Trichinella in wildlife with the first record in a small felid, *Leopardus guigna*, and a mustelid, *Galictis cuja*, in South America

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Background. Trichinellosis is a worldwide distributed disease caused by *Trichinella* species and also considered neglected and emerging. *Trichinella* spp. are transmitted by predation or carrion consumption and present a domestic and a sylvatic cycle. Human trichinellosis occurs due to the consumption of raw or poorly cooked infected meat, mainly associated with the household slaughter of pigs or the hunting of wild animals without veterinary inspection, a cultural practice that is difficult to resolve. Therefore, the knowledge of the reservoir of this parasite is relevant. The aim of this study was to assess the presence of *Trichinella* sp. in several carnivore and omnivore vertebrates in Central-Southern Chile.

Methods. A total of 61 animals, either found run over or that died in rescue/rehabilitation centers, encompassing 15 species, were examined by artificial digestion for detection of *Trichinella* larvae, and larvae were molecularly analyzed to identify the species.

Results. *Trichinella* larvae were found in a *Leopardus guigna* Molina, 1782 (Felidae) and a *Galictis cuja* Molina, 1782 (Mustelidae). Only those of *L. guigna* could be identified as *Trichinella spiralis* Owen, 1835. This is the first record of *Trichinella* in a native mustelid of South America and the first record of *T. spiralis* in *L. guigna*. These results increase the number of hosts, enhancing the need to identify the role of these animals in the reservoir for humans, and highlighting the priority that the study of the rural-sylvatic interphase represents.

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Abstract

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Introduction

Trichinellosis is a disease with worldwide distribution caused by *Trichinella* species (*Korhonen et al., 2016*), and also considered neglected and emerging (*Dupouy-Camet, 1999; Murrell & Pozio, 2000; Bruschi, 2012; Boutsini et al., 2014*). *Trichinella* is transmitted by predation and carrion consumption and circulates among carnivore and omnivore mammals; thus, human trichinellosis is mainly associated to cultural factors, especially the household slaughter of pigs without veterinary inspection and the consumption of raw or poorly cooked meat (*Pozio, 2014*), conditions that are difficult to improve. Another source of infection is the consumption of game animals (*Tryland et al., 2014; Fichi et al., 2015; Kärssin et al., 2017*). Thus, the knowledge of the cycle is relevant for proposing control measures. *Trichinella* circulates among carnivore and omnivore vertebrates and in both the domestic (encompassing mainly pigs, rats, dogs and cats) and the sylvatic environments (encompassing free-range vertebrates); which can be connected by invasive rats and other synanthropic animals (*Pozio, 2000*). At present, there are 10 recognized species of *Trichinella* around the world, as well as three genotypes that have not yet been demonstrated to be distinct species (*Korhonen et al., 2016; Sharma et al., 2020*). Since most *Trichinella* species infect only mammals, the sylvatic cycle encompasses mainly mammal hosts (*Klun et al., 2019; Bilska-Zając et al., 2020*), including marine mammals (*Tryland et al., 2014; Pasqualetti et al., 2018*). However, *Trichinella pseudospiralis* Garkavi, 1972 also infects birds, and *Trichinella zimbabwensis* Pozio et al., 2002 and *Trichinella papuae* Pozio et al., 1999 infect reptile hosts (*Korhonen et al., 2016*). Thus, the ecological and epidemiological knowledge of the cycle are relevant for reducing the incidence. Hence, the study of the hosts participating in the reservoir of *Trichinella* is valuable.

In South America, *Trichinella* is present in Bolivia, Argentina, Brazil, Ecuador and Chile, and most studies have focused on the domestic cycle (*Ribicich et al., 2020*). Four *Trichinella* species have been reported in this continent, mainly in Argentina: *Trichinella spiralis* Owen, 1835, *Trichinella patagoniensis* Krivokapich et al. 2012, *Trichinella britovi* Pozio et al. 1992 and *T. pseudospiralis* (*Krivokapich et al., 2006; Krivokapich et al., 2012; Krivokapich et al., 2015; Krivokapich et al., 2019*), as well as cougars, wild boars, foxes, opossums, sea lions, armadillos and a cricetid rodent, *Graomys centralis* Thomas, 1902 (*Minoprio, Hipólito & Dantis, 1967; Ribicich et al., 2020*) are the described wild host of *Trichinella*. In Chile, the domestic cycle is fairly well-studied (*Alcaíno & Arenas, 1981; Schenone et al., 2002*), but the sylvatic cycle is mostly unknown. *Trichinella spiralis* is the sole species that has been reported in Chile (*Schenone et al., 2002; Landaeta-Aqueveque et al., 2015; Hidalgo et al., 2019*). Cougars and wild boars are the sole wild/feral host species reported (*Landaeta-Aqueveque et al., 2015; Hidalgo et al., 2019*), and, in addition to those reports, other studies did not find infected animals (*Alvarez et al., 1970; González-Acuña et al., 2010; Ramirez-Pizarro et al., 2019*). Thus, the aim of this study was to assess the presence of *Trichinella* sp. in several carnivore and omnivore vertebrates in central-southern Chile.

Materials & Methods

The present study includes three administrative regions of Chile, the Maule, Ñuble and Biobío Regions (Fig. 1). These regions feature a transitional climate that falls somewhere between the classifications of warm Mediterranean (Csb, after Köpen classification) and wet temperate oceanic (Cfb, after Köpen classification), and lay within the limits between Central and Southern Chile (34° 47' 24.37" S to 38° 29' 6.08" S; 71° 2' 13.52" W to 73° 57' 36.74" W).

This study considered animals that were found dead, mainly run over by a vehicle, or that died in wild animal rescue/rehabilitation centers (Fauna Rehabilitation Center of the Universidad de Concepción; Wild Fauna Rehabilitation Center of the Universidad San Sebastián) from 2013 to 2020. At least 1 g of muscle (10 g when possible) of these animals were examined for *Trichinella* larvae. Muscles selected for parasitological examination were diaphragm, masseter, tongue, quadriceps (in mammals), pectoral (in birds) and intercostal. Examined animals were: Birds-Strigiformes: *Bubo magellanicus* Gmelin, 1788 (n=2), *Glaucidium nana* King, 1828 (1), *Strix rufipes* King, 1828 (2), *Tyto furcata* Temminck, 1827 (5), Birds-Accipitriformes: *Parabuteo unicinctus* Temminck, 1824 (11), *Cathartes aura* Linnaeus, 1758 (1), *Coragyps atratus* Bechstein, 1793 (2); Birds-Pelecaniformis: *Pelecanus thagus* Molina, 1782 (1); Mammals-Artiodactyla: *Grampus griseus* Cuvier, 1812 (1); Mammals-Carnivora: *Otaria flavescens* Shaw, 1800 (1), *Leopardus guigna* Molina, 1782 (6), *Galictis cuja* Molina, 1782 (17), *Lycalopex culpaeus* Molina, 1782 (9); Mammals-Microbiotheria: *Dromiciops bozinovici* D'Elía, Hurtado and D'Anatro, 2016 (1); and Reptile-Squamata: *Philodryas chamissonis* Wiegmann, 1834 (1).

Artificial digestion of the muscles was performed following Gajadhar et al. (*2019*) and larvae were conserved in 96% ethanol. For the molecular identification, the DNA was extracted from a pool of 10 *Trichinella* larvae with DNeasy Blood & Tissue Kit (Qiagen®) and 10 ng of DNA was used for identification at the species level by nested PCR following a modification of the protocol of Zarlengaet al. (*1999*). Reactions were performed in 25 µl final volume. Primers *Ne* forward (5'-TCTTGGTGGTAGTAGC-3') and reverse (5'-GCGATTGAGTTGAACGC-3') were used in the first PCR (0.5 µM of each primer) and 12.5 µl of GoTaq Green Master Mix (Promega®). DNA was amplified in a thermocycler (MultiGene™ OptiMax Thermal Cycler, Labnet) under the following cycling conditions: 95°C x 1 min for initial denaturation; followed by 40 cycles of 95°C x 30 seg; 56°C x 1 min and 72°C x 1 min; and final extension of 72°C x 2 min. Then, 0.5 µM of each Primers *I* forward (5'-GTTCCATGTGAACAGCAG-3') and reverse (5'- CGAAAACATACGACAACTGC-3') were used in a second PCR under same conditions with an annealing temperature of 55°C. The PCR products were submitted to electrophoresis in 2% agarose gel.

Bioethical considerations: This study meets the International Guiding Principles for Biomedical Research Involving Animals. The Comité de Ética of the Facultad de Ciencias Veterinarias of the Universidad de Concepción approved the study (CBE-47-2017).

Results

Trichinella larvae was isolated only from one *L. guigna* (güiña. 52 larvae per gram) and one *G. cuja* (lesser grison. 0.3 larvae per gram), both from the Ñuble Region (Fig. 1). The DNA of larvae isolated from the grison was degraded and did not output a PCR product. The DNA of larvae isolated from the güiña output a PCR product of 173 bp, corresponding to *T. spiralis*.

Discussion

Trichinella larvae in Chile is difficult to study in wild fauna because most carnivore vertebrates are protected by law (*SAG, 2012*), either due to conservationist concerns or because they support pest control. Only invasive animals can be hunted for assessing the *Trichinella* infection and the larvae isolation. Given that, few studies have assessed the presence of *Trichinella* infection in native carnivores in Chile: Alvarez *et al*. (*1970*) included two güiñas and 24 lesser grisons, in addition to other mammals, and González-Acuña *et al*. (*2010*) included two güiñas, with negative results in both cases. Other studies in Argentina included another wild cat (*Leopardus geoffroyi* D' Orbigny and Gervais, 1844) and the lesser grison with negative results (*Ribicich et al., 2010; Winter et al., 2018*). Thus, this is the first record of *Trichinella* larvae in a native mustelid in South America, and the first record of *T. spiralis* in the güiña, with the güiña being the second South American felid host for this parasitic species. Previously, other mustelids have been reported to host *Trichinella* elsewhere, as *Neovison vison* Schreber, 1777 (American mink) hosting *T. spiralis*, *T. britovi* and *T. pseudospiralis* in Poland (*Hurníková et al., 2016*), as well as *Meles meles* Linnaeus, 1758 (European badger) hosting *T. britovi* in Romania (*Boros et al., 2020*). Similarly, other felids have been reported harboring *Trichinella* in Chile, as *Puma* *concolor* Linnaeus, 1771 harboring *T. spiralis* (*Landaeta-Aqueveque et al., 2015*), and elsewhere, as *P. concolor* harboring *T. patagoniensis* in Argentina (*Krivokapich et al., 2012*), *P. concolor coryi* harboring *T. spiralis* and *T. pseudospiralis* in USA (*Reichard et al., 2015*), *P. concolor cougar* harboring *Trichinella nativa* Britov and Boev, 1972, *T. pseudospiralis*, *Trichinella murrelli* Pozio and La Rosa, 2000 and *Trichinella* T6 (*Gajadhar & Forbes, 2010*), *Lynx rufus* Schreber, 1777 harboring *T. britovi* and *Felis silvestris* Schreber, 1777 harboring *T. britovi* and *T. spiralis* (*Pozio et al., 2009*).

The güiña is one of the smallest felids in the world, with a distribution in Chile and Argentina between 33º and 48º of latitude S (*Napolitano et al., 2014*). This felid consumes micromammals preys as primary items (*Delibes-Mateos et al., 2014; Figueroa, Corales & Rau, 2018*), in such a way that this prey, especially rodents, can be the source of infection. However, rodents have been recognized as hosts of *T. spiralis* mainly in the domestic environment in Chile (*Schenone et al., 1967; Schenone et al., 2002*). This record is in accordance with the fact that güiñas have been frequently infected by micro-organisms spilled from free-range domestic animals (*Ortega et al., 2020; Sacristán et al., 2020*); and, although *T. spiralis* is not an important pathogen for the health of non-human animals, its presence in the güiña highlights the need of surveyance of pathogens in the rural-sylvatic interphase.

The lesser grison is a Neotropical mustelid that inhabit an area from southern Peru, Uruguay and Paraguay to southern Chile and Argentina, encompassing several environments (*Prevosti & Travaini, 2005*), and is a generalist predator, including rodents as an important part of its diet (*Ebensperger, Mella & Simonetti, 1991; Zapata et al., 2005*). Given that, this species most likely can be infected in the domestic environment; however, a further identification of the *Trichinella* species that the lesser grison harbors allows to better understand the source of infection, given that not all *Trichinella* species identified in South America have been reported in the domestic cycle. For instance, *T. patagoniensis* has been reported only in cougars (*Krivokapich et al., 2008; Krivokapich et al., 2012*).

To the best of our knowledge, there is no report of the güiña as prey of larger predators while *B. magellanicus* (Magellanic horned owl) is the sole predator reported for the lesser grison (*Prevosti & Travaini, 2005*). Regarding that, *T. pseudospiralis*, also zoonotic, is the only species of the genus reported to infect birds. This species has not been reported in Chile with the record of a single pig from Argentina being the sole report in South America (*Krivokapich et al., 2015*). Thus, it is most unlikely that this owl could be a way for *Trichinella* to be transmitted from the grison to another species. Hence, whether güiña and lesser grison participate in the reservoir or constitute dead-end hosts is unknown, and the most likely way for *Trichinella* larvae to be transmitted from these hosts seems to be their consumption by carrion-consuming mammal. Therefore, further studies are necessary to test this hypothesis. On the other hand, human trichinellosis after the direct consumption of a free-range mammal has been also reported worldwide (*García et al., 2005; Fichi et al., 2015*); however, neither güiñas nor grisons are typical prey for hunters to eat, nor is their hunting permitted by law in Chile (*SAG, 2012*).

It is noteworthy that the two host species herein reported correspond to the mammal species with the larger sample size, which suggests that larger samples in the other mammals could output new hosts for *Trichinella*. The contrasting lack of finding of Alvarez *et al*. (*1970*) could be due to a real absence of larvae in their samples, as well as to the parasitological technique, trichinoscopy, which is of lower sensitivity (*Forbes, Parker & Scandrett, 2003*).

Conclusions

This is the first record of *Trichinella* larvae in a native mustelid in South America, and the first record of *T. spiralis* in the güiña. Thus, this study raises the number of mammals infected with *Trichinella* larvae in the Neotropics, enhancing the need of identify the role of neotropical animals in the reservoir for humans, and underlying the priority that the study of the rural-sylvatic interphase represents.

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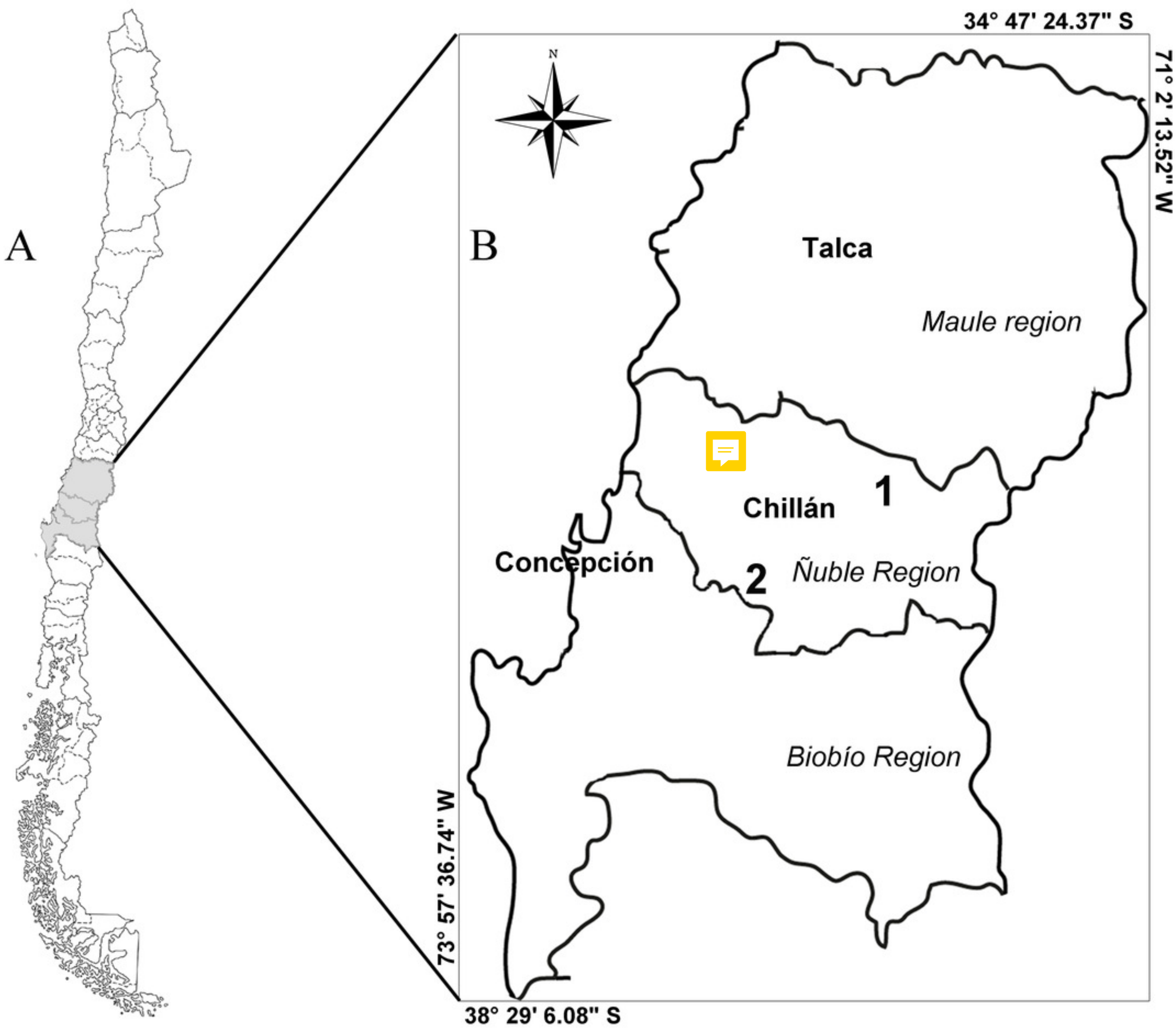
Figure 1

Figure 1: Map of Chile (A) and the studied administrative regions (B).

Italicized text indicates the name of the regions and roman text indicates their capitals. Infected animals are presented with numbers; 1: *Galictis cuja*, 2: *Leopardus guigna*.



Abstract

Background. Trichinellosis is a zoonotic disease with a worldwide distribution.. It is caused by several species of nematodes in the genus *Trichinella.* . *Trichinella* spp. are transmitted by predation or carrion consumption and occur in a domestic and a sylvatic cycle. In humans trichinellosis occurs due to the consumption of raw or undercooked infected meat, and is mainly associated with the household slaughter of pigs or the consumption of wild game.. Therefore, the knowledge of the reservoir of this parasite is relevant to identifying hosts to better implement public health strategies. The aim of this study was to assess the presence of *Trichinella* sp. in several wild carnivore and omnivore vertebrates in Central-Southern Chile.

Methods. We collected a total of 61 animals, encompassing 15 species. We digested muscle tissue to detect *Trichinella* larvae and further characterized larvae using molecular techniques.

Results. We detected *Trichinella* larvae found in a *Leopardus guigna* (Felidae) and a *Galictis cuja* (Mustelidae). We identified the larvae collected from *L. guigna* as *T. spiralis* but were unable to molecularly characterize the larvae from *G. cuja*.

This is the first record of *Trichinella* in a native mustelid of South America and the first record of *T. spiralis* in *L. guigna*. This study identified two novel hosts of *Trichinella* spp. in Chile; however, further work needs to be done to identify the role these and other hosts play in the sylvatic cycle of *Trichinella* spp. in Chile.