Biota Neotropica 22(4): e20221382, 2022
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
Article

Phylogeography of the white-crowned parrot (*Pionus senilis*)

***Ricardo Canek Rivera-Arroyo*[1], *Patricia Escalante-Pliego*[1]*, *Damián Aguilar-Torres*[2] & *Milton F. Úbeda-Olivas*[3]**

[1]*Universidad Nacional Autónoma de México, Instituto de Biología, Ciudad de México, México.*
[2]*Universidad Autónoma Metropolitana, Unidad Xochimilco, Ciudad de México, México.*
[3]*URKU Estudios Amazónicos, Tarapoto, Peru.*
**Corresponding author: tilmatura@ib.unam.mx*

RIVERA-ARROYO, C., ESCALANTE-PLIEGO, P., AGUILAR-TORRES, D., ÚBEDA-OLIVAS, M.F. **Phylogeography of the white-crowned parrot (*Pionus senilis*)**. Biota Neotropica 22(4): e20221382. https://doi.org/10.1590/1676-0611-BN-2022-1382

Abstract: The white-crowned parrot *Pionus senilis* (von Spix, 1824) is distributed throughout Middle America, inhabiting the Gulf of Mexico coastal area from Tamaulipas (Mexico) to northern Panama. We used mitochondrial data (COI, ND2 and ND4) from 55 specimens to infer phylogenetic relationships, and analyzed the phylogeographic structure, genetic diversity, divergence periods, and historical demography to explore phylogeographic patterns. We found three divergent lineages: two geographically separated by the Isthmus of Tehuantepec, and the third, in Costa Rica by the Nicaragua Depression. The analysis of molecular variance and statistical analyses were consistent with genetically distinct populations. The Central American lineage diverged 1.33 million years ago, whereas the other two lines branched off 1.19 million years ago. This phylogenetic pattern has been reported in other species of Middle American birds.
Keywords: *Psittacidae; genetic structure; conservation genetics.*

Filogeografia do maitaca-de-testa-branca (*Pionus senilis*)

Resumo: A curica-de-testa-branca *Pionus senilis* (von Spix, 1824) está distribuída por toda a América Central, habitando a área costeira do Golfo do México de Tamaulipas (México) ao norte do Panamá. Usamos dados mitocondriais (COI, ND2 e ND4) de 55 espécimes para inferir relações filogenéticas e analisamos a estrutura filogeográfica, diversidade genética, períodos de divergência e demografia histórica para explorar padrões filogeográficos. Encontramos três linhagens divergentes: duas geograficamente separadas pelo Istmo de Tehuantepec, e a terceira, na Costa Rica pela Depressão da Nicarágua. A análise de variância molecular e as análises estatísticas foram consistentes com populações geneticamente distintas. A linhagem da América Central divergiu há 1.33 milhão de anos, enquanto as outras duas linhas se ramificaram há 1.19 milhão de anos. Este padrão filogenético foi relatado em outras espécies de aves da América Central.
Palavras-chave*: Psittacidae; estrutura genética; genética da conservação.*

Introduction

A comprehensive study of biodiversity must not only include ecological variability, but genetic variability as well, since it is essential for the persistence and evolutionary continuum of a species or lineage (Frankham et al. 2002). The International Union for Conservation of Nature places genetic diversity as one of the three global priorities for conservation (IUCN 1980). The order Psittaciformes has the highest number of endangered species among groups of birds, with 111 (28%) of the 360 species listed as in danger of extinction and shrinking population sizes in 56% of its species (Berkunsky et al. 2017). Given the current extinction processes, the genetic studies needed to support conservation programs are of utmost importance (Avise 2002, Frankham et al. 2002, Olah et al. 2022).

Phylogeography can be considered a theoretical bridge between population genetics and phylogenetic biology. Ever since phylogeography was conceived and has been applied, it has made valuable contributions to the comprehension and protection of biodiversity (Avise et al. 2016). Information thus obtained may be useful to determine possible evolutionarily significant units (ESUs), which are needed to apply conservation plans. Phylogeographic studies have been applied to solve the existence of several cryptic Psittacidae taxa (Russello and Amato 2004; Joseph et al. 2011; Murphy et al. 2011). They have made it possible to observe the effect of fragmentation and bottlenecks on population structure (Ringler et al. 2012; Miller et al. 2013; Bergner et al. 2016), as well as elucidate how evolutionary history has woven the current diversity patterns in the species (Murphy et al. 2007; Caparroz et al. 2009;

Murphy et al. 2011). These studies have fostered awareness of how important the preservation of a species' evolutionary potential is for it to persist, thus improving our understanding of factors associated with habitat fragmentation, the effect of endogamy, and loss of populations. Since species lacking robust information are in particular need of such studies, it is fundamental that genetic data be obtained to distinguish their phylogeographic patterns.

The genus *Pionus* has eight species of parrots, four of which are allopatrically distributed in mountain forests from northern Mexico to South America, and four others are in the lowlands (Juniper & Parr 1998, Ribas et al. 2007). This is consistent with speciation patterns caused by vicariance, since mountain groups are limited by boundaries related to topographic diversity isolating the mountain forests surrounded by lower, warmer lands (Ribas et al. 2007). The genus *Pionus* diversified in the late Miocene and early Pliocene, approximately 4.7 to 5.8 million years ago (Ribas et al. 2007). The white-crowned parrot is the only species in the genus that is distributed in northern Middle America, and it expands from Mexico (from southern Tamaulipas along the Gulf of Mexico coastline to the Yucatan Peninsula and Chiapas) to the west of the Isthmus of Panama. Mexican laws have cataloged it as a Threatened species (NOM-059, SEMARNAT 2010), whereas the International Union for Conservation of Nature has it listed as of Least Concern (IUCN 2016).

Spix described the species *Pionus senilis* in 1824 from a specimen that was later determined to have been obtained from Veracruz, Mexico. The species was considered monotypic until Griscom (1929) described *P. s. decoloratus* from the populations in southern Quintana Roo (Mexico), Guatemala, Honduras, and western Panama. The latter is distinguished by the darker purple, less blue, plumage on the chest and throat; the abdomen and sides are an olive green instead of a bright green, and the primary feathers are mostly bright blue with little green. The typical *P. senilis* is greener and has a green stripe between the blue and dark areas of the inner vanes of the external primary feathers. The above description explains that the southern populations (southeastern Nicaragua, Costa Rica and Panama) are differentiated by these characteristics, and that those in Quintana Roo (Mexico), Belize, Honduras, and northern Nicaragua are intermediate.

This species' populations have dwindled in Mexico and have even vanished from western Quintana Roo and several areas along the Gulf of Mexico (Salinas-Melgoza & Renton 2008). This is mostly due to the impact that human activity has had on its habitat (evergreen tropical forest, oak forest, and lower mountain forest), which has been highly deforested for farming. As a result, it inhabits only 48% of its original area, and only 16.2% of this surface is protected (Monterrubio-Rico et al. 2016). Further, this species is illegally sold as a pet (Cantú et al. 2007). There is little data on the species in Central American countries.

Given its progressive depletion and possible population fragmentation, a conservation plan for the species is important because it would then be possible to keep the populations comprising evolutionary units safe and flag them as of high conservation priority (Ryder 1986). To achieve this goal, we investigate population differentiation patterns with three mitochondrial markers applied at the intraspecific level with genealogy analyses. These data would facilitate the study of population evolution, deductions concerning lineage colonization, diversification and extinction, and the identification of geological or ecological causes that influenced the populations (Avise 1998, Domínguez-Domínguez and Vázquez-Domínguez 2009).

Following this need to learn more about the white-crowned parrot (*Pionus senilis* [Von Spix 1824]), we decided to perform a phylogeographic analysis with these main objectives: a) identifying genetic lineages within the entire distribution area of the species, b) revealing the likely geological events that allowed the current distribution of the distinct haplotypes, and c) identifying the geographic barriers that have influenced the distribution of genetic lineages.

Material and Methods

1. Biological samples

Blood samples were taken from white-crowned parrot specimens between 2017 and 2019 using collection permit SGPA/DGVS/05058/17. Each sample was georeferenced. One 0.1-ml blood sample was taken from each parrot, conserved on an FTA card, and placed in the National Bird Collection at the Institute of Biology of the National Autonomous University of Mexico (Universidad Nacional Autónoma de México, UNAM). We collected and analyzed samples from 59 individuals from 11 localities ranging from northern Mexico (state of Tamaulipas) to Costa Rica (Table 1, Figure 1). The capture of wild samples was unsuccessful because of the challenge of catching the parrots while foraging, the difficulties in finding and climbing to their nests, and time constraints. We resorted to collecting samples of captive individuals in the locations studied with the certainty that these individuals were captured in the area. This conclusion was based on the information provided by owners, and on the fact that many rural people collect nestling's locality. They keep them as pets and perhaps sell them to people that look for them illegally, but they cannot afford to buy them in the illegal trade, a situation more likely in cities. Additional samples were facilitated by the Macaw Mountain Bird Park (Honduras), and Rescate Animal Zoo Ave (Costa Rica). Each sample was georeferenced. One 0.1-ml blood sample was taken from each parrot, conserved on an FTA card, and placed in the National Bird Collection at the Institute of Biology of the National Autonomous University of Mexico (Universidad Nacional Autónoma de México, UNAM). We collected and analyzed samples from 59 individuals from 11 localities ranging from northern Mexico (state of Tamaulipas) to Costa Rica (Table 1, Figure 1).

2. DNA extraction, PCR amplification and marker sequencing

Genome DNA was obtained using a modified technique to extract DNA from animal tissue using phenol-chloroform. We amplified mitochondrial fragments *NADH dehydrogenase II (ND2), NADH dehydrogenase 4 (ND4),* and *cytochrome oxidase I (COI).* We were able to amplify 55 individuals with the three genes. The ND2 gene was amplified using L5215 (Hackett 1996) and H6313 primers (Bonaccorso et al. 2010), ND4 and LEU primers were used for ND4 (Arévalo et al. 1994), and COI was amplified using COIbird F1 and COIbird R1 primers (Hebert et al. 2004).

The amplification reactions caused by the polymerase chain reaction (PCR) (12.5 µL) were prepared with 6 µl 10% trehalose,

Table 1. Localities and number of collected the white-crowned parrot (*Pionus senilis*) samples.

Country	State	Locality	Samples	ID	Latitude	Longitude
Mexico	Tamaulipas	El Cielo	9	RCRA1 – RCRA3, RCRA25 – RCRA30	23.024	–99.148
Mexico	San Luis Potosí	El Naranjo	2	RCRA4, RCRA5	22.572	–99.343
Mexico	San Luis Potosí	Aquismon	1	RCRA6	21.624	–99.028
Mexico	San Luis Potosí	Xilitla	2	RCRA7, RCRA8	21.375	–99.990
Mexico	San Luis Potosí	Santos	1	RCRA9	21.572	–99.961
Mexico	Oaxaca	Chalchijalpan	3	RCRA14 – RCRA16	17.057	–94.656
Mexico	Chiapas	Tecpatan	3	RCRA17 – RCRA19	17.137	–93.318
Mexico	Tabasco	Tenosique	5	RCRA20 – RCRA24	17.256	–91.133
Honduras	Copán	Macaw Mountain	11	Hon-23 – Hon-33	14.851	–89.154
Nicaragua	Jinotega	Wiwili	10	Nica 13-1, Nica 14-2, Nica 15-4, Nica 16-3, Nica 17-5, Nica 18-6, Nica 19-7, Nica 20-8, Nica 21-9, Nica 22-10	13.584	–85.803
Costa Rica	Alajuela	Zoo Ave	12	Avezoo1 – Avezoo 12	10.012	–88.276

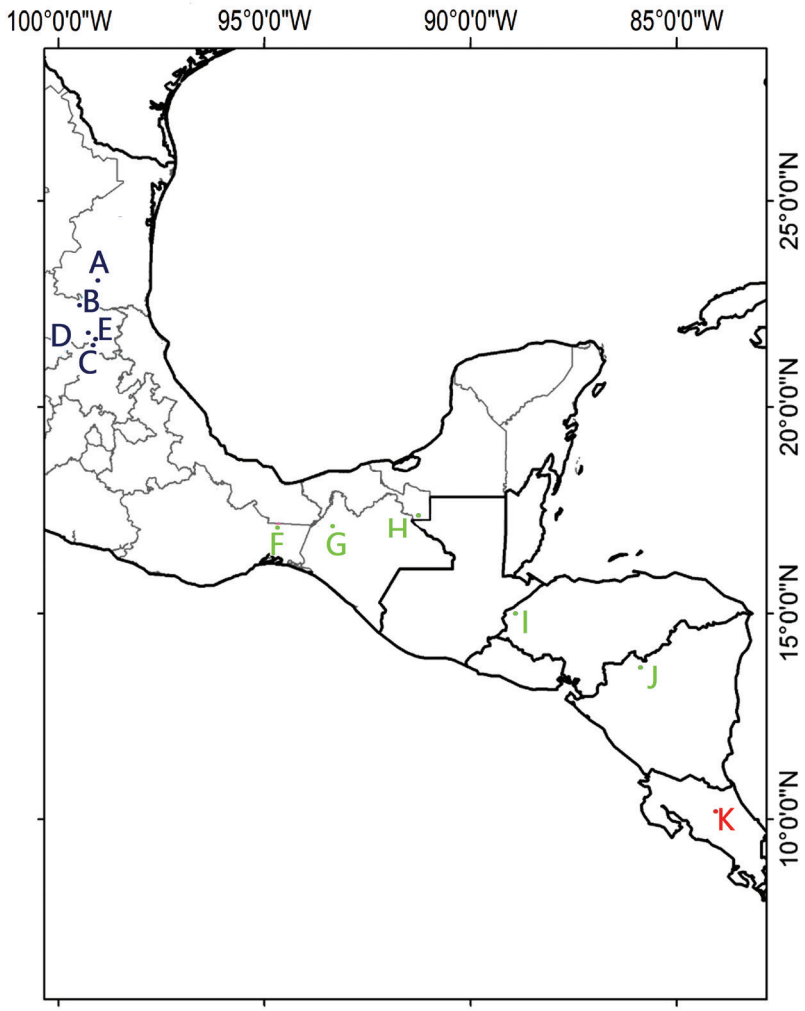

Figure 1. Sampling areas of the white-crowned parrot (*Pionus senilis*) along its distribution Centroamerica. A. El Cielo, Tamaulipas; B. El Naranjo SLP; C. Xilitla SLP; D. Aquismon, SLP; E. Santos, SLP; F. Chalchijalpan, Oaxaca; G. Tecpatan, Chiapas; H. Tenosique, Tabasco; I. Macaw Mountain, Copán; J. Wiwili, Jinotega and K. Zoo Ave, Alajuela. The colors represent the three unique genetic lineages of this work: Northern (blue), Center (green) and Southern (red).

2 µl distilled water, 10 mM PCR buffer, 0.2 mM of each dNTP, 1.5 mM MgCl2, 0.3 µM of each primer, 0.5 units of Taq DNA polymerase, and approximately 50 ng of genome DNA. Both DNA chains were sequenced using the amplification primers and the dideoxi method (Sanger et al. 1977). The PCR products were sent for sequencing to the Biodiversity and Human Health Genome Sequencing Laboratory (Laboratorio de Secuenciación de la Biodiversidad y de la Salud) at the UNAM Institute of Biology and to Macrogen (Maryland, USA).

3. *Sequence analysis*

Sequences were edited using BioEdit v7.2 software (Hall 1999), and chromatograms were manually checked using FinchTV v1.4 software. In addition, each sequence was compared to the information available at GenBank using the basic local alignment search tool (BLAST), which made it possible to establish a 96–99% identity in each sequenced case. MEGA v10 software was used to read sequences (Tamura et al. 2011).

Genetic diversity indicators, such as number of mitochondrial haplotypes (h), number of polymorphic sites (Sn), genetic diversity (H), average number of differences between pairs of sequences (π), and nucleotide diversity (k), were calculated using DNAsp v10 software (Rozas & Rozas 1999). Different summary statistics were also implemented to determine whether the markers assume a neutral evolution model: Tajima's D (Tajima 1989), Fu (Fu & Li 1993) and Fs (Fu 1997). We used MEGA v10 software to identify the genetic distances between groups (Tamura et al. 2011) and individuals were assembled into groups established by phylogenetic trees using the Kimura two-parameter substitution model.

An analysis of molecular variance (AMOVA) (Excoffier et al. 2005) was conducted to determine whether genetic variation and differentiation between the groups had a structured distribution. This analysis breaks variance down into (a) differences in haplotype composition among individuals from individual populations (variance within a population); (b) differences in haplotype composition of individuals from different populations (variance between populations); and (c) differences in haplotype composition between groups of populations (variance between FST regions). We performed this analysis using GenAIEx v6.0 software (Peakall & Smouse 2006) with 9999 permutations. To know whether a species' gene flow follows an isolation by distance model (i.e., whether the geographic distances and genetic distances between different pairs of populations are correlated), we performed a Mantel test using GenAlEX v6.0 software (Peakall and Smouse 2012). This analysis assumes that genetic distances increase with geographic distance. The test computes the correlation between a geographic distance matrix versus a genetic distance matrix, then permutes the matrices and computes the same test statistic under each permutation and compares the original test statistic with the distribution of the test statistic from the permutation to generate the p-value.

4. *Relationships between haplotypes and genealogical analyses*

We constructed a haplotype network in PopART v1.7 (Leigh & Bryant 2015) using the MJN algorithm, to represent the relationships between haplotypes at each sampling locality. PopART v1.7 software starts from the number of paired substitutions to introduce medium vectors that represent the haplotypes the concatenated 55 individuals. An analysis was also performed with Split Tree v4 software (Huson & Bryant 2006) using Neighbor Net with the GTR substitution model and 400 bootstrap replicas.

We reconstructed genealogical relationships using Bayesian inference (BI) and maximum likelihood (ML). The matrix for these analyses included 55 sequences of COI, ND2 and ND4 from the samples collected in the field, in addition to sequences belonging to *Pionus chalcopterus* (GenBank Access: MF784450.1) that we included as an external group. Molecular evolution models were estimated with jModelTest v2.1.1 (Posadas 2008), using the corrected Akaike's Information Criterion (AICc) (Alfaro and Huelsenbeck 2006). TN93 was the best model for ND2 (Tamura and Nei 1993 + 3 rates), TIM1 for ND4 (Posada, 2008), and HKY for COI (Hasegawa et al. 1985). The best model for the concatenated sequences (COI + ND2 + ND4) was TIM3 (Posada 2008). We used MrBayes v3.2 (Ronquist and Huelsenbeck 2003) and RAxML v7.8 software (Stamatakis 2014), respectively, to reconstruct Bayesian inference and maximum likelihood. MrBayes made two independent runs of 30,000,000 generations and four Markov chains (Markov Chain Monte Carlo), testing a tree every 2,000 generations at a temperature of 0.3 and burning 30% of the generated data. The remaining trees were summarized as majority consensus. In RAxML, the ML+ through bootstrap search was performed with 10 searches and 10,000 replicas. Trees were displayed in FigTree v1.4.0 (Rambaut 2014).

5. *Molecular clock*

We analyzed the molecular clock by implementing the BEAST v.1.6.1 program (Drummond & Rambaut 2007) to estimate the divergence time in a tree of species. We used the 55 concatenated sequences of the three-mitochondrial markers, calibrating with *Pionus chalcopterus* to 2.2 million years in the past when the two species branched off (Ribas et al. 2007). We ran a simulation to determine when the three white-crowned parrot lineages separated, using GTR as the substitution rate, with estimated base frequencies, gamma shape distribution (with 4 categories), proportion of invariant sites, a relaxed molecular clock with uncorrelated lognormal distribution and a Yule tree prior. We performed the BEAST analysis three times with 100 million generations each time and took a sample every 1000 steps using the Yules speciation tree, an uncorrelated relaxed clock model with log-normal distribution. After running the analyses through BEAST, we used TRACER v.1.6 to observe the parameters of the results. We then combined the tree files (.tree) using LOGCOMBINER and summarized them as a maximum clade credibility tree produced by TREEANNOTATOR (Drummond & Rambaut 2007) after burning 30%. This tree was displayed in Figtree.

6. *Historical demography*

To evaluate whether the data were consistent with the occurrence of selection at a molecular level or with past demographic expansion, we calculated the observed distribution of the number of differences between pairs of haplotypes using DnaSP v5.10 software to distinguish whether the populations, Northern (14), Central (30), and Southern (11) were in demographic equilibrium (Librado & Rozas 2009).

Results

1. Analysis of genetic diversity and differentiation

We obtained amplification products with the COI gene from 59 individuals, with the ND2 gene from 55 individuals, and with the ND4 gene from 58 individuals, though only 55 individuals simultaneously exhibited all three fragments. No insertions or deletions were found, and the start and stop codons were at the expected sites. Base composition patterns were those expected for avian mtDNA. No compositional bias was found in the bases. The three genes were concatenated in 55 individuals, resulting in a total dataset of 2222 base pairs. Seventeen variable sites,16 informative sites and five haplotypes were found. General haplotype diversity (h) was 0.706 and nucleotide diversity (π) was 0.00254. Tajima's D was D = 1.412 (P > 0.10). Statistical data Fu and Li's F = 1.64 and Fu's Fs 1.65 (P > 0.10) were not significant (Table 2).

We detected four haplotypes with COI and ND2, three with ND4, and five with the concatenated sequences when all of the samples were analyzed as a single group. Almost every population exhibited a single haplotype, even those with a larger sample size (Nicaragua, Costa Rica, and Honduras). Nucleotide diversity was also low (Pi = 0.00254) (Table 2). The genetic distances between groups were less than 1%: 0.23% between populations in northern Mexico and Central America, 0.58% between northern Mexico and Costa Rica, and finally 0.54% between Central America and Costa Rica.

The comparison by pairs of the FST values showed high differentiation between the population groups (Table 3). The comparison of central populations (southern Mexico, Honduras, and Nicaragua) with those from the south (Costa Rica) had the highest FST value (0.992), while the comparison of populations from the north (northeastern Mexico) with those from the south had an FST value of 0.956. The lowest FST value was obtained when the populations from the north were compared with those from the center (0.904). AMOVA results showed that the greatest genetic difference occurs between the population groups with 99.5% of differences, whereas the difference within the populations is 0.18%. The correlation between the geographic distance matrix and the genetic distance matrix was moderately significant (r = 0.568, p < 0.05, Figure 2), indicating that partly a process of isolation by distance undergone by this species' populations (Table 4).

2. Relationships between haplotypes and their geographic distribution

The haplotype network showed the relationships of the three haplogroups and their frequencies found in the 55 individuals (Figure 3). In the network, the green haplogroups of the Costa Rican specimens are separated by 12 mutations from the other two groups (Hap 1, southern group). All of the specimens from the populations in northeastern Mexico are included in a single haplogroup (blue) (Hap 5, northern group). The populations in southern Mexico, Honduras and Nicaragua are in the pink-cherry-purple haplogroup and are widely distributed (Haps 2-4, central group). The latter group has three haplotypes: haplotype 2, which is common, haplotype 4 in three individuals from Tenosique, and haplotype 3 in a specimen from Nicaragua (Figures 3 and 4).

The Red Split Tree analysis also produced three groups (Figure 5), which are similar to those reported by prior analyses. The first southern group included individuals from Costa Rica, the second central group had individuals from southern Mexico (Tecpatan, Tenosique, and Chalchijalpal localities) and northern Central America (Honduras and Nicaragua), and specimens from northeastern Mexico (states of Tamaulipas and San Luis Potosí) comprised the third northern group.

3. Genealogical analysis

Phylogenetic analyses were performed to estimate the genealogical relationships between the detected groups (Figure 6). The tree constructed from Bayesian inference coincides with the topology obtained by maximum likelihood. The analyzed samples were grouped into three clades: the first with 11 specimens from Costa Rica; the second

Table 2. Genetic diversity indices obtained for the white-crowned parrot (*Pionus senilis*).

Gene fragment	N	Nt	H	S	Hd	Pi	Dt	Fu y Li	Fs fu
COI	60	677	4	12	0.655	0.00297	0.90837	1.23	1.53
ND2	56	767	4	12	0.674	0.00222	0.90837	1.04	1.23
ND4	58	778	3	10	0.627	0.00283	2.488	1.14	1,21
Concatenated	55	2222	5	34	0.706	0.00254	1.412	1.64	1.65

N = sample size, Nt = base pairs, H = haplotypes, S = polymorphic sites, Hd = haplotype diversity, Pi = nucleotide diversity, Dt = D Tajima significant at p < 0.10.

Table 3. Genetic differentiation for the white-crowned parrot (*Pionus senilis*) groups.

		%	F_{ST}	G_{ST}	N_{ST}
Northern	Southern	0.4%	0.956	0.785	0.956
Northern	Central	0.3%	0.904	0.793	0.904
Southern	Central	0.5%	0.992	0.894	0.992

% genetic distance F_{ST}, genetic differentiation between populations; G_{ST}, genetic differentiation between populations; N_{ST} nucleotide diversity between populations.

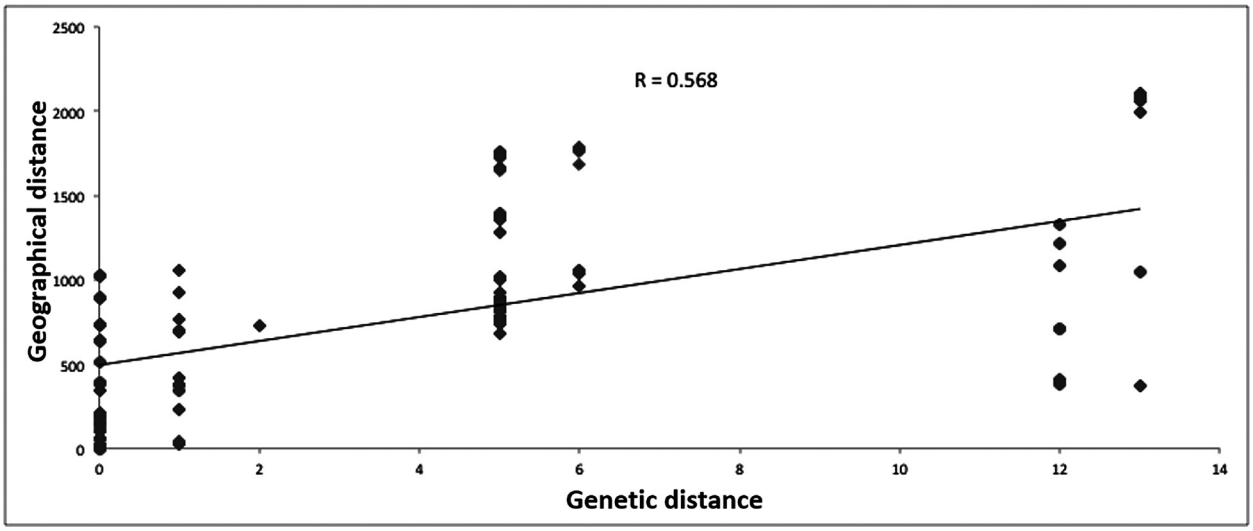

Figure 2. Concatenated Mantel test for all populations of the white-crowned parrot (*Pionus senilis*). The AMOVA test produced genetic diversity distribution values of 99.5% between the groups; the rest is distributed among the populations.

Table 4. AMOVA summary for the white-crowned parrot (*Pionus senilis*) populations using concatenated mitochondrial gene sequences.

Source	df	SS	Components variation	% variation
Among groups	2	1687.759	45.610	99.5
Among populations within groups	8	4.136	0.085	0.32
Within groups	49	6.889	0.14	0.18
Total	59	1698.78	45.835	100%

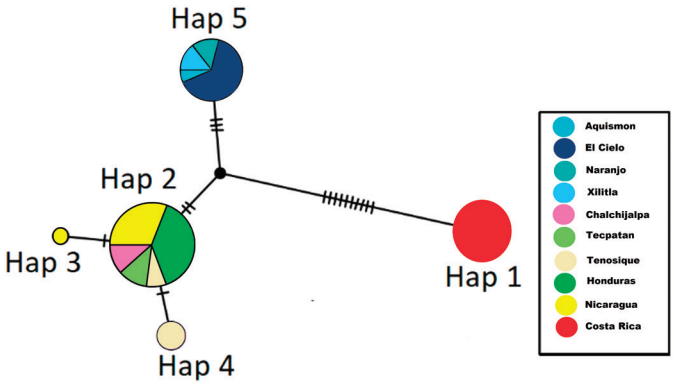

Figure 3. Concatenated haplotype network of the white-crowned parrot (*Pionus senilis*) for populations in Mexico, Honduras, Nicaragua, and Costa Rica using nucleotide sequences ND2, ND4 and COI. Dashes on haplotype network branches indicate mutations between haplotypes and the sizes of circles are proportional to the number of samples for each haplotype.

with 30 specimens from Nicaragua, Honduras, and the localities of Tecpatan, Tenosique, and Chalchijalpa, Mexico; and the third with 14 individuals from the populations in northern Mexico, namely El Cielo, El Naranjo, Xilitla and Santos.

4. Molecular clock

The BEAST analyses produced a high effective sample size (ESS) (200) for all parameters, indicating that the posterior distribution

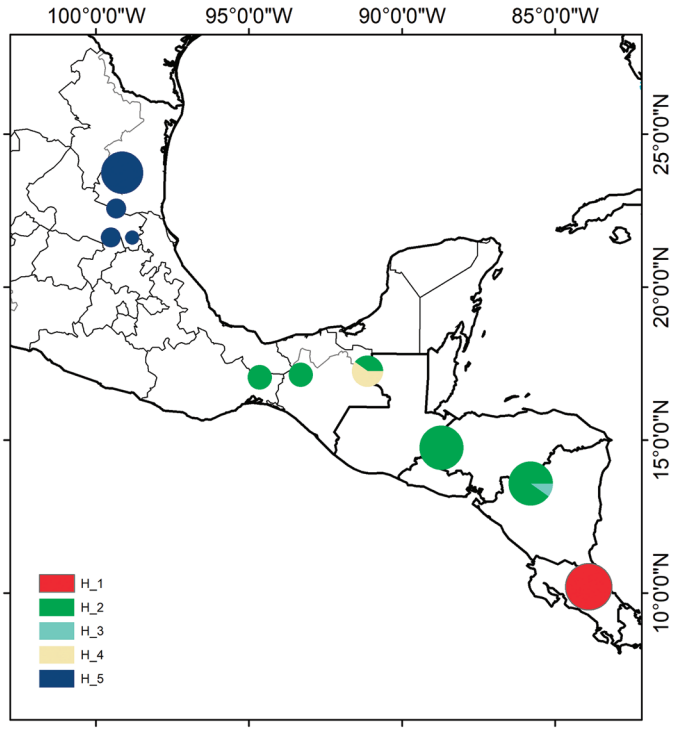

Figure 4. Geographical distribution and statistical parsimony network of concatenated haplotypes of white-crowned parrot (*Pionus senilis*) overlaid on a map of Centro America. Pie charts represent haplotypes found in each sampling locality.

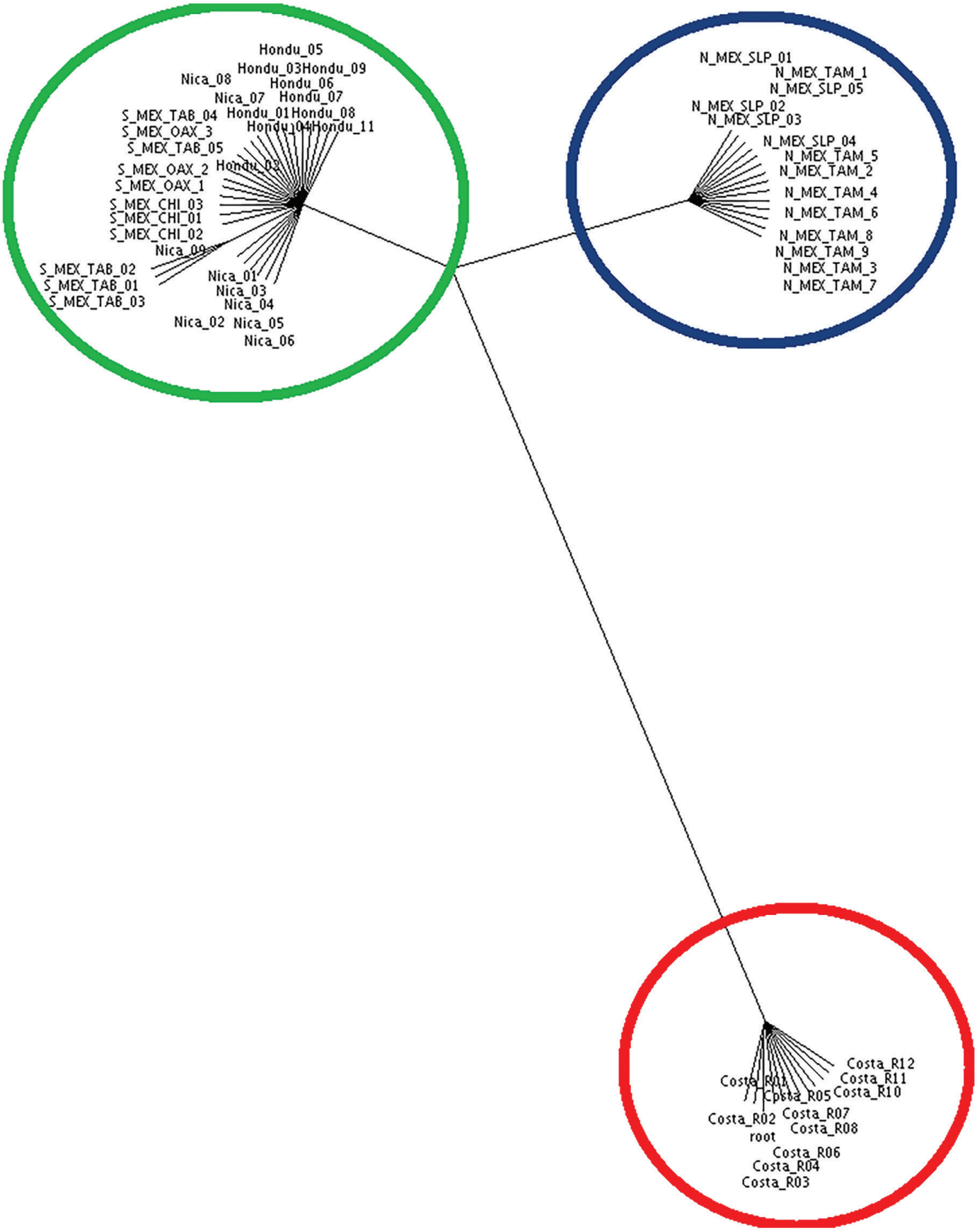

Figure 5. Concatenated Split Tree of the white-crowned parrot (*Pionus senilis*) for populations in Mexico, Honduras, Nicaragua, and Costa Rica using nucleotide sequences ND2, ND4 and COI. The distance between taxa represents the sum of weights of all splits that separate taxa.

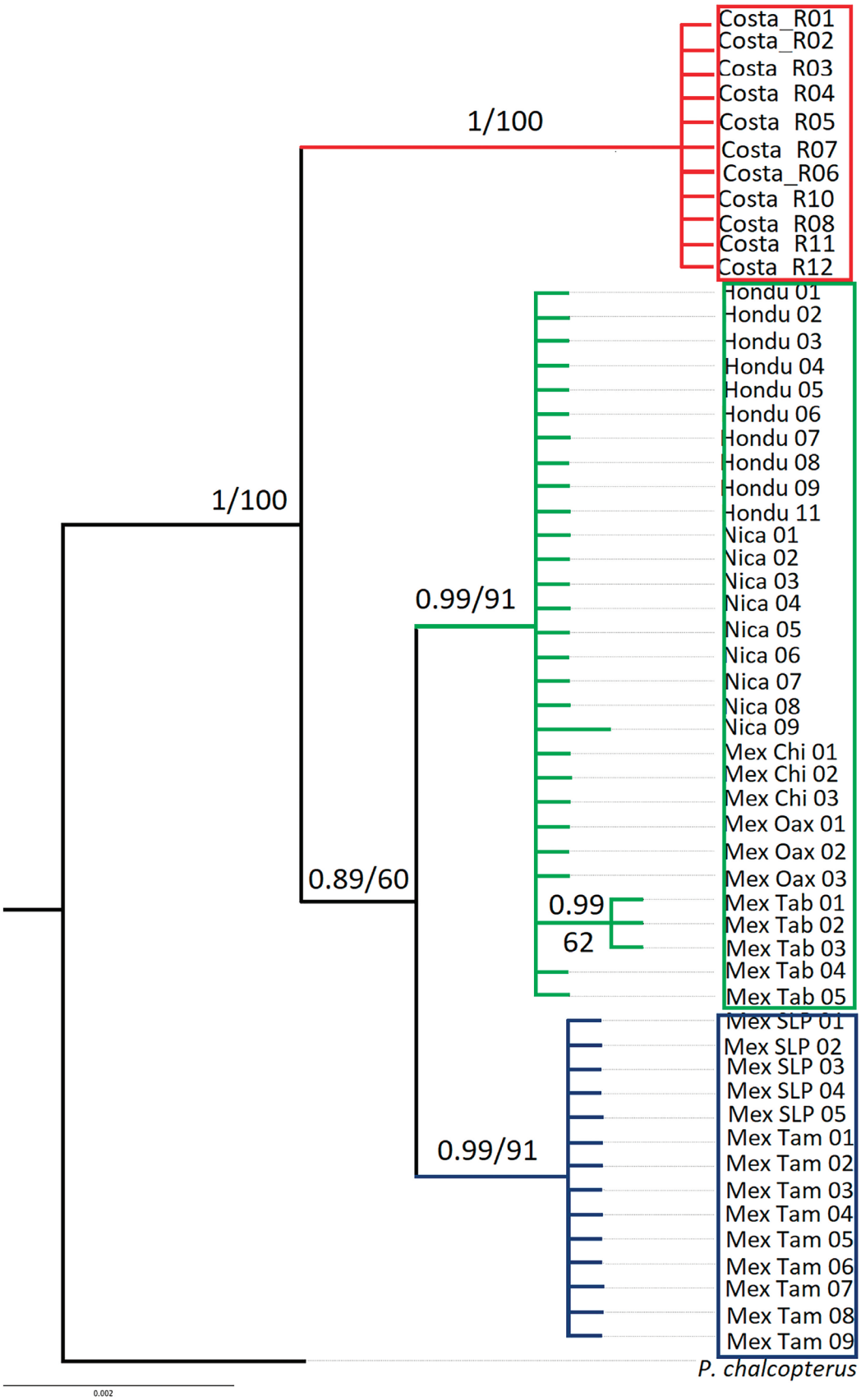

Figure 6. Consensus (ND4, COI and ND2) tree showing the genealogical relationships between 55 individuals of white-crowned parrot individuals (*Pionus senilis*) with *Pionus chalcopterus* as outgroup obtained with Bayesian inference (BI) and maximum likelihood analysis (ML). Node values represent posterior probabilities and bootstrap values (PP / BP). The scale bar below is a reference of branch length, and branch length is proportional to the amount of evolutionary change. Populations groups: Northern (blue), Center (green) and Southern (red).

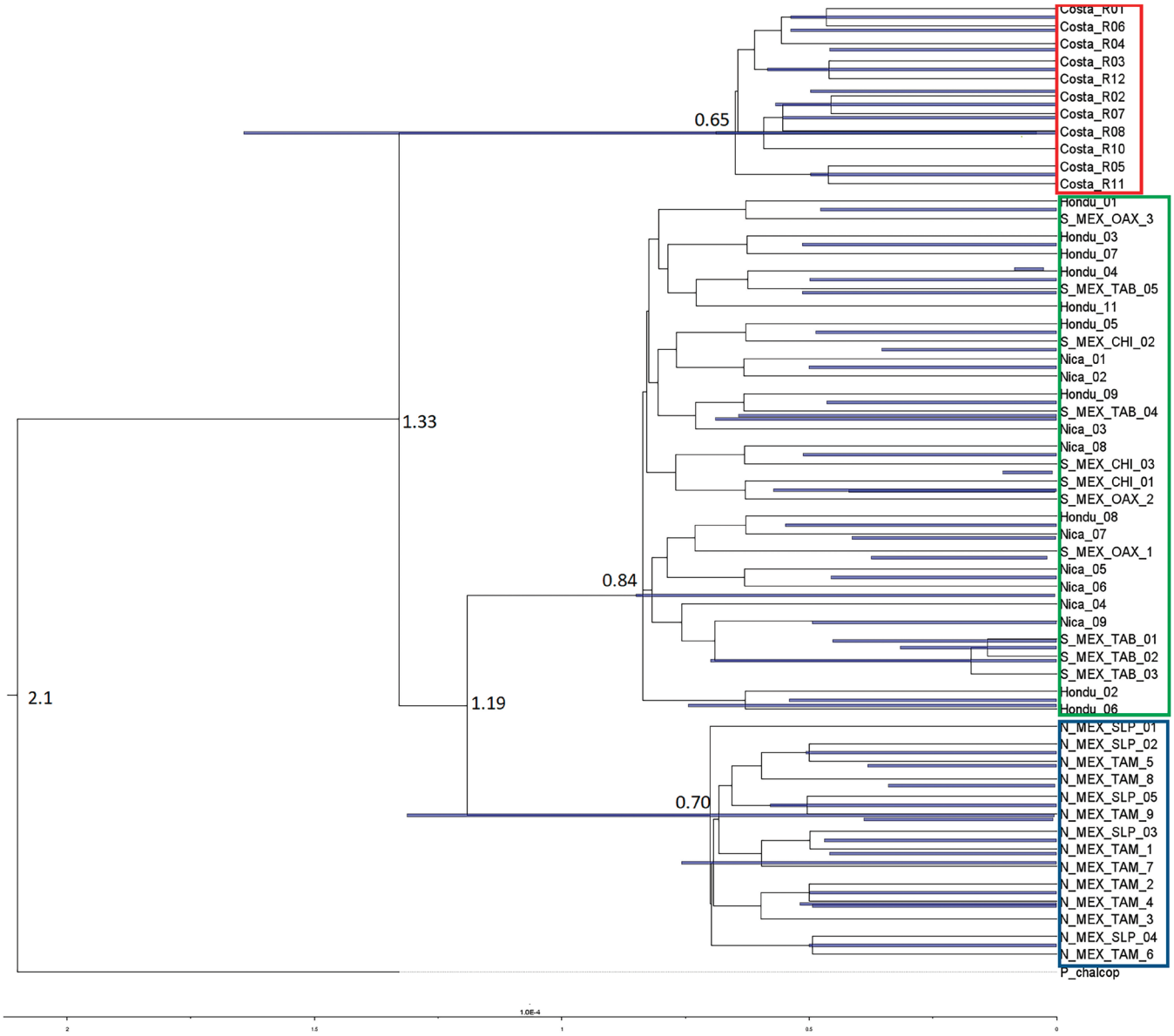

Figure 7. Estimates of divergence time in years shown by lineages of the white-crowned parrot (*Pionus senilis*) populations based on the concatenated 2222 bp data from mtDNA sequences. Blue bars on the tree correspond to the 95% credibility intervals of the estimated node ages. The colors represent the three outstanding genetic lineages of this work: Northern (blue), Central (green) and Southern (purple).

was adequately sampled. This result concurred with the Bayesian inference and maximum likelihood analyses. The tree from BEAST (Figure 7) with mtDNA data strongly supported (PP = 1.0) a division between the Costa Rican (Southern) lineage and the other two lineages that occurred 1.33 Mya (95% highest posterior density [HPD]), as well as the differentiation between the other two lineages (PP = 0.53) at 1.19 Mya (95% HPD). The Central lineage was dated at 840,000 years and the Northern lineage at 700,000 years. Results from the mitochondrial data suggest that both lineages branched away during the Pleistocene.

5. *Historical demography*

Distribution analysis of concatenated paired differences (Figure 8) showed a distribution that would be expected of a constant population size. This result fits the population expansion of the global sample analyzed for populations of the white-crowned parrot. Specifically, for haplogroups only the central group fits a stable distribution model.

Discussion

We conducted the first complete phylogeographic study for a charismatic, but least studied, parrot in Middle America. Our mtDNA data revealed three genetic groups. The first group corresponded to individuals from the states of Tamaulipas and San Luis Potosí in northern Mexico (Northern), the second to populations in southern Mexico, Honduras, and Guatemala (Central), and the third exclusively to specimens from Costa Rica (Southern). These white-crowned parrot groups are separated by a short genetic distance (0.5–0.3%, Table 3). *P. senilis* branched away from *P. cyanescens/P. chalcopterus* about 2.2 – 1.2 million years ago (Ribas et al. 2007). This finding, and the

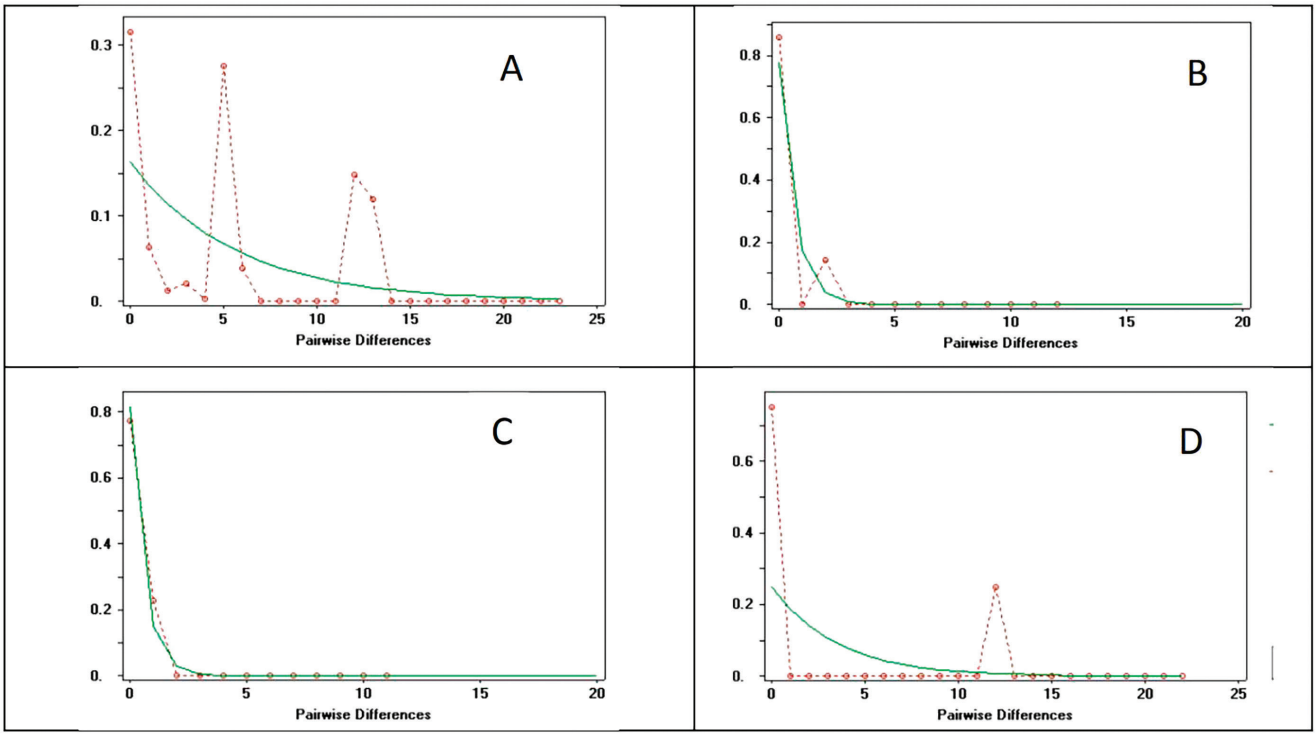

Figure 8. Mismatch distributions of the white-crowned parrot (*Pionus senilis*) populations: concatenated (A), Northern (b), Central (C), Southern (D). Dashes lines indicates the observed distribution of pairwise differences (red and solid lines show the expected distribution (green) under a model of sudden expansion.

fact that *P. senilis* is mostly distributed in lowlands at present, strongly implies an ancestral distribution across Central American lowlands (Ribas et al. 2007).

White-crowned parrots have a wide geographic distribution, yet no studies have been made of the connectivity between its populations. Our mtDNA data show consistent levels of phylogeographic structure among the three population groups, though it must be determined whether this result stems from their historical biogeography (i.e., it is caused by geographic boundaries) or from gaps in sampling. We discovered that the genetic structure in all three groups was consistent, even when the isolation by distance analysis was taken into consideration. Moreover, the highest Fst values point to a greater genetic differentiation between the Central and Southern groups (0.992), which are geographically closer to each other, and the Fst values (0.956) for the Northern-Southern and Northern-Central groups are extremely high compared to any other species (Table 3).

Although the Northern and the Southern groups each presented a unique haplotype, three unique haplotypes were found in the Central populations, indicating high haplotype diversity (Hd) in total (h = 0.706). These data are similar to those reported by other studies on Psittacidae, such as *Cyanoliseus patagonus* with a haplotype diversity of 0.943, *Eolophus roseicapilla* with Hd: 0.817, *Anodorhynchus hyacinthinus* with Hd: 0.604, *Eclectus roratus* with Hd: 0.500 and *Lophochroa leadbeateri* with Hd: 0.602 (Masello et al. 2011; Engelhard et al. 2015; Presti et al. 2015; Astuti 2020; Ewart et al. 2021). Low nucleotide diversity values ($\pi < 0.0025$) and a small number of haplotypes are characteristic of the effects of population demographic expansion over a relatively recent period (Hamilton 2009). This has been attributed to expansion following a small effective size period in the population, since rapid growth in a population increases its retention of new mutations (Avise et al. 1984, Watterson 1984).

Tajima's D and Fu's Fs neutrality tests were performed to measure the effect of the population's demographic changes on sequences of mtDNA. The Fs test is more powerful at detecting recent or selected population growth and demographic expansion than Tajima's D (Tajima 1989), and it usually produces high negative values. The analyses showed that both Fu's, Fs, and Tajima's D tests had non-significant values (Table 2), indicating that the mutations neither favor nor hinder the organism and thus do not exert selection pressure (Ramos-Onsins and Rozas 2002).

We recovered a clear phylogeographic structure even though the genetic groups are separated by few mutations. The geographic distribution of genetic variation is not entirely random (as indicated by the AMOVA and F_{ST} values), suggesting a scenario in which the groups have been diverging in isolation and then expanding their distribution range. Furthermore, the low nucleotide diversity but high haplotype diversity we found in *P. senilis* is consistent with populations with small effective sizes that undergo rapid population growth (Grant and Bowen 1998). Although they are similar, the different haplotypes in the populations suggest that there is no detectable gene flow between the three *P. senilis* population groups. This is also supported by the AMOVA results, which indicate that the largest percentage of genetic variation is spread throughout the population groups (99.5). There is little variation within lineages.

The F_{ST} and R_{ST} values (Table 2) and the haplotype networks (Figure 3) show that the populations of *P. senilis* are separated into three lineages with geographic distributions that do not overlap. In this study, the pattern observed in the haplotype network and the results from

molecular dating point to the diversification of the different haplotype groups 1.3 million years ago, even when a small number of haplotypes exist in the geographically distant groups (e.g., samples from southern Mexico and Nicaragua) with low levels of nucleotide diversity. Since the displacement range of this species has not been studied, it would be interesting to determine the displacement range of the populations throughout their territory, showing how dispersion capacity affects the genetic structure of their populations.

An analysis of endemism using four different groups of fauna found that after the Trans-Mexican Volcanic Belt separates the highest amount of biota between N and S Mexico and Central America, the Isthmus of Tehuantepec and the Nicaraguan depression are the following marked barriers (Miguez-Gutierrez et al. 2013). Correlating geologic and genetic studies to elucidate the patterns of biogeographic and evolutionary history in Central America, four tectonic blocks appear, Maya, Chortis, Chorotega, and Chocó, and three evolutionary groups separated by the barriers between these tectonic blocks emerge, the Mayan, Mid-CA, and Panamian. After the Great American Biotic Interchange (3.1 to 2.5 Ma) after the formation of the Isthmus of Panama, new migrations or colonizations prompted by the Pleistocene climatic fluctuations and local volcanic activity followed (Gutiérrez-García and Vázquez-Domínguez, 2013).

Prior studies based on mtDNA have revealed a strong differentiation between the populations of different vertebrates inhabiting Middle America. In the case of the glass frog *Hyalinobatrachium fleischmanni* six concordant genetic lineages have been described with geographical barriers of the Sierra Madre del Sur, the Tehuantepec isthmus, Motagua–Polochic–Jocotán fault system, Hess escarpment, and the Panama Isthmus (Mendoza et al. 2019). Regarding some clades of snakes, genetic divergences correspond to the depression of Nicaragua, the Chortis block, the Maya block, and the Isthmus of Tehuantepec. (Daza et al. 2010).

The Isthmus of Tehuantepec is undoubtedly an important barrier in the distribution of genetic lineages, as is the case of rodents of the genus *Reithrodontomys* (Sullivan et al. 2000*), Peromyscus* with 5.7% of divergence between its different groups (Kilpatrick et al. 2021), salamanders (Rovito et al. 2012; Rovito and Parra-Olea 2016), the toad *Incilus valliceps* (Mulcahy et al. 2006) and bats (Guevara-Chumacero et al. 2013, Hernández-Canchola and León-Paniagua 2017). Even the genetic structure of mammals with a higher dispersion capacity has been influenced by the Isthmus of Tehuantepec, as is the case of the coati *Nasua narica* (Nigenda-Morales et al. 2019).

Geological information (Barrier et al. 1998; Manea and Manea 2006) suggests that a highland corridor spanned the Isthmus of Tehuantepec during the Miocene, then collapsed due to extreme Pliocene tectonic activity (about 3.5 million years ago). This event separated the mountain ranges on either side of the isthmus. The genetic differences between the white-crowned parrot individuals on both sides of the isthmus match a more recent divergence after the geological events causing lowlands to form in the Isthmus of Tehuantepec; as observed in local mountain species (González et al. 2011; Ortiz-Ramírez et al. 2016).

The Nicaragua Depression determines a site that marks significant changes in the communities of Middle American birds (Patten and Smith-Patten 2008; Sánchez-González et al. 2008), acting as a barrier for other taxa that inhabit Central American rainforests. This pattern suggests that the dispersion and vicariance of lineages due to this barrier have occurred numerous times, even after the Pliocene (Bonaccorso et al. 2010; Gutiérrez-García and Vázquez-Domínguez 2012). This area also holds sway over vicariant events in Central America, being proposed as a region of numerous changes in the distribution patterns of many groups that it bars from the Middle American highlands (Marshall and Liebherr 2000). The Nicaragua Depression has also played a relevant role in the distribution of the bat *Sturnira hondurensis*, separating the continuous Mexico-Nicaragua area from another ideal habitat in the mountain regions of Costa Rica and Panama (Torres-Morales 2019). This pattern is evident in the rodent *Ototylomys phyllotis* since the depression acted as a barrier that influenced the rodent's phylogeographic pattern (Gutiérrez-García and Vázquez-Domínguez 2012). Daza et al. (2010) propose that hypotheses be generated and tested by combining phylogeographic studies with geological-tectonic data.

Throughout the distribution area of the white-crowned parrot, its phylogeographic structure matches that of other species of birds in this area of Middle America (Barber and Klicka 2010; Barrera-Guzmán et al. 2012; Rocha-Méndez et al. 2018; Castillo-Chora et al. 2021). The Middle American *Turdus assimilis* populations bared low genetic differentiation with a difference of 0.03 between individuals from northern Mexico and Central America, including the state of Chiapas, Mexico; this indicates that the Isthmus of Tehuantepec may be the boundary between these populations (Nuñez-Zapata et al. 2016). However, many examples of similarly distributed bird species exhibit a more pronounced genetic differentiation in almost the same range. The magnificent hummingbird *Eugenes fulgens*, whose phylogeographic pattern associated with Middle American highlands revealed three principal lineages: *E. f. fulgens* to the west of the Isthmus of Tehuantepec, and two groups of *E. f. viridiceps* to the east of the Isthmus, isolated from each other by the Motagua-Polochic-Jocotán fault system (Zamudio-Beltrán et al. 2020).

Not only geographical barriers can determine the population structure of Central American birds but also foraging ecology. Species that depend on seasonally variable plant reproductive parts present less geographic genetic differentiation compared to those that rely primarily on insectivorous diets (Miller et al. 2021). However, for the white-crowned parrot, being a seedeater mainly, they still exhibit a divergence pattern in these areas. We can also see a division between the white-crowned parrot individuals from Costa Rica and the other populations, which coincides with the lowlands of the Nicaragua Depression.

The evolutionary impact of the Nicaragua Depression on birds is also evident in several sets of taxa found on either side of the region. Such is the case of *Lepidocolaptes affinis*, whose phylogeographic pattern indicates that the Nicaragua Depression has prevented gene flow, even though the populations on both sides of the Isthmus of Tehuantepec did not show high genetic differentiation (Arbeláez-Cortés et al. 2010). On the other hand, *Catharus frantzii* at the Isthmus of Tehuantepec and populations on either side of the Nicaragua Depression presented a phylogeographic pattern (Ortiz-Ramírez et al. 2016). Another case is *Habia fuscicauda*, which has two clades: one that genetically corresponds to the region from southeastern Mexico to the Nicaragua Depression, and the other from the Nicaragua Depression to Central Panama (Castillo-Chora et al. 2021).

The estimated mean divergence time produced by BEAST analysis indicates that white-crowned parrot branched away from *P. chalcopterus* 1.33 Mya and that the other two clades differentiated

1.19 Mya. The Central lineage is dated at 840,000 years, and the Northern at 700,000 ys. Results from mitochondrial data suggest that these lineages parted during the Pleistocene, whose paleoclimatic dynamics and geological events were the main cause for swift, recent diversification in neotropical biota (Hackett and Lehn 1997; Haffer 1997).

Our work enabled us to reconstruct the phylogeographic pattern and evolutionary history of the white-crowned parrot, whose populations form into three distinct lineages. The Southern lineage is located to the south of the Nicaragua Depression, a geographic barrier for different species of birds. Comparison of the genetic variation in the populations from southern Mexico to Nicaragua against those from northern Mexico leads us to infer that the Isthmus of Tehuantepec is the barrier that keeps these two lineages apart. Our results prove that even though this divergence occurred during the Pleistocene, geographic barriers play an active role in the genetic structuring of a species.

Given the differences in plumage detected when the subspecies were named, and the marked genetic structure found with an absence of gene flow in the last 0.7–1.7 million years between the three metapopulations, it would be relevant to complete a study that included the vocalizations, morphology, and nuclear sequences of these three groups to determine whether they must continue to be treated as one species.

The strong genetic differentiation of the white.crowned parrot populations in three genetic lineages at the mitochondrial level requires the need to use independent conservation status for each. It is critical considering that the Northern and Central groups have decreased to the point of being eliminated in western Quintana Roo and some areas in the Gulf of Mexico (Salinas-Melgoza and Renton 2002). It is well-known that deforestation rates in tropical moist forests, oak groves, and lower montane forests are high, mainly because of the land transformation into agricultural activities (Rosete-Verges et al. 2014). In addition, it is unknown how biological mechanisms, physiological traits, phenotypic plasticity, local adaptations, interactions between species, dispersal capabilities, or food availability influence the permanence of populations (Hoffmann and Sgro 2011).

The three genetic groups detected in this study have a geographic concordance, which indicates that each group can be considered an Evolutionarily Significant Unit (ESU). ESUs are based on sequence marker-based phylogeographic analyses, defining ESUs as reciprocally monophyletic mitochondrial DNA (mtDNA) clusters with divergence (Moritz 1994). They can also be explained as intraspecific lineages with highly restricted gene flow between them, allowing the delimitation of the ESUs without reciprocal monophyly (Fraser y Bernatchez 2001). Our results on the genetic structure of the populations of the white-crowned parrot have implications for conservation since most of the sites we studied represent very isolated and small populations, which need efficient protection actions at the regional level to preserve them, along with their habitats, and its genetic heritage. We propose that these three groups be considered a reference for the conservation programs of the white-crowned parrot in the countries where they are distributed including the maintenance of genetic connectivity between different populations with its effects on sustaining gene flow, to preserve this ESUs. It is also necessary to carry out a study in which the impact of habitat fragmentation is evaluated and that allows the establishment of more natural protected areas.

Acknowledgments

This study was conducted thanks to support provided by the Graduate Program at the National Autonomous University of Mexico (UNAM), the Support Program for Research and Technological Innovation Projects (PAPIIT) issued by the Office of Support Services for Academic Personnel at the UNAM (IN208220), the National Council of Science and Technology (CONACYT Mexico), the Rufford Foundation, and Idea Wild, who provided funds to cover the financial expenses and material necessary to perform our fieldwork. We also thank Macaw Mountain Bird Park in Honduras and Rescate Animal Zoo Ave in Costa Rica for their contribution to white-crowned parrots sampling, which were vital for this work.

We are grateful to all of the field guides who contributed valuable information that enabled us to track white-crowned parrot populations: Lázaro Chavarría, German de Catazaja, Mario Álvarez, Chankin Wiliam Garcia, Esteban Berrones Benitez, Juan Carlos Orraca Corona, Neydy Perez, Leoni Zepol, Limberg Perez Benavente, Luis Armando Ferman Cortez, and Saúl García Rivera, as well as Hostal del Café and Hotel El Salto del Meco in Xilitla, San Luis Potosí. We are grateful to the state of San Luis Potosí for their warm welcome and excellent services, which allowed us to conduct this research.

Associate Editor

Luis Fabio Silveira

Author Contributions

Canek Rivera-Arroyo: conceptualization; resources; methodology; writing – original draft; writing – review & editing.

Patricia Escalante-Pliego: conceptualization; resources; methodology; writing – review & editing.

Damián Aguilar-Torres: methodology.

Milton F. Úbeda-Olivas: methodology.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

Ethics

This study did not involve human beings and/or clinical trials that should be approved by one Institutional Committee.

Data Availability

The raw data for the samples collected under this study are available in this link: https://doi.org/10.48331/scielodata.TYSDUZ, SciELO Data.

All the gene sequences have been submitted to GenBank under accession numbers OP491996-OP492050, OP566431-OP566485, OP583596-583650.

References

ALFARO, M.E. & HUELSENBECK, J.P. 2006. Comparative performance of Bayesian and AIC-based measures of phylogenetic model uncertainty. Systematic Biology. 55:89–96. https://doi.org/10.1080/10635150500433565

ARBELÁEZ-CORTÉS, E., NYÁRI, A.S. & NAVARRO-SIGÜENZA, A.G. 2010. The differential effect of lowlands on the phylogeographic pattern of a Mesoamerican montane species (Lepidocolaptes affinis, Aves: Furnariidae). Molecular Phylogenetics and Evolution. 57(29):658–668. DOI: 10.1016/j.ympev.2010.06.013

ARÉVALO, E., DAVIS, S.K. & SITES, J.W. 1994. Mitochondrial DNA Sequence Divergence and Phylogenetic Relationships among Eight Chromosome Races of the *Sceloporus grammicus* Complex (Phrynosomatidae) in Central Mexico. Systematic Biology. 43:387–418. https://doi.org/10.1093/sysbio/43.3.387

ASTUTI, D.A. 2020. Genetic diversity of Indonesian protected eclectus parrot (*Eclectus roratus*) based on mitochondrial gene sequences. IOP Conf. Series: Earth and Environmental Science. 591. doi:10.1088/1755-1315/591/1/012037

AVISE, J.C. 1998. The Genetic Gods: Evolution and Belief in Human Affairs. Harvard University Press, Cambridge, MA. (279 pp.). https://doi.org/10.1073/pnas.1604338113

AVISE, J.C. 2002. Genetics in the Wild. Washington, DC: Smithsonian Inst. Press

AVISE, J.C., BOWEN, W.B. & AYALA, F.J. 2016. In the light of evolution X: Comparative phylogeography. Proceedings of the National Academy of Sciences. 113(29):7957–7961. DOI: 10.1007/BF02257369

AVISE, J.C., NEIGEL, J.E. & ARNOLD, J. 1984. Demographic Influences on Mitochondrial DNA Lineage Survivorship in Animal Populations. Journal of Molecular Evolution. 20:99–105.

BARBER, B.R. & KLICKA, J. 2010. Two pulses of diversification across the Isthmus of Tehuantepec in a montane Mexican bird fauna. Proceedings of the Royal Society B: Biological Sciences. 277:2675–2681. doi:10.1098/rspb.2010.0343

BARRERA-GUZMAN, A.O., MILÁ, B., SÁNCHEZ-GONZÁLEZ, L.A. & NAVARRO-SIGÜENZA, A.G. 2012 Speciation in an avian complex endemic to the mountains of Middle America (*Ergaticus*, Aves: Parulidae). Molecular Phylogenetics and Evolution. 62(3):907–920. https://doi.org/10.1016/j.ympev.2011.11.020

BARRIER, E., VELASQUILLO, L., CHÁVEZ, M. & GOULON, R. 1998, Neotectonic evolution of the Isthmus of Tehuantepec (southeastern Mexico). Tectonophysics. 287:77–96. https://doi.org/10.1016/S0040-1951(98)80062-0

BERGNER, L.M., DUSSEX, N., JAMIESON, I.G. & ROBERTSON, B.C. 2016. European Colonization, Not Polynesian Arrival, Impacted Population Size and Genetic Diversity in the Critically Endangered New Zealand Kākāpō. Journal of Heredity. 107(7):593–602. https://doi.org/10.1093/jhered/esw065

BERKUNSKY, I., QUILLFELDT, P., BRIGHTSMITH, D.J., ABBUD, M.C., AGUILAR, J.M.R.E., ALEMÁNZELAYA, U., & MASELLO, J.F. 2017. Current threats faced by Neotropical parrot populations. Biological Conservation. 214:278–287. https://doi.org/10.1016/j.biocon.2017.08.016

BONACCORSO, E., GUAYASAMIN, J., PETERSON, T. & NAVARRO-SIGÜENZA, A. 2010. Molecular phylogeny and systematics of Neotropical toucanets in the genus *Aulacorhynchus* (Aves, Ramphastidae). Zoologica Scripta. 40(4):336–349. DOI:10.1111/j.1463-6409.2011.00475.x

CANTÚ, J.C., SÁNCHEZ, M.E., GROSSELET, M. & SILVA, J. 2007. Tráfico Ilegal de Pericos en México. Una Evaluación Detallada. Defenders of Wildlife. 75 pp.

CAPARROZ, R., MIYAKI, C.Y. & BAKER, A.J. 2009. Contrasting phylogeographic patterns in mitochondrial DNA and microsatellites: evidence of female philopatry and male-biased gene flow among regional populations of the blue-and-yellow macaw (Psittaciformes: *Ara ararauna*) in Brazil. The Auk. 126(2):359–370. https://doi.org/10.1525/auk.2009.07183

CASTILLO-CHORA, V.J., ZAMUDIO-BELTRÁN, L.E., POZO, C. & HERNÁNDEZ-BAÑOS, B.E. 2021. Phylogeography of *Habia fuscicauda* (Cardinalidae) indicates population isolation, genetic divergence and demographic changes during the Quaternary climate shifts in the Mesoamerican rainforest. Journal of Ornithologyv. 162:961–976. DOI:10.1007/s10336-021-01904-x

DAZA, J.M., CASTOE, T. & PARKINSON, C.L. 2010. Using regional comparative phylogeographic data from snake lineages to infer historical processes in Middle AmericA. Ecography. 33(2):343–354. DOI:10.1111/j.1600-0587.2010.06281.x

DOMÍNGUEZ–DOMÍNGUEZ, O. & VÁZQUEZ–DOMÍNGUEZ, E. 2009. Filogeografía: aplicaciones entaxonomía y conservación. Animal Biodiversity and Conservation. 32(1):59–70.

DRUMMOND, A.J. & RAMBAUT, A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology. 7(214). https://doi.org/10.1186/1471-2148-7-214

ENGELHARD, D., JOSEPH, L., TOON, A., PEDLER, L. & WILKE, T. 2015. Rise (and demise?) of subspecies in the Galah (*Eolophus roseicapilla*), a widespread and abundant Australian cockatoo. Emu. 115(4):289–301. DOI: 10.1071/MU15018

EWART, K.M., JOHNSON, R.N., JOSEPH, L., OGDEN, R., FRANKHAM, G.J. & LO, N. 2021. Phylogeography of the iconic Australian pink cockatoo, *Lophochroa leadbeater*. Biological Journal of the Linnean Society. 132(3): 704–723. https://doi.org/10.1093/biolinnean/blaa225

EXCOFFIER, L., LAVAL, G. & SCHNEIDER, S. 2005. Arlequin ver. 3.1: an integrated software package for population genetics dataanalysis. Evolutionary Bioinformatics Online. 1:47–50.

FRANKHAM, R., BALLOU, J.D. & BRISCOE, D.A. 2002. Introduction to Conservation Genetics. Cambridge University Press, Cambridge, UK.

FRANKHAM, R., BALLOU, J.D., ELDRIDGE, M.D., LACY, R.C., RALLS, K., DUDASH, M.R. & FENSTER, C.B. 2011. Predicting the probability of outbreeding depression. Conservation Biology. 25:465–475. DOI: 10.1111/j.1523-1739.2011.01662.

FRASER, D.J. & BERNATCHEZ, L. 2001 Adaptive evolutionary conservation: towards a unified concept for defining conservation units. Molecular Ecology. 10(12):2741–2752.

FU, Y.X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics. 147:915–925. DOI: 10.1093/genetics/147.2.915

FU, Y.X. & LI, W.H. 1993. Statistical tests of neutrality of mutations. Genetics. 133:693–709. DOI: 10.1093/genetics/133.3.693

GONZÁLEZ, C., ORNELAS, J.F. & GUTIÉRREZ-RODRÍGUEZ, C. 2011. Selection and geographic isolation influence hummingbird speciation: genetic, acoustic and morphological divergence in the wedgetailed sabrewing (*Campylopterus curvipennis*). BMC Evolutionary Biology. 11:38. DOI:10.1186/1471-2148-11-38

GRANT, W.A.S. & BOWEN, B.W. 1998. Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. Journal of Heredity. 89(5):415–426. https://doi.org/10.1093/jhered/89.5.415

GRISCOM, L. 1929. Studies from the Dwight collection of Guatemala birds. I. American Museum Novitates; no.379.

GUEVARA-CHUMACERO, L.M., LÓPEZ-WILCHIS, R., JUSTE, J., IBAÑEZ, C., MARTÍNEZ-MÉNDEZ, L.A. & BARRIGA-SOSA, I. 2013. Conservation units of *Pteronotus davyi* (Chiroptera: Mormoopidae) in Mexico based on phylogeographical analysis. Acta Chiropterologica. 15(2):353–363. https://doi.org/10.3161/150811013X678973

GUTIÉRREZ-GARCÍA, T.A. & VÁZQUEZ-DOMÍNGUEZ, E. 2013. Consensus between genes and stones in the biogeographic and evolutionary history of Central America. Quaternary Research. 79(3):311–324. https://doi.org/10.1016/j.yqres.2012.12.007

GUTIÉRREZ-GARCÍA, T.A. & VÁZQUEZ-DOMÍNGUEZ, E. 2012. Biogeographically dynamic genetic structure bridging two continents in the monotypic Central American rodent *Ototylomys phyllotis*. Biological Journal of the Linnean Society of London. 107:593–610. https://doi.org/10.1111/j.1095-8312.2012.01966.x

HACKETT, S.J. 1996. Molecular phylogenetics and biogeography of tanagers in the genus *Ramphocelus* (Aves). Molecular Phylogenetics and Evolution. 5:368–382. DOI: 10.1006/mpev.1996.0032

HACKETT, S.J. & LEHN, C.A. 1997. Lack of genetic divergence in a genus (*Pteroglossus*) of Neotropical birds: The connection between life-history characteristics and levels of genetic divergence. Pages 267–279 in Studies in Neotropical Ornithology Honoring Ted Parker (J.V. Remsen, Jr., Ed.). Ornithological Monographs, no. 48.

HAFFER, J. 1997. Alternative models of vertebrate speciation in Amazonia: an overview. Biodiversity & Conservation. 6:451–476.

HALL, T.A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/96/NT. Nucleic Acids Symposium. 41:95–98.

HAMILTON, M.B. 2009. Populations genetics. Wiley-Blackwell, Oxford, 424 pp.

HASEGAWA, M., KISHINO, K. & YANO, T. 1985. Dating the human-ape splitting by a molecular clock of mitochondrial DNA, Journal Molecular Evolution. 22:160–174.

HEBERT, P.D.N, STOECKLE, M.Y., ZEMLAK, T.S. & FRANCIS, C.M. 2004. Identification of birds through DNA barcodes. PLoS Biol. 2(e312): 1657–1663. https://doi.org/10.1371/journal.pbio.0020312

HERNÁNDEZ-CANCHOL, G. & LEÓN-PANIAGUA, L. 2017. Genetic and ecological processes promoting early diversification in the lowland Mesoamerican bat *Sturnira parvidens* (Chiroptera: Phyllostomidae). Molecular Phylogenetics and Evolution. 114:334.345. DOI:10.1016/j.ympev.2017.06.015

HOFFMANN, A.A. & SGRO, C.M. 2011. Climate change and evolutionary adaptation. Nature. 470:479–485. DOI: 10.1038/nature09670

HUSON, D.N. & BRYANT, D. 2006. Application of Phylogenetic Networks in Evolutionary Studies. Molecular Biology and Evolution. 23(2):254–67. DOI:10.1093/molbev/msj030

IUCN, 1980. Estrategia Mundial para la conservación. UICN PNUMA WWF. Primera edición. 71pp.

IUCN (INTERNATIONAL UNION FOR CONSERVATION OF NATURE). 2019 http://www.iucnredlist.org/details/22685548/0. (last access on 21/01/2022).

JOSEPH, L., TOON, A., SCHIRTZINGER, E. & WRIGHT, T. 2011 Molecular systematics of two enigmatic genera *Psittacella* and *Pezoporus* illuminate the ecological radiation of Australo-Papuan parrots. Molecular Phylogenetics and Evolution. 59:675–684. https://doi.org/10.1016/j.ympev.2011.03.017

JUNIPER, T. & PARR, M. 1998. Parrots. A guide to parrots of the world. London, UK.: Yale University Press.

KILPATRICK, C.W., PRADHAN, N. & NORRIS, R.W. 2021. A re-examination of the molecular systematics and phylogeography of taxa of the *Peromyscus aztecus* species group, with comments on the distribution of *P. winkelmanni*. Therya. 12(2):331–346. DOI: 10.12933/therya-21-1115

LEIGH, J.W. & BRYANT, D. 2015. PopArt: full-feature software for haplotype network construction. Methods in Ecology and Evolution. 6:1110–1116. DOI:10.1111/2041-210X.12410

LIBRADO, P.J.R. & J. ROZAS. 2009. DnaSP v5: A Software for Comprehensive Analysis of DNA Polymorphism Data. Computer applications in the biosciences: CABIOS. 25(11):1451–1452. DOI:10.1093/bioinformatics/btp187

MANEA, V.C. & MANEA, M. 2006. The origin of modern Chiapan volcanic arc in Southern Mexico inferred from thermal models. Geological Society of America. 412:27–38. DOI:10.1130/2006.2412(02)

MARSHALL, C. & LIEBHERR, J. 2000. Cladistic biogeography of the Mexican Transition Zone. Journal of Biogeography. 27:203–216. DOI:10.1046/j.1365-2699.2000.00388.x

MASELLO, J.F., QUILLFELDT, P., MUNIMANDA, G.K., KLAUKE, N., SEGELBACHER, G., SHAEFER, H.M. ET AL. 2011. The high Andes, gene flow and a stable hybrid zone shape the genetic structure of a wideranging South American parrot. Frontiers in Zoology. 8:16. doi:10.1186/1742-9994-8-1

MENDOZA, A.M., BOLÍVAR-GARCÍA, W. VÁZQUEZ-DOMÍNGUEZ, E. IBÁÑEZ, R. & PARRA, G. 2019. The role of Central American barriers in shaping the evolutionary history of the northernmost glassfrog, *Hylanobatrachium fleischmanni* (Anura: Centrolenidae). PeerJ. 7:e6115. https://doi.org/10.7717/peerj.6115

MIGUEZ-GUTIÉRREZ, A., CASTILLO, J., MÁRQUEZ, J. & GOYENECHEA, I. 2013. Biogeografía de la Zona de Transición Mexicana con base en un análisis de árboles reconciliados. 84:215–224. DOI: 10.7550/rmb.32119

MILLER, A.D., GOOD, R.T., COLEMAN, R.A., LANCASTER, M.L., & WEEKS, A.R. 2013. Microsatellite loci and the complete mitochondrial DNA sequence characterized through next generation sequencing and de novo genome assembly for the critically endangered orange-bellied parrot, *Neophema chrysogaster.* Molecular Biology Reports. 40:35–42. DOI: 10.1007/s11033-012-1950-z

MILLER, M.J., BERMINGHAM, E., TURNER, B.L., TOUCHON, J.C., JOHNSON, A.B., & WINKER, K. 2021. Demographic consequences of foraging ecology explain genetic diversification in Neotropical bird species. Ecology Letters. 24(3):563–571.

MONTERRUBIO-RICO, T.C., CHARRE-MEDELLÍN, J.F., PACHECO-FIGUEROA, C., ARRIAGA-WEISS, S., VALDEZ-LEAL, J., CANCINO-MURILLO, R., ESCALONA-SEGURA, G., BONILLA-RUZ, C. & RUBIO-ROCHA, Y. 2016. Distribución potencial histórica y contemporánea de la familia Psittacidae en México. Revista Mexicana de Biodiversidad. 87:1103–1117. https://doi.org/10.1016/j.rmb.2016.06.004

MORITZ, C. 1994. Defining 'Evolutionarily Significant Units' for conservation. Trends in Ecology & Evolution. 9(10):373.375. https://doi.org/10.1016/0169-5347(94)90057-4

MULCAHY, D.G., MORRILL, B.H. & MENDELSON, J.R. 2006. Historical biogeography of lowland species of toads (*Bufo*) across the Trans-Mexican Neovolcanic Belt and the Isthmus of Tehuantepec. Journal of Biogeography. 33:1889–1904. http://dx.doi.org/10.1111/j.1365-2699.2006.01546.x

MURPHY, S.A., JOSEPH, L., BURBIDGE, A.H. & AUSTIN, J. 2011. A cryptic and critically endangered species revealed by mitochondrial DNA analyses: the Western Ground Parrot. Conservation Genetics. 12: 595–600. DOI:10.1007/s10592-010-0161-1

MURPHY, S.A., DOUBLE, M.C. & LEGGE, S.M. 2007. The phylogeography of palm cockatoos, *Probosciger aterrimus*, in the dynamic Australo-Papuan region. Journal of Biogeography. 34:1534–1545. DOI:10.1007/s10592-010-0161-1

NIGENDA-MORALES, S.F., GOMPPER, M.E., VALENZUELA-GALVÁN, D., LAY, A.R., KAPHEIM, K.M., HASS, C. ET AL. 2017. Phylogeographic and diversification patterns of the white-nosed coati (*Nasua narica*): Evidence for south-to-north colonization of North America. Molecular phylogenetics and evolution.131:149–163. https://doi.org/10.1016/j.ympev.2018.11.011

NUÑEZ-ZAPATA, J., PETERSON, A.T. & NAVARRO-SIGÜENZA, A.G. 2016. Pleistocene diversification and speciation of White-throated Thrush (*Turdus assimilis*; Aves: Turdidae). Journal of Ornithology. 157:1073–1085. DOI:10.1007/s10336-016-1350-6

OLAH, G., SMITH, B.T., JOSEPH, L., BANKS, S.C. & HEINSOHN, R. 2021. Advancing genetic methods in the study of parrot biology and conservation. *Diversity* **2021**. 13(11):521. https://doi.org/10.3390/d13110521

ORTIZ-RAMÍREZ, M.F., ANDERSEN, M.J., ZALDÍVAR-RIVERÓN, A. & ORNELAS, J.F. 2016. Geographic isolation drives divergence of uncorrelated genetic and song variation in the Ruddy-capped NightingaleThrush (*Catharus frantzii*; Aves: Turdidae).Molecular Phylogenetics and Evolution. 94:74–86. DOI:10.1016/j.ympev.2015.08.017

PATTEN, M.A. & SMITH-PATTEN, B.D. 2008. Biogeographical Boundaries and Monmonier's Algorithm: A Case Study in the Northern Neotropic. Journal of Biogeography. 35(3):407–416. https://www.jstor.org/stable/30054704

PEAKALL, R. & SMOUSE, P.E. 2006. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology. Notes. 6:288–295. https://doi.org/10.1111/j.1471-8286.2005.01155.x

PEAKALL, R. & SMOUSE, P.E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics. 28(19):2537–2539. doi: 10.1093/bioinformatics/bts460

POSADA, D. 2008. jModelTest: phylogenetic model averaging. Molecular Biology and Evolution. 25(7):1253–1266. doi: 10.1093/molbev/msn083.

PRESTI, F.T., GUEDES, N.M.R., ANTAS, P.T.Z. & MIYAKI, C.Y. 2015. Population Genetic Structure in Hyacinth Macaws (*Anodorhynchus hyacinthinus*) and Identification of the Probable Origin of Confiscated Individuals. Journal of Heredity. 106:491–502. doi:10.1093/jhered/esv038

RAMBAUT, A. 2014. FigTree v1.4.2, A Graphical Viewer of Phylogenetic Trees. Available from http://tree.bio.ed.ac.uk/software/figtree/

RAMOS-ONSINS, S.E. & ROZAS, J. 2003. Statistical Properties of New Neutrality Tests Against Population Growth. Molecular Biology and Evolution. 19(12):2092–100. DOI:10.1093/oxfordjournals.molbev.a004034

RIBAS, C.C., MOYLE, R.G., MIYAKI, C.Y. & CRACRAFT, J. 2007. The assembly of montane biotas: linking Andean tectonics and climatic oscillations to independent regimes of diversification in *Pionus* parrot. The Royal Society. 2742399–2408. http://doi.org/10.1098/rspb.2007.0613

RINGLER E. 2012. The use of cross-species testing of microsatellite markers and sibship analysis in ex situ population management. Conservation Genetics Resources. 4:815–819. DOI:10.1007/s12686-012-9642-5

ROCHA-MÉNDEZ, A., SÁNCHEZ-GONZÁLEZ, L.A., ARBALÁEZ-CORTÉS, E. & NAVARRO-SIGÜENZA, A.G. 2018. Phylogeography indicates incomplete genetic divergence among phenotypically differentiated montane forest populations of *Atlapetes albinucha* (Aves, Passerellidae). ZooKeys. 809(3):125–148. DOI:10.3897/zookeys.809.28743

RONQUIST, F. & HUELSENBECK, J.P. 2003. MRBAYES 3: Bayesian phylogenetic inferenceunder mixed models. Bioinformatics. 19:1572–1574. https://doi.org/10.1093/bioinformatics/btg180

ROVITO, S.M. & PARRA-OLEA, G. 2016. Neotropical Plethodontid Biogeography: Insights from Molecular Phylogenetics. Copeia. 104(1): 222–232. DOI: http://dx.doi.org/10.1643/CH-14-190

ROVITO, S.M., PARRA-OLEA, G., VAZQUEZ-ALMAZÁN, G.R., LUNA-REYES, R. & WAKE, D.B. 2012. Deep divergences and extensive phylogeographic structure in a clade of lowland tropical salamanders. BMC Evolutionary Biology. 12:255. DOI: 10.1186/1471-2148-12-255

ROZAS, J. & ROZAS, R. 1999. DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics. 15:174–175. DOI:10.1093/bioinformatics/15.2.174

RUSSELLO, M.A. & AMATO, G. 2004. A molecular phylogeny of Amazona: implications for Neotropical parrot biogeography, taxonomy, and conservation. Molecular Phylogenetics and Evolution. 30:421–437. https://doi.org/10.1016/S1055-7903(03)00192-1

RYDER, O.A. 1986. Species conservation and systematics: the dilemma of subspecies. Trends in Ecology and Evolution. 1:9–10. http://linkinghub.elsevier.com/retrieve/pii/0169534786900595

SALINAS-MELGOZA, A. & RENTON, K. 2008. Ficha técnica de *Pionus senilis.* En: Escalante-Pliego, P. (compilador). "Fichas sobre las especies de Aves incluidas en el Proyecto de Norma Oficial Mexicana PROY-NOM-ECOL-2000. Parte 2". Instituto de Biología, Universidad Nacional Autónoma de México. Bases de datos SNIB-CONABIO. Proyecto No. W042. México, D.F.

SÁNCHEZ-GONZÁLEZ, L.A., MORRONE, J.J. & NAVARRO-SIGÜENZA, A.G. 2008. Distributional patterns of the Neotropical humid montane forest avifaunas. Biological Journal of the Linnean Society. 94:175–194. DOI:10.1111/j.1095-8312.2008.00979.x

SANGER, F., NICKLEN, S. & COULSON, A.R. 1977. DNA sequencing with chain-terminating inihibitors. Proceedings of the National Academy of Sciencies of the United States America. 74(12):5463–5467.

SEMARNAT (2010). Norma Oficial Mexicana NOM-059-SEMARNAT-2010. Protección ambiental—Especies nativas de México de flora y fauna silvestres—Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio—Lista de especies en riesgo (77 663 pp.). Secretaría de Medio Ambiente y Recursos Naturales. http://biblioteca.semarnat.gob.mx/janium/Documentos/Ciga/agenda/DOFsr/DO2454.pdf.

ROSETE-VERGES, F.A., PÉREZ-DAMIÁN, J.L., VILLALOBOS-DELGADO, M., NAVARRO-SALAS, E.N., SALINAS-CHÁVEZ, E. & REMOND-NOA, R. 2014. El avance de la deforestación en México 1976-2007. Madera Bosques. 20(1):21–35. http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S1405-04712014000100003

STAMATAKIS, A. 2014. RAxML version 8: a tool for phylogenetic analysis andpost-analysis of large phylogenies. Computer applications in the biosciences: CABIOS. 30(9):1312–1313. DOI:10.1093/bioinformatics/btu033

SULLIVAN, J., ARELLANO, E. & ROGERS, D.S. 2000. Comparative Phylogeography of Mesoamerican Highland Rodents: Concerted versus Independent Response to Past Climatic Fluctuations. The american naturalist. 155(6):755–768. DOI:10.1086/303362

TAJIMA, F. (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics. 123, 585–595. DOI: 10.1093/genetics/123.3.585

TAMURA, K. & NEI, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution. 10:512–526.

TAMURA, K., PERERSON, D. & PETERSON, N. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution. 28(10):2731–2739. DOI:10.1093/molbev/msr121

TORRES-MORALES, L. 2019. Límites de distribución actual de *Sturnira hondurensis*. Revista Mexicana de Biodiversidad. 90: e902644. http://dx.doi.org/10.22201/ib.20078706e.2019.90.2644

WATTERSON, G.A. 1984. Lines of descent and the coalescent. Theoretical Population Biology. 26:77–92. https://doi.org/10.1016/0040-5809(84)90025-X

ZAMUDIO-BELTRÁN, L.E., ORNELAS, J.F., MALPICA, A. & HERNÁNDEZ-BAÑOS, B.E. 2020. Genetic and morphological differentiation among populations of the Rivoli's Hummingbird (*Eugenes fulgens*) species complex (Aves: Trochilidae). The Auk. 137:1–20. DOI: 10.1093/auk/ukaa032

Received: 24/06/2022
Accepted: 02/12/2022
Published online: 13/01/2023