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Helminth infracommunity of the cane toad *Rhinella marina* (Anura: Bufonidae) within its native distribution range

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ABSTRACT

During August 2010, a total of 66 cane toad *Rhinella marina* were collected in Laguna de Coyuca, Guerrero, Mexico and examined for the presence of helminths. Ninety-two percent were parasitized by 1 or more parasite species. A total of 4624 individuals were found, belonging to 8 helminth taxa (1 digenean, 1 cestode and 6 nematodes). Infracommunities harbored by this host species are highly variable (mean Jaccard similarity index 0.31 ± 0.27); mean percent similarity index 0.26 ± 0.32) and depauperate (mean richness 1.91 ± 0.92; mean abundance 64.4 ± 100.34); almost 50% of them were dominated by *Rhabdias fuelleborni*, a skin-penetrating nematode. Differences related to host gender and size were found, since males registered infracommunities with lower species richness and diversity than females. The reproductive condition of females (collected during the breeding season) might explain the results obtained; during this season, females invest less in resistance to infection in favor of investing resources in reproduction. However, confirmation of this statement can only be made by sampling in the dry season, when the toads do not reach their reproductive peak.

1. Introduction

The cane toad, *Rhinella marina* (Linnaeus 1758) is a large terrestrial anuran with a native range from southern Texas to central Brazil [1], but introduced worldwide [2]. This bufonid species is one of the most tolerant amphibians to distinct environments, since inhabiting forested areas and semi-deserts, disturbed habitat or areas surrounding urbanization and roadways [3]. Adults spend most of the time out of water, but utilize permanent or temporary ponds in breeding season [4].

Members of this species are nocturnal; daytime hiding among rocks or dark places. Their primarily diet consist of terrestrial arthropods [5]; however, they also prey on small worms, mollusks, and less frequently birds, lizards, and snakes [6].

The wide range of native and introduced distribution of this species, along with their opportunistic feeding habits, exposes it to numerous helminth species worldwide. In consequence, the helminthological record of *R. marina* is constituted by a total of 131 helminth taxa through its entire distribution range; 51.1% of these taxa correspond to nematodes, 31.6% to digeneans, 9.8% to cestodes, 5.3% to acanthocephalans, and 0.7% to monogeneans [6]. Particularly in Mexico, fifty-one taxa have been recorded as parasites of the cane toad [7]. In spite of all this information, studies dealing with the helminth community harbored by this bufonid species have been neglected; to the best of our knowledge, only one work, conducted outside its native distribution range, i.e., Australia, analyzed some attributes of the helminth community associated with the cane toad [8].

The main objectives of this study were: 1) to determine the richness and diversity of helminth community of *R. marina* within its native distribution range; 2) to examine the factors involved in the structuring processes of this community; 3) to analyze differences in a related to sex and size of toads, and 4) to compare the helminth infracommunity found in this anuran species with those described for other bufonid species.

2. Materials and methods

The specimens of *R. marina* studied, were collected by hand on the shore of Laguna de Coyuca, Guerrero, Mexico (16°52′59″N,

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100°04′31″W), in August 2010, during the wet season. Located 35 km northwest of Acapulco, this lagoon is predominantly oligohaline (1.5 to 5 ppm) during most of the year; has a warm humid climate, with the maximum rain (43–55%) in summer and the lower (5%) during the winter [9].

Vegetation around the margins of the lagoon is represented by tulles, reeds and mangroves. Aquatic vegetation includes floating plants and grass [10]. Sampling was conducted under permit FAUT 0084 issued by Secretaría del Medio Ambiente y Recursos Naturales, Mexico. Animals were stored at 4 °C, and killed with an overdose of intraperitoneal sodium pentobarbital, necropsied within the following 48 h, and examined using a stereo-microscope Leica EZ4. The snout-vent length (SVL) and sex of each host were recorded.

Helminths were removed from the internal organs, counted in situ and placed in saline solution (0.65%), fixed by suddenly immersion in hot 4% formaldehyde or 70% ethanol, and preserved in 70% ethanol. Platyhelminths were stained with Mayer's paracarmine, dehydrated, cleared in methyl salicylate, and mounted in Canada balsam. Nematodes were cleared with Amman's lactophenol, and temporarily mounted for morphological study. Specimens of all helminth species were deposited at Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México (UNAM), Mexico City (Table 1).

The use of descriptive ecological terms at population and infracommunity levels follows Bush et al. [11]. Cumulative species richness curve was constructed to determine if the number of examined hosts was sufficient to represent the helminth infracommunities. Analyses of helminth infracommunity, irrespective of their site of infection, included measurements of mean number of helminths (abundance) and species of helminths (richness); attributes of the infracomminities were calculated using PAST 3.0; mean diversity and evenness per toad (infected and uninfected) were calculated using Brillouin's index (H0), and evenness (J0) as H0/H0 maximum. The Berger-Parker index (d) was used to determine the numerically dominant species. Quantitative and qualitative similarities were calculated for 2178 pairs of infracommunities, using percent similarity and Jaccard's index, respectively [12].

Multivariate analysis (Principal Component Analysis, PCA) was conducted to identify association between some host traits (size and sex), and the abundance of each helminth specie, and some infracommunity attributes (richness and diversity).

3. Results

A total of 66 adult specimens of *R. marina* (33 males, mean SVL = 11.3 cm ± 2.1; 33 females, mean SVL = 11.6 cm ± 1.9) were examined, whereby 61 were found infected by one or more helminth species. The cumulative species richness curves indicate that the inventory presented here for *R. marina* can be considered complete since 100% of the helminth species found were recovered from only 28 toads.

The 4624 individual parasites collected belong to 8 helminth taxa, including 1 digenean [*Rauschiella poncedeleoni* (Razo-Mendivil and León-Règagnon 2001], 1 cestode [*Nematotaenia dispar* (Goeze 1782)] and 6 nematodes (*Oswaldocruzia lamotheargumedoi* Ruiz-Torres, García-Prieto, Osorio-Sarabia and Violante-González 2013; *Foleyellides rhinellae* García-Prieto, Ruiz-Torres, Osorio-Sarabia and Merlo-Serna 2014; *Rhabdias fuelleborni* Travassos 1926; Cosmocercinae gen. sp., *Physocephalus* sp., and *Hysterothylacium* sp.).

Nematodes were the most abundant group (3873 worms, representing 75% of individuals), followed by cestodes (827) and digeneans (14). Digeneans infected 4 hosts, cestodes 32, and nematodes were found in 57 of the toads. Of the 66 cane toad examined, 29% harbored 0–1 species of helminth, 46.9% were infected by 2 helminth species and 24.2% had more than 2 taxa.

The most parasitized site in this host was the digestive tract, housing 50% of the taxa; *Rhabdias fuelleborni* and *F. rhinellae* infected lungs and body cavity, respectively; *Physocephalus* sp., and *Hysterothylacium* sp. were found encapsulated in the stomach wall. Six of the 8 taxa were collected in adult stage and 2 as larvae. Two of the species recorded here have not been found parasitizing other host species (*O. lamotheargumedoi* and *F. rhinellae*) one represents new host records (*Hysterothylacium* sp.) and seven represent new locality records. The nematodes *R. fuelleborni* and Cosmocercinae gen. sp., as well as the cestode *N. dispar* were the most prevalent and abundant species. The other helminth species identified herein infected cane toads with lower prevalence (Table 1).

At infracommunity level, the mean richness and abundance recorded in the cane toad were 1.91 ± 0.92 (0–4) and 64.4 ± 100.34 (0–707), respectively; mean diversity (Brillouin index) was low [0.47 ± 0.41 (0–1.37)] because *R. fuelleborni* dominated almost 50% of the infracommunities with a Berger Parker index of 0.79 ± 0.49 (0–1). The relative abundance of the remaining helminth species was heterogeneous, which is why evenness values were also low [0.42 ± 0.36 (0–1)]; in addition, 29% of the infracommunities were free of infection or parasitized by only 1 helminth species. Overall qualitative and quantitative similarities between helminth infracommunities, indicated a low similarity at both levels, since only 9.5% and 22% of the pairs compared, respectively, displayed values higher than 0.5. The average value for both indices was 0.31 ± 0.27 (qualitative) and 0.26 ± 0.32 (quantitative).

The PCA applied to determine possible influence between the host traits and the abundance of helminth species, indicated that the eigenvalues of the first correlations matrix varied of 1.270 to 1.815 and allowed the extraction of three components with a total explained variance of 46.67%. The first component indicated a strong positive association between *R. poncedeleoni* and *F. rhinellae*, as long as the second component showed positive association among the host size and the abundance of *O. lamotheargumedoi* and *Hysterotylacium* sp. (i.e., the larger host registered a higher abundance of both helminths), but negative association with *N. dispar* (Fig. 1). The third component showed

Table 1
Infection parameters of helminths parasitizing *Rinella marina* in Laguna de Coyuca, Guerrero, Mexico.

Helminth species	%	MI	MA	I-R	Site of infection
Digenea	6.06	3.50	0.21	1–10	Intestine
Rauschiella poncedeleoni (CNHE-9644)[g, i]					
Cestoda	48.40	25.80	12.53	1–170	Intestine
Nematotaenia dispar (CNHE-9645)[g, i]					
Nematoda	9.09	10.66	0.96	2–33	Intestine
Oswaldocruzia lamotheargumedoi (CNHE-8260-8262)[s, p]					
Cosmocercinae gen. sp. (CNHE-9646)[g, i, p]	37.87	32.88	12.45	1–248	Intestine
Rhabdias fuelleborni (CNHE-9647)[g, p]	66.60	45.84	30.60	1–647	Lungs
Foleyellides rhinellae (CNHE-8907-8909)[s, p]	9.09	4.66	0.42	1–8	Body cavity
Hysterothylacium sp. (CNHE-9649)[g, l, i]	10.60	104.71	11.10	14–383	Stomach wall
Physocephalus sp. (CNHE-9648)[g, l, i]	21.21	8.50	1.80	2–86	Stomach wall

% = Prevalence; MA = Mean abundance; MI = Mean intensity; I-R = Intensity range; [g] = Generalist; [s] = Specialist for amphibian hosts; [l] = larva; [p] = penetration; [i] = Ingetion.