ANIMAL SCIENCE

Ecological characterization of fleas on small mammals in natural and disturbed landscapes in the Atlantic Forest ecoregion, Argentina

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Abstract: To understand the ecoepidemiology of fleas and their interactions with domestic and wild animals, it is necessary to decode the cycles of flea abundance in relation to host species and environmental factors. The province of Misiones, known for its biodiversity and land use changes, provides an excellent study location. We assessed the diversity, prevalence, abundance, and parasitic intensity of fleas collected from 1153 rodents and opossums in various environments in the Upper Paraná Atlantic Forest of Argentina, considering phytogeography and land use. Our findings were consistent with previous studies conducted in humid regions of Argentina and the Atlantic Forest in Brazil. Hosts with high population density exhibited greater flea prevalence and abundance, and widely distributed hosts showed higher flea species richness and diversity. Significant similarities in flea similarity indices among phytogeographic areas, especially in forest. Environments with an anthropogenic gradient exhibited marked dissimilarities, with higher species richness and diversity in less disturbed sites. These parameters may be influenced by the presence of polyxenous *Ctenocephalides*, *Polygenis*, and *Adoratopsylla* fleas co-infesting opossum in urban areas. These findings provide valuable insights into the ecoepidemiology of fleas, their relationships with host species, and the impact of environmental factors in a strategic Atlantic forest ecoregion.

Key words: Diversity, landscape gradient, opossum, rodent, Siphonaptera.

INTRODUCTION

Fleas (Siphonaptera) are holometabolous insects whose adult fleas are highly specialized parasites that feed on blood, while the egg, larva, and pupa stages develop on the ground or in host nests. Over than 94% of flea species parasitize mammals, with the highest number of species associated with cricetid rodents (Marshall 1981). In a population of rodents, individual variations such as sex, age, reproductive status, physiology, and ecology can affect their interactions with parasites, including fleas (Linardi & Krasnov 2012). However, the composition of flea species in a habitat is not only determined by the characteristics of the host, but also by environmental factors such as temperature and relative humidity. These factors can significantly impact the survival of fleas, particularly during their preimaginal stages (Krasnov et al. 1997, Linardi & Krasnov 2012).

There are currently almost 3000 flea species and subspecies belonging to 16 families known worldwide (Lewis 1998, Whiting et al. 2008). In Argentina, a total of 137 flea species and subspecies belonging to 11 families have been recorded, of which 48 are endemic (Lareschi et al. 2016). The flea fauna of the Atlantic Forest ecoregion in Misiones province, northeastern Argentina, comprises 14 species and subspecies

belonging to four families, most of which are associated with cricetid rodents (Urdapilleta et al. 2019). However, some species also parasitize marsupials (Didelphimorphia) and other mammals, including humans, domestic and synanthropic animals, presenting low host specificity (Lareschi et al. 2016, 2018, Sánchez et al. 2018). Additionally, some species are involved in the maintenance and transmission of pathogens that can cause diseases in humans and domestic animals (Schott et al. 2019, Urdapilleta et al. 2020, 2021).

In Argentina, there are 25 known species of marsupials and 108 of cricetid rodents, of which 15 and 24 respectively are found in Misiones province (Lanzone et al. 2018, Galliari & Pardiñas 2021). These mammals are distributed throughout the Atlantic Forest areas of Paraguay and Brazil (Patton et al. 2015). These animals are not only important for the biodiversity of the region but also serve as hosts for a wide variety of flea species, making them potential reservoirs of parasites, bacteria, and viruses (Berrizbeitia et al. 2024). This aspect is particularly relevant when considering the potential implications of these microorganisms for human and animal health (Bezerra-Santos et al. 2021 Gonçalves-Oliveira et al. 2020). Therefore, fleas are of epidemiological importance at the interface between wildlife, domestic animals, and humans (Gutiérrez et al. 2015).

Changes in land use can modify the populations of mammals that serve as hosts for fleas, thereby influencing the transmission dynamics of diseases such as plague and murine typhus, which are transmitted by fleas. Ecotonal habitats may be pivotal areas where fleas can find suitable hosts and where wildlife-human interactions occur (Gibb et al. 2020). To gain a deeper understanding of flea ecoepidemiology and the potential for spillover and spillback between domestic and wild animals, it is essential to comprehend flea abundance cycles and prevalence in relation to host species and environmental characteristics. In this study, the objective was to investigate the diversity, prevalence, and abundance of flea species associated with rodents and opossums in different environments of the Atlantic Forest ecoregion of Argentina, according to their phytogeography and land uses.

Abbreviations

AE: agricultural environment
AFD: Arborescent Ferns District
AFE: anthropized forest environment
CI: Confidence Interval
df: degree freedom
H´: Shannon-Wiener diversity index
KOH: potassium hydroxide
L: sampling sites
LD: Laurels District
MA: mean abundance
MI: mean intensity
MUE: mixed use environment
n: individual
NFE: natural forest environments
P: prevalence
PS: Planaltense Sector
RD: Rosewood District
S: species richness
s.e.: standard error
SD: standard deviation
UE: urban environment
UPAF: Upper Parana Atlantic Forest
X^2: Chi-square

MATERIALS AND METHODS
Study area

This research was focused on Misiones province, northeastern Argentina, which harbors the largest remnant surface of the Atlantic Forest ecoregion complex, including the Araucaria

Forest and the Upper Parana Atlantic Forest (UPAF) ecoregions, the most biodiverse ecoregion of the complex and the most affected by forest loss and fragmentation (Di Bitetti et al. 2003). This ecoregion has a subtropical climate, with a mean annual temperature of 16-22°C and rainfall of 1,000 to 2,200 mm per year (Peel et al. 2007). The UPAF ecoregion, with an original area of about 470,000 km^2 distributed between Argentina, Brazil and Paraguay, limits with Araucaria Forests ecoregion to the east (Martínez Crovetto 1963), on an undulating plateau topography that descends from the southern Brazilian Planalto in a northeast-southwest direction, along the Misiones sierras. In Misiones province, monocultures such as pine, yerba mate and tobacco are the main agricultural activities that increase deforestation and pressure on the scarce forest remnants (Izquierdo et al. 2008, 2011). Habitat loss and fragmentation that these activities generate are linked to biodiversity loss and variations in species composition and ecosystem dynamics (Iezzi et al. 2018, 2019).

Following the phytogeographic scheme of Martinez Crovetto (1963), sampling sites were grouped in four phytogeographic areas. The Misionero sector encompasses the western portion of the Argentine province of Misiones. It is characterized by the presence of hygrophilous forests dominated by Lauraceae. This sector includes the Laurels District (LD), where the black laurel (*Nectandra saligna*) predominates, associated with the yellow laurel (*Nectandra lanceolata*); the Rosewood District (RD) (*Aspidosperma polyneuron*); and the Arborescent Ferns District (AFD), with the presence of five species of "tree ferns" belonging to the Cyatheaceae family. The Planaltense sector (PS) occupies the northeastern part of Misiones and is distinguished by the presence of *Araucaria angustifolia* (Figure 1, Table I). Fleas collected in forest environments of the four phytogeographic areas were used to analyze differences between these areas.

Sampling sites within LD, AFD and PS corresponded only to forest environments, while sampling sites located within RD included: 1) urban environment (UE), urbanized landscape with at least some of the basic public services; 2) mixed use environment (MUE), characterized by subsistence agriculture often mixed with small pasture, shrub and forest patches, contiguous to protected natural forests: 3) agricultural environment (AE), dominated by perennial crops of yerba mate, cassava, sugar cane and monospecific plantations of *Pinus*, *Eucalyptus* and *Araucaria* used for wood and pulp production; 4) anthropized forest environment (AFE), primary and secondary forest with mild anthropogenic disturbance, inhabited by a Guaraní Community; and 5) natural forest environments (NFE), protected areas with primary and riparian forest. Samples collected within RD were used to conduct a different comparative analysis based on a landscape gradient (Izquierdo et al. 2008) (Figure 1, Table I).

Sample collection

For comparison of fleas among phytogeographic areas, fleas collected from cricetid rodents during other research projects in 2005 (May), 2007 (April), 2009 (September), 2012 (September), and 2013 (May/September) were utilized. Additionally, fleas from *Didelphis* opossums captured in a project conducted in Iguazu National Park from February 2015 to October 2016 were included in the analysis. Specimens collected from forest environments in a seasonal study conducted in Rosewood District (RD) from July 2017 to November 2018 were also studied. The samples collected in this seasonal sampling were used to compare fleas on a landscape gradient. The methodology employed for the collection of ectoparasites was consistent across all studies.
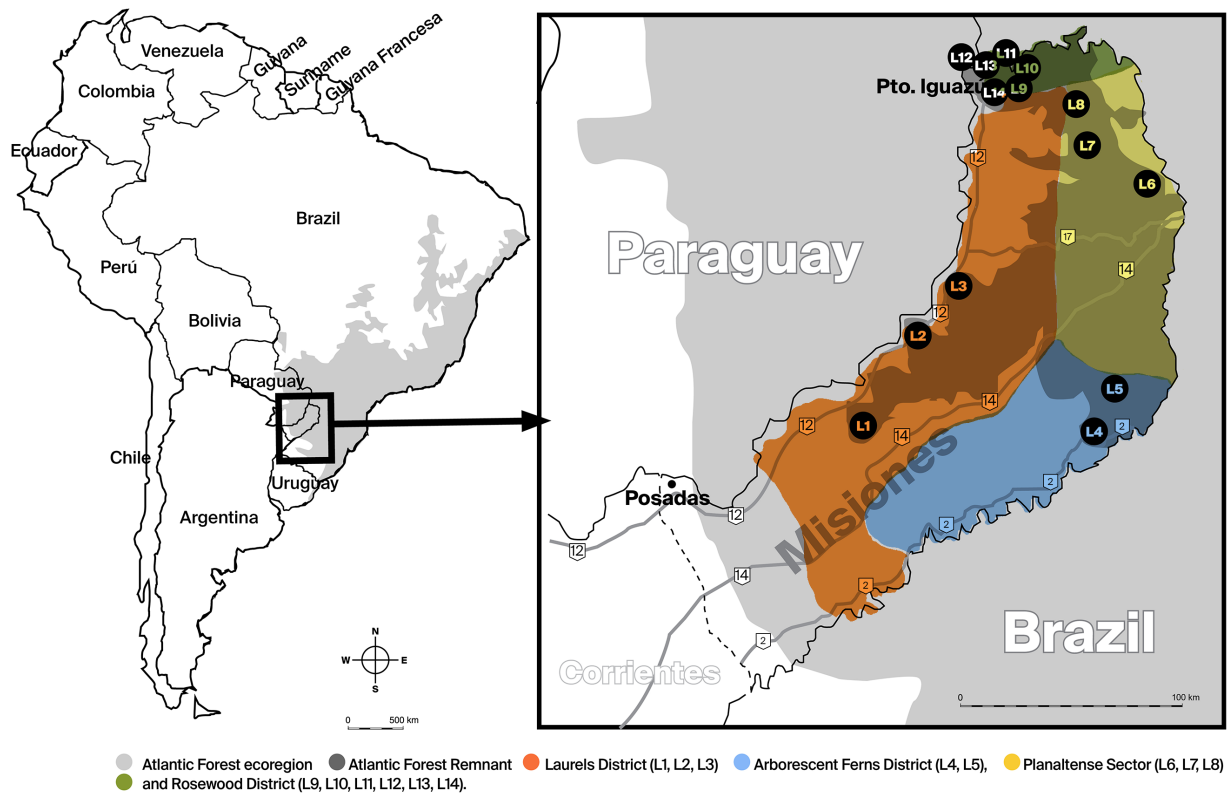
Figure 1. Phytogeographic areas and flea sampling sites in the Atlantic rainforest of Misiones Province, Argentina.

During the seasonal sampling, capture of small and medium mammals was carried out using 200 Sherman-type live traps (24x9x8cm) baited with a preparation of fat, oats and peanut paste wrapped in cotton, and 90 cage-type live traps (40x15x15cm) baited with meat and fruit. Sherman and cage traps were placed in transects in each environment, with a separation of at least 10m between traps. All traps remained active for 72 hours, and were checked every morning. Captured animals were transported to a processing area and traps and baits were replaced as necessary.

The field processing of mammals was conducted in accordance with the "Biosafety Guidelines for Working with Small and Medium-sized Mammals" (Carroll et al. 2017) and the "Guidelines of the American Society of Mammalogists for the Use of Wild Mammals in Research and Education" (Sikes et al. 2016).

The sampling and associated procedures conducted in Misiones province were carried out in accordance with permits issued by the National Parks Administration, Northeastern Argentine Delegation (N°IF-2018-09413013-APN-DRNEA#APNAC) and the Misiones Ministry of Ecology and Renewable Natural Resources (No. 34/2017 and No. 26/2018). For the collection of ectoparasites, wild rodents were sedated by inhalation of a low dose of isoflurane, while synanthropic rodents were first sedated and then euthanized in accordance with the protocol set forth in Mills et al. (1995). In all cases, weight was recorded with an OHAUS Traveler® electronic scale, which is accurate to 0.1 g. Standardized body measurements (total body, tail, foot, and ear lengths) were taken for subsequent identification. A comprehensive flea search was conducted for a duration of five minutes per host, with the time allotted

Table I. Phytogeographic areas with definition of environment types and flea sampling sites (L).

Phytogeographic area	Environment	Sites	Geographical coordinates
Laurels District	Forest	L1, Aristóbulo del Valle on Cuña Pirú stream. Dto. Cainguás	27°3'42.11"S 55°2'35.07"W
	Forest	L2, Fishing Club Paraná-í Guazú. Dto. Montecarlo	26°40'27.97"S 54°48'47.65"W
	Forest	L3, "Ernesto Che Guevara" Provincial Park. Dto. Montecarlo	26°36'53.69"S 54°46'50.82"W
Arborescent Ferns District	Forest	L4, PR N°2, 6 km NE Paraíso stream. Dto. Guaraní	27°12'47,7"S 54°01'59,9"W
	Forest	L5, Moconá Refuge. Dto. Guaraní	27°08'29.36"S 53°55'29.05"W
Planaltense Sector	Forest	L6, Annex Field M. Belgrano, INTA. Dto. Gral. Manuel Belgrano	26°02'54.21"S 53°46'32.40"W
	Forest	L7, Urugua-í Wildlife Reserve. Dto. Gral. Manuel Belgrano	25°58'32.29"S 54°07'00.08"W
	Forest	L8, Urugua-í Provincial Park. Dto. Gral. Manuel Belgrano	25°51'39.62"S 54°10'02.51"W
Rosewood District	Forest	L9, Puerto Península Provincial Park. Dto. Iguazú	25°43'00"S 54°35'00"W
	Forest	L10, Apepú, Iguazú National Park. Dto. Iguazú	25°34'18.09"S 54°18'6. 18"W
	Forest	L11, Iguazú National Park. Dto. Iguazú	25°40'59"S 54°27'17"W
	Urban	L12, Puerto Iguazú City. Dto. Iguazú	25°36'39"S 54°34'49"W
	Mixed use	L12, 2000 Hectares, Puerto Iguazú. Dto. Iguazú	25.65'2149"S 54°56'1695"W
	Anthropized forest	L13, Yriapú Guaraní Community Reserv. Dto. Iguazú	25°36'51.0"S 54°32'46.1"W
	Agriculture	L14, Colonia Wanda City. Dto. Iguazú	25°58'S 54°34"W

for each sample standardized. The majority of sigmodontine rodents were subsequently released at the same capture site once sampling was completed. Identification was based on the external characteristics of the rodents, which were carried out by trained personnel. Necropsy was performed in the field on synanthropic rodents to collect spleen and liver samples, which were placed in tubes containing 96° ethanol and stored at -20°C for subsequent analysis.

The sedation of captured opossums involved the intramuscular injection of 20-25 mg/kg ketamine (5%) and 10 mg/kg xylazine (2%) (West et al. 2007). This was conducted for the purpose of an exhaustive search for ectoparasites over a 15-minute period, followed by weighing and body measurements. Once recovered from anesthesia, the opossums were released at their respective capture sites. The opossums were identified to the species level for the personal trainer following Massoia et al. (2000) & Bárquez et al. (2006).

The trapping effort for rodents and opossums was quantified as the number of traps deployed per sampling period. The sampling unit was

defined as a trap-night, as proposed by Jones et al. (1996).

To obtain fleas, the tegument of each captured mammal was examined by hair and against the hair using needles, tweezers and brushes. The ectoparasites were placed in individual tubes per host with 96% alcohol, labeled with the corresponding field number and placed in a freezer at -20°C until taxonomic determination at the National Institute of Tropical Medicine (INMeT), in Puerto Iguazú, Misiones.

Taxonomic determination of fleas

Fleas were taxonomically determined according to their morphological characters, following the keys and descriptions presented by Johnson (1957), Smit (1987) and Linardi & Guimarães (2000). They were prepared following Linardi & Guimarães (2000): they were rinsed in 10% potassium hydroxide (KOH), in an estimated time between three and seven days according to the specimen. They were washed in distilled water and subsequently dehydrated in an increasing series of ethanol (from 80 to 100%) (24 hours in each graduation), diaphanized in eugenol (24 hours), and finally mounted in Canada balsam, dried in an oven at 50ºC and observed under an optical microscope (Carl Zeiss, Axiostar plus 176045 USA, Leica DM 2500, Olympus BX51). Some of the specimens were deposited in the Entomology Collection of the Museo de La Plata, Argentina, while others were stored at the National Institute of Tropical Medicine.

Statistical analysis

Flea samples collected on each host species in each phytogeographic area and environment were described using the following parasitological indices: mean intensity (MI = number of individuals of a flea species/ number of infested hosts), mean abundance (MA = number of specimens of a flea species/ number of hosts examined), and prevalence (P = number of host specimens parasitized by a flea species/number of hosts examined*100) (Bush et al. 1997). Differences in these parameters between areas, host species and flea species were evaluated with Chi-square and Bootstrap t-tests (number of bootstrap replicates=2000) using Quantitative Parasitology 3.0 software (Reiczigel & Rózsa 2005). In addition, seasonal prevalence of fleas collected on *Didelphis albiventris* in urban environment and mixed use environment were analyzed using Chi-square test, since fleas were abundant on this species in these environments.

In order to evaluate completeness of each sample, rarefaction and extrapolation curves for the first two orders of Hill numbers (q=0 and q=1) were obtained (Chao et al. 2014) using the iNEXT package for R (Chao et al. 2016, R-Core Team 2021).

To assess alpha-diversity, species richness (S) of each flea assemblage was estimated for comparative purposes by taking into account data on rare species abundance in the total sample, through the Chao1-bc richness estimator, which includes possible undetected species (Chao et al. 2015):

$$S_{Chao1-bc} = S_{obs} + [(n-1)/n][(f_1(f_1-1))/(2(f_2+1))]$$

where S_{obs} is the total number of species observed, n is the total number of flea specimens collected, f_1 is the number of *singletons* (species with only one specimen collected) and f_2 is the number of *doubletons* (species with only two specimens collected).

To assess the uncertainty in predicting to which species a randomly selected individual from each assemblage would belong, the Shannon-Wiener species diversity index was calculated using the Diversity Profile Estimation

program of the SpadeR online platform (Chao et al. 2015):

$$H_e' = -\Sigma p_{ie} * \ln p_{ie}$$

where H_e' is the Shannon entropy index of each flea assemblage e, and p_{ie} is the relative abundance of species i in assemblage e (Begon et al. 1988).

To assess beta-diversity, two different statistical analyses were performed, 1) one to compare flea assemblages between native forest environments of different phytogeographic areas, and 2) one to compare flea assemblages present on a landscape gradient located in Rosewood District (RD). Beta diversity was assessed using SpadeR to determine the degree of similarity or dissimilarity among multiple communities with Horn index (q=1), which weights species by abundance without focusing on dominant species, with a bootstrap of 1000 replicates. Index values are between 0 and 1, with values close to one being the most similar assemblages.

RESULTS
Samples collected

The total trap-night effort was 2745, 2883, 1883, 4500, and 5883 in urban (UE), mixed-use (MUE), anthropized forest (AFE), agricultural (AE), and natural forest (NFE) environments, respectively. A total of 914 rodents and 38 opossums were captured in NFE, 34 rodents and 5 opossums in AFE, 24 rodents and 43 opossums in UE, 48 rodents and 24 opossums in MUE, and 23 rodents in AE.

A total of 447 fleas were obtained from 1153 hosts (1043 rodents and 110 opossums), of which 173 flea specimens were found in NFE, 16 in AFE, 196 in UE and 62 in MUE. No fleas were recorded in rodents captured in AE.

Fleas of the 11 following species and subspecies were identified, considering all hosts: Pulicidae: Pulicinae: *Ctenocephalides felis felis* (Bouché, 1835); Stephanocircidae, Craneopsyllinae: *Craneopsylla minerva minerva* (Rothschild, 1909); Rhopalopsyllidae: Rhopalopsyllinae: *Polygenis* (*Polygenis*) *platensis* (Jordan and Rothschild, 1908), *Polygenis* (*Polygenis*) *rimatus* (Jordan, 1932), *Polygenis* (*Polygenis*) *roberti beebei* (Fox, 1947), *Polygenis* (*Polygenis*) *roberti roberti* (Rothschild, 1905), *Polygenis* (*Neopolygenis*) *pradoi* (Wagner, 1937), *Polygenis* (*Neopolygenis*) *pygaerus* (Wagner, 1937); Ctenophthalmidae: Doratopsyllinae: *Adoratopsylla* (*Adoratopsylla*) *antiquorum antiquorum* (Rothschild, 1904), *Adoratopsylla* (*Adoratopsylla*) *antiquorum ronnai* (Guimaraes, 1954), and *Adoratopsylla* (*Tritopsylla*) *intermedia intermedia* (Wagner, 1901) (Supplementary Material Table SI, and Table II).

Statistical analysis

The tables below (Tables SI and II) present the numbers of collected fleas and the parasitological parameters. In forest environments, approximately 90.17% of the fleas collected were identified as belonging to the genus *Polygenis*, which is associated with eight species of sigmodontine rodents. The most abundant rodent species in the communities under study was *Akodon montensis*. *Polygenis* (*P.*) *rimatus* was the most prevalent and abundant flea species (P=12.7%; AM=0.13), with the largest host range, recorded in most of the sites parasitizing seven host species (87.5% of host species). Furthermore, significant differences were observed in infestation rates compared to the remaining species registered in Laurels district (LD) (X^2=42.25, df=3, p-value<0.001) and RD (X^2=9.38, df=3, p-value=0.02). In turn, *P.* (*P.*) *platensis*, *P.* (*N.*) *pradoi*, *P.* (*P.*) *r. roberti*, *P.* (*P.*) *pygaerus* exhibited prevalence rates ranging

Table II. Fleas collected from rodents and marsupials in different environments of the province of Misiones, Argentina. I: Infested hosts. E: Examined hosts. N: Number of fleas. MA: Mean Abundance. MI: Mean Intensity. P: Prevalence (%). Natural Forest in RD.

ENVIRONMENT/ HOST	I/E	Ctenocephalides f. felis				Adoratopsylla a. antiquorum				Adoratopsylla a. ronnai				Adoratopsylla i. intermedia				Polygenis (P.) rimatus				Polygenis (P.) r. roberti				Polygenis (P.) pradoi			
		N	MA	MI	P	N	MA	MI	P	N	MA	MI	P	N	MA	MI	P	N	MA	MI	P	N	MA	MI	P	N	MA	MI	P
URBAN																													
Mus musculus	0/2																												
Rattus rattus	0/22																												
Didelphis albiventris	28/43	189	4.40	6.75	60.50	2	0.05	0.07	4.65	1	0.02	0.03	2.32					4	0.09	0.14	4.65								
Total	**28/67**	**189**	**4.40**	**6.75**	**60.50**	**2**	**0.05**	**0.07**	**4.65**	**1**	**0.02**	**0.03**	**2.32**					**4**	**0.09**	**0.14**	**4.65**								
MIXED USE																													
Akodon montensis	0/2																												
Mus musculus	0/13																												
Rattus rattus	2/33																	5	0.15	2.5	6.06								
Monodelphis dimidiata	1/2																	4	2.00	4	50								
Didelphis albiventris	15/22	40	1.82	0.27	63.30	1	0.05	0.07	4.54									12	0.55	0.8	27.27								
Total	**18/72**	**40**	**1.82**	**0.27**	**63.30**	**1**	**0.05**	**0.07**	**4.54**									**21**	**0.29**	**1.17**	**29.17**								
ANTHROPIZED FOREST																													
Akodon montensis	4/24																	5	0.21	0.21	16.66								
Mus musculus	0/9																												
Rattus rattus	0/1																												
Didelphis albiventris	1/1	2	2.00	2	100																								
Philander quica	3/4													8	2.00	2	75	1	0.25	0.25	25								
Total	**8/39**	**2**	**2.00**	**2**	**100**									**8**	**2.00**	**2**	**75**	**6**	**0.15**	**0.75**	**15.40**								
AGRICULTURE																													
Akodon montensis	0/2																												
Calomys tener	0/13																												
Oligoryzomys nigripes	0/1																												
Mus musculus	0/6																												
Rattus rattus	0/1																												
Total	**0/23**																												
NATURAL FOREST																													
Akodon montensis	7/94																	11	0.12	1.6	11.70								
Oligoryzomys nigripes	0/6																												

Table II. Continuation.

Brucepatersonius sp.	0/1																						
Thaptomys nigrita	0/7																						
Mus musculus	0/1																						
Rattus rattus	0/1																						
Didelphis aurita	8/36			1	0.03	0.125	2.77				6	0.17	0.75	11.1	6	0.17	0.75	5.55		0.22	13.80		
Monodelphis americana	1/1			1	1.00	1	1																
Philander quica	1/1										1	1	1	100									
Vegetation																							1
Total	**17/149**			**2**	**0.01**	**0.12**	**1.34**				**7**	**0.05**	**0.41**	**4.70**	**17**	**0.1**	**1**	**11.04**		**0.05**	**5.36**	**1**	
TOTAL	**71/350**	**231**		**5**	**0.43**	**0.1**	**1.43**	**1**	**0**	**0.01**	**0.28**	**15**	**0.04**	**0.21**	**4.30**	**48**	**0.14**	**0.68**	**13.70**		**0.02**	**2.30**	**1**

from 0% to 7%, with parasitism occurring in one to six species of sigmodontine rodents. In contrast, *P. r. beebei* was found only in *Thaptomys nigrita*. With regard to host species, *A. montensis* and *T. nigrita* exhibited the highest flea infestation frequencies and prevalences (n=111, P=10.5%; n=26, P=42%, respectively). With regard to opossums, *D. aurita* was the species with the highest number of captured individuals and the one presenting the highest flea species richness, with a prevalence of infested individuals of 22.2% in Rosewood districts (RD).

A significant difference in overall prevalence (P) and mean intensity (MI) was observed among environments in landscape gradient (RD). These parameters were higher in the urban environment (UE) (42% and 7, respectively), while they were lower in the mixed-use (MUE) (25% and 3.5), anthropized forest (AFE), and natural forest (NFE) environments. The results indicated that the NFE exhibited a significant difference between the UE and the MUE (X^2=25.7, df=3, p-value=0.0001) (Table II). Upon analysis of these parameters between pairs of environments, it was observed that the UE exhibited a significant difference from all other environments, while the differences between MUE and AFE, as well as the latter and NFE, were not found to be significant (X^2=0.284, df=1, p-value=0.594).

There was a significant difference between seasons in fleas collected on *D. albiventris* in the UE (X^2=15.0; df = 3, p-value= 0.002), with a higher prevalence recorded in spring (82.8%) and summer (44.4%), but this association was not observed in the MUE (X^2=2.71; df=2, p-value= 0.26).

Diversity analysis of phytogeographic areas

Considering the total number of individuals collected in each area, rarefaction and extrapolation curves showed that sample coverage (completeness) estimates for each set were in the range of 0.9702 to 1, meaning that increasing the number of individuals in the samples would not increase the number of species or species completeness.

Flea species richness estimated was similar in forest environments being 5.5 species in Laurels District (LD), Arborescent Ferns District (AFD) and Rosewood District (RD), and 4.5 species in Planaltense sector (PS). The Shannon-Wiener diversity indices (H') were 4.461 (SD=0.522) in AFD, 3.844 (SD=0.533) in RD, 2.783 (SD=0.439) in LD and 1.404 (SD=0.187) in PS. The beta-diversity analysis revealed a high global similarity among

phytogeographic areas (Horn= 0.7189, SD=0.044, 95%CI=0.6327-0.8050), with pairwise similarity of assemblages ranging from 0.57 to 0.90 (Table III). This indicates a notable degree of overlap in species composition across the areas. The flea species that were shared between the various areas were *Polygenis (Polygenis) rimatus, Polygenis (Neopolygenis) pradoi* and *Craneopsylla minerva minerva.*

Diversity analysis of landscape gradient

Within the study area RD, species richness estimates were 5.0 in natural forest (NFE), 4.0 in urban (UE), and 3.0 in mixed-use (MUE) and anthropized forest (AFE) environments. In agricultural environment (AE), no fleas were collected. The Shannon-Wiener diversity index (H´) was 3.84 (SD=0.55) in NFE, 2.83 (SD=0.32) in AFE, 2.18 (SD=0.16) in MUE, and 1.22 (SD=0.08)

in UE. The beta-diversity analysis revealed a lower overall similarity among areas (Horn= 0.61, SD=0.02, CI95%=0.55-0.66), with pairwise similarity values ranging from 0.14 to 0.83 (Table IV), indicating distinct species assemblages in the different areas. The pairwise similarity indices approaching one indicate a higher presence of shared species, as exemplified by the occurrences of *C. f. felis, A. a. antiquorum,* and *P. rimatus* in UE and MUE. Conversely, values approaching zero provide evidence of species dissimilarities between areas, such as the presence of *A. intermedia* and *P. roberti* species in forest environments, which are absent in urban areas. To prevent the overestimation of dissimilarity among environments, agroforestry crops were excluded from this analysis due to the absence of collected fleas.

Table III. Similarity indices between flea assemblages collected in different phytogeographic areas. s.e.: standard error. 95%CI: 95% Confidence Interval. LD: Laurels District, AFD: Arborescent Ferns District, PS: Planaltense Sector, RD: Rosewood District.

Areas	Estimate	s.e.	95% CI
LD / AFD	0.78	0.09	(0.61- 0.95)
LD / PS	0.90	0.05	(0.80-0.99)
LD / RD	0.61	0.06	(0.49-0.73)
AFD / PS	0.82	0.11	(0.59-1.00)
AFD / RD	0.57	0.09	(0.39-0.75)
PS / RD	0.71	0.09	(0.54-0.87)

Table IV. Similarity indices of flea assemblages collected in different environments. s.e.: standard error. 95%CI: 95% Confidence Interval

Environment pairs	Estimate	s.e.	95% CI
Forest / Urban	0.14	0.04	(0.05-0.23)
Forest / Mixed use	0.46	0.05	(0.35-0.56)
Forest / Anthropized forest	0.74	0.08	(0.58-0.89)
Urban / Mixed use	0.83	0.05	(0.74-0.93)
Urban / Anthropized forest	0.31	0.11	(0.10-0.53)

DISCUSSION

Phytogeographic areas

The Atlantic Forest ecoregion of Argentina exhibits a high diversity of flea species associated with cricetid rodents and opossums, particularly within the Rhopalopsyllidae and Ctenophthalmidae families, as highlighted by previous research (Horta et al. 2007, Barros-Battesti 2008, Cáceres 2012, Lareschi et al. 2016, Urdapilleta et al. 2019, 2021). Notably, 86.5% of the fleas reported in our study belong to the family Rhopalopsyllidae, subfamily Rhopalopsyllinae, genus *Polygenis*, associated with 14 host species. This higher proportion could be attributed to the species diversity, higher abundances, and wide host range among the small mammals of the Upper Parana Atlantic Forest (UPAF) (D'Elía & Pardiñas 2015, Pardiñas et al. 2003).

In line with findings reported by Moraes et al. (2003), *A. montensis* is the most prevalent host, exhibiting the highest number of collected flea species, predominantly parasitized by *P. (P.) rimatus*. In other regions of the Atlantic Forest, its congener, *Akodon cursor*, demonstrates a notable prevalence of parasitism by flea species of this genus (Carvalho et al. 2001). Notably, *P. (P.) rimatus*, the most widely distributed species, is observed across all phytogeographic areas, followed by *P. (P.) platensis* and *P. (N.) pradoi*, respectively. When examining variations among flea species, *P. (P.) rimatus* showed significantly higher abundances and prevalence. This is likely attributed to its high prevalence on *A. montensis, T. nigrita* and *Brucepattersonius* sp., the three rodent species parasitized by this flea in Laurels District (LD), as well as on *A. montensis* in Rosewood District (RD).

The phenomenon of low or no flea infestations observed in various rodent species in our study can be explained by the concept of parasite aggregation, which reflects the uneven distribution of parasites among hosts. This distribution is characterized by the presence of a significant number of parasites in a few hosts, while the majority of hosts have few or no parasites (Anderson & May 1978).

The rodents and opossums in our study exhibited mean abundance values for fleas similar to those documented in various locations within the Atlantic Forest, such as the investigations on Foz do Iguaçu, Paraná, Ilha Grande in Rio de Janeiro and Cantareira State Park, São Paulo (Barros et al. 1993, Bittencourt & Rocha 2003, Nieri-Bastos et al. 2003). However, these values were comparatively lower than those reported in other sites (Moraes et al. 2003; Pinto et al. 2009). The total prevalences within the phytogeographic areas, as reported, are notably lower when compared to studies in southeastern Brazil (Carvalho et al. 2001, Moraes et al. 2003). Particularly, the prevalence in LD (26.31%) aligning closely with values documented in the Central Atlantic Forest Corridor in Caratinga, Minas Gerais, and in Cantareira State Park, São Paulo (Pinto et al. 2009, Nieri-Bastos et al. 2004). According to Krasnov et al. (2005), prevalence is positively correlated with mean abundance; however, in this case, both parameters were proportionally low in comparison to other regions. Contrary to the typical trend where parasitism increases at lower latitudes, it appears that other environmental factors may play a significant role in influencing host parasitism in our study area.

Considering the natural forest sampling sites, the Arborescent Ferns District (AFD) presented similar specific richness to the other phytogeographic areas, and it was the one with the highest diversity evidenced by the presence of unique species, such as *P. (P.) pygaerus* and *P. (N.) pradoi*. The LD showed the highest number of flea-host associations, being the only area where *Polygenis (P.) r. beebei* was recorded. *Polygenis (P.) platensis* was the second most

frequent species, and the most distributed among the rodent specimens.

In Planaltense Sector (PS), the Shannon-Wiener diversity index (H´) was lower than the rest of the areas. In this area, most of collected flea individuals were *P.* (*P.*) *rimatus*, mostly recorded on *A. montensis*, which impacted the relative abundance of this species, reducing the equitability of the sample. The high number of captured *A. montensis* is probably related to the higher relative abundance of *P.* (*P.*) *rimatus* recorded in PS.

On the other hand, the RD presented equal specific richness than LD and AFD and was the second area characterized by the highest H´ index. Opossums were only collected in the RD, adding species richness to the sample, since almost none of the rodent hosts were found parasitized by fleas (except *A. montensis* only parasitized by *P.* (*P.*) *rimatus*). The species *P. r. roberti* was recorded parasitizing only opossums *D. aurita*, however, this flea species was also described to be strongly associated with *O. nigripes*, *Nectomys squamipes* and *Oryzomys russatus* in areas of the Atlantic Forest in Southeastern Brazil (Barros et al. 1993, Moraes et al. 2003, Barros-Battesti et al. 2008). For this reason, the richness and diversity of the sample depends on flea species found on opossums. When calculating Chao-bc species richness and H´ index only for rodent hosts in RD, these values drop to 1 and 0, respectively, representing the lowest species richness and diversity index of all phytogeographic areas. This district together with LD have the lowest flea diversity indices.

Regarding the similarity indices of flea collections between pairs of phytogeographic areas, all of them showed high similarity, mainly because these assemblages correspond to forest environments represented by almost the same flea species collected on similar host species. It should be noted that pairs including

RD recorded the lowest similarity values (<0.75), as the other pairs of phytogeographic areas showed higher similarity values (>0.75), this is due to the presence of marsupial-associated species, which were only recorded in RD.

In center-southern Misiones province, there is a transition zone between Atlantic Forest and Fields and Grasslands ecoregions. This zone exhibits distinct biotic and abiotic conditions when compared to the northern part of the province, including variations in temperature, humidity, and vegetation structure. It is important to note that the sampling years may potentially influence these parameters. In general, each flea species and population are found in environments that present certain characteristics and ranges of those variables that are adequate for their survival and reproduction, being determinant for their distribution and abundance (Krasnov et al. 2006, 2023). Thus, the differences in flea diversity found in the northern and central-southern part of Misiones province can be explained by the different preferences of each species towards a specific set of environmental conditions that define its abiotic niche, with fleas being able to parasitize different hosts in different environments (Urdapilleta et al. 2019, Lareschi et al. 2019, Savchenko et al. 2021).

The family Ctenophthalmidae was mainly associated with opossums, although they also parasitize sigmodontine rodents, such as *Abrawayaomys chebezi* or *A. montensis* and *Nectomys squamipes* in southeastern Brazil (Carvalho et al. 2001, Moraes et al. 2003, Pardiñas et al. 2016). In this study, the highest proportion of *Adoratopsylla* fleas was represented by three subspecies associated with opossums in the RD. The finding of the subspecies *A. a. ronnai* in association with *D. albiventris* and *A. i. intermedia* with *P. quica* and *D. aurita* reinforce the high flea/host specificity hypothesis suggested by

Pinto et al. (2009) and Urdapilleta et al. (2023). In turn, *A. a. antiquorum* is frequently found both on opossums and cricetid rodents, which indicates that not all flea species of the same genus have the same host specificity (Oliveira et al. 2010, Urdapilleta et al. 2019).

Landscape gradient

In the analysis between environments, our findings align with the conclusions drawn by Santos et al. (2018), suggesting that the reduction of forest cover intensifies parasitism rates, impacting flea abundance and species richness. As anticipated, host species identified in environments with higher levels of anthropization (urban (UE) and mixed-use (MUE)) exhibited elevated infestation rates compared to their counterparts in forested environments (natural forest (NFE) and anthropized forest (AFE)). This disparity resulted in a higher abundance of fleas in anthropized areas, consistent with the patterns observed in the study conducted by Santos et al. (2018).

Significant differences were observed in both prevalence and mean intensity between the analyzed environments, with values being higher in anthropized environments and lower in forested environments. This underscores the potential role of forest habitat reduction in influencing the dynamics and health of small mammals in the Atlantic Forest.

Regarding the significantly higher overall prevalence (P) and mean intensity (MI) of fleas collected in UE, this may be attributed to the high prevalence and abundance of the common cat flea, *C. f. felis*, on *D. albiventris* in this environment. The higher flea infestation values on *D. albiventris* occurred during the spring and summer seasons, coinciding with the seasonal variation in population density of neotropical opossums, characterized by a defined reproductive season (Cáceres 2012). During this period, increased dispersal and contact rates among opossums may contribute to higher flea-host contact and, consequently, increased flea infestation.

With regard to the environmental types within the Rosewood District area, similar values of host and flea diversity and richness were observed in mixed-use (MUE) and anthropized forest (AFE) environments. The highest richness and H´ values were observed in the natural forest environment (NFE), followed by the AFE, MUE, and urban (UE) environments. This is consistent with the presumption that species diversity is higher in sites with lower anthropogenic disturbance, as stated by Friggens & Beier (2010). In this sense, regarding Horn indices, the most similar pairs were UE and MUE, and NFE and AFE, given the abiotic and host similarities in these environments, which are essential factors that determine the presence and abundance of flea species. In agreement, the lowest similarity pair included NFE and UE, primarily due to the collections of specific flea species on opossums (Table IV).

These findings show that environments with different anthropogenic disturbance that share some host species and ecological variables can generate niche overlaps, where different species of hosts and parasites cohabit. Generally, in tropical anthropized environments, the life cycles of fleas associated with domestic and wild animals coincide, partially or completely, depending on the context, and they can act as zoonotic pathogen vectors according to local patterns of coexistence between humans and animals (Feldmeier et al. 2014).

As previously stated by Gibb et al. (2020), alterations in land use have the effect of homogenizing global biodiversity and influencing the composition of zoonotic host communities. This results in variability of responses by different host species, which in

turn can influence flea ecology. For instance, an increase in the abundance of rodents in certain disturbed habitats could facilitate flea proliferation and, consequently, elevate the risk of zoonotic diseases. In essence, the process of urbanization and agriculture can alter the habitats of flea hosts, such as rodents, opossums, and other mammals. This can lead to changes in the dynamics of disease transmission. Furthermore, the increased proximity of humans to these species in urban areas may increase the risk of flea-borne diseases.

Increased deforestation has caused some rodent and opossum species to acquire synanthropic habits in response to the availability of resources, favored by their omnivorous diet (Hassell et al. 2017). For this reason, *D. albiventris* was the most abundant synanthropic species found in the UE and the most abundant opossum in the MUE, while *P. quica* and *M. dimidiata* were captured in AFE and MUE adjacent to native forest patches. On the other hand, *D. aurita* was found only in NFE, unlike the scenario described by Bezerra-Santos et al. (2020) for Minas Gerais, Brazil. Flea species richness and diversity patterns can be affected by the presence of infested synanthropic hosts, which is demonstrated by the presence of *P.* (*P.*) *rimatus* parasitizing synanthropic rodents such as *R. rattus* and opossums such as *D. albiventris* captured close to patches of native forest in the MUE.

Regarding fleas of the Rhopalopsyllidae and Stephanocircidae families, studies on opossums and rodents from the states of Paraná and Santa Catarina in Brazil showed that some species such as *P.* (*N.*) *pradoi*, *P.* (*P.*) *rimatus* and *C. m. minerva*, are frequently found in sympatry with fleas of the family Pulicidae (*C. f. felis* and *Pulex irritans*) (Linardi et al. 1991, Carvalho et al. 2001). This leads to infer that pathogens could be transmitted between different flea species by

cohabiting and cofeeding on the same hosts in areas of sympatry, and that some flea genus as *Polygenis* could play a key role in maintaining pathogens in wild rodents (Abbot et al. 2007, Buffet et al. 2013, Linardi 2017, Melis et al. 2020).

In the same way, the presence of *D. albiventris* in UE was associated with high infestations of *C. f. felis*, which suggests that opossums play an important role in the circulation of this flea species between UE, MUE and AFE, and therefore in the dissemination of associated zoonotic pathogens. In Brazil, similar infestation patterns occurred with *C. f. felis* on *D. aurita* captured in anthropized environments (Bezerra-Santos et al. 2020) reinforcing that the polyxenous behavior of *C. f. felis* favours its presence on synanthropic opossums (Dobler & Pfeffer 2011, Hassell et al. 2021). On the other hand, the lack of infestations by the flea *Tunga penetrans* in synanthropic animals in this study is not in agreement with the high infestation values observed in dogs and cats in MUE (Urdapilleta et al. in process). In relation to this, *T. penetrans* infestations in humans and domestic animals in northeastern Argentina are lower than those found in other studies carried out in Brazil, where opossum and rodent infestations with *T. penetrans* are greater and more frequent, probably due to climatic and edapohological variables (Harvey et al. 2021).

The research findings have contributed to the understanding of species richness, diversity, and distribution of fleas in the Argentinean Upper Parana Atlantic Forest ecoregion. It is worth noting that fleas in tropical and subtropical regions exhibit lower local diversity compared to those found in arid regions (Lareschi et al. 2004, Linardi & Krasnov 2012, Sanchez & Lareschi 2019). The prevalence of flea infestation in the phytogeographic areas of this study was similar to that found in wetlands of the Rio de La Plata, Argentina, and in Belo Horizonte and Santa Catarina, Brazil, where the

prevalence was less than 25% (Linardi et al. 1991, Lareschi & Krasnov 2010). Concurrently, changes in land use, the proximity of different socio-environmental landscapes, and climate trends may alter species richness and dispersal profiles, posing a risk of changes in pathogen transmission patterns (Friggens & Beier 2010, Clark et al. 2018). Anthropogenic disturbance creates conditions that promote the spread of flea-borne diseases. These conditions include higher flea abundance and prevalence due to increased host utilization.

Acknowledgments

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Author contributions

Data collection was performed by Mara Urdapilleta, Eliana F. Burgos, Daniela Lamattina, and Marcela Lareschi. Data analysis was conducted out by Mara Urdapilleta and Daniela Lamattina. The initial manuscript was drafted by Mara Urdapilleta, and all authors contributed to the final version. The project was supervised by Marcela Lareschi and Oscar D. Salomón, who also assumed directorial responsibilities.