Gabriel Jorgewich Cohen

Genética de populações aplicada à biologia da invasão: um panorama da
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Population genetics applied to invasion biology: a panorama of the
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Introdução geral

Os processos e conceitos relacionados às introduções biológicas vem sendo reconhecidos e estabelecidos na literatura científica das últimas décadas (revisado por Catford, Jansson, & Nilsson, 2009). Alguns autores (e.g. Williamson, 1993; Levine, Adler and Yelenik, 2004; Lockwood, Cassey and Blackburn, 2005) já descreveram o processo de invasão através de quatro principais fases semelhantes, aqui definidas como: I. Importação (podendo ser intencional ou não), II. Introdução, III. Fixação e IV. Dispersão.

O desenvolvimento tecnológico e os processos de globalização atingidos pelo homem nos últimos séculos, tiveram como consequência a crescente facilitação de processos de invasão biológica. Mesmo com inúmeros casos de importação de novas espécies, como em zoológicos, espécies de interesse agropecuário, animais de estimação e para diversas outras finalidades, incluindo casos de introdução acidental, relativamente poucas espécies atingiram os dois últimos níveis de uma invasão. Mesmo que introduções biológicas bem-sucedidas sejam eventos muito menos frequentes que o total de espécies importadas para novas regiões, já reconhecemos diversos exemplos de sucesso, com inúmeros impactos causados sobre populações nativas (Both & Grant, 2012; Didham, Tylianakis, Gemmell, Rand, & Ewers, 2007; Molnar, Gamboa, Revenga, & Spalding, 2008; Pearson et al., 2013; Shine, 2012). Em muitos casos, espécies invasoras são consideradas as principais responsáveis pela extinção de espécies nativas (Clavero & García-Berthou, 2005; Lowe, Browne, Boudjelas, & De Poorter, 2000), mesmo que este ainda seja um tema em discussão (Gurevitch & Padilla, 2004). Por outro lado, as invasões também funcionam como experiências evolutivas que podem ser estudadas em curto e médio prazo (Schlaepfer, Sherman, Blossey, & Runge, 2005), gerando respostas interessantes para entender e conservar populações nativas, assim como evitar a introdução de novos propágulos (García-Berthou, 2007).

A pesquisa relacionada à biologia da invasão é ampla e apresenta diversas vertentes. A aplicação de recursos genéticos nesse tipo de estudo pode ser muito valiosa, e vem crescendo muito nos últimos anos (Hoshino, Bravo, Nobile, & Morelli, 1999; Kamath, Sepulveda, & Layhee, 2016; Turner, 2013). Uma ferramenta útil para entender os processos de invasão e sua dinâmica é a genética de populações. Apesar de existirem algumas limitações metodológicas da área (Selkoe & Toonen, 2006), especialmente quando aplicadas a populações com baixa diversidade gênica (Robertson & Gemmell, 2004), assim como se espera em populações invasoras (Estoup et al., 2011), salvo exceções (Kolbe et al., 2004); esse tipo de estudo tem o potencial de responder perguntas muito relevantes para esforços de controle de populações invasoras.

Entre as espécies invasoras mais notórias do planeta está a Rã-touro-americana, *Lithobates catesbeianus* (Shaw, 1802), classificada como uma das 100 piores espécies invasoras do mundo (Lowe et al., 2000). Apesar de outras espécies de anfíbios apresentarem populações invasoras consolidadas em diversos locais do planeta, como a *Rhinella marina* (Linnaeus 1758) na Austrália, *Xenopus laevis* (Daudin, 1802) em

países da Europa e América do Sul, e *Eleutherodactylus coqui*, Thomas, 1966, no Havaí e em algumas áreas da América do Sul; a rã-touro é a espécie mais amplamente distribuída no planeta. Nativa da América do Norte, esta espécie foi introduzida em mais de 40 países (Kraus, 2009; Lever, 2003) para diversos fins, sendo a ranicultura a maior motivação. Existem muitos relatos de escapes de rãs confinadas em criatórios e, alguns trabalhos já associaram o aparecimento e estabilidade de populações ferais com ranários comerciais próximos (Liu & Li, 2009). A sucessiva soltura de novos propágulos é consensualmente considerada uma das características importantes para o sucesso de uma invasão biológica (Kolbe et al., 2004; Lockwood et al., 2005).

Ranário científico da faculdade de zootecnia da UNESP - Campus Botucatu. (Foto: Gabriel J. Cohen)

Algumas das características físicas, fisiológicas e reprodutivas da rã-touro podem ter facilitado o processo de invasão da espécie em diversos locais. Além de sua área de

ocorrência nativa possuir grande variação latitudinal, com consequente variação climática, esta é uma espécie de grande porte e voracidade. Os maiores exemplares podem exceder 150mm de comprimento (Kaefer, Boelter, & Cechin, 2007) com uma dieta muito flexível, capaz de predar diversos animais, incluindo pequenos vertebrados como mamíferos, aves, répteis, peixes e anfíbios (Boelter & Cechin, 2007; McCoy, 1967; Teixeira, Silva, Pinto, Filho, & Feio, 2011), muitas vezes com comportamentos canibais (Lima, Casali, & Agostinho, 2003). Tem grande fecundidade, podendo produzir até 20.000 ovos por desova (Howard, 1978b), sendo até duas desovas por período reprodutivo (Howard, 1978a); e um crescimento muito rápido, alcançando a maturidade sexual em um ano após a metamorfose, quando em condições favoráveis (Howard, 1978b).

Os impactos causados pela introdução desta espécie já foram relatados em algumas pesquisas, como a interferência sonora nas vocalizações de anfíbios nativos (Both & Grant, 2012) e a contaminação de *Batrachochytrium dendrobatidis*, um fungo que dizima várias espécies de anfíbios no mundo, das quais a Rã-touro é considerada um vetor (Schloegel et al., 2010), além da competição e predação de espécies nativas (Ferreira & Lima, 2012; Li, Ke, Wang, & Blackburn, 2011).

Indivíduo adulto de Lithobates catesbeianus predando um adulto de Boana faber no parque estadual do Turvo, Derrubadas – RS. (Foto: Gabriel J. Cohen)

Alguns trabalhos foram feitos com a intenção de entender, controlar ou erradicar esta espécie de algumas regiões onde foi introduzida, com poucos casos de sucesso

(Descamps & Vocht, 2017; Louette, 2012; Louette, Devisscher, & Adriaens, 2014; Snow & Witmer, 2010). Pesquisas focadas em genética de populações invasoras da rã-touro também já ocorreram localmente nos Estados Unidos (Funk, Garcia, Cortina, & Hill, 2011; Kamath et al., 2016) e China (Bai, Ke, Consuegra, Liu, & Yiming, 2012); e internacionalmente na Europa (Ficetola, Bonin, & Miaud, 2008); levantando informações úteis para compreender as características dessas invasões, e o local de origem de seus propágulos. Não faltam razões para se desenvolver novos estudos na área da biologia da invasão, especialmente aqueles que podem trazer novos conhecimentos aplicáveis de forma direta ou indireta no controle das populações invasoras da Rã-touro. Com o intuito de unificar os conhecimentos já existentes sobre a invasão da rã-touro no Brasil e no mundo, e entender os processos de invasão dessa espécie de forma global, este trabalho foi realizado através do uso de ferramentas da genética de populações.

Através de dois capítulos interligados, exploramos a dinâmica da invasão da rã-touro no Brasil e nas principais linhagens do planeta. O primeiro capítulo discute a estruturação genética da espécie no país, abordando a literatura e uso de marcadores nucleares do tipo microsatélite. No segundo capítulo discutimos a estruturação das populações, os caminhos percorridos nos processos de invasão, e a provável origem nativa dessas populações, incluindo amostras de diversos países da América do Sul, Ásia e Caribe. Diversas inferências puderam ser feitas através do uso da literatura específica, marcadores nucleares e mitocondriais, e eventos históricos relacionados às questões econômicas e sociais que regem a dinâmica desta espécie que apresenta grande interesse econômico. Os dois capítulos foram escritos em formato de submissão para revistas científicas, sendo o primeiro para *Biol invasions*, e o segundo para *Molecular ecology*. Nesta dissertação ambos serão apresentados com a mesma formatação, por motivos de padronização. As referências contidas no segundo capítulo que aparecem como *Cohen and Grant*, CHAPTER 1, se referem ao primeiro capítulo desta dissertação.

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Capítulo 1

Genetic structure of introduced American bullfrog populations (*Lithobates catesbeianus*) in Brazil

Gabriel Jorgewich Cohen^{1,*} ; Taran Grant¹

1 Instituto de Biociências, Universidade de São Paulo. Rua do Matão, 101, Cidade Universitária, CEP 05508-090, São Paulo, SP, Brazil.

*Corresponding author. Email: gabrieljcohen2@gmail.com - Orcid: 0000-0001-9807-6297

Abstract

Alien species are a major problem affecting numerous biomes around the globe. Population genetics can be a powerful tool to understand invasions, providing insights into the underlying processes and providing useful information for control efforts. Here we evaluate the population structure of the invasive species *Lithobates catesbeianus* in Brazil. 300-600 animals were imported to Brazil at the 1930s as a governmental breeding program, resulting in several feral populations spread at the country. Tissue samples were collected from feral and captive populations at 38 different sites in different parts of the country. Using nuclear microsatellite loci, we assessed the degree of differentiation between populations with Jost's D index of differentiation and discriminant analysis of principal components. We found a single gene pool encompassing all distribution but one location, that must have received a new importation or suffered differentiation by isolation resulting on different haplotypes.

Keywords Alien species; Amphibia; Anura; invasion biology; invasive species management; propagule pressure; population establishment; Ranidae

Introduction

One of the most significant environmental problems that affect several biomes in the world is the introduction of exotic species. This phenomenon is directly associated with human action and globalization, being considered one of the major causes of extinction (Clavero & García-Berthou, 2005; Lowe, Browne, Boudjelas, & De Poorter, 2000). There are several problems associated with this process, that can represent great biological and economic losses (Holmes, Aukema, Von Holle, Liebhold, & Sills, 2009; Pimentel, Zuniga, & Morrison, 2005; Xu et al., 2006). Precautionary policies for new invasion events are essential to avoid dissemination of species with high adaptive capacities and a potential subsequent reduction of native populations (Gregory & Long, 2009). Efforts to control already established alien species should not be left behind, in order to remedy the environmental damage these species may cause.

Population genetics studies can be an efficient tool to understand population structure and other useful information for conservation programs in general (Schwartz, Luikart, & Waples, 2007), being applicable on studies of introduced species (Kolbe et al., 2004; Rollins, Woolnough, Wilton, Sinclair, & Sherwin, 2009). This type of study has the potential to raise critical information for the success of efforts to control invasive species (Rollins et al., 2009), which imply a high cost and cannot always be applied more than once (Hulme, 2006).

The target species of the present work is the American Bullfrog, *Lithobates catesbeianus*, (Shaw, 1802). Native from Eastern North America, this species has been introduced in more than 40 countries (Lever, 2003) for frog farming and other purposes. The American Bullfrog is a large and voracious species, that can exceed 150mm of snout-vent length (Kaefer, Boelter, & Cechin, 2007) with a very flexible diet, capable to predate on various animal taxa, including small vertebrates (Boelter & Cechin, 2007; McCoy, 1967; Teixeira, Silva, Pinto, Filho, & Feio, 2011). It has great fecundity, being able to produce up to 20.000 eggs by clutch (Howard, 1978), and a very fast growing, reaching sexual maturity in one year after metamorphosis, if in favorable conditions (Howard, 1978). These are some of the characteristics that may favor this species when invading different environments.

Impacts caused by the introduction of this species have already been reported, such as sound interference on native amphibians' vocalizations (Both & Grant, 2012), and *Batrachochytrium dendrobatidis* contamination, a fungus that is decimating several amphibian species in the world, of which the Bullfrog is considered a vector (Schloegel et al., 2010). Few efforts have been made to control this species (Snow & Witmer, 2010) or to understand its genetic structure (Bai, Ke, Consuegra, Liu, & Yiming, 2012; Ficetola, Bonin, & Miaud, 2008; Funk, Garcia, Cortina, & Hill, 2011; Kamath, Sepulveda, & Layhee, 2016), but it is crucial that several studies be carried out to better understand its dynamics and ensure that there is no recolonization, so the eradication program can be considered successful (Abdelkrim, Pascal, Calmet, & Samadi, 2005). The study of population genetics can be an excellent tool to generate useful information for invasive species management and control programs (Rollins et al.,

2009; Schwartz et al., 2007), and for understanding invasions history and its dynamics (Ficetola, Thuiller, & Miaud, 2007; Funk et al., 2011).

In 1935, 300 individuals (Cunha & Delariva, 2009) or 300 couples (Ferreira, Pimenta, & Neto, 2002) of unknown origin were introduced into Brazil by a Canadian named Tom Cyril Harrison. The animals were taken to the country's first breeding farm in the State of Rio de Janeiro, where their numbers increased, and the tadpoles produced were sent to new breeding facilities in several States of the country, in an agricultural program encouraged by the government (Fontanello & Ferreira, 2007). Motivated by economic issues, several facilities were opened and closed until the present time due to economic issues. Nowadays, it is very common that, when initiating businesses, a breeder will buy matrices from different places of the country, in order to ensure the greatest possible genetic diversity (Romar Bullfrog farm's owner, personal communication). However, due to expected low differentiation, breeders may not achieve their intentions since they often do not know the former origin of the specimens or its introduction history, probably not reaching their intentions due to expected low differentiation (Prim, Padua, & Bataus, 2003). Many escapes have occurred since the species has been bred in the country, and even some cases of intentional release by breeders who closed their businesses (Pedrinhas Bullfrog farm's owner, personal communication). Leakage and releases are highly correlated to the presence of Bullfrogs' feral populations (Bai et al., 2012; Liu & Li, 2009a; Santos-pereira & Rocha, 2015) which are now spread at the South and Southeast regions, but present in some sites of the Central-west, Northeast, and North of Brazil (Both et al., 2011).

Our goal is to assess Bullfrog population's genetic structure and possible gene flow between them, so future measures can be taken to control this species. Based on the facts that only one successful introduction has been documented on literature, and highly genetic flow is expected due to agricultural reasons, our hypothesis is that all populations present in the country belong to only one genetic population, without any significant differentiation.

Materials and Methods

We sampled a total of 321 bullfrog skin, liver, or muscle tissues between November 2014 and March 2017, of which 128 were purchased from eleven farms and 194 were collected in feral populations (Table 1). Samples were stored in 99% ethanol and kept at -20 °C until DNA extraction, which we performed with the DNeasy Blood and Tissue extraction kit (Quiagen, Valencia, CA, USA) following the manufacture's guidelines. We amplified seven nuclear microsatellite loci using the library developed by Austin et al. (2003). PCR was performed on a Veriti™ thermal cycler (Applied Biosystems) with a thermal profile consisting of 95°C for 7 min followed by 10 cycles of 95°C for 30 s, touchdown from 62–57°C for 45 s, and 72°C for 30 s, followed by 30 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 30s, and final extension at 72°C for 7 min. The reaction mix, with a total volume of 10 µl, contained 1.0 µl of buffer, 0.5 µl 2 mM dNTPs, 0.5 µl

fluorescent dye (VIC for RcatJ11 and RcatJ44b; NED for RcatJ21 and RcatJ41; PET for RcatJ54 and Rcat3-2b or 6-FAM for RcatJ8; applied biosystems), 0.5 µl of mixed forward and reverse primers (5 µM), 0.125 µl Taq polymerase, 3.375 µl distilled deionized water, and 3.0 µl template DNA. Later, we diluted the PCR products to a proportion of 1:4 and submitted them to sequencing by a third party. We scored results using Gene Marker ver. 2.6.3 (SoftGenetics).

We tested for the presence of null alleles, allele dropout, and stuttering using Microchecker (Van Oosterhout, Hutchinson, Wills, & Shipley, 2004). We used the gstudio package (Dyer, 2014), on platform R (R Core Team, 2017), to calculate the following diversity indices: expected (H_e) and observed (H_o) heterozygosity, number of alleles (A) per locus, effective number of alleles (A_e) per locus, and size corrected Wright's inbreed coefficient (F_{is}). Through a permutation procedure with 100 batches of 1000 iterations, we checked for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) deviations by performing a probability test in Genepop ver. 3.4 (Rousset, 2008). We applied the results to the Benjamini and Hochberg procedure (Hochberg & Benjamini, 1990) to control for false discovery rates (FDR) and avoid type I error. The *p.adjust* function from stats package (R Core Team, 2017) was used for this purpose.

We used Jost's D index (Jost, 2008) to quantify pairwise genetic differentiation between populations, which was calculated with the mmod R package (Winter, 2012). Next, we tested the significance of differentiation between pairs of populations using the DEMETics R package (Gerlach, 2010) with 10000 permutations. P values were corrected with the Benjamini and Hochberg procedure to avoid type I error. To assess current genetic structure, we performed a discriminant analysis of principal components (DAPC; Jombart, Devillard, & Balloux, 2010) in the adegenet R package (Jombart, 2008). To determine if there is any genetic differentiation between groups defined by feral or captive origin, and avoid any possible bias in the total data analysis, we performed one DAPC for states in which both groups are present (viz., Paraíba, Rio de Janeiro, and São Paulo) and a second DAPC of all samples grouped by state.

Results

The presence of null alleles was indicated for all loci, and several pairwise comparisons indicated significant linkage (S_1) and Hardy-Weinberg disequilibrium even after FDR corrections (Table 2). The mean number of alleles per locus (A) among all populations was 3–7, while the effective number of alleles (A_e) presented a considerably lower number due to the strong dominance of one or more alleles at most loci (dominant alleles account for 20–50% of the total at each locus). The observed heterozygosity did not meet the expectation (H_e) and all populations showed positive values of inbreeding (Table 2). Pairwise multilocus Jost's D revealed comparatively low differentiation between groups, with Minas Gerais being the group with the highest degree of differentiation at all comparisons but one (Table 3). The DAPC (S_2) that

tested the differentiation between all feral and captive groups presented almost complete overlap between all sample units, leading to the conclusion that there is no significant differentiation between feral and captive animals in the sampled states, so the analysis of the total data could be performed safely without distinguishing between feral and captive origins. The total data DAPC (Fig. 1) showed great overlap between all sample units except Minas Gerais, which presents some degree of differentiation.

Discussion

Our analyses suggest that Brazilian bullfrogs have undergone a strong bottleneck and intense inbreeding. These findings are expected, since all current bullfrogs are believed to descend from a small number of founders introduced 82 years ago (Cunha & Delariva, 2009; Ferreira et al., 2002), representing a little more than 50 generations. The introduction history of this species resulted in specific genetic characteristics for this populations, such as high rates of homozygosity, high inbreeding coefficient values, low allele richness, and consequent overestimations of linkage disequilibrium and Hardy-Weinberg disequilibrium.

Although null alleles are known to inflate measures of genetic differentiation and create false homozygotes (Chapuis and Estoup 2007; Carlsson, 2008), the common practice of discarding loci inferred to possess null alleles (e.g. Consuegra, Phillips, Gajardo, & Leaniz, 2011; Peacock, Beard, O'Neill, Kirchoff, & Peters, 2009; Rollins et al., 2009; Santos, Jamieson, Santos, & Nakagawa, 2013) is counterindicated by the invasion history of this species. Specifically, Microchecker indicates the presence of null alleles on the basis of excess homozygotes evenly distributed across the homozygote classes (van Oosterhout et al., 2004), which is precisely the situation expected to obtain in recent invasions from subjected to high levels of inbreeding (e.g., Robertson & Gemmell, 2004). Thus, we included all loci in our analyses, despite indications of the presence of null alleles.

The structure analysis of the captive and feral samples from the states of São Paulo, Rio de Janeiro, and Paraíba presented almost completely overlapping groups. This result can be explained not only by biological processes, like the bottleneck effect and intense inbreeding experienced by the species when it was introduced in the country, but also by the common practice among breeders of purchasing breeding specimens from different farms in different states with the intention of increasing the gene pool among the frogs bred in their facilities (GJC, personal communication with several farm managers). Although this practice might not be effective in the present context since all frogs in the country are believed to descend from the same initial population (Cunha & Delariva, 2009; Ferreira et al., 2002), it might be responsible for preventing frogs from different locations from undergoing an isolation by distance process. That is, the breeding facilities exchange "migrants" with each other, and effectively

replenish feral clusters with constant leaks (Liu & Li, 2009), keeping contact across the country in an infinite island model.

As predicted in the initial hypothesis, pairwise Jost's D distance analyses and the DAPC revealed little genetic differentiation between the Brazilian bullfrog localities. The Minas Gerais population was most differentiated in DAPC, although the degree of difference is not always the most expressive between comparisons at Jost's D analysis. Besides the possibility that is due to sampling bias, we propose two hypotheses to explain this observation: (1) the population from MG underwent differentiation by isolation, or (2) at least one additional, undocumented invasion event occurred in this region, thereby increasing the gene pool. Both hypotheses fit the patterns found in the DAPC, where there is still a great overlap between MG group and the others. The first hypothesis, however, requires that this population has been isolated after being introduced to the state, and has suffered mutations that have been fixed and increased their allelic frequency in less than fifty generations, reducing the likelihood of this hypothesis in comparison to the other. Further research is needed to elucidate this issue.

The information about the population genetics of the Bullfrog can be useful for future control efforts in the country, avoiding different gene pool mixing. The first steps to be taken for this purpose should be the application of methods to avoid leakage of new propagules, and a region limitation for the purchase of matrices. The hypothetical fragmentation of the population structure caused by isolation by distance after the application of these suggestions could facilitate control efforts, since smaller populations are the most promising targets for the success of these efforts (Robertson & Gemmell, 2004) and would become easier to identify sources that replenish feral populations, although time needed for establishment of genetic structure cannot be predicted.

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Tables and Figures

Table 1. Captive (C) and feral (F) individuals sampled from the states of Paraíba (PB), Minas Gerais (MG), Rio de Janeiro (RJ), São Paulo (SP), Paraná (PR), Santa Catarina (SC) and Rio Grande do Sul (RS).

Locality	Number of specimens	Origin
Bananeiras (PB)	22	C
Alfenas (MG)	21	F
Magé (RJ)	16	F
Cach. de Macacu (RJ)	10	F
Cach. de Macacu (RJ)	16	C
Guapimirim (RJ)	25	C
Botucatu (SP)	20	C
Campos do Jordão (SP)	4	F
Embu das Artes (SP)	4	F
Iporanga (SP)	1	F
Jaboticabal (SP)	4	C
Juquitiba (SP)	1	F
Matão (SP)	10	C
Mogi das Cruzes (SP)	5	F
Piedade (SP)	9	F
Pindamonhangaba (SP)	7	C
Sta. Bárbara D'oeste (SP)	3	F
Santa Isabel (SP)	4	F
S. Luiz do Paraitinga (SP)	3	F
São Paulo (SP)	3	C
São Roque (SP)	9	C
Francisco Beltrão (PR)	15	F
Maringá (PR)	16	F
Quatro Barras (PR)	5	F
Águas Mornas (SC)	1	F
Blumenau (SC)	2	F
Pinhalzinho (SC)	1	F
Pomerode (SC)	5	F
Cotipora (RS)	1	F
Derrubadas (RS)	7	F
Dois Lageados (RS)	1	F
Dona Francisca (RS)	1	F
Eldorado do Sul (RS)	2	F
Faxinal do Soturno (RS)	26	F
Ivora (RS)	2	F
Nova Palma (RS)	16	F
Santa Cruz do Sul (RS)	25	F
Serafina Correa (RS)	1	F

Table 2. Summary results on genetic survey of Bullfrog populations in Brazilian states of Paraíba (PB), Minas Gerais (MG), Rio de Janeiro (RJ), São Paulo (SP), Paraná (PR), Santa Catarina (SC) and Rio Grande do Sul (RS). False discovery rates (FDR) corrected *p* values that still out of Hardy-Weinberg equilibrium appears in bold

Site	Heterozygosity		Diversity			Hardie-Weinberg probability test <i>P</i> values						
	He	Ho	Fis	A	Ae	RcatJ11	RcatJ21	RcatJ54	RcatJ8	RcatJ44b	RcatJ41	Rcat3-2b
PB	0.6349	0.5300	0.18118	4.4285	2.8871	0.2636	0.2720	0.7035	0.1124	0	0	0.3162
MG	0.6321	0.4643	0.26973	4.0000	2.8979	0.6784	0.6492	0.0060	0.4413	0.0003	0.0117	0.5275
RJ	0.6599	0.5487	0.19568	6.5714	3.4379	0.0060	0.0055	0	0.0377	0	0	0.1294
SP	0.7072	0.5623	0.21753	7.1428	3.6488	0.0700	0.0436	0.0060	0.0006	0	0	0.0966
PR	0.6456	0.4848	0.25740	4.5714	2.8863	0	0.0024	0.3128	0.0026	0	0	0.0026
SC	0.6135	0.4603	0.29527	3.8571	2.7383	0.4800	0.0033	0.1039	0.4002	0.0703	0.0099	0.1294
RS	0.6825	0.5021	0.26817	7.1428	3.2535	0.0048	0	0	0	0	0	0.0500

Table 3. Pairwise Jost's *D* differentiation test between introduced populations from states of Paraíba (PB), Minas Gerais (MG), Rio de Janeiro (RJ), São Paulo (SP), Paraná (PR), Santa Catarina (SC) and Rio Grande do Sul (RS).

	SP	RS	PR	SC	MG	PB	RJ
SP	*	0.032374491	0.160078344	0.194865876	0.26294595	0.041451175	0.080926836
RS	0.032374491	*	0.141818858	0.143177652	0.231685042	0.05262367	0.16777656
PR	0.160078344	0.141818858	*	0.243617546	0.399831722	0.182059441	0.293166234
SC	0.194865876	0.143177652	0.243617546	*	0.406628496	0.192199951	0.334281257
MG	0.26294595	0.231685042	0.399831722	0.406628496	*	0.307043982	0.292442324
PB	0.041451175	0.05262367	0.182059441	0.192199951	0.307043982	*	0.123119557
RJ	0.080926836	0.16777656	0.293166234	0.334281257	0.292442324	0.123119557	*

Figure 1. Discriminant analysis of principal components of invasive populations of Bullfrogs in Brazilian states of Paraíba (PB), Minas Gerais (MG), Rio de Janeiro (RJ), São Paulo (SP), Paraná (PR), Santa Catarina (SC) and Rio Grande do Sul (RS).

Supplementary Tables and Figures

Table S1. Locus pairwise linkage disequilibrium test. FDR corrected P values (q) that still in linkage disequilibrium appear in bold

Pop	Locus.1	Locus.2	P.Value	S.E.	Switches	q (FDR)
SP	Rcat J11	Rcat J21	0,2691	0,036431	2718	0,545539
SP	Rcat J11	Rcat J54	0,16108	0,031976	1326	0,417684
SP	Rcat J21	Rcat J54	0,05105	0,016065	1944	0,196278
SP	Rcat J11	Rcat J8	0,07754	0,016025	6365	0,261991
SP	Rcat J21	Rcat J8	0,13392	0,019349	6697	0,382874
SP	Rcat J54	Rcat J8	0,07628	0,019217	4179	0,261905
SP	Rcat J11	Rcat J44b	0,0623	0,018637	1635	0,224079
SP	Rcat J21	Rcat J44b	0	0	2232	0
SP	Rcat J54	Rcat J44b	0	0	1156	0
SP	Rcat J8	Rcat J44b	0,42179	0,032689	3811	0,686563
SP	Rcat J11	Rcat J41	0,10887	0,018962	4988	0,32021
SP	Rcat J21	Rcat J41	0,70344	0,025306	7359	0,951277
SP	Rcat J54	Rcat J41	0,50262	0,037916	3542	0,783806
SP	Rcat J8	Rcat J41	0,18304	0,017629	10602	0,445346
SP	Rcat J44b	Rcat J41	0,37439	0,034036	3969	0,652258
SP	Rcat J11	Rcat3-2b	0,02336	0,007149	4237	0,106312
SP	Rcat J21	Rcat3-2b	0,08732	0,016225	4667	0,280407
SP	Rcat J54	Rcat3-2b	0,14187	0,024601	3140	0,395463
SP	Rcat J8	Rcat3-2b	0,38609	0,025795	7291	0,652258
SP	Rcat J44b	Rcat3-2b	0,07919	0,016581	3703	0,263573
SP	Rcat J41	Rcat3-2b	0,52871	0,02835	7741	0,812843
SC	Rcat J11	Rcat J21	0,36197	0,009749	13743	0,650946
SC	Rcat J11	Rcat J54	0,23058	0,014676	5889	0,494417
SC	Rcat J21	Rcat J54	0,17751	0,00563	16339	0,441912
SC	Rcat J11	Rcat J8	1	0	6549	1
SC	Rcat J21	Rcat J8	0,00751	0,001114	18347	0,044072
SC	Rcat J54	Rcat J8	0,72534	0,009051	8833	0,970908
SC	Rcat J11	Rcat J44b	0,68121	0,013322	6585	0,947736
SC	Rcat J21	Rcat J44b	0,03787	0,002842	18643	0,156719
SC	Rcat J54	Rcat J44b	0,72766	0,010448	8685	0,970908
SC	Rcat J8	Rcat J44b	0,56246	0,011078	9693	0,84749
SC	Rcat J11	Rcat J41	0,36488	0,011344	8390	0,650946
SC	Rcat J21	Rcat J41	1	0	19715	1
SC	Rcat J54	Rcat J41	0,79129	0,007552	8799	1

SC	Rcat J8	Rcat J41	1	0	14392	1
	Rcat					
SC	J44b	Rcat J41	1	0	11482	1
SC	Rcat J11	Rcat3-2b	1	0	4268	1
SC	Rcat J21	Rcat3-2b	0,53582	0,011074	13730	0,812843
SC	Rcat J54	Rcat3-2b	0,77065	0,011934	5794	0,999157
SC	Rcat J8	Rcat3-2b	0,10913	0,010574	6503	0,32021
	Rcat					
SC	J44b	Rcat3-2b	0,69274	0,01613	6464	0,947736
SC	Rcat J41	Rcat3-2b	0,19308	0,008153	8807	0,455624
RS	Rcat J11	Rcat J21	0,15921	0,027391	3645	0,417684
RS	Rcat J11	Rcat J54	0	0	1751	0
RS	Rcat J21	Rcat J54	0,05835	0,012757	2809	0,216091
RS	Rcat J11	Rcat J8	0,33047	0,026473	6670	0,613176
RS	Rcat J21	Rcat J8	0,01446	0,004174	8964	0,080552
RS	Rcat J54	Rcat J8	0,24181	0,023752	5399	0,510503
	Rcat					
RS	Rcat J11	J44b	0,02057	0,010911	2051	0,09972
	Rcat					
RS	Rcat J21	J44b	0,0015	0,0015	3951	0,011946
	Rcat					
RS	Rcat J54	J44b	0	0	1508	0
	Rcat					
RS	Rcat J8	J44b	0,00059	0,000416	5469	0,00572
RS	Rcat J11	Rcat J41	0,30381	0,02836	4117	0,584798
RS	Rcat J21	Rcat J41	0,68485	0,026405	5525	0,947736
RS	Rcat J54	Rcat J41	0	0	3494	0
RS	Rcat J8	Rcat J41	0,40728	0,023677	10196	0,672766
	Rcat					
RS	J44b	Rcat J41	0,01818	0,007127	3620	0,094282
RS	Rcat J11	Rcat3-2b	0,00826	0,003033	3975	0,04723
RS	Rcat J21	Rcat3-2b	0,04196	0,008902	5329	0,170129
RS	Rcat J54	Rcat3-2b	0,02558	0,009277	2809	0,114087
RS	Rcat J8	Rcat3-2b	0,12952	0,016621	10819	0,375103
	Rcat					
RS	J44b	Rcat3-2b	0,0439	0,012647	3244	0,174816
RS	Rcat J41	Rcat3-2b	0,37139	0,028426	6765	0,652258
RJ	Rcat J11	Rcat J21	0,00043	0,00043	3813	0,004359
RJ	Rcat J11	Rcat J54	0,10484	0,023067	2090	0,315937
RJ	Rcat J21	Rcat J54	0,03795	0,015302	2790	0,156719
RJ	Rcat J11	Rcat J8	0,01873	0,007879	6236	0,094927
RJ	Rcat J21	Rcat J8	0	0	5493	0
RJ	Rcat J54	Rcat J8	0	0	3547	0
	Rcat					
RJ	Rcat J11	J44b	0,06493	0,013456	5292	0,229832
	Rcat					
RJ	Rcat J21	J44b	0,00034	0,00034	5564	0,00361
	Rcat					
RJ	Rcat J54	J44b	0	0	3521	0

		Rcat				
RJ	Rcat J8	J44b	8,00E-05	0,00008	6423	0,000939
RJ	Rcat J11	Rcat J41	0	0	1874	0
RJ	Rcat J21	Rcat J41	0,00107	0,000753	2357	0,009177
RJ	Rcat J54	Rcat J41	0,3103	0,031677	1609	0,591426
RJ	Rcat J8	Rcat J41	0,02026	0,006165	3025	0,09972
	Rcat					
RJ	J44b	Rcat J41	0,41864	0,034395	3144	0,686446
RJ	Rcat J11	Rcat3-2b	0,17835	0,023024	5514	0,441912
RJ	Rcat J21	Rcat3-2b	0,00104	0,00104	7246	0,009177
RJ	Rcat J54	Rcat3-2b	0,00145	0,00142	4320	0,011946
RJ	Rcat J8	Rcat3-2b	0,0049	0,001738	8413	0,032138
	Rcat					
RJ	J44b	Rcat3-2b	0,00447	0,002015	7834	0,030206
RJ	Rcat J41	Rcat3-2b	0,0022	0,001024	3806	0,015826
PR	Rcat J11	Rcat J21	0	0	6068	0
PR	Rcat J11	Rcat J54	0,00675	0,003304	6082	0,041813
PR	Rcat J21	Rcat J54	0,0072	0,002703	6096	0,043395
PR	Rcat J11	Rcat J8	0,00207	0,001322	8659	0,015387
PR	Rcat J21	Rcat J8	0,03374	0,008805	8052	0,144693
PR	Rcat J54	Rcat J8	0,44234	0,017793	7329	0,709653
	Rcat					
PR	Rcat J11	J44b	0	0	9889	0
	Rcat					
PR	Rcat J21	J44b	0	0	12260	0
	Rcat					
PR	Rcat J54	J44b	0,00672	0,001638	10874	0,041813
	Rcat					
PR	Rcat J8	J44b	0,00012	0,00011	16127	0,001338
PR	Rcat J11	Rcat J41	0	0	7257	0
PR	Rcat J21	Rcat J41	0	0	8505	0
PR	Rcat J54	Rcat J41	0	0	7534	0
PR	Rcat J8	Rcat J41	0	0	11799	0
	Rcat					
PR	J44b	Rcat J41	0	0	9047	0
PR	Rcat J11	Rcat3-2b	0,01481	0,003838	10778	0,080552
PR	Rcat J21	Rcat3-2b	0,00276	0,001105	12424	0,019234
PR	Rcat J54	Rcat3-2b	0,00171	0,000814	9251	0,013149
PR	Rcat J8	Rcat3-2b	0,0222	0,002929	16558	0,10402
	Rcat					
PR	J44b	Rcat3-2b	0,05582	0,004145	14267	0,210981
PR	Rcat J41	Rcat3-2b	0	0	17044	0
PB	Rcat J11	Rcat J21	0,73475	0,023743	3344	0,970908
PB	Rcat J11	Rcat J54	0,25713	0,013207	6621	0,530926
PB	Rcat J21	Rcat J54	0,89227	0,007301	7816	1
PB	Rcat J11	Rcat J8	0,39803	0,019038	7675	0,667374
PB	Rcat J21	Rcat J8	0,86633	0,008316	10078	1
PB	Rcat J54	Rcat J8	0,89462	0,005146	12785	1

PB	Rcat J11	Rcat J44b	0,95192	0,008698	3586	1
PB	Rcat J21	Rcat J44b	0,56881	0,022294	5172	0,851306
PB	Rcat J54	Rcat J44b	0,045	0,005053	9032	0,176053
PB	Rcat J8	Rcat J44b	0,33101	0,014727	10129	0,613176
PB	Rcat J11	Rcat J41	0,18373	0,013418	6426	0,445346
PB	Rcat J21	Rcat J41	0,37205	0,016161	7328	0,652258
PB	Rcat J54	Rcat J41	0,66797	0,013017	7069	0,942768
PB	Rcat J8	Rcat J41	0,8373	0,007928	13948	1
PB	J44b	Rcat J41	0,81324	0,012258	7418	1
PB	Rcat J11	Rcat3-2b	0,20179	0,023909	3332	0,45608
PB	Rcat J21	Rcat3-2b	0,89218	0,012735	4768	1
PB	Rcat J54	Rcat3-2b	0,53444	0,012662	7671	0,812843
PB	Rcat J8	Rcat3-2b	0,35736	0,015874	9607	0,647897
PB	J44b	Rcat3-2b	0,21655	0,01919	5009	0,478125
PB	Rcat J41	Rcat3-2b	0,07634	0,010933	6724	0,261905
MG	Rcat J11	Rcat J21	0,09923	0,0089	8281	0,307337
MG	Rcat J11	Rcat J54	0,34858	0,020732	4636	0,637159
MG	Rcat J21	Rcat J54	0,64657	0,016151	8190	0,918376
MG	Rcat J11	Rcat J8	0,94815	0,004243	12353	1
MG	Rcat J21	Rcat J8	0,63961	0,012787	11161	0,914314
MG	Rcat J54	Rcat J8	0,7358	0,012774	8768	0,970908
MG	Rcat J11	Rcat J44b	0,46472	0,015341	7324	0,738367
MG	Rcat J21	Rcat J44b	0,18677	0,006592	14662	0,447846
MG	Rcat J54	Rcat J44b	1	0	8870	1
MG	Rcat J8	Rcat J44b	1	0	10994	1
MG	Rcat J11	Rcat J41	0,20452	0,008893	17559	0,45608
MG	Rcat J21	Rcat J41	0,08802	0,004493	27244	0,280407
MG	Rcat J54	Rcat J41	0,83421	0,005285	22371	1
MG	Rcat J8	Rcat J41	0,14678	0,005167	27277	0,404098
MG	J44b	Rcat J41	0,26311	0,006273	16653	0,538289
MG	Rcat J11	Rcat3-2b	0,76245	0,016846	4815	0,999157
MG	Rcat J21	Rcat3-2b	0,51543	0,014612	10137	0,798201
MG	Rcat J54	Rcat3-2b	0,99945	0,000332	7780	1
MG	Rcat J8	Rcat3-2b	0,6904	0,008697	15103	0,947736
MG	J44b	Rcat3-2b	1	0	7649	1
MG	Rcat J41	Rcat3-2b	0,16086	0,006951	21062	0,417684

S2. Discriminant analysis of principal components between feral (F) and captive (C) populations of invasive *Lithobates catesbeianus* from São Paulo (SP) and Rio de Janeiro (RJ).

Capítulo 2

Paths of introduction: assessing global colonization history of the most successful amphibian invader

Gabriel Jorgewich Cohen^{1,*} ; Taran Grant¹

1 Instituto de Biociências, Universidade de São Paulo. Rua do Matão, 101, Cidade Universitária, CEP 05508-090, São Paulo, SP, Brazil.

*Corresponding author. Email: gabrieljcohen2@gmail.com

Orcid: 0000-0001-9807-6297

Abstract

International socioeconomic relationships form the background that underlies the invasion histories of invasive species. Species with economic value, such as the American bullfrog (*Lithobates catesbeianus*), are more likely to become internationally distributed and, consequently, more difficult to control. We studied the relationships between global introduced Bullfrog populations using population genetics methods (analysis based on seven microsatellite nuclear loci and the mitochondrial cytochrome b locus) and historical inferences in order to investigate the connectivity between countries, invasion pathways, and native populations of origin. Three main lineages are identified, one first reported here, and analyzed in comparisons with previous findings on specialized literature. This species' capability to colonize several different countries from few starting lineages highlights the necessity to control new propagule pressure to ensure successful management programs, as high inbreed and bottleneck effects seem not to diminish its invasive success.

Keywords. Amphibia; Anura; invasion biology; globalization; population establishment

Introduction

Human actions influence natural environments and biological processes in many ways, often with unintended consequences. Political issues may represent changes not only on people interaction, but also in many biological scales. One of the most significant phenomena related to human actions that substantially increased with globalization is the invasion of alien species. Together with deforestation, this is the main reasons for native species population decreases or extinctions (Lowe, Browne, Boudjelas, & De Poorter, 2000). Invasive species control efforts are expensive and commonly ineffective (Ficetola & Scali, 2010; Hulme, 2006), which rises the necessity to understand invasion pathways to avoid the colonization of new species or the release of new propagules that might increase genetic variability and fitness of already established populations (Kolbe et al., 2004).

Sometimes, alien species are intentionally introduced for economic reasons, such as agriculture, biological control, pet trade (Chiaverano, Wright, & Holland, 2014; Ng & Lim, 2010). In these cases, human history can help identify and explain translocation events. It is also common to find information in the lay and gray literature that can help elucidate the origin and pathways of introduction. However, gathering and interpreting information from such literature around the globe can be difficult due to access limitations, language differences, and contradictory and misleading information. Population genetics can be a useful tool for reconstructing and understanding the history behind the events of invasions.

The world's most spread invasive amphibian species is the American bullfrog, *Lithobates catesbeianus*, which is native from eastern North America but currently occurs in many countries in the Americas, Europe, and Asia, where the species was introduced for frog farming and subsequently became established (Kraus, 2009). Despite recognized impacts on native populations (Both & Grant, 2012; Schloegel et al., 2010), there is a small number of eradication attempts (Adams & Pearl, 2007; Snow & Witmer, 2010), possibly as a reflection of the high cost and low rate of success of these attempts (Adams & Pearl, 2007). The lack of information on history and paths of invasion, connectivity and dispersal between populations and their genetic structure, is one of the main reasons why management strategies tend to fail (Rollins, Woolnough, Wilton, Sinclair, & Sherwin, 2009), as this kind of information is important for conservation in general (Rollins et al., 2009; Schwartz, Luikart, & Waples, 2007).

Using population genetics data from within the native range (Austin et al., 2004), some efforts were made to understand alien populations' native source and genetic structure in Europe (Ficetola, Bonin, & Miaud, 2008), western USA (Funk, Garcia, Cortina, & Hill, 2011; Kamath, Sepulveda, & Layhee, 2016) (Funk et al., 2011; Kamath et al., 2016), China (Bai, Ke, Consuegra, Liu, & Yiming, 2012), and Brazil (Prim, Padua, & Bataus, 2003; Cohen and Grant, CHAPTER 1), but none of these studies aimed to reconstruct global pathways of introduction. Our goal is to reveal the paths of invasion for the main bullfrog alien populations around the globe, combining our findings with genetic information from previous works and historical literature.

Materials and Methods

Along with all samples previously collected and analyzed for the study of populations genetics of the bullfrog in Brazil (Cohen and Grant, CHAPTER 1), 61 new bullfrogs samples from six different countries were collected between February 2015 and November 2016 (Table 1). Tissue samples from liver, muscle or skin were removed and stored in 90% ethanol at -20°C until analysis. We used the DNeasy Blood and Tissue extraction kit (Quiagen, Valencia, CA, USA) following the manufacture's guidelines for DNA extraction. Seven microsatellite loci were amplified following Austin et al. (2003), with same PCR conditions from Cohen and Grant (CHAPTER 1).

We analyzed a total of 382 specimens, which were submitted to the same data analysis procedures previously done on bullfrog population genetics study in Brazil (Cohen et al., unpublished data). Populations that shared relative small values of differentiation at Jost's D (Jost, 2008) index pairwise analysis and showed graphic overlap at the discriminant analysis of principal components (DAPC) were considered the same genetic population for further analysis. On the basis of those results, we selected several of the most variable samples from each genetic population (Table 2) and amplified a 1047pb segment of the mitochondrial cytochrome b gene (cytb) that includes the fragment used by Austin et al. (2004). We used a combination of the primers MVZ15L (Moritz, Schneider, & Wake, 1992) and cyt-bAR-H (Goebel, Donnelly, & Atz, 1999) and a thermal profile for polymerase chain reaction that consisted of 95°C for 10 min, followed by 45 cycles at 95°C for 30 s, 50°C for 40 s, and 72°C for 40 s, with a final extension step at 72°C for 5min. The reaction mix, with a total volume of 25 µl, contained 0.15 µl Go Taq G2 Flexi DNA Polymerase (Promega corporation), 2.5 µl of Go Taq flexi Buffer, 1.0 µl 2 mM dNTPs, 2.0 µl 25 mM MgCl₂, 1.0 µl of each primer (10 pM), 15.35 µl distilled deionized water, and 2.0 µl template DNA. PCR amplification products were cleaned using Agencourt AMPure XP DNA Purification and Cleanup kit (Beckman Coulter Genomics, Brea, CA, USA), and they were sequenced by a third party using fluorescent-dye labelled terminators (ABI Prism Big Dye Terminators v. 1.1 cycle sequencing kits; Applied Biosystems, Foster City, CA, USA) with an ABI 3730XL (Applied Biosystems, Foster City, CA, USA).

We used Geneious ver. 10.2.3 (Kearse et al., 2012) for sequence edition and contig formation of the cytb sequences based on the chromatograms obtained from the automated sequencer. All samples were sequenced in both directions to check for potential errors. The sequences were aligned with the MAFFT (Katoh & Standley, 2013) plugin in Geneious 10.2.3 (Biomatters) with the G-INS-I strategy. We trimmed the sequence alignment to a length of 937pb.

We used Arlequin 3.5 (Excoffier & Lischer, 2010) for most genetic analysis. Two diversity indices were calculated: H_d and π , described by Austin et al. (2004) respectively as "the relative frequencies of haplotypes in a population, without consideration of their relationships" and the "weighted mean of pairwise divergence

among haplotypes". We also calculated the pairwise differences between the introduced populations using θ_{ST} index and evaluated its significance by performing 10.000 permutations. Using the *p.adjust* function from stats package (R Core Team, 2014), we performed the Benjamini and Hochberg procedure (Hochberg & Benjamini, 1990) for false discovery rates control. Significant differentiations between populations were interpreted as cases of different native populations descendants, as it has been done before (Funk et al., 2011; Kamath et al., 2016). We used analysis of molecular variance (AMOVA) to assess the native origin of introduced populations. Information on native range is available at Austin et al. (2004), where four groups were delimited through a nested clade analysis. To perform AMOVA, we gather populations that show non-significant differentiation on θ_{ST} analysis and compared them with each of four source populations. We also performed a significance test with 10.000 permutations. The smallest value found between the four AMOVAs was considered the indication of origin for the tested population (Bai et al., 2012; Ficetola et al., 2008; Funk et al., 2011; Kamath et al., 2016).

For better understanding haplotype relationship, we performed a phylogenetic analysis under the phylogenetic parsimony optimality criteria, under which the hypothesis that minimizes the number of transformation events required the data is chosen and optimal (Kluge & Grant, 2006). We used unique haplotypes from our data and added haplotypes from Austin et al. (2004; we used all of their haplotypes, although only sequences with 925 base pairs were analyzed in their work), Ficetola et al. (2008), Bai et al. (2012), and Kamath et al. (2016) to compare the relationships among haplotypes from introduced and native populations, and between those haplotypes that have not been sampled in the native range. Tree rooting was based on the outgroup method (Farris, 1982; K. C. Nixon & Carpenter, 1993), and outgroup selection was based on a previous phylogenetic analysis (Pyron, 2014). We used *Lithobates septentrionalis* to root the tree and *L. clamitans*, *L. okaloosae*, and *L. heckscheri* as additional outgroup species (respective GenBank accession numbers: AY083273, AY083281, AY083286, AY083299). We used TNT (Goloboff, Farris, & Nixon, 2008) to perform the phylogenetic analysis. Analyses were carried out with Driven Search using new technologies (Sectorial Searches, Fusing, Ratchet, and Tree-drifting) (Goloboff, 1999; C. K. Nixon, 1999), with gaps as fifth state, and weighting all transformations equally, until the best score was reached 20 times. The strict consensus was constructed based on all most parsimonious trees (Nelson, 1979) Goodman-Bremer (GB; Bremer, 1988) values were calculated in TNT by performing TBR in the most parsimonious trees and holding trees until 50 steps longer than the optimum.

Results

Microsatellite analyses: All sampled populations have a recent history of introduction along with a small number of founders. Although analyses using Microchecker (Van Oosterhout, Hutchinson, Wills, & Shipley, 2004) indicated the presence of null alleles

for all loci, and most pairwise comparisons indicated significant linkage and Hardy-Weinberg disequilibrium (Table S1, S2 and S3, respectively), this is expected for recent invaders, so employed all microsatellite loci in our analyses (see also Cohen and Grant, CHAPTER 1). Calculation of pairwise Jost's D index values (Table 3) revealed greater differentiation between Asian countries and all others, with small distances among each other. Cuba also differed greatly from all the other countries, but distances between all South American countries are short. DAPC presented almost entire overlap between all South American countries and a marginal link between them and Cuba, which partially overlapped with Singapore. Japan also had a marginal overlap with the Singapore population (Fig. 1). We identified four major groups on the basis of DAPC, but we chose to treat the Brazilian Minas Gerais (MG) samples as a separate population in further analysis (see also Cohen and Grant, CHAPTER 1).

Cytochrome b analyses: We identified three cytb haplotypes among all sampled populations. South America includes two haplotypes, whereas each of the other populations has only one (Table 4). The population differentiation test was significant following the Benjamini and Hochberg procedure for all comparisons except MG–Cuba and Singapore–Japan (Table 5). These results suggest that each of these pairs of populations have the same native origin and, for this reason, they were pooled for the AMOVA. AMOVA results showed similar covariance results between native range areas (Cuba + MG: 26.01 - 34.22; Singapore + Japan: 30.24 – 39.00; South America: 40.09 – 47.26; Table 6). Our phylogenetic analysis resulted in 149 most parsimonious trees of 263 steps. The strict consensus is shown in Figure 2. Goodman-Bremer values ranged from 1–18.

Discussion

Several invasive species are known to have populations with low genetic diversity (Bai et al., 2012; Rollins et al., 2009; Santos, Jamieson, Santos, & Nakagawa, 2013). Besides cases where individuals from multiple native sources are put together (Kolbe et al., 2004), low genetic diversity and increased rates of inbreeding are expected due to sequential bottleneck effect (Estoup et al., 2011). Countries that have been colonized by the American Bullfrog not always received them directly from native sources, resulting in populations with poor genetical diversity, which turns to be little informative. Although it can make questions such as the source of the population hard to answer, it is possible to track their introduction pathway within invaded locations by genetic similarity. The association between genetic data and information from specific literature along with historical events can be very helpful on understanding introduction pathways.

Although microsatellite analysis has shown some gradient of differentiation among most sampled populations, a more conservative locus was not so sensible. There is a consensus between markers that most areas of South America belong to one population, except for the samples collected at Minas Gerais, in Brazil. Even though

the DAPC showed total overlap between MG and all other South American sites, we interpreted it as a scale issue and treated them as separated populations based on previous studies (Cohen et al., unpublished data. We also performed a DAPC that does not include countries from Southeast Asia to verify this effect (S4), and we found a much smaller degree of overlap between MG and other South American samples). Mitochondrial locus sequencing provided information about the haplotypes present in each area and, together with θ_{ST} differentiation index, we were able to indicate which areas have the same source population. We identified only three haplotypes among all sampled populations. None of them have been sampled at native range by Austin et al. (2004). This analysis confirmed that samples from MG differ from most South American samples. MG has only one haplotype, which is also the only one found in Cuba. This is the same haplotype found by Kamath et al. (2016) in Grand Teton National Park (GenBank Accession number KX344492), which is also present in lower frequency in the rest of South America. The most frequently found haplotype in South America is closely related to the haplotype found by Ficetola et al. (2008) in Belgium and Greece (GenBank Accession number EF221759), with only three different base pairs. This is the first report for this haplotype. The only haplotype present in Japan and Singapore is the same found by Bai et al. (2012) in China (GenBank Accession number JQ241268).

In South America, 10 countries are known to possess invasive bullfrog populations (Akmentins & Cardozo, 2009; Urbina-cardona & Nori, 2011), but no evidence of persistent invasive populations exists in Chile and Paraguay (F. Bauer, personal communication). Brazil was the first country to be invaded, with two legal importations reported. The first happened in 1935, while Brazil was facing a turbulent political period known as the “1930s revolution”. There was a pressure from some segments of society for the diversification of the economy (Fonseca, 2012). The government started a breeding program with 300 couples introduced by a Canadian technician named Tom Cyril Harrison (Ferreira, Pimenta, & Neto, 2002). Apart from speculation about the Canadian origin of these animals, only Rueda-Almonacid (1999) reported the United States as the native source population, although they did not state the basis for that assertion. As this work has been cited previously due to mistakes made (Measey et al., 2017), and there is no source of this information at the work, we prefer to disregard this information. The second importation was in the mid-1980s (Kraus, 2009) with a smaller number of founders, that produced no offspring (C. F. Maris, personal communication). All other countries from South America appear to have received founders exclusively from Brazil (G. Laufer, personal communication; Gallardo, 2004; Kraus, 2009; Pereyra, Baldo, & Krauczuc, 2006) between the 1980s and 1990s (Table 7) as part of a general effort to improve the economy after the “lost decade” of the 1980s (Ghirardi, López, Scarabotti, Steciow, & Perotti, 2011; Laufer, Canavero, Núñez, & Maneyro, 2008). This information is consistent with our results.

Bullfrog breeders in Brazil customarily purchase breeding frogs from different states to enhance genetic diversity (Cohen and Grant, CHAPTER 1). Taking this into consideration, along with the introduction history, it is not expected specimens from

one location to show as much differences as the MG samples compared to the rest of the continent. The similarity with the Cuban population raise the hypothesis that a more recently introduction event happened in Brazil, with individuals coming from Cuba or its same source population at native range. The small proportion of this haplotype in Brazil indicates that this is a recent introduction, as we expect a high degree of gene flow between populations due to agricultural practices.

Cuba was one of the first countries in the world where the Bullfrog was introduced. The first known introduction happened in 1915-1916 (Kraus, 2009; Santos-barrera et al., 2009), with other two events of introduction in 1927 and 1946. All animals came from the USA (Borroto-Páez, Bosch, Fabres, & Osmany, 2015), as Cuba was its protectorate since the treaty of Paris. Although none information about the native origin of these introductions are given (besides brief and weak conjectures based on morphotype variation by Hoffman & Noble, 1927), it is expected that multiple events of introduction would increase genetic variation at Cuban population; besides on the case that all founders belong to the same source. Only one haplotype was found in Cuba, what can indicate a single source of origin or a great loss of variability due to genetic drift since the last introduction event in 1946.

After the Cuban revolution, Cuba started to relate with the communist countries during the cold war and suffered the USA embargo. At the same period, the political split between the URSS and China due to ideological differences on Marxism-Leninism interpretation caused an increase of tension and rupture between Cuba and China from 1959 to 1966. Literature mentions two events of bullfrog exportation from Cuba to China (Fig. 3), both during this period (Li & Xie, 2002; Bai et al., 2012; Liu & Li, 2009; Xuan, Yiming, & Mcgarrrity, 2010). At the same time, China's economic isolation caused the necessity to reconnect with Japan. At late 1950s, China received some bullfrogs from Japan, but these animals died without producing offspring (Li & Xie, 2002; Bai et al., 2012; Liu & Li, 2009; Xuan, Yiming, & Mcgarrrity, 2010).

Given the close ties between Cuba and China, one might expect that Chinese and Cuban bullfrog populations to exhibit same haplotypes that differ from those in Japan, since the only documented introduction from Japan to China were reported to have failed. However, haplotypes found in all sampled Asian countries and Cuba differ only by three base pairs, which both supports a kinship hypothesis and contradicts the first hypothesis of consecutive spread. Japan's history of introduction is complex and not well documented, with a few different events starting in 1907 with the importation of a few breeding pairs from New Orleans, USA by the biologist Shozaburo Watase (Takeshi Igawa, personal communication) after being an exchange student in USA. New events occurred in 1918 (Hirai, 2004; Maeda & Matsui, 1999), 1920s (Minowa, Senga, & Miyashita, 2008), 1952 (Goris, 1967; Kraus, 2009; Ota, 2002), 1953, 1954 and late 1950s (Kraus, 2009; Ota, 1983) from undocumented sources. In 1924 and 1951 the bullfrog was introduced into Taiwan coming from Japan, during the period of the treaty of Shimonoseki, in which Taiwan turned to a Japanese dependency, after the first Sino-Japanese war. Currently, Taiwan is one of the greatest bullfrog producers and

exporters of the world, having been the source of many introductions in Southeast Asia. Some of these introductions happened in Singapore (from the 1980s to present) (Ng & Lim, 2010); Indonesia (1984) (Indo Prima Bullfrog's owner personal communication); and Malaysia (Hardouin, 1997), which was one of the sources for the Cambodian population (Neang, 2010).

Since all countries of Asia seem to have Japan as their main source population and genetic data corroborates this information, it is possible that prior literature is mistaken about the Cuban source of Chinese population, or have a lack of information about other events of introduction. Several different situations are likely to have happened to cause this genetic homogeneity in Southeast Asia. The most parsimonious hypothesis is that other translocations happened from Japanese lineage to China. There are few reports that it may have happened at different periods. One during the Japanese invasion of China at the second Sino-Japanese war in between 1937 and 1945 (Takeshi Igawa, personal communication), and another in 1959 with three other events of exportation from Japan to China (Yang Yi, personal communication). These events might have taken a greater number of founders of the Asian haplotype to China, which caused the loss of Cuban haplotype. Another possibility is that the Asian haplotype was also present in Cuba, being introduced from the same native source as Japan at one of the many events of introduction. It may have colonized China alone by a bottleneck effect, later disappearing from Cuba. This hypothesis might seem less probable, but the low number of divergent sites between these haplotypes and the shared positioning on the Western clade of the phylogenetic tree indicates, at least, similar native origin between haplotypes.

Although only Western lineage (Austin et al., 2004) was recovered as a distinct and monophyletic clade at our analysis, we can diagnose this as a missing data impact caused by samples with only 408 base pairs. Consensus trees made with the same methodology but without 408 bp fragments, or with all sequences trimmed at 410 bp, recover both Western and Eastern lineages described by Austin et al. (2004) (S5 and S6 respectively). Based on these analyzes, it is possible to conclude that Cuban and Southeast Asian haplotypes might share native distribution, which is apart from South American's haplotype in Eastern lineage. However, the native origin of these haplotypes cannot be accurately found. AMOVAs results were quite inconclusive, since none of these haplotypes were found by Austin et al. (2004) at native range. Finer sampling with more loci sequencing is needed to elucidate the native population structure (Bai et al., 2012; Kamath et al., 2016), including all haplotypes that have not been sampled before. This way, it will be possible to better understand the origin and the paths of the introduced populations of the world, as well as the invasion processes, so they can be mitigated on future events.

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Tables and Figures

Table 1. Samples from invasive populations. Brazilian samples are the same found in Cohen et al (not published) and they are not included in this table.

n	Local	Country
2	Punta de la Sierra, arroyo	Cuba
1	Soroa	Cuba
1	Camino al Cerrado	Cuba
2	El Moncada, Viñales	Cuba
10	Acegua	Uruguay
1	bizcocho	Uruguay
6	San Carlos	Uruguay
5	San Juan	Argentina
7	Río frio	Colombia
2	Buga	Colombia
20	Hiroshima	Japan
4	Kent Ridge	Singapore

Table 2. Selected samples for cytochrome b mitochondrial locus sequencing.

n	Local	Population
2	Punta de la Sierra, arroyo	Cuba
1	Soroa	Cuba
1	Camino al Cerrado	Cuba
2	El Moncada, Viñales	Cuba
8	Alfenas	MG
1	Derrubadas, RS	South America
1	Quatro Barras, PR	South America
1	São Luiz Paraitinga, SP	South America
3	Jaboticabal, SP	South America
1	Magé, RJ	South America
1	Guapimirim, RJ	South America
1	Francisco Beltrão, PR	South America
1	Faxinal Soturno, RS	South America
4	Kent Ridge	Singapore
20	Hiroshima	Japan

Table 3. Jost's D pairwise distance matrix of the Brazilian states of Paraíba (PB), Minas Gerais (MG), Rio de Janeiro (RJ), São Paulo (SP), Paraná (PR), Santa Catarina (SC) and Rio Grande do Sul (RS); Uruguay (URU); Singapore (SIN); Japan (JAP); Colombia (COL); Cuba (CUB); and Argentina (ARG).

	SP	MG	RJ	URU	SIN	JAP	RS	PR	SC	COL	CUB	ARG	PB
SP	*												
MG	0.262530406	*											
RJ	0.082334826	0.292442324	*										
URU	0.263438128	0.551795715	0.364352382	*									
SIN	0.734781939	0.782901971	0.675762675	0.856617571	*								
JAP	0.750421428	0.810859488	0.696864264	0.880514588	0.464612267	*							
RS	0.031987913	0.231685042	0.16777656	0.333430556	0.77851856	0.767929197	*						
PR	0.160255958	0.399831722	0.293166234	0.338863003	0.799553083	0.784841963	0.141818858	*					
SC	0.190952254	0.406628496	0.334281257	0.489108938	0.8222549	0.81116098	0.143177652	0.243617546	*				
COL	0.094647248	0.236576779	0.123331572	0.320804617	0.806128955	0.88203627	0.118148509	0.260456908	0.366865468	*			
CUB	0.635673184	0.673670865	0.606060561	0.819937754	0.647385154	0.638041706	0.675342727	0.716391645	0.734337541	0.781950836	*		
ARG	0.121029571	0.372750773	0.203147994	0.34705564	0.645999778	0.675583311	0.151438675	0.174390613	0.396081144	0.122903818	0.65294878	*	
PB	0.041544913	0.307043982	0.123119557	0.319774226	0.722758444	0.734230062	0.05262367	0.182059441	0.192199951	0.084488728	0.669117305	0.087623398	*

Table 4. Population genetic diversity indices (haplotype diversity (Hd), and nucleotide diversity (π) of the cytochrome b locus within introduced Bullfrog populations.

Population	Hd (SD)	π (SD)
Cuba	0.000 (0.000)	0.000 (0.000)
Minas Gerais	0.000 (0.000)	0.000 (0.000)
South America	0.3556 (0.159)	0.005324 (0.003)
Singapore	0.000 (0.000)	0.000 (0.000)
Japan	0.000 (0.000)	0.000 (0.000)

Table 5. Pairwise ϕ_{ST} (lower triangle) and associated P values (upper triangle) between introduced populations. Significant P values after FDR corrections appears in bold.

	Cuba	MG	South Am.	Japan	Singapore
Cuba	*	0.99990	0.01015	0.00000	0.00678
Minas Gerais	0.00000	*	0.00147	0.00000	0.00363
South Am.	0.72727	0.75535	*	0.00000	0.00218
Japan	1.0	1.0	0.85240	*	0.99990
Singapore	1.0	1.0	0.69989	0.00000	*

Table 6. AMOVAs results evaluating the degree of covariance between groups of native and introduced populations. Non-significant P values and smallest values of genetic covariance between populations appears in bold.

Non-native groups		Native groups			
		Northeast	East	Overlap	West
Cuba + MG	Percentage of covariation	34.22	32.20	26.01	32.44
	P	0.02366	0.01624	0.05634	0.03426
Singapore + Japan	Percentage of covariation	39.00	36.07	30.24	38.54
	P	0.00079	0.00901	0.01634	0.00267
South America	Percentage of covariation	41.85	40.09	40.48	47.26
	P	0.47634	0.39545	0.34802	0.20624

Figure 1. Discriminant analysis of principal components of *Lithobates catesbeianus* invasive populations of the Brazilian states of Paraíba (PB), Minas Gerais (MG), Rio de Janeiro (RJ), São Paulo (SP), Paraná (PR), Santa Catarina (SC) Rio Grande do Sul (RS); and Uruguay (URU); Singapore (SIN); Japan (JAP); Colombia (COL); Cuba (CUB); and Argentina (ARG).

Table 7. Introduction history of invasive populations of the American Bullfrogs

Date	Origin	Destination	Responsible	Comment	Reference
1907	New Orleans (USA)	Japan	Shozaburo Watase		Takeshi Igawa, personal communication; Tanizu 1940
1918		Honshu (Japan)			Maeda e Matsui 1999; Hirai 2004
1920s		Japan			Matsui 1989; Minowa et al. 2008
1952		Izu islands (Japan) Ogasawara islands (Japan)			Goris 1967; Ota 2002b; Kraus 2009
1953/1954/Late 1950s		Ryukyu islands (Japan)			Toda and Yoshida 2005; Kraus 2009
1980s - today	Taiwan, Malasia	Singapore	Jurong Frog Farm		Ota 1983; Kraus 2009
1950s		South Korea		Non-successful - frogs died	Ng and Lim 2010
1959	Japan	South Korea		Many events - same source	Amaël, personal communication
Early 1970s	Japan	South Korea	Hyun Kyu Kim		Kim 1972; Park et al. 2014
1973	USA	South Korea	Jung Hwan Sup and Song Byung Ik		Amaël personal communication (1973, 12. 17. Donga); Kim 1975; Shim et al. 2005; Park et al. 2014
1937	Japan	China			Amaël personal communication (1976.3.31., Donga)
Late 1950s	Japan	China	Shanghai fisheries university	Non-successful - frogs died	Takeshi Igawa, personal communication
1959	Japan	China			Li and Xie 2002; Liu et al. 2009; Xuan et al. 2010; Bai et al. 2012
Late 1950s	Cuba	Guandong (China)	Guangdong provincial fisheries office	Non successfull - frogs died	Yang Yi, personal communication
1960s	Cuba	Guandong (China)	Guangdong provincial fisheries office	1980s-Expanded to mainland China	Li and Xie 2002; Liu et al. 2009; Xuan et al. 2010; Bai et al. 2012
1924	Japan	Taiwan			Hou 2006; Kraus 2009; Santos-Barrera 2009
1951	Japan	Taiwan			Hou 2006; Kraus 2009; Santos-Barrera 2009
1970		Indonesia			Kusrini and Alford 2006; Kraus 2009; Santos-Barrera 2009
1980s		Indonesia			Kusrini and Alford 2006
1984	Taiwan	Indonesia			Indo Prima Bullfrog personal communication
1975		Thailand			Hardouin 1997

1980		Thailand	Commonly bred in the North	Neang 2010; Pariyanonth & Daorerk, 1995; McLeod et al. 2008
1986	USA	Phillipines		Hardouin 1991
	Canada, Thailand and Malaysia	Cambodia	more than one species	Neang 2010
"long time ago"	Taiwan	Malaysia		Hardouin 1997
1915	USA	Cuba	Coronel Charles Hernández	Kraus 2009
1916/1917	USA	Cuba		Paéz et al. 2015
1927	USA	Cuba		Paéz et al. 2016
1946	USA	Cuba		Paéz et al. 2017
1935		Brazil	Tom Cyrril Harrison	Ferreira et al. 2002; Cunha and Rosilene 2009
Mid 1980s		Brazil		
1980s	Brazil	Uruguay	Many events - same source	Laufer, personal communication; Laufer 2008
1935		Argentina	Non successfull - frogs died	Ghirardi 2017
1980s	Brazil	Argentina		Pereyra et al. 2006; Arkmentins e Cardozo 2009; Nori et al. 2011; Ghirardi 2017
1990		Argentina		Pereyra et al. 2006
1986		Colombia		Kraus 2009; Santos-Barrera 2009; Urbina and Nori 2011
1985	Brazil	Ecuador		Gallardo 2004; Una and Nativa 2004; Iñiguez and Morejón 2012
Late 1990s		Ecuador		Baker 1995; Kraus 2009
1990s	Brazil	Venezuela		Kraus 2009; Santos-Barrera 2009

Figure 3. Bullfrog invasion pathways diagram based on literature information. The yellow arrow represents genetic flow described in the literature that was not corroborated in the genetic analysis. The green arrow indicates genetic flow in disagreement with the literature. The symbol of the cross indicates populations that were introduced but left no descendants.

Supplementary Tables and Figures

Table S1 Microchecker test for presence of null alleles. Four analytical corrections are available (Brookfield, 1996; Chakraborty, De Andrade, Daiger, & Budowle, 1992; Van Oosterhout et al., 2004).

Locus	Null Present	Oosterhout	Chakraborty	Brookfield 1	Brookfield 2
RcatJ11	yes	0.0892	0.0987	0.0808	0.316
RcatJ21	yes	0.1112	0.1344	0.1043	0.2892
RcatJ54	yes	0.0957	0.1097	0.0892	0.4153
RcatJ8	yes	0.1093	0.1323	0.0931	0.2703
RcatJ44b	yes	0.2592	0.3755	0.235	0.4933
RcatJ41	yes	0.257	0.3873	0.2188	0.4565
Rcat3-2b	yes	0.0745	0.0823	0.0638	0.2996

Table S2. Locus pairwise linkage disequilibrium test. FDR corrected P values (q) that still in linkage disequilibrium appear in bold

Pop	Locus.1	Locus.2	P.Value	S.E.	q (FDR)
SP	Rcat J11	Rcat J21	0.2691	0.036431	0.545539090909091
SP	Rcat J11	Rcat J54	0.16108	0.031976	0.417684186046512
SP	Rcat J21	Rcat J54	0.05105	0.016065	0.196278448275862
SP	Rcat J11	Rcat J8	0.07754	0.016025	0.261991212121212
SP	Rcat J21	Rcat J8	0.13392	0.019349	0.382873846153846
SP	Rcat J54	Rcat J8	0.07628	0.019217	0.261904923076923
SP	Rcat J11	Rcat J44b	0.0623	0.018637	0.224079032258065
SP	Rcat J21	Rcat J44b	0	0	0
SP	Rcat J54	Rcat J44b	0	0	0
SP	Rcat J8	Rcat J44b	0.42179	0.032689	0.686563284671533
SP	Rcat J11	Rcat J41	0.10887	0.018962	0.320210394736842
SP	Rcat J21	Rcat J41	0.70344	0.025306	0.951277454545455
SP	Rcat J54	Rcat J41	0.50262	0.037916	0.783806013986014
SP	Rcat J8	Rcat J41	0.18304	0.017629	0.445345543478261
SP	Rcat J44b	Rcat J41	0.37439	0.034036	0.652257578125
SP	Rcat J11	Rcat3-2b	0.02336	0.007149	0.106311836734694
SP	Rcat J21	Rcat3-2b	0.08732	0.016225	0.280406571428571
SP	Rcat J54	Rcat3-2b	0.14187	0.024601	0.395462625
SP	Rcat J8	Rcat3-2b	0.38609	0.025795	0.652258106060606
SP	Rcat J44b	Rcat3-2b	0.07919	0.016581	0.263572686567164
SP	Rcat J41	Rcat3-2b	0.52871	0.02835	0.812842585034014
SC	Rcat J11	Rcat J21	0.36197	0.009749	0.65094592
SC	Rcat J11	Rcat J54	0.23058	0.014676	0.494416730769231
SC	Rcat J21	Rcat J54	0.17751	0.00563	0.441911666666667
SC	Rcat J11	Rcat J8	1	0	1
SC	Rcat J21	Rcat J8	0.00751	0.001114	0.0440718421052632
SC	Rcat J54	Rcat J8	0.72534	0.009051	0.970907692307692

SC	Rcat J11	Rcat J44b	0.68121	0.013322	0.947736319018405
SC	Rcat J21	Rcat J44b	0.03787	0.002842	0.1567194444444444
SC	Rcat J54	Rcat J44b	0.72766	0.010448	0.970907692307692
SC	Rcat J8	Rcat J44b	0.56246	0.011078	0.847490405405405
SC	Rcat J11	Rcat J41	0.36488	0.011344	0.65094592
SC	Rcat J21	Rcat J41	1	0	1
SC	Rcat J54	Rcat J41	0.79129	0.007552	1
SC	Rcat J8	Rcat J41	1	0	1
SC	Rcat J44b	Rcat J41	1	0	1
SC	Rcat J11	Rcat3-2b	1	0	1
SC	Rcat J21	Rcat3-2b	0.53582	0.011074	0.812842585034014
SC	Rcat J54	Rcat3-2b	0.77065	0.011934	0.999156686046512
SC	Rcat J8	Rcat3-2b	0.10913	0.010574	0.320210394736842
SC	Rcat J44b	Rcat3-2b	0.69274	0.01613	0.947736319018405
SC	Rcat J41	Rcat3-2b	0.19308	0.008153	0.455624210526316
RS	Rcat J11	Rcat J21	0.15921	0.027391	0.417684186046512
RS	Rcat J11	Rcat J54	0	0	0
RS	Rcat J21	Rcat J54	0.05835	0.012757	0.216090655737705
RS	Rcat J11	Rcat J8	0.33047	0.026473	0.613176280991736
RS	Rcat J21	Rcat J8	0.01446	0.004174	0.0805519512195122
RS	Rcat J54	Rcat J8	0.24181	0.023752	0.510503271028037
RS	Rcat J11	Rcat J44b	0.02057	0.010911	0.0997197826086957
RS	Rcat J21	Rcat J44b	0.0015	0.0015	0.0119464285714286
RS	Rcat J54	Rcat J44b	0	0	0
RS	Rcat J8	Rcat J44b	0.00059	0.000416	0.0057204347826087
RS	Rcat J11	Rcat J41	0.30381	0.02836	0.584798275862069
RS	Rcat J21	Rcat J41	0.68485	0.026405	0.947736319018405
RS	Rcat J54	Rcat J41	0	0	0
RS	Rcat J8	Rcat J41	0.40728	0.023677	0.6727662222222222
RS	Rcat J44b	Rcat J41	0.01818	0.007127	0.0942823255813954
RS	Rcat J11	Rcat3-2b	0.00826	0.003033	0.0472302564102564
RS	Rcat J21	Rcat3-2b	0.04196	0.008902	0.170128727272727
RS	Rcat J54	Rcat3-2b	0.02558	0.009277	0.1140868
RS	Rcat J8	Rcat3-2b	0.12952	0.016621	0.375103376623377
RS	Rcat J44b	Rcat3-2b	0.0439	0.012647	0.174816071428571
RS	Rcat J41	Rcat3-2b	0.37139	0.028426	0.652257578125
RJ	Rcat J11	Rcat J21	0.00043	0.00043	0.00435863636363636
RJ	Rcat J11	Rcat J54	0.10484	0.023067	0.315936756756757
RJ	Rcat J21	Rcat J54	0.03795	0.015302	0.1567194444444444
RJ	Rcat J11	Rcat J8	0.01873	0.007879	0.0949270454545455
RJ	Rcat J21	Rcat J8	0	0	0
RJ	Rcat J54	Rcat J8	0	0	0
RJ	Rcat J11	Rcat J44b	0.06493	0.013456	0.229831587301587
RJ	Rcat J21	Rcat J44b	0.00034	0.00034	0.00361047619047619
RJ	Rcat J54	Rcat J44b	0	0	0
RJ	Rcat J8	Rcat J44b	0.00008	0.00008	0.000938947368421053
RJ	Rcat J11	Rcat J41	0	0	0
RJ	Rcat J21	Rcat J41	0.00107	0.000753	0.00917730769230769

RJ	Rcat J54	Rcat J41	0.3103	0.031677	0.591426495726496
RJ	Rcat J8	Rcat J41	0.02026	0.006165	0.0997197826086957
RJ	Rcat J44b	Rcat J41	0.41864	0.034395	0.686446470588235
RJ	Rcat J11	Rcat3-2b	0.17835	0.023024	0.441911666666667
RJ	Rcat J21	Rcat3-2b	0.00104	0.00104	0.00917730769230769
RJ	Rcat J54	Rcat3-2b	0.00145	0.00142	0.0119464285714286
RJ	Rcat J8	Rcat3-2b	0.0049	0.001738	0.0321382352941176
RJ	Rcat J44b	Rcat3-2b	0.00447	0.002015	0.0302063636363636
RJ	Rcat J41	Rcat3-2b	0.0022	0.001024	0.0158258064516129
PR	Rcat J11	Rcat J21	0	0	0
PR	Rcat J11	Rcat J54	0.00675	0.003304	0.0418125
PR	Rcat J21	Rcat J54	0.0072	0.002703	0.0433945945945946
PR	Rcat J11	Rcat J8	0.00207	0.001322	0.015387
PR	Rcat J21	Rcat J8	0.03374	0.008805	0.144692692307692
PR	Rcat J54	Rcat J8	0.44234	0.017793	0.709653381294964
PR	Rcat J11	Rcat J44b	0	0	0
PR	Rcat J21	Rcat J44b	0	0	0
PR	Rcat J54	Rcat J44b	0.00672	0.001638	0.0418125
PR	Rcat J8	Rcat J44b	0.00012	0.00011	0.001338
PR	Rcat J11	Rcat J41	0	0	0
PR	Rcat J21	Rcat J41	0	0	0
PR	Rcat J54	Rcat J41	0	0	0
PR	Rcat J8	Rcat J41	0	0	0
PR	Rcat J44b	Rcat J41	0	0	0
PR	Rcat J11	Rcat3-2b	0.01481	0.003838	0.0805519512195122
PR	Rcat J21	Rcat3-2b	0.00276	0.001105	0.01923375
PR	Rcat J54	Rcat3-2b	0.00171	0.000814	0.0131493103448276
PR	Rcat J8	Rcat3-2b	0.0222	0.002929	0.104020208333333
PR	Rcat J44b	Rcat3-2b	0.05582	0.004145	0.210980677966102
PR	Rcat J41	Rcat3-2b	0	0	0
PB	Rcat J11	Rcat J21	0.73475	0.023743	0.970907692307692
PB	Rcat J11	Rcat J54	0.25713	0.013207	0.530925833333333
PB	Rcat J21	Rcat J54	0.89227	0.007301	1
PB	Rcat J11	Rcat J8	0.39803	0.019038	0.667373609022556
PB	Rcat J21	Rcat J8	0.86633	0.008316	1
PB	Rcat J54	Rcat J8	0.89462	0.005146	1
PB	Rcat J11	Rcat J44b	0.95192	0.008698	1
PB	Rcat J21	Rcat J44b	0.56881	0.022294	0.851306241610738
PB	Rcat J54	Rcat J44b	0.045	0.005053	0.176052631578947
PB	Rcat J8	Rcat J44b	0.33101	0.014727	0.613176280991736
PB	Rcat J11	Rcat J41	0.18373	0.013418	0.445345543478261
PB	Rcat J21	Rcat J41	0.37205	0.016161	0.652257578125
PB	Rcat J54	Rcat J41	0.66797	0.013017	0.942767784810126
PB	Rcat J8	Rcat J41	0.8373	0.007928	1
PB	Rcat J44b	Rcat J41	0.81324	0.012258	1
PB	Rcat J11	Rcat3-2b	0.20179	0.023909	0.4560796
PB	Rcat J21	Rcat3-2b	0.89218	0.012735	1
PB	Rcat J54	Rcat3-2b	0.53444	0.012662	0.812842585034014

PB	Rcat J8	Rcat3-2b	0.35736	0.015874	0.647896585365854
PB	Rcat J44b	Rcat3-2b	0.21655	0.01919	0.478125247524752
PB	Rcat J41	Rcat3-2b	0.07634	0.010933	0.261904923076923
MG	Rcat J11	Rcat J21	0.09923	0.0089	0.307337361111111
MG	Rcat J11	Rcat J54	0.34858	0.020732	0.637158524590164
MG	Rcat J21	Rcat J54	0.64657	0.016151	0.918376496815287
MG	Rcat J11	Rcat J8	0.94815	0.004243	1
MG	Rcat J21	Rcat J8	0.63961	0.012787	0.914314294871795
MG	Rcat J54	Rcat J8	0.7358	0.012774	0.970907692307692
MG	Rcat J11	Rcat J44b	0.46472	0.015341	0.738367234042553
MG	Rcat J21	Rcat J44b	0.18677	0.006592	0.447846344086021
MG	Rcat J54	Rcat J44b	1	0	1
MG	Rcat J8	Rcat J44b	1	0	1
MG	Rcat J11	Rcat J41	0.20452	0.008893	0.4560796
MG	Rcat J21	Rcat J41	0.08802	0.004493	0.280406571428571
MG	Rcat J54	Rcat J41	0.83421	0.005285	1
MG	Rcat J8	Rcat J41	0.14678	0.005167	0.404098024691358
MG	Rcat J44b	Rcat J41	0.26311	0.006273	0.538289266055046
MG	Rcat J11	Rcat3-2b	0.76245	0.016846	0.999156686046512
MG	Rcat J21	Rcat3-2b	0.51543	0.014612	0.798200625
MG	Rcat J54	Rcat3-2b	0.99945	0.000332	1
MG	Rcat J8	Rcat3-2b	0.6904	0.008697	0.947736319018405
MG	Rcat J44b	Rcat3-2b	1	0	1
MG	Rcat J41	Rcat3-2b	0.16086	0.006951	0.417684186046512
ARG	Rcat J11	Rcat J21	1	0	1
ARG	Rcat J11	Rcat J54	NA	NA	NA
ARG	Rcat J21	Rcat J54	NA	NA	NA
ARG	Rcat J11	Rcat J8	0.31495	0.006788	0.595202118644068
ARG	Rcat J21	Rcat J8	1	0	1
ARG	Rcat J54	Rcat J8	NA	NA	NA
ARG	Rcat J11	Rcat J44b	0.3042	0.007067	0.584798275862069
ARG	Rcat J21	Rcat J44b	1	0	1
ARG	Rcat J54	Rcat J44b	NA	NA	NA
ARG	Rcat J8	Rcat J44b	0.10153	0.003859	0.310153287671233
ARG	Rcat J11	Rcat J41	1	0	1
ARG	Rcat J21	Rcat J41	1	0	1
ARG	Rcat J54	Rcat J41	NA	NA	NA
ARG	Rcat J8	Rcat J41	0.59917	0.004839	0.874695779220779
ARG	Rcat J44b	Rcat J41	0.59652	0.00479	0.874695779220779
ARG	Rcat J11	Rcat3-2b	0.20024	0.005022	0.4560796
ARG	Rcat J21	Rcat3-2b	1	0	1
ARG	Rcat J54	Rcat3-2b	NA	NA	NA
ARG	Rcat J8	Rcat3-2b	0.60248	0.005103	0.874695779220779
ARG	Rcat J44b	Rcat3-2b	0.60405	0.00528	0.874695779220779
ARG	Rcat J41	Rcat3-2b	1	0	1
COL	Rcat J11	Rcat J21	1	0	1
COL	Rcat J11	Rcat J54	1	0	1
COL	Rcat J21	Rcat J54	1	0	1

COL	Rcat J11	Rcat J8	1	0	1
COL	Rcat J21	Rcat J8	1	0	1
COL	Rcat J54	Rcat J8	NA	NA	NA
COL	Rcat J11	Rcat J44b	NA	NA	NA
COL	Rcat J21	Rcat J44b	NA	NA	NA
COL	Rcat J54	Rcat J44b	NA	NA	NA
COL	Rcat J8	Rcat J44b	NA	NA	NA
COL	Rcat J11	Rcat J41	NA	NA	NA
COL	Rcat J21	Rcat J41	NA	NA	NA
COL	Rcat J54	Rcat J41	NA	NA	NA
COL	Rcat J8	Rcat J41	NA	NA	NA
COL	Rcat J44b	Rcat J41	NA	data	NA
COL	Rcat J11	Rcat3-2b	1	0	1
COL	Rcat J21	Rcat3-2b	0.20278	0.005944	0.4560796
COL	Rcat J54	Rcat3-2b	1	0	1
COL	Rcat J8	Rcat3-2b	1	0	1
COL	Rcat J44b	Rcat3-2b	NA	NA	NA
COL	Rcat J41	Rcat3-2b	NA	NA	NA
CUB	Rcat J11	Rcat J21	1	0	1
CUB	Rcat J11	Rcat J54	0.40648	0.008803	0.6727662222222222
CUB	Rcat J21	Rcat J54	1	0	1
CUB	Rcat J11	Rcat J8	1	0	1
CUB	Rcat J21	Rcat J8	1	0	1
CUB	Rcat J54	Rcat J8	1	0	1
CUB	Rcat J11	Rcat J44b	1	0	1
CUB	Rcat J21	Rcat J44b	1	0	1
CUB	Rcat J54	Rcat J44b	1	0	1
CUB	Rcat J8	Rcat J44b	NA	NA	NA
CUB	Rcat J11	Rcat J41	NA	NA	NA
CUB	Rcat J21	Rcat J41	NA	NA	NA
CUB	Rcat J54	Rcat J41	NA	NA	NA
CUB	Rcat J8	Rcat J41	NA	NA	NA
CUB	Rcat J44b	Rcat J41	NA	NA	NA
CUB	Rcat J11	Rcat3-2b	NA	NA	NA
CUB	Rcat J21	Rcat3-2b	NA	NA	NA
CUB	Rcat J54	Rcat3-2b	NA	NA	NA
CUB	Rcat J8	Rcat3-2b	NA	NA	NA
CUB	Rcat J44b	Rcat3-2b	NA	NA	NA
CUB	Rcat J41	Rcat3-2b	NA	NA	NA
URU	Rcat J11	Rcat J21	0.61394	0.006352	0.883281419354839
URU	Rcat J11	Rcat J54	0.38324	0.010088	0.652258106060606
URU	Rcat J21	Rcat J54	0.09828	0.012329	0.3073373611111111
URU	Rcat J11	Rcat J8	0.68965	0.005035	0.947736319018405
URU	Rcat J21	Rcat J8	0.05911	0.005603	0.216090655737705
URU	Rcat J54	Rcat J8	0.24495	0.015605	0.510503271028037
URU	Rcat J11	Rcat J44b	0.46686	0.004606	0.738367234042553
URU	Rcat J21	Rcat J44b	0.60329	0.009566	0.874695779220779
URU	Rcat J54	Rcat J44b	0.27473	0.010263	0.551935045045045

URU	Rcat J8	Rcat J44b	0.76788	0.005868	0.999156686046512
URU	Rcat J11	Rcat J41	0.27943	0.007254	0.556365089285714
URU	Rcat J21	Rcat J41	0.14014	0.010176	0.395462625
URU	Rcat J54	Rcat J41	0.37747	0.021966	0.652258106060606
URU	Rcat J8	Rcat J41	0.2439	0.009715	0.510503271028037
URU	Rcat J44b	Rcat J41	0.16586	0.009669	0.425135402298851
URU	Rcat J11	Rcat3-2b	0.02814	0.002885	0.123043529411765
URU	Rcat J21	Rcat3-2b	0.22095	0.015672	0.483057352941176
URU	Rcat J54	Rcat3-2b	0.2008	0.020997	0.4560796
URU	Rcat J8	Rcat3-2b	0.29506	0.009139	0.577178771929825
URU	Rcat J44b	Rcat3-2b	0.28682	0.011848	0.566025309734513
URU	Rcat J41	Rcat3-2b	0.0167	0.003473	0.0886690476190476
SIN	Rcat J11	Rcat J21	NA	NA	NA
SIN	Rcat J11	Rcat J54	NA	data	NA
SIN	Rcat J21	Rcat J54	NA	data	NA
SIN	Rcat J11	Rcat J8	1	0	1
SIN	Rcat J21	Rcat J8	NA	NA	NA
SIN	Rcat J54	Rcat J8	NA	data	NA
SIN	Rcat J11	Rcat J44b	1	0	1
SIN	Rcat J21	Rcat J44b	0.33271	0.001952	0.613176280991736
SIN	Rcat J54	Rcat J44b	NA	data	NA
SIN	Rcat J8	Rcat J44b	0.50195	0.003364	0.783806013986014
SIN	Rcat J11	Rcat J41	NA	NA	NA
SIN	Rcat J21	Rcat J41	NA	NA	NA
SIN	Rcat J54	Rcat J41	NA	data	NA
SIN	Rcat J8	Rcat J41	NA	NA	NA
SIN	Rcat J44b	Rcat J41	NA	NA	NA
SIN	Rcat J11	Rcat3-2b	1	0	1
SIN	Rcat J21	Rcat3-2b	NA	NA	NA
SIN	Rcat J54	Rcat3-2b	NA	data	NA
SIN	Rcat J8	Rcat3-2b	1	0	1
SIN	Rcat J44b	Rcat3-2b	1	0	1
SIN	Rcat J41	Rcat3-2b	NA	NA	NA
JAP	Rcat J11	Rcat J21	NA	NA	NA
JAP	Rcat J11	Rcat J54	NA	NA	NA
JAP	Rcat J21	Rcat J54	0.42981	0.00624	0.694548043478261
JAP	Rcat J11	Rcat J8	NA	NA	NA
JAP	Rcat J21	Rcat J8	0.83448	0.003837	1
JAP	Rcat J54	Rcat J8	0.70386	0.006512	0.951277454545455
JAP	Rcat J11	Rcat J44b	NA	NA	NA
JAP	Rcat J21	Rcat J44b	0.22775	0.002661	0.493089805825243
JAP	Rcat J54	Rcat J44b	0.00071	0.000164	0.006597083333333333
JAP	Rcat J8	Rcat J44b	0.1538	0.002715	0.417684186046512
JAP	Rcat J11	Rcat J41	NA	NA	NA
JAP	Rcat J21	Rcat J41	1	0	1
JAP	Rcat J54	Rcat J41	0.15674	0.002884	0.417684186046512
JAP	Rcat J8	Rcat J41	0.08334	0.002074	0.273306176470588
JAP	Rcat J44b	Rcat J41	0.16865	0.001764	0.427374431818182

JAP	Rcat J11	Rcat3-2b	NA	NA	NA
JAP	Rcat J21	Rcat3-2b	0.02239	0.002107	0.1040202083333333
JAP	Rcat J54	Rcat3-2b	0.38428	0.007646	0.652258106060606
JAP	Rcat J8	Rcat3-2b	0.85205	0.003841	1
JAP	Rcat J44b	Rcat3-2b	0.1941	0.002984	0.455624210526316
JAP	Rcat J41	Rcat3-2b	1	0	1

Table S3. Weir and Cockerham probability tests of Hardy-Weinberg equilibrium. FDR corrected P values that shows WHE disequilibrium are shown in bold

RcatJ11					
POP	P-val	S.E.	W&C	R&H	q(FDR)
SP	0.0292	0.0044	0.1094	0.1130	0,07008
SC	0.4000	0.0142	0.1111	-0.0099	0,48
RS	0.0012	0.0010	0.0978	0.1784	0,0048
RJ	0.0020	0.0007	0.0378	0.0367	0,006
PR	0.0000	0.0000	0.4916	0.1759	0
PB	0.1977	0.0069	0.0097	0.0642	0,2636
MG	0.6219	0.0074	0.1220	0.0976	0,678436
ARG	0.1372	0.0057	0.4667	0.3000	0,2124
COL	0.0880	0.0037	-0.0870	0.1000	0,176
CUB	0.1416	0.0045	0.2857	0.1611	0,2124
URU	1.0000	0.0000	-0,1	-0.0591	1
SIN					
JAP	0.0000	0.0000	-1	-1.0000	0
RcatJ21					
POP	P-val	S.E.	W&C	R&H	q(FDR)
SP	0.0218	0.0029	0.0373	0.0296	0,0436
SC	0.0011	0.0005	-0.0370	0.0625	0,0033
RS	0.0000	0.0000	0.2912	0.2045	0
RJ	0.0023	0.0013	0.1417	0.1080	0,00552
PR	0.0006	0.0003	0.3166	0.2178	0,0024
PB	0.1587	0.0040	0.2041	0.1837	0,272057
MG	0.5156	0.0044	-0,0788	-0.0166	0,6492
ARG	0.5410	0.0045	0.2000	0.2188	0,6492
COL	0.2015	0.0045	0.4118	0.5125	0,30225
CUB	1.0000	0.0000	-0,1364	-0.1200	1
URU	0.0006	0.0005	0.5604	0.2013	0,0024
SIN					
JAP	0.6457	0.0014	-0,1484	-0.1516	0,7044
RcatJ54					
POP	P-val	S.E.	W&C	R&H	q(FDR)
SP	0.0017	0.0012	0.1580	0.2027	0,006
SC	0.0693	0.0049	0.4545	0.4972	0,10395

RS	0.0000	0.0000	0.1546	0.1714	0
RJ	0.0000	0.0000	0.2289	0.1709	0
PR	0.2346	0.0093	-0,0575	-0.0160	0,3128
PB	0.6024	0.0070	-0,0769	-0.0846	0,703527
MG	0.0020	0.0008	0.3472	0.4228	0,006
ARG	1.0000	0.0000	0.1111	0.0417	1
COL	0.6449	0.0102	-0,2	-0.1250	0,703527
CUB	0.0088	0.0011	0.3750	0.6133	0,018686
URU	0.0109	0.0034	-0,0799	-0.0357	0,018686
SIN					
JAP	0.0098	0.0009	-0,4382	-0.4079	0,018686
RcatJ8					
POP	P-val	S.E.	W&C	R&H	q(FDR)
SP	0.0001	0.0001	0.3147	0.2877	0,00065
SC	0.2771	0.0035	0.2157	0.2497	0,400256
RS	0.0000	0.0000	0.2488	0.1054	0
RJ	0.0116	0.0030	0.1933	0.1596	0,0377
PR	0.0006	0.0003	0.1561	0.4606	0,0026
PB	0.0519	0.0022	0.1779	0.3250	0,11245
MG	0.3395	0.0070	0.0857	0.1102	0,44135
ARG	0.0483	0.0022	0.6923	0.5000	0,11245
COL	0.7748	0.0032	0.0789	0.0204	0,839367
CUB	1.0000	0.0000	-0,2121	-0.0833	1
URU	0.0976	0.0030	0.3293	0.3941	0,1586
SIN	0.7591	0.0059	-0,2632	-0.2222	0,839367
JAP	0.0621	0.0027	-0,168	-0.2168	0,115329
RcatJ44b					
POP	P-val	S.E.	W&C	R&H	q(FDR)
SP	0.0000	0.0000	0.3618	0.2611	0
SC	0.0469	0.0027	0.2727	0.4420	0,07035
RS	0.0000	0.0000	0.5877	0.3983	0
RJ	0.0000	0.0000	0.2612	0.0876	0
PR	0.0000	0.0000	0.6732	0.4743	0
PB	0.0000	0.0000	0.6335	0.4896	0
MG	0.0002	0.0001	0.7217	0.5052	0,000343
ARG	0.3310	0.0107	0.2727	0.0714	0,361091
COL					
CUB	0.0749	0.0072	0.4000	0.1667	0,099867
URU	0.0000	0.0000	0.8485	0.5750	0
SIN	0.1442	0.0020	1.0000	1.3333	0,17304
JAP	1.0000	0.0000	-0,0556	-0.0569	1
RcatJ41					
POP	P-val	S.E.	W&C	R&H	q(FDR)
SP	0.0000	0.0000	0.5417	0.5641	0
SC	0.0054	0.0007	1.0000	1.1429	0,0099
RS	0.0000	0.0000	0.3633	0.2630	0
RJ	0.0000	0.0000	0.4859	0.3429	0
PR	0.0000	0.0000	0.6123	0.4938	0

PB	0.0000	0.0000	0.6260	0.4503	0
MG	0.0075	0.0005	0.6330	0.6586	0,011786
ARG	0.0481	0.0023	0.7143	0.5750	0,058789
COL					
CUB					
URU	0.2636	0.0084	-0,0543	-0.0320	0,28996
SIN	1.0000	0.0000	0.0000	-0.0278	1
JAP	0.0366	0.0023	0.5000	0.0455	0,050325
<hr/>					
Rcat3-2b					
POP	P-val	S.E.	W&C	R&H	q(FDR)
SP	0.0223	0.0040	0.0566	0.0883	0,096633
SC	0.0476	0.0032	0.3786	0.2002	0,12948
RS	0.0077	0.0017	0.1850	0.1304	0,05005
RJ	0.0498	0.0052	0.1102	0.0457	0,12948
PR	0.0002	0.0002	-0,2737	-0.2291	0,0026
PB	0.1703	0.0077	-0,1369	-0.0538	0,316271
MG	0.4464	0.0069	0.1185	0.1166	0,527564
ARG	0.3657	0.0018	0.6667	0.8000	0,47541
COL	0.2614	0.0072	0.3571	0.2338	0,424775
CUB	0.5314	0.0064	0.3750	0.3444	0,575683
URU	0.3352	0.0070	0.2734	0.1252	0,47541
SIN	0.6585	0.0038	0.0000	-0.0278	0,6585
JAP	0.1435	0.0033	-0,1538	-0.2168	0,310917

S4. Discriminant analysis of principal components of invasive populations of *Lithobates catesbeianus* from South America and Cuba

56. Parsimony consensus tree with all published haplotypes trimmed at 410 bp

Conclusão

A genética de populações é uma área explorada e consolidada, mas vem sendo aplicada à biologia da invasão há relativamente pouco tempo. Com metodologias aplicadas para esse tipo de contexto biológico, é possível alcançar grandes quantidades de informações úteis para compreender as dinâmicas que regem as invasões, e aplica-las diretamente em esforços mais eficazes para controlar populações invasoras. O presente trabalho é uma demonstração da importância do uso de ferramentas moleculares para o estudo e compreensão das dinâmicas de invasões biológicas. Encontramos resultados destoantes com aqueles levantados na literatura específica, aumentando o conhecimento sobre o tema no Brasil e no mundo.

Alguns trabalhos de genética de populações já foram aplicados a populações invasoras, incluindo algumas espécies da herpetofauna. Poucos trabalhos, porém, focaram esforços em entender a dinâmica das populações invasoras da rã-touro. O primeiro capítulo deste trabalho equivale ao primeiro estudo sobre o tema com amplitude nacional com abordagem de populações invasoras desta espécie no Brasil, e o quinto trabalho do mundo a esclarecer a estruturação genética de uma população invasora de *Lithobates catesbeianus*.

O segundo capítulo trata globalmente a dinâmica das principais frentes de invasão da rã-touro no planeta, dando um aspecto histórico e descrevendo os caminhos pelos quais a espécie seguiu até invadir dezenas de países, se tornando a espécie de anfíbio invasora mais amplamente distribuída do mundo. Esse capítulo engloba todos os trabalhos mencionados no parágrafo anterior, além de incluir novas localidades chave para responder as perguntas propostas. Novas interpretações sobre o histórico de invasão foram propostas para algumas linhagens importantes, como por exemplo para todo o sudeste asiático. Esse tipo de resultado somente reforça a importância do uso de ferramentas moleculares, além de uma amostragem ampla para compreender diversos aspectos das populações estudadas.

Os resultados aqui obtidos podem ser úteis não só para uma aplicação direta nos esforços de controle das populações, como por exemplo evitando que linhagens diferentes sejam misturadas e aumentem seu fitness através do aumento da variabilidade, mas também para entender os processos pelos quais as populações invasoras se expandem e se fixam em novas localidades. Através dessas respostas podemos aplicar políticas que minimizem as chances de novos propágulos de novas linhagem sejam introduzidos, ou até mesmo evitar que novas espécies invasoras passem pelo mesmo processo e alcancem sucesso em se fixar.

Resumo

Invasões biológicas tem um papel cada vez mais importante nas políticas ambientais, visto que espécies invasoras desempenham uma crescente influência sobre novos ambientes onde são introduzidas, podendo gerar grandes impactos naturais e financeiros. Estudos na área da biologia da invasão se fazem extremamente necessários para remediar e evitar novas introduções. Dentre as metodologias aplicadas ao estudo das invasões biológicas, a genética de populações apresenta diversas ferramentas úteis para responder perguntas relevantes nos esforços de controle de espécies invasoras. No presente trabalho usamos recursos moleculares aplicados à genética de populações da rã-touro (*Lithobates catesbeianus*), o anfíbio invasor mais disseminado no planeta. Através deste estudo foi possível compreender mais sobre a estrutura genética das populações invasoras do Brasil e do mundo, além de seu histórico de invasão e sua população nativa de origem. Entender e contextualizar as características e motivos que levam ao sucesso de uma invasão biológica é importante para esforços de combate a pragas e para evitar que outros invasores se fixem em novos ambientes. Este trabalho levantou novos conhecimentos que podem e devem ser usados em políticas de combates à invasão da rã-touro.

Palavras-chave: Amphibia; Anura; Gestão de espécies invasoras; Danos ambientais

Abstract

Biological invasions play an increasingly important role in environmental policies as invasive species represent a growing impact in new environments where they are introduced, potentially causing large natural and financial problems. Studies in the field of invasion biology are extremely necessary to remedy and prevent new introductions. Among the methodologies applied to the study of biological invasions, population genetics presents several useful tools to answer relevant questions in efforts to control invasive species. In the present work we used molecular resources applied to the genetics of populations of the American Bullfrog (*Lithobates catesbeianus*), the most widespread invasive amphibian on the planet. Through this study it was possible to understand more about the genetic structure of the invasive populations in Brazil and in the world, and its history of invasion and its native population of origin. Understanding and contextualizing the characteristics and motives that lead to the success of a biological invasion is important for pest control efforts and to prevent other invaders from focusing on new environments. This work has raised new knowledge that can and should be used in policies to combat Bullfrog invasion.

Key-words: Amphibia; Anura; Invasive species management; environmental damage