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Reproductive Biology of *Ameivula nigrigula* (Squamata: Teiidae) in Caatinga Domain, Northeastern Brazil

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***Abstract*.—*Ameivula nigrigula* is an endemic species to the transition zone Caatinga between the Caatinga and Cerrado Domain in Brazil in Caatinga vegetation. It was recently described, but little is known about its biology and ecology. We studied the reproductive biology of this species in Caatinga vegetation in Bahia state, Brazil. The minimum size at maturity in females was 65.5 mm snout-vent length (SVL), whereas in males it was 54.2 mm SVL. Clutch size of *A. nigrigula* varied from 1 to 2 vitellogenic follicles (median = 2); it was not significantly related to female body size. A simultaneous presence of vitellogenic follicles and corpora lutea suggest that *A. nigrigula* produces more than one clutch per breeding season. The reproductive period was synchronous for both sexes and occurred during the rainy season and likely was associated with the presence of microclimate conditions favorable to the embryonic development and survival of the offspring. This study revealed important information about the reproductive biology of *A. nigrigula* that may contribute to an adequate consevation plan for this speciess.**

Key Words.—endemic; natural history; São Francisco River; rainy season; reproduction

Introduction

Reproductive patterns in squamates are often influenced by the environment, reproductive mode (oviparous or viviparous), and phylogeny (Fitch 1970; Méndez-de la Cruz et al. 1998; Mesquita et al. 2016). These patterns are important enhancing knowledge about the of ecological and biological characteristics of the life history of these organisms (Verrastro and Krause 1999; Balestrin et al. 2010). According to Sherbrooke (1975), lizards have three types of reproductive patterns: (1) continuous reproductive activity occurs throughout the year; (2) continuous with variation as individuals show gonad volume or fertility variations in some months of the year; and (3) non-continuous where there is a period when all individuals in the population are reproductively inactive.

Environmental factors, such as precipitation, temperature, photoperiod, and availability of resources have been associated with the reproductive patterns of lizards (Fitch 1970; Wiederhecker et al. 2002; Ribeiro et al. 2012). In temperate and tropical regions environmental variability influences mating, egg development, and hatching (Fitch 1970; Méndez-de la Cruz et al. 1998; Mojica et al. 2003). In tropical regions, reproductive patterns can vary from continuous to seasonal (Ribeiro et al. 2012). Rainfall is one of the main factors for these patterns, as it allows favorable conditions for reproduction, stimulating gonadal activity, egg development, preventing their desiccation, and greater availability of food resources for the survival of the species (Ramírez-Bautista et al. 2000; Valdéz-Gonzáles and Ramírez-Bautista 2002; Mesquita and Colli 2003b; Ribeiro et al. 2012). Continuous reproductive patterns are observed in species of the family Teiidae in tropical regions, such as *Calango-verde* (*Ameiva ameiva*; Vitt 1982), *Lagartinho-de-linhares* (*Ameivula nativo*; Menezes et al. 2004), *A. ocellifera* (no common name [NCN]; Vitt 1983; Mesquita and Colli 2003a; Zanchi-Silva et al. 2014), and *Calango-listrado* (*Cnemidophorus lemniscatus*; Mojica et al. 2003).

The genus *Ameivula* comprises 11 species (e.g., Rocha et al. 1997; Colli et al. 2009; Cabrera 2012; Silva and Ávila-Piris 2013; Arias et al. 2018). *Ameivula nigrigula* (NCN; Fig. 1) was recently described, though little is known about its biology and ecology. For this species there are studies on cannibalism (Travassos et al. 2017), habitat use and diet (Xavier et al. 2019a), parasitism (Xavier et al. 2019b), and predation (Leite et al. 2019). It is endemic to the transition zone between the Caatinga and Cerrado domains where it occupies sandy herbaceous-shrubby environments (Arias et al. 2011; Da

Figure 1. (A) An adult *Ameivula nigrigula* (no common name) from Serra de Santo Inácio, municipality of Gentio do Ouro, Bahia state, northeastern Brazil. (B) General view of a sandy herbaceous-shrubby environments, the main habitat used by *A. nigrigula* in the study site. (Photographed by Maria Aldenise Xavier).

Silva and Soares 2018; Sousa et al. 2019; Fig. 1) in Bahia and Minas Gerais states. The Caatinga is a rich domain that provides a refuge for some endemic species (Leal et al. 2003); however, the progressive anthropization of the Caatinga has resulted in a continuous loss of habitat quality (Leal et al. 2003; Brasil. 2018. Livro Vermelho da Fauna Brasileira Ameaçada de Extinção. Instituto Chico Mendes de Conservação da Biodiversidade. Available from https://www.icmbio.gov.br/portal/images/stories/comunicacao/publicacoes/publicacoes-diversas/livro_vermelho_2018_vol1.pdf [Accessed 29 August 2021]). Thus, many of these species are nearing extinction even before they have been studied (Xavier et al. 2021). According to the Brazilian Red List, *A. nigrigula* has been categorized as Data Deficient (DD) due to lack of knowledge about the species (Brasil *op. cit*.). We analyzed the reproductive biology of *A. nigrigula* to determine (1) the reproductive phases of *A. nigrigula* in the rainy season, (2) the minimum maturation size for males and females of this species, (3) the clutch size of *A. nigrigula*, and (4) the clutch size of females.

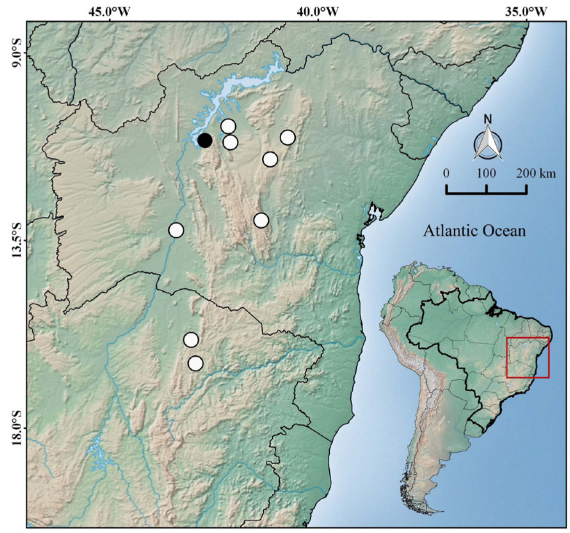

Figure 2. Geographic distribution of *Ameivula nigrigula* (no common name) in Brazil. Solid circle represents the population examined in this study at Caatinga Santo Inácio, Gentio do Ouro, Bahia state, Brazil.

Materials and Methods

Study area.—The Serra de Santo Inácio (10°45’41’’S, 37°20’2’’W; datum: WGS84; 520 m elevation) is located in the municipality of Gentio do Ouro, Bahia state, Brazil. The study site is located in the Caatinga (Fig. 2), an environment with herbaceous arboreal vegetation in the sand dune region along the lower-mid course of the São Francisco River. The Caatinga is known for its quartzitic rock outcrops, sandy shallow soils, and vegetation with an abundance of plant species within the Bromeliaceae, Cactaceae, Euphorbiaceae, and Leguminoseae (Arias et al. 2011). The regional climate is hot semi-arid (BSh in the Köppen classification system) with low rainfall rates throughout the year (annual average of 687 mm) and a mean annual temperature of 24.9° C (Fig. 3). This Caatinga presents two well-defined seasons with rainfall occurring between November and April followed by an extended dry season between May and October (http://pt.climate-data.org).

Methodological procedures.—We collected 23 lizards (15 males and eight females) of *A. nigrigula* during 2 mo within the rainy season (November 2016 and April 2017). We captured lizards using the rubber band technique (Franco and Salomão 2002), recorded their snout-vent length (SVL) with digital calipers (to the nearest 0.01 mm), and euthanized them in accordance with the standards of the Conselho Nacional de Controle de Experimentação Animal (Brasil. 2013. Diretrizes da Prática de Eutanásia do Conselho Nacional de Controle de Experimentação Animal - CONCEA. Available

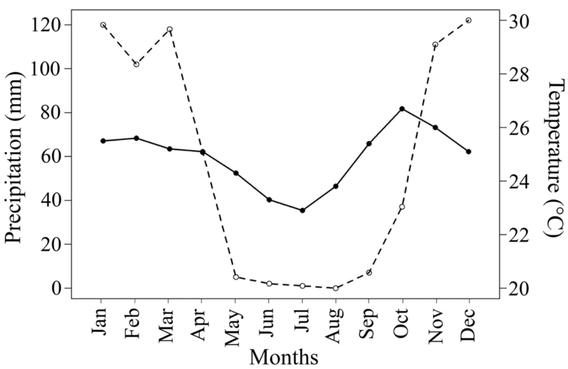

Figure 3. Climatic diagram with average air temperature (in °C; solid line) and precipitation (in mm; dashed line) for the Caatinga Santo Inácio, Gentio do Ouro, Bahia state, Brazil. The data were based on monthly verages from 1982–2012 (https://en.climate-data.org/).

from https://www.in.gov.br/materia/-/asset_publisher/Kujrw0TZC2Mb/content/id/31061978/do1-2013-09-26-resolucaonormativa-n-13-de-20-de-setembro-de-2013-31061974 [Accessed 29 August 2021]) and immediately fixed specimens with 10% formalin. We deposited specimens in the Laboratório de Biologia e Ecologia de Vertebrados (LABEV) at the Universidade Federal de Sergipe, Brazil.

We dissected individuals to examine their gonads. We recorded the number of vitellogenic follicles in each ovary (yellow follicles and > 1.4 mm in diameter in in accordance with Menezes and Rocha 2014) in each ovary, number of eggs in each oviduct, and presence of corpora lutea in each ovary. We estimated clutch size by the number of eggs, when present, or vitellogenic follicles. We estimated the minimum size at sexual maturation based on the size of the smallest female with eggs in the oviduct or vitellogenic follicles in the ovaries. We based the morphological characterization of the follicular cycle, to assess the reproductive condition of females, on the stages proposed by Santos et al. (2015). We considered the simultaneous presence of vitellogenic follicles, eggs in the oviducts and/or corpora lutea as indicative of more than one clutch per breeding season (Galdino et al. 2003).

For histological analysis, we fixed the testes and ovaries (right gonads) of *A. nigrigula* in 10% formalin, dehydrated gonads in a graded series of ethanol, cleared in xylol, and embedded them in paraffin. We sectioned tissues at 5 µm thick, and we prepared slides using the hematoxylin-eosin (HE) staining technique. The method we used to fix tissues resulted in shrinkage, however, and because of this, we could not make measurements of the tissues, such as germinative epithelium height and seminiferous tubule diameter. We photographed the slides with a digital camera (Model ICC50 W; Leica Microsystems, Heerbrugg, Switzerland), coupled to the bright field illumination microscope (Model DM750; Leica Microsystems) and the Leica Aplication Suite (LAS) EZ version 3.4 to characterize the reproductive phases of each individual.

The classification of testicular activity was based on the five reproductive stages defined by Vieira et al. (2001). The minimum size at sexual maturity was determined as the SVL of the smallest male with spermatozoa. According to Vieira et al. (2021), throughout spermatogenesis, germ cells varied in seminiferous tubules at different stages. Stage I is characterized by having spermatogonial cells and not having a luminal opening in seminiferous tubules. Stage II, on the other hand, is characterized by its luminal opening and the seminiferous epithelium being dominated by spermatogonia and spermatocytes. Individuals in stage IV are at the peak of reproductive spermiogenesis, in which all types of spermatogenic cells are observed (spermatogonia, spermatocytes and spermatids) and a high concentration of mature spermatozoa is found in the seminiferous tubules and in the epididymal ducts.

Statistical analysis.—We evaluated the effect of female SVL on clutch size using the Spearman's Rank Correlation Coefficient (Zar 2010). We evaluated sexual dimorphism for SVL and weight using the Mann-Whitney test (Zar 2010). We tested assumptions of parametric parameters using the Shapiro-Wilk test (Shapiro and Wilk 1965) and used non-parametric tests when the normality criteria were not met (Zar 2010). The significance value was set at $P \leq 0.05$. We performed the statistical analyses and prepared the graphs using R (v3.4.2; R Development Core Team 2020).

Results

We examined 23 *A. nigrigula*, including 15 (65.2% of total) males (11 adults and four juveniles) and eight (34.8%) females (seven adults and one juvenile). The mean SVL of males was 61.47 ± 11.71 (standard deviation) mm (range, 46.1–80.4 mm), and for females was 68.96 ± 10.95 (range, 43.90–76.70 mm), and means did not differ significantly between sexes (U = 39.0, n = 8, 15; $P = 0.087$). The mean weight of males was 8.1 ± 5.0 (range, 3.0–17.5 g) and of females was 8.8 ± 2.9 (range, 3.5–12.0 g), which also were not significantly different (U = 46.0, n = 8, 15; $P = 0.183$). We collected reproductively active males and females within the same season indicating synchronic reproduction.

Seven females of *A. nigrigula* collected in November and April were reproductive, but without oviductal eggs; only one was nonreproductive. The minimum size at maturity for females was 65.5 mm based on April collections. We collected one juvenile female (SVL

= 43.9 mm) at the beginning of the rainy season. The clutch size of *A. nigrigula* varied from 1–2 vitellogenic follicles (median = 2), which was not significantly correlated with SVL ($P = 0.282$).

Histological examination of the ovaries revealed three gonadal phases for the species: pre-vitellogenic, vitellogenic, and corpora lutea (Fig. 4). In the nonreproductive period, characterized by the pre-vitellogenic phase, we observed a thick granular layer composed of cuboid and pyriform folicular cells, in addition to showing little deposition of vitelline granules in the oocyte cytoplasm (Fig. 4). At this phase, it was also possible to observe a small cell layer and the vitelline membrane. In the reproductive period, represented by the vitellogenic phase (Fig. 4), the granular membrane had a lesser thickness and was characterized by a large number of vitelline granules in the oocyte cytoplasm. The post-ovulatory or luteal phase was characterized by the presence of granulosa cells deposited uniformly throughout the follicular cavity forming a mass of heterogeneous cells (Fig. 4). We found six females with both vitellogenic follicles and corpora lutea at the beginning and end of the rainy season (November and April, respectively), indicating the production of multiple clutches throughout the year.

Eleven males of *A. nigrigula* collected in November and April were reproductive (showing seminiferous tubules and epididymis with mature spermatozoa) and only four (26.7%) were nonreproductive (without spermatozoa). The minimum size at maturity for males was 54.2 mm collected in November. We collected three juvenile males (SVL = 46.1, 47.3, and 47.6 mm) at the beginning of the rainy season. All adult males we collected were reproductively active. The histology of the testes revealed three reproductive stages for the species (Fig. 4): I (n = 1), II (n = 3) and IV (n = 11).

Discussion

Ameivula nigrigula has small clutch size, similar to other congeneric species from different geographical areas, such as *A. jalapensis* (NCN), *A. nativo*, *A. mumbuca* (NCN), *A. ocellifera*, and *A. xacriaba* (NCN; e.g., Colli et al. 2003, 2009; Arias et al. 2014; Menezes and Rocha 2014). Clutch sizes in reptiles are influenced by factors, such as body morphology, foraging mode,

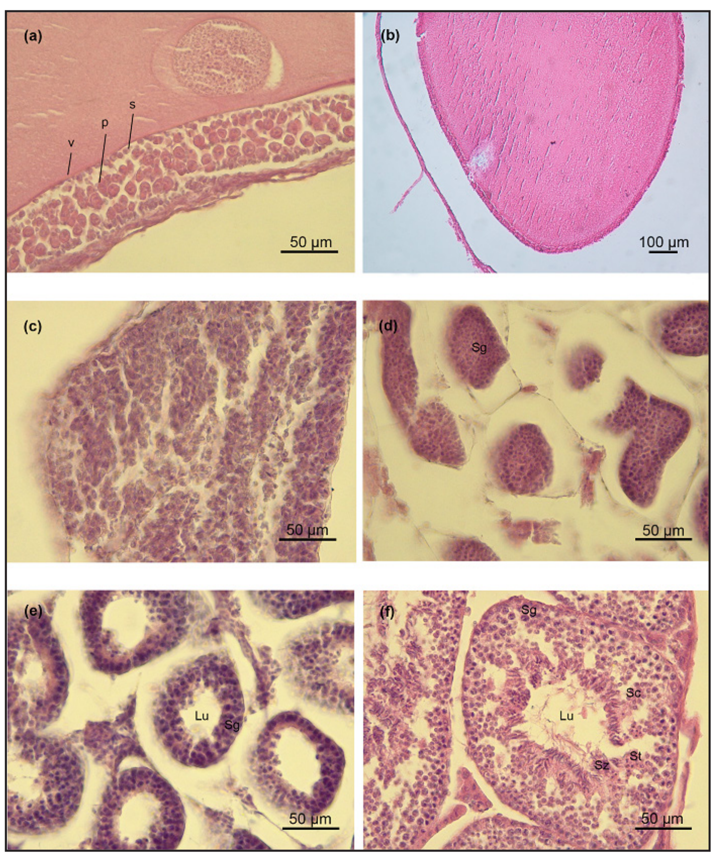

Figure 4. Histological sections of gonadal phases (A-C) of seminiferous tubules (D-F) of *Ameivula nigrigula* (no common name). Gonadal phases: (A) pre-vitellogenic; (B) vitellogenic; (C) corpora lutea. Seminiferous tubules: (D) Stage I; (E) Stage II; (F) Stage IV. Abbreviations: vitelline membrane (v), granulosa layer with the presence of pyriform cells (p), small cells (s); Lumen (Lu), spermatogonia (Sg), spermatocytes (Sc), spermatids (St), and spermatozoa (Sz).

climactic factors (e.g., precipitation, temperature, photoperiod) and food availability (Pianka and Vitt 2003; Kiefer et al. 2008; Ribeiro et al. 2012; Vitt and Caldwell 2014). The lack of a relationship between clutch size and SVL in the present study may be associated with the species morphology and foraging mode. *Ameivula nigrigula* and congeners (Family Teiidae), comprised of species with long, thin, and streamlined bodies, are active foragers (Pianka and Vitt 2003). Active foraging species actively seek their food, and consequently, are particularly exposed to predators, so a reduced clutch is advantageous, as it favors escape behavior (Pianka and Vitt 2003; Menezes and Rocha 2014).

The presence of post-ovulatory and vitellogenic follicles in the ovaries of most *A. nigrigula* females indicate the production of multiple clutches during the breeding season (Galdino et al. 2003; Santana et al. 2010; Ribeiro et al. 2012; Galina-Tessaro et al. 2021). We found corpora lutea in some females, which indicated that oviposition had occurred. Absence of eggs in the oviducts and the presence of the corpora lutea in ovaries indicate that vitellogenesis and ovulation have previously occurred (Galina-Tessaro et al. 2021). In addition, the evidence of vitellogenic follicles indicates that a new maturation of follicles has begun, and a second oviposition will possibly occur (Galina-Tessaro et al. 2021).

All adult males were collected during peak reproductive activity, with testes in stage IV, containing spermatozoa in the lumen (Vieira et al. 2001). Males and females of *A. nigrigula* were reproductively active in the rainy season (November 2016 and April 2017). The reproductive period coincided with the rainy season in many tropical lizards, such as *A. ameiva*, *Lagartinho-branco-da-areia* (*Liolaemus lutzae*), *Tropidurus etheridgei* (NCN), *T. guarani* (NCN), *T. itambere* (NCN), *T. oreadicus* (NCN), *Lagarto-de-parede* (*T. semitaeniatus*), and *T. spinulosus* (NCN; Colli 1991; Rocha 1992; Ferreira et al. 2011; Santos et al. 2015). The importance of rainfall is associated the greater avalability of food resources (as higher abundance of arthropods) and the higher humidity, that prevent desiccation of the eggs (Rocha 1996; Botes et al. 2006; Vasconcellos et al. 2010; Ferreira et al. 2013). In addition, temperature is an important factor in regulating the testicular activity of lizards, contributing to the cycle of spermatogenesis (Licht 1971; Ferreira et al. 2011).

The effect of rainfall on reproduction is known in lizards that inhabit seasonal areas, such as the Cerrado, Caatinga, and Restinga (Rocha 1992; Ribeiro et al. 2012; Santos et al. 2015). In seasonal environments, there is apparently a tendency to concentrate reproductive activity during the rainy season (Wiederhecker et al. 2002; Ferreira et al. 2011; Ribeiro et al. 2012). Some lizards have similar reproductive patterns within the same family, however, regardless of the environmental conditions in which they are exposed (Vitt 1986).

Continuous reproductive patterns are known in species of the family Teiidae in tropical regions (Vitt 1982, 1983; Mesquita and Colli 2003a; Menezes et al. 2004; Zanchi-Silva et al. 2014). Species of the genus *Ameivula* (*A. ocellifera* and *A. nativo*) have continuous reproduction (Menezes et al. 2004; Zanchi-Silva et al. 2014). Although under different environmental effects, populations of *A. ocellifera* from the Caatinga, Cerrado, and coastal zone reproduce throughout the year (Vitt 1983; Mesquita and Colli 2003a; Zanchi-Silva et al. 2014). We found juveniles in the population of *A. nigrigula* at the beginning of the rainy season, which suggests that there was oviposition and recruitment in the previous season (Wiederhecker et al. 2002) and, possibly, that reproduction of this species is continuous.

We found important information about the reproductive biology of *A. nigrigula*. Due to the loss of natural habitat, it is necessary to develop conservation measures for this species. Therefore, more detailed studies on the biology and ecology of this species may to contribute for an adequate consevation plan.

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