First record of *Trichinella* in *Leopardus guigna* (Carnivora, Felidae) and *Galictis cuja* (Carnivora, Mustelidae): New hosts in Chile

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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 through predation or carrion consumption and occur in domestic and sylvatic cycles. 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 game animals without veterinary inspection, a cultural practice that is difficult to resolve. Therefore, knowledge of this parasite's reservoir is relevant for better implementing public health strategies. The aim of this study was to assess the presence of *Trichinella* sp. in several carnivore and omnivore vertebrates in central-southern Chile.

Methods. We collected muscle tissue from a total of 53 animals from 15 species and were digested to detect *Trichinella* larvae which were further identified to species level using molecular techniques.

Results. We detected *Trichinella* larvae in *Leopardus guigna* (Felidae) and *Galictis cuja* (Mustelidae). We identified the larvae collected from *L. guigna* as *Trichinella spiralis*, but we 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; however, further work is needed to identify the role that these and other hosts play in the cycle of *Trichinella* in Chile.

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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 through predation or carrion consumption and occur in domestic and sylvatic cycles. 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 game animals without veterinary inspection, a cultural practice that is difficult to resolve. Therefore, knowledge of this parasite's reservoir is relevant for better implementing public health strategies. The aim of this study was to assess the presence of *Trichinella* sp. in several carnivore and omnivore vertebrates in central-southern Chile.

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Introduction

Trichinellosis is a disease that is distributed worldwide and is caused by nematodes in the genus *Trichinella* (*Korhonen et al., 2016*). It is considered neglected and emerging in some regions (*Dupouy-Camet, 1999; Murrell & Pozio, 2000; Bruschi, 2012; Boutsini et al., 2014*). *Trichinella* nematodes are transmitted from animals to humans by the ingestion of raw or undercooked infected meat.

Trichinella is transmitted among non-human animals via predation and carrion consumption; therefore, it circulates among carnivorous and omnivorous vertebrates. Two cycles have been described: the domestic (encompassing mainly pigs, rats, dogs, and cats) and the sylvatic (encompassing free-range vertebrates) cycles (*Pozio, 2000; Pozio, 2007; Loutfy et al., 1999*). These cycles can be connected and fed back by invasive rats and other synanthropic animals (*Pozio, 2000*). The domestic cycle was the primary cause of human infections; however, improvements in pork production have reduced outbreaks globally (*Devleesschauwer et al., 2015; Murrell, 2016*). The improvements to pork production changed the epidemiology of trichinellosis in human populations. *Trichinella* infections now primarily occur during the consumption of meat from unregulated sources, mainly backyard pork production and the consumption of game animals (*Pozio, 2014; Tryland et al., 2014; Fichi et al., 2015; Kärssin et al., 2017*).

At present, there are 10 recognized species of *Trichinella* around the world and three additional genotypes that have not yet been identified as distinct species (*Korhonen et al., 2016; Sharma et*

57   *al., 2020*). Most species infect only mammals (*Klun et al., 2019; Bilska-Zając et al., 2020*),
58   including marine mammals (*Tryland et al., 2014; Pasqualetti et al., 2018*). However, *Trichinella*
59   *pseudospiralis* Garkavi, 1972 also infects birds, and *Trichinella zimbabwensis* Pozio et al., 2002
60   and *Trichinella papuae* Pozio et al., 1999 infect reptile hosts (*Korhonen et al., 2016*). Thus,
61   obtaining ecological and epidemiological knowledge of the transmission cycle is relevant for
62   reducing the incidence of this parasite.
63   In South America, *Trichinella* spp. infections have been detected in Argentina, Bolivia, Chile
64   (larvae isolation), Brazil, and Ecuador (antibody detection) with most studies focusing on the
65   domestic cycle (*Bjorland et al., 1993; Ribicich et al., 2020*). Four species have been reported:
66   *Trichinella spiralis* Owen, 1835, *Trichinella patagoniensis* Krivokapich et al. 2012, *Trichinella*
67   *britovi* Pozio et al. 1992, and *T. pseudospiralis* (*Krivokapich et al., 2006; Krivokapich et al.,*
68   *2012; Krivokapich et al., 2015; Krivokapich et al., 2019*). Additionally, *Trichinella* infections
69   have been documented from eight wild species: cougar (*Puma concolor* Linnaeus, 1771), wild
70   boar (*Sus scrofa* Linnaeus, 1758), fox (*Lycalopex gymnocercus gracilis* Fischer, 1814), opossum
71   (*Didelphis albiventris* Lund, 1840), sea lion (*Otaria flavescens* Shaw, 1800), pecarí (*Tayassu*
72   *tajacu* Palmer, 1897), armadillo (*Chaetophractus villosus* Desmerest, 1804), and pericote
73   (*Graomys centralis* Thomas, 1902) (*Minoprio, Abdon & Abdon, 1967; Ribicich et al., 2020;*
74   *Soria et al., 2010*).
75   In Chile, the domestic cycle is fairly well-studied (*Alcaíno & Arenas, 1981; Schenone et al.,*
76   *2002*), but the sylvatic cycle is largely unknown. *Trichinella spiralis* is the sole species that has
77   been reported in Chile (*Schenone et al., 2002; Landaeta-Aqueveque et al., 2015; Hidalgo et al.,*
78   *2019; Echeverry et al., 2021; Espinoza-Rojas et al., 2021*). Among non-domestic animals,
79   cougars, American minks (*Neovison vison* Schreber, 1777) and wild boar are the only wild/feral
80   hosts with documented infections (*Landaeta-Aqueveque et al., 2015; Hidalgo et al., 2019;*
81   *Echeverry et al., 2021; Espinoza-Rojas et al., 2021*). In addition to those reports, other studies
82   have not found infected animals (*Alvarez et al., 1970; González-Acuña et al., 2010; Ramirez-*
83   *Pizarro et al., 2019*). Therefore, the objective of this study was to assess the presence of
84   *Trichinella* sp. in carnivorous and omnivorous wild vertebrates from south-central Chile.
85
86   **Materials & Methods**
87   The study area includes four administrative regions of Chile: the O'Higgins, Maule, Ñuble, and
88   Biobío regions (Figure 1). These regions feature a transitional climate that falls somewhere
89   between the classifications of warm Mediterranean (Csb, after Köpen classification) and wet
90   temperate oceanic (Cfb, after Köpen classification). These regions lie within the limits between
91   central and southern Chile.
92   This study considered animals that were found dead, mainly run over by a vehicle, or that died in
93   wild animal rescue/rehabilitation centers (Fauna Rehabilitation Center of the Universidad de
94   Concepción; Wild Fauna Rehabilitation Center of the Universidad San Sebastián) from 2013 to
95   2020. We examined at least 1 g of muscle (10 g, when possible) of these animals to determine
96   the presence of *Trichinella* spp. larvae. We then selected the following muscles for

97   parasitological examination: the diaphragm, masseter, tongue, quadriceps (in mammals), pectoral
98   (in birds), and intercostals (in all animals).
99   We performed artificial digestion of the muscles following the method described by Gajadhar et
100   al. (*2019*) and preserved the larvae in 96% ethanol. For molecular identification, we extracted
101   DNA from a pool of 10 *Trichinella* larvae isolated from each positive animal using the DNeasy
102   Blood & Tissue Kit (Qiagen, Hilden, Germany) and used 10 ng of DNA for identification at the
103   species level by nested polymerase chain reaction (PCR), following a modification of the
104   protocol of Zarlenga et al. (*1999*). We performed the reactions at a final volume of 25 μL. We
105   used the following primers: *Ne* forward (5'-TCTTGGTGGTAGTAGC-3') and reverse (5'-
106   GCGATTGAGTTGAACGC-3') in the first PCR (0.5 μM of each primer), and 12.5 μL of
107   GoTaq Green Master Mix (Promega Corporation, Madison, WI, USA). We amplified the DNA
108   in a thermocycler (MultiGene™ OptiMax Thermal Cycler; Labnet International, Inc., Edison,
109   NJ, USA) under the following cycling conditions: 95°C × 1 minute for initial denaturation,
110   followed by 40 cycles of 95°C × 30 sec; 56°C × 1 minute, and 72°C × 1 minute; and a final
111   extension of 72°C × 2 minutes. Then, we used 0.5 μM of each Primers *I* forward (5'-
112   GTTCCATGTGAACAGCAG-3') and reverse (5'- CGAAAACATACGACAACTGC-3') in a
113   second PCR under same conditions with an annealing temperature of 55°C. The PCR products
114   were subjected to electrophoresis in 2% agarose gel. We used master mix without the DNA as
115   the negative control, and *T. spiralis* larvae obtained from a previous study (*Landaeta-Aqueveque*
116   *et al., 2015*) as a positive control of the PCR.
117   Bioethical considerations: This study met the International Guiding Principles for Biomedical
118   Research Involving Animals. The Comité de Ética of the Facultad de Ciencias Veterinarias of
119   the Universidad de Concepción approved the study (CBE-47-2017).
120

121   Results

122   We collected samples from 53 animals. The sample was composed of 28 mammals, 24 birds and
123   one reptile (Table 1). The weight of the examined muscle samples were at least 10 g with the
124   exception of *D. bozinovici* and *P. chamissonis* with samples sizes of 3 g and 1 g, respectively.
125   *Trichinella* larvae were isolated only from one *Leopardus guigna* Molina, 1782 (güiña; 52 larvae
126   per gram of muscle) and one *Galictis cuja* Molina, 1782 (lesser grison; 0.3 larvae per gram of
127   muscle), both from the Ñuble region (Figure 1). We were unable to amplify DNA from the
128   larvae isolated from the grison. However, we were able to amplify a PCR product of 173 bp from
129   the güiña which is consistent with our *T. spiralis* positive control (Figure 2) and the size
130   described for this species (*Pozio & Zarlenga, 2019*).
131

132   Discussion

133   Detecting *Trichinella* infection is a challenge in wild fauna of Chile because most carnivore
134   vertebrates are protected by law (*SAG, 2012*). This protection is due to conservation concerns or
135   because these animals aid in pest control. Therefore, only invasive animals can be hunted to
136   assess *Trichinella* infection (*Hidalgo et al., 2019; Ramirez-Pizarro et al., 2019; Espinoza-Rojas*

137 *et al., 2021*). This has resulted in few studies that have assessed the presence of *Trichinella*
138 infection in native wildlife in Chile (*Alvarez et al. 1970; González-Acuña et al. 2010; Hidalgo et*
139 *al., 2013; Landaeta-Aqueveque et al., 2015; Echeverry et al., 2021*). Although one of these
140 studies sampled a broad range of mammalian species including güiñas and lesser grisons, it did
141 not detect *Trichinella* spp. (*Alvarez et al. 1970*).
142 Studies in Argentina examined another wild felid, the Geoffroy's cat (*Leopardus geoffroyi* D'
143 Orbigny and Gervais, 1844), and the lesser grison with negative results (*Ribicich et al., 2010;*
144 *Winter et al., 2018*). Thus, this is the first record of *Trichinella* spp. larvae in a native mustelid in
145 South America, and the first record of *T. spiralis* in the güiña. The güiña is the second reported
146 South American felid host for this species.
147 Previously, other mustelids have been reported to host *Trichinella* infections: American mink
148 infected with *T. spiralis* in Chile (*Espinoza-Rojas et al., 2021*) and with *T. spiralis*, *T. britovi*,
149 and *T. pseudospiralis* in Poland (*Hurníková et al., 2016*) and the European badger (*Meles meles*
150 Linnaeus, 1758) infected with *T. britovi* in Romania (*Boros et al., 2020*). Similarly, other felids
151 have reportedly harbored *Trichinella* larvae. *Trichinella* infections have been reported in cougars
152 across most of their range including with *T. spiralis* in Chile (*Landaeta-Aqueveque et al., 2015;*
153 *Echeverry et al., 2021*), *T. patagoniensis* in Argentina (*Krivokapich et al., 2012*), *T. spiralis* and
154 *T. pseudospiralis* in the United States (*Reichard et al., 2015*), *Trichinella nativa* Britov and
155 Boev, 1972, *T. pseudospiralis*, *Trichinella murrelli* Pozio and La Rosa, 2000, and *Trichinella* T6
156 in Canada (*Gajadhar & Forbes, 2010*). Additionally, infections have been reported in Canadian
157 lynx (*Lynx canadensis* Kerr, 1792) with *Trichinella* T6 in Canada (*Gajadhar & Forbes, 2010*),
158 Eurasian lynx (*Lynx lynx* Schreber, 1777) with *T. britovi*, and the European wildcat (*Felis*
159 *silvestris* Schreber, 1777) with *T. britovi* and *T. spiralis* (*Pozio et al., 2009*).
160 The güiña is one of the smallest felids in the world. It is distributed across Chile and Argentina
161 between latitudes of 33º S and 48º S (*Napolitano et al., 2014*). This felid consumes
162 micromammals such as rodents as primary prey (*Delibes-Mateos et al., 2014; Figueroa, Corales*
163 *& Rau, 2018*); consequently, rodents could be the source of infection. Rodents have been
164 recognized as hosts of *T. spiralis*, mainly in the domestic environment in Chile (*Schenone et al.,*
165 *1967; Schenone et al., 2002*). This record is in accordance with the fact that güiñas have been
166 frequently infected by pathogens from free-roaming domestic animals (*Ortega et al., 2020;*
167 *Sacristán et al., 2020*); although *T. spiralis* is not an important pathogen for the health of non-
168 human animals, its presence in the güiña highlights the need for pathogen surveillance in the
169 rural–sylvatic interphase.
170 The lesser grison is a neotropical mustelid that inhabits an area spanning southern Peru,
171 Uruguay, and Paraguay to southern Chile and Argentina, encompassing several environments
172 (*Prevosti & Travaini, 2005*). It is a generalist predator and rodents comprise an important part of
173 its diet (*Ebensperger, Mella & Simonetti, 1991; Zapata et al., 2005*). Given that, and considering
174 how other pathogens have spilled from domestic animals (*Megid et al., 2013; Pedrassani et al.,*
175 *2018*), this species might most likely be infected in domestic environments. However,
176 identification of the *Trichinella* species harbored by the lesser grison helps to better understand

177   the source of infection, given that not all *Trichinella* species identified in South America have
178   been reported in the domestic cycle. For instance, *T. patagoniensis* has been reported only in
179   cougars (*Krivokapich et al., 2008; Krivokapich et al., 2012*).
180   To the best of our knowledge, there are no reports of the güiña as prey of larger predators,
181   whereas the lesser horned owl (*Bubo magellanicus*) is the sole predator to be reported for the
182   lesser grison (*Prevosti & Travaini, 2005*). In that respect, *T. pseudospiralis*, also zoonotic, is the
183   only species of the genus that has reportedly infected birds, and this may be the only species of
184   *Trichinella* that could be transmitted from the grison to the owl. However, this species has not
185   been reported in Chile and one record of a single pig from Argentina represents the only report in
186   South America (*Krivokapich et al., 2015*). Therefore, it is unlikely that this owl could play a role
187   in the sylvatic cycle of *Trichinella* in Chile. Hence, whether güiña and lesser grison participate in
188   the reservoir or constitute dead-end hosts is unknown, and the most likely way for *Trichinella*
189   larvae to be transmitted from these hosts seems to be their consumption by carrion-consuming
190   mammals. Furthermore, human trichinellosis resulting from the direct consumption of a wild
191   mammal has also been reported worldwide (*García et al., 2005; Fichi et al., 2015*); however,
192   neither güiñas nor grisons are typical prey for hunters to eat, nor is their hunting permitted by
193   law in Chile (*SAG, 2012*). However, further studies are needed to evaluate these hypotheses.
194   It is worth noting that the two types of mammal host species reported herein had the largest
195   sample sizes, suggesting that larger samples of other mammals could represent new hosts for
196   *Trichinella*. In contrast, the lack of findings identified by Alvarez *et al.* (*1970*) may have been
197   due to the real absence of larvae in their samples, as well as to the parasitological technique
198   (trichinoscopy) used, which is of lower sensitivity (*Forbes, Parker & Scandrett, 2003*).
199

Conclusions

201   This is the first record of *Trichinella* larvae in a native mustelid, *G. cuja*, in South America, as
202   well as the first record of *T. spiralis* in *L. guigna*. Thus, this study increased the number of
203   mammals infected with *Trichinella* larvae in the neotropics, enhancing the need to identify the
204   role played by neotropical animals in the reservoir for humans. This underlies how studying the
205   rural–sylvatic interphase is of utmost importance.
206

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208   In memoriam: The authors dedicate this article to Daniel González-Acuña, who died during the
209   writing of this manuscript prior to submission, and who made significant contributions to this
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211

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421

Details of examined animals

1 Table 1: Details of examined animals.

Species	Infected/Analyzed (%)	Class
Glaucidium nana King, 1828 (Austral pygmy owl)	0/1 (0)	Aves
Bubo magellanicus Gmelin, 1788 (Magellanic horned owl)	0/2 (0)	Aves
Tyto furcata Temminck, 1827 (American barn owl)	0/5 (0)	Aves
Strix rufipes King, 1828 (Rufous-legged owl)	0/2 (0)	Aves
Parabuteo unicinctus Temminck, 1824 (Harris' hawk)	0/11 (0)	Aves
Coragyps atratus Bechstein, 1793 (Black vulture)	0/1 (0)	Aves
Cathartes aura Linnaeus, 1758 (Turkey vulture)	0/1 (0)	Aves
Pelecanus thagus Molina, 1782 (Peruvian pelican)	0/1 (0)	Aves
Grampus griseus Cuvier, 1812 (Risso's Dolphin)	0/1 (0)	Mammalia
Otaria flavescens Shaw, 1800 (South American sealion)	0/1 (0)	Mammalia
Leopardus guigna Molina, 1782 (Güiña)	1/6 (16.67)	Mammalia
Lycalopex culpaeus Molina, 1782 (Culpeo fox)	0/2 (0)	Mammalia
Galictis cuja Molina, 1782 (Lesser grison)	1/17 (5.88)	Mammalia
Dromiciops bozinovici D'Elía, Hurtado and D'Anatro, 2016 ('Monito del monte')	0/1 (0)	Mammalia
Philodryas chamissonis Wiegmann, 1834 (Long-tailed snake)	0/1 (0)	Reptilia

Figure 1

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

The italicized text indicates the name of the regions, and the Roman text indicates the name of the communes. Infected animals are presented with the symbols "**+**" (*Leopardus guigna*) and "***" (*Galictis cuja*). The numbers indicate the number of animals examined in each commune. Thick lines indicate the regional limits, while thin lines indicate the limits of the communes.
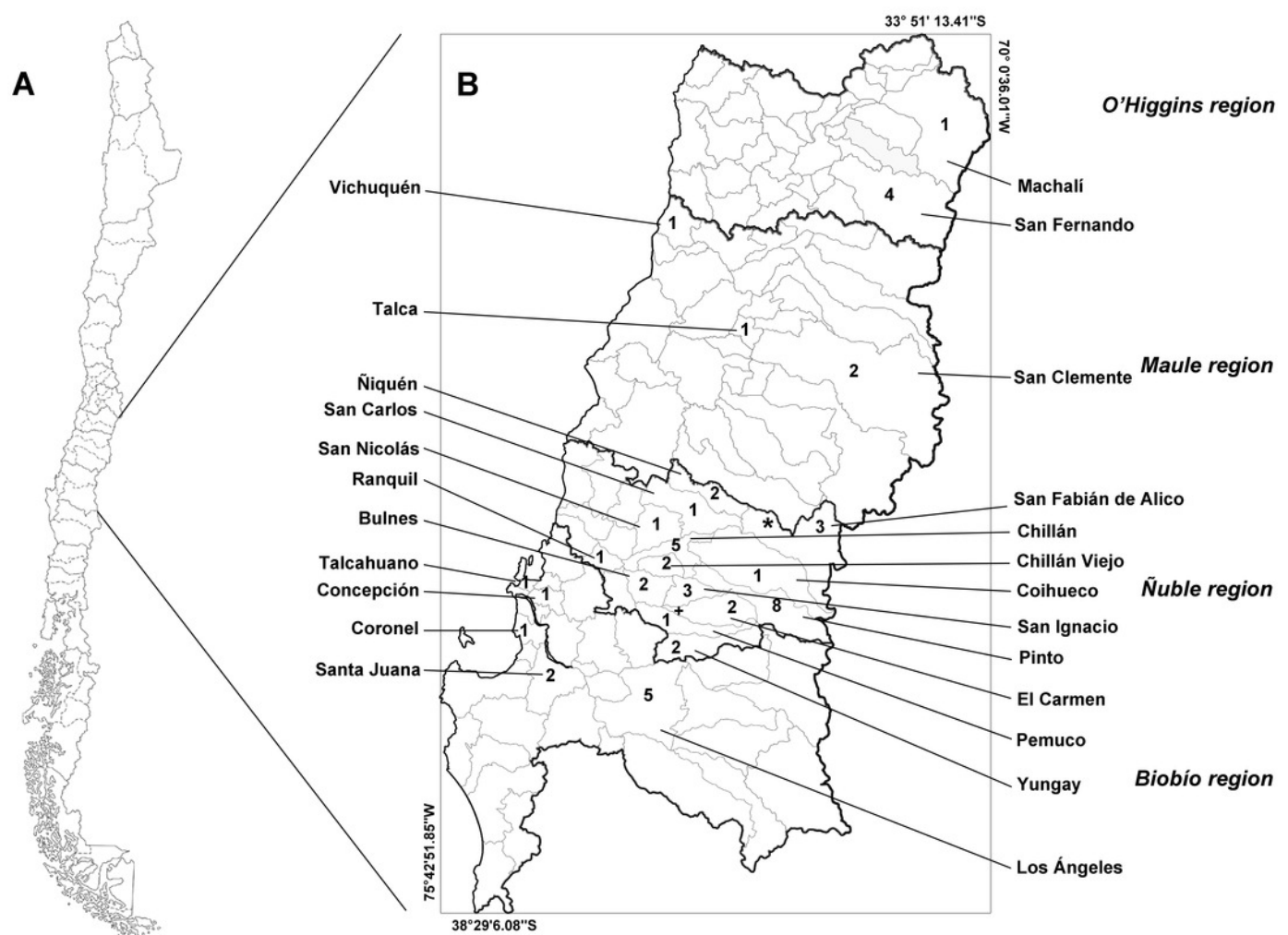
Figure 2

a) Larva of *Trichinella* sp. isolated from a *Galictis cuja*. b) Gel electrophoresis of PCR
products

(b) MW: Marker of 50 bp. C-: negative control. C+: *Trichinella spiralis* positive control. Lanes 1

– 4: isolates from *Leopardus guigna*.