



# Conservation genetics of the threatened catfish *Conorhynchos conirostris* (Siluriformes: *incertae sedis*), an evolutionary relict endemic to the São Francisco River Basin, Brazil

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## Abstract

Analysis of genetic datasets can be particularly useful in providing guidelines for conservation management of understudied species targeted by commercial activities. Here we used population genetic approaches to inform on the conservation status of the Neotropical long-nose pirá catfish, *Conorhynchos conirostris*. Pirá is a large migratory fish endemic to the São Francisco River Basin (SFRB). It is an evolutionarily divergent and relict species, being the sole representative of an *incertae sedis* family. The species is considered locally extinct in the upper and lower SFRB, listed as vulnerable on the IUCN Red List and as endangered on the Brazilian Red List (ICMBIO). Fishing prohibition has received severe criticism from middle SFRB fisheries that claim that this understudied species is relatively abundant in that region. We used information from 13 microsatellite markers and COI mitochondrial sequences to clarify the genetic diversity of this enigmatic species in the middle SFRB, to estimate contemporary effective population size ( $N_e$ ), and to assess its conservation status. Results from bottleneck analyses indicated that the species has experienced recent reductions in population size, which is consistent with small estimates of contemporary  $N_e$ . The predicted amount of heterozygosity loss ( $H_t$ ) in  $t$  generations ranged from 0.1152 (for an estimated  $N_e$  of 26.4;  $t=100$ ) to 0.7573 (for an estimated  $N_e$  of 169.9;  $t=10$ ). Our study supports the conservation status proposed by the ICMBIO to the remaining pirá population. Moreover, we highlight the need for demographic data and the re-assessment of the current IUCN classification for this evolutionary relict lineage.

**Keywords** Neotropical catfish · Endangered species · Fisheries statistics · Demographic changes · Conservation status · Red List

## Introduction

The rich Neotropical fish fauna represents over 25% of all described freshwater fish species of the world (Buckup et al. 2007), encompassing over 4000 species. Threats such as over-exploitation, pollution, habitat fragmentation, and invasive species have had major impacts on Neotropical

fishes, leading to the inclusion of several species on Red Lists (ICMBio 2014). Unfortunately, conservation biologists in the Neotropics often do not have enough biological data or resources to assess the conservation status of aquatic biodiversity (Pinheiro et al. 2015). This can lead to disputed management recommendations and conflicts between governmental agencies, conservationists, and the fisheries industry (Di Dario et al. 2015; Pelicice et al. 2017). Genetic datasets can provide useful and inexpensive information for clarifying the conservation status and prioritizing management actions for aquatic biodiversity, especially for understudied species (e.g. Schwartz and Beheregaray 2008; Lean et al. 2017).

The long-nose catfish, pirá (*Conorhynchos conirostris* Valenciennes, 1840), is a large migratory fish endemic to the São Francisco River Basin (SFRB), Brazil. Pirá was included in the Minas Gerais State Red List as vulnerable under the ‘B2ab(iii)c(ii)’ IUCN criterion (Alves and Bockmann 2008).

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In the 2014 Brazil Red List, pirá was classified as endangered based on criterion 'A2cd' (ICMBio 2014), as it is now restricted to the middle SFRB and suffered population reductions associated with dam constructions and intensive fishing pressure (ICMBio 2014). Moreover, it has been estimated that the population size of pirá has reduced in 50% in the last 30 or so years (around three generations), simultaneously with the construction of large dams in the lower SFRB (ICMBio 2014). Pirá has been a substantial component of the artisanal fisheries of the region (Godinho et al. 1997). It ranked in the top 10 most fished species in the middle SFRB at the beginning of the 21st century (FUNDEP 2000). Because of its protected status, fishing prohibition has been subjected to criticism from middle SFRB fishermen who claim that pirá is still relatively abundant in that region.

Biological data on pirá is scarce, especially those relevant to its systematics, biology and conservation management. The species represents a dramatic example of systematic uncertainty amongst Neotropical catfishes. Although it was traditionally classified within the family Pimelodidae, pirá shares no synapomorphies with other members of the Pimelodidae, and based on molecular data it has been removed from this family and classified as *incertae sedis* (Ferraris 2003; Sullivan et al. 2006). Molecular phylogenetic analysis suggested that pirá is sister to Pimelodidae, Pseudopimelodidae, and Heptapteridae, and included the species as the sole member of a potentially new family within the monophyletic superfamily Pimelodoidea (Sullivan et al. 2006). Therefore, pirá represents a relict and divergent evolutionary lineage that is possibly representative of an entire fish family, has a relatively restricted distribution for a large migratory catfish, and is a targeted fisheries resource. These factors reinforce the need of studies that generate guidelines for the conservation management of this iconic species.

Conservation genetics datasets provide information about key demographic parameters, such as contemporary effective population size and estimates of genetic diversity (Frankham et al. 2014). Although genetic tools have not yet reached the forefront of conservation policy and management (Hoban et al. 2013), metrics based on genetic parameters have been proposed for recommendations regarding IUCN conservation status (Willoughby et al. 2015; Frankham et al. 2014). For instance, Willoughby et al. (2015) developed a novel approach for identifying species in need of conservation using the predicted amount of heterozygosity loss in the population after 10, 50, and 100 generations. Using this approach, the conservation status may be determined according to expected heterozygosity loss reduction of 25% correlated with the number of generations that it is expected to occur. If the expected heterozygosity reduction occurs in 10 generations or less, the species is ranked as critically endangered, in 50 generations as endangered and 100 generations as vulnerable.

No data concerning the remaining genetic diversity or population structure are available for pirá, but highly variable molecular markers have been developed for this species (Carvalho and Beheregaray 2011). In this study, we assessed the remaining genetic diversity of pirá in the middle SFRB using mitochondrial DNA sequences and nuclear microsatellite markers. We used these datasets to estimate genetic diversity indices, rate of heterozygosity loss, and contemporary effective population size of the remaining pirá population inhabiting the middle SFRB. Our central aim is to generate information that can be used to assess if pirá should be included in red lists of threatened species, a mechanism that enables legal protection for this enigmatic species.

## Materials and methods

### Genetic methods

#### Samples, DNA extraction, PCR amplification, and sequencing

We collected 50 tissue samples from pirá captured with gill nets within 300 km of the main free flowing São Francisco River (SFR) channel, between the towns of Três Marias and Januária ( $n = 41$ ). We collected nine samples from the Paracatú River, an affluent of the SFR that flows into the main river channel 180 km below Três Marias town (ICMBIO collection permit number: 37298-1). Fin clips were removed from the caudal fin and fixed in ethanol 90% for further DNA isolation by homogenization and digestion with proteinase K at 37 °C overnight, followed by salting-out purification (Sunnucks and Hales 1996).

#### mtDNA

Mitochondrial sequences were obtained to assess genetic diversity and to gauge information about the demographic history of the remaining pirá population. A fragment of the cytochrome oxidase subunit I (COI, 658 bp) was amplified using the primers FishF1 and FishR1 (Ward et al. 2005). The 25 µl PCR mixes included 19.5 µl of ultrapure water, 2.5 µl of 10× PCR buffer, 2.5 µM of MgCl<sub>2</sub>, 0.35 µl of each primer (10 µM), 2.5 µl of dNTP (1 mM), 0.25 µl of Taq DNA polymerase (5 U/µl), and 1.0 µl of DNA template (50–100 ng/µl). Thermal cycling conditions consisted of an initial denaturation step at 94 °C for 2 min, 35 cycles at 94 °C for 30 s, at 54 °C for 30 s, and at 72 °C for 1 min, with a final extension at 72 °C for 10 min. The DNA sequences were sequenced bi-directionally using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), following the manufacturer's protocol on an 3130 genetic analyzer.

## Microsatellites

To assess contemporary genetic diversity, effective population size and structure we used a set of 13 microsatellites previously described for pirá (Carvalho and Beheregaray 2011) to genotype all 50 pirá samples. We used the amplification method in which PCR products are fluorescently labeled through the inclusion of a third (fluorescent M13) primer in each reaction. PCRs had a final volume of 10  $\mu$ l containing 1 $\times$  Flexi Buffer GoTaq (Promega), 2.5 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 0.2 U Go-Taq Flexi DNA polymerase (Promega), BSA (0.1%), 0.05  $\mu$ M forward primer, 0.2  $\mu$ M reverse primer and 0.2  $\mu$ M fluorescent M13 primer. The PCR amplifications followed the 63–55 °C touchdown of Beheregaray et al. (2004). Amplification products were detected on an ABI 3130 genetic analyzer (Applied Biosystems). Resulting microsatellite profiles were examined using GeneMapper 4.0 (Applied Biosystems) and the scoring was done manually.

## DNA data analysis

### mtDNA

Chromatograms were inspected by eye and aligned using default parameters in the ClustalW software included in the MEGA software (Tamura et al. 2013). We estimated summary statistics such as haplotype diversity ( $h$ ), nucleotide diversity ( $\pi$ ), segregating sites, and departures from neutrality tested with Tajimas's  $D$  and Fu and Li's  $D$  indices (Tajima 1989; Fu and Li 1993) using DNASP version 5 (Librado and Rozas 2009) in order to compare pirá's genetic diversity with other endangered and non-endangered species and to test for historical demographic changes. To depict the genealogical relationships among mtDNA sequences, we used tcs version 1.06 (Clement et al. 2000) to constructing a haplotype network with the parsimony method. tcs estimates the maximum number of substitutions to connect parsimoniously two haplotypes with 95% confidence interval. Each DNA sequence was deposited in GenBank (Accession numbers MH448604–MH448653).

### Microsatellites

The average number of alleles ( $N_a$ ), observed heterozygosity ( $H_o$ ) and expected heterozygosity ( $H_e$ ) were obtained using GENAL EX 6.41 (Peakall and Smouse 2006). We used the program BOTTLENECK (Cornuet and Luikart 1996) to test for recent demographic reductions. Three different statistical tests were used to calculate the probability of heterozygosity excess, which is indicative of a bottleneck in the population (Cornuet and Luikart 1996): the Wilcoxon sign-rank, the sign, and the standardized differences test. The analyses

were carried out under three different mutation models: infinite allele model (IAM), stepwise mutation model (SMM), and two-phase mutation (TPM), using default parameters.

In order to investigate if there is population genetic structure within pirá sampled in the middle SFR, without allocating individuals to populations prior to analysis, we used the Bayesian clustering methods implemented in STRUCTURE v.2.2 (Pritchard et al. 2000). To determine the number of populations ( $K$ ) within the complete data set, five independent simulations for  $K = 1-5$  with 100,000 burn in iterations and 500,000 data iterations were run. We used the admixture model of population structure where allele frequencies are correlated among populations.

### Contemporary effective population size

The contemporary effective population size ( $N_e$ ) was estimated with NEESTIMATOR v.2 (Do et al. 2014) using the linkage disequilibrium and heterozygote excess (Waples and Do 2010) based on the 13 microsatellite dataset and the jackknife to determine 95% confidence intervals, screen out alleles with frequencies below values of 0.01, and 0.05 ( $P_{\text{Crit}}$ ). We also used the same approach to estimate contemporary  $N_e$  for two large migratory Neotropical fishes for which microsatellite data are available: the surubim *Pseudoplatystoma corruscans* (Carvalho et al. 2012), and the curimba *Prochilodus lineatus* (Perini 2013). The surubim population from the SFRB consists of an evolutionarily significant unit (ESU) thought to be threatened (Carvalho et al. 2012), but is not included in the Red List. The curimba, on the other hand, typically shows large population sizes and is one of the most abundant fish species in southeast Brazil (Castro and Begossi 1995). These analyses were carried out to obtain contemporary  $N_e$  estimates from large Neotropical species with contrasting demographics to be compared with estimates obtained for pirá.

### Genetic conservation status

The genetic conservation status of pirá was estimated using the predicted amount of heterozygosity loss ( $H_t$ ) in the population after  $t$  generations using Eq. (1), as proposed in Wiloughby et al. (2015):

$$H_t = H_o \left( 1 - \frac{1}{2N_e} \right)^t \quad (1)$$

In this approach, the conservation status of a species is determined by the decrease in the observed heterozygosity ( $H_o$ ) and contemporary effective population size ( $N_e$ ) estimations, according to the reduction in the estimated  $H_t$  in 10, 50, and 100 generations ( $t$ ). To control for distinct  $N_e$  estimations recovered from the two methods implemented

in the software NEESTIMATOR v.2 (Do et al. 2014), we applied the lower and higher mean  $N_e$  value obtained using the heterozygote method ( $N_e = 26.4$  and  $42.8$ ) and the linkage disequilibrium method ( $N_e = 98.2$ – $169.9$ ), to estimate  $H_t$  loss in Eq. (1).

The conservation status may be determined as: critically endangered if  $H_t$  is reduced by 25% in 10 generations or less; endangered if  $H_t$  is reduced by 25% in 50 generations; and as vulnerable if the 25% reduction is estimated to occur in 100 generations.

## Results

### mtDNA

After trimming ambiguous ends, mtDNA sequences of 620 bp from all 50 specimens were obtained. Overall, 11 haplotypes were detected with genetic diversity of  $0.66 \pm 0.07$  (mean  $\pm$  standard) and  $\pi$  of  $1.99 \pm 0.36 \times 10^{-4}$ . The Tajimas's  $D$  and Fu and Li's  $D$  indices recovered were  $-1.52$  and  $-0.86$  respectively, but not statistically significant (Table 1). The haplotype network suggested a single historical population in the region (i.e. all haplotypes are closely related) and showed no star like shape indicative of historical demographic growth (Fig. 1).

### Microsatellites

All loci were polymorphic, ranging from 6 to 19 alleles per loci ( $11.6 \pm 1.4$ ). The  $H_e$  per locus ranged from 0.24 to 0.92 ( $0.76 \pm 0.05$ ; Table 1). Since there was no evidence of population structure using the Bayesian clustering methods implemented in STRUCTURE V.2.2 (Pritchard et al. 2000) (Fig. 2), which is consistent with the absence of physical barriers between sites in this migratory species, all individuals were considered as members of the same genetic population.

The analysis of recent demographic changes indicated a bottleneck effect within pirá under IAM for the Wilcoxon sign-rank test, sign test and the standardized differences test ( $P = 0.01$ ,  $0.01$  and  $0.02$ , respectively—Table 2). For the SMM, the sign test and the standardized difference test were significant ( $P = 0.00$  for both tests). Using the TPM model, no statistically significant result was obtained ( $P > 0.23$ ).

### Contemporary effective population size

The contemporary  $N_e$  for pirá estimated by the linkage disequilibrium method (LD) ranged from 98.2 (69.9–155.7) to 169.9 (126.2–250.9), and from 26.4 (11.5– $\infty$ ) to 42.8 (18.0– $\infty$ ) when using the heterozygote excess method. The contemporary  $N_e$  of the putatively threatened surubim (*Pseudoplatystoma corruscans*) resulted in  $N_e$  ranging from 15.3

**Table 1** Summary statistics estimated using the COI mtDNA and 13 microsatellite loci for 50 specimens of pirá catfish *Conorhynchus conirostris* from the middle São Francisco River Basin, Brazil

Index	Value
mtDNA	
Sample size	50
Segregating sites	11
Haplotypes	11
Haplotype diversity ( $h$ )	$0.66 \pm 0.07$
Nucleotide diversity ( $\pi$ )	$1.99 \times 10^{-3} \pm 0.36 \times 10^{-3}$
Tajima's $D$	$-1.52^{ns}$
Fu and Li's $D^*$	$-0.86^{ns}$
Microsatellites	
Sample size (n)	50
Average number of alleles ( $N_a$ )	$11.60 \pm 1.40$
Observed heterozygosity ( $H_o$ )	$0.78 \pm 0.06$
Expected heterozygosity ( $H_e$ )	$0.76 \pm 0.05$

Mean  $\pm$  standard error,  $ns$  non-significant ( $P > 0.05$ )

to 34.5 when applying the LD method and infinite when using the heterozygote excess method. The non-threatened Neotropical fish species *Prochilodus lineatus* contemporary  $N_e$  estimation resulted in a  $N_e$  ranging from 315 to 9317.9 (LD method) and infinite (heterozygote excess method) (Table 3).

### Genetic conservation status

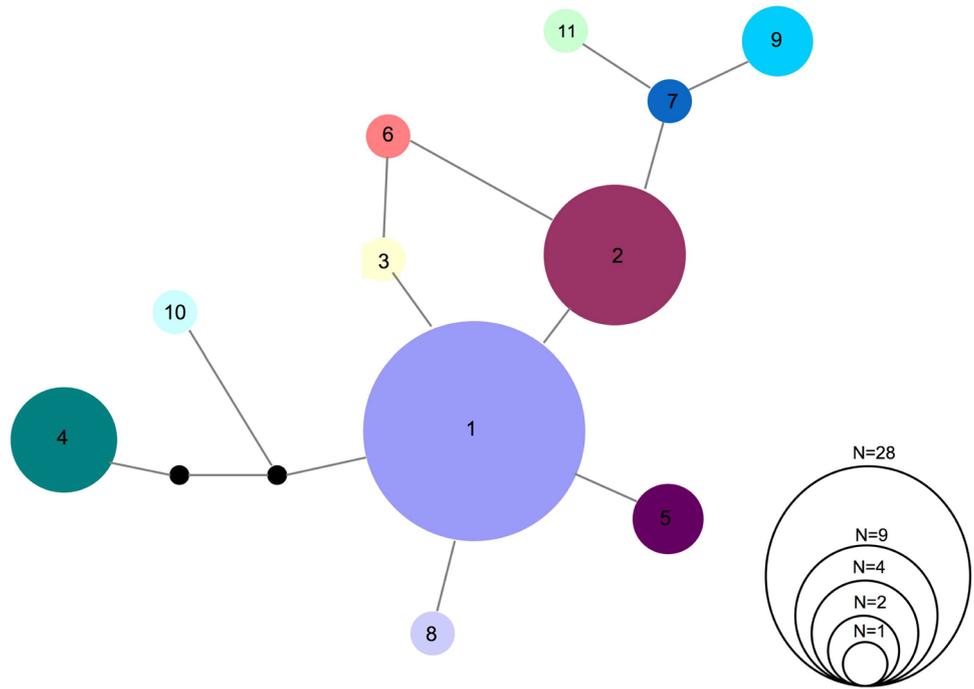
The conservation status of pirá was determined from the decrease in the observed heterozygosity ( $H_o$ ), which ranged from 0.1152 ( $N_e = 26.4$ ;  $t = 100$ ) to 0.7573 ( $N_e = 169.9$ ;  $t = 10$ ) (Table 4). Thus, the genetic conservation status of pirá was considered as vulnerable for all estimates of  $H_t$  over 100 generations ( $t$ ), since we observed a 25% reduction of  $H_t$  within this time period (Table 4; Fig. 3). Moreover, a  $H_t$  reduction of 25% was observed in 50 generations for pirá (estimated  $N_e$  of 26.4), which suggests a ranking of endangered for pirá (Table 4; Fig. 3).

Using Eq. (1) to estimate when  $H_t$  would reach the threshold of 25% reduction, we observed that a  $H_t$  reduction of 25.53% would be reached within 100 generations with a  $N_e$  equal to 169.9 (Table 4; Fig. 3). Considering 10 generations only, all  $H_t$  estimates were lower than 25% (i.e. non-threatened).

## Discussion

The estimated genetic diversity of the remaining pirá population in the middle SFRB was higher ( $H_e = 0.76$ ;  $h = 0.66$ ) than for the putatively threatened surubim (i.e.  $H_e = 0.648$ ;

**Fig. 1** Network of 11 mitochondrial haplotypes of the mtDNA gene COI for the pirá catfish *Conorhynchos conirostris*. Each line indicates one mutation between haplotypes. Each circle represents one mitochondrial haplotype. Circle size is proportional to the haplotype frequency. Small dark circle (without numbers) represents missing haplotype



**Fig. 2** Barplot of estimated membership coefficient (X axis) based on 10 polymorphic microsatellite loci for each individual from *C. conirostris* showing lack of genetic structure considering two (k=2) and three populations (k=3)



**Table 2** Results of Bottleneck tests for the pirá catfish *Conorhynchos conirostris* from the middle São Francisco River basin, Brazil

Microsatellite mutation model	Wilcoxon sign-rank test (one tail for H excess)	Sign test	Standardized differences test
Infinite allele (IAM)	0.01	0.01	0.02
Stepwise (SMM)	1.00	0.00	0.00
Two-phase (TPM)	0.23	0.33	0.37

*P* values for heterozygosity excess test are based on three microsatellite mutation models

**Table 3** Contemporary effective population size ( $N_e$ ) and 95% confidence interval (between brackets) determined by two single-sample estimators, sample sizes, and number of molecular markers (micro-atellites) for the pirá catfish *Conorhynchos conirostris* and two other Neotropical fish species

Species	$N_e$ estimation method		Sample size	Number of molecular markers	References
	Linkage disequilibrium	Heterozygote excess			
Pirá ( <i>Conorhynchos conirostris</i> )	98.2 (69.9–155.7) to 169.9 (126.2–250.9)	26.4 (11.5–∞) to 42.8 (18.0–∞)	50	13	Present work
Surubim ( <i>Pseudoplatystoma corruscans</i> )	15.3 (10.0–23.9) to 34.5 (20.9–68.1)	Infinite–Infinite (∞–∞)	47	8	Carvalho et al. (2012)
Curimba ( <i>Prochilodus lineatus</i> )	315.4 (128.7–∞) to 9317.9 (374.7–∞)	Infinite–Infinite (∞–∞)	56	10	Perini (2013)

Alleles screened out below  $P_{\text{crit}} = 0.05$  and  $0.01$ ; 95% confidence interval based on jackknife on loci**Table 4** Conservation status estimates for the pirá catfish *Conorhynchos conirostris* based on heterozygosity reduction ( $H_t$ ), contemporary effective population size ( $N_e$ ), and observed heterozygosity ( $H_o$ ) in 10, 50 and 100 generations (t)

$H_t$ reduction (%)	$H_t$ reduction	t	$N_e$	$H_t$	Conservation status
3.57	0.0227	10	169.9	0.7573	Non-threatened
4.987	0.0389	10	98.2	0.7411	Non-threatened
11.09	0.0865	10	42.8	0.6935	Non-threatened
13.71	0.1069	50	169.9	0.6731	Non-threatened
17.41	0.1358	10	26.4	0.6442	Non-threatened
22.32	0.1741	50	98.2	0.6059	Non-threatened
<b>25.53</b>	<b>0.1991</b>	<b>100</b>	<b>169.9</b>	<b>0.5809</b>	<b>Vulnerable</b>
<b>39.67</b>	<b>0.3094</b>	<b>100</b>	<b>98.2</b>	<b>0.4706</b>	<b>Vulnerable</b>
<b>44.44</b>	<b>0.3466</b>	<b>50</b>	<b>42.8</b>	<b>0.4334</b>	<b>Endangered</b>
<b>61.56</b>	<b>0.4802</b>	<b>50</b>	<b>26.4</b>	<b>0.2998</b>	<b>Endangered</b>
<b>69.12</b>	<b>0.5391</b>	<b>100</b>	<b>42.8</b>	<b>0.2408</b>	<b>Vulnerable</b>
<b>85.23</b>	<b>0.6648</b>	<b>100</b>	<b>26.4</b>	<b>0.1152</b>	<b>Vulnerable</b>

Conservation status was determined as: critically endangered if  $H_t < 25\%$  in 10 generations or less; endangered if  $H_t < 25\%$  in 50 generations; and as vulnerable if  $H_t < 25\%$  in 100 generations (highlighted in bold)

$N_e$  values ranged from 26.4 to 42.8 (heterozygote method) and from 98.2 to 169.9 (linkage disequilibrium method)

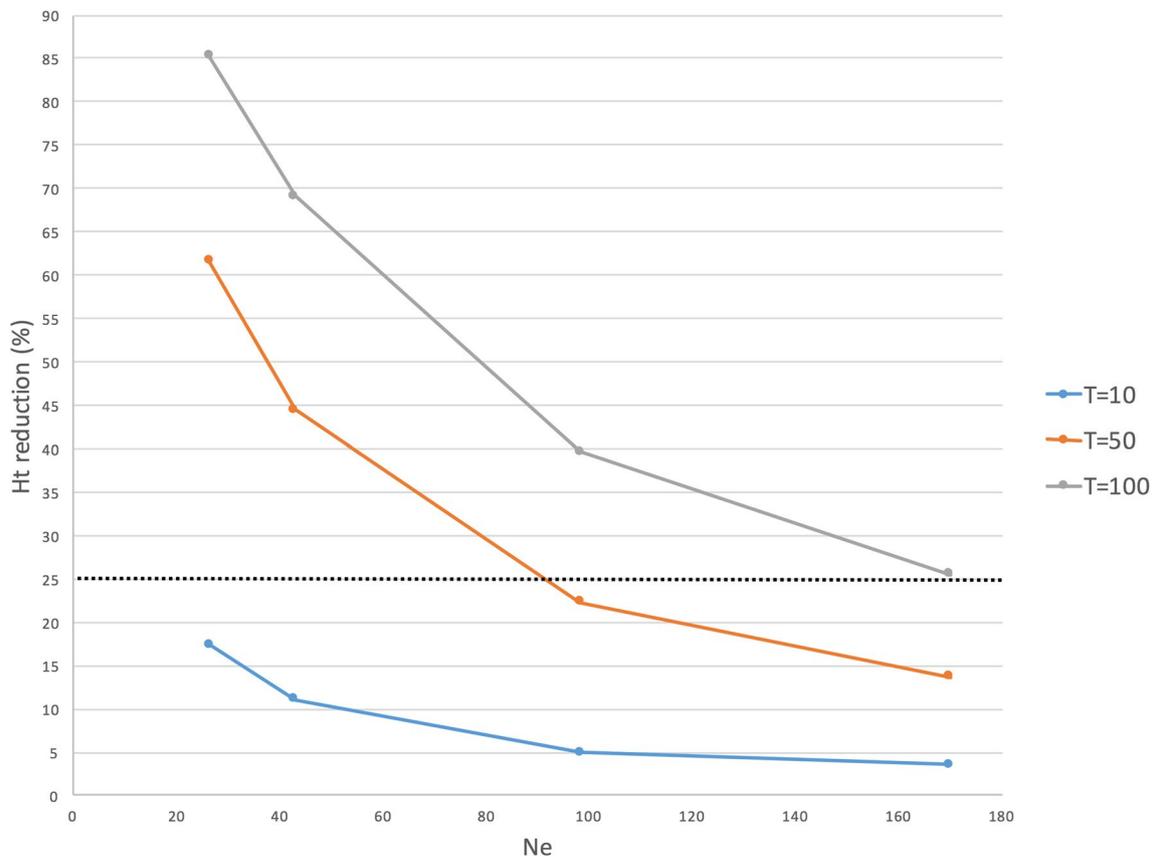
$h = 0.569$ ; Carvalho et al. 2012) and other threatened fish species (e.g. Mekong giant catfish *Pangasianodon gigas*  $h = 0.350$ ; Na-Nakorn et al. 2006). Pirá's estimated  $H_e$  was also similar to the abundant curimba, a non-threatened fish species ( $H_e = 0.784$ ; Perini 2013). These results, together with the bottleneck tests indicated recent demographic reductions in pirá (Tables 1 and 2), consistent with the recent history of overfishing and habitat degradation in the region (Godinho et al. 2007). The impact on the observed levels of genetic diversity should come in the future, after multiple consecutive generations. The same can be suggested for the threatened surubim, another long-lived catfish found in the region that, together with pirá, was the focus of intensive

fisheries in the middle SFRB during the late twentieth century (FUNDEP 2000). Pirá was maintained in the 2014 Brazilian Red List due to its currently narrow endemic distribution (restricted to the middle SFRB) and population reductions associated with dam constructions and intensive fishing pressure (ICMBio 2014).

No evidence of spatial genetic structure was detected for Pirá in the Middle São Francisco River using Bayesian clustering methods (Fig. 2). The absence of spatial genetic structure was expected due to the lack of physical or environmental barriers between sampling sites. However, populations composed of individuals with different reproductive times may be isolated by time (namely Isolation by Time—IBT) (Hendry and Day 2005). IBT is usually related to reproduction waves of different shoals, and may limit gene flow. IBT has been reported for the fish *Prochilodus costatus*, a Neotropical migratory species species also habiting the Middle São Francisco River (Braga-Silva and Galetti 2016). Thus, further investigations using a broader sample size and appropriated temporal sampling may shed light on pirá spatial or temporal genetic structure.

The contemporary  $N_e$ , which is applied to recent past generations and estimates the effective number of breeders directly from the parents of a sampled cohort (Waples and Do 2010). We detected a greater  $N_e$  value for the abundant curimba when compared to surubim, and with pirá (Table 2). Although the comparison of contemporary  $N_e$  between species might be compromised by idiosyncrasies in the evolutionary history of each species (Rodríguez-Zárate et al. 2013), Frankham et al. (2014) estimated that a  $N_e$  value higher than 100 is required to limit inbreeding depression to 10% over five generations. Our estimates of pirá's  $N_e$  recovered low values, ranging from 26.4 to 169, indicating that pirá could perhaps be at risk of the negative consequences of inbreeding in the short term.

Regarding conservation status, our molecular data indicates that pirá should be ranked on the Red List as a vulnerable or as threatened species, in agreement with its current



**Fig. 3** Estimated heterozygosity reduction ( $H_t$ ) and effective population size in 10, 50 and 100 generations ( $t$ ) for the pirá catfish *Conorhynchos conirostris* according with Eq. 1. The solid line indicates the threshold of 25%  $H_t$  reduction

classification by ICMBio and IUCN. However, the genetic diversity estimated for pirá has revealed that its remaining population still holds evolutionary potential, and therefore it may be able to adapt to future environment changes, if critical habitat and the free-flowing river are maintained.

Creation of protected areas in the middle SFRB are needed to establish in situ gene banks and to conserve critical habitat to avoid potential evolutionary loss. Targeted restocking (i.e. human mediated release of fish fry or fingerlings bred in hatcheries) into the lower and upper SFRB, where pirá seems to be currently extinct, may also help fisheries restoration. Stocking the middle SFRB with pirá, however, should be done using approaches that avoid the erosion of genetic diversity of host populations (García-Marín et al. 1999), such as genetically-informed captive breeding followed by stocking. Furthermore, any stocking should be preceded by careful analyses as to the quality of the donor population and should be followed by long-term monitoring (*sensu* Attard et al. 2016).

Additional population genetic surveys covering the current restricted range of the species are needed to monitor its genetic status and to inform conservation management strategies for this evolutionarily relict catfish. Until this

information is gathered, we recommend the maintenance of pirá on the Red List as a “threatened” species, thereby prohibiting its fishing under Brazilian environmental law. Furthermore, habitat preservation and the reconnection of Upper and Middle São Francisco River Basin by building a fish ladder at the Três Marias hydroelectric dam as well as avoiding the construction of new dams may help guarantee the survival of this species endemic to the São Francisco River Basin.

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