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Genetic Characterization of Jaguars (*Panthera onca*) in Captivity in Zoological Parks of Colombia

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Abstract

The construction of the pedigree of captive jaguars (*Panthera onca*) in zoological parks of Colombia was done using the analysis of the Regional Studbook for Jaguars and DNA analysis of 9 microsatellites of 20 Jaguars (n=20). The assignments for paternities and maternities were done with for the program CERVUS and the relationship between animals were established with the KINSHIP program. The analysis of the Studbook was done with SPARKS and PM2000 software generating the following values: genetic diversity for the population (GD=0.7832), potential genetic diversity (GD=0.9113), genic value (GV=0.7846), mean coefficient of inbreeding (F=0.0179), and the Mean KINSHIP (MK) for each individual. The averages of the observed and expected heterozygosity were 0.687 and 0.684 respectively. Nevertheless, a wild jaguar sample of 156 individuals obtained in Colombia substantially showed a higher degree of gene diversity (H = 0.87) than the Colombian captive jaguar population. Thus, the captive jaguar population retained 78 % of the gene diversity of the Colombian wild jaguar population. With this study the pedigree of the captive population of jaguars was built in order to develop an ex situ conservation plan for the species in the Colombian zoological parks.

Keywords: jaguar, *Panthera onca*, genetics, microsatellites, DNA, Studbook

1. Introduction

During the last decades, genetic analyses have been conducted using microsatellites, on different species of wild felids, in order to solve questions regarding the origin and evolution of members of the *Felidae* family. DNA microsatellites are composed of tandem repetitive units of two to six base pairs in length [1]. Microsatellites are randomly distributed, highly polymorphic, and frequently found inside eukaryotic genomes. One of the first reports about the use of microsatellites was done in order to determine the time when the first great depletion of cheetahs (*Acinonyx jubatus*) was proven, as evidence was found, of a "bottle neck" effect in the population, approximately 10,000 years ago [2]. Subsequently, they were employed by the same group of investigators, to determine the level of genetic diversity in four species of felids: domestic cats (*Felis catus*), cheetahs, pumas (*Puma concolor*), and African lions (*Panthera leo*) [3]. In 2001 a study was conducted on pumas that enabled to confirm the presence of homoplasy in 10 microsatellites in pumas [4]. Microsatellites have also been employed to detect differences among subspecies of clouded leopards (*Neofelis nebulosa nebulosa*) found in Thailand and the clouded leopard (*N. nebulosa diardi*) found in the Island of Borneo [5]. In this way, microsatellites have also become a fundamental tool to differentiate subspecies of captive tigers (*Panthera tigris*), at world level, that could be employed as important genetic reservoirs [6]. Also microsatellites have been applied to different Neotropical cat species, several studies have been carried out with the ocelot (*Leopardus pardalis*). These studies with microsatellites revealed the non-existence of agreement between the putative morphological subspecies of ocelots and the molecular results obtained [7, 8]. Contrarily, microsatellites have been usefully employed in the expansion determination of significant molecular subspecies or evolutionary units in the pampas cat, *Leopardus pajeros*, and in the Andean cat, *L. jacobita* [9–11]. As for jaguars (*Panthera onca*), the first analysis with microsatellites employed 29 loci analyzed, finding molecular evidence of a recent population expansion and suggesting differences due to the geographic barriers like the *Tapon del Darien* and the Amazon river [12]. Nevertheless, more recent studies with jaguars and microsatellites have shown the non-existence of significant geographical barriers to the dispersion of the jaguars [13, 14]. Likewise, the variance in the size of the alleles of the microsatellites has permitted to develop genomic estimates for phenomena that occurred in ancestral times, such as the founding effects presented in the North American pumas, Asiatic lions (*P. leo persicus*), and cheetah [15], as well as some studies have shown the existence of mutation constrictions or some selective pressures on the microsatellite evolution in felids [16]. The first reports about the phyllogeography and the natural history of jaguars were conducted with the analysis of microsatellites and of mitochondrial DNA by Eizirik [12]. The employment of these is suggested to characterize specimens of different origins, thus determining possible crossbreeding of specimens in captivity, which represent the same genetic legacy. In a subsequent study, great genetic diversity was reported and a small genetic heterogeneity between the subspecies was found in Colombia, composed mainly by Central American jaguars (*P. onca centralis*) and Amazonic jaguars (*P. onca onca*) [13]. Lately, other works determined the non-existence of bottlenecks, different temporal splits in the diversification of mitochondrial lineages, and some estimation of effective numbers in the jaguar populations of Colombia and other Latin American regions [7, 14].

In Colombia, the genetic structure of jaguars held captive in zoological parks is unknown, as is their pedigree, their importance as possible genetic reservoir, and their feasible participation in a conservation plan *ex situ*, which might consider the viability and the vitality of the population at a genetic level as well as reproductive. Therefore, the main aims of the current work are as follows: (1) Conducting a retrospective genetic analysis from the records of the regional jaguar (*P. onca*) Studbook for Colombia and knowing the current genetic characteristics of the population in captivity, future sustainable projections can be established at a reproductive level, for the captive population. (2) The construction of a pedigree of the captive Colombian jaguar population through the identification of individuals, using microsatellites, could help to determine the genetic structure of this captive population. It could be useful to identify specimens which have a high genetic and reproductive potential in a conservation plan that may contribute to the conservation *ex situ*, in the zoological parks of Colombia, as a genetic reservoir, and in the same way that may contribute to the conservation *in situ* of the species in their natural habitat. (3) We compared the gene diversity levels found in the Colombian jaguar captive population with the gene diversity found in wildlife jaguars sampled in Colombia.

2. Materials and methods

2.1. Genetic analysis with microsatellites

2.1.1. Samples

Seeking to construct the pedigree of the population, blood samples were obtained from captive jaguars (*n* = 15) under general anesthesia, in three Colombian zoological parks: Fundación Zoológico Santacruz (*n* = 8) located in the San Antonio del Tequendama municipality in the department of Cundinamarca with coordinates Latitude 6°13′23.93″N Longitude 75°34′48.81″W, Zoológico Matecaña (*n* = 6) located in the city of Pereira in the department of Risaralda with coordinates Latitude 4°49′0.30″N Longitude 75°44′15.04″W, Parque Recreativo y Zoológico Piscilago (*n* = 1) located in the Nilo Municipality in the department of Cundinamarca coordinates Latitude 11° 0′39.84″N Longitude 74°47′53.69″W; hair follicles were also obtained from the jaguars in the Fundación Zoológico Santafé (*n* = 5) located in the city of Medellin, in the department of Antioquia with coordinates Latitude 6°13′23.93″N Longitude 75°34′48.81″W. The information to complete the construction of the pedigree, regarding the animals present in the Piscilago Zoological Park (*n* = 2), Barranquilla Zoological Park (*n* = 3), Bioparque los Ocarros (*n* = 2), Fundación Zoológico de Cali (*n* = 2), and Fundación Zoológico Parque Jaime Duque (*n* = 3), was obtained from the Regional Jaguar Studbook for Colombia.

The samples of the 156 jaguars sampled in the wild in Colombia for comparative purposes were from the following localities as follows: representing the putative subspecies *centralis*, the samples were from the Departments of Atlantico, Bolivar (Colorado), Magdalena (Sierra Nevada de Santa Marta, Antioquia (Apartadó, Santa Fé de Antioquia), Norte de Santander, Chocó (St. Maria

de Condoto, Utría), Risaralda and Cauca, and representing the putative subspecies *onca*, the samples were from the Departments of Arauca (Lipa River), Meta (La Macarena), Caquetá (Caguán River), Vichada (Tuparro National Park), Guainía (Inirida River, Isana River), Vaupés (Miraflores, Itilla River, Apaporis River, Yuruparí), Guaviare (San José del Guaviare, Itilla River, Guayabero River), Putumayo and Amazonas (from Leticia to San Juan de Atacuarí).

2.1.2. Anesthesia

Following a 24-h fast, an anesthesia protocol was conducted, using a combination of xylazine–ketamine. Xylazine, an agonist alfa 2 adrenergic sedative, was administered with an initial injection. After 5 min of its application, and once signs of sedation were observed, a second injection with a fixed dissociative anesthetic, derived from cyclohexylamines (ketamine), was administered. The administration of these medications was conducted using devices of drug injections at a distance (DIDD). The site of the impact of the dart was localized in the muscular mass of the upper and lower limbs; the former being preferred, seeking that the dart or the injection be applied in the most caudal muscular mass and avoiding a possible impact on the femoral bone or the sciatic nerve. The doses of the medications administered were calculated, bearing in mind the reports on weight recorded on the medical history and the doses reported for jaguars [17, 18].

2.1.3. Sample collection

Blood samples were obtained by puncturing the femoral vein or the saphenous vein, having previously disinfected the area with alcohol. The samples were collected in vials with ethylenediaminetetraacetic acid (EDTA) (10 ml) and were maintained at 4°C, until their arrival to the laboratory.

2.1.4. Microsatellites employed

The microsatellite primers selected were developed for domestic cats [3] (**Table 1**). The criteria for the selection for the microsatellites were based on the fact that all microsatellites should be in different chromosomes and that their employment be reported on jaguars [12, 13]. The microsatellites selected were marked by fluorescence with fluorochromes that do not overlap among themselves, depending on the microsatellite selected. Only the first of the forward sequence was fluoro-marked for the wild jaguars analyzed for comparative purposes, 12 microsatellites were employed, being them, *Fca08*, *Fca24*, *Fca43*, *Fca45*, *Fca96*, *Fca126*, *Fca136*, *Fca176*, *Fca225*, *Fca294*, *Fca391*, and *Fca506*.

2.1.5. Extraction and quantification of DNA

The extraction of DNA was conducted from leucocytes isolated from samples of blood, through the salting-out technique. The concentration of DNA was quantified through spectrophotometry at 260 nm. As for the temperature for each microsatellite, it was calculated through the thermodynamic formula, obtaining that the average annealing temperature needed to conduct the coupling of bases was of 55.5°C. As for the hair samples, the extraction kit DNeasy Blood and Tissue of QIAGEN was employed, following the steps given.

Name	Forward	Reverse	Fluorochrome	Temperature °C	Molecular weight NCBI
FCA075	ATGCTAATCAGTGGCATTTGG	GAACAAAAATTCCAGACGTGC	PET	57.0	104-146
FCA043	AGACGGGATTGCATGAAAAG	GAGCCACCCTAGCACATATACC	6-FAM	57.0	106-128
FCA441	ATCGGTAGGTAGGTAGATATAG	GCTTGCTTCAAAATTTTCAC	VIC	58.0	113–137
FCA008	ACTGTAAATTTCTGAGCTGGCC	TGACAGACTGTTCTGGGTATGG	NED	57.0	114-148
FCA224	CTGGGTGCTGACAGCATAGA	TGCCAGAGTTGTATGAAAGGG	6-FAM	57.0	148-180
FCA096	CACGCCAAACTCTATGCTGA	CAATGTGCCGTCCAAGAAC	NED	58.0	180–220
FCA736	TCAATGTCTTGACAACGCATAA	AGGATTGCATGACCAGGAAC	6-FAM	53.0	196-280
FCA220	CGATGGAAATTGTATCCATGG	GAATGAAGGCAGTCACAAACTG	VIC	57.0	210-224
FCA391	GCCTTCTAACTTCCTTGCAGA	TTTAGGTAGCCCATTTTCATCA	NED	57.0	222-238

Table 1. Microsatellites employed in the captive population of jaguars.

2.1.6. Polymerase chain reaction

The reaction for all the markers were carried to a total volume of 20 µl; these consisted of 1.5 mM of $MgCl_2$; 1.25 mM for each one of the dNTPs (dATP dGTP dCTP y dTTP) plus 0.4 units of DNA polymerase *Thermus thermophilus* (Tth) and 10 pmol of each first, 0.5 mg/ml of BSA just as 4 µl of genomic DNA (10 ng). The samples were amplified under the following conditions: initial denaturizing at 95°C for 5 min; 35 cycles of denaturizing at 95°C for 1 min; banding temperature corresponding to each marker for 2 min; extension at 72°C for 2 min and later 1 cycle of final extension at 72°C for 5 min employing a polymerase chain reaction (PCR) Multigene Gradient (Labnet, International) Thermal Cycler.

2.1.7. Agarose

After having conducted the PCR procedures, the products of amplification were evaluated in agarose gel at 1.5% stained with SYRB-GREEN (Invitrogen, USA). Once the amplification of the products was verified by direct visualization, they were taken to electrophoresis in capillary.

2.1.8. Electrophoresis in capillary

Electrophoresis in capillary was conducted in ABI PRISM® 310 Genetic Analyzer. The results of the electrophoresis in capillary and the naming of the alleles are determined by direct visualization of the chromatograms in the Softgenetics Gene Marker Version 1.97 program. All samples were amplified and Genotypified at least twice to minimize problems of no assignation.

2.2. Statistical analysis

2.2.1. Genetic analysis from records

Records from captive jaguars were analyzed with the information compiled in the Regional Studbook for Jaguars (*P. onca*) for Colombia, through the Single Population Analysis and

Record Keeping System (SPARKS 1.5) program, developed by the International Species Information System (ISIS). Later, seeking to conduct the genetic analysis from records, information was exported and analyzed in the program Population Management 2000 (PM2000) [19].

Statistics of individual jaguars, founders of the population, and of their descendants was calculated. The allelic **retention**, which is defined as the probability that a gene originated from a founding animal could be found in the existing current population, was calculated. The current **value of gene diversity (GD)** of the population that corresponds to the heterozygosity expected, and which is defined as the variance of allelic frequencies in a determined locus and is equal to the heterozygosity expected for a population in balance. Hardy-Weinberg with coupling of gametes at random was obtained [20–22]. The gene diversity level found in the Colombian captive jaguar population at the zoos of this country was compared with the gene diversity level obtained for the set of wildlife Colombian jaguars analyzed by Ruiz-García et al. [14]. For this, the differences among estimates were statistically analyzed with a Student *t*-test. The heterozygosity data were arcsine transformed prior to analysis, as proposed by Archie [23].

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The **average inbreeding coefficient *F*** for the population was calculated over time, being this the descent of the heterozygosity observed, relative to the heterozygosity expected of the founding population [24]. The **genetic value (GV)** corresponding to the heterozygosis expected in the following generations, providing all animals reproduce and have a progeny equal to the values expected, based on the **fertility rate (Mx), mortality rate (Px)**, and **reproductive value (Vx)**, was also obtained [19].

The value of the **equivalent of the current and potential founding genome** was found; this is defined as the number of founders equally represented in the population without the random loss of alleles in the offspring awaited to produce the same genetic diversity in the population under study. Likewise, the value of the **equivalent of the current surviving founding and potential genome** was obtained [25].

Individual statistics of captive jaguars with a known pedigree were calculated, and the average kinship **(MK)** is defined as the average of coefficients of relationships that exist between an individual (including itself) and the live population of animals born in captivity. The average of kinship is equal to the proportional loss of genetic diversity of the offspring born in captivity relative to the founders. It was also calculated: the **value of kinship (KV)**, which forecasts the loss of diversity of the awaited gene of the following generation, if all animals were reproduced at random and all would produce the number of offspring awaited for each kind of age; **total genome singularity** (GU–all genome uniqueness), which is the possibility of taking an allele at random from an individual not present and that this allele be identical by means of

descendants, in another live individual in the population. **Descendants genome singularity** (genome singularity relative to the population of descendants of non-founding animals); **probability of loss of genome singularity, equivalent of first-degree relatives (FOKE**, First-Order Kin Equivalent), which is the number of first-degree relatives (siblings, offspring) that should contain the number of copies of the alleles of the individuals (identical by progeny) present in the population born in captivity. For example: a son or a brother contributes 1 to FOKE; each grandson contributes ½ to FOKE; each cousin contributes ¼ to FOKE. FOKE being = 4*N*MK. The size of the effective population or **effective population number (Ne)** based on the history of the population was also calculated; the **current population number (N)** shows the number of live animals in the population, including males as well as females [19].

2.2.2. Genetic analysis from DNA

For the analysis of the information obtained, the *Genepop on the web* was employed [26, 27]. For each microsatellite the allelic frequencies, heterozygosity expected and observed, estimate of balance Hardy-Weinberg, linkage imbalance, determination of inbreeding through F_{is}. Calculations were performed. For assignation of paternity and maternity, and for the construction of the pedigree in the population, the programs CERVUS 3.0.3 version and KINSHIP 1.2. were employed. For the final construction of the pedigree, the following variables were taken into account: information obtained from the Studbook (gender, date of birth, previous knowledge of fathers and mothers), values of natural logarithm of relations of verisimilitude (LOD) assigned by CERVUS, with flexible intervals of assurance of 80% and strict to 95%. Likewise, the probabilities of relationship and family kinship assigned by KINSHIP for H_1 Rp = 0.5 and Rm = 0.5 versus H_0 Rp = 0 and Rm = 0 (α = 0.05, 0.01 and 0.001) where Kinship is used to prove the hypothesis of relationship of pedigree among individuals, employing data of analysis of genetic markers, after specifying the hypothesis of relationships using two variables Rp and Rm and specifying the different levels of significance Rp and Rm. The variables define the probabilities that a couple share an allele by direct line of descent coming from their father or their mother, respectively. For example, if the hypothetical relationship of two complete siblings, both values would be of 0.5. After giving the hypothesis, Kinship uses the values *r*, the allelic frequencies, and the genotypes of two individuals under consideration, to calculate the verisimilitude that such combination of genotypes could have been produced as it had been specified [28].

3. Results

3.1. Genetic analysis from the regional Studbook of jaguars for Colombia

3.1.1. Demographic and individual statistics

The demographic statistics that determine the genetic characteristics of the population of captive jaguars in Colombia were calculated. By means of the genetic analysis from records, conducted with the PM2000 program, we found that the origin of 28.9% of the population starting in the year 1968 is known, and that there have been five founding animals (T1, T2, T5, T6, T27) for

the population; likewise, three possible potential founders were the individuals T32 and T33 present at the Barranquilla zoo, and the T20 in the Piscilago zoological park. These founding animals, coming from wildlife, were either captured or seized and would represent the closest genetics to that that could be found among jaguars in the wild. In this way, individual statistics were obtained, of the jaguars founders of the population, where the number is presented on the Studbook, as well as the gender, age, representation (proportion of genes within the direct line living descendants of that founder of the population), contribution (number of copies of the founder's genome that are present in the living descendants; each new generation of offspring contributes 0.5, each generation of grandchildren 0.25, etc.), allelic retention (the probability that a gene present in a founding individual exist in the living descendant animals), potential retention, and descendants. The jaguars T1 and T2 are the oldest for which there is any kind of information, they were part of the Santafé Zoo and their remains are kept in the museum of natural history of the Universidad de Antioquia; their genetic representation in the population is of 8.9% and have contributed with two descendants. Jaguars T5 and T6 are animals that remained in the Santacruz Zoological Foundation, until their death. Their genetic representation in the population is of 28 and 46% and has contributed with four and six descendants respectively. Jaguar T2, situated at the Jaime Duque Park, has a genetic representation of 7% in the population, and has contributed with a descendant.

It was also found that five founding animals have produced descendants and that jaguar T6 the one that presented the greatest number of descendants ($n = 7$), followed by T5 ($n = 5$). Thus, the genetic contribution of these founders ranges between 0.5 and 3.5%.

The current and potential allelic retention was calculated. Regarding the current allelic retention, it can be observed that this depends mainly on whether the founders have produced descendants and if there has been a genetic contribution from them. It can be observed, in the case of the potential allelic retention, the capacity of allelic retention of the population with the genes coming from those animals that still have not reproduced themselves within the population. (T33, T32, and T20). It can also be observed that individuals T1 and T2, although having contributed to the allelic retention, do not contribute to the potential allelic retention, due to the fact that they have died.

The average value of the current genetic diversity, of the population (GD = 0.7832), was obtained. Similarly, the value of the potential genetic diversity (GDp = 0.9113) was calculated, which is determined when conducting control on the production of cubs in the population, and if there is control over which genes are transmitted to the descendants by means of programs of the management of reproduction toward the future. In this way, it was possible to determine the variations of genetic diversity, during a span of time which permits appreciating that between 1995 and 1996, the genetic diversity of the population increased from 50 to 75%, due to a birth and other reproductive events that took place at that time. A slight increase from the years 1999–2002 can also be appreciated. A genetic value (GV) of 0.7846 was obtained, as was also obtained the value of the equivalent for the current founding genome (FGE = 2.31) and the potential (FGEp = 5.64). In the same way, the value of the equivalent of the current surviving founding genome (FGS = 3.14) and potential (FGSp = 5.64) was obtained. In the graph, an increment of FGE between the years 1995 and 2002 can be observed.

The average coefficient of F inbreeding [24] for the population was calculated through time, and it was found on average an $F = 0.0179$, being this the descent of heterozygosity observed, relative to the heterozygosity expected from the founding population. It was found that the coefficient of inbreeding was different from 0, starting in the year 1998.

The size of the effective population or the effective demographic number was also calculated (Ne) based on the history of the population; the current demographic number (N) points out the number of live animals within the population, including males as well as females. The primary use of N predicts future genetic changes, and its first estimate is based on the amount of diversity of lost genes through generations of cubs in captivity. The second estimate of the Ne is simply based on the number of live males and live females who have produced the current offspring. The proportion of Ne and N is employed during the addressing phase of the population, estimating the size of the population that will be needed to achieve genetic goals [19]. Considering the earlier, for the first estimate, the following was found: Ne = 5.26 over the last 1.39 generations, and for the second estimate: Ne = 2.667 for 1.0 male for each 2.0 breeding females; as with the relationship Ne/N = 0.2667 and the average value of MK was 0.217.

3.2. Genetic analysis with microsatellites

3.2.1. Genotypes

The genotypes of the jaguars sampled were stabilized from the analysis of the chromatograms obtained after the capillary electrophoresis. It could be determined that the microsatellite, which amplified in fewer individuals, was FCA008 showing the highest index of presentation of null alleles (+0.16). For the 20 jaguars analyzed and the 9 microsatellites, an average of alleles of 5.67 per locus was found, finding the highest number of alleles for the locus FCA 224 (eight alleles) and the lowest for the FCA736 (two alleles). In the wild Colombian jaguar sample for 156 individuals and 12 microsatellites, the average allele number was 13 ± 2.523, being this value significantly higher than that estimated in the captive jaguar population. *Fca136* yielded 18 alleles, whereas *Fca24* and *Fca45* presented 10 alleles.

The average gene diversity (expected heterozygosity) found for the Colombian captive jaguar population was $H = 0.684 \pm 0.230$ by means of microsatellites. It is noteworthy to mention that this average gene diversity value was significantly lower to that estimated in the Colombian wild jaguar population ($H = 0.867 \pm 0.0588$; $t = 2.86$, $p < 0.02$). Identically, the value obtained for the Colombian captive jaguar population was also significantly lower from the wild jaguar populations of Peru and Bolivia ($H = 0.883 \pm 0.045$ and $H = 0.883 \pm 0.043$, respectively; [14]). Thus, the captive jaguar population of Colombia retained about 78% of the genetic diversity found in the north-western South-American wild jaguar population.

In the captive population, FCA 391 and +FCA 220 presented the highest values of heterozygosity and information of polymorphic content (PIC) and the locus with least heterozygosity and PIC was the FCA736. In the wild sample, *Fca176* and *Fca136* were the two markers with the highest values of expected heterozygosity and PIC, meanwhile *Fca08* and *Fca45* were those with the lowest values of gene diversity and PIC. As for the probability of the presentation

of null alleles, the highest probability was found for the locus FCA 008. The values of the heterozygous and the homozygous alleles were found for each locus, as well as the frequency of presentation including and excluding the probability of presentation of null alleles. For microsatellite Fca 075, seven (7) alleles were found; being el alelo de 114 pares de bases (bp) the most frequent. For microsatellite Fca 44, five (5) alleles were found; being 146 bp the most frequent. For microsatellite Fca 220, six (6) alleles were found; being 212 the most frequent. For microsatellite Fca 043, three (3) alleles were found, being the 114 bp. This microsatellite was also genotyped for the wild sample showing 13 alleles. For the microsatellite Fca 224, eight (8) alleles were found; being 152 bp the most frequent. For microsatellite Fca 736, two (2) alleles were found; being 125 the most frequent. For microsatellite Fca 008, seven (7) alleles were found; being 110 bp the most frequent. This microsatellite was also genotyped for the wild sample showing 15 alleles. For microsatellite Fca 096, seven (7) alleles were found; being 189 bp the most frequent. This microsatellite was also genotyped for the wild sample showing 14 alleles. For microsatellite Fca 391, six (6) alleles were found, 208 bp being the most frequent. This microsatellite was also genotyped for the wild sample showing 15 alleles. Therefore, in those microsatellites simultaneously genotyped in both jaguar samples, in all the cases the wild jaguar sample yielded a significantly higher number of alleles.

3.2.2. The Hardy-Weinberg equilibrium

In this study, the test of probabilities for each locus was conducted; finding excess of homozygotes for the loci FCA 441, FCA 008, FCA 224, and FCA096 through the estimates F_{is} de Weir & Cockerham's (W&C) [29], and Robertson and Hill's (R&H) [30]; nevertheless, the Fisher method resulted in not being significant for the excess of homozygotes (χ^2: 18.9872 GL: 18.0000, $p = 0.3926$).

3.2.3. Endogamy

When assuming the Hardy-Weinberg equilibrium, the demographic F_{is} statistic was calculated. An excess of heterozygotes was found for this population in the loci FCA075, FCA043, FCA736, FCA220, and FCA391 through the estimates of F_{is} of Weir & Cockerham's (1984) (W&C), and Robertson & Hill's (1984) (R&H); likewise, the result obtained for the F_{is} average for the population was (F_{is} W&C= −0.0157) and (F_{is} R&H = −0.0109). Contrarily to that found in the Colombian captive jaguar population, the Colombian wild jaguar sample showed a significant positive value of F_{is} ($F_{is} = 0.344 \pm 0.042$), which agrees quite well with a large genetic subdivision in this population than in the captive jaguar population of this South America country.

3.2.4. Disequilibrium by linkage

No significant differences for the de test of disequilibrium by linkage were found, which means that each locus, being independent from the others, can be employed as determiners of the genetic diversity of the population. The same was found for the 12 microsatellites applied in the wild jaguar sample.

3.2.5. Pedigree

3.2.5.1. Maternity

The analysis of verisimilitude obtained in CERVUS resulted with assignments of maternity with no knowledge of the father by 34% for the strict confidence interval at 95% (LOD critical = 4) and with a 100% for the assignments for the flexible interval at 85%. The assignments of maternity with knowledge of the father were of 84% for the strict confidence interval at 95% (LOD = 2.05). Only the father (T5) of individuals T7, T8, and the father (T3) of individual T10 were known. Assignments are conducted according to the coupled LOD scores.

3.2.5.2. Paternity

The analysis of verisimilitude obtained in CERVUS resulted with assignments of paternity with no knowledge of the mother of 32% for the strict confidence interval at 95% (LOD critical = 4) and with 100% of assignments for the flexible interval at 85%. Assignments of paternity with knowledge of the mother were of 81% for the strict confidence interval at 95% (LOD = 2.05). Only the mother (T6) of individuals T7, T8, T10 was known. The assignments were conducted according to the coupled LOD scores.

3.2.5.3. Kinship

The probabilities of relationship and kinship assigned by KINSHIP were obtained to a prove H_1 Rp = 0.5 and Rm = 0.5 versus H_0 Rp = 0 and Rm = 0 at a significant level of α = 0.05, 0.01, and 0.001, which means that when finding significant and highly significant relationships, it is accepted H_1 where Rp and Rm = 0.5 are the probability that two individuals present a first-degree relationship, be it father and/or mother of son/daughter or brother-sister/-sister/brother. In the results obtained in the simulations, they found significant relationships ($p < 0.05$) in 6 couples of jaguars, highly significant relationships in 3 couples ($p < 0.01$), and significant relationships ($p < 0.001$) for 11 couples.

Taking into account the information of the records of the Studbook, the assignation of paternities and maternities for the CERVUS program and the relationships of kinship obtained by KINSHIP, the construction of the pedigree was conducted, for the population of captive jaguars in the zoological parks of Colombia (**Figure 1**). When conducting the construction of the pedigree, a great association was found, between the animals present at the Matecaña and the Santafe zoological parks. A high genetic relation among the jaguars present at the Santacruz zoological park could be determined, and additionally there were connections in the records of the Studbook between this zoo and the Piscilago, Barranquilla, and Jaime Duque. As for the *fundacion zoologico de Cali*, relationships were found from the records in its historical collections. Regarding the jaguars that are in the Ocarros, no associations were found with other individuals of the captive population in Colombia.

To sum it up, through the CERVUS program, four maternities (T15, T25, and T19 in two occasions) and five paternities (T17, T26, T3, and T16 in two occasions) were assigned. They were assigned by CERVUS as well as by KINSHIP four paternities ($p < 0.001$) (T17, T18, and T5 in
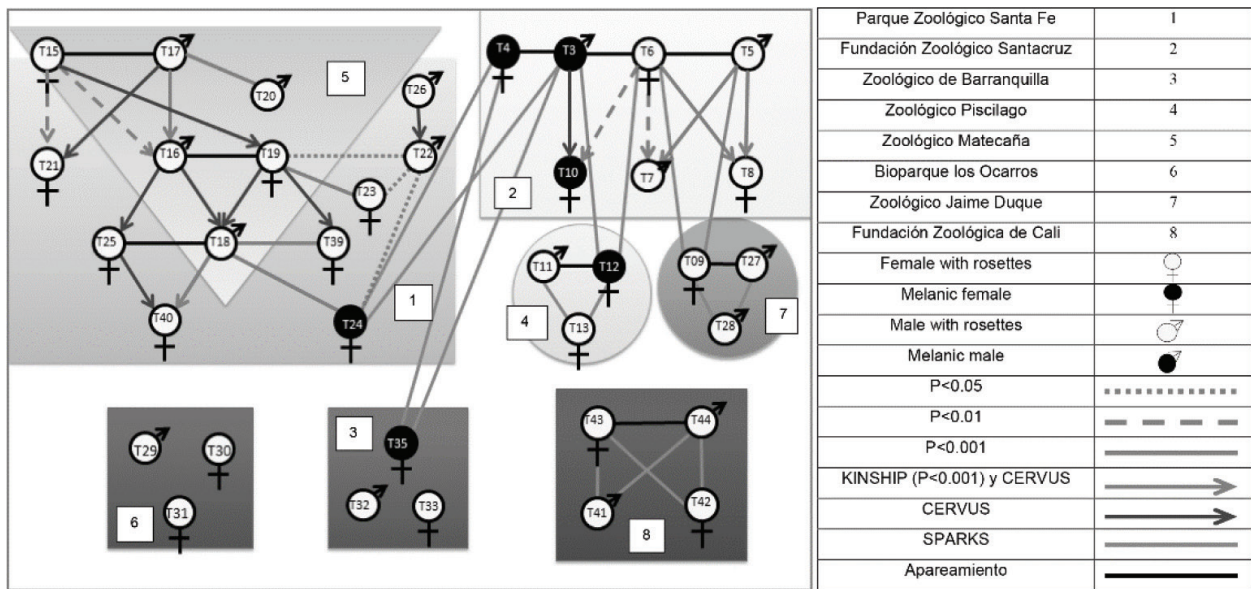
Figure 1. Pedigree was of the population of captive jaguars in the zoological parks of Colombia.

two occasions) and one maternity (T6). In this manner, four maternities were assigned by KINSHIP ($p < 0.01$) for two individuals (T15 in two occasions and T6 two occasions). A significant ($p < 0.05$) relationship was found through KINSHIP, between jaguar T17 and jaguars T23, T19, and T24.

In **Figure 2**, it is possible to observe as an example a paternity and maternity test in jaguars (*P. onca*), employing nine microsatellites, assigned by CERVUS with a significant relationship ($p < 0.001$) found in the KINSHIP program, between father and son and significant ($p < 0.001$) between mother and son. The results are presented graphically through the chromatograms of each system of microsatellites employed and numerically highlighting in grey the size of the alleles inherited.
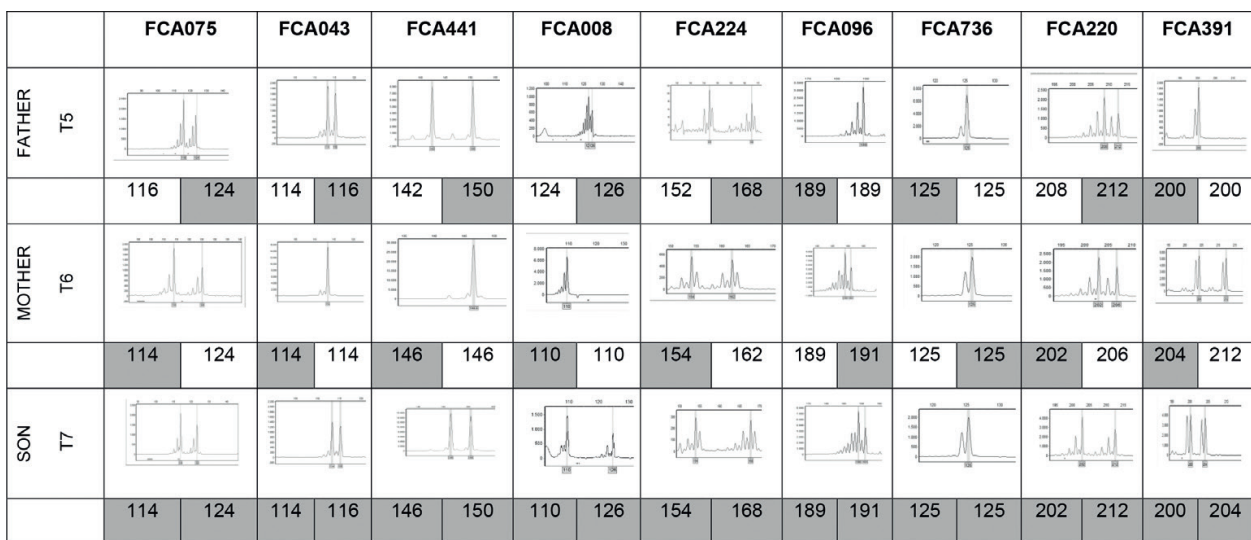

Figure 2. Paternity and maternity of a triplet of captive jaguars.

4. Discussion

In this study, it could be proven that the population of captive jaguars in zoological parks in Colombia is a population that presents a high genetic variability and that is a population with the necessary conditions to be an important model as a genetic reservoir to revitalize natural populations given the case that they might be isolated and present low levels of genetic diversity. Nevertheless, this study also shows that the Colombian zoological captive jaguar population only has a fraction of the overall genetic diversity found in the wild jaguar population of this South-American country [7, 13, 14, 31].

As for the genetic analysis from the regional Jaguar Studbook, it could be determined that regarding the mean kinship (MK) on average (MK = 0.217), the values found for the captive population in Colombia are less than the MK reported by Drury [32] for captive jaguars in Europe (MK = 0.238), which offers a large indicative as future potential to reduce and/or events of inbreeding. It was found that there exists representation and genetic contribution of founders from wildlife in the current population due to breeding in captivity.

Regarding the genetic diversity according to the records found in the regional Studbook, a clear tendency was found, of an increase that went from 50% in the year 1994 to 72% in the year 1996 and reaching nearly 78% in the year 2002, due to the reproductive processes and the births that took place in this lapse of time.

As for the genetic diversity in the analysis of microsatellites, it was found that it is in the order of 68%. Such a result is found in the levels reported by Eizirik et al. [12], where he studied levels of genetic diversity between 62 and 73%. This last study analyzed a large fraction of jaguars living in the periphery of the geographical distribution of this species (Mexico and Central America and Southern Brazil and Argentina). However, these gene diversities were considerably minor than those found by Ruiz-García et al. [7, 13], who reported a gene diversity of 84%, or the gene diversity showed in this study and Ruiz-García et al. [14] for a larger Colombian wild jaguar sample around 87% [14]. Henceforth, the Colombian captive jaguar sample contained around 78% of the gene diversity found in wild conditions. Due to this, it is possible to determine that the genetic diversity of captive jaguars in Colombia is at a high level; nevertheless, it is necessary to follow the alignments of reproductive population management for the species and thus reach a level close to 90% and maintain it for a 100-year period as it is proposed in the plans of survival of species [33].

As for the analysis conducted of the microsatellites, a smaller number of alleles were found, for locus FCA96 (7 alleles), FCA08 (7 alleles), and FCA391 (6 alleles), than the number reported by Ruiz-García et al. [34] where the number of alleles found was greater for FCA96 (15 alleles), FCA08 (15 alleles), and FCA391 (13 alleles) [34], and by Ruiz-García et al. [13], where it was found for Fca96 (13 alleles), Fca08 (13 alleles), and Fca391 (13 alleles) [13]. Regarding locus Fca96, alleles of 185pb 187pb, and 189pb have been reported in Amazonian jaguars of the subspecies (*P. onca onca*) and of 183pb in Guatemala of the subspecies (*P. onca goldmani*) [13], which in this investigation were found with the following allelic frequency: 185 pb = 0.05, 187pb = 0.175, 189 pb = 0.35 and for the 183 pb = 0.075, found only in the T15, T16, and T19 at the Matecaña zoo. As for loci T15, T16, and T19 located at Matecaña zoo, an allele of 205 pb

was reported only in jaguars of Peru [13] found in this study in eight specimens with a high allelic frequency of 0.21. Regarding locus Fca008, an allele of 124pb was reported, only in jaguars from the central Amazonia [13], found through this study, two specimens with a high allelic frequency of 0.09 in specimens T5 and T8 located at *Fundación Zoológico Santacruz*. This could mean that the Colombian captive jaguar population could be composed by animals proceeding to three different putative subspecies (although the subspecies concept applied to the jaguar is discussed by Ruiz-García et al. [14]), with greater predominance of the Amazonian jauar *P. onca onca* [35], followed by the Peruvian jaguar *P. onca ucayale* [36] and lastly by *P. onca centralis*, not ruling out the *P. onca goldmani*, having found alleles of this subspecies.

In this study, it was found that for the loci analyzed, there exists Hardy-Weinberg equilibrium, which contrasts with the findings of Ruiz-García et al. [13, 31] who reported the inexistence of the Hardy-Weinberg equilibrium for excess of homozygotes which was attributed to an effect of the demographic subdivision or Wahlund effect in the total wild jaguar population of Colombia and north-western South America. On the other hand, Eizirik [12] reported deviations from the Hardy-Weinberg equilibrium in 6 loci of the 29 analyzed from the population of jaguars studied. Thus, the estimated equilibrium Hardy-Weinberg is a point that needs greater approach in order to clarify and determine if the disequilibrium found by Ruiz-García is due to the effect of demographic subdivisions, or Wahlund effect, or if this finding is a dependent reflection of loci analyzed. However, notwithstanding that in this study we find the existence of equilibrium for the loci analyzed, the size of the sample is reduced; therefore, inference could not be extensively extrapolated to the populations of jaguars in the wild. In this way, it would be important to continue this kind of analysis in future investigations.

The results of the genetic analysis of the Regional Jaguar Studbook for Colombia in the PM2000 program clearly show that according to the records regarding inbreeding, the maximum value was found for F in jaguars in Europe ($F = 0.08$), a more superior value to that found in the Jaguar Studbook for Colombia ($F = 0.0179$), event that has only been registered since the year 1998. Nevertheless, posterior to the analysis employing microsatellites, it could be determined through statistic calculation F_{is} as a determiner of inbreeding of the population based on analysis data of DNA, contrasts with the results obtained in the analysis of records for the coefficient of inbreeding F.

As for the value found for F_{is}, it represents that in the population, an excess of heterozygotes is found, which when averaged in all the loci analyzed and being a negative value, it is an indicator that the population is not in endogamy; on the contrary, it is in exogamy (the F_{is} value is negative if the heterozygosity observed is greater than expected, which means that the average observed is greater than the one expected). The inbred populations are characterized by an increment in their homozygosis or a diminishment in their heterozygosis; thus, a negative value of F_{is} signifies that there exists an increment in heterozygosis and that the population is in exogamy [37].

Before conducting the study, the origin of the pedigree population of jaguars was known by 28.9% ($n = 11$) after the construction of the pedigree of the population through the analysis with the microsatellites, 53% ($n = 21$) could be known following an assignation of paternities

and maternities through the CERVUS and KINSHIP programs, which proves an optimization of the information in 98%, offering great advantages from the use of microsatellites as a complement in the actualization of the Studbook.

From this, it was possible to obtain a precise register of the origin of the live specimens within the population, which can be employed in the future programs of population management, involving controlled crossings in order to avoid the presence of inbreeding and thus maintain the jaguar population as a variable population, in genetic terms, as a model of investigation.

For some species where populations in the wild are highly diminished, the survival of the species depends on the propagation of captive animals. In these cases, the entire genetic pool of a species represented by the genetic contribution of the captive founders to the following generations of individuals bred and raised in captivity is presented as a closed system. In these cases, the new mutations that occur within the population provide the only new source of genetic variation. This process takes place at an extremely low rate and provides significant changes only at long intervals of time or when populations are extremely large. In the cases where the wild populations are stable in number and distribution so that potentially they can be sampled and could provide immigrants to the captive population, this access to new sources of variation provides the opportunity to preserve larger proportions of wild genetic pool, employing fewer captive individuals in a greater way than in closed populations. It is at this point where starting from the historical knowledge of animal populations is possible to pose concrete solutions with the purpose of conserving all the species that mankind has placed under threat, as is the jaguar. The implementation of programs of genetic analysis in the collections present in captivity contributes to the conservation of the species threatened, through the maintenance of viable populations as genetic reservoirs that can be used periodically to reinforce, revitalize, or reestablish captive populations and when necessary in cases of wild populations with low genetic variability. It also permits the identification of subspecies that even though phenotypically might seem similar, they have different geographical origins and have suffered a particular process of speciation. Although in the case of the jaguar, both morphological and molecular studies put in doubt the existence of subspecies [12–14, 34, 38, 39].

The scope of the instauration of programs of genetic investigation in the zoological parks and centers of rehabilitation of fauna, contributor to the ordering of captive collections, provides clear tools in the face of the movement of specimens from one place to another, in order to guarantee efficient reproductive and viable processes.

In addition, it contributes to the liberation of animals after the processes of rehabilitation in zones corresponding to the natural genetic origin of their congeners. The implementation of a genetic analysis of a population in captivity requires systematic steps in order to obtain the greatest quantity of possible information, such as the construction of a "pedigree" in the population, the identification of founding parental, the genetic contribution of each parental to the population, the estimate of the loss of alleles due to endogamy or "bottleneck" effects, and estimates of coefficients of inbreeding and making of tests employing molecular markers. In this way, adequate genetic techniques can be implemented to clarify the blanks of information about unknown specimens.

Therefore, this was the first study of records of captive jaguars in Colombia where it was possible to obtain relevant information regarding the identification of individual's founders of the population and their descendants. This kind of studies could be carried out in other Latin American countries, where the captive populations of jaguars are large (for instance, Peru, Bolivia, and Brazil), and thus these captive populations could be decisive to the conservation of the largest wild cat of the Americas.

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