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FELINE IMMUNODEFICIENCY VIRUS AND FELINE LEUKEMIA VIRUS INFECTION IN FREE-RANGING GUIGNAS (*LEOPARDUS GUIGNA*) AND SYMPATRIC DOMESTIC CATS IN HUMAN PERTURBED LANDSCAPES ON CHILOÉ ISLAND, CHILE

Mónica Mora,[1,4] Constanza Napolitano,[2] René Ortega,[3] Elie Poulin,[2] and José Pizarro-Lucero[1]

[1] Laboratorio de Virología Animal, Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Avda. Santa Rosa 11735, La Pintana, Santiago, Chile
[2] Laboratorio de Ecología Molecular, Instituto de Ecología y Biodiversidad, Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Las Palmeras 3425, Ñuñoa, Santiago, Chile
[3] Laboratorio de Virología, Departamento de Patología y Medicina Preventiva, Facultad de Ciencias Veterinarias, Universidad de Concepción, Avda. Vicente Méndez 595, Chillán, Chile
[4] Corresponding author (email: mpmora1@uc.cl)

ABSTRACT: Feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) are two of the most common viruses affecting domestic cats (*Felis catus*). During the last two decades, reports show that both viruses also infect or affect other species of the family Felidae. Human landscape perturbation is one of the main causes of emerging diseases in wild animals, facilitating contact and transmission of pathogens between domestic and wild animals. We investigated FIV and FeLV infection in free-ranging guignas (*Leopardus guigna*) and sympatric domestic cats in human perturbed landscapes on Chiloé Island, Chile. Samples from 78 domestic cats and 15 guignas were collected from 2008 to 2010 and analyzed by PCR amplification and sequencing. Two guignas and two domestic cats were positive for FIV; three guignas and 26 domestic cats were positive for FeLV. The high percentage of nucleotide identity of FIV and FeLV sequences from both species suggests possible interspecies transmission of viruses, facilitated by increased contact probability through human invasion into natural habitats, fragmentation of guigna habitat, and poultry attacks by guignas. This study enhances our knowledge on the transmission of pathogens from domestic to wild animals in the global scenario of human landscape perturbation and emerging diseases.

Key words: Emerging diseases, *Felis catus*, FeLV, FIV, human landscape perturbation, *Leopardus guigna*.

INTRODUCTION

Infectious diseases are, along with reproductive success and predation, one of the main demographic and evolutionary drivers in natural free-ranging populations (Altizer et al. 2003). The recent increase in diseases affecting wild animals is associated with ecologic changes in the environment, the host, or the pathogen (Daszak et al. 2000). Human landscape perturbation (i.e., habitat loss and fragmentation, human settlements, and domestic animals) raises the probabilities of contact and transmission of infections between domestic and wild animals and is one of the main causes of emerging diseases (Dobson and Foufopoulos 2001; Foley et al. 2013). Pathogens that most frequently participate in interspecific host jumping are RNA viruses that are transmitted through direct contact (Woolhouse et al. 2005). Feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) are two of the most common pathogens affecting the immune system of domestic cats (*Felis catus*), causing significant morbidity and death (O'Brien et al. 2012). During the last two decades, infections by these viruses have been reported in free-ranging wild species from the family Felidae (Olmsted et al. 1992; Meli et al. 2009; O'Brien et al. 2012). Feline immunodeficiency virus (*Retroviridae*: *Lentivirus*) is associated with immunosuppression and opportunistic diseases in domestic cats (Teixeira et al. 2012), whereas FeLV (*Retroviridae*: *Gammaretrovirus*) causes neoplastic diseases and, in most cases, leads to immunosuppression in domestic cats (Cunningham et al. 2008). Dissemination of both viruses occurs via direct contact (e.g., fighting, sexual contact, and

mother–offspring transmission) (Cunningham et al. 2008; Troyer et al. 2008). Given that human population density, deforestation, and fragmentation are increasing in the Chilean temperate rainforest (Echeverría et al. 2006), human invasion into natural habitats and subsequent contact and exposure of wild animals with domestic pathogens might be an increasing threat.

The guigna (*Leopardus guigna*) (Carnivora: Felidae), a small wild cat, inhabits a restricted distribution range in central and southern Chile and a narrow strip of land in southwestern Argentina (Napolitano et al. 2014). Closely associated with temperate rainforests of southern South America, threats to the guigna include habitat loss and fragmentation and direct persecution as retaliation for poultry depredation (Sanderson et al. 2002; Silva-Rodríguez et al. 2007; Gálvez et al. 2013). It is currently classified by the International Union for Conservation of Nature (2014) Red List as vulnerable. There is only one study of FIV antigen western blot screening in a captive-born guigna, finding no FIV infection (Troyer et al. 2005). There is no known record of FIV or FeLV infection in free-ranging guignas or any other wild felid in Chile. There is also no published record of FIV or FeLV infection in domestic cats on Chiloé Island. Records of FIV and FeLV prevalence for domestic cats in Chile include 4% (2/50) FIV antibody prevalence in Concepción, Bío Bío Region (Troncoso et al. 2013), 4% (2/55) FeLV and 9% (5/55) FIV prevalence by PCR and sequencing in Bío Bío Region (Bilbao 2008), and 10% FeLV and 15% FIV antibody prevalence in Santiago (Troncoso et al. 2013). We investigated FIV and FeLV infection in free-ranging guignas and sympatric domestic cats in human-perturbed landscapes on Chiloé Island, Chile.

MATERIALS AND METHODS

Study site and sample collection

This study was conducted on Chiloé Island (8,394 km^2), belonging to Los Lagos Region in southern Chile (42–43°S, 73–74°W; urban population 86,646; rural population 68,120; Instituto Nacional de Estadísticas, Ministerio de Vivienda y Urbanismo, República de Chile 2002). The island's southern area has predominantly low-perturbation landscapes, continuous pristine native temperate rainforest, two protected areas, and lower human densities, whereas the island's northern area has a highly perturbed landscape with higher human densities, where fragments of remnant temperate rainforest are surrounded by a matrix of agricultural, livestock, and human activities (Napolitano 2012). In these perturbed landscapes, guignas occasionally attack poultry within human settlements, facilitating casual encounters and possible contact probabilities with domestic cats (Sanderson et al. 2002). No other felid species inhabit Chiloé. There are no population size or niche overlap estimates for guigna or domestic cats on Chiloé Island. There is no record of feral cats on Chiloé or any domestic cat population control.

Samples of domestic cats and guignas were collected from 2008 to 2010 in 11 rural localities in northern Chiloé Island and on four rural localities in the southern area (Fig. 1). We collected 10 blood samples from captured free-ranging guignas and four tissue samples of bone marrow and Peyer's patches from recent roadkills found opportunistically (Holznagel et al. 1997). Additionally, we included one blood sample from a free-ranging adult male guigna confiscated and released on December 2008 by the Agriculture and Livestock Service (SAG) in Chile in the locality of Molina (35°07′S, 71°17′W), Maule Region, in central Chile. Guigna captures were carried out using Tomahawk live traps (Tomahawk Live Trap Company, Tomahawk, Wisconsin, USA), baited with chicken and fish, checked twice a day, and following proven and successful handling protocols (Sanderson et al. 2002; Napolitano et al. 2014). Total trapping effort was 2,875 trap nights (similar efforts for both northern and southern areas). Captured guignas were anesthetized intramuscularly with 15 mg/kg ketamine hydrochloride (Ketamina 100®, Chemie©, Santiago, Chile) and released once completely recovered at the capture site. Blood samples were obtained by cephalic or jugular venipuncture using 25-gauge needles and 2-mL vacuum tubes with anticoagulant. Small passive microchips (8.5×2.12 mm) were implanted subcutaneously to identify captured guignas. Guigna captures and tissue collection were carried out with permission from SAG (capture permits 814/13 February 2008; 109/9 January 2009; 1220/22 February 2010; and

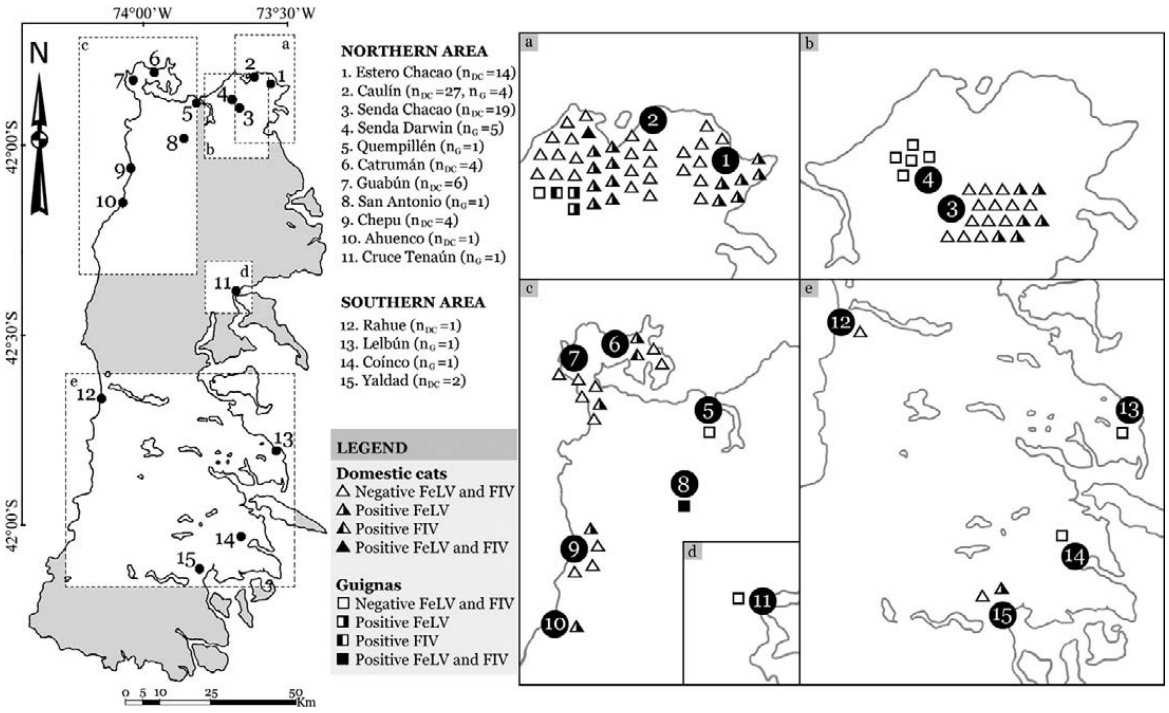

FIGURE 1. Sampling localities for domestic cats (*Felis catus*) and guignas (*Leopardus guigna*) on Chiloé Island, Chile, 2008–10. n_{DC}=domestic cat sample size, n_G=guigna sample size, FeLV=feline leukemia virus, FIV=feline immunodeficiency virus; areas a–e in the left-hand map are detailed in maps on the right-hand side.

1708/26 March 2010), and following handling and supervision protocols within bioethical and animal welfare frameworks (National Research Council 2011). Blood samples from 78 domestic cats were collected in rural areas near guigna capturing sites, with the owner's consent and manual restraint using no anesthesia (Fig. 1). Domestic cats had no known history of disease and were not vaccinated. For guignas and domestic cats, sex, age, physical condition, and possible clinical signs of disease were assessed through direct inspection, and locality was recorded. Age was estimated from dentition. All blood samples were centrifuged for 10 min at 1,200 × G; buffy coats (leukocytes) were extracted and stored at −20 C along with guigna tissue samples. Analyses were carried out at the Laboratorio de Virología Animal, Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile.

Molecular analysis

DNA extraction was performed using the commercial QIAGEN DNeasy Blood and Tissue kit following manufacturer's instructions (Qiagen, Valencia, California, USA). For enhanced sensibility and specificity, nested PCR were performed in a PTC-100™ Programmable Thermal Controller (MJ Research Inc., Waltham, Massachusetts, USA). We amplified a 291–base-pair (bp) fragment of proviral DNA from the FIV *gag* genomic region using external primers (SF-1s and SF-2a; Cammarota et al. 1996) and internal primers (FIV-2N: 5′-AAGGCAAGAGAAGGACTAGGAG-3′ and FIV-4M: 5′-TACACTGCATCCTAGCTGGTG-3′) designed within conserved regions. We amplified a 211-bp fragment of proviral DNA from FeLV *U3 LTR* genomic region using external primers (LF-1s and LF-2a; Tandon et al. 2005) and internal primers (FLV-F18: 5′-GTCTCCAGGCTCCCCAGT-3′ and FLV-R20: 5′-ACCGAGACCACGAGTCAGAT-3′) designed within conserved regions, present on the three retroviral subtypes (A, B, and C). Conditions for nested PCR first and second rounds for both genomic regions were performed following Bilbao (2008). At least three independent PCR amplifications were performed for each DNA extraction, all yielding consistent results. Positive controls in PCR reactions were FIV- and FeLV-viremic domestic cats diagnosed by PCR and confirmed by nucleotide sequencing. PCR products were separated by electrophoresis in 2% agarose gels (Ultra Pure™ Agarose, Invitrogen, Carlsbad, California, USA) at 120 volts for 60 min using ethidium bromide staining for visualiza-

TABLE 1. Origin and characteristics of guignas (*Leopardus guigna*) sampled in Chile, 2008–10, for this study.[a]

ID	Locality	Sex	Estimated age (mo)	Sample type	PCR result FIV	PCR result FeLV
1	Senda Darwin	F	8	Blood	−	−
2	Senda Darwin	M	36	Blood	−	−
3	Caulín	M	18	Blood	Positive[b]	−
4	Senda Darwin	M	18	Blood	−	−
5	Caulín	M	12	Blood	−	Positive[b]
6	Caulín	M	24	Blood	−	Positive[b]
7	Caulín	F	12	Blood	−	−
8	Senda Chacao	M	12	Blood	−	−
9	Coínco	M	24	Tissue	−	−
10	Senda Darwin	M	36	Blood	−	−
11	San Antonio	M	12	Blood	Positive[c]	Positive[c]
12	Quempillén	F	12	Tissue	−	−
13	Lelbún	M	12	Tissue	−	−
14	Cruce Tenaún	F	18	Tissue	−	−
15	Molina, Maule Region	M	24	Blood	−	Positive[c]

[a] F = female; M = male; FIV = feline immunodeficiency virus; FeLV = feline leukemia virus; − = negative.

[b] Good-quality sequence included in phylogenetic analysis.

[c] Not included in phylogenetic analysis.

tion. The PCR products with the expected fragment sizes were purified using a commercial purification kit (HiYield Gel/PCR DNA Fragment Extraction Kit, RBC Bioscience, Chung Ho, Taipei, Taiwan) following manufacturer's instructions and sequenced by Biogenetics (Santiago, Chile). DNA sequences were aligned using CLUSTALW v.2.0 (Larkin et al. 2007), and compared to nucleotide sequences available using Basic Local Alignment Search Tool (National Center for Biotechnology Information 2014). A neighbor-joining tree was constructed with MEGA v.3.0 (Kumar et al. 2004) for both viruses using reference database sequences.

RESULTS

Ten free-ranging guignas were captured and bled in northern Chiloé Island; there were no captures in the southern area. Four guigna tissue samples from roadkills were collected, two from northern Chiloé Island and two from southern Chiloé Island (Fig. 1). From the total 14 guignas sampled, two were positive for FIV (13%; both males from the northern area) and three were positive for FeLV (20%; all males from the northern area); one of them was coinfected with both viruses (7%) (Fig. 1 and Table 1). The guigna sample from Maule Region (male) was positive for FeLV. All FIV- and FeLV-positive tests were from blood samples. Ages of guignas positive for FIV were 12–18 mo; ages of guignas positive for FeLV were 12–24 mo. All guignas were in good physical condition, with no evident clinical signs of disease. Roadkills had no evident pathologic signs at gross necropsy. From the 78 domestic cat samples, two were positive for FIV (3%; from the northern area; one female, one male) and 26 were positive for FeLV (33%; 25 from the northern area, one from the southern area; 12 females, 14 males) (Fig. 1 and Table 2). One cat was coinfected with both viruses. Ages of FIV-positive cats were 24–48 mo; FeLV-positive cats were 6–84 mo. All were in good physical condition, with no evident clinical signs of disease.

Only good-quality nucleotide sequences from guignas (one FIV; three FeLV) and domestic cats (no FIV; six FeLV) were further analyzed. The highest percentage of nucleotide identity (PNI) for the guigna FIV sequence (94–98%) was calculated against FIV sequences obtained from

domestic cats, especially of the B subtype from Brazil, Canada, and Italy. The highest PNIs for guigna FeLV sequences (93–99%) were calculated against FeLV sequences obtained from domestic cats, particularly strains of the A subtype from Brazil.

In phylogenetic analysis (Fig. 2) guigna FIV nucleotide sequence (FIV-Guigna Chiloe1) grouped more closely with sequences of FIV B subtype viruses from domestic cats from Chile and Brazil (FIV Santiago 1 and 2 and Sao Paulo-Brazil 452 and 428). Guigna FeLV nucleotide sequences from Chiloé (FeLV-Guigna Chiloe-1) and Maule Region (FeLV-Guigna Maule) grouped more closely with nucleotide sequences of FeLV A subtype viruses isolated from domestic cats from Chiloé Island (this study; FeLV Chiloe-1, 3, 5, 6, 7, 20), Santiago (FeLV Santiago-17), Brazil (FeLV 1179MG, 1286MG, 1235MG, 1182MG), and one virus from the US (FeLV-FAIDS) (Fig. 3). Nevertheless, one guigna FeLV nucleotide sequence obtained in this study from Chiloé (FeLV-Guigna Chiloe-2) grouped more closely with nucleotide sequences of FeLV isolated from domestic cats in Chile (FeLV Santiago-17) and FeLV C subtype virus Sarma.

DISCUSSION

The high nucleotide identities among sequences of FIV and FeLV isolated from guignas and domestic cats suggest possible interspecies transmission (Troyer et al. 2005). Domestic cats as sources of FIV and FeLV infections in wild felids have been widely recorded in different species and contexts (Nishimura et al. 1999; Cunningham et al. 2008; Guimaraes et al. 2009). Environmental and behavioral barriers under natural conditions may hinder interspecific FIV and FeLV transmission (Troyer et al. 2008; VandeWoude et al. 2010). However, transmission may be facilitated in perturbed landscapes, where human invasion of natural habitats leading to fragmentation of guigna habitat and occasional poultry attacks by guignas within human settlements could increase aggressive encounters with domestic cats (Sanderson et al. 2002; Silva-Rodríguez et al. 2007; Gálvez et al. 2013). Residents of the study area report occasional aggressive encounters between domestic cats and guignas (J. Hetz pers. comm.). Considering that FIV and FeLV are shed in high concentrations in saliva and that the major mode of transmission is through bites (Sykes 2014a, b), these aggressive encounters could increase transmission. In this study, all FIV- and FeLV-infected guignas, as well as all FIV- and almost all FeLV-(25/26) infected domestic cats, belonged to the human-perturbed landscapes of northern Chiloé Island.

Once the species barrier has been crossed, it is possible, under some conditions, for the virus to propagate among individuals of the new host species (Webby et al. 2004; Denner 2007). Thus, an aggressive encounter between a domestic cat and a guigna leading to virus transmission may be enough to potentially spread the infection within guigna populations. Guignas are solitary felids pairing only when mating (Sanderson et al. 2002; Napolitano 2012); thus, transmission of FIV and FeLV among conspecifics may be low, but likely occurs through fighting (bites), sexual contact, and mother–offspring transmission. In domestic cats, males have an increased risk of FIV (up to 4.7 times higher than females) and FeLV infection (Sykes 2014a,b) compared to females. In this study, only male guignas were infected with FIV and FeLV, which could be because of their more aggressive and daring behavior, being the dispersing sex and having larger home ranges than females, thus increasing infection probabilities (Liberg and Von Schantz 1985; Sanderson et al. 2002; Napolitano 2012). Conversely, male and female domestic cats had similar FIV and FeLV prevalences, suggesting no behavioral or infection differences between sexes.

Exposure and infection with FIV and FeLV via direct contact may occur early

TABLE 2. Origin and characteristics of domestic cats (*Felis catus*) sampled for this study in Chile.[a]

ID	Locality	Sex	Estimated age (mo)	PCR result FIV	PCR result FeLV
1	Caulín	F	24	–	–
2	Caulín	F	8	–	–
3	Caulín	F	24	–	–
4	Caulín	F	10	–	–
5	Caulín	F	10	–	–
6	Caulín	F	12	–	Positive[b]
7	Caulín	M	24	–	Positive[b]
8	Caulín	F	12	–	–
9	Caulín	M	12	–	–
10	Caulín	F	12	–	–
11	Caulín	F	48	–	–
12	Caulín	M	24	–	Positive[b]
13	Caulín	M	36	–	Positive[b]
14	Caulín	F	72	–	–
15	Caulín	M	24	–	–
16	Caulín	F	36	–	–
17	Caulín	F	12	–	–
18	Caulín	F	12	–	Positive[b]
19	Caulín	M	48	–	Positive[b]
20	Caulín	M	24	–	–
21	Caulín	F	96	–	–
21	Caulín	M	36	–	–
23	Caulín	M	48	Positive[b]	Positive[b]
24	Caulín	F	48	–	Positive[c]
25	Caulín	F	24	–	–
26	Caulín	M	24	–	–
27	Caulín	M	24	–	Positive[b]
28	Senda Chacao	F	18	–	–
29	Senda Chacao	F	7	–	Positive[b]
30	Senda Chacao	M	48	–	–
31	Senda Chacao	M	36	–	–
32	Senda Chacao	F	72	–	Positive[b]
33	Senda Chacao	F	36	–	–
34	Senda Chacao	M	36	–	–
35	Senda Chacao	F	36	–	–
36	Senda Chacao	F	24	–	–
37	Senda Chacao	F	36	–	Positive[b]
38	Senda Chacao	M	7	–	Positive[b]
39	Senda Chacao	M	24	–	–
40	Senda Chacao	M	24	–	–
41	Senda Chacao	M	12	–	–
42	Senda Chacao	F	48	–	–
43	Senda Chacao	M	36	–	Positive[b]
44	Senda Chacao	F	7	–	Positive[b]
45	Senda Chacao	M	24	–	–
46	Senda Chacao	F	24	–	–
47	Estero Chacao	F	24	–	Positive[b]
48	Estero Chacao	F	48	–	Positive[c]
49	Estero Chacao	F	24	–	–
50	Estero Chacao	F	48	–	–
51	Estero Chacao	M	24	–	Positive[b]
52	Estero Chacao	F	12	–	–
53	Estero Chacao	F	6	–	Positive[b]
54	Estero Chacao	F	12	–	–
55	Estero Chacao	F	24	–	–
56	Estero Chacao	F	84	–	Positive[b]

TABLE 2. Continued.

ID	Locality	Sex	Estimated age (mo)	PCR result FIV	PCR result FeLV
57	Estero Chacao	M	36	–	–
58	Estero Chacao	M	18	–	Positive[b]
59	Estero Chacao	F	60	–	–
60	Estero Chacao	M	4	–	–
61	Guabún	F	24	–	–
62	Guabún	F	12	–	–
62	Guabún	F	36	–	–
63	Guabún	M	36	–	Positive[c]
64	Guabún	F	36	–	–
66	Guabún	F	48	–	–
67	Catruman	F	24	Positive[b]	–
68	Catruman	M	36	–	–
69	Catruman	M	48	–	Positive[b]
70	Catruman	M	24	–	–
71	Chepu	F	36	–	–
72	Chepu	F	12	–	–
73	Chepu	M	18	–	–
74	Chepu	M	24	–	Positive[c]
75	Ahuenco	F	36	–	Positive[c]
76	Rahue	M	60	–	–
77	Yaldad	F	48	–	–
78	Yaldad	M	12	–	Positive[c]

[a] F = female; M = male; FIV = feline immunodeficiency virus; FeLV = feline leukemia virus; – = negative.

[b] Not included in phylogenetic analysis.

[c] Good-quality sequence included in phylogenetic analysis.

(mother–offspring transmission) or later (fighting, sexual contact) in life. Regarding the age of FIV- (12–18 mo) and FeLV- (12–24 mo) infected guignas, and FIV- (24–48 mo) and FeLV- (6–84 mo) infected domestic cats, all types of possible direct contact exposure and infection could be operating. In domestic cats, FIV and FeLV infections are associated with older age (Sykes 2014a,b) because possible transmission events accumulate over time. In this study, coinfection with FIV and FeLV was low for guignas (7%) and domestic cats (1%). Similarly low coinfection prevalences have been recorded for domestic cats in Chile: 2% (Bilbao 2008) and 5% (Troncoso et al. 2013).

Whether FIV and FeLV are causing disease in wild felid species has been the subject of debate (O'Brien et al. 2012). However, clinical signs, hematologic abnormalities, and even mortality with postmortem lesions compatible to FIV and FeLV disease have been recorded in free-ranging African lions (*Panthera leo*), Florida cougars (*Puma concolor coryi*), and Iberian lynx (*Lynx pardinus*) (Packer et al. 1999; Roelke et al. 2009; Troyer et al. 2011). Recent studies facilitated by current technologies, in addition to increased wild felid surveillance, have allowed paradigm shifts regarding the real pathology of these two infections in nondomestic felids (O'Brien et al. 2012). We did not find clinical signs of disease in guignas or domestic cats or evident pathologic signs at gross necropsy of road-killed guignas. Future investigators should consider elucidating the potential disease that FIV and FeLV may produce in guigna populations. Studies are also needed to assess population sizes, behavioral patterns, and niche overlap between guignas and domestic cats, all potentially important aspects lacking supporting evidence. Responsible pet ownership and spay–neuter programs to reduce free-ranging domestic cats contacting guignas in human perturbed

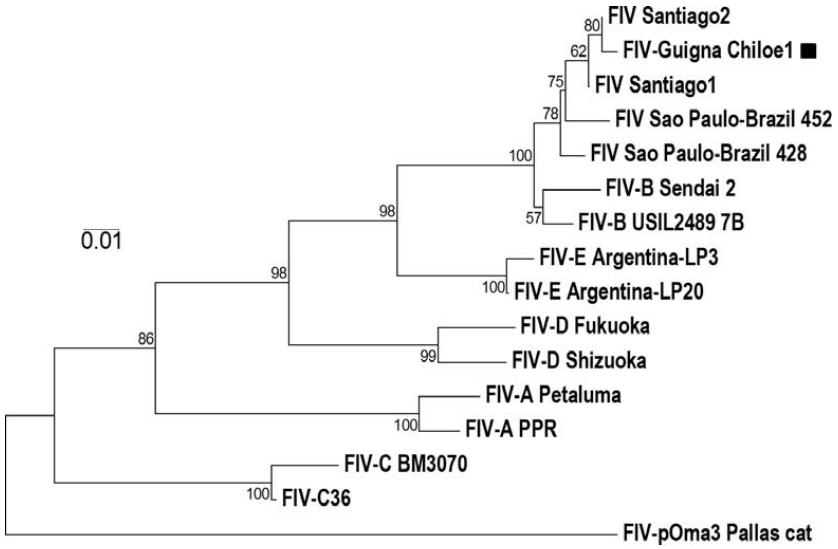

FIGURE 2. Neighbor-joining phylogenetic tree of feline immunodeficiency virus (FIV) *gag* nucleotide region from one guigna (*Leopardus guigna*) from Chiloé Island, Chile, along with other Chilean (FIV Santiago1, FIV Santiago2) and non-Chilean isolates of domestic cats (*Felis catus*) from reference databases. Numbers in nodes indicate percentages of 1,000 replicates. Only values ≥50 are shown. One Pallas's cat (*Otocolobus manul*) sequence (FIV-pOma3 Pallas cat) was used as an outgroup. (■) FIV-Guigna Chiloe1 corresponds to guigna ID 3 in Table 1.

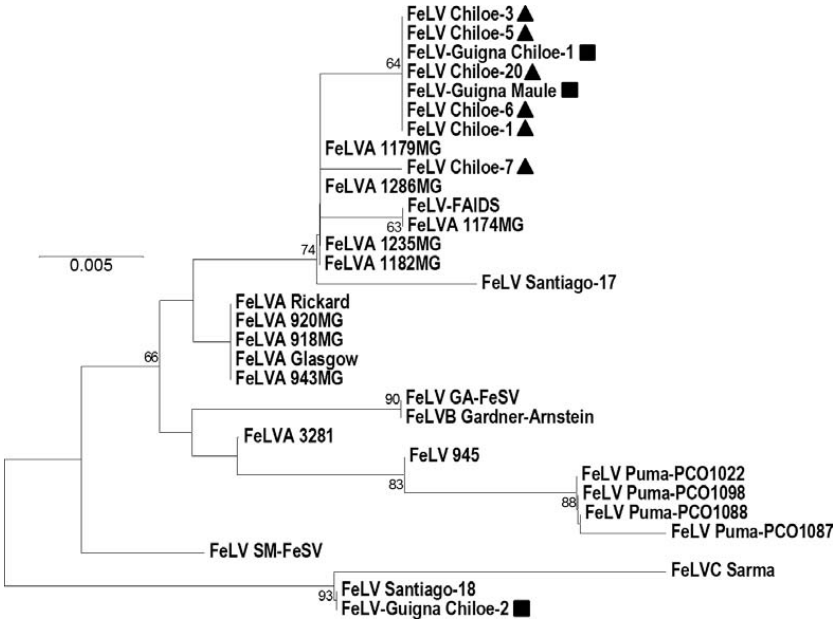

FIGURE 3. Neighbor-joining phylogenetic tree of feline leukemia virus (FeLV) *U3 LTR* nucleotide region from three guignas (*Leopardus guigna*) and six domestic cats (*Felis catus*) from Chiloé Island, Chile, along with other Chilean (FeLV Santiago-17, FeLV Santiago-18) and non-Chilean isolates of domestic cats and pumas from reference databases. Numbers in nodes indicate percentages of 1,000 replicates. Only values ≥50 are shown. (■) FeLV-Guigna Chiloe-1, FeLV-Guigna Maule, and FeLV-Guigna Chiloe-2 correspond to guignas ID 5, 15, and 6 in Table 1, respectively. (▲) FeLV Chiloe-3, FeLV Chiloe-5, FeLV Chiloe-20, FeLV Chiloe-6, FeLV Chiloe-1, and FeLV Chiloe-7 correspond to domestic cats ID 48, 63, 78, 75, 24, and 74 in Table 2, respectively.

landscapes, vaccination and health monitoring of domestic cat populations, poultry raised within secure coops to prevent guigna attacks in human settlements, and reduction in habitat loss, fragmentation, and human invasion into natural habitats are proposed measures to control potential risks threatening long-term persistence of guigna populations.

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