

Helminths infecting the cat-eyed snake *Leptodeira annulata* Linnaeus 1758 (Squamata: Dipsadidae) in a semiarid region of Brazil

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Summary

Snakes have diverse feeding and living habits, being exposed to a variety of endoparasite communities. However, more studies are still necessary to document these relationships. We examined 18 specimens of the cat-eyed snake *Leptodeira annulata* from a semi-arid region in Northeast Brazil. Eight taxa of parasites were found, with higher prevalence of cystacanths (Acanthocephala). Five nematode species (*Hexametra boddaertii*, *Oswaldocruzia* sp., *Oxyascaris* sp., *Physaloptera* sp. and *Raillietiema spectans*) and the pentastome *Raillietiella furcocerca* represent a new parasitism record for the host studied. Our results also showed that *L. annulata* could act as paratenic host for acanthocephalans. These results contribute to the knowledge of the helminth fauna of *L. annulata*.

Keywords: parasites, nematoda, neotropical, Pentastomida, snakes, reptiles

Introduction

Parasitism is one of the most common life styles with parasites representing a considerable portion of the world's biomass, but these organisms were for a long time neglected in biodiversity surveys (Poulin & Morand, 2004; Dobson *et al.*, 2008; Kuris, 2008). Given the importance of these organisms structuring communities in ecosystems, as well provide data on ecology of the host (Poulin, 1999; Brooks & Hoberg, 2000), there has been a recent increase of studies on the fauna of endoparasites especially of reptiles in Brazil (Anjos *et al.*, 2011; Albuquerque *et al.*, 2012; Ávila *et al.*, 2012; Teles *et al.*, 2015). Such studies provide information about the ecology, natural history, life cycle, and evolution of host-parasite systems. However, the lack of studies on helminths associated with vertebrate organisms is still evident, being necessary more studies in the area (Mati *et al.*, 2015).

The endoparasite fauna can be related, among other factors, to the diet and microhabitat of hosts (Brito *et al.*, 2014; Ribas *et al.*,

1998). Snakes have very diverse feeding habits, being exposed to a wide variety of parasites (Aho, 1990; Jiménez-Ruiz, *et al.*, 2002). *Leptodeira annulata* (Linnaeus 1758) is a semi-arboreal reptile, distributed from Mexico to eastern of South America (Duellman, 1958) and along all biomes of Brazil, such as the Amazon, Atlantic forest, Cerrado, and Caatinga (Bertoluci *et al.*, 2009; Bernarde *et al.*, 2012; Cole, *et al.*, 2013; Mesquita *et al.*, 2013). Studies on *L. annulata* address aspects like foraging, diet and reproduction (Martins & Oliveira, 1998; Mesquita *et al.*, 2013; Silva-Neta *et al.*, 2015), but data on the parasitic fauna are scarce with records only *Ophidascaaris trichuriformis* Vaz, 1935 (Sprent, 1988) and *Renifer heterocoelium* Travassos, 1921 (Pinto *et al.*, 2012).

In this context, species inventory are important tools serving as a base for ecological studies, enabling the knowledge of what and how many species are part of an ecosystem and providing essential information about the diversity of organisms (Poulin *et al.*, 2015). Aiming at filling the gap in the knowledge of the parasite fauna of *L. annulata*, this study analyzed the helminth fauna as-

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sociated with individuals from the Southern region of Ceará State, Brazil.

Material and Methods

This study was carried out with samples from the Herpetological Collection of Universidade Regional do Cariri (URCA-H- 1981; 3279; 4532; 4907; 4910; 4911; 4913; 4914; 4915; 5541; 5631; 6742; 6847; 7521; 7889; 7900; 8014; 11228). The specimens were collected from 2012 to 2014 in the municipality of Aiuaba (n=4) (6° 34' 25" S, 40° 07' 25" W, WGS84), Barro (n=4) (7° 10' 36" S, 38° 46' 54" W, WGS84), Farias Brito (n=8) (6° 55' 50" S, 39° 33' 56" W, WGS84), Jati (n=1) (7° 41' 10" S, 39° 00' 57" W, WGS84) and Mauriti (n=1) (7° 23' 21" S, 38° 46' 28" W, WGS84) all located in the Southern region of Ceará State, Brazil (Fig. 1). Study area is characterized by hot semi-arid tropical climate and mild hot semi-arid tropical climate (IPECE, 2016).

A total of 18 specimens of *L. annulata* being eight females (mean snout-vent length 541.2 mm) and eleven males (447.5 mm SVL) were euthanized with a lethal injection of sodium thiopental (CFMV, 2013) necropsied and had the liver, lung, heart, mouth, larynx, stomach, large and small intestine, coelomic cavity, and kidneys checked for presence of parasites under the stereomicroscope. The parasites found were processed to separate them completely from the host tissue and stored in 70 % ethanol.

Aiming to perform the taxonomic identification of the helminths obtained, different preparation methods were carried out according to the taxonomic group. Cystacanths were, stained with carmine and preserved in 70 % ethanol. The cestode was also stained with carmine and fixed between slide and coverslip. The nematodes were mounted in temporary slides with Amman's lactophenol or lactic acid. The pentastomes were cleared using Hoyer's solution and preserved in 70 % ethanol. The slides were examined with optical microscope and the specimens were identified using the keys for identification of Yamaguti (1959, 1961, 1963), Vicente *et al.* (1993), Gibbons (2010), Rego (1983). Samples of all parasites were deposited in the Helminthological Collection of the laboratory of Zoology of Universidade Regional do Cariri, URCA, Ceará State, Brazil.

The parasitological descriptors of prevalence (P), mean abundance (MA), mean intensity of infection (MI), richness, and range of intensity of infection (RI) were calculated according to Bush *et al.* (1997).

Ethical Approval and/or Informed Consent

The collection of specimens was authorized by Instituto Chico Mendes de Conservação da Biodiversidade-ICMBio (Authorization number 29613-1) and by the ethics committee of Universidade Regional do Cariri (CEUA/URCA, process No. 00260/2016.1), the

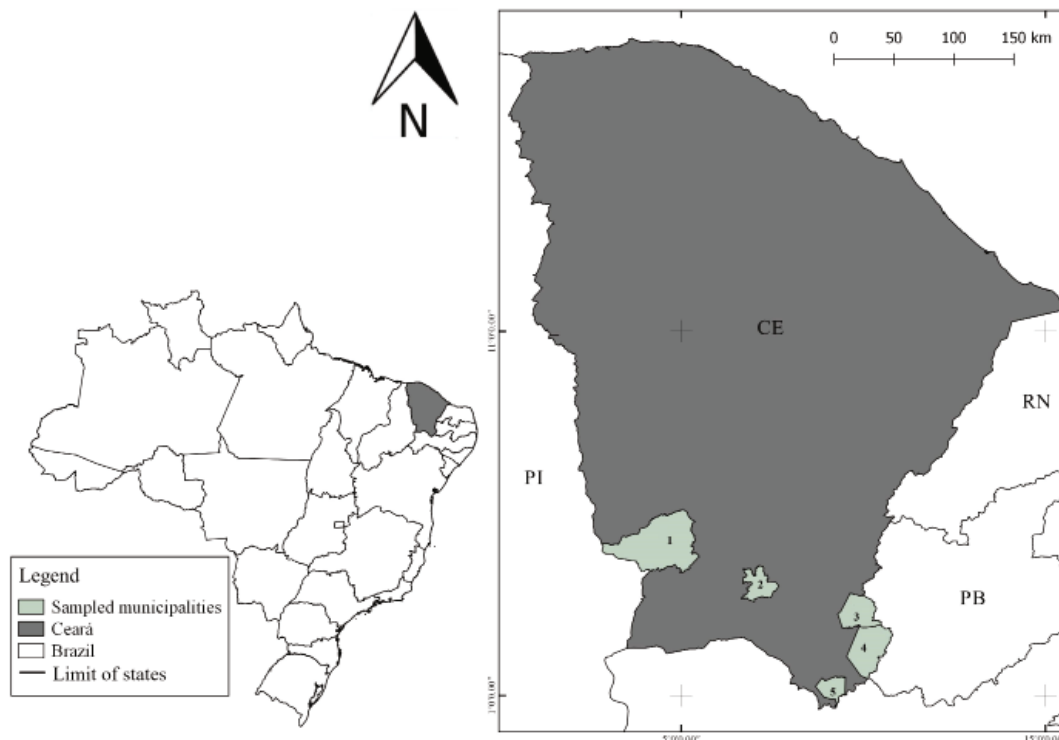


Fig. 1 Municipalities where the specimens of *Leptodeira annulata* were collected in the state of Ceará, Brazil. 1-Aiuaba, 2-Farias Brito, 3- Barro, 4- Mauriti, 5- Jati.

research related to animals has been complied with all the relevant national regulations and institutional policies for the care and use of animals.

Results

A total of 153 parasite specimens were collected with total prevalence of 78.9 % and mean intensity of infection of 10.20 ± 2.81 . The component community associated with *L. annulata* was composed of eight taxa: 18 specimens of nematodes distributed in five taxa (*Hexametra boddaertii* Baird 1860, *Raillietnema spectans* Gomes 1964, *Oswaldocruzia* sp., *Oxyascaris* sp., and *Physaloptera* sp.), three pentastomes of the species *Raillietiella furcocerca* Diesing, 1863, one unidentified cestode, and 131 unidentified cystacanths (Acanthocephala) (Table 1). The cystacanths showed the highest prevalence (63.2 %), intensity (10.92 ± 3.28), and mean abundance (6.9). The cysts could not be identified at species level because the shape and number of the hooks in the proboscis could not be determined. The cestode was found in one female host specimen (SVL = 544.36), showing the lowest values of prevalence (5 %), intensity (1), and mean abundance (0.05), but the parasite specimen could not be identified at species level due to poor conditions of preservation.

Discussion

Studies that investigated the helminth fauna of some snake species from the Neotropical region such as McAllister *et al.* (2010a, 2010b), Bursley and Brooks (2011) did not record any infection in *L. annulata*. This fact may be due to the low number of individuals studied, because in the present study, *L. annulata* presented higher richness (8 parasite taxa) compared to studies of endoparasites for other snake species (Ávila *et al.*, 2013; Nasiri *et al.*, 2014).

Raillietnema spectans is a common parasite in amphibians (Vicente *et al.*, 1990). The low prevalence of this parasite in the snake *L. annulata* could represent an accidental infection, since this nematode have a monoxenic life cycle and infection occurs through ingestion of eggs and/or larvae penetrating actively in host skin (Anderson, 2000). *Leptodeira annulata* is an active forager feeding mainly on amphibians (Martins & Oliveira, 1998; Bernarde & Abe, 2010; Mesquita *et al.*, 2013) and could be exposed to this parasite while foraging.

The unidentified species of Acanthocephala found in this study showed the highest prevalence and were present in the coelomic cavity of 12 specimens of *L. annulata*. Similar results were found by Smales (2007) in nine colubrid species which also presented acanthocephalans encysted in coelomic cavity. The presence of cystacanths in the coelomic cavity of the snakes analyzed indicates that they are acting as paratenic hosts. The cysts were probably acquired from amphibians that are part of the diet of *L. annulata* (Mesquita *et al.* 2013). According to Baker (2007), acanthocephalans have indirect life cycle, with intermediate forms in arthropods and crustaceans, reaching their adult stage in fish or aquatic birds. In the case of infecting an unsuitable host, the parasites can encyst again until reaching a definite host.

The correct identification of the acanthocephalans found in the present study was not possible due to encysted form of the specimens which prevents the visualization of morphological characteristics necessary for identification, such as proboscis rows of hooks and reproductive organs (Smales, 2007).

The pentastome *R. furcocerca* belongs to a parasite genus commonly found in the respiratory system of squamates from South America (Almeida *et al.*, 2008a). This parasite is known to infect snakes with Neotropical distribution, being already recorded in *Boa constrictor* (Linnaeus, 1758), *Clelia clelia* (Daudin, 1803), *Crotalus durissus* (Linnaeus, 1758), *Drymarchon corais* (Boie, 1827),

Table 1. Prevalence (P), mean intensity of infection (MII) with standard error (SE), (MA) mean abundance, (IS) infection site, and (RII) range of intensity of infection of the helminths associated with the snake *Leptodeira annulata* from the South region of Ceará State, Brazil.

	P (%)	MI \pm SE	MA	IS	RII
Acanthocephala					
Cystacanth	66.7	10.92 ± 3.28	7.28	BC	2 – 37
Cestoda					
Unidentified cestode	5.6	1	0.05	SI	1 – 1
Nematoda					
<i>Hexametra boddaertii</i>	5.6	1	0.05	L	1 – 1
<i>Oswaldocruzia</i> sp.	5.6	2	0.11	LI	1 – 2
<i>Oxyascaris</i> sp.	5.6	2	0.11	SI	1 – 2
<i>Physaloptera</i> sp.	5.6	1	0.11	ST	1 – 2
<i>Raillietnema spectans</i>	5.6	11	0.61	LI	1 – 11
Pentastomida					
<i>Raillietiella furcocerca</i>	11.1	1.5 ± 0.5	0.17	L	1 – 3

Infection sites: body cavity (BC), large intestine (LI), small intestine (SI), stomach (ST), lung (L).

Lachesis sp. (Motta, 1963; Rego, 1983) *Mastigodryas bifossatus* (Raddi, 1820), *Philodryas nattereri* (Steindachner, 1870), *Pseudoboa nigra* (Duméril, Bibron and Duméril, 1854), *Thamnodynastes chaquensis* (Bergna and Alvarez, 1993), *Thamnodynastes chaquensis* (Bergna and Alvarez, 1993), *Xenodon merremii* (Wagler, 1824), (Alcantara *et al.*, 2014; Almeida *et al.*, 2008b; Esslinger, 1986). The present study represents the first record of *R. furcocerca* infecting *L. annulata*.

This study presents new records for the nematodes *H. boddaertii*, *Oswaldocruzia* sp., *Oxyascaris* sp. and *R. spectans* in *L. annulata*, the first record of a cestode in *L. annulata*, and the first record of infection by the pentastome *R. furcocerca* in this snake species. These records have the importance of being part of the first studies for the Caatinga area in Northeast of Brazil with this species, and also contribute significantly to the knowledge of the parasitic fauna of *L. annulata* in the Neotropical region providing data on the helminths associated with this snake species.

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Conflict of interest

Authors state no conflict of interest.

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