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Differential population history in the migratory catfishes *Brachyplatystoma flavicans* and *Pseudoplatystoma fasciatum* (Pimelodidae) from the Bolivian Amazon assessed with nuclear and mitochondrial DNA markers

J. S. Coronel*†, G. E. Maes*, S. Claus*‡, P. A. Van Damme*† and F. A. M. Volckaert*§

*Laboratory of Aquatic Ecology, Katholieke Universiteit Leuven, Ch. de Bériotstraat 32, B-3000 Leuven, Belgium and †Programa de Conservación y Manejo de Recursos Hidrobiológicos, Centro de Limnología y Recursos Acuáticos, Universidad Mayor de San Simón, Cochabamba, Bolivia

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The catfishes *Brachyplatystoma flavicans* ($n = 49$) and *Pseudoplatystoma fasciatum* ($n = 69$) showed comparable low allozyme diversities ($H_e = 0{\cdot}012$ and 0·009–0·028, respectively), but contrasting PCR-RFLP restriction site mitochondrial DNA diversities (three haplotypes: $\pi = 0{\cdot}034$–$0{\cdot}092$ and five haplotypes: $\pi = 0{\cdot}001$–$0{\cdot}023$, respectively) in the Rio Ichilo and Beni (Bolivia). Genetic homogeneity between samples was high for *B. flavicans* and lower for *P. fasciatum*. Based on mitochondrial diversity, both species probably experienced a historic population reduction but at different time scales.

Key words: allozymes; evolution; Mamoré basin; PCR-RFLP; restriction sites; Teleostei.

INTRODUCTION

The catfish family of the Pimelodidae (Siluroidei), comprising >300 species, represents one of the largest freshwater taxa in the neotropics (Teugels, 1996). It includes remarkable long-distance migrants between the downstream and upstream reaches of large rivers such as the Amazon and Orinoco (Barthem & Goulding, 1997; Ruffino *et al.*, 2000). *Brachyplatystoma flavicans* (Castelnau) and *Pseudoplatystoma fasciatum* (L.), which were the focus of the present study, are among the five most important commercial catfishes in the Amazon Basin.

Brachyplatystoma flavicans, known as 'plateado' or 'dorado' (Bolivia) and 'dourada' (Brazil), occurs in the Amazon, Orinoco and Paraná river basins, including their estuaries. These fish reach sizes of >140 cm total length (L_T) and

§Author to whom correspondence should be addressed. Tel.: +32 16 32 39 66; fax: +32 16 32 45 75; email: filip.volckaert@bio.kuleuven.ac.be

‡Present address: Flanders Marine Institute, Vismijn, Pakhuizen 45-52, B-8400 Oostende, Belgium.

masses of 30 kg. The fish prefers the pelagic of large river channels and preys on fishes. It probably makes the longest migrations known among freshwater fishes, traveling *c.* 3300 km from the Amazonian estuary to the spawning grounds in the upper reaches of the basin (Barthem & Goulding, 1997; Ruffino *et al.*, 2000).

Pseudoplatystoma fasciatum, known as 'surubí' (Bolivia) and 'surubim-lenha' (Brazil), can be found in a much wider range of habitats, including main river channels, floodplain lakes (várzea) and larger rainforest streams, in both running and still waters. It is widespread in the Amazon, Corintijns, Essequibo, Orinoco and Paraná basins, but rare or absent from the estuary (Barthem & Goulding, 1997). Surubí feed on fishes and crabs (Burgess, 1989) and migrate between the spawning grounds in the headwaters of the Andes and the middle Amazon (Rio Negro) (Barthem & Goulding, 1997; Loubens & Panfili, 2000).

Genomic variability reflects the population history of each species, including the distinction between various demographic histories (Grant & Bowen, 1998). The few studies on the genetic features of Amazon fishes (Lovejoy & De Araujo, 2000; Calcagnotto *et al.*, 2001; Farias *et al.*, 2003), and even fewer on catfishes (Almeida & Sodré, 1998; Almeida *et al.*, 2001) have focused on diversity and to a lesser extent genetic population structure. Using nuclear and mitochondrial (mt) DNA markers, the genetic characteristics of *B. flavicans* and *P. fasciatum* were assessed in the Bolivian part of the Amazon basin in relation to their historical and contemporary population dynamics. Discrimination was made between the influence of vicariance and fishing pressure. The relationship between the high dispersal capabilities and the genetic structures of both species was then tested.

MATERIALS AND METHODS

All specimens were caught at maturity, assuming a single stock, by gillnet in the main channel of the Rio Ichilo and Beni, as well as in some Ichilo-floodplain lakes (distances of 0·1 to 0·5 km separated the river from the lakes), between August 1999 and February 2000, possibly during their upstream spawning migration at the beginning of the high-water season and close to the spawning grounds (Fig. 1 and Table I). Fish enter the floodplain to feed and occasionally are trapped in the lakes until the next flood pulse (pers. obs.). Samples of liver and muscle were collected and flash frozen in liquid nitrogen. Additional samples of muscle or fin tissue were preserved in salt saturated dimethylsulphoxide.

Allozyme mobility differences were detected by cellulose acetate gel electrophoresis (CAGE) using two buffer systems: tris-maleate pH 7·8 and tris-glycine pH 8·8 (Hebert & Beaton, 1989). Isozymes were visualized using the agar overlay method (Richardson *et al.*, 1986; Hebert & Beaton, 1989). Locus nomenclature followed Shaklee *et al.* (1990). Nine enzyme systems coding for 18 loci were screened: aspartate aminotransferase (*AAT-1**, *AAT-2**, E.C.2.6.1.1), creatine kinase (*CK-1**, *CK-2**, E.C.2.7.3.2), isocitrate dehydrogenase (*IDH-1**, *IDH-2**, E.C.1.1.1.42), lactate dehydrogenase (*LDH-1**, *LDH-2**, E.C.1.1.1.27), malate dehydrogenase (*MDH-1**, *MDH-2**, E.C.1.1.1.37), mannose-6-phosphate isomerase (*MPI-1**, *MPI-2**, E.C.5.3.1.8), glucose phosphate isomerase (*GPI-1**, *GPI-2**, E.C.5.3.1.9), phosphoglucomutase (*PGM-1**, *PGM-2**, E.C.2.7.5.1) and L-iditol dehydrogenase (*IDDH-1**, *IDDH-2**, E.C.1.1.1.14). Alleles were denoted according to their relative mobility.

Genomic DNA was isolated from fin tissue following Winnepenninckx *et al.* (1993). Two mitochondrial DNA regions were amplified: the ND5/6 region (*c.* 2·4 kb) was amplified using the primers ND5/6H T0586B10 and ND5/6L T0586B09 (Cronin *et al.*, 1993) together with the combined cytochrome *b* and control region (D-Loop) (*c.* 2·25 kb)

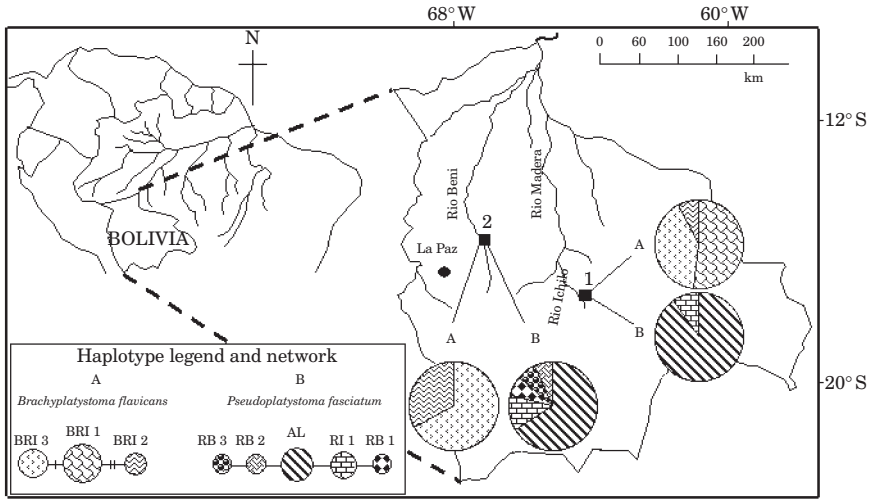

FIG. 1. *Brachyplatystoma flavicans* and *Pseudoplatystoma fasciatum*: sampling sites and RFLP-PCR haplotype frequencies (pies) in the Bolivian Amazon basin; 1 and 2, the villages of 'Puerto Villarroel' on the Rio Ichilo, and 'Rurrenabaque' on the Rio Beni. Inset: median joining network of RFLP-PCR restriction fragments.

using the primers CND5/6 and HN20 (Bernatchez & Danzman, 1993). Each fragment was amplified by the polymerase chain reaction (PCR) in 25 µl reaction cocktails containing 2·5 µl of PCR-buffer, 2·5 µl of dNTPs (1 mM), 1 µl of $MgCl_2$ (50 mM), 0·1 µl (5 U) of *Taq*-polymerase, 16 µl of dH_2O and 1 µl of each primer. One µl of DNA solution was added to each reaction. The PCR conditions were as follows: 95° C (3 min), 35 cycles of: 95° C (45 s), 50° C (45 s), 72° C (150 s), followed by a final elongation step at 72° C (7 min) and a cooling period at 4° C. Restriction enzyme digestion was performed in 10 µl of solution containing 5 µl of resulting DNA-PCR, 1 µl of buffer, 3·5 µl of double-distilled H_2O and 0·5 µl of restriction enzyme. The following restriction enzymes were used: *Mbo I, Hinf I, Hpa II, Alu I, Hae III, Taq I* and *Rsa I* (MBI Fermentas). The restriction fragments were electrophoretically separated on a 2% agarose gel (1 g of agarose and 1 g of Nusieve agarose diluted in 100 ml of 0·5X TBE buffer), stained with ethidiumbromide and scored. Only the complementary primers CND5/6 and HN20 (2·25 kb) yielded any level of polymorphism.

Allozyme mean number of alleles (MNA), level of polymorphism (P) and observed and expected heterozygosity (H_O, H_E) were calculated with the software GENETIX version 4.03 (Belkhir *et al*., 1998). Departures from Hardy–Weinberg expectations were evaluated with GENEPOP version 3.1 (Raymond & Rousset, 1995). The *F*-statistics (Weir & Cockerham, 1984) were quantified in the GENETIX software and tested for significance with 1000 permutations. Significance levels of simultaneous tests were corrected with sequential Bonferroni adjustments to probabilities (Rice, 1989). Mitochondrial haplotype and nucleotide diversity and F_{ST} values (Weir & Cockerham, 1984) were calculated from the restriction fragment frequencies using the ARLEQUIN version 2.0 (Schneider *et al*., 2000). A median joining network was constructed using with NETWORK version 4.0 (Bandelt *et al*., 1999).

RESULTS

All *B. flavicans* were adults with average sizes of 99 (males) to 114 cm L_T (females). The nine allozymes scored for the Rio Ichilo sample yielded 16 loci of which five (*IDH-1**, *MPI-1**, *MDH-1**, *MDH-2** and *GPI-2**) were polymorphic,

TABLE I. Allozyme and mitochondrial DNA genetic diversity (means ± S.D.) of *Brachyplatystoma flavicans* and *Pseudoplatystoma fasciatum*. Allozymes: *n*, number of individuals; H_O, observed mean heterozygosity per locus; H_E, unbiased expected heterozygosity (Nei, 1978); P(0·95), level of polymorphism at the 95% criterion; P(0·99), level of polymorphism at the 99% criterion; *MNA*, mean number of alleles per locus. PCR-RFLP restriction site haplotypes at the cytochrome *b* and control region (2·25 kb): *h*, haplotype diversity; π, nucleotide diversity; number of haplotypes and number of polymorphic sites are also given

	Allozymes						Mitochondrial DNA				
Species and collection site	*n*	$H_O \pm$ S.D.	$H_E \pm$ S.D.	P(0·95)	P(0·99)	*MNA*	*h*	π	Number of haplotypes	Polymorphic sites
Brachyplatystoma flavicans										
Ichilo	46	0·012 ± 0·021	0·012 ± 0·020	0·000	0·312	1·3	0·551 ± 0·048	0·034 ± 0·025	3	5
Beni	3	–	–	–	–	–	0·667 ± 0·314	0·092 ± 0·080	2	5
Pseudoplatystoma fasciatum										
Ichilo	37	0·013 ± 0·041	0·014 ± 0·048	0·056	0·167	1·2	0·054 ± 0·051	0·001 ± 0·003	2	1
Ichilo-lakes	17	0·010 ± 0·030	0·009 ± 0·028	0·056	0·111	1·1	0·118 ± 0·101	0·003 ± 0·005	2	1
Beni	15	0·026 ± 0·069	0·028 ± 0·079	0·111	0·167	1·2	0·562 ± 0·143	0·023 ± 0·019	5	4

having common allele frequencies of >0·95. Mean ± S.D. heterozygosity per locus was low $H_O = 0{\cdot}012 \pm 0{\cdot}021$, even though the level of polymorphism was 31·2% at P(0·99). These statistics point to the presence of many rare alleles (Table I). There were no deviations from Hardy–Weinberg expectations (Appendix I).

Three composite mtDNA haplotypes based on three polymorphic enzymes (*HinfI, AluI* and *RsaI*) were found in the main channel of the Rio Ichilo (BRI1, BRI2 and BRI3). These haplotypes differed at two to five restriction sites (Appendix II). Haplotypes BRI2 and BRI3, found in the Rio Ichilo, were also present in the main channel of the Rio Beni. The average haplotype diversities (H_O) in the Rio Beni ($0{\cdot}667 \pm 0{\cdot}314$, mean ± S.D.) and Rio Ichilo ($0{\cdot}551 \pm 0{\cdot}048$) were similar. Nucleotide diversities (π) were moderate to large ranging from 0·03 (Ichilo) to 0·09 (Beni). Geographical differences in haplotype frequencies between the two populations of *B. flavicans* were not significant, and the non-significant fixation index ($F_{ST} = 0{\cdot}078$, $P = 0{\cdot}229$) suggests one breeding unit. The median joining network, constructed from the matrix of the restriction sites, yielded a central (ancestral) haplotype BRI1 and two terminal haplotypes separated by two to three mutations (BRI2 and BRI3) (Fig. 1).

All *P. fasciatum* were adult with average L_T of 78–92 cm (males) and 83–98 cm (females), being significantly larger in the channel of the Rio Beni than fish from the floodplain lakes and main channel of the Rio Ichilo (*t*-test, d.f. = 50, $P < 0{\cdot}001$). Out of 18 loci scored, five loci (*AAT-2**, *IDH-1**, *MDH-2**, *GPI-1** and *PGM-1**) were polymorphic in the three populations at the 99% criterion (Appendix 1). The population in the main channel of the Rio Beni had the highest observed heterozygosity ($0{\cdot}026 \pm 0{\cdot}069$, mean ± S.D.) and a higher level of polymorphism than the other populations 0·111 at P(0·95) and 0·167 at P(0·99). The population in the main channel of the Rio Ichilo had a mean ± S.D. observed heterozygosity of $0{\cdot}013 \pm 0{\cdot}041$, and a level of polymorphism of 0·056 and 0·167 at P(0·95) and P(0·99), respectively (Table I). The population in the Ichilo-floodplain lakes had a mean ± S.D. observed heterozygosity of $0{\cdot}010 \pm 0{\cdot}030$, with a level of polymorphism of 0·056 and 0·111 at P(0·95) and P(0·99), respectively, attributed to the loci *GPI-1** and *PGM-1**. The number of alleles per locus ranged from one to three with an average of 1·2. There was little or no population differentiation among the three populations of *P. fasciatum* ($F_{ST} = 0{\cdot}010$, $P > 0{\cdot}05$).

Five composite haplotypes (RI1, AL, RB1, RB2 and RB3) were found in *P. fasciatum* with the enzymes *MboI*, *HinfI*, *HaeIII* and *RsaI* (Appendix II). Haplotypes RI1 and AL were present at the three sites sampled, while haplotypes RB1, RB2 and RB3 were unique to the main channel of the Rio Beni. The most common haplotype (AL) had a frequency >66% in each population. In total 97% of the individuals from the main channel of the Rio Ichilo had the AL haplotype and only 3% RI1. Haplotype diversity was very low, namely $0{\cdot}054 \pm 0{\cdot}050$ (Table I). The population of the Ichilo-floodplain lakes had the same haplotypes as the river itself with a predominance of the AL haplotype (94%). Haplotype diversity was higher, $h = 0{\cdot}118 \pm 0{\cdot}101$. The population of the Rio Beni had the highest diversity ($0{\cdot}562 \pm 0{\cdot}143$) (Table I), where again, haplotype AL was most frequent (66%) followed by RI1 (13%) and RB1, RB2 and RB3 with 7% each. Nucleotide diversity (π) was low to moderate, ranging from 0·001 (Ichilo) to 0·02 (Beni). A significant but low amount of between-population

variation was observed among populations of *P. fasciatum* (mitochondrial $F_{ST} = 0{\cdot}146$, $P = 0{\cdot}021$). Most of this variation was attributed to the difference between the populations in the main channels of the Rio Ichilo and Beni ($F_{ST} = 0{\cdot}135$, $P = 0{\cdot}004$), which points to a low level of maternal gene flow. The median joining network puts the very common haplotype AL centrally, with RI1 and the singletons RB1, RB2 and RB3 being located externally (Fig. 1).

DISCUSSION

Allozyme diversity patterns differed between species: a high value in *P. fasciatum* (Beni) and low values in *B. flavicans* (Ichilo) and *P. fasciatum* (Ichilo). This might reflect a historic reduction in population size in the River Ichilo, and most obviously for *P. fasciatum*. A comparison of the allozyme genetic diversity of both species shows that the observed heterozygosities are lower than those in the sister family of the Pimelodidae, including *Pinirampus pirinampu* (Spix & Agassiz) ($H_O = 0{\cdot}043$; $n = 8$), *Pimelodus maculatus* Lacepède (0·060; 87) and in *Iheringichthys labrosus* (Lütken) (0·083; 17) (Almeida & Sodré, 1998), and much lower than the average for 49 species of freshwater fishes ($H_O = 0{\cdot}046$) (Ward *et al.*, 1994). Mitochondrial haplotypes provide more details and a higher resolution than allozymes as they enabled the detection of a maternal structure in *P. fasciatum*. More important, they allow for some critical differences between the population histories of both species to be made. The relation between haplotype and nucleotide diversities enables the discrimination between several scenarios of population history at different time scales (Grant & Bowen, 1998). *Brachyplatystoma flavicans* exhibits a large mitochondrial haplotype diversity in general and moderate nucleotide diversity mainly in the Ichilo River, which points to a population bottleneck followed by a rapid population growth and accumulation of mutations (Grant & Bowen, 1998). Recent data suggest that this species suffers the highest fishing pressure, possibly accounting for the missing and the lower number of haplotypes. On the other hand, *P. fasciatum* exhibits small (Ichilo) to large (Beni) mtDNA haplotype diversities and very small nucleotide diversities, which point to either (1) a recent population bottleneck or founder event by a few mtDNA lineages or (2) a population bottleneck followed by rapid population growth and accumulation of mutations respectively (Grant & Bowen, 1998). Nucleotide diversities, however, differ by a factor 10 between the Rivers Ichilo and Beni (Table I).

Populations in the Ichilo River seem to have suffered historical (*B. flavicans*) or recent (*P. fasciatum*) population reductions. The genetic markers indicate that the Beni River has maintained the highest level of genetic variability through history and that a restricted amount of female gene flow connects most populations, having important consequences for conservation and fisheries management.

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APPENDIX I. Allele frequencies at eight polymorphic allozyme loci of *Brachyplatystoma flavicans* and *Pseudoplatystoma fasciatum*. H_O, observed heterozygosity; H_E, unbiased expected heterozygosity; F_{IS}, average inbreeding coefficient (no value is significant at $P < 0{\cdot}05$)

		Brachyplatystoma flavicans	*Pseudoplatystoma fasciatum*		
		Ichilo	Ichilo	Ichilo-lakes	Beni
Locus	Allele	(46)	(35)	(17)	(15)
	100	1·000	1·000	1·000	0·970
*AAT-2**	110	–	–	–	0·030
	H_E	0·000	0·000	0·000	0·064
	H_O	0·000	0·000	0·000	0·066
	F_{IS}	–	–	–	0·000
*IDH-1**	090	0·970	–	–	–
	100	0·030	0·990	1·000	1·000
	110	–	0·010	–	–
	H_E	0·043	0·028	0·000	0·000
	H_O	0·044	0·029	0·000	0·000
	F_{IS}	0·011	0·000	–	–
*MPI-1**	080	0·010	–	–	–
	100	0·990	1·000	1·000	1·000
	H_E	0·021	0·000	0·000	0·000
	H_O	0·021	0·000	0·000	0·000
	F_{IS}	0·000	–	–	–
*MDH-1**	080	0·010	–	–	–
	100	0·990	1·000	1·000	1·000
	H_E	0·021	0·000	0·000	0·000
	H_O	0·021	0·000	0·000	0·000
	F_{IS}	0·000	–	–	–
*MDH-2**	080	0·030	0·010	–	–
	100	0·970	0·990	1·000	1·000
	H_E	0·063	0·028	0·000	0·000
	H_O	0·065	0·028	0·000	0·000
	F_{IS}	0·023	0·000	–	–
*GPI-1**	080	1·000	0·100	0·060	0·200
	100	–	0·890	0·940	0·800
	150	–	0·010	–	–
	H_E	0·000	0·205	0·111	0·320
	H_O	0·000	0·171	0·117	0·267
	F_{IS}	–	0·179	0·032	0·200
*GPI-2**	080	0·980	–	–	–
	100	0·020	1·000	1·000	1·000
	H_E	0·044	0·000	0·000	0·000
	H_O	0·044	0·000	0·000	0·000
	F_{IS}	0·011	–	–	–
*PGM-1**	100	1·000	1·000	0·970	0·930
	110	–	–	0·030	0·070
	H_E	0·000	0·000	0·057	0·124
	H_O	0·000	0·000	0·058	0·133
	F_{IS}	–	–	0·000	0·037
Multilocus F_{IS}		0·013	0·140	−0·021	0·117

APPENDIX II. PCR-RFLP haplotypes and restriction sites of the cytochrome *b* and control region locus for five restriction enzymes in *Brachyplatystoma flavicans* and *Pseudoplatystoma fasciatum*. *n*, number of individuals. *P*, level of polymorphism at 95%

Population	Code	*n*	*MboI*	*Hinf I*	*Alu I*	*Hae III*	*Rsa I*	*P*
Brachyplatystoma flavicans								
Ichilo	BRI 1	14	11111	110011	01111	1111111	0111111	0·540
	BRI 2	11	11111	110001	11111	1111111	0111111	0·420
	BRI 3	1	11111	111111	01111	1111111	1111111	0·040
Beni	BRI 2	2	11111	110001	11111	1111111	0111111	0·670
	BRI 3	1	11111	111111	01111	1111111	1111111	0·330
Pseudoplatystoma fasciatum								
Ichilo	AL	36	11001111	0111111	111111	111	1011111	0·970
	RI 1	1	11001111	0111111	111111	111	1111111	0·030
Ichilo lakes	AL	16	11001111	0111111	111111	111	1011111	0·940
	RI 1	1	11001111	0111111	111111	111	1111111	0·060
Beni	AL	10	11001111	0111111	111111	111	1011111	0·660
	RI 1	2	11001111	0111111	111111	111	1111111	0·130
	RB 1	1	11001111	0111111	111111	110	1111111	0·070
	RB 2	1	10001111	0111111	111111	111	1011111	0·070
	RB 3	1	10001111	0110111	111111	111	1011111	0·070