Mastozoología Neotropical, 31(1):e0989, Mendoza, 2024
Versión on-line ISSN 1666-0536
https://doi.org/10.31687/saremMN.24.31.01.09.e0989

Sección Especial

LOS MAMÍFEROS COMO HOSPEDADORES DE PARÁSITOS

Editores de Sección: Cavia, R., Gómez Villafañe, I. E. y Sánchez, J. P.

Artículo

INTESTINAL HELMINTH COMMUNITY STRUCTURE OF WHITE-EARED OPOSSUM (*Didelphis albiventris*) THAT INHABIT RURAL AREAS OF BUENOS AIRES, ARGENTINA

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ABSTRACT. The white-eared opossum (*Didelphis albiventris*) is a neotropical marsupial that inhabits various ecoregions and highly modified environments. However, there is limited research on host-parasite interactions and the structure of helminth communities in *D. albiventris*. Therefore, the aim of this study is to investigate the intestinal helminths of *D. albiventris* inhabiting a rural area in Buenos Aires, Argentina, and assess the relationship between host age and sex and helminth community structure. Opposums were captured in 2004 and intestines were examined to detect helminths. By analyzing 22 intestines, we identified a total of 8 978 helminths, including *Cruzia tentaculata, Turgida turgida, Rhopalias coronatus, Brachylaima* sp., individuals of the family Diplostomidae, and a single specimen of a cestode. Among these, *C. tentaculata* and *R. coronatus* were the most prevalent species, with *C. tentaculata* exhibiting the highest levels of abundance, mean intensity, and aggregation index. To assess the influence of host sex and age on parasite abundance and probability of occurrence, we conducted Generalized Linear Models. The results revealed that host juveniles had lower helminth prevalence and abundance compared to other age categories. Host sex was only significant in interaction with host age for parasite abundance in *C. tentaculata* and *R. coronatus*. Most captured juveniles relied on maternal feeding, potentially reducing exposure to consuming infected intermediate hosts with indirect life cycles. Understanding the parasite ecology of this opossum species in modified environments provides valuable information about their role at the wildlife-human interface in anthropogenic areas.

Recibido: 31 mayo 2023. Aceptado: 4 septiembre 2023. Editora invitada: Dra. Juliana Sánchez.

RESUMEN. ESTRUCTURA DE LA COMUNIDAD HELMÍNTICA INTESTINAL DE LAS COMADREJAS OVERAS (*Didelphis albiventris*) QUE HABITAN ÁREAS RURALES DE BUENOS AIRES, ARGENTINA. La comadreja overa *Didelphis albiventris* es un marsupial neotropical que habita en diversas ecorregiones, y con frecuencia se halla en ambientes peridomésticos. Si bien existen estudios que describen los parásitos de *D. albiventris*, los que contemplan asociaciones entre estos y las características intrínsecas del hospedador son escasos. El objetivo de este estudio fue describir los helmintos intestinales de *Didelphis albiventris* en un área rural de Buenos Aires, y evaluar la influencia del sexo y la edad del hospedador sobre la presencia y abundancia de parásitos. Se analizaron 22 intestinos provenientes de la captura de ejemplares de *D. albiventris* y se realizaron Modelos Lineales Generalizados. Se hallaron 8 978 helmintos: *Cruzia tentaculata, Turgida turgida, Rhopalias coronatus, Brachylaima* sp.; familia Diplostomidae y un cestode. *Cruzia tentaculata* y *R. coronatus* fueron las especies más prevalentes; de ellas, *C. tentaculata* presentó mayor abundancia, intensidad media e índice de agregación. Los helmintos hallados son heteroxenos y fueron registrados previamente para esta especie. Los resultados indican que los individuos juveniles poseen una menor presencia y abundancia de helmintos, lo cual estaría asociado su modo de alimentación. El sexo solo fue significativo en interacción con la edad para la abundancia de *C. tentaculata* y *R. coronatus*. Estudiar la estructura de la comunidad parasitaria de *D. albiventris* y los factores que la determinan proporciona información sobre su papel en el ambiente, particularmente en ambientes modificados, donde la interacción fauna silvestre-humanos es mayor. Futuros estudios que contemplen características ambientales y tamaño corporal del hospedador complementarán los resultados hallados en este estudio.

Palabras clave: Didelphimorphia, Ecología parasitaria, Neotropical, Parasitología.
Key words: Didelphimorphia, Parasite Ecology, Neotropical, Parasitology.

Cite as: Illia G., I. E. Gómez Villafañe, M. C. Ezquiaga, G. Cassini, & A. C. Gozzi. 2024. Intestinal helminth community structure of white-eared opossum (*Didelphis albiventris*) that inhabit rural areas of Buenos Aires, Argentina. Mastozoología Neotropical, 31(1):e0989. https://doi.org/10.31687/saremMN.24.31.01.09.e0989

INTRODUCTION

Parasitism is one of the most common lifestyles on Earth (Windsor 1998). Parasites are key components of biodiversity (Lafferty et al. 2008) and can play essential roles in the ecosystem (Carlson et al. 2020). In addition, parasites live in constant interaction with their hosts and can influence their way of life and their relationships with the environment while using them as a source of food and shelter (Lucius et al. 2017). Thus, the majority of organisms are likely to be parasitized by at least one parasite species. Knowledge of parasites in a host species can provide crucial information for understanding its behavior, diet, and habitat use (Poulin 2021).

The structure of a parasite community results from the interaction between parasites and their hosts, which is shaped by evolutionary, physiological, ecological, geographical, and stochastic factors (Bush et al. 1997; Morand et al. 2006; Poulin 2011, 2021). Thus, the host-parasite association occurs within a complex network of interactions (Kaltz & Shykoff 1998; Morand et al. 2006) and reflects the interactions between environmental conditions and the intrinsic factors of both parasite and host (such as age, sex, longevity, body size, and home range) (Beldomenico & Begon 2015). For example, males and females of a host species often differ in physiological, morphological, and behavioral traits, and this can result in differences in the structure of parasite communities between them (Klein 2004; Krasnov et al. 2006; Duneau & Ebert 2012; Bellay et al. 2020). Similarly, host age can also influence the diversity of the parasite community. Young hosts may have a lower initial parasite load compared to older individuals. As hosts age, they are exposed to a greater number of parasite species and accumulate a higher parasite burden through increased contact with the environment and other individuals (Hämäläinen et al. 2015). With increasing age, hosts are more likely to have encountered and developed immunity to specific parasites, potentially reducing the prevalence or intensity of certain infections and altering the balance between different parasite species (Galvani 2005). Changes in host life history traits, such as diet, metabolism, or reproductive activity, as they age can indirectly impact the parasite community by creating new opportunities for parasites or modifying host susceptibility (Thomas et al. 1995). Understanding baseline levels of parasitic infections is essential to recording any changes in the parasite community structure due to different anthropogenic and environmental activities.

The white-eared opossum (*Didelphis albiventris* Lund, 1840) is a neotropical marsupial widely distributed in South America (Emmons & Feer 1997; Cáceres 2002; Carrera & Udrizar Sauthier 2014). In Argentina, it can be found from the North of the country up to the South (Rio Negro province) (Bianchini 2018). The species inhabits different ecoregions, and it can also be found in highly modified environments such as urban and rural areas (Flores 2006; Almeida et al. 2008; Pérez Carusi et al. 2009; Chemisquy & Martin 2019). Due to its peridomestic habits and its potential sanitary risk, diverse zoonotic and parasitological studies have been carried out in Brazil (Vicente et al. 1997; Quintão E Silva & De Araújo Costa 1999; Gomes et al. 2003; Thatcher 2006; Santiago et al. 2007; Arruda Gimenes Nantes et al. 2019); and Argentina (Mazza & Romaña 1932; Navone & Suriano 1992; Schweigmann et al. 1999; Mirope Santa Cruz 2006). Studies in Argentina, and particularly in the province of Buenos Aires, are focused mainly on the record of specific zoonotic intestinal helminths such as *Trichinella* or aim to investigate the presence of pathogens such as *Salmonella* and *Leptospira* (Gómez Villafañe et al. 2004; Pérez Carusi et al. 2009; Castaño Zubieta et al. 2014).

Moreover, information regarding host-parasite interactions and the structure of helminth communities in *D. albiventris* is limited. Cirino et al. (2022) evaluated the influence of host sex, body mass, and age on helminth species richness and the influence of the locality on the abundance and prevalence of the helminth species in Brazil. For Argentina, the work published by Navone & Suriano (1992) in the province of Santiago del Estero is the only one that describes the community structure of the helminths in the white-eared opossum, taking into account extrinsic factors such as seasonal fluctuations, but there are no studies aiming to describe the helminth parasites of the white-eared opossum in relation to intrinsic host factors. The aim of this study was to determine the intestinal helminths of *Didelphis albiventris* inhabiting a rural area of the Pampean region of Argentina and to evaluate the relationship between the age and sex of the hosts and the abundance and prevalence of the parasite species.

MATERIALS AND METHODS

The study was conducted in Exaltación de la Cruz (34°17'S, 59°07'W), province of Buenos Aires, Argentina. The study area belongs to the Neotropical biogeographic region, Chacoan subregion, Pampean province (Morrone 2014), with a mean annual temperature between 14 °C and 20 °C, and a mean annual precipitation of 1000 mm (Matteuchi 2012).

Opossums were captured by manual extraction from their shelters by local people during the daytime in February and March 2004 and euthanized by anesthetic overdose via intramuscular injection of ketamine (24 mg/kg) and acepromazine maleate (2.4 mg/kg). The removal method was conducted to collect parasitological samples. The animal handling protocol was approved by the Provincial Department of Wild Fauna and Flora and followed the guidelines established by the American Society of Mammalogists (Sikes & The Animal Care And Use Committee Of The American Society Of Mammalogists 2016). For each animal captured, we recorded sex, body length, total length (body-tail), and age class. Opossums were classified as juveniles, preadults, and adults by assigning one of the seven age classes proposed by Schweigmann et al. (1999) based on tooth eruption, replacement, and wear.

In the laboratory, the anterior, medium, and posterior small intestines, large intestine, and cecum were examined to detect helminths. Stomachs were not analyzed because they were used for other purposes. Helminth species were collected and fixed in formaldehyde at 10% for further identification. Nematodes were cleared with lactophenol, and cestodes and digeneans were stained in acetic carmine, dehydrated in a series of alcohol dilutions (70% to 100%), and mounted in Canada balsam following Navone & Suriano (1992). Helminth specimens were observed under an optic microscope (Numak XSZ 100 BNT) and identified the following taxonomic keys and specifically related bibliography (Dubois 1976; Anderson 1978; Vicente et al. 1997; Mirope Santa Cruz 2006; Haverkost & Gardner 2008; Fernandes et al. 2015). The host-studied specimens were deposited in the Colección Nacional de Mastozoología from the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN-Ma 23298-23307), Buenos Aires, Argentina.

Data analyses were performed using the statistical analysis software package R Studio 4.0.0 (R Core Team 2020). Prevalence (P), mean abundance (MA), mean intensity (MI), and aggregation index ($S^2/\bar{x}$) for each intestinal parasite species or taxa were calculated following Reiczigel et al. (2019). Based on the prevalence of the helminth, species were classified as core (P > 70%), secondary (30% ≤ P ≤ 70%), and satellite species (P < 30%) (Bush & Holmes 1986; Bush et al. 2001). Generalized Linear

Models (GLM) were used to evaluate the effect of host sex (male/female), age (categorical ordinal sequential: juvenile, preadult, adult), and their interaction (age×sex) on both responses: Probability of occurrence (presence/absence data) and abundance of each parasite species (count data). The nematode *Turgida turgida* Rudolphi, 1819, and the cestode were excluded from this analysis. This decision was made because only a single cestode was found and because stomachs, which are the site of infection for *T. turgida* (Anderson 2000), were not analyzed in this study. A binomial family was used for presence/absence data. The models with both independent variables were tested first. Then, using the log-likelihood ratio test (LRT) non-significant terms were eliminated (Zuur et al. 2009). The models that fit better than the null model were selected utilizing the Akaike Information Criterion (AIC) using the LRT. Tukeys posthoc test was used to perform pairwise comparisons between categories in selected models with the agricolae 1.3-5 R package (De Mendiburu 2021). For count data, to evaluate error distribution and overdispersion of abundances (Poisson or negative binomial) and/or zero data (zero-inflated), each parasite species count was visualized in Cleveland dot plots and histograms. Whenever graphical analyses were not conclusive, models using different error distributions and accounted for zero-inflated data were obtained and later compared using LRT (Zuur et al. 2009). In a zero-inflated GLM, a constant was used to model false-zero probability on the abundances. The GLM with a lower AIC and that fit better than the null model (△AIC>2) was selected. All GLM and statistical analyses were performed with the lmtest 0.9-33 R package (Zeileis & Hothorn 2002), emmeans 1.8.6 R package (Lenth 2023), and pscl 1.04.4 R package (Zeileis et al. 2008) and lme4 1.1-12 (Bates et al. 2015).

RESULTS

A total of 22 opossums were captured during the fieldwork. From those, 13 individuals were juveniles (three females and 10 males), five preadults (three females and two males), and four adults (three females and one male). Helminth species were found in 45.5% (10/22) of the samples analyzed. A total of 8 978 helminths were recovered. We identified two nematode species, *Cruzia tentaculata* Rudolphi, 1819 (Ascaridida; n = 6 796) and *Turgida turgida* Rudolphi, 1819 (Spirurida; n = 10); two digenean species, *Rhopalias coronatus* Rudolphi, 1819 (Echinostomida; n = 702), *Brachylaima* sp. (Diplostomida; n = 196); and individuals of the family Diplostomidae (n = 1 273); and a single specimen of a cestode (not determined). As mentioned before, *T. turgida* is a common stomach parasite in opossums. Because stomachs were not analyzed in this study, this finding could be related to a postmortem migration of these parasites to the intestine. For this reason, *T. turgida* is mentioned, but it is not considered part of the intestinal helminth community.

Based on the prevalences, none of the species were classified as a core species (P > 70%), whereas *C. tentaculata*, *R. coronatus, Brachylaima* sp., and the family Diplostomidae were classified as secondary species, and the cestode as satellite species (**Fig.** 1). *Cruzia tentaculata* presented the highest mean intensity, mean abundance, and aggregation index, followed by Diplostomidae and *R. coronatus* (**Table** 1).

The GLM showed that the probability of occurrence was significant for host age in *C. tentaculata, R. coronatus*, *Brachylaima* sp., and Diplostomidae (**Table** 2). Juvenile hosts have less probability to present these parasite species compared to the other host age categories (**Fig.** 2). Additionally, host preadults showed a higher probability of being parasitized with *Brachylaima* sp. than juveniles and adults. Regarding host sex, GLM did not show any significant influence on the presence of any of the parasite species.

When the effect of host age on the helminth intensity was analyzed, host juveniles showed less *Brachylaima* sp. abundance (**Fig.** 3). Host sex was only significant in interaction with host age for *C. tentaculata* and *R. coronatus* (**Fig.** 3, **Table** 2). *Cruzia tentaculata* was more abundant in preadult females than in other sex and age categories. *Rhopalias coronatus* was significantly more abundant in preadult and adult females than in males of the same age categories, and adult females presented more abundance of this parasite than preadults.

DISCUSSION

Although the helminths found in this study have been recorded previously in *D. albiventris* along its distribution (Cirino et al. 2022), this is the first record of *Rhopalias coronatus* and *Brachylaima* sp. parasitizing this opossum species in the province of Buenos Aires. Within the helminth species recorded in this study, *C. tentaculata* and *R. coronatus* were the most frequent species, followed by *Brachylaima* sp. and Diplostomidae. *Cruzia tentaculata* and *R. coronatus* are commonly found in different species of marsupials of the genus *Didelphis* along its distribution, such as *D. virginiana, D. marsupialis, D. aurita* and

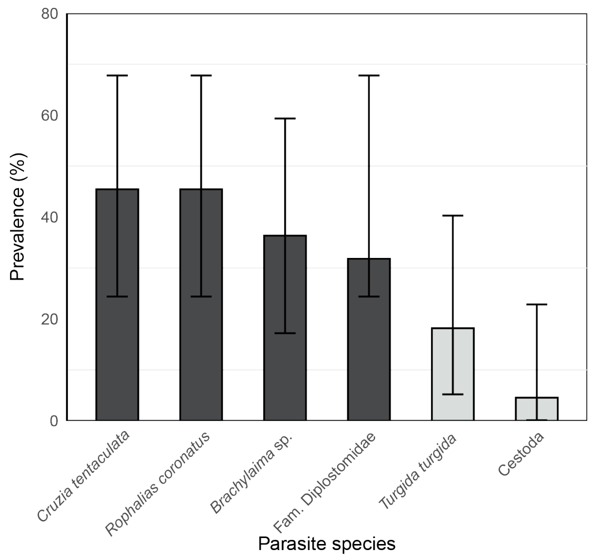

Fig. 1. Intestinal helminth community structure of *Didelphis albiventris* in Exaltación de la Cruz, province of Buenos Aires, Argentina. Prevalence with 95% confidence interval (Clooper Pearson method). Dark gray: Secondary species (prevalence between 30%-70%) Gray: Satellite species (prevalence below 30%).

Table 1

Helminths recovered from 22 intestinal samples of *Didelphis albiventris* captured between February and March 2004 in Exaltación de la Cruz, province of Buenos Aires, Argentina. Prevalence (P%) with 95% confidence interval (CI, Clooper Pearson Method); mean intensity (MI); range (Min-Max); mean abundance (MA); aggregation index ($S^2/\bar{x}$), and infection site of the helminths found. SIa = anterior small intestine; SIm = medium small intestine; SIp = posterior small intestine; C = Cecum, LI = large intestine.

	P%	95% CI	MI (±S.E.)	Range	MA	Aggregation index ($S^2/\bar{x}$)	Infection site
Nematoda Ascaridida: Kathlaniidae *Cruzia tentaculata*	45.45 (10/22)	22.40-67.80	679.40 (613.55)	71-2038	308.90	910.59	SIa, SIm, SIp, C, LI
Trematoda, Digenea Echinostomida, Rhopaliidae *Rhopalias coronatus*	45.45 (10/22)	22.40-67.80	70.20 (76.53)	1-201	31.91	118.30	SIa, SIm, SIp, C
Diplostomida, Brachylaimidae *Brachylaima* sp.	36.36 (8/22)	17.2-59.3	24.50 (33.38)	1-95	8.73	58.05	SIp, C, LI
Fam. Displostomidae	32.82 (7/22)	13.9-54.9	181.90 (211.61)	5-584	57.86	351.01	SIa, SIm, SIp
Cestoda Cestoda	4.55 (1/22)	1-22.8	1	-	0.05	1.00	SIm

D. albiventris (Vicente et al. 1997; Adnet et al. 2009; Tantaleán et al. 2010; Jiménez et al. 2011; Acosta-Virgen et al. 2015; Chero et al. 2017; Costa-Neto et al. 2019; Mollericona & Nallar 2021; Cirino et al.

Table 2

Statistical parameters of General Linear Models of probability of occurrence (presence/absence) and abundance vs. host-related factor (age, sex, and age×sex) of *D. albiventris* in Exaltación de la Cruz, province of Buenos Aires, Argentina. pR^2: pseudo$\triangle R^2$; AIC, Akaike information criterion; AICn, AIC of the null model; Theta: Overdispersion. Only significant models are shown (p-value < 0.05). NB: negative binomial; ZINB: zero-inflated negative binomial.

	Variable	pR^2	AICn	AIC	△AIC	Theta	Family
Probability of occurrence	*C. tentaculata* ∼ age	76.74	32.32	13.05	19.27	0.37	Binomial
	Diplostomidae ∼age	33.58	29.52	24.28	15.24	0.96	Binomial
	R. coronatus ∼ age	76.74	32.32	13.05	19.27	0.37	Binomial
	Brachylaima sp. ∼ age	56.33	30.84	18.6	12.24	0.66	Binomial
Abundance	*C. tentaculata* ∼ age×sex	20.71	186.687	184.38	2.307	1.93	ZINB
	R. coronatus ∼ age×sex	12.28	139.51	133.10	6.41	2.09	ZINB
	Brachylaima sp. ∼ *age*	78.5	96.99	92.92	4.07	0.22	NB

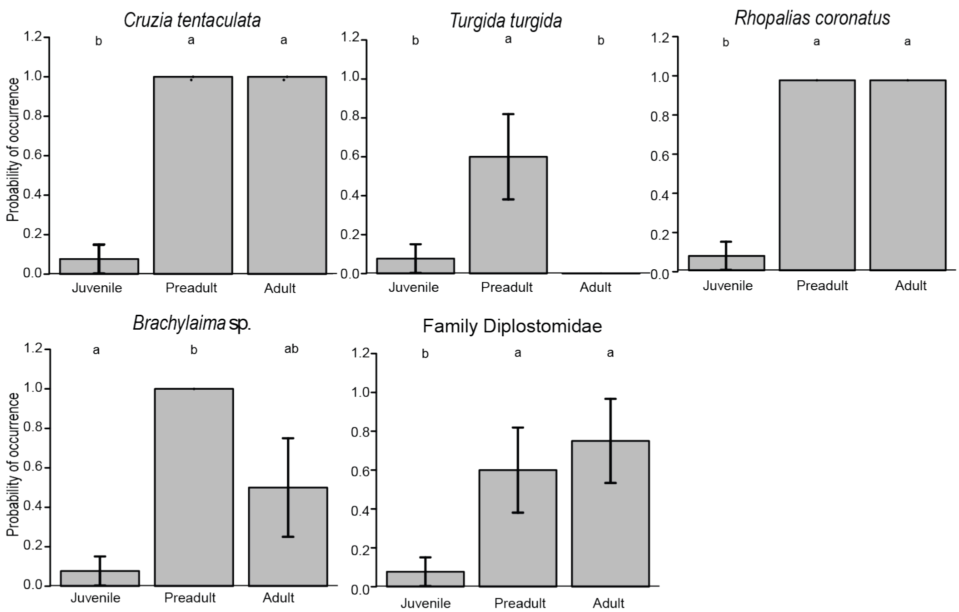

Fig. 2. Significant General Linear Models of probability of occurrence (presence/absence data) vs. host age of *Didelphis albiventris* in Exaltación de la Cruz, province of Buenos Aires, Argentina.

2022). In Argentina, *C. tentaculata* and *R. coronatus* have previously been recorded in *D. albiventris* in the provinces of Chaco, Corrientes, and Formosa (Lombardero & Moriena 1973; Martínez 1986, 1987; Mirope Santa Cruz et al. 1999; Mirope Santa Cruz 2006). Additionally, *C. tentaculata* and the genus *Rhopalias* have also been recorded in the province of Buenos Aires (Illia 2019).

Although *C. tentaculata* and *R. coronatus* presented the highest prevalence, they were both classified as secondary species. These results are in partial agreement with other studies that found *C. tentaculata* also in high prevalence, abundance, and/or mean intensity and being classified as core species (Navone & Suriano 1992; Mirope Santa Cruz 2006; Cirino et al. 2022).

Species of Diplostomidae, such as *Bursotrema tetracotyloides* Szidat, 1960, and *Tylodelphys nunezae* Dubois, 1976 (Achatz et al. 2022), were found in *D. albiventris* in previous studies, including Argentina (Dubois 1976). Also, *B. migrans* Dujardin, 1845 has been previously reported for *D. albiventris* in

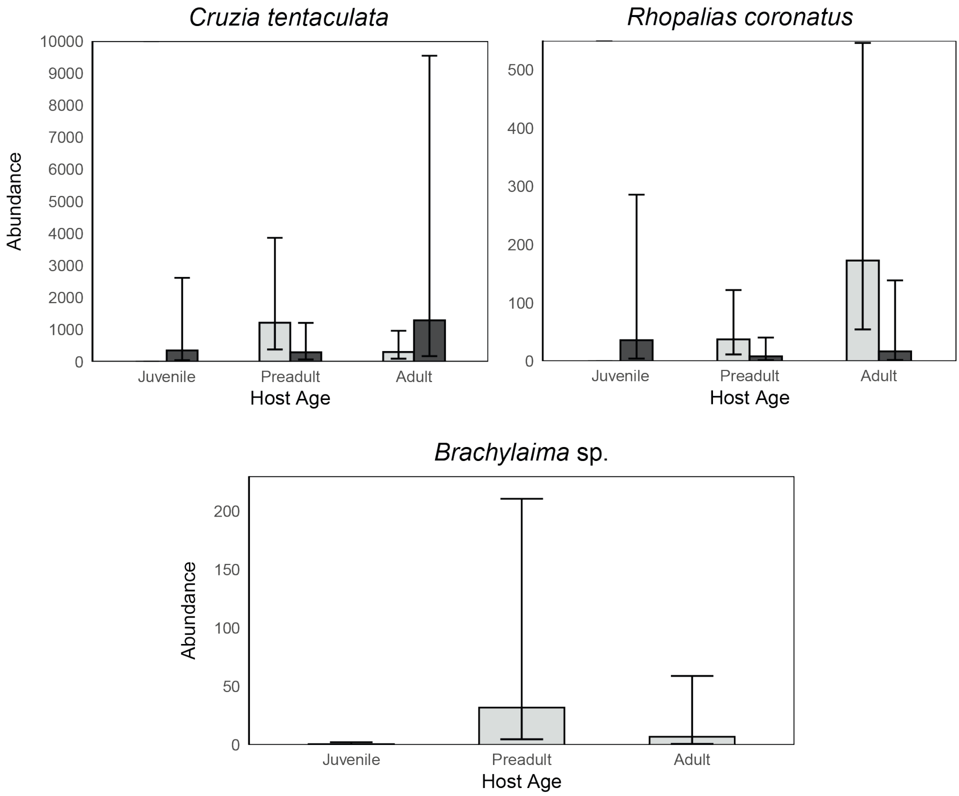

Fig. 3. Significant General Linear Models of abundance data vs. host-related factor (age, and age×sex) of *Didelphis albiventris* in Exaltación de la Cruz, province of Buenos Aires, Argentina. Dark gray: Male, Gray: Female.

Argentina in the provinces of Corrientes, Chaco, and Formosa with a prevalence higher than 50% (Mirope Santa Cruz 2006). Although the specimens found in this work could not be identified at the species level, some morphological characteristics observed differ from those described for *D. albiventris* previously (Mirope Santa Cruz et al. 1999). More studies should be done with a detailed morphological and molecular approach to determine the digenean species found in this work.

Cestodes species (Platyhelminthes, Cestoda) were also recorded from *D. albiventris* in Argentina and Brazil, two species from the genus *Mathevotaenia* and one species from the order Pseudophyllidea, respectively (Komma 1972; Komma & Alves 1974; Navone & Suriano 1992; Campbell et al. 2003). The single specimen found in this study could not be related to any of the species mentioned above.

Turgida turgida is commonly found in several didelphid species throughout its distribution, including *D. albiventris* (Travassos 1922; Vicente et al. 1997; Gomes et al. 2003; Richardson & Campo 2005; Tantaleán et al. 2010; Humberg et al. 2011; Pinto et al. 2014; Acosta-Virgen et al. 2015), and is usually found in higher prevalences (Cirino et al. 2022). In Argentina, it was found in *D. albiventris* in the provinces of Buenos Aires, Corrientes, Chaco, Formosa, and Santiago del Estero (Navone & Suriano 1992; Mirope Santa Cruz et al. 1999; Illia 2019). As mentioned before, *T. turgida* resides attached to the stomach wall of its host (Anderson 2000). For that reason, we did not consider *T. turgida* as part of the intestinal parasite community, and it was not analyzed.

All the parasites found in this study have indirect life cycles. *Brachylaima* species include terrestrial mollusks as intermediate hosts (Lunaschi & Drago 2012) and, although *C. tentaculata* was considered to be a monoxenous species, a recent study indicates that gastropods may act as intermediate hosts, indicating a heteroxenous life cycle for the species (Ramos de Souza et al. 2021). In this sense, the findings of these parasite species are related to the opportunistic omnivorous feeding habit of the opossums, which may feed on invertebrates, fruits, birds, and small mammals (Cantor et al. 2010), favoring the consumption of a wide range of parasitized intermediate hosts.

GLMs show that juveniles had a lower probability of having the parasite species and lower abundances compared to the other host age categories. Juvenile hosts may have lower exposure to parasites because they spend less time in environments where parasites are present or engage in less risky behaviors than adults or preadults, such as mating or foraging behavior, having less time to accumulate parasites (Kołodziej-Sobocińska 2019). Cirino et al. (2022) found in Brazil the same pattern in helminth abundance. Additionally, this variable might be important to include in the analysis in the future.

Based on tooth eruption, replacement, and wear, most of the juveniles analyzed in this study were up to three-four months that were still feeding from their mother (Schweigmann 1994; Schweigmann et al. 1999), preventing the acquisition of heteroxenous parasites. The higher probability of infection and higher parasite abundance of *Brachylaima* sp. observed in preadult hosts compared to adults and juveniles may be linked to increased exposure to the parasite through the consumption of its intermediate hosts. This could also explain the significantly higher abundance of *C. tentaculata* and *R. coronatus* observed in preadult females than in preadult males. Although studies done in mammals usually show that males have higher nematode prevalence and abundance than females, a higher abundance of *C. tentaculata* in female hosts was also found in *D. albiventris* in Brazil (Cirino et al. 2022), showing that females are more susceptible to this species, and in this case, probably also due to hormonal changes that preadult females undergo as they reach sexual maturity.

Despite opossums can harbor a wide range of pathogens that have implications for human health, as well as for domestic animals [e.g., *Trypanosoma cruzi*, *Toxoplasma gondii*, *Trichinella spiralis*, *Toxocara cati*, ticks, and fleas (Schweigmann et al. 1999; Fornazari et al. 2011; Castaño Zubieta et al. 2014; Pinto et al. 2014)], none of the parasite species found in this study have been recorded as potentially hazardous for public health (Bezerra-Santos et al. 2021). Studying the parasite ecology of this opossum species in modified environments provides information about the sanitary risk that this species harbors at the wildlife–livestock–human interface in anthropogenic areas, such as the agrosystem ones. Further studies that consider different host age classes, other types of samples, body mass, and environmental conditions in different landscapes would allow a better comprehension of the factors that determine the distribution of the parasites in *D. albiventris*.

CONFLICT OF INTEREST

The authors declare no competing interests.

ACKNOWLEDGMENTS

We would like to thank Lorena Perez Carusi, Pablo de Paz Sierra, and the local people from Exaltación de la Cruz for their contributions to the field. We are grateful to Graciela Navone (CEPAVE) for her important contribution to helminth identification and improvement of this work. We thank Isabel Gómez Villafañe, Regino Cavia, and Juliana Sanchez editors of this special section. This research was supported by the Universidad Nacional de Luján (CDD-CB 264/17), the Universidad de Buenos Aires, and the Consejo Nacional de Investigaciones Científicas y Técnicas.

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