Trabajo

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Contributions to the knowledge of parasitic nematodes of amphibians from the Dry Chaco ecoregion in Argentina.

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ABSTRACT

In the Argentinean Gran Chaco, numerous investigations have dealt with the diversity of vertebrates, including amphibians and reptiles. The parasitofauna of these organisms, has been more studied in some groups such as amphibians; however, from a biogeographic perspective, most of these studies were carried out in the Humid Chaco. The goal of this work is to present the nematofauna found in six species of amphibians, *Rhinella diptycha*, *Chacophrys pierottii*, *Ceratophrys cranwelli*, *Scinax nasicus*, *Leptodactylus bufonius*, and *Odontophrynus lavillai*, collected in different locations within the Dry Chaco, in the department of Matacos, Formosa, Argentina. In a total of 34 amphibians analyzed, we found 1,526 parasitic nematodes (total prevalence: 61.7%; total mean intensity: 72.6±90.3; total mean abundance: 44.9±78.9), belonging to 9 taxa: adults of *Rhabdias* sp., *Oswaldocruzia* sp., *O. subauricularis*, *Schulzia travassosi*, *Aplectana hylambatis*, *Cosmocerca podicipinus*, *Oxyascaris caudacutus*, *Schrankiana formosula*, and larvae of *Physaloptera* sp. These nematodes were collected from the lungs, small intestine, large intestine, gastric mucosa, and gallbladder of hosts. This work is the first record of helminths from *C. pierottii* and *O. lavillai* and the first record of nematodes from *Ce. cranwelli* throughout its geographical range. The species *Schrankiana formosula* (Atractidae) is recorded for the first time in Argentinean amphibians. Thus, we expand the knowledge of the biological diversity of helminth parasites of amphibians from the Dry Chaco ecoregion. This base-line information, would allow to understand the factors that influence the patterns of host and geographic distribution of vertebrate parasites in an area with extreme characteristics such as the Dry Chaco.

Key Words: Anurans; Chaco; Nematode Parasites.

RESUMEN

Contribuciones al conocimiento de nematodos parásitos de anfibios de la ecorregión Chaco Seco de Argentina. En el Gran Chaco Argentino, numerosas investigaciones han tratado la diversidad de los vertebrados, incluida su fauna herpetológica. En cuanto a la parasitofauna de estos organismos, algunos grupos como el de los anfibios han sido más estudiados; sin embargo, biogeográficamente, la mayor parte de estos estudios fueron realizados en el Chaco Húmedo. El objetivo de este trabajo es presentar la nematofauna hallada en seis especies de anfibios, *Rhinella diptycha*, *Chacophrys pierottii*, *Ceratophrys cranwelli*, *Scinax nasicus*, *Leptodactylus bufonius* y *Odontophrynus lavillai*, colectadas en distintos puntos del Chaco Seco en el departamento de Matacos, Formosa, Argentina. En un total de 34 anfibios analizados encontramos 1.526 nematodes parásitos (prevalencia total: 61,7%; intensidad media total: 72,6±90,3; abundancia media total: 44,9±78,9), pertenecientes a 9 taxones, adultos de *Rhabdias* sp., *Oswaldocruzia* sp., *O. subauricularis*, *Schulzia travassosi*, *Aplectana hylambatis*, *Cosmocerca podicipinus*, *Oxyascaris caudacutus*, *Schrankiana formosula* y larvas de *Physaloptera* sp. Estos nematodos se colectaron de

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los pulmones, el intestino delgado, el intestino grueso, la mucosa gástrica y la vesícula biliar de los hospedadores. Este trabajo constituye el primer registro de helmintos para *C. pierottii* y *O. lavillai* y el primero de nematodes para *Ce. cranwelli* en toda su área de distribución geográfica. La especie *Schrankiana formosula* (Atractidae) es registrada por primera vez en anfibios argentinos. De este modo, ampliamos el conocimiento de la diversidad biológica de los helmintos parásitos de anfibios de la ecorregión Chaco Seco. Esta información permitirá conocer cuáles son los factores que influyen en los patrones de ocurrencia en hospedadores y distribución geográfica de helmintos parásitos de vertebrados, en un área con características tan extremas como el Chaco Seco.

Palabras claves: Anuros; Chaco; Nematodos Parásitos.

Introduction

Studies on amphibian parasites are relevant because they can provide information regarding the structure and relationships of trophic networks, as many parasites may pass from one host to another through predator-prey relationships. Besides, parasitological data may provide indirect information about the host diet, as the presence of certain parasites with known indirect cycles would indicate the consumption of certain type of prey. The study of parasites may indicate the state of ecosystems, as their presence or absence makes possible to infer the richness of their intermediate and definitive hosts, and provide also information related to host stress because some agrochemicals are known to depress the immune system and turn amphibians more susceptible to certain parasites (see for instance Marcogliese and Cone, 1997; Lafferty *et al.*, 2008; Koprivnikar and Redfern, 2012; Koprivnikar *et al.*, 2012), among other aspects. However, despite the fact that their study may give us a wealth of information, parasites are not generally considered in biodiversity studies (Gómez and Nichols, 2013; Rocha *et al.*, 2016).

In recent investigations, Campião *et al.* (2014; 2015) compiled data on helminth parasites of amphibians from South America; one of the results of their investigation was that the most studied hosts were anurans of the families Bufonidae, Hylidae and Leptodactylidae, mainly *Leptodactylus latrans* (Steffen, 1815) and *Rhinella marina* (Linnaeus, 1758). In addition, they found that Brazil was the origin of most studies with over 50% of the total output. This evidence large information gaps regarding several host species and large geographical areas that remain unsurveyed.

Particularly for the Argentinean Chaco, parasitological studies have focused on diverse vertebrate hosts, including mammals such as monkeys (Perea-Rodriguez *et al.*, 2010), wolves (Orozco *et al.*, 2014), and armadillos (Ríos *et al.*, 2020), and sauropsids such as birds (Dueñas Díaz *et al.*, 2018; Lunaschi *et al.*, 2018), snakes (Lunaschi and Drago, 2007; 2010), lizards (Lamas and Zaracho, 2006; Zaracho and Lamas, 2006), caimans and turtles (Lunaschi and Drago, 2007). Regarding amphibians, the majority of helminth studies were carried out in the Humid Chaco, mainly in the province of Corrientes (Hamann *et al.*, 2013a; 2020; Draghi *et al.*, 2015a, b; González and Hamann, 2015a; González *et al.*, 2019), while such studies are only incipient for the Dry Chaco region (Hamann and González, 2015; González and Hamann, 2015b; González *et al.*, 2020).

The aim of the present work is to provide records of nematode parasites for six amphibian species collected in a locality within the Dry Chaco ecoregion, Argentina.

Materials y methods

Thirty-four adult amphibians belonging to six species of five families, namely *Rhinella diptycha* (Cope, 1862), *Scinax nasicus* (Cope, 1862), *Chacophrys pierottii* (Vellard, 1948), *Ceratophrys cranwelli* Barrio, 1980, *Leptodactylus bufonius* Boulenger, 1894, and *Odontophrynus lavillai* Cei, 1985, were collected for this study. Table 1 presents the number of specimens for each amphibian species analyzed, with the respective collection date, locality, and collection accession number.

Amphibians were collected within the Dry Chaco ecoregion (Morello *et al.*, 2012), approximately 32 km south from the city Ingeniero Juárez, Matacos Department, in Formosa, Argentina. In this area the vegetation cover is adapted to dry conditions (xerophytic deciduous forest), with predominance of small leaves deciduous and thorny species tolerant to large fluctuations in water availability, as well as to seasonal thermal variations. Woody vegetation is sparse, and the herbaceous species are predominantly grasses. The landscape is flat, gently sloping

toward the east. The climate is characterized by low rainfall (mean annual precipitation <700 mm), and high temperatures, at times exceeding 47°C since this area comprises part of the South American Heat Pole (Maldonado *et al*., 2006).

Amphibians were hand-captured between 18 and 21 hours using the sampling technique defined as "visual encounters survey" (Crump and Scott Jr., 1994) and "Audio Strip Transect" method (Zimmerman, 1994) in the most favorable environments such as shallow lakes, temporary puddles and ditches. Frogs were subsequently transported alive to a field-mounted laboratory, euthanized topically using 10% lidocaine cream and necropsied. Their snout-vent length (SVL) and body weight (g) were recorded. We examined under a stereoscopic microscope the digestive tract, lungs, liver, gallbladder, kidneys, urinary bladder, coelomic cavity, and musculature. Parasites were observed *in vivo*, counted, and then fixed in hot 70% ethyl alcohol. Posteriorly, they were clarified in Amman's lactophenol, mounted on temporary slides and examined under a light microscope. For morphological examination and measurements, a Leica DM2500 microscope equipped with a drawing tube was used. Amphibian taxonomy followed Frost (2020). Specimens were preserved in 70% ethyl alcohol and deposited in the Helminthological Collection of Centro de Ecología Aplicada del Litoral (CECOAL) Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET),

Table 1. Amphibian species from the Dry Chaco Ecoregion analyzed for nematode parasites: collection date, locality and accession numbers.

Family	Species and number of specimens analyzed	Collection date	Locality - Geographical coordinates	Accession number
Bufonidae	*Rhinella diptycha* (n= 3)	Dec. 2016; May 2017; Feb. 2018	-24.215401, -61.981015	UNNEC 13601
Ceratophryidae	*Chacophrys pierottii* (n= 8)	Dec. 2016; Feb. 2018; Dec. 2019	-24.216001, -61.979434	UNNEC 13602
	Ceratophrys cranwelli (n= 1)	Dec. 2016	-24.216001, -61.979434	UNNEC 13603
Hylidae	*Scinax nasicus* (n= 8)	Dec. 2016; Feb. 2017; Jul. 2017	-24.223394, -61.958458	UNNEC 13604
Leptodactylidae	*Leptodactylus bufonius* (n= 12)	Dec. 2016; April 2018; Jul. 2018	-24.223394, -61.958458	UNNEC 13605
Odontophrynidae	*Odontophrynus lavillai* (n= 2)	Dec. 2016; Feb. 2017; May 2017; Jul. 2017	-24.191161, -62.04896	UNNEC 13606

Corrientes city, Corrientes, Argentina. Amphibian specimens were deposited in the Colección Herpetológica of the Universidad Nacional del Nordeste (UNNEC); accession numbers are shown in Table 1.

Prevalence, mean intensity (MI) and mean abundance (MA) of infection for parasites were calculated following Bush *et al.* (1997).

Results

We found 21 amphibians parasitized by nematodes (total prevalence: 61.7%), from which we recovered 1,526 nematode specimens (total MI: 72.6±90.3; total MA: 44.9±78.9) of the following species and families: Rhabdiasidae: *Rhabdias* sp. (CECOAL 18021944, 1 hermaphrodite female); Molineidae: *Oswaldocruzia* sp. (CECOAL 16122115; 3 FF), *O. subauricularis* (Rudolphi, 1819) (CECOAL 17050809; 5 FF, 5 MM), *Schulzia travassosi* Durette-Desset, Baker and Vaucher, 1985 (CECOAL 18071603; 2 FF, 2 MM); Cosmocercidae: *Aplectana hylambatis* (Baylis, 1927) Travassos, 1931 (CECOAL 18042419; 10 FF, 10 MM), *Cosmocerca podicipinus* Baker and Vaucher, 1984 (CECOAL 18042402; 1 F, 1 M), *Oxyascaris*

Table 2. Nematode parasites found in six amphibian species from the Dry Chaco ecoregion, Argentina. n: number of nematode parasites collected; %: prevalence of infection; MI±SD: mean intensity (± 1 standard deviation); MA±SD: mean abundance (± 1 standard deviation).

Amphibian species	Nematode parasites	Stage	n	%	MI±SD	MA±SD	Site of infection
R. dyptycha	*Rhabdias* sp.	A	2	33.3 (1/3)	-	0.66±1.1	LU
	O. subauricularis	A	62	33.3 (1/3)	-	20.6±35.7	SI
	A. hylambatis	A	112	66.6 (2/3)	56±49.4	37.3±47.6	LI
C. pierottii	*A. hylambatis*	A	55	37.5 (3/8)	20.6±29.8	7.75±19.2	LI
	C. podicipinus	A	4	25.0 (2/8)	2.0±1.4	0.5±1.1	LI
	Physaloptera sp.	L	3	12.5 (1/8)	-	0.37±1.1	GM
Ce. cranwelli	*Oswaldocruzia* sp.	A	3	100.0 (1/1)	-	-	SI
	A. hylambatis	A	202	100.0 (1/1)	-	-	LI
	C. podicipinus	A	3	100.0 (1/1)	-	-	SI
S. nasicus	*O. caudacutus*	A	25	25.0 (2/8)	12.5±12.0	3.12±7.35	SI
L. bufonius	*Rhabdias sp.*	A	1	8.3 (1/12)	-	0.08±0.2	LU
	S. travassosi	A	6	33.3 (4/12)	1.5±0.5	0.5±0.7	SI-GB
	A. hylambatis	A	835	83.3 (10/12)	83.5±80.1	69.5±79.4	LI
	C. podicipinus	A	6	25.0 (3/12)	2±1	0.5±1	SI-LI
	S. formosula	A	150	16.6 (2/12)	75±59.3	12.5±34.2	LI
	Physaloptera sp.	L	13	8.3(1/12)	-	1.08±3.7	GM
O. lavillai	*A. hylambatis*	A	44	100.0 (2/2)	22±15.5	11±15.5	LI

Stage: A: adult; L: larval; Site of infection: LU: lung; SI: small intestine; LI: large intestine; GM: gastric mucosa; GB: gallbladder.

caudacutus (Freitas, 1958) Baker and Vaucher, 1984 (CECOAL 17072407; 5 FF, 1 M); Atractidae: *Schrankiana formosula* Freitas, 1959 (CECOAL 16122124; 10 FF; 10 MM); Physalopteridae: *Physaloptera* sp. (CECOAL 16122136; 5 larvae).

A more precise identification of the specimens of genus *Rhabdias* Stiles and Hassall, 1905 obtained from *R. diptycha* and *L. bufonius*, and those of *Oswaldocruzia* Travassos, 1917 identified in *Ce. cranwelli* was not possible because the number of worms was very low.

Table 2 presents the number of nematode parasites found in each host species, as well as quantitative parasitological descriptors, stage (larval or adult), and site of infection.

Discussion

To our knowledge, no parasites have been reported for *C. pierottii* or *O. lavillai* throughout their geographical ranges. For *Ce. cranwelli*, there is one report about the finding of *Haematoloechus longiplexus* (Trematoda, Plagiorchiidae) from Santa Fe province, Argentina (Hamann and Pérez, 1999). In contrast, *Rhinella diptycha*, *S. nasicus* and *L. bufonius* have been previously analyzed for their parasitic helminths several times and from different localities of their geographical ranges (see Lunaschi and Drago, 2007; Hamann *et al.*, 2013a; Campião *et al.*, 2014; González and Hamann, 2015a).

All the nematode taxa found in this study correspond to generalist species, i.e. they can parasitize a broad range of amphibians, even from different families (Table 3). This was not surprising as generalist parasites are the major components of helminth parasitic communities in amphibians (Aho, 1990). One of these nematode species, *S. formosula*, has not been reported in amphibians from Argentina until the present work.

Schrankiana formosula is an atractid nemato-

de that has only been found in leptodactylid hosts; it was found in *Leptodactylus fuscus* (Schneider, 1799) and *L. syphax* Bokermann, 1969 from Brazil (Campião *et al*., 2014; Souza Linz *et al*., 2017), and in *L. fuscus* and *L. elenae* Heyer, 1978 from Paraguay (Baker and Vaucher, 1988). Our record is the first for *L. bufonius* throughout the distribution area of this species.

Campião *et. al.* (2015) established that gastrointestinal roundworms of the family Cosmocercidae are the most reported helminth parasites in South American amphibians. Concurrently, Gon-

Table 3. Amphibian nematode parasites found in this study and previous reports from Argentina, after the checklist of González and Hamann (2015a). Adult nematodes not identified at species level were not included in this table.

Nematode species	Amphibian species	Locality	References
O. subauricularis	*R. diptycha*£	Matacos Department, Formosa	Present study
	Rhinella major (Müller and Hellmich, 1936)	Corrientes, Corrientes	Hamann et al., 2013b
S. travassosi	*R. major*	Las Lomitas, Formosa; Concepción del Bermejo, Chaco	Hamann and González, 2015
A. hylambatis	*R. major*	Las Lomitas and Ingeniero Juárez, Formosa; Concepción del Bermejo and Taco Pozo, Chaco	Hamann and González, 2015; González et al., 2019
	Trachycephalus typhonius (Linnaeus, 1758)	Pirané, Formosa	Draghi et al., 2015b
	L. bufonius	Las Lomitas, Formosa; Taco Pozo, Chaco	González et al., 2019
		Matacos Department, Formosa	Present study
	Dermatonotus muelleri (Boettger, 1885)	Las Lomitas, Formosa	González et al., 2019
	D. muelleri	Ingeniero Juárez, Formosa	González et al., 2020
	R. diptycha	Matacos Department, Formosa	Present study
	*C. pierottii**	Matacos Department, Formosa	Present study
	Ce. cranwelli *	Matacos Department, Formosa	Present study
	*O. lavillai**	Matacos Department, Formosa	Present study
C. podicipinus	*Leptodactylus elenae; L. podicipinus* (Cope, 1862)	Corrientes, Corrientes	González and Hamann, 2016
	R. major	Las Lomitas, Formosa	Hamann and González, 2015
	P. albonotatus	Corrientes, Corrientes	González et al., 2019
	D. muelleri	Ing. Juárez, Formosa	González et al., 2020
	C. pierottii *	Matacos Department, Formosa	Present study
	Ce. cranwelli *	Matacos Department, Formosa	Present study
	L. bufonius	Matacos Department, Formosa	Present study
O. caudacutus	*S. nasicus*£	Matacos Department, Formosa	Present study
S. formosula	*L. bufonius**£	Matacos Department , Formosa	Present study
Physaloptera sp.	*R. major*	Las Lomitas and Ing. Juárez, Formosa; Concepción del Bermejo, Chaco	Hamann and González, 2015
	Melanophryniscus klappenbachi Prigioni and Langone, 2000	Resistencia, Chaco	Hamann et al., 2014
	*C. pierottii**	Matacos Department, Formosa	Present study
	L. bufonius	Matacos Department, Formosa	Present study

* new host record; £ new geographical record

zález and Hamann (2015a) found that *Cosmocerca parva* Travassos, 1925 and *C. podicipinus* (Cosmocercidae), collected as adults, are the most commonly occurring nematodes in Argentinean amphibians; each of them was found parasitizing 14 amphibian species. In the present study, cosmocercids were also the most common nematodes; they were represented by the greatest number of species (*A. hylambatis*, *C. podicipinus*, and *O. caudacutus*), and in general, the highest quantitative descriptors. In addition, they parasitized all the amphibian species analyzed, except for the hylid *S. nasicus*. However, it was not a species of the genus *Cosmocerca* Diesing, 1861 the one most frequently occurring, but *A. hylambatis* which presented the highest values of abundance and infection prevalence in the amphibians analyzed (see Table 2). This is in agreement with recent studies on the helminth fauna of anurans from the Dry Chaco ecoregion (Hamann and González, 2015; González *et al.*, 2019; 2020). We herein expand the hosts list of *A. hylambatis* in Argentina to thirteen species (see González and Hamann, 2015a and Table 3).

Nematodes of the families Rhabdiasidae, Cosmocercidae, and Molineidae have direct life cycles (Anderson, 2000); infection may occur in some cases by active penetration of the infective larvae (L3) that occur in the soil and penetrates through the skin, or through the mucosa or eye cavity (e.g., *Rhabdias*, *Oswaldocruzia*, *Cosmocerca*); in others, amphibians ingest the larvae present in the environment and acquire the infection (e.g., *Aplectana*). For *Oxyascaris* Travassos, 1920, no information is available about their life cycle yet.

Nematodes of the family Atractidae have been found in amphibians, reptiles, mammals and fishes. Eggs develop to third-stage larvae in utero and autoinfect the host; these autoinfections are generally accompanied by high intensities, low pathogenesis and large larvae. Their transmission from host to host is not understood for most species (Anderson, 2000).

Of all the nematodes found in this study, only those of the genus *Physaloptera* Rudolphi, 1819 present an indirect life cycle. Amphibians become infected when they consume intermediate hosts (e.g. orthopterans and coleopterans) that contain L3. In amphibians, these nematodes do not develop or grow, and, only complete their life cycle when consumed by the definitive hosts, which include all types of terrestrial vertebrates (Anderson, 2000; de Quadros *et al.*, 2014). Accordingly, the amphibians analyzed in this study are the definitive hosts in the life cycle of eight nematode taxa, while only in the case of genus *Physaloptera* found as larvae, the frogs *L. bufonius* and *C. pierottii* represent paratenic hosts.

In recent years, the Gran Chaco ecoregion has been the focus of relevant studies spanning related topics such as the biodiversity of amphibians, the loss of that diversity as a consequence of human activities, the regional distribution of biodiversity according to land-use and land-cover, and the determination of priority conservation areas (see Medina *et al.*, 2016; Nori *et al.*, 2016; Torres *et al.*, 2014). At this point, we would like to highlight that the loss of amphibian species also represents the loss of a whole other dimension of diversity that is usually not taken into account, i.e. the diversity of organisms that parasitize them. Future studies in new host species, will provide further knowledge of the helminth fauna of these vertebrates in one of the most threatened subtropical woodland savannas in the world.

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