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Understanding patterns of diversity and evolution in mainland Anolis lizards

Levi Gray
University of New Mexico

Levi Nathaniel Gray
_____

Candidate

Department of Biology
_____

Department

This dissertation is approved, and it is acceptable in quality and form for publication:

Approved by the Dissertation Committee:

Dr. Steve Poe , Chairperson
_____

Dr. Mike Andersen
_____

Dr. Joe Cook
_____

Dr. Ian Wang
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Understanding patterns of diversity and evolution in mainland *Anolis* lizards

By

Levi Nathaniel Gray

B.S., Evolution, Ecology, and Biodiversity, University of California at Davis, 2006

DISSERTATION

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I have a lot of aunts and uncles, and all of them played important roles in my development as a person and as a scientist. Uncle Mark was like a second father, and often let me go to work with him so that I could go herping while he worked. I can't understate how lucky I was to be surrounded by so many people that cared about me as I grew up.

Every single one of my cousins and three brothers contributed to who I am today. My definition of a cousin certainly extends to more distant relatives (Mason, Vincent, Robbie, and Sarah) as well as honorary cousins like Kim and Kevin McNally. Our creative, often very competitive, and at times violent games taught me a lot. Or screwed me up. All I know is that each of you is very important to me and taught me something individually. If I can arrange a cousins/sibling weekend in Chiapas at some point, I will be honored to get the chance to share it with you. Even though I know damn well some of you will never go. (Prove me wrong.)

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I've had some great friends and I attribute a fair amount of my positive qualities to things I have learned from them. I want to address some individually. John DeSoto, you were always a great friend and I was really lucky to have a curious and non-

judgmental friend interested in herps. Ned Boehme, you always impressed the hell out of me with your insight, empathy, and moral compass. I sometimes worry about what opinions I would have to this day if you hadn't been patient with me. Joe Gulden, you were bold enough to go to Chiapas with me when I had zero experience outside the US!

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UNDERSTANDING PATTERNS OF DIVERSITY AND EVOLUTION IN MAINLAND *ANOLIS* LIZARDS

Levi N. Gray

B.S. Evolution, Ecology, and Biodiversity, University of California at Davis
Ph.D Biology, University of New Mexico

ABSTRACT

Patterns of organismal diversity and evolution are often difficult to interpret with a high level of confidence. The number of mechanisms and processes that contribute to shaping patterns of diversity is extensive and is reflected in the many methods researchers have used to infer causation. Taxonomic groups that are well-studied can offer more precise interpretation of pattern and process due to the considerable amount of research addressing ecology, natural history, and behavior of the organisms.

In this dissertation, I explore patterns of phylogenetic and phenotypic variation in *Anolis* lizards (anoles) by testing hypotheses that could have led to the observed variation. Anoles are prime test subjects to address my questions due to extensive background research on their ecology and evolution. I tested hypotheses at multiple scales. In my first chapter, I studied evolutionary, ecological, and geographic patterns in a closely related species complex, the silky anoles (*A. sericeus* group). For my second chapter, I examined broad patterns of sexual trait variation among distantly related anole species. Finally, in my third chapter I test the phylogenetic utility of a restriction-site associated DNA (RAD) molecular marker set on a selected group of anoles. Though anoles are well-studied relative to most taxonomic groups, my work reveals that there is

still a lot to learn about evolutionary patterns in *Anolis*, particularly in less studied

taxonomic groups in mainland North and Central America.

INTRODUCTION

Determining patterns in the natural world and inferring underlying processes is an important but challenging part of understanding evolution. *Anolis* lizards (anoles) have been the focus of a disproportionate amount of evolutionary research (Losos 2009), though the bulk of that research has been on island species. However, mainland anoles offer similar opportunities for answering questions relating to the origin and maintenance of diversity and are vastly understudied in comparison to their island relatives (Poe et al. 2017). This dissertation examines patterns of biological diversity at multiple levels; first of phylogeographic relationships of a species complex that occurs broadly throughout Mexico and Central America, then at sexual trait variation among the species present in Mexico, and finally at the evolutionary history of anoles in general. In an attempt to address these issues, I accumulated large morphological and molecular data sets from mainland anoles that I hope will contribute significantly to the growing body of knowledge of this under-studied system.

In *Chapter 1*, my coauthors and I set out to understand phylogeographic patterns in the silky anoles (*Anolis sericeus* complex). Silky anoles have long been a difficult system taxonomically (Stuart 1955; Lee 1980; Köhler & Vesely 2010; Lara-Tufiño et al. 2016) that belied significant potential for the evolutionary biologist. Silky anoles occur continuously, or nearly so, in all lowland habitats from northern Costa Rica to northern Mexico. A handful of described forms have been synonymized (Lee 1980, Köhler & Vesely 2010), as relatedness of populations of these lizards was confounded by morphological conservatism overall while exhibiting some signs of local adaptation in certain environments (Lee 1980). Their distribution allows for rigorous testing of several

phylogeographic hypotheses, which was the primary focus of the chapter. I generated a large restriction-site associated DNA (RAD) marker set for the sampled specimens to infer phylogeographic relationships within the lineage. I found two deep divergences that, upon further investigation, were likely the result of past geographic isolation. I also used a coalescent modeling approach to address the demographic context of divergence, which suggested recent gene flow has occurred between populations of deeply divergent lineages that are parapatrically distributed. Two such lineages were found to have diverged in environmental niche metrics, which may be contributing to the maintenance of each lineage as independent evolutionary lineages.

In *Chapter 2*, I set out to test a longstanding hypothesis for the evolution of the male dewlap, a sexual signaling organ, in anoles (Fitch & Hillis 1984). In the original study, the authors gathered a large data set of male dewlap size composed entirely of mainland anoles. They found a significant statistical association between increased dewlap size and seasonality of the environment, which they related to sexual selection. They hypothesized that, in accordance with their results, anole species that lived in environments that constrained the breeding season would have larger dewlaps due to enhanced sexual selection on male signaling (Fitch & Hillis 1984). Two potentially damning problems were present in their data and analyses, however: they did not account for shared evolutionary history and their non-random sampling was biased towards species with large dewlaps in seasonal areas. I re-assessed the Fitch-Hillis Hypothesis (FHH) at two scales with entirely new data sets that I and collaborators collected throughout Mexico. With more complete sampling and accounting for phylogenetic relatedness of species, we found no support for the FHH. Our results are consistent with

other studies that have shown that macro-analyses investigating hypotheses generating dewlap diversity rarely find support (Losos & Chu 1998; Nicholson et al. 2007; Ingram et al. 2017). It is likely that the many factors playing a role in dewlap evolution confound the ability to find strong support for any one hypothesis (Losos & Chu 1998).

Chapter 3 aims to test the phylogenetic utility of a fairly new Next Generation DNA Sequencing marker type, restriction-site associated DNA (RAD) libraries, for resolving the evolutionary history of *Anolis* lizards. Thus far, phylogenetics of *Anolis* has depended heavily on signal in mtDNA sequences. Next Generation Sequencing methods that generate hundreds (or thousands) of loci for resolving phylogenetic problems are widely being used for a variety of taxonomic groups (McCormack et al. 2013) but had not yet been tested on a broad group of anoles. We selected species representing important anole lineages and built RAD libraries for sequencing. The resulting data set consisted of 396 loci that were utilized in a Bayesian framework to infer phylogenetic relationships of anoles. The consensus trees from analyses of the RAD data resolved a majority of nodes with high support, some for the first time in anole phylogenetics. These results suggest that, contrary to some expectations (Rubin et al. 2012), RAD markers may be a useful, cheap method for resolving evolutionary history in fairly old groups.

Inference of mechanisms for generating and maintaining patterns of diversity is difficult. With each investigation described above, some level of failure was apparent due to a number of complicating factors. But through excessive data collection, careful analysis, and logic, I hope that each chapter contains some wisdom regarding why these lizards exhibit the patterns they do in nature.

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CHAPTER 1

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

AUTHORS
Levi N. Gray[1], Anthony J. Barley[2], Steven Poe[1], Robert C. Thomson[2], Adrián Nieto-Montes de Oca[3], and Ian J. Wang[4]

[1]Department of Biology and Museum of Southwestern Biology, University of New Mexico, Albuquerque, NM 87131, USA
[2]Department of Biology, University of Hawai'i at Mānoa, Honolulu, HI 96822, USA
[3]Departamento de Biología Evolutiva, Facultad de Ciencias, Universidad Nacional Autónoma de México, México, D.F. 04510, México
[4]Department of Environmental Science, Policy, and Management, University of California, Berkeley, CA 94705, USA

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 a deep genetic break is likely associated with historical geographic isolation, while a more recent break is associated with the contemporary environment. Moreover, comparisons of parapatric sister lineages suggest that recent niche divergence contributed to isolation by environment in this system, reflecting the natural history differences among populations in divergent environments.

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 (Avise 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 et al. 2008; Zellmer et al. 2012; Zink 2014; Paz et al. 2015). 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 (Wang et al. 2013; Paz et al. 2015), relatively few studies have explicitly examined the relative roles of geographic and ecological factors in explaining phylogeographic patterns (Sexton et al. 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 (Mayr 1963; Coyne & Orr 2004; 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 byproduct 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 modeling and coalescent modeling 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 hotspot with complex topography and substantial environmental variation (Ramamoorthy et al. 1993; Morrone 2014), 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 (Stuart 1966; Flores-Villela & Martínez-Salazar 2009). 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 (Hulsey et al. 2004; Rovito et al. 2012; Zaldivar-Riveron et al. 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 (Fig. 1). 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 et al. 2016) are continuously distributed in nearly all types of lowland habitat from northern Mexico to northern Costa Rica (Stuart 1955; Henderson & Fitch 1975; Lee 1980). Silky anoles exhibit substantial external morphological conservation overall (Köhler & Vesely 2010) but considerable within- and between-population variation in certain traits surrounding the mountains of Chiapas, Mexico and Guatemala (Stuart 1955; Lee 1980; Köhler & Vesely 2010). 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 (Malhotra & Thorpe 1997; Calsbeek et al. 2006). These environment-associated differences expressed by silky anole populations raise the interesting 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 et al. 2008; Warren et al. 2010). Research on niche divergence in birds (Peterson et al. 1999) and Cuban *Anolis* lizards (Warren et al. 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 niche or are not distributed across environments that are variable enough to detect differences using available methods. The silky anoles of Central America and Mexico fulfill the requirements for 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 datasets, phylogenetic reconstruction, coalescent model testing, ecological niche modeling, and explicit tests of niche divergence. We consider three hypotheses that describe the phylogeographic structure in this system: 1) phylogeographic structure reflects only geographic isolation (barriers) separating populations, 2) phylogeographic structure is associated with ecological niche divergence that is the byproduct of divergence in allopatry followed by secondary contact, and 3) phylogeographic structure resulted from environmental isolation due to ecological divergence between lineages that occurred either in allopatry or parapatry. In the first scenario, phylogenetic breaks occur in concert with geographic barriers rather than environmental transitions. Support for the second and third hypotheses can be informed by inference of past distributions, the degree of ecological niche divergence between lineages, and coalescent modeling—the distinction between these hypotheses is whether ecological divergence contributed to lineage divergence or was merely a byproduct of lineage divergence. 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 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. For example, if a model for past divergence and secondary contact is preferred over a model for past gene flow and recent isolation between lineages abutting one another, it suggests that the genomic divergence likely occurred in allopatry. We weigh evidence from both the ENM analyses and coalescent analyses to evaluate whether ecological isolation has played a role in the diversification of a widespread, lowland, generalist lizard that experiences extreme differences in environment and habitat.

METHODS

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 (Fig. 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 (Fig. S1, Supporting Information). GPS coordinates for each sample were collected in the field using Garmin GPS devices with accuracy of ~3-5 m. Sampling in the northwestern portion of the range (primarily southern Mexico) was more dense than sampling in the southeastern portion of the range (Fig. 2). One sample of *A. laeviventris* from Costa Rica was used as an outgroup for phylogenetic analyses. *Anolis laeviventris* has been identified as a closely related species within the *Draconura* clade in past phylogenetic studies (Nicholson et al. 2012; Poe et al. 2017).

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 et al. 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 2100 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. The library was sequenced in one lane of an Illumina HiSeq 2500 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 et al. 2008). We initially used default values for all other parameters, 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.

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 dataset, we used PartitionFinder v1.1 (Supporting Information). 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 et al. 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 (>1000) using Tracer v1.4 (Rambaut & Drummond 2007). In the Maximum-likelihood analyses, we assigned a GTR + Γ nucleotide substitution model to each partition and analyzed the dataset using RAxML, with support determined by 1000 bootstrap replicates.

Coalescent Analyses

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In order to identify the primary mode of divergence among the lineages identified by our phylogenetic analyses, we used a coalescent modeling approach based on the joint allele frequency spectrum between populations (Gutenkunst et al. 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). We analyzed 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: 1) divergence with no gene flow, 2) divergence with constant (symmetrical) gene flow between populations, 3) divergence with historical migration, and 4) secondary contact following divergence in isolation (see Results for model parameters). We assembled separate SNP datasets for each comparison (composed of 2715 and 2770 SNPs for the Pacific-Caribbean and North-South comparisons, respectively), selecting loci for each comparison that had the least missing data. We also chose to use only 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).

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.

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 et al. 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", Fig. 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 (Fig. 1) they occurred. There were several localities that were therefore left out of the niche modeling 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 1) a perceived geographic gap between lineages where none actually exists and 2) a tendency for Maxent to overfit data (Peterson et al. 2007; Phillips 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. Despite these concerns, only a single coordinate was included in construction of 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.

Ecological Niche Models

Niche Models were constructed in Maxent using climate and elevation data from Worldclim (Hijmans et al. 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. Default settings were utilized, with 25% of presence points withheld to train the model (Syfert et al. 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 et al. 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 group and projected them onto two series of historical climate layers: mid-Holocene (~6000 ya) and last inter-glacial (~120-140,000 ya; Otto-Bliesner et al. 2006). 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. These two sets of climate layers 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 2008), but our broad sampling in our study should lead to the niche being appropriately characterized for our purposes (Peterson et al. 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.

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 1) relative divergence in ecological niche space and 2) 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 zero 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 et al. 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 4992 (North) to 7855 (Caribbean).

RESULTS

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 dataset 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 dataset on downstream phylogenetic analyses, so we used a minimum read depth of 10 and only included loci in the final dataset if they had data for at least 70 individuals. Our final molecular data set consisted of 520 loci for 76 individuals (75 silky anole samples plus 1 outgroup sample). Loci were on averages ~94 bp in length resulting in a total of 48,947 aligned nucleotide positions.

Our analyses reveal three relatively deeply divergent, geographically coherent, and well-supported clades (Fig. 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 (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 (Fig. 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.

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 clade occupies, with few exceptions (Fig. 3). The North clade shows suitable habitat extending far into the distribution of the South clade and vice versa, suggesting that either group would be able to expand its distribution in the absence of the other. Based on both Schoener's *D* and Warren's *I*, there is much stronger niche overlap (weaker niche divergence) between North and South clades (Schoener's $D = 0.32759$; Warren's $I = 0.61622$) than between the Pacific and Caribbean clades (Schoener's $D = 0.16267$; Warren's $I = 0.37839$; Fig. 3). There is also substantial overlap among the environmental variables that describe the ecological niches in both models for the North and South clades (Table 1). The Pacific and Caribbean clade ENMs 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 (Fig. S1, Supporting Information).

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 ~120-140K years (Fig. S2, Supporting Information). Background tests for every clade pairing demonstrate higher niche similarity than expected due to chance (p<0.01; Fig. 4).

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, T1 = 4.27; Table 2) and the Pacific and Caribbean clades (unscaled time, T1 = 3.88; Table 2) and the beginning of secondary contact, which occurred relatively recently (unscaled time, T2 = 0.55 and T2 = 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 geographical area.

DISCUSSION

Phylogeography of silky anoles

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Our phylogenetic analyses reveal strong phylogeographic structure, which may be unexpected *a priori* for a widespread and abundant 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; Fig. 2). The mountains of Nuclear Central America have strongly influenced biogeographic patterns in the region for a wide range of taxa (Wake 1987; Ramamoorthy et al. 2009; Flores-Villela & Martínez-Salazar 2009), and they appear to have been a key factor in the early diversification of silky anoles as well.

The North clade 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 toward year-round wet forests and the majority of the Pacific dominated by seasonally dry forests (Morrone 2014; Halffter & Morrone 2017). 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 (Fig. S1, Supporting Information).

Within the Caribbean clade, we also found evidence of strong phylogeographic structure. For instance, the Yucatan sample is deeply divergent from the rest of the Caribbean clade (Fig. 2). Although the Yucatan is not a region of particularly high endemism when compared to other regions of Central America (Estrada-Loera 1991; Morrone 2014), it does harbor some endemic lineages (Ibarra-Manríquez et al. 2002; Lee 2000). The strong overlap in species diversity between the Yucatan and lowland Caribbean forests of southern Mexico, Belize, and northern Guatemala indicates similarity in biotic communities (Estrada-Loera 1991; Morrone 2014), so an endemic lineage of silky anole in the Yucatan might not be expected. How this divergence originated could be related to different environmental regimes and plant communities or due to past geographic isolation at the Maya Mountains region during periods of higher sea levels (Fig. 1). Another phylogeographic split occurs 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 (Zaldívar-Riverón et al. 2004; Morrone 2010; Bryson et al. 2011; Meza-Lázaro & Nieto-Montes de Oca 2015), although the estimated zone of contact does not obviously appear to be associated with any geographic barriers.

Within the Pacific clade, populations from the Central Depression of Chiapas are closely related to nearby Pacific coastal populations (Fig. 2). This result is consistent with commonly recognized biogeographic patterns (Johnson 2015; 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).

The Isthmus of Tehuantepec 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 studied plants and animals throughout the region (Morrone 2014; Halffter & Morrone 2017). 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 et al. 2007; Morrone 2014). The Pacific versant of the Isthmus, however, appears to be a strong dispersal barrier for lowland species (Bryson et al. 2011). The *Anolis sericeus* group is unlike any other anole species complex in Mexico in that it occurs throughout the Pacific versant east and west of the Isthmus (one possible exception, *A. boulengerianus*, has a distribution that does not extend far to the east and ends before reaching the state of Chiapas; Henderson & Fitch 1975). The anole communities on the west side are composed entirely of species from Mexican endemic clades and are deeply divergent from communities on the east side, which come from groups that are mostly Central American in origin (Gray, pers. obs.; Poe et al. 2017). The silky anole populations on either side 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.

Phylogeographic patterns in the South clade are more difficult to interpret than those of the North clade due to sparse sampling, but a pattern of fine-scale structure near the mountains of Nuclear Central America is clear (Fig. 2). The paraphyly and deep divergence between populations in the Pacific of Guatemala is worth investigating since some other taxa have diversified within this region (Wake 1987; Barber & Klicka 2010; Gutiérrez-García & Vázquez-Domínguez 2012; Ornelas et al. 2014; Fig. 2). However, most taxa in those studies have more restricted habitat or niche requirements, and thus may be geographically isolated more easily. Given the topographic complexity of the region, both geographic and ecological processes could have contributed to the observed population divergence within the South clade. Some mountain valleys of Guatemala (such as the Motagua Valley) have notably drier environments than surrounding areas and could contribute to isolation among lineages.

Ecological isolation and phylogeographic structure

Two major divergence events stand out in the phylogeographic history of the silky anoles—one at the base of Nuclear Central America (North vs. South clades) and another associated with different environmental regimes in southern Mexico (Pacific vs. Caribbean clades; Fig. 2). The first of these divergence events (the North/South break) is consistent with past geographic isolation between two lineages (Dobzhansky 1937; Mayr 1963; Avise 2000) and subsequent secondary contact, a scenario that was the best supported model in the coalescent analyses. Despite being on separate evolutionary trajectories for a longer period of time than the Pacific and Caribbean clades, niche divergence between North and South clades is not apparent. Background tests further demonstrate strong niche conservatism between the two groups (Fig. 4; Table 1). The

geographic distributions of the North and South lineages near mountains of Nuclear Central America are congruent with a role for geographic isolation in the past for these lowland-dwelling lizards. These results therefore support the hypothesis that the two lineages diverged in allopatry, maintained relatively conserved niches during isolation, and are now parapatric after coming back into secondary contact (Hypothesis 2).

The mode of divergence between the Pacific and Caribbean clades is difficult to infer without the niche divergence and coalescent analyses. There are no known or suggested physical barriers likely to have cut off gene flow in the region inhabited by the North clade (Kesler 1971; Morrone 2014). A widespread lowland generalist occurring in southern Mexico over the last few million years should have had a continuous distribution spanning the Pacific and Caribbean versants in the Chimalapas region of Oaxaca and Ocote region of northwestern Chiapas, even in the event of raised sea levels and the closing of the Isthmus of Tehuantepec (Kesler 1971; Bryson et al. 2011; Blakey 2011). None of the smaller Sierras present in the region reach high enough elevations to be uninhabitable for silky anoles based on their current distribution. Results from historical projections of the distribution of the silky anoles confirm the long-term stability of suitable habitat throughout the region (Fig. S2, Supporting Information). The deep genomic divergence between the Pacific and Caribbean lineages is surprising given the geographic history of the region and difficult to explain outside of ecologically-mediated divergence. Niche divergence between these two lineages is substantially more pronounced than between the North and South lineages (Fig. 3), and there is little overlap among the environmental variables that contributed most to the ENMs (Table 1), suggesting that these lineages may have experienced divergent niche evolution. This niche divergence is not merely a consequence of these clades being found in geographic regions with different environmental backgrounds. Although niche divergence is strong (Schoener's D = 0.16267; Warren's I = 0.37839), it is actually less than expected by chance based on the environmental backgrounds experienced by the two clades. This result suggests that these clades experienced differential natural selection between environments, leading to niche divergence despite some constraints on niche evolution. Our investigation into finer-scale niche overlap near the contact zone of the two lineages yielded similar or lower values relative to the comparisons between the entire clades (Fig. S3, Supporting Information), lending additional support for this finding. The results are consistent with niche divergence between Pacific and Caribbean clades, which could have evolved in parapatry, as suggested by the niche models and topography of the region (Fig. S2, Supporting Information), or in allopatry, as suggested by the coalescent analyses (Fig. 5).

Whether the observed niche divergence between the Pacific and Caribbean lineages occurred in parapatry or in allopatry is difficult to answer using current methods. Silky anoles presently occur in every lowland habitat we have searched, often in great abundance and regardless of the amount of disturbance. This kind of "generalist" natural history would make it difficult for the lizards to become geographically isolated in lowlands in the absence of physical barriers. Historical projections of the distribution of the group under different climatic regimes provide further evidence that divergence could have occurred in parapatry. Yet the coalescent analyses strongly prefer the secondary contact model, which would most easily be explained by a period of allopatry for the Pacific and Caribbean lineages. While the coalescent models tested in our study are likely

overly simplistic with respect to the variety of complex demographic scenarios potentially experienced by silky anole lineages, the preferred model suggests more gene flow now than in the recent past. Given the body of evidence for divergence in allopatry, it would be unsurprising for the initial divergence to have occurred with geographic separation between the populations. One of the lines of evidence (the ENMs or the coalescent analyses) is likely misleading. However, reciprocal monophyly of the Pacific and Caribbean lineages suggests that the niche divergence, whether it evolved in allopatry or parapatry, is potentially playing a role in maintaining some level of reproductive isolation between the two lineages (Hypothesis 3).

There are several avenues by which natural or sexual selection can lead to the pattern observed in the North clade. One possible mechanism underlying the environmentally associated isolation found between the Pacific and Caribbean clades 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 (Smith et al. 1972; Fitch 1973a; Losos 2009). However, in the seasonally dry Pacific of southern Mexico, silky anoles may have a significantly shortened breeding season, a pattern that has been found in other anole species inhabiting drier parts of the tropics (Fitch 1973b; Fleming & Hooker 1975). The effect these differences would have on population dynamics could be profound, possibly initiating divergence (Räsänen & Hendry 2008) and certainly limiting reproductive opportunities that could lead to admixture between the two clades. 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 (Lambert et al. 2013) at the contact zones is likely to be informative. These factors have been documented in another morphologically conserved anole species group, the *Anolis brevirostris* complex in the Greater Antilles (Webster & Burns 1973; Lambert et al. 2013).

We still know relatively little about rates of environmental niche evolution and what drives them (Warren et el. 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 at 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 et al. 2008; Thorpe et al. 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 et al. 2010) than between lineages that had previously been isolated for up to 8 million years on separate paleo-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 et al. 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 Fig. 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 similar niche requirements but be too similar to coexist, effectively preventing the invasion of one lineage into the distribution of the other.

CONCLUSIONS

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. 2013; 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), none have demonstrated environmental niche divergence as a major factor promoting broader phylogeographic divergence or speciation. Here we find evidence for lineage diversification related to divergent environmental niche evolution in an abundant and widespread lizard group that otherwise exhibits fairly strong niche and morphological conservatism. Coalescent modeling suggests that major divergence events were facilitated, at least in part, by periods of geographic isolation. 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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FIGURE CAPTIONS

Figure 1. Topographic map of study region with important geographical features highlighted.

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. The North clade is composed of the Pacific + Caribbean clades, as that group is sister to the South clade. Photo for Yucatan by Brittney White, photo for Honduras by Tom Kennedy. Photos for Pacific and Caribbean by Levi Gray.

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.

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)

TABLES

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

North Clade (AUC = 0.850)

Variable	PERM	PCT
Elevation	24.4	13.4
Precip seasonality	21.1	12.2
Temp seasonality	20.1	9.3
Temp annual range	5.3	13.9
Max temp of warmest month	4.4	4.5
Min temp of coldest month	4.1	19.7
Isothermality	3.3	1.3
Mean diurnal range	2.1	3.3

South Clade (AUC = 0.918)

Variable	PERM	PCT
Temp seasonality	55.8	35.5
Elevation	19.2	5.3
Precip seasonality	5.8	4.4
Mean temp of wettest qtr	5.1	0.6
Precip of warmest qtr	3.5	4.5
Min temp of coldest month	3.4	13.2
Mean diurnal range	2.2	1.6
Precip of coldest qtr	1.9	5.5

Pacific Clade (AUC = 0.961)

Variable	PERM	PCT
Isothermality	23.5	4.4
Precip of coldest qtr	17.7	18.5
Elevation	12.2	9.2
Temp seasonality	11.8	2
Mean diurnal range	8.4	5.5
Precip seasonality	7.7	19.3
Mean temp of wettest qtr	6.6	0.2
Mean temp of driest qtr	5.3	3.2

Caribbean Clade (AUC = 0.833)

Variable	PERM	PCT
Temp seasonality	28.5	39.2
Precip seasonality	23.4	5.3
Precip of driest month	15.8	22.3
Elevation	12.2	0.4
Temp annual range	6.3	0.3
Min temp of coldest month	4.4	1.3
Precip of driest qtr	3.7	0.2
Annual precip	2.9	12.1

Table 2. Results of coalescent simulations using δaδi.

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

FIGURES

Figure 1.

Figure 2.

Figure 3.

NORTH CLADE

SOUTH CLADE

Niche Overlap
Schoener's *D*: **0.32759**
Warren's *I*: **0.61622**

CARIBBEAN CLADE

PACIFIC CLADE

Niche Overlap
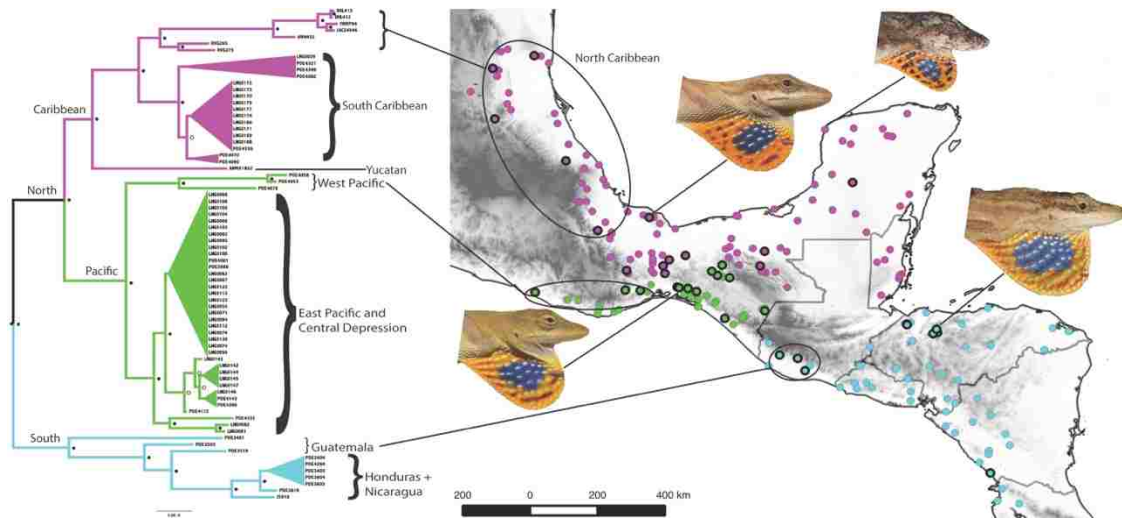**Schoener's** *D*: **0.16267**
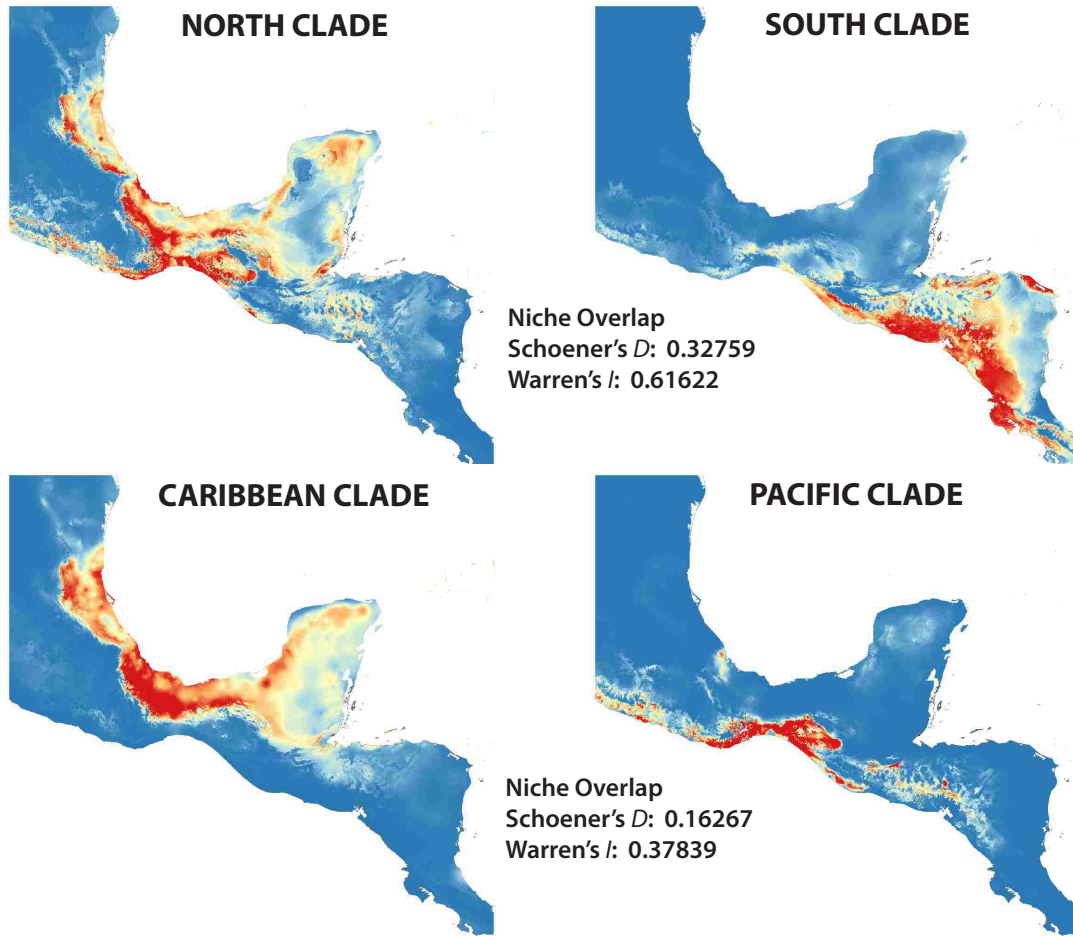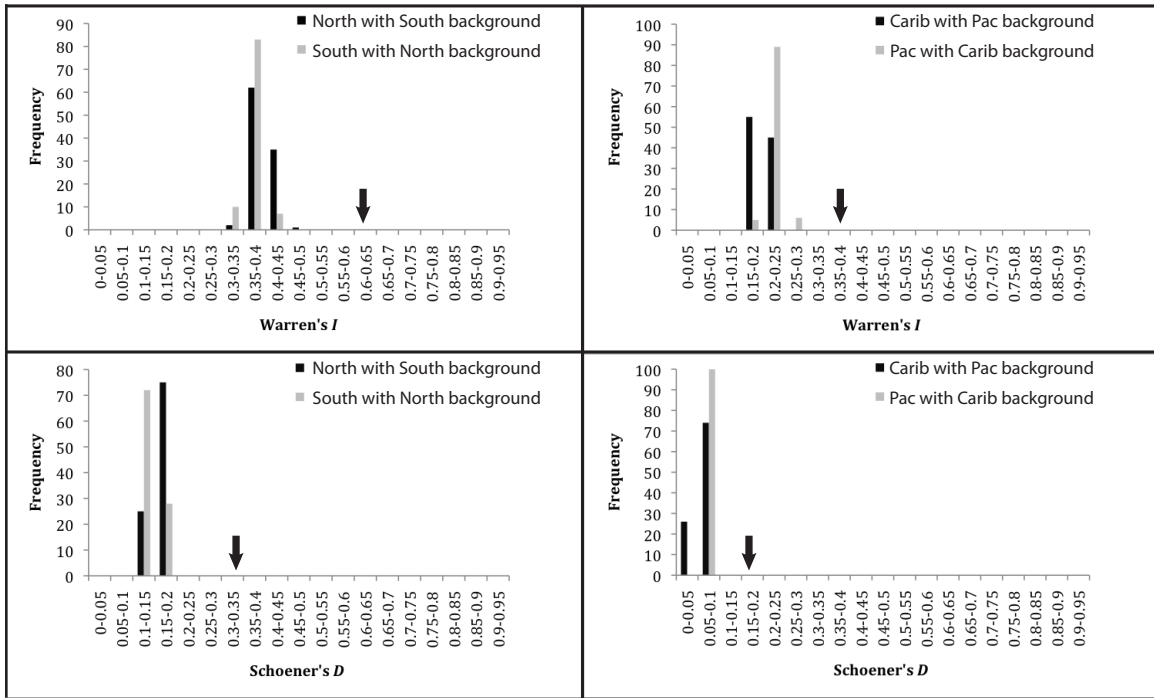**Warren's** *I*: **0.37839**

Figure 4.

SUPPLEMENTARY FIGURE CAPTIONS

Figure S1. Map showing annual precipitation throughout the distribution of the *Anolis sericeus* group. Note the Tehuantepec region and the rapid transition from wet to dry environments, which correspond with the broad zone of parapatry between the Pacific and Caribbean lineages.

Figure S2. Historical projections of ENMs for North clade. (a) is a projection of the niche model on climatic layers representing conditions during the last inter-glacial ~120-140,000 years ago. (b) is a projection during the mid-Holocene ~6000 years ago.

Figure S3. Ecological niche models constructed in Maxent and niche overlap for populations near the contact zone.
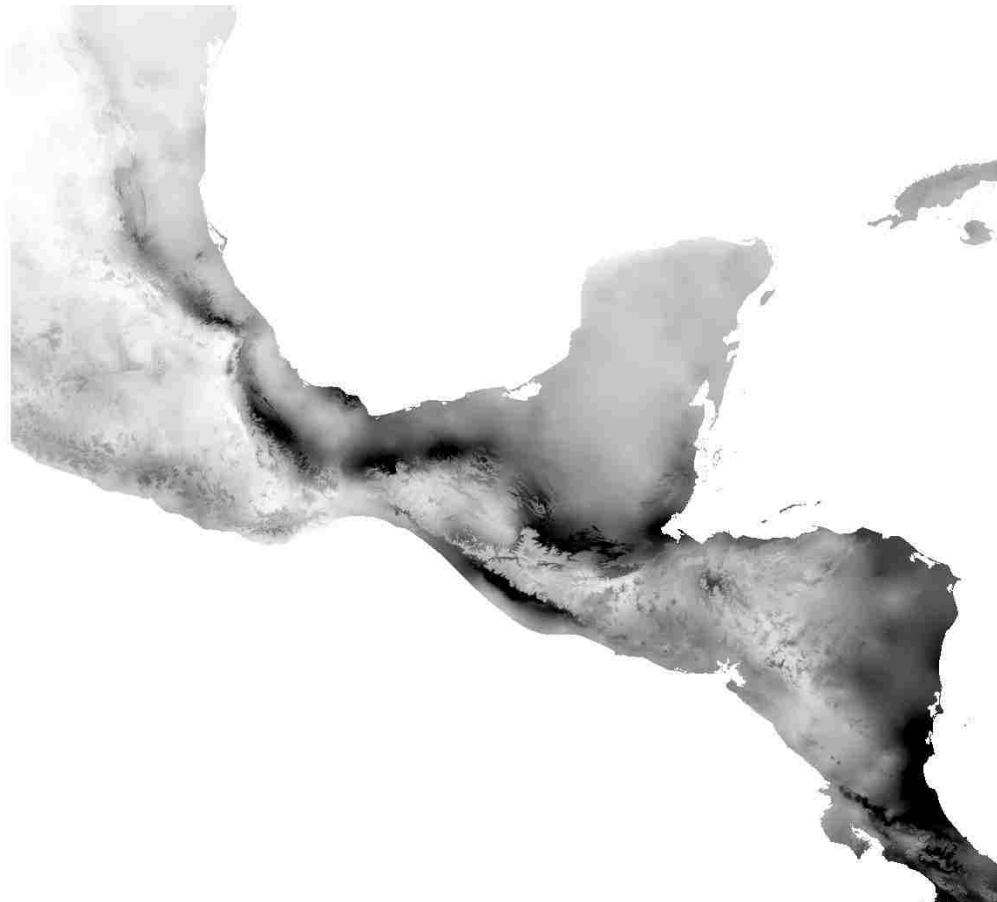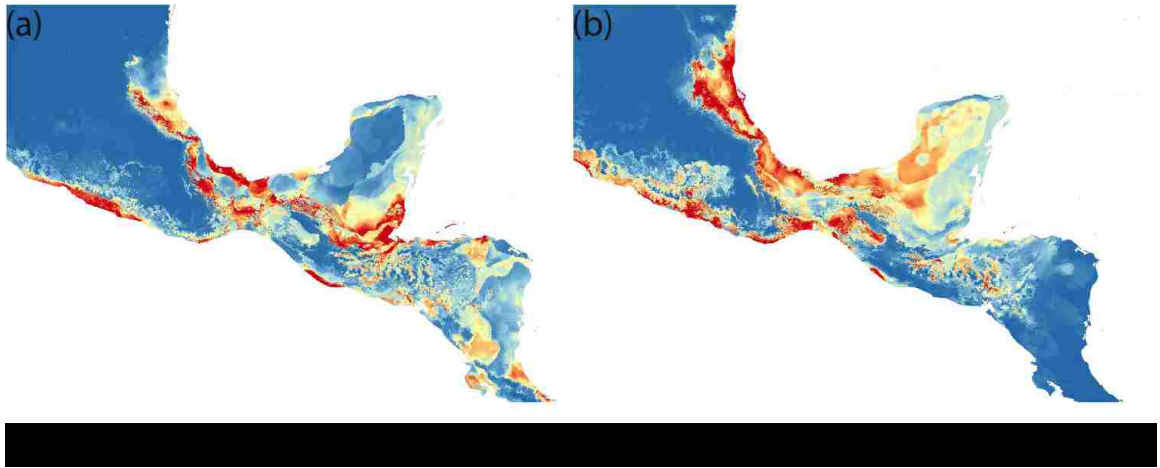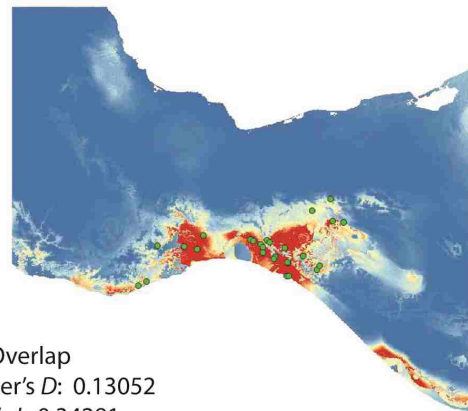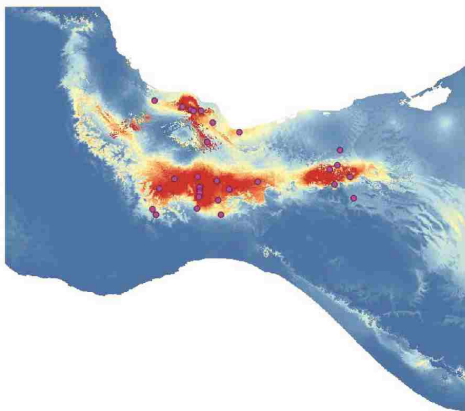
SUPPLEMENTARY FIGURES

Figure S1.

Figure S2.

Figure S3.

Niche Overlap
Schoener's *D*: 0.13052
Warren's *I*: 0.34281

CHAPTER 2

Can the length of the breeding season explain evolution of a sexual signaling trait in a tropical lizard clade?

AUTHORS
Levi N. Gray[1], Anthony J. Barley[2], David M. Hillis[3], Carlos J. Pavón-Vázquez[4], Steven Poe[1], and Brittney A. White[1]

[1]Department of Biology and Museum of Southwestern Biology, University of New Mexico, Albuquerque, NM 87131, USA
[2]Department of Biology, University of Hawai'i at Mānoa, Honolulu, HI 96822, USA
[3]Department of Integrative Biology, University of Texas, Austin, TX 78705, USA
[4]Research School of Biology, Australian National University, Acton, ACT 2601, Australia

ABSTRACT

Sexually selected traits are expected to increase in importance when the period of sexual behavior is constrained. Seasonality may shorten breeding seasons and escalate competition for mates. *Anolis* lizard male dewlaps are classic examples of multifaceted signaling traits, with demonstrated reproductive function, and are the only known example of temporal constraint driving evolution of a sexual signal. A previous study by Fitch and Hillis suggested a correlation between dewlap size and seasonality in mainland *Anolis*. Here, we present two tests of the Fitch-Hillis Hypothesis using new phylogenetic and morphological data sets for Mexican *Anolis*. Once phylogenetic relationships are accounted for, the relationship between dewlap size and seasonality erodes. This loss of statistical support for a significant relationship between dewlap size and seasonality occurs at both a macroscale of most Mexican species and a microscale of the only species group to occur broadly over both environmental extremes: the silky anoles (*sericeus* group). Our results suggest that seasonality is not a strong driver of evolution of *Anolis* dewlap size in Mexico. Perhaps due to the remarkable variability in natural and sexually selective forces driving complex signaling traits such as the *Anolis* dewlap, a single, general mechanism is unlikely to explain their evolution. Although theoretically plausible, evidential support for temporal constraint driving evolution of sexually selected traits currently is lacking.

KEYWORDS
Anolis, sexual selection, species recognition, sensory drive, dewlap, signaling trait evolution

INTRODUCTION

Signaling traits such as nuptial color in fish and bird songs are the focus of much research in evolutionary biology (1,2). Postulated mechanisms for the evolution of these traits span a variety of hypotheses of natural and sexual selection. Since many "successful" radiations exhibit such traits, they are also regularly identified as "key innovations" (3). Despite their apparent importance for evolution, determining selective drivers for signaling traits has proven difficult (2).

Anolis lizards are common research subjects in evolution (4). Among traits associated with *Anolis*, dewlaps are perhaps the most discussed and least understood. Males of almost all ~400 species have dewlaps, which are (usually) colorful flaps of skin used for species recognition, territorial behaviors, predator deterrence, and courtship (4). Dewlap size and color are incredibly variable in *Anolis*, and several attempts have been made to characterize general patterns (5-8). To date, these attempts have largely failed to find strong support for hypotheses expected to drive dewlap evolution.

Mechanisms suggested to play important roles in creating dewlap variation include species recognition (9), sensory drive (10,11), and sexual selection (5). The species recognition hypothesis suggests that dewlap variation evolved as a means for recognition of conspecifics (4). This hypothesis garners support from the observation that many anole assemblages are composed of multiple species with disparate dewlaps (4). Experimental and phylogenetic support for this hypothesis, however, has been elusive. Losos & Chu (6) and Nicholson et al. (7) tested for dewlap correlation with environmental, lineage, and assemblage factors across *Anolis* and found limited (nonsignificant; [6]) support for sensory drive and no support for other hypotheses.

Sexual selection is the only hypothesis that has been strongly supported in driving dewlap size across a broad sample of anoles. Fitch & Hillis (5) tested the hypothesis that dewlap size is correlated with length of the breeding season. They suggested that species with short breeding seasons have heightened levels of sexual selection relative to species that occupy aseasonal environments and breed almost year-round (5). Using data for 37 species, they found that anoles in seasonal environments had larger dewlaps than aseasonal species (5). Additionally, species with larger dewlaps also exhibited stronger male-biased sexual size dimorphism, providing more support for their hypothesis. The one species (*Anolis sericeus*; now considered a species complex (12,14]) found in both seasonal and aseasonal habitats also exhibited the dewlap pattern, with populations in seasonal environments possessing significantly larger dewlaps than those from aseasonal environments. This study constitutes the only empirical support for temporal constraint driving the evolution of a sexually selected trait.

There are two potentially confounding factors in the Fitch & Hillis (5) study. First, they did not apply a phylogenetic correction to their data, as a reasonable phylogenetic hypothesis for their sampled species did not exist then. Additionally, their

incomplete and biased sampling may have misled results. For instance, 9 of the 16 "seasonal" species were from a particular clade of west Mexican anoles that exhibits large dewlaps and occurs exclusively in seasonal areas, and there was also a notable lack of aseasonal species included.

Here, we aim to assess support for the Fitch-Hillis Hypothesis (FHH) of temporal constraint driving evolution of a sexual signaling trait. We do this at two scales. First, we test for a positive correlation between seasonality and dewlap size in Mexican anole species using phylogenetic regression. Second, we test for the same correlation within silky anoles (*Anolis sericeus* complex), the only species group that occurs broadly throughout highly seasonal and aseasonal environments in the region. We find no evidence for a significant relationship between seasonality and dewlap size once accounting for phylogenetic effects.

METHODS

Data collection and measurements

We took digital photographs of individuals collected in the field between 2010 and 2017. Photos were taken by multiple investigators throughout Mexico, spanning all habitat types inhabited by anoles. We included images for which 1) a method of calibration for proper measurement was in the photo, 2) the lizard's head was laid out flat, and 3) the individual was unquestionably a sexually mature male. As a proxy for lizard body size, we used head length (HL; 8). Measurements for HL (in mm) and dewlap area (mm^2) were taken using ImageJ (16). Coordinates were taken at collection sites (error: 3-12 m).

We extracted seasonality data from the seasonality of precipitation (BIO15) layer available through WORLDCLIM (17) for each collection site using QGIS (18). These values represent the coefficient of variation of the precipitation data and should be tightly correlated with length of breeding season for anoles (Fig. 1).

Macroanalyses

Mexican anole taxonomy has a long history of uncertainty (19,20). In our analyses, we included all Mexican species for which we had data, and for which previous research strongly supports their recognition (see supplementary material for details). To account for phylogeny in our macroanalyses, we used a recently published tree (15). We used the maximum clade credibility tree from the combined analysis of 46 morphological traits and 50 loci, trimming the tips to match the species for which we collected dewlap size data using the 'ape' package in R (21).

For each species, we averaged the relative dewlap size and seasonality values from the localities where a species was sampled. Because body size is a confounding variable for relative dewlap size (6), we ran a phylogenetic regression using the "gls" function in R (22) on log-transformed dewlap size with log-transformed HL for all species. We then used the residual values from that regression as relative dewlap size for each species. We performed another phylogenetic regression on the relative dewlap size against seasonality to test for a positive correlation between the two variables. We also

repeated the above analysis via standard (i.e., phylogenetically uncorrected) Ordinary Least Squares regressions.

Microanalyses

In Mexico, the *Anolis sericeus* group consists of three divergent clades that may be considered separate species (but are monophyletic; 13; see Fig. 1 and online supplement). We averaged relative dewlap size for speciemens from each locality and performed a standard OLS regression of dewlap size and head length using all silky anole localities. Using the residuals from that regression as measures for relative dewlap size, we then ran a regression of those values against seasonality. Subsequently, we performed another regression analysis using localities from only the Pacific and Caribbean clades. The Yucatan population males are diagnosed by their small dewlaps, so by removing them, we tested whether the Yucatan is strongly influencing the results of the analysis when the group is analyzed as a whole. We also ran each lineage on its own to test for signal within each group.

RESULTS

Macroanalyses

Our sampling resulted in data for 181 adult male dewlaps representing 41 non-silky anole species. Sampling within species ranged from 1-25 individuals from 1-19 localities. Within-species variation in dewlap size and seasonality was minimal for species represented by multiple individuals and localities (online supplements; Table S1).
Our standard regression with no phylogenetic correction showed a significant positive correlation between relative dewlap size and seasonality ($p = 0.02165$; Fig. 2). Once accounting for the phylogenetic relationships, the correlation was nonsignificant ($p = 0.3111$; Fig. 2).

Microanalyses

Our sampling for the silky anoles included 137 adult males representing 65 populations/localities (20 Pacific, 27 Caribbean, and 18 Yucatan). The regression on the entire group resulted in a significantly positive correlation between relative dewlap size and seasonality ($p = 0.00667$, adjusted $r^2 = 0.097$; Fig. 2). The regression incorporating only the Pacific and Caribbean lineages, however, fails to find a significant positive correlation between relative dewlap size and seasonality ($p = 0.2498$; Fig. 2). None of the single lineage analyses resulted in significant positive correlations between relative dewlap size and seasonality (online supplements; Fig. S1).

DISCUSSION

In our analyses at two evolutionary scales, the null hypothesis that dewlap size variation is unrelated to seasonality is accepted once shared evolutionary history is taken into account. In our macroanalysis, support without phylogenetic correction was much

weaker than in Fitch & Hillis (p = 0.02165 vs p < 0.001; 5), possibly reflecting more complete sampling in this study. The Yucatan silky anole lineage strongly influenced results of the micro-analysis because the lineage has a small dewlap and inhabits a region that is primarily aseasonal. The Caribbean lineage (sister to the Yucatan; 13) does not have a reduced dewlap and occurs in statistically indistinguishable seasonality environments (Table 1). If seasonality plays a large role in the evolution of dewlap size, we should see reduced dewlaps in both lineages found in relatively aseasonal environments or a significant increase in dewlap size in the lineage inhabiting highly seasonal environments. Neither of those observations obtains.

Our results and those of others (6,7) suggest that sexual selection, species recognition, and sensory drive hypotheses are not supported as sole explanations driving dewlap variation in macroanalyses of anoles. However, certain undeniably important aspects of dewlap variation—in particular, color pattern and display mechanics—have been difficult to incorporate on a broad scale. Incorporating those factors is likely to be challenging. The variability in dewlap color, pattern, size, and use is truly remarkable in *Anolis*, and unlikely to be the result of a single selective force (6).

With our finding of no strong association between dewlap size and seasonality, there currently is no support for temporal constraint driving evolution of sexual traits in any clade. Though Fitch & Hillis (5) suggested dewlap color could be a mitigating factor for size, our preliminary data do not support this idea. For instance, species with small dewlaps in seasonal environments do not have categorically "more bright" dewlaps than those with large dewlaps (supplementary material; Table S1). Mechanisms shaping the evolution of sexual traits are often difficult to determine in empirical systems (2). Perhaps approaches that incorporate more fine-scale inference of causal mechanisms (23) will find evidence for the FHH. Currently, however, the FHH remains theoretically plausible but is empirically unsupported.

ACKNOWLEDGEMENTS

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FIGURE CAPTIONS

Figure 1. (A) Map of sampling localities for the 41 species, with background raster reflecting seasonality (BIO15). (B) Sampling of the silky anoles, colored by clade. The Pacific clade (blue) occurs almost exclusively in the Pacific versant of southern Mexico, the Caribbean clade (purple) occurs south and west of the Yucatan in the Caribbean versant of Mexico, and the Yucatan clade (red) is only known to occur in the Yucatan Peninsula. These three clades form a monophyletic group to the exclusion of the rest of the silky anoles, which occur east and/or south of Mexico.

Figure 2. (A) Plot showing standard Ordinary Least Squares (OLS) regression (black line; $p = 0.02165$) and Phylogenetic Least Squares regression (dotted red line; $p = 0.26$) of macro-analyses. (B) Plot showing OLS regression of all silky anole populations (black line; $p = 0.00667$) only Pacific and Caribbean silky anole populations (dotted red line; $p = 0.1514$).

TABLE

Table 1. Silky anole sampling, along with average and range of seasonality experienced by each lineage. The Caribbean and Yucatan lineages occur in indistinguishable seasonality environments (t-test, p = 0.6377).

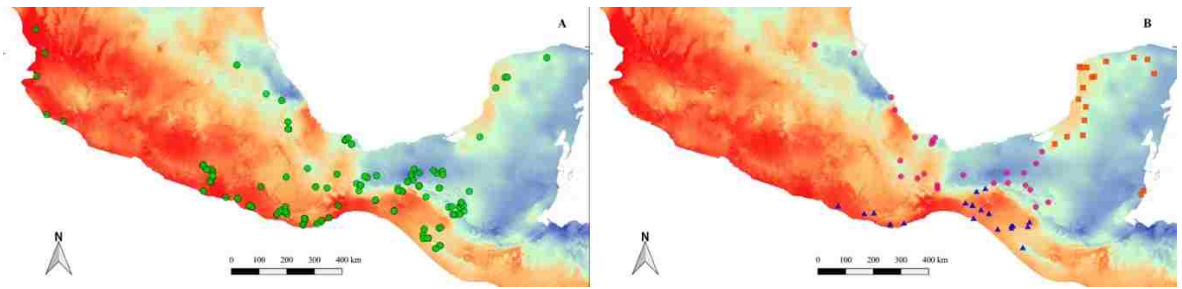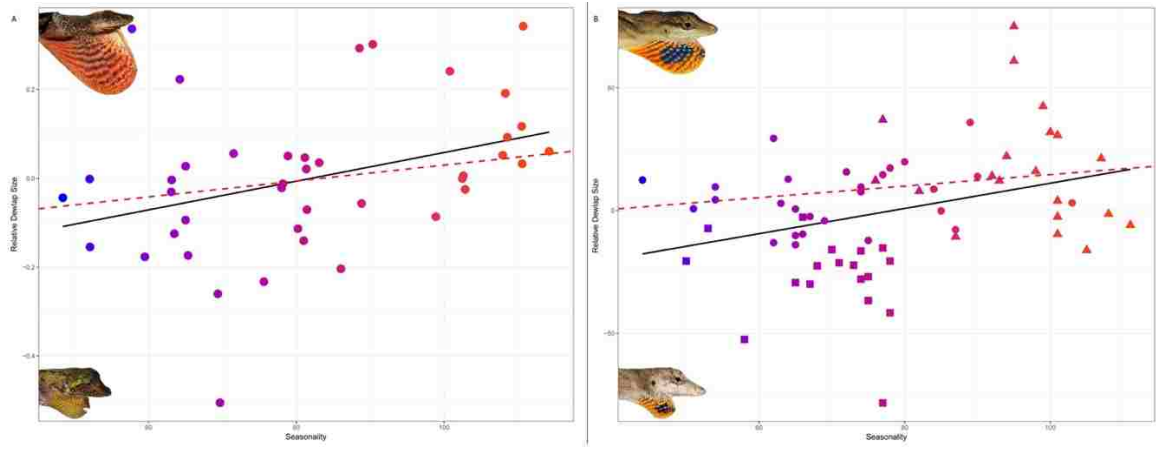| Lineage	Sample Number	Number of Localities	Average Seasonality	Range of Seasonality
Caribbean	58	27	71.1	44-103
Pacific	39	20	96.2	76-111
Yucatan	40	18	69.4	50-78

FIGURES

Figure 1.

Figure 2.

SUPPLEMENTAL TEXT

Taxonomic decisions

Excluded taxa in macroanalyses

The authors decided it would be premature to recognize several recently-described Mexican anole species that lacked strong evidence supporting their recognition. The main concern is that in recognizing lineages that do not properly reflect species-level diversity, we would potentially bias the results of our analyses.

We excluded taxa that were described primarily on the basis of differences in hemipenial morphology between populations (Köhler & Veseley 2010; Köhler et al. 2010; Köhler et al. 2014). The reasoning for delimiting the populations as species by the authors is that hemipenial traits are likely to be associated with reproductive isolation. In the species groups that have been investigated with multiple lines of molecular and/or morphological evidence, this hypothesis has not been supported. For instance, there are now several documented examples of within-species and within-population variation in hemipenial morphology (Köhler et al. 2012; Phillips et al. 2015; Lara-Tufiño et al. 2016; Gray et al. 2018). Finding variation in hemipenial morphology within species is to be expected due to high rates of hemipenial evolution (Klaczko et al. 2015) and the resulting likelihood of multiple hemipenial forms to exist prior to speciation (as expected with other traits not associated with reproductive isolation; Lara-Tufiño et al. 2016). Gray et al. (2018) used a large multilocus RAD data set to infer phylogeographic relationships in the silky anoles (*Anolis sericeus* complex) and found no association between population divergence and hemipenial morphology. The two taxa described based on hemipenial morphology that we excluded from analyses, *A. carlliebi* and *A. sacamecatensis*, were poorly sampled throughout the group's continuous distribution, making it difficult to make a strong case in support of recognizing the putative species as valid at this point in time (Köhler et al. 2014).

We also excluded taxa that were described solely on the basis of varying levels of divergence among mitochondrial haplotypes (Köhler et al. 2014). There is an abundance of evidence that mtDNA haplotypes can exhibit strong patterns of divergence without barriers to gene flow (Irwin 2002; Funk & Omland 2003; Petit & Excoffier 2009), and anole systems have been known to show significant mitochondrial divergence between freely-interbreeding populations (Thorpe et al. 2008; Thorpe et al. 2010; Ng & Glor 2011; Ng et al. 2016). Anole populations delimited as separate species by Köhler et al. (2014) exhibit far lower levels of mtDNA divergence than other populations known to maintain evolutionary cohesion (Thorpe et. al. 2008; Ng et al. 2011). Taxonomic conclusions were further confounded by not sampling near putative contact zones of the populations they described as separate species. A standard isolation-by-distance model explains the resulting phylogenetic structure in their analyses, and in the absence of evidence for reduced or absent gene flow it would be questionable to assume the populations are on independent trajectories.

For further information on species not recognized in our study, see Table S2.

Microanalyses

We grouped populations by clades recovered via phylogenetic analysis of a large multilocus genomic data set (Gray et al. 2018) rather than species delimited using hemipenial morphology (Köhler & Vesely 2010). The molecular data used in that study consists of over 500 restriction-site associated DNA (RAD) markers and attained strong resolution for clades within the silky anole group. The populations present in Mexico represent a monophyletic group that is deeply divergent from populations in Guatemala, Honduras, and to the southeast. Only the Yucatan lineage is morphologically distinct, which can be diagnosed by a smaller dewlap in males and a slightly larger dewlap in females (Lara-Tufiño et al. 2016).

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SUPPLEMENTAL FIGURE CAPTION

Figure S1. Figure showing best-fit Ordinary Least Square regression lines for each silky anole lineage. Red squares represent the Yucatan lineage, purple circles represent the Caribbean lineage, and blue triangles represent the Pacific lineage. The Caribbean regression line shows a positive slope, unlike the other two groups, but with a p-value of 0.3964 it remains nonsignificant.

SUPPLEMENTAL FIGURE
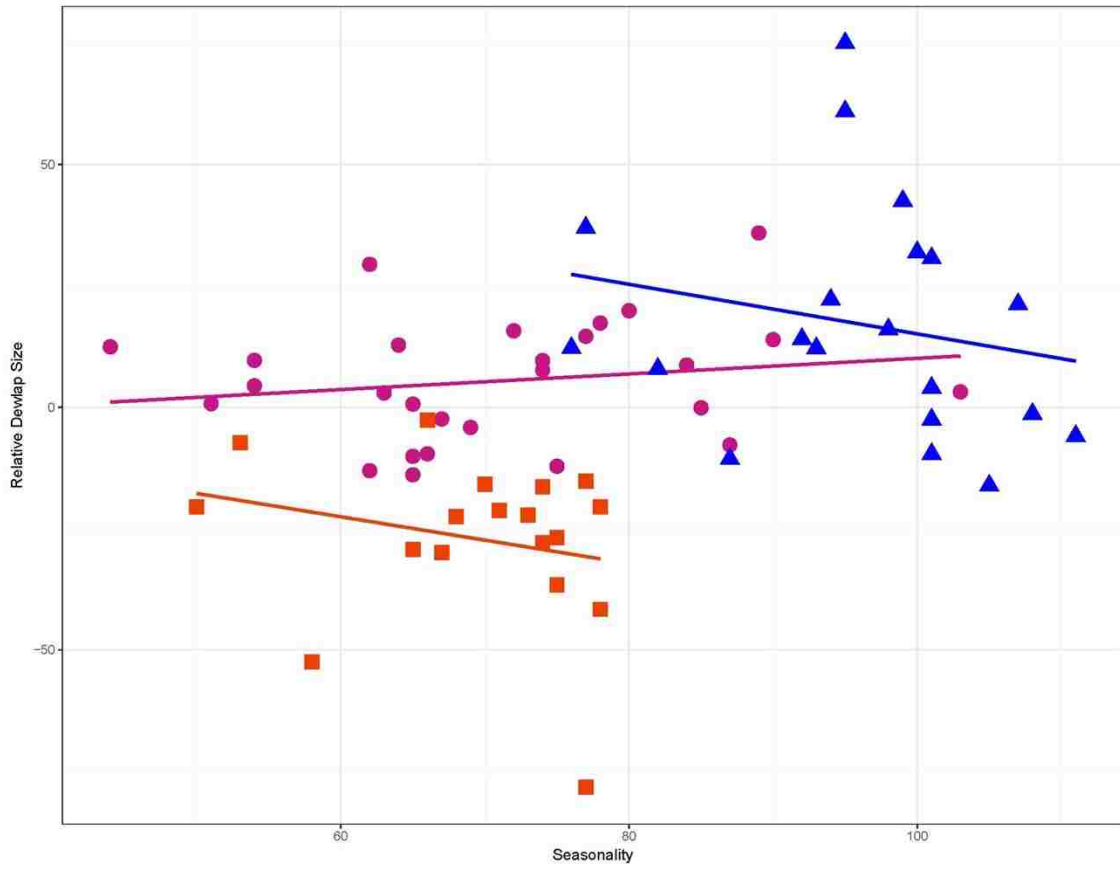
SUPPLEMENTARY TABLES

Table S1. Table providing details on per species sampling, range in dewlap size, seasonality of sampled localities, and dewlap color. For dewlap color, up to three colors are listed: primary color, secondary color, and tertiary color are ranked by proportion of each color in the dewlap.

Anolis Species	No. of Samples	No. of Localities	Range logdsize	Range Seasonality	Color
alvarezdeltoroi	3	2	2.51-2.62	55-71	Red
barkeri	3	3	2.69-3.13	44-65	Red/purple/white
beckeri	6	5	1.68-2.36	44-54	Pink
biporcatus	2	2	2.62-2.76	64-74	Blue/orange/white
boulengerianus	10	8	1.94-2.46	106-115	Orange/yellow
campbelli	2	1	2.4	65	Pink
capito	3	3	2.01-2.22	68-71	Yellow
compressicauda	3	3	2.37-2.41	44-57	Purple/yellow
crassulus	4	3	2.13-2.47	75-85	Orange
cristifer	1	1	2.27	86	Red
cymbops	1	1	2.37	79	Pink
dollfusianus	3	2	2.05-2.07	75-81	Yellow
duellmani	7	3	1.97-2.37	63	Purple
dunni	2	2	2.29-2.48	106-111	Red/yellow
gadovii	1	1	2.78	108	Purple/pink
hobartsmithi	2	2	2.23-2.25	47-50	Purple
laeviventris	15	12	1.71-2.15	50-100	White
lemurinus	16	12	1.84-2.4	53-76	Red/orange
liogaster	2	2	2.21-2.52	102-103	Purple
macrinii	1	1	3.14	90	Orange/white
matudai	3	3	2.24-2.31	83-99	Purple
megapholidotus	2	2	1.94-2	110-111	Pink
microlepidotus	1	1	2.28	102	Orange/yellow
milleri	1	1	2	81	Pink-purple
naufragus	6	3	2.15-2.38	68-78	Orange-red
nebuloides	13	10	1.97-2.63	93-109	Pink
nebulosus	8	8	1.72-2.28	110-126	Orange
omiltemanus	4	3	2.04-2.29	102-104	Orange
parvicirculatus	2	2	2.38-2.41	80-83	Red/orange
petersi	4	2	2.14-2.66	63-88	Red/black
peucephilus	1	1	1.93	99	Orange
purpuronectes	1	1	2.96	64	Purple

quercorum	3	2	2.26-2.41	87-90	Pink
rodriguezii	25	19	1.67-2.03	44-89	Orange-yellow/red
rubiginosus	1	1	2.04	81	Pink
schiedii	2	1	2.4-2.44	78	Orange
serranoi	3	3	2.51-2.61	75-88	Red/black
subocularis	6	6	2.46-2.87	107-113	Pink/yellow
taylori	2	1	2.64-2.69	111	Red/mint
tropidonotus	3	2	2.09-2.3	65	Yellow/red
uniformis	3	2	1.95-1.97	53-64	Purple/pink

Table S2. Table summarizing evidence for and against recently described species not recognized in this study.

Species not recognized	Synonymous with	Evidence for validity	Evidence against
Anolis nietoi	*Anolis nebuloides*	mtDNA clustering	Phylogenetic trees not consistent using their markers; morphology and continuous distribution of population does not suggest reproductive isolation
Anolis stevepoei	*Anolis nebuloides*	mtDNA clustering	Phylogenetic trees not consistent using their markers; morphology and continuous distribution of population does not suggest reproductive isolation
Anolis zapotecorum	*Anolis nebuloides*	mtDNA clustering	Phylogenetic trees not consistent using their markers; morphology and continuous distribution of population does not suggest reproductive isolation
Anolis carlliebi	*Anolis quercorum*	mtDNA clustering/hemipenial morphology	Morphology and only slight hemipenial differences. Molecular sampling missing many important localities.
Anolis sacamecatensis	*Anolis quercorum*	mtDNA clustering/hemipenial morphology	Morphology and only slight hemipenial differences. Molecular sampling missing many important localities.
Anolis immaculogularis	*Anolis subocularis*	Distinct in dewlap coloration from one species and in mtDNA divergence from another	Phylogenetic trees show a clustering of a mix of *immaculogularis* and *subocularis*; the population described is not distinct in morphology or in mtDNA.

CHAPTER 3

Using RAD markers for phylogenetic inference of an old and diverse clade of lizards

AUTHORS
Levi N. Gray[1], Anthony J. Barley[2], and Steve Poe[1]

[1] Department of Biology and Museum of Southwestern Biology, 1 University of New Mexico, MSC03, Albuquerque, NM 87131 USA
[2] Department of Biology, University of Hawai'i at Mānoa, Honolulu, HI 96822, USA

ABSTRACT
 Anolis lizards (anoles) are common study subjects in ecology and evolution. They have been the focus of a large number of phylogenetic studies incorporating many different molecular and morphological data sets. Results from analyses of these data sets have been questioned based on problems of random and selective convergence, weak clade support, and the suspected inadequacy of the incorporated data types for resolving deep phylogenetic splits. Restriction-site associated DNA (RAD) markers are widely cited as being useful for phylogenetic inference of relatively young groups, not unlike mtDNA markers. However, few "old" groups have been investigated using these markers, and the sheer number of loci in RAD data sets has at times been used to useful phylogenetic effect. In this chapter, we attempt to assess the utility of RAD markers in resolving the phylogeny of a select group of anoles from across the clade and compare results from RAD analyses with phylogenetic inference of morphological and mtDNA data sets. We found that Bayesian phylogenetic analyses of our RAD markers produces a tree that is nearly completely resolved for most shallow and deep splits in anoles. Furthermore, the results are broadly congruent with our mtDNA analysis, suggesting, for the first time, some confidence in hypothesized deep phylogenetic relationships in anoles. Analysis of the morphological data set resulted in mostly unresolved relationships and produced no strong conflict with the molecular results. Our results suggest that RAD markers may be useful for lineages ~60 million years old, even when data matrices contain ~35% missing data.

KEYWORDS
 Anolis, phylogenetics, restriction-site associated DNA libraries, morphology, NADH dehydrogenase subunit 2, convergence

INTRODUCTION

Anolis lizards have long been popular research subjects in biology. This popularity is due in part to *Anolis* being a classic example of an adaptive radiation, but other factors such as ease of study and relevance for sexual selection research are also responsible (Losos 2009). Two synapomorphic morphological traits—the dewlap, a sexual signaling organ, and toepads, expanded scales that facilitate climbing—are not unique to anoles but have nonetheless likely contributed to the diversification of the clade. Despite this extensive focus from researchers, known diversity within the genus is still underestimated. New species continue to be described, making it even clearer that a well-resolved, complete phylogeny of the group is not likely to be published soon.

Phylogenetics studies of *Anolis* have an extensive history and began, as studies of most groups have, with inference based on morphological traits (Etheridge 1959). The Etheridge work (1959) used skeletal traits to categorize groups of anoles, which were later expanded upon by Williams (1976). One influential finding of Etheridge (1959) was the discovery of a trait uniting the "Beta" anoles—transverse processes on the caudal vertebrae. "Alpha" anoles were species that lacked that trait. The first quantitative morphological phylogenetic analyses performed on a broad sampling of anoles incorporated a total of 27 species (Guyer & Savage 1992; see also Guyer & Savage 1986) and resulted in the suggested erection of new genera to accommodate what was clearly a diverse group of lizards. The genus *Norops* was applied to the monophyletic Beta anoles, a genus name that is still used by some workers (e.g. Nicholson et al. 2012). Morphological traits continue to be incorporated into phylogenetic analyses, though the characters used have changed a fair amount through time (Poe 2004; Poe et al. 2017).

The first molecular phylogenetic analyses of anoles were aimed at resolving various subgroups of *Anolis* (Gorman 1973; Shochat & Dessauer 1981; Burnell & Hedges 1990), and greatly improved our understanding of evolutionary relationships. But the work that set the baseline for what marker would be sequenced during the Sanger sequencing period is Jackman et al.'s (1999) attempt at addressing broad relationships among anoles. In that work, they presented a new mtDNA data set composed primarily of the NADH dehydrogenase subunit 2 (ND2) and found that the several recent splits were strongly resolved in their analyses (Jackman et al. 1999). Moving forward, researchers built on the ND2 dataset (as well as the morphological data set) in attempts to resolve the phylogenetic relationships of anoles (Poe 2004; Nicholson et al. 2012), of which the recent culmination is Poe et al's (2017) work that estimated phylogeny for all 379 species of *Anolis* that those authors judged to be valid at the time of that work. Although many other molecular markers, both mitochondrial and nuclear, have been added through time, the phylogenetic signal in ND2 has played an important role in phylogenetic inference of *Anolis* to date, and indeed of squamates in general (Townsend et al. 2004).

With the advent of Next Generation Sequencing (NGS) technologies, the ability to amass large nuclear genomic data sets has advanced research in phylogenetics (McCormack et al. 2013). Though various NGS data sets have been incorporated into multiple *Anolis* studies (Campbell-Staton et al. 2016; Manthey et al. 2016a; Tollis et al. 2018), a data set has not yet been used to attempt to resolve broad phylogenetic relationships using Restriction-site Associated DNA (RADseq) or Ultra-Conserved

Elements (UCEs). Considering the dependence on the signal exhibited by mtDNA markers for understanding phylogenetic relationships of anoles, use of a large data set of highly variable and unlinked nuclear markers for anole phylogeny is warranted. Furthermore, such a data set could be very informative regarding the utility of the mtDNA signal for resolving older splits in this diverse group.

The aim of this paper is to assess the utility of a RAD marker data set in resolving evolutionary relationships of 43 species of *Anolis* in a Bayesian framework. Given expressed and demonstrated concerns about RAD data sets (Rubin et al. 2012), the RAD analysis may prove to have the same apparent limitation of the ND2 analysis—difficulty in finding support for branching order of deep splits (but see Harvey et al. 2016; Manthey et al. 2016). We also compared the consensus RAD tree to results of morphological and mtDNA analyses. The morphological analysis was not expected to provide much resolution with only 46 traits, but the mtDNA has been shown to provide reasonable support for the recent splits within *Anolis*. Comparison of the RAD and ND2 data sets should be informative for determining which recovered relationships are likely to reflect the true history of the group (Swofford 1991), and for testing the utility of these data sets for resolving deep splits in an old, diverse group (~46-64 mya; Poe et al. 2017).

METHODS

Samples chosen for sequencing were selected to maximize resolving deep splits in the *Anolis* tree based on Poe et al. (2017). Raw sequence reads were demultiplexed, quality filtered, and de novo assembled using pyRAD v3.0.66 (Eaton 2014). We followed the same methods as in Gray et al. (2018), exploring changes in parameters (minimum read depth, minimum coverage across samples, and maximum proportion of shared heterozygous sites) to see how they affected downstream results.

Two data sets were compiled—one for all species for which we had >20% of the selected loci, and another for only the species that we also had both ND2 and morphological data.

ND2 and morphological data

The morphological data set of 46 traits and ND2 mtDNA sequences were taken from the Poe et al. (2017) data matrix. The ND2 data set was trimmed to 1037 bps shared by most samples.

Phylogenetic analyses in MrBayes

For the morphological analysis, 42 of the traits were ordered and 4 unordered. Gamma-distributed rate variation was allowed with six categories, as in Poe et al. (2017).

For the ND2 and RAD analyses, we used PartitionFinder v1.1 to find an optimal partitioning scheme. There were three partitions for the ND2 analysis (one for each codon) and each locus was treated as a potential partition in the RAD data set. The best substitution model was selected via Akaike Information Criterion (AICc) from the models available in MrBayes. We also used "model-averaging" for RAD analyses in MrBayes (Stamatakis 2006; Moyle et al. 2012; Poe et al. 2017).

We ran all analyses for 10 million generations, sampling every 1000 generations, and used Tracer v1.4 (Rambaut & Drummond 2007) to verify convergence.

Comparing trees

Support for shared clades among consensus phylogenetic estimates was compared at nodes supported in the RAD tree by Posterior Probabilities (PP) of 0.95 and higher and at 0.99 and higher.

RESULTS

The 43 taxon RAD tree was very well-resolved, with 32/40 nodes resolved at >0.99 PP and 34/40 resolved at >0.95 (Figure 1; Table 1). A series of very short internodes exposes the difficulty of resolving branching order early on in the lineage sister to *Anolis auratus*.

The Bayesian analysis of the morphological traits, as expected, did not lead to a well-supported tree. Two clades were recognized with very weak support (PP > 0.8), one clade with PP > 0.95 (Fig. 1), and none of those groups were supported by the ND2 or RAD trees.

The ND2 tree had weak support for most clades (only 9 out of 24 nodes resolved with high PP > 0.95) and exhibited two major polytomies where branching order could not be resolved. One of these polytomies represents an inability to resolve branching order deep in the anole tree, while a second polytomy has seven branches and occurs at a shallower portion of the tree.

DISCUSSION

RAD markers and their utility in phylogenetic inference of old, diverse groups

The most surprising result of this study is the strength of phylogenetic resolution of anole relationships for the 43 (mostly) distantly-related species. In Figure 3, we highlight the clades that are for the first time well-supported (PP > 0.99) in a broad sampling of anoles. Jackman et al. (1999) discussed evidence for a hard polytomy deep in the anole tree, but our RAD results do not support this inference. The most difficult to resolve portion of anole evolutionary history based on the RAD tree does not appear to be deep in the tree, but rather sorting the branching order of a series of mainland *Draconura* clades (Fig. 1). This result might be expected due to the large number of Mexican and Central American species included here and our sparse sampling of Caribbean lineages. Regardless of sampling effects, the deep branches in the anole tree appear, for the first time, to be strongly-supported.

Patterns of Anolis *phylogeny*

Our findings are significant in that the diversification of *Draconura* (the *Norops* lineage that re-invaded the mainland) is understudied but might represent fertile ground for testing several general evolutionary patterns. For instance, a large portion of the

species diversity within *Anolis* is in the *Draconura* clade, and these forms might constitute an adaptive radiation as shown classically among anoles in the Greater Antilles (Poe et al. 2018).

Biogeographic patterns pointing to further testing also are evident in our RAD tree. Species represented in the strongly supported clades within that radiation are found throughout the mainland, from South America to Mexico. The sister to this putative rapid radiation event is *Anolis auratus*, a species with the bulk of its distribution in South America but reaches Central America in Panama. This relationship was found in Poe et al. (2017) but with weaker support (PP = 0.81). The finding suggests that after reinvading the mainland (believed to be within modern Mexico and Central America; Nicholson et al. 2005; Poe et al. 2017) from Jamaica, the next divergence event took place between Central and South America. Without a terrestrial bridge between Central and South America, the lineage would need to disperse over the ocean to reach South America. Once there, the ancestor of *Anolis auratus* and relatives (such as the *A. chrysolepis* series) diversified to a much lesser extent than its sister group in Central America. It is possible that the presence of anoline lizards in South America hindered the diversification of the *auratus-chrysolepis* lineage as compared to the rest of the *Draconura* in Central America and Mexico, where a significant portion of anoline diversity occurs.

Phylogenetic analyses of mtDNA vs RAD markers

Analyses of both ND2 and RAD markers supported a deep split between *Dactyloa* lineage anoles and the rest (Fig. 3). Generally, there is strong concordance between the two preferred trees. Nearly all clades supported with PP of >0.99 in the RAD analyses were either supported or not contradicted in the ND2 analyses (24 nodes out of 25 total). Only one node was strongly contradicted: in the RAD tree, *Anolis macrolepis* and *A. lionotus* were sister species, with *A. gaigei* as sister to them. In the ND2 tree, *A. macrolepis* was strongly supported as sister to *A. gaigei*, with *A. lionotus* as the outgroup. Since both analyses have PP above 0.99, the correct biological interpretation of this inconsistency may be past mitochondrial introgression between *A. macrolepis* and *A. gaigei*. Analyses of both data sets struggle to resolve the cluster of mainland lineages (marked *Draconura* on Fig. 3) that correspond with the re-invasion of the mainland from the Greater Antilles. However, it is worth noting that the polytomy in the 34 taxon RAD tree is partially resolved with the addition of more taxa in the 43 taxon tree (Figure 1). This result provides evidence that the addition of more taxa might further resolve branching order issues in the tree.

The major difference between the RAD and ND2 trees is in overall node support (Fig. 3; Table 1). Analyses of the RAD data set provided much stronger resolution to clades at all levels of divergence. Relationships that had previously been weakly supported (Nicholson et al. 2012; Poe et al. 2017), such as *A. extremus* as being sister to a group of mainland *Dactyloa* anoles, are very strongly supported for the first time in our RAD tree. Matters of support notwithstanding, ND2 appears not to have been particularly misleading in phylogenetic studies of *Anolis*, as analysis of our nuclear data set consisting of hundreds of loci is largely congruent with the ND2 analysis.

CONCLUSIONS

This first attempt at resolving phylogeny of anoles using an NGS data set suggests that RAD markers may be more useful than expected for phylogenetic inference of old and diverse groups (Rubin et al. 2012). It also verifies that ND2 has been a very useful and informative marker for inferring phylogeny of anoles, as the majority of supported relationships inferred from that gene are supported by analysis of a large number of nuclear loci. The comparatively cheap cost of RAD library preparation and sequencing may make this approach an appealing option for researchers aiming to uncover evolutionary relationships of relatively old lineages. In this study, we have found strong support for several evolutionary relationships in a difficult taxonomic group for the first time despite the old evolutionary history and high species diversity of this group.

ACKNOWLEDGEMENTS

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FIGURE CAPTIONS

Figure 1. Consensus tree of the 43 anole taxa from MrBayes analysis of RAD markers.

Figure 2. (A) Consensus tree of 34 anole taxa based on MrBayes analysis of 46 morphological traits, with monophyletic lineages from morphological analyses highlighted on ND2 (B) and RAD (C) trees.

Figure 3. (A) Consensus tree of the 34 anole taxa based on MrBayes analysis of ND2 and (B) consensus of RAD analysis. Red dots indicate PP > 0.99, orange dots indicate PP $0.99 > x > 0.95$, and blue dots indicate PP < 0.95.

TABLE

Table 1. Table summarizing congruence between 34-taxon RAD and ND2 trees.

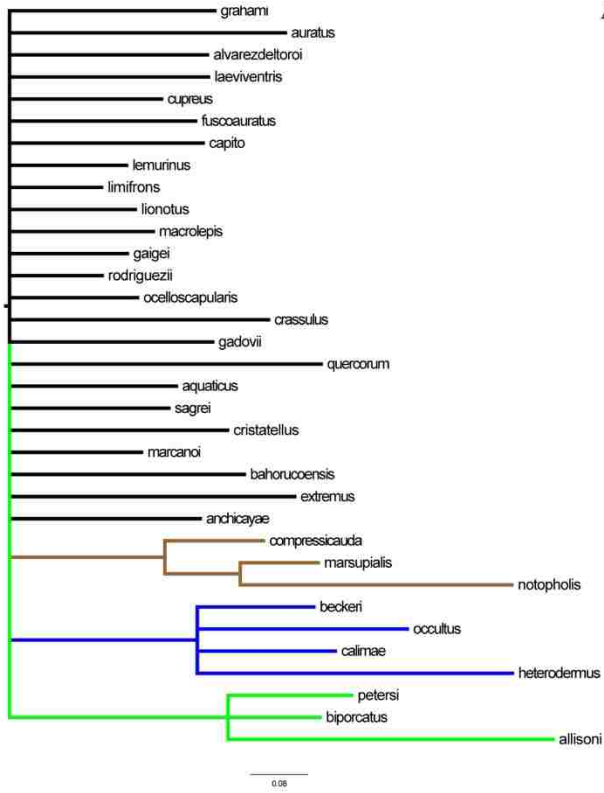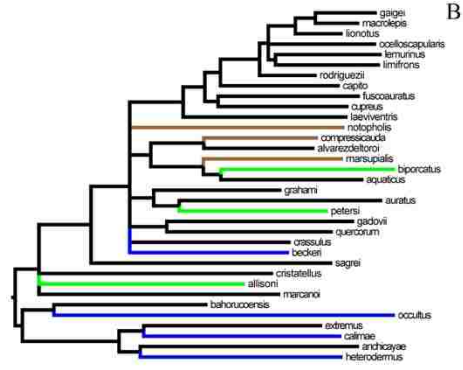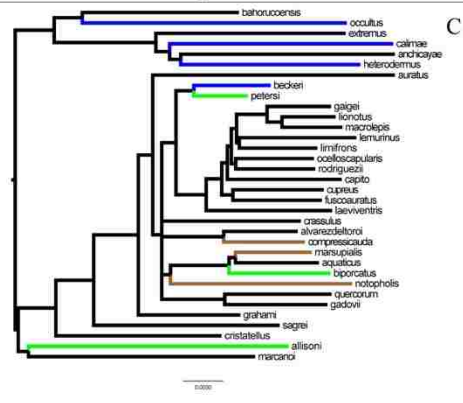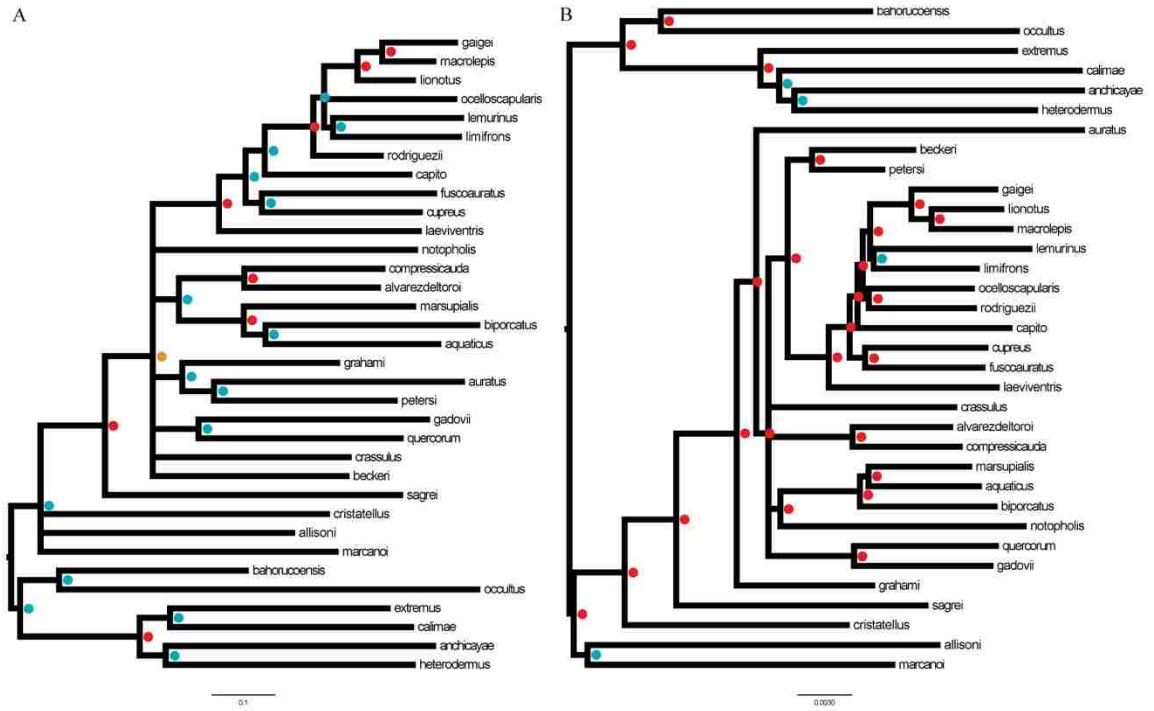|	RAD nodes PP > 0.99
ND2 supports	8
ND2 no contradiction	16
ND2 contradicts	1

FIGURES

Figure 1.
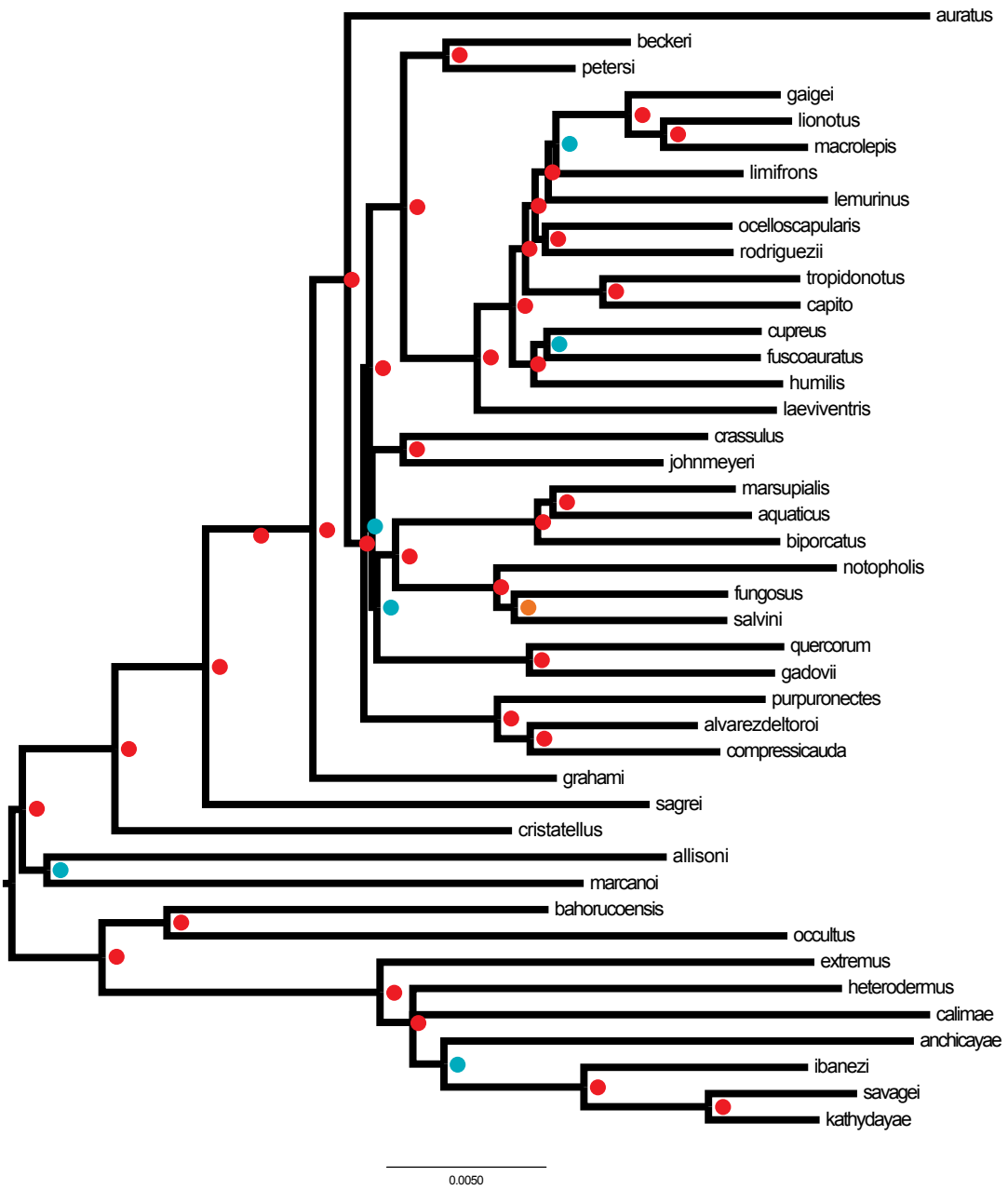
Figure 2.

Figure 3.

CONCLUSIONS

       In this dissertation I examined patterns of diversity and evolution in mainland *Anolis* lizards (anoles). In chapter one, I inferred phylogeographic relationships of a widespread species complex and delved into mechanisms of divergence. In chapter two, I tested a commonly-cited hypothesis for dewlap evolution using new data sets and methodology. In chapter three, I tested a new molecular marker set for phylogenetic utility in an old and speciose group. Each chapter, in its own way, demonstrated the potential for evolutionary investigations using mainland anoles as study subjects.