







New records of *Gigantolaelaps wolffsohni* (Mesostigmata: Laelapidae) in Chile, an ectoparasite of *Oligoryzomys longicaudatus* (Rodentia: Cricetidae): ecological aspects and relation to body size and sex of their host

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Original research

ABSTRACT

The *Gigantolaelaps* mites (Mesostigmata: Laelapidae) are easily recognized by their large size. In Chile, *Gigantolaelaps wolffsohni* is the only recognized species *Gigantolaelaps* and it has only been reported from four localities, always in association with *Oligoryzomys longicaudatus*. Our research study aimed to contribute to the severely limited information about the ecology and distribution of *G. wolffsohni* in Chile and determine whether factors such as season, ecological region and weight-and sex of the host influence the abundance of this mite. We captured and assessed 329 *O. longicaudatus* in different seasons and localities. We classified sampled localities as being part of the Mediterranean, Template Forest, or Magellanic Forest ecoregion due to reported genetic and morphological differences of *O. longicaudatus* between ecoregions. Information about the season, ecoregion, and weight and sex of the host were recorded during mite sampling. These variables were assessed to determine their influence on mite abundance and prevalence through negative binomial and logistic regression models. We found 19 new localities where *G. wolffsohni* is distributed in Chile. These mites displayed an aggregated distribution in all ecoregions. We found that biogeography, weight, and sex of the host are not related to the abundance and prevalence of *G. wolffsohni*. Nonetheless, we found a strong association between the season and the abundance and prevalence of mites. All mites found were females; additionally, we found larva stage inside some female mites. We discussed the relationship between different ecological aspects and the abundance and prevalence of ectoparasites. Also, we discussed the finding of the larvae of *G. wolffsohni*, which means that this specie is at least larviparous, and finally, we discuss the specificity of Laelapidae mites.

Keywords host sex; host weight; long-tail pygmy rice rat; mite ecology; parasite ecology

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Introduction

The Laelapidae family (Acari: Mesostigmata) is a big and cosmopolitan group of mites with diverse habitats and lifestyles (Dhooria 2016; Lindquist *et al.* 2009; Wall and Shearer 2001). They are the most common ectoparasites of marsupials and rodents (Lareschi 2017). Usually, mites from this family are associated with New-World rodents (Cricetidae: Sigmodontinae: Oryzomyini) and Old-World rodents (Muridae: Murinae) (Gettinger *et al.* 2011). The Neotropical region holds a wide diversity of Laelapidae mites (Gettinger *et al.* 2005), including those from the genus *Gigantolaelaps* (Abba *et al.* 2001).

Members of the genus *Gigantolaelaps* can be easily identified thanks to their large size, known as the largest representatives of the Laelapidae family (Fonseca 1939), and the length of their idiosoma being more than 1400 µm long (Furman 1972). As diagnostic characteristics, these mites have a sternal plate narrow at the front with wide anterior projection, which occupies most or all of the presternal area; a genito-ventral plate slightly expanded posteriorly with only one pair of setae, and their posterior setae of the leg II always longer than the other setae of the legs (Furman 1939). These mites are described as ectoparasite exclusive of Oryzomyini tribe rodents (Gettinger 1987; Lareschi *et al.* 2004), with male mites inhabiting their host's nest and females living on their host (Radovsky 1994; Gettinger *et al.* 2011; Lareschi 2017). Because of the latter, only female *Gigantolaelaps* are dispersed by their hosts (Radovsky 1994).

In Chile, *Gigantolaelaps wolffsohni* (Oudemans) has been found in the long-tail pygmy rice rat *Oligoryzomys longicaudatus* Bennet in only four localities: Valparaíso (Valparaíso region), Río Melado (Maule region), Concepción (Biobío region) and Chiloé island (Los Lagos region) (Lareschi and González-Acuña 2010). The first locality belongs to the Mediterranean ecoregion, while the other three localities belong to the Template Forest ecoregion. This rodent is a native species distributed in Chile and Argentina. In Chile, it can be found from Atacama to Tierra del Fuego (Weksler and Bonvicino 2015). Infestation with Laelapidae mites can lead from minor skin irritation in their hosts to transmission of pathogenic microorganisms. Moreover, the role of this mite in epizootics and the maintenance of disease among rodents may be significant but unknown (Carmichael *et al.* 2007; Lareschi 2017). These effects could vary with different factors such as prevalence and abundance (Carmichael *et al.* 2007). To date, there is limited information about the distribution of the laelapid *G. wolffsohni* in Uruguay, Venezuela, and Chile, but the characteristics of its ecology are yet to be studied (Furman 1972; Lareschi *et al.* 2006; Lareschi and González-Acuña 2010).

The purpose of this research was to provide an update on the distribution of *G. wolffsohni* mites on *O. longicaudatus* in Chile and describe the ecological aspects of this parasite and its host. The latter included whether factors such as the ecoregion, season, and weight, and sex of the host influence the abundance and prevalence of the mite *G. wolffsohni*. These factors were included based on previous studies indicating their influence on (Strandtmann and Wharton 1958; Carmichael *et al.* 2007; Fernandes *et al.* 2012, 2015).

Material and methods

Rodent trapping and mite collecting

Rodents were captured from Arica to Magallanes regions of Chile (18°28'60"S – 70°19'60"W to 53°10'0"S – 70°55'60"W) for 10 years (2010-2019) in different seasons (summer, autumn, winter, spring). Most of the captures were in National Parks (NP) or National Reserves (NR). The captures were separated in three of the main ecoregions present in Chile: Mediterranean (from latitude 30°S to 38°S) (Villagrán and Hinojosa 2005; Armesto *et al.* 2007), Template Forest (from latitude 38°S to 47°S) (Villagrán and Hinojosa 2005), and Magellanic Forest (from latitude 47°S to south) (Morrone 2001) (Figure 1). The Mediterranean has high temperatures in summer (from 14 °C to 29 °C) and lower in winter (from 3.7 °C to 13 °C), with relative humidity from 45% to 80%. Likewise, the Temperate Forest has warm temperatures in summer

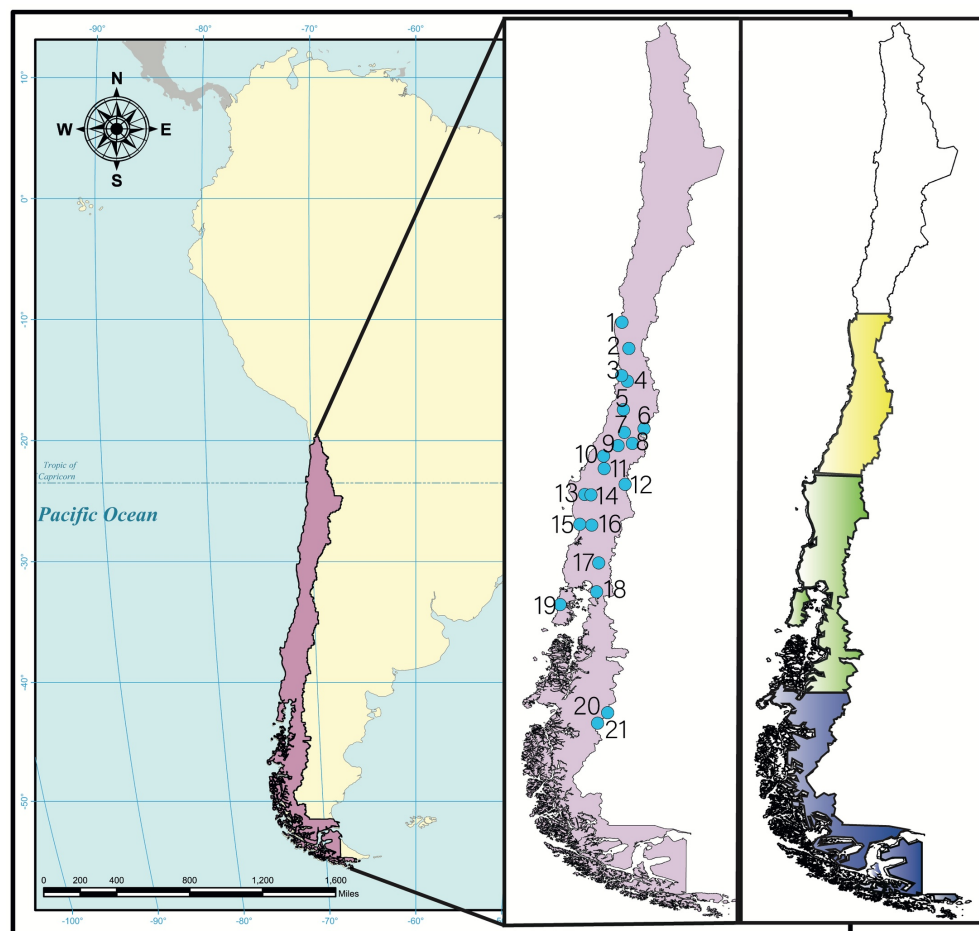


Figure 1 Localities where *G. wolffsohni* were found and ecoregions in Chile. 1. National Park (NP) Bosque Fray Jorge; 2. NP Las Chinchillas; 3. National Reserve (NR) Lago Peñuelas; 4. NP La Campana; 5. Sierras de Bellavista; 6. Termas del Flaco; 7. NR Altos de Lircay; 8. Parque Inglés; 9. Quilmo; 10. NP Nonguén; 11. Santa Elena; 12. NP Laguna del Laja; 13. NP Nahuelbuta; 14. Angol; 15. Bosque San Martín experimental station; 16. Gorbea; 17. Puyehue; 18. Hornopirén; 19. NP Chiloé; 20. NP Patagonia; 21. NP Tamango. All localities represent a new distributional record except the locality number 10 and 19. Yellow: Mediterranean ecoregion, green: Template Forest ecoregion, blue: Magellanic ecoregion.

(from 11 °C to 24 °C) and lower in winter (from 3.5 °C to 9 °C), with relative humidity from 66% to 90%. Meanwhile, the Magellanic Forest (in the latitude 47°S) has temperatures from 6 °C to 25 °C in summer and -1.7 °C to 9.6 °C in winter, with relative humidity from 51% to 70% (Dirección Meteorológica de Chile 2021).

Rodents were captured with Sherman traps and oatmeal was used as bait (Mills *et al.* 1995). Traps remained open throughout the night and they were checked in the early morning. For the handling of rodents, we followed the biosafety standards established by the Pan-American Health Organization (Organización Panamericana de la Salud 1999).

Rodents captured were sedated with xylazine 2% (5mg/kg) and ketamine 100% (75mg/kg) mixed together in the same syringe and injected intramuscularly (Mayer 2013). Then, rodents were identified (Patton *et al.* 2015), measured (with digital caliper Mitutoyo®), sexed, and weighted (with portable digital scale Pesolac®). Finally, rodents were brushed with a toothbrush over a white tray looking for mites and then were released where they were captured once they woke up from sedation.

Mites' identification

All mites found were stored in microtubes of 1,5 ml with ethanol 96% inside until laboratory examination. Mites were examined under a stereo microscope in the Parasite Ecology Laboratory of Zoology Department in Universidad de Concepción, Concepción, Chile. The mites were separated from other ectoparasites and were cleared in a microtube of 1,5 ml with Nesbitt solution for 15 minutes to 80 °C. After that, they were put in a new microtube with deionized water for 24 hours. Finally, mites were mounted in Berlese medium on a slide where they were identified following the descriptions of Lee (1966) and Furman (1972).

Statistical analysis

A database was established with the following data by rodent specimen: the amount of *G. wolffsohni* mites per rodent, weight and sex of rodents, season, and the ecoregion.

We used Quantitative Parasitology 3.0 online software (Q.P.3.0) (Reiczigel *et al.* 2019) to determine ecological indexes (prevalence, abundance, mean intensity, median intensity, crowding mean, and aggregation indexes (variance/mean, Poulin's index and negative binomial exponent k)).

Then, we used negative binomial regressions (NBR) to assess the association of the abundance of *G. wolffsohni* with body weight, sex, season, and ecoregional distribution of their host, and logistic regressions (LR) to assess the association of the same variables with the presence of the parasite. These analyzes were performed using STATA/BE 17 software. In the case of season and ecoregion of distribution, autumn and Magellanic Forest, respectively, were the base level and the other categories were the dummy comparing variables.

In order to select the best model, we were eliminating the less significant variables and we compared models with likelihood ratio tests. The best model was that in which no variable could be removed without significant loss of likelihood. A probability value $p < 0.05$ was used to indicate statistical significance.

Results

All mites analyzed were identified as *G. wolffsohni* which were founded in 21 localities in Chile (Figure 1). We captured 4723 rodents of different species which 329 were identified as *O. longicaudatus* (204 female and 125 male). We found 273 *G. wolffsohni* mites on *O. longicaudatus* rodents (150 mites on 62 female rodents and 123 mites on 49 male rodents). All the mites found were female.

We collected *G. wolffsohni* mites from the hind legs of their host, specifically in the medial side of the thigh. Additionally, a larval stage inside an adult female were observed under microscopical examination (Figure 2). These were visible mainly from June to November.

The ecological indexes are presented in Table 1. The total prevalence of *G. wolffsohni* was 34% (range: 29% - 39%). These mites displayed an aggregated distribution in all ecoregions (Table 1). We did not find association between weight, sex, and biogeographical distribution of host with the presence and the abundance of *G. wolffsohni* mites. Nonetheless, we determined that the abundance of mites is associated with the season (Table 2). Similar results were found in the analyses of the presence, with season being the sole variable that significantly associated with presence. In both, abundance and presence, the autumn presented parasitism rates significantly lower than the other seasons (Table 2).

Discussion

This research expands the known distribution of *G. wolffsohni* from latitude 30°S to 47°S in Chile with 19 new localities of distribution. All *G. wolffsohni* mites found were associated with *O. longicaudatus*, except for six mites found in one specimen of *Rattus rattus* in Ocoa,

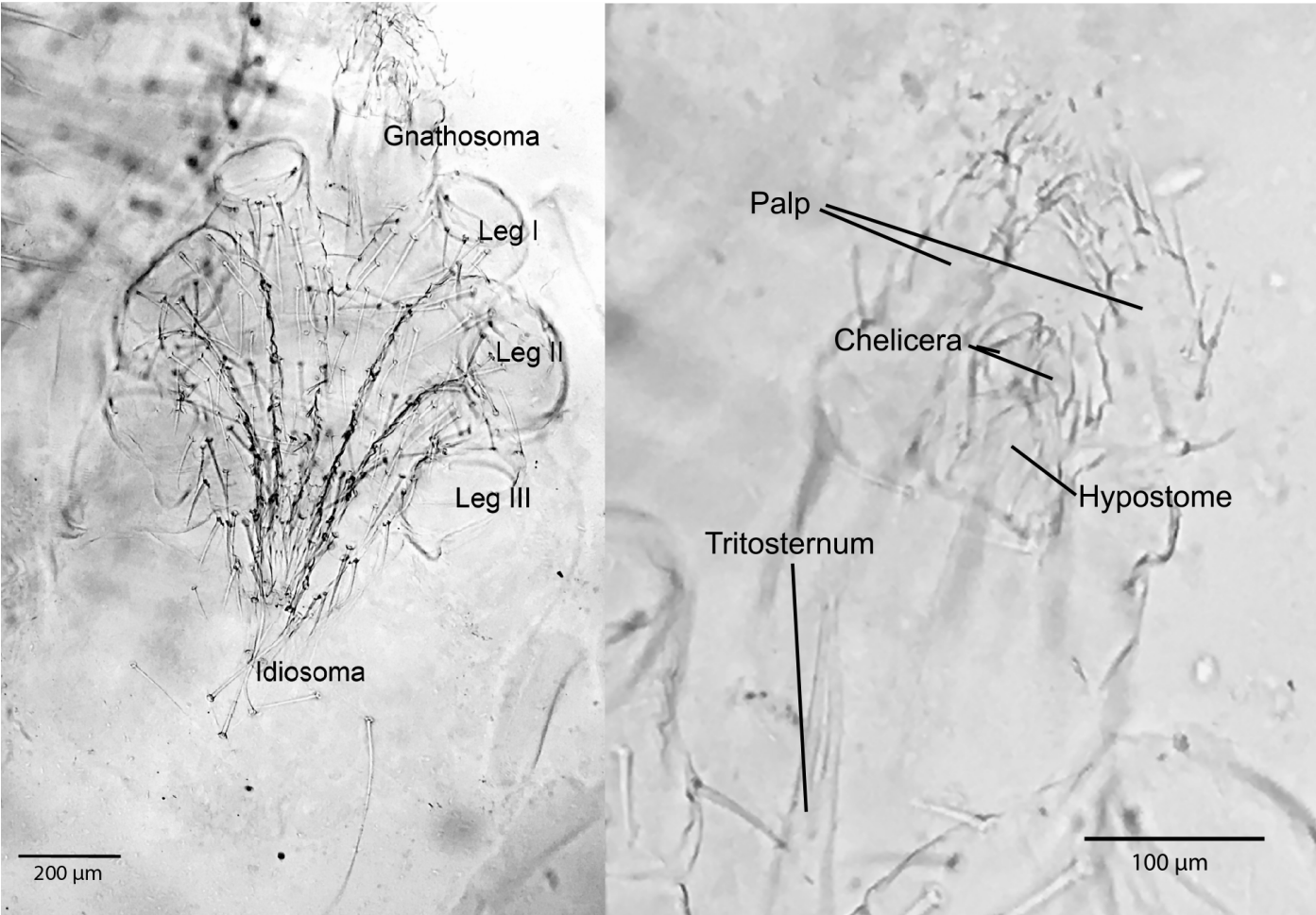


Figure 2 Larva of *Gigantolaelaps wolffsohni* from NP Chiloé, Chile. A. Full body of larvae *G. wolffsohni* (10x). B. Zoom to gnathosoma of larvae *G. wolffsohni* (40x).

Valparaíso region (32°51'0"S – 71°4'60"O). Since *R. rattus* has an opportunistic nidification (Coto 2015) and it could share some arboreal habits with *O. longicaudatus* (González-Ittig *et al.* 2010; Coto 2015), we believe that this finding is accidental and *R. rattus* should not

Table 1 Ecological indexes of *Gigantolaelaps wolffsohni* in Chile divided by ecoregions.

	N	N Inf	P (%)	MI	ml	MA	MC	Var/med	D	K
Mediterranean	69	31	44.90%	1.9	2	0.855	2.53	1.69	0.664	1.056
			(32.9%-57.4%)	(1.58 - 2.39)	(1- 2)	(0.609 - 1.16)	(1.98 - 3.67)		(0.569 - 0.755)	
Template Forest	152	68	44.70%	2.44	2	1.09	3.43	2.36	0.695	0.6019
			(36.7%-53%)	(2.1 - 3.85)	(2 - 3)	(0.855 - 1.36)	(2.93 - 4.49)		(0.636 - 0.751)	
Magallanic Forest	108	12	11.10%	4	3.5	0.444	5.83	5.44	0.92	0.0539
			(5.9%-18.6%)	(2.75 - 5.92)	(2 - 5)	(0.231 - 0.88)	(3.89 - 8.79)		(0.874 - 0.951)	
Chile	329	111	33.7	2.46	2	0.83	3.66	2.84	0.776	0.3497
			(28.6%-39.1%)	(2.16 - 2.82)	(2 - 2)	(0.67 - 0.99)	(30.8 - 4.71)		(0.742 - 0.814)	

N: number of total hosts captured. N inf: number of infected hosts. P: prevalence. MI: mean intensity. ml: median intensity. MA: mean abundance. MC: mean crowding. Var/me: dispersion index of variance/mean. D: Poulin's index. K: Negative binomial exponent k. (): confidence limits with the 95% of confidence.

Table 2 Final model of logistic regression for the presence / absence (above), and binomial negative regression for the abundance (below) of *Gigantolaelaps wolffsohni*.

Presence/Absence of <i>G. wolffsohni</i>	Coefficient	Standard error	<i>p</i> Value	95% Confidence Interval
Winter	2.428	0.399	0.0001	1.644 - 3.212
Spring	2.991	0.412	0.0001	2.182 - 3.8
Summer	3.056	0.509	0.0001	2.057 - 4.055
Abundance of <i>G. wolffsohni</i>	Odds ratio	Standard error	<i>p</i> Value	95% Confidence Interval
Winter	16.134	8.7	0.0001	5.605 - 46.436
Spring	35.116	19.7	0.0001	11.652 - 105.829
Summer	30.625	21.3	0.0001	7.819 - 119.948

be considerate as a new host for *G. wolffsohni*. Our research states that *G. wolffsohni* is parasite-specific to *O. longicaudatus* in Chile. The host specificity for Laelapidae mites had been reported before through an association of a highest and specific prevalence in exclusive hosts (Gettinger 1987; Lareschi *et al.* 2004; Correia 2015; Gettinger and Owen 2016). *Gigantolaelaps wolffsohni* has been found in the others no Oryzomyini rodents such as *Lundomys molitor* (Winge), *Akodon azarae* Fischer, and *Bolomys obscurus* Waterhouse in Uruguay (Lareschi *et al.* 2006), however, these authors found almost all the mites associated with *Oligoryzomys* rodents. Previously, *G. wolffsohni* was found on a single *Abrothrix sanborni* (Osgood) in Chile (Lareschi and González-Acuña 2010). Nevertheless, the authors that reported it, mentioned that it corresponds to an accidental finding. Our results, added to other findings, suggest that *G. wolffsohni* might use occasionally no Oryzomyini rodents for transportation and dispersion.

We only found female mites which agrees with the hypothesis that only female disperses on rodents (Radovsky 1994; Martins-Hatano *et al.* 2002). Since the host's nest provides a large and diverse food supply, it is likely that males and nymphs stay in the host nest (Radovsky 1994).

Some laelapines mites have an abbreviation of the general lifecycle by the retention of the egg by the female, or by the retention of both the egg and larva (Radovsky 1994; Cakmak and Da Silva 2018). The suppression of the egg by retention in the female protects the developing mite from mortality factors that otherwise would affect it. However, this suppression could reduce the reproductive capacity of the female since the individual offspring requires more time in uterus (Radovsky 1994). In this research, we observed that *G. wolffsohni* are, at least, larviparous. The results do not allow establishing that these mites are exclusively larviparous and not nymphiparous. Moreover, the type of reproduction has not been described for this species. Parthenogenesis is a type of asexual reproduction well documented in other mites of the order Mesostigmata (Błoszyk *et al.* 2004). More research is needed to understand the life cycle of species of the genus *Gigantolaelaps*.

Even though we did not find association between the abundance and prevalence of *G. wolffsohni* with the ecoregions, previous studies have determined that these factors can affect its, since different host's densities in different areas influence the frequency of intraspecific contacts (Fernandes *et al.* 2012, 2015). In this aspect, *O. longicaudatus* has been reported as a rodent with high vagility (Palma *et al.* 2007), which means a higher level of dispersion for their parasites (Poulin 2007). Probably, this high dispersion rate of *O. longicaudatus* maintains *G. wolffsohni* with low variation in its abundance and prevalence through different ecoregions. Additionally, we do not find an association between the abundance and prevalence of *G. wolffsohni* and the sex and weight of their host. This agrees with other authors who found that *Gigantolaelaps peruviana* (Ewing) abundance was not related to the sex and weight of its host; however, they found an association in other Laelapidae mites (Fernandes *et al.* 2012, 2015).

An important factor of this variation is the distribution of its host and the environmental conditions throughout the ecoregions. It has been reported that the lifecycle of mites is sensitive to humidity and temperature variations (Strandtmann and Wharton 1958). Additionally, it has been observed that these mites prefer warm temperature (23 °C to 35 °C) and high humidity conditions (above 50%) (Strandtmann and Wharton 1958). In this aspect, all ecoregions have high humidity, however the Mediterranean and the Template Forest have upper humidity than Magellanic Forest (Dirección Meteorológica de Chile 2021), which is consistent with the higher prevalence in Mediterranean and Template Forest (Table 1). *Gigantolaelaps wolffsohni* was not found in the NP. Torres del Paine and NR. Magallanes localities, nonetheless, its host was found there. This may be due to the fact that in these localities the temperatures do not exceed 7 °C in winter, nor 15 °C in summer, and despite presenting high relative humidity (60% - 76%) (Dirección Meteorológica de Chile 2021), the temperature would be a limiting factor for its development (Strandtmann and Wharton 1958).

The distribution of *G. wolffsohni* is aggregated through ecoregions, which agrees with the most common distribution of parasites where some hosts have several parasites and most of the hosts have none or few parasites (Poulin 2007). The aggregation value is higher in Magellanic Forest than in other ecoregions. This may be due to the fact that in the areas of Patagonia, population densities of 5.4 individuals per hectare have been reported for *O. longicaudatus*, unlike the template forest, where the population density is from 24 to 47 individuals per hectare (Spotorno *et al.* 2000). Hence, mites of Magellanic Forest have less possibility to find other hosts, since they are more dispersed in the environment. As a consequence, mites would tend to pass a long time on the same host and it is less likely that they find other individuals to parasitize (Poulin 2007).

We determine that the season influences the abundance and prevalence of *G. wolffsohni* in Chile, which is consistent with the findings of other authors (Carmichael *et al.* 2007; Lareschi and Krasnov 2010). They found that *Gigantolaelaps mattogrossensis* has a variation of its abundance related to the humidity and precipitation factors in different seasons (Carmichael *et al.* 2007). Meanwhile, a variation in the abundance of *G. wolffsohni* throughout the season was found in Buenos Aires province, Argentina (Lareschi and Krasnov 2010). Humidity and precipitation are important factors for the host *O. longicaudatus* as well because they are highly dependent on water, moreover it is one of the rodents with lowest survival rate in drought conditions (Palma *et al.* 2007). As a consequence, if the environmental conditions are unfavorable for the host, it would not be favorable for the parasite (Poulin 2007).

This research represents a contribution to the parasitology and acarology studies of Chile and South America. Additionally, this study provides a better understanding of ecological aspects of *G. wolffsohni* and their relation with its host *O. longicaudatus*. This rodent represents an important reservoir Hantavirus in Chile (Belmar-Lucero *et al.* 2009; Spotorno *et al.* 2000), consequently, the knowledge about this rodent has importance in public health. An important part of understanding the population and community dynamics around *O. longicaudatus* is knowing its parasites, to which this research contributes.

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