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Autecology of neotropical lizard species Anotosaura vanzolinia (Squamata, Gymnophthalmidae) in a Caatinga region, north-eastern Brazil

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Here we investigate the autecology of the poorly known lizard Anotosaura vanzolinia (Squamata: Gymnophthalmidae) and describe diet, reproductive biology and morphological aspects, testing hypotheses of seasonality and ontogenetic differences. We collected 154 specimens (44 males, 41 females and 69 juveniles) from April 2011 to June 2014, where 101 were found buried in soil. Their diet consists mainly of arthropods found within its microhabitat, including ants and termites, but differences were found between adults and juveniles, and between seasons. Reproduction occurs during the wet season, even though reproductive males could be found in almost all months of the year. Females have fixed clutch size of two eggs, producing more than one clutch during the reproductive season; the incubation period is about 43 to 49 days.

Key words: microhabitat use, diet, reproduction, sexual dimorphism, hatchling size, semiarid.

INTRODUCTION

he family Gymnophthalmidae contains approximately 235 species distributed in 48 genera (Uetz, 2017). These species comprise small lizards (from 40 to 150 mm of snout-vent length) that occur from southern Mexico to Argentina, in Central and South America, and in the West Indies and a number of continental islands (Presch, 1980). They display a wide variety of ecological patterns, inhabiting varied Neotropical habitats, and consisting of terrestrial (e.g. Vanzosaura spp.) and fossorial (e.g. Bachia spp.) species, as well as semi-aquatic (e.g. Potamites spp.) and semi-arboreal (e.g. Placosoma spp.) species (Pianka & Vitt, 2003).

In Brazil, there are approximately 93 gymnophthalmid species distributed in 33 genera (Costa & Bérnils, 2015). Anotosaura, one of those genera, comprises two species: A. vanzolinia Dixon, 1974 (Fig. 1) and A. collaris Amaral, 1933, both inhabiting mesic environments in the Caatinga region. However, A. vanzolinia seems to be more widely distributed within the biome than A. collaris, which currently has only been recorded in mountain regions from northern Bahia State (Rodrigues et al., 2013). To date, A. vanzolinia has been recorded in the Brazilian states of Alagoas (Gonçalves et al., 2012), Bahia (Freitas & Silva, 2007; Garda et al., 2013), Pernambuco (Dixon, 1974; Pedrosa et al., 2014), Rio Grande do Norte (Gogliath et al., 2010) and Paraíba (Rodrigues, 1986; Delfim & Freire, 2007; Oliveira & Pessanha, 2013). Currently, we know that A. vanzolinia is a semifossorial

Figure 1. Adult A. vanzolinia from Caatinga in Campina Grande, Paraíba, Brazil.

lizard that lives in leaf litter and feeds mainly on soil arthropods (Delfim & Freire, 2007; Freire et al., 2009; Oliveira & Pessanha, 2013). However, detailed ecological studies of this species are rare. Oliveira & Pessanha (2013) described both the microhabitat use and the diet of A. vanzolinia, and Oliveira et al. (2017) examed its endoparasitic fauna. Despite these, some important information, like reproductive ecology, morphological aspects and sexual dimorphism are still completely unknown for this species.

Here we perform a detailed study examining the

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Figure 2. Collecting sites in Paraíba State (a) in North-east Brazil, Campina Grande municipality; (b) Study areas; (c) Forest Fragment at São José da Mata (SJM) and Forest Park Complexo Aluízio Campos (CAC).

autecology of population of the lizard *A. vanzolinia* from the Caatinga Region in north-east Brazil. Specifically, we describe the diet, reproductive biology and morphological aspects of this species. We also tested the hypothesis that dietary composition is affected by seasonality and ontogetic differences. Lastly, we looked for sexual dimorphism in body size and shape.

MATERIALS AND METHODS

Study area and collecting procedures

We collected the lizards from the "Parque Florestal Complexo Aluízio Campos" and from a forest fragment in São José da Mata district, both in Paraiba State, northeastern Brazil (Fig. 2), about 20 km away from each other. The "Parque Florestal Complexo Aluízio Campos" (7°16'34"S, 35°53'7"W) is a Caatinga area at an altitude of approximately 500 m, with shrubby vegetation represented mainly by Bromeliaceae and Cactaceae, and a large number of rock outcrops and accumulated leaf litter (Alves et al., 2010; Silva et al., 2010). São José da Mata district (7°11'2.85"S, 35°59'6.17"W), with an altitude of approximately 700 m, is located between the arboreal formation locally known as Brejo and consists of a typical Caatinga vegetation. It is probably one of the last remaining transitional arboreal vegetation in Paraíba State, containing typical plant species from both Caatinga and Atlantic Forest (MMA, 2003).

This region has a tropical climate with a mean temperature of 22.9 °C; January being the hottest (about

24.5 °C) and July the coldest (about 20.7 °C) months of the year. The highest precipitation rates occur in April (around 115 mm) and the lowest in October (about 13 mm) and November (about 7 mm) (Climate-Date, 2016). Here, we consider the wet season from March to August and the dry season from September to February, based on monthly rainfall indices (Climate-Date, 2016).

Sampling from "Parque Florestal Complexo Aluízio Campos" occurred once a month from July 2013 to June 2014. At these times, we searched for and hand collected animals by revolving the leaf litter, rocks, termite nests, and fallen logs, and by digging in the soil with gardening shovels. Sampling was always carried out during the day (from 0800 h to 1700 h). Sampling from the Forest Fragment at São José da Mata occurred monthly from May 2013 to April 2014. At these times, we used only pitfall-traps installed on six sampling units at the site. Each sampling unit contained one central and three external 20L buckets arranged in a Y-shape and joined by 3 m long plastic fences. The traps remained open for 15 days per month. In addition, we also analysed the lizards collected by Oliveira & Pessanha (2013), from April-May, July–August 2011.

All collected lizards were euthanised with a lethal injection of 2% lidocaine hydrochloride, preserved with 10% formalin and stored in 70% alcohol. All collected specimens were housed in Coleção Herpetológica da Universidade Federal da Paraíba (CHUFPB, João Pessoa, Brazil).

Microhabitat use

We defined three categories to describe the microhabitat used by this species: (1) buried in soil, (2) between leaf litter and (3) under rocks. We did not categorise the microhabitats of lizards collected from pitfall-traps. To describe solar incidence at the collecting site, we used three categories: (1) sunny, (2) shaded, and (3) cloudy.

Diet

We dissected the lizards and analysed their gastrointestinal tracts under a stereomicroscope. We identified prey items to (usually) order level. Prey volume was estimated using the ellipsoid formula:

$$V = \frac{4}{3}\pi \left(\frac{length}{2}\right)^2 \left(\frac{width}{2}\right)$$

To determine the relative contribution of each prey category in the lizards diet, we calculated a Relative Importance Index (RII) introduced by Pinkas et al. (1971):

$$RII = F\% \times (N\% + V\%)$$

where *F* represents frequency of prey category, *N* is the total number of prey category and *V* is the total volume of prey category.

We calculated volumetric and numeric niche breadth for individual and pooled stomachs using the inverse of the Simpson's diversity index (Simpson, 1949):

$$B = \frac{1}{\sum_{i=1}^{n} p_i^2}$$

where *i* represents prey category, *p* is the proportion of category *i*, and *n* is the total number of categories.

To investigate dietary overlap between males and females and between adults and juveniles, we calculated numeric and volumetric niche overlap indices using the overlap equation (Pianka, 1973):

$$O_{jk} = \frac{\sum_{i}^{n} p_{ij} p_{ik}}{\sqrt{\sum_{i}^{n} p_{ij}^{2} \sum_{i}^{n} p_{ik}^{2}}}$$

where *p* is the proportion of the prey category *i*, *n* is the number of categories, and *j* and *k* represent the groups being compared (males/females; adult/juveniles). The overlap φ_{jk} ranges from 0 (no overlap) to 1 (complete overlap). To investigate differences among groups, based on the presence of non-random patterns in the niche overlap, we used the Niche Overlap Module of EcoSim.

We compared sexual, ontogenetic and seasonal dietary differences with the nonparametric Wilcoxon test, using the five prey categories with the highest values of Relative Importance Index (*RII*).

Sexual dimorphism

We measured the snout-vent length (SVL), tail length, head length, width and height, body width and height, and

forelimb and hindlimb length of each lizard using a digital calliper. We log-transformed (base 10) all morphometric variables prior to analysis to meet the requirements of normality. We considered the body size to be an isometric size variable defined a *priori* by the multiplication of $p^{.0.5}$, with the $n \ge p$ matrix of log-transformed data, where *p* is the number of variables and *n* is the number of observations (Jolicoeur, 1963; Somers, 1986). To remove the effects of body size from the log-transformed variables, we used Burnaby's method (Burnaby, 1966) defined as:

$$L = I_{n} - V(V^{T}V)^{-1}V^{T},$$

where I_p is the identity matrix $p \ge p$, V is the isometric size eigenvector defined above and V^T is the transpose of matrix V (Rohlf & Bookstein, 1987). Hereafter, we refer to the resulting size adjusted variables as shape variables. To test the null hypothesis that there is no morphological difference between males and females, we conducted a separate analysis of variance in body size (ANOVA), and we created an empty model based on logistic regressions and included the significant shape variables with the lowest AIC values to create the best model that explains the variation between shape variables among sexes. *A posteriori*, we performed a discriminant analysis based on 9999 bootstrap replications to determine the misclassification error based on the selected variables from logistic regression models.

Reproduction

The sex of each lizard was determined by direct observation of the gonads. We defined the minimum size at sexual maturity based on the smallest reproductive male and female. Therefore, all lizards with equal or bigger SVL were considered adults.

We described females as reproductive by the presence of vitellogenic follicles and/or oviductal eggs. We considered the simultaneous presence of vitellogenic follicles and oviductal eggs to indicate the production of more than one clutch per reproductive season. We considered males to be reproductively active when they presented enlarged testis and convoluted epididymis.

In females, we measured the width and length of vitellogenic follicles and oviductal eggs when present. In males, we measured the width and length of the testis. We estimated the volume of eggs, follicles and testis using the ellipsoid formula described above. We also conducted regressions between SVL and testis/egg volume. For males, we used an ANCOVA to consider the effect of SVL on individuals and analysed monthly variations in testis volume.

In order to obtain information about hatchling size and incubation time, five clutches were collected and maintained in terrariums under natural temperature and humidity conditions. After hatching, the hatchlings were euthanised, measured and submitted to the same procedures described previously.

We conducted all statistical analyses (except Niche Overlap) using the software R (R Development Core Team 2015), with a significance level of 5% to reject null hypotheses. **Table 1**. Dietary composition of *A. vanzolinia* (n= 120) from Caatinga. F = frequency; N = number; V = volume; RII = relative importance index.

Prey Items	Occurance		Pooled stomachs					
	F	F%	N	N%	v	V%	RII	RII%
GASTROPODA								
Pulmonata	1	0.83	1	0.15	8.00	0.40	0.46	0.01
DIPLOPODA	1	0.83	1	0.15	3.00	0.15	0.25	0.01
ARACHNIDA								
Araneae	10	8.33	14	2.12	68.88	3.46	46.47	1.03
MALACOSTRACA								
Isopoda	6	5.00	15	2.27	19.53	0.98	16.25	0.36
INSECTA								
Blattaria	7	5.83	11	1.66	58.99	2.96	26.99	0.60
Coleoptera	30	25.00	45	6.81	189.78	9.53	408.41	9.04
Coleoptera (larvae)	18	15.00	38	5.75	445.43	22.36	421.69	9.34
Diptera	3	2.50	3	0.45	3.00	0.15	1.51	0.03
Eggs	11	9.17	121	18.31	140.07	7.03	232.27	5.14
Hemiptera	1	0.83	3	0.45	37.43	1.88	1.94	0.04
Hymenoptera (Formicidae)	59	49.17	158	23.90	213.18	10.70	1701.48	37.67
Hymenoptera (non-Formicidae)	2	1.67	2	0.30	14.38	0.72	1.71	0.04
Isoptera	32	26.67	196	29.65	505.64	25.39	1467.72	32.50
Larvae	15	12.50	26	3.93	152.36	7.65	144.73	3.20
Lepidoptera (larvae)	1	0.83	2	0.30	56.00	2.81	2.60	0.06
Orthoptera	3	2.50	3	0.45	21.17	1.06	3.79	0.08
PLANT MATERIAL	6	5.00	7	1.06	8.49	0.43	7.43	0.16
NON-IDENTIFIED	8	6.67	15	2.27	46.47	2.33	30.68	0.68
Total	-	-	661	-	1991.8	-	-	-
Niche breadth	-	-	5.26	-	6.68	-	-	-

Table 2. Morphological variables of *A. vanzolinia* from Caatinga. SVL = snout-vent length, TL = tail length, HL = head length, HW = head width, HH = head height, BW = body width, BH = body height, FLL = forelimb length and HLL = hindlimb length. Size-adjusted values are in parentheses. Values in millimetres. *Isometric size variable, see methods.

Variable	Mean ± Standard Deviation					
	Males (n=39)	Females (n=41)				
Body size*	2.60±0.08	2.65 ± 0.07				
SVL	38.56±2.21 (0.72±0.02)	42.61±2.21 (0.75±0.03)				
TL	49.33±12.87 (0.81±0.11)	47.71±15.87 (0.76±0.16)				
HL	5.91±0.40 (-0.30±0.05)	6.05±0.35 (-0.27±0.06)				
HW	3.97±0.28 (-0.41±0.03)	4.02±0.31 (-0.40±0.04)				
HH	2.88±0.26 (-0.24±0.04)	3.02±0.27 (-0.22±0.04)				
BW	4.21±0.54 (-0.09±0.03)	4.62±0.45 (-0.10±0.03)				
BH	3.69±0.55 (-0.27±0.03)	4.16±0.59 (-0.28±0.03)				
FLL	4.10±0.37 (-0.25±0.04)	4.08±0.40 (-0.27±0.04)				
HLL	8.12±0.39 (0.04±0.03)	8.28±0.59 (0.04±0.04)				

RESULTS

Microhabitat use

A total of 154 specimens of *A. vanzolinia* were identified, of which 66% (101) were buried in soil, 21% (33) were present in leaf litter and 3% (5) were found under rocks. Considering solar incidence, 103 specimens (74%) were located in shaded environments and only seven (5%) in sunny environments. Twenty one percent (29) of lizards

were found on cloudy days, when it was not possible to characterise solar incidence on the lizard.

Diet

Seventeen prey categories were identified (Table 1). The most frequent prey categories were Formicidae (ants), Isoptera (termites) and Coleoptera (beetles). Numerically, Isoptera was the most commonly consumed, followed by Formicidae and insect eggs. Volumetrically, Isoptera dominated the diet, followed by Coleoptera (larvae) and Formicidae. The five most important prey items (based on *RII*) were Formicidae, Isoptera, Coleoptera (larvae), Coleoptera (adult) and insect eggs. The diet niche breadth for pooled stomachs was 5.26 based on prey number and 6.68 based on prey volume.

Adult males and females exhibited a relatively high dietary overlap based on number (φ_{jk} =0.77) and volume (φ_{jk} =0.64). Adults and juveniles also showed a similar overlap based on number (φ_{jk} =0.59) and volume (φ_{ik} =0.68).

We did not find sexual differences in the five most important prey in adults. Both sexes exhibited highest *RII* values for Isoptera and Formicidae (Appendix 1). However, juveniles showed larger values than adults for Formicidae and Coleoptera (larvae) (Appendix 2). In the dry season, Formicidae and Coleoptera (larvae) were the most important prey, and in the wet season, Isoptera and Formicidae were the most important (Appendix 3).

We found significant differences in the numeric and volumetric consumption of Isoptera ($W_n = 2167, p = 0.008$; $W_v = 2194.5, p = 0.004$) and insect eggs ($W_n = 2067, p = 0.002$;



Figure 3. Monthly distribution of reproductive adult males and females of *A. vanzolinia* from Caatinga. Numbers on top of bars indicate sample sizes.

 $W_v = 2067$, p = 0.002) between adults and juveniles; and Isoptera ($W_n = 2185$, p = 0.002; $W_v = 2206.5$, p = 0.001) and insect eggs ($W_n = 2009$, p = 0.004; $W_v = 2009$, p = 0.004) between seasons.

Sexual dimorphism

In total, 44 adult males, 41 adult females and 69 juveniles were analysed. The SVL of the smallest adult male was 34.9 mm and of the smallest adult female was 38.4 mm. The SVL of the largest adult male was 42.9 mm and of the largest adult female was 47.7 mm.

We found a significant difference in the body size between males and females (ANOVA $F_{1,78}$ =9.08; p=0.003), with females being larger than males (Table 2). The model selection analysis indicated that snout-vent length, body height and hindlimb length presented the best discrimination index between sexes, with males being taller and having longer hindlimbs, while females had longer bodies (Model = sex ~ SVL + BH + HLL, AIC = 75.960, p=0.003). The linear discriminant function using the three selected variables had a misclassification error of 0.20.

Reproduction

In this species, reproduction occurred during the wet season, from March to August, with reproductive activity declining in September and October (Fig. 3) and hatchlings emerging mainly in April and August (Fig. 4). Females had the highest volume of vitellogenic follicles in March and April (Fig. 5), and egg presence from March to August.



Figure 4. Monthly distribution of individuals of *A. vanzolinia* from Caatinga according to snout-vent length



Figure 5. Monthly variation (mean + SE) in follicle volume of *A. vanzolinia* from Caatinga



Figure 6. Monthly variation (mean + SE) in adjusted testis volume of *A. vanzolinia* from Caatinga. The adjusted volume was calculated by summing the mean volume of the testis with regression residues between CRC and testis volume.

The mean egg length and width were 7.17±2.19 mm and 3.72±0.66 mm, respectively, and the average egg volume was 55.68±26.09 mm³. We did not find relationship between average egg volume with female SVL (R²=0.003; p=0.840). Gravid females had a fixed clutch size of two eggs. Five females contained vitellogenic follicles and oviduct eggs simultaneously, indicating the presence of more than one clutch during the reproductive season.

The reproductive activity of males showed peaks in March, April and August, coinciding with the period of highest female reproductive activity. However, reproductive males could be identified in almost all months of the year (Fig. 3). In males, testis volume was positively correlated with male SVL (R^2 =0.268; p<0.001). We did not find significant differences between monthly testis volume (ANCOVA $F_{11,39}$ =1.911; p=0.068) (Fig. 6).

We collected five egg clutches of *A. vanzolinia*, one in July 2013, two in September 2013 and more two in April 2014. The eggs were collected from under leaf litter, always in pairs. Two eggs hatched in seven and nine days, respectively. The incubation period for the other eggs was from 43 to 49 days. The mean size of hatchlings was 18.85±0.91 mm (Appendix 4).

DISCUSSION

Anotosaura vanzolinia is a lizard species that inhabits semi-arid Caatinga areas, but it seems to be strictly located in the milder microhabitats within the biome, characterised by large amounts of leaf litter and organic matter with low direct solar incidence. The use of milder microhabitats, such as forest fragments within the Caatinga, or sites with accumulated leaf litter, was previously described for this species (e.g. Rodrigues, 1986; Delfim & Freire, 2007; Gonçalves et al., 2012). Rodrigues (1986) suggested that this species have relictual distribution, and here we show that it was found only within these specific microhabitats. In open sites in same study area, with high solar incidence and little leaf litter, we did not find the species.

Besides that, it is a wide foraging lizard that feeds on a variety of arthropods, both mobile and sedentary preys, such as ants and termites. The consumption of termites by active foragers is very common (Huey & Pianka, 1981). On the other hand, the high consumption of ants, not only by A. vanzolinia but also by other active foragers, is still uncertain, since ants and other Hymenopterans can possess chemical compounds that are harmful to lizards (Vitt & Pianka, 1994; Vitt et al., 2003). Since these lizards, like other autarchoglossans, can chemically discriminate their prey, they should avoid them (Vitt & Pianka, 1994; Vitt et al., 2003). Despite this, A. vanzolinia seems not to avoid eating ants, reflected in the high rates of ants in our data. Another interesting point is that juveniles also eat lots of ants, which indicate that the resistance from ant toxins seems to be something innate.

Apparently, some autarchoglossans could have developed a way to get rid of the effects of the toxins produced by ants, because ants also were described as important prey for other gymnophthalmids, like *Dryadosaura nordestina*, *Ecpleopus gaudichaudii* and *Leposoma scincoides*, from Atlantic forest, and Andean lizards of genus *Proctoporus* (Teixeira & Fonseca, 2003; Doan, 2008; Maia et al., 2011; Garda et al., 2014). From this reason, we suggest that in some gymnophthalmids (e.g. Ecpleopodini), the feeding ecology can be influenced by their phylogenetic history (i.e. niche conservatism).

Furthermore these data shows that ant consumption not occurs only or more within Iguania, as previously stated (e.g. Vitt & Pianka, 1994; Vitt et al., 2003; Sites et al., 2011).

Adults feed on more termites than juveniles, which

could be explained by the size incompatibility between prey and predator (Costa et al., 2008), since termites are larger than ants, or simply that for juveniles may be easier to catch ants than other prey types. Vitt (2000) suggested that ontogenetic differences in lizard diets are important for population balance and juvenile survivorship, because juveniles can compete with adults if they continue feeding on small prey.

We observed differences in the consumption of termites and insect eggs during different seasons. The difference on prey availability may result from changes in resource availability for season (Van Sluys, 1995; Wiederhecker et al., 2002). For example, insect eggs are absent during the dry season and termites are more abundant during the wet season (Vasconcellos et al., 2007; Araujo et al., 2010).

The diet niche breadth of *A. vanzolinia* is similar to that of other gymnophthalmids, such as *Micrablepharus maximiliani* and *Colobosaura modesta* from Cerrado, which show niche breadth values of 5.0 and 4.0, respectively (Mesquita et al., 2006; Dal Vechio et al., 2014), or Amazonian species, like *Alopoglossus* genus, which show values around 7.0 (Vitt et al., 2007). In the present study, we also observed that females have a larger niche breadth than males, which is likely a result of different energetic requirements between the sexes, due to the high costs of egg production and maintenance by females (Shine, 1980; Shine & Schwarzkopf, 1992).

The greater body size and snout-vent length observed in females of *A. vanzolinia* can be likely related to sexual differences in reproductive success, since they possess a larger space in the peritoneal cavity for the development of eggs (Olsson et al., 2002; Cox et al., 2003). Most female gymnophthalmids show a larger body size than males, including the close related species *A. collaris* (Rodrigues et al., 2013). Therefore, this trend is probably a general pattern within the family (Vitt, 1982; Balestrin et al., 2010; Rodrigues et al., 2013). Furthermore, males *A. vanzolinia* reached sexual maturity at a smaller body size than females, which may simply reflect the largest female size, similar to *Cercosaura schreibersii* (Balestrin et al., 2010).

The reproduction of *A. vanzolinia* in the Caatinga occurs during the wet season, with gravid females appearing from March to August. Both males and females present higher reproductive activity in April – the month with the highest precipitation values (Climate-Date, 2016) – with reproductive activity decreasing in September and October. The amount of rainfall appears to be an important regulatory factor in reproductive seasonality of tropical lizards (Rocha, 1994), which may be related to large prey abundance during the wet season or their low levels in the dry season (Van Sluys, 1995), or to avoid desiccation of eggs in dry season (Overall, 1994). Andrews & Sexton (1981) stated that dampness in the wet season can produce more appropriate conditions for egg deposition and embryo development.

Anotosaura vanzolinia has a fixed clutch of two eggs – a recurrent pattern in Gymnophthalmidae which is considered a synapomorphy of the family (Fitch, 1970; Vitt, 1992) – and produces at least two clutches per reproductive season. Lizards with fixed clutch size can increase their reproductive effort by investing in multiple clutches per reproductive season (Vitt, 1986; Selcer, 1990), similar to other gymnophthalmids, such as *C. schreibersii* (Balestrin et al., 2010), *D. nordestina* (Garda et al., 2014), *M. maximiliani* (Dal Vechio et al., 2014) and *Vanzosaura multiscutata* (Vitt, 1982).

In summary, *A. vanzolinia* is a small semifossorial lizard that inhabits forest fragments in semi-arid Caatingas, foraging in the leaf litter and eating small arthropods, especially ants and termites. They are small elongate lizards with reduced eyes and limbs, a long tail and no ears. Female *A. vanzolinia* are larger than males. They reproduce in the wet season, when females deposit their eggs, two eggs per reproductive event, twice a year.

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