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Aspects of the natural history of the Sertao lancehead *Bothrops lutzi* from Brazil

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ABSTRACT - *Bothrops lutzi* is a secretive species mainly associated with the Cerrado, Carrasco and ecotonal areas of Cerrado-Caatinga vegetation. We gathered information about habitat use, diet and the parasites of this elusive species. The species was found exclusively in Carrasco vegetation habitats in the states of Piaui and Ceará, but not in the core Caatinga region. *Bothrops lutzi* appears to have a diet composed of invertebrates and small vertebrates, not changing during its ontogeny. Three endoparasites were identified, one of them, *Oswaldofilaria* sp., is the first record for a *Bothrops* spp.

INTRODUCTION

The secretive snake *Bothrops lutzi* Miranda-Ribeiro, 1915, is of the *Bothrops neuwiedi* complex (Silva & Rodrigues, 2008). The species is endemic to Brazil, occurring in the states of Bahia, Ceará, Goiás, Minas Gerais, Pernambuco, Piauí and Tocantins (Nogueira et al., 2019). *Bothrops lutzi* is mostly associated with the vegetation of Cerrado, Carrasco and ecotonal areas of Caatinga-Cerrado, with an isolated record in the Atlantic Forest of the state of Bahia (Loebmann, 2009; Guedes et al., 2014; Nogueira et al., 2019).

Brothrops lutzi is a locally rare snake (Loebmann & Haddad, 2010; Dal Vechio et al., 2013), consequently most information about this species deals with geographical distribution and taxonomy (Campbell & Lamar, 2004; Nogueira et al., 2019). In the present study, we present data on habitat use, diet and the parasites of *B. lutzi*.

MATERIALS & METHODS

Study area

We collected data during four field surveys, in three different areas (Table 1S, see Supplementary Material). Each area had four sampling points, following a gradient from Caatinga to Carrasco phytophysiognomies across east Piaui and west Ceará states in north-east Brazil (Table 1S). The four study areas were at least 20 km apart, and each sampling point was at least 1 km apart. Area 1 is mostly covered by arboreal Caatinga, with presence of 10 m canopy tree, shallow and clayey, rocky soils (Rocha et al., 2017). Areas 2 and 3 have Carrasco vegetation, a deciduous forest, with dense scrubland, sparse, short trees and white sandy soil (Araújo et al., 1999). Area 2 is more degraded than Area 3, with denser scrubland and fewer trees. In Area 3, there is a vegetation transitional gradient between Caatinga and Carrasco. The climate of the region is semi-arid (BSh, Koeppen's system), with a rainy season from January to May, and an extended dry season from June to December. The temperature ranges from 22 °C in the rainy season to 39 °C in the dry season, with a mean precipitation of 600 mm (Silva et al., 2013).

Sampling design

There were three field trips in the rainy season: 15 April–2 May 2014, 13–29 February 2016, and 8–22 May 2017; and one field survey in the dry season, 23 October–6 November 2014. In each area we collected data on the occurrence of *B. lutzi* (activity, habitat, microhabitat) using nocturnal visual constrained searches (VCS) (using four researchers), between 18:00 h and 22:00 h. Additionally, we installed pitfall traps with drift fences: there were four traps (60-litre buckets) per sampling point, separated by 1 m tall plastic fencing; incidental encounters were also recorded. For each field survey, in each area we had a standardise sampling effort of 64 pitfall traps/area, and 16 person/hours of VCS.

The *Bothrops lutzi* (Fig. 1) collected were euthanised according to ethical procedures of the Federal Council of Veterinary Medicine - CFMV (2013) and fixed according to Calleffo (2002), after authorisation by the Instituto Chico Mendes de Conservação da Biodiversidadel CMBio/SISBIO, number 66099-2, and approval by the Ethics Committee of the Universidade Regional do Cariri (CEUA/URCA, process #00260/2016.1). Specimens of *B. lutzi* were weighed on an electronic scale (precision 0.1 g) and the snout-vent length (SVL) and tail length (TL) measured with a Mitutoyo® digital caliper (precision 0.01 mm). Specimens were later deposited in the Herpetological Collection of the Regional University of Cariri (for accession codes see Table 1) state of Ceará, Brazil.

Figure 1. Adult female of *Bothrops lutzi* (URCA-H 11598), collected in Area 2, municipality of Pio IX, state of Piauí, north-eastern Brazil

We also gathered data of specimens of *B. lutzi* deposited in the Herpetological Collection of the Federal University of Ceará (UFC). They were all from the locality of Serra das Almas (RPPN Serra das Almas), municipality of Crateús, state of Ceará (for area description see Borges-Nojosa & Cascon, 2005). In the laboratory, all specimens were necropsied, sexed, and the contents of the gastrointestinal tract analysed under a stereoscopic microscope to investigate food content and/or endoparasites. The following organs - digestive tract, lungs, heart, liver and kidneys were analysed according to Amato et al. (1991). All prey items were counted and identified to the lowest taxonomic level possible, using specialised literature (Oliveira-Costa, 2011). Parasite fixation and conservation techniques were applied as recommended for each taxonomic group (Vidal-Martínez et al., 2001). For identification of parasite species, we referred to Vicente et al. (1993) and Gibbons (2010). The infection parameters analysed following Bush et al. (1997) were - % prevalence (number of parasitised host individuals divided by the total number of individuals), mean intensity of infection (number of helminths divided by the number of parasitised individuals) and mean abundance (number of helminths divided by total number of individuals in the sample).

RESULTS

In each sampling area, we undertook a total of 64 hours of visual active search and deployed a total 960 pitfall traps*days of effort. Individuals of *B. lutzi* (n = 10) were found only in Carrasco vegetation in Areas 2 and 3 (Table 1S). The visual active survey sampling effort resulted in an encounter rate of 0.05 snakes/hour (n = 3) in Area 2, where all the individuals were found in the rainy season (February and April) between 18:00 h and 20:00 h. Two adults were found foraging in sandy soil, and one juvenile was found coiled in scrubland. The pitfall traps captured five individuals of *B. lutzi*: three adults (455–550 mm SVL) in Area 2, and two in Area 3 (one juvenile and one adult). Two adult individuals of *B. lutzi* were recorded by opportunistic encounter, when crossing the roads at night.

Investigation of five *B. lutzi* (2 males, 2 females and 1 juvenile) collected during the field study (Table 1) revealed only one specimen URCA-H 13161 (male, SVL 495 mm) that had food in its digestive tract, a centipede (*Scolopendra* sp). Of the preserved, UFC specimens (n = 5), only two had food remains. UFC-2249 (male, SVL 302 mm) had parts of a teiid lizard, and UFC-2250 (female, SVL 205mm) had a *Scolopendra* sp. The other specimens had no evidence of stomach contents.

Regarding parasitism, three parasitic taxa were identified, namely: *Ophidascaris* sp., *Oswaldofilaria* sp. and *Physaloptera* sp., with an overall infection prevalence of 40 %, with four specimens infected with at least one parasite species, more information on infection can be found in Table 1.

DISCUSSION

In the states of Piauí and Ceará, we found *B. lutzi* exclusively in Carrasco and Cerrado vegetation, at altitudes above 600 m, in sandy soils (see Araújo & Martins, 1999, for the definition of Carrasco). Using a similar sampling effort in an area with arboreal Caatinga vegetation, at lower altitudes 200–300 m (Area 1), we found no *B. lutzi*, only specimens of *Bothrops erythromelas*. The occurrence of *B. lutzi* in Carrasco follows a clear distribution pattern along the Ibiapaba plateau in the state of Ceará, where the species is found exclusively in these areas (Borges-Nojosa & Cascon, 2005; Loebmann, 2009; Roberto & Loebmann, 2016). In the humid forests of Ibiapaba plateau only *Bothrops* gr. *atrox* occurs, whereas in the lower altitude areas in the Caatinga forest only *B. erythromelas* is found, showing the habitat specificity for each *Bothrops* species in the Serra da Ibiapaba Mountain range (Roberto & Loebmann, 2016). However, there are populations of *B. lutzi* found in the state of Bahia in a Cerrado enclave in Atlantic Forest (Marques et al., 2017), ecotone areas of Caatinga-Cerrado (Rodrigues & Prudente, 2011; Cavalcanti et al., 2014) and in the Cerrado of central Brazil (Recoder & Nogueira, 2007; Recoder et al., 2011).

Most inventories of herpetofauna show few records of *B. lutzi* despite being associated with long periods of sampling efforts. Amaral (1925), when describing *Bothrops neuwiedi piauiensis* (*B. lutzi*), mentioned that the species was locally abundant in Jurumenha and Fazenda Grande do Piauí, regions located in the middle Gurguéia river basin, in the state of Piauí, an area mostly covered by Caatinga-Cerrado ecotone vegetation. Almost one century later, Madella-Auricchio et al. (2017) sampled a larger region in the middle Gurguéia river basin, despite a sampling effort of two years of active visual search, did not find any *B. lutzi*, indicating the rarity of the species.

In the state of Piauí, in similar habitats from which the species was described, Rodrigues & Prudente (2011) employed 912 hours of active visual search and found only three *B. lutzi* (0.003 specimens/hour). Additionally, the authors installed pitfall traps (a total of 6,468 traps*days effort) without any captures of *B. lutzi*. In Serra da Capivara National Park, Cavalcanti et al. (2014) after 960 survey hours, found only two *B. lutzi* (0.002 specimens/hour), with 1,110 pitfall traps*days effort without any captures. In the state of Ceará, Borges-Nojosa & Cascon (2005) sampled the Carrasco

Table 1. Information on *Bothrops lutzi* specimens from a preserved collection (UFC) or collected from the field in this study (URCA): SVL-snout-vent length; TL- Tail length; NP- Number of parasites; P%- Prevalence; MII±SD- Mean Intensity of Infection ± standard deviation, and MA-Mean abundance

Voucher	Sex	Life stage	SVL	TL	NP	Infection site	Parasites	P%	MII±SD	MA	Stomach contents
UFC-2268	male	juvenile	271	45							
UFC-2249	male	adult	302	51							teiid lizard
UFC-2250	male	juvenile	205	30							*Scolopendra* sp.
UFC-2251	female	adult	440	79							
UFC-2252	male	adult	350	57	2	stomach	*Physaloptera* sp.				
					2	small intestine	*Physaloptera* sp.				
URCA-H-13161	male	adult	495	71	3	stomach	*Physaloptera* sp.	30	5 ± 0.8	1.5	*Scolopendra* sp.
					2	small intestine	*Physaloptera* sp.				
URCA-H-11598	female	adult	691.95	64.62	6	large intestine	*Physaloptera* sp.				
					15	coelomic cavity	*Oswaldofilaria* sp.	10	15	1.5	
URCA-H-11596	female	adult	455	70	2	small intestine	*Ophidascaris* sp.	10	2	0.2	
URCA-H-13162	male	adult	303	52							
URCA-H-11597	female	juvenile	187	33							

vegetation in Serra das Almas Mountain and had an encounter rate of 0.004 specimens/hour (243 hours/1 specimen). The other areas of Cerrado in the states of Tocantins (Recoder et al., 2011) and Bahia (Marques et al., 2017) also yielded few records, despite the intense sampling effort. Our sampling effort in the Carrasco had a higher encounter rate of 0.05 specimens/hour, and we also captured five individuals in pitfall traps with a total 960 traps*days effort, being the area with the highest encounter rate for the species so far. Regarding the differences within the two successfully sampled areas in the Carrasco, the higher encounter rate of Area 2 can reflect habitats preferences of *B. lutzi*, since Area 3 is more influenced by the less favourable Caatinga vegetation.

Most species of the *Bothrops neuwiedi* group have a generalist diet, reflecting an ancestral state (Martins et al., 2002), consuming mammals, lizards, amphibians, chilopods, and birds (Martins et al., 2002; Valdujo et al., 2002; Nogueira et al., 2003; Sawaya et al., 2008). Most species also undergo an ontogenetic shift in their diets, changing from ectotherms in juvenile stage to endotherms in adults (Martins et al., 2002). We found lizards in the digestive trait of a *B. lutzi* juvenile, and centipedes in both juvenile and larger adults. However, due to the small sample size, it is not possible to confirm whether *B. lutzi* follows this pattern of ontogenetic change, as observed in its phylogenetic sister species *B. erythromelas* (Machado et al., 2014) necessitating further studies on this topic.

Centipedes are an important food source for *Bothrops* spp, being part of the diet of at least 15 species (Parker, 1926; Dixon & Soini, 1986; Greene, 1992; Marques et al., 2002; Martins et al., 2002; Valdujo et al., 2002; Nogueira et al., 2003; Boada et al., 2005; Monteiro et al., 2006; Sawaya et al., 2008; Barbo et al., 2016; Silva et al., 2017; Bisneto & Kaefer, 2019; Barbo et al., 2021). Most records of predation of centipedes were associated with juvenile snakes (Martins et al., 2002; Barbo et al., 2016; Silva et al., 2017). However, *B. alcatraz* and *B. germanoi*, small insular species, prey mostly on centipedes through all life stages (Marques et al., 2002; Barbo et al., 2021). Moreover, only a few records of centipede predation by adults are available for *B. asper*, and *B. mattogrossensis* (Boada et al., 2005; Monteiro et al., 2006). For *B. lutzi*, centipedes (*Scolopendra* spp.) could be an important part of the diet of both juvenile and adult stages.

The observed parasitic fauna of *B. lutzi* were all nematodes (*Ophidascaris* sp., *Oswaldofilaria* sp. and *Physaloptera* sp.), a common pattern in Neotropical snakes (Vicente et al., 1993). A more precise identification of the nematodes from this study was not possible because only female specimens were found that do not have taxonomic characters for identification to species level. These parasites are known to have a heteroxen life cycle, and use invertebrates, amphibians, and rodents as intermediate and/or paratenic hosts (Bush et al., 2001), thus snakes are probably infected by ingesting intermediate hosts with infectious larvae encysted in the musculature or viscera (Anderson, 2000).

There are approximately 21 species of *Ophidascaris* parasitising snakes worldwide, with nine species occurring

in Brazil (Vicente et al., 1993; Siqueira et al., 2005). This nematode has been reported parasitising the gastrointestinal tract of *Bothrops jararaca* and *B. atrox* (Vicente et al., 1993; Siqueira et al., 2005). Herein, we present the first record for *B. lutzi*. Individuals of *Ophidascaris* can cause damage to the host, due to the migration of larvae through the viscera (Jacobson, 2007).

Oswaldofilaria Travassos 1933, comprises thirteen species that parasitise mainly crocodilians and lizards from Australia, Africa, and South America (Pereira et al., 2010; Bursey et al., 2014; Vieira et al., 2019). Previously, in Brazil only *Oswaldofilaria carinii* has been found parasitising a snake, *Phalotris tricolor* Sonin 1975. This genus does not seem to be a common snake parasite, and this study presents the first case of it infecting a *Bothrops* sp. as a host.

The genus *Physaloptera* Rudolphi 1819, comprises 100 species. They are commonly found infecting snakes (Goldberg et al., 2004) in Brazil, such as: *Micrurus surinamensis* (Ávila et al., 2013), *Philodryas nattereri* (Oliveira et al., 2019), *Philodryas olfersii* (Araújo et al., 2020), *B. neuwiedi* (Gouveia et al., 2012), *B. jararaca*, (Vicente et al., 1993) and *B. moojeni* (Silva, 2014). In this study, there was only larval infection, suggesting that the hosts were either intermediate or paratenic. This is the first record of *Physaloptera* sp. using *B. lutzi* as a host.

Bothrops lutzi is classified as Least Concern (IUCN, 2022). However, the habitat of the species is being severely impacted by habitat loss, especially deforestation that has made way for the expansion of crop monocultures such as soy, which has severely impacted the Cerrado domain and ecotone areas (Grecchi et al., 2014). This could have had a direct impact on populations of *B. lutzi*, a rare species, with rather specific habitat requirements. We clearly need to know much more about the natural history and biology of this elusive and endemic Brazilian species.

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