UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

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Analysis of the genetic diversity of the Ecuadorian Andean bear (*Tremarctos ornatus*) using species-specific mitochondrial markers in individuals from Pichincha, Imbabura, and Zamora Chinchipe, Ecuador

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Natalia Carolina Herrera Torres

Ingeniería en Biotecnología

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Natalia Carolina Herrera Torres

Nombre del profesor, Título académico	**María de Lourdes Torres, PhD.**
Nombre del profesor, Título académico	**María José Pozo, MBS.**

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Nombres y apellidos: Natalia Carolina Herrera Torres

Código: 00216763

Cédula de identidad: 1726694035

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RESUMEN

El oso andino (*Tremarctos ornatus*), endémico de la región andina, es el último representante vivo de la subfamilia Tremarctinae en América del Sur. Esta especie desempeña un papel importante en la biodiversidad de los Andes, contribuyendo a la dispersión de semillas y renovación de la vegetación. Los análisis genéticos son fundamentales para comprender la estructura poblacional de especies en peligro como *T. ornatus,* que está catalogada como una especie vulnerable por la IUCN, y permiten evaluar su capacidad de respuesta ante presiones ambientales o antropogénicas. En este estudio se evaluó la diversidad genética de individuos de *T. ornatus* provenientes de las provincias de Pichincha, Imbabura y Zamora Chinchipe en Ecuador, mediante el análisis de cinco marcadores mitocondriales (HVR1 D-Loop, COI-secuencia parcial, COI-COII-ATP8, ATP6-COIII y ND6-CytB). Los resultados obtenidos en esta investigación se sumaron a los de estudios previos realizados en el Laboratorio de Biotecnología Vegetal (USFQ), identificando así 10 haplotipos y 37 sitios polimórficos. Se encontró una alta diversidad de haplotipos ($Hd = 0.8619 \pm 0.0321$) y una baja diversidad de nucleótidos ($\pi = 0.00534 \pm 0.00270$). El AMOVA reveló que el 93.33% de la variación genética se debe a diferencias entre poblaciones, mientras que el índice de fijación determinó una alta diferenciación genética entre provincias. Estos hallazgos respaldan investigaciones anteriores que evidencian una estructura poblacional diferenciada del oso andino entre el norte y el sur del Ecuador, probablemente causada por barreras geográficas y la fragmentación del hábitat. Se recomienda que las poblaciones del oso andino sean tratadas como unidades de conservación separadas y se sugiere complementar esta información con estudios genómicos para profundizar el conocimiento sobre esta especie.

Palabras clave: *Tremarctos ornatus*, oso andino, Ecuador, diversidad genética, marcadores mitocondriales, estructura poblacional.

ABSTRACT

The Andean bear (*Tremarctos ornatus*), endemic to the Andean region, is the last surviving member of the subfamily Tremarctinae in South America. This species plays a vital role in the biodiversity of the Andes, contributing to seed dispersal and vegetation renewal. Genetic analyses are essential for understanding the population structure of endangered species such as *T. ornatus*, which is classified as vulnerable by the IUCN, and for evaluating its ability to respond to environmental and anthropogenic pressures. In this study, the genetic diversity of *T. ornatus* individuals from the provinces of Pichincha, Imbabura, and Zamora Chinchipe in Ecuador was assessed using five mitochondrial markers (HVR1 D-Loop, COI-partial sequence, COI-COII-ATP8, ATP6-COIII, and ND6-CytB). The results were integrated with previous studies from the Plant Biotechnology Lab - USFQ, identifying 10 haplotypes and 37 polymorphic sites. A high haplotype diversity ($Hd = 0.8619 \pm 0.0321$) and low nucleotide diversity ($\pi = 0.00534 \pm 0.00270$). AMOVA revealed that 93.33% of genetic variation is due to differences between populations, while the fixation index indicated significant genetic differentiation among provinces. These findings support previous studies that highlights a differentiated population structure of the Andean between northern and southern Ecuador, likely due to geographic barriers and habitat fragmentation. It is recommended that Andean bear populations be treated as separate conservation units, and it is suggested to complement this information with genomic studies to deepen the understanding of this species.

Key words: *Tremarctos ornatus*, Andean bear, Ecuador, genetic diversity, mitochondrial markers, population structure.

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1. INTRODUCTION

1.1. General characteristics of the Andean Bear (*Tremarctos ornatus*)

1.1.1. Morphology, behavior and diet

The Andean Bear (*Tremarctos ornatus*) is the only bear species found in South America, making it endemic to the Andes region (Maslak et al., 2013; Gonzales et al., 2016). *T. ornatus* is a medium-sized bear, ranging from 1.2 to 2.2 meters in length, and exhibits noticeable sexual dimorphism, with males weighing up to 200kg, while females are significantly smaller, with a maximum weight of 65kg. Their fur is typically black, but can sometimes include shades of dark red-brown (Peyton, 1999, as cited in Cavelier et al., 2011). Both adult males and females have short necks, small ears and a brown snout (Albarracín et al., 2013). This species is commonly known as the spectacled bear due to a unique pattern of white to yellow fur around their eyes, which often extends to their foreheads, cheeks, necks and chests. This characteristic fur pattern is useful for identifying individual bears (Sandoval-Guillén & Yánez-Moretta, 2019).

In terms of behavior, *T. ornatus* is known for its solitary and shy nature, though they occasionally come together for mating. In captivity, males usually reach sexually mature at four years, while females mature between four and seven years. The breeding season occurs from February to June, and the gestation period ranges from 160 to 255 days (Albarracín et al., 2013). Males are known to mark their territory to define boundaries and communicate with other spectacled bears, whereas females do not exhibit this behavior. *T. ornatus* is mainly active during the day but can also show some nocturnal activity. Their climbing skills allow them to seek food or shelter in trees (Vela-Vargas et al., 2021).

The Andean Bear is classified as a large carnivore, known for preying on species such as vicuñas, deer, and cattle (Stucchi et al., 2009). There have been multiple reports of *T. ornatus*

attacking mammals like horses, cows, goats, and sheep, which provides them a higher level of protein and energy (Figueroa, 2013). Additionally, the Andean bear diet includes a variety of plants such as bromeliads, fruits and cacti (Maslak et al., 2013).

1.1.2. Taxonomy and distribution

The spectacled bear belongs to the Carnivora order, Ursidae family and Tremarctinae subfamily (Brandstaetter, 2020; Saremi et al., 2021). While the Ursidae family is globally distributed, only the Ursinae, Ailuropodinae, and Tremarctinae subfamilies still have living representatives. In fact, *Tremarctos ornatus* is the only surviving member of the Tremarctinae subfamily in South America (Trevisan et al., 2023).

T. ornatus, endemic to the Andes region, is distributed from Bolivia to Argentina and occupies habitats ranging from lowlands to paramos above 4000 meters (Kattan et al., 2004). Its range extends along the Andes mountains, which is 4600km in length and 200-650km in width. These bears primarily inhabit cloud forests but can also be found in dry forests, tropical rainforests and montane forests (Meza et al., 2020). Human activities, particularly agriculture and construction, significantly affect the distribution of the Andean bear (García-Rangel, 2012).

1.1.3. Ecological role

The Andean bear plays a crucial role in seed dispersal, as seeds remain viable after passing through its digestive system. This process benefits specific plant species in the Andean forest and paramo –high-altitude tropical grasslands– since the bear deposits seeds in optimal locations, promoting greater germination (Gonzales et al., 2016). *T. ornatus* is capable of toppling trees to feed and rest, which allows more sunlight and water to reach the ground, fostering vegetation renewal. Similarly, the bears can break branches, which eventually contributes to the generation of organic matter and compost (Sandoval-Guillén & Yánez-

Moretta, 2019). Their feces contribute to soil fertilization by adding organic matter and nutrients, which in turn support the growth of plants and serve as a food source for small organisms such as beetles and insect larvae (García-Rangel, 2012).

1.2. Population density and conservation status in Ecuador

In Ecuador, various population density estimates have been reported for *T. ornatus*. Viteri (2007) recorded a density of 3 to 7 bears per 100 km^2, while Morrell (2014) estimated a density of 3.9 bears per 100 km^2, and Molina et al. (2014), provided a higher estimate of 7.45 bears per 100 km^2 (Rodríguez et al., 2020). According to studies by Ruiz-García in 2003, the global population of *Tremarctos ornatus* ranged between 19,000 and 24,000 individuals (Vela-Vargas et al., 2021). These discrepancies arise primarily from uncertainties in population estimates using camera traps, which are influenced by capture probabilities and the spatial placement of the traps (Pettigrew et al., 2021).

One of the most critical aspects of the spectacled bear is its conservation status. According to the International Union for Conservation of Nature (IUCN), *T. ornatus* is classified as a vulnerable species globally (Velez-Liendo & García-Rangel, 2017). In Ecuador, the Andean bear is considered endangered, largely due to habitat fragmentation. Reports indicate that paramo and cloud forest habitats have lost around 40% of their original extent in the country, mainly due to deforestation (Enríquez et al., 2023; Peralvo et al., 2005). Additional threats include the expansion of agricultural activities, illegal hunting, forest fires, and mining (Rodríguez et al., 2019).

1.3. Analysis of genetic diversity using mitochondrial markers

Mitochondrial DNA (mtDNA) in mammals is a circular, double-stranded molecule of 16.6kb that encodes 11 mRNAs, 2rRNAs, and 22 tRNAs (Gustafsson et al., 2016). It produces critical proteins for oxidative phosphorylation, such as seven subunits of the NADH

dehydrogenase complex (ND1, ND2, ND3, ND4, ND4L, ND5, ND6), the cytochrome b subunit (CytB), three subunits of the cytochrome c oxidase complex (COI, COII, COIII), and two ATP synthase subunits (ATP6, ATP8) (Da Fonseca et al., 2008). Additionally, its longest non-coding region, the D-Loop, functions as a promoter for the heavy and light strands of mtDNA, which are named based on their differing guanine content. It contains two hypervariable regions (HVRI and HVRII) which exhibit moderate mutation rates (Nikbakhsh et al., 2023).

Mitochondrial DNA is particularly useful for genetic diversity and evolutionary research due to its characteristics, including near neutrality (where most mutations have little or no effect on fitness), maternal inheritance, high variability, and a stable mutation rate (Sharma et al., 2015). For this reason, it is frequently used as an informative molecular marker in animal population studies, as it mutates faster than nuclear DNA (Nabholz et al., 2008).

Given the vulnerable status of this species, various genetic approaches have been suggested to study its populations, including genetic diversity analysis using mitochondrial markers. This data is essential for effective conservation efforts, as the genetics of *T. ornatus* are not well understood. Few studies have focused on the Andean bear, partly due to the lack of species-specific genetic markers (Cueva et al., 2024). Therefore, the main goal of this research is to determine the genetic diversity of Ecuadorian Andean bear individuals from Pichincha, Imbabura, and Zamora Chinchipe in Ecuador by analyzing five mitochondrial markers: HVRI D-Loop, COI-partial sequence, COI-COII-ATP8, ATP6-COIII, and ND6-CytB. This information will be integrated with results from previous studies performed at the Plant Biotechnology Lab - USFQ.

2. METHODS

2.1. Sample collection and selection of individuals

Hair samples from *Tremarctos ornatus* were collected from various locations in Pichincha, Imbabura, and Zamora Chinchipe between 2020 and 2024 (Figure 1a) using a non-invasive collection technique. These samples were then sent to the Plant Biotechnology Lab - USFQ, where they were stored at -20ºC. Video footage from camera traps was reviewed to verify that each sample belonged to a unique individual. Based on this, hair samples from 28 different *T. ornatus* individuals were analyzed, with 14 samples from Pichincha, 1 from Imbabura, and 13 from Zamora Chinchipe.

2.2. DNA extraction and quantification

DNA extraction from all samples was performed at the Plant Biotechnology Lab - USFQ using the DNA IQ System kit according to the manufacturer's protocol (Promega Corporation, 2016), with the single modification: the elution volume was adjusted to 40µl. The extracted DNA samples were then stored at -20ºC. Subsequently, DNA concentration and quality were assessed using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, 2009).

2.3. Selection of mitochondrial markers

The mitochondrial markers used in this study were HVRI D-Loop, COI-partial sequence, COI-COII-ATP8, ATP6-COIII, and ND6-CytB. The primers used to amplify these regions (Table 1) were previously designed and validated for Andean bears by the Plant Biotechnology Lab - USFQ and synthesized by Macrogen (Seoul, South Korea). These markers were chosen because they contain at least three variable sites and were proven to be informative for *T. ornatus* in prior studies conducted in the Plant Biotechnology Lab - USFQ (Cueva, 2018; Guallasamín, 2023; Barragán, 2023).

2.4. Amplification of mtDNA regions and amplicon sequencing

The Polymerase Chain Reaction (PCR) technique was used to independently amplify all mitochondrial markers for each individual in the study. The final reagent concentrations were as follows: 1X Buffer, 1.5mM $MgCl_2$, 0.25mg/ml BSA, 0.5µM of each primer (forward and reverse), 0.2mM dNTPs, 1U of Taq Polymerase (Invitrogen, MA, USA), and 2ng of DNA. Each reaction had a final volume of 25µl.

The thermocycling program varied according to the mitochondrial marker. An initial denaturation step was performed at 94ºC for 5 minutes, followed by denaturation at 94ºC for 30 seconds for all markers. The annealing step lasted 30 seconds, with temperatures specific to each marker: 68ºC for HVRI D-Loop; 60ºC for COI-partial sequence, COI-COII-ATP8, and ND6-CytB; and 58ºC for ATP6-COIII. The extension step was set at 72ºC, with times varying: 1 minute for HVRI D-Loop and COI-COII-ATP8, and 45 seconds for the other markers. The denaturation, annealing, and extension steps were repeated for 40 cycles, followed by a final extension at 72ºC for 5 minutes for all markers. For samples that did not successfully amplify, the first approach was to increase the DNA volume in the PCR reactions. If amplification still failed, the second approach involved purifying the DNA using the Ligation Sequencing gDNA kit from Oxford Nanopore Technologies (Oxford Nanopore Technologies, 2019). Amplicons were visualized on a 1.5% agarose gel using electrophoresis, run for 30 minutes at 100V. Finally, all amplicons were sent to Macrogen in Seoul, South Korea, for sequencing using the Sanger method on ABI3730XLs.

2.5. Sequence processing and data analysis

Raw sequence cleaning was performed using Geneious Prime v.2023.1.1 to generate consensus sequences for each sample from both the forward and reverse sequences. Using the same software, all samples were aligned for each mitochondrial marker with the Clustal Omega

algorithm. To ensure consistency, sequence lengths across all samples were standardized (Geneious Prime, 2024; Kearse et al., 2012).

Sequences from previous studies conducted at the Plant Biotechnology Lab - Lab were retrieved for all mtDNA markers from Pichincha, Loja, and Zamora Chinchipe (Guallasamín, 2023; Barragán, 2023). A concatenated region was then created by merging and aligning the sequences for each of the five mtDNA markers per individual (HVRI D-Loop, COI-partial sequence, COI-COII-ATP8, ATP6-COIII, and ND6-CytB) (Geneious Prime, 2024; Kearse et al., 2012).

Haplotype networks were constructed individually for each mitochondrial marker and for the concatenated region using R Studio v.4.3.0, following the script developed by Toparslan et al., (2020) and employing the *haploNet* function form the *pegas* package. For each mitochondrial marker and the concatenated region, the haplotype diversity index (Hd), nucleotide diversity index (π), Tajima's D (Tajima, 1989), Fu's Fs (Fu, 1997), and Analysis of Molecular Variance (AMOVA) were calculated. All statistical analyses were performed using DnaSP v.6.12.03 (Rozas et al., 2003) and Arlequin v.3.5.2.2 (Excoffier et al., 2005). Imbabura, represented by only a single individual, was excluded from the Hd, π, Tajima's D, and Fu's Fs analyses because it is not possible to infer population's dynamics based on just one individual. Lastly, a phylogenetic tree was built using the Maximum-Likelihood method with the Hasegawa-Kishino-Yano substitution model in MEGA v.11 (Hasegawa et al., 1985; Tamura et al., 2021).

3. RESULTS

3.1. DNA extraction and quantification

The DNA concentration obtained from the 28 samples ranged from 0.1 ng/ul to 553.4 ng/ul, with an average concentration of 65.42 ng/µl. Among these, 75% of the samples had a concentration below 20 ng/µl. Regarding the quality indices, the overall averages were 1.58 ng/ul for A260/A280 and 1.25 ng/ul for A260/A230 (Appendix 1).

3.2. Amplification and sequencing of the mtDNA markers

Out of the 28 samples initially processed, 14 successfully amplified for all five mitochondrial markers (HVRI D-Loop, COI-partial sequence, COI-COII-ATP8, ATP6-COIII, and ND6-CytB; Appendix 2) following the protocol mentioned in section 2.4. Each sequence was processed as outlined in section 2.5. After sequence cleaning and alignment, the HVRI D-Loop resulted in a length of approximately 600 bp, reduced to 430 bp after cleaning. For COI-partial sequence, the sequence length was 617 bp, reduced to 552 bp after processing. Similarly, COI-COII-ATP8 generated 1082 bp amplicons, which were reduced to 972 bp after editing. ATP6-COIII had an initial length of 680 bp, reduced to 618 bp post-cleaning, while ND6-CytB started at 604 bp and measured 553 bp after processing.

3.3. Haplotype identification and polymorphic sites analysis

Incorporating previous studies by Guallasamín (2023) and Barragán (2023), sequences for the same mitochondrial markers for samples from Pichincha, Loja and Zamora Chinchipe were included. Consequently, 22 individuals from these prior studies were added, bringing the total number of individuals analyzed to 36. Across individual markers, 8 polymorphic sites were observed in HVRI D-Loop, resulting in 8 haplotypes (Figure 2b). For the COI-partial sequence, 3 polymorphic sites were identified, resulting in the definition of 2 haplotypes among

the individuals (Figure 2c). In the COI-COII-ATP8 marker, 7 polymorphic sites were identified, resulting in 4 haplotypes (Figure 2d). ATP6-COIII also had 7 polymorphic sites, leading to the formation of 5 haplotypes (Figure 2e). Lastly, ND6-CytB contained 12 polymorphic sites, forming 3 haplotypes (Figure 2f).

When analyzing the concatenated region, 37 polymorphic sites were identified, resulting in 10 haplotypes. Among these polymorphic sites, there was 1 insertion/deletion, 35 transitions (C⇔T; A⇔G), and 1 transversion (C⇔A) (Table 2). The haplotypes were as follows: 3 from Pichincha (HTOP1, HTOP2, and HTOP3), 1 from Imbabura (HTOI1), 2 from Loja (HTOL1 and HTOL2), and 4 from Zamora Chinchipe (HTOZ1, HTOZ2, HTOZ3, and HTOZ4) (Table 2, Figure 3). Seven of these haplotypes were previously reported in earlier studies (Guallasamín, 2023; Barragán, 2023), however, this analysis also revealed three new haplotypes, underscoring additional genetic variation within the population. Additionally, no haplotypes were shared between the individuals from Pichincha, Imbabura, Loja, and Zamora Chinchipe.

3.4. Genetic diversity statistics, neutrality tests, and AMOVA

Considering the entire population of 36 individuals, the concatenated region (3125 bp) was selected, as it represents a larger portion of the Andean bear's mitochondrial genome. Among populations represented by more than one individual, multiple haplotypes were found for each province (Pichincha, Loja, and Zamora Chinchipe). Overall, without dividing by geographical region, the haplotype diversity (Hd) was 0.8619 ± 0.0321, and the nucleotide diversity (π) was 0.005339 ± 0.002704 (Table 3).

When segmented by province, Pichincha exhibited the highest haplotype diversity (n = 13; Hd = 0.6410 ± 0.09667), followed by Zamora Chinchipe (n = 18; Hd = 0.6340 ± 0.0926), and Loja (n = 4; Hd = 0.5000 ± 0.2652). In terms of nucleotide diversity, Loja had the highest

value (0.001120 ± 0.000856), followed by Zamora Chinchipe (0.000443 ± 0.000320) and Pichincha (0.000238 ± 0.000211) (Appendix 4).

In terms of neutrality tests, the analysis of the concatenated region (3125 bp) without geographical division yielded a Tajima's D of 3.085 (p-value = 1) and Fu's Fs of 9.213 (p-value = 0.988) (Table 3). When analyzed by province, negative Tajima's D values were recorded for Pichincha (-0.27429; p-value = 0.295) and Loja (-0.80861; p-value = 0.149). In contrast to Zamora Chinchipe, which presented a positive Tajima's D of 0.03225 (p-value = 0.580). Fu's Fs values were positive across all provinces, with the highest value in Loja at 3.25086 (p-value = 0.912), followed by Zamora Chinchipe at 0.64207 (p-value = 0.665) and Pichincha at 0.18471 (p-value = 0.425) (Appendix 4).

The AMOVA analysis revealed that 93.33% of the variation was due to differences between populations, while 6.67% was attributable to variation within populations, resulting in a fixation index (FST) of 0.933 (Table 4). Lastly, the phylogenetic tree, constructed using the Maximum-Likelihood method and the Hasegawa-Kishino-Yano (HKY) substitution model, revealed two distinct groups: one containing the individuals from Pichincha and the other containing individuals from Imbabura, Loja, and Zamora Chinchipe. Notably, the individual from Imbabura was closely related to those from Pichincha (Figure 4).

4. DISCUSSION

4.1. Genetic diversity and population structure inferences based on mitochondrial DNA

One approach to studying genetic diversity is to examine the number of haplotypes, which represent sets of shared polymorphisms among individuals. The number of haplotypes is influenced by mutation events, recombination, marker selection, and demographic factors (Stumf, 2004). In this study, 3 new haplotypes were identified by incorporating additional samples, bringing the total number of distinct haplotypes to 10 across 36 individuals from four provinces in Ecuador (Pichincha, Imbabura, Loja, and Zamora Chinchipe) (Figure 3). Previous studies have observed a similar pattern of distinct haplotypes across provinces, with haplotypes generally grouped by provinces (Cueva et al., 2014; Barragán, 2023). This analysis included both coding (COI-partial sequence, COI-COII-ATP8, ATP6-COIII, and ND6-CytB) and a non-coding region (HVRI D-Loop), where non-coding regions generally accumulate more mutations due to lower selective pressure, as coding regions contain sequences essential for protein production (Scacheri & Scacheri, 2015). By analyzing mitochondrial markers individually for each province, a consistent pattern of regional differentiation was observed, with individuals grouped either by province or, in some cases, by their geographical region (north or south of Ecuador) (Figure 2c, 2d, 2e, 2f).

Key genetic diversity metrics like haplotype diversity (Hd) and nucleotide diversity (π) are valuable for assessing variation within populations. These statistics can be influenced by population size, age, and connectivity between populations (Goodall-Copestake et al., 2012). Haplotype diversity measures the probability that two randomly selected individuals belong to different haplotypes; values near 1 indicate high diversity, while values near 0 indicate low diversity (Fan et al., 2021). In this study, a high haplotype diversity (0.8619 ± 0.0321) was recorded, with 10 haplotypes identified. This value exceeds those reported in previous studies

that focused only on the HVRI D-Loop, where haplotype diversity values were 0.71, 0.52, and 0.50 (Cueva et al., 2018; Moreta, 2020; Vallejo, 2021, respectively). However, it was similar to values found by Guallasamín (2023) and Barragán (2023) (Hd = 0.857). When analyzed by province, Pichincha and Zamora Chinchipe showed the highest haplotype diversity, contrasting with earlier studies (Cueva, 2018; Moreta, 2020; Vallejo, 2021) that reported lower diversity for Zamora Chinchipe (Appendix 4). However, these earlier studies only analyzed HVRI D-Loop and included a larger sample size.

Nucleotide diversity measures the average number of nucleotide differences among sequences, directly reflecting genetic variability (Nei & Li, 1979). This study found low nucleotide diversity (0.00534 ± 0.00270), indicating low genetic variability. Thus, although there is a wide variety of haplotypes, differentiation among them is minimal, with some haplotypes differing by only one polymorphism (Figure 3). This trend is also observed in province-separated analyses (Appendix 4) which may be indicative of populations that may have undergone a genetic bottleneck, a pattern often seen in populations with reduced genetic diversity (Grant & Bowen, 1998). Cueva et al. (2018), also, reported lower nucleotide diversity, but their analysis focused exclusively on the HVR1 D-Loop, which covers a smaller portion of the mitochondrial genome and thus provides less comprehensive information than the additional mitochondrial markers used in this study. Although these genetic diversity statistics help understand the studied *T. ornatus* populations, results can be over- or underestimated due to relatively small sample size (Goodall-Copestake et al., 2012).

4.2. Demographic events

For demographic events, our analyses focused solely on the overall 36 individuals without dividing them by province, as the small sample sizes from each province are insufficient to adequately represent the dynamic of *T. ornatus* populations. Neutrality tests,

such as Tajima's D and Fu's Fs are useful for assessing demographic changes within a population by indicating whether a population has experienced expansion or contraction. Specifically, Tajima's D examines the frequency of polymorphisms, while Fu's Fs assesses haplotype distribution (You et al., 2023). Negative values in these tests indicate population expansion, whereas positive values suggest a population bottleneck or population reduction (Oussal et al., 2021).

In this study, both Tajima's D and Fu's Fs yielded positive values, which may suggest a population reduction in the analyzed *T. ornatus* populations. However, these results lack statistical significance ($p > 0.05$) (Table 3), and should therefore be interpreted cautiously, as the observed values may occur by random chance. Therefore, no definitive conclusions can be drawn, likely due to the limited sample size used in the study. Positive values in these tests suggest an excess of intermediate-frequency alleles (Tajima's D) or a lack of new haplotype (Fu's Fs), which are often the results of genetic drift in populations that have experienced reductions (Nei et al., 1975). However, the limited sample size may reduce the statistical power of the neutrality tests, making it difficult to detect subtle demographic changes reliably. Nevertheless, the findings of this study are consistent with those of Guallasamín (2023) and Barragán (2023), who also suggested a possible population contraction but did not provide strong conclusions. Thus, the positive but non-significant values observed in this study suggest preliminary indications that would benefit from further data to confidently confirm these demographic trends.

4.3. Genetic structure and conservation implications for *Tremarctos ornatus*

Studying genetic structure is essential for understanding conservation implications, as it helps develop strategies to protect species (Tan et al., 2005). Populations with higher genetic diversity are better equipped to adapt to environmental changes and selective pressures

(Mondol et al., 2013). Genetic structure can be interpreted by analyzing genetic variation between and within populations using statistical methods like the Analysis of Molecular Variance (AMOVA), which helps determine the percentage of differentiation (Wang, 2020). In this study, when analyzing the concatenated region, most of the genetic variation (93.33%) was found between provinces – specifically between Pichincha, Imbabura, Loja, and Zamora Chinchipe – while a smaller proportion (6.67%) was found within populations (Table 4). This level of differentiation is slightly lower than the 96.28% variation between populations and 3.72% within populations reported by Barragán (2023). This could be explained by the presence of multiple haplotypes within each population analyzed in this study, which could increase the proportion of genetic variation attributed to differences within populations, thereby slightly reducing the percentage of variation observed between provinces (Gardner et al., 2023.

Another important metric for assessing genetic differentiation is the fixation index, FST, which ranges from 0 to 1. A value closer to 1 indicates strong genetic separation between subpopulations or provinces, reflecting limited or no gene flow (Hasan et al., 2021). In the present study, the fixation index FST was 0.93, suggesting significant genetic differentiation among the provinces studied, likely driven by geographic barriers, reproductive isolation, or habitat fragmentation due to anthropogenic activities affecting the Andean bear (Guerrero-Casado & Zambrano, 2020). Given this significant differentiation, each *T. ornatus* population should be managed as an independent conservation unit, with distinct conservation measures tailored to each subpopulation's needs (Bradshaw et al., 2018).

5. CONCLUSION

The genetic analysis of the concatenated region (HVR1 D-Loop, COI-partial sequence, COI-COII-ATP8, ATP6-COIII, and ND6-CytB) revealed a sequence length of 3125 bp containing 37 polymorphic sites. Among the 36 individuals analyzed, 10 distinct haplotypes were found, with 3 newly documented in this study. Haplotype diversity was high (0.8619 ± 0.0321), whereas nucleotide diversity was low (0.00534 ± 0.00270), indicating limited differentiation between haplotypes, as some differed by only one polymorphic site.

Neutrality tests (Tajima's D and Fu's Fs) yielded positive values, suggesting a possible population reduction. However, these results lacked statistical significance, likely due to the limited sample size. Despite this, the results are consistent with previous studies, which also reported evidence of contraction in Andean bear (*Tremarctos ornatus*) populations. According to the AMOVA test, most genetic variation was found between populations rather than within them (93.33%), and the fixation index (FST) indicated substantial genetic separation among provinces.

Consequently, it is recommended to consider the population structure of *Tremarctos ornatus* when implementing conservation strategies, as Andean bear subpopulations should be managed as independent conservation units. Future research should aim to increase the sample size, number of sampling locations, and mitochondrial DNA regions analyzed to achieve more robust results, ideally supported by genomic studies to enhance the scope of these findings.

6. TABLES

Table 1. Sets of primers utilized for the amplifying the five mtDNA markers.

Mitochondrial marker	Expected length (bp)	Final length (bp)		Primer sequence (5' – 3')	Tm (ºC)	Ta (ºC)	GC (%)	Off-targets	Comments
HVRI D-Loop	600	430	Forward	TAGCTCCACCATCAACACCC	61	68	55	None	No dimers / hairpins
			Reverse	ACTGCGACGAGACCTTTACG	60		55		
COI-partial sequence	617	552	Forward	GACCGATGACTATTTTCCACA	56	60	43	None	No dimers / hairpins
			Reverse	AGCATAGTAATCCCAGCTGCC	60		52		
COI - COII - ATP8	1082	972	Forward	GATGCCCTCCTCCGTATCAC	60	60	60	None	No dimers / hairpins
			Reverse	GGTGGAAAAGGTTTTAGTTCGGG	61		48		
ATP6 - COIII	680	618	Forward	CAACATCACTGCAGGTCACT	59	58	50	None	No dimers / hairpins
			Reverse	AGTGATAAGTAGAGCCTGGAGT	59		45		
ND6-CytB	604	553	Forward	AGTACGATACCACATCCAACA	57	60	43	None	No dimers / hairpins
			Reverse	GGACGTACCCCATGAATGCT	60		55		

HVRI D-Loop primers were designed by Gabriela Bruque. COI-partial sequence, COI-COII-ATP8, ATP6-COIII, and ND6-CytB were designed by Darío Cueva. Tm, melting temperature. Ta, annealing temperature. GC, guanine-cytosine content.

Table 2. Identification of polymorphic sites in the sequence of each haplotype within the concatenated region.

Haplotype	n	Nucleotide position in concatenated region													
		1[+]		16	75	138	148	149	182	198	230	461	559	784	1127	1222
HTOP1	7	tRNA-Pro		C	-	T	C	T	T	T	A	C	G	C	C	C
HTOP2	4	tRNA-Pro		C	T	T	C	T	T	T	A	C	G	C	C	C
HTOP3	2	tRNA-Pro		C	-	T	C	T	T	T	G	C	G	C	C	C
HTOI1*	1	tRNA-Pro		C	-	T	C	T	T	T	A	C	G	C	C	C
HTOL1	3	tRNA-Pro		T	-	T	T	C	C	C	G	A	A	T	T	T
HTOL2	1	tRNA-Pro		C	T	T	C	T	T	T	A	A	A	T	T	T
HTOZ1	10	tRNA-Pro		T	-	C	T	T	C	C	G	A	A	T	T	C
HTOZ2	5	tRNA-Pro		T	T	C	T	T	C	C	G	A	A	T	T	C
HTOZ3*	1	tRNA-Pro		T	-	T	T	T	C	C	G	A	A	T	T	C
HTOZ4*	2	tRNA-Pro		T	T	T	T	T	C	C	G	A	A	T	T	C
		1283	1444	1464	1738	1891	1964	2005	2076	2168	2223	2471	2565	2596	2796	2806
HTOP1	7	A	C	G	C	G	G	T	A	T	C	A	A	G	G	T
HTOP2	4	A	C	G	C	G	G	T	A	T	C	A	A	G	G	T
HTOP3	2	A	C	G	C	G	G	T	A	T	C	A	A	G	G	T
HTOI1*	1	G	C	G	C	G	G	C	A	T	C	G	A	G	G	T
HTOL1	3	G	T	A	T	A	A	C	A	T	T	G	G	A	A	C
HTOL2	1	G	T	A	T	A	A	C	A	T	T	G	G	A	A	C
HTOZ1	10	G	T	A	T	A	A	C	G	T	T	G	G	A	A	C
HTOZ2	5	G	T	A	T	A	A	C	G	T	T	G	G	A	A	C
HTOZ3*	1	G	T	A	T	A	A	C	A	C	T	G	G	A	A	C
HTOZ4*	2	G	T	A	T	A	A	C	A	C	T	G	G	A	A	C

		2873	2931	2977	2992	3007	3031	3058	3070	3094
HTOP1	7	A	C	A	C	A	G	T	T	T
HTOP2	4	A	C	A	C	A	G	T	T	T
HTOP3	2	A	C	A	C	A	G	T	T	T
HTOI1*	1	A	C	G	C	A	G	T	T	T
HTOL1	3	G	T	A	T	G	A	C	C	C
HTOL2	1	G	T	A	T	G	A	C	C	C
HTOZ1	10	G	T	A	T	G	A	C	C	C
HTOZ2	5	G	T	A	T	G	A	C	C	C
HTOZ3*	1	G	T	A	T	G	A	C	C	C
HTOZ4*	2	G	T	A	T	G	A	C	C	C

n Number of individuals

+ The starting position is located within the coding sequence for the proline tRNA in the mtDNA, with the 37 identified polymorphic sites detailed per mitochondrial marker (**HVRI D-Loop**, **COI-partial sequence**, **COI-COII-ATP8**, **ATP6-COIII**, and **ND6-CytB**).

* New haplotype(s) found in this study for the *T. ornatus* populations in Imbabura and Zamora.

Table 3. Number of haplotypes, haplotype diversity, nucleotide diversity, and results of neutrality tests (Tajima's D and Fu's Fs) for five mitochondrial markers and the concatenated region of the analyzed *Tremarctos ornatus* individuals.

mtDNA marker (bp)	Population (n)	Number of haplotypes	Haplotype diversity (Hd ± sd)	Nucleotide diversity (π ± sd)	Tajima's D**	p-value	Fu's Fs**	p-value
HVRI D-Loop (430)	Overall (36)	8	0.8444 ± 0.0309	0.00835 ± 0.00482	2.42293	0.992	1.10266	0.720
COI-partial sequence (552)	Overall (36)	2	0.4889 ± 0.0408	0.00266 ± 0.00183	2.30057	0.990	4.99700	0.970
COI-COII-ATP8 (972)	Overall (36)	4	0.6238 ± 0.0489	0.00321 ± 0.00190	2.40695	0.996	4.82454	0.960
ATP6-COIII (618)	Overall (36)	5	0.6952 ± 0.0460	0.00497 ± 0.00294	2.32436	0.993	3.33132	0.919
ND6-CytB (553)	Overall (36)	3	0.5095 ± 0.0530	0.00983 ± 0.00538	2.75843	0.997	11.08895	0.999
Concatenated region (3125)* HVRI D-Loop + COI-partial sequence + COI-COII-ATP8 + ATP6-COIII + ND6-CytB	Overall (36)	10	0.8619 ± 0.0321	0.00534 ± 0.00270	3.08465	1.000	9.21257	0.988

bp, final size of the sequence after trimming.

* The concatenated region was created from the combination of the sequences of the five mitochondrial markers analyzed in *T. ornatus* populations.

** None of the values of the neutrality tests (Tajima's D and Fu's Fs) were statistically significant ($p>0.05$).

Table 4. Analysis of Molecular Variance (AMOVA) of the concatenated region.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation (%)	p-value
Between populations	1	255.82	14.89 Va	93.33	0.00
Within populations	34	36.15	1.06 Vb	6.67	
Total	35	291.97	15.95		
Fixation index			FST: 0.93		

The concatenated region was created from the combination of the sequences of the five mitochondrial markers analyzed in *T. ornatus* populations.

d.f., degrees of freedom.

FST, Fixation index.

7. FIGURES

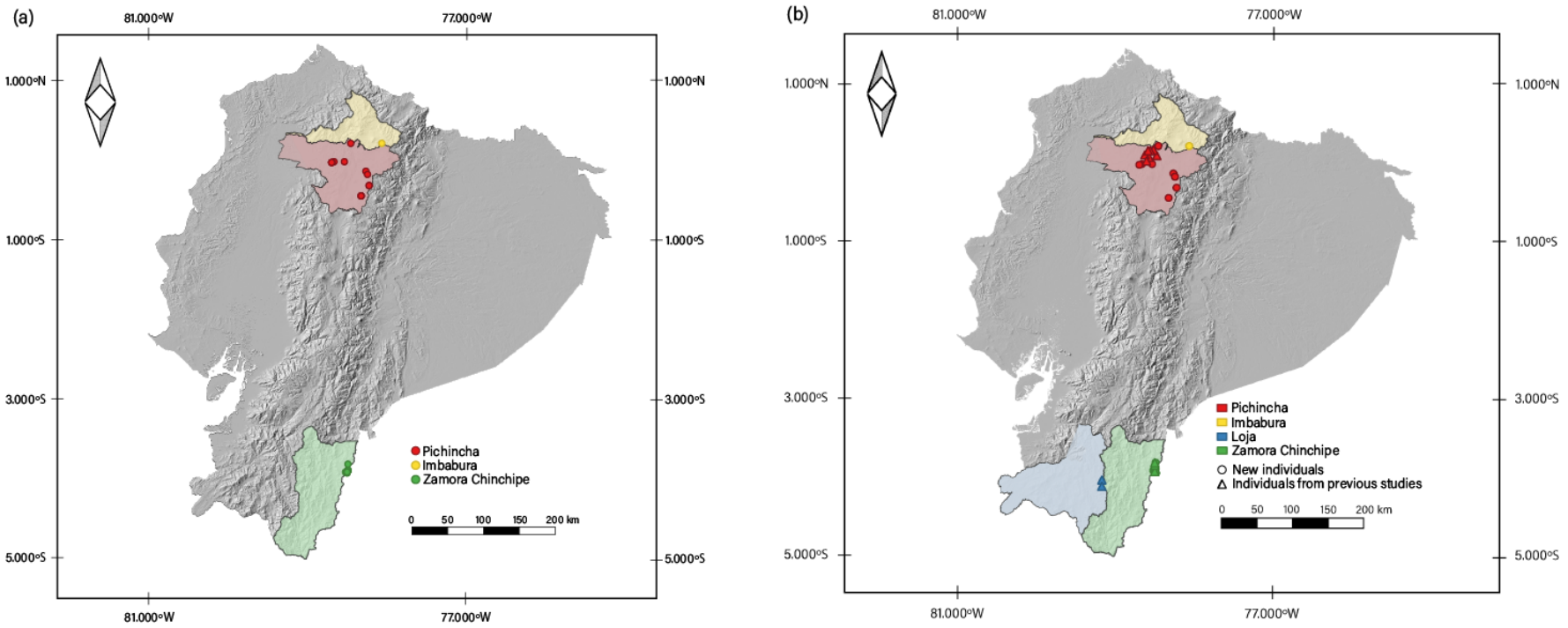

Figure 1. Map showing the locations where samples from *T. ornatus* individuals were collected in Ecuador.

(a) Map of the geographic locations of all 28 individuals collected for this research, regardless of their inclusion in the analyses (Appendix 1).

(b) Map of the geographic locations of all individuals included in the analyses, 14 from this study (Appendix 1) and 22 from previous studies by Guallasamín (2023) and Barragán (2023). Both maps were created using QGis v.3.34 (Moyroud & Portet, 2018).

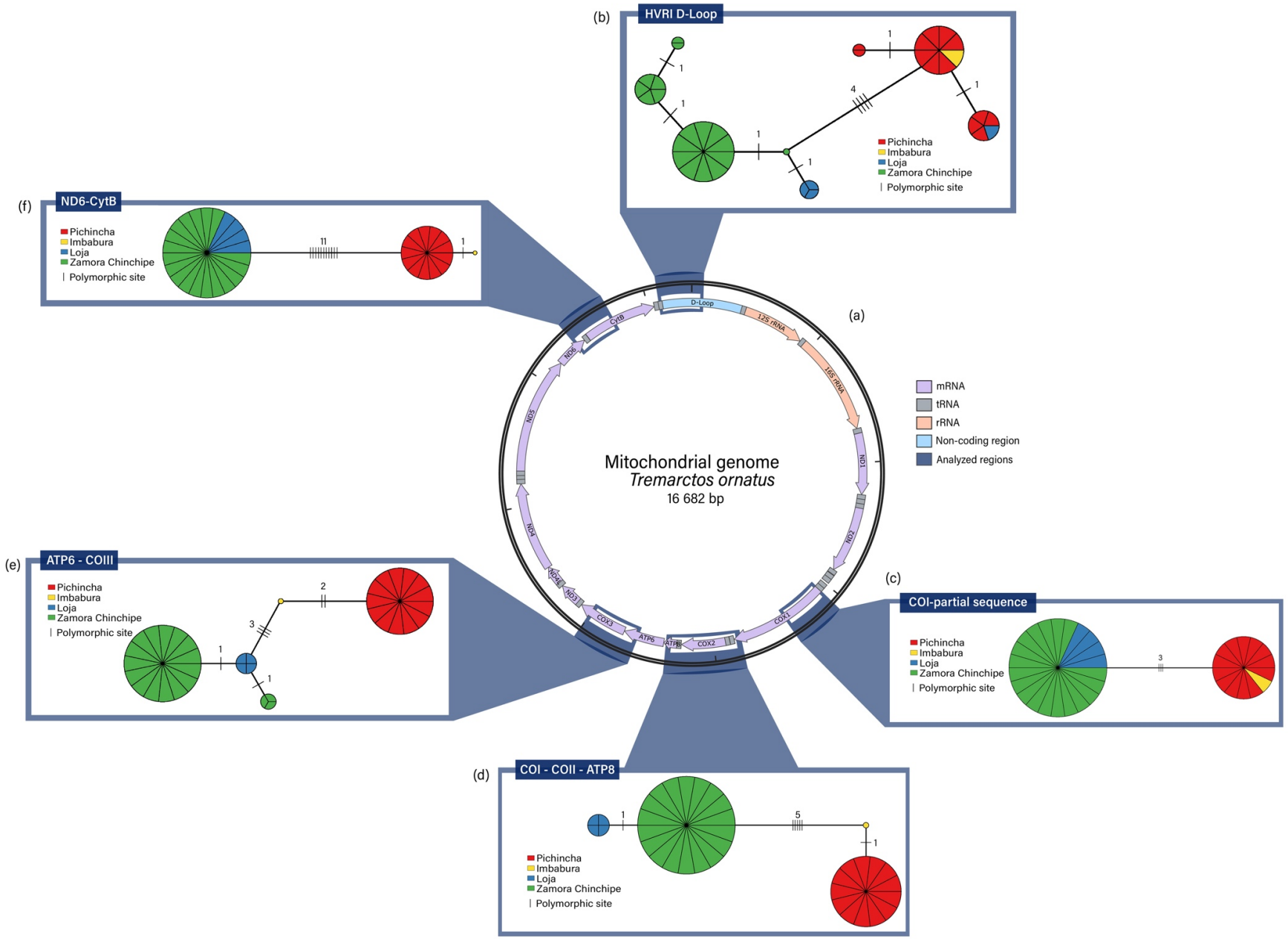
(a)
Mitochondrial genome
Tremarctos ornatus
16 682 bp
D-Loop
12S rRNA
16S rRNA
ND1
ND2
COX1
COX2
ATP8
ATP6
COX3
ND3
ND4L
ND4
ND5
ND6
CytB
mRNA
tRNA
rRNA
Non-coding region
Analyzed regions
(b)
HVRI D-Loop
(c)
COI-partial sequence
(d)
COI - COII - ATP8
(e)
ATP6 - COIII
(f)
ND6-CytB
Pichincha
Imbabura
Loja
Zamora Chinchipe
Polymorphic site

Figure 2. Haplotype networks representing the individual mtDNA markers.

(a) Mitochondrial genome map of *Tremarctos ornatus* (NCBI accession MW556430.1), showing its respective genes (**mRNA**, **tRNA**, **rRNA**) and the **non-coding region**. The regions analyzed in this study are highlighted: HVRI D-Loop, COI-partial sequence, COI-COII-ATP8, ATP6-COIII, and ND6-CytB. (b) Haplotype network of HVRI D-Loop. (c) Haplotype network of COI-partial sequence. (d) Haplotype network of COI-COII-ATP8. (e) Haplotype network of ATP6-COIII. (f) Haplotype network of ND6-CytB. Each circle represents a distinct haplotype, with its size proportional to the number of individuals it includes, which is further indicated by the number of segments within the circle. Colors represent the province of origin of individuals (**Pichincha**, **Imbabura**, **Loja**, and **Zamora Chinchipe**). Vertical lines indicate polymorphic sites present among haplotypes.

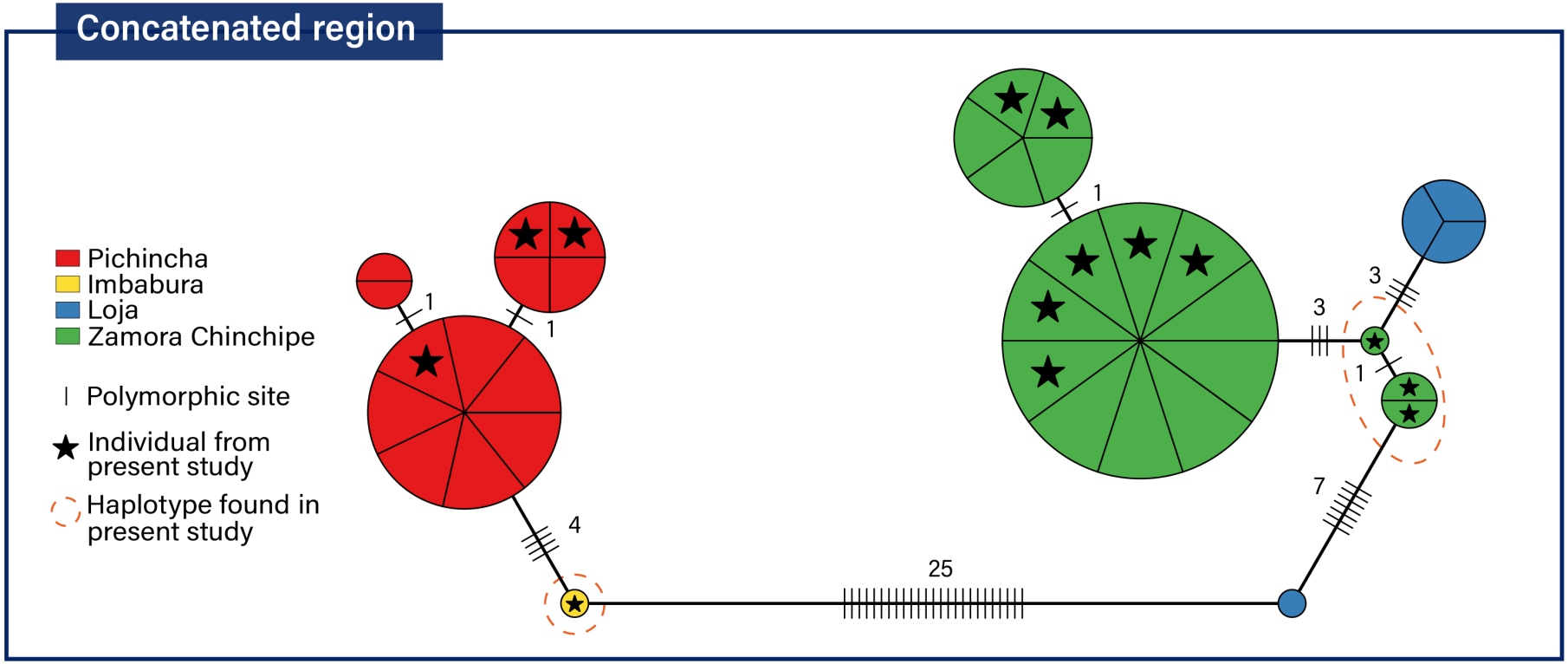

Figure 3. Haplotype networks representing the concatenated region.

The concatenated region haplotype network includes sequences from the five mitochondrial markers used in this study: HVRI D-Loop + COI-partial sequence + COI-COII-ATP8 + ATP6-COIII + ND6-CytB. Each circle represents a distinct haplotype, with its size proportional to the number of individuals it includes, which is further indicated by the number of segments within the circle. Colors represent the province of origin

of individuals (**Pichincha**, **Imbabura**, **Loja**, and **Zamora Chinchipe**). Vertical lines indicate polymorphic sites present among haplotypes. Star icons indicate the 14 new individuals included in this study.

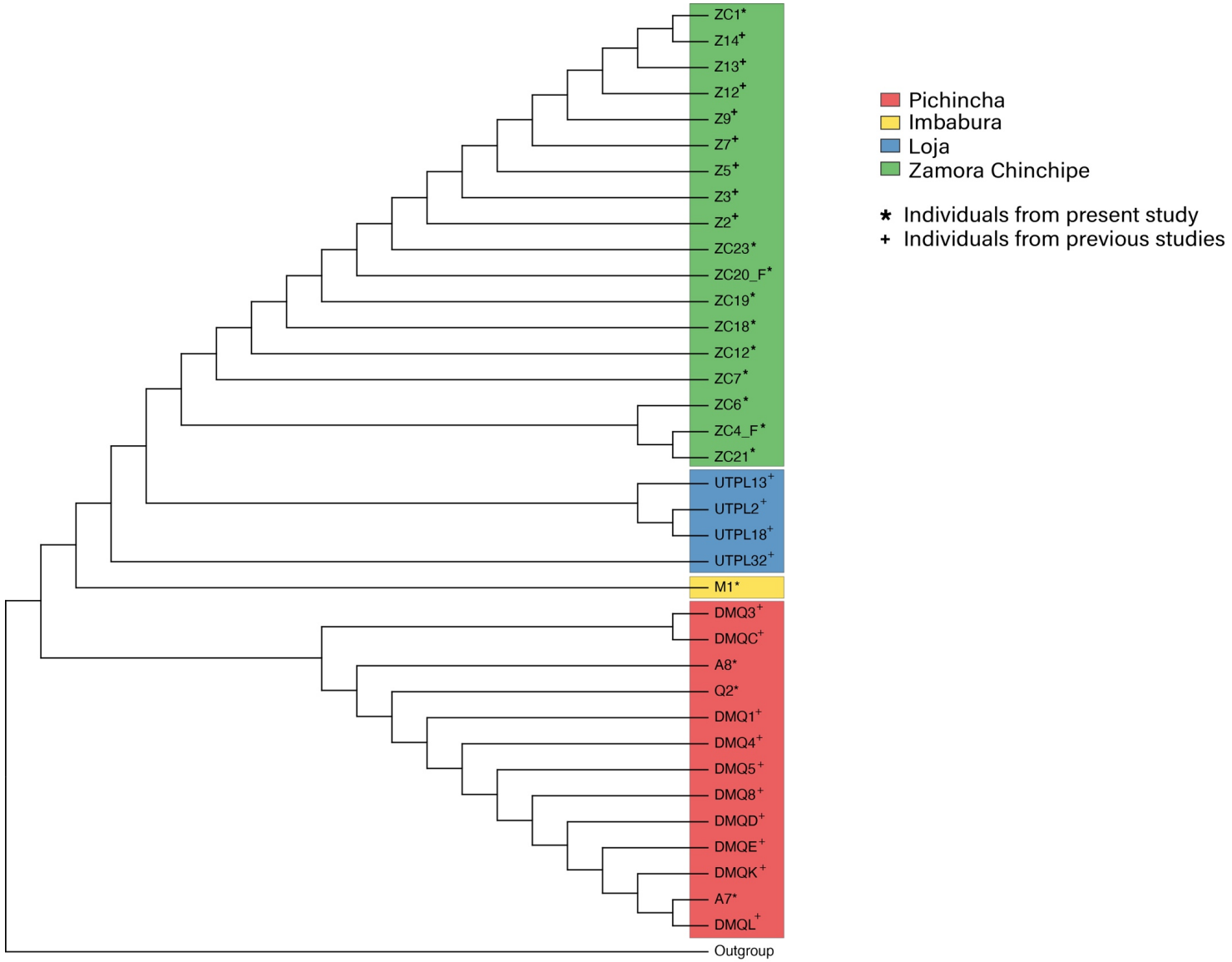
ZC1*
Z14+
Z13+
Z12+
Z9+
Z7+
Z5+
Z3+
Z2+
ZC23*
ZC20_F*
ZC19*
ZC18*
ZC12*
ZC7*
ZC6*
ZC4_F*
ZC21*
UTPL13+
UTPL2+
UTPL18+
UTPL32+
M1*
DMQ3+
DMQC+
A8*
Q2*
DMQ1+
DMQ4+
DMQ5+
DMQ8+
DMQD+
DMQE+
DMQK+
A7*
DMQL+
Outgroup
Pichincha
Imbabura
Loja
Zamora Chinchipe
* Individuals from present study
+ Individuals from previous studies

Figure 4. Phylogenetic tree of all 36 individuals included in this study, constructed using the Maximum-Likelihood method and the Hasegawa-Kishino-Yano (HKY) substitution model.

The phylogenetic tree reveals two groups: individuals from Pichincha are found in the first group, while individuals from Zamora Chinchipe, Loja, and Imbabura form the second. The mitochondrial DNA genome sequence of *Ursus americanus* (GenBank accession: NC_003426.1) was used as an outgroup.

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APPENDICES

Appendix 1. Information of the 28 sampled individuals: Sample ID, location of sampling site, longitude and latitude of sampling site, nucleic acid concentration, A260/A280 index, A260/A230 index (indicating DNA extraction quality), and assigned haplotype.

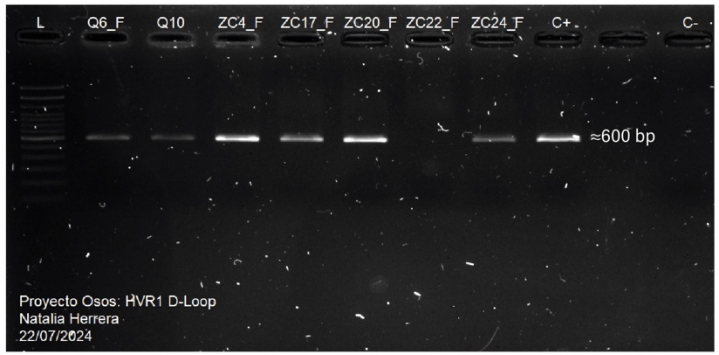
Province	Sample ID	Sampling site	Longitude	Latitude	DNA concentration (ng/ul)	A260/A280	A260/A230	Haplotype
Pichincha	A7	Antisana Chakana Isco	-78.33	-0.45	6.9	3.05	0.02	HTOP2
	A8	Antisana Itulcachi Niguanchi	-78.23	-0.32	8.90	2.59	0.01	HTOP1
	A9	Antisana Chakana Isco	-78.33	-0.45	388.20	1.30	6.16	NA
	Q1_F	Quito Pahuma	-78.67	-0.02	108.50	1.67	2.40	NA
	Q2	Quito Bellavista, San Luis	-78.70	-0.03	11.80	2.21	0.01	HTOP2
	Q5	Quito Bellavista, San Luis	-78.70	-0.03	332.20	1.41	6.73	NA
	Q6_F	Quito Bellavista, San Luis	-78.70	-0.03	1.40	0.46	0.18	NA
	Q8	Quito Yunguilla El Guarumal	-78.54	-0.02	553.40	1.33	12.46	NA
	Q9	Checa	-78.27	-0.14	0.80	0.54	0.03	NA
	Q10	Lalagachi Alto, Checa	-78.25	-0.18	2.90	0.68	0.09	NA
	Q11	Minaschupa, San José de Minas	-78.46	0.21	2.00	0.67	0.06	NA
	L1	Lava/Isco	-78.33	-0.45	2.20	0.63	0.22	NA
	L2	Lava	-78.33	-0.45	2.60	0.66	0.22	NA
	N1	Niguanchi	-78.23	-0.32	163.60	1.55	3.07	NA
Imbabura	M1	Manzano	-78.07	0.21	61.80	1.74	0.12	HTOI1
	ZC1	Paquisha, Río Blanco	-78.49	-3.90	2.70	2.68	0.27	HTOZ1
	ZC4_F	Paquisha, Río Blanco	-78.49	-3.90	0.60	0.28	0.02	HTOZ3

Zamora Chinchipe	ZC6	Paquisha, Río Blanco	-78.49	-3.90	3.50	2.34	0.30	HTOZ4
	ZC7	Paquisha, Río Blanco	-78.49	-3.90	9.50	2.35	0.01	HTOZ1
	ZC12	Paquisha, Río Blanco	-78.52	-3.92	16.20	2.37	0.03	HTOZ1
	ZC17_F	Paquisha, Río Blanco	-78.49	-3.90	0.70	0.75	0.03	NA
	ZC18	Paquisha, Río Blanco	-78.49	-3.90	13.90	2.46	0.02	HTOZ2
	ZC19	Paquisha, Río Blanco	-78.49	-3.90	12.20	2.66	0.02	HTOZ1
	ZC20_F	Paquisha, Río Blanco	-78.50	-3.93	0.40	0.40	0.01	HTOZ1
	ZC21	Paquisha, Río Blanco	-78.50	-3.93	117.10	1.89	2.42	HTOZ4
	ZC22_F	Paquisha, Río Blanco	-78.50	-3.92	0.10	1.54	0.03	NA
	ZC23	Paquisha, Río Blanco	-78.50	-3.92	4.80	3.44	0.01	HTOZ2
	ZC24_F	Paquisha, Río Blanco	-78.49	-3.82	2.90	0.60	0.18	NA

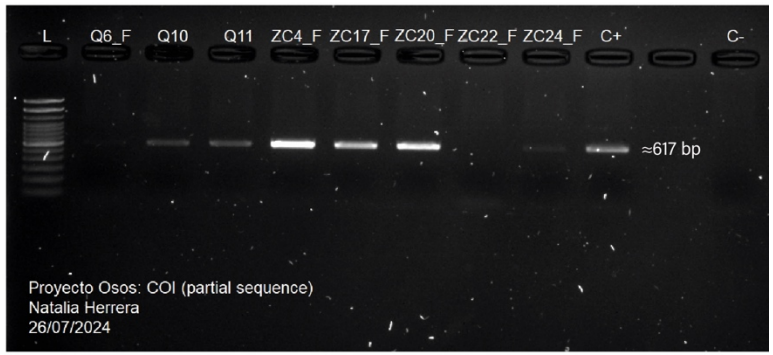
NA, the individual does not belong to any haplotype because amplification failed for one or more mitochondrial markers, avoiding inclusion in the analyses.

Appendix 2. Images of electrophoresis gel of PCR products obtained from the amplification of the five mitochondrial markers.

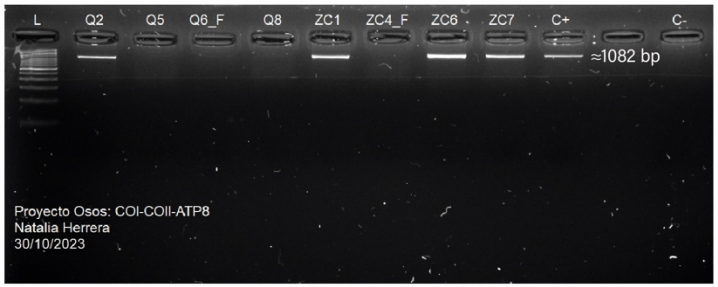
(a)



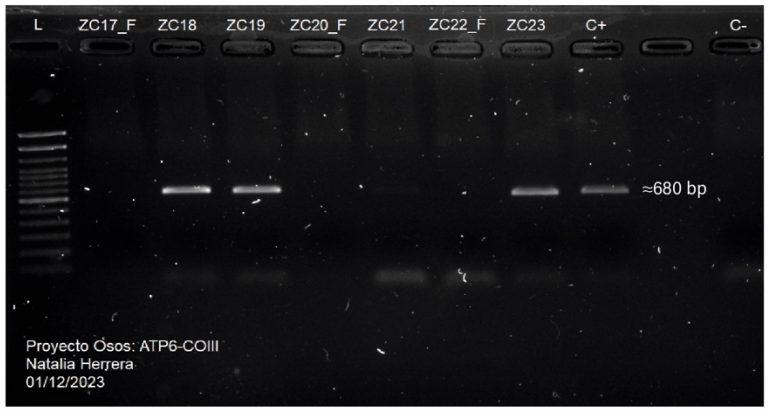
(b)



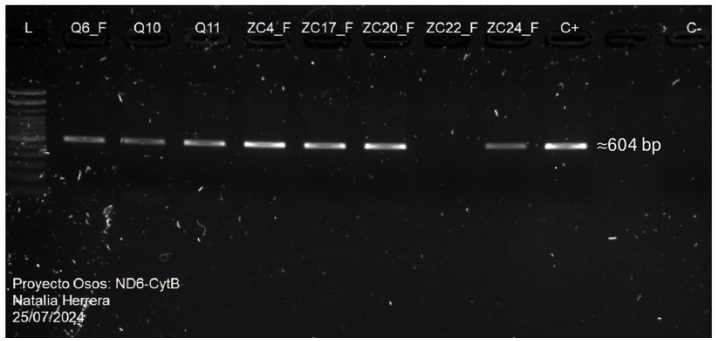
(c)



(d)



(e)



DNA bands indicate successful amplification of the regions a) HVRI D-Loop, b) COI-partial sequence, c) COI-COII-ATP8, d) ATP6-COIII, and e) ND6-CytB for samples from Pichincha and Zamora Chinchipe, with expected amplicon lengths of 600, 617, 1082, 680, and 604 bp, respectively.

Appendix 3. Haplotype sequences of the concatenated region and the number of individuals per haplotype.

Haplotype	Population (n)	Concatenated region
HTOP1	Pichincha (7)	TTTACTTATTTCATACATATCATCCCACGTACTGTAGCATCCTAGTATGTCCCCGAACAAGGAAACCTTTCTTT- TTTTTCCCCCCTATGTACGTCGTGCATTAATGGCGTGCCCCATGCATATAAGCATGTACATATCTTGCTTGGCTTT ACATGAGGACATGGACTTCAAAAACTCGTTTTGAAGACGTAGTCTGTAAGCATGTATTTCACTTAGTCCGGGAG CTTAATCACCAGGCCTCGAGAAACCAGCAACCCTTGCGAGTACGTATACCTCTTCTCGCTCCGGGCCCATAGAA ACGTGGGGGTTTCTATACTGAAACTATACCTGGCATCTGGTTCTTACTTCAGGGCCATGATAGCTCTAGATTCCA ATCCTACTAACCCTTCAAATGGGACATCTCGATGGACTAATGACTAATCAGCCCATACAAACCATAAAGACATT GGTACTCTCTATCTCTTATTCGGTGCATGAGCCGGAATAGTAGGTACTGCCCTCAGCCTTCTAATCCGCGCTGAA CTAGGTCAACCCGGAGCCCTGTTAGGGGATGATCAGATCTATAACGTGGTCGTAACTGCCCATGCATTCGTAAT AATCTTCTTTATAGTAATGCCTATTATAATCGGAGGTTTTGGAAACTGATTAGTGCCTTTAATAATTGGCGCTCC CGATATAGCATTCCCTCGAATAAACAATATGAGTTTCTGATTACTGCCACCATCCTTCCTACTTCTTCTAGCCTC TTCCATAGTAGAAGCAGGTGCAGGGACTGGTTGAACCGTTTACCCCCCTCTAGCGGGCAATCTAGCCCATGCAG GAGCATCAGTAGACCTAACAATTTTTTCCCTACATTTAGCAGGCGTTTCCTCCATTCTAGGAGCTATTAACTTTA TTACCACTATTATCAATATGAAGCCTCCTGCAATATCTCAATACCAAACTCCTCTGTTCGTATGATCCGTCCTAA TTACGGCGGTAAGGAGTCGAACCCCCTGAAATTGGTTTCAAGCCAATACCATAACCACTATGTCTCTCTCAATA AAGAGATATTAGTAAAAATTACATAACTTCGTCAGGGTTAAATTATAGGTGAAAATCCTTTATATCTCTATGGC GTATCCCCTTCAAATAGGCCTTCAAGACGCAACTTCTCCCATTATAGAAGAGCTCCTACACTTCCACGACCATA CATTAATGATTGTATTCCTGATTAGCTCCTTAGTTCTCTACATTATTTCAACTATGCTAACTACTAAACTAACGC ACACAAACACAATAAATGCACAGGAAGTAGAAACGGTATGAACCATTCTACCAGCCATTATCCTGGTTCTAATT GCACTCCCATCATTACGAATCCTCTATATAATGGATGAAATCAACAATCCTTTACTGACTGTAAAAACTATAGG CCATCAATGATACTGAAGTTACGAATACACGGACTATGAAGATTTGAGCTTTGATTCTTACATGATTCCAACAC AAGAATTAAAGCCTGGAGAACTACGACTATTAGAAGTAGACAATCGAGCGGTACTACCCATAGAAATGACTAT TCGCATGCTAATTTCATCAGAAGATGTCTTGCACTCATGAGCTGTACCATCCCTAGGACTAAAAACTGATGCAA TTCCGGGACGACTAAACCAAACGACTCTCATGGCCATGCGACCAGGGCTGTATTATGGCCAATGCTCGGAAATC TGTGGCTCTAACCACAGCTTCATGCCCATTGTTCTCGAACTAGTCCCACTGTCCTATTTCGAAAAATGATCCGCT TCAATATTATAAAATCATTAAGAAGCTATATAGCATTAACCTTTTAAGTTAAAGACTGAGAGTGCAAGTCTCTC CTTAATGGAGATGCCACAACTAGACACGTCGACATGGTTTATCACAATTCTATCTATAACTCTAACACTATTTAT TGTATTCCAACTAAAAATCTAAACATCAGCATCGCCACAGCCCTCATCACTTTCTCTATTCTAGTACTATTCACT ATTCTTGAACTCGCCGTGGCTCTCATCCAAGCCTATGTCTTTGCTCTACTAGTAAGTCTATACTTACATGACAAC

		ACCTAATGGCCCACCAAACACATGCATATCACATAGTCAATCCAAGCCCATGACCACTAACGGGAGCCCTTTCA GCCCTTCTTATAACATCAGGACTAATCATATGATTCCACTTCAACTCAACATCCTTGCTATTACTAGGCCTTACA ACTAATATACTTACCATATATCAATGATGACGGGACATTATCCGAGAAAGTACTTTTCAAGGCCACCACACTCC TGTCGTTCAAAAGGGGTTGCGGTATGGGATAGTCCTATTTATTGTATCGGAGGTATTTTTCTTCACAGGATTCTT CTGAGCTTTTTACCACTCAAGTCTAGCACCTACTCCCGAACTAGGAGCATGCTGACCACCCACAGGCATCACCC CCCTAAACCCATTAGAAGTGCCGCTTCTTAATACCTCAGTACTCCTAGCATCTGGAGTATCTATTACTTGAGCCC ACCATAGCTTAATAGAAGGAAATCGCAATCATATACTCCAACAATTAAAACTAAACCCCCGTAGACAGGAGAG GGCTTAGAAGAAAAGCCTACAAAACCTATTACAAAAACAACACTTAAAATGAACACAGTATATGTTACCATTA TTCCCACATGGAATCTAACCATGACTAATGACATGAAAAATCACCGTTGTATTTCAACTATAAGAATCTTAATG ACCAACATCCGAAAAACTCACCCACTAGCTAAAATCATCAACAGCTCATTCATTGACCTCCCAACACCATCAAA TATCTCAGCATGATGAAACTTCGGGTCCCTTCTTGGGGTGTGCCTGATCCTACAAATCCTAACGGGCCTATTCCT GGCCATACACTATACAGCAGACACGACTACAGCCTTCTCATCAGTCGCCCATATCTGTCGAGACGTTAACTACG GATGAGTTATCCGATACATACACGCGAACGGAGCTTCAATATTCTTTATCTGCTTGTTCATACACGTGGGACGG GGTCTGTATTACGGCTCATATCTATTTTCAGAAACATGAAACATTGGAATTATTCTCCTACTCACAGTTATAGCC A
HTOP2	Pichincha (4)	TTTACTTATTTCATACATATCATCCCACGTACTGTAGCATCCTAGTATGTCCCCGAACAAGGAAACCTTTCTTTTT TTTTCCCCCCTATGTACGTCGTGCATTAATGGCGTGCCCCATGCATATAAGCATGTACATATCTTGCTTGGCTTT ACATGAGGACATGGACTTCAAAAACTCGTTTTGAAGACGTAGTCTGTAAGCATGTATTTCACTTAGTCCGGGAG CTTAATCACCAGGCCTCGAGAAACCAGCAACCCTTGCGAGTACGTATACCTCTTCTCGCTCCGGGCCCATAGAA ACGTGGGGGTTTCTATACTGAAACTATACCTGGCATCTGGTTCTTACTTCAGGGCCATGATAGCTCTAGATTCCA ATCCTACTAACCCTTCAAATGGGACATCTCGATGGACTAATGACTAATCAGCCCATACAAACCATAAAGACATT GGTACTCTCTATCTCTTATTCGGTGCATGAGCCGGAATAGTAGGTACTGCCCTCAGCCTTCTAATCCGCGCTGAA CTAGGTCAACCCGGAGCCCTGTTAGGGGATGATCAGATCTATAACGTGGTCGTAACTGCCCATGCATTCGTAAT AATCTTCTTTATAGTAATGCCTATTATAATCGGAGGTTTTGGAAACTGATTAGTGCCTTTAATAATTGGCGCTCC CGATATAGCATTCCCTCGAATAAACAATATGAGTTTCTGATTACTGCCACCATCCTTCCTACTTCTTCTAGCCTC TTCCATAGTAGAAGCAGGTGCAGGGACTGGTTGAACCGTTTACCCCCCTCTAGCGGGCAATCTAGCCCATGCAG GAGCATCAGTAGACCTAACAATTTTTTCCCTACATTTAGCAGGCGTTTCCTCCATTCTAGGAGCTATTAACTTTA TTACCACTATTATCAATATGAAGCCTCCTGCAATATCTCAATACCAAACTCCTCTGTTCGTATGATCCGTCCTAA TTACGGCGGTAAGGAGTCGAACCCCCTGAAATTGGTTTCAAGCCAATACCATAACCACTATGTCTCTCTCAATA AAGAGATATTAGTAAAAATTACATAACTTCGTCAGGGTTAAATTATAGGTGAAAATCCTTTATATCTCTATGGC GTATCCCCTTCAAATAGGCCTTCAAGACGCAACTTCTCCCATTATAGAAGAGCTCCTACACTTCCACGACCATA CATTAATGATTGTATTCCTGATTAGCTCCTTAGTTCTCTACATTATTTCAACTATGCTAACTACTAAACTAACGC ACACAAACACAATAAATGCACAGGAAGTAGAAACGGTATGAACCATTCTACCAGCCATTATCCTGGTTCTAATT GCACTCCCATCATTACGAATCCTCTATATAATGGATGAAATCAACAATCCTTTACTGACTGTAAAAACTATAGG

		CCATCAATGATACTGAAGTTACGAATACACGGACTATGAAGATTTGAGCTTTGATTCTTACATGATTCCAACAC AAGAATTAAAGCCTGGAGAACTACGACTATTAGAAGTAGACAATCGAGCGGTACTACCCATAGAAATGACTAT TCGCATGCTAATTTCATCAGAAGATGTCTTGCACTCATGAGCTGTACCATCCCTAGGACTAAAAACTGATGCAA TTCCGGGACGACTAAACCAAACGACTCTCATGGCCATGCGACCAGGGCTGTATTATGGCCAATGCTCGGAAATC TGTGGCTCTAACCACAGCTTCATGCCCATTGTTCTCGAACTAGTCCCACTGTCCTATTTCGAAAAATGATCCGCT TCAATATTATAAAATCATTAAGAAGCTATATAGCATTAACCTTTTAAGTTAAAGACTGAGAGTGCAAGTCTCTC CTTAATGGAGATGCCACAACTAGACACGTCGACATGGTTTATCACAATTCTATCTATAACTCTAACACTATTTAT TGTATTCCAACTAAAAATCTAAACATCAGCATCGCCACAGCCCTCATCACTTTCTCTATTCTAGTACTATTCACT ATTCTTGAACTCGCCGTGGCTCTCATCCAAGCCTATGTCTTTGCTCTACTAGTAAGTCTATACTTACATGACAAC ACCTAATGGCCCACCAAACACATGCATATCACATAGTCAATCCAAGCCCATGACCACTAACGGGAGCCCTTTCA GCCCTTCTTATAACATCAGGACTAATCATATGATTCCACTTCAACTCAACATCCTTGCTATTACTAGGCCTTACA ACTAATATACTTACCATATATCAATGATGACGGGACATTATCCGAGAAAGTACTTTTCAAGGCCACCACACTCC TGTCGTTCAAAAGGGGTTGCGGTATGGGATAGTCCTATTTATTGTATCGGAGGTATTTTTCTTCACAGGATTCTT CTGAGCTTTTTACCACTCAAGTCTAGCACCTACTCCCGAACTAGGAGCATGCTGACCACCCACAGGCATCACCC CCCTAAACCCATTAGAAGTGCCGCTTCTTAATACCTCAGTACTCCTAGCATCTGGAGTATCTATTACTTGAGCCC ACCATAGCTTAATAGAAGGAAATCGCAATCATATACTCCAACAATTAAAACTAAACCCCCGTAGACAGGAGAG GGCTTAGAAGAAAAGCCTACAAAACCTATTACAAAAACAACACTTAAAATGAACACAGTATATGTTACCATTA TTCCCACATGGAATCTAACCATGACTAATGACATGAAAAATCACCGTTGTATTTCAACTATAAGAATCTTAATG ACCAACATCCGAAAAACTCACCCACTAGCTAAAATCATCAACAGCTCATTCATTGACCTCCCAACACCATCAAA TATCTCAGCATGATGAAACTTCGGGTCCCTTCTTGGGGTGTGCCTGATCCTACAAATCCTAACGGGCCTATTCCT GGCCATACACTATACAGCAGACACGACTACAGCCTTCTCATCAGTCGCCCATATCTGTCGAGACGTTAACTACG GATGAGTTATCCGATACATACACGCGAACGGAGCTTCAATATTCTTTATCTGCTTGTTCATACACGTGGGACGG GGTCTGTATTACGGCTCATATCTATTTTCAGAAACATGAAACATTGGAATTATTCTCCTACTCACAGTTATAGCC A
HTOP3	Pichincha (2)	TTTACTTATTTCATACATATCATCCCACGTACTGTAGCATCCTAGTATGTCCCCGAACAAGGAAACCTTTCTTT- TTTTTCCCCCCTATGTACGTCGTGCATTAATGGCGTGCCCCATGCATATAAGCATGTACATATCTTGCTTGGCTTT ACATGAGGACATGGACTTCAAAAACTCGTTTTGAAGACGTAGTCTGTAAGCATGTATTTCACTTAGTCCGGGAG CTTAGTCACCAGGCCTCGAGAAACCAGCAACCCTTGCGAGTACGTATACCTCTTCTCGCTCCGGGCCCATAGAA ACGTGGGGGTTTCTATACTGAAACTATACCTGGCATCTGGTTCTTACTTCAGGGCCATGATAGCTCTAGATTCCA ATCCTACTAACCCTTCAAATGGGACATCTCGATGGACTAATGACTAATCAGCCCATACAAACCATAAAGACATT GGTACTCTCTATCTCTTATTCGGTGCATGAGCCGGAATAGTAGGTACTGCCCTCAGCCTTCTAATCCGCGCTGAA CTAGGTCAACCCGGAGCCCTGTTAGGGGATGATCAGATCTATAACGTGGTCGTAACTGCCCATGCATTCGTAAT AATCTTCTTTATAGTAATGCCTATTATAATCGGAGGTTTTGGAAACTGATTAGTGCCTTTAATAATTGGCGCTCC CGATATAGCATTCCCTCGAATAAACAATATGAGTTTCTGATTACTGCCACCATCCTTCCTACTTCTTCTAGCCTC

TTCCATAGTAGAAGCAGGTGCAGGGACTGGTTGAACCGTTTACCCCCCTCTAGCGGGCAATCTAGCCCATGCAG
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TTACGGCGGTAAGGAGTCGAACCCCCTGAAATTGGTTTCAAGCCAATACCATAACCACTATGTCTCTCTCAATA
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GTATCCCCTTCAAATAGGCCTTCAAGACGCAACTTCTCCCATTATAGAAGAGCTCCTACACTTCCACGACCATA
CATTAATGATTGTATTCCTGATTAGCTCCTTAGTTCTCTACATTATTTCAACTATGCTAACTACTAAACTAACGC
ACACAAACACAATAAATGCACAGGAAGTAGAAACGGTATGAACCATTCTACCAGCCATTATCCTGGTTCTAATT
GCACTCCCATCATTACGAATCCTCTATATAATGGATGAAATCAACAATCCTTTACTGACTGTAAAAACTATAGG
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AAGAATTAAAGCCTGGAGAACTACGACTATTAGAAGTAGACAATCGAGCGGTACTACCCATAGAAATGACTAT
TCGCATGCTAATTTCATCAGAAGATGTCTTGCACTCATGAGCTGTACCATCCCTAGGACTAAAAACTGATGCAA
TTCCGGGACGACTAAACCAAACGACTCTCATGGCCATGCGACCAGGGCTGTATTATGGCCAATGCTCGGAAATC
TGTGGCTCTAACCACAGCTTCATGCCCATTGTTCTCGAACTAGTCCCACTGTCCTATTTCGAAAAATGATCCGCT
TCAATATTATAAAATCATTAAGAAGCTATATAGCATTAACCTTTTAAGTTAAAGACTGAGAGTGCAAGTCTCTC
CTTAATGGAGATGCCACAACTAGACACGTCGACATGGTTTATCACAATTCTATCTATAACTCTAACACTATTTAT
TGTATTCCAACTAAAAATCTAAACATCAGCATCGCCACAGCCCTCATCACTTTCTCTATTCTAGTACTATTCACT
ATTCTTGAACTCGCCGTGGCTCTCATCCAAGCCTATGTCTTTGCTCTACTAGTAAGTCTATACTTACATGACAAC
ACCTAATGGCCCACCAAACACATGCATATCACATAGTCAATCCAAGCCCATGACCACTAACGGGAGCCCTTTCA
GCCCTTCTTATAACATCAGGACTAATCATATGATTCCACTTCAACTCAACATCCTTGCTATTACTAGGCCTTACA
ACTAATATACTTACCATATATCAATGATGACGGGACATTATCCGAGAAAGTACTTTTCAAGGCCACCACACTCC
TGTCGTTCAAAAGGGGTTGCGGTATGGGATAGTCCTATTTATTGTATCGGAGGTATTTTTCTTCACAGGATTCTT
CTGAGCTTTTTACCACTCAAGTCTAGCACCTACTCCCGAACTAGGAGCATGCTGACCACCCACAGGCATCACCC
CCCTAAACCCATTAGAAGTGCCGCTTCTTAATACCTCAGTACTCCTAGCATCTGGAGTATCTATTACTTGAGCCC
ACCATAGCTTAATAGAAGGAAATCGCAATCATATACTCCAACAATTAAAACTAAACCCCCGTAGACAGGAGAG
GGCTTAGAAGAAAAGCCTACAAAACCTATTACAAAAACAACACTTAAAATGAACACAGTATATGTTACCATTA
TTCCCACATGGAATCTAACCATGACTAATGACATGAAAAATCACCGTTGTATTTCAACTATAAGAATCTTAATG
ACCAACATCCGAAAAACTCACCCACTAGCTAAAATCATCAACAGCTCATTCATTGACCTCCCAACACCATCAAA
TATCTCAGCATGATGAAACTTCGGGTCCCTTCTTGGGGTGTGCCTGATCCTACAAATCCTAACGGGCCTATTCCT
GGCCATACACTATACAGCAGACACGACTACAGCCTTCTCATCAGTCGCCCATATCTGTCGAGACGTTAACTACG
GATGAGTTATCCGATACATACACGCGAACGGAGCTTCAATATTCTTTATCTGCTTGTTCATACACGTGGGACGG
GGTCTGTATTACGGCTCATATCTATTTTCAGAAACATGAAACATTGGAATTATTCTCCTACTCACAGTTATAGCC
A

HTOI1*	Imbabura (1)	TTTACTTATTTCATACATATCATCCCACGTACTGTAGCATCCTAGTATGTCCCCGAACAAGGAAACCTTTCTTT- TTTTTCCCCCCTATGTACGTCGTGCATTAATGGCGTGCCCCATGCATATAAGCATGTACATATCTTGCTTGGCTTT ACATGAGGACATGGACTTCAAAAACTCGTTTTGAAGACGTAGTCTGTAAGCATGTATTTCACTTAGTCCGGGAG CTTAATCACCAGGCCTCGAGAAACCAGCAACCCTTGCGAGTACGTATACCTCTTCTCGCTCCGGGCCCATAGAA ACGTGGGGGTTTCTATACTGAAACTATACCTGGCATCTGGTTCTTACTTCAGGGCCATGATAGCTCTAGATTCCA ATCCTACTAACCCTTCAAATGGGACATCTCGATGGACTAATGACTAATCAGCCCATACAAACCATAAAGACATT GGTACTCTCTATCTCTTATTCGGTGCATGAGCCGGAATAGTAGGTACTGCCCTCAGCCTTCTAATCCGCGCTGAA CTAGGTCAACCCGGAGCCCTGTTAGGGGATGATCAGATCTATAACGTGGTCGTAACTGCCCATGCATTCGTAAT AATCTTCTTTATAGTAATGCCTATTATAATCGGAGGTTTTGGAAACTGATTAGTGCCTTTAATAATTGGCGCTCC CGATATAGCATTCCCTCGAATAAACAATATGAGTTTCTGATTACTGCCACCATCCTTCCTACTTCTTCTAGCCTC TTCCATAGTAGAAGCAGGTGCAGGGACTGGTTGAACCGTTTACCCCCCTCTAGCGGGCAATCTAGCCCATGCAG GAGCATCAGTAGACCTAACAATTTTTTCCCTACATTTAGCAGGCGTTTCCTCCATTCTAGGAGCTATTAACTTTA TTACCACTATTATCAATATGAAGCCTCCTGCAATATCTCAATACCAAACTCCTCTGTTCGTATGATCCGTCCTAA TTACGGCGGTAAGGAGTCGAACCCCCTGAAATTGGTTTCAAGCCAATACCATAACCACTATGTCTCTCTCAATA AAGAGATATTAGTAAAAATTACATAACTTCGTCAGGGTTAAATTATAGGTGAAAATCCTTTATATCTCTATGGC GTATCCCCTTCAAATAGGCCTTCAAGACGCAACTTCTCCCATTATAGAAGAGCTCCTACACTTCCACGACCATA CATTAATGATTGTATTCCTGATTAGCTCCTTAGTTCTCTACATTATTTCAACTATGCTAACTACTAAACTAACGC ACACAAACACAATAGATGCACAGGAAGTAGAAACGGTATGAACCATTCTACCAGCCATTATCCTGGTTCTAATT GCACTCCCATCATTACGAATCCTCTATATAATGGATGAAATCAACAATCCTTTACTGACTGTAAAAACTATAGG CCATCAATGATACTGAAGTTACGAATACACGGACTATGAAGATTTGAGCTTTGATTCTTACATGATTCCAACAC AAGAATTAAAGCCTGGAGAACTACGACTATTAGAAGTAGACAATCGAGCGGTACTACCCATAGAAATGACTAT TCGCATGCTAATTTCATCAGAAGATGTCTTGCACTCATGAGCTGTACCATCCCTAGGACTAAAAACTGATGCAA TTCCGGGACGACTAAACCAAACGACTCTCATGGCCATGCGACCAGGGCTGTATTATGGCCAATGCTCGGAAATC TGTGGCTCTAACCACAGCTTCATGCCCATTGTTCTCGAACTAGTCCCACTGTCCTATTTCGAAAAATGATCCGCT TCAATATTATAAAATCATTAAGAAGCTATATAGCATTAACCTTTTAAGTTAAAGACTGAGAGTGCAAGTCTCTC CTTAATGGAGATGCCACAACTAGACACGTCGACATGGTTTATCACAATTCTATCTATAACTCTAACACTATTTAT TGTATTCCAACTAAAAATCTAAACATCAGCATCGCCACAGCCCTCATCACTTTCTCTATTCTAGTACTACTCACT ATTCTTGAACTCGCCGTGGCTCTCATCCAAGCCTATGTCTTTGCTCTACTAGTAAGTCTATACTTACATGACAAC ACCTAATGGCCCACCAAACACATGCATATCACATAGTCAATCCAAGCCCATGACCACTAACGGGAGCCCTTTCA GCCCTTCTTATAACATCAGGACTAATCATATGATTCCACTTCAACTCAACATCCTTGCTATTACTAGGCCTTACA ACTAATATACTTACCATATATCAATGATGACGGGACATTATCCGAGAAAGTACTTTTCAAGGCCACCACACTCC TGTCGTTCAAAAGGGGTTGCGGTATGGGATAGTCCTATTTATTGTATCGGAGGTATTTTTCTTCACAGGATTCTT CTGAGCTTTTTACCACTCAAGTCTAGCACCTACTCCCGAACTAGGAGCATGCTGACCACCCACAGGCATCACCC CCCTAAACCCATTGGAAGTGCCGCTTCTTAATACCTCAGTACTCCTAGCATCTGGAGTATCTATTACTTGAGCCC

		ACCATAGCTTAATAGAAGGAAATCGCAATCATATACTCCAACAATTAAAACTAAACCCCCGTAGACAGGAGAG GGCTTAGAAGAAAAGCCTACAAAACCTATTACAAAAACAACACTTAAAATGAACACAGTATATGTTACCATTA TTCCCACATGGAATCTAACCATGACTAATGACATGAAAAATCACCGTTGTATTTCAACTATAAGAATCTTAATG ACCAACATCCGAAAAACTCACCCACTAGCTAAAATCATCAACAGCTCATTCATTGACCTCCCAACACCATCAAA TATCTCAGCATGATGAAACTTCGGGTCCCTTCTTGGGGTGTGCCTGATCCTACAAATCCTAACGGGCCTATTCCT GGCCATACACTATACAGCAGACACGACTACAGCCTTCTCATCAGTCGCCCATATCTGTCGAGACGTTAACTACG GGTGAGTTATCCGATACATACACGCGAACGGAGCTTCAATATTCTTTATCTGCTTGTTCATACACGTGGGACGG GGTCTGTATTACGGCTCATATCTATTTTCAGAAACATGAAACATTGGAATTATTCTCCTACTCACAGTTATAGCC A
HTOL1	Loja (3)	TTTACTTATTTCATATATATCATCCCACGTACTGTAGCATCCTAGTATGTCCCCGAACAAGGAAACCTTTCTTT- TTTTTCCCCCCTATGTACGTCGTGCATTAATGGCGTGCCCCATGCATATAAGCATGTACATATCTTGCTTGGTCTT ACATGAGGACATGGACTTCAAAAACTCGTTCTGAAGACGTAGTCTGCAAGCATGTATTTCACTTAGTCCGGGAG CTTAGTCACCAGGCCTCGAGAAACCAGCAACCCTTGCGAGTACGTATACCTCTTCTCGCTCCGGGCCCATAGAA ACGTGGGGGTTTCTATACTGAAACTATACCTGGCATCTGGTTCTTACTTCAGGGCCATGATAGCTCTAGATTCCA ATCCTACTAACCCTTCAAATGGGACATCTCGATGGACTAATGACTAATCAGCCCATACAAACCATAAAGACATT GGTACTCTCTATATCTTATTCGGTGCATGAGCCGGAATAGTAGGTACTGCCCTCAGCCTTCTAATCCGCGCTGAA CTAGGTCAACCCGGAGCCCTGTTAGGGGATGATCAAATCTATAACGTGGTCGTAACTGCCCATGCATTCGTAAT AATCTTCTTTATAGTAATGCCTATTATAATCGGAGGTTTTGGAAACTGATTAGTGCCTTTAATAATTGGCGCTCC CGATATAGCATTCCCTCGAATAAACAATATGAGTTTCTGATTACTGCCACCATCCTTCCTACTTCTTCTAGCCTC TTCCATAGTAGAAGCAGGTGCAGGGACTGGTTGAACTGTTTACCCCCCTCTAGCGGGCAATCTAGCCCATGCAG GAGCATCAGTAGACCTAACAATTTTTTCCCTACATTTAGCAGGCGTTTCCTCCATTCTAGGAGCTATTAACTTTA TTACCACTATTATCAATATGAAGCCTCCTGCAATATCTCAATACCAAACTCCTCTGTTCGTATGATCCGTCCTAA TTACGGCGGTAAGGAGTCGAACCCCCTGAAATTGGTTTCAAGCCAATACCATAACCACTATGTCTCTCTCAATA AAGAGATATTAGTAAAAATTACATAACTTCGTCAGGGTTAAATTATAGGTGAAAATCCTTTATATCTCTATGGC GTATCCCTTTCAAATAGGCCTTCAAGACGCAACTTCTCCCATTATAGAAGAGCTCCTACACTTCCACGACCATAC ATTAATGATTGTATTCCTGATTAGCTCTTTAGTTCTCTACATTATTTCAACTATGCTAACTACTAAACTAACGCAC ACAAACACAATAGATGCACAGGAAGTAGAAACGGTATGAACCATTCTACCAGCCATTATCCTGGTTCTAATTGC ACTCCCATCATTACGAATCCTCTATATAATGGATGAAATCAACAATCCTTTACTGACTGTAAAAACTATAGGCC ATCAATGATACTGAAGTTACGAATATACGGACTATGAAGATTTGAACTTTGATTCTTACATGATTCCAACACAA GAATTAAAGCCTGGAGAACTACGACTATTAGAAGTAGACAATCGAGCGGTACTACCCATAGAAATGACTATTC GCATGCTAATTTCATCAGAAGATGTCTTGCACTCATGAGCTGTACCATCCCTAGGACTAAAAACTGATGCAATT CCGGGACGACTAAACCAAACGACTCTCATGGCCATGCGACCAGGGCTGTATTATGGCCAATGCTCGGAAATCT GTGGCTCTAACCACAGCTTCATGCCTATTGTTCTCGAACTAGTCCCACTGTCCTATTTCGAAAAATGATCCGCTT CAATATTATAAAATCATTAAGAAGCTATATAGCATTAACCTTTTAAGTTAAAGACTGAGAGTGCAAGTCTCTCC

		TTAATGGAGATGCCACAACTAGACACGTCAACATGGTTTATCACAATTCTATCTATAACTCTAACACTATTTATT GTATTCCAACTAAAAATCTAAACATCAACATCGCCACAGCCCTCATCACTTTCTCTATTCTAGTACTACTCACTA TTCTTGAACTCGCCGTGGCTCTCATCCAAGCCTATGTCTTTGCTCTACTAGTAAGTCTATACTTACATGACAACA CCTAATGGCCCACCAAACACATGCATATCACATAGTCAATCCAAGCCCATGACCACTAACGGGAGCCCTTTCAG CCCTTCTTATAACATCAGGACTAATCATATGATTCCACTTCAACTCAACATCCTTGCTATTATTAGGCCTTACAA CTAATATACTTACCATATATCAATGATGACGGGACATTATCCGAGAAAGTACTTTTCAAGGCCACCACACTCCT GTCGTTCAAAAGGGGTTGCGGTATGGGATAGTCCTATTTATTGTATCGGAGGTATTTTTCTTCACAGGATTCTTC TGAGCTTTTTACCACTCAAGTCTAGCACCTACTCCCGAACTAGGAGCATGCTGACCACCCACAGGCATCACCCC CCTAAACCCATTGGAAGTGCCGCTTCTTAATACCTCAGTACTCCTAGCATCTGGAGTATCTATTACTTGAGCCCA CCATAGCTTAATAGAAGGAAATCGCAATCATGTACTCCAACAATTAAAACTAAACCCCCGTAAACAGGAGAGG GCTTAGAAGAAAAGCCTACAAAACCTATTACAAAAACAACACTTAAAATGAACACAGTATATGTTACCATTATT CCCACATGGAATCTAACCATGACTAATGACATGAAAAATCACCGTTGTATTTCAACTATAAGAATCTTAATGAC CAACATCCGAAAAACTCACCCACTAGCTAAAATCATCAACAACTCATTCATCGACCTCCCAACACCATCAAATA TCTCAGCATGATGAAACTTCGGGTCCCTTCTTGGGGTGTGCCTGGTCCTACAAATCCTAACGGGCCTATTCCTGG CCATACACTATACAGCAGACACGACTATAGCCTTCTCATCAGTCGCCCATATCTGTCGAGACGTTAACTACGGA TGAGTTATCCGATATATACACGCGAACGGGGCTTCAATATTCTTTATCTGCTTATTCATACACGTGGGACGGGGT CTGTACTACGGCTCATACCTATTTTCAGAAACATGAAACATCGGAATTATTCTCCTACTCACAGTTATAGCCA
HTOL2	Loja (1)	TTTACTTATTTCATACATATCATCCCACGTACTGTAGCATCCTAGTATGTCCCCGAACAAGGAAACCTTTCTTTTT TTTTCCCCCCTATGTACGTCGTGCATTAATGGCGTGCCCCATGCATATAAGCATGTACATATCTTGCTTGGCTTT ACATGAGGACATGGACTTCAAAAACTCGTTTTGAAGACGTAGTCTGTAAGCATGTATTTCACTTAGTCCGGGAG CTTAATCACCAGGCCTCGAGAAACCAGCAACCCTTGCGAGTACGTATACCTCTTCTCGCTCCGGGCCCATAGAA ACGTGGGGGTTTCTATACTGAAACTATACCTGGCATCTGGTTCTTACTTCAGGGCCATGATAGCTCTAGATTCCA ATCCTACTAACCCTTCAAATGGGACATCTCGATGGACTAATGACTAATCAGCCCATACAAACCATAAAGACATT GGTACTCTCTATATCTTATTCGGTGCATGAGCCGGAATAGTAGGTACTGCCCTCAGCCTTCTAATCCGCGCTGAA CTAGGTCAACCCGGAGCCCTGTTAGGGGATGATCAAATCTATAACGTGGTCGTAACTGCCCATGCATTCGTAAT AATCTTCTTTATAGTAATGCCTATTATAATCGGAGGTTTTGGAAACTGATTAGTGCCTTTAATAATTGGCGCTCC CGATATAGCATTCCCTCGAATAAACAATATGAGTTTCTGATTACTGCCACCATCCTTCCTACTTCTTCTAGCCTC TTCCATAGTAGAAGCAGGTGCAGGGACTGGTTGAACTGTTTACCCCCCTCTAGCGGGCAATCTAGCCCATGCAG GAGCATCAGTAGACCTAACAATTTTTTCCCTACATTTAGCAGGCGTTTCCTCCATTCTAGGAGCTATTAACTTTA TTACCACTATTATCAATATGAAGCCTCCTGCAATATCTCAATACCAAACTCCTCTGTTCGTATGATCCGTCCTAA TTACGGCGGTAAGGAGTCGAACCCCCTGAAATTGGTTTCAAGCCAATACCATAACCACTATGTCTCTCTCAATA AAGAGATATTAGTAAAAATTACATAACTTCGTCAGGGTTAAATTATAGGTGAAAATCCTTTATATCTCTATGGC GTATCCCTTTCAAATAGGCCTTCAAGACGCAACTTCTCCCATTATAGAAGAGCTCCTACACTTCCACGACCATAC ATTAATGATTGTATTCCTGATTAGCTCTTTAGTTCTCTACATTATTTCAACTATGCTAACTACTAAACTAACGCAC

		ACAAACACAATAGATGCACAGGAAGTAGAAACGGTATGAACCATTCTACCAGCCATTATCCTGGTTCTAATTGC ACTCCCATCATTACGAATCCTCTATATAATGGATGAAATCAACAATCCTTTACTGACTGTAAAAACTATAGGCC ATCAATGATACTGAAGTTACGAATATACGGACTATGAAGATTTGAACTTTGATTCTTACATGATTCCAACACAA GAATTAAAGCCTGGAGAACTACGACTATTAGAAGTAGACAATCGAGCGGTACTACCCATAGAAATGACTATTC GCATGCTAATTTCATCAGAAGATGTCTTGCACTCATGAGCTGTACCATCCCTAGGACTAAAAACTGATGCAATT CCGGGACGACTAAACCAAACGACTCTCATGGCCATGCGACCAGGGCTGTATTATGGCCAATGCTCGGAAATCT GTGGCTCTAACCACAGCTTCATGCCTATTGTTCTCGAACTAGTCCCACTGTCCTATTTCGAAAAATGATCCGCTT CAATATTATAAAATCATTAAGAAGCTATATAGCATTAACCTTTTAAGTTAAAGACTGAGAGTGCAAGTCTCTCC TTAATGGAGATGCCACAACTAGACACGTCAACATGGTTTATCACAATTCTATCTATAACTCTAACACTATTTATT GTATTCCAACTAAAAATCTAAACATCAACATCGCCACAGCCCTCATCACTTTCTCTATTCTAGTACTACTCACTA TTCTTGAACTCGCCGTGGCTCTCATCCAAGCCTATGTCTTTGCTCTACTAGTAAGTCTATACTTACATGACAACA CCTAATGGCCCACCAAACACATGCATATCACATAGTCAATCCAAGCCCATGACCACTAACGGGAGCCCTTTCAG CCCTTCTTATAACATCAGGACTAATCATATGATTCCACTTCAACTCAACATCCTTGCTATTATTAGGCCTTACAA CTAATATACTTACCATATATCAATGATGACGGGACATTATCCGAGAAAGTACTTTTCAAGGCCACCACACTCCT GTCGTTCAAAAGGGGTTGCGGTATGGGATAGTCCTATTTATTGTATCGGAGGTATTTTTCTTCACAGGATTCTTC TGAGCTTTTTACCACTCAAGTCTAGCACCTACTCCCGAACTAGGAGCATGCTGACCACCCACAGGCATCACCCC CCTAAACCCATTGGAAGTGCCGCTTCTTAATACCTCAGTACTCCTAGCATCTGGAGTATCTATTACTTGAGCCCA CCATAGCTTAATAGAAGGAAATCGCAATCATGTACTCCAACAATTAAAACTAAACCCCCGTAAACAGGAGAGG GCTTAGAAGAAAAGCCTACAAAACCTATTACAAAAACAACACTTAAAATGAACACAGTATATGTTACCATTATT CCCACATGGAATCTAACCATGACTAATGACATGAAAAATCACCGTTGTATTTCAACTATAAGAATCTTAATGAC CAACATCCGAAAAACTCACCCACTAGCTAAAATCATCAACAACTCATTCATCGACCTCCCAACACCATCAAATA TCTCAGCATGATGAAACTTCGGGTCCCTTCTTGGGGTGTGCCTGGTCCTACAAATCCTAACGGGCCTATTCCTGG CCATACACTATACAGCAGACACGACTATAGCCTTCTCATCAGTCGCCCATATCTGTCGAGACGTTAACTACGGA TGAGTTATCCGATATATACACGCGAACGGGGCTTCAATATTCTTTATCTGCTTATTCATACACGTGGGACGGGGT CTGTACTACGGCTCATACCTATTTTCAGAAACATGAAACATCGGAATTATTCTCCTACTCACAGTTATAGCCA
HTOZ1	10	TTTACTTATTTCATATATATCATCCCACGTACTGTAGCATCCTAGTATGTCCCCGAACAAGGAAACCTTTCTTT- TTTTTCCCCCCTATGTACGTCGTGCATTAATGGCGTGCCCCATGCATATAAGCATGTACATACCTTGCTTGGTTTT ACATGAGGACATGGACTTCAAAAACTCGTTCTGAAGACGTAGTCTGCAAGCATGTATTTCACTTAGTCCGGGAG CTTAGTCACCAGGCCTCGAGAAACCAGCAACCCTTGCGAGTACGTATACCTCTTCTCGCTCCGGGCCCATAGAA ACGTGGGGGTTTCTATACTGAAACTATACCTGGCATCTGGTTCTTACTTCAGGGCCATGATAGCTCTAGATTCCA ATCCTACTAACCCTTCAAATGGGACATCTCGATGGACTAATGACTAATCAGCCCATACAAACCATAAAGACATT GGTACTCTCTATATCTTATTCGGTGCATGAGCCGGAATAGTAGGTACTGCCCTCAGCCTTCTAATCCGCGCTGAA CTAGGTCAACCCGGAGCCCTGTTAGGGGATGATCAAATCTATAACGTGGTCGTAACTGCCCATGCATTCGTAAT AATCTTCTTTATAGTAATGCCTATTATAATCGGAGGTTTTGGAAACTGATTAGTGCCTTTAATAATTGGCGCTCC

CGATATAGCATTCCCTCGAATAAACAATATGAGTTTCTGATTACTGCCACCATCCTTCCTACTTCTTCTAGCCTC
TTCCATAGTAGAAGCAGGTGCAGGGACTGGTTGAACTGTTTACCCCCCTCTAGCGGGCAATCTAGCCCATGCAG
GAGCATCAGTAGACCTAACAATTTTTTCCCTACATTTAGCAGGCGTTTCCTCCATTCTAGGAGCTATTAACTTTA
TTACCACTATTATCAATATGAAGCCTCCTGCAATATCTCAATACCAAACTCCTCTGTTCGTATGATCCGTCCTAA
TTACGGCGGTAAGGAGTCGAACCCCCTGAAATTGGTTTCAAGCCAATACCATAACCACTATGTCTCTCTCAATA
AAGAGATATTAGTAAAAATTACATAACTTCGTCAGGGTTAAATTATAGGTGAAAATCCTTTATATCTCTATGGC
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ATTAATGATTGTATTCCTGATTAGCTCCTTAGTTCTCTACATTATTTCAACTATGCTAACTACTAAACTAACGCA
CACAAACACAATAGATGCACAGGAAGTAGAAACGGTATGAACCATTCTACCAGCCATTATCCTGGTTCTAATTG
CACTCCCATCATTACGAATCCTCTATATAATGGATGAAATCAACAATCCTTTACTGACTGTAAAAACTATAGGC
CATCAATGATACTGAAGTTACGAATATACGGACTATGAAGATTTGAACTTTGATTCTTACATGATTCCAACACA
AGAATTAAAGCCTGGAGAACTACGACTATTAGAAGTAGACAATCGAGCGGTACTACCCATAGAAATGACTATT
CGCATGCTAATTTCATCAGAAGATGTCTTGCACTCATGAGCTGTACCATCCCTAGGACTAAAAACTGATGCAAT
TCCGGGACGACTAAACCAAACGACTCTCATGGCCATGCGACCAGGGCTGTATTATGGCCAATGCTCGGAAATCT
GTGGCTCTAACCACAGCTTCATGCCTATTGTTCTCGAACTAGTCCCACTGTCCTATTTCGAAAAATGATCCGCTT
CAATATTATAAAATCATTAAGAAGCTATATAGCATTAACCTTTTAAGTTAAAGACTGAGAGTGCAAGTCTCTCC
TTAATGGAGATGCCACAACTAGACACGTCAACATGGTTTATCACAATTCTATCTATAACTCTAACACTATTTATT
GTATTCCAACTAAAAATCTAAACATCAACATCGCCACAGCCCTCATCACTTTCTCTATTCTAGTACTACTCACTA
TTCTTGAACTCGCCGTGGCTCTCATCCAAGCCTATGTCTTTGCTCTACTAGTAAGTCTATACTTGCATGACAACA
CCTAATGGCCCACCAAACACATGCATATCACATAGTCAATCCAAGCCCATGACCACTAACGGGAGCCCTTTCAG
CCCTTCTTATAACATCAGGACTAATCATATGATTCCACTTCAACTCAACATCCTTGCTATTATTAGGCCTTACAA
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GTCGTTCAAAAGGGGTTGCGGTATGGGATAGTCCTATTTATTGTATCGGAGGTATTTTTCTTCACAGGATTCTTC
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CCTAAACCCATTGGAAGTGCCGCTTCTTAATACCTCAGTACTCCTAGCATCTGGAGTATCTATTACTTGAGCCCA
CCATAGCTTAATAGAAGGAAATCGCAATCATGTACTCCAACAATTAAAACTAAACCCCCGTAAACAGGAGAGG
GCTTAGAAGAAAAGCCTACAAAACCTATTACAAAAACAACACTTAAAATGAACACAGTATATGTTACCATTATT
CCCACATGGAATCTAACCATGACTAATGACATGAAAAATCACCGTTGTATTTCAACTATAAGAATCTTAATGAC
CAACATCCGAAAAACTCACCCACTAGCTAAAATCATCAACAACTCATTCATCGACCTCCCAACACCATCAAATA
TCTCAGCATGATGAAACTTCGGGTCCCTTCTTGGGGTGTGCCTGGTCCTACAAATCCTAACGGGCCTATTCCTGG
CCATACACTATACAGCAGACACGACTATAGCCTTCTCATCAGTCGCCCATATCTGTCGAGACGTTAACTACGGA
TGAGTTATCCGATATATACACGCGAACGGGGCTTCAATATTCTTTATCTGCTTATTCATACACGTGGGACGGGGT
CTGTACTACGGCTCATACCTATTTTCAGAAACATGAAACATCGGAATTATTCTCCTACTCACAGTTATAGCCA

HTOZ2	5	TTTACTTATTTCATATATATCATCCCACGTACTGTAGCATCCTAGTATGTCCCCGAACAAGGAAACCTTTCTTTTT TTTTCCCCCCTATGTACGTCGTGCATTAATGGCGTGCCCCATGCATATAAGCATGTACATACCTTGCTTGGTTTT ACATGAGGACATGGACTTCAAAAACTCGTTCTGAAGACGTAGTCTGCAAGCATGTATTTCACTTAGTCCGGGAG CTTAGTCACCAGGCCTCGAGAAACCAGCAACCCTTGCGAGTACGTATACCTCTTCTCGCTCCGGGCCCATAGAA ACGTGGGGGTTTCTATACTGAAACTATACCTGGCATCTGGTTCTTACTTCAGGGCCATGATAGCTCTAGATTCCA ATCCTACTAACCCTTCAAATGGGACATCTCGATGGACTAATGACTAATCAGCCCATACAAACCATAAAGACATT GGTACTCTCTATATCTTATTCGGTGCATGAGCCGGAATAGTAGGTACTGCCCTCAGCCTTCTAATCCGCGCTGAA CTAGGTCAACCCGGAGCCCTGTTAGGGGATGATCAAATCTATAACGTGGTCGTAACTGCCCATGCATTCGTAAT AATCTTCTTTATAGTAATGCCTATTATAATCGGAGGTTTTGGAAACTGATTAGTGCCTTTAATAATTGGCGCTCC CGATATAGCATTCCCTCGAATAAACAATATGAGTTTCTGATTACTGCCACCATCCTTCCTACTTCTTCTAGCCTC TTCCATAGTAGAAGCAGGTGCAGGGACTGGTTGAACTGTTTACCCCCCTCTAGCGGGCAATCTAGCCCATGCAG GAGCATCAGTAGACCTAACAATTTTTTCCCTACATTTAGCAGGCGTTTCCTCCATTCTAGGAGCTATTAACTTTA TTACCACTATTATCAATATGAAGCCTCCTGCAATATCTCAATACCAAACTCCTCTGTTCGTATGATCCGTCCTAA TTACGGCGGTAAGGAGTCGAACCCCCTGAAATTGGTTTCAAGCCAATACCATAACCACTATGTCTCTCTCAATA AAGAGATATTAGTAAAAATTACATAACTTCGTCAGGGTTAAATTATAGGTGAAAATCCTTTATATCTCTATGGC GTATCCCTTTCAAATAGGCCTTCAAGACGCAACTTCTCCCATTATAGAAGAGCTCCTACACTTCCACGACCATAC ATTAATGATTGTATTCCTGATTAGCTCCTTAGTTCTCTACATTATTTCAACTATGCTAACTACTAAACTAACGCA CACAAACACAATAGATGCACAGGAAGTAGAAACGGTATGAACCATTCTACCAGCCATTATCCTGGTTCTAATTG CACTCCCATCATTACGAATCCTCTATATAATGGATGAAATCAACAATCCTTTACTGACTGTAAAAACTATAGGC CATCAATGATACTGAAGTTACGAATATACGGACTATGAAGATTTGAACTTTGATTCTTACATGATTCCAACACA AGAATTAAAGCCTGGAGAACTACGACTATTAGAAGTAGACAATCGAGCGGTACTACCCATAGAAATGACTATT CGCATGCTAATTTCATCAGAAGATGTCTTGCACTCATGAGCTGTACCATCCCTAGGACTAAAAACTGATGCAAT TCCGGGACGACTAAACCAAACGACTCTCATGGCCATGCGACCAGGGCTGTATTATGGCCAATGCTCGGAAATCT GTGGCTCTAACCACAGCTTCATGCCTATTGTTCTCGAACTAGTCCCACTGTCCTATTTCGAAAAATGATCCGCTT CAATATTATAAAATCATTAAGAAGCTATATAGCATTAACCTTTTAAGTTAAAGACTGAGAGTGCAAGTCTCTCC TTAATGGAGATGCCACAACTAGACACGTCAACATGGTTTATCACAATTCTATCTATAACTCTAACACTATTTATT GTATTCCAACTAAAAATCTAAACATCAACATCGCCACAGCCCTCATCACTTTCTCTATTCTAGTACTACTCACTA TTCTTGAACTCGCCGTGGCTCTCATCCAAGCCTATGTCTTTGCTCTACTAGTAAGTCTATACTTGCATGACAACA CCTAATGGCCCACCAAACACATGCATATCACATAGTCAATCCAAGCCCATGACCACTAACGGGAGCCCTTTCAG CCCTTCTTATAACATCAGGACTAATCATATGATTCCACTTCAACTCAACATCCTTGCTATTATTAGGCCTTACAA CTAATATACTTACCATATATCAATGATGACGGGACATTATCCGAGAAAGTACTTTTCAAGGCCACCACACTCCT GTCGTTCAAAAGGGGTTGCGGTATGGGATAGTCCTATTTATTGTATCGGAGGTATTTTTCTTCACAGGATTCTTC TGAGCTTTTTACCACTCAAGTCTAGCACCTACTCCCGAACTAGGAGCATGCTGACCACCCACAGGCATCACCCC CCTAAACCCATTGGAAGTGCCGCTTCTTAATACCTCAGTACTCCTAGCATCTGGAGTATCTATTACTTGAGCCCA

		CCATAGCTTAATAGAAGGAAATCGCAATCATGTACTCCAACAATTAAAACTAAACCCCCGTAAACAGGAGAGG GCTTAGAAGAAAAGCCTACAAAACCTATTACAAAAACAACACTTAAAATGAACACAGTATATGTTACCATTATT CCCACATGGAATCTAACCATGACTAATGACATGAAAAATCACCGTTGTATTTCAACTATAAGAATCTTAATGAC CAACATCCGAAAAACTCACCCACTAGCTAAAATCATCAACAACTCATTCATCGACCTCCCAACACCATCAAATA TCTCAGCATGATGAAACTTCGGGTCCCTTCTTGGGGTGTGCCTGGTCCTACAAATCCTAACGGGCCTATTCCTGG CCATACACTATACAGCAGACACGACTATAGCCTTCTCATCAGTCGCCCATATCTGTCGAGACGTTAACTACGGA TGAGTTATCCGATATATACACGCGAACGGGGCTTCAATATTCTTTATCTGCTTATTCATACACGTGGGACGGGGT CTGTACTACGGCTCATACCTATTTTCAGAAACATGAAACATCGGAATTATTCTCCTACTCACAGTTATAGCCA
HTOZ3*	1	TTTACTTATTTCATATATATCATCCCACGTACTGTAGCATCCTAGTATGTCCCCGAACAAGGAAACCTTTCTTT- TTTTTCCCCCCTATGTACGTCGTGCATTAATGGCGTGCCCCATGCATATAAGCATGTACATATCTTGCTTGGTTTT ACATGAGGACATGGACTTCAAAAACTCGTTCTGAAGACGTAGTCTGCAAGCATGTATTTCACTTAGTCCGGGAG CTTAGTCACCAGGCCTCGAGAAACCAGCAACCCTTGCGAGTACGTATACCTCTTCTCGCTCCGGGCCCATAGAA ACGTGGGGGTTTCTATACTGAAACTATACCTGGCATCTGGTTCTTACTTCAGGGCCATGATAGCTCTAGATTCCA ATCCTACTAACCCTTCAAATGGGACATCTCGATGGACTAATGACTAATCAGCCCATACAAACCATAAAGACATT GGTACTCTCTATATCTTATTCGGTGCATGAGCCGGAATAGTAGGTACTGCCCTCAGCCTTCTAATCCGCGCTGAA CTAGGTCAACCCGGAGCCCTGTTAGGGGATGATCAAATCTATAACGTGGTCGTAACTGCCCATGCATTCGTAAT AATCTTCTTTATAGTAATGCCTATTATAATCGGAGGTTTTGGAAACTGATTAGTGCCTTTAATAATTGGCGCTCC CGATATAGCATTCCCTCGAATAAACAATATGAGTTTCTGATTACTGCCACCATCCTTCCTACTTCTTCTAGCCTC TTCCATAGTAGAAGCAGGTGCAGGGACTGGTTGAACTGTTTACCCCCCTCTAGCGGGCAATCTAGCCCATGCAG GAGCATCAGTAGACCTAACAATTTTTTCCCTACATTTAGCAGGCGTTTCCTCCATTCTAGGAGCTATTAACTTTA TTACCACTATTATCAATATGAAGCCTCCTGCAATATCTCAATACCAAACTCCTCTGTTCGTATGATCCGTCCTAA TTACGGCGGTAAGGAGTCGAACCCCCTGAAATTGGTTTCAAGCCAATACCATAACCACTATGTCTCTCTCAATA AAGAGATATTAGTAAAAATTACATAACTTCGTCAGGGTTAAATTATAGGTGAAAATCCTTTATATCTCTATGGC GTATCCCTTTCAAATAGGCCTTCAAGACGCAACTTCTCCCATTATAGAAGAGCTCCTACACTTCCACGACCATAC ATTAATGATTGTATTCCTGATTAGCTCCTTAGTTCTCTACATTATTTCAACTATGCTAACTACTAAACTAACGCA CACAAACACAATAGATGCACAGGAAGTAGAAACGGTATGAACCATTCTACCAGCCATTATCCTGGTTCTAATTG CACTCCCATCATTACGAATCCTCTATATAATGGATGAAATCAACAATCCTTTACTGACTGTAAAAACTATAGGC CATCAATGATACTGAAGTTACGAATATACGGACTATGAAGATTTGAACTTTGATTCTTACATGATTCCAACACA AGAATTAAAGCCTGGAGAACTACGACTATTAGAAGTAGACAATCGAGCGGTACTACCCATAGAAATGACTATT CGCATGCTAATTTCATCAGAAGATGTCTTGCACTCATGAGCTGTACCATCCCTAGGACTAAAAACTGATGCAAT TCCGGGACGACTAAACCAAACGACTCTCATGGCCATGCGACCAGGGCTGTATTATGGCCAATGCTCGGAAATCT GTGGCTCTAACCACAGCTTCATGCCTATTGTTCTCGAACTAGTCCCACTGTCCTATTTCGAAAAATGATCCGCTT CAATATTATAAAATCATTAAGAAGCTATATAGCATTAACCTTTTAAGTTAAAGACTGAGAGTGCAAGTCTCTCC TTAATGGAGATGCCACAACTAGACACGTCAACATGGTTTATCACAATTCTATCTATAACTCTAACACTATTTATT

		GTATTCCAACTAAAAATCTAAACATCAACATCGCCACAGCCCTCATCACTTTCTCTATTCTAGTACTACTCACTA TTCTTGAACTCGCCGTGGCTCTCATCCAAGCCTATGTCTTTGCTCTACTAGTAAGTCTATACTTACATGACAACA CCTAATGGCCCACCAAACACATGCATATCACATAGTCAATCCAAGCCCATGACCACTAACGGGAGCCCTTTCAG CCCTTCTCATAACATCAGGACTAATCATATGATTCCACTTCAACTCAACATCCTTGCTATTATTAGGCCTTACAA CTAATATACTTACCATATATCAATGATGACGGGACATTATCCGAGAAAGTACTTTTCAAGGCCACCACACTCCT GTCGTTCAAAAGGGGTTGCGGTATGGGATAGTCCTATTTATTGTATCGGAGGTATTTTTCTTCACAGGATTCTTC TGAGCTTTTTACCACTCAAGTCTAGCACCTACTCCCGAACTAGGAGCATGCTGACCACCCACAGGCATCACCCC CCTAAACCCATTGGAAGTGCCGCTTCTTAATACCTCAGTACTCCTAGCATCTGGAGTATCTATTACTTGAGCCCA CCATAGCTTAATAGAAGGAAATCGCAATCATGTACTCCAACAATTAAAACTAAACCCCCGTAAACAGGAGAGG GCTTAGAAGAAAAGCCTACAAAACCTATTACAAAAACAACACTTAAAATGAACACAGTATATGTTACCATTATT CCCACATGGAATCTAACCATGACTAATGACATGAAAAATCACCGTTGTATTTCAACTATAAGAATCTTAATGAC CAACATCCGAAAAACTCACCCACTAGCTAAAATCATCAACAACTCATTCATCGACCTCCCAACACCATCAAATA TCTCAGCATGATGAAACTTCGGGTCCCTTCTTGGGGTGTGCCTGGTCCTACAAATCCTAACGGGCCTATTCCTGG CCATACACTATACAGCAGACACGACTATAGCCTTCTCATCAGTCGCCCATATCTGTCGAGACGTTAACTACGGA TGAGTTATCCGATATATACACGCGAACGGGGCTTCAATATTCTTTATCTGCTTATTCATACACGTGGGACGGGGT CTGTACTACGGCTCATACCTATTTTCAGAAACATGAAACATCGGAATTATTCTCCTACTCACAGTTATAGCCA
HTOZ4*	2	TTTACTTATTTCATATATATCATCCCACGTACTGTAGCATCCTAGTATGTCCCCGAACAAGGAAACCTTTCTTTTT TTTTCCCCCCTATGTACGTCGTGCATTAATGGCGTGCCCCATGCATATAAGCATGTACATATCTTGCTTGGTTTT ACATGAGGACATGGACTTCAAAAACTCGTTCTGAAGACGTAGTCTGCAAGCATGTATTTCACTTAGTCCGGGAG CTTAGTCACCAGGCCTCGAGAAACCAGCAACCCTTGCGAGTACGTATACCTCTTCTCGCTCCGGGCCCATAGAA ACGTGGGGGTTTCTATACTGAAACTATACCTGGCATCTGGTTCTTACTTCAGGGCCATGATAGCTCTAGATTCCA ATCCTACTAACCCTTCAAATGGGACATCTCGATGGACTAATGACTAATCAGCCCATACAAACCATAAAGACATT GGTACTCTCTATATCTTATTCGGTGCATGAGCCGGAATAGTAGGTACTGCCCTCAGCCTTCTAATCCGCGCTGAA CTAGGTCAACCCGGAGCCCTGTTAGGGGATGATCAAATCTATAACGTGGTCGTAACTGCCCATGCATTCGTAAT AATCTTCTTTATAGTAATGCCTATTATAATCGGAGGTTTTGGAAACTGATTAGTGCCTTTAATAATTGGCGCTCC CGATATAGCATTCCCTCGAATAAACAATATGAGTTTCTGATTACTGCCACCATCCTTCCTACTTCTTCTAGCCTC TTCCATAGTAGAAGCAGGTGCAGGGACTGGTTGAACTGTTTACCCCCCTCTAGCGGGCAATCTAGCCCATGCAG GAGCATCAGTAGACCTAACAATTTTTTCCCTACATTTAGCAGGCGTTTCCTCCATTCTAGGAGCTATTAACTTTA TTACCACTATTATCAATATGAAGCCTCCTGCAATATCTCAATACCAAACTCCTCTGTTCGTATGATCCGTCCTAA TTACGGCGGTAAGGAGTCGAACCCCCTGAAATTGGTTTCAAGCCAATACCATAACCACTATGTCTCTCTCAATA AAGAGATATTAGTAAAAATTACATAACTTCGTCAGGGTTAAATTATAGGTGAAAATCCTTTATATCTCTATGGC GTATCCCTTTCAAATAGGCCTTCAAGACGCAACTTCTCCCATTATAGAAGAGCTCCTACACTTCCACGACCATAC ATTAATGATTGTATTCCTGATTAGCTCCTTAGTTCTCTACATTATTTCAACTATGCTAACTACTAAACTAACGCA CACAAACACAATAGATGCACAGGAAGTAGAAACGGTATGAACCATTCTACCAGCCATTATCCTGGTTCTAATTG

	CACTCCCATCATTACGAATCCTCTATATAATGGATGAAATCAACAATCCTTTACTGACTGTAAAAACTATAGGC CATCAATGATACTGAAGTTACGAATATACGGACTATGAAGATTTGAACTTTGATTCTTACATGATTCCAACACA AGAATTAAAGCCTGGAGAACTACGACTATTAGAAGTAGACAATCGAGCGGTACTACCCATAGAAATGACTATT CGCATGCTAATTTCATCAGAAGATGTCTTGCACTCATGAGCTGTACCATCCCTAGGACTAAAAACTGATGCAAT TCCGGGACGACTAAACCAAACGACTCTCATGGCCATGCGACCAGGGCTGTATTATGGCCAATGCTCGGAAATCT GTGGCTCTAACCACAGCTTCATGCCTATTGTTCTCGAACTAGTCCCACTGTCCTATTTCGAAAAATGATCCGCTT CAATATTATAAAATCATTAAGAAGCTATATAGCATTAACCTTTTAAGTTAAAGACTGAGAGTGCAAGTCTCTCC TTAATGGAGATGCCACAACTAGACACGTCAACATGGTTTATCACAATTCTATCTATAACTCTAACACTATTTATT GTATTCCAACTAAAAATCTAAACATCAACATCGCCACAGCCCTCATCACTTTCTCTATTCTAGTACTACTCACTA TTCTTGAACTCGCCGTGGCTCTCATCCAAGCCTATGTCTTTGCTCTACTAGTAAGTCTATACTTACATGACAACA CCTAATGGCCCACCAAACACATGCATATCACATAGTCAATCCAAGCCCATGACCACTAACGGGAGCCCTTTCAG CCCTTCTCATAACATCAGGACTAATCATATGATTCCACTTCAACTCAACATCCTTGCTATTATTAGGCCTTACAA CTAATATACTTACCATATATCAATGATGACGGGACATTATCCGAGAAAGTACTTTTCAAGGCCACCACACTCCT GTCGTTCAAAAGGGGTTGCGGTATGGGATAGTCCTATTTATTGTATCGGAGGTATTTTTCTTCACAGGATTCTTC TGAGCTTTTTACCACTCAAGTCTAGCACCTACTCCCGAACTAGGAGCATGCTGACCACCCACAGGCATCACCCC CCTAAACCCATTGGAAGTGCCGCTTCTTAATACCTCAGTACTCCTAGCATCTGGAGTATCTATTACTTGAGCCCA CCATAGCTTAATAGAAGGAAATCGCAATCATGTACTCCAACAATTAAAACTAAACCCCCGTAAACAGGAGAGG GCTTAGAAGAAAAGCCTACAAAACCTATTACAAAAACAACACTTAAAATGAACACAGTATATGTTACCATTATT CCCACATGGAATCTAACCATGACTAATGACATGAAAAATCACCGTTGTATTTCAACTATAAGAATCTTAATGAC CAACATCCGAAAAACTCACCCACTAGCTAAAATCATCAACAACTCATTCATCGACCTCCCAACACCATCAAATA TCTCAGCATGATGAAACTTCGGGTCCCTTCTTGGGGTGTGCCTGGTCCTACAAATCCTAACGGGCCTATTCCTGG CCATACACTATACAGCAGACACGACTATAGCCTTCTCATCAGTCGCCCATATCTGTCGAGACGTTAACTACGGA TGAGTTATCCGATATATACACGCGAACGGGGCTTCAATATTCTTTATCTGCTTATTCATACACGTGGGACGGGGT CTGTACTACGGCTCATACCTATTTTCAGAAACATGAAACATCGGAATTATTCTCCTACTCACAGTTATAGCCA

The different colors indicate each marker's sequence beginning and end: **HVRI D-Loop**, **COI-partial sequence**, **COI-COII-ATP8**, **ATP6-COIII**, and **ND6-CytB**. n represents the number of individuals.

* New haplotype(s) found in this study for the *T. ornatus* populations in Imbabura and Zamora.

Appendix 4. Number of haplotypes, haplotype diversity, nucleotide diversity, and neutrality tests (Tajima's D and Fu's Fs) for the five mitochondrial markers and the concatenated region of the 36 *T. ornatus* individuals, separated by province.

mtDNA Marker (bp)	Population (n)	Number of Haplotypes	Haplotype diversity (Hd ± sd)	Nucleotide diversity (π ± sd)	Tajima's D**	p-value	Fu's Fs**	p-value
HVRI D-Loop (430)	Pichincha (13)	3	0.6410 ± 0.0967	0.00173 ± 0.00153	-0.27429	0.299	0.18471	0.427
	Imbabura (1)	1	NA	NA	NA	NA	NA	NA
	Loja (4)	2	0.5000 ± 0.2652	0.00814 ± 0.00622	-0.80861	0.155	325.086	0.918
	Zamora Chinchipe (18)	4	0.6340 ± 0.0926	0.00185 ± 0.00157	0.02193	0.745	-0.63177	0.272
COI-partial sequence (552)	Pichincha (13)	1	0.0000 ± 0.0000	0.00000 ± 0.00000	0.00000	1.000	0.00000	NA
	Imbabura (1)	1	NA	NA	NA	NA	NA	NA
	Loja (4)	1	0.0000 ± 0.0000	0.00000 ± 0.00000	0.00000	1.000	0.00000	NA
	Zamora Chinchipe (18)	1	0.0000 ± 0.0000	0.00000 ± 0.00000	0.00000	1.000	0.00000	NA
COI-COII-ATP8 (972)	Pichincha (13)	1	0.0000 ± 0.0000	0.00000 ± 0.00000	0.00000	1.000	0.00000	NA
	Imbabura (1)	1	NA	NA	NA	NA	NA	NA
	Loja (4)	1	0.0000 ± 0.0000	0.00000 ± 0.00000	0.00000	1.000	0.00000	NA
	Zamora Chinchipe (18)	1	0.0000 ± 0.0000	0.00000 ± 0.00000	0.00000	1.000	0.00000	NA
ATP6-COIII (618)	Pichincha (13)	1	0.0000 ± 0.0000	0.00000 ± 0.00000	0.00000	1.000	0.00000	NA
	Imbabura (1)	1	NA	NA	NA	NA	NA	NA
	Loja (4)	1	0.0000 ± 0.0000	0.00000 ± 0.00000	0.00000	1.000	0.00000	NA
	Zamora Chinchipe (18)	2	0.2941 ± 0.1193	0.00095 ± 0.00090	0.02839	0.638	162.832	0.742

ND6-CytB (553)	Pichincha (13)	1	0.0000 ± 0.0000	0.00000 ± 0.00000	0.00000	1.000	0.00000	NA
	Imbabura (1)	1	NA	NA	NA	NA	NA	NA
	Loja (4)	1	0.0000 ± 0.0000	0.00000 ± 0.00000	0.00000	1.000	0.00000	NA
	Zamora Chinchipe (18)	1	0.0000 ± 0.0000	0.00000 ± 0.00000	0.00000	1.000	0.00000	NA
Concatenated region (3125)* HVRI D-Loop + COI-partial sequence + COI-COII-ATP8 + ATP6-COIII + ND6-CytB	Pichincha (13)	3	0.6410 ± 0.0967	0.00024 ± 0.00021	-0.27429	0.295	0.18471	0.426
	Imbabura (1)	1	NA	NA	NA	NA	NA	NA
	Loja (4)	2	0.5000 ± 0.2652	0.00112 ± 0.00086	-0.80861	0.149	325.086	0.912
	Zamora Chinchipe (18)	4	0.6340 ± 0.0926	0.00044 ± 0.00032	0.03225	0.58	0.64207	0.665

bp, final size of the sequence after trimming. NA, these values could not be calculated because there was only one individual.

* The concatenated region was created from the combination of the sequences of the five mitochondrial markers analyzed in *T. ornatus* populations (HVR1 D-Loop, COI-partial sequence, COI-COII-ATP8, ATP6-COIII, and ND6-CytB).

** None of the values of the neutrality tests (Tajima's D and Fu's Fs) were statistically significant ($p>0.05$).