Utilizing Geometric Morphometrics to Characterize Head-Shape Variation in the Brazilian Lance-head Viper (*Bothrops moojeni*)

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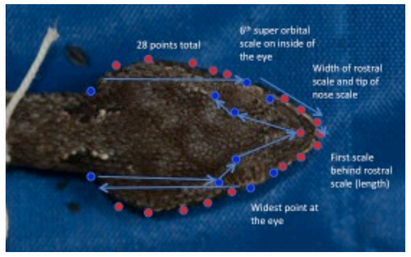
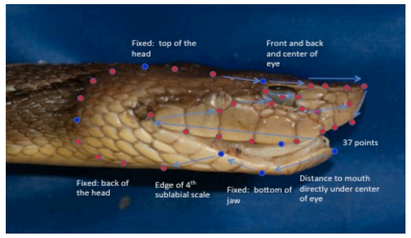
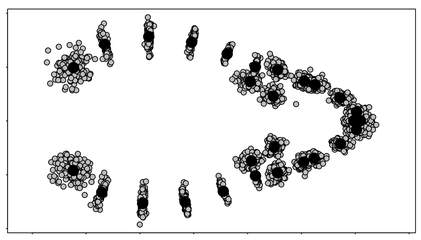
Background: The accurate delimitation and diagnosis of biodiversity is a prerequisite for effective protection. It is becoming increasingly clear that even the best studied vertebrate groups harbor complex patterns of genetic diversity that hinder species delimitation and disguise a large number of unrecognized taxa. The Brazilian Lancehead (*B. moojeni)* is a putative species complex that is both widespread and endemic to the Cerrado, is comprised of populations that are morphologically similar yet have been shown to exhibit wide genetic variation, and is member of the group of snakes responsible for more than half of the snake bite fatalities in Brazil (Assakura et al. 1992; Nogueira et al. 2003; Fenwick et al. 2008). Previous genetic studies have shown that individuals classified as *B. moojeni* based on morphology are often genetically more similar to other *Bothrops* species (i.e. *B. atrox*) and toxicity analyses of *B. moojeni* venom have shown significant variation among populations (Assakura et al. 1992; Wüster et al. 1999; Dutra et al. 2008).

The focus of this research is to use computer-aided geometric morphometric image analyses of *B. moojeni* to determine if there are significant phenotypic differences that correspond to species level variation. Images were taken of specimens housed in museum collections and represent populations across the species' range (Fig. 1). If shown to be true, such differences could aid in species identification and delimitation for this putative species complex.

Data







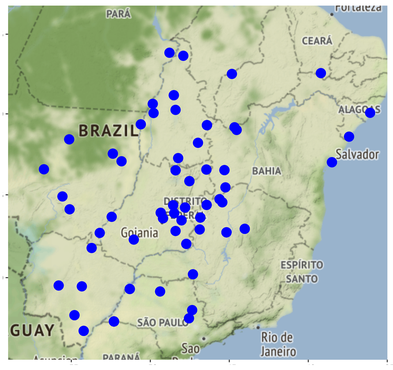


Figure 1 (above). Landmakrs used to characterize head shape variation. Dorsal view above, left; lateral view above, right. Model of *B. moojeni* head shape based on 145 specimens across all populations (bottom, left). Populations included in the sampling mapped in blue on bottom right.

Figure 3 (right). Head shape variation within *B. moojeni* coded by sex (left) and body size (right).

Methods: Data consisted of >1000 raw images of *Bothrops moojeni* specimens and several outgroup species (Fig 2). Images were processed and organized by museum collection, specimen ID and angle of orientation. Relabeled images were then landmarked using the Geomporph package in the program R. Landmarks (Fig. 1, shown in the bottom left) are placed on fixed features common to all of the samples and are used to reproduce shape variation. Landmarked images are translated into 2 dimensional models which can be statistically evaluated and used in total-evidence species delimitation.

Preliminary Results

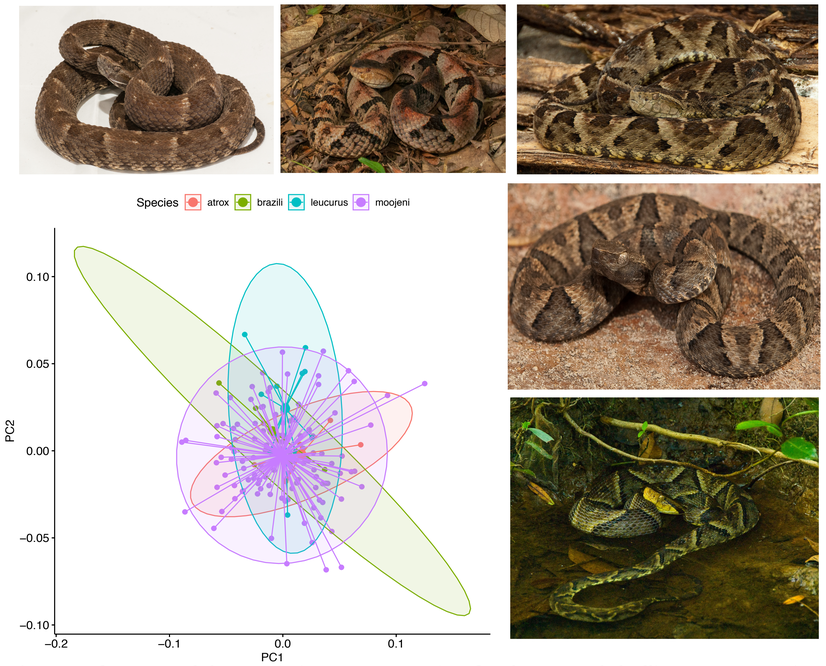
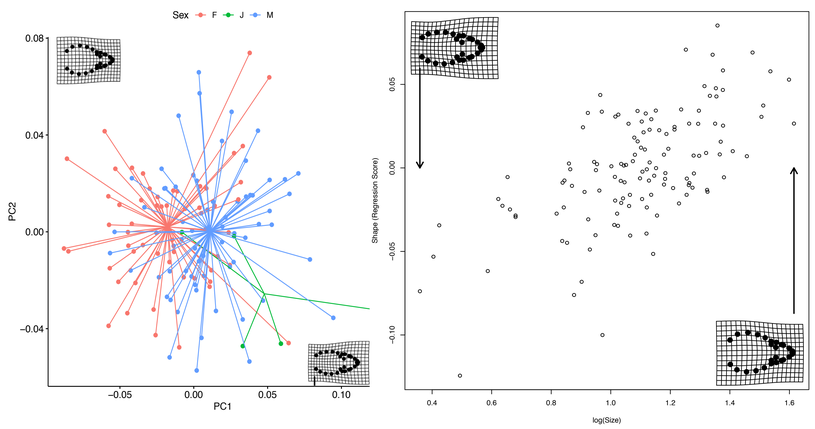


Figure 2 (above). Head shape variation among 4 species of *Bothrops* sampled. Ellipses represent 95% CI. *Bothrops moojeni* head dorsal head shape spans nearly all variation present in the three outgroup species. Photos: *B. atrox* (top, left); *B. brazili* (top, center); *B. leucurus* (top, right); *B. moojeni* (right, above and below). Photos by TJC.



Discussion & Future Directions: Analyses of head shape variation across species suggests that *Bothrops moojeni* has a higher degree of morphometric variance compared to other closely related species (Fig. 2). This is further evidence that *Bothrops moojeni* could consist of multiple cryptic species.

Preliminary results show dorsal head shape shares significant correlation to sex and overall body size (Figs. 3, 4) with males and smaller individuals possessing narrower heads. Diet may explain the difference in head shape, as a broader head allows for predation of larger mammalian prey. Future studies will incorporate morphology (35+ morphological and meristic characters), habitat, and other niche characteristics with genome wide genetic data (1000's of nuclear SNPs) from all populations to use a total evidence approach to species delimitation in this putative species complex.

While this research is ongoing, our preliminary results recovered statistically significant differences in dorsal head shape and other factors. With better species recognition comes better informed conservation and effective snakebite management practices.

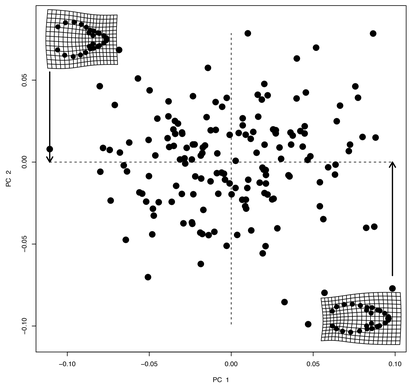


Figure 4 (left). Dorsal head shape variation within *B. moojeni* populations. Total shape varies from individuals with broad, short heads to individuals with narrow, longer heads. Below: typical Cerrado habitat depicting dry savannahs punctuated with riverine gallery forests, primary habitat for *B. moojeni*.

Acknowledgements

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