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SNAKES AS HOSTS FOR *CENTRORHYNCHUS* (ACANTHOCEPHALA) CYSTACANTHS IN THE BRAZILIAN PAMPA

SERPIENTES COMO HOSPEDEROS DE *CENTRORHYNCHUS* (ACANTHOCEPHALA) CISTACANTOS EN LA PAMPA BRASILEÑA

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Resumen.— Las serpientes pueden actuar como hospederos paraténicos de *Centrorhynchus* spp., pero hay pocos registros en Brasil. Por lo tanto, este estudio tuvo como objetivo registrar cistacantos de *Centrorhynchus* y sus índices de infección en diferentes especies de serpientes del bioma Pampa, en el sur de Brasil. Se examinaron 42 individuos pertenecientes a once especies. Veintisiete (64.29 %) serpientes estuvieron parasitadas por cistacantos de *Centrorhynchus*. La intensidad media de la infección osciló entre 5.50 y 139.33 helmintos/hospedero. *Pseudablabes patagoniensis*, *Philodryas olfersii* y *Erythrolamprus poecilogyrus* tuvieron mayores intensidades de infección: 836, 248 y 235 cistacantos, respectivamente. *Atractus reticulatus*, *Phalotris lemniscatus* y *Dipsas ventrimaculata* no se encontraron parasitados. *Thamnodynastes strigatus*, *Philodryas aestiva*, *Erythrolamprus jaegeri*, *Helicops infrataeniatus* y *Bothrops alternatus* se registraron por primera vez como hospederos de cistacantos de *Centrorhynchus*. Las serpientes representan un puente trófico para que el parásito llegue al hospedador final y se desarrolle hasta adulto para completar su ciclo vital.

Palabras clave.— Dipsadidae, hospedero paraténico, índices de infección, Viperidae.

Abstract.— Snakes may act as paratenic hosts for *Centrorhynchus* species, but there are few records in Brazil. Therefore, this study aimed to record *Centrorhynchus* cystacanths and their infection indices in different snake species from the Pampa biome in southern Brazil. Forty-two individuals belonging to eleven species were examined. Twenty-seven (64.29 %) snakes were parasitized by *Centrorhynchus* cystacanths. Mean intensities of infection ranged from 5.50 to 139.33 helminths/host. *Pseudablabes patagoniensis*, *Philodryas olfersii* and *Erythrolamprus poecilogyrus* were the species with the highest intensities of infection: 836, 248 and 235 cystacanths, respectively. *Atractus reticulatus*, *Phalotris lemniscatus* and *Dipsas ventrimaculata* were not parasitized. *Thamnodynastes strigatus*, *Philodryas aestiva*, *Erythrolamprus jaegeri*, *Helicops infrataeniatus* and *Bothrops alternatus* were recorded for the first time as hosts for *Centrorhynchus* cystacanths. Snakes represent a trophic bridge for the parasite to reach the final host and develop into an adult to complete its life cycle.

Keywords.— Dipsadidae, infection indices, paratenic host, Viperidae

INTRODUCTION

The diversity of snakes is represented by 4,145 species worldwide (Uetz et al., 2024), and 435 species have been recorded for Brazil (Guedes et al., 2023). Snakes participate in complex trophic webs since they may act as predators of various animals (vertebrates and invertebrates) and as prey for several species of birds, mammals and reptiles (Bernarde, 2012; Bellini et al., 2015). Snakes may be found in different habitats, such as aquatic, semi-aquatic, fossorial, terrestrial, cryptozoic, semi-arboreal

and arboreal ones (Marques et al., 2001; Bernarde, 2012; Bellini et al., 2015). These characteristics enable them to act as hosts for a rich variety of helminths, but there is a large gap in knowledge about species associated with snakes and aspects related to parasite-host interactions.

Centrorhynchus Lühe, 1911 (Palaeacanthocephala: Centrorhynchidae) species parasitize intestines of birds and mammals, which are infected when they ingest arthropods (intermediate hosts), amphibians or reptiles (paratenic hosts) that carry infective forms (cystacanths) (Petrochenko, 1971; Amato et al., 2003; Kennedy, 2006). Oliveira et al. (2024) listed vertebrate hosts in South America; in Brazil, Argentina, Paraguay and Peru, 16 species of snakes (mainly Colubridae and Dipsadidae) were recorded as hosts for *Centrorhynchus* cystacanths.

The Pampa biome extends across Uruguay, Argentina and Brazil. The Brazilian Pampa, which covers 2.3 % of the country (193,836 km2) in Rio Grande do Sul (RS) state (60 % of its area), is the second smallest biome in the country (IBGE, 2019). It constitutes the largest temperate grassland ecosystem in South America (Bencke et al., 2016) with pastures, forests and woodlands, savannah-type parks, palm groves, rocky outcrops, dunes, different types of wetlands and bodies of water (Chomenko & Bencke, 2016; IBGE, 2019; Andrade et al., 2023; Farias et al., 2023). In the Pampa biome, there are only records of *Centrorhynchus* sp. (cystacanths) in snakes, one in *Philodryas olfersii* (Lichtenstein, 1823); in the Brazilian Pampa (Silva & Müller, 2012), and one in *Paraphimophis rusticus* (Cope, 1878); in the Argentinian Pampa (Vizcaíno, 1993). Therefore, this study aimed to record *Centrorhynchus* cystacanth and their infection indices in snake species in the Pampa biome, southern Brazil, and contribute to knowledge about their parasitic fauna in the country.

MATERIALS AND METHODS

Forty-two specimens of the following species were examined: *Atractus reticulatus* (Boulenger, 1885) (n = 1), *Phalotris lemniscatus* (Duméril, Bibron & Duméril, 1854) (n = 1), *Philodryas aestiva* (Duméril, Bibron & Duméril, 1854) (n = 1), *Erythrolamprus jaegeri* (Günther, 1858) (n = 2), *Thamnodynastes strigatus* (Günther, 1858) (n = 2), *Dipsas ventrimaculata* (Boulenger, 1885) (n = 3), *Philodryas olfersii* (n = 4), *Pseudablabes patagoniensis* (Girard, 1858) (n = 6), *Helicops infrataeniatus* Jan, 1865 (n = 6), *Erythrolamprus poecilogyrus* (Wied-Neuwied, 1825) (n = 10) (Dipsadidae) and *Bothrops alternatus* (Duméril, Bibron & Duméril, 1854) (n = 6) (Viperidae). Snakes were collected in Capão do Leão (31° 45' 48" S, 52° 29' 02" W), Pelotas (31° 46' 19 " S, 52° 20' 33" W), Rio Grande (32° 02' 06" S, 52° 05' 55" W), Encruzilhada do Sul (30° 32' 38" S, 52° 31' 19" W) and Dom Pedrito (30° 58' 58" S, 54° 40' 23" W), RS, Brazil. Thirty-four were found dead on roads from March 2017 to June 2019. Collections were licensed by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio - SISBIO No. 38913). Four snakes were donated by the Núcleo de Reabilitação da Fauna Silvestre and Centro de Triagem de Animais Silvestres at the Federal University of Pelotas (NURFS-CETAS/UFPel), where they died after a rehabilitation attempt. Four *E. poecilogyrus* were donated by the Vertebrate Zoology Laboratory at the Federal University of Pelotas, where specimens were fixed in formalin and conserved in 70° GL ethanol.

Hosts were necropsied for the analysis of infection sites. Acanthocephalans were removed from cysts, compressed and fixed in AFA, conserved in 70 °GL ethanol, stained with Langeron carmine or Delafield hematoxylin, cleared with creosote and mounted with Canada balsam (Amato et al., 1991). Systematic determination of helminths was carried out in agreement with Petrochenko (1971), Vizcaíno (1993) and Santos & Amato (2010). Representative specimens were deposited in the Helminthological Collection in the Oswaldo Cruz Institute (CHIOC 39735 and 39191). Ecological parameters were calculated in agreement with Bush et al. (1997).

RESULTS

Twenty-seven snakes (64.29 %) were parasitized by *Centrorhynchus* cystacanths, which were found in the coelom cavity of the hosts. Results show that 1,597 helminths (mean abundance was 38.02; mean intensity of infection was 59.15) were associated with eight snake species (Table 1). *Atractus reticulatus* (n = 1), *P. lemniscatus* (n = 1) and *D. ventrimaculata* (n = 3) were not parasitized by acanthocephalans. Mean intensities of infection ranged from 5.50 to 139.33 helminths/host (Table 1). *Pseudablabes patagoniensis*, *P. olfersii* and *E. poecilogyrus* exhibited the highest infection rates: 836, 248 and 235 cystacanths, respectively.

DISCUSSION

Interactions between snakes and acanthocephalans are little known in South America, especially the ones that occur in the Pampa biome. Regarding *Centrorhynchus*, there are records of 10 species of Dipsadidae snakes and one of Viperidae as cystacanths hosts in South America (Oliveira et al., 2024), where two records correspond to the Pampa biome (Vizcaíno, 1993; Silva & Müller, 2012). Concerning snakes investigated by this study, there are reports of *Centrorhynchus* cystacanths in *P. patagoniensis* and *E. poecilogyrus* in Paraguay (Smales, 2007) and in *P. olfersii* in the

Tabla 1. Prevalencia (P %), intensidad media de infección (MII), abundancia media (MA) y range (R) de cistacantos de *Centrorhynchus* parásitos de serpientes (Dipsadidae y Viperidae) en diferentes sitios de colecta en el bioma Pampa, sur de Brasil. n - número de especímenes examinados.

Table 1. Prevalence (P %), mean intensity of infection (MII), mean abundance (MA) and range (R) of *Centrorhynchus* cystacanths parasites of snakes (Dipsadidae and Viperidae) in different collection sites in the Pampa biome, southern Brazil. n – number of examined specimens

Host	P%	MII	MA	R	Collection sites
Dipsadidae					
Erythrolamprus poecilogyrus (n = 10)	50.00	47.00	23.50	1 – 169	Capão do Leão and Pelotas
Pseudablabes patagoniensis (n = 6)	100.00	139.33	139.33	1 – 542	Capão do Leão and Encruzilhada do Sul
Helicops infrataeniatus (n = 6)	100.00	17.40	14.50	3 – 39	Capão do Leão and Pelotas
Philodryas olfersii (n = 4)	100.00	62.00	62.00	3 – 117	Capão do Leão and Pelotas
Erythrolamprus jaegeri (n = 2)	100.00	36.00	36.00	17 – 55	Capão do Leão
Thamnodynastes strigatus (n = 2)	100.00	29.50	29.50	21 – 38	Capão do Leão
Philodryas aestiva (n = 1)	100.00	49.00	49.00	49	Capão do Leão
Viperidae					
Bothrops alternatus (n = 6)	33.33	5.50	1.83	4 – 7	Capão do Leão

study area (Silva & Müller, 2012). Therefore, this is the first report of *Centrorhynchus* cystacanths in *T. strigatus, P. aestiva, E. jaegeri, H. infrataeniatus* and *B. alternatus*.

Many parasitological studies with South American snakes have been carried out with a low number of hosts per species (as in the present study), so it's important to consider this issue when interpreting infection indices. However, even with only a few hosts, investigations of this nature act as a starting point for complementary studies with a greater number of hosts, to expand information on parasite loads. Smales (2007) investigated 115 snakes of different species from Paraguay and southeast Brazil and reported that the prevalence of *Centrorhynchus* cystacanths ranged from 5.3 % to 100 % and mean intensities ranged from 1 to 7 helminths/host. On the other hand, Lamas & Lunaschi (2009), in Argentina, recorded a high number of parasites (600 cystacanths) in a single Colubridae host (*Leptophis ahaetulla* [Linnaeus, 1758]). Vizcaíno (1993) found cystacanths (*Centrorhynchus* sp.) in *P. rusticus* (Dipsadidae) in the Province of Buenos Aires, Argentina (included in the Pampa biome), but he did not report the intensity of infection. Regarding the species investigated by this study, Silva & Müller (2012) examined two *P. olfersii* snakes, which were parasitized at intensities of infection of 109 and 131 cystacanths (*Centrorhynchus* sp.), i.e., mean intensity of infection was higher (120 helminths/host) than that recorded by this study (62.0 helminths/host), in which all four examined snakes were parasitized. On the other hand, in the cases of *P. patagoniensis* and *E. poecilogyrus*, Smales (2007) recorded mean intensities of infection that were much lower (3 helminths/host per snake) than those found by this study. Regarding records of Viperidae species in South America, there is only the report of *Centrorhynchus tumidulus* (Rudolphi, 1919) cystacanths in *Lachesis lanceolatus* Lacépède, 1789 (= *Bothrops lanceolatus* [Bonnaterre, 1790]) in Brazil, but there is information neither on the site of the record nor on its infection rates (Travassos, 1926). This viperid may have been *Bothrops jararaca* (Wied-Neuwied, 1824) since *B. lanceolatus* is an insular species native to Central America (Lesser Antilles: Martinique) (Tanasov et al., 2003; Uetz et al., 2024). According to Tanasov et al. (2003), at the beginning of the 20th century, *B. lanceolatus* was not known to be exclusive to the insular condition and the name (*L. lanceolatus*) was adopted to designate other continental representatives.

Infection rates observed in different species may reflect aspects related to the diet and habitat of hosts since snakes under investigation use several habitats and food resources that may lead to infection by *Centrorhynchus* spp. In general, transmission of *Centrorhynchus* spp. involves prey-predator interactions, i.e., it depends on the trophic chain to develop its life cycle. Cystacanths (infective forms of the parasite) develop in an obligate intermediate host (e.g., isopod crustaceans) and may use facultative paratenic hosts (e.g., amphibians and reptiles), which ingest the arthropods and act as carriers of infective forms. Birds and mammals (definitive hosts) get infected by ingesting cystacanths through predation on them (Petrochenko, 1971; Amato et al., 2003; Kennedy, 2006).

Complex trophic webs involving a wide diversity of hosts may enhance transmission of cystacanths since several paratenic *Centrorhynchus* hosts have been recorded (Oliveira et al. 2024).

Thus, it may be hypothesized that the parasite uses more than a facultative host to reach definitive hosts. *Centrorhynchus* sp. cystacanths have been reported in several anuran species (Oliveira et al., 2024); in the study area of this work, there are records in *Boana pulchella* (Duméril & Bibron, 1841) (Hylidae) (Silveira et al., 2022), *Rhinella dorbignyi* (Duméril & Bibron, 1841) (Bufonidae) (Coimbra et al., 2023) and *Aquarana catesbeiana* (Shaw, 1802) (Ranidae) (Oliveira et al., 2024), in which prevalence and intensities of infection ranged from 51.1 % to 61 % and from 4.85 to 17.1 helminths/host, respectively (Silveira et al., 2022; Coimbra et al., 2023; Oliveira et al., 2024). These anurans may be potential preys and transmitters of cystacanths to snakes investigated in the Pampa biome. Most snakes in this study feed on anurans (Bernarde et al., 2000; Aguiar & Di-Bernardo, 2004; Hartmann & Marques, 2005; Quintela & Loebmann, 2009; Bellini et al., 2015; Corrêa et al., 2016; Thaler et al., 2018; Quintela & Loebmann, 2019). Among the species analyzed, *P. patagoniensis* has a generalist diet that includes anurans and snakes (including its own species) (Hartmann & Marques, 2005; Bellini et al., 2015; Quintela & Loebmann, 2019). *Phalotris lemniscatus* feeds on amphisbaenians and other snakes, while *D. ventrimaculata* is specializes in mollusks and *A. reticulatus* feeds on annelids (Achaval & Olmos, 2003; Balestrin et al., 2007; Quintela & Loebmann, 2009; Bellini et al., 2015). Therefore, it may be suggested that the occurrence and infection rates of cystacanths in snakes under study may be directly related to the ingestion of anurans and even snakes, which are important food resources for these species. Likewise, absence of cystacanths in *A. reticulatus* and *D. ventrimaculata* reflects their diet. Parasitized snakes may act as potential transmitters of cystacanths to mammals and birds through the trophic chain since they are an important food resource for several species. Hosts for *Centrorhynchus* spp. in South America, such as *Lycalopex gymnocercus* (Fischer, 1814), *Cerdocyon thous* (Linnaeus, 1766) and *Chrysocyon brachyurus* (Illiger, 1815) (Canidae), and birds, such as *Rupornis magnirostris* (Gmelin, 1788), *Urubitinga urubitinga* (Gmelin, 1788) (Accipitridae), *Guira guira* (Gmelin, 1788) (Cuculidae) and *Athene cunicularia* (Molina, 1782) (Strigidae) (Oliveira et al., 2024), include snakes in their diets (Panasci & Whitacre, 2000; Silva & Talamoni, 2003; Vieira & Teixeira, 2008; Varela et al., 2008; Soave et al., 2008; Abegg et al., 2015; Porto & Rui, 2019; Frota et al., 2021). Two of these records were in the Pampa biome: *C. tumidulus* in *G. guira* (Cordero, 1933) and *Centrorhynchus* sp. in *L. gymnocercus* and *C. thous* (Ruas et al., 2008). Thus, snakes represent a trophic bridge for the parasite to reach the final host and develop into an adult, a fact that enables the life cycle of the parasite to continue. However, further studies of a larger number of snakes and integrative taxonomic studies of cystacanths are important to understand parasite-host interactions and aspects related to transmission and life cycle of *Centrorhynchus* species.

Finally, it should be emphasized that the investigation into parasites associated with vertebrates that have been run over is a viable alternative for helminthological research, as highlighted by Mascarenhas et al. (2022), who expanded knowledge about helminths associated with freshwater turtles (dead specimens found on highways in the Brazilian Pampa). One of the anthropogenic disturbances caused by urbanization is the construction of highways, which, even after construction, cause impacts on biodiversity since they represent barriers to the habitat of many species and even pose risks to those that try to cross them. Thus, several organisms end up dying, the case of most snakes investigated by this study. Therefore, the study of parasites and their relations with the environment and their hosts may provide fundamental tools for biodiversity conservation programs since parasites are important environmental indicators (Marcogliese, 2005; Vidal-Martinez et al., 2010).

Among all Brazilian biomes, the Pampa is the one with the lowest coverage of protected areas, i. e., only 0.6 % of its area (Ribeiro et al., 2021). The area covered by non-forest natural formations fell by 30 % between 1985 and 2022 (MapBiomas, 2024). Land use activities in the biome include agriculture, livestock production (on natural grasslands), forest plantations and urbanization (Souza et al., 2020), with agriculture and livestock production accounts for 43.5 % of the area of the Brazilian Pampa (MapBiomas, 2024). Biodiversity of the Brazilian Pampa includes approximately 12,000 species (Andrade et al., 2023) that interact in complex life cycles, which include *Centrorhynchus* species and their various hosts. Snakes play a significant role in the transmission of these parasites, which use prey-predator interactions to complete its life cycle.

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