Pentastomid infection in *Philodryas nattereri* Steindachner, 1870 and *Oxybelis aeneus* (Wagler, 1824) (Squamata: Colubridae) in a caatinga of northeastern Brazil

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(With 2 figures)

Abstract

The relationship between pentastomids and two Colubridae species, *Phillodryas nattereri* Steindachner, 1870 and *Oxybelis aeneus* (Wagler, 1824), were investigated in the federal government's reserve Estação Ecológica do Seridó (ESEC, Seridó) situated at lat 6° 35'-40' S and long 37° 15'-20 W in the municipality of Serra Negra do Norte, state of Rio Grande do Norte, Northeast Brazil and run by IBAMA (the Brazilian Institute for the Environment and Natural Resources). Throughout 2005, 26 specimens of snakes, 13 of *P. nattereri* and 13 of *O. aeneus* were collected. After anatomical dissection and laboratorial examination of the snakes' respiratory tracts, *P. nattereri* was found to be parasitized by two species of pentastomids: *Cephalobaena tetrapoda* Heymons, 1922 with a prevalence of 30.8% and a mean intensity of infection of 51.5 ± 32.7 (range 3-147), and *Raillietiella furcocerca* (Diesing, 1863) which had a prevalence of 7.7% and a mean intensity of infection of 1.0. Only one female of *O. aeneus* was found to be infected by *C. tetrapoda*, with a prevalence of 7.7% and mean intensity of infection of 2.0. There was no significant relationship between size of snout-vent length (SVL) and intensity of infection in the specimens investigated here. The two individuals of *P. nattereri* infected by more than 40 specimens of pentastomids had their lungs completely infected including the pulmonary peritoneum and trachea. It is noteworthy that the hosts had their lung tissues partially destroyed with apparent haemorrhage, and the trabecular structure of their lungs was also destroyed. The contrasting rates of infection estimated here may be related to differences in foraging strategies, in diet, and habitat selection carried out by individuals of *P. nattereri* and *O. aeneus*.

Keywords: Endoparasites, *Cephalobaena*, *Raillietiella*, neotropical region.

Infecção por pentastomídeos em *Philodryas nattereri* Steindachner, 1870 e *Oxybelis aeneus* (Wagler, 1824) (Squamata: Colubridae) em uma Caatinga do nordeste do Brasil

Resumo

As relações entre pentastomídeos e duas espécies de Colubridae, *Phillodryas nattereri* Steindachner, 1870 e *Oxybelis aeneus* (Wagler, 1824), foram investigadas na Estação Ecológica do Seridó (ESEC-Seridó) situada em 6° 35' - 40'S e 37°15' - 20'W no munício de Serra Negra do Norte, Estado do Rio Grande do Norte, Nordeste do Brasil, mantida pelo IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis). Ao longo de 2005 foram coletados 26 espécimes de serpentes, sendo 13 de *P. nattereri* e 13 de *O. aeneus*. Depois da dissecação dos espécimes e exame em laboratório do trato respiratório, foram encontradas duas espécies de pentastomídeos parasitando *P. nattereri*: *Cephalobaena tetrapoda* Heymons, 1922 com prevalência 30.8%, intensidade média de infecção 51.5 ± 32.7 (amplitude de 3-147) e *Raillietiella furcocerca* (Diesing, 1863) com prevalência 7.7% e intensidade média de infecção 1.0. Apenas uma fêmea de *O. aeneus* estava infectada por *C. tetrapoda* com prevalência 7.7% e intensidade de infecção 2.0. Não houve relação significativa entre o comprimento rostro-anal (SVL) e a intensidade de infecção nos espécimes coletados. Os dois indivíduos de *P. nattereri* com infecção por pentastomídeos em número superior a 40 tinham seus pulmões completamente infectados por esses parasitas, incluindo a traquéia e peritônio pulmonar. Esses indivíduos possuíam seus pulmões gradualmente destruídos, hemorragias aparentes e partes da estrutura trabecular dos pulmões destruída. As taxas de infecção contrastantes estimadas aqui podem estar relacionadas com as diferenças de estratégias de forrageamento, dieta e seleção de habitats por *P. nattereri* e *O. aeneus*.

Palavras-chave: Endoparasitas, *Cephalobaena*, *Raillietiella*, região neotropical.

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1. Introduction

Pentastomids are pulmonary parasites of vertebrates that act as population regulators (Riley, 1986). Cases of acute infections with serious pulmonary lesions, reduction of egg production, and increased mortality have been reported in lizard communities (Riley, 1986; Pence and Selcer, 1988; Zhang et al., 1988, Riley et al., 1991). However, such consequences have not been investigated in snakes, mainly in the South America ecosystems.

In Brazil, only four species of pentastomids have been reported until now, as snake parasites: *Cephalobaena tetrapoda* Heymons, 1922; *Kiricephalus coarctatus* (Diesing, 1850); *Porocephalus crotali* (Humboldt, 1808) and *Raillietiella furcocerca* (Diesing, 1863). These species may be considered as generalists because their hosts belong to different snake families, genera, and species, whose diets vary from arthropods to vertebrates (Sambon, 1922; Heymons, 1935; Motta, 1963a, 1963b; Rego, 1981, 1983, 1984; Almeida and Christoffersen, 2002; Almeida et al., 2006, 2007).

Most of the above-mentioned studies have only reported the occurrence of pentastomids, except for the ones carried out by Almeida et al. (2006, 2007) who estimated for the first time pentastomids infecting snakes of the family Colubridae *Liophis lineatus* (Linnaeus, 1758), *Philodryas nattereri* Steindachner, 1870, and Elapidae *Micrurus ibiboboca* (Merrem, 1820), inhabiting the ecosystems of the semi-arid region of Ceará state, northeastern Brazil. Almeida et al. (2006, 2007) also confirmed *C. tetrapoda* as a generalist parasite of the colubrids *L. lineatus* and *P. nattereri* that feed on small vertebrates, which are likely to be intermediate hosts of pentastomids. It is quite relevant, as reported by Almeida et al. (2007) that the occurrence of *R. furcocerca* parasitizing *P. nattereri*, indicating a prevalence and mean intensity of infection similar to the ones estimated by the authors in the case of *C. tetrapoda* within the same collection area, therefore confirms that different species of pentastomids share the same host. Almeida et al. (2006, 2007) emphasized the importance of further studies in other regions of Brazil, in order to research rates and patterns of infection of these parasites in South America ecosystems.

In the present study, the rates of infection evaluated in snakes from the microregion of Seridó, in the state of Rio Grande do Norte are compared with those obtained from snakes from the microregion of Cariri, in the state of Ceará, both regions located in northeastern Brazil. The aim here was to search for similarities and differences with respect to: i) composition of pentastomid fauna, ii) patterns of infection (prevalence and intensity), iii) probable diseases these parasites cause to their hosts, and iv) the relationship between infection, sex, and body size of the host.

2. Material and Methods

The snakes were captured throughout 2005, using standard herpetological methods such as active patterned collection (Auricchio and Salomão, 2002) in the federal government's reserve Estação Ecológica do Seridó (ESEC, Seridó) situated at lat 6° 35'-40'S and long 37° 15'-20 W in the municipality of Serra Negra do Norte, state of Rio Grande do Norte, Northeast Brazil and run by IBAMA (the Brazilian Institute for the Environment and Natural Resources) (Figure 1). The region is characterized by a hot, semi-arid climate, with precipitation between 400 and 700 mm/year (average 497 mm/year) occurring predominantly from March to May. The average air temperature is from 28 to 30° C and average air
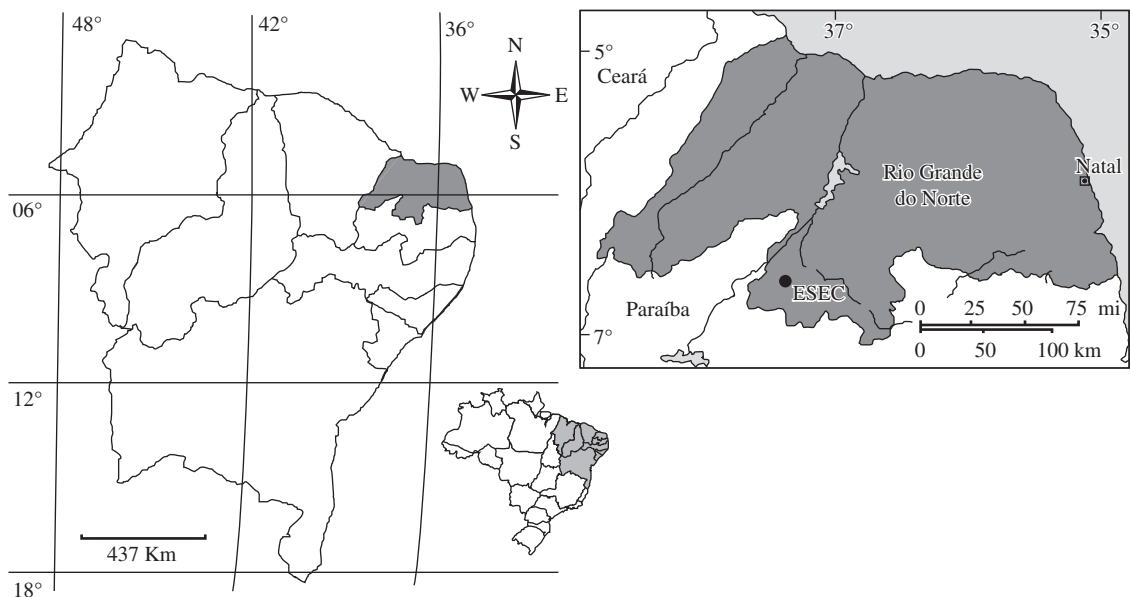
Figure 1. Location of the study area in Northeast Brazil. The federal government's reserve Estação Ecológica do Seridó (ESEC, Seridó), State of Rio Grande do Norte, Northeast Brazil, is indicated by the black dot.

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humidity is between 30 and 50% in the dry season. The vegetation is characterized as hyperxerophile Caatinga, consisting of deciduous xerophytic bushes and trees of up to 8-10 m height, under which a herbaceous layer is well developed, at least during the rainy season (Varela-Freire, 1995; 2002).

Upon collection, the snakes were frozen-euthanized and their snout-vent length (SVL) was measured with a ruler (to the nearest 1 mm). The snakes were fixed in formalin 10% and preserved in ethanol 70%. They were subsequently identified according to Vanzolini et al. (1980). After preservation treatment, they were housed in the Herpetological Collection of the Departamento de Botânica, Ecologia e Zoologia (CHBEZ) of the Universidade Federal do Rio Grande do Norte, and numbered as: CHBEZ 628, 1041, 1084, 1102, 1099-103, 1126-129, 1181, 1216, 1217, 1231, 1238, 1239, 1240, 1246-1249, 1311, 1313, 1314.

The respiratory tracts of the snakes were removed and the search for pentastomids was performed under a stereomicroscope. The pentastomids that were found were cleared in Hoyer's medium, temporarily slide-mounted and preserved in ethanol 70%. Identification was based on the dimensions of the hooks and male copulatory spicules (see Rego, 1983; 1984; Ali et al., 1984a; 1984b; 1985). During the identification of the individuals we also compared the specimens with material from the Helminthological Collection of the Instituto Oswaldo Cruz (CHIOC): *C. tetrapoda* (CHIOC 29185a-d, 29190a-l), *R. furcocerca* (CHIOC 31899a-c); and with material from the Coleção Zoológica da Universidade Regional do Cariri (LZ-URCA): *C. tetrapoda* (LZ-URCA 0043-0044; 0157-167) and *R. furcocerca* (LZ-URCA 168-170a-e).

The eco-parasitological terms used in this study followed the recommendations of Bush et al. (1997). The proportion of infected individuals was compared between sexes for *P. nattereri* using the Z-test for proportions (Zar, 1999).

3. Results

A total of 26 specimens of snakes were collected during the study period, 13 being *Philodryas nattereri* and 13 *Oxybelis aeneus*. The parasite species found in *P. nattereri* were *Cephalobaena tetrapoda* and *Raillietiella furcocerca*. In *O. aeneus* we only found *C. tetrapoda*, which represents a new host record for this pentastomid.

Of the 26 snakes collected, six (23.1%) were infected with pentastomids. They were adults, as we noticed the uterus of the females full of eggs and their simple hooks with no chitinous accessories, which differentiates them from nymphs with their double hooks and chitinous accessories (Riley, 1986).

Of the 13 specimens of *Philodryas nattereri* we collected, 38.5% (5/13) were infected with pentastomids, being *C. tetrapoda* the most frequent species, representing a prevalence of 30.8% (4/13) and the highest mean of intensity of infection (51.5 ± 32.7; range of 3-147). The two individuals of *P. nattereri* infected by more than 40 specimens of pentastomids had their lungs completely infected with these parasites including the pulmonary peritoneum and trachea. It is noteworthy that the hosts had their lung tissues partially destroyed with apparent haemorrhage, and the trabecular structure of their lungs was also destroyed (Figure 2). *Raillietiella furcocerca* was another species of pentastomid that we found parasitizing only one specimen of *P. nattereri* (prevalence 7.7%) with a very low mean intensity of infection (1.0). We also observed only one specimen of *O. aeneus* infected with *C. tetrapoda* (prevalence 7.7%) with a fairly low mean intensity of infection (2.0).
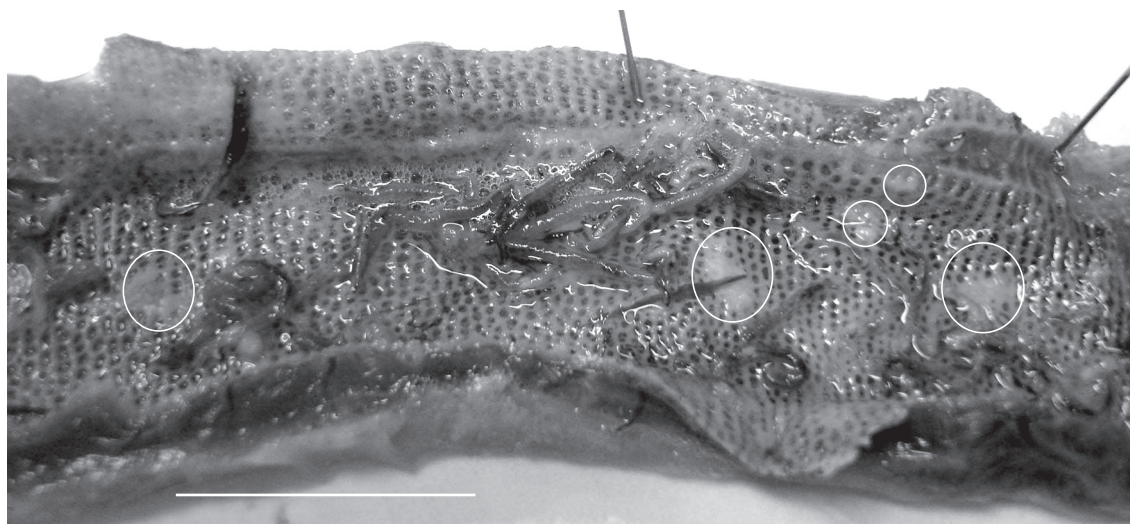
Figure 2. Opened lung of a specimen of *Philodryas nattereri*, the snake with the highest intensity of infection by the pentastomid *Cephalobaena tetrapoda* (147 individuals). The circles indicate points of destruction of pulmonary tissue. Bar = 3 cm.

The proportion of infected individuals was not different between males (1/7) and females (4/6) of *P. nattereri* (Z = 1.21; P = 0.173). The snout-vent length had no significant variation among males (mean SVL = 82.47 ± 15.04 cm) and females (mean SVL = 93.28 ± 20.56) (t = 1.09; df = 11; P = 0.29). There was not any SVL variation among host individuals (mean SVL = 88.58 ± 28.79 cm) nor among non-host individuals (mean SVL = 8.65 cm) (t = 0.17; df = 11; P = 0.86).

4. Discussion

The rates of infection of *Micrurus ibiboboca*, *Liophis lineatus* and *P. nattereri* obtained by Almeida et al. (2006, 2007) were compared in this study, since they were snakes parasitized by pentastomids, in the microregion of Cariri, in the semi-arid northeastern Brazil. In both investigations (Almeida et al., 2006, 2007) and in the present study no difference of rates of infection between males and females was found nor any difference of size between infected and non-infected snakes. The pentastomid species (*C. tetrapoda* and *R. furcocerca*) parasitizing *P. nattereri* in the microregion of Cariri (Ceará State) (Almeida et al., 2007) are the same species we detected in hosts thriving at the ESEC in the microregion of Seridó (Rio Grande do Norte state).

According to Almeida et al. (2007) a higher prevalence of *C. tetrapoda* in *P. nattereri* (36.4% 4/11) was detected than in those results of Almeida et al. (2006) to *L. lineatus* (16.7% 1/6), still both hosts presented similar values of mean intensity of infection (*P. nattereri* 1.5 ± 0.28 and *L. lineatus* 2.0). However, differently from the population of *L. lineatus* in Almeida et al. (2006), *P. nattereri*'s population was parasitized simultaneously by another pentastomid: *R. furcocerca*, presenting a prevalence of 27.3% (3/11) and mean intensity of infection of 2.3 ± 1.32 (1-5), which were similar to the values obtained from *C. tetrapoda* (Almeida et al., 2007). These results indicate that the generalist pentastomid species share the same host with no predominance of either. Otherwise, the investigation performed on *Micrurus ibiboboca* (Elapidae) in the same area of the *P. nattereri*'s population, Almeida et al. (2007) reported for elapid a prevalence of 25% (1/4), a value similar to that of the infected *P. nattereri*. However, the pentastomid observed was probably a new species of *Raillietiella* and the mean intensity of infection was also different (1.0). Such similarities and differences, as observed by Almeida et al. (2007) result from differing forage and dietary habits of the studied snakes. *Philodryas nattereri* are free-ranging snakes that prey on small vertebrates, whereas *M. ibiboboca* is a fossorial and ophiphagous species (Vanzolini et al., 1980; Martins and Oliveira, 1999; Hartmann and Marques, 2005).

In the present study the prevalence of infection in *P. nattereri* and *C. tetrapoda* are similar (30.8%, 4/13) while it is low for *R. furcocerca* (7.7% 1/13). Such results are even more relevant when the values of mean inten-sity of infection are compared; for example *C. tetrapoda* infected strongly the hosts at the ESEC, Seridó (mean 51.5 ± 32.7; range 3-147), causing serious damage to *P. nattereri*'s lungs (Figure 2). *Raillietiella furcocerca*, however, was found parasitizing only one specimen of *P. nattereri* (7.7%) with a very low mean intensity of infection (1.0). We do not have any explanation at the moment for this observation.

Oxybelis aeneus and *Philodryas nattereri* live in sympatry and feed on similar small vertebrates (Vanzolini et al., 1980; Martins and Oliveira, 1999; Hartmann and Marques, 2005). However, as *C. tetrapoda* parasitizing *O. aeneus* at the ESEC, Seridó, presented low prevalence (7.7%, 1/13) and low mean of intensity of infection (2.0), this may have been the result of habit differences, since these snakes are predominantly arboreal species. Hence, phyllogenetic and ecological factors should be intimately related to patterns of infection determined by the pentastomids in the snakes studied here and by Almeida et al. (2006, 2007). Environmental or anthropic interferences probably must have a significant influence on the rates of pentastomid infection in snakes, leading us to agree with Marcogliese (2004) with respect to the need for associating all ecological studies on parasites and hosts. This would contribute towards better identification and better knowledge that would be useful for management and conservation plans, mainly in neotropical regions, where such kinds of information are still scarce.

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