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Phylogeography of the Neotropical catfish *Pimelodus albicans* (Siluriformes: Pimelodidae) from río de la Plata basin, South America, and conservation remarks

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Pimelodus albicans Valenciennes, 1840 (common name "moncholo" or "bagre blanco") is an endemic species of the family Pimelodidae in the río de la Plata basin. Phylogenetic approach based on cytochrome b sequences was performed to test the existence of a unique evolutionary lineage in *P. albicans* and to discriminate populations units or subpopulations related to a migration behavior of this taxon in the río de la Plata basin. This study included 34 samples of *P. albicans* of different collecting sites in the río de la Plata estuary and in the río Arrecifes belonging to the río Paraná basin. Among 614 base pairs in the cytochrome b sequence data set, 203 were variable and 120 were phylogenetically informative sites in *P. albicans*. A total of twenty haplotypes, nucleotide diversity (π) = 0.032 and haplotype diversity = 0.941 were found. Tajima's test showed significant value D= -1.88 ($p<0.05$) rejecting the neutral mutation hypothesis for the *P. albicans* data set. All phylogenetic approaches showed that *P. albicans* included four monophyletic assemblages that were supported by high bootstrap and Bayesian posterior probability values. Minimum spanning network corroborated these groups for *P. albicans* haplotypes. High genetic structure was found in *P. albicans* by means of AMOVA analysis showing that the río Arrecifes samples constitute an isolated lineage. Moreover, the high value of genetic divergence (10%) between the río de la Plata and the río Arrecifes populations could suggest that *P. albicans* may be conformed by a sibling species complex. On the other hand, a degree of genetic structuring was detected among different sites of the río de la Plata. A partial isolation of the 760 site may suggest that *P. albicans* could migrates to different tributaries for reproduction, generating different schools of haplotypes which could mix in the río de la Plata estuary. The high nucleotide diversity found in the 765 site and the existence of gene flow with the remaining collecting sites would be concordant with the outlined hypothetic scenarios of the mixing populations in the middle of the río de la Plata estuary.

Pimelodus albicans Valenciennes, 1840 (popularmente conhecida como moncholo ou bagre branco) é uma espécie endêmica da família Pimelodidae na bacia do rio da Prata. Estudos filogeográficos baseados nas seqüências do citocromo b mitocondrial foram realizados para testar a existência de uma única linhagem evolutiva in *P. albicans* e para discriminar unidades populacionais relacionadas ao comportamento migratório desse táxon na bacia do rio da Prata. Um total de 34 amostras de *P. albicans* provenientes de diferentes lugares de coleta no estuário do rio da Prata e rio Arrecifes na bacia do rio Paraná foram analisados. Entre as 614 pares de bases do citocromo b no conjunto de dados, 203 deles variaram e 120 foram sítios filogeneticamente informativos para *P. albicans*. No presente estudo foi encontrado um total de vinte haplótipos, diversidade de nucleotídeos (π) = 0,032 e diversidade de haplótipos = 0,941. O teste de Tajima mostrou valores significativos D= -1,88 ($p<0,05$) rejeitando a hipótese de mutação neutra para os dados de *P. albicans*. Todas as análises filogenéticas mostraram que o clado *P. albicans* apresenta quatro grupos monofiléticos com um forte suporte estatístico e uma elevada probabilidade posterior Bayesiana. A rede de haplótipos para *P. albicans* mostrou uma forte estrutura desses quatro grupos. Uma grande estruturação genética foi observada em *P. albicans* nas análises de AMOVA mostrando que as amostras do rio Arrecifes constituem uma linhagem isolada. A alta divergência (10%) encontrada entre as populações do rio da Prata e rio Arrecifes sugere que *P. albicans* pode constituir um complexo de espécies crípticas. Foi verificada também a ocorrência de estrutura genética na bacia do rio da Prata. A localidade 760 apareceu parcialmente isolada sugerindo que *P. albicans* migra para procriar em os afluentes do rio da Prata gerando diferentes cardumes de haplótipos que poderiam se misturar no estuário do rio da Prata. A alta diversidade nucleotídica encontrada na localidade 765 e a existência de fluxo gênico entre os demais sítios de coleta são concordantes com o cenário hipotético de existência de populações intercruzantes no meio do estuário do rio da Prata.

Key words: Population structure, Cytochrome b gene.

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Introduction

Catfishes comprise 38 families and include more than 3000 species (Sullivan *et al*., 2006), representing an important component of the global fish fauna. Several authors concluded that catfishes constitute a monophyletic group of fishes (Fink & Fink, 1981, 1986; de Pinna, 1998; Saitoh *et al.*, 2003). The Siluriformes have a wide distribution, living in freshwater, estuarine and marine environments, from inland or coastal waters of all continents, excluded Antarctica where they have been present in the past (Grande & Eastman, 1986). Probably, the high radiation of those fishes begun in the Late Cretaceous (de Muizon *et al.*, 1983; Cione *et al.*, 1985). Most members of the order inhabit freshwater environments and the highest catfish diversity occurs in the Neotropical Region. Neotropical catfishes are included in fifteen families: Ariidae, Astroblepidae, Aspredinidae, Auchenipteridae, Callichthyidae, Cetopsidae, Diplomystidae, Doradidae, Heptapteridae, Loricariidae, Nematogenyidae, Pimelodidae, Pseudopimelodidae, Scoloplacidae and Trichomycteridae. However, the relationships among catfish families are poorly understood. Three recent papers showed the difficulties to resolve interfamilial phylogenetic relationships with morphology information (Rodiles-Hernández *et al*., 2005) and molecular studies (Hardman, 2005; Sullivan *et al.*, 2006).

Lundberg *et al.* (1991a, 1991b) showed that the traditional family Pimelodidae did not form a monophyletic group. This assemblage, including more than 300 species was separated in three monophyletic units (de Pinna, 1998) subsequently considered at family level, Pimelodidae (Lundberg & Littmann, 2003), Heptapteridae (Bockman & Guanzelli, 2003) and Pseudopimelodidae (Shibatta, 2003). Combining information of several authors and based on morphological characters de Pinna (1998) implemented a phylogenetic analyses, concluding that these families appeared to be more closely related to other catfishes than to each other (Fig.1). However, newly phylogenetic reconstruction based in molecular markers showed that these families conformed a monophyletic entity in which Heptapteridae clade appeared to be a sister group of the clade integrated by Pimelodidae and Pseudopimelodidae (Fig. 1, Hardman, 2005; Sullivan *et al.*, 2006).

Molecular studies carried out during last years, involving Siluriformes, were focussed to resolve the complex phylogenetic relationships of catfishes at higher taxonomic levels, among or within families. However, more recent studies have characterized the catfish diversity at intrageneric level. A recently published paper about Mesoamerican populations of the genus *Rhamdia* using a strong molecular marker, the mitochondrial cytochrome b gene, found high level of intrageneric diversity among populations of *Rhamdia* from Central and South America concluding that this taxon includes two major sibling species complex (Perdices *et al.*, 2002).

The cytochrome b gene has proven to be an excellent molecular marker. It is widely used as a tool in molecular phylogeny of fishes, since this gene has both conservative and variable regions which contain signals that may be used in phylogeny at many different taxonomic levels (Kocher *et al*., 1989; Meyer *et al*., 1990; Cantatore *et al*., 1994; Martin & Bermingham, 1998; Renesto *et al.,* 2000; Garcia *et al*., 2000, 2002; So *et al.*, 2006).

The río de la Plata basin is the second major one basin in South America. A family Pimelodidae member, *Pimelodus albicans* Valenciennes, 1940 (common name "moncholo" or "bagre blanco") is an endemic species of that basin, especially living in the río de la Plata and the lower areas of the rivers Paraná and Uruguay. This taxon seems to be a seasonal species of the assemblage living in the inner río de la Plata estuary (Jaureguizar *et al*., 2004). Adult fishes reach a maximum size of 65 cm, occupying freshwater benthopelagic environments. This species represent a high value resource on the artisanal and commercial fisheries from estuarine and river areas. However, population dynamics and genetic structure are unknown. As other fishes from the río de la Plata basin, *P. albicans* carries out reproductive migrations from lower parts of rivers to the headwaters (Svertlij *et al.*, 1998). The knowledge about spawning and breeding sites, movements and migrations of larvae and juveniles are almost completely unknown along its wide distribution.

Here we present a preliminary phylogeographic approach based on mitochondrial cytochrome b sequences to test the existence of a unique evolutionary lineage or a possible sibling species complex in *P. albicans* and to discriminate populations units or subpopulations related to migrations behaviour of this taxon in the río de la Plata basin. The knowledge of the dynamic and structure of *P. albicans* population are prerequisites for the design of appropriate resource management and conservation strategies.

Material and Methods

Samples and DNA extraction. The samples of *P. albicans* were obtained from specimens belonging to 5 collecting sites in the río de la Plata, between Argentina and Uruguay countries, during the travel of the BIP Dr. E. Holmberg cruiser, conducted by the FREPLATA Project (GEF, 2001). The voucher specimens of this Project were not preserved. Moreover, specimens from the río Arrecifes, an affluent of the Paraná river, in Argentina, were included (Fig. 2). In order to compare the level of the genetic divergence among sibling species complex several sequences of the genus *Rhamdia* were included as a part of the ingroup taxa: *Rhamdia* sp., *R. cinerascens* (AY036736), *R. rogersi* (AY036734), *R. cabrerae* (AY036726), *R. nicaraguensis* (AY036719), *R. laticauda* (AY036710, AY036704, AY036699, AF287456), *R. wagneri* (AY036695, AY036693, AY036678), and *R. guatemalensis* (AY036670, AY036667, AY036641, AY036634). Additionally in order to determinate the divergence at family level other species of the families Pimelodidae (*Parapimelodus valenciennis, "Pimelodus" ornatus* and *Pimelodus pictus*; AY458896) and Heptapteridae (*Imparfinis* sp., *Pimelodella* sp., and *Pimelodella chagresi* AY036751) were included. The outgroup analysis included cytochrome b sequences

belonging to *Olivaichthys viedmensis* and *O. mesembrinus*. Tissues and voucher specimens from the aforementioned Siluriformes genera were deposited in the Sección Genética Evolutiva Collection, Facultad de Ciencias, Montevideo, Uruguay (Catalog identification number is indicated in Table 1).

DNA was extracted from small pieces of liver and muscle tissues fixed in EtOH, using proteinase K digestion, protein precipitation through sodium chloride and DNA total precipitation with ethanol (modified from Medrano *et al.*, 1990).

Mitochondrial cytochrome b (cyt b) sequences. Amplification of approximately 800-bp fragment of cyt b gene was performed by means of PCR experiments using GludgL and CB3H primers (Palumbi *et al*., 1991). The reaction occurs in 15µL, 1.5µL of core buffer 10x, 0.45µL of $MgCl_2$ (50mM), 0.3µL of dNTPs (10mM), 0.25µL of each primer (10µM), 0,15 (5U/µL) of Taq DNA polimerase (Invitrogen™), 1.5µL of template DNA (100-400 ng/ml) and 10.6µL of H_2O. PCR cycle profile was as follow: 94ºC for 45s, 45ºC for 45s, 72º for 1 min; iterated during

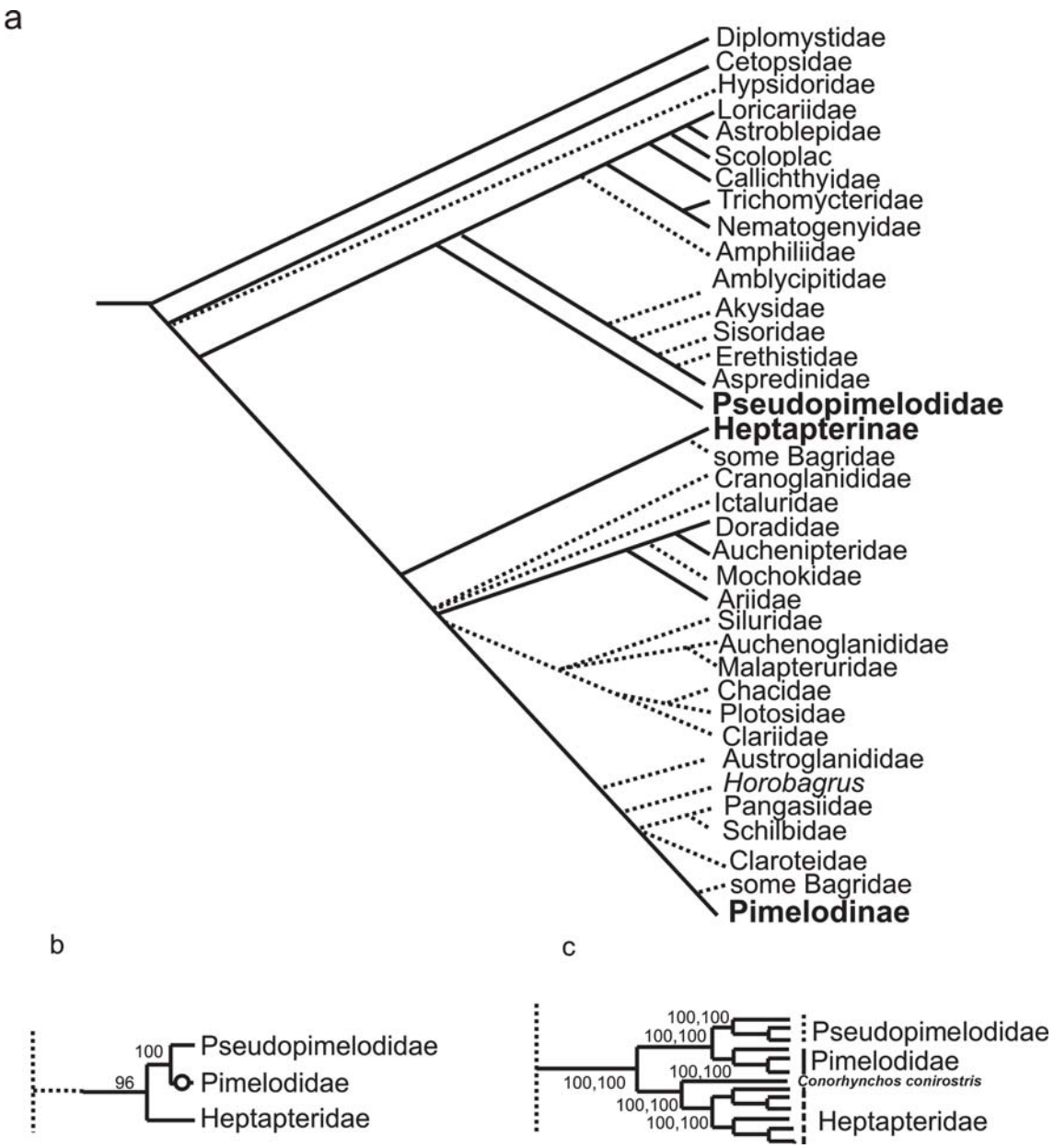

Fig. 1. Hypotheses of phylogenetic relationship among three Siluriformes families: Pimelodidae, Heptapteridae, Pseudopimelodidae. **a-** Cladogram presented by de Pinna (1998). **b-** Consensus tree among three most parsimonious topologies based on cyt b sequences modified from Hardman (2005). Numbers above nodes are posterior probabilities recovered by the Bayesian analysis for those clades common to both parsimony and likelihood topologies. Nodes with 0 failed to reject the null hypothesis of zero length, and are considered falsely resolved. **c-** Maximum parsimony analysis of *rag1* and *rag2* sequences showing likelihood bootstrap values and Bayesian posterior probabilities (as %) modified from Sullivan *et al.* (2006).

Table 1. Specimens list included in this study. The corresponding number of laboratory catalog identification for each sample and cyt b haplotype in *P. albicans* are indicated. The number of specimens corresponding to each haplotype, their collecting sites GenBank Accession Numbers and Number of Voucher Specimens and Collection are included.

Family	Species	cyt b haplotype	Catalog number	n	Collecting sites	Accession umber to GenBank	Voucher Specimens and Collection
Pimelodidae	*Pimelodus albicans*	Pa.hap1	P606	5	756	EF564718	
			P619		760		
			P627		779		
			P630		765		
			P631		765		
		Pa.hap2	P609	3	756	EF564719	
			P610		756		
			P618		760		
		Pa.hap3	P607	1	756	EF564720	
		Pa.hap4	P622	1	760	EF564721	
		Pa.hap5	P614	1	771	EF564722	
		Pa.hap6	P604	1	756	EF564723	
		Pa.hap7	P616	1	771	EF564724	
		Pa.hap8	P617	1	771	EF564725	
		Pa.hap9	P605	5	756	EF564726	
			P613		771		
			P623		779		
			P624		779		
			P625		779		
		Pa.hap10	P633	1	756	EF564727	
		Pa.hap11	P629	1	765	EF564728	
		Pa.hap12	P628	1	765	EF564729	
		Pa.hap13	P621	5	760	EF564730	
			P632		765		
			P634		765		
			P636		760		
			P637		760		
		Pa.hap14	P635	1	760	EF564731	
		Pa.hap15	P611	1	760	EF564732	
		Pa.hap16	P612	1	760	EF564733	
		Pa.hap17	P294	1	Argentina, Buenos Aires, río Arrecifes, Jul 2005	EF564734	AI 219, Coll. Sergio Bogan
		Pa.hap18	P296	1		EF564735	
		Pa.hap19	P293	1		EF564736	
		Pa.hap20	P295	1		EF564737	
	Parapimelodus valenciennis		P96	1	Uruguay	EF564738	P96, Coll. Sección Genética Evolutiva
			P97	1		EF564739	P97, Coll. Sección Genética Evolutiva
	"Pimelodus" ornatus		P279	1	Argentina, Misiones, río Paraná, Posadas	EF564741	uncat., Coll. M. Azpelicueta
Heptapteridae	*Pimelodella sp.*		P116	1	Uruguay	EF564740	P116, Coll. Sección Genética Evolutiva
	Imparfinis sp.		P283	1	Argentina, río Iguazú basin, Feb 2002	EF564742	AI 211, Coll. M. Moreno
	Rhamdia sp.		P284	1	Argentina, Misiones, Cuñapirú Oct 2004	EF564743	uncat., Coll. M. Azpelicueta
Diplomystidae	*Olivaichthys viedmensis*		P280	1	Argentina, río Negro, lago Moreno Este, Mar 2000	EF564744	unact., Coll. Liliana Semenas
	Olivaichthys mesembrinus		P282	1	Argentina	EF564745	Cenpat 1999/1-1, A. E. Gosztonyi

38 cycles. The resulting products were purified for direct sequencing with the kit: CONCERT™ Rapid PCR Purification System (Gibcoâ). Sequences were obtained using a Perkin-Elmer ABI Prisma 337 automatic sequencer in the Centro Técnico de Análisis Genéticos (CTAG) from Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay.

The final sequence data set resulted by reconciling chromatograms for the light and heavy DNA strands. Sequence alignment was performed using CLUSTAL X program, version 1.8 (Thompson *et al.,* 1997).

Statistical analyses, DNA polymorphism. Nucleotide composition and substitution patterns were calculated using MEGA (Kumar *et al*., 2004) and DnaSP4 (Rozas *et al.,* 2003) software packages. The corrected estimation of pairwise sequence divergence was obtained using Kimura two-parameter algorithm (K2P, Kimura, 1980) implemented with MEGA software. DNA polymorphism was measured calculating the proportion of segregating sites (S), the haplotype diversity (Nei, 1987: 179) and the nucleotide diversity π (Nei, 1987: 257) using ARLEQUIN (Excoffier *et al*.,

2005) and DNASP4 (Rozas *et al*., 2003) software packages.

Tajima´s test (Tajima, 1989) was performed to estimate the significant excess of low-frequency haplotypes in order to evaluate the hypothesis of population expansion for all data set using the DnaSP4 (Rozas *et al*., 2003).

ARLEQUIN 3.01 software package (Excoffier *et al*., 2005) was used to compute Tajima´s D (Tajima, 1989) and Fu´s Fs (Fu, 1997) neutrality tests grouping río de la Plata and río Arrecifes samples separately.

Phylogenetic Analyses. In order to access to the phylogeographic association among mitochondrial haplotypes, different methods of phylogenetic reconstruction using PAUP*4.0b8 (Swofford, 1998) were performed: maximum-parsimony (MP), neighbour-joining (NJ) and maximum-likelihood (ML). An equally weighted maximum-parsimony analysis was carried out through heuristic search (MULPARS option, stepwise addition, tree-bisection-reconnection [TBR] branch swapping, 100 replications). A strict consensus between rival trees was computed to found equally parsimonious topologies. Distance trees were generated under Hasenawa, Kishino & Yano model (1985) which considers differences among transversion and transition substitutions and among base frequencies. The phylogenetic reconstruction was subjected to the neighbour-joining method (Saitou & Nei, 1987). The degree of confidence assigned to nodes in the trees was assessed by bootstrapping with 500 replicates for both methods (MP and NJ). ML analysis was based on the optimal model of nucleotide substitution through the hierarchical likelihood ratio test (hLRTS) and additionally based on the Akaike Information Criterion (AIC); both analyses were implemented in the Modeltest v3.7 (Posada & Crandall, 1998). General time reversible model whit gamma distribution (GTR+G) resulted to be the best model for nucleotide substitution for these cyt b sequences. The ML tree topologies were inferred using that model. All trees were rooted by outgroup criterion using the Diplomystidae taxa. Bayesian inference (Rannnala & Yang, 1996) was implemented to access to the posterior probability of ML clades using Mr. Bayes 3.1 (Ronquist & Huelsenbeck, 2003).

Fig. 2. Map showing the collecting sites of *Pimelodus albicans*. The square represents the río Arrecifes, the remaining localities are indicated in the río de la Plata.

Analysis of Molecular Variance and Minimum Spanning Network. The hierarchical partitions of the genetic variance components in the data set of *P. albicans* were assessed through the Analysis of Molecular Variance (AMOVA) developed by Excoffier *et al.* (1992). The Euclidean metric of Excoffier *et al.* (1992) was used to construct the matrix of pairwise distances. Each haplotypes was assigned to their corresponding collecting site. The genetic variation was partitioned into three components: among groups, among populations (collecting sites) within groups and among individuals, disregarding either their original populations or their groups.

The relationships among the cyt b haplotypes were inferred through a minimum spanning tree based on the mean number of pairwise differences between haplotypes using the ARLEQUIN 3.01 software package (Excoffier *et al*., 2005).

Population structure was measured assuming the infinite mutation model (Kimura & Crow, 1964) and calculating the F_{ST} (Slatkin, 1991) for the whole population. Pairwise estimates of F_{ST} were calculated using ARLEQUIN 3.01 (Excoffier *et al*., 2005) to generate pairwise estimates of gene flow with the following formula: $N_f m \approx \frac{1}{2} [(1/F_{ST}) - 1]$ (Wright, 1951).

Results

Sequence Analysis and DNA Polymorphism. Present data set included approximately 614 bp of mitochondrial cyt b sequences belonging to *P. albicans* and other genera of the family Pimelodidae, Heptapteridae, and Diplomystidae. All sequences of *P. albicans* (N=34) as well as those of other pimelodids (N=3), heptapterids (N=3), and diplomystids (N=2) constitute new data set from catfishes (GeneBank accessions numbers are shown in Table 1).

Table 2 shows the pairwise genetic corrected distance values among the ingroup and the outgroup taxa using Kimura two-parameter model (K2P). The average nucleotide divergence between outgroup and ingroup was 17.5%.

The *P. albicans* nucleotide sequences analysis showed 203 variables and 120 phylogenetically informative sites. Base frequencies were 27.7% A, 27.7% T, 28.0% C, and 16.5% G. Similar values were observed in other Siluriformes (Peng *et al.*, 2004; Shimabukuro-Dias *et al.*, 2004). The average ratio of Ts:Tv (R) for cyt b sequence within *P. albicans* samples was 0.7. No indels of nucleotide sequences were detected. The obtained data set resulted in 204 amino acids: 111 were variables and 59 of them were phylogenetically informative sites.

Twenty haplotypes, nucleotide diversity (π) = 0.032 and

haplotype diversity = 0.941 were found in the *P. albicans* data set. Table 3 shows nucleotide diversity (π) for each collecting site. The average of genetic divergence among samples from all collecting sites was 5.7% and the pairwise genetic distances between them are presented in Table 4. Remarkably, río Arrecifes samples were separated by 10% of the genetic distance from all other localities. Additionally, the locality 760 was the most distant (2%) among all other remaining sites in the río de la Plata.

The Tajima's test has showed significative value D= -1.88 ($p<0.05$) which rejected the neutral mutation hypothesis for all these data set. Additionally, Tajima´s D and Fu´s Fs tests were not significant considering río Arrecifes and río de la Plata like separated sites (Table 3).

Phylogenetic analyses. All the phylogenetic approaches yielded to the same tree topology and showed two major clades: the first one includes the monophyletic family Heptapteridae and several members of the family Pimelodidae and the second one corresponds to *P. albicans* haplotypes. Nonetheless, each of them received different bootstrap support in MP, but they were well resolved in the ML tree topology and through Bayesian Inference (Fig. 3). Remarkably, the genus *Pimelodus* appears as a paraphyletic taxon and *P. ornatus* collapsed in a basal position of the tree.

Pimelodus albicans forms a monophyletic group showing high bootstrap support and bayesian posterior probability (Fig. 3). In both analyses *P. albicans* included four monophyletic assemblages supported by high bootstrap and Bayesian posterior probability values. The first clade comprised all río Arrecifes samples (1 in Fig. 3). The other clades were composed by río de la Plata samples as follows: the second clade (2 in Fig. 3) included samples from collecting site 771, the third clade (3 in Fig. 3) was predominantly integrated by samples from 760 and 765 sites, and the fourth clade (4 in Fig. 3) included a mix of individuals from four collecting sites (756, 765, 771, and 779). The remaining *P. albicans* haplotypes (1, 2, 3, 4, 5, 6, 10 and 11) collapsed in a basal polytomy joining the above mentioned clades. The first and second clades of *P. albicans* were the most divergent (see Figs. 3-4).

AMOVA analyses, Haplotype Network and Mantel test. To analyse the hierarchical partition of the genetic variation, four different hypotheses were tested: 1) all haplotypes of *P. albicans* considering their corresponding collecting sites as population were included in a single group; 2) the haplotypes included in their corresponding collecting sites were distributed in two groups: one of them including the río de la Plata samples and the other one with specimens of the río Arrecifes; 3) the haplotypes were distributed in five groups according to present phylogeographic results; 4) the haplotypes were separated in three groups corresponding to río Arrecifes, locality 760 from río de la Plata and the remaining río de la Plata samples.

Table 5 shows the results of the hierarchical partition of the variance components. This analysis revealed that under five groups hypothesis the percentage of genetic variation (80%) among groups was higher than other alternative hypotheses. Moreover, the fixation index (Φ_{ST}) was 0.79. These values were similar under the hypotheses 2 and 3.

Minimum spanning network based on cyt b sequences (Fig. 4) showed a strong structure among haplotypes of *P. albicans* conforming four groups. Haplotype 1 was the most

Table 2. Pairwise corrected genetic distances among Siluriformes taxa (below diagonal) and values of the corresponding standard error computed by bootstrap (500 replicates) (above diagonal).

	1	2	3	4	5	6	7	8
1 *Rhamdia* sp.		[0.013]	[0.029]	[0.021]	[0.020]	[0.022]	[0.030]	[0.025]
2 *Pimelodella* sp.	0.061		[0.030]	[0.025]	[0.024]	[0.022]	[0.032]	[0.027]
3 *Imparfinis* sp.	0.269	0.276		[0.027]	[0.027]	[0.027]	[0.041]	[0.031]
4 *Pimelodus albicans*	0.170	0.197	0.254		[0.013]	[0.019]	[0.028]	[0.017]
5 *Parapimelodus valenciennis*	0.154	0.172	0.252	0.059		[0.017]	[0.028]	[0.019]
6 *Pimelodus pictus*	0.176	0.155	0.248	0.125	0.091		[0.031]	[0.025]
7 *Diplomystes* spp.	0.261	0.262	0.411	0.223	0.206	0.242		[0.030]
8 *Pimelodus ornatus*	0.222	0.219	0.297	0.103	0.121	0.180	0.224	

Table 3. Nucleotide diversity (π) for *Pimelodus albicans* for each locality from the río de la Plata and río Arrecifes. D = Neutrality test (Tajima, 1989). Fs = Neutrality test (Fu, 1997) corresponding to río de la Plata and río Arrecifes sites.

Locality		π	Fu's F	Tajima's D
río de la Plata	756	0.083056 +/- 0.050820	0.33330 (p>0.05)	0.61823 (p>0.05)
	760	0.139671 +/- 0.080120		
	765	0.187879 +/- 0.112971		
	771	0.119565 +/- 0.082529		
	779	0.042017 +/- 0.030791		
río Arrecifes		0.173516 +/- 0.118929	0.60661 (p>0.05)	0.57082 (p>0.05)

Table 4. Pairwise corrected genetic distances for *Pimelodus albicans* among all the collecting sites (below diagonal) and values of the corresponding standard error computation by bootstrap (500 replicates) (above diagonal).

	756	760	765	779	771	Río Arr.
756		(0.005)	(0.003)	(0.003)	(0.004)	(0.015)
760	0.025		(0.004)	(0.005)	(0.005)	(0.015)
765	0.016	0.022		(0.003)	(0.004)	(0.015)
779	0.010	0.023	0.012		(0.004)	(0.015)
771	0.016	0.029	0.019	0.012		(0.015)
río Arrecifes	0.103	0.116	0.106	0.100	0.106	

frequent and central in the network analysis, representing perhaps the most ancestral one among the río de la Plata samples.

The pairwise F_{ST} and Nm values among collecting sites were presented in Table 6. Noticed, the samples from río Arrecifes presented the highest pairwise F_{ST} values (>0.7) and they showed absence of gene flow from all other collecting sites. In the río de la Plata, the 760 collecting site only shows genetic exchange with locality 765 and it appeared to be an isolated site from the rest. Moreover, 779 and 756 sites represent the most distant ones in the río de la Plata, showing absence of genetic exchange.

Discussion

High level of genetic diversity in the family Pimelodidae. In the present work, the genetic divergence among species of the family Pimelodidae ranged from 6% to 12%. In fact, the family Pimelodidae and the genus *Pimelodus* were paraphyletic (Fig. 3). Even though our analyses included a very limited number of taxa compared with previous molecular phylogenetic analyses in Siluriformes, our present data showed that two members of the family Pimelodidae resulted sister groups of the monophyletic Heptapteridae family

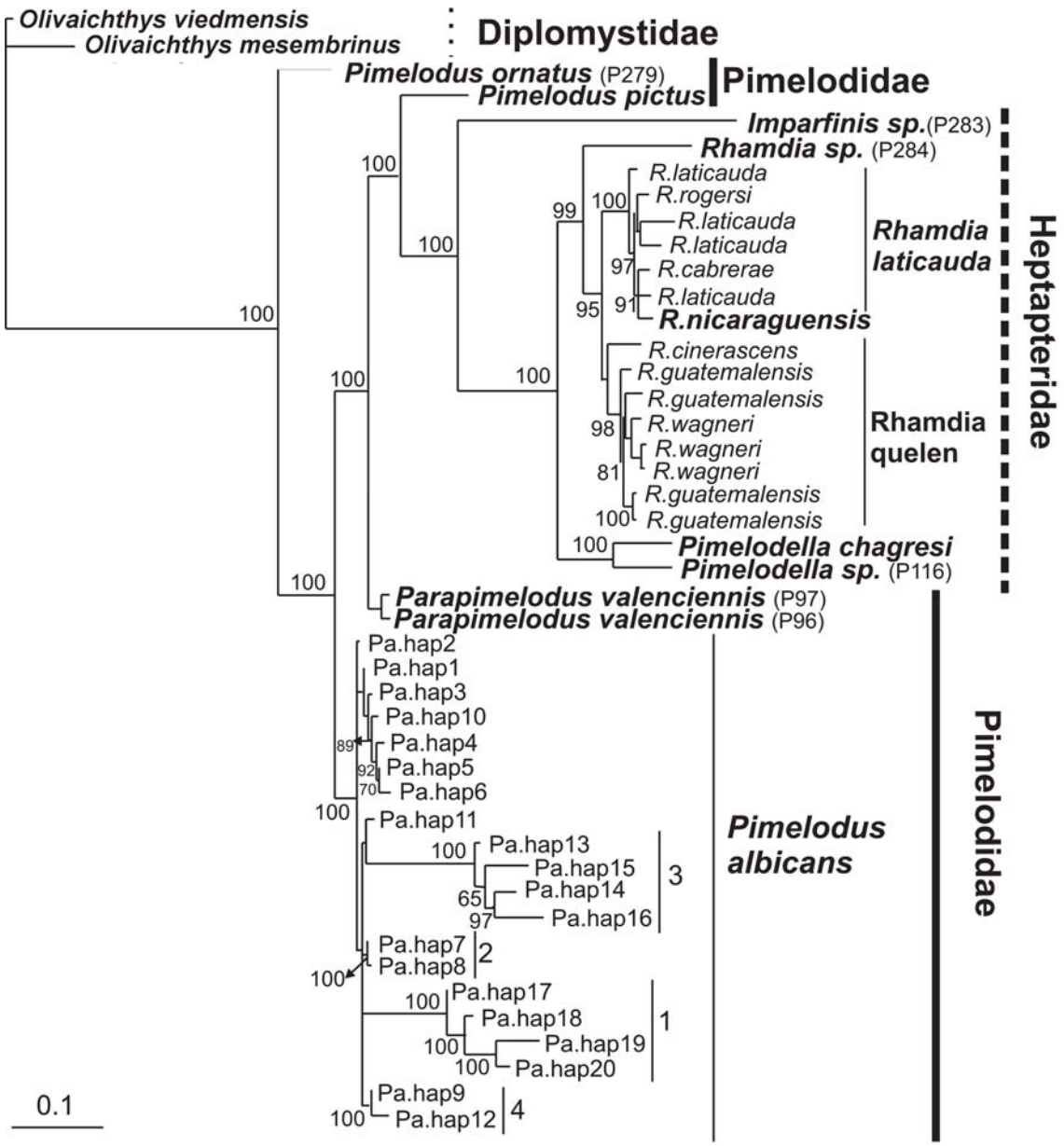

Fig. 3. Maximum likelihood tree based on GTR+G model, showing Bayesian support values in the nodes. Pimelodidae family appears basal and paraphyletic whereas *P. albicans* conforms as a monophyletic assemblage. *Pimelodus albicans* clade shows four monophyletic groups with strong clade support.

Table 5. Hierarchical partition of the variance components for *Pimelodus albicans* haplotypes under four different hypotheses: 1) a single group; 2) two groups including the río de la Plata samples and the río Arrecifes samples; 3) five groups according to present phylogeographic results; 4) three groups from río Arrecifes, locality 760, and remaining localities in the río de la Plata.

Hyp	Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation	Φ statistics
1	Among groups	5	149.663	4.72332 Va	55.68	-
	Among population within groups	-	-	-	-	-
	Within populations	28	105.278	3.75992 Vb	44.32	Φ_{ST} = 0.55678
2	Among groups	1	106.225	13.77550 Va	73.47	Φ_{CT} = 0.73473
	Among population within groups	4	43.439	1.21364 Vb	6.47	Φ_{CS} = 0.24402
	Within populations	28	102.278	3.75992 Vc	20.05	Φ_{ST} = 0.79946
3	Among groups	4	204.233	8.16359 Va	83.79	Φ_{CT} = 0.83789
	Among population within groups	8	8.958	-0.40861 Vb	-4.19	Φ_{CS} = -0.25870
	Within populations	21	41.750	1.98810 Vc	20.41	Φ_{ST} = 0.79595
4	Among groups	2	134.294	6.79562 Va	62.80	Φ_{CT}= 0.62803
	Among population within groups	3	15.369	0.26505 Vb	2.45	Φ_{CS}= 0.06585
	Within populations	28	105.278	3.75992 Vc	34.75	Φ_{ST} = 0.65252

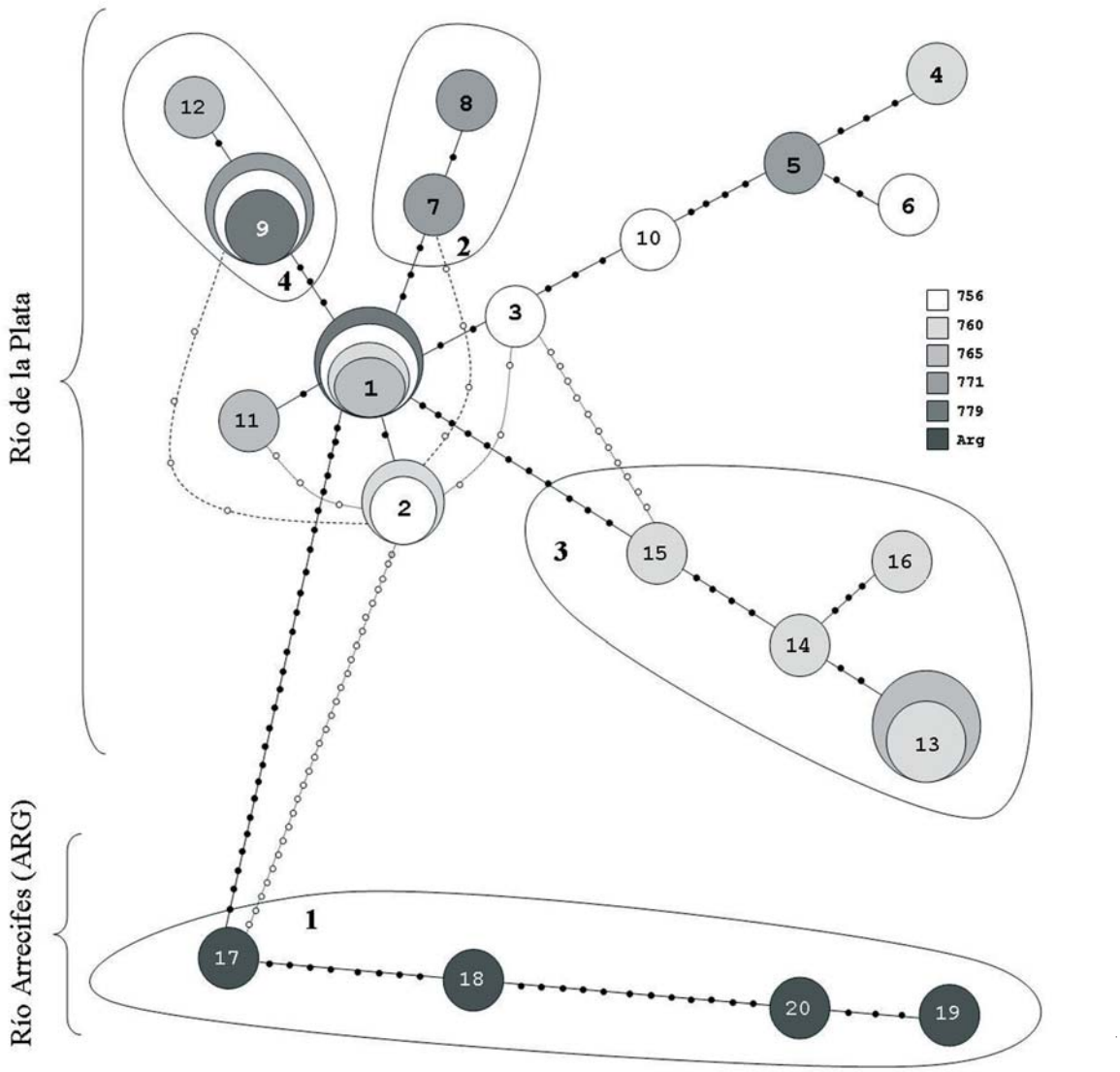

Fig. 4. Minimum spanning network of *P. albicans* haplotypes. The number of mutational steps separating each haplotype were represented by dots. Pointed branches represent alternatives links. The size of the circles represents the frequency of each haplotype.

(Hardman, 2005, Sullivan *et al*., 2006). Our results were at odds from those presented by Hardman (2005) in which the Heptapteridae family appears as a basal group in the clade integrated by Pimelodidae and Pseudopimelodidae. In Sullivan *et al.* (2006) Heptapteridae and Pimelodidae represent sister groups (Fig. 1). Remarkably, the paraphyly of the genus *Pimelodus* was previously mentioned by Lundberg and Littman (2003).

***Pimelodus albicans* phylogeographic pattern in the río de la Plata basin.** The present work represents the first phylogeographic approach within the genus *Pimelodus* using mtDNA sequences.

Pimelodus albicans has high levels of genetic variation among cyt b haplotypes in the río de la Plata basin. Genetic divergence of *P. albicans* from different collecting sites revealed low values among río de la Plata localities (range 1% -3%) and considerable divergence between río Arrecifes and río de la Plata collecting sites (10%). Similar values of genetic divergence (8.3%) were found among specimens of *Rhamdia quelen* collected in several rivers of Central and South America representing a sibling species complex (Perdices *et al.*, 2002). The values of nucleotide diversity were higher than those reported in others Asian siluriforms (So *et al.*, 2006).

All the phylogenetic reconstructions showed that *P. albicans* constitutes a monophyletic entity including four well supported monophyletic clades (Fig. 3). In addition, the minimum spanning network tree topology showed the same four assemblages (Fig. 4). Among them, there are two groups formed by samples from the same locality, one group is río Arrecifes (1 in Figs. 3 and 4) and the other one is the 771 collecting site in the río de la Plata (2 in Fig.3 and 4). The two remaining assemblages included haplotypes from more than one locality. The localities 760 and 765 (3 in Fig.3 and 4) were partially isolated sharing only the haplotype 13 (Fig. 4).

High level of genetic structuring was found in *P. albicans* by means of AMOVA analyses (Table 4). Under two groups and five groups hypotheses, the percentage of genetic variation (>70%) among groups were higher than other alternative ones. Both grouping hypotheses were consistent in showing that río Arrecifes samples constitute an isolated lineage. Moreover, these results were corroborated by the pairwise F_{ST} and Nm values (Table 6) which also considered the río Arrecifes samples isolated from río de la Plata ones. All present analyses corroborated that río Arrecifes population is the most divergent population within *P. albicans* data set, representing an unexpected separate lineage. In fact, the genetic divergence (10 %) between populations of these two regions was similar to those found at intrageneric and intergeneric level in the Pimelodidae data set (Table 2). Based on the present results it is possible to hypothesize that *P. albicans* may be conformed by a sibling species complex as it was previously described for the genus *Rhamdia* (Perdices *et al.*, 2002).

In all present analyses, the locality 760 was the most partially isolated among río de la Plata collecting sites, although there are no physical barriers separating areas of the estuary. These results support the idea that *P. albicans* has a genetic structure inside the río de la Plata basin. Up to this moment, the isolation of the locality 760 in the río de la Plata is difficult to explain. Temperature, salinity, and seasonal migrations are factors that influence the composition of the fish community from the inner río de la Plata (Jaureguizar *et al.*, 2004). But we did no rule out that *P. albicans* could migrates for reproduction segregating into different tributaries and generating different schools of haplotypes which could mix in the río de la Plata estuary during a considerable portion of their life cycle. The high nucleotide diversity found in the 765 collecting site (Table 3) and the existence of the gene flow with the remaining collecting sites (Table 6) could be concordant with the outlined hypothetic scenarios of the mixing populations in the middle of the río de la Plata estuary. This peculiar reproductive behavior was found in *Brachyplatystoma rousseauxii* in the Amazon River (Batista & Alves-Gomes, 2006).

On the other hand, environmental factors such as salinity could influence population structuring in *P. albicans* since low genetic diversity found in the 779 collecting site could be related to the increase of the salinity. This site represent the most distantly station in the río de la Plata estuary toward the border of the Maritime Front (Lasta *et al.*, 2002).

The data presented herein, were partially in agreement with information about population structure found in the congeneric taxa *Pimelodus maculatus* from the Tietê and Paranapanema Rivers (Brazil). In such study, genetic diversity (GST) and Nm estimate showed that the population from the Tietê River was genetically homogeneous, whereas population structuring was detected in the Paranapanema River (de Almeida *et al*., 2003). On the other hand, Batista and Alves-Gomes (2006) found non genetic structuring in population of dourada *Brachyplatystoma rousseauxii* among three collecting sites in the Amazon River. Moreover, low level of genetic differentiation was found in two species of catfish in Cambodian Mekong River by So *et al.* (2006).

Historical demography and conservation remarks. Tajima´s D statistic for all samples rejects the hypothesis of neutral mutation indicating that the total population could be under influence of an expansion process from the ancestral and central haplotype 1. The star-like network topology could

Table 6. Population pairwise estimates of F_{ST} values (below) and the corresponding indirect estimations of gene flow values (above) found among different collecting sites in *Pimelodus albicans*. Numbers in bold indicate the significant Fst P values (a=0.05).

FSTs**Nm**	1	2	3	4	5	6
1: 756	0	3.7	0.8	1.6	23.3	0.1
2: 765	0.119	0	13.3	2.1	3.9	0.2
3: 760	**0.391**	0.036	0	0. 6	0.9	0.2
4: 779	**0.239**	0.190	**0.455**	0	2.4	0.1
5: 771	0.021	0.115	**0.366**	0.171	0	0.2
6: río Arrecifes	**0.815**	**0.738**	**0.754**	**0.821**	**0.755**	0

support this population demographic scenario in *P. albicans*. These types of demographic events were also reported in the Asian catfish *Pangasius bocourti* from Cambodian Mekong River (So *et al*., 2006).

Taking into account that present analyses detected two different and genetically distantly lineages in *P. albicans* this results could be suggesting that both phylogroups may represent different Evolution Management Units (Moritz, 1994) in a further conservation program implemented for this species complex.

Further analyses including additional samples from coastal and tributaries rivers of the río de la Plata basin and new data set from nuclear and mitochondrial sequences, could clarify the pattern of the genetic structuring and homing behavior in the populations of *P. albicans*.

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