

Biological aspects of Ameivula nigrigula

**BIOLOGICAL ASPECTS OF *Ameivula nigrigula* (Squamata: Teiidae):
FEEDING HABIT, SEXUAL DIMORPHISM, REPRODUCTION AND
MATURATION OF FEMALES**

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Abstract: Life history information plays a crucial role in comprehending the evolutionary processes that shape ecological patterns within species, but there is limited information available on the reproductive and other aspects of tropical Squamata. In this study, we present a comprehensive analysis of dietetic and morphometric data, specifically related to the size and sex determination of *Ameivula nigrigula*, aiming to investigate if there is sexual dimorphism and identify the onset of sexual maturity in female individuals. By thoroughly examining the stomach contents, we observed that the majority of specimens retain food remnants, with a prevalence of small prey items such as Hymenoptera and Isoptera. Our findings reveal a lack of sexual dimorphism in terms of body size, indicating that males and females possess similar dimensions and proportions. Moreover, our investigations suggest that different color patterns do not

correspond to distinct stages of female development, as chromatic variations are observed in both juvenile and ovigerous females. Consequently, dorsal coloration may not serve as a reliable indicator of sexual maturity. The observed chromatic variation is likely a form of polymorphism within the population, lacking the potential to determine life stages. Furthermore, our examination of female fertility reveals the presence of one to two eggs per female, consistent with findings in other Teiidae species.

Keywords: Caatinga, Lizard, Morphology, Trophic-ecology, Whip-Tail-Lizards

INTRODUCTION

Understanding life history parameters such as age of sexual maturity, growth rate, and reproductive mode is crucial for comprehending the evolution of species' ecological patterns (Almeida-Santos *et al.* 2014, Ramiro 2015). In addition, the timing of sexual maturation is an essential part of a species' life history strategy and is influenced by the duration of activity seasons (Adolph & Porter 1996, Brandt 2010). Lizard species that have shorter activity seasons, for instance, are known to have high annual survival rates, delayed sexual maturity, and long lifespan as a result of cooler thermal environments. On the other hand, longer-active lizard species have the opposite traits (Dunham *et al.* 1988, Brandt 2010). Early reproduction usually offers increased fitness for the offspring population during favorable times, but it also reduces the growth and size that the individual can reach later, as energy that could be used for growth is diverted for reproduction (Brandt 2010). Nevertheless, there is still limited information available on the reproductive aspects of tropical Squamata (Tsai & Tu 2000, Ramiro 2015, Xavier *et al.* 2022).

Sexual dimorphism (SD), which refers to the morphological differences between males and females, is a widespread phenomenon observed across various animal species, including reptiles (Srichairat 2016). Lizards, in particular, provide an excellent model for studying SD due to their remarkable variation in size (Cox *et al.* 2003). When it comes to sexual size dimorphism, two

fundamental hypotheses have been proposed. The first is the intrasexual selection hypothesis, which suggests that sexual selection favors larger males (Thompson & Withers 2005). This hypothesis is based on the idea that larger males may have a competitive advantage in securing mates and defending territories against rivals. The second hypothesis is the fertilization advantage hypothesis, which proposes that natural selection favors larger females (Thompson & Withers 2005). According to this hypothesis, larger females may have increased reproductive success, as they can produce larger clutch sizes or invest more resources into individual offspring. These two hypotheses provide contrasting explanations for the evolution of sexual size dimorphism in lizards. Furthermore, studying sexual dimorphism in lizards contributes to our broader understanding of the evolutionary processes shaping the diversity and ecological dynamics of animal species (Thompson & Withers 2005).

In addition to the aspects of their natural history, lizards exhibit diverse trophic ecologies that encompass two fundamental foraging strategies: sedentary foraging ambush and active foraging (Huey & Pianka 1981, Stephen *et al.* 2007, Verrastro & Ely 2015). The diet of lizards is influenced by morphological aspects such as head and jaw size, resulting in differences in quantity and diversity (Fitch 1978). Different species of lizards have evolved distinct feeding adaptations, allowing them to exploit specific food sources more efficiently (Fitch 1978). Thus, by studying a wide range of lizard species, particularly those with limited available information, such as those within the genus *Ameivula*, we can gain a deeper understanding of the intricate trophic ecologies exhibited by these reptiles.

The genus *Ameivula* comprises 11 species (Silva *et al.* 2020) and exhibits a wide distribution ranging from the Brazilian Northeast to northern Argentina (Arias *et al.* 2014). These authors also among the members of this genus, *Ameivula nigrigula* (Arias, de Carvalho, Rodrigues e Zaher 2011) (Squamata: Teiidae) (Figure 1) stands out due to its distinct anatomic features, including a dark black gular region and occasional sublabial region. This species is endemic to the Caatinga and is known to undergo ontogenetic alterations in the color pattern of

males, display sexual dimorphism in size, and exhibit marked sexual dichromatism (Arias *et al.* 2011). Despite its unique features, no studies have been conducted to investigate any aspect of its natural history.

Considering all these aspects, we aim to provide a comprehensive description of the biological aspects of *Ameivula nigrigula*, by examined its diet, conducting morphometrical analyses and providing chromatic patters on the gender of individuals collected in Sento Sé (10°27'36"S, 41°27'00"W) in the Campo Largo Phase II Wind Complex in the state of Bahia, Brazil. Additionally, we evaluate the presence of sexual dimorphism in this species and determine the sexual maturation of females by correlating the presence of eggs in the oviduct with ontogenetic changes in dorsal coloration.

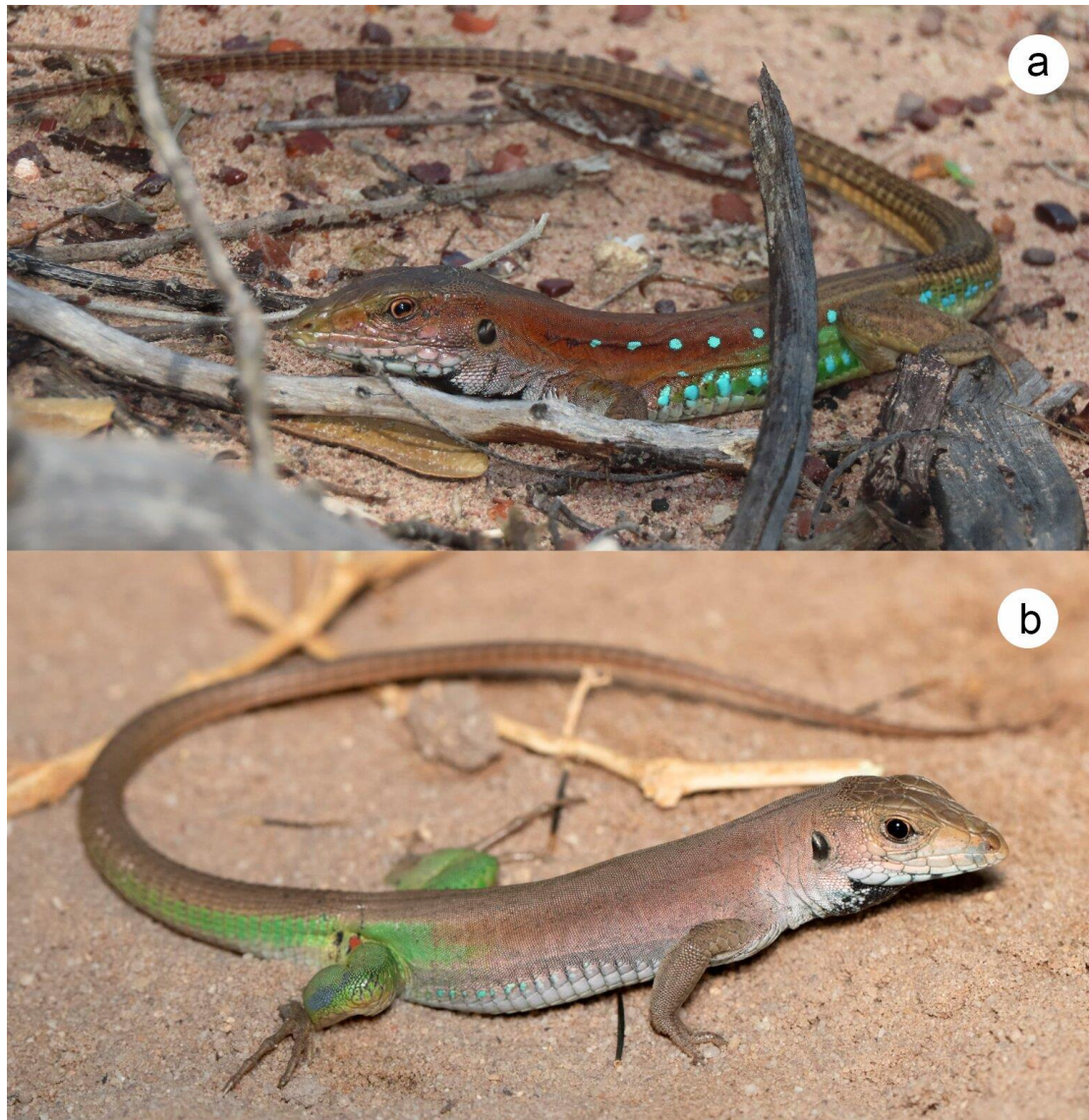


Figure 1. 1a – Male of *Ameivula nigrigula* from Umburanas municipality, state of Bahia, Brazil, and 1b – Female of *Ameivula nigrigula* from Sento Sé municipality, state of Bahia, Brazil.

MATERIALS AND METHODS

We collected the lizards (*Ameivula nigrigula*) in August of 2019, November of 2020, February and April of 2021 in Sento Sé (10°27'36"S, 41°27'00"W) in the Campo Largo Phase II Wind Complex in the state of Bahia, Brazil. The specimens were collected from pitfall traps, the method was employed at five sampling points, each composed of two lines of pitfalls located at least 1.000 m apart. Each station consisted of 20 buried buckets of 20 liters, set 5 m apart and

connected by drift fences (50 cm high). We checked the buckets daily and kept them open for eight days per expedition, resulting in a total of 6.400 buckets-day (200 buckets x 8 days x 4 expeditions). We fixed organisms in formaldehyde 10%, preserved in 70% ethanol and stored in the Zoological Collection of the Federal University of Mato Grosso do Sul (ZUFMS-REP).

We dissected and analyzed the stomach contents of all individuals under a stereomicroscope to identify the dietary prey up to the order level. We classified highly digested items as undefined. With the aim of estimating the importance of different prey in *A. nigrigula* diet, we calculated the Relative Importance Index (IRI) by summing the numerical (N) and volumetric (V) percentage values and multiplying them by the frequency of occurrence percentage value (F), where $IRI = (N + V) F$ according to Pinkas et al (1971).

To measure the frequency of occurrence (F), we estimated the number of stomachs in which a certain prey was found in relation to the total number of stomachs. The volume (V) was calculated by measuring each item present in the stomach and applying the proposed ellipsoid formula by Mayhew (1963): $V = 4/3 \pi ab^2$, where “a” is 1/2 of the longest prey length and “b” is 1/2 of the shortest prey length. The percentage of elements consumed by each lizard was also performed. The volume of sand, dust and rocks found were ignored in these calculations.

For sexual dimorphism, we analyzed the presence of the hemipenis and testes in males, and the presence of eggs, ovary and corpus luteum in females. We then measured the individuals using a digital caliper (mm), resulting in a total of 19 raw measures: snout-cloacal length (SCL): measured from the tip of the snout to the anterior margin of the cloaca; snout-forelimb length (SFL): tip of muzzle to shoulder; forelimb length (FL): axilla to the tip of the fourth finger extended at right angles; axilla-groin length (AGL): distance between the forelimb and hindlimb; tail length (Tail): measured from the posterior edge of the cloaca to the tip of the tail; cloacal length (CL): greater width of the base of the tail; head width (HW): largest cross-sectional measurement of the head; internal distance (ID): distance between the nostrils of the snout; head length (HL): measured from the tip of the snout to the posterior margin of the mandible; head

height (HD): distance between the top of the head and the deepest part; mouth length (ML): maximally extended from the ventral edge of the lower jaw; snout to nostril (SN): the tip of the snout to the anterior edge of the nostril; distance between the nostril and the anterior edge of the eye (DBNE); distance from the posterior border of the eye to the anterior border of the eardrum (DBET); eye length (EL): the anterior edge of the orbit to the posterior edge of the orbit; snout to anterior edge of eye (SL1): the tip of the snout to the anterior edge of the eye; snout to back edge of eye (SL2): the tip of the snout to the back edge of the eye; snout to anterior border of eardrum (ST): the tip of the snout to the anterior border of the eardrum; tympanum diameter in vertical (TDV): from the dorsal edge of the tympanum to the ventral edge of the tympanum; tympanum diameter in horizontal (TDH): from the anterior border to the posterior border of the eardrum (Nattawut *et al.* 2016).

To assess sexual dimorphism in size, we employed a principal component analysis (PCA) and extracted the first three principal components for ordination. Subsequently, a multivariate analysis of variance (MANOVA) was conducted using these components. Individuals with autotomy or those that did not develop gonads were excluded from these calculations. All statistical analyses were performed using the R software (R Core Team 2022) with the Vegan package (Oksanen 2007, Ganci *et al.* 2018). Our analyzes of coloration in relation to life stage followed the patterns established by Arias (2011).

The spawning size was estimated based on the number of eggs present in the females. Egg volume was calculated using the ellipsoid formula (Mayhew 1963, Verrastro & Ely 2015): volume: $4/3 (1/2 \text{ length}) (1/2 \text{ width})^2$. After confirmation of data normality by the Shapiro-Wilk analysis, we performed a linear regression between the snout-cloacal length (SCL) and the volume of the eggs, to verify the influence of the size of the females on egg volume.

RESULTS

From the 80 individuals analyzed, we found 29 empty stomachs. A total of 51 prey items were identified and classified at the order, suborder (Isoptera), and family (Hymenoptera) levels, (Rafael *et al.* 2012): Hymenoptera (Formicidae and non-Formicidae), Isoptera, Coleoptera and Eudoscordion (Table I). The most frequent items in the diet were Hymenoptera – Formicidae (36.92%) and Isoptera (23.08%). The preys with the highest total volume were Isoptera (11.72%) and Hymenoptera – not Formicidae (2.44%). The relative importance index (IRI) demonstrated that Hymenoptera - Formicidae (IRI = 81.07), and Isoptera (IRI = 62.97) were the preferred items in the diet. Nematode parasites of the genus *Physaloptera* were found in the intestines of two individuals.

Table I. Prey consumed by *Ameivula nigrigula* in Sento Sé, Bahia - Brazil (n = 80).

Prey categories	F	F (%)	N	N (%)	V	V (%)	IRI
Hymenoptera Formicidae	24	36.92	91	42.72	18949.45	1.42	81.07
Not Formicidae	5	7.69	9	4.23	32572.37	2.44	14.36
Isoptera	15	23.08	60	28.17	156234.06	11.72	62.97
Coleoptera	2	3.08	2	0.94	186.26	0.01	4.03
Eudoscordion	2	3.08	2	0.94	29.14	0.00	4.02
Unidentified	17	26.15	49	23.00	1125152.03	84.40	133.56

Frequency of occurrence in stomach contents (F), number of items (N), volume (V) and index of relative importance index (IRI) by prey item.

A total of 59 analyzed individuals were females (73.75%), while 21 were males (26.25%). In our sample, 11 individuals had autotomy (13.75%) and 29 lacked fully developed gonads (43.75%), which we did not include in the morphometric analysis. Morphological measures are shown in Table II. The males had a SCL with Mean = 71.91 and SD = 7.76, while females had a mean SCL of 68.43 and SD = 5.76. Despite male *Ameivula nigrigula* being longer, the p-value obtained for the individual coefficients of the MANOVA model for sexual dimorphism was not statistically significant (F= 1.6; p > 0.05) (Figure 2).

Table II. Morphometric variation of *Ameivula nigrigula* in Sento Sé, Bahia – Brazil (n = 40).

Morphometric character	Male (N=17)			Female (N=23)		
	Mean ± SD	Min	Max	Mean ± SD	Min	Max
SCL	71.91 ± 7.76	60.66	85.01	68.43 ± 5.76	58.57	83.22
SFL	21.26 ± 2.89	17.14	27.73	19.34 ± 2.71	16.16	25.82
FL	5.63 ± 0.97	4.25	7.28	5.77 ± 1.47	3.46	8.31
AGL	35.56 ± 4.88	27.71	44.63	35.22 ± 4.45	27.45	43.97
fiTail	135.22 ± 32.34	88.96	186.2	131.66 ± 28.24	59.09	175.46
CL	6.35 ± 1.19	4.29	8.02	5.66 ± 1.10	3.56	7.97
HW	10.60 ± 1.78	7.83	13.81	9.33 ± 1.35	7.48	12.75
ID	2.30 ± 0.31	1.77	2.85	1.92 ± 0.35	1.08	2.56
HL	20.63 ± 2.64	16.92	25.83	18.98 ± 2.20	15.82	25.12
HD	8.36 ± 1.44	6.12	10.87	7.15 ± 1.08	5.84	10.12
ML	15.58 ± 2.44	12.38	20.32	14.29 ± 1.59	11.44	18.08
SN	1.94 ± 0.23	1.6	2.28	1.82 ± 0.24	1.28	2.32
DBNE	6.15 ± 0.81	5.22	8.35	5.58 ± 0.70	4.38	6.8
DBET	3.92 ± 0.96	2.44	5.54	3.25 ± 0.86	2.04	4.88
EL	7.20 ± 0.70	5.48	8.13	6.76 ± 0.76	5.18	8.26
SL1	8.69 ± 1.05	7.08	10.88	8.03 ± 0.76	6.71	9.89
SL2	14.94 ± 1.54	12.04	17.37	14.04 ± 1.27	12.05	16.66
ST	18.14 ± 2.42	14.25	22.64	16.23 ± 1.98	13.62	20.29
TDV	2.90 ± 0.38	2.37	3.7	2.72 ± 0.35	2.2	3.45
TDH	2.98 ± 0.69	2.1	4.16	2.57 ± 0.42	1.88	3.47

SCL = snoutevent length; SFL = snoutforelimb length; FL = forelimb length; AGL = axilla-groin length; Tail = tail length; CL = cloacal length; HW = head width; ID = internarial distance; HL = head length; HD = head depth; ML = mouth length; DeL = dewlap length; SN = snout to nostril; DBNE = distance between nostril to anterior edge of eye; DBET = distance between posterior edge of eye to anterior edge of tympanum; EL = eye length; SL1 = snout to anterior edge of eye (snout length 1); SL2 = snout to posterior edge of eye (snout length 2); ST = snout to anterior edge of tympanum; TDV = tympanum diameter in vertical; TDH = tympanum diameter in horizontal.

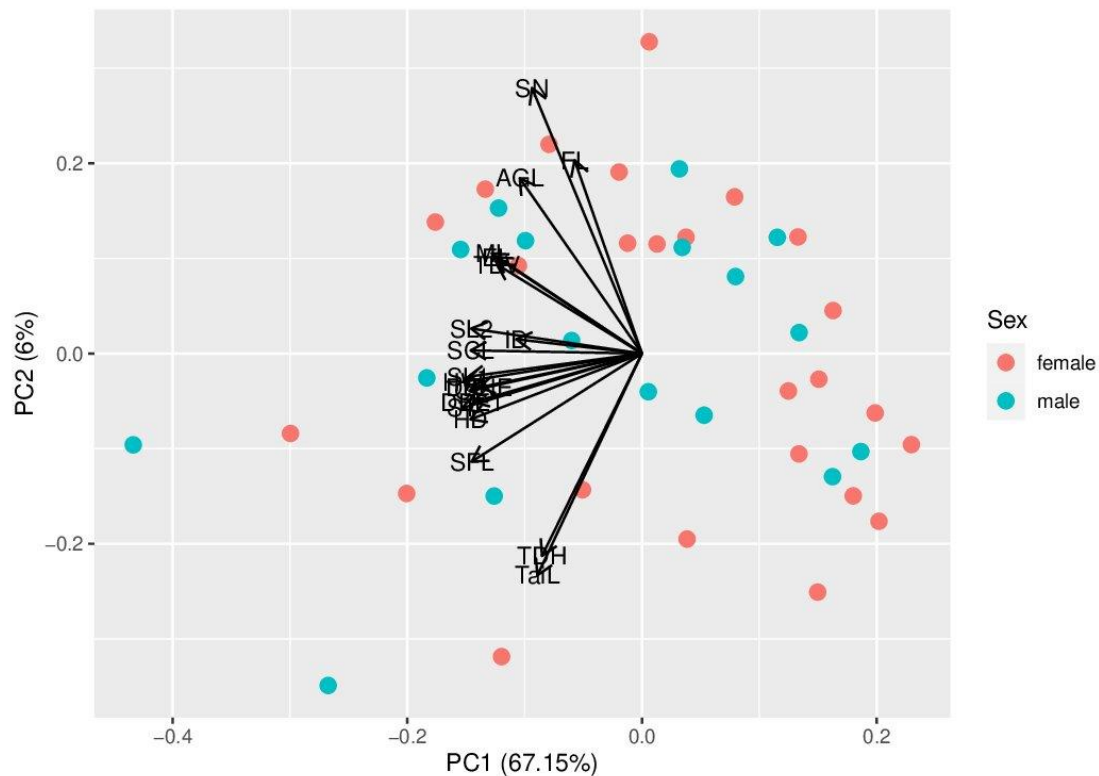


Figure 2. Principal Component Analysis of morphological differences between males (M) (n = 17) and females (F) (n=23) of *A. nigrigula* from Sento Sé, Bahia – Brazil. SCL = snoutevent length; SFL = snoutforelimb length; FL = forelimb length; AGL = axilla-groin length; Tail = tail length; CL = cloaca length; HW = head width; ID = internarial distance; HL = head length; HD = head depth; ML = mouth length; DeL = dewlap length; SN = snout to nostril; DBNE = distance between nostril to anterior edge of eye; DBET = distance between posterior edge of eye to anterior edge of tympanum; EL = eye length; SL1 = snout to anterior edge of eye (snout length 1); SL2 = snout to posterior edge of eye (snout length 2); ST = snout to anterior edge of tympanum; TDV = tympanum diameter in vertical; TDH = tympanum diameter in horizontal.

From the 59 females, 17 individuals had eggs inside their oviducts (28.73%). In particular, 12 of them carried two eggs (70.56%), while five individuals carried a single egg within their oviducts (29.40%). Notably, all females that exhibited eggs demonstrated considerable variability in their size, with the smallest female measuring SCL at 59.30 mm, while the largest ovigerous female reaching an SCL of 75.39 mm (Figure 3). Additionally, we observed a variable coloration pattern among the females, noting that mature individuals did not exhibit a strict pattern. Through our linear regression analysis, we found that the increase in the female size did not significantly influence the egg volume ($F = 0.728$; $p = 0.406$) (Figure 4).

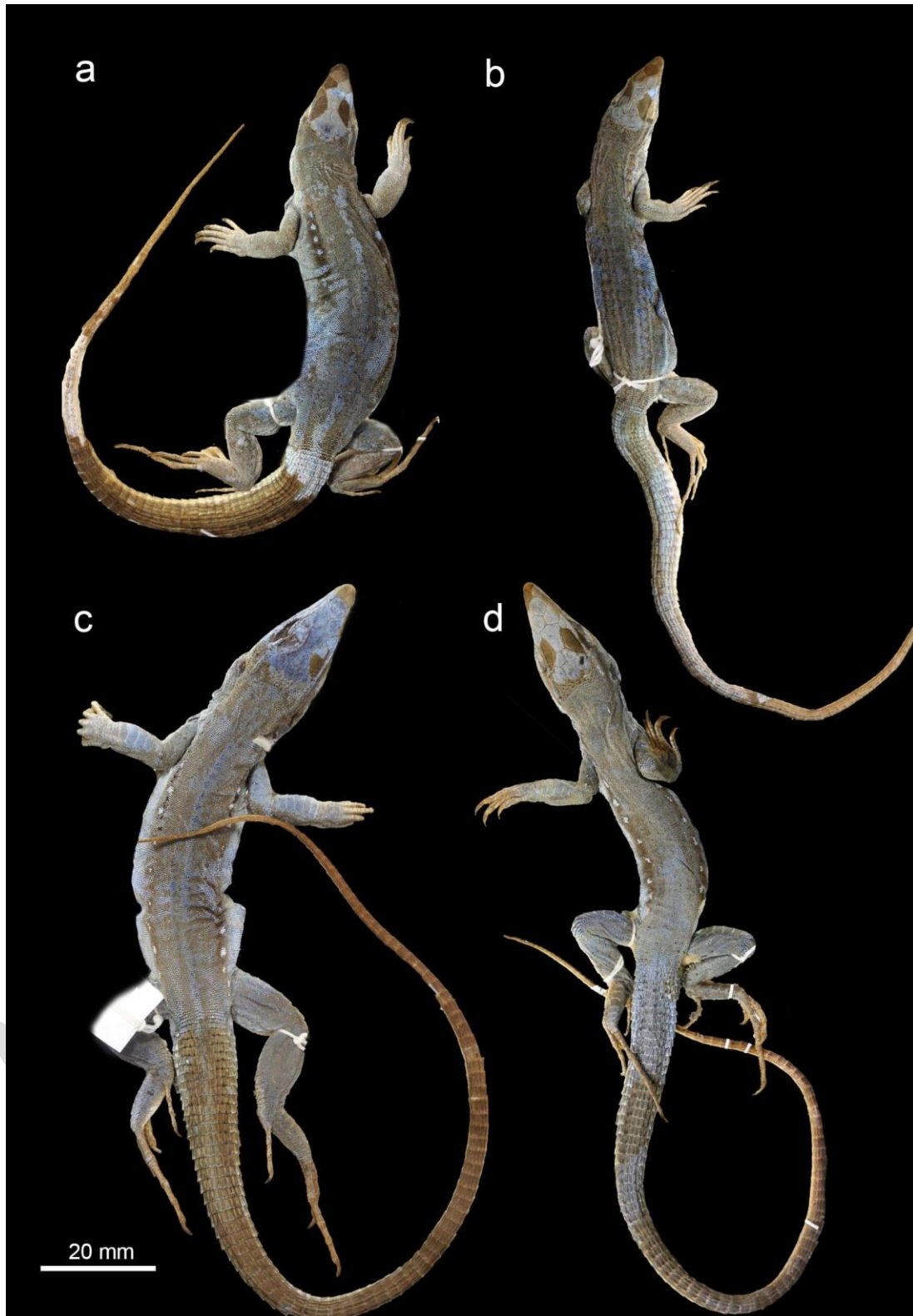


Figure 3. *Ameivula nigrigula* dorsal pattern mature females from Sento Sé, Bahia - Brazil. a - Female with adult dorsal pattern, SCL = 75.39 mm, 2 eggs; b - Juvenile female, SCL = 59.30 mm, 1 egg; c - Semi-adult female, SCL= 67.04mm, 1 egg; d - Semi-adult female, SCL = 66.36mm, 2 eggs (n = 23).

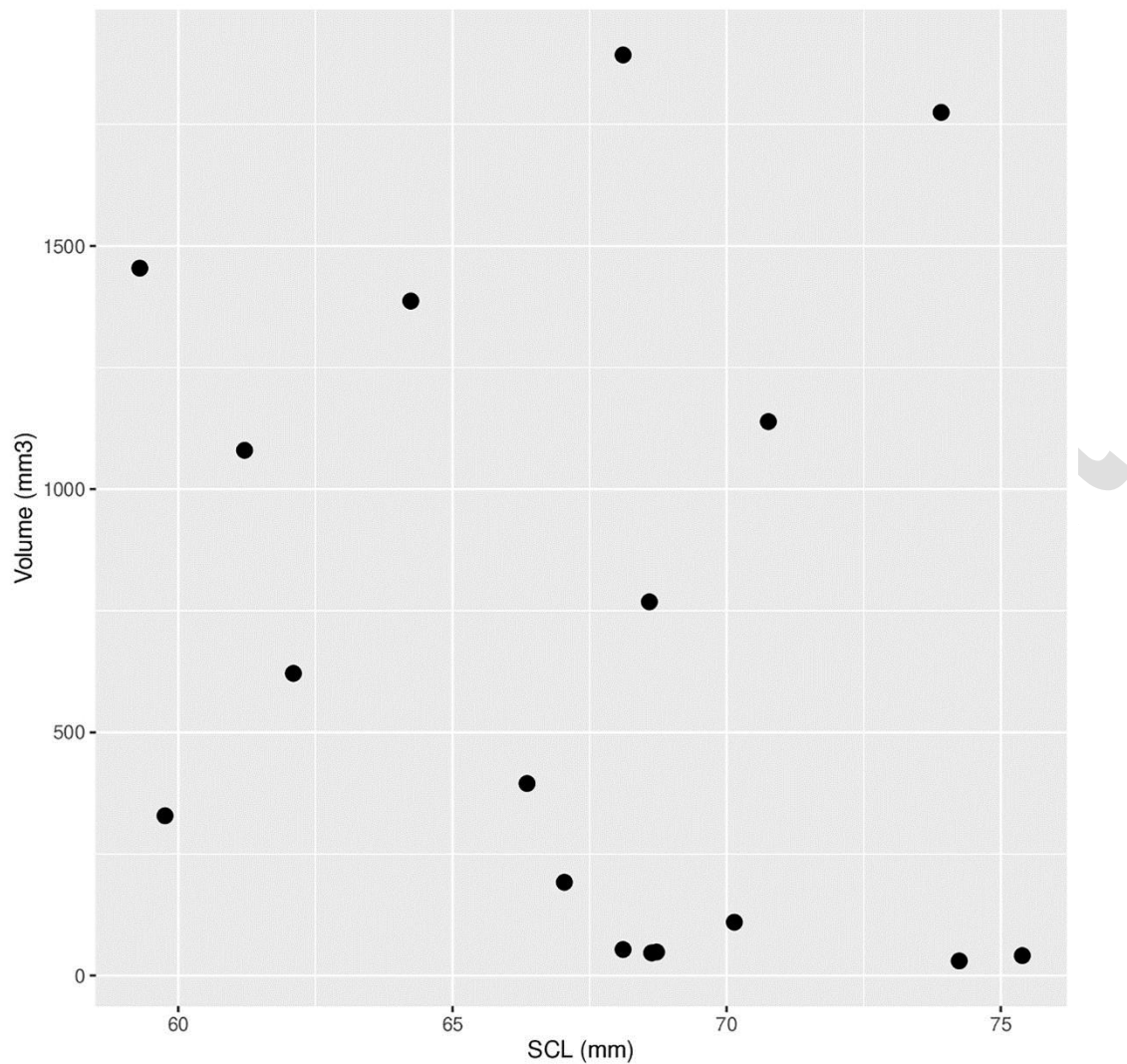


Figure 4. Linear regression analysis of the snout-cloacal length (SCL; mm) and the egg volume (mm³) of *A. nigrigula* from Sento Sé, Bahia – Brazil (n = 23).

DISCUSSION

The dietary composition of *Ameivula nigrigula* predominantly consisted of small insects, with Hymenoptera and Isoptera comprising the most frequently consumed prey items. Nevertheless, larger prey items such as Coleoptera were observed only in a limited number of large adult individuals. As discussed by Verrastro & Ely (2015) for *Liolaemus occipitalis* in Southern Brazil, this pattern can be attributed to the selective feeding behavior for this species, likely driven by the need to acquire essential nutrients while minimizing energy expenditure during foraging activities. In addition, the presence of nematode parasites found in only two

individuals may indicate that lizards of *A. nigrigula* are not major hosts of *Physaloptera* (Dorigo *et al.* 2014), once the frequency was low.

The morphological data, combined with the examination of character proportions between males and females, strongly suggest that there is no significant sexual dimorphism in terms of overall size. Males of *A. nigrigula* exhibit a slightly higher SCL than females, but it was not statistically significant ($p > 0.05$), as observed in *Cnemidophorus lacertoides* (Feltrim 2002). This pattern differs from other genus and other Teiids such as *Ameivula ocellifera*, *Ameiva ameiva* and *Cnemidophorus tigris* (Anderson & Vitt 1990).

The suggested correlation between the color patterns on the dorsal region and the reproductive investment of females, as proposed by Arias *et al.* (2011), was not corroborated in our findings. Our observations revealed the presence of ovigerous females displaying both juvenile and semi-adult color patterns on their backs (Figure 4). Consequently, it is plausible to hypothesize that these color patterns are potentially associated with sexual recognition, with males exhibiting more prominent chromatic variations compared to females. Therefore, the observed chromatic variation is characterized as polymorphism within the population and cannot be utilized as a determinant of different life stages in *Ameivula nigrigula*.

The genus *Ameivula* activity levels are commonly related to different temperature levels, and in the Caatinga, reproduction is continuous, where distinct seasons are identified (Henriques 2014). These characteristics of the genus are present in the species with evidence of maturation and multiple spawning in smaller individuals, considered juveniles. Males and females of *A. nigrigula* are reproductively active in the rainy season (November 2016 and April 2017) (Xavier *et al.* 2022). The reproductive period coincided with the rainy season in many tropical lizards, such as *A. ameiva*, *Tropidurus etheridgei*, *T. guarani*, *T. itambere*, *T. oreadicus* (Colli 1991, Ferreira *et al.* 2011, Santos *et al.* 2015, Xavier *et al.* 2022). The importance of rainfall is associated with the greater availability of food resources, such as greater abundance of arthropods, and greater humidity that prevent eggs from desiccating (Xavier *et al.* 2022). A small, fixed clutch size (one

to two eggs) generally allows females to produce litters quickly and successively, as well as continuously (Diehl 2007). *Ameivula nigrigula* has a small clutch size, similar to other congeners from different geographic areas, such as *A. jalapensis*, *A. ocellifera* and *A. xacriaba* (Colli *et al.* 2009, Arias *et al.* 2014, Menezes & Rocha 2014, Xavier *et al.* 2022). Due to the fixed number of the clutch size (two eggs), females would not be under pressure for sexual selection. Thus, the increase in female body size would not be pressured by a potential increase in the number of eggs per spawning, and we would expect the absence of differential selective pressure between the sexes (Anjos 2004, Diehl 2007).

In conclusion, our study on *Ameivula nigrigula* provides valuable insights into its dietary composition, morphology, color patterns, and reproductive behavior. The data reveals a diet predominantly consisting of small insects, with selective feeding behavior and sexual dimorphism in head size. Contrary to previous suggestions (Arias *et al.* 2011), color patterns do not correlate with reproductive investment in females but likely serve as sexual recognition cues. The species exhibits a small, fixed clutch size, indicating no selective pressure on female body size.

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