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Original Article

Gastrointestinal parasites in wild rodents in Chiloé Island-Chile

Parasitos gastrointestinais em roedores selvagens na Ilha de Chiloé-Chile

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Abstract

Gastrointestinal parasites are well-documented in small mammals from north-central Chile, but little is known about endoparasites of rodents in southern Chile. A survey was conducted between January and February 2018 to evaluate gastrointestinal parasites and risk factors of wild rodents that live in rural areas in Northern Chiloé Island, Chile. A total of 174 fecal samples from rodents of six native and one introduced species were collected and examined using the Mini-FLOTAC method. Also, 41 individuals of four native wild rodent species were examined furtherly to determinate adult parasites from gastrointestinal tracts. The overall prevalence of endoparasites was 89.65% (156). Helminth egg types included: *Rodentolepis* spp., Capillariidae, *Trichuris* sp., *Syphacia* sp., oxyurid-type eggs, *Strongyloides* sp., Spirurid-type eggs, Strongilid-type eggs, *Moniliformis* sp., and an unidentified nematode egg and larvae. Protozoa comprised coccidia, amoeba, and unidentified cysts. From necropsies, adult parasites involved *Syphacia* sp. *Trichuris* sp., *Protospirura* sp. and *Physaloptera* sp. In *Abrothrix olivacea,* individuals with low-body-mass index exhibited reduced infection probability for Spirurid-type and Strongilid-type eggs. Some parasites in this study may affect human health. In rural settings where environmental conditions are changing, more research should be undertaken to understand parasitic infections in wildlife and implications for public health and conservation.

Keywords: Small-mammals, helminth, protozoa, parasites, Chile, Austral.

Resumo

Parasitos gastrointestinais são bem documentados em pequenos mamíferos do centro-norte do Chile, mas pouco conhecido sobre endoparasitos de roedores no sul do Chile. Uma pesquisa foi realizada entre janeiro e fevereiro de 2018, avaliando parasitas gastrointestinais e fatores de risco de roedores selvagens vivendo em áreas rurais no norte da Ilha de Chiloé, Chile. Um total de 174 amostras fecais de roedores de seis espécies nativas e uma introduzida foi coletado e examinado pelo método Mini-FLOTAC. Ademais, 41 indivíduos de quatro espécies nativas de roedores selvagens foram examinados para determinar parasitas adultos do trato gastrointestinal. A prevalência geral de endoparasitos foi de 89,65% (156). Os tipos de ovos de helmintos incluíram: *Rodentolepis* spp., Capillariidae, *Trichuris* sp., *Syphacia* sp.; dos tipos Oxyurideos, *Strongyloides* sp., dos tipos Spirurideos e Estrongilideos, *Moniliformis* sp.; e um ovo e larvas de nematoides não identificados. Os protozoários compreendiam coccídios, amebas e cistos não identificados. Nas necropsias, os parasitos adultos envolveram *Syphacia* sp. *Trichuris* sp., *Protospirura* sp. e *Physaloptera* sp. Em *Abrothrix olivacea,* indivíduos com baixo índice de massa corporal apresentaram probabilidade de infecção reduzida para ovos Spirurideos e Estrongilideos. Alguns parasitos, neste estudo, podem afetar a saúde humana. Em ambientes rurais, onde as

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condições ambientais mudam, mais pesquisas são necessárias para entender as infecções parasitárias na vida selvagem e as implicações para a saúde pública e conservação.

Palavras-chave: Mamíferos, helmintos, protozoários, parasitas, Chile, Austral.

Introduction

Human-induced changes on the environment modify balancing ecological processes with negative repercussions on ecosystems and wildlife (Daszak et al., 2000). Habitat disturbance through anthropogenic activities (i.e., agriculture, resource extraction, and urbanization) generate variations in populations sizes, genetics, and immune competence that can alter host-parasite interactions (Daszak et al., 2000). Specifically, in fragmented and degraded habitats, wildlife populations are likely to show higher densities with higher intra and inter-specific contacts and depressed immune function due to chronic stress and low genetic diversity (Anderson & May, 1979; May & Anderson, 1979; Daszak et al., 2000; Meyer-Lucht et al., 2010). Such modifications in host factors related to environmental conditions favor infectious agents to increase in host ranges, virulence and pathogenicity, and promote the occurrence of previously unrecognized infectious diseases (Holmes, 1996; Patz et al., 2004; Jones et al., 2008; Suzán et al., 2012)

Rodents are considered reservoirs and carriers for a variety of infectious diseases that can also be transmitted to humans (Han et al., 2015). Out of 2277 extant rodent species worldwide, 217 have been identified as reservoirs for zoonoses, including viral (e.g., Hantavirus pulmonary syndrome), bacterial (e.g., leptospirosis), fungal (eg., *Aspergillus* sp.), and parasitic infections (Suzuki et al., 2004; Thomas et al., 2012; Han et al., 2015; Fantozzi et al., 2018; Luna et al., 2020). In particular, rodents are hosts of a range of gastrointestinal parasites that include helminths such as *Rodentolepis* spp. and *Calodium hepaticum*, and protozoa such as *Cryptosporidium* spp. and *Giardia* spp. (Wells et al., 2007; Perec-Matysiak et al., 2015; Fantozzi et al., 2018; Hurtado et al., 2021; Sáez-Durán et al., 2021). Gastrointestinal helminths and protozoal infections can affect survival and reproduction directly by pathological effects (e.g., blood loss, tissue damage) and indirectly through reduction of host condition (e.g., malabsorption of nutrients, predator escape, energetic costs) (Lyles & Dobson, 1993; Scantlebury et al., 2007; Taylor et al., 2016).

Parasitological parameters (e.g., prevalence, burden, richness) of helminths and protozoa can be influenced by host attributes, such as sex, age, and body condition, and tend to be different according to each ecological setting (Morand, 2015; Seifollahi et al., 2016; Dos Santos Lucio et al., 2021). Overall, males are more prone to exhibit higher parasite infection rates than females apparently due to testosterone-mediated effects in behavior and immune response (Zuk & McKean, 1996; Biard et al., 2015; Morand, 2015). Also, adult individuals can be more parasitized because of the cumulative exposure, whereas juveniles can be more affected given that their adaptive immunity is still under-developed (Wilson et al., 2002; Wells et al., 2007). Likewise, individuals with high body mass can harbor more parasites since a larger habitat favor parasite colonization, while individuals with low body condition can exhibit reduced immune competence related to nutrients deficiency and an increase in parasitosis (Wilson et al., 2002; Morand, 2015). In addition, environmental characteristics that influence parasitic infections include altitude, climate (e.g., temperature, humidity), habitat quality, among others (Wells et al., 2007; Barelli et al., 2021; Deak et al., 2020; Kiene et al., 2021). Effects of helminths and protozoa infections on rodents living in human-modified habitats can be intensified with detrimental impact at host population scale (Santicchia et al., 2015). Consequently, helminth and protozoal infections are considered to be good indicators of host and environmental conditions (Marcogliese & Pietrock, 2011).

In Chile, many endoparasites have been reported in native and introduced rodent species, in which parasite infection ranged from 0.5 to 88% (Alba & Jarpa, 1951; Olsen, 1966; Schenone et al., 1967; Babero et al., 1975; Babero et al., 1976; Babero & Murua, 1987; Landaeta-Aqueveque et al., 2014; Digiani et al., 2017; Seguel et al., 2017; Landaeta-Aqueveque et al., 2018; Yáñez-Meza et al., 2019; Riquelme et al., 2021). Although well-documented information is available about the taxonomy and ecological features of endoparasites in rodents mainly from Central Chile, little work has been done on gastrointestinal parasites and host determinants in rodents in southern Chile (Landaeta-Aqueveque et al., 2021). Recently, it was described the infestation of trombiculid mites in wild rodents from Chiloé Island and the presence of *Orientia* spp., that causes the Scrub typhus in humans (Acosta-Jamett et al., 2020; Weitzel et al., 2020). However, information about the gastrointestinal parasitic fauna in wild rodents from rural Chiloé Island is still limited. Therefore, the aim of this study was to determine the gastrointestinal parasites in wild rodents inhabiting rural locations from Chiloé Island and assess host determinants (i.e., sex, age, and body mass) on the parasite infection probability, and thus establishing a baseline data on gastrointestinal parasites from wild rodents to better understand how habitat disturbance will impact host-parasite interactions.

Materials and Methods

Study site

A cross-sectional survey was carried out at six rural sites in the north-eastern area of Chiloe Island in Los Lagos Region, during the austral summer (January-February) in 2018. Study locations were detailed previously in Acosta-Jamett et al. (2020) (see Figure 1). The study area comprises a highly fragmented rural mosaic of remnant old-growth and secondary forest, bogs, shrublands, exotic plantations, and artificial landscapes, which was shaped by a 200-year fire history of degradation due to logging and forest fire (Willson & Armesto, 1996; Gutiérrez et al., 2009). Climate is wet-temperate, with an annual mean temperature ranging from 9.1 to 10.8 C (coast: 6.9 -17.6 C; inland: 4.2 - 14.4 C) and annual rainfall of approximately 2000 mm (summer: 13-25% [January-March]) (Gutiérrez et al., 2009).

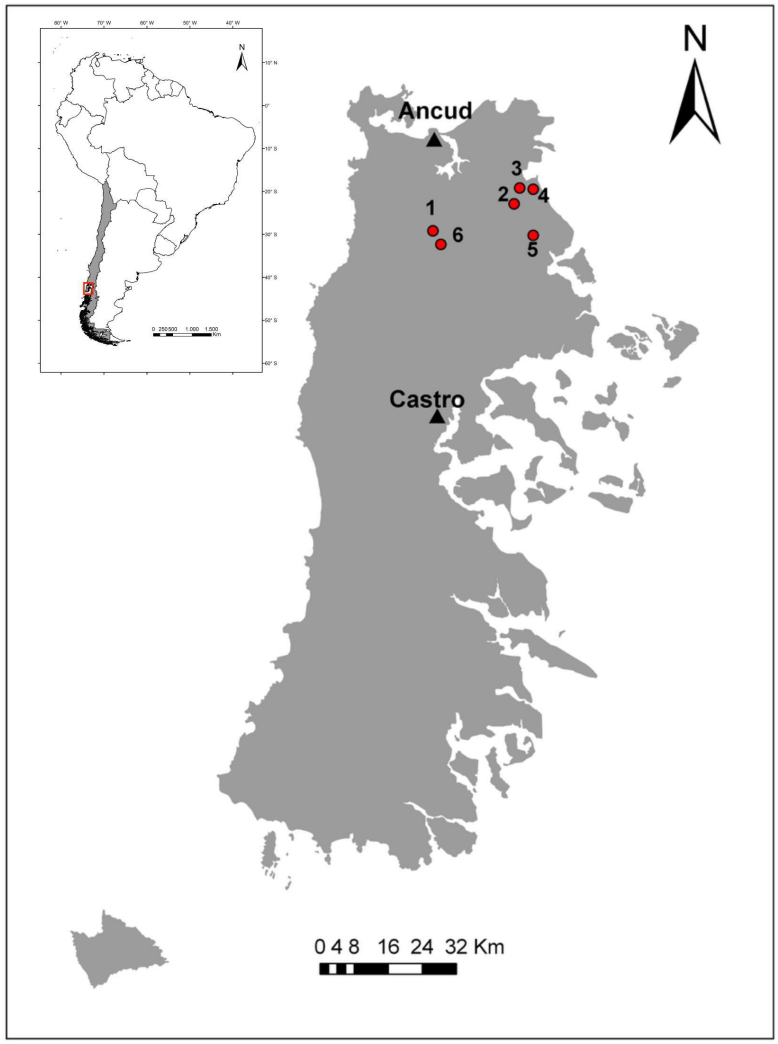

Figure 1. Sampling sites and study area in rural localities of the north-eastern Chiloé Island, Los Lagos Region, Chile.

Sampling sites were selected by convenience under the availability of native forest patches and the nearest human settlements where cases of Scrub typhus had been previously reported (see Acosta-Jamett et al. 2020 for further details).

Host capture and fecal sampling

Rodents were live-trapped under the approval and supervision of the Agricultural and Livestock Service of Chile in 2017 (Nº 7034-2017) and the Scientific Ethics Committee Resolution for the Animals and Environment Care

of the Pontificia Universidad Católica de Chile (Nº 160816007-2017). Animal capture and sampling followed the Standard Operating Procedures for Biosafety by the Center for Disease Control and Prevention (CDC), and for the Use of Wild Mammals in Research and Education by the American Veterinary Medicine Association and American Society of Mammologists (ASM) (CDC, 2012; Sikes & Animal Care and Use Committee of the American Society of Mammalogists, 2016).

Rodents were captured in Sherman-type live traps (n = 148-175; dimensions = 300 x 100 x 110 mm) baited with oat flakes and vanilla essence. Traps were placed under vegetal material (i.e., scrubs, fallen logs) with a distance of at least 5 meters from each other. Traps were set in the late afternoon and checked in the early morning of the next day. Trapping was conducted for 4/5 nights in each study site, ranging from 668 to 895 trap-night per site. Captured rodents were moved to a central processing tent installed at the sampling site.

After capture, each rodent was placed inside an induction chamber and anesthetized with isoflurane (1 mL isoflurane/500 mL chamber volume). Once individuals were induced (after 1-2 min), animals were classified according to species, sex, age (i.e., juvenile and adults), and reproductive status (Muñoz-Pedreros & Gill, 2009). Morphometric measurements (i.e., body length, tail, and hind-foot [mm]) and weight (gr) were recorded by using a digital caliper (Uberman®, precision 0,01 mm) and a precision digital scale (Pesamatic Newton Series®, Model EJ1500; +0.1 gr SD). Fecal samples (0.05 to 2.0 g.) were opportunistically taken from the anus or collected from the previously disinfected trap base during each morning (08 am – 12 pm) to minimize the effects of temporal variation in parasitic eggs/oocyst shedding (Filipiak et al., 2009). Samples were placed in clean plastic vials with 1.5 mL ethanol (70%) and stored at 4°C. Finally, adult female rodents were marked by a haircut and released at the respective capture points. Male rodents (juveniles or adults) and juvenile females were euthanized by cervical dislocation under anesthetic plane for further chigger collection. Forty-one euthanized rodents were frozen temporally until the extraction of the gastrointestinal tract. Then, the gastrointestinal tract was preserved in ethanol 96% (approximately 70% final dilution) until parasitological examination.

Coprologic examination

A parasitological examination was carried out at the Ecology and Evolution of Infectious Diseases Lab at the Universidad Austral de Chile. Parasite infection of helminth and protozoa was assessed qualitatively and quantitatively by the Mini-FLOTAC method (Mini-FLOTAC®, University of Naples Federico II) (Cringoli et al., 2017; Catalano et al., 2019). Briefly, fecal samples were weighed (Pesamatic Newton Series®, Model EJ1500; +0.1 gr SD) after ethanol removal by centrifugation (5 min at 1200 rpm), and an aliquot of feces (0.1 – 0.3 g) was poured into the Fill FLOTAC® 2 device. Zinc sulfate solution ($ZnSO_4$*$7H_2O$; FS7) was chosen given its previous validation in rodents (Cringoli et al., 2010; Catalano et al., 2019), in which the density of 1.35 was confirmed with a hydrometer (EISCO, New York 14564, US). To determine the multiplication factor, zinc sulfate solution was added in the Fill FLOTAC® until the final volume of 15 mL, as previously described (Catalano et al., 2019). Subsequently, the mixture was homogenized, dispensed in each of the two chambers of the Mini-FLOTAC® apparatus, and examined after 10 minutes by a microscope (Carl Zeiss 183858 Axiostar, Fisher Scientific, Schwerte DE 58239 Germany) with a digital camera (Axiocam ERc 5s, Carl Zeiss Microscopy, Göttingen 37081, Germany).

Helminth eggs and protozoa cysts/oocysts were identified to genus levels when possible, according to morphological keys (Thienpont et al., 2003; Zajac & Conboy, 2012; Taylor et al., 2016; Cordeiro et al., 2018). A maximum of ten related morphotypes of eggs/oocysts in each positive sample were digitally measured and photographically recorded (length and width; µm) (Zen 3.2 Blue Edition, ZEISS Group, München 81379, Germany). Finally, related morphotypes of eggs and oocyst were counted in each of the positive samples and multiplied by the multiplication factor to obtain fecal egg/oocyst per gram of feces (EPG, OPG), as previously described (Catalano et al., 2019).

Post-mortem examination

Gastrointestinal tracts of euthanized individuals were examined furtherly under a stereomicroscope (Leica S6D, Leica Microsystems, Heerbrugg CH-9435, Switzerland), and each segment was studied separately (i,e., stomach, small intestine, caecum and large intestine). The presence of Nematodes were examined, and any parasitic form was cleared with lactophenol or ethanol-glycerine and identified under a microscope (Leica DM 1000, Heerbrugg CH-9435, Switzerland) following keys (Anderson et al., 2009).

Data analysis

Parasitological parameters such as prevalence, mean intensity, and mean abundance were interpreted and calculated according to Bush et al. (1997) for both coprologic and post-mortem examination. In this regard, Prevalence (p) is the number of individuals of a host species infected with a specific parasite type divided by the number of hosts examined, and the 95% confidence interval [CI] was calculated with the Clopper–Pearson method (Clopper & Pearson, 1934).

For the coprologic examination, a proxy for adult parasite intensity was calculated using the number of eggs per gram (EPG) and oocyst per gram (OPG) given that fecal samples were obtained non-invasively, as carried out elsewhere (Wells et al., 2007; Hodder & Chapman, 2012; Barelli et al., 2020). The mean intensity ($_M$I) corresponds to the sum of eggs per gram (EPG) and oocysts per gram (OPG) in feces for each parasite type divided by the number of hosts infected with that parasite. The intensity range ($_R$I) was the minimum and the maximum value of load (EPG, OPG) for each morphotype. The mean abundance ($_M$A) is the total number of each isolated parasite egg or oocyst types (EPG, OPG) divided by the number of total analyzed hosts. Also, the mean helminth species richness ($_M$HR) was calculated as the average number of simultaneously present helminth egg types (EPG) in the feces of individual hosts of each species.

For post-mortem examination, the $_M$I corresponds to the number of helminths of a species divided by the number of infected hosts, and the $_M$A is the number of helminths of a species divided by the number of examined hosts (Bush et al., 1997). Given the sample size of host species (n >40) (Shvydka et al., 2018), the CI for $_M$I and $_M$A was calculated only for *A. olivacea* by the bootstrapping method (2000 replications) using the Quantitative Parasitology online application, QPweb (Reiczigel et al., 2019). Additionally, for *A. olivacea*, the aggregation of adult parasites was assessed by parasite species with the Poulin's Discrepancy Index using the QPweb.

To establish an approximation for body condition, the Scaled Mass Index (SMI) was calculated (Peig & Green, 2009), in which the individual measurements of body weight (*Mi*) and body length (*Li*) were inserted into the formula [SMI = $Mi \times (L0/Li)^{bSMA}$]; *L0* was the arithmetic mean of body length for each rodent species (i.e., *A. olivacea* = 81.4; *A. manni* = 91.9; *G. valdivianus* = 90.5; *I. tarsalis* = 81.4; *L. micropus* = 112; and *O. longicaudatus* = 82.1), and *bSMA* was the slope estimate of a standardized major axis (SMA) regression of the mass-length relationship (i.e., *bSMA* = β OLS/r = 0.48/0.60 = 0.75). Subsequently, the SMI of individuals was categorized into three groups: Low (<40), Medium (40-60), and High (>60) (thereafter called SMI categories), as carried out elsewhere (Pannoni et al., 2022; Valenzuela et al., 2022).

Given the sample size of each host species, parasite infection probability of most frequent parasite egg morphotypes (>5% total prevalence) were assessed in relation to host factors (sex, age, SMI, and SMI categories) only for *Abrothrix olivacea*, by using Generalized linear mixed models (GLMERs) (Bates et al., 2015). Dependent variables comprised the presence-absence (binomial) of the most prevalent parasite types (>5% total prevalence; ie., *Moniliformis* sp., Capillariidae, *Trichuris* sp., Spirurid type eggs, Strongylid type eggs, *Rodentolepis* sp., coccidia, amoeba, and unidentified protozoa cysts). Fixed effects included host age (juveniles, adults), host sex (male, female), and host body condition (SMI categories [low, medium, high]). Trapping site was included as a random factor to account for the variation due to environmental conditions. The strategy for building the model consisted of an initial screening of each fixed effect (i.e., host age, host sex, and SMI) by obtaining unconditional models. Only variables associated with the outcome ($p<0.05$) (i.e., host sex and SMI) were eligible for inclusion in the conditional model that was built using a forward variable selection method, including potential interactions. A comparison of the goodness-of-the-fit between different models was assessed using the Akaike Information Criteria (AIC) index. Finally, confounders were evaluated based on their biological significance. Statistical significance was set at $p<0.05$. The "lme4" and "MASS" packages were used for calculation of models using the software R (R Foundation for Statistical Computing, Vienna, Austria) (R Development CoreTeam, 2013) and RStudio (RStudio Team, 2020). Odds Ratio (OR) were calculated by raising the estimates of variables to the exponent using the software R and RStudio (Sommet & Morselli, 2017).

Results

Captured rodent community

Fecal samples were obtained from 174 captured rodents, 134 of which belonged to olive grass mouse (*Abrothrix olivacea* Waterhouse, 1837), 16 were Chilean climbing mouse (*Irenomys tarsalis* Philippi, 1900), 12 were

Valdivian mole mouse (*Geoxus valdivianus* Philippi, 1858), five were Mann's Soft-haired Grass mice (*Abrothrix manni* D'Elía et al., 2015), three were long-tailed colilargo (*Oligoryzomys longicaudatus* Bennett, 1832), two were southern pericote (*Loxodontomys micropus* Waterhouse, 1837), one was the brown rat (*Rattus norvegicus* Berkenhout, 1769), and one could not be classified to species (see Table 1). Of 174 sampled individuals, 112 (64.4%) were males (*A. olivacea* = 85, *I. tarsalis* = 12, *G. valdivianus* = 9, *A. manni* = 4, *L. micropus* = 1, and *R. norvegicus* = 1) and 62 (35.6%) were females (*A. olivacea* = 49, *I. tarsalis* = 4, *G. valdivianus* = 3, *O. longicaudatus* = 3, *A. manni* = 1, *L. micropus* = 1, and non-identified = 1). Likewise, of 174 individuals, 100 (57.5%) were adults (*A. olivacea* = 77, *I. tarsalis* = 7, *G. valdivianus* = 7, *A. manni* = 3, *L. micropus* = 2, *O. longicaudatus* = 2, and *R. norvegicus* = 1, and non-identified = 1) and 74 (42.5%) were juveniles (*A. olivacea* = 57, *I. tarsalis* = 9, *G. valdivianus* = 5, *A. manni* = 2, and *O. longicaudatus* = 1). Body weight of native rodents ranged from 8.5 to 52 g. (weight mean 23.73 ± standard error = 0.9, n = 171). Scaled body mass index of native rodents ranged from 10.5 to 95 (23.34 ± 0.7, n=170), in which 71 individuals were categorised as Low, 66 as Medium, and 33 as High.

Table 1. Captured rodents in North-eastern Chiloé Island, Chile. $_{M}$SMI (SE) = Mean of scaled body mass index (standard error).

Host Species	*N*	Female	Male	Juvenile	Adult	$_{M}$SMI (SE)
Site 1						
A. olivacea	13	2	11	5	8	20.0 (1)
G. valdivianus	4	1	3	2	2	26 (3.5)
Site 2						
A. olivacea	30	9	21	12	18	20.9 (0.9)
G. valdivianus	5	1	4	2	3	24.5 (3.1)
L. micropus	1	-	1	-	1	43.6
O. longicaudatus	3	3	-	1	2	25.7 (0.1)
Site 3						
A. olivacea	24	9	*15*	*8*	16	24.3 (5.1)
G. valdivianus	1	-	1	1	-	30.7
I. tarsalis	8	3	5	5	3	17.4 (2.9)
Site 4						
A. olivacea	21	6	15	14	7	19.9 (1.5)
A. manni	2	-	2	1	1	26.2 (6.7)
G. valdivianus	1	-	1	-	1	22.4
L. micropus	1	1	-	-	1	53.0
R. norvegicus	1	-	1	-	1	-
Non-identified	1	1	-	-	1	-
Site 5						
A. olivacea	33	14	19	13	20	25.5 (2.5)
A. manni	2	1	1	1	1	27.6 (9.7)
G. valdivianus	1	1	-	-	1	23.9
I. tarsalis	7	1	6	4	3	18.4 (2.6)
Site 6						
A. olivacea	13	9	4	2	11	28.5 (2.1)
A. manni	1	-	1	-	1	30.6
I. tarsalis	1	-	1	-	1	26.0

General gastrointestinal parasitism in rodent community

Of the 174 captured individuals, 156 (89.65%) were infected with any gastrointestinal parasite by coprologic and post-mortem examination. One-hundred-twelve individuals (64.4%) harbored helminths, 119 (68.4%) were infected with protozoa, and 74 (42.5%) showed mixed parasitic infection.

Coprologic findings

The overall parasite prevalence, mean intensity, intensity range, mean abundance, and mean helminth richness are shown in Table 2.

Table 2. Parasitological parameters of gastrointestinal morphotypes obtained from fecal samples of rodents collected in Chiloé Island. Prevalence (P) and 95% confidence intervals (CI), mean intensity ($_M$I), intensity range ($_R$I), mean abundance ($_M$A), and mean helminth richness ($_M$HR) are reported.

Morphotype[a]	P % (CI 95%)	$_M$I [b]	$_R$I [b]	$_M$A
***A. olivacea*:**127/134; $_M$HR: 2				
Acanthocephala				
Moniliformis sp.	5.9 (2.6–11.2)	2098	25–5850	125.2
Nematoda				
Capillariidae	5.9 (2.6–11.42)	444	38–2250	26.5
Trichuris sp.	6.7 (3.1–12.3)	558	38–3563	37.5
Syphacia sp.	3.7 (1.2–8.4)	110	25–365	4.1
Other oxyurids	2.2 (0.4–6.4)	287.6	75-750	6.4
Strongyloides sp.	1.5 (0.1–5.2)	50	25–75	0.7
Spirurid-type	33.6 (25.6–42.2)	832	2525–13500	279.4
Strongylid-type	35 (27–43.7)	238	1–17	82.9
Larvae	3.7 (1.2–8.4)	872	75–3375	58.5
Platyhelminth				
Rodentolepis spp.	20.1 (13.7–27.9)	638	25 –3450	138
Protozoa				
Coccidia	62.6 (53.9–70.8)	18784	25–253800	11774.7
Amoeba	8.2 (4.1–14.2)	146	38–750	11.9
Unidentified	20.1 (13.7–27.1)	325.7	25-5213	54
***I. tarsalis*:**13/16; $_M$HR: 1.1				
Nematoda				
Strongyloides sp.	12.5 (1.5–38.3)	2025	1050–3000	253.1
Spirurid-type	30 (9.0–61.4)	319	75–675	79.6
Strongylid-type	43.7 (19.7–70.1)	191	75–300	83.5
Protozoa				
Coccidia	50 (24.6–75.3)	78738	150–621100	39368.7
Unidentified	6.25 (0.1–30.2)	3000	3000	187.5

[a]One individual was not identified to species level, which harboured helminths such as oxyurids, *Strongyloides* sp., Strongilid-type eggs, and Spirurid-type ova; [b]EPG, eggs per gram; OPG, oocyst/cysts per gram.

Table 2. Continued...

Morphotype[a]	P % (CI 95%)	$_{M}I$ [b]	$_{R}I$ [b]	$_{M}A$
***G. valdivianus*:** 9/12; $_{M}$HR:1.3				
Acanthocephala				
Moniliformis sp.	16.6 (2–48.4)	1294	150–2438	215.6
Nematoda				
Strongyloides sp.	8.3 (0.2–38.4)	125	125	10.4
Spirurid-type	8.3 (0.2–38.4)	150	150	12.5
Strongylid-type	33.3 (9.9–65.1)	225	75–375	75
Non-identified	8.3 (0.2–38.4)	38	38	3.1
Platyhelminth				
Rodentolepis sp.	8.3 (0.2–38.4)	375	375	31.25
Protozoa				
Coccidia	75 (42.8–94.5)	1120	38–6125	840.6
Amoeba	25 (5.4–57.1)	58.3	38–100	14.6
Unidentified	16.6 (2–48.4)	43.7	38–50	7.2
***A. manni*:** 3/5; $_{M}$HR: 2				
Acanthocephala				
Moniliformis sp.	20 (0.5–71.6)	1725	1725	345
Nematoda				
Spirurid-type	20 (0.5–71.6)	6000	6000	1200
Protozoa				
Coccidia	20 (0.5–71.6)	2100	2100	420
Amoeba	20 (0.5–71.6)	38	38	7.5
Unidentified	20 (0.5–71.6)	38	38	7.6
***O. longicaudatus*:** 2/3; $_{M}$HR:1				
Nematoda				
Strongylid-type	33.3 (9.4–99.1)	38	38	12.6
Protozoa				
Coccidia	33.3 (9.4–99.1)	210750	210750	936.6
***L. micropus*:** 1/2				
Protozoa				
Coccidia	50 (1.2–98)	3900	3900	1950
***R. norvegicus*:** 1/1				
Protozoa				
Coccidia	100	75	75	75
Amoeba	100	113	113	113

[a]One individual was not identified to species level, which harboured helminths such as oxyurids, *Strongyloides* sp., Strongilid-type eggs, and Spirurid-type ova; [b]EPG, eggs per gram; OPG, oocyst/cysts per gram.

Morphotypes of helminth eggs included *Rodentolepis* spp. (size mean ± standard error = 42.9 ± 0.6 µm length, 27.2 ± 0.5 µm width; *n* =118; Fig. 2ab), Capillariidae eggs (65.3 ± 0.9 µm, 30.6 ± 0.6 µm; n = 22; Figure 2c), *Trichuris* sp. (65.6 ± 0.6 µm, 33.9 ± 0.6 µm; *n* =20; Figure 2d), *Syphacia* sp. (121.7 ± 7.8 µm, 39.5 ± 4.6 µm; *n* =10; Figure 2e), oxyurid-type 1 egg (100.5 µm, 28 µm width; n = 1; Figure 2f), oxyurid-type 2 eggs (128 ± 5.6 µm, 35.6 ± 11.1 µm; *n* = 3; Figure 2g), *Strongyloides* sp. (51.7 ± 3.2 µm, 28.3 ± 0.7 µm; *n* =29; Figure 2i), Spirurid-type eggs (42.8 ± 0,4 µm, 26.9 ± 0,3 µm; *n* =242; Figure 2j), Strongylid-type eggs (56.5 ± 0.7 µm, 32.5 ± 0.5 µm; *n* =184; Figure 2k), *Moniliformis* sp. (59.5 ± 0.5 µm, 34.4 ± 0.4 µm; *n* = 81; Figure 2l), and unidentified nematode egg (137.23 µm x 21.45 µm; n=1; Figure 2h) and larvae. Protozoa comprised sporulated and non-sporulated coccidia oocysts (19.1 ± 0.2 µm, 15.5 ± 0.2 µm; *n* =446; Figure 2m-q), amoeba cysts (38.9 ± 2.3 µm, 24.8 ± 1.4 µm; *n* = 22; Figure 2r), and unidentified protozoa cysts (30 ± 1.4 µm, 24.6 ± 1.3 µm; *n* = 65) (see Figure 2).

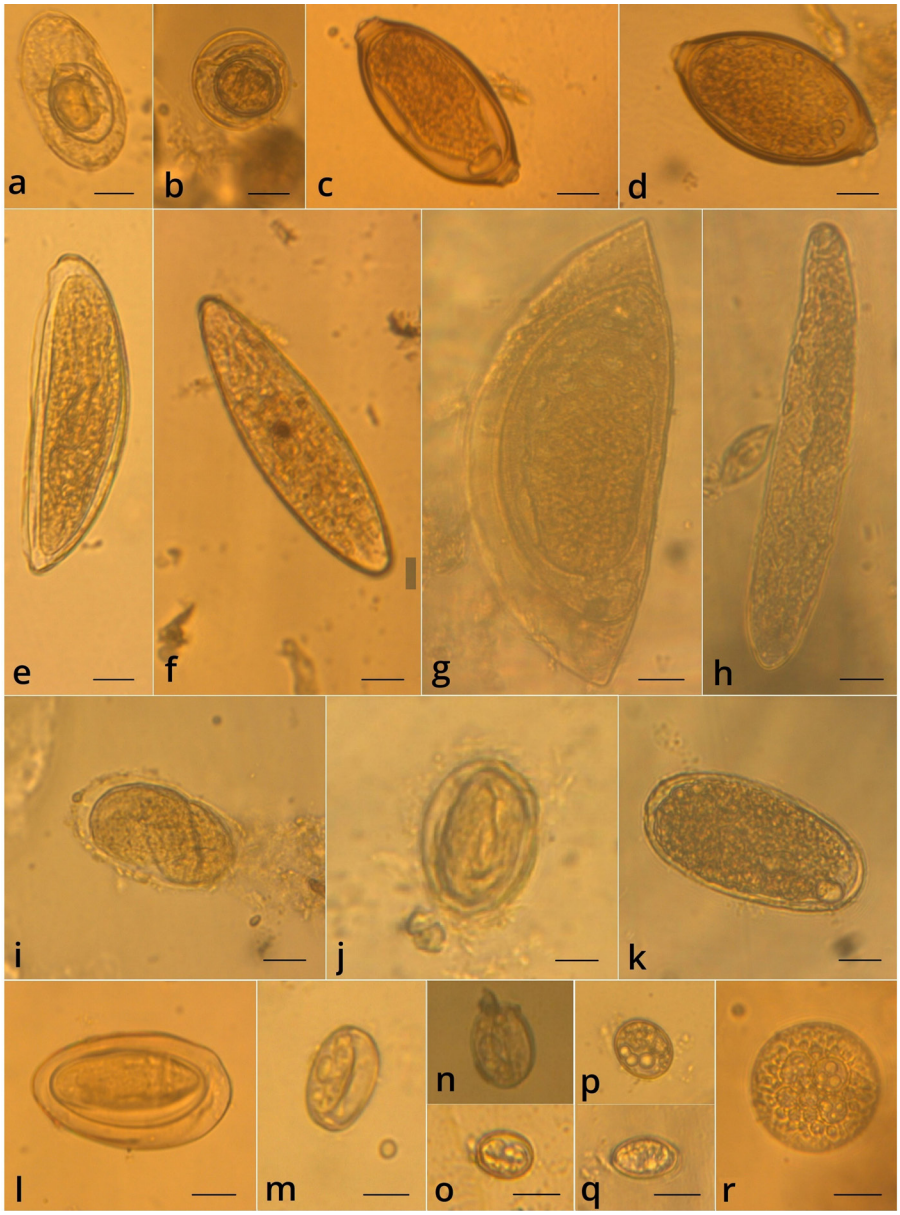

Figure 2. Gastrointestinal parasites in wild rodents in Chiloé Island, Chile (400x): **(a-b)** *Rodentolepis* spp. in *Abrothrix olivacea* and *Geoxus valdivianus,* **(c)** Capillariidae in *A. olivacea*, **(d)** *Trichuris* sp. in *A. olivacea*, **(e)** *Syphacia* sp. in *A. olivacea*, **(f-g)** oxyurid type eggs in *A. olivacea,* **(h)** Unidentified nematode egg in *G. valdivianus*, **(i)** *Strongyloides* sp., in *A. olivacea*, *G. valdivianus,* and *Irenomys tarsais*, **(j)** Spirurid-type egg in *A. olivacea*, *A. manni, G. valdivianus,* and *I. tarsalis*, **(k)** Strongylid-type eggs in *A. olivacea*, *G. valdivianus, I. tarsalis*, and *Oligoryzomys longicaudatus*, **(l)** *Moniliformis* sp. in *A. olivacea*, *A. manni* and *G. valdivianus*, **(m-o)** sporulated coccidia oocysts in *A. olivacea*, *A. manni, G. valdivianus* and *Loxodontomys micropus*, **(p-q)** unsporulated coccidia oocysts in all studied species. **(r)** *Amoeba* cyst in *A. olivacea*, *G. valdivianus*, and *Rattus norvegicus.* Bar 10 µm.

In *A. olivacea*, the most frequent helminth egg morphotypes were Spirurid-type eggs, and the most abundant was *Moniliformis* sp. In *I. tarsalis*, the most frequent helminth eggs were strongilid-type eggs, and the most abundant was *Strongyloides* sp. In *G. valdivianus*, strongilid-type eggs were the most frequent, and *Moniliformis* sp. eggs were the most abundant. Concerning protozoa, coccidia oocysts were the most frequent and abundant in all rodent species, with exception of *R. norvegicus*, in which amoeba cysts were more abundant. *A. olivacea* and *A. manni* exhibited the highest mean helminth richness (2).

Post-mortem findings

A total of 41 rodents were euthanized and examined for gastrointestinal parasites, 33 of which involved *A. olivacea*, six *G. valdivianus*, one *L. micropus*, and one *O. longicaudatus*.

Four helminth taxa were found: *Protospirura* sp., *Physaloptera* sp., *Syphacia* sp. and *Trichuris* sp. by post-mortem examination of gastrointestinal tracts (Figure 3). The estimation of the ecological parameters (p% [Confidence interval], $_{M}$I, and $_{M}$A is shown in Table 3. Confidence intervals for intensity and abundance and the Poulin's discrepancy

Figure 3. Gastrointestinal helminths extracted from *Abrothrix olivacea* in Chiloé Island, Chile: **(a)** *Physaloptera* sp. anterior end, ventral view; **(b)** *Protospirura* sp. anterior end, lateral view; **(c)** *Trichuris* sp. posterior end, lateral view; **(d)** *Syphacia* sp. anterior end, lateral view. Bar: (a) = 0.15mm; (b,c,d) = 0.3 mm.

index were estimated only for *A, olivacea* due to sample size (n>30) (Shvydka et al., 2018). In *G. valdivianus* one *Physaloptera* sp. and four *Protospirura* sp. were found in the same individual, and 12 *Syphacia* sp. were found in another individual. A single *Syphacia* sp. specimen was found in one *O. longicaudatus* and no worms were found in the *L. micropus*.

Table 3. Parasitological descriptors of gastrointestinal adult parasites of rodents subjected to post-mortem examination in Chiloé, Chile. Sample size (N), number of positive individuals (+), percent prevalence (P) and 95% confidence intervals (CI), mean intensity ($_M$I), mean abundance ($_M$A), and the Poulin's Discrepancy Index (PDI) are reported.

Morphotype	P % (CI 95%)	$_M$I (CI 95%)	$_M$A (CI 95%)	PDI
A. olivacea [a]**:** 15/33				
Physaloptera sp.	6.1 (0.7–20)	2 (1–2)	0.12 (0–0.42)	0.93
Protospirura sp.	30.3 (15.6–48.7)	3 (2.2–4.5)	0.9 (0.45–1.69)	0.76
Syphacia sp.	36.4 (20.4–54.9)	26.5 (11.9–58.3)	9.6 (3.8–24)	0.84
Trichuris sp.	27.3 (13.3–45.5)	1.89 (1.22–2.56)	0.52 (0.21–0.94)	0.79
G. valdivianus**:** 2/6				
Physaloptera sp.	16.6 (0.4–64.1)	1	0.16	–
Protospirura sp.	16.6 (0.4–64.1)	4	0.66	–
Syphacia sp.	16.6 (0.4–64.1)	12	2	–
O. longicaudatus**:** 1/1				
Syphacia sp.	100	1	1	–
L. micropus**:** 0/1	–	–	–	–

[a]Confidence interval and PDI was calculated only for *A. olivacea* due to sample size (n>30).

Host factors associated with gastrointestinal parasite infection probability of parasite egg/cyst morphotypes in *Abrothrix olivacea*

Prevalence of most frequent parasite eggs/cysts morphotypes (>5% total prevalence) in *A. olivacea* by age, sex, and scaled body mass index categories (SMI) are shown in Table 4. For GLMER unconditional models to assess the probability of infection, the presence/absence of Strongylid-type eggs were associated with age ($p<0.001$) and SMI category ($p<0.001$). Also, Spirurid-type eggs were associated with age ($p<0.01$) and SMI categories ($p<0.05$). The infection status with Capillariidae, *Trichuris* sp., *Rodentolepis* spp., *Moniliformis* sp., coccidia, *Amoeba*, and unidentified protozoa cysts were not statistically related to any host factor (i.e., sex, age, and SMI categories) (GLMER, $p>0.05$). The GLMER conditional models included the variables primarily associated with the infection status (i.e., host age

Table 4. Prevalence of most frequent parasite eggs/cysts morphotypes (>5% total prevalence) in *Abrothrix olivacea* in Chiloe Island by age, sex, and scaled body mass index categories (SMI).

Parasite	Sex		Age		Scaled Mass Index [a]		
	Female	Male	Juvenile	Adult	High (>60)	Medium (40-60)	Low (<40)
	[n =49]	[n=85]	[n=57]	[n=77]	[n=24]	[n=50]	[n=59]
***Moniliformis* sp.**	10.2%	2.2%	0.8%	5.2%	2.2%	3.7%	-
Capillariidae	4.1%	4.7%	1.8%	9.0%	4.2%	10.0%	3.4%
***Trichuris* sp.**	4.1%	8.2%	1.8%	10.4%	8.3%	12.0%	1.7%
Spirurid-type	26.5%	37.6%	21.0%	42.9%	41.6%	50.0%	16.9%
Strongylid-type	40.8%	31.8%	19.3%	46.8%	54.2%	46.0%	18.6%
***Rodentolepis* spp.**	24.5%	20.0%	14.0%	16.2%	29.2%	28.0%	11.9%
Coccidia oocysts	63.3%	62.4%	70.2%	57.1%	54.2%	56.0%	71.1%
Amoeba	4.1%	10.6%	8.8%	7.8%	8.3%	10.0%	6.8%
Protozoa cysts	24.5%	17.6%	21.1%	19.5%	12.5%	24.0%	20.3%

[a]Excluding one individual that could not be measured morphometrically.

and SMI) as fixed effects and the trapping site as random effect. After simplification of models, results exhibited statistical significance only for the SMI categories (p<0.05) for both Strongylid-type eggs and Spirurid-type eggs. No interaction or confounding effects were found to be statistically significant (e.g., SMI*AGE; SMI+AGE). Values of Odds ratio, CI 95%, and AIC index regarding the SMI categories are shown in Table 5. For Strongilid-like eggs and Spirurid-like eggs, results suggest that individuals with low Scaled Mass Index exhibited a lower risk of infection in comparison with the other SMI categories.

Table 5. Generalized linear mixed models with binomial error showing the scaled mass index (SMI) categories as a factor for the parasitic infection with Spirurid-type eggs and Strongylid-type eggs in *Abrothrix olivacea* (n = 134). Random factor = trapping site.

Parasite	Factor		OR	CI 95%	p	AIC
Spirurid-type	SMI	High				163.6
		Medium	1.4	0.68- 2.84	0.50	
		Low	0.28	0.14-0.58	0.02*	
Strongilid-type	SMI	High				166.9
		Medium	0.72	0.35- 1.46	0.511	
		Low	0.19	0.09-0.39	0.00*****	

OR, odds ratio; CI 95%, confidence interval 95%; AIC = Akaike Information Criteria. ***** p < 0.05.

Discussion

In the present study, it was found gastrointestinal parasites of six native (Cricetidae family: *A. olivacea, I. tarsalis, G. valdivianus, A. manni, L. micropus, O. longicaudatus*) and one introduced rodent species (Muridae family: *R. norvegicus*) in rural areas from Northern Chiloe, Chile. All native rodent species in this study were expected to be sampled according to species distribution (Muñoz-Pedreros & Gill, 2009; D'Elía et al., 2015).

Helminth egg morphotypes and adult specimens found in this study have been previously recorded in rodents in Chile, with exception of *Trichuris* sp. in *A. olivacea*, *Strongyloides* sp. in *A. olivacea*, *G. valdivianus*, and *I. tarsais*, *Physaloptera* sp., *Protospirura* sp., and *Rodentolepis* sp. in *G. valdivianus*; and, *Moniliformis* sp. in *A. manni* taking into consideration that the latter rodent species was recently described (Ruiz del Río, 1939; Babero et al., 1975; Babero et al., 1976; Babero & Murua, 1987; Cattan et al., 1992; Landaeta-Aqueveque et al., 2007a, 2007b; Landaeta-Aqueveque et al., 2014; Seguel et al., 2017; Digiani et al., 2017; Landaeta-Aqueveque et al., 2018; Yáñez-Meza et al., 2019; Riquelme et al., 2021; D'Elía et al., 2015). Overall, the prevalence of each parasite type eggs described in this study was higher than those detailed previously, with exception of *Syphacia* sp. in *A. olivacea* (i.e, 3.7% < *Syphacia phyllotios* = 13.6%) (Yáñez-Meza et al., 2019). Variations in parasite prevalence among studies may be related to different sample size, geographical features, season sampling, and diagnosis methods (Lyles & Dobson, 1993; Morand, 2015; Barelli et al., 2021). To the best author's knowledge, this is the first time that the Mini-FLOTAC® method was used in rodents in Chile, which have shown to have increased sensitivity (i.e., 90%) in comparison to other non-invasive coprological techniques such as formol-ether concentration (60%) (Barda et al., 2013; Catalano et al., 2019). However, comparisons of infection prevalence of parasite species in each host should be made with caution due to the reduced sample size per rodent species in the present study.

Spirurid-type eggs were observed in *A. olivacea, A. manni, G. valdivianus,* and *I. tarsalis.* Spirurid-type eggs were elliptical with thick shells containing well-formed larvae (Figure 2j). Regarding post-mortem analysis from 41 rodents, Spiruridae: *Physaloptera* sp. and *Protospirura* sp. were determined in *A. olivacea* and *G. valdivianus*. *Physalotera* sp. was identified based on the presence of two pseudolabia with a group of dentiform lobes on their middle superior margin and submedian papillae at their base (Figure 3a), and *Protospirura* sp. in accordance with the two large pseudolabia, each divided in three lobes (Figure 3b) and a pharynx slightly lined with chitin (Anderson et al., 2009). On previous records, *Physaloptera calnuensis* was identified in *A. olivacea* and *Mus musculus* in Santiago, Chile (Landaeta-Aqueveque et al., 2007a, b). Also, eggs of *Physaloptera* sp. were identified in *A. longipilis*, *O. longicaudatus*, and *P. darwini* in Central Chile (Riquelme et al., 2021). Additionally, *Protospirura numidicola* was reported in *A. longipilis* at Las Chinchillas National Reserve (Landaeta-Aqueveque et al., 2018) and *Protospirura* sp. in *A. olivacea* and *A. longipilis* in Lago Peñuelas, Auco and Fray Jorge National Park (Cattan et al., 1992). Other Spiruridae genera previously reported in Chile include *Gongylonema neoplasticum* in introduced *Rattus* sp. in Concepción

(Landaeta-Aqueveque et al., 2021), as well as *Gongylonema* sp. in *A. longipilis* at the Fray Jorge National Park, and *Pterygodermatites* sp. in *A. olivacea* in Lago Peñuelas (Cattan et al., 1992). The life cycle of Spiruridae requires arthropods as intermediate hosts (e.g., coprophagous beetles or cockroaches), in which once eggs are ingested, they hatch and become infective, and rodents are infected through ingestion of the intermediate hosts (Taylor et al., 2016). In addition, the acanthocephalan *Moniliformis* sp. was found in *A. olivacea*, *G. valdivianus,* and *A. manni*. Eggs were featured with elongated-oval shape with three membranes (size = 59.5 × 34.4 µm) (Figure 2l). Dimensions agree with *M. clarki* (50–90 × 30–50 µm) and *M. spiralis* (60 × 30 µm) found in Muridae rodents in Missouri-US, and Birmania, respectively (Amin & Pitts, 1966; Guerreiro Martins et al., 2017), and were slightly larger than *M. amini*, described in *A. olivacea* in Santa Cruz, Argentina (Guerreiro Martins et al., 2017). *Moniliformis* spp. require arthropods as intermediate hosts to develop the infective cystacanth stage that subsequently is ingested by the definitive rodent host (Taylor et al., 2016). Also, *Rodentolepis* spp. (syn. *Hymenolepis*) was found in *A. olivacea* and *G. valdivianus*. Eggs of *Rodentolepis* spp. were elliptical with a smooth clear shell wall that contain an hexacanth embryo with six hooks (Figure 1ab). The length of observed *Rodentolepis* spp. eggs were in accordance with dimensions for *R. nana* (i.e., 40–45 µm), but the width was slightly smaller (34–37 µm ≠ 27.2 µm) (Zajac & Conboy, 2012). Also, observed *Rodentolepis* spp. eggs were slightly longer and thinner in comparison to that reported on *R. octocoronata* (37.3 um; 30.6 um) in *Myocastor coypus* in Argentina (Sutton, 1974). The *R. nana* can exhibit direct and indirect life cycles, in which flour beetles or fleas can serve as intermediate hosts (Taylor et al., 2016). Rodent species in this study may have become infected with Spiruridae, *Moniliformis* sp. and *Rodentolepis* spp., since their diet include arthropods, insect larvae, annelids, and other invertebrates (Meserve et al., 1988; Silva, 2005; Muñoz-Pedreros & Gill, 2009).

Trichuris sp. and Capillariidae were found only in *A. olivacea*. The former was identified in both coprologic and post-mortem examination. The shape and dimensions of *Trichuris* sp. and Capillarid-like eggs (Figure 2cd) agree with those cited in the literature, exhibiting bipolar plugs with thick shell (Zajac & Conboy, 2012; Taylor et al., 2016). Adults specimens of *Trichuris* sp. were identified based on a body with a thin anterior part presenting the stichosoma and a thick posterior portion presenting the reproductive and digestive organs, and a characteristic long spicula covered by a specular sheet (Figure 3c) (Anderson et al., 2009). Previously, *Capillaria* sp. had been reported in *A. olivacea* in Chile Central, and *Calodium hepaticum* in *R. norvegicus* (Landaeta-Aqueveque et al., 2021; Riquelme et al., 2021). Conversely, no reports are found about *Trichuris* sp. in *A. olivacea* in Chile, but they have been found in other native and introduced species (e.g., *T. chilensis* in *A. longipilis*, and *Trichuris muris* in *Mus musculus*) (Landaeta-Aqueveque et al., 2021). *Trichuris* spp. and some *Capillaria* spp. show direct life cycles, in which eggs require optimal environmental conditions to embryonate and reach the infective stage (Taylor et al., 2016). Rodents become infected by eating the infective stages on the ground, by cannibalism, or predation in case of *C. hepaticum* (Taylor et al., 2016).

Syphacia sp. was found in *A. olivacea* by examination of both feces and gastrointestinal tracts, and in *G. valdivianus* only by post-mortem analysis. Moreover, two oxyurid-type eggs (Figure 2f-g) were isolated from fecal samples of *A. olivacea*. *Syphacia* sp. eggs (Figure 2e) exhibited smooth clear shell walls with dimensions that concur with morphological keys (*Syphacia* = 100–142 × 30–40 µm) (Thienpont et al., 2003; Zajac & Conboy, 2012), being slightly smaller than *S. obvelata* previously reported in *Mus musculus* in Chile (Landaeta-Aqueveque et al., 2007b), but similar to *S. obvelata* previously described in *A. olivacea* (Landaeta-Aqueveque et al., 2007a), and *S. phyllotios* in *Phyllotis darwini* (Quentin et al., 1979) and in *A. olivacea* (Yáñez-Meza et al., 2019). On post-mortem examination, *Syphacia* sp. was identified in *A. olivacea* and *G. valdivianus* based on the presence of a muscular oesophagus ended in a oesophageal bulb, three labia on the mouth, vulva in the anterior part of the body short after the oesophageal bulb (figure 3d) and characteristic banana-shaped eggs with a subterminal operculum. The life cycle of oxyurids is direct, in which females deposit embryonated eggs on the perineal skins of hosts, and transmission occurs through ingestion of eggs in the perineum, by contaminated food, or when eggs hatch in the perineal region and migrate back via the anus (Taylor et al., 2016). Additionally, *Strongyloides* sp., was determined in *I. tarsalis, A. olivacea* and *G. valdivianus*. Eggs of *Strongyloides* sp. exhibited fully formed larvae with a thin shell (Figure 2i). Dimensions of observed eggs agree with reports for the species (40-60 × 32–40 µm) (Zajac & Conboy, 2012). *Strongyloides ratti* has been previously recorded in *Rattus* sp. in Concepción (Ruiz del Río, 1939; Landaeta-Aqueveque et al., 2021). *Strongyloides* spp. has a direct life cycle which involves both parasitic and free-living reproductive cycles (Taylor et al., 2016). Eggs pass in feces and hatch in the environment where first-stage larvae are released and can develop into the infective third/stage larvae (termed homogonic development) or develop into free-living males and females. Subsequently, adults can mate and their progeny complete moults in the environment until they reach the infective larval stage (L3) (heterogonic development) (Viney, 1999). Hosts become infected via ingestion or penetration of the skin. Although, trans mammary infection also occurs (Zajac & Conboy, 2012). *I. tarsalis* may be inhabiting

locations where environmental conditions are appropriate for the development and survival of parasitic stages of *Strongyloides* sp., thus promoting high parasite burden in individuals. Also, strongilid morphotype eggs were isolated in *G. valdivianus, I. tarsais, A. olivacea* and *O. longicaudatus.* Observed eggs of strongilids were thin-shelled and contain morula (Figure 2k). In Chile, records of Strongylida in Cricetidae rodents are available including *Inglamidium akodon* (*A. olivacea*), *Stilestrongylus manni* (*A. olivacea* and *O. longicaudatus*), and *Stilestrongylus valdivianus* (*L. micropus*) (Durette-Desset et al., 1976; Denke & Murua, 1977; Durette-Desset & Murua, 1979; Landaeta-Aqueveque et al., 2021). Regarding life cycle of Strongylida, eggs are released in feces and develop the infective larvae (L3) in the environment that hosts have to ingest to become infected (Taylor et al., 2016).

Coccidia was common in all studied rodent species. Although most of the observed coccidia oocysts were unsporulated which made it difficult to identify to species level, some oocysts and sporocysts did show sporulation. A coccidia cyst morphotype (Figure 2m) found in *A. olivacea*, *A. manni*, *G. valdivianus* and *L. micropus* presented clear cyst wall with banana-shaped sporozoites and residual body (19.2 × 13.5 µm), which resemble *Sarcocystis* sp. sporocysts (Size = 7–22 × 3–15 µm) (Taylor et al., 2016). Also, oocysts (Figure 2n-o) found in *A. olivacea* contained two sporocysts with no evident stieda body, which suggest *Isospora*-like oocysts (Zajac & Conboy, 2012). In Valdivia Chile, *Giardia muris* (36.8%), *Hexamita muris* (38.6%), *Trichomonas muris* (15.8%), and *Eimeria* sp. (26.3%) have been recorded in synanthropic rodents (n=57) (Franjola T. et al., 1995). Extraintestinal stages of Sarcocystidae have been found in *Thylamys* spp. opossums in northern Chile (Santodomingo et al., 2022). However, to the best authors' knowledge, *Sarcocystis* sp. and *Isospora* sp. oocysts have not been reported in fecal samples of native rodents in Chile. The life cycle of *Sarcocystis muris* (Blanchard, 1885), for example, requires rodents as intermediate hosts and felines as definitive hosts (Powell & McCarley, 1975). Though, *Sarcocystis* bradyzoites can also replicate in rodents, which indicates that transmission also occurs due to cannibalism between rodents (dihomoxenous life cycle) (Koudela & Modrý, 2000). It could be possible that *Sarcocystis*-like sporocyst in rodents in this study may be spurious findings (i.e., cyst that passed through rodents' gastrointestinal tracts), but their life cycles involve carnivore hosts. Additionally, *Isospora peromysci* (Davis 1967) (Protozoa: Eimeriidae) was reported in white-footed mice *Peromyscus maniculatus* (Wagner, 1845) in California, US (Davis, 1967). *Isospora* spp. have a direct life cycle with asexual and sexual reproduction, and hosts become infected by eating infective sporulated oocysts (Taylor et al., 2016). Recent studies on *Isospora* spp. in fecal samples of bank voles (*Myodes glareolus* Schreber, 1780) probed that such finding was a pseudoparasite since it was phylogenetically related to birds and did not replicate in rodents by experimental infections (Trefancová et al., 2019). Thus, *Isospora* oocysts in the present study is likely to be also a spurious finding from bird feces, passing through rodent gastrointestinal tracts. More research should carry out to clarify the origin of these findings. Finally, amoeba cysts were found in *R. norvegicus, A. olivacea* and *G. valdivianus.* Cysts contained a varied number of nuclei (Zajac & Conboy, 2012). *Amoeba* spp. can be transmitted directly by ingestion of viable cysts in contaminated food or water (Taylor et al., 2016).

Interestingly, only *Trichuris* sp. and *Syphacia* sp. were found in both coprologic and post-mortem examination. *Syphacia* sp. was the most prevalent parasite in the post-mortem analysis, which contrasts with the low copro-prevalence, suggesting that the fecal examination have a low sensitivity in detecting their eggs. Maybe, the sensitivity of detection of *Syphacia* sp. in live rodents could be improved with the tape (Graham's) test, which was designed to find eggs added to the anus. The other species found by both techniques was *Trichuris* sp., which was also found more frequently in necropsy than in coprological examination, which agrees with the recommendation of examining at least three serial stool samples per individual (Knopp et al., 2008). This could also be due to the low abundances which mean low loads of eggs. Furthermore, post-mortem examination allowed to identify genera of Spiruridae: *Physaloptera* sp. and *Protospirura* sp, which were difficult only by egg morphological features. Indeed, the second most prevalent parasite obtained through the necropsy was *Protospirura* sp. in *A. olivacea*, which was in accordance with findings in fecal samples in the same species (see Table 2 and 3). Nematodes including Strongylid-type eggs, Capillariidae, and *Strongyloides* sp., could not be determined by post-mortem examination. It is likely that adult parasites degraded due to freezing condition during preservation of gastrointestinal tracts. Also, adult parasites in necropsies could not be identified to species level since some features are still debatable, and there may be some new records for the studied rodent species. For future assessment, molecular diagnosis should be carried out to determinate parasite species and their phylogenetic relationships.

Coprological examination is a useful noninvasive diagnosis method to study endoparasites in wild species. Though, it may have limitations in determining the actual parasite load in comparison to other methods such as necropsy (Zajac & Conboy, 2012; Taylor et al., 2016). Therefore, information in the present study about parasite intensity should be evaluated with caution. Long-term surveys of rodents may be essential to assess endoparasites in eventual mortality to comply with animal welfare standards.

Contrary to what was expected, in *A. olivacea*, individuals classified as low body mass showed less infection probability for Spirurid-type eggs and Strongilid-type eggs. Low body mass individuals may be eating fewer arthropods and contaminated items with infective free-living larvae that might reduce the risks of parasite infection with Spiruridae and Strongylida, respectively.

Findings of the present study serve as preliminary epidemiologic information for future surveys. Several inherent factors of the study limit its explanatory power including a low sample size and the arbitrary time of the year at which the study was carried out. Due to low sample size, the scaled body mass could not be calculated in accordance with age differences (i.e., adults and juveniles). Also, environmental conditions, such as habitat type, vegetation clearance, or arthropod density, were not assessed in this study. Long-term surveys in different seasons with a higher number of sites and rodents may be required to evaluate the influence of habitat degradation on helminth infections and body condition of wild rodents inhabiting Chiloé Island.

Finally, helminths including *Moniliformis* sp., *Rodentolepis* spp., *Strongyloides* spp., *Capillaria* spp. and protozoa such as *Amoeba* sp. have the potential to be transmitted to humans and cause zoonotic diseases (Molavi et al., 2006; Salehabadi et al., 2008; Taylor et al., 2016). Therefore, further studies aiming to identify the species of these parasites should be performed to assess their zoonotic potential. Given that natural environments are continually reducing due to human-induced activities, more research should be carried out to enhance our understanding of parasitic infections in wild rodents inhabiting rural areas and the impact on public health and wildlife conservation.

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Ethics declarations

The project that gave rise to the present data was approved by the Scientific Ethics Committee Resolution for the Animals and Environment Care of the Pontificia Universidad Católica de Chile (Nº 160816007-2017) and the Agricultural and Livestock Service of Chile SAG in 2017 (Nº 7034-2017).

Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship or publication of this article.

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