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Article

Genetic Diversity and Genealogical Relationships of *Anolis nebulosus* and *Anolis microlepidotus*

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Abstract

Clouded Anole (*Anolis nebulosus*) and Guerreran Oak Anole (*Anolis microlepidotus*), there are two endemic species of Mexico. They can be found in tropical deciduous forest, semi-evergreen forest, xeric scrub, and pine-oak forests. It has been reported that *Anolis* species present a rapid adaptation to their habitat, which occurs in a few generations, and episodes of natural selection that lead to evolution on a contemporary time scale have also been observed. The aim of this study was to evaluate the genetic diversity and the genealogical relationships of groups of *A. nebulosus* and *A. microlepidotus* individuals. The study was carried out on specimens collected from locations in central Mexico, through the markers of 16S ribosomal RNA (16S rRNA). Both species showed a high haplotype and nucleotide diversity, suggesting a large and stable population with an ancient history. However, low haplotype and nucleotide diversity were observed in both species, alongside high genetic differentiation among groups of individuals from different localities; this suggest low vagility and supports the proposal to consider multiple phylogeographic lineages as evolutionarily significant units.

Keywords: *Anolis*; genetic diversity; genealogical relationships; phylogenetic lineages

1. Introduction

Anoles are a highly diverse group of vertebrates made of 429 species that make up the largest clade of terrestrial vertebrates [1]. With a range that extends from the northern half of South America, through Central America and until North America [2]. Anoles are typical lizards with two main distinguishing features, the possession of dewlap and subdigital pads on their toes. The dewlap is an extensible and often brightly colored structure located on the throat, it is used for communication in a variety of contexts, including courtship and intrasexual encounters [2]. The subdigital toe pads are composed of several laterally expanded scales called lamellae, which are covered by microscopic setae [2].

In Mexico, anoles have a wide distribution, and they are relatively abundant in the localities where they are found. For this country between 54 and 60 species of *Anolis* are recognized, of which 37 are endemic [3,4]. Twenty-seven species are protected by law, of which, 25 are endemic and are found in the Official Mexican Norm NOM-059, under the categories of special protection or threatened [5]. Most of *Anolis* species in Mexico belong to the *Norups auratus* Species Group, and there are representatives of the groups, *Norups valencienni*, *Norops sagrei*, *Anolis carolinensis*, and *Ctenonotus cristatellus* (proposed by Nicholson et al. [1,6]). However, due to the microenvironmental complexity that occurs in Mexico, several authors mention that species diversity and endemism are underestimated at the regional level and assert that there are still more undescribed anole species [7-11]. Therefore, it

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is important to undertake regional efforts that contribute to the knowledge of biological diversity at all levels, genetic, species, and ecosystem diversity.

Within the *Norups auratus* Species Group [1,6], genetic information has been obtained from different *Anolis* species for various purposes. Some explore genomic variation and phenotypic diversity [12-14], but most seek to establish phylogenetic relationships. This type of effort is reflected in publications where phylogenetic relationships between different groups or between different species within groups are proposed [7,15,16,17-21], or as a complement in the description of new species [9,11,22,23].

In conservation genetics, molecular characters, are useful for formulating conservation proposal, and in recent decades, an increasing number of articles have been published for many taxa. For anoles distributed in North America, despite their utility, there is a notable lack of studies using molecular data for conservation purposes. Its exclusion from conservation plans, biases our understanding of the underlying processes governing global vertebrate biodiversity. In this sense, Clouded Anole (*Anolis nebulosus*) and Guerreran Oak Anole (*Anolis microlepidotus*), there are two endemic species whose genetic diversity and genealogical relationships are unknown.

A. nebulosus is distinguished by its small size (snout-vent length of 43.0 mm in males and 39.5 mm in females), in males the dewlap is pale orange yellow with oblique reddish Pratt lines and between 8-9 horizontal sternal rows on throat (cream white), in female the dewlap is very small [9]. This species was the first described Mexican anole and is endemic to western Mexico, with a wide distribution along the Pacific Slope from the Mexican states of Sinaloa and Chihuahua to Oaxaca [22]. It can be found from sea level to 2,100 meters above sea level (masl), in habitats such as tropical deciduous forest, semi-evergreen forest, xeric scrub and pine-oak forests, mangrove swamps and disturbed areas [24]. It has been proposed that the species may be made up of a complex of several species, so studies are recommended to clarify the taxonomy of this endemic species [9].

A. microlepidotus is a small species with a maximum recorded snout-vent length of 46.5 mm in males and 44.0 mm in females, the males are distinguished by having a dewlap dull orange yellow with paler semicircular streaks and blotches [9]. The species is endemic to Mexico, with a distribution in the states of Guerrero, Puebla an Oaxaca [4,9]. Can be found at intermediate elevations, between 1245 and 1900 masl, in different habitats, in dry forest, mesic forest edge, in areas with sparse vegetation or in the edge of a small village [9].

Species conservation programs require the assessment of intraspecific diversity levels; therefore, the analysis of genetic diversity and the genealogical relationships of *A. nebulosus* and *A. microlepidotus* constitutes an initial contribution to the future understanding of the ecological and evolutionary processes of these endemic species of Mexico. In this study, we report the genetic diversity and explore genealogical relationships of a group of individuals of *A. nebulosus* and a group of *A. microlepidotus* from central Mexico.

2. Materials and Methods

2.1. Biological Samples

A total of 33 samples were used in the analyses: 17 belong to *A. nebulosus* and 16 to *A. microlepidotus*. The samples were collected in central Mexico: five in El Tuito (Jalisco), nine in Huetamo/Chihuero (Michoacan), 12 in La Tzararacua (Michoacan), and seven from an unknown location, but within the distribution area that corresponds to the state of Michoacan (Figure 1, Table 1). The samples from La Tzararacua and those from unknown locality are complete specimens preserved in formaldehyde, belonging to the Herpetology collection of the Faculty of Biology at the Universidad Michoacana de San Nicolas de Hidalgo.

In addition, tissue samples were collected from *Anolis* tails in the locations of El Tuito, Huetamo/Chihuero in 2015, 2018, and 2019 (Figure 1, Table 1). Tail samples were taken for each individual and placed in 2 mL vials with 0.5 mL of storage and lysis buffer (100 mM Tris pH 8.0, 100 mM EDTA pH 8.0, 10 mM NaCl and 2% SDS), saturated with salt-DMSO for tissue samples [26].

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Then, the samples were stored at room temperature during transport and subsequently stored at 4 °C until analysis. The samples were deposited in the biological sample collection of the laboratory of Biologia y Evolucion Molecular de Eucariotas, Centro Multidisciplinario de Estudios en Biotecnologia, FMVZ, Universidad Michoacana de San Nicolas de Hidalgo.

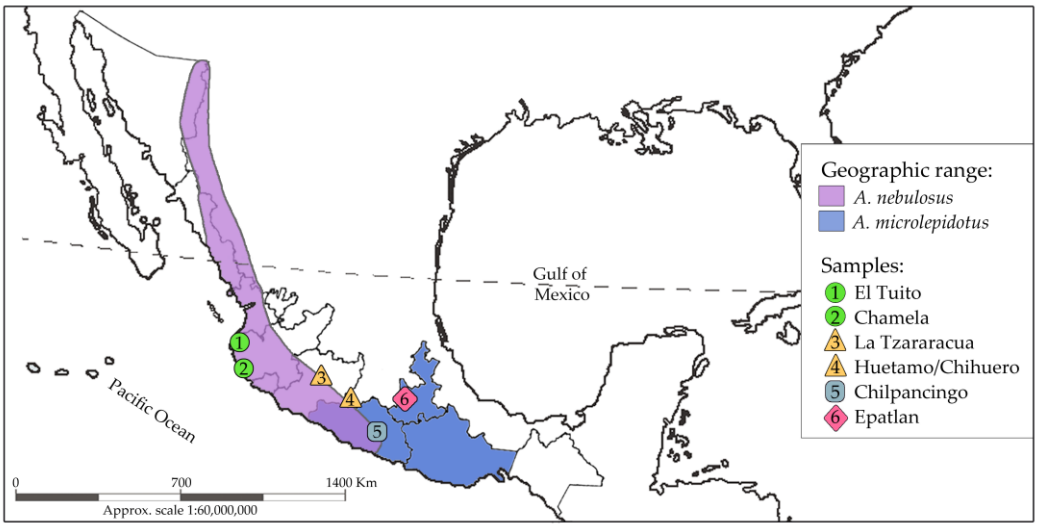


Figure 1. Map of geographic range and sampling locations of Clouded Anole (*Anolis nebulosus*) and Guerreran Oak Anole (*Anolis microlepidotus*) [4,9,25].

Table 1. Biological samples of Clouded Anole (*Anolis nebulosus*) and Guerreran Oak Anole (*Anolis microlepidotus*) from this study. Locality numbers are indicated on the map (Figure 1).

Species	Individual key in this study	Locality/Source (access number)	Locality number on the map
Anolis nebulosus	Anne_2014_J01	El Tuito, Jalisco	1
	Anne_2014_J02	El Tuito, Jalisco	1
	Anne_2014_J03	El Tuito, Jalisco	1
	Anne_2018_M05	Huetamo, Michoacan	4
	Anne_FBH-483	Unknown	-
	Anne_FBH-589	La Tzararacua, Michoacan	3
	Anne_FBH-598	La Tzararacua, Michoacan	3
	Anne_FBH-599	La Tzararacua, Michoacan	3
	Anne_FBH-603	La Tzararacua, Michoacan	3
	Anne_FBH-606	La Tzararacua, Michoacan	3
	Anne_FBH-610	La Tzararacua, Michoacan	3
	Anne_FBH-611	La Tzararacua, Michoacan	3
	Anne_FBH-612	La Tzararacua, Michoacan	3
	Anne_FBH-616	La Tzararacua, Michoacan	3
	Anne_FBH-626	La Tzararacua, Michoacan	3
	Anne_FBH-628	La Tzararacua, Michoacan	3
	Anne_FBH-659	La Tzararacua, Michoacan	3
	Anne_NCBI_218	Chamela, Jalisco/NCBI (KJ606218)	2
	Anne_NCBI_219	Chamela, Jalisco/NCBI (KJ606219)	2
	Anne_NCBI_220	Chamela, Jalisco/NCBI (KJ606220)	2
	Anne_NCBI_402	Chilpancingo, Guerrero/NCBI (MK621402)	5
	Anne_NCBI_404	Chilpancingo, Guerrero/NCBI (MK621404)	5

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A. microlepidotus	Anmi_2015_J01	El Tuito, Jalisco	1
	Anmi_2015_JD	El Tuito, Jalisco	1
	Anmi_2018_C02	Huetamo, Michoacan	4
	Anmi_2018_C03	Huetamo, Michoacan	4
	Anmi_2018_C04	Huetamo, Michoacan	4
	Anmi_2018_C06	Huetamo, Michoacan	4
	Anmi_2018_C08	Huetamo, Michoacan	4
	Anmi_2018_D01	Huetamo, Michoacan	4
	Anmi_2019-02	Chihuero, Michoacan	4
	Anmi_2019-03	Chihuero, Michoacan	4
	Anmi_FBH-0042	Unknown	-
	Anmi_FBH-0144	Unknown	-
	Anmi_FBH-0388	Unknown	-
	Anmi_FBH-0397	Unknown	-
	Anmi_FBH-0398	Unknown	-
	Anmi_FBH-0400	Unknown	-
	Anmi_NCBI_242	Epatlan, Puebla/NCBI (KP178242)	6
	Anmi_NCBI_243	Epatlan, Puebla/NCBI (KP178243)	6
	Anmi_NCBI_244	Epatlan, Puebla/NCBI (KP178244)	6
	Anmi_NCBI_245	Epatlan, Puebla/NCBI (KP178245)	6
	Anmi_NCBI_272	Chilpancingo, Guerrero/NCBI (KP178272)	5

2.2. DNA Extraction, Amplification and Sequencing

DNA was extracted using the phenol-free method described by FitzSimmons [27]. We amplified the DNA mitochondrial fragments of RNAr16S (16S) using the primers reported by Sumida et al. [28]. The PCR reactions for amplification of 16S were performed in a total volume of 25 µl as follows: 20 mM Tris-HCl pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$, 200 mM of each dNTP, 10 pmol of each oligonucleotide, 1.5 U Platinum Taq polymerase (Invitrogen, Grand Island, NY, USA), and 50 ng of DNA. The reaction mixtures were placed in a thermocycler (Gene Amp 2700; Applied Biosystems, Foster City, CA, USA) under the following conditions: 94 °C for 1 min; followed by 30 cycles of 95 °C for 45 s, 55 °C for 40 s, and 72 °C for 40 s; and a final extension at 72 °C for 7 min.

The sequencing of both DNA strands was performed using the amplification primers and the dideoxy method [29], and the service was provided by Macrogen (Rockville, MD, USA).

2.3. Genetic Diversity and Differentiation

Sequence editing, alignments, and the construction of data matrices were performed using Sequencher v.4.1 (Gene Codes Corporation, Ann Arbor, MI, USA) and PhyDE [30]. Two data matrices were constructed: one containing 17 sequences obtained in this study for *A. nebulosus*, supplemented with five sequences from GenBank-NCBI database (n = 22); and the other containing 16 sequences obtained for *A. microlepidotus*, supplemented with five sequences from GenBank-NCBI database for the species (n = 21). For both matrices, the number of haplotypes (H), polymorphic sites (S), the nucleotide (Pi) and haplotype diversity were estimated using ARLEQUIN v3.5 [31]. In addition, to estimate the genetic differentiation between individuals of both species, we computed pairwise comparisons of F_{ST} values with 1000 permutations using ARLEQUIN v3.5 [31].

2.4. Genealogical Relationships

Haplotype networks were built for both species to examine the haplotype frequencies and genealogical relationships among individuals. Sequences from each species were analyzed separately with NETWORK v10.2.0.0 software [32] under the median-joining method. To build genealogical trees two data matrices were analyzed as follows: for *A. nebulosus*, the alignment included 11 unique haplotypes and 376 characters, five haplotypes were obtained in the present study and six were from

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the GenBank-NCBI database, including *Anolis auratus* as outgroup (Access: MH140489). For *A. microlepidotus*, the data matrix included five unique haplotypes and 318 characters, of which two were obtained in the present study and tree were from the GenBank-NCBI database, including *Anolis liogaster* as outgroup (No. access: KP178257).

Genealogic trees were performed under maximum likelihood (ML) and Bayesian Inference (BI) using RAxML v7.8 [33] and MrBayes v3.2.6 [34] software, respectively. Molecular evolution models were estimated using jModelTest v2.1.10 [35] and selected using the corrected Akaike Information Criterion (AICc) [36]. The best model for *A. nebulosus* matrix was TIM2+G (Transition Model-Posada 2003 + Gamma distribution). For *A. microlepidotus* was TPM2uf (Kimura 1981, unequal base frequencies). The branch-support values were estimated by bootstrap analysis (BP) of 500 replicates for ML analyses, and by posterior probabilities (PP) for BI. MrBayes runs were performed using the following parameters: four independent runs for each of four chains (one cold chain and three hot chains) for 10 million generations, sampling one tree every 1,000 generations. Trees and parameters were summarized after discarding 25% of the data (burn-in). The remaining trees were summarized as a majority consensus tree and visualized using FigTree v1.4.4 [37].

3. Results

3.1. Genetic Diversity and Differentiation

In this study, 33 sequences were obtained and deposited in GenBank-NCBI (Access numbers: QB131700-QB131716 and QB131721-QB131736). Despite the sample size, some interesting data were retrieved from the analysis. For *A. nebulosus*, the total group of individuals analyzed (n = 22) showed a high haplotype and nucleotide diversity with 10 haplotypes and 39 polymorphic sites (Table 2). However, an analysis of the individuals for each locality showed low diversity within the groups, suggesting high diversity between groups of different localities. Thus, in the three individuals from El Tuito (Jalisco) one haplotype was detected, while in the 12 individuals from La Tzararacua (Michoacan) two haplotypes with a polymorphic site were identified (Table 2). The five sequences from the database (Chamela n = 3 and Chilpancingo n = 2) represented five haplotypes with two and one polymorphic sites between them (Table 2). These reports consider unique haplotypes, without specifying the number of individuals with each haplotype.

Table 2. Indices of genetic diversity in individuals of *Anolis nebulosus*. N = number of sequences; Nt = number of characters; H = number of haplotypes; S = polymorphic sites; Hd = haplotype diversity; *Pi* = nucleotide diversity; Tajima's *D* significance, not significant, $P > 0.1$.

	Group		N	Nt	H	S	Hd	Pi	Tajima's *D* (*P* value)
A. nebulosus	Jalisco	El Tuito	3	376	1	0	0.0 (+/-0.0)	0.0 (+/-0.0)	0.0 (0.0)
		Chamela	3	376	3	2	1 (+/-0.2722)	0.0035 (+/-0.0036)	0.0 (0.941)
	Michoacan	La Tzararacua	12	376	2	1	0.303 (+/-0.1475)	0.0008 (+/-0.0010)	-0.1949 (0.327)
		Huetamo	1	376	1	0	0.0 (+/-0.0)	0.0 (+/-0.0)	0.0 (0.0)
		Unknown	1	376	1	0	0.0 (+/-0.0)	0.0 (+/-0.0)	0.0 (0.0)
	Guerrero	Chilpancingo	2	376	2	1	1.0 (+/-0.5)	0.0026 (+/-0.0037)	0.0 (0.0)
	Total		22	376	10	39	0.7879 (+/-0.0834)	0.0256 (+/-0.0136)	-0.3848 (0.38)

On the other hand, we analyzed a total of 21 samples of *A. microlepidotus*, which showed four haplotypes with 37 polymorphic sites. These samples show high genetic and nucleotide diversity (Table 3).

When analyzing the samples by locality, a low or null diversity haplotypes and nucleotides was observed. The samples from El Tuito (Jalisco) showed one haplotype, whereas those from Huetamo/Chihuero (Michoacan) exhibit two distinct haplotypes, and those of unknown origin showed two haplotypes. However, in general, for both species, the results should be taken with caution due to the sample size.

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Table 3. Indices of genetic diversity in individuals of *Anolis microlepidotus*. N = number of sequences; Nt = number of characters; H = number of haplotypes; S = polymorphic sites; Hd = haplotype diversity; *Pi* = nucleotide diversity; Tajima's *D* significance, not significant, $P > 0.1$.

	Group		N	Nt	H	S	Hd	Pi	Tajima's *D* (*P* value)
A. microlepidotus	Jalisco	El Tuito	2	318	1	0	0.0 (+/-0.0)	0.0 (+/-0.0)	0.0 (0.0)
	Michoacan	Huetamo/Chihuero	8	318	2	1	0.4286 (+/-0.1687)	(0.0013+/-0.0015)	0.3335 (0.8010)
		Unknown	6	318	2	1	0.600 (+/-0.1291)	0.0018 (+/-0.0019)	1.4451 (0.9840)
	Guerrero	Chilpancingo	1	318	1	0	0.0 (+/-0.0)	0.0 (+/-0.0)	0.0 (0.0)
	Puebla	Epatlan	4	323	1	0	0.0 (+/-0.0)	0.0 (+/-0.0)	0.0 (0.0)
	Total		21	324	4	37	0.661 (+/-0.0782)	0.0385 (+/-0.0202)	0.8235 (0.818)

In *A. nebulosus*, pairwise distances showed the lowest values between haplotypes of individuals from the same locality. For example, between haplotypes H6 and H7-H8 from Chamela (Jalisco) a low value of 0.0026 was detected; as well as between H4 and H5 of La Tzararacua (Michoacan). While the highest pairwise distances values were identified among the haplotypes from Chilpancingo (H9 and H10) and those from Chamela (H6, H7 and H8), El Tuito (Jalisco) (H1) and Huetamo - Unknown (Michoacan) (H2 and H3) (values = 0.0544 to 0.0675) (Table 4).

Table 4. Pairwise distances among haplotypes of *Anolis nebulosus*. Ch = Chilpancingo (Guerrero), Cha = Chamela (Jalisco), Hu = Huetamo (Michoacan), Tu = El Tuito (Jalisco), Tz = La Tzararacua (Michoacan), Unk = Unknown (Michoacan); H1-H10 = Haplotypes.

	H1 (Tu)	**H2 (Hu)**	**H3 (Unk)**	**H4 (Tz)**	**H5 (Tz)**	**H6 (Cha)**	**H7 (Cha)**	**H8 (Cha)**	**H9 (Ch)**	**H10 (Ch)**
H1 (Tu)	0									
H2 (Hu)	0.0363	0								
H3 (Unk)	0.0364	0.0305	0							
H4 (Tz)	0.0305	0.0306	0.0247	0						
H5 (Tz)	0.0305	0.0277	0.0247	0.0026	0					
H6 (Cha)	0.0307	0.0421	0.0276	0.0304	0.0304	0				
H7 (Cha)	0.0337	0.0452	0.0305	0.0333	0.0333	0.0026	0			
H8 (Cha)	0.0337	0.0452	0.0305	0.0333	0.0333	0.0026	0.0053	0		
H9 (Ch)	0.0642	0.0671	0.0640	0.0576	0.0576	0.0642	0.0675	0.0675	0	
H10 (Ch)	0.0610	0.0638	0.0608	0.0544	0.0544	0.0609	0.0642	0.0642	0.0026	0

The pairwise distance values for the *A. microlepidotus* showed the lowest value between the haplotype 1 (H1) from localities in Jalisco and Michoacan, and haplotype 2 (H2) from Michoacan. The highest distance value was between the Guerrero haplotype (H4) and the Jalisco and Michoacan haplotypes (H1 and H2) (Table 5).

Table 5. Pairwise distances among haplotypes of *Anolis microlepidotus*. Ch = Chilpancingo (Guerrero), Chi = Chihuero (Michoacan), Ep = Epatlan (Puebla), Hu = Huetamo (Michoacan), Tu = El Tuito (Jalisco), Unk = Unknown (Michoacan); H1-H4 = Haplotypes.

	H1 (Tu, Hu, Chi, Unk)	**H2 (Hu, Unk)**	**H3 (Ep)**	**H4 (Ch)**
H1 (Tu, Hu, Chi, Unk)	0			
H2 (Hu, Unk)	0.0032	0		
H3 (Ep)	0.0893	0.0893	0	
H4 (Ch)	0.0978	0.0978	0.0300	0

On the other hand, the F_{ST} analyzes carried out for the five localities of *A. nebulosus* show the greatest differentiation between individuals of Michoacan and Guerrero, and the lowest between Jalisco and Michoacan (Table 6). For *A. microlepidotus*, the greatest differentiation was between Jalisco, Guerrero and Puebla, and between Guerrero and Puebla, with the maximum value indicating that

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they do not share haplotypes (Table 7). While the lowest value was between Jalisco and Michoacan, indicating that they share the same haplotypes. It must be emphasized that the results should be considered with caution due to the sample size, and the database reports consider unique haplotypes.

Table 6. Pairwise genetic differentiation (F_{ST}) among groups of *Anolis nebulosus*.

	Jalisco	Michoacan	Guerrero
Jalisco	0		
Michoacan	0.6156	0	
Guerrero	0.7113	0. 8609	0

Table 7. Pairwise genetic differentiation (F_{ST}) among groups of *Anolis microlepidotus*.

	Jalisco	Michoacan	Guerrero	Puebla
Jalisco	0			
Michoacan	-0.008	0		
Guerrero	1	0.9829	0	
Puebla	1	0.9874	1	0

3.2. Genealogical Relationships

The haplotype network built with the sequences of 22 individuals of *A. nebulosus* shows multiple polygons (Figure 2). It was possible to identify the sites that produce the homoplasy reflected in the polygons, and several hypothetical haplotypes are proposed through the network (mv1-mv10).

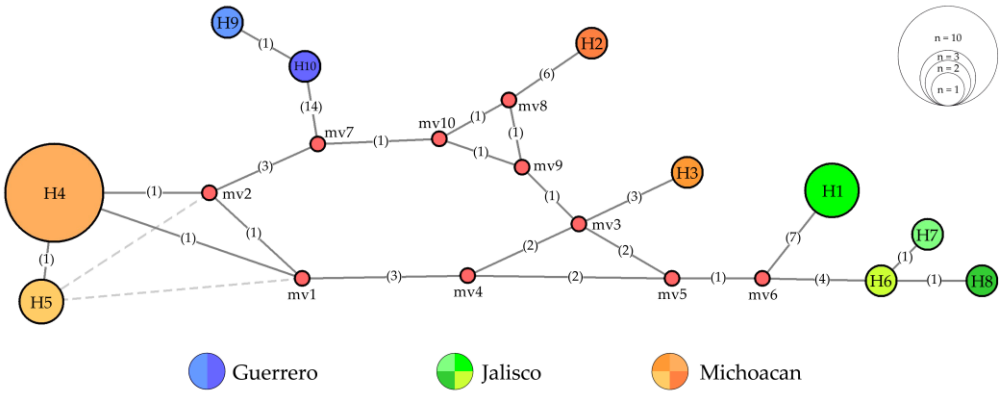


Figure 2. Haplotype network of the *Anolis nebulosus*. Median-Joining network based on 16S mitochondrial marker. The size of the circles is proportional to the frequency of each haplotype; numbers on the branches represent the mutations.

However, it is worth mentioning that the H9 and H10 haplotypes from Chilpancingo (Guerrero) that correspond to the GenBank-NCBI database presented the highest number of mutations that separates them from the rest of the analyzed individuals. In Jalisco there seem to be two distinct groups, one in Chamela and the other in El Tuito, separated by 11 mutational steps. Also noteworthy is the case of Michoacan, where there are apparently at least three different lineages, separated by nine, 11, or 12 mutational steps (haplotypes H2, H3 and H4) (Figure 2).

The genealogical tree shows two main clades; clade 1 (C1) contains haplotypes from Michoacan and Jalisco, and clade 2 (C2) contains the haplotypes from Guerrero (Figure 3). Within C1, a polytomy with low nodal support (PB = 82; PP = 0.52) can be identified. This is composed of the subclade 1 (SC1), the subclade 2 (SC2), Anne_FBH_483 (haplotype H3), and Anne_2018_M05 (haplotype H2). The SC1 is integrated by individuals from Jalisco (PB = 45; PP = 0.98). The individuals from Chamela (Anne_NCBI_218, Anne_NCBI_219 and Anne_NCBI_220) are close related to each other (PB = 99; PP = 1.0). While Anne_2015_J02 (haplotype H1), identified in three individuals from Tuito, is the most

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distant within SC1. For individuals from Michoacan, three lineages were identified, represented by Anne_FBH-589 (H4) - Anne_FBH-599 (H5), Anne_FBH-483 (H3), and Anne_2018_M05 (H2). The Guerrero individuals, Anne_NCBI_402 and Anne_NCBI_404, shows a relationship as sister haplotypes (PB = 100; PP = 1).

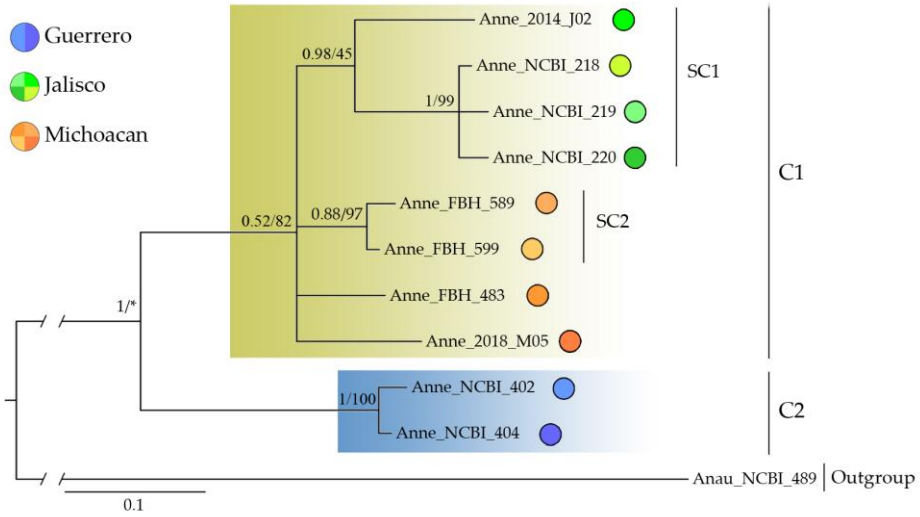


Figure 3. Genealogical relationships of *Anolis nebulosus* built with Bayesian inference and maximum likelihood analyses. Consensus tree built with 11 unique haplotypes and 376 characters of mitochondrial gen 16S. Values at the nodes represent posterior probabilities and bootstrap values (PP/BP); (*) values < 50%. The scale bar under the tree is a reference for the length of the branches (proportional to the amount of evolutionary change).

Otherwise, for the species *A. microlepidotus*, the haplotype network built with the sequences of 21 individuals, shows the relationship of four haplotypes, separated by two hypothetical haplotypes (mv1 and mv2) (Figure 4). Individuals from Guerrero (haplotype H4) and Puebla (haplotype H3) are separated by 8 mutations. While in Jalisco and Michoacan the haplotype H1 was shared by individuals from El Tuito (Jalisco) and Huetamo/Chihuero and Unknown (Michoacan). The haplotype H2 is separated of H1 by one mutation, and was identified in individuals from Huetamo and Unknown (Michoacan). It should be noted that, between mv1 and mv2 (that separated Guerrero – Puebla and Jalisco – Michoacan) there are 21 mutational steps.

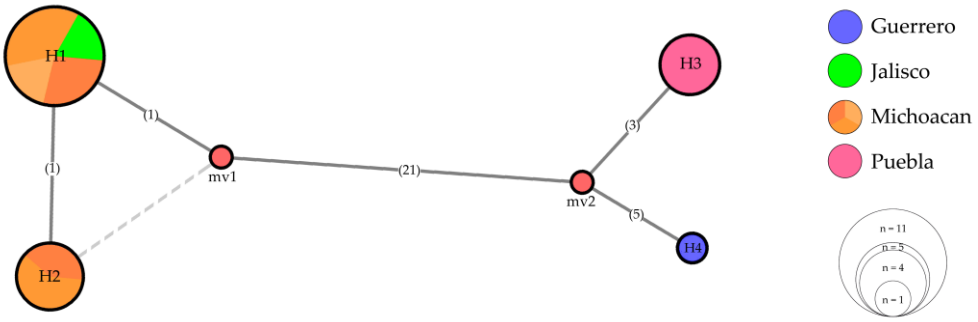


Figure 4. Haplotype network of the *Anolis microlepidotus*. Median-Joining network based on 16S mitochondrial marker. The size of the circles is proportional to the frequency of each haplotype; numbers on the branches represent the mutations.

The genealogical tree confirms the separation of haplotypes in two clades. The tree topology shows the clades C1 and C2. The clade C1 contain individuals from Jalisco and Michoacan, represented by Anmi_2015_J01 (haplotype H1) and Anmi_2018_C03 (haplotype H2) in a relationship

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closed (PB = 97; PP = 1.0). In the C2, are Anmi_NCBI_242 (haplotype H3) from Epatlan (Puebla) and Anmi_NCBI_272 (haplotype H4) from Chilpancigo (Guerrero) (PB = 100; PP = 1.0).

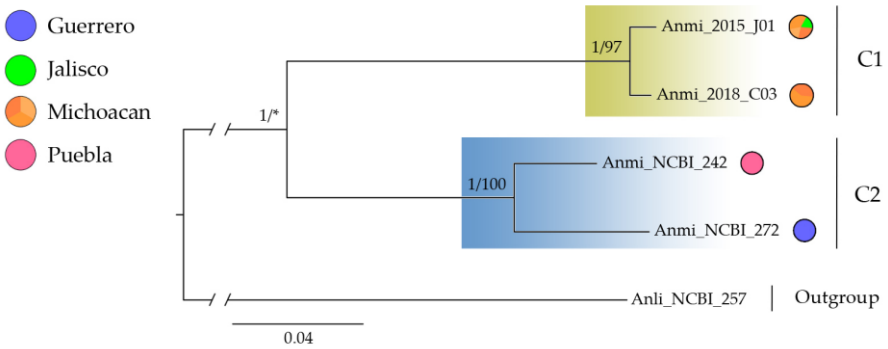


Figure 5. Genealogical relationships of *Anolis microlepidotus* built with bayesian inference and maximum likelihood analyses. Consensus tree built with five unique haplotypes and 324 characters of mitochondrial gen 16S. Values at the nodes represent posterior probabilities and bootstrap values (PP/BP); (*) values < 50%. The scale bar under the tree is a reference for the length of the branches (proportional to the amount of evolutionary change).

4. Discussion

4.1. Genetic Diversity and Differentiation

We present the first estimates of genetic diversity and genealogical relationships for two Anolis species endemic to Mexico. Both species, *A. nebulosus* and *A. microlepidotus*, showed high haplotype and nucleotide diversity when all individuals were analyzed together, but low or null diversity when subdivided by collection locality. This pattern partially matches that reported by Rodriguez-Robles et al. [38], who found high haplotype diversity (Hd = 0.89) and low nucleotide diversity (pi = 0.0013) in the species *Anolis desechensis*. In the same study, high haplotype diversity (Hd = 0.63 and 0.98) and low or high nucleotide diversity (pi = 0.0019 and 0.0065) are reported for two populations of the species *Anolis monensis*. In another study published by Jezkova et al. [39], variable haplotype diversity (Hd range = 0.0 to 0.62) and low nucleotide diversity (pi range = 0.0 to 0.0012) were reported for five populations of *Anolis poncensis*; whereas variable haplotype and nucleotide diversity (Hd ranges = 0.33 to 0.97; pi = 0.00045 to 0.0064) were reported for six populations of *Anolis cooki* [39]. This compilation of values, including our own, reflects the diversity within *Anolis* that, according to several authors, has been underestimated and requires further research to reveal the evolutionary processes within populations [40].

Genetic diversity indices (Hd and Pi) reflect processes that have occurred within populations. According to Grant and Bowen [41], high values of haplotype and nucleotide diversity (Hd > 0.5 and pi > 0.005) indicate a large, stable population with an ancient evolutionary history; alternatively, they may represent secondary contact between lineages. Analysis of all individuals revealed high haplotype and nucleotide diversity in both species, suggesting stable populations with an ancient history. However, we emphasize that, to gain greater certainty regarding this proposal, it is necessary to conduct additional analyses involving a larger number of individuals and characters per population, extending sampling across the geographic range of each species.

Furthermore, when analyzing the samples by locality, low genetic diversity was identified in both species. For example, in the case of *A. nebulosus*, only two haplotypes were found among the 12 individuals from La Tzararacua, showing low haplotype and nucleotide diversity (Hd = 0.303, Pi = 0.0008), whereas a single haplotype was detected in the three individuals from El Tuito, with zero haplotype and nucleotide diversity. Similarly, for *A. microlepidotus*, two haplotypes were identified in eight individuals from Huetamo/Chihuero, showing low haplotype and nucleotide diversity (Hd

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= 0.4286, Pi = 0.0013), whereas a single haplotype with zero haplotype and nucleotide diversity was detected in four individuals from Epatlan. Low genetic diversity (Hd < 0.5 and pi < 0.005) may indicate a recent bottleneck, founder effect, metapopulation structure, etc. [41]. However, to determine the most likely hypothesis, it is necessary to collect a larger sample of individuals and molecular markers, conduct complementary analyses, and incorporate evidence from other sources, such as biogeography, ecology, or ethology.

For example, regarding *A. nebulosus,* it can be argued that if its populations underwent a founder effect, this may have been reinforced by the species' behavior. Losos [2] notes that, despite the limited number of studies on dispersal, the existing ones point relatively low vagility among individuals of different *Anolis* species. Furthermore, in the vast majority of species, both females and males are territorial and aggressively defend their territories, especially during the breeding season. Likewise, females disperse even less than males, and *A. nebulosus*, in particular, is monogamous [2]. This combination of low mobility, territoriality, and monogamy may be contributing to the low genetic diversity observed among individuals from the same locality and promoting differentiation among them. This could also explain the values obtained through the analysis of pairwise distances between haplotypes; specifically, the lowest values corresponded to individuals from the same locality (0.0026), whereas the highest values were recorded between haplotypes from different localities (identified in individuals from Guerrero and those from Jalisco – Michoacan) ranging from 0.0544 to 0.0675.

Although the same pattern of low haplotype and nucleotide diversity was identified in the analyzed localities, a unique situation arises in the case of *A. microlepidotus*; a haplotype is shared between individuals from Michoacan and Jalisco. This could be the result of the incomplete lineage sorting; it that case, the previously proposed hypothesis (supported by low vagility and territoriality), fails to explain the presence of shared polymorphisms and even appears almost contradictory to it. Consequently, pairwise distance values indicate a distance of zero; the next highest value is between individuals from Jalisco and Michoacan (0.0032), and the highest values were recorded between the haplotypes identified in individuals from Guerrero and those from Jalisco-Michoacan (0.0978).

Regarding the genetic differentiation values (F_{ST}), it is reported that the groups of both species are well differentiated. In *A. nebulosus*, all F_{ST} values exceeded 0.6; the lowest genetic differentiation was observed between individuals from Jalisco and Michoacan (F_{ST} = 0.6156), and the highest between Michoacan and Guerrero (F_{ST} = 0.8609). This occurs despite the fact that the greater geographical distance between the sampled locations in Jalisco and Michoacan is greater than between Michoacan and Guerrero. Specifically, the Euclidean distance between Chamela (Jalisco) and La Tzararacua (Michoacan) is 318 km, whereas the distance between Huetamo (Michoacan) and Chilpancingo (Guerrero) is approximately 187 km. However, it is worth noting that Chilpancingo and Huetamo are separated by the Sierra Madre del Sur (SMS), which reaches elevations exceeding 2000 masl. Although the species has been found at altitudes of up 2100 masl [24], the Sierra Madre del Sur appears to constitute an effective barrier to the species' dispersal toward the center of the country, as has been observed in other taxa [42-44]. In *A. microlepidotus*, F_{ST} values indicate genetic differences among three groups: Puebla, Guerrero, and Michoacan-Jalisco. These differences are not directly proportional to geographic distance. The Euclidean distance between El Tuito (Jalisco) and Huetamo/Chihuero (Michoacan) is estimated at approximately 495 km, whereas the distance from Huetamo (Michoacan) to Chilpancingo (Guerrero) is approximately 187 km, and the distance between Epatlan (Puebla) and Chilpancingo (Guerrero) is 170 km.

4.2. Genealogical Analysis

The genealogical analyses performed reveal two distinct evolutionary histories. Although deep divergences are observed in both species, the warrant attention and future research, as they could potentially different species. The *A. nebulosus* haplotype network reveals that the greatest number of mutations was observed between the haplotypes identified in Guerrero and the other haplotypes analyzed. Although the sample size is limited, two groups are observed in Jalisco (one in Chamela

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and another in El Tuito), whereas three distinct lineages are observed in Michoacan: one in La Tzararacua, another in Huetamo, and one of unknown origin but from Michoacan.

The separation into two main groups can be clearly identified through the genealogical trees. The clade (C1) is composed of haplotypes identified in individuals from Michoacan and Jalisco, whereas the basal clade (C2) contains haplotypes from individuals from Guerrero. Within C1, a subclade (SC1) formed by individuals from Jalisco is observed. In the case of the individuals from Michoacan, a polytomy is observed involving three lineages that possibly migrated from the coast to the state's interior in three separate events, utilizing the vegetation along the rivers that drain into the Balsas River Basin.

Based on an overview F_{ST} values, the haplotype network, and the genealogical trees, the following points can be proposed: 1) the species' possible origin lies in the southern part of its geographic range; 2) from the south, the species expanded its range toward the northwest, following the Pacific slope; and 3) from the coast, it dispersed into the continental interior, utilizing riparian vegetation.

For *A. microlepidotus*, the haplotype network and genealogical trees clearly show two groups or clades. In the haplotype network, these two groups are separated by 21 mutational steps; one group comprises the haplotypes from Puebla and Guerrero, while the other includes those from Jalisco and Michoacan. The network reveals the presence of a shared haplotype among individuals from Michoacan and Jalisco; this could be due to the incomplete lineage sorting, a more recent divergence between these populations, or even gene flow, although, given the species' behavior, the latter possibility seems less likely. Similarly, this separation was detected in the pedigrees within clade 1 (C1), composed of haplotypes present in individuals from Jalisco and Michoacan, whereas clade 2 (C2) groups those from Guerrero and Puebla.

4.3. Conservation Applications

To date, few studies have analyzed molecular data for conservation purposes in different Anolis populations. In the specialized literature, studies estimating genealogical relationships and genetic diversity indices for *Anolis* populations focus primarily on proposing evolutionary histories or describing biogeographic patterns [39,40,45-47]. However, few studies used this information as a tool to formulate proposals in conservation genetics, despite the high diversity, endemism, and threatened status of many populations within the group.

In conservation genetics, various concepts have been proposed to define conservation units, including species, evolutionarily significant units (ESUs), and management units (MUs) [48]. Specifically, the ESU concept aims to recognize and protect the evolutionary heritage and potential of groups [49,50]. To this end, it uses evolutionary information derived from molecular data, analyzed with phylogenetic or genealogical tools, to differentiate groups and inform conservation decisions. In genealogic trees, these groups can be identified as monophyletic clades, that may correspond to phylogeographic lineages [49-51].

In some studies, the ESU concept has been applied to identify conservation units within the genus *Anolis*. For example, regrading *A. cooki*, Rodríguez-Robles et al. [52] analyzed mitochondrial molecular markers (Cytb and ND2) from individuals of six populations. Based on this analysis, the authors found these populations are genetically distinct, with limited gene flow. Using this information, they identified the six populations as independent lineages and proposed them as ESUs warranting special conservation attention. Furthermore, in *A. heterodemus*, Vargas-Ramírez and Moreno-Arias [53] identified three independent evolutionary lineages through the analysis of nuclear and mitochondrial molecular markers (RAG1, ND2, and COI) in the species. The authors propose these three lineages as ESUs and candidate species. Additionally, they identify two populations proposed as MUs based on their demographic independence [53].

Based on our results regarding genetic differentiation, haplotype networks, and genealogical tree, we identified lineages within *A. nebulosus* that can be proposed as ESUs or phylogeographic lineages. Our analyses distinguish at least three lineages in Michoacan, two in Jalisco, and one in Guerrero. Among these, the lineages found within a Protected Natural Area are; the one distributed

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in Chamela (Jalisco), located in the "Chamela-Cuixmala" Biosphere Reserve; the one in Chilpancingo (Guerrero), situated in the "Agua de Obispo" Conservation Area; and the lineage present in La Tzararacua (Michoacan), located in the "Barranca del Cupatitzio" National Park. However, the remaining lineages do not coincide with any protected area. In the case of *A. microlepidotus*, three distinct lineages were observed: Puebla, Guerrero, and Michoacan-Jalisco. None of these lineages coincides with a protected natural area. Particularly noteworthy is the diversity found in the Huetamo/Chihuero region, where two groups represented by two haplotypes were identified.

5. Conclusions

We present data on genetic diversity and explore the genealogical relationships of a group of *A. nebulosus* individuals and another of *A. microlepidotus* from central Mexico, species endemic to the country. As species, *A. nebulosus* and *A. microlepidotus* exhibited high levels of diversity; however, when the individuals were analyzed according to their collection locality, they showed low levels of haplotype and nucleotide diversity. Furthermore, marked genetic differentiation was observed among the collection sites, indicating the existence of independently evolving groups or phylogeographic lineages. This observation was also evidenced by haplotype networks and genealogical trees, in which the divisions were clearly identified, particularly for *A. nebulosus*. We highlight the need for further research in both cases, as they may consist of different species. Based on the results of this study, we propose the conservation of the following evolutionary units of *A. nebulosus*: one lineage distributed in Huetamo (Michoacan) and one in El Tuito (Jalisco); neither is found within protected natural areas. None of the lineages identified in *A. microlepidotus* are found in protected natural areas; therefore, it is recommended that the protection status of these areas be reviewed, particularly in Huetamo/Chihuero (Michoacan).

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Data Availability Statement: All sequences were deposited in GenBank-NCBI. Access numbers: QB131700-QB131716 and QB131721-QB131736.

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Conflicts of Interest: The authors declare no conflict of interests.

References

1. Nicholson, K.E.; Crother, B.I.; Guyer, C.; Savage, J.M. Translating a clade based classification into one that is valid under the international code of zoological nomenclature: the case of the lizards of the family Dactyloidae (Order Squamata). *Zootaxa* **2018**, *4461*, 573–586. DOI: 10.11646/zootaxa.4461.4.7
2. Losos, J.B. Lizards in an evolutionary tree: ecology and adaptive radiation of anoles; Univ of California Press: London, England, 2009; pp. 507.
3. Balderas-Valdivia, C.J.; González-Hernández, A. Inventario de la Herpetofauna de México 2024: taxones biodiversos. *Herpetol Mex* **2024**, *8*, 71–135. DOI: https://doi.org/10.69905/r0jana70

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Preprints.org | NOT PEER-REVIEWED | Posted: 26 October 2026 https://doi.org/10.20944/preprints202610.2436.v1

4. Uetz, P.; Freed, P.; Aguilar, R.; Reyes, F.; Kudera, J.; Hošek, J. The Reptile Database. Available online: http://www.reptile-database.org (accessed on 04 August 2026).
5. DOF. 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; Secretaría de Medio Ambiente y Recursos Naturales: Ciudad de México, México, **2010**.
6. Nicholson, K.E.; Crother, B.I.; Guyer, C.; Savage, J.M. It is time for a new classification of anoles (Squamata: Dactyloidae). *Zootaxa* **2012**, *3477*, 1–108. DOI: https://doi.org/10.11646/zootaxa.3477.1.1
7. Castiglia, R.; Annesi, F.; Bezerra, A.M.R.; García, A.; Flores-Villela, O. Cytotaxonomy and DNA taxonomy of lizards (Squamata, Sauria) from a tropical dry forest in the Chamela-Cuixmala Biosphere Reserve on the coast of Jalisco. *Zootaxa* **2010**, *2508*, 1–29. DOI: https://doi.org/10.11646/zootaxa.2508.1.1
8. Flores-Villela, O.; García-Vázquez, U.O. Biodiversity of reptiles in Mexico. *Rev Mex Biodivers* **2014**, *85*, S467–S475. DOI: 10.7550/rmb.43236
9. Köhler, G.; Pérez, R.G.T.; Petersen, C.B.P.; De La Cruz, F.R.M. A revision of the Mexican *Anolis* (Reptilia, Squamata, Dactyloidae) from the Pacific versant west of the Isthmus de Tehuantepec in the states of Oaxaca, Guerrero, and Puebla, with the description of six new species. *Zootaxa* **2014**, *3862*, 1–210. DOI: https://doi.org/10.11646/zootaxa.3862.1.1
10. Gray, L.; Meza-Lázaro, R.; Poe, S.; Nieto-Montes de Oca, A. A new species of semiaquatic *Anolis* (Squamata: Dactyloidae) from Oaxaca and Veracruz, Mexico. *Herpetol J* **2016**, *26*, 253–262.
11. Köhler, G.; Vargas, J. A new species of anole from Parque Nacional Volcán Arenal, Costa Rica (Reptilia, Squamata, Dactyloidae: Norops). *Zootaxa* **2019**, *4608*, 261–278. DOI: https://doi.org/10.11646/zootaxa.4608.2.4
12. Alföldi, J.; Di Palma, F.; Grabherr, M.; Williams, C.; Kong, L.; Mauceli, E.; Russell, P.; Lowe, C.B.; Glor, R.E.; Jaffe, J.D.; et al. The genome of the green anole lizard and a comparative analysis with birds and mammals. *Nat Methods* **2011**, *477*, 587–591. DOI: https://doi.org/10.1038/nature10390
13. Geneva, A.J.; Park, S.; Bock, D.G.; de Mello, P.L.; Sarigol, F.; Tollis, M.; Donihue, C.M.; Reynolds, R.G.; Feiner, N.; Rasys, A.M.; et al. Chromosome-scale genome assembly of the brown anole (*Anolis sagrei*), an emerging model species. *Commun Biol* **2022**, *5*, 1126. DOI: https://doi.org/10.1038/s42003-022-04074-5
14. Araya-Donoso, R.; Baty, S.M.; Johnson, J.E.; Lasku, E.; Taft, J.M.; Fisher, R.E.; Losos, J.B.; Dolby, G.A.; Kusumi, K.; Geneva, A.J. Structural rearrangements and selection promote phenotypic evolution in *Anolis* lizards. *Genome Biol Evol* **2025**, *17*, 1–18. DOI: https://doi.org/10.1093/gbe/evaf196
15. Castañeda, M.D.R.; De Queiroz, K. Phylogenetic relationships of the Dactyloa clade of *Anolis* lizards based on nuclear and mitochondrial DNA sequence data. *Mol Phylogenet Evol* **2011**, *61*, 784–800. DOI: https://doi.org/10.1016/j.ympev.2011.07.004
16. Castañeda, M.D.R.; De Queiroz, K. Phylogeny of the Dactyloa clade of *Anolis* lizards: new insights from combining morphological and molecular data. *Bull Mus Comp Zool* **2013**, *160*, 345–398. https://doi.org/10.3099/0027-4100-160.7.345
17. Mulcahy, D.G.; Ibáñez, R.; Jaramillo, C.A.; Crawford, A.J.; Ray, J.M.; Gotte, S.W.; Jacobs, J.F.; Wynn, A.H.; Gonzalez-Porter, G.P.; McDiarmid, R.W.; et al. DNA barcoding of the National Museum of Natural History reptile tissue holdings raises concerns about the use of natural history collections and the responsibilities of scientists in the molecular age. *PLoS One* **2022**, *17*, e0264930. DOI: https://doi.org/10.1371/journal.pone.0264930
18. Nicholson, K.E. Phylogenetic analysis and a test of the current infrageneric classification of Norops (Beta Anolis). *Herpetological Monographs* **2002**, *16*, 93–120.
19. Poe, S.; Nieto-Montes de Oca, A.; Torres-Carvajal, O.; De Queiroz, K.; Velasco, J.A.; Truett, B.; Gray, L.N.; Ryan, M.J.; Köhler, G.; Ayala-Varela, F.; et al. A phylogenetic, biogeographic, and taxonomic study of all extant species of *Anolis* (Squamata; Iguanidae). *Syst Biol* **2017**, *66*, 663–697. DOI: https://doi.org/10.1093/sysbio/syx029
20. Poe, S. Phylogeny of anoles. *Herpetological Monographs* **2004**, *18*, 37–89.

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Preprints.org | NOT PEER-REVIEWED | Posted: 26 October 2026 https://doi.org/10.20944/preprints202610.2436.v1

21. Prates, I.; Rodrigues, M.T.; Melo-Sampaio, P.R.; Carnaval, A.C. Phylogenetic relationships of Amazonian anole lizards (Dactyloa): taxonomic implications, new insights about phenotypic evolution and the timing of diversification. *Mol Phylogenet Evol* **2015**, *82*, 258–268. DOI: https://doi.org/10.1016/j.ympev.2014.10.005
22. Köhler, G.; Pérez, R.G.T.; Petersen, C.B.P.; De La Cruz, F.R.M. A new species of pine anole from the Sierra Madre del Sur in Oaxaca, Mexico (reptilia, Squamata, Dactyloidae: Anolis). *Zootaxa* **2014**, *3753*, 453–468. DOI: https://doi.org/10.11646/zootaxa.3753.5.4
23. Poe, S.; Latella, I.; Ayala-Varela, F.; Yañez-Miranda, C.; Torres-Carvajal, O. A new species of Phenacosaur *Anolis* (Squamata; Iguanidae) from Peru and a comprehensive phylogeny of Dactyloa-clade Anolis based on new DNA sequences and morphology. *Ichthyol Herpetol* **2015**, *103*, 639–650. DOI: 10.1643/CH-14-127
24. Flores-Villela, O.; Hammerson, G.A. *Anolis nebulosus*. Available online: https://dx.doi.org/10.2305/IUCN.UK.2007.RLTS.T64207A12752241.en (accessed on 04 August 2026).
25. IUCN. The IUCN Red List of Threatened Species. Version 2023-1. Available online: https://www.iucnredlist.org. (accessed on 21 February 2024).
26. Dutton, P.H. Methods for collection and preservation of samples for sea turtle genetic studies. In Proceedings of the Proceedings of International Symposium on Sea Turtle Conservation Genetics, Forida, USA, 1996; p. 17–24.
27. FitzSimmons, N.N. Male marine turtles: gene flow, philopatry and mating systems of the green turtle (*Chelonia mydas*). Ph.D. Thesis, University of Queensland, Queensland, Australia, 1997.
28. Sumida, M.; Kondo, Y.; Kanamori, Y.; Nishioka, M. Inter-and intraspecific evolutionary relationships of the rice frog *Rana limnocharis* and the allied species *R. cancrivora* inferred from crossing experiments and mitochondrial DNA sequences of the 12S and 16S rRNA genes. *Mol Phylogenet Evol* **2002**, *25*, 293–305. DOI: https://doi.org/10.1016/S1055-7903(02)00243-9
29. Sanger, F.; Nicklen, S.; Coulson, A.R. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* **1977**, *74*, 5463–5467. DOI: https://doi.org/10.1073/pnas.74.12.5463
30. Müller, J.; Müller, K.; Neinhuis, C.; Quandt, D. PhyDE-Phylogenetic data editor. Available online: http//www.phyde.de/index.htm (accessed on 13 March 2015).
31. Excoffier, L.; Lischer, H.E.L. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Resour* **2010**, *10*, 564–567. DOI: https://doi.org/10.1111/j.1755-0998.2010.02847.x
32. Fluxus Technology Ltd. Network v10.2.0.0 Software: Network publisher and DNA alignment software. Fluxus Technology Ltd., Clare, England. Available online: https://www.fluxus-engineering.com/sharenet.htm (accessed on 25 January 2025).
33. Stamatakis, A. RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. *Bioinformatics* **2014**, *30*, 1312–1313. DOI: https://doi.org/10.1093/bioinformatics/btu033
34. Ronquist, F.; Teslenko, M.; van der Mark, P.; Ayres, D.L.; Darling, A.; Höhna, S.; Larget, B.; Liu, L.; Suchard, M.A.; Huelsenbeck, J.P. MrBayes 3.2: efficient Bayesian Phylogenetic inference and model choice across a large model space. *Syst Biol* **2012**, *61*, 539–542. DOI: 10.1093/sysbio/sys029
35. Darriba, D.; Taboada, G.; Doallo, R.; Posada, D. jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods* **2012**, *9*, 772. DOI: https://doi.org/10.1038/nmeth.2109
36. Alfaro, M.E.; Huelsenbeck, J.P. Comparative performance of Bayesian and AIC-based measures of phylogenetic model uncertainty. *Syst Biol* **2006**, *55*, 89–96. DOI: 10.1080/10635150500433565.
37. Rambaut, A. FigTree v1.4.4. Available online: http://tree.bio.ed.ac.uk/software/figtree/ (accessed on 26 March 2025).
38. Rodriguez-Robles, J.A.; Jezkova, T.; García, M.A. Evolutionary relationships and historical biogeography of Anolis desechensis and *Anolis monensis*, two lizards endemic to small islands in the eastern Caribbean Sea. *J Biogeogr* **2007**, *34*, 1546–1558. DOI: https://doi.org/10.1111/j.1365-2699.2007.01718.x
39. Jezkova, T.; Leal, M.; Rodriguez-Robles, J.A. Living together but remaining apart: comparative phylogeography of *Anolis poncensis* and *A. cooki*, two lizards endemic to the aridlands of Puerto Rico. *Biol J Linn Soc Lond* **2009**, *96*, 617–634. DOI: https://doi.org/10.1111/j.1095-8312.2008.01147.x

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40. Hofmann, E.P.; Townsend, J.H. Origins and biogeography of the *Anolis crassulus* subgroup (Squamata: Dactyloidae) in the highlands of Nuclear Central America. *BMC Evol Biol* **2017**, *17*, 267. DOI: https://doi.org/10.1186/s12862-017-1115-8
41. Grant, W.; Bowen, B. Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. *J Hered* **1998**, *89*, 415–426. DOI: https://doi.org/10.1093/jhered/89.5.415
42. Ramírez-Reyes, T.; Piñero, D.; Flores-Villela, O.; Vázquez-Domínguez, E. Molecular systematics, species delimitation and diversification patterns of the Phyllodactylus lanei complex (Gekkota: Phyllodactylidae) in Mexico. *Mol Phylogenet Evol* **2017**, *115*, 82–94. DOI: https://doi.org/10.1016/j.ympev.2017.07.008
43. Ramírez-Reyes, T.; Blair, C.; Flores-Villela, O.; Piñero, D.; Lathrop, A.; Murphy, R. Phylogenomics and molecular species delimitation reveals great cryptic diversity of leaf-toed geckos (Phyllodactylidae: Phyllodactylus), ancient origins, and diversification in Mexico. *Mol Phylogenet Evol* **2020**, *150*, 106880. DOI: https://doi.org/10.1016/j.ympev.2020.106880
44. Zarza, E.; Reynoso, V.H.; Emerson, B.C. Diversification in the northern neotropics: mitochondrial and nuclear DNA phylogeography of the iguana *Ctenosaura pectinata* and related species. *Mol Ecol* **2008**, *17*, 3259-3275. DOI: https://doi.org/10.1111/j.1365-294X.2008.03826.x
45. Guarnizo, C.E.; Werneck, F.P.; Giugliano, L.G.; Santos, M.G.; Fenker, J.; Sousa, L.; D'Angiolella, A.B.; Dos Santos, A.R.; Strüssmann, C.; Rodrigues, M.T.; et al. Cryptic lineages and diversification of an endemic anole lizard (Squamata, Dactyloidae) of the Cerrado hotspot. *Mol Phylogenet Evol* **2016**, *94*, 279–289. DOI: https://doi.org/10.1016/j.ympev.2015.09.005
46. Phillips, J.G.; Deitloff, J.; Guyer, C.; Huetteman, S.; Nicholson, K.E. Biogeography and evolution of a widespread Central American lizard species complex: Norops humilis, (Squamata: Dactyloidae). *BMC Evol Biol* **2015**, *15*, 1–13. DOI: https://doi.org/10.1186/s12862-015-0391-4
47. Tollis, M.; Ausubel, G.; Ghimire, D.; Boissinot, S. Multi-locus phylogeographic and population genetic analysis of *Anolis carolinensis*: historical demography of a genomic model species. *PLoS One* **2012**, *7*, e38474. DOI: https://doi.org/10.1371/journal.pone.0038474
48. Allendorf, F.W.; Luikart, G.H. *Conservation and the genetics of populations*; Blackwell Publishing: Oxford, UK., 2007; pp. 642.
49. Moritz, C. Defining 'Evolutionarily Significant Units' for conservation. *Trends Ecol Evol* **1994**, *9*, 373–375, DOI: https://doi.org/10.1016/0169-5347(94)90057-4
50. Moritz, C. Strategies to protect biological diversity and the evolutionary processes that sustain it. *Syst Biol* **2002**, *51*, 238–254. DOI: https://doi.org/10.1080/10635150252899752
51. Blair, C.; Pavón-Vázquez, C.J.; Rana, Q.; Farleigh, K.; Crispo, E.; Panzera, T.; de Medeiros Magalhães, F.; White, B.; Garcia-Vazquez, U.O. Isolation and divergence of Greater Earless Lizards (Phrynosomatidae: Cophosaurus) in western North America support multiple diversification processes and a complex demographic history. *Mol Phylogenet Evol* **2026**, *214*, 108442. DOI: https://doi.org/10.1016/j.ympev.2025.108442
52. Rodríguez-Robles, J.A.; Jezkova, T.; Leal, M. Genetic structuring in the threatened "Lagartijo del Bosque Seco"(Anolis cooki) from Puerto Rico. *Mol Phylogenet Evol* **2008**, *46*, 503–514. DOI: 10.1016/j.ympev.2007.11.006
53. Vargas-Ramírez, M.; Moreno-Arias, R. Unknown evolutionary lineages and population differentiation in Anolis heterodermus (Squamata: Dactyloidae) from the eastern and central Cordilleras of Colombia revealed by DNA sequence data. *South Am J Herpetol* **2014**, *9*, 131–141. DOI: https://doi.org/10.2994/SAJH-D-13-00013.1

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