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MORPHOLOGICAL VARIATION IN *BOTHROPS JARARACA* AND *B. INSULARIS*: SEXUAL DIMORPHISM AND ONTOGENY

São José do Rio Preto
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Tese apresentada como parte dos requisitos para obtenção do título de Doutor em Biologia Animal, junto ao Programa de Pós-Graduação Biologia Animal, do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista “Júlio de Mesquita Filho”, Câmpus de São José do Rio Preto.

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Dedico a minha família e meus amigos mais próximos que foram pilares sem os
quais este trabalho não estaria de pé

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“[...] Venha, o amor tem sempre a porta aberta

E vem chegando a primavera

Nosso futuro recomeça

Venha, que o que vem é perfeição.”

(Legião Urbana, 1993)

RESUMO

A morfologia é um dos traços mais variáveis nas serpentes. Ela é altamente correlacionada a vários traços biológicos e também a pressões ambientais. Usei duas técnicas complementares, morfometria linear e geométrica, para avaliar a variação morfológica em *Bothrops jararaca* e *B. insularis* dentro, e entre populações e espécies, para testar o efeito do dimorfismo sexual, distribuição geográfica e tendências microevolutivas. Medii entre 11 a 17 variáveis lineares de cada indivíduo. Além disso, 19 landmarks anatômicas foram posicionadas na cabeça, usando uma imagem fotográfica da vista dorsal. Em *B. jararaca*, fêmeas foram geralmente maiores para as medidas do corpo e da cabeça, ao passo que machos foram maiores para as variáveis da cauda. Encontrei efeito significativo da população, sendo que a população do planalto alcançou maiores tamanhos do que no litoral, e fêmeas apresentaram a cabeça com uma região pós-occipital em formato de flecha. Ambas as populações mostraram marcada alometria ontogenética, e a trajetória variou para cada traço medido. Um padrão de dimorfismo sexual similar ocorreu em *B. insularis*, mas não houve diferença no formato da cabeça, porém machos apresentaram olhos maiores. Os sexos tiveram trajetória ontogenética sobreposta para o formato do corpo, mas com inclinação diferente para o formato da cabeça. Comparações interespecíficas indicaram uma cabeça mais comprida e com focinho mais proeminente em *B. insularis*, mais similar à da população do planalto. A trajetória ontogenética também foi paralela com a do planalto e convergente com a população do litoral. A partição de nicho é uma explicação a para algumas das diferenças dos padrões aqui detectados. Da mesma forma, a disponibilidade de presas e ecologia comportamental podem produzir diferentes fenótipos em cada população ou espécie. Atribuo as diferenças na trajetória ontogenética principalmente à eventos de maturação heterocrônica e variação temporal nas mudanças ontogenéticas.

Palavras-chave: Jararaca. Forma. Morfometria geométrica. Alometria. Jararaca-Ilhoa.

ABSTRACT

Morphology is one of the most variable traits in snakes. It is highly related to several biological traits and also environmental pressures. I used two complementary techniques, linear and geometric morphometrics, to evaluate morphological variation in *Bothrops jararaca* and *B. insularis* within and among populations and species, to test the effects of sexual dimorphism, geographic distribution and microevolutionary trends. I measured from 11 to 17 linear variables from each individual. Moreover, 19 anatomical landmarks were placed in the head using a photographed image of the dorsal view. In females were generally larger than males for body and head measures, while males were larger for tail variables. I found a significative effect of population, being that the highland population reached larger sizes than coastal population, and females presented a larger post-ocular region and a more arrow shaped head. Both populations showed a marked ontogenetic allometry and ontogenetic trajectory varied depending on each variable. A similar sexual dimorphism pattern occurred in *B. insularis* body, but with no difference in head shape, however males had larger eyes than females. Sexes had overlapped ontogenetic trajectory in body shape, but with different slopes in head shape. Interspecific comparisons indicated a longer head and prominent snout in *B. insularis*, closer to the highland population. Ontogenetic trajectory also was parallel with highland and convergent with coastal population. Niche partitioning is an explanation for the differences in the observed patterns. Accordingly, prey availability and behavioral ecology may produce different phenotypes on each population or species. I attribute differences in ontogenetic trajectories mainly due heterochronic maturation events and different onset on ontogenetic changes.

Keywords: Pitviper. Shape. Geometrical morphometrics. Allometry. Golden Lancehead.

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LIST OF ABBREVIATIONS AND ACRONYMS

A.S.L. – Above sea level

IBSP – Instituto Butantan São Paulo

SVL – Snout-vent length

VS – Ventral scales

SS – Subcaudal scales

TL – Tail length

TW – Tail width

MW – Middle width

HW – Head width

DBE – Distance between eyes

DBL – Distance between loreals

DBN – Distance between nasals

DEN – Distance eye to nasal

DEL – Distance eye to loreal

DLN – Distance loreal to nasal

HL – Head length

DRL – Distance rostral to labial

HH – Head height

ED – Eye diameter

F – Female

M – Male

SDI – Sexual dimorphism index

LDA – Linear discriminant analysis

ANOVA – Analysis of variance

ANCOVA – Analysis of covariance

PCA – Principal component analysis

CS – Centroid size

MANOVA – Multivariate analysis of variance

SSD – Sexual shape dimorphism

BC – Body circumference

RLD - Rostrum-labial Distance

QGI – Queimada Grande island

Regscore – Regression score

RTL – Relative tail length

MAT – Mean annual temperature

MAP – Mean annual precipitation

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GENERAL INTRODUCTION

Extant snakes are characterized by a unique morphology related to other vertebrates. It has an elongated and cylindrical body associated to a multiplication in body vertebrae and an almost total reduction on limbs and girdle elements, which are most attributed to an evolution to a more terrestrial than aquatic habits (Apesteguías and Zaher 2006; Müller et al. 2010). Thereby, one may mistakenly think that snakes have a simplified morphology with little variation, however several studies have shown otherwise, with a large variation in size and shape mainly attributed to their functional biology, phylogenetic relationships and ecological pressures (Gans 1961; França et al. 2008; Hampton 2011; Esquerré and Keogh 2016).

In reptiles, morphological variation is expected not only on large scale, but occur in closely related species (Zamudio 1998; Wüster et al. 2005), and even intraspecifically, among sexes (Camilleri 1990; Shine and Shetty 2001; Brown et al. 2017) or populations (Hoge et al. 1976; Zamudio 1998; Shine et al. 2012). Body size, the most prominent morphological trait, is often biased toward the sex that receive advantage for being larger (Shine 1994). For example, females are larger due an increase in fecundity, and males are the large sex when male-male combat is present (Shine 1978; Shine 1993; Shine 1994). On the other hand, the ecological hypothesis also provides good explanation for sexual dimorphism. Sexes often diverge due a niche partitioning, such as diet or habitat use, accordingly, the sex that consume larger prey often attain larger sizes (Shine 1986; Shine and Fitzgerald 1991; Shine 1998; Cox 2007). Although body size be the most studied trait, dimorphism also occur in a variety of other traits, such as tail and head size and shape, scalation and coloration (Shine 1993; Shine 1991; Shine and Shetty 2001).

The hypothesis stated above also account for morphological variation between populations of the same sex and also for sexual dimorphism degree. In Australia the python *Morelia spilota* is widespread all over mainland and islands and, unusually among reptiles, populations diverge in mating systems, being that populations of northeast, males present combat and are larger than females, whereas in southeast, no evidence of combat are known and females grew twice than males and reach

almost 10 times their mass (Shine and Fitzgerald 1995; Pearson et al. 2002a). Furthermore, comparing populations with a single mating system (no combat), females were always larger than males, however the degree of sexual dimorphism greatly varies, associated mainly with prey resources in each population (Pearson et al. 2002b).

Sexual dimorphism is rather studied in samples composed only by adult specimens, however a comprehensive analysis on postnatal ontogenetic growth is important to understand the onset of diversification as sexes may have different growth rates and size/age at maturation (Beaupre et al. 1998; Taylor and Denardo 2005; Pearson et al. 2002a). Additionally, several traits exhibit a significative allometric association with size, and differences in sex, population or species may rise as differences in ontogenetic trajectories (Scanferla 2016; Strong et al. 2019). Accordingly, patterns of allometry are strongly related to species phylogeny and ecology, such as diet, foraging behavior and habitat use (Taylor and Denardo 2005; Urosevic et al. 2013; Sherrat et al. 2019).

Notwithstanding, phenotypes may diverge even in overlapped allometric trajectory, through heterochronic events. In its seminal review, Klingenberg (1998) argued about the concept of heterochrony, and although it is still under discussion, from a developmental point of view, heterochrony may be summarized as changes in rate and/or timing of ontogenetic allometries between groups. Heterochrony is the proximate cause responsible for several cases of morphological variation, being that groups may be paedomorphic or peramorphic in relation to each other (Klingenberg 1998; Piras et al. 2011). In snakes, heterochronic processes are known to drive phenotypical convergence or divergence even in megadiverse clades, as the skull shape of microcephalic sea snakes (Sherrat et al. 2019) and the body and head shape of pythons (Esquerré et al. 2017).

In Brazil, the pit vipers of the genus *Bothrops* are one of the most diverse. About 30 species are recognized and are widespread in all country (Costa and Bérnils 2018; Nogueira et al. 2019). The rapid diversification rates and radiation of the pit vipers to the New World in late Oligocene and early Miocene (Alencar et al. 2016), enabled the occupation of several niches, and consequently, morphological adaptations (Alencar et al. 2016; Alencar et al. 2017). Species with enhanced arboreal habits are generally in intermediate sizes, are slender bodied and present larger tails than terrestrial

species (Martins et al. 2001; Alencar et al. 2017). Also, there is a broader variation in diet of pit vipers, with a wider range of prey types, generalist or specialist species, and presence or absence of a conspicuous ontogenetic change (Martins et al. 2002). Females are usually larger, and no male-male combat are rare (Barros et al. 2020). In this group, two species have been the subject of several researches, the common lancehead *B. jararaca* and its sister clade *B. insularis*, yet, little is known about morphological variation and developmental processes in these two species.

BOTHROPS JARARACA

The common jararaca *Bothrops jararaca* is a medium sized pit viper that reaches until 1,600 mm in total size (Campbell and Lamar 2004). It is widely distributed in South America, occurring in Paraguay, Argentina, and in Brazil, from Rio Grande do Sul to southern Bahia (Campbell and Lamar 2004; Nogueira et al. 2019). This species horizontally, inhabits mainly the Atlantic Forest, however may occur in open areas and even in modified and highly urbanized regions, and vertically from sea level up to 1,200m A.S.L. (Puerto et al. 1991; Marques et al. 2019; Nogueira et al. 2019). *B. jararaca* is largely a nocturnal species and may be found almost the entire year, although show a seasonal peak of activity during the rainy season (Sazima 1992; Campbell and Lamar 2004; Siqueira et al. 2021).

The species pass through a marked ontogenetic change in diet, with juveniles feeding mostly on frogs such as *Hylids* and *Leptodactylids*, however small rodents, lizards and centipedes are also eaten less frequently, and as adults rely mainly on small rodents (Sazima 1992; Marques et al. 2019). Ontogenetic changes are also evident in some morphological traits, such as tail tip color, that are often white or yellowish contrasting with the body color in juveniles and are used as a bait to attract ectothermic prey such as frogs and lizards, and the tail become suffused as snake grows (Sazima 1991; Sazima 1992; Martins et al. 2002). *B. jararaca* is an ambush predator that probably actively forage occasionally (Sazima 1992). Juveniles usually bite to envenom and hold their harmless prey to avoid them to scape (frogs jumps and chemical clues became difficult to follow), however, adults face usually more dangerous species, therefore release the prey after the bite, and follow chemical clues to find them after subjugation (Sazima 1989; Sazima 1991; Hartman et al. 2003).

Females are larger and heavier than males (Sazima 1992; Matias et al. 2011), have a larger head and smaller tail (Wüster et al. 2005). A previous study also indicates that morphology may vary between population, with a trend of larger females in a small and urbanized fragment than in larger and connected one (Siqueira et al. 2018). Females usually mature at larger sizes than males (750 mm for females and 650 mm for males), and growth rates vary from 5 to 18 mm monthly, but no sex differences are known, and life span are estimated from 10 to 12 years (Sazima 1992).

BOTHROPS INSULARIS

The golden lancehead *Bothrops insularis* is an insular species endemic on Queimada Grande island, located about 33 kilometers far from the Southwestern Brazilian coast (24° 30' S, 43° 42' W; Duarte et al. 1995). The island is considered an inhospitable place due its inaccessibility and hostile environment with no fresh water spring and the presence of the venomous and snake (Amaral 1921b; Duarte et al. 1995). Although *B. insularis* appears to be abundant relatively with any other continental snake, the first populational estimative was below those once speculate in literature (less than 2500 individuals in the island; Martins et al 2008). Unfortunately, few years later a detailed demographic survey found evidence for a populational decline trend (Guimarães et al. 2014), which brought new concerns about the species knowledge and conservation. The endemism, small island area (430,000 m²) with suitable habitat, populational decline, and biopiracy make this snake one of the most threatened species in the entire world (Marques et al. 2004).

The most accepted hypothesis for the origin of the species is that *B. insularis* share a common ancestor with its sister clade *B. jararaca*. About 11,000 years ago, in the quaternary period, a glaciation has enhanced the sea level, isolating one population of the ancestor on what would be today, the Queimada Grande island, and due different selective pressures of the mainland, the island population suffered an allopatric speciation (Marques et al. 2002; Wüster et al. 2005). The golden lancehead has a more diurnal and arboreal habits than the general in the genus *Bothrops* (Amaral 1921; Amaral 1921b; Marques et al. 2019). It feeds on small ectotherms as juvenile, such as centipedes and small frogs, and whereas adults, rely most on migratory

passerine birds that visit the island twice a year, moreover, its ontogenetic shift in diet is less conspicuous than *B. jararaca* (Martins et al. 2002; Marques et al. 2012).

Several morphological adaptations have accompanied the increased arboreal habits, such as smaller size, larger tail and slender body than the mainland sister species (Martins et al. 2001; Alencar et al. 2017). Due to morphological constraint, this species matures at small sizes and produces small clutches (Marques et al. 2013). Also, the feeding apparatus is adapted to its feeding habits, such as the larger head and smaller fangs than *B. jararaca* (Wüster et a. 2005). Sexual dimorphism includes a female biased body, head and fang length, and also an anteriorly positioned hearts, and males had a larger tail (Wüster et a. 2005; Marques et al. 2013).

In this work we use linear and geometric morphometric techniques to analyze morphological variation in *B. jararaca* and *B. insularis*. Specifically, we test hypothesis that there are morphological disparities in adult body and head shape between sexes, populations and species. Also, we seek for ontogenetic scaling in several morphological traits and test for differences in allometric trajectories between groups.

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CHAPTER 1

MORPHOLOGICAL VARIATION ON THE COMMON LANCEHEAD *BOTHROPS JARARACA* POPULATIONS: SEXUAL DIMORPHISM AND ONTOGENETIC PATTERNS.

ABSTRACT

The common lancehead *Bothrops jararaca* is widespread in the Atlantic Forest in Brazil. The species is known to show a marked sexual dimorphism pattern, with the female being larger than males. However, most efforts in clarifying morphological variation between sexes are often focused on a single population. In this paper we investigate the effect of populational variation on the sexual dimorphism and ontogenetic trajectories of *B. jararaca*. We measured 17 morphological traits, including linear and meristic characters, and the analysis revealed a clear but variable effect of sex and population. Females were larger than males in all evaluated populations. Furthermore, females in the coastal population were generally smaller than in the highland population, but had significantly more scales. Widespread species often suffer from differential environmental pressure even in biotic and abiotic factors. We attribute the results found herein to specificities in prey availability and climatic conditions, which affect the ontogenetic pattern between sexes and populations resulting in specific sexual dimorphism pattern.

KEYWORDS

Geographic variation; Allometry; Growth; Morphometry.

2.1. INTRODUCTION

In snakes, sexual dimorphism is a character with ecological impact, being widely shared in the *Viperidae* family (Hendry et al. 2014). Two hypothesis that possibly explain the difference between sexes acquire notable prominence in the last decades. The sexual selection hypothesis predicts that being larger for one sex carries certain advantages. In this case, males are larger in species that have combat behavior and females are larger in species where fecundity rate or offspring size is strongly correlated with maternal size (Shine, 1993; Shine, 1994). On the other hand, the niche partition hypothesis predicts that morphological differences are due to ecological differences between the sexes such as habitat use or diet (Camilleri and Shine, 1990; Shine, 1986).

The relationship between ecology and morphology in snakes is so complex that significant adaptations may emerge in a short time period after a drastic change in local dynamics (e.g. introduction of new species, environmental change). A striking example is the change in body measures of Australian snakes associated with the occurrence of the invasive toxic frog *Rhinella marina*. Over time, batracophagous snakes vulnerable to the toxin showed a reduction in the size of the mouth opening and an increase in body size (increasing in toxicity tolerance), changes that limit the intake of larger and potentially more toxic frogs and enhance survival probabilities (Phillips and Shine, 2004).

The same species interaction can be applied to the allometric relationship between morphological traits of the prey and the predator. The parotoid gland of the frog grows disproportionately (larger frogs are relatively more toxic), while the snakes' head relatively decreases with size, and this two allometric patterns compensate each other (Phillips and Shine, 2006). This means that snakes with relative smaller heads, younger individuals, or conspecific of the smaller sex, may become more vulnerable to the *R. marina* poison (Phillips and Shine, 2006).

Widely distributed species generally exhibit morphological variations among different populations. This pattern is mainly associated with differences in the environmental pressures to which each population is subject. These pressures can be of biotic origins such as eating habits in different types of prey (Fabien 2004), or of abiotic origins linked to climate, geography (e.g. altitude, latitude), or phytophysiology (Cruz -Elizalde et al. 2017; Zhong et al. 2017; Nóbrega et al. 2016).

Morphometric studies are often focused only on adult individuals, however the morphological pattern studied may have resulted from ontogenetic development. Allometric hypotheses contrast growth rates in a given variable with body growth, and size dimorphism may arise i) early, if groups are already born in different sizes and maintain a parallel trajectory, ii) late, if a group grows for a longer time or iii) late, if the groups have different rates of intrinsic growth (see Klingenberg, 1996; Sanger et al. 2013).

The common lancehead *Bothrops jararaca* is one of the most emblematic snakes in Brazil, associated with the Atlantic Forest. This forest is located on the coast of Brazil at altitudes between 0 and 1,200 A.S.L. Juveniles feed mainly on anuran amphibians, while adults eat mostly small rodents (Campbell and Lamar, 2004; Marques et al. 2019; Sazima, 1992). Some study has shown populations with larger body size or marked sexual dimorphism (e.g., Matias et al. 2011, Siqueira and Marques, 2018). Although studies addressing morphological divergences in snakes have been published extensively, many are focused only on one population, sympatric species or address only one age group (e.g. adults). Therefore, the aim of this work was to test the hypothesis that populations of *B. jararaca* subject to different environmental conditions may present divergent morphologies. Specifically, we tested the influence of population variation on: i) direction and degree of intra and interpopulation sexual dimorphism and ii) ontogenetic allometry as a mechanism of morphological divergence.

2.2. MATERIAL AND METHODS

We choose to use an arbitrary but clear criterion for populational categorization. Populations found between 0 to 80 m A.S.L., were classified as coastal population, and above 800 m A.S.L., as highland population (Fig. 1). We analyzed 211 specimens of *Bothrops jararaca*: 1) 59 females and 51 males from coastal populations; and 2) 50 females and 51 males the highland populations. All individuals were housed in the Herpetological Collection Richard Alphonse Hoge (IBSP), at the Instituto Butantan, São Paulo, Brazil. We measured 17 morphological traits, being 15 linear measures and 2 meristic traits in all individuals (see Fig. 2 for head schematics and Tab. 1 for variables descriptions). All measures were made using a ruler (precision on 1 mm) or digital caliper (precision of 0.5 mm). Raw data were used to present mean value and

standard deviation. Then, all measures were log transformed in order to achieve the normality requirement before the statistical analysis.

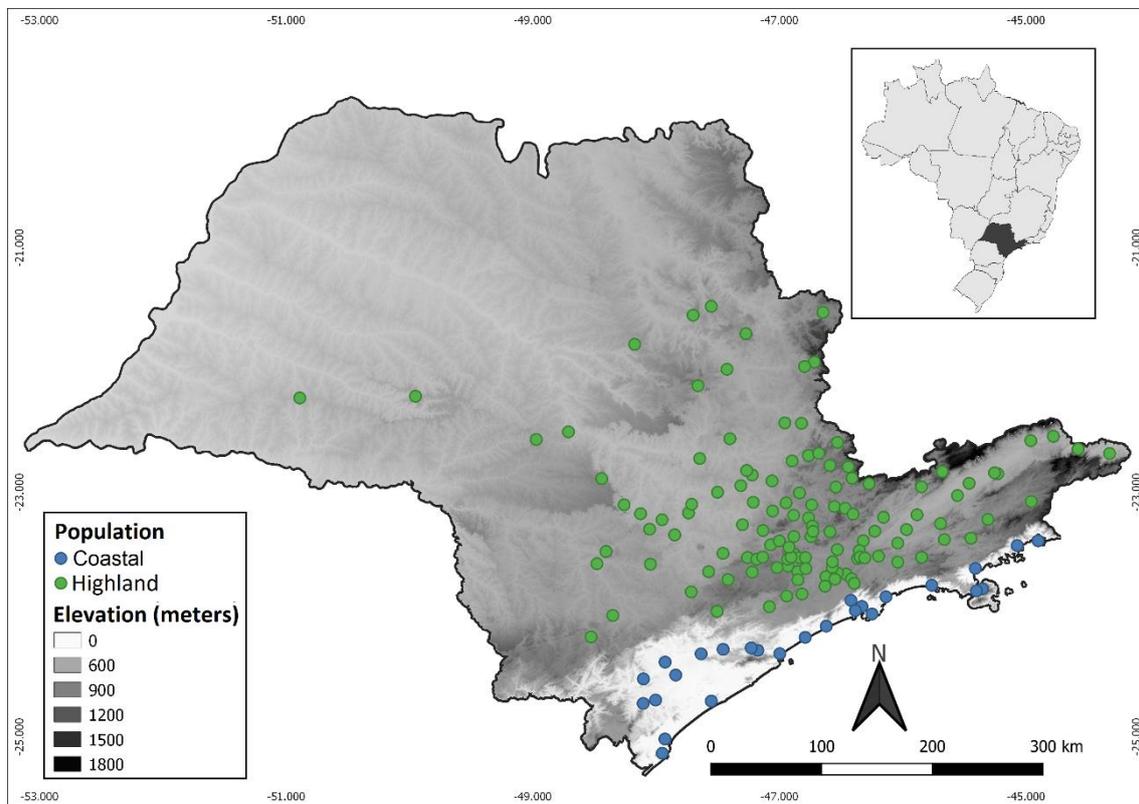


Fig. 1. Localities of *Bothrops jararaca* included in the study.

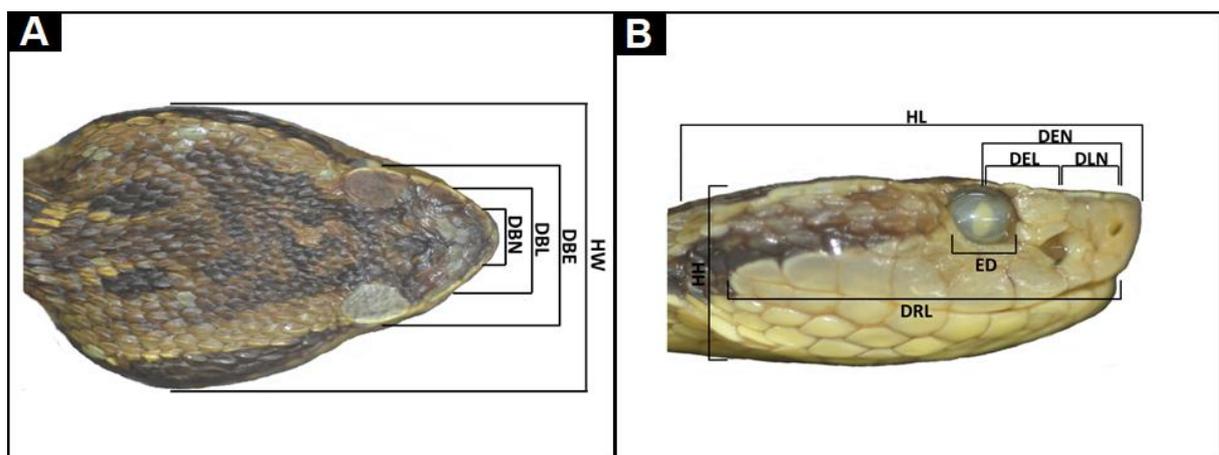


Fig. 2. Schematics illustration showing the variables measured in snakes' head to analyze morphological variation among and within populations of *Bothrops jararaca* in A) dorsal and B) lateral view. Head width (HW), Distance between eyes (DBE), Distance between Loreals (DBL), Distance between nasals (DBN), Distance eye to

nasal (DEN), Distance eye to loreal (DEL), Distance loreal to nasal (DLN), Head length (HL), Distance rostral to labial (DRL), Head height, and Eye diameter.

Table1: Names and description of the morphological variables used to access *Bothrops jararaca* morphological variation. Schematics in Fig. 1.

| VARIABLE | DESCRIPTION |
|----------|---|
| SVL | Snout-Vent Length; Measured from the tip of the nose to the anal scale |
| VS | Ventral Scales; Counted from the first scale post-quadrata bone |
| SS | Subcaudal Scales; Counted from the first post-anal scale to the tip of the tail |
| TL | Tail Length; Measured from the cloaca to the tip of the tail |
| TW | Tail Width; Measured post-cloaca |
| MW | Middle Width; Total circumference in mid-body |
| HW | Head Width; Measured in the larger portion of the head (quadrata bone) |
| DBE | Distance Between Eyes; Measured from right to left subocular scales |
| DBL | Distance Between Loreals; Measured from right to left loreal pit |
| DBN | Distance Between Nasals; Measured from right to left nasal scales |
| DEN | Distance Eye to Nasal; Measured from eye to nasal scales in the right side |
| DEL | Distance Eye to Loreal; Measured from eye to loreal pit in the right side |
| DLN | Distance Loreal to Nasal; Measured from loreal pit to nasal scale in the right side |
| HL | Head Length; Measured from the neck to the tip of the nose |
| DRL | Distance Rostral to Labial; Measured from the tip of the nose to the last labia scale in the right side |
| HH | Head height; Measured in parietal region; |
| ED | Eyes Diameter; Measured horizontally in the middle of the eye |

2.2.1. Sexual dimorphism

We included only adults in the analyses: females larger than 750 mm SVL and males larger than 650 mm SVL (Sazima, 1992). We analyzed 109 specimens in total, 43 from the coastal population (25F and 18M), and 66 from the highland population (35F and 31M). Variation on SVL, VS and SS between sexes and populations were tested using ANOVA with sex, population and interactions as factors. The size dependent variables were tested using ANCOVA (Table 3 details the variables and covariables). Significant triple interactions were further clarified using linear models with each dependent variable and its covariate to eliminate the effect of size. Then, the residuals of the regression were extracted and an ANOVA were performed with sex and population as factors with paired Tukey *post-hoc* test.

The Sexual Dimorphism Index (SDI) for each variable was computed as the (mean of female/mean of male) -1 (Shine, 1994). This arbitrary index varies from -1 to 1, and expresses the relative size difference between sexes, being positive when female-biased, negative when male-biased, and zero when sexes are equal sized. Additionally, a Linear Discriminant Analysis (LDA) was used in order to observe the degree of separation or overlap of the sexes in each population, as well as which variables have higher discriminating scores between classes (male or female).

2.2.2. Ontogenetic allometry

Linear models were built for each sex and population separately, using 14 variables that co-varied with size. The aim of these models was to test the hypothesis of presence of allometry or isometry in each one, and observe the size variation along the individual growth. Then an ANCOVA was performed to test the homogeneity of the slope. The presence of a significant result in the interaction is indicative of a difference in the growth trajectory. Similar slopes with intercept statistically significant, indicate parallel trajectories, with premature differentiation between groups. Significantly different slopes indicate divergent trajectory, with late differentiation between groups. Equal slopes and intercepts indicate no difference in allometric trajectory and any difference between groups simply appearing as a device of size magnitude (Sanger et al. 2013).

Finally, a Principal Component Analysis (PCA) was used to visualize the relationship between groups in the tangent space. Only the variables that showed significant results were kept in the analysis. and to avoid bias due to scaling in allometric variables, the residuals of the linear models were used.

2.3. RESULTS

2.3.1. Sexual dimorphism

In general, females were morphologically larger than males, except for the tail variables. Between populations, the coastal population exhibited smaller values than the highland, except for meristic traits (mean and standard deviations in Table 2). In the same way, statistical analysis revealed a great variation in morphological patterns, both between sexes and populations (Table 3, Fig. 3). Six variables were significantly different only between sexes, while eight were different only between populations. We found significant effect of the triple interaction for HH and ED. However, those results did not hold after ANOVA performed in the residuals of linear models and Tukey *post-hoc* tests. There was no effect of sex, populations nor interactions for the variables HW, DBL, DBN and DLN.

Females were larger than males for SVL, VS and HL, whereas males were larger for SS, TL and TW. Considering populations, coastal females had more ventral scales (VS) than males and females from the highland, while coastal males had more subcaudal scales (SS) than males and females from the highland. Both sexes from the coastal population were smaller for TW, MW and HL, and were larger for DBE, DEN and DRL.

In the coastal population SDI varied from 0 to 0.39, and in the highland, from 0 to 0.3 (Table 4). The size disparity was larger in the coastal population only for MW, suggesting higher equitability between sexes in this population. In the LDA all females and males were correctly classified in both populations, and no overlap occurred (Fig, 4). The best discriminant variables for the coastal population were HL (with negative values on the “x” axis) and TL (with positive values in the “x” axis). In the highland population the best discriminant variables were VS (with positive values in the “x” axis) and SS (with positive values in the “x” axis).

Table 2: Raw data of morphological variables of *Bothrops jararaca* on coastal and highland populations. F = females; M = males; *sd* = standard deviation. see abbreviations in Fig. 1)

| VARIABLE | MEAN \pm <i>sd</i> | | | |
|----------|----------------------|-------------------|--------------------|------------------|
| | F COASTAL | M COASTAL | F HIGHLAND | M HIGHLAND |
| SVL | 987.1 \pm 128.9 | 815.4 \pm 110.3 | 1038.2 \pm 124.6 | 800.4 \pm 82.1 |
| VS | 204.9 \pm 5.7 | 199.2 \pm 5.9 | 196.7 \pm 5.4 | 191.7 \pm 4.9 |
| SS | 59.1 \pm 2.1 | 63.5 \pm 4.6 | 56.6 \pm 3.3 | 62.1 \pm 2.9 |
| TL | 146.7 \pm 22.4 | 129.6 \pm 13.2 | 147.7 \pm 18.2 | 129.8 \pm 16 |
| TW | 10.3 \pm 2.1 | 9.4 \pm 1.6 | 11.8 \pm 2.4 | 10.6 \pm 1.4 |
| MW | 68.9 \pm 37.8 | 41.7 \pm 26.1 | 104.6 \pm 24 | 73.5 \pm 9.9 |
| HW | 27 \pm 5.9 | 21.3 \pm 3.3 | 30.8 \pm 3.6 | 22.2 \pm 3.1 |
| DBE | 16.1 \pm 2.1 | 14.1 \pm 1.8 | 17.7 \pm 1.7 | 14.1 \pm 1.6 |
| DBL | 12.6 \pm 1.9 | 10.7 \pm 1.6 | 14.4 \pm 1.6 | 11.2 \pm 1.3 |
| DBN | 7.6 \pm 1.1 | 6.2 \pm 0.8 | 8.9 \pm 1.5 | 7.2 \pm 1 |
| DEN | 11 \pm 1.4 | 9.1 \pm 1.2 | 11.8 \pm 1.6 | 9.3 \pm 1.2 |
| DEL | 6 \pm 1 | 5.2 \pm 0.8" | 7.3 \pm 1.1 | 5.6 \pm 0.8 |
| DLN | 4.8 \pm 0.7 | 3.9 \pm 0.4" | 5.3 \pm 1.1 | 4.1 \pm 0.6 |
| HL | 43.1 \pm 5.3 | 32.8 \pm 3.7 | 49.4 \pm 5.8 | 35.8 \pm 4.2 |
| DRL | 34.4 \pm 4.7 | 27.3 \pm 2.9 | 38.9 \pm 5.4 | 28.3 \pm 3.4 |
| HH | 15.6 \pm 2.9 | 12.5 \pm 2 | 18.2 \pm 2.4 | 13.2 \pm 2 |
| ED | 4.9 \pm 0.7 | 4.4 \pm 0.5 | 5.2 \pm 0.5 | 4.4 \pm 0.5 |

Table 3: ANOVA and ANCOVA results of the morphological variation between sex and populations (Coastal and Highland) of *Bothrops jararaca* (dependent variables and predictors shown). *F* = *F*-test; *P* = *P*-value. (See abbreviations in Fig. 1)

| Dependent | Predictors | <i>F</i> | <i>P</i> |
|-----------|--------------------|----------|------------------|
| | Sex | 99.55 | <0.001 |
| SVL | Population | 0.88 | 0.34 |
| | Sex:Population | 1.96 | 0.16 |
| | Sex | 27.80 | <0.001 |
| VS | Population | 54.27 | <0.001 |
| | Sex:Population | 0.05 | 0.823 |
| | Sex | 55.34 | <0.001 |
| SS | Population | 9.01 | 0.003 |
| | Sex:Population | 1.06 | 0.30 |
| | SVL | 160.62 | <0.001 |
| | Sex | 6.41 | 0.01 |
| TL | Population | 0.05 | 0.82 |
| | SVL:Sex | 0.22 | 0.63 |
| | SVL: Population | 0.53 | 0.46 |
| | Sex: Population | 1.70 | 0.19 |
| | SVL:Sex:Population | 1.03 | 0.31 |
| | TL | 47.20 | <0.001 |
| | Sex | 0.72 | 0.39 |
| | Population | 12.09 | <0.001 |
| TW | TL:Sex | 0.30 | 0.58 |
| | TL: Population | 0.34 | 0.55 |
| | Sex: Population | 1.08 | 0.3 |
| | TL:Sex:Population | 1.67 | 0.19 |

Table 3: Continuation

| | | | |
|-----|--------------------|--------|------------------|
| | SVL | 21.62 | <0.001 |
| | Sex | 0.36 | 0.54 |
| | Population | 43.00 | <0.001 |
| MW | SVL:Sex | 1.26 | 0.26 |
| | SVL: Population | 1.59 | 0.21 |
| | Sex: Population | 0.16 | 0.68 |
| | SVL:Sex:Population | 1.33 | 0.25 |
| | HL | 364.95 | <0.001 |
| | Sex | 0.01 | 0.90 |
| | Population | 0.81 | 0.36 |
| HW | HL:Sex | 0.34 | 0.56 |
| | HL: Population | 0.30 | 0.58 |
| | Sex: Population | 2.58 | 0.11 |
| | HL:Sex:Population | 0.40 | 0.52 |
| | HL | 387.69 | <0.001 |
| | Sex | 0.34 | 0.55 |
| | Population | 6.09 | 0.01 |
| DBE | HL:Sex | 0.20 | 0.64 |
| | HL: Population | 0.35 | 0.55 |
| | Sex: Population | 0.90 | 0.34 |
| | HL:Sex:Population | 0.06 | 0.79 |

Table 3: Continuation

| | | | |
|-----|-------------------|--------|------------------|
| | HL | 393.43 | <0.001 |
| | Sex | 1.19 | 0.27 |
| | Population | 0.62 | 0.43 |
| DBL | HL:Sex | 0.00 | 0.97 |
| | HL: Population | 0.02 | 0.88 |
| | Sex: Population | 0.82 | 0.36 |
| | HL:Sex:Population | 0.22 | 0.63 |
| | HL | 215.46 | <0.001 |
| | Sex | 3.18 | 0.07 |
| | Population | 3.90 | 0.05 |
| DBN | HL:Sex | 0.03 | 0.85 |
| | HL: Population | 1.04 | 0.31 |
| | Sex: Population | 0.29 | 0.58 |
| | HL:Sex:Population | 0.04 | 0.83 |
| | HL | 301.17 | <0.001 |
| | Sex | 0.27 | 0.60 |
| | Population | 6.91 | 0.009 |
| DEN | HL:Sex | 2.40 | 0.12 |
| | HL: Population | 0.01 | 0.92 |
| | Sex: Population | 0.02 | 0.87 |
| | HL:Sex:Population | 0.03 | 0.85 |
| | HL | 269.92 | <0.001 |
| DEL | Sex | 4.52 | 0.03 |
| | Population | 1.42 | 0.23 |

| | | | |
|-----|--------------------|---------|------------------|
| | HL:Sex | 0.00 | 0.98 |
| | HL: Population | 1.33 | 0.25 |
| | Sex: Population | 0.19 | 0.65 |
| | HL:Sex:Population | 0.00 | 0.97 |
| | HL | 167.84 | <0.001 |
| | Sex | 0.10 | 0.74 |
| | Population | 1.46 | 0.230 |
| DLN | HL:Sex | 0.34 | 0.5 |
| | HL: Population | 0.17 | 0.68 |
| | Sex: Population | 2.53 | 0.11 |
| | HL:Sex:Population | 0.35 | 0.55 |
| | SVL | 634.97 | <0.001 |
| | Sex | 25.50 | <0.001 |
| | Population | 45.24 | <0.001 |
| HL | SVL:Sex | 0.12 | 0.72 |
| | SVL: Population | 1.08 | 0.29 |
| | Sex: Population | 0.38 | 0.53 |
| | SVL:Sex:Population | 0.78 | 0.37 |
| | HL | 1341.79 | <0.001 |
| | Sex | 0.96 | 0.32 |
| | Population | 8.09 | 0.005 |
| DRL | HL:Sex | 2.36 | 0.12 |
| | HL: Population | 1.57 | 0.21 |
| | Sex: Population | 2.70 | 0.10 |
| | HL:Sex:Population | 0.00 | 0.94 |

| | | | |
|----|-------------------|--------|------------------|
| | HL | 429.74 | <0.001 |
| | Sex | 0.44 | 0.50 |
| | Population | 0.25 | 0.61 |
| HH | HL:Sex | 0.33 | 0.56 |
| | HL: Population | 0.03 | 0.85 |
| | Sex: Population | 4.39 | 0.03 |
| | HL:Sex:Population | 2.69 | 0.10 |
| | HL | 155.19 | <0.001 |
| | Sex | 0.53 | 0.46 |
| | Population | 5.09 | 0.02 |
| ED | HL:Sex | 2.64 | 0.10 |
| | HL: Population | 1.44 | 0.23 |
| | Sex: Population | 2.75 | 0.10 |
| | HL:Sex:Population | 4.47 | 0.03 |

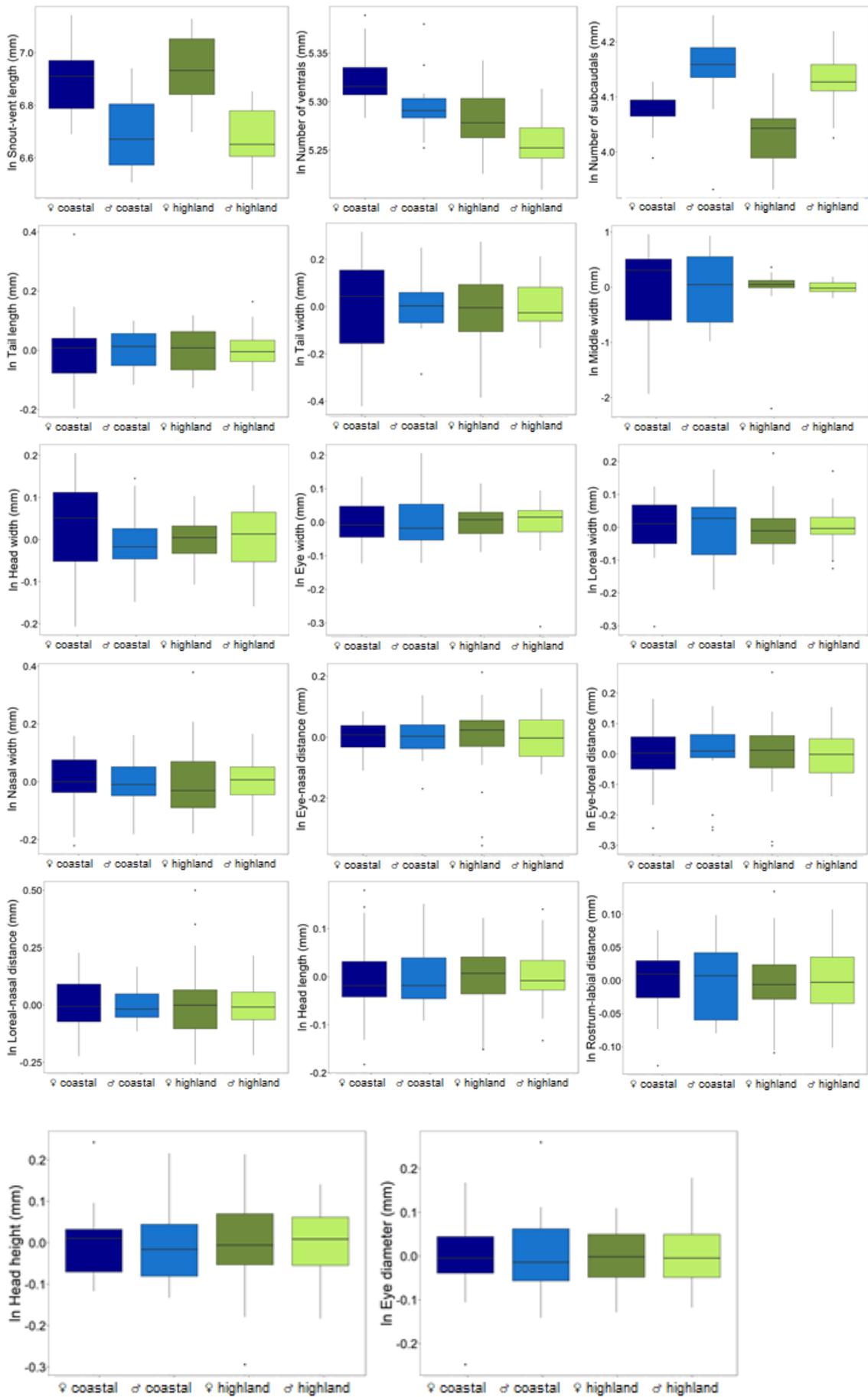


Fig. 3. Boxplots showing sexual dimorphism in two populations of *Bothrops jararaca*. Top panels are raw data, and other plots are residuals extracted from linear models between target variable and covariable (see table 3) to exclude the effect from size. All variables were previously log-transformed.

Table 4: Sexual Dimorphism Index (SDI) for morphological disparity in two *Bothrops jararaca* populations (calculated as (mean of female / mean of male) - 1). Positive values indicate female bias, negative values male bias, and 0 absence of dimorphism.

| VARIABLE | SDI | |
|----------|---------|----------|
| | COASTAL | HIGHLAND |
| SVL | 0.17 | 0.23 |
| VS | 0.12 | 0.12 |
| SS | 0.03 | 0.03 |
| TL | -0.07 | -0.1 |
| TW | 0.08 | 0.1 |
| MW | 0.39 | 0.3 |
| HW | 0.21 | 0.28 |
| DBE | 0.13 | 0.2 |
| DBL | 0.16 | 0.23 |
| DBN | 0.18 | 0.2 |
| DEN | 0.18 | 0.21 |
| DEL | 0.14 | 0.23 |
| DLN | 0.2 | 0.23 |
| HL | 0.24 | 0.27 |
| DRL | 0.21 | 0.27 |
| HH | 0.2 | 0.28 |
| ED | 0.1 | 0.25 |

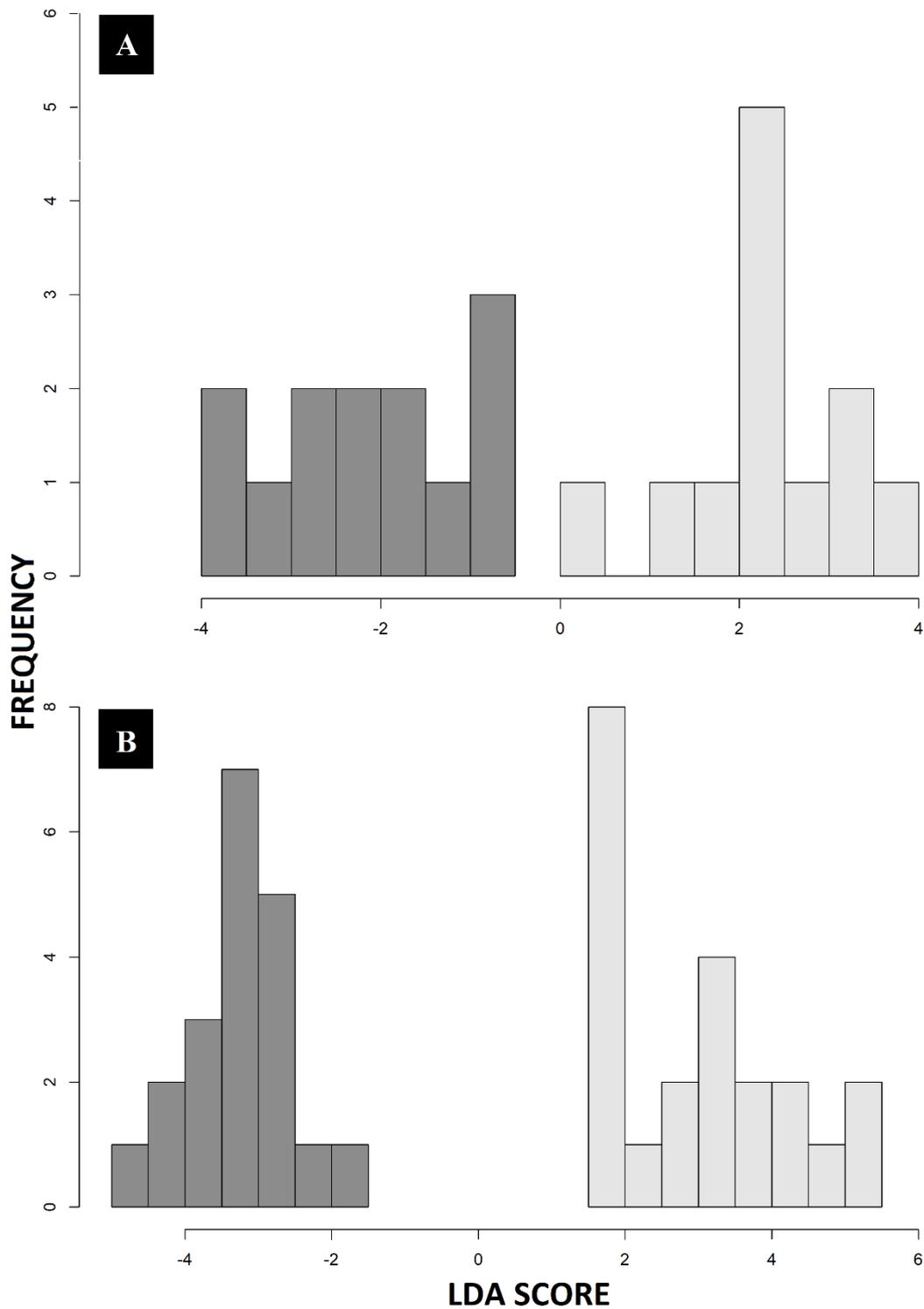


Fig. 4. Linear Discriminant Analysis between females and males *Bothrops jararaca* based on morphological data. A) coastal population and B) highland population. Dark grey bar = females; Light grey bar = males.

2.3.2 Ontogenetic allometry

Allometry hypothesis were rejected once for MW in coastal males ($r^2 = 0.07$, $p = 0.08$). In general, the percentage of variation explained by the size scaling is quite close in both sexes and populations. Only in two of the 56 models built, the percentage of variation explained by size was below 70% (64% for DLN and 42% for ED in males from the coast).

Significant effects in the triple interaction between the covariate and the fixed factors sex and population were not found, indicating parallel trajectories between the groups (Fig. 5). Nevertheless, a significant effect on double interactions occurred in six variables, indicating a difference in the inter- or intra-population allometric trajectory. For the variables HW, DBL, DEL, and DRL, there was no significant effect of sex, population or interactions, with equivalent intercepts, and females reach higher values just because they have longer duration of systemic growth. The variable TL had significant intercept for the sex factor, with males being the larger one. The variables TW, DBE, DBN and ED had significant intercept for the population factor, where the coastal snakes being larger and indicating parallel trajectories with early morphological divergence.

The variable HH showed a significant interaction between the factors sex and population, which points to parallelism between the trajectories, however with alternation of the larger sex, that is, on the coastal population, males are larger whereas on the highland population the opposite occurs. The variables MW, DEN and DLN showed significant interactions between the covariate and population, indicating late divergence between populations. Finally, HL showed significant interactions for the covariate and sex and for the covariate and population, indicating late divergence between these two factors.

For the PCA, we used the residuals of the linear models for the ten variables above that presented significant results. The first two axes were responsible for capturing 48.6% of the data variation (Fig. 6). We found great overlap in the distribution of specimens in the tangent space, still, it is possible to observe a clear separation between males from the coast and females from the highland. The variables HL and MW had the highest negative values on the PC1 axis, while ED and DEN were higher

in the positive direction. This axis is responsible for the segregation on the distribution among adults, with males from the coast having mainly greater eye diameter and greater distance between eye and loreal pit and females from the highlands were more robust and had larger heads. On the PC2 axis, ED and DEN are higher in the negative direction and MW and TL in the positive direction. This axis is responsible for the greater separation between young and adult specimens, with the former having a larger diameter of the eye and distance between the eye and nostrils. Overall adults were more robust and had a relatively larger tail.

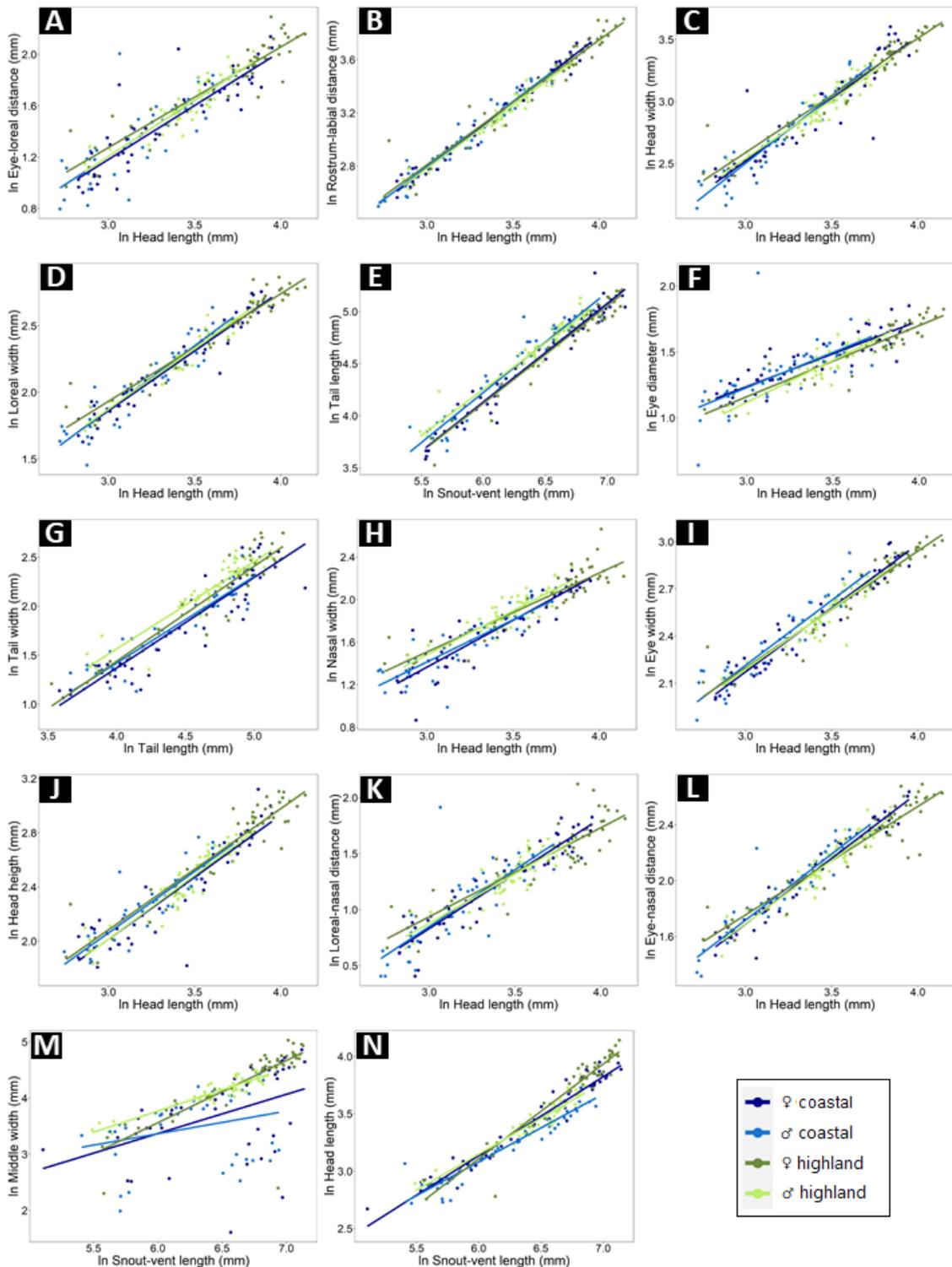


Fig. 5. Ontogenetic allometry of morphological traits of females and males *Bothrops jararaca* from the coastal and highland populations. A-D) Equal intercepts and parallel trajectory; E-J) different intercepts and parallel trajectory; and K-N) different intercepts and non-parallel trajectory.

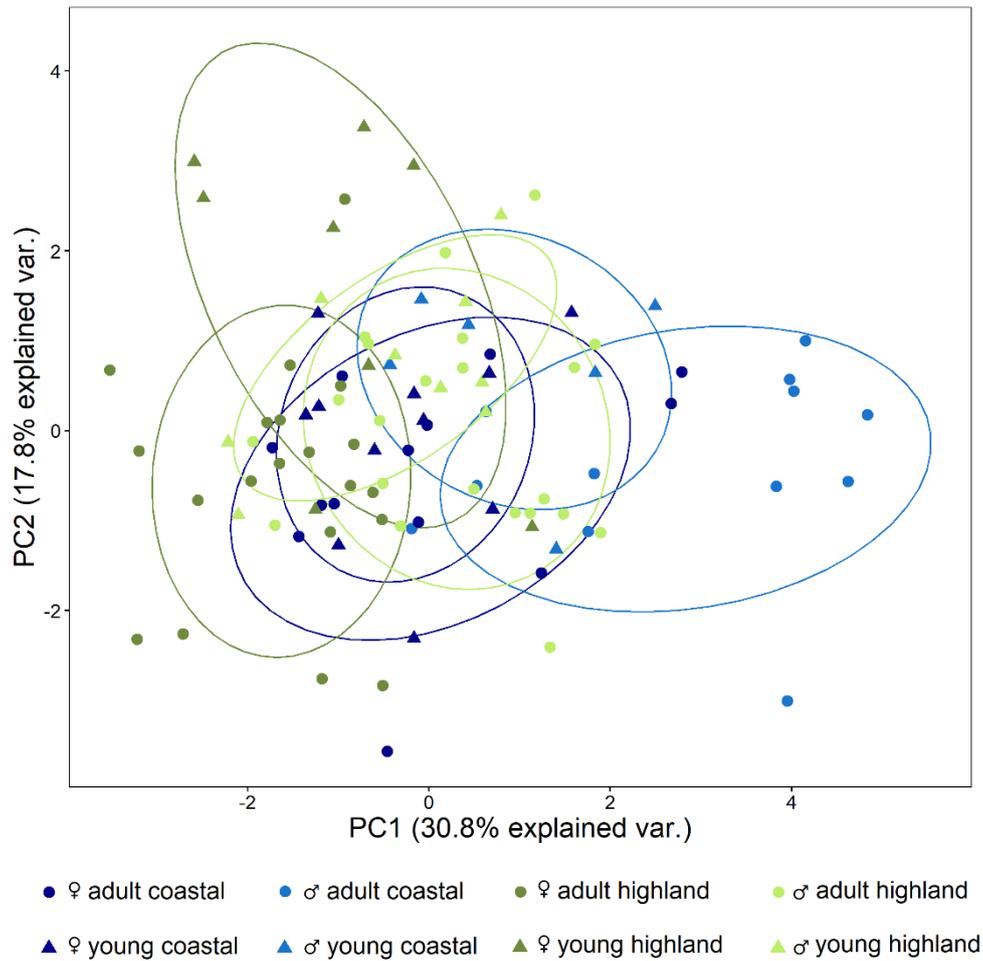


Fig. 6. Principal Component Analysis of the ontogenetic morphological variation between females and males of *Bothrops jararaca* from the coastal and highland populations.

2.4. DISCUSSION

2.4.1. Sexual dimorphism

The results above clearly point to a great morphological difference both intra and intersexual, but the direction of variation often alternated between groups depending on each variable. These results are consistent with other species of viperids (Hoyos et al. 2003; Matias et al. 2011; Sasa, 2002; Zhong et al. 2017), showing that the sexes have different mechanisms of divergence and are influenced by different factors along its distribution.

A previous study carried with *Bothrops jararaca* in southern Brazil showed that several morphological traits differ between the sexes (Matias et al. 2011), with females being generally larger than males. However, the average values presented by the females were lower than the females of the highland population and similar to the coast population. Therefore, different populations of this species are likely to have morphological characteristics strongly associated with local environmental pressures.

The morphological archetype of females with larger body size and smaller tails than males is the most common among snakes that lack combat behavior between males (King, 1989; Shine, 1993; Shine, 1994) and is largely consistent with the hypothesis of sexual selection. Larger females are able to produce more offspring, which provides great adaptive advantages. Tail characteristics (size, width and number of scales) greater in males, is probably a consequence of the accommodation of copulatory organs, an evidence that it is extensively found in snakes (King, 1989).

Nevertheless, not all variations may be explained by the hypothesis of sexual selection, especially considering the trophic morphology (e.g. traits of the head). Females of the marine species *Laticauda colubrina* usually feed on few large eels, whereas males feed on multiple smaller eels (Shetty and Shine, 2002). The females of *Acrochordus arafunae* forage in deeper waters than the males feeding on more bulky fishes (Shine, 1986). Such ecological divergences are accompanied by adaptive variations in morphology. Thus, differences in body size and stoutness of *B. jararaca* could be associated with differences in trophic ecology between the sexes.

Morphological traits may be a result of genetic variation. The number of temporal and ventral scales, and color pattern associated with anti-predatory behavior in species of *Thamnophis* sp. is known to have genetic correlation (Broadie III, 1983; Dohm and Garland-Jr, 1993). Although the populations of the study belong to the same phylogroup (e.g., North clade; Graziotin et al. 2006) and are geographically close, the abrupt altitudinal difference imposed by the Serra do Mar, and consequent physiographic variations, possibly represents a barrier, isolating and preventing part of gene flow.

Of the findings reached in this study, the divergence in the number of ventral scales is one of the most exceptional. Hoge et al. (1976) reported to *B. jararaca* a large range in the number of ventral scales, however, the latitudinal, and consequently climatic, influence is well marked, with specimens from the southern part of the distribution having a considerably smaller number ventral scales in relation to those of

the north. Still, individuals in the State of São Paulo have an intermediate number of scales, being responsible for most of the overlap in data distribution. Accordingly, the most interesting about the populations in this study is the fact that they are very close, with little latitudinal, but most altitudinal variation, which may change the climatic conditions and consequently the number of scales.

A macroecological study found a positive correlation between the scale count and geographical elevation in the *Bothrops* genus (Jadin et al. 2019). However, we find an opposite intraspecific variation, which means that the scale count may possibly vary on smaller geographic scales. The number of ventral and subcaudal scales is strongly related to the number of vertebrae, and consequently to the macrohabitat, with the density of vertebrae increasing with the arboreal habit (Hamptom, 2011). Thus, the largest number of scales for the coastal population suggest a most accentuated use of arboreal habitat, however, observational and/or experimental studies are needed to better elucidate this issue.

Several traits of the head varied between populations. The highland snakes have a longer head but the distance between eyes, distance from eye to nostril and distance from rostral to the last labial scale is greater in the coast population. In snakes with generalist diet habits, the type of prey consumed may lead to variations in the shape of the head. In the *Notechis scutatus*, the population that preyed on species with greater mass, size and circumference also had a larger jaw and mouth (Fabien et al. 2004). In this sense, differences found here may be an artifact of prey choice.

We found no effect of sex or population on the eye size of adults. Although some individuals of *B. jararaca* can be found actively foraging, this species is known to be an ambush predator (Sazima, 1992). Thus, relying on other senses, such as thermoreception for hunting. Experiments with naturally blind snakes or partially deprived of vision, had no impaired biological traits, such as body condition, prey capturing rate, and sexual partner meeting (Bonnet et al. 1999; Young and Morain, 2002), indicating that the size of the eye per se may not undergo strong natural selection.

Coastal females and males are less differentiated from each other than the highland population (e.g., degree of sexual dimorphism). The morphological disparity between the sexes can be more or less accentuated due to the spectrum of the ecological niche occupied by each one in different populations. In sea snakes, for example, in regions where large prey are less abundant, the degree of sexual

dimorphism is often reduced (Shine et al. 2002). This suggests a niche partition among the populations of this study.

The linear discriminant analysis shows the marked dimorphism between the sexes in both populations. The lack of overlap, however, may have occurred for the reduced sample, since the sample used contained only individuals with all the variables present. Even so, it is possible to notice that different variables were responsible for the separation, which indicates morphological adaptation in each environment. It is still necessary to keep in mind that many other factors can contribute to sexual segregation, especially demographic ones, which may cause bias in the male-female ratio and expression of the SDI, such as parasitism, nutritional stress or physical exhaustion (Giery and Layman, 2019).

2.4.2. Ontogenetic allometry

Females reached larger sizes (with the exception of the tail attributes) by different mechanisms. The ontogenetic growth patterns found in this work are very similar to the population of southern Brazil (Matias et al. 2011) and other species of the genus (e.g., *Bothrops atrox*; Silva et al. 2017). Thus, it seems to be a very conserved trait in the genus *Bothrops*. In some traits, females are larger from birth. This fact also appears to be common in snakes, as in some Natricines where females are larger and most sexual differences appear soon in newborns instead of being fixed in adults (Gregory, 2004).

For some traits, even though males were initially equivalent or even larger, female growth rate was faster, culminating in relatively larger sizes. In snakes, the growth rate is rapid initially and decreases after sexual maturity, and in many cases where sexual dimorphism tends towards larger females, they tend to mature later, which can result in the observed allometric pattern (Brown and Weatherhead, 1999; Shine, 1978; Webb et al. 2003).

The skewed survival rate for one sex may culminate in size disparities. Although this factor has not been explored in the present study, the discrepant allometric trajectory between the sexes suggests that the smaller growth rate in males is the most likely factor causing dimorphism, rather than a higher mortality rate. Similar results were found for *Morelia spilota*, where females showed extreme values of size, however, the recapture rate was equivalent between sexes (Pearson et al. 2002), which supports the growth rate hypothesis instead survival rate.

Since snakes have undetermined growth, life expectancy may cause morphological differences between populations. Specimens of *Elaphe quadrivirgata* from the island of Tadanae-Jima, Japan, are considered gigantic in relation to those of other populations, and take twice as long to reach their maximum size, with a constant growth rate (Hasegawa and Mori 2008). The analysis of the ontogenetic trajectories together with the PCA helps to illustrate the difference between sexes and populations throughout development and supports the hypothesis that specific ecological pressures act in each population, considering that the sexes in both are different earlier in life.

Prey availability often fluctuates according climatic variation. Growth rate of *Liasis fuscus* born in years with more food available was higher and constant throughout development (Madsen and Shine, 2008). Likewise, prey availability (e.g. anurans) and snake fecundity also co-vary annually, and the positive correlation between maternal size and litter size makes larger females more sensitive to variation in prey availability (Brown and Shine, 2007). Considering that *B. jararaca* feeds on anurans at least in juvenile stages (Sazima, 1992), differences in prey availability between populations may induce important ecological variations.

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CHAPTER 2

SEXUAL DIMORPHISM AND ONTOGENETIC VARIATION ON THE HEAD SHAPE OF TWO NEIGHBOURING POPULATIONS OF THE COMMON LANCEHEAD *BOTHROPS JARARACA*: A GEOMETRIC MORPHOMETRIC APPROACH

ABSTRACT

The head are the most important element in trophic ecology among snakes. Head shape is affected by several factors both intrinsic as size and sex, as extrinsic such as geographic variation. Often different populations are subject do different environmental conditions that modulate ecology and are reflected in morphology. Here we investigate sexual dimorphism and ontogenetic allometry on *Bothrops jararaca* head shape in two populations. We found a significant effect of sex and population, being that females from the highland had a large post-ocular region e more arrow shaped head. Size accounted for most variation in shape but diverged between populations. Sexual dimorphism in head shape were not found in juveniles, however, ontogenetic trajectory varied greatly between populations. In the *Bothrops* genus, except in early stages, females often grow faster and attain larger sizes than males, which may be affecting head shape. Also, possible differences in diet and ontogenetic variations between populations may be a reasonable cause to specific allometric trajectories.

3.1. INTRODUCTION

The head figure as one of the most important elements in snakes bauplan. Since these animals have an elongated body with almost no limbs, the head becomes fundamental for performing many essential functions of the snake biology. In some species the triangular shape of the head may act like an antipredator signal, such as in vipers and their mimics (Valkonem et al 2011). But most important is the role as a feeding apparatus, responsible for capture, holding, manipulating and swallowing of the prey (Gans 1961; Cundall 1983).

As gape-limited predators, the snake's diet is constrained by the mouth opening, and therefore, it is reasonable to assume that the head represents a source of great variation in snake fitness. In these group of reptiles, body and head size are widely correlated, and in the same way, head size is also correlated with swallowing performance. This indicates that longer snakes may take larger prey, but snakes with larger relative head size are capable to eat even heavier prey than those with smaller heads but with same body size, and which are translated into different energy intake (Forsman and Lindell 1993). Following the same idea, head shape may be also affected by the prey-predator ratio trade-off. Head shape varies among snakes of different size, which allows that different head shaped snakes explore different diets (Vincent 2003). Therefore, due to this intricate relationship, the morphology of the head becomes a target of strong selective pressures.

Differential niche exploitation may be quite advantageous mostly because is a major source of competition reduction. Juveniles often shows different patterns then adults, both spatially and temporally, having specific diet habits, habitat selection or daily activity (Lind and Welsh Jr 1994; Shine et al. 2003; Webb et al. 2005; Székely et al. 2020). Furthermore, these partitioning may occur even in the same age class. Male and females of different body sizes are able to explore specific prey types or sizes, associated or not to different foraging sites (Shine 1986; Shetty and Shine 2002; Vincent et al. 2004). Accordingly, in the cottonmouth *Agkistrodon piscivorus*, males are mostly piscivorous, whereas females rely mostly on reptiles, such as snakes, and prey size increase as the snake grows (Vincent et al. 2003; Vincent et al. 2004).

Nonetheless, both sexes are also subjected to strong sexual selection. Larger females generally present a higher reproductive output (e. g. Higher frequency, larger litters, larger neonates or higher relative clutch mass) while larger males gain more

access to females in species where male-male combat is common (Luiselli et al. 1996; Shine 2003). Nevertheless, some morphological traits such as body and head size, may be more or less affected by trophic or reproductive ecology than others (Ford and Seigel, 1989; Bonnet et al. 2000), hence, drivers of this sex-biased size and their cause-consequence relation are often difficult to determine.

The developmental processes that result into given final adult sexual dimorphism can be often complex. One sex will be larger than the other if it already born larger, or grows during the same time interval, but with a higher growth rate, or if it grows at a similar growth rate, but during a longer time period. Understanding the mechanistic strategies involved in generating those patterns is important, since the degree of sexual dimorphism may be expressed differently across species, populations or even among different traits in an organism (Badyaev, 2002). For this purpose, studying ontogenetic growth that may result in sexual divergences is crucial.

In studies regarding morphology, the usual linear morphometrics are extensively used. However, even though its indubitable value, this method may eventually become less appropriate when comparing shape variation, providing poor descriptors mainly in studies where the focus are functional traits (Sidlauskas et al 2011; Fabre et al 2014). In this regard, geometric morphometrics rises as a complementary approach that improves significantly the morphological analyses. In reptiles this method has been applied as a powerful analytic tool to shed light on several important biological questions concerning species delimitation (Ruane 2015), evolutionary trends (Davis et al. 2016), ecological drivers (Manier 2004) and developmental and allometric trajectories (Kaliontzoupoulou et al. 2008).

The common lancehead *Bothrops jararaca* is a widespread Atlantic Forest dweller that occurs from sea level to 1200 m altitude (Sazima 1992; Campbell and Lamar 2004), and has a well-known natural history. In this species several biological traits pass through a marked ontogenetic change, such as venom action and composition (Zelanis et al. 2010), habitat use, and behavior (Sazima 1992; Marques et al 2019). Ontogenetic variation is also conspicuous in the diet, as juveniles feed mostly on ectothermic vertebrates, such as anurans (*e.g. Hylids*) and lizards, whereas adults rely almost completely on small endotherms like rodents (Sazima 1992; Hartman et al. 2003). In the Atlantic Forrest, richness, composition, abundance and biomass of anurans are often driven by local environmental factors and generally vary in relation to the altitudinal gradient (Giaretta et al 1999; Vasconcelos et al 2014). In

the Serra do Mar Region (Southeastern Coast-Brazil) assemblage composition of the *Hylid* family was found to be greatly variable among the sampling sites at different altitudes (Silva et al 2017), which may affect prey availability for *B. jararaca*.

Therefore, based on the premises that *B. jararaca* i) presents a considerable geographic distribution, ii) shows an ontogenetic variation in the diet, iii) depends on the prey availability which may vary among sites, and iii) presents singular both static, and ontogenetic allometric trajectories presumably driven by environmental pressure, we aim to explore the hypothesis that *B. jararaca* shows sexual and populational differences in the individual head shape, and that allometric pattern vary both intra and interpopulationally.

3.2. METHODS

3.2.1 Data sampling

Photographs of the dorsal view of the head were taken from 163 *B. jararaca* specimens. Snout-vent Length (SVL) were also measured to the nearest mm using a tape. From those, 74 individuals belonged to the coastal population (42 females and 32 males), and 89 to the highland population (42 females and 47 males). All specimens were housed at the Herpetological Collection “Alphonse Richard Hoge” in the Butantan Institute, São Paulo, Brazil.

Nineteenth anatomical landmarks in the right side of the head (to avoid pseudo replication) were digitized using the software TPSdig 2 (positioning and landmarks types are indicated in Figure 1). The criteria used to select the landmarks focused on optimally defining the shape variation among sexes and populations, including most relevant morphological characters mainly focused on trophic anatomy. All configurations were then subjected to Generalized Procrustes Superimposition analysis. This procedure was used to standardize all specimens subtracting the effect of size, positioning and orientation of the coordinates, remaining only shape-derived variation. A size variable was extracted from each landmark configuration as the Centroid Size (CS). This measure was estimated as the square root of the sum of square distances of the landmarks from their barycenter, and were largely used in geometric morphometric analysis (Tamagnini et al, 2018; Loebens et al, 2019).

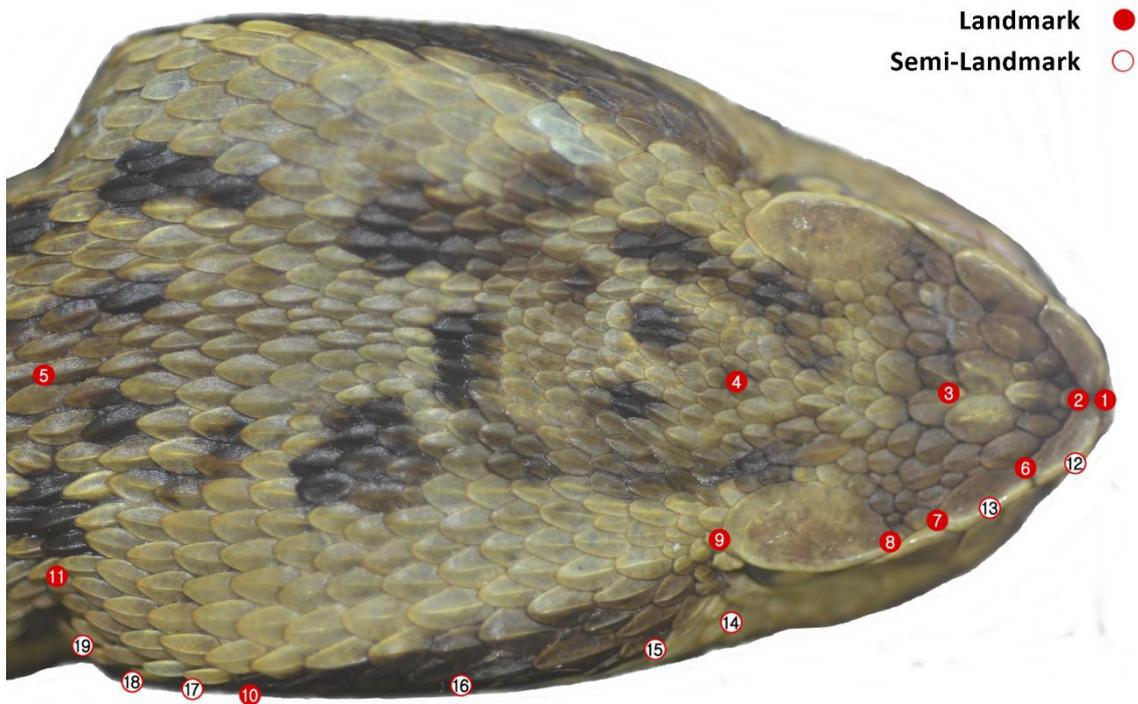


Figure 1: Schematic illustration indicating the position of the 19 landmarks used to analyze head shape variation in *Bothrops jararaca*.

3.2.2. Sexual dimorphism

A nested subset containing only adults was used to assess sexual dimorphism. Females were considered adult when larger than 750 mm, and males when larger than 650 mm length (Sazima 1992). Linear models were used to test the relation between CS and SVL in each group, and to test for head size variation a one-way ANOVA was carried on CS. Principal Component Analyses (PCA) were performed on the Procrustes Coordinates to visualize groups relationship in the morphospace. The presence of intersexual and interpopulation variance on head shape was tested with a three-way Procrustes MANOVA using the Procrustes coordinates as independent shape variables, with CS as covariate and sex and population as fixed factors. This procedure was used to account for the impact of size in the head shape due to static allometry.

In order to analyze the degree of sexual dimorphism in the two populations, an Index of sexual shape dimorphism (SSD) was computed as the Procrustes distances between mean female and mean male measurements divided by the maximum Procrustes distances between males and females (Tamagnini et al 2018). Static

allometry was tested regressing shape coordinates onto CS using multivariate linear models in each sex separately, and wireframes were built to visualize specific shape change along CS gradient.

3.2.3. Ontogenetic allometry

First, a three-way Procrustes MANOVA was performed to test if head shape was different between sex and populations among the juveniles, as well as accounting for CS variation. A PCA was then performed with the complete dataset to observe the relation of age classes, sex and populations in the morphospace.

Ontogeny was investigated using a very similar approach as the described in the previous section but using the complete dataset (including juveniles). Another three-way Procrustes MANOVA was performed to test the significance of the interaction between CS, sex and population across all individuals. Significant results in the interactions mean different slopes, and consequently different ontogenetic trajectories. To verify the assumption of ontogenetic allometry, the impact of CS variation in head shape was tested using multivariate linear models separately in each sex. Ontogenetic trajectory were visualized using wireframes.

3.3. RESULTS

3.3.1. Sexual dimorphism

In the coastal population, larger snakes also had higher CS values (females $r^2 = 0.43$, $p = 0.002$; males $r^2 = 0.83$, $p \gg 0.05$), however no relation was found in the Highland population (females $r^2 = 0.005$, $p = 0.29$; males $r^2 = -0.02$, $p = 0.5$). The ANOVA results showed a significant interaction between SVL and population ($F = 10.38$, $df = 1$, $p = 0.001$), being that in smaller highland snakes the CS was greater, whereas in larger highland snakes the CS was smaller (Figure 2).

The first two PCs captured 47.6% of the shape variation (Figure 3). The PC1 (25.5% of the variation) explains mostly the post-ocular length, specifically in landmarks 9 and 10. The positive values on this axis indicates shorter post-ocular region, with landmarks 9 and 10 close to each other and a longer snout (pre-ocular region), and the opposite occurring along the negative values. We found great overlap in specimen distributions on this axis, however, the coastal males concentrated more in the positive region having a relatively larger pre-ocular region. The PC2 (22.1% of the variation) explains head width, mainly in landmarks 5 and 17. The positive values

on this axis indicates broader and most rounded (arrow-shaped) head, with landmarks 5 and 17 far from each other, whereas negative values, a thinner head. No discrimination pattern was clear in this axis. Based on Procrustes distances, SSD on coastal population was 0.20, while in highland population SSD was 0.16, which means that the morphological displacement on the former was more considerable.

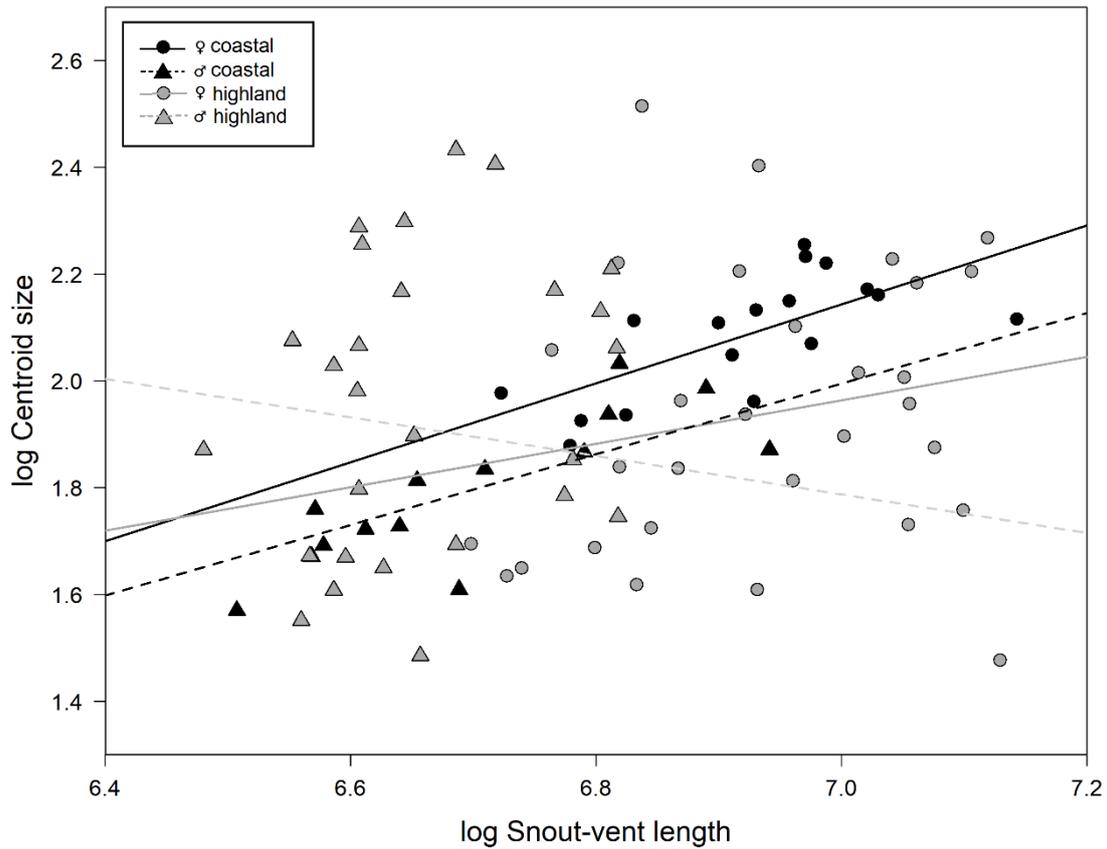


Figure 2: Regression models for Centroid size derived from Procrustes coordinates of the dorsal view of the head by snake snout-vent length in *Bothrops jacacara* populations.

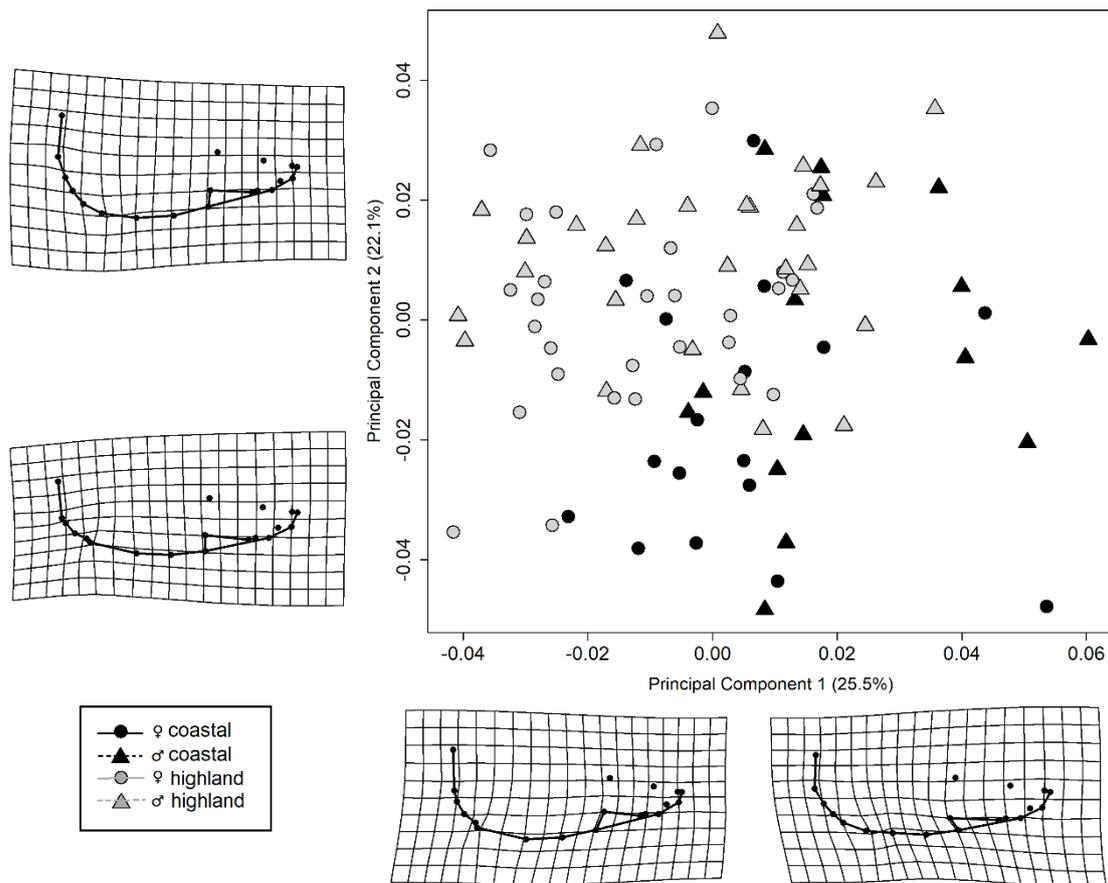


Figure 3: Principal Component Analysis based on Procrustes coordinates showing head shape variation in adults *Bothrops jararca*.

Size variation explained a higher percentage of shape variation in the coastal population but had no effect on shape variation in the highland population (table 1). The three-way Procrustes MANOVA for adults was found to be significant for all factors and the double interactions CSxPopulation even after Bonferroni correction (table 2), which means that head shape changes with increasing CS, and were different, and parallel between sexes but with different slopes between populations. The general static allometry pattern suggests that snakes with smaller CS had thinner heads, while greater CS corresponds to more rounded arrow-shaped head (figure 4). Specific wireframes highlighted distinct shape variation between populations. The overall shape variation pattern indicated a more elongated and rounded head when CS was greater. However, the most apparent difference between populations was found in the backward and lateral displacement of the facial landmarks (e.g. landmarks 5 and 9) in individuals of the coastal population, and the forward displacement of the same

landmarks producing greater post-occipital region in individuals of the highland population (Figure 5).

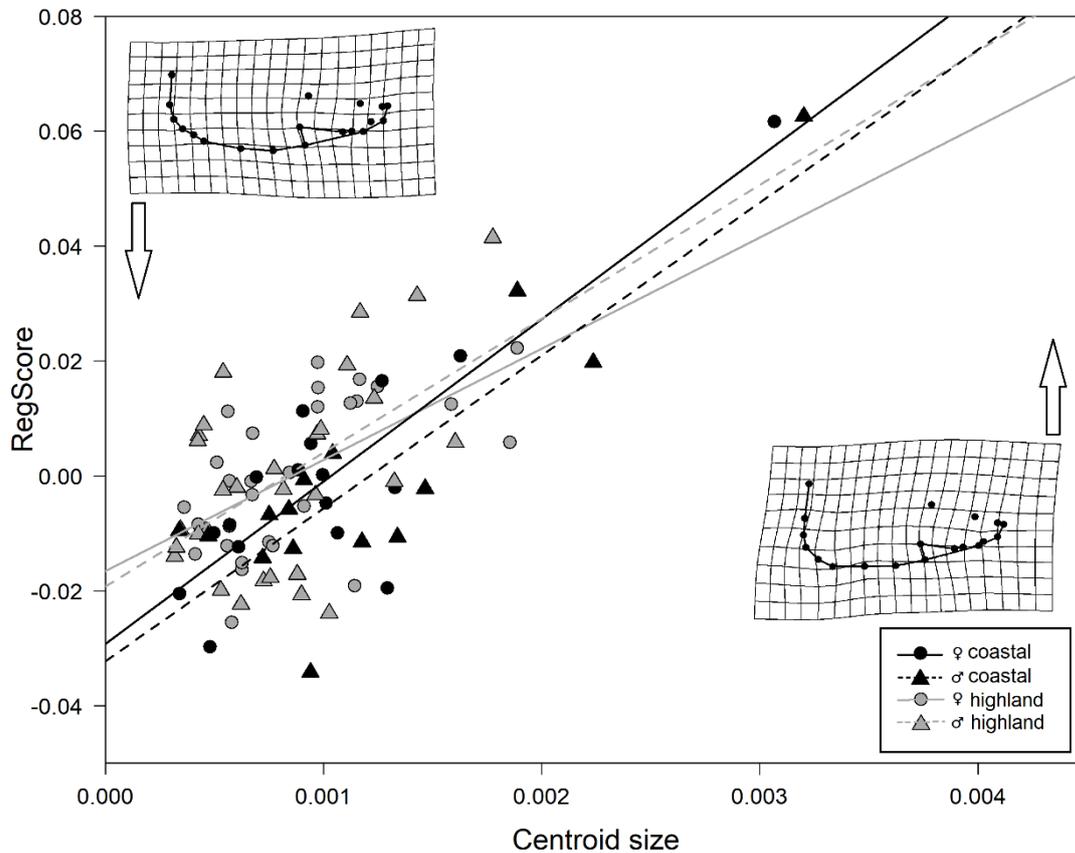


Figure 4: Static allometry (adults) of head shape in *Bohtrops jararaca* sexes and populations based on the regression of Procrustes coordinates on Centroid Size.

Table 1: Static (Adult) and ontogenetic (All sample) allometric models for *Bothrops jararaca* populations with the head shape variation predicted by size. Bold indicates significant size effect.

| Data | Population | Sex | Predicted | <i>F</i> | <i>P</i> |
|-------|------------|--------|-----------|----------|--------------|
| Adult | coastal | female | 19% | 3.53 | 0.002 |
| | | male | 19% | 3.1 | 0.008 |
| | highland | female | 3% | 1.14 | 0.31 |
| | | male | 5% | 1.51 | 0.16 |

| | | | | | |
|------------|----------|--------|-----|------|--------------|
| All sample | coastal | female | 13% | 6.01 | 0.001 |
| | | male | 19% | 7.16 | 0.001 |
| | highland | female | 3% | 1.62 | 0.14 |
| | | male | 5% | 2.47 | 0.02 |

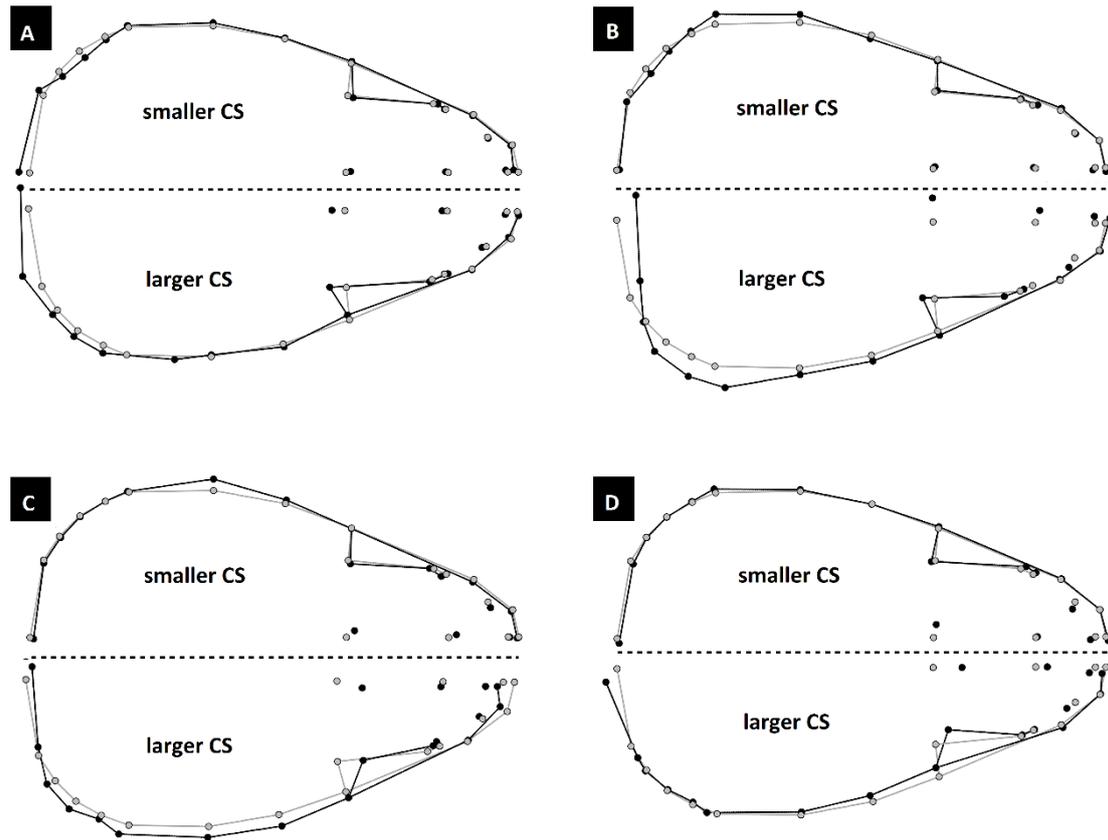


Figure 5: Wireframes providing visualization of static allometry trajectory using Centroid size (CS) as predictor in *Bothrops jararaca* a) coastal females, b) coastal males, c) highland females and d) highland males.

Table 2: Three-way Procrustes MANOVA of three datasets, accounting for size, sex and population effects on head shape of *Bothrops jararaca*. Bold indicates significant effects after *Bonferroni* correction.

| Data | Variable | df | SS | MS | RSQ | F | Z | P- adjusted |
|---------------|-------------------|----|-------|-------|-------|-------|-------|----------------|
| Adults | CS | 1 | 0.006 | 0.006 | 0.035 | 4.025 | 2.960 | 0.007 |
| | sex | 1 | 0.005 | 0.005 | 0.030 | 3.536 | 2.734 | 0.014 |
| | population | 1 | 0.017 | 0.017 | 0.102 | 11.72 | 4.996 | 0.007 |
| | CS:sex | 1 | 0.001 | 0.001 | 0.007 | 0.849 | -0.14 | 1.000 |
| | CS:population | 1 | 0.011 | 0.011 | 0.067 | 7.739 | 4.497 | 0.007 |
| | sex:population | 1 | 0.003 | 0.003 | 0.021 | 2.487 | 2.141 | 0.105 |
| | CS:sex:population | 1 | 0.001 | 0.001 | 0.011 | 1.331 | 0.789 | 1.000 |
| | residuals | 83 | 0.124 | 0.001 | 0.723 | | | |
| | total | 90 | 0.171 | | | | | |
| Juveniles | CS | 1 | 0.019 | 0.019 | 0.123 | 11.9 | 4.82 | 0.007 |
| | sex | 1 | 0.001 | 0.001 | 0.012 | 1.18 | 0.59 | 1.00 |
| | population | 1 | 0.019 | 0.019 | 0.119 | 11.6 | 4.93 | 0.007 |
| | CS:sex | 1 | 0.002 | 0.002 | 0.018 | 1.75 | 1.42 | 0.588 |
| | CS:population | 1 | 0.006 | 0.006 | 0.043 | 4.17 | 3.15 | 0.007 |
| | sex:population | 1 | 0.001 | 0.001 | 0.012 | 1.19 | 0.65 | 1.00 |
| | CS:sex:population | 1 | 0.003 | 0.003 | 0.022 | 2.16 | 1.89 | 0.196 |
| | residuals | 63 | 0.104 | 0.001 | 0.649 | | | |
| | total | 70 | 0.160 | | | | | |
| All Sample | CS | 1 | 0.070 | 0.070 | 0.187 | 40.6 | 7.10 | 0.007 |
| | sex | 1 | 0.002 | 0.002 | 0.005 | 1.27 | 0.77 | 1.00 |
| | population | 1 | 0.026 | 0.026 | 0.069 | 15.1 | 5.80 | 0.007 |
| | CS:sex | 1 | 0.002 | 0.002 | 0.007 | 1.68 | 1.32 | 0.71 |
| | CS:population | 1 | 0.004 | 0.004 | 0.011 | 2.44 | 2.16 | 0.091 |

| | | | | | | | |
|-------------------|-----|-------|-------|-------|------|------|------|
| sex:population | 1 | 0.001 | 0.001 | 0.003 | 0.77 | -0.2 | 1.00 |
| CS:sex:population | 1 | 0.001 | 0.001 | 0.003 | 0.86 | -0.1 | 1.00 |
| residuals | 161 | 0.267 | 0.001 | 0.710 | | | |
| total | 164 | 0.376 | | | | | |

3.3.2. Ontogenetic allometry

We found a significant population effect when analyzing the juveniles, although there was no effect for sex (table 2). Thus, females and males were pooled together on the PCA analysis. PC1 and PC2 explained 51.3% of the total ontogenetic variation (Figure 6). Overall, ontogenetic PCA followed a similar pattern of the static PCA, that is, PC1 controlled mainly the positioning of the landmarks 9 and 10, stressing a large pre-ocular and short post-ocular region in the positive way and the opposite in the negative way. Despite a small overlap on this axis, both populations appeared separated, being that the coastal individuals occupy mostly the positive portions, with juveniles in the extreme of the gradient whereas the Highland individuals were distributed mostly in the negative portion despite the ontogenetic trend of variation being less evident. PC2 controlled most for head width, and landmarks 5 and 17 (quadrate bone region) had a major importance on this axis. Individuals had relatively broader head (lance-shaped) in the positive way and a thinner head in the negative way. We found no clear pattern in this axis.

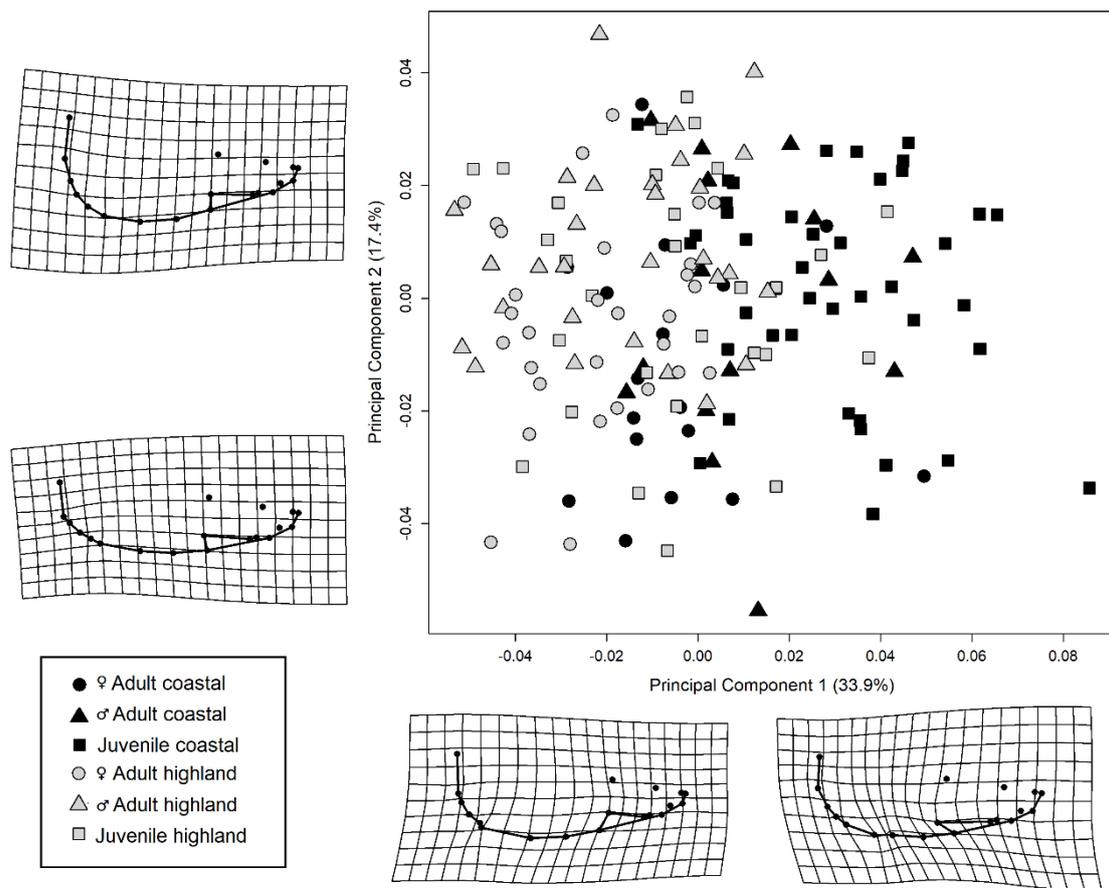


Figure 6: Principal Component Analysis based on Procrustes coordinates showing head shape variation during ontogeny in *Bothrops jararaca* individuals of two populations.

Size variation also explained a higher percentage of shape variation in the Coastal population (table 1), but it resulted statistically significant only among the males from the highland population. The three-way Procrustes MANOVA performed in the complete sample confirmed PCA pattern. We found a significant effect of CS and population on head shape variation, but no effect for sex or any interactions (table 2). Ontogenetic allometry shown that sex variation where equal, but different between populations. However, the lack of significance in interactions indicates parallelism between ontogenetic trajectories. In general juveniles had a thinner head and larger pre-ocular region, and adults had broader heads and larger post-ocular region (figure 7). Specific wireframes show a similar head in the juveniles however the most prominent change was that in the coastal population landmarks in the ocular region moved backward with increasing CS while in the Highland population the opposite occurred (Figure 8).

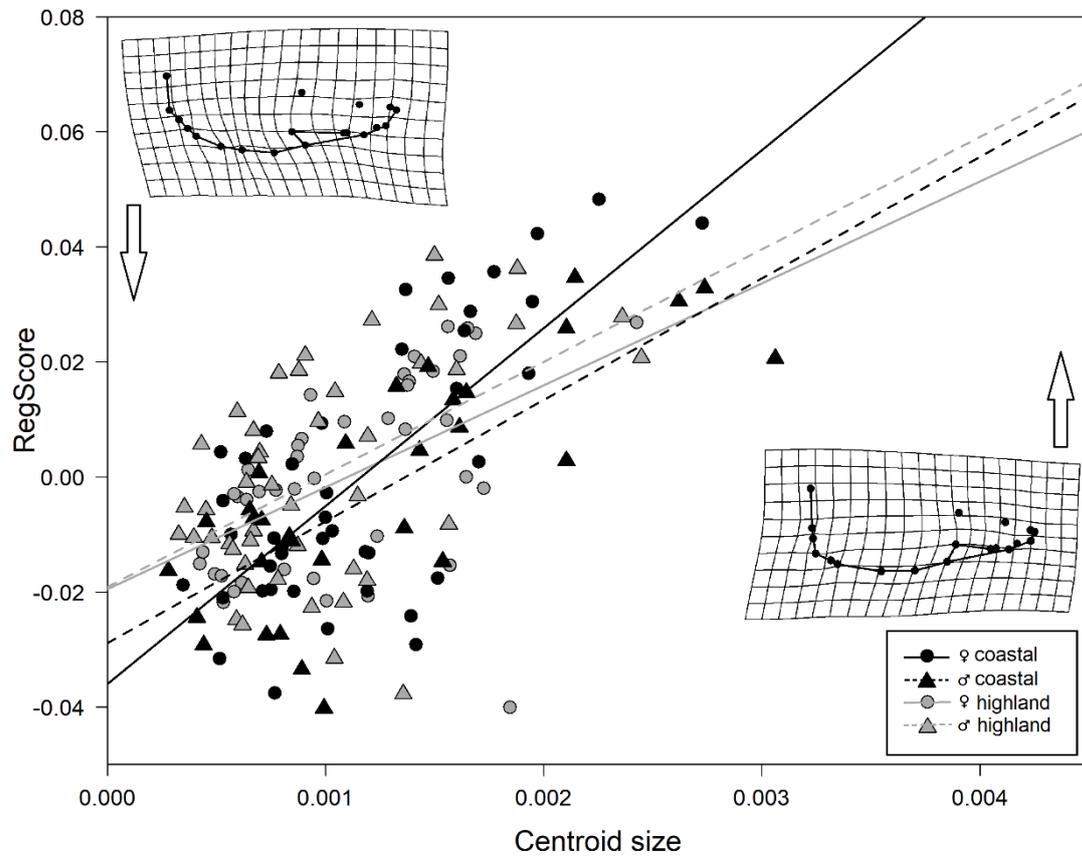


Figure 7: Ontogenetic allometry of head shape in *Bothrops jararaca* sexes and populations based on the regression of Procrustes coordinates on Centroid Size.

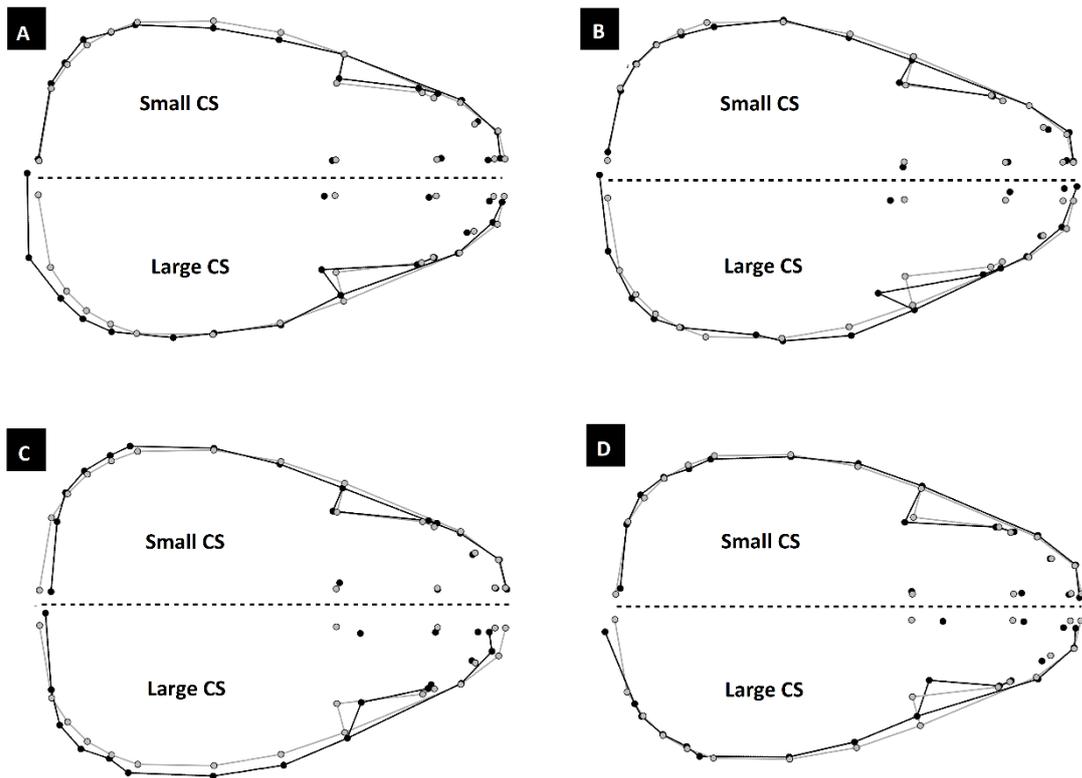


Figure 8: Wireframes providing a visualization of the ontogenetic allometry trajectory using Centroid size (CS) as a predictor in *Bothrops jararaca*: a) coastal females, b) coastal males, c) highland females and d) highland males.

3.4. DISCUSSION

3.4.1. Sexual dimorphism

Our results showed a distinct pattern of size and shape differences between sexes and populations. Since *Bothrops jararaca* had a clear sexual size dimorphism in morphological traits of the head (Matias 2011), shape disparities were not surprising. Highland females shown relatively longer pre-ocular region than sympatric males and coastal males and females. However, distinct from the coastal, the highland population showed no effect of shape explained by size variation (represented by the centroid size), thus, this difference in shape is probably an outcome of other sources, as ecological pressures. We cannot discard though, a displacement between head and body measures. Highland females were the longest in body size (chapter 1), and head shape may be reflecting this pattern, which suggest a different co-variation between morphological traits and head shape among populations.

Similar patterns of sexual shape dimorphism were previously reported for the genus. Females *Bothrops atrox* presented relatively more robust heads than males

(Silva et al 2017). However, populational differences remain unknown. These results are congruent with several other linear traits both of the head and the body, as females are generally larger in almost all variables measured (Sasa et al 2003; Hartmann et al 2004; Matias 2011; Leão et al 2014; Bisneto and Kaefer 2019).

The PCA suggested that neither population was completely sexually dimorphic. This appears to be a trend in snakes. For example, a study in two sympatric and closely related species *Tomodon dorsatus* and *Thamnodynastes strigatus* indicated that there was some degree of head shape dimorphism, besides a relevant overlap in both species (Loebens et al 2019). There was also a significant difference between the two species studied, which may be due to specificities in their ecology, such as diet or habitat use. The same premise (e. g. niche partitioning in prey size) may be applied to sex divergence.

In reptiles, jaw musculature may evolve differently in males and females as a result of sexual selection (male-male combat) and/or functional ecology (bite force applied to crush different prey types), and this particularity may be accompanied by an enlargement of the head (Herrell et al 1999). This association may explain sexual dimorphism on *T. dorsatus* and *T. strigatus*, since those species are both active forager that hold and manipulates prey (Bernardes et al 2000; Bizerra et al 2005; Loebens et al 2019). On the other hands, adults *B. jararaca* are ambush hunters that bite, release the prey and then follow chemical clues to found and ingest the dead prey (Sazima 1989). The predation strategy used in this case may explain the lack of differences in head width between sexes within both populations.

Feeding behavior is probably the most important factor influencing trophic morphology on snakes (Shine 1989). Prey size increase with snake size, and often, sexes diverge in prey dimensions, which result in different head morphology (Vincent et al. 2003; Vincent et al. 2004). Feeding habits analysis indicates that the highland populations consume relatively larger prey than coastal population (Moraes 2008). Feeding ecology associated with sexual size dimorphism patterns (e. g. female-biased) may explain why highland females showed a larger pos-occipital and more rounded head.

However, there is no evidence indicating that head is affected by sexual selection in snakes. Shine (1991) has examined a big data-set with several snake species of different families and found no evidence that head size positively affected reproductive success. He found that females had larger heads in most species and

when male-male combat was present in a species, males seem to present larger heads. The higher values of Centroid Size in *B. jararaca* females and the lack of male-male combat indicate that sexual selection plays a negligible role in head shape dimorphism. The common gecko *Woodworthia maculatus*, that generally present a weak pre-copulatory selection, also show no sexually dimorphic head shapes (Kelly 2015).

Static allometric trajectory also varied between sexes and populations. Allometric regression performed on six cranium elements in adults *Natrix natrix* and *Natrix tessellata*, species ecologically close, but with different feeding strategies, shown a significant different allometry within (males versus females) and across species, however each element were affected by size in different degrees (Andjelković et al 2016). Another example with species that occupy overlapping niches but with different feeding strategies are the sympatric *Natrix Helvetica* and *Vipera berus*. Tamagnini et al. (2018), found a significant amount of shape predicted by four size variables in adults of *N. Helvetica* but not in *V. berus* (except head shape predicted by Centroid Size in females). This finding indicates that allometric trajectories are species-specific and are possibly driven by feeding behavior.

Notwithstanding, head shape variance between populations may rise as a function of environmental factors. The Ceasar's Lizard *Gallotia caesaris* populations living in regions with significant different substrate types and vegetation showed a marked shape difference between sexes and among populations (Molina-Borges et al. 2010). Also, the Rural population of the common Lizard *podarcis muralis* significantly diverged from the Urban population in head shape and allometric trajectories (Lazié et al. 2015). In this sense, differences in shape and allometry in *B. jararaca* is probably an artifact of different ecological pressures that males and females are subjected in each region.

3.4.2. Ontogenetic allometry

Juveniles presented a similar head shape between sexes. Diet (e.g. amount of food, prey type or size) is one of the most cited factor inducing sexual dimorphism in snakes head, however experimental studies that controlled for food intake in males and females, besides a strong impact in body measures, shown little effect of a higher feeding treatment on head measures in early stages of ontogeny (Queral-Regil and

King 1998; Schuett et al. 2005). Sexual size dimorphism usually appears later in juvenile stages, probably because sexes grew in different rates (Chiaraviglio et al. 2003; Schuett et al. 2005). In this way, the equally head shaped sexes in our study corroborates the absence of sexual dimorphism in early stages.

Head shape were strongly correlated to size (except females from highland). Commonly in snakes, a given sex may grow faster than other, nevertheless, the difference may not be significant in early stages of life (Gregory and Prelypchan 1994; Lerner and Mason 2001). In *Bothrops fonsecai*, another pitviper showing female-biased sexual dimorphism, newborns were equally sized after birth, however tests repeatedly performed showed that females grew faster until the 15th month even when fed in the same proportions than the males (Stuginsky et al. 2017). Accordingly, differential growth rates may explain why shape differences were present only in adults *B. jararaca*.

Contrasting the strike and release strategy used for prey capturing in adult *B. jararaca*, juveniles often bite and holds their prey possibly due the fact that frogs or lizards are less dangerous than small rodents (Sazima 1989; Sazima 1991). A study carried with four snake species that vary greatly in head morphology and prey capturing methods revealed different muscle recruitment during feeding (Culdall 1983). Thus, we suggest that an association between diet and feeding behavior related to muscle performance may explain the difference in head shape between juveniles and adults while growing.

The genus *Bothrops* is known to present a marked pattern of ontogenetic variation in diet (Sazima 1992; Martins et al. 2002; Valdujo et al. 2002; Nogueira et al. 2003). Also, prey availability or preference often vary between populations (Fabien 2004; Manjarrez et al. 2013; Siqueira and Marques 2018). Thus, a possible displacement in diet habits by the populations of the study may be causing differences in head shape. Yet, the onset of ontogenetic shifts may be different among populations (Natusch and Lyons 2012). Indeed, the highland population showed a drastic reduction in the frequency of ectothermic prey frequency in early sizes, contrasting with a smoother pattern on coastal population (Moraes 2008). In this sense we believe that timing in diet shift from ectotherms to endothermic prey in are probably heterochronic, which are reflected in different ontogenetic trajectories in head shape.

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CHAPTER 3

MORPHOLOGICAL VARIATION IN THE GOLDEN LANCEHEAD *BOTHROPS INSULARIS*: SEXUAL DIMORPHISM, ONTOGENY AND MICROEVOLUTIONARY TRENDS.

ABSTRACT

The golden lancehead *Bothrops insularis* presents several morphological aspects that diverge between sexes and closely related species such as body size, stoutness and head traits. Those differences are often attributed to specificities in reproductive or ecological requirements and frequently reflect microevolutionary patterns. In this study we evaluate sexual dimorphism and ontogenetic allometry in *B. insularis* and also compare head morphology within two populations of *B. jararaca* using linear and geometric morphometrics, in a mechanistic approach to understand patterns involved in morphological divergence. Adult females are generally larger than males, except for the tail length, but no difference was found in head shape. Both sexes presented allometric growth. Ontogeny of body shape are similar, but a convergent pattern was found in head shape. Interspecific comparisons also showed marked differences in head shape and ontogenetic trajectories for both sexes. Larger females produce bigger clutches, which may explain this sexual dimorphism. The similar head shape indicates similar functions in both sexes. Difference in the onset of ontogenetic change in diet may explain non-parallel trajectory of head shape, and we suggest that difference in final body shape is probably a result of heterochronic growth. The island isolation and concomitant adaptations in feeding and habitat use are probably the major causes in head shape divergence between the two species.

4.1. INTRODUCTION

Morphology is one of the most important traits in reptiles' biology, and are intimately related to functional ecology, such as locomotion, and feeding mechanics, and also natural history aspects, such as foraging habits, habitat use and reproduction (Seigel et al. 1989). Due to its high degree of specialization, snakes evolved as a megadiverse group, and is expected that different lineages differ morphologically even in closely related species (Alencar et al. 2017; Harrington and Reeder 2017; Sherrat et al. 2018). However, divergence may occur even intraspecifically often driven by different acting selective pressure (Baird et al. 1997; Zamudio 1998; Kaliontzopoulou et. 2007).

In snakes, sexes often diverge morphologically. In species that exhibit male-male combat, body size is often male-biased, whereas in species without such behavior body size is female-biased, since larger size confers greater fecundity (Shine 1993; Shine 1994). Additionally, sexual divergence may occur due the necessity of reduce competition (e.g. diet and habitat use; Shine et al. 2002; Shetty and Shine 2002; Bonnet et al. 2000; Shine et al. 2012). However, other morphological traits are sexually dimorphic, mainly those subjected to a great selective pressure, as those related to trophic ecology, namely head size or shape (Shine 1991; Pearson et al. 2002; Meik et al. 2012; Tamagnini et al. 2018). Other less studied factors, such as island isolation may also account for morphological variation in reptiles, with insular species being considered generally more paedomorphic as their continental counterparts, and also affecting sexual size dimorphism degree (Natusch et al. 2012; Anaya-Meraz and Escobedo-Galván 2020)

Besides the factors mentioned above, a mechanistic one causing morphological disparity is the allometry itself. Lineages often possess a quite variable allometric pattern, sometimes converging, or diverging, and also overlapping, which produces a great number of phenotypes (Esquerré and Keogh 2016; Murta-Fonseca and Fernandes 2016; Sherrat et al. 2018; Parłci et al. 2019). Those post-natal ontogenetic and/or static allometric patterns may also vary intraspecifically, enhancing or diminishing size and shape distances between sexes that grow differently (Gregory 2004; Piras et al. 2011; Andjelković et al. 2016; Tamagnini et al. 2018).

The golden lancehead *Bothrops insularis* is a threatened species endemic to the Queimada Grande Island, about 33 kilometers far from the Southeastern Brazilian coast (Duarte et al. 1995), that are probably separated of the mainland after the last glaciation for about 11.000 years (Vanzolini 1973). Evolutionists suggest that *B.*

insularis have originated from a common ancestor of the mainland congeneric *B. jararaca* (Wüster et al. 2005; Grazziotin et al. 2006). Even though this time interval seems small, specially thinking about evolution, it was enough to produce several ecological adaptations such as a diet specialized in birds and a more pronounced arboreal and diurnal habit than its sister clade (Amaral 1921; Amaral 1921b; Martins et al. 2002; Marques et al. 2012a; Marques et al. 2012b; Marques et al. 2019).

A previous study provided interesting data about in *B. insularis* morphology, such as body size, head length, and fang length female biased, and also a heart more anteriorly positioned than males (Wüster et al. 2005). In comparison with *B. jararaca*, *B. insularis* had relatively larger tail, larger head and shorter fangs and anteriorly positioned hearts (Wüster et al. 2005). Additionally, Amaral (1921) mentioned a narrower and shorter snout than *B. jararaca*, however no statistics was provided. Despite those data, we lack a more detailed study with a mechanistic approach. In this sense, the aim of the present study was to test morphological variation within *B. insularis* and also compare head morphology with two populations of *B. jararaca*. Specifically, we seek to address the following questions: 1) There is intersexual and interspecific differences in size and/or shape morphology? 2) Ontogenetic scaling occurs for the species? And 3) how allometric trajectories differ between sexes and species?

4.2. MATERIAL AND METHODS

4.2.1. DATA SAMPLING

We used in this study 158 *B. insularis* individuals from the Queimada Grande Island (hereafter QGI population; 44 adult females, 37 juvenile females, 55 adult males and 22 juvenile males). All specimens were housed in the “Coleção Herpetológica Alphonse Richard Hoge”, Brazil. From each specimen 11 linear measurements were taken: Snout-vent Length (SVL), Tail Length (TL), Number of Ventral Scales (VS), Number of Subcaudal Scales (SS), Tail Width (TW), Body Circumference (BC; measured at mid-body), Head Length (HL), Head Width (HW), Rostrum-labial Distance (RLD; measures from the tip of the nose to the last supralabial scale), Head Height (HH), and Eye Diameter (ED). All measures were taken with a flexible ruler to the nearest 1 millimeter or digital caliper to the nearest 0.5 millimeter. Before the analysis

all variables were log-transformed. Sex was determined by sexual dimorphism or inspection of gonads or presence/absence of the hemipenis erector muscle.

For each *B. insularis* specimen that were considered preserved (31 adult female, 28 juvenile female, 37 adult male and 20 juvenile male), a photograph of the dorsal view of the head were taken. Additionally, 163 *B. jararaca* from two populations were included for interspecific analysis: coastal population (42 females and 32 males) and highland population (42 females and 47 males). Nineteen anatomical landmarks were then placed in strategical locations that encompass all relevant trophic morphology of the head. The set of landmarks were the same used to analyses head morphology of *B. jararaca* to enable direct comparison with the sister clade (see cap 2). The landmarks were digitized using the TPSdig 2.0 software. All specimens were aligned with a Generalized Procrustes Superimposition analysis in order to extract the effect of positioning, rotation and size, remaining only shape-derived variation. The Centroid Size (CS) was the variable used as covariate in all analysis performed herein.

4.2.2. INTRASPECIFIC COMPARISONS

Sexual dimorphism was tested for each linear variable and head shape for adults and juveniles separately. Females were considered adult SVL > 555 mm, and males SVL > 505 mm (Marques et al. 2013). For the variables SVL, VS and SS, morphological variation were tested using a t-test, and for the reminder variables that scale with size, Analysis of Covariance (ANCOVA) was used, with sex as factor and SVL as covariate for TL, TW, BC, HL, and CS, and HL as covariate for HW, RLD, HH, and ED. To remove the effect of size, all linear variables were regressed against its covariate, the residuals were then extracted and used in a Principal Component Analysis (PCA).

To test for sexual dimorphism of head shape a Procrustes multivariate analysis of variance (MANOVA) was used on shape coordinates, with CS as covariate and Sex as factor. A PCA was used to visualize the specimen's distribution in the morphospace, and deformation grids was built to illustrate morphological divergence. An Index of sexual shape dimorphism (SSD) was computed as the Procrustes distances between mean female and mean male measurements divided by the maximum Procrustes distances between males and females (Tamagnini et al 2018).

To test for variation in ontogenetic allometry, residuals extracted from linear models of the linear measures with the complete sample were then used as a

multivariate dataset, representing body shape hereafter. Allometry were than tested separately for each sex using the two multivariate datasets as response variable (body shape and head shape) and SVL and CS respectively, as predictor using the *procD.lm* function from the *geomorph* 4.0.0 package (Adams et al. 2021), being that non-significant results accepts the null hypothesis (isometry). Homogeneity of slopes (HOS) was tested also using the *procD.lm* function with the multivariate datasets as response and covariate, Sex and the interaction as predictors, being that non-significance for the interaction accepts the null hypothesis for equal slopes. Following, a complementary trajectory analysis was carried out using the function *trajectory.analysis* using stage (juvenile and adult) as start and ending points. This analysis test for differences in the angle and magnitude (path distances) between male and female ontogenetic trajectory in the morphospace. Body shape and head shape were tested separately and all permutation procedures were made with 10000 interactions.

4.2.3. INTERSPECIFIC COMPARISONS

Due to sexual dimorphism in *B. jararaca* head shape (Cap 2), males and females were tested separately. After controlling for size (CS), a MANOVA were used to test for difference in adult head shape between species with the Procrustes coordinates as response and Population (Coast, Highland and QGI) as predictors. Additionally, pairwise comparisons based in Procrustes distances were carried. Principal Component Analysis (PCA) was used to visualize the distribution of specimens in the morphospace. Differences in ontogenetic trajectories were then tested with the HOS test with CS as covariate and Population and interactions as predictors, following by a pairwise test based on vector correlation. After, a trajectory analysis was carried as mentioned above. Ontogenetic trajectory analysis made with all sample.

4.3. RESULTS

4.3.1. INTRASPECIFIC COMPARISONS

Raw data for all variables tested can be found on the table 1. In general, adult females were larger than males for SVL, VS, and HL, while males were larger for TL, SS and ED. No difference was found in the remaining variables. These sexual differences were already present in juveniles for VS, TL, HL, and ED, following the

same pattern verified in adult. Males had larger TW only when juveniles (table 2, figure 1).

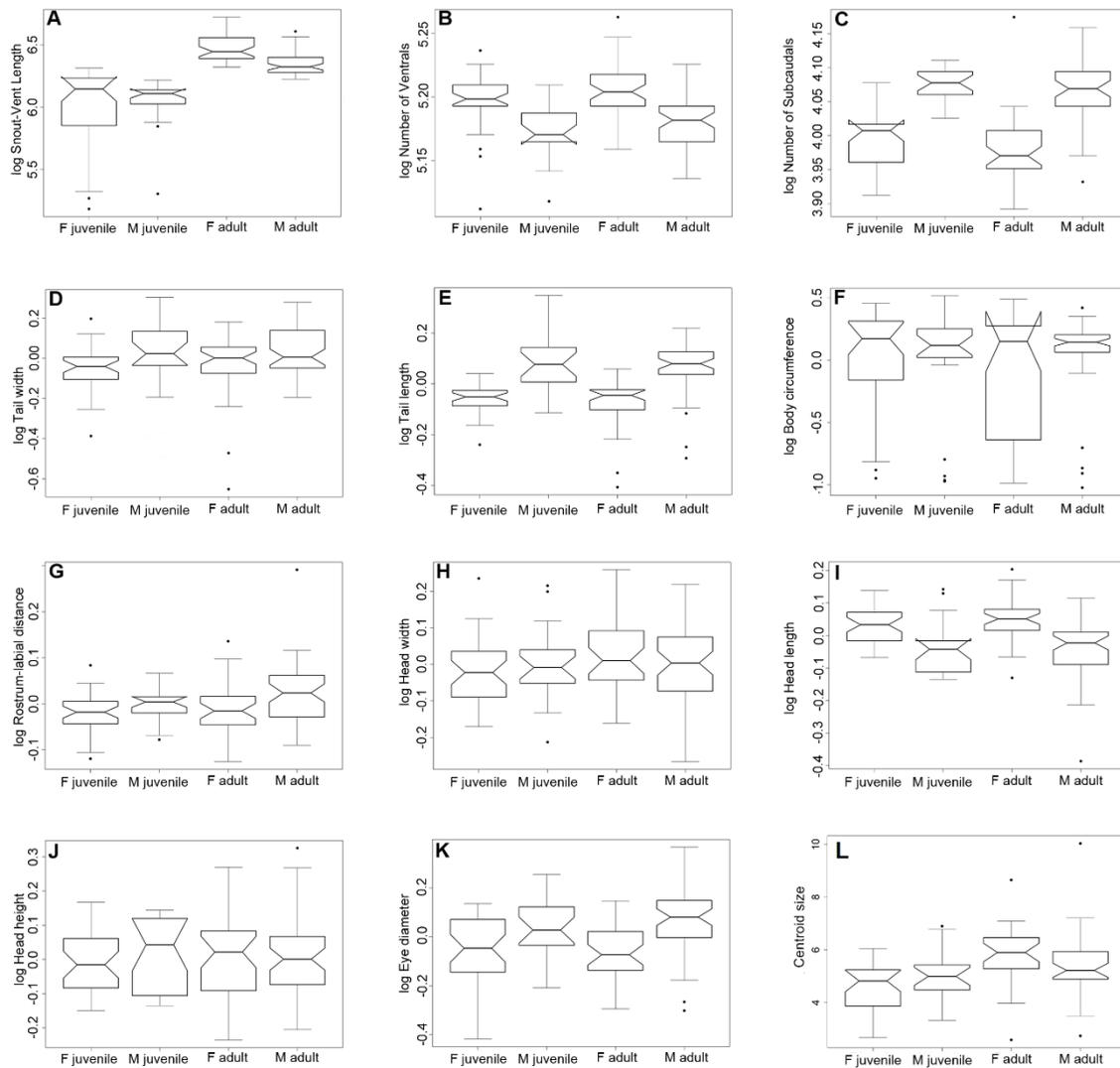


Figure 1: Boxplots for morphological variation in *Bothrops insularis* sexes and stages. D – L) are residuals from linear regressions. F = female, M = male, Notch indicates Confidence Interval, points are outliers.

Table 1: Means (SVL, VS, and SS), adjusted means, standard deviation (se) and sample size (N) for eleven morphological variables of adult *Bothrops insularis*.

| VARIABLE | FEMALE | | MALE | |
|----------|---------------|---|---------------|---|
| | Mean \pm se | N | Mean \pm se | N |
| | | | | |

| | | | | |
|-----|----------------|----|---------------|----|
| SVL | 652.59 ± 11.31 | 44 | 576.25 ± 7.87 | 55 |
| VS | 182.27 ± 0.60 | 44 | 177.77 ± 0.49 | 54 |
| SS | 53.62 ± 0.45 | 44 | 58.47 ± 0.38 | 54 |
| TL | 96.31 ± 1.59 | 40 | 106.49 ± 1.49 | 48 |
| TW | 8.21 ± 0.19 | 42 | 8.64 ± 0.17 | 54 |
| BC | 45.47 ± 2.64 | 36 | 50.38 ± 2.12 | 54 |
| HW | 17.93 ± 0.35 | 37 | 18.38 ± 0.29 | 53 |
| HL | 32.85 ± 0.44 | 37 | 30.17 ± 0.36 | 53 |
| RLD | 25.92 ± 0.33 | 35 | 26.25 ± 0.25 | 53 |
| HH | 11.83 ± 0.30 | 36 | 11.29 ± 0.25 | 47 |
| ED | 3.38 ± 0.09 | 36 | 3.85 ± 0.07 | 50 |
| CS | 5.43 ± 0.24 | 29 | 5.29 ± 0.18 | 37 |

Table 2: T-tests (SVL, VS, and SS) and ANCOVA tables for sexual dimorphism in 11 morphological traits of *Bothrops insularis*. Numbers in parenthesis are number of comparisons and df of the residuals. Significance was highlighted in bold.

| VARIABLE | STAGE | DF | STATISTICS | P-VALUE |
|----------|----------|------------|------------|------------------|
| SVL | adult | 78.48 4 | -8.176 | <0.001 |
| | juvenile | 56.30 8 | -0.474 | 0.637 |
| VS | adult | 88.14 7 | 5.728 | <0.001 |
| | juvenile | 45.53 6 | 3.917 | <0.001 |
| SS | adult | 78.48 4 | -8.176 | <0.001 |

| | | | | |
|-----|----------|--------|--------|------------------|
| | juvenile | 49.97 | -8.380 | <0.001 |
| TL | adult | (1-85) | 21.365 | <0.001 |
| | juvenile | (1-53) | 37.35 | <0.001 |
| TW | adult | (1-93) | 2.599 | 0.11 |
| | juvenile | (1-53) | 8.714 | <0.001 |
| BC | adult | (1-87) | 2.071 | 0.153 |
| | juvenile | (1-52) | 0.048 | 0.828 |
| HW | adult | (1-86) | 0.908 | 0.343 |
| | juvenile | (1-55) | 0.41 | 0.525 |
| HL | adult | (1-87) | 21.96 | <0.001 |
| | juvenile | (1-56) | 13.35 | <0.001 |
| RLD | adult | (1-85) | 0.485 | 0.488 |
| | juvenile | (1-55) | 3.331 | 0.073 |
| HH | adult | (1-81) | 4.548 | 0.036 |
| | juvenile | (1-53) | 0.544 | 0.464 |
| ED | adult | (1-82) | 14.256 | <0.001 |
| | juvenile | (1-55) | 9.594 | 0.003 |
| CS | adult | (1-63) | 0.008 | 0.931 |
| | juvenile | (1-45) | 2.30 | 0.137 |

In the PCA, the two first axis explained together 77.30% of all morphological variation, and a clear distinction of sexes and stages was clear (figure 2). In the PC1 (57.58%), SVL and HL were the most important variables in the negative way, and TL in the positive way. Females were most distributed in the negative region, being larger and with larger heads, while males had larger tails. In the PC2 (19.72%), VS was the most important variable in the negative way, and ED in the positive way. Females were

distributed in the negative region, presenting more ventral scales and males in the positive region with bigger eyes. Juveniles were separated from the adults for being small sized.

After corrected for size scaling, the MANOVA showed that the head shape of sexes did not differ for juveniles ($F_{(1-47)} = 0.98$, $p = 0.44$), nor for adults ($F_{(1-65)} = 1.01$, $p = 0.41$). The two first PC axes explained 44.33% of head shape variation (figure 3). The negative side of the PC1 stressed out a shorter head, and the positive side a longer head, mainly because the landmarks 4 and 9 came closer to 1 (snout), and came distant to 5 and 9 (neck), enhancing the post-occipital region. Adults had the head longer than juveniles, but no clear separation of sexes was found. In the PC2, the negative side pointed to a more rounded head, and the positive side a thin head, mainly due the approximation of the landmark 10 to 5. No clear separation was found in this axis, however juveniles had a higher shape variation. The SSD index for head shape was 0.07.

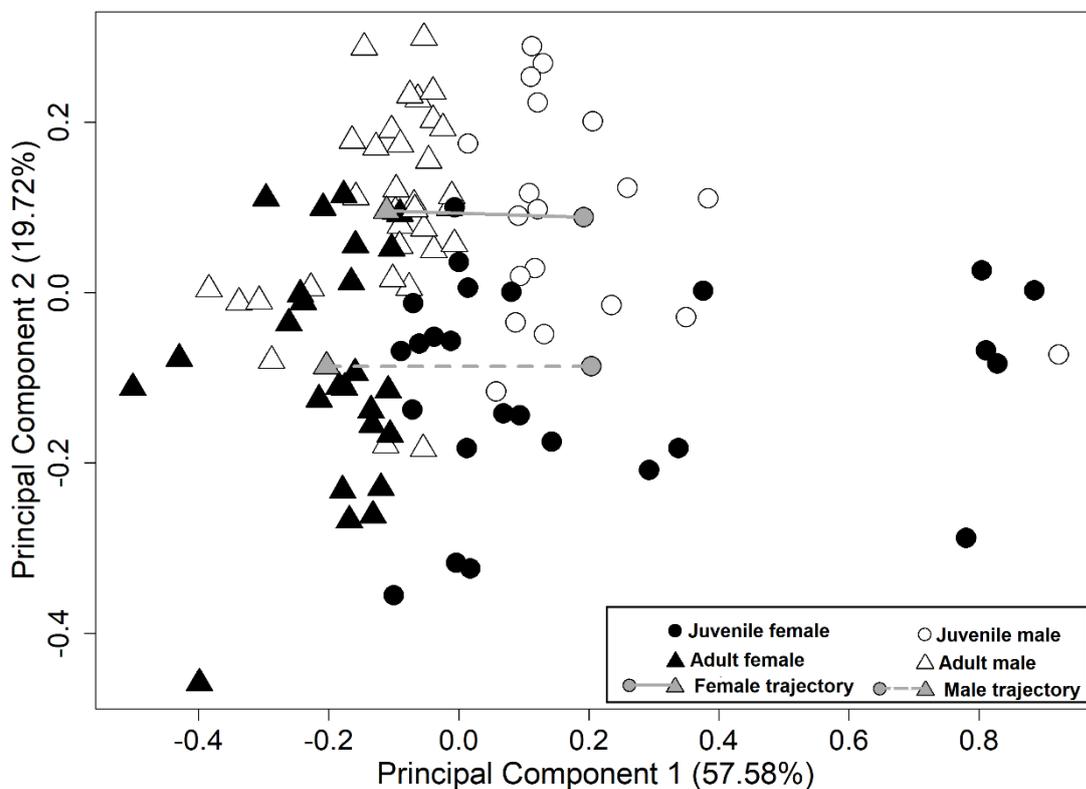


Figure 2: Principal Component Analysis for morphological traits in *Bothrops insularis*.

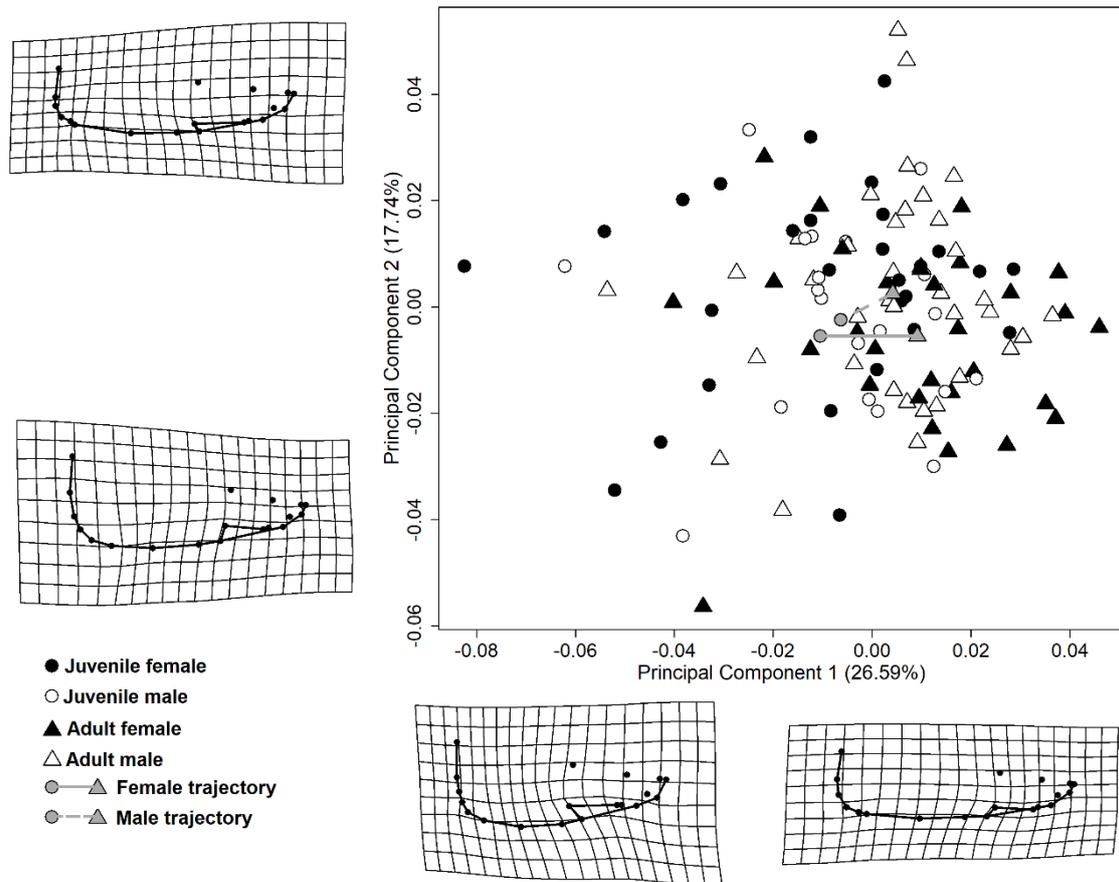


Figure 3: Principal Component Analysis of head shape for *Bothrops insularis*. Deformation grids represents the shape variation along the two first axis.

For body shape, the alternative hypothesis for ontogenetic allometry was accepted for both females ($F_{(1-49)} = 133.63$, $r^2 = 73.57$, $p = 9.999e-05$) and males ($F_{(1-51)} = 47.61$, $r^2 = 48.77$, $p = 9.999e-05$). The HOS test show that slope for sexes were homogenous (Interaction SVL:Sex; $F_{(1-98)}$, $p = 0.196$, figure 4), and the trajectory analysis showed that ontogenetic trajectory for sexes did not differ for magnitude ($z = 0.88$, $p = 0.22$) nor angle ($z = -0.71$, $p = 0.76$, figure 2). Both HOS and trajectory analysis indicates parallelism for sex ontogeny.

As the body shape, the alternative hypothesis for ontogenetic allometry in head shape was accepted for both females ($F_{(1-56)} = 11.395$, $r^2 = 17.16$, $p = 0.001$), and males ($F_{(1-56)} = 4.150$, $r^2 = 7.01$, $p = 0.001$). The HOS test show a significant result in the interaction CS: Sex ($F_{(1-113)} = 3.074$, $p = 0.002$, figure 5), indicating a general non-

parallel trajectory. Finally, the trajectory analysis show that sexes did not differ for magnitude ($z = -1.124$, $r^2 = 48.77$, $p = 0.84$) nor angle ($z = 0.050$, $p = 0.48$, figure x) in the morphospace, being that only size increment account for head shape variation.

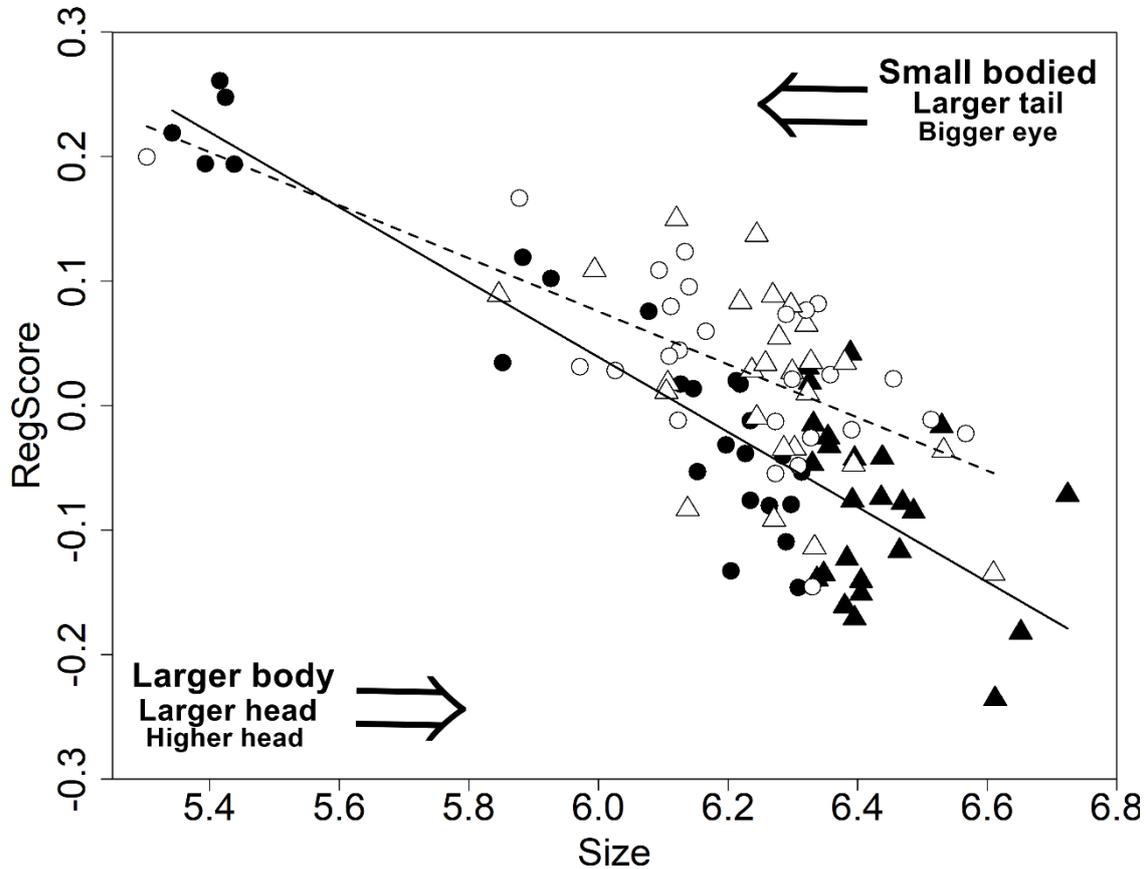


Figure 4: Body shape represented for the regression scores (RegScore) plotted against a general body size variation illustrating ontogenetic allometry for a multivariate linear data in *Bothrops insularis*. Circles = juveniles, triangles = adults, black = females, and white = males.

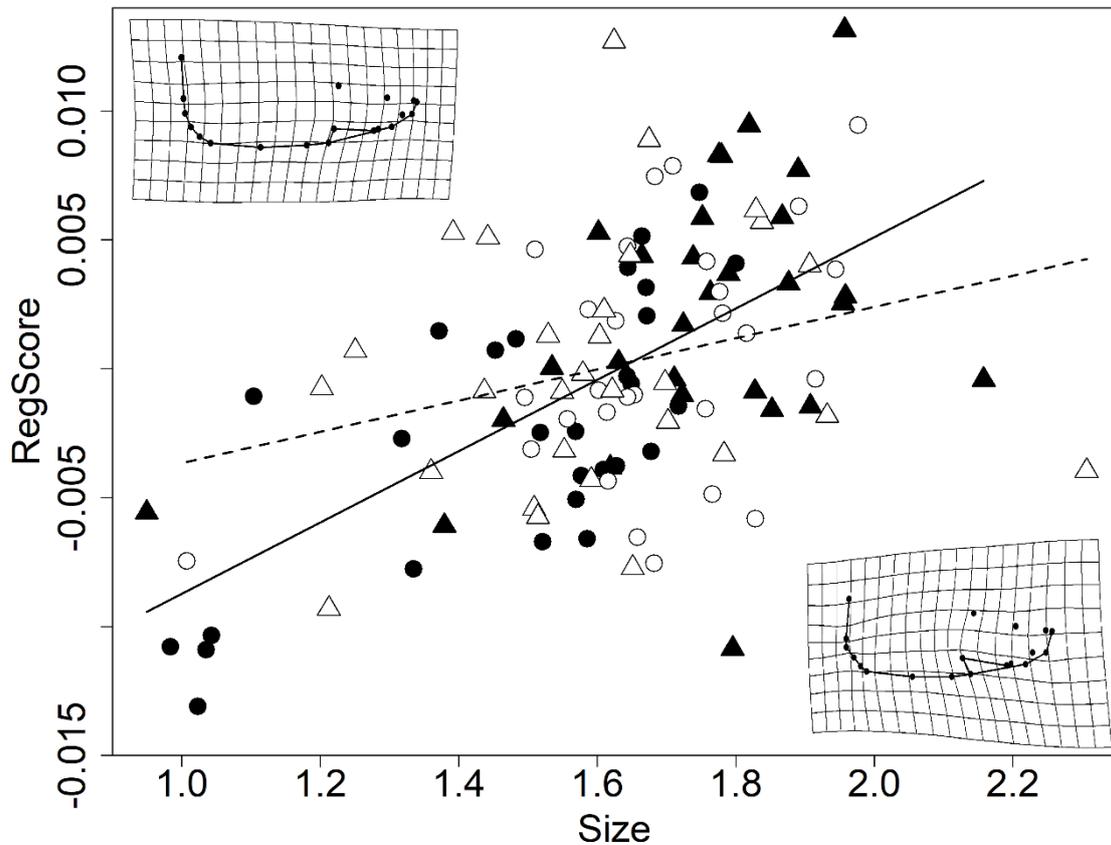


Figure 5: Head shape represented for the regression scores (RegScore) plotted against a general size variation (Centroid size) illustrating ontogenetic allometry for a set of landmark coordinates in *Bothrops insularis*. Deformation grids represents shape variation with size. Circles = juveniles, triangles = adults, black = females, and white = males.

4.3.2. INTERSPECIFIC COMPARISONS

Bothrops insularis had different head shape from the two *B. jararaca* populations for females ($F_{(2-73)} = 7.84$, $p = 0.001$), and males ($F_{(2-73)} = 10.24$, $p = 0.001$). However, pairwise comparison indicates that females were closer to the highland ($d = 0.039$; $p = 0.001$), than the coastal population ($d = 0.049$; $p = 0.001$). The same occurred to males, that were closer to the highland ($d = 0.044$; $p = 0.001$), than from the coastal population ($d = 0.054$; $p = 0.001$). In general, *B. insularis* had a longer and narrower head, mainly in the post occipital region, and *B. jararaca* had a more robust head and slightly shorter and narrower snout (Figure 6).

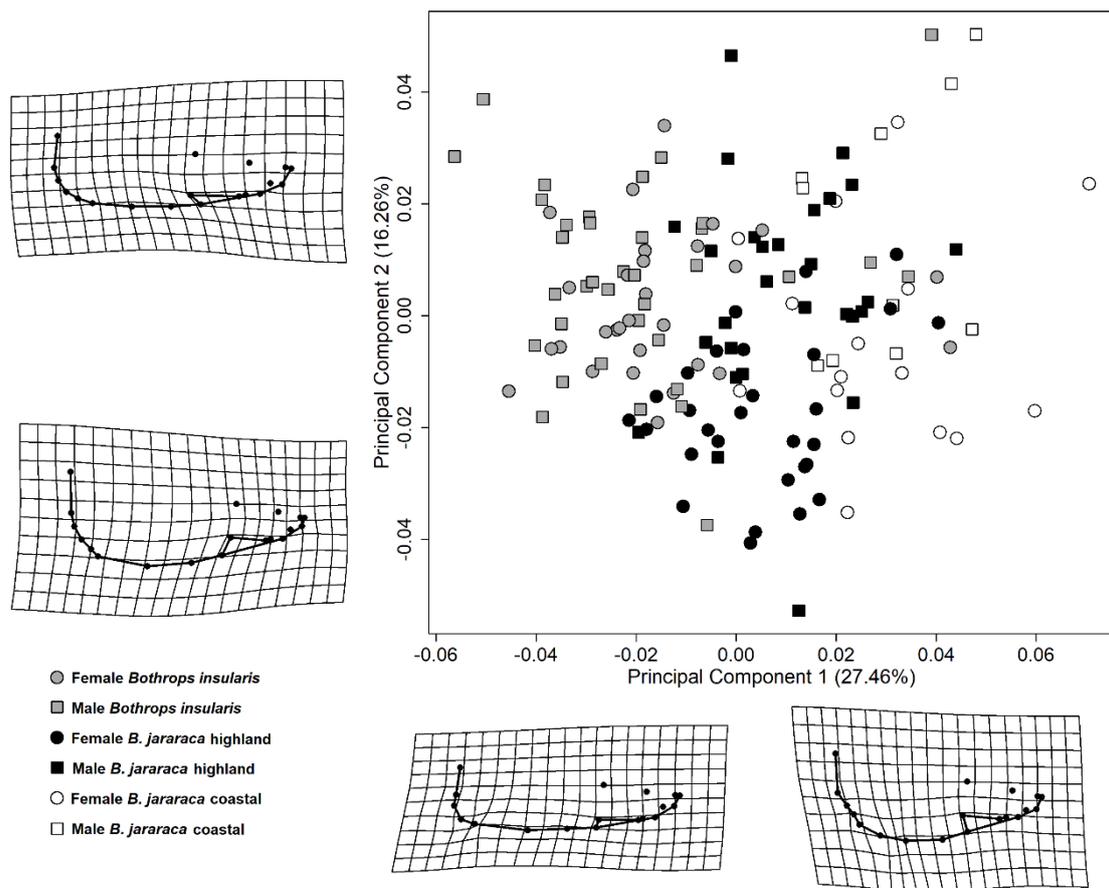


Figure 6: Principal Component Analysis of head shape for *Bothrops insularis* and two populations of *B. jararaca*. Deformation grids represents the shape variation along the two first axis.

The HOS test showed that ontogenetic trajectory of females had different slopes (interaction CS x population; $F_{(2-133)} = 2.69$, $p = 0.002$), and the pairwise comparison indicates that *B. insularis* ontogeny are parallel to highland ($r = 0.82$, $p = 0.09$) and convergent with coastal *B. jararaca* population ($r = 0.66$, $p = 0.001$; Figure 7a). Males presented equal slopes (interaction CS x population; $F_{(2-131)} = 1.49$, $p = 0.089$), and *B. insularis* ontogeny were parallel with highland ($r = 0.66$, $p = 0.12$), and coast *B. jararaca* population ($r = 0.75$, $p = 0.26$, Figure 7b).

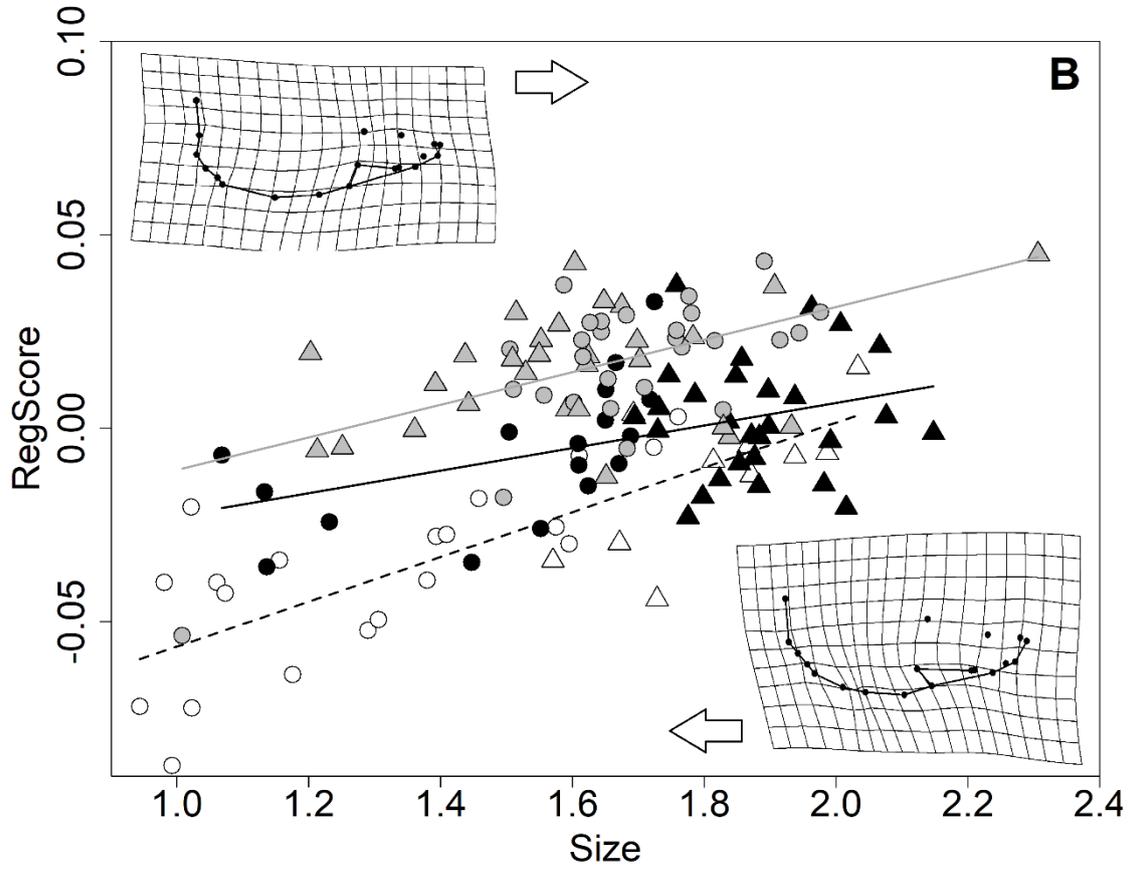
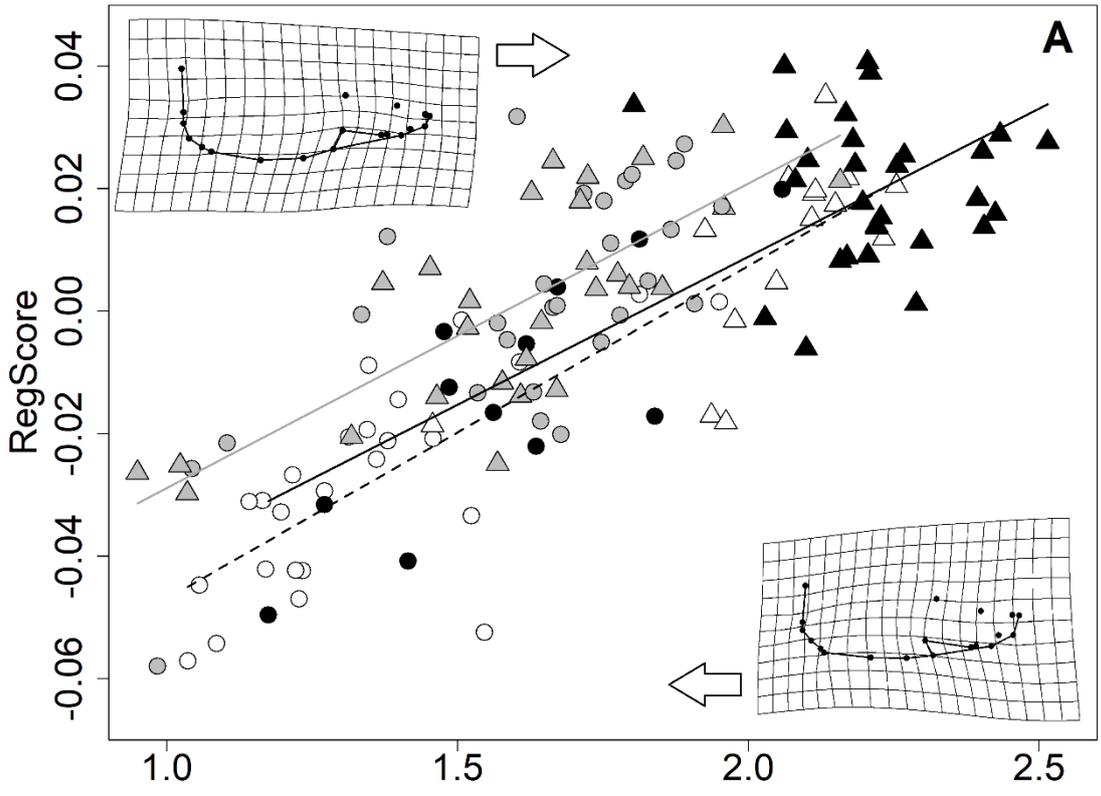


Figure 7: Head shape represented for the regression scores (RegScore) plotted against a general size variation (Centroid size) illustrating ontogenetic allometry for a set of landmark coordinates in *Bothrops insularis* and *B. jararaca* A) females and B) males. Deformation grids represents shape variation with size. Circles = juveniles, triangles = adults, grey = *B. insularis*, black = *B. jararaca* from highland and white = *B. jararaca* from coastal population.

4.4. DISCUSSION

4.4.1. INTRASPECIFIC COMPARISONS

Sexual selection hypothesis states that females are larger in species which reproductive outputs correlates with maternal body sizes (Shine 1994; Cox 2007). Previous studies clearly indicates that *B. insularis* female biased SSD are in agreement with this hypothesis since larger females produce larger clutches, and further comparisons with the conspecific *B. jararaca* reiterates this conclusion, as the smaller sizes attained by the former are accompanied by smaller average number of follicles (Marques et al. 2013). On the other hand, ecological hypothesis, states that morphological differences may be caused by niche partitioning. Although these two hypotheses are not mutually exclusive (Shine 1993), we have no evidence that is the case, since no difference were found in adult trophic morphology (e.g. head shape), and no divergence in diet are known (Marques et al. 2012a). Sexually dimorphic morphology may arise by other ecological reasons, for example, if males and females are using different vertical substrates. However further investigations are needed to verify this supposed difference.

Once the number of scales did not vary with size, differences in adults and juveniles were expected. In snakes, the number of scales is correlated with number of vertebrae (Alexander and Gans 1966; Arnold 1988; Lindell et al. 1993). Additionally, the number of vertebrae determines the maximum size achieved, and may vary between sexes (Lindell 1994). In this regard, the larger number of ventral scales in females and subcaudals in males may be explained by the larger sizes (body and tail sizes respectively) achieved by each sex.

In contrast, the tail was larger in males, but wider only in males as juveniles. This is probably a consequence of an adaptation to accommodate copulatory organs and associated muscles (King 1989). Interestingly, the lack of difference in tail width as adults may be a result of the development of the hemyclitoris present in females

(Hoge et al. 1953; Marques et al. 2002). Although some evidences show that larger tailed males may be more successful in female access, it occurs in species presenting reproductive aggregations (Shine et al. 1999). In this sense, caudal pattern in *B. insularis* is rather a consequence of morphological constraint.

Eye diameter was sexually dimorphic (larger in males) in juveniles and adults. In snakes there is a strong correlation between eye size and habits, being that diurnal and arboreal species had larger eye than nocturnal and terrestrial and semi-aquatic snakes (Liu et al. 2002; Liu et al. 2016). Considering this relationship true for Viperid, it is possible that male *B. insularis* had larger eyes due an increase in arboreal use relative to females, to foraging and mating search. This seems consistent considering that our species achieve larger maximum eye diameter (5.21 mm) than for similar body sized pit vipers such as *B. itapetingae*, and *B. neuwiedii* (3.45 mm and 4.44 mm, respectively), that are species more terrestrial and nocturnal that inhabits open areas (França et al. 2008).

Interestingly, females had larger heads than males, but were equal for centroid size, and for head shape. Data on diet of adults *Agkistrodon piscivorus* indicates that males eat taller prey and sexes consumed different proportions of prey, with are accompanied by a significant difference in head shape, but not in linear measures (Vincent et al. 2004a). Thus, our data suggests that female *B. insularis* may eat larger prey, however no difference in feeding frequency is expected.

Females and males showed significative ontogenetic allometry for body and head shape. However, a higher amount of shape variance was attributed to size variation in females in both cases. This seems to be the trend for the larger sex. For example, in the *A. piscivorus* (male-biased), slopes for head measures were higher for males (Vincent et al. 2004b), while for *Bothrops atrox* (female-biased), the opposite occurred (Silva et al. 2017). Therefore, the female-biased sexual size dimorphism in *B. insularis* (Marques et al. 2013; this study) may explain patterns found herein.

The eye diameter was one of the most variant characters through ontogeny. In Crotaline, the eye is at least in part, responsible to recognition of mates, prey and predators (Berson and Hartline 1988; Young and Morain 2002; Chen 2012). Liu et al. (2016) reported a strong effect of habitat on eye and loreal pit area regardless of phylogeny, additionally there was a negative correlation between them, implying a complementary function between these two sensitive organs. In this sense, a possible

niche partitioning between sexes, and/or males relying most on visual than infrared clues, may explain the slightly difference in *B. insularis* ontogenetic trajectory.

Also, sexes differed slightly for head length trajectory, but significantly for head shape. If sexes diverge in feeding habits (e.g. prey types), head dimensions if often affected (Vincent et al. 2004b). Accordingly, the small range of prey types in *B. insularis* (Martins et al. 2002; Marques et al. 2012a) probably constrain feeding habits which results in an unpaired ontogenetic trajectory. On the other hand, divergent ontogenetic patterns are commonly an artifact of ontogenetic changes on diet and sexual maturation (Natusch and Lyons 2012). As snakes grow, energy requirements change (Mushinsky et al. 1982), therefore, it is possible that sexes rely on different prey categories as juveniles, but change to larger prey as adults to compensate energy cost.

Trajectory analysis indicates similar ontogeny between sexes. Equal trajectory between sexes is common even in a highly diverse taxa such as Pythons (Esquerré et al. 2016). In some cases, even in overlapped trajectories, variation may occur through heterochronic changes in size increment (Piras et al. 2011; Silva et al. 2017). Growth curves based on mark recapture data for *B. insularis* showed that males and females had different growth pattern, with females growing faster, with a small delay in age of maturation (K. Banci pers. com), which is highly consistent with ontogenetic patterns on body and head shape, and any shape variation is probably an effect of heterochrony.

4.4.2. INTERSPECIFIC COMPARISONS

Bothrops insularis head shape was different from *B. jararaca* for males and females. This pattern is strongly consistent with feeding ecology, since the former are a bird eater and the second prey upon small rodents (Sazima 1992; Martins et al. 2002; Marques et al. 2012a; Marques et al. 2019). Our findings are in accordance with previous studies of Wüster et al. (2005) since *B. insularis* presenting a longer head than *B. jararaca*. On the other hand, we found no evidence for the Amaral (1921) statement, since *B. insularis* had larger and broader snout than its counterpart. One explanation for this difference, is the feeding behavior of the adult Golden lancehead, that usually hold their bird prey to prevent them to escape (Marques et al. 2002; Martins et al. 2002; Marques and Sazima 2009). In this sense, the more robust snout of this species may allow more efficient retention of the prey. In contrast, adult *B. jararaca*

usually bites and release their prey to prevent injuries (Sazima 1992). Considering those findings, we suggest a displacement in head morphology, being that different selective forces may act in different head structures with different functions (holding prey or swallowing).

Its presumable that at least some morphological traits may be genetically correlated, such as size, shape or scalation traits (Dohm and Garland-Jr 1993; Webb et al. 2001). Molecular analysis indicates that *B. insularis* are genetically closer to a São Bernardo do Campo – Brazil, *B. jararaca* specimen (Grazziotin et al. 2006), locality that belongs to the highland population, and our Procrustes distance bases pairwise analysis corroborates phylogenetic expectations, since *B. insularis* head shape resemble more the highland population. In this sense, some traits may be conserved for the genus. Our ontogenetic analysis is also in agreement, since females of *B. insularis* and *B. jararaca* from highland had parallel trajectories. Males also presented this pattern, but were also parallel to the Coast population. This may be due an under-representation of small males in our sample.

Boback (2003) investigates body size change among insular snake species and found a general dwarfism pattern for Viperids. Island area, and distance from mainland exert no effect on snake's body size change, and Boback (2003) argued a "diet change hypothesis" to explain the results found, since island snakes usually consume larger or smaller (in case of viperids) prey, and also, those species that present ontogenetic change in diet may retain juvenile diets related to their mainland counterparts. The differences in head shape and ontogeny found herein are highly consistent with the diet change hypothesis, considering as the source population a common ancestor of the two species studied here and others members of the Jararaca group, such as *B. alcatraz*, *B. otavioi* and *B. sazimai*, that are all insular species, smaller body sized and present diets that may be considered paedomorphic in relation than mainland population (Sazima 1992; Martins et al. 2002; Barbo et al. 2012; Marques et al. 2012a; Marques et al. 2012b; Barbo et al. 2016). For instance, geometric morphometric analysis indicates that *B. sazimai* have a smaller head and larger eyes than *B. jararaca* and feeds on centipedes and small lizards, prey found in *B. jararaca* almost exclusively when juveniles (Sazima 1992; Barbo et al. 2016).

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APPENDIX A – Complementary paper to the thesis

INTRINSIC AND GEOGRAPHIC INFLUENCES ON THE PROBABILITY OF BORN AND RETAIN CONSPICUOUS TAIL TIP COLOR (TAIL LURING) IN THE COMMON LANCEHEAD *BOTHROPS JARARACA*

ABSTRACT

Tail luring risen as a supplementary strategy to attract prey in snakes that present ambush behavior. The wriggling movement of the snakes' tail resembles a worm or insect larvae, and are used to attract ectothermic prey. In some species, some individuals may present a conspicuous bright color in the tail tip, frequently losing this trait along ontogeny. Some authors argue that this character enhances the resemblance with a potential lure. In this paper we test the influence of population, sex, relative tail length, and environmental variables in the probability of *Bothrops jararaca* individuals being born with conspicuous tail luring. Also, if populations may retain this trait until higher sizes. None of the predictors used shown any impact in the probability of being born with tail luring, however individuals in the coastal population indeed retain the colored tail tip until higher sizes them the highland population. The lack of differences found herein indicates that there are other factors influencing tail tip color, probably phylogenetic inertia or genetic correlates rather than intrinsic or environmental pressure. A higher frequency of endothermic prey on the coastal diet may explain why this population keep the tail luring to higher sizes.

INTRODUCTION

Throughout their life story, snakes evolved a series of intriguing strategies for hunting and capture their prey. The major methods vary from active foragers, that generally searches the prey and simply seize, constrict, or uses the venom to immobilize and them swallow it, to ambush predators that, following chemical cues, found a local potentially used by their prey and then “sit and wait” to the moment that they reach a striking distance (Greene, 1997; Beaupre and Montgomery, 2007; McDiarmid 2012). Besides the main hunting strategy, other supplementary methods have emerged, like in *Hydrodynastes gigas*, a semi-aquatic species that uses its tail to swipe the vegetation in flooded habitats, scaring frogs and inducing to flee, and thus, possibly enhancing the chances to find them (Strüssman and Sazima, 1990).

However, another curious behavior is the use of the tail as a bait, tactic known as “tail luring” (also “aggressive mimicry”; Pasteur 1982), which, contrasting the former has the objective of trick the prey, bringing them toward the snake. This behavior consists in waving movement of the tail, resembling a potential prey (e.g., mimicry of an insect larvae or worm), and are frequently related to the attraction of ectothermic prey, such as frogs and lizards, that approaches the bait looking for a meal, and became a meal themselves (Neil, 1960; Heatwole and Davison, 1976). Additionally, the tip of the tail often presents a conspicuous bright color, contrasting the cryptic color of the body (Heatwole and Davison, 1976; Carpenter 1978), and some authors argue that the colored tail tip is advantageous because enhances its resemblance with a target worm or insect larvae, and diminish the probability of a prey detect the attachment of the lure with the snake body than non-colored tail tips (Rabastky 2008), although a cautionary posture have been suggested when interpreting this assumption.

The presence of this characteristic bright color in the tail tip is relatively common in snakes and appear to be affected by intrinsic factors like sexual dimorphism, where a given pattern (color of the tail tip) is more related to males or females (Burger and Smith, 1950; Heatwole and Davison, 1976; Tesler et al. 2019), age/size, as the tendency of the contrasting color to disappear as the snake grows (Burger and Smith, 1950; Murphy et al. 1978; Andrade et al. 1996), and geographic variation, as the difference in the presence/absence of the conspicuous markings between populations (Neil 1960). Still, there is an interesting discussion about the correlation of the size of tail/lure with efficacy of prey attraction (Hagman et al. 2008; Pisani and Riedle, 2018). Therefore, if the presence of the colored tail luring brings more advantages to a sex,

age/size class, or population than others, modifying individual fitness, is presumable that this trait became a fixed character along evolution.

Tail luring is widespread in snakes, and it seem to be frequent in the genus *Bothrops* (Burger and Smith, 1950; Greene and Campbell, 1972; Andrade et al. 1996; Martins et al. 2002; Andrade 2010; Fonseca et al. 2019). *Bothrops jararaca* is an Atlantic Forest dweller in Brazil (Campbell and Lamar 2004). This species has a markedly ontogenetic change in diet, with juveniles feeding most on frogs and lizards (ectotherms) and adults mainly on small rodents (endotherms). Also, it is known to present tail luring behavior (Sazima 1992, Sazima 2006) and at least three morphotypes of tail tips: two conspicuous colors, whitish, or blackish, and one inconspicuous mottled brownish similar to the body color (Sazima 2001). All three morphotypes were recorded performing tail luring behavior, and the conspicuous tail tips becomes suffused with the body colors as the snake grows (Sazima 2001).

In the Neotropical region, juvenile *B. jararaca* potential preys' activity was positively correlated with climate factors, mainly temperature and rainfall, for both anurans (Boquimpani-Freitas et al. 2007; Canelas and Bertoluci 2007; Vasconcelos et al. 2010; Ximenes and Tozetti 2015; Schalk and Saenz 2016), and lizards (Bergallo and Rocha, 1993; Vrcibradic and Rocha, 1998; Filogonio et al. 2010; Maia-Carneiro 2012). Additionally, juveniles from a coastal population consume more ectothermic prey than the highland population, and the ontogenetic shift from ectothermic to endothermic prey occurred earlier on the former (Moraes 2008; figure 1). Accordingly, we expect that frogs and lizards from areas with hotter temperatures and higher precipitation became more active, and *B. jararaca* individuals probably born with conspicuous tail luring with a higher frequency.

Despite the large number of studies about prey luring in snakes, most of them aimed to elicit issues related to predator-prey behavior. In this paper we explored two main hypotheses focused on adaptations that possibly have risen between *B. jararaca* populations, specifically: i) There are different chances of an individual born with conspicuous tail tip; ii) and/or may retain this trait until higher sizes. Additionally, we evaluated the influence of sex, relative tail length, and climatic factors on the presence-absence of conspicuous tail tip.

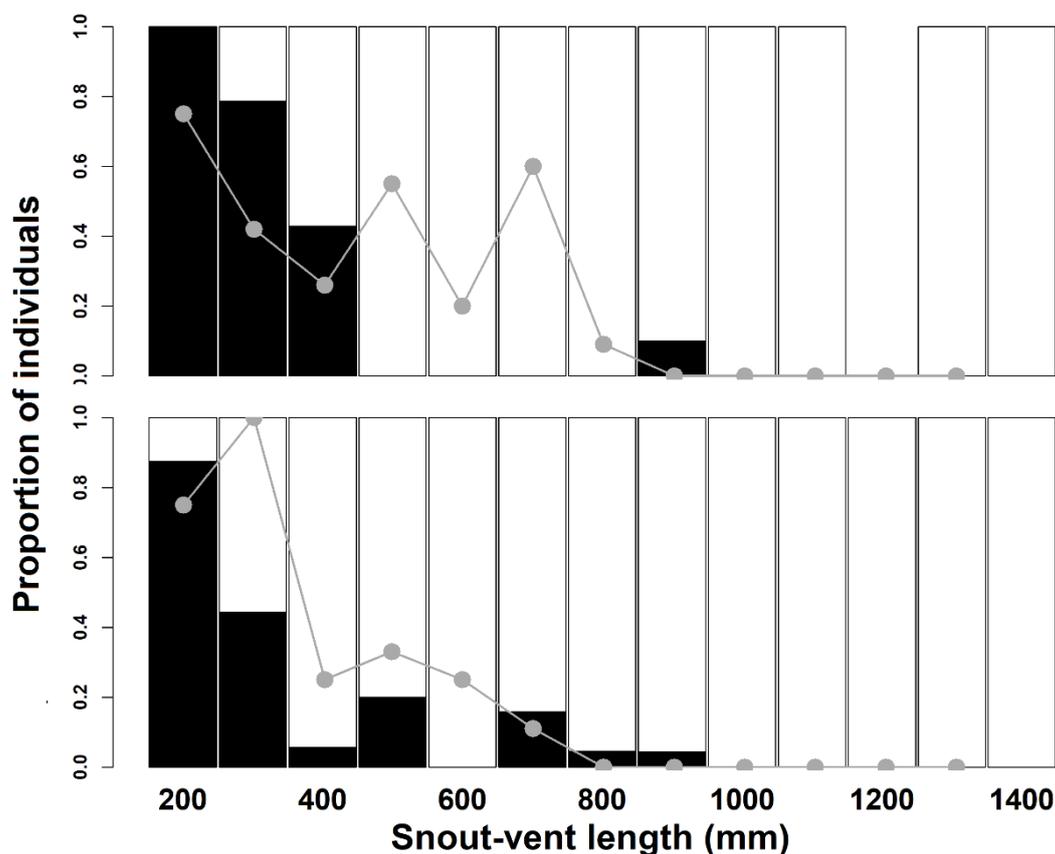


Figure 1: Frequency of prey type by size class in two populations of *Bothrops jararaca*. Top = coastal, bottom = highland, black = ectotherms, white = endotherms (modified from Moraes 2008). Grey dots and lines are frequency of conspicuous tail tip (tail luring; this study).

METHODS

We used in this study 209 *B. jararaca* individuals. 110 were from coastal population (59 females and 51 males) and 99 were from the highland population (50 females and 49 males). The snout-vent length (SVL) of the specimens included ranged from 165 to 1,265 millimeters in the coastal and from 242 to 1,248 millimeters in the highland populations.

From each individual, we recorded four morphological variables: sex (determined by sexual size dimorphism, inspection of the gonads or the hemipenis erector muscle); snout-vent length (SVL), measured from the tip of the nose to the last scale before the cloacal scale; tail length (TL), measured from the cloacal scale to the tip of the tail, and tail tip color, that was categorized as tail luring absent (the same color as the body) or present (conspicuous tail tip, lighter or darker than the body). The

relative tail length (RTL) was then computed as the TL divided by the SVL. Specimens with incomplete tail were not considered. We classified the specimens belonging to each population based on an elevational criterion described in (cap 1). The environmental data were Mean Annual Temperature (MAT) and Mean Annual Precipitation (MAP), and were extracted from the Wordclim database at a resolution of 30 arc-seconds based on the geographic coordinates for each specimen (see cap 1).

The best way to analyzing differential chances of having or not the contrasting color on the tail is to look upon newborns. However, because of the lack of litters in both populations we arbitrarily nested our dataset on individuals up to 350 millimeters (juvenile dataset). Previous works found that *B. jararaca* often born about 250 mm (range 190 to 275 mm, Sazima 1992), and in this sense, we expect that in the size range we use in our juvenile dataset, the chance of losing tail luring will be negligible.

Our hypothesis was then tested by two approaches: First, the effect of intrinsic (sex, population and RTL) and environmental predictors (MAT and MAP) on the probability of born with contrasting color on the tail were tested by means of logistic regression due the binomial nature of our response variable (absent = 0 and present = 1, colored tail tip) using only the juvenile data set. Secondly, the impact of sex and population, plus the SVL on the probability of keep the colored tail tip was tested also by means of logistic regression, however using the complete data set. All analysis were carried out with R software version 4.03 (R Core Team 2020).

RESULTS

In the coastal population 33.6% (11 females and 26 males) of the individuals presented contrasting color on the tail. The larger male in this group were 776 mm, and female 814 mm. In the highland population 17.1% (4 females and 13 males) of the individuals had contrasting color on the tail. The larger male in this group were 711 mm and female 467 mm (Figure 1).

The juvenile (SVL < 322 mm for males and < 347 mm for females) dataset comprises 37 individuals, 27 from the Coast (12 females and 15 males), and 10 from the highland (5 females and 5 males). The logistic model shows that none predictors had significant effect in the probability of present conspicuous tail luring (Table 1, figure 2), which means that there is the same chance of individuals born with this trait, and were independent from any factor used.

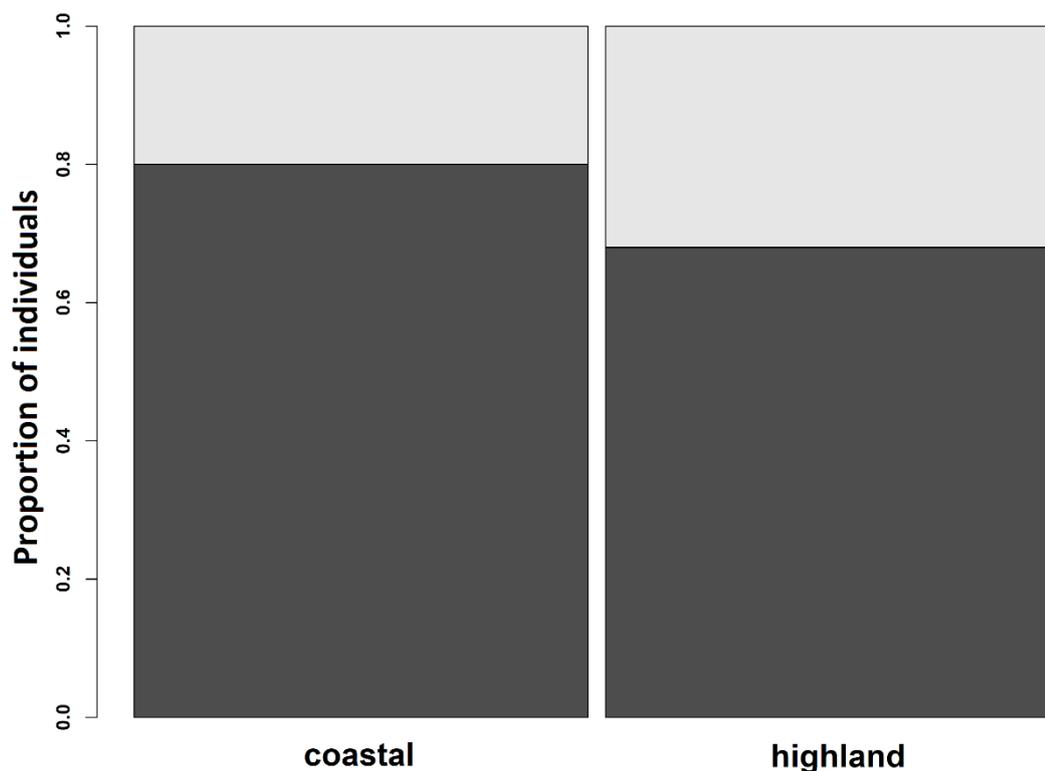


Figure 2: Proportion of *Bothrops jararaca* individuals that born with or without contrasting tail luring in the coastal or highland populations. Present (dark grey) and absent (light grey), sex pooled together.

Table 1: Logistic model table for the Juvenile and Complete data to test the probability of males and females of two *Bothrops jararaca* populations having tail luring. In Complete model sex were pooled together. Statistical significance is highlighted in bold. RTL = relative tail length, MAP = mean annual precipitation, MAT = mean annual temperature, SVL = snout-vent length.

| Model | factor | Estimate | Std. error | Z value | P value |
|----------|------------|----------|------------|---------|---------|
| | Intercept | 5.472 | 16.835 | 0.325 | 0.745 |
| | Sex | 0.784 | 1.006 | 0.779 | 0.436 |
| Juvenile | Population | 1.537 | 3.105 | 0.495 | 0.621 |
| | RTL | -20.292 | 18.685 | -1.086 | 0.277 |

| | | | | | |
|----------|-----------------|--------|-------|--------|--------------|
| | MAP | 0.039 | 0.026 | 1.492 | 0.136 |
| | MAT | -0.437 | 0.675 | -0.647 | 0.517 |
| | Intercept | 1.391 | 0.531 | 2.619 | 0.008 |
| complete | SVL | -0.003 | 0.000 | -3.882 | 0.000 |
| | Population | 2.392 | 1.263 | 1.894 | 0.058 |
| | SVL: Population | -0.004 | 0.002 | -2.179 | 0.029 |

In the same fashion that previous model, sex had no impact in keeping conspicuous tail luring ($z = -0.4$, $df = 207$, $p = 0.65$), therefore this factor was removed and a second and simpler model were then built. We found a significant interaction between SVL and population (Table 1). In other words, in early sizes there is a higher probability to find a snake with conspicuous tail luring in the highland population, at about 460 mm the probability will be the same, and above this size the probability will be higher in the coast population. That is, snakes in the coast retain tail luring until higher sizes (figure 3).

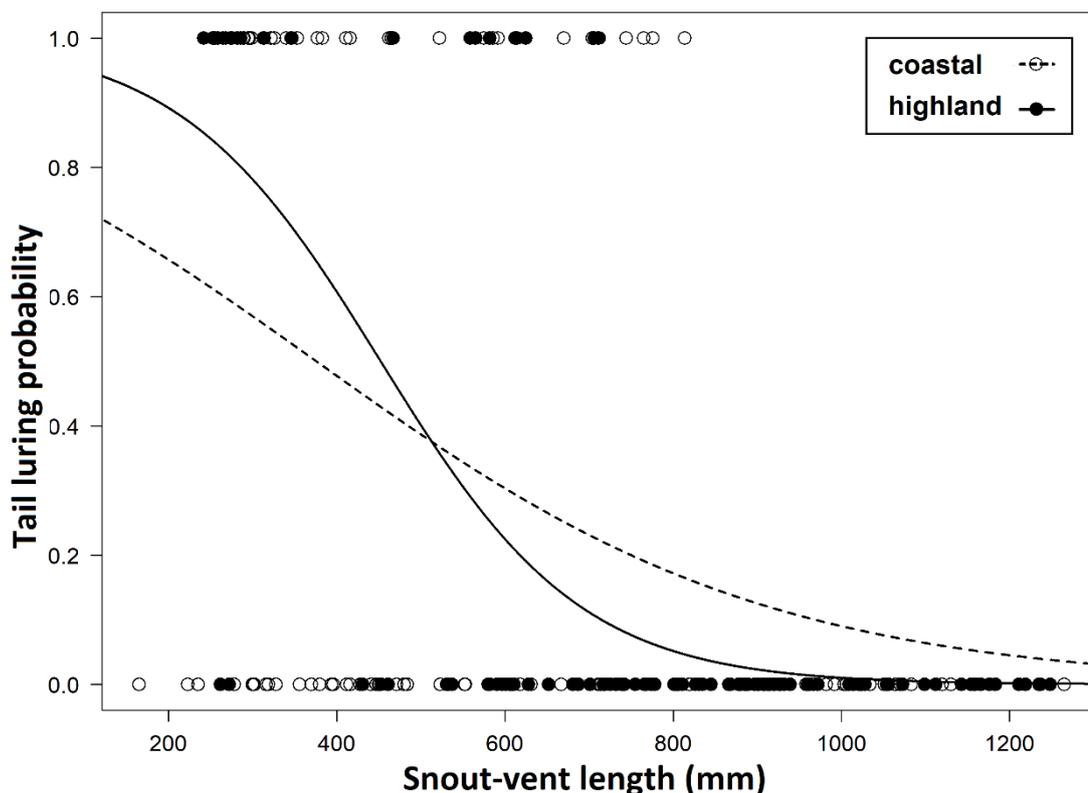


Figure 3: Probability of present conspicuous tail tip color (tail luring) of *Bothrops jararaca* individuals from two populations.

DISCUSSION

We found conspicuous tail tip in adults male and female in the coastal, but only in adult male in the highland population. Although conspicuous color has been reported mainly in juveniles, this trait may persist into adulthood in some species, such as *Acanthophis antarcticus* (Carpenter et al. 1978), *Vipera latasti* (Parellada and Santos 2002), and the arboreal pit viper *B. bilineatus* (Greene and Campbell, 1972). Thus, the results found herein are expected. However, if the tail luring behavior is performed in such large specimens in our study populations remains unknown.

Morphology and climate are known to control several ecological aspects of reptiles. In the worm lizard *Amphisbaena vermicularis*, larger individuals, and those from regions with hotter temperatures and less precipitation had a higher probability of presenting urotomy, associated with a longer exposure to, and higher activity of their predators (Guedes et al. 2020). Indeed, in a previous study we found that seasonal

activity of *B. jararaca* are synchronic with their potential preys and no synchronic with predators, and are higher in the most rainy and warmer period of the year (Siqueira et al. 2021). However, none of the variables (relative tail length, Mean Annual Temperature and Mean Annual Precipitation) had no effect in frequency of colored tail tip.

The lack of differences in conspicuous tail tip frequency between sexes and populations may be explained by the food habits of the species. Besides *B. jararaca* being considered as an ambush predator, other feeding tactics were previously reported, such as the necrophagy or scavenging habits (Sazima and Strüssmann, 1990) and active foraging lacking ambush behavior (Sazima 1989; Sazima 1992). For this reason, the color of the tail may be not exerting enough evolutive pressure in order to become a selected trait across any group studied herein.

Martins et al. (2002) made interesting character reconstructions about tail tip color in the genus *Bothrops*. First of all, colored color is a plesiomorphic character, present in the ancestor. Secondly, this character is variable, with some species having it, while other don't (even in mammal specialists). And finally, lack of colored tail tip is autapomorphic for *B. alternatus* (mammal specialist). These generalizations provide valuable data about the importance of the phylogeny for the presence/absence of distinct colored tail tip in neotropical pitvipers, and we suggest that the lack of difference in the probability of presenting this trait between our population study may be a result of phylogenetic inertia.

The probability of having colored tail tip decreased with snake body size in both populations studied. That appears to be a common trend, being frequently found during snakes' growth (Neil, 1960; Heatwole and Davison, 1976). A study carried in a wetland region found that *Agkistrodon piscivorous* present a marked ontogenetic shift in several ecological characteristics as foraging strategy, habitat use and diet composition, concomitantly, that change was also accompanied by a loss of the characteristic yellowish tail tip of the juveniles (Eskew et al. 2009). Accordingly, juveniles *B. jararaca* are commonly found in stream edges probably due the abundance of frogs in these habitats, and which are usually found in their diet, and sometimes these individuals were seen performing caudal luring behavior (Hartman et al. 2003). Adults, however, feed mostly on small rodents, and never were recorded performing caudal luring behavior elicited by this prey type (Sazima 1988; Sazima 1992). In this sense we believe

that there is a strong ontogenetic association between tail color, behavior, and ecological features.

There are several evidences that species with wide geographic distribution and broad diet range show variation not only in diet composition but also in ontogenetic shift patterns between populations, mainly attributed to habitat use and prey availability (King, 1993; Daltry et al. 1998; Luiselli et al. 2001; Queiroz et al. 2001; Luiselli et al. 2005). Considering the close relation between morphology and feeding habits in snakes, differences in prey availability possibly explain why individuals in the coast population may retain conspicuous tail luring at longer sizes. The most gradual inclusion of endothermic prey on the diet of coastal population is highly consistent with the pattern of tail tip suffusion found herein. Notwithstanding, the onset of ontogenetic changes on color patterns and prey selection along growth may be asynchronous between populations (Natusch et al. 2012).

Another hypothesis that possibly may explain the differences in the tail tip color between populations is the differential predation rate across regions. Some authors argue that the conspicuous color may act not only to attract prey, but also as an anti-predatory signal (Greene 1973; Booth 1990). Experiments carried with the sidewinder rattlesnake *Crotalus cerastes*, showed that juveniles that engaged caudal luring behavior (although lacking the bright colored tail tip) quickly change the characteristics wriggling movement to a more rapid and audible tail vibration when a potential predator is presented (Reiserer and Schuett, 2008), indicating that snakes may have a good recognition of prey and predator species. In a previous study, we provide empirical evidences that *B. jararaca* may be subject to a different predation pressure and predator types between populations (Siqueira and Marques 2018), and in this sense, retention of the conspicuous tail tip, may be advantageous to populations subjected to a more visual orientated predator. However, the higher efficacy of conspicuous colored tail in defensive display remains merely speculative.

We must consider that the color of the lure itself may not be a sufficient influence to determine prey capture. An experiment carried with *Acanthophis praelongus*, species that exhibit a brown tail but with ventral yellowish color on the tail tip, show that tails with painted lures (all brown, and all yellow versus natural ones) had the same efficacy in attracting lizards (Hagman et al. 2008). Another field experiment that controlled tail tip color in *Sistrurus miliaris*, also show that conspicuous tail tips had no effect on foraging success (Farrel et al. 2011). Therefore, we suggest that the color of

the tail tip may play an important role in *B. jararaca* ecology, however, this trait alone may not be the only one driving feeding ecology in this species.

In this study we clarify important questions about fixed adaptations on the presence of conspicuous color of tail luring in *B. jararaca*, bringing novel evidences that enhances populational differences in this species. However, when these adaptations are related with individual behavior still be an issue for further researches.

ACKNOWLEDGEMENTS

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