

Genetic evidence of a recent successful colonization of introduced species on islands: *Boa constrictor imperator* on Cozumel Island

Ella Vázquez-Domínguez · Marco Suárez-Atilano ·
Warren Booth · Cristopher González-Baca ·
Alfredo D. Cuarón

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Abstract Only one study has described the population genetic structure of *Boa constrictor imperator* and only in a single naturally colonized island. *B.c. imperator*, a snake with a naturally wide distribution across the Neotropics, was introduced to Cozumel Island, Mexico, in 1971. At present, it is one of the most abundant, widely distributed, terrestrial vertebrate on the island. We analysed the genetic diversity and structure, based on seven microsatellite loci, of 76 individuals of *B.c. imperator* from Cozumel. We also

included 96 samples from different localities across mainland Mexico, to review the potential origin of the founder individuals. We identified two genetically differentiated populations on Cozumel that showed moderate levels of genetic diversity ($H_o = 0.590\text{--}0.620$), with a low but significant level of genetic structure ($F_{ST} = 0.032$). Individuals were highly unrelated (71 %) and the majority of genetic variation was distributed within individuals (84 %). We detected a signal of reduction in population size and evidence of genetic bottleneck. The genetically closest mainland populations, indicating potential source of island founders, are localities from the Gulf of Mexico and Yucatan peninsula. Results are in agreement with a recently introduced population, founded by a few individuals originating from several sources, which has been successful. *B.c. imperator* is jeopardizing a highly vulnerable insular ecosystem, hence its eradication from Cozumel is urgently needed. Our information is also valuable for other introduced-boa cases or as a basis for its conservation elsewhere given its classification as a threatened species.

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E. Vázquez-Domínguez (✉) · M. Suárez-Atilano
Departamento de Ecología de la Biodiversidad, Instituto de Ecología, Universidad Nacional Autónoma de México, Ap. Postal 70-275, Ciudad Universitaria, 04510 Mexico, DF, Mexico
e-mail: evazquez@ecologia.unam.mx

W. Booth
Department of Entomology and W. M. Keck Center for Behavioral Biology, North Carolina State University, Campus Box 7613, Raleigh, NC 27695-7613, USA

C. González-Baca · A. D. Cuarón
SACBÉ – Servicios Ambientales, Conservación Biológica y Educación, Pelicanos 75, Colonia Flamingos II, 77660 Cozumel, Quintana Roo, Mexico

A. D. Cuarón
Multicriteria SC, Torre Uxmal 18, Unidad Independencia IMSS, 10100 Mexico, DF, Mexico

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Introduction

Exotic or non-native species are recognised as major threats to biodiversity (Allendorf and Luikart 2007;

Ficetola et al. 2008; Gurevitch and Padilla 2004), many of which have been responsible of species extinctions on island ecosystems (Abdelkrim et al. 2005; Sax and Gaines 2008; Vázquez-Domínguez et al. 2004). When a species is introduced to a new habitat, it acts as a new selection agent over native taxa, while it experiences different selective pressures compared to those from its original distribution (Ficetola et al. 2008; Suarez and Tsutsui 2008). There are general characteristics that increase the potential of a species to become a successful invasive, like predisposition to be transported by humans, high abundance, high dispersal capacity, among others (Gurevitch and Padilla 2004; Suarez and Tsutsui 2008). In particular, effective competitor or predator status, dietary breadth or multiple introductions are some relevant features for invasive reptiles (see Bomford et al. 2009; Kraus 2009, 2010).

Geographical or ecological islands are particularly vulnerable to species introductions, mainly because these ecosystems are characterised by high levels of endemism and high susceptibility to extinction (Berglund et al. 2009; Roemer et al. 2002; Sax et al. 2005). Many examples have been documented of the severe impact that introduced species have had on islands, like the brown tree snake, *Boiga irregularis*, introduced to Guam shortly after World War II, which caused the extinction of virtually all the native terrestrial vertebrates (Fritts and Rodda 1998; Rodda et al. 2002). Wilson et al. (2011) documented deaths due to cane toad (*Bufo marinus*) poisoning on the endemic and threatened Jamaican boa (*Epicrates subflavus*). The Norway rat (*Rattus norvegicus*) is considered one of the worst invasive species, known to have caused extinctions of many insular species (Abdelkrim et al. 2005; Lowe et al. 2001). The effect of a small number of invasive predators can have devastating consequences, for example a single feral cat (*Felis silvestris catus*) completely extirpated the endemic rodent *Peromyscus guardia*, from Angel de la Guardia Island, Baja California (Vázquez-Domínguez et al. 2004).

Boa constrictor imperator (Serpentes: Boidae) is believed to have been introduced to Cozumel Island, in the Mexican Caribbean, from a small number of individuals (6–30), which were set loose at two geographically separate locations on the island after the filming of the movie “El jardín de tía Isabel” in 1971 (Martínez-Morales and Cuarón 1999). Evidence

to support this include (Martínez-Morales and Cuarón 1999; Romero-Nájera et al. 2007): (1) in a rich and well-documented zooarchaeological study of Cozumel (Hamblin 1984) there are no records of boas, despite the importance of snakes in Maya culture; (2) prior to 1991, no records exist of boa presence on the island notwithstanding numerous collecting or herpetological expeditions during the nineteenth and twentieth centuries; (3) local inhabitants indicate the absence of boas on Cozumel before 1971; and (4) local people and key members of staff of the aforementioned movie acknowledge the liberation of boas on Cozumel after the filming on the island ended. The geographic source of the introduced individuals is however unknown (Martínez-Morales and Cuarón 1999).

The boa, naturally distributed throughout most of the Neotropics and inhabiting different vegetation types from deciduous forests to semi-deserts, is a prolific viviparous snake and a successful predator with generalist habits (Bertona and Chiaraviglio 2003). At present, *B.c. imperator* is not only amply distributed on Cozumel, but it is one of the most abundant terrestrial vertebrates on the island. With no natural enemies, *B.c. imperator* has become a key-stone generalist predator (Romero-Nájera et al. 2007), and its presence has been directly linked to the collapse of populations of diverse native species, many of them endemic to Cozumel (e.g. Cuarón et al. 2004, 2009; Fuentes-Montemayor et al. 2009; Martínez-Morales and Cuarón 1999; Martínez-Morales et al. 2009; Vega et al. 2007). Although the ecology (e.g. abundance, distribution, feeding habits) of the boa has been well studied (González-Baca 2006; Martínez-Morales and Cuarón 1999; Romero-Nájera et al. 2007), no genetic or evolutionary surveys have been performed. In the present study, our aim was to evaluate the genetic diversity and structure of *B.c. imperator* on Cozumel. If this population was derived from such a reduced number of individuals, we expected to see low genetic levels of variability, low or null genetic structure as a result of the recent introduction (40 years), and potentially signs of a recent genetic bottleneck. We also explored the possible source of the founding population. Our results support the boa is a recently introduced, non-native species that has successfully established and spread on the island. Also, our results provide relevant information to be considered in designing the management needed for the boa in order to protect the

native biota of the island. Its relevance also comes from the boa's duality in terms of conservation: it is classified as a threatened species and is legally protected in many countries, like Mexico (Semarnat 2010) but, simultaneously, it is an introduced predator threatening a highly vulnerable insular ecosystem.

Materials and methods

Population sampling and DNA extraction

Cozumel is an oceanic island of approximately 478 km², located 17.5 km off the Yucatán peninsula in the Mexican Caribbean (20°16'18.2"–20°35'32.8"N; 86°43'23.3"–87°01'31.1"W). The island was formed between the Oligocene and the Pleistocene, for which no land bridge is recognised, thus its

classification as oceanic (Weidie 1985). It is separated from the mainland by the Cozumel Channel, more than 400 m deep, with yearly predominant strong currents. The dominant vegetation type is semi-evergreen tropical forest and roughly 90 % of the island is covered with native vegetation (Cuarón 2009; Romero-Nájera et al. 2007). We analyzed a total of 76 *Boa constrictor* individuals, 49 from Romero-Nájera et al. (2007) collected in 2001–2002, and 27 samples collected during 2003, 2004 and 2008. We had 13 males, 17 females and 46 of indeterminate sex. Despite intensive and extensive fieldwork throughout the island, due to logistical restrictions and, especially, to the cryptic and secretive behaviour of *B.c. imperator*, most live or dead individuals were found on paved or dirt roads (Fig. 1). Samples are from different sources: blood or shed skin from live animals (obtained by non-invasive techniques) or bone or tail

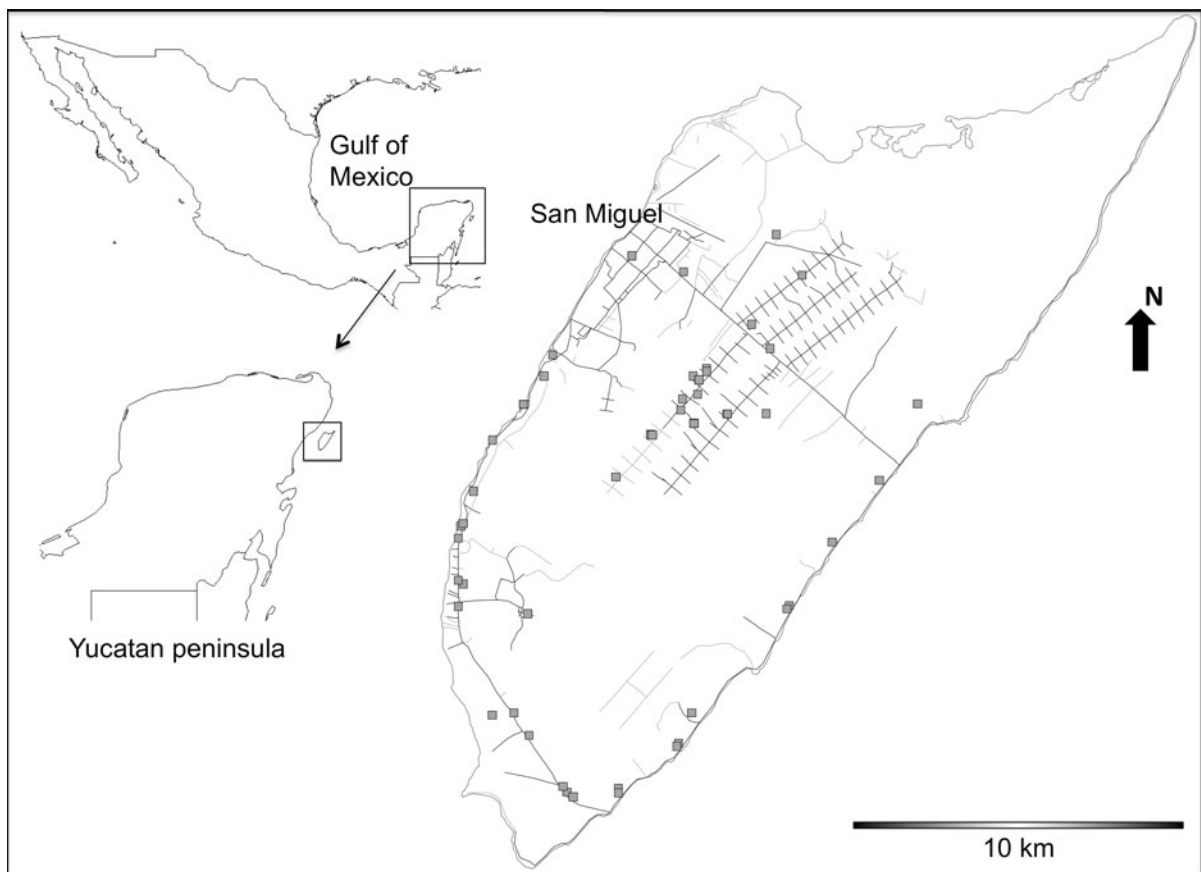


Fig. 1 Map depicting sampling localities (filled squares) for *Boa constrictor imperator* from Cozumel Island. The location of the human populated area is indicated (San Miguel); dark lines represent the main roads, while light lines are secondary, unpaved roads

tissue when found dead. Blood was taken from the caudal vein and stored on 500 μ l lysis buffer (Longmire et al. 1997), tail tissue was stored on 96 % ethanol, while bone and shed skin were dehydrated and kept refrigerated at -20 °C.

We performed DNA extraction with different commercial kits depending on the nature of the sample: Quick Gene DNA Tissue Kit and Quick Gene DNA Whole Blood Kit (Fujifilms Life Sciences) for bone or tail tissue and blood, respectively, and Dneasy Blood and Tissue Kit (Qiagen) for shed skin, following the manufacturer's instructions. We assessed DNA quantity and quality with 1 % agarose gels stained with 0.5 μ g/ml ethidium bromide and visualized with UV light.

Microsatellite typing

We tested 16 fluorescently labelled microsatellite primers, six (*Ns μ 2*, *Ns μ 3*, *Ts1*, *Ts4*, *Hb2*, *Hb30*) reported by Hille et al. (2002) as positively cross-amplifying in *B.c. imperator*; four (*μ sat1*, *μ sat20*, *μ sat24*, *μ sat36*) developed for the Jamaican yellow boa *E. subflavus* (Booth et al. 2011a; Tzika et al. 2008a, 2009); and six (*Bci-14*, *Bci-15*, *Bci-18*, *Bci-19*, *Bci-21*, *Bci-23*) designed for *B.c. imperator* (Booth et al. 2011a).

We amplified DNA in a 7.5 μ l reaction volume containing the following: approximately 25 ng template DNA, 0.5 units of Taq DNA polymerase, 1.5–2.5 mM MgCl₂, 0.2 μ M dNTPs and 0.5–1.5 μ M of each primer. Polymerase chain reaction (PCR) conditions were as follows: initial 1 min denaturation at 94 °C, followed by 35 cycles consisting of 94 °C denaturing for 30 s, annealing for 30 s at 50–60 °C and extension at 72 °C for 30 s, with a final 72 °C for 3 min, with a PTC-100 thermocycler (M. J. Research). After amplification, microsatellite products were run on an ABI Prism3730xl and 3100 Genetic Analyzer (Applied Biosystems) and allele size determined with the software GENE Mapper 4.0 (Applied Biosystems). Multiple samples were sized at least twice to assure reproducibility and correct readings.

Genetic diversity and structure

Given the wide distribution of *B.c. imperator* on Cozumel and the predominance of records along roads (Fig. 1), we could not define a priori populations;

instead, we obtained a partition of the island population defined by the data itself. We used a Bayesian model implemented in a Markov chain Monte Carlo scheme that considers genetic data and geographical coordinates, and which infers and locates genetic discontinuities between populations in space from individual multilocus genotypes (GENELAND v.3.1.4; Guillot et al. 2005, 2009; Guillot 2009). Accordingly, one can estimate the number of populations in the studied area (i.e. populations that are genetically maximally differentiated). Another advantage of this model is that it takes null alleles into account. To run GENELAND, we chose the most adequate combination of models for our data: we assumed a correlated allelic frequencies model and a true spatial model (Guillot et al. 2005), with a coordinate uncertainty value of 1.5 km. This combination is recommended for data that suggest a spatial differentiation due to landscape features and a low genetic differentiation related to a recent ecological event (Guillot et al. 2009). Considering 57 individuals for which we had geographic coordinates, we next performed 10 independent runs with 500,000 iterations, thinning = 100 and burnin = 100, using $K = 10$, $K = 5$ and $K = 3$ in each trial. Once we obtained the maximum number of possible populations (in this case $K = 2$, see “Results”), we proceeded with the assigning of individuals, using 20 independent runs with 1,000,000 iterations (thinning = 100, burnin = 1,000). We had 19 individuals with no geographical coordinates, thus in addition we did an analysis of assignment with the program STRUCTURE v.2.3.3 (Pritchard et al. 2000). We estimated the probability of assignment of each individual to the populations of origin—in this case the two populations obtained with GENELAND. We performed 100 simulations with 500,000 iterations and burnin = 10,000, using the admixture model and the correlated allele frequency.

We examined possible departures from Hardy–Weiberg equilibrium (HWE) with an exact test and calculated the unbiased P value with a Markov chain algorithm (Guo and Thompson 1992) with 10,000 dememorizations, 1,000 batches and 10,000 iterations per batch. We also evaluated linkage disequilibrium by a log-likelihood ratio statistic (G test) and estimated allelic frequencies and F_{IS} statistics to evaluate heterozygote deficit or excess (Weir and Cockerham 1984). All tests were carried out using GENEPOP v.4.0 (Raymond and Rousset 1995). A sequential Bonferroni correction for multiple comparisons (Rice 1989) was

applied where necessary. We assessed genetic variability in each population by estimating the observed (n_o) and effective number of alleles (n_e ; estimated as the reciprocal of homozygosity), observed (H_o) and expected heterozygosity (H_e) and Nei's unbiased expected heterozygosity (H_{Nei} ; Nei 1972), using the program GENALEX v.6 (Peakall and Smouse 2006). To evaluate statistical differences between populations we used Nei and Kumar's (2000) method.

To assess if populations were genetically different and estimate their degree of genetic structure, we used several approaches. We calculated F_{ST} based on Weir and Cockerham (1984) using ARLEQUIN v3.5 (Excoffier and Lischer 2010). To analyze the distribution of the genetic variance between and within populations, we used a molecular analysis of variance (AMOVA; Excoffier and Lischer 2010) based on F_{ST} . Significance was calculated using a non-parametric test with 30,000 permutations of genotypes among populations, with ARLEQUIN. Finally, we did a factorial correspondence analysis of the microsatellite data using GENETIX v.4.05 (Belkhir 2004), a test that graphically projects the individuals in the factor space defined by the similarity of their allelic states.

We evaluated the presence of null alleles with the program MICRO-CHECKER v.2.2.3 (van Oosterhout et al. 2004), which corrects the allelic and genotypic frequencies if null alleles are detected, using a 95 % confidence interval, 1,000 repetitions and the Brookfield 1 estimator. In addition, with the program FREE-NA (Chapuis and Estoup 2007) we estimated F_{ST} corrected for null alleles by means of the ENA (Excluding Null Alleles) method (F_{ST-ENA}). We also obtained a database of genotypes corrected for null alleles using the INA method (Including Null Alleles). We estimated the Cavalli-Sforza and Edwards (1967) genetic distance (D_{CS}) using the software GENECLASS v2.0 (Piry et al. 2004), together with corrected distance values (D_{CS-ENA}) calculated from the corrected database. Both F_{ST} and F_{ST-ENA} , and genetic distance values (D_{CS} and D_{CS-ENA}), were subsequently compared in order to assess whether significant differences existed with and without taking null alleles into consideration.

Population size and genetic bottlenecks

To test for a genetic signature of recent historical reduction in the effective population size, i.e. a bottleneck, we used three methods. First, we tested

for heterozygote excess under mutation-drift equilibrium with the program BOTTLENECK v5.1.26 (Cornuet and Luikart 1996; Piry et al. 1999), by comparing observed and expected heterozygosity values estimated under three mutational models: infinite allele (IAM), stepwise mutation (SMM) and two-phase (TPM) models. TPM is an intermediate model of evolution that is considered more appropriate for microsatellites; we used the default setting values (70 % SMM, 30 % IAM and 10 % variance) and also 90 % SMM, 10 % IAM and 10 % variance (Cornuet and Luikart 1996). Models were run with 10,000 replicates and excess heterozygosity was tested using a Wilcoxon test. Second, we used a graphical method to detect a mode-shift distortion in the distribution of allele frequencies (a collapsed L-shaped; Luikart and Cornuet 1998), under the premise that recently bottlenecked populations are likely to have lost more rare alleles than abundant ones. We used BOTTLENECK to obtain allele frequency distributions and to evaluate if they matched a collapsed L-shape distribution. Lastly, we calculated the Garza-Williamson index (M), an empirical value of the ratio of number of alleles to range in allele size, and the critical value (M_c), a value obtained by simulations based on the empirical data, using the program Critical_M (<http://swfsc.noaa.gov/textblock.aspx?Division=FEDandid=3298>). M values lower than the critical number are indicative of more historical (e.g. decades) population declines (Garza and Williamson 2001). To evaluate significant differences between M and M_c , we used 10,000 simulations with the following parameters, the proportion of one step mutations (p_s), the average size of non one-step mutations (Δg) and θ ($\theta = 4 Ne\mu$); the latter was obtained with ARLEQUIN.

We calculated the effective population size (Ne) by a method that estimates Ne from linkage disequilibrium (LD), using a Jackknife method with LD values between pairs of loci and a random mating system, with the program LDNE v.1.31 (Waples 2006; Waples and Do 2008). We estimated the relatedness among individuals using maximum likelihood tests (Blouin 2003) with the program ML-RELATE (Kalinowski et al. 2006), which has the advantages that it is designed for microsatellites and considers null alleles. Finally, overall relatedness for Cozumel, and for clusters CMZ1 and CMZ2 (see "Results"), were calculated using Relatedness 5.0.8 (Queller and Goodnight 1989).

Potential continental source of Cozumel's *Boa constrictor imperator*

To evaluate the potential continental source for the *B.c. imperator* founder population on Cozumel, we also obtained microsatellite genetic data for 95 individuals from 16 mainland localities in Mexico and one in Central America (Table 2). Samples were obtained from a museum collection (Museo de Zoología Alfonso Herrera, Facultad de Ciencias, UNAM) and from direct sampling. We included in the analysis a subsample from Cozumel consisting of individuals from the two populations identified in the results, and followed two approaches to explore the data: by estimating genetically maximally differentiated populations using GENELAND (as explained above) and by constructing a Neighbour-joining (NJ)

tree. For the latter we estimated different genetic distances in accordance with a SMM mutation model: ASD (Goldstein et al. 1995a), $\delta\mu^2$ (Goldstein et al. 1995b) and D_{SW} (Shriver et al. 1995). NJ trees with 1,000 replicates to estimate bootstrap values were constructed with the program POPULATIONS v.1.2.30 (Langella 2002). Finally, for comparative purposes, we estimated the number of alleles and average heterozygosity for the complete continental sample.

Results

Genetic diversity and structure

Of the 16 microsatellite loci tested, seven proved polymorphic (Table 1, Online Resource 1, Online

Table 1 Genetic diversity values for Cozumel 1 (CZM1), Cozumel 2 (CZM2) and the entire island (Cozumel) populations of *Boa constrictor imperator*

Population	Locus	n_a	n_e	n_{ex}	H_o	H_e	H_{NEI}	F_{IS}
CZM1 $N = 30$	<i>Bci-14</i>	11	7	2	0.90	0.86	0.87	0.11*
	<i>Bci-21</i>	11	6.5	4	0.70	0.85	0.86	0.18*
	msat1	6	1.91	1	0.33	0.48	0.49	0.32*
	msat36	7	5.22	2	0.87	0.81	0.82	0.01
	<i>Bci-15</i>	9	3.31	5	0.40	0.70	0.71	0.29*
	<i>Bci-18</i>	8	4.24	1	0.53	0.76	0.78	0.33*
	msat20	10	6.02	2	0.60	0.83	0.85	0.15
Average		8.86	4.89	2.43	0.62	0.76	0.77	0.20
CZM2 $N = 46$	<i>Bci-14</i>	11	4.57	2	0.63	0.78	0.79	0.11*
	<i>Bci-21</i>	10	4.63	3	0.67	0.78	0.79	0.14
	msat1	5	3.14	0	0.54	0.68	0.69	0.21
	msat36	7	4.17	2	0.59	0.76	0.77	0.11*
	<i>Bci-15</i>	4	2.07	0	0.46	0.52	0.52	0.27*
	<i>Bci-18</i>	7	4.58	0	0.50	0.78	0.79	0.32*
	msat20	9	5.03	1	0.63	0.80	0.81	0.31*
Average		7.57	3.93	1.14	0.57	0.73	0.74	0.21
Cozumel $N = 76$	<i>Bci-14</i>	13	5.53		0.74	0.82	0.82	0.11*
	<i>Bci-21</i>	14	5.43		0.68	0.82	0.82	0.17*
	msat1	6	2.61		0.46	0.62	0.62	0.26*
	msat36	9	4.94		0.70	0.80	0.80	0.13*
	<i>Bci-15</i>	9	2.50		0.43	0.60	0.60	0.28*
	<i>Bci-18</i>	8	4.52		0.51	0.78	0.78	0.35*
	msat20	11	5.62		0.62	0.82	0.83	0.25*
Average		10	4.45		0.59	0.75	0.76	0.22

n_a allelic diversity, n_e effective number of alleles, n_{ex} exclusive alleles, H_o observed heterozygosity, H_e expected heterozygosity, H_{NEI} Nei's expected heterozygosity (Nei 1972), F_{IS} fixation index

* $P < 0.05$

Resource 2). None of the six heterologous loci reported by Hille et al. (2002) amplified with our samples, despite multiple assays and diverse PCR protocol testing. We therefore advise caution using those primers for any study with *B.c. imperator*. Two populations ($K = 2$) were defined with GENELAND, based on the results of 20 runs ($[\text{Ln Pr}(K = 2) = -1124.112]$), in all of which individuals were systematically distributed in the same groups. We defined these populations as Cozumel 1 (CZM1) and Cozumel 2 (CZM2). The assignment analysis of samples with no geographic reference assigned 8 and 11 individuals to CZM1 (58–98 % probability) and CZM2 (77–96 %), respectively, for a total of 30 individuals in CZM1 and 46 in CZM2 (Fig. 2).

We found a total of 70 alleles across the seven loci, with a range of 6–14 (average 10) alleles per locus for the entire island population (Cozumel): CZM1 had 6–11 (8.9) and CZM2 had 4–11 (7.6) (Table 1). *Bci-14* and *Bci-21* were the most variable loci. Expected and observed heterozygosities in Cozumel showed intermediate values ($H_o = 0.590$, $H_e = 0.750$, and $H_{Nei} = 0.760$), while CZM1 had slightly higher ($H_o = 0.620$, $H_e = 0.760$, and $H_{Nei} = 0.770$) and CZM2 slightly lower values ($H_o = 0.570$, $H_e = 0.730$, and $H_{Nei} =$

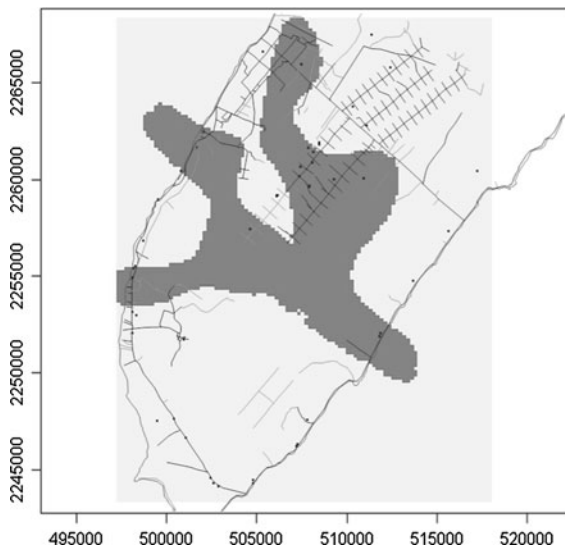


Fig. 2 Distribution of the two populations of *Boa constrictor imperator* identified with GENELAND (see “Results”). The shaded area depicts the Cozumel 1 population (CZM1), whereas the unshaded island area represents Cozumel 2 (CZM2). GENELAND does not consider physical boundaries when building distribution polygons, thus the shading out of the limits of the island

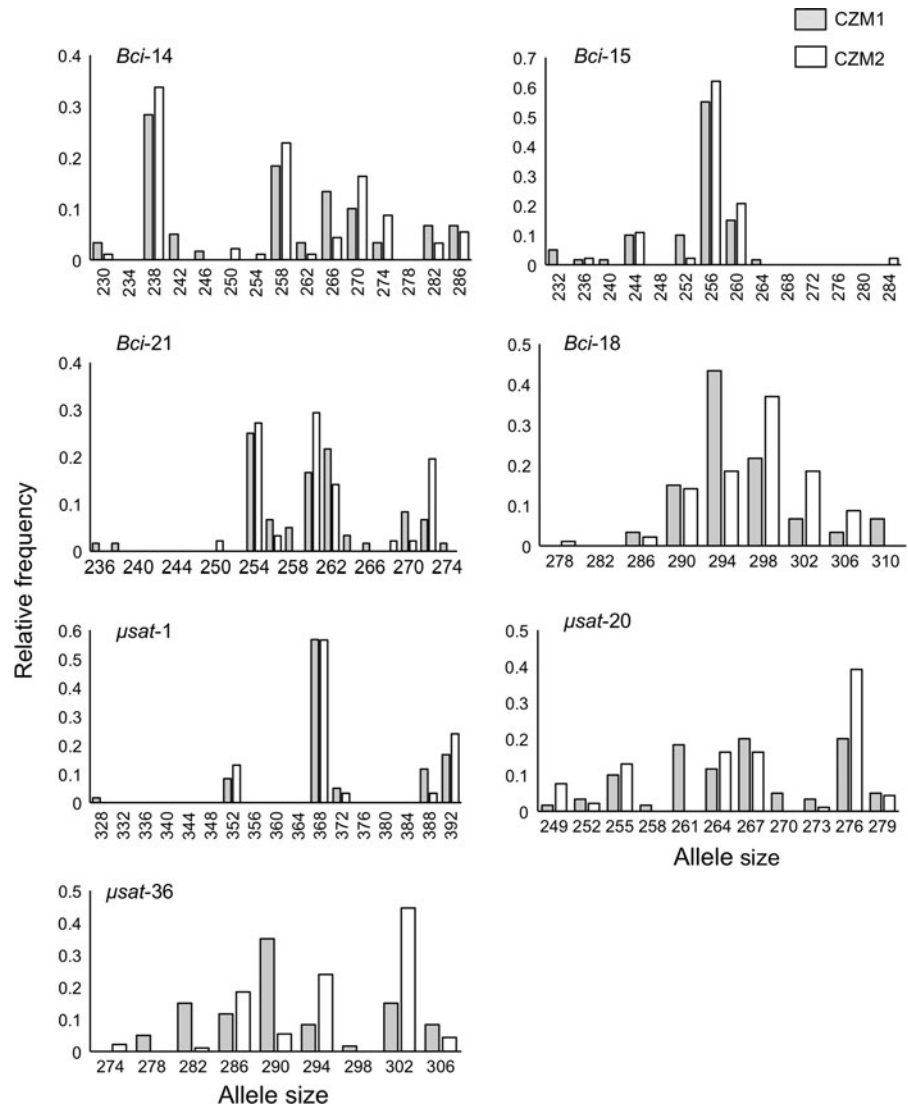
0.740) (Table 1). After Bonferroni correction, exact tests found departures from HWE in five loci in both populations due to heterozygote deficiency, but at different loci: $\mu\text{sat}36$ and $\mu\text{sat}20$ loci conformed to HW expectations in CZM1, while *Bci-21* and $\mu\text{sat}1$ did for CZM2. All seven loci were in linkage equilibrium in each population. Most loci showed exclusive alleles in each population (CZM1 = 17 and CZM2 = 8; Table 1, Fig. 3).

Results regarding genetic differentiation showed low but significant values ($F_{ST} = 0.032$; 0.005–0.066, 95 % C.I.), in agreement with the AMOVA results that revealed that the majority of total genetic variation resided within individuals (77 %; $P < 0.01$), followed by that among individuals within populations (19.7; $P < 0.01$), while the lowest was between populations (3.3; $P < 0.01$). The presence of genetic structuring was confirmed by the factorial correspondence analysis, in which genotypes form a continuous distribution along the axes but in which the two populations can be clearly distinguished (Fig. 4). Null alleles were detected only in two loci, *Bci-15* and *Bci-18*; the F_{ST-ENA} value (corrected for null alleles) was 0.028, which was not significantly different from the F_{ST} without correction ($F_{ST} = 0.032$, $t = -0.45$, g.l. = 12, $P > 0.05$). Likewise, the difference between the Cavalli-Sforza genetic distance with ($D_{CS-ENA} = 0.292$) and without ($D_{CS} = 0.281$) correction was not significant ($t = -1.76$, g.l. = 12, $P > 0.05$).

Genetic bottlenecks, population size and relatedness

Evidence of a recent genetic bottleneck associated with a heterozygote excess (BOTTLENECK results) was observed for both populations (CZM1 and CZM2) when analyzed independently and when combined, under the IAM model ($P < 0.05$). However, none showed a significant heterozygote excess with either the TPM or the SMM models. The IAM has been reported to be better able to detect subtle population bottlenecks (Luikart and Cornuet 1998). Allele frequencies followed an L-shaped distribution and no evidence of a mode shift in allele frequencies toward intermediate values was observed. The results from the Garza-Williamson test showed empirical M values (CZM1 = 0.79; CZM2 = 0.67) significantly lower than M_c for both CZM1 ($p_s = 0.1$ and 0.2 ; $\Delta g = 2.0$ and 2.5) and CZM2 ($p_s = 0.1$ and 0.2 ; $\Delta g = 2.0$, 2.5

Fig. 3 Allelic relative frequencies for each locus for *B.c. imperator* from Cozumel 1 (CZM1) and Cozumel 2 (CZM2) populations



and 3.0), indicative of a more historical bottleneck. The effective population size (N_e) estimated from linkage disequilibrium was $N_e = 255.5$ (88.5–520.3, 95 % C.I.) for CZM1, $N_e = 749$ (140.2–1,847.2) for CZM2 and $N_e = 455.1$ (156.2–874.2) for the entire island. Finally, proportion of relatedness of individuals within each population was similar: most individuals were unrelated (CZM1 = 84 % and CZM2 = 88 %), followed by half-siblings (9 and 12 %), siblings (2 and 2.5 %), and parent/offspring (0.5 and 1 %). Overall relatedness (r) on Cozumel was 0.065 (S.E. 0.029): CMZ1 = -0.011 (S.E. 0.034), CMZ2 = 0.117 (S.E. 0.034).

Potential continental source

The estimation of genetically maximally differentiated populations for the *B.c. imperator* samples from continental localities showed seven populations ($K = 7$) consistently obtained in 12 of 20 runs ([Ln Pr($K = 6$) = $-2,458.24$]). Given the continental scale of the analysis, Cozumel was selected as one population (i.e. six continental clusters and one island cluster; Table 2, Fig. 5). The Neighbour-joining trees, including the six mainland populations plus the two from Cozumel, showed a similar topology for the different genetic distance measures used: the two

Fig. 4 Three-dimensional representation of the factorial correspondence analysis based on microsatellite genotypes for *B.c. imperator*. Eigenvalues are shown along the axes. Axis 1 explains 100 % of the differences because no genotypes are shared between the two populations. Light squares and dark squares are Cozumel 1 (CZM1) and Cozumel 2 (CZM2) genotypes, respectively

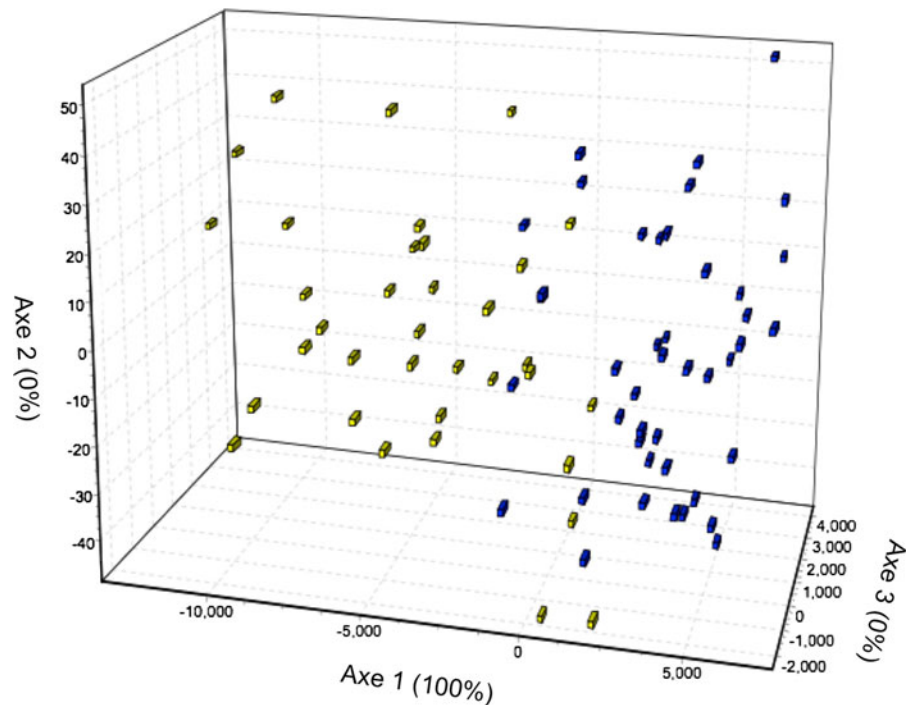


Table 2 Clusters of the genetically maximally differentiated populations (as in Fig. 5) obtained for 95 *B. constrictor imperator* individuals from 16 mainland localities from Mexico and Central America, including 42 individuals from Cozumel island

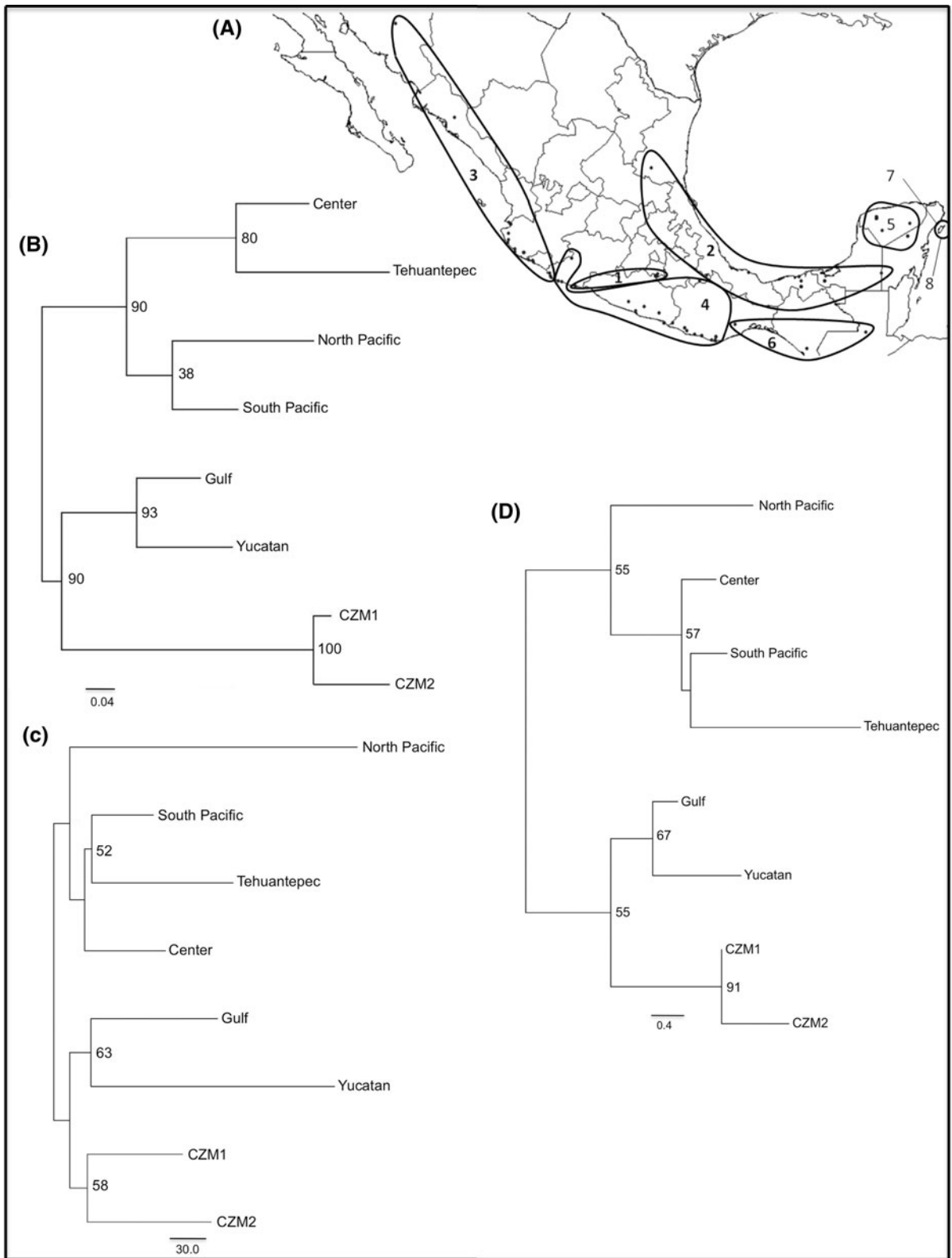
Cluster (sample size)	Locality (sample size)
1. Center (13)	Morelos (12), Michoacán (1)
2. Gulf (13)	Tamaulipas (1), Veracruz (2), Puebla (1), Tabasco (5), Campeche (4)
3. North Pacific (19)	Sonora (1), Sinaloa (1), Colima (3), Jalisco (11), Michoacán (3)
4. South Pacific (25)	Michoacán (3), Guerrero (3), Oaxaca (18), Puebla (1)
5. Yucatan peninsula (22)	Yucatán (20), Quintana Roo (2)
6. Tehuantepec (3)	Oaxaca (1), Chiapas (1), Guatemala (1)
7. Cozumel (42)	Cozumel, QR 1 (20), Cozumel, QR 2 (22)

The localities belonging to each cluster are shown

Cozumel populations are the closest genetically, which form a group with the populations from the Gulf of Mexico and the Yucatan peninsula. Comparable estimates of pairwise genetic differentiation (F_{ST}) were observed between the two potential mainland origins, and the two genetic subpopulations observed on Cozumel (data not shown). The remaining populations are more distantly related. The number of alleles per locus for the entire continental population ranged from 9 to 28 (average 16.9) and the expected and observed heterozygosity values were $H_o = 0.623$, $H_e = 0.875$ and $H_{Nei} = 0.880$.

Discussion

The geographic isolation of oceanic islands has facilitated the establishment and evolution of distinct and limited arrays of species as compared with continental systems. Also, the presence of the surrounding marine environment and physical distance from the mainland limit the number and taxa that can naturally reach and colonize islands (Reaser et al. 2007). In accordance, given the paucity of species on islands, it is believed that islands may be more susceptible to the introduction of nonindigenous taxa



◀ **Fig. 5 a** Geographical representation of the genetic clusters obtained for 79 *B.c. imperator* individuals from Mexico and Central America. Clusters (with sample size in *parenthesis*), in which Cozumel is depicted as the two populations identified genetically, are: 1 Center (13), 2 Gulf (13), 3 North Pacific (19), 4 South Pacific (25), 5 Yucatan peninsula (22), 6 Tehuantepec (3), 7 Cozumel 1 (20), 8 Cozumel 2 (22). Neighbor-joining (NJ) trees constructed using **b** *ASD* (Goldstein et al. 1995a), **c** $\delta\mu^2$ (Goldstein et al. 1995b) and **d** D_{SW} (Shriver et al. 1995) genetic distances. Bootstrap values (>50) are shown along branches

that are dominant in many mainland ecosystems (Elton 1958; Berglund et al. 2009; Oppel et al. 2011; Sax and Gaines 2008). While this hypothesis may lack universal application, a number of studies have highlighted the ability of vertebrates to successfully invade islands and establish viable breeding populations (Christy et al. 2007; Kraus 2009, 2010; Long 2003; Sax and Gaines 2008). *Boa constrictor* is no exception, having colonized at least 43 marine islands throughout their range (neotropical spanning 68° of latitude), either naturally or through human-mediated movement (Henderson et al. 1995; Porras 1999; Quick et al. 2005). Despite their widespread distribution across marine islands in the Caribbean, only a single study has described the population genetic structure of *B.c. imperator* (Green 2010), and only in a single naturally colonized island. The present work therefore represents the first genetic study of *B.c. imperator* from Mexico (and the northern Neotropics), and to our knowledge, the first using microsatellites to evaluate genetic diversity and structure of a recently colonized (<~40 years) wild population of this species. It represents a unique opportunity to investigate the rapid evolutionary processes associated with the early stages of island colonization by this species under natural conditions.

Contrary to the expectation of limited genetic diversity associated with a recent introduction of a small founding propagule to an island (Frankham et al. 2005), we observed relatively high levels of allelic diversity and moderately high heterozygosity values. While moderate to high, these were similar to values observed in some reptilian populations known to have suffered recent habitat fragmentation, population size reductions, and/or genetic bottlenecks associated with founder effects (Jansen et al. 2008; Prosser et al. 1999, 2000). The observed departure from Hardy–Weinberg equilibrium as an outcome of heterozygote deficiency is not uncommon in species with fragmented

populations (Castañeda-Rico et al. 2011; Degner et al. 2007; Spear and Storfer 2010). A significant decrease in observed heterozygosity suggests non-random mating and genetic drift (Loew et al. 2005), which would lead to allelic fixation and loss within populations, with an increase in differentiation between populations. The latter can explain the observed heterozygosity deficiency, considering that the boa population on Cozumel is originated from individuals from multiple origins—not a natural population—and that it is differentiating into two genetically different subpopulations (CMZ1 and CMZ2). Another explanation for the observed HW disequilibrium could be related to the Wahlund effect. When a group of subdivided populations with different allele frequencies is considered and analyzed as one population, the result can be an excess of homozygotes (Wahlund 1928).

Multiple introductions or the introduction of propagules originating from different geographic, and thus potentially different genetic sources, may elevate diversity values, mainly because there is a short-term increment of the effective population size, which in turn promotes the maintenance of nearly 80 % of the genetic diversity in the introduced population compared to its population of origin (Dlugosh and Parker 2008; Kaeuffer et al. 2007). While the number of introduced propagules may have been small [6–30 individuals at two geographically separate locations on the island (Martínez-Morales and Cuarón 1999)], the observed high levels of genetic diversity are likely a consequence of both genetically diverse founding propagules and the inherent characteristics and reproductive biology of this species. While genetic studies examining the reproductive biology of *B.c. imperator* are scarce (Rivera et al. 2006), we offer a number of predictions based on reproductive biology in serpents. First, the presence of gravid females during introduction and thus the production of neonates soon after release is highly plausible, considering that mating in *B.c. imperator* occurs from December to March and the Cozumel introduction occurred in April (Martínez-Morales and Cuarón 1999). Second, sperm storage is known to occur in reptiles, including some snake families (Booth and Schuett 2011; Sever and Hamlett 2002; Uller and Olsson 2008). Lastly, the introduction of unrelated individuals can slow down the effect of inbreeding during the initial generations thus minimizing the loss of genetic diversity. While the exact

origin of the individual snakes introduced to Cozumel has yet to be confirmed, relatedness estimates generated for individuals within the two genetic subpopulations suggest that most were unrelated. As a result, if the initial introductions consisted of multiple unrelated individuals, both male and female, with gravid females having stored viable sperm from previous mating events, the initial founder effects may have been reduced and the effective population size elevated. The recent discovery of facultative parthenogenesis in New World boid snakes (Booth et al. 2011a, b), including *B.c. imperator*, suggests that alternate reproductive strategies may also be utilized in the early stages of island colonization. The lack of individuals homozygous across all loci in our dataset, however, indicates that parthenogenetic reproduction, if utilized in the wild, was not a prevalent reproductive strategy on Cozumel Island.

In light of the detection of two genetically identified subpopulations (CMZ1 and CMZ2) and the moderate to large effective population size estimated, it is likely that *B.c. imperator* on Cozumel had been introduced at two geographically separate localities, as suggested by Martínez-Morales and Cuarón (1999). Given the limited number of generations since introduction (average generation time = ~ 5 years), the observation of low but significant genetic subdivision suggests that populations expanded in separate locations, followed by population admixture through dispersal. Bertona and Chiaraviglio (2003) documented that males of *B.c. occidentalis* can move an average distance of 1.5 km in a couple of months. Two alternate scenarios could unfold regarding future genetic subdivision on Cozumel if the species persists. In the absence of barriers to reproduction, assuming population size remains large, the genetic signature of population subdivision would likely diminish as generation number increases. Alternatively, the east–west structuring of the two populations delimited by the urban area (San Miguel) and the main road traversing the island (Fig. 2) suggests that human presence may have an important effect in their genetic separation. The latter is supported by significant differentiation values (F_{ST}) and the presence of exclusive alleles in each subpopulation. Despite the fact that the establishment of *B.c. imperator* is recent, genetic differentiation could be maintained through the physical or ecological separation of populations due to urban sprawl. Similar F_{ST} values have been

found in snake populations structured by roads that impacted their seasonal migration, genetic diversity and gene flow (Clark et al. 2010). Regardless, weak genetic differentiation has been found in amphibian and reptile populations introduced to other islands: *Rana ridibunda*, introduced to England in 1935, and *A. cristatellus* to two Caribbean islands in 2000, both show markedly low genetic differentiation and structure (Eales et al. 2008; Zeiseet and Beebee 2003). On the contrary, native reptile populations inhabiting oceanic islands commonly show high levels of structure, like the snake *E. subflavus*, endemic to Jamaica, that has values of $F_{ST} = 0.120$ (Tzika et al. 2008a), whereas iguanas of the genus *Conolophus* in Galapagos show values between 0.170 and 0.700 (Tzika et al. 2008b).

Average genetic diversity is often lower within island populations when compared with their mainland equivalents for species with limited dispersal opportunities (Frankham et al. 2005), thus the detection of diversity values higher for the mainland populations than to those on the island (as much as twice the number of alleles), is not unexpected. As populations that recently suffered a bottleneck lose alleles more rapidly than heterozygosity (Luikart and Cornuet 1998), the failure to detect significant evidence of a recent genetic bottleneck suggests that genetic drift was negligible. This would result from either a rapid population expansion following initial introduction, a large founding propagule(s) and/or a limited number of generations at a small population size. While support for a recent genetic bottleneck is weak, our genetic data suggests a bottleneck occurred historically (i.e. decades), likely a genetic signature of the initial founding population(s). The moderate to large effective population size estimated (455.1; 156.2–874.2, 95 % C.I.) supports a rapid population expansion from genetically diverse initial propagules, given the estimated number initially released and the year of potential introduction (Martínez-Morales and Cuarón 1999). The effective population size is defined as the number of breeding individuals in an idealized population that would show the same amount of dispersion of allele frequencies under random genetic drift or the same amount of inbreeding as the population under consideration. Regardless, it is important to note that this value is usually considerably smaller than the absolute population size (N), and as a result strongly influences a population's ability to

respond to microevolutionary forces, such as genetic drift and bottlenecks. In accordance, *B.c. imperator* on Cozumel may consist of a significantly large population. Its successful establishment is likely related to its broad dietary habits on the island, including several endemic vertebrate species (González-Baca 2006), the lack of carnivores or raptors preying up it, and its apparent lack of habitat specialization in the absence of anthropogenic disturbance (Romero-Nájera et al. 2007). A population successful establishment will be unlikely outside the species life-history variable limits.

Very few examples exist that have evaluated the introduction of snakes into oceanic islands and identified their geographic origin. One example is *Natrix maura*, an exotic snake on the island of Mallorca, which was introduced by the Romans during the first century. Guicking et al. (2006) identified its origin, using molecular analyses, from populations in Spain and France. While Cozumel is geographically proximate to mainland Mexico, the deep channel (~400 m deep), strong oceanic currents, and strong predominant winds in counter direction to the mainland, are likely to preclude natural colonization from mainland populations, thus human-mediated introduction is the most plausible explanation for *B.c. imperator* on Cozumel. Our results reveal that the closest mainland populations, from a genetic perspective, to subpopulations detected on Cozumel, are from the Gulf of Mexico and the Yucatan peninsula, which suggests these localities are potential sources of the island founders. We are aware that these results are preliminary; we are conducting a more complete evaluation that includes mitochondrial and nuclear DNA sequences and samples from the complete geographical distribution of the species complex in Mexico and Central America (Suárez-Atilano et al. unpublished data).

The high abundance, extensive distribution, ample diet and size diversity of *B.c. imperator* in Cozumel (González-Baca 2006; Martínez-Morales and Cuarón 1999; Romero-Nájera et al. 2007) confirm it now constitutes a large, reproductive population. While its population on Cozumel has increased significantly and spread throughout the island after only 40 years from introduction (i.e. approximately eight generations), it has become a successful top predator with no natural enemies other than humans (Romero-Nájera et al. 2007). It is important to highlight that the time frame

over which the sharp population decline and, in some cases virtual extinction of endemic taxa, like the rodent *Peromyscus leucopus cozumelae* and the Cozumel Thrasher *Toxostoma guttatum*, has occurred (Cuarón 2009; Cuarón et al. 2004, 2009; Cuarón et al. unpublished data; Fuentes-Montemayor et al. 2009; Martínez-Morales et al. 2009; McFadden et al. 2010; Perdomo 2006; Vega et al. 2007), is directly correlated with the introduction of the boa onto Cozumel. During this time however, substantial feral populations of non-native predator species and potential competitors have also established on Cozumel, namely cats (*Felis catus*) and dogs (*Canis familiaris*) (Cuarón et al. 2004, 2009; Martínez-Morales et al. 2009; Mena 2007; Torres Villegas 2006), as well as house rats (*Rattus rattus*) and mice (*Mus musculus*) (Sotomayor 2009), which also have contributed to the collapse of endemic species populations.

Our results provide relevant information that supports *B.c. imperator* is a recently introduced, non-native species that has successfully established and spread on the island. The unique status of *B.c. imperator*, both as an invasive exotic predator on Cozumel and as a threatened species that is legally protected in different countries (including Mexico), represents a legal conservation paradox. This genetic information, in concert with what is known about the ecology and distribution of *B.c. imperator* on the island, should be considered when designing and implementing management strategies. In particular, the urgent need to eradicate *B.c. imperator* from Cozumel and the essential consideration that specimens from Cozumel should not be released elsewhere in reintroduction, repopulation or other conservation management programs. Our results might also provide valuable information as a reference for other boa-introduced cases, as well as for conservation purposes of native boa populations.

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