Received: 11 March 2024 | Accepted: 18 June 2024

DOI: 10.1111/mve.12739

SHORT COMMUNICATION

Medical and Veterinary Entomology

Are *Rattus rattus* fleas invasive? Evaluation of flea communities in invasive and native rodents in Chile

Nicol Lizama-Schmeisser[1] | Elaine Serafin de Castro[1] | Mario Espinoza-Carniglia[2] | Yessica Herrera[1] | María Carolina Silva-de La Fuente[3] | Marcela Lareschi[2] | Lucila Moreno[1]

[1]Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Concepción, Chile

[2]Centro de Estudios Parasitológicos y de Vectores CEPAVE (CONICET CCT-La Plata-UNLP), La Plata, Argentina

[3]Departamento de Ciencias Agrarias, Facultad de Ciencias Agrarias y Forestales, Universidad Católica del Maule, Curicó, Chile

Correspondence
Lucila Moreno, Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Barrio Universitario S/N, Casilla 160C Concepción, Chile.
Email: lumoreno@udec.cl

Funding information
National Fund for Scientific and Technological Development, Grant/Award Numbers: 11150875, 1130948, 1170972; Vicerrectoría de Investigación y Desarrollo-Universidad de Concepcion, Grant/Award Numbers: 220.113.099-INV, 2023000985INT

Associate Editor: María Soledad Leonardi

Abstract

Co-invasion, characterized by the simultaneous introduction of hosts and parasites with the latter establishing themselves in native hosts, is a phenomenon of ecological concern. *Rattus rattus*, a notorious invasive species, has driven the extinction and displacement of numerous avian and mammalian species and serves as a key vector for diseases affecting both humans and wildlife. Among the parasites hosted by *R. rattus* are fleas, which exhibit obligate parasitic behaviour, a generalist nature and high prevalence, increasing the likelihood of flea invasion. Simultaneously, invasive species can serve as hosts for native parasites, leading to potential amplification or dilution of parasite populations in the environment. In Chile, *R. rattus* has been present since the 17th century because of the arrival of the Spanish colonizers through the ports and has spread throughout urban, rural and wild Chilean territories. This study aims to evaluate whether co-invasion of native fleas of invasive rats occurs on native rodents in Chile and to determine whether black rats have acquired flea native to Chile during their invasion. For this, we captured 1132 rodents from 26 localities (20° S–53° S). *Rattus rattus* was found coexisting with 11 native rodent species and two species of introduced rodents. Among the native rodents, *Abrothrix olivacea* and *Oligoryzomys longicaudatus* exhibited more extensive sympatry with *R. rattus*. We identified 14 flea species associated with *R. rattus*, of which only three were native to rats: *Xenopsylla cheopis*, *Leptopsylla segnis* and *Nosopsyllus fasciatus*. These three species presented a higher parasite load in black rats compared to native fleas. *Leptopsylla segnis* and *N. fasciatus* were also found associated with native rodent species that cohabit with *R. rattus*. The remaining species associated with *R. rattus* were fleas of native rodents, although they were less abundant compared to those associated with native rodents, except for *Neotyphloceras pardinasi* and *Sphinctopsylla ares*. Although there has been evidence of flea transmission from rats to native species, the prevalence and abundance were relatively low. Therefore, it cannot be definitively concluded that these fleas have established themselves in native rodent populations, and hence, they cannot be classified as invasive fleas. This study underscores *R. rattus*' adaptability to diverse environmental and geographical conditions in Chile, including its capacity to acquire fleas from native rodents. This aspect has critical

implications for public health, potentially facilitating the spread of pathogens across various habitats where these rats are found.

KEYWORDS
Chile, fleas, invasive species, parasites, *Rattus rattus*

INTRODUCTION

Invasive species, originating outside their native range and frequently introduced by human activities, establish self-sustaining populations in new, sometimes distant, environments. These organisms disperse, survive and reproduce in diverse habitats, contributing to alter ecological dynamics (Blackburn et al., 2011; Chinchio et al., 2020). Their global proliferation has led to significant economic and ecological consequences, as they act as predators and disease vectors, impacting native plants and animals, ultimately diminishing biodiversity and modifying trophic networks (Britton, 2013; Pejchar & Mooney, 2009).

Invasion into new habitats may cause changes in the parasite fauna of invaders. These changes range from the loss of parasites from their native habitats due to stochastic events or selection processes to the acquisition of new parasites in the invaded habitats (Lymbery et al., 2014; White et al., 2006). The loss of native parasites provides invaders with a competitive advantage, a phenomenon known as the 'enemy release hypothesis'. Meanwhile, the acquisition of local parasites by invaders can have two outcomes: the invader may not be a suitable host, reducing infection in native species (dilution effect) or it may act as a reservoir, amplifying the parasite and increasing infection in native species (spillback; Hulme, 2014; Poulin et al., 2011; White et al., 2006). Additionally, invasive species can transport parasites outside their natural range (co-introduction), which can then spread to indigenous hosts in the new habitat. This process, known as spillover, can lead to co-invasion and plays a crucial role in the emergence of new diseases (Lymbery et al., 2014; Pettersen et al., 2016; Vancheva et al., 2020).

Co-introduced parasites are more likely to become invaders when closely related native hosts are available in the new environment, allowing for parasite 'jumps' from one host to another (Schatz & Park, 2023; Tompkins & Poulin, 2006). If this situation does not occur, parasites could remain exclusively associated with their original host (Taraschewski, 2006). However, if a co-introduced parasite has low host specificity, is transmitted vertically (from parent to offspring) and has a direct life cycle (no intermediate hosts), it is more probable to successfully colonize native host populations in the new region (Lymbery et al., 2014; Prenter et al., 2004; Smith & Carpenter, 2006; Taraschewski, 2006). In such case, if the invasive host becomes extinct or is eradicated (in the case of being considered a pest or plague species), the co-introduced parasite may remain in the environment (Sayyadzahed et al., 2016). Additionally, the success of parasite establishment will depend on interactions with other parasite species and environmental conditions in the new area of introduction (Taraschewski, 2006). Also, physiological and immunological issues may prevent a parasite from surviving in a new host (Plowright et al., 2017).

Parasitic species with direct life cycles, fewer intermediate hosts, generalist behaviour and high prevalence, but low virulence, in natural hosts, tend to fare better in co-invasion scenarios (MacLeod et al., 2010; Morand et al., 2015; Torchin et al., 2003). Among these, fleas (Siphonaptera) are noteworthy obligate parasites, primarily infesting rodents, exhibiting generalist tendencies towards their hosts and displaying high prevalence with low virulence (Hastriter & Whiting, 2009). The understanding of flea invasion is crucial due to their dual impact: direct effects on the host and their role as reservoirs and transmitters of various parasitic diseases affecting wildlife, domestic animals and even humans, thus posing a significant public health concern (Kim et al., 2010; Luce-Fedrow et al., 2015; Troyo et al., 2016; Wiggers et al., 2005). Within this group of flea species, *Xenopsylla cheopis* stands out as the main vector of plague (caused by *Yersinia pestis*) and murine typhus (caused by *Rickettsia typhi*), among other pathogens (Blanton & Walker, 2017), whose main host is the black rat or *Rattus rattus* (Muridae), a rodent considered one of the main invasive species with the widest global distribution (Aplin et al., 2011).

The black rat has inadvertently introduced and dispersed its associated parasites to new regions worldwide, resulting in a complex web of parasitic relationships (Aplin et al., 2011; Morand et al., 2015). For instance, studies have documented 38% of native rat parasites in introduced populations, while the black rat has also acquired parasitic species from local wildlife (Torchin et al., 2003).

In Chile, the black rat is believed to have arrived with the Spanish conquerors in the 17th century, adapting to diverse urban, rural and wild environments, where it coexists with native rodent species, mainly from the family Cricetidae (Lobos et al., 2005). Notably, in Chile, a wide diversity of flea species has been documented in association with black rats; however, most of these species are derived from native rodents (Moreno et al., 2019). Only three flea species have been identified as native to black rats, and it remains uncertain whether these flea species have dispersed to native rodents (co-invasion), or whether the black rats have been colonized by fleas from native rodents (Landaeta-Aqueveque et al., 2018; Landaeta-Aqueveque et al., 2021; Moreno et al., 2019).

Therefore, the main aim of this study was to evaluate whether co-invasion of native fleas of invasive rats occurs on native rodents in Chile. Additionally, we aimed to determine whether black rats have acquired flea native to Chile during their invasion. Given the absence of native rodent species within the Muridae family in Chile and the fact that co-introduced parasites are more likely to become invaders when closely related native hosts are present in the new environment, we expected to predominantly observe co-introduction rather than co-invasion processes in flea populations.

MATERIALS AND METHODS

One thousand one hundred thirty-two rodents were captured from 26 locations along Chile (Figure 1), between 2016 and 2018. Each locality was classified as cities, rural areas or natural areas depending on the number of inhabitants (INE, 2005): (1) city: an urban entity with more than 5000 inhabitants; (2) rural area: an urban entity with a population ranging from 2001 to 5000 inhabitants or between 1001 and

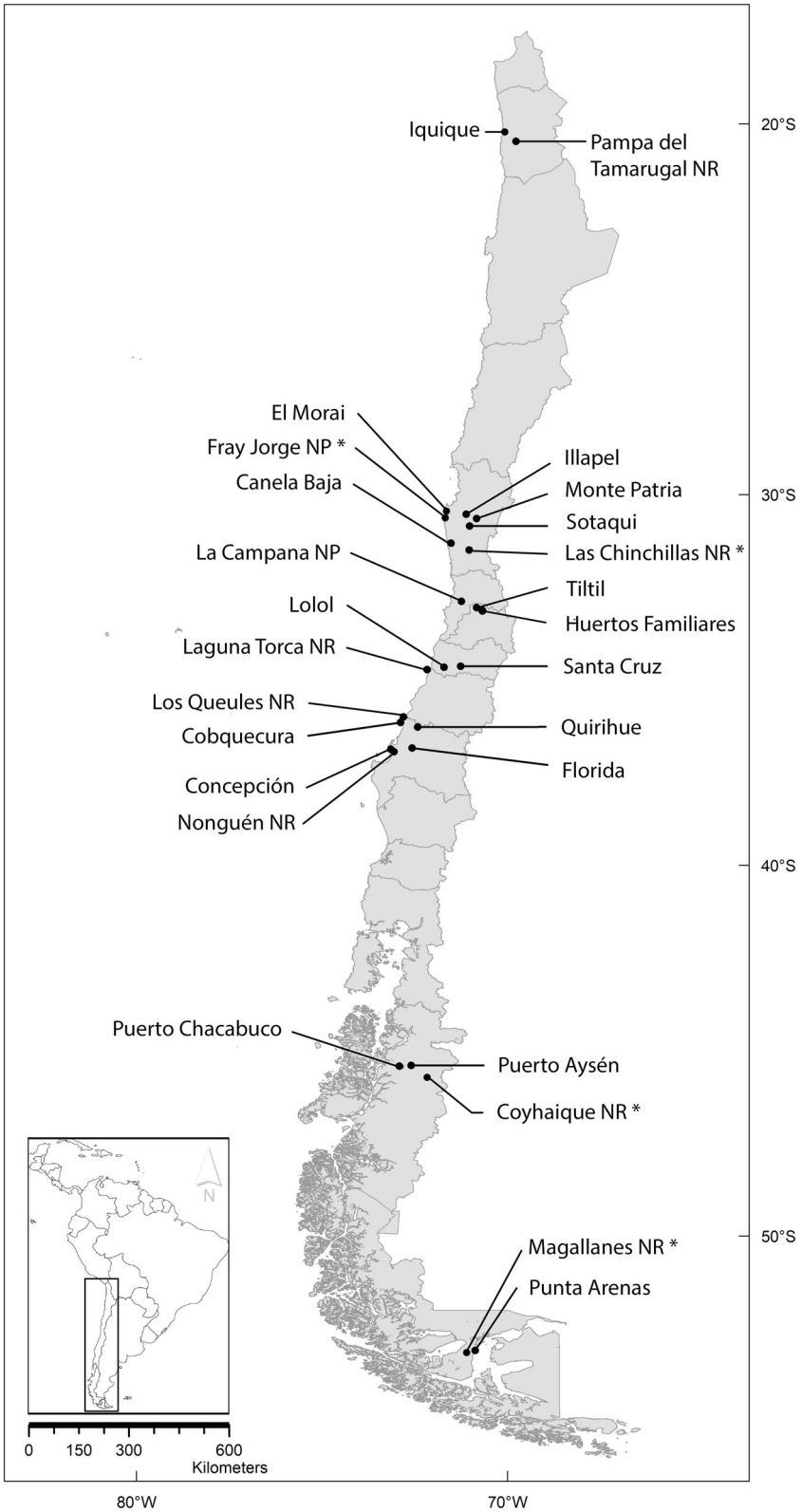

FIGURE 1 Map of sampling localities throughout Chile. The asterisk indicates the localities where no rats were captured.

2000 people and met the economic activity requirement and (3) natural area: a protected area without human settlement.

Each location was sampled for two consecutive nights, using live capture traps (23 × 7.5 × 9 cm, Sherman Co., Tallahassee, USA) and wire-mesh traps (30 × 10 × 11 cm; Forma Ltd., Santiago, Chile), baited with oats and placed in 100 m transects, 5 m apart each. Once captured, the rodents were handled under standard techniques and anesthetized with Ketamine:Xylazine (1:1) to extract the fleas (Mills et al., 1995; Carpenter et al., 2018). After anaesthesia, the rodents' fur was brushed on a white base, collecting the fleas by hand or with forceps, which were individually stored in sterile cryovials with 95% ethanol.

After extracting the fleas from the native rodents, we identified them in accordance with Iriarte's guidelines (Iriarte, 2008). Subsequently, these rodents were tagged and released in the same place of capture. In contrast, black rats were humanely euthanized through cervical dislocation, following the methods described by Mills et al. (1995) and then stored individually in bags with 95% alcohol. It's important to note that all animal handling and capture procedures were conducted with full authorization from the Universidad de Concepción Bioethics Committee, the Servicio Agrícola y Ganadero (SAG R.E: 8968-2015, 1657.2016, 73-2016, 23-2017) and Corporación Nacional Forestal (CONAF No.018-2015).

To identify the fleas, specimens were mounted on glass slides and examined under a microscope. The classification to the species level was carried out using taxonomic keys and descriptions provided by Hopkins and Rothschild (1956, 1962, 1966), Smit (1987), Schramm (1987), Beaucournu et al. (1988), Beaucournu and Gallardo (1988), Beaucournu and Kelt (1990), Beaucournu et al. (2011), Sánchez et al. (2012) and Sánchez and Lareschi (2014).

For each rodent, the total number of collected fleas (abundance) was recorded. The following parasitological descriptors were estimated for each rodent species in each locality: richness (number of parasitic species), prevalence (P%: number of parasitized hosts/number of hosts examined, × 100), mean abundance (MA: number of individuals of a parasitic species/number of hosts examined) and intensity of infection (MI: number of individuals of a parasitic species/number of parasitized hosts) with their respective confidence intervals (95%), following Bush et al. (1997). Confidence intervals (alpha = 0.05) were constructed by Sterne's exact method for prevalences and bootstrap for mean intensities and abundances. The set of individuals captured in the same locality was considered a host community, and statistical analyses were performed in 14 of the 26 communities sampled, where black rats and native rodents were present.

To evaluate the co-invasion, we tested for variation in prevalence (i.e., presence and absence), abundance and intensity of fleas between native and introduced rodents, for each species of fleas introduced and native, using t-bootstrap test and Fisher's exact test. The analyses were performed with the Quantitative Parasitology program (Reiczigel et al., 2019). Co-invasion will be considered if fleas of the invasive rodents are found in the native rodents with prevalence and abundances equal to or greater than those of the invasive rodents. The same will be considered for invasive rodents when they present native fleas. If this is not observed, it will be considered that the fleas are only accidentally present in the host or in an initial invasion process (not yet established).

Selected parasitological indices are commonly used to quantify parasites in hosts, as they help predict the potential rate of parasite spread and provide valuable information regarding variability in host susceptibility. They also serve as indirect indicators of the success of parasites in populations (Jovani & Tella, 2006; Rózsa et al., 2000). Our choice was based on the recommendations by Catford et al. (2012) for quantifying invasion levels.

RESULTS

In the 26 localities analysed, *R. rattus* ($n = 262$), *Abrothrix olivacea* ($n = 407$) and *Abrothrix hirta* ($n = 160$) were the most common species of rodents captured. *Rattus rattus* was found in sympatry with 11 species of native rodents and two species of introduced rodents (*Mus musculus* and *Rattus norvegicus*). Among the native rodents, *A. olivacea* and *Oligoryzomys longicaudatus* were found in 14 and 12 localities in sympatry with *R. rattus*, respectively. In four of the locations sampled, no black rats were captured (Table 1).

A total of 1132 fleas' specimens were examined, of these, 1099 specimens were identified at the species level, identifying a total of 20 species of fleas, while the other 33 specimens could only be recognized up to the genus level (4 genera recognized; Table 1). Between one to eight fleas' species were recorded in each of the localities studied. In three localities, *R. rattus* was not parasitized by fleas. Overall, the species of rodents that registered the highest flea species richness were *A. olivacea* (15 species, range 1–7), *R. rattus* (14 species, range 1–4) and *A. hirta* (11 species, range 2–8; Table 1).

In *R. rattus*, of the 14 species of fleas, only 3 correspond to species commonly found in rats: *Leptopsylla segnis* (range P%: 2.6–78, MA: 0.0–2.2, MI: 1.0–2.8), *Nosopsyllus fasciatus* (range P%: 2.6–64.7, MA: 0.1–1.3, MI: 1–5) and *X. cheopis* (P%: 50%, MA: 5.5, MI: 11), which were collected from eight, eight and one locality, respectively.

Leptopsylla segnis was found in two native rodents in only two of the eight localities (*O. longicaudatus* from Canela Baja and *Phyllotis darwini* from Las Chinchillas National Reserve), *N. fasciatus* in three species of rodent native (*A. olivacea*, *A. hirta* and *O. longicaudatus*) from four of the eight localities (Cobquecura, Concepción, Puerto Aysén and Puerto Chacabuco) and *X. cheopis* was not found in native rodents. In these localities the prevalences, abundances and mean intensities were low. Six *L. segnis* were found in one specimen of *O. longicaudatus* (P%: 14.3, MA: 0.9, MI: 6) in Canela Baja, and one *L. segnis* was found in one *P. darwini* in Las Chinchillas National Reserve (P%: 1.9; MA: 0.0, MI: 1), the latter was the only locality where a black rat flea was recorded without having captured any rat specimens. *Nosopsyllus fasciatus* was found in two *A. olivacea* in Cobquecura (P%: 5.3, MA: 0.1, MI: 1) and Puerto Aysén (P%: 5.3, MA: 0.1, MI: 1), one *A. hirta* from Concepción (P%: 25, MA: 0.3, MI: 1) and one *O. longicaudatus* from Puerto Chacabuco (P%: 16.7, MI: 0.2, MI: 1). No significant

TABLE 1 Number of rodents and fleas collected, prevalence (%) of infection, mean intensities and mean abundances of infections at each locality and host.

Localities (type)	Rodent species	Number of rodents analysed	Number of fleas analysed	Prevalence (CI 95%)	Mean intensity (CI 95%)	Mean abundance (CI 95%)	Species of fleas (number of fleas collected)
Iquique (C)	*Rattus norvegicus*	1	0	—	—	—	
	Rattus rattus	2	11	50.0 (25.4–97.5)	11.0 (0.0–0.0)	5.5 (0.0–5.5)	*Xenopsylla cheopis* (11)
Pampa del Tamarugal N.R. (N)	*Phyllotis* spp.	2	0	—	—	—	—
	Rattus rattus	10	0	—	—	—	—
Fray Jorge N.P. (N)	*Abrothrix longipilis*	2	6	100.0 (22.4–100)	3.0 (2.0–3.0)	3.0 (2.0–3.0)	*Hectopsylla* sp. (5) and *Tetrapsyllus corfidi* (1)
	Abrothrix olivacea	71	60	60.6 (49.0–71.2)	2.3 (1.9–2.7)	1.4 (1.1–1.8)	*Ectinorus cocyti* (1), *Hectopsylla* sp. (7), *Neotyphloceras chilensis* (6), *Neotyphloceras crassispina* (2), *Neotyploceras pardinasi* (2), *Sphictopsylla ares* (2) and *Tetrapsyllus tantillus* (40)
	Phyllotis darwini	41	22	31.7 (19.3–47.5)	2.4 (1.6–3.2)	0.8 (0.4–1.3)	*Hectopsylla* sp. (13), *Delostichus phyllotis* (1), *Neotyphloceras chilensis* (3), *Sphinctopsylla ares* (2) and *Tetrapsyllus tantillus* (3)
El Morai (R)	*Abrothrix olivacea*	5	4	60.0 (18.9–92.3)	1.3 (1.0–1.7)	0.8 (0.0–1.4)	*Neotyphloceras crassispina* (2) and *Tetrapsyllus tantillus* (2)
	Octodon degus	1	5	100 (5–100)	5.0 (0.0–0.0)	5.0 (0.0–0.0)	*Delostichus coxalis* (2) and *Delostichus smiti* (3)
	Phyllotis darwini	5	3	40.0 (7.6–81)	2.0 (1.0–2.0)	0.8 (0.0–2.0)	*Neotyphloceras crassispina* (1), *Neotyphloceras chilensis* (1) and *Tetrapsyllus tantillus* (1)
	Rattus rattus	8	4	25.0 (4.6–63.5)	2.0 (1.0–2.0)	0.5 (0.0–1.5)	*Leptopsylla segnis* (3) and *Neotyphloceras pardinasi* (1)
Monte Patria (C)	*Octodon degus*	9	28	100 (67–100)	8.1 (4.2–16.8)	8.1 (4.2–17.3)	*Delostichus degus* (16), *Delostichus smiti* (10) and *Tetrpsyllus corfidii* (2)
	Oligoryzomys longicaudatus	4	0	—	—	—	—
	Phyllotis darwini	6	1	16.7 (0.0–58)	1.0 (0.0–0.0)	0.2 (0.0–0.3)	*Tunga* sp. (1)
	Rattus rattus	13	7	38.5 (16.0–65.0)	1.4 (1.0–1.6)	0.54 (0.15–0.9)	*Leptopsylla segnis* (6) and *Nosospsullus fasciatus* (1)
Sotaquí (R)	*Mus musculus*	5	2	40.0 (7.6–81)	1.0 (0.0–0.0)	0.4 (0.0–0.6)	*Leptopsylla segnis* (2)
	Oligoryzomys longicaudatus	1	0	—	—	—	—
	Rattus rattus	14	40	85.7 (57.4–97.4)	5.25 (3.3–8.2)	4.5 (2.6–7.1)	*Leptopsylla segnis* (31), *Nosopsyllus fasciatus* (6) and *Hectopsylla* sp. (3)
Canela Baja (R)	*Abrothrix olivacea*	31	59	77.4 (59.8–89.2)	3.3 (2.5–4.1)	2.5 (1.7–3.4)	*Hectopsylla* sp. (1), *Neotyphloceras chilensis* (40), *Neotyphloceras crassispina* (1), *Sphinctopsylla ares* (1), *Tetrapsyllus amplus* (6) and *Tetrapsyllus tantillus* (10)
	Octodon degus	12	36	66.7 (37–87.7)	16 (10.7–28.4)	10.7 (5.7–20.3)	*Delostichus coxalis* (1), *Delostichus degus* (3), *Delostichus phyllotis* (1), *Delostichus smiti* (28) and *Neotyphloceras chilensis* (3)

(Continues)

TABLE 1 (Continued)

Localities (type)	Rodent species	Number of rodents analysed	Number of fleas analysed	Prevalence (CI 95%)	Mean intensity (CI 95%)	Mean abundance (CI 95%)	Species of fleas (number of fleas collected)
	Oligoryzomys longicaudatus	7	20	71.4 (34.1–96.6)	3.2 (1.8–4.6)	2.3 (0.9–3.9)	*Leptopsylla segnis* (6), *Neotyphloceras chilensis* (11), *Neotyphloceras pardinasi* (1), *Ectinorus chilensis* (1) and *Tetrapsyllus amplus* (1)
	Phyllotis darwini	8	15	75.0 (36.5–95.4)	2.8 (1.8–3.7)	2.1 (0.9–3.1)	*Delostichus smiti* (1), *Hectopsylla* sp. (1), *Neotyphloceras chilensis* (11), *Sphinctopsylla ares* (1) and *Tetrapsyllus tantillus* (1)
	Rattus rattus	6	7	50.0 (15.3–84.6)	3.7 (1.8–3.7)	1.8 (0.3–4.5)	*Leptopsylla segnis* (5) and *Neotyphloceras chilensis* (2)
Illapel (C)	*Oligoryzomys longicaudatus*	1	0	–	–	–	–
	Rattus rattus	17	35	76.5 (51.1–91.5)	3.4 (2.2–5.0)	2.6 (1.5–4.1)	*Leptopsylla segnis* (13), *Nosopsyllus fasciatus* (21) and *Delostichus smiti* (1)
Las Chinchillas N.R. (N)	*Abrothrix longipilis*	1	2	100 (5.0–100)	2.0 (0.0–0.0)	2.0 (0.0–0.0)	*Neotyphloceras chilensis* (2)
	Abrothrix olivacea	25	59	76.0 (56.1–89.1)	3.2 (2.4–3.8)	2.4 (1.6–3.2)	*Neotyphloceras chilensis* (38), *Neotyphloceras pardinasi* (3), *Tetrapsyllus amplus* (6), *Tetrappsyllus corfidii* (1) and *Tetrapsyllus tantillus* (11)
	Octodon degus	31	72	74.2 (56.5–87.4)	3.2 (2.5–3.7)	2.4 (1.7–3.0)	*Delostichus coxalis* (7), *Delostichus degus* (5), *Delostichus phyllotis* (1), *Delostichus smiti* (41), *Ectinorus chilensis* (8), *Notyphloceras chilensis* (4) and *Tetrapsyllus corfidii* (6)
	Phyllotis darwini	52	63	46.2 (32.6–59.7)	2.9 (2.3–3.5)	1.3 (0.9–1.8)	*Leptopsylla segnis* (1), *Neotyphloceras chilensis* (55), *Neotyphloceras crassispina* (2), *Sphinctopsylla ares* (2) and *Tetrapsyllus tantillus* (3)
Tiltil (C)	*Abrothrix olivacea*	2	1	50.0 (2.5–97.5)	1.0 (0.0–0.0)	0.5 (0.0–0.5)	*Tetrapsyllus tantillus* (1)
	Octodon degus	4	9	100 (47.0–100)	4.5 (1.0–7.0)	4.5 (1.0–7.0)	*Delostichus coxalis* (8) and *Delostichus phyllotis* (1)
	Phyllotis darwini	1	0	–	–	–	–
	Rattus rattus	3	1	33.0 (1.7–86)	1.0 (0.0–0.0)	0.3 (0.0–0.7)	*Plocopsylla* sp. (1)
La Campana N.P. (N)	*Abrocoma bennetti*	1	5	100 (5.0–100)	5.0 (0.0–0.0)	5.0 (0.0–0.0)	*Delostichus phyllotis* (1), *Delostichus coxalis* (2), *Ectinorus chilensis* (1) and *Tertrapsyllus corfidii* (1)
	Octodon degus	11	37	90.9 (59.5–99.5)	3.7 (2.8–4.3)	3.4 (2.2–4.1)	*Delostichus coxalis* (29), *Delostichus phyllotis* (1), *Delostichus smiti* (1), *Ectinorus chilensis* (1), *Tetrapsyllus corfidii* (3) and *Tetrapsyllus tantillus* (2)
	Rattus rattus	22	17	22.7 (9.4–45.3)	2.6 (1.0–3.0)	0.6 (0.2–1.1)	*Delostichus coxalis* (9), *Neotyphloceras pardinasi* (6) and *Tetrapsyllus rhombus* (2)
Huertos Familiares (R)	*Mus musculus*	2	0	–	–	–	–
	Rattus rattus	2	0	–	–	–	–
Santa Cruz (C)	*Abrothrix olivacea*	4	0	–	–	–	–
	Mus musculus	2	0	–	–	–	–

TABLE 1 (Continued)

Localities (type)	Rodent species	Number of rodents analysed	Number of fleas analysed	Prevalence (CI 95%)	Mean intensity (CI 95%)	Mean abundance (CI 95%)	Species of fleas (number of fleas collected)
	Rattus norvegicus	1	0	–	–	–	–
	Rattus rattus	5	1	20.0 (1.0–65.7)	1.0 (0.0–0.0)	0.2 (0.0–0.4)	*Nosopsyllus fasciatus* (1)
Lolol (R)	*Abrothrix olivacea*	5	8	20.0 (1–65.7)	8.0 (0.0–0.0)	1.6 (0.0–3.2)	*Tetrapsyllus tantillus* (8)
	Rattus rattus	1	4	100 (5.0–100)	4.0 (0.0–0.0)	4.0 (0.0–0.0)	*Leptopsylla segnis* (2) and *Nosospsyllus fasciatus* (2)
Laguna Torca N.R. (N)	*Abrothrix longipilis*	2	1	50.0 (2.5–97.4)	1.0 (0.0–0.0)	0.5 (0.0–0.5)	*Neotyphloceras* sp. (1)
	Abrothrix olivacea	2	3	100 (22.3–100)	4.0 (0.0–0.0)	4.0 (0.0–0.0)	*Sphinctopsylla ares* (2) and *Tetrapsyllus amplus* (1)
	Oligoryzomys longicaudatus	2	7	50.0 (2.5–97.4)	3.0 (0.0–0.0)	1.5 (0.0–1.5)	*Sphinctopsylla ares* (6) and *Neotyphloceras crassispina* (1)
	Rattus rattus	2	5	50.0 (2.5–97.4)	5.0 (0.0–0.0)	2.5 (0.0–2.5)	*Sphinctopsylla ares* (2) and *Neotyphloceras pardinasi* (3)
Los Queules N.R. (N)	*Abrothrix hirta*	80	56	28.7 (19.8–39.9)	2.1 (1.52–2.7)	0.6 (0.36–0.9)	*Chiliopsylla allophyla* (1), *Ctenoparia inopinata* (9), *Ctenoparia jordani* (1), *Neotyphloceras crassispina* (4), *Neotyphloceras pardinasi* (6), *Sphinctopsylla ares* (24), *Tetrapsyllus rhombus* (9) and *Tetrapsyllus tantillus* (2)
	Abrothrix olivacea	77	38	35.1 (25.2–46.7)	2.2 (1.6–2.7)	0.8 (0.5–1.1)	*Ctenoparia inopinata* (6), *Ctenoparia jordani* (1), *Neotyphloceras crassispina* (6), *Sphinctopsylla ares* (12), *Tetrapsyllus rhombus* (9) and *Tetrapsyllus tantillus* (4)
	Irenomys tarsalis	1	0	–	–	–	–
	Oligoryzomys longicaudatus	20	0	–	–	–	–
	Phyllotis darwini	4	0	–	–	–	–
	Rattus rattus	4	0	–	–	–	–
Cobquecura (R)	*Abrothrix hirta*	18	32	66.7 (41.4–84.3)	2.9 (1.8–5.3)	1.9 (1.1–3.7)	*Ctenoparia inopinata* (4), *Neotyphloceras crassispina* (6), *Neotyphloceras pardinasi* (4), *Sphinctopsylla ares* (10), *Tetrapsyllus amplus* (2) and *Tetrapsyllus rhombus* (6)
	Abrothrix olivacea	19	39	52.6 (31.0–74.3)	2.7 (1.6–4.4)	1.4 (0.7–2.6)	*Ctenoparia inopinata* (6), *Neotyphloceras crassispina* (1), *Neotyphloceras pardinasi* (1), *Nosopsyllus fasciatus* (1), *Sphinctopsylla ares* (11), *Tetrapsyllus rhombus* (6) and *Tetrapsyllus tantillus* (13)
	Octodon bridgesi	1	1	100 (5.0–100)	1.0 (0.0–0.0)	1.0 (0.0–0.0)	*Delostichus phyllotis* (1)
	Oligoryzomys longicaudatus	5	10	100 (5.0–100)	2.2 (1.0–2.6)	2.2 (1.0–2.6)	*Neotyphloceras crassispina* (4) and *Sphinctopsylla ares* (6)
	Phyllotis darwini	1	2	100 (5.0–100)	1.0 (0.0–0.0)	1.0 (0.0–0.0)	*Tetrapsyllus rhombus* (1) and *Ctenoparia inopinata* (1)

(Continues)

TABLE 1 (Continued)

Localities (type)	Rodent species	Number of rodents analysed	Number of fleas analysed	Prevalence (CI 95%)	Mean intensity (CI 95%)	Mean abundance (CI 95%)	Species of fleas (number of fleas collected)
	Rattus rattus	1	2	100 (5.0–100)	1.0 (0.0–0.0)	1.0 (0.0–0.0)	*Neotyphloceras pardinasi* (1) and *Sphinctopsylla ares* (1)
Quirihue (C)	*Abrothrix olivacea*	2	0	—	—	—	—
	Oligoryzomys longicaudatus	6	0	—	—	—	—
	Rattus rattus	77	14	13.0 (6.9–22.5)	1.6 (1.1–2.4)	0.2 (0.1–0.4)	*Leptopsylla segnis* (2), *Nosopsyllus fasciatus* (2), *Neotyphloceras pardinasi* (9) and *Sphinctopsylla ares* (1)
Concepción (C)	*Abrothrix hirta*	4	13	100 (47.3–100)	4.3 (2.5–5.8)	4.3 (2.3–6.0)	*Chiliopsylla allophyla* (1), *Ctenoparia inopinata* (7), *Neotyphloceras pardinasi* (1), *Nosopsyllus fasciatus* (1), *Tetrapsyllus amplus* (1) and *Tetrapsyllus rhombus* (2)
	Abrothrix olivacea	31	8	25.8 (12.6–43.4)	1.0 (0.0–0.0)	0.3 (0.1–0.4)	*Ctenoparia inopinata* (4), *Neotyphloceras crassispina* (1) and *Sphinctopsylla ares* (3)
	Oligoryzomys longicaudatus	4	1	25.0 (1.3–75.8)	1.0 (0.0–0.0)	0.3 (0.0–0.5)	*Sphinctopsylla ares* (1)
	Rattus rattus	22	8	22.7 (9.4–45.3)	1.6 (1.0–2.2)	0.4 (0.1–0.8)	*Nosopsylla fasciatus* (5), *Sphinctopsylla ares* (1), *Neotyphloceras pardinasi* (1) and *Ctenoparia inopinata* (1)
Florida (R)	*Abrothrix hirta*	1	0	—	—	—	—
	Abrothrix olivacea	1	0	—	—	—	—
	Oligoryzomys longicaudatus	2	1	50.0 (2.5–97.4)	1.0 (0.0–0.0)	0.5 (0.0–0.5)	*Neotyphloceras* sp. (1)
	Phyllotis darwini	1	7	100 (5.0–100)	7.0 (0.0–0.0)	7.0 (0.0–0.0)	*Sphinctopsylla ares* (7)
	Rattus rattus	18	6	22.2 (7.9–47.1)	1.5 (1.0–2.0)	0.33 (0.1–0.8)	*Nosopsyllus fasciatus* (5) and *Plocopsylla wolffsohni* (1)
Nonguén N.R. (N)	*Abrothrix hirta*	40	59	60.0 (43.7–73.9)	2.3 (1.8–2.9)	1.4 (1.0–1.9)	*Chiliopsylla allophyla* (4), *Ctenoparia inopinata* (18), *Ctenoparia jordani* (1), *Ctenoparia topali* (1), *Neotyphloceras crassispina* (1), *Neotyphloceras pardinasi* (2), *Sphinctopsylla ares* (22) and *Tetrapsyllus rhombus* (10)
	Abrothrix olivacea	12	13	50.0 (24.2–75.7)	2.0 (1.0–3.8)	1.0 (0.3–2.4)	*Tetrapsyllus rhombus* (1), *Sphinctopsylla ares* (8), and *Ctenoparia inopinata* (4)
	Oligoryzomys longicaudatus	16	24	31.3 (13.2–56.4)	2.0 (1.2–2.4)	0.6 (1.0–1.1)	*Tetrapsyllus rhombus* (1) and *Sphinctopsylla ares* (23)
	Rattus rattus	25	9	48.0 (29.6–68.3)	1.7 (1.2–2.6)	0.8 (0.4–1.4)	*Tetrapsyllus rhombus* (1), *Ctenoparia jordani* (1), *Sphinctopsylla ares* (6) and *Plocopsylla wolffsohni* (1)
Puerto Aysén (C)	*Abrothrix hirta*	2	8	100 (22.0–100)	4.5 (4.0–4.5)	4.5 (4–4.5)	*Ctenoparia inopinata* (7) and *Sphinctopsylla ares* (1)

TABLE 1 (Continued)

Localities (type)	Rodent species	Number of rodents analysed	Number of fleas analysed	Prevalence (CI 95%)	Mean intensity (CI 95%)	Mean abundance (CI 95%)	Species of fleas (number of fleas collected)
	Abrothrix olivacea	19	8	42 (22.0–65.0)	1.1 (1.0–1.4)	0.5 (0.2–0.7)	*Nosopsylla fasciatus* (1), *Ctenoparia inopinata* (4), *Sphinctopsylla ares* (2) and *Tetrapsyllus amplus* (1)
	Rattus rattus	4	5	25.0 (1.2–75.0)	5.0 (0.0–0.0)	1.3 (0.0–2.5)	*Nosopsylla fasciatus* (5)
Puerto Chacabuco (R)	*Abrothrix lanosus*	1	1	100 (5–100)	1.0 (0.0–0.0)	1.0 (0.0–0.0)	*Sphinctopsylla ares* (1)
	Abrothrix olivacea	18	23	50.0 (27.1–72.8)	3.7 (2.3–5.6)	1.8 (0.9–3.2)	*Ctenoparia inopinata* (12), *Sphinctopsylla ares* (6) and *Tetrapsyllus rhombus* (5)
	Oligoryzomys longicaudatus	6	3	50.0 (15.3–84.6)	1.67 (1.0–2.3)	0.83 (0.2–1.8)	*Nosopsyllus fasciatus* (1) and *Sphinctopsylla ares* (2)
	Rattus rattus	3	2	100 (5.0–100)	2.0 (0.0–0.0)	2.0 (0.0–0.0)	*Sphinctopsylla ares* (1) and *Neotyphloceras pardinasi* (1)
Coyhaique N.R. (N)	*Oligoryzomys longicaudatus*	1	1	100 (0.0–1.0)	1.0 (0.0–0.0)	1.0 (0.0–0.0)	*Sphinctopsylla ares* (1)
	Loxodontomys micropus	1	1	100 (0.0–1.0)	4.0 (0.0–0.0)	4.0 (0.0–0.0)	*Sphinctopsylla ares* (1)
	Abrothrix olivacea	15	30	73.3 (46.6–90.3)	2.5 (1.7–3.2)	1.8 (1.1–2.5)	*Sphinctopsylla ares* (19), *Tetrapsyllus rhombus* (4), *Ctenoparia inopinata* (5) and *Neotyphloceras crassispina* (2)
	Abrothrix hirta	15	16	66.7 (39.7–86.0)	1.7 (1.2–2.4)	1.1 (0.6–1.8)	*Sphinctopsylla ares* (8), *Tetrapsyllus rhombus* (5), *Ctenoparia inopinata* (2) and *Neotyphloceras crassispina* (1)
Punta Arenas (C)	*Abrothrix olivacea*	2	0	–	–	–	–
	Mus musculus	1	0	–	–	–	–
	Rattus rattus	5	1	20.0 (1.0–65.7)	1.0 (0.0–0.0)	0.2 (0.0–0.4)	*Sphinctopsylla ares* (1)
Magallanes N.R. (N)	*Abrothrix olivacea*	66	9	25.8 (16.5–37.9)	1.5 (1.1–1.9)	0.4 (0.2–0.6)	*Sphinctopsylla ares* (2), *Plocopsylla lewisi* (1), *Tetrapsyllus rhombus* (4) and *Agastopsylla boxi* (2)
	Oligoryzomys longicaudatus	15	2	40.0 (19.1–66.8)	1.2 (1.0–1.3)	0.5 (0.1–0.7)	*Sphinctopsylla ares* (1) and *Agastopsylla boxi* (1)

Note: Confidence intervals (alpha = 0.05) were constructed using Sterne's exact method for prevalences and bootstrap methods for mean intensities and abundances. The type of locality is indicated in parentheses next to the locality name.
Abbreviations: C, cit; N, natural area; R, rural area.

TABLE 2 Results of statistical analyses (Fisher's exact test, bootstrap *t*-test and diversity *t*-test) for comparing prevalences, mean intensity, mean abundance and diversity between *Rattus rattus* and native rodents.

	Cricetidae						Octodontidae		Abrocomidae
Localities	**A.o.**	**A.h.**	**A.l.**	**A.la.**	**O.l.**	**P.d.**	**O.d.**	**O.b.**	**A.b.**
p-values for Fisher's test (2-sided) for comparing prevalence									
El Morai	0.293	–	–	–	–	1	0.333	–	–
Monte Patria	–	–	–	–	–	–	**0.006**	–	–
Canela Baja	0.313	–.	–	–	0.592	0.580	0.627	–	–
Tiltil	1	–	–	–	–	–	0.143	–	–
La Campana N.P.	–		–	–	–	–	**<0.000**	–	0.261
Lolol	0.333	–	–	–	–	–	–	–	–
Laguna Torca N.R.	1	–	1	–	1	–	–	–	–
Los Queules N.R.	0.296	0.571	–	–	–	–	–	–	–
Cobquecura	1	1	–	–	1	1	–	1	–
Florida	–	–	–	–	–	0.263	–	–	–
Concepción	1	**0.008**	–	–	1	–	–	–	–
Nonguén N.R.	1	0.443	–	–	0.344	–	–	–	–
Puerto Aysén	1	0.400	–	–	–	–	–	–	–
Puerto Chacabuco	1	–	–	1	1	–	–	–	–
p-values for bootstrap *t*-test for comparing mean intensities									
El Morai	0.500	–	–	–	–	1	1	–	–
Monte Patria	–	–	–	–	–	–	0.219	–	–
Canela Baja	0.791	–	–	–	0.790	0.647	0.075	–	–
Tiltil	1	–	–	–	–	–	1	–	–
La Campana N.P.	–		–	–	–	–	0.089	–	1
Lolol	1	–	–	–	–	–	–	–	–
Laguna Torca N.R.	1	–	1	–	1	–	–	–	–
Los Queules N.R.	1	1	–	–	–	–	–	–	–
Cobquecura	1	1	–	–	1	1	–	1	–
Florida	–	–	–	–	–	1	–	–	–
Nonguén N.R.	0.704	0.158	–	–	0.499	–	–	–	–
Concepción	0.275	0.099	–	–	1	–	–	–	–
Puerto Aysén	1	1	–	–	–	–	–	–	–
Puerto Chacabuco	1	–	–	1	1	–	–	–	–
p-values for bootstrap *t*-test for comparing mean abundances									
El Morai	0.585	–	–	–	–	0.698	1	–	–
Monte Patria	–	–	–	–	–	–	0.166	–	–
Canela Baja	0.581	–.	–	–	0.763	0.822	0.063	–	–
Tiltil	0.782	–	–	–	–	–	0.127	–	–
La Campana N.P.	–		–	–	–	–	**0.001**	–	1
Lolol	1	–	–	–	–	–	–	–	–
Laguna Torca N.R.	0.51	–	0.488	–	0.758	–	–	–	–
Los Queules N.R.	**<0.000**	**0.002**	–	–	–	–	–	–	–
Cobquecura	1	1	–	–	1	1	–	1	–
Florida	–	–	–	–	–		–	–	–
Concepción	0.594	0.086	–	–	0.718	–	–	–	–
Nonguén NR	0.727	0.073	–	–	0.639	–	–	–	–
Puerto Aysén	0.563	0.339	–	–	–	–	–	–	–

TABLE 2 (Continued)

Localities	Cricetidae						Octodontidae		Abrocomidae
	A.o.	A.h.	A.l.	A.la.	O.l.	P.d.	O.d.	O.b.	A.b.
Puerto Chacabuco	1	—	—	1	1	—	—	—	—
p-values for diversity *t*-test for comparing Shannon's index									
El Morai	0.550					0.622	0.685	—	—
Monte Patria	-	—	—	—		0.230	**0.023**	—	—
Canela Baja	**0.003**	—	—	—	0.410	0.219	**0.022**	—	—
Tiltil		—	—	—	—		**0.004**	—	—
La Campana N.P.	-	—	—	—	—	—	0.490	—	0.936
Lolol	**0.032**	—	—	—	—	—	—	—	—
Laguna Torca N.R.	0.505	—	—	**0.019**	0.407	—	—	—	—
Los Queules N.R.	-	—	—	—	-	—	—	—	—
Cobquecura	0.067	0.059	—	—	0.666	1	—	1	—
Florida			—	—	0.225	0.225	—	—	—
Concepción	0.923	0.161	—	—	**0.023**	—	—	—	—
Nonguén N.R.	0.320	0.055	—	—	**0.005**	—	—	—	—
Puerto Aysén	**0.004**	0.237	—	—	—	—	—	—	—
Puerto Chacabuco	0.170	—	0.337	—	0.389	—	—	—	—

Note: Bold values of $p < 0.05$ was considered statistically signicant.

differences were found in the prevalence, MA and mean intensity of *L. segnis* and *N. fasciatus* between black rats and native rodents. In Puerto Chacabuco and Cobquecura, black rats were only infected with native fleas.

Of the 11 native flea species recorded on black rats, the most frequently collected species were: *Neotyphloceras pardinasi* and *Sphinctopsylla ares*, from eight and seven localities, respectively, but with less abundance concerning native rodents. *Delostichus coxalis* collected at the La Campana National Reserve on *Octodon degus* (P%: 72.7, MI: 3.6, MA: 2.6) and *Abrocoma benneti* (P%: 100, MI: 1, MA: 2) presented significantly higher values of prevalence (*p*-value: 0.01), abundance (*p*-value: 0.01) and mean intensity (*p*-value: 0.03) than the black rat (P%: 27.3, MI: 1.5, MA: 0.4). In the Nonguén National Reserve, *Tetrapsyllus rhombus* collected on *A. hirta* alone had a significantly higher MA (P%: 20, MI: 1.3, MA: 0.3) than black rat (P%: 4, MI: 1, MA: 0.0, *p*-value: 0.04; Table 2).

DISCUSSION

Of the 14 flea species found on *R. rattus*, 3 were typical rat fleas (*L. segnis*, *N. fasciatus* and *X. cheopis*). These species were also found in native rodents, but at consistently low intensities and prevalences. Similar patterns were observed with native rodent fleas collected from *R. rattus*, which were found in low intensity and prevalence. No significant differences were found in the prevalence, MA and mean intensity of fleas between black rats and native rodents in most of the localities studied. In addition, it was observed that black rats were only infected with native fleas in some localities, while in others, they were not parasitized by fleas. Therefore, it cannot be concluded that there is co-invasion of fleas associated with black rats on native rodents based on the results provided in the study. This finding could indicate that fleas are not actively colonizing native rodents after being introduced by black rats. However, it is important to consider some limitations in the interpretation of these results.

The absence of fleas in some locations where black rats were found could be due to environmental, seasonal or sampling factors, which may influence the presence of fleas. Our sampling covered a climatic gradient, from absolute desert to hyper-humid regions. These environmental differences could be affecting the establishment of fleas in some extreme areas of Chile. For example, *X. cheopis* was only collected from a city located in the extreme north of Chile characterized by an arid environment without large seasonal fluctuations (between 25 and 26°C), with hot summers and dry winters. *Xenopsylla cheopis* is distributed in tropical and subtropical zones throughout the world; therefore, the climatic conditions present in the rest of the country would limit the establishment of this flea (Boyer et al., 2022). In contrast, *N. fasciatus* and *L. segnis* are found especially in temperate and northern regions of the world (Durden & Hinkle, 2019), which resemble the environmental conditions of Chile. In our study, *N. fasciatus* had the widest distribution of the three native rat species, ranging from the arid zone (Montepatria) to the per-humid zone (Puerto Aysén and Puerto Chacabuco). Meanwhile, the distribution of *L. segnis* was more limited, ranging from the Arid zone (El Morai) to the sub-humid zone (Quirihue). Although it has been described that *L. segnis* and *N. fasciatus* show stable seasonal abundance and prevalence, which could be explained by the lower population fluctuations suffered by rodent populations inhabiting

urban versus rural areas (Nakazawa et al., 1957), seasonal differences in the prevalence of both species have also been observed (Fitzgerald et al., 2023; Yeh & Davis, 1950).

Flea behaviour could also have influenced the sampling. For example, *L. segnis* remains attached to the host hair for long periods of time, whereas *N. fasciatus* and *X. cheopis* leave the host after feeding, spending more time in rodent burrows (Boyer et al., 2022; Fitzgerald et al., 2023; Krampitz 1980). These differences in behaviour could bias flea collection, however, it has been observed that the mean number of fleas collected from the host body correlates positively with the number of fleas in the host nests/burrows, so abundance would be a good indicator of population size (Krasnov, Mouillot, et al., 2004).

The finding of native fleas on black rats indicates the close contact that this invader has with native rodents, but the low intensity and prevalence found indicate that there is a certain degree of specificity (the number of host species used or their taxonomic diversity) among the fleas that parasitize these rodents (Poulin et al., 2006). This study suggests a 'filter effect' regulating different phases of invasion (Drake, 2003). One such filter is phylogenetic proximity, which indicates that success in colonizing new hosts is related to phylogenetic proximity between the original host and new hosts, as well as co-occurrence and compatibility with other hosts in the same habitat (Krasnov et al., 2012; Krasnov et al., 2014). Most of the host species used by a flea usually belong to the same taxon (e.g., the same subfamily of rodents), and only rarely one or two host species belong to other taxa (e.g., another order, such as insectivores; Poulin et al., 2006). Therefore, the success of fleas on a new host is low on hosts that are newly colonized and have no proximity to the original host (Krasnov, Shenbrot, et al., 2004). In this study, rodent species sympatric with *R. rattus* often belonged to the family Cricetidae, subfamily Sigmodontinae, rodents exclusive to the Neotropics, which has an evolutionary history distinct from Muridae (Steppan & Schenk, 2017).

In addition to this phylogenetic distance, the colonization success of rodent fleas is influenced by various factors, for example, biotic factors related to physiology of parasites and/or hosts (e.g., immune response of rodents, nutrition requirements of fleas) and abiotic factors (e.g., humidity, temperature; Krasnov et al., 2022). The biological and ecological differences between sigmodontine rodents and Muridae contribute to a certain degree of specificity in flea–host relationships. Among the species most frequently found in *R. rattus* sympatry are *A. olivacea*, *A. hirta*, *P. darwini* and *O. longicaudatus*, all species widely distributed in Chile, also corresponding to species where rat flea specimens were found. For instance, considering the habitat use of some of the rodent species mentioned, such as *A. olivacea*, which is characterized by making caves with simple nests for reproduction, prefers subway caves for shelter and makes galleries between bushes. While *O. longicaudatus* has arboreal tendencies, building arboreal nests or using abandoned bird nests. In turn, *R. rattus* is an arboreal species, so direct interaction through fights or the use of the same nests could lead to the exchange of fleas. Moreover, *O. longicaudatus* was the only native species in which the two species of rat fleas were isolated: *L. segnis* and *N. fasciatus*.

The adaptation of fleas to different habitats, especially at the larval stage, is another potential explanation. *Rattus rattus* is usually more associated with human urban habitats, where human waste is present, providing food and shelter for rodents and fleas. In contrast, fleas of native rodents may be more adapted to natural habitats. Flea larvae need to feed on organic matter in the environment, and organic matter from urban sites is different from organic waste from rural or natural areas. Different feeding stage requirements of flea larvae may be a factor in their selection (Khokhlova et al., 2012; Yin et al., 2020). This habitat-specific adaptation could constrain host-switching by parasites, leading to high levels of host specificity (Poulin et al., 2006).

Co-introduced parasites may face competition from native parasites that are better adapted to the local host species and environmental conditions. This competition can result in the exclusion of the co-introduced parasites. In fleas, there is evidence of larval interspecific competition between species that parasitize the same rodent, producing paratopic distributions (Krasnov et al., 2005).

Wells et al. (2015) demonstrated that globally invasive *R. rattus* and *R. norvegicus* exhibit significantly higher overall parasite species diversity outside their geographical origins (i.e., the Orient). This observation implies that local parasites from wild or domestic animals have infected the invasive rodents. However, even though in our results *R. rattus* hosts fleas of native species, these are found in very low prevalence and intensity, less than in native species. This could indicate that a dilution effect is occurring, where the extended availability of hosts results in a decreased impact on any specific host species. The dilution effect is a phenomenon in which, if there are several species with varying susceptibility to being infected by a pathogen, parasites will 'dilute' among them. This effect can occur directly or indirectly and can be caused by competitors, predators or species with a slow life history that disappears from disturbed areas, whereas reservoir hosts tend to be habitat generalists, have fast life histories and tolerate disturbance (Khalil et al., 2016; Schmidt & Ostfeld, 2001). Both transshipment and local acquisition of parasites and pathogens can have significant consequences for ecosystems, wildlife and domestic species (Wood & Johnson, 2015).

Co-invasion is a complex process that has significant implications for rodent health and ecosystem ecology. The presence of invasive rodent parasites in native rodents could affect the health of native populations and zoonotic disease transmission. However, it is essential to recognize the limitations of this study, including the sampling duration (only two nights per locality) and varied localities sampled (cities, rural areas and natural areas), which causes the abundance of rats and fleas to change between these types of environments, resulting in differences in the probability of contact between rodents and flea transmission. Laboratory studies would be necessary to evaluate whether there is any host-dependent effect on flea reproduction or survival.

In summary, while co-introductions can occur, the combination of ecological mismatch, environmental constraints, evolutionary adaptations, competition, predation, limited genetic diversity and empirical evidence all suggest that co-introductions often face significant challenges that prevent them from persisting indefinitely in new

environments. The results of this study pave the way for future research, such as exploring the long-term effects of co-invasion on ecosystems, flea genetics and host–host relationships and disease transmission dynamics between rodents and humans.

AUTHOR CONTRIBUTIONS

Nicol Lizama-Schmeisser: Data curation; formal analysis; investigation; methodology. **Elaine Serafin de Castro:** Writing – original draft; writing – review and editing. **Mario Espinoza-Carniglia:** Project administration; data curation; formal analysis; investigation; writing – review and editing. **Yessica Herrera:** Data curation; formal analysis; investigation; methodology. **María Carolina Silva-de La Fuente:** Conceptualization; methodology; investigation; writing – review and editing. **Marcela Lareschi:** Writing – review and editing; data curation; investigation; validation. **Lucila Moreno:** Conceptualization; funding acquisition; supervision; writing – original draft; writing – review and editing.

ACKNOWLEDGEMENTS

Thanks to the support provided by the Servicio Agrícola y Ganadero (SAG) and Corporación Nacional Forestal (CONAF).

FUNDING INFORMATION

The National Fund for Scientific and Technological Development (FONDECYT 11150875, 1130948 and 1170972) and the Vicerrectoría de Investigación y Desarrollo-Universidad de Concepcion (VRID 220.113.099-INV, 2023000985INT) supported this study.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships, which could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

ORCID

Mario Espinoza-Carniglia https://orcid.org/0000-0001-6786-3271
Lucila Moreno https://orcid.org/0000-0003-3159-4916

REFERENCES

Aplin, K.P., Suzuki, H., Chinen, A.A., Chesser, R.T., ten Have, J., Donnellan, S.C. et al. (2011) Multiple geographic origins of commensalism and complex dispersal history of black rats. *PLoS One*, 6, e26357. Available from: https://doi.org/10.1371/journal.pone.0026357

Beaucournu, J.-C. & Gallardo, M.N. (1988) Puces nouvelles d'Argentine (Insecta, Siphonaptera). *Revue Suisse de Zoologie (1988)*, 95(1), 99–112. Available from: https://doi.org/10.5962/bhl.part.79641

Beaucournu, J.C. & Kelt, D.A. (1990) Contribution à la faune du Chili: puces nouvelles ou peuconnues de la partie sud (Insecta, Siphonaptera). *Revue Suisse de Zoologie (1990)*, 97(3), 647–668. Available from: https://doi.org/10.5962/bhl.part.79755

Beaucournu, J.-C., Moreno, L. & González-Acuña, D. (2011) Deux espèces nouvelles de puces (Siphonaptera: Ctenophthalmidae & Rhopalopsyllidae) du Chili. *Parasite*, 18(3), 241–246. Available from: https://doi.org/10.1051/parasite/2011183241

Beaucournu, J.-C., Torres Mura, J.C. & Gallabdo, M.N. (1988) Description de la femelle de *Ctenoparia topali* Smit, 1963 et clef dichotomique du genreCtenopariaRothschild, 1909 (Siphonaptera, Hystrichopsyllidae). *Annales de Parasitologie Humaine et Comparée*, 63(5), 380–383. Available from: https://doi.org/10.1051/parasite/1988635380

Blackburn, T.M., Pyšek, P., Bacher, S., Carlton, J.T., Duncan, R.P., Jarošík, V. et al. (2011) A proposed unified framework for biological invasions. *Trends in Ecology & Evolution*, 26, 333–339. Available from: https://doi.org/10.1016/j.tree.2011.03.023

Blanton, L.S. & Walker, D.H. (2017) Flea-borne rickettsioses and Rickettsiae. *The American Journal of Tropical Medicine and Hygiene*, 96(1), 53–56. Available from: https://doi.org/10.4269/ajtmh.16-0537

Boyer, S., Gillespie, T.R. & Miarinjara, A. (2022) *Xenopsylla cheopis* (rat flea). *Trends in Parasitology*, 38(7), 607–608. Available from: https://doi.org/10.1016/j.pt.2022.03.006

Britton, J.R. (2013) Introduced parasites in food webs: new species, shifting structures? *Trends in Ecology & Evolution*, 28, 93–99. Available from: https://doi.org/10.1016/j.tree.2012.08.020

Bush, A.O., Lafferty, K.D., Lotz, J.M. & Shostak, A.W. (1997) Parasitology meets ecology on its own terms: Margolis Shostak AW revisited. *Journal of Parasitology*, 83, 575–583. Available from: https://doi.org/10.2307/3284227

Carpenter, J.W. & Marion, C.J. (2018) *Exotic Animal Formularu: Fifth Edition*.

Catford, J.A., Vesk, P.A., Richardson, D.M. & Pyšek, P. (2012) Quantifying levels of biological invasion: towards the objective classification of invaded and invasible ecosystems. *Global Change Biology*, 18(1), 44–62. Available from: https://doi.org/10.1111/j.1365-2486.2011.02549.x

Chinchio, E., Crotta, M., Romeo, C., Drewe, J.A., Guitian, J. & Ferrari, N. (2020) Invasive alien species and disease risk: an open challenge in public and animal health. *PLoS Pathogens*, 16, e1008922. Available from: https://doi.org/10.1371/journal.ppat.1008922

Drake, J.M. (2003) The paradox of the parasites: implications for biological invasion. *Proceedings of the Royal Society of London Series B: Biological Sciences*, 270, S133–S135.

Durden, L.A. & Hinkle, N.C. (2019) Chapter 10 Fleas (Siphonaptera). In: *Medical and Veterinary Entomology*. Berkeley, CA: Elsevier Inc, pp. 145–169. Available from: https://doi.org/10.1016/b978-0-12-814043-7.00010-8

Fitzgerald, B.M., Efford, M.G. & Karl, B.J. (2023) The fleas of house mice (*Mus musculus* L.) and ship rats (*Rattus rattus* L.) in forest of the Orongorongo Valley, New Zealand. *New Zealand Journal of Zoology*, 1–16. Available from: https://doi.org/10.1080/03014223.2023.2270433

Hastriter, M.W. & Whiting, M.F. (2009) Siphonaptera: (fleas). In: *Encyclopedia of insects*. Berkeley, CA: Elsevier Inc, pp. 924–928. Available from: https://doi.org/10.1016/B978-0-12-374144-8.00245-9

Hopkins, G. & Rothschild, M. (1956) *An illustrated catalogue of the Rothschild collection of fleas (Siphonaptera) in the British museum (natural history). Vol II, Coptopsyllidae, Vermipsyllidae, Stephanocircidae, Ischnopsyllidae, Hypsophthalmidae and Xiphiopsyllidae*. London: British-Museum (Natural History), p. xi, 445.

Hopkins, G. & Rothschild, M. (1962) *An illustrated catalogue of the Rothschild collection offleas(Siphonaptera) in the British museum (natural history). Vol III, Hystrichopsyllidae (Acedestiinae, Anomiosyllinae, Hystrichopsyllinae, Neopsyllinae, Rhadinopsyllinae and Stenoponiinae)*. London: British Museum (Natural History), p. xi,600.

Hopkins, G. & Rothschild, M. (1966) *An illustrated catalogue of the Rothschild collection offleas(Siphonaptera) in the British museum (natural history). Vol IV, Hystrichopsyllidae (Ctenophthalminae, Dinopsyllinae, Doratopsyllinae and Listropsyllinae)*. London: BritishMuseum (Natural History), p. viii, 549.

Hulme, P.E. (2014) Invasive species challenge the global respose to emerging diseases. *Trens in Parasitology*, 30, 267–270. Availabre from: https://doi.org/10.1016/j.pt.214.03.005

INE. (2005) *Chile: Ciudades, Pueblos, Aldeas y Caserios*. Chile: Instituto Nacional de Estadísticas.

Iriarte A. Los mamíferos de Chile. (2008) pp 417. https://doi.org/10.1007/s13398-014-0173-7.2

Jovani, R. & Tella, J.L. (2006) Parasite prevalence and sample size: misconceptions and solutions. *Trends in Parasitology*, 22(5), 214–218. Available from: https://doi.org/10.1016/j.pt.2006.02.011

Khalil, H., Ecke, F., Evander, M., Magnusson, M. & Hörnfeldt, B. (2016) Declining ecosystem health and the dilution effect. *Scientific Reports*, 6, 31314. Available from: https://doi.org/10.1038/srep31314

Khokhlova, I., Fielden, L., Degen, A. & Krasnov, B. (2012) Digesting blood of an auxiliary host in fleas: effect of phylogenetic distance from a principal host. *The Journal of Experimental Biology*, 215, 1259–1265. Available from: https://doi.org/10.1242/jeb.066878

Kim, H.-C., Yang, Y.-C., Chong, S.-T., Ko, S.-J., Lee, S.-E., Klein, T.A. et al. (2010) Detection of *Rickettsia typhi* and seasonal prevalence of fleas collected from small mammals in the Republic of Korea. *Journal of Wildlife Management*, 46, 165–172.

Krampitz, H.E. (1980) Host preference, sessility and mating behaviour of *Leptopsylla segnis* reared in captivity. In: Traub, R. & Starcke, H. (Eds.) *Fleas. Proceedings of the international conference on fleas*. Rotterdam: A.A. Balkema, pp. 371–378.

Krasnov, B.R., Burdelova, N.V., Khokhlova, I.S., Shenbrot, G.I. & Degen, A. (2005) Larval interspecific competition in two flea species parasitic on the same rodent host. *Ecological Entomology*, 30, 146–155. Available from: https://doi.org/10.1111/j.0307-6946.2005.00686.x

Krasnov, B.R., Mouillot, D., Khokhlova, I.S., Shenbrot, G.I. & Poulin, R. (2012) Compositional and phylogenetic dissimilarity of host communities drives dissimilarity of ectoparasite assemblages: geographical variation and scale-dependence. *Parasitology*, 139, 338–347.

Krasnov, B.R., Mouillot, D., Shenbrot, G.I., Khokhlova, I.S. & Poulin, R. (2004) Geographical variation in host specificity of fleas (Siphonaptera) parasitic on small mammals: the influence of phylogeny and local environmental conditions. *Ecography*, 27, 787–797.

Krasnov, B.R., Pilosof, S., Stanko, M., Morand, S., Korallo-Vinarskaya, N.P., Vinarski, M.V. et al. (2014) Co-occurrence and phylogenetic distance in communities of mammalian ectoparasites: limiting similarity versus environmental filtering. *Oikos*, 123, 63–70.

Krasnov, B.R., Shenbrot, G.I. & Khokhlova, I.S. (2022) Dark diversity of flea assemblages of small mammalian hosts: effects of environment, host traits and host phylogeny. *International Journal for Parasitology*, 52(2–3), 157–167. Available from: https://doi.org/10.1016/j.ijpara.2021.08.003

Krasnov, B.R., Shenbrot, G.I., Khokhlova, I.S. & Poulin, R. (2004) Relationships between parasite abundance and the taxonomic distance among a parasite's host species: an example with fleas parasitic on small mammals. *International Journal for Parasitology*, 34, 1289–1297.

Landaeta-Aqueveque, C., del Robles, M.R., Henríquez, A., Yáñez-Meza, A., Correa, J.P., González-Acuña, D. et al. (2018) Phylogenetic and ecological factors affecting the sharing of helminths between native and introduced rodents in Central Chile. *Parasitology*, 145, 1570–1576. Available from: https://doi.org/10.1017/S0031182018000446

Landaeta-Aqueveque, C., Moreno, L., Henríquez, A., Silva-de la Fuente, M.C. & González-Acuña, D. (2021) Parasites of native and invasive rodents in Chile: ecological and human health needs. *Frontiers in Veterinary Science*, 8, 643742. Available from: https://doi.org/10.3389/fvets.2021.643742

Lobos, G., Ferres, M. & Palma, R.E. (2005) Presencia de los géneros invasores Mus y Rattus en áreas naturales de Chile: Un riesgo ambiental y epidemiológico. *Revista Chilena de Historia Natural*, 78, 113–124. Available from: https://doi.org/10.4067/s0716-078x2005000100008

Luce-Fedrow, A., Maina, A.N., Otiang, E., Ade, F., Omulo, S., Ogola, E. et al. (2015) Isolation of *Candidatus* rickettsia asemboensis from *Ctenocephalides* fleas. *Vector-Borne and Zoonotic Diseases*, 15, 268–277. Available from: https://doi.org/10.1089/vbz.2014.1744

Lymbery, A.J., Morine, M., Kanani, H.G., Beatty, S.J. & Morgan, D.L. (2014) Co-invaders: the effects of alien parasites on native hosts. *International Journal for Parasitology: Parasites and Wildlife*, 3, 171–177. Available from: https://doi.org/10.1016/j.ijppaw.2014.04.002

MacLeod, C.J., Paterson, A.M., Tompkins, D.M. & Duncan, R.P. (2010) Parasites lost - do invaders miss the boat or drown on arrival? *Ecology Letters*, 13, 516–527. Available from: https://doi.org/10.1111/j.1461-0248.2010.01446.x

Mills, J.N., Yates, T.L., Childs, J.E., Parmenter, R.R., Ksiazek, T.G., Rollin, P.E. et al. (1995) Guidelines for working with rodents potentially infected with hantavirus. *Journal of Mammalogy*, 76, 716. Available from: https://doi.org/10.2307/1382742

Morand, S., Bordes, F., Chen, H.W., Claude, J., Cosson, J.F., Galan, M. et al. (2015) Global parasite and *Rattus* rodent invasions: the consequences for rodent-borne diseases. *Integrative Zoology*, 10(5), 409–423. Available from: https://doi.org/10.1111/1749-4877.12143

Moreno, L., Espinoza-Carniglia, M., Schmeisser, N.L., Torres, L.G., Silva-De La Fuente, M.C., Lareschi, M. et al. (2019) Fleas of black rats (*Rattus rattus*) as reservoir host of Bartonella spp. in Chile. *PeerJ*, 7, e7371. Available from: https://doi.org/10.7717/peerj.7371

Nakazawa, K., Oisi, I. & Kumi, S. (1957) Seasonal prevalence of rodent fleas. *Medical Entomology and Zoology*, 8, 11–13.

Pejchar, L. & Mooney, H. (2009) The impact of invasive alien species on ecosystem services and human well-being. In: *Bioinvasions and globalization: ecology, economics, management, and policy*. Oxford, England: Oxford University Press. Available from: https://doi.org/10.1093/acprof:oso/9780199560158.003.0012

Pettersen, R.A., Østbye, K., Holmen, J., Vøllestad, L.A. & Mo, T.A. (2016) *Gyrodactylus* spp. diversity in native and introduced minnow (*Phoxinus phoxinus*) populations: No support for "the enemy release" hypothesis. *Parasites and Vectors*, 9, 51. Available from: https://doi.org/10.1186/s13071-016-1306-y

Plowright, R.K., Parrish, C.R., McCallum, H., Hudson, P.J., Ko, A.I., Graham, A.L. et al. (2017) Pathways to zoonotic spillover. *Nature Reviews Microbiology*, 15(8), 502–510. Available from: https://doi.org/10.1038/nrmicro.2017.45

Poulin, R., Krasnov, B.R. & Morand, S. (2006) Chapter 13. Patterns of host specificity in parasites exploiting small mammals. In: Morand, S., Krasnov, B.R. & Poulin, R. (Eds.) *Micromammals and macroparasites from evolutionary ecology to management*. Tokyo Berlin Heidelberg New York: Springer-Verlag.

Poulin, R., Paterson, R.A., Townsend, C.R., Tompkins, D.M. & Kelly, D.W. (2011) Biological invasions and the dynamics of endemic diseases in freshwater ecosystems. *Freshwater Biology*, 56, 676–688. Available from: https://doi.org/10.1111/j.1365-2427.2010.02425.x

Prenter, J., MacNeil, C., Dick, J.T.A. & Dunn, A.M. (2004) Roles of parasites in animal invasions. *Trends in Ecology & Evolution*, 19, 385–390. Available from: https://doi.org/10.1016/j.tree.2004.05.002

Reiczigel, J., Marozzi, M., Fábián, I. & Rózsa, L. (2019) Biostatistics for Parasitologists – a primer to quantitative parasitology. *Trends in Parasitology*, 35, 277–281. Available from: https://doi.org/10.1016/j.pt.2019.01.003

Rózsa, L., Reiczigel, J. & Majoros, G. (2000) Quantifying parasites in samples of hosts. *The Journal of Parasitology*, 86(2), 228–232. Available from: https://doi.org/10.1645/0022-3395(2000)086[0228:QPISOH]2.0.CO;2

Sánchez, J., Amor, V., Bazán-León, E., Vásquez, R. & Lareschi, M. (2012) Redescription of *Neotyphloceras chilensis* Jordan, new status (Siphonaptera: Ctenophthalmidae: Neotyphloceratini). *Zootaxa*, 3259, 51–57. Available from: https://doi.org/10.5281/zenodo.280702

Sánchez, J. & Lareschi, M. (2014) Two new species of *Neotyphloceras* (Siphonaptera:Ctenophthalmidae) from Argentinean Patagonia. *Zootaxa*, 3784(2), 159–170. Available from: https://doi.org/10.11646/zootaxa.3784.2.5

Sayyadzahed, G., Esmaeili, H.R., Ghasemian, S., Mirghiyasi, S., Parsi, B., Zamanpoore, M. et al. (2016) Co-invasion of anchor worms *Lernaea cyprinacea* (Copepoda: Lernaeidae) in some freshwater fishes of the Kor River basin, southwest of Iran with some remarks on the ecological aspects of lernaeosis in the country. *Iranian Journal of Fisheries Sciences*, 15, 369–389.

Schatz, A.M. & Park, A.W. (2023) Patterns of host–parasite coinvasion promote enemy release and specialist parasite spillover. *Journal of Animal Ecology*, 92, 1029–1041. Available from: https://doi.org/10.1111/1365-2656.13910

Schmidt, K. & Ostfeld, R. (2001) Biodiversity and the dilution effect in disease ecology. *Ecology*, 82, 609–619. Available from: https://doi.org/10.2307/2680183

Schramm, B.A. (1987) *A taxonomic revision of the genus Plocopsylla Jordan, 1931 (Siphonaptera: Stephanocircidae). PhD retrospective theses and dissertations*. Ames: Iowa State University, p. 227. Available from: https://doi.org/10.31274/rtd-180813-11700

Smit, F.G.A.M. (1987) *An illustrated catalogue of the Rothschild collection of fleas (Siphonaptera) inthe British museum (natural history) 7: Malacopsylloidea (Malacopsyllidae and Rhopalopsyllidae)*. UK: Oxford University, p. 380.

Smith, K.F. & Carpenter, S.M. (2006) Potential spread of introduced black rat (*Rattus rattus*) parasites to endemic deer mice (*Peromyscus maniculatus*) on the California Channel Islands. *Diversity and Distributions*, 12, 742–748. Available from: https://doi.org/10.1111/j.1472-4642.2006.00279.x

Steppan, S.J. & Schenk, J.J. (2017) Muroid rodent phylogenetics: 900-species tree reveals increasing diversification rates. *PLoS One*, 12(8), e0183070. Available from: https://doi.org/10.1371/journal.pone.0183070

Taraschewski, H. (2006) Hosts and parasites as aliens. *Journal of Helminthology*, 80(2), 99–128. Available from: https://doi.org/10.1079/joh2006364

Tompkins, D.M. & Poulin, R. (2006) Parasites and biological invasions. In: Allen, R.B. & Lee, W. (Eds.) *Biological invasions in New Zealand*. Berlin: Springer-Verlag, pp. 67–84.

Torchin, M.E., Lafferty, K.D., Dobson, A.P., McKenzie, V.J. & Kuris, A.M. (2003) Introduced species and their missing parasites. *Nature*, 421, 628–630. Available from: https://doi.org/10.1038/nature01346

Troyo, A., Moreira-Soto, R.D., Calderon-Arguedas, Ó., Mata-Somarribas, C., Ortiz-Tello, J., Barbieri, A.R.M. et al. (2016) Detection of rickettsiae in fleas and ticks from areas of Costa Rica with history of spotted fever group rickettsioses. *Ticks and Tick-Borne Diseases*, 7, 1128–1134. Available from: https://doi.org/10.1016/j.ttbdis.2016.08.009

Vancheva, N., Bobeva, A., Pehlivanov, L., Stefanov, T. & Georgiev, B.B. (2020) Alien parasites on an alien fish species: monogeneans from the black bullhead *Ameiurus melas* (Siluriformes) in the Lake Srebarna biosphere reserve, Bulgaria, with the first record of *Gyrodactylus nebulosus* in the Palaearctic. *Journal of Parasitology Research*, 119, 2105–2112. Available from: https://doi.org/10.1007/s00436-020-06699-z

Wells, K., O'Hara, R.B., Morand, S., Lessard, J.-P. & Ribas, A. (2015) The importance of parasite geography and spillover effects for global patterns of host-parasite associations in two invasive species. *Diversity and Distributions*, 21, 477–486. Available from: https://doi.org/10.1111/ddi.12297

White, E.M., Wilson, J.C. & Clarke, A.R. (2006) Biotic indirect effects: a neglected concept in invasion biology. *Diversity and Distributions*, 12, 443–455. Available from: https://doi.org/10.1111/j.1366-9516.2006.00265.x

Wiggers, R.J., Martin, M.C. & Bouyer, D. (2005) Rickettsia felis infection rates in an east Texas population. *Texas Medicine*, 101, 56–58.

Wood, C.L. & Johnson, P.T. (2015) A world without parasites: exploring the hidden ecology of infection. *Frontiers in Ecology and the Environment*, 13, 425–434. Available from: https://doi.org/10.1890/140368

Yeh, J. & Davis, D.E. (1950) Seasonal changes in abundance of fleas on rats at Baltimore, Md. *Public Health Reports (1896–1970)*, 65(10), 337–342. Available from: https://doi.org/10.2307/4587270

Yin, J.X., Cheng, X.O., Luo, Y.Y., Zhao, Q.F., Wei, Z.F., Xu, D.D. et al. (2020) The relationship between fleas and small mammals in households of the Western Yunnan Province, China. *Scientific Reports*, 10(1), 16705. Available from: https://doi.org/10.1038/s41598-020-73690-0

How to cite this article: Lizama-Schmeisser, N., de Castro, E.S., Espinoza-Carniglia, M., Herrera, Y., Silva-de La Fuente, M.C., Lareschi, M. et al. (2024) Are *Rattus rattus* fleas invasive? Evaluation of flea communities in invasive and native rodents in Chile. *Medical and Veterinary Entomology*, 1–15. Available from: https://doi.org/10.1111/mve.12739