

# Original Contribution

# Helminth Diversity in Synanthropic Rodents from an Urban Ecosystem

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Abstract: Richness and diversity of parasites depend on a set of interrelated factors related to the characteristics of the host, the environment and the parasites itself. In the City of Buenos Aires, rodent communities vary according to landscape structure. The goal of this paper was to study the variations of helminth richness and diversity among invasive rodent species in different landscape units of the City of Buenos Aires. 73% of the rodents were parasitized with at least one of the 10 identified helminth species. Each rodent species presented its own characteristics in terms of richness, diversity and helminth composition, keeping these characteristics still occupying more than one landscape unit. The infracommunities with greater diversity corresponded to R. norvegicus due to its high values of parasitic richness, proportion of infected hosts and parasite prevalence. Instead, R. rattus and M. musculus infracommunities had lower diversity since a high percentage of them presented a unique helminth species. Within the city, the inhabitants of shantytowns would be the most exposed to zoonotic diseases transmitted by rodents due to high abundance of rodents harboring a high parasite load, including species like Hymenolepis nana and H. diminuta, recognized worldwide from a zoonotic aspect.

Keywords: Urban environments, Helminth, Diversity, Synanthropic rodents, Zoonosis

### **INTRODUCTION**

The structure of parasite diversity of many free-living animals is affected at individual, population or species level by ongoing global changes such as climate change, habitat fragmentation or bioinvasions (Bordes and Morand [2009\)](#page-9-0). Processes such as biological invasions may lead to novel parasite–host interactions and transmission opportunities,

with the potential to affect human, wildlife, and ecosystem health and resilience (Dunn and Hatcher [2015\)](#page-9-0). Urbanization is a phenomenon that accelerates and intensifies these impacts on the interactions of zoonotic diseases and their hosts (Mackenstedt et al. [2015](#page-10-0); Neiderud [2015\)](#page-10-0). Particularly for urban rodents, the risk of disease spread should be higher in human-dominated habitat, like cities, due to the synanthropic behavior of these invasive animals (Morand et al. [2015](#page-10-0)).

Mus musculus, Rattus rattus, and R. norvegicus are the Correspondence to: Diego Hancke, e-mail: diegohancke@ege.fcen.uba.ar most urban adapted rodents. Original from Southeast Asia,

various aspects of their biology, such as an enormous reproductive potential, feeding behavior, and adaptations to urban environments, contribute to the success of a worldwide dissemination (Kosoy et al. [2015](#page-10-0)). Synanthropic rodents are the main reservoirs or host for different human pathogens, including zoonotic helminth species (Himsworth et al. [2013](#page-10-0)). The success of murine rodents in anthropogenic habitats could be the reason for many parasitological studies done worldwide. In general, studies have focused primarily on list of all parasites species and analyzed biotic and abiotic factors influencing composition and parasite burden (see Battersby et al. [2002;](#page-9-0) Milazzo et al. [2010](#page-10-0); Easterbrook et al. [2007;](#page-10-0) Kataranovski et al. [2008,](#page-10-0) [2011](#page-10-0); Hancke et al. [2011](#page-10-0)). In recent decades, the detection of diseases associated with rodents as reservoirs has increased (see Meerburg et al. [2009](#page-10-0)), which gives them a significant role in disease transmission to humans.

In disturbed environments like cities, animals and their parasites would be exposed to a different combination of factors than those of less disturbed environments. By one side, the replacement of natural environments with buildings could break up host-parasite interactions, whereas higher environmental (substrate) diversity allows the survival of a wider range of intermediate hosts and vectors and their associated parasites (Deplazes et al. [2004;](#page-9-0) Calegaro-Marques and Amato [2014](#page-9-0)). In the City of Buenos Aires, rodent richness and composition vary according to habitat characteristics. The matrix of the city is composed by buildings, houses, and paved streets, with patches formed by parks, green spaces, and shantytowns inside. These characteristics affect the establishment and proliferation of animal populations. According to Cavia et al. [\(2009](#page-9-0)), in highly urbanized environments, such as residential or industrial areas, the dominant species is Rattus rattus. In environments with a lower degree of urbanization, as in the case of shantytowns, the dominant species are R. norvegicus and Mus musculus (Fernández et al. [2007](#page-10-0); Cavia et al. [2009](#page-9-0)). In parks and green spaces, R. norvegicus and M. musculus are the most abundant species, but accompanied by native rodents (Cavia et al. [2009](#page-9-0)).

Although geographic variations in the richness of parasites for the same host species are observed, studies of parasites of mammal populations from 3 continents showed a strong influence of host identity in parasite species richness (Bordes and Morand [2008\)](#page-9-0). At a global level, Wells et al. [\(2015](#page-10-0)) observed that richness and composition of helminth assemblages in R. norvegicus and R. rattus vary together at geographical scales and such patterns can only

be understood in relation to complex interactions linking synanthropic host species with local wildlife, humans, and domestic animals. Bordes et al. ([2015\)](#page-9-0) recommended further studies about the parasites transmission modes and evaluation of human disease risk, focusing on three issues: parasites transmission between different rodent species, their distribution across rodent species in space and time, and the rodent–human contact.

The aim of this paper was to study the variations of helminth richness and diversity among invasive rodent species in different landscape units of an urban environment. For this, rodent trapping and parasitological screening were performed in the City of Buenos Aires to test two hypotheses. First, parasite diversity at host population level is affected by the local pool of wildlife biodiversity (e.g., alternative definitive hosts, more intermediate host species). Therefore, we expected higher helminth diversity in rats and mouse populations from parklands compared to those from shantytowns and residential areas. Second, in very heterogeneous environments within a host's home range, the exploitation of available resources tends to be more diversified by increasing infection with a greater number of parasite species (Wells et al. [2007;](#page-10-0) Bordes et al. [2009\)](#page-9-0). Shantytowns may present a greater variety of microhabitats than other landscape units because they are built in an unplanned manner, where houses constructed of various types of material, water bodies, vacant lots, dumps, and small patches of spontaneous vegetation could be found (Cavia et al. [2009](#page-9-0)). In contrast, green spaces and residential neighborhoods, despite being environmentally contrasting, may be more homogeneous within host's home range. So we expected a higher helminth species richness and diversity at host individual scale in R. norvegicus and M. musculus from shantytowns compared to those of other landscape units, respectively. As murid rodents and humans coexist in cities worldwide, it is hoped that the knowledge generated in this paper will be useful to improve prevention of zoonotic diseases, especially of the most vulnerable groups.

# MATERIALS AND METHODS

#### Study Area

Buenos Aires (34°37'S; 58°24'W) is the main city of Argentina in terms of population, with an area of around 200 km<sup>2</sup> and is one of the largest metropolises in the world (United Nations [2012\)](#page-10-0). The climate is temperate with a mean annual temperature of 17.48°C, seasonal amplitude of 13.28°C, and mean annual precipitation of 1014 mm. Rodent samples were examined from three landscape units including three parklands, four shantytowns, and three industrial–residential sites. The sites were not contiguous and were spaced by at least 1 km and separated by barriers such as railroad tracks, avenues, and highways (Figure 1).

Parklands refer to public areas of recreation, where areas of spontaneous vegetation and woodlots with planted species are included in a matrix of grass or ornamental lawn (Cavia et al. [2009](#page-9-0); Vadell et al. [2010](#page-10-0)). The parklands studied were located on the banks of the Río de la Plata River and the Riachuelo River, both of which allow the entry of native flora and fauna to an urban ecosystem (Cavia et al. [2009\)](#page-9-0).

Shantytowns refer to areas inhabited by a very lowincome population that lives in precarious dwellings with an inadequate supply of basic urban services, such as garbage removal, sanitation networks, electricity, telephones, and plumbing (Fernández et al. [2007](#page-10-0); Vadell et al. [2010;](#page-10-0) Hancke and Suárez [2014](#page-10-0)).

Industrial–residential areas refer to neighborhoods where buildings and pavement are the dominant elements in the landscape unit. In these neighborhoods, the dominant types of construction are houses of no more than two stories, but in some sections, there are also industries and

apartment block buildings. Different kinds of stores may be found along avenues (Cavia et al. [2009\)](#page-9-0).

## Collection and Examination of Rodents

Rodents were collected from surveys carried out as part of a rodent control program in the city of Buenos Aires. The disadvantage of working with urban rodents is that they are very difficult to capture and quantify during field research (Himsworth et al. [2014](#page-10-0)). So, to achieve an acceptable number of samples, it was necessary to consider an extended period of time, from 2004 to 2011. Rodents from the three landscape units were collected during the spring and summer (between October and March), when mean temperature was 17°C or more and mean precipitation above 100 ml and during the autumn and winter (between April and September), when mean temperature was below 17°C and mean precipitation 100 ml or less. The animals were captured using live cage traps, wire mesh traps of 15  $\times$  16  $\times$  31 cm with a door that is locked open with a pin connected to a trigger device holding the bait and Sherman trap, an aluminum box-trap  $(8 \text{ cm} \times 9 \text{ cm} \times 23 \text{ cm})$  with a door open at one end leading to a weight-sensitive treadle. Traps were placed inside houses and in their yards, in stores or factories, or on lines in sites dominated by vegetation. Sherman traps were baited with a mixture of peanut butter and cow fat, and cage traps with carrot and raw meat and were monitored every morning



Figure 1. Map of the city of Buenos Aires showing the ten sites where rodent was sampled. Black arrows indicate the location of the sampled sites. PL 1–3: parklands 1–3, SH 1–4: shantytowns 1–4 and IR 1–3: industrial–residential neighborhoods 1–3 a. Gray polygons indicate the location of parklands and open green spaces, black polygons correspond to shantytowns and the white foreground corresponds to the matrix of blocks with buildings and pavement.

Season	Host sex	Rat species/Landscape unit						Total by sex Total by season
		Resident. neighb. Shantytowns Parklands Shantytowns Parklands						
Spring–Summer	Female	7	14	4	13	$\overline{4}$	42	
	Male	7	17	6	21	$\overline{4}$	55	
	Combined 14		31	10	34	8		97
Autumn-Winter	Female	6	16	8	$\overline{4}$	5	39	
	Male	8	18	9	2	12	49	
	Combined 14		34	17	6	17		88
Total by sex	Female	13	30	12	17	9		
	Male	15	35	15	23	16		
Total by landscape unit		28	65	27	40	25		
Grand total								185

<span id="page-3-0"></span>Table 1. Number of Rattus norvegicus, R. rattus, and Mus musculus Examined Relative to Host Sex, Season, and Landscape Unit.

for four consecutive days. Captured animals were removed, deeply anaesthetized, and sacrificed by cervical dislocation. R. norvegicus individuals were anaesthetised with an intramuscular dose of ketamine hydrochloride (40 mg/kg) and acepromazine (2.5 mg/kg), while M. musculus individuals were anaesthetised by means of inhalation of isoflurane. All animals were sexed, measured, weighed, fixed in formaldehyde and a week later preserved in 70% ethanol and deposited in the collection of the Laboratory of Urban Rodent Ecology of the Buenos Aires University.

Only sexually mature rodents were kept for this study since juvenile is less likely to harbor parasites due to reduced times of exposure to infection (Chaisiri et al. [2015\)](#page-9-0). A total of 28 R. rattus, 92 R. norvegicus, and 65 M. musculus were analyzed for parasitological screening (Table 1). The entire alimentary tract was removed and carefully scrutinized for helminth parasites. When found, these were removed carefully, identified, counted, and preserved in 70% ethanol.

#### Data Analysis

In parasitological studies, when ecological aspects as richness and diversity are addressed, it is recommended to define the limits of parasite populations or communities at different scales. According to Bush et al. [\(1997](#page-9-0)):

Infracommunities A community of infrapopulations, which include all individuals of a parasite species in a single host at a particular time.

Component community It refers to all infrapopulations of parasites associated with a subset of hosts in a particular time and place (or in a given ecosystem).

Compound community It refers to all parasitic forms within an ensemble of hosts.

Within the rodent assembly of Buenos Aires, 5 different groups of hosts (or component communities) could be defined: R. rattus of residential neighborhoods (Rr); R. norvegicus of shantytown (Rn/ST); M. musculus of shantytown (Mm/ST); R. norvegicus of parklands (Rn/PL); M. musculus of parklands (Mm/PL). Prevalence, mean intensity (I) and abundance (A) were calculated for each component community following (Bush et al. [1997\)](#page-9-0). The degree of aggregation in the worm counts was calculated by the index of discrepancy (D) (Poulin [1993](#page-10-0)), where a value of 0 indicates an even distribution and a value of 1 indicates that all parasites aggregated in a single host. These terms were estimated for the total sample and for each landscape unit by using the software Quantitative Parasitology 3.0 (Rózsa et al.  $2000$ ). Finally, specific importance index (I) was calculated as a measure of the influence of parasitic species in an ecological assembly  $(I = Prevalence + (aver$ age abundance  $\times$  100)) (Bursey et al. [2001](#page-9-0)).

Parasite species were classified, according to their distribution and abundance in core, satellites or secondary species (Bush and Holmes [1986](#page-9-0)). To identify core species, the prevalence in the X-axis and the average intensity on the Y-axis were scattered for all the parasite species in each component communities.

#### Richness and Diversity

Two methods were used to estimate helminth richness: a nonparametric estimator, the first-order Jackknife (according to Walther and Morand ([1998\)](#page-10-0), one of the most suitable species richness estimators for parasitological data), and extrapolation of species accumulation curves. Both were calculated using the EstimateS software (v. 9.0, available from Colwell at [www.viceroy.eeb.uconn.edu/esti](http://www.viceroy.eeb.uconn.edu/estimates) [mates](http://www.viceroy.eeb.uconn.edu/estimates)). To calculate the expected number of helminth species in each component community, EstimateS calculate the expected number of species that would be found in an augmented sample using nonparametric methods described at Colwell et al. [\(2012](#page-9-0)).

To analyze variations of helminth richness and diversity at infracommunity level among component communities, generalized linear mixed models (GLMM) were performed considering each host as an experimental unit and the component community as fixed factors. Also gender, season, and year of capture were included in the model as fixed factors since these could affect helminth abundance. Rodent abundance was also included as covariable. It was measured by trap success, and this estimator was the proportion of traps with captures out of the total number of trap nights for each site in each trapping survey. To include possible variations among sampling sites in each landscape unit, these were included in the models as random factor.

Helminth richness in all infracommunities (including not parasited rodents) was modeled. As species richness an integer count number is, a distribution with Poisson errors and log as link function was considered (Zuur et al. [2009\)](#page-10-0). To check the validity of the model, residuals and the predicted values by the model, they were plotted. Overdispersion was calculated from Pearson residues to estimate the existence of an extra variation in the response variable that could not be explained by the model. To estimate diversity in each infected host (infracommunity), the Brillouin index (HB) was calculated (Magurran [2013\)](#page-10-0) and considered as response variable in a linear mixed model. As diversity a continuous variable represents, a normal distribution of errors was assumed (therefore, general linear mixed models were performed). The assumptions of homogeneity of variance and normality were controlled by applying the varident residual heteroscedasticity function. All models were progressively simplified, eliminating nonsignificant terms, testing the model again and keeping only the significant terms ( $p < 0.05$ ). The selection of models was performed by Chi-square tests and using the Akaike information criterion (AIC). Comparisons between factor levels were done using Fisher's LSD test. All calculations were performed using the R version 2.15.1 (R Development Core Team [2013](#page-10-0)).

## **RESULTS**

A total of 13,367 parasites from 10 different helminth species were recovered, being 73% of the prospected rodents ( $N = 185$ ) parasitized at the moment of capture. The 10 species included 1 acanthocephalan (Monoliformis moniliformis), 3 cestoda (Taenia taeniaeformis, Hymenolepis. nana and H. diminuta), and 6 nematoda (Gongylonema neoplasticum, Nippostrogylus brasiliensis, Capillaria sp., Heterakis spumosa, Aspicularis tetraptera and Syphacia obvelata) (Table [2\)](#page-5-0). Among them, both  $Hy$ menolepis species are zoonotic, although M. moniliformis, T. taeniaeformis, and G. neoplasticum are also mentioned as human parasites.

Nippostrongylus brasiliensis and H. spumosa were found in the three rodent species in the three landscape units, being the most generalist species in the compound community (Table [1](#page-3-0)). After them, T. taeniaeformis absent only in R. rattus, while H. nana, H. diminuta, and M. moniliformis were present in both rat species. In general, all helminth species showed high aggregation levels except N. brasiliensis and H. spumosa in R. norvegicus (even in shantytowns or in parklands) (Table [1\)](#page-3-0). In these cases, both species showed high prevalence and high mean intensity values, which allowed us to classify both helminth as core species for these components communities (Figure [2](#page-6-0)). In contrast, no core species were observed in R. rattus or in M. musculus (both, shantytowns or parklands).

At component community level, the species richness estimator first-order Jackknife (Table [2\)](#page-5-0) and the extrapolated species accumulation curves (Figure [3](#page-6-0)) showed that the highest helminth richness was found in the populations of R. norvegicus from parklands (at least 8 species) and the total helminth richness was lower in R. norvegicus from shantytowns and R. rattus (5 and 6 species, respectively). In contrast, the populations of M. musculus from shantytowns and parklands, as well as being the less parasitized, harbored both 4 helminth species. In spite the unbalanced number of host in the different component communities, the extrapolated accumulation curves showed that the number of helminth species detected was close to the plateau in most of the cases (Figure [3\)](#page-6-0).

At infracommunity level, the only variable that had a significant effect on the helminth species richness and the diversity index (Brillouin index) was the identity of component community in both cases ( $\chi^2$  = 38.68; *df* = 4;  $p < 0.01$  and *F*-value = 14.70;  $df = 4$ ;  $p < 0.01$ , respec-

<span id="page-5-0"></span>

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Figure 3. Extrapolated species accumulation curves for each of the combination of host species and landscape unit observed in the city of Buenos Aires for murine rodents (asterisk Rr; open diamond Rn/ST; open circle Mm/ST; filled diamond Rn/PL; filled circle Mm/PL).

tively). The variables dropped out were host abundance, host gender, season, and year ( $p > 0.05$  in all cases). Sampling sites were included as random factor to consider

the variance between them in each landscape unit. The comparisons between component communities showed that infracommunities from R. norvegicus from parklands



Figure 4. Analysis of differences in mean of helminth species richness found at the individual host level among the five categorized component communities with Fisher's LSD test (\*  $P < 0.05$ ) (bars represents the standard errors).





# **DISCUSSION**

Our results showed that three from every four murine rodents captured in the city of Buenos Aires were parasitized with at least one helminth species. Ten different species were identified, and many of them were recorded in more than one host species, being H. spumosa, N. brasiliensis, and T. taeniaeformis the most generalist. However, each rodent species featured its own parasite composition, richness, and diversity, even when occupying more than one landscape unit.

Environmental conditions play a key role in parasite– host interaction. The biotic and abiotic characteristics that define the 3 landscape units considered in this paper would affect not only the rodent community's attributes, but also the composition and abundance of intermediate hosts and the survival of free-living stages of different helminths species. We expected higher helminth richness at infracommunity's scale in animals trapped in shantytowns than other landscape units. However, the greatest values of richness and diversity were observed in brown rats from shantytowns and parklands, both. The environmental characteristics that act as predictors for the presence of R. norvegicus (water availability, vegetated cover, permeable surface, see Cavia et al. ([2009\)](#page-9-0) for more details) characterized these two landscape units, and this would explain the high similarity of the infracommunities of both groups. The high abundance of N. brasiliensis and H. spumosa indicates that R. norvegicus exploit sites (in both landscape units) with favorable conditions for the development of soil-transmitted helminthes (STH) and with direct life cycle, respectively (Arneberg [2002](#page-9-0); Weaver et al. [2010;](#page-10-0) Pullan and Brooker [2012](#page-10-0)).

However, the total helminth species richness was higher, in concordance with the first hypothesis, in the component community from brown rats captured in parklands than in shantytowns. Parklands are mentioned as important for conservation of animal and plant biodiversity in an urban context. Urban parks and open green areas include in many cases line coasts of rivers or lakes and patches of native vegetation which allows the presence of local wild species, including small mammals like rodents (Mahan and O'Connell [2005](#page-10-0)). These characteristics could favor higher parasite richness at host population level (Morand et al. [2015](#page-10-0)).

Compared to R. norvegicus, the total number of helminth species observed in R. rattus from residential and industrial neighborhoods was similar (6 species vs. 5 and 8 from shantytowns and parklands, respectively) but at infracommunity scale, lower levels of helminth infection were recorded. Black rats exploit successfully the higher strata like roofs, trees, and power lines) and exhibit more

aerial movements, less populated colonies, and a minor contact with soil, vegetation, domestic animals or others possible sources of helminthes infective stages (Marsh [1994](#page-10-0)). This could affect parasite loads of infracommunities, reducing species richness of each host as well as its abundances.

These differences between infracommunity's helminth diversity of both rat species were not detected by Zain et al. ([2012\)](#page-10-0) in Kuala Lumpur (Malaysia), probably because of the absence of core species in both hosts. The two main species of R. norvegicus found in our study, N. brasiliensis and H. spumosa, have a worldwide distribution, but prevalences greater than 80%, as our case, are uncommon to observe. For example, in urban and suburban areas of Belgrade, Serbia, the estimated prevalence for N. brasiliensis and H. spumosa varies between 15 and 40% (Kataranovski et al. [2011](#page-10-0)). Instead, H. spumosa was a central species in R. norvegicus captured in poultry farms of Argentina as well as in urban environments from Palermo, Italy (Gómez Vil-lafañe et al. [2008](#page-10-0); Milazzo et al. [2010](#page-10-0)).

Mus musculus harbored the same four helminth species in shantytowns and parklands, and no differences were detected at infracommunity level between both groups. In shantytowns, M. musculus was preferably captured inside the houses, mainly rooms or kitchens (Cavia et al. [2009\)](#page-9-0). Hence, M. musculus exploits microhabitats in shantytowns and parklands with different conditions and this was reflected in the level of infection of N. brasiliensis whose life cycles (STH) occur under certain environmental conditions. A fewer contact with permeable and vegetated surfaces would explain the lower prevalence of this parasite in shantytowns. Probably the pressure of competition and predation by R. norvegicus on M. musculus (see Caut et al. [2007](#page-9-0)) and the presence of domestic animals force the mice to seek refuge inside the houses.

According to Palmeirim et al. [\(2014](#page-10-0)), differences in body size and life strategies among murine rodent species determine the richness of its infracommunities because individuals would be exposed differently to helminth infections. In our study, each species of rodent presented its own characteristic of richness, diversity, and helminth parasites species, keeping these characteristics even still occupying more than one landscape unit. High values of parasite richness (8 species), of percentage of infestation  $(>95%)$  and prevalence and the presence of core species, explain the higher average helminths diversity of brown rats from parklands and shantytowns. Rodents that were caught in more than one landscape unit (R. norvegicus and <span id="page-9-0"></span>M. musculus) showed no statistical differences in the values of diversity of its components communities.

Within Buenos Aires City, shantytowns exhibited the greatest abundance of rodents, and the parasitological results of this paper, together with previous studies (Cueto et al. 2008; Cavia et al. 2015, Hancke and Suárez [2016](#page-10-0)) indicate that their inhabitants would be the most exposed to zoonotic diseases transmitted by rodents. The high parasite burden of the captured rats reflects a high environmental contamination of helminths infectious stages (eggs, larvae) present in these sites. Shantytowns are characterized by high human densities and a deficit in urban basic services (Fernández et al. [2007](#page-10-0)) that facilitate the spread of infectious diseases, not only in Buenos Aires but also in other large cities.

Shantytowns are not included in urban planning programs, so it is essential to focus efforts on individual and community actions to improve environmental quality. The high helminth diversity and load at individual scale, compared to residential neighborhoods, is indicating a high pressure of infection from the environment. This trend was suggested in a previous study of the abundance of H. diminuta (see Hancke and Suárez [2016](#page-10-0)), and the approach in this paper of the whole compound community of the rodent assemblage of the city of Buenos Aires confirms it. Taking into account that other animals are present, like dog, cats, horses, the risk of zoonotic infection in humans, especially children, is high. Zoonotic diseases can be managed through the reduction in human exposure patterns, reducing environmental pollution, improving hygiene practices of community members, and changing the behavior and attitudes (Nguyen-Viet et al. [2009\)](#page-10-0). We suggest that environmental health education campaigns are a feasible starting point for the incorporation of concepts related to environmental improvement and healthy hygiene practices, indispensable to obtain a preventive attitude about the problem posed by the presence of rodents in such environments (Hancke and Suárez [2014\)](#page-10-0).

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