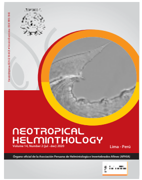
ISSN Versión impresa 2218-6425 ISSN Versión Electrónica 1995-1043

Neotropical Helminthology, 2020, 14(2), jul-dic:129-141.

APHIA

Neotropical Helminthology



ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

ENDOPARASITES OF *PHILODRYAS OLFERSII* (LICHTENSTEIN, 1823) IN RESTINGA ENVIRONMENTS OF THE PARNAÍBA RIVER DELTA, NORTHEASTERN BRAZIL

ENDOPARÁSITOS DE *PHILODRYAS OLFERSII* (LICHTENSTEIN, 1823) EN AMBIENTES DE RESTINGA DEL DELTA DEL RÍO PARNAIBA, NORDESTE DE BRASIL

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ABSTRACT

Endoparasites checklists might contribute to biodiversity understanding. Endoparasites infection might be associated with the host evolutionary history or vary according to host diet and habitat. It was important to investigate these infections in distinct environments since environmental conditions and resource availability might influence the helminth composition and structure in the snake assemblages. We aimed to investigate the endoparasites associated with *Philodryas olfersii* (Lichtenstein, 1823), and we present a bibliographic revision of the endoparasites associated with the genus *Philodryas*. We surveyed the endoparasites of the snake *P. olfersii* in the Parnaíba River Delta, coastal zone of Piauí state, northeastern Brazil. We analyzed the presence of ectoparasites under the epidermis and oral cavity and endoparasites of the digestive tract, lungs, heart, liver, and kidneys, and we used the parasitological descriptors: prevalence, abundance, mean abundance, and mean infection intensity to describe the endoparasite community. We recorded 312 endoparasite specimens belonging to nine taxa: *Kalicephalus costatus* Rudolphi, 1819, *Strongyloides ophidiae* Pereira 1929, *Physaloptera* sp., *Physalopteroides venancioi* Lent, Freitas & Proença, 1946, Cosmocercidae larvae, *Raillietiella furcocerca* (Diesing, 1835), *Ophiotaenia* sp., cystacanths, and a digenetic trematode not identified. Nematodes and Pentastomids were the most abundant taxa infecting *P. olfersii*. Our results contribute to the increasing knowledge base concerning the endoparasites of *P. olfersii.* This is the first research about parasitism in reptiles from the Parnaíba River Delta. We encourage additional parasitic studies in order to improve the understanding of the host-parasite relationships in Brazilian snakes, mainly in coastal environments.

Keywords: Checklist – Delta do Parnaíba – Neotropical – Parasites – Snakes

doi:10.24039/rnh2020142720

RESUMEN

Lista de endoparásitos son fundamentalmente importantes porqué contribuyen para la comprensión de la biodiversidad. Las infecciones endoparásitas pueden estar asociadas a la historia evolutiva del huésped del parásito o variar según la dieta y el hábitat; así, es importante investigar tales infecciones en distintos ambientes, a la vez que las condiciones ambientales y disponibilidad de recursos pueden influir en la composición y estructura de los helmintos en ensambles de serpientes. Aquí, investigamos los endoparásitos asociados con *Philodryas olfersii* (Lichtenstein, 1823), y presentamos la revisión bibliográfica de los endoparásitos asociados con el género *Philodryas*. Buscamos los endoparásitos relaccionados con la serpiente *P. olfersii* en el Delta del Parnaíba, región costera del estado de Piauí, noreste de Brasil. Hemos analizado la presencia de ectoparásitos en la epidermis y la cavidad oral, y endoparásitos en los siguientes órganos: tracto digestivo, pulmones, corazón, hígado y riñones, utilizando los descriptores parasitológicos: prevalencia, abundancia, abundancia promedio e intensidad promedio de infección, para describir la comunidad de endoparásitos. Registramos 312 especímenes de endoparásitos pertenecientes a nueve tasas: *Kalicephalus costatus* Rudolphi, 1819*, Strongyloides ophidiae* Pereira 1929, *Physaloptera* sp*., Physalopteroides venancioi* Lent, Freitas & Proença, 1946, larva de Cosmocercidae, *Raillietiella furcocerca* (Diesing, 1835), *Ophiotaenia* sp., cistacantos y un trematodo digenea no identificado. Nematodos y pentastómidos fueron los taxones más abundantes infectando *P. olfersii*. Nuestros resultados contribuyen a la expansión del conocimiento acerca de los endoparásitos de *P. olfersii*, con destaque para la naturaleza pionera del presente estudio, el primero en investigar el parasitismo en reptiles del Delta del Parnaíba; por consiguiente, alentamos más estudios parasitológicos con vista a mejorar la comprensión de la relación parásito-huésped en serpientes brasileñas, sobre todo en ambientes costeros.

Palabras clave: Inventario – Delta del Parnaíba – Neotropical – Parásitos – Serpientes

INTRODUCTION

Endoparasites checklists are fundamentally important because they might contribute to biodiversity understanding and also are considered environmental quality bioindicators (Galli *et al*., 2001; Poulin & Morand, 2004; Brandão, 2017). Furthermore, due to the considerable number of dead snakes by parasitic infection (Klingenberg, 1993; Sánchez *et al*., 2004; Chinnadurai *et al*., 2008; Vimalaraj *et al*., 2015) and the role of endoparasites on distinct levels of environmental organizations (Wood & Johnson, 2015), it is essential to study the endoparasites associated with Brazilian snakes. Nematodes, digenetic and cestodes are the main causes of these infections (Benson, 1999; Wilson & Carpenter, 1996; Silva, 2000). These endoparasites produce negative effects on the host as anemia, anorexia, survival and fecundity reduction (Vitt & Caldwell, 2009; Matias *et al*., 2018).

Endoparasites associated with snakes are commonly acquired through their diet or direct infection (Aho, 1990) and these endoparasites might be associated with the host evolutionary history or vary according to the host diet and habitat (Fontenot & Font, 1996; Jiménez-Ruiz *et al*., 2002). Therefore, it is important to investigate the endoparasites infection in distinct environments, since environmental conditions and resource availability might influence the helminth composition and structure in snake assemblages. The restinga environments are coastal sandy ecosystems with floristical and physiognomical distinct communities (Falkenberg, 1999); however, the endoparasite loads associated with reptiles in these environments is still undersampled (Van Sluys *et al*., 2000; Menezes *et al*., 2004; Dias *et al*., 2005; Almeida *et al*., 2009; Viana *et al*., 2013).

Brazil harbors a rich snake fauna (405 species), being the Family Dipsadidae the most diverse

(Costa & Bérnils, 2018). Despite this diversity, endoparasites associated with Brazilian snakes have been neglected, with an increase in number of studies recently (*e.g*. Kuzmin *et al*., 2014; Mati *et al*., 2015; Mendoza-Roldan & Fiorillo, 2016; Borges-Nojosa *et al*., 2017; Carvalho *et al*., 2018; Emmerich *et al*., 2018; Matias *et al*., 2018). Among Dipsadidae, the genus *Philodryas* Wagler, 1830 is widely distributed and diverse in Brazil, with 13 species recorded (Costa & Bérnils, 2018). However, endoparasites have been reported to only five species: *Philodryas aestiva* Rossellini, 2007, *P. nattereri* Tavares *et al*. 2017, *P. patagoniensis* Sprent, 1978, *P. psammophidea* Lunaschi & Drago, 2007 and *P. olfersii* Travassos, 1917. Furthermore, there are endoparasites records for *P. baroni* Berg, 1895 in North America (Hartdegen & Gamble, 2002) and *P. chamissonis* Wiegmann, 1835 in Chile (Travassos, 1917; Fredes & Raffo, 2005).

For *Philodryas olfersii* a semi-arboreal snake with diurnal habits preying on small vertebrates (Hartmann & Marques, 2005), there are eight parasites recorded. Herein, we aimed to investigate the endoparasites associated with *P. olfersii* from a restinga environment in the Parnaíba River Delta, Northeastern Brazil, and we present a bibliographic revision of endoparasites associated with the genus *Philodryas*.

MATERIAL AND METHODS

Study area

Fieldwork took place in the Environmental Protection Area (EPA) Delta do Parnaíba, Ilha Grande municipality, Piauí State, Northeastern Brazil (2°50'9.62"S and 41°48'46.20"W, 5 masl). The study area presents quaternary habitats characterized by sandy soils with high salt concentrations covered predominantly by herbaceous and shrubby xerophytic vegetation (Santos-Filho *et al*., 2010).

Sampling

Five specimens of *P. olfersii* were collected manually at the end of rain season (July/2017). The specimens were euthanized following ethical procedures of Federal Council of Veterinary Medicine - CFMV (2013) after approval by the Ethics Committee of Universidade Regional do Cariri (CEUA/URCA, process #00260/2016.1). We analyzed the presence of ectoparasites under the epidermis and oral cavity and endoparasites in the following organs: digestive tract, lungs, heart, liver and kidneys, according to Amato *et al*. (1991).

The fixation techniques and parasite preservations were conducted according to each taxonomic group (Vidal-Martínez *et al*., 2001). Nematodes were clarified with lactic acid (Andrade, 2000), Trematoda and Cestoda were dehydrated in an increasing series of alcohol, stained with the hydrochloric carmine technique and diaphanized in eugenol. Then, we mounted on temporary slides and examined in light microscope. For species identification, we used the following literature: Yamaguti (1961), Sprent (1978), Vicente *et al*. (1993), Smales (2007), Gibbons (2010), and Gamil (2012). The *P. olfersii* specimens were deposited in the Coleção Herpetológica of Universidade Regional do Cariri (voucher specimens URCA-H 13474-13478) and the endoparasites specimens were deposited in the Helminthological Collection of the laboratory of Zoology of Universidade Regional do Cariri, URCA, Ceará State, Brazil.

The parasitological descriptors prevalence, abundance, mean abundance, and mean infection intensity were measured according to Bush *et al*. (1997), using the software Quantitative Parasitology 3.0 (Rózsa *et al*., 2000).

RESULTS

All *P. olfersii* specimens analyzed (three females and two males, both adults) were infected by at least one parasite taxon. We recorded 312 endoparasite specimens belonging to nine taxa from the following taxonomic groups: Acanthocephala, Cestoda, Nematoda, Pentastomida, and Trematoda (Table 1).

The highest endoparasite abundance of *P olfersii* was recorded in stomach (n = 137), small intestine (n = 72) and large intestine (n = 55). Adult endoparasites were recorded at all these organs analyzed, whereas cystacanths were recorded only in stomach (n = 3). The most abundant taxa infecting *P. olfersii* were Nematoda (n = 256), followed by Pentastomida (*Raillietiella furcocerca*, n = 48, Table 1).

The endoparasite species most abundant were *Physaloptera* sp. (n = 170), *Strongyloides ophidiae* (n = 54), *R. furcocerca* (n = 48), and Cosmocercidae larvae (n = 28). These species also had the highest values of prevalence, being *S. ophidiae* recorded for all specimens studied. The mean infection intensity in *P. olfersii* ranged from 1 to 42.5, in which *Physaloptera* sp. also was the most infectious species, and the endoparasites infection amplitude ranged 1 to 46 (Table 1).

Table 1. Endoparasites associated with *Philodryas olfersii*. Values of prevalence (P %), number of endoparasites (NE), mean infection intensity and standard deviation (MII ± SD), mean abundance (MA), site of infection (SI) and range (R). Sto= stomach; Lun=lung; Lgi= large intestine; Smi= small intestine; * new record for *P. olfersii*.

Endoparasites	P%	NE	MII± DP	AM	SI	R
Acanthocephala						
Cystacanth	20	3	3	0.6	Sto	3
Cestoda						
Ophiotaenia sp.*	40	3	1.5 ± 0.7	0.6	Smi	1 – 2
Nematoda						
Cosmocercidae larvae	80	28	7 ± 2.5	5.6	Smi/Lgi	3 – 8
*Kalicephalus costatus**	20	2	2	0.4	Smi	2
Strongyloides ophidiae	100	54	10.8 ± 13	10.8	Sto/Smi/Lgi	1 – 21
Physaloptera sp.	80	170	42.5 ± 6	34	Sto/Smi/Lgi	2 – 46
*Physalopteroides venancioi**	40	2	1±0	0.4	Smi	1
Pentastomida						
*Raillietiella furcocerca**	80	48	12 ± 12.3	9.6	Lun	4 – 30
Trematoda						
Unidentified digenetic	20	2	2	0.4	Lgi	2

DISCUSSION

Despite the low sample size of the present study, we recorded a relative abundance and richness of endoparasites, since at least 41 endoparasites species are recorded for the genus *Philodryas* and about 20 % of these were listed in the present study (Table 2). We present the first record of the following endoparasites associated with *P. olfersii*: *Ophiotaenia* sp., *Kalicephalus costatus*, *Physalopteroides venancioi*, and *R. furcocerca*. In addition, we present the first record of *P. venancioi* infecting the genus *Philodryas*. However, considering its wide distribution in South American (Uetz *et al*., 2020) we believe that this genus is able to be a host for further endoparasite diversity.

Regarding nematodes, we registered two specimens of *P. venancioi* in the host small intestine. The *Physalopteroides* genus is distributed worldwide (Bursey & Goldberg, 1994; Al-Moussawi, 2016); however, only *P. venancioi* is registered in South America (Vrcibradic *et al*., 2000; Ávila & Silva, 2010; Cabral *et al*., 2018). Despite the *P. venancioi* life-cycle remains poorly known (Bursey *et al*., 2005), this genus has heteroxenic life-cycle, with an intermediated invertebrate host and a vertebrate (amphibian, lizard, bird or small rodent) as a final host (Anderson, 2000; Al-Moussawi, 2016). Thus, as

Table 2. Bibliographic revision of endoparasites associated with the genus *Philodryas*.

Host	Endoparasites	References
Philodryas aestiva (Duméril, Bibron & Duméril, 1854)		
	Caryospora brasiliensis Carini, 1932	Lainson & Shaw (1973)
	Hastospiculum digiticaudum Freitas, 1956	Rosselini (2007)
	Infidum infidum Faria, 1910	Noronha *et al*. (2009)
P. baroni Berg, 1895		
	Cephalobaena tetrapoda Heymons, 1922	Haffner & Rack (1971)
	Hexametra boddaertii Baird, 1860	Hartdegen & Gamble (2002)
P. chamissonis (Wiegmann, 1835)		
	Raillietiella sp.	Fredes & Raffo (2005)
P. nattereri Steindachner, 1870		
	Caryospora brasiliensis Carini, 1932	Lainson & Shaw (1973)
	Cephalobaena tetrapoda	Almeida *et al*. (2008a)
	Hepatozoon musa Miller, 1908	Borges-Nojosa *et al*. (2017)
	Oligacanthorrhynchus sp.	Araújo-Filho *et al*. (2018)
	Physaloptera sp.	Oliveira *et al*. (2019)
	Raillietiella furcocerca	Almeida *et al*. (2008a)
P. olfersii (Linchtenstein, 1823)		
	Caryospora brasiliensis	Lainson & Shaw (1973)
	Caryospora olfersii Viana *et al*. 2013	Viana *et al*. (2013)
	Catadiscus longicoecalis Poumarau, 1965	Caubisens-Paumorau (1965)
	Centrorhynchus sp.	Silva & Muller (2012)
	Cosmocercidae larvae	Present study
	Cystacanth	Present study
	Infidum similis Travassos, 1916	Caubisens-Paumorau (1968)
	Isospora decipiens Lainson & Shaw 1973	Lainson & Shaw (1973)
	Kalicephalus costatus	Present study
	Opisthogonimus lecithonotus Lühe, 1900	Silva & Muller (2012)
	Ophiotaenia sp.	Present study
	Physaloptera sp.	Oliveira *et al*. (2020)
	Physalopteroides venancioi	Present study
	Raillietiella furcocerca	Present study
	Strongyloides ophidiae	Mati & Melo (2014)
	Westella serpentis Artigas, Ruiz & Leão, 1943	Rosselini (2007)
	Unidentified digenetic	Present study
P. patagoniensis (Girard, 1858)		
	Caryospora brasiliensis	Lainson & Shaw (1973)
	Dioctophyma renale Goeze,1782	Mascarenhas *et al*. (2018)
	Hepatozoon philodryasi Carini, 1910	Carini (1910)
	Hexametra boddaerti	Sprent (1978)

	Kalicephalus costatus	Vicente *et al*. (1993)
	Kalicephalus inermis Molin, 1861	Vicente *et al*. (1993)
	Ophiotaenia sp.	Ammann & Chambrier (2008)
	Ophidascaris sicki Freitas, 1951	Sprent (1988)
	Opisthogonimus afranioi Pereira, 1929	Rosselini (2007)
	Opisthogonimus fonsecai Ruiz & Leão, 1942	Rosselini (2007)
	Opisthogonimus interrogativus Nicoll, 1914	Rosselini (2007)
	Opisthogonimus lecithonotus	Lunaschi & Drago (2007)
	Opisthogonimus pereirai Ruiz & Leão, 1942	Rosselini (2007)
	Ochetosoma heterocoelium Travassos, 1921	Rosselini (2007)
	Paradistomus parvissimum Travassos, 1918	Noronha *et al*. (2009)
	Renifer heterocoelium Travassos, 1921	Pinto *et al*. (2012)
	Rhabdias vellardi Pereira, 1928	Rosselini (2007)
	Styphlodora condita Faria, 1911	Lunaschi & Drago (2007)
	Travtrema stenocotyle Cohn, 1902	Lunaschi & Drago (2007)
	Westella philodryadum West, 1896	Rosselini (2007)
P. psammophidea Gunther, 1872		
	Infidum infidum	Lunaschi & Drago (2007)
	Opisthogonimus artigasi Ruiz & Leao, 1942	Lunaschi & Drago (2007)
Philodryas sp.		
	Catadiscus dolichocotyle Cohn, 1903	Lunaschi & Drago (2016)
	Infidum similis	Lunaschi & Drago (2016)
	Travtrema stenocotyle	Lunaschi & Drago (2016)

supposed in previous studies with other snake hosts (Al-Moussawi, 2016; Matias *et al*., 2018) and considering that *P. olfersii* feed on small vertebrates (Leite *et al*., 2009), it also may have acquired this helminth through ingestion of infected prey.

Likewise, we also registered only two *K. costatus* specimens parasitizing one individual host. It is a digestive tract helminth of snakes, being recorded for different species such as *Crotalus durissus* Linnaeus, 1758, *Erythrolamprus miliaris* Linnaeus, 1758, *Macrelaps microlepidotus* Gunther, 1860, and *Xenodon* sp. (Dias *et al*., 2004; Ramallo, 2005; McAllister *et al*., 2010; Mati *et al*., 2015; Tavares *et al*., 2017). Thus, this is the first record of *K. costatus* infecting *P. olfersii*. This nematode has direct life-cycle (Anderson, 2000), adhering to the mucosa, feeding on blood (Mitchell, 2007). This endoparasite might be considered unhealthy because it was already registered obstructing the snakes' intestinal tract (Matos-Junior *et al*., 2004).

Strongyloides infection on snakes also occurs through cutaneous penetration, ingestion of infected preys and contaminated water (Mitchell, 2007; Mihalca *et al*., 2010; Taylor *et al*., 2010). Despite not having a detailed biology (Santos *et al*., 2010), it is known that this parasite presents direct and indirect lifecycle, being the first one most common (Mati & Melo, 2014). This helminth was already recorded infecting birds, mammals, amphibians, reptiles and occasionally humans around the world (Little, 1966; Urquhart *et al*., 1998). Nevertheless, only *S. ophidiae* has been recorded for Neotropical snakes (*e.g*. Pereira, 1929; Santos *et al*., 2010; Mati & Melo, 2014). Among the Brazilian Squamata, the helminth *S. ophidiae* was registered infecting the snakes *Crotalus durissus*, *Erythrolamprus miliaris*, *Oxyrhopus guibei* Hoge & Romano, 1977, and also

P. olfersii (see: Pereira, 1929; Santos *et al*., 2010; Mati & Melo, 2014; Mati *et al*., 2015).

Nematodes belong to *Physaloptera* Rudolphi, 1819 genus are commonly registered infecting snakes (Goldberg *et al*., 2004; Barbosa *et al*., 2006), being recorded parasitizing distinct serpents in Brazil, counting *Philodryas* species (*e.g*. Ávila *et al*., 2013; Silva, 2014; Oliveira *et al*., 2019; 2020). *Physaloptera* spp. use invertebrates as intermediate hosts, which are consumed by amphibians and lizards (Duellman & Trueb, 1986; Werneck *et al*., 2009), common preys of *P. olfersii* (Hartmann & Marques, 2005). Infection by *Physaloptera* spp. may cause hemorrhages and excessive digestive secretions (Schell, 1952; Widmer, 1970; Goldberg & Bursey, 1989; Araújo *et al*., 1999).

About 80% of the hosts were infected by Cosmocercidae larvae, however, these helminths were not identified at species level due to their immature stage, although are usually registered parasitizing amphibian and reptile species (Avila *et al*., 2010; Rizvi, 2009; Rizvi & Bursey, 2014; Bursey *et al*., 2015). Likewise, the digenetics recorded in the large intestine were not identified at the species level due to their development stage. Trematodes were frequently reported infecting snakes (see. Travassos *et al*., 1969; Rossellini, 2007; Pinto *et al*., 2012; Fernandes & Kohn, 2014), including *Philodryas olfersii* (Silva & Müller, 2012).

We also recorded cystacanths of Acanthocephala on the snake stomach, which was extensively reported for other snakes (Smales, 2007; Carvalho *et al*., 2018; Matias *et al*., 2018). Acanthocephala species belonging to the genus *Centrorhynchus* Lühe, 1911 and *Oligacanthorhynchus* Travassos, 1915 were already registered parasitizing individuals of *P. olfersii* and *P. nattereri*, respectively (Silva & Muller, 2012; Araújo-Filho *et al*., 2018). Silva & Muller (2012) considered *P. olfersii* as a paratenic host for acanthocephalans, reaching their adult stage mainly in birds (Baker, 2007).

The genus *Ophiotaenia* La Rue, 1911 has six species which five were reported infecting snakes (*O. calmetti* Barrois, 1898, *O. elongate* Fuhrmann, 1927, *O. flava* Rudin, 1917, *O. hyaline* Rudin, 1917 and *O. macrobothria* Rudin, 1917) and *O. lopesi* Rego, 1967 was found infecting lizards (Schmidt, 1986; Silva *et al*., 2006). These endoparasites have wide distribution, being reported for different snake hosts (*e.g*. Chambrier *et al*., 2010; McAllister *et al*., 2010), including records for Brazilian snakes (*e.g*. Silva *et al*., 2006; Matias *et al*., 2018). This species is a small intestine endoparasite and it could difficult the nutrient absorption by the host, obstructing their intestinal tract (Tantaleán & Gozalo, 1985), and there are important records of snake death due to possible cestodes intensive infection (Murvanidze, 2013; Sánchez *et al*., 2004; Vimalaraj *et al*., 2015).

Pentastomids are reported to different snakes belong to genus *Philodryas* (*e.g*. Almeida *et al*., 2008a; 2008b; Brito *et al*., 2012). The species *R. furcocerca* infected about 80% of *P. olfersii* hosts in present study. The higher infection levels reported here might be considered unhealthy because pentastomids are specific lung parasites, which could cause serious lung injuries (Riley, 1986; Almeida *et al*., 2007; 2008b).

The Environmental Protection Area Delta do Parnaíba, northeastern Brazil harbors rich herpetofauna (Loebmann & Mai, 2008; Andrade *et al*., 2016, Araújo *et al*., 2018), however, there is a lack of information about the reptile parasite load in restinga environments, mainly in northeastern region. Our results contribute to increase the knowledge of endoparasites infecting *P. olfersii*, and this is the first research about parasitism in reptiles from the Parnaíba River Delta; thus, we encourage further parasitological studies in order to improve the understanding of the parasite-host relationships in Brazilian snakes, mainly in coastal environments.

ACKNOWLEDGEMENTS

We are grateful to the ICMBio for the collection license (Permit: ICMBio 72384, process: 29613). We are grateful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) and the FUNCAP (Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico) for partial financial support (PROTAX-Processes 440511/2015-1; 5574685/2017; 88882.156872/2016-01). R.W.A thanks CNPq for the research grant (PQ#303622/2015-6; 305988/2018-2).

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Received June 11, 2020.
Accepted July 27, 2020.