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Genetic diversity and structure of the commercially important native fish pacu (*Piaractus mesopotamicus*) from cultured and wild fish populations: relevance for broodstock management

Felipe del Pazo[1] · Sebastián Sánchez[2] · Victoria Posner[1] · Andrés A. Sciara[1] · Silvia E. Arranz[1] · Gabriela V. Villanova[1]

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Abstract
Pacu (*Piaractus mesopotamicus*) is one of the most important Neotropical freshwater fish species produced by aquaculture in South America. This study is the first attempt to inquire about aquaculture stocks in Argentina regarding genetic diversity and structure. Neither genetic characterization nor pedigree records are available for pacu stocks in farms in Argentina. The presence of hybrids in both natural environment (Lower Paraná River) and farms has not been evaluated yet at the southern region of pacu distribution. Genetic characterization of pacu broodstocks, corresponding to 8 farms, and wild individuals from four areas at Lower Paraná River was performed. Pacu hybrids were not detected neither in wild nor in farm stocks analyzed. In general, similar levels of genetic diversity were observed between cultured and wild fish populations. Global genetic differentiation ($F_{st} = 0.055$) indicated a low level of structure and AMOVA showed that genetic variation was mostly within populations. Reduced contemporary effective population size (Ne) was observed, and probably reflects the bottleneck by founder effect in farmed fish populations. Moreover, kinship analysis showed that in fish farms, on average, 43.00% of the individuals were genetically related, whereas in wild population it was 36.40%. We recommend that broodstock management practices, such as using large Ne, single pair mating, precise records, and tagging of brood fish, should be implemented to avoid unintentional mismanagement.

Keywords Aquaculture · Genetic structure · Inbreeding · Microsatellites · Serrasalmidae

✉ Gabriela V. Villanova
gvillanova@fbioyf.unr.edu.ar; villanova@inv.rosario–conicet.gov.ar

Extended author information available on the last page of the article

Introduction

According to the Food and Agriculture Organization of the United Nations (FAO) data, aquatic food production has transitioned from being primarily based on capture of wild fish to culture, increasing numbers of farmed species. In Latin America, the contribution of aquaculture to the regional economy has grown substantially in the last 10 years. Until now, most of South American aquaculture production have been based on non-native species such as salmon, trout, turbot, abalones, tilapia, white shrimp, carps, and catfishes. However, in the last decade, expansion and diversification efforts were focused on the development of new technologies to farm native species (FAO 2017, 2018, Valladão et al. 2018). Among them, pacu (*Piaractus mesopotamicus* (Holmberg, 1887)) is one of the most important cultivated species in South America. It is farmed mainly in Argentina, Brazil, and Paraguay (Valladão et al. 2018) with a total production of 17,252 tons in 2017 (data obtained from FIGiS/FAO). Recently, pacu production has been extended to Asian countries such as China, Myanmar, Thailand, and Vietnam (Flores Nava 2007; FAO 2010; Honglang 2007). In addition, hybrids between species of the *Serrasalmidae* family, i.e., *Colossoma macropomum* (commonly known as tambaqui, cachama blanca, or black pacu), pacu, and *Piaractus brachypomus* (commonly known as pirapitinga, cachama, or red pacu), are very popular in Brazilian aquaculture (IBGE 2017). However, hybrid fish constitute a potential biological and environmental risk, whose impact could affect the aquaculture industry and threaten native species, as previously observed for other species, such as tilapia, catfish, and trout (Bradbeer et al. 2019; McKelvey et al. 2016; Silva et al. 2009). It has been reported that fertile pacu hybrids are sold as pure species due to misidentification, (Hashimoto et al. 2014). Moreover, hybrid fish have been detected in the natural environment at the Upper Paraná River basin, probably as a consequence of aquaculture activities (do Prado et al. 2017; Hashimoto et al. 2014).

Pacu is a migratory species found throughout the Paraná-Paraguay River basin in South America. Pacu wild stocks have been reduced in the last decades, possibly due to overfishing (Agostinho et al. 2003; Resende 2003) and large-scale habitat alterations (Smith et al. 2003) that prevent its migration.

In Argentina, pacu production has shown a sustained growth since it was first farmed in the 1990s, nowadays being Argentina's main aquaculture crop (Panné Huidobro 2016). It has been consolidated in the country as a product of aquaculture of excellent flavor and texture (Wicki and Wiltchiensky 2017). Pacu aquaculture is carried out in semi-intensive systems within land-based excavated ponds, and it is restricted to the warm-subtropical region of the country. Currently, farming in suspended cages is being attempted as well as the pacu-rice rotation culture system (Corvalán Romero et al. 2014; Luchini 2017). Pacu aquaculture is based on unimproved strains and relies on wild fish for broodstock. According to anecdotal evidence, fish stocks used for culture in Argentina have been introduced from wild populations from the Lower Paraná and Lower Paraguay basins, as well as from Brazilian farms.

From an aquaculture perspective, genetic diversity studies and the implementation of genotyping in early stages of domestication have a significant impact to prevent or slow down inbreeding, and to improve broodstock management (Zhang et al. 2017). The genetic consequences of inbreeding, domestication, genotype-environment interactions, and selection are well known in many species, so preliminary studies are needed for setting up suitable guidelines for creating and maintaining cultured stocks.

No description is available about the genetic diversity and structure of wild pacu populations at the Lower Paraná River basin, the Southern region pacu distribution. This basin

comprises the region downstream from the Itaipú Dam, which was naturally isolated from the Upper Paraná River basin by the Guayra Falls until 1983. Until now, genetic population studies were restricted to the Upper-Paraguay and Upper Paraná River basins (Calcagnotto and DeSalle 2009; Iervolino et al. 2010), as well as to stocking programs implemented in Brazil (Povh et al. 2011). In those, wild populations of pacu were characterized as a panmictic stock with high gene flow among rivers at Upper-Paraguay and Upper Paraná River basins (Calcagnotto and DeSalle 2009; Iervolino et al. 2010).

Microsatellite markers have demonstrated to be suitable for genetic structure and parentage assignment of important species for aquaculture (Vandeputte and Haffray 2014; Gonçalves et al. 2019). A parentage assignment tool based on microsatellites has been standardized and validated previously using pacu families (Posner 2016), as well as used to estimate genetic diversity in Brazilian fish farms (Mastrochirico-Filho et al. 2019).

Thus, the main objective of the present study was to determine the genetic diversity of cultivated stocks of pacu in Argentina, and the possible presence of hybrids in farmed and wild fish stocks. Moreover, we aimed to compare the genetic diversity between cultivated and wild stocks from the same region. All this information will be essential to evaluate the genetic status of existing pacu broodstocks and the genetic consequences of using brood fish without pedigree records. In addition, based on present results, we aim to establish recommendations on good genetic management practices in order to create and maintain cultured stocks for future breeding programs.

Material and methods

Ethic statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the Animal Ethics Committee at Facultad de Ciencias Bioquímicas y Farmacéuticas-Universidad Nacional de Rosario approval has been received (protocol no. 302/2013). The US National Research Council's guidelines for the Care and Use of Laboratory Animals were followed. Fin fragments were collected from each fish under benzocaine anesthesia and all efforts were made to minimize suffering.

Experimental population, DNA extraction, and microsatellite analysis

Genetic analysis was performed through fin sampling of 302 individuals (adults) identified morphologically as *P. mesopotamicus* from eight different farms (H1–H8) in Argentina, and four sampling cities/areas (W1–W4) of wild fish population at Lower Paraná River (Table 1; Fig. 1). For wild fish population sampling, the presence of hydroelectric dams was considered. W1 was in Puerto Rico, which is placed upstream Yacyretá Dam and downstream Itaipú Dam, which separates the Upper and Lower Paraná River. W2 was in the confluence of Paraná and Paraguay Rivers. Paraguay River does not present dams and drains water from the Pantanal, the world's largest tropical wetland. W3 and W4 were at the southern area of current pacu natural distribution. In W1 and W4, a lower sampling size was obtained despite the high fishing efforts employed. Broodstocks from hatcheries were individually tagged and kept alive at the fish farm stations for subsequent management. Farm stations are placed in the North-Eastern Region of Argentina (NEA region) covering different states (Table 1, Fig. 1) where

Table 1 Collection details of pacu samples

Code	Sampling place	Sampling area (province/river)	Sample size	Stock source
H1	Farm 1	Santa Fe	88	Cultured
H2	Farm 2	Misiones	20	Cultured
H3	Farm 3	Formosa	16	Cultured
H4	Farm 4	Corrientes	21	Cultured
H5	Farm 5	Formosa	21	Cultured
H6	Farm 6	Chaco	16	Cultured
H7	Farm 7	Corrientes	13	Cultured
H8	Farm 8	Misiones	12	Cultured
W1	Puerto Rico	Paraná River	9	Wild
W2	Paso de la Patria	Paraná River	39	Wild
W3	Bella Vista	Paraná River	34	Wild
W4	Esquina	Paraná River	13	Wild

pacu is currently being cultured. One of the farms, H5, started its activities in the 1990s; H2, H3, and H4 started in the 2000s; and H1, H6, H7, and H8 started during the last 8 years. Farm fish stocks were generated from wild fish populations from the rivers Pilcomayo, Paraná, and Paraguay as well as other farms, including farms from Brazil. Identity and exact location of fish farm stations were kept confidential. Fin samples from all individuals were stored in ethanol 96% at − 20 °C.

DNA was extracted from fin fragments following Villanova et al. (2015) and quantified using Nanodrop 2000 (Thermo Scientific). Genotyping was performed using eight

Fig. 1 Sampling sites of pacu from farms (H1–H8) and localities at Lower Paraná River for wild population (W1–W4). Aquaculture farm exact localization is not shown. However, the states where farms are placed are shown in pink. Sampling locations of the wild populations are shown as black dots (W1–W4). Dams at Paraná River are shown with bars and named D1, Yacyretá Dam, and D2, Itaipú Dam. Map created using QGIS (QGIS 2.8 Las Palmas)

microsatellite markers (Pm1, Pm3, Pm5, Pm11, Pm4, Pm6, Pm9, and Pm13) in two multiplex PCR reactions previously standardized (Posner 2016) using fluorescent-labeled primers by CONICET Service (Stan CONICET No. 2353, CCT Rosario, Argentina; http://vinculacion.conicet.gov.ar/buscador-de-oferta-tecnologica/?id_ot=2353tipo=3) and a 3730XL DNA analyzer (Macrogen Korea). Fragment analysis was completed using Peak Scanner software (Applied Biosystems) and GeneScan 500LIZ Size Standard.

Hybrid determination

Samples were analyzed by a multiplex PCR based on nuclear a-Tropomyosin (tpm1) (Hashimoto et al. 2011). This method provides different electrophoretic fragment lengths for each parental species: 269 bp for *P. mesopotamicus*, 172 bp for *C. macropomum*, and 131 for *P. brachypomus*. The interspecific hybrids present a combination of two bands depending on parental species. Primer sequences and reaction conditions were used as previously described (Hashimoto et al. 2011). DNA samples from pure parental species as well as DNA samples from hybrids were used as controls for reaction specificity. Control samples were previously identified through morphological and molecular analyses. PCR products were analyzed by electrophoresis on a 2.5% agarose gel stained with GelGreen (Biotium, USA) using a 50-bp size standard (PB-L, Argentina).

Genetic diversity and structure analysis

The presence of null alleles (*F*null) and allelic dropout in microsatellite loci were tested using MICRO-CHECKER 2.2.3Van Oosterhout et al. 2004). The number of alleles per locus (Na) and observed (Hobs) and expected (Hexp) heterozygosity were estimated using CERVUS 3.0 (Kalinowski et al. 2007). The exact tests for deviation from the Hardy-Weinberg equilibrium (HWE) (Markov Chains of 100,000 steps), inbreeding coefficient (F_{is}), and linkage disequilibrium (LD) ($p < 0.05$) were performed using GENEPOP 4.0.11 (Raymond and Rousset 1995; Rousset 2008). Allele richness (AR) and number of private alleles (Np) were estimated through FSTAT 2.9.3.2 (Goudet 2001). Significant difference hypotheses between AR, Hobs, and Hexp means of each population with the reference population (W1, W2, W3, and W4) were tested through the non-parametric Wilcoxon test (p value < 0.050). The effective population size (Ne) was estimated by the linkage disequilibrium method implemented in NeESTIMATOR V2.01 (Do et al. 2014) with confidence intervals estimated with the parametric method (which were highly similar to those estimated by the jackknife method). Populations with sample size below 20 individuals were not analyzed for Ne. Low-frequency alleles (≤ 0.02) were excluded from the analysis to minimize potential bias caused by rare alleles.

Genetic evidence for a recent reduction in local population size was tested by heterozygosity excess (Cornuet and Luikart 1996) and *M*-ratio (Garza and Williamson 2001) methods. Heterozygosity excess tests were performed with the program BOTTLENECK 1.2.02 (Cornuet and Luikart 1996) by the two-phased mutation model (TPM), using conditions suggested by authors that correspond to sensible parameter values for most microsatellites: a proportion of SMM in the TPM = 0.00 and a variance of the geometric distribution for TPM = 0.36. Statistical significance was evaluated by Wilcoxon signed-rank test from 10,000 simulation replicates. Arlequin v. 3.5.2.2 (Excoffier and Lischer 2010) was used to trace bottleneck signatures by the modified *M*-ratio method. The total number of alleles (k) divided by overall

range in allele size (r) produces the ratio (M), which is expected to be smaller in recently reduced populations than in populations under mutation-drift equilibrium (Garza and Williamson 2001). Based on simulations and experimental data, populations that have experienced recent bottlenecks presented the mean value of $M < 0.68$ (Garza and Williamson 2001; Reid et al. 2008).

To estimate genetic differentiation between stocks, global and pairwise F_{ST} values were calculated with the Weir and Clark Cockerham (1984) method using Arlequin v. 3.5.2.2 (Excoffier and Lischer 2010). The significance of these values was estimated with 10,000 permutations. In addition, hierarchical analysis of molecular variance (AMOVA), based on allele frequency information, was carried out using Arlequin v. 3.5.2.2 (Excoffier and Lischer 2010). For AMOVA, two-group hypotheses were tested, in which populations were grouped according to their origin, wild or cultured stocks. In addition, the presence of three groups was tested, considering cultured stock as one group and wild fish population as two groups (it was separated in two groups taking into account the Yacyretá Dam between W1, and the other three areas (W2, W3, and W4)). Levels of admixture among stocks were inferred by estimating the optimum number of population clusters (K) using STRUCTURE version 2.3.4 (Pritchard et al. 2000). Cluster number estimation (K) was completed using 10 independent runs with $K = 1$ to 12 at 500,000 MCMC repetitions combined with a 100,000 burn-in period. Admixture ancestry model with LOCPRIOR was used in order to enable maximum resolution, as recommended to identify a subtle population structure (Pritchard et al. 2000). Results from STRUCTURE were processed with the online program STRUCTURE HARVESTER 0.3 (Earl and vonHoldt 2012) to estimate the ideal number of K based on the ΔK method described by Evanno et al. (2005). The results of independent STRUCTURE runs were summarized and corrected for the best K using CLUMPP software version 1.1.2 (Jakobsson and Rosenberg 2007). Individual genotypes were also clustered by discriminant analysis of principal components (DAPC) implemented in R (www.r-project.org). Data was first transformed using principal component analysis (PCA) and retained an appropriate number of PCs and discriminant functions. DAPC was loaded using the ADEGENET package (Jombart 2008) for the R software. Kinship coefficients (r_{xy}) were estimated by COANCESTRY v. 1.0.1.8 (Wang 2011). TrioML (r_{ML}) (Wang 2007) and QuellerGt (r_{QG}) (Queller and Goodnight 1989) pairwise relatedness estimators were selected among the options given by COANCESTRY v. 1.0.1.8 based on a previous work in which all estimators available through this program were tested in pacu individuals with known genealogical relationships (Posner 2016). Threshold values of r_{xy} coefficient were adopted as lower values ($r_{xy} < 0.125$) corresponding to unrelated individuals; intermediate values ($0.126 \leq r_{xy} \leq 0.375$) were considered as half siblings; and $r_{xy} > 0.376$ were considered full siblings.

Results

Hybrid determination

In order to discard the presence of hybrid fish samples before genetic diversity analyses, DNA samples of 302 individuals from 8 farms and 4 wild fish populations were analyzed by tpm1 PCR. An amplicon of 269 bp (Sup. Figure 1) was observed in all samples analyzed on agarose gels indicating that all evaluated individuals were pure *P. mesopotamicus*.

Genetic diversity

All individuals (302) were successfully genotyped at eight microsatellite loci. Micro-Checker analysis suggested consistent genotyping at all loci. The genetic variability parameters for pacu populations (over all loci) are shown in Table S1. The mean values of the population parameters, F_{is} values, and overall locus p values of HWE are shown in Table 2. A total of 44 alleles were detected in the analyzed populations, and the number of alleles per locus ranged between 2 and 9. Average population values ranged between 3.125 ± 1.126 (H7) and 4.000 ± 1.309 (H1), with a mean value of 3.563 ± 0.298 in cultured fish population, whereas in wild fish populations, average population values ranged between 3.175 ± 1.282 (W2) and 4.125 ± 1.125 (W3), and mean value was 3.718 ± 0.295. Allelic richness ranged from 1.000 to 5.034, with average population values ranging between 2.948 ± 0.956 (H7) and 3.364 ± 1.356 (H4), and mean value of 3.131 ± 0.129 in cultured fish population, while in wild fish population, average population values ranged between 3.085 ± 0.960 (W2) and 3.500 ± 1.069 (W1) and mean value was 3.247 ± 0.178. Private alleles were detected in individuals belonging to cultured (H2, H4, H5) and wild (W1, W3, W4) fish populations. Cultured fish population analyses revealed that mean Hobs per population ranged from 0.453 ± 0.236 (H3 and H6) to 0.543 ± 0.276 (H8), with a mean value of 0.481 ± 0.036. Meanwhile, wild population analyses showed that mean Hobs per sampling locality ranged from 0.389 ± 0.243 (W4) to 0.507 ± 0.212 (W2), and mean value was 0.450 ± 0.051. Mean Hexp in cultured fish population ranged from 0.446 ± 0.221 (H6) to 0.540 ± 0.229 (H4), with a mean value of 0.496 ± 0.034, while in wild fish populations, Hexp ranged from 0.460 ± 0.238 (W4) to 0.564 ± 0.174 (W1), with a mean value of 0.522 ± 0.044. In general, genetic diversity parameters were similar between cultured and wild fish populations. There was no significant difference in mean Hobs between wild and cultured fish populations, after Wilcoxon test ($p = 0.401$). Only two cultured fish populations (H5 and H6) presented lower Hexp values than wild fish sampling points after comparison by Wilcoxon test ($p < 0.050$). Mean Hexp in H5 (0.463 ± 0.193) was lower than mean Hexp in W1 (0.564 ± 0.174), and mean Hexp in H6 (0.446 ± 0.221) was lower than mean Hexp in W1 (0.564 ± 0.174) and W3 (0.529 ± 0.199).

Four fish farms (H1, H2, H4, and H5) and one wild fish population (W3) departed from HWE ($p < 0.050$) when applying global test (Table 2). Some *loci* departed from HWE (Table S1), such as *locus* Pm1 in H5; Pm3 in H1 and H4; Pm4 in H1, H2, H4, W2, W3, and W4; Pm6 in H1; Pm9 in H2; and Pm13 in H1. Null alleles were detected in the *locus* Pm3 in H1, Pm4 in H1, H2, W2, W3 and W4, and Pm6 in H1. Neither scoring errors nor allele dropout was detected.

Overall population F_{is} values varied between − 0.051 (H8) and 0.247 (W1). Significant deviations from 0 ($p < 0.050$) of F_{is} values were found in the fish farms H1 ($F_{is} = 0.116$; $p = 0.015$) and H2 ($F_{is} = 0.120$; $p = 0.002$), as well as in the wild fish populations W1 ($F_{is} = 0.247$; $p = 0.000$), W3 ($F_{is} = 0.104$; $p = 0.019$), and W4 ($F_{is} = 0.160$; $p = 0.010$) (Table 2). Probably, the observed deviations from HWE in H1, H2, and W3 could be caused by heterozygote deficiency.

Genetic structure

Global F_{ST} value suggested low genetic differentiation among populations ($F_{ST} = 0.0557$, $p < 0.050$). When the parameter was estimated only including samples from wild population, the obtained value was lower ($F_{ST} = 0.04452$, $p = 0.000$). Pairwise F_{ST} values were also

Table 2 Summary statistics for genetic variation of pacu at each sampling site, and mean farmed (H) and wild (W) values, showing sample size (*n*), total number of alleles observed in the stock (Na), number of private alleles (Np), mean number of alleles per locus (*N*/locus), allelic richness (AR), expected (Hexp) and observed (Hobs) heterozygosity, fixation index (F_{is}), and departure from Hardy-Weinberg equilibrium (*p*(HWE))

Sampling site	*n*	Na/Np	AR (mean ± SD)	*N*/locus (mean ± SD)	Hobs (mean ± SD)	Hexp (mean ± SD)	pHWE	F_{is}
H1	88	33/0	3.162 ± 0.971	4.000 ± 1.309	0.470 ± 0.190	0.531 ± 0.186	*0.0000*	*0.116*
H2	20	31/2	3.234 ± 1.350	3.750 ± 1.908	0.457 ± 0.260	0.517 ± 0.236	*0.0004*	*0.120*
H3	16	28/0	3.102 ± 0.886	3.500 ± 1.069	0.453 ± 0.236	0.480 ± 0.203	0.3338	0.057
H4	21	30/1	3.364 ± 1.356	3750 ± 1.669	0.536 ± 0.275	0.540 ± 0.229	*0.0065*	0.009
H5	21	30/1	3.080 ± 0.833	3.750 ± 1.035	0.476 ± 0.267	0.463 ± 0.193	*0.0341*	0.0341
H6	16	27/0	3.012 ± 0.918	3.375 ± 1.060	0.453 ± 0.247	0.446 ± 0.221	0.7521	−0.016
H7	13	25/0	2.948 ± 0.956	3.125 ± 1.126	0.462 ± 0.260	0.476 ± 0.185	0.6159	0.032
H8	12	27/0	3.147 ± 1.162	3.375 ± 1.165	0.543 ± 0.276	0.517 ± 0.243	0.8328	−0.051
Mean H		28.50 ± 2.39	3.131 ± 0.129	3.563 ± 0.298	0.481 ± 0.036	0.496 ± 0.034		
W1	9	28/1	3.500 ± 1.069	3500 ± 1.070	0.431 ± 0.218	0.564 ± 0.174	0.1391	*0.247*
W2	39	30/0	3.085 ± 0.960	3.175 ± 1.282	0.507 ± 0.212	0.535 ± 0.187	0.3637	0.054
W3	34	33/1	3.229 ± 0.978	4.125 ± 1.125	0.474 ± 0.249	0.529 ± 0.199	*0.0006*	*0.104*
W4	13	28/1	3.175 ± 1.261	3.500 ± 1.603	0.389 ± 0.243	0.460 ± 0.238	0.2112	*0.160*
Mean W		29.75 ± 2.36	3.247 ± 0.178	3.718 ± 0.295	0.450 ± 0.051	0.522 ± 0.044		

In italics, $p < 0.050$. *SD*, standard deviation

calculated and most of them were significant ($p < 0.050$) ranging between − 0.0015 (H5–W3) and 0.1320 (H7–W4) (Table 3). In general, low to moderate genetic differentiation was observed among the population pairs. In cultured fish populations, significant pairwise F_{ST} values ranged between 0.0210 (H2–H4) and 0.1300 (H7–H8) suggesting low to moderate genetic differentiation. In wild fish populations, W2–W3 (pairwise $F_{st} = 0.0400$; $p < 0.050$) and W2–W4 (pairwise $F_{st} = 0.0890$; $p < 0.050$) presented pairwise F_{st} values that were significant but low, suggesting low differentiation between populations.

To assess the level of admixture among samples, Bayesian model-based clustering analyses were performed based on the ΔK distribution. Analysis conducted in the program STRUCTURE identified three genetic groups ($K = 3$) (Supplementary Figure 2). STRUCTURE results suggested an admixed population in which two of the three genetic groups were mainly represented (clusters 1 and 2; green and red colors respectively) in all farmed populations, except for H7, which displayed a third genetic group (cluster 3; blue color) in a higher proportion. Regarding wild fish population, the three genetic groups were represented in each sample. W2 presented a slightly different distribution of each genetic group. This pattern was also evident in the DAPC analysis (Fig. 2), in which H7 and W2 were the most distant groups, and cultured stocks were very close to wild fish samples. AMOVA did not support differentiation due to the origin of the samples (wild and farm stocks) in any of the two evaluated hypotheses (two-group test: F_{CT}: 0.00645, $p = 0.21069$; three-group test: F_{CT}: 0.00525, $p = 0.28822$). The genetic variance was explained by variation within groups and sampling points (two-group test: F_{SC}: 0.04938, $p = 0.00000$; F_{ST}: 0.05551, $p = 0.00000$; three-group test: F_{SC}: 0.04986, $p = 0.00000$; F_{ST}: 0.05484, $p = 0.00000$).

Effective population size (Ne), bottleneck estimation, and kinship estimation

The Ne of pacu fish farms ranged from 9.9 in H1 to 130.4 in H5 where the upper confidence limit reached infinity in most cases (Table 4). Potential genetic bottleneck analysis performed by Wilcoxon sign-rank test showed that H1, H4, and H8 presented deviations from mutation-drift balance under TPM ($p < 0.050$). Almost all the stocks had normal L-shaped distribution, but H7 and H8 presented shift distribution mode. In addition, modified *M*-ratio index revealed that most of the stocks had recently experienced reduction in effective size since it can be assumed that a population suffers a recent size reduction when $M < 0.68$, indicating bottleneck

Table 3 Pairwise F_{st} values

	H1	H2	H3	H4	H5	H6	H7	H8	W1	W2	W3
H1											
H2	*0.0494*										
H3	*0.0582*	0.0117									
H4	*0.0232*	*0.0210*	*0.0320*								
H5	*0.0439*	*0.0311*	0.0122	0.0132							
H6	*0.0550*	*0.0298*	0.0139	*0.0428*	*0.0343*						
H7	*0.1294*	*0.0547*	*0.0793*	*0.1017*	*0.0883*	*0.1147*					
H8	*0.0445*	*0.0433*	*0.0707*	0.0113	*0.0479*	*0.0371*	*0.1303*				
W1	0.0169	*0.0426*	*0.0513*	0.0112	0.0194	*0.0391*	*0.0890*	− 0.0024			
W2	*0.0861*	*0.0641*	*0.0952*	*0.0908*	*0.1133*	*0.1020*	*0.1233*	*0.1251*	*0.0821*		
W3	*0.0205*	0.0178	*0.0245*	0.0103	*0.0155*	*0.0226*	*0.0773*	*0.0389*	− 0.0033	*0.0404*	
W4	0.0145	*0.0317*	*0.0393*	0.0132	0.0225	0.0039	*0.1318*	0.0139	0.0217	*0.0892*	0.0107

In italics, $p < 0.050$

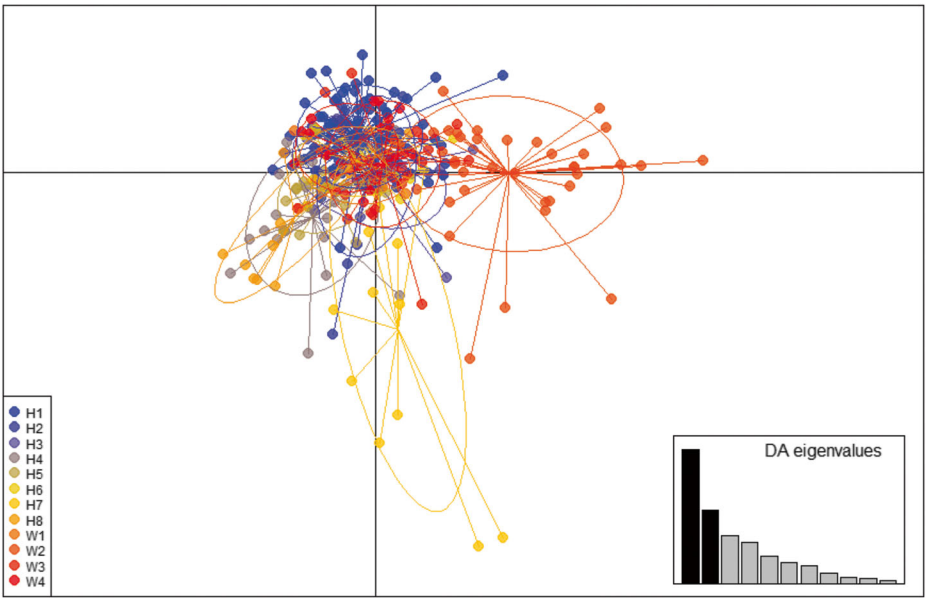

Fig. 2 Scatterplots of the discriminant analysis of principal components (DAPC) for pacu individuals from cultured ($N = 207$) and wild ($N = 95$) populations. Plots represent individual genotypes and colors represent populations. The sample origin is labeled within their 95% inertia ellipses and individuals are connected to the corresponding group centroids. The first two principal components are represented by *X* and *Y* axes, respectively

events (Garza and Williamson 2001). Only the H1 and wild fish population presented *M*-ratio indexes > 0.68. However, *M*-ratio in stock H1 was just slightly higher than 0.68 (0.6921 ± 0.1886) (Table 4). Regarding wild fish population, Ne, bottleneck and relatedness coefficients were estimated by clustering wild fish samples as one population (W), based on the results of genetic structure analyses (W = W1 + W2 + W3 + W4), and taking into account the low sampling size in two sampling points (W1 and W4). Ne value for W was 278.4, and wild

Table 4 Bottleneck results of one-tailed Wilcoxon test for heterozygote excess under TPM and observed values of the *M*-ratio averaged over the number of polymorphic microsatellite loci. Estimates of effective population size (Ne) with 95% confidence intervals (CI) for farm and wild fish stocks ($N \geq 20$)

Stock (number of individuals)	*M*-ratio (mean ± sd)	*p* value for Wilcoxon sign-rank test TPM	Graphical representation of the mode-shift indicator	Ne (95% CI)
H1 (88)	0.6921 ± 0.1886	*0.0273*	L-shaped	9.9 (5.1–15.7)
H2 (20)	0.6160 ± 0.2229	0.1250	L-shaped	31.9 (3.2–inf)
H3 (16)	0.6244 ± 0.2299	0.2304	L-shaped	ND
H4 (21)	0.6462 ± 0.2393	*0.0136*	L-shaped	25.1 (6.2–inf)
H5 (21)	0.6608 ± 0.1981	0.5273	L-shaped	130.4 (14–inf)
H6 (16)	0.5994 ± 0.2187	0.5273	L-shaped	ND
H7 (13)	0.5483 ± 0.2069	0.2734	Shift mode	ND
H8 (12)	0.5744 ± 0.2298	*0.0371*	Shift mode	ND
W (95)	0.8279 ± 0.1193	0.1250	L-shaped	278.4 (61.2–inf)

In italics, $p < 0.050$. *ND*, not determined due to $N < 20$

population (W) did not show signs of bottleneck events (Table 4). In addition, Ne was estimated for W2 and W3, which presented sampling size higher than 20 individuals. Ne = 113 in W2, and Ne = 50.7 in W3. These values were lower than the obtained for W (W1 + W2 + W3 + W4), but they were higher than the observed for most farmed populations.

Kinship evaluation showed that most of the fish farms had related individuals (full sibling and half siblings) in a mean proportion of 43.25% or 51.88%, using r_{ML} or r_{QG} respectively (Table 5 and Fig. 3). The highest percentage of related individuals was observed in H7 (63% r_{ML}/70% r_{QG}), with a high proportion of full sibling individuals (25.64% r_{ML}/29.49% r_{QG}). In contrast, H2 farm showed the highest percentage of unrelated individuals (66.84% r_{ML}, 60.53% r_{QG}) (Fig. 3). Related individuals were found in wild fish populations as well, although at a lower level than in most farm stocks (36.40% r_{ML}/43.48% r_{QG}). In five of the eight fish farms under study, mean r_{xy} (r_{ML} and r_{QG}) were significantly different from W's mean r_{xy} after the Mann-Whitney test ($p < 0.050$) (Table 5).

Discussion

Molecular genetic tools and genetic diversity studies have been developed through the years for aquaculture species contributing to aquaculture expansion for new emerging species. Several practices associated with Neotropical fish production in emerging species, especially those related to the management of broodstock, may reduce the effective population size Alarcón et al. 2004). These practices are generally linked to the lack of registration and control of broodstock, such as information on its origin, kinship, and mating record, which could result in increased susceptibility to inbreeding depression over generations (Duncan et al. 2013; Naish et al. 2013). This study can be considered the first diagnosis of pacu genetic diversity in aquaculture farms in Argentina and the state of natural populations in the Lower Paraná River pacu.

As previously mentioned, hybrid fish of pacu, pirapitinga, and cachama are very popular in Brazilian aquaculture and have been detected in the natural environment at the Upper Paraná River basin, probably as a consequence of aquaculture activities (Hashimoto et al. 2014). Our results did not evidence hybrid's presence neither on Argentinian fish farms under study nor in

Table 5 Kinship analysis in farmed (H) and wild (W) fish populations of pacu according to TrioML and Queller and Goodnight r_{xy} coefficients

	r_{xy} TrioML				r_{xy} Queller and Goodnight			
	% Unrelated	% Half-sib	% Full-sib	Mean r_{xy}	% Unrelated	% Half-sib	% Full-sib	Mean r_{xy}
H1	59.64	24.63	15.73	0.1514*	56.58	25.94	17.48	0.0731
H2	66.84	17.89	15.26	0.1349	60.53	22.11	17.37	0.0843
H3	62.50	18.33	19.17	0.1670*	44.17	35.83	20.00	0.1699
H4	61.90	24.76	13.33	0.1368	60.00	26.19	13.81	0.0517
H5	48.57	24.76	26.67	0.2124*	42.38	27.62	30.00	0.2083
H6	54.17	21.67	24.17	0.1851*	37.50	34.17	28.33	0.2140
H7	39.74	33.33	26.92	0.2353*	30.77	38.46	30.77	0.2359
H8	60.61	22.73	16.67	0.1621	53.03	30.30	16.67	0.1030
W	63.60	22.61	13.79	0.1334	56.52	27.54	15.94	0.0772

* Indicates that mean r_{xy} value was significantly different from W's mean r_{xy} after Mann Whitney test ($p < 0.050$)

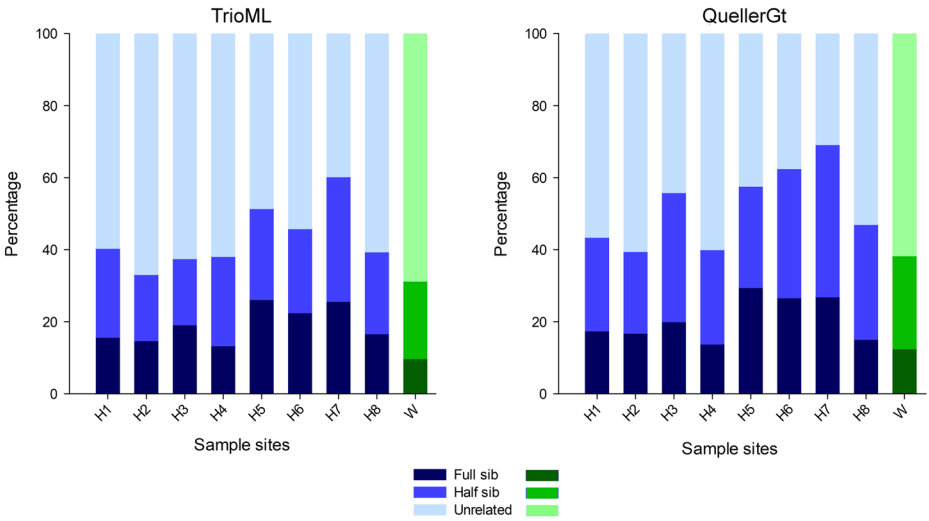

Fig. 3 Relatedness estimates for cultured and wild fish populations as determined by the TrioML r_{ML} (Wang 2011) (a) and Queller and Goodnight (1989) r_{QG} (b) estimators. Average values are shown for the wild population. Plots represent the percentage of individuals at each category of relatedness: unrelated, half sib, and full sib

wild populations from the Lower Paraná River. It was important to discard the hypothesis that hybrids could be present among brood fish in Argentine farms because hybrids could have been informally introduced as brood fish by mistake, due to misidentification as pure individuals (Hashimoto et al. 2014).

The analysis of genetic diversity parameters estimated for pacu broodstocks showed low values for AR (ranging around 3) and Hexp (ranging around 0.500) in all the cultured populations analyzed. Low genetic diversity values were expected for cultivated stocks, as was observed for pacu farms (Mastrochirico-Filho et al. 2019) and for *P. brachypomus* farms (Jorge et al. 2018), in Brazil. Low genetic diversity values characterize populations with genetic drift events due to low effective population sizes and, consequently, recent bottleneck effects or founder events. However, the low levels of genetic diversity found in pacu farmed stocks were also shared by wild fish populations from the Lower Paraná River. Only three farm fish stocks (H4, H5, and H6) presented lower mean AR or mean Hexp values than wild fish populations. Moreover, Hexp values for wild populations (W1, W2, W3, and W4) were slightly lower than those observed in previous studies of wild pacu populations in the Pantanal and Upper Paraná River (Calcagnotto and DeSalle 2009), which reported higher heterozygosity values (mean He between 0.558 and 0.638). Probably, pacu wild fish population from the Lower Paraná River may be negatively affected by overfishing, as well as by habitat modification, such as deforestation, urbanization, and industrialization near rivers, causing water pollution, among others. Historically, pacu distribution was extended up to higher latitudes (34.677° S, Río de la Plata estuary, Argentina; Ringuelet et al. 1967), but over the last 60 year, its distribution has been reduced to the northern region of the Lower Paraná River (30.413° S). Probably the low diversity observed in farmed stocks was not only the result of populations with low effective population sizes and founder events but also the result of the use of brood fish from an impacted wild fish population. The low levels of genetic diversity in farmed and wild fish populations in Argentina should be warned for fisheries management.

Generally, domesticated stocks show evidence of inbreeding, and selection, as well as genetic differentiation between wild and cultured stocks if sufficient time for domestication has elapsed (Kohlmann et al. 2005). Moderate differentiation was observed for farm H7, supported by pairwise F_{st}, STRUCTURE, and DAPC analysis. This fish farm population showed the highest percentage of related individuals too, suggesting inbreeding and lack of broodstock management. In addition, most farms showed moderate genetic differentiation with W2 sampling point, supported by pairwise F_{st}, STRUCTURE, and DAPC analysis. In contrast, considerable gene flow was observed among farm and wild individuals in most other cases. The lack of significant genetic differentiation among farm and wild stocks supported by AMOVA, STRUCTURE, and DAPC analyses suggested a relatively short domestication history and could be associated with the practice of introducing individuals from the river as brood fish into aquaculture stocks.

Departure from HWE was observed in four fish farms (H1, H2, H4, and H5) and one of the wild fish sampling points (W3). The Hardy-Weinberg disequilibrium is common in many fishes, and deviations from the equilibrium generally prevail over heterozygote deficits resulting from factors involving reproductive systems, presence of null alleles, and a Wahlund effect (reduction of heterozygosity in a population caused by subpopulation structure) (Allendorf and Luikart 2009). In the case of wild fish population, at W3 sampling point, heterozygote deficiency was detected. Heterozygote deficiency, when compared to Hardy-Weinberg expectations, is common in fish populations and these deficiencies could arise either by population subdivision (Wahlund effect) (Wilson et al. 2004), by inbreeding (O'Connell and Wright 1997), or by bottleneck caused by founder effect. Probably the heterozygote deficiency observed at W3 was the combination of more than one factor, since W3 is placed at the southern area of current pacu distribution at Lower Paraná River and it is one of the favorite areas for pacu fishing. In addition, W1 and W4 showed heterozygote deficiency. However, a low sample number could be the reason for this observation in these two sampling points.

Regarding farm fish populations, probably bottleneck by founder effect could be the cause of Hardy-Weinberg disequilibrium. In these four farm stocks, population size reduction was supported by bottleneck analysis, and three of them showed low Ne values. Indeed, most estimated Ne values were low for cultured stocks. It has been proposed that the minimum effective population size to avoid severe short-term inbreeding depression is in the order of $Ne \approx 70$ for a wide range of specie's reproductive rates (Caballero et al. 2016). In this study, most Ne values obtained were smaller than the ideal values proposed. Moreover, significant recent bottlenecks were detected for all farm stocks. It is likely that most farm stocks were founded with a small number of individuals, due to the high fecundity of pacu females (300,000 eggs/female; Criscuolo-Urbinati et al. 2012); thus, a sufficient number of fingerlings could be obtained from few females. Regarding kinship analysis, our results showed that all fish farms showed a substantial number of related individuals (half sibling + full sibling). On average, 51% (r_{QG})/43% (r_{ML}) of individuals were relatives in farm populations analyzed. This outcome results in a higher probability of mating between relatives, which means a higher inbreeding risk, that can affect morphological and viability traits (Kincaid 1983).

Considering the increasing importance of pacu to South American aquaculture, our results are aimed to provide initial knowledge about the genetic profile of pacu stocks in different fish farms and highlight the necessity of improving broodstock management and mating design in order to reduce the potential negative effects of inbreeding. Molecular identification of individuals is necessary to monitor the genetic variability of the stocks and to assess how this variation could be maintained through selective mating (Beaumont and Hoare 2003; Gjedrem

and Baranski 2009). Moreover, we recommend serious consideration of selection methods and hatchery practices for pacu brood fish to reduce inbreeding levels. Broodstock management practices, such as using large Ne, single pair mating, and precise records and tagging of brood fish, should be promoted to avoid unintentional mismanagement.

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Author contribution GVV, SA, and FdP conceived the study; FdP, SS, and VP conducted the experiments; FdP, AS, and GVV analyzed the data; and FdP, SA, and GVV wrote the manuscript. All authors read and approved the final version.

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Compliance with ethical standards

Ethical approval This study was conducted in strict accordance with the recommendations of the Animal Ethical Committee at Facultad de Ciencias Bioquímicas y Farmacéuticas-Universidad Nacional de Rosario. Protocol approval has been received (protocol no. 302/2013).

Conflict of interest The authors declare that there is no conflict of interest.

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Affiliations

Felipe del Pazo[1] · Sebastián Sánchez[2] · Victoria Posner[1] · Andrés A. Sciara[1] · Silvia E. Arranz[1] · Gabriela V. Villanova[1]

[1] Laboratorio Mixto de Biotecnología Acuática-Facultad de Ciencias Bioquímicas y Farmacéuticas-Universidad Nacional de Rosario-Centro Científico, Tecnológico y Educativo "Acuario del río Paraná", Carrasco Av and Cordiviola, 2000 Rosario, Santa Fe, Argentina

[2] Instituto de Ictiología del Nordeste-Facultad de Ciencias Veterinarias-Universidad Nacional del Nordeste, Sargento Cabral 2139, 3400 Corrientes, Corrientes, Argentina