



Phylogeography of a widespread lizard complex reflects patterns of both geographic and ecological isolation

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Abstract

A primary challenge for modern phylogeography is understanding how ecology and geography, both contemporary and historical, shape the spatial distribution and evolutionary histories of species. Phylogeographic patterns are the result of many factors, including geology, climate, habitat, colonization history and lineage-specific constraints. Assessing the relative influences of these factors is difficult because few species, regions and environments are sampled in enough detail to compare competing hypotheses rigorously and because a particular phylogeographic pattern can potentially result from different evolutionary scenarios. The silky anoles (*Anolis sericeus* complex) of Central America and Mexico are abundant and found in all types of lowland terrestrial habitat, offering an excellent opportunity to test the relative influences of the factors affecting diversification. Here, we performed a range-wide statistical phylogeographic analysis on restriction site-associated DNA (RAD) markers from silky anoles and compared the phylogeographic patterns we recovered to historical and contemporary environmental and topographic data. We constructed niche models to compare niche overlap between sister lineages and conducted coalescent simulations to characterize how the major lineages of silky anoles have diverged. Our results revealed that the mode of divergence for major lineage diversification events was geographic isolation, resulting in ecological divergence between lineages, followed by secondary contact. Moreover, comparisons of parapatric sister lineages suggest that ecological niche divergence contributed to isolation by environment in this system, reflecting the natural history differences among populations in divergent environments.

KEYWORDS

ecological niche model, habitat isolation, isolation-by-environment, Nuclear Central America, phylogeography, silky anoles

1 | INTRODUCTION

Patterns of evolutionary divergence result from many historical and contemporary factors. Phylogeography has traditionally focused on geographic factors—distance, topography and physical landscape

barriers—that can shape lineage diversification and the spatial distribution of lineages on a landscape (Avice, 2000; Kidd & Ritchie, 2006). However, isolating mechanisms that drive lineage divergence range from strictly abiotic to biotic (Coyne & Orr, 2004; Mayr, 1963; Nosil, 2008), and thus, environmental and ecological variation

between lineages can also generate phylogeographic structure (Kozak, Graham, & Wiens, 2008; Paz, Ibáñez, Lips, & Crawford, 2015; Zellmer, Hanes, Hird, & Carstens, 2012; Zink, 2014). For instance, divergent natural selection in different environments can lead to isolation due to local adaptation, variation in reproductive timing tied to different environments can generate reproductive barriers, and novel habitat avoidance can lead to isolation between divergent environments (Coyne & Orr, 2004). Although these alternative drivers of phylogeographic and population genetic structure are becoming more widely appreciated (Paz et al., 2015; Wang, Glor, & Losos, 2013), relatively few studies have explicitly examined the relative roles of geographic and ecological factors in explaining phylogeographic patterns (Sexton, Hangartner, & Hoffman, 2014), particularly at different stages of diversification.

Moreover, geographic and ecological isolating factors can often act in concert, making it difficult to identify the primary mode of diversification (Coyne & Orr, 2004; Mayr, 1963; Nosil, 2008; Wang & Bradburd, 2014). For example, a pattern of ecologically divergent lineages occupying different parts of geographic space could result from several different processes. Allopatric lineages may diverge ecologically as a by-product of evolving independently in different environments while geographically isolated (Mayr, 1963), or lineages may become geographically isolated because ecological divergence causes their distributions to shift apart due to the spatial structure of habitats and environmental variables (Wang & Bradburd, 2014). Hence, populations may diverge ecologically during geographic isolation or may become geographically isolated due to ecological divergence, and the resulting spatial patterns may be indistinguishable. Therefore, identifying whether diversification results primarily from geographic isolation or ecological isolation (e.g., habitat isolation; Mayr, 1942) has long been extremely difficult (Coyne & Orr, 2004). Now, however, advances in ecological niche modelling and coalescent modelling make it possible to reconstruct the geographic distributions of lineages at different stages of their diversification. Species that are distributed widely across environmentally and geographically heterogeneous landscapes provide the power to distinguish between the processes driving phylogeographic patterns and are particularly valuable for understanding the geography and ecology of lineage diversification.

Nuclear Central America is composed of the mountainous region east of the Isthmus of Tehuantepec (Mexico) to Honduras. This region is a biodiversity hot spot with complex topography and substantial environmental variation (Morrone, 2014; Ramamoorthy, Bye, Lot, & Fa, 1993), providing an excellent study landscape for phylogeographic analyses. Mountain chains of varying ages effectively separate lowland communities in the “core” of the region and contribute to the formation of disparate environmental regimes scattered across the area (Flores-Villela & Martínez-Salazar, 2009; Stuart, 1966). Species inhabiting low elevations in southern Mexico and northern Central America encounter a variety of potential isolating mechanisms both ecological (in the form of environmental gradients and habitat transitions) and topographical (Hulseley, García de León, Johnson, Hendrickson, & Near, 2004; Rovito, Parra-Olea,

Vásquez-Almazán, Luna-Reyes, & Wake, 2012; Zaldívar-Riverón, León-Regagnon, & Nieto-Montes de Oca, 2004). The presence of these environmental and topographical barriers might explain the paucity of forms that occur on both the Caribbean and Pacific versants of Nuclear Central America (Morrone, 2014). Habitat connectivity in lowlands between both versants occurs primarily around the margins of the southeastern and northwestern boundaries of Nuclear Central America: the “porous” and increasingly continuous lowlands of Honduras to the east and the low-lying Isthmus of Tehuantepec to the west (Figure 1). Hence, the topography and environmental variation exhibited in this region offers excellent opportunities to investigate patterns of geographic and environmental isolation.

Phylogeographic studies of species groups distributed widely and continuously across diverse environments play an important part in understanding how geographic and ecological isolation shape evolutionary histories. Few terrestrial vertebrate species are as widely and abundantly distributed in and around Nuclear Central America as the silky anoles (*Anolis sericeus*, *A. unilobatus*, *A. ustus* and *A. wellbornae*). The four species currently recognized (Köhler & Vesely, 2010; Lara-Tufiño, Nieto-Montes de Oca, Ramirez Bautista, & Gray, 2016) are continuously distributed in nearly all types of lowland habitat from northern Mexico to northern Costa Rica (Henderson & Fitch, 1975; Lee, 1980; Stuart, 1955). Silky anoles exhibit substantial external morphological conservatism overall (Köhler & Vesely, 2010) but considerable within- and between-population variation in certain traits surrounding the mountains of Chiapas, Mexico and Guatemala (Köhler & Vesely, 2010; Lee, 1980; Stuart, 1955). Lee's (1980) in-depth look at scale traits in the silky anoles suggested a pattern of local adaptation and concluded that “morphological similarity in populations is largely independent of geographical proximity.” Some geographically distant populations exhibited convergence in scale traits, which have been linked to variation in humidity or precipitation in other anole species (Calsbeek, Knouft, & Smith, 2006; Malhotra & Thorpe 1997). These environment-associated differences expressed by silky anole populations raise the possibility that ecological divergence could contribute to genetic isolation. Recent methods for assessing niche divergence through the use of ecological niche models (ENMs) provide an opportunity to test relative niche divergence between populations or clades (Warren, Glor, & Turelli, 2008, 2010). Research on niche divergence in birds (Peterson, Soberon, & Sanchez-Cordero, 1999) and Cuban *Anolis* lizards (Warren, Glor, & Turelli, 2008) has suggested environmental niche traits can be remarkably labile and may be associated with speciation events. However, the ability to investigate the importance of niche evolution for population divergence can be obstructed by difficulties related to sampling and environmental variation present within the distribution of the focal group (Peterson, 2011). For instance, some organisms either do not have enough occurrence data to allow for accurate characterization of their environmental niches or are not distributed across environments that are variable enough to detect differences using available methods. The silky anoles of Central America and Mexico, however, fulfil the requirements for

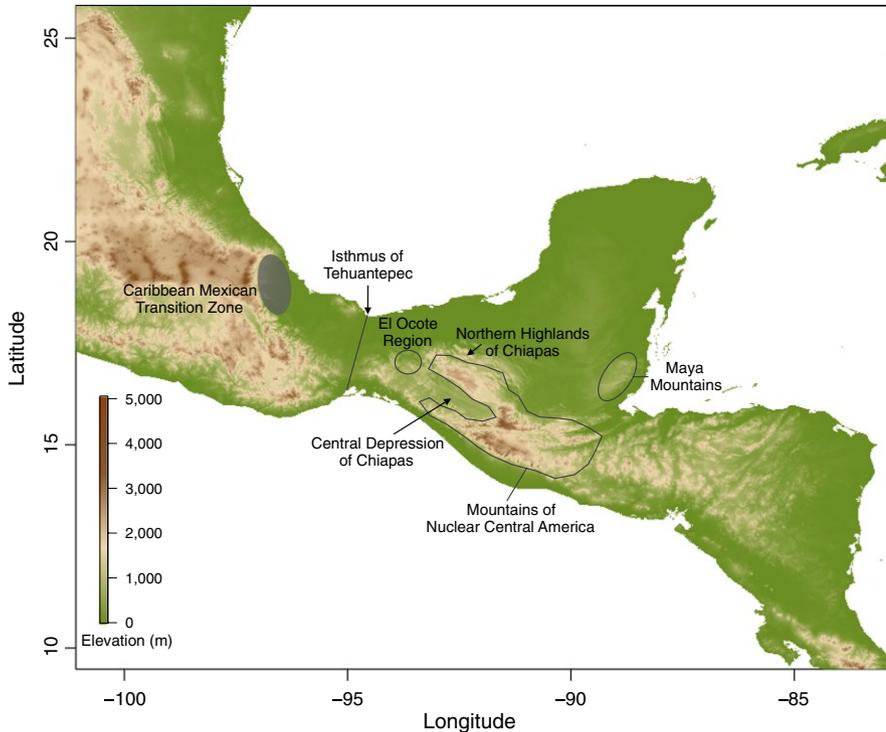


FIGURE 1 Topographic map of study region with important geographic features highlighted [Colour figure can be viewed at wileyonlinelibrary.com]

accurate, informative ENM comparisons that can shed light on whether niche divergence can be an important factor contributing to genetic isolation between phylogeographic lineages.

In this study, we investigate the factors driving patterns of phylogeographic divergence in silky anoles based on large restriction site-associated DNA sequence (RADseq) and GIS data sets, phylogenetic reconstruction, coalescent model testing, ecological niche modelling and explicit tests of niche divergence. We consider a set of hypotheses to describe the phylogeographic structure in this system: (1) phylogeographic structure reflects only geographic isolation, (2) phylogeographic structure is associated with ecological niche divergence that is the by-product of divergence in allopatry, and (3) phylogeographic structure is associated with ecological niche divergence that resulted in environmental isolation between lineages. To test these hypotheses, we consider past and present physical geographic barriers and infer past environmental niche suitability to determine the likelihood of past geographic isolation events due to dramatic changes in climatic regimes. Using a diffusion approximation-based method ($\delta a \delta i$) for inferring demographic model parameters from single nucleotide polymorphism (SNP) allele frequency data, we evaluated the demographic context of divergence between lineages to determine the likelihood of a past geographic isolation event. We also collectively weigh evidence from both the ENM analyses and coalescent analyses to evaluate whether ecological isolation played a role in the diversification of this system. If phylogeographic structure reflects only geographic isolation (Hypothesis 1), then phylogenetic breaks should occur in concert with geographic barriers rather than environmental transitions, and we expect $\delta a \delta i$ coalescent analyses to support a model of no (or greatly limited) gene flow. If phylogeographic structure is associated with ecological divergence,

then we expect to find phylogenetic breaks associated with environmental transitions and low overlap in the modelled niches of different lineages. If ecological divergence occurred as a by-product of allopatric isolation (Hypothesis 2), then coalescent analyses should identify support for a model of past isolation followed by secondary contact over models that shows constant or reduced gene flow over time. If, on the other hand, ecological divergence led to lineage isolation (Hypothesis 3), then coalescent analyses should support a model of divergence with historical gene flow (that was reduced as niches diverged) over models with constant gene flow (historical and present) or with a period of allopatry (either no migration or past isolation followed by secondary contact).

2 | MATERIALS AND METHODS

2.1 | Sampling for phylogeographic analyses

We sampled 90 individuals from 46 localities throughout the distribution of the four species currently recognized in the *Anolis sericeus* complex (Figure 2), from near the northwestern extent of the group in northern Mexico to near the southeastern extent close to the Nicaragua–Costa Rica border. Species boundaries for this clade are currently in flux (Lara-Tufiño et al., 2016); therefore, we focused more on geographic than taxonomic coverage. We were able to include samples spanning a broad range of habitats and environmental conditions inhabited by silky anoles. Some of the wettest and driest portions of the distribution were sampled, as both extremes occur in southern Mexico (Supporting Information Figure S1). GPS coordinates for each sample were collected in the field using Garmin GPS devices with accuracy of ~3–5 m. Sampling in the northwestern

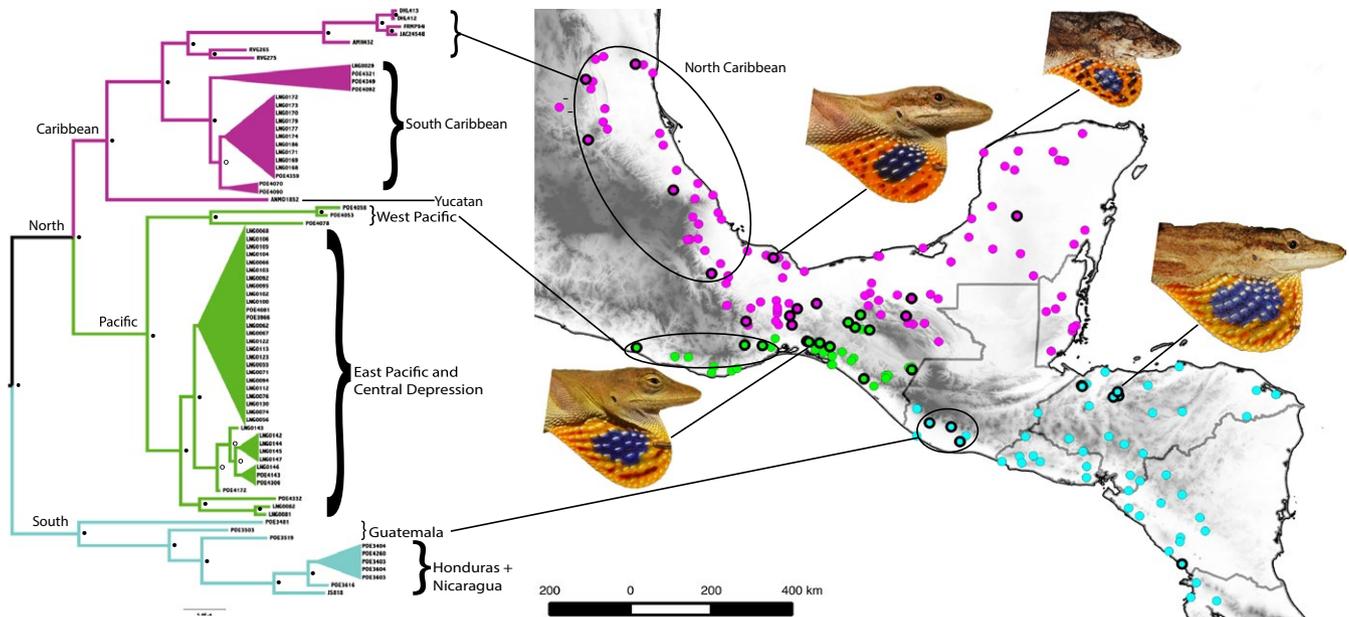


FIGURE 2 Phylogenetic relationships of the silky anoles, highlighted by clade membership. Closed circles at nodes in phylogeny indicate posterior probability of 1 in Bayesian analysis; open circles indicate PP > 0.8. Circles on the map with dark margins represent localities represented in the phylogenetic analyses, and other circles represent localities used in the ENM analyses. The North clade is composed of the Pacific + Caribbean clades, as that group is sister to the South clade. Photograph for Yucatan by Brittney White, photograph for Honduras by Tom Kennedy. Photographs for Pacific and Caribbean by Levi Gray [Colour figure can be viewed at wileyonlinelibrary.com]

portion of the range (primarily southern Mexico) was more dense than sampling in the southeastern portion of the range (Figure 2). One sample of *A. laevis* from Costa Rica was used as an out-group for phylogenetic analyses. *Anolis laevis* has been identified as a closely related species within the *Draconura* clade in a recent phylogenetic study (Poe et al., 2017).

2.2 | Genomic library preparation and bioinformatics

We extracted genomic DNA from liver and muscle tissue using Qiagen DNeasy Blood and Tissue kits (Qiagen, Valencia, CA). DNA was quantified using a Qubit 2.0 Fluorometer and diluted to a concentration of 5 ng/μl. We utilized a multiplexed shotgun genotyping protocol (Andolfatto et al., 2011; Monnahan, Colicchio, & Kelly, 2015) to generate a genomic library with 90 individuals (96 samples, with 6 duplicated due to lower concentrations of DNA present). DNA was digested with NdeI restriction enzyme (New England BioLabs, Ipswich, MA, USA), and unique barcode adapters (Monnahan et al., 2015) were ligated onto each of the samples in the library. The library was then “size selected” in order to increase likelihood of sequencing homologous loci across samples. Fragments of size 475–525 bp were selected using a Pippin Prep (Sage Science, Beverly, MA, USA) and verified using an Agilent 2,100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Libraries were then amplified by PCR using Phusion High-Fidelity PCR Master Mix to increase quantities for final sequencing (Monnahan et al., 2015). The library was sequenced in one lane of an Illumina HiSeq 2,500 sequencer using a single-end 100-bp read protocol.

All raw data were demultiplexed, quality-filtered and de novo assembled using PYRAD v.3.0.66 (Eaton, 2014). Briefly, PYRAD uses the program USEARCH (Edgar, 2010) to cluster reads and identify consensus loci using a predefined similarity threshold, and subsequently aligns the sequences for each locus using MUSCLE (Edgar, 2004). We identified an optimal clustering threshold of 0.9 using the clustering threshold series approach described in Ilut et al. (2014). pyRAD jointly estimates the mean heterozygosity and sequencing error rates using maximum likelihood (Lynch, 2008), which are used to call SNPs (Li, Ruan, & Durban, 2008). We initially used default values for all other parameters and then explored the impact of changing the values for the minimum read depth (6–12), minimum coverages across samples (50–72) and maximum proportion of shared heterozygous sites (0.1–0.5) on our downstream phylogenetic analyses.

2.3 | Phylogenetic tree estimation

We estimated phylogeographic relationships within the *Anolis sericeus* group in a likelihood framework using RAXML v8.0 (Stamatakis, 2014) and a Bayesian framework using MrBayes (Ronquist et al., 2012). In order to identify the optimal partitioning scheme, and models of molecular evolution for each partition in our data set, we used PARTITIONFINDER v1.1. We used Akaike information criterion (AICc) to select a substitution model from among the 24 common models implemented in MrBayes. Each locus was input as a potential partition, and we used the “rcluster” algorithm option (Lanfear, Calcott, Kainer, Mayer, & Stamatakis, 2014). We ran the MrBayes analysis for 20 million generations, sampling every 2,000 generations, and assessed convergence by assuring that all parameters had reached

stationarity and sufficient effective sample sizes (>1,000) using TRACER v1.4 (Rambaut & Drummond, 2007). In the maximum-likelihood analyses, we assigned a GTR + Γ nucleotide substitution model to each partition and analysed the data set using RAXML, with support determined by 1,000 bootstrap replicates.

2.4 | Coalescent analyses

In order to identify the primary mode of divergence among the lineages identified by our phylogenetic analyses, we used a coalescent modelling approach based on the joint allele frequency spectrum between populations (Gutenkunst, Hernandez, Williamson, & Bustamante, 2009). This approach (implemented in the program *δaδi*) computes the expected frequency spectrum for a candidate model using a diffusion approximation to the one-locus, two-allele Wright–Fisher process and estimates model parameters under a likelihood framework (Gutenkunst et al., 2009). On the basis of the results of our phylogeographic analyses (see below), we defined two major lineages (North and South) and two sublineages (Pacific and Caribbean) within the North lineage. We analysed the two-dimensional joint site frequency spectrum for the divergence events between the Pacific and Caribbean lineages and between the North and South lineages. For each comparison, we examined four alternative demographic models: (a) divergence with no gene flow, (b) divergence with constant (symmetrical) gene flow between populations, (c) divergence with historical migration and (d) secondary contact following divergence in isolation (see Results for model parameters). We assembled separate SNP data sets for each comparison (composed of 2,715 and 2,770 SNPs for the Pacific–Caribbean and North–South comparisons, respectively), selecting loci for each comparison that had the least missing data. We randomly selected a single SNP per locus and assumed loci were unlinked so that we could use the log-likelihood values as true likelihood values in our model comparisons (Portik et al. 2017). We projected allele sample sizes down to account for missing data in our analyses, maximizing the number of segregating sites for each population (Gutenkunst et al., 2009). Allele sample sizes were projected to 5 (North) and 10 (South) in South/North analyses and 20 (Caribbean) and 30 (Pacific) in Caribbean/Pacific analyses.

We performed initial optimizations of the demographic parameters by generating 50 sets of threefold randomly perturbed parameters, optimizing each using the Nelder–Mead method (Gutenkunst et al., 2009). We ran each optimization step (three in total) for a maximum of 100 iterations. We then used these optimized parameter sets to simulate the joint site frequency spectrum for each model. We used the parameters from the replicate with the highest likelihood as starting values to run the second round of twofold perturbed parameter optimizations with 50 replicates and used the optimal parameter values from this second round as starting parameters for a final onefold perturbed parameter optimization with 100 replicates. We compared models using the Akaike information criterion and considered the model with the lowest AIC score the best model (Burnham & Anderson, 1998).

2.5 | Sampling for niche model analyses

To characterize the niche of monophyletic lineages inferred from our phylogenetic analyses within the silky anoles, we set out to assemble a minimum of 30 localities for each lineage (Proosdij, Sosef, Wieringa, & Raes, 2016). Monophyletic sister lineages were selected for further analyses rather than species assignments because of uncertainty in taxonomy and species boundaries within silky anoles. We assembled point data for the *Anolis sericeus* group by pooling localities from museum collections with our own collection records. When georeferencing localities from museums, a record was only included in downstream analyses when LNG deemed the coordinates accurate and easy to interpret based on the description of the locality. Localities that were not sufficiently described by the collectors and thus not dependable were excluded from further analysis. Every coordinate from a museum was double-checked, and new coordinates were georeferenced if the coordinate from the museum did not match the description of the locality. Sampling for each group is well within the range for which ENM production is considered robust (Proosdij et al., 2016): 47 samples for the Pacific, 105 for the Caribbean, 64 for the South, and 153 for the North lineages, respectively (see below). All available localities were used for the construction of a model for the entire group.

For each lineage inferred by the phylogeographic analyses (assigned “North,” “South,” “Pacific” and “Caribbean,” Figure 2; see below for justification), we included a coordinate only if it was assignable to the known distribution of the group. We acknowledge that the names of the clades are somewhat subjective, but believe they generally describe the distribution of each clade with respect to one another. For instance, the North lineage occurs the farthest north but the southern extent of the distribution overlaps significantly with the northern limits of the South clade. In several instances of locality assignment, such as near the eastern break between Caribbean and Pacific lineages, localities were assigned to lineage based on which side of the Northern Highlands of Chiapas (Figure 1) they occurred. There were several localities that were therefore left out of the niche modelling analyses because the exact boundaries of each lineage are unknown, and the localities fell near a potential contact zone between two lineages. The exclusion of localities near contact zones will likely have the downstream effect of decreasing niche overlap values due to (a) a perceived geographic gap between lineages where none actually exists and (b) a tendency for Maxent to overfit data (Peterson, Papeş, & Eaton, 2007; Phillips & Dudík, 2008). This decrease in niche overlap should more strongly affect the North–South comparisons because of less thorough genomic sampling of the South clade and near the putative contact zones in Guatemala. Only a single coordinate was used for the North ENM but not assignable to Pacific or Caribbean. Samples from the eastern portion of the Yucatan Peninsula were confidently placed within the Caribbean lineage based on a recent study (Lara-Tufiño et al., 2016), which found that the Yucatan lineage is morphologically distinct from other silky anoles and occurs widely throughout Belize.

Finally, we used a subset of localities from a broad zone of parapatry between the Pacific and Caribbean lineages in order to examine niche divergence at a finer scale. For the estimated extent of the potential contact zone, which lies primarily at the Isthmus of Tehuantepec but continues to the east and west, we included all known localities that could be confidently assigned to one lineage or the other based on geographic distance and similarity in habitat type to nearby sequenced individuals. We compiled 30 localities for the Caribbean and 28 for the Pacific lineages, respectively.

2.6 | Ecological niche models

Niche models were constructed in Maxent using climate and elevation data from Worldclim (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005). The first 19 “Bioclim” layers, reflecting aspects of precipitation and temperature, were used in addition to elevation—all are commonly used in ENM construction and have been deemed biologically relevant for a wide range of organisms. Raster layers were clipped to an appropriate extent surrounding the distribution of the group prior to analysis (−104.24 to −80.86 degrees longitude and 9.33 to 25.2 degrees latitude). Default settings were utilized, with 25% of presence points withheld to train the model (Syfert, Smith, & Coomes, 2013). We withheld 25% of the localities rather than 10% to train the model because we had significantly more presence points than the minimum needed for accurate niche inference (Proosdij et al., 2016) and we wanted to minimize the known problem of model overfitting in Maxent (Warren & Seifert, 2011). The model performance was based on the area under the receiver operating characteristic curve (AUC). With no absence data for these lizards, AUC scores represent the model's effectiveness at distinguishing presence from the background (Phillips, Anderson, & Schapire, 2006).

To examine the long-term distributional stability of silky anoles in the lowlands surrounding Nuclear Central America, we also produced models for the North lineage and projected them onto two series of historical climate layers: mid-Holocene (~6,000 ya) and last interglacial (~120–140,000 ya; Otto-Bleisner, Marshall, Overpeck, Miller, & Hu, 2006). We performed these projections to determine whether the North lineage would be expected to face distributional contraction under different climate regimes, which would be evidence for past geographic isolation. Only the North group was used for this analysis, as the goal was to investigate whether lizards in that lineage might have been isolated geographically during different climate regimes. The North group was also well-sampled and we wanted to investigate the possibility of divergence in parapatry between the Pacific and Caribbean sublineages. These two sets of climate layers used in these projections (mid-Holocene and last interglacial) span the longest segment of time currently available at a high resolution.

Concerns of model overfitting are warranted in the construction of ENMs in Maxent (Peterson et al., 2007; Phillips & Dudík, 2008), but our broad sampling in our study should lead to the niche being appropriately characterized for our purposes (Peterson, 2011; Proosdij et al., 2016). Overfitting the input data should have the

effect of reducing overall niche overlap metrics in our group ENM comparisons. Since we focus on relative niche divergence between groups in this study, biases affecting all groups equally should not be problematic.

2.7 | Niche overlap/divergence

To evaluate niche divergence/overlap between sister clades inferred in the phylogeographic analyses, we compared ENMs produced for sister clades using ENMTools (Warren et al., 2008). Comparisons between well-supported, deeply divergent sister clades uncovered in the phylogenetic analyses were conducted so that only groups of equal age were compared. Niche overlap tests were combined with Background tests to determine (a) relative divergence in ecological niche space and (b) whether there is detectable niche conservation within the lineages we investigate.

In ENMTools, we utilized two metrics for calculating niche overlap from the Maxent niche models: Schoener's D (Schoener, 1968) and Warren's I statistic (Warren et al., 2008). Both metrics range 0 to 1, with 0 representing no niche overlap and 1 corresponding to identical niches between the two compared groups. Two sets of comparisons were done to evaluate levels of niche divergence/overlap. The first compares sister clades representing the deepest phylogenetic split within the group, which we named “North” and “South.” The second compares the more recent phylogenetic split involving subclades of the North group, named “Caribbean” and “Pacific” (after the versant in which each monophyletic group occurs).

To determine the strength of niche conservatism in silky anoles, we used Background tests (Warren, Glor, & Turelli, 2010). Background tests are commonly used to quantify whether two lineages are more or less similar to one another in niche space based on the environmental conditions available to them. Background tests produce a null distribution that can be compared to niche overlap metrics, yielding a two-tailed test demonstrating whether the compared lineages are exhibiting niche conservation or divergence. We generated artificial occurrence points for the Background tests for each clade using the Resample from raster tool on ENMTools. We used a linear sampling function and provided the raster from our Maxent output, starting with 10,000 points for each clade and subsequently removed points that could be considered to fall too far from the known distribution of the group. Final artificial occurrence points for clades ranged from 4,992 (North) to 7,855 (Caribbean).

3 | RESULTS

3.1 | Phylogeography of the *Anolis sericeus* complex

Summaries of genomic data quality were examined using FASTQC (Andrews 2012), which showed that sequence quality was generally high across the entire lengths of the 190,398,747 sequencing reads (average Phred score ~38). We removed 14 individuals from the

data set that were only sequenced for a very small number of reads. Removed samples were likely due to degraded DNA, as the DNA extraction process demonstrated variability in quantity and quality of DNA. An average of 14,800 loci were assembled for each of the remaining individuals before filtering. There was no noticeable effect of changing the default values of the assembly/filtering parameters or the composition of the data set on downstream phylogenetic analyses, so we used a minimum read depth of 10 and only included loci in the final data set if they had data for at least 70 individuals. Our final molecular data set consisted of 510 loci for 76 individuals (75 silky anole samples plus 1 outgroup sample). Loci were on average ~94 bp in length resulting in a total of 47,885 aligned nucleotide positions.

Our analyses reveal three relatively deeply divergent, geographically coherent and well-supported clades (Figure 2): one distributed from at least Honduras and Guatemala to Nicaragua and likely Costa Rica (South clade), another associated largely with the Caribbean versant of southern and central Mexico (Caribbean clade), and one found along the Pacific versant of southern Mexico including the Central Depression of Chiapas (Pacific clade). The oldest divergence event occurs near the mountains of Nuclear Central America (Sierra de los Cuchumatanes, Sierra de las Minas, Sierra Madre de Guatemala), separating the North and South clades. Another strongly supported divergence occurs between the Caribbean and Pacific Versant-inhabiting populations within the North lineage, which appear to be parapatrically distributed from the Isthmus of Tehuantepec region to northwestern Chiapas. All relevant nodes discussed below have high support values, most with a posterior probability of 1.0 (Figure 2). RAXML analyses yielded identical results to the MrBayes analyses with respect to the nodes and relationships discussed below.

Though sampling was not as dense in the South clade, some interesting relationships were revealed in the phylogeny. Despite being relatively close geographically, samples from the Pacific lowlands of Guatemala and northwestern Honduras are relatively deeply divergent. The sample from the farthest south (southern Nicaragua) was sister to a clade of Honduran populations.

We can draw stronger conclusions about phylogeographic structure within the North clade due mainly to more thorough geographic sampling. First, there is a strongly supported Caribbean clade that includes the sample from the Yucatan Peninsula as sister to all other samples in the clade. After the split of the Yucatan sample, there is a split dividing populations roughly north and south in the western part of the distribution, with the contact zone occurring slightly south of the eastern extent of the Mexican Transvolcanic Belt. The Isthmus of Tehuantepec does not appear to be a barrier east-west for the Caribbean populations. In the Pacific clade, there is strong support for a break at the Isthmus of Tehuantepec. There is also a well-supported sister relationship between populations of the Ocote region of Chiapas and the rest of the Pacific plus the Central Depression of Chiapas.

3.2 | Ecological niche models in maxent and niche overlap

All four lineage ENMs (North, South, Pacific and Caribbean) were characterized by high AUCs (>0.8), suggesting good performance of the models (Table 1). As expected, the models largely predicted high suitability for lowland areas throughout the region each lineage occupies, with few exceptions (Figure 3). Based on both Schoener's *D* and Warren's *I*, there is stronger niche overlap (weaker niche divergence) between the North and South lineages (Schoener's *D* = 0.32759; Warren's *I* = 0.61622) than between the Pacific and Caribbean lineages (Schoener's *D* = 0.16267; Warren's *I* = 0.37839;), but the North and South lineages do still exhibit a moderate degree of niche divergence. This is reflected in the projections of habitat suitability for each lineage (Figure 3). The South lineage ENM shows some regions of suitable habitat extending into the distribution of the North lineage distribution, primarily along the coast near the Isthmus of Tehuantepec, and vice versa. The Pacific and Caribbean clade ENMs, on the other hand, show little to no overlap into each other's distribution. The models representing samples from the zone of parapatry between Pacific and Caribbean lineages show similar levels of niche overlap to their broader clades (Supporting Information Figure S1 and S3).

Historical projections for the habitat suitability of the region immediately north and west of Nuclear Central America for silky anoles suggest a broad distribution over the last ~120–140 K years (Supporting Information Figure S2). Background tests for every clade pairing demonstrate higher niche similarity than expected due to chance, based on their environmental backgrounds ($p < 0.01$; Figure 4).

3.3 | Coalescent analyses

For both North–South and Pacific–Caribbean comparisons, model selection based on the Akaike information criterion (AIC) identified secondary contact following divergence in isolation as the best-supported model (Table 2). The inferred parameters of these models suggest a relatively long time between the divergence of the North and South clades (unscaled time, $T_1 = 4.27$; Table 2) and the Pacific and Caribbean clades (unscaled time, $T_1 = 3.88$; Table 2) and the beginning of secondary contact, which occurred relatively recently (unscaled time, $T_2 = 0.55$ and $T_2 = 0.12$, respectively; Table 2). Migration following secondary contact was inferred to be relatively high between the North and South clades ($m = 0.14$; Table 2) and fairly low between the Pacific and Caribbean clades ($m = 0.04$; Table 2), even though the Pacific and Caribbean clades are currently likely to be parapatric over a broad geographic area.

4 | DISCUSSION

Our phylogenetic analyses reveal strong phylogeographic structure, which may be unexpected a priori for a widespread and abundant

TABLE 1 Top eight variables for each Maxent clade niche model arranged by permutation importance (PERM) and including per cent contribution to the model (PCT). Variables that overlapped in sister clade comparisons are shaded in grey

Variable	PERM	PCT	Variable	PERM	PCT
North Clade (AUC = 0.850)			South Clade (AUC = 0.918)		
Elevation	24.4	13.4	Temp seasonality	55.8	35.5
Precip seasonality	21.1	12.2	Elevation	19.2	5.3
Temp seasonality	20.1	9.3	Precip seasonality	5.8	4.4
Temp annual range	5.3	13.9	Mean temp of wettest qtr	5.1	0.6
Max temp of warmest month	4.4	4.5	Precipt of warmest qtr	3.5	4.5
Min temp of coldest month	4.1	19.7	Min temp of coldest month	3.4	13.2
Isothermality	3.3	1.3	Mean diurnal range	2.2	1.6
Mean diurnal range	2.1	3.3	Precip of coldest qtr	1.9	5.5
Pacific Clade (AUC = 0.961)			Caribbean Clade (AUC = 0.833)		
Isothermality	23.5	4.4	Temp seasonality	28.5	39.2
Precip of coldest qtr	17.7	18.5	Precip seasonality	23.4	5.3
Elevation	12.2	9.2	Precip of driest month	15.8	22.3
Temp seasonality	11.8	2	Elevation	12.2	0.4
Mean diurnal range	8.4	5.5	Temp annual range	6.3	0.3
Precip seasonality	7.7	19.3	Min temp of coldest month	4.4	1.3
Mean temp of wettest qtr	6.6	0.2	Precip of driest qtr	3.7	0.2
Mean temp of driest qtr	5.3	3.2	Annual precip	2.9	12.1

lizard capable of living in a variety of habitats under a wide range of environmental conditions. Our results suggest that the *Anolis sericeus* complex may have originated near the mountains of Nuclear Central America (Sierra de los Cuchumatanes, Sierra de las Minas, etc.) and spread south and east into lower Central America and north and west into Mexico, becoming two major clades (North and South; Figure 2). The North clade further exhibits relatively deep divergence between Caribbean and Pacific versant populations, despite the broad zone of parapatry between these lineages. The divide largely mirrors the environmental regimes associated with either versant—the Caribbean with its tendency towards year-round wet forests and the majority of the Pacific dominated by seasonally dry forests (Halffter & Morrone, 2017; Morrone, 2014). The transition of wet-to-seasonally dry habitats occurs rather abruptly across Pacific and Caribbean versants from the west side of the Isthmus of Tehuantepec to northern Chiapas and corresponds with the abrupt transition from the Caribbean clade to the Pacific clade (Supporting Information Figure S1). Hence, the conditions exist for geographic or ecological divergence in both pairs of lineages.

For both major diversification events (North–South and Caribbean–Pacific), the results of our coalescent modelling and ecological niche divergence analyses are broadly consistent with a period of geographic isolation, which resulted in ecological divergence between lineages, followed by secondary contact (Hypothesis 2). Our coalescent analyses identified this as the most likely mode of

diversification, finding strongest support for a model of allopatry followed by secondary contact (Table 2). In both cases, niche similarity between lineages was more than expected purely by chance, based on different environmental backgrounds (Figure 4), but was still low to moderate overall (Schoener's $D = 0.328$ and 0.163 and Warren's $I = 0.616$ and 0.378 , respectively; Figure 3). This suggests some constraint on niche evolution but indicates that the niches for these pairs of lineages have still diverged to some degree (niche similarity should be near 1, for both metrics, for highly constrained niches). These results stand in contrast to what would be expected for diversification driven by ecological divergence, under which we would expect to find greater levels of niche divergence between contemporary populations and support for a model of “historical migration” that was reduced over time as niches diverged, or diversification associated only with geographic isolation, in which we would expect to find support for a model of “no migration” or “constant migration” from the coalescent analyses.

Although our results suggest that the ecological divergence we observed between major lineages in the *Anolis sericeus* complex was likely a by-product of isolation in different environments, they also suggest an important role for niche divergence during secondary contact. Despite being on separate evolutionary trajectories for a shorter period of time than the North and South lineages, niche divergence between the Caribbean and Pacific lineages is substantially more pronounced (Figure 3), and there is little overlap among

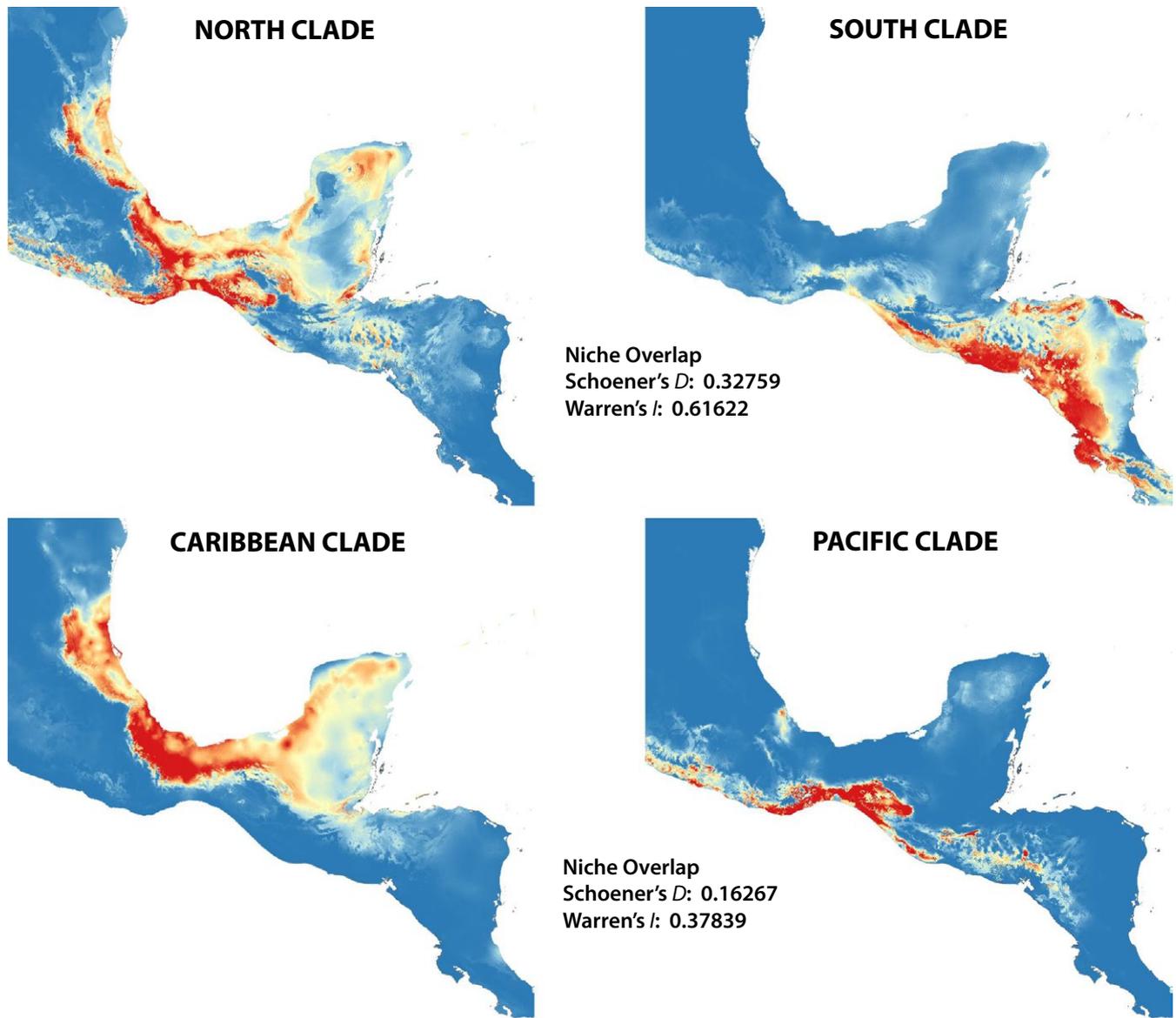


FIGURE 3 Ecological niche models constructed in Maxent for each group, along with niche overlap values for sister clade comparisons (South vs. North and Pacific vs. Caribbean) comparisons [Colour figure can be viewed at wileyonlinelibrary.com]

the environmental variables that contributed most to the ENMs (Table 1), suggesting that these lineages may have diverged along different environmental axes. Consistent with a role for ecological divergence limiting admixture, our coalescent analyses identified relatively high levels of gene flow between the North and South lineages ($m = 0.14$) and relatively low levels between the Pacific and Caribbean lineages ($m = 0.04$). Moreover, the restricted gene flow between the Pacific and Caribbean lineages occurs despite a broad zone of parapatry between them (~twice as wide as the contact zone between the North and South lineages). Thus, our coalescent estimates of demographic parameters and analyses of ecological niche divergence suggest that niche divergence leads to a reduction in gene flow upon secondary contact in this system. Altogether, our results suggest a primary role for geographic isolation driving phylogeographic structure in the silky anoles with important contributions from ecological isolation in maintaining distinct lineages.

The mechanisms underlying ecological isolation in most systems are still largely unknown. Although our study did not include explicit tests of different mechanisms, the phylogeographic and environmental patterns we observed in our data suggest some potentially interesting possibilities. For instance, there are several avenues by which natural or sexual selection can lead to the pattern of environmentally associated isolation found between the Pacific and Caribbean clades. One potential mechanism is the distinct difference in reproductive timing between lizard populations in the two regions. In the relatively aseasonal environment experienced by the Caribbean lineage, populations are able to breed throughout most of the year, like many tropical anoles (Fitch, 1973, 1973a; Losos, 2009; Smith, Sinelnik, Fawcett, & Jones, 1972). However, in the seasonally dry Pacific of southern Mexico, silky anoles likely have a significantly shortened breeding season, a pattern that has been found in other anole species inhabiting drier parts of the tropics (Fitch,

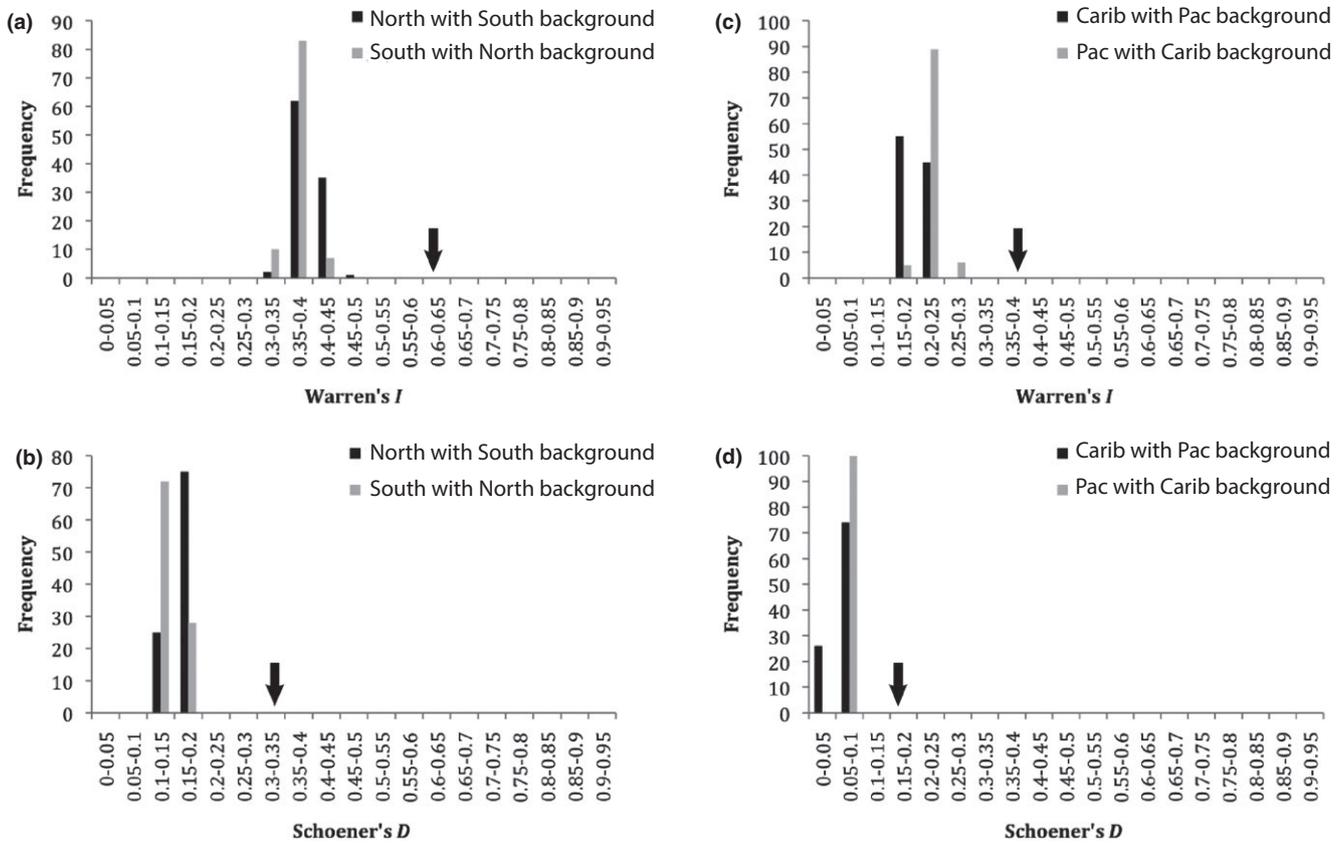


FIGURE 4 Background similarity tests for each sister clade comparison, summarized from 100 models generated from randomly drawn localities within the range of the appropriate clade. (a) and (b) represent North/South comparisons; (c) and (d) represent Pacific/Caribbean comparisons. All observed measures of niche overlap (indicated by arrows) were significantly higher than the null distributions, indicating niche conservatism in each group ($p < 0.01$)

TABLE 2 Results of coalescent simulations using $\delta a\delta i$

Species Complex	Model	Log-likelihood	AIC	ΔAIC	AIC ω	nu1	nu2	T1	T2	m
Pacific versus Caribbean	No migration	-472.86	951.72	161.30	9.4e-36	3.74	2.71	1.52	-	-
Pacific versus Caribbean	Constant Migration	-403.35	814.70	24.28	5.3e-6	9.77	6.61	6.07	-	0.02
Pacific versus Caribbean	Historical Migration	-405.66	821.32	30.90	2.0e-7	14.1	10.1	9.88	0.06	0.01
Pacific versus Caribbean	Secondary Contact	-390.21	790.42	-	0.9999	7.72	5.13	3.88	0.55	0.04
North versus South	No migration	-94.74	195.48	24.68	3.9e-6	1.14	5.85	1.89	-	-
North versus South	Constant Migration	-83.53	175.06	4.26	0.1061	3.63	16.9	8.86	-	0.01
North versus South	Historical Migration	-87.28	184.56	13.76	0.9e-4	2.18	10.5	4.31	0.59	0.02
North versus South	Secondary Contact	-80.40	170.80	-	0.8930	2.03	9.73	4.27	0.12	0.14

Notes. Log-likelihood, AIC values and Akaike weights (AIC ω) given for each model. nu1 and nu2 correspond to the relative population sizes for population 1 and 2. T1 refers to the time between initial population divergence and the present (in $2N$ generations). T2 refers to the time between the present and the initial secondary contact or the time migration stopped (for the historical migration model). m refers to the symmetrical migration rate between the two populations in units of $2Nm$. We did not transform these parameter estimates to biologically interpretable values here because our goal was not to perform parameter estimation, which should be done in combination with a procedure to obtain confidence intervals.

1973; Henderson & Fitch, 1975). The effect these differences would have on population dynamics could be profound, possibly initiating divergence and certainly limiting reproductive opportunities that could lead to admixture between the two clades (Räsänen & Hendry, 2008). The existence of multiple contact zones between morphologically indistinguishable populations with varying levels of genomic divergence exhibiting little to no gene flow makes the silky anole system attractive for uncovering mechanisms leading to speciation (Coyne & Orr, 2004). Seeking evidence for reinforcement or reproductive character displacement at the contact zones is likely to be informative (Lambert, Geneva, Mahler, & Glor, 2013). These factors have been documented in another morphologically conserved anole species group, the *Anolis breviostris* complex in the Greater Antilles (Lambert et al., 2013; Webster & Burns, 1973).

Our results also contribute to a growing body of literature on rates of niche evolution (Arteaga, McCormack, Eguiarte, & Medellín, 2011; Nunes & Pearson, 2017; Warren et al., 2008). In general, we still know relatively little about rates of environmental niche evolution and what drives them (Warren et al., 2008), and there is much to learn about the role of the environment in the diversification of lineages. While revisiting a large data set on birds across the Isthmus of Tehuantepec (Peterson et al., 1999), Warren et al. (2008) found significant levels of environmental niche differentiation between most species pairs. Whether this is a taxonomically broad trend remains to be seen, as few studies have investigated rates of niche evolution or niche lability, especially at recent evolutionary timescales. In this study, we find that strong environmental niche evolution and divergence can occur despite some background level of niche conservatism in a species group as a whole. Yet just how important differences in environmental selection regimes can be in producing or strengthening reproductive isolation between populations is an open question. Thorpe and colleagues (Thorpe, Surget-Groba, & Johansson, 2008, 2010) found evidence for increased levels of reproductive isolation between populations experiencing different environments (xeric vs. mesic) in the *Anolis roquet* group on Martinique. Fine-scale molecular investigations revealed that stronger isolation occurred between populations at the xeric/rainforest ecotone (Thorpe, Surget-Groba, & Johansson, 2010) than between lineages that had previously been isolated for up to 8 million years on separate palaeo-islands that make up the currently inhabited island of Martinique. Thus, evidence exists for environmental transitions playing an isolating role in anoles.

In the silky anoles, the abrupt change in climate regimes between the Pacific and Caribbean versants of southern Mexico may have created a situation similar to *Anolis roquet* (Thorpe, Surget-Groba, & Johansson, 2008; Thorpe et al., 2010) for which near-complete reproductive isolation resulted. However, there is an important difference between the *A. roquet* group on Martinique and the *A. sericeus* group: in the *A. roquet* group, there are clear morphological differences between the two populations, most noticeably coloration, likely reflecting local adaptation. Silky anoles possess no obvious morphological differences associated with the starkly different environmental regimes the Pacific and Caribbean populations

experience. How these populations have maintained such similar morphologies despite a broadly parapatric distribution (even in the conservation of a rather complex dewlap arrangement; see Figure 2) remains to be investigated. Williams' (1965) niche incumbency hypothesis might help explain why the two lineages have not invaded each other's distributions. That is, the two lineages may exhibit differentiated requirements but still be too similar to coexist, effectively preventing the invasion of one lineage into the distribution of the other.

At finer scales of divergence, within the major clades, our phylogeographic results also reveal some evidence of both geographic and environmental isolation. For instance, the Isthmus of Tehuantepec, potentially a major geographic barrier, appears to have played little role in shaping phylogeographic structure in the Caribbean populations, whereas in the Pacific, there is reciprocal monophyly of populations on the east and west sides of the Isthmus. This same biogeographic pattern is reflected in the majority of plants and animals studied throughout the region (Halfpeter & Morrone, 2017; Morrone, 2014). In the Caribbean portion of the Isthmus, the environment and plant communities tend to be stable and continuous, with strong overlap in species diversity on the east and west sides (Escalante, Rodríguez, Cao, Ebach, & Morrone, 2007; Morrone, 2014). The Pacific versant of the Isthmus, however, appears to be a strong dispersal barrier for lowland species (Bryson, García-Vázquez, & Riddle, 2011). The silky anole populations on either side of the Isthmus could have been isolated in the past due to higher sea levels (Bryson et al. 2011), but the maintenance of strong phylogeographic structure after coming back into contact is noteworthy when there are no obvious morphological traits distinguishing the two sets of populations. Future work on this group should include locating the contact zone and investigating population dynamics between the eastern and western Pacific populations, as well as examining the dynamics of these populations relative to the presumably parapatric Caribbean lineage to the north.

The phylogeographic patterns we uncovered in the *Anolis sericeus* complex are generally consistent with broader biogeographic patterns from this region as well. At a broad scale, the mountains of Nuclear Central America have strongly influenced biogeographic patterns for a wide range of taxa (Flores-Villela & Martínez-Salazar, 2009; Ramamoorthy et al., 1993; Wake, 1987), and they appear to have been a key factor in the early diversification of silky anoles as well (for discussion of the taxonomic implications of our results, see Supporting Information Appendix S1: "Taxonomic implications for the *A. sericeus* group"). At a finer scale, we found evidence for a phylogeographic split within the Caribbean clade near the Mexican Transition Zone at the Transvolcanic Belt (Morrone, 2010). The location of the contact zone is very close to where other researchers have noted a biotic transition (Bryson et al. 2011; Meza-Lázaro & Nieto-Montes de Oca 2015; Morrone, 2010; Zaldívar-Riverón et al., 2004), although the estimated zone of contact does not obviously appear to be associated with any geographic barriers. In the Pacific clade, populations from the Central Depression of Chiapas are closely related to nearby Pacific coastal populations (Figure 2).

This result is consistent with commonly recognized biogeographic patterns (Johnson, 1990; Morrone, 2014), though it is noteworthy that individuals from the Ocote region of Chiapas appear to be members of the Pacific lineage. The Ocote region is particularly diverse, in some ways resembles Caribbean lowland communities in species composition (Urbina-Cardona & Flores-Villela, 2010), and does not contain many of the species found in the drier Central Depression and Pacific coastal regions of Chiapas (Johnson, 1990). In very general terms, silky anoles may be representative of the prevailing biogeographic trends in southern Mexico and Nuclear Central America, although comparative analyses are needed to assess this possibility.

5 | CONCLUSIONS

Overall, in our molecular assessment of the evolutionary relationships of the silky anoles, we found evidence for both geographic factors and ecological processes shaping phylogeographic patterns. Recent studies highlighting the strength and ubiquity of isolation by environment (Sexton et al., 2014; Wang & Bradburd, 2014) have typically focused on finer scales, mostly at the level of recent population differentiation. Although some studies have demonstrated relatively high lability in environmental niche traits between closely related species (Peterson et al., 1999; Warren et al., 2008), few have demonstrated environmental niche divergence as a major factor promoting broader phylogeographic divergence or speciation (but see Cooney, Seddon, & Tobias, 2016; Pitteloud et al., 2017). Here, we find evidence for lineage diversification related to both geographic isolation and environmental niche evolution in an abundant and widespread lizard group that otherwise exhibits fairly strong niche and morphological conservatism. Future work on other widespread clades is needed to assess whether such cryptic environment-driven divergence events are common in nature at the phylogeographic scale.

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AUTHOR CONTRIBUTIONS

L.N.G. initiated and designed the project; L.N.G., A.J.B., A.N.M.O., S.P. and R.C.T. made field collections; L.N.G. and A.J.B. collected molecular data and performed analyses; and all authors helped write the manuscript.

DATA ACCESSIBILITY

DNA alignments, phylogenetic trees, raw Illumina reads and sampling locality coordinates used in this study are archived in the Dryad digital repository (<https://doi.org/10.5061/dryad.p65mc58>).

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