

Genetic analysis of a novel invasion of Puerto Rico by an exotic constricting snake

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Abstract The tropical island Puerto Rico is potentially vulnerable to invasion by some species of exotic snakes; however, until now no established populations had been reported. Here we report and genetically characterize the nascent invasion of Puerto Rico by an exotic constricting snake of the family Boidae (*Boa constrictor*) using mtDNA and microsatellite data. Over 150 individual *B. constrictor* have been removed from Mayagüez municipality since May 2011, and our results from the genetic analysis of 32 individuals suggest that this population was recently founded by individuals of one subspecies from a genetic lineage common to zoo and breeding collections, but that the potential propagule pool consists of two subspecies. We also suggest that anthropogenic long-distance

dispersal within the island of Puerto Rico may be occurring from the established population, with implications for further establishment across the island. This study represents the first report of the naturalization of an invasive species of boid snake in Puerto Rico and will be important in determining mitigation strategies for this invasion as well as providing a basis for comparison to other on-going studies of invasive snakes.

Keywords *Boa constrictor* · Boidae · Effective population size · Genetic diversity · Invasive species · Microsatellite

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Introduction

Recently, much attention has been given to the impact and spread of invasive snakes; and the Boa Constrictor (*Boa constrictor*), native to Central and South America, has established invasive populations on the islands of Cozumel (Vázquez-Domínguez et al. 2012) and Aruba (Quick et al. 2005), as well as in a small area of south Florida (Snow et al. 2007). Previous climate-matching exercises based on conditions in the extensive native range of this species suggest that *B. constrictor* represents a high establishment risk to large areas of the globe, including most or all of the island of Puerto Rico (Bomford et al. 2005; Reed and Rodda 2009; van Wilgen et al. 2009).

Puerto Rico is a large (8,900 km²) tropical island in the Greater Antilles. At least four exotic reptiles have established naturalized populations on the island (Mayer 2012), and many more species are encountered as waifs or seized by wildlife officers. Large constricting snakes, including Indian (*Python molurus*), Burmese (*P. bivittatus*), African (*P. sebae*), and Reticulated (*Broghammerus reticulatus*) Pythons, Dumeril's Boa (*Acrantophis dumerili*), and *B. constrictor* are regularly found as waifs or are confiscated from private homes on the island (authors, pers. obs.; Mayer 2012), but until recently none had become reproductively established in the wild. However, since May 2011, over 150 *B. constrictor* of all size classes (738–3,015 mm total length) have been removed from the wild in an expanding area of the western Puerto Rican municipality of Mayagüez. Anecdotal reports from local conservation officials, based on their discussion with parties involved in the incident, suggest that this population may have been established around 1992 by the accidental release of neonates, though boas have regularly been reported and removed only in the last few years.

Here, we provide the first report of the establishment of a large (>2.5 m total length) constricting snake in the United States outside of south Florida. We show that the invasion likely resulted from the introduction of *B. c. constrictor* from a genetic lineage common to zoo and breeder collections and not from genetically divergent individuals or multiple subspecies of *B. constrictor*. Furthermore, our findings suggest that waif individuals discovered in other parts of the island are the result of anthropogenic long-distance movement from the introduced population in Mayagüez, and not from introductions of novel genetic stock.

Materials and methods

Sample collection

Between May 2011 and March 2012, we obtained 32 samples of *B. constrictor* (625–2,785 mm snout-vent length; SVL) from an established population in Mayagüez, Puerto Rico (Online Resource 1). Sampling periods were separated in space and time during this interval. Most samples were obtained by rangers from the Departamento de Recursos Naturales y Ambientales (DRNA) and officials from the Dr. Juan A. Rivero

Zoo responding to calls from local residents who had seen *B. constrictor* from localities throughout Mayagüez. We also conducted focused sampling at different locations in Mayagüez at intervals throughout the sampling period, where the authors searched for snakes both diurnally and nocturnally in forest patches.

Since 2011, individual boas have occasionally been found in other municipalities of northern Puerto Rico, usually along the main highways (PR-2, PR-22). These animals are thought to represent waifs, as no established populations are known outside of Mayagüez. To investigate whether these waifs are genetically differentiated animals (and hence originating from possible independent introductions) or are consistent with jump dispersal from Mayagüez, we obtained samples of three waif individuals captured during March of 2012 outside of the established population. As a preliminary investigation of whether genetic diversity exists within the pet trade in Puerto Rico, we also sampled two individuals confiscated from a private home in San Juan. As it is illegal to keep these animals in captivity, it is quite challenging to obtain samples from the pet trade outside of confiscated individuals. We obtained samples from all five of these animals at a DRNA animal holding facility at Cambalache State Forest (Online Resource 2).

Genetic analysis

Our 37 samples consisted of muscle tissue from dissected animals or tail tips from live individuals. We used PCR to amplify a portion of the mitochondrial genome (1,067 base pairs of cytochrome B; *CYTB*) using primers and conditions from Burbrink et al. (2000). We purified and resolved sequencing reactions on an automated sequencer (Applied Biosystems Inc.; ABI 3730XL) at Massachusetts General Hospital DNA Core Facility, Cambridge, MA. We edited sequences manually using SEQUENCHER 5.0 and then performed multiple sequence alignment using CLUSTALW 2.1 (Larkin et al. 2007) in MESQUITE 2.75 (Maddison and Maddison 2011). To identify the subspecies represented in Puerto Rico, we downloaded all 120 available *CYTB* sequences of *B. constrictor* and one sequence of the outgroup *Corallus enydris* (= *C. hortulanus*) from GenBank (March 9, 2012). We then aligned these 121 sequences with the only two unique haplotypes found among the 37 sequences in the present study; we then estimated a Bayesian consensus

tree using MRBAYES 3.2 (Ronquist et al. 2012). The alignment consisted of 123 sequences 1,065 base pairs in length (alignment submitted to TreeBASE) and each codon was partitioned and unlinked. The best-fit models of molecular evolution for each partition (1st codon: HKY + I + G; 2nd codon: HKY + I; 3rd codon: TIM + G) were selected using the Akaike's Information Criterion implemented in Modeltest Server (Posada 2006). A six-chain MCMC was run for 10 million generations with 25 % burn-in and we examined the resultant trace files to confirm convergence of the chains. We deposited novel representative sequences in GenBank (JX026897-98).

We genotyped all individuals sampled from Mayagüez at six microsatellite loci (Online Resource 3) using primers and conditions from Booth et al. (2011) and Tzika et al. (2008). We resolved genotypes on the above equipment using GeneScanTM 500 LIZ size standard and GENEMAPPER 4.0 software (ABI) with manual verification of peak calling. To ensure consistency in allele calling, we re-genotyped from the PCR stage any locus with more than two alleles. We estimated the number of alleles (N_A), effective number of alleles (N_E), observed heterozygosity (H_O), and expected heterozygosity (H_E), using GENALEX 6.4 (Peakall and Smouse 2006). We tested for departures from Hardy–Weinberg equilibrium (HWE) using exact tests with 10,000 dememorizations, 2,500 batches, and 20,000 iterations per batch implemented in GENEPOP 4.0 (Raymond and Rousset 1995). We estimated genetic relatedness of individuals from the introduced population at Mayagüez using a maximum-likelihood estimator implemented in the program ML-RELATE (Kalinowski et al. 2006), which has the advantage of controlling for null alleles.

Finally, we estimated the genetic effective population size (N_e), which approximates the effective number of breeders (N_b) when generations overlap (Waples 2006). This was accomplished by calculating linkage (gametic) disequilibrium (LD) using a parametric method implemented in the program LDNE 1.31 (Waples 2006; Waples and Do 2008).

Results

We observed a single mtDNA haplotype among 32 individuals in the introduced population of *B. constrictor* in Mayagüez, corresponding to the northern

South American *B. constrictor* clade and the subspecific trinomial name *B. c. constrictor* (Fig. 1). This haplotype is common to 14 sequences in GenBank from European breeding collections (Fig. 1; Hynková et al. 2009) and is also found in zoo specimens (e.g. Knoxville Zoological Gardens, Knoxville, TN, USA; GenBank JX126860). Three waifs from elsewhere in Puerto Rico and one individual confiscated from a private home in San Juan shared this haplotype, while one confiscated individual was 6.7 % divergent and corresponded to the Central American *B. constrictor* clade and the trinomen *B. c. imperator* (Fig. 1).

All six microsatellite markers were polymorphic for the 32 individuals from Mayagüez (Online Resource 4); however, one locus (μ sat 20; Tzika et al. 2008) proved unreliable when repeated and was excluded from the analyses. We found a total of 20 alleles (avg. 4 per locus, allelic richness = 0.13) across the five remaining loci (range 2–6 per locus; Table 1) with no error in allele calling. Only one locus (Bci-15) did not conform to HWE (Table 1) due to heterozygosity deficiency, a situation which is common to populations subject to non-random mating and genetic drift (Loew et al. 2005). Given the low number of loci and the fact that this locus did not appear to influence the results, we include it in our analyses, which is consistent with similar studies (e.g. Vázquez-Domínguez et al. 2012). Average F_{IS} (an index of the inbreeding coefficient of individuals relative to the population) across loci was 0.15 (0.07–0.25). The number of breeders ($\approx N_e$ when generations overlap; Waples 2006) was estimated as $N_b = 15.7$ (5.2–57.9, 95 % CI), and mean pairwise relatedness (r) was 0.16 (SE 0.007; 0–0.83). Three waifs and one confiscated individual belonging to the *B. c. constrictor* mtDNA genetic lineage shared the same alleles as the individuals from Mayagüez (Online Resource 4).

Discussion

Though the use of population genetics in invasion biology has some notable shortcomings, forensic genetic questions can yield important insights into the invasion process and the origin of the invasion (Fitzpatrick et al. 2012). Our analysis of 32 *B. constrictor* from an established population in Mayagüez, three waifs from elsewhere in Puerto Rico, and two confiscated animals revealed that the individuals sampled from the

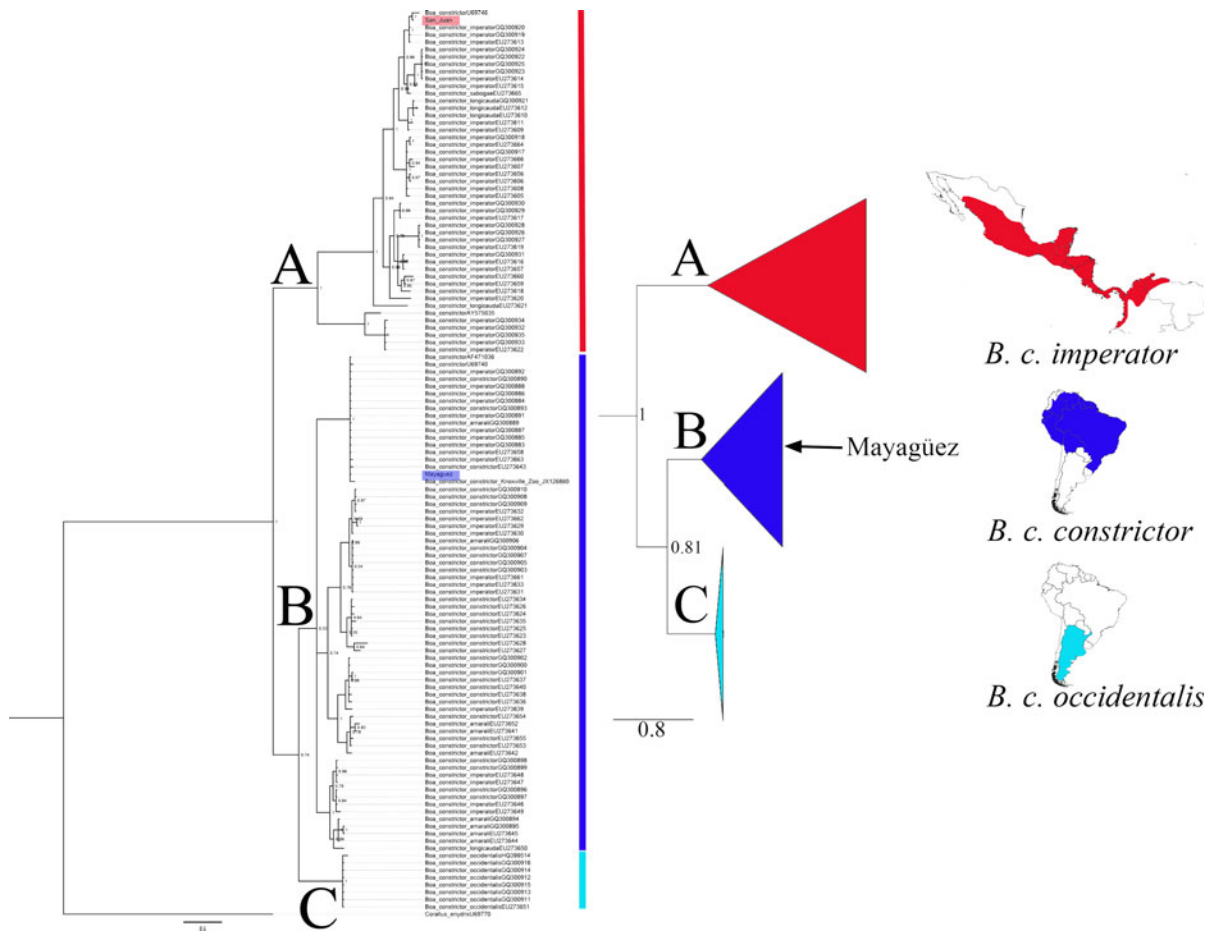


Fig. 1 Full (left) and collapsed (right) consensus *CYTB* gene tree for all available sequences on Gen Bank. One sequence of *Corallus enydris* is included as an outgroup. Three clades are recovered: **a** *B. c. imperator* from Central America, **b** *B. c. constrictor* from northern South America; and **c** *B. c. occidentalis* from Argentina. The introduced population in Mayagüez, Puerto Rico falls in the northern South American *B. c. constrictor* clade. Note that the tip names on left are drawn directly from GenBank with accession numbers provided for

reference, and that some of the tip names are probably erroneous with respect to taxonomy and geographic origin of the sample owing to ambiguous origins or admixture in captivity (see: Hynková et al. 2009). Nodal values are Bayesian posterior probabilities. Two haplotypes recovered in this study are highlighted on the left: the introduced population in Mayagüez municipality (“Mayagüez,” in blue) and the haplotype from an individual found in San Juan municipality (“San Juan,” in red). (Color figure online)

established population share a single mitochondrial haplotype from the northern South American *B. c. constrictor* clade, despite the fact that at least two subspecies constitute the potential propagule pool (Fig. 1). Microsatellite data revealed that the individuals from Mayagüez have a mean relatedness ($r = 0.16$) approaching half-sibship ($r = 0.25$). Because we obtained samples near what was likely the start of the invasion approximately 20 years ago, we expect that fewer than four generations (5-year generation time)

have passed in the invaded range and hence that little inbreeding has occurred. This expectation is corroborated by our estimate of the fixation index ($F_{IS} = 0.15$). However, it will be interesting to monitor temporal changes in genetic relatedness and genetic effective population size. Two individuals had alleles (240, 244, 248) at locus Bci-15 which were not found to associate with other alleles at this locus. This is likely an artifact of sampling and the low frequency of these alleles in the population (Online Resource 4).

Table 1 Genetic diversity at five loci for 32 individual *Boa constrictor* sampled from the established population in Mayagüez

Locus	Repeat motif	N _A	N _E	H _O	H _E	F _{IS}	HWE
Bci-14	(AAGA) _n	4	3.02	0.63	0.67	0.08	0.87
Bci-15	(TATC) _n	6	3.28	0.81	0.69	0.15	<0.001*
Bci-18	(TCCT) _n	5	2.67	0.59	0.63	0.07	1.0
Bci-21	(AG) _n	2	1.99	0.41	0.50	0.20	0.30
Bci-23	(TCTG) _n (TC) _n	3	2.14	0.41	0.53	0.25	0.14
Average		4	2.62	0.57	0.60	0.15	

N_A number of alleles, N_E effective number of alleles, H_O observed heterozygosity, H_E expected heterozygosity, F_{IS} fixation index, HWE *P* value for test of departure from Hardy–Weinberg equilibrium

* Significant at *P* ≤ 0.05

It is important to note that estimates of *N_e* based on LD assume population genetic (mutation-migration-drift) equilibrium. Under this assumption, the expected value of the correlation between alleles at different loci (*r*²) is inversely proportional to the product of the effective population size (*N_e*) and the recombination rate (*c*):

$$E(r^2) \approx \frac{1}{1 + 4N_e c}$$

between two loci (Hill and Robertson 1968; Ohta and Kimura 1969). The rate of recombination might be reduced in the case of inbreeding or genetic bottlenecks, both common in introduced populations, owing to an excess of homozygotes producing identical gametes during recombination, and yielding a reduction in the rate of decay of LD (Hedrick 2005). Importantly for biological invasions, a recent rapidly expanding population will experience little *new* LD at tightly linked loci as the influence of drift is reduced (rate of decay of LD is not slowed) and mutations occurring on the tips of gene trees have not had the opportunity to recombine. Thus, the calculation of *N_e* by this approach in non-equilibrium populations (biological invasions) can serve as a rough estimate of *N_b* in the parental generation, but should not be regarded as a precise estimate of *N_e*. Furthermore, it is important to contextualize the meaning of *N_e* in biological invasions—because estimates of *N_b* are sampling residual LD in the parental population, little relationship is expected between *N_e* and the census population size (*N_c*; actual number of individuals in the introduced population) of a rapidly expanding population. Although we measured a very small genetic effective population size in this study, over

150 individuals of this cryptic and secretive species have been found (mostly without targeted searches) in a single year, indicating that the census population size is likely quite high.

To our knowledge, very few published studies of population genetics in alien snakes exist. Serially introduced populations of dice snakes (*Natrix tessellata*) in Switzerland revealed a significant loss of allelic diversity and heterozygosity (Gautschi et al. 2002). Analysis of mtDNA and ISSR (between repeat-region) fragments in an introduced population of viperine snakes (*N. maura*) on Mallorca suggested a recent non-natural arrival to the island from France and not from Africa or the adjacent Iberian coastline (Guicking et al. 2006). The only other study characterizing genetic variation in an introduced population of boid snakes (Vázquez-Domínguez et al. 2012) found a much greater degree of genetic structure in *B. constrictor* (including two genetic populations), a higher number of alleles using the same loci targeted in this study (*N_A* = 70, avg. allelic richness = 0.16–0.29), and a larger effective population size (*N_b* = 455); as well as a much lower degree of relatedness (*r* = 0.065) among individuals on the island of Cozumel, Mexico. Also, in contrast to our study, the initial introduction in Cozumel included individuals of the subspecies *B. c. imperator* that appear to have originated from several distinct geographic areas (Vázquez-Domínguez et al. 2012).

Establishing the origin of *B. constrictor* waifs in Puerto Rico is important for designing eradication and prevention strategies, as the observation that boas are possibly being moved long distances along roads indicates the potential for rapid colonization of the rest of Puerto Rico and suggests means of conveyance

(e.g., in agricultural goods). It is important to note that as yet we have found no evidence of the introduction of additional genetic diversity from zoo specimens or the illegal pet trade, even though genetically differentiated animals are present in these industries both in Puerto Rico and elsewhere (Fig. 1). This, in addition to anecdotal evidence suggesting that the introduced population is of recent origin and our finding of low genetic diversity relative to another introduced population of *B. constrictor*, indicates that immediate awareness outreach measures might help prevent introduction of new genetic material, eliminate additional anthropogenic spread of the established population, and slow or prevent the spread of exotic boas from Mayagüez.

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