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GENETIC DIVERSITY AND STRUCTURE OF THE WHITE-FRONTED PARROT (*AMAZONA ALBIFRONS)* IN MEXICO

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Abstract · We used mitochondrial DNA markers to assess the genetic diversity, genetic differentiation, and genealogical relationships among individuals of *Amazona albifrons* sampled across the Pacific Slope and the Yucatán Peninsula in México. In both regions, the species showed high genetic diversity, suggesting population expansion from a small effective population size. However, genealogical relationships revealed the presence of two genetic groups that probably went through different demographic events, one on the Yucatán Peninsula and the other on the Pacific Slope. Considering that the individuals of the Yucatán Peninsula formed a genetic group exclusive to the region because of isolation events and unique evolutionary history, we suggest the recognition of this group as an evolutionarily significant unit (ESU). In addition, considering the presence of unique haplotypes in the localities of Sinaloa and Michoacán, we recommended that conservation plans for *A. albifrons* focus on these two regions.

Resumen · Diversidad y estructura genética del Loro Frenti-blanco (*Amazona albifrons*) en México

En el presente estudio se examinaron la diversidad genética, la diferenciación genética y las relaciones genealógicas entre individuos de (*Amazona albifrons)* muestreados en la vertiente del Pacífico mexicano y en la Península de Yucatán, México, mediante el uso de marcadores de ADN mitocondrial. En ambas regiones de su distribución, la especie muestra una diversidad genética alta que sugiere una expansión de la población a partir de un tamaño efectivo de la población pequeño. Sin embargo, las relaciones genealógicas revelaron la presencia de dos grupos genéticos que probablemente pasaron por eventos demográficos distintos, uno en la Península de Yucatán y el otro en la vertiente del Pacífico. Considerando que los individuos de la Península de Yucatán formaron un grupo genético exclusivo de la región, debido a eventos de aislamiento y una historia evolutiva única, sugerimos su reconocimiento como Unidad Evolutivamente Significativa (ESU, por sus siglas en inglés). Además, considerando la presencia de haplotipos únicos en las localidades de Sinaloa y Michoacán, recomendamos que los planes de conservación se enfoquen en estas dos regiones.

Key words: *Genealogical relationships · Genetic groups · Haplotypes · Mitochondrial DNA · Psittacidae*

INTRODUCTION

The White-fronted Parrot (*Amazona albifrons,* Sparman 1788), is a medium-sized psittacine with generally green plumage, distributed in México and Central America. It is sexually dimorphic; in males, the alula and upper primary covert feathers are red, while the females lack any red on the wings and have a white patch on the forehead that is less extensive than that of males (Howell & Webb 1995). These parrots can be found in tropical dry or semiarid forests, in arid tropical scrublands, around agricultural areas, and even in pine forests (Forshaw 1989).

The historical range of *A. albifrons* was estimated to be approximately 438,793 km^2 (records up to 1995), including a continuous range along the Pacific Slope, from southern Sonora in northern México to Costa Rica. On the Atlantic side, it had a continuous distribution along the Gulf of México, from southern Veracruz to Tabasco and across the Yucatán Peninsula (Howell & Webb

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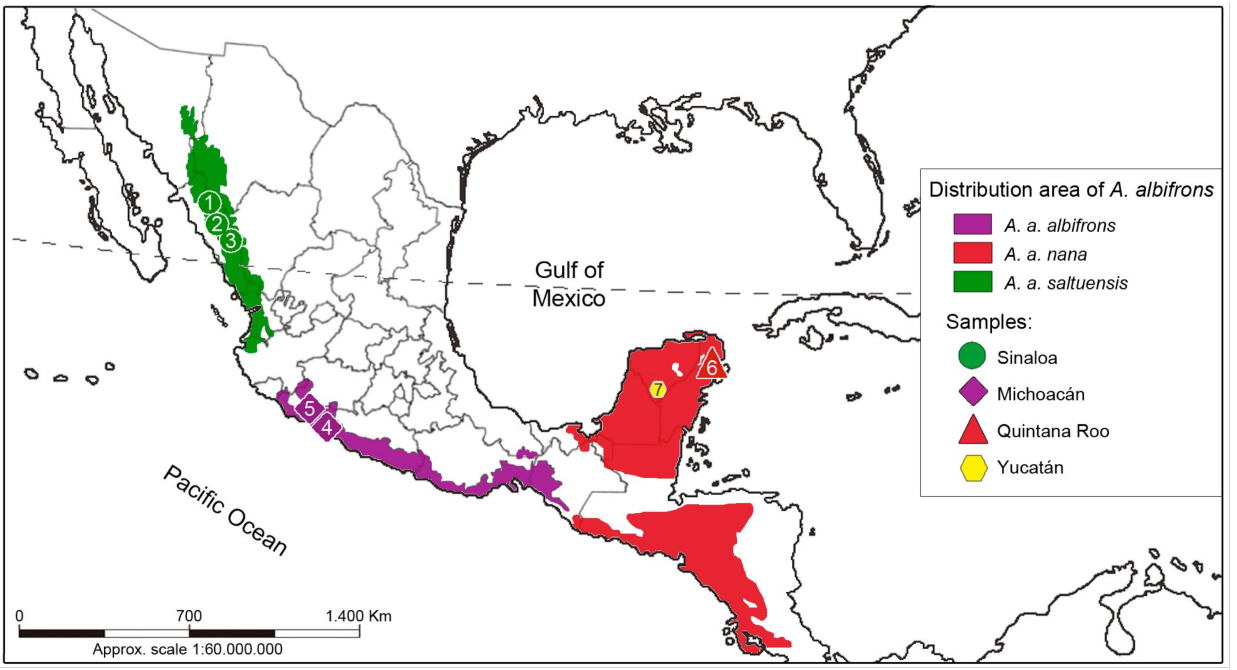

Figure 1. Distribution area and sampling locations of the White-fronted Parrot *Amazona albifrons*. The contemporary distribution area for subspecies is shown (Forshaw 2010, BLI 2015, Monterrubio-Rico et al. 2016). The numbers on the geometric figures indicate the collection locations included in the present study (see Table 1).

1995, Monterrubio-Rico et al. 2016). Based on its geographic distribution and some variation in its morphological traits, three subspecies of *A. albifrons* are recognized: *A. a. albifrons* (Sparman 1788), *A. a. saltuensis* (Nelson 1899) and *A. a. nana* (Miller 1905). The geographic distribution of *A. a. albifrons* ranges from Nayarit, in west-central México, south along the Pacific Slope to southern Chiapas and to southwestern Guatemala. Subspecies *A. a. saltuensis* is distributed across northwestern México in Sinaloa, western Durango, and southern Sonora. Finally, *A. a. nana* is distributed across southeastern Veracruz and northeastern Chiapas, in southern México, and from northwestern to southern Costa Rica (Figure 1) (Forshaw 1989).

Habitat loss and poaching for the pet trade are the major threats to parrots in the Neotropics (Berkunsky et al. 2017), and in México, both factors have been documented as the main ones causing the decline in psittacine populations (Collar & Juniper 1992, Cantú-Guzmán et al. 2007, Monterrubio-Rico et al. 2016). Because of exploitation by humans, species of the genus *Amazona* rank thirteenth among psittacines in the number of seizures at international customs checkpoints (UNODC 2016). In México, *A. albifrons* ranks second in terms of species suffering the greatest pressure from the illegal pet trade. It is estimated that 8,000 individuals are captured each year to supply the main domestic market (Cantú-Guzmán et al. 2007). Therefore, this species has been given special protection in the Official Mexican Norm NOM-059 and under Appendix II of CITES (DOF 2010, CITES 2022). Currently, the range of *A. albifrons* has contracted, with estimates indicating a 40% loss of range over approximately 18 years, from 438,793 km^2 in 1995 to 263,173 km^2 in 2013 (Monterrubio-Rico et al. 2016). The species distribution on the Mexican Pacific Slope is discontinuous since *A. albifrons* is not found in the states of Jalisco and Colima (Figure 1). Additionally, the study of the presence/absence of individuals per area unit and niche modeling shows that this species has a reduction in its distribution area in southern Sinaloa, Nayarit, Chiapas, Veracruz, and in the Yucatán Peninsula (Figure 1) (Marín-Togo et al. 2012, Monterrubio-Rico et al. 2016).

From the conservation perspective, recognition of subspecies-level differentiation is often used in the establishment of conservation strategies. With the support of molecular phylogeny and population genetics obtained from DNA sequences, it has become possible to document intraspecific evolutionary diversity and to obtain information about the historical and current processes at work in populations of a variety of organisms (Avise et al. 1987, Avise & Nelson 1989, Zink 2004, Johnson et al. 2005, Zink et al. 2005, Phillimore & Owens 2006, Pruett & Winker 2010). This kind of supporting information is fundamental to the design of conservation strategies focused on preserving the processes that allow a species to persist and evolve (Moritz 1994, 1995, 2002). The concept of evolutionarily significant units (ESUs) has been used in the management and conservation prioritization of intraspecific units and provides a phylogenetic framework for deciding which population unit is the most distinctive (Avise 2000). Molecular markers (particularly mtDNA) are useful for identifying ESUs in different species of birds, and in some cases, the analyses of molecular data identify groups that match a named subspecies (e.g., Hackett 1996, Barry & Tallmon 2010, Draheim et al. 2010, Russello et al. 2010, Wenner et al. 2012, Wu et al. 2012).

Despite the conservation vulnerability of *A. albifrons*, no molecular analyses have been performed to date to assess the diversity or genetic structure of the species. In this study, we used data from mitochondrial sequences to evaluate the genetic diversity and differentiation in this species, and to examine genealogical relationships among the subspecies of *A. albifrons* throughout its distribution in México. This genetic information can be useful in the design of conservation strategies

Table 1. Biological samples from individuals of White-fronted Parrot (*Amazona albifrons*) from this study and those reported in GenBank. Locality numbers are indicated on the map (Figure 1). List of accession numbers of sequences obtained in this study and those reported in GenBank.

Subspecies	Code	Locality	Map locality	12S	16S	COI	ND2	Source
A. a. saltuensis	Amal2005_11	Rancho Viejo, Mocorito, Sinaloa	1	–	–	OP297903	OP297914	This study
A. a. saltuensis	Amal2005_12	Rancho Viejo, Mocorito, Sinaloa	1	–	OP297891	–	OP297915	This study
A. a. saltuensis	Amal2005_13	Rancho Viejo, Mocorito, Sinaloa	1	–	OP297892	OP297904	OP297916	This study
A. a. saltuensis	Amal2005_24	Imala, Sinaloa	2	–	OP297893	–	–	This study
A. a. saltuensis	Amal2005_25	Imala, Sinaloa	2	–	–	–	OP297917	This study
A. a. saltuensis	Amal2005_30	Cosala, Sinaloa	3	–	OP297894	OP297905	OP297918	This study
A. a. saltuensis	Amal2005_31	Cosala, Sinaloa	3	–	OP297895	–	–	This study
A. a. saltuensis	Amal2005_34	Culiacan, Sinaloa	2	–	OP297896	–	–	This study
A. a. saltuensis	Amal2005_42	La Tasajera, Cosala, Sinaloa	3	–	OP297897	OP297906	OP297919	This study
A. a. saltuensis	Amal2005_44	La Tasajera, Cosala, Sinaloa	3	–	OP297898	–	–	This study
A. a. saltuensis	Amal2005_46	La Tasajera, Cosala, Sinaloa	3	OP297887	–	–	OP297920	This study
A. a. saltuensis	Amal2005_47	La Tasajera, Cosala, Sinaloa	3	–	OP297899	–	OP297921	This study
A. a. albifrons	Amal2006_89	Cuilala, Lázaro Cárdenas, Michoacán	4	OP297888	OP297900	OP297907	OP297922	This study
A. a. albifrons	Amal2006_90	Cuilala, Lázaro Cárdenas, Michoacán	4	OP297889	OP297901	OP297908	OP297923	This study
A. a. albifrons	Amal2007_15	Rancho los Pozos, Arteaga, Michoacán	5	OP297890	OP297902	OP297909	OP297924	This study
A. a. nana	Amal2012_01	Playa del Carmen, Quintana Roo (poached individual).	6	–	–	OP297910	OP297925	This study
A. a. nana	Amal2012_02	Playa del Carmen, Quintana Roo (poached individual).	6	–	–	OP297911	OP297926	This study
A. a. nana	Amal2012_03	Playa del Carmen, Quintana Roo (poached individual).	6	–	–	OP297912	OP297927	This study
A. a. nana	Amal2012_04	Playa del Carmen, Quintana Roo (poached individual).	6	–	–	OP297913	OP297928	This study
A. albifrons	Amsp	Yucatán	7	KU605663	KU605664	KU605665	–	GenBank
A. albifrons	Amalvo	Unknown	–	–	–	HQ629750	HQ629715	GenBank
A. a. albifrons	Amala	Central America	–	AY301331	AY01379	AY301427	–	GenBank
A. a. nana	Amaln	Central America	–	AY301332	AY301380	AY301428	–	GenBank
A. a. saltuensis	Amals	Central America	–	AY301333	AY301381	AY301429	–	GenBank
A. ventralis	Amve	–	–	KX925977	KX925977	KX925977	KX925977	GenBank

such as those based on identifying ESUs.

METHODS

Biological samples. Blood or feather samples from 19 *A. albifrons* individuals were obtained from nestlings in 2005, 2006, and 2007 without harming the birds. The geographic coordinates of each collection location were recorded. The samples came from specimens found in six locations in the north and center of the Pacific Slope of México (Sinaloa and Michoacán states) (Table 1 and Figure 1). In addition, through a donation, we obtained feather samples from poached parrots that were seized by Mexican authorities in 2009 in the state of Quintana Roo in the Yucatán Peninsula (Table 1 and Figure 1) and deposited in the Xaman Ha Aviary, located at Playa del Carmen, Quintana Roo, México. All samples were collected and preserved using the method described by Padilla-Jacobo et al. (2016) and were deposited in the wildlife samples collection at *Centro Multidisciplinario de Estudios en Biotecnología* (CMEB), at the Universidad Michoacana de San Nicolás de Hidalgo (UMSNH) in Morelia, Michoacán, México.

DNA extraction, PCR amplification, and marker sequencing. DNA extraction was performed using the phenol-free method of FitzSimmons (1997). We amplified fragments of the 12S (four individuals), 16S (13 individuals), COI (11 individuals), and ND2 genes (15 individuals) (Table 1). The 12S and 16S fragments were amplified using the primers and PCR conditions described by Miyaki et al. (1998). The COI fragment was amplified using the primers and PCR conditions described by Palumbi et al. (1991), and the ND2 fragment was amplified using the PCR conditions and primers described by Hackett (1996). The sequencing of both DNA strands was performed using the amplification primers and the dideoxy method (Sanger et al. 1977) at Macrogen (Rockville, MD, USA).

Sequence analysis. Sequence editing and alignment were carried out with Clustal W using BioEdit 7.09 (Thompson et al. 1994, Hall 1999). Analyses were carried out using sequences generated in this study, along with sequences available in GenBank-NCBI (https://www.ncbi.nlm.nih.gov/genbank/). The total number of sequences analyzed was as follows: 12S (8), 16S (17), COI (16), and ND2 (16) (see Table 1).

Table 2. Indices of genetic diversity in individuals of White-fronted Parrot (*Amazona albifrons*). N = number of sequences; Nt = number of characters; H = number of haplotypes; S = polymorphic sites; Hd = haplotype diversity; *Pi* = nucleotide diversity; Tajima´s *D* value and corresponding *P* values, not significant, *P* > 0.1.

Marker	N (this study/ GenBank-NCBI)	Nt	H	S	Hd	*Pi*	Tajima´s *D* (*P* value)
12S	8 (4/4)	370	3	2	0.4643 (+/–0.200)	0.00183 (+/–0.001)	–0.44794 (0.3)
16S	16 (12/4)	439	5	5	0.666 (+/–0. 113)	0.00315 (+/–0.002)	–0.7804 (0.23)
COI	16 (11/5)	532	7	7	0.850 (+/–0.059)	0.00346 (+/–0.002)	–0.44746 (0.38)
ND2	16 (15/1)	972	9	15	0.925 (+/–0.038)	0.00398 (+/–0.002)	–0.5665 (0.3)

Table 3. Pairwise genetic differentiation (F_{ST}) among groups of the White-fronted Parrot (*Amazona albifrons*).

	Michoacán	Sinaloa	Quintana Roo
Michoacán	–		
Sinaloa	0.17444	–	
Quintana Roo	0.69827	0.63294	–

Genetic diversity and differentiation. The number of haplotypes (H), polymorphic sites (S), the nucleotide (Pi) and haplotype (Hd) diversity, and Tajima's *D* (a neutrality test index) were estimated using DnaSP v.5.10 (Librado & Rozas 2009). To estimate the genetic differentiation among the three groups corresponding to the recognized distributions for the three subspecies (*A. a. albifrons* – Michoacán, *A. a. saltuensis* – Sinaloa, and *A. a. nana* – Quintana Roo) (Figure 1), we computed pairwise comparisons of F_{ST} values with 1000 permutations using ARLEQUIN v3.1 (Excoffier et al. 2005).

An analysis of molecular variance (AMOVA) (Excoffier et al. 2005) was carried out to detect the genetic structure in the pattern of genetic variation across samples. The analysis was performed with 10,000 permutations, and the samples were grouped as follows: (1) without a priori grouping and (2) samples grouped by sampling location (either Pacific Slope or Quintana Roo).

Haplotype relationships and genealogical analyses. Haplotype networks were built under the median-joining method with NETWORK v5 (Fluxus Technology Ltd. 2017). Genealogical relationship reconstructions were estimated under the maximum likelihood (ML) criterion and by means of Bayesian inference (BI), with RaxML (Stamatakis 2014) and Mr Bayes v3.1 (Ronquist & Huelsenbeck 2003) software, respectively. For each reconstruction method, we ran two sets of analyses: the first for each marker independently and the second for the concatenated sequences (16S + COI + ND2). Unique haplotypes were included in the data matrices. Sequences from the Hispaniolan Parrot, *Amazona ventralis* Müller et al. 1776, were included as an outgroup (Table 2). To determine the molecular evolution model that best describes the data, we used JModeltest v2.1.1 (Posada 2008) and carried out model selection using the corrected Akaike Information Criterion (AICc) (Alfaro & Huelsenbeck 2006). The best model of nucleotide substitution for 12S, 16S, and COI was HKY (Hasegawa et al. 1985), and for ND2 was TrN+I (Tamura & Nei 1993 + Invariant sites). The best model of nucleotide substitution for the concatenated sequences (16S + COI + ND2) was GTR+G+I (General Time Reversible + Gamma distribution of rates among sites + Invariant sites; Tavaré 1986). For the ML analysis, the branch-support values were estimated by means of bootstrap analysis with 500 replicates. Bayesian analyses were run with 10 x 10^6 generations using two Monte Carlo Markov Chains (MCMCs). The trees were sampled every 1,000 generations, and 10% (burn-in trees) were discarded to obtain a majority consensus tree. Posterior probabilities were calculated on trees sampled after burning. To view and edit the trees, we used the program FigTree v1.4.0 (Rambaut 2012).

RESULTS

Genetic diversity and differentiation. From the DNA samples of *A. albifrons*, we obtained sequences for the mitochondrial genes 12S, 16S, COI, and ND2. All sequences were deposited in GenBank-NCBI (see Table 1). To examine the genetic diversity of the samples, we used a data matrix that included the sequences obtained in this study and those reported in GenBank-NCBI for each marker. The markers ND2 and COI presented the highest number of polymorphic sites, while 12S and 16S presented the lowest number (Table 2). Haplotype diversity and nucleotide diversity values were compared to thresholds suggested by Grant and Bowen (1998): haplotype diversity values above 0.5 are considered high and those below this value are considered low, while nucleotide diversity values above 0.005 are high and those below this value are considered low. In our results, haplotype diversity (Hd) was high for 16S, COI and ND2 and was low for 12S, while nucleotide diversity (Pi) was low for all markers (Table 2).

Since we had a greater number of ND2 sequences (15) and more characters for this analysis (972 bp), we carried out the following analyses based on data for this gene alone. Analysis by pairwise genetic comparison (F_{ST}) revealed low to high genetic differentiation between groups (Table 3). The greatest genetic differentiation was found between the individuals from Michoacán and those from Quintana Roo (F_{ST} = 0.69827). Meanwhile, low genetic differentiation was observed between individuals from Michoacán and Sinaloa (F_{ST} = 0.17444). The

Table 4. Molecular variance analysis (AMOVA) for the White-fronted Parrot (*Amazona albifrons)*. A) without a priori defined groups, B) QuRo = Quintana Roo, Pacific = Pacific Slope.

Source of variation	df	Sum of squares	Variance components	Percentage of variation	Fixation index	*P*-value
A) Without *a priori* grouping						
Among populations	2	16.358	1.459	60.02		
Within populations	13	12.642	0.972	39.98	F_{ST} = 0.60015	0.0
Total	15	29	2.432	100		
B) QuRo / Pacific						
Among groups	1	14.855	1.921	63.72	F_{CT} = 0.63718	0.315
Among populations within groups	1	1.504	0.121	4.04	F_{SC} = 0.11128	0.001
Within populations	13	12.642	0.972	32.24	F_{ST} = 0.67755	0.0
Total	15	29	3.015	100		

AMOVA analysis showed that without groups defined a priori, 60% of the genetic variation is explained by differences between populations and 39.9% by differences within populations, suggesting that the genetic variation is geographically structured (Table 4A). When the data were analyzed defining two groups, one from the Pacific Slope (Michoacán and Sinaloa) and the other from Quintana Roo, the results indicated that most of the variation (63.72%) was explained by differences between groups. Differences within the groups represented 32.24% of the variation. The F_{CT} fixation index showed a high value (F_{CT} = 0. 63718), which indicates genetic differentiation between the Pacific Slope group and Quintana Roo (Table 4B).

Genealogical relationships. Consistently, the haplotype networks with the 16S, COI, and ND2 markers, revealed a separation into two genetic groups, one integrated with haplotypes identified in individuals from the Yucatán Peninsula and the other with haplotypes identified in individuals from Pacific Slope (Figure 2). Furthermore, the haplotype networks of all markers revealed a dominant haplotype detected in individuals from each region, while other individuals showed less frequent haplotypes derived from the dominant haplotype in a star-like topology; individuals with these haplotypes were associated with specific localities within regions (Figure 2). Interestingly, all haplotype networks revealed a dominant haplotype shared by individuals from Michoacán and Sinaloa, indicating the presence of a central to northward distributed genetic group on the Pacific Slope (Figure 2).

The genealogical tree with the 12S marker showed a sin-

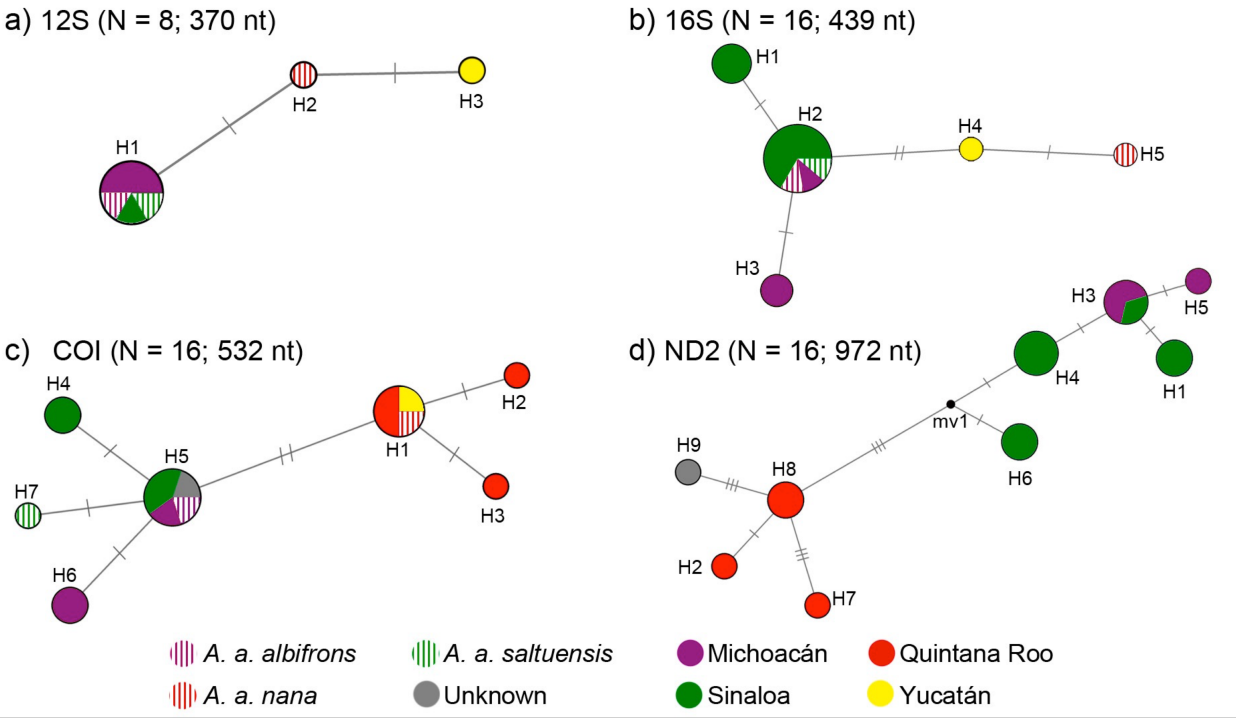

Figure 2. Haplotype networks of the White-fronted Parrot (*Amazona albifrons*). Median-joining network based on; a) 12S, b) 16S, c) COI, and d) ND2. The size of the circles is proportional to the frequency of each haplotype; transversal lines on the branches represent the mutations. N = number of sequences, nt = nucleotides.

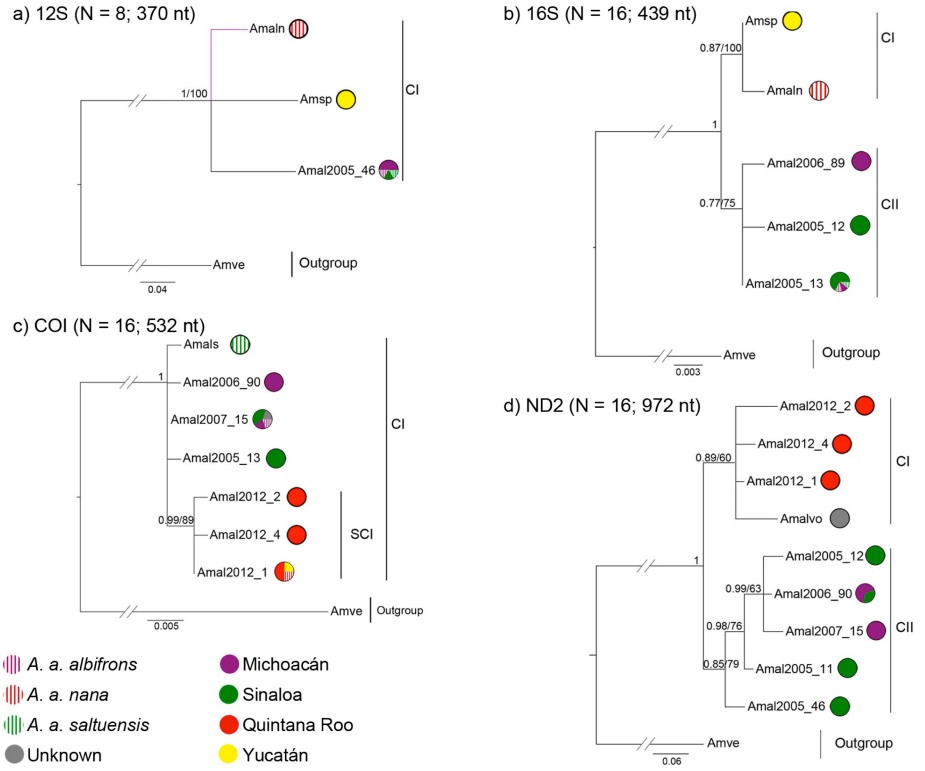

Figure 3. Genealogical relationships of the White-fronted Parrot (*Amazona albifrons*) obtained using BI and ML analyses. Consensus tree were built with a) 12S, b) 16S, c) COI, and d) ND2. The species *Amazona ventralis* was used as an outgroup. For abbreviations of each sample see Table 1. Values at the nodes represent posterior probabilities and bootstrap values (PP/BP). The scale bar under the tree is a reference to the length of the branches (proportional to the amount of evolutionary change). N = number of sequences, nt = nucleotides.

gle clade with all three haplotypes in a polytomy (Figure 3a), whereas in the genealogical trees with the 16S and ND2 markers, the haplotypes were separated into two clades (CI and CII) (Figure 3b). The CI clade included haplotypes identified in individuals from the Yucatán Peninsula and the CII clade included haplotypes identified in individuals from the Pacific Slope.

Regarding the ancestor-descendant relationships with COI marker, in the consensus tree, the haplotypes of the individuals from Michoacán and Sinaloa come together in a soft polytomy, consistent with their close relationship observed in the haplotype network (Figure 3c). Furthermore, haplotypes

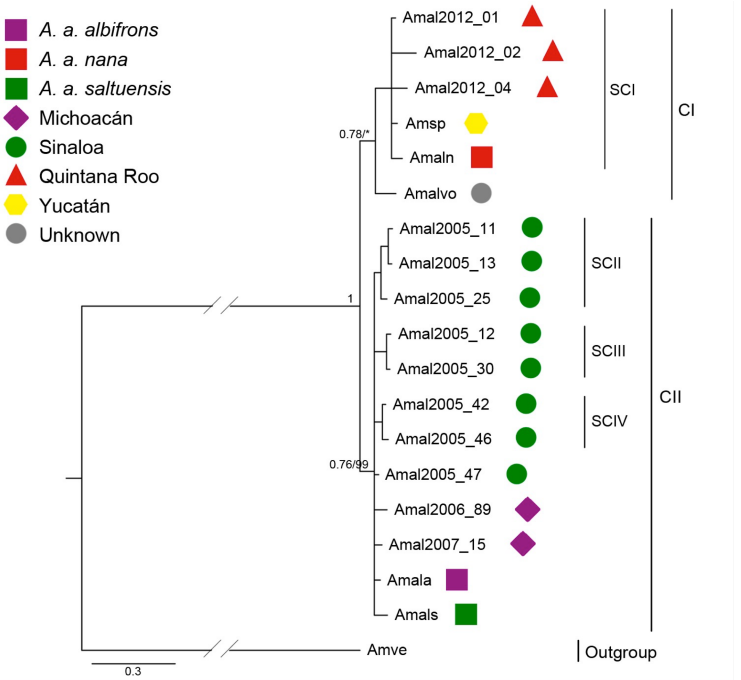

Figure 4. Genealogical relationships of the White-fronted Parrot *Amazona albifrons* obtained with Bayesian inference and maximum likelihood analyses. Consensus tree built with 19 samples and 1943 characters of concatenated sequences from tree mitochondrial genes (16S + COI + ND2). The species *Amazona ventralis* was considered as an outgroup. For abbreviations of each sample see Table 1. Values at the nodes represent posterior probabilities and bootstrap values (PP/BP); (*) values < 50%. The scale bar under the tree is a reference for the length of the branches (proportional to the amount of evolutionary change).

from individuals from the Yucatán Peninsula were included in a subclade (SCI) with high support values at the node (Figure 3c).

In addition to the genealogical analysis for each marker, we built a consensus tree under BI and ML with the dataset of concatenated sequences (16S + COI + ND2) using 19 samples and 1943 characters. Individuals with repeated haplotypes and with 77.4% missing data were excluded. The data matrix included 13 individuals with missing data and six with complete sequences. This implies that the tree was estimated with 68.4% of individuals missing data. The percentage of missing data was as follows; 22.6% in five individuals, 27.4% in two, and 50% in six. In the consensus tree topology, two clades (CI and CII) were detected (Figure 4). Although relationships within clades were not well supported, they were consistently found in both analyses (BI and ML). The CI clade included a haplotype from an individual of unknown origin and haplotypes from individuals from the Yucatán Peninsula grouped into a subclade (SC1) with soft polytomy (Figure 4). However, the consensus trees of the 16S, COI, and ND2 markers did not show a similar subclade (Figure 2). Haplotypes from individuals from the Pacific Slope were included in the CII clade. This clade also showed a soft polytomy; however, it revealed three subclades (SCII, SCII, and SCIV) composed of haplotypes from individuals from Sinaloa (Figure 4), consistent with what was observed in the consensus tree with the ND2 marker (Figure 3d). Although missing data introduced some uncertainty, the topology of the consensus tree using the concatenated sequences was consistent with that of the consensus trees of the individual 16S and ND2 markers.

DISCUSSION

Genetic diversity and differentiation. The set of individuals sampled in our study showed high haplotype diversity and low nucleotide diversity for the 16S, COI, and ND2 markers (Table 2). These results suggest a growth from a small effective population size, perhaps due to a bottleneck followed by rapid expansion, during which growth promotes the retention of new mutations (Grant & Bowen 1998). Similarly, although Tajima´s *D* values were not significant (P = 0.1), they were all negative, possibly indicating an excess of rare haplotypes, which is consistent with the hypothesis of a recent expansion from a small effective population size (Hamilton 2011, Hedrick 2011) (Table 2).

Pairwise genetic comparison revealed low genetic differentiation (F_{ST} = 0.17444) between the group of individuals from Michoacán and the group from Sinaloa, indicating that they belong to the same genetic group. On the other hand, the high genetic differentiation observed among the group of individuals from Quintana Roo and the groups from Michoacán and Sinaloa (F_{ST} = 0.69827 and 0.63294, respectively) indicate the presence of two genetic groups in the species: one on the Pacific Slope and another in Quintana Roo (Table 3). This result was corroborated by the results of the AMOVA, which showed that the genetic variation and the F_{CT} fixation index between the Pacific Slope group of and the Quintana Roo group were high (Table 4).

Genealogical relationships. Except for the 12S haplotype network, each of the dominant haplotypes detected was related by one to three mutational steps to two, or three haplotypes (Figure 2). Although sample size limitations do not allow us to draw strong conclusions, the topologies associated with each region (Pacific Slope and the Yucatán Peninsula) suggest a trend toward star patterns, which are typical of a population that has expanded recently. Furthermore, this recent expansion is consistent with the high Hd and low Pi detected. In agreement with Avise (2009), the common and widespread haplotype in each region is probably the ancestral condition

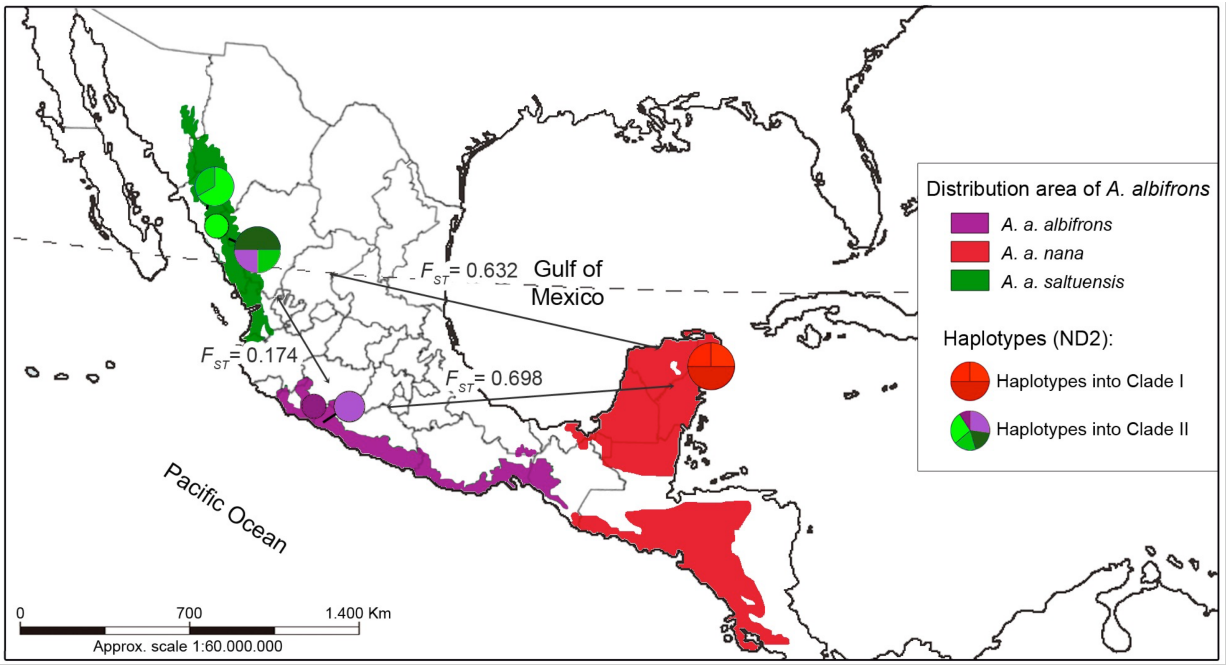

Figure 5. Geographic distribution of ND2 haplotypes of the White-fronted Parrot (*Amazona albifrons*). The circles indicate the frequency of occurrence in each locality; colors within circles indicate different haplotypes identified in the present analysis. Numbers accompanying arrows are F_{ST} values among groups.

from which the rare haplotypes were recently derived by separate mutations. However, in the ND2 haplotype network, the topology of the Pacific Slope haplotype group does not show a star pattern, suggesting a lack of rare haplotypes (Hedrick 2011).

In addition, the COI haplotype network showed a dominant haplotype (H1) shared by individuals from the Yucatán Peninsula and an individual of unknown origin (Figure 2c). In the ND2 haplotype network, the haplotype of this individual of unknown origin (H9) showed differentiation of three mutational steps from the dominant haplotype (H8) from Quintana Roo; thus, it can be assigned to the Yucatán Peninsula.

Haplotype networks of all markers revealed a dominant haplotype shared by individuals distributed in the north and center of the Pacific Slope (Sinaloa and Michoacán) (Figure 2). In addition, the 16S, COI, and ND2 networks show that one haplotype from Michoacán and one from Sinaloa, were consistently differentiated by a single mutation from the dominant haplotype (Figures 2b, 2c, and 2d). Considering the neotropical origin of the species, it probably expanded its range from the south to the north of the Pacific Slope, establishing and diversifying in each locality. A similar pattern was found in the Orange-fronted Parakeet (*Eupsittula canicularis*), which also has a distribution on the Pacific Slope (Padilla-Jacobo et al. 2018b). The distribution of dominant haplotypes and their derivatives in the Mexican Pacific Slope may indicate that diversification processes occurred in specific areas after the arrival and establishment of the populations (Padilla-Jacobo et al. 2018b).

The genealogical analysis also showed a separation of haplotypes into two genetic groups: one in the Yucatán Peninsula and the other on the Pacific Slope. The consensus trees for 16S, ND2, and the concatenated sequences (16S + COI + ND2) were consistent in showing that individuals from the center and north of the Pacific Slope (Michoacán and Sinaloa) form one clade (CII), while those from Yucatán Peninsula (Quintana Roo and Yucatán) are grouped into a separate clade (CI) (Figures 3b, 3d, and 4). The result from the genealogic analyses is supported by the F_{ST} values, with the lowest degree of differentiation found between individuals from Michoacán and Sinaloa, and the greatest differentiation detected between individuals from Michoacán and Sinaloa and those from Quintana Roo (Table 3, Figure 5). In addition, the AMOVA showed a marked genetic differentiation between the Pacific Slope group of and the Quintana Roo group (Table 4). However, for future analyses, we recommend expanding the sampling to the entire distribution range of the species and increasing the sample size, particularly including individuals from southern México and Central America.

In our study, we detected the presence of individuals of *A. albifrons* that share the same haplotype, both in the north and in the center of the Pacific Slope, which does not coincide with the designation of two subspecies for this region. Analyses of genealogical relationships and genetic structure allow the identification of genetic lineages that do not necessarily coincide with previously described subspecies. Some examples have been reported by Ball & Avise (1992) in Downy Woodpeckers (*Dryobates pubescens*), Mourning Doves (*Zenaida macroura*), Brown-headed Cowbirds (*Molothrus ater*), and Song Sparrows (*Melospiza melodia*); by Zink et al. (2000) in California Gnatcatchers (*Polioptila californica*); by Draheim et al. (2010) in Least Terns (*Sternula antillarum*); by Wu et al. (2012) in Black-throated Laughing Thrushes (*Pterorhinus chinensis*)**;** by Padilla-Jacobo et al. (2018a) in Wild Turkeys (*Meleagris gallopavo*); and by Padilla-Jacobo et al. (2018b) in Orange-fronted Parakeets (*E. canicularis*). In a genealogical tree that describes intraspecific relationships, several genetic lineages can be observed as subclades. Studies describing the phylogeographic histories of birds that inhabit the tropical regions of México are scarce, and even more scarce are works that describe patterns in birds that inhabit the tropical dry forests of the region. For the Mexican Pacific Slope, a pattern similar to the genealogical relationships found in *A. albifrons* has recently been reported for some species. In *E. canicularis*, a widely distributed haplotype shared by individuals from localities of Sinaloa, Nayarit, and Michoacán was detected, as were star patterns indicating a recent expansion (Padilla-Jacobo et al. 2018b). In the Military Macaw (*Ara militaris*), at least two genetic lineages were detected using mitochondrial and nuclear (microsatellites) DNA in the subspecies *A. m. mexicanus*, which represents two clades associated with two areas of its geographic distribution (Eberhard et al. 2015, Rivera-Ortíz et al. 2016). In the Streak-backed Oriole (*Icterus pustulatus*) a genetic group encompassing individuals from Sinaloa to Guerrero has been reported (Cortés-Rodríguez et al. 2008a).

Conservation implications. In México, the range of *A. albifrons* has been reduced by approximately 40% over the last 18 years (Monterrubio-Rico et al. 2016) and only 11 % of it is protected; consequently the short- medium-term future of the species is uncertain. Under these circumstances, the genetic differentiation that we identified must be recognized and considered in the design of conservation strategies. Our results support the designation of the Yucatán Peninsula population as an evolutionarily significant unit. While the Pacific Slope populations are genetic groups distinct from the Yucatán Peninsula birds, genetic evidence supporting further subdivision (e.g., separate ESUs from those of Sinaloa and Michoacán) is not as strong. In addition, the relationships among the haplotypes identified with 16S, COI, and ND2 on the Pacific Slope populations suggest an *in situ* diversification, a pattern observed in other species of birds in the tropical forests of Mexico.

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