

Analysis of morphological variability and heritability in the head of the Argentine Black and White Tegú (*Salvator merianae*): undisturbed vs. disturbed environments



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ABSTRACT

The heterogeneity of biotic and abiotic factors influencing fitness produce selective pressures that promote local adaptation and divergence among different populations of the same species. In order for adaptations to be maintained through evolutionary time, heritable genetic variation controlling the expression of the morphological features under selection is necessary. Here we compare morphological shape variability and size of the cephalic region of *Salvator merianae* specimens from undisturbed environments to those of individuals from disturbed environments, and estimated heritability for shape and size using geometric morphometric and quantitative genetics tools. The results of these analyzes indicated that there are statistically significant differences in shape and size between populations from the two environments. Possibly, one of the main determinants of cephalic shape and size is adaptation to the characteristics of the environment and to the trophic niche. Individuals from disturbed environments have a cephalic region with less shape variation and also have a larger centroid size when compared to individuals from undisturbed environments. The high heritability values obtained for shape and size in dorsal view and right side view indicate that these phenotypic characters have a great capacity to respond to the selection pressures to which they are subjected. Data obtained here could be used as an important tool when establishing guidelines for plans for the sustainable use and conservation of *S. merianae* and other species living in disturbed areas.

1. INTRODUCTION

1.1. Anthropogenic alteration of the environment

The primary effects of anthropogenic modification of undisturbed environments are alterations in the structure and composition of vegetation, affecting environmental conditions of microhabitats (Saunders et al., 1991). This may particularly influence reptiles, which, due to their ectothermy, are susceptible to changes in the thermal environment (Schlaepfer and Gavin, 2001). In general, the term “disturbance” usually refers to physical changes in the environment, and can range from very local alterations of the microclimate or microhabitat to phenomena that have effect at the continental scale (Brown, 2001). The fate of a large part of terrestrial biodiversity will depend on the ability of its constituent species to survive within agroecosystems,

because of the increasing geographic expansion of these, and the intrinsic environmental disturbances associated with them (Tilman et al., 2001). The natural environment where *Salvator merianae* is found in Argentina has been highly transformed as a result of the continued expansion of cultivated areas. This has led to deforestation, drainage of wetlands to obtain more land for agriculture, and pollution of natural environments through the application of pesticides (Schaumburg et al., 2012). Therefore the purpose of this work is to understand how the species responds to environmental disturbances and based on this, to decide if action should be taken for the conservation of the species and which should be these action.

1.2. Studies of the lizard head

The lizard head could be a paradigm for mosaic evolution because

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of the numerous selective forces acting on it, because it is involved in a variety of functions (feeding, territory acquisition and defense, habitat and refuge use, mating) which provide opportunities for the action of selective forces on the size and shape of the head (Kaliontzopoulou et al., 2008). Geometric morphometric techniques may be ideally suited to quantify the effect of selection on different functional features of the lacertilian skull (Fabre et al., 2014b). Geometric morphometrics provide data for which a solid statistical framework exists, permitting the investigation of organismal shape while preserving the geometrical properties of the structures analyzed, and thus improving our understanding of ecological and historical factors explaining patterns of morphological variation (Kaliontzopoulou, 2011).

1.3. Phenotypic variation

The phenotypic variation observed in natural populations results from evolutionary processes that predominantly reflect interactions between selective pressures inherent in the environments where the populations evolve. The selection of an environment by an organism is directly influenced by the interaction between its morphological and physiological capacities and ecological factors such as climate, structural complexity of the habitat or availability of food (Barros et al., 2011). It is expected that the heterogeneity of biotic and abiotic factors influencing fitness will result in selection that promotes local adaptation and divergences between different populations of the same species (Laugen et al., 2003).

1.4. Morphological evolution and heritability

To understand morphological evolution, one must first document patterns of variation and then examine the relative influences that environmental and genetic factors exert upon the development and maintenance of these patterns. Although natural and sexual selection are important forces that impose immediate patterns upon morphology, for these patterns to be realized through evolutionary time, the morphological features under selection must exhibit heritable variation (Myers et al., 2006). The contribution of heritable genetic variance to total phenotypic variance is a critical parameter controlling the ability of a phenotype to respond to selection (Monteiro et al., 2002). Heritability is the proportion of the phenotypic variance attributable to genetic variation that parents can pass on to their offspring; thus, the heritability of a character in a population determines its evolutionary potential (Frankham et al., 2002). Given the expectation of a straightforward relationship between shape, function, and fitness, it is often assumed that measuring heritability of shape traits provides an acceptable means of estimating the response of those traits to selection (Chaves-Campos et al., 2012).

1.5. Niche variation hypothesis

Another relevant concept is the “niche variation hypothesis” (Van Valen, 1965), which suggests that populations with larger niches are more phenotypically variable than populations with narrow niches. The amplitude of a particular niche can be achieved in two ways by a population: by all individuals using the entire set of available resources or, alternatively, by each individual using a narrower range of resources, differing from those used by their conspecifics, thus minimizing the intraspecific overlap of resource use and competition. Thus, the amplitude of the dietary niche of a population is achieved by dietary variation among the individuals comprising that population, which additively encompasses the limits of the dietary niche (Bolnick et al., 2007; Giri and Loy, 2008).

1.6. Biological characteristics of *Salvator merianae*

The family Teiidae is distributed from the northeastern United

States to Argentina, and its species occupy a wide variety of ecosystems. The black and white tegu, *Salvator merianae*, is a widely distributed species, being found from southern Brazil to northern Patagonia, east of the Andes (Lima dos Santos, 2007). It is found in both natural and human-disturbed habitats (Schaumburg et al., 2016). Lizards of this species are active foragers and have generalist habits; the diet is comprised of insects, fruits, small vertebrates, eggs and carrion. Adults exhibit sexual size dimorphism, males attaining greater body size than do females. During the fall and winter months, individuals undergo torpor; the active season is spring and summer (Fitzgerald, 1992).

There are few geometric morphometric studies of the head of *Salvator merianae* (Monteiro and Abe, 1997; Fabre et al., 2014a, 2014b). None of these integrated morphometric data and quantitative genetics. In recent years, an increasing interest in combining quantitative genetics and geometric morphometric methods has become evident, although this has not been emphasized in studies of reptiles (Myers et al., 2006; Leaché et al., 2009; Adams, 2011; Imhoff et al., 2015; Sacchi et al., 2016).

1.7. Legal and commercial framework

Historically, the black and white tegu lizard has been harvested commercially for its skin; since 1977 it has been included in Appendix II of the “Convention on International Trade in Endangered Species of Wild Fauna and Flora” (CITES), being placed within the category of “Least Concern” (Porini, 2006). In addition, this species has been under management as part of a sustainable use program in Santa Fe province (Argentina), known as the Iguana Project (PI – Secretary of State for Environment and Sustainable Development of the Province of Santa Fe. Resolution Number 0031/07). This program is based on ranching techniques, which entails the collection of eggs from undisturbed environment, subsequent artificial incubation, hatching and maintenance of the animals under controlled conditions until they reach an appropriate size to be released into the wild, in order to avoid predation or the influence of low temperatures (Schaumburg et al., 2012).

1.8. Main objective and hypotheses

The main objective of this work is to evaluate the influence of the environment, undisturbed vs. anthropogenically disturbed, on shape and size variation of the head of *Salvator merianae*, and to evaluate the capacity of individuals to respond to environmental variation. Our hypothesis is that individuals will present variation in the shape and size of the head associated with environment type, because the different selection pressures acting in the two types of environments will have different influences upon both analyzed characters. Our results would allow us to relate morphological variability of the head of individuals of *S. merianae* to different environmental conditions, and to calculate the heritability of cephalic shape and size. All of this relies upon the theoretical basis that the tegu lizard is considered to be a generalist and opportunistic species (Winck et al., 2011), so it could adapt rapidly to changing environmental conditions. These data could also be used as an important tool when establishing the plans for the sustainable use and conservation of *S. merianae* and other species living in disturbed areas.

2. MATERIALS AND METHODS

2.1. Sampling

Our sample consisted of sixty specimens of *Salvator merianae* hatched from eggs collected in the wild in different localities of Santa Fe Province: Reconquista (29°14'00"S, 59°56'00"W), Romang (29°30'00S, 59°46'00W), Sa Pereira (31°34'18.25S, 61°23'22.02W), Alejandra (29°57'6.24S, 59°49'53.40W), San Cristóbal (30°18'21.81S, 61°15'48.40W) and Costa del Salado (29°40'37.57'S, 60°54'44.00" W) (Fig. 1). We select the sampling sites based on dispersal ability of the

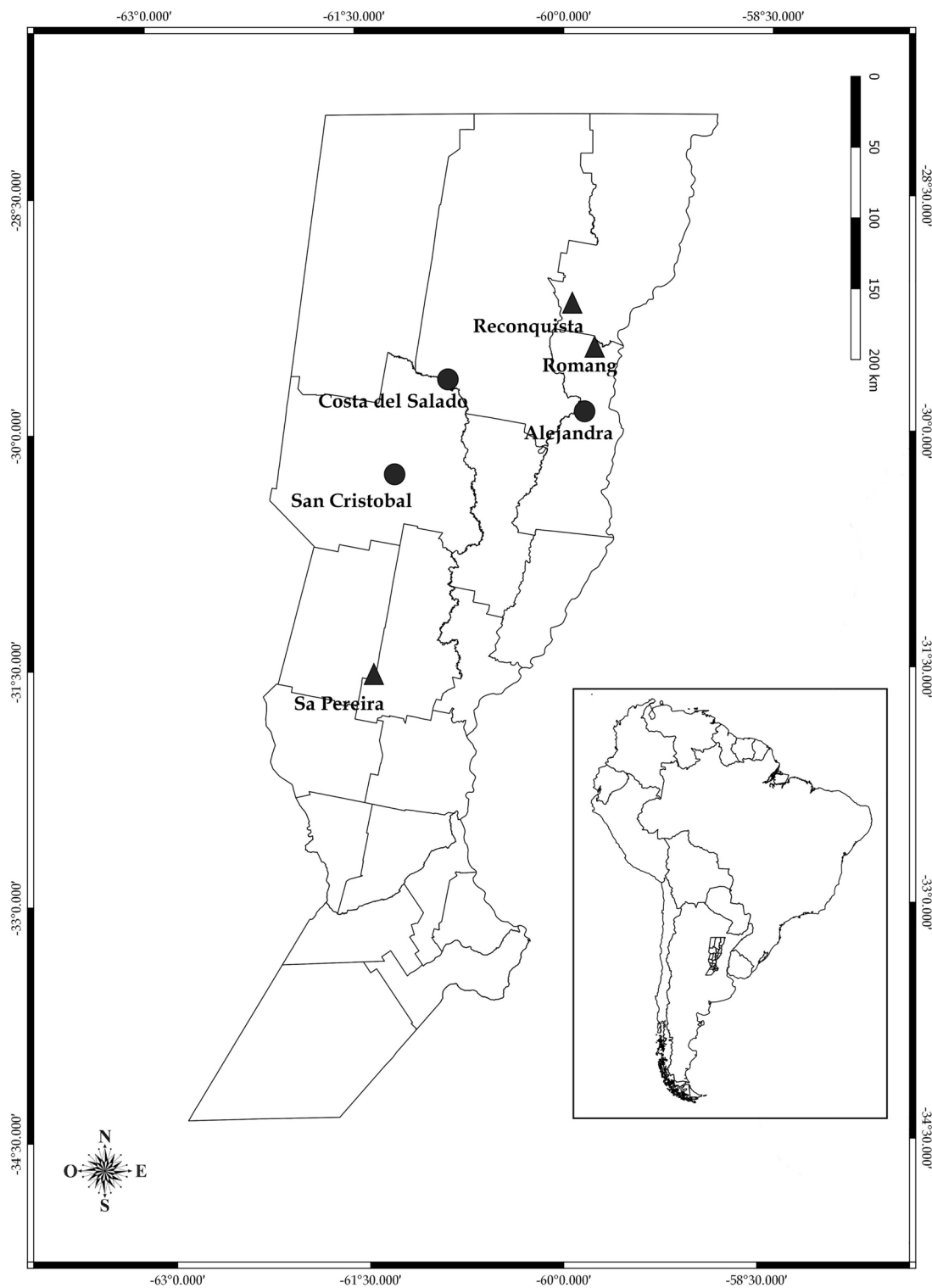


Fig. 1. Sampling sites selected. Circles represent the sampling sites of undisturbed environments and triangles represent the sampling sites of disturbed environments.

species, in order to obtain samples from different populations (Winck et al., 2011). We sampled one nest from each sampling site and, approximately one week after hatching, from each nest we randomly selected ten individuals for data collection. As part of the ranching program “PI”, the eggs were artificially incubated under controlled conditions of temperature (29 – 32 °C) and (< 20 %).

We considered the first three locations (Reconquista, Romang and Sa Pereira) to be disturbed habitats because the nests were harvested from sown fields (soya and sorghum fields) with marked alterations of

endemic flora; and the other three (Alejandra, San Cristóbal and Costa del Salado), as undisturbed environments, because the nests were harvested from fields that lacked such anthropic alteration, with little or no modification of the local flora. Furthermore, we consider it unlikely that individuals at any location move between disturbed and undisturbed environments. Females have small home ranges, centred on nesting sites (Manes et al., 2003), and while males have larger home ranges, they are territorial and tend to associate with females (Winck et al., 2011).

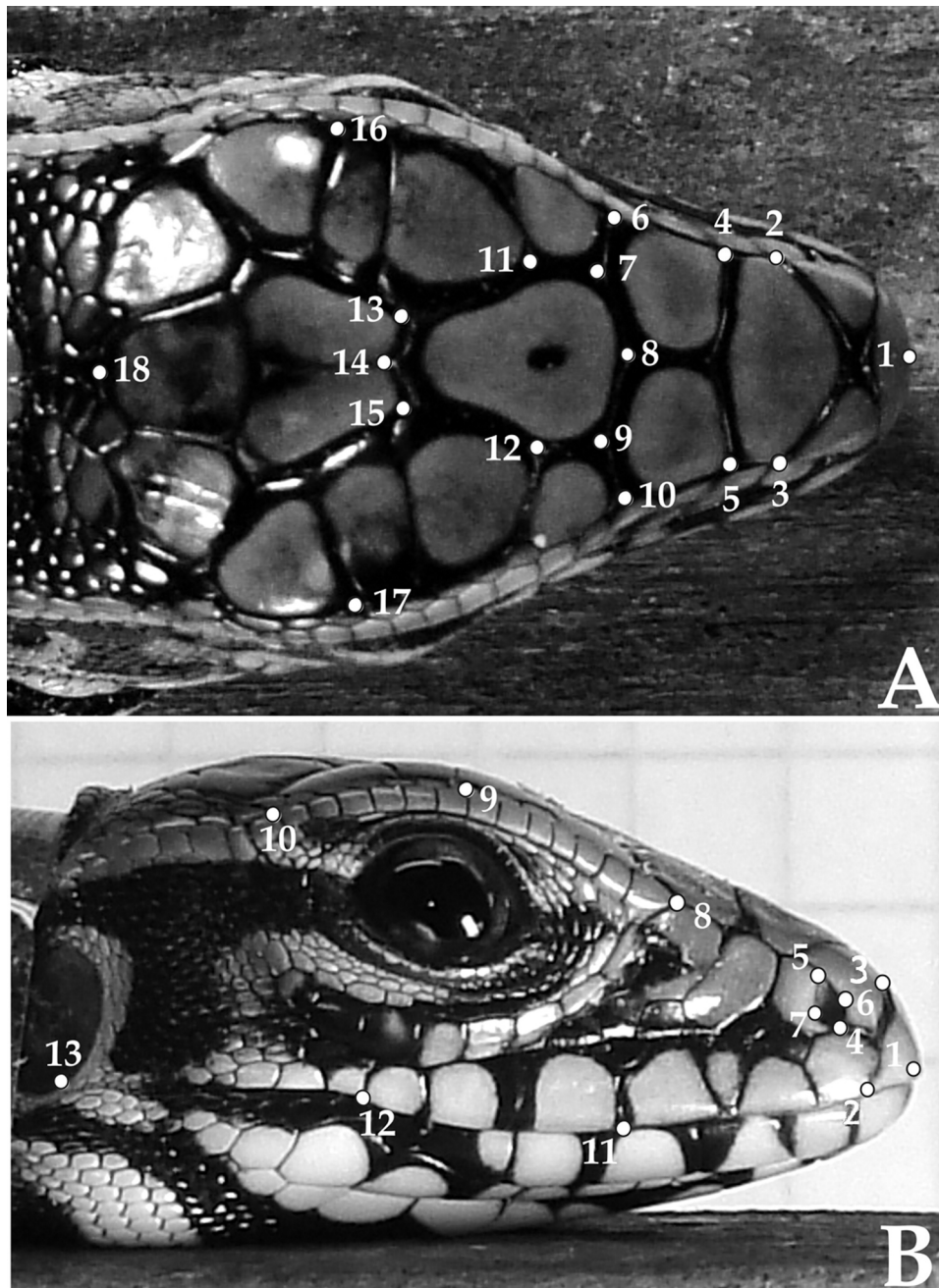


Fig. 2. Head of *Salvator merianae*, showing placements of landmarks. a) Dorsal view. b) Right side (see also supplementary Table A for descriptions).

2.2. Photography

We performed photography and data analysis following the methodology of Imhoff et al. (2015). We photographed the dorsal view and the right side of the head of each lizard using a digital camera (SONY Cyber-shot DSC-H20, Full HD 1080, with 10.1 Mp resolution). To immobilize each animal we placed a ribbon around neck that held it to a wooden stand located beneath it, so that it lay flat upon this, without possibility of movement. We placed the camera perpendicular to the center of the head, and to the right lateral side of the head, using a tripod, at a focal distance of about 30 centimeters between the individual and the lens. We used a sheet of graph paper with a grid space of 0.5 cm as background, serving as a metric reference to compute size (Fig. 2).

2.3. Landmarks

We took three photographs in dorsal view and three in right-side lateral view for each individual. Then, using TPSDig2 program (Rohlf, 2004), a single person placed 18 and 13 landmarks (Lm) for each configuration respectively on each of the three replicate photographs of each lizard, that is to say that each individual has three photographs in dorsal view and three photographs in right side view, and in each one of these photographs we placed the Lm. We selected the points for the placement of the landmarks with the aim of achieving complete coverage of the shape of the head. To do this we placed the Lm in the sutures of the large, regular cephalic scales covering the dorsal and lateral aspects of the head, since they are useful in the investigation of the evolution and morphogenesis of lizards due to their biomechanical relationships with the underlying bones and the muscular anatomy (Bruner and Costantini, 2007). The locations of the landmarks used are

Table 1

Average values of the environmental variables analyzed for each population for the incubation period prior to egg collection. NDEA: Number of days exposed to ambient conditions; PP: Precipitation; RH: Relative ambient humidity; Mst10: Median soil temperature at 10 cm depth; MaxAT: Maximum air temperature; MinAT: Minimum air temperature; MeanAT: Mean air temperature; MVP: Median vapor pressure.

Sampling site	NDEA	PP (ml)	RH (%)	Mst10 (°C)	MaxAT (°C)	MinAT (°C)	MeanAT (°C)	MVP (hPa)
Alejandra	10	3.23	69.1	22.51	30.33	16.6	22.98	18.26
San Cristóbal	5	0.96	76.6	24.02	29.76	14.3	21.38	18.2
Costa del Salado	21	0.64	73.74	25.93	32.69	17.72	24.67	21.99
Romang	5	13.08	84.2	22.7	28.56	16.16	21.9	21.34
Reconquista	33	4.98	75.82	22.66	29.76	16.27	22.8	20.15
Sa Pereira	28	2.24	59.04	24.25	31.9	15.95	23.49	15.85

shown in Fig. 2(A–B); for descriptions see supplementary: Table A: A–B.

2.4. Environmental variables chosen

The environmental variables measured during the incubation period prior to collection were: precipitation (PP), relative ambient humidity (RH), median vapor pressure (MVP), maximum air temperature (MaxAT), minimum air temperature (MinAT), mean air temperature (MeanAT), and median soil temperature at 10 cm depth (Mst10). All environmental variable values were obtained from the “Sistema de Información y Gestión Agrometeorológico” of the “Instituto Nacional de Tecnología Agropecuaria” (SIGA-INTA: www.siga2.inta.gov.ar). Based upon the number of days that nests were exposed to the original environment (calculated as (65 days – (known number of days incubated)), we calculated the means from the values of the environmental variables (Table 1). It is worth mentioning that initially we also included the days of exposure to the environment like covariate, although then we decide to remove it from the analysis because there are much variation of the absolute valor in the data, therefore we consider that the number of exposure to the environment does not work as a good covariate because it forces too much the results, although it is not an irrelevant fact since it give accounts of the great influence exerted by the environment on the clutch prior to its collection.

2.5. Data analysis

For all analyses we used the MorphoJ program (Klingenberg, 2011). First we selected a subset of seven individuals upon which we performed a generalized Procrustes fit of all replicate landmark configurations. We then subjected these data to a Procrustes ANOVA (Goodall, 1991; Klingenberg, 2011) to assess the relative magnitudes of measurement error from repeated measurements (to know more details of the procedure for obtaining the error see supplementary Table B). The ANOVA indicated that the individual variation was greater than both the error among replicate photos and the error of placement of Lm (see results in supplementary Table C). This justified further analysis for the total set of the sixty individuals using the mean of the three photographs, that is to say that an average position for each Lm is calculated from its position in each of the three photographs taken of each individual in order to reduce placement error of landmarks.

The coordinates of Lm in all specimens were superimposed on a common coordinate system using Generalized Procrustes Analysis (GPA), to remove translation, rotation, and scale effects (Rohlf and Slice, 1999). We performed a multivariate regression analysis of Procrustes coordinates (Drake and Klingenberg, 2008) on landmark configuration centroid size to detect static allometry in populations. We performed a single multivariate regression of the dorsal view landmark configuration using the data from all six populations pooled, and a single multivariate regression of the right side landmark configuration using the data from all six populations pooled. In morphometrics, regression is most frequently used to correct for the effects of size on shape (allometry), the residuals from that regression being shape values from which the variance due to allometry has been removed. From

these regressions we derived the covariance matrices of the regression residuals, which were used for all subsequent analyses. We first explored shape variation using a Principal Component Analysis (PCA) for each of the configurations and environments, that is to say that we performed four PCAs (dorsal view landmark configuration: undisturbed and disturbed environment; right side landmark configuration: undisturbed and disturbed environment). Subsequently, we performed discriminant analyses using the dorsal view landmark configurations and the right side landmark configurations from all six populations, pooled into disturbed versus undisturbed environments, to determine shape differences between populations from undisturbed environment versus disturbed environment. The discriminant function also yields classification/misclassification tables, with cross-validation to test the acuity of the discrimination.

We evaluated the presence of fluctuating asymmetry for the dorsal view landmark configuration for both types of environments. We first employed a Procrustes ANOVA, which is a method proposed by Klingenberg (2011) that among other functions can evaluate the asymmetric variation between the right and left sides of a structure considered symmetrical (in our case the cephalic region in dorsal view). Then we carried out regression and discriminant function analysis on the asymmetric component derived from the Procrustes procedure. Fluctuating asymmetry is used as an indicator of instability in development caused by disturbances in the environment. A clear example of such disturbances would be habitat modification by anthropogenic activities, which would generate stress in animals inhabiting such environments (Laia et al., 2015).

We used Two-block Partial Least Squares method (PLS) to study the relationships between dorsal view landmark configuration and right side landmark configuration, and between each of the two landmark configurations and environmental variables for all six populations pooled. PLS is a method used for exploring patterns of covariation and correlation between two (and potentially more) blocks of variables (Zelditch et al., 2004). Also, this method provided the *RV coefficient* which measures overall strength of association between blocks, and its associated p-value. To avoid spatial autocorrelation, we performed a Trend Surface Analysis (TSA) to examine the relationship of cephalic shape to geographical coordinates (latitude and longitude). TSA is a non-linear model that can take into account non-linearities in the relationship between independent and dependent variables, in order to avoid spatial autocorrelation (Legendre and Legendre, 1998). Finally, we performed the Two-block PLS on shape variables and we used as covariate the results for the third degree polynomial of latitude and longitude obtained by the TSA.

To examine differences in centroid size of the cephalic landmark configurations, we performed ANOVAs with the centroid sizes (CS), obtained through MorphoJ (Klingenberg, 2011) for all six populations for each of the configurations in two separate ANOVAs, that is to say that we performed one ANOVA for CS in dorsal configuration and another ANOVA for CS in right side configuration.

2.6. Heritability

We calculated the heritability values of head for shape (shape Procrustes distances) and size. We calculated the heritability values for the dorsal view landmark configuration and for the right side landmark configuration for each of the populations. To calculate heritability we used the method of Monteiro et al. (2002), who used a combination of the model of multiple group analyses of variance from Goodall (1991) and the intraclass correlation coefficient (Sokal and Rohlf, 1995) to obtain the different components that contribute to the final calculation of heritability (Eqs. No. 3, 4, and 5 in Monteiro et al., 2002). The degree of relationship (r) among siblings was taken as $r = 0.50$ for all nests, as it was assumed that individuals were full siblings (see Amavet et al., 2009, 2012). Heritability for each univariate character was calculated as $h = t/r$ (Falconer and Mackay, 1996).

2.7. Legal Permits and Ethical Considerations

Collection and handling of animals was in accordance with legal permissions secured as part of the terms of PI. All animals were treated following the Ethical Reference Framework for Biomedics Researches: ethical principles for research with laboratory, farm and wild animals (National Scientific and Technical Research Council, 2005), minimizing stress and suffering by suitable management methods. After data collection lizards were released into the wild at their points of collection.

3. RESULTS

3.1. Allometry and Principal Components Analysis

The regressions were carried out for all six populations in dorsal view, for all six populations in lateral view, for each type of environment and configuration separately and for each sampling site; because most of the analyzes were statistically significant (see Table 2) we decided to perform the rest of the analyzes on the residuals of the regression obtained from the analysis of the six populations together for the dorsal view Lm configuration on one hand and right side Lm configuration on the other.

The first two principal components (PCs) explained 68.83 % of the total size-corrected shape variance for the undisturbed environment, and 51.38 % of the total size-corrected shape variance for the disturbed environment, in the dorsal view landmark configuration. In the lateral view landmark configuration, the first four PCs explained 49.15 % and 43.71 % of the total size-corrected shape variance, respectively, for each type of environment.

3.2. Linear Discriminant Function

The discriminant analysis indicated a statistically significant difference in the dorsal view landmark configuration between the

Table 2
Results of the regression analyzes. Allometry was present in seven of the nine analysis performed.

	Dorsal view Lm configuration		Right side Lm configuration	
	<i>p</i> -value	R-Squared	<i>p</i> -value	R-Squared
Reconquista	0.1645	0.1677	0.9356	0.0580
Romang	0.0062	0.3426	0.6784	0.0868
Sa Pereira	0.0144	0.3537	0.3396	0.1244
Alejandra	0.0447	0.2795	0.3091	0.1313
Costa del Salado	0.6461	0.0835	0.4208	0.1146
San Cristóbal	0.0276	0.2670	0.0586	0.2189
Undisturbed	0.0001	0.2056	0.0044	0.1039
Disturbed	0.0001	0.2073	0.0978	0.0547
Six sites together	0.0001	0.1731	0.0001	0.1066

individuals from undisturbed environments and those from disturbed environments. Both tests were statistically significant: Procrustes distances, $p = 0.0010$, and Mahalanobis distance, $p = 0.0001$ (permutation tests = 1000). For the right side landmark configuration, the difference between the two types of environments was not statistically significant: Procrustes distances, $p = 0.429$, and Mahalanobis distance, $p = 0.938$ (permutation tests = 1000). The mean shapes were obtained from mean values of negative scale factor (values to the left of zero: Shape variation A) and from mean values of positive scale factor (values to the right of zero: shape variation B) in the X axis of the bar graph obtained by the discriminant function (see Fig. 3). In the histogram it can be observed that for the dorsal view landmark configuration there is a clear division between the shapes from the two types of environment, whereas for the right side landmark configuration the distributions of the individuals overlap considerably. The characteristics of these configurations for undisturbed environments are summarized in Table 3.

For the dorsal view landmark configuration, individuals were accurately classified by type of environment: 22 individuals from the 30 belonging to group 1 (disturbed) were correctly grouped into group 1, and for group 2 (undisturbed), 23 individuals were grouped within this group and only 7 within group 1 (Table 4). For the right side landmark configuration, shape variation was not accurately classified by the type of environment: 17 individuals belonging to group 1 were grouped within group 2 and 21 of group 2 were located in group 1. This is consistent with the non-significance of the tests for classification of the right side landmark configuration.

3.3. Asymmetry

The Procrustes ANOVA indicated a slight asymmetry in individuals from both types of environments (Undisturbed environment: p -value = 0.0001; Disturbed environment: p -value = 0.0001). We performed a multivariate regression analysis on the asymmetric component of the dorsal view landmark configuration for all six populations on centroid size; this analysis indicated the absence of allometry (R-squared = 0.014; p -value = 0.4502). Hence for the regression we used the asymmetric component in a discriminant analysis, instead of the regression residuals. The discriminant function showed that there are no statistically significant differences in the asymmetric component between the two types of environments: Procrustes distances, $p = 0.579$, and Mahalanobis distance, $p = 0.367$ (permutation tests = 1000).

3.4. Covariation Between Dorsal view Landmark Configuration and Right side Landmark Configuration

The Two-block PLS analyzing the covariation between the dorsal view landmark configuration and the right side landmark configuration indicated that there is no significant statistical covariation between the two landmark configuration within individuals when data from all localities are pooled (RV coefficient = 0.1305; p -value = 0.1402), although there is a statistically significant correlation in PLS1 (Correlation PLS1: 0.68, $p = 0.0202$). It was observed that individuals belonging to the disturbed environments have less morphological variability and are more similar to one other (smallest ellipse) than individuals sampled from undisturbed environments (largest ellipse). The ellipses represents 95 % confidence limits (Fig. 4). The RV coefficient indicates the overall strength of association between blocks (Fig. 4: Block1 PLS1 vs Block 2 PLS 1).

For the Two-block PLS performed for each type of environment separately, specimens from the undisturbed environment exhibited significant covariation between the two configurations (RV coefficient = 0.2713; $p = 0.0163$; % total covariation PLS1: 68.66, $p = 0.0187$; Correlation PLS1: 0.68, $p = 0.0388$), while for the sample from the disturbed environment this covariation was not statistically

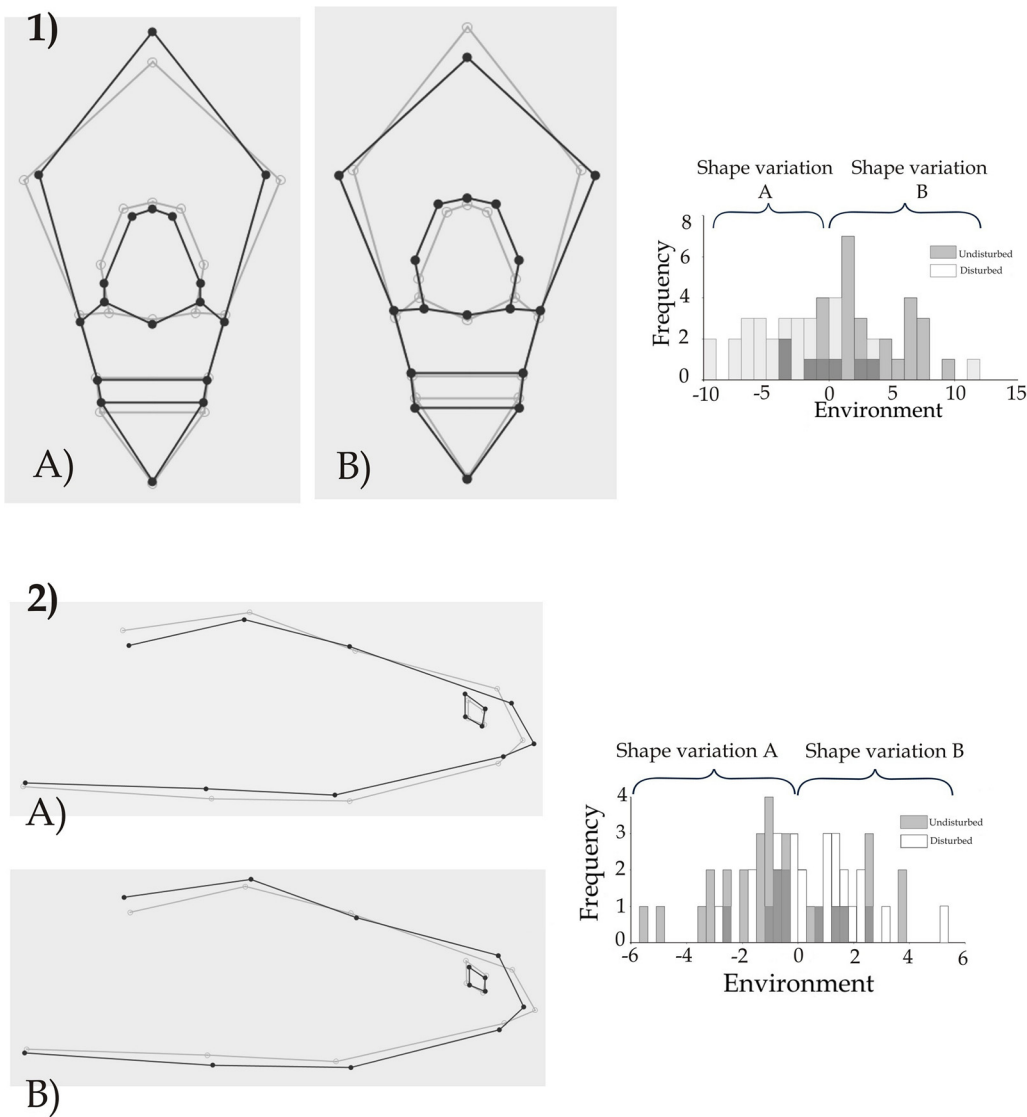


Fig. 3. Mean shapes for the two types of environments. 1- Dorsal view landmark configuration- A: Mean shape A (Negative scale factor: values to the left of the zero in the bar graph)- B: Mean shape B (Positive scale factor: Values to the right of the zero in the bar graph). 2- Right side landmark configuration- A: Mean shape A (Negative scale factor): B: Mean shape B (Positive scale factor). The black line corresponds to the undisturbed environment, and the gray line corresponds to the disturbed environment, for the Lm configurations. Histograms on the right show how the individuals are distributed, taking into account the environment from which they come. The gray bars correspond to the individuals belonging to the undisturbed environments, the white bars correspond to the individuals belonging to the disturbed environments, dark gray bars correspond to the overlap of individuals of both types of environments.

Table 3
Description of the mean shapes for dorsal view landmark configuration and right side landmark configuration determined by discriminant function.

Configuration	Mean Shape A (Negative scale factor)	Mean shape B (Positive scale factor)
Dorsal view landmark configuration	<ul style="list-style-type: none"> - General shape narrow - Set of rostral and nasal scales more elongated and narrow - Small frontonasal scale - Frontal scale of “pyramidal” shape - Posterior region of the cranium less developed (lower development of frontoparietal and interparietal scales) 	<ul style="list-style-type: none"> - General shape widened - Set of rostral and nasal scales shorter and wider - Large frontonasal scale - Frontal scale of “rectangular” shape - Posterior region of the cranium more developed (greater development of the frontoparietal and interparietal scales)
Right side landmark configuration	<ul style="list-style-type: none"> - Considerable development in the superciliar scale region resulting in a concave shape pronounced of the cranium - Larger nostrils - Less developed rostral scale - Line of supralabial scales with flat shape, which implies a poor development of the mandibular region 	<ul style="list-style-type: none"> - Less development in the superciliar scale region resulting in a more flattened cranium - Smaller nostrils - More developed rostral scale - Line of supralabial scales with convex shape, which implies a greater development of the mandibular region

significant (RV coefficient = 0.1913; $p = 0.6424$) (see supplementary Table D).

3.5. Covariation of Shape-Geographical variables (TSA)

We obtained statistically significant covariations between the dorsal view landmark configuration and geographical coordinates (third

degree polynomial of latitude and longitude obtained by the TSA) for both types of environments for the dorsal view (RV coefficient = 0.1104; p -value = 0.0135) and for the right side landmark configurations (RV coefficient = 0.1269; p -value = 0.0069). In both landmark configurations, the covariation explained by PLS 1 was statistically significant, explaining 80-99 % of the covariance, except for the PLS1 of the dorsal view landmark configuration of the sample from

Table 4

Cross validation tables for dorsal view landmark configuration and for right side landmark configuration. Group 1 corresponds to disturbed environments; Group 2: corresponds to undisturbed environments.

True	Allocated to		
	Group 1	Group 2	Total
Dorsal View Landmark configuration			
Group 1	22	8	30
Group 2	7	23	30
Right Side Landmark Configuration			
Group 1	13	17	30
Group 2	21	9	30

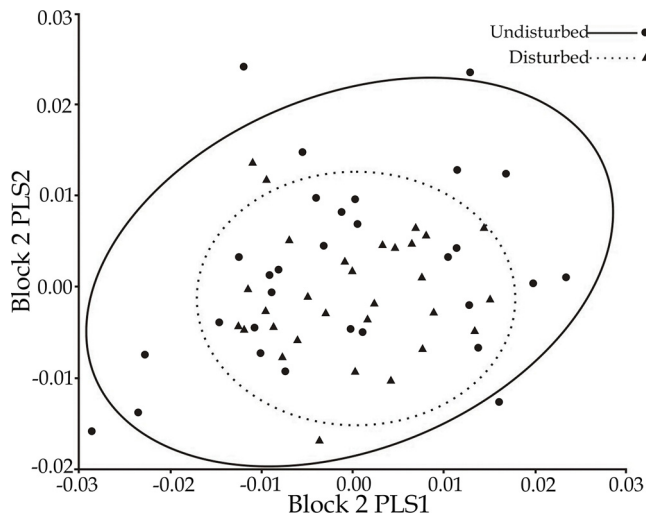


Fig. 4. Graph resulting from the Two-block PLS evaluating the covariation between the dorsal view landmark configuration and the right side landmark configuration for the set of the six sampling sites. The smaller ellipse of the disturbed environment indicates that the morphology of the individuals in this environment is more homogeneous than in the undisturbed environment. Circles represent the individuals belonging to the undisturbed environment and triangles represent the individuals belonging to disturbed environments. The ellipses represent the 95 % confidence limits.

disturbed environment: p -value = 0.0876 (see supplementary Table E). The correlation also was statistically significant in PLS 1 for all the landmark configurations and environments; furthermore, the most influential geographical variable is latitude. The Sa Pereira location, which is located furthest south (Fig. 1), is the most widely separated from the remainder of the locations for both types of landmark configurations, but retains the division between sampling sites from disturbed environments vs. undisturbed environments (Fig. 5A–B). Due to the great influence exerted by the Sa Pereira site as a consequence of its geographical location, we decided to exclude the Sa Pereira in a second PLS analysis. The results indicate that significant covariation remains in the dorsal view landmark configuration (RV coefficient = 0.1168; p -value = 0.0216), but that the covariation is not significant for the right side landmark configuration (RV coefficient = 0.0543; p -value = 0.4163). PLS 1 explains more than 95 % of the variance in both landmark configurations. However, in the graphs it can be observed that latitude remains the variable with the greatest influence, and in this analysis it is the Reconquista site, the most northerly of the sites sampled, that is the most widely separated from the remainder of the sites (Fig. 5C–D). Again, it is important to note that despite the great influence of the geographic variables, the division between the sites is maintained according to whether they come from disturbed or undisturbed environments.

3.6. Covariation shape-environmental variables

With respect to the environmental variables, covariation with shape

was statistically significant for the dorsal view landmark configuration and the right side landmark configuration when we analyzed each environment separately (Table 5).

For both landmark configurations and the two types of environments, covariation was statistically significant in PLS 1, which explains more than 90 % of the total covariation, with p -values ranging from 0.0001 to 0.0497 (see supplementary Table E).

In the analysis for both types of environments together (i.e. the six sampling sites pooled), covariation was significant for the dorsal view landmark configuration (RV coefficient = 0.2187; p = 0.0001) and the right side landmark configuration (RV coefficient = 0.2508; p = 0.0001). What varied in this analysis was the percentage of variance explained by the PLS; for the dorsal view landmark configuration, PLS1 explains almost 67 % and PLS2 22 % of the shape variation, while for the right side landmark configuration PLS1 explains almost 56 % and PLS2 33 % of the total variation; that is, PLS1 and PLS2 together explain higher percentages of the total variation. Fig. 6A–B shows that, for both landmark configurations, for the specimens from the undisturbed environment, the variables that exert the most influence are: MinAT, MeanAT, MVP (for dorsal view landmark configuration) and Mst10 (for right side landmark configuration). Whereas, for the specimens from the disturbed environment the most influential variables are: PP, RH, Mst10 (for dorsal view landmark configuration) and MaxAT (for right side landmark configuration) (Fig. 6A–B).

3.7. ANOVA for centroid size

The ANOVA comparing the cephalic CS of individuals from undisturbed environments versus those from disturbed environments indicated statistically significant differences between the means (dorsal view landmark configuration: df = 1, F = 6.529, p = 0.013; right side landmark configuration: df = 1, F = 18.149, p < 0.0001) (Fig. 7A–B), individuals from disturbed environments having larger centroid sizes than those from undisturbed environments. In the regression plot the differences between the CS for both types of environments are clear (Fig. 8A–B). It can be seen that for both landmark configurations, individuals from disturbed environments are located to the right side of the graph; they have larger centroid sizes than those from the undisturbed environment. In addition it can be seen that for the right side there is less centroid size variability in individuals from the disturbed environment (smaller 95 % CI ellipse). It should be noted that in both graphs (box plot and regression) it can be seen that the size difference is more accentuated in the right side landmark configuration, which is consistent with the p -values obtained by the ANOVA.

3.8. Heritability of shape and size

The heritability estimates for the shape of the head, as defined by landmark configurations, indicated high values for both dorsal view landmark configuration and right side landmark configuration. With regard to centroid size, the values were even higher and similar to each other, since centroid size is the same in both dorsal and lateral view landmark configurations (Table 6).

4. DISCUSSION

4.1. Summary of results obtained

Geometric morphometric tools, in combination with quantitative genetics, allowed us to obtain information regarding cephalic shape and size variation of *Salvator merianae* in relation to the environmental characteristics to which the populations from which the individuals were sampled are subjected in undisturbed and in disturbed environments. We found significant differences in the morphology of the head between lizards from the two types of environments. The most striking result of the present study was that individuals from undisturbed

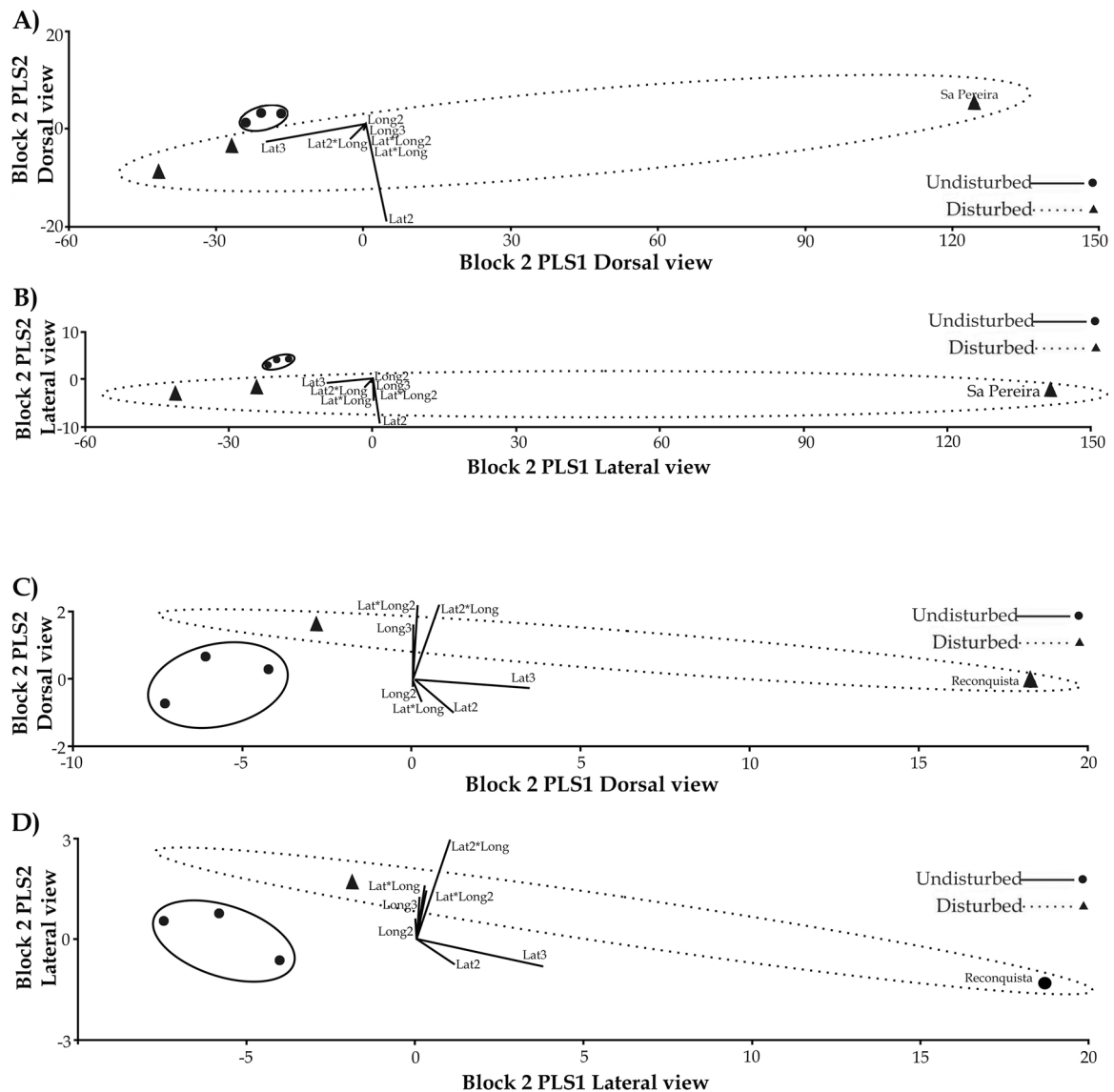


Fig. 5. Graph obtained from the Two-block PLS evaluating the covariation between head shape and geographical variables. A) Graph for dorsal view landmark configuration for the six sampling sites. B) Graph for right side landmark configuration for the six sampling sites. C) Graph for dorsal view landmark configuration for five sampling sites (omitting Sa Pereira Site). D) Graph for right side landmark configuration for five sampling sites (omitting Sa Pereira Site). The X axis represents changes in skull shape in the PLS 1. The Y axis represents changes in skull shape in the PLS 2. The ellipses in the graph represents the distribution of individuals of each population corresponding to shape variation. Circles represent the sampling sites of undisturbed environments and the triangles represent the sampling sites of disturbed environments. The ellipses represent the 95 % confidence limits.

Table 5

Values of the RV coefficient for each of the configurations and environments with their corresponding *p*-values.

	RV coefficient for dorsal view Lm configuration	<i>p</i> -value	RV coefficient for right side Lm configuration	<i>p</i> -value
Undisturbed	0.4526	0.0001	0.3244	0.0005
Disturbed	0.1957	0.0217	0.3430	0.0001

environments presented greater phenotypic variability, whereas the shape of the animals from disturbed environments was more homogeneous and exhibited a larger mean centroid size. We hypothesize that these differences are related mainly to intraspecific competition occurring as a consequence of the type and amount of food available in both environments studied. The high values of heritability obtained for shape and size of the head would indicate that the species has a great ability to respond to selection pressures exerted by the environment.

4.2. Hypothesis of trophic niche in undisturbed environments

Metzger and Herrel (2005) demonstrated that there is a strong relationship between diet and cranial morphology in lepidosaurs, and that the trophic apparatus included in the skull (jaws and areas of attachment for associated musculature) shows strong correlation with the trophic niche. The availability of food items depends on the seasonal variation of flora and fauna (Metzger and Herrel, 2005), and lizards of the genus *Salvator* are active and opportunistic foragers (Rocha et al., 2009; Winck et al., 2011). Possibly, in the undisturbed environments, the variety of food types available and the energy cost involved in obtaining them could lead to the shape variation found in the sampling sites, resulting from the specialization of individuals upon different trophic items, consequent intraspecific partitioning of the trophic niche ensuring the availability of food for the populations in such environments.

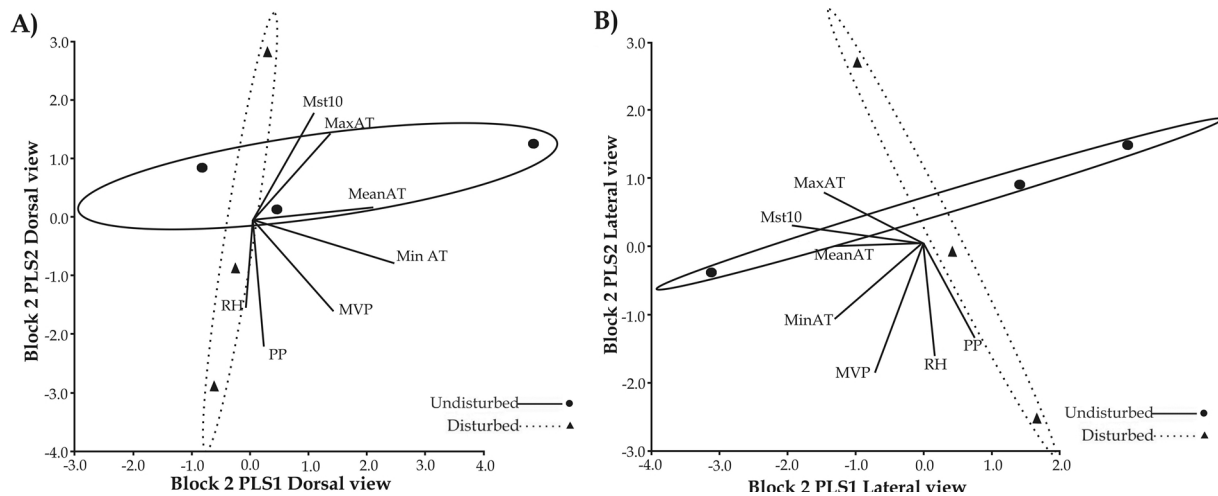


Fig. 6. Plots obtained from the Two-block PLS evaluating the covariation between cephalic shape and environmental variables. A) Dorsal view landmark configuration – both types of environments. B) Right side landmark configuration – both types of environments. The X axis represents the variation of the environmental data in PLS1. The Y axis represents the variation of the environmental data in PLS 2. The ellipses in the graph represent the distribution of the types of environments along Block 2 and the ratio of the distribution with environmental variables (black lines). PP: precipitation, RH: relative ambient humidity, MVP: medium vapor pressure, MaxAT: maximum air temperature, MinAT: minimum air temperature, MeanAT: mean air temperature, Mst10: medium soil temperature at 10 cm depth. The X axis represents changes in skull shape in PLS 1. The Y axis represents changes in skull shape in PLS 2. The ellipses in the graph represents the distribution of individuals of each population corresponding to shape variations. Circles represent the sampling sites of undisturbed environments and triangles represent the sampling sites of disturbed environments. The ellipses represents 95 % confidence limits.

4.3. Hypothesis of trophic niche in disturbed environments

Disturbed environments are much more homogeneous in terms of flora and fauna, since modern agriculture implies the simplification of the structure of the environment over large areas, replacing the diversity of nature with a small number of cultivated plants and domesticated animals, which may directly affect the abundance and diversity of endemic species (Altieri, 1999). The homogeneity and abundance of available food could lead to a reduction in the need for partitioning of the dietary niche among individuals, which would explain the decreased shape and size variation of the head in disturbed environments. This conclusion is supported by the findings of Barraco (2015), who evaluated the risk of invasion of *S. merianae* in southern Florida (United States of America). Gut content analysis indicated that most differences in the items between individuals in undisturbed and disturbed areas were due to the degree to which plants and gastropods were consumed; also; it was also found that the taxonomic range of dietary items taken by individuals from disturbed environments was much less varied than in the undisturbed environment (Barraco, 2015).

Winck et al. (2011) investigated aspects of the ecology of a *Salvator*

merianae population located in an ecological Station in the south of Brazil. There were no differences in dietary behavior among age groups in this population, which Winck et al. (2011) attributed to the area's low ecological complexity (homogeneity in food items and shelter type), due to the presence of rice plantations. This is consistent with our hypothesis from above.

4.4. Specialization by trophic niche

There is abundant evidence that ecological generalist species, which use a wide variety of resources, are in fact heterogeneous collections of relatively specialized individuals (Bolnick et al., 2007). In addition, competition for resources is known to be one of the main causes of physical dispersion of individuals in a population (McPeck and Holt, 1992; Winck et al., 2011). Furthermore, it has been shown that differentiation of trophic morphology function as a way of avoiding competition (Verwajen et al., 2002).

González et al. (2007) found two morphotypes differing in mandible width in a population of the lizard *Ameiva ameiva*; one of these morphotypes presents a narrower mandible, the other presents a wider jaw,

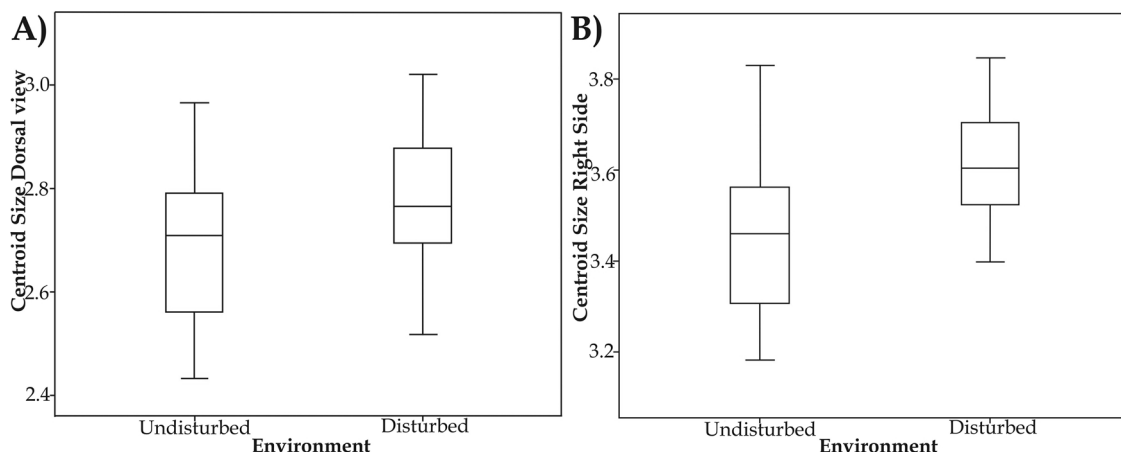


Fig. 7. Distribution of individuals related to their centroid size and the type of environment from which they come. A) Dorsal view landmark configuration. B) Right side landmark configuration. It can be seen for both landmark configurations that the sampling sites belonging to the disturbed environment have larger centroid sizes than those of undisturbed environment.

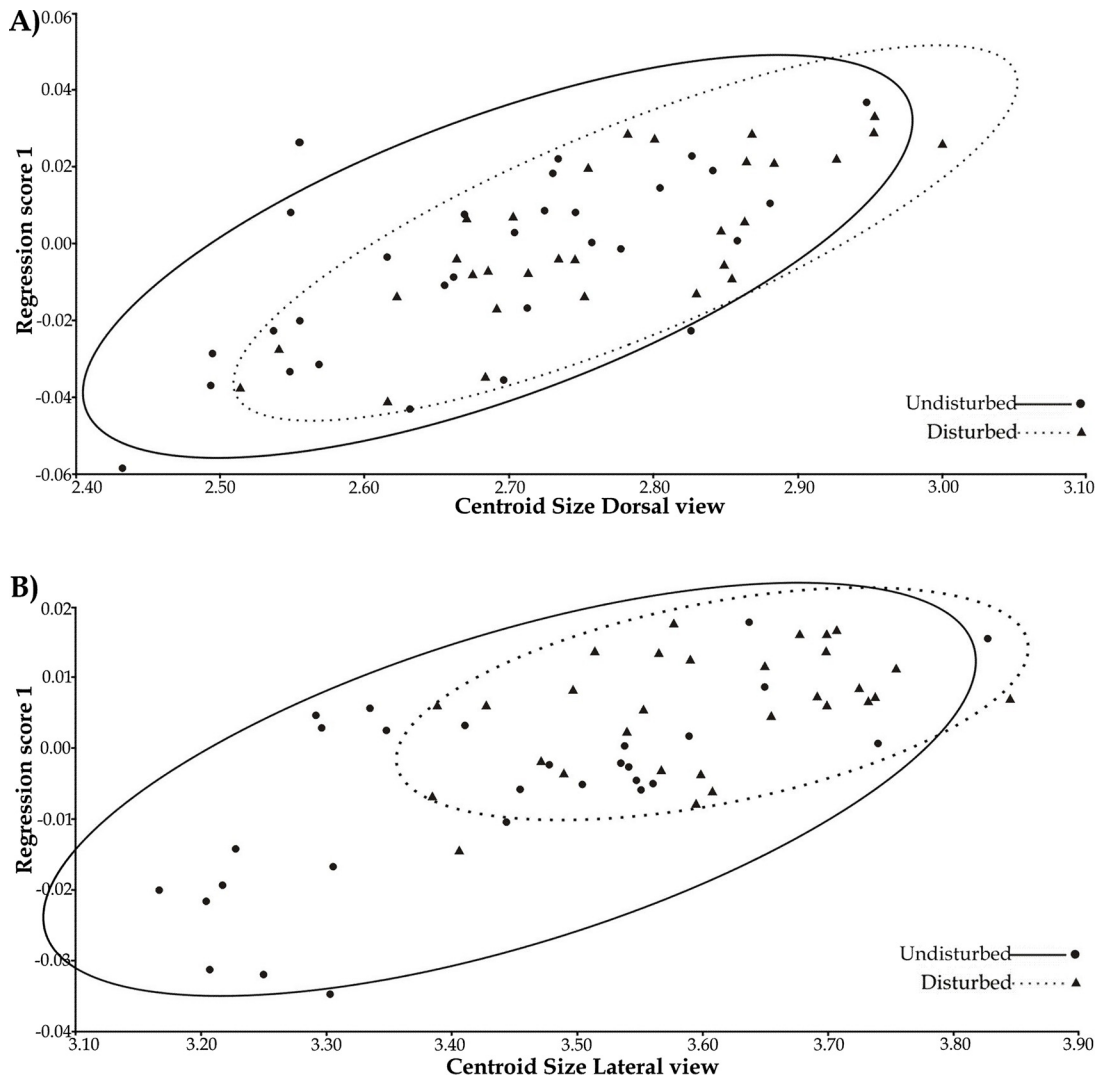


Fig. 8. Allometric relationship between size and shape. A) Dorsal view landmark configuration. B) Right side landmark configuration. “Regression score” represents the regression coefficients obtained from the regression model of the shape with respect to size. Circles represent the individuals belonging to the undisturbed environment and triangles represent the individuals belonging to disturbed environments. The ellipses represent the 95 % confidence limits.

which is more pronounced in males. The authors proposed that this is due to the greater body size of males, and to the greater foraging activity that males exhibit in the search for larger prey, which could lead to different biomechanics in the mandible, suggesting a possible sympatric divergence in the trophic morphology of individuals as a consequence of partitioning of the dietary niche by prey size. Huyghe et al. (2009) analyzed differences in bite force in the lizard *Podarcis melisellensis* and found three bite force levels corresponding with different coloration patterns in male individuals; they proposed that this could be due to ecological selection resulting in a dietary divergence among the

color morphs. The authors found that orange males bite harder than white and yellow ones, and white males bite harder than yellow ones, with orange males being able to handle and crush harder prey items. Indeed, orange males had a larger proportion of hard prey items in their stomachs than the other two morphs

Adams and Rohlf (2000) examined skull shape of individuals from sympatric populations corresponding to two species of salamanders of the genus *Plethodon*, using a combination of traditional and geometric morphometry techniques in order to determine if competition for resources could explain the current geographical distributions of the

Table 6
Heritability values of cephalic shape and centroid size for each of the sampling sites, for dorsal view landmark configuration and right side landmark configuration.

		Shape		Centroid size	
		Dorsal view	Right side	Dorsal view	Right side
Undisturbed	Alejandra	1.0764	1.0803	1.1098	1.1099
	Costa del Salado	1.0646	1.0446	1.1101	1.1101
	San Cristóbal	1.0906	1.0827	1.1099	1.1101
Disturbed	Reconquista	1.0809	1.0864	1.1103	1.1107
	Romang	1.0820	1.0953	1.1101	1.0953
	Sa Pereira	1.0629	1.0750	1.1103	1.1103

species. Adams and Rohlf (2000) found significant morphological differences among the sympatric populations, which was associated with a partitioning of the type of prey consumed according to prey size; furthermore, in the allopatric populations of the studied species they found no differences in the trophic morphology or in the dietary niche, indicating that the morphological differences found resulted from competition for food in the zones of sympatry.

Bolnick et al. (2007) reviewed five case studies: three-spine sticklebacks (*Gasterosteus aculeatus*), Eurasian perch (*Perca fluviatilis*), Anolis lizards, intertidal gastropods (*Nucella* spp.), and a community of neotropical frogs (*Adenomera* sp., *Eleutherodactylus* sp., *Leptodactylus fuscus*, *Proceratophrys* sp.), and found that dietary variation within populations increases with the niche amplitude of the population as a whole in all taxa examined, which would act to decrease intraspecific competition. Bolnick et al. (2007) concluded that the more generalist populations tend to be, intraspecifically, ecologically more heterogeneous.

Therefore, it could be said that the differences in the type and availability of trophic items, depending on the type of environment, are likely to be the cause of significant differences found among individuals from undisturbed environments, as opposed to those in disturbed environments, in our study. However, because there is probably high food availability in disturbed environments, it could be assumed that competition is reduced and that individuals would not be required to travel great distances to meet their energy needs, which in turn would favor the specialization and morphological differentiation of individuals living in disturbed environments compared to those in undisturbed environments.

It is important to mention that cephalic scales are used as useful indicators of head morphology, as they cover the entire cephalic volume and possibly homologous anatomical references. In reptiles can provide tools for the intra and interspecific study of morphological variations (Bruner et al., 2005; Bruner and Costantini, 2007). In addition, the close relationship between the dermal, muscular and skeletal systems makes the study of scales very useful in the investigation of growth and morphogenesis patterns (Bruner and Costantini, 2007).

Bruner and Costantini (2007) investigated the underlying causes of the morphological variation of the cephalic scales of two species of lacertids (*Podarcis muralis* and *P. sicula*) and they concluded that the head shape in both species is characterized by a common allometric pattern in which the bone growth in the frontoparietal suture and the development of the occipito-parietal muscular system shaping the dermal structures, probably representing the structural determinant more important of the morphology of cephalic scales. In addition, *P. muralis* shows a high degree of variation and some features associated with the reduction of the occipital area and the anterior elongation of the frontoparietal area may be species-specific. According to the authors two blocks can be recognized: a group of anterior Lm that converge towards the center of the frontal scale and a group of posterior Lm that involve the compression of the posterior surface; the border between both regions covers the frontoparietal suture that separates the frontal bone of the parietal bone and is considered a significant source of morphogenetic variation of the head of the lizards. In addition, the posterior area (interparietal and occipital region) is associated with the muscular system, characterized by the development of the parietal muscles, the masticatory functions and the nuchal muscles.

Bruner et al. (2005) analyzed how size and sex influence the head shape using cephalic scales patterns in the lizard *Lacerta bilineata*. They found that in males the scales are generally larger, with a relative shortening of the frontal area, frontoparietal and occipital elongation, and bulging of the parietal scales, which compresses and narrows the interparietal and occipital areas. The authors also noted that the suture between the frontal and parietal bones is under the frontoparietal scales and that the area behind this suture shows a marked elongation. They propose that the development of the parietal scales can be considered a consequence of the anteroposterior allometric growth, coupled with a hypertrophy related to the sex. Functionally, these processes are related

to the development of the adductor muscle, which may be related to minimizing the overlap of the trophic niche in females and males or may be necessary during male fighting for territory and during mating behavior.

In our work, although we did not specifically study the shape of the cephalic scales, the overall pattern of the head shape obtained in *S. merianae* is similar to that found in the work of Bruner et al. (2005) and Bruner and Costantini (2007), since in the average shapes of the dorsal configuration in the two types of environments can be observed the two blocks of Lm mentioned in the works cited above. These blocks could be assumed to be produced by the division of the frontal and parietal bones, which would allow the *S. merianae* head to vary from one shape with a short anterior region linked with a developed posterior region to another shape with a elongate anterior region associated with a less developed posterior region. This shape variations would indicate a specialization by trophic niche in the species. In support of this hypothesis may be cited also González et al. (2007), who propose that variations in the width of head for the lizard *Ameiva ameiva* would be the consequence of differences in the manipulation of food items according to the type of prey consumed. Furthermore, Metzger and Herrel (2005) investigated whether there is a correlation between the general cranial shape and the dietary niche in the lepidosaur lizards, for which they measured cranial and postcranial structures of individuals belonging to 104 genera and 246 species of lepidosaurs. The authors postulated that higher skulls and shorter snouts are related to the need for greater bite force because they have greater areas of insertion for the adductor muscles; on the other hand, longer snouts permit the more efficient capture and processing of evasive prey.

Based on all of the above, we can posit that in undisturbed environments there would be a large variety of food items that would promote individual variation in the shape of the head, decreasing intraspecific competition, which would favor the persistence of the populations. In disturbed environments, less morphological variation of the cephalic region is observed (smallest ellipse in Fig. 4), which leads us to infer two possible causes: a) that there is a smaller diversity of food items due to the homogeneity of the environment, which would promote decreased disparity in the shape of the cephalic region, and b) that the available prey is low in disparity, but present in great quantity, reducing the necessity for competition for food, and reducing the necessity for partitioning of the trophic niche.

4.5. Hypothesis of trophic niche and size

Another of the phenotypic characteristics analyzed in the present study which can be explained through the hypothesis of trophic niche partitioning was the centroid size of the head, which was used as a proxy of the body size of individuals. We decided to use CS as a proxy for body size because we rely on the assumption that a particular feature in study can be used as a proxy for another trait of which no information is available (Gordon, 2004; Cardini et al., 2007; Coleman, 2008). Individuals of *Salvator merianae* from disturbed environments exhibited a larger mean centroid size than those from undisturbed environments, so it could be assumed that they also have a larger body size (Fig. 7A–B).

Barraco (2015) concluded that the habitat type influences the composition of the diet and the general body condition of *Salvator merianae*. The tegus with better corporal condition were collected from disturbed/agricultural areas, which they attributed to the availability of snails in agricultural fields, resulting in lizards consuming more calories with less effort (Barraco, 2015). Al-Hashem and Brain (2009), in a study of the effects of oil pollution on body size and weight of the lizard *Acanthodactylus scutellatus* in an oil field in Kuwait, found that adult males were generally larger at contaminated sites than at control sites. One possible explanation proposed by Al-Hashem and Brain (2009) was that the food resources are affected by oil contamination, and only prey with high levels of fat are available. This could be because the residues

of oil pollution are lipophilic and would accumulate in the adipose tissue and in the liver of the prey; therefore, this accumulation would function as a mechanism of defense, reducing the risk of immediate intoxication on the part of the predator; lizards would accumulate more adipose tissue when restricted to prey contaminated in this way (Al-Hashem and Brain 2009). It is possible that the prey available for *S. merianae* in the disturbed environments are those that tolerate the conditions under which crops are raised, such as the presence of agrochemicals, and that these in turn contain a high caloric content, which would contribute to the increase of size of *S. merianae* that consume them.

Therefore, as mentioned above, in disturbed environments there would be some prey types that would favour disturbed environmental conditions; in addition, it is likely that these prey would be found in great quantity and would have a high caloric value, all of which would lead to the difference of sizes found between the populations of *S. merianae* found in undisturbed environments compared to those occupying disturbed environments.

It is important to mention that the reproductive success for lizards of the genus *Salvator* depends on the availability of food in two seasons. In the first season the chance of reproduction of an adult female depends upon its nutritional status at the time of initiating the previous hibernation, if before hibernating there is a failure to accumulate energetic reserves in fatty bodies (that constitute the vitello of the oocytes) the female will not reproduce. In the second season it is extremely important that the hatchlings have food that allows them to accumulate reserves to endure their first hibernation (Quintana, 2000). Thus it is possible that in the disturbed environments the high availability of food will allow the *S. merianae* females to accumulate a large amount of energy reserve that will be transmitted to the progeny, thus giving rise to large individuals; furthermore, at hatching, these hatchlings would have access to a large amount of foods rich in calories that would allow them not only survive the first hibernation but also reach larger sizes than individuals in living in natural environments.

4.6. Another possible adaptive advantage of size difference

The difference in mean centroid size between the two types of environments can be attributed to other adaptive advantages, provided that CS be considered as a proxy for body size: possibly there is an environmental pressure upon individuals living in disturbed environments to develop a larger body size which could be associated with greater resistance to the agrochemicals used on crops. It is known that small animals have a relatively larger total capillary area and thus will absorb proportionately more environmental contaminants (Ellgehausen et al., 1980). A small body size is thus associated with increased exposure through the absorption of contaminated food (Weir et al., 2010), which would lead to a positive selection towards larger body size in disturbed environments.

4.7. Analysis of asymmetry in dorsal view

Another possible explanation for the difference in dorsal view shape associated with the type of environment was thought to originate in the asymmetry of right side versus left side. Fluctuating asymmetry (FA), namely, small random deviations from perfect bilateral symmetry, is used to assess environmental influences upon development, since it is assumed that it does not have a heritable component but is a developmental response to environmental pressures, being found in organisms exposed to some type of environmental stress during the development (Longson et al., 2007). There are several studies which examine the association of environmental disturbances with FA of individuals, and therefore their fitness. Some have found a direct association between environmental stress and FA (for examples see Cadée, 2000; Herczeg et al., 2005; Tull and Brussard, 2007) while in other works (such as Longson et al., 2007; Bellaagh et al., 2010; Velo-Antón et al.,

2011) no direct relationship was found. In the present study, the analysis of asymmetry in the sampled animals indicated that there is a slight asymmetry in both the undisturbed and disturbed environments and that there is no difference between the types of environment with regard to asymmetry. However, it may be that the effects of disturbances in the habitat are only observed in individuals that have developed and grown under such circumstances, whereas the individuals studied here were still incubating at time of sampling. Future work would be necessary to follow up on ontogenetic development to confirm whether or not there are significant differences in asymmetry between older individuals from undisturbed environments and those from disturbed environments.

4.8. The most influential environmental variables on the shape depend on the type of environment

With regard to the covariation of shape with environmental variables, it is known that the phenotypic variability observed in natural lineages results from evolutionary processes that reflect the interactions between selective pressures exerted by the environment where these populations evolve (Kaliontzopoulou et al., 2010). The biophysical environment affects the evolution of most ecological characteristics of ectothermic animals, such as preferred body temperature, activity levels, growth rate, reproduction modes and survival (Medina et al., 2009).

An important characteristic of these lizards is that they have non-cleidoic eggs; in this type of eggs, in addition to the gas exchange, liquid water flow and water vapor flow from the environment to the egg and in the reverse direction. This establishes a dynamic balance between egg and environment that determines the conditions in which the embryo develops (Quintana, 2000). Many experimental studies on a variety of sauropsids have shown that important characteristics of the offspring depend on the physical conditions that the embryo experiences prior to hatching (Imhoff et al., 2015). Thus, environmental conditions during embryogenesis can induce phenotypic variation (Braña and Ji, 2000). Furthermore, the environmental conditions experienced during early ontogeny affect embryos (Arnqvist and Johansson, 1998). For this reason, even though the incubation period was not completed under natural conditions, the days that the eggs were exposed to the characteristics of each site at the beginning of ontogenetic development (an average of 12 days for the undisturbed environment and 22 days for disturbed) were sufficient to exert their influence on the shape of the trait studied. In a work that we previously did in broad snouted caiman (*Caiman latirostris*), we obtained similar results in terms of covariation with environmental variables. In this work we studied through geometric morphometry the shape and size of the cephalic region of neonates of *C. latirostris*, which were also subjected to ranching methodology and, although some of the sampled nests were exposed a few days to the environmental conditions of the nesting site chosen by the female, a statistically significant covariation was found between the environmental variables of the place and the head shape, RH, PP and Mst10 being the variables that most influence (see Imhoff et al., 2015).

With regard to *Salvator merianae*, our results indicated that there are differences in the type of variables exerting the most influence, depending upon the type of environment. In the undisturbed environments the most influential variables are mainly related to the temperature (Ej: MinAT, MeanAT, MaxAT). These results agree with those obtained by Lanfri et al. (2013), who studied the ecological niche to elucidate the spatial strategies of the lizards *S. merianae* and *S. rufescens* (spatial strategies refer to niche differentiation and divergence of distribution patterns at a regional scale). Lanfri et al. (2013) conclude that temperature, precipitation, plant biomass and altitude define the ecological niche of both species, with environmental temperature being the most important factors limiting the probability of the presence of these species.

With regard to the disturbed environments, the variables that exert the most influence are mainly related to the ambient humidity (Ej: PP, RH), and, to a lesser extent, temperature (Ej: Mst10, MaxAT). This may be due to the characteristics of cultivated environments, in which the crop type strongly affects the humidity regime. Winck et al. (2011) stated that the characteristics of their sampling sites were strongly determined by the type of anthropic exploitation of the area, which was located in a rice plantation with a high humidity that favors plant species adapted to this type of environment (Apiaceae: *Eringium* sp.), which further resulted in good shelters for lizards (especially hatchlings) as well as for their prey.

The different biophysical conditions in the two types of environments thus also have a great influence upon the shape of the cephalic region. In the disturbed environments conditions are modified in accordance with the requirements of the crops. Thus, despite our finding a significant covariation between shape and geographical variables, the type of environment exerted a greater influence in the shape of the cephalic region. For example, the sampling site Sa Pereira is the furthest south of our sampling locations, but the discriminant function groups it with the populations from disturbed environment, despite its geographic location.

4.9. Heritability

The variation in head shape that we document must be heritable if it is to be of evolutionary significance. Because specialist individuals compete with a subset of the population to which they belong, intraspecific competition may lead to disruptive selection that can lead to evolutionary changes such as polymorphisms, sexual dimorphism, or speciation if the variation has a heritable base (Bolnick et al., 2007).

There has recently been a series of studies that combine geometric morphometrics and quantitative genetics in order to evaluate which fraction of the phenotypic variation is determined genetically (Myers et al., 2006; Adams, 2011; Imhoff et al., 2015; Sacchi et al., 2016). The information obtained is of utmost importance, since selection promotes evolutionary adaptation only if the trait under selection has sufficient genetic variation (i.e. is heritable) upon which selection can work (Lande, 1979).

Therefore, the high heritability values obtained here for the shape and size of the head of *Salvator merianae* indicate that in this species heritable variation is present, which would allow the evolutionary changes necessary to reduce intraspecific competition. In addition, this heritable variation would allow populations of *S. merianae* to respond phenotypically to the variety of environments with differing characteristics that they inhabit, such as, in this case, the undisturbed and disturbed environments.

Our results agree with those obtained by Sacchi et al. (2016), who studied heritability patterns of shape and size of the head in the model lizard *P. muralis*, finding high values of heritability. Sacchi et al. (2016) concluded that the variability in the shape of lizards' heads has a large genetic component and that the morphological differentiation within and between species can be explained as a result of adaptive processes driven by selection. Likewise, Adams (2011) evaluated the patterns of variation inheritable of head shape of two species of salamanders of the genus *Plethodon*. The high heritability values obtained for all studied populations suggest that these salamanders exhibit sufficient genetic variation for response to ecological selective forces (such as the use of trophic resources and behavioral interactions) influencing on the shape of the head (Adams, 2011). In our previous work (Imhoff et al., 2015), also we found high heritability values for cranial shape and size in the broad snouted caiman (*C. latirostris*) and we proposed that the high values obtained could allow great ability to respond quickly to selection pressures in a changing environment.

Finally, it is important to mention that the human impact on the environment increased the problem of the persistence of populations and species because it often causes rapid environmental changes. One

resource that organisms possess is the ability to respond adaptively to environmental change, thus increasing fitness, population size and probability of subsistence (Crispo et al., 2010). Conservation biology focuses on maintaining a viable population against environmental change, which will depend in part on the adaptive fit of the phenotypes to their selective environment (Hendry et al., 2008). The evolution of phenotypic plasticity requires two components; one is the correlation between plasticity and fitness and the other is the genetic variation of plasticity (Crispo et al., 2010).

As mentioned above, *Salvator merianae* has a great capacity to adapt to changes in the environment, at least in regard to variations in the shape and size of the head. Furthermore, due to its status as a generalist and opportunist, its populations can survive successfully in disturbed environments; because of their great plasticity, the impact on the fitness of the individuals would seem to be positive at least in the characteristics under study and with respect to ecological challenges. In addition, variation in the shape and size of the head, because it has high heritability values, can be transmitted quickly to the following generations, enabling rapid evolutionary response to the abrupt changes characteristic of the disturbances resulting from human activities. However, future work would be needed to assess the long-term impact of disturbances in these environments (such as the use of pesticides) throughout the life cycles of individuals and through successive generations.

5. CONCLUSIONS

Our findings lead to the conclusion that *Salvator merianae* exhibits heritable phenotypic variability in head shape and size, allowing it to respond to environmental conditions.

The type and abundance of available prey could influence head morphology. The variation in morphology among individuals is a possible method of reducing intraspecific competition for prey. The possible high food availability in disturbed environments would greatly reduce intraspecific competition. We hypothesize that lower variance in shape and size of the head could be a consequence of the greater homogeneity of the microhabitats exhibited by the lands dedicated to agriculture in lizards inhabiting such areas.

The high heritability values of the shape and size of the head indicate that the morphological traits analyzed in this study have a heritable base that allows populations to respond to the pressures of selection of the environment according to the characteristics of their habitat.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.zool.2018.02.002>.

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