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# Helminth fauna of *Norops fuscoauratus* (D'Orbigny, 1837) (Squamata: Dactyloidae) in the Atlantic Forest, northeastern Brazil

## Helmintofauna de *Norops fuscoauratus* (D'Orbigny, 1837) (Squamata: Dactyloidae) na Mata Atlântica, Nordeste, Brasil

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**Abstract**

The composition of macro endoparasites associated with the lizard *Norops fuscoauratus* (Squamata) was analysed in two localities in the Atlantic Forest on the northeast of Brazil between December 2012 and July 2015. 74 specimens of *N. fuscoauratus* were examined and five species of helminths were reported, being: (a) for the population of Pernambuco: Cystacanth (Prevalence=37.5%), *Physaloptera retusa* Rudolphi, 1819 (Prevalence=4.16%), larva of flatworm (Prevalence=2.08%), *Rhabdias* sp. (Prevalence=2.08%) and *Strongyluris oscari* Travassos, 1923 (Prevalence=2.08%), and (b) of Alagoas: *S. oscari* (Prevalence=17.85%) and *Rhabdias* sp. (Prevalence=3.57%). The differences in the composition of endoparasites in the two populations are attributed to individualities of environments occupied by the lizards. The collection period does not influence the abundance of parasites, but when associated with sex, there was a positive correlation with the abundance of helminths, with more females than males being infected with parasites in the rainy season.

**Keywords:** lizards, anole, endoparasites, Pernambuco, Alagoas.

**Resumo**

A composição de macro endoparasitas associada com o lagarto *Norops fuscoauratus* (Squamata) foi analisada em duas localidades da Mata Atlântica no nordeste do Brasil, entre dezembro de 2012 e julho de 2015. 74 espécimes foram examinados e cinco espécies de helmintos foram encontradas, sendo: (a) para a população de Pernambuco: Cistacanto (Prevalência=37.5%), *Physaloptera retusa* Rudolphi, 1819 (Prevalência=4.16%), larva de platelminto (Prevalência=2.08%), *Rhabdias* sp. (Prevalência =2.08%) e *Strongyluris oscari* Travassos, 1923 (Prevalência =2.08%) e (b) Alagoas: *S. oscari* (Prevalência=17.85%) e *Rhabdias* sp. (Prevalência =3.57%). As diferenças na composição dos endoparasitas nas duas populações pode ser atribuída as individualidades dos ambientes ocupados por esses lagartos. O período de coleta não influenciou na abundância de parasitas, mas quando associado com o sexo, houve uma correlação positiva com a abundância de helmintos, com mais fêmeas do que machos, infectadas na estação chuvosa.

**Palavras-chave:** lagartos, anole, endoparasitas, Pernambuco, Alagoas.

## 1. Introduction

The disdain for parasites is usually a result of not understanding the complex biology and ecology of these organisms, neglecting their important role in the ecosystems (Marcogliese, 2004).

Just over two decades ago, helminth fauna associated with reptiles was characterized as poor and unrepresentative (Aho, 1990). However, Avila and Silva (2010) emphasized studies conducted in the neotropics and classified the community of macro endoparasites associated with reptiles as expressive and diverse. This diversity among brazilian lizards has been increasing considerably as new species of helminths and new hosts for known species are described (Ávila and Silva, 2010). Apart from the revision held by Ávila and Silva (2010), other studies developed in Brazil and equally important have been published in recent years, like Anjos et al. (2011), Ávila et al. (2012), Ribeiro et al. (2012),

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Brito et al. (2014a, b), Araújo-Filho et al. (2014) for the populations from the semi-arid; Cabral et al. (2018), Amorim and Ávila (2019) and Santos Mesquita et al. (2020) for humid forest ("Brejos-de-altitude") and Almeida-Gomes et al. (2012), Barreto-Lima et al. (2012) and Teixeira et al. (2020) for lizards from the Atlantic Forest. In addition to these contributions, the descriptive study by Albuquerque et al. (2012) should be highlighted. This study was conducted in the Amazon rainforest as a collaboration to investigate the helminth fauna associated with lizards, including additional research on *Norops fuscoauratus* as the host of the nematode *Physaloptera retusa*. Avila and Silva (2013), Teixeira et al. (2020) and Santos Mesquita et al. (2020) also present important data on parasites of this lizard.

The genus *Norops* Wagler (1830), one of eight genera reallocated to reclassify the anole (Nicholson et al., 2012), is supported by three major groups and represented by 175 species. Of these, 150 belong to the group *N. auratus* (Daudin, 1802), including *N. fuscoauratus*, which is an anole with greater distribution in South America that lives in areas of primary and secondary forests as well as forest edges (Vitt et al., 2003). Their wide distribution allows them to enjoy a least concern conservation status and makes it a good object of study because, despite this privilege, ecological aspects of the Atlantic Forest populations are unknown, since there is a concentration of information restricted to the Amazon Forest (e.g., Bursey et al., 2005; Goldberg et al., 2006a). In this study we investigated the composition of macro endoparasites associated with *N. fuscoauratus* in the Atlantic Forest on the northeast of Brazil and indicated the infection rates and their relations with seasonality, sex and size of the host.

## 2. Material and Methods

### *2.1. Data collection*

The lizards were collected by active search (manually), pitfall traps (196 buckets arranged in 49 sets), and glue traps (100 adhesives distributed per campaign) in Atlantic Forest fragments located in Centro de Instrução Marechal Newton Cavalcanti (CIMNC) (7°49'54" S, 35°6'10" W), in Pernambuco, Brazil, between August 2014 and July 2015, with a total of twelve excursions; and in Mata da Bananeira, Ecological Station of Murici (ESEC de Murici) (9°12'47" S, 35°52'9" W), Alagoas, Brazil, between December 2012 and June 2015, with eighteen excursions. The forest fragment in Pernambuco is composed of secondary vegetation of tropical rain forest and semi deciduous forest, with elevation between 50 and 100 m. In Alagoas the fragment is composed of primary vegetation of dense forest, open and seasonal forest, with elevation between 200 and 640 m. The *N. fuscoauratus* collected were euthanized with lidocaine hydrochloride 2%, weighed by a scale type Pesola ® and had recorded with the aid of a digital callipers, measures snout-vent length (SVL), later had their body cavities analyzed in search of parasites, which occurred on a stereomicroscope, which when found form preserved in 70% ethanol. After dissection, the lizards were fixed in 10% formalin and preserved in 70% alcohol. All parasites found were mounted on slides with the aid of lactophenol and analyzed under light microscope.

### *2.2. Statistical analysis*

Infection rates (prevalence, mean intensity and probably ranges) were calculated according to Bush et al. (1997) and the discrepancy index (D) was calculated according Poulin (1993). This index is the minimum value of 0 (D=0) when all hosts have the same number of parasites and a maximum value of 1 (D=1) when all the parasites are aggregated into a single host. The index was calculated using the Quantitative Parasitology 3.0 (Rózsa et al., 2000). A simple linear regression was performed to verify the relationship between the abundance of parasites and the size (SVL) of the host (Zar, 1999). To investigate the existence of seasonal variation in the abundance of endoparasites in *N. fuscoauratus* between the sexes and the interaction between seasonality and sexes we used a Generalized Linear Model (GLM) assuming a Poisson distribution. To conduct all analyses, we used only the adult hosts aiming to avoid the influence of ontogenetic factors. Were also excluded data flatworm larvae. The software used for analysis was Statistica version 8.0. For all tests, the significance level was 5%. Voucher specimens of hosts were deposited in the Coleção Herpetológica da Universidade Federal do Pernambuco (CHUFPE), and Coleção Herpetológica do Museu de História Natural da Universidade Federal de Alagoas (MHN-UFAL), and parasites in the Coleção de Parasitas do Laboratório de Biologia e Ecologia de Animais Silvestres (LABEAS), Universidade Federal do Cariri- UFCA (CHERP-P-UFCA).

### *2.3. Ethical approval*

We authors state that our research has followed all ethical and policy regulations on the use of animals with proper authorizations and permits of national organs. The collecting methods were defined and authorized by regulamentory agency in Brazil (ICMBio/SISBio: 33507-1, 43750-1, and 43750-2). The research related to animal's use has been complied with all the relevant national regulations and institutional policies for the care and use of animals.

## 3. Results

We examined 74 specimens of *Norops fuscoauratus*: 33 adult males (SVL= 38.8 ± 6.3 mm, weight= 1.0 ± 0.3 g), 33 adult females (SVL= 41.2 ± 7.1 mm; weight= 1.5 ± 0.6 g), and 8 juveniles (SVL= 28.5 ± 3.8 mm, weight= 0.4 ± 0.1 g). Overall the prevalence of endoparasites was 35.1% (26/74), being 53.9% adult females, 34.6% adult males, and 11.5% juveniles. Five species of helminths were reported to the lizard *N. fuscoauratus* (Table 1).

The abundance of macro endoparasites analysed was higher in the population of *N. fuscoauratus* of Pernambuco (N= 48) compared to the Alagoas (N= 26). In the first, we find five categories of helminths: Cystacanth (D= 0.768; range= 1-4), Larva of flatworm (D= 1), *Physaloptera retusa* (D= 0.95, range= 3-60), *Rhabdias* sp. (D= 1) and *Strongyluris oscari* (D= 1). Already in anoles of Murici (AL), we obtained

**Table 1.** Review of helminth fauna associated to *Norops fuscoauratus* indicating prevalence rates P (%) and mean intensity of infection (MI) versus the respective sites of infection (SI).

| Species | R1 | R2 | R3 | R4 | R5 | R6 | R7 | This study | SI |
|---|---|---|---|---|---|---|---|---|---|
| | P(%)/MI | P(%)/MI | P(%)/MI | P(%)/MI | P(%)/MI | P(%)/MI | P(%)/MI | P(%)/MI | |
| Cystacanth larvae | - | - | - | - | - | - | - | 37.5/2.15±2.2[1] | Fc, Al, I, S, Aw |
| *Centrorhynchus* sp. | - | - | - | - | - | 4 | - | - | Aw |
| *Mesocoelium monas* | - | - | - | - | - | 4 | 25/8 | - | I |
| Larvae (Monogenic) | - | - | - | - | - | - | - | 2.08/93[1] | S, I |
| *Urotrema shirleyae* | - | 5.4 /1 | - | - | - | - | - | - | I |
| *Ophiotaenia* sp. | - | 3.6/1 | - | - | - | - | - | - | I |
| *Aplectana* sp. | - | - | - | - | 50/2 | - | - | - | L |
| *Cyrtosomum* sp. | - | - | - | - | - | 4 | - | - | I |
| *Strongyluris oscari* | 4/1 | 28.6/3.2 | - | - | 50/9 | - | 50/1 | 2.08/1[1] | S, I |
| | | 38/3.2 | | | | | | 17.85/2.2±1.3[2] | |
| *Cosmocerca vrcibradici* | - | 3.6/1.5 | - | - | - | - | - | - | I |
| *Oswaldocruzia vitti* | - | 48.2/2.9 | - | - | - | - | 25/4 | - | I |
| | | 31/2.8 | | | | | | | |
| *Oswaldocruzia bainae* | - | - | * | - | - | - | - | - | I |
| *Pharyngodon travassosi* | - | - | - | - | - | 8 | - | - | I |
| *Strongyloides* sp. | - | - | - | - | - | 4/24 | - | - | I |
| *Physaloptera retusa* | - | 1.8/1 | - | * | - | - | 50/3±1.4 | 4.16/30±37[1] | S; I |
| *Physaloptera* sp. | - | - | - | - | - | 40/3.3±1.1 | - | - | - |
| *Rhabdias* sp. | - | 1.8/2 | - | - | - | 8/2.5 ± 1.50 | - | 2.08/1[1] | L; S |
| | | | | | | | | 3.57/0.5[2] | |
| *Skrjabinellazia* sp. | - | - | - | - | - | 4 | - | - | I |
| *Skrjabinellazia intermedia* | - | - | - | - | 50/5 | - | - | - | S |
| *Skrjabinellazia galliardi* | - | - | - | - | - | - | 25/2 | - | I |

Fc = free in the body cavities; Al = adhered to the liver; I = intestine; S = stomach; Aw = adhered to the walls of the body; L = lung. The asterisk (*) indicates the occurrence of the parasite without data. Reference1 (R1) = Bursey et al. (2005); R2 = Goldberg et al. (2006a); R3 = Ben-Slimane and Durette-Desset (1996); R4 = Albuquerque et al. (2012); R5 = Teixeira et al. (2020); R6 = Santos Mesquita et al. (2020). Localities: [1]CIMNC, Pernambuco (Brazil); [2]Murici, Alagoas (Brazil).

**Table 2.** Result of the GLM analysis, for the relationship between helminth abundance, and the collection period (dry vs. rainy), helminth abundance versus sex, and sex versus collection period of *Norops fuscoauratus* in the Atlantic Forest, northeastern Brazil.

| | Wald | *P* |
|---|---|---|
| **General abundance** | | |
| Periods | 2.242 | 0.13 |
| Sex | 13.636 | 0.001 |

Degrees of freedom = 1.

two species of nematodes associated with the population of *N. fuscoauratus: Rhabdias* sp. (D= 1) and *Strongyluris oscari* (D= 0.84; range= 1-3). In general, the sex of the lizards influenced the abundance of endoparasites (Table 2), with more females than males being infested with parasites.

The collection period, by itself, did not influence the abundance of parasites, but when associated with the sex of the lizards, there was a positive relationship with the abundance of helminths, with more females than males being infested with parasites during the rainy season. For both populations the intensity of infection was not correlated with the size (SVL) of the host (F= (1.22) 0.1545, P <0.69).

## 4. Discussion

*Norops fuscoauratus* is appointed for second time intermediate host of Acanthocephala in Cystacanth phase (Santos Mesquita et al., 2020). In this study, these parasites were found in the body cavity and gastrointestinal organs and confirm the relationship between the site of infection in the host and the way of transmission of endoparasites. Acanthocephala phylum comprises generalists parasites which infect insectivores (e.g., lizards and frogs), in different environments, suggesting a life story without extensive restrictions to the intermediate host and the site of infection (Yamaguti, 1963; Ribas et al., 1995; Goldberg et al., 2014). Representatives of this group of parasites were recorded infecting anoles from *N. auratus* group as *N. sagrei* (Duméril and Bibron, 1837) (Goldberg and Bursey, 2000), *N. limifrons* (Cope, 1862) (Bursey and Goldberg, 2003), *N. uniformis* (Cope, 1885) (Cabrera-Guzman and Garrido-Olvera, 2014), *N. chrysolepis* (Duméril and Bibron, 1837) (*N. nitens*) (Goldberg et al., 2010), *N. capito* (Peters, 1863), *N. humilis* (Peters, 1863), and *N. pachypus* (Cope, 1875), presenting the latter, their prevalence rates: 15.2%, 5.7% and 14.3% (Bursey et al., 2012), to another group of the same genus, as *N. lineatopus* (Gray, 1840) (Vogel and Bundy, 1987). Once infected, lizards become paratenic to be preyed upon by a definitive host (e.g., birds and mammals) (Anderson, 2000). The high prevalence rate of cystacanths (37.5%), the largest ever recorded for anoles lizards, in *N. fuscoauratus* collected in the Atlantic Forest of the state of Pernambuco and the absence of these helminths in lizards collected in the state of Alagoas, suggest that the environment should influence the level of infection of the parasite, and the Atlantic Forest region of CIMNC (PE) more inclined to the spread of the identified acanthocephalan.

*Physaloptera retusa* (Nematoda: Physalopteridae), one helminth using lizards as one of their hosts, it was found strongly adhered to the internal tissue of the stomach. This intense connection with the mucosal surface epithelium cause injuries and even total loss of gastric glands in the anchoring region, which may hinder the host digestion capacity (Goldberg and Bursey, 1989). We thus evidenced the importance of these organisms in the population dynamics, therefore, to complete their life cycles, parasites can act affecting behaviour, intraspecific relations and various other aspects of the biology of the hosts (Marcogliese, 2004). In the *N. auratus* group, this nematode had already been reported to *Norops nitens* (McAllister et al., 2010), *N. lionotus* (Cope, 1861), *N. humilis* and *N. cupreus* (Hallowell, 1860) (Bursey and Brooks, 2010), *N. biporcatus* (Wiegmann, 1834) and *N. capito* with prevalence of 2.3% and 6.1% for the last two, respectively (Bursey et al., 2012) and *N. brasiliensis* (Vanzolini and Williams, 1970; Amorim and Ávila, 2019). Relative to *N. fuscoauratus*, three studies were carried out reporting the occurrence of this species in the digestive tract. Goldberg et al. (2006a) observed a prevalence rate of 1.8% for *P. retusa*, while this study identified the value of 4.16% (Table 1). Avila and Silva (2013) and Albuquerque et al. (2012) did not provide data on infection rates for comparison, but the present study was similar as to the sites of infection, such as intestine and especially stomach.

*Strongyluris oscari* (Nematoda: Heterakidae), despite being one of the few representatives for the genus in Brazil, is an endoparasite associated with lizards in different ecosystems of the country (Ávila et al., 2011, 2012; Barreto-Lima and Anjos, 2014; Cabral et al., 2018). Their occurrence has been cited previously in anoles (Goldberg et al., 2006b). To *N. fuscoauratus* of the Amazon Forest, this nematode is also a relatively common parasitic association (Ávila and Silva, 2010, 2013) and reached different prevalence rates, such that the presented in Bursey et al. (2005) (4%) and Goldberg et al. (2006a) (28.6% and 38%, respectively). Teixeira et al. (2020) also register this parasite in the Atlantic Forest of Northeast Brazil with a 50% prevalence (N=7). Its occurrence in the two populations of *N. fuscoauratus* in this study only confirms the extent of the parasite-host relationship, which was more evident in lizards from Murici (AL) (P=17.85%) compared to the CIMNC (PE) (P=2.08%).

Anderson (2000) supports that abiotic factors in general interfere in the development and act as the main responsible factor for the type of infection caused by parasites, especially when we talk about *Rhabdias* spp. (Nematoda: Rhabdiasidae) that alternate between the free and parasite life cycles. Nematodes of this genus, different from the diet-spinoff helminths (heteroxenous) were detected in the respiratory tract of *N. fuscoauratus*, the default location for these worms when compared with studies conducted for other hosts (Tkach et al., 2014). *Rhabdias* spp. are usually reported parasitizing lizards, particularly the anoles as well represented by Torres-Ortiz (1980), Bundy et al. (1987), Dobson et al. (1992), Bursey et al. (2003, 2005), Goldberg et al. (2006b, 2010), Vrcibradic et al. (2007, 2008), Ávila et al. (2011) and Ribeiro et al. (2012). For *N. fuscoauratus*, these helminths are relatively common, and associations have been

discovered in two stages: in the first, a prevalence rate of 1.8% was expressed (Goldberg et al., 2006a) - similar to the rates obtained for the analysed population in this study (PE=2.08% and AL=3.57%) - and the second time, were located both inhabiting the lung and the stomach of lizards (Avila and Silva, 2010). Recently, Santos Mesquita et al. (2020) also registered these parasites, with slightly higher prevalence (8%).

The larvae of monogenic flatworms are reported for the first time infecting *N. fuscoauratus* because, until then, only *Ophiotaenia* sp. (Platyhelminthes: Cestoda) and *Urotrema shirleyae* Zamparo, Brooks and Tkach, 2005 (Platyhelminthes: Digenea) were identified parasitizing this species of lizard (Goldberg et al., 2006a; Avila and Silva, 2010). The site of infection (digestive tract) for these helminths also suggests contamination acquired by food.

In specific studies, size, sex, and age of the lizards tend to influence prevalence rates, amplitude and mean intensity of infection (Anjos et al., 2005; Amorim and Ávila, 2019). In *Enyalius perditus* Jackson, 1978, for example, greater host and therefore older showed the highest rates evaluated and the males of the species, which were more parasitized than females (Sousa et al., 2007). Although Vogel and Bundy (1987) have obtained the same result for *N. lineatopus* – males more parasitized than females - the inverse was seen in this study: in general, the endoparasites found were more abundant in females than in males of *N. fuscoauratus*. Amorim and Ávila (2019) studying *N. brasiliensis* did not obtain in their study significant relationship between abundance of the trematode *Mesocoelium monas* (Rudolphi, 1819) and host sex or season, although the abundance of this parasite increased significantly with host body size and mass, while abundance of nematodes was related to season and host mass.

The difference in the abundance of parasites between the sexes and between collection period related to the sexes is due to intrinsic ecological and physiological factors (Aho, 1990) and/or, especially, sexual dimorphism, because studies show the relationship positive between body size and parasitic infection rates (Ribas et al., 1995; Sousa et al., 2007). It is known that the females of *N. fuscoauratus* are significantly larger than males on the snout-vent length (SVL) and the width of the body (Vitt et al., 2003; and unpublished data) so it is consistent to suggest that sexual dimorphism in relation to body size may cause such differences in the incidence of parasites.

Although there is a smaller range of associated endoparasites, the *N. fuscoauratus* from Murici (AL) had a greater range of the nematodes *Rhabdias* sp. and *S. oscari* compared to the CIMNC (PE) and a highly added Poulin discrepancy index (D=0.84) for the latter parasite. Based on the claim submitted by Poulin (1993), lower values in the prevalence rate accentuate the aggregation of parasites in the host, but this was not observed for the population of *N. fuscoauratus* from Alagoas, because it had a higher prevalence rate (17.85%) among the other hosts listed in Table 1. Probably this result was a reflection of the sample size difference between populations.

Poulin (2007) affirmed that the evolution of parasites takes into account the strategies developed for better success of their life cycles. The helminth categories identified in this study appear to conform more to the non-favouritism strategy because they are not specific to *N. fuscoauratus* and already notified to other host species in other areas surveyed. The differences in the diversity of macro endoparasites in two populations of *N. fuscoauratus* in the Atlantic Forest are attributed to individuals' characteristics of environments occupied by animals, allowing slightly different diets and contacts with different species of helminths. Even though parasitological studies were previously developed for *N. fuscoauratus*, we contribute to the literature presenting new parasite associated with the species and increasing its geographical extent of activity, including the Atlantic Forest, a natural area with high biological diversity (Myers et al., 2000).

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