# UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

**Colegio de Ciencias Biológicas y Ambientales**


# Analysis of the genetic diversity of the Andean bear (*Tremarctos ornatus*) using three new mitochondrial markers in populations from Quito, Loja and Zamora Chinchipe, Ecuador.


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# UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

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**HOJA DE CALIFICACIÓN**
**DE TRABAJO DE FIN DE CARRERA**

**Analysis of the genetic diversity of the Andean bear (*Tremarctos ornatus*) using three new mitochondrial markers in populations from Quito, Loja and Zamora Chinchipe, Ecuador.**

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## RESUMEN

El oso andino (*Tremarctos ornatus*) es el único representante vivo de la subfamilia de úrsidos Tremarctinae. Esta especie tiene un rol importante en sus ecosistemas, ya que es un excelente dispersor de semillas y podador de la vegetación. A nivel regional, la UICN cataloga al oso andino como una especie vulnerable y con una tendencia poblacional decreciente. Los estudios genéticos en especies amenazadas son cruciales para entender su ecología y modelar su resiliencia a presiones ambientales o antropogénicas. Por esta razón, en el presente estudio se diseñaron tres nuevos marcadores moleculares para analizar la diversidad genética mitocondrial de 23 ejemplares de oso andino de Quito, Loja y Zamora Chinchipe, en Ecuador. Las nuevas secuencias obtenidas se concatenaron con información de la región hipervariable 1 (HVR1) del D–loop del ADN mitocondrial. Se encontraron 7 haplotipos; 6 descritos en trabajos previos y se reporta un nuevo haplotipo para la población de Loja (HTOL2). Se obtuvo un índice de diversidad de haplotipos alto ($H_d = 0.8617 \pm 0.0352$) y un índice de diversidad de nucleótidos bajo ($\pi = 0.0036 \pm 0.0019$). El análisis de varianza molecular (AMOVA) determinó que la mayoría de la variabilidad genética encontrada se atribuye a las diferencias entre poblaciones. Estos resultados sugieren la existencia de diferenciación y estructura genética entre las tres poblaciones de osos analizadas, lo cual podría deberse a barreras geográficas, fragmentación del hábitat o diferencias en las preferencias alimenticias de cada grupo. Se recomienda la realización de estudios genómicos, con el fin de obtener más información sobre la diversidad de esta especie.


**Palabras clave:** *Tremarctos ornatus*, marcadores moleculares, diversidad genética, estructura poblacional.

## ABSTRACT

The Andean bear (*Tremarctos ornatus*) is the sole living representative of the ursid subfamily Tremarctinae. This species plays a crucial role in its ecosystems, as an excellent seed disperser and vegetation pruner. At the regional level, the IUCN classifies the Andean bear as a vulnerable species with a declining population trend. Genetic studies on threatened species are crucial for understanding their ecology and modeling their resilience to environmental or anthropogenic pressures. Therefore, this study designed three new molecular markers to analyze the mitochondrial genetic diversity of 23 Andean bear specimens from Quito, Loja, and Zamora Chinchipe, in Ecuador. The new obtained sequences were combined with information of the hypervariable region 1 (HVR1) of the D–loop in the mitochondrial DNA. Seven haplotypes were found; six were described in previous works, and a new haplotype was reported for the Loja population (HTOL2). A high haplotype diversity index ($H_d$ = 0.8617±0.0352) and a low nucleotide diversity index ($\pi = 0.0036 \pm 0.0019$) were obtained. The analysis of molecular variance (AMOVA) indicated that most of the genetic variability is attributed to differences among populations. These findings suggest the existence of differentiation and genetic structure in the three bear populations analyzed, which may be caused by geographic barriers, habitat fragmentation, or differences in the food preferences of each group. Genomic studies are recommended in order to obtain more information on the diversity of this species.


**Keywords**: *Tremarctos ornatus*, molecular markers, genetic diversity, population structure.

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# 1. INTRODUCTION

## 1.1. General information about the Andean Bear

### 1.1.1. Description and morphology.

Andean bears, *Tremarctos ornatus* (Cuvier, 1825), are medium-sized ursids with rounded heads and ears, and short necks and snouts. Their head-body length is within 1.12 to 2.10 m, and they present sexual dimorphism in which males are larger than females (Velez-Liendo et al., 2020). Adults weigh around 60 to 175 kg. They are plantigrades with front limbs longer than hind limbs and have five forward-pointing toes with non-retractable curved claws. Their long and thick coat varies in color, from black to blackish-brown, and they have white or cream marks around their eyes down to the chest (García-Rangel, 2012; Vela-Vargas et al., 2021). This phenotypic attribute, known as ornament, is distinctive of the species and is highly variable between individuals, which is why it is used as an identification method (Horn et al., 2014). Compared to other bears, additional specific characteristics of *T. ornatus* are a smaller cranial bone, deep pre-masseteric fossa in the jaw, and 13 pairs of ribs (Castellanos, 2010).

### 1.1.2. Distribution, habitat and biology.

The Andean bear is distributed throughout the South American Andes, from Venezuela to Argentina, in an area of 4,600 km in length and 200 – 650 km in width (Kattan et al., 2004; Teta et al., 2018). Its altitudinal range of distribution is 200 – 4,750 masl. This species inhabits diverse habitats, including coastal desert scrub, submontane and montane forests, cloud forests, paramos, and puna grasslands (García-Rangel, 2012; Peyton, 1981).

*T. ornatus* is characterized by being solitary and forming temporary groups in the reproductive and parental care periods (Appleton et al., 2018; Parra-Romero et al., 2019). These bears are elusive and shy when they come in contact with humans, although they can behave aggressively when injured, threatened or if they have cubs (Castellanos & Boada, 2022). Their activity is mainly diurnal, terrestrial, and partially arboreal; they can climb trees to feed, rest,

or escape. The Andean bear is an opportunistic omnivore that feeds predominantly on plants (314 identified species) and soft fresh fruits. The animal protein for bears comes from various sources, such as ingesting worms, insects, larvae, and eggs, feeding on dead animals, or hunting other animals such as rodents, birds, rabbits, and tapirs. However, some bears may attack domestic livestock (Figueroa, 2013; Velez-Liendo et al., 2020). The species can act as a seed disperser, pruner of the vegetation, and promoter of the renewal of the vegetal cover. These characteristics make it a modulator of the structure and composition of their habitats (Vela Vargas et al., 2011).

### 1.1.3. Taxonomy and evolutionary relationships.

The Andean bear belongs to the Carnivora order, Ursidae family and Tremarctinae subfamily; it is the only living species of this last taxonomic category. The Tremarctinae subfamily includes all the endemic bears of the American continent and comprises four genera: *Plionarctos*, *Arctodus*, *Arctotherium*, and *Tremarctos* (Brandstaetter, 2020). The genus *Tremarctos* diverged from *Arctotherium* 5.66 – 4.1 Ma ago, resulting in the appearance of Florida's spectacled bear (*T. floridanus*) in North America. When *T. ornatus* separated from *T. floridanus*, it became the youngest bear species (Mitchell et al., 2016). Genetic studies support the existence of two Evolutionarily Significant Units (ESU) throughout the geographic distribution of the Andean bear: the first, located in the north, is distributed throughout Venezuela, Colombia, Ecuador, and the north-central part of Peru; and the second, present in the south, encompasses the populations of southern Peru and north-central Bolivia. The southern ESU has a slightly higher genetic diversity than the northern ESU, which might suggest that the southern ESU may have originated first and then expanded to the north (Ruiz-García et al., 2020b; Velez- Liendo et al., 2020). However, the traditional theory states that *T. ornatus* originated in North or Central America and reached South America during the Great American Biotic Interchange (GABI) (Ruiz-García, 2003; Tedford & Martin, 2001).

### 1.2. Population status and threats in Ecuador

The population size throughout its distribution range is estimated at 13,000 – 18,000 bears, approximately 5 – 7 bears per 100 $km^2$. In Ecuador, the population of Andean bears is estimated at 1,200 – 2,000 individuals, and their population density is 0.03 – 0.074 individuals per $km^2$ (Molina et al., 2017; Viteri, 2007). At the regional level, the species is listed as "Vulnerable" in the A3c+4c criteria by the IUCN. At the local level, its status is "Endangered" in the A4acd,C1+2a criteria (Tirira, 2021; Velez-Liendo & García-Rangel, 2017). The main threats to Ecuadorian bear populations are habitat degradation and fragmentation, illegal hunting and traffic, and conflicts with humans (Ruiz-García et al., 2020a).

The destruction of habitats in the Andean region of Ecuador, which occurs in part due to urban development and the expansion of the agricultural frontier, leads to the isolation of populations of bears (Sandoval-Guillén et al., 2019). It has been estimated that there has been a loss of 42% of the original range of the Andean bear in the northern Andes (Venezuela, Colombia, and Ecuador) (Kattan et al., 2004). They are often hunted illegally by locals for their meat and body fat and in retaliation for damaged crops and attacks on livestock (Tirira, 2017). Throughout the distribution range of the Andean bear, there are 58 protected areas, but only a limited number have the necessary tools to protect and monitor bear populations (Kattan et al., 2004). Climate change is also a major threat to the Andean bear due to its impact on high-altitude ecosystems, which are the preferred habitats of the species. In the next 30 years, a rate of decline of more than 30% of its population is calculated in each country within its range of distribution and, in the next 20 years, its risk of extinction will increase faster than other bear species (Kattan et al., 2004; Vélez-Liendo & García-Rangel, 2017).

### 1.3. Mitochondrial molecular markers

Molecular markers provide a deeper understanding of the ecology and diversity of populations (Dong et al., 2021). Mammalian mitochondrial DNA (mtDNA) is a closed circular

molecule of about 16.6 kb, whose genes, and their order (a.k.a. synteny), are highly conserved across species (Bowmaker et al., 2003). Characteristics of mtDNA, such as its maternal inheritance, lack of recombination, near neutrality, high copy number, and rapid rate of evolution compared to nuclear DNA, make it a useful genetic marker (Galtier et al., 2009). Mammalian mtDNA contains 37 genes: 2 ribosomal RNA genes, 22 transfer RNA genes, and 13 genes encoding protein subunits of the electron transport chain (Chinnery & Hudson, 2013). These subunits form groups according to the protein complex in which they are: ND1, ND2, ND3, ND4L, ND4, ND5, and ND6 in complex I; Cyt b in complex III; COI, COII, and COIII in complex IV; and ATP6 and ATP8 in complex V (Kato, 2001). This molecule also contains a non-coding region called D–loop, which presents two hypervariable segments (HVR1 and HVR2) and has a moderate mutation rate and a high degree of sequence variation (Hudson, 2017; Myćka et al., 2022).

To date, ten population genetics studies have been performed for *T. ornatus* (Cueva et al., 2018; Ruiz-García, 2003, 2007, 2012; Ruiz-García et al., 2003, 2005, 2020a, 2020b, 2020c; Viteri & Waits, 2009). Most of these investigations used nuclear non–species–specific microsatellite markers to assess genetic diversity, except Ruiz-García et al. (2020a, 2020b, 2020c) and Cueva et al. (2018), which also incorporate information from mitochondrial markers. Therefore, the main objective of this research is to study nucleotide and haplotype diversity of the Andean bear in populations from Quito, Loja, and Zamora Chinchipe in Ecuador, through the analysis of three mtDNA regions: ND1–ND2, COI (partial sequence), and COI–COII–ATP8.

# 2. METHODS

## 2.1. Sampling and selection of individuals

The samples used in this study were collected by Santiago Molina in Quito and Zamora Chinchipe, and by researchers from the Universidad Técnica Particular de Loja in Loja. In Quito and Zamora Chinchipe, hair samples were obtained during 2015–2017 and 2020, respectively. The sampling methodology consisted of installing barbed wire corrals inside which a vanilla scent lure was placed, and camera traps were installed in areas near the corral to obtain a photographic record of each bear entry. The hair trapped in the wire of the corral was collected every 15 days. The photos and videos obtained were used for bear identification based on its facial features. The hairs were placed in paper envelopes labeled with the code of the individual. In Loja, between 2014 and 2015, fecal and saliva samples were collected using non-invasive techniques.

All samples were transported to the Laboratorio de Biotecnología Vegetal at USFQ and stored at –20°C. After checking the photographic record and the field data, only the samples that belonged to different individuals were selected. Twenty-three samples were used for molecular analysis: 10 from Quito, 5 from Loja, and 8 from Zamora Chinchipe (Appendix 1).

## 2.2. DNA extraction and quantification

DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions with some modifications. The concentration and quality of the extracted DNA were determined using a Nanodrop 2000$^{TM}$ (Thermo Fisher Scientific, 2009). DNA amplification was assessed using the hypervariable region 1 (HVR1) of the D–loop in mtDNA, as previously described (Cueva et al., 2018).

## 2.3. Primer design and mitochondrial marker amplification

Four complete mitochondrial genome sequences of *T. ornatus* (GenBank accessions: NC_009969.1, EF196665.1, FM177764.1, and MW556430.1), and two other mitochondrial

genomes assembled from short–read sequencing data (GenBank accessions: ERX1025773 and ERX1025774) were aligned with the program CodonCode Aligner v.10.0.2 (Codon Code Corporation, 2021), to identify and target variable sites. As a result of this analysis, three of the most variable mtDNA regions: 1) ND1–ND2, 2) COI, and 3) COI–COII–ATP8 were selected as templates for primer design (Figure 1a). The target regions were selected considering a minimum length of 600 bp, at least three variable sites, and the absence of long homopolymeric sequences to reduce the possibility of noise appearance during deoxy-dinucleotide sequencing. Primer3 v.4.1.0 (Kõressaar et al., 2018) was used for primer design with the following parameters: a length of 18–22 nucleotides for each primer, a GC content between 40–60%, and a melting temperature (Tm) between 55–65°C. The amplification of non-target regions was evaluated with Primer-BLAST (Ye et al., 2012). Then, the potential formation of primer dimers was checked with MFE primer v. 3.1 (Wang et al., 2019). Finally, the three pairs of primers with the best features were chosen, one for each marker, and their sequences were sent to Macrogen (Seoul, South Korea) for synthesis (Appendix 2).

The three markers were independently amplified by the polymerase chain reaction (PCR) using a T100$^{TM}$ thermocycler (Bio–Rad Laboratories, CA, USA). The final volume of the PCR reaction was 25 µL, which included 1X PCR Buffer, 1.5 mM $MgCl_2$, 0.25 BSA mg/mL, 0.5 µM of each primer, 0.2 mM dNTPs, 1 U *Taq* DNA polymerase (Invitrogen, MA, USA), and 2 ng of DNA. The thermocycling conditions for all markers consisted of an initial denaturation step at 94°C for 5 min, followed by 35 cycles (denaturation: 30 s at 94°C, annealing: 30 s at 60°C, extension: 1 min at 72°C), and a final extension at 72°C for 5 min. PCR products were visualized on 1.5% (w/v) agarose gel through electrophoresis at 100 V for 30 min.

**2.4. Sequencing and data analysis**

The amplification products were sent for bidirectional Sanger sequencing with an ABI 3730XLs sequencer to Macrogen (Seoul, South Korea). A consensus sequence was generated

from the information of both strands (forward and reverse) for each sample using the programs PreGap4 and Gap4 from the Staden software package (Staden, 1996). Consensus sequences belonging to the same mtDNA region were aligned in MEGA v.11 (Tamura et al., 2021) with the Clustal algorithm, and non-informative ends were trimmed to equalize the length (bp) of the sequences. Two concatenated mtDNA sequences were obtained by joining the sequences of the markers: the first region includes ND1–ND2, COI (partial sequence), and COI–COII–ATP8, and the second region includes the HVR1 of the D–loop plus the three markers of the first region. Sequences from the HVR1 of the D–loop of bears analyzed in the DMQ were retrieved from Cueva et al. (2018) (GenBank accessions: KX812512–KX812515). Sequences from the same mtDNA region of individuals from Loja (GenBank accessions: MT425202) and Zamora Chinchipe (GenBank accessions: MZ191063 y MZ191064) were retrieved from Moreta (2020) and Vallejo (2021), respectively. The samples analyzed in the present study were previously analyzed in those studies. The concatenation of the sequences was performed in Geneious Prime 2023.1.1 software (Kearse et al., 2012).

Haplotype networks were obtained for the three markers and the concatenated regions in R v4.3.0 (R core Team, 2018), as previously described (Toparslan et al., 2020). The network generation method applied was statistical parsimony or TSC (Templeton et al., 1992) using the *haploNet* function of the *pegas* v.1.2 package (Paradis, 2023). The haplotype ($H_d$) and nucleotide ($\pi$) diversity indexes, the Fu's Fs (Fu,1997) and Tajima's D (Tajima, 1989) tests, and an analysis of molecular variance (AMOVA) were performed in Arlequin v.3.5.2.2 (Excoffier et al., 2005). A Maximum–Likelihood phylogenetic tree was constructed using MEGA v.11 (Tamura et al., 2021) with a Hasegawa–Kishino–Yano (HKY) substitution model (Hasegawa et al., 1985) as it was predicted to be the best substitution model. Finally, the DNA sequences were translated in Geneious Prime 2023.1.1 (Kearse et al., 2012) to evaluate if variations in nucleotides produced changes in the amino acid chains.

# 3. RESULTS

## 3.1. DNA concentration and quality

The concentration of the extracted DNA was low, ranging from 0 to 45.40 ng/µL, with an average of 8.82 ng/µL for all samples. Of all the samples, 78% had a concentration below 10 ng/µL. The average DNA concentration for the samples from Quito, Loja, and Zamora Chinchipe was 7.08 ng/µL, 15.68 ng/µL, and 6.71 ng/µL, respectively (Appendix 1).

Regarding the DNA quality indexes, the average value for the A260/A280 and A260/A230 ratios for all samples was 1.02 and 0.25, respectively. The average A260/A280 ratio was not within the established range (1.8–2.0) for a DNA sample free of protein contaminants or aromatic compounds (Thermo Fisher Scientific, 2016). However, the average A260/A230 ratio was significantly low, not within the range of 2.0–2.2, suggesting a high presence of organic contaminants of chaotropic nature or carbohydrates in the samples (Thermo Fisher Scientific, 2016). Despite the low concentrations and qualities of the obtained DNA, successful amplification of the samples for HVR1 of the D–loop was obtained.

## 3.2. Amplification of the three mitochondrial markers and sequencing of PCR products

In 19 of the 23 samples, all three markers were successfully amplified with the designed primers (Appendix 3). In samples where amplification was not achieved following the initial PCR protocol, bovine serum albumin (BSA) was added at a concentration of 0.25 mg/mL.

For the first marker, ND1–ND2, the expected fragment length was 805 bp, and after editing, all sequences remained at 745 bp. The second marker, COI (partial sequence), had an expected fragment length of 617 bp, but editing reduced it to 552 bp. The third marker, COI–COII–ATP8 had an expected fragment length of 1,082 bp, and after editing, the length was 972 bp (Table 1).

## 3.3. Genetic diversity and population genetic statistics

The sequence analysis of each mtDNA region revealed 2, 3, and 7 variable sites for ND1–ND2, COI (partial sequence), and COI–COII–ATP8, respectively (Figure 1a). For ND1–ND2 and COI (partial sequence), these nucleotide differences resulted in 2 haplotypes: one exclusive to the population of Quito and another shared by the populations of Loja and Zamora Chinchipe (Figure 1b, c). For COI–COII–ATP8, 3 haplotypes were obtained, one for each population. For this third marker, the population of Loja and Zamora Chinchipe exhibited a distinct haplotype; the haplotype of the Loja population differed from the haplotype of the Zamora Chinchipe by a single nucleotide change (Figure 1d). The Concatenated Region 1, which combines the sequences of these three regions (ND1–ND2 + COI (partial sequence) + COI–COII–ATP8), also presented 3 haplotypes (Figure 1e).

To obtain the Concatenated Region 2, the HVR1 sequences (HVR1 + ND1–ND2 + COI (partial sequence) + COI–COII–ATP8) were added to the Concatenated Region 1. The Concatenated Region 2 had 2,731 bp, contained 20 polymorphic sites, and resulted in 7 haplotypes (Tables 1–3). Three haplotypes were found in Quito (n=10), 2 in Loja (n=5), and 2 (n=8) in Zamora Chinchipe. No shared haplotypes were found among the three populations (Figure 2a). Of the total polymorphisms observed in the Concatenated Region 2, 17 were transitions (C⇔T or A⇔G), 2 were transversions (A⇔T and A⇔C), and one was an insertion/deletion (Table 3).

The genetic diversity analyses were conducted using the Concatenated Region 2, as this region revealed the presence of more than one haplotype within each population. The haplotype diversity ($H_d$) was higher for the bears of Quito (0.6222 ± 0.1383) compared to the Loja (0.4000 ± 0.2373) and Zamora Chinchipe (0.5714 ± 0.0945) bears. The population of Loja exhibited a higher nucleotide diversity ($\pi$) than the other two populations (0.0010 ± 0.0008). When considering all three populations as a single set representing Ecuadorian bears (Overall), the haplotype diversity ($H_d$) was 0.8617 ± 0.0352, and the nucleotide diversity ($\pi$) was 0.0036 ±

0.0019. The average of pairwise genetic differences ($\theta_\pi$) was 9.8103, which indicates that when analyzing the entire sequence of the Concatenated Region 2 (2,731 bp) from at least two individuals, an average of 10 differences between the nucleotides would be expected. The Tajima's D test resulted in a value of 3.0015 (p-value = 1), while the Fu's Fs test resulted in a value of 5.5330 (p-value = 0.9730). These statistics are used to evaluate population expansion or bottlenecks (Table 1). When analyzing the Concatenated Region 1, Tajima's D and Fu's Fs values could not be calculated for each population because all three populations exhibited only one haplotype. Only when adding the HVR1, some differences were found within populations as reflected in the Analysis of Molecular Variance (AMOVA). The AMOVA revealed that when analyzing only the three new mtDNA regions (Concatenated Region 1) the 100% of molecular variance was found between populations. When adding the HVR1 in the Concatenated Region 2, 92.33% of variation was found between populations and 7.67% within populations.

Finally, the Maximum–Likelihood phylogenetic tree indicated the existence of two monophyletic groups: the first included the populations of Loja and Zamora Chinchipe, and the second the population of Quito (Figure 3).

### 3.4. Amino acid substitutions

To assess if the polymorphisms identified in the ND1, ND2, COI, COII, and ATP8 genes have an impact on the protein amino acid sequence, the nucleotide sequences of all individuals were translated into amino acids using the mammalian mitochondrial genetic code as the mitochondria uses their own tRNA for translation. In the first analyzed region, which included only partial sequences of ND1 and ND2 genes, the two polymorphisms led to modifications in the amino acid sequence of the protein subunit ND2. Regarding the partial sequence of the COI gene, only one of the three polymorphisms identified resulted in modifications to the amino acid sequence of the protein subunit COI. Finally, within the COI–COII–ATP8 region, three

out of the seven polymorphisms detected caused changes in the residues of the protein subunit COII (Table 4).

# 4. DISCUSSION

## 4.1. Genetic diversity and population structure

In this study, three haplotypes were found in 23 bears from three different populations in Ecuador (Quito, Loja, and Zamora Chinchipe). One haplotype was found per population by concatenating three regions of mtDNA (Concatenated Region 1) which covered five genes (ND1, ND2, COI, COII, and ATP8) (Figure 1e). This result suggests a lack of genetic diversity within each population, as haplotype and nucleotide diversity were equal to zero (Table 1). However, it may not be appropriate to conclude based on this result due to the number of individuals analyzed in each population.

To enhance the analysis of mitochondrial genetic diversity, the HVR1 sequence of the D–loop previously studied by Cueva et al. (2018), Moreta (2020), and Vallejo (2021) was added in this study. As a result, the Concatenated Region 2 was obtained, which revealed a larger number of haplotypes within each population. This allowed for subsequent calculations of population genetic diversity and structure (Table 1).

The appearance of additional haplotypes when including HVR1 sequences can be attributed to its higher mutation rate when compared to coding parts of the mitochondrial DNA. The D–loop region, which contains HVR1, is characterized by rapid evolution and high genetic variation, as there is no selection pressure due to its lack of coding function. Thus, it is a useful marker for detecting genetic diversity within populations (Gatt et al., 2000). In contrast, the sequences of the three mitochondrial markers designed in this study included fragments from 5 genes (ND1, ND2, COI, COII, and ATP8) involved in cellular respiration. These genes have a low rate of evolution, given their importance for the viability of an organism (Rackham & Filipovska, 2022; Sari et al., 2015; Huang et al., 2008). Therefore, the number of observable polymorphisms may be significantly lower as important genes are subject to purifying selection (Ma et al., 2014; Palozzi et al., 2018; Stewart et al., 2008).

Haplotype diversity ($H_d$) is the probability that two individuals will have a different haplotype if selected at random (Lowe et al., 2004). Previous studies conducted in the populations of Quito, Loja, and Zamora Chinchipe, which only analyzed the HVR1 of D–loop in mtDNA, reported haplotype diversities of 0.71, 0.53, and 0.50, respectively (Cueva et al., 2018; Moreta, 2020; Vallejo, 2021). These values are higher than ones reported in this study (Quito: $H_d = 0.62$; Loja: $H_d = 0.40$), except for the Zamora Chinchipe population ($H_d = 0.57$). However, it is important to note that the number of individuals sampled in the three mentioned studies was larger compared to this study (Quito: 38 vs. 10 samples; Loja: 26 vs. 5 samples; Zamora Chinchipe: 14 vs. 8 samples). Nevertheless, when all samples were analyzed as a single set (Overall), the haplotype diversity is close (previous studies: $H_d = 0.83$; this study: $H_d = 0.86$) (Cueva et al., 2018; Moreta, 2020; Vallejo, 2021).

Nucleotide diversity ($\pi$) refers to the average number of differences per nucleotide site between pairs of analyzed DNA sequences (Nei & Li, 1979). Regarding this parameter, the three analyzed bear populations showed lower nucleotide diversity values compared to previous studies (Cueva et al., 2018; Moreta, 2020; Vallejo, 2021). Similarly, when all individuals were treated as a single group, the nucleotide diversity was also lower than the previously reported values (previous studies: $\pi = 0.0078$; this study: $\pi = 0.0036$) (Cueva et al., 2018; Moreta, 2020; Vallejo, 2021).

The differences observed in haplotype and nucleotide diversity between previous studies that exclusively examined the HVR1 and this study that analyzed a region that combines sequences from the HVR1, ND1–ND2, COI (partial sequence), and COI–COII–ATP8 (Concatenated Region 2) markers could be attributed to the number of polymorphic sites identified and the sample size. Estimates of $H_d$ and $\pi$ can be artificially inflated or deflated due to a low number of sampled individuals, as seen in this study (Nei & Li, 1979). For example, for the COI gene, $H_d$ may be biased by the limited number of samples when the sequence

variation among individuals in the focal population is low, while π may be affected by subsampling when the focal population is highly diverse (Goodall-Copestake et al., 2012).

Despite the high haplotype diversity found ($H_d$ = 0.86) in this study when analyzing the Concatenated Region 2 without distinguishing populations, the low nucleotide diversity value ($\pi$ = 0.0036) indicates few differences among haplotypes. The results also suggest the presence of genetic structure within the populations of the northern and southern parts of the country. This is because no haplotype was shared between populations, suggesting that they are genetically different from each other. This claim is supported by the AMOVA test, in which more than 92% of the observed variation is distributed among populations, rather than within them (8%).

**4.2. Demographic events**

Neutrality tests allow for the evaluation of possible scenarios of population expansion or decline. Evolutionary neutrality states that most mutations that affect genetic variability are neutral and not adaptive (Li et al., 2003). The statistics most used are Tajima's D and Fu's Fs tests. In both statistics, positive values indicate a possible population decline, while negative values indicate a possible population expansion (de Jong et al., 2011). For the analyzed region, positive values were obtained for both Tajima's D (3.0015) and Fu's Fs (5.5330) when analyzing all the individuals. However, no value was statistically significant, and it is not possible to establish accurate conclusions based on these results.

**4.3. Changes in the amino acid sequence**

In the present study, it was found that out of the 12 polymorphisms found in regions that encompass 5 mitochondrial genes (ND1, ND2, COI, COII, and ATP8), only 7 produced alterations in the amino acid sequence coded by the ND2, COI, and COII genes. The amino acid substitutions described in this study are not expected to affect the enzymatically active part of the proteins, where mutations could be lethal (Pentinsaari et al., 2016).

The genes that code for mitochondrial proteins are mostly under purifying selection (Ma et al., 2014; Palozzi et al., 2018; Stewart et al., 2008). In the case of the cytochrome C oxidase (COX) protein, it is a dimer formed by two identical parts, which are composed of several amino acid chains, 3 of mitochondrial coding (COI, COII, and COIII) and 11 of nuclear coding in mammals (Balsa et al., 2012; Fernández-Vizarra et al., 2009). COX is the last enzyme in the electron transport chain, and thus alterations in its amino acid sequence can affect the energy metabolism, especially if they are close to the enzymatically active sites of the protein (Pesole et al., 1999). However, studies that have analyzed the differences between the amino acid chain of this protein show that of the 219 amino acids, 99 are highly variable within the metazoans (Pentinsaari et al., 2016). Most of the variation occurs in sites far from the active site of the protein and therefore it is unlikely to affect its functionality (Pentinsaari et al., 2016).

### 4.4. Genetic structure and its implications for the conservation of the Andean bear

The analysis of genetic diversity within a population is crucial for understanding its ecology and establishing effective conservation plans.

The results obtained in this research suggest that each bear population analyzed (Quito, Loja, and Zamora Chinchipe) has a different genetic structure. The evidence of population structure within a species can affect the its conservation programs since it implies that, due to their differences, each population should be managed as a separate conservation unit (Dalapicolla et al., 2021). Possible explanations for the genetic structure among populations could include: the fidelity of females to a single site for much of their lives, the presence of geographic barriers, the habitat fragmentation due to human activities, or the presence of different types of predominant vegetation in the areas inhabited by each population (García-Rangel, 2012; Hernani-Lineros et al., 2020; Kattan et al., 2004; Ruiz-García et al., 2020a).

# 5. CONCLUSIONS

A new haplotype is reported for the population of Andean bears in Loja through the analysis of the Concatenated Region 2, which includes the sequences of the markers HVR1, ND1–ND2, COI (partial sequence), and COI–COII–ATP8. These three regions, plus the HVR1 of the D–loop, produced a concatenated region of 2,731 bp that showed 20 variable sites in the sequences of all the individuals analyzed.

A high genetic diversity was obtained at the haplotype level ($H_d = 0.86 \pm 0.04$), but a low nucleotide diversity ($\pi = 0.0036 \pm 0.0019$). The AMOVA test determined that most of the variation found in the analyzed sequences was due to interspecific differences between populations.

Neutrality tests were positive for Tajima's D and Fu's Fs, with values of 3.0015 and 5.5330, respectively. However, none of the values were statistically significant.

It is suggested to improve conservation efforts for this species by taking into consideration the genetic differences of each population, as well as the variability of the ecosystems where it inhabits. Additionally, for future studies, it is recommended to increase sampling efforts in other populations of Andean bears throughout the country and to implement genomic studies.

## 6. TABLES

**Table 1. Genetic diversity parameters and neutrality tests for each mitochondrial marker and two concatenated regions.**

| Mitochondrial Marker (bp) | Population (n) | H | Haplotype diversity ($H_d \pm sd$) | Nucleotide diversity ($\pi \pm sd$) | Tajima's $D^+$ | p-value | Fu's $Fs^+$ | p-value |
|---|---|---|---|---|---|---|---|---|
| *ND1–ND2* (732) | Overall (23) [X] | 2 | 0.514 ± 0.041 | 0.001 ± 0.001 | 2.000 | 0.983 | 3.170 | 0.919 |
| *COI (partial sequence)* (565) | Overall (23) | 2 | 0.514 ± 0.041 | 0.003 ± 0.002 | 2.286 | 0.993 | 4.526 | 0.977 |
| *COI–COII–ATP8* (972) | Overall (23) | 3 | 0.672 ± 0.045 | 0.004 ± 0.002 | 2.571 | 0.997 | 5.969 | 0.985 |
| *Concatenated Region 1* (2269)* ND1–ND2 + COI (partial sequence) + COI–COII–ATP8 | Overall (23) | 3 | 0.672 ± 0.045 | 0.003 ± 0.001 | 2.939 | 0.998 | 9.708 | 0.997 |
| *Concatenated Region 2* (2731)* HVR1 + ND1–ND2 + COI (partial sequence) + COI–COII–ATP8 | DMQ (10) | 3 | 0.6222 ± 0.1383 | 0.0003 ± 0.0002 | 0.0150 | 0.7360 | –0.1561 | 0.3340 |
| | Loja (5) | 2 | 0.4000 ± 0.2373 | 0.0010 ± 0.0008 | –1.1455 | 0.0500 | 3.3664 | 0.9180 |
| | Zamora (8) | 2 | 0.5714 ± 0.0945 | 0.0002 ± 0.0002 | 0.0000 | 1.0000 | 0.9664 | 0.6180 |
| | Overall (23) | 7 | 0.8617 ± 0.0352 | 0.0036 ± 0.0019 | 3.0015 | 1.0000 | 5.5330 | 0.9730 |

**bp**, final length after trimming the sequences.

*****The two concatenated regions resulted from the union of the sequences of the three and four mitochondrial markers analyzed in the Andean bears mtDNA, respectively.

[X]Analysis of the three markers separately and the Concatenated Region 1 for the three populations resulted in zero haplotype and nucleotide diversity; the values changed by not separating the samples by population (Overall).

[+] None of the values of the Tajima's D and Fu's Fs tests presented statistical significance.

**Table 2. Sequence of the haplotypes found in Concatenated Region 2, and number of individuals per haplotype in each population.**

| Haplotype | Population (n) | Concatenated Region 2 |
|---|---|---|
| HTOQ1 | Quito (6) | TTTACTTATTTCATACATATCATCCCACGTACTGTAGCATCCTAGTATGTCCCCGAACAAGGAAACCTTTCTTTTTTTT–CCCCCCTATGTACGTCGTG<br>CATTAATGGCGTGCCCCATGCATATAAGCATGTACATATCTTGCTTGGCTTTACATGAGGACATGGACTTCAAAAACTCGTTTTGAAGACGTAGTCTG<br>TAAGCATGTATTTCACTTAGTCCGGGAGCTTAATCACCAGGCCTCGAGAAACCAGCAACCCTTGCGAGTACGTATACCTCTTCTCGCTCCGGGCCCA<br>TAGAAACGTGGGGGTTTCTATACTGAAACTATACCTGGCATCTGGTTCTTACTTCAGGGCCATGATAGCTCTAGATTCCAATCCTACTAACCCTTCAA<br>ATGGGACATCTCGATGGACTAATGACTAATCAGCCCATGATCACACATAACTGTGGTGTCATGCATTTGGGCATATGGTACGTAACTCTACCTATTAT<br>TACAGCAAGCATCCCTCCCCAAACATAAGAAATATGTCTGACAAAAGAGTTACTTTGATAGAGTAAATAATAGAGGTTTAAATCCTCTTATTTCTAGA<br>ATTATAGGAATCGAACCTAATCTTAAGAATCCAAAAATCTTCGTGCTACCTGATTACACCACATCCTAGAGTAAGGTCAGCTAAATAAGCTATCGGGC<br>CCATACCCCGAAAATGTTGGTTTATCCCCTTCCCATACTAATAAAACCTCCCATTCTTACTATTATTATATCCACCATCGCTCTAGGAACCATAATCGT<br>TCTATTTAGCTCCCACTGATTTATAATCTGAATCGGTTTTGAAATAAATATACTGGCAATTATTCCCATTTTAATGAAAAAATTTAACCCACGAGCCGT<br>AGAAGCCTCCACAAAATATTTTCTAACACAGGCCACTGCATCCATGCTCCTCATACTAGGCATTATTATCAACTTACTATACTCAGGACACTGAACAA<br>TCTCAACAACTCTCAACCCAATCGCATCAACCGTGATCACCATTGCCCTAGCGATAAAACTTGGCCTATCTCCCTTCCACTTCTGAGTTCCAGAAGTT<br>ACACAAGGCATTCCCCTATCCTCAGGAGTAATCCTACTAACATGACAGAAAATCGCACCCTTGTCCGTTCTATACCAGATCTCACCATTCATTAACCC<br>AAACTTATTAATAACAATGGCTATCGCATACAAACCATAAAGACATTGGTACTCTCTATCTCTTATTCGGTGCATGAGCCGGAATAGTAGGTACTGCC<br>CTCAGCCTTCTAATCCGCGCTGAACTAGGTCAACCCGGAGCCCTGTTAGGGGATGATCAGATCTATAACGTGGTCGTAACTGCCCATGCATTCGTAA<br>TAATCTTCTTTATAGTAATGCCTATTATAATCGGAGGTTTTGGAAACTGATTAGTGCCTTTAATAATTGGCGCTCCCGATATAGCATTCCCTCGAATA<br>AACAATATGAGTTTCTGATTACTGCCACCATCCTTCCTACTTCTTCTAGCCTCTTCCATAGTAGAAGCAGGTGCAGGGACTGGTTGAACCGTTTACCC<br>CCCTCTAGCGGGCAATCTAGCCCATGCAGGAGCATCAGTAGACCTAACAATTTTTTCCCTACATTTAGCAGGCGTTTCCTCCATTCTAGGAGCTATTA<br>ACTTTATTACCACTATTATCAATATGAAGCCTCCTGCAATATCTCAATACCAAACTCCTCTGTTCGTATGATCCGTCCTAATTACGGCGGTAAGGAGT<br>CGAACCCCCTGAAATTGGTTTCAAGCCAATACCATAACCACTATGTCTCTCTCAATAAAGAGATATTAGTAAAAATTACATAACTTCGTCAGGGTTAA<br>ATTATAGGTGAAAATCCTTTATATCTCTATGGCGTATCCCCTTCAAATAGGCCTTCAAGACGCAACTTCTCCCATTATAGAAGAGCTCCTACACTTCC<br>ACGACCATACATTAATGATTGTATTCCTGATTAGCTCCTTAGTTCTCTACATTATTTCAACTATGCTAACTACTAAACTAACGCACACAAACACAATAA<br>ATGCACAGGAAGTAGAAACGGTATGAACCATTCTACCAGCCATTATCCTGGTTCTAATTGCACTCCCATCATTACGAATCCTCTATATAATGGATGAA<br>ATCAACAATCCTTTACTGACTGTAAAAACTATAGGCCATCAATGATACTGAAGTTACGAATACACGGACTATGAAGATTTGAGCTTTGATTCTTACAT<br>GATTCCAACACAAGAATTAAAGCCTGGAGAACTACGACTATTAGAAGTAGACAATCGAGCGGTACTACCCATAGAAATGACTATTCGCATGCTAATT<br>TCATCAGAAGATGTCTTGCACTCATGAGCTGTACCATCCCTAGGACTAAAAACTGATGCAATTCCGGGACGACTAAACCAAACGACTCTCATGGCCA<br>TGCGACCAGGGCTGTATTATGGCCAATGCTCGGAAATCTGTGGCTCTAACCACAGCTTCATGCCCATTGTTCTCGAACTAGTCCCACTGTCCTATTTC<br>GAAAAATGATCCGCTTCAATATTATAAAATCATTAAGAAGCTATATAGCATTAACCTTTTAAGTTAAAGACTGAGAGTGCAAGTCTCTCCTTAATGGA<br>GATGCCACAACTAGACACGTCGACATGGTTTATCACAATTCTATCTATAACTCTAACACTATTTATTGTATTCCAACTAAAAATC |
| HTOQ2 | Quito (2) | TTTACTTATTTCATACATATCATCCCACGTACTGTAGCATCCTAGTATGTCCCCGAACAAGGAAACCTTTCTTTTTTTT–CCCCCCTATGTACGTCGTG<br>CATTAATGGCGTGCCCCATGCATATAAGCATGTACATATCTTGCTTGGCTTTACATGAGGACATGGACTTCAAAAACTCGTTTTGAAGACGTAGTCTG<br>TAAGCATGTATTTCACTTAGTCCGGGAGCTTAGTCACCAGGCCTCGAGAAACCAGCAACCCTTGCGAGTACGTATACCTCTTCTCGCTCCGGGCCCA<br>TAGAAACGTGGGGGTTTCTATACTGAAACTATACCTGGCATCTGGTTCTTACTTCAGGGCCATGATAGCTCTAGATTCCAATCCTACTAACCCTTCAA<br>ATGGGACATCTCGATGGACTAATGACTAATCAGCCCATGATCACACATAACTGTGGTGTCATGCATTTGGGCATATGGTACGTAACTCTACCTATTAT<br>TACAGCAAGCATCCCTCCCCAAACATAAGAAATATGTCTGACAAAAGAGTTACTTTGATAGAGTAAATAATAGAGGTTTAAATCCTCTTATTTCTAGA<br>ATTATAGGAATCGAACCTAATCTTAAGAATCCAAAAATCTTCGTGCTACCTGATTACACCACATCCTAGAGTAAGGTCAGCTAAATAAGCTATCGGGC<br>CCATACCCCGAAAATGTTGGTTTATCCCCTTCCCATACTAATAAAACCTCCCATTCTTACTATTATTATATCCACCATCGCTCTAGGAACCATAATCGT<br>TCTATTTAGCTCCCACTGATTTATAATCTGAATCGGTTTTGAAATAAATATACTGGCAATTATTCCCATTTTAATGAAAAAATTTAACCCACGAGCCGT |

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| | | AGAAGCCTCCACAAAATATTTTCTAACACAGGCCACTGCATCCATGCTCCTCATACTAGGCATTATTATCAACTTACTATACTCAGGACACTGAACAA<br>TCTCAACAACTCTCAACCCAATCGCATCAACCGTGATCACCATTGCCCTAGCGATAAAACTTGGCCTATCTCCCTTCCACTTCTGAGTTCCAGAAGTT<br>ACACAAGGCATTCCCCTATCCTCAGGAGTAATCCTACTAACATGACAGAAAATCGCACCCTTGTCCGTTCTATACCAGATCTCACCATTCATTAACCC<br>AAACTTATTAATAACAATGGCTATCGCATACAAACCATAAAGACATTGGTACTCTCTATCTCTTATTCGGTGCATGAGCCGGAATAGTAGGTACTGCC<br>CTCAGCCTTCTAATCCGCGCTGAACTAGGTCAACCCGGAGCCCTGTTAGGGGATGATCAGATCTATAACGTGGTCGTAACTGCCCATGCATTCGTAA<br>TAATCTTCTTTATAGTAATGCCTATTATAATCGGAGGTTTTGGAAACTGATTAGTGCCTTTAATAATTGGCGCTCCCGATATAGCATTCCCTCGAATA<br>AACAATATGAGTTTCTGATTACTGCCACCATCCTTCCTACTTCTTCTAGCCTCTTCCATAGTAGAAGCAGGTGCAGGGACTGGTTGAACCGTTTACCC<br>CCCTCTAGCGGGCAATCTAGCCCATGCAGGAGCATCAGTAGACCTAACAATTTTTTCCCTACATTTAGCAGGCGTTTCCTCCATTCTAGGAGCTATTA<br>ACTTTATTACCACTATTATCAATATGAAGCCTCCTGCAATATCTCAATACCAAACTCCTCTGTTCGTATGATCCGTCCTAATTACGGCGGTA**AGGAGT**<br>**CGAACCCCCTGAAATTGGTTTCAAGCCAATACCATAACCACTATGTCTCTCTCAATAAAGAGATATTAGTAAAAATTACATAACTTCGTCAGGGTTAA**<br>**ATTATAGGTGAAAATCCTTTATATCTCTATGGCGTATCCCCTTCAAATAGGCCTTCAAGACGCAACTTCTCCCATTATAGAAGAGCTCCTACACTTCC**<br>**ACGACCATACATTAATGATTGTATTCCTGATTAGCTCCTTAGTTCTCTACATTATTTCAACTATGCTAACTACTAAACTAACGCACACAAACACAATAA**<br>**ATGCACAGGAAGTAGAAACGGTATGAACCATTCTACCAGCCATTATCCTGGTTCTAATTGCACTCCCATCATTACGAATCCTCTATATAATGGATGAA**<br>**ATCAACAATCCTTTACTGACTGTAAAAACTATAGGCCATCAATGATACTGAAGTTACGAATACACGGACTATGAAGATTTGAGCTTTGATTCTTACAT**<br>**GATTCCAACACAAGAATTAAAGCCTGGAGAACTACGACTATTAGAAGTAGACAATCGAGCGGTACTACCCATAGAAATGACTATTCGCATGCTAATT**<br>**TCATCAGAAGATGTCTTGCACTCATGAGCTGTACCATCCCTAGGACTAAAAACTGATGCAATTCCGGGACGACTAAACCAAACGACTCTCATGGCCA**<br>**TGCGACCAGGGCTGTATTATGGCCAATGCTCGGAAATCTGTGGCTCTAACCACAGCTTCATGCCCATTGTTCTCGAACTAGTCCCACTGTCCTATTTC**<br>**GAAAAATGATCCGCTTCAATATTATAAAATCATTAAGAAGCTATATAGCATTAACCTTTTAAGTTAAAGACTGAGAGTGCAAGTCTCTCCTTAATGGA**<br>**GATGCCACAACTAGACACGTCGACATGGTTTATCACAATTCTATCTATAACTCTAACACTATTTATTGTATTCCAACTAAAAATC** |
| HTOQ3 | Quito (2) | **TTTACTTATTTCATACATATCATCCCACGTACTGTAGCATCCTAGTATGTCCCCGAACAAGGAAACCTTTCTTTTTTTTTCCCCCCTATGTACGTCGTG**<br>**CATTAATGGCGTGCCCCATGCATATAAGCATGTACATATCTTGCTTGGCTTTACATGAGGACATGGACTTCAAAAACTCGTTTTGAAGACGTAGTCTG**<br>**TAAGCATGTATTTCACTTAGTCCGGGAGCTTAATCACCAGGCCTCGAGAAACCAGCAACCCTTGCGAGTACGTATACCTCTTCTCGCTCCGGGCCCA**<br>**TAGAAACGTGGGGGTTTCTATACTGAAACTATACCTGGCATCTGGTTCTTACTTCAGGGCCATGATAGCTCTAGATTCCAATCCTACTAACCCTTCAA**<br>**ATGGGACATCTCGATGGACTAATGACTAATCAGCCCATGATCACACATAACTGTGGTGTCATGCATTTGG**GCATATGGTACGTAACTCTACCTATTAT<br>TACAGCAAGCATCCCTCCCCAAACATAAGAAATATGTCTGACAAAAGAGTTACTTTGATAGAGTAAATAATAGAGGTTTAAATCCTCTTATTTCTAGA<br>ATTATAGGAATCGAACCTAATCTTAAGAATCCAAAAATCTTCGTGCTACCTGATTACACCACATCCTAGAGTAAGGTCAGCTAAATAAGCTATCGGGC<br>CCATACCCCGAAAATGTTGGTTTATCCCCTTCCCATACTAATAAAACCTCCCATTCTTACTATTATTATATCCACCATCGCTCTAGGAACCATAATCGT<br>TCTATTTAGCTCCCACTGATTTATAATCTGAATCGGTTTTGAAATAAATATACTGGCAATTATTCCCATTTTAATGAAAAAATTTAACCCACGAGCCGT<br>AGAAGCCTCCACAAAATATTTTCTAACACAGGCCACTGCATCCATGCTCCTCATACTAGGCATTATTATCAACTTACTATACTCAGGACACTGAACAA<br>TCTCAACAACTCTCAACCCAATCGCATCAACCGTGATCACCATTGCCCTAGCGATAAAACTTGGCCTATCTCCCTTCCACTTCTGAGTTCCAGAAGTT<br>ACACAAGGCATTCCCCTATCCTCAGGAGTAATCCTACTAACATGACAGAAAATCGCACCCTTGTCCGTTCTATACCAGATCTCACCATTCATTAACCC<br>AAACTTATTAATAACAATGGCTATCGCATACAAACCATAAAGACATTGGTACTCTCTATCTCTTATTCGGTGCATGAGCCGGAATAGTAGGTACTGCC<br>CTCAGCCTTCTAATCCGCGCTGAACTAGGTCAACCCGGAGCCCTGTTAGGGGATGATCAGATCTATAACGTGGTCGTAACTGCCCATGCATTCGTAA<br>TAATCTTCTTTATAGTAATGCCTATTATAATCGGAGGTTTTGGAAACTGATTAGTGCCTTTAATAATTGGCGCTCCCGATATAGCATTCCCTCGAATA<br>AACAATATGAGTTTCTGATTACTGCCACCATCCTTCCTACTTCTTCTAGCCTCTTCCATAGTAGAAGCAGGTGCAGGGACTGGTTGAACCGTTTACCC<br>CCCTCTAGCGGGCAATCTAGCCCATGCAGGAGCATCAGTAGACCTAACAATTTTTTCCCTACATTTAGCAGGCGTTTCCTCCATTCTAGGAGCTATTA<br>ACTTTATTACCACTATTATCAATATGAAGCCTCCTGCAATATCTCAATACCAAACTCCTCTGTTCGTATGATCCGTCCTAATTACGGCGGTA**AGGAGT**<br>**CGAACCCCCTGAAATTGGTTTCAAGCCAATACCATAACCACTATGTCTCTCTCAATAAAGAGATATTAGTAAAAATTACATAACTTCGTCAGGGTTAA**<br>**ATTATAGGTGAAAATCCTTTATATCTCTATGGCGTATCCCCTTCAAATAGGCCTTCAAGACGCAACTTCTCCCATTATAGAAGAGCTCCTACACTTCC**<br>**ACGACCATACATTAATGATTGTATTCCTGATTAGCTCCTTAGTTCTCTACATTATTTCAACTATGCTAACTACTAAACTAACGCACACAAACACAATAA**<br>**ATGCACAGGAAGTAGAAACGGTATGAACCATTCTACCAGCCATTATCCTGGTTCTAATTGCACTCCCATCATTACGAATCCTCTATATAATGGATGAA**<br>**ATCAACAATCCTTTACTGACTGTAAAAACTATAGGCCATCAATGATACTGAAGTTACGAATACACGGACTATGAAGATTTGAGCTTTGATTCTTACAT** |

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| | | GATTCCAACACAAGAATTAAAGCCTGGAGAACTACGACTATTAGAAGTAGACAATCGAGCGGTACTACCCATAGAAATGACTATTCGCATGCTAATT<br>TCATCAGAAGATGTCTTGCACTCATGAGCTGTACCATCCCTAGGACTAAAAACTGATGCAATTCCGGGACGACTAAACCAAACGACTCTCATGGCCA<br>TGCGACCAGGGCTGTATTATGGCCAATGCTCGGAAATCTGTGGCTCTAACCACAGCTTCATGCCCATTGTTCTCGAACTAGTCCCACTGTCCTATTTC<br>GAAAAATGATCCGCTTCAATATTATAAAATCATTAAGAAGCTATATAGCATTAACCTTTTAAGTTAAAGACTGAGAGTGCAAGTCTCTCCTTAATGGA<br>GATGCCACAACTAGACACGTCGACATGGTTTATCACAATTCTATCTATAACTCTAACACTATTTATTGTATTCCAACTAAAAATC |
| HTOL1 | Loja (4) | TTTACTTATTTCATATATATCATCCCACGTACTGTAGCATCCTAGTATGTCCCCGAACAAGGAAACCTTTCTTTTTTTT–CCCCCCTATGTACGTCGTG<br>CATTAATGGCGTGCCCCATGCATATAAGCATGTACATATCTTGCTTGGTCTTACATGAGGACATGGACTTCAAAAACTCGTTCTGAAGACGTAGTCTG<br>CAAGCATGTATTTCACTTAGTCCGGGAGCTTAGTCACCAGGCCTCGAGAAACCAGCAACCCTTGCGAGTACGTATACCTCTTCTCGCTCCGGGCCCA<br>TAGAAACGTGGGGGTTTCTATACTGAAACTATACCTGGCATCTGGTTCTTACTTCAGGGCCATGATAGCTCTAGATTCCAATCCTACTAACCCTTCAA<br>ATGGGACATCTCGATGGACTAATGACTAATCAGCCCATGATCACACATAACTGTGGTGTCATGCATTTGGGCATATGGTACGTAACTCTACCTATTAT<br>TACAGCAAGCATCCCTCCCCAAACATAAGAAATATGTCTGACAAAAGAGTTACTTTGATAGAGTAAATAATAGAGGTTTAAATCCTCTTATTTCTAGA<br>ATTATAGGAATCGAACCTAATCTTAAGAATCCAAAAATCTTCGTGCTACCTGATTACACCACATCCTAGAGTAAGGTCAGCTAAATAAGCTATCGGGC<br>CCATACCCCGAAAATGTTGGTTTATCCCCTTCCCATACTAATAAATCCTCCCATTCTTACTATTATTATATCCACCATCGCTCTAGGAACCATAATCGT<br>TCTATTTAGCTCCCACTGATTTATAATCTGAATCGGTTTTGAAATAAATATACTGGCAATTATTCCCATTTTAATGAAAAAATTTAACCCACGAGCCGT<br>AGAAGCCTCCACAAAATATTTTCTAACACAGGCCACTGCATCCATGCTCCTCATACTAGGCATTATTATCAACTTACTATACTCAGGACACTGAACAA<br>TCTCAACAACTCTCAACCCAATCGCATCAACCGTGATCACCATTGCCCTAGCGATAAAACTTGGCCTATCTCCCTTCCACTTCTGAGTTCCAGAAGTT<br>ACACAAGGCATTCCCCTATCCTCAGGAGTAATCCTACTAACATGACAGAAAATCGCACCCTTGTCCGTTCTATACCAGATCTCACCATTCATTAACCC<br>AAACTTATTAATAACAATGGCTATCACATACAAACCATAAAGACATTGGTACTCTCTATATCTTATTCGGTGCATGAGCCGGAATAGTAGGTACTGCC<br>CTCAGCCTTCTAATCCGCGCTGAACTAGGTCAACCCGGAGCCCTGTTAGGGGATGATCAAATCTATAACGTGGTCGTAACTGCCCATGCATTCGTAA<br>TAATCTTCTTTATAGTAATGCCTATTATAATCGGAGGTTTTGGAAACTGATTAGTGCCTTTAATAATTGGCGCTCCCGATATAGCATTCCCTCGAATA<br>AACAATATGAGTTTCTGATTACTGCCACCATCCTTCCTACTTCTTCTAGCCTCTTCCATAGTAGAAGCAGGTGCAGGGACTGGTTGAACTGTTTACCC<br>CCCTCTAGCGGGCAATCTAGCCCATGCAGGAGCATCAGTAGACCTAACAATTTTTTCCCTACATTTAGCAGGCGTTTCCTCCATTCTAGGAGCTATTA<br>ACTTTATTACCACTATTATCAATATGAAGCCTCCTGCAATATCTCAATACCAAACTCCTCTGTTCGTATGATCCGTCCTAATTACGGCGGTAAGGAGT<br>CGAACCCCCTGAAATTGGTTTCAAGCCAATACCATAACCACTATGTCTCTCTCAATAAAGAGATATTAGTAAAAATTACATAACTTCGTCAGGGTTAA<br>ATTATAGGTGAAAATCCTTTATATCTCTATGGCGTATCCCTTTCAAATAGGCCTTCAAGACGCAACTTCTCCCATTATAGAAGAGCTCCTACACTTCC<br>ACGACCATACATTAATGATTGTATTCCTGATTAGCTCTTTAGTTCTCTACATTATTTCAACTATGCTAACTACTAAACTAACGCACACAAACACAATAG<br>ATGCACAGGAAGTAGAAACGGTATGAACCATTCTACCAGCCATTATCCTGGTTCTAATTGCACTCCCATCATTACGAATCCTCTATATAATGGATGAA<br>ATCAACAATCCTTTACTGACTGTAAAAACTATAGGCCATCAATGATACTGAAGTTACGAATATACGGACTATGAAGATTTGAACTTTGATTCTTACAT<br>GATTCCAACACAAGAATTAAAGCCTGGAGAACTACGACTATTAGAAGTAGACAATCGAGCGGTACTACCCATAGAAATGACTATTCGCATGCTAATT<br>TCATCAGAAGATGTCTTGCACTCATGAGCTGTACCATCCCTAGGACTAAAAACTGATGCAATTCCGGGACGACTAAACCAAACGACTCTCATGGCCA<br>TGCGACCAGGGCTGTATTATGGCCAATGCTCGGAAATCTGTGGCTCTAACCACAGCTTCATGCCTATTGTTCTCGAACTAGTCCCACTGTCCTATTTC<br>GAAAAATGATCCGCTTCAATATTATAAAATCATTAAGAAGCTATATAGCATTAACCTTTTAAGTTAAAGACTGAGAGTGCAAGTCTCTCCTTAATGGA<br>GATGCCACAACTAGACACGTCAACATGGTTTATCACAATTCTATCTATAACTCTAACACTATTTATTGTATTCCAACTAAAAATC |
| HTOL2* | Loja (1) | TTTACTTATTTCATACATATCATCCCACGTACTGTAGCATCCTAGTATGTCCCCGAACAAGGAAACCTTTCTTTTTTTTTCCCCCCTATGTACGTCGTG<br>CATTAATGGCGTGCCCCATGCATATAAGCATGTACATATCTTGCTTGGCTTTACATGAGGACATGGACTTCAAAAACTCGTTTTGAAGACGTAGTCTG<br>TAAGCATGTATTTCACTTAGTCCGGGAGCTTAATCACCAGGCCTCGAGAAACCAGCAACCCTTGCGAGTACGTATACCTCTTCTCGCTCCGGGCCCA<br>TAGAAACGTGGGGGTTTCTATACTGAAACTATACCTGGCATCTGGTTCTTACTTCAGGGCCATGATAGCTCTAGATTCCAATCCTACTAACCCTTCAA<br>ATGGGACATCTCGATGGACTAATGACTAATCAGCCCATGATCACACATAACTGTGGTGTCATGCATTTGGGCATATGGTACGTAACTCTACCTATTAT<br>TACAGCAAGCATCCCTCCCCAAACATAAGAAATATGTCTGACAAAAGAGTTACTTTGATAGAGTAAATAATAGAGGTTTAAATCCTCTTATTTCTAGA<br>ATTATAGGAATCGAACCTAATCTTAAGAATCCAAAAATCTTCGTGCTACCTGATTACACCACATCCTAGAGTAAGGTCAGCTAAATAAGCTATCGGGC<br>CCATACCCCGAAAATGTTGGTTTATCCCCTTCCCATACTAATAAATCCTCCCATTCTTACTATTATTATATCCACCATCGCTCTAGGAACCATAATCGT<br>TCTATTTAGCTCCCACTGATTTATAATCTGAATCGGTTTTGAAATAAATATACTGGCAATTATTCCCATTTTAATGAAAAAATTTAACCCACGAGCCGT |

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| | | AGAAGCCTCCACAAAATATTTTCTAACACAGGCCACTGCATCCATGCTCCTCATACTAGGCATTATTATCAACTTACTATACTCAGGACACTGAACAA<br>TCTCAACAACTCTCAACCCAATCGCATCAACCGTGATCACCATTGCCCTAGCGATAAAACTTGGCCTATCTCCCTTCCACTTCTGAGTTCCAGAAGTT<br>ACACAAGGCATTCCCCTATCCTCAGGAGTAATCCTACTAACATGACAGAAAATCGCACCCTTGTCCGTTCTATACCAGATCTCACCATTCATTAACCC<br>AAACTTATTAATAACAATGGCTATCACATACAAACCATAAAGACATTGGTACTCTCTATATCTTATTCGGTGCATGAGCCGGAATAGTAGGTACTGCC<br>CTCAGCCTTCTAATCCGCGCTGAACTAGGTCAACCCGGAGCCCTGTTAGGGGATGATCAAATCTATAACGTGGTCGTAACTGCCCATGCATTCGTAA<br>TAATCTTCTTTATAGTAATGCCTATTATAATCGGAGGTTTTGGAAACTGATTAGTGCCTTTAATAATTGGCGCTCCCGATATAGCATTCCCTCGAATA<br>AACAATATGAGTTTCTGATTACTGCCACCATCCTTCCTACTTCTTCTAGCCTCTTCCATAGTAGAAGCAGGTGCAGGGACTGGTTGAACTGTTTACCC<br>CCCTCTAGCGGGCAATCTAGCCCATGCAGGAGCATCAGTAGACCTAACAATTTTTTCCCTACATTTAGCAGGCGTTTCCTCCATTCTAGGAGCTATTA<br>ACTTTATTACCACTATTATCAATATGAAGCCTCCTGCAATATCTCAATACCAAACTCCTCTGTTCGTATGATCCGTCCTAATTACGGCGGTA**AGGAGT**<br>**CGAACCCCCTGAAATTGGTTTCAAGCCAATACCATAACCACTATGTCTCTCTCAATAAAGAGATATTAGTAAAAATTACATAACTTCGTCAGGGTTAA**<br>**ATTATAGGTGAAAATCCTTTATATCTCTATGGCGTATCCCTTTCAAATAGGCCTTCAAGACGCAACTTCTCCCATTATAGAAGAGCTCCTACACTTCC**<br>**ACGACCATACATTAATGATTGTATTCCTGATTAGCTCTTTAGTTCTCTACATTATTTCAACTATGCTAACTACTAAACTAACGCACACAAACACAATAG**<br>**ATGCACAGGAAGTAGAAACGGTATGAACCATTCTACCAGCCATTATCCTGGTTCTAATTGCACTCCCATCATTACGAATCCTCTATATAATGGATGAA**<br>**ATCAACAATCCTTTACTGACTGTAAAAACTATAGGCCATCAATGATACTGAAGTTACGAATATACGGACTATGAAGATTTGAACTTTGATTCTTACAT**<br>**GATTCCAACACAAGAATTAAAGCCTGGAGAACTACGACTATTAGAAGTAGACAATCGAGCGGTACTACCCATAGAAATGACTATTCGCATGCTAATT**<br>**TCATCAGAAGATGTCTTGCACTCATGAGCTGTACCATCCCTAGGACTAAAAACTGATGCAATTCCGGGACGACTAAACCAAACGACTCTCATGGCCA**<br>**TGCGACCAGGGCTGTATTATGGCCAATGCTCGGAAATCTGTGGCTCTAACCACAGCTTCATGCCTATTGTTCTCGAACTAGTCCCACTGTCCTATTTC**<br>**GAAAAATGATCCGCTTCAATATTATAAAATCATTAAGAAGCTATATAGCATTAACCTTTTAAGTTAAAGACTGAGAGTGCAAGTCTCTCCTTAATGGA**<br>**GATGCCACAACTAGACACGTCAACATGGTTTATCACAATTCTATCTATAACTCTAACACTATTTATTGTATTCCAACTAAAAATC** |
| HTOZ1 | Zamora Chinchipe (5) | **TTTACTTATTTCATATATATCATCCCACGTACTGTAGCATCCTAGTATGTCCCCGAACAAGGAAACCTTTCTTTTTTTT–CCCCCCTATGTACGTCGTG**<br>**CATTAATGGCGTGCCCCATGCATATAAGCATGTACATACCTTGCTTGGTTTTACATGAGGACATGGACTTCAAAAACTCGTTCTGAAGACGTAGTCTG**<br>**CAAGCATGTATTTCACTTAGTCCGGGAGCTTAGTCACCAGGCCTCGAGAAACCAGCAACCCTTGCGAGTACGTATACCTCTTCTCGCTCCGGGCCCA**<br>**TAGAAACGTGGGGGTTTCTATACTGAAACTATACCTGGCATCTGGTTCTTACTTCAGGGCCATGATAGCTCTAGATTCCAATCCTACTAACCCTTCAA**<br>**ATGGGACATCTCGATGGACTAATGACTAATCAGCCCATGATCACACATAACTGTGGTGTCATGCATTTGG**GCATATGGTACGTAACTCTACCTATTAT<br>TACAGCAAGCATCCCTCCCCAAACATAAGAAATATGTCTGACAAAAGAGTTACTTTGATAGAGTAAATAATAGAGGTTTAAATCCTCTTATTTCTAGA<br>ATTATAGGAATCGAACCTAATCTTAAGAATCCAAAAATCTTCGTGCTACCTGATTACACCACATCCTAGAGTAAGGTCAGCTAAATAAGCTATCGGGC<br>CCATACCCCGAAAATGTTGGTTTATCCCCTTCCCATACTAATAAATCCTCCCATTCTTACTATTATTATATCCACCATCGCTCTAGGAACCATAATCGT<br>TCTATTTAGCTCCCACTGATTTATAATCTGAATCGGTTTTGAAATAAATATACTGGCAATTATTCCCATTTTAATGAAAAAATTTAACCCACGAGCCGT<br>AGAAGCCTCCACAAAATATTTTCTAACACAGGCCACTGCATCCATGCTCCTCATACTAGGCATTATTATCAACTTACTATACTCAGGACACTGAACAA<br>TCTCAACAACTCTCAACCCAATCGCATCAACCGTGATCACCATTGCCCTAGCGATAAAACTTGGCCTATCTCCCTTCCACTTCTGAGTTCCAGAAGTT<br>ACACAAGGCATTCCCCTATCCTCAGGAGTAATCCTACTAACATGACAGAAAATCGCACCCTTGTCCGTTCTATACCAGATCTCACCATTCATTAACCC<br>AAACTTATTAATAACAATGGCTATCACATACAAACCATAAAGACATTGGTACTCTCTATATCTTATTCGGTGCATGAGCCGGAATAGTAGGTACTGCC<br>CTCAGCCTTCTAATCCGCGCTGAACTAGGTCAACCCGGAGCCCTGTTAGGGGATGATCAAATCTATAACGTGGTCGTAACTGCCCATGCATTCGTAA<br>TAATCTTCTTTATAGTAATGCCTATTATAATCGGAGGTTTTGGAAACTGATTAGTGCCTTTAATAATTGGCGCTCCCGATATAGCATTCCCTCGAATA<br>AACAATATGAGTTTCTGATTACTGCCACCATCCTTCCTACTTCTTCTAGCCTCTTCCATAGTAGAAGCAGGTGCAGGGACTGGTTGAACTGTTTACCC<br>CCCTCTAGCGGGCAATCTAGCCCATGCAGGAGCATCAGTAGACCTAACAATTTTTTCCCTACATTTAGCAGGCGTTTCCTCCATTCTAGGAGCTATTA<br>ACTTTATTACCACTATTATCAATATGAAGCCTCCTGCAATATCTCAATACCAAACTCCTCTGTTCGTATGATCCGTCCTAATTACGGCGGTA**AGGAGT**<br>**CGAACCCCCTGAAATTGGTTTCAAGCCAATACCATAACCACTATGTCTCTCTCAATAAAGAGATATTAGTAAAAATTACATAACTTCGTCAGGGTTAA**<br>**ATTATAGGTGAAAATCCTTTATATCTCTATGGCGTATCCCTTTCAAATAGGCCTTCAAGACGCAACTTCTCCCATTATAGAAGAGCTCCTACACTTCC**<br>**ACGACCATACATTAATGATTGTATTCCTGATTAGCTCCTTAGTTCTCTACATTATTTCAACTATGCTAACTACTAAACTAACGCACACAAACACAATAG**<br>**ATGCACAGGAAGTAGAAACGGTATGAACCATTCTACCAGCCATTATCCTGGTTCTAATTGCACTCCCATCATTACGAATCCTCTATATAATGGATGAA**<br>**ATCAACAATCCTTTACTGACTGTAAAAACTATAGGCCATCAATGATACTGAAGTTACGAATATACGGACTATGAAGATTTGAACTTTGATTCTTACAT** |

| | | |
|---|---|---|
| | | GATTCCAACACAAGAATTAAAGCCTGGAGAACTACGACTATTAGAAGTAGACAATCGAGCGGTACTACCCATAGAAATGACTATTCGCATGCTAATT<br>TCATCAGAAGATGTCTTGCACTCATGAGCTGTACCATCCCTAGGACTAAAAACTGATGCAATTCCGGGACGACTAAACCAAACGACTCTCATGGCCA<br>TGCGACCAGGGCTGTATTATGGCCAATGCTCGGAAATCTGTGGCTCTAACCACAGCTTCATGCCTATTGTTCTCGAACTAGTCCCACTGTCCTATTTC<br>GAAAAATGATCCGCTTCAATATTATAAAATCATTAAGAAGCTATATAGCATTAACCTTTTAAGTTAAAGACTGAGAGTGCAAGTCTCTCCTTAATGGA<br>GATGCCACAACTAGACACGTCAACATGGTTTATCACAATTCTATCTATAACTCTAACACTATTTATTGTATTCCAACTAAAAATC |
| HTOZ2 | Zamora Chinchipe (3) | TTACTTATTTCATATATATCATCCCACGTACTGTAGCATCCTAGTATGTCCCCGAACAAGGAAACCTTTCTTTTTTTTTCCCCCCTATGTACGTCGTG<br>CATTAATGGCGTGCCCCATGCATATAAGCATGTACATACCTTGCTTGGTTTTACATGAGGACATGGACTTCAAAAACTCGTTCTGAAGACGTAGTCTG<br>CAAGCATGTATTTCACTTAGTCCGGGAGCTTAGTCACCAGGCCTCGAGAAACCAGCAACCCTTGCGAGTACGTATACCTCTTCTCGCTCCGGGCCCA<br>TAGAAACGTGGGGGTTTCTATACTGAAACTATACCTGGCATCTGGTTCTTACTTCAGGGCCATGATAGCTCTAGATTCCAATCCTACTAACCCTTCAA<br>ATGGGACATCTCGATGGACTAATGACTAATCAGCCCATGATCACACATAACTGTGGTGTCATGCATTTGGGCATATGGTACGTAACTCTACCTATTAT<br>TACAGCAAGCATCCCTCCCCAAACATAAGAAATATGTCTGACAAAAGAGTTACTTTGATAGAGTAAATAATAGAGGTTTAAATCCTCTTATTTCTAGA<br>ATTATAGGAATCGAACCTAATCTTAAGAATCCAAAAATCTTCGTGCTACCTGATTACACCACATCCTAGAGTAAGGTCAGCTAAATAAGCTATCGGGC<br>CCATACCCCGAAAATGTTGGTTTATCCCCTTCCCATACTAATAAATCCTCCCATTCTTACTATTATTATATCCACCATCGCTCTAGGAACCATAATCGT<br>TCTATTTAGCTCCCACTGATTTATAATCTGAATCGGTTTTGAAATAAATATACTGGCAATTATTCCCATTTTAATGAAAAAATTTAACCCACGAGCCGT<br>AGAAGCCTCCACAAAATATTTTCTAACACAGGCCACTGCATCCATGCTCCTCATACTAGGCATTATTATCAACTTACTATACTCAGGACACTGAACAA<br>TCTCAACAACTCTCAACCCAATCGCATCAACCGTGATCACCATTGCCCTAGCGATAAAACTTGGCCTATCTCCCTTCCACTTCTGAGTTCCAGAAGTT<br>ACACAAGGCATTCCCCTATCCTCAGGAGTAATCCTACTAACATGACAGAAAATCGCACCCTTGTCCGTTCTATACCAGATCTCACCATTCATTAACCC<br>AAACTTATTAATAACAATGGCTATCACATACAAACCATAAAGACATTGGTACTCTCTATATCTTATTCGGTGCATGAGCCGGAATAGTAGGTACTGCC<br>CTCAGCCTTCTAATCCGCGCTGAACTAGGTCAACCCGGAGCCCTGTTAGGGGATGATCAAATCTATAACGTGGTCGTAACTGCCCATGCATTCGTAA<br>TAATCTTCTTTATAGTAATGCCTATTATAATCGGAGGTTTTGGAAACTGATTAGTGCCTTTAATAATTGGCGCTCCCGATATAGCATTCCCTCGAATA<br>AACAATATGAGTTTCTGATTACTGCCACCATCCTTCCTACTTCTTCTAGCCTCTTCCATAGTAGAAGCAGGTGCAGGGACTGGTTGAACTGTTTACCC<br>CCCTCTAGCGGGCAATCTAGCCCATGCAGGAGCATCAGTAGACCTAACAATTTTTTCCCTACATTTAGCAGGCGTTTCCTCCATTCTAGGAGCTATTA<br>ACTTTATTACCACTATTATCAATATGAAGCCTCCTGCAATATCTCAATACCAAACTCCTCTGTTCGTATGATCCGTCCTAATTACGGCGGTAAGGAGT<br>CGAACCCCCTGAAATTGGTTTCAAGCCAATACCATAACCACTATGTCTCTCTCAATAAAGAGATATTAGTAAAAATTACATAACTTCGTCAGGGTTAA<br>ATTATAGGTGAAAATCCTTTATATCTCTATGGCGTATCCCTTTCAAATAGGCCTTCAAGACGCAACTTCTCCCATTATAGAAGAGCTCCTACACTTCC<br>ACGACCATACATTAATGATTGTATTCCTGATTAGCTCCTTAGTTCTCTACATTATTTCAACTATGCTAACTACTAAACTAACGCACACAAACACAATAG<br>ATGCACAGGAAGTAGAAACGGTATGAACCATTCTACCAGCCATTATCCTGGTTCTAATTGCACTCCCATCATTACGAATCCTCTATATAATGGATGAA<br>ATCAACAATCCTTTACTGACTGTAAAAACTATAGGCCATCAATGATACTGAAGTTACGAATATACGGACTATGAAGATTTGAACTTTGATTCTTACAT<br>GATTCCAACACAAGAATTAAAGCCTGGAGAACTACGACTATTAGAAGTAGACAATCGAGCGGTACTACCCATAGAAATGACTATTCGCATGCTAATT<br>TCATCAGAAGATGTCTTGCACTCATGAGCTGTACCATCCCTAGGACTAAAAACTGATGCAATTCCGGGACGACTAAACCAAACGACTCTCATGGCCA<br>TGCGACCAGGGCTGTATTATGGCCAATGCTCGGAAATCTGTGGCTCTAACCACAGCTTCATGCCTATTGTTCTCGAACTAGTCCCACTGTCCTATTTC<br>GAAAAATGATCCGCTTCAATATTATAAAATCATTAAGAAGCTATATAGCATTAACCTTTTAAGTTAAAGACTGAGAGTGCAAGTCTCTCCTTAATGGA<br>GATGCCACAACTAGACACGTCAACATGGTTTATCACAATTCTATCTATAACTCTAACACTATTTATTGTATTCCAACTAAAAATC |

The colors indicate the beginning and end of the sequences of each marker: **HVR1**, **ND1-ND2**, **COI (partial sequence)**, **COI-COII-ATP8**. **n,** number of individuals.

*New haplotype found in this study for the Andean bear population in Loja.

**Table 3. Polymorphisms found in the sequence of each haplotype of the Concatenated Region 2.**

| Haplotype | n | Nucleotide position in Concatenated Region 2 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **1** | | **16** | **80** | **138** | **148** | **149** | **182** | **198** | **230** | **700** |
| HTOQ1 | 6 | tRNA-Pro | | C | – | T | C | T | T | T | A | A |
| HTOQ2 | 2 | tRNA-Pro | | C | – | T | C | T | T | T | G | A |
| HTOQ3 | 2 | tRNA-Pro | | C | T | T | C | T | T | T | A | A |
| HTOL1 | 4 | tRNA-Pro | | T | – | T | T | C | C | C | G | T |
| HTOL2* | 1 | tRNA-Pro | | C | T | T | C | T | T | T | A | T |
| HTOZ1 | 5 | tRNA-Pro | | T | – | C | T | T | C | C | G | T |
| HTOZ2 | 3 | tRNA-Pro | | T | T | C | T | T | C | C | G | T |
| | | **1172** | **1206** | **1304** | **1529** | **1872** | **1967** | **2028** | **2189** | **2209** | **2483** | **2636** |
| HTOQ1 | 6 | G | C | G | C | C | C | A | C | G | C | G |
| HTOQ2 | 2 | G | C | G | C | C | C | A | C | G | C | G |
| HTOQ3 | 2 | G | C | G | C | C | C | A | C | G | C | G |
| HTOL1 | 4 | A | A | A | T | T | T | G | T | A | T | A |
| HTOL2* | 1 | A | A | A | T | T | T | G | T | A | T | A |
| HTOZ1 | 5 | A | A | A | T | T | C | G | T | A | T | A |
| HTOZ2 | 3 | A | A | A | T | T | C | G | T | A | T | A |

The initial position starts in the sequence of the coding region for the proline tRNA within the mtDNA, and the 20 polymorphic sites found are detailed. **n,** number of individuals.

*****New haplotype found in this study for the Andean bear population in Loja.

**Table 4. Amino acid substitutions in the chains of the proteins encoded by three genes.**

| Haplotype | n | Gene | | | | | |
|---|---|---|---|---|---|---|---|
| | | ND2 | | COI | COII | | |
| | | 2 | 160 | 20 | 5 | 57 | 117 |
| HTOQ1 | 6 | Lys | Ala | Leu | Leu | Asn | Ser |
| HTOQ2 | 2 | Lys | Ala | Leu | Leu | Asn | Ser |
| HTOQ3 | 2 | Lys | Ala | Leu | Leu | Asn | Ser |
| HTOL1 | 4 | Asn | Thr | Ile | Phe | Asp | Asn |
| HTOL2* | 1 | Asn | Thr | Ile | Phe | Asp | Asn |
| HTOZ1 | 5 | Asn | Thr | Ile | Phe | Asp | Asn |
| HTOZ2 | 3 | Asn | Thr | Ile | Phe | Asp | Asn |

For each gene, the positions are numbered from the amino acid methionine (Met) at position 1. **n,** number of individuals.

*****New haplotype found in this study for the Andean bear population in Loja.

# 7. FIGURES

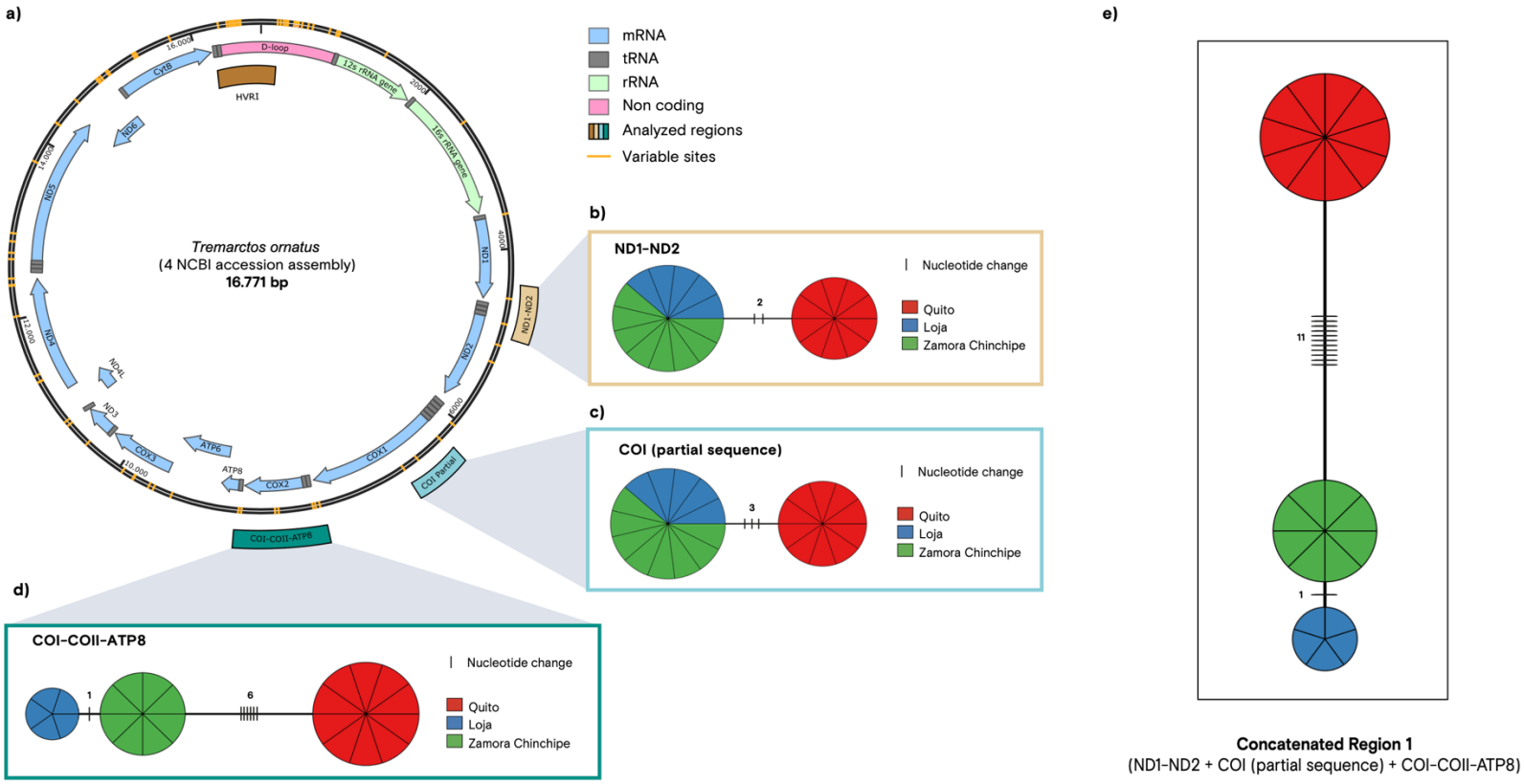


**Figure 1. Mitochondrial haplotype networks for the markers studied.**

**a)** The schematic mitochondrial genome of *T. ornatus* resulting from the alignment of four NCBI accessions showing the genes (light blue, gray, and light green), non-coding region D–loop (pink), analyzed regions in this study (**HVR1**, **ND1–ND2**, **COI (partial sequence)**, and **COI–COII–**

**ATP8**), and variable sites (orange) resulted by comparing the four sequences. **b)** Haplotype network of ND1–ND2. **c)** Haplotype network of COI (partial sequence). **d)** Haplotype network of COI–COII–ATP8. **e)** Haplotype network of the Concatenated Region 1, which includes the sequences of ND1–ND2, COI (partial sequence), and COI–COII–ATP8. A haplotype is represented by a circle whose size is proportional to the number of individuals showing that haplotype, and the colors refer to the sampling site. Each division between the lines represents a nucleotide change between the haplotypes.

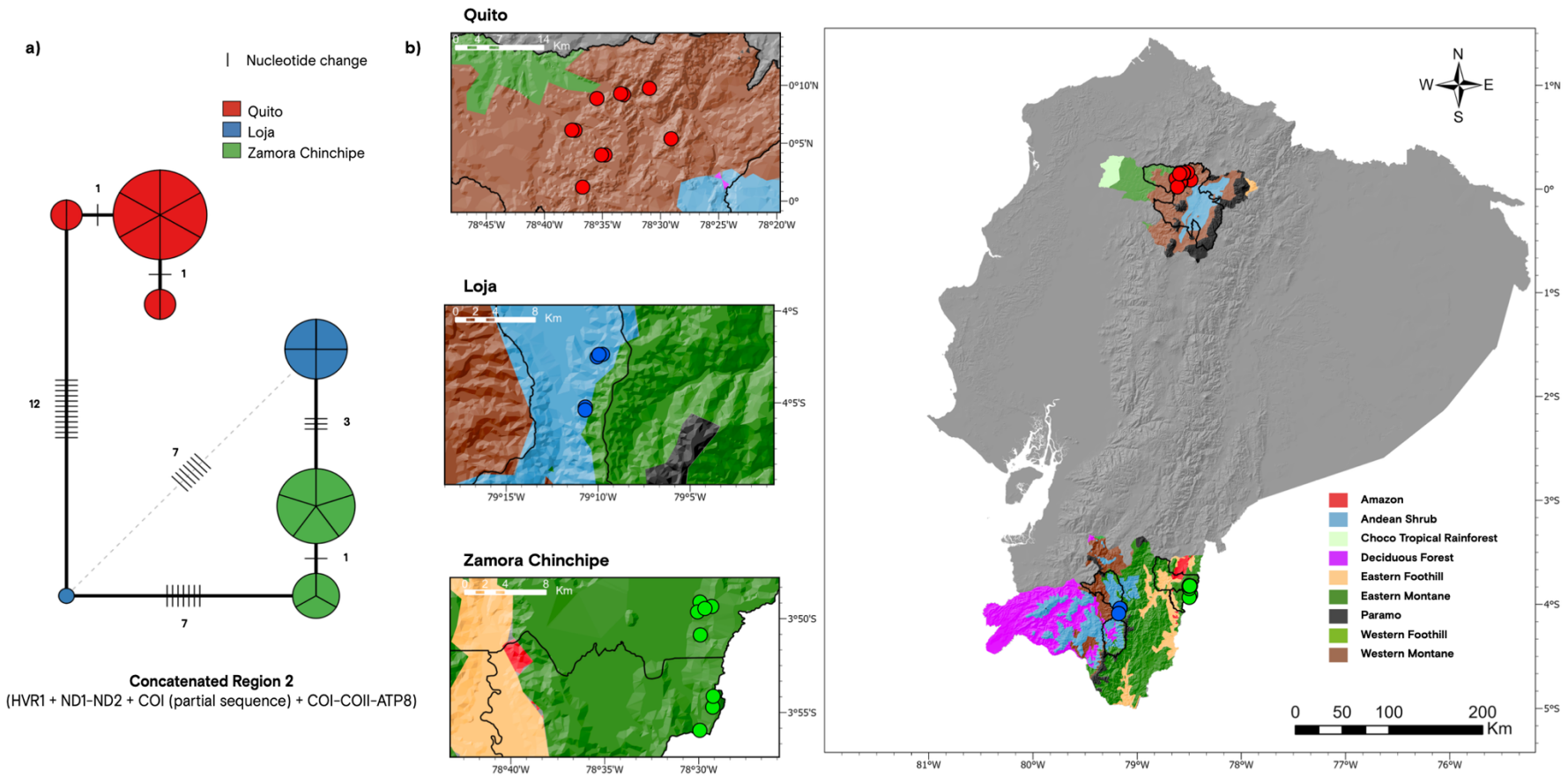


**Figure 2. Haplotype network and distribution of sampling points in the three sites.**

**a)** Haplotype network of the Concatenated Region 2, which includes the sequences of HVR1, ND1–ND2, COI (partial sequence), and COI–COII–ATP8. **b)** Map of the geographic points (Appendix 1) where samples were collected, drawn in ArcGis Pro v.10.8.2 (ESRI, 2023). The colors on the map represent the different types of ecosystems present in each province, according to Ron (2020).

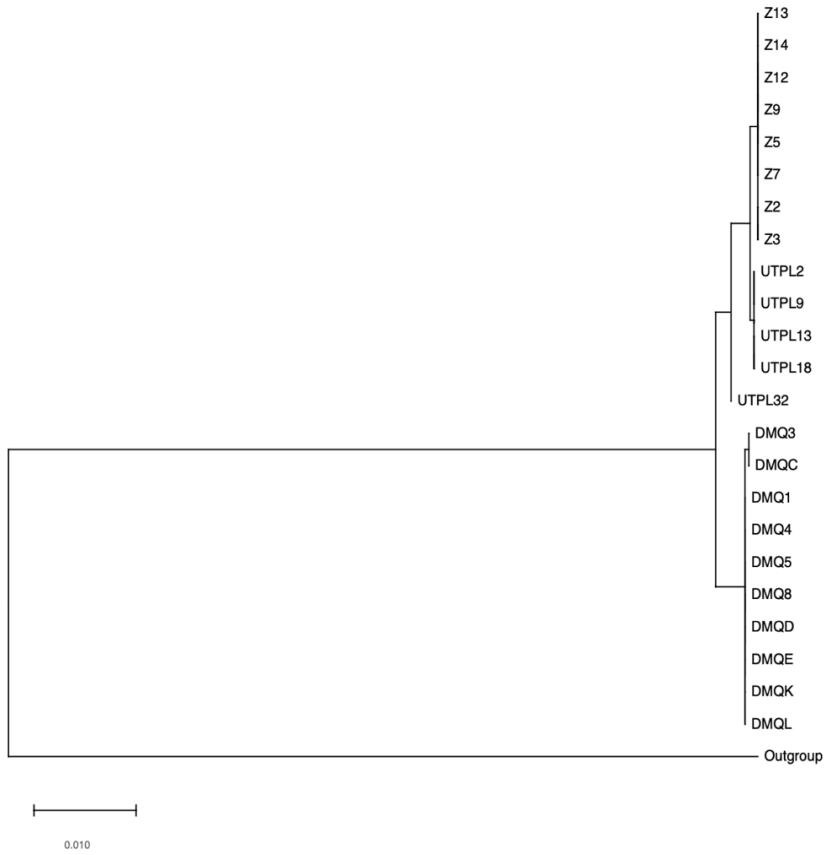


**Figure 3. Phylogenetic tree using the Maximum-Likelihood method with a Hasegawa–Kishino–Yano (HKY) substitution model.**

The populations of bears from Loja and Zamora Chinchipe are grouped into the same phylogenetic group, and all individuals from Quito belong to the same monophyletic group. The *Ursus americanus* mitochondrial genome sequence (GenBank accession: NC_003426.1) was used as an outgroup.

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# APPENDICES

**Appendix 1. Information of the sampled individuals used in this study in Quito, Loja, and Zamora Chinchipe, and concentration of the DNA extracted and absorbance indexes of each sample.**

| Population | Individual ID | Sample ID | Locality | Latitude | Longitude | Type of sample | DNA concentration | A260/A280 | A260/A230 | Haplotype |
|---|---|---|---|---|---|---|---|---|---|---|
| Quito | O1H3M | DMQ1 | Lulumbamba | 0.09 | –78.48 | Hair | 9.5 | 2.0 | 0.8 | HTOQ1 |
| | 23M8 | DMQ3 | Cambugan | 0.16 | –78.52 | Hair | 0.7 | 1.2 | –0.2 | HTOQ2 |
| | Ind2 | DMQ4 | Bellavista 3 | 0.15 | –78.56 | Hair | 4.7 | 1.5 | –1.4 | HTOQ1 |
| | 25M9 | DMQ5 | Gavilanes | 0.07 | –78.58 | Hair | 1.9 | –11.5 | 0.4 | HTOQ1 |
| | 20I | DMQ8 | Gavilanes | 0.07 | –78.58 | Hair | 6.7 | 5.0 | 0.2 | HTOQ1 |
| | 5M5 | DMQC | Campamento | 0.10 | –78.63 | Hair | 0.1 | 0.2 | 0.0 | HTOQ2 |
| | 21M7 | DMQD | Bellavista 3 | 0.15 | –78.56 | Hair | 0.0 | –0.7 | 0.0 | HTOQ3 |
| | 35I | DMQE | Campamento | 0.10 | –78.63 | Hair | 1.3 | 1.9 | –0.2 | HTOQ3 |
| | YUMBO | DMQK | Santa Lucía | 0.02 | –78.61 | Hair | 43.5 | 2.0 | 1.0 | HTOQ1 |
| | Ind1 | DMQL | Curipogrio | 0.15 | –78.59 | Hair | 2.4 | 12.8 | 0.3 | HTOQ1 |
| Loja | UTPL002 | UTPL2 | Reserva Madrigal | –4.04 | –79.17 | Fecal | 5.7 | 1.9 | 0.2 | HTOL1 |
| | UTPL009 | UTPL9 | Reserva Madrigal | –4.04 | –79.17 | Fecal | 45.4 | 1.6 | 0.5 | HTOL1 |
| | UTPL013 | UTPL13 | Reserva Madrigal | –4.04 | –79.17 | Fecal | 16.2 | 2.1 | 0.4 | HTOL1 |
| | UTPL018 | UTPL18 | Parque Nacional Podocarpus | –4.12 | –79.17 | Saliva | 1.9 | –11.1 | 0.1 | HTOL1 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | UTPL032 | UTPL32 | Parque Nacional Podocarpus | –4.12 | –79.17 | Saliva | 9.2 | 2.2 | 0.4 | HTOL2 |
| **Zamora Chinchipe** | C5LB | Z2 | Paquisha, Río Blanco | –3.91 | –78.49 | Hair | 7.2 | 1.9 | 1.4 | HTOZ2 |
| | D5LBC | Z3 | Paquisha, Río Blanco | –3.90 | –78.49 | Hair | 2.5 | 1.0 | 0.2 | HTOZ2 |
| | A4LA | Z5 | Paquisha, Río Blanco | –3.93 | –78.50 | Hair | 2.7 | 1.3 | 0.5 | HTOZ1 |
| | I4C | Z7 | Paquisha, Río Blanco | –3.85 | –78.50 | Hair | 14.6 | 1.9 | 0.5 | HTOZ2 |
| | A2C | Z9 | Paquisha, Río Blanco | –3.82 | –78.50 | Hair | 6.3 | 1.6 | 0.5 | HTOZ1 |
| | A2A2B2 | Z12 | Paquisha, Río Blanco | –3.82 | –78.50 | Hair | 1.8 | 1.1 | –2.2 | HTOZ1 |
| | A2A2B1 | Z13 | Paquisha, Río Blanco | –3.82 | –78.50 | Hair | 17.0 | 1.9 | 0.8 | HTOZ1 |
| | R2LA2I | Z14 | Paquisha, Río Blanco | –3.82 | –78.50 | Hair | 1.6 | 1.8 | 1.6 | HTOZ1 |

**Appendix 2. Sets of primers used for the amplification of the three mitochondrial markers.**

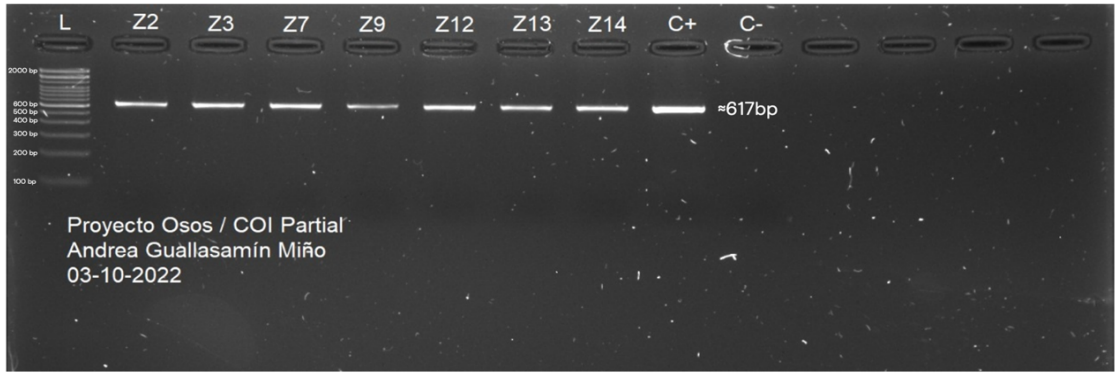
| Mitochondrial Marker | Expected length (bp) | Final length (bp) | Primer sequence (5'– 3') | Tm (°C) | Ta (°C) | GC (%) | Off-targets | Comments |
|---|---|---|---|---|---|---|---|---|
| **ND1–ND2** | 805 | 745 | F: CTTTCTACCTCTCACATTAGCC | 57 | 60 | 46 | *Bos taurus* mtDNA | No dimers/hairpins |
| | | | R: TGGTTTAGTCCTCCTCAGCCT | 61 | | 52 | | |
| **COI (partial sequence)** | 617 | 552 | F: GACCGATGACTATTTTCCACA | 56 | 60 | 43 | None | No dimers/hairpins |
| | | | R: AGCATAGTAATCCCAGCTGCC | 60 | | 52 | | |
| **COI–COII–ATP8** | 1082 | 972 | F: GATGCCCTCCTCCGTATCAC | 60 | 60 | 60 | None | No dimers/hairpins |
| | | | R: GGTGGAAAAGGTTTTAGTTCGGG | 61 | | 48 | | |

The primers were designed by Darío F. Cueva and validated by Andrea Guallasamín. **Tm**, melting temperature. **Ta**, annealing temperature. **GC**, guanine-cytosine content.

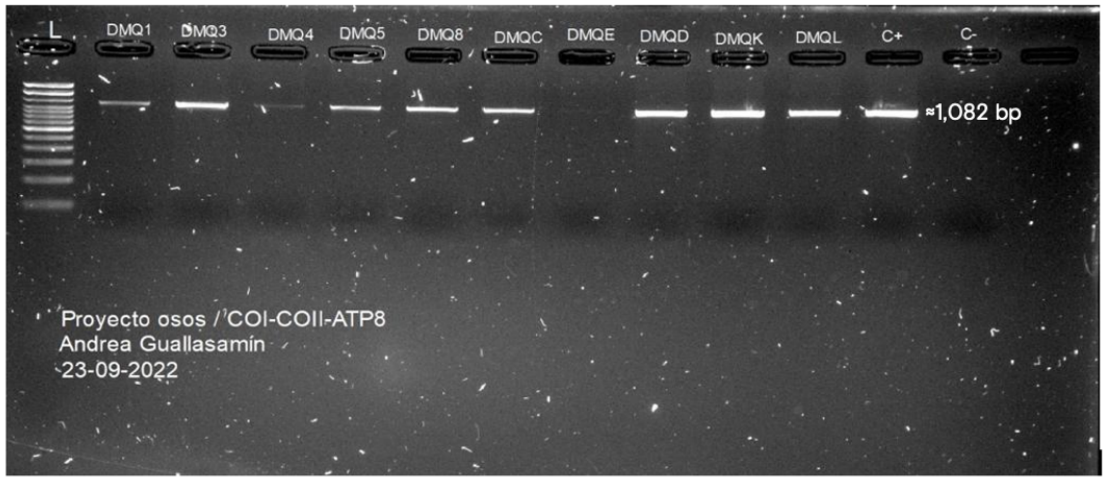
**Appendix 3. Electrophoresis gel of the PCR products obtained from the amplification of the molecular markers used in this study.**

**a)**


**b)**



**c)**




DNA bands that show the successful amplification of the **a)** ND1-ND2, **b)** COI (partial sequence), and **c)** COI-COII-ATP8 regions for the samples from Quito and Zamora Chinchipe. The expected length of the amplicons was 805, 617, and 1,082 bp, respectively.