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CONTRIBUTIONS TO THE KNOWLEDGE OF SEXUAL DIMORPHISM IN *LIOLAEMUS DARWINII* (SQUAMATA, LIOLAEMIDAE) IN THE MONTE DESERT OF ARGENTINA

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Contributions to the Knowledge of Sexual Dimorphism in *Liolaemus darwini* (Squamata, Liolaemidae) in the Monte Desert of Argentina. Castillo, G. N., Gonzalez-Rivas, C. J., Acosta, J. C. —The lizard *Liolaemus darwini* (Liolaemidae) is a typical species with wide distribution in Monte environments of Argentina. The objective of this study is contribute to knowledge of sexual dimorphism in a population of *L. darwini* (Bell, 1843). We evaluated sexual shape variation of the cephalic region through procustes analyses with geometric morphometry. We predict that the heads in males will have differences in shape with respect to females. The results showed significant sexual differences in shape, mainly in the region around the eyes. There were no significant differences in sizes. Geometric morphometry analyses are a useful tool for addressing sexual differences in Monte lizards. This constitutes the first study for the center-west of Argentina in San Juan province that implements these geometric morphometry analyses. Key words: Argentina, San Juan, Monte, *Liolaemus darwini*, geometric morphometry.

Introduction

Reptiles are important in dimorphism research, due to the considerable variety of dimorphisms and life histories observed within this group (Cox et al., 2007). One aspect analyzed in dimorphism is size, although the difference between male and female adults is very common in organisms with separate sexes (Fairbairn, 2007). In addition, in most lizards, males tend to be larger than females (Cox et al., 2007; Cabrera et al., 2013). Different hypotheses have been proposed to explain this phenomenon, associated with sexual, natural (ecological interactions) and fertility selection (Hedrick & Temeles, 1989; Fairbairn, 1997; Cox et al., 2007; Vincent & Herrel, 2007). The problem is that these mechanisms lead to the same phenomenon and can be problematic (Anderson & Vitt, 1990; Pincheira-Donoso, 2012).

Another interesting aspect to analyze is the variation in shape between sexes (Benitez, 2013). So far, shape variation with geometric morphometry in lizards has been little explored in Argentina, only with contributions of Minoli et al. (2016) and González-Marín et al. (2016). Geometric morphometry (GM) is a statistical approach to study shape variation and its co-variation with other variables, it allows visualizing both the size and direction of shape change among groups. This methodology has considerable power to detect small differences in shape. Such shapes refer to the geometric properties of an object that do not vary with changes in its location, rotation or size (Aguirre & Prado, 2018). GM is based in landmarks with coordinates in two or three dimensions defined in pictures or scans. In GM, landmarks are used to define the shape, and identify the same anatomical point in all the individuals of the study. Thus, landmarks with coordinates allow describing such variation keeping the shape of the geometric structure intact (Aguirre & Prado, 2018).

Geometric morphometry is a useful tool to detect differences among reptiles species, as well as shape variation among sexes (González-Marín et al., 2016; Minoli et al., 2016; Murta-Fonseca et al., 2019). Its frequent use is due to Geometric morphometry helps to elucidate differences among species with a poor morphological variation or scarce sexual variation among individuals (González-Marín et al., 2016).

Liolaemus darwini (Bell, 1843) is a lizard species distributed in many provinces of Argentina (Abdala et al., 2012), with a mean size of 65 mm (Ceí, 1986; Acosta et al., 2017). It is a typical Monte form to the point that it is almost an indicator (Ceí, 1986). It is an oviparous species, with two layings per year and a mean clutch size of 3–6 eggs (Ceí, 1986; Acosta et al., 2017). Its conservation status is non-threatened (Abdala et al., 2012).

To date, morphometric studies with GM have not been carried out in *L. darwini*, although data on the size of the head with lineal morphometry have been reported (Cabrera et al., 2013). Due to this, the objective is to analyze the shape variation in *L. darwini*'s head through geometric morphology.

We analyzed the cephalic region of 30 adult specimens of *L. darwini* (16 females, 14 males) (fig. 1). Lizard were captured at El Encón locality, Department 25 de mayo, San Juan province (32°12' S, 67°47' W) (fig. 2). This sector is represented by the phytogeographic province of Monte, covering extensive arid areas with an average rainfall of less than 100 mm / year, with years without any records. It covers an approximate area of 40,499 km², corresponding to 45 % of the total province. Predominantly xerophytic plants adapted to a warm and dry climate,



Fig. 1. Female specimen of *L. darwini*.

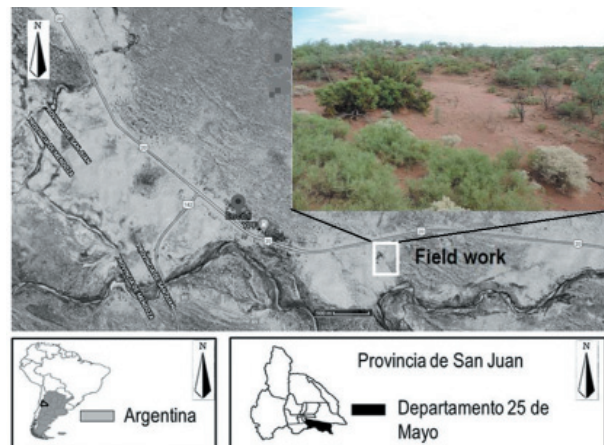


Fig. 2. Sampling area, locality of the Encón, dpto. Mayo 25, province of San Juan, Argentina.

with little summer rainfall. Vegetation responds to wet and dry cycles. It is characterized by the presence of shrub steppe exceeding 3 m high, which branch from the base (Morello, 1958).

Sampling was conducted during 3 years 2017, 2018 and 2019. 16 pitfall traps were placed randomly (width 25 cm, height 37 cm). The traps were buried at a depth of 37 cm. Captured individuals were sacrificed by intraperitoneal administration of sodium thiopental, fixed with 10 % formalin and preserved in 70 % alcohol. All the specimens were deposited in the Herpetological Collection, Department of Biology, Faculty of Exact, Physical and Natural Sciences of the National University of San Juan (*Liolaemus darwini*: UNSJ, 4033–4052).

Image captures were taken with a Nikon® COOLPIX P520 42x digital camera. Pictures of all the specimens were taken inside a light box (Minoli et al., 2016). The photographic camera was mounted on a tripod with the same focal distance length (40 cm), which remained constant in each photography. The dorsal view of the head of each lizard was captured, and the scale was recorded with a grid. Subsequently, images were downloaded in a computer and a tps. file was created using tpsUtil 1.58 (Rohlf, 2013 b). Reference points were selected to quantify any detectable difference between lizards, to meet the following assumptions: homology, cover, repeatability, consistency in the relative position and coplanarity (Toro-Ibacache et al., 2010).

Eight Type 1 landmarks (landmarks in discrete points formed by the intersection of

tissues) of adult specimens were included, and all the reference points were digitized on the right dorsal side of the head for each sample (fig. 3) using tpsDig v. 2.17 (Rohlf, 2013 a). Lizards showing anomalies (n= 10) (e. g., lumps, scars, bites, twisted head) were excluded, since in those cases landmarks could not be accurately established (Minoli et al., 2016). Pictures were taken in the instant euthanasia was carried out. To avoid the precision error, all the photomontages were performed by the same person. The consensus shapes, partial wraps and relative wraps were generated with the tpsRelw software version 1.69.

Using the MorphoJ software v. 1.06 (Klingenberg, 2011), a generalized procrustes analysis was carried out. This allowed the elimination of the individual variation components (in images) that did not correspond to the shape. Landmark alienation of all the individuals under study was carried out, in such way that the two-dimensional coordinates (x, y) represent shape variation (Aguirre & Prado, 2018). To model how shape varied between specimens, deformation grids were used. Additionally, an outlier test was performed to control and exclude lizards that widely diverted from the mean.

To explore shape variation patterns identifying the directions of higher variance, principal component analyses were carried out. The information on size was retained as centroid size (CS). To explore size variations, an ANOVA with centroid size as dependent variable, and sex (male/female) as independent variable was carried out. For a better visualization of the data, a boxplot diagram with the centroid size was performed.

A regression test between the Procrustes coordinate data (which reflect individuals shape) and centroid size was carried out to correct the differences related with allometry. "Procrustes coordinates" was selected as the dependent variable, and "Log Centroid Size" as the independent variable. A "Perform permutation test" was carried out with 10.000 permutations. The CVA (Canonical Variates Analysis) analysis was performed, and "Residuals, Regression" was selected to carry out the analysis independently of allometry.

Results

The principal components 1 (28 %), 2 (20 %) and 3 (17 %) were the axes with the highest variation in the new space, explaining 65 % of the original variance associated to female and male variation (table 1) (fig. 4). Differences in the cephalic region shape between *L. darwini* males and females were found

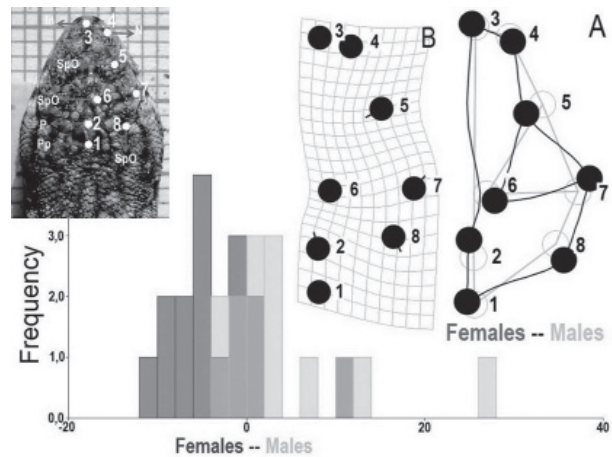


Fig. 3. Landmark type 1 in *L. darwini*. Cephalic scales (1): Pp — postparietal (2); P — pineal (3); In — internasal, (4); N — nasal (5, 6, 7, 8); SpO — supraocular. Females and males of *L. darwini*, shape comparison. Deformation grids, showing the sectors where the shape differs most between sexes. Cross-validation scores: The residual form of the regression was used to assess sexual dimorphism (corrected size) by discriminant analysis. The difference in shapes between females and males of *L. darwini* is observed in the dorsal cephalic region. Fisher's discriminant rule (x-axis), with the cut-off point at a value zero: males with positive values and females with negative values.

Table 1. Main components for the variation of shape in *L. darwini*

	Autovalues	Variance, %	Accumulation, %
1	0.00119	28.03	28
2	0.00086	20.184	48
3	0.000759	17.812	66
4	0.000442	10.381	76
5	0.000353	8.284	84.69
6	0.000239	5.607	90
7	0.000108	3.143	93.4
8	0.0000782	2.536	95.9

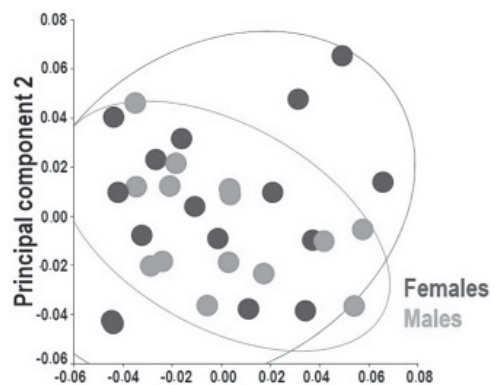


Fig. 4. Morphological separation of the cephalic region of females and males using main components.

Table 2. Generalized analysis of Procrustes, ANOVA, for shape variation. sexual, individual variation and measurement error is observed

Effect	SS	MS	df	F	p
Sexes	0.0164	0.001	12	2.19	0.01
Individual	0.2175	0.0006	348	8.44	0.0001
Error	0.02	0.00007	348		

Table 3. Generalized analysis of Procrustes, ANOVA. Sexual variation in size, individual and measurement error are observed

Effect	SS	MS	df	F	p
Extra 1	2948.7	2948.77	1	0.16	0.6
Individual	544670.31	18781.734	29	20.07	0.0001
Error 1	27137.224	935.766	29		

Note. Extra 1 — variation between sexes; individual — individual variation.

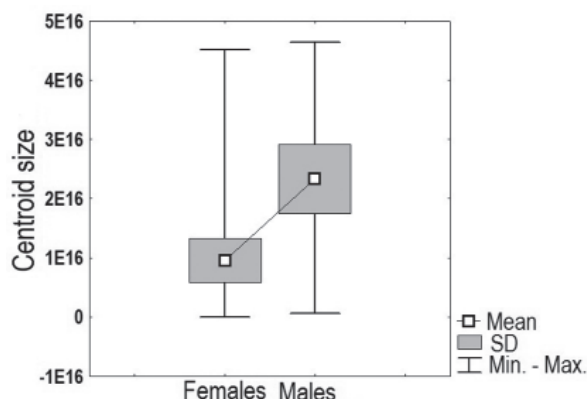


Fig. 5. Variation in size of the cephalic region between males and females of *L. darwinii*, where it is observed an absence of significant differences between both sexes.

interactions); and a third one based on reproductive roles, named by Fairbairn et al. (2007) as “fertility selection”, which favors the size of larger females. However, several phenomena could lead to the same result. In the case of sexual selection and ecological interactions may interact synergistically during the evolution of sexual dimorphism (Bolnick & Doebeli, 2003).

Our results indicated that *L. darwinii* presented head shape variation, specifically exhibited statistical differences in the shape of the eye orbit. These correspond to supraocular scales, indicated by landmarks 5, 6, 7 and 8. To date, in *L. darwinii* there was only head information based on linear morphology, with the head of males larger than females (Cabrera et al., 2013). Sexual dimorphism with linear morphology has been frequently evaluated for other species of the desert of the mount indicating similar results such as *L. olongasta* Etheridge, 1993, *L. riojanus* Cei, 1979, *L. cuyanus* Cei & Scolaro, 1980 and *L. acostai* (= *L. pseudoanomalous*) Abdala & Juárez-Heredia, 2013 (Villavicencio et al., 2003; Cánovas et al., 2006; Laspiur et al., 2006; Laspiur & Acosta, 2007).

(ANOVA, Procrustes $F = 2.29$; $df = 12$; $p = 0.01$) (table 2). Deformations in the grids, supraocular scales (landmarks 5, 6, 7, 8) were observed. Such deformation regions corresponded to the sector where configuration differed the most (fig. 3). Males showed a laterally more widened shape, observed in landmarks 1, 2, 7 and 8; as well as higher longitudinal arrangement. In figure 3, the cross-validation analysis is observed (cross-validation scores), showing shape differences in the cephalic region between males and females of *L. darwinii*. An overlap between males and females can be seen, determining subtle although existent differences in shape (figs 3 and 4). Males and females did not show significant differences considering centroid size, although males presented higher sizes (table 3) (fig. 5).

Discussion and conclusion

Morphological sexual variations (sexual dimorphism) act in a differential way in males and females, and they are, thus, the evolutionary result of selective pressures (Pianka, 1982). To date, three mechanisms have been proposed that could explain the evolution of sexual dimorphism (Cox et al., 2007): one based on sexual selection mechanisms (i. e., males have competition advantages); a second one based on intersexual competition related to resource use (i. e., ecological

With regard to the difference in shape in *L. darwini*, Vidal et al. (2011) analyzed the eye orbit in *Liolaemus tenuis* and found significant differences between sexes. Males have a more rounded and extended orbit shape than females. These authors suggested that they correspond to a potentially adaptive character associated with social condition (Vidal et al., 2011). In our case study in *L. darwini*, to date, we do not have relevant information to associate it to a social system. However, the shape of the orbit could be related to a polygynous social system in reptiles (Vidal et al., 2011). In addition, Vidal et al. (2005) mention that morphological differences in eye orbits are probably also related to territorial defense.

Regarding our results, we believe that the shape differences found between sexes in *L. darwini* could be related to sexual selection. Sexual selection is related in a context where male size confers an advantage in male-male competition (Cox et al., 2007). That is, body size determines success in agonistic encounters between males and territoriality (Shine et al., 1989; Cox et al., 2007). Territorial species show a marked dimorphism in size (Cox et al., 2007). Shine (1989) mentions other types of sexual interactions; exhibition and courtship, forced insemination, couple transport and provision of nuptial gifts between males and females. Although not necessarily these types of interactions occur in reptiles. Regarding the ecological hypothesis for sexual dimorphism (Cox et al., 2007), an ecological effect on dimorphism is likely when it occurs in a character that is free from sexual selection (Shine, 1989; Bolnick & Doebeli, 2003). There is evidence that morphological dimorphism is not related to trophic resource (Shine, 1989). For other mount species in Argentina, it has been reported that the largest head size in males, might be related to aggressive interactions between males for access to females and territory defense (Cánovas et al., 2006). Thus, sexual selection could favor large size in males (due to combat between males) but not in females (Shine, 1989).

We emphasize that all three processes; selection of fertility, sexual and ecological divergence can operate in the same population. Size can be a consequence of any of these factors acting alone or together (Shine, 1989).

As a conclusion, sexual dimorphism is a common phenomenon in lizards, and it is frequent in *Liolaemus* spp. (Valdecantos & Lobo, 2007; Cabrera et al. 2013). Thus, in our study, females and males of *L. darwini* showed a differential adaptation to the environment, and consequently, shape variation in males and females could interact differentially in their habitat.

In summary, the utility of geometric morphometry as a quantitative tool to differentiate sexes in *L. darwini* is observed. Geometric morphometry analysis is an excellent tool to distinguish sexes and can be complemented with lineal morphometry studies.

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