Global Ecology and Conservation 3 (2015) 487–502

Contents lists available at ScienceDirect

Global Ecology and Conservation

journal homepage: www.elsevier.com/locate/gecco

Original research article

Large divergence and low diversity suggest genetically informed conservation strategies for the endangered Virgin Islands Boa (*Chilabothrus monensis*)

R. Graham Reynolds [a,b,*], Alberto R. Puente-Rolón [c], Renata Platenberg [d], R. Kirsten Tyler [b], Peter J. Tolson [e], Liam J. Revell [b]

[a] *Department of Organismic and Evolutionary Biology & Museum of Comparative Zoology, Harvard University, 26 Oxford St., Cambridge, MA 02138, USA*

[b] *Department of Biology, University of Massachusetts Boston, 100 Morrissey Blvd., Boston, MA, 02125, USA*

[c] *Departamento de Ciencias y Tecnología, Universidad Interamericana de Puerto Rico, Recinto Arecibo, Arecibo, Puerto Rico 00614, USA*

[d] *College of Science and Mathematics, University of the Virgin Islands, #2 John Brewers Bay, St. Thomas, US Virgin Islands, 00802, USA*

[e] *Toledo Zoo, PO Box 140130, Toledo, OH, 43614, USA*

ARTICLE INFO

Article history:
Received 12 August 2014
Received in revised form 2 February 2015
Accepted 3 February 2015
Available online 11 February 2015

Keywords:
Boidae
Chilabothrus monensis
mtDNA
Multilocus
Simulations
Translocation

ABSTRACT

The Virgin Islands boa (*Chilabothrus monensis*) was listed as critically endangered by the U.S. Fish and Wildlife Service in 1979, and is presently known to occur in two disjunct regions: Isla de Mona and the eastern Puerto Rico Bank. Populations of the species are highly vulnerable and are hypothesized to have contracted considerably from their former range. Here we conduct intraspecific genetic analyses for this species using mitochondrial and nuclear loci as well as population genetic simulations. In so doing, we characterize nine microsatellite markers for *C. monensis* and demonstrate their potential usefulness for in situ or ex situ conservation genetic analysis. We find that populations on the Puerto Rico Bank are highly divergent (3.03% sequence divergence; 2.10 Mya temporal divergence) from Isla de Mona animals and that little genetic diversity exists within or among these sampling sites. Furthermore, we provide recommendations and an assessment of translocation/reintroduction potential for this species based on the genetic data presented herein. Our study also highlights the usefulness of simulations for assessing small sample size in conservation genetic studies. We anticipate that these results and genetic tools will be useful in formulating a comprehensive conservation genetic approach for Virgin Island boas.
© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

To protect a threatened island-dwelling species, conservation planners must know the extent and distribution of genetic variation within and among populations across a species' range so that appropriate conservation measures might be implemented (Lande, 1988; Allendorf et al., 2012; Frankham et al., 2014). If nonrandom mating, limited genetic diversity, and susceptibility to extirpation are characteristic of an endangered insular species, protecting only a few islands might not be sufficient for the species to persist. When populations are subdivided (into demes) and connected by limited gene flow or

* Corresponding author at: Department of Organismic and Evolutionary Biology & Museum of Comparative Zoology, Harvard University, 26 Oxford St., Cambridge, MA 02138, USA. Tel.: +617 495 2460; fax: +617 495 5667.
E-mail address: robertreynolds@fas.harvard.edu (R.G. Reynolds).
URL: http://www.caribbeanboas.org (R.G. Reynolds).

http://dx.doi.org/10.1016/j.gecco.2015.02.003
2351-9894/© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

dispersal, extinction of local demes and subsequent loss of unique alleles might greatly influence global genetic diversity (Holsinger, 2000; Frankham, 2006). These considerations are of particular importance when establishing reserves designed to protect sensitive species composed of subdivided demes, as might occur in island archipelagos (e.g. Michaelides et al., 2014). Furthermore, captive breeding, reintroduction, and translocation should be informed by an understanding of both global and intra/interdemic genetic diversity (Storfer, 1999; Avise, 2004; Allendorf et al., 2012).

A serious concern of conservation genetic studies on endangered species is obtaining sufficient sample sizes for population genetic inference (e.g. Kim et al., 2011; Emel and Storfer, 2012). Many endangered species exist at low densities or in remote areas (IUCN, 2014), meaning that we cannot always anticipate fulfilling a research design calling for dozens of individual samples per population. Recent work (e.g., Hale et al., 2012; see also: Crandall and Templeton, 1993 and Crandall et al., 2000) suggests that sampling fewer than 10 individuals per population for microsatellite analysis will result in high error rates for estimates such as expected heterozygosity (H_e), and that sampling designs should aim for 25–30 individuals per population. Unfortunately, population sample sizes this large are often unfeasible for many threatened vertebrates. Furthermore, population-level genetic summary statistics such as deviation from Hardy–Weinberg equilibrium (HWE) rely on estimates of population-level allele frequencies; consequently the inability to accurately capture these frequencies will in turn bias these estimates (Allendorf et al., 2012). As such, studies with small sample size should not report measures of F_{ST} (or analogs), as these statistics rely on an assumption of mutation–migration–drift (population genetic) equilibrium and are calculated as the amount of genetic variation within populations relative to the amount of genetic variation among populations (see: Meirmans and Hedrick, 2011 for a recent review). Endangered species populations may not be in mutation–migration–drift equilibrium (e.g., Fitzpatrick et al., 2012), especially if the populations have been steadily declining (a severe form of a "relaxation" population genetic model; Brown, 1971). Other measures relevant to conservation genetic studies include the calculation of effective population size (N_e), often using an observed measure of linkage disequilibrium (LD; e.g. Waples, 2006 and Waples and Do, 2008), Inference of N_e is an extremely meaningful component of genetic assessments of threatened or endangered species; however, recent work has indicated that many studies likely fail to meet the sampling requirements for obtaining unbiased estimates of this parameter (England et al., 2006). Additionally, the LD method of estimating N_e is influenced by the rate of recombination at each locus (Hill and Robertson, 1968; Ohta and Kimura, 1969). This recombination rate is expected to be reduced in the presence of inbreeding (non-random mating) and genetic bottlenecks, leading to a lower rate of decay of linkage disequilibrium and contributing another source of bias to estimates of LD and N_e (Hedrick, 2009).

In spite of these limitations, here we combine multiple types of genetic data with population genetic simulations to show how even a small sample size of endangered species can significantly contribute to genetically-informed conservation management. We provide an empirical example focusing on an endangered boid snake from the Greater Antilles which is threatened with extirpation from the majority of its native range.

As a global biodiversity hotspot, the Greater Antilles contain an important and imperiled reptile assemblage (Myers et al., 2000; Hailey et al., 2011). Snakes, especially boids in the genus *Chilabothrus* (formerly *Epicrates*; Reynolds et al., 2013a), face a variety of threats across much of their range in this region, including but not limited to habitat loss, invasive predators and competitors, and direct persecution (Tzika et al., 2008; Reynolds, 2011; Tolson and Henderson, 2011; Reynolds and Gerber, 2012; Reynolds et al., 2013b; Puente-Rolón et al., 2013). One such example, the Virgin Islands boa (*C. monensis*; Fig. 1), is currently protected under two United States Federal programs: The Endangered Species Act (1973; species listed 1979) and the Coastal Zone Management Act (1972), the latter of which supports enforcement of federal regulations. The species has also been listed as endangered by the International Union for Conservation of Nature (IUCN) and is listed on Appendix A of the Convention on International Trade in Endangered Species (CITES). In Puerto Rico, the species is protected under the Regulation to Govern the Management of Threatened and Endangered Species in the Commonwealth of Puerto Rico (Tolson and Henderson, 1993). *Chilabothrus monensis* is currently considered to include two subspecies: the Mona boa (*C. monensis monensis*) and the Virgin Islands (VI) boa (*C. monensis granti*). Recovery plans for the two subspecies were established in 1984 (*C. m. monensis*) and 1986 (*C. m. granti*). The latter identified three main objectives for implementation: captive breeding, reintroduction of extirpated populations, and studies of the remnant population on St. Thomas (USFWS, 1984, 1986). Subsequent five-year reviews in 1991 and 2006 indicated that the use of genetic tools would benefit all three objectives. Major impediments to this work have included the lack of species-specific and sufficiently polymorphic genetic markers, and the limited availability of samples. Here we use genetic data to specifically address the aforementioned objectives. We characterize nine novel polymorphic microsatellite markers, examine nuclear and mitochondrial diversity, and use computer simulations to assess the potential utility of our markers, sample, and analytical tools in diagnosing genetic diversity in *C. m. granti* on the Puerto Rico Bank (PRB). We also provide a time-calibrated measure of divergence between *C. m. monensis* and *C. m. granti* across the greater Puerto Rican region and evaluate translocation potential from a genetic perspective for islands on the PRB under the jurisdiction of the Commonwealth of Puerto Rico.

2. Materials and methods

2.1. Study area and sample collection

Virgin Islands boas exist in two highly disjunct regions (Nellis et al., 1983; Mayer and Lazell, 1988; Tolson and Henderson, 1993; Mayer, 2011). One population is isolated on Isla de Mona west of Puerto Rico in the Mona Passage (*C. m. monensis*).

Fig. 1. Subadult female Virgin Islands boa, *Chilabothrus m. granti*, from Rio Grande, Puerto Rico. Photo by RGR.

Remaining populations are found on some of the Spanish (Passage), US, and British Virgin Islands as far east as Virgin Gorda and Necker Island on the partially submerged eastern Puerto Rican Bank (*C. m. granti*; Fig. 2). In addition, an extremely localized population of *C. m. granti* occurs on the main island of Puerto Rico in the municipality of Río Grande. Isla de Mona is an isolated bank with an emergent area of 55.81 km^2 which has never been connected to another landmass. Though harboring many introduced vertebrates (Campbell, 1991; Tolson, 1996), the island is otherwise well protected as part of the Mona Island Natural Reserve and administered by the Puerto Rico Department of Natural Resources. Virgin Island boas on Puerto Rico and the eastern Puerto Rico Bank are highly endangered and remaining populations are thought to be remnants resulting from the decline or extirpation of populations within a broader historical range (USFWS, 1986; Mayer and Lazell, 1988; Tolson, 1996; DRNA, 2010). When the initial review of *C. monensis* was conducted (USFWS, 1986), the subspecies *C. m. granti* was known from only 71 recorded specimens (USFWS, 1986, 2009) and a few years prior only 12 specimens were known (Nellis et al., 1983). It is estimated that only 1300–1500 boas remain in this region (USFWS, 2009), though these data are based on encounters with a cryptic and secretive species and hence should be considered minimum and/or highly uncertain estimates. We sampled individuals of *C. m. granti* from three regions with the most highly imperiled populations: the last remaining population on the island of St. Thomas, US Virgin Islands (Harvey and Platenberg, 2009; Platenberg and Harvey, 2010; Platenberg and Boulon, 2011); a native remnant population on the island of Cayo Diablo, Puerto Rico; and the only known population on the main island of Puerto Rico in Río Grande Municipality (Table 1; exact locality intentionally obfuscated). On St. Thomas, boas are presently restricted to the extreme eastern end of the island, where they occur in small numbers in a few localized areas (Fig. 2). Between 1982 and 2006, only 114 boa sightings (live or dead) were verified on St. Thomas by the Division of Fish and Wildlife (Platenberg and Harvey, 2010), and the long-term survival of the species on that island is in question (Tolson and Henderson, 1993; Platenberg and Harvey, 2010; Platenberg and Boulon, 2011). Cayo Diablo is a two hectare island located ∼9 km off of the east coast of Puerto Rico. It is the southernmost island of La Cordillera, an oolitic formation of islands geologically similar to the Bahamian Archipelago and dating to the late Pleistocene (Kaye, 1959a). The population of *C. m. granti* on Cayo Diablo is apparently naturally occurring (as opposed to introduced), and is one of the densest reported populations of West Indian boas, with recent estimates of 100–150 individuals per hectare (Tolson, 1996; USFWS, 2009). Nonetheless, given the small size and low elevation (<6 m) of the island it is vulnerable to stochastic effects such as overwash or arboreal habitat destruction during hurricanes (Tolson, 1996), invasion by non-native predators (Tolson, 1996) such as rats (*Rattus* sp.), mongoose (*Herpestes auropunctatus*), and cats (*Felis silvestris*), rising sea levels, and attrition of genetic diversity (e.g. Frankham, 2005, among other dangers. The population in Río Grande is extremely localized (<2 km^2), and recent searches have yielded encounter estimates of 0.05–0.33 snakes/person hour (USFWS, 2009; this study), with fewer than eight individual snakes having been found in recent searches (authors pers. ob.). This population is completely surrounded by urban development and is likely quite small in total size.

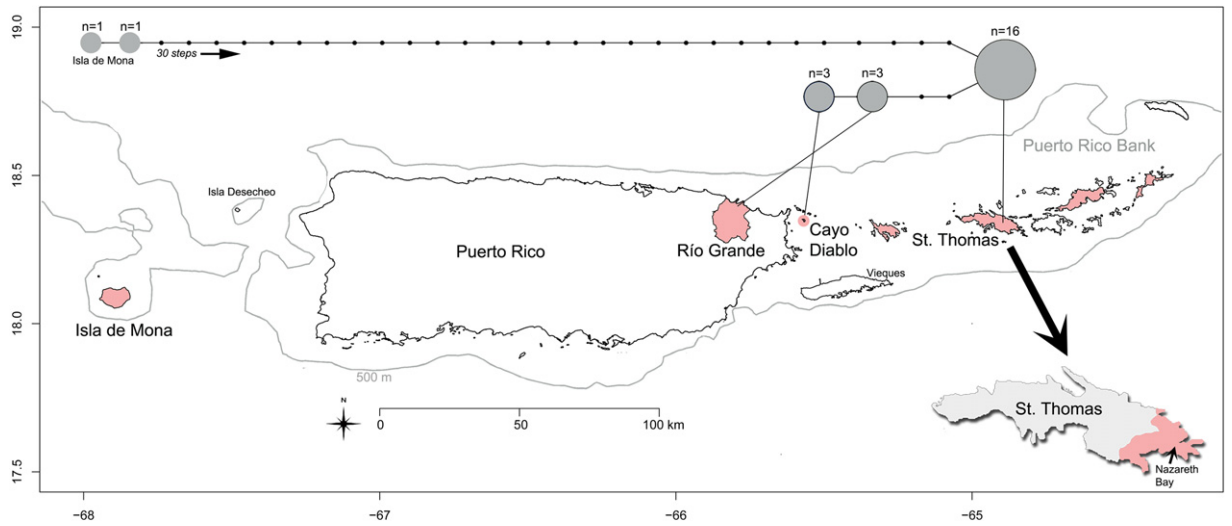

Fig. 2. Phylogeography of *Chilabothrus monensis* on the Puerto Rico Bank and Isla de Mona Bank. The approximate extent of the 500 m depth line is shown in gray, and island outlines are in black. Current range of the species is shown in pink, with the island of St. Thomas expanded in lower-right inset. Note that on St. Thomas the species is restricted to the extreme eastern end of the island, and that most boa road kills are found in the neighborhood of Nazareth Bay. A haplotype network for the mtDNA locus is shown above the map, where haplotypes are matched to sampling location and un-sampled inferred haplotypes are shown as small black circles. Haplotype circles are proportional to sample size, and the number of sequences in each haplotype is given. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Sampling locations and information for material included in this study. GenBank accession numbers reference mtDNA *CYTB* sequences, and GenSeq designations are given *sensu* Chakrabarty et al. (2013).

Specimen #	Island	Locality	Date	Origin	GenBank IDs
268382	St. Thomas	–	–	Shed skin	KP116239
268383	St. Thomas	–	–	Shed skin	KP116240
269976	St. Thomas	Nazareth	09/29/2008	Whole body, salvaged	KP116241
269977	St. Thomas	Nazareth	10/02/2008	Whole body, salvaged	KP116242
269978	St. Thomas	Red Hook	07/19/2006	Whole body, salvaged	KP116243
269979	St. Thomas	Nazareth	06/14/2006	Whole body, salvaged	KP116244
269980	St. Thomas	Nazareth	12/29/2006	Whole body, salvaged	KP116245
269981	St. Thomas	Nazareth	12/09/2006	Whole body, salvaged	KP116246
269982	St. Thomas	Red Hook	08/09/2005	Whole body, salvaged	KP116247
269983	St. Thomas	Friedenhoj	06/03/2007	Whole body, salvaged	KP116248
269984	St. Thomas	Secret Harbour, Nazareth	07/22/2010	Whole body, salvaged	KP116249
269985	St. Thomas	Ridge Rd Nazareth	03/25/2011	Whole body, salvaged	KP116250
269986	St. Thomas	Ridge Rd Nazareth	09/28/2009	Whole body, salvaged	KP116251
269987	St. Thomas	Secret Harbour, Nazareth	08/29/2009	Whole body, salvaged	KP116252
269988	St. Thomas	Ridge Rd., Nazareth	03/02/2010	Whole body, salvaged	KP116253
269989	St. Thomas	Ridge Rd., Nazareth	11/25/2009	Whole body, salvaged	KP116254
UMFS 14871	Cayo Diablo	–	2/18/1998	Wild caught	KP116255; genseq-4
UMFS 14872	Cayo Diablo	–	6/2/1998	Wild caught	KP116256; genseq-4
UMFS 14873	Cayo Diablo	–	8/12/1980	Captive born, known lineage	KP116257; genseq-4
PR Grt1	Puerto Rico	Río Grande[a]	2012	salvaged	KP116260
PR Grt2	Puerto Rico	Río Grande[a]	01/7/2013	Wild caught	KP116261, genseq-5
PR Grt3	Puerto Rico	Río Grande[a]	01/7/2013	Wild caught	KP116262, genseq-5
UMFS 14869	Mona	Playa Pajaros	7/22/1999	Wild caught	KP116258; genseq-4
UMFS 14870	Mona	Playa Coco	11/29/1997	Wild caught	KP116259; genseq-4

UMFS = University of michigan field series.
[a] Locality intentionally obfuscated.

Samples from St. Thomas ($n = 16$) were collected opportunistically as roadkills reported to wildlife authorities over a period of six years (2005–2011). No live boa has ever been found under organized survey effort on St. Thomas (RJP pers. ob.) and surveys are becoming increasingly difficult owing to development of remaining habitat and the increasing danger to researchers from illegal human activity near the population (RJP pers. ob.; Platenberg and Harvey, 2010). Samples from Puerto Rico ($n = 3$) were obtained during one survey night in March 2011 and four consecutive nights of focused nocturnal surveys conducted during January 2012 (other surveys did not yield samples). Samples from Cayo Diablo ($n = 3$) were collected in 1980 (a captive born individual from sire and dam of known origin) and 1998 (wild caught, focused nocturnal

Table 2
Genes and selected best-fit models of evolution, after Reynolds et al. (2013a).

Gene	Abbreviation	Length	Ploidy	Selected Model
Cytochrome B	CYTB	1077	N	HKY + I + G
Oocyte maturation factor	c-mos	465	2n	K80
Brain-derived neurotrophic factor	bdnf	711	2n	K80 + I
Neurotrophin-3	ntf3	525	2n	K80 + I
Bone morphogenetic protein 2	bmp2	660	2n	K80 + I
Recombination activating protein 1	rag1	678	2n	K80 + I
NADH dehydrogenase subunit 4	ND4	636	N	HKY + I + G
Prostaglandin E receptor 4	ptger4	507	2n	TPM3 + G
Protein tyrosine phosphatase non-receptor type 12	ptpn12	387	2n	K80
Ornithine decarboxylase	odc	610	2n	TPM3uf + G

survey). To compare divergence across a large portion of the range of *C. monensis*, we also sampled two individuals from Isla de Mona in 1997 and 1999.

Samples from boas consisted of 3–10 mm tail clips or dissected muscle or liver tissue (dead specimens) preserved in 95% ethanol. We sanitized ventral surfaces and tails of live animals before and after clipping and applied antiseptic dermal adhesive to prevent infection. Any boa found with a clipped or damaged tail tip was not sampled to prevent repeated sampling. Live boas were returned to the exact capture location within 24 h of sampling. We extracted whole genomic DNA using the Promega Wizard® SV DNA purification system according to the manufacturer's protocol and stored the extracts at −20 ° C.

2.2. Genetic divergence in C. monensis

We used the polymerase chain reaction (PCR) to amplify each of 10 loci (two mtDNA loci and eight nuclear loci) using primers and conditions in Reynolds et al. (2013a) (Table 2). All reactions were conducted in either an Eppendorf Mastercycler Pro or a TC9639 non-gradient thermocycler. We purified and sequenced products on an automated sequencer (ABI 3730XL) at Massachusetts General Hospital DNA Core Facility, Cambridge, MA. We assembled contigs and manually verified ambiguous base calls using Geneious 7.1.2 (Biomatters, Auckland, New Zealand). We resolved heterozygous sequences using Phase 2.1 (Stephens et al., 2001; Stephens and Donnelly, 2003) implemented in DnaSP v5.10.1 (Librado and Rozas, 2009) using default parameters for 100 iterations with a burnin of 100. We then aligned sequences using the ClustalW 2.1 (Larkin et al., 2007) algorithm implemented in Geneious with a subset of two individuals from each *Chilabothrus* species (except *C. gracilis*) as well as outgroup taxa from the 10-gene alignment in Reynolds et al. (2013a). We also separately aligned just the cytochrome B (*CYTB*) mtDNA locus, which has been shown to be useful in species identification in boas (Campbell, 1997; Burbrink, 2004). We estimated models of nucleotide substitution using BIC in jModelTest2 (Guindon and Gascuel, 2003 and Darriba et al., 2012; Table 2). We deposited representative sequences in GenBank (Table 1) with associated GenSeq nomenclature (Chakrabarty et al., 2013).

We used the mtDNA *CYTB* locus to examine phylogeographic structuring in *C. monensis*. We created a statistical parsimony network using a connection limit of 40 steps in the program TCS 1.21 (Clement et al., 2000). We calculated pairwise genetic distances (p-distances) between populations using MEGA6 (Tamura et al., 2013). To temporally contextualize subspecies divergence in *C. monensis*, we estimated a time-calibrated mitochondrial coalescent tree for 9 species of West Indian *Chilabothrus*, including all unique haplotypes found in the present study. Prior studies have shown that mtDNA loci, including *CYTB*, evolve at similar rates in snakes (Kuch et al., 2005), and that these rates vary between 0.65% and 1.77% per lineage per million years in squamate reptiles (Zamudio and Greene, 1997; Macey et al., 1998; Malhotra and Thorpe, 2000; Wüster et al., 2002; Morando et al., 2003; Fontanella et al., 2012). These rates are potentially idiosyncratic for particular taxa (e.g. Lynch, 2010), so we chose to estimate a substitution rate for the mtDNA locus from the alignment of West Indian boas. We constrained the root node of *Chilabothrus* using a normal prior with a mean of 21.7 Mya and a standard deviation of 1.8 Mya, derived from a fossil-calibrated divergence time analysis of the larger Neotropical boid phylogeny (Reynolds et al., 2013a). We ran the MCMC for 100 million generations in the program Beast v1.8 (Drummond et al., 2012) using a HKY+I+G substitution model (Reynolds et al., 2013a), a Yule speciation prior, and an uncorrelated lognormal relaxed clock model. We repeated the analyses three times with different starting parameter values, sampling every 1000 generations and discarding the first 25% of generations as burn-in, to generate effective sample sizes (ESS) for all parameters larger than 200. We assessed convergence of the independent runs by a comparison of likelihood scores and model parameter estimates in TRACER v1.5 (Rambaut et al., 2013). We combined results from the three analyses using LogCombiner and generated a maximum clade credibility tree using TreeAnotator.

In order to simultaneously examine the multilocus species-tree topology as well as divergence times, we analyzed the 10-gene dataset with two representatives per species (treating *C. m. monensis* and *C. m. granti* as separate operational taxonomic units) using the Bayesian MCMC method *Beast (Heled and Drummond, 2010) implemented in Beast v1.8. We partitioned sequence data by locus and assigned a locus-specific model of nucleotide substitution (Table 2). We unlinked nucleotide substitution models, clock models, and gene trees in all analyses. We employed an uncorrelated lognormal (UCLN) relaxed molecular clock model of rate variation for each locus, and we used a Yule process speciation prior for the branching rates.

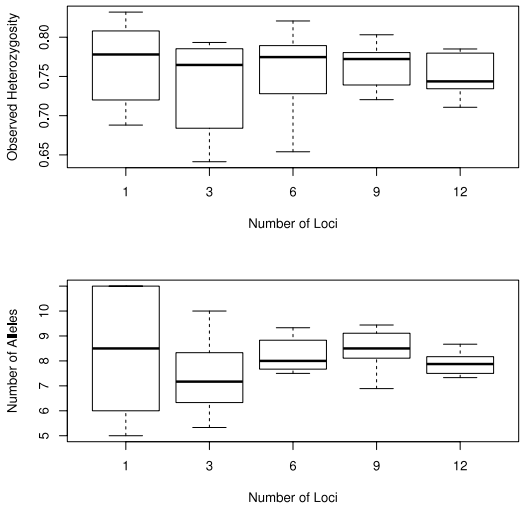

Fig. 3. Decreasing variance for two summary statistics with increasing number of loci sampled in a simulation of a relaxed population genetic model. The values are drawn from 10 independent simulations for each parameter set, with values representing the average for a population of 250 individuals after 1600 generations of drift. All other simulation conditions are as in the text. Number of alleles is number of alleles per locus in the population.

We assigned calibrations to the nodes as in Reynolds et al. (2013a). We ran the MCMC for 400 million generations and we repeated the analyses three times with different starting numbers, sampling every 10,000 generations and discarding the first 20% of generations as burn-in. We assessed ESS and convergence, combined posterior trees from across runs, and generated an MCC tree as above.

2.3. Novel genetic markers

Since we are only able to obtain a limited number of individuals, we elected to use a multilocus approach whereby we sampled multiple presumably independently segregating regions of the genome experiencing the same demographic history. In this way we are able to obtain a higher genetic sample size in spite of having a low number of individuals (Pluzhnikov and Donnelly, 1996), thus reducing variance in our estimates of population genetic parameters (Fig. 3; Nei, 1978; Wakeley and Hey, 1997 and Kuhner et al., 2000), even while our sample sizes of this species are very low relative to appropriate sample sizes ascertained through simulation (Hale et al., 2012). As no species-specific genetic markers exist for VI boas, we opted to characterize novel genetic markers for *C. m. granti*. We screened 32 tetra- to hexa-nucleotide microsatellite primer pairs developed from >5 million next generation sequencing reads from the genome of the sister species, *C. inornatus*, (Reynolds et al., 2014c) to test for amplification in *C. m. granti*. *Chilabothrus inornatus* is ∼10 Mya divergent from *C. monensis* (Reynolds et al., 2013a) and interspecific amplification of other microsatellites in this genus has met with mixed success (e.g., Reynolds et al., 2011 and Booth et al., 2011). Hence, we wanted to assess whether cross-species amplification of microsatellite loci developed from next generation sequencing runs for other endangered species (*C. inornatus*) might be useful and represent a significant cost savings compared to developing loci de novo. Of these 32 loci, nine were successfully amplified and generated repeatable genotypes.

We modified the forward primer from each primer pair on the 5′ end with a 19 bp sequence tag (5′-CACGACGTTG TAAAACGAC-3′) to allow for the use of a third fluorescently-labeled PCR primer (M13 method; Schuelke, 2000). PCR conditions for each reaction were as in Reynolds et al. (2014c), and we included an additional forward primer labeled on the 5′ end with one of four dyes (6-FAM, PET, VIC, or NED) in each PCR. We visualized PCR products by gel electrophoresis, multiplexed PCR products with different dyes, and resolved genotypes on an automated sequencer (ABI 3730XL) at Massachusetts General Hospital DNA Core Facility, Cambridge, MA using GeneScan™ 500 LIZ size standard and Peak Scanner 1.0 software (ABI) with manual verification of peak calling. We tested for genotyping errors by randomly selecting 20% of the samples for repeated genotyping from the PCR stage. In addition, we used Micro-Checker 2.2.3 (Van Oosterhout et al., 2004) to investigate whether our genotype profiles showed evidence of allele-dropout or null alleles; though we do not exclude loci which deviated from HWE as here we are not attempting to obtain robust population genetic parameters due to our small sample sizes (Dharmarajan et al., 2013). We calculated 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).

2.4. Simulations and dealing with small sample size

To examine the effects of possible demographic histories and small sample size on estimates of H_E and H_O, we conducted individual-based, forward-time population genetic simulations for the Cayo Diablo and St. Thomas populations. We chose

these populations because we have some prior information about these sites that we can use to reasonably parameterize the simulations. At the present time, we know far too little about the potential demographic history of Rio Grande (e.g., is it a remnant from a former range across PR, has a small population existed there for millennia, or was the population introduced from the Virgin Islands?) to reliably parameterize a simulation for that population. Here, the intention is to use simulations under realistic genetic and demographic conditions to explore the robustness of our population genetical parameter estimates to the very small sample sizes of the present study.

For the Cayo Diablo population we do not a priori expect the signature of a population bottleneck, given that the population is almost certainly at carrying capacity. A bottleneck followed relatively quickly by complete population recovery would obfuscate any signal of that event in the genetic data (Nei et al., 1975; Reynolds and Fitzpatrick, 2013). Instead, we expect that this population might conform to a relaxation model, whereby the relative influences of drift and mutation have been acting independently of other populations since the most recent fragmentation of the PRB owing to post-Pleistocene sea level rise. Our assumption here is that VI boas on La Cordillera (and emergent PRB) existed in panmixia up until the isolation of Cayo Diablo during the end of the last glacial maximum approximately 8000 years ago (Kaye, 1959a; Donn et al., 1962; Heatwole and MacKenzie, 1966; Lighty et al., 1982; Fairbanks, 1989). Since boas likely experience a mean generation time of ∼5 years (3 years USFWS, 2009; 5 years Reynolds et al., 2013b; 8.5 years in captivity Earnhardt et al., 2004), this translates to approximately 1600 generations of drift. We simulated this relaxation model in the absence of gene flow using Easypop v2.0.1 (Balloux, 2001) to observe the expected degree of heterozygosity and allele loss through time owing to drift. Given that we know almost exactly how many boas currently exist on the island (census population size $N_C \approx 200$–250; Tolson, 1996 and USFWS, 2009) we simulated three realistic genetic effective population sizes based on prior empirical census to effective population size ratios: $N_e = 25$, 50, and 100 ($N_C \approx N_e/0.15$; Earnhardt et al., 2004 and Frankham et al., 2014). We initiated the simulations with the following starting conditions: nine microsatellite loci, free recombination, maximum of 20 alleles/locus, equal sex ratios, maximal initial variation (var in = max), and equal single-stepwise mutation rates. We used a mutation rate scaled by N_e ($\sim 1/N_e$) such that $\Theta \approx 4$ (Reynolds and Fitzpatrick, 2013), as well as a slower overall mutation rate (0.001 mutations/locus/generation). We ran the simulations for 1600 generations and generated summary statistics averaged across 10 independent simulations. To investigate our ability to obtain reliable estimates using small sample size, we randomly drew (with replacement) three individuals from a simulation 100 times using R v3.0.3 (R Development Core Team, 2013) and calculated summary statistics in GenAlEx as above. These subsample sets were then compared to the true means from the simulation and the empirical data.

For St. Thomas, we expect that severe demographic declines and a deviation from population-genetic equilibrium best characterize this population. Hence we conducted forward-time simulations in R v3.0.3 of a genetic bottleneck (scripts modified from Reynolds and Fitzpatrick, 2013) to estimate population genetic parameters which would result from this demographic scenario. We parameterized our simulation based on our empirical data: sampling 14 individuals at nine microsatellite markers per simulation. We started with an initial effective population size $N_{e0} = 1000$, followed by a moderate bottleneck to $N_e = 72$ or a severe bottleneck to $N_e = 8$. Because the duration of the bottleneck can influence the magnitude of heterozygosity loss at the sampling period (Cornuet and Luikart, 1996), we simulated a range of event times from 2 to 64 generations to encompass the expectation for recent large-scale development of St. Thomas (Platenberg and Harvey, 2010; Platenberg and Boulon, 2011). To provide a frame of reference for our non-equilibrium simulations, we also conducted simulations of an equilibrium scenario whereby no demographic events occur (Reynolds and Fitzpatrick, 2013) and $N_{e0} = 1000$. Each simulation begins with $10 * N_{e0}$ generations at mutation–drift equilibrium to establish starting parameters prior to simulating the demographic scenario. We simulated 1200 independent populations for each of the initial conditions, and calculated summary statistics for each simulation.

2.5. Conservation and translocation

While in situ conservation management should generally be preferred (Sax et al., 2013), translocation and reintroduction can also be useful conservation strategies in certain circumstances so long as combined with genetic information (e.g. Michaelides et al., 2014). Some previous assessment has been performed to characterize critical habitat in Puerto Rico for *C. m. granti* (DRNA, 2010), including for possible translocation, and we build on this work by assessing the translocation potential of islands on the PRB informed by our genetic analyses. We assessed islands identified in DRNA (2010), as well as other offshore cays under the jurisdiction of the Commonwealth of Puerto Rico. We qualitatively characterize islands as being of high, moderate, or low suitability for translocation (or reintroduction) of *C. m. granti* based on the following criteria: availability of prey, diversity of other squamate reptiles already present, relative island isolation, island size, presence of forest canopy or suitable arboreal habitat (Chandler and Tolson, 1990; DRNA, 2010), and protected status of the island. We do not account for the presence of introduced vertebrate predators, as we expect that all islands contain at least one species (*Rattus*) and that islands slated for translocation programs would be subjected to invasive vertebrate removal programs prior to introduction (e.g., García et al., 2002 and Savidge et al., 2012).

3. Results

We obtained an alignment of 1095 bp of the cytochrome B [CYTB] locus (near-complete coding DNA sequences [cds]) from 24 individuals across four sampling sites of *C. monensis*. We observed only a single haplotype for each of the three

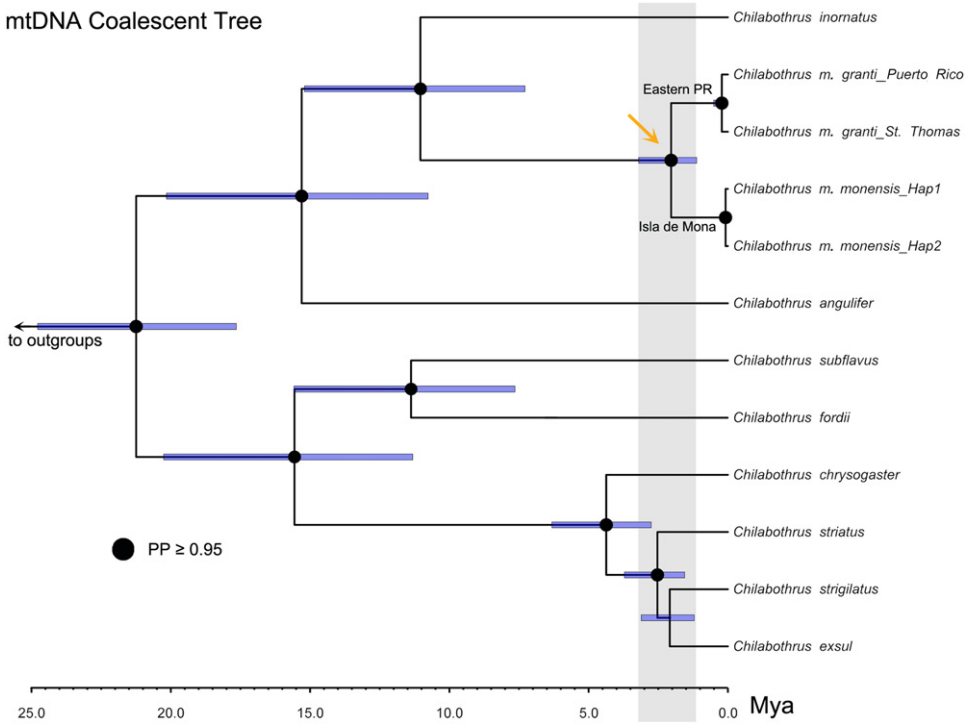

Fig. 4. Time-calibrated phylogenetic coalescent tree for the mitochondrial locus *CYTB* for species of West Indian *Chilabothrus*. Representative haplotypes recovered during this study are included in the analysis. 95% HPD intervals are shown as nodal bars, while black circles at nodes indicate posterior probabilities (PP) $\geq$0.95. A vertical gray bar spans the 95% HPD interval for the split between *C. m. monensis* and *C. m. granti*, which is indicated by an orange arrow. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

sampling sites of VI boas on Puerto Rico and the Puerto Rico Bank (Fig. 2). A maximum of only four mutational steps separate the most distant VI haplotypes (St. Thomas and Cayo Diablo), and a single step distinguishes Río Grande and Cayo Diablo sequences (Fig. 2). We found two haplotypes among the two samples from *C. m. monensis* on Isla de Mona, and a minimum of 30 mutational steps, corresponding to a mean of 3.03% (range 2.8%–3.2%) sequence divergence, separating Mona boas (*C. m. monensis*) from VI boas (*C. m. granti*). In our phylogenetic analysis of *CYTB*, we recovered a well-supported tree with an estimated coalescent time of 2.1 Mya (95% highest posterior density interval [95% HPD]: 1.12–3.20 Mya) between Mona and VI boas (Fig. 4). Other coalescent times among *Chilabothrus* in the *CYTB* gene tree were largely consistent with previous multilocus fossil-calibrated estimates (Reynolds et al., 2013a); Table A.1).

For the 5731 bp multilocus dataset, we obtained a fairly well resolved species tree for the WI boa clade, with the majority of nodes (7/10) showing high (>0.95) posterior probability (Fig. 5) and topological congruence with previous studies (Reynolds et al., 2014a). The species tree analysis supports the distinction between the two *C. monensis* subspecies ($PP = 0.99$), with a mean estimated divergence time of 2.1 Mya (95% HPD 0.24–3.55 Mya).

For the microsatellite data, we successfully resolved 20 genotypes at nine polymorphic loci among *C. m. granti* with an allele-calling error rate of 2.7% (two miscalled alleles out of 72 replications). We found no evidence for allelic dropout or stutter, though we did find evidence for homozygosity excess at loci *Ci18*, *Ci24*, *Ci37*, and *Ci43*. We found between four and seven alleles per locus, with an average of 5.2 alleles and 3.2 effective alleles per locus (N_E) across all nine loci (Table 3). We found that five of nine loci deviate from multilocus HWE expectations owing to a deficiency of heterozygotes. At the population level, we found an average of between 2.33 and 2.78 alleles per locus, per population, with the Río Grande population exhibiting the highest allelic richness (Table 4). Among islands, Río Grande had the highest effective number of alleles ($N_E = 2.30 \pm 0.23$) relative to the other populations.

Under a relaxation simulation for Cayo Diablo, our empirical estimate for H_O (0.52) corresponds to that expected when $N_e = 50$; while our empirical estimate of N_A (2.33) is slightly lower than estimates from the simulations, with the simulations of $N_e = 25$ yielding a mean of $N_A = 2.89$ (Fig. 6(A), (B); Table 5). Under a slower rate of mutation, we underestimate our empirical H_O across all effective population sizes, while our estimates of N_A (2.29) when $N_e = 100$ are similar to our empirical estimate (Fig. 6(C), (D)). Resampling demonstrates that three samples genotyped at nine loci are sufficient to recover a similar mean H_O (0.54 ± 0.01) and mean N_A (2.59 ± 0.03) to the observed empirical values for Cayo Diablo when $N_e = 50$ (Fig. 7(A), (B)). When sampling draws are considered independently, we recover a value for H_O within 0.1 units in 68/100 draws, and a value for H_O within the first and third quartiles of the distribution 58/100 times (Fig. 7(A)). For N_A, we recover a value within the first and third quartiles of the distribution 63/100 times (Fig. 7(B)). For St. Thomas, we find that our empirical

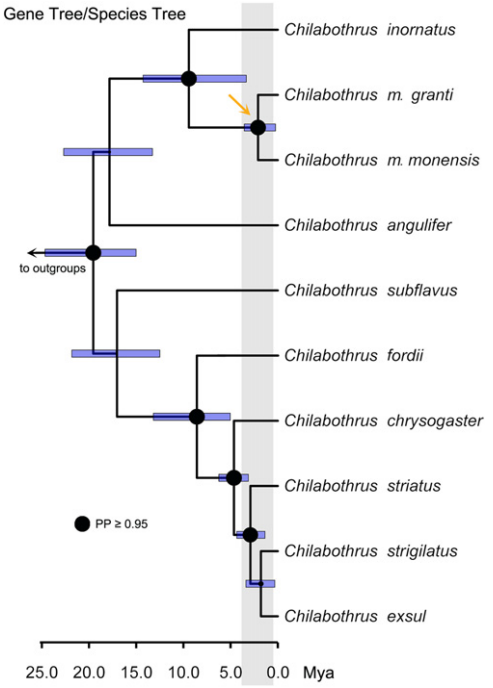

Fig. 5. Fossil-calibrated multilocus species tree for the West Indian *Chilabothrus*. 95% HPD intervals are shown as nodal bars, while black circles at nodes indicate posterior probabilities (PP) ≥0.95. A vertical gray bar spans the 95% HPD interval for the split between *C. m. monensis* and *C. m. granti*, which is indicated by an orange arrow. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Summary statistics (N = sample size; N_A = number of alleles; N_E = effective number of alleles; H_O = observed heterozygosity; H_E = expected heterozygosity; HWE = P-value from Hardy–Weinberg equilibrium test for heterozygote deficit) for nine microsatellite loci. Standard error is in parenthesis and significant values ($P \leq 0.05$) are in **bold**.

Locus	N	Allelic range (bp)	N_A	N_E	H_O	H_E	*HWE*
Ci18	20	259–279	5	2.19	0.35	0.54	**0.00**
Ci24	17	269–281	4	3.19	0.35	0.69	**0.00**
Ci25	18	296–324	6	3.70	0.50	0.73	**0.00**
Ci34	15	267–287	6	3.02	0.67	0.67	0.16
Ci35	16	333–353	5	2.49	0.44	0.60	**0.02**
Ci36	19	200–240	5	2.43	0.47	0.59	0.06
Ci37	19	238–270	7	5.47	0.11	0.82	0.06
Ci41	14	413–441	4	2.39	0.43	0.58	**0.00**
Ci43	16	329–345	5	2.28	0.31	0.56	0.11
Avg.	17.1 (0.68)	–	5.22 (0.32)	3.02 (0.35)	0.40 (0.05)	0.64 (0.03)	–

Table 4
Summary statistics by population for the microsatellite data. N_A = number of alleles, N_E = effective number of alleles, H_O and H_E, observed and expected heterozygosity.

Population	N	N_A	N_E	H_O	H_E
St. Thomas	14	2.78 ± 0.28	1.84 ± 0.22	0.35 ± 0.06	0.40 ± 0.06
Cayo Diablo	3	2.33 ± 0.24	2.13 ± 0.19	0.52 ± 0.15	0.49 ± 0.07
Río Grande	3	2.89 ± 0.26	2.30 ± 0.23	0.54 ± 0.05	0.53 ± 0.05

observations are similar to those expected under a severely reduced effective population size ($N_e = 8$; Fig. 7(C), (D)). Our estimates of the summary statistics for the empirical data ($H_O = 0.35 \pm 0.06$; $H_E = 0.40 \pm 0.06$; $N_A = 2.78 \pm 0.28$) are similar to those obtained from the simulated datasets when $N_e = 8$ ($H_O = 0.36 \pm 0.00$; $H_E = 0.35 \pm 0.00$; $N_A = 2.40 \pm 0.01$), but not when $N_e = 72$ ($H_O = 0.58 \pm 0.00$; $H_E = 0.57 \pm 0.00$; $N_A = 3.93 \pm 0.01$) (Table 5).

We identified a total of 13 islands for assessment of translocation potential (Table A.2). Of these, we consider six to be of high potential for translocation of the VI boa, including Caja de Muertos, Cayo Icacos, Cayo Afuera, Cayo de Tierra, Islote

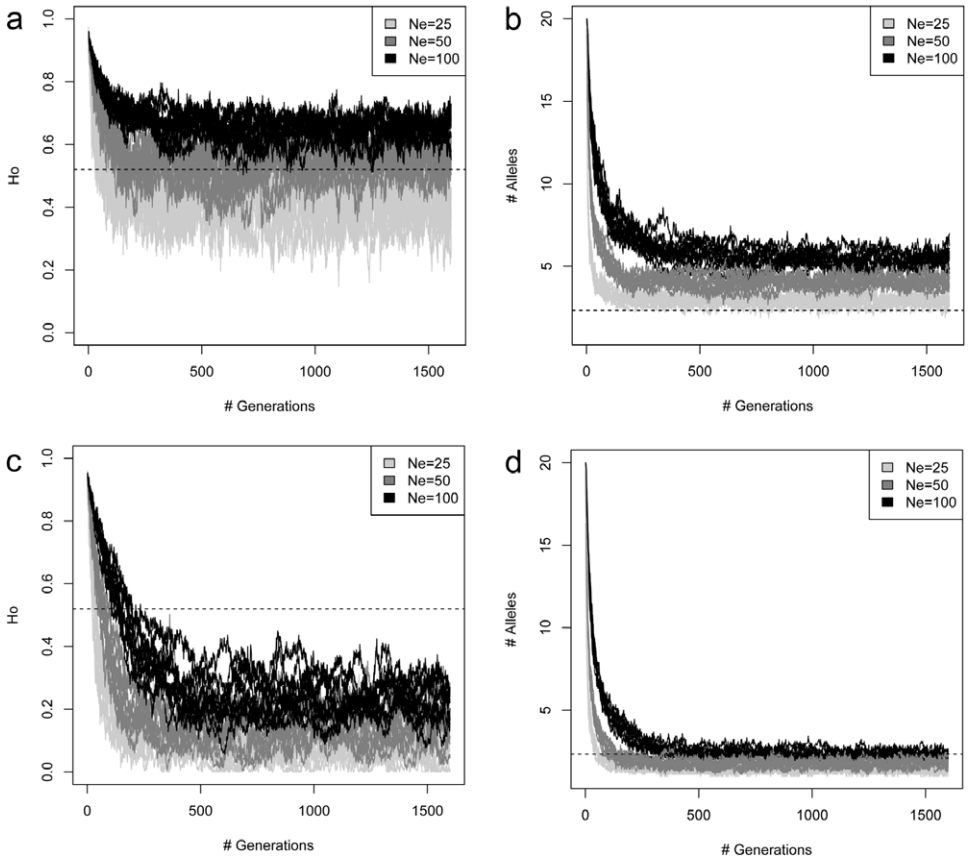

Fig. 6. Relaxation population genetic model for Cayo Diablo, Puerto Rico based on 10 simulations per parameter set for theta-scaled (panels A and B) and fast (panels C and D) mutation rates. Panels A and C show H_0 through time, and panels B and D show the loss of alleles through time owing to drift. All simulations were begun with maximal genetic diversity. The dotted line denotes the empirical estimate for Cayo Diablo.

Table 5
Comparison of summary statistics (N_A = number of alleles, H_O and H_E, observed and expected heterozygosity) for empirically-derived data (Cayo Diablo and St. Thomas) and simulated data for each population. Note that the Cayo Diablo data are from the Θ-scaled mutation rate.

Dataset	N_A	H_O	H_E
Cayo Diablo	2.33 ± 0.24	0.52 ± 0.15	0.49 ± 0.07
Ne = 25	2.89	0.37	–±
Ne = 50	4.10	0.57	–±
Ne = 100	5.71	0.64	–±
Resampling Ne = 25	2.08 ± 0.03	0.42 ± 0.01	0.35 ± 0.01
Resampling Ne = 50	2.59 ± 0.03	0.54 ± 0.01	0.46 ± 0.01
Resampling Ne = 100	3.06 ± 0.03	0.64 ± 0.01	0.54 ± 0.01
St. Thomas	2.78 ± 0.28	0.35 ± 0.06	0.40 ± 0.06
Ne = 72	3.93 ± 0.01	0.58 ± 0.00	0.57 ± 0.00
Ne = 8	2.40 ± 0.01	0.36 ± 0.00	0.35 ± 0.00
Ne = 1000	5.17 ± 0.01	0.70 ± 0.00	0.67 ± 0.00

Monito, and Isla Desecheo. In particular, Cayo Afuera, and Cayo Icacos appear to be the most promising candidates for future translocation of the VI boa.

4. Discussion

4.1. Island phylogeography

Prior to our work no species-specific genetic markers existed for Virgin Islands boas, and genetic analysis of the species was limited to using only four individuals of one subspecies (*C. m. granti*) in phylogenetic analyses of the West Indian clade

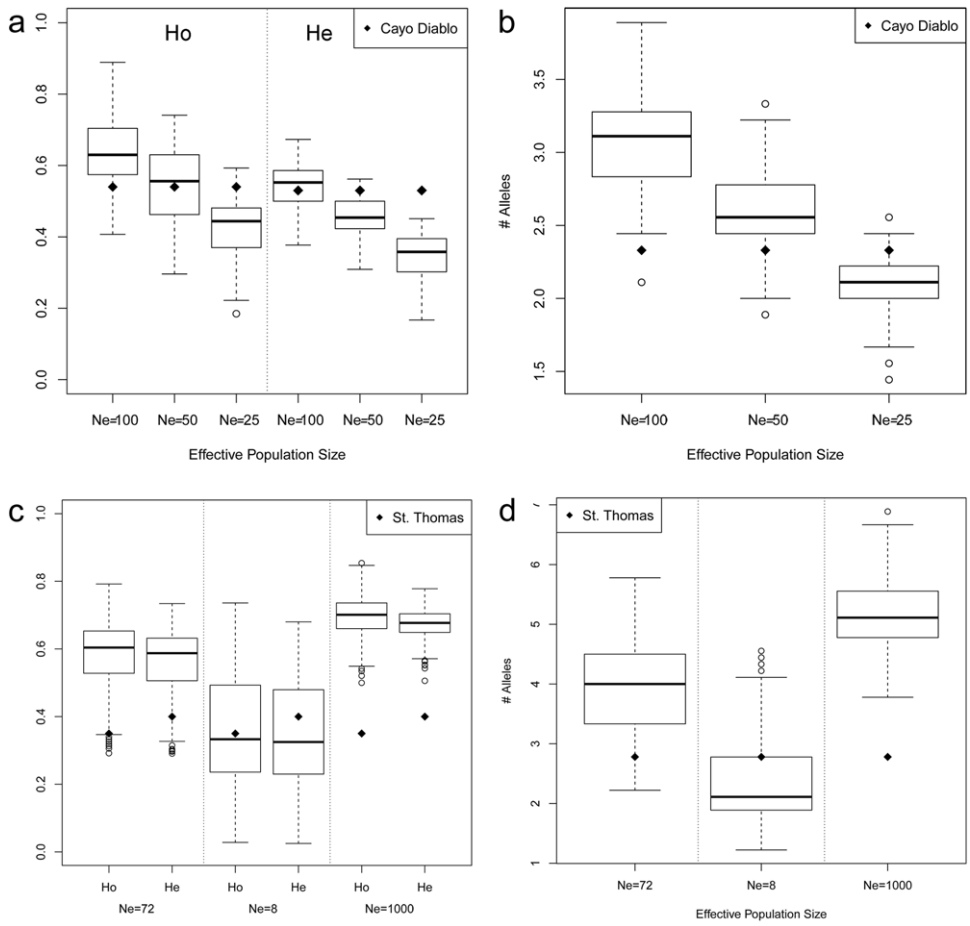

Fig. 7. Panels A and B represent summary statistics calculated from repeated draws of 3 individuals from a relaxation simulation for Cayo Diablo. The resampling scheme was repeated 100 times to obtain parameter distributions. Panel A shows H_E and H_O for three different parameter sets and Panel B shows N_A for each parameter set. The empirical estimate for Cayo Diablo is shown as a black diamond. Panels C and D represent summary statistics for 14 individuals drawn from each of 1200 simulated populations for each parameter set based on a bottleneck model for St. Thomas. Panel A shows H_E and H_O for two bottleneck levels ($N_e = 72$; $N_e = 8$) in comparison to an equilibrium simulation ($N_e = 1000$); while Panel B shows calculations of N_A for each set. The empirical estimate for St. Thomas is shown as a black diamond.

(e.g., Reynolds et al., 2013a). Other phylogenetic studies included only individuals from the Puerto Rico bank (Reynolds et al., 2014a) or captive individuals with unclear origins (e.g. Campbell, 1997; Tzika et al., 2008; Lynch and Wagner, 2009; Rivera et al., 2011 and Pyron et al., 2013), but not individuals from both Isla de Mona and the Puerto Rico Bank. Here we provide an intraspecific genetic analysis for these boas using data from multilocus nucleotide sequences and several novel microsatellite loci. Among VI boas on the PRB, we found a single mtDNA haplotype apparently unique to each island, with a maximum of four mutational steps separating our sampling sites (Fig. 2). Other intraspecific phylogeographic studies of West Indian boas (see Puente-Rolón et al., 2013 for a recent review) have found a mixture of relatively deep and relatively shallow divergence. For example, a similar amount of genetic divergence (though not diversity) to *C. m. granti* was found in the same mtDNA locus in the Turks Island boa (*C. chrysogaster*), whereby populations on the Caicos bank exhibited minimal divergence among islands (Reynolds et al., 2011). However, Turks Island boas showed evidence of intrademic diversity and haplotype sharing across islands, contrary to our finding in the VI boa.

Using a larger phylogenetic dataset across the West Indian boa clade, we generated a time-calibrated coalescent tree for the mtDNA *CYTB* gene (Fig. 4) and a fossil-calibrated multilocus species tree (Fig. 5). Our overall tree topologies are slightly different than Reynolds et al. (2013a), though similar to Reynolds et al. (2014a,b), likely owing to differences in genetic loci used and inference methods (species-tree versus single gene or concatenated analyses). Both phylogenetic analyses support the distinction of *C. m. monensis* and *C. m. granti* with a similar estimate of divergence times in the mid Pleistocene. The mtDNA coalescent analysis provides a slightly older minimum divergence estimate (1.12 Mya for mtDNA, 0.24 Mya for multilocus), which is to be expected given that any single-gene inferred coalescent time must naturally predate (and thus overestimate) the actual time of lineage separation (e.g., Degnan and Rosenberg, 2009).

Since the estimated divergence time of *C. monensis* lineages around the mid Pleistocene, the Puerto Rico bank has been periodically inundated and exposed, allowing potential connections among subpopulations presently restricted to islands (Donn et al., 1962; Heatwole and MacKenzie, 1966). However, Isla de Mona has never been connected to the main island of Puerto Rico since its uplift in the Miocene (Kaye, 1959b), suggesting that boas dispersed westward across the Mona Passage from Puerto Rico. Overwater dispersal has been inferred in other West Indian boid species (Reynolds et al., 2013a), and dispersal in this direction would be concordant with prevailing ocean surface currents (Iturralde-Vinet and MacPhee, 1999; Griffin et al., 2001). Other studies of endemic squamates (*Anolis* and *Sphaerodactylus* lizards) on Isla de Mona have inferred a similar dispersal event from Puerto Rico around the same time period (e.g., Brandley and de Quieroz, 2004; Rodríguez-Robles et al., 2007 and Díaz-Lameiro et al., 2013).

4.2. Novel markers and conservation genetics

While we were able to develop polymorphic microsatellite markers for *C. m. granti* which will be useful in conservation genetic studies of this species, we caution that we have extremely small sample sizes, particularly for two of our populations: Cayo Diablo and Río Grande. Unfortunately the addition of more samples from these locations might not prove to be practical or feasible owing the extreme rarity of this species. Obtaining additional individuals from Río Grande would involve significant field work in an area that is not entirely safe for researchers at night, and our collective observations suggest that finding unique animals is exceedingly difficult.

Somewhat reassuringly, our simulations of a relaxation scenario for Cayo Diablo suggest that some of our estimates are similar to expectations under this demographic history. For instance, our estimate for H_O is consistent with the expectation for a genetic effective population size of $N_e = 50$ (Fig. 6(A)), and our resampling analysis demonstrates that we can frequently recover similar estimates for population genetic summary statistics with only 3 samples and 9 loci, as 68% of our resampling trials at $N_e = 50$ resulted in values within a range of ± 0.1 units around the true mean. Nonetheless, our other simulated estimates were not as robust, indicating that such a low sample size will potentially bias estimates even when using nine polymorphic loci. Furthermore, our simulations using a slower rate of mutation recover a similar N_A and lower H_O relative to observations from the Cayo Diablo population (Fig. 6(C), (D); Table 5). This might be expected owing to our sampling of only three individuals. By sampling so few individuals, we have effectively under-sampled allelic diversity, missing rare alleles in the population and producing an effect similar to a bottleneck (loss of rare alleles at a faster rate than loss of heterozygosity; Nei et al., 1975). Other studies of observed heterozygosity in threatened snake populations (see Table 3 in Jansen et al., 2008) have found a wide range of values for H_O, indicating that idiosyncratic demographic processes are likely influencing these measures as well.

For the St. Thomas population, our simulations show that our estimated population genetic parameters correspond to the values that we would expect to obtain under the scenario of a significant bottleneck ($N_e = 8$; Fig. 7(C), (D)). Using a mean N_e/N_c ratio of 0.15 this would translate to a census population size of fewer than 100 individuals (i.e., Frankham et al., 2014), though we again note that our estimates of H_O and H_E are likely biased owing to an undersampling of these parameters. Other studies of threatened snakes have similarly measured low effective population sizes, ranging from $N_e = 15$ to $N_e = 2528$ (Madsen et al., 1996; Manier and Arnold, 2005; Clark et al., 2008; Marshall et al., 2009; Gibbs and Chiucchi, 2012). Given the continuing disappearance of critical habitat on St. Thomas (Tolson and Henderson, 1993; USFWS, 2009; Platenberg and Harvey, 2010), it is unlikely that census population size will increase in the near future.

In spite of the limitations of sampling endangered species, we report population genetic summary statistics (H_O, H_E, N_A) with the above caveats in mind in the hope that while not conforming to population genetic expectations, they will serve as heuristic measures of these parameters for this species and a basis for comparison to future studies. In addition, some of our samples are temporally separated, with a maximum of approximately 3–4 generations (though still spanning the potential lifespan of any individual snake) separating sampling intervals on Cayo Diablo. Interpretation of population genetic parameters is difficult in this situation, as the expectation would be that a loss of alleles owing to drift would influence estimates between sampling intervals in non-overlapping populations.

Virgin Islands boas also occur on Culebra Island, (a Spanish Virgin [Passage] island) which lies between Cayo Diablo and St. Thomas. We were unable to sample this population; however, it will be important for future studies to do so using the genetic markers described herein. Additionally, we were unable to obtain samples from the British Virgin Islands (BVI). Little is known about the status of VI boas in the BVI aside from the known historical range and the occasional sighting (Lazell, 2005; USFWS, 2009; Perry and Gerber, 2011). Boas are sometimes reported from the other large Passage Island of Vieques, and interestingly an adult female Hispaniolan boa (*C. striatus*) was recently found there (Reynolds et al., 2014b). No contemporary reliable VI boa sightings have been reported from Vieques, though ample suitable habitat now exists. A long history of severe anthropogenic modification, including sugar cane plantations, introduced predators (e.g., Wetmore, 1916), and near-complete deforestation might have destroyed any population of VI boas that once may have occurred on the island

4.3. Conservation, captive breeding, and translocation

Our results suggest some directly applicable conservation strategies. Firstly, Mona and VI boas, while presently conspecific, should be managed separately as distinct evolutionarily significant units (ESUs *sensu*: Crandall et al., 2000;

Moritz, 1994; Ryder, 1986 and Waples, 1991). Though some authors already recognize *C. granti* separately (Platenberg and Harvey, 2010), full elevation to specific status for the VI boa should await a more comprehensive analysis which would include populations on Culebra and the BVI (currently underway; Rodríguez-Robles in litt.). Nevertheless, VI boas should be immediately evaluated for potential elevation to critically endangered status.

Secondly, our findings suggest that translocation should be accomplished with genetically appropriate source populations and that any translocated populations should be genetically monitored (Michaelides et al., 2014; Wright et al., 2014). For instance, translocation to other Cordillera islands off the east coast of Puerto Rico, such as Isla Icacos (DRNA, 2010), should be undertaken with propagules from the Cayo Diablo population following eradication of introduced predators. Any translocations within northeastern Puerto Rico (such as to La Reserva Natural de las Cabezas de San Juan; DRNA, 2010) should be undertaken with propagules from the native population at Río Grande, though realistically it appears that there are so few animals at this site that it would be difficult to initiate a breeding program. We identified at least six islands which warrant further investigation for translocation potential (Table A.2). In particular, Islote Monito and Isla Desecheo might represent translocation targets for the Isla de Mona lineage (*C. m. monensis*). Islote Monito, though a tiny island (0.16 km^2), has some emergent tropical dry forest on the leeward side, is elevated up to 66 m above sea level, and is difficult to access (Kepler, 1978). These islands are important seabird colonies and have been subjected to a rat removal campaigns (García et al., 2002; USFWS, 2011). Both islands are protected by the Commonwealth of Puerto Rico and have robust populations of *Anolis* lizards, though the presence of endemic species and breeding birds means that exploratory studies should evaluate any potential impacts on these populations. Previous studies have identified Cayo Icacos as a suitable target for translocation of *C. m. granti* (DRNA, 2010). We expand on this by suggesting that Caja de Muertos and the Vieques satellites of Cayo de Afuera and Cayo de Tierra represent potential sites for translocation following invasive species removal. The latter two are heavily forested (tropical dry forest), have abundant *Anolis* populations (Revell et al., 2007), and are seldom disturbed by people; aside from the occasional daytime visitor. While Cayo de Afuera is physically separated from Vieques, Cayo de Tierra is periodically connected by a sand spit which could allow dispersal of *Rattus, Herpestes,* and *Felis* to the island. A simple trapping survey could determine whether these predators use this corridor.

Translocations have been successful on two islands: Cayo Ratones (∼5 km northeast of Cayo Diablo), and an undisclosed cay close to St. Thomas (Platenberg and Boulon, 2011). Introduced predators (*Rattus* sp.) were removed from both of these islands prior to translocation. Indeed, VI boas are thought to be highly susceptible to introduced predators (Tolson and García, 1997; Platenberg and Boulon, 2011), and any translocation or reintroduction efforts should account for this. Rat eradication campaigns have met with mixed success in the US Virgin Islands (Savidge et al., 2012); however, campaigns in Puerto Rico have been successful (García et al., 2002; USFWS, 2011) and hence it is plausible that additional islands could be ecologically restored such that VI boas could be reintroduced. However, any introduction campaign should account for potential impacts on native animals and hence be undertaken with the utmost care.

While translocation or reintroduction can be a useful safeguard against loss of remaining native populations (Germano and Bishop, 2009), we suggest that in situ conservation practices be undertaken to focus on preserving extant native populations. For example, it is known that boas on St. Thomas can survive in human-modified habitat, provided some forest remains (Platenberg and Harvey, 2010). Conservation measures could include restriction on damaging development practices (clear-cutting) as well as campaigns to reduce the number of invasive vertebrate predators through spaying of feral cats and trapping of mongoose.

Finally, the genetic tools developed herein will likely prove valuable for assessing current ex situ captive breeding programs for the species. Colonies of *C. m. monensis* and *C. m. granti* are currently maintained by a number of zoological societies, and are actively bred at the Toledo Zoo (Tolson, 1989, 1991). Using these molecular markers breeders might genotype their animals and design breeding pedigrees based on relatedness of individuals which will supplement an existing American Association of Zoological Parks and Aquariums regional studbook. Development of new genetic tools is crucial to conservation planning for VI boas and other endangered species. De novo microsatellite characterization from next generation sequencing can cost between five and seven thousand US dollars (authors' pers. ob.) per species and can represent a significant technical and financial barrier for endangered species researchers, many of whom sadly operate with limited funding. We have demonstrated the utility of these microsatellite markers for cross-species amplification, and we emphasize that if necessary this approach to microsatellite isolation and characterization can yield thousands of potential loci (Castoe et al., 2012), many of which might amplify in other species owing to conserved priming regions. Future studies might also make use of emerging technologies such as Restriction-site Associated DNA makers (RADseq) to generate additional genetic markers for phylogeographic and conservation genetic analyses given the reality of low sample sizes.

Acknowledgments

We are grateful to the Puerto Rico Departamento de Recursos Naturales y Ambientales (DRNA) for permits and assistance. All samples were collected under DRNA permits 2012-EPE-001 (to RGR); 2011-EPE-015, 97-EPE-2, 97 EPE-11, 96 EPE-11, DRN 95-74; US Virgin Islands Department of Planning and Natural Resources (DPNR) permits DRN-93-84, DRN 93-104, and DRN 86-80 (to PJT); US Fish and Wildlife Service Native Endangered Species Recovery Permit # TE63270A-0 (to RGR); US Fish and Wildlife Service Permits SA 86-14, SA 88-2, SA 90-13, and SA 93-15 (to PJT); and a DPNR Section 6 Cooperative Agreement with the United States Fish and Wildlife Service (USFWS). We are grateful for funding from the USFWS Southeast Region Endangered Species Recovery Implementation for related work and for support from the University of Massachusetts

Boston. We would like to thank Rosario Castañeda, Ambika Kamath, and the Losos Lab at Harvard for comments on earlier versions of the manuscript, and Ben Fitzpatrick for assistance with simulation scripts. We thank S. Blair Hedges for discussion related to this work and for assistance with sample curation. We also thank the participants of the 2013 Caribbean and Latin American Boa Specialist Group meeting, held in Arecibo, Puerto Rico, for excellent discussions related to this project. This work has been approved by the University of Massachusetts Boston Institutional Animal Care and Use Committee (IACUC) Protocol no. 2011006, and reviewed annually. We thank the editor and three anonymous reviewers for excellent comments on a previous version of the manuscript.

Appendix A. Supplementary data

Supplementary material related to this article can be found online at http://dx.doi.org/10.1016/j.gecco.2015.02.003.

References

Allendorf, F.W., Luikart, G.H., Aitken, S.A., 2012. Conservation and the Genetics of Populations, second ed. Wiley-Blackwell, United Kingdom.
Avise, J.C., 2004. Molecular Markers, Natural History, and Evolution, second ed. Sinauer Associates, Massachusetts.
Balloux, F., 2001. EASYPOP (Version 1.7): a computer program for population genetics simulations. J. Hered. 92, 301–302.
Booth, W., Million, L., Reynolds, R.G., Burghardt, G.M., Vargo, E.L., Schal, C., Tzika, A.C., Schuett, G.W., 2011. Consecutive virgin births in the new world boid snake, the Colombian Rainbow Boa, *Epicrates maurus*. J. Hered. 102, 759–763.
Brandley, M.C., de Quieroz, K., 2004. Phylogeny, ecomorphological evolution, and historical biogeography of the *Anolis cristatellus* series. Herpetol. Monogr. 18, 90–126.
Brown, J.H., 1971. Mammals on mountaintops: non-equilibrium insular biogeography. Am. Nat. 105, 467–478.
Burbrink, F.T., 2004. Inferring the phylogenetic position of *Boa constrictor* among the Boinae. Mol. Phylogenet. Evol. 34, 167–180.
Campbell, E.W., 1991. The effect of introduced roof rats on bird diversity of Antillean Cays. J. Field Ornithol. 62, 343–348.
Campbell, B.N., 1997. Hic sunt serpentes- molecular phylogenetics and the Boidae (Serpentes: Booidae) (Ph.D. dissertation), Queen's University, Kingston, Ontario, Canada.
Castoe, T.A., Poole, A.W., Jason de Koning, A.P., Jones, K.L., Tomback, D.F., Oyler-McCance, S.J., Fike, J.A., Lance, S.L., Streicher, J.W., Smith, E.N., Pollock, D.D., 2012. Rapid microsatellite identification from Illumina paired-end genomic sequencing in two birds and a snake. PLoS One 7, e30953.
Chakrabarty, P., Warren, M., Page, L.M., Baldwin, C.C., 2013. GenSeq: an updated nomenclature and ranking for genetic sequences from type and non-type sources. ZooKeys 346, 29–41.
Chandler, C.R., Tolson, P.J., 1990. Habitat use by a boid snake, *Epicrates monensis*, and its anoline prey, *Anolis cristatellus*. J. Herpetol. 24, 151–157.
Clark, R.W., Brown, W.S., Stechert, R., Zamudio, K.R., 2008. Integrating individual behavior and landscape genetics: the population structure of timber rattlesnake hibernacula. Mol. Ecol. 17, 719–730.
Clement, M.D., Posada, D., Crandall, K., 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9, 1657–1660.
Cornuet, J.M., Luikart, G., 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144, 2001–2014.
Crandall, K.A., Bininda-Edmonds, O.R.P., Mace, G.M., Wayne, R.K., 2000. Considering evolutionary processes in conservation biology: an alternative to evolutionarily significant units. Trends Ecol. Evol. 15, 290–295.
Crandall, K.A., Templeton, A.R., 1993. Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. Genetics 134, 959–969.
Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9, 772.
Degnan, J.H., Rosenberg, N.A., 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. Trends Ecol. Evol. 24, 332–340.
Departamento de Recursos Naturales y Ambientales (DRNA). 2010. Designación hábitat natural crítico y hábitat natural crítico esencial para la boa de Islas Vírgenes (*Epicrates monensis granti*) en Puerto Rico. http://www.drna.gobierno.pr/boa-islas-virgenes/designacion-habitat-epicrates-monensis-granti. (Accessed January 2014).
Dharmarajan, G., Beatty, W.S., Rhodes, O.E., 2013. Heterozygote deficiencies caused by a Wahlund effect: dispelling unfounded expectations. J. Wildl. Manag. 77, 226–234.
Díaz-Lameiro, A.M., Oleksyk, T.K., Bird-Picó, F.J., Martínez-Cruzado, J.C., 2013. Colonization of islands in the Mona Passage by endemic dwarf geckoes (genus *Sphaerodactylus*) reconstructed withy mitochondrial phylogeny. Ecol. Evol. 3, 4488–4500.
Donn, W.L., Farrand, W.R., Ewing, M., 1962. Pleistocene ice volumes and sea-level lowering. J. Geol. 70, 206–214.
Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUTi and the BEAST 1.7. Mol. Evol. Biol. 29, 1969–1973.
Earnhardt, J.M., Thompson, S.D., Schad, K., 2004. Strategic planning for captive populations: projecting changes in genetic diversity. Anim. Conserv. 7, 9–16.
Emel, S.L., Storfer, A., 2012. A decade of amphibian population genetic studies: synthesis and recommendations. Conserv. Genet. 13, 1685–1689.
England, P.R., Cornuet, J.-M., Berthier, P., Tallmon, D.A., Luikart, G., 2006. Estimating effective population size from linakge disequilibrium: severe bias in small samples. Conserv. Genet. 7, 303–308.
Fairbanks, R.G., 1989. A 17,000-year glacio-eustatic sea level record: influence of glacial melting rates on the Younger Dryas event and deep-ocean circulation. Nature 342, 637–641.
Fitzpatrick, B.M., Fordyce, J.A., Niemiller, M.L., Reynolds, R.G., 2012. What can DNA tell us about biological invasions? Biol. Invasions 14, 245–253.
Fontanella, F.M., Feltrin, N., Avila, L.J., Sites Jr., J.W., Morando, M., 2012. Early stages of divergence: phylogeography, climate modeling, and morphological differentiation in the South American lizard *Liolaemus petrophilus* (Squamata: Liolaemidae). Ecol. Evol. 2, 792–808.
Frankham, R., 2005. Genetics and extinction. Biol. Conserv. 126, 131–140.
Frankham, R., 2006. Evolutionary conservation genetics. In: Fox, C.W., Wolf, J.B. (Eds.), Evolutionary Genetics. Oxford University Press, New York.
Frankham, R., Bradshaw, C.J.A., Brook, B.W., 2014. Genetics in conservation management: revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. Biol. Conserv. 170, 56–63.
García, M.A., Diez, C.E., Álvarez, A.O., 2002. Eradication of *Rattus rattus* from Monito Island, West Indies. In: Veitch, C.R., Clout, M.N. (Eds.), Turning the Tide: The Eradication of Invasive Species (Proceedings of The International Conference On Eradication of Island Invasives). IUCN—The World Conservation Union, Switzerland.
Germano, J.M., Bishop, P.J., 2009. Suitability of amphibians and reptiles for translocation. Conserv. Biol. 23, 7–15.
Gibbs, H.L., Chiucchi, J.E., 2012. Inbreeding, body condition, and heterozygisity-fitness correlations in isolated populations of the endangered eastern massasauga rattlesnake (*Sistrurus c. catenatus*). Conserv. Genet. 13, 1133–1143.
Griffin, D.W., Kellogg, C.A., Shin, E.A., 2001. Dust in the wind: long range transport of dust in the atmosphere and its implications for global public and ecosystem health. Glob. Change Hum. Health 2, 20–33.
Guindon, S., Gascuel, O., 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Syst. Biol. 52, 696–704.
Hailey, A., Wilson, B.S., Horrocks, J.A. (Eds.), 2011. Conservation of Caribbean Island Herpetofaunas, Vol.1. Brill, Netherlands.
Hale, M.L., Burg, T.M., Steeves, T.E., 2012. Sampling for microsatellite-based population genetic studies: 25 to 30 individuals per population is enough to accurately estimate allele frequencies. PLoS One 7, e45170.

Harvey, D.S., Platenberg, R.J., 2009. Predicting habitat use from opportunistic observations: a case study of the Virgin Islands tree boa (*Epicrates granti*). Herpetol. J. 19, 111–118.
Heatwole, H., MacKenzie, F., 1966. Herpetogeography of Puerto Rico. IV. Paleogeography, faunal similarity an endemism. Evolution 21, 429–438.
Hedrick, P.W., 2009. Genetics of Populations, fourth ed. Jones and Bartlett, Massachusetts.
Heled, J., Drummond, A.J., 2010. Bayesian inference of species trees from multilocus data. Mol. Biol. Evol. 27, 570–580.
Hill, W.G., Robertson, A., 1968. Linkage disequilibrium in finite populations. Theor. Appl. Genet. 38, 226–231.
Holsinger, K.E., 2000. Demography and extinction in small populations. In: Young, A.C., Clarke, G.M. (Eds.), Genetics, Demography, and Viability of Fragmented Populations. Cambridge University Press, United Kingdom.
Iturralde-Vinet, M., MacPhee, R., 1999. Paleogeography of the Caribbean region, implications for Cenozoic biogeography. Bull. Am. Mus. Nat. Hist. 238, 1–95.
IUCN. 2014. IUCN Red List of Threatened Species, Version 2014.2. http://www.iucnredlist.org. (Accessed 24 October 2014).
Jansen, K.P., Mushinsky, H.R., Karl, S.A., 2008. Population genetics of the mangrove saltmarsh snake, *Nerodia clarkii compressicauda*, in a linear, fragmented habitat. Conserv. Genet. 9, 401–410.
Kaye, C.A., 1959a. Shoreline Features and Quaternary Shoreline Changes in Puerto Rico. In: US Geol. Surv. Prof Pap, vol. 317-B. United States Government Printing Office, Washington, DC, pp. 49–140.
Kaye, C.A., 1959b. Geology of Isla Mona Puerto Rico, and Notes on Age of Mona Passage. In: US Geol. Surv. Prof Pap, vol. 317-C. United States Government Printing Office, Washington, DC.
Kepler, C.B., 1978. Breeding ecology of sea birds on Monito Island, Puerto Rico. Condor 80, 72–87.
Kim, S., Carbone, L., Becquet, C., Mootnick, A.R., Jiang Li, D., de Jong, P.J., Wall, J.D., 2011. Patterns of genetic variation within and between gibbon species. Mol. Biol. Evol. 28, 2211–2218.
Kuch, U., Koegh, J.S., Wigel, J., Smith, L.A., Mebs, D., 2005. Phylogeography of Australia's king brown snake (*Pseudechis australis*) reveals Pliocene divergence and Pleistocene dispersal of a top predator. Naturwissenschaften 92, 121–127.
Kuhner, M.K., Beerli, P., Yamato, J., Felsenstein, J., 2000. Usefulness of single nucleotide polymorphism data for estimating population parameters. Genetics 156, 439–447.
Lande, R., 1988. Genetics and demography in biological conservation. Science 241, 1455–1460.
Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., et al., 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948.
Lazell, J.D., 2005. Island: Fact and Theory in Nature. University of California Press, California.
Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451–1452.
Lighty, R.G., Macintyre, I.G., Stuckenrath, R., 1982. *Acropora palmata* reef framework: a reliable indicator of sea level in the Western Atlantic for the past 10,000 years. Coral Reefs 1, 125–130.
Lynch, M., 2010. Evolution of the mutation rate. Trends Genet. 26, 345–352.
Lynch, V.J., Wagner, G.P., 2009. Did egg-laying boas break Dollo's law? Phylogenetic evidence for reversal to oviparity in sand boas (*Eryx*: Boidae). Evolution 64, 207–216.
Macey, J.R., Schulte II, J.A., Ananjeva, N.B., Larson, A., Rastegar-Pouyani, N., Shammakov, S.M., Papenfuss, T.J., 1998. Phylogenetic relationships among agamid lizards of the *Laudakia caucasia* species group: testing hypotheses of biogeographic fragmentation and an area cladogram for the Iranian Plateau. Mol. Phylogenet. Evol. 10, 118–131.
Madsen, T., Stille, B., Shine, R., 1996. Inbreeding depression in an isolated population of adders *Vipera berus*. Biol. Conserv. 75, 113–118.
Malhotra, A., Thorpe, R.S., 2000. The dynamics of natural selection and vicariance in the Dominican anole: patterns of within-island molecular and morphological divergence. Evolution 54, 245–258.
Manier, M.K., S.J., Arnold, 2005. Population genetic analysis identifies source-sink dynamics for two sympatric garter snake species (*Thamnopphis elegans* and *Thamnophis sirtalis*). Mol. Ecol. 14, 3965–3976.
Marshall Jr., J.C., Kingsbury, B.A., Minchella, D.J., 2009. Microsatellite variation, population structure, and bottlenecks in the threatened copperbelly water snake. Conserv. Genet. 10, 465–476.
Mayer, G.C., 2011. Puerto Rico and the Virgin Islands. In: Powell, R., Henderson, R.W. (Eds.), Island Lists of West Indian Amphibians and Reptiles. In: Bull. Florida Mus. Nat. Hist., vol. 51. pp. 85–166.
Mayer, G.C., Lazell Jr., J.D., 1988. Distributional records for reptiles and amphibians from the Puerto Rico Bank. Herpetol. Rev. 19, 23–24.
Meirmans, P.G., Hedrick, P.W., 2011. Assessing population structure: F_{ST} and related measures. Mol. Ecol. Resour. 11, 5–18.
Michaelides, S., Cole, N., Funk, S.M., 2014. Translocation retains genetic diversity iof a threatened endemic reptile in Mauritius. Conserv. Genet. http://dx.doi.org/10.1007/s10592-014-0691-z.
Morando, M., Avila, L.J., Sites Jr., J.W., 2003. Sampling strategies for delimiting species: genes, individuals, and populations in the *Liolaemus elongatus-kriegi* complex (Squamata: Liolaemidae) in Andean-Patagonian South America. Syst. Biol. 52, 159–185.
Moritz, C., 1994. Defining evolutionarily significant units for conservation. Trends Ecol. Evol. 9, 373–375.
Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Ke, J., 2000. Biodiversity hotspots for conservation priorities. Nature 403, 853–858.
Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89, 583–590.
Nei, M., Maruyama, T., Chakraborty, R., 1975. The bottleneck effect and genetic variability in populations. Evolution 29, 1–10.
Nellis, D.W., Norton, R.L., MacLean, W.P., 1983. On the biogeography of the Virgin Islands boa, *Epicrates monensis granti*. J. Herpetol. 17, 413–417.
Ohta, T., Kimura, M., 1969. Linkage disequilibrium due to random genetic drift. Genet. Res. 13, 47–55.
Peakall, R., Smouse, P.E., 2006. Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes 6, 288–295.
Perry, G., Gerber, G.P., 2011. Conservation status of amphibians and reptiles in the British Virgin Islands: status and patterns. In: Hailey, A., Wilson, B.S., Horrocks, J.A. (Eds.), Conservation of Caribbean Island Herpetofaunas, Volume 2: Regional Accounts of the West Indies. Brill, Netherlands.
Platenberg, R.J., Boulon Jr., R.H., 2011. Conservation status of reptiles and amphibians in the US Virgin Islands. In: Hailey, A., Wilson, B.S., Horrocks, J.A. (Eds.), Conservation of Caribbean Island Herpetofaunas, Volume 2: Regional Accounts of the West Indies. Brill, Netherlands.
Platenberg, R.J., Harvey, D.S., 2010. Endangered species and land use conflicts: a case study of the Virgin Islands boa (*Epicrates granti*). Herpetol. Conserv. Biol. 5, 548–554.
Pluzhnikov, A., Donnelly, P., 1996. Optimal sequencing strategies for surveying molecular genetic diversity. Genetics 144, 1247–1262.
Puente-Rolón, A.R., Reynolds, R.G., Revell, L.J., 2013. Preliminary genetic analysis supports cave populations as targets for conservation in the endemic, endangered Puerto Rican boa (Boidae: *Epicrates inornatus*). PLoS One 8, e63899.
Pyron, R.A., Burbrink, F.T., Wiens, J.J., 2013. A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. BMC Evol. Biol. 13, 93.
R Development Core Team, 2013. R: a language and environment for statistical computing. R. Foundation for Statistical Computing, Vienna, Austria.
Rambaut, A., Suchard, M.A., Xie, D., Drummond, A.J., 2013. Tracer v1.5, Available from http://beast.bio.ed.ac.uk/Tracer.
Revell, L.J., Harmon, L.J., Langerhans, R.B., Kolbe, J.J., 2007. A phylogenetic approach to determining the importance of constraint on phenotypic evolution in the Neotropical lizard *Anolis cristatellus*. Evol. Ecol. Res. 9, 261–282.
Reynolds, R.G., 2011. Status, conservation, and introduction of amphibians and reptiles in the Turks and Caicos Islands, British West Indies. In: Hailey, A., Wilson, B.S., Horrocks, J.A. (Eds.), Conservation of Caribbean Island Herpetofaunas, Volume 2: Regional Accounts of the West Indies. Brill, Netherlands.
Reynolds, R.G., Fitzpatrick, B.M., 2013. Tests of two methods for identifying founder effects in metapopulations reveal substantial type II error. Genetica 141, 119–131.
Reynolds, R.G., Gerber, G.P., 2012. Ecology and conservation of the Turks Island Boa (*Epicrates c. chrysogaster*: Squamata: Boidae) on Big Ambergris Cay. J. Herpetol. 46, 578–586.
Reynolds, R.G., Gerber, G.P., Fitzpatrick, B.M., 2011. Unexpected shallow genetic divergence in Turks Island Boas (*Epicrates c. chrysogaster*) reveals single evolutionarily significant unit for conservation. Herpetologica 67, 477–486.

Reynolds, R.G., Niemiller, M.L., Hedges, S.B., Dornburg, A., Puente-Rolón, A.R., Revell, L.J., 2013a. Molecular phylogeny and historical biogeography of West Indian boid snakes (*Chilabothrus*). Mol. Phylogenet. Evol. 68, 461–470.
Reynolds, R.G., Niemiller, M.L., Revell, L.J., 2014a. Toward a Tree-of-Life for the boas and pythons: multilocus species-level phylogeny with unprecedented taxon sampling. Mol. Phylogenet. Evol. 71, 201–213.
Reynolds, R.G., Puente-Rolón, A.R., Barandiaran, M., Revell, L.J., 2014b. Hispaniolan boa (*Chilabothrus striatus*) on Vieques Island, Puerto Rico. Herpetol. Notes 7, 121–122.
Reynolds, R.G., Puente-Rolón, A.R., Kolodzaike, K., Butler-Smith, T., 2014c. Isolation and characterization of 23 novel polymorphic microsatellite markers from the endangered Puerto Rican boa (*Chilabothrus inornatus*) using paired-end Illumina shotgun sequencing. Conserv. Genet. Res. 6, 107–109.
Reynolds, R.G., Puente-Rolón, A.R., Reed, R.N., Revell, L.J., 2013b. Genetic analysis of a novel invasion of Puerto Rico by an exotic constricting snake. Biol. Invasions 15, 953–959.
Rivera, P.C., Di Cola, V., Martínez, J.J., Gardenal, C.N., Chiaraviglio, M., 2011. Species delimitation in the continental forms of the genus *Epicrates* (Serpentes, Boidae) integrating phylogenetics and environmental niche models. PLoS One 6, e22199.
Rodríguez-Robles, J.A., Jezkova, T., García, M.A., 2007. Evolutionary relationships and historical biogeography of *Anolis desechensis* and *Anolis monensis*, two lizards endemic to small islands in the eastern Caribbean Sea. J. Biogeogr. 34, 1546–1558.
Ryder, O.A., 1986. Species conservation and systematics: the dilemma of subspecies. Trends Ecol. Evol. 1, 9–10.
Savidge, J.A., Hopken, M.W., Witmer, G.W., Jojola, S.M., Pierce, J.J., Burke, P.W., Piaggio, A.J., 2012. Genetic evaluation of an attempted *Rattus rattus* eradication on Congo Cay, US Virgin islands, identifies importance of eradication units. Biol. Invasions 14, 2343–2354.
Sax, D.F., Early, R., Bellemare, J., 2013. Niche syndromes, species extinction risks, and management under climate change. Trends Ecol. Evol. 28, 517–523.
Schuelke, M., 2000. An economic method for the fluorescent labeling of PCR fragments. Nat. Biotechnol. 18, 233–234.
Stephens, M., Donnelly, P., 2003. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. Am. J. Hum. Genet. 73, 1162–1169.
Stephens, M., Smith, N.J., Donnelly, P., 2001. A new statistical method for haplotype reconstruction from population data. Am. J. Hum. Genet. 68, 978–989.
Storfer, A., 1999. Gene flow and endangered species translocations: a topic revisited. Biol. Conserv. 87, 173–180.
Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729.
Tolson, P.J., 1989. Breeding the Virgin Islands boa (*Epicrates monensis granti*) at the Toledo Zoological Gardens. Int. Zoo Yearb. 28, 163–167.
Tolson, P.J., 1991. Captive breeding and reintroduction: recovery efforts for the Virgin Islands boa (*Epicrates monensis granti*). Endanger. Species Updat. 8, 52–53.
Tolson, P.J., 1996. Conservation of *Epicrates monensis* on the satellite islands of Puerto Rico. In: Powell, R., Henderson, R.W. (Eds.), Contribution to West Indian Herpetology: A Tribute to Albert Schwartz. Society for the Study of Amphibians and Reptiles.
Tolson, P.J., García, M.A., 1997. Mona/Virgin Islands Boa: A US/Puerto Rico Partnership Seeks to Recover an Endangered Boa. AZA Species Survival Plan Profile. American Zoo and Aquarium Association.
Tolson, P.J., Henderson, R.W., 1993. The Natural History of West Indian Boas. R&A Publishing Ltd., England.
Tolson, P.J., Henderson, R.W., 2011. An overview of snake conservation in the West Indies. In: Hailey, A., Wilson, B.S., Horrocks, J.A. (Eds.), Conservation of Caribbean Island Herpetofaunas. Vol. 1. Brill, Netherlands.
Tzika, A.C., Koenig, S., Miller, R., Garcia, G., Remy, C., Milinkovitch, M.C., 2008. Population structure of an endemic vulnerable species, the Jamaican boa (*Epicraes subflavus*). Mol. Ecol. 17, 533–544.
US Fish and Wildlife Service (USFWS), 1984. Mona Boa Recovery Plan. USFWS, Southeast Region, Atlanta.
US Fish and Wildlife Service (USFWS), 1986. Virgin Islands Tree Boa Recovery Plan. USFWS, Southeast Region, Atlanta.
US Fish and Wildlife Service (USFWS), 2009. Virgin Islands Tree Boa (*Epicrates monensis granti*) 5-Year Review: Summary and Evaluation. USFWS, Southeast Region, Boquerón.
US Fish and Wildlife Service (USFWS), 2011. Final Environmental Assessment, Desecheo National Wildlife Refuge Rat Eradication Project. USFWS, Southeast Region, Boquerón.
Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., Shipley, P., 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4, 535–538.
Wakeley, J., Hey, J., 1997. Estimating ancestral population parameters. Genetics 145, 847–855.
Waples, R.S., 1991. Pacific salmon, *Oncorhynchus* spp., and the definition of species under the Endangered Species Act. Mar. Fish. Rev. 53, 11–22.
Waples, R.S., 2006. A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked loci. Conserv. Genet. 7, 167–184.
Waples, R.S., Do, C., 2008. LDNE: a program for estimating effective population size from data on linkage disequilibrium. Mol. Ecol. Res. 8, 753–756.
Wetmore, A., 1916. The birds of Vieques Island, Porto Rico. Auk 33, 403–419.
Wright, D.J., Spurgin, L.G., Collar, N.J., Komdeur, J., Burke, T., Richardson, D.S., 2014. The impact of translocations on neutral and functional genetic diversity within and among populations of the Seychelles warbler. Mol. Ecol. 23, 2165–2177.
Wüster, W., Salomão, M.G., Quijada-Mascareñas, J.A., Thorpe, R.S., BBBSP, , 2002. Origins and evolution of the South American pitviper fauna: evidence from mitochondrial DNA sequence analysis. In: Schuett, G.W., Höggren, M., Douglas, M.E., Greene, H.W. (Eds.), Biology of the Vipers. Eagle Mountain Publishing, Utah.
Zamudio, K.R., Greene, H.W., 1997. Phylogeography of the bush-master (*Lachesis muta*: Viperidae): implications for neotropical biogeography, systematics, and conservation. Biol. J. Linn. Soc. 62, 421–442.