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Origin of the Invasive Boa Constrictor population in St. Croix, U.S. Virgin Islands

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Abstract

Boa constrictors (*Boa constrictor*) are an extremely diverse species of snake with a Neotropical range stretching from Mexico to Argentina. Their generalist diet, high fecundity, and live birth strategy have made them extremely adaptable to a wide variety of environments. Due to these exceptionally adaptive traits they have great potential to become an invasive species. In the summer of 2013 the first *B. constrictor* sightings were reported on the U.S. Virgin Island of Saint Croix. As of the spring of 2016 twenty-one individuals, including three juveniles and three adults exceeding 2.25 meters in length, have been found on the west coast of the island, north of the city of Frederiksted. Due to the presence of juveniles it is believed that a successful invasive population has been established on the island. Despite their abundance, the geographic origin and subspecies of the St. Croix population was unknown. Through analysis of the *CYTB* gene from the shed-skin of these snakes, their subspecies, *B. c. imperator* was determined; their geographic origin can then be inferred. This information has important implications for management, prevention of further introductions, and further study of the invasive population. This study represents a unique opportunity to observe the onset of an invasion by a constricting snake in an island context.

Keywords: Invasive, Boa Constrictor, Population

1. Introduction

Boa constrictors (*Boa constrictor*) inhabit the Neotropics from Northern Mexico to Argentina, the widest range of any terrestrial snake species[6]. There are eight subspecies of *B. constrictor*, each uniquely adapted to its habitat[9, 13]. *B. constrictor* can inhabit a variety of non-native environments because of its broad diet, high fecundity, and live birth strategy. Given its potential for adaptation, *B. constrictor* has become an established invasive species on islands such as Aruba, Puerto Rico, and Cozumel[3, 12]. Invasive species pose a great threat to endemic ecosystems as they can destroy habitat and become fierce competitors with native biota[11]. With little natural competition and limited chance for migration, endemic island species are particularly susceptible to extirpation as a result of invasion[3].

Depending on their age and size invasive, *B. constrictor* can be a competitor with native biota for multiple food sources and thus, disrupt ecosystem function. Juvenile *B. constrictor* will typically feed on amphibians, reptiles, small mammals, and birds. The endangered St. Croix ground lizard (*America polops*) as well as the endemic St. Croix Anole (*Anolis acutus*) could be directly threatened by the presence of *B. constrictor*. Due to its similar diet, invasive *B. constrictor* on St. Croix could also represent a threat and source of competition for the endangered Virgin Island Tree Boa (*Epicrates monensis*) on the neighboring island of St. Thomas if it were to spread there. While it is characteristic of invasive species to lack natural predators, smaller *B. constrictor* are often the prey of non-native mongoose (*Herpestes javanicus*), another deleterious invasive species found on many Neotropical islands, including St. Croix[5].

Unlike juveniles, adult boas have no natural predators and will often prey upon medium-sized mammals[11]. Thus, an invasive *B. constrictor* population represents a threat to a wide variety of endemic and introduced island biota.

Invasions and subsequent population establishments of *B. constrictor* have been observed on many islands including Aruba, Puerto Rico, and Cozumel[9, 11, 12]. In the summer of 2013, an unspecified subspecies of *B. constrictor* sighting was reported on the island of St. Croix in the U.S. Virgin Islands. As of the spring of 2016, twenty-one boas have been found on the island of St. Croix. The boas found have mostly been adults but represent a wide variety of life stages: three have been over 2.25 meters long; three have been juveniles, less than one meter; and the remaining 15, between 1.5-2 meters in length. These numbers were procured from William Coles, chief of wildlife from the St. Croix U.S. Fish and Wildlife Service. The presence of such a variety of boas, particularly juveniles, suggests that there is an introduced breeding population on St. Croix.

The boas found on St. Croix thus far have been highly variable in terms of physical appearance and size. Indeed, many of the individuals have a mixture of *B.c. imperator* and *B.c. constrictor* traits. For example, a range of saddle counts has been found on the island between 20 and 24, characteristic of *B.c. constrictor* and *B.c. imperator* respectively[13]. Isolated and fused saddle patterns indicative of *B.c. constrictor* and *B.c. imperator* respectively have also been observed in the St. Croix population. Interestingly most *B.c. imperator* found in Mexico and Central America rarely exceed 1.83m in length, while a number of individuals on St. Croix have been observed to exceed 2.4m in length. Such sizes are characteristic of either *B.c. constrictor* or Colombian *B.c. imperator*. The large size of Colombian *B.c. imperator* is believed to stem from gene flow between subspecies with the neighboring *B.c. constrictor* subspecies[13]. Thus the phenotypic mixture of *B.c. imperator* and *B.c. constrictor* observed in the St. Croix *B. constrictor* population could be explained if they were Colombian *B.c. imperator*. While this could provide an explanation for the ambiguous identity, it is also likely that many different subspecies have been introduced to the island. If the St. Croix *B. constrictor* population comes from an extremely diverse gene pool, its heterozygosity could aid in its invasive potential.

In spite of their abundance, the origin of the invasive boas on St. Croix remains a mystery. Knowledge of the subspecies and geographic origin can be important tools for management and prevention of further introductions as the life history traits of different populations may vary. In the summer of 2016, nine shed *B. constrictor* skins were collected from the St. Croix U.S. Fish and Wildlife Service and brought to the University of North Carolina at Asheville for genetic analysis. The aim of the genetic analysis was to determine the subspecies and thus the geographic region of origin of the invasive population. In order to determine the subspecies the *CYTB* gene, a highly conserved mitochondrial gene involved in the electron transport chain, was isolated and amplified from the shed skin DNA. Once sequenced, said DNA can be used to infer the subspecies and region of origin from the matrilineal line[1]. This information could be the key to understanding how the boas were introduced, how further introductions could be avoided, and management of the existing population on St. Croix.

2. Methodology

2.1 DNA Extraction

DNA samples were obtained from shed boa skins of captive boas provided by the St. Croix U.S. Fish & Wildlife Service. The method of DNA extraction was informed by the work of James W. Fetzner, Jr.'s paper *Extracting High-Quality DNA from Shed Reptile Skins: A Simplified Method*[8]. Methods were modified to accommodate the amount of shed skin available. Three-inch square skin samples were cut from each of the nine samples. These skins were then placed in liquid nitrogen to increase their rigidity. Once brittle, the skins were ground to a powder using a mortar and pestle, leaving a few pieces of skin intact.

The ground samples were placed into individual 2700 µL solutions of cell lysis buffer in order to lyse the cells, break down unwanted proteins, and release DNA into the solution. The lysis buffer was a solution of 10mM Tris-base, 10mM EDTA, 2% sodium dodecyl sulfate (SDS), and 27 µL of proteinase K (20mg/mL) at pH 8.0. The samples were mixed and inverted several times and then placed into a 55ºC water bath over night.

With DNA released from the cells into the solution the samples were taken out of the water bath and allowed to cool to room temperature. Once the samples reached room temperature, 12 µL of RNAse A (10 mg/mL) were added to each sample to break down any RNA in the solution. The samples were then placed into a 37ºC bath for 1 hour to facilitate the breakdown of RNA. After the bath, the samples were cooled to room temperature and 900 µL of 7.5 M ammonium acetate were added to each sample. To ensure even mixing, each sample was then vortexed for 10 seconds

and placed on ice for fifteen minutes. After the ice bath each sample was centrifuged at 14,000 rpm for 3 minutes to pellet any remaining skin fragments, SDS, or cell debris.

Upon the formation of a pellet the supernatant was drawn from each sample and pipetted into a new, appropriately labeled 1.5 mL microcentrifuge tube. The new microcentrifuge tube was centrifuged again for 3 minutes at 14,000 rpm to pellet any remaining particles within the supernatant. After the second centrifugation the supernatant was transferred to a new, appropriately labeled 1.5 mL microcentrifuge tube with 900 µL of isopropanol to precipitate the DNA. The microcentrifuge tube containing isopropanol and the DNA solution was then inverted 20 times and centrifuged at 16,000x *g* for 2 minutes to pellet DNA precipitate.

Once DNA had been pelleted the supernatant was gently poured off. Five hundred microliters of 70% ethanol were then added to the sample to wash the pellet. Next the samples were centrifuged for an additional 2 minutes at 16,000x *g* to pellet the DNA. Upon completion of centrifugation the ethanol supernatant was poured off. Each microcentrifuge tube containing a DNA pellet was then inverted and allowed to dry for 20 minutes until all remaining ethanol evaporated. Finally the DNA pellet was resuspended in 30 µL of TE buffer for storage (10mM Tris-base, 0.1 MM EDTA at pH 8.0) (Fetzner method).

The resuspended DNA pellet solution was purified via phenol-chloroform purification. To each DNA solution an equal volume (~30µL) of phenol: chloroform was added. The tube was then inverted 20 times. Each sample was centrifuged at 12,000x *g* for 1 minute. Next the samples were removed from the centrifuge and a division between the aqueous and the white organic phase of the sample was visible in the microcentrifuge tube. The top, aqueous phase was pipetted from the sample and placed into a new, appropriately labeled microcentrifuge tube. The phenol chloroform protocol was repeated until no white, organic phase was visible within the sample.

2.2 Mitochondrial Locus Amplification

Once DNA was extracted and purified, a polymerase chain reaction (PCR) was then used to amplify the *cytochrome b* gene (*CYTB*, 1112 bp)[2]. The primers used in amplifying the *CYTB* sequence were: H (forward) 5'-CTT TGG TTT ACA AGA ACA ATG CTT TA-3' and L (reverse) 5'-GAC CTG TGA TMT GAA AAC CAY CGG TGT-3'[4].

The following solution was used for the 25µL PCR reaction: 10.4µL of deionized water, 5µL of Flexi buffer, 2.5µL of $MgCl_2$, 1.25µL of primer H, 1.25µL of primer L, 0.125µL of *Taq* polymerase and 3 µL of template DNA (Reynolds personal communication). Each tube containing this mixture was placed into a thermocycler with the following program: 1) 94ºC for 4 minutes, 2) 94ºC for 40 s, 3) 55ºC for 30s, 4) 72ºC for 1 minute, 5) return to step 2 39x, and 6) 72ºC for 5 minutes[4].

2.3 Sequencing

Upon completion of PCR, DNA bands were visualized on a 1% agarose gel and prepared for Sanger sequencing. Sequencing reaction preparation began with purification of PCR samples using the Qiagen QIAquick purification kit. Then, for each individual, two samples were prepared for sequencing. Each sample was prepared in a 1.5 mL centrifuge tube with 25.5µL of deionized water and 6µL of purified DNA template. For each individual, one centrifuge tube contained 4.5µL of primer H and the other had the same volume of primer L. Once sequenced, the results were contiguously aligned and analyzed using Basic Local Alignment Search Tool (BLAST) to determine their similarity to known *B. constrictor* subspecies sequences.

3. Results

Only two of the nine *B. constrictor* samples yielded quality results: individuals JV1 and JV2, both juvenile boas. The BLAST search revealed that the *CYTB* sequences of these two individuals were likely from the *B.c. imperator* lineage. Individual JV1 shared a 99% identity with 772 out of 775 nucleotide identities matching the *B.c. imperator* sequence. Further analysis revealed that this individual shared a 98% match to the Central American and Mexican population of *B.c. imperator* paired to NCBI accession number KJ621476[10]. JV2 shared a 98% identity with 359 out of 366 nucleotides matching the *B.c. imperator* sequence. Interestingly, further analysis of its sequence revealed a 100% match to a Colombian population of *B.c. imperator* with the accession number KX150405[2].

Table 1. Percent identity of individuals between subspecies

Individual	% Similarity to *B.c. imperator*	% Similarity to *B.c. constrictor*
JVI	99%	91%
JV2	98%	91%

4. Discussion

It is clear that these two individuals have mitochondrial DNA that belongs to the subspecies *B.c. imperator*. *B.c. imperator* inhabit a range spanning from Mexico to northern Colombia and thus it can be inferred that this is the original geographic region of origin for these individuals[7]. Interestingly, the genetic lineage of both individuals can be traced to distinct populations in different parts of the native region. The *CYTB* sequence for JV1 is 98% similar to a Central American and Mexican population described by Suárez-Atilano et al.[10]. Likewise JV2 shares a 100% identity match with a Colombian population described by Card et al.[2]. Based on these results it seems likely that the *B. constrictor* population of St. Croix is of mixed heritage and it is possible that there was more than one introduction.

The findings of this study confirm the hypothesis that the *B. constrictor* population is at least in part of Colombian origin. The boas of St. Croix have a phenotype that resembles a blend of *B.c. imperator* in terms of saddle pattern and saddle number and *B.c. constrictor* in terms of size. The saddle pattern and number of the St. Croix population of *B. constrictor* is emblematic of the classic, highly defined pattern of *B.c. imperator*. However, despite the similarity of the saddle pattern, several individuals from the St. Croix *B. constrictor* population have been found that are between 2.4m and 2.7m in length. Such lengths greatly exceed the standard length of Central American *B.c. imperator* with the exception of the larger, Colombian populations of *B.c. imperator*[13]. All of this data suggests a Colombian origin of the St. Croix *B. constrictor* population. The analysis of the *CYTB* gene in JV2 confirms that Colombian *B.c. imperator* is present within the St. Croix *B. constrictor* gene pool. The presence of Central American *B.c. imperator* observed in JV1 also suggests a varied gene pool and can explain the phenotypic ambiguity of the St. Croix *B. constrictor* population. The snakes seem to be coming from different gene pools and breeding with one another. This would give them a very diverse gene pool and would make them well adapted to colonize the island.

While these are interesting results there is still much to be determined about the St. Croix *B. constrictor* population. The first step to uncovering the origin of the island's *B. constrictor* population would be to determine the matrilineal origin of more individuals in the population through analysis of the *CYTB* sequence. At the moment of the study only two of the 20+ individuals found thus far have had their *CYTB* gene successfully sequenced. There has been great difficulty in extracting quality DNA from the shed skins of the individual boas. It is likely that this low success rate be related to the degradation of shed skin over time. In the future blood samples of the *B. constrictor* individuals may provide a more reliable source of quality DNA. In short, it is impossible to make a reliable declaration about the origin of the entire population of *B. constrictor* without more comprehensive data.

Further, analysis of other DNA fragments should be performed as *CYTB* can only reveal the matrilineal heritage of an individual. The *CYTB* gene, involved in the function of the electron transport chain, is located mitochondrial DNA. While this makes it a useful sequence for comparison and study, mitochondrial DNA is inherited exclusively from an individual's mother. Thus while the analysis of the individuals JV1 and JV2 reveals both are of matrilineal *B.c. imperator* descent it says nothing of the patrilineal line. This is a subject of interest due to the ambiguous phenotype of the St. Croix *B. constrictor* population. Due to the large size of a number of individuals found that exceeds typical *B.c. imperator* length it is entirely possible that there are *B.c. constrictor* genes circulating within the population. Further analysis of microsatellite DNA should be conducted to determine the origin of the patrilineal line. Sequence data from the microsatellite regions could also allow the number of introductions and mating patterns within the *B. constrictor* population to be determined.

5. Conclusion

The analysis of the *CYTB* gene in the individuals JV1 and JV2 suggest that the *B. constrictor* population on St. Croix is descended from the *B.c. imperator* lineage. The individuals JV1 and JV2 can be traced back to specific *B.c. imperator* populations in Central America and Colombia respectively. While these sequences only reveal the matrilineal descent of the two individuals from the *B. constrictor* population on St. Croix their differing geographic origins point toward a genetically diverse population. This diversity in the St. Croix *B. constrictor* gene pool has the potential to make them highly adaptable to new environments and thus, a greater threat as an invasive species. The presence of a young Colombian *B.c. imperator* in the population implies that there are Colombian adult *B.c. imperator* individuals contributing to the gene pool. The existence of adult Colombian *B.c. imperator* explains the large size of some of the boas observed on St. Croix.

In conclusion, further research is needed to determine which subspecies and to which specific population the remainder of the boa constrictors on St. Croix belongs. This can be done through analysis of the CYTB gene in the remaining boas. However, microsatellite sequences of the boas must be determined if a more comprehensive understanding of the St. Croix population's origins is to be obtained. Analysis of microsatellites like those in Reynolds et al. can illuminate the patrilineal origin of the boas[12]. From microsatellite data the mating patterns and the number of introductions onto the island an also be inferred. The addition of this microsatellite data will allow for determination of mating patterns of the existing *B. constrictor* populations and for tracking introductions of this invasive species. This more comprehensive understanding of the patterns of population growth can support control of this species and protection of the existing biota on St. Croix.

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