

# Trichinella in Wildlife in Chile With the First Record in Leopardus Guigna and Galictis Cuja

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## Short report

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# Abstract

**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 without veterinary inspection, a cultural practice that is difficult to resolve. Therefore, the knowledge of the reservoir of this parasite is relevant.

**Methods:** The aim of this study was to assess the presence of *Trichinella* sp. in several carnivore and omnivore vertebrates in Central-Southern Chile. 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* and a *Galictis cuja*. Only those of *L. guigna* could be identified as *Trichinella spiralis*.

**Conclusions:** 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.

# Background

Trichinellosis is a disease with worldwide distribution caused by *Trichinella* species [1], and also considered neglected and emerging [2, 3, 4, 5]. *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 [6], conditions that are difficult to improve. Another source of infection is the consumption of game animals [7, 8, 9, 10]. 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 [11]. 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 [1, 12]. Since most *Trichinella* species infect only mammals, the sylvatic cycle encompasses mainly mammal hosts [13, 14], including marine mammals [9, 15]. However, *T. pseudospiralis* also infects birds, and *T. zimbabwensis* and *T. papuae* infect reptile hosts [1]. 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 [16]. Four *Trichinella* species have been reported in this continent, mainly in Argentina: *T. spiralis*, *T. patagoniensis*, *T. britovi* and *T. pseudospiralis* [17, 18, 19, 20], as well as in cougars, wild boars, foxes, opossums, sea lions, armadillos and a cricetid rodent, *Graomys centralis* [16, 21]. These are the described wild host of *Trichinella* in the sylvatic environment. In Chile, the domestic cycle is fairly well-

studied [22, 23], but the sylvatic cycle is mostly unknown. *Trichinella spiralis* is the sole species that has been reported in Chile with few larvae identified to the species level [23, 24, 25]. Cougars and wild boars are the sole reported wild/feral host species reported [24, 25], and, in addition to those reports, other studies did not find infected animals [26, 27, 28]. Thus, the aim of this study was to assess the presence of *Trichinella* sp. in several carnivore and omnivore vertebrates in central-southern Chile.

## 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), 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* (n = 2), *Glaucidium nana* (1), *Strix rufipes* (2), *Tyto furcata* (5), Birds-Accipitriformes: *Parabuteo unicinctus* (11), *Cathartes aura* (1), *Coragyps atratus* (2); Birds-Pelecaniformis: *Pelecanus thagus* (1); Mammals-Artiodactyla: *Grampus griseus* (1); Mammals-Carnivora: *Otaria flavescens* (1), *Leopardus guigna* (6), *Galictis cuja* (17), *Lycalopex culpaeus* (9); Mammals-Microbiotheria: *Dromiciops gliroides* (1); and Reptile-Squamata: *Philodryas chamissonis* (1).

Artificial digestion of the muscles was performed in the Laboratory of Parasitology Dr. Luis Rubilar of the Facultad de Ciencias Veterinarias of the Universidad de Concepción following Gajadhar *et al.* [29] 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 Pozio and Zarlenga [30]. 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. Primers I forward (5'-GTTCCATGTGAACAGCAG-3') and reverse (5'-CGAAAACATACGACAACTGC-3') were used in a second PCR under same conditions with a annealing temperature of 55 °C. The PCR products were submitted to electrophoresis in 2% agarose gel. The Comité de Ética of the Universidad de Concepción approved the study (Certify 112017).

## 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 the grison was degraded and did

not output a PCR product. The DNA of the guiña 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 [31], 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.* [26] included two guiñas and 24 lesser grisons, in addition to other mammals, and González-Acuña *et al.* [27] included two guiñas, with negative results in both cases. Other studies in Argentina included another wild cat (*Leopardus geoffroyi*) and the lesser grison with negative results [32, 33]. 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 guiña, with the guiña being the second South American felid host for this parasitic species. Previously, other mustelids have been reported to host *Trichinella* elsewhere, as *Neovison vison* (American mink) hosting *T. spiralis*, *T. britovi* and *T. pseudospiralis* in Poland [34], as well as *Meles meles* (European badger) hosting *T. britovi* in Romania [35]. Similarly, other felids have been reported harboring *Trichinella* in Chile, as *Puma concolor* harboring *T. spiralis* [25], and elsewhere, as *Puma concolor* harboring *T. patagoniensis* in Argentina [20], *Puma concolor coryi* harboring *T. spiralis* and *T. pseudospiralis* in USA [36], *Puma concolor cougar* harboring *Trichinella nativa*, *Trichinella pseudospiralis*, *Trichinella murrelli* and *Trichinella* T6 [37], *Lynx rufus* harboring *T. britovi* and *Felis silvestris* harboring *T. britovi* and *T. spiralis* [38].

The guiña is one of the smallest felids in the world, with a restricted distribution in Chile and Argentina between 33° and 48° of latitude S [39]. This felid consumes micromammals preys as primary items [40, 41], 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 [23, 42]. This record is in accordance with the fact that guiñas have been frequently infected by micro-organisms spilled from free-range domestic animals [43, 44]; and, although *T. spiralis* is not an important pathogen for the health of non-human animals, its presence in the guiña 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 [45], and is a generalist predator, including rodents as an important part of its diet [46, 47]. 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 [20, 48].

To the best of our knowledge, there is no report of the guiña as prey of larger predators while *B. magellanicus* (Magellanic horned owl) is the sole predator reported for the lesser grison [45]. 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 [18]. 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 guiña 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 [7, 8]; however, neither guiñas nor grisons are typical prey for hunters to eat, nor is their hunting permitted by law in Chile [31].

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.* [26] 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 [49].

## 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 guiña. Thus, this study raises the number of mammals infected with *Trichinella* larvae, enhancing the need of identify the role of these animals in the reservoir for humans, and underlying the priority that the study of the rural-sylvatic interphase represents.

## Abbreviations

DNA  
deoxyribonucleic acid  
PCR  
polymerase chain reaction

## Declarations

**Ethic approval:** The Comité de Ética of the Universidad de Concepción approved the study (Certify 112017).

**Competing interests:** The authors declare that they have no competing interests.

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**Author contribution:** Each author has contributed individually and significantly to the development of the manuscript. DME, CS-d, RO and DS performed laboratory analysis. AH and DG-A collected/provided the samples. CL-A designed the study and wrote the first draft. All authors revised and expanded upon the original draft and approved of the submitted manuscript.

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**Consent for publication:** Not applicable

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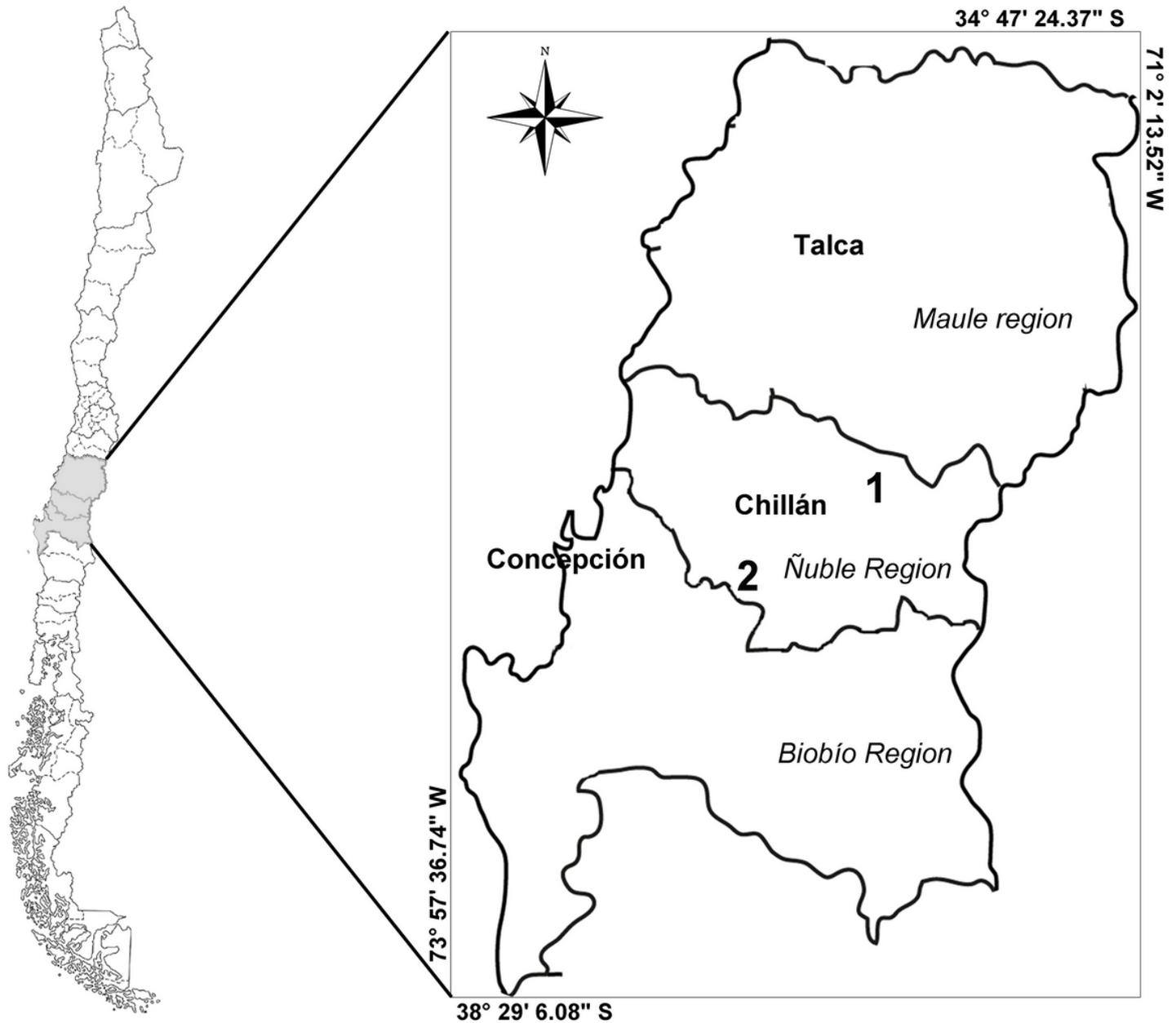
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## Figures



**Figure 1**

Map of Chile (left) and the studied administrative regions (right). 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*.

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