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RESEARCH ARTICLE

Phylogeography and population history of *Leopardus guigna*, the smallest American felid

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Abstract The guigna (*Leopardus guigna*) is the smallest and most-restricted New World cat species, inhabiting only around 160,000 km^2 of temperate rain forests in southern South America and is currently threatened by habitat loss, fragmentation and human persecution. We investigated phylogeographic patterns of genetic diversity, demographic history and barriers to gene flow with 116 individuals sampled across the species geographic range by analyzing 1,798 base pairs of the mtDNA (496 bp HVSI region, 720 bp NADH-5 gene, 364 bp from 16S gene and 218 bp from ATP-8 gene) and 15 microsatellite loci. Mitochondrial DNA data revealed a clear phylogeographic pattern with moderate separation between northern and southern Chilean populations supporting recognized subspecific partitions based on morphology. A recent demographic expansion was inferred for the southern-most group (San Rafael Lake), presumably due to the complete coverage of this area during the last glacial period, 28000–16000 years BP. Geographical barriers such as the Andes Mountains and the Chacao Channel have partially restricted historic and more-recent gene flow and the Chiloé Island population has diverged genetically since being separated from the mainland 7000 years BP. This is the first study of the genetic structure of this threatened species throughout its whole geographic range.

Keywords *Leopardus guigna* · Felid · Phylogeography · Demographic history · Dispersal barriers

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Introduction

Contemporary geographical distribution and genetic diversity of species are influenced by historical and ecological processes that cause population expansions and contractions (Hewitt 2004). Historical climatic changes, specifically during the late Pleistocene glacial periods, have had a large impact on current geographical distribution and genetic diversity of vertebrates in regions of high and middle latitudes through habitat shifts, transient fragmentation of the ancestral range of a widely distributed species, variation in gene flow, intensity of genetic drift, bottlenecks and loss of genetic diversity, genetic divergence between refugial small isolated populations during periods of increased ice cover and expansion of geographic ranges of previously isolated populations during ice cover shrinkage (Klicka and Zink 1997; Taberlet et al. 1998; Bennett 2004; Hewitt 2004).

In southern South America, during the Last Glacial Maximum (LGM) approximately 28000–16000 years BP, ice sheets covered most of upper elevation areas from 56° to 35°S and most of the current coastline south of 41°S (Clapperton 1993; Heusser et al. 1999; McCulloch et al. 2000). Global sea levels dropped approximately 120 m below current levels, exposing much of the continental shelf and connecting Chiloé Island and the mainland 26000–7000 years BP (Villagrán et al. 1986; Moreno et al. 1994; Vidal et al. 2012). Chiloé Island is currently separated from the mainland by the Chacao channel (2.3–6 km wide, 50–100 m deep) (Formas and Brieva 2000). There are no migration records of mammals across this channel.

Ice-free regions to the west, such as the continental coastal area around 41°S, the northwest portion of Chiloé Island and the intermediate area of exposed continental shelf were possible refugia for terrestrial biota during the late Pleistocene glacial periods (Villagrán 1988; Sérsic et al. 2011; Vidal et al. 2012). Biological connections across the Andes may also have existed during the warmest interglacial periods (Moreno et al. 1994; Moreno 2000), as has been suggested for a variety of trans-Andean mammals (Smith et al. 2001; Palma et al. 2002, 2005; Himes et al. 2008). The evolutionary history and current genetic diversity of many Patagonian species have also been linked with Quaternary glacial cycles and colonization from lower latitudes after glacial retreat (Lessa et al. 2010; Sérsic et al. 2011; Pardiñas et al. 2011).

The temperate rainforests of southern South America (35–55°S) originated during the Middle Tertiary (Hinojosa and Villagrán 1997) and were broadly impacted by the Miocene rise of the Andes (Garzione et al. 2008). The Andes became a barrier with sharp altitudinal climatic zones that restricted the distribution of most biota to valleys both east and west (Webb 1991). The stark climatic and vegetational contrast that formed between the eastern and western slopes of the Andes along the arid diagonal of South America, coincident with the gradual isolation of the forests in southern South America, was one of the major evolutionary and biogeographic events in southern South America (Villagrán and Hinojosa 1997; Abraham et al. 2000; Villagrán and Hinojosa 2005). This extended period of biogeographical isolation resulted in numerous endemic flora and fauna species (Armesto et al. 1996; Arroyo et al. 1996; Villagrán and Hinojosa 1997) and these isolated and unique temperate rainforests are recognized as the "Chilean winter rainfall-Valdivian forests Hotspot" of biodiversity (Myers et al. 2000; Arroyo et al. 2004), a "frontier forest" by the World Resources Institute (Armesto et al. 1996; Arroyo et al. 1996), and as a Global 200 priority ecosystem of the World Wildlife Fund (Olson et al. 2001).

The guigna (*Leopardus guigna*), the smallest felid in the American continent and one of the smallest in the world (weight 1.5–3.0 kg) (Nowell and Jackson 1996), is closely associated with this unique forest ecosystem. It has the most-restricted distribution of New World cat species, inhabiting around 160,000 km^2 of Chile (30°–48°S) and a narrow strip of south-western Argentina (39°–46°S west of 70°W), including some offshore islands such as Chiloé Island, from sea level to 2,500 meters (Nowell and Jackson 1996; Quintana et al. 2000) (Fig. 2). Two guigna subspecies are generally recognized based on morphological data (Cabrera 1957). *L. g. tigrillo* (from 30° to 38°S in Chile) inhabits mediterranean matorral and sclerophyll woodlands and forests in northern and central Chile and has a lighter coat and larger body size (Figs. 1, 2; Table 1). *L. g. guigna* (from 38° to 48° in Chile and from 39° to 46°S in Argentina west of 70°W) inhabits more-dense Valdivian temperate rainforest and north Patagonian forest in southern Chile and the Andean Patagonian forest in southwestern Argentina and is darker and smaller (Osgood 1943) (Figs. 1, 2; Table 1). Melanistic individuals occur within the range of *L. g. guigna* (Dunstone et al. 2002; Sanderson et al. 2002).

Vegetation cover is an important ecological requirement for this species (Sanderson et al. 2002; Acosta-Jamett and Simonetti 2004; Gálvez et al. 2013). Home range size of guignas is 0.3–2.2 km^2 in Torres del Paine and Queulat National Parks (Dunstone et al. 2002), and 1.3–22.4 km^2 in a fragmented landscape on the northeastern coast of Chiloé Island (Sanderson et al. 2002). The species' maximum dispersal distance is 1.83 km in Torres del Paine and Queulat National Parks (Dunstone et al. 2002), and 13.9 km in a fragmented landscape on the northeastern coast of Chiloé Island (Sanderson et al. 2002), revealing its high and facultative dispersal ability (Napolitano 2012).

Guignas are classified as Vulnerable with a decreasing population on the IUCN Red List, and along with the

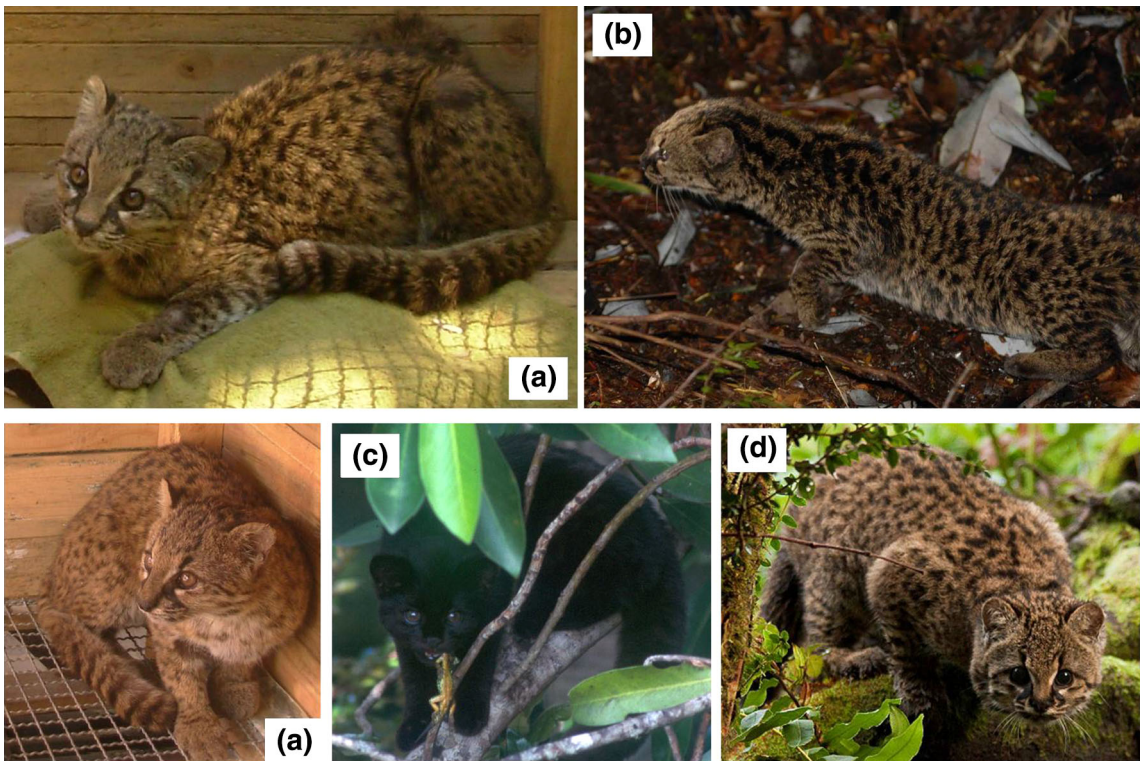

Fig. 1 Morphological features of guigna subspecies. **a** *L. guigna tigrillo* (Molina, Maule Region, this study); **b** *L. guigna guigna* (Chiloé Island, this study); **c** *L. guigna guigna* (Chiloé Island, melanistic individual); **d** *L. guigna guigna* (Quetruleufu, Araucania Region). Photo credits: **a** Luis Villanueva, **b** Andrés Charrier, **c** Jim Sanderson, **d** Fauna Australis

Andean cat (*Leopardus jacobita*) is the most-threatened South America cat species (Wozencraft 1993; Nowell and Jackson 1996). In Chile, guignas are classified as Vulnerable (from Los Ríos Region to the north) and as Near Threatened (from Los Lagos Region to the south) (CONAMA 2011). Current threats include severe habitat loss, fragmentation and direct persecution (Nowell and Jackson 1996; Sanderson et al. 2002; Dunstone et al. 2002; Acosta-Jamett et al. 2003; Acosta-Jamett and Simonetti 2004; Freer 2004; Silva-Rodriguez et al. 2007; Napolitano 2012; Gálvez et al. 2013; Herrmann et al. 2013).

Wild cats generally require large areas over which to forage, thus are particularly affected by land use change and the resulting loss of prey species and habitat (Lindenmayer and Fischer 2006). The spatial extent of protected areas alone is usually not enough for the long-term viability of many cat species (Woodroffe and Ginsberg 1998). Given this, the conservation of cats in private lands outside protected areas has gained relevance (Simonetti and Acosta 2002). In the end, wild cat conservation hinges in how rural people perceive and know them, being human attitudes among the most important issues when cat conservation is the intended goal (Sillero-Zubiri and Laurenson 2001; Silva-Rodriguez et al. 2007; Herrmann et al. 2013). Human-felid conflicts are one of the most urgent wild cat conservation issues worldwide, affecting over 75 % of the world's felid species (Inskip and Zimmermann 2009). These conflicts for small cats usually involve livestock depredation and felids killed in retaliation (Woodroffe et al. 2005).

As reported by Zorondo (2005) and Silva-Rodriguez et al. (2007), most people in rural landscapes of central and southern Chile had negative attitudes toward the guigna, arguing livestock and poultry losses. Given this scenario, long-term conservation challenges for the guigna outside protected areas will depend on the increase of local awareness to reduce conflict in areas where they are considered poultry pests, highlighting the services provided by its role as controller of mice and European hares (*Lepus europaeus*) (Silva-Rodriguez et al. 2007; Gálvez et al. 2013) and also improving chicken coops (IUCN 2013). Another important challenge is preserving native vegetation corridors to provide connectivity between forest fragments or larger forested areas (Dunstone et al. 2002; Sanderson et al. 2002; Gálvez et al. 2013), since human populations and deforestation are increasing in the Chilean temperate rainforest (Willson et al. 2005) and climate change may be an emerging additional threat (Malcolm et al. 2005).

From an evolutionary perspective, guignas are closely related to six other small Neotropical cats of the genus

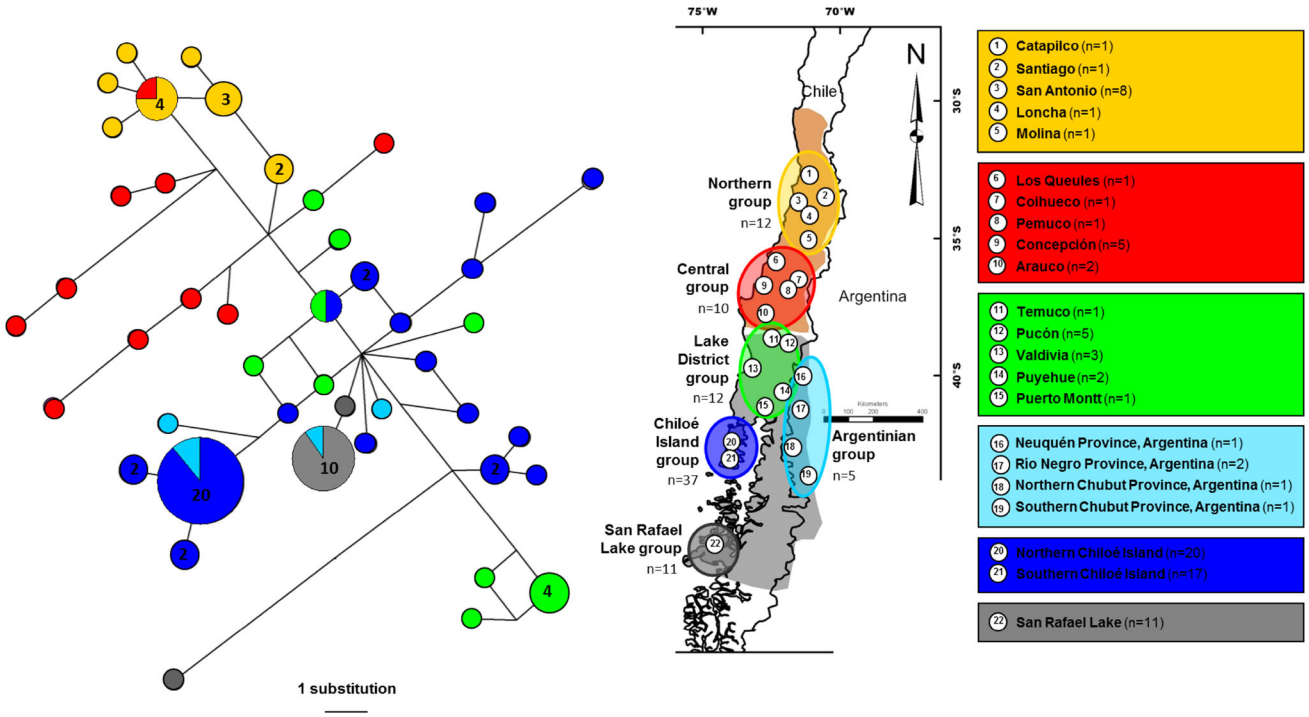

Fig. 2 Mitochondrial haplotype network of guignas. Each *circle* in the network corresponds to a different haplotype, the size of the *circles* correspond to haplotype frequencies, the *numbers* associated to each *circle* correspond to the *number* of individuals displaying that haplotype, and the *colors* of the *circles* correspond to the different geographical groups shown in the map along with the sample size per group. Sampling localities are shown with sample size per locality. The *brown-shaded area* in the map corresponds to the geographical distribution of the northern subspecies *L. g. tigrillo* and the *grey-shaded area* corresponds to the southern subspecies *L. g. guigna*

Leopardus belonging to the Ocelot Lineage (Johnson et al. 2006). This exclusive Neotropical lineage diverged from a common ancestor around the formation of the Panamanian land bridge 2.8 million years (Ma) (Johnson et al. 1998, 1999, 2006). Guignas' sister species, the Geoffroy's cat (*L. geoffroyi*), which last shared a common ancestor less than 1 Ma (Johnson et al. 2006), is found along the eastern side of the Andes mountain range, generally exhibiting a disjunct distribution (Nowell and Jackson 1996).

The objective of this study was to investigate population evolutionary history, uncover phylogeographic patterns of genetic diversity and to understand demographic partitions relevant to the conservation of guignas by addressing: (i) Patterns of genetic structure relative to historic climatic events in southern South America and (ii) the influence of historic and contemporary geographic barriers to gene flow, specifically testing the influence of the Andes and the Chacao channel.

More generally, phylogeographical inferences, applied in a comparative framework across multiple species at a regional scale, enables the detection of regional and landscape-level patterns of biodiversity, which are important for understanding macroecology, evolution, broad impacts of geological events and areas of high conservation priority (Bermingham and Moritz 1998; Moritz 2002). In recent years, phylogeographical knowledge in southern South America has been accumulating for both aquatic and terrestrial organisms (Sérsic et al. 2011). Concordant demographic or spatial patterns across major organismal groups bring light into past geological events occurred in this region, and these types of comparisons require intraspecific data from multiple species (Lessa et al. 2010). This study intends to build upon this knowledge and facilitate further regional comparative phylogeographic hypotheses.

It is likely that the geological and environmental history of a certain region produced similar impacts on a regional biota with comparable natural history and ecological requirements. Thus, although species-specific, our results might be extrapolable to other carnivores in southern South America with vegetation cover as their main ecological requirement.

This study is the first to describe the genetic diversity and demographic partitions of guignas, hence providing much-needed information to guide suitable conservation strategies for the long-term preservation of these populations.

Table 1 Morphological measurements of guigna subspecies from captured individuals in this study

Subspecies	*n*	Locality	Sex	Coat colour	Estimated age (months)	Age range classification	Weight (kg)	Body length[a] (cm)	Tail length (cm)	Head circumference (cm)	Neck circumference (cm)	Hind foot length (cm)	Ear length (cm)
L. g. tigrillo	1	Molina, Maule Region	M	Spotted	24	Adult	3.0	56.0	25.0	22.0	16.5	11.0	3.5
L. g. guigna	10	Chiloé Island	F	Spotted	5	Juvenile	0.8	34.0	18.0	17.0	12.0	9.5	3.5
		Chiloé Island	F	Spotted	12	Subadult	1.4	46.0	22.0	17.0	17.0	8.0	3.5
		Chiloé Island	F	Spotted	8	Subadult	1.8	45.0	20.0	16.0	13.0	10.0	3.0
		Chiloé Island	M	Spotted	12	Subadult	1.8	45.0	22.0	20.0	15.5	10.0	4.3
		Chiloé Island	M	Spotted	12	Subadult	1.9	51.0	21.0	20.0	17.0	10.0	3.0
		Chiloé Island	M	Spotted	12	Subadult	1.7	49.0	21.0	19.0	15.0	8.0	3.0
		Chiloé Island	M	Spotted	18	Adult	1.8	47.0	22.0	19.0	17.0	9.5	4.0
		Chiloé Island	M	Spotted	18	Adult	1.8	48.0	22.0	18.0	16.0	11.0	3.5
		Chiloé Island	M	Spotted	24	Adult	1.9	49.0	21.0	20.0	17.0	10.0	4.2
		Chiloé Island	M	Spotted	36	Adult	1.7	45.0	21.0	18.0	13.5	9.5	4.0

[a] Not including tail length. *M* male, *F* female. Individuals were classified as juvenile, subadult or adult on the basis of size, weight, dentition, and reproductive condition. Independent animals that had not yet reached the age of full sexual maturity were classed as subadults

Materials and methods

Sample collection

Samples of 116 guignas were obtained from 22 sites ($n = 1$–20 samples per site) across most of its distribution (Fig. 2). To test barriers and subspecific partitions, individuals were placed into six groups: Northern, Central, Lake District, Argentinian, Chiloé Island and San Rafael Lake. The Northern and Central groups correspond to the range of *L. g. tigrillo* while the others correspond to the range of *L. g. guigna*). Three *L. geoffroyi* and one *L. wiedii* were used as outgroups for the phylogenetic analyses.

Laboratory procedures

Genomic DNA was extracted from whole blood from wild-caught individuals, tissues (liver, kidney or muscle) from recent road-kills, skin fragments from pelts found in local communities and museums (Table S3) and faeces (only one faeces sample; preserved in 95 % ethanol, 4° or −20 °C) using commercially available kits (DNeasy Blood and Tissue kit; QIAamp DNA Stool Mini Kit) following the manufacturer's suggested protocol. Nucleotide sequences of four mtDNA gene segments encompassing 1,798 base pairs (bp), were obtained by PCR amplification (Saiki et al. 1985) from genomic DNA for: (i) NADH dehydrogenase subunit 5 (NADH-5, 720 bp) using primers ND5-DF1 and ND5-DR1 (Trigo et al. 2008), (ii) the 16S rDNA gene (364 bp) as in Hoelzel and Green (1992) and Johnson et al. (1998), (iii) the adenosine triphosphate (ATP-8) and part of the ATP-6 gene (275 bp) using primers ATP8-DF1 and ATP6-DR1 (Trigo et al. 2008) and (iv) the 5′ portion of the Control Region (CR), containing the First Hypervariable Segment (HVS-I) (439 bp) using primers CHF3 and CHR3 (Freeman et al. 2001). These four particular mtDNA gene segments were chosen because they are polymorphic, well described, have a good collection of reference sequences and are broadly used in felid and mammal studies so represent an excellent source for comparative purposes. PCR reactions were performed in a 25 μL volume containing 1.5 μL 10× PCR buffer, 1.5–2.0 mm MgCl2, 0.2 mm each dNTP, 0.5 U of Taq DNA polymerase (Invitrogen), and 0.2 μm of each primer. Thermocycling parameters consisted of an initial denaturation at 94 °C for 5 min, 35 cycles of 94 °C for 1 min, 53–55 °C for 1 min (pending primer sets), 72 °C for 1 min and a final extension of 72 °C for 10 min. PCR amplifications of faecal samples were repeated at least twice for each gene fragment to ensure repeatability of species identification and haplotype assignment. PCR products were checked using ethidium-bromide stained 1.5 % agarose gels. Forward and reverse strands were sequenced using an ABI 3730XL Analyzer by Macrogen Inc. (Korea).

Fifteen nuclear DNA microsatellite loci [twelve tetra-nucleotide repeat loci (FCA441, F124, F41, FCA424, F141, F146, FCA391, FCA453, F42, F98, F164, F27) and three dinucleotide repeat loci (FCA008, FCA176, FCA698)] developed originally for the domestic cat (Menotti-Raymond et al. 1999) and partially tested in guignas (Johnson et al. 1999; Ruiz-García et al. 2001) were amplified separately by PCR in a 15 μL volume containing 1.5 μL 10× PCR Buffer, 1.5–2.0 mm MgCl2, 0.2 mm each dNTP, 0.5 U of Taq DNA polymerase (Invitrogen), and 0.16 μm of the reverse primers, 0.064 μm of the forward primer and 0.12 μm of the fluorescent dye-labeled M13 tails (Schuelke 2000). Thermocycling parameters consisted of an initial denaturation at 95 °C for 5 min, 35 cycles of 95 °C for 30 s, 53–63 °C for 30 s (annealing temperature varied among loci, Table S1), 72 °C for 30 s, and a final extension of 72 °C for 10 min. To ensure that allelic dropout or other genotyping errors have not compromised our microsatellite data, we used: (i) multiple tubes approach, were each amplification was repeated twice per locus (Taberlet et al. 1996, 1999; Navidi et al. 1992; Creel et al. 2003; Frantz et al. 2003; Bellemain and Taberlet 2004; Smith et al. 2006); (ii) randomly reanalysed 30 % of the samples per locus (Bonin et al. 2004; Smith et al. 2006); (iii) the program Micro-Checker 2.2.3 (Van Oosterhout et al. 2004) to identify genotyping errors, null alleles or allele dropout in the data. PCR products were checked on an ethidium-bromide stained 1.5 % agarose gel and sent for direct fragment analysis using an ABI Prism 3730xl DNA Analyzer at the University of Illinois DNA Core Sequencing Facility (USA).

MtDNA data analysis

Sequences (forward and reverse) were aligned using ProSeq 2.91 (Filatov 2002) against reference sequences and checked by eye. The four mtDNA gene segments were concatenated (Huelsenbeck et al. 1996). The number of haplotypes and polymorphic sites, gene diversity, differences between pairs of sequences (Π) and nucleotide diversity (π per nucleotide site) were estimated with Arlequin 3.5.1.2 (Excoffier and Lischer 2010). Rarefaction analysis with PAST (Hammer et al. 2001) was used to adjust for unequal samples sizes for comparisons among geographic groups.

A maximum-likelihood (ML) tree was constructed with MEGA 5.05 (Tamura et al. 2011) using the Tamura-Nei model of sequence evolution. Node support was obtained by bootstrap analysis using 1,000 resampling steps.

A haplotype network was generated using the median-joining approach method (Bandelt et al. 1999) implemented in Network 4.6.

Patterns of phylogeographic structure were tested using G_{ST} and N_{ST} coefficients (Burban et al. 1999) as implemented in PERMUT (Pons and Petit 1995, 1996). Both of these parameters will estimate the relationship between average intrapopulation and total genetic diversity, but only G_{ST} uses haplotype frequencies while N_{ST} uses differences between haplotypes. The distribution of N_{ST} from 1,000 permutations was compared with observed N_{ST} values. N_{ST} values significantly larger than G_{ST} indicate haplotype phylogeographic structure because similar haplotypes are more likely within a population than between populations. Measures of genetic distance among geographic units (pairwise F_{ST}) were estimated with Arlequin 3.5.1.2 (Excoffier et al. 1992).

Two different clustering methods were used to infer the spatial genetic structure of guigna populations. First, we used SAMOVA 1.0 (Dupanloup et al. 2002), which defines groups of populations that are geographically homogeneous and maximally differentiated from each other, to investigate population subdivision using analysis of molecular variance in a geographical context and to help identify genetic barriers between groups. This method uses a simulated annealing procedure, maximizing the proportion of total genetic variance between groups of populations. Statistical significance was tested using 1,000 permutations. Second, we estimated the number of clusters, as well as the spatial boundaries among them using a Bayesian model computed with the GENELAND package, version 4.0.0 (Guillot et al. 2005) in the R environment (R, version 3.0.2; Ihaka and Gentleman 1996). This software implements a Markov chain Monte Carlo (MCMC) procedure to determine the best clustering of samples using genetic and geographical information. Geographical information is taken into account at the Bayesian prior level. 5,000,000 MCMC iterations sampled each 1,000 steps with a 4,900 burn-in period, using both the correlated and uncorrelated frequency models and a maximum number of clusters $K = 10$ were run to estimate the model parameters and posterior probabilities of group membership. The correlated frequency model sets a more realistic scenario because most often, allele frequencies tend to be similar in different populations (Nicholson et al. 2002; Balding 2003). Described as a more biologically grounded way to make inference, it has been observed that using the correlated frequency model could be more powerful at detecting subtle differentiations (Guillot et al. 2005).

To test for isolation by distance, we assessed the correlation between genetic and geographic distances among samples (mean genetic distance for each unit), performed in Arlequin 3.5.1.2 with 100,000 permutations. In addition, to test for patterns of geographic distribution of genetic distances, a surface of genetic landscape was constructed using Alleles in Space (Miller 2005; Miller et al. 2006), which applies a Delaunay triangulation connectivity network to all sample sites and assigns a genetic distance to the middle point for each pair of sites. Genetic distances over the landscape surface were then interpolated (on a 50×50 grid of uniformly spaced cells) throughout the sampled geographic range, providing a qualitative graphic representation of genetic distances.

Inferences of population expansion or contraction were based on mismatch distribution analyses (Rogers and Harpending 1992) and estimates of neutrality tests such as Tajima's D (Tajima 1989), Fu and Li's F* and D* (Fu and Li 1993), and Fu's Fs (Fu 1997) were computed in DnaSP 5.1 (Librado and Rozas 2009). Time estimates were calculated using a divergence rate of 0.67 %/MY, assessed from a felid-specific mitochondrial divergence rate as in Johnson et al. (1999). We acknowledge the possibility of some inaccuracy in estimated dates when using divergence rates. Effective population sizes for each geographic group (N_e) and migration rates between groups (N_em) were estimated with LAMARC (Kuhner 2006).

Microsatellite data analysis

Microsatellite genotypes were scored with GeneScan 3.7 (ABI) and Peak Scanner 1.0 (ABI) to precisely calibrate allele sizes. Microsatellite data was analyzed with Genepop 4.0.10 (Raymond and Rousset 1995; Rousset 2008) for general diversity estimates including heterozygosity, number and allele size range per locus and to test for deviations from Hardy–Weinberg equilibrium. Rarefaction analysis was used to adjust for unequal samples sizes for comparisons among geographic groups. Genetic distances among geographic units (pairwise F_{ST}) were estimated with Arlequin 3.5.1.2. We used SAMOVA 1.0 to investigate population subdivision using analysis of molecular variance in a geographical context, identifying the most likely position (using 1,000 permutations) of inferred historical genetic barriers. Isolation by distance was assessed through correlation of genetic and geographic distances among samples (mean genetic distance value for each unit) performed in Arlequin 3.5.1.2 with 100,000 permutations.

We used Structure 2.3.3 (Pritchard et al. 2000) to define the contemporary population structure through the definition of clusters or groups and assignment of individuals to these clusters using 100,000 iterations, 100,000 MCMC and an admixed ancestry model. We discarded individuals with <50 % data ($n = 16$). We evaluated $K = 1$–8 populations and results from 10 replicates to evaluate the variance and stability of likelihood values. We also used BayesAss 1.3 (Wilson and Rannala 2003) to estimate

recent migration rates (during the last several generations) between populations, defined as the proportion of individuals in each generation that are not migrants.

Results

We obtained 1,798 bp of mtDNA sequence for 87 individuals from four mitochondrial genes: NADH-5 (720 bp), 16S rDNA (364 bp), CR HVS-I (439 bp), ATP-8 and part of the ATP-6 (275 bp), excluding 29 individuals without complete sequence data. Polymorphic sites (total = 55) are mostly found in the CR HVS-I (37 sites, 67.3 %), followed by NADH-5 (16 sites, 29.1 %) and 16S rDNA (2 sites, 3.6 %) genes. No polymorphic sites were found in the ATP-8 and part of ATP-6 gene. To assess possible Numt content, we checked the reading frames for the whole data set of protein coding (ND5) or RNA (16S) genes. No insertion/deletion or stop codons were detected.

Regarding microsatellite loci, we obtained complete multilocus genotypes for 102 individuals. Locus F164 was monomorphic and F27 resulted in unreliable genotyping without concordance between independent amplifications, so they were not included in the subsequent analyses. There was 100 % concordance among replicates from the 13 microsatellite loci included in the analyses. With F124 and F98, a few groups deviated from Hardy–Weinberg Equilibirum with heterozygous deficiency, but since this was not observed in most populations we included them in our analyses. Micro-Checker 2.2.3 did not identify genotyping errors, null alleles or allele dropout in our data. The 102 individuals included in the analysis had >50 % loci data, most of them displaying 80–100 %.

All 87 individuals with mtDNA sequences correspond to individuals with known and different microsatellite multilocus genotypes, so there is no overrepresentation of samples. Reasons for having less individuals with complete concatenated mtDNA sequences (87 individuals) compared to individuals with complete microsatellite multilocus genotypes (102 individuals) may include longer sequences in mtDNA gene segments (275, 364, 439, 720 bp after editing) compared to microsatellite loci (129–304 bp), which makes the former harder to amplify when DNA is degraded. Besides, only individuals with the four mtDNA gene segments were included in the final analysis to improve definition (more information).

Phylogeography and evolutionary history

The mtDNA haplotype network (Fig. 2) showed a non-random association among the 45 haplotypes that corresponded with their geographic origin, with *L. g. tigrillo* in the north (Northern and Central groups) and *L. g. guigna* in the south (Lake District, Chiloé Island, Argentinian and San Rafael Lake groups). N_{ST} coefficients (0.421 ± 0.122) were significantly higher than G_{ST} values (0.177 ± 0.0988) ($p < 0.001$), suggesting that there was a phylogeographic structuring of haplotypes and that within a population it is more likely to find genetically related than genetically distant haplotypes. There were fewer haplotypes in the Northern group ($n = 7$) compared with Central, Lake District and Chiloé Island groups, which had a higher number of variable and divergent haplotypes ($n = 16$). The four haplotypes from the Argentinian group did not cluster independently, but grouped with Lake District, Chiloé Island and most of San Rafael Lake haplotypes.

There is a shared haplotype among Northern and Central groups, suggesting a possible historical connection (Fig. 2). There is a shared haplotype among Lake District and Chiloé Island groups, specifically between Puerto Montt (locality 15) and Northern Chiloé Island (locality 20), supporting historical connectivity across the land bridge during the last glacial period (Figs. 2, 3). Chiloé Island and Argentinian groups share their most common haplotype, supporting a recent connection of the island with the continent and also suggesting that the Andes was not a complete barrier for gene flow (e.g. low-elevation pass exist at several points in this area). Also, the Argentinian and San Rafael Lake groups share their most common haplotype, specifically between Southern Chubut Province (locality 19) and San Rafael Lake (locality 22), suggesting a possible post-glacial recolonization route and glacial refugia (Figs. 2, 3).

The maximum likelihood tree revealed no significant phylogenetic separations among different geographic groups (Fig. 4). A highly divergent haplotype from San Rafael Lake group was the most basal guigna haplotype, with good bootstrap support (84 %). Other sequences located in basal positions were from the Lake District and Chiloé Island groups. Haplotypes from the Northern group were the most recently derived.

Population structure

To explore population subdivision and genetic structure in a geographical context, we performed a spatial analysis of molecular variance (SAMOVA) using mtDNA and microsatellite loci data (Table 2). The mtDNA variation among groups was largest (48.8 %) and the percentage of variation within groups lowest (−0.70 %) when guigna samples were divided into five groups (48.08 %): (i) Northern, (ii) Central, (iii) Lake District, (iv) Chiloé Island and Argentinian, and (v) San Rafael Lake (Table 2).

SAMOVA analysis for the microsatellite data showed that variation among groups was the largest (27.30 %) and

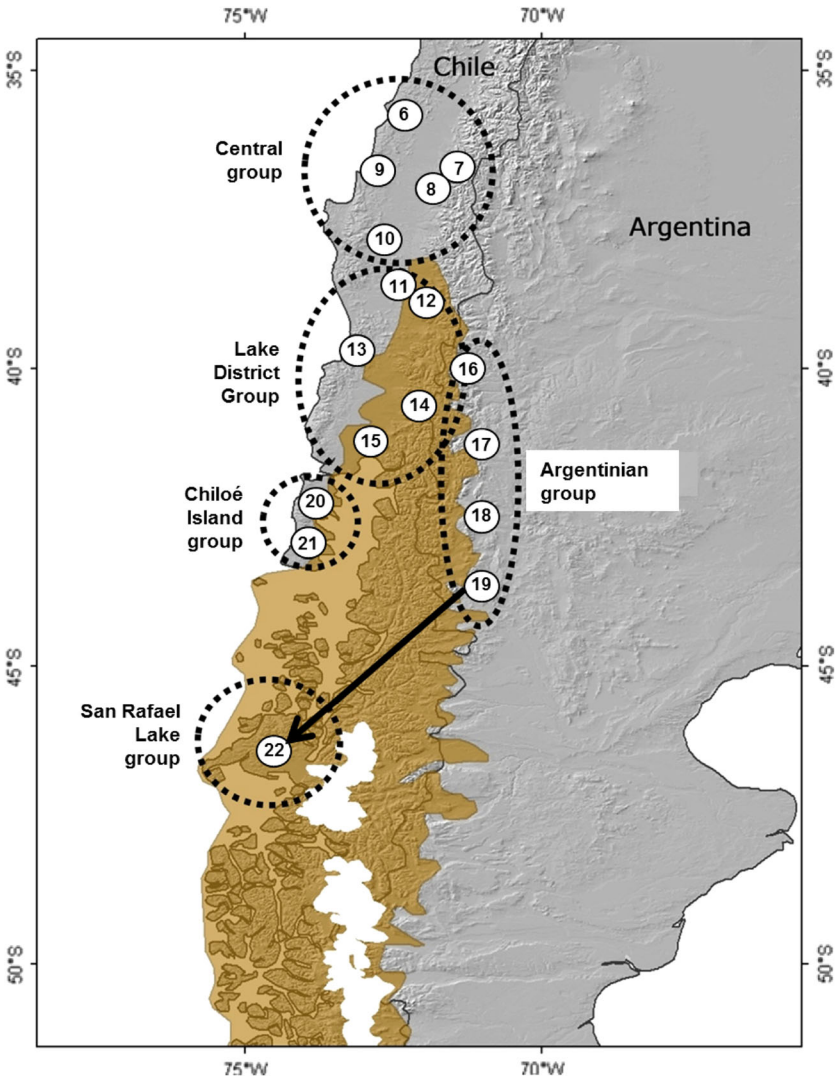

Fig. 3 Maximum extension of ice sheets during the last glacial event in southern South America shaded in brown, modified from Denton et al. (1999). Location *numbers* correspond to sampling localities in Fig. 2. *Arrow* shows proposed recolonization route

the percentage of variation within groups the lowest (−3.37 %) with four groups: (i) Northern, (ii) Central, (iii) Lake District, Argentinian, and San Rafael Lake, and (iv) Chiloé Island (Table 2).

Based on the mtDNA data, the Bayesian clustering algorithm implemented in Geneland using the correlated frequency model, detected five main clusters (K = 5): (i) Northern, (ii) Central, (iii) Lake District, (iv) Chiloé Island and Argentinian, and (v) San Rafael Lake (Fig. 5). The existence of these five clusters is supported by the results of the SAMOVA analysis. Using the uncorrelated frequency model, the Bayesian clustering detected only two clusters (K = 2): (i) Northern and Central, (ii) Lake District, Chiloé Island, Argentinian and San Rafael Lake (Figure S4). These two clusters coincide with subspecific partitions, showing the boundary between them runs approximately across latitude 38°S. The correlated frequency model was more powerful at detecting subtle differentiations in a biologically realistic scenario.

Bayesian structure analyses of microsatellite data (Fig. 6) suggested that the existing population is composed of 3 clusters or groups (K = 3). Log likelihood values for each K were the following: K = 2 LnP(D) = −2,604.3; K = 3 LnP(D) = −2,500.0; K = 4 LnP(D) = −2,518.4; K = 5 LnP(D) = −2,582.0; K = 6 LnP(D) = −2,535.6; K = 7 LnP(D) = −2,630.5. The 3 identified clusters correspond to: (1) the Northern, Central, and Lake District and some individuals from the Argentinian group, (2) Chiloé Island and some individuals from the Argentinian group, and (3) San Rafael Lake. Individuals from the Argentinian group were assigned to the Northern, Central and Lake District or to Chiloé Island. There were some individuals of "mixed" heritage suggesting limited gene flow among these three geographical regions.

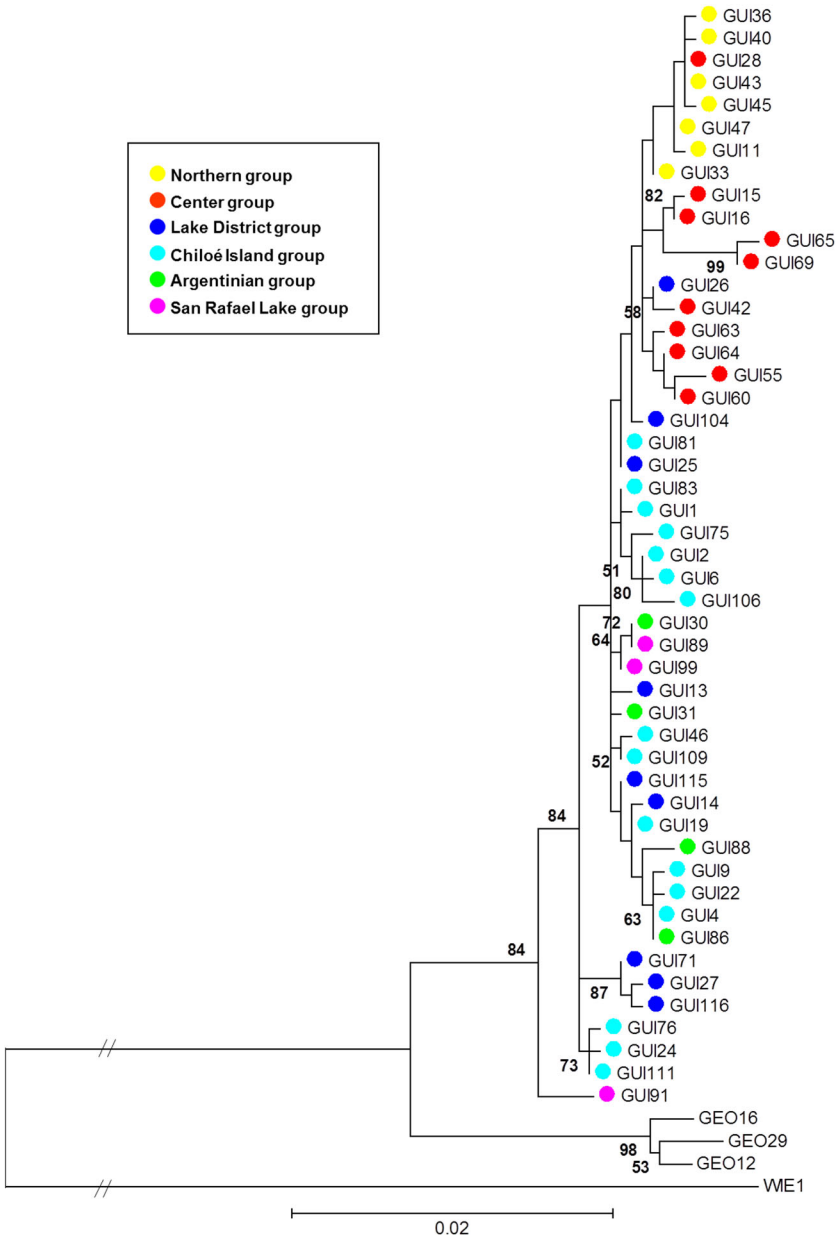

Fig. 4 Maximum likelihood tree for guigna haplotypes, three sequences of *L. geoffroyi* (GEO) and one sequence of *L. wiedii* (WIE) are used as outgroups. Bootstrap support values are shown and the *colors* on haplotype labels correspond to different geographic groups

We explored the distribution of genetic diversity through an analysis of landscape shape interpolation of genetic distances in geographic space (Figure S2) which illustrated two peaks of genetic distance (greatest genetic discontinuity in the landscape). These correspond with areas near Concepción (36°46′S, 72°53′W) and Los Angeles (38°11′S, 72°17′W) which are in the transition area between the two subspecies. To the north, maximum genetic distances decreased with decreasing latitude, suggesting increased genetic connectivity.

The mtDNA genetic differentiation increased with geographic distance between groups. There were low levels of genetic differentiation between the Argentinian and Chiloé Island groups, and between the Lake District and Argentinian groups. Genetic differentiation between the Northern and Central groups (*L. g. tigrillo*) ($F_{ST} = 0.26494$, $p < 0.001$) was lower than between the Central and Lake District groups (representing the transitional zone between *L. g. tigrillo* and *L. g. guigna*, respectively) ($F_{ST} = 0.34856$, $p < 0.001$) (Table 3).

Patterns of pairwise F_{ST} estimates using microsatellite loci were similar (Table 3), but the San Rafael Lake and Chiloé Island groups had a significant degree of differentiation with most of the other geographical groups, except with the Argentinian and San Rafael Lake groups. Genetic differentiation between the Central, Lake District and Argentinian groups was low and not significant. Genetic differentiation between the Northern and Central groups of

Table 2 Spatial analysis of molecular variance of mtDNA and microsatellite loci for different guigna geographic groups

Clusters	mtDNA			Microsatellite loci	
	% Variance between groups	% Variance between populations within groups	Clusters	% Variance between groups	% Variance between populations within groups
2 groups	37.25*	18.45**	2 groups	20.50 n.s	12.34 n.s
1+2; 3+4+5+6			1; 2+3+4+5+6		
3 groups	37.06*	14.37**	3 groups	22.24 n.s	6.45 **
1+2; 3+4+5; 6			1; 2; 3+4+5+6		
4 groups	37.58*	10.29**	4 groups	**27.30***	**−3.37 n.s**
1+2; 3; 4+5; 6			1; 2; 3+5+6; 4		
5 groups	**48.08***	**−0.70****	5 groups	26.95 n.s	−4.28 n.s
1; 2; 3; 4+5; 6			1; 2; 3+5; 4; 6		

1 Northern group, *2* Central group, *3* Lake District group, *4* Chiloé Island group, *5* Argentinian group, *6* San Rafael Lake group

* Significant ($p < 0.05$); ** significant ($p < 0.001$); *n.s.* non significant ($p > 0.05$)

In bold, largest variation among groups and lowest variation within groups

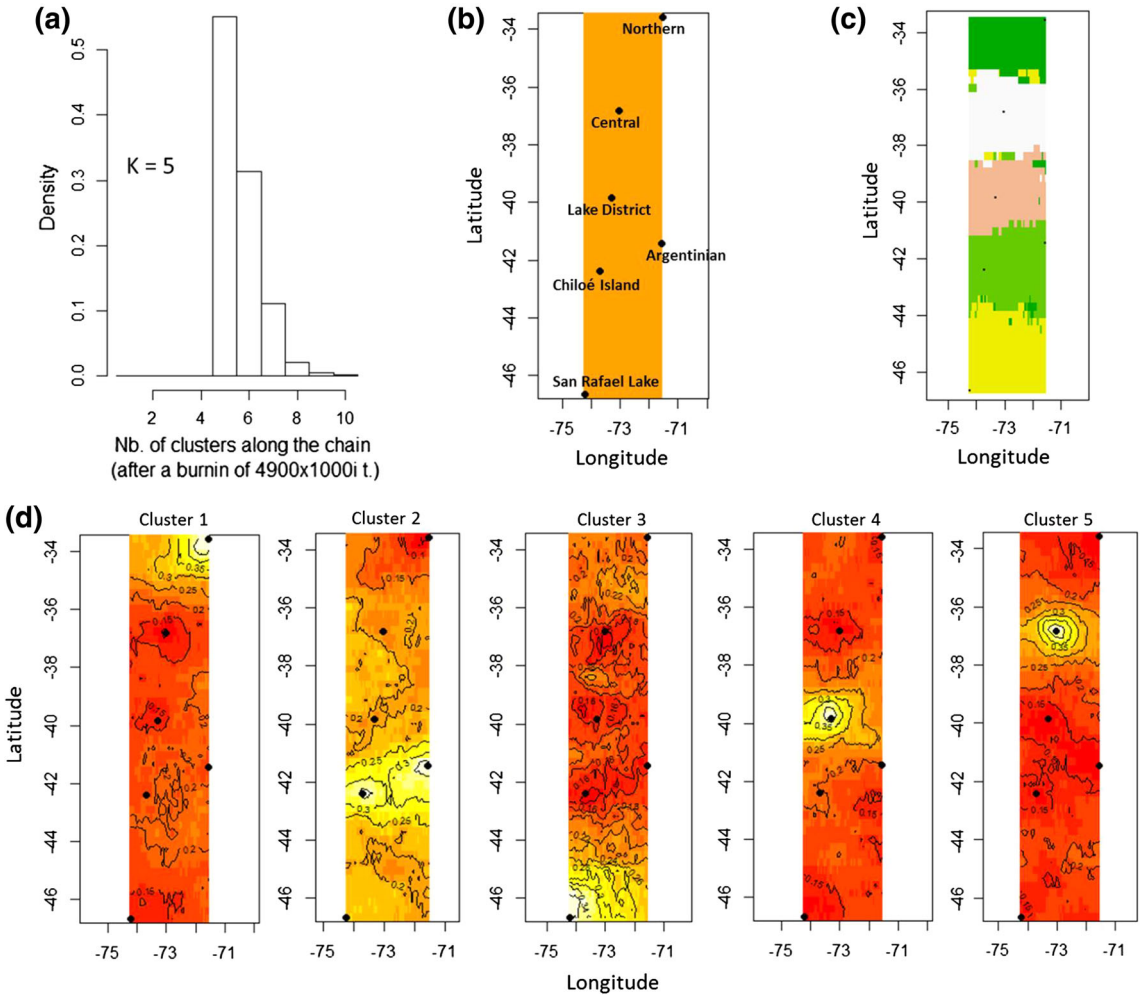

Fig. 5 Spatial output from Geneland using the correlated frequency model for mtDNA data of all guigna samples. **a** Number of populations (K = 5); **b** relative position of sampled populations; **c** map of population membership; **d** map of posterior probabilities to belong to the different clusters. *Black circles* indicate the relative position of the sampled populations. *Darker* and *lighter shading* are proportional to posterior probabilities of membership in clusters, with *lighter* (*yellow*) areas showing the highest probabilities of clusters

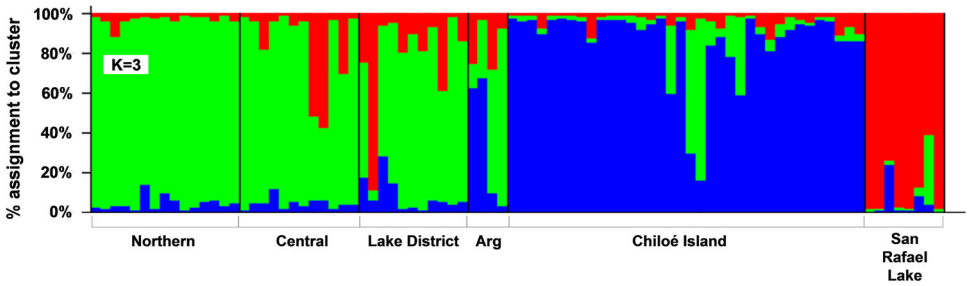

Fig. 6 Genetic population structure and assignment of individuals to clusters for different guignas geographic groups based on microsatellite loci information. Each *vertical line* represents an individual. Individuals are grouped according to their location of origin. Clusters are encoded with 3 different *colors* and the fraction of each individual *color* represents the probability of assignment to the cluster of that *color*. Log likelihood values, K = 2 LnP(D) = −2,604.3; K = 3 LnP(D) = −2,500.0; K = 4 LnP(D) = −2,518.4; K = 5 LnP(D) = −2,582.0; K = 6 LnP(D) = −2,535.6; K = 7 LnP(D) = −2,630.5

Table 3 Measures of genetic distance (pairwise F_{ST}) using mtDNA and microsatellite loci between guigna geographic groups

Geographic groups	Northern	Central	Lake District	Argentinian	Chiloé Island	San Rafael Lake
Northern (32.5–35° S)	–	0.101**	0.132*	0.158*	0.203**	0.334**
Central (36–37.5° S)	0.26494**	–	0.027 n.s	0.011 n.s	0.110**	0.153**
Lake District (38.5–41.5° S)	0.61506**	0.34856**	–	0.063*	0.053*	0.215**
Argentinian (39–44° S)	0.71665**	0.35911**	0.16568*	–	0.078*	0.003 n.s
Chiloé Island (41.7–43.5° S)	0.64245**	0.45663**	0.26057**	$0.03932^{n.s.}$	–	0.202 **
San Rafael Lake (46.5° S)	0.75299**	0.46474**	0.36240**	0.36646**	0.42667**	–

mtDNA information below diagonal, microsatellite loci information above diagonal

* Significant ($p < 0.05$); **significant ($p < 0.001$); *n.s.* non significant ($p > 0.05$)

L. g. tigrillo were higher ($F_{ST} = 0.101$, $p < 0.001$) than the differentiation between Central and Lake District groups (corresponding to the transitional zone between *L. g. tigrillo* and *L. g. guigna*, respectively) ($F_{ST} = 0.027$, $p > 0.05$).

There was a highly significant positive correlation between geographic distance and genetic distance among all guigna samples for both mtDNA (r = 0.8084, $p = 0.001$) and microsatellite data (r = 0.762, $p = 0.006$). Genetic differentiation increased with geographic distance (Fig S1) throughout the species' distribution.

Gene flow among populations

The Central group had the highest effective population size and the Northern and San Rafael Lake groups the lowest using mtDNA data (Table 4). Migration rates from the Northern group to the Central group ($N_em = 0.000424$) were approximately one order of magnitude higher than in the opposite direction ($N_em = 0.0000127$). There were high migration rates from the Lake District to the Central group and from the Argentinian to Chiloé Island group. Low migration rates were observed from most geographic groups to the Northern and the San Rafael Lake groups.

Microsatellite analyses reflected more-contemporary barriers to gene flow and migration (Table 5). Rates from the Lake District to the Northern and Central groups were the highest. San Rafael Lake and Chiloé Island groups had very low rates of migration with the other geographic groups and very high intra-population migration rates. Migration rates were unequal between the Northern and Lake District groups, between the Central and Lake District groups, and among Lake District, Argentinian and San Rafael Lake groups.

Differences in bidirectional migration rates may be affected by smaller sample sizes (e.g. between Argentinian group and all the other groups), because they increase the variance of the posterior probabilities distribution, decreasing its accuracy when compared to larger sample sizes (Wilson and Rannala 2003).

Intrapopulation diversity and bottleneck events

Overall, guignas had medium to high mtDNA genetic diversity, with 45 haplotypes, 55 polymorphic sites, haplotype diversity of 0.94 (±0.02) and an average 7.014 number of nucleotide differences between pairs of sequences (Table 6). The Central, Lake District and

Table 4 Effective population size (N_e) and bidirectional migration rates (N_em) among guigna geographic groups using mtDNA information

Geographic groups	N_e	To					
From		Northern	Central	Lake District	Chiloé Island	Argentinian	San Rafael Lake
Northern	90,000	–	0.000424	2.245	0.00016	0.000035	0.506
Central	1,800,000	0.0000127	–	1.32	0.271	0.000018	0.000006
Lake District	500,000	0.7154	9.892	–	0.181	0.1448	0.000007
Chiloé Island	360,000	0.00032	1.127	0.314	–	0.7253	0.182
Argentinian	290,000	0.0000844	0.00016	3.9	7.0124	–	0.188
San Rafael Lake	97,000	0.000029	0.00029	0.000073	0.00006	4.345	–

Table 5 Recent bidirectional migration rates (N_em) among guigna geographic groups using microsatellite loci information

From	To					
	Northern	Central	Lake District	Argentinian	Chiloé Island	San Rafael Lake
Northern	**0.750 ± 0.05**	0.024 ± 0.03	0.008 ± 0.01	0.024 ± 0.03	0.002 ± 0.00	0.005 ± 0.01
Central	0.014 ± 0.02	**0.719 ± 0.04**	0.009 ± 0.02	0.028 ± 0.03	0.003 ± 0.01	0.007 ± 0.01
Lake District	0.201 ± 0.05	0.203 ± 0.05	**0.917 ± 0.07**	0.089 ± 0.06	0.002 ± 0.00	0.006 ± 0.01
Argentinian	0.011 ± 0.02	0.012 ± 0.02	0.008 ± 0.01	**0.713 ± 0.04**	0.002 ± 0.00	0.004 ± 0.01
Chiloé Island	0.014 ± 0.02	0.014 ± 0.02	0.014 ± 0.02	0.100 ± 0.06	**0.989 ± 0.01**	0.005 ± 0.01
San Rafael Lake	0.010 ± 0.01	0.029 ± 0.03	0.044 ± 0.06	0.046 ± 0.04	0.002 ± 0.00	**0.973 ± 0.03**

Main diagonal (in bold): Migration rates from one population to the same population corresponds to the proportion of individuals in each generation who are not migrants

Argentinian groups had the highest genetic variability and Chiloé Island and San Rafael Lake groups, the lowest. The Northern group had the lowest average number of differences between pairs of sequences, while the Central group had the highest number of pairwise differences. Guignas had both moderate levels of microsatellite loci heterozygosity and number of alleles per locus (Table 6). Average heterozygosity decreased from north to south. The Argentinian group had no private alleles and the Central group had the most.

We inferred historic demographic changes for each geographical group. The San Rafael Lake group showed evidence of a contraction–expansion demographic event supported by Tajima ($D = -1.93$, $p < 0.05$) and Fu and Li ($D = -2.21$, $p < 0.05$; $F = -2.42$, $p < 0.05$) tests (Table S2). Results for all other geographic groups were not significant. The Central, Lake District, Argentina and Chiloé Island groups had multimodal mismatch distribution patterns (Fig. 7), suggesting populations in demographic equilibrium (Rogers and Harpending 1992). In contrast, the San Rafael Lake group had a main peak (Fig. 7a) suggestive of a recent demographic expansion around 9,800 years BP (after removing the highly divergent haplotype from the analysis, Tau = 0.2) (Fig. 7b). The Northern group had a unimodal mismatch distribution pattern (Fig. 7c), suggesting a demographic expansion event (Tau = 1.82) around 89000 years BP. The mismatch distribution for all guigna sequences had a unimodal distribution representing a demographic expansion event an estimated 318000 years BP (Tau = 4.115) (Fig. 7d).

To further explore our results, we unlinked the sequence data and carried out mismatch distribution analysis exclusively with D-loop, the most informative segment. We also performed a Bayesian skyline plot (BSP) analysis implemented in BEAST, version 1.7 (Drummond and Rambaut 2007) exclusively for D-loop sequences, using the GTR+G+I model previously estimated with MrModeltest version 2.3 (Nylander 2004). The D-loop mismatch distribution analysis showed similar patterns of population expansion as for the populations in the total concatenate analysis (Figure S3). In the case of the BPS analysis, it did not show clear signals of population size change in any of the populations (Figure S3). We believe that low sample size and sampling strategy (local or scattered sampling) may be affecting the BPS analysis, impeding us to detect recent population size changes, as has been recorded in simulations by Heller et al. (2013). Recent (Holocene or Pleistocene) population size changes were unobservable in scenarios under local sampling or scattered sampling, leading to the false negative of failing to detect a true population expansion towards the present (Heller et al. 2013). Other studies have also highlighted the danger of

Table 6 Measures of genetic diversity for mtDNA and microsatellite loci in different guigna geographic groups

Geographic groups	*n*	Number of haplotypes (K)	Number of polymorphic sites (S)	Haplotype diversity (H)	Average number of nucleotide differences between pairs of sequences (Π)	Nucleotide diversity (π)	Rarefaction[a]: Number of haplotypes (K^a)
mtDNA							
Total	87	45	55	0.94 ± 0.02	7.014	0.00461	
Northern (32.5–35°S)	12	7	7	0.89 ± 0.06	1.818	0.00119	6.59 ± 0.52
Central (36–37.5°S)	10	10	23	1.00 ± 0.03	8.026	0.00527	10.00 ± 0.00
Lake District (38.5–41.5°S)	12	9	14	0.91 ± 0.08	5.667	0.00372	7.96 ± 0.64
Argentinian (39–44°S)	5	4	10	0.90 ± 0.16	4.800	0.00315	
Chiloé Island (41.7–43.5°S)	37	16	20	0.76 ± 0.08	4.204	0.00276	8.32 ± 0.92
San Rafael Lake (46.5°S)	11	3	14	0.35 ± 0.17	2.691	0.00177	3.00 ± 0.00

Geographic groups	*n*	Average Heterozygosity	Average number of alleles per locus	Average allele size range	Number of private alleles	Rarefaction[a]: Average number of alleles per locus
Microsatellite loci						
Total	102	0.49 ± 0.1	6.54 ± 2.0	21.39 ± 11.5		
Northern (32.5–35°S)	16	0.58 ± 0.2	4.31 ± 1.6	14.77 ± 10.0	5	3.99 ± 0.1
Central (36–37.5°S)	17	0.52 ± 0.2	4.08 ± 1.3	14.77 ± 11.2	7	3.99 ± 0.1
Lake District (38.5–41.5°S)	12	0.51 ± 0.2	4.08 ± 1.7	11.69 ± 6.3	2	4.08 ± 1.7
Argentinian (39–44°S)	5	0.48 ± 0.3	3.17 ± 1.1	9.67 ± 7.0	0	_
Chiloé Island (41.7–43.5°S)	38	0.49 ± 0.1	4.54 ± 1.7	13.54 ± 8.1	4	3.94 ± 0.2
San Rafael Lake (46.5°S)	14	0.39 ± 0.2	2.92 ± 0.8	9.0 ± 4.3	1	3.0 ± 0

[a] Rarefaction curves to compare the average number of alleles per locus in geographical groups with different sample sizes

violating the panmixia assumption when inferring population size changes for BSP inference in natural populations (Peter et al. 2010; Ho and Shapiro 2011).

Discussion

Subspecific partitions

A lack of reciprocal monophyly was revealed in the mtDNA phylogenetic tree, with no major partitions or statistically different lineages within guignas. This pattern reflects the relatively recent evolutionary history of guignas (400000 BP, Johnson et al. 1999; 318000 BP, this study), which is likely insufficient time to cause complete separation of intraspecific lineages. Moreover, the large home ranges and dispersal ability of guignas (Dunstone et al. 2002; Sanderson et al. 2002), exemplified by evidence of gene flow between major mainland populations, might also have contributed to less differentiation among groups.

Contrasting patterns between subspecies defined phenotypically and their phylogenetic clusters identified using modern molecular techniques is not uncommon (Burbrink et al. 2000; Zink 2004). Reciprocal monophyly is less likely to be observed in species with high gene flow in natural environments (e.g. birds or medium to large mammals) (Waits et al. 1998; Wayne et al. 1992; Haig et al. 2001), or in recently diverged subspecies at an early stage of differentiation (Phillimore and Owens 2006). Discordant results may also suggest that morphological differences used to define subspecies could represent phenotypic plasticity in differing environments rather than long-term genetic isolation (Waits et al. 1998), or may be due to historical processes such as introgression or incomplete lineage separation (Funk and Omland 2003).

Despite the lack of reciprocal monophyly, a phylogeographic structuring of haplotypes according to their geographic origin is shown in the mtDNA haplotype network. Individuals from the northern (*L. g. tigrillo*) and southern (*L. g. guigna*) subspecies are generally distributed into distinct groups, although with a low degree of genetic differentiation (1–11 substitutions). Geneland analysis (uncorrelated model, Fig S4), landscape shape interpolation analysis (Fig S2) and population

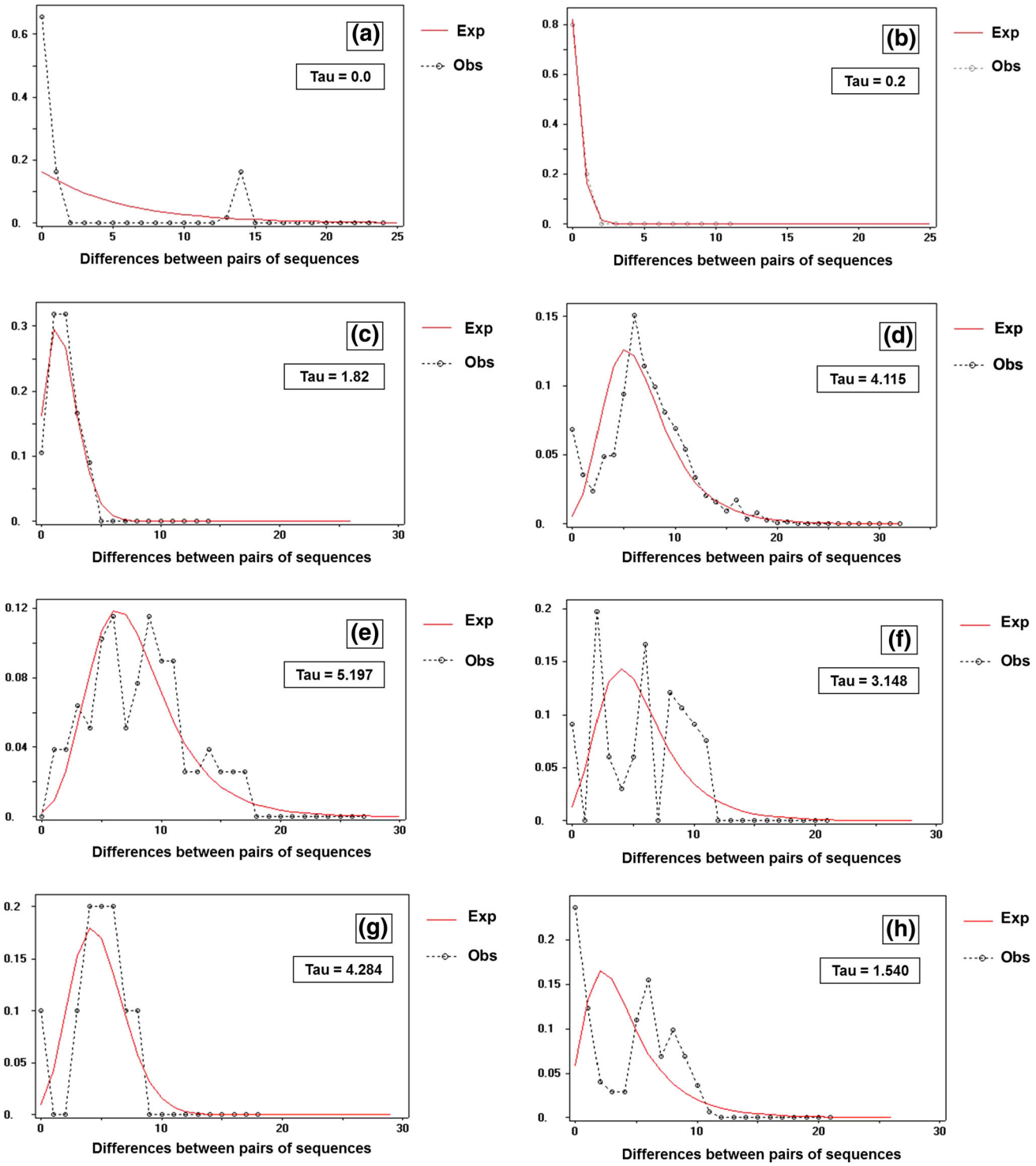

Fig. 7 Mismatch distribution of guigna sequences in different geographical groups. **a** San Rafael Lake group, all sequences; **b** San Rafael Lake group, all sequences after removing the highly divergent haplotype; **c** northern group; **d** all guigna sample sequences; **e** central; **f** Lake District; **g** Argentinian; **h** Chiloé Island. *Dotted black line* observed values; *continuous red line* expected values under a model of population growth-decline

distinctiveness analysis to define Management Units for Conservation (Fig. 8) spatially separate the two subspecific groups.

Incipient divergence between both guigna subspecies may reflect physiological adaptation of ecotypes to different environmental conditions, given that the patterns of

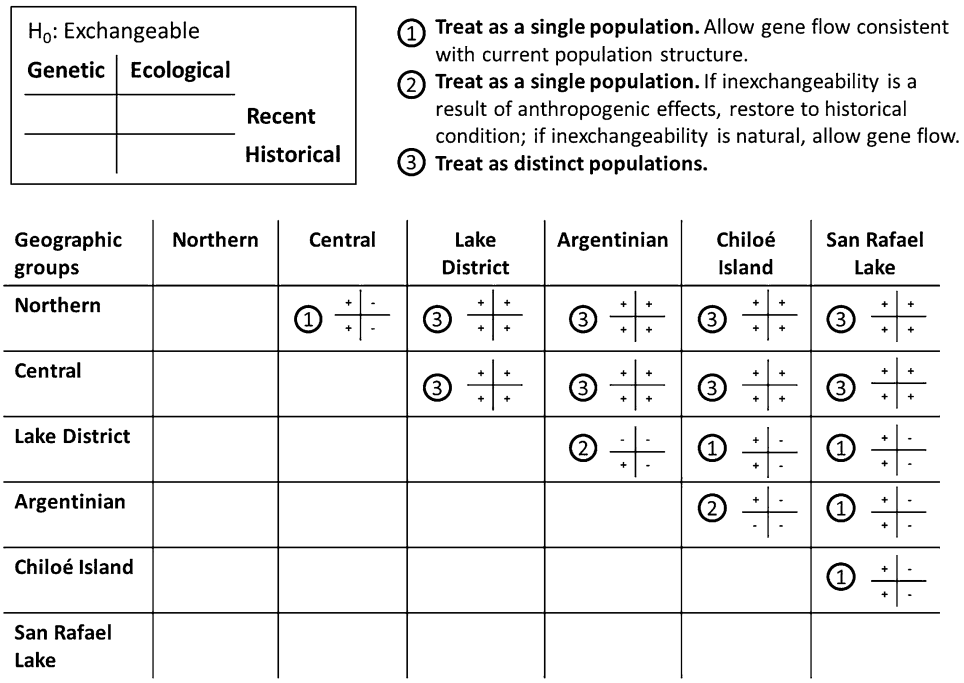

Fig. 8 Categories of population distinctiveness to define management units for conservation in guignas. Null hypotheses of genetic and ecological exchangeability are scored as rejected (+) or not rejected (−) for both recent and historical time frames, as in Crandall et al. (2000)

genetic differentiation between subspecies coincide with underlying ecosystem discontinuities. The northern biogeographical macroregion (31°–38°S), is temperate and mesomorphic, has a mediterranean climate with a distinct dry season (Kottek et al. 2006), and is mainly dominated by sclerophyll scrub and forest vegetation (Gajardo 1994). The southern biogeographical macroregion (38°–43°S), is temperate and higromorphic, with humid and cold rainy climates (Kottek et al. 2006), dominated by dense evergreen temperate rainforests and north-Patagonian forests (Gajardo 1994).

Phylogeographic and evolutionary patterns

A south to north latitudinal pattern is present in the mtDNA phylogenetic tree, with the most-basal and most-divergent haplotypes found in the southern regions (San Rafael Lake, Chiloé Island and Lake District groups), while the most derived haplotypes belong to northern regions (Northern and Central groups). The most ancestral lineage within guignas is the highly divergent haplotype from San Rafael Lake group, which would be consistent with the divergence of guignas from Geoffroy's cat haplotypes following migration from the west and subsequent isolation by the Andes. Movement across the Andes would have been facilitated at higher latitudes given that the Andes average elevation decreases from around 5,000 m to around 2,000 m southwards (Aragón et al. 2011). The most derived haplotypes from the Northern group suggest a more-recent geographic expansion, as was also inferred by mismatch distribution analysis (89000 BP). Low intraspecific nucleotide differentiation and the smallest estimated effective population size ($N_e = 90{,}000$) of all guigna geographic groups also points out to this more recent demographic history of the Northern group. This south to north latitudinal pattern may be linked to the consecutive glacial events during the late Pleistocene (Mercer 1983), which may have promoted population migration to lower latitudes in search of non-glaciated more favorable areas (Mercer 1983; Moreno et al. 1999). Habitat tracking has been exemplified by various studies (Eronen and Rook 2004; Parmesan 2006; Devictor et al. 2008; Hofreiter and Stewart 2009; Reside et al. 2013), even though a lack of postglacial habitat tracking was shown for a mid-sized Northern Hemisphere carnivore (Dalén et al. 2007). The LGM-to-present climate-change velocity exhibits marked geographic variation, with peaks in northeast North America and north-central Eurasia, while velocities tended to be lower in the Southern Hemisphere (Sandel et al. 2011). Thus, a certain pattern for a Northern Hemisphere species may not be comparable to a Southern Hemisphere species. In addition, strong dispersers like the guigna may track climate fairly closely avoiding extinction (Graham et al. 2010; Sandel et al. 2011). The individualistic response of species to climate change suggests that it is very difficult to precisely predict the responses of individual species to rapid climate change, as each species responds differently (Bennett and Provan 2008; Stewart 2008, 2009; Hofreiter and Stewart 2009; Graham et al. 2010).

A major phylogeographic break was inferred around 38°S, corresponding with the transitional area between biogeographical macroregions and subspecies geographic distributions. For guignas, the major phylogeographical break separated clades latitudinally, rather than longitudinally from east to west as would be suggested by the organization of macrohabitats or segregating units east and west of the Andes (Patterson 2010). With prevailing westerlies at temperate latitudes, the north–south orientation of the Andes creates temperate and sub-antarctic rainforests on the Pacific slopes but a rain shadow on the eastern side of Patagonia. Thus, major floristic associations in southern South America are often oriented in north–south strips, rather than the latitudinal bands observed in most other regions. The general latitudinal organization of the phylogeographical breaks in southern South America, as observed with guignas, suggests the predominance of historical signal over ecological determinism (Patterson 2010); although a mosaic of phylogeographical patterns occurs in Patagonia (Sérsic et al. 2011). Similar phylogeographic breaks have been observed in other species, including the marsupial monito del monte (*Dromiciops gliroides*) (39°S; Himes et al. 2008), several Patagonian-Fueguian rodents (39–42°S; Lessa et al. 2010), three lizard species of the genus *Liolaemus* (38°S; Victoriano et al. 2008), and several Patagonian terrestrial vertebrates (33°S, 35°S and 38°S; Sérsic et al. 2011). Similar patterns are also apparent in some plant species in the temperate rainforests of southern Chile, including the *tineo* (*Weinmannia trichosperma*) (39°–40°S; Montenegro 2011), the *ulmo* (*Eucryphia cordifolia*) (40°S; Segovia et al. 2012), the *tepa* (*Laureliopsis philippiana*) (40°S; Bosshard 2011) and the *lenga beech* (*Nothofagus pumilio*) (38°S, 40°S and 43°S; Mathiasen and Premoli 2010).

We acknowledge that additional sampling within the unsampled area (40°S–45°S in continental Chile) of guigna geographic range may contribute to complete the evolutionary patterns described here. Limited sampling can lead to the erroneous diagnosis of distinct populations when sampling intermediate populations would show ongoing gene flow (Crandall et al. 2000). Therefore, we believe the main genetic patterns showed in this study are unlikely to change significantly, given that the unsampled area belongs to the same ecoregion as other thoroughly sampled areas like Lake District and Chiloé Island, the Valdivian forest (Cofre and Marquet 1999).

The Andes range is not a barrier to gene flow

The Andes mountain range was neither a historical nor a current effective barrier to gene flow for guignas. Historically, high genetic connectivity between both sides of the Andes is supported by the mtDNA haplotype network, the mtDNA SAMOVA, correlated frequency model Geneland analysis and historical migration-rate estimates. Since elevation of the Andes decreases at higher latitudes (Aragón et al. 2011), movement across the Andes would have been facilitated in this region. Similarly, other authors have reported the existence of connectivity and dispersal of individuals between both sides of the Andes through low-elevation mountain passes in southern latitudes (Smith et al. 2001; Palma et al. 2002, 2005; Himes et al. 2008; Victoriano et al. 2008; Sérsic et al. 2011). On the other hand, recent high genetic connectivity between both sides of the Andes is supported by microsatellite SAMOVA, migration-rate estimates, and genetic assignment analysis in Structure, where individuals from the Argentinian group do not constitute an independent cluster.

The lack of private alleles or distinct genetic structure of the Argentinian group supports a scenario of high levels of connectivity between the Andes, perhaps combined with the recent establishment of Argentinian populations from Chile. Either way, during the last glacial event, populations of guignas persisted in ice-free regions in Argentina, participating later in the recolonization of southern regions (i.e. San Rafael Lake) after the retreat of the ice sheets. The sharing of the most common haplotype between the southernmost Argentina locality and San Rafael Lake supports this postglacial recolonization route. Peripheral glacial refugia have been described in the eastern foothills of the Andes between 39°S and 43°S (Sérsic et al. 2011). At those latitudes, the ice sheet was confined to the higher-elevation summits with most of the foothill forests remaining unglaciated, thus allowing persistence of different taxa through Quaternary climate shifts (Heusser et al. 1999). Similar southward postglacial colonization routes have been proposed from the east towards the southwest at different latitudes for terrestrial vertebrates such as the rodents *Loxodontomys micropus* (Sérsic et al. 2011), *Abrothrix olivaceus* (Lessa et al. 2010) and *Oligoryzomys longicaudatus* (Palma et al. 2005), and the lizards *Liolaemus pictus* and *Liolaemus lemniscatus* (Sérsic et al. 2011), all potential or described guigna prey-species (Correa and Roa 2005).

The Chacao channel as a recent barrier to gene flow

During the last glacial event in southern South America, between 0.026 and 0.007 Ma, a land bridge was formed between Chiloé Island and the mainland, allowing the movement of many fauna species and effective gene flow (Vidal et al. 2012). High historical connectivity between Chiloé Island and mainland populations is supported by the mtDNA haplotype network, mtDNA SAMOVA, correlated frequency model Geneland analysis and historical migration rate estimates. The modern insularity of Chiloé Island

was reached only about 7000 BP. The current isolation of Chiloé Island is evident from the assignment analysis in Structure, where all individuals from Chiloé Island group to a unique, independent cluster. Recent limited migration rates between Chiloé Island and all other geographic groups, along with microsatellite SAMOVA results also support this demographic isolation scenario. Overall, the Chacao channel is a recent effective barrier to gene flow for guignas, but was not a historical effective barrier to gene flow in the past.

San Rafael Lake

The San Rafael Lake group experienced a significant demographic bottleneck during the last glacial event in southern South America, when much of its current geographic range was covered by ice. This is likely reflected in the relatively low levels of genetic diversity compared with the other geographic groups, one of the smallest estimated effective population sizes ($N_e = 97{,}000$), and in both demographic inference tests (Tajima, Fu and Fu and Li) and the mismatch distribution analysis suggesting a contraction and expansion event around 9800 BP. Genetic footprints of demographic expansions following the retreat of ice sheets during the last glacial event have been described in species located along previously glaciated areas in the Patagonian Andean cordillera (41°S–52°S) (Sérsic et al. 2011), in several Patagonian rodent species (Lessa et al. 2010), and also the rodents *A. olivaceus* (Smith et al. 2001; Rodríguez-Serrano et al. 2006) and *O. longicaudatus* (Palma et al. 2005; Belmar-Lucero et al. 2009) along the southern Andes.

More generally, the observed genetic footprints of late Quaternary climate change in the genetic diversity patterns of guignas coincide with other fauna and plant population genetic patterns, supporting a common geological history in southern South America.

Conservation implications and definition of management units

Population management should aim to preserve adaptive diversity and evolutionary processes across the geographic range of a species by maintaining the natural network of genetic connections between populations (gene flow) (Taylor and Dizon 1999; Crandall et al. 2000, Palsbøll et al. 2006).

Criteria based exclusively on molecular phylogenies are not adequate for determining appropriate Management Units. As suggested by Crandall et al. (2000), incorporating both ecological data and genetic variation of adaptive significance, are more relevant for conservation. Following Crandall et al. (2000) criteria, we used a broader categorization of population distinctiveness based on concepts of ecological and genetic exchangeability (Fig. 8). This approach provides better insights into the conservation units that can best maintain evolutionary processes and the potential for evolutionary change in the future (Crandall et al. 2000).

We propose at least two Management Units for Conservation: (i) Northern group + Central group, (ii) Lake District group + Argentinian group + Chiloé Island group + San Rafael Lake group (Fig. 8). Each Management Unit warrants separate priority conservation and should be monitored and managed independently (Taylor and Dizon 1999; Crandall et al. 2000; Palsbøll et al. 2006). Within Management Units, populations should be treated as connected by various degrees of gene flow (Crandall et al. 2000). The two identified Management Units roughly coincide with the current conservation status classification groups for guignas in Chile: Vulnerable from Los Ríos Region to the north, and Near Threatened from Los Lagos Region to the south (CONAMA 2011).

The two identified Management Units correspond to the traditional morphological subspecies. Evidence for rejection of recent or historical genetic exchangeability alone is not sufficient to warrant separate priority conservation unless it is accompanied by adaptive divergence (Crandall et al. 2000) (Case 1 and 2, Fig. 8). Evidence for recent ecological nonexchangeability is indicative of the adaptive divergence necessary for distinct population persistence (Crandall et al. 2000) (Case 3, Fig. 8).

Overall, levels of genetic diversity in guignas are relatively high for mtDNA data and moderate for microsatellites, compared to other South American felids (Eizirik et al. 1998, 2001; Johnson et al. 1999; Culver et al. 2000; Sinclair et al. 2001; Uphyrkina et al. 2001; Cossios et al. 2009). However, patterns of genetic variation, biogeographic history, and conservation threats vary significantly among guigna geographic groups, suggesting different particular situations for each population within the two Management Units.

The Northern group, situated in the northernmost limit of the distribution range of guignas, is quite isolated from other groups, displaying genetic uniqueness. The rich Chilean Matorral ecosystem it inhabits, where more than half of the country's total human population inhabits, has been dramatically reduced by habitat conversion to pine plantations and agricultural lands (Nowell and Jackson 1996). Moreover, direct human pressure over native fauna is not uncommon. Guigna populations in these areas have been negatively impacted, currently subsisting in fragmented and restricted populations of variable size, considered to be severely endangered (Nowell and Jackson 1996). Connectivity between these groups inhabiting fragmented landscapes through habitat corridors is a

critical issue to maintain and secure viable long term populations, favoring metapopulation dynamics to assist demographic and genetic interchange between populations (Taylor et al. 1993; Hanski et al. 1995; Hanski and Simberloff 1997).

The Central group displays the highest genetic variability, the highest pairwise differences, the highest number of private alleles, and the highest estimated effective population size among all guigna geographic groups. Coinciding with our results, a pattern of higher vertebrate richness at mid-latitudes in Chile (33–43°S) has been proposed by Samaniego and Marquet (2009), attributed to the interaction between historical processes associated with desertification in the north and ice ages in the south. The location of the Central group just north of the last glacial icefield probably determined its persistence throughout the last glacial maxima, thus harbouring a longer demographic history. In addition, high latitude populations may have migrated to lower latitudes avoiding areas covered by ice during the last glacial period, probably resulting in the admixture of different genetic lineages in this ice-free area.

We estimated the number of guignas that could theoretically inhabit the total surface area for each studied zone (Table S4). The Central group displays a pattern of $N_e \gg N$, suggesting it may be going through a current population size reduction (Table S4). In practice, the N_e is usually less than the number of breeding adults, because they deviate from the assumptions of an idealized population (Frankham et al. 2005). When population size (N) decreases, the effective population size (N_e) also decreases but remains higher than N during a bottleneck event. This area has a high prevalence of human-related threats: habitat conversion, fragmentation and direct human persecution (Acosta-Jamett et al. 2003; Acosta-Jamett and Simonetti 2004; Silva-Rodriguez et al. 2007; Herrmann et al. 2013).

The Argentinian group shows a lack of genetic structure in relation to populations from the western side of the Andes in Chile, along with its current high connectivity with Lake District group. Inhabiting only a narrow strip of land in southwestern Argentina, these populations are likely exposed to competition from its more abundant and bigger sized sister species, *L. geoffroyi*, a frequent resident on the eastern side of the Andes.

The Chiloé Island group, situated in complete isolation from mainland populations, harbours a unique genetic identity. Although the land may be sparsely populated in Chiloé Island, native forests have been largely cleared to support domestic fowl, grazing, and farming (Sanderson et al. 2002) and genetic diversity has been negatively impacted by habitat loss and fragmentation, and also human persecution (Napolitano 2012). This may be reason why the group also displays a pattern of $Ne \gg N$, suggesting it may be going through a current population size reduction (Table S4).

The San Rafael Lake group, the southernmost limit of guignas, is geographically isolated on the Taitao Peninsula flanked to the east by extensive ice fields. This group displays unique genetic identity and demographic isolation.

Conservation challenges for the long-term viability of guigna populations are the maintenance of adequate population sizes and effective dispersal, especially in human dominated landscapes. Future research should focus on new hypothesis and molecular approaches, widening the studied area and using different molecular markers for a clearer picture. Most importantly, future directions should consider comparative phylogeographic perspectives in southern South America, as a way to bring light into more general questions in biogeography, evolution and conservation in the region.

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References

Abraham EM, Garleff K, Liebricht H et al (2000) Geomorphology and Paleoecology of the Arid Diagonal in southern South America. Zeitschrift für Angewandte Geologie SH1:55–61

Acosta-Jamett G, Simonetti JA (2004) Habitat use by *Oncifelis guigna* and *Pseudalopex culpaeus* in a fragmented forest landscape in central Chile. Biodivers Conserv 13:1135–1151

Acosta-Jamett G, Simonetti JA, Bustamante RO et al (2003) Metapopulation approach to assess survival of Oncifelis guigna in fragmented forests of central Chile: a theoretical model. J Neotrop Mammal 10:217–229

Aragón E, Goin FJ, Aguilera YE et al (2011) Palaeogeography and palaeoenvironments of northern Patagonia from the Late Cretaceous to the Miocene: the Palaeogene Andean gap and the rise of the North Patagonian High Plateau. Biol J Linn Soc 103:305–315

Armesto JJ, León-Lobos P, Arroyo MTK (1996) Los bosques templados de Chile y Argentina: una isla biogeográfica. In: Armesto JJ, Villagrán C, Arroyo MTK (eds) Ecología de los bosques nativos de Chile. Editorial Universitaria, Santiago, pp 23–28

Arroyo MTK, Cavieres L, Peñaloza A et al (1996) Relaciones fitogeográficas y patrones regionales de riqueza de especies en la flora del bosque lluvioso templado de Sudamérica. In: Armesto JJ, Villagrán C, Arroyo MTK (eds) Ecología de los bosques nativos de Chile. Editorial Universitaria, Santiago, pp 71–99

Arroyo MTK, Marquet PA, Marticorena C et al (2004) Chilean winter rainfall-Valdivian forests. In: Mittermeier RA, Gil PR, Hoffmann M et al (eds) Hotspots Revisited: Earth's Biologically Wealthiest and most Threatened Ecosystems. CEMEX, México D.F., pp 99–103

Balding DJ (2003) Likelihood-based inference for genetic correlation coefficients. Theor Popul Biol 63:221–230

Bandelt H-J, Forster P, Rohl A (1999) Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 16:37–48

Bellemain E, Taberlet P (2004) Improved noninvasive genotyping method: application to brown bears (Ursus arctos) feces. Mol Ecol Notes 4:519–522

Belmar-Lucero S, Godoy P, Ferrés M et al. (2009) Range expansion of *Oligoryzomys longicaudatus* (Rodentia, Sigmodontinae) in Patagonian Chile, and first record of Hantavirus in the region. Rev Chil Hist Nat 82:265-–275

Bennett KD (2004) Continuing the debate on the role of Quaternary environmental change for macroevolution. Philos Trans R Soc Lond B 359:295–303

Bennett KD, Provan J (2008) What do we mean by refugia? Quat Sci Rev 27:2449-–2455

Bermingham E, Moritz C (1998) Comparative phylogeography: concepts and applications. Mol Ecol 7:367–369

Bonin A, Bellemain E, Bronken Eidesen P et al (2004) How to track and assess genotyping errors in population genetics studies. Mol Ecol 13:3261–3273

Bosshard M (2011) Evidencias genéticas de la historia glacial de *Laureliopsis philippiana*. Dissertation, Universidad de Chile, Santiago, Chile. p 33

Burban C, Petit RJ, Carcreff E et al (1999) Rangewide variation of the maritime pine bast scale *Matsucoccus feytaudi* Duc (Homoptera: Matsucoccidae) in relation to the genetic structure of its host. Mol Ecol 8:1593–1602

Burbrink FT, Lawson R, Slowinski JB (2000) Mitochondrial DNA phylogeography of the polytypic North American rat snake (*Elaphe obsoleta*): a critique of the subspecies concept. Evolution 54:2107–2118

Cabrera A (1957) Catálogo de los mamíferos de América del Sur. Parte 1, Metatheria-Unguiculata-Carnivora. Revista del Museo Argentino de Ciencias Naturales 4:1–37

Clapperton CM (1993) Nature of environmental changes in South America at the Last Glacial Maximum. Palaeogeogr Palaeoclim Palaeoecol 101:189–208

Cofre H, Marquet PA (1999) Conservation status, rarity, and geographic priorities for conservation of Chilean mammals: an assessment. Biol Conserv 88:53–68

CONAMA (2011) Reglamento para la Clasificación de las Especies Silvestres. Séptimo proceso para la clasificación de especies Acuerdo N°9/2011. Ministerio Secretaría General de la Presidencia de la República de Chile

Correa P, Roa A (2005) Relaciones tróficas entre *Oncifelis guigna, Lycalopex culpaeus, Lycalopex griseus* y *Tyto alba* en un ambiente fragmentado de la zona central de Chile. J Neotrop Mammal 12:57–60

Cossios D, Lucherini M, Ruiz-García M et al (2009) Influence of ancient glacial periods on the Andean fauna: the case of the pampas cat (*Leopardus colocolo*). BMC Evol Biol 9:1–12

Crandall KA, Bininda-Emonds ORP, Mace GM et al (2000) Considering evolutionary processes in conservation biology. TREE 15:290–295

Creel S, Spong G, Sands J et al (2003) Population size estimation in Yellowstone wolves with errorprone noninvasive microsatellite genotypes. Mol Ecol 12:2003–2009

Culver M, Johnson WE, Pecon-Slattery J et al (2000) Genomic ancestry of the American puma (*Puma concolor*). J of Heredity 91:186–197

Dalén L, Nyström V, Valdiosera C et al (2007) Ancient DNA reveals lack of postglacial habitat tracking in the arctic fox. Proc Natl Acad Sci USA 104:6726–6729

Denton GH, Heusser CJ, Lowell TV et al (1999) Interhemispheric linkage of paleoclimate during the last glaciation. Geogr Ann 81:107–153

Devictor V, Julliard R, Couvet D, Jiguet F (2008) Birds are tracking climate warming, but not fast enough. Proc R Soc B 275:2743–2748

Drummond A, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol 7:214

Dunstone N, Durbin L, Wyllie I, Freer RA, Acosta JG, Mazzolli M, Rose S (2002) Spatial organization, ranging behaviour and habitat use of the kodkod (*Oncifelis guigna*) in southern Chile. J Zool 257:1–11

Dupanloup I, Schneider S, Excoffier L (2002) A simulated annealing approach to define the genetic structure of populations. Mol Ecol 11:2571–2581

Eizirik E, Bonatto SL, Johnson WE et al (1998) Phylogeographic patterns and evolution of the mitochondrial DNA control region in two Neotropical cats (Mammalia, Felidae). J Mol Evol 47:613–624

Eizirik E, Kim J-H, Menotti-Raymond M et al (2001) Phylogeography, population history and conservation genetics of jaguars (*Panthera onca*, Mammalia, Felidae). Mol Ecol 10:65–79

Eronen JT, Rook L (2004) The Mio-Pliocene European primate fossil record: dynamics and habitat tracking. J Hum Evol 47:323–341
Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Res 10:564–567
Excoffier L, Smouse P, Quattro J (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491
Filatov DA (2002) Proseq: a software for preparation and evolutionary analysis of DNA sequence data sets. Mol Ecol Notes 2:621–624
Formas JR, Brieva LM (2000) Population genetics of the Chilean frog *Batrachyla leptopus* (Leptodactylidae). Genet Mol Biol 23:43–48
Frankham R, Ballou JD, Briscoe DA (2005) Introduction to conservation genetics. Cambridge University Press, Cambridge, p 617
Frantz AC, Pope LC, Carpenter PJ et al (2003) Reliable microsatellite genotyping of the Eurasian badger (*Meles meles*) using faecal DNA. Mol Ecol 12:1649–1661
Freeman AR, Machugh DE, Mckeown S et al (2001) Sequence variation in the mitochondrial DNA control region of wild African cheetahs (*Acinonyx jubatus*). Heredity 86:355–362
Freer RA (2004) The Spatial Ecology of the Güiña (Oncifelis guigna) in Southern Chile. D.Phil. Dissertation. University of Durham, UK. p 219
Fu Y-X (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915–925
Fu Y-X, Li W-H (1993) Statistical tests of neutrality of mutations. Genetics 133:693–709
Funk DJ, Omland KE (2003) Species-level paraphyly and polyphyly: frequency, Causes, and Consequences, with Insights from Animal Mitochondrial DNA. Annu Rev Ecol Evol Syst 34:397–423
Gajardo R (1994) La vegetación natural de Chile: Clasificación y distribución geográfica. Editorial Universitaria, Chile
Gálvez N, Hernández F, Laker J et al (2013) Forest cover outside protected areas plays an important role in the conservation of the Vulnerable guiña *Leopardus guigna*. Oryx 47:251–258
Garzione CN, Hoke GD, Libarkin JC et al (2008) Rise of the Andes. Science 320:1304–1307
Graham CH, VanDerWal J, Phillips SJ et al. (2010) Dynamic refugia and species resistence: Tracking spatial shifts in habitat trough time. Ecography 33:1062-–1069
Guillot G, Mortier F, Estoup A (2005) Geneland: a computer package for landscape genetics. Mol Ecol Notes 5:712–715
Haig SM, Wagner RS, Forsman ED et al (2001) Geographic variation and genetic structure in Spotted Owls. Conserv Genet 2:25–40
Hammer Ø, Harper DAT, Ryan PD (2001) PAST: paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4:1–9
Hanski I, Simberloff D (1997) The metapopulation approach, its history, conceptual domain, and application to conservation. In: Hanski IA, Gilpin ME (eds) Metapopulation biology: ecology, genetics, and evolution. Academic Press, San Diego, pp 5–26
Hanski I, Pöyry J, Pakkala T et al (1995) Multiple equilibria in metapopulation dynamics. Nature 377:618–621
Heller R, Chikhi L, Siegismund HR (2013) The confounding effect of population structure on bayesian skyline plot inferences of demographic history. PLoS ONE 8(5):e62992
Herrmann TM, Schüttler E, Benavides P et al (2013) Values, animal symbolism, and human-animal relationships associated to two threatened felids in Mapuche and Chilean local narratives. J Ethnobiol Ethnomed 9:41–56
Heusser CJ, Heusser LE, Lowell TV (1999) Paleoecology of The Southern Chilean Lake District-Isla Grande de Chiloé during Middle-late Llanquihue Glaciation and Deglaciation. Geogr Ann Phys Geogr 81:231–284
Hewitt GM (2004) Genetic consequences of climatic oscillations in the Quaternary. Phil Trans Roy Soc Lond B 359:183–195
Himes CMT, Gallardo MH, Kenagyg J (2008) Historical biogeography and postglacial recolonization of South American temperate rain forest by the relictual marsupial *Dromiciops gliroides*. J Biogeog 35:1415–1424
Hinojosa LF, Villagrán C (1997) Historia de los bosques del sur de América, I: antecedentes paleobotánicos, geológicos y climáticos del Terciario del Cono Sur de América. Rev Chil Hist Nat 70:225–239
Ho SYW, Shapiro B (2011) Skyline-plot methods for estimating demographic history from nucleotide sequences. Mol Ecol Resour 11:423–434
Hoelzel AR, Green A (1992) Analysis of population-level variation by sequencing PCR-amplified DNA. In: Hoelzel AR (ed) Molecular genetic analysis of populations. Oxford University Press, Oxford, pp 159–187
Hofreiter M, Stewart J (2009) Ecological change, range fluctuations and population dynamics during the Pleistocene. Curr Biol 19:R584–R594
Huelsenbeck JP, Bull JJ, Cunningham CW (1996) Combining data in phylogenetic analysis. TREE 11:152–158
Ihaka R, Gentleman R (1996) R: a language for data analysis and Graphics. J Comput Graphical Statistics 5:299–314
Inskip C, Zimmermann A (2009) Human-felid conflict: a review of patterns and priorities worldwide. Oryx 43:18–34
IUCN (2013) IUCN Red List of Threatened Species. Version 2013.2. <www.iucnredlist.org>. Accessed 29 Nov 2013
Johnson WE, Culver M, Iriarte AJ et al (1998) Tracking the evolution of the elusive Andean Mountain Cat (*Oreailurus jacobita*) from mitochondrial DNA. J Hered 89:227–232
Johnson WE, Pecon-Slattery J, Eizirik E et al (1999) Disparate phylogeographic patterns of molecular genetic variation in four closely related South American small cat species. Mol Ecol 8:S79–S94
Johnson WE, Eizirik E, Murphy WJ et al (2006) The Late Miocene radiation of modern Felidae: a genetic assessment. Science 311:73–77
Klicka J, Zink RM (1997) The importance of recent ice ages in speciation: a failed paradigm. Science 277:1666–1669
Kottek M, Grieser J, Beck C et al (2006) World map of the Köppen-Geiger climate classification updated. Meteorol Z 15:259–263
Kuhner MK (2006) LAMARC 2.0: maximum likelihood and Bayesian estimation of population parameters. Bioinformatics 22:768–770
Lessa EP, D'Elia G, Pardiñas UFJ (2010) Genetic footprints of late Quaternary climate change in the diversity of Patagonian-Fueguian rodents. Mol Ecol 19:3031–3037
Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452
Lindenmayer DB, Fischer J (2006) Habitat Fragmentation and Landscape Change: An Ecological and Conservation Synthesis. Island Press, Washington
Malcolm JR, Liu C, Neilson RP et al (2005) Global warming and extinctions of endemic species from biodiversity hotspots. Conserv Biol 20:538–548
Mathiasen P, Premoli AC (2010) Out in the cold: genetic variation of *Nothofagus pumilio* (Nothofagaceae) provides evidence for latitudinally distinct evolutionary histories in austral South America. Mol Ecol 19:371–385

McCulloch RD, Bentley MJ, Purves RS et al (2000) Climatic inferences from glacial and palaeoecological evidence at the last glacial termination, southern South America. J Quat Sci 15:409–417

Menotti-Raymond M, David VA, Lyons LA et al (1999) A genetic linkage map of microsatellites in the domestic cat (*Felis catus*). Genomics 57:9–23

Mercer JH (1983) Cenozoic glaciation in the Southern Hemisphere. Annu Rev Earth Planet Sci 11:99–132

Miller MP (2005) Alleles In Space: computer software for the joint analysis of interindividual spatial and genetic information. J Hered 96:722–724

Miller MP, Haig SM, Wagner RS (2006) Phylogeography and spatial genetic structure of the Southern Torrent Salamander: implications for conservation and management. J Hered 97:561–570

Montenegro PN (2011) Filogeografía de *Weinmannia trichosperma*: Herencia genética de una historia glacial en el sur de Sudamérica. Dissertation, Universidad De Chile. pp 52

Moreno PI (2000) Climate, fire, and vegetation between about 13000 and 9200 ^{14}C yr B.P. in the Chilean Lake District. Quat Res 54:81–89

Moreno PI, Villagrán C, Marquet PA et al (1994) Quaternary paleobiogeography of northern and central Chile. Rev Chil Hist Nat 67:487–502

Moreno PI, Lowell TV, Jacobson JR Jr et al (1999) Abrupt vegetation and climate changes during the last glacial maximum and last termination in the Chilean Lake District: a case study from Canal de la Puntilla (41°S). Geogr Ann 81:285–311

Moritz C (2002) Strategies to protect biological diversity and the processes that sustain it. Systematic Biol 51:238–254

Myers N, Mittermeier RA, Mittermeier CG et al (2000) Biodiversity hotspots for conservation priorities. Nature 403:853–858

Napolitano C (2012). Filogeografía, inferencia demográfica y genética de la conservación del felino *Leopardus guigna* en el sur de Sudamérica. Dissertation, Universidad de Chile. pp 255

Navidi W, Arnheim N, Waterman MS (1992) A multiple-tube approach for accurate genotyping of very small DNA samples by using PCR: statistical considerations. Am J Hum Genet 50:347–359

Nicholson GA, Smith V, Jónsson F et al (2002) Assessing population differentiation and isolation from single nucleotide polymorphism data. J R Stat Soc B 64:695–715

Nowell K, Jackson P (1996) Status survey and conservation action plan: wild cats. IUCN/SSC Cat Specialist Group, Gland

Nylander JAA (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University

Olson DM, Dinerstein E, Wikramanayake ED et al (2001) Terrestrial ecoregions of the world: a new map of life on earth. Bioscience 51:933–938

Osgood WH (1943) The mammals of Chile. Field Mus Nat Hist Zool 30:1–268

Palma RE, Rivera-Milla E, Yates TL et al (2002) Phylogenetic and biogeographic relationships of the mouse opossum *Thylamys* (Didelphimorphia, Didelphidae) in southern South America. Mol Phylogenet Evol 25:245–253

Palma RE, Rivera-Milla E, Salazar-Bravo J et al (2005) Phylogeography of *Oligoryzomys longicaudatus* (Rodentia: sigmodontinae) in temperate South America. J Mammal 86:191–200

Palsbøll PJ, Bérubé M, Allendorf FW (2006) Identification of management units using population genetic data. TREE 22:11–16

Pardiñas UFJ, Teta P, D'Elía G et al (2011) The evolutionary history of sigmodontine rodents in Patagonia and Tierra del Fuego. Biol J Linn Soc 103:495–513

Parmesan C (2006) Ecological and evolutionary responses to recent climate change. Rev Ecol Evol Syst 2006(37):637–669

Patterson BD (2010) Climate change and faunal dynamics in the uttermost part of the earth. Mol Ecol 19:3019–3021

Peter MB, Wegmann D, Excoffier L (2010) Distinguishing between population bottleneck and population subdivision by a Bayesian model choice procedure. Mol Ecol 19:4648–4660

Phillimore AB, Owens IPF (2006) Are subspecies useful in evolutionary and conservation biology? Proc Royal Soc B 273:1049–1053

Pons O, Petit RJ (1995) Estimation, variance and optimal sampling of gene diversity. I: haploid locus. Theor Appl Genet 90:462–470

Pons O, Petit RJ (1996) Measuring and testing genetic differentiation with ordered versus unordered alleles. Genetics 144:1237–1245

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Quintana V, Yáñez J, Valdebenito M (2000) Orden Carnivora. In: Muñoz A, Yáñez J (eds) Mamíferos de Chile. CEA Ediciones, Valdivia, pp 155–187

Raymond R, Rousset M (1995) GENEPOP: (Version 4) population genetics software for exact tests and ecumenicism. J Hered 86:248–249

Reside AE, VanDerWal J, Phillips BL et al (2013) Climate change refugia for terrestrial biodiversity: defining areas that promote species persistence and ecosystem resilience in the face of global climate change. National Climate Change Adaptation Research Facility, Gold Coast, p 216

Rodríguez-Serrano E, Cancino RA, Palma RE (2006) Molecular phylogeography of *Abrothrix olivaceus* (Rodentia: Sigmodontinae) in Chile. J Mammal 87:971–-980

Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. Mol Biol Evol 9:552–569

Rousset F (2008) GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Mol Ecol Res 8:103–106

Ruiz-García M, Luengos E, Lucherini M et al (2001) Distribución alélica para los microsatélites FCA96, FCA45, FCA43, FCA08, FCA126 para los félidos neotropicales *Oncifelis geoffroyi, Oncifelis guigna* y *Lynchailurus colocolo* en Argentina, Paraguay y Bolivia: Intento de diferenciación de especies mediante excrementos y pelos. Acta Biol Colomb 6:79

Saiki RK, Scharf S, Faloona F et al (1985) Enzimatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 230:1350

Samaniego H, Marquet PA (2009) Mammal and butterfly species richness in Chile: taxonomic covariation and history. Rev Chil Hist Nat 82:135–151

Sandel B, Arge L, Dalsgaard B et al (2011) The influence of Late Quaternary climate-change velocity on species endemism. Science 334:660–664

Sanderson J, Sunquist ME, Iriarte A (2002) Natural history and landscape-use of guignas (*Oncifelis guigna*) on Isla Grande de Chiloé, Chile. J Mammal 83:608–613

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. Nat Biotech 18:233–234

Segovia RA, Pérez MF, Hinojosa LF (2012) Genetic evidence for glacial refugia of the temperate tree *Eucryphia cordifolia* (Cunoniaceae) in Southern South America. Am J Bot 99:121–129

Sérsic AN, Cosacov A, Cocucci AA et al (2011) Emerging phylogeographical patterns of plants and terrestrial vertebrates from Patagonia. Biol J Linn Soc Lond 103:475–494

Sillero-Zubiri C, Laurenson MK (2001) Interactions between carnivores and local communities: conflict or co-existence? In: Gittleman JL, Funk SM, Macdonald D, Wayne RK (eds) Carnivore Conservation. Cambridge University Press, Cambridge, pp 282–312

Silva-Rodriguez EA, Ortega-Solís GR, Jiménez JE (2007) Human attitudes toward wild felids in a human-dominated landscape of Southern Chile. Cat News 46:19–21

Simonetti JA, Acosta G (2002) Conservando biodiversidad en tierras privadas: el ejemplo de los carnívoros. Ambiente y Desarrollo 18:51–59

Sinclair EA, Swenson EL, Wolfe ML et al (2001) Gene flow estimates in Utah's cougars imply management beyond Utah. Anim Conserv 4:257–264

Smith MF, Kelt DA, Patton JL (2001) Testing models of diversification in mice in the *Abrothrix olivaceus/xanthorhinus* complex in Chile and Argentina. Mol Ecol 10:397–405

Smith DA, Ralls K, Hurt A et al (2006) Assessing reliability of microsatellite genotypes from kit fox faecal samples using genetic and GIS analyses. Mol Ecol 15:387–406

Taberlet P, Griffin S, Goossens B et al (1996) Reliable genotyping of samples with very low DNA quantities using PCR. Nucleic Acids Res 24:3189–3194

Stewart JR (2008) The progressive effect of the individualistic response of species to Quaternary climate change: an analysis of British mammalian faunas. Quat Sci Rev 27:2499-–2508

Stewart JR (2009) The evolutionary consequence of the individualistic response to climate change. J Evol Biol 22:2363-–2375

Taberlet P, Fumagalli L, Wust-Saucy A-G et al (1998) Comparative phylogeography and postglacial colonization routes in Europe. Mol Ecol 7:453–464

Taberlet P, Waits LP, Luikart G (1999) Noninvasive genetic sampling: look before you leap. Trends Ecol Evol 14:323–327

Tajima F (1989) Statistical Method for Testing the Neutral Mutation Hypothesis by DNA Polymorphism. Genetics 123:585–595

Tamura K, Peterson D, Peterson N et al (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739

Taylor BL, Dizon AE (1999) First policy then science: why a management unit based solely on genetic criteria cannot work. Mol Ecol 8:S11–S16

Taylor PD, Fahrig L, Henein K et al (1993) Connectivity is a vital element of landscape structure. Oikos 68:571–573

Trigo TC, Freitas TRO, Kunzler G et al (2008) Inter-species hybridization among Neotropical cats of the genus *Leopardus*, and evidence for an introgressive hybrid zone between *L. geoffroyi* and *L. tigrinus* in southern Brazil. Mol Ecol 17:4317–4333

Uphyrkina O, Johnson WE, Quigley H et al (2001) Phylogenetics, genome diversity and origin of modern leopard, *Panthera pardus*. Mol Ecol 10:2617–2633

Van Oosterhout C, Hutchinson WF, Wills DPM et al (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Victoriano PF, Ortíz JC, Benavides ER et al (2008) Comparative phylogeography of codistributed species of Chilean *Liolaemus* (Squamata: Tropiduridae) from the central-southern Andean range. Mol Ecol 17:2397–2416

Vidal MA, Moreno PI, Poulin E (2012) Genetic diversity and insular colonization of *Liolaemus pictus* (Squamata, Liolaeminae) in north-western Patagonia. Aust Ecol 37:67–77

Villagrán C (1988) Expansion of Magellanic Moorland during the late Pleistocene: palynological evidence from northern Isla de Chiloé, Chile. Quat Res 30:304–314

Villagrán C, Hinojosa LF (1997) Historia de los bosques del sur de Sudamérica, II: análisis fitogeográfico. Rev Chil Hist Nat 70:241–267

Villagrán C, Hinojosa LF (2005) Esquema Biogeográfico de Chile. In: Morrone JJ, Llorente Bousquets J (eds) Regionalización Biogeográfica en Iberoámeríca y tópicos afines. Ediciones de la Universidad Nacional Autónoma de México, Jiménez Editores, México, pp 551–577

Villagrán C, Armesto JJ, Leiva R (1986) Recolonización postglacial de Chiloé insular: evidencias basadas en la distribución geográfica y los modos de dispersión de la flora. Rev Chil Hist Nat 59:19–39

Waits LP, Paetkau D, Strobeck C (1998) The genetics of the bears of the world. In: Servheen C (ed) Bear Conservation Act. IUCN, Gland, pp 25–32

Wayne RK, Lehman N, Allard MW et al (1992) Mitochondrial DNA variability of the gray wolf: genetic consequences of population decline and habitat fragmentation. Conserv Biol 6:559–569

Webb SD (1991) Ecogeography and the great American interchange. Paleobiology 17:266–280

Willson K, Newton A, Echeverría C et al (2005) A vulnerability analysis of the temperate forests of south central Chile. Biol Conserv 122:9–21

Wilson GA, Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163:1177–1191

Woodroffe R, Ginsberg JR (1998) Edge effects and the extinction of wildlife inside protected areas. Science 280:2126–2128

Woodroffe R, Thirgood S, Rabinowitz A (2005) People and wildlife: conflict or coexistence?. Cambridge University Press, UK

Wozencraft WC (1993) Order Carnivora. In: Wilson DE, Reeder DM (eds) Mammal species of the world: a taxonomic and geographic reference. Smithsonian Institution Press, Washington, DC, pp 279–348

Zink RM (2004) The role of subspecies in obscuring avian biological diversity and misleading conservation policy. Proc R Soc Lond B 271:561–564

Zorondo F (2005) Conservación de carnívoros en Chile central: el factor social. Thesis, Facultad de Ciencias, Universidad de Chile, Santiago, Chile. pp 41