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Fluctuating asymmetry and environmental stress in a reptile under different levels of anthropogenic disturbance

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Abstract. In the Monte region of Argentina, the local population is causing severe habitat degradation by extracting native vegetation and domestic animal grazing. To assess whether disturbed environments have higher levels of asymmetry than control environments, we examined morphological variation and fluctuating asymmetry in the cephalic region of the longtail whiptail lizard *Aurivela longicauda* (Teiidae) using a Procrustes analysis with geometric morphometry. This is the first study of asymmetry using geometric morphometry in a lizard from Argentina. While there was no difference in the size of the cephalic region between the two environments, there were differences in shape between the right and left side of the lizard's head (object symmetry), the differences being greater at disturbed sites (fluctuating asymmetry), suggesting that anthropogenic activities may act as stressors driving alterations in the fitness (reproduction) of reptile populations. Fluctuating asymmetry analysis is an excellent conservation biology and environmental monitoring tool for measuring stress in different organisms.

Key words: Argentina, development instability, geometric morphometrics, habitat degradation, reptile, San Juan

Introduction

Not only is human-induced habitat degradation leading to a decline in global biodiversity (Newbold et al. 2016), human activities are increasingly affecting natural resources and acting as stressors to many different species (Leamy & Klingenberg 2005, Lauck 2006, Söderman et al. 2007, Tull & Brussard 2007, Klingenberg 2015, Eisemberg & Bertoluci 2016). While there have been several studies on the impacts of human activity and habitat degradation on the local biota of the Chaco region of Argentina, particularly

as regards the abundance, species richness and microhabitat use of reptiles (Leynaud & Bucher 2005, Pelegrin et al. 2009, Pelegrin & Bucher 2012, Coria et al. 2017), there has been far less research on reptile populations of the Monte region. In a recent study, however, Cabrera (2021) examined three lizard species inhabiting sites altered through the destruction of shrubs by truck traffic and solid waste dumping. His results show the disappearance of one species. Additionally, Vega et al. (2000) observed variation in the abundance and microhabitat use of two *Liolaemus* species at a site before and after

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disturbance. In the Monte region, and specifically in San Juan Province, forests have historically provided rural communities with a wide range of products, including food, medicinal items, aromatics, fibres, ornamental items, forage products and firewood, which are either used by the inhabitants themselves or sold. In a disturbed environment such as the Monte region, obtaining data on the life history and ecology of reptile populations will be essential for developing suitable conservation plans (Acosta et al. 2016).

Reptiles inhabiting disturbed environments are constantly exposed to stress, defined as “the effect of a force that extends a homeostatic process beyond its normal limit” (Esch et al. 1975), which can then affect normal phenotypic development, causing developmental instability (Palmer & Strobeck 1986). One way of determining developmental instability is through fluctuating asymmetry, which has proven to be a good tool for detecting stress responses in animal populations inhabiting disturbed environments (Tull & Brussard 2007). Simply put, an organism’s left and right sides can be viewed as independent replicas of the same development, in which the body sides of an individual share the same genotype in a homogeneous environment (Van Valen 1962, Benítez & Püschel 2014). Fluctuating asymmetry is defined as a random deviation between the left and the right sides in a perfect bilateral symmetry plane in a population of organisms (Graham et al. 2010). Fluctuating asymmetry can be a response to both environmental and genetic stress, with genetic stress representing the natural susceptibility condition of organisms leading to fluctuating asymmetry. In contrast, environmental stress represents the pressure exerted by the environment in which organisms develop (Naugler & Ludman 1996). Therefore, such changes can be used as an indicator of the capacity of organisms to cope with environmental changes that affect their development, i.e. the adaptation capacity of an organism (Knierim et al. 2007).

In Argentina, fluctuating asymmetry based on geometric morphometrics has yet to be used to evaluate reptile populations in conservation studies. Here, therefore, we set out to determine 1) shape and size variations in the head region, and 2) fluctuating asymmetry in the cephalic region of two populations of the longtail whiptail lizard *Aurivela longicauda* (Bell, 1843) (Family Teiidae) from disturbed and control environments in the Monte region of Argentina. We predict higher fluctuating asymmetry in *A. longicauda* inhabiting disturbed environments relative to those inhabiting natural, undisturbed areas.

Material and Methods

This study took place in Encón, department 25 de Mayo San Juan Province, Argentina (32°12'55.72" S, 67°47'43.28" W; Fig. 1). This sector is represented by the phytogeographic province of Monte, which includes extensive arid areas (ca. 40.499 km², corresponding to 45% of the province) with an average rainfall of less than 100 mm/year (some years with no records). The local vegetation responds to wet and dry cycles and is characterised by predominantly xerophytic plants adapted to warm and dry climates with little summer rainfall and shrub steppe exceeding 3 m high (Morello 1958).

Aurivela longicauda is an insectivorous species with a unimodal activity pattern widely distributed throughout Argentina. It inhabits primarily sandy environments and is agile and an excellent excavator (Acosta et al. 2017). It is a medium-sized species (62 mm) with a long, red tail and large cephalic plates. Both males and females have five alternating red and white lines as dorsal colouration, with no dimorphism or sexual dichromatism (Acosta et al. 2017). The females are oviparous, with a single posture per season producing two eggs (Acosta et al. 2017).

For this study, we analysed the cephalic regions of 23 adult male *A. longicauda* from two populations, 13 from a disturbed environment and ten from an undisturbed control environment (Fig. 1). Sampling took place in the summer of 2017, 2018 and 2019 (10 sampling sessions in all) using 16 pitfall traps (25 cm wide × 37 cm high) buried to a depth of 37 cm, with eight traps placed in the disturbed environment and eight in the control environment (Fig. 2). Sampling sites were defined based on the criteria proposed by Hannah et al. (1994) and Sanderson et al. (2002). In short, disturbed environment site selection was associated with anthropogenic disturbance, defined as human influence or footprint through geographic proxies, such as population density, settlement, roads and other points of access, size and remoteness of the area. In this case, the disturbed site chosen was very close to a road, had very degraded soil and had lost 70% of its vegetation due to anthropogenic pressure, such as the establishment of tracks and the presence and activity of farmyard and domestic animals (e.g. goats, dogs, horses, donkeys). The control environment was situated 200 m away from the disturbed site and was considered unimpacted, and was characterised by 80% vegetation coverage and an absence of tracks and domestic animals (Fig. 2).

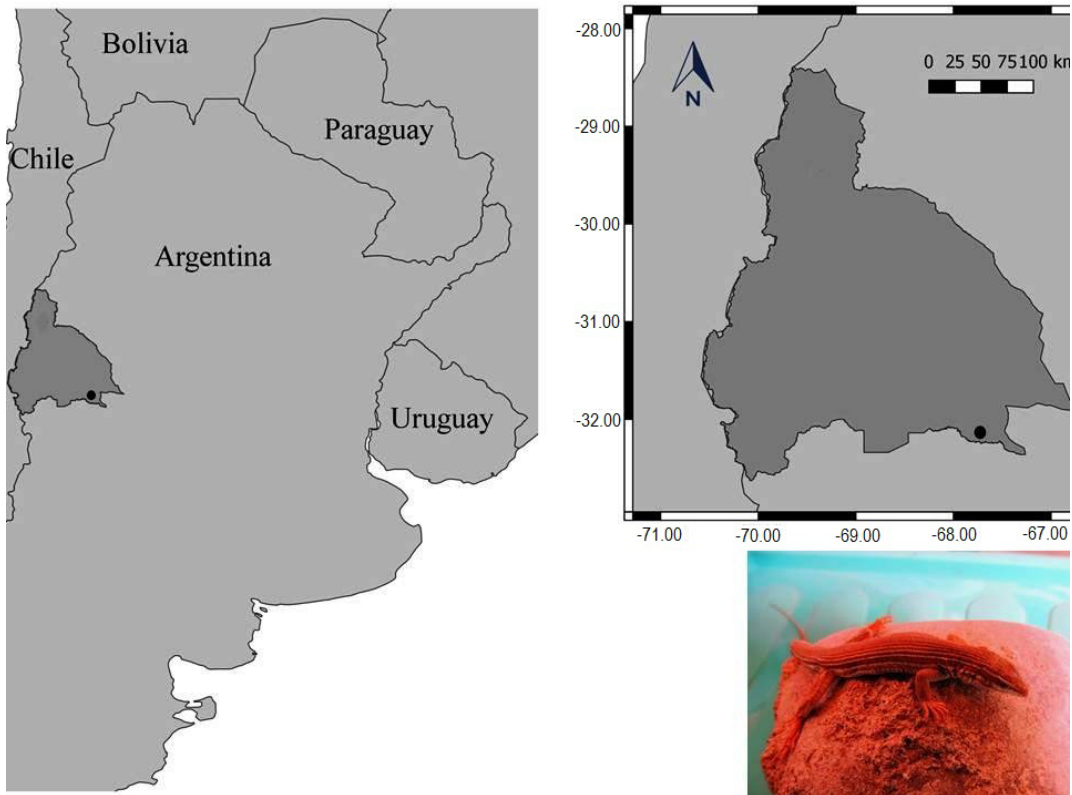


Fig. 1. The Encón study area (25 de Mayo District, San Juan Province, Argentina), with a specimen of *Aurivela longicauda* inset.

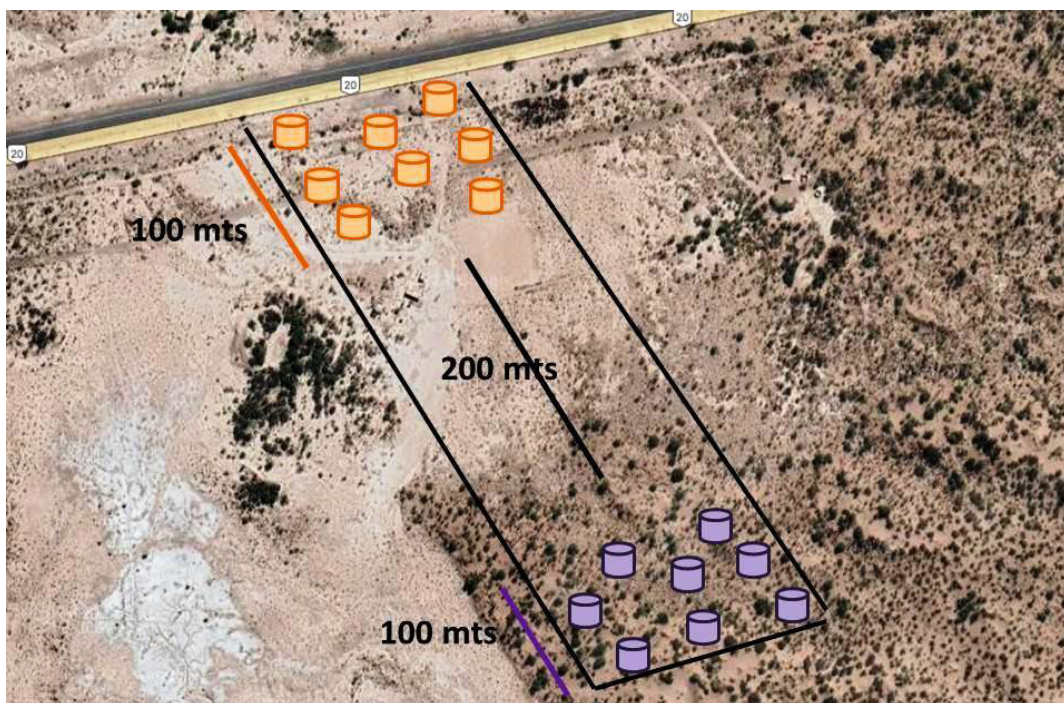


Fig. 2. Detailed layout of the Monte desert sampling site (25 de Mayo District, San Juan Province, Argentina). Pitfall traps in the disturbed environment ($n = 8$) are shown in orange, and those in the control environment ($n = 8$) in purple.

The lizards were immediately transported to the laboratory, where they were placed inside a lightbox (Minoli et al. 2016), and images were taken at the

point of euthanasia using a Nikon® COOLPIX P520 42X digital camera mounted on a tripod, the focal distance (40 cm) remaining constant throughout.

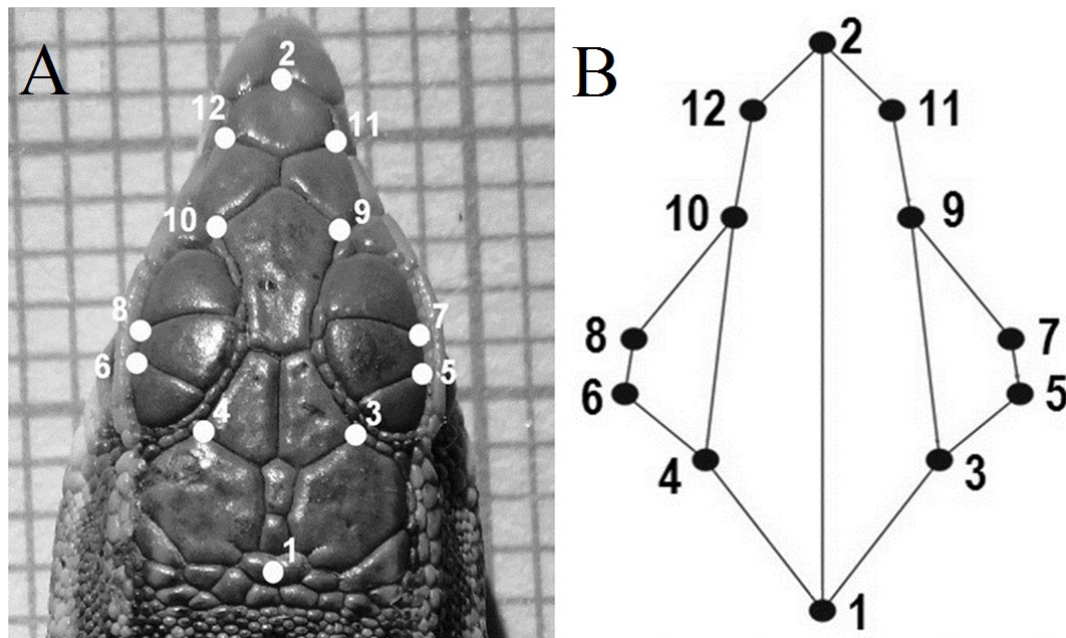


Fig. 3. (A) Cephalic region of *Aurivela longicauda*, (B) configuration of landmarks.

A dorsal view of the head of each lizard was then digitised, and a standardised grid scale was added. To quantify any detectable difference between the lizards, 12 type-one landmark reference points were selected (Fig. 3) to meet assumptions of homology, cover, repeatability and consistency in relative position and coplanarity (Toro-Ibacache et al. 2010, Minoli et al. 2016, Aguirre & Prado 2018). Lizards showing anomalies (e.g. lumps, scars, bites or twisted heads) were excluded as the landmarks could not be accurately established (Minoli et al. 2016). To avoid precision error, all photomontages were performed by the same person. Consensus shapes, partial wraps and relative wraps were then generated using the tpsRelw v.1.69 software package.

For fluctuating asymmetry analysis, the object of interest was considered symmetrical (Klingenberg 2015), and asymmetry was considered a deviation between measurements from the right and left sides of a structure within a plan of bilateral symmetry. A generalised Procrustes analysis was then carried out using the MorphoJ v.1.06 software package (Klingenberg 2011), which eliminates the individual variation components that do not correspond to the shape. The Procrustes analysis method was first modified for landmark configurations with object symmetry, following which asymmetry was quantified through landmark deviations of the original configuration from the symmetric consensus of the original and its mirror image. An additional outlier test was also performed to control for and exclude lizards that diverged widely from the mean. To explore deviations in landmark configuration from the symmetric consensus patterns

of shape variation, a Procrustes ANOVA test was carried out, followed by a principal component analysis to identify directions of the highest variance. Size information was retained as centroid size and shape difference visualised with deformation grids. Finally, an ANOVA test with centroid size as the dependent variable and environment (altered habitat/control) as an independent variable was carried out to explore size variation and a regression test between the Procrustes coordinate data (reflecting individual shape) and centroid size to correct for differences related to allometry.

A boxplot graph subsequently expressed asymmetry values between the disturbed and control environments. In addition, variation between disturbed and control sites were presented using

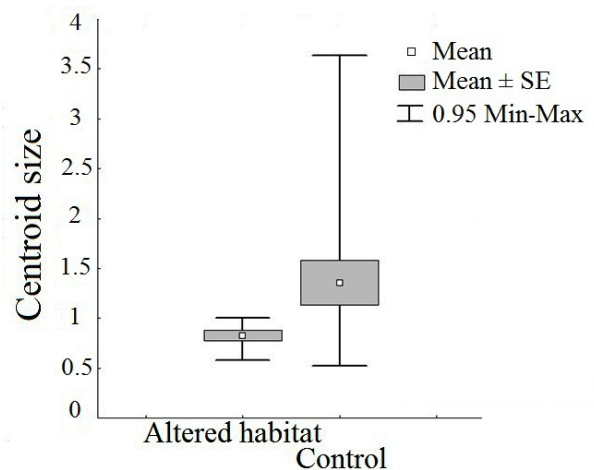


Fig. 4. Variation in size of the cephalic regions of *Aurivela longicauda* from disturbed and control environments. Mean values, standard error and minimum/maximum values are shown.

Table 1. Procrustes ANOVA for shape variation and developmental instability (fluctuating asymmetry; object symmetry) in *Aurivela longicauda* from different environments (disturbed/control).

| Effect | SS | MS | df | F | P |
|-----------------------|-------|-----------|-----|-------|--------|
| Environments | 0.019 | 0.001 | 10 | 2.37 | 0.0112 |
| Individual | 0.17 | 0.000814 | 210 | 4.65 | 0.0001 |
| Directional asymmetry | 0.008 | 0.00089 | 10 | 5.13 | 0.0001 |
| Fluctuating asymmetry | 0.03 | 0.000175 | 220 | 14.74 | 0.0001 |
| Error | 0.005 | 0.0000118 | 460 | | |

Note: Environments = variation in shape between environments, Individual = variation of forms between individuals, Directional asymmetry = (side), Fluctuating asymmetry = (Ind* Side). Sums of squares (SS) and mean squares (MS) are in Procrustes distance units (dimensionless).

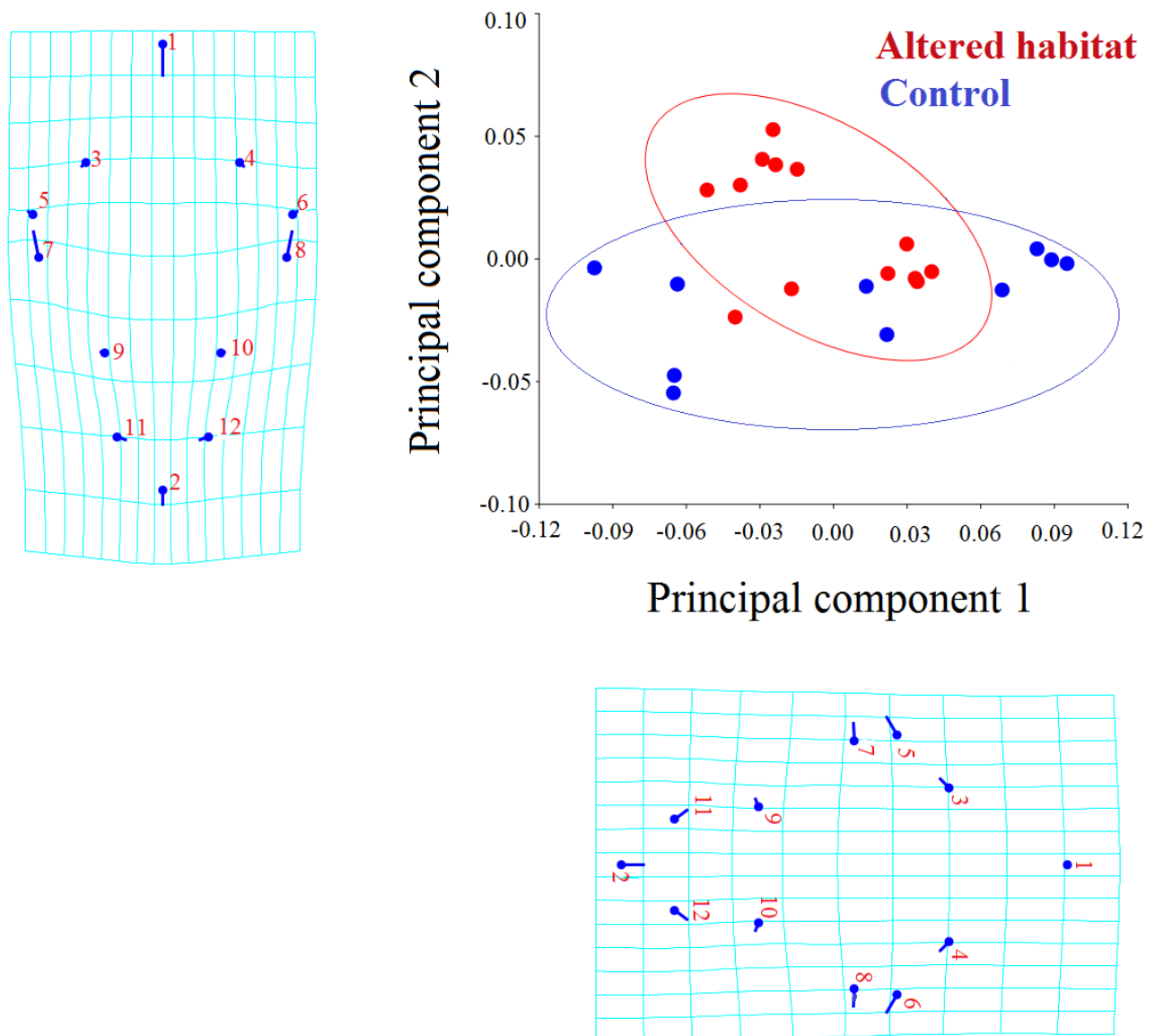


Fig. 5. Principal component analysis for shape variation in *Aurivela longicauda* from disturbed and control environments. Procrustes analysis indicates differences between types of environment ($P = 0.01$).

the “shape FA score”, with the main effects analysed being between environment asymmetry (disturbed/control), individual variation, directional asymmetry

(side) and fluctuating asymmetry (individual × side), the residual term representing measurement error.

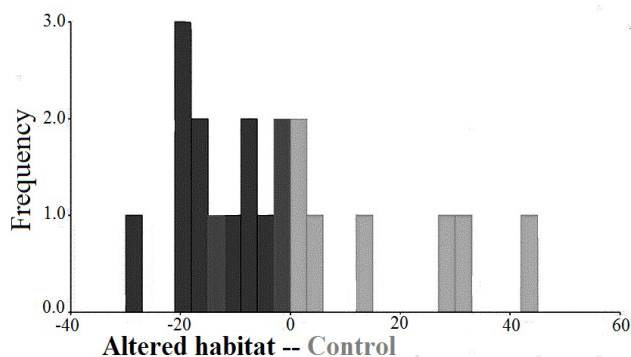


Fig. 6. Cross-validation scores. Frequencies observed for *Aurivela longicauda* in disturbed and control environments. Fisher's discriminant rule (x-axis) with a cut-off point at zero; control with positive values, disturbed with negative values.

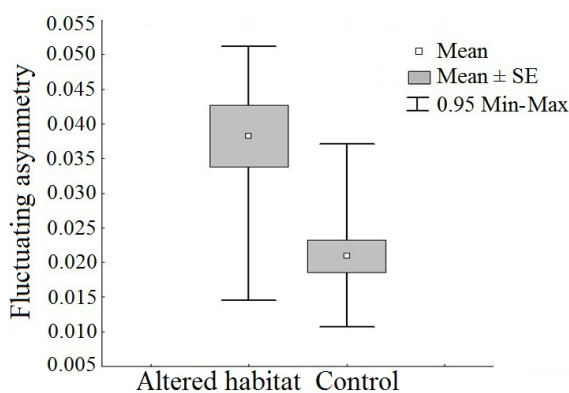


Fig. 7. Fluctuating asymmetry values for disturbed and control habitats.

Results

Regression analysis indicated no significant allometry in *A. longicauda* from different habitats ($P > 0.05$), indicating that allometry did not influence the shape of the evaluated structures. Likewise, we observed no significant difference in the cephalic region between disturbed and control environments (ANOVA, Procrustes $F = 1.6$, $P > 0.05$; Fig. 4).

On the other hand, shape variation analysis indicated significant differences in *A. longicauda* from the two environments, with components 1 and 2 explaining 65% and 16% of shape variation, respectively (ANOVA, Procrustes $F = 2.37$, $df = 10$, $P < 0.05$; Fig. 5). A crossed validation analysis (Fig. 6), using the residuals of the regressions to evaluate morphological variation (i.e. shape variation) in the two habitats, indicated minimal shape overlap between, confirming shape differentiation between the disturbed and control environments.

Analysis of development instability (fluctuating asymmetry) confirmed deviations in the left and right

side in the cephalic region (object symmetry), with higher levels of asymmetry observed in the disturbed environment (Fig. 7; Table 1), confirming an impact from environmental perturbation on *A. longicauda*.

Discussion

Over the last century, increasing anthropogenic impacts on the environment have led to widespread damage to biodiversity and species extinctions (Chapin et al. 2000). Consequently, information must be obtained on variations in species' conditions in their various environments. Fluctuating asymmetry, represented by slight random developmental differences between right and left sides, has previously been used as an indicator of environmental stress (Benítez et al. 2020) in animal populations disturbed by factors such as parasitism (Almeida et al. 2008), temperature (Savage & Hogarth 1999), chemical pollution (Shadrina & Vol'pert 2018) or deforestation (Anciaes & Marini 2000). Having been used in various studies, fluctuating asymmetry is now widely accepted as a good measure (biomarker) of phenotypic response to environmental stress (Benítez et al. 2020).

In this study, we quantified the effects of habitat degradation on *A. longicauda* in the Monte desert of San Juan Province, Argentina, by determining fluctuating asymmetry in the shape and size of the cephalic region in two populations from disturbed and undisturbed (control) environments. We observed no significant difference in head size (centroid size) between the two sites, though those from the control site were slightly larger. On the other hand, there were significant differences in the shape of the cephalic region between control and disturbed sites, in addition, the selected characters were not associated with allometric changes.

This shape variation in the cephalic region of *A. longicauda* could be attributed to the effects of anthropogenic environmental perturbation (Matías-Ferrer & Escalante 2015, Bonner et al. 2019, Mirč et al. 2019), the stress induced by such disturbances causing shape variations (morphological variation) during the organism's early development (Lazić et al. 2013). There is now a lot of information available indicating that external factors can produce changes in an organism's shape, including the effects of urbanisation (Lazić et al. 2013, Mirč et al. 2019), parasitism (Møller 1992) and contamination (Zhelev et al. 2019). Nevertheless, further field studies are required to confirm and evaluate specific sources of



shape variation. In the present study, it would appear highly likely that the high level of habitat modification at the disturbed site is the best explanation for the morphological variation observed.

Several studies have shown that stress in reptiles causes an increase in glucocorticoid, which acts as an inhibitor of the immune system, and that this can result in an increase in parasitism, disease or other pathologies (Warwick 1991, Romero 2004, Trompeter & Langkilde 2011). This situation would support the hypothesis that the asymmetry observed in our study could be a response to less suitable environmental conditions, as also detected in other reptile and amphibian species (Bosch & Márquez 2000, Delgado-Acevedo & Restrepo 2008, Vervust et al. 2008, St-Amour et al. 2010, Lazić et al. 2016, Zhelev et al. 2019). For example, our results for *A. longicauda* are similar to those reported by Tull & Brussard (2007), who observed an increase in fluctuating asymmetry in western fence lizards *Sceloporus occidentalis* (Baird & Girard, 1852) close to roads with high road traffic. Similarly, Lazić et al. (2013) recorded higher levels of asymmetry in common wall lizards *Podarcis muralis* (Laurenti, 1768) in urban environments compared with those from rural areas.

In the present study, the differences in levels of asymmetry observed could be attributed to differences in vegetation patches and bare soil in disturbed and control environments. Rural communities in the study region have a long history of using a broad range of forestry products as sources of food, medicine, fibre, high-quality timber or forage, whether for self-consumption or sale. Furthermore, domestic animals, such as goats and horses, are constantly left to graze in the disturbed environment and dogs, which act as predators, wander freely. Consequently, while

wood extraction may be the main anthropogenic activity in the disturbed environment, microhabitat changes caused by this and other activities are likely to play an essential role in the observed asymmetry. In reptiles, for example, microhabitat selection is strongly influenced by both presence of vegetation, which helps reduce predation risk, and vegetation structure, which influences microhabitat use through impacts on thermal conditions and shelter behaviour (Labra et al. 2001, Amo de Paz 2005, Mella 2007, Pelegrin et al. 2009, Lara-Reséndiz et al. 2014).

In summary, the results obtained in this study provide important information for the study of asymmetry, both from a biological and methodological perspective. Not only does this study provide the first results for asymmetry in the dorsal cephalic region of a common lizard species in the Monte region of Argentina, but it also highlights the utility of geometric morphometry as a tool for identifying populations in different types of environment and the study of fluctuating asymmetry as a helpful conservation biology tool for measuring stress in animal populations.

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Author Contributions

G.N. Castillo – conception, study design, writing the manuscript, acquisition and collation of data; C.J. González-Rivas – analysis, interpretation of data, acquisition and collation of data.



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