Investigating the evolutionary relationships of species within the *Boa constrictor* species complex

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Abstract

The taxonomic history of the *Boa constrictor* group is very complex because there are nine subspecies currently recognized. In this research project, we explored the genetic similarities and differences of four of the subspecies (*Boa constrictor constrictor, Boa constrictor amarali, Boa constrictor sabogae* and *Boa constrictor imperator*), to assess whether any of these subspecies should actually be raised to species-level status. Genomic DNA was extracted using the Qiagen DNEasy kit from shed skin samples, and two genes (one mitochondrial (COI) and one nuclear (PRLR)) were amplified and sequenced. Our results showed that *B. c. amarali* falls within the *B. c. constrictor* clade and *B. c. sabogae* falls within *B. c. imperator* clade. We also found that *B. c. imperator* is rendered paraphyletic using both trees, and that *B. c. constrictor* always falls outside of *B. c. imperator*. While there is high genetic variation within *B. constrictor*, more data is needed to elevate any of the boa subspecies to species level. Our results do provide evidence that *B. c. sabogae* should be classified as the same subspecies as *B. c. imperator*, and that *B. c. amarali* should be classified as the same species as *B. c. constrictor*.

B. c. imperator *B. c. constrictor* *B. c. amarali* *B. c. sabogae*

Introduction

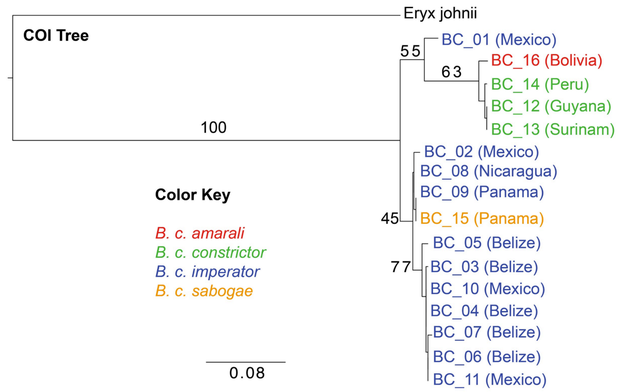
Boa constrictors are nonpoisonous constrictors that have a wide distribution (tropical Central and South America – see red on map to the right) and that vary in appearance. Boa constrictors wear some of the most distinctive markings of all reptiles. These markings are usually work well to blend the snakes in with their habitat. The patterns can consist of jagged lines, ovals, diamonds and circles, as well as colors including tan, green, red, or yellow. Boas are also excellent swimmers but prefer to stay on dry land. While the species is well known in the pet trade because they are easily captive bred and are morphologically variable, few scientific studies have confirmed the species status of *Boa constrictor* and the extent of genetic variation within the species. The four main subspecies we will be working with are *B. c. constrictor, B. c. amarali, B. c. sabogae* and *B. c. imperator*. While *Boa constrictor* is still formally considered a single species, extensive genetic variation has been reported (Reynolds et al., 2013) and previous studies have also suggested that *Boa constrictor* should be split into at least two species (Hynková et al., 2009). Reynolds et al. (2014) recommended that boas be split into *B. constrictor* and *B. imperator*, but did not have sufficient geographic or genetic sampling to formally describe the new species. The main goal of our project was to see if these subspecies are different species by first reconstructing their evolutionary relationships, and then by determining percent genetic differences between the subspecies. We related our data to the 5% threshold. This "rule of thumb" says if there is at least a 5% disparity between members of a species or between subspecies, then they may be different species overall.

Geographic distribution of *Boa constrictor*. Numbers indicating samples used in this experiment and their locations.

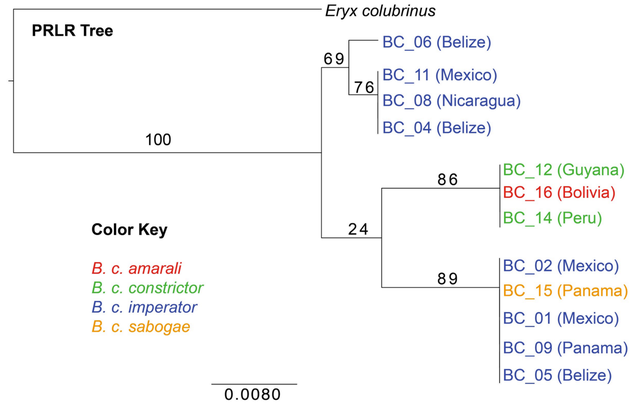
Materials & Methods

- We first obtained specimen from the NY Reptile Expo in White Plains, NY
- We extracted the DNA of sixteen different samples of our target subspecies (*B. c. constrictor, B. c. amarali, B. c. sabogae,* and *B. c. imperator* using a Qiagen DNEasy kit.
- The mitochondrial COI gene and the nuclear PRLR gene were amplified using PCR under locus–specific parameters.
- PCR success was confirmed with gel electrophoresis, and then successful PCRs were sent to Genewiz for Sanger sequencing.
- Our sequences were then edited and analyzed using Geneious, and a multiple sequence alignment was carried out using MUSCLE.
- We created a percentage comparison matrix for both the COI gene and the PRLR genes.
- We checked for a five percent difference between the different samples to determine whether there was evidence that a subspecies should be raised to species level.
- Finally, phylogenetic trees were reconstructed using raxML and bootstrap support values were generated using 500 random addition replicates. The genus *Eryx* was used as an outgroup for each tree.

Results



- The majority of the *B. c. imperator* samples fall into one clade, but one sample BC_01 falls outside of all the other samples.
- All *B. c. constrictor* samples fall within one clade, along with the one *B. c. amarali* sample.
- *B. c. sabogae* falls within the "mostly" *B. c. imperator* clade
- Excluding BC_01, the difference between *B. c imperator* + *B. c. sabogae* and *B. c. constrictor* + *B. c. amarali* is about 10%.
- The difference between *B. c. imperator* samples is low (i.e., BC1 and BC2 are only 3% genetically different), even though the mitochondrial tree shows these samples as separate.



- *B. c. imperator* is rendered paraphyletic with respective to *B. c. constrictor*
- *B. c. sabogae* is falling with one clade of *B. c. imperator*
- *B. c. amarali* is falling within the clade of *B. c. constrictor*
- There is moderate support (86%) for the monophyly of *B. c. constrictor* + *B. c. amarali*
- There is a maximum of 2% difference between all the samples using the PRLR gene.

Discussion

Our project used both nuclear and mitochondrial DNA to determine the evolutionary relationships of boa constrictors, and whether or not there are cryptic species nested within this group. We used 5% greater as a threshold value to quickly assess whether there is potential cryptic species nested within *B. constrictors*. Our goal was to answer the following two hypotheses:

Hypothesis 1: We hypothesize that there are multiple species nested within *Boa constrictor*.

- With our data, we cannot support that there are multiple species nested with *B. constrictor*.
- It may be the case that *B. c. imperator* and *B. c. constrictor* are in the process of speciation, but our data is not sufficient enough to prove that they are the same species yet because there is conflict between mitochondrial (COI) and nuclear (PRLR) data. While COI shows a 9-10% variation between these subspecies, PRLR reveals only a 2% genetic difference between *B. c. imperator and B. c. constrictor*.
- This relates to the work of Reynolds et al. (2012), since he stated in his work as well that although it seems as though *B. c. imperator* and *B. c. constrictor* should be classified into two separate species, yet he also stated that more data is required in order to support this hypothesis.

Hypothesis 2: We hypothesize that *B. c. sabogae* should be classified as the same subspecies as *B. c. imperator*, and that *B. c. amarali* should be classified as the same species as *B. c. constrictor*.

- *B. c. sabogae* should be classified as the same subspecies as *B. c. imperator*. This is because both phylogenetic trees reconstructed using COI and PRLR show that *B. c. sabogae* is almost nested within one of the *B. c. imperator* clades. Additionally, there is low genetic differences (PRLR = 0.002 - .135%; COI = 0 – 2%) between the *B. c. sabogae* sample and *B. c. imperator* samples
- *B. c. amarali* should be classified as the same species as *B. c. constrictor*. Both of the COI and PRLR phylogenetic trees have shown *B. c. amarali* to always be present within the *B. c. constrictor* clade. Importantly, the PRLR comparison matrix shows no difference between the PRLR gene of the *B. c. amarali* and the *B. c. constrictor* samples and the COI comparison matrix showed a small percent difference (2 – 2.5%) between the *B. c. amarali* and the *B. c. constrictor* samples.

References

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