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New haplotypes found in specimens of *Puma concolor* (Carnivora: Felidae) from Argentina

Hallazgo de nuevos haplotipos en ejemplares de *Puma concolor* (Carnivora: Felidae) de Argentina

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Abstract

The puma is a mammal with a wide geographic distribution across the Americas, serving as an apex predator in different ecosystems. However, the available genetic and population information about this species is limited, as are many aspects of its biology and ecology. Habitat reduction and fragmentation have intensified conflicts between pumas and humans, leading to a decline in their populations in Argentina,

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primarily due to retaliatory hunting for livestock attacks. In Santa Fe province, numerous pumas are confiscated during wildlife checks or due to conflict situations and transferred to the Centro de Rescate, Investigación e Interpretación de Fauna (CRIIF) in Santa Fe city. After their recovery, some individuals are reintroduced into the wild without considering their genetic information. This study analyzed the mitochondrial haplotypes (ND5 gene) of puma specimens housed at the CRIIF and in a museum to infer their possible population of origin based on haplotypes previously described for the species in Argentina. Morphometric and hair (trichological) characteristics, previously undocumented in the country, were also recorded. Four previously unreported haplotypes and five that coincided with previous records were identified. Morphometric measurements were characterized by sex and age, and trichological data were obtained, providing relevant information for the development of management plans to conserve this species.

Keywords: Mammals, population of origin, ND5 gene, puma-human conflicts, conservation genetics.

Resumen

El puma es un mamífero con extensa distribución geográfica en el continente americano y que cumple el rol de depredador tope en numerosos ecosistemas. Sin embargo, la información genética y poblacional disponible acerca de esta especie es limitada, al igual que muchos aspectos de su biología y ecología. La reducción y fragmentación de su hábitat han intensificado conflictos del puma con humanos, generando una disminución de sus poblaciones en Argentina, principalmente debido a la caza de ejemplares, en represalia por ataques al ganado. En la provincia de Santa Fe, numerosos ejemplares son confiscados en controles de fauna o por situaciones de conflicto con humanos, y derivados al Centro de Rescate, Investigación e Interpretación de Fauna (CRIIF) La Esmeralda de la ciudad de Santa Fe. Tras su recuperación, algunos individuos son reintroducidos a la naturaleza sin considerar su información genética. Este estudio analizó los haplotipos mitocondriales (gen ND5) de ejemplares alojados en el CRIIF y en un museo, con el fin de inferir su posible población de origen a partir de haplotipos previamente descritos para la especie en Argentina. Asimismo, se registraron características morfométricas y de sus pelos (tricológicas), hasta ahora no documentadas en el país. Se identificaron cuatro haplotipos no reportados previamente y cinco coincidentes con registros anteriores, además de caracterizar medidas morfométricas por sexo y por edad, y de obtener datos tricológicos, aportando información relevante para la gestión de planes de manejo orientados a la conservación de esta especie.

Palabras clave: Mamíferos, población de origen, gen ND5, conflictos puma-humanos, genética de la conservación.

INTRODUCTION

The puma or cougar (*Puma concolor* Linnaeus, 1771) is the mammal with the greatest geographical range in America, inhabiting Canada to southern Argentina and Chile. This species is categorized as "Least concern" by the IUCN (International Union for Conservation of Nature), and it is included in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), which limits its international traffic and marketing. In Argentina is categorized as "Least concern" by the Argentine Society for the Study of Mammals (SAREM) (De Angelo et al., 2019), and it is protected by the National Law 22421.

The puma plays a major ecological role as top predator, controlling prey species, maintaining trophic cascades, and promoting ecosystem balance (Ripple et al., 2014). It has a great capacity to adapt to anthropogenic changes; however, the advance of urbanization, livestock and agricultural activities, and deforestation cause the loss and degradation of its habitat. These reasons, combined with the hunting of the puma and its prey, lead to a decrease in the species' abundance. (Llanos, 2017; Paviolo et al., 2018). The degree to which different threats affect this species varies by region. In general, it is considered by rural inhabitants to be harmful to livestock (Quiroga et al. 2016), and for this cause, preventive hunting is a common practice, although it is illegal in many provinces. This includes direct hunting, trapping, and poisoning (Guerisoli et al. 2017; Llanos, Andrade, Travaini, 2020).

There is little general data for the species in Argentina regarding its conservation status, and it is not included in national conservation plans. Currently, there is limited information on the population genetics of the puma, based on various molecular markers, including the mitochondrial gene ND5. Some of these studies have been conducted in Argentina, primarily examining specimens from the south of the country (Tintorelli, 2017; Mac Allister, 2019; Gallo et al., 2021; Mac Allister et al., 2024), and few haplotypes of the species have been described in Argentina. Understanding the genetic structure of the species is fundamental for developing an optimal reintroduction strategy, which includes knowing the population of origin of each individual.

Morphometric studies in this species are also very scarce, with some published data about body measures and weight from adults determined by Pereira and Aprile (2012) and weight from specimens studied in Misiones and in the Parana Forest in Argentina (Gonçalves et al., 2018). Regarding its fur, the literature describes it as having a uniform coloration of gray, brown, tawny, or reddish, and that this varies significantly depending on the environment the individuals occupy (Pereira and Aprile, 2012). However, there are no specific trichological studies, that is, on the color pattern or hair structure in this species.

In Santa Fe province, the possession, sale, and hunting are prohibited because it is a native species protected by Provincial Wildlife Law No. 4830. However, it is common to find pumas confiscated at roadside checkpoints or due to conflicts with humans, and they are housed at the La Esmeralda Wildlife Rescue, Research and Interpretation Center (CRIIF) in the city of Santa Fe. After their recovery, some of these animals are reintroduced into their natural range.

This work aimed to determine the mitochondrial haplotypes of captive puma specimens in the CRIIF La Esmeralda and from a collection of the Museo de Ciencias Naturales (MCN) Florentino Ameghino in Santa Fe city. In addition, morphometric measurements and trichological characteristics of the specimens were recorded to provide data for the biological characterization of the species in Argentina.

MATERIALS AND METHODS

We analyzed nine pumas found in different locations in the province of Santa Fe (Table 1). Seven were kept in captivity at the CRIIF La Esmeralda, and two specimens were preserved at the MCN “Florentino Ameghino”, both institutions located in the city of Santa Fe, Argentina. We sampled the animals at the CRIIF within the framework of the periodic checks carried out by veterinary staff on the animals, with the corresponding permits for obtaining samples; while the taking of samples from the museum was authorized by request to the curator of the collection.

Obtaining samples

Specimens kept in the CRIFF: We used a combination of xylazine HCl 2% and ketamine base 5% to sedate the animals. Then, we extracted 100 μl of blood and guard hairs from the dorsal scapular region of each specimen. Besides, we weighed and took their morphometric measurements using a caliper, a tape measure, and a string.

Specimens preserved in the MCN: We obtained bone samples from the zygomatic arch of each skull using a cordless drill. We extracted leather and muscle tissue samples using a scalpel and stored them at - 20 °C.

Table 1. General characteristics of the sampled individuals, location of discovery, and site where the samples were obtained.

Tabla 1. Características generales de los individuos muestreados, lugar de hallazgo y sitio donde se obtuvieron las muestras.

N°	Gender	Age (years)	Sample	Place where it was found	Geographic coordinates	Sample collection site
1	male	0.5	Blood, hairs	Gregoria Perez de Denis, 9 de Julio Department, Santa Fe province.	28°14′00″ S 61°32′00″ W	CRIIF
2	female	0.5	Blood, hairs	Gregoria Pérez de Denis, 9 de Julio Department, Santa Fe.	28°14′00″ S 61°32′00″ W	CRIIF
3	female	7	Blood, hairs	Cañada de Gómez, Iriondo Department, Santa Fe province.	32°49′00″ S 61°24′00″ W	CRIIF
4	female	6	Blood, hairs	Cañada de Gómez, Iriondo Department, Santa Fe province.	32°49′00″ S 61°24′00″ W	CRIIF
5	male	3	Muscle, hairs	San Genaro, San Jerónimo Department, Santa Fe province.	32°22′00″ S 61°19′00″ W	CRIIF
6	No data	No data	Leather, bone	Monte Negro, Vera Department, Santa Fe province.	28°35′30″ S 60°14′ 8″ W	MCN
7	No data	No data	Muscle, hairs	Sauce Viejo, La Capital Department, Santa Fe province.	31°46′00″ S 60°51′00″ W	MCN
8	female	3	Blood, hairs	San Cristóbal, San Cristóbal Department, Santa Fe.	29°57′53″ S 61°31′04″ W	CRIIF
9	female	3	Blood, hairs	San Javier, San Javier Department, Santa Fe province.	30°35′00″ S 59°56′00″ W	CRIIF

Molecular studies

DNA isolation.— Eighteen samples were obtained from different tissues to perform DNA analysis (nine from bone, six from blood, two from muscle, and one from leather and bone). We processed blood samples following Amavet, Vilardi, Rosso, Saidman (2009) protocols, and leather and muscle samples using Ojeda, Amavet, Rueda, Siroski (2012) methods. We used the DNA PuriPrep-T kit (INBIO HIGHWAY S.A.®) in the processing of bone and some leather samples.

PCR protocols.— To amplify a fragment of 669 bp of the ND5 gene, we used the primers designed by Trigo et al. (2008) (ND5-DF1: TTGGTGCAACTCCAAATAAAAGT and ND5-DR1: AGGAGTTGGGCCTTCTATGG), as well as protocols proposed by Culver, Johnson, Pecon-Slattery, O'Brien (2000) with some modifications. We prepared a master mix of 25 μL of final volume constituted by 2,5 μL 10X Buffer; 1 μL MgCl2 (50 mM); 2,5 μL dNTP mix (2 mM); 3 μL (10 pMol) of each primer; 0,15 μL Taq polymerase (Genbiotech®) and 50 ng DNA. We used an initial denaturation of 5 min to 94 °C, followed by 30 cycles of 1 min to 94 °C, 1 min to 49 °C, and 1:30 min to 72 °C, and a final extension of 10 min to 72 °C.

To verify if PCR products were of the appropriate molecular size, we used agarose gel electrophoresis, and then, we sequenced them on an ABI 3730 sequencer (Applied Biosystems®, Macrogen Inc., Korea). We confirmed that the gene studied was the ND5 gene by comparison with the available information in GenBank using BLAST software ((https://blast.ncbi.nlm.nih.gov/Blast.cgi). We performed the sequence alignments using the web version of Mafft (Katoh, Rozewicki, Yamada, 2019) and edited the sequences using Mega 7.0 (Kumar, Stecher, Li, Knyaz, Tamura, 2018). We analyzed the genetic diversity, the number of haplotypes (n), haplotype diversity (h), number of segregating sites between sequences (S), and nucleotide diversity (π) using the DnaSP 6 program (Librado and Rozas 2009). We estimated these parameters for the sequences obtained in this study and, on the other hand, compared with sequences downloaded from genetic databases belonging to the Matte et al. (2013), Gallo et al. (2021), and Mac Allister et al. (2024) studies (Table 2). For the latter analysis, we used single haplotypes. Finally, we performed haplotype networks using NETWORK v. 4.6.0.0 2 (Bandelt and Forster,1999).

Morphometric data analysis

We obtained morphometric measurements (Table 3) according to Palacios (2007), López Romero (2008), and Ferreira, Allemann, Dias, dos Santos Honshoet (2014) methods. To standardize the measurements, we chose the right side of the body for those measurements corresponding to paired sections, such as the limbs. We estimated the age of each individual from their dental wear (Beck et al., 2007). The sample size for the morphological analysis was seven because the two museum specimens were not found complete. We conducted all data analyses with the statistical environment R (R Core Team, 2023). We estimated the arithmetic mean and variance of each variable, studying adults and juvenile specimens separately.

Hair analysis

We analyzed hairs from the seven individuals sampled at the CRIIF, as well as one specimen sampled at the MCN. In this analysis, we did not separate juveniles from adults since the former already possessed a smooth and uniform coat. We randomly selected ten hairs from each individual (n= 80), and we performed a macroscopic description of the hairs and the color bands along the longitudinal axis of the hair, from the root to the tip, as described by Palma Pecho (2019).

Subsequently, we prepared the hairs for microscopic analysis of the medulla and scales. We performed the medulla analysis using the technique described by Pech-Canché, Sosa–Escalante, Koyoc Cruz (2009) and Baca Ibarra, Sánchez-Cordero, Stoner (2010), while for the scale analysis we employed methods suggested by Dávila Flores and Matos Suclupe (2018).

Table 2. Source and place of origin of the haplotypes obtained by other authors and downloaded from GenBank.

Tabla 2. Fuente y lugar de origen de los haplotipos obtenidos por otros autores y descargados de GenBank.

Accession Number	Designation by the authors	Found in	Source
KF460496.1	Hap 01	Central-North-Eastern South America (CNESA)	Matte et al. (2013)
KF460497.1	Hap 02	Central-North-Eastern South America (CNESA)	Matte et al. (2013)
KF460498.1	Hap 03	Central-North-Eastern South America (CNESA), and Eastern South America (ESA)	Matte et al. (2013)
KF460499.1	Hap 04	CNESA	Matte et al. (2013)
KF460500.1	Hap 05	Central-Southern South America (CSSA)	Matte et al. (2013)
KF460501.1	Hap 06	CNESA and CSSA	Matte et al. (2013)
KF460502.1	Hap 07	CNESA, CSSA and Southwestern South America (SWSA)	Matte et al. (2013)
KF460503.1	Hap 08	CSSA	Matte et al. (2013)
KF460504.1	Hap 09	Southern Central America (SCA), CNESA, and CSSA	Matte et al. (2013)
KF460505.1	Hap 10	CNESA	Matte et al. (2013)
KF460506.1	Hap 11	CNESA	Matte et al. (2013)
KF460507.1	Hap 12	CSSA	Matte et al. (2013)
KF460508.1	Hap 13	CNESA	Matte et al. (2013)
KF460509.1	Hap 14	CNESA	Matte et al. (2013)
KF460510.1	Hap 15	CNESA	Matte et al. (2013)
KF460511.1	Hap 16	Northern South America (NSA) and CNESA	Matte et al. (2013)
KF460512.1	Hap 17	CNESA	Matte et al. (2013)
KF460513.1	Hap 18	SWSA	Matte et al. (2013)
KF460514.1	Hap 19	SWSA	Matte et al. (2013)
KF460515.1	Hap 20	NSA	Matte et al. (2013)
KF460516.1	Hap 21	CNESA	Matte et al. (2013)
KF460517.1	Hap 22	CNESA	Matte et al. (2013)
MZ241238.1	ND1	Rio Negro and Neuquén provinces, Argentina	Gallo et al. (2021)
MZ268112.1	ND2	Río Negro province, Argentina	Gallo et al. (2021)
PP952688.1	ND1	Neuquén, Santa Cruz, Río Negro, Chubut, Santa Fe, Buenos Aires, La Pampa, San Luis and Mendoza provinces, Argentina	Mac Allister et al. (2024)
PP952689.1	ND2	Río Negro, Santa Fe, Buenos Aires, La Pampa, San Luis, and Córdoba provinces, Argentina	Mac Allister et al. (2024)
PP952691.1	ND3	Santa Fe province, Argentina	Mac Allister et al. (2024)
PP952692.1	ND4	Santa Fe, Buenos Aires, La Pampa, San Luis provinces, Argentina	Mac Allister et al. (2024)
PP952693.1	ND5	Río Negro province, Argentina	Mac Allister et al. (2024)
PP952694.1	ND6	La Pampa province, Argentina	Mac Allister et al. (2024)
PP952695.1	ND7	Córdoba province, Argentina	Mac Allister et al. (2024)
PP952696.1	ND8	Córdoba province, Argentina	Mac Allister et al. (2024)
PP952697.1	ND9	Córdoba province, Argentina	Mac Allister et al. (2024)
PP952698.1	ND10	Córdoba province, Argentina	Mac Allister et al. (2024)

Table 3. Data obtained for each of the morphological quantitative variables analyzed in adults and juveniles (Min-Max: minimum and maximum values; A: Average; AM: Arithmetic mean; V: variance).

Tabla 3. Datos obtenidos para cada una de las variables cuantitativas morfológicas analizadas en adultos y juveniles (Mín-Máx: Valores mínimos y máximos; A: Media; AM: Media aritmética; V: Varianza).

Variable	Adult Females (N=4)			Adult male	Juveniles (N=2)	
	Min-Max	AM	V		A	Min-Max
Age (years)	3 – 7	4.75	4.25	3	0.5	0.5
Weight (kg)	25.5 – 31	27.875	5.895	45	15	15
Head length (cm)	24 – 34	28	18.166	40	34.25	29 – 39.5
Total length (cm)	173 – 184	176.75	24.916	202	143.25	136.5 – 150
Body length (cm)	82 – 89	85.375	9.229	101	56	56
Tail length (cm)	58 – 70	63.375	24.562	61	53	51.5 – 54.5
Height at the shoulder (cm)	48 – 55	52.75	10.25	64.5	49.25	48.5 – 50
Height at the hip (cm)	54 – 60	57.25	6.25	62.5	50	49 – 51
Chest diameter (cm)	59 – 63	60.625	2.895	74.5	48.5	48.5
Neck diameter (cm)	36 – 39	37.25	1.583	48.3	29.5	28.5 – 30.5
Wishkers length (cm)	9.12 – 11	10.23	0.654	8	8.55	7.1 – 10
Foreleg length (upper region) (cm)	21 – 25	23.5	3	35	26	21 – 31
Foreleg length (lower region) (cm)	24.5 – 38	33.375	36.562	26	23.75	19 – 28.5
Hind leg length (upper region) (cm)	25 – 32	29.75	10.916	33	25.75	22.5 – 29
Hind leg length (lower region) (cm)	22 – 24.5	23.125	1.0625	24	24	21.5 – 26.5
Distance between upper canines (cm)	4.83 – 5	4.897	0.005	5.6	4.05	4 – 4.1
Distance between lower canines (cm)	3 – 3.6	3.265	0.072	4	3	2.73 – 3.27

For microscopic analysis, we randomly selected three hairs from each individual (n=24), and each hair was divided longitudinally into four sections: base, proximal section, shield, and tip. We took measurements of the total hair width and medullary width for the proximal section and shield, because the medulla is absent at both the base and tip of the hair in *Puma concolor*. We also measured the width and length of the scales in the proximal section and the middle part of the shield. We took all measurements using an optical microscope Nikon E200 at 400x magnification, and analyzed the data obtained using R (R Core team, 2020) software.

In medulla measures (Table 4), we considered the medullary index defined as the ratio of the medulla diameter to the total hair diameter. Within the scale measurements (Table 5), we obtained the scale index by randomly selecting 10 scales from the proximal section and shield of every 24 hairs (n=240 measurements).

In the statistical descriptive analysis of the obtained measures, we estimated the arithmetic mean and variance of each variable using R (R Core Team 2020).

Table 4. Data obtained for the studied variables in the medulla hair: Min-Max: minimum and maximum values; AM: arithmetic mean, V: variance. Three hairs from each individual (n=24) were measured.

Tabla 4. Datos obtenidos para las variables estudiadas en la médula de los pelos: Mín-Máx: valores mínimo y máximo; AM: media aritmética; V: varianza. Se midieron tres pelos de cada individuo (n=24).

Variable	Min-Max (mm)	AM	V
Medulla width in the proximal section (mm)	9.6×10^{-3} – 6.72×10^{-2}	3.49×10^{-2}	1.97×10^{-4}
Medulla width in the shield (mm)	4.32×10^{-2} – 0.123	6.87×10^{-2}	3.55×10^{-4}
Total hair width at the base (mm)	1.92×10^{-2} – 8.88×10^{-2}	4.99×10^{-2}	1.89×10^{-4}
Total hair width in the proximal section (mm)	2.88×10^{-2} – 9.12×10^{-2}	5.74×10^{-2}	2.72×10^{-4}
Total hair width at the shield (mm)	6.72×10^{-2} – 0.144	0.101	5.34×10^{-4}
Total hair width at the tip (mm)	2.4×10^{-3} – 2.64×10^{-2}	1.27×10^{-2}	3.71×10^{-2}
Medullary index of the proximal section (mm)	0.330 – 0.820	0.592	1.38×10^{-2}
Medullary index of the shield (mm)	0.500 – 0.920	0.681	1.32×10^{-2}
Total hair length (mm)	13.500 – 44.000	21.375	72.390

Table 5. Data obtained for the studied variables in the hair scales: Min-Max: minimum and maximum values; AM: arithmetic mean, V: variance. Three hairs from each individual (n=24) were measured, and the scale index was calculated by randomly selecting 10 scales from the proximal section and shield of every 24 hairs (n=240 measurements).

Tabla 5. Datos obtenidos para las variables estudiadas en las escamas de los pelos: Mín-Máx: valores mínimo y máximo; AM: media aritmética, V: varianza. Se midieron tres pelos de cada individuo (n=24) y se calculó el índice de escamas seleccionando aleatoriamente 10 escamas de la sección proximal y el escudo de cada 24 pelos (n=240 mediciones).

Variable	Min-Max (mm)	AM	V
Scale width from the proximal section (mm)	0.016 – 0.075	0.041	1.23×10^{-4}
Scale length from the proximal section (mm)	0.007 – 0.032	0.014	8.07×10^{-3}
Scale index from the proximal section (mm)	0.292 – 7.009	2.788	1.740
Scale width from the shield (mm)	0.036 – 0.119	0.079	3.34×10^{-4}
Scale length from the shield (mm)	0.006 – 0.029	0.014	1.16×10^{-2}
Scale index from the shield (mm)	2.614 – 11.052	6.038	3.350

RESULTS

Genetic analysis

High-quality DNA was extracted from 12 samples, and nine of these DNA samples (one for each sampled individual) were amplified successfully to analyze the ND5 gene. These sequences showed a genetic similarity greater than 99% with *P. concolor* sequences in BLAST analysis. Diversity index were: $h = 0.944$, number of haplotypes = 7, $S = 9$, and $\pi = 0.005$.

By including sequences corresponding to Matte et al. (2013), Gallo et al. (2021), and Mac Allister et al. (2024) in a joint analysis (Figure 2), we discovered that four haplotypes found in our study (H20, H30, H31, and H32) were not registered previously in genetic databases.

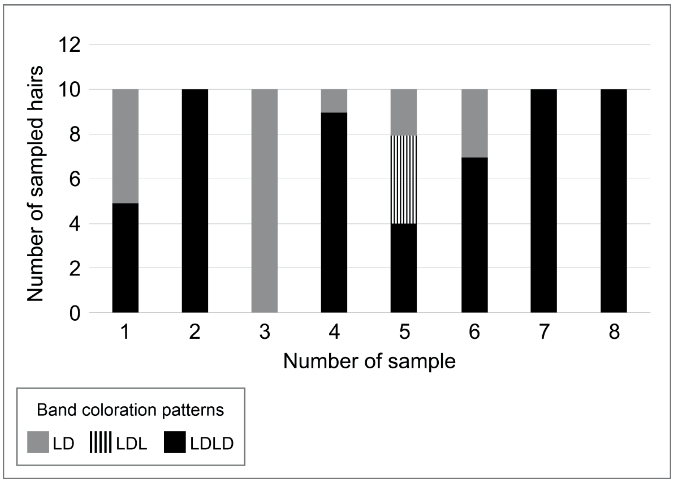

Figure 1. Bands coloration patterns found in each sampled individual, as well as the number of hairs corresponding to each pattern. LD: Light-Dark bands, LDL: Light-Dark-Light bands, LDLD: Light-Dark-Light-Dark bands.

Figura 1. Patrones de coloración de bandas en cada individuo analizado, así como el número de pelos correspondientes a cada patrón. LD: bandas clara-oscura, LDL: bandas clara-oscura-clara, LDLD: bandas clara-oscura-clara-oscura.

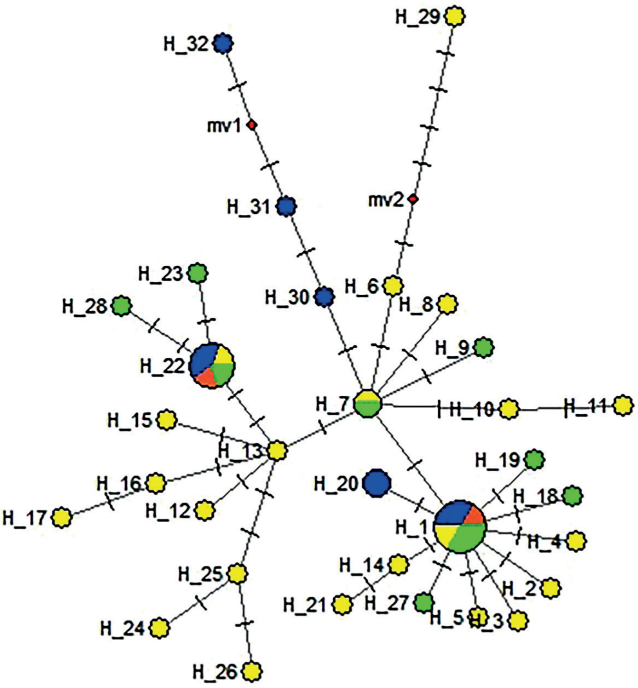

Figure 2. Haplotype network including the 9 sequences obtained in the present study (blue), plus 22 sequences (yellow) corresponding to Matte et al. (2013), 2 sequences (orange) from Gallo et al. (2021), and 10 sequences (green) from Mac Alister et al. (2024) data. mv: median vectors. The circle size is in relation to the number of sequences that share each haplotype.

Figura 2. Red de haplotipos que incluye las 9 secuencias obtenidas en el presente estudio (azul), además de las 22 secuencias (amarillo) correspondientes a datos de Matte et al. (2013), 2 secuencias (naranja) del trabajo de Gallo et al. (2021) y 10 secuencias (verde) correspondientes a datos de Mac Allister et al. (2024). mv: median vectors. El tamaño del círculo tiene relación con el número de secuencias que comparten cada haplotipo.

The two haplotypes that include the most sequences in the haplotype network are H1 and H22. H1 includes 6 sequences: two sequences obtained in this study and four obtained by other authors: the Hap 07 described by Matte et al. (2013) for much of South America -Central-North-Easter South America, Central-South South America, and Southwestern South America-, the Hap ND2 found by Gallo et al. (2021) in the east of Rio Negro Province, and finally, two haplotypes described by Mac Allister et al. (2024): ND9 found in Cordoba province, and ND2 found in several provinces from the central region of Argentina including Santa Fe. The other most represented haplotype (H22) included 5 sequences: two sequences obtained in this study, together with Hap 18 described by Southwestern South America (Matte et al., 2013), ND1 found by Gallo et al. (2021) in the Argentine Patagonia, and the haplotype ND1 described by Mac Allister et al. (2024) in several provinces from Argentina, including Santa Fe.

Morphological analysis

The only adult male we studied, 3 years old, showed higher values compared to the females in almost all body measurements (Table 3). The exceptions were “tail length”, “whiskers length”, “Foreleg length (lower region)”, and “Hind leg length (lower region)”. The traits that showed more variance among adult females were “Foreleg length (lower region)”, “total length”, and “tail length”. The traits with the least variance were “distance between upper canines,” “distance between lower canines,” and “whiskers length”. It is noticeable in the measurements taken of the two 6-month-old juveniles that, on average, their head length and Foreleg length (upper region) values are higher than those of the adult females. One of the two sampled juveniles was male (Table 1) and evidently already possessed, in these variables, a size similar to that of the adult male.

Trichological analysis

The variation in band coloration was observed in the hairs within each individual and between individuals (Figure 1). Three band coloration patterns were registered: LD (Light-Dark bands), LDL: (Light-Dark-Light bands), and LDLD: Light-Dark-Light-Dark bands)

The results of the hair analysis at the level of medulla showed that the character with higher variance was the Total hair length (Table 4), and in the hair scales were the two scale indexes (Table 5).

DISCUSSION

In this study, the haplotype diversity (h) was 0,944 and the number of haplotypes was 7, a high value compared to that obtained by other authors studying the same mitochondrial gene in pumas. In Argentina, Tintorelli (2017) analyzed 18 individuals from Neuquén and found only one haplotype, and Gallo et al. (2021) analyzed 13 individuals from Neuquén and Río Negro and found an h value of 0,462, a nucleotide diversity (π) value of 0,003, and only two haplotypes. Besides, Mac Allister et al. (2024) studied 24 individuals from the Center of Argentina founding 8 haplotypes.

Of the 22 haplotypes found by Matte et al. (2013) in South America, two of them were shared by sequences obtained in this study (H1 and H22; Figure 2). Haplotype H1 was shared by 6 sequences: two from this study, one described by Matte et al. (2013) in Brazil, Paraguay, and northern Argentina; one found by Gallo et al. (2021) in Río Negro province, as well as two sequences found by Mac Allister et al (2024): one from Cordoba province, and one found in the central region of Argentina (Río Negro, Santa Fe, Buenos Aires, La Pampa, San Luis, and Córdoba provinces). On the other hand, Haplotype H22 was shared by 5 sequences: two from this study, as well as one found by Matte et al. (2013) in Chile and southern Argentina, one found by Gallo et al. (2021) from Río Negro and Neuquén provinces, and one sequence obtained by Mac Allister et al. (2024) in a wide region from Argentina (Neuquén, Santa Cruz, Río Negro, Chubut, Santa Fe, Buenos Aires, La Pampa, San Luis, and Mendoza provinces).

Besides, four new haplotypes were found in this study: H20 (shared by two individuals), H30, H31, and H32, which are not registered in the databases. H20 derives from H1 (described above), which contains sequences from different origin places. The other three haplotypes described here for the first time derive from H7, which includes Hap 09 described by Matte et al. (2013) in Southern Central America, Central North East South America, and Central South South America, and ND4 found by Mac Allister et al. (2024) from Santa Fe, Buenos Aires, La Pampa, and San Luis provinces.

The genetic data obtained are very important because they provide data on pumas from the province of Santa Fe, where only 5 samples had been obtained up to that point by Mac Allister et al. (2024). On the other hand, we believe that new sampling and the use of nuclear markers are necessary to complete the genetic data of pumas in the central area of the country. Supporting this, the appearance of medium-vectors (mv) in the haplotype network indicates hypothetical (unsampled or extinct) sequences needed to connect existing haplotypes with maximum parsimony (Bandelt and Forster,1999). Pumas appear to have a significant genetic structure between Patagonia and the rest of the country (Mac Allister et al. 2024), but in this latter area, there are many shared haplotypes in individuals from different provinces, so this structure does not appear to be so marked.

Based on the results, the origin of the studied pumas can only be inferred with a certain degree of probability. The two sampled pumas (1 and 8) that carry the H1 haplotype probably belong to a population in central Argentina, since they share this haplotype with pumas primarily sampled in that region. Similarly, the sampled pumas 2 and 7 (which share H20, which derives from H1), and pumas 5, 6, and 9, which have new haplotypes (which derive from H7), could come from the same region. On the other hand, pumas 3 and 4, which possess the H22 haplotype, share it with animals from a wide region (mainly located further south of central Argentina), making it more difficult to establish their original population. These animals were found in Cañada de Gómez, in the south of Santa Fe province, and based on this data, their region of origin could be that area which includes Santa Fe.

For this reason, it could be assumed that the release of animals recovered at the CRIIF after being hit, injured, or seized could take place in the central area of Argentina or a little further south, since there does not seem to be a defined genetic structure in the populations of those areas.

The morphological measures obtained in this study (Table 3) are similar to the data described by other authors, since the body length maximum obtained in this study was 101 cm, while Pereira and Aprile (2012) described a range between 85 and 150 cm. In the tail length, our maximum measure was 70 cm, and the mentioned authors described a range between 45 and 97 cm. Similarly, the weight in the adult females in this study showed a range of 25.5-31 kg, while other authors described a range of 34-48 kg (Pereira and Aprile, 2012) and 31-33 kg (Gonçalves et al., 2018). The only adult male analyzed in this study weighed 45 kg, while Pereira and Aprile (2012) described a range between 53-72 kg, and Gonçalves et al. (2018) established a range between 40,5 and 57 kg. Besides, Palacios (2007) observed in the prey of pumas from Argentina that the distances between the puncture marks coming from their upper and lower canines were similar to those found in this study. The rest of the obtained measures do not have antecedents in *Puma concolor*; for this reason, it is important to carry out further studies, mainly because the place of origin, as well as the possibility of having been bred in captivity, of our studied specimens, is unknown.

Three bands' coloration patterns were found in this study: LD, LDL, and LDLD, a very similar result to those obtained by Palma Pecho (2019), who found four bands' coloration patterns, three of which were observed in this study. Besides, hair length obtained here showed similar values to those obtained by Chehébar and Martín (1989). It is important to note that different banding patterns were found in the hairs of four of the individuals analyzed (1,4,5 and 6- Fig.1). Therefore, it can be concluded that the banding pattern in the hairs of the studied pumas does not represent a characteristic that allows the classification of the specimens, but rather is a clearly individual characteristic. On the other hand, the measures obtained from the hairs of the studied individuals showed variations in all analyzed variables.

This work provides important data for characterizing pumas in Argentina, a species not included in national conservation plans due to the complexity of its management resulting from conflict with humans. Further genetic analysis are needed to study the genetic structure of the puma in central Argentina more deeply, as well as to characterize the populations in their biological aspects and to optimize decisions regarding the reintroduction of confiscated individuals.

Coexistence with wild species must be rethought in light of new environmental conditions, which requires comprehensive information on their life history.

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CONTRIBUTION STATEMENT

Ailén Dumont: writing (review and editing) original draft, methodology, investigation, formal analysis, data curation, conceptualization. **Pablo Siroski:** writing and correction original draft, supervision, conceptualization. **Antonio Sciabarrasi**: writing and correction original draft, methodology, conceptualization. **Gisela Poletta**: writing and correction original draft, methodology, conceptualization. **Patricia Amavet:** writing (review and editing) original draft, supervision, methodology, investigation, formal analysis, data curation, conceptualization.

DECLARATION OF COMPETING INTEREST

No potential conflict of interest was reported by the authors.

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