Neotropical Ichthyology, 12(1):105-116, 2014

DOI: 10.1590/S1679-62252014000100011

Analysis of propagule pressure and genetic diversity in the invasibility of a freshwater apex predator: the peacock bass (genus *Cichla*)

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An important step in invasive biology is to assess biological variables that could be used to predict invasion success. The study of genetics, evolution, and interactions of invasive and native species in invaded ranges provides a unique opportunity to study processes in population genetics and the capability of a species' range expansion. Here, we used information from microsatellite DNA markers to test if genetic variation relates to propagule pressure in the successful invasion of an apex predator (the Amazonian cichlid *Cichla*) into Southeastern Brazilian River systems. Invasive populations of *Cichla* have negatively impacted many freshwater communities in Southeastern Brazil since the 1960s. Reduction of genetic variation was observed in all invasive populations for both *Cichla kelberi* (CK) and *Cichla piquiti* (CP). For instance, heterozygosity was lower in the invasive range when compared to native populations from the Amazon basin (CP H_E = 0.179/0.44; CK H_E = 0.258/0.536 respectively). Therefore, despite the successful invasion of *Cichla* in southeast Brazil, low genetic diversity was observed in the introduced populations. We suggest that a combination of factors, such as *Cichla*'s reproductive and feeding strategies, the "evolutionary trap" effect and the biotic resistance hypothesis, overcome their depauperete genetic diversity, being key aspects in this apex predator invasion.

Uma importante etapa na biologia da invasão é acessar variáveis biológicas que podem predizer o sucesso de invasão. O estudo da genética, evolução e interações entre invasores e espécies nativas no ambiente invadido pode prover uma oportunidade única para o estudo dos processos em genética de populações e a capacidade de uma espécie ampliar seu habitat. Nesse trabalho, nos utilizamos dados de marcadores de DNA microssatélites para testar se a variação genética é relacionada a pressão de propágulo na invasão bem sucedida do predador de topo (o ciclídeo Amazônico *Cichla*) nos rios do Sudeste Brasileiro. Populações invasoras de *Cichla* vem impactando negativamente diversas comunidades de água doce no Sudeste brasileiro deste 1960. A redução da variação genética foi observada em todas populações invasoras, tanto para *Cichla kelberi* (CK) como *Cichla piquiti* (CP). Por exemplo, a heterozigose foi menor no ambiente invadido quando comparada com as populações nativas da bacia Amazônica (CP HE = 0.179/0.44; CK HE = 0.258/0.536 respectivamente). Assim, apesar do sucesso da invasão de *Cichla* no sudoeste do Brasil, baixa diversidade genética foi observada nas populações introduzidas. Nós sugerimos que uma combinação de fatores, como as estratégias reprodutivas de *Cichla*, o efeito de "armadilha evolutiva" e a hipótese de resistências biótica superam o efeito que a diversidade genética depauperada exerce, sendo aspectos-chave na invasão desse predador de topo de cadeia.

Key words: Invasion biology, Neotropical, Population genetics, Translocation, Tucunaré.

Introduction

Invasive species could be treated as natural experiments that provide a unique opportunity to study basic processes in population genetics and the capability of range expansion (Sakai *et al.*, 2001; Sax *et al.*, 2005). Introduction of a small number of individuals into a new environment has been considered a potential limitation to the establishment of a species, since adaptive evolutionary changes required by novel selection pressures need enough genetic variation

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to take place (Okada *et al.*, 2009; Suarez & Tsutsui, 2008). This fact leads to the genetic paradox in invasion biology: how invasive species with low genetic variation due to bottlenecks after founder effects are able to persist and adapt to novel conditions and evolutionary challenges (Allendorf & Lundquist, 2003; Kolbe *et al.*, 2004; Sakai *et al.*, 2001). Kolbe *et al.* (2004) have shown that multi-introductions are key to invasion success. However, the influence of multiple introductions (*i.e.*, intense propagule pressure) and the invasive genetic paradigm have not yet been resolved since spatial and temporal aspects of multiple introductions were also associated with propagule pressure and the ability of a species to spread (Dlugosch & Parker, 2008). Genetic data have been useful to provide information on the frequency in which a species is introduced into a specific area, the size of introductions and the subsequent pattern of genetic structure in the new range (Banks *et al.*, 2010; Carvalho *et al.*, 2009b; Dlugosch & Parker, 2008; Henry *et al.*, 2009). Nonetheless, it is yet unclear what role genetics may have in the propagule pressure effect (Allendorf & Lundquist, 2003).

An important step in invasive biology is to assess which biological variables are useful to predict which species could potentially become invasive (Lockwood *et al.*, 2005). The genetic diversity of an invasion measured by neutral markers (*i.e.*, parts of the genome that are not under natural selection) vary from unexpected low (Gaskin *et al.*, 2012; Valiente *et al.*, 2010) to high (Kolbe *et al.*, 2004a), and therefore may not be a good predictor of invasion success. However, genetic diversity can be useful in management and control decisions (Sakai *et al.*, 2001; Gaskin, 2012).

The *Cichla* (peacock bass or *tucunaré*) is an indigenous Amazonian cichlid fish that was introduced into river basins outside their native range, both in Brazil and in other countries, whereupon they have often become invasive and implicated in local extinctions (Latini & Petrere, 2004; Zaret & Paine, 1973). Established invasive populations of *Cichla* were first recorded in southeastern Brazil in the 1960s (Agostinho *et al.*, 1994), and since then several impacts of this apex predator on indigenous fish populations have been reported (Latini & Petrere, 2004; Pompeu & Godinho, 2001), such as biotic homogenization (Zaret & Paine, 1973; Latini & Petrere, 2004) and impacting native fish diversity (Godinho *et al.*, 1994; Santos *et al.*, 1994). Moreover, the biological characteristics of *Cichla* species have made their populations capable of expanding rapidly in the invasive range, especially in modified ecosystems such as hydroelectric reservoirs, pristine floodplains and lagoons (Espinola *et al.*, 2010; Zaret & Paine, 1973). These include reproductive activity during almost the entire year (Vieira *et al.*, 2009), bi-parental care of eggs and capability to rear young in oxygen-depleted lakes (Zaret, 1980), opportunistic feeding behavior - it preys on whatever it can swallow (Resende *et al.*, 2008), cannibalism (Gomiero & Braga, 2004), and phenotypic plasticity that allowed adaptation to harsh ecological conditions (Chellappa *et al.*, 2003). While *Cichla* species were apparently introduced into northeastern Brazil in the late 1940s by government agencies for establishing fish breeding farms, no information was available regarding their origin. Applying a molecular phylogenetic analysis based on mitochondrial 16S ribosomal DNA and control region sequences (Carvalho *et al.*, 2009b) identified the source of *Cichla* species introduced in four different river basins in Southern Brazil. Introductions were likely carried out from a single river, the Tocantins River (Amazon basin). A low diversity of maternal lineages was detected in the invasive populations, except for the reservoir closest to the source population, suggesting different propagule pressures in the invasive range (Carvalho *et al.*, 2009b).

Karyological evidence for interspecific hybridization, intermediate morphology and hybrids have been detected in the peacock bass native range, suggesting hybridization between the sympatric species (Brinn *et al.*, 2004). Natural fertile hybrids between *C.* cf. *monoculus* and *Cichla* sp. in the invasive range (Itaipu hydroelectric reservoir and in the floodplain of the upper Paraná River) together with multiple introductions of *Cichla* species in the Paraná and Parapanema River basin was also reported (Oliveira *et al.*, 2006).

Several aspects of why *Cichla*'s invisibility was so successful in Southeastern Brazil have been investigated, but no correlation with propagule pressure (Espinola *et al.*, 2010) or with the prey naiveté hypothesis (*i.e.*, prey are unable to recognize and respond to predators due to lack of previous ontogenetic contact to a sympatric predator (Kovalenko *et al.*, 2010) have been found. In the latter work, the different co-evolutionary history with a certain predator archetype was not found to explain local extinctions after introduction of *C. kelberi*. The latter was proposed based on behavioral experiments conducted in laboratory where non-native peacock bass could be recognized as a predator by native species (Kovalenko *et al.*, 2010).

Here, we used microsatellite DNA markers to assess genetic diversity in native and introduced populations of two *Cichla* species: *C. kelberi* (Kullander & Ferreira, 2006) and *C. piquiti* (Kullander & Ferreira, 2006) and address the following questions: (1) Are there genetic differences between invasive and non-invasive populations of these species? (2) Is hybridization occurring in the native or invaded range? (3) Is genetic diversity correlated with invasiveness of *Cichla* in non-pristine (*i.e.*, reservoirs) and pristine areas (*i.e.*, natural lakes)? Assessing how multiple introductions (*i.e.*, intense propagule pressure), hybridization and genetic diversity are related to the invasive genetic paradigm might provide insights into the high invasibility success and adaptability of *Cichla* into new habitats and assist in management decisions.

Material and Methods

Sampling

Fin clips from *Cichla piquiti* (CP) and *Cichla kelberi* (CK) were collected and preserved in 90% ethanol from the Amazon basin (Tucuruí reservoir, n = 38) in the easternmost native range of the species and from five populations in the invasive range in Southeastern Brazil (non-Amazonian rivers, n = 84). Samples from the invasive range were collected in four different river systems and include populations from impacted sites (*i.e.*, hydroelectrical reservoirs) and pristine sites (one natural lake and one marginal lagoon) for both species (Table 1; Fig. 1).

Genetic methods

Tissue samples were digested using proteinase K, and DNA isolated by phenol/chloroform purification (Sambrook *et al.*, 1989). A set of eight microsatellite previously described for CP and successfully cross-amplified in CK (Carvalho *et al.*, 2009a) were amplified by the Polymerase Chain Reaction (PCR) for all samples. Amplification followed the method described by (Schuelke, 2000) in which PCR products are fluorescently labeled through the inclusion of a third (fluorescent M13) primer in each reaction. Reactions were performed in a final volume of 10 µl containing 1X Flexi Buffer GoTaq (Promega), 2.5 mm $MgCl_2$, 0.2 mm dNTPs, 0.2 U Go-Taq Flexi DNA polymerase (Promega), BSA (0.1%), 0.05 µM forward primer, 0.2 µm reverse primer and 0.2 µm fluorescent M13 primer. The PCR amplifications followed the 63°C-55°C touchdown of (Beheregaray & Sunnucks, 2000). Amplification products were separated on an ABI 3130 genetic analyzer (Applied Biosystems) at the DNA Sequencing Facility of Macquarie University. The resulting microsatellite profiles were examined using genemapper 4.0 (Applied Biosystems) and peaks were scored manually.

Data analysis

We used genepop v3.4 (Rousset, 2008) to estimate expected (H_E) and observed (H_O) heterozygosities, inbreeding coefficient (F_{IS}), number of alleles (N_A) and Hardy-Weinberg proportions. Allelic richness (R_A) was estimated in Fstat 2.9 (Goudet, 1995). Bonferroni corrections were applied when conducting multiple statistical tests (Rice 1989). Scoring errors, large allele dropout and null alleles were checked employing the program Microcheker (Van Oosterhout *et al.*, 2004) and Inest (Chybicki & Burczyk, 2009).

To assess possible reductions in diversity related to the process of introduction we first compared allelic richness (R_A) and Nei's gene diversity averaged (H_E. Differences in R_A and H_E were assessed using the Kruskal-Wallis test to compare native *vs* invasive population samples; whereas the Dunn's procedure (two tailed test) was used to estimate significant differences between populations using the software Xlstat^{Ò}.

Levels of population genetic structure in each species were estimated by computing Weir's F_{ST} using Genepop 3.4 (Rousset, 2008) and statistical significance estimated with

Table 1. Measures of genetic diversity for the two species of *Cichla*. Number of samples (N), mean allele richness (R_A), mean Nei's genetic diversity (H_E), mean number of private alleles per population (N_{PA}), mean Inbreed coefficient (F_{IS}), percentage of polymorphic *loci* (% poli *Loci*), probability (p-value) of rejecting the HWE equilibrium for each population and number of Control region mtDNA haplotypes (*h*), data from Carvalho *et al.* (2009). Statistical significance differences between populations of H_E and R_A are presented. Identical letters within species indicate similar statistical values, whereas, distinct letters indicate values with significantly statistical difference.

Pop	Location	River Basin	Species	Type	N	Mean H_E	Mean R_A	Mean N_{PA}	Mean Fis (GENEPOP/Inest)	% poli Loci	HWE (p-value)	h
Native												
TO	Tocantins	Amazonas	*C. kelberi*	Reservoir	18	0.536^{A}	3.68^{A}	2.55	0.425/0.046	77.78	0.000*	3
TO	Tocantins	Amazonas	*C. piquiti*	Reservoir	20	0.44	2.99^{C}	3.33	0.279/0.039	88.89	0.000*	6
Invasive												
ITU	Itumbiara	Upper Paraná	*C. kelberi*	Reservoir	18	0.393^{A}	2.63^{AB}	1.11	0.28/0.046	77.78	0.000*	3
TRM	Três Marias	São Francisco	*C. kelberi*	Reservoir	16	0.2^{B}	1.64^{B}	0.33	0.44/0.046	66.67	0.0002*	1
RD	Rio Doce	Doce	*C. kelberi*	Lake	15	0.182^{B}	1.5^{B}	0.11	0.23/0.052	55.56	0.2165	1
ITU	Itumbiara	Paraná	*C. piquiti*	Reservoir	14	0.07^{D}	1.28^{D}	0.56	0.161/0.036	33.33	0.2636	1
ML	Marginal lake - São Francisco	São Francisco	*C. piquiti*	Lake	11	0.028^{D}	1.12^{D}	0.11	-0.0714/0.037	11.11	1.000	1
FUR	Furnas	Upper Paraná	*C. piquiti*	Reservoir	10	0.169^{CD}	1.59^{CD}	0.44	0.307/0.034	50.00	0.0195*	1

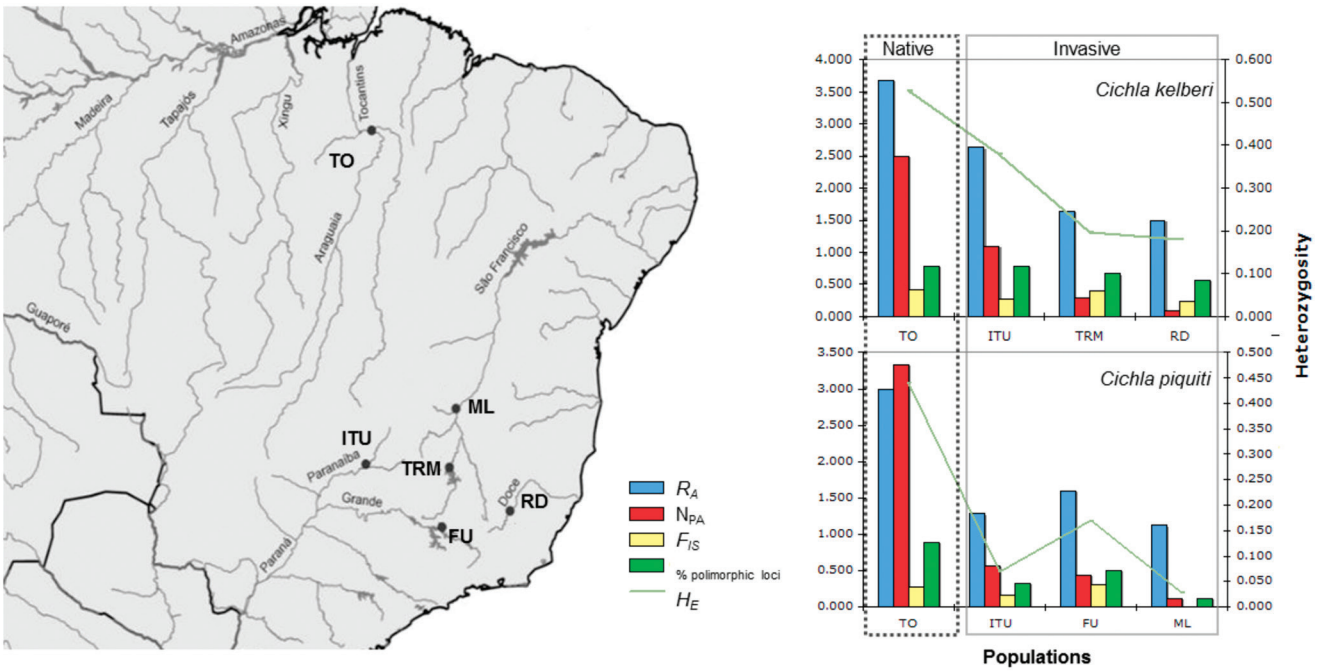

Fig. 1. River basins in eastern and northern Brazil showing sample sites and genetic parameters of native and introduced populations: Allelic richness (R_A), Nei's genetic diversity (H_E), Number of private alleles (N_{PA}) and percentage of polymorphic *loci*. Samples sites: TRM - Três Marias reservoir and ML - Marginal Lake (both in the São Francisco River); ITU - Itumbiara reservoir (Paraná River - upper Paraná River basin); RD - Rio Doce (Dom Helvécio Lake - Doce basin); FU - Furnas reservoir (Grande River - upper Paraná River basin); TO - Tucuruí reservoir (Tocantins River - Amazon basin).

Fstat 2.9 (Goudet, 1995). To better assess levels of genetic structure in situations where null alleles were identified, we used F_{ST}ENA estimated using the "ENA" (Excluding Null Alleles) adjustment as described in (Chapuis & Estoup, 2007) and implemented in Freena (http://www.montpellier.inra.fr/URLB/). Since several introductions events are likely to increase the genetic divergence among populations (Wade & Mccauley, 1988), F_{ST} values are expected to indicate to what extent a colonization process may globally modify the partition of genetic diversity, within and between populations. Low F_{ST} values may be consequence of a high number of founder specimens and/or high propagule pressure mediated by human dispersal (Le Corre & Kremer, 1998), whereas high F_{ST} values suggest low propagule pressure and cumulative founder effects.

A Bayesian model-based clustering method implemented in Structure (Pritchard *et al.*, 2000) was conducted to determine if there was evidence for hybridization between CP and CK in the native and in introduced populations. The q-values (*i.e.*, the ancestry of individual fish which estimates the proportion of an individual's genotype) were inferred based on genotyped specimens assigned to two species groups (k=2; *i.e.*, CK and CP) using 500 000 burn-ins and 1 000 000 repetitions. We used a conservative *q*-value threshold of ≤ 0.05 to identify individuals (*i.e.*, q = 0.95 for CP; q = 0.05 for CK) following (Schwartz & Beheregaray, 2008).

Two methods implemented in Bottleneck version 1.2.02 (Piry *et al.*, 1999) were used to detect population bottlenecks. The first method is based on the detection of 'heterozygosity excess'. In a recently bottlenecked population, the observed heterozygosity is higher than the heterozygosity expected from the observed number of alleles under the assumption of a population at mutation-drift equilibrium (Cornuet & Luikart, 1996). The results obtained separately for each *locus* were combined using the Wilcoxon test (Cornuet & Luikart, 1996; Piry *et al.*, 1999). Second, we used a qualitative descriptor of allele frequency distribution (the mode-shift indicator), which discriminates between bottlenecked and stable populations. For a stable population, it is assumed that the rare allele is the most common, whereas in a recently bottlenecked population, intermediate classes are better represented (Luikart & Cornuet, 1998; Luikart *et al.*, 1998). A shift in the mode of the distribution of allelic frequency classes is thus expected.

Results

Genetic diversity and population structure

Cichla kelberi

For CK, a mean of 5 alleles *per locus* was observed in the invasive populations, compared to 5.6 alleles in the natives

Table 2. Pairwise Fixation Index for native and invasive populations of *C. kelberi* (A) and *C. piquiti* (B). The first value is the estimate F_{ST} of Weir (1996) and the second is the F_{ST}^{ENA} using ENA correction (Chapuis & Estoup, 2007).

(A) *C. kelberi*

	TO	ITU	TRM	RD	
TO	0				
ITU	0.3020/0.298 (P=0.002)	0			
TRM	0.3472/0.329 (P=0.002)	0.5469/0.522 (P=0.002)	0		
RD	0.3335/0.335 (P=0.001)	0.5418/0.519 (P=0.002)	0.3143/0.277 (P=0.002)	0	Mean = 0.40/0.38

(B) *C. piquiti*

	TO	ITU	ML	FU	
TO	0				
ITU	0.4376/0.432 (P=0.002)	0			
ML	0.5716/0.578 (P=0.002)	0.8061/0.799 (P=0.002)	0		
FUR	0.4937/0.476 (P=0.002)	0.6631/0.652 (P=0.002)	0.0884/0.13 (P=0.03)	0	Mean= 0.51/0.51

range. When considering introduced and native populations a low number of alleles *per locus* (2.36 in average) and low Nei's genetic diversity were recovered (mean of 0.33). The percentage of polymorphic *loci* ranged from 56% to 89% and allelic richness ranged from 1.5 to 3.68. Significant deviations from Hardy-Weinberg genotypic proportions were always associated with positive F_{IS} values (Table 1). The highest F_{ST} value was observed between Três Marias and Itumbiara, and the lowest between Itumbiara and Tocantins (average F_{ST} of 0.40) (Table 2A). F_{ST}^{ENA}, using ENA correction (Chapuis & Estoup, 2007), were consistent with F_{ST} values estimated according with (Weir, 1996) (Table 2A).

Cichla piquiti

For CP, a low mean allelic richness of 1.75 and Nei's genetic diversity (mean of 0.177) were recovered. The percentage of polymorphic *loci* ranged from 11% to 50%. As in CK, the significant deviations from Hardy-Weinberg genotypic proportions were always associated with positive F_{IS} values (Table 1). An average F_{ST} and F_{ST}^{ENA} of 0.51 were recovered (Table 2B). The highest value (80%) was observed between Itumbiara and Marginal Lake (São Francisco River), and the lowest (9%) between Furnas and Marginal Lake (Table 2B).

Null alleles

Microcheker detected the presence of null alleles but rejected the existence of large allele dropout or scoring errors within CP. Only the native population showed evidence for null alleles (*loci* Tuc 10, 2, 18 – Supplement 1). For CK, only *locus* Tuc 13 consistently showed null alleles in more than one population. However, the F_{ST}^{ENA} estimation with ENA adjustment had no differences in F_{ST} for both species (Table 2). Using the software Inest, which estimates inbreeding and null allele frequencies to account for deviations from HWE (Supplement 1), five *loci* were positive for null alleles (Tuc9, Tuc 8, Tuc 3, Tuc 13, Tuc 16), but none of them had consistent results over all populations.

Our adjustment of Weir's F_{ST} carried out with ENA in the present data (F_{ST}^{ENA}) did not show any bias due to null alleles. Therefore, the high values of F_{ST} recovered could reliably

Table 3. Bottleneck tests. Results of two methods assessing bottlenecks in *Cichla* populations in the invasive range for both species (ITU = Itumbira; TRM = Três Marias; RD = Rio Doce; ML = Marginal Lake; FUR = Furnas). *significant values (P=0.05).

Sample	Sample size	Mode-shift[§]	Heterozygosity excess[†]
C. kelberi			
ITU	18	Normal	0.71094
TRM	16	Normal	0.34375
RD	15	Normal	0.59375
C. piquiti			
ITU	20	Normal	0.06250
ML	11	Normal	0.25000
FUR	10	shifted	0.01563*

indicate low gene flow between introduced sites, suggesting that only one introduction act might be responsible for the establishment of a new invasive population of peacock bass at each site.

Bottleneck

Statistical evidence of a bottleneck was detected within CP for the introduced population at Furnas Reservoir, based on the Wilcoxon test under the two-phase, stepwise mutation model and shifted distribution of allelic frequency classes. Within CK populations, no statistical support for bottleneck was recovered (Table 3).

Native *vs* invasive range

A clear pattern of reduction of genetic diversity was observed in all invasive populations for both *Cichla* species (Fig. 1). The percentage of polymorphic *loci* were high in native populations of CK and CP (78 and 89%, respectively) but varied widely in the invasive range (77.8-11.1%) (Table 1). A mean of 2.7 alleles *per locus* were observed in invasive populations, compared to 4.6 in the native populations of CP. Alleles detected in the introduced range were not always detected in native populations, but the great majority of private alleles were exclusive to the native populations (30 alleles or 75%). Mean F_{IS} varied from -0.07 to 0.279 (Table 1).

Mean allelic richness was significantly lower in populations in the invasive range (CK = 1.923; CP = 1.33) compared with the native range (CK = 3.68; CP = 2.63) ($z = 3.543$, $P<0.0001$; $z = 2.131$. $P = 0.033$) (Table 1). Nei's genetic diversity (H_E) was marginally significant in the invasive range when compared to the native populations considering CP (H_E=0.179/0.44 respectively; z=1.972, P=0.049), but not significant for CK (H_E=0.258/0.536 respectively; z=0.641, P=0.521). The highest values of H_E and R_A (H_E=0.393; R_A=2.63; Table 1) for CK in the invasive range were detected in the Itumbiara reservoir (upper Paraná River basin), the closest site to the native species. In contrast, a significant reduction in H_E and R_A was detected for CP at the same site (H_E=0.07; R_A=1.28; Table 1), possibly due to lower propagule pressure. For CP, the highest values of H_E and R_A were detected in the invasive population of Furnas reservoir (H_E=0.169; R_A=1.59), which is also part of the upper Paraná River Basin, despite the small number of specimens analyzed (N=10). Interestingly, within the invasive range, populations from non-impacted sites (pristine lakes) had the lowest H_E, R_A and F_{IS} values (RD and ML, Table 1). Strong genetic structure was detected within invasive populations and also when comparing invasive with natives. The overall F_{ST} and F_{ST}^{ENA} had identical or very similar values for both species (CK = 0.40/0.38 and CP = 0.51/0.51, respectively).

A significant deviation from Hardy-Weinberg equilibrium was found at *locus* Tuc 18, but only in the native population. Only one *locus* (Tuc 4) out of nine was monophorphic in the native population, whereas several *loci* were monomorphic in the invasive range (Supplement 1). The *locus* Tuc 18 had significant deviation from Hardy-Weinberg genotypic proportions associated with positive F_{IS} values.

Admixture analysis

Our analysis identified two putative hybrids between CP and CK in the native population (fish 152, $q<0.95$ and fish 63 $q>0.05$, Fig. 2). No evidence of hybridization was detected in the invasive populations, even in the Itumbiara reservoir - the only site within the introduced populations where CK and CP were detected in sympatry.

Discussion

Genetic diversity in native vs invasive populations

The genetic consequences of a range expansion depend on the relative magnitudes of the number of colonists and migrants, similar to the extinction and colonization process (Wade & Mccauley, 1988). These consequences are far

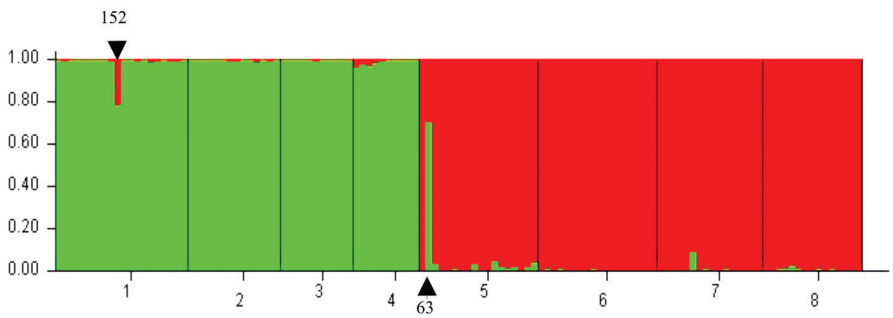

Fig. 2. Structure bar plots of probabilities of assignment of each individual from populations of CP in green (1 - TOC, 2 - ITU, 3 - ML and 4 - FU) and CK in red (5 - TOC, 6 - ITU, 7 - TRM, 8 - RD). Probabilities of assignment (q) of each individual to each cluster are shown along the x-axis.

more pronounced and lasting in the linear stepping-stone model than in the island model. Therefore, comparison of the genetic composition of native species with their recently established populations provide valuable information about the process of invasion (Kirkpatrick & Barton, 1997). Here, we show a heterogeneous pattern of genetic diversity in the invasive range of two species of the apex predator genus *Cichla* (Fig. 1).

Nei's genetic diversity (H_E) was lower in the invasive range when compared to the native populations (CP H_E=0.179/0.44; CK H_E=0.258/0.536 respectively), probably due to founder effects during introduction or lower propagule pressure. Despite the reduction in genetic diversity, the population from the Itumbiara reservoir (the only site where the two species are found in sympatry) and the Furnas reservoir had levels of genetic diversity not significantly different to the native population (Table 1). The similarity in the genetic composition in Itumbiara can be attributed to multiple introductions at this site with fish from Tocantins River (Carvalho *et al.*, 2009b).

Interestingly, both sites with no significant reduction in genetic diversity are located in the same river basin (upper Paraná River). However, Furnas is the only one out of these sites that shows a bottleneck signature (Table 3). This probably relates to its greater distance from the native populations, with bottleneck signal still persisting due to a likely recent population foundation.

Evidence of hybridization was detected in the native population (Fig. 2). This is in accordance with our morphological observations, since intermediate morphotypes were observed within the native range only. Carvalho *et al.* (2009b) also did not find any evidence of shared haplotypes within CP and CK invasive populations, even in areas of sympatry. Besides the relatively small number of fish analyzed (mean=15.3, Table 1), evidence suggests that hybridization is not an important factor in the invasive capability of *Cichla*.

Since cumulative founding events are predicted to lead to allele losses, the percentage of polymorphic *loci* and allelic richness might decrease within each translocation of a species (Le Corre & Kremer, 1998). The heterogeneous pattern of genetic diversity in the invasive range (Fig. 1) together with high F_{ST} values, suggests that the introduction of peacock bass agrees with a pattern of dispersal named as "jump dispersal" (Wilson *et al.*, 2009). Here, long-distance dispersal may occur over substantial distances, but a connection (*i.e.*, gene flow) between the new and original ranges could remain with differentiated propagule pressure (Wilson *et al.*, 2009). For instance, average F_{ST} for both species were high, with mean values of 0.4 and 0.5 for CK and CP (Table 2) respectively, suggesting low gene flow between introduced sites, but different propagule pressure between introduced and source populations appears to be occurring (Fig. 1). The initial propagule size and the occurrence of limited gene flow between established populations, in the present case, seem to be an important factor explaining the genetic patterns observed in the invasive range.

Pristine *vs.* impacted sites

Interestingly, the lowest value of F_{IS}, H_E, R_A and percentage of polymorphic *loci* were detected in the marginal lake in the São Francisco reservoir. Marginal lakes are temporary natural lakes formed by river flooding, which might completely dry out during long drought periods. Moreover, the income of new species into marginal lakes is not frequent, limiting the propagule pressure of invasive species due to human introductions. The marginal lake analyzed here is also far from the urban centers, which might prevent recurrent introductions. At this site, the presence of only one polymorphic marker indicated that only a few specimens were responsible for the establishment of this population, with no further new introductions, leading to extremely low genetic diversity. Similar results were observed for the brown trout (*Salmo trutta*) introduced into Patagonia National Parks (Argentina). In this case, plasticity in life-history such as flexibility in migratory behavior and spawning, seem to be more important to the invasive process than genetic variability (Valiente *et al.*, 2010).

Using human density as a proxy for propagule pressure, (Espinola *et al.*, 2010) did not found any association between invasibility with human density in an analysis of 38 reservoirs invaded by CK. Likewise, we found that propagule pressure appears unimportant in the peacock bass establishment, even in pristine sites where greater resilience to invasions would be expected (*i.e.*, Doce River and Marginal Lake). Is interesting to note that in the Pantanal (the world's largest wetland ecosystem), where more than 260 fish species occur (Britski *et al.*, 1997) evidence suggest that a co-existence among CP and native fishes will be reached in the long term (Resende *et al.*, 2008). Other examples of biotic resistance have been reported in marine (Stachowicz *et al.*, 1999; Hunt & Yamada, 2003), freshwater (Harvey *et al.*, 2004; Yonekura *et al.*, 2004) and terrestrial (Lake & O'Dowd, 1991; Parker *et al.*, 2006) ecosystems.

Since neutral genetic diversity appear unimportant in *Cichla*'s invasive capability, what makes *Cichla* an invasive species?

We speculate that the lack of previous ontogenetic exposure of native species to a sympatric invasive predator, due to the distinct co-evolutionary history, may unable prey to recognize and respond to predators, an effect named "prey naiveté" (Cox & Lima, 2006). The lack of avoidance behavior of native prey will therefore lead to increase predation and mortality of native species. For example, in New Zealand after the introduction of brown trout (approximately 120 years ago), crayfish (*Paranephrops zealandicus*) were unable to recognize chemical

cues of the invasive species (Shave *et al.*, 1994). On the other hand, prey may recognize predators without prior exposure, what may explain induced habitat shifts in native prey (*Galaxias auratus*, Stuart-Smith *et al.*, 2008). However, (Kovalenko *et al.*, 2010) showed that prey naiveté might not be an important factor in the invasibility of peacock bass, since native prey could recognize *Cichla kelberi* as a predator, showing avoidance behavior. However, since prey was collected from the wild, and experiments were conducted in laboratory conditions, it is possible that prey might have previously encountered peacock bass and learned to avoid it, as pointed out by the authors (Kovalenko *et al.*, 2010).

Many studies have shown that invaders have a superior ability to exploit local resources when compared with native residents (Byers, 2000; Kupferberg, 1997; Petren & Case, 1996). Competitive ability is, therefore, a trait that may confer an advantage for invasive species during establishment. Differences between the competing species in the home range and those in the new range may influence an invader's success and their ability to dominate a community. Decision-making rules, also named "Darwinian algorithms" (Cosmides & Tooby, 1987), are predicted to be adaptive, since they depend on cues that might change over evolutionary time, interfering in the survival and reproductive success of species (Williams & Nichols, 1984). Particularly important, are some biological aspects (*e.g.*, intense reproductive activity, biparental care of eggs and capability of adaptation to harsh ecological conditions) that make *Cichla* a potential invasive apex predator (Munoz *et al.*, 2006; Resende *et al.*, 2008; Vieira *et al.*, 2009; Zaret, 1980), as also shown for the brown trout introduced in Patagonia (Valiente *et al.*, 2010).

When considering environments that have rapidly changed (*i.e.*, reservoirs) or received invasive species, preliminary characteristics and adaptations of native species might no longer be advantageous. In such cases, native organisms can become "trapped" by their evolutionary responses and unable to rapidly adapt and compete with the invasive species, resulting in reduced survival and in long term extinction of native species (Schlaepfer *et al.*, 2005).

Another important aspect in the adaptability to new environmental conditions that should be considered is *Cichla*'s opportunistic feeding behavior (Resende *et al.*, 2008) and cannibalism (Gomiero & Braga, 2004). For example, it was observed that cannibalism is more accentuated during *Cichla*'s spawning periods and due to scarcity of alternative food items, such as small individuals of small native fish species (Gomiero & Braga, 2004; Villares Junior & Gomiero, 2010). On the other hand, low rates of cannibalism occur in natural environments and in invaded sites mainly due to high availability of prey (Resende *et al.*, 2008; Winemiller *et al.*, 1997; Zaret, 1977).

Our work indicates that in *Cichla*, invasiveness capability is not influenced by genetic diversity (as a proxy to propagule pressure within distinct environments conditions) and hybridization. It is suggested that a combination of other factors, such as *Cichla*'s reproductive strategies, feeding opportunistic behavior, cannibalism of young, the "evolutionary trap" effect and relatively low species richness in the invaded native ecosystems, are key in this apex predator invasion phenomenon to overcome low propagule pressure.

Acknowledgments

The authors are grateful to several artisanal fishermen for their help with the sampling and to Arno Soares, José Enemir Santos, Daniel Crepaldi and CEMIG (Companhia Energética de Minas Gerais – Programa Peixe Vivo) for providing samples. This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq (process numbers INCT 573899/2008-8 and 482852/2011-9) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais - FAPEMIG (process number INCT APQ-0084/08).

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Submitted July 1, 2013

Accepted October 2, 2013 by Claudio Oliveira

Published March 31, 2014

Supplement 1. Data estimated by each locus of microsatellite loci considering every locus in all populations for both species. The follow indexes are presented: Number of alleles (N_A); Inbreed coefficient (F_{IS}), Observed heterozygosity (Ho), Expected heterozygosity (He) and null alleles. "Mono" stands for monomorphic *locus*. * Indicate *locus* out of HWE equilibrium (P=0.05) after Bonferroni adjustment.

	C. *kelberi*				**C. *piquiti***			
***Loci*/Pop**	TO	ITU	TRM	RD	TO	ITU	ML	FUR
Tuc12								
N_A	4	2	2	2	2	1	1	1
Ho	0.8333	0.6000	0.3846	0.3333	0.0500	Mono	Mono	Mono
He	0.7833	0.4286	0.3205	0.5000	0.0500	-	-	-
Fis	-0.0638	-0.4000	-0.2000	0.3333	0.0000	-	-	-
Null allele	no	no	no	no	no	-	-	-
INEST	no	no	no	no	no	no	no	no
Tuc10								
N_A	1	1	1	1	2	1	1	1
Ho	Mono	Mono	Mono	Mono	0.0000	Mono	Mono	Mono
He	-	-	-	-	0.1000	-	-	-
Fis	-	-	-	-	1.0000	-	-	-
Null allele	-	-	-	-	no	-	-	-
INEST	no	no	no	no	no	no	no	no
Tuc9								
N_A	3	1	1	1	3	1	1	1
Ho	0.0000*	Mono	Mono	Mono	0.2500	Mono	Mono	Mono
He	0.2279	-	-	-	0.3333	-	-	-
Fis	1.0000	-	-	-	0.2500	-	-	-
Null allele	yes	-	-	-	no	-	-	-
INEST	yes	no	no	no	no	no	no	no
Tuc3								
N_A	8	4	3	1	6	1	1	2
Ho	0.3889*	0.5000*	0.0667*	Mono	0.6500	Mono	Mono	0.0000
He	0.8072	0.7680	0.5381	-	0.6474	-	-	0.2000
Fis	0.5182	0.3489	0.8761	-	-0.0041	-	-	1.0000
Null allele	yes	no	yes	-	no	-	-	no
INEST	yes	no	yes	yes	no	no	no	yes
Tuc13								
N_A	6	9	1	1	9	1	1	2
Ho	0.1765*	0.5556*	Mono	Mono	0.4500	Mono	Mono	0.0000
He	0.8162	0.8856	-	-	0.6895	-	-	0.2000
Fis	0.7838	0.3727	-	-	0.3473	-	-	1.0000
Null allele	yes	yes	-	-	yes	-	-	no
INEST	yes	yes	no	yes	no	no	no	yes
Tuc4								
N_A	1	2	2	2	1	2	3	2
Ho	Mono	0.0000*	0.0667	0.0769	Mono	0.0714	0.2727	0.1000
He	-	0.1111	0.0667	0.0769	-	0.0714	0.2545	0.1000
Fis	-	1.0000	-0.0000	-0.0000	-	0.0000	-0.0714	0.0000
Null allele	-	no	no	no	-	no	no	no
INEST	no	no	no	no	no	no	no	no
Tuc5								
N_A	8	5	2	3	6	4	1	3
Ho	0.6667	0.4444	0.0000	0.0769*	0.5000	0.1429	Mono	0.2000
He	0.8366	0.5327	0.1250	0.3462	0.7013	0.2720	-	0.3667
Fis	0.2031	0.1656	1.0000	0.7778	0.2871	0.4747	-	0.4545
Null allele	no	no	no	no	no	no	-	no
INEST	no	no	no	no	no	no	no	no
Tuc16								
N_A	7	2	3	2	4	1	1	2
Ho	0.3333*	0.1111*	0.2500	0.4000	0.4500	Mono	Mono	0.2000
He	0.6029	0.1078	0.4167	0.4619	0.6132	-	-	0.1889
Fis	0.4472	-0.0303	0.4000	0.1340	0.2661	-	-	-0.0588
Null allele	yes	no	no	no	no	-	-	no
INEST	yes	no	no	no	no	no	no	no

Supplement 1. Cont. Data estimated by each locus of microsatellite loci considering every locus in all populations for both species. The follow indexes are presented: Number of alleles (N_A); Inbreed coefficient (F_{IS}), Observed heterozygosity (Ho), Expected heterozygosity (He) and null alleles. "Mono" stands for monomorphic *locus*. ***** Indicate *locus* out of HWE equilibrium (P=0.05) after Bonferroni adjustment.

Tuc18								
N_A	12	5	2	2	8	2	1	2
Ho	0.7059	0.3889*	0.3333	0.4167	0.5263*	0.2857	Mono	0.5000
He	0.9081	0.7059	0.3939	0.3409	0.8626	0.2527	-	0.3889
Fis	0.2227	0.4491	0.1538	-0.2222	0.3898	-0.1304	-	-0.2857
Null allele	yes	no	no	no	yes	no	-	no
INEST	no	no	no	no	yes	no	no	no
Average								
N_A	5.6	3.4	1.8	1.6	4.5	1.6	1.2	1.8
Ho	0.3082	0.2830	0.1128	0.1402	0.3200	0.0588	0.0303	0.1176
He	0.5365	0.3926	0.2002	0.1824	0.4443	0.0701	0.0283	0.1699
Fis genpop	0.4255	0.2792	0.4368	0.2315	0.2797	0.1613	-0.0714	0.3077
Fis inest	0.076	0.046	0.046	0.046	0.039	0.036	0.037	0.034