Introgressive hybridization in a Spiny-Tailed Iguana, *Ctenosaura pectinata*, and its implications for taxonomy and conservation (#13787)

First submission

Please read the **Important notes** below, and the **Review guidance** on the next page.
When ready **submit online**. The manuscript starts on page 3.

Important notes

Editor and deadline

Tomas Hrbek / 6 Nov 2016

Files	5 Figure file(s) 5 Table file(s) 1 Raw data file(s) 1 Other file(s) Please visit the overview page to **download and review** the files not included in this review pdf.
Declarations	**One or more DNA sequences were reported.** **Involves vertebrate animals.** **Involves a field study on animals or plants.**

Review guidelines

Please in full read before you begin

How to review

When ready **submit your review online**. The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

You can also annotate this **pdf** and upload it as part of your review

To finish, enter your editorial recommendation (accept, revise or reject) and submit.

BASIC REPORTING

- Clear, unambiguous, professional English language used throughout.
- Intro & background to show context. Literature well referenced & relevant.
- Structure conforms to **PeerJ standard**, discipline norm, or improved for clarity.
- Figures are relevant, high quality, well labelled & described.
- Raw data supplied (See **PeerJ policy**).

EXPERIMENTAL DESIGN

- Original primary research within **Scope of the journal**.
- Research question well defined, relevant & meaningful. It is stated how research fills an identified knowledge gap.
- Rigorous investigation performed to a high technical & ethical standard.
- Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

- Impact and novelty not assessed. Negative/inconclusive results accepted. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
- Data is robust, statistically sound, & controlled.
- Conclusion well stated, linked to original research question & limited to supporting results.
- Speculation is welcome, but should be identified as such.

The above is the editorial criteria summary. To view in full visit **https://peerj.com/about/editorial-criteria/**

Introgressive hybridization in a Spiny-Tailed Iguana, *Ctenosaura pectinata*, and its implications for taxonomy and conservation

Eugenia Zarza [Corresp., 1, 2], **Victor Hugo Reynoso** [3], **Christiana M.A. Faria** [4, 5], **Brent C Emerson** [6]

[1] Moore Laboratory of Zoology, Occidental College, Los Angeles, California, United States
[2] Senckenberg Biodiversity and Climate Research Centre, Frankfurt am Main, Germany
[3] Departamento de Zoología, Universidad Nacional Autónoma de México, Ciudad de México, Mexico
[4] School of Biological Sciences, University of East Anglia, Norwich, United Kingdom
[5] Department of Biology/School of Science, Federal University of Ceara, Fortaleza, Ceara, Brazil
[6] Island Ecology and Evolution Research Group, Instituto de Productos Naturales y Agrobiología, La Laguna, Spain

Corresponding Author: Eugenia Zarza
Email address: eugenia.zarza@gmail.com

Introgression, the transmission of genetic material of one taxon into another through hybridization, can have various evolutionary outcomes. Previous studies have detected signs of introgression between western populations of the Mexican endemic and threatened spiny-tailed iguana, *Ctenosaura pectinata*. However, the extent of this phenomenon along the geographic distribution of the species is unknown. Here we use multilocus data together with detailed geographic sampling to (1) define genotypic clusters within *C. pectinata*; (2) evaluate geographic concordance between maternally and biparentally inherited markers; (3) examine levels of introgression between genotypic clusters, and (4) suggest taxonomic modifications in light of this information. Applying clustering methods to genotypes of 341 individuals from 49 localities of *C. pectinata* and the closely related *C. acanthura*, we inferred the existence of five genotypic clusters. Contact zones between genotypic clusters with signatures of interbreeding were detected, showing different levels of geographic discordance with mtDNA lineages. In northern localities, mtDNA and microsatellites exhibit concordant distributions, supporting the resurrection of *C. brachylopha*. Similar concordance is observed along the distribution of *C. acanthura*, confirming its unique taxonomic identity. Genetic and geographic concordance is also observed for populations within southwestern Mexico, where the recognition of a new species awaits in depth taxonomic revision. Contrarily, in western localities a striking pattern of discordance was detected where up to six mtDNA lineages co-occur with only two genotypic clusters. Given that the type specimen originated from this area, we suggest that individuals from western Mexico keep the name *C. pectinata.* Our results have profound implications for conservation, management, and forensics of Mexican

iguanas.

Introgressive Hybridization in a Spiny-Tailed Iguana, *Ctenosaura pectinata*, and its Implications for Taxonomy and Conservation.

Short title: Introgression in *Ctenosaura pectinata*

Eugenia Zarza[1,2#a] ,Víctor H. Reynoso[3], Christiana M.A. Faria[4#b] and Brent C. Emerson[5]

[1] Moore Laboratory of Zoology, Occidental College, Los Angeles, USA

[2] LOEWE-Biodiversity and Climate Research Centre, Frankfurt am Main, Germany

[3] Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, Distrito Federal, México

[4] School of Biological Sciences, University of East Anglia, Norwich, United Kingdom

[5] Island Ecology and Evolution Research Group, Instituto de Productos Naturales y Agrobiología (IPNA-CSIC), La Laguna, Spain

Corresponding author:

Eugenia Zarza

Email address: eugenia.zarza@gmail.com

[#a] Current Address: Laboratorio Nacional de Ciencias de la Sostenibilidad. Instituto de Ecología. Universidad Nacional Autónoma de México. AP 70-275, Ciudad Universitaria, UNAM, 04510, Ciudad de México, México

[#b] Current Address: Departamento de Biologia, Universidade Federal do Ceará, Campus do Pici, Bloco 906, Fortaleza, Ceará, CEP 60455-760, Brasil

Abstract

Introgression, the transmission of genetic material of one taxon into another through hybridization, can have various evolutionary outcomes. Previous studies have detected signs of introgression between western populations of the Mexican endemic and threatened spiny-tailed iguana, *Ctenosaura pectinata*. However, the extent of this phenomenon along the geographic distribution of the species is unknown. Here we use multilocus data together with detailed geographic sampling to (1) define genotypic clusters within *C. pectinata*; (2) evaluate geographic concordance between maternally and biparentally inherited markers; (3) examine levels of introgression between genotypic clusters, and (4) suggest taxonomic modifications in light of this information. Applying clustering methods to genotypes of 341 individuals from 49 localities of *C. pectinata* and the closely related *C. acanthura*, we inferred the existence of five genotypic clusters. Contact zones between genotypic clusters with signatures of interbreeding were detected, showing different levels of geographic discordance with mtDNA lineages. In northern localities, mtDNA and microsatellites exhibit concordant distributions, supporting the resurrection of *C. brachylopha*. Similar concordance is observed along the distribution of *C. acanthura*, confirming its unique taxonomic identity. Genetic and geographic concordance is also observed for populations within southwestern Mexico, where the recognition of a new species awaits in depth taxonomic revision. Contrarily, in western localities a striking pattern of discordance was detected where up to six mtDNA lineages co-occur with only two genotypic clusters. Given that the type specimen originated from this area, we suggest that individuals from western Mexico keep the name *C. pectinata.* Our results have profound implications for conservation, management, and forensics of Mexican iguanas.

45

46 Introduction

47 The role of introgression, the transmission of genetic material from one taxon into
48 another through hybridization, in shaping biodiversity is receiving increasing attention in
49 different taxa and geographic areas (e.g. Abbott et al., 2013; Haus, Roos & Zinner, 2013; Mallet,
50 Besansky & Hahn, 2016). There is evidence suggesting that introgression can increase the risk of
51 extinction in endangered species through genetic swamping, have deleterious effects in hybrids,
52 lead to adaptation by the emergence of novel genotypes or have no effect on the fate of a species
53 (Seehausen, 2004; Mallet, 2005; Frankham, 2006; Kronforst, 2012; Pardo-Diaz et al., 2012).
54 Given these various outcomes, it is particularly important to study the extent and impact of
55 introgression in biologically rich areas like Mesoamerica, where general patterns of genetic
56 diversity are just starting to be uncovered. This may have direct implications for species
57 delimitation and, ultimately, conservation and wildlife management (Gompert, 2012). The term
58 introgression has been primarily used to refer to gene flow between species, however more
59 recent works have applied the term to gene flow occurring between young divergent lineages
60 (Streicher et al., 2014). Here we used 'introgression' as in the latter instance.

61 The dry tropical forests of the western lowlands of Mexico are part of the Mesoamerica
62 Hot Spot (Myers et al., 2000). ~~Although~~ ~~m~~any phylogeographic studies have focused on this
63 area, only a few of them have employed a multilocus approach that can ~~allow for the detection of~~
64 introgression (e.g. Daza et al., 2009; Greenbaum, Smith & de Sá, 2011; Pringle et al., 2012;
65 Arbeláez-Cortés, Milá & Navarro-Sigüenza, 2014; Arbeláez-Cortés, Roldán-Piña & Navarro-
66 Sigüenza, 2014). In the spiny-tailed iguana *Ctenosaura pectinata*, distributed in the lowlands of
67 the Pacific slope and the Balsas Depression in Mexico (Smith & Taylor, 1950; Köhler, Schroth

& Streit, 2000), initial phylogeographic studies ~~uncovered~~ eight mtDNA lineages: North A, North B, North C, Colima, Balsas, Guerrero, Oaxaca and South (Fig. 1, Fig. S1; Zarza, Reynoso & Emerson, 2008). *Ctenosaura acanthura,* found in the lowlands of the Gulf of Mexico, ~~appeared~~ nested within the South lineage, whereas *C. hemilopha* and *C. similis* ~~appeared~~ as ~~clearly~~ distinct lineages (Zarza, Reynoso & Emerson, 2008).

Genetic distances (Tamura & Nei, 1993) between *C. pectinata* mtDNA lineages range from 4.11 to 11.57%, ~~these distances are~~ similar to ~~those~~ estimated among species of Iguanas of the genus *Cyclura* (Malone et al., 2000). The largest distances measured among *C. pectinata* lineages occur between the North and Colima ~~lineages~~ (Zarza, Reynoso & Emerson, 2008). This phylogeographic break occurs in the vicinity of the Trans-Mexican Volcanic Belt (TMVB; Fig. 1)~~,~~ on the central western coast of Mexico and ~~probably~~ occurred between 1.1 and 3.1 million years ago (Zarza, Reynoso & Emerson, 2008). This geological feature has attracted many biogeographers because ~~several highland and lowland taxa find their distribution limits here~~ (e.g. (Mastretta-Yanes et al., 2015; Zaldivar-Riverón, Leon-Regagnon & de Oca, 2004; Devitt, 2006; Mulcahy, Morrill & Mendelson, 2006; Bryson, García-Vázquez & Riddle, 2012; Blair et al., 2015)~~)~~. ~~Further~~ multilocus research and detailed geographic sampling of *C. pectinata* in this area revealed a ninth mtDNA lineage occurring between North C and Colima lineages: North D (Zarza, Reynoso & Emerson, 2011; Fig. 1). Interestingly, the North C, North D, Colima and Balsas mtDNA lineages show geographically discordant patterns with two clusters ~~defined~~ with microsatellite ~~nuclear~~ markers (Zarza, Reynoso & Emerson, 2011). The discordance ~~possibly~~ resulted from contemporary and/or past introgression among lineages coupled with male sex biased dispersal (Zarza, Reynoso & Emerson, 2011). It is unknown if geographic discordance

between mtDNA and microsatellite markers, and introgression are restricted to this part of *C. pectinata* distribution or if it is prevalent ~~between~~ other ~~neighboring lineages.~~

It is clear that molecular studies in *C. pectinata* have uncovered diversity that had been overlooked or not detected by the ~~latest~~ morphological revisions of the species and closely related taxa (Köhler, Schroth & Streit, 2000; Köhler, 2002). This is in contrast to ~~the very early~~ studies of the genus. Bailey (1928), ~~in a revision of the genus *Ctenosaura*~~ recognized five species (~~C.~~ *brachylopha, C. pectinata, C. acanthura, C. brevirostris, C. parkeri*) within the range of what ~~we currently know~~ as *C. pectinata*. He stated that *C. acanthura* was the most widely distributed in Mexico. *Ctenosaura pectinata* was restricted to Colima (Wiegmann, 1834) and Jalisco together with *C. brevisrostris*. *Ctenosaura brachylopha* was described as inhabiting the northern states of Nayarit and Sinaloa. Without giving any justification, Smith and Taylor (~~Smith & Taylor,~~ 1950) lumped *C. brachylopha, C. brevirostris* and *C. parkeri* with *C. pectinata* and restricted the name *C. acanthura* for iguanas from the Gulf of Mexico area. More recent morphological revisions have not recovered *C. brachylopha, C. brevirostris* or *C. parkeri* as distinct entities (Köhler, Schroth & Streit, 2000; Köhler, 2002).

In light of recent molecular studies and previous morphological classifications, ~~a revisit of~~ *C. pectinata* taxonomy is warranted. This species, threatened by hunting and habitat loss (Aguirre-Hidalgo & Reynoso, 1998; Faria et al., 2010), ~~may not~~ receive proper protection without a clear definition of its boundaries and genetic composition (Frankham, 2006). Taxonomic modifications should rely on a multilocus approach and comprehensive geographic sampling (Leaché & Fujita, 2010; Rittmeyer & Austin, 2012). This, in turn, can facilitate the identification of genotypic clusters: groups of individuals that have few or no intermediates when in contact (Mallet, 1995). Such groups may inter-grade freely at their boundaries, but be strongly

differentiated and relatively ~~constant~~ in morphology, genetics and ecology. This implies that species can be affected by gene flow, selection and history, but they are not necessarily defined by these processes (Mallet, 1995). Defining genotypic clusters is useful in cases where gene flow between otherwise differentiated clusters occurs, for example in contact zones, as might be the case of *C. pectinata*.

Here we use multilocus data from individuals sampled across the ranges of *C. pectinata* and the closely related *C. acanthura*. Our specific aims are to: (1) define genotypic clusters; (2) investigate the levels of geographic concordance between mtDNA lineages and genotypic clusters; (3) evaluate evidence for introgression between clusters, and; (4) re-define taxonomic entities based on maternally and biparentally inherited markers, and compare these to previous ~~proposals~~ (Bailey, 1928).

Materials and Methods

Sampling and Laboratory procedures

Spiny-tailed iguanas were collected between 2004 and 2006 using tomahawk traps, noosing or by hand within the distribution ~~range~~ of *C. pectinata* and *C. acanthura.* The narrow area of sympatry between *C. pectinata* and *C. hemilopha* in northern Mexico was excluded to avoid the inclusion of *C. hemilopha* alleles in the analyses (Zarza Franco, 2008; Fig. 1). All samples have been analyzed in previous studies (Zarza, Reynoso & Emerson, 2016, 2008, 2011; Faria, 2008; Zarza Franco, 2008; Faria et al., 2010) to obtain microsatellite and/or mtDNA data (see File S1 for details). Except for the mtDNA sequences and microsatellite genotypes produced by ~~(~~Zarza Franco~~,~~ 2008), data from previous studies ~~had~~ been deposited ~~in~~ GenBank (File S1) or

as supplementary material in (Zarza, Reynoso & Emerson, 2016). Here we make available two previously unpublished mtDNA sequences (GenBank accession numbers KT003209-KT003210) and microsatellite data (File S1) produced by (Zarza Franco, 2008) from three localities in northern Mexico (Fig. 1).

We gathered all the data available to us to create microsatellite and mtDNA datasets that are mostly overlapping regarding sample content. This study comprises samples from 53 of the localities sampled in the above-mentioned studies; individuals from 49 of these localities were included in the microsatellite dataset. In some instances, individuals failed to amplify for mtDNA in earlier studies, but were successfully genotyped (24 out 341 samples; File S1). All mtDNA lineages described in previous publications were represented in the mtDNA dataset analyzed herein (File S1; Fig. 1).

A thorough description of the methods can be found in (Zarza, Reynoso & Emerson, 2016, 2008, 2011; Faria et al., 2010); however a summarized version follows. From each individual, a 0.15 µl blood sample was taken from the caudal vein or a tail clip and preserved in ethanol. DNA samples were purified using a modified salt precipitation protocol (Aljanabi & Martinez, 1997). A 561 bp fragment of the mitochondrial ND4 gene was PCR amplified and sequenced using primers ND4, ND4Rev (Arèvalo, Davis & Sites, 1994), ND4F1 (Zarza, Reynoso & Emerson, 2008) and ND4R623 (Hasbún et al., 2005) with conditions described in (Zarza, Reynoso & Emerson, 2008). Individuals were genotyped with nine microsatellite markers. Loci Cthe12, Cthe37 (Blázquez, Rodríguez Estrella & Munguía Vega, 2006), Pec01, Pec03, Pec16, Pec20, Pec25, Pec73, and Pec89 (Zarza et al., 2009), were PCR amplified using conditions described in (2011) and run in two multiplexes that allow for loci separation by color

and size in an automated ABI prism 3730. Fragment size was visualized with the GeneMapper software version 4.0 (Applied Biosystems, Foster City, CA, USA).

The School of Biological Sciences Ethical Review Committee at the University of East Anglia approved this study as stated in an "Approval letter" to EZ. All efforts were made to minimize stress when taking blood samples, which were obtained under the permits SEMARNAT SGPA/DGVS/08239, SGPA/DGVS/ 02934/06, 03563/06 to VHR.

Data Analyses

Mitochondrial DNA data

A median joining haplotype network was calculated with Network (Bandelt, Forster & Rohl, 1999) to update previously proposed haplotype networks (Zarza, Reynoso & Emerson, 2008, 2011). SAMOVA 2.0 (Dupanloup, Schneider & Excoffier, 2002) was used to define groups of populations that are geographically homogeneous and maximally differentiated from each other and to estimate their hierarchical differentiation. One hundred initial independent processes were tested followed by 10,000 steps of the simulated annealing process, which maximizes the proportion of total genetic variance among groups. Analyses were run under scenarios of 2 to 15 groups (K) without geographic restrictions. The FCT index was used to select the best grouping, i.e. the most suitable K. This index reflects the among-group component of the overall genetic variance. We selected the number of groupings that maximizes ~~such~~ component, ~~meaning that under that scenario~~ the groups of populations are maximally differentiated from each other (Dupanloup, Schneider & Excoffier, 2002). To accomplish this, the most suitable K value was selected based on the observed changes of FCT among consecutive K values. We considered arbitrarily that the most suitable value of K is observed when there is a FCT change <1%

between two consecutive Ks. We refer to this as Δ FCT obtained as $FCT_{K+1} - FCT_K$, reflecting changes in the percentage of variation explained by FCT. Bar plots were created with R 2.15 (R Core Team, 2012) to show the mtDNA lineage of each individual as determined by the haplotype network (Fig. S1) and to illustrate the results of SAMOVA.

Microsatellite data

The software GENEPOP 4.1 (Rousset, 2008) was used to estimate allele and null allele frequencies, to perform tests for linkage disequilibrium between pairs of loci and, to detect deviations from Hardy-Weinberg equilibrium. FST values between localities were calculated with Arlequin 3.5 with the pairwise differences distance method (Excoffier & Lischer, 2010).

The possible number of genotypic clusters under a scenario of admixture was inferred with STRUCTURE 2.3.2 (Pritchard, Stephens & Donnelly, 2000). Simulations were run assigning a uniform prior for the parameter Alpha (degree of admixture) and estimating the allele frequency parameter (Lambda) assuming correlated allele frequencies. Ten iterations for each value of K (from K=2 up to K=10) were performed with ten million MCMC replicates after a burn-in period of 1,000,000. The most likely number of clusters was inferred with the method of Evanno et al. (2005) implemented in Structure Harvester (Earl & vonHoldt, 2012).

In addition to the STUCTURE analyses, SAMOVA 2.0 (Dupanloup, Schneider & Excoffier, 2002) was used to define groups of populations and to estimate their hierarchical differentiation applying the same criteria and parameters used for the mtDNA data. Bar plots were created with R to show STRUCTURE and SAMOVA results for each individual. Expected and observed heterozygosity, number of alleles and FST values between the resulting groups were calculated with Arlequin 3.5. Effective population size was estimated with the coalescent

method implemented in NeEstimator v2 (Do et al., 2014). Allelic richness and private allelic richness were calculated applying the rarefaction method implemented in ADZE 1.0 (Szpiech, Jakobsson & Rosenberg, 2008). The standardized sample size for the calculation was set to be equal to the smallest sample size across populations. The largest allowed fraction of missing data at any given group for a locus was 50%.

The software NewHybrids (Anderson & Thompson, 2002; Anderson, 2008) was used to calculate hybrid indices between the SAMOVA defined genotypic clusters. This method employs a Bayesian model in which parental and various classes of hybrids form a mixture from which the sample is drawn. Throughout the manuscript we apply the terminology used by NewHybrids when referring to 'hybrid' categories and indices calculated with this software. However, the individuals involved are not necessarily 'hybrids' in strict sense (i.e offspring resulting from inter-species mating).

We estimated the posterior probability P(z) that each individual in a pair of clusters (*X* and *Y*) falls into each of six hybrid classes: pure cluster *X*, pure cluster *Y*, F_1, F_2, cluster *X* backcross, cluster *Y* backcross. Five independent Markov chain Monte Carlo (MCMC) analyses were run for each pair of neighboring clusters with at least 300,000 sweeps after 10,000 burnin sweeps. Convergence of the MCMC was assessed visually with the variable plots generated by NewHybrids. P(z) values were averaged among the five independent runs. An individual was considered as belonging to a ~~pure or hybrid~~ class if it ~~had been~~ assigned with P(z) >0.8 (Anderson & Thompson, 2002).

Results

Mitochondrial data

Out of the 368 individuals included in this study, 344 were sequenced for a fragment of the ND4 mtDNA locus. An updated haplotype network is shown in Fig. S1, which includes the previously unpublished haplotypes KT003209- KT003210. In the SAMOVA analyses, a change of less than 1% in FCT was observed at K=10 (Table A and Fig. A in File S2). Under this K, 79% of the variation can be explained by variation among groups (Table 1). These groups (mt1-mt10 from now onwards) coincide almost entirely with the haplotype groups previously recovered by the mtDNA locus, and that were defined based on a haplotype network and nested clade analysis (Zarza, Reynoso & Emerson, 2008; Fig. 1, Fig. 2, Fig. 4 A-B). SAMOVA detected a subdivision (mt4, mt5) within the Colima mtDNA lineage, whereas individuals forming the North B mtDNA lineage were assigned to two different groups (mt1 and mt2). The Oaxaca mtDNA lineage was not ~~identified~~.

Microsatellite data

Genotypes of a total of 341 individuals from 49 localities were used. Number of samples per locality ranged from 1-15 (File S1; Fig. 2). Locus Pec25 suffered from null alleles at a frequency higher than 20% in twelve localities, thus it was not included in further analyses. Other loci are possibly affected by null alleles but in less than 10% of the localities, which may reflect local phenomena leading to homozygous excess, ~~or null alleles.~~ The remaining loci exhibited ~~from~~ 9 to 27 alleles among the sampled localities. The null hypothesis of random union of gametes was rejected in twelve localities, but only in one location (La Fortuna, see File S1) was deviation from Hardy-Weinberg equilibrium detected in more than one locus (Pec01, Pec03). After Šidák correction ($p<0.00007$), the null hypothesis of independence of genotypes at one locus from

genotypes at another locus could not be rejected. Pairwise FST values showed a wide range of genetic differentiation among localities, from non-differentiation (FST =0) to ~~great~~ differentiation (~~M~~aximum significant FST =0.66; Table S1).

SAMOVA analyses with microsatellite data showed a FCT change <1% under K=5 (from now onwards *Nuc1-*Nuc5; Table A ~~in File S2; Fig. B~~ in File S2). Under this scenario, around 22% of the variation is explained by variation among groups, whereas 71% of the variation was explained by variation within individuals (Table 1). These clusters differ from the mtDNA grouping schemes obtained with SAMOVA, but coincide with the clustering resulting from the STRUCTURE analysis as explained below. Allele number, observed heterozygosity, expected heterozygosity, inbreeding coefficient, effective population size for *Nuc1-*Nuc5 are shown in Table 2. The standardized sample size for the allelic and private allelic richness was 14. Locus Cthe12 was removed from these calculations because it had at least one grouping (i.e. groupings 4 and 5) with more than 50% missing data. Allelic and private richness mean and variance values are shown in Table 2. Genetic differentiation (FST values) between the SAMOVA groups is shown in Table 3.

STRUCTURE ~~analyses~~ suggest that the most likely number of genotypic clusters is seven, based on the Delta-K (ΔK) value. However we suspect that ΔK under K=7 is an artifact resulting from the large variation in likelihood values obtained with the previous K, K=6 (SD = 1231.92; Fig. C in File S2). After removing two runs that seemed to be outliers due to lower likelihood values, the SD under K=6 was greatly reduced (90.45). We then recalculated K. This time K=4 showed the highest ΔK (Fig. D in File S2; Fig. 3 D). Individuals were consistently assigned among runs. However these results differ from the clustering obtained with SAMOVA where further substructure in the southern part of the distribution was detected resulting in K=5.

Thus to establish the most likely number of K in the southern part of the distribution, further analyses were performed on a subset of individuals that included only iguanas collected south of Manzanillo (M in Fig. 2) and along the Gulf of Mexico. We refer to these analyses as South-SS from now onwards. Simulations for 10 million generations were run with K=2 – K=6, with 10 replicates each. K=4 showed the highest ΔK with consistent results among runs (Fig. E in File S2). When analyzing the entire dataset, only one cluster was detected between Manzanillo and Las Negras (between M and N in Fig. 2; Nuc 3 in Fig. 3 D), whereas two clusters were recognized in the South-SS analyses (Nuc 3a and Nuc 3b in Fig. 3 E). However, several individuals of Nuc 3a and 3b showed admixed ancestry, indicating weak geographic structure (Fig. 3 E). The division between Nuc 3a and 3b was not detected with SAMOVA. Two other clusters were identified with the South-SS analyses, one equivalent to *Nuc4 and the other comprising individuals identified as *C. acanthura* and equivalent to *Nuc5 (Fig. 2 and Fig. 3 C-E). Individuals forming these two clusters were consistently assigned among runs and in accordance with the assignment observed when analyzing the entire dataset.

Given the weak geographic structure observed between Nuc 3a and Nuc 3b and the lack of support for such subdivision with SAMOVA, we take a conservative approach and consider these as forming only one genotypic cluster (equivalent to *Nuc3 and Nuc 3). Both SAMOVA and STRUCTURE support the distinction between *Nuc 4 (Nuc 4) and Nuc *5 (Nuc 5, in the South-SS analyses). Taking into account the results of SAMOVA and STRUCTURE we recognize a total of five microsatellite genotypic clusters within the entire distribution of *C. pectinata* + *C. acanthura* (Fig. 2).

The microsatellite genotypic clusters detected with STRUCTURE (Nuc 1-Nuc 5) and SAMOVA (*Nuc1-*Nuc5) are geographically localized (Fig. 2). The limits of the clusters

296 defined with SAMOVA appear sharp, as this algorithm does not take admixture into account.
297 However, the presence of introgression is supported by the hybrid indices calculated with
298 NewHybrids between SAMOVA genotype clusters (Table 4). Sharp limits of clusters are not
299 observed in the genotypic clusters defined with STRUCTURE but admixed individuals and
300 zones of overlap are clearly observed (Fig. 2 and Fig. 3).

301 There are different levels of geographic concordance between the distribution of mtDNA
302 lineages North A, North B, North C, North D, Colima, Balsas, Guerrero, Oaxaca, and South as
303 described by (Zarza, Reynoso & Emerson, 2008, 2011; Fig. 1) and genotypic clusters (Fig. 2 and
304 Fig. 3). In northern Mexico, the distributions of genotypic cluster Nuc 1 (and *Nuc1) and the
305 North A mtDNA lineage are almost entirely concordant. Further south, in Central Mexico, Nuc 1
306 overlaps with Nuc 2. Most of the samples in the SAMOVA equivalent genotypic clusters (*Nuc1
307 and *Nuc2) were assigned to a 'pure' category with NewHybrids (Table 4). Only one F2 was
308 detected and 13 individuals could not be assigned to any category. However four of these
309 individuals had a posterior probability <0.2 of being a 'pure' individual. Thus, given the data and
310 the assumptions of the model, those four individuals have a posterior probability >0.8 of being
311 hybrids of some sort. Indeed, STRUCTURE plots show signs of interbreeding in the contact
312 zone (Fig. 3 D).

313 Individuals forming Nuc 2 have mtDNA haplotypes belonging to North A, North B,
314 North C, North D and Colima mtDNA lineages. Genotypic cluster Nuc 2 forms a contact zone
315 with Nuc 3. Individuals in this last cluster carry mtDNA haplotypes of Colima, Balsas and
316 Guerrero lineages. The geographically discordant patterns between mtDNA (North C-D, Colima,
317 Balsas) and microsatellite markers in this area (Nuc 2 and Nuc 3) have been previously detected
318 and described (Zarza, Reynoso & Emerson, 2011). In the equivalent SAMOVA clusters, 83

319 individuals were assigned to *Nuc2 pure class. Pure individuals of *Nuc3 were not found,
320 however 37 and 4 individuals were assigned to the F2 and *Nuc3 backcross hybrid classes
321 respectively (Table 4). Almost 50% of the individuals forming these clusters could not be
322 assigned to any category. Among these, 83 individuals showed a posterior probability <0.2 of
323 belonging to any of the pure classes, thus they might be hybrids of some sort. FST values
324 between these genotypic clusters are the lowest observed in the pairwise comparisons (Table 3).
325 Genotypic cluster Nuc 3 overlaps with Nuc 4, which is formed by individuals collected in
326 southeast Mexico with mtDNA haplotypes belonging to the Guerrero, Oaxaca and South mtDNA
327 lineages. Most of the individuals were assigned to one of the pure categories in the SAMOVA
328 equivalents *Nuc3 and *Nuc4 (Table 4). Only one F2 individual was found and 27 were not
329 assigned to any category. None of them had posterior probability <0.2 of belonging to any pure
330 class.
331 Nuc 4 and Nuc 5 do not seem to overlap. All individuals in the SAMOVA equivalents
332 *Nuc4 and *Nuc5 were assigned to a pure category with a posterior probability >0.8. Nuc 5
333 includes individuals described as *C. acanthura*, collected in eastern Mexico. It is geographically
334 concordant with the distribution of a mtDNA lineage closely related to the Southern mtDNA
335 lineage (2008). Admixture between *C. acanthura* and *C. pectinata* is only evident in Zapotitlán
336 de las Salinas (Fig. 2), with individuals carrying *C. acanthura* mtDNA haplotypes but with
337 nuclear ancestry of Nuc 3 and Nuc 5. The NewHybrids analysis between *Nuc3 and *Nuc5
338 detected two F2 individuals. One was collected in Zapotitlán de las Salinas, and the other in
339 Apatzingán (Fig. 2). The latter locality is not geographically close to the distribution limits of
340 Nuc 5 (or *Nuc5). Thus the potential of long distance dispersal, perhaps human mediated, should

be investigated. The remaining of the individuals was assigned to one of the pure categories and only four were not assigned to any hybrid or pure category.

Discussion

Introgression and geographic discordance between mtDNA and nuclear markers

Different degrees of discordance are observed in the geographic distribution of mtDNA lineages and microsatellite genotypic clusters across the range of *C. pectinata*. At one end of the spectrum, mtDNA North A lineage is almost entirely concordant with Nuc 1 cluster. At the opposite end of the spectrum, mtDNA lineages distributed along the central western coast of Mexico exhibit a striking discordant pattern where up to six geographically distinct mtDNA lineages (North A, North B, North C, North D, Colima, Balsas) co-occur with only two nuclear clusters (Nuc 2 and Nuc 3). This discordance between maternally and biparentally inherited markers in *C. pectinata* might be the result of several processes acting alone, in concert or at different points in time. For example under a scenario of short term refugia where populations decline throughout the range, resulting in isolation, followed by recent range expansion and male biased dispersal (Dubey et al., 2008; Johansson, Surget-Groba & Thorpe, 2008; Ujvari, Dowton & Madsen, 2008; Zarza, Reynoso & Emerson, 2011; Toews & Brelsford, 2012). The discordant pattern can also be the result of coalescence stochasticity (Irwin, 2002; Hickerson et al., 2010), selection of mtDNA (Dowling, Friberg & Lindell, 2008), differences in effective population size between mtDNA and nuclear markers.

Introgression, along current and past contact zones, ~~probably~~ also contributed to the patterns of geographic discordance in conjunction with other demographic phenomena. For example, it has been suggested that, in contact zones, selection and genetic drift can lead to mtDNA introgressing further and faster than nDNA. This is because mitochondrial genomes are less likely to hitchhike with a region under selection that prevents introgression (Ballard & Whitlock, 2004; Petit & Excoffier, 2009; Milá et al., 2013). Additionally, in small populations, genetic drift can allow the fixation of slightly deleterious alleles in the mtDNA of one population resulting in lower fitness than a related species in the same area. Selection could then drive introgression of mtDNA from the more fit population into the less fit population (Ballard & Whitlock, 2004). Furthermore, it is possible that some contact zones have changed location (Barton & Hewitt, 1985; Buggs, 2007), or that others have disappeared entirely as a result of complex climate mediated cycles of range expansion and contraction, or due to other phenomena. It is difficult to disentangle the effect of these processes with the currently available data. Sampling more finely along contact zones, and sequencing additional nuclear markers may permit coalescence analyses (Singhal & Moritz, 2012). Behavioral studies may also be informative to evaluate the effects of ecological, demographic, historical, and stochastic factors shaping the discordant patterns.

Interestingly, pairs of inter-breeding nuclear clusters with different levels of divergence occur throughout the distribution of *C. pectinata*. For example, allele frequency divergence between *Nuc1 and *Nuc2 is 0.18952, whereas it is 0.14815 between *Nuc2 and *Nuc3 (Table 3). Assignment of individuals to pure and hybrid classes also shows that contact zones have different hybrid compositions. A higher proportion of individuals were assigned to a pure class when analyzing *Nuc1 and *Nuc2 (89%) than when analyzing *Nuc2 and *Nuc3 (36%). This is

385 also observed in the STRUCTURE plots which reveal Nuc 2 and Nuc 3 admixed individuals
386 more frequently than admixed Nuc 1 and Nuc 2.
387 Thus *C. pectinata* constitutes an excellent system to better understand the process of
388 speciation by studying the effects of introgression between genotypic clusters at different stages
389 of divergence. Furthermore, this system potentially allows for the comparison of evolutionary
390 patterns and processes with contact zones in temperate and other tropical regions of the world
391 (Leaché & McGuire, 2006; McGuire et al., 2007; Singhal & Moritz, 2012; Miraldo et al., 2013;
392 Milá et al., 2013).
393

394 Implications for *Ctenosaura pectinata* taxonomy and conservation

395 Our results suggest that there are five nuclear genotypic clusters forming what is
396 currently considered *C. pectinata*. Individuals forming the Nuc 1 cluster belong to the North A
397 mtDNA lineage. Thus Nuc 1 and North A mtDNA lineages are geographically concordant. The
398 distribution of this genotypic cluster coincides with the distribution of *C. brachylopha* as revised
399 by Bailey (1928) using morphological data (i.e. states of Sinaloa, Nayarit, North of Jalisco and
400 Isla Isabel).
401 The observed concordance in the geographic distribution of nuclear and mtDNA might be
402 the result of stochastic coalescent processes, which is particularly true in taxa with low dispersal
403 rates, as is the case for iguanas (Irwin, 2002). Other phenomena such as natural selection could
404 be shaping the observed pattern, however this cannot be evaluated with the currently available
405 data. Another possibility is that the formation of a biogeographic barrier affected the distribution
406 of Nuc 1 and North A. Their southern distribution limit coincides approximately with the
407 TMVB. This geographic feature has been proposed as a geographic barrier for several lowland

taxa (Devitt, 2006; Mulcahy, Morrill & Mendelson, 2006; De-Nova et al., 2012; Arbeláez-Cortés, Milá & Navarro-Sigüenza, 2014; Arbeláez-Cortés, Roldán-Piña & Navarro-Sigüenza, 2014; Suárez-Atilano, Burbrink & Vázquez-Domínguez, 2014; Blair et al., 2015). However, given the complex geological history of the area, the TMVB barrier might not have affected all taxa equally (Mastretta-Yanes et al., 2015). Indeed, despite this barrier, gene flow has occurred in the recent past between Nuc 1 and the neighboring Nuc 2 at the limits of their distribution in the vicinity of the TMVB.

Gene flow has also been observed in a contact zone between Nuc 1 and *C. hemilopha* in the northern edge of Nuc 1 distribution (Zarza, Reynoso & Emerson, 2008). ~~In both,~~ northern and southern edges, gene flow seems to be limited to a narrow area. According to hybrid zone theory, several factors affect the extent, maintenance and shifting of hybrid zones: dispersal, selection, recombination rates and time since secondary contact (Barton & Hewitt, 1985).

The paradigm that lack of gene flow is a prerequisite to maintain species integrity is shifting (Abbott et al., 2013). In recent years evidence has accumulated suggesting that gene flow is an integral part of the process of speciation and that divergence can occur in the presence of gene flow (Mallet, 1995; Pinho & Hey, 2010; Leaché et al., 2014; Zarza, et al 2016). Indeed, if reproductive barriers have emerged in secondary contact zones, it is uncertain whether barriers to gene flow will be strengthened or broken down due to recombination and admixture (Barton & Hewitt, 1985; Abbott et al., 2013).

Despite the levels of gene flow detected and given the geographic concordance in the distribution of mtDNA and nuclear markers, the geographic limits that coincide with the geographic limits of other species, and the morphological signal detected by Bailey (Bailey,

1928), we suggest the resurrection of the name *Ctenosaura brachylopha* for populations inhabiting northwestern Mexico.

The distribution of Nuc 2 and Nuc 3 genetic clusters are geographically discordant with the distribution of mtDNA lineages in central Mexico (North A-D, Colima, Balsas). Maternal lineages seem to be more deeply structured than the genotypic clusters. The distribution of the maternally and paternally inherited markers and the high number of sampled admixed individuals suggest that, although there is some substructure in the area, gene flow among populations has been on going. Given that the holotype locality is labeled as "Colima" (Wiegmann, 1834) we suggest that these genotypic clusters keep the historical name *Ctenosaura pectinata*.

Iguanas described as *C. acanthura* also form a coherent nuclear cluster (Nuc 5) that is concordant with a mtDNA lineage closely related to the South lineage (Zarza, Reynoso & Emerson, 2008). Thus the name *Ctenosaura acanthura* should continue to be applied to populations of spiny-tailed iguanas in the coast of the Gulf of Mexico. Introgression seemed to have occurred in the area of Zapotitlán de las Salinas (Fig. 2), where individuals carry mtDNA haplotypes typical of *C. acanthura* and some alleles of Nuc 3 and Nuc 5.

Nuc 4 is almost entirely geographically concordant with the South mtDNA lineage, with some signs of mitochondrial introgression with the Oaxaca and Guerrero lineages. Thus Nuc 4 probably deserves taxonomic recognition at the species level, and awaits full description until morphological data is gathered and analyzed. In the meantime, we propose that these populations are recognized as an independent Evolutionary Significant Unit (Moritz, 1994) within *C. pectinata*.

We are aware that the modifications in taxonomy proposed in this paper are based entirely on molecular and geographic evidence. Morphological data have not revealed the existence of divisions within *C. pectinata*, at least with the approaches applied so far (Köhler, Schroth & Streit, 2000), except for the work of Bailey (Bailey, 1928). He realized that *C. brachylopha* resembles *C. pectinata* but may be distinguished from it by having a median dorsal crest that does not extend over the sacral region and that it is formed by 65 to 75 scales. He also noticed that the first seven whorls of spinous caudal scales are separated from each other by three rows of small flat scales. In *C. pectinata* the first five whorls of spinous scales are separated from each other by three rows of small flat scales, but subsequent whorls of spinous scales are separated by two rows of flat scales up the middle of the length of the tail (Bailey, 1928). These and other morphological characters need to be studied in depth, with a large sample and with more modern statistical methods to validate their utility to distinguish *C. brachylopha* from *C. pectinata,* and between groups within *C. pectinata* based on morphology. Color may be an important character too. ~~We have noticed that individuals~~ from northern Mexico ~~have~~ yellow coloration (Fig. S2), those in central Mexico show blue and orange patterns ~~whereas~~ individuals from the south are black and white. Bailey studied stuffed or ~~alcoholic~~ specimens that most likely lost their original color, so he did not address this character.

Our molecular approach has uncovered several genotypic clusters. However this may present challenges for the field biologist working in areas with high levels of admixture (i.e. central western Mexico) and with only morphological data at hand. Further research is needed to determine if coloration patterns or morphological characters of individuals outside the contact zones provide information for their assignment to a specific genotypic cluster.

This work provides important knowledge with profound implications in conservation, wildlife management and forensics. *Ctenosaura pectinata sensu lato* is a threatened species under the Mexican law (SEMARNAT, 2002). Measurements have been taken to protect its populations, however there are still gaps regarding re-introduction of confiscated individuals and/or their offspring. Ideally, the genetic origin of iguanas should be recognized before re-introduction to avoid admixture in populations that may lead to loss of diversity through hybridization, reduced viability or fertility in the case of genetic incompatibilities, reduced population fitness due to selective disadvantage of intermediate genotypes or loss of advantageous parental traits (Lynch, 1991; Burke & Arnold, 2001). Furthermore, our results suggest that *C. pectinata*, a species already recognized as threatened, is actually composed of multiple genotypic clusters that might be at a higher risk than previously thought, given their reduced geographical distributions and effective population sizes (Bickford et al., 2007).

Acknowledgements

EZ thanks the Mexican Council for Science and Technology (CONACyT) for funding this research during her PhD studies at the University of East Anglia where the data was generated. EZ and VHR would like to dedicate this work to the memory of Wendoli Medina Mantecón, a close collaborator and friend who will be remembered, among other things, for her efforts and dedication to the conservation of iguanas and other endangered species. Analyses and manuscript drafting by EZ were carried out during a postdoctoral position at the Biodiversity and Climate Research Centre funded by the 'LOEWE-Landes-Offensive zur Entwicklung Wissenschaftlich-ökonomischer Exzellenz' of Hesse's Ministry of Higher Education, Research, and the Arts.

References

Abbott R., Albach D., Ansell S., Arntzen JW., Baird SJE., Bierne N., Boughman J., Brelsford A., Buerkle CA., Buggs R., Butlin RK., Dieckmann U., Eroukhmanoff F., Grill A., Cahan SH., Hermansen JS., Hewitt G., Hudson AG., Jiggins C., Jones J., Keller B., Marczewski T., Mallet J., Martinez-Rodriguez P., Möst M., Mullen S., Nichols R., Nolte AW., Parisod C., Pfennig K., Rice AM., Ritchie MG., Seifert B., Smadja CM., Stelkens R., Szymura JM., Väinölä R., Wolf JBW., Zinner D. 2013. Hybridization and speciation. *Journal of Evolutionary Biology* 26:229–246. DOI: 10.1111/j.1420-9101.2012.02599.x.

Aguirre-Hidalgo V., Reynoso VH. 1998. Aspectos reproductivos de la iguana negra (Ctenosaura pectinata), en una población sujeta a alta incidencia de caza en el Istmo de Tehuantepec

Aljanabi SM., Martinez I. 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research* 25:4692–4693. DOI: 10.1093/nar/25.22.4692.

Anderson EC. 2008. Bayesian inference of species hybrids using multilocus dominant genetic markers. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 363:2841–2850. DOI: 10.1098/rstb.2008.0043.

Anderson EC., Thompson EA. 2002. A Model-Based Method for Identifying Species Hybrids Using Multilocus Genetic Data. *Genetics* 160:1217–1229.

Arbeláez-Cortés E., Milá B., Navarro-Sigüenza AG. 2014. Multilocus analysis of intraspecific differentiation in three endemic bird species from the northern Neotropical dry forest.

519 *Molecular Phylogenetics and Evolution* 70:362–377. DOI:
520 10.1016/j.ympev.2013.10.006.
521 Arbeláez-Cortés E., Roldán-Piña D., Navarro-Sigüenza AG. 2014. Multilocus phylogeography
522 and morphology give insights into the recent evolution of a Mexican endemic songbird:
523 Vireo hypochryseus. *Journal of Avian Biology* 45:253–263. DOI: 10.1111/j.1600-
524 048X.2013.00335.x.
525 Arèvalo E., Davis SK., Sites JW. 1994. Mitochondrial DNA Sequence Divergence and
526 Phylogenetic Relationships among Eight Chromosome Races of the Sceloporus
527 Grammicus Complex (Phrynosomatidae) in Central Mexico. *Systematic Biology* 43:387–
528 418. DOI: 10.1093/sysbio/43.3.387.
529 Bailey JW. 1928. A revision of the lizards of the genus Ctenosaura. *Proceedings of the United*
530 *States National Museum* 73:1–55.
531 Ballard JWO., Whitlock MC. 2004. The incomplete natural history of mitochondria. *Molecular*
532 *Ecology* 13:729–744. DOI: 10.1046/j.1365-294X.2003.02063.x.
533 Bandelt HJ., Forster P., Rohl A. 1999. Median-joining networks for inferring intraspecific
534 phylogenies. *Molecular Biology and Evolution* 16:37–48.
535 Barton NH., Hewitt GM. 1985. Analysis of hybrid zones. *Annual review of Ecology and*
536 *Systematics* 6:113–148.
537 Bickford D., Lohman DJ., Sodhi NS., Ng PKL., Meier R., Winker K., Ingram KK., Das I. 2007.
538 Cryptic species as a window on diversity and conservation. *Trends in ecology &*
539 *evolution* 22:148–155. DOI: 10.1016/j.tree.2006.11.004.
540 Blair C., Méndez de la Cruz FR., Law C., Murphy RW. 2015. Molecular phylogenetics and
541 species delimitation of leaf-toed geckos (Phyllodactylidae: Phyllodactylus) throughout

542 the Mexican tropical dry forest. *Molecular Phylogenetics and Evolution* 84:254–265.
543 DOI: 10.1016/j.ympev.2015.01.003.
544 Blázquez MC., Rodríguez Estrella R., Munguía Vega A. 2006. Characterization of 10
545 microsatellite loci in the spiny-tailed iguana Ctenosaura hemilopha. *Molecular Ecology*
546 *Notes* 6:753–755.
547 Bryson RW., García-Vázquez UO., Riddle BR. 2012. Relative roles of Neogene vicariance and
548 Quaternary climate change on the historical diversification of bunchgrass lizards
549 (Sceloporus scalaris group) in Mexico. *Molecular Phylogenetics and Evolution* 62:447–
550 457. DOI: 10.1016/j.ympev.2011.10.014.
551 Buggs RJA. 2007. Empirical study of hybrid zone movement. *Heredity* 99:301–312. DOI:
552 10.1038/sj.hdy.6800997.
553 Burke JM., Arnold ML. 2001. Genetics and the fitness of hybrids. *Annual Review of Genetics*
554 35:31–52. DOI: 10.1146/annurev.genet.35.102401.085719.
555 Daza JM., Smith EN., Páez VP., Parkinson CL. 2009. Complex evolution in the Neotropics: The
556 origin and diversification of the widespread genus Leptodeira (Serpentes: Colubridae).
557 *Molecular Phylogenetics and Evolution* 53:653–667. DOI:
558 10.1016/j.ympev.2009.07.022.
559 De-Nova JA., Medina R., Montero JC., Weeks A., Rosell JA., Olson ME., Eguiarte LE.,
560 Magallón S. 2012. Insights into the historical construction of species-rich Mesoamerican
561 seasonally dry tropical forests: the diversification of Bursera (Burseraceae, Sapindales).
562 *New Phytologist* 193:276–287. DOI: 10.1111/j.1469-8137.2011.03909.x.

563 Devitt TJ. 2006. Phylogeography of the Western Lyre snake (Trimorphodon biscutatus): testing
564 arid land biogeographical hypotheses across the Nearctic-Neotropical transition.
565 *Molecular Ecology* 15:4387–4407.
566 Do C., Waples RS., Peel D., Macbeth GM., Tillett BJ., Ovenden JR. 2014. NeEstimator v2: re-
567 implementation of software for the estimation of contemporary effective population size
568 (Ne) from genetic data. *Molecular Ecology Resources* 14:209–214. DOI: 10.1111/1755-
569 0998.12157.
570 Dowling DK., Friberg U., Lindell J. 2008. Evolutionary implications of non-neutral
571 mitochondrial genetic variation. *Trends in Ecology & Evolution* 23:546–554.
572 Dubey S., Brown GP., Madsen T., Shine R. 2008. Male-biased dispersal in a tropical Australian
573 snake (Stegonotus cucullatus, Colubridae). *Molecular Ecology* 17:3506–3514. DOI:
574 10.1111/j.1365-294X.2008.03859.x.
575 Dupanloup I., Schneider S., Excoffier L. 2002. A simulated annealing approach to define the
576 genetic structure of populations. *Molecular Ecology* 11:2571–2581.
577 Earl DA., vonHoldt BM. 2012. STRUCTURE HARVESTER: a website and program for
578 visualizing STRUCTURE output and implementing the Evanno method. *Conservation*
579 *Genetics Resources* 4:359–361. DOI: 10.1007/s12686-011-9548-7.
580 Evanno G., Regnaut S., Goudet J. 2005. Detecting the number of clusters of individuals using the
581 software STRUCTURE: a simulation study. *Molecular Ecology* 14:2611–2620.
582 Excoffier L., Lischer HEL. 2010. Arlequin suite ver 3.5: a new series of programs to perform
583 population genetics analyses under Linux and Windows. *Molecular Ecology Resources*
584 10:564–567. DOI: 10.1111/j.1755-0998.2010.02847.x.

585 Faria C. 2008. Conservation genetics of the Black Iguana - testing for multiple paternity and
586 assessing the use of microsatellites for forensic genetics. Norwich: Univeristy of East
587 Anglia,.
588 Faria CMA., Zarza E., Reynoso VH., Emerson BC. 2010. Predominance of single paternity in
589 the black spiny-tailed iguana: conservation genetic concerns for female-biased hunting.
590 *Conservation Genetics* 11:1645–1652. DOI: 10.1007/s10592-010-0047-2.
591 Frankham R. 2006. Evolutionary conservation genetics. In: Fox CW, Wolf JB eds. *Evolutionary*
592 *Genetics, Concepts and Case Studies*. New York: Oxford University Press, 502–512.
593 Gompert Z. 2012. Population genomics as a new tool for wildlife management. *Molecular*
594 *Ecology* 21:1542–1544. DOI: 10.1111/j.1365-294X.2012.05471.x.
595 Greenbaum E., Smith EN., de Sá RO. 2011. Molecular systematics of the Middle American
596 genus Hypopachus (Anura: Microhylidae). *Molecular Phylogenetics and Evolution*
597 61:265–277. DOI: 10.1016/j.ympev.2011.07.002.
598 Hasbún CR., Gómez A., Köhler G., Lunt DH. 2005. Mitochondrial DNA phylogeography of the
599 Mesoamerican spiny tailed lizards (Ctenosaura quinquecarinata complex): historical
600 biogeography, species status and conservation. *Molecular Ecology* 14:3095–3107.
601 Haus T., Roos C., Zinner D. 2013. Discordance Between Spatial Distributions of Y-
602 Chromosomal and Mitochondrial Haplotypes in African Green Monkeys (Chlorocebus
603 spp.): A Result of Introgressive Hybridization or Cryptic Diversity? *International*
604 *Journal of Primatology* 34:986–999. DOI: 10.1007/s10764-013-9717-5.
605 Hickerson MJ., Carstens BC., Cavender-Bares J., Crandall KA., Graham CH., Johnson JB.,
606 Rissler L., Victoriano PF., Yoder AD. 2010. Phylogeography's past, present, and future:

10 years after Avise, 2000. *Molecular Phylogenetics and Evolution* 54:291–301. DOI: 10.1016/j.ympev.2009.09.016.

Irwin DE. 2002. Phylogeographic breaks without geographic barriers to gene flow. *Evolution; international journal of organic evolution* 56:2383–2394.

Johansson H., Surget-Groba Y., Thorpe RS. 2008. Microsatellite data show evidence for male-biased dispersal in the Caribbean lizard Anolis roquet. *Molecular Ecology* 17:4425–4432.

Köhler G. 2002. *Schwarzleguane - Lebensweise, Haltung, Zucht,*. Offenbach: Herpeton.

Köhler G., Schroth W., Streit B. 2000. Systematics of the Ctenosaura group of lizards (Reptilia : Sauria : Iguanidae). *Amphibia-Reptilia* 21:177–191.

Kronforst MR. 2012. Mimetic Butterflies Introgress to Impress. *PLoS Genet* 8:e1002802. DOI: 10.1371/journal.pgen.1002802.

Leaché AD., Harris RB., Rannala B., Yang Z. 2014. The Influence of Gene Flow on Species Tree Estimation: A Simulation Study. *Systematic Biology* 63:17–30. DOI: 10.1093/sysbio/syt049.

Leaché AD., Fujita MK. 2010. Bayesian species delimitation in West African forest geckos (Hemidactylus fasciatus). *Proceedings of the Royal Society B: Biological Sciences* 277:3071–3077. DOI: 10.1098/rspb.2010.0662.

Leaché AD., McGuire JA. 2006. Phylogenetic relationships of horned lizards (Phrynosoma) based on nuclear and mitochondrial data: Evidence for a misleading mitochondrial gene tree. *Molecular Phylogenetics and Evolution* 39:628–644.

Lynch M. 1991. The Genetic Interpretation of Inbreeding Depression and Outbreeding Depression. *Evolution* 45:622–629. DOI: 10.2307/2409915.

629 Mallet J. 1995. A species definition for the modern synthesis. *Trends in Ecology & Evolution*
630 10:294–299. DOI: 10.1016/0169-5347(95)90031-4.
631 Mallet J. 2005. Hybridization as an invasion of the genome. *Trends in Ecology & Evolution*
632 20:229–237.
633 Mallet J., Besansky N., Hahn MW. 2016. How reticulated are species? *BioEssays: News and*
634 *Reviews in Molecular, Cellular and Developmental Biology* 38:140–149. DOI:
635 10.1002/bies.201500149.
636 Malone CL., Wheeler T., Taylor JF., Davis SK. 2000. Phylogeography of the Caribbean rock
637 iguana (Cyclura): Implications for conservation and insights on the biogeographic history
638 of the West Indies. *Molecular Phylogenetics and Evolution* 17:269–279. DOI:
639 10.1006/mpev.2000.0836.
640 Mastretta-Yanes A., Moreno-Letelier A., Piñero D., Jorgensen TH., Emerson BC. 2015.
641 Biodiversity in the Mexican Highlands and the interaction of geology, geography and
642 climate within the Trans-Mexican Volcanic Belt. *Journal of Biogeography* 42:1586-
643 1600.
644 McGuire JA., Linkem CW., Koo MS., Hutchison DW., Lappin AK., Orange DI., Lemos-Espinal
645 J., Riddle BR., Jaeger JR. 2007. Mitochondrial introgression and incomplete lineage
646 sorting through space and time: Phylogenetics of crotaphytid lizards. *Evolution* 61:2879–
647 2897. DOI: 10.1111/j.1558-5646.2007.00239.x.
648 Milá B., Surget-Groba Y., Heulin B., Gosá A., Fitze PS. 2013. Multilocus phylogeography of the
649 common lizard Zootoca vivipara at the Ibero-Pyrenean suture zone reveals lowland
650 barriers and high-elevation introgression. *BMC Evolutionary Biology* 13:192. DOI:
651 10.1186/1471-2148-13-192.

652 Miraldo A., Faria C., Hewitt GM., Paulo OS., Emerson BC. 2013. Genetic analysis of a contact
653 zone between two lineages of the ocellated lizard (Lacerta lepida Daudin 1802) in south-
654 eastern Iberia reveal a steep and narrow hybrid zone. *Journal of Zoological Systematics*
655 *and Evolutionary Research* 51:45–54. DOI: 10.1111/jzs.12005.
656 Moritz C. 1994. Defining "Evolutionarily Significant Units" for conservation. *Trends in Ecology*
657 *& Evolution* 9:373–375. DOI: 10.1016/0169-5347(94)90057-4.
658 Mulcahy DG., Morrill BH., Mendelson JR. 2006. Historical biogeography of lowland species of
659 toads (Bufo) across the Trans-Mexican Neovolcanic Belt and the Isthmus of
660 Tehuantepec. *Journal of Biogeography* 33:1889–1904.
661 Myers N., Mittermeier RA., Mittermeier CG., da Fonseca GAB., Kent J. 2000. Biodiversity
662 hotspots for conservation priorities. *Nature* 403:853–858. DOI: 10.1038/35002501.
663 Pardo-Diaz C., Salazar C., Baxter SW., Merot C., Figueiredo-Ready W., Joron M., McMillan
664 WO., Jiggins CD. 2012. Adaptive Introgression across Species Boundaries in Heliconius
665 Butterflies. *PLoS Genet* 8:e1002752. DOI: 10.1371/journal.pgen.1002752.
666 Petit RJ., Excoffier L. 2009. Gene flow and species delimitation. *Trends in Ecology & Evolution*
667 24:386–393. DOI: 10.1016/j.tree.2009.02.011.
668 Pinho C., Hey J. 2010. Divergence with Gene Flow: Models and Data. *Annual Review of*
669 *Ecology, Evolution, and Systematics* 41:215–230. DOI: 10.1146/annurev-ecolsys-
670 102209-144644.
671 Pringle EG., Ramírez SR., Bonebrake TC., Gordon DM., Dirzo R. 2012. Diversification and
672 phylogeographic structure in widespread Azteca plant-ants from the northern Neotropics.
673 *Molecular Ecology* 21:3576–3592. DOI: 10.1111/j.1365-294X.2012.05618.x.

674 Pritchard JK., Stephens M., Donnelly P. 2000. Inference of population structure using multilocus
675 genotype data. *Genetics* 155:945–959.
676 R Core Team 2012. *A language and environment for statistical computing.* Vienna, Austria: R
677 Foundation for Statistical Computing.
678 Rittmeyer EN., Austin CC. 2012. The effects of sampling on delimiting species from multi-locus
679 sequence data. *Molecular Phylogenetics and Evolution* 65:451–463. DOI:
680 10.1016/j.ympev.2012.06.031.
681 Rousset F. 2008. GENEPOP â€™ 007: a complete re-implementation of the GENEPOP software
682 for Windows and Linux. *Molecular Ecology Resources* 8:103–106.
683 Seehausen O. 2004. Hybridization and adaptive radiation. *Trends in Ecology & Evolution*
684 19:198–207. DOI: 10.1016/j.tree.2004.01.003.
685 SEMARNAT. 2002. NOM-059-ECOL-2001. *Diario Oficial de la Federación (segunda sección),*
686 *Miércoles 6 de marzo*.
687 Singhal S., Moritz C. 2012. Testing hypotheses for genealogical discordance in a rainforest
688 lizard. *Molecular ecology* 21:5059–5072. DOI: 10.1111/j.1365-294X.2012.05747.x.
689 Smith HM., Taylor EH. 1950. An annotated check list and key to the reptiles of Mexico
690 exclusive of snakes. *United States National Museum Bulletin* 199:vi, 253.
691 Streicher JW., Devitt TJ., Goldberg CS., Malone JH., Blackmon H., Fujita MK. 2014.
692 Diversification and asymmetrical gene flow across time and space: lineage sorting and
693 hybridization in polytypic barking frogs. *Molecular Ecology* 23:3273–3291. DOI:
694 10.1111/mec.12814.

695 Suárez-Atilano M., Burbrink F., Vázquez-Domínguez E. 2014. Phylogeographical structure
696 within Boa constrictor imperator across the lowlands and mountains of Central America
697 and Mexico. *Journal of Biogeography* 41:2371–2384. DOI: 10.1111/jbi.12372.
698 Szpiech ZA., Jakobsson M., Rosenberg NA. 2008. ADZE: a rarefaction approach for counting
699 alleles private to combinations of populations. *Bioinformatics (Oxford, England)*
700 24:2498–2504. DOI: 10.1093/bioinformatics/btn478.
701 Tamura K., Nei M. 1993. Estimation of the number of nucleotide substitutions in the control
702 region of mitochondrial DNA in humans and chimpanzees. *Molecular biology and*
703 *evolution* 10:512–526.
704 Toews DPL., Brelsford A. 2012. The biogeography of mitochondrial and nuclear discordance in
705 animals. *Molecular ecology* 21:3907–3930. DOI: 10.1111/j.1365-294X.2012.05664.x.
706 Ujvari B., Dowton M., Madsen T. 2008. Population genetic structure, gene flow and sex-biased
707 dispersal in frillneck lizards (Chlamydosaurus kingii). *Molecular Ecology* 17:3557–3564.
708 DOI: 10.1111/j.1365-294X.2008.03849.x.
709 Wiegmann AF. 1834. *Herp. Mex.* Berlin.
710 Zaldivar-Riverón A., Leon-Regagnon V., de Oca ANM. 2004. Phylogeny of the Mexican coastal
711 leopard frogs of the Rana berlandieri group based on mtDNA sequences. *Molecular*
712 *Phylogenetics and Evolution* 30:38–49.
713 Zarza E., Pereyra RT., Reynoso VH., Emerson BC. 2009. Isolation and characterisation of
714 polymorphic microsatellite markers in the black spiny tailed iguana (Ctenosaura
715 pectinata) and their cross-utility in other Ctenosaura. *Molecular Ecology Resources*
716 9:117–119.

717 Zarza Franco GE. 2008. Phylogeography and genetic analysis of secondary contact zones of
718 Ctenosaura pectinata and related species. Ph.D. dissertation Thesis. Norwich, UK:
719 Univeristy of East Anglia.
720
721 Zarza E., Reynoso VH., Emerson BC. 2008. Diversification in the northern neotropics:
722 mitochondrial and nuclear DNA phylogeography of the iguana Ctenosaura pectinata and
723 related species. *Molecular Ecology* 17:3259–3275. DOI: 10.1111/j.1365-
724 294X.2008.03826.x.
725 Zarza E., Reynoso VH., Emerson BC. 2011. Discordant patterns of geographic variation between
726 mitochondrial and microsatellite markers in the Mexican black iguana (Ctenosaura
727 pectinata) in a contact zone. *Journal of Biogeography* 38:1394–1405. DOI:
728 10.1111/j.1365-2699.2011.02485.x.
729 Zarza E., Reynoso VH., Emerson BC. 2016. Genetic tools for assisting sustainable management
730 and conservation of the spiny-tailed iguana, Ctenosaura pectinata. *Herpetology*
731 *Conservation and Biology* 11 (Monograph 6): 255-264.
732 Zarza, E., Faircloth, BC., Tsai, WLE., Bryson, RW., Klicka, J, McCormack, JE. 2016. Hidden
733 histories of gene flow in highland birds revealed with genomic markers. *Molecular*
734 *Ecology,* 25: 5144–5157. Doi: 10.1111/mec.13813

Figures

Figure 1. Geographic distribution of mtDNA lineages within *Ctenosaura pectinata* and *C. acanthura*. Localities are color-coded according to the SAMOVA group they form (mt1-mt10). Haplotype groups defined by Zarza et al (Zarza, Reynoso & Emerson, 2008) are delimited with lines. H: Area of overlap between *C. pectinata* and *C. hemilopha*, which was not included in the analyses. Data produced by (Zarza Franco, 2008) from three localities (numbered consecutively north to south 1-3) is made publicly available in this study for the first time (see File S1). Map generated using 'World Imagery' base map from ESRI ArcMap 10.1. World Imagery source: Esri, DigitalGlobe, GeoEye, i-cubed, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community. Modified from (Zarza, Reynoso & Emerson, 2008, 2011).

Figure 2. Geographic distribution of genotypic clusters in Mexico as estimated with SAMOVA (*Nuc1-*Nuc5) and STRUCTURE (Nuc 1 – Nuc 5). A: Apatzingán, M: Manzanillo, N: Las Negras, Z: Zapotitlán de las Salinas. Map created as in Fig. 1.

Figure 3. Bar plots showing population assignment and ancestry for individuals according to different methods. (A) MtDNA lineage of each individual as inferred from haplotype networks (Zarza, Reynoso & Emerson, 2008, 2011); (B) SAMOVA mtDNA groups detected under K=10; (C) microsatellite genotypic cluster defined with SAMOVA under K=5 and (D) STRUCTURE under K=4; (E) substructure estimated with STRUCTURE in a reduced data set (South-SS analyses). In STRUCTURE plots, the Y-axis represents proportion of ancestry. As this cannot be calculated with SAMOVA, values are always shown as 1. Each bar represents an individual. White bars are missing data.

Tables

Table 1. Sources of variation for mtDNA and microsatellite data calculated with SAMOVA under K=10 and K=5 respectively. Bold font indicates statistically significant values ($p<0.05$).

Table 2. Summary statistics per locus for genotypic clusters (*Nuc1-*Nuc5) defined with SAMOVA.

Table 3. Differentiation between SAMOVA clusters (FST values) as estimated with Arlequin 3.5. All values are statistically significant ($p<0.05$).

Table 4. Number of individuals assigned to each hybrid class according to NewHybrids. In all cases, SAMOVA defined clusters were compared.

Supplemental information

Figure S1. MtDNA haplotype network. Modified from (Zarza, Reynoso & Emerson, 2008, 2011); Haplotypes produced by (Zarza Franco, 2008) were added to the North A lineage and are highlighted with a red circle.

File S1. Sampling localities, geographic coordinates, haplotype accession numbers and genotype data of individuals included in this study, and summary of previous research outcomes.

File S2. SAMOVA K associated FCT values, Δ FCT plots; STRUCTURE K likelihoods and Δ K plots.

Table S1. FST values between pairs of localities estimated with Arlequin 3.5.

778 **Figure S2. A male spiny-tailed iguana from Sinaloa, northern Mexico, with the**
779 **characteristic yellow coloration. Here we propose that populations from northern Mexico**
780 **are referred as *Ctenosaura brachylopha*. Photo Credit: Eugenia Zarza.**
781

Table 1(on next page)

Sources of variation for mtDNA and microsatellite data calculated with SAMOVA under K=10 and K=5 respectively.

Bold font indicates statistically significant values ($p<0.05$).

Table 1. Sources of variation for mtDNA and microsatellite data calculated with SAMOVA under K=10 and K=5 respectively. Bold font indicates statistically significant values ($p<0.05$).

Marker	Source of variation	d.f.	Sum of squares	Variance components	% variation	Fixation indices
mtDNA	Among groups	9	3061.309	9.779	79.28	**FCT=0.793**
	Among populations within groups	43	332.944	1.002	8.12	**FSC=0.392**
	Within populations	291	452.013	1.553	12.59	**FST=0.874**
	Total	343	3846.266	12.334		
microsatellites	Among groups	4	268.732	0.517	21.66	**FCT=0.217**
	Among populations within groups	44	164.372	0.145	6.08	**FSC=0.078**
	Among individuals within populations	292	513.913	0.034	1.42	FIS=0.02
	Within individuals	341	577	1.692	70.84	**FIT=0.292**
	Total	681	1524.018	2.389		

Table 2(on next page)

Summary statistics per locus for genotypic clusters (*Nuc1-*Nuc5) defined with SAMOVA.

Table 2. Summary statistics per locus for genotypic clusters (*Nuc1-*Nuc5) defined with SAMOVA.

	*Nuc1				*Nuc2				*Nuc3			
L	**A**	**H_O**	**H_E**	**FIS**	**A**	**H_O**	**H_E**	**FIS**	**A**	**H_O**	**H_E**	**FIS**
1	4	0.48	0.49	0.01	13	0.86	0.86	0.00	12	0.79	0.82	0.05
2	2	0.41	0.50	0.19	6	0.33	0.35	0.05	7	0.44	0.45	0.01
3	8	0.85	0.80	-0.06	15	0.78	0.85	0.08	25	0.83	0.91	0.09
4	2	0.52	0.50	-0.03	7	0.45	0.45	0.00	8	0.74	0.80	0.07
5	3	0.78	0.68	-0.15	7	0.51	0.55	0.06	6	0.25	0.28	0.10
6	3	0.15	0.14	-0.05	11	0.77	0.84	0.09	10	0.50	0.66	0.25
7	6	0.59	0.62	N.A.	10	0.63	0.74	N.A.	14	0.55	0.74	N.A.
8	8	0.67	0.82	0.19	13	0.77	0.80	0.04	13	0.83	0.86	0.03
M	4.5	0.56	0.57		10.3	0.64	0.68		11.9	0.62	0.69	
s.d.	2.5	0.22	0.22		3.3	0.19	0.20		6.0	0.21	0.22	
n	27				105				131			
AR	3.29(2.64)				5.14(3.98)				5.54(4.65)			
PA	0.29(0.07)				0.62(0.18)				0.96(0.60)			
Ne	35.1 (0-176)				8.5 (1.8-20.5)				15.8 (5.8-30.8)			

L= Locus; A = Allele number; H_O = Observed heterozygosity; H_E = Expected heterozygosity; FIS = inbreeding coefficient; N.A. = missing data; m = monomorphic locus; n = number of individuals; M = Mean; s.d. = standard deviation; AR = Allele richness; PA = Private alleles mean (variance); Ne = Effective population size (Jackknife CI)

Table 2 continued.

	*Nuc4				*Nuc5			
L	**A**	**H_O**	**H_E**	**FIS**	**A**	**H_O**	**H_E**	**FIS**
1	14.	0.68	0.84	0.19	3	0.14	0.52	0.73
2	6	0.36	0.49	0.27	4	0.14	0.51	0.72
3	13	0.54	0.79	0.32	5	0.64	0.75	0.15
4	5	0.14	0.16	0.14	3	0.64	0.63	-0.02
5	5	0.63	0.71	0.13	2	0.07	0.07	0.00
6	11	0.72	0.84	0.14	2	0.29	0.48	0.41
7	4	0.07	0.58	0.10	m	m	m	N.A.
8	8	0.67	0.74		m	m	m	N.A.
M	8.3	0.48	0.65		3.2	0.32	0.49	
s.d.	3.9	0.26	0.23		1.2	0.26	0.23	
n	64				14			
AR	4.7(3.19)				2.56(1.38)			
PA	0.76(0.40)				0.53(0.29)			
Ne	22.5 (0-112.8)				1.9 (1.3-2.7)			

L= Locus; A = Allele number; H_O = Observed heterozygosity; H_E = Expected heterozygosity; FIS = inbreeding coefficient; N.A. = missing data; m = monomorphic locus; n = number of individuals; M = Mean; s.d. = standard deviation; AR = Allele richness; PA = Private alleles mean (variance); Ne = Effective population size (Jackknife CI)

Table 3(on next page)

Differentiation between SAMOVA clusters (FST values) estimated with Arlequin 3.5. All values are statistically significant ($p<0.05$).

Table 3. Differentiation between SAMOVA clusters (FST values) as estimated with Arlequin 3.5. All values are statistically significant ($p<0.05$).

	*Nuc1	*Nuc2	*Nuc 3	*Nuc 4
***Nuc1**	0			
***Nuc2**	0.18952	0		
***Nuc3**	0.26536	0.14815	0	
***Nuc4**	0.28768	0.18468	0.15797	0
***Nuc5**	0.44634	0.36999	0.32849	0.34052

Table 4(on next page)

Number of individuals assigned to each hybrid class according to NewHybrids. In all cases, SAMOVA defined clusters were compared.

Table 4. Number of individuals assigned to each hybrid class according to NewHybrids. In all cases, SAMOVA defined clusters were compared.

X,Y	Pure *NucX	Pure *NucY	F1	F2	*NucX Bc.	*NucY Bc.	Un-assigned	n (X+Y)
***Nuc1,*Nuc2**	26	92	0	1	0	0	13	132
***Nuc2,*Nuc3**	83	0	0	37	0	4	112	236
***Nuc3,*Nuc4**	110	56	0	0	2	0	27	195
***Nuc3,*Nuc5**	125	14	0	2	0	0	4	145
***Nuc4,*Nuc5**	14	64	0	0	0	0	0	78

X,Y = SAMOVA-defined Genotypic cluster compared. As in the main text, tables and figures, the *Nuc prefix denotes SAMOVA defined genotypic cluster. Bc = backcross

Figure 1

Geographic distribution of mtDNA lineages within *Ctenosaura pectinata* and *C. acanthura*.

Localities are color-coded according to the SAMOVA group they form (mt1-mt10). Haplotype groups defined by Zarza et al (2008) are delimited with lines. H: Area of overlap between *C. pectinata* and *C. hemilopha*, which was not included in the analyses. Data produced by (Zarza Franco, 2008) from three localities (numbered consecutively north to south 1-3) is made publicly available in this study for the first time (see File S1). Map generated using 'World Imagery' base map from ESRI ArcMap 10.1. World Imagery source: Esri, DigitalGlobe, GeoEye, i-cubed, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community. Modified from (Zarza, Reynoso & Emerson, 2008, 2011)

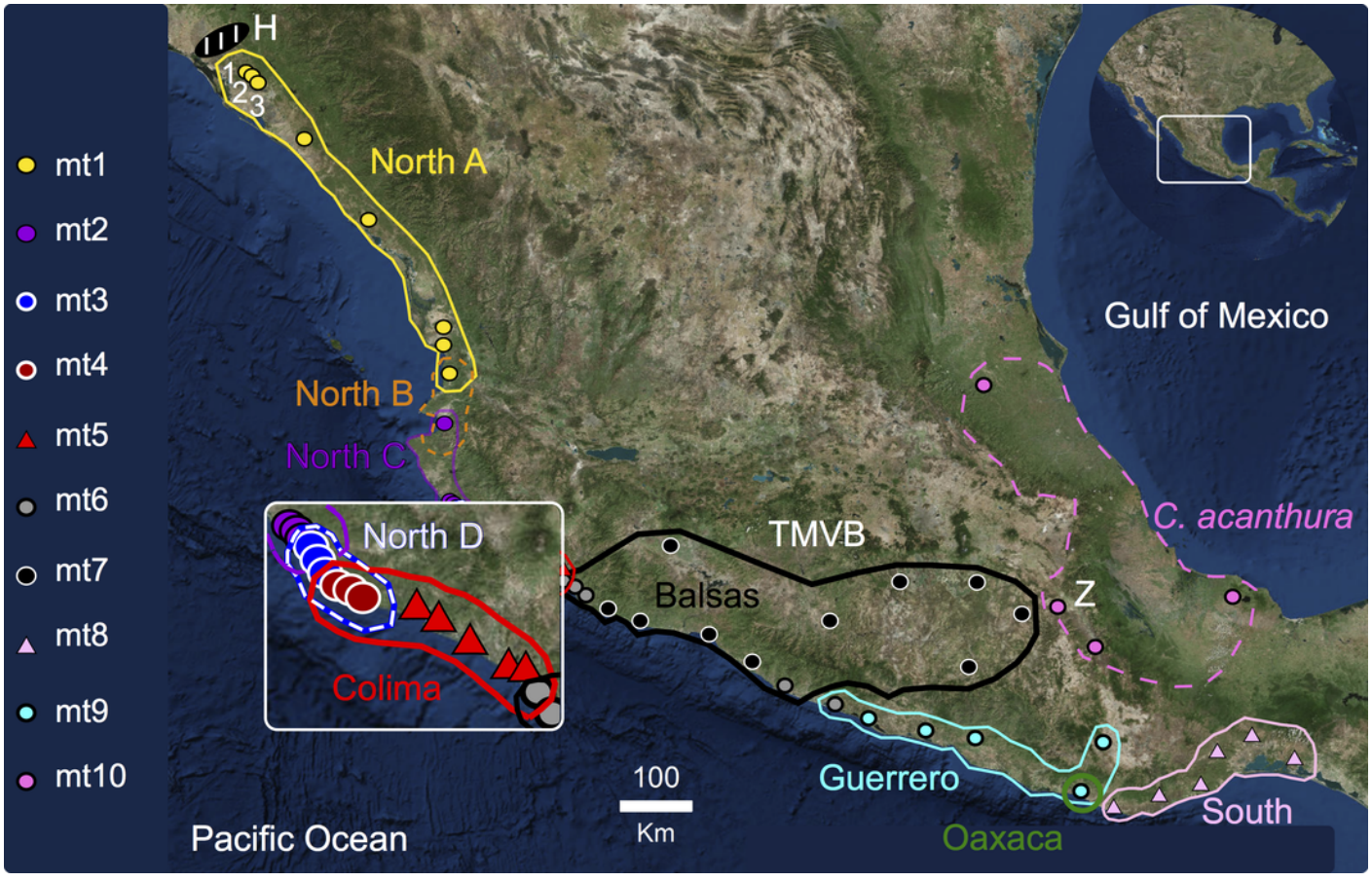

Figure 2

Geographic distribution of genotypic clusters in Mexico as estimated with SAMOVA (*Nuc1-*Nuc5) and STRUCTURE (Nuc 1 – Nuc 5)

A: Apatzingán, M: Manzanillo, N: Las Negras, Z: Zapotitlán de las Salinas. Map created as in Fig. 1.

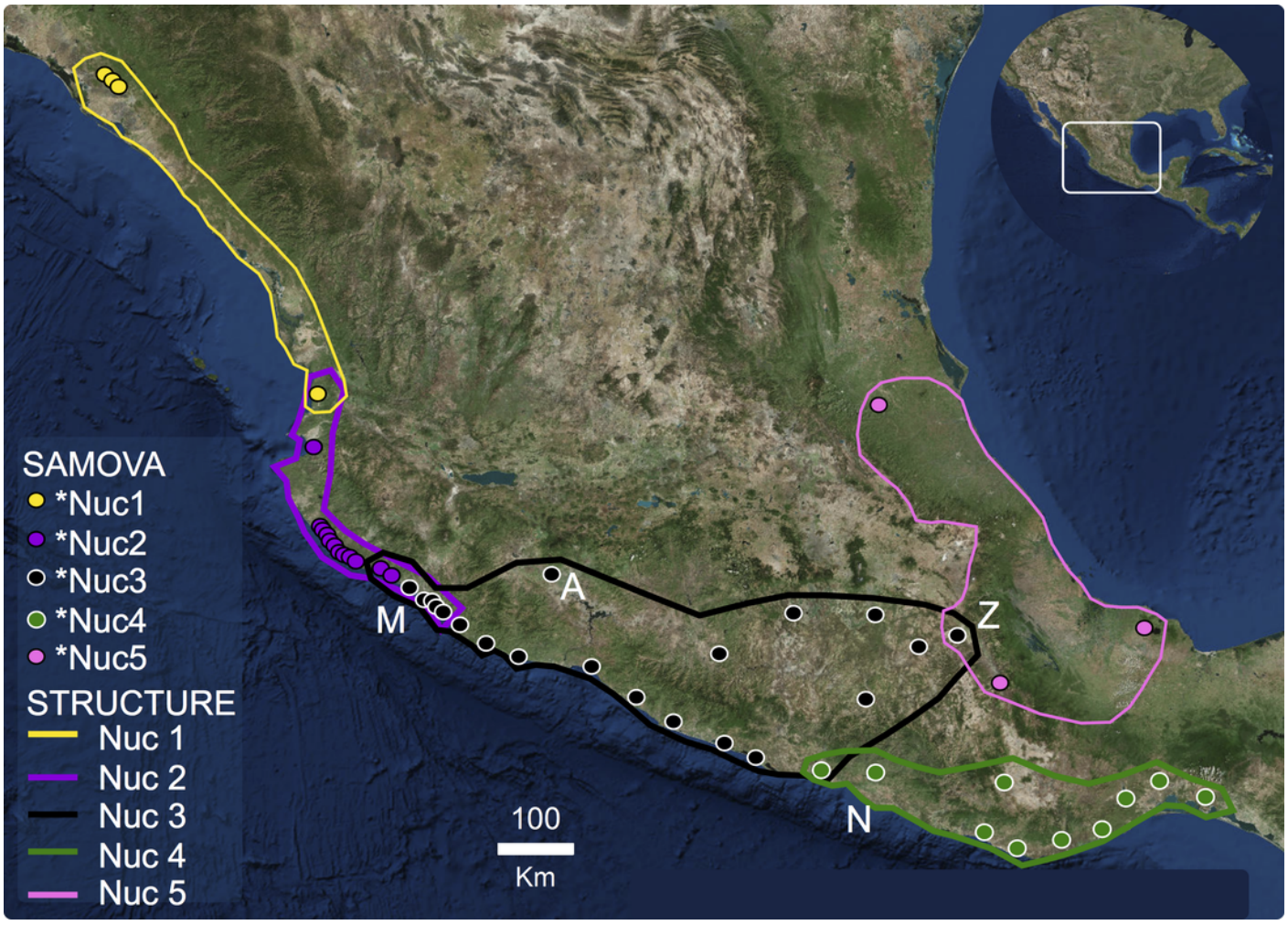

Figure 3

Bar plots showing population assignment and ancestry for individuals according to different methods.

(A) MtDNA lineage of each individual as inferred from haplotype networks (Zarza, Reynoso & Emerson, 2008, 2011); (B) SAMOVA mtDNA groups detected under K=10; (C) microsatellite genotypic cluster defined with SAMOVA under K=5 and (D) STRUCTURE under K=4; (E) substructure estimated with STRUCTURE in a reduced data set (South-SS analyses). In STRUCTURE plots, the Y-axis represents proportion of ancestry. As this cannot be calculated with SAMOVA, values are always shown as 1. Each bar represents an individual. White bars are missing data.