Mammalian Biology 80 (2015) 255–259

Contents lists available at ScienceDirect

Mammalian Biology

journal homepage: www.elsevier.com/locate/mambio

Original Investigation

Parasitic infection alters rodent movement in a semiarid ecosystem

Carolina Jiménez[a,1], Francisco E. Fontúrbel[a,1], Esteban Oda[a,1], Patricia A. Ramírez[b], Carezza Botto-Mahan[a,*]

[a] Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile
[b] School of Biological Sciences, Victoria University of Wellington, Wellington, New Zealand

ARTICLE INFO

Article history:
Received 4 November 2014
Accepted 18 January 2015
Handled by Danilo Russo
Available online 25 January 2015

Keywords:
Movement behavior
Octodon degus
Parasite-mediated alteration
Phyllotis darwini
Trypanosoma cruzi

ABSTRACT

Parasite-mediated behavioral changes in their hosts have been documented in many species, but field evidence is scarce. The protozoan *Trypanosoma cruzi* is transmitted by insect vectors to several mammal species. Although previous studies have shown high levels of infection in hosts and vectors, it is unknown if this protozoan affects movement behavior of mammal reservoirs. Here we examine, under natural conditions, the existence of movement alterations in two species of rodents (*Octodon degus* and *Phyllotis darwini*) when infected with *T. cruzi*, evaluated for four consecutive years. We found that infected *O. degus* traveled shorter distances than those non-infected, the opposite was found for *P. darwini*. We also detected a strong inter-annual effect for both species. Our results show that rodent species respond differentially to *T. cruzi* infection in regard to their movements, which may have implications in disease spreading.

Introduction

Host behavioral alterations mediated by parasites have been largely documented in the literature (Poulin and Thomas, 1999; Moore, 2002). Specifically, infected intermediate hosts may change their reaction to ecological conditions such as the presence of predators (i.e., parasite definitive hosts). For example, rodents infected with *Toxoplasma gondii* do not escape under the presence of feline urine odor; therefore, they are less likely to avoid a predator attack and more likely to be consumed by felines, the definitive hosts (Vyas et al., 2007). Behavioral changes can also be detected in systems involving hosts and vectors, usually upraising the probability of host–vector contact. This phenomenon has been described on rodents infected with *Plasmodium*, which become lethargic and do not avoid mosquito bites, increasing the number of infected vectors (Day and Edman, 1983). Nevertheless, most studies come from laboratory experiments, and evidence under natural field conditions is scarce.

North-central Chile is considered as a hyper-endemic zone of Chagas disease, where the flagellated parasite *Trypanosoma cruzi*, transmitted by the wild vector *Mepraia spinolai*, infects many native and introduced mammal species (Botto-Mahan et al., 2005b, 2009, 2010, 2012). However, scarce information is available about the effect this protozoan can cause on rodents under natural conditions. We hypothesized that *T. cruzi* infection causes negative physiological alterations on mammals (as Chagas disease in humans), therefore we expect infected sylvatic host mammals to reduce their movement capabilities, related to deteriorated body conditions. In this study, we examine the correlation between *T. cruzi*-infection and movement capabilities of two native rodent species in a semiarid ecosystem, in four consecutive years.

Material and methods

Study area

This study was carried out in Las Chinchillas National Reserve (northern Chile; 31°30′ S, 71°06′ W), a protected area with a semiarid Mediterranean climate and scarce rainfall concentrated between June and August. This Reserve is part of a hyper-endemic zone of Chagas disease in Chile (Botto-Mahan et al., 2010).

Chagas is a vector-borne disease caused by the flagellated protozoan *Trypanosoma cruzi*, and transmitted by triatomine insects (Hemiptera: Reduviidae) to several mammal species (Coura and Vinas, 2010), involving both the domestic and wild transmission cycles (Xavier et al., 2012). The triatomine *Mepraia spinolai* is the main wild vector of *T. cruzi* in Chile, showing infection levels up to

* Corresponding author. Tel.: +56 2 29787392; fax: +56 2 22727363.
E-mail address: cbotto@uchile.cl (C. Botto-Mahan).
[1] Tel.: +56 2 29787392; fax: +56 2 22727363.

http://dx.doi.org/10.1016/j.mambio.2015.01.006

46% (Botto-Mahan et al., 2005b). Several mammal species may act as reservoir hosts of *T. cruzi*, including native rodents, carnivores, marsupials and introduced lagomorphs (Botto-Mahan et al., 2009, 2010, 2012) with prevalence levels up to 71% (Muñoz-Pedreros and Gil, 2009; Botto-Mahan et al., 2010, 2012), with large temporal variability (Botto-Mahan et al., 2010).

The study area is characterized by stony slopes, mainly inhabited by native rodent species such as *Octodon degus* (Octodontidae), *Phyllotis darwini*, *Abrothrix olivaceus*, *Oligoryzomys longicaudatus* (Cricetidae), *Abrocoma bennetti* (Abrocomidae), and the marsupial *Thylamys elegans* (Didelphidae) (Botto-Mahan et al., 2005a), whose *T. cruzi*-infection levels range from 46 to 71% (Botto-Mahan et al., 2010). As *O. degus* and *P. darwini* were the most abundant species (89.4% of the total captures) compared to the other mammal species, we focused our analyses on these two rodent species.

Small mammal trapping and blood sample collection

Small mammal trapping was performed using 300 live-animal-traps (collapsible Sherman traps of 24 cm × 8 cm × 9 cm; FORMA: Products and Services, Santiago, Chile), distributed in three sites (100 traps per site) at the study area. Live-traps were baited with oatmeal flakes and provided with cotton balls for bedding. Traps were arranged on a grid of 100 traps at each site. Each grid consisted of two lines of 50 traps separated 10 m one from each other, covering an area of ~4900 m^2. Small mammal collection was carried out for four to five nights from 19:00 to 09:00 h during the austral summer for four consecutive years, from 2010 to 2013 during the first week of January, which coincides with rodent abundance peak. Capturing and handling procedures met the guidelines of the American Society of Mammalogists (Sikes and Gannon, 2011).

Each *O. degus* and *P. darwini* captured was sexed, weighed, and measured (total and tail length) under short-term isoflurane anesthesia (Jekl et al., 2011). On these individuals, 0.2 ml of blood was withdrawn by (i) saphenous vein puncture for *O. degus* (method recommended for large-bodied rodents), with 21G needle, and (ii) masseteric vein puncture for *P. darwini* (method recommended for small-bodied rodents), with 21G needle (Johnson-Delaney, 2006). Blood sample collection took between 5 and 10 min per subject. All animals were released within 5 h after trap checking; during waiting for release all individuals were provided with fresh vegetables (carrots and cucumber) to avoid dehydration. Individuals were ear-tagged (National Band Tag Co., Newport, KY, model 1005-1, 2.36 mm and 0.25 g) with a unique combination of numbers to exclude recaptures from the analyses, and released in the capture point. Blood extraction procedure was conducted following the international recommendations (Johnson-Delaney, 2006), and authorized by the Chilean Agriculture and Livestock Bureau permits Nos. 0048 and 7462, and National Forest Corporation permits Nos. 32/2009 and 61/2010.

PCR detection of Trypanosoma cruzi *in blood samples*

Whole genomic DNA was isolated from blood samples and stored at −20 °C. The PCR assay was performed as previously reported using primers 121 and 122 to amplify the variable region of minicircle DNA (Veas et al., 1991). Samples were tested in triplicate and to be considered positive, at least two out of the three assays should give amplifications. Samples with only one positive assay were considered negatives or doubtful and repeated three additional times (39% of the *O. degus* samples and 35% of the *P. darwini* samples were repeated). Each trial included positive and negative controls. The PCR products were analyzed by electrophoresis on 2% agarose gels and visualized by staining with ethidium bromide. A 330-basepair product indicated a positive sample.

Data analyses

To quantify the movement of the two rodent species, we estimated the average distance (AD) traveled by rodent subjects. This measure corresponds to the average distance used by an individual per year, based on the geographical coordinates of each trap in which an animal was captured. This was calculated by correlatively adding the lineal distances between each pair of traps ($d_{i,j}$) in which the specimen was captured (for example, if an individual was captured first in trap # 35, then in trap # 4, then in trap # 99, and then in trap # 16, we calculated $d_{35,4}$, $d_{4,99}$, and $d_{99,16}$ distances), divided by the number of captures minus one ($N-1$):

$$\mathrm{AD} = \frac{\sum d_{i,j}}{N-1}$$

This procedure was used for all the rodents showing at least two recaptures (i.e., three or more captures in total). For individuals with only one recapture (i.e., two captures in total), the distance between the two traps were used instead. This study included only specimens of *P. darwini* and *O. degus* with complete characterization, allowing us to calculate their body condition indexes (BCI) as:

$$\mathrm{BCI} = \frac{\text{mass}}{(\text{total length} - \text{tail length})^2}$$

The average distance (Box-Cox transformed), recorded for each individual with two or more captures, was used as response variable for fitting Generalized Linear Models (GLM) that included status (positive/negative) and sex (male/female). BCI and the number of recaptures were included as covariates (histograms of the number of recaptures per species are presented in Fig. S1, available online as Supplementary Material), and year was included as a categorical factor. We also included the status × sex and status × year interactions in the models. We ran separate GLM for *O. degus* and *P. darwini*.

Results

During the sampling period (2010–2013) six small mammal species were captured: *P. darwini*, *O. degus*, *A. olivaceus*, *A. bennetti*, *T. elegans* and *O. longicaudatus* (Table S1, available online as Supplementary Material), being *O. degus* and *P. darwini* the most abundant species. From the 599 *O. degus* and 575 *P. darwini* individuals captured during the four-year sampling period, we obtained a full molecular characterization of 272 *O. degus* and 210 *P. darwini* individuals. Most of those individuals were adults with a few juveniles sampled, the proportion of males varied from 34.1 to 62.5% across years as well as the *T. cruzi* prevalence that varied from 18.0 to 70.4% in *O degus*, and from 18.0 to 61.2% in *P. darwini* (Table 1; detailed information per infection status available at Table S2). Further, *T. cruzi* prevalence in *Mepraia spinolai* (i.e., the vector) colonies was 49.3 ± 6.1% in 2010, 14.9 ± 4.3% in 2011, 47.3 ± 7.0% in 2012, and 20.6 ± 5.6 in 2014.

Infection status has a significant but contrasting effect on *O. degus* and *P. darwini* (Table 2). While infected *O. degus* individuals showed shorter movement distances respect to the non-infected ones, infected *P. darwini* individuals showed larger movement distances (Fig. 1a and c). Further, infected *P. darwini* individuals showed larger BCI values (i.e., weighted more respect to their size) compared to non-infected individuals, but this difference was not found for *O. degus* (Fig. 1b and d). There was no effect of sex or the number of recaptures in any case (Table 2). However, a year effect was noted in both species (Fig. 2).

Discussion

After four years of assessment, we detected a significant association between *T. cruzi* infection status and the movement behavior of the native rodents *O. degus* and *P. darwini* from a semiarid zone of Chile. However, such association was contrasting between species, since movement distances of infected hosts were decreased for *O. degus* as we initially predicted, but in the case of *P. darwini* movement distances have increased. This association also showed a strong inter-annual variation for both species, but there was no correlation with sex.

Table 1
Descriptive information for (a) *Octodon degus* and (b) *Phyllotis darwini*, including the percentage of males and adults in the studied populations for each sampling year. We also present the mean *Trypanosoma cruzi*-infection levels.

	2010	2011	2012	2013
(a) *Octodon degus*				
Males (%)	53.2	37.5	51.3	34.1
Adults (%)	100.0	98.2	92.9	100.0
Infection (%)	70.4	24.4	18.0	64.6
(b) *Phyllotis darwini*				
Males (%)	49.1	62.5	53.3	55.0
Adults (%)	100.0	100.0	71.1	100.0
Infection (%)	61.2	11.4	18.0	20.6

Infected *O. degus* individuals showed shorter movement distances compared to non-infected individuals. This species is colonial and has a social behavior that tends to restrict their movement around family burrows (Vasquez, 1997; Ebensperger

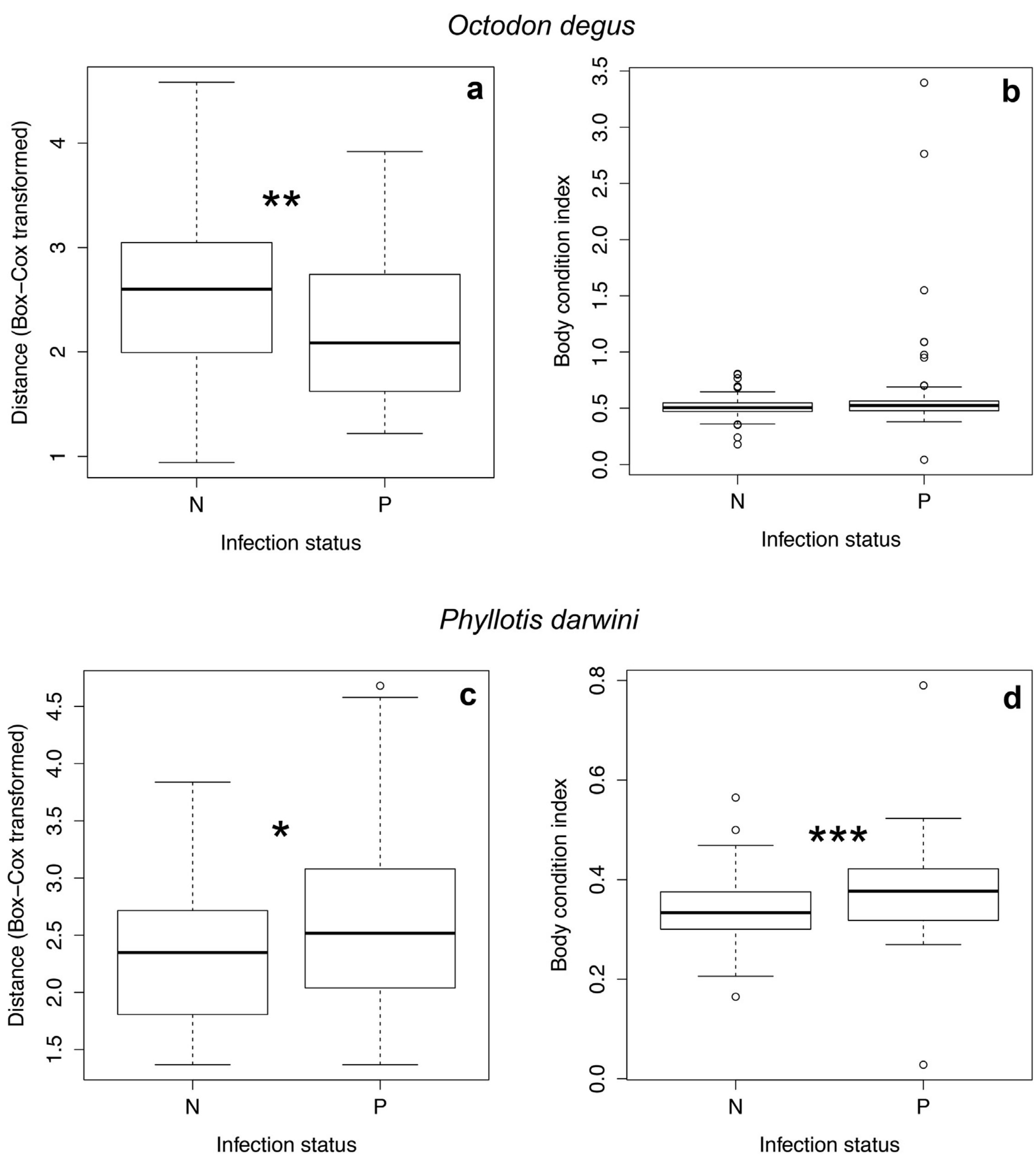

Fig. 1. Effect of infection status (N = negative, P = positive) on average distances and body condition indexes of *Octodon degus* and *Phyllotis darwini* (all years combined). Significance: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 2
Generalized Lineal Models fitted for (a) *Octodon degus* and (b) *Phyllotis darwini* using the average distance as response variable. Factors: infection status (positive/negative), and sex (male/female). Covariates: body condition index (BCI) and the number of recaptures.

	Estimate	Std. error	*t*-Value	*P*-value
(a) *Octodon degus* (*N* = 272)				
Intercept	−679.41	79.28	−8.57	<0.01
Status (P)	−0.22	0.08	−2.75	0.01
Sex (M)	−0.06	0.08	−0.70	0.48
BCI	0.25	0.16	1.58	0.12
Year	0.34	0.04	8.60	<0.01
Recaptures	0.08	0.05	1.77	0.08
Status × sex	0.04	0.18	0.21	0.83
Status × year	0.07	0.08	0.89	0.37
(b) *Phyllotis darwini* (*N* = 210)				
Intercept	334.43	89.41	3.74	<0.01
Status (P)	0.20	0.09	2.10	0.04
Sex (M)	0.03	0.09	0.35	0.73
BCI	2.47	0.66	3.74	<0.01
Year	−0.17	0.04	−3.73	<0.01
Recaptures	0.07	0.04	1.56	0.12
Status × sex	0.20	0.21	1.01	0.32
Status × year	−0.23	0.08	−2.74	0.01

and Bozinovic, 2000; Ebensperger et al., 2012). Similarly, rodents infected with *Plasmodium* became more lethargic and did not avoid mosquito bites (Day and Edman, 1983). In the case of *O. degus*, this reduction in movement distances may modify foraging areas, or impair their capabilities to patrol territory boundaries. Although it is known that *T. cruzi* infection does not affect survival probabilities in this species (Botto-Mahan et al., 2012), we cannot discard the possibility that infected rodents with high parasitemia have high mortality rates. Moreover, they may be quickly purged from the population, as was observed during laboratory experimental infection trials in which those individuals with high parasitemia died within the week after inoculation (Wallace et al., 2001). Therefore, the infected individuals that we captured and examined might correspond to a more tolerant group with low parasite loads. However, this aspect remains to be assessed.

In contrast, infected *P. darwini* individuals showed larger movement distances and greater body mass compared to non-infected individuals. Probably the latter is due to collateral infection effects caused by inflammatory processes, which may produce liquid retention in those organs invaded by the parasites (Roque et al., 2005). Such body mass increase may have two non-mutually exclusive effects on movement behavior: (1) infected individuals might need to travel farther to find food because of an increased energetic demand (Magnanou et al., 2006), and (2) they might need to move beyond their normal range because they cannot maintain a stable territory when occupied by non-infected conspecifics (Rau, 1983). This increase in rodent movement distances may also alter the spatial dynamics of *T. cruzi* infection, accessing to non-infected vector colonies that are beyond the normal movement range of non-infected rodents.

Future studies should consider determining the host threshold parasitic load that triggers changes in movement behavior. Experiments under controlled conditions may shed light on this issue, although this could be challenging to conduct under field conditions. To our best knowledge, this is the first study reporting an association between *T. cruzi* and host movement behavior in natural conditions. Nevertheless, this correlational evidence may be influenced by a large-scale temporal variability related to climate and primary productivity.

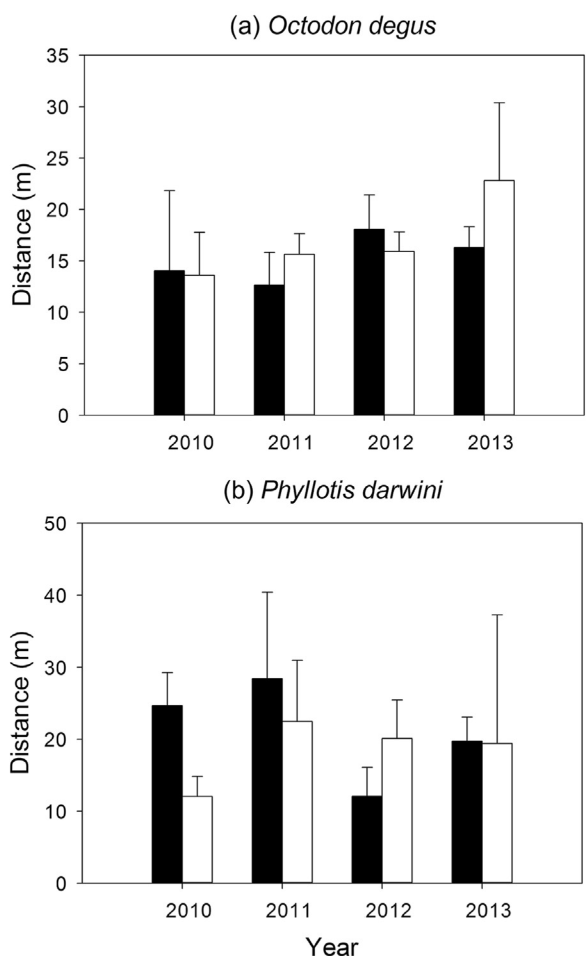

Fig. 2. Temporal variation in movement distance (median ± 1 SE), for infected (black bars) and non-infected (white bars) individuals of (a) *Octodon degus* and (b) *Phyllotis darwini*.

Acknowledgements

We thank all the people who assisted in field and laboratory, and CONAF-Illapel for logistic support. Comments of C. González-Browne, J. Kuzmicic, and two anonymous reviewers improved an early version of the manuscript. Financial support was obtained from FONDECYT 11090086 and 1140521 (CBM). EO was supported by CONICYT doctoral fellowship. A CONICYT-Becas Chile Doctoral Scholarship supported PAR.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.mambio.2015.01.006

References

Botto-Mahan, C., Cattan, P.E., Canals, M., Acuña, M., 2005a. Seasonal variation in the home range and host availability of the blood-sucking insect *Mepraia spinolai* in wild environment. Acta Trop. 95, 160–163.

Botto-Mahan, C., Ortiz, S., Rozas, M., Cattan, P.E., Solari, A., 2005b. DNA evidence of *Trypanosoma cruzi* in the Chilean wild vector *Mepraia spinolai* (Hemiptera: Reduviidae). Mem. Inst. Oswaldo Cruz 100, 237–239.

Botto-Mahan, C., Acuña-Retamar, M., Campos, R., Cattan, P.E., Solari, A., 2009. European rabbits (*Oryctolagus cuniculus*) are naturally infected with different *Trypanosoma cruzi* genotypes. Am. J. Trop. Med. Hyg. 80, 944–946.

Botto-Mahan, C., Bacigalupo, A., Correa, J.P., Oda, E., Solari, A., 2012. Field assessment of *Trypanosoma cruzi* infection and host survival in the native rodent *Octodon degus*. Acta Trop. 122, 164–167.

Botto-Mahan, C., Campos, R., Acuña-Retamar, M., Coronado, X., Cattan, P.E., Solari, A., 2010. Temporal variation of *Trypanosoma cruzi* infection in native mammals in Chile. Vector Borne Zoonotic Dis. 10, 317–319.

Coura, J.R., Vinas, P.A., 2010. Chagas disease: a new worldwide challenge. Nature 465, S6–S7.

Day, J.F., Edman, J.D., 1983. Malaria renders mice susceptible to mosquito feeding when gametocytes are most infective. J. Parasitol. 69, 163–170.

Ebensperger, L.A., Bozinovic, F., 2000. Communal burrowing in the hystricognath rodent, *Octodon degus*: a benefit of sociality? Behav. Ecol. Sociobiol. 47, 365–369.

Ebensperger, L.A., Sobrero, R., Quirici, V., Castro, R.A., Tolhuysen, L.O., Vargas, F., Burger, J.R., Quispe, R., Villavicencio, C.P., Vasquez, R.A., et al., 2012. Ecological drivers of group living in two populations on the communally rearing rodent, *Octodon degus*. Behav. Ecol. Sociobiol. 66, 261–274.

Jekl, V., Hauptman, K., Knotek, Z., 2011. Diseases in pet degus: a retrospective study in 300 animals. J. Small Anim. Pract. 52, 107–112.

Johnson-Delaney, C.A., 2006. Common procedures in hedgehogs, prairie dogs, exotic rodents, and companion marsupials. Vet. Clin. N. Am. Exot. Anim. Pract. 9, 415–435.

Magnanou, E., Fons, R., Feliu, C., Morand, S., 2006. Physiological responses of insular wild black rat (*Rattus rattus*) to natural infection by the digenean trematode *Fasciola hepatica*. Parasitol. Res. 99, 97–101.

Moore, J., 2002. Parasites and the Behavior of Animals. Oxford University Press, New York.

Muñoz-Pedreros, A., Gil, C.C., 2009. Orden Rodentia. In: Muñoz-Pedreros, A., Yáñez, J. (Eds.), Mamíferos de Chile. CEA Ediciones, Valdivia, Chile, pp. 93–157.

Poulin, R., Thomas, F., 1999. Phenotypic variability induiced by parasites: extent and evolutionary implications. Parasitol. Today 15, 28–32.

Rau, M.E., 1983. Establishment and maintenance of behavioural dominance in male mice infected with *Trichinella spiralis*. Parasitology 86, 319–322.

Roque, A.L.R., D'Andrea, P.S., de Andrade, G.B., Jansen, A.M., 2005. *Trypanosoma cruzi*: distinct patterns of infection in the sibling caviomorph rodent species *Thrichomys apereoides laurentius* and *Thrichomys pachyurus* (Rodentia, Echimyidae). Exp. Parasitol. 111, 37–46.

Sikes, R.S., Gannon, W.L., 2011. Care and Use Committee Guidelines of the American Society of Mammalogists for the use of wild mammals in research. J. Mammal. 92, 235–253.

Vasquez, R.A., 1997. Vigilance and social foraging in *Octodon degus* (Rodentia: Octodontidae) in central Chile. Rev. Chil. Hist. Nat. 70, 557–563.

Veas, F., Breniere, S.F., Cuny, G., Brengues, C., Solari, A., Tibayrenc, M., 1991. General procedure to construct highly specific kDNA probes for clones of *Trypanosoma cruzi* for sensitive detection by polymerase chain reaction. Cell. Mol. Biol. 37, 73–84.

Vyas, A., Kim, S.K., Giacomini, N., Boothroyd, J.C., Sapolsky, R.M., 2007. Behavioral changes induced by *Toxoplasma infection* of rodents are highly specific to aversion of cat odors. Proc. Natl. Acad. Sci. U. S. A. 104, 6442–6447.

Wallace, A., Ortiz, S., Sánchez, G., Villagra, R., Muga, M., Solari, A., 2001. Studies on parasitemia courses and mortality in mice infected with genetically distant *Trypanosoma cruzi* clonets. Biol. Res. 34, 83–90.

Xavier, S.C.D., Roque, A.L.R., Lima, V.D., Monteiro, K.J.L., Otaviano, J.C.R., da Silva, L.F.C.F., Jansen, A.M., 2012. Lower richness of small wild mammal species and Chagas disease risk. PLoS Negl. Trop. Dis. 6, e1647.