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Primary Research Paper

Determining management units in southeastern Brazil: the case of *Astyanax bimaculatus* (Linnaeus, 1758) (Teleostei: Ostariophysi: Characidae)

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Abstract

Characterization, management and protection of biodiversity are the most prominent challenges in conservation biology. Analyses on molecular similarity (Random Amplified Polymorphic DNA-Polymerase Chain Reaction, RAPD-PCR) and morphologic patterns (morphometric and meristic characters) were performed to evaluate the existence of population structuring in the Doce river basin, in a widespread small-sized characin, *Astyanax bimaculatus*. Three hundred (300) individuals were collected from six locations isolated by waterfalls or dams, in the Doce river basin, Minas Gerais State, Brazil. Genetic differentiation among tributaries was significant ($p < 0.00001$) and accounted for 21% of total variance ($p < 0.00001$). Only the Grande waterfall of the Casca river was an effective factor for differentiation of populations ($p < 0.00001$), and meristic characters were also consistent with molecular data. We concluded that genetic and morphologic variation of this species was not necessarily associated with waterfalls and that molecular and meristic data are effective predictors of population divergence in this basin. The consequences of these findings for the protection and management of aquatic biodiversity were discussed.

Introduction

The Neotropical region is the richest in numbers of freshwater fishes, and may represent 25% of all species worldwide (Schaefer, 1998). This richness is dwindling due to many factors, such as habitat destruction, introduction of exotic species and pollution. Most of these changes occur in a Sentinel fashion (Meffe & Carroll, 1997), where unknown species are extirpated before they are formally described. Typically, sampling efforts are performed shortly before implementation of irrigation projects, hydroelectric dam building or other environmentally damaging activities. As an attempt to reduce these impacts, the State of Minas Gerais, Brazil has reinforced environmental laws allowing fish transposition to the upper sections of the river (Martins & Tamada, 2000). However, at least some fish scales are ineffective and species-selective (e.g., Bazzoli et al., 1991), mainly because they are designed for game (such as large- or medium-sized characids) or commercially valuable species (e.g., *Prochilodus* spp.).

Current impact evaluation does not consider estimates of gene flow among populations occurring in different river locations (down and upriver). It is known, however, that dams may promote a halt in the gene flow process between populations of aquatic organisms, with subsequent alterations in gene frequencies of fish species (Avise & Felley, 1979; Revaldaves et al., 1997; Neeras & Spruell, 2001). In this study, we explored and compared the information obtained by two methods that characterize biological distinctness among populations isolated by recently built dams or by natural obstacles such as waterfalls. A small

characin, *A. bimaculatus*, was studied as it is a widespread and abundant Neotropical species complex. Populations of small characins diagnosed by a round humeral spot and a caudal spot extending to the end of caudal rays were traditionally considered as subspecies of *Astyanax bimaculatus* (Eigenmann, 1921: 254–259). Current taxonomy acknowledges specific status for six former subspecies and four recently described species (Lima et al., 2003) that share the two spots pattern. Because *A. bimaculatus* of the Doce river has not been included in recent revisions, we considered it as a member of the *A. bimaculatus* species complex. *A. bimaculatus* is considered a generalist (Costa & Braga, 1993; Esteves & Galetti, 1995) well adapted to both running and stagnant waters (Agostinho et al., 1997). It is migratory (Uieda, 1984; Garutti, 1988), although its partial spawning reproductive regime and adhesive eggs (Bazzoli et al., 1991) are shared with other non-migratory Neotropical freshwater fishes (Lamas, 1993). In the Neotropical region, *A. bimaculatus* may be considered as representative of a large number of small sized and widespread taxa that are usually neglected during ecological impact assessment studies. The genetic structure of this widespread characin may provide information on natural patterns of geographic isolation and will be a baseline for managing programs. Genetic divergence was assessed using Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) markers (Welsh & McClelland, 1990; Williams et al., 1990) and phenotypic variation included meristic and morphometric characters. Phenotypic variation is the most inexpensive approach but it must be applied on populational levels for meaningful informative value (e.g., Salducci et al., 2004). RAPD-PCR markers are cost-effective, and there is no need for previous knowledge of the species genome. These markers have been successfully applied to understand population genetics, systematics and ecology of many organisms (Hadrys et al., 1992; Fritsch & Rieseberg, 1996; Hsiao & Liao, 1998; Bartish et al., 1999; Lacerda et al., 2001). Despite being dominant markers, RAPD-PCR are considered very effective as long as they are: (1) performed on relatively large samples; (2) contamination problems are overcome; (3) repeatability tests are included in the pilot test; and (4) care is exercised to minimize statistical bias in estimating allelic frequencies. In comparative population studies, the effectiveness of these markers has been considered as satisfactory as isoenzymes, microsatellites, minisatellites and mitochondrial DNA (Naish et al., 1995; Aagaard et al., 1998; Nesbo et al., 1998; Sun et al., 1998).

The objective of this study was to conduct a survey on the genetic variability and population structuring presumably caused by natural barriers of *A. bimaculatus* in the rio Doce basin, and its reflection on the morphology of the species. The detected pattern represents baseline data for management, implementation or revision of current hydrologic practices that affect migration levels among populations of this species, considering the potential deleterious effects of reduced genetic diversity and the population viability.

Materials and methods

A total of 300 *Astyanax bimaculatus* individuals from six populations were seined or collected with

Table 1. Locales and sample sizes for molecular (*N*1) and morphologic (*N*2) analyses of *A. bimaculatus* in the Doce Basin

Samples (rivers)	Locale	*N*1	*N*2
Casca (A)	Downstream from Escura Waterfall	50	30
Casca (B)	Between Escura and Grande Waterfalls	50	30
Santana (C)*	Upstream from Grande Waterfall	50	30
Matipó (D)	Upstream from Emboque Dam	50	30
Matipó (E)	Downstream from Emboque Dam	50	30
Sto.Antônio (F)	Near city of Ferros/MG, Brazil	50	30
Total		300	180

*Small tributary of Casca River.

gillnets in regions comprising most of the upper reaches of the Doce river basin, Minas Gerais State, Brazil (Table 1 and Fig. 1). These locales are characterized by abundant waterfalls and are therefore potential sites for future dam building projects. All individuals initially sampled were used in molecular analysis and 180 voucher specimens were used for morphologic analyses. All specimens were deposited in the Zoology Museum of the Universidade Federal de Viçosa. Epaxial muscle or gill filaments were sampled in live individuals larger than 50 mm, while smaller specimens were fixed whole in 95% ethanol. DNA extraction was carried out with CTAB (Boyce et al., 1989). DNA pellets were resuspended in sterile TE (0.01 M Tris–HCl, pH 7.5, 0.001 M EDTA) and stored at −20 or −80 °C until use.

Random amplified polymorphic DNA-polymerase chain reactions were processed in 20 μl volume: 13.8 μl H_2O; 2 μl 10× buffer (500 mM KCl, 100 mM Tris–HCl pH 8.3); 0.8 μl 40 mM $MgCl_2$; 0·2 μl dNTPs 20 mM; 2 μl 3.3 μM primer (Operon Technologies) 1 unit *Taq* polymerase and 1 μl of 25–100 ng DNA. Reactions were carried out in a PTC100 thermocycler (MJ Research) with 40 cycles 95 °C 15 s; 35 °C 30 s and 72 °C 1 min with a final extension at 72 °C 7 min. Fragments were visualized on 6% polyacrylamide gels using silver staining (Hiss et al., 1994) (Fig. 2), and all reactions included a negative control where template DNA was substituted by water.

Pilot tests were conducted with 66 primers, allowing for selection of polymorphic and sharp bands (loci). Loci were identified across gels by

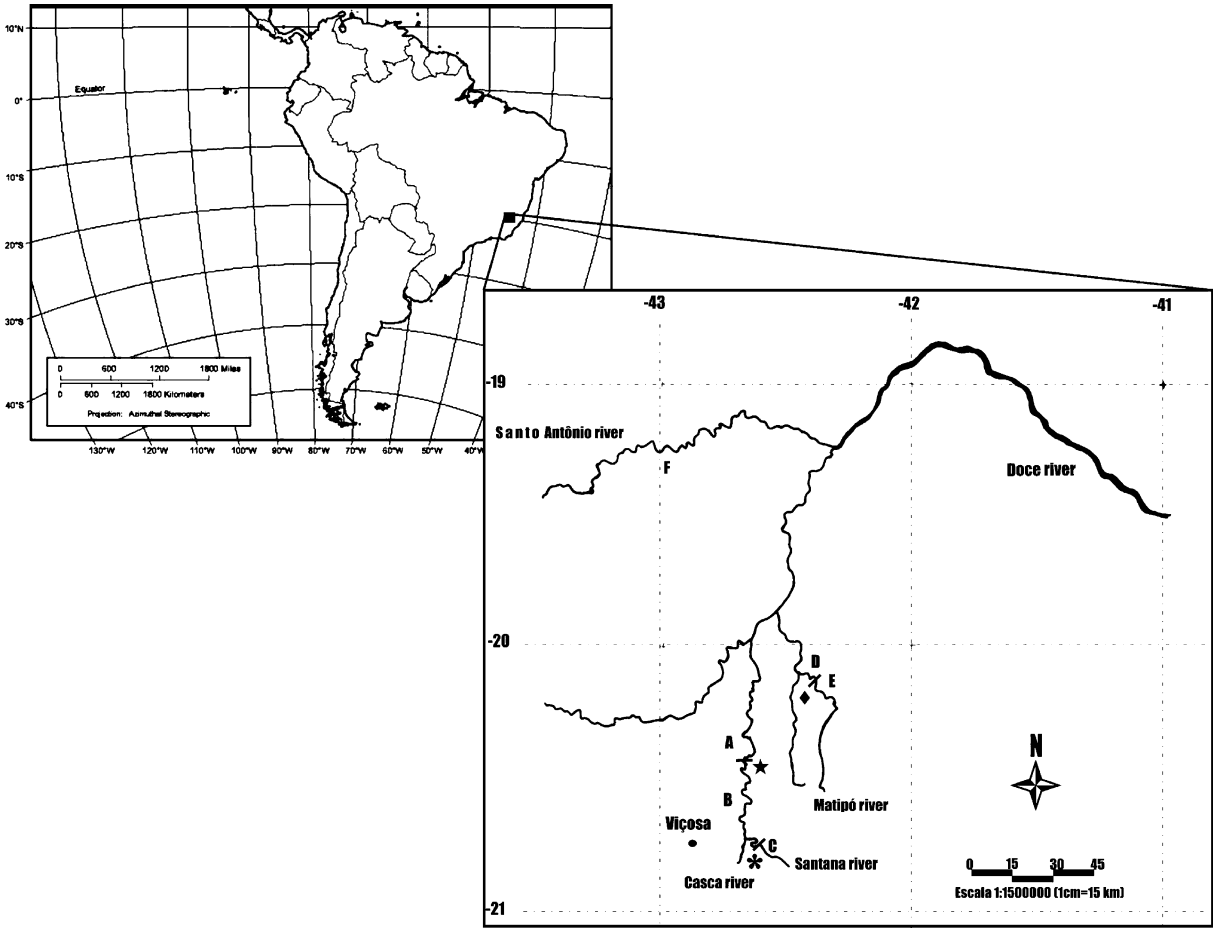

Figure 1. South America, indicating the Doce river basin in Brazil and the study area. Collecting sites and waterfalls: downstream Escura waterfall-A; river section between Escura and Grande waterfalls-B; upstream Grande waterfall-C; upstream Emboque dam-D; downstream Emboque dam-E; Ferros-F; ♦, Emboque dam; ✲, Grande waterfall; ★, Escura waterfall.

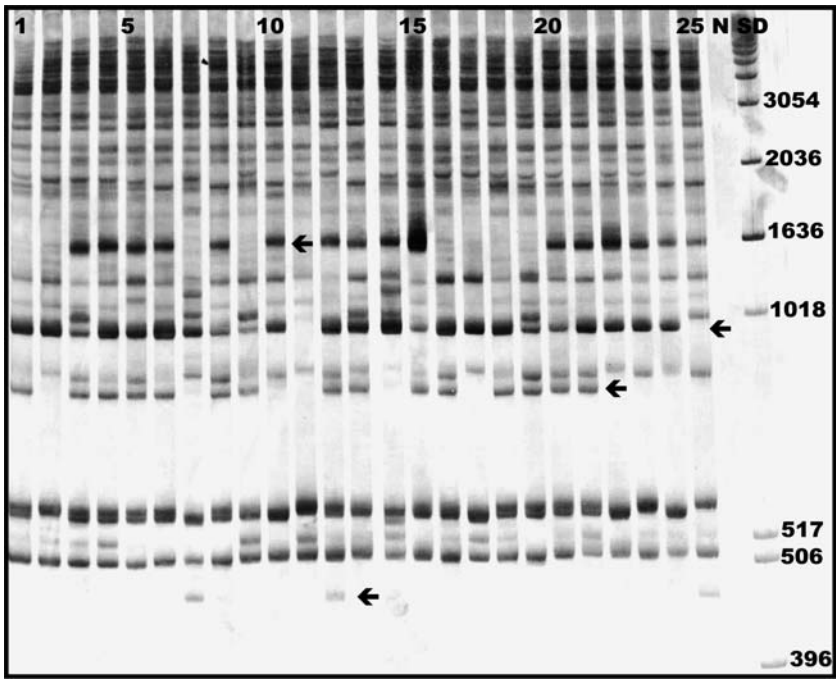

Figure 2. Example of RAPD amplifications (6% polyacrylamide gels using silver staining) with primer OPD18 in sample of Santo Antonio river (F). Each lane (1–25) represents an individual. N, negative control; SD, 1 kb DNA Ladder (Gibco). Arrows represent polymorphic bands.

their appearance and position on the gel, according to a regression function (Schaffer & Sederoff, 1981) relative to 1 kb ladder (BRL, Gibco). Repeatability tests were carried out on all selected loci in 20 randomly chosen individuals. Fragments with 90% repeatability rates were selected for analyses (Bartish et al., 1999; Vucetich et al., 2001). Approximately 70% of the primers used in the pilot study amplified successfully *A. bimaculatus* DNA and six primers (OPA01, OPA03, OPA09, OPD15, OPD18, and OPD20) were selected. Twenty-eight fragments were chosen within a size range between 460 and 1744 base pairs (estimated from gel positions).

Presence and absence were recorded on a binary matrix, and before analyses were conducted, fragments with a frequency higher than $[1-(3/N)]$ were discarded (where N is the sample in each population) for a less biased allelic estimate (Lynch & Milligan, 1994). Statistic analyses were carried out with TFPGA – Tools For Population Genetic Analysis (Miller, 1997), Arlequin (Schneider et al., 2000) and IBD – Isolation by Distance (Bohonak, 2002). Allelic frequency was estimated from null allele (absence of fragment) frequency as $q = x^{-1/2}(1-[x\ (1-x)/N]/8x^2)$ (Lynch & Milligan, 1994), where x is the population proportion lacking the fragment and N is sample size. Within-population variation was estimated assuming expected Hardy–Weinberg frequencies and the proportion of polymorphic loci. Bands were screened as 'diagnostic' when they were unique for some populations, and as 'characteristic' when they occurred in more than 5% of individuals of each population.

Genetic structure was estimated with Fisher's Exact Test (Raymond & Rousset, 1995) and F_{ST}, under null hypothesis of panmixia. A sequential Bonferroni correction (Holm, 1979) was applied to the exact p-values to correct critical significance levels. Effective number of migrants were estimated as $N_m = 0.25\ (1/F_{ST}-1)$ (Wright, 1951). Hierarchical analysis was conducted with Analysis of Molecular Variance – AMOVA (Excoffier et al., 1992) where variation was partitioned among major tributaries of the Doce river (1-Casca/Santana, 2-Matipo and 3-Santo Antônio), among samples within major tributaries (Casca/Santana

tributary – samples A, B and C; Matipo tributary – samples D and E; Santo Antônio tributary-sample F) and within populations (samples). Nei's distance (1972) was used to determine among-population variability, and it was represented with an UPGMA phenogram. The collection localities were used as experimental units in this analysis. Bootstrap values (Efron, 1985) were obtained from 1000 repetitions. To determine the effects of migratory behavior of the species, a Mantel test between genetic and geographic distances was carried out (Sokal & Rohlf, 1995).

Fifteen morphometric and two meristic characters were selected, according to Lagler et al. (1977) and Garutti & Britski (1997). These included Standard length (SL); head length (HL); body height (BH); caudal peduncule height (CPH); interorbital width (IW); ocular diameter (OD); pre-anal distance (PD); pre-dorsal distance (PDD); dorsal-pectoral distance (DPD); pectoral-pelvic distance (PPD); pelvic-anal distance (PAD); dorsal-anal distance (DAD); anal-adipose distance (AAD); dorsal-adipose distance (DADP); head height (HH); lateral line scales (LL), and number of rays in anal (RAN). Other meristic characters were used, but only those ones were shown because were the most variable within this species complex (Garutti, 1993; Garutti & Britski, 1997). Measures were carried out with a 0·1 mm digital precision caliper, transformed to base 10 logarithm to maintain allometry and to standardize variances (Jolicoeur, 1963). Statistic analyses were performed with SAS (1997) and Matlab (v.6). Data were subject to size-free canonical discriminant analysis CDA (Bookstein et al., 1985; Strauss, 1985; Reis et al., 1990; Peres-Neto, 1995). This analysis consists of removing the effect of within-group size variation by the regression of each character separately on the first pooled within-group principal component and then applying canonical discriminant analysis to the residues obtained from the regressions.

The first step in the analysis consists of centering the values for each character by the sample mean. Then the effect of size on each character was removed by regressing the values of each morphometric character on the first principal component (PC-1), thus obtaining the residuals that express variation after the removal of the within-group size effect. Canonical discriminate analysis was then performed with the residuals obtained by the regression of each character on PC-1. The computations were continued by multiplying the intergroup covariance matrix by the inverse of the within-group covariance matrix and extracting the eigenvalues and eigenvectors from the resulting matrix. As the eigenvectors express the axes of greatest variation, they were linearly combined with the values of the morphometric characters to compose the canonical variables that produce the individual's scores. The individual's scores for each sample were plotted in the space of the canonical variables that determine the patterns of discrimination among populations. The contribution of characters to population differences was obtained using correlation vectors from the first two canonic eigenvalues for each character (Strauss, 1985).

Among-population variability of morphometric characters was estimated with D2 Mahalanobis distances, and they were depicted in a UPGMA phenogram. Phenograms obtained with molecular and morphometric data were compared using a Mantel test. Among-population variation of meristic characters histograms were plotted and significance of differences was assessed with ANOVA and contrasted with Tukey test ($\alpha = 5\%$).

Results

Molecular data

Genic Diversity (H) and proportion of polymorphic loci (P) values were lowest in samples from Santo Antônio and Santana rivers, respectively (Table 2). Qualitative analysis of fragments indicated a 25% loss of alleles in Casca samples, which also displayed only one diagnostic allele. On the other extreme, Matipó samples showed the highest values of H and P and 11 diagnostic loci (Table 2). Most samples showed significant levels of genetic structure even after applying sequential Bonferroni correction (Table 3). Besides samples B and C, isolated by the Cachoeira Grande ($\chi^2 = 92.4307$, $p = 0.00001$), samples isolated by the Escura waterfall on the Casca tributary and Emboque dam on the Matipó tributary were not significantly divergent ($\chi^2 = 36.8373$, $p = 0.99$, and $\chi^2 = 51.4704$, $p = 0.64$, respectively), as also expressed by F_{ST}

Table 2. Within-population variation in populations of *A. bimaculatus* in the Doce basin

Samples	*H*	*P*
Casca (A)	0.2613	67.8571
Casca (B)	0.2537	67.8571
Santana (C)	0.2832	67.8571
Matipó (D)	0.3245	100
Matipó (E)	0.3579	96.4286
Sto.Antônio (F)	0.2441	85.7143

H, heterozigosity; P, percentage of polymorphic loci (99% criterium).

values (Table 3). Most of the genetic variation occurred within samples (76.35%, $p < 0.00001$); 21.04% ($p = 0.00001$) of the genetic variance occurred among major tributaries and 2.61% ($p < 0.00001$) of the variance occurred among samples within major tributaries.

UPGMA phenograms based on Nei's (1972) distance were consistent with previous analyses, such as a high divergence of sample C from the Casca tributary (Fig. 3). A positive correlation was observed ($r = 0.5862$, $p < 0.00001$) using Mantle's test between genetic and geographic distances.

Morphologic data

Principal component 1 (CP-1) explained 92.97% of overall variation of the covariance matrix among the six samples. This component may be interpreted as a general size variable since all characters showed positive and statistically significant correlation coefficients (*r*) (Table 4). This first eigenvector may be applied to size free Canonical Discriminant Analysis as a generalized size estimate. A regression analysis between scores of each character and CP-1 was carried out and a Size free Canonical Discriminant Analysis was applied to all samples using residuals of each character. In the resulting analysis, the two first canonic variables were responsible for 79% of observed variation ($p < 0.00001$). Canonical variables showed major overlap among populations (Fig. 4) except for some individuals from populations from the Casca (A), Santana (C), and Santo Antônio tributaries (F) (data not shown).

Correlation vectors between the values of each character and the first two canonical axes (data not shown), and CP and CV values (Table 4) indicate the characters that most contributed to levels of variation among populations. The Santo Antônio sample differed from all others in eye diameter (OD) and head length (HL); Matipó specimens were characterized by higher body height (BH), whereas high values of pre-dorsal distance (PDD), anal adipose distance (AAD) and interorbital width (IW) characterized the Casca samples.

In contrast to the evident geographic (divergence) structure of molecular markers (Fig. 3), UPGMA dendrogram of morphometric data failed to separate samples (data not shown), and this pattern was also evident in the lack of correlation between geographic and morphologic variation (Mantel's test $r = 0.0937$, $p = 0.28$).

Meristic data corroborated the patterns observed with RAPD-PCR markers (Fig. 5). The *F* statistics calculated in ANOVA was significant for both characters ($p < 0.05$). Tukey Test was applied to verify sample means that were significantly different (Fig. 5). For example, samples C and B from Santana and Casca tributaries, isolated by the Grande waterfall, differed in molecular $p < 0.01$

Table 3. Estimated migrant numbers between populations of *A. bimaculatus* in the Doce basin

Samples	A	B	C	D	E	F
Casca (A)	_	23.765	2.305	0.521	0.523	0.731
Casca (B)	0.01041	_	3.477	0.647	0.630	1.024
Santana (C)	0.09783*	0.06707*	_	0.955	0.960	1.478
Matipó (D)	0.32419*	0.27876*	0.20749*	_	76.437	1.841
Matipó (E)	0.32331*	0.28404*	0.20659*	0.00326	_	1.549
Sto.Antônio (F)	0.25492*	0.19617*	0.14465*	0.11956*	0.13893*	_

*$p < 0.00001$.
N_m (upper matrix) and F_{ST} values (lower matrix).

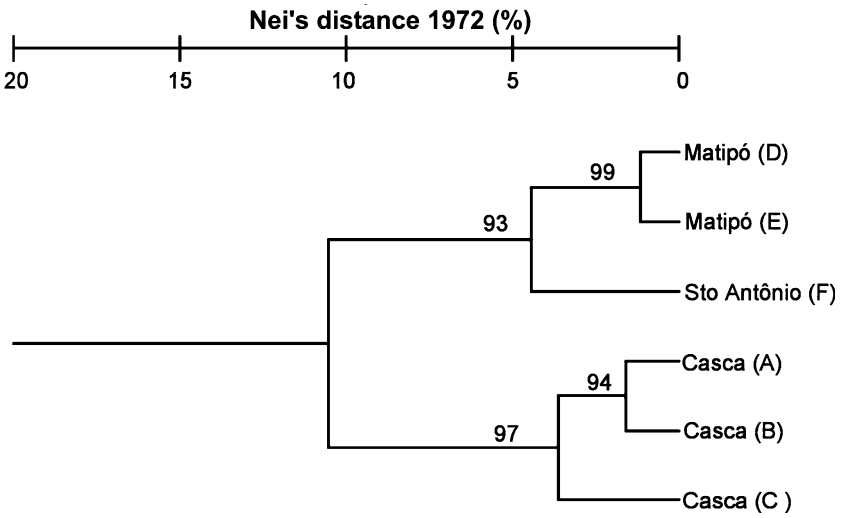

Figure 3. UPGMA dendrogram showing genetic relationships among populations of *A. bimaculatus* from the Doce river basin inferred from Nei's genetic distances matrix. Bootstrap values indicate consistency of data.

and meristic data $p < 0.05$ for both lateral line scales and anal ray counts (Fig. 5). In another comparison, molecular and meristic patterns were partially congruent in the Matipó samples, despite they were similar in RAPD markers and meristic Ran character, these samples were statistically different in LL counts. The Santo Antônio river sample, regardless its geographic origin, was more

Table 4. First principal component (CP-1) coefficients and size-free canonical variation (CV) analyses for six samples of *A. bimaculatus* in the Doce river basin. The latter values are expressed as Pearson correlation coefficients between morphometric characters values and scores obtained by canonical analysis; r is the correlation coefficient between the first eigenvector (CP-1) and each morphometric character

Character	CP-1	*r*	CV-1	CV-2
SL	0.253862	0.99262*	−0.09301^{ns}	−0.35720*
HL	0.211542	0.94172*	−0.34816*	0.38096*
BH	0·261556	0·97641*	0·35401*	−0.02866^{ns}
CPH	0.293413	0.94871*	0.05358^{ns}	−0.06030^{ns}
IW	0.283489	0.94647*	−0.27077*	−0.17607^{ns}
OD	0.163794	0.90663*	0.08637^{ns}	0.70928*
PD	0.254093	0.96096*	−0.08564^{ns}	0.15470^{ns}
PDD	0.246453	0.98616*	−0.44525*	0.03255^{ns}
DPD	0·257711	0·98819*	0·07734^{ns}	0·0398^{ns}
PPD	0.256592	0.96833*	0.15620^{ns}	0.09242^{ns}
PAD	0.321645	0.94249*	0.24308*	−0.14449^{ns}
DAD	0.266810	0.98469*	0.25018*	−0.34370*
AAD	0.284521	0.98782*	−0.34943*	−0.05588^{ns}
DADP	0·271176	0·98588*	−0·14722^{ns}	−0·32459*
HH	0·205957	0·91312*	0·12004^{ns}	0·10898^{ns}

*$p < 0.01$; ns, nonsignificant; SL, standard length; CC, head length; BH, body height; CPH, caudal peduncule height; IW, interorbital width; OD, ocular diameter; PD, pre-anal distance; PDD, pre-dorsal distance; DPD, dorsal-pectoral distance; PPD, pectoral-pelvic distance; PAD, pelvic-anal distance; DAD, dorsal-anal distance; AAD, anal-adipose distance; DADP, dorsal-adipose distance; HH, head height.

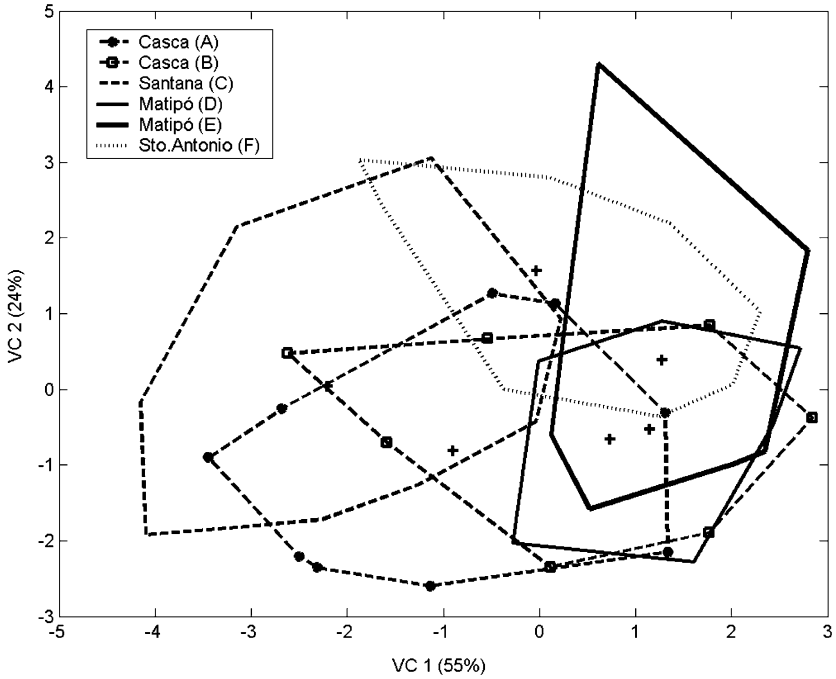

Figure 4. Projection of first and second canonical variables (CV) 1 and 2 taken from a discriminant analysis of the size-independent residuals of morphometric data among six populations of *A. bimaculatus* in the Doce river basin. Convex polygons minimally enclose sets of points for each group; crosses indicate centroids.

similar to Matipó samples in lateral line scales and more similar to Casca samples in anal ray numbers.

Discussion

In freshwater fish, hierarchic analyses of genetic data indicate variable levels of population structure. Tributary-related levels of variation are reported for *Etheostoma flabellare* (Faber & White, 2000), and in *Salmo trutta*, microsatellite variation seems related to waterfalls and tributaries (Carlsson et al., 1999). In continental species of fish, isoenzyme variation in tributaries is apparently responsible for 22.2% (Ward et al., 1994) to 32.8% (Gyllensten, 1985) of total genetic variation. Low levels of variation among samples within major tributaries (2.61% of AMOVA) and high levels of variation among major tributaries (21.04% of AMOVA) suggested that *A. bimaculatus* of the Doce basin also behaved as a short distance migrant, as reported by Uieda (1984) and Garutti (1988) for other drainages. Although in the Doce river basin, large numbers of *A. bimaculatus* are usually caught while swimming upstream at waterfalls during the rainy season, large rivers are also considered as ecological barriers for these small-sized characins (Garutti & Britski, 1997; Garutti, 1988).

Quantitative morphometric characters were uniform among samples, suggesting the occurrence of a single species in the basin, a condition already reported for other basins (Garutti & Britski, 1997). Populations of *A. bimaculatus* seem amenable to morphologic approaches as the same morphometric characters that were informative in this study, such as body height, ocular diameter, interorbital width and head length, have been successfully applied in ecologic studies of this species complex (Garutti & Figueiredo-Garutti, 1992). Meristic variation such as number of anal fin rays in *A. altiparanae* (a species that belongs to the same species complex) in the Paraná basin has been interpreted as a result of the action of environmental factors (Garutti, 1993).

The use of simultaneous approaches using independent data sets allows for a more thorough

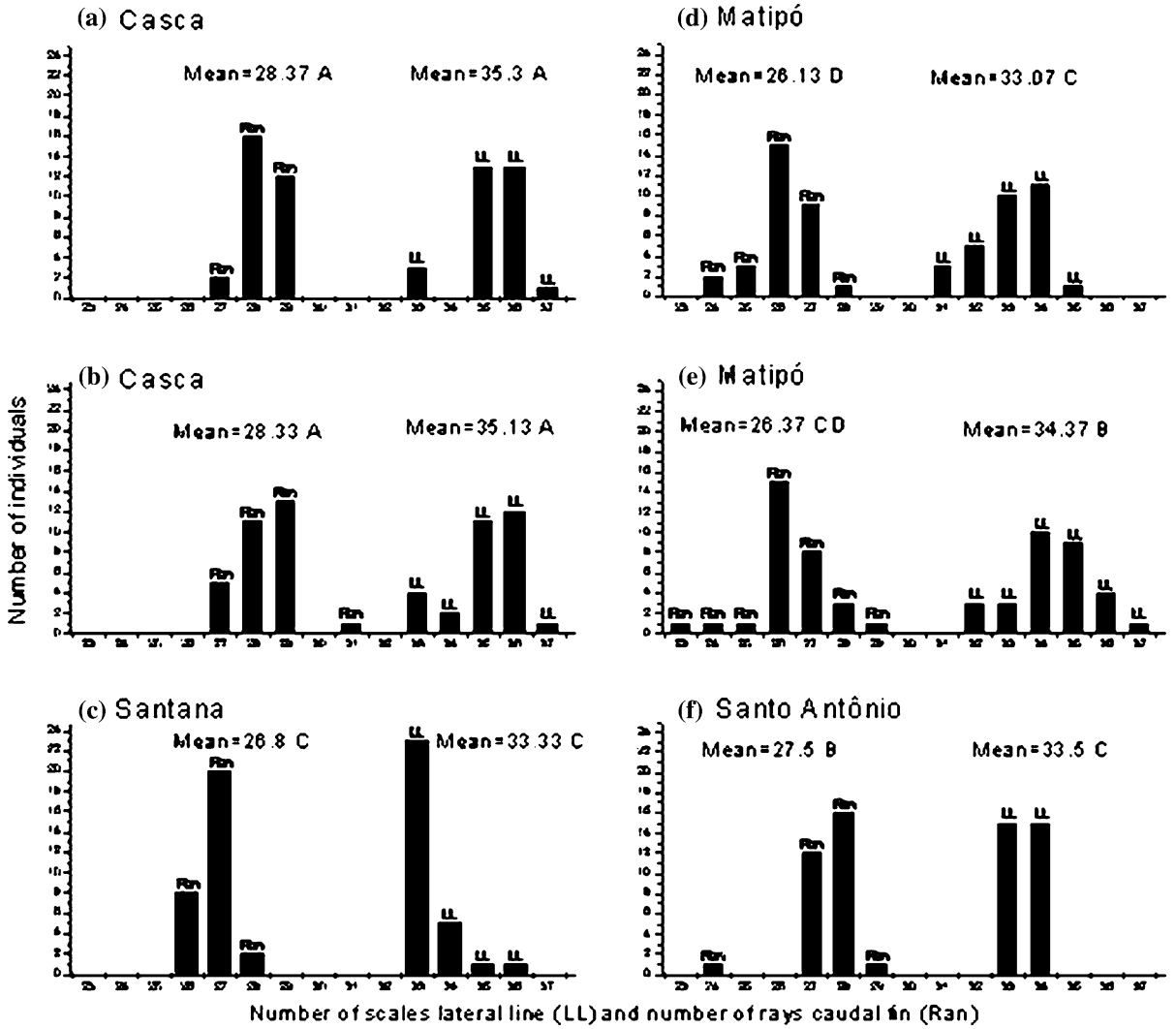

Figure 5. Variation of lateral-line scale (LL) and caudal fin ray numbers (RAN) in samples of *A. bimaculatus* in the Doce basin. Mean values followed by the same letter are not significantly different (Tukey, $\alpha = 0.05$).

understanding of evolutionary patterns, as each technique has its own resolving power (Reis et al., 1990; Hillis et al., 1996; Boero, 2001) and it has been applied to studies in taxonomy and conservation biology assessments (e.g., Agnèse et al., 1997; Rognon et al., 1998; Salducci et al., 2004). In the Neotropical region, studies with these techniques have included cytogenetics and morphology (Moreira-Filho & Bertollo, 1991; Mizoguchi & Martins-Santos, 1998).

Our data were compatible with an isolation-by-distance model, also affected by higher-order vicariant processes and independent ecological or evolutionary histories, evidenced by lower levels of genetic diversity in the Casca river samples and high levels of variation in the Matipó river. In the Santo Antônio sample, sampling conditions may have biased our results, because most specimens were collected in a marginal pond. In Casca river samples, similar RAPD-PCR marker frequencies in locales A and B was unexpected. The Escura waterfall is an effective barrier for a medium-sized characin, *Leporinus copelandii*, a strong swimmer and highly migratory species. This pattern would be expected either if (a) the waterfall were not a selective obstacle for the Astyanax species, (b) the

waterfall was of relatively geological recent age. Waterfalls in this basin are usually considered as Plio-Pleistocene (A. Saadi, University Federal of Minas Gerais, Belo Horizonte/MG, Brazil, pers. com.) and it may not have allowed for genetic differentiation between upriver and downriver populations. Finally, (c) populations isolated by the waterfalls may be large enough to overcome random loss of alleles. In Matipó (locales D and E), all species are present at both sides of the former waterfall of Emboque, now substituted by a dam. Because dams are efficient barriers to gene flow in any direction (Avise & Felley, 1979; Revaldaves et al., 1997; Neeras & Spruell, 2001), our data are relevant for suggesting the need for restitution of historical migration in the Matipó river. In the Casca river, the Grande waterfall was the only locale where populations showed the large morphologic and molecular differences. This waterfall is also the highest (15 m) and probably affects survival of fish swimming downstream. Considering all the evidences, it may be that in the Matipó river, as well as the lower reaches of the Casca rivers, migrants from upstream populations may be the strongest factor in maintaining similarities between populations. Maintenance of large populations would be guaranteed by the generalist behavior of this species (Costa & Braga, 1993; Esteves & Galetti, 1995). Divergent molecular frequencies qualify each tributary as a management unit (Moritz, 1994), as also suggested by divergent patterns of meristic characters. Although this species behaves as an *r*-strategist, ecological studies have also reported population differentiation in a related form, *A. altiparanae* (Orsi et al., 2004). Within the Doce basin, molecular differences accumulate at a fast rate in lacustrine habitats. Significant variation in RAPD-PCR alleles has been reported for a sedentary species (*Hoplias malabaricus*) inhabiting Quaternary lakes (Dergam et al., 2002). Likewise, Nesbo et al. (1998) observed molecular differences among populations of *Perca fluviatilis* isolated for 100 years in the Baltic Sea.

Conclusion

Genetic and morphologic variation of *A. bimaculatus* in the Doce river basin was not necessarily associated with waterfall and dam location. For the major waterfalls, both sets of data were congruent and suggested high levels of population divergence. Tributaries of this river basin may be considered as management units for this species, and overall levels of morphologic variation suggest the existence of a single taxon.

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