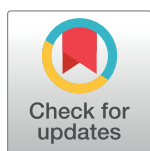RESEARCH ARTICLE

Identification of trombiculid mites (Acari: Trombiculidae) on rodents from Chiloé Island and molecular evidence of infection with *Orientia* species

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Abstract

Background

Scrub typhus is an emerging vector-borne zoonosis, caused by *Orientia* spp. and transmitted by larvae of trombiculid mites, called chiggers. It mainly occurs within a region of the Asia-Pacific called the tsutsugamushi triangle, where rodents are known as the most relevant hosts for the trombiculid vector. However, the reservoir(s) and vector(s) of the scrub typhus outside Asia-Pacific are unknown. The disease has recently been discovered on and is considered endemic for Chiloé Island in southern Chile. The aim of the present work was to detect and determine the prevalence of chiggers on different rodent species captured in probable sites for the transmission of orientiae responsible for scrub typhus on Chiloé Island in southern Chile and to molecularly examine collected chiggers for the presence of *Orientia* DNA.

Methodology/Principal findings

During the austral summer 2018, rodents were live-trapped in six sites and examined for chigger infestation. All study sites were rural areas on Chiloé Island, previously identified as probable localities where human cases acquired the scrub typhus. During a total of 4,713 trap-nights, 244 rodents of seven species were captured: the most abundant was *Abrothrix olivacea*. Chiggers were detected on all seven rodent species with a 55% prevalence rate. Chiggers showed low host specificity and varied according to site specific host abundance. Three genera of trombiculids were identified. *Herpetacarus* was the most abundant genus (93%), prevalent in five of the six sites. Infestation rates showed site specific differences, which were statistically significant using a GLM model with binomial errors. Molecular analyses proved that 21 of 133 (15.8%) mite pools were positive for *Orientia* species, all of them belonged to the genus *Herpetacarus*.

Conclusions/Significance

This study firstly reports the presence of different rodent-associated chigger mites positive for *Orientia* sp., in a region endemic for scrub typhus in southern Chile. *Herpetacarus* and two other genera of mites were found with high infestation rates of rodents in sites previously identified as probable exposure of scrub typhus cases. A substantial percentage of mite pools were positive for *Orientia* DNA, suggesting that chigger mites serve as vectors and reservoirs of this emerging zoonosis in South America.

Author summary

Scrub typhus is a chigger-transmitted zoonotic infection caused by *Orientia* species, which is endemic to the tsutsugamushi triangle in Asia-Pacific region. Recently, a focus of scrub typhus in South America has been confirmed on Chiloé Island in southern Chile. However, the vectors of scrub typhus in this region remain unknown. We undertook a survey to study the presence of chiggers on different rodent species in areas identified as probable sites of exposure to scrub typhus on Chiloé Island. The study showed that 55% of rodents were infested by trombiculids. Three mite genera were identified, of which *Herpetacarus* was the most abundant. Chiggers showed low host specificity, but spatial differences. Using molecular techniques, the trombiculid mites were found to be infected with *Orientia* species. These findings suggest that chigger mites play a role in the life cycle and transmission of this emerging infectious disease in Chile.

Introduction

Scrub typhus is a zoonotic disease caused by bacteria of the genus *Orientia*, which causes significant morbidity and mortality [1]. The disease was previously thought to be restricted to a certain region, known as the tsutsugamushi triangle, in Asia-Pacific, but recent cases from the Arabian Peninsula and southern Chile have called this paradigm into question [2–4]. This recent expansion of the endemic region is supported by serological data and studies in animals mainly from Africa [5].

In the Asia-Pacific area, the orientia infection is transmitted by larvae of trombiculid mites known as chiggers, which are also the reservoir of the orientiae through transovarial and transstadial transmission. Small vertebrates, usually rodents, serve as main hosts of the chiggers and are a critical part of the epidemiology of scrub typhus in the Asia-Australia-Pacific region [6]. Cases mainly occur in rural areas, where populations of infected trombiculid mites are present with a patchy distribution (mite islands) [1]. Surveillance of chiggers has been used as a proxy for the spatial risk of scrub typhus in humans [7, 8]. In Asia-Pacific, bacteria is transmitted by different species of *Leptotrombidium* [9], the transmission by other genera in Korea (*Euschoengastia*, *Neotrombicula*), Japan (*Schoengastia*), and India (*Schoengastiella*) has been suggested, but remains controversial [10, 11]. A key factor to understand the distribution and emergence of scrub typhus in these regions is the knowledge of the local chigger fauna, their rodent hosts, and their interaction with environmental and climatic factors [eg. 12, 13].

Recently, the first endemic focus of scrub typhus in South America has been confirmed on Chiloé Island in southern Chile [2, 3]. Still, the vectors and reservoirs of scrub typhus in regions outside the tsutsugamushi triangle remain unknown. In Chile, 18 species of trombiculid mites have been reported so far, mostly from reptiles [14–16]. Still, none of them belong to the genus *Leptotrombidium* or other genera associated with orientia, and up to now, no studies regarding the rodent-associated chigger fauna have been performed in scrub typhus endemic regions in southern Chile. The aim of the present work was to study the presence, prevalence, and distribution of chiggers and analyse their infestation pattern on different rodent species captured in sites, which were identified in previous studies as probable hot spots of scrub typhus on Chiloé Island. To study them as possible vectors of scrub typhus in Chile, chigger mite samples were molecularly examined for the presence of *Orientia* DNA.

Methods

Study sites

Chiloé Island belongs to Los Lagos region in southern Chile and is the second largest island in Chile with an area of 8,394 km^2. The climate is oceanic temperate, with mean annual precipitations of 2,090 mm and an average annual temperature of 12˚C [17]. The original vegetation is Valdivian temperate rain forest, which has been highly fragmented by clearing for livestock rising and timber extraction [18], and the current matrix involves mainly pastures and secondary scrublands [19].

During the austral summer months of January and February 2018, small mammals were live-trapped at six sites in the northern part of Chiloé Island (Fig 1). Study sites had been identified as possible areas of exposure of scrub typhus cases [3]. All sites consisted of partially cleared forest due to timber activities, with remaining native lower vegetation. Localities were geo-referenced by GPS and located into a digitalized map using Arc Gis 10.1 (Esri, New York).

Trapping and parasite sampling

A total of 148 to 175 Sherman-like traps (300 x 100 x 110 mm) were set up during four to five consecutive nights at each site. Trapping was operated for a total of 4,713 trap-nights and ranged from 668 to 895 trap-nights per site (average 785.5). Traps were situated ≥5 meters apart and placed under scrub, fallen logs, understory or burrows, baited with oat flakes and vanilla essence, and conditioned both inside and outside with vegetal material to protect animals from cold and rain. Traps were activated at sunset, checked early the next morning, and closed during the day to avoid capturing non-target species. Captured rodents were moved to a central processing tent installed at the sampling site (Fig 2), where they were chemically immobilized using an induction chamber containing cotton embedded with isoflurane (1 ml of
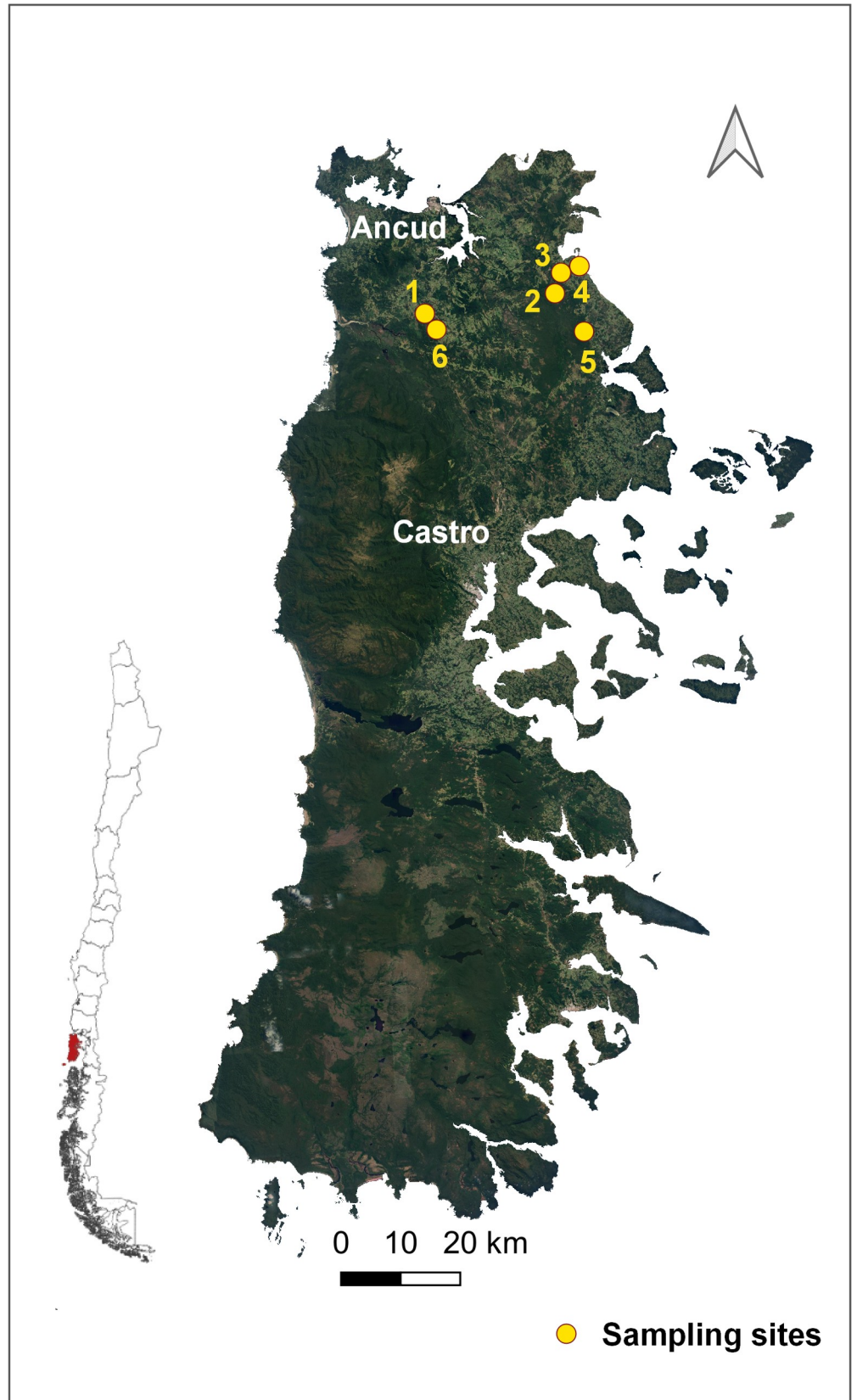
Fig 1. Study area in rural localities of the north-eastern area of Chiloé Island, Los Lagos Region, Chile. (Map made in QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org. Shapes downloaded from an open source from the Biblioteca del Congreso Nacional, Available at https://www.bcn.cl/siit/mapas_vectoriales/index_html).

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isoflurane per 500 ml of chamber volume). After anaesthesia, male and juvenile female rodents were euthanized by cervical dislocation; adult females were marked by haircut and released at the respective capture points. Rodents species were identified by morphological criteria following Iriarte [20]. Each rodent was thoroughly examined for the presence of ectoparasites by brushing the body with a fine comb over a plate covered with water. In adult females with high mite loads, we also conducted a careful scraping of the perianal zone. Chigger mites were collected from the water surface and placed in tubes with 95% ethanol. The skin of euthanized rodents, including ears and perianal zones, was dissected, stored in falcon tubes with 95% ethanol, and later revised for additional chiggers in the entomological laboratory.

Identification of mites

Taxonomic analyses of trombiculid mites were performed at the Laboratorio de Parásitos y Enfermedades de Fauna Silvestre, Universidad de Concepción in Chillán, Chile. Firstly, mite specimens of the individual rodents were pooled by their macroscopic appearance (morphotypes). Preserved rodent samples of skin and ears were checked for additional mites, which were added to the respective pools. One individual of each pool was cleared in Nesbitt's solution and mounted in Berlese's medium [21]. Specimens were then identified under an optical microscope (Leica DM 1000 LED) at 400x magnification, following nomenclature and methodology of Brennan & Goff [22].

Southern Chile is endemic for Andes virus, a rodent-borne virus causing hantavirus cardiopulmonary syndrome [23]. Therefore, the handling of captured animals strictly followed the guidelines of the Centers for Disease Control and Prevention (CDC) [24] and the American Society of Mammalogists [25] for such regions. Personal protective equipment included masks with HEPA filters as well as disposable gowns and gloves (Fig 2). The study also adhered to the guidelines from the American Veterinary Medicine Association [26] and American Society of Mammalogists for the use of wild mammals in research and education [27].

Ethics statement

The animal protocol used in this study was approved by the Chilean Animal Health Service (permit number 7034/2017), and by the Scientific Ethics Committee for the Care of Animals and the Environment, Pontificia Universidad Católica de Chile (N°160816007, 07-Nov-2017). All members of the field team were advised and clinically followed for five weeks post-exposure for signs and symptoms of scrub typhus and hantavirus; chemoprophylaxis with doxycycline was not used.

Analysis of rodent data

A descriptive analysis of the rodent host community and its infestation pattern with ectoparasites was carried out. The percentage of infected individuals with trombiculid mites per species was estimated. Then, we performed a Generalised Lineal Model (GLM) with binomial errors to assess effects of rodent species, and sites on the chigger prevalence using R, version 3.4.1 [28].

Fig 2. Central processing tent installed at each sampling site. Biosafety conditions during rodent handling were adapted to the risk of hantavirus cardiopulmonary syndrome.

Molecular testing for *Orientia* DNA

Morphologically identical mites from individual rodents were tested as pools of 6–20 individuals. Pools were washed in distilled water and dried in 37˚C for 3 hours. Then, mites were disrupted with a freeze-thaw cycle of 30 minutes at -40˚C and 30 minutes at 70˚C in 100μL distilled water and 100μL lysis buffer (Cat. 04659180001, Roche). Pooled total DNA was automatically extracted by MagNA Pure System (Roche, Pleasanton, California, Roche Molecular Diagnostics), following the manufacturer´s instructions, to a final elution volume of 50 μL [29]. Mite pools were tested by a newly designed quantitative real-time PCR (qPCR) assay targeting the *rrs* gene (16S RNA), which successfully detects all known *Orientia* species including *Candidatus* O. chuto and Chilean *Orientia* isolates [30].

Results

Of the captured animals, 244 rodents were included, belonging to seven species, most abundantly *Abrothrix olivacea* (Table 1). Among the trapped animals, 55% (133/244) were infested with trombiculid mites, mainly found in the ears and anogenital region (Fig 3). Other collected ectoparasites included ticks, fleas, and non-trombiculid mites (data not presented). Chigger infestation was observed among all rodent species, with prevalence rates ranging from 77% in *G. valdivianus* to 32% in *Irenomys tarsalis*, without significant species-specific differences (GLM, p>0.05). Overall, the most abundant species (*Abrothrix olivacea*) represented 77% (102/133) of all infected animals.

Table 1. Infestation with different genera of trombiculid mites and infection of mite pools with *Orientia* species per rodent species in 244 rodents captured between January and February 2018 on Chiloé Island.

Rodent species	Trapped	Infested by trombiculid mites				Mite pools		
	n (%)[1]	Any (%)[2]	*Herpetacarus*	*Quadraseta*	*Paratrombicula*	Total	*Orientia* pos.	%
Abrothrix olivacea	185 (76)	102 (55)	94	8	8	102	17	16.7
Abrothrix sanborni	13 (5)	9 (69)	9	0	0	9	3	33.3
Geoxus valdivianus	13 (5)	10 (77)	10	0	0	10	1	10
Irenomys tarsalis	25 (10)	8 (32)	7	1	1	8	0	0
Oligoryzomys longicaudatus	3 (1)	1 (33)	1	0	0	1	0	0
Loxodontomys micropus	3 (1)	2 (67)	2	0	0	2	0	0
Rattus norvegicus	2 (1)	1 (50)	1	0	0	1	0	0
Total	244	133 (55)	124	9	9	133	21	15.8

[1] Percentage of rodent species (among trapped rodent)

[2] Percentage of infested rodents (among trapped rodents)

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Among the collected trombiculids, three morphotypes were observed, which were identified as *Herpetacarus* sp., *Quadraseta* sp., and *Paratrombicula* sp.; details of the detected species will be published elsewhere. *Herpetacarus* sp. was predominate (93% of infested rodents) and occurred on all rodent species and in all sites except Site 3, whereas the other two species co-parasitized on few rodents only in Site 3 (Table 2). The overall prevalence of chiggers in different sites showed significant variations and ranged from 25% to 78% (Table 2). These spatial differences were also presented in a Generalized Lineal Model, demonstrated that it was less likely to find positive rodents in Sites 3 and 5 than in other sites (Table 3).

Orientia DNA was detected by a genus-specific qPCR assay in 21 of 133 (15.8%) mite pools (Table 1). All of orientia-positive mite pools belonged to the genus *Herpetacarus* (21 of 124, 16.9%), whereas pools of other mite genera were orientia-negative (Table 1). Three of the most abundant rodent species, *Abrothrix olivacea*, *Abrothrix sanborni*, *Geoxus valdivianus* harbored all *Orientia* positive mites (Table 1). *Orientia*-positive *Herpetacarus* pools were found in four of six sites with prevalence rates ranging from 7.1% to 41.7% (Table 2).

Discussion

The presented study aimed to explore the trombiculid fauna on Chiloé Island, an endemic area of scrub typhus in southern Chile. Although scrub typhus in Asia-Pacific is transmitted by chiggers, in Chile and other possible newly identified endemic regions, the vector is unknown. Interestingly, a study from 2018 found the first molecular evidence for *Candidatus* Orientia chuto infection of chiggers collected from a rodent in Kenya [31], another region outside the tsutsugamushi triangle, where scrub typhus might occur as suggested by two recent retrospective surveys, detecting antibodies to *Orientia* spp. in 3% to 5% of febrile patients [5, 31]. In the endemic regions in Asia-Pacific, the disease is transmitted by different *Leptotrombidium* species; however, mites of this genus are not endemic in Chile or any other region of the Neotropics [32]. Since the first Chilean scrub typhus patient suffered several terrestrial leech bites prior to his infection, it was speculated that *Orientia* was leech-transmitted [2]. This hypothesis cannot be corroborated by our data. None of the cases diagnosed by our group reported leech bites, at least one had symptoms compatible with trombiculidiasis, and most had nature activities with increased risk of arthropod exposure [3, 33, 34], suggesting that a transmission by chiggers is more likely.
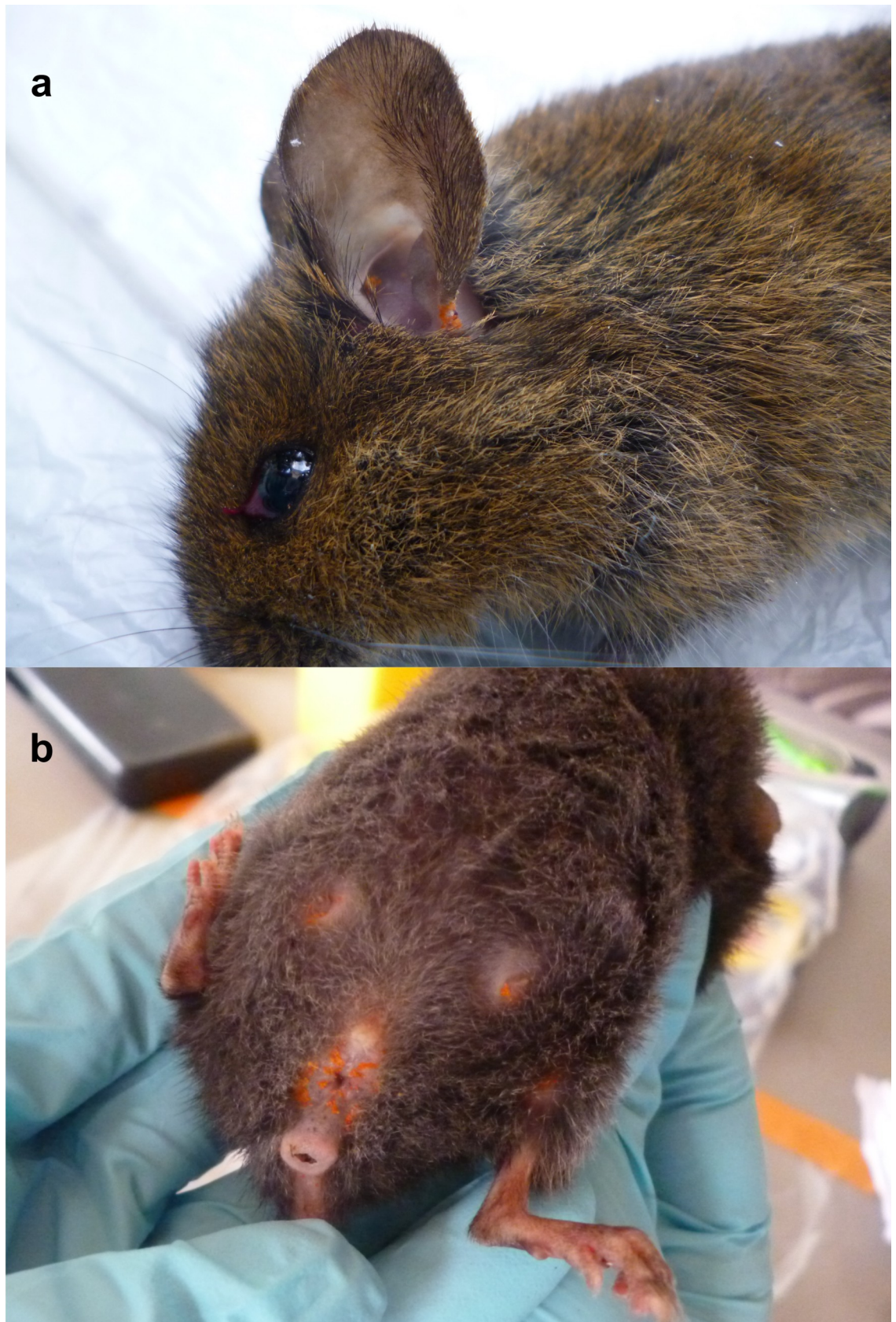
Fig 3. Multiple orange colored trombiculid mites on ear (a) of a *Loxodontomys micropus*, and on the genital region and tits of a *Geoxus valdivianus* (b), captured on Chiloé Island.

The study was conducted during the typical scrub typhus season in southern Chile, i.e. the summer months of January and February, and locations were chosen according to our previous studies as possible hot spots of *Orientia* transmission [3, 35]. We could demonstrate that rodent-associated chigger mites were present in all those sites. The three detected trombiculid genera have been described before in the Neotropics. *Herpetacarus* was most abundant and parasitized all of the captured rodent species. This genus currently includes four subgenera (*Abonnencia*, *Cricacarus*, *Herpetacarus*, and *Arisocerus*), which are found on reptiles, mammals, and occasionally birds in Africa, Asia, Oceania, and Latin America [36, 37]. Members of

Table 2. Prevalence of infestation with trombiculid mites and infection of mite pools with *Orientia* species per site and rodent species.

Site and host species	Trapped rodents	Mite infested rodents					Mite pools		
		Total		*Herpetacarus*	*Quadraseta*	*Paratrombicula*	Total	*Orientia* pos.	
	n	n	%	n	n	n	n	n	%
Site 1	18	12	67	12	0	0	12	0	0
Abrothrix olivacea	14	9	64	9	0	0	9	0	0
Geoxus valdivianus	4	3	75	3	0	0	3	0	0
Site 2	43	31	72	31	0	0	31	6	19.4
Abrothrix olivacea	34	23	67	23	0	0	23	5	21.7
Geoxus valdivianus	5	5	100	5	0	0	5	1	20.0
Oligoryzomys longicaudatus	3	2	67	2	0	0	2	0	0
Loxodontomys micropus	1	1	100	1	0	0	1	0	0
Site 3	36	9	25	0	9	9	9	0	0
Abrothrix olivacea	26	8	31	0	8	8	8	0	0
Geoxus valdivianus	1	0	0	0	0	0	0	0	0
Irenomys tarsalis	9	1	11	0	1	1	1	0	0
Site 4	37	24	65	24	0	0	24	10	41.7
Abrothrix olivacea	29	20	69	20	0	0	20	9	45.0
Abrothrix sanborni	3	2	67	2	0	0	2	1	50.0
Geoxus valdivianus	2	1	50	1	0	0	1	0	0
Irenomys tarsalis	1	1	100	1	0	0	1	0	0
Loxodontomys micropus	1	0	0	0	0	0	0	0	0
Rattus norvegicus	1	0	0	0	0	0	0	0	0
Site 5	73	28	38	28	0	0	28	2	7.1
Abrothrix olivacea	54	20	37	20	0	0	20	1	5.0
Abrothrix sanborni	6	3	50	3	0	0	3	1	33.3
Geoxus valdivianus	1	1	100	1	0	0	1	0	0
Irenomys tarsalis	10	3	30	3	0	0	3	0	0
Loxodontomys micropus	1	0	0	0	0	0	0	0	0
Rattus norvegicus	1	1	100	1	0	0	1	0	0
Site 6	37	29	78	29	0	0	29	3	10.3
Abrothrix olivacea	28	22	79	22	0	0	22	2	9.1
Abrothrix sanborni	4	4	100	4	0	0	4	1	25.0
Irenomys tarsalis	5	3	60	3	0	0	3	0	0
Total	**244**	**133**	**55**	**124**	**9**	**9**	**133**	**21**	**15.8**

Table 3. Generalized Lineal Model with binomial error indicating trapping site as a factor for trombiculid infestation in rodents (n = 244) on Chiloé Island.

Sites	OR*	IC95%	p
1	1.00		
2	1.29	0.39–4.22	0.672
3	0.17	0.05–0.57	0.005
4	0.92	0.28–3.03	0.895
5	0.31	0.10–0.92	0.035
6	1.81	0.52–6.35	0.353

*OR = Odd ratio

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this genus have not been detected in Chile, although a closely related genus (*Proschoengastia*) was found in Chile's far south [14]. The other two morphotypes (*Quadraseta* sp. and *Paratrombicula* sp.) were less prevalent. *Quadraseta*, which comprises 14 species found on rodents and birds [38–45], has not been reported previously in Chile. *Paratrombicula* includes six species isolated from lizards and rodents; two of those (*P. chilensis* and *P. goffi*) have been described in central Chile, both on lizards [14, 15, 46]. Results of detailed morphological analysis and descriptions of probable new species will be presented elsewhere.

Rodents are a main reservoir of chiggers and important determinant of the distribution of scrub typhus in endemic regions in Asia [6]. In our study, *A. olivacea* was the most abundant rodent species and also represented the highest (absolute) number of infested rodents. This species is a typical inhabitant of Valdivian temperate forests; showing a large numerical response during bamboo blossom (*Chusquea* spp.) [47–49]. Other less abundant host species were infested in similar rates, indicating low host-specificity of trombiculids. This is in accordance with studies from Asia-Pacific, where feeding on small mammals was non-specific and depended on host species abundance in the community [1, 7, 8].

Although chiggers were detected on rodents in all six surveyed areas, the prevalence rates differed geographically. Variations in the distribution of trombiculids are a known phenomenon; in fact, within suitable habitats, the mites usually have a patchy distribution, forming so called "mite islands" [9, 50]. Our findings might indicate a high prevalence of chiggers on Chiloé Island, although the selection of sites as probable "hot spots" of exposure to *Orientia* spp. could be a bias towards overestimation. The infestation rates reported in this study are comparable to those reported in the Asia-Pacific region where prevalence rates on small mammals ranged from 45% to 95% [7, 8, 51, 52].

Trombiculid mites live in moist soil covered with vegetation and are mostly found in grassy and weedy areas [eg. 50]. Optimal living conditions depend on various factors such as air humidity, soil composition, temperature, and light intensity. Habitat fragmentation seems to affect mite survival by modifying their ecological niche [53]. Recently, a large-scale research found higher infestation (prevalence, mean abundance, and intensity) with vector mites on small mammals in areas with lower biodiversity compared to those with higher biodiversity [54]. As documented in various studies, forest fragmentation on Chiloé Island reduces the biodiversity of small mammals, non-raptorial birds, and the predator assemblage and increases the abundance of generalist species [55–58]. In a similar manner, fragmentation might affect rodent populations and their associated trombiculid ectoparasites.

This first demonstration of rodent-associated chiggers in probable hot spots of scrub typhus suggests that chiggers might serve as vectors of this infection in Chile. The study detected high chigger prevalence in the summer season, during which up to now all cases of scrub typhus in

Chiloé have occurred [59]. The hypothesis was further supported by the detection of *Orientia* DNA in 15.8% of mites, all of which belonged to the *Herpetacarus* genus. Final proof, however, of the vector competence of the detected trombiculid mites in this new endemic region requires further studies. To understand the risk of human exposure to trombiculid mites, further investigations are necessary, which should include environmental, anthropogenic, and climatic variables influencing the epidemiology of these potential vectors in southern Chile.

Conclusions

Our study firstly documented the presence of rodent-associated trombiculid mites infected with *Orientia* sp. on Chiloé Island, a region endemic for scrub typhus in South America. Three different mite genera were identified on the rodents with the genus *Herpetacarus* the most abundant and the only infected with *Orientia* sp. Overall, we detected a high rate of *Orientia*-infected chigger infestation independent of host species, but with significant spatial variations.

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Author Contributions

Conceptualization: Gerardo Acosta-Jamett, Thomas Weitzel, Katia Abarca.

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Writing – review & editing: Gerardo Acosta-Jamett, Constanza Martínez-Valdebenito, Esperanza Beltrami, María Carolina Silva-de La Fuente, Ju Jiang, Allen L. Richards, Thomas Weitzel, Katia Abarca.

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