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Prevalence, infected density or individual probability of infection? Assessing vector infection risk in the wild transmission of Chagas disease

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Vector-borne infectious disease dynamics result mainly from the intertwined effect of the diversity, abundance, and behaviour of hosts and vectors. Most studies, however, have analysed the relationship between host–species diversity and infection risk, focusing on vector population instead of individuals, probably dismissing the level at which the transmission process occurs. In this paper, we examine the importance of the host community in accounting for infection risk, at both population and individual levels, using the wild transmission of the protozoan that causes Chagas disease as a vector-borne disease model. Chagas disease is caused by *Trypanosoma cruzi*, transmitted by triatomine insects to mammals. We assessed if *T. cruzi* infection in vectors is explained by small mammal diversity and their densities (total and infected), when infection risk is measured at population level as infection prevalence (under a frequency-dependent transmission approach) and as density of infected vectors (density-dependent transmission approach), and when measured at individual level as vector infection probability. We analysed the infection status of 1974 vectors and co-occurring small mammal hosts in a semi-arid-Mediterranean ecosystem. Results revealed that regardless of the level of analysis, only one host rodent species accounted for most variation in vector infection risk, suggesting a key role in the transmission cycle. To determine the factors explaining vector-borne disease dynamics, infection risk should be assessed at different scales, reflecting the factors meaningful from the vector's perspective and considering vector class-specific features.

1. Introduction

Vector-borne infectious diseases have been recognized as a major worldwide threat, and their dynamics are most immediately affected by the diversity, abundance, and behaviour of hosts, vectors, and parasites [1,2]. Several studies have suggested that host species diversity may regulate the emergence and prevalence of infectious diseases [3,4], because disease transmission could be reduced as a result of a dilution effect when species diversity increases [2,5,6]. However, not all zoonotic diseases have shown this association [7].

Most studies have assessed disease risk based on infection prevalence at vector population level instead of using vector individual infection probability [5,8–10], precluding to disclose existing associations. A second limitation is that most vector-borne diseases are described by a frequency-dependent rather than

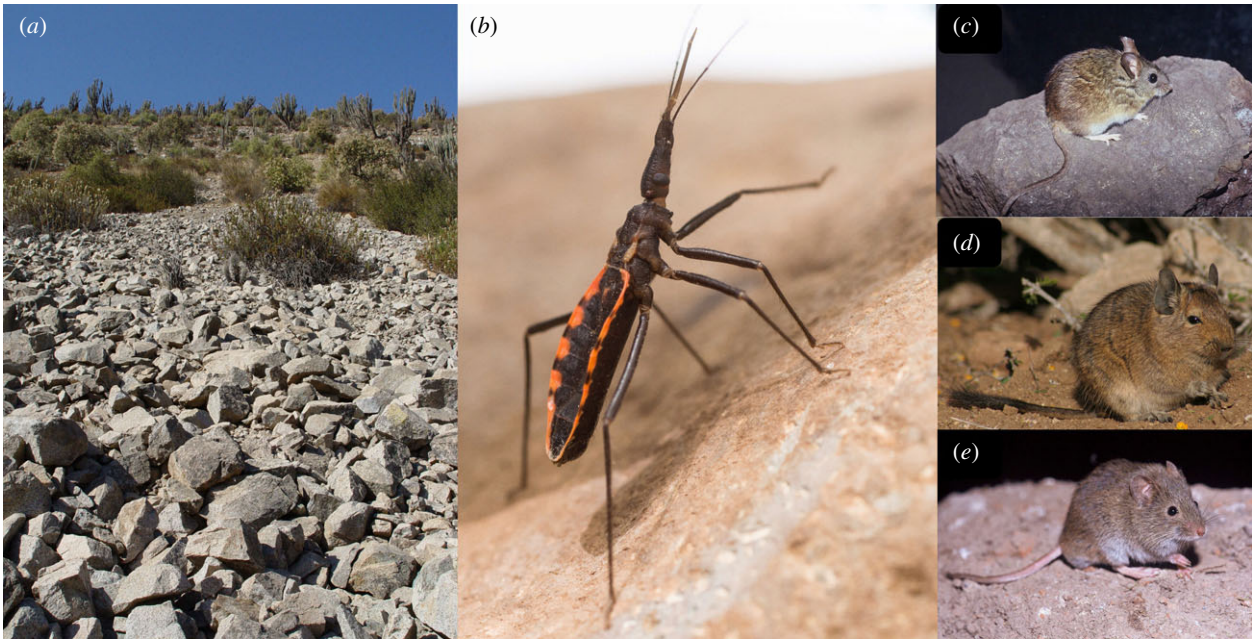

Figure 1. (*a*) Panoramic view of the study site at Las Chinchillas National Reserve, Chile; (*b*) adult male of *Mepraia spinolai*; (*c*) *Phyllotis darwini*; (*d*) *Octodon degus*, and (*e*) *Abrothrix olivaceus*. Photographs by Rodrigo Medel (*a*), Vicente Valdés (*b*), Mariana Acuña-Retamar (*c–e*). (Online version in colour.)

density-dependent transmission. The former is reasonable when vectors actively search for their hosts and compensate for increased host spacing by increasing their travelling distances [3,11]. Unfortunately, most studies on vector-borne diseases do not explicitly consider the classes of vector (e.g. ticks, mosquitoes, or kissing bugs) [12]. Instead, they rely on a frequency-dependent function to represent vector dynamics, causing discrepancies between the expected and observed responses [12]. Therefore, parasite transmission in vector-borne diseases should be examined considering the level of analysis that better represents vector infection risk, taking into account vector-specific life-history traits and behaviour.

Chagas disease, a neglected tropical disease occurring mainly in America, is a zoonotic infection caused by the flagellated protozoan *Trypanosoma cruzi*, transmitted by domestic and sylvatic triatomine vectors (Hemiptera: Reduviidae) to several mammalian species through contact with their infected faeces and urine (dejections) [7,13]. Mammal infection can also occur by congenital transmission and orally when feeding on infected triatomines or dejections. In turn, triatomine vectors become infected when feeding on infected mammals, and by cannibalism and coprophagy [13].

The South American Mediterranean ecosystem (29°–35° S) presents low terrestrial mammal species richness compared to other areas with similar climate [14], and within this ecosystem, approximately 25 wild mammal species geographically overlap with the wild triatomine vector *Mepraia spinolai*. This hemimetabolous insect is diurnal and a restricted-movement species, spatially aggregated in rocky outcrops and bromeliads [15,16], where it exhibits a sit-and-wait predation strategy [16]. Populations of this vector species and native mammal hosts can reach over 70% of *T. cruzi* infection in some areas, with spatial variation in infection prevalence [15–19]. Rodents are represented by nine species, corresponding to the most common and abundant mammal Order [20]. They mainly use similar ecotopes to those used by triatomine vectors, translating into higher chances to be used as blood meals by *M. spinolai* [16,20]. In fact, some rodent species such as *Phyllotis darwini* and *Octodon degus* are frequent blood sources for *M. spinolai*, reaching up to 56% of its diet [21,22], and act as important *T. cruzi* reservoirs, acquiring and maintaining the infection [23–27].

Studies examining the association between host species diversity and Chagas infection risk are scarce and inconsistent. On one side, a study reported a dilution effect for *T. cruzi* infection rate in the triatomine vector *Rhodnius pallescens* in a tropical ecosystem [9]. Conversely, no association was detected between small mammal species diversity and *T. cruzi* infection prevalence in *M. spinolai* [10]. Both studies were based on a vector population approach (i.e. infection prevalence in a point or area), without considering that infection dynamics for a restricted-movement vector may be better represented by a density-dependent transmission model [12]. The goal of our study was to assess if infection risk, measured as: (i) vector infection prevalence (i.e. at population level under frequency-dependent transmission), (ii) density of infected vectors (i.e. at population level under density-dependent transmission), and (iii) vector infection probability (i.e. at individual level), can be explained by host species diversity and host species densities, considering their spatial distribution and relative abundances.

2. Material and Methods

(a) Study system

This study was carried out in Las Chinchillas National Reserve (31°30′ S, 71°06′ W; Chile), a protected area located approximately 60 km east from the Pacific coast, where the wild transmission cycle of *T. cruzi* has been widely documented [10,16–18,28–30]. The climate is semiarid-Mediterranean, with most rainfall concentrated between June and August.

Mepraia spinolai is the only vector of *T. cruzi* described in the study site (figure 1), with infection prevalence ranging from 15% to 76% [17,18]. Its home range shows strong seasonal variation, increasing almost eightfold in summer compared to winter season [16]. Several native small mammal species infected with *T. cruzi* inhabit the study site, such as the rodents *O. degus* (Octodontidae), *P. darwini*, *Abrothrix olivaceus*, *Abrothrix longipilis*, *Oligoryzomys longicaudatus* (Cricetidae), *Abrocoma*

bennetti (Abrocomidae), and the marsupial *Thylamys elegans* (Didelphidae) [18]. *Octodon degus*, *P. darwini*, and *A. olivaceus* are the most abundant rodent species in the study site (figure 1) [18,31], with infection prevalence fluctuating between 46 and 71%, depending on the host species and sampling year [18,29].

(b) Capture and processing of triatomine insects

During the austral summer (January) over four consecutive years (2010 to 2013), we collected *M. spinolai* individuals from a total of 45 colonies (9 colonies in 2010 and 12 colonies per year from 2011 to 2013). Colonies were beneath rocky outcrops composed of hundreds of rocks exposed to hot and dry surface conditions. At each colony, the same trained researcher manually collected triatomine insects during 1-hour span between 1100 and 1600 h, the period of *M. spinolai* maximum activity [32], only on sunny days to ensure equal trapping conditions among colonies. Sampled colonies were separated by at least 30 m to ensure independence, following a previous study reporting a mean maximum travelling distance of 12.3 m during summertime [16]. Therefore, the number of captured insects is an appropriate indicator of colony density. Each colony was geo-referenced in united transverse mercator (UTM) coordinates (WGS84 19S, precision: ±3 m) using a handheld global positioning system (GPS) device (Garmin® Vista Cx). The captured insects were individually stored to avoid potential cross-contamination with *T. cruzi*-infected faeces. In the laboratory, insects were euthanized with a cold shock (−20°C for 48 h) and subjected to abdominal extrusion to obtain intestinal content samples. Each sample was mixed with 200 µl of bidistilled water and stored at −20°C.

(c) Capture and processing of small mammals

Small mammal trapping was performed with wire mesh live-animal-traps (trap dimension: 24 cm × 8 cm × 9 cm; FORMA: Products and Services, Santiago, Chile) baited with rolled oats and equipped with cotton bedding. Traps were numbered, placed in three grids in the surroundings of the sampled *M. spinolai* colonies, and geo-referenced as previously described. Each grid consisted of two lines of 50 traps each; each trap was set 10 m apart with a separation of 10 m between lines from the same grid, covering a total area of 3.39 ha (the three grids combined). This sampling procedure was carried out during four to five nights from 1900 to 0900 h during January 2010, 2011, 2012, and 2013, the month with high mammal activity [33]. We recorded the species and weight of each captured mammal, and blood was withdrawn (0.2–0.5 ml) and preserved from a subset of the captured individuals under short-term isoflurane anaesthesia, using a methodology previously described [34]. Blood extraction procedures followed the international recommendations for mammals [35]. All individuals were ear-tagged and released at the point of capture after full recovery from anaesthesia.

(d) *Trypanosoma cruzi* detection

Whole genomic DNA was extracted from intestinal content (AXYGEN, AxyPrep Multisource Genomic DNA Miniprep Kit, California, USA) and blood samples (AXYGEN, AxyPrep Blood Genomic DNA Miniprep Kit, California, USA), and stored at –20° C for molecular analyses. Parasite detection was performed by conventional PCR with an estimated sensitivity limit of 0.01 parasite equivalent/PCR assay [36]. The amplification reaction was performed in triplicate with oligonucleotides 121 and 122, which anneal to the four conserved regions of the kinetoplast minicircles of all the discrete typing units of *T. cruzi*, as previously described [28,37,38]. A total of 5 µl of the extracted sample was used as the DNA template in 50 µl of final volume. Each experiment included a negative control that contained water instead of DNA and a positive control that contained purified kinetoplast DNA of *T. cruzi* as the template. The PCR products were run by electrophoresis in a 2% agarose gel and visualized in a UV transilluminator by ethidium bromide staining. A sample was considered positive when at least two of the three assays showed amplification of a 330 bp fragment. Samples with only one positive assay were considered doubtful and repeated three additional times.

(e) Overlapping between small mammals and vector colonies

For each year, we introduced the geographic coordinates of the mammal traps and *M. spinolai* colonies into the Geographic Information System QGIS v. 2.18 Las Palmas (http://qgis.org) [39]. To consider the spatial distribution of hosts in relation to vector colonies, we examined the degree of overlapping between the colonies and the captured small mammals. First, we constructed 12.13 m buffers around each vector colony (462 m^2 of area), which corresponds to the mean maximum dispersal distance reported for this triatomine species during the summer season [16]. Then, we calculated the distance travelled by each mammal captured two or more times (recapture distances) in different traps. We excluded from this calculation the small mammals captured several times only in the same trap. With that information, we estimated the maximum recapture distances per small mammal species, grid, and year. Then, we constructed a buffer around every trapping location of each small mammal, using the maximum recapture distances considering the species, grid, and year. After that, we overlapped those buffers with the vector colony buffers to determine if they intersected (irrespective of the extent of the overlapping area). We considered intersecting small mammal individuals as inside the vector colony influence area and therefore potential hosts for that specific colony; otherwise, they were considered not to be influencing that colony. Because we were mostly interested in reflecting blood meal availability to *M. spinolai*, only the intersecting small mammal individuals were considered meaningful from the vector's perspective. Graphical explanation of the overlapping procedure is shown in the electronic supplementary material, electronic supplementary material, figure S1.

(f) Predictor and response variables

Predictor variables were host species diversity, densities of small mammals, and density of infected small mammals. Host species diversity was estimated using the Shannon–Wiener index, including all trapped small mammal species associated with each vector colony area. For the densities of small mammal species to be included as predictors, we considered the most abundant species using a rank-abundance diagram to visualize community composition, and a Biodiversity-gram (BDG hereafter) analysis [40] to assess dominance and diversity relationships in this community. The density of infected small mammals also considered the sum of the most abundant species only, relevant to the vector colony.

As response variables, we used: (i) infection prevalence, calculated as the proportion of infected vectors per colony, (ii) density of infected vectors, calculated as the number of infected vectors captured in a colony occupying a standard area (462 m^2) [16], and (iii) vector infection probability, calculated using the infection status of each vector (0 for uninfected vectors, 1 for those infected).

(g) Statistical analyses

We constructed three models to explain *M. spinolai* infection risk. In the first model, with the prevalence of infection as the response variable (at population level with frequency-dependent transmission), we fitted a mixed-effects generalized linear model (GLMM hereafter) with a Gaussian error distribution and an identity link function, using: (i) host species diversity, (ii) density of *P. darwini*, (iii) density of *O. degus*, (iv) density of *A. olivaceus*, and (v) density of the most abundant infected small mammals as

Table 1. Summary of mixed-effects generalized linear models fitted to assess vector infection prevalence, density of infected vectors (both at population level), and vector infection probability (individual level) (s.e. = standard error; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). See complete information for each model in electronic supplementary material, tables S5, S6, and S7.

	models (estimate ± s.e.)		
predictors	vector infection prevalence	density of infected vectors	vector infection probability
intercept	0.695 ± 0.243**	4.036 ± 0.942***	1.605 ± 1.374
host species diversity	−0.438 ± 0.253	−1.203 ± 1.026	−2.823 ± 1.477
density of *P. darwini*	−0.006 ± 0.003*	−0.038 ± 0.011*	−0.049 ± 0.016**
density of *O. degus*	0.001 ± 0.004	−0.008 ± 0.014	0.022 ± 0.022
density of *A. olivaceus*	0.038 ± 0.027	−0.027 ± 0.110	0.066 ± 0.159
density of infected rodents	0.004 ± 0.005	0.031 ± 0.011*	0.037 ± 0.022

fixed factors, including year as a random factor to account for interannual variation [41]. In the second model, with density of infected vectors as the response variable (at population level with density-dependent transmission), we fitted a GLMM with a negative binomial error distribution and a log link function, the previously mentioned variables as fixed factors, and year as a random factor to account for interannual variation. The third model included the infection probability (either infected or uninfected) of each vector as the response variable (at the individual level), we fitted a GLMM with a binomial error distribution and a logit link function, the previously mentioned variables as fixed factors, and year and vector colony identification as random factors to account for temporal and spatial variation. Additional models tested for each response variable are shown in the electronic supplementary material, table S1.

In addition, we fitted GLM models with a Poisson error distribution to test for interannual variation in the densities of the most abundant small mammal species and *M. spinolai* (total and infected only). GLMM and GLM analyses were performed using R v. 3.5.1 [42] with the *lme4* [43], *mgcv* [44], and *gamm4* [45] packages.

3. Results

(a) Collection of vectors and *Trypanosoma cruzi* infection

We collected and analysed a total of 1974 *M. spinolai* individuals, including all developmental stages from 45 colonies, recording a range of 14.9–49.3% of *T. cruzi* infection prevalence, depending on the year (electronic supplementary material, table S2 and figure S2). We detected statistically significant differences among years in the total number of *M. spinolai* and in the number of individuals infected with *T. cruzi* (electronic supplementary material, table S3).

(b) Capture of small mammals

We captured specimens of seven small mammal species: *P. darwini*, *O. degus*, *A. olivaceus*, *T. elegans*, *O. longicaudatus*, *A. bennetti*, and *A. longipilis*, with a total of 1034 individuals. The rank-abundance and the BDG diagrams showed an uneven pattern, resembling a lognormal distribution, indicating that the small mammal community in the study site had low diversity, dominated by three abundant species (*P. darwini*, *O. degus*, and *A. olivaceus*), which constituted 96.9% of the small mammal community. From those, *P. darwini* and *O. degus* largely dominated the community (electronic supplementary material, figure S3 and table S2), but the three species showed similar capture probabilities (electronic supplementary material, table S4). Then, only these three host species were included as input for the densities of rodents (*O. degus*, *P. darwini*, and *A. olivaceus*, separately) and the density of infected rodents (infected *O. degus*, *P. darwini*, and *A. olivaceus*, combined).

A total of 613 small mammal specimens were spatially associated with vector colonies, in a range of 19–99 individuals of 2–5 small mammal species per colony. Host species diversity (H') ranged from 0.360 to 1.171 in these colonies. Based on weight and an efficient blood withdrawal procedure, we assessed *T. cruzi* infection in 548 of these small mammal specimens, detecting a range of 14.2–74.4% of infection in *O. degus*, 14.7–60.0% in *P. darwini*, and 00.0–60.0% in *A. olivaceus*, depending on the year. Descriptive information on these rodent species by year is shown in electronic supplementary material, table S2. For all three rodent species, we detected statistically significant interannual variation in the total and the *T. cruzi*-infected number of individuals associated with the vector colonies (electronic supplementary material, table S3).

(c) Prevalence, infected density, and individual probability of infection models

Mepraia spinolai infection risk, at population level under a frequency-dependent transmission, showed that density of *P. darwini* had a negative and significant effect on vector infection prevalence ($p = 0.032$; table 1 and electronic supplementary material, table S5). When assessed at population level under a density-dependent transmission, while the density of *P. darwini* also had a negative and significant effect ($p = 0.012$; table 1 and electronic supplementary material, table S6), the density of infected rodents of the most abundant species had a positive and significant effect on the density of infected vectors ($p = 0.028$; table 1 and electronic supplementary material, table S6). At the individual level, again the density of *P. darwini* had a negative and significant effect on the vector infection probability ($p = 0.003$; table 1 and electronic supplementary material, table S7).

4. Discussion

In this study, we assessed the association between *T. cruzi* infection in the wild vector *M. spinolai* and host species diversity, host

densities, and infected host density. Overall, our study site corresponded to a mammal host community with a natural and stable low richness and diversity, mainly dominated by three rodent species. Their densities varied temporally, as well as the density of the most abundant infected rodents. Temporal variation was also detected in the total density of vectors, and in that of infected ones. Therefore, the transmission cycle of *T. cruzi* in this Mediterranean-type ecosystem is highly variable.

Contrary to our expectations, the three models tested did not differ considerably in the relevant predictors explaining vector infection risk. At population level—both in the frequency and density-dependent transmission models—and at individual level, the density of the rodent *P. darwini* explained infection risk. However, only in the density-dependent model the density of infected hosts was a significant predictor. Unlike other studies on movement-restricted vectors [2,3,9], here, we did not detect in any model significant relationships between host species diversity (measured as *H*′) and vector infection risk.

Under the premise of vector-borne transmission as the main mechanisms of *T. cruzi* transmission from mammals to vectors, in our study, we included densities of hosts with relevant features: (i) the most abundant mammal species, (ii) those small mammals reported as the most represented vertebrates in the diet of this generalist and opportunistic bloodsucking insect, (iii) those small mammals infected by *T. cruzi*, and (iv) those using vector-preferred ecotopes [18,21,22,29,30]. At all levels, we detected that vector infection risk relates to the total density of *P. darwini*. This rodent is mainly nocturnal, solitary, and a dispersing species, with herbivorous, granivorous, and insectivorous feeding habits [20]. Under favourable conditions, i.e. high primary productivity as a result of rainfall, this rodent's population size increases [46], implying a high number of uninfected defenceless newborns enter the system, which reduces vector infection risk [29]. Furthermore, the nocturnal behaviour of *P. darwini* would turn this host species into a highly accessible prey for the diurnal vector *M. spinolai* [19]. This hypothetical scenario assumes low or null *T. cruzi* congenital transmission in *P. darwini*, a still untested issue.

As expected, the density of infected rodents (of the most abundant species combined) had a positive effect, but only on the density of infected vectors. The discrepancy between the two tested models at population level may be caused by the use of an appropriate density-dependent approach for this movement-restricted vector. Interestingly, a previous study also detected the densities of the most abundant infected rodent species (separately) as relevant predictors to explain vector infection prevalence [10]. Regarding the infected mammal species included in our study, most of the infected rodents belonged to *O. degus* (56.9%), followed by *P. darwini* (35.5%), and *A. olivaceus* (7.6%). A previous study showed that infected *P. darwini* travels a larger distance than those uninfected, and the opposite occurs for *O. degus* [47]. This could imply that infected *P. darwini* may influence several vector colonies, meanwhile infected *O. degus* would remain as an available blood source for a smaller area. Even though *A. olivaceus* is a less abundant species, some studies have reported extremely high *T. cruzi* infection prevalence [10,19,29], probably with a disproportionate influence on vector infection risk. At this point, we cannot dismiss the possibility that vector preference for infected hosts could be influencing infection in vectors, as reported for *Triatoma dimidiata* [48]. There are reports of feeding preferences in other triatomine species [49], which would modulate the effect of host species, with a probable change in their expected relative importance in relation to their abundances. Some hosts are less competent than others in *T. cruzi* transmission, as their probability of acquiring, maintaining, and transmitting the infection is lower because: (i) they are less frequent blood sources (due to their contact rate and vector preference) and/or (ii) they possess species-specific characteristics (e.g. immune system, parasitaemia, attractant cues, skin thickness, fur density and length, grooming behaviour, repulsion behaviour to vector bites, among others) [12]. To our best knowledge, there are no studies reporting host competence for *T. cruzi* in the Mediterranean of South America, but studies in Argentina suggest that some mammals from the same Subfamily Sigmodontinae as *P. darwini* may be less infectious to vectors than others [50,51]. Future studies should consider the whole potential spectrum of host availability and competence—including other vertebrate groups—and vector preferences in host species diversity and density calculations.

Our proposed methodology comprised buffer areas to discern the relevant hosts for vector colonies. This measure used travelled distances of vectors and small mammals, which may have been underestimated, influencing our results by leaving some relevant hosts out of the analyses. New studies may improve this caveat by including more accurate measurements of vector and hosts' home ranges, or distance-dependent space use probabilities instead of fixed areas. In addition, it has not been determined in the field if all developmental stages of *M. spinolai* move the same distances or with the same frequency, and triatomine nymphs are expected to be movement restricted when compared to adults [52]. This fact could affect the probability of stage-specific vector–host contact.

Given that our study site is embedded in a protected area, with almost null domestic mammal species influence, we considered only—but not all—native small mammal species to estimate our predictors. For example, due to conservation status, we were unable to capture the endangered species *Chinchilla lanigera*, which has also been reported to be infected with *T. cruzi* in the study site [53]. On the other hand, the free-ranging invasive European rabbit, *Oryctolagus cuniculus*, might be an important host species, due to its high abundance and *T. cruzi* infection in the Mediterranean ecosystem of South America [24]. Unfortunately, rabbits were not captured with the trap type used.

To assess vector infection risk in the transmission of infectious diseases, different scales of analyses and the correct transmission model should be considered to detect the appropriate process level at which transmission is occurring. We suggest triatomine density (total or infected), and the density of mammals coexisting with triatomine bugs would be an appropriate metric to compare our findings across different sylvatic habitats (e.g. rocky outcrops, bromeliads, palm trees, among others) with different host and vector species, especially considering the dispersal-restricted feature of most individuals in a colony [19,54,55], and that most triatomine bugs would take blood meals from what is offered nearby their colonies [10]. Our results support the selection of epidemiologically more relevant host species when studying the transmission of vector-borne infectious diseases. In our study, we were able to unveil *P. darwini* as a relevant host species, at population and individual levels of analysis, explaining the reduction of *T. cruzi* infection risk in the Mediterranean ecosystem of South America.

Ethics. The Ethical Committee of the Faculty of Science of the University of Chile, the Chilean Agriculture and Livestock Bureau (SAG, permit resolutions 0048 and 7462) and the National Forest Corporation (CONAF, permit resolutions 32/2009, 61/2010, 105/2012) reviewed and approved the animal-handling protocol for this study.

Data accessibility. The datasets (Botto-Mahan_figures_tables_ESM1.pdf Botto-Mahan_data_ESM2.xlsx) supporting this article have been uploaded as electronic supplementary material.

Authors' contributions. C.B.M. conceived and designed the study, coordinated the study, collected field data, and wrote the manuscript. A.B. and J.P.C. participated in the design of the study, collected field data, participated in data analysis, and wrote the manuscript. F.E.F. carried out the statistical analyses, wrote part of the manuscript, and critically revised the manuscript. P.E.C. and A.S. helped to conceive the study and critically revised the manuscript. All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Competing interests. The authors have no competing interests.

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