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SHORT COMMUNICATION

Leptodactylus luctator (Anura: Leptodactylidae) as a host for *Centrorhynchus* sp. (Acanthocephala) cystacanths in the Pampa biome, southern Brazil

Carolina S. Mascarenhas,[1] Juliana H. Wolter,[2] Ana Beatriz D. Henzel,[3] Marco Antonio A. Coimbra,[4] and Gertrud Müller[5]

[1] Independent researcher. Capão do Leão, Brazil. E-mail: phrybio@hotmail.com.

[2] Universidade Federal de Pelotas, Instituto de Biologia, Departamento de Ecologia, Zoologia e Genética, Programa de Pós-Graduação em Biodiversidade Animal. Campus Universitário, s/n, 96160-000, Capão do Leão, RS, Brazil. E-mail: julianahwolter@hotmail.com.

Universidade Federal de Pelotas, Programa de Pós-Graduação em Sistemas de Produção Agrícola Familiar. Campus Universitário, s/n., 96160-000, Capão do Leão, RS, Brazil. E-mail: biahenzel@hotmail.com.

[4] Universidade Federal de Pelotas, Núcleo de Reabilitação da Fauna Silvestre e Centro de Triagem de Animais Silvestres (NURFS-CETAS). Campus Universitário, s/n., 96160-000, Capão do Leão, RS, Brazil. E-mail: coimbra.nurfs@gmail.com.

[5] Independent researcher. Pelotas, RS, Brazil. E-mail: gertrudmuller40@gmail.com.

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Anurans utilize both aquatic and terrestrial environments. Most species have aquatic larval stages in the form of tadpoles (with herbivorous diets) and adult terrestrial stages (with carnivorous diets based mainly on arthropods) (Duellman and Trueb 1994). These characteristics enable anurans to maintain interactions as both predator and prey with a wide variety of vertebrates and invertebrates throughout the trophic chain, thus helping to regulate biological communities in both terrestrial and aquatic ecosystems (Toft 1980, Kupfer *et al.* 2006, Bernarde 2012, Blanco-Torres *et al.* 2020). Many helminths also spend part of their development in aquatic environments and part in terrestrial environments, a fact that is essential for maintaining their life cycles (Poulin and Morand 2004). Anurans therefore provide a link that enables parasites to make this transition, since both helminths and their vertebrate hosts use the same environments (Aho 1990, Poulin and Cribb 2002).

Anuran amphibians have been associated with a rich diversity of helminths. They act as intermediate, definitive, and paratenic hosts for nematodes, cestodes, digeneans, and acanthocephalans (Campião *et al.* 2014, Fernandes and Kohn 2014, Olivera and Campião 2024, Oliveira *et al.* 2024). Regarding acanthocephalans, adult and infective forms (cystacanths) parasitize anurans in South America, where most reports mention cystacanths of the genus *Centrorhynchus* Lühe, 1911 (Palaeacanthocephala: Centrorhynchidae) associated with species of the anuran families

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Hylidae, Leptodactylidae, and Bufonidae (Campião *et al.* 2014, Olivera and Campião 2024, Oliveira *et al.* 2024; Santos *et al.* 2024). In the Pampa biome (which includes Uruguay, Argentina, and southern Brazil), *Centrorhynchus* cystacanths have been found in *Leptodactylus ocellatus* Girard, 1853 (Leptodactylidae) in Uruguay (Cordero 1933), as well as in species of Hylidae, Bufonidae, and Ranidae in the Brazilian Pampa (Silveira *et al.* 2022, Coimbra *et al.* 2023, Oliveira *et al.* 2024). Anurans play a role in maintenance and transmission of cystacanths to birds and mammals (definitive hosts) through the trophic chain (Petrochenko 1971, Kennedy 2006), since they represent a significant food resource for those predators (e.g. Silva and Talamoni 2003, Porto and Rui 2019, Brentano *et al.* 2020, Frota *et al.* 2021).

The family Leptodactylidae includes species that only occur in the Americas, from southern Texas (USA), southern Sonora (Mexico), and the northern Antilles to southern Brazil, Argentina, and Chile (Frost 2024). *Leptodactylus* Fitzinger, 1826 is comprised of 84 small- to large-sized species (Frost 2024) that may be found in a wide range of environments, including lowland dense rainforest (primary and secondary rainforest), open habitats, disturbed forests, and areas currently used for agriculture and livestock farming (de Sá *et al.* 2014). *Leptodactylus luctator* (Hudson, 1892) occurs from south-central Bahia state and east-central Goiás state to western Mato Grosso state and from southern Brazil, Uruguay, and Paraguay to eastern Bolivia and Argentina (from the south to northern Buenos Aires and southern Córdoba Provinces) (Magalhães *et al.* 2020, Frost 2024). Magalhães *et al.* (2020) provided a taxonomic revision of all species included in the *Leptodactylus latrans* group based on acoustic data, morphological / chromatic variation, and molecular phylogenetics.

Despite many records of *Centrorhynchus* sp. cystacanths in leptodactylid anurans in South America (Campião *et al.* 2014, Olivera and Campião 2024, Oliveira *et al.* 2024, Santos *et al.* 2024), few studies have analyzed infections in relation to body parameters of hosts (Hamann *et al.* 2012). Male and female hosts in species of Bufonidae and Ranidae have similar rates of helminth infections (Santos and Amato 2010a, Coimbra *et al.* 2023, Oliveira *et al.* 2024); however, abundance of cystacanths varied with the snout-vent length of the hosts in different regions (Santos and Amato 2010a, Coimbra *et al.* 2023). Helminthological studies provide data on biodiversity and parasite–host interactions and generate information that may be used by conservation programs. Because the Pampa is considered one of the least protected Brazilian biomes (Ribeiro *et al.* 2021), all knowledge generated in the region provides valuable support for its conservation. This study examined *Centrorhynchus* sp. cystacanths in *L. luctator* and analyzed their infection indices regarding gender, size, and mass of hosts in the Pampa biome, southern Brazil.

Forty-two adult specimens of *L. luctator* (23 females and 19 males) were collected in an urban area with wet, dry, and flooded fields on the west bank where the Laguna dos Patos meets the São Gonçalo Channel in Pelotas (31°46'38.0" S, 52°13'57.2" W), Rio Grande do Sul state, Brazil. Anurans were collected by hand in September ($N = 12$), October ($N = 2$), and December 2017 ($N = 10$), and in April ($N = 17$) and August 2018 ($N = 1$); active searches with the aid of headlamp took place at the beginning of twilight. The frogs were kept in individual plastic containers and taken to the Laboratório de Parasitologia de Animais Silvestres at the Universidade Federal de Pelotas (LAPASIL / UFPel) where they were sacrificed using lidocaine 2% and frozen for later necropsy. Anurans were weighed and measured (snout–vent length, SVL) after thawing, using an electronic precision scale and a digital caliper to the nearest 0.1 mm. Gender was confirmed by analyzing gonads during dissection. The study was approved by the Instituto Chico Mendes de Conservação da Biodiversidade (SISBIO, no. 47397) and by the Ethics and Animal Experimentation Committee at UFPel (CEEA, no. 1859/2015).

During necropsy of the frogs, the following potential sites of infection were examined: oral cavity, esophagus, stomach, small and large intestines, liver, lungs, heart, spleen, pancreas, kidneys, urinary bladder, and coelomic cavity. Helminths were removed from cysts, placed in distilled water, and kept in a refrigerator to induce the proboscis to evert. Subsequently, acanthocephalans were compressed and fixed in AFA (ethanol 70° GL, 93 parts; formalin 37%, 5 parts; glacial acetic acid, 2 parts). Samples were preserved in 70° GL ethanol, stained with Langeron's Carmine, and mounted in Canada Balsam (Amato *et al.* 1991). Acanthocephalans were identified based on Petrochenko (1971), Santos and Amato (2010b) and Oliveira *et al.* (2024). Representative specimens were deposited in the Helminthological Collection at the Oswaldo Cruz Institute (CHIOC 40435, 40436a-b, 40437–40441).

Prevalence (P%), mean intensity of infection (MII), and mean abundance (MA) of helminths were calculated based on Bush *et al.* (1997). P% and MII of cystacanths were compared between the two collection periods (2017 and 2018) and between male and female hosts (using the total sample as well as the two sampling periods). Fisher's Exact test ($p \leq 0.05$) and the bootstrap *t*-test ($p \leq 0.05$) were used to compare P% and MII, respectively, by the Quantitative Parasitology (QPweb) program (Reiczigel *et al.* 2019). The Mann-Whitney *U* test was used to check for differences in body mass (BM) and SVL between males and females. Spearman's correlation coefficient (r_s) was used to estimate the influence of BM and SVL of hosts on the abundance of helminths. Analyses were carried out regardless of the sampling period by Jamovi® version 2.2 (The Jamovi Project 2023).

Thirty-nine individuals (92.9%) were parasitized with cystacanths of *Centhrorynchus* sp. A total of 440 specimens was removed from the coelomic cavity, external surface of the liver, serosa of the stomach, musculature of small and large intestines, and external surface of the urinary system. Mean intensity of infection was 11.3 cystacanths/host (Table 1). Prevalence of *Centhrorynchus* sp. was similar in the two sampling periods (2017 and 2018); however, the MII was significantly ($p = 0.036$) higher in 2018 (16.3 cystacanths/host) compared to the first period (7.41 cystacanths/host) (Table 1). Prevalence and mean intensity of infection in females were 91.3% and 12.8 cystacanths/host, respectively, while in males, values for those parameters were 94.7% and 9.6 cystacanths/host, with no significant intersexual differences (Table 2). Similarly, infections in females and males did not show significant differences between the two sampling periods (Table 2).

Regarding host size, mean SVL of *L. luctator* individuals was 73.6 ± 13.1 mm (range 46.0–102.0 mm) and mean BM was 49.9 ± 23.6 g (range 12.1–113 g). Even though mean SVL of females was significantly ($p = 0.028$) larger than that of males, abundance of cystacanths was significantly correlated only with SVL and BM of males (Table 3).

In South America, records of cystacanths of the genus *Centhrorynchus* infecting species of *Leptodactylus* had lower P% and MII values than those recorded by us in *L. luctator.* These studies revealed P% values ranging from 1.1% to 46.05% and MII between 1.0 and 8.7 cystacanths/host in Argentina (Corrientes, Formosa, and Chaco Provinces; González and Hamann 2006, Hamann *et al.* 2006, Schaefer *et al.* 2006, Hamann *et al.* 2012, Zaracho *et al.* 2012), in Paraguay (Alto Paraguay and Itapua Provinces; Smales 2007) and in Brazil (Ceará state; Oliveira *et al.* 2022). In the Brazilian Cerrado biome, the MII by *Centhrorynchus* cystacanths in *Leptodactylus macrosternum* Miranda-Ribeiro, 1926 and *Leptodactylus vastus* Lutz, 1930 was 4 and 111 helminths/host, respectively; however, only one anuran of each species was parasitized, leading to prevalences of 4% and 7.69%, respectively (Santos *et al.* 2024). Some investigations carried out in the north, central-west, and northeast of Brazil have recorded the occurrence of cystacanths of undetermined genera associated with *Leptodactylus*

Table 1. Prevalence (P%), mean intensity of infection (MII), mean abundance (MA), and range of *Centrorhynchus* sp. cystacanths parasitizing *Leptodactylus luctator* from the Brazilian Pampa, southern Brazil, in 2017 and 2018. **p*-value = 0.036.

Parasitological indexes	**2017 (*N* = 24)**	**2018 (*N* = 18)**	**Total (*N* = 42)**
P%	91.7	94.4	92.9
MII ± SE	7.41 ± 1.37*	16.3 ± 3.45*	11.3 ± 1.81
MA ± SE	6.79 ± 1.32	15.4 ± 3.37	10.5 ± 1.74
Range	1–26	1–46	1–46

Table 2. Prevalence (P%), mean intensity of infection (MII), mean abundance (MA), and range of *Centrorhynchus* sp. cystacanths parasitizing males and females of *Leptodactylus luctator* from the Brazilian Pampa, southern Brazil, in 2017 and 2018.

Parasitological indexes	**Total**		**2017**		**2018**	
	Females (*N* = 23)	Males (*N* = 19)	Females (*N* = 12)	Males (*N* = 12)	Females (*N* = 11)	Males (*N* = 7)
P%	91.3	94.7	91.7	91.7	90.9	100
MII ± SE	12.8 ± 2.81	9.6 ± 2.16	6.36 ± 1.62	8.45 ± 2.25	19.80 ± 4.82	11.29 ± 4.48
MA ± SE	11.7 ± 2.68	9.1 ± 2.11	5.83 ± 1.57	7.75 ± 2.17	18.0 ± 4.72	11.3 ± 4.48
Range	1–46	1–36	1–20	2–26	3–46	1–36

Table 3. Snout–vent length (SVL) and body mass (BM) of *Leptodactylus luctator* and the Spearman's correlation coefficient (r_s) between *Centrorhynchus* sp. cystacanths abundance and body parameters (SVL and BM) in relation to the total sample of anuran males and females from the Brazilian Pampa, southern Brazil. ^{a}Mann-Whitney U = 131 (*p* = 0.028); ^{b}Mann-Whitney U = 144 (*p* = 0.061); ^{c}Significant correlation.

	Total sample (*N* = 42)		**Females (*N* = 23)**		**Males (*N* = 19)**	
	SVL (mm)	BM (g)	SVL (mm)	BM (g)	SVL (mm)	BM (g)
Mean ± SD	73.6 ± 13.1	49.9 ± 23.6	77.6 ± 9.27	55.9 ± 19.83	68.8 ± 15.5	42.7 ± 26.2
Range	46.0–102.0	12.1–112.8	65.0–95.0^{a}	29.3–112.8^{b}	46.0–102.0^{a}	12.1–100.5^{b}
r_s	-0.032	-0.065	-0.147	-0.206	0.452	0.436
p-value	0.840	0.682	0.502	0.345	0.030^{c}	0.037^{c}

spp., also with lower P% (Goldberg *et al.* 2009, Campião *et al.* 2016, Lins *et al.* 2017, Santos *et al.* 2024) than that found in this study. Among the records of cystacanths of undetermined genus, Santos *et al.* (2024) reported an MII (8.27–17 helminths/host) that is similar to the present study, and Lins *et al.* (2017) reported a higher mean intensity (37 cystacanths/host) than that observed in *L. luctator* in the Pampa biome. Cordero (1933) reported *Centrorhynchus tumidulus* (Rudolphi, 1919) cystacanths in *L. ocellatus* in Uruguay; however, the number of hosts examined and the parasite infection indices were not reported. In anurans of the families Bufonidae (Santos and

Amato 2010a, Coimbra *et al.* 2023), Hylidae (Silveira *et al.* 2022), and Ranidae (Oliveira *et al.* 2024) in the Brazilian Pampa, records of cystacanths of *Centhrorynchus* sp. with infection rates were close to those found in this study, i.e., P% ranging between 55.1% and 84% and MII, from 4.79 to 17.1 helminths/host.

The influence of length and body mass of *Leptodactylus bufonius* Boulenger, 1894 on the abundance of *Centrorhynchus* sp. cystacanths was investigated in Argentina (Corrientes Province), where Hamann *et al.* (2012) observed that the larger the anurans, the more cystacanths they bore (considering the total sample, $N = 66$), a fact that was not observed in this study. Analyses of body parameters of males and females have shown that abundance was significantly correlated with SVL in *L. luctator* males (this study) and *L. bufonius* males (Hamann *et al.* 2012); in the case of *L. luctator,* BM and helminth abundance were also correlated. In both species, larger males tend to have a higher abundance of cystacanths, while body parameters of females showed no significant correlation with abundance of parasites (Hamann *et al.* 2012, this study). It should be noted that in *L. bufonius,* males were larger than females (Hamann *et al.* 2012), which is the opposite of *L. luctator.* In other groups of anurans, such as *Rhinella dorbignyi* (Duméril and Bibron, 1841), larger hosts had greater abundances of cystacanths of *Centrorhynchus* sp. (Santos and Amato 2010a, Hamann *et al.* 2013). Larger size is related to longer host life and, consequently, provides more time to be infected, a fact that favors higher parasitism rates (Hamann *et al.* 2013). Oliveira *et al.* (2022) argued that microhabitat use, physiology, behavior, and seasonality may exert more influence on parasite load than anuran body size. Although *L. luctator* males had a higher abundance of cystacanths, there was no difference in P% and MII between males and females, corroborating studies with other anuran species (Santos and Amato 2010a, Coimbra *et al.* 2023, Oliveira *et al.* 2024). In many vertebrate host groups (mainly mammals), the high parasite load in males compared to females has been associated with hormonal and immunological factors (Klein 2004). Lees and Bass (1960) recorded higher parasite loads (including acanthocephalans) in males than in females of naturally infected *Rana temporaria* Linnaeus, 1758. The authors associated this difference with the hormone oestradiol after carrying out an experiment in which the hormone was injected into a group of male *R. temporaria,* which had a drop in parasitism compared to the control group (Lees and Bass 1960). Further studies, not only on cystacanth infections, but also on other groups of helminths associated with *L. luctator,* are needed to assess other aspects of parasite-host interactions.

It is worth mentioning that the diet of anurans influences *Centrorhynchus* infections directly. In general, transmission of *Centrorhynchus* spp. involves prey-predator interactions, i.e., the trophic chain is necessary 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) that ingest arthropods and act as carriers of infective forms; birds and mammals (definitive hosts) get infected by ingesting cystacanths while preying on them (Petrochenko 1971, Amato *et al.* 2003, Kennedy 2006). Isopods are part of the diet of *Leptodactylus* spp., which may also prey on other anurans, including other leptodactylids (Teixeira and Vrcibradic 2003, França *et al.* 2004, Maneyro *et al.* 2004, Sanabria *et al.* 2005, Pazinato *et al.* 2011, Solé *et al.* 2019) and thus increase participation of these vertebrates in the *Centrorhynchus* transmission chain. Anurans are valuable food sources for reptiles, birds, and mammals (Panasci and Whitacre 2000, Silva and Talamoni 2003, Aguiar and Di-Bernardo 2004, Hartmann and Marques 2005, Soave *et al.* 2008, Corrêa *et al.* 2016, Thaler *et al.* 2018, Quintela and Loebmann 2019, Porto and Rui 2019, Brentano *et al.* 2020, Frota *et al.* 2021). Adult parasites have been recorded in several species of birds and mammals in South America (Oliveira *et* *al.* 2024), including the Pampa (Cordero 1933, Ruas *et al.* 2008, Gomes *et al.* 2012), reinforcing the idea that the region has favorable conditions for the development and maintenance of the parasites. A diverse network of trophic interactions is involved in the transmission of acanthocephalan parasites. Parasite-host interactions are constantly under the influence of both abiotic (e. g., temperature, humidity, and anthropogenic changes) and biotic factors (e. g., reproductive and physiological aspects of hosts and parasites), which may influence the life cycle of parasites and, as a result, affect parasitism rates.

This is the first record of *Centrorynchus* sp. cystacanths in *L. luctator* in the Brazilian Pampa, where infection indices suggest that this anuran plays a role in maintaining the infective forms, thus contributing to the life cycle of the acanthocephalan. The occurrence of a heteroxenic parasite (i.e., one that necessarily requires more than one host) reinforces the need for conserving ecosystems. Parasites are a natural part of all ecosystems and each species is a potentially useful unit of information (Marcogliese 2005). Helminthological studies carried out in the Pampa biome have helped generate data that may assist in its conservation since it supports complex life cycles.

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