GENETIC TOOLS FOR ASSISTING SUSTAINABLE MANAGEMENT AND CONSERVATION OF THE SPINY-TAILED IGUANA, *CTENOSAURA PECTINATA*

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Abstract.—The System of Management Units for the Conservation of Wildlife (Sistema de Unidades de Manejo para la Conservación de la Vida Silvestre, SUMA), regulated by the Mexican Ministry of Environment and Natural Resources, seeks the correct management and sustainable use of wildlife. The main tool used by SUMA is wildlife management units (UMAs) that harbor wildlife for several purposes (e.g., production, education, research, conservation, and exhibition). *Ctenosaura pectinata,* a threatened Mexican endemic spiny-tailed iguana, is legally managed through UMAs, some of them harboring individuals that were unwanted pets or confiscations of unknown origin. Facilities may become overcrowded, as iguanas cannot be returned to the wild without knowledge of their origins. This may lead to irresponsible or accidental releases of individuals or their captive-born offspring into the wild; a potential source of genetic contamination to local populations. To promote proper management of captive individuals and to evaluate their potential reintroduction into the wild, we created and tested a distribution-wide database to identify the origin of 24 confiscated individuals harbored in two UMAs in México. We compiled mtDNA and microsatellite data derived from previous studies, including 341 individuals from 49 localities. We applied two Bayesian methods for population genetic assignment using the database as a baseline. In several cases, it was possible to narrow down the potential geographic region of origin when considering mtDNA together with the microsatellite data. The database is potentially a useful resource for authorities and conservation organizations.

Resumen.—El Sistema de Unidades de Manejo para la conservación de la Vida Silvestre (SUMA), regulado por la Secretaría del Medio Ambiente y Recursos Naturales en México, busca el manejo correcto y el uso sustentable de la vida silvestre. El instrumento principal que usa SUMA son las unidades de manejo de vida silvestre (UMAs) que albergan vida silvestre con diferentes propósitos (e.g., producción, educación, investigación, conservación, y exibición). La iguana negra *Ctenosaura pectinata* es una especie amenazada y endémica de México que es manejada a través de UMAs, algunas de las cuales albergan iguanas que fueron mascotas indeseadas o decomisadas, de origen desconocido. Las instalaciones pueden llegar a superpoblarse al no poder regresar las iguanas a su hábitat sin saber de dónde vienen. Esto puede llevar a liberaciones irresponsables o escapes de individuos o su descendencia nacida en cautiverio, constituyendo así una fuente potencial de 'contaminación genética' para las poblaciones locales. Para promover el manejo adecuado de los individuos en cautiverio y evaluar la posibilidad de su liberación en su hábitat, creamos una base de datos que cubre la distribución entera de la especie, para identificar el sitio de origen de 24 individuos confiscados y albergados en dos zoológicos en México. Recopilamos datos de DNA mitocondrial y microsatelital derivados de estudios previos que incluyen 341 individuos de 49 localidades. Empleamos dos métodos bayesianos de asignación genética de poblaciones usando la base de datos como referencia. En algunos casos fue posible reducir el número de potenciales regiones geográficas de origen cuando se usa la información mitocondrial y microsatelital conjuntamente. La base de datos es potencialmente un recurso útil para las autoridades y organizaciones involucradas en la conservación de la especie.

Key Words.—Garrobo de Roca; genetic assignment; Guerreran iguanas; México; microsatellites; mtDNA; wildlife management units.

INTRODUCTION

Conservation genetics involves the application of evolutionary and molecular genetics to biodiversity conservation (Frankham 2010). Among other applications, conservation genetics contributes to: (1) resolving taxonomic uncertainties; (2) defining evolutionary divergent units that require separate management; (3) managing to minimize inbreeding, loss of genetic diversity, and extinction risk; (4) obtaining important information for species conservation (e.g., demographic parameters and history, mating system, gene flow, parentage, etc.); and (5)

forensics (Frankham 2010). Conservation genetics takes advantage of molecular and analytical techniques to quantify and understand the distribution and dynamics of genetic diversity. One such analytical approach is genetic population assignment. This consists of assigning reference populations as possible origins of individuals on the basis of multilocus genotypes (Piry et al. 2004; Latch et al. 2006). This procedure has been widely used in conservation genetics of iguanas and other organisms to understand patterns of migration (Paetkau et al. 2004; Lanterbecq et al. 2010; Colosimo et al. 2014; Wang 2014), to identify hybrid individuals (Pierpaoli et al. 2003; Barilani et al. 2005; Vähä and Primmer 2006), to address illegal species trading, hunting and fishing (Frantz et al. 2006; Wasser et al. 2008; Nielsen et al. 2012; Gentile et al. 2013), to identify past translocations and inform repatriation of individuals (Tzika et al. 2008), and to identify the origin of captive individuals to inform release of species to their habitat (Russello et al. 2007). Baseline information on the genetic composition and structure of the species populations is thus required to obtain reliable results.

Here we apply genetic population assignment methods to identify potential source populations of captive individuals of unknown origin, of the threatened spiny-tailed iguana, *Ctenosaura pectinata*. We aim to improve management of captive individuals and to advise on their potential release into the wild. In the following we summarize background information and the outcomes of evolutionary and molecular genetic studies on *C. pectinata*. The latter of which provide the baseline genetic information for this study.

Ctenosaura pectinata.—Our model species is one of the nearly 500 reptilian species endemic to México (Flores-Villela and García-Vazquez 2014). It is found from 0 to 1,000 m above sea level within the tropical lowlands along the Pacific coast and in the Río Balsas basin in central México, with populations also on the Marías and Isabel islands in the Pacific Ocean. It is found primarily in the seasonally dry tropical deciduous forest (SDTDF), but it can also be found in thorny forests, grasslands, oak forest, mangrove, and coastal dunes, and is sometimes associated with human settlements (Suazo and Alvarado 1994). This species exhibits sexual dimorphism, with males bearing a dorsal spiny crest and being generally bigger than females, reaching up to one meter in total length. They show ontogenetic color change, with hatchlings being green and becoming darker when adults (Köhler 2002), who exhibit a vast range in coloration (Fig. 1).

Currently, Mexican Law NOM-059-ECOL-2010 (SEMARNAT 2010) lists *C. pectinata* as threatened ('amenazada'), meaning that it may be at risk of extinction in the short or medium term, if action to mitigate threats is not taken. This species is economically and culturally important for some rural communities, as they are used as food, traditional medicine, pets, and their skin is used to produce handicrafts. The traditional practice of hunting gravid female *C. pectinata* just before they lay their eggs is particularly threatening because it leads to rapid population decline (Aguirre-Hidalgo 2007). This is aggravated by the predominance of single paternity, which makes the species susceptible to rapid loss of genetic variation (Faria et al. 2010). Additionally, the anthropogenic transformation of 71% of the original area of the SDTDF in México, and the protection of only 0.2% of the remaining area (Portillo-Quintero and Sánchez-Azofeifa 2010) challenge the survival of this species.

Ctenosaura pectinata is comprised of nine cryptic mtDNA lineages: North A, North B, North C, North D,

FIGURE 1. Examples of color variation in *Ctenosaura pectinata* across its geographic distribution in México. (Photographed by Víctor H. Reynoso and Eugenia Zarza).

Colima, Balsas, Guerrero, Oaxaca and South (Zarza et al. 2008, 2011). Genetic distances (Tamura and Nei 1993) between these lineages range from 4.11 to 11.57% (Zarza et al. 2008). The deepest genetic distance occurs between overlapping clades from the Central Western Coast of México (North and Colima clades). Further, multilocus studies uncovered two microsatellite genotypic clusters that are geographically discordant with the distribution of four mtDNA lineages (i.e., North C, North D, Colima, and Balsas; Zarza et al. 2011). Several processes might account for this discordance. Differences in effective population size between mtDNA and nuclear markers could lead to faster lineage sorting of maternal lineages than of nuclear markers, resulting in the observed discordance. However, after correcting for effective population size, Zarza et al. (2011) could not entirely account for the differences in genetic structure. The observed pattern is consistent with historic secondary contact and hybridization of once genetically distinct lineages. Previously isolated lineages of other taxa have come into secondary contact in central western México (Devitt 2006), suggesting that introgressive hybridization might not be a rare process in the evolution of species in this region.

These recently described genetic clusters within *C. pectinata* have restricted distributions and divergent evolutionary histories, however when individuals from different clusters come into contact they may mate and produce fertile offspring (Zarza et al. 2008, 2011). Accidental or intentional introductions could lead to mating of individuals with different genetic backgrounds and thus introgression. While recent research suggests that introgression can sometimes have adaptive outcomes (Kronforst 2012; Pardo-Diaz et al. 2012), it can also lead to a loss of local adaptation and outbreeding depression (Gentile et al. 2013). Conservation strategies that utilize genetic information alongside the biology of the species and its threats are urgently needed to protect the unique evolutionary history, adaptations, and ecological role of *C. pectinata*.

The Mexican Ministry of Environment and Natural Resources (SEMARNAT) implemented the System of Management Units for the Conservation of Wildlife (Sistema de Unidades de Manejo para la Conservación de la Vida Silvestre, SUMA), that aims to reach a balance between wildlife conservation and the needs of the people. Within this system, Wildlife Management Units (UMAs) function as breeding, research, education, and training centers among other things (SEMARNAT, Sistema de Unidades de Manejo para la Conservación de la Vida Silvestre. Available from http://www.semarnat.gob.mx/temas/gestion-ambiental/vida-silvestre/sistema-de-unidades-de-manejo [Accessed 1 December 2015]). For example, all zoos and iguana breeding facilities (iguanarios) are UMAs. SUMA guidelines discourage the inclusion of animals from geographic regions outside of where a given UMA is located, however this is not always adhered to. Some UMAs harbor individuals that were unwanted pets or confiscations, lacking origin information. In some cases these facilities become overcrowded or unsuitable, and irresponsible or accidental releases of individuals or their captive-born offspring could occur. This could in turn result in genetic contamination of local populations through interbreeding between individuals with different genetic backgrounds, leading to outbreeding depression.

To promote proper management of *C. pectinata* captive individuals, and to provide information for their potential release into the wild, we created a genetic database using data generated by previous studies (Zarza et al. 2008, 2011; Faria et al. 2010). We then tested the utility of our database for genetic population assignment, which consists of assigning reference populations as possible origins of individuals on the basis of multilocus genotypes (Piry et al. 2004; Latch et al. 2006). We focused on *C. pectinata* individuals held in two UMA zoos in México and applied two Bayesian clustering approaches. This is a direct application of a large-scale genetic survey that could serve as a model in other vertebrate species facing similar threats.

MATERIALS AND METHODS

Field and laboratory work.—We created a geo-referenced database comprised of microsatellite genotypes for 341 individuals across eight loci, and mtDNA sequence data (ND4) for 317 individuals, collected across 49 sites covering the entire distribution of *C. pectinata* and part of the distribution of *C. acanthura*, which appears nested within *C. pectinata* (see Zarza et al. 2008). We excluded localities in northern México where *C. pectinata* and *C. hemilopha* co-occur and hybridize (Zarza Franco 2008) to avoid the inclusion of *C. hemilopha* alleles that may confound population genetic assignment. Previous molecular and evolutionary studies detail the protocols used to purify, genotype, sequence, and analyze these samples (Zarza et al. 2008, 2009, 2011; Zarza Franco 2008; Faria et al. 2010).

To test the utility of our database for population assignment we collected 0.15 ml blood samples from the caudal vein of *C. pectinata* individuals harbored in the Zoológico de Morelia, Morelia, Michoacán (n = 15; 100% of the population in the zoo) and in Zoológico Zoochilpan in Chilpancingo, Guerrero (n = 9; approximately 50% of the adult population in the zoo), in 2006. Iguanas were captured by hand or by noosing. We applied the same laboratory and analytical procedures to these 24 samples as those that were used for the reference database, and provide a brief summary below.

We extracted genomic DNA using a modified salt precipitation protocol (Aljanabi and Martinez 1997). We PCR amplified and sequenced a 561 bp fragment of the mitochondrial ND4 gene using the primers ND4,

ND4Rev (Arèvalo et al. 1994), ND4F1 (Zarza et al. 2008) and ND4R623 (Hasbún et al. 2005) with conditions described in Zarza et al. (2008). We genotyped the captive samples with twelve microsatellite markers. We individually PCR amplified loci Pec01, Pec03, Pec20, Pec21, Pec24, Pec25, Pec89, TNB1 with conditions described by Zarza et al. (2009). We PCR amplified locus Cthe37 using protocols described by Blázquez et al. (2006). We PCR amplified loci Pec16 and Pec73 (Zarza et al. 2009) together with Cthe12 (Blázquez et al. 2006) in a multiplex reaction described in Zarza et al. (2011). We combined the PCR products of the 12 loci in two different mixes that allow loci to be distinguished according to fluorescent dye and allele size (Zarza et al. 2011). The two mixes were run independently in an automated ABIprism 3730 and peaks were visualized with GeneMapper software version 4.0 (Applied Biosystems, Foster City, California, USA).

Data analyses.—We took a phylogenetic tree estimation approach to infer the mtDNA haplotype relationships of the zoo individuals. The analyses included all the haplotypes currently known for *C. pectinata* available from GeneBank plus the haplotypes of the confiscated individuals. We aligned the mtDNA sequences with MUSCLE (Edgar 2004). We searched for the best tree with maximum likelihood inference and performed 1,000 bootstrap replicates with RAxMLv8 (Stamatakis 2014) to assess the statistical support for each node.

We performed genetic population assignment of individuals based on microsatellite frequencies with two Bayesian clustering approaches that do not require *a priori* grouping of individuals: BAPS (Corander and Marttinen 2006; Corander et al. 2008) and STRUCTURE (Pritchard et al. 2000; Hubisz et al. 2009). These methods allow individuals to be of mixed ancestry, proportionally assigning an individual genome to clusters defined while minimizing Hardy-Weinberg and linkage disequilibrium (Latch et al. 2006).

We ran simulations with STRUCTURE v2.3.2 under the admixture model assigning a uniform prior for the parameter Alpha (degree of admixture) and estimating the allele frequency parameter (Lambda) assuming correlated allele frequencies and without taking into account knowledge on locality. We ran preliminary analyses including the entire dataset (i.e., 341 individuals) plus the 24 individuals of unknown origin, under $K = 2 - K = 10$ for one million MCMC iterations and 10 replicates. Likelihood values plateaued after $K = 4$, with some $K = 4 - K = 6$ runs giving alternative but biologically meaningful clustering
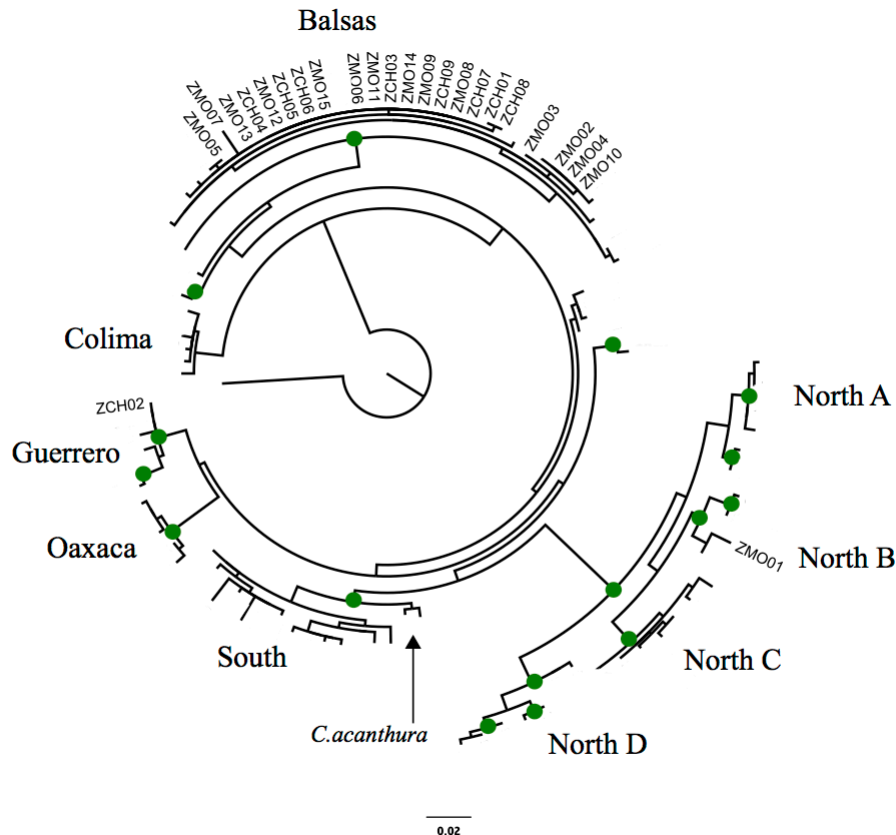

FIGURE 2. Maximum likelihood tree showing the relationship of *Ctenosaura pectinata* mtDNA haplotypes sampled in wild populations (unlabeled tips) and each captive individual (labeled tips). Statistically supported nodes (bootstrap > 80%) are marked with green circles. Haplotypes of captive individuals are labeled as ZM- (Zoológico de Morelia) and ZCH- (Zoológico Zoochilpan). Clade names correspond to those used in Zarza et al. (2008).

patterns (e.g., concordance with the distribution of some mtDNA clades, presence of geographical barriers). However, in the very north and very south of the distribution, clustering was not consistent among all the runs for each K, perhaps as a result of isolation by distance (Frantz et al. 2006). This will be further investigated in a future publication. As the zoo samples were consistently not assigned to any of these 'problematic' regions, we removed the most northern and southern localities (Sinaloa, Oaxaca, and Veracruz samples) and worked with a reduced data set (Fig. 2; supplementary material available on request) and the captive individuals, however the entire database is available upon request. This allowed us to investigate potential substructure in the central coastal and inland area of the distribution with the benefit of reduced computation time required for the assignment. We performed ten

iterations for each value of K (K = 2 – K = 4) with one million MCMC replicates after a burn-in period of 100,000. We chose the most likely K using the Evanno et al. (2005) method. For each confiscated and wild individual, we averaged their proportion of ancestry (Q value) among the results of the ten iterations under the most likely K. This served to genetically characterize each locality and to compare its composition with the proportion of ancestry of the confiscated individuals and infer their potential origin. Previous studies have employed Q values between 0.75 and 0.90 to assign individuals to a population (Vähä and Primmer 2006; Schwartz and Karl 2008; Wilkinson et al. 2011; Winkler et al. 2011). In these studies, individuals with lower Q values were assigned to different hybrid categories. We used a stringent value of Q = 0.9 as the threshold for population assignment.

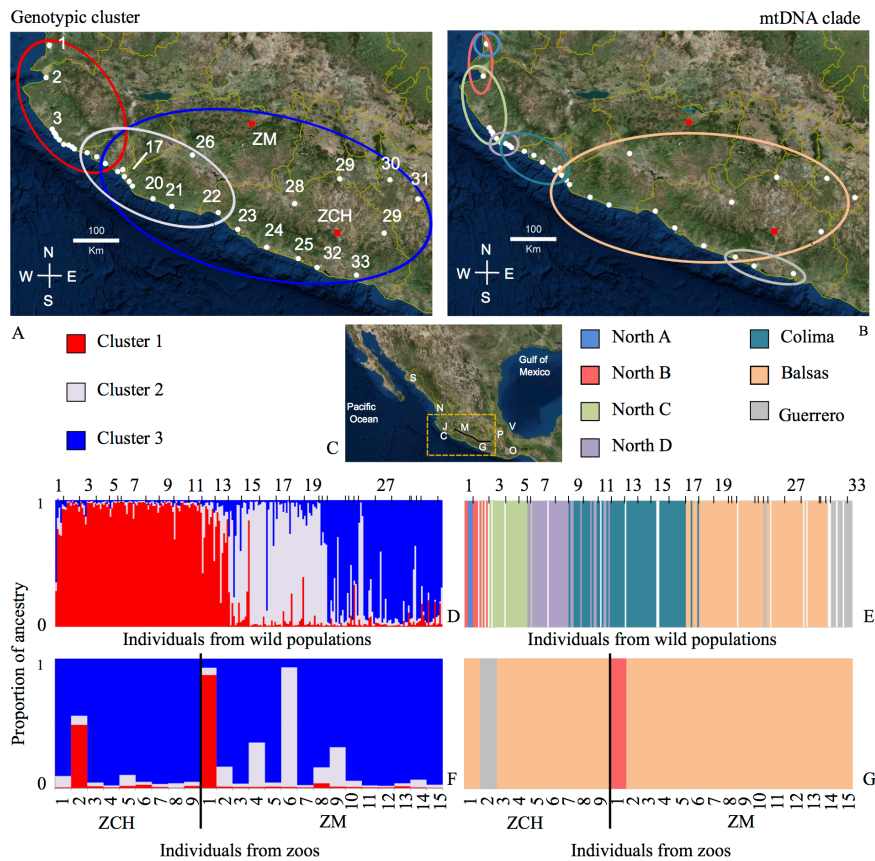Additionally, we applied the trained clustering

FIGURE 3. Geographic setting, sampling, and genetic population assignment of *C. pectinata* captive individuals from México. (A) Geographic distribution (ovals) of genotypic clusters inferred with STRUCTURE, and wild individual sampling localities (white dots). For simplicity only some localities are numbered. The location of Zoológico de Morelia (ZM) and Zoológico Zoochilpan (ZCH) are marked as red squares. Details of localities are listed in supplementary material available on request. (B) mtDNA clade distribution and sampling localities as in A. (C) Map of México with state names relevant for this study (S: Sinaloa; N: Nayarit; J: Jalisco; C: Colima; M: Michoacán; G: Guerrero; O: Oaxaca; P: Puebla; V: Veracruz), reduced data set geographic area (yellow square, as highlighted in A and B) and location of the Balsas Depression (black line). (D) STRUCTURE plot showing three clusters in the wild populations reduced dataset, each bar represents an individual's proportion of ancestry (Q value). For simplicity only some localities are numbered. (E) Bar plot showing mtDNA haplotype clade of each individual collected in wild populations; white bars are missing data. For simplicity only some localities are numbered. (F) Proportion of ancestry of the confiscated individuals harbored in two UMA zoos. (G) mtDNA haplotype of confiscated individuals harbored in zoos. In Fig. F and E, individuals collected in Zoológico Zoochilpan are shown on the left side of the black line (ZCH 1–9); individuals collected in Zoológico de Morelia are shown on the right side of the black line (ZM 1–15). Map sources: Esri, DigitalGlobe, GeoEye, i-cubed, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, the GIS User Community, INEGI, and CONABIO.

algorithm implemented in BAPS v6 (Corander et al. 2008), which uses baseline populations that include individuals of known origin to perform population assignment of individuals of unknown origin. To create the training data set, we combined microsatellite genotypes and mtDNA clade information of the 341 reference individuals, where each mtDNA clade was coded as diploid data with one unknown allele. We then input genotype and mtDNA information of the confiscated individuals to perform the assignment to any of the baseline populations. We performed five replicates and confirmed assignment consistency and likelihood variation among runs.

RESULTS

Individuals of unknown origin carried mtDNA haplotypes of the clades North B, Balsas, or Guerrero (Fig. 2). The STRUCTURE analyses suggest that there are three genetic clusters in the reduced dataset (Fig. 3A). These clusters are found from the state of Nayarit to Guerrero on the Pacific lowlands and the Balsas Depression (Fig. 3A, 3C). Ten of the 15 individuals harbored at Zoológico de Morelia were assigned to clusters 1, 2, or 3, with Q > 0.90 of ancestry, and three more individuals would have been assigned if we had used a less stringent, but acceptable threshold (Q > 0.80; Table 1). With this method alone it was not possible to pinpoint

a specific locality due to the inherent genetic homogeneity of the genetic clusters. However, given the geographic discordance between mtDNA and nuclear markers, when using both types of information, it is possible to narrow down the potential localities of origin. For example individual ZM01 has an mtDNA haplotype that is only found in localities 1 and 2 (Fig. 3B, 3E, 3G) with a genotype matching that of individuals in those localities. This interpretation is consistent with the outcome of BAPS, which assigns this individual to locality 2 (Table 1). Similarly, individual ZM06 has an mtDNA haplotype belonging to the Balsas clade, which is widespread in central México (localities 17–30; Fig. 3B), with its genotype belonging to cluster 2. This narrows down the possible localities of origin to sites where the combination of individuals with cluster 2 genotypes and Balsas clade mtDNA haplotypes occurs (i.e., localities 17–21 and 25–26; Fig. 3A). BAPS assigns this individual to localities 21 and 30 with equal probability. The assignment to locality 30 is probably due to a relatively low number of individuals collected in this locality ($n = 3$).

Individuals ZM04 and ZM09 show mixed ancestry with around 0.34 of their genome belonging to cluster 2 and around 0.64 to cluster 3 (Table 1). Although in the wild there are individuals with mixed ancestry, there are no localities with a high number of individuals with the above-described combination of ancestry, only some individuals collected in localities 22–26 and 30 (Fig. 3;

TABLE 1. Genetic population assignment of *Ctenosaura pectinata* individuals harbored in UMA zoos, in México, based on: (1) their proportion of ancestry (Q value) estimated with STRUCTURE (Clusters 1–3); (2) mtDNA clade they belong to as inferred with RAxML; and (3) locality number of origin assigned with BAPS. Cluster 1 is depicted in red, Cluster 2 in pink, and Cluster 3 in blue, and shown in Fig. 2A, 2D, and 2F. ZCH = samples collected in Zoochilpan Zoo; ZM = samples collected in Zoológico de Morelia.

Sample	Cluster 1 ancestry	Cluster 2 ancestry	Cluster 3 ancestry	mtDNA clade	BAPS assigned locality
ZM01	0.87	0.06	0.07	North B	2
ZM02	0.01	0.15	0.83	Balsas	26, 27
ZM03	0.01	0.03	0.96	Balsas	27, 28
ZM04	0.01	0.34	0.65	Balsas	22
ZM05	0.01	0.03	0.96	Balsas	28
ZM06	0.01	0.92	0.07	Balsas	21, 30
ZM07	0.01	0.02	0.98	Balsas	27
ZM08	0.04	0.12	0.84	Balsas	25, 26
ZM09	0.01	0.31	0.68	Balsas	21
ZM10	0.01	0.05	0.94	Balsas	22
ZM11	0.01	0.01	0.98	Balsas	27
ZM12	0.01	0.01	0.98	Balsas	26, 27
ZM13	0.02	0.02	0.96	Balsas	27
ZM14	0.01	0.06	0.93	Balsas	26, 27
ZM15	0.01	0.02	0.97	Balsas	26, 27
ZCH01	0.01	0.09	0.91	Balsas	27
ZCH02	0.49	0.07	0.44	Guerrero	33
ZCH03	0.02	0.03	0.96	Balsas	27
ZCH04	0.01	0.01	0.98	Balsas	27
ZCH05	0.02	0.08	0.90	Balsas	27
ZCH06	0.03	0.02	0.95	Balsas	26, 27
ZCH07	0.01	0.02	0.97	Balsas	22, 27
ZCH08	0.01	0.03	0.96	Balsas	26, 27
ZCH09	0.02	0.03	0.95	Balsas	27

supplementary material available on request). BAPS assigns ZM04 to locality 22 whereas ZM09 is assigned to locality 21 and 27. The latter individual carries a Balsas clade mtDNA haplotype (i.e., Genbank accession number EU246769; supplementary material available on request) that is not found in locality 21. Therefore, it is not clear if ZM09 originated in captivity or in the wild.

Eight of the sampled individuals harbored in Zoológico Zoochilpan were assigned to cluster 3 (Q > 0.90; Table 1; Fig. 3). Only one individual showed mixed ancestry and was assigned to cluster 1 and 3 with mtDNA haplotype belonging to the Guerrero clade. Cluster 1 and 3 do not have areas of overlap in nature that we know of. BAPS assigned this individual to locality 33 (Table 1). None of the genotyped individuals from locality 33 or from anywhere else in the wild showed this pattern and proportion of mixed ancestry, thus it is likely that this individual is a hybrid that originated in captivity. However, it is uncertain if the hybridization occurred in Zoochilpan. In general, the locality assignments with BAPS are consistent with the STRUCTURE cluster assignment.

DISCUSSION

The genetic database created and tested herein includes the genotype and mtDNA haplotype of hundreds of individuals sampled throughout the geographic distribution of *C. pectinata*. This database has proved useful in narrowing down potential areas of origin of confiscated individuals. BAPS allowed for assignment at the locality level that was consistent with cluster assignment performed with STRUCTURE. In other words, the locality assigned by BAPS was within the cluster assigned by STRUCTURE. Most of the individuals were assigned when setting a high threshold (i.e., Q > 0.90), or a slightly less stringent threshold (Q > 0.80). However, it was more challenging to assign individuals to specific localities when the individual demonstrated admixture or when there are several admixed wild populations. Indeed, previous studies have pointed out that population assignment with any method is less accurate when levels of gene flow between localities reflect an F_{ST} value of 0.05 or less (Latch et al. 2006; Vähä and Primmer 2006).

The strength of our study comes from the range-wide sampling and multilocus approach that allowed for the characterization of the geographic structure of this species throughout its more than 1,000 km long distribution. To further explore the utility of the database, more individuals with known origin and different levels of admixture should be used to verify the robustness of their genetic assignment.

Our results have direct implications for the management of captive populations. We show that UMA zoos in México do harbor individuals from various origins, sometimes having a genetic composition that is native to areas far away from the location of the UMA. It has been shown that even largely differentiated genotypic clusters (Zarza et al. 2011) and even *Ctenosaura* species (Gutsche and Köhler 2008; Zarza Franco 2008; Pasachnik et al. 2009) can exchange genes when in contact. Thus accidental or intentional releases may have unforeseen consequences in the local populations. Until the effects of hybridization and introgression on the adaptation of different genetic clusters are studied, translocation and releases should be avoided.

With the creation of a database including nuclear genotypes and mtDNA data, we aim to provide a resource for Mexican authorities and conservation organizations to identify the origin of confiscated individuals to either return the individuals to their area of origin or to place them in a UMA within a genetically similar area (Schwartz and Karl 2008). Genetic population assignment methods have assisted in determining the origin of confiscated Galápagos iguanas using mtDNA (Gentile et al. 2013). However, in the case of *C. pectinata*, given the discordant geographic distribution between maternally and paternally inherited markers, it is not possible to rely on mtDNA sequences alone to assign individuals to their population of origin. The use of maternally and bi-parentally inherited markers helps to discriminate among possible localities of origin and to improve genetic assignment as has been carried out in other iguana species (Tzika et al. 2008; Lanterbecq et al. 2010; Colosimo et al. 2014). The compiled database will also be useful in inferring the origin of the individuals introduced in non-natural ranges, e.g., USA and Grenada (Townsend et al. 2003; VHR pers. obs.). With this information, locations of illegal trade could be detected and special efforts made to stop poaching for the pet trade (Wasser et al. 2008).

Future genetic work, aided with new sequencing technologies, should aim to detect adaptive genomic variation associated with particular ecological or morphological traits (Allendorf et al. 2010; Angeloni et al. 2012). This will serve to identify regions under selection and associated single nucleotide polymorphisms. Recent conservation genomic developments suggest that genetic markers associated with genes under selection are especially powerful for population genetic assignment given their high degree of genetic differentiation (Nielsen et al. 2012).

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