

# Mitochondrial DNA diversity of feral pigs from Karukinka Natural Park, Tierra del Fuego Island, Chile

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**ABSTRACT.** Control or eradication of exotic species is one of the greatest challenges facing biodiversity and ecosystem conservation. Domestic pigs (*Sus scrofa domestica*) were released and became feral in the southern region of Chilean Tierra del Fuego Island in the 1900s. Currently, they inhabit part of Karukinka Natural Park, an area of global conservation concern. To gain insight into the control of this invasive species, we analyzed genetic variation in the mitochondrial DNA control region to determine the origin and population subdivision of feral pigs in Tierra del Fuego. Sequences from a sample of 42 feral pigs, 10 domestic pigs from local farms, and references from other countries and commercial breeds revealed 2 highly differentiated populations, 1 in the western and the other in the eastern area of the park, each

harboring a different haplotype, suggesting no connectivity between populations. Comparison of these haplotypes with reference sequences from other countries and commercial breeds indicated that feral pigs from Chilean Tierra del Fuego are of European origin, very likely from 2 separate introduction events. The haplotype found in the western feral population was also identified in domestic pigs from a farm. This raises concerns regarding the possible connectivity between stocks from local farms and the wild population. Based on these results, we recommend the development of strategies for controlling the population of this invasive species in Karukinka Natural Park.

**Key words:** Feral pigs; Invasive species; mtDNA control region; *Sus scrofa*; Tierra del Fuego Island

#### INTRODUCTION

Invasive species can cause considerable damage to the environment, particularly on islands where most species have evolved in the absence of major competitors, herbivory, and predation (Gong et al., 2009). In general, the best solution for protecting insular ecosystems from invaders is control and, if possible, eradication. This represents an interesting yet challenging field of research for the development of strategies to optimize management measures. Some essential measures for the success of both population control and eradication include 1) obtaining basic information about the invasive population, 2) evaluating the participation of the local community, and 3) optimizing time and economic costs (Carrion et al., 2011). Horwitz and Bar-Gal (2006) as well as others found that genetic analysis is effective for providing basic information about the historical origin and spatial structure of invasive species populations. Furthermore, such an approach can help reduce the response time for taking appropriate actions and, thus, decrease the cost of preventing and controlling biological invasions.

In the early stages of Spanish and Portuguese colonization of South America, several plant and domestic animal species were intentionally introduced, including the domestic pig (Sus scrofa domestica), which had a significant feral impact on the ecosystem (Mittermeier et al., 2002). The success of the domestic pig as food support for navigators and local people prompted the introduction of animals in wild areas located further south of the continent (Cuadra, 1866). In 1773, during his second voyage, Captain Cook released pigs in some of the islands of New Zealand, and then sailed to Tierra del Fuego (TDF), leaving pigs on board, where he remained for 3 days (Kippis, 1795). Although the release of pigs in TDF is not documented, it is possible that some were introduced by Cook.

Domestic pigs were bred in TDF as early as the late 19th century (Vera, 1897). Subsequently, in the first half of the 20th century, some domestic pigs of undescribed origin and number were introduced from abroad and released into the wild southwestern forest habitat, currently named Timaukel (Fuentes, 1923). This feral population increased in size over time until it was reduced by hunting (Skewes, 1990). According to Skewes (1990), from 1970-1973 a new stock of 9 pregnant female pigs was released in the same region. Currently this population has persisted despite hunting pressure. Far from Timaukel (125 km southeast), feral pigs of unknown origin were seen near Vicuña in 1988 (Skewes, 1990) and again in 2005-2007 (Reveillaud et al., 2008). Approximately 50 km north of Timaukel, small herds

(<10 individuals) are farmed. The areas of Timaukel and Vicuña are parts of the Karukinka Natural Park and are dominated by a wild landscape of subantarctic forest of *Nothofagus pumilio*, where biodiversity is of great interest for global conservation (Silva and Saavedra, 2008). Given the value of the native TDF forest, the need for control or eradication of this wild pig population is urgent (Soto and Cabello, 2007).

Although eradication of feral species on islands is possible, a good control strategy may be affected or even fail if there is no information regarding the basic dynamics of the introduction or participation from the local community in its maintenance (Horwitz and Bar-Gal, 2006). If the local community uses wild pigs as a food source or for recreational hunting, there may be multiple episodes of release into the wild and/or reintroduction after eradication (Weeks and Packard, 2009). The temporal lag between feral pig sightings in the 2 areas of the Karukinka Natural Park raises questions regarding the source of the invasion in TDF, connectivity between both localities, and human participation in translocation events. Understanding this dynamic is critical for developing effective management or surveillance strategies (Choquenot et al., 1996; Gongora et al., 2004).

In recent years, advances in the phylogenetic analysis of the origin of the domestic pig using the hyper-variable mitochondrial DNA (mtDNA) control region (D-loop) has made available pig sequences from many regions worldwide, enabling researchers to relate sequences of introduced populations with a geographic source (Gongora et al., 2004; Larson et al., 2010). Thus, the number and pattern of polymorphisms in mtDNA sequences can be used to infer the history of the introduction of wild populations, as well as provide information regarding population structure and gene flow in a relatively short time period (Gongora et al., 2004; Horwitz and Bar-Gal, 2006). In this study, we analyzed genetic variation in the mtDNA control region to 1) identify the geographic origin of the TDF feral pig population, 2) elucidate whether there are 1 or more populations in Karukinka Natural Park, and 3) identify genetic relationships between feral pigs and domestic pigs from local farms. This information will provide a reference point for managing this invasive population in TDF.

#### MATERIAL AND METHODS

#### Study area

The Karukinka Natural Park (54°04'06"S, 68°42'57"W) is located in the southwestern region of the main island of TDF in Chile (Figure 1). Its current area includes 297.6 km² of private protected area, which contains highly physio-, oro-, and geomorphologically differentiated areas, with a variety of subantarctic ecosystems.

## **Tissue samples**

Liver, muscle, and skin samples were collected from 37 adult feral pigs (20 males, 16 females, 1 unknown) of 72 animals sighted in the southwestern area of Timaukel in Chilean TDF (53°45'35"S, 69°58'39"W; SW Timaukel) and 5 (1 male, 3 females, 1 unknown) from the southeastern area of Vicuña (54°07'42"S, 68°40'44"W; SE Vicuña). Samples were collected by shooting during the 2010-2011 period in the Karukinka Natural Park. Feral pigs were hunted with permission of the park administration and according to the Chilean law. In addition, muscle samples from 10 dead animals from 4 farms located in the northern Chilean TDF were

taken: 6 from Porvenir1 (53°16'S, 70°17'W), 1 from Porvenir2 (53°19'S, 70°07'W), 2 from Onaissin (53°20'S, 69°21'W), and 1 from Cerro Sombrero (52°40'S, 69°25'W). These domestic pig samples were provided by the local agricultural government agency (SAG, Porvenir). All sampling sites are shown in Figure 1.

### DNA extraction, polymerase chain reaction (PCR) amplification, and sequencing

DNA was extracted from all tissue samples using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to manufacturer instructions. A fragment of mtDNA from the control region (D-loop) was amplified by PCR using the primers L-Strand 5'-CTCCGCCATCAGCACCCAAAG-3' and H-Strand 5'-GCACCTTGTTTGGATTRTCG-3' (Larson et al., 2005) anchoring at positions 15387 and 16108 of the complete pig mtDNA genome (GenBank accession No. AJ002189; Ursing and Arnason, 1998). PCR was performed in a 25-μL reaction mix containing 10 ng total DNA, 1X NH<sub>4</sub> Biolase Buffer [16 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 67 mM Tris-HCl, pH 8.8], 2.8 mM MgCl<sub>2</sub>, 0.4 mM each dNTP, 0.4 μM of each primer, and 1 U Biolase DNA Polymerase (Bioline, London, UK). Thermocycling conditions were: denaturating at 95°C for 10 min, followed by 35 cycles at 94°C for 30 s, 54°C for 30 s and 72°C for 1 min, with a final extension of 72°C for 7 min. The PCR products were purified with the ISOLATE PCR and Gel Kit (Bioline), and sequenced using BigDye termination by Macrogen (Seoul, South Korea).

# Data analysis

Sequences of the TDF pigs were edited using GENIOUS® 5.5.3 (Biomatters, Ltd., Auckland, New Zealand), and aligned using CLUSTAL W (Thompson et al., 2002) with 34 sequences of European and Asian pigs and 5 sequences of commercial breeds distributed worldwide. Details regarding sequence origin, including Genbank accession numbers, are shown in Table 1. To determine the origin of *Sus scrofa* in TDF, a phylogenetic tree was built using the neighbor-joining method and the maximum likelihood distance with MEGA 5.0 (Tamura et al., 2011). Statistical confidence for each branch was estimated with 1000 bootstrap replications. The relationships between haplotypes were visualized using a haplotype network generated using HAPSTAR (Teacher and Griffiths, 2011).

## **RESULTS**

A total of 54 sequences from feral and domestic pigs were analyzed in this study. Only 5 haplotypes from the Chilean TDF were found, among which 22 sites were variable (Tables 1 and 2). Two haplotypes, Hap\_1 in the SW area and Hap\_2 in the SE area, belonged to the feral pig population (Figure 1 and Table 1). They differed by only 1 transversion and 1 transition at loci 15.558 and 15.741, respectively (Table 2). The Hap\_1 sequence was composed of C: 24.92%, T: 27.48%, A: 33.07%, and G: 14.54%, while the Hap\_2 sequence contained C: 24.76%, T: 27.48%, A: 33.23%, and G: 14.54%. No genetic variation was found within the SW Timaukel or within the SE Vicuña sampled sites. Four haplotypes were found in the pool of domestic pigs: Hap\_1 in the Porvenir1 farm, Hap\_3 in Porvenir2, Hap\_4 in Onaissin, and Hap\_5 in Cerro Sombrero (Figure 1 and Table 1). Therefore, the haplotype Hap\_1 was present in feral pigs from the SW area and in domestic pigs from the farm of Porvenir1.

16 10,11,12

AB041496, AB041486, AB041492, AY230820

AY 884758

GQ169776

Hap\_22

Hap\_14, Hap\_16 to Hap\_18

Domestic

Large White, Landrace, Duroc, Pietrain

Meishan

Cosmopolitan pig

Euroam England

\*Hap\_1

Domestic

Hampshire

AB041481, DQ779440

Hap\_15 Hap\_12

Hap\_14 'Hap\_3

**Domestic Domestic** Domestic Domestic

Fanna Island (Kapia)

Vanuatu Vietnam

Welsh

Lao Cai, Moncai

9,10,11 10,11,12 2,3,4,5 AF276923, AF276927, AF276930, AF276931, AF276932, AY486118, GQ141900, AY230818, AF276929 HM747222, HM74722 AY463089, AY463085 AY463079, AY463076 AY 232865, AY 232861 GenBank accesion No JQ668032, JQ668033, JQ668034, JQ668035 AM779910 AF276937 DO779372 AY884674 AB015093 AB015091 AF276933 AY 232891 JO668030 AY884751 Hap\_6 to Hap\_10, Hap\_20 <sup>‡</sup>Hap\_3 (1), Hap\_5 (1) to Hap\_23, Hap\_25 Asian Clade Hap\_19 Hap\_30 Hap\_15 Hap\_11 Hap\_24 Hap\_15 Haplotypes \*Hap\_1 (6), Hap\_4 (2) \*Hap\_1, Hap\_27, Hap\_28 European Clade 'Hap\_2, Hap\_26 <sup>k</sup>Hap 1 (39) 'Hap 2 (5) \*Hap\_1 Hap 29 \*Hap\_1 Hap\_13 Hap 2 Table 1. Haplotypes of mtDNA control region analyzed in this study. Feral domestic Feral Domestic Domestic Condition Domestic Feral Liaoning Wild Erhualian, Neijang Putian, Wanhua, Guizhou Xiang, Iberian Black Hairy, Iberian Red SW Timaukel population (TDF) FongCheng, Yanxin, Jinghua, SE Vicuña population (TDF) Pig breeding farms (TDF) Auckland Island Kune kune South Korea (Cheju Island) Dirranbandi, Tooraweenah Chiang Mai (Thai Native) Breed o local distribution Bisaro, Alentejano inderodssvin **Ambikapur** Polynesia Satsuma *(*ucatan Basque Iberian Peninsula N Zealand Thailand Australia ortugal Country Mexico Sweden China Korea Chile pain India lapan

Ref

Kim et al., 2002; (4) Yue and Wang, 2003; (5) Huang et al., unpublished data; (6) Larson et al., 2007; (7) Alves et al., 2003; (8) Larson et al., 2005; (9) Okumura et al., 1996; (10) Watanobe et al., 1999; (11) Watanobe et al., 2001; (12) Okumura et al., 2001; (13) Alves et al., 2010; (14) Charoensook et al., 2011; (15) Lum et al., 2006; (16) Wu et al., unplublished data. \*Shared Hap\_1 haplotypes, †Shared Hap\_2 haplotypes, †Shared Hap\_3 haplotypes. The proportion of haplotypes in each of the Chilean sites is reported in parentheses next to the haplotype number. a(1) This study; (2) Gongora et al., 2004; (3)

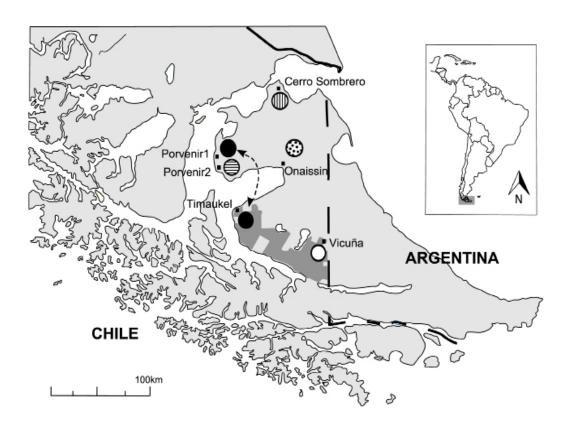
**Table 2.** Position of variable sites among 30 haplotypes of the control region from TDF and worldwide pig samples.

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Haplotypes	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	6	6	6	6
	5	5	5	5	5	5	5	5	5	5	5	5	5	6	6	6	6	7	7	7	7	7	7	7	8	8	8	8	8	8	8	9	9	9	0	0	0	0
	1	2	4	4	5	6	7	7	7	8	9	9	9	1	7	8	9	1	1	2	3	4	5	7	2	4	4	7	7	8	9	3	5	9	0	0	1	7
	1	1	3	4	8	5	1	6	9	7	2	3	7	5	5	7	4	4	5	0	0	1	9	9	5	0	1	5	9	8	4	6	9	5	0	8	0	5
AJ002189	С	Т	Т	G	A	G	С	A	С	С	A	С	A	Т	Т	Т	С	Т	T	Т	A	С	Т	A	С	Т	Т	A	A	С	Т	A	A	Т	G	Т	A	
Hap 1*				-	T								-					C			-		C			-			-		-							
Hap_2*																		C				T	C			-												
Hap_3*			C			A	-		T	T	G			C							G	T			T	-						G					G	
Hap_4*	T			Α																			C											C				
Hap_5*		$\mathbf{C}$	C			A			T	T	G	T									G	T	C		T				G								G	T
Hap_6			C			A	-		T													_			T						٠						G	
Hap_7			C			A	-		T	T	G		٠	C		٠					G	T			T						٠	G		C			G	
Hap_8	٠	٠	C	-		A	-		T	T	G	٠	-	C					٠		G	T			T	-		٠	-		-	٠	G			٠	G	
Hap_9			C			A	-		T	T	G		٠	C		٠					G	T			T						٠					$\boldsymbol{C}$	G	
Hap_10			C			A	-		T	T	G		٠	C		٠					G	T			T						٠						G	
Hap_11			C			A	-	T	T	T	G		٠	C	C	٠					G	T			T			G			٠						G	
Hap_12	٠	٠	C	-		A	-		T	T	G	٠	-	C				C	٠		G	T			T		C		G	T	-	٠				٠	G	
Hap_13	٠	٠		-	T							٠	-					C	٠		-		C						-		-	٠				٠	G	
Hap_14	٠	٠		-	T							٠	-					C	٠		-		C			C			-		-	٠				٠		
Hap_15	٠	٠	C	-		A	-		T	T	G	٠	-	C	C			C	٠		G	T			T	C			-	T	-	٠				٠	G	
Hap_16	٠		٠		T	٠	٠						٠	٠		٠	٠	-			-		C	٠	٠			٠			٠	G	٠		٠		G	
Hap_17	٠	٠	٠		T	٠	٠	٠	٠		٠	٠		٠	٠	٠	٠	C					C	٠	٠		٠	٠					٠					٠
Hap_18	T	٠	٠	Α		٠	٠	٠	٠		٠	٠		٠	٠	٠	٠	٠					C	T	٠		٠	٠					٠					٠
Hap_19	٠	٠	٠		٠	٠	٠	٠	٠		٠	٠		٠	٠	٠	٠	C					C	٠	٠		٠	٠					٠		T			٠
Hap_20	٠	٠	C		٠	A	-	٠	T	T	G	٠		C	٠	٠	٠	C		C	G				T		٠			-			٠				G	٠
Hap_21	٠	٠	C		٠	A	-	٠	T	T	G	٠		C	C	٠	٠	٠				T	٠	٠	T	C	٠	٠		T			٠				G	٠
Hap_22	٠	٠	C		٠	A	-	٠	T	T	G	٠													T					T			٠				G	٠
Hap_23	٠	٠	C		٠	A	-	٠	T	T	G	٠													T					T	Α		٠				G	
Hap_24	٠	٠	C	-		A	-		T	T	G	٠	-	C	C			C	٠		G	T			T	-			G	T	-	٠				٠	G	
Hap_25	٠	٠	C	-		A	-		T	T	G	٠	-	C		C		C	٠		G	T			T	-			G	T	-	٠				٠	G	
Hap_26	٠	٠		Α								٠	-						C		-		C			-			-		-	٠				٠		
Hap_27	٠	٠		-								٠	-					C	٠		-		C			-			-		-	٠				٠		
Hap_28	٠	٠							-			٠	٠				T	C	٠			٠	C					٠		٠	٠	٠				٠		
Hap_29	T	٠							-			٠	٠					-	٠			٠	C					٠		٠	٠	٠				٠		
Hap_30	٠	C	C	٠	٠	A	٠	٠	T	T	G	T	٠	٠		٠	٠	٠	٠	٠	G	T	C	٠	T	٠	٠	٠	G	٠	٠	٠	٠	C	٠	٠	G	T

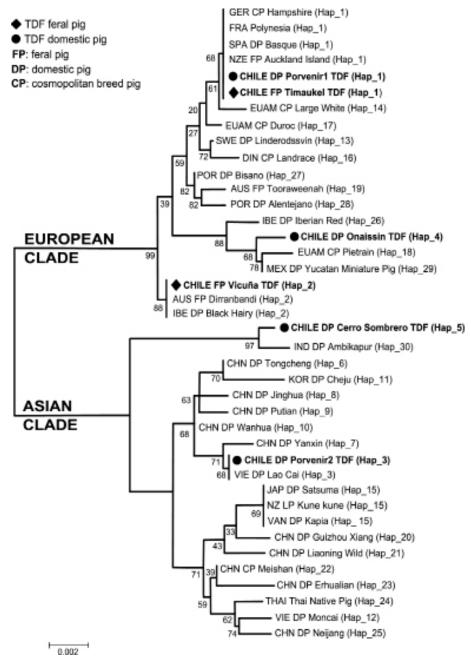
Asterisks indicate pig squences generated in the present study. Dots  $(\cdot)$  and daches  $(\cdot)$  indicate matches and gaps, respectively, compared to the reference pig sequence GeneBank accession No. AJ002189 (Ursing and Arnason, 1998).

Phylogenetic analysis, including additional sequences from other feral, domestic, and commercial breeds, revealed that TDF pig sequences were divided into 2 highly differentiated clades: a European clade and an Asian clade (Figure 2). The same marked difference was also observed in the haplotype network, with 11 mutational events separating each clade (Figure 3). Haplotypes Hap\_1, Hap\_2, and Hap\_4 found in feral and domestic pigs belonged to the European clade, while Hap\_3 and Hap\_5 found only in domestic pigs belonged to the Asian clade. Within the European clade, the 3 TDF haplotypes appeared to be well-differentiated. The Hap\_1 haplotype also shared with the Hampshire breed (one of the oldest breed of hogs), Polynesian French pig, Basque Spanish pig, and New Zealand feral hog (Table 1), clustered with sequences from common

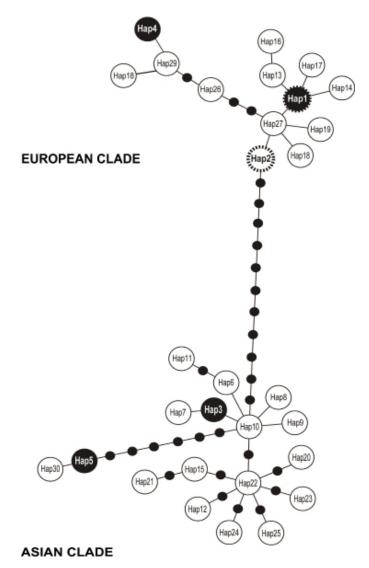
European domestic and commercial breed pigs (Figure 2). The haplotype Hap\_2 was shared with the Iberian Black Hairy pig and Australian feral pig (Table 1), and constituted a separate subclade supported by a relatively high bootstrap value of 88% (Figure 2). Hap\_4 belonged to a different subclade and showed similarities with sequences from the Yucatan Miniature pig, Pietrain commercial breed, and Iberian Red pig (Figure 2). For the Asian clade, Hap\_3 and Hap\_5 were grouped in different subclades: Hap\_3, also found in Vietnamese domestic pigs (Table 1), was clustered with a sequence from domestic Chinese pigs from Yanxin (bootstrap support 71%; Figure 2), and Hap\_5 showed more genetic similarity with an Indian domestic pig (bootstrap support 97%; Figure 2). No homologous sequence was found in the Genbank database for the haplotypes Hap\_4 and Hap\_5 from domestic pigs from the farms of Onaissin and Cerro Sombrero, respectively. They have been given the following Genbank accession Nos. Hap\_4 JQ668034 and Hap\_5 JQ668035.



**Figure 1.** Map of the sampling sites and geographic distribution of the mtDNA haplotypes found in feral and domestic pigs from the Chilean TDF. Black-colored circles represent Hap\_1; full white, Hap\_2; horizontal black lines, Hap\_3; black dots, Hap\_4; and vertical black lines, Hap\_5. The area colored in dark gray corresponds to Karukinka Natural Park. The dashed arrows indicate sample sites with shared haplotypes.



**Figure 2.** Phylogenetic tree based on variation of 652 bp in the mtDNA control region. Haplotype numbers are reported in parentheses and the name of the country of origin is abbreviated as follows: GER = Germany; FRA = France; SPA = Spain; NZE = New Zealand; EUAM = cosmopolitan breeds originating from Europe or America; SWE = Sweden; POR = Portugal; AUS = Australia; IBE = Iberian Peninsula; MEX = Mexico; IND = India; KOR = Korea; CHN = China; VIE = Vietnam; JAP = Japan; VAN = Vanuatu; THAI = Thailand.



**Figure 3.** Network of the 30 haplotypes in the mtDNA control region described in Table 1. In black: TDF domestic pig; with dashed border: TDF feral pig; and in black with dashed border: shared haplotype between domestic and feral pigs.

## **DISCUSSION**

# Origin and population structure of feral pigs in TDF

According to our results, the feral pig populations of TDF have a European origin only, but from 2 different lineages. This supports the hypothesis of different introduction events in TDF, 1 in the natural ecosystem of southwestern TDF near Timaukel and the other in

the southeastern part near Vicuña on the border between Chile and Argentina (Fuentes, 1923; Skewes, 1990). Indeed, the Timaukel feral pigs constitute a single population harboring a unique haplotype also found in European ancient breeds. Therefore, this population may have originated from a single or very few introduction events, directly from Europe in the late 19th century. This hypothesis seems the most parsimonious as it is consistent with the observations of Fuentes (1923) and with the record of the massive arrival of European immigrants in Tierra del Fuego in the late 19th century following the boom in local gold mining and livestock farming. However, a New Zealand origin of the SW Timaukel population cannot be ruled out given the relationship with haplotypes from Oceania, and the common release of the now rare old European pig breeds in the Pacific between the 18th and the 19th centuries. However, we found no evidence to support more than 1 introduction event of pigs into the SW forest of TDF, as suggested by Skewes (1990). A more extensive study including continental samples from South America and other Subantarctic islands would clarify this question.

The identification of the same haplotype (Hap\_2) in the wild pig population of SE Vicuña and a rustic strain of Iberian pig suggests that the origin of the SE Vicuña population is related to the first introductions from Europe (Gade, 1987). In wild pig populations from Australia, the presence of the same haplotype has been associated with the first stages of colonization by Europeans in the 18-19th centuries (Gongora et al., 2004). Moreover, although there is no precise description of the feral pig populations in Latin America, studies have identified descendants of the first introduced animals distributed from Mexico to the southern tip of Argentina (Benítez, 2001).

The large difference in the mtDNA haplotype composition between Timaukel (SW TDF) and Vicuña (SE TDF) feral pigs suggests that there is no connectivity between populations, and that they have evolved independently since their introduction. This is consistent with personal observations in the field and information provided by local people indicating the absence of feral pigs in the forest separating these 2 distant areas of the Karukinka Natural Park (Aravena P, unpublished data). While *Sus scrofa* is capable of occupying a large range of habitats and can cover distances of up to 50 km at an average speed of 1 km/day searching for food, water, and/or shelter (Choquenot et al., 1996), feral pigs have been described as rather sedentary animals, not moving far from their home territory in the absence of major disturbances such as hunting, low resource availability, or predation (e.g., Sparklin et al., 2009), which may be occurring in Karukinka Natural Park. Such sedentary behavior has been described in feral pigs in Australia, even when populations were separated by just a few kilometers (Hampton et al., 2004). Nevertheless, only a complementary analysis with a larger number of samples from Vicuña and with nuclear markers will reveal whether there is an incipient male flow between these populations.

# Connectivity between domestic and feral pigs of TDF

Haplotypes from 2 distinct lineages, Asian and European, were found in the domestic pigs sampled from the TDF farms. The presence of mtDNA of Asian origin in Chilean domestic pigs is not surprising as some modern commercial breeds were created through the introgression of Asian germplasm into European domestic pigs in the 18th century (Larson et al., 2005). After this, they were introduced worldwide for intensive production, arriving in Chile in the mid-19th century (Cuadra, 1866). Descendants of the cross between commercial modern races and pigs of antique Iberian lines are found in rural parts of Latin America

(Grossi et al., 2006). However, our study showed no evidence of genetic introgression of Asian mtDNA into the feral pig populations of TDF, suggesting that the escape of domestic pigs from the farms of Cerro Sombrero and Porvenir2 is very rare or nonexistent. The fact that pigs from the farm of Onaissin harbor a European haplotype found in neither of the populations of wild pigs also indicates that domestic pigs from this farm did not contribute to the wild population gene pool. Although our results suggest the absence of connectivity between domestic and feral pigs in TDF, the same haplotype observed in domestic pigs from the farm of Porvenir1 and the feral pig population of SW Timaukel proves a direct connection between the 2 sites. This situation is more likely the result of the capture and transfer of some feral pigs to be raised in this specific farm. Indeed, it is considered probable that any pigs escaping from Porvenir1 would have been caught or have died from lack of food and shelter because of the topographic and climatic characteristics of the central zone of TDF (Pisano, 1977). Intentional translocation of animals is not thought to be customary among locals, as observed by Spencer and Hampton (2005) in Australia.

## Implications for feral pig population control in Chilean TDF

According to our results, the feral pigs of SW Timaukel form an independent and bounded population within the natural forest of Karukinka Natural Park in Tierra del Fuego. Given the great value of this native forest, there is an urgent need to manage or eradicate this invasive species (Soto and Cabello, 2007). However, because the population of feral pigs is located next to sheep ranches and a small urban center, it appears crucial that any control strategy in this area should include a social focus in order to reduce the risk of new releases in the future and limit potential disease transmission to humans or livestock.

In the Argentinean part of Tierra del Fuego Island, for which there is no published data, there is no geographic barrier at the border that can limit the movement of pigs from one country to another. Some local Argentineans indicate the existence of a feral pig population in areas adjacent to San Justo, also adjacent to Vicuña (southeast TDF), and El Carmen in the south central part of the Argentinean TDF (Aravena P, personal communication). Therefore, we recommend developing a bi-national action plan to control or eradicate this population and prevent new invasions.

According to Novak and Mack (2005) as well as others, new genetic combinations may promote the feral populations' success and put the Fuegian environment even more at risk. Therefore, although we have no evidence that the wild population received genetic variability from domestic pigs, we suggest initiating a control plan to prevent this possibility.

Furthermore, the feral populations derived from the first introductions are generally well-adapted to their local environment and represent a valuable resource for science and live-stock management on a global scale (e.g., Guo et al., 2014). This constitutes an interesting and challenging field of research for the development of strategies that promote the preservation of natural ecosystems without reducing the genetic resources that these remote feral populations may represent.

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