Evolutionary Biology
https://doi.org/10.1007/s11692-025-09661-y

Morphological Variation in the Golden Lancehead *Bothrops Insularis*: Sexual Dimorphism, Ontogeny and Microevolutionary Trends

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Received: 27 April 2025 / Accepted: 20 October 2025

Abstract

The insular golden lancehead *Bothrops insularis* presents several morphological features that diverge between sexes and closely related species such as body size, stoutness, and head traits. These differences are often attributed to specificities in reproductive or ecological requirements and frequently reflect microevolutionary patterns. In this study, we evaluated sexual dimorphism and ontogenetic allometry in *B. insularis* and compared head shape with two mainland populations of *B. jararaca* using linear and geometric morphometrics to understand the patterns involved in morphological divergence. Females were larger than males for almost all body parameters analyzed, except for tail traits, and also had a larger and wider head. We found no difference in model slopes for body and head shape ontogenetic trajectories, indicating that both sexes shared common trajectories in postnatal development. Interspecific comparisons revealed marked differences in males' head shape and ontogenetic trajectories. The head of *B. insularis* was phenotypically closer to the highland *B. jararaca* population which is in accordance with the phylogenetic affinity of *B. insularis* with this population. On the other hand, *B. insularis* showed a snout size similar to that of the coastal population. The resemblance in snout shape to the coastal population may represent an evolutionary divergence from a shared ancestor with the highland population as a consequence of island isolation and diet consisting of birds.

Keywords Allometry · Geometric morphometrics · Head · Island · Pitviper

Introduction

Morphology is one of the most important aspects of reptile biology, intimately related to functional ecology, such as locomotion and feeding mechanics, and natural history, such as diet, habitat use, reproduction and phylogeny (Seigel et al., 1989). Due to their high degree of specialization, snakes evolved as a megadiverse lineage, and it is expected that different lineages differ morphologically even among closely related species (Alencar et al., 2017; Harrington & Reeder, 2017; Sherrat et al., 2018a). Moreover, divergence may also occur intraspecifically often driven by distinct selective pressures acting at the population level (Baird et al., 1997; Kaliontzopoulou et al., 2007; Zamudio, 1998).

In snakes, sexes differ 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, 1994). Additionally, sexual divergence may arise due to resource partitioning between the sexes (e.g. prey spectrum and habitat use; Shine et al., 2002; Shetty & Shine, 2002; Bonnet et al., 2000; Shine et al., 2012). Accordingly, other morphological traits may also be sexually dimorphic, mainly those subjected to substantial selective pressure, such as those related to trophic ecology, namely head size or shape (Meik et al., 2012; Pearson et al., 2002; Shine, 1991; Tamagnini et al., 2018).

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Published online: 04 November 2025

Island isolation often influences morphological variation in reptiles, with insular species frequently showing more paedomorphic traits compared to continental relatives, as well as altered degrees of sexual size dimorphism (Anaya-Meraz & Escobedo-Galván, 2020; Natusch et al. 2012). Allometry — the effect of size increase on shape — also contributes to variation, as lineages often possesses variable patterns, sometimes converging, diverging, or overlapping, resulting in a great range of phenotypes (Esquerré & Keogh, 2016; Murta-Fonseca & Fernandes, 2016; Palci et al., 2019; Sherrat et al., 2018b). Post-natal ontogenetic and/or static allometric patterns may also vary intraspecifically, enhancing or reducing size and shape distances between sexes that grow differently (Andjelkovíc et al., 2016; Gregory, 2004; Piras et al., 2011; Tamagnini et al., 2018).

The Golden Lancehead *Bothrops insularis* (Amaral, 1921a) is a threatened species endemic to Queimada Grande Island, located about 30 km from the southeastern Brazilian coast (Martins et al., 2019; Marques, 2021). Its insular status makes conservation especially critical, highlighting the importance of understanding its morphology for conservation efforts. The island was likely isolated from the mainland at least six times in the last 420,000 years (Barbo et al., 2022; Vanzolini, 1973),which has influenced the unique evolutionary trajectory of its endemic snake, *B. insularis*. This species likely originated from a common ancestor shared with the mainland congeneric *B. jararaca* (Wied-Neuwied, 1824) (Barbo et al., 2022; Grazziotin et al., 2006; Wüster et al., 2005). Although this time interval seems short in terms of evolutionary processes, it was sufficient to drive several morphological modifications associated with a shift toward a specialized bird diet and more pronounced arboreal habits (Amaral, 1921; Amaral, 1951b; Martins et al., 2002; Marques, 2021).

Amaral (1921) reported that *B. insularis* possesses a narrower and shorter snout in addition to a wider head (in the temporal region) compared to *B. jararaca*. However, he did not perform any statistical analysis or provide comparative numerical data between the two species. Additionally, Wüster et al. (2005) provided data on *B. insularis* morphology, such as female-biased body size, head, and fang length, and anteriorly positioned heart compared to males. Besides, compared to *B. jararaca*, *B. insularis* has relatively larger tails, larger heads, shorter fangs, and anteriorly positioned hearts (Wüster et al., 2005). Given these data, and assuming that *B. insularis* and *B. jararaca* share a common ancestor, we still lack a more accurate comparison of intra- and inter-specific morphological traits. Therefore, we gathered linear and geometric morphometrics data to describe ontogenetic and sexual morphological variation in *B. insularis*, testing the hypothesis of intra- and interspecific divergent allometric patterns in body and head shape. We also provide further comparisons with two populations of *B. jararaca* to evaluate microevolutionary trends.

Materials and Methods

Data Sampling

We examined 158 individuals of *Bothrops insularis* 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 (Supplementary file: "B_insularis_raw_data"). From each specimen, we took 11 linear measurements: snout-vent length (SVL), tail length (TL), number of ventral scales (VS), number of subcaudal scales (SS), tail width (TW) measured immediately after the cloacal scale, body circumference (BC; measured at mid-body), head length (HL), head width (HW; measured as the widest portion of the head), rostrum-labial distance (RLD; measured from the tip of the snout to the last supralabial scale), head height (HH; measured in the same point that HW), and eye diameter (ED). All measures were taken with a flexible ruler to the nearest 1 mm or digital caliper to the nearest 0.5 mm. Before the analyses, all variables were log-transformed. Sex was determined by sexual dimorphism in ventral and subcaudal scales or inspection of gonads (presence of testicles or ovaries) or the presence/absence of the hemiclitores erector muscle whenever possible.

For each preserved specimen of *B. insularis* (59 females, and 57 males), a photograph of the dorsal view of the head was taken. Additionally, 162 *B. jararaca* from two mainland populations were included for interspecific analysis: the coastal population (42 females and 32 males) and the highland population (41 females and 47 males), determined by elevational gradient according to Siqueira et al. (2022). Nineteen anatomical landmarks were placed in strategic locations according to Siqueira et al. (2024) to allow direct comparisons. The landmarks were digitized using the TPS-dig v.2 (Rohlf, 2015). All specimens were aligned with a Generalized Procrustes Superimposition analysis to remove the effect of positioning, rotation, and size, retaining only shape-derived variation. The centroid size (CS) or SVL were used as covariates in the analyses performed herein.

Measurement Error

We randomly sampled 18 individuals from our data. From each specimen the landmarks were digitized in the same configuration used in the shape analysis (replicate 1). After 10 days the landmarks were digitized again to avoid time

correlation (replicate 2). We test the hypothesis of differences between pairs of individuals induced by Systematic Error Measurement using the function *gm.measurement.error*, from the *Geomorph* package (Collyer & Adams, 2024).

Intraspecific Comparisons

Sexual dimorphism (SD) was tested for body and head shape in adults and juveniles separately, both in univariate and multivariate contexts. Ontogenetic stages were determined based on body size at sexual maturity, following Marques et al. (2013). Females were considered juveniles when SVL < 555 mm, and males when SVL < 505 mm. Individuals above these thresholds were classified as adults. Morphological variation was tested using a *t*-test (for SVL, VS, and SS), and Analysis of Covariance (ANCOVA; the remaining linear measurements were analyzed individually as dependent variables and scaled with size using sex as a factor, SVL as a covariate for body, and HL as a covariate for head variables). Principal Component Analysis was used for visualization of the morphospace, and all linear variables that showed significant results in previous analyses were kept (TL, SS, VS, HH and ED), except for SLV and HL, that was used as covariates to extract the allometric component. A size-free PCA was performed using the residuals of the linear measurements regressed against their covariate (SVL for body and HL for head measures; hereafter "size-free PCA dataset"). The allometric component was estimated as the R^2 of each model, and then the effect of size in the specimen's distribution in the morphospace was observed by comparing the PCA performed with raw data and the size-free PCA. A Procrustes non-parametric multivariate analysis of variance (np-MANCOVA) considered shape coordinates as dependent variables, CS and SVL as covariate separately, Sex and and interaction factor (CS x Sex; or SVL x Sex) as predictors to assess SD in head shape. To visualize the specimen's distribution in the morphospace and the allometric component, we first performed a size-free PCA using the residuals of Procrustes coordinates regressed against CS, followed by a second overall PCA using the Procrustes coordinates.

Ontogenetic allometry was tested in body and head shape using the *procD.lm* function from the *Geomorph* 4.0.0 package (Adams et al., 2021). The dependent variables for body shape were the same used in the PCA, and for head shape the Procrustes coordinates were used. The two multivariate datasets were regressed against SVL (for body shape allometry) and CS (for head shape allometry) with the complete sample for each sex separately. To compare ontogenetic trajectory a test of homogeneity of slopes (HOS) was performed with the *procD.lm* function using the multivariate datasets as response and covariates, sex, and their interaction factors as predictors. Non-significant results for the interaction factor indicate equal slopes. For visualization of the trajectory slopes, raw data were used to perform a multivariate regression against SVL for body shape, and Procrustes coordinates were regressed against CS for head shape (RegScores) To address issues related to an uneven sample in juveniles, a complementary trajectory analysis was carried out using the function *trajectory.analysis*, following Esquerré et al. (2017). To do this we performed a model using Procrustes coordinates as dependent variable, SVL (for body shape) or CS (for head shape) as covariates, sex, stages and all interactions as factors (covariate x sex, covariate x stage, sex x stage, and covariate x sex x stage). Juvenile and adult individuals were used as trajectory points. To test for differences, this model was used as the input in the *trajectory.analisys* function, and sex was used as the grouping parameter. This analysis tests for differences in the position, angle and magnitude (path distances) between male and female ontogenetic trajectories in the morphospace, and visualization was provided as the trajectory between the centroid of the distribution of each group. All models were performed with 10,000 permutations under residual randomization to ensure robustness of significance testing.

Interspecific Comparisons

Bothrops jararaca presents SD in head shape (Siqueira et al. 2024), thus, males and females were compared separately. A MANCOVA controlling for CS was used to test variance in adult head shape between species with the Procrustes coordinates as dependent variable, population (the mainland coastal, highland, and the insular QGI) and interaction CS x population as predictors. Pairwise comparisons were carried out to elucidate whether groups were significant different based on the distance between means. Principal Component Analysis (PCA) on the Procrustes coordinates was used to visualize the distribution of specimens in the morphospace. Differences in ontogenetic trajectories were then tested with a HOS test, considering CS as covariate, population and the interaction factor (CS x population), followed by a pairwise comparison based on the results of vector correlation to test whether populations diverge in ontogenetic trajectory. The HOS and ontogenetic trajectory analysis were done with the complete sample. All statistical analyses (see below) were carried out with Geomorph Package and R-base Packages in the R v.4.2 environment (R Core Team, 2022).

Table 1 Morphological summary for Raw data. Means for SVL, VS, and SS, or adjusted means (adjusted to SLV or HL for head measures), ± standard error (SE), and sample size (N) of eleven morphological variables of adult *Bothrops insularis*: Snout-vent length (SVL), ventral scales (VS), subcaudal scales (SS), tail length (TL), tail width (TW), body circumference (BC), head width (HW), head length (HL), rostrum-labial distance (RLD), head height (HH), eye diameter (ED), and centroid size (CS)

Variable	Female	*N*	Male	*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 Comparisons of 11 morphological traits between juveniles and adults of both sexes of *Bothrops insularis* using *t*-tests and ANCOVA (F-test): Snout-vent length (SVL), ventral scales (VS), subcaudal scales (SS), tail length (TL), tail width (TW), body circumference (BC), head width (HW), head length (HL), rostrum-labial distance (RLD), head height (HH), eye diameter (ED), and centroid size (CS).

Variable	Stage	df	t-test	F-test	*P*-value
SVL	adult	78.484	−8.176	–	**<0.001**
	juvenile	56.308	−0.474	–	0.637
VS	adult	88.147	5.728	–	**<0.001**
	juvenile	45.536	3.917	–	**<0.001**
SS	adult	78.484	−8.176	–	**<0.001**
	juvenile	49.97	−8.380	–	**<0.001**
TL	adult	85	–	21.365	**<0.001**
	juvenile	53	–	37.35	**<0.001**
TW	adult	93	–	2.599	0.11
	juvenile	53	–	8.714	**<0.001**
BC	adult	87	–	2.071	0.153
	juvenile	52	–	0.048	0.828
HW	adult	86	–	0.908	0.343
	juvenile	55	–	0.41	0.525
HL	adult	87	–	21.96	**<0.001**
	juvenile	56	–	13.35	**<0.001**
RLD	adult	85	–	0.485	0.488
	juvenile	55	–	3.331	0.073
HH	adult	81	–	4.548	0.036
	juvenile	53	–	0.544	0.464
ED	adult	82	–	14.256	**<0.001**
	juvenile	55	–	9.594	**0.003**
CS	adult	64	–	20.8	**<0.001**
	juvenile	44	–	3.10	0.08

Degree of freedom (df); significant results were highlighted in bold

Fig. 1 Size-free Principal Component Analysis (**A**), and overall Principal Component Analysis (**B**) of *Bothrops insularis* body shape. Segments represent ontogenetic trajectories estimated from the centroids of the distributions of each group

Results

Measurement Error

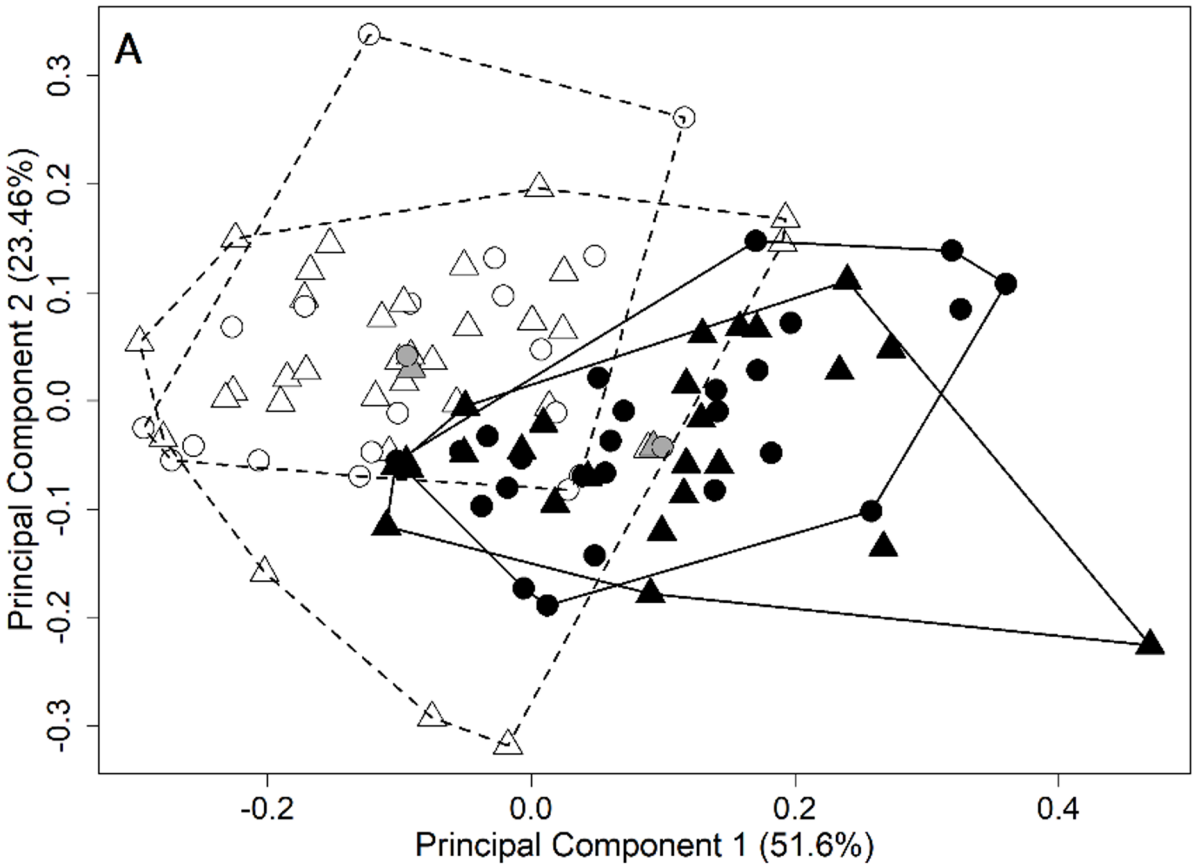
We found no evidence of systematic measurement error ($Z=0.90$, $P=0.18$), rejecting the hypothesis of significant differences attributed to landmark digitization.

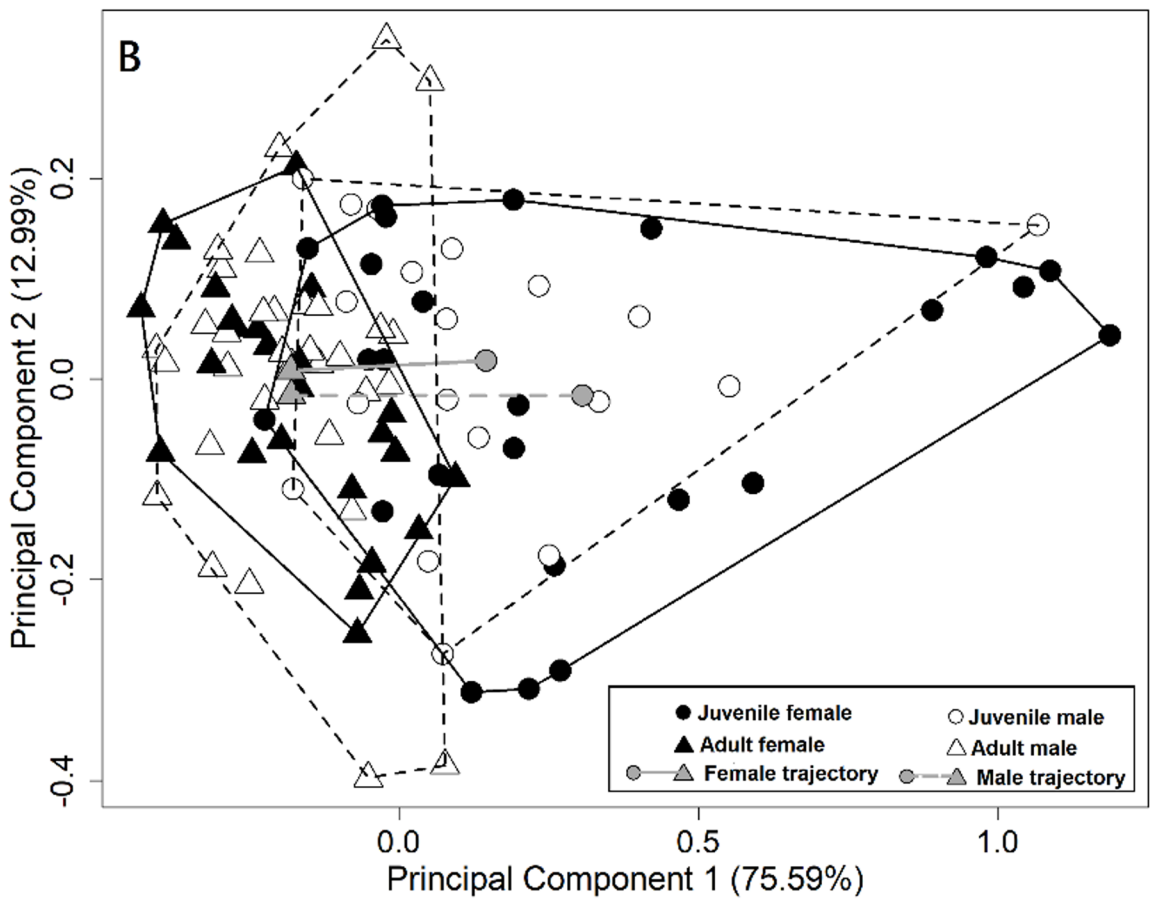
Intraspecific Comparisons

Means and adjusted means are in Table 1. Adult females were larger than males for all body and head measures (except HH), while males were larger for tail traits and eye diameter (ED), including scalation. No difference was found in the remaining variables (Table 2, Online Resource 1). These sexual differences were already present in juveniles for tail length, head length and eye, but not for SLV. Males had larger tail width only when juveniles (Table 2, Online Resource 2–3). The coefficients of linear regressions used to extract the residuals used in the size-free PCA are found in Online Resource 4. In the body measures size-free PCA, the two first axes explained together 75.06% of all morphological variation (Fig. 1A). In PC1, ED and TL were the most significant variables in the negative direction, and VS in the opposite. Females had more ventral scales, while males had larger eyes and tails. In PC2 ED was the most important variable in the negative direction, and TL in the opposite. Males had more extreme values in this axis. In a second PCA performed with raw data, the first two axis explained 88,58% of the total variation, indicating that more than 11% of the variation were attributed to size (Fig. 1B).

We found significant effect of SVL in the multivariate data of body measures, indicating ontogenetic allometry for body shape in females ($F_{(1-49)}=133.63$, $P>0.001$) and males ($F_{(1-51)}=47.61$, $P>0.001$). The SVL was responsible for 79% of body shape variation in females and 48% in males. As size increased head became shorter and head height decreased, while tail and eye size increased (Online Resource). The HOS test showed that the vectors of both sexes are parallel (Interaction SVL x Sex; $F_{(1-98)}=1.54$, $P=0.196$), reflecting a common trajectory, being that 9% of the variation were attributed to sex ($F_{(1-98)}=13.65$, $P>0.001$; Fig. 2). The trajectory analysis showed that ontogenetic trajectory for sexes did not differ for path lengths (magnitude; $z=0.88$, $P=0.20$) nor direction (trajectory correlation in angles; $z=-0.71$, $P=0.76$, Fig. 1).

The MANCOVAs performed on Procrustes coordinates showed no effect of sex in head shape for juveniles, but it was


A
Principal Component 2 (23.46%)
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.2
0.0
0.2
0.4
Principal Component 1 (51.6%)


B
Principal Component 2 (12.99%)
0.2
0.0
-0.2
-0.4
0.0
0.5
1.0
Principal Component 1 (75.59%)
Juvenile female
Juvenile male
Adult female
Adult male
Female trajectory
Male trajectory

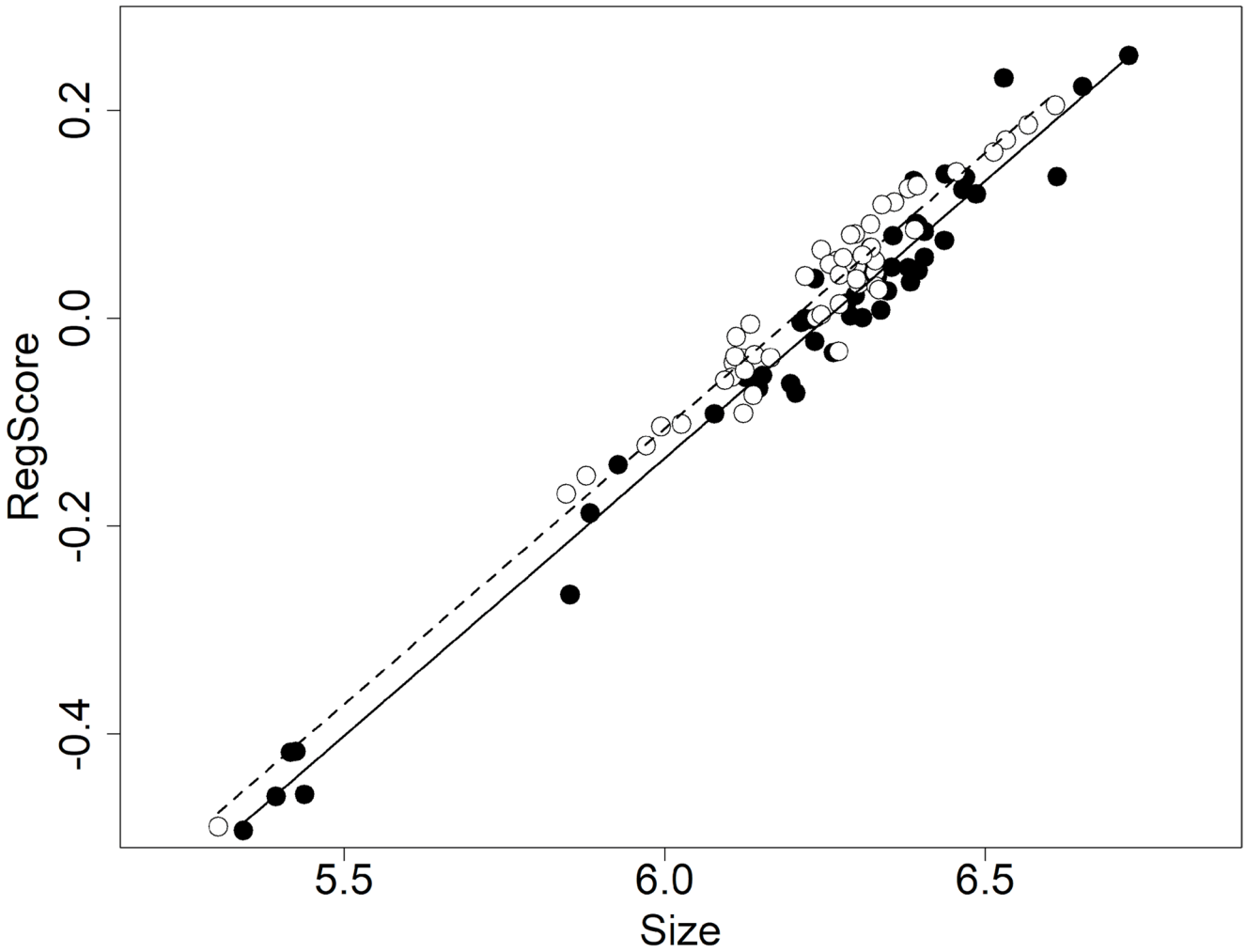


Fig. 2 Multivariate analysis of raw body measurements represented as RegScores plotted against size (snout–vent length), illustrating ontogenetic allometry for linear data in *Bothrops insularis*. Black circles = females; white circles = males

significant for adults (Table 3). The allometric component explained 11,2% of head shape variation. In the size-free PCA performed with the residuals of the allometric regression, the two first axes explained 39.75% of the variation (Fig. 3) showing that adult females had a longer and slightly narrower head. Adult males and juveniles had apparently a shorter and broader head, with a more pronounced snout, however there is a great overlap in the specimen's distribution in PC2 (Fig. 3A). In the overall PCA performed with the Procrustes coordinates, the first two axes explained a large amount of shape variance (44.35%), indicating a more pronounced longer and narrower head in adult females (Fig. 3B).

Ontogenetic allometry in head shape was also significant for both females ($F_{(1-54)}=11.76$, $P=0.001$), and males ($F_{(1-56)}=2.74$, $P=0.01$). The CS was responsible for 17% of head shape variation in females and 4% in males. Larger sizes accounted for a relatively larger and wider head with a smaller snout surface (Fig. 4). Despite an apparent difference in slopes, the HOS test indicates a non-significant interaction CS x Sex ($F_{(1-110)}=1.36$, $P=0.19$), pointing to parallel trajectories. Ultimately, the trajectory analysis showed that sexes did not differ for path lengths (magnitude z = −0.32, $P=0.62$) nor direction (trajectory z = −0.78, $P=0.77$, Fig. 2).

Interspecific Comparisons

Overall, we found a significant effect of population ($F_{(2-172)}=18.96$, $P=0.001$), indicating different head shapes between species. When comparing sexes separately, we found a significant effect of the interaction CS x population on head shape for females ($F_{(2-77)}=1.83$, $P=0.022$), however, only population was significant for males ($F_{(2-89)}=8.09$, $P=0.001$). Pairwise comparisons indicated that heads of *B. insularis* females more closely resembled the highland population (d = 0.034, $P=0.73$) than the coastal population (d = 0.044, $P=0.65$), although differences were

Table 3 Results from MANOVAs performed on Procrustres coordinates for juveniles and adults showing the effect of size and sex on head shape of *Bothrops insularis*

Data	Predictor	df	SS	MS	R^2	F-test	Z	*P*-value
Juveniles	log(CS)	1	0.0197	0.0197	0.1892	10.2686	4.9023	**<0.001**
	sex	1	0.0006	0.0006	0.0059	0.3176	-2.0349	0.98
	log(CS):sex	1	0.0013	0.0013	0.0125	0.6782	-0.5735	0.709
	Residuals	43	0.0824	0.0019	0.7924			
	Total	46	0.104					
	log(SVL)	1	0.0201	0.0201	0.1937	10.6495	4.8876	**<0.001**
	sex	1	0.0012	0.0012	0.0119	0.6527	-0.6494	0.737
	log(SVL):sex	1	0.0013	0.0013	0.0125	0.6871	-0.4724	0.678
	Residuals	43	0.0813	0.0019	0.782			
	Total	46	0.104					
Adults	log(CS)	1	0.0087	0.0087	0.0793	5.8763	4.2338	**<0.001**
	sex	1	0.0055	0.0055	0.05	3.7087	3.2836	**<0.001**
	log(CS):sex	1	0.0023	0.0023	0.0207	1.5338	1.146	0.129
	Residuals	63	0.0928	0.0015	0.85			
	Total	66	0.1092					
	log(SVL)	1	0.0058	0.0058	0.0534	3.9085	3.2802	**<0.001**
	sex	1	0.0069	0.0069	0.0632	4.6232	3.8093	**<0.001**
	log(SVL):sex	1	0.0024	0.0024	0.022	1.61	1.2394	0.111
	Residuals	63	0.0941	0.0015	0.8613			
	Total	66	0.1092					

Degree of freedom (df); significant results were highlighted in bold

not statistically significant. The same pattern occurred in males, which were phenotypically closer to the highland population (d = 0.029 $P = 0.001$), than to the coastal population (d = 0.041, $P = 0.001$). Considering PCA analysis, PC1 indicated that *B. insularis* had the most positive values, with a longer and narrower head compared to *B. jararaca*, while PC2 emphasized a large amount of head-shape variation (Fig. 5), with *B. insularis* individuals more concentrated in the upper region of the morphospace, except for one individual below −0.02, indicating a slightly larger snout than those of *B. jararaca* populations.

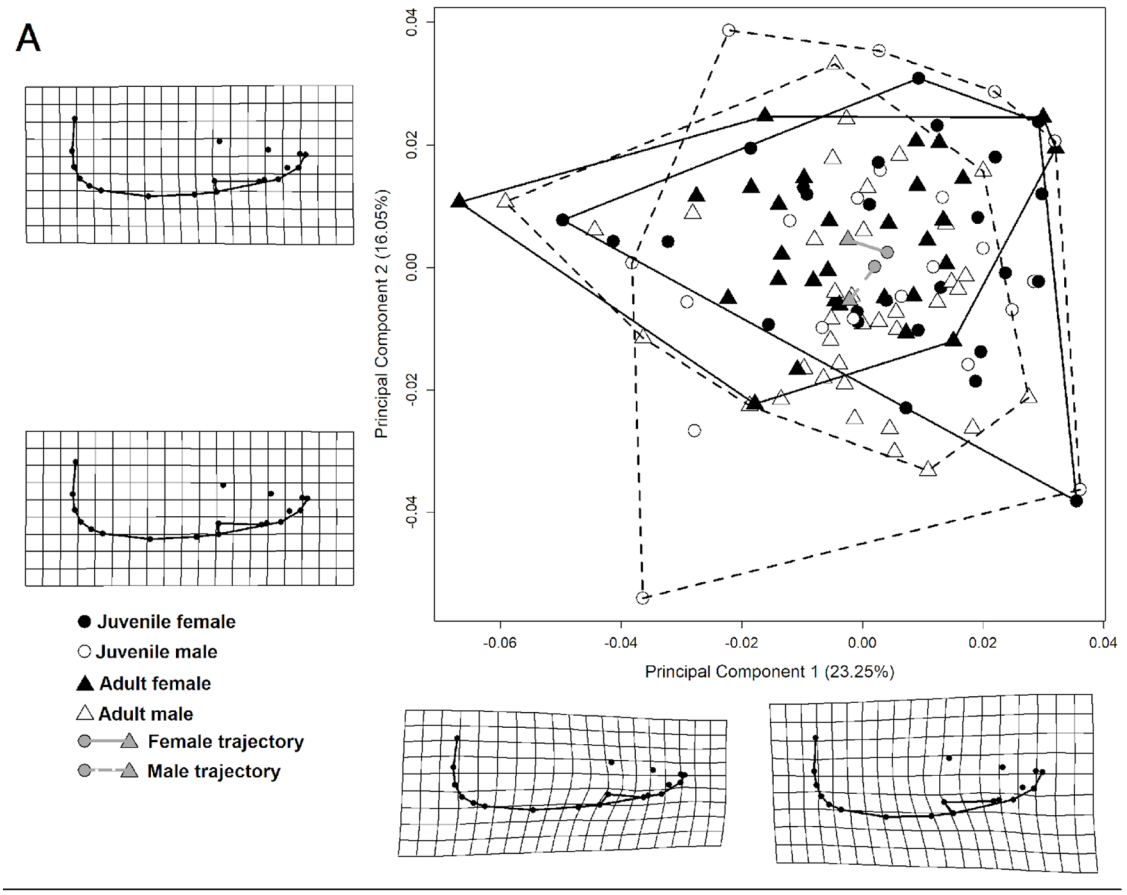
Ontogenetic trajectories also indicated a relatively larger and wider head with smaller (arrow-shaped) snouts as size increased (Fig. 6), and the HOS test performed on Procrustes coordinates using CS, population, and their interactions showed that ontogenetic trajectories for females had different slopes (interaction CS x population; $F_{(2-133)} = 1.79$, $P = 0.023$). Pairwise comparison indicated that *B. insularis* ontogenetic trajectory was parallel to the highland population ($r = 0.89$, $P = 0.29$) and convergent with the coastal *B. jararaca* population, as adults showed close phenotypes ($r = 0.78$, $P = 0.007$; Fig. 6A). Males' slopes were only marginally significant (interaction CS x population; $F_{(2-131)} = 1.51$, $P = 0.065$), and ontogeny was also parallel to the highland population ($r = 0.80$, $P = 0.68$), and convergent with coastal *B. jararaca* population ($r = 0.49$, $P = 0.045$, Fig. 6B).

Discussion

Intraspecific Comparisons

The fecundity selection hypothesis states that females are larger in species where reproductive outputs correlates positively with maternal body sizes (Cox, 2007; Shine, 1994). Previous studies indicated that the female-biased sexual size dimorphism (SSD) in *Bothrops insularis* is in agreement with this hypothesis, since larger females produce larger clutches, and further comparisons with the congeneric *B. jararaca* reiterate this conclusion, as the smaller sizes attained by the former are associated with smaller average numbers of reproductive follicles (Marques et al., 2013). On the other hand, the ecological hypothesis proposes that morphological differences result from niche partitioning. Although these two hypotheses are not mutually exclusive (Shine, 1993), ecological selection appears more plausible given the observed differences in body and head morphology. However, further investigations are needed to verify this hypothesis, as no evidence of niche partitioning in this species is currently available (cf. Marques, 2012a).

In snakes, the number of ventral scales correlates with the number of vertebrae (Alexander & Gans, 1966; Arnold, 1988; Lindell et al., 1993). Additionally, the number of vertebrae determines maximum body size 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 body and tail size, respectively attained by


A
Principal Component 2 (16.05%)
Principal Component 1 (23.25%)
0.04
0.02
0.00
-0.02
-0.04
-0.06
-0.04
-0.02
0.00
0.02
0.04
Juvenile female
Juvenile male
Adult female
Adult male
Female trajectory
Male trajectory

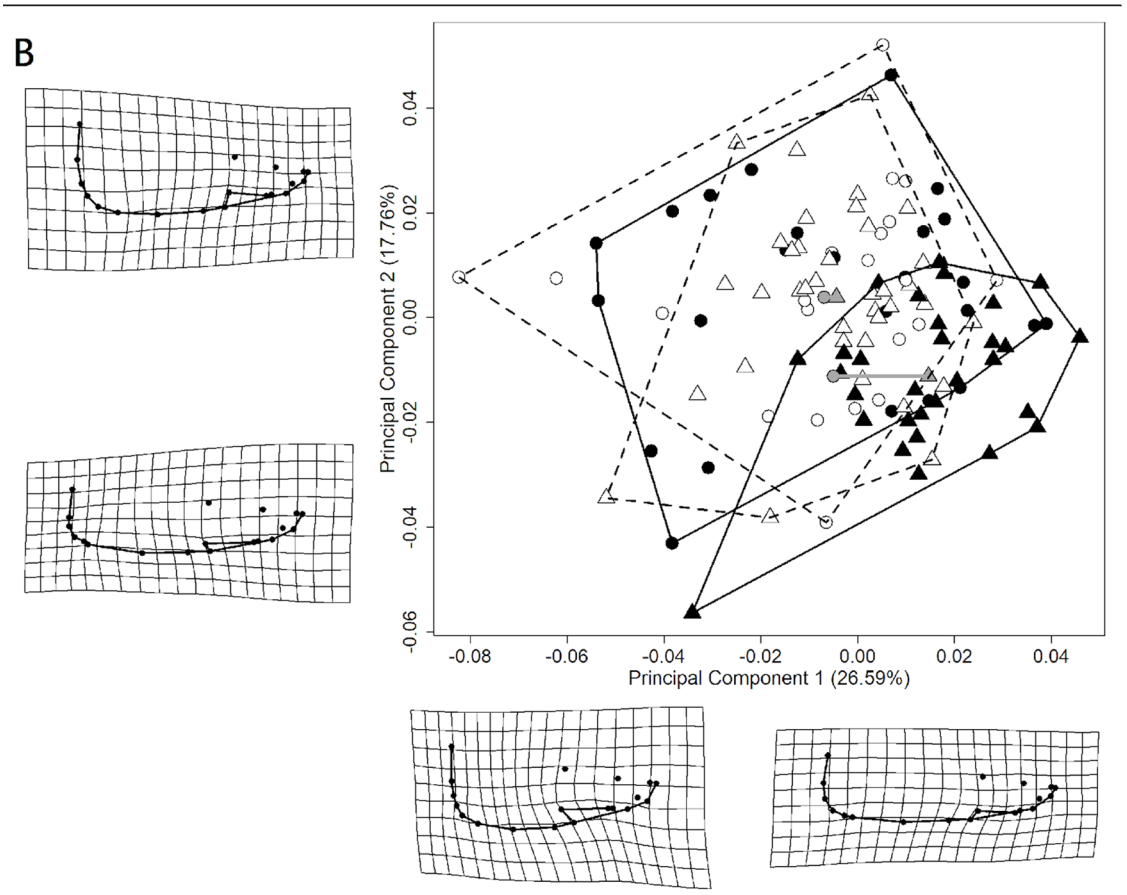

B
Principal Component 2 (17.76%)
Principal Component 1 (26.59%)
0.04
0.02
0.00
-0.02
-0.04
-0.06
-0.08
-0.06
-0.04
-0.02
0.00
0.02
0.04

Fig. 3 Size-free Principal Component Analysis (**A**) and overall Principal Component Analysis (**B**) of *Bothrops insularis* head shape. Deformation grids represent predicted head-shape variation along the first two axes. Segments represent ontogenetic trajectories

each sex. In contrast, the tail was longer in males, but wider only in juvenile males. This is probably a consequence of the presence of copulatory organs and associated muscles (King, 1989). Interestingly, the absence of sexual dimorphism in tail width in adults may result from the hemiclitores present in females (Hoge et al., 1953; Marques et al., 2002). In *B. insularis*, hemiclitores appear as a triangular structure and, although they do not show the level of ossification observed in hemipenial spines (Garcial et al. 2022), they may still influence tail width. Although some evidence suggests that males with larger tails are more successful in gaining access to females, this pattern usually occurs in species showing reproductive aggregations (Shine et al., 1999). In this sense, the caudal pattern in *B. insularis* is rather a consequence of morphological constraint.

Eye diameter was larger in males among both juveniles and adults. In snakes, there is a strong correlation between eye size and ecological habits, with diurnal and arboreal species exhibiting larger eyes than nocturnal, terrestrial, or semi-aquatic species (Liu et al., 2002, 2016). However, recent in situ evidence indicates that *B. insularis* is predominantly nocturnal, and sex apparently has no effect on activity (Banci et al., 2025). Additionally, we are not aware of studies showing that males are more arboreal than females, and this statement remains speculative.

Sexes also differed in head size and shape. Dietary data from adult *Agkistrodon piscivorus* (Lacepède, 1789) indicate that males consume taller prey and that the sexes differ in prey proportions, correlating with significant differences in head shape (Vincent et al., 2004a). Thus, these data suggest that female *B. insularis* may consume larger prey, which is supported by observations that only females have been reported to feed on the relatively large bird *Turdus flavipes* (cf. Kasperoviczus et al., 2023). Ontogenetic allometry for body and head shape was parallel, although a slightly larger amount of shape variation per unit change of the covariate was attributed to size in females. If the sexes diverge in feeding habits (e.g., prey types), head dimensions are often affected (Meik et al. 2012; Vincent et al., 2004b). Accordingly, the small range of prey types in *B. insularis* (Martins et al., 2002; Marques et al., 2012a) probably constrains feeding habits, resulting in a paired trajectory for head length.

Trajectory analysis indicated similar ontogeny between sexes. Equal trajectories between sexes are common even in highly diverse taxa such as Pythonidae (Esquerré et al. 2016). In some cases, even when trajectories overlap, 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 patterns, with females growing faster and maturing slightly later (Banci et al., 2024), which is highly consistent with ontogenetic variation in body and head shape. Shape variation is likely an effect of heterochrony. We acknowledge that a small gap in our juvenile sample could bias our results; however, a more specific approach at this stage may help address this issue.

Interspecific Comparisons

Our findings are consistent with those of Wüster et al. (2005), since B. insularis has a generally longer head than *B. jararaca*; nevertheless, *B. insularis* head shape was significantly different from that of *B. jararaca* for males. However, we found no support for Amaral's (1951b) statements, since *B. insularis* has a narrower head in the temporal region as well as a larger and stouter snout than its counterpart, at least for one of the populations studied (highland population). This pattern is strongly consistent with feeding ecology, since adult *B. insularis* are predominantly bird-eaters, whereas *B. jararaca* prey upon small rodents (Martins et al., 2002; Marques et al., 2012a, 2019; Sazima, 1992). Feeding behavior may explain this trend, as adult Golden lanceheads and juvenile *B. jararaca* usually hold their prey to prevent escape (Marques et al., 2002; Martins et al., 2002; Marques & Sazima, 2009). In this sense, the more robust snout of *B. insularis* may allow more efficient prey capture. The fact that this difference was detected only in males is consistent with this interpretation, since females already have larger heads that may facilitate prey retention, while males might compensate their smaller head size with a proportionally longer snout to improve grasping efficiency on birds.

The heads of juvenile *B. insularis* are smaller and more rounded. Throughout postnatal development, head shape becomes larger and wider, with a smaller snout surface. This species exhibits an ontogenetic dietary shift, with juveniles primarily consuming ectothermic prey (e.g., centipedes, frogs, and lizards; Marques et al., 2012a), which may explain the pattern found. Ontogenetic trajectories differed from those of *B. jararaca* for both sexes, at least when considering the coastal population. In this sense, we suggest that, although both species undergo ontogenetic dietary shifts, differences in prey types consumed by juveniles and adults between species may influence ontogeny in head shape.

It is plausible that some morphological traits are genetically correlated, such as size, shape, or scalation (Dohm & Garland Jr., 1993; Webb et al., 2002). Molecular analyses indicate that *B. insularis* is genetically closer to a *B. jararaca* specimen from São Bernardo do Campo, Brazil

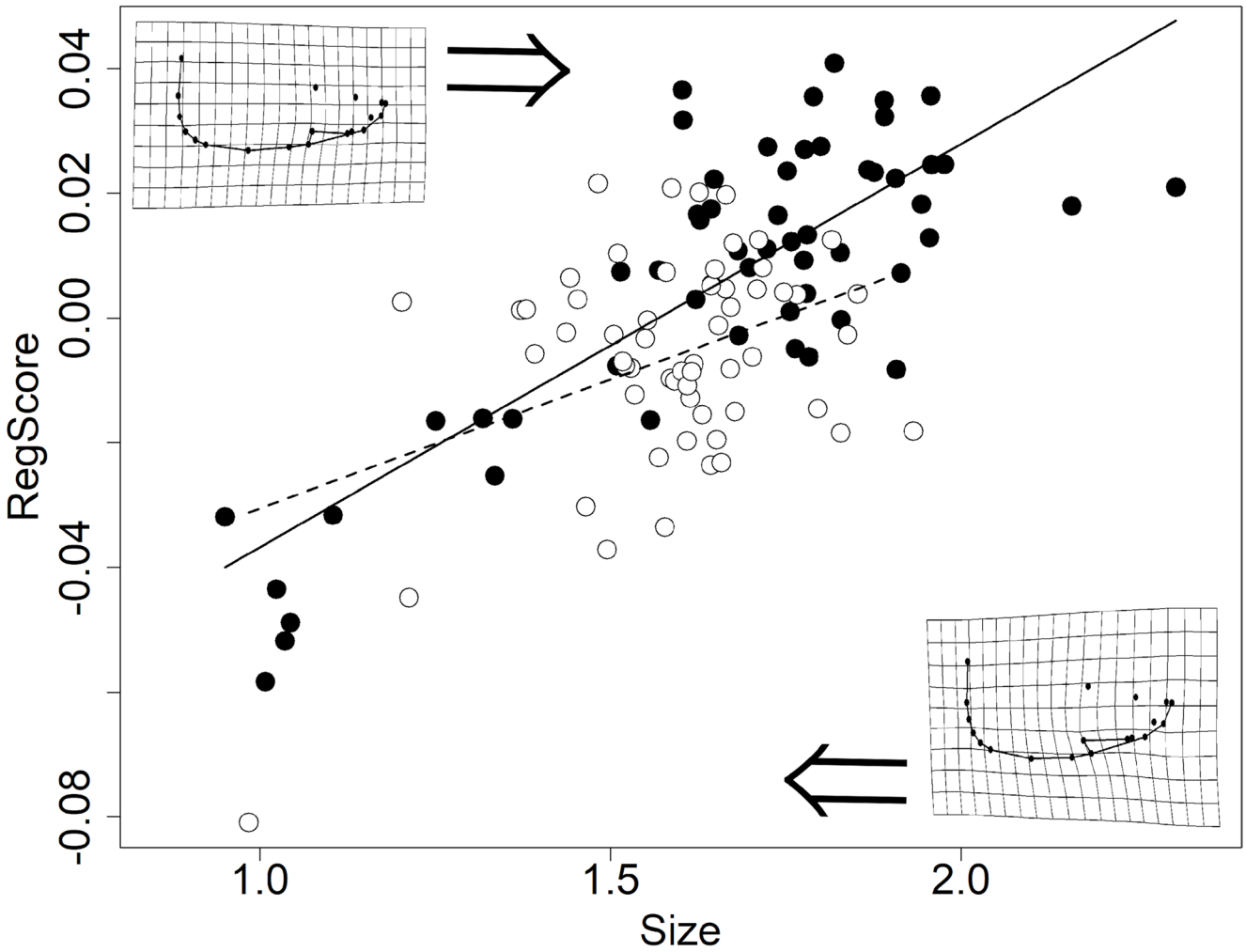


Fig. 4 Head-shape variation represented by regression scores (RegScores) plotted against a general size variable (centroid size), illustrating ontogenetic allometry for a set of landmark coordinates in *Bothrops insularis*. Deformation grids represent predicted head-shape variation with size. Circles = juveniles; triangles = adults; black = females; white = males

(Grazziotin et al., 2006) — a highland locality — and our pairwise analysis corroborates phylogenetic expectations, since *B. insularis* head shape is more similar to the highland than to the coastal population. Our ontogenetic analysis is also consistent, since *B. insularis* and highland *B. jararaca* had parallel trajectories. Nonetheless, considering the distribution of specimens in the interspecific PCA, we found a similar variance in both species. For instance, Barbo et al. (2022) showed broader variance across all mainland *B. jararaca* populations compared with a much smaller variance in *B. insularis*. Thus, the relatively high variance found for this insular and restricted species is unexpected. This may result from the different approach to phenotype measurement used here or even from the preservation process, which could introduce error into the sample. Measuring traits with microCT scanning or directly on the skull would be useful to address this hypothesis, since both techniques are reliable for evaluating phenotypic variation (Souto et al., 2019).

In this study, we included only a fraction of the *B. jararaca* distribution range and considered altitude difference as a potential geographic barrier to categorize populations. Therefore, we cannot rule out gene flow between coastal and highland populations. Additionally, some lineages defined by Barbo et al. (2022) were lumped in our analysis, which may have masked variability in our data, since regional patterns may be more complex than expected. A more specific approach is needed to address the role of genetic aspects in phenotypic variation and ontogeny.

Boback (2003) investigated body size changes among insular snake species and found a general dwarfism pattern in viperids. Island area and distance from the mainland exerted no effect on snake body size, and Boback (2003) proposed a "diet change hypothesis" to explain these results, since island snakes usually consume either larger or smaller prey (in the case of viperids), and species that undergo ontogenetic dietary shifts may retain juvenile patterns relative

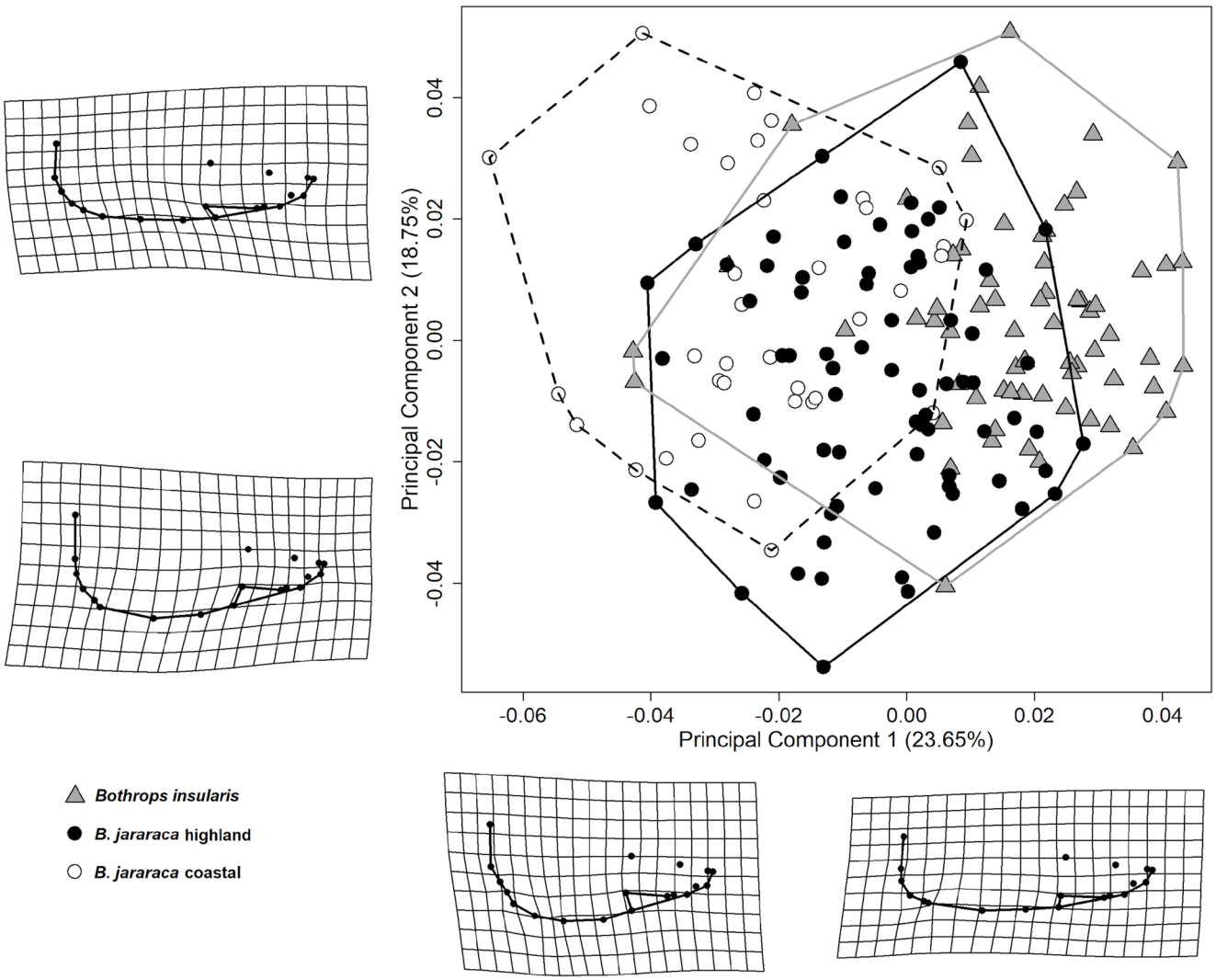


Fig. 5 Principal Component Analysis of head-shape variation showing the distribution in shape space of *Bothrops insularis* and two populations of *B. jararaca*. Deformation grids represent predicted head-shape variation along the first two axes

to their mainland counterparts. Indeed, other insular species from the *jararaca* group, such as *B. alcatraz* (Marques et al., 2002), *B. otavioi* (Barbo et al., 2012)d *sazimai* (Barbo et al., 2016), show smaller body sizes and diets similar to those of juvenile *B. jararaca* from the mainland (Barbo et al., 2012; Martins et al., 2002; 2016; Marques et al., 2012a, b; Sazima, 1992). On the other hand, *B. insularis* feeds on a prey item (birds) also consumed by adult *B. jararaca* from the mainland and reaches a larger size than its insular counterparts (Sawaya et al., 2023).

Conclusion

In this study, we combined two complementary morphometric approaches to address key aspects of sexual dimorphism and morphological variation in *Bothrops insularis*, one of Brazil's most threatened insular snakes. The sexes showed significant divergence in both body and head shape, probably reflecting unequal resource exploitation. Ontogenetic trajectories were similar in both angle and magnitude, but size increasing explained a larger percentage of shape variance in females. Thus, we suggest that intrinsic factors such as growth rate and heterochrony play a fundamental role in phenotypic disparity in this species.

Overall, *B. insularis* males have a longer and narrower head, with a stouter snout than *B. jararaca*. Nonetheless, this species was phenotypically closer to the highland population, indicating a feasible phylogenetic association. However, our results contradict Amaral's (1951b) predictions, since *B. insularis* does not have smaller snouts than its congener. Its larger snout can be interpreted as an evolutionary consequence of local pressures such as prey availability. This difference being evident only in males suggests that

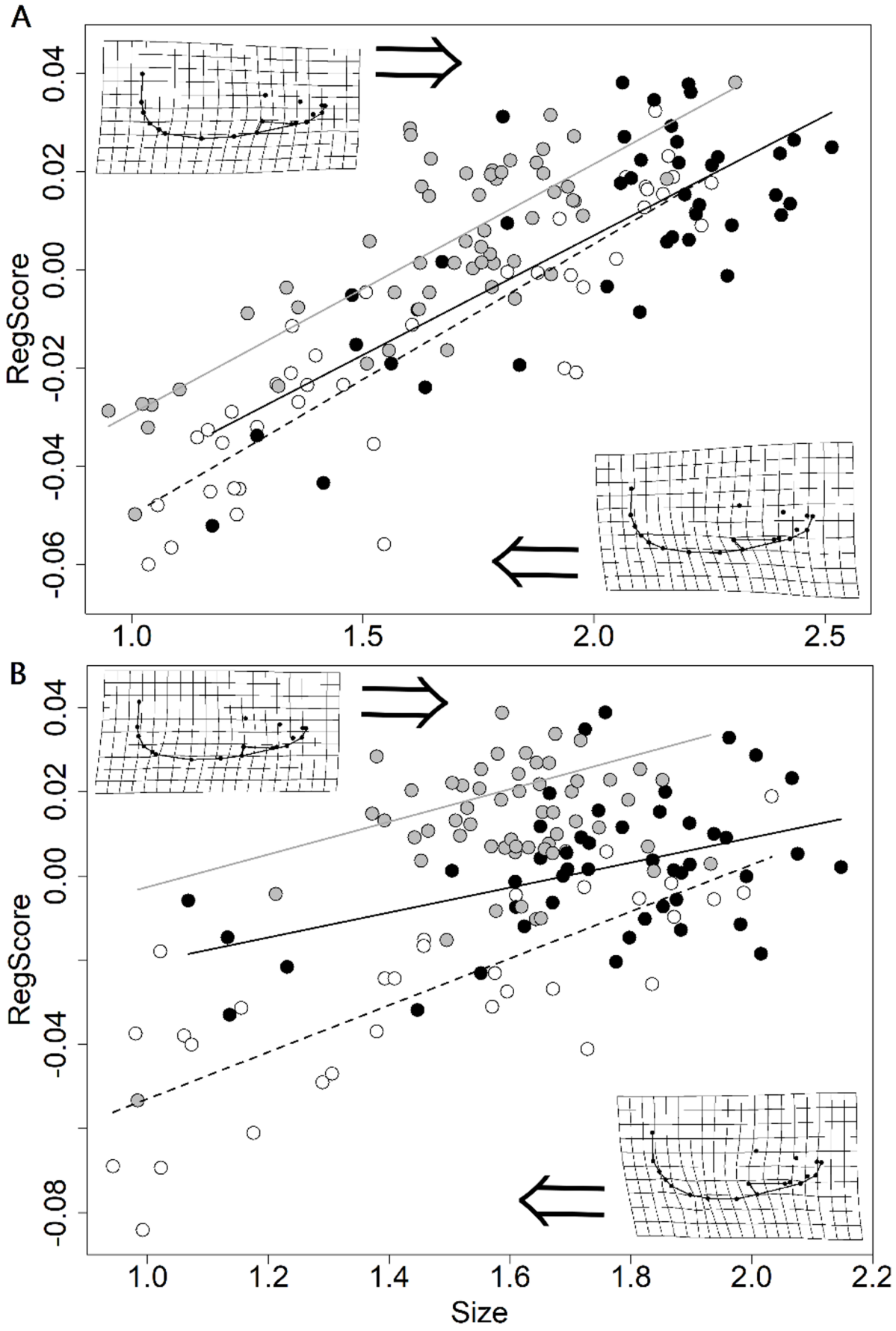

A
RegScore
0.04
0.02
0.00
-0.02
-0.04
-0.06
1.0
1.5
2.0
2.5
B
RegScore
0.04
0.02
0.00
-0.04
-0.08
1.0
1.2
1.4
1.6
1.8
2.0
2.2
Size

Fig. 6 Head-shape variation represented by regression scores (Reg-Scores) plotted against centroid size, illustrating ontogenetic allometry for a set of landmark coordinates comparing *Bothrops insularis* and two *B. jararaca* populations. **A** females; **B** males. Deformation grids represent predicted head-shape variation with size. White circles = *B. jararaca* from the coastal population; black circles = *B. jararaca* from the highland population; grey circles = *B. insularis*

females, with their naturally larger heads, already handle avian prey efficiently, while males may rely on a proportionally longer snout to improve grasping performance. This work emphasizes the importance of using geometric morphometrics in comparative studies, as it provides not only novel insights into the species' morphology but also offers evidence supporting previously proposed hypotheses.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11692-025-09661-y.

Acknowledgements We thank K.R.S.B. for her personal communications, and G. P. and F. G. G. for granting access to the specimens in the zoological collection under their care. We especially thank V. G. for his collaboration in preparing the material and for the kindness in sharing his knowledge. We thank the editor and anonymous reviewers for providing valuable insights and suggestions that greatly improved our manuscript quality.

Author Contributions Lucas Henrique Carvalho Siqueira (conceived and designed the study, collected and analyzed the data and wrote the manuscript), Otavio Augusto Vuolo Marques (conceived and designed the study, supervised the study and wrote the manuscript), and Carla Piantoni (supervised the study and wrote the manuscript).

Funding This study was funded by the "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (CAPES). Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) [grant number 20/12658-4].

Data Availability Raw data, supplementary material and R scripts are available online.

Declarations

Conflict of interest There is no conflict of interest.

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