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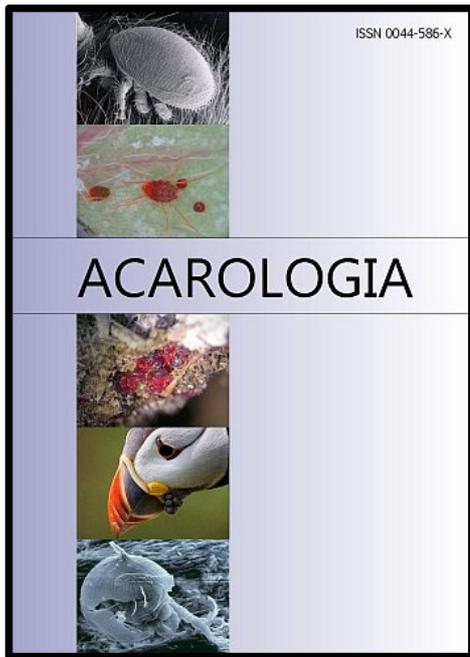
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Fragmented host distribution and trombiculid parasitic load: *Eutrombicula araucanensis* and *Liolaemus pictus* in Chile

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ABSTRACT — Habitat fragmentation produced by human impacts can generate changes in the distribution and abundance of populations across their geographic ranges. The chigger mites are ectoparasites that are widely distributed on a wide range of species. The distribution depends of the characteristics of the host and of the habitat where said species is found. As the host distribution is highly dependent on environmental factors, chigger mites are good study models to test geographical distribution patterns. The main objective of this study was to determine variation in abundance of *Eutrombicula araucanensis*, parasite on *Liolaemus pictus*, by testing three hypotheses: abundant centre hypothesis, abundance optimum hypothesis and latitudinal distribution pattern. For this purpose, mites from 147 individuals from 23 localities along the distribution of *L. pictus* were extracted. The three distribution models evaluated in the present study were not adequate to represent the geographical structure of parasitic mites on *L. pictus*. No association between the distance from the area of greatest species abundance and relative intensity, or association with the distance to the mite's distribution centre was observed. In addition, no latitudinal distribution pattern was observed. Temperate forest fragmentation may influence the population density of mites, with each forest patch having different optimal conditions for development. The heterogeneity makes it difficult to find a clear distribution pattern.

KEYWORDS — *Eutrombicula araucanensis*; density; distribution; *Liolaemus pictus*; fragmentation

INTRODUCTION

The abundance of species varies across their geographical distribution, where high densities in some localities are observed, while in other places they are sparsely scattered (Hengeveld and Haeck 1982; Rapoport 1982; Sagarin *et al.* 2006). Three parameters, prevalence, intensity, abundance of parasites vary among the different host populations, but within species-specific bounds (Arneberg

et al. 1997; Krasnov *et al.* 2006; Bordes *et al.* 2010). Different models were used to explain the distributional patterns. The "central-marginal" or "abundant-centre" hypothesis (ACH) when the highest abundance is in the centre of their geographical range, where the environmental conditions are supposed favourable, decreasing towards the periphery, when habitat conditions became sub-optimal (Brown 1984; Sagarin and Gaines 2002). Because a complex of limiting factors determines

geographic ranges, the geometric centre of a geographic range does not necessarily coincide exactly with supposed most favourable conditions. Inside this area of distribution, several localities may present highly favourable conditions (Brown 1995; Brown *et al.* 1996; Sagarin *et al.* 2006; Tuya *et al.* 2008). The "abundance optimum" model (AOH) assumes that the greatest abundance of species occurs in these localities under the most favourable conditions and abundance is decreasing with the distance from these favourable sites (Sagarin *et al.* 2006; Krasnov *et al.* 2008a). This model has been supported by different parasites like fleas and mites, but not for intestinal helminthes (Poulin and Dick 2007; Krasnov *et al.* 2008b). Furthermore, latitudinal patterns, traducing the climatic gradient, have been frequently demonstrated in a larger number of taxa of free-living organisms and parasites (Krasnov *et al.* 2006; Poulin and Dick 2007; Merino *et al.* 2008; Salkeld *et al.* 2008; Bordes *et al.* 2010). Some studies have documented that the abundance is a species-specific attribute and would not present any variation among the different populations (Arneberg *et al.* 1997; Krasnov *et al.* 2006; Vinarski *et al.* 2007; Korallo-Vinarskaya *et al.* 2009; Krasnov and Poulin 2010).

Moreover, the distribution and abundance of species across geographic range can be affected by habitat fragmentation, because that is a process where a large continuous area of habitat is reduced and divided into smaller patches. The remaining patches no only differ in size from the original area by being smaller, they also have a greater proportion of edge in relation to the total area, and that the edges are closer to another edge than the previous habitat (Paterson 2012). As a result, the landscape fragmentation reduced host local population, decreasing the availability and the probability of parasite transmission between hosts, affecting the population dynamics of parasites (Arneberg *et al.* 1998), which may lead local extinction of parasites (Dobson and Pacala 1992).

The south-central Chile, for over 50 years, has undergone habitat fragmentation product of land-cover change for agricultural and forest crops (Echeverria *et al.* 2006), this has caused many

natural populations of animals change over time. Among the species affected by fragmentation is *Liolaemus pictus* Duméril and Bibron, 1837 (Liolaemidae) which product of habitat fragmentation has reduced its population size and geographic distribution and currently not recorded in the southern Chilean intermediate depression. This lizard inhabits temperate and cold zones (Kubisch *et al.* 2011) in the *Nothofagus* forest in Chile and the conifer woodland in Argentina. Chigger (larval Trombiculidae) often parasitizes *L. pictus*. The chigger are obligate ectoparasites in larval instar, whereas nymphs and adults are free-living predators (Wharton and Fuller 1952; Shatrov and Kudryashova 2006), therefore changes in microhabitat by fragmentation can affect their survival. In the present study, we examined the variation in infestation patterns of *Eutrombicula araucanensis* Stekol'nikov & González-Acuña, 2010 on the lizard *L. pictus* across the geographical range in Chile, to evaluate distribution pattern in a fragmented habitat.

MATERIALS AND METHODS

Sample collection.

Liolaemus pictus is distributed in Argentina (Río Negro Province) and in Chile, from the Vilches Alto (35°35'S 71°05'W) to the Futaleufú National Reserve (43°15'S 71°47'W), and from the islands of the Chiloé Archipelago and Mocha Island (Veloso and Navarro 1988; Pincheira-Donoso and Núñez 2005; Elgueta *et al.* 2006). We analysed a total of 147 specimens of *L. pictus*, collected at 23 localities in different periods and years (Table 1) across the latitudinal range of the species (Figure 1). After collection, lizards were weighted, measured in their snout-vent length (SVL) using a digital calliper (to the nearest 0.1 mm). In the field, the lizards were fixed in 96% ethanol in order to kill the chigger mites immediately before they could detach from the host. Voucher specimens were catalogued in the Museo de Zoología de la Universidad de Concepción (MZUC-UCC, Chile). Geographical coordinates for each site were taken with a hand-held Global Positioning System receiver (ETrex, Garmin Ltd, Olathe, USA).

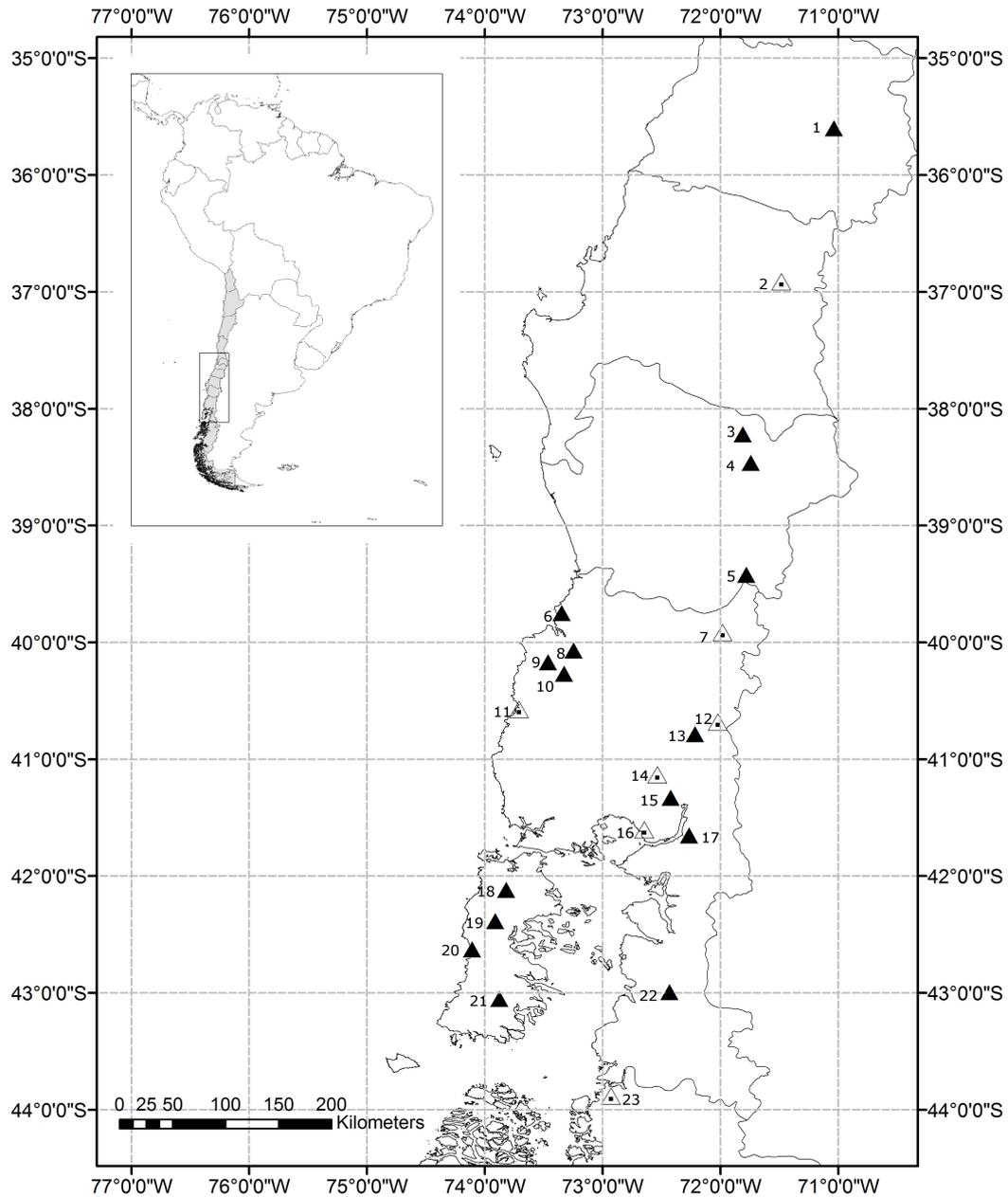


FIGURE 1: Sample sites of *Liolaemus pictus*. White triangles show the localities where none were recorded *Eutrombicula araucanensis*. 1. Altos de Lircay National Park (N. P.); 2. Las Trancas; 3. Curacautín; 4. Malalcahuello; 5. Villarrica N. P.; 6. Oncol Park; 7. Panguipulli; 8. Valdivia Reserve; 9. Alerce Costero; 10. Hueicolla; 11. San Juan de la Costa; 12. Puyehue N. P.; 13. Puyehue; 14. Osorno Vulcano; 15. Llanquihue National Reserve (N. R.); 16. Puerto Montt; 17. Río Puelo basin; 18. Ancud; 19. La Chacra; 20. Chonchi; 21. Tantauco Park; 22. Chaitén; 23. Cisnes.

TABLE 1: Number and date of capture *Liolaemus pictus* from 23 locations in Chile. Bold show the localities where were recorded *Eutrombicula araucanensis*.

Locality name	<i>n</i>	date capture
Altos de Lircay N. P.	13	april-2013
Las Trancas	4	april-2009
Curacautín	7	march-2007
Malalcahuello	4	january-2008
Oncol Park	5	march-2008
Villarrica N. P.	3	march-2007
Panguipulli	3	november-2010
Valdivia Reserve	5	january-2008
Alerce Costero	8	december-2008
Hueicolla	5	december-2008
San Juan de la Costa	5	march-2007
Puyehue N. P.	12	november-2011
Puyehue	7	march-2007
Osorno Vulcano	12	november-2011
Llanquihue N. R.	9	november-2011
Puerto Montt	10	march-2007
Río Puelo basin	2	march-2008
Ancud	10	march-2007
La Chacra	4	march-2007
Chonchi	3	march-2007
Tantauco Park	7	january-2008
Chaitén	8	march-2007
Cisnes	1	march-2007

Each lizard's body was completely and carefully checked for mites under stereomicroscope. 940 mites were collected using forceps and all were cleared in Nesbitt's solution and mounted on permanent slides in Berlese medium (Krantz and Walter 2009), for subsequent identification using taxonomic key and description from Brennan and Reed (1974), Hoffmann (1990), Krantz and Walter (2009) and Stekol'nikov and González-Acuña (2010).

For each locality, three parameters were calculated: the mean abundance (= mean number of parasites per host, including uninfested ones), the prevalence (= proportion infested hosts) and mean intensity of infestation (mean number of parasites per infested host) (Bush *et al.* 1997). Each value was

expressed as proportion of the maximum value, in order to obtain relative and comparable measures.

Data analyses.

Statistical tests were executed in the software JMP 7.0.1 (SAS Institute Inc.). To satisfy the assumption of normality relative abundance and prevalence were log-transformed. Even when transformed, they were not normally distributed. Consequently, we applied non-parametric statistics. Spearman's rank correlation coefficient was used to assess the relationships between sample size and the population density of mites. The population density was not affected by the sample size (prevalence: $\rho = 0.03$, $p = 0.89$; mean abundance: $\rho = 0.07$, $p = 0.79$; mean

TABLE 2: Prevalence, abundance mean and intensity mean of *Eutrombicula araucanensis* at different localities in Chile and distance geographic (km) to the centre of the region with maximum abundance (A), maximum intensity (B) and to the centre of the geographic range (C).

Locality name	<i>n</i>	Prevalence	Mean	Mean	A	B	C
		(%)	abundance	intensity			
Altos de Lircay N. P.	13	77	8.54	11.10	817.52	644.11	426.03
Malalcahuello	4	25	3.25	13.00	505.49	323.13	107.95
Curacautín	7	100	22.29	22.43	527.61	349.23	134.66
Oncol Park	5	40	1.80	4.50	326.07	191.92	126.29
Villarrica N. P.	3	100	6.33	6.33	407.77	218.4	13.3
Valdivia Reserve	5	20	0.80	3.00	293.67	155.83	133.23
Alerce Costero	8	75	4.25	5.50	269.44	155	155.2
Hueicolla	5	40	7.60	19.00	273.28	140.08	151.13
Puyehue	7	57	7.71	12.75	259.94	62.37	152.98
Río Puelo basin	2	50	3.50	7.00	186.57	38.7	248.26
Llanquihue N. R.	9	78	25.11	32.00	191.82	0	212.75
Chaitén	8	13	0.25	2.00	142.67	184.43	396.76
Ancud	10	50	2.20	4.40	60.97	145.81	338.03
La Chacra	4	50	3.50	7.00	31.79	171.04	368.06
Chonchi	3	100	26.67	26.67	0	200.4	398.3
Tantauco Park	7	100	22.43	22.43	50.47	224.13	433.17

intensity: $\rho = -0.08$, $p = 0.75$). We compared mean intensity and mean abundance between localities by the Kruskal-Wallis test and prevalence between localities using on Chi-square test.

AOH was tested following Poulin and Dick (2007). Prevalence was not analysed, because the value of 100% was recorded in several sites. The locality with highest abundance and maximal intensity was used as a reference point for the other localities. The geographical distance between each locality and the site of maximum intensity or abundance was estimated by the linear distance obtained from a topographic map. Intensity and abundance in each locality are expressed by the relative value. Spearman's rank correlations coefficients were computed between the relative values and the log-transformed distance to the site of maximum intensity or abundance (Poulin and Dick 2007) but the values from the region of maximum abundance or intensity generate a spurious negative relationship due to the occurrence of the highest recorded value at a zero distance and were not included (Krasnov *et al.* 2008b).

To test the abundant-centre hypothesis (ACH), the latitudinal position of the centre of the geographic range from distribution maps using the Diva-Gis 7.5.0 software was determined (Krasnov

et al. 2008a). The remoteness between localities and the centre of the geographic range was calculated as the linear distance measured on a topographic map. Spearman's rank correlations coefficients were computed between the relative values (prevalence, abundance and intensity) and the log transformed distance to centre of the geographic range

To account for possible latitudinal gradient, we evaluate too the correlation between prevalence, intensity and abundance of infestation, and the latitude of the sampled locality.

RESULTS

Of the 147 lizard examined, 37.41% ($n=55$) were infested with *E. araucanensis* and 8.84% ($n=13$) with *Pterygosoma* sp. (Trombidiformes: Pterygosomatidae). A total of 940 mites were collected, of which 797 corresponded to *E. araucanensis*. Parasitological descriptors are given in Table 2. The mites occurred in 16 at 23 localities included in our dataset (Fig. 1). Intensity and abundance varied significantly among localities (KW test, $p < 0.0004$). The highest values of abundance and intensity of infestation occurred in Chonchi (Chiloé) and Llanquihue National Reserve, respectively. The maximal value

of prevalence (100%) was recorded in three localities: Curacautín, Tantauco Park and Villarrica National Park. There was no correlation between either relative intensity ($\rho = 0.3363$, $p = 0.2203$) or relative abundance ($\rho = 0.1144$, $p = 0.684$) and the distance from the locality showing the maximum value. There is no association between either relative intensity ($\rho = 0.3208$, $p = 0.2257$) or relative abundance ($\rho = 0.1694$, $p = 0.5306$) and distance to centre of the geographic range. No latitudinal gradient was observed for mite prevalence ($\rho = 0.0159$, $p = 0.9534$), intensity ($\rho = -0.0082$, $p = 0.9758$) or abundance ($\rho = 0.0255$, $p = 0.9255$).

DISCUSSION

The proportion of infested hosts and the mean number of parasites per host are not fixed values across the geographic range of a parasitic species and distribution of species depends on a pool of environmental factors (Krasnov and Poulin 2010). As a result, the presence of *L. pictus* did not guarantee the presence of the parasite, absent from seven sampling sites. The three tested models were not adequate to represent the geographical structure of the distribution of the mites on *L. pictus*. In literature, AOH model was previously evaluated as for both endoparasites and ectoparasites (Poulin and Dick 2007; Krasnov *et al.* 2008b), but without finding a clear distribution pattern. The plausible explanation would be that the distribution and fitness of the ectoparasites depend on the host and on the environmental conditions of the host's habitats. Despite this, the AOH was not supported by ectoparasites either (Krasnov *et al.* 2008b), with solely a significant negative association between the distance from the locality with the greatest abundance and relative abundance in one species of flea (*Catallagia dacenkoi*) and two parasitic mites of mammals (*Echinonyssus eusoricis* and *Hyperlalelaps amphibius*) has been found.

The CMH (central-marginal hypothesis) has been tested by some authors, being supported only by a 39% of the studies (Sagarin and Gaines 2002). This model suggests that the decrease in the density of the hosts in the marginal areas of distribution could result in limited contact between infected

and uninfected hosts. For example, González and Moreno (2005) mention that the fish population in the central areas could be much more interactive, leading to higher rates of infection.

The latitude has been considered as the main factor influencing the diversity and the distribution of parasites, because of the variation of the temperature, which increases often abundance of parasites from the coldest to the warm conditions (south to north in the South Hemisphere) or vice versa (Poulin and Dick 2007). This pattern was not observed in mites of *L. pictus*, despite the temperature increases along the distribution of *E. araucanensis* in Chile, on average 4.2°C from the southern distribution limit (Tantauco Park entrance) to the northern limit (Altos de Lircay National Reserve) whereas relative humidity decreases in 10% (Di Castri 1968); therefore, the mite abundance is expected to be affected. Larval population densities of *Eutrombicula alfreddugesi* (Oudemans 1910) a parasite of lizards have been observed favoured under forest environments with high relative humidity, mild temperature, low incidence of sunlight and an increase in the vegetation of the substrate in south-central Chile (Clopton and Gold 1993; Murcia 1995).

On *L. pictus* differences in prevalence, abundance and intensity of infestation among localities were observed, related by other authors to the oscillations that mites' populations suffer from the different habitat of lizards (Carvalho *et al.* 2006). The localities with high abundance of parasites were from the extreme northern and southern points, as in localities nearby to the geographical distribution centre of the mite. The geographic centre does not necessarily coincide with the location of maximum abundance as in Chonchi, in correlation with the fact that no geographical distribution pattern was found, this supports and affirms that the biological "characteristics" of the parasites can cancel out the effect of local environmental conditions in the population dynamics of the parasite (Krasnov *et al.* 2006). The abundance of the parasites strongly depends of the availability of their hosts, which in turn, is spatially variant (Krasnov *et al.* 2002; Stanko *et al.* 2006): the distribution of *L. pictus* is discontinuous and fragmented partially due to the an-

thropogenic disturbances. The fragmentation of the habitat has modified the ecological factors (temperature, humidity and light incidence) which differ in each patch, and may disturb the theoretical regular distribution (Rubio and Simonetti 2009). Differences in the parasite load in lizards have been found, with a lower intensity on the edge of the forest and fragments, attributed to the differences in the microclimatic conditions with higher maximum temperature and lower humidity in fragment edges when compared to the interior or the edges of the forest (Rubio and Simonetti 2009). Similar results were found by on *Ameiva festiva* Lichtenstein 1856 (Squamata: Teiidae) in meadows compared with secondary forest and between urban and suburban environments in Brazil on *E. alfreddugesi* hosted by lizards (*T. torquatus*) (Carvalho *et al.* 2006; Ramirez-Morales *et al.* 2012); the difference is explained by the susceptibility of this mite to the environmental variations, introducing changes in density as the environment is more degraded (Clopton and Gold 1993).

Did the seasonality have affected the result? The specimens were collected from November to April and local abiotic conditions may regulate the variation in abundance, prevalence, and intensity in a parasite species (Pietroock and Marcogliese 2003; Waltari and Perkins 2010). Nevertheless, variation in intensity and abundance among parasite populations of the same species are sufficiently repeatable to be considered as a character of the species (Krasnov *et al.* 2006; Krasnov and Poulin 2010). By contrast, prevalence does not appear to be a repeatable characteristic among populations (Arneberg *et al.* 1997).

In conclusion, it is evident that in some areas, the parasite abundance is higher than in other places, but a clear pattern of the distribution has not been possible to establish.

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