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Molecular Survey of Parvoviruses and *Mycoplasma s*pp. in Invasive American Mink (*Neovison vison*) from Southern Chile

María Belén Zapararte,[1,5] Francisco Ramírez-Pizarro,[2,5] Carlos Landaeta-Aqueveque,[2] Elie Poulin,[1,3] René Ortega,[2,6] and Constanza Napolitano[3,4,6] [1]Laboratorio de Ecología Molecular, Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Las Palmeras 3425, Ñuñoa, Santiago, Chile; [2]Departamento de Patología y Medicina Preventiva, Facultad de Ciencias Veterinarias, Universidad de Concepción, Avenida Vicente Méndez 595, Chillán, Chile; [3]Instituto de Ecología y Biodiversidad, Las Palmeras 3425, Ñuñoa, Santiago, Chile; [4]Departamento de Ciencias Biológicas y Biodiversidad, Universidad de Los Lagos, Avenida Fuchslocher 1305, Osorno, Chile; [5]These authors contributed equally to this article; [6]Corresponding authors (emails: constanza.napolitano@ulagos.cl, reortega@udec.cl)

ABSTRACT: Using PCR, we evaluated the presence of parvoviruses and *Mycoplasma* spp. in 123 American mink (*Neovison vison*), an introduced invasive carnivore in Chile. Our results showed all analyzed animals were negative for both pathogen groups. We cannot completely dismiss their presence, but if present, their prevalence should be lower than 2%.

The American mink (*Neovison vison*) was introduced in Chile in 1930 for the fur industry and then escaped or was released from mink farms, becoming an important invasive species (no mink farms currently operate in Chile). Its high plasticity has allowed American mink to inhabit a wide range of habitats, being currently distributed from the Araucanía to the Magallanes Regions, where they lack natural predators (Schüttler et al. 2010). No study has evaluated the presence of parvoviruses or *Mycoplasma* species in mink populations across Chile, although they have been detected in mink introduced in other South American countries (Martino et al. 2017) and in their native range.

Hemotrophic mycoplasmas (hemoplasmas) are epierythrocytic bacteria that can be transmitted by vectors, vertically or by direct contact with blood or saliva (Millán et al. 2015). In Chile, mycoplasmas have been reported in 25% of domestic dogs (*Canis lupus familiaris*) from the Los Ríos Region (Soto et al. 2017), 15% of domestic cats and 24% of guignas (*Leopardus guigna*) across its range (Sacristán et al. 2019), and 57% of Darwin's foxes (*Lycalopex fulvipes*) from Chiloé Island (Cabello et al. 2013).

Parvoviruses are environmentally stable viruses whose transmission can be direct or indirect (Buonavoglia et al. 2001). A high prevalence of parvoviruses has been reported in domestic and wild carnivores in Chile: 78% canine parvovirus (CPV) in dogs from the Araucanía Region (Acosta-Jamett et al. 2015) and 6% and 13% of CPV and feline parvovirus (FPV) in domestic cats and guignas, respectively, across the range of the guigna (Sacristán 2019).

Mink have high abundance in Chile, and they can potentially share habitats with native and domestic carnivores, thus enhancing pathogen transmission or acting as bridge hosts. Therefore, we hypothesized that populations of invasive American mink in Chile are infected by parvoviruses and *Mycoplasma* spp.,harbor new introduced variants, and thus represent a threat for native carnivores such as guigna, three species of fox, marine otter (*Lontra felina*), southern river otter (*L. provocax*), and lesser grison (*Galictis cuja*). We used molecular methods to detect infection with these pathogens in invasive American mink in Chile.

The study was conducted at four study sites in southern Chile (Fig. 1). In Valdivia (Los Ríos region), animals were captured and euthanized by the Servicio Agrícola y Ganadero as part of the American mink eradication program. At Maullín, Ancud and Navarino Island, captures were conducted as described by Ramírez-Pizarro et al. (2019). We obtained a total of 123 mink carcasses (Table 1), which were preserved at –20 C. During necropsy, we collected 1.5 mL volume of the first

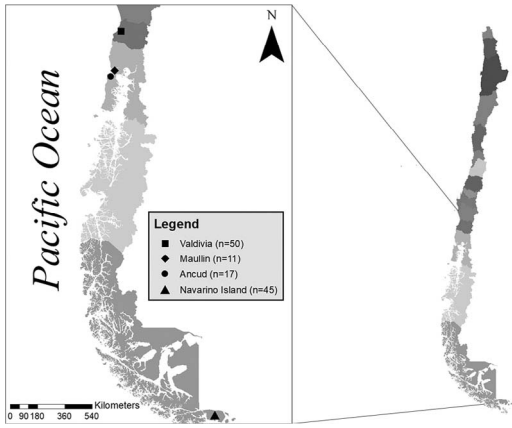

FIGURE 1. Geographic location of study sites in Chile, and mink (*Neovison vison*) sample sizes collected in each of them: square, Valdivia, Los Ríos Region (region XIV), *n*=50; diamond, Maullín, Los Lagos Region (region X), *n*=11; circle, Ancud, Los Lagos Region (region X), *n*=17; triangle, Navarino Island, Magallanes Region (region XII), *n*=45. Different shades in the map show different administrative regions across Chile.

portion of the small intestine and feces as the target tissue for parvoviruses, and spleen samples as the target tissue for *Mycoplasma* spp. (Table 1). External analyses (morphometric measures, age class, sex) was obtained for only 88% of the bodies (*n*=108). All samples were preserved at –20 C until molecular analysis.

Total DNA from all tissues and feces collected (Table 1) were extracted using the DNeasy® Blood & Tissue kit or QIAamp DNA Stool Mini kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. We conducted PCR for amplification of: 1) internal control for DNA quality (mtDNA control region); 2) the *VP2* gene of canine parvovirus and mink enteritis virus (Wang et al. 2013) and the *VP1* gene (Buonavoglia et al., 2001); 3) the *VP2* and *NS1* genes for Aleutian mink disease virus (Jensen et al. 2011); and 4) *Mycoplasma* species rRNA16S gene (Millán et al. 2015). Each PCR was run at least twice, including negative (ultrapure water) and positive controls (DNA from PCR-positive domestic cats and dogs confirmed by sequencing).

TABLE 1. Number of invasive American mink (*Neovison vison*) from southern Chile sampled for parvoviruses and *Mycoplasma* spp., by type of tissue analyzed across the different study sites.

Study site	No. animals	Sample types		
		Spleen	Intestine	Feces
Valdivia	50	50	50	0
Maullín	11	0	11	11
Ancud	17	0	17	17
Navarino Island	45	0	45	45
Totals	123	50	123	73

Sex proportions in all samples were 79.6% (86/108) males and 20.4% (22/108) females; age classes were 33.3% (36/108) juveniles and 66.7% (72/108) adults. The quality of DNA was high (internal controls amplified correctly), and PCR were successful with no contamination. No evidence of infection with the pathogens was found in the animals. None of the animals showed pathological signs during necropsies.

The prevalence and confidence intervals were estimated using the online platform Winepi® (de Blas 2006) with the Maximum Possible Prevalence function, which, based on the number of samples available, determines the maximum possible prevalence that could exist in a population when all collected samples are negative. We calculated that the lower limit of detectable prevalence (95% CI) was 5.82% for Valdivia, 23.84% for Maullín, 16.16% for Ancud, 6.44% for Navarino Island, and 2.4% for the total sample size.

Our sampling extended across a large geographic range, with an appropriate overall sample size. Our results suggested that both *Mycoplasma* spp. and parvoviruses may be absent from Chilean mink populations or, alternatively, that prevalence is low enough not to be detected with the number of samples available. When prevalence is low, the number of samples necessary to detect pathogens increases exponentially; for example, 300 is the minimum number of mink to evaluate 1% prevalence with 5% type II error. Absence or low prevalence (at least less than

2.4%) in the Chilean mink population might be related to the lower population densities reported in Chile compared to their counterpart in Europe (Bonesi and Palazon 2007), assuming transmission is density-dependent, as reported in other studies (Acosta-Jamett et al. 2015; Millán et al. 2015). The higher number of native mustelids in Europe (13 species), might also facilitate the possibility for infectious agents to maintain their populations in a broader range of hosts (Temple and Terry 2017). In contrast, there are only four native mustelid species inhabiting Chile and their interactions are limited because of their distributions.

Our results suggest that American mink are not yet a threat for cross-species transmission of the studied pathogens to native carnivores in Chile. Future studies should consider a larger sample size and more sensitive molecular techniques (i.e., real-time PCR), to increase the probability of detecting pathogens with low prevalence. We recommend continuing with these studies, due to the relevance of mink as invasive species potentially threatening Chilean native wildlife.

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