of nuclear DNA and molecular analysis of variance of mitochondrial DNA (Ott, 2002; Lázaro *et al.*, 2004) indicated no significant differences between franciscana populations from these two FMAs.

Mendez *et al.* (2007) analysed the mitochondrial DNA of franciscana samples from distinct locations of Argentina and compared them to data published by Lázaro *et al.* (2004), finding evidence for a small-scale structuring. They

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recommended that, for management purposes, animals from FMA IV should be split further into at least two distinct units named San Clemente and Claromecó populations, both from the northern Buenos Aires coast. Therefore, genetic and morphological studies on a finer scale and with samples from Rio Grande do Sul, Uruguay (FMA III) and distinct locations within Argentina (FMA IV) are needed for improving our understanding about small-scale population structures.

This study aimed at providing additional information about the franciscana phenotype, by examining geographical variation in external morphology and evaluating whether the results are consistent with the proposed FMAs.

MATERIALS AND METHODS

Data

A total of 259 franciscanas caught in gillnets between October 1989 and August 2006 in Rio de Janeiro, São Paulo and Rio Grande do Sul States, Brazil, and Buenos Aires Province, Argentina were classified, respectively, as FMA I to IV, as proposed by Secchi *et al.* (2003) (Figure 1). Up to 47 external metric characters were measured using standardized methods based on Norris (1961). Measurements were taken to the nearest 0.1 cm or 0.2 cm by trained researchers from each location using a commercial measuring tape.

The probable effects of sexual differences were excluded by analysing sexes separately. To exclude the effects of ontogenetic variation, only adults were considered. Prior information about reproductive status allowed the classification of specimens from Rio Grande do Sul, Rio de Janeiro and Argentina into adults and juveniles. For the samples from São Paulo State, adults were selected based on an estimate of body length at attainment of sexual maturity (Rosas & Monteiro-Filho, 2002).

After excluding immature animals, the sample size was reduced to 79 individuals (Table 1). The sample from Argentina was composed of one male and ten females (Table 1). Therefore, FMA IV was included in the analyses of variation in external morphology among the putative populations, only for females.

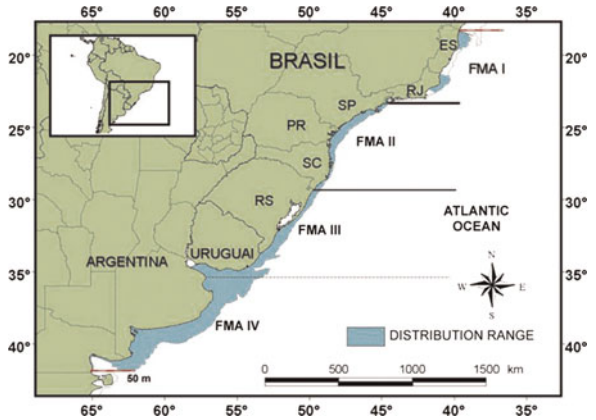

Fig. 1. Map showing the southern and northern limits of the franciscana range and the four proposed Franciscana Management Areas (FMAs) (modified from Secchi *et al.*, 2003). Unshaded areas represent gaps in franciscana distribution.

The number of measured characters and the way in which measurements were performed (e.g. on both sides of the body, or just on the left side; as an axial projection or point-to-point) varied among and, sometimes, within locations. Furthermore, measurements were taken by different observers without prior calibration, which could introduce biases in the analyses and result in confounding between systematic differences in measurement procedures and variation among locations. To minimize the risk of possible confounding results due to 'observer-effect', we decided to: (1) only use the measurements referring to the left body side and which were clearly undertaken in the same way by researchers from FMA I, FMA II and FMA III (Figure 2; Table 2); and (2) exclude all metric characters considered subjective and prone to different interpretations regarding the exact position of limiting points of a measurement (e.g. snout to apex of melon, snout to anterior insertion of dorsal fin, anterior length of flipper and basal length of dorsal fin).

From twenty-three external morphometric characters initially selected, fourteen measurements were finally used in the analyses to examine the existence of variation among the first three FMAs (Table 2; Figure 2). The measurements 'snout to external auditory meatus length' and 'snout to midpoint of umbilicus' were not taken from the specimens from Argentina and, therefore, were excluded from the analyses when FMA IV was included in the analyses.

Statistical analyses

The statistical analyses were carried out in *R* version 2.2.1 (R Development Core Team, 2005). All selected characters, when examined individually, showed approximate normality. Hence, it was considered reasonable to assume a joint multivariate normal distribution for all 14 characters (Manly, 1994). Each of the 14 characters was tested individually for differences among localities and between sexes using a two-way analysis of variance (ANOVA).

Before performing multivariate analyses, and to avoid exclusion of animals with only a few missing measurements, absent values for a given specimen were replaced with the expected values of the multivariate normal distribution after conditioning on all observed characters for that specimen (Chattfield & Collins, 1980). In the data matrix with 14 characters and 68 individuals, that is, for the analysis carried out for males and females from FMAs I, II and III, 99 cells were filled in missing values. A maximum of 9 missing values were necessary per individual. For the analysis carried out only with females from the four areas, similar methods were employed to estimate the 45 missing values in the matrix of data with 43 specimens and 12 characters. A maximum of 7 missing values were necessary to complete per individual.

A canonical variate analysis (CVA) (Reyment *et al.*, 1984), was employed to assess variation between males and females from FMA I, FMA II and FMA III, and among the females from all four FMAs. This method maximizes between-group variation in relation to the within-group variation, producing maximal separation between groups. The function 'lda' (linear discriminant analysis) from the MASS library of *R* was applied for this purpose. The multiple-group principal component analysis (MGPCA) was used complementarily. It works similarly to the principal component analysis (PCA) but, instead of starting with the global covariance matrix, it uses the within-group covariance matrix (Thorpe, 1988).

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Table 1. Number of adult specimens by location, sex and putative population used in the analyses. The only male from Franciscana Management Area (FMA) IV was not included.

Putative population	Location	Number of specimens	Sex		Total
			Males	Females	
FMA I	RJ	19	9	10	19
FMA II	Ubatuba—SP	14	8	6	19
	Central coast of SP	5	2	3	
FMA III	North coast of RS	8	4	4	30
	South coast of RS	22	12	10	
FMA IV	San Clemente and Canal 15	7	1	6	11
	Cabo San Antonio	4	-	4	
		Total	36	43	79

RJ, Rio de Janeiro; RS, Rio Grande do Sul; SP, São Paulo.

The advantage of MGPCA is its direct relationship to CVA (Thorpe, 1983a). When a MGPCA is run on a set of characters and their component scores are used as inputs for CVA, identical results to those of CVA on the original set of characters will be obtained. However, only the former allows for assessing the contribution of within-group components to the between-group discrimination (Thorpe *et al.*, 1982; Thorpe, 1983a, b; Thorpe & Leany, 1983).

In this study a MGPCA was initially carried out to describe the relationships of growth and size among males and females from FMAs I, II and III and also to describe the relationship among females from all four areas. Variables were standardized to have a mean of zero and variance of one before starting the analysis (Reyment *et al.*, 1984; Manly, 1994). Each of the scores was subject to a two-way ANOVA to evaluate if individual components showed significant differences between sexes and locations. The relative contribution of each of the components for discrimination among groups was verified for both males and females through a one-way ANOVA (see details in Malhotra & Thorpe, 1997).

A significance level of $\alpha = 0.05$ was adopted for all analyses. The main differences and similarities among the groups were graphically portrayed by 95% confidence contours (Reyment *et al.*, 1984) for the estimated mean for each group on the first three canonical variates.

Assessment of 'observer-effect'

In order to examine whether observed differences between centroids of canonical variates (CV1, CV2 and CV3) could have derived from systematic differences in measurements taken by researchers in different locations, a simulation study was carried out using the results obtained with females from the four areas as if they were 'true' data. Simulated data were produced with the same number of specimens (N = 43), divided among locations as in the original data.

Hypothetical systematic errors were defined for each area as known deviations in the mean vectors of the 12 characters. FMA III was the reference location, without systematic errors. In FMA I all the measurements were overestimated, with means 10% higher than the correspondent means in FMA III. In FMA II all the measurements were underestimated, with means 10% lower than the correspondent means in FMA III. Finally, in FMA IV half of the measurements were overestimated by 10% while the other half was underestimated by 10%. A total of 300 Monte Carlo simulations were produced. For each simulated data set a CVA was carried out and Mahalanobis distances were calculated among the four

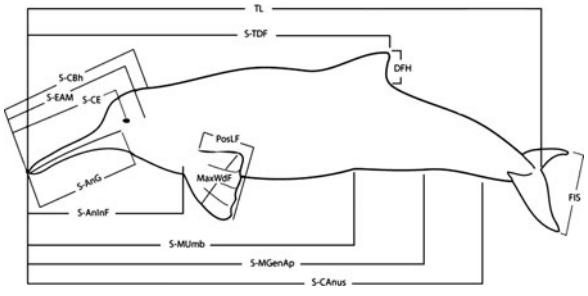

Fig. 2. Schematic description of the measurements used in the morphometric analysis of franciscanas.

Table 2. External morphometric characters common to Franciscana Management Areas (FMAs) I, II and III.

External measurements	Code
Total length*	TL
Snout to apex of melon length	S-ApM
Snout to centre of eye length*	S-CE
Snout to external auditory meatus length*	S-EAM
Snout to angle of gape length*	S-AnG
Snout to centre of blowhole length*	S-CBh
Snout to anterior insertion of dorsal fin length	S-AnInDF
Snout to tip of dorsal fin length*	S-TDF
Snout to anterior insertion of flipper length*	S-AnInF
Snout to midpoint of umbilicus*	S-MUmb
Snout to midpoint of genital aperture*	S-MGenAp
Snout to centre of anus*	S-CAnus
Centre of eye to external auditory meatus length	CE-EAM
Anterior length of flipper	AnLF
Posterior length of flipper*	PosLF
Maximum width of flipper*	MaxWdF
Dorsal fin height*	DFH
Basal length of dorsal fin	BLDF
Fluke span*	FlS
Notch length	NL
Fluke notch to centre of anus	FlN-CAnus
Fluke notch to midpoint of genital aperture	FlN-MGenAp
Fluke notch to midpoint of umbilicus	FlN-MUmb

*, measurements used in the analyses with males and females from FMA I, II and III; for definitions of codes see text.

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Table 3. Summary of external morphological measurements of mature males and females from Rio de Janeiro. FMA, Franciscana Management Area; Min, minimum; Max, maximum; SE, standard error; N, number. For definitions of codes see text.

	FMA I									
	Males					Females				
Measurement	Min	Mean	Max	SE	N	Min	Mean	Max	SE	N
TL	115.0	119.0	123.0	2.46	9	129.5	136.6	147.5	5.51	10
S-CE	25.00	26.97	29.00	1.24	9	29.50	30.89	32.00	0.89	9
S-EAM	31.50	32.94	35.00	1.04	9	36.30	37.70	39.50	1.10	10
S-AnG	22.00	23.26	25.50	1.14	9	20.00	26.63	28.50	2.44	10
S-Bh	28.00	29.54	31.00	0.84	9	32.00	33.85	35.50	1.00	10
S-TDF	70.50	73.83	80.00	3.08	9	84.00	86.94	90.00	2.16	9
S-AnInF	39.00	40.89	43.50	1.67	9	42.50	45.44	49.50	1.89	9
S-MUmb	64.00	66.89	70.00	2.15	9	70.50	76.85	85.00	4.43	10
S-MGenAp	73.00	75.11	79.00	2.09	9	88.00	97.00	106.0	5.80	9
S-CAnus	85.00	86.94	91.50	1.93	9	92.50	101.5	110.0	6.00	10
PosLF	12.50	14.50	15.50	1.00	9	15.00	16.35	17.50	0.67	10
MaxWdF	8.80	10.29	11.00	0.66	9	10.50	11.65	12.50	0.71	10
DFH	6.50	8.50	10.00	1.32	9	7.50	8.96	10.00	0.80	10
FlS	27.00	32.87	38.00	3.51	9	30.50	34.70	42.00	3.35	10

centroids (distances I–II, I–III, I–IV, II–III, II–IV and III–IV). Finally, an average Mahalanobis distance was calculated to describe the distance between centroids. The 'true' average distance was compared to the simulated distribution of mean distances. A 'true' average distance similar in line to the simulated distribution would indicate a possible 'observer-effect' as a confounding factor with differences among locations.

RESULTS

The means, standard errors, ranges and the number of specimens for each one of the external morphometric characters analysed for both males and females from FMAs I, II and III and for females from FMA IV are given in the Tables 3, 4, 5 and 6.

To facilitate reference to each of two analyses, we will refer to the analysis with males and females from FMA I, II and III as 'B' (for *both* sexes) and the analysis with females only but from all four areas as 'F' (for *females*).

For case 'B', four characters, when evaluated individually for approximate normality: S-AnG, S-CBh, S-AnInF, S-CAnus failed the Shapiro-Wilk test ($P < 0.05$; Table 7). The same occurred for two other characters in case 'F': PosLF and MaxWdF ($P < 0.05$; Table 8). Since a visual inspection of qq-plots indicated that they were only slightly deviated from normality, they were analysed without transformation.

The two-way ANOVA for each one of 14 external characters showed significant differences between areas and between sexes for all characters. There was significant sex–area interaction for S-EAM, S-TDF and PosLF, suggesting that observed differences between sexes for these measurements vary

Table 4. Summary of external morphological measurements of mature males and females from São Paulo. FMA, Franciscana Management Area; Min, minimum; Max, maximum; SE, standard error; N, number. For definitions of codes see text.

	FMA II									
	Males					Females				
Measurement	Min	Mean	Max	SE	N	Min	Mean	Max	SE	N
TL	111.0	117.1	124.0	5.30	10	112.0	130.2	142.0	9.12	9
S-CE	22.00	24.00	26.00	1.28	8	26.00	26.83	28.00	0.98	6
S-EAM	28.00	29.25	32.00	1.39	8	31.00	32.67	34.00	1.21	6
S-AnG	19.00	21.00	23.00	1.31	8	22.00	23.50	25.00	1.05	6
S-Bh	23.00	25.61	29.00	1.57	10	20.50	28.61	36.00	4.44	9
S-TDF	71.00	75.88	81.00	3.44	8	80.00	83.17	86.00	2.32	6
S-AnInF	35.50	38.00	41.00	1.94	10	31.00	41.67	48.00	4.79	9
S-MUmb	58.00	61.88	65.00	2.69	8	62.00	69.00	73.00	3.74	6
S-MGenAp	65.00	70.50	76.00	3.78	8	81.00	90.33	94.00	4.84	9
S-CAnus	73.00	83.00	90.00	5.05	10	70.00	93.63	108.00	11.30	8
PosLF	13.00	14.83	17.00	1.17	9	13.00	14.50	16.00	1.19	7
MaxWdF	9.00	10.56	13.50	1.22	10	10.00	10.88	12.00	0.83	8
DFH	6.00	7.63	9.00	0.95	10	8.00	8.87	10.00	0.83	8
FlS	26.00	31.63	37.00	3.69	8	30.00	33.50	36.00	2.17	6

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Table 5. Summary of external morphological measurements of mature males and females from Rio Grande do Sul. FMA, Franciscana Management Area; Min, minimum; Max, maximum; SE, standard error; N, number. For definitions of codes see text.

	FMA III									
	Males					Females				
Measurement	Min	Mean	Max	SE	N	Min	Mean	Max	SE	N
TL	128.0	133.8	138.7	3.24	16	131.9	144.1	154.6	5.73	14
S-CE	24.70	26.54	28.60	1.08	16	27.50	28.97	31.20	1.11	14
S-EAM	30.20	32.92	34.60	1.28	15	33.00	35.02	38.10	1.38	13
S-AnG	21.00	22.96	25.50	1.22	16	23.30	25.14	27.20	1.13	14
S-Bh	16.50	26.42	30.30	4.69	11	29.40	30.76	33.10	1.05	10
S-TDF	84.50	87.18	91.00	2.46	10	86.10	93.25	98.10	3.49	10
S-AnInF	34.50	42.82	45.40	2.65	16	42.20	45.59	48.50	1.91	13
S-MUmb	69.50	74.59	79.50	2.75	14	76.20	80.58	85.50	2.81	12
S-MGenAp	79.00	84.45	90.00	3.06	15	96.50	104.8	112.0	4.24	13
S-CAnus	87.50	99.05	107.3	5.25	13	100.3	109.4	117.5	4.88	13
PosLF	16.10	16.73	18.10	0.69	12	16.10	17.36	19.40	1.07	10
MaxWdF	10.30	12.25	13.30	0.87	13	11.70	12.74	14.10	0.74	12
DFH	7.20	8.95	10.50	1.06	11	7.60	9.16	10.50	0.89	10
FlS	33.30	36.06	39.80	1.90	16	33.50	38.54	46.00	3.83	14

geographically (Table 7). In the ANOVA carried out with females from all four areas, significant differences also occurred. S-MGenAp, DFH and S-TDF showed the best contributions to discriminating females from the four areas (Table 8).

Multiple-group principal component analysis carried out for case 'B' showed that 70.94% of overall within-group variation can be explained by the first three components (Table 9). PC1 assumes high positive correlations with all characters analysed. This is frequently interpreted as an index of general size. The other components show positive and negative correlations of varied magnitude, and are indicative of shape variation. PC2 and PC3 describe the most important characters related to shape, to explain the variability between animals within groups. PC2 can be interpreted as a contrast between DFH and two other measurements, since the latter have positive correlations and DFH, negative correlation with PC2. For PC3 contrasts involve mainly S-CAnus, S-MUmb and S-AnG. The first two characters show positive correlations, while S-AnG shows negative correlation with PC3.

The relative contributions from PC1, PC2 and PC3 to discriminating among groups are smaller than from other PCs. The components that best explain the between-group variation were PC8, PC9 and PC12, for males and PC4, PC10 and PC12 for females (Table 9). These components contribute, respectively, with 50.38% and 52.37% of between-group variation for males and females. PC12, which shows a relationship between S-MUmb and S-CAnus, is the component that best explains the between-groups variation for males (22.98%). It also has a high contribution for between-group variation for females (13.76%). Nevertheless, for within-group variation, its contribution is 0.34%. PC9 and PC10 can be interpreted as a component related to beak size/shape for males and females, respectively. The former

Table 6. Summary of external morphological measurements of mature females from Argentina. FMA, Franciscana Management Area; Min, minimum; Max, maximum; SE, standard error; N, number. For definitions of codes see text.

	FMA IV				
	Females				
Measurement	Min	Mean	Max	SE	N
TL	125.0	138.2	149.0	7.43	10
S-CE	26.00	29.02	31.30	1.99	10
S-EAM	–	–	–	–	–
S-AnG	23.00	25.53	27.80	1.72	10
S-Bh	28.30	32.55	38.70	2.85	10
S-TDF	75.70	81.28	88.00	4.98	10
S-AnInF	41.00	44.44	45.80	1.50	10
S-MUmb	–	–	–	–	–
S-MGenAp	86.80	89.26	91.20	1.56	10
S-CAnus	88.30	90.69	92.70	1.49	10
PosLF	15.00	16.77	22.20	2.88	10
MaxWdF	8.50	9.84	12.00	1.50	10
DFH	5.80	6.69	7.30	0.48	10
FlS	35.00	38.60	42.00	2.31	10

Table 7. Results of a two-way analysis of variance and Shapiro–Wilk test for each one of the 14 external morphometric characters (2 df for area and 1 df for sex). For definitions of codes see text.

Measurement	Between area	Between sex	Area × sex	Shapiro–Wilk
TL	50.85*	99.32*	2.55	0.0839
S-CE	45.35*	110.07*	2.59	0.3657
S-EAM	58.64*	100.83*	6.38*	0.3876
S-AnG	16.87*	50.51*	0.99	4.034e-05
S-Bh	14.30*	27.94*	0.35	9.773e-05
S-TDF	80.55*	120.25*	7.65*	0.4936
S-AnInF	16.01*	29.13*	0.64	4.958e-05
S-MUmb	72.06*	84.68*	2.25	0.9471
S-MGenAp	67.68*	396.78*	0.31	0.4665
S-CAnus	40.85*	58.37*	0.77	0.0027
PosLF	29.41*	9.05*	5.65*	0.8401
MaxWdF	27.36*	10.38*	2.03	0.1206
DFH	3.77*	5.43*	1.40	0.0817
FlS	13.36*	7.31*	0.08	0.5001

Reference value for F ratio: * = $\alpha < 0.05$.

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Table 8. Results of analysis of variance and Shapiro–Wilk test for each one of 12 external morphometric characters (3 df). For definitions of codes see text.

Measurement	F ratio	Shapiro–Wilk
TL	7.62*	0.429
S-CE	11.11*	0.333
S-AnG	4.45*	0.0002
S-Bh	6.85*	0.0003
S-TDF	20.73*	0.663
S-AnInF	4.25*	0.0004
S-MGenAp	24.95*	0.252
S-CAnus	19.08*	0.023
PosLF	4.04*	4.051e-05
MaxWdF	16.37*	0.004
DFH	22.69*	0.289
FlS	6.06*	0.055

Reference value for F ratio: * = $\alpha < 0.05$

establishes high correlations with S-AnG and S-AnInF and the latter establishes correlations mostly with S-CE, S-EAM and S-AnG. PC8 can be interpreted in terms of contrast between two measurements related to flipper shape, PosLF and MaxWdF and PC4 can be explained by contrast between MaxWdF and DFH, also being a component related to appendix shape.

For the analysis carried out for case 'F', MGPCA showed that the first three components together explain 77.98% of overall within-group variation (Table 10). All PCs explain shape variation. Characters S-TDF, PosLF, MaxWdF and DFH, were the least correlated with PC1, reflecting the small importance of measurements related to appendix shape in defining this component. PC2 can be interpreted as a component related to appendix shape, since the highest correlations were established with PosLF, MaxWdF, FlS and DFH, all of them positive.

Like in the analysis carried out with case 'B', the first three components obtained for case 'F' contribute most to explain within-group variation. The importance of PC1, PC2 and PC3 to explain between-groups variation is reduced when compared to the importance of PC8, PC10 and PC12 (Table 10). PC3 does not allow for discrimination between areas ($F = 0.74$, $P = 0.53$) and PC1 and PC2 explain only 1.70% and 4.06% of between = groups variation, respectively. In contrast, PC8, PC10 and PC12 contribute together with 66.21% of between-groups variation. Individual scores calculated for PC8, allows for discriminating FMA IV from other areas. All females from FMA I establish positive scores in this component, as well as most females from FMAs II and III, contrasting with negative scores for females from FMA IV. These results suggest that dorsal fins of FMA IV females are shorter than those of the females of the other areas. Thus, the differences between FMA I and FMA IV are more

Table 9. Analysis of variance (ANOVA) of multiple-group principal component analysis (MGPCA) scores. Within-group variance is the percentage of within-group variation summarized by the multiple group principal components. Between-group variance is the percentage that each component contributes to between-group variance (for males and females separately). The F ratios are derived from a two-way ANOVA of the MGPCA scores, and show how individual components differentiate between sexes and localities.

PC	Within-group variance	Between-group variance				F ratios
		Male	Female	Area	Sex	Area × sex
1	43.01	8.88	9.67	39.1859*	83.3797*	1.5514
2	17.95	1.49	3.56	10.1442*	27.1798	0.0936
3	9.98	4.12	10.58	29.1578*	1.3723	0.7151
4	8.55	3.81	16.36	26.1518*	10.1101	7.5954*
5	4.80	2.31	3.10	10.0062*	47.9672	1.6943
6	4.53	2.74	2.41	11.3036	10.4011	0.7642
7	3.80	0.91	1.66	4.7944*	0.1755	0.6575
8	3.10	12.60	1.46	28.4760*	39.7648	9.0605*
9	1.91	14.80	12.60	51.8887*	1.6450	6.9697*
10	1.42	11.66	22.25	69.3330*	19.3495	1.3554
11	0.82	1.94	0.27	2.8896	32.5584	3.3355*
12	0.34	22.98	13.76	83.6882*	0.4464	9.6537*
13	0.20	4.39	0.54	8.6366*	14.5231	2.3709
14	0.05	7.35	4.82	11.382*	2994.53	15.199*

Reference value for F ratio: * = $\alpha < 0.05$

Table 10. Analysis of variance (ANOVA) of multiple-group principal component analysis (MGPCA) scores. Within-group variance is the percentage of within-group variation summarized by the multiple group principal components. Between-group variance is the percentage that each component contributes to between-group variance. The F ratios derived from ANOVA of the MGPCA scores show how individual components differentiate between localities.

PC	Within-group variance	Between-group variance	F ratio	*P* values
1	46.64	1.70	5.60	0.003
2	20.09	4.06	13.35	3.838-06
3	11.25	0.22	0.74	0.53
4	6.86	3.22	10.59	3.156e-05
5	4.55	0.18	0.58	0.63
6	3.36	11.30	37.14	1.632e-11
7	2.47	4.15	13.64	3.129e-06
8	2.05	20.67	68.01	1.496e-15
9	1.52	1.17	3.86	0.017
10	0.66	19.71	64.82	3.265e-11
11	0.46	7.78	25.59	2.550e-09
12	0.04	25.83	84.98	2.2e-16

Table 11. Standardized component scores from canonical variates analysis. The three components summarize, respectively, 79.31%, 15.65% and 4.01% of overall variation for males and females analysed together. For definition of codes see text.

Measurement	CV1	CV2	CV3
TL	−2.044	0.191	0.003
S-CE	1.498	−2.644	1.270
S-EAM	0.608	1.721	1.515
S-AnG	−0.434	−0.346	−0.751
S-Bh	−0.186	−0.843	0.002
S-TDF	1.173	2.821	−0.999
S-AnInF	−0.499	0.754	−0.330
S-MUmb	−3.644	1.897	2.164
S-MGenAp	14.902	−2.320	−2.751
S-CAnus	−5.665	0.199	0.879
PosLF	0.562	0.883	0.009
MaxWdF	−1.058	0.148	0.195
DFH	−0.640	−0.360	0.115
FlS	0.329	−0.479	−0.492

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Table 12. Standardized component scores from canonical variates analysis. The three components summarize, respectively, 67.21%, 22.19% and 10.61% of overall variation for females. For definitions of codes see text.

Measurement	CV1	CV2	CV3
TL	−0.122	−0.435	−1.882
S-CE	−0.428	1.253	2.476
S-AnG	−0.726	−1.294	−0.710
S-Bh	1.343	0.692	0.697
S-TDF	0.315	1.627	0.441
S-AnInF	−0.028	0.107	−0.760
S-MGenAp	7.316	4.652	0.509
S-CAnus	−7.347	−2.854	0.119
PosLF	1.398	0.867	0.884
MaxWdF	−1.613	−0.323	−0.914
DFH	−2.224	−1.028	0.171
FlS	1.151	−0.458	−0.143

conspicuous. PC10 establishes a similar pattern to PC8 in discriminating between areas but with opposite signs: positive for FMA IV and negative for all the other FMAs. This shows that the measure which establishes the higher correlation with PC10, R-BH, assumes the largest size in FMA IV. Individual scores obtained for PC12 allowed for discriminating females from FMA II and IV, the former presenting negative and the latter, positive values. The position of the genital slit in relation to the anus must present significant variation between areas allowing for their discrimination.

Canonical variate analysis allowed maximizing discrimination between areas in both analyses, cases 'B' and 'F' (Tables 11 & 12). These differences are visualized by the graphical display of 95% confidence contours of estimated mean individual scores (Figures 3, 4, 5, 6 & 7). In case 'B', while CV1 × CV2 showed the differences between sexes (Figure 3), CV2 × CV3 clearly separated areas (Figure 4). In the CV1 axis, all females assume positive scores and all males assume negative scores. This is related to position of umbilicus, genital slit and anus. CV2 discriminates FMA III from FMA I and II. It is probable that in specimens from FMA III the dorsal fin is further backwards since the sampled specimens from FMA III show positive scores to S-TDF and the sampled specimens of other areas assume negative scores. In contrast, the anterior portion of the body seems to be longer in specimens from FMA I. CV3 discriminates mainly FMA I from FMA II, but when plotted against CV2, differences between three areas can be observed.

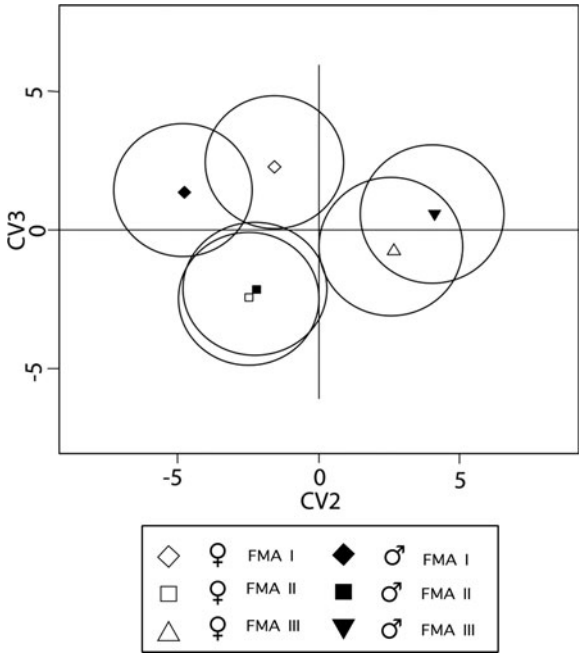

Fig. 4. 95% confidence contours from individual scores of canonical variates CV2 × CV3.

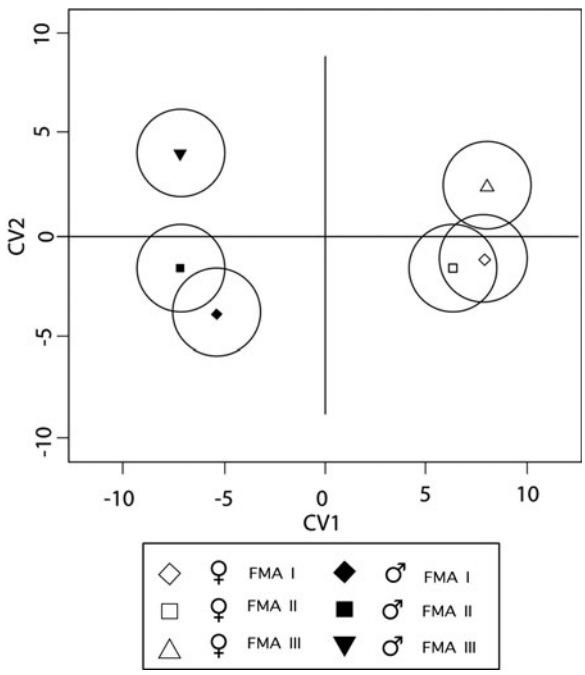

Fig. 3. 95% confidence contours from individual scores of canonical variates CV1 × CV2.

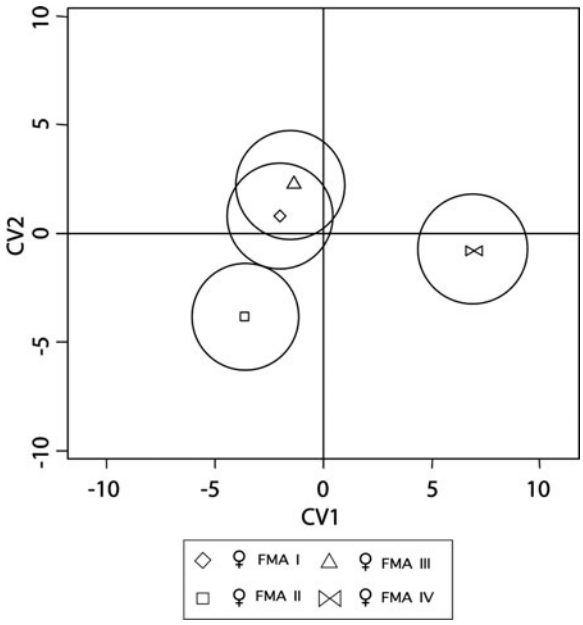

Fig. 5. 95% confidence contours from individual scores of canonical variates CV1 × CV2.

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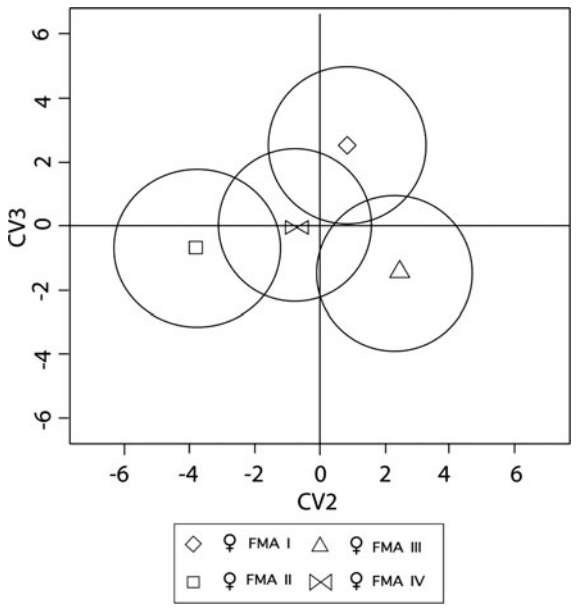

Fig. 6. 95% confidence contours from individual scores of canonical variates CV2 × CV3.

Specimens from FMA I assume positive scores on CV3 while specimens from FMA II, negative scores, suggesting that the characteristics separating FMA I franciscanas from the others are related to beak size.

For case 'F', CV1 clearly discriminates females from FMA IV from all others, but CV2 does not. Individual scores from FMA IV on CV1 were all positive, while for other areas scores are negative or near null (Figures 5 & 7). Similar results were obtained with PC12, the component of MGPCA that mostly contributed to the discrimination between areas. CV2 allowed for discriminating females from FMA II from the others (Figure 6). The differences detected by CV2 suggest that the beak of females from FMA II is shorter than females from other areas. CV3 establishes positive coefficients of high magnitude with S-CE and negative coefficients with TL. This suggests the same pattern of morphological differences found in case 'B' analyses. Females from FMA I assume positive scores and females from FMA III negative scores (Figures 6 & 7), indicating that the former have larger S-CE, suggesting that the beak in this area is longer than in the other areas. On the other hand, females from FMA III attain the longest body lengths. Females from FMA IV have an intermediate total length between FMA I and III, which also can to be verified by mean absolute values of body size to these areas. Females from FMA II are smaller than females from all other areas.

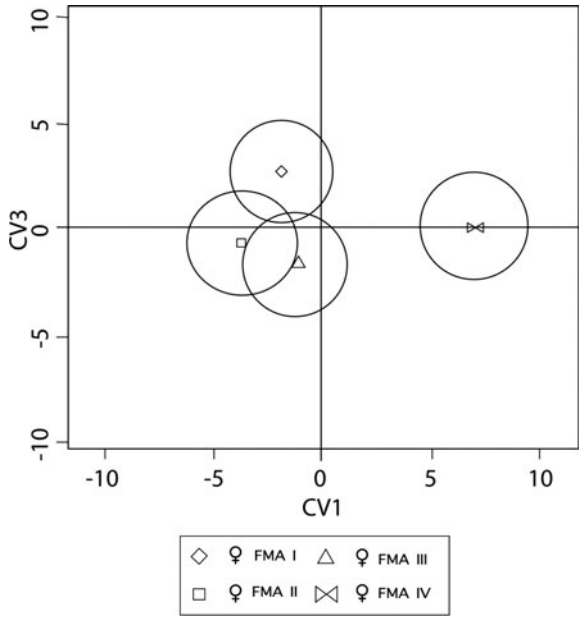

Fig. 7. 95% confidence contours from individual scores of canonical variates CV1 × CV3.

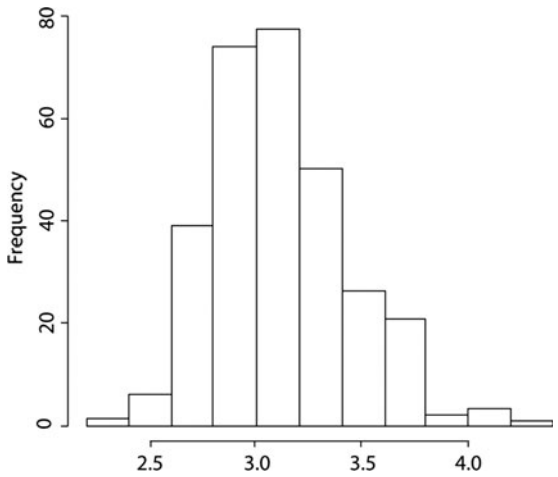

Fig. 8. Frequency distribution of mean Mahalanobis distance between centroids, derived through 300 Monte Carlo simulations.

Monte Carlo simulation

The distribution of the average Mahalanobis distances between the defined centroids for CV1, CV2, and CV3, obtained from Monte Carlo simulation, showed a mean of 3.11 with the minimum and maximum values of 2.23 and 4.38, respectively (Figure 8). The real data average distance was 7.71. This value is considerably higher than the highest simulated value, suggesting that the observer-effect is relatively small and that between-group differences obtained in the analyses are likely to represent true spatial variation.

DISCUSSION

Differences in external morphology of franciscanas between distinct locations, as well as, between sexes are significant. Individual scores calculated for PC1 produced higher values for females than males in FMA I, II and III, indicating that females are larger than males. This has already been observed in other studies throughout most of the species range (e.g. Kasuya & Brownell, 1979; Pinedo, 1995; Ramos *et al.*, 2000, Barreto & Rosas, 2006), demonstrating the existence of sexual dimorphism in size for this species. Larger females are also observed in other species of small cetaceans, such as harbour porpoise (*Phocoena phocoena*), the

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vaquita (*Phocoena sinus*) and the dolphins of the genus *Cephalorhynchus* (Hohn *et al.*, 1996; Read & Tolley, 1997; Dawson, 2002). In franciscanas, the selection pressure associated with larger females might be related to the need of giving birth to larger calves which would increase their chances of survival (Slooten, 1991; Chivers, 2009).

Besides the differences in total length, other differences between males and females included body shape and a more elongated anterior body in females. Females in FMAs I, II and III had larger mean body shape values than males, corroborating other studies (Pinedo, 1995; Ramos *et al.*, 2002). Marked differences were found between males and females from FMA III, including a more elongated anterior portion of the body, which may reflect a relatively longer beak in females. Pinedo (1995) observed that females from Rio Grande do Sul had larger body size, beak length and distance between the blowhole and dorsal fin basis than males. Males, on the other hand, showed taller dorsal fins. Other male odontocetes have taller dorsal fins than females (e.g. *Orcinus orca* (Ford *et al.*, 2000) and *Phocoena dioptrica* (Goodall & Schiavini, 1995)). However, the differences in dorsal fin height between males and females in franciscanas are not as evident as that observed in the other species, and should not be regarded as functional sexual dimorphism.

The relative position of umbilicus, genital aperture and anus were the characteristics that allowed splitting markedly the sexes. However, the position of genital aperture, occupying a more forward position in males is a common characteristic in cetaceans (e.g. Sergeant, 1962; Perrin, 1975; Yonekura *et al.*, 1980) and most mammals (Pough *et al.*, 2005).

Graphical representation of 95% confidence contours established for the mean scores, obtained with CVA, allowed documenting the main similarities and differences between populations. CV2 × CV3 produced similar results to those observed with MGPCA scores. Morphological difference related to dorsal fin position and beak length were the most evident results (Figure 4). The dorsal fin is located further backwards in individuals from FMA III than FMA I, and the beak was longest in animals from FMA I. Variations in the anterior measurements of the body strongly affected by condylobasal length (Perrin, 1975), can be related to changes in oral apparatus and feeding habits. In fact, snout to centre of eye length and snout to external auditory meatus length contribute to explain CV2 and CV3. Besides PC9 and PC10, the principal components that best discriminate areas, for males and females, respectively, are defined by the measurements snout to angle of gape, snout to anterior insertion of flipper, snout to centre of eye and snout to external auditory meatus. These differences could indicate that franciscanas from different areas show morphological differences related to distinct feeding habits. The diet of individuals from Rio de Janeiro and Espírito Santo (FMA I) is considerably different from that of animals from Rio Grande do Sul, Uruguay and Argentina. Franciscanas in the southernmost portion of their distribution feed mainly on demersal species (Fitch & Brownell, 1971; Rodriguez *et al.*, 2002; Bassoi, 2005), while franciscanas from Rio de Janeiro prey more often upon pelagic species (Di Beneditto & Ramos, 2001). Morphological differences related to anterior dimensions of the body detected in this study discriminate, mainly, FMA I and FMA III. Although morphological differences have been detected in FMA II, a biological explanation for such differences could not be determined.

Morphological differences between females from FMA IV and females from the other areas (Figures 5, 6 & 7) provide support for the hypothesis that the population from northern Argentina can be treated as a distinct unit as proposed by Secchi *et al.* (2003). Females from FMA IV show intermediary values between FMA I and FMA III in regard to anterior measurements of the body, which are related to condylobasal length and body size. Although the absolute mean values of the measurements associated with condylobasal length (with the exception of snout to centre of blowhole length) in females from FMA IV are close to those from FMA III and lower than those from FMA I, graphical representation of 95% confidence contours of the mean individual scores to CV2 shows more similarities between FMA IV and FMA I (Figures 5 & 6). This difference can be explained by the character snout to tip of dorsal fin length, which also influences CV2. This measure points to differences in position of the dorsal fin among all areas.

Differences in size and shape constitute valuable information that can be used along with genetic, parasite and contaminant loads, feeding ecology and reproductive biology data to help identify discrete units for management and taxonomic purposes (e.g. Dizon *et al.*, 1992; Moritz, 1994; Avise, 2000; Wang, 2002). Congruent differences were found by Wang *et al.* (2000a, b) and Baker *et al.* (2002), using genetic, osteological and morphological data between two sympatric forms of bottlenose dolphins in the waters of China and between populations of Hector's dolphin from New Zealand, respectively.

In the southern portion of the species range, a high genetic similarity among populations from Argentina, Uruguay and the States of Rio Grande do Sul and southern Santa Catarina was observed (Ott, 2002), suggesting the existence of only one large genetic population throughout this area, which spreads out from the southernmost part of FMA II to FMA IV. The greatest levels of genetic differentiation that were observed within this region were around ten times lower than differences between these areas altogether and populations from São Paulo and Paraná States, Brazil (Ott, 2002). Lázaro *et al.* (2004) also detected substantial levels of gene flow between populations from Rio Grande do Sul, Uruguay and Argentina. Like Ott (2002) and Lázaro *et al.* (2004), our findings do not support the split of populations from Argentina, Uruguay and Rio Grande do Sul into two distinct management areas as proposed by Secchi *et al.* (2003). Our results, however, constitute morphological evidence that supports splitting part of FMA IV from FMA III as distinct management areas. The sampled specimens in San Clemente and Cabo San Antonio, located in northern Buenos Aires Province, are morphologically different from those of FMA III and more similar to individuals from FMA I in terms of body size and measurements related to the anterior portion of the body (Figures 5, 6 & 7). Furthermore, Mendez *et al.* (2007) compared mitochondrial DNA from distinct locations in Argentina with data from Lázaro *et al.* (2004), finding evidence for the presence of at least two genetically different populations within FMA IV: San Clemente and Claromecó. The population from northern Buenos Aires Province constitutes the most isolated population from Argentina and this is in agreement with morphological differences found here.

The specimens from Argentina used in this study were mostly the same individuals as those sampled for the genetics study by Mendez *et al.* (2007) and had been incidentally

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caught in gillnet fisheries operating in estuarine waters of the La Plata River (Samborombón Bay). A similar pattern of genetic differentiation between estuarine and marine coastal individuals has recently been documented by Costa *et al.* (2008) in Uruguayan waters.

Morphological differences found in this study and the genetic differences found by Mendez *et al.* (2007) between populations from Argentina and the other populations could be reflecting a possible ecological separation or habitat partitioning, at least around the boundary between oceanic and estuary waters near the La Plata River mouth. Such segregation could be related to the use of different feeding resources. The diet and parasite infestation levels were more similar between Rio Grande do Sul and Uruguay than between any of these two areas and Argentina (Fitch & Brownell, 1971; Aznar *et al.*, 1995; Andrade *et al.*, 1997; Rodriguez *et al.*, 2002; Bassoi, 2005). Differences in diet were also important between individuals collected in the estuary waters of Samborombón Bay and those sampled in the oceanic coastal waters of Argentina (Rodriguez *et al.*, 2002).

Systematic errors from the observer when following measurement protocols can be considered to have had, at most, only minor effects on the morphological differences observed here. Inter-observer systematic errors were shown by the simulation study to be negligible when compared to variations between FMAs. However, if feasible, calibration experiments prior to the study are recommended in order to increase accuracy of the analyses. To reduce the effects of stochasticity larger sample sizes are also desirable. The level of morphological differentiation observed between the sampled populations in this study, agrees with some results of genetic studies and gives additional support for the separation of the proposed FMA I, II and III and, at least, part of FMA IV. Populations from these areas represent distinct demographic entities and, therefore, must be managed independently to guarantee the maintenance of intraspecific genetic variability.

Furthermore, the occurrence of unique haplotypes in the population from Rio de Janeiro, negligible gene flow with adjacent areas, the distinct reproductive pattern, the difference in growth and demographic parameters and the indications of a geographical isolation (Secchi *et al.*, 1998; Di Beneditto & Ramos, 2001; Ott, 2002; Siciliano *et al.*, 2002), constitute evidence indicating that FMA I is a distinct evolutionarily significant unit (*sensu* Ryder, 1986). Franciscanas from FMA I might be considered a distinct subspecies, if the same criteria used by Baker *et al.* (2002) are adopted, which are congruent lines of morphological and genetic evidences and a relatively short time of divergence to the occurrence of an event of speciation. Phylogenetic analysis of haplotypes carried out by Lázaro *et al.* (2004) pointed out that one of the haplotypes found in the population from Rio de Janeiro is more closely related to the haplotypes found in the southern populations than those of the population from Rio de Janeiro, suggesting that these populations separated recently in their evolutionary paths.

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REFERENCES

Andrade A.L.V., Pinedo M.C. and Pereira J. Jr (1997) The gastrointestinal helminths of franciscana, *Pontoporia blainvillei*, in Southern Brazil. *Reports of the International Whaling Commission* 47, 669–673.

Avise J.C. (2000) *Philogeography: the history and formation of species*. London: Harvard University Press.

Aznar F.J., Raga J.A., Corcuera J. and Monzon F. (1995) Helminths as biological tags for franciscanas *Pontoporia blainvillei* (Cetacea: Pontoporiidae) in Argentinian and Uruguayan waters. *Mammalia* 59, 427–435.

Baker A.L., Smith A.N.H. and Pichler F.B. (2002) Geographical variation in Hector's dolphin: recognition of new subspecies of *Cephalorhynchus hectori*. *Journal of the Royal Society of New Zealand* 32, 713–727.

Barreto A.S. and Rosas F.C.W. (2006) Comparative growth analysis of two populations of *Pontoporia blainvillei* on the Brazilian coast. *Marine Mammal Science* 22, 644–653.

Bassoi M. (2005) *Feeding ecology of franciscana dolphin*, Pontoporia blainvillei *(Cetacea: Pontoporiidae), and oceanographic processes on the Southern Brazilian coast.* PhD thesis. University of Southampton, Southampton, UK.

Chatfield C. and Collins A.J. (1980) *Introduction to multivariate analysis*. London: Chapman & Hall.

Chivers S.J. (2009) Cetacean life history. In Perrin W.F., Würsig B. and Thewissen J.G.M. (eds) *Encyclopedia of marine mammals*. San Diego, CA: Academic Press, pp. 215–220.

Christensen I., Haug T. and Wiig O. (1990) Morphometric comparison of minke whales *Balaenoptera acutorostrata* from different areas of the North Atlantic. *Marine Mammal Science* 6, 327–338.

Costa P., Lessa E.P. and Secchi E.R. (2008) Microestrutura poblacional del delfin franciscana, *Pontoporia blainvillei.* In *XIII Reunión de Trabajo de Especialistas en Mamíferos Acuáticos de América del Sur, SOLAMAC, Montevideo, 13–17 Octubre*, p. 126.

Dawson S.M. (2002) *Cephalorhynchus* dolphins *Cephalorhynchus heavisidii*, *C. eutropia*, *C. hectori* and *C. commersonii*. In Perrin W.F., Würsig

https://doi.org/10.1017/S0025315411000725 Published online by Cambridge University Press

B. and Thewissen J.G.M. (eds) *Encyclopedia of marine mammals*. San Diego, CA: Academic Press, pp. 200–203.

Di Beneditto A.P.M. and Ramos R.M.A. (2001) Biology and conservation of the franciscana (*Pontoporia blainvillei*) in the north of Rio de Janeiro State, Brazil. *Journal of Cetacean Research and Management* 3, 185–192.

Dizon A.E., Lockyer C., Perrin W.F., Demaster D.P. and Seisson J. (1992) Rethinking the stock concept: a phylogeographic approach. *Conservation Biology* 6, 24–36.

Fitch J.E. and Brownell R.L Jr. (1971) Food habits of the franciscana *Pontoporia blainvillei* (Cetacea: Platanistidae) from South America. *Bulletin of Marine Science* 21, 626–636.

Ford J.K.B., Ellis G.M. and Balcomb K.C. (2000) *Killer whales: the natural history and genealogy of* Orcinus orca *in the waters of British Columbia and Washington*. 2nd edition. Vancouver: UBC Press.

Gao A., Zhou K. and Wang Y. (1995) Geographical variation in morphology of bottlenose dolphins (*Tursiops* sp.) in Chinese waters. *Aquatic Mammals* 21, 121–135.

Goodall N.R.P. and Schiavini A.C.M. (1995) On the biology of the spectacled porpoise, *Australophocaena dioptrica*. *Reports of the International Whaling Commission* Special Issue 16, 411–453.

Heyning J.E. and Perrin W.F. (1994) Evidence for two species of common dolphins (genus *Delphinus*) from the eastern North Pacific. *Contributions in Science, Natural History Museum of Los Angeles County* 442, 1–35.

Higa A., Hingst-Zaher E. and De Vivo M. (2002) Size and shape variability in the skull of *Pontoporia blainvillei* (Cetacea: Pontoporiidae) from Brazillian Coast. *Latin American Journal of Aquatic Mammals* Special Issue 1, 145–152.

Hohn A.A., Read R.J., Fernandez S., Vidal O. and Findley L.T. (1996) Life history of the vaquita, *Phocoena sinus* (Phocoenidae, Cetacea). *Journal of Zoology* 239, 235–251.

Jefferson T.A. and Van Waerebeek K. (2004) Geographic variation in skull morphology of humpback dolphins (*Sousa* spp.). *Aquatic Mammals* 30, 3–17.

Kasuya T. and Brownell R.L. (1979) Age determination, reproduction and growth of the franciscana dolphin, *Pontoporia blainvillei*. *Scientific Reports of the Whales Research Institute* 31, 45–67.

Kasuya T., Miyashita T. and Kasamatsu F. (1988) Segregation of two forms of short-finned pilot whales off the Pacific coast of Japan. *Scientific Reports of the Whales Research Institute* 39, 77–90.

Lázaro M., Lessa E.P. and Hamilton H. (2004) Geographic genetic structure in the franciscana dolphin (*Pontoporia blainvillei*). *Marine Mammal Science* 20, 201–214.

Malhotra A. and Thorpe R.S. (1997) Size and shape variation in a Lesser Antillean anole, *Anolis oculatus* (Sauria: Iguanidae) in relation to habitat. *Biological Journal of the Linnean Society* 60, 53–72.

Manly B.F.J. (1994) *Multivariate statistical methods: a primer*. 3rd edition. London: Chapman & Hall.

Mendez M., Rosembaum H.C. and Bordino P. (2007) Conservation genetics of the franciscana dolphin in Northern Argentina: population structure, by-catch impacts and management implications. *Conservation Genetics* 9, 419–435.

Moritz C. (1994) Defining evolutionarily 'significant units' for conservation. *Trends in Evolutionary Ecology* 9, 373–375.

Norris K.S. (1961) Standardized methods for measuring and recording data on the smaller cetaceans. *Journal of Mammalogy* 42, 471–476.

Ott P.H. (2002) *Diversidade genética e estrutura populacional de duas espécies de cetáceos do Atlântico Sul Ocidental*: Pontoporia blainvillei *e* Eubalaena australis. PhD thesis. Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.

Perrin W.F. (1975) Variation and taxonomy of spotted and spinner porpoise (genus *Stenella*) in the eastern tropical Pacific and Hawaii. *Bulletin of the Scripps Institution of Oceanography* 21, 1–206.

Perrin W.F., Akin P.A. and Kashiwada J.V. (1991) Geographic variation in external morphology of the spinner dolphin *Stenella longirostris* in the Eastern Pacific and implications for conservation. *Fishery Bulletin* 89, 411–428.

Perrin W.F., Dolar M.L.L. and Amano M. (2003) Cranial sexual dimorphism and geographic variation in Fraser's dolphin, *Lagenodelphis hosei*. *Marine Mammal Science* 19, 484–501.

Pinedo M.C. (1991) *Development and variation of the franciscana*, Pontoporia blainvillei. PhD thesis. University of California, Santa Cruz, USA.

Pinedo M.C. (1995) Development and variation in external morphology of the franciscana, *Pontoporia blainvillei*. *Revista Brasileira de Biologia* 55, 85–96.

Pough F.H., Janis C.M. and Heiser J.B. (2005) *Vertebrate life*. 7th edition. New York: Prentice-Hall.

Ramos R.M.A., Di Beneditto A.P.M. and Lima N.R.W. (2000) Growth parameters of *Pontoporia blainvillei* and *Sotalia fluviatilis* (Cetacea) in northern Rio de Janeiro, Brazil. *Aquatic Mammals* 26, 65–75.

Ramos R.M.A., Di Beneditto A.P.M., Siciliano S., Santos M.C.O., Zerbini A.N., Bertozzi C., Vicente A.F.C., Zampirolli E., Alvarenga F.S. and Lima N.R.W. (2002) Morphology of the franciscana (*Pontoporia blainvillei*) off Southeastern Brazil: sexual dimorphism, growth and geographic variation. *Latin American Journal of Aquatic Mammals* 1, 129–144.

Read A.J. and Tolley K.A. (1997) Postnatal growth and allometry of harbour porpoises from the Bay of Fundy. *Canadian Journal of Zoology* 75, 122–130.

R Development Core Team (2005) *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. ISBN 3-900051-07-0, URL: http://www.R-project.org

Reyment R.A., Blackith R.E. and Campbell N.A. (1984) *Multivariate morphometrics*. 2nd edition. London: Academic Press.

Rodriguez D., Rivero L. and Bastida R. (2002) Feeding ecology of the franciscana (*Pontoporia blainvillei*) in marine and estuarine waters of Argentina. *Latin American Journal of Aquatic Mammals* Special Issue 1, 77–94.

Rosas F.C.W. and Monteiro-Filho E.L.A. (2002) Reproductive parameters of *Pontoporia blainvillei* (Cetacea, Pontoporiidae) on the coast of São Paulo and Paraná States, Brazil. *Mammalia* 66, 231–245.

Ryder O. A. (1986) Species conservation and systematics: the dilemma of subspecies. *Trends in Ecology and Evolution* 1, 9–10.

Secchi E.R., Danilewicz D. and Ott P.H. (2003) Applying the phylogeographic concept to identify franciscana dolphin stocks: implications to meet management objectives. *Journal of Cetacean Research and Management* 5, 61–68.

Secchi E.R., Wang J.Y., Murray B.W., Rocha-Campos C.C. and White B.N. (1998) Population differentiation in the franciscana (*Pontoporia blainvillei*) from two geographic locations in Brazil as determined from mitochondrial DNA control region sequences. *Canadian Journal of Zoology* 76, 622–627.

Sergeant D.E. (1962) On the external characters of the blackfish or pilot whales (genus *Globicephala*). *Journal of Mammalogy* 32, 395–413.

Siciliano S., Di Beneditto A.P.M. and Ramos R.M.A. (2002) A toninha, *Pontoporia blainvillei* (Mammalia, Cetacea, Pontoporiidae), nos estados do Rio de Janeiro e do Espírito Santo, costa sudeste do

https://doi.org/10.1017/S0025315411000725 Published online by Cambridge University Press

Brasil: caracterizações dos habitats e fatores de isolamento das populações. *Boletim do Museu Nacional, Zoologia* 47, 1–15.

Slooten E. (1991) Age, growth, and reproduction in Hector's dolphins. *Canadian Journal of Zoology* 69, 1689–1700.

Thorpe R.S. (1983a) A review of the numerical methods for recognizing and analyzing racial differentiation. In Felsenstein J. (ed.) *Numerical taxonomy: Proceedings of a NATO Advanced Studies Institute*. NATO ASI Series, pp. 404–423.

Thorpe R.S. (1983b) A biometric study on the effects of growth on the analyis of geographic variation: tooth number in green geckos (Reptilia: Phelsuma). *Journal of Zoology* 201, 13–26.

Thorpe R.S. (1988) Multiple group principal component analysis and population differentiation. *Journal of Zoology* 216, 37–40.

Thorpe R.S. and Leany L. (1983) Morphometric studies in inbred and hybrid house mice (*Mus* sp): multivariate analysis of size and shape. *Journal of Zoology* 199, 421–432.

Thorpe R.S., Corti M. and Capanna E. (1982) Morphometric divergence of Robertsonian population/species of *Mus*: a multivariate analysis of size and shape. *Experientia* 38, 920–923.

Wang J.Y. (2002) Stock identity. In Perrin W.F., Würsig B. and Thewissem J.G.M. (eds) *Encyclopedia of marine mammals*. San Diego, CA: Academic Press, pp. 1189–1192.

Wang J.Y., Chou L.S. and White B.N. (1999) Mitochondrial DNA analysis of sympatric morphotypes of bottlenose dolphins (genus *Tursiops*) in Chinese waters. *Molecular Ecology* 8, 1603–1612.

Wang J.Y., Chou L.S. and White B.N. (2000a) Osteological differences between two sympatric forms of bottlenose dolphins (genus *Tursiops*) in Chinese waters. *Journal of Zoology* 252, 147–162.

Wang J.Y., Chou L.S. and White B.N. (2000b) Differences in the external morphology of two sympatric species of bottlenose dolphins (genus *Tursiops*) in the Waters of China. *Journal of Mammalogy* 81, 1157–1165.

and

Yonekura M., Matsui S. and Kasuya T. (1980) On the external characters of *Globicephala macrorhynchus* off Taiji, Pacific coast of Japan. *Scientific Reports of the Whales Research Institute* 32, 67–95.

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