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# EAR MITES, *OTODECTES CYNOTIS*, ON WILD FOXES (*PSEUDALOPEX* SPP.) IN CHILE

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ABSTRACT: We found the ear mite parasite (*Otodectes cynotis*; Acari: Psoroptidae) in two distant insular endangered fox populations in Chile. We identified *O. cynotis* in both the Darwin's fox (*Pseudalopex fulvipes*) from Chiloé and the Fuegian culpeo (*Pseudalopex culpaeus lycoides*) in Tierra del Fuego. These populations are approximately 2,000 km apart. Infestation rates were high for both endemic foxes: 76% (19/25) of Darwin's foxes were affected, and 73% (11/15) of Fuegian culpeos had ear mites. Two Darwin's foxes had abundant ear discharge, and one of these also exhibited secondary infections of *Morganella morganii* and *Geotrichum* sp. fungi. Mites were characterized molecularly as *Otodectes* spp. for the Fuegian culpeo samples. Genetic analyses of two mites found the *O. cynotis* genotype I, as well as what appeared to be a new allele sequence for *O. cynotis*. These results confirmed the hypothesis of a worldwide distribution species of ear mite. Introduced chilla foxes (*Pseudalopex griseus*; *n*=11) on Tierra del Fuego Island and domestic dogs (*Canis lupus familiaris*; *n*=379) from both islands were also sampled, but they showed no signs of infection. Our findings provided insight into the genetic diversity, the origins, and the possible impact of this globally distributed mite on endemic free-ranging populations of foxes.

*Key words:* Chiloé, ear mite, Darwin's fox, Fuegian culpeo fox, *Otodectes cynotis*, *Pseudalopex culpaeus lycoides*, *Pseudalopex fulvipes*, Tierra del Fuego.

## INTRODUCTION

*Otodectes cynotis* has a worldwide distribution and is an ear mite of domestic and wild cats (*Felis catus*) and dogs (*Canis lupus familiaris*). Mites affect the ear canals of their hosts, leading to otodectic mange and otitis externa (Urquhart 1996). Mites also affect wild species, mainly carnivores including red foxes (*Vulpes vulpes*), island foxes (*Urocyon littoralis*), arctic foxes (*Alopex lagopus*), ferrets (*Mustela putorius furo*), and raccoons (*Procyon lotor*; Mullen and OConnor 2002; Moriarty et al. 2015). Mite occurrence, distribution, and impact on individual health is difficult to estimate and is almost completely unknown for wild, endangered species and isolated populations (McCallum 2012). Although mite infestations are not normally lethal, otodectic mange has been associated with high levels of mortality (over 90%) in cubs of endangered Mednyi Island Arctic foxes (*Alopex lagopus semenovi*; Goltsman et al. 1996). Thus, ectoparasites are mostly sublethal, but they may contribute to population decline and might lead to extinction of small populations.

Three species of South American foxes of the genus *Pseudalopex* are found in Chile. The culpeo fox (*Pseudalopex culpaeus*) is the largest of the three and is distributed throughout the Andes (Novaro 1997; Jiménez and Novaro 2004; Lucherini 2016a). The chilla fox (*Pseudalopex griseus*) is abundant and mostly found in shrubby open habitats along the Pacific coast of the Southern Cone (Lucherini 2016b). The Darwin's fox (*Pseudalopex fulvipes*) is the smallest of the three species. It is endangered and endemic to Chile. The largest population lives on Chiloé Island, estimated to consist of fewer than 500 individuals. It was the only canid on Chiloé until introduction of domestic dogs, which currently roam unleashed (Jiménez and McMahon 2004). Smaller populations of Darwin's foxes also exist on mainland Chile, in Nahuelbuta National Park, and some smaller populations have been identified in territory between Nahuelbuta and Chiloé Island (Jiménez and McMahon 2004; D'Elía et al. 2013; Farias et al. 2014; Silva-Rodríguez et al. 2018).

The Fuegian culpeo fox is considered a distinct subspecies (Jiménez and Novaro 2004; Tadich et al. 2018). It is restricted to the Isla Grande of Tierra del Fuego (TdF), the largest island in South America, which is shared by Argentina and Chile (Osgood 1943). The population has been classified as vulnerable (Ministerio del Medio Ambiente 2011). The Fuegian culpeo fox was once the sole endemic fox on TdF, until introduction of domestic dogs and chilla foxes. The latter were introduced in 1951 to control rabbits (Jaksic and Yanez 1983).

Relatively little is known about ectoparasites found on isolated *Pseudalopex* canids. White-ribbed dog ticks (*Amblyomma tigrinum*) have been found on culpeos, chillas, and dogs in central and southern continental regions, north of Chiloé Island (González-Acuña and Guglielmone 2005). *Trichodectes canis* lice have been observed on Darwin's foxes from Chiloé Island, but their source has not been identified (González-Acuña et al. 2007). Sarcoptic mange, produced by the *Sarcoptes scabiei* mite, has been described in inland chillas and dogs, at the approximate latitude of Chiloé Island. Verdugo et al. (2016) suggest that *S. scabiei* may have been transferred to chillas from introduced rabbits.

The ear mite is distributed throughout the world and is a common ectoparasite of domestic dogs and cats, both of which are known reservoirs for cross-species parasite transmission. In South America, this mite has been described in ocelot (*Leopardus pardalis*; Murray and Gardner 1997) and urban cat populations in Brazil (Mendes-de-Almeida et al. 2011; Machado et al. 2018). There is no information on ear mite infection among wild canids in Chile (Tagle 1966; Alcaíno and Gorman 1999) or elsewhere in South America.

Our objective was to survey for *O. cynotis* in wild foxes and domestic dogs on Chiloé and TdF. Here we report on ear mite infestations, identify the species using a genetic marker, and assess the potential impact in wild insular populations.

## MATERIALS AND METHODS

Darwin's foxes were captured using baited cage traps baited with canned fish, on Chiloé Island between March 2003 and April 2005. Fuegian culpeo and chilla foxes were similarly captured using Tomahawk traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin, USA) on TdF during August 2008 and December 2009, respectively. All captures took place under permits 1350 (2003), 5292 (2005), 3607 (2007), 4053 (2007), 1845 (2008), 4468 (2009), and 8009 (2011) issued by Servicio Agrícola y Ganadero (the Chilean Bureau for Agriculture and Environment). All foxes were restrained in the trap using a squeeze panel and sedated by intramuscular injection of 20 mg/kg of ketamine (Imalgene 1000®, 100 mg/mL, Merial, Lyon, France) and 1 mg/kg of xylazine (Rompun®, 20 mg/mL, Bayer, Leverkusen, Germany) or 2.5 mg/kg ketamine and 0.05 mg/kg medetomidine (Domitor®, 1 mg/mL, Orion, Espoo, Finland). Once sedated and safe to handle, each animal was removed from the trap and placed on a canvas tarp. Heart and respiratory rate and body temperature were monitored every 5 min to ensure sedation was safe for the animal. Each individual was given a physical examination, including ear duct inspection using an otoscope and an ear tag. Occurrence of dirty ear canals and crusts and presence of acari-like invertebrates were noted. Otic material was sampled using cotton-covered ear forceps. The individually labeled cotton was preserved in 10% buffered formalin (Chiloé) or 70% ethanol (TdF). Following sample collection, each animal's ears were cleaned. Sedation was reversed using intravenous injection of 0.2 mg/kg yohimbine (Yohimbine, 2 mg/mL, Laboratorio Chile, Santiago, Chile) for xylazine or intramuscular injection of 0.25 mg/kg atipamezole (Antisedan®, 5 mg/mL, Orion, Espoo, Finland) for medetomidine after handling for about 35 to 45 min. Following full physical recovery and lack of signs of neurological impairment, animals were released near their trapping sites.

During the capture seasons, 346 dogs from Chiloé and 33 dogs from TdF were inspected for otitis and ear mites by veterinarians (Fig. 1). The

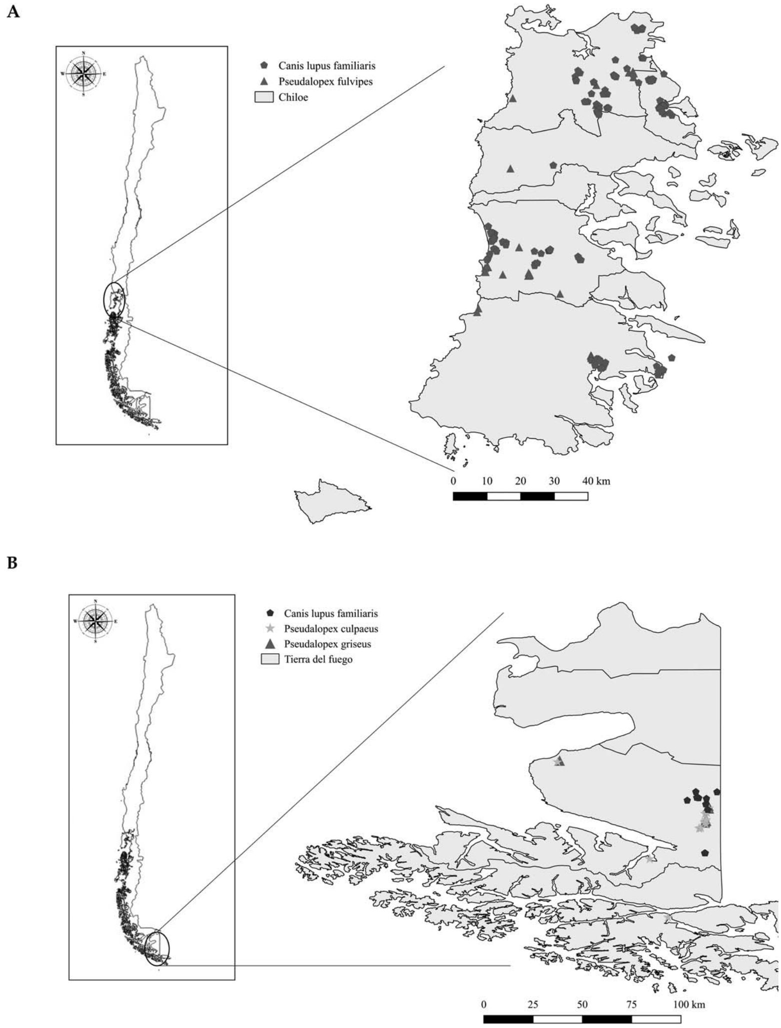


FIGURE 1. Maps depicting fox capture and dog sampling places for (A) Chiloé and (B) Tierra del Fuego islands during 2003–05 and 2008–09, respectively.

dog health inspections were part of a disease surveillance program that included dogs from villages and ranches. All dog owners gave information about dog management and consent during these inspections.

All mites obtained from foxes were isolated for identification. Morphometric variables were recorded for nine samples using electron microscopy images (Fig. 2). For genetic studies, mites were individually pressed between two frozen glass slides, and, later, biological material was retrieved by pipetting 200 µL ATL buffer (QIAmp® DNA mini kit, Qiagen Ltd., West Sussex, UK) onto the rim of the slides and collecting it by capillary action. Extraction was completed using the QIAmp® DNA mini kit following the manufacturer's instructions.

To identify the mite species, the second internal transcribe spacer (ITS 2) of the ribosomal DNA was amplified using primers RIB-4CB (5′-ATC GGT GTG AAA TGC AGG ACA-3′ (modified from Zahler et al. 1995) and RIB-31 (Lohse et al. 2002); both primers also incorporated an M13 sequencing primer on the 5′ end. The PCR reactions consisted of 30-40 ng genomic DNA, 1× Qiagen Multiplex mix (Qiagen), 0.5× Qiagen Q solution, and 0.2 mM of each primer and double-distilled water for a final volume of 24 µL. Cycling conditions consisted of one initial denaturation at 95 C for 120 s followed by 40 cycles as described by Lohse et al. (2002) and a final elongation step of 72 C for 10 min. Amplicons were separated on a 1.5% agarose gel containing ethidium bromide

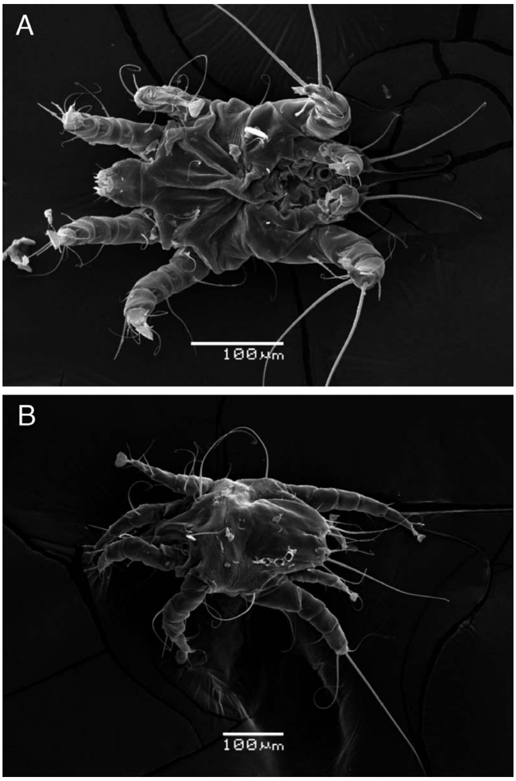


FIGURE 2. Electron microscopy images of adult ear mites (*Otodectes cynotis*). (A) Ventral view of a male specimen found on a Darwin's fox (*Pseudalopex fulvipes*) ear canal. (B) Dorsal view of a deutonymph (nymphal stage; smaller rear legs), found inside the ear canal of a Fuegian culpeo fox (*Pseudalopex culpaeus lycoides*).

and visualized under ultraviolet light. Amplicons of the size expected for the ITS 2 primer pair (324 base pairs) were excised from the gel, purified, and directly sequenced (ABI3730XL, Macrogen Inc., Seoul, Republic of Korea), using the M13 forward and reverse primers. We edited DNA sequences manually by reviewing chromatograms and aligned them using MEGA 6 (Tamura et al. 2013). Consensus sequences were compared with five mite sequences previously published in GenBank (accession nos. AF367699 to AF367703; Lohse et al. 2002).

Cary-Blair medium (Winkler, Santiago, Chile) was inoculated with brown exudate swabbed from the ear duct of one Darwin's fox and processed at Laboratorio de Química Clínica Especializada (Santiago, Chile). Presence of fungi was assessed using morphologic examination and culturing on Sabouraud agar. Bacilli were tested for resistance to beta-lactam antibiotics and for cefradine, doxycycline, and metronidazole, using the Kirby-Bauer method (Poirel et al. 1999).

## RESULTS

A total of 25 Darwin's foxes were captured on Chiloé Island (Fig. 1A), and 14 Fuegian culpeos and 11 chilla foxes were captured on TdF (Fig. 1B). Excluding three individuals, where occurrence of mite infection remained undetermined, 83% (19/23) Darwin's foxes, 79% (11/14) culpeos, and no (0/11) chilla foxes exhibited ear mite infestation (Table 1). There was no significant difference in infectious rate between Darwin's foxes and culpeos (Fisher's exact test, $P>0.999$). Crusty brown material appeared in both dry and moist conditions (Fig. 2) and was sometimes accompanied by a strong foul smell. Otitis appeared more severe among Darwin's foxes than among culpeos. The more severe infestation was indicated by the presence of the exudative ear canal in two Darwin's foxes. Mites from Darwin's foxes were 428 µm (female, $n$=1) and a mean (SD) 283 (26.9) µm (males, $n$=3) in body length, while the gnathosoma measured 62.2 µm and 51.9 µm in male ($n$=1) and female ($n$=1). Mites from culpeos had body lengths of 442 (0) µm for females ($n$=2), 302.5 (2.1) µm ($n$=2)

TABLE 1. Number of ear mite infestation cases on three species of foxes: Darwin's fox (*Pseudalopex fulvipes*), Fuegian culpeo (*Pseudalopex culpaeus*), and chilla (*Pseudalopex griseus*) surveyed in Chiloé Island, Chile, during 2003–05, and Tierra del Fuego Island (TdF), Chile, during 2008–09.

| Common name | *n* | Location | Positive | Negative | Undetermined |
|---|---|---|---|---|---|
| Darwin's fox | 25 | Chiloé | 19[a] | 4 | 2 |
| Fuegian culpeo | 15 | TdF | 11 | 3 | 1 |
| Chilla fox | 11 | TdF | 0 | 11 | 0 |

[a] Two of these foxes showed exudative ear discharge.

TABLE 2. Base differences depicting polymorphisms in the ITS-2 ribosomal RNA sequence (324 base pairs) of the ear mite (*Otodectes cynotis*). Genotypes I–V obtained from Lohse et al. (2002) and MK032887-8 (this study) obtained from ear mites from Fuegian culpeos (*Pseudalopex culpaeus*) sampled in Tierra del Fuego. Bold type represents differences found in the two obtained sequences in this study. M (A or C) and Y (C or T) represent mixed base pair positions.

| | Base pair positions[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| | 89 | 92 | 105 | 149 | 165 | 178 | 179 |
| Genotype I | G | G | A | C | T | A | — |
| Genotype II | A | A | C | T | — | — | G |
| Genotype III | G | G | A | C | T | A | G |
| Genotype IV | A | A | A | C | T | A | G |
| Genotype V | A | A | C | T | T | A | G |
| MK032887 | G | G | **M** | C | T | A | — |
| MK032888 | G | G | C | **Y** | T | A | — |

[a] — = base pair deletion.

for males, and 386 µm for a deutonymph ($n$=1). For these mites, their gnathosoma measured 58.8 (9.8) µm in females ($n$=2), 55.65 (6.1) µm ($n$=2), in males, and 66.5 µm in the deutonymph. None of the domestic dogs from Chiloé ($n$=346) or TdF ($n$=33) exhibited ear mite infestation or exudative cerumen.

We extracted DNA separately from two mites removed from two Fuegian culpeos on TdF. Two PCR amplicons were directly sequenced in both directions. Analyses revealed one sequence with 100% identity to *O. cynotis* genotype I (Lohse et al. 2002). We also identified two other sequences with 99% identity to *O. cynotis* genotype I, accession MK032887 with 1 mutation; A→M(A or C) at nucleotide position 105, and accession MK032888 with 2 mutations: A→C and C→Y(C or T) at nucleotide positions 105 and 149, respectively (Table 2). Heterozygous polymorphisms in the ITS-2 ribosomal RNA sequence were observed as peaks visualized on the chromatograms for each of the two mites from which fragments were amplified.

We identified the Gram-negative bacillus *Morganella morganii* and the fungus *Geotrichum* sp. from the Darwin's fox exudate. The *Morganella morganii* bacillus was resistant not only to beta-lactam antibiotics, but also to doxycycline and metronidazole.

## DISCUSSION

To date, there have been no reports of *O. cynotis* in wild mammals in Chile. Our survey of foxes and domestic dogs throughout Chiloé, and on the Chilean side of TdF, identified two species of native foxes, living approximately 2,000 km apart, infected with ear mites. This parasite often produces moistened and brown exudates in the ear canal, and diagnosis can be confirmed with otoscopic examination (Mullen and OConnor 2002). Our survey of dogs from Chiloé and TdF, and chilla foxes from TdF, showed no sign of otodectic mites or other ear infections.

Our DNA analyses of two mites from culpeo foxes found sequences that were identical and some that were very similar to genotype I, which was previously described by Lohse et al. (2002). Although we were limited by sample number and the narrow scope of our preliminary genetic analyses, our results suggested that we have identified several new genotypes in *O. cynotis*. Additionally, since we isolated DNA from single individuals and obtained multiple, but similar, sequences, we interpret this as supporting evidence for the intra-isolate variation of *O. cynotis* described by Lohse et al. (2002). Furthermore, our results supported the argument that there is just one species of ear mite (Lohse et al. 2002), which has worldwide distribution.

In general, parasites that are not lethal occur at higher frequency compared to those that are highly pathogenic (McCallum and Dobson 1995; McCallum 2012). Macroparasites, such as mites, tend to be enzootic in host populations, and their impact is more difficult to assess than microparasites (i.e., viruses, bacteria, and protozoa), which may lead to epidemics (McCallum and Dobson 1995). In small or endangered populations, like the Darwin's fox, infectious diseases may be important drivers of extinction (Jiménez and McMahon 2004). However, the impact of infection is difficult to measure and, therefore, may be underestimated (McCallum 2012). Acari mites, for example, had a detrimental effect on native animal populations when red foxes (*Vulpes vulpes*) were introduced in 1910 in Western Australia. These mites were believed to result in mange, which led to large-scale mortality in native mammals (Abbott 2006).

*Otodectes cynotis* produce inflammation and pruritus of the ear canal and accumulation of cerumen and brown exudate that may obstruct the ear canal and produce ceruminous gland hyperplasia (Mullen and OConnor 2002; Moriarty et al. 2015). This ceruminous thickened exudate has the potential to be carcinogenic (Moriarty et al. 2015). Severe untreated cases may cause emaciation, self-induced trauma, and even seizures (Mullen and OConnor 2002). Thus, they should be considered a genuine threat to wildlife (Gómez and Aguirre 2008). Mite infestation resulted in a 96% mortality rate for cubs of the endangered arctic fox population of the Mednyi Island (*Vulpes lagopus*). More recently, ear mites were found on captive Patagonian mara (*Dolichotis patagonum*), where domestic cats were identified as the source of multiple infections, even after treatment (Lopes da Cruz et al. 2017).

Given the wide distribution and monotypic characteristics of this mite (Lohse et al. 2002) as well as the wide distribution and abundance of dogs (Hughes and Macdonald 2013) and cats (Long 2003) that serve as carriers, it is likely that *O. cynotis* has spread in parallel with human population expansion. The mite's ability to parasitize more than one host suggests that it is less pathogenic to some species and, thus, has gained a wider geographic distribution (McCallum and Dobson 1995). Support for this argument comes from the observation that domestic cats on Chiloé Island are affecting the health of sympatric native guigna (*Leopardus guigna*) through interspecific transmission of pathogens resulting from human invasion of natural habitats and fragmentation (Mora et al. 2015).

This parasite may be a real threat to the survival of endangered populations that encounter it if they have had no prior exposure (McCallum 2012). Perhaps this is why endangered native foxes exhibited such high rates of infection and apparent intensity. Importantly, even if this ear mite infection has little impact on the survival, or fitness, of endangered insular foxes in Chile, it may have ecological implications since chilla foxes and dogs, which seem to lack the parasite, could have a competitive advantage (McCallum and Dobson 1995).

Our relatively limited survey found that Darwin's foxes compared to culpeos were more affected in intensity by ear mites. Nineteen Darwin's foxes exhibited inflammation and exudate in their ear canals. For one fox, the ear discharge was sampled, and bacterial and fungal infections were detected. Further, the isolated bacilli had multiple resistance to tetracycline (doxycycline) antibiotics.

It is unclear why some foxes exhibited a secondary infection, but it could be due to the fact that Chiloé Island is rainier and more humid than the colder and drier Tierra del Fuego Island, which may provide a wetter environment, leading to more ear canal infections (Dalziel 1981; Yoshida et al. 2002; Smith-Ramírez 2004). Darwin's foxes have limited genetic diversity of critical immune genes, due to their small population size (Jiménez and McMahon 2004), and this may also affect susceptibility to infection. For instance, the louse *Trichodectes canis* has been also found in Darwin's foxes from Chiloé but not yet in dogs (González-Acuña et al.

2007). We encourage further research on ectoparasites and assessment of their prevalence in other carnivores, such as cats. This could not only help determine more sources of infection, but it could contribute to our understanding of the long-term impact on endangered and native insular fox populations.

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