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Population ecology of Ameivula ocellifera (Spix, 1825) (Reptilia, Squamata), in an area of caatinga in Pernambuco, Brazil

The Caatinga is an exclusively Brazilian biome, which has many endemic life forms and species adapted to semi-arid conditions. Spix's whiptail Ameivula ocellifera is amply distributed in the semi-arid polygon of South America, and is common in anthropogenic environments. The present study investigated the ecology and genetic variability of A. ocellifera in an area of Caatinga in the Brazilian state of Pernambuco. A total of 12 field trips were conducted between November 2015 and October 2016, with three environments being surveyed: pasture, a prickly pear plantation, and secondary Caatinga vegetation. All the specimens collected were measured and samples were obtained for the analysis of the ISSR molecular marker. A total of 32 A. ocellifera specimens, which presented a sex ratio typical of the species, with a continuous reproductive period, which reflects the adaptive success of this species to the Caatinga environment. Based on similarity indices, it was possible to confirm that all the specimens collected were closely related. However, the larger number of individuals and the greater genetic variability found in the specimens collected from the farmland indicate that this environment constitutes a refuge for the lizards, providing shelter and feeding resources. However, this anthropogenic microenvironment also represents a risk to the species due to increasing intra- and interspecific competition, and a reduction of genetic variability through endogamy.

Palavras-chave: Anthropogenic impact; ISSR; Lizard.

Ecologia populacional de Ameivula ocellifera (Spix, 1825) (Reptilia, Squamata), em uma área de caatinga em Pernambuco, Brasil

A Caatinga é um bioma exclusivo do Brasil, onde pode-se identificar formas de vida endêmicas ou adaptadas a região. A espécie de lagarto Ameivula ocellifera é amplamente distribuída na américa e bastante associado a ambientes antrópicos. Este trabalho teve como objetivo investigar a ecologia e variabilidade genética de A. ocellifera em uma área de Caatiga no estado de Pernambuco. Foram realizadas 12 saídas a campo durante o período de novembro de 2015 até outubro de 2016, abrangendo três pontos de coleta: pastagem, plantação de palma-forrageira e vegetação secundária de Caatinga. Todos os espécimes coletados foram medidos e amostras foram obtidas para análise do marcador molecular ISSR. Foram coletados 32 exemplares de A. ocellifera, que apresentaram razão sexual típica da espécie, com período reprodutivo contínuo, o que reflete o sucesso adaptativo desta espécie ao ambiente da Caatinga. Com base nos índices de similaridade, foi possível confirmar que todos os espécimes coletados estavam intimamente relacionados. Entretanto, o maior número de indivíduos e maior variabilidade genética detectados na área de cultivo indicam que este ambiente funcione como um refúgio para os lagartos, uma vez que oferece abrigo, alimento e proteção. Contudo, esse microambiente antropizado, representa um risco à espécie em virtude do aumento na competição intra/interespecífica e redução da variabilidade genética por endogamia.

Keywords: Impacto antrópico; ISSR; Lagarto.

Topic: **Conservação da Biodiversidade**

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INTRODUCTION

The Caatinga is an exclusively Brazilian biome located within this country's semi-arid zone (Izabel et al., 2011), where relatively high levels of endemism can be found (ABÍLIO et al., 2010). While this biome is still considered to be relatively poorly known, there have been a number of major studies, such as those of Vanzolini et al. (1980) and Leal et al. (2003, 2005), although the relative paucity of scientific data does constitute a potential problem for the adequate management of the biome's biodiversity in the face of ongoing anthropogenic impacts (SILVA et al., 2008).

Spix's whiptail, *Ameivula ocellifera* is found in Argentina, Brazil, Bolivia, and Paraguay (HARVEY et al., 2012). In Brazil, *A. ocellifera* is amply distributed in the Caatinga, where this diurnal lizard is most active during the hottest parts of the day (RIBEIRO et al., 2011). These lizards forage actively for arthropods and larvae (MENEZES et al., 2011) and some populations are sexually dimorphic in body size and shape, with males being larger than females, with proportionately larger heads and bodies (MESQUITA et al., 2003). In the Caatinga, *A. ocellifera* reproduces continuously (SANTOS et al., 2008), which may reflect its high degree of adaptation to the environment.

Lizards are amply used as models for ecological studies, given their ample distribution in the environment (ROCHA, 2009). Genetic variability is fundamental to the capacity of a population to adapt to the environment (CANUTO, 2009), and is the basis of biodiversity (FREITAS et al., 2005). Studies of the genetic variation both within and between natural populations are important for the understanding of their structure (SOUZA, 2017). Molecular markers are basic tools in conservation genetics, given their capacity for the description of the genetic variability and structure of populations, as well as estimating coefficients of endogamy (RAMALHO et al., 2016).

The Inter Simple Sequence Repeat (ISSR) markers amplify regions between microsatellite loci by Polymerase Chain Reaction (PCR) (BRAGA, 2013). These low-cost markers are simple and rapid to use, and do not require any prior knowledge of the target genome (SANTOS et al., 2011). The ISSRs can provide a high level of polymorfism, and are easily reproduced using only a small quantity of DNA (BRAGA, 2013).

The present study focused on the reptiles of an area of Caatinga in the municipality of Paranatama, in the southern backlands of the Brazilian state of Pernambuco. The objective of this study was to evaluate the *A. ocellifera* adaptation to anthropic impacts and different types of vegetation using biometric indices and molecular markers to understand its population structure.

MATERIALS AND METHODS

Study Area

The study area is located in the rural zone of the municipality of Paranatama in Pernambuco State, which has an economy based on farming and services[1]. Three locations approximately 0.4 km apart were selected for data collection: (A) a pasture planted predominantly with digitgrass (Digitaria decumbens); (B) a

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[1] https://cidades.ibge.gov.br/brasil/pe/paranatama/panorama

plantation of prickly pear (*Opuntia cochenillifera*), used as cattle feed, and (C) an area of secondary Caatinga vegetation (Fig. 1).

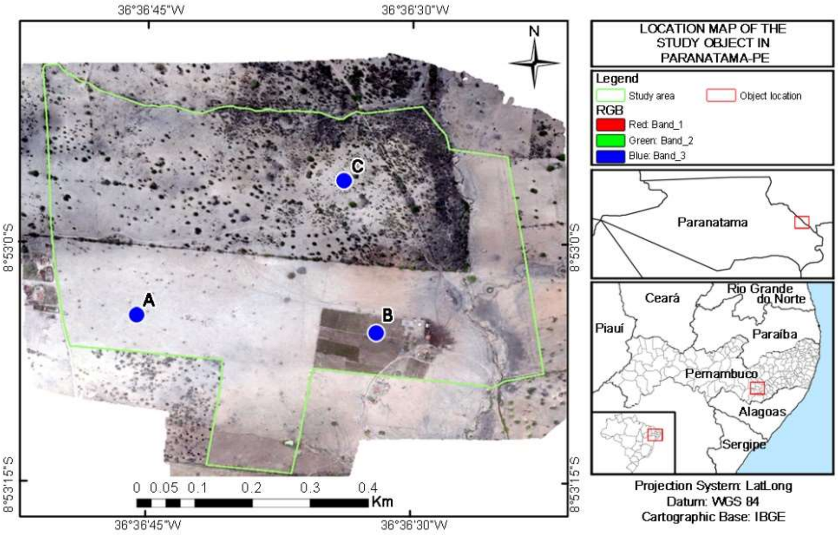

Figure 1: Location of the study area in the municipality of Paranatama, Pernambuco, Brazil. Sampling points: A – pasture; B – prickly pear plantation; C – secondary Caatinga vegetation.

Specimen Collection and Morphometry

The specimens were collected monthly between November 2015 and October 2016, using a set of pitfall traps made of four 24-liter buckets, which were buried in the ground in a Y shape, connected by a drift fence to funnel ground-dwelling animals toward the traps (CAMPBELL et al., 1982). After 36 hours, the traps were checked to remove the captured animals. The animals were processed, sexed, and measured in the field using a digital caliper, which was used to determine: the Snout-Vent Length (SVL); Tail Length (TL); Mandibular-Lip Length (MLL), and Mandible Length (ML). Some of the individuals collected during each field trip were fixed in 4% formaldehyde and sent to the zoology laboratory of the Garanhuns campus of the University of Pernambuco, where they were included in the zoology teaching collection (CDZ/GUS). The collection of specimens for scientific research was authorized by SISBIO licensed number 50621-1.

Statistical Analyses

For the analysis of the structure of the A. ocellifera populations, the individuals were grouped in three age classes (juveniles, and adult males and females) and divided into size classes. These data were analyzed as follows:

Distribution of size class frequencies

This analysis was conducted monthly over the study period (one year), in order to determine the possible seasonal variation in the age structure of the populations. The normality of the frequency distributions was evaluated using the Shapiro-Wilk test (ZAR, 1996).

Body size

The mean body sizes of the adult males and females were compared using Student's t, with a 5% significance level (ZAR, 1996).

Sex ratio

Possible deviations from an expected 1:1 proportion of males:females were assessed using Chi-square (X^2), with a 5% significance level (ZAR, 1996).

Relative growth patterns

The allometric equation $y = a.x^b$ (HUXLEY, 1950) was applied to determine the relative growth rates of the SVL, as the independent variable, and the TL, MLL, and ML, as the dependent variables.

Interpretation of the results

The results of the analyses were considered in the context of certain habitat characteristics, such as the typical composition and structure of the vegetation, the temperature of the air and the ground, and environmental impacts. These analyses were run in the Excel 2010, BioEstat 5.0, and PAST programs.

Collection of Biological Samples

During the processing of the lizards in the field, approximately 2.0 cm of the distal portion of the tail of each A. ocellifera specimens was collected (SISBIO, number 50621-1). The tail was chosen for the collection of tissue sample due to the capacity of these lizards to regenerate this organ, thus minimizing the potential damage to the individuals. Each tailpipe was stored in 70% ethanol in a 2 mL microtube, and preserved at -20°C for the subsequent extraction of the genomic DNA in the Cellular and Molecular Biology Laboratory at the Garanhuns campus of the University of Pernambuco.

Extraction of the DNA and the Molecular Analyses

The DNA was extracted using the protocol described by Sambrook et al. (2001). For this, the tails were macerated in lysis buffer (10 mM of Tris-HCl pH 8.0, 1 mM of EDTA pH 8.0, 1% SDS, 3 M of sodium acetate, and 100 µg of proteinase K) and incubated in a water bath at 60°C for 1 hour. The DNA was then purified using phenol-chloroform-isoamyl alcohol (25:24:1), precipitated in isopropanol, and rehydrated in 40 µL of ultra-pure water. The integrity of the extracted DNA was evaluated in 1% agarose gel stained with 0.1% ethidium bromide, which was viewed under a UV trans-illuminator. The DNA was quantified using a spectrophotometer.

The molecular analysis was based on six polymorphic ISSR markers developed by the University of British Columbia (UBC primers, Vancouver, Canada; Table 1). The samples were amplified using the protocol described by Faria et al. (2006), which consists of one cycle of 4 min at 94°C, followed by 40 cycles of 40 s at

94°C, 40 s at the annealing temperature of each primer (Table 1), and 1 min at 72°C, with a final extension of 7 min. All the reactions were accompanied by a negative control.

Table 1: Description of the ISSR primers used in the molecular analysis of the *Ameivula ocellifera* population from the municipality of Paranatama, Pernambuco, Brazil.

ISSR primer	Sequence	Annealing temperature (°C)
ISSR1	(AG)8T	**50.4**
ISSR5	(CT)8G	**52.0**
ISSR17	(GGAC)4	**51.0**
UBC808	(AG)8C	**55.0**
UBC816	(CA)8T	**55.0**
UBC842	**(GA)8YG**	**55.0**

Following amplification, the samples were electrophoresed in 2% agarose gel stained with 0.1% ethidium bromide, using a 100-bp DNA ladder as a reference scale. Following electrophoresis, the gels were photographed under a UV trans-illuminator. The amplified bands identified by this electrophoresis were used to compile a binary matrix of presence (1) or absence (0). This matrix was used to calculate the Sørensen-Dice coefficient of genetic similarity among the samples. A cladogram of the genetic similarities of the *A. ocellifera* populations was plotted in NTSYSpc 2.1 using the UPGMA algorithm.

RESULTS

A total of 32 *A. ocellifera* specimens – 16 males and 16 females – were collected during the present study. Clearly, the sex ratio of the lizards did not deviate significantly from 1:1. The biometric data on the male and female *A. ocellifera* are presented in Table 3, and the variation in body size, based on the Snout-Vent Length (SVL), the independent variable selected for the assessment of body size (Fig. 2). This curve has two peaks, at 36.65 mm and 58.65 mm, a clear bimodal distribution.

Table 2: Biometry of the *Ameivula ocellifera* specimens collected in an area of Caatinga in the municipality of Paranatama, Pernambuco, Brazil. SVL = Snout-Vent Length; MLL = Mandibular-Lip Length; ML = Mandible Length; TL = Tail Length

Parameter	SVL (mm)	MLL (mm)	ML (mm)	TL (mm)
Minimum	33.10	4.66	6.88	**24.61**
Mean	55.41	8.62	11.20	**99.16**
Standard Deviation	10.17	1.49	2.13	**24.46**
Maximum	**76.04**	**13.90**	**16.45**	**153.35**

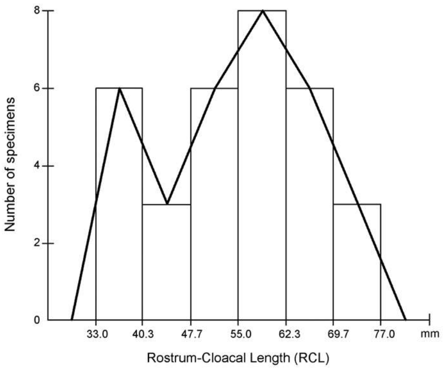

Figure 2: Frequency of *Ameivula ocellifera* individuals in the different body size classes in the study area in the municipality of Paranatama, Pernambuco, Brazil.

Despite having sexual dimorphism, it cannot affect a significant difference in the size of the independent variable between males and females. (t = 0.34, p = 0.37). Although the individuals analyzed showed a pattern of body uniformity, some degree of variation was evident. The relationship between the independent variable SVL and the dependent variable ML (Fig. 3) has b = 0.89, which indicates that the SVL grows at a faster rate than the ML, that is, that these animals have negative allometric growth. The relationship between the SVL and the MLL is closely similar (Fig. 4), with a b value of 0.83, once again, indicating that the SVL tends to grow at a faster rate than the MLL. The relationship between the SVL and the TL is characterized by a b value of 0.55, with y = 10.29 (Fig. 5), which probably reflects the potential for the autotomy of the tail, which is a predator-defense mechanism in these lizards.

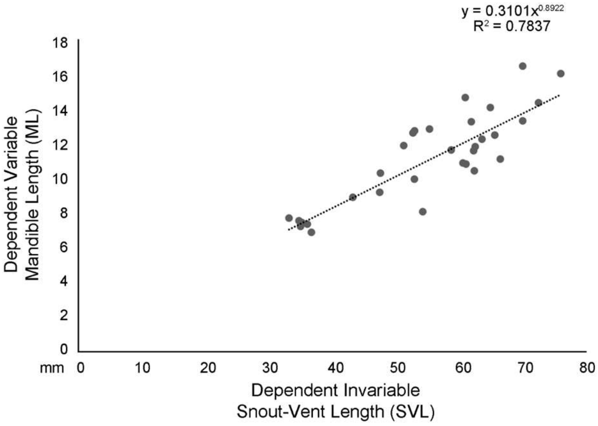

Figure 3: Grow curve of *Ameivula ocellifera* from the municipality of Paranatama, Pernambuco, Brazil, comparing the Snout-Vent Length (SVL) and Mandible Length (ML).

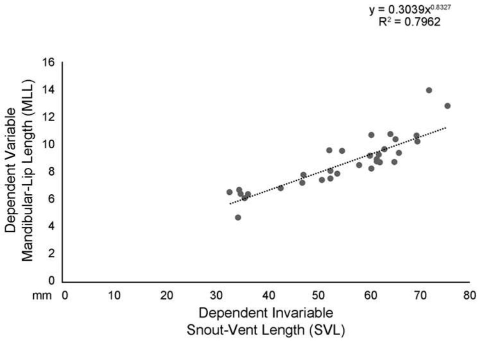

Figure 4: Grow curve of *Ameivula ocellifera* from the municipality of Paranatama, Pernambuco, Brazil, comparing the Snout-Vent Length (SVL) and Mandibular-Lip Length (MLL).

The molecular analysis was based on the sequencing of 25 A. ocellifera samples, including five from point A, 13 from point B, and seven from point C. Four of the six ISSR primers applied in the present study (ISSR1, ISSR5, UBC816, and UBC84) were selected for this analysis, given their greater polymorphism and the clear definition of the bands amplified.

Only 18 of the 35 bands obtained in this analysis were polymorphic, however, which indicates a low degree of genetic variability in the study population. Despite this, it was possible to plot a cladogram, which revealed two well-formed clusters, using a similarity coefficient of 0.6 as the cutoff point (Fig. 6). When the cutoff point is increased to 0.7, the individuals are distributed in six intimately-related genotype profiles I, II, III, IV, V, and VI (Fig. 6). Profile V is amply distributed among the three sampling points, with a predominance of 60% (15/25). However, the genetic variability recorded in the areas of pasture (point A – II and V) and secondary vegetation (point C – V and VI) is clearly much lower than that recorded in the O. cochenillifera plantation (point B), where all the genotypes were recorded.

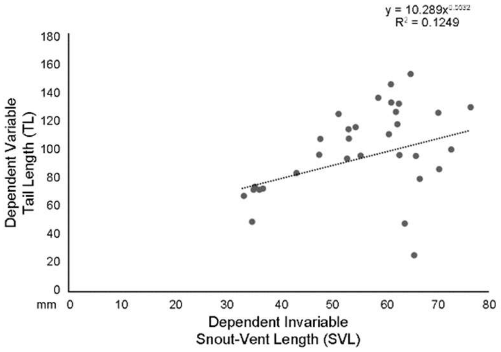

Figure 5: Grow curve of *Ameivula ocellifera* from the municipality of Paranatama, Pernambuco, Brazil, comparing the Snout-Vent Length (SVL) and Tail Length (TL).

DISCUSSION

The present study revealed a clear balance between the male and female *A. ocellifera* population, which is consistent with the reproductive biology of the species. Most lizards are polygynous (VITT et al., 2014), with the males defending a territory that encompasses a number of receptive females. In non-territorial species, however, such as those of the Family Teiidae, polygyny is sequencial, with the males protecting a single receptive female at a time (VITT et al., 2014).

The teiid lizards vary considerably in their body size, with some species, such as Salvator merianae, reaching a Snout-Vent Length (SVL) of 600 mm, whereas others, such as those of the genus Aurivela, which grow to approximately 55 mm (HARVEY et al., 2012). The maximum SVL recorded in the present study was 76 mm (in an adult male), while the mean value was 55 mm.

The bimodal distribution of the SVL values indicates the occurrence of more than one reproductive peak per year (Fig. 2). A number of different reproduction patterns have been recorded in Caatinga populations. Sales et al. (2016) found that *A. ocellifera* has a prolonged reproductive period, and it's strictly associated with periods of increased precipitation. By contrast, Zanchi et al. (2014) concluded that the species has distinct reproductive seasons, with reproductive peaks during the rainy season. The body size data collected in the present study (Fig. 2) are consistent with a seasonal pattern, with more than one reproductive peak per year.

The analyses indicate significant sexual dimorphism in the study *A. ocellifera* population, with the males having a larger body, head, and mandible, and hypertrophied musculature (SANTOS et al., 2008; SALES et al., 2012). Larger *A. ocellifera* males are known to have larger territories and higher reproductive success than smaller males (ANDERSON, 1990; ZALDÍVAR et al., 2007).

The morphometric analyses indicate that *A. ocellifera* has negative allometric growth. Pinto et al. (2004) found evidence of negative allometric growth in their study of five Mabuya species, with variation in growth rates relative to the SVL, in particular in the members and the tapering of the head. These authors related the observed growth patterns to the environments inhabited by the species, including areas of dense grassland and leaf litter, where a more snakelike form, with smaller members may facilitate movement. In *A. ocellifera*, however, the allometry was relatively subtle, and the lizards tended to have relatively uniform growth.

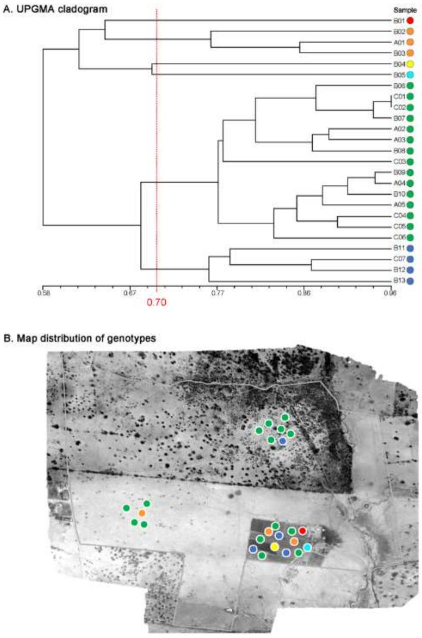

Figure 6: The UPGMA Cladogram based on the coefficient of genetic similarity (A) and the distribution of the genotype profiles (B) in the 25 specimens of *Ameivula ocellifera* collected from an area of Caatinga in the municipality of Paranatama, Pernambuco, Brazil.

When threatened by predators or other males, these lizards will detach their tails as a mechanism of distraction, which allows them to escape. In the present study, four (12.5%) of the specimens collected presented autotomy of the tail, and three of these were males, which may reflect the typical male behavior of disputing females, as observed in other lizard species (VITT et al., 1975). All the other individuals (15 females and 13 males) either had intact tails or organs with the characteristic darker coloration of parts under reconstruction.

The loci amplified by the ISSR primers revealed 35 bands, with a mean of 8.5 bands per primer. This is a relatively high level of variability in comparison with similar previous studies, such as Brito (2012), who observed 28 bands per primer. This variability depends on the primers used, the study species, the DNA integrity and the amplification protocol.

Despite the limited genetic diversity of the study population, the cladogram analysis revealed that individual's transit freely among the different sampling points, a pattern consistent with the behavior of the species, which is active throughout most of the day. Teiids are almost exclusively terrestrial (only a few species have arboreal habits) and the non-territorial species tend to have relatively large home ranges (PIANKA et al., 2003). However, the larger number of individuals and the greater genetic diversity of *A. ocellifera* at point B indicated that this plantation may function as a type of refuge for this lizard. Menezes et al. (2011) and Ribeiro et al. (2011) concluded that habitats of this type may constitute "oasis" environments, which guarantee a greater availability of food, refuge, and breeding opportunities. This oasis effect may be especially accentuated in the study area, which is highly degraded, with no reserves of primary Caatinga vegetation.

The high degree of similarity among the individuals in this environment is related directly to the fragmentation and anthropogenic degradation of the areas populated by the species. Habitat fragmentation may also be a factor determining the genetic similarity among the Ameivula species (SILVA et al., 2008).

CONCLUSION

Spix's whiptail, *A. ocellifera*, is well adapted to the environmental conditions found in the Caatinga biome, where it may adopt a seasonal breeding pattern and maintain a balanced sex ratio. The species is also able to adapt to anthropogenic environments, where they may use plantations as refuges. While this may seem favorable to the species, it also has negative aspects, such as an increase in both intra- and inter-specific competition. Over the long term, in addition, there may be a reduction in genetic variability as a result of endogamy in the individuals isolated in these microsystems. Molecular studies using STR markers, the monitoring of foraging patterns, and the analysis of sexual behavior may all contribute to the better understanding of the ecology of the species, both in natural environments and in anthropogenic habitats.

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