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Helminths Associated with Three *Physalaemus* Species (Anura: Leptodactylidae) from Caatinga Biome, Brazil

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Abstract

Objectives and Design Knowledge of which and how many species are part of an ecosystem is fundamental to understanding the diversity of organisms, including parasitic organisms that vary widely among host populations. This study describes the composition, infection patterns, and similarity in composition of helminths associated with *P. albifrons*, *P. cicada*, and *P. cuvieri*.

Materials and Methods Four samplings were carried out in two caatinga areas in the southern region of Ceará State, Brazil. The specimens were collected by active search, necropsied, and surveyed for helminths. The infection patterns for all helminth species found were estimated through prevalence, mean abundance, and mean intensity of infection.

Results A total of 242 helminth specimens were collected from 264 hosts—100 *Physalaemus albifrons* with overall prevalence of 20%, 93 *Physalaemus cicada* with overall prevalence of 27%, and 71 *Physalaemus cuvieri* with overall prevalence of 15%. Ten parasite taxa with a mean of one–three parasite species per host were identified. The nematode *Raillietnema spectans* was present in the three host species and had the highest prevalence.

Conclusions The results of this study represent 38.4% of known parasites for the species of the subfamily Leiuperinae, and 52.6% for the species of *Physalaemus*. Four parasite taxa (*Oswaldocruzia* cf. *mazzai*, *Raillietnema spectans*, *Schrankiana schranki*, and *Cylindrotaenia americana*) are new records for the host species studied, contributing to the knowledge of the parasitic interactions of amphibians of the genus *Physalaemus*.

Keywords Inventories · Parasitism · Amphibians · Neotropical

Introduction

Species inventories are the base for scientific studies since the knowledge of which and how many species are part of an ecosystem is fundamental to understanding the diversity and function of the organisms. The parasitism is part of this diversity, being the most common life strategy in the planet [69]. Parasites are diverse organisms that are an integral part of nature, but were neglected for a long time in biodiversity inventories [32, 69].

The helminth fauna associated with amphibians is rich and diverse. The latest checklist of helminths of amphibians in South America state that only 8% of all amphibian species have had their helminth fauna surveyed, in which representatives of the Hylidae and Leptodactylidae are the most studied hosts [25].

Despite the efforts to survey the helminth fauna of the Neotropical region, many areas and hosts remain neglected regarding the study of helminths. The composition of parasite communities can vary significantly among host populations of the same species [11, 70]. This way, data on occurrence and distribution of parasite species broaden the database used to understand species richness patterns of helminth associated with amphibians [26].

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According to Campião et al. [25], anurans have patterns of helminth parasite communities with wide distribution, high diversity, and low host specificity. These communities are influenced by several factors, like host size, sex, diet, infection site, species, and behavior [13, 26, 42]. Besides, the characteristics of host habitats are a key factor for the probability of parasite colonization [41].

Leptodactylid frogs show rich parasite communities, with wide taxonomic diversity, composed mainly of nematodes [27, 74, 77, 84, 85]. Most of the records are adult nematodes parasitizing the gastrointestinal tract of the hosts, with members of the Cosmocercidae, Kathlaniidae, Molineidae, Physalopteridae, and pulmonary Rhabdiasidae among the most recorded helminths [25, 26].

The genus *Physalaemus* [37] is characterized as a heterogeneous taxon [66]. From the 42 species recorded in Brazil [79], only ten had their helminth fauna surveyed: *Physalaemus albonotatus* [24, 46, 52], *Physalaemus biligonigerus* [49, 88], *Physalaemus cuvieri* [2, 31, 60, 75, 85], *Physalaemus lopesi* [8], *Physalaemus marmoratus* [89], *Physalaemus nattereri* [31], *Physalaemus olfersii* [85], *Physalaemus santafecinus* [45, 52], *Physalaemus signifer* [36, 88], and *Physalaemus soaresi* [35, 88].

Species of *Physalaemus* have wide geographical distribution in the Neotropical region, from Mexico to South America [66]. In Brazil, *Physalaemus albifrons* (Spix 1824) can be found in open areas from Maranhão State to the south of Minas Gerais State [5]; *Physalaemus cicada* [12] inhabit open areas of Caatinga, Cerrado, and domains of Atlantic forest [21, 62, 81, 82]; and *P. cuvieri* (Fitzinger 1826) is widely distributed in the South, Southeast, Northeast, and Central West, besides some areas in Uruguay, Paraguay, and Argentina [39, 50].

The aim of this study is to describe the composition, infection patterns, and similarity in composition of helminths associated with *P. albifrons*, *P. cicada*, and *P. cuvieri* from a Caatinga area in the south of Ceará State, Brazil.

Materials and Methods

This study was carried out in two sampling sites, 'Sítio Pedra Preta' (06°46′57.7″S, 39°33′11.2″W) and 'Sítio Várzea' (06°51′55.7″S, 39°32′08.0″W), both located at the Municipality of Farias Brito, Ceará State, Brazil. The sites have vegetation typical of Caatinga, with rainy season from January to April and mean precipitation of 896.5 mm^3 [55]. The samplings were performed in temporary ponds on January 20th and 27th, and March 4th and 28th, 2017, with a total of 48 sampling hours.

The specimens were collected by visual search [10], individualized in plastic containers, and euthanized with lethal injection of Sodium thiopental [29]. Afterwards, all host specimens were weighed and had their snout-vent length (SVL) taken with a digital caliper Mitutoyo® (0.01 mm of precision). Host samples were fixed in 10% formalin according to Franco and Salomão [38], preserved in 70% ethanol and deposited at the Herpetological Collection of the Universidade Regional do Cariri (URCA-H), Crato, Ceará (URCA-H 12749-13003).

For parasitological examination, the hosts were necropsied while fresh, and the organs like gastrointestinal tract, lungs, liver, and kidneys were separated and checked under stereomicroscope. The helminths found were collected following the methodology of Amato et al. [3]. For identification of the helminths, the nematodes were clarified in lactic acid, and cestodes, trematodes, and acanthocephalans were stained with hydrochloric carmine. Thereafter, the helminths were mounted in temporary slides and examined under the light microscope ZEISS Axio Imager M2 [4].

The infection patterns for all helminth species found were estimated through prevalence (number of parasitized host individuals divided by the total number of individuals, expressed in percentage), mean abundance (number of helminths divided by total number of individuals in the sample), and mean intensity of infection (number of helminths divided by the number of parasitized individuals), followed by their respective standard errors [20]. Simpson's dominance index was used to estimate which parasite community showed the greatest diversity among the host species *P. albifrons*, *P. cicada*, and *P. cuvieri*. A correlation test using Pearson's coefficient was carried out to verify whether there was correlation between SVL of the hosts and abundance of endoparasites. Jaccard's index for qualitative similarity, based on presence and absence of helminths, was used to evaluate the similarity of the parasitic infra-communities among the host species *P. albifrons*, *P. cicada*, and *P. cuvieri*.

The analyses were all carried out using the R software, with the package "R commander" [73].

Ethical Approval and/or Informed Consent

The sampling of the anuran specimens was authorized by the 'Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis—IBAMA' (Brazilian Institute of Environment and Renewable Natural Resources), authorization # 56612-1, and by the Ethical Committee of Universidade Regional do Cariri (CEUA/URCA, process # 00260/2016.1).

Results

A total of 264 hosts were sampled and necropsied—100 specimens of *P. albifrons* with prevalence of 20% and mean intensity of infection 2.15 ± 3.06, 93 *P. cicada*

with prevalence of 27% and mean intensity of infection 4.42 ± 5.93, and 71 *P. cuvieri* with prevalence of 15% and mean intensity of infection 7.63 ± 14.27.

A total of 242 helminths were recovered from the hosts analyzed, with a mean of one to three species by host. The compound community associated with the three anuran species comprised ten taxa: *Rhabdias* cf. *breviensis*, *Oswaldocruzia* cf. *mazzai*, *Cosmocerca parva*, *Raillietnema spectans*, *Schrankiana schranki*, *Oxyascaris oxyascaris*, *Physaloptera* sp., *Cylindrotaenia americana*, *Acanthocephalus* cf. *saopauloensis*, and cystacanths. The nematode *Raillietnema spectans* [44] showed the highest prevalence, being present in the three host species (Table 1).

Simpson diversity index showed *P. cicada* (ID = 0.37) with the greatest diversity, *P. albifrons* (ID = 0.5) with intermediate, and *P. cuvieri* (ID = 0.62) with the lowest.

There was no significant correlation between parasite abundance (AB) and host size (SVL) for *P. albifrons* ($r = 0.29$; $p = 0.1$), *P. cicada* ($r = 0.2$; $p = 0.13$), and *P. cuvieri* ($r = 0.005$; $p = 0.96$).

The similarity index (*S*) of the compound community (10 taxa) showed the greatest similarity (57%) between *P. albifrons* and *P. cuvieri* ($S = 0.57$; DE = 1.73), while *P. cicada* showed the lowest similarity compared to *P. albifrons* ($S = 0.22$; DE = 2.64), and *P. cuvieri* ($S = 0.37$; DE = 2.44).

Discussion

Nematodes and trematodes are commonly recorded parasitizing amphibians of the Leptodactylidae, with nematodes of the Cosmocercidae, Molineidae, Physalopteridae, Rhabdiasidae, and trematodes of the Diplodiscidae, Glyptthelminthidae, Gorgoderidae, and Plagiorchiidae the taxa most frequently found [25, 26, 33, 84].

According to Campião et al. [25], Silva et al. [80] and Teles et al. 84], 26 helminth species are recorded for members of the subfamily Leiuperinae, from which approximately 68% belong to the phylum Nematoda. Anurans of *Physalaemus* have 20 helminth species recorded, from which 69% are nematodes [2, 25, 60, 85].

The results of the present study represent 38.4% of the known parasite species for members of the subfamily Leiuperinae, and 52.6% for members of *Physalaemus*. This study also recovered nematodes and acanthocephalans as predominant components, following the same infection patterns of other congeneric hosts. Low host specificity is common for some helminth species [26], which justifies the high number of infection by nematodes of the Cosmocercidae.

The lowest richness among the three host species of this study ($R = 5$) was observed for *P. albifrons*, although it showed a great diversity of parasite communities, according to the Simpson index (ID = 0.5). Campião et al. [26] affirm that the geographical locality in association with ecology and life history of host, influence parasite richness, making

Table 1 Helminths recorded in the host species: *Physalaemus albifrons*, *Physalaemus cicada*, and *Physalaemus cuvieri* from the municipality of Farias Brito

Host	Parasite	P%	NH	$I \pm$ EP	Min–Max	IS
Physalaemus albifrons	Acanthocephalan (cystacanth)	2	2	1	1	St/C
	Oswaldocruzia cf. *mazzai*	3	4	1.33 ± 0.33	1–2	Si
	Physaloptera sp.	5	7	1.40 ± 0.24	1–2	St
	Raillietnema spectans	9	29	3.22 ± 1.47	1–12	Si/Li
	Rhabdias cf. *breviensis*	1	1	1	1	L
Physalaemus cicada	Acanthocephalan (cystacanth)	2.15	4.30	2	1–2	St
	Cylindrotaenia americana	1.07	2.10	2	2	Si
	Cosmocerca parva	2.15	2.10	1	1	Si
	Oxyascaris oxyascaris	7.52	13.90	1.85 ± 0.55	1–5	Si
	Raillietnema spectans	17.20	96.70	5.60 ± 1.80	1–22	Si/Li
	Schrankiana schranki	1.07	4.30	4	4	Li
Physalaemus cuvieri	Acanthocephalan (cystacanth)	1.40	32.39	23	3–23	C
	Acanthocephalus cf. *saopauloensis*	1.40	2.81	2	3	Li
	Oswaldocruzia cf. *mazzai*	4.22	7.04	1.66 ± 0.66	1–3	Si
	Physaloptera sp.	2.81	8.45	3	2–3	St
	Raillietnema spectans	5.63	61.97	11 ± 10	1–41	Li
	Schrankiana schranki	1.40	1.40	1	1	Si

P% prevalence, *NH* number of helminths, *I, EP* mean intensity and standard error, *Min–Max* minimum and maximum values of parasite specimens, *IS* infection site, *St* stomach, *C* cavity, *L* lung, *Li* large intestine, *Si* small intestine

difficult the contact with infectious forms, which can result in colonization by a less number of parasite species. Nevertheless, the composition of helminth communities of *P. albifrons* followed the same pattern of *P. signifer* ($R=4$) and *P. soaresi* ($R=4$), which were also composed mainly of nematodes [35, 88].

Among the eight species of *Physalaemus* already studied, *P. cicada* ($R=6$) was the only one that had parasites belonging to more than two phyla—Nematoda, Platyhelminthes (Cestoda), and Acanthocephala (see [25]). Moreover, *P. cicada* showed high parasite richness, like *P. albonotatus* ($R=7$; [46]) and *P. santafecinus* ($R=7$; [45]). *P. cicada* also showed parasite diversity (ID = 0.37) superior to *P. cuvieri* and *P. albifrons*, suggesting that the form of habitat exploitation is associated with richness and parasite diversity of *P. cicada* [69]. Seasonal changes, biotic factors, like immune system and host age, may also affect parasitism, influencing the life of the parasite and its host [67]. *Physalaemus cicada* follows the same infection patterns as other Neotropical anurans, in which infections formed almost exclusively by nematodes can be observed [25]. Up to date, this is the only surveyed *Physalaemus* species that showed members of Cestoda as a component of the helminthfauna.

Physalaemus cuvieri ($R=6$), showed the lowest diversity (ID = 0.62), but had the same infection patterns ($R=8$) as observed by Aguiar et al. [2], Leivas et al. [60], Santos and Amato [75] and Toledo et al. [85] for component helminth fauna. The present study shows two new records for this host (*R. spectans* and *S. schranki*), increasing the known parasite richness for *P. cuvieri* (10 taxa).

The values of parasite richness found for *P. albifrons* ($R=5$), *P. cicada* ($R=6$), and *P. cuvieri* ($R=6$) in the present study are similar to the results in the literature for the eight *Physalaemus* species already surveyed [2, 25, 60, 85]. These values indicate that *Physalaemus* species show low richness of parasite taxa, which seems like a common pattern for this genus. According to Campião et al. [26], parasite richness is linked to geographical distribution, host size, and transmission mode of the parasite.

Inventories of parasites associated with members of the Leptodactylidae, like *Pseudopaludicola boliviana* from Argentina ($R=9$; [33, 47]) *Leptodactylus chaquensis* and *Leptodactylus podicipinus* from the Brazilian Pantanal ($R=14$; $R=11$, [23]), and [65] for 21 species of leptodactylids from Mato Grosso State, Brazil (mean richness = 10 species), suggest infection patterns with high parasite richness for this group. However, such patterns were not xobserved in the present study for *P. albifrons*, *P. cicada*, and *P. cuvieri*, similarly to the results of Teles et al. [84] in *Pleurodema diplolister* and Silva et al. [80] in *Pseudopauludicola pocoto*. Thus, leptodactylids from Caatinga might harbor lower richness of endoparasites compared to leptodactylids from other localities.

Lins et al. [61] surveyed the helminths of *Leptodactylus syphax* from the same locality as the present study, and found a richness of six parasite species (*Aplectana membranosa*, *Schrankiana formosula*, *Physaloptera* sp., *Lophosicyadiplostomum* sp., cystacanths, and no identified larvae). Such richness is similar to what was found in this study for *P. albifrons*, *P. cicada*, and *P. cuvieri*, demonstrating that the environment is a key factor to predict parasite richness in Neotropical anurans. However, the parasitic composition differs from the observed for the *Physalaemus* spp. of this study. The composition of helminth communities in anurans depend on several factors, like body size, gender, diet, infection site, behavior, host species, and interactions with the environment [18, 26]. According to Barton [9] and Bursey et al. [19], such communities are composed of generalist species of direct transmission, favoring parasites with infectious stage in soil, like nematodes [22]. Communities richness can also vary according to environmental conditions, due to the way that species respond to biotic factors [71]. The absence of trematode species in our study is noteworthy, since species of *Physalaemus* are semiaquatic. Trematodes in general require aquatic intermediate hosts in addition to snails. Therefore, anurans which live in aquatic habitats are more easily infected with digeneans than terrestrial species [48]. The Caatinga has a marked water deficit and this may be related to the low diversity or even the absence of trematodes in parasitological studies conducted in this domain.

The results of the present study follow the common pattern of parasite communities found in leptodactylids of South America, with dominance of nematodes [25, 47, 65, 76, 84]. For the total richness ($R=10$) of *P. albifrons*, *P. cicada*, and *P. cuvieri* studied herein, only the cosmocercid *R. spectans* and cystacanths were present in the three host species.

Raillietnema spectans had the highest prevalence (11%), similar to the patterns found by Morais [65] for 21 species of leptodactylids from Mato Grosso State, Brazil. This result shows that *Physalaemus* spp. present an interaction web of parasites composed of generalist species of direct infection, a common pattern found in the literature for Neotropical anurans.

According to Baker [7], acanthocephalans are parasites with indirect life cycle, with arthropods (usually crustaceans) as intermediate host, and fishes or aquatic birds as final hosts. The presence of cystacanths in *P. albifrons*, *P. cicada*, and *P. cuvieri* indicate that these species are serving as paratenic hosts of acanthocephalans. The cystacanths were probably acquired through the diet of *Physalaemus* spp. that is composed mainly of ants and coleopterans [30, 60].

Given the increase of studies on parasites of amphibians [6, 22–28, 51, 60, 61, 74], new host records are becoming common, as well as records of parasite taxa that are still new to science [1, 6].

The parasite *R. spectans* was reported before in *Rhinella crucifer*, *R. icterica*, *Leptodactylus latrans* [25], and *Leptodactylus mystaceus* [65]. The present study is a new host record of *R. spectans* parasitizing *P. albifrons*, *P. cicada*, and *P. cuvieri* from Caatinga area.

Schrankiana schranki [86] was reported before in *Leptodactylus latinasus*, *L. pentadactylus*, *L. rhodomystax*, *L. vastus*, *L. labyrinthicus*, *L. mystaceus*, *L. fuscus*, and *L. pustulatus* [25, 43, 65], which is a new record of *S. schranki* parasitizing *P. cuvieri* and *P. cicada* from the Caatinga area. *Oswaldocruzia* cf. *mazzai* [87] was reported before by Morais [65] in *Rhinella icterica*, *R. margaritifera*, *R. marina*, *Leptodactylus bufonius*, *L. fuscus*, *L. chaquensis*, *L. knudseni*, *L. labyrinthicus*, *L. latrans*, and *L. rhodomystax*. In the present study, this nematode is a new host record for *P. albifrons* and *P. cuvieri*.

The cestode *Cylindrotaenia americana* [56] was recorded before in the amphibians *R. icterica* [83] and *P. olfersii* [85] in Brazil, *R. marina* in Colombia [17], *L. latrans* in Argentina [78], *Lithobates septentrionalis* in the United States [14], *Hyla arborea* in the former Czechoslovakia [72], and in the reptile *Scincella lateralis* [16, 54]. In the present study, we present the first record of *C. americana* parasitizing *P. cicada* from the Caatinga area in Brazil. The low mean abundance found in this study for *S. schranki* corroborate the findings of Hamann et al. [53] for *L. latinasus* in Argentina, but not the findings of Dyer and Altig [34] for *L. pentadactylus* in Ecuador. For mean abundance of the cestode *C. americana*, this study corroborates the findings of Bouchard [14], Brooks [16, 17], Harwood [54], Prokopic [72], Savazzini [78], and Stumpf [83] that also presented low mean abundance values for this parasite. Low abundance of parasite can be an occasional finding since there is not a common pattern among amphibians. Infection abundance is related to environmental factors, influencing parasite or host species in different ways, which can alter infection rates [57, 68]. The ecology, life history, and geographical location of the hosts can also influence parasite infection rates [26].

Host body size is suggested as a determinant to predict species richness, affecting the composition of parasite communities [26]. However, the values of SVL and parasite abundance did not show any correlation in the present study. As proposed by George-Nascimento et al. [40], larger hosts (body mass and SVL) can harbor greater parasitic loads and species richness [26] because they can offer more diversity of microhabitat, favoring the development and reproduction of parasites. Even though body size influences the acquisition of parasites, other aspects like physiology, behavior, and seasonality can also explain variations in the abundance of parasites [15].

In the present study, the analyses of similarity of parasite composition showed that the greatest similarity was between *P. albifrons* and *P. cuvieri* (S = 0.57; DE = 1.73), while *P.*

cicada was more similar to *P. cuvieri* (S = 0.37; DE = 2.44) than *P. albifrons* (S = 0.22; DE = 2.64). Such proximity can be explained by the phylogenetic relationship among species of this group [63]. Krasnov et al. [59] affirm that phylogeny is a determinant factor for structuring webs between parasites and hosts since the phylogenetic proximity is reflected on phenotypic similarities, leading parasite species to explore particular hosts. This result can also be explained by niche occupation, given that hosts sharing the same habitat are exposed to the same web of interactions [58]. Moreover, Martin et al. [64] suggest that host species with similar diet show more similarity of parasite composition, as observed for *P. albifrons* and *P. cuvieri*.

The present study evaluated the helminth associated with three species of *Physalaemus* from the Caatinga area recovering four new host records, which highlights the importance of parasite inventories for host species and poorly researched areas. The pattern presented in this study indicates that the parasite communities associated with the studied hosts are composed mainly of generalist parasites with direct life cycle, which is a common standard for the species of *Physalaemus*. No relation was observed between mean host size and average parasite abundance. In addition, the highest similarity of the parasite composition associated with *P. albifrons* and *P. cuvieri* in relation to *P. cicada* shows this to be related to the phylogenetic proximity of the species.

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