

## Morphological analysis of three *Inia* (Cetacea: Iniidae) populations from Colombia and Bolivia

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We present new morphological data on three Colombian and Bolivian *Inia* populations. Analysis of morphometric data strongly suggests that *Inia* are sexually dimorphic, with males smaller and more heterogeneous than females. Our morphological data supported that Colombian Amazon males had the greatest total body length as well as greatest fluke values (tip to tip) compared to the males from the Orinoco and Bolivia Rivers. There were also significant differences in females among populations. The two Colombian groups (Amazon and Orinoco basin) presented greater total body length than the Bolivian array, while distance from jaw tip to tip of dorsal fin was greater in the Orinoco basin females than in the other two populations. A Canonic Population Analysis showed that the confidence intervals of the Colombian Amazon and Orinoco males were partially superposed. However, the Bolivian male sample was remarkably differing from these two populations. Two long standing debates exist in the systematic literature on *Inia*: (1) the number of extant species (one, *Inia geoffrensis*, or two *I. geoffrensis* and *I. boliviensis*) and (2) the number of extant subspecies of *I. geoffrensis* (*I. geoffrensis geoffrensis*, *I. geoffrensis humboldtiana* and *I. geoffrensis boliviensis*). Using recent collections of both molecular and morphometric data, we suggest the existence of two distinct species of *Inia* with no subspecific distinction among *I. geoffrensis* populations.

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### Introduction

Several morphological studies have attempted to determine the systematic relationship of *Inia* (pink dolphin) populations present in South American rivers (Casinos and Ocaña

1979, Da Silva 1994, Hershkovitz 1966, Pilleri 1969, 1979, Pilleri and Gihir 1969, 1976, 1977, 1981, Trebbau and Van Bree 1974, Van Bree and Robineau 1973). There is no consensus concerning either the number of extant species of *Inia* or the number of subspecies within one of the species, *Inia geoffrensis*. However, all stud-

ies have restricted the number of distinct species to either one species *Inia geoffrensis* (De Blainville, 1817) in the Orinoco basin and in the Amazon basin from Perú, Ecuador, Colombia, Brazil and Bolivia with three subspecies, *I. g. geoffrensis* in the Amazon basin from Perú, Ecuador, Colombia and Brazil, *I. g. humboldtiana* in the Orinoco basin from Venezuela and Colombia and *I. g. boliviensis* in the Bolivian Amazon basin) or two species *Inia boliviensis* (D'Orbigny, 1834) in Bolivia as a different species in regard to *I. geoffrensis*.

Until the work of Pilleri and Ghir (1977), the main point of view followed the contention of Hershkovitz (1966) that *Inia* is a monotypic species. In fact, the study of Pilleri and Ghir (1969) resulting from their first expedition to Bolivia was based on the taxonomic assessments of Hershkovitz (1966). Nevertheless, these authors noted certain important differences between the Bolivian and the Amazon forms of *Inia*. Casinos and Ocaña (1979) and Best and da Silva (1993) recognized one species (*Inia geoffrensis*) and two subspecies (*I. geoffrensis geoffrensis* and *I. geoffrensis boliviensis*) or three (*I. g. geoffrensis*, *I. g. boliviensis* and *I. g. humboldtiana*), respectively. Conversely, Pilleri and Ghir (1977, 1981), Da Silva (1994), and Grabert (1984) recognized two species (*I. geoffrensis* and *I. boliviensis*) based on color, length-mass ratio, cranial characters, number of teeth, brain size and cephalic index, shape of sternum and several biochemical characters. However, these last studies were criticized because they were based on sample sizes deemed too small for rigorous analyses and on characters too variable to be taxonomically useful.

To try to resolve these taxonomic problems, we recently analyzed the molecular composition and diversity of the mitochondrial *Cyt-b* (600 bp) and D-loop (570 bp) genes (Banguera-Hinestroza *et al.* 2002) of 96 pink dolphins caught in the Colombian Orinoco, Colombian Amazon and Bolivian Amazon Rivers and concluded that: (1) there are two distinct species of *Inia* (*I. geoffrensis* and *I. boliviensis* – average genetic differentiation for the mitochondrial D-loop and the *Cyt-b* genes of 3.6%), and (2) there appear to be no subspecific divisions

within the populations of *I. geoffrensis* from Amazon and Orinoco basins (average genetic differentiation for the mitochondrial D-loop and the *Cyt-b* genes of 0.95%).

Our conclusions based on molecular data were not completely congruent with any previous morphometric study. Therefore, we endeavored to find validation for our molecular results by collecting morphometric data from 66 of the 96 individuals captured for the molecular study. This sample size represents the largest sample of *Inia* used for morphometric analysis. Until now, the largest available data set analyzed consisted of only 16–20 animals collected from 1790 to 1985. In addition, it is likely that some of these individuals were used more than once in the previous analysis. These records were few and scattered; they included: one specimen from Guayabero River (Meta, Colombia – Olivares 1962), one from Dudita Caño (tributary Gueyar River, eastern side of the Macarena Mountains, Colombia; Mohr 1964), six individuals from Leticia (Colombian Amazon – Layne 1956, and Cadwell 1966), and two from Medem in 1953 (in Guaviare River, Meta-Vaupés, Colombia, and reported by Pilleri and Ghir 1977), and five specimens belonging to the Instituto de Ciencias Naturales of Universidad Nacional from Bogotá, Colombia (Casinos and Ocaña 1979), some of which were obtained from collections listed above.

The objective of our study was twofold. First, within a morphometric context, we wanted to determine the number of species and subspecies that were represented by the currently available collections of *Inia*. Second, to compare these results with those of our recently completed molecular studies on many of these same specimens which may strengthen our systematic conclusions by using multiple data sets.

## Material and methods

### Specimens examined

We examined a total of 66 adult *Inia* from three distinct geographic regions. They were as follows: 16 males and 11 females captured in several Bolivian Amazon lagoons in the Mamoré River and in two locations along the Ipurupuru (af-

fluent of the Guaporé River) and the Tijamuchi rivers, 13 males and 14 females from the Colombian Amazon (Putumayo River, a transect of 443 km from Puerto Leguizamo to Puerto Encanto), and 5 males and 7 females from the Colombian Orinoco basin (Orinoco, Guaviare, Inirida and Arauca rivers – Fig. 1). This represents the largest sample of this taxon available for analysis to date measured by the same researchers in a considerable area of the geographic distribution of this dolphin. All specimens were captured using special fishing nets (length of 300 meters and length of 10 meters) taking special care to ensure the physical safety of each dolphin. Dolphins were brought on board and measured with a flexible ruler. The males and the females were independently studied to avoid variation due to sexual dimorphism, although Da Silva (1994) claimed that the sexual dimorphism is relatively low among *Inia* individuals.

Nine external measurements were taken from all dolphins; three additional measurements were taken for males and one for females. The height of dorsal fin, the length from the tip of jaw to middle of eye and the position of genital aperture are highly dimorphic between sexes (Pilleri and Ghir 1969, Da Silva 1994). Therefore, measurements of these traits differed between sexes. Measurements included: all dolphins – total length of the body (TL), length from the tip of jaw to melon (LTJM), length from the tip of jaw to mouth origin (LTJMO), length from the tip of jaw to spiracle (LTJS), width of left pectoral flipper (WPF), length

of left pectoral flipper (LLF), length of dorsal fin (LDF), width of fluke (WF), and flukes, tip to tip (FIT); males only – length from the tip of jaw to middle of eye (LTJME), length from tip of jaw to the genital aperture (LTJGA), and girth in front of pectoral flippers (GPF), although this last variable was deleted in several analyses; females only – length from the tip of jaw to the tip of the dorsal fin (LTJTDF) (Fig. 2).

### Quantitative analysis

Several statistical analyses were used to detect possible differences from a morphological perspective among the three *Inia* populations. First, elemental statistics such as means and standard deviations were calculated. To determine significant differences between the means for the three different populations studied, an ANOVA and a Kruskal-Wallis one way ANOVA on ranks were carried out. Second, all the variables were included in a multiple linear regression analysis (least square method). The Mahalanobis metric matrix was calculated as well as the mean square distances of each point to the regression hyperplanes,  $R_0^2/n$ , being  $R_0^2 = \sum (y_i - \hat{y}_i)^2$  where  $y_i$  is the prediction of  $y_i$  in function of the regression hyperplane. In addition, the square multiple correlation coefficient  $P^2$  was obtained. Third, to determine homogeneity or heterogeneity (if each one of these populations represented a differ-

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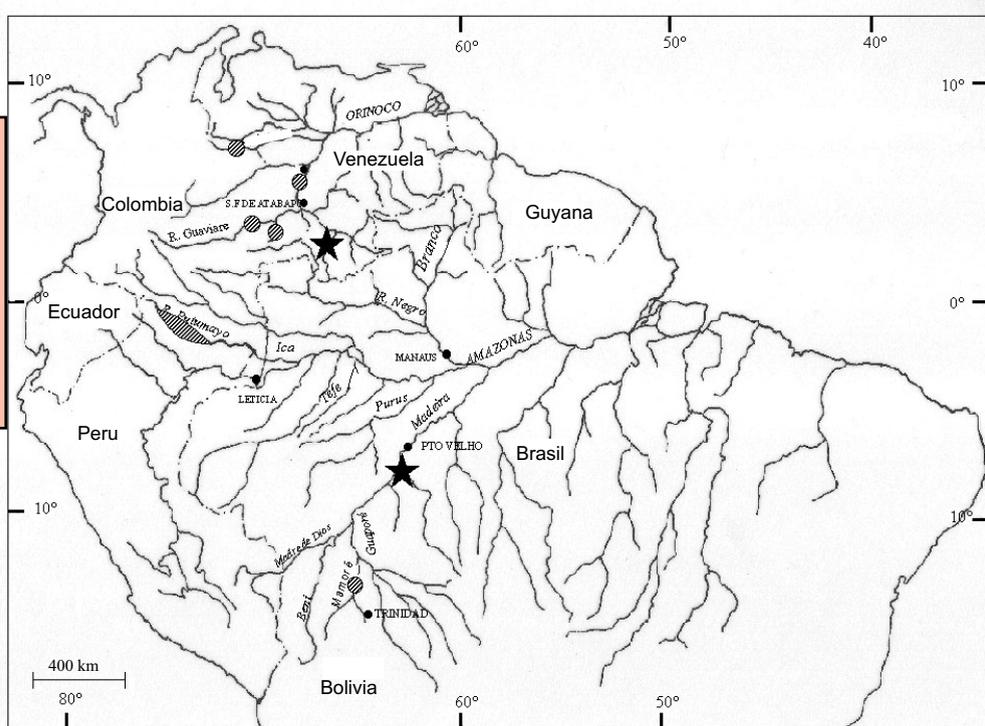


Fig. 1. Map of the Orinoco and Amazon Basins where pink river dolphins were sampled from 1998 to 2003. The shaded areas indicate where dolphins were captured in Colombia and Bolivia. The stars represent the location of obstacles within the Orinoco and Amazon Rivers (and their tributaries) as well as within the Bolivian Amazon and the rest of the Amazon.

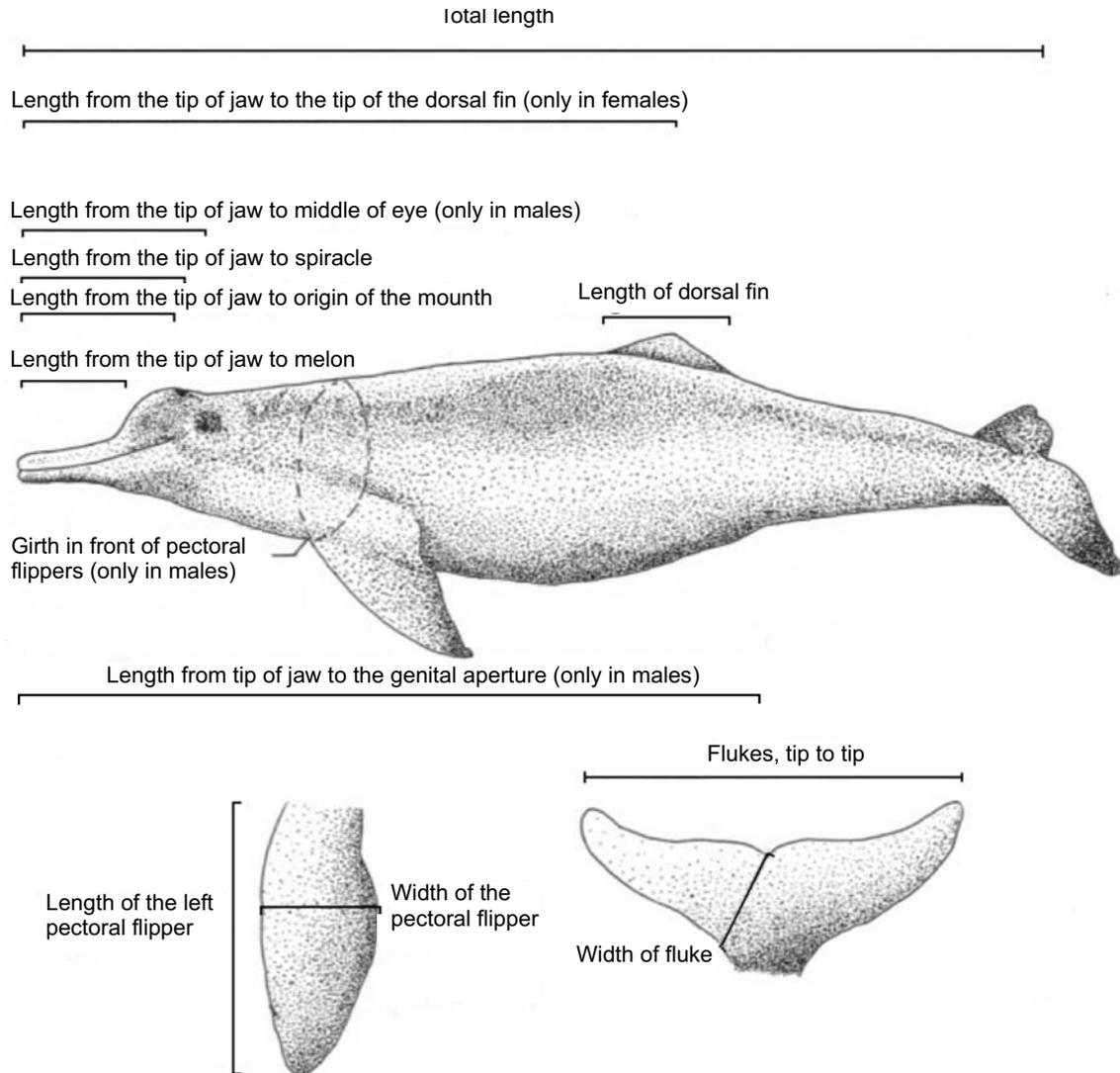


Fig. 2. Morphometric measures taken in pink river dolphins (*Inia*) captured in the Colombian Orinoco and Amazon Rivers and in the Bolivian Amazon from 1998 to 2003. Measures taken in all dolphins studied – total length of the body (TL), length from the tip of jaw to melon (LTJM), length from the tip of jaw to mouth origin (LTJMO), length from the tip of jaw to spiracle (LTJS), width of left pectoral flipper (WPF), length of left pectoral flipper (LLF), length of dorsal fin (LDF), width of fluke (WF), and flukes, tip to tip (FTT); measures taken in males only – length from the tip of jaw to middle of eye (LTJME), length from tip of jaw to the genital aperture (LTJGA), and girth in front of pectoral flippers (GPF), although this last variable was deleted in several analyses; measures taken in females only – length from the tip of jaw to the tip of the dorsal fin (LTJTDF)

ent species or subspecies) of the variance-covariance matrices, the determinant of each one of the variance-covariance matrix was obtained along with the product of the variances for each morphological variable studied. The ratio of both quantities was employed as a homogeneity test as follows:  $\chi^2 = [1 - ((2p^2 + 3p - 1)/(6(p + 1)(s - 1)) (\sum 1/n_i - (1/\sum n_i)))] (\sum n_i \ln |S| - \sum n_{ij} \ln |S_i|)$  with  $1/2(s - 1)p(p + 1)$  degree of freedom, where  $s$  is the number of populations studied,  $n_i$  is the sample size of the  $i$ th population,  $p$  is the number of variables,  $|S|$  is the generalized

determinant and  $|S_i|$  is the determinant of each  $i$ th population.

Lastly, several multivariate procedures were applied to ascertain if the morphological variance within groups was less than the variance among the individuals of the overall *Inia* population. Diverse multivariate approaches were developed to accomplish this task. Factorial (FA) (Harman 1976) and Principal Coordinate Analyses (Gower 1966) were used to determine the feasible relationships among all the individuals and variables studied. We used the iterative

main factor procedure with a precision of  $10^{-6}$  to obtain the factorial matrix and convergence. The factorial matrix was rotated with varimax orthogonal (Kaiser 1958; Harman 1980) and oblique promax (exponent equal to 3) rotations and the communalities were provided.

In addition, the varimax rotation was applied to maximize the factor variance. Each column of the rotated factorial matrix produced several very high weights while others were near zero. This procedure tends to minimize the variable number which have high saturations in a given factor, which facilitates the interpretation of results especially if the factors are uncorrelated. This means that the rotations do not maintain  $90^\circ$  angles. This analysis was employed to analyze the differential influence of size and shape in the three *Inia* populations and in the overall population (Jolicœur and Mosimann 1960, Mardia *et al.* 1979) throughout the variance-covariance and correlation matrices. On the other hand, the communalities explained the contribution of the different factors to the variability of each one of the morphological variables analyzed.

In the second analysis (PCA), we used three different matrix criteria (correlation, variance-covariance and Manhattan distance matrices) with the Gower's (1966) procedure. A Q type PCA was carried out to analyze the relationship among the individuals. Meanwhile an R type of PCA was obtained to analyze the relationship among the variables. For each one of these PCA's, the corresponding eigenvectors and eigenvalues were calculated from the transformed matrices. Various tests of significance were performed to discover whether the first dimensions (coordinates) were significantly different among them. For this, the results of an Anderson's (1963) modification of Bartlett's (1950) test, which measured for the equality of roots, is provided (dimensions corresponding to homogenous roots were ignored). Likewise, the proportions of the variance expected under the broken stick model (Jolliffe 1986) were compared with the observed dimension variances. Those dimensions with higher observed proportions than those of the broken stick model explained more variance than expected due to chance alone.

Furthermore, a canonical analysis of populations (CAP) was used to obtain conclusive differences among the three *Inia* populations analyzed. This method discriminates populations along axes with high power (canonic axes) by using the Mahalanobis distance (Cuadras 1991) and is based on two hypotheses. One hypothesis is that there is homogeneity between all covariance matrices corresponding to the population groups (tested with a maximum-likelihood test). Although several differences were noted with the previously quoted homogeneity test, the violation of this rule was not severe. The second hypothesis, that the populations analyzed were significantly different, was tested by the calculation of Wilk's L and the associate value of the Fisher-Snedecor F test by means of the approximation. Subsequently, the canonical transformation, the eigenvalues and the significance of the first canonical axes by using the Bartlett's test were obtained. In addition, the factorial structure and representation of the canonical variables and the radius of the confidence intervals, for a 90% level, were constructed. The expression for calculating the radius was  $R / N^{1/2}$ , where  $R^2 = F (N - s) n / (N - s - n + 1)$  with  $P (F >$

$F) = 1 - e$  for the Fisher-Snedecor distribution with  $n$  and  $N - s - n - 1$  degrees of freedom ( $N$  - Overall sample size,  $s$  - number of populations considered, and  $n$  - number of variables employed). All of the analyses were independently applied to males and females.

## Results

The morphometric means and standard deviations of the three *Inia* populations studied are provided in Table 1. There were several significant differences in means of the geographical origin of the animals studied. In the case of the males, those coming from the Colombian Amazon showed the greatest length for several measurements and significant heterogeneity was detected among the three populations studied. These were the cases of TL (ANOVA:  $F = 3.84$ ,  $p = 0.043$ ; Kruskal-Wallis ANOVA on ranks ( $H = 5.69$ , 2 df,  $p = 0.05$ ), LTJMO ( $F = 3.99$ ,  $p = 0.032$ ;  $H = 6.10$ , 2 df,  $p = 0.047$ ), LTJGA ( $F = 9.34$ ,  $p = 0.0014$ ;  $H = 8.03$ , 2 df,  $p = 0.018$ ), WPF ( $F = 4.66$ ,  $p = 0.019$ ;  $H = 4.69$ , 2 df,  $p = 0.096$ ), LDF ( $F = 8.70$ ,  $p = 0.0013$ ;  $H = 10.92$ , 2 df,  $p = 0.0042$ ) and FIT ( $F = 5.67$ ,  $p = 0.0093$ ;  $H = 6.89$ , 2 df,  $p = 0.032$ ). The measures which did not show any evidence of statistical heterogeneity among the three male populations were LTJM, LTJS, LTJME, LLF, WF and GPF. Thereby, in general, for an outstanding number of morphological variables the males from the Colombian, especially, and Bolivian Amazon were greater than males from the Orinoco Basin.

The situation among the three female groups of *Inia* was different. In general, females exhibited a greater size than males and had a slightly greater homogeneity compared to males. The morphological measures which did show significant heterogeneities among the three female populations studied were LTJM ( $F = 6.76$ ,  $p = 0.0034$ ;  $H = 9.79$ , 2 df,  $p = 0.0075$ ), LTJMO ( $F = 9.77$ ,  $p = 0.0005$ ;  $H = 13.84$ , 2 df,  $p = 0.001$ ), LTJS ( $F = 9.23$ ,  $p = 0.0007$ ;  $H = 12.66$ , 2 df,  $p = 0.0018$ ), WPF ( $F = 3.43$ ,  $p = 0.044$ ) and WF ( $F = 5.07$ ,  $p = 0.012$ ;  $H = 8.93$ , 2 df,  $p = 0.011$ ). The morphometric variables which did not show heterogeneity were TL, LTJTDF, LLF, LDF and FIT. The Colombian Amazon female group presented the greater measures for the major frac-

Table 1. Morphometric means (cm) and standard deviations of three *Inia* populations studied in the Colombian Orinoco and Amazon Rivers and in the Bolivian Amazon (Mamoré, Tijamuchí and Ipurupuru Rivers) from 1998 to 2003. See text for explanations of abbreviations of morphological variables. \*\* Morphological variables which presented significant heterogeneity among the three populations studied for an ANOVA and for a Kruskal-Wallis one way ANOVA on ranks. \* Significant heterogeneity only for an ANOVA.

Variables	Colombian Orinoco Rivers	Colombian Amazon Rivers	Bolivian Amazon Rivers	Total morphological mean
Males				
TL**	189.67 ± 5.77	203.00 ± 24.93	193.77 ± 9.15	200.05 ± 19.57
LTJM	17.12 ± 1.92	21.92 ± 6.15	20.97 ± 4.30	20.77 ± 5.55
LTJMO**	23.36 ± 3.78	28.08 ± 6.39	29.00 ± 4.47	27.82 ± 6.43
LTJS	29.60 ± 3.31	35.15 ± 9.26	35.13 ± 5.47	34.32 ± 7.76
LTJME	25.40 ± 4.64	31.42 ± 6.81	30.06 ± 4.15	29.90 ± 6.17
LTJGA**	67.50 ± 0.71	92.15 ± 19.42	89.00 ± 9.20	88.44 ± 17.88
WPF*	19.50 ± 4.30	18.65 ± 3.04	16.61 ± 1.83	17.81 ± 3.11
LLF	35.10 ± 2.56	38.35 ± 6.67	37.28 ± 4.15	37.36 ± 5.54
LDF**	6.40 ± 2.36	8.31 ± 2.38	5.85 ± 0.88	6.87 ± 1.94
WF	14.75 ± 3.09	16.40 ± 3.04	14.53 ± 1.39	15.36 ± 2.27
FIT**	43.80 ± 10.31	48.31 ± 10.76	40.88 ± 4.48	44.15 ± 8.51
GPF	84.50 ± 19.79	88.15 ± 14.69	83.61 ± 10.83	85.71 ± 13.00
Females				
TL	205.28 ± 9.16	214.60 ± 13.06	202.20 ± 14.82	205.48 ± 14.98
LTJM**	21.29 ± 2.29	25.29 ± 3.88	24.82 ± 2.71	24.25 ± 3.46
LTJMO**	26.29 ± 2.29	30.57 ± 2.95	33.09 ± 3.75	30.50 ± 4.04
LTJS**	34.29 ± 2.69	39.79 ± 4.14	38.91 ± 3.56	38.28 ± 4.16
LTJTDF	120.40 ± 3.78	115.30 ± 12.73	116.20 ± 9.71	116.70 ± 10.52
WPF*	19.00 ± 1.29	20.07 ± 3.04	18.09 ± 2.02	19.16 ± 2.37
LLF	38.43 ± 3.69	40.93 ± 3.08	40.45 ± 4.03	40.22 ± 3.64
LDF	6.86 ± 1.50	8.21 ± 1.13	6.64 ± 0.97	7.38 ± 1.42
WF**	14.71 ± 0.76	17.00 ± 1.70	15.36 ± 2.50	15.94 ± 2.15
FIT	43.43 ± 3.69	45.36 ± 6.67	44.64 ± 3.38	44.69 ± 4.75

tion of the variables studied. LTJTDF was greater in the Orinoco basin females than in the other two populations ( $p < 0.02$ ).

### Multiple regression equations among the morphological variables studied in three *Inia* populations

In males, most variance of the morphological variables LTJS ( $P^2 = 0.96$ ), LTJM ( $P^2 = 0.94$ ), LTJME ( $P^2 = 0.92$ ) and LTJMO ( $P^2 = 0.91$ ) was attributed to the remaining variables (Table 2). The relationships among the morphological variables within females were dissimilar compared to males. Moreover, in females, the multiple square correlation coefficient of each variable against the remaining variables was dissimilar compared to that observed in males.

In general, the values of  $P^2$  were consistently lower than those observed in males. The variables, which were more conspicuously associated with the remaining morphological variables, were TL ( $P^2 = 0.92$ ) and LTJTDF ( $P^2 = 0.94$ ), which were different to those observed in the males (Table 2).

### Homogeneity tests of Variance-Covariance Matrices

The sample size of the Orinoco male group was not sufficiently large to measure the homogeneity of its variance-covariance regarding these same matrices for the Bolivian and the Colombian Amazon samples. The comparison between both populations showed a determinant ([S]) of 0.014 and 0.000001 for the Colombian

Table 2. Multiple regression equations for each morphological variables with regard to all other variables analyzed in three pink river dolphin populations (*Inia*) studied in the Colombian Orinoco and Amazon Rivers and in the Bolivian Amazon (Mamoré, Tjiamuchi and Ipurupuru Rivers) from 1998 to 2003. TL, LTJM, LTJMO, LTJS, LTLME, LTJGA, WPF, LLF, LDF, WF, FIT, and GPF are the abbreviations of the morphological variables studied. See text for explanations.

Males	
TL	= 0.7438LTJM - 0.1974 LTJMO + 0.4981 LTJS - 3.312 LTJME - 0.07625LTJGA - 0.1844 WPF - 2.239 LLLF + 0.7683 LDF - 1.370 WF + 0.00265 FIT + 15.61
LTJM	= 0.01030TL - 0.3148 LTJMO - 0.5945 LTJS - 0.08944 LTJME - 0.05395LTJGA + 0.03932WPF + 0.03959LLF - 0.3983 LDF - 0.1535 WF - 0.03149 FIT + 3.511
LTJMO	= 0.05168TL - 0.5953 LTJM - 0.06109LTJS - 0.2928 LTJME - 0.05142LTJGA - 0.04002WPF - 0.1513LLF + 0.1505 LDF - 0.2636 WF + 0.1372 FIT - 3.956
LTJS	= 0.008242TL - 0.7104LTJM - 0.03861 LTJMO - 0.3238LTJME - 0.08096LTJGA - 0.1694 WPF - 0.1774 LLLF + 0.6584 LDF + 0.07665WF - 0.03645FIT + 2.505
LTJME	= 0.08004TL + 0.1561LTJM - 0.2702 LTJMO - 0.4729 LTJS - 0.02555LTJGA + 0.2762 WPF + 0.1739 LLLF - 0.6020 LDF + 0.1869 WF - 0.08768 FIT - 4.40
LTJGA	= 0.02904TL + 1.484LTJM - 0.7479 LTJMO - 1.863LTJS + 0.4026 LTJME + 0.9124 WPF - 0.7674 LLLF - 2.504 LDF - 0.02540 WF - 0.1052 FIT - 6.962
WPF	= 0.006141TL + 0.09457LTJM - 0.05090 LTJMO - 0.3409 LTJS - 0.3807 LTJME - 0.07978LTJGA - 0.1187LLF - 0.5861 LDF - 0.1030 WF - 0.1850 FIT - 5.852
LLF	= 0.06252 TL + 0.07677LTJM - 0.1551 LTJMO - 0.2879 LTJS + 0.1932 LTJME - 0.05410LTJGA - 0.09568WPF + 0.07186 LDF - 0.5713 WF + 0.0048665FIT - 4.983
LDF	= 0.008017TL - 0.3001LTJM + 0.05999 LTJMO + 0.4152 LTJS - 0.2599 LTJME - 0.06860LTJGA - 0.1836 WPF + 0.02793LLF - 0.2789 WF - 0.02042 FIT + 3.338
WF	= 0.01964TL - 0.1588LTJM + 0.1443 LTJMO + 0.06638 LTJS + 0.1108 LTJME - 0.0009556LTJGA - 0.04432 WPF - 0.3049 LLLF - 0.3830 LDF - 0.006208 FIT - 3.174
FIT	= 0.0003572TL - 0.3935LTJM - 0.9062 LTJMO - 0.3811 LTJS - 0.6277 LTJME - 0.04779LTJGA - 0.9607 WPF - 0.03135 LLLF - 0.03135 LDF - 0.3386 WF + 0.07494FIT - 3.1411
Females	
TL	= 2.508LTJM - 1.329 LTJMO + 1.542 LTJS - 1.542 LTJTDf - 1.144WPF - 0.03928LLF - 1.337 LDF + 0.02752WF - 0.3319FIT - 17.13
LTJM	= 0.1676TL - 0.4704 LTJMO + -0.4198 LTJS - 0.2938 LTJTDf + 0.1392WPF - 0.08870LLF - 0.8591 LDF + 0.03302WF - 0.01234FIT + 2.719
LTJMO	= 0.1710TL - 0.9059LTJM + 0.2604 LTJS - 0.3760 LTJTDf - 0.2797WPF + 0.05951LLF - 1.007 LDF - 0.3039WF - 0.04754FIT - 7.692
LTJS	= 0.1898TL - 0.7733LTJM + 0.2491LTJMO - 0.3163 LTJTDf - 0.2231WPF - 0.2637LLF - 0.1398 LDF + 0.1121WF - 0.1552FIT - 3.932
LTJTDf	= 0.5542TL + 1.580LTJM - 1.050 LTJMO + 0.9234 LTJS - 0.6910 WPF - 0.09434LLF - 1.349 LDF + 0.1041WF + 0.1331FIT + 6.785
WPF	= 0.1689 TL + 0.3077LTJM - 0.3209LTJMO - 0.2676 LTJS + 0.2839 LTJTDf - 0.1073LLF - 0.4489 LDF - 0.3839WF - 0.1240FIT - 0.5708
LLF	= 0.01226 TL + 0.4142LTJM + 0.1443 LTJMO + 0.6683LTJS - 0.08190LTJDF - 0.2267 WPF + 0.5939 LDF - 0.5781WF - 0.03342FIT - 11.09
LDF	= 0.03224 TL - 0.3100LTJM + 0.1886 LTJMO + 0.02739LTJS - 0.09054LTJDF - 0.0733 WPF + 0.0459 LLLF + -0.1092WF + 0.04370FIT - 0.5551
WF	= 0.002504 TL + 0.04496LTJM - 0.2149 LTJMO + 0.08287LTJS + 0.02636LTJDF - 0.2365 WPF - 0.1686 - 0.04119 LLLF - 0.1452 FIT + 3.654
FIT	= 0.2054 TL - 0.1143LTJM + 0.2286 LTJMO - 0.7804 LTJS + 0.2292LTJDF + 0.5197 - 0.06629 WPF + 1.122 LLLF - 0.987WF - 7.943

and Bolivian Amazon samples respectively. The test for homogeneity of variance-covariance matrices between both samples supported a significant difference of matrices between populations ( $\chi^2 = 103.45$ ,  $df = 78$ ,  $p = 0.03$ ). The inclusion of the Orinoco males enhanced the differences among the variance-covariance matrices regarding the Bolivian male array ( $\chi^2 = 125.48$ ,  $df = 78$ ,  $p = 0.0005$ ).

For an identical procedure in females to that of males, the determinant ( $|S|$ ) was 0.005 and 0.0000005 for the Colombian and Bolivian Amazon groups respectively. The homogeneity among both variance-covariance matrices was rejected ( $\chi^2 = 87.6$ , 55  $df$ ,  $p = 0.0034$ ). When the Orinoco females were pooled with the other Colombian array, the homogeneity was rejected as well ( $\chi^2 = 92.5$ , 55  $df$ ,  $p = 0.0012$ ).

### Factorial analysis

The factorial analysis, using the iterative principal factor, showed the highest communalities on the variables LTJS (0.9488), LTJMO (0.9197), LTJM (0.8946) and LTJME (0.8837) for the overall sample (Table 3). This means that the main factors account for a very high proportion of the variance of these morphological variables. A considerable fraction of all morphological variables had high values with the first factor. The variables that correlated less with this factor were WPF (0.6383) and LDF (0.6819). The fact that all variables showed high positive values on the first factor, and no negative values indicated that this factor represented size. Hence, the variables which greatly influenced the differences in male size were LTJS, LTJM, LLP, LTJME and TL. In addition,

Table 3. Factorial Matrices and communalities from a Factorial Analysis carried out independently for pink river dolphin males and females captured in the Colombian Orinoco and Amazon Rivers and in the Bolivian Amazon (Mamoré, Tijamuchí and Ipurupuru Rivers) from 1998 to 2003. For males, the two main factors were extremely important, whereas for females the four first factors explained an important degree of the variance of the system. See text for explanation of abbreviations.

	Communalities	Factor I	Factor II	Factor III	Factor IV
Males					
TL	0.8172	0.9002	0.0821		
LTJM	0.8946	0.9339	0.1495		
LTJMO	0.9197	0.8846	0.3704		
LTJS	0.9488	0.9448	0.2369		
LTJME	0.8837	0.9130	0.2237		
LTJGA	0.7902	0.8827	0.1050		
WPF	0.5916	0.6383	-0.4291		
LLF	0.8682	0.9317	-0.0095		
LDF	0.6176	0.6819	-0.3906		
WF	0.7050	0.7409	-0.3950		
FIT	0.7095	0.7956	-0.2768		
Females					
TL	0.9037	0.70560	-0.62700	-0.05676	-0.09738
LTJM	1.0000	0.81820	0.26070	-0.52850	-0.07314
LTJMO	0.4891	0.56880	0.09915	-0.22090	0.32710
LTJS	0.8063	0.82230	0.27660	0.01184	0.23120
LTJTDF	0.9647	0.60480	-0.75680	0.06583	0.14800
WPF	0.5085	0.56380	0.09469	0.11130	-0.41140
LLF	0.7031	0.59390	0.16250	0.53410	0.19700
LDF	0.5970	0.65480	0.02814	-0.17330	-0.37070
WF	0.7119	0.73460	0.25890	0.24090	-0.21730
FIT	0.3515	0.54310	0.08164	0.19360	0.11140

the fact, that the second main factor was represented by positive and negative values simultaneously, was a clear indication that this second factor represented shape. The variables which contributed most to the shape were WPF, LDF, WF and LTJMO. The Varimax procedure enhances the most prominent saturations on main factors. Using this procedure, LTJMO, LTJS and LTJME were the variables more related to size. Similarly, the variables more highly related with shape were WPF, LDF and WF. The factorial oblique rotation Promax matrix showed similar results. With this last procedure the correlation among the two main factors was 0.6562. The factorial analysis with the iterative main procedure showed the highest communalities, for the females, on the variables LTJM (1.0000), LTJTDF (0.9647) and TL (0.9037).

In general, the female communalities were considerably smaller than those observed in the males. Several of them were considerably low, such as WPF (0.5085) and FTT (0.3515). Table 3 presents the factorial matrix for the global female array. A similar trend to that detected in the males was discovered. Positive relative elevated scores were presented on the first factor, indicating that this factor describes a size pattern, although the values did not reach the high values found in males. The two highest values were for LTJM (0.8182) and LTJS (0.8223). The second main factor presented positive and negative scores, which could suggest a relationship with the shape although this was not as pronounced to that found in males. The variables most related (though negatively), with shape in females were TL (-0.6270) and LTJTDF (-0.7568). The Varimax procedure showed that LTJM (0.9041) is the most noteworthy variable explaining size and LTJTDF (0.9520) in explaining shape in the females. A similar situation is found for the oblique rotation. Note that the correlation between the two first factors was only 0.3756 in females, while in the males it was 0.6562, thus supporting that relation between size and shape is stronger in males compared to females.

### Principal Coordinates Analysis (PCA)

Table 4 and Fig. 3 show the Q type PCA (individuals related to the principal coordinates) applied to the correlation, variance-covariance and Manhattan distance matrices for the males. The first PCA applied to the correlation matrix showed that the first coordinate explained 51.66% of the variance, meanwhile the three first coordinates explained 87.81% of the variance. Clearly, the first two coordinates explained more variance than that expected in a model by chance (broken-stick model, first coordinate: 12.11% and second coordinate: 9.17%), which suggests very important diverse evolutionary influences on size and shape on all the pink river dolphin individuals studied. For the variance-covariance matrix, the first coordinate explained 74.17% of the variance, meanwhile the three first coordinates reached 92.85% of the overall variance. In this case, the first coordinate presented an extraordinarily higher value with regard to that expected in the broken-stick model (12.11%). Thus, all the individuals were highly differentiated by an epigenetic factor related to size and clearly not at random. Nevertheless, with this procedure the second main coordinate (epigenetic shape factor) was not significant. A similar situation was found by using the Manhattan distance matrix.

For the R-type PCA (morphological variables related to the principal coordinates), the analysis of the significance of the eigenvalues associated to each eigenvector against the remainder eigenvalues from the correlation and the variance-covariance matrices, measured by the Anderson's (1963) modification of Bartlett's (1950) test, offered a clear perspective. For the correlation matrix, the test that analyzed whether all eigenvalues were equal (that is, that all correlations are equal to zero) rejected the null hypothesis ( $\chi^2 = 481.11$ , 66 df,  $p < 0.0001$ ). An additional test (ln of determinate) showed that only the first eigenvalue was different from the rest ( $\chi^2 = 170.03$ , 60 df,  $p < 0.0001$ ). This again demonstrates the greater discriminative power of the size factor compared to shape and other factors. The Manhattan distance showed that the first two principal coordinates (size and

Table 4. Q Principal Coordinates analyses of the pink river dolphins *Inia* from correlation, variance-covariance and Manhattan distance matrices for males and females analyzed independently. The specimens studied were captured in the Colombian Orinoco and Amazon Rivers and in the Bolivian Amazon (Mamoré, Tijamuchí and Ipurupuru Rivers) from 1998 to 2003. The main eigenvalues, the percentage of the variances and the cumulative percentage explained by these eigenvalues, a test for significance of these eigenvalues and a comparison of the percentage of variance found by the eigenvalues detected in regard to the broken-stick model, are shown. df – degree of freedom,  $p$  – probability, EV – eigenvalue.

Eigenvalues	Percentage (%)	Cumulative percentage (%)	Test of equality of roots			Proportion of EV using broken-stick model	
			$\chi^2$	df	$p$	Eigenvalues	EV (%)
Males							
Q PCA from correlation matrix							
0.197	51.662	51.662	1262.256	594	0.000	1	12.122
0.010	26.067	77.729	598.280	560	0.127	2	9.171
0.038	10.084	87.813	0.000	527	1.000	3	7.700
0.022	5.884	93.697	0.000	495	1.000	4	6.720
Q PCA from variance-covariance matrix							
258.842	85.242	85.242	7240.983	594	0.000	1	12.122
178.069	7.610	92.852	5905.574	560	0.000	2	9.171
83.723	3.579	96.431	5434.711	527	0.000	3	7.700
41.116	1.757	98.188	4818.048	495	0.000	4	6.720
Q PCA from Manhattan distance matrix							
1624.450	87.113	87.113	3423.094	594	0.000	1	12.122
137.030	7.348	94.461	1839.305	560	0.000	2	9.171
59.939	3.214	97.675	1532.304	527	0.000	3	7.700
46.236	2.479	100.000	1383.760	495	0.000	4	6.720
Females							
Q PCA from correlation matrix							
0.028	32.088	32.088	0.000	527	1.000	1	12.683
0.023	26.399	58.487	0.000	495	1.000	2	9.558
0.014	16.247	74.735	0.000	464	1.000	3	7.995
0.010	12.039	86.774	0.000	434	1.000	4	6.953
0.006	6.498	93.271	0.000	405	1.000	5	6.172
0.003	3.529	96.801	0.000	377	1.000	6	5.547
Q PCA from variance-covariance matrix							
829.384	77.650	77.650	6861.952	527	0.000	1	12.683
77.851	7.289	84.934	5552.143	495	0.000	2	9.558
59.070	5.530	90.469	5287.279	464	0.000	3	7.995
40.843	3.824	94.293	4980.755	434	0.000	4	6.954
27.535	2.578	96.871	4642.131	405	0.000	5	6.172
17.418	1.631	98.502	4248.200	377	0.000	6	5.547
Q PCA from Manhattan distance matrix							
435.480	67.063	67.063	1870.810	527	0.000	1	12.683
121.575	18.722	85.785	1178.422	495	0.000	2	9.558
43.742	6.736	92.521	852.967	464	0.000	3	7.995
34.214	5.269	97.790	726.067	434	0.000	4	6.954
28.883	4.448	100.000	606.864	405	0.000	5	6.172
25.490	3.925	100.000	480.152	377	0.000	6	5.547

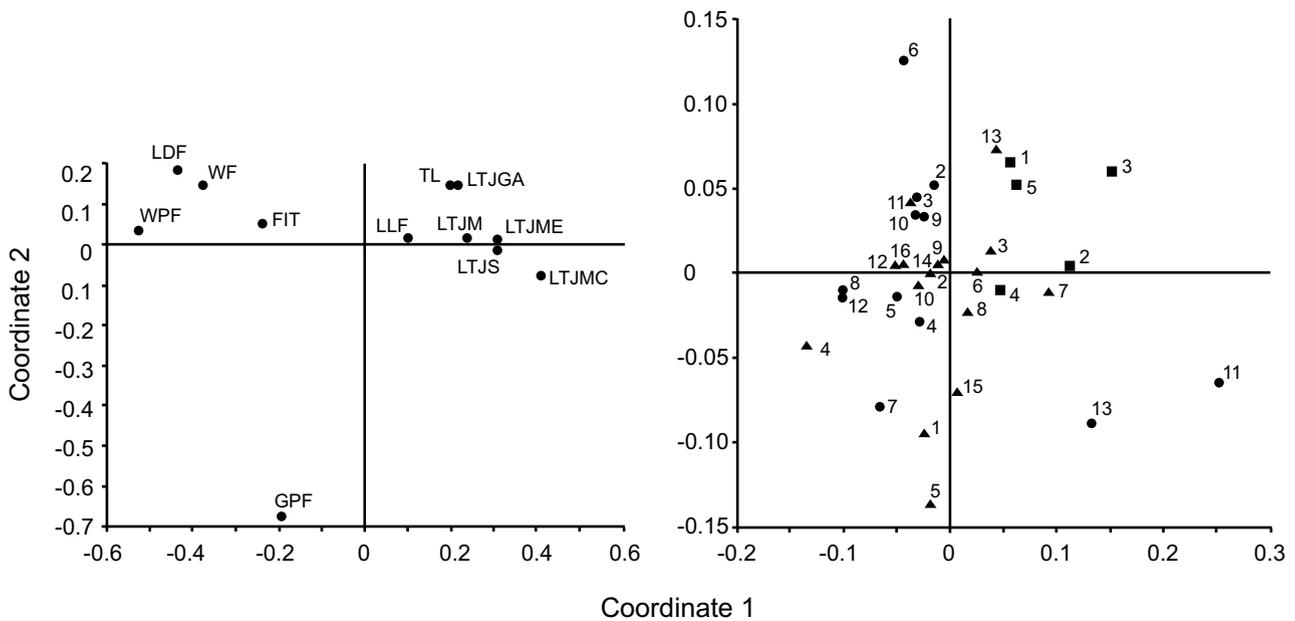


Fig. 3. R type Principal Coordinate analysis of the morphological variables studied in pink river dolphin males (Inia) (left panel) and Q type Principal Coordinate analysis of the pink river dolphin males (right panel) studied in the Colombian Orinoco and Amazon Rivers and in the Bolivian Amazon (from 1998 to 2003) throughout the correlation matrix (circle – Colombian Amazon individuals, triangle – Bolivian Amazon individuals, square – Colombian Orinoco individuals. LDF, WPF, WF, FIT, etc are the abbreviations of the morphological variables analyzed).

shape) were significant (first coordinate:  $\chi^2 = 2432.96$ , 77 df,  $p < 0.0001$ ; second coordinate:  $\chi^2 = 419.42$ , 65 df,  $p < 0.0001$ ), while with the variance-covariance matrix, the first six principal coordinates were significant and hardly differed from the other eigenvalues.

The Q-type PCA results obtained throughout the correlation, variance-covariance and Manhattan distance matrices are shown in Table 4 and Fig. 4. As observed for the first matrix type, the first eigenvalue explained 32.09% of the overall variance, while the first five principal coordinates explained 93.27% of the total variance. For this procedure, the male percentages for the same first coordinates were clearly higher than in the females. The first four principal coordinates explained more variance than that expected in the random broken-stick model (first coordinate: 12.68%, second coordinate: 9.55%, third coordinate: 7.99% and fourth coordinate: 6.95%). Nevertheless, the findings for the variance-covariance matrix showed similar results to that observed for males. The first eigenvalue explained 77.65% of the overall vari-

ance, while 90.47% of the variance was explained by the first three coordinates. In this case, only the first coordinate showed an explained variance higher than that expected in the broken-stick model (12.68%). For the Manhattan distance, the eigenvalue of the first coordinate explained 67.06% of the variance, while the first three coordinates explained 92.52% of the variance. Again, such as in the case of correlations, the eigenvalue of the first principal coordinate explained less variance than in males (87.11%). The first two coordinates presented an explained variance significantly higher than that expected in the broken-stick model (12.68% and 9.55%, respectively). The variance-covariance and the Manhattan distance more clearly differentiated the relationships between female and male individuals than did correlations. Also, the differences among the overall male sample was more striking than those detected among the overall female sample. For this reason, the association of the males individuals to the first coordinates produced eigenvalues, which explained higher

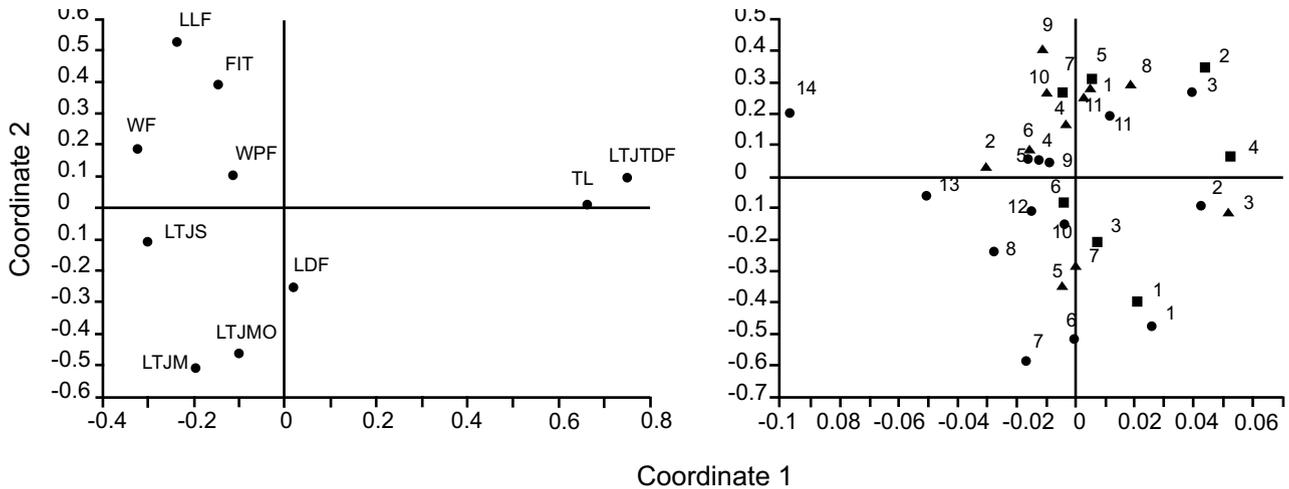


Fig. 4. R type Principal Coordinate analysis of the morphological variables studied in the pink river dolphin females (*Inia*) (left panel) and Q type Principal Coordinate analysis of the pink river dolphin females (right panel) studied in the Colombian Orinoco and Amazon Rivers and in the Bolivian Amazon (from 1998 to 2003) throughout the correlation matrix (circle – Colombian Amazon individuals; triangle – Bolivian Amazon individuals; square – Colombian Orinoco individuals). See text for explanation of abbreviations.

variance percentages than in the females, especially, by using the correlation and the Manhattan distance matrices.

The R-type PCA analysis supported that all eigenvalues were not equal ( $\chi^2 = 200.23$ , 45 *df*,  $p < 0.0001$ ). Likewise, it was demonstrated that only the first eigenvalue was significantly different from the rest ( $\chi^2 = 106.78$ , 38 *df*,  $p < 0.0001$ ). Similar to males, the eigenvalues from the correlation matrix determined the importance of the first coordinate (size) relative to the remaining coordinates in females. As well as in the males, the eigenvalues from the variance-covariance matrix showed that all eigenvalues were significant. Lastly, the eigenvalues obtained from the Manhattan distance matrix showed that the first two coordinates (size and shape) have a greater significance on the evolutionary morphologic traits analyzed than do the remaining coordinates ( $\chi^2 = 717.72$ , 54 *df*,  $p < 0.0001$  and  $\chi^2 = 92.84$ , 44 *df*,  $p < 0.0001$ , respectively). The same trend was detected among males.

### Canonic population analysis

The Canonic population analysis clearly showed that the variance-covariance matrices among the three populations analyzed were sig-

nificantly different (Bartlett's test:  $\chi^2 = 67.9$  with 30 *df*, critical value = 43.77 with a significance level of 0.05), whereas the Wilks's L was equal to 0.39 with a  $F = 3.21$  with 10 and 54 *df*. The critical region with a significance level of 0.05 was  $F = 2.01$ . Therefore, the population means of the three dolphin samples were significantly different from each other. Two eigenvalues (3.537 and 0.6474) explained 100% of variance. The canonic coordinates and the confidence interval ratios (90%) for the males support that the confidence regions of the Colombian Amazon and Orinoco were partially superposed (Fig. 5a). However, the Bolivian sample was clearly differentiated from both populations. Therefore, while there were no significant differences for the male Colombian Amazon and the Orinoco populations, the Bolivian population represented a distinct population.

Like males, the variance-covariance matrices among the three female populations were significantly different (Bartlett's test:  $\chi^2 = 70.77$ , 42 *df*,  $p = 0.0001$ ). Similarly, the population means of the three females populations were significantly different (Wilks's L = 0.41,  $F = 2.26$ , *df* = 12 and 48,  $p = 0.002$ ). The two first eigenvalues explained 100% of variance (3.588 and 0.4880, respectively). Figure 5b represents the canonic

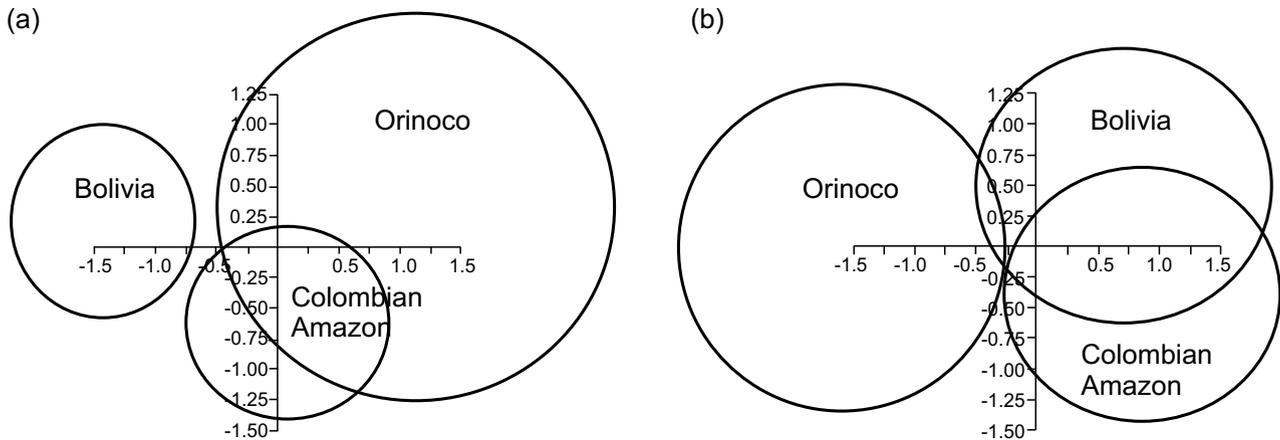


Fig. 5. Canonic population analysis of the pink river male (a) and female (b) dolphins (*Inia*) analyzed in the Colombian Orinoco and Amazon Rivers and in the Bolivian Amazon (from 1998 to 2003).

coordinates and the confidence interval ratios (90%) for the females. The differentiation among the three female populations was not as clear as that observed in males.

## Discussion

### Morphological differentiation among the three *Inia* populations studied

The morphological results obtained in the present study in combination with previous molecular results (Banguera-Hinestroza *et al.* 2002) support several findings. For males there is evidence that the Colombian Amazon group showed several morphological variables with values greater than the other two populations. Definitely, the Orinoco males were significantly smaller than the animals from the Colombian and the Bolivian Amazon, but this last population was the most differentiated. Several significant differences in variables were also found among the three female groups. For example, the Orinoco group showed higher value from jaw tip to the tip of the dorsal fin than the remaining populations, although for all the other variables were smaller. Comparisons of the variance-covariance matrices supported significant differences between the Colombian and Bolivian Amazon arrays (indistinguishably for males and

females). These results indicated that there are morphological differences for males and for females among the groups. In strong agreement with this affirmation were the results obtained from the canonic population analysis. At the 90% confidence intervals, the Orinoco and Colombian Amazon males had superposed areas, whereas the Bolivia confidence interval was clearly outside. For females the situation was not as clear and therefore we are uncertain as to what level these morphological differences correspond to specific and/or subspecific differences. Our analysis of mitochondrial *Cyt-b* and the D-loop regions revealed divergence sequence levels as follows. For the hypervariable D-loop region, the divergence between the Bolivian and the Colombian Amazon populations and between the Bolivian and the Orinoco populations were 4.1% and 4.72%, respectively, whereas the difference for the Colombian Amazon and the Orinoco was as little as 1.86%. For the *Cyt-b* gene, which has a lower evolutionary rate than the D-loop region, the genetic divergences were 2.99%, 2.48% and 0.059%, respectively. These previous values agree quite well with the present results, especially, those obtained with the morphological analysis of males. These divergence genetic values were transformed into divergence times, assuming several mutation rates for two mitochondrial regions analyzed. The upper and lower time divergence estimates

for *Inia boliviensis* and *Inia geoffrensis* were around 5.96 and 2.7 million of years ago, respectively. This is sufficient time for the aforementioned species to appear by allopatry and neither mitochondrial haplotypes presented in the Bolivia population were found in the Colombian animals. From a geographical point of view, the Bolivian rivers, where the *I. boliviensis* lives, are separated from the Abuña Pass, which is an open door to the Amazon basin, by an insuperable obstacle: the 400 km of waterfalls between Guayará-Mirim and Porto Velho on the Madeira River. This geographical obstacle is an important barrier, which could cause allopatric speciation between the two *Inia* taxa analyzed. In addition to this geographical barrier, the ecological conditions of the Bolivian Amazon considerably differ from those typical of the Colombian Orinoco and Amazon. From a biotope standpoint, for instance, the waters of the Mamoré-Beni system have a higher carbonate hardness than the Colombian Amazon waters. Differential prey sources probably had some influence on speciation of the *Inia* taxa as well (Pilleri 1969). Therefore, the morphological differences observed by Pilleri and Ghir (1977) among the Bolivian and remaining *Inia* populations, in conjunction with the biometric and molecular differences presented in this study, support the existence of two *Inia* species. Furthermore, plasma proteins analyzed by De Monte and Pilleri (1979) showed important differences between Bolivian and Amazon Basin individuals.

On the other hand, the divergence times between the two supposed *Inia geoffrensis* subspecies (*I. geoffrensis geoffrensis* and *I. g. humboldtiana*) were relatively minor when we used the cited molecular markers. The upper time divergence estimate obtained with the D-loop region was 930,000 years ago, whereas the lower time divergence estimate obtained with the *Cyt-b* gene was only 118,000 years. Even, these values could be an over estimation due to gene drift especially in those populations with small effective sizes, possibly a characteristic of several Orinoco populations. In addition, there are several mitochondrial haplotypes simultaneously presented in the Orinoco and Am-

azon basins. This finding, along with the morphological results obtained herein, especially for males, do not support the existence of both subspecies, *Inia geoffrensis geoffrensis* and *Inia geoffrensis humboldtiana*. In this case, contrary to the above, our results agree quite well with those claimed by Casinos and Ocaña (1979). This does not mean that the Orinoco population does not have any particular features and specificities from a morphological point of view. Probably, a relatively strong natural selection pressure on a limited number of morphological genes could develop the morphological differences recorded in this population without affecting the neutral evolution of molecular markers.

It is well documented that the characteristics of rivers cause differential selection pressures on the river dolphins (Pilleri 1972). This means that the limited morphologic differences among the *Inia geoffrensis* from the Orinoco and Amazon would be attributed to ecological selection differences, or simply to different environmental influences due to quality of habitat exploited. One noteworthy example to this is seasonal water depth, which is for more pronounced in the Orinoco than in the Amazon River. During the dry season in the Orinoco, water flow is only 1/25 to 1/30 than that during the wet season whereas in the Amazon the water depth during the dry-season is only 1/2 or 1/3 of its wet-season level. A strong seasonal variation in the water levels of the Orinoco could cause morphological modification in its dolphin population, such as a smaller body and a more developed melon (more developed echoacoustic system). Moreover, water depth oscillations could generate greater seasonal migrations of animals in the Orinoco than in the Amazon in search of food sources and optimal habitats. This could require several morphologic adaptations as, for instance, an enhanced swimming ability for the Orinoco specimens. One possible explanation for the greater differences in males compared to females in the Orinoco population is that males are more mobile than females and travel more frequently between different groups. Several of these biometric differences between the Orinoco array and the other groups could reflect only different proportions of the age-class of the adults be-

tween the diverse areas analyzed. However, many of the measurements of flippers, fluke, and other structures are connected to propulsion and swimming capacity and therefore could be adaptations for swimming in rivers with fast or slow moving waters. Additionally, *I. boliviensis* lives primarily in rivers with gallery forests that are relatively deforested, whereas *I. geoffrensis* from the Amazon inhabit rivers of the evergreen rain forest and *I. geoffrensis* from the Orinoco basin primarily inhabit rivers from the Colombian and Venezuelan Llanos with very different flora and soil properties.

Thus far, it is easy to imagine that several different natural selection pressures are acting on the body structures of these three dolphin populations. Anyway, the molecular differences found, which certainly have a more neutral evolution, yielded relatively small differences between the Colombian Amazon and the Colombian Orinoco *Inia* populations. From a geographical and geological point of view, the subspecific differentiation between both Colombian populations is not supported. The Orinoco and the Amazon were extensively connected with each other throughout the Tertiary Era until the Pleistocene. Moreover, both basins are currently connected by the Casiquiari system. Although Pilleri and Ghr (1977, 1981) have emphatically stated that pink dolphins do not use the Casiquiari system and do not connect the Orinoco and Amazon populations, this assertion is unsustainable. Best and da Silva (1983, 1989a, 1989b) and Meade and Koehnken (1991), have observed dolphins in the Casiaquiari Canal and between San Fernando de Atabapo and La Esmeralda. Meade and Koehnken (1991) reported at least eight dolphin sightings between San Fernando and La Esmeralda. In addition, during the low-water season the Casiaquiari Canal can decrease its depth by six meters compared to the high-water season. The average discharge of the Casiquiari is about  $300 \text{ m}^3/\text{sec}$  which provides 20% of the average flow of diverse points of the Orinoco and  $2000 \text{ m}^3/\text{sec}$  into the upper Negro River. Even in the dry-season, 75% of lower Casiquiari and 20% of upper

Casiquiari have depths greater than three meters, which provide sufficient depth for pink dolphins to travel.

Moreover, *Inia* has been recorded throughout the main stem of the Orinoco River, being common in whitewater rivers that drain from the Andes as well as in clearwater rivers that drain the Colombian and Venezuelan Llanos and in the backwaters that drain the upland Guyana Shield (Meade and Koehnken 1991). The dolphins are also commonly found in acid waters (pH 4–5), such as the Negro River, the Casiquiari Canal, up-stream on the Orinoco as far as La Esmeralda (Best and da Silva 1983), and the Atabapo River. Therefore, the morphological differences detected by Pilleri and Ghr (1977) among the specimens from the Amazon and Orinoco basins, such as the rostrum length/zygomatic width index and the number of teeth per ramus, are morphological characters, which may not necessarily have taxonomic value. Moreover, many of these differences have a clinal character such as was claimed by Casinos and Ocaña (1979). Several length variables increased from north to south, whereas some width variables decreased. These slight differences within populations are insufficient to support that these populations are different subspecies.

#### **Importance of sexual dimorphism in the analysis of taxonomic differences**

Many authors claim that the morphological sexual dimorphism in *Inia* is not extraordinarily important (Pilleri and Ghr 1969, Da Silva, 1994). Our results support other conclusions. For instance, the females were generally greater in size and considerably more homogeneous than the males. This last record was observed by Da Silva (1994) in Central Amazonian pink dolphins as well. This situation has been observed in other organisms (Ruiz-García and Alvarez, 1997).

On the contrary, Da Silva (1994) found, that with the exception of the measurement from tip of the jaw to the midpoint of the genital aper-

ture, males exhibited the largest means in all measurements.

The multiple regression analyses supported important differences among males and females. In males, the morphological variables LTJS, LTJM, LTJME and LTJMO showed the highest amount of variance explained by the other variables compared to TL and LTJTDF in females. Similarly, there were contrasting differences among sexes for the variables which were more highly correlated with the factors representing size (first factor) and shape (second factor). The variables more related with size in males were TTJS, LTJM, LLP, LTJME and TL, while in females they were LTJM and LTJS. For shape, the variables more related in males were WPF, LDF, WF and LTJMO whereas for females they were TL and LTJTDF.

A large segment of our results show an important influence of sex in several biometric measures such as dorsal fin height and flipper width, in contrast to findings by Pilleri and Ghir (1969) and da Silva (1994). Our next investigations will focus on sampling specimens from the Casiquiari Canal, Negro River and from San Fernando de Atabapo to La Esmeralda. Analyses from a molecular (mitochondrial and MHC gene sequences and microsatellite nuclear loci) and from a morphologic perspective are planned. The characteristics of these local *Inia* populations are extraordinarily important in order to understand the evolution within the *I. geoffrensis* species.

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