2018

Unearthing the Past and Present of a Semi-fossorial Lizard: Conservation Genetics, Phylogeography, and Taxonomy of Plestiodon egregius.

Kathryn Mercier
University of Central Florida

UNEARTHING THE PAST AND PRESENT OF A SEMI-FOSSORIAL LIZARD: CONSERVATION GENETICS, PHYLOGEOGRAPHY, AND TAXONOMY OF *Plestiodon egregius*

by

KATHRYN P. MERCIER
B.S. University of Central Florida, 2012

A thesis submitted in partial fulfilment of the requirements
for the degree of Master of Science in Biology
in the Department of Biology
in the College of Sciences
at the University of Central Florida
Orlando, Florida

Summer Term
2018

Major Professors: Christopher L. Parkinson and Anna E. Savage

ABSTRACT

Characterizing an organism's evolutionary history and population structure as well as understanding the forces shaping that divergence is crucial to conservation biology. A clear understanding of the patterns of diversity and divergence are imperative for the best management of the organism, while an awareness of what drives these patterns can lead to better predictions of how organisms will respond to future climate change. Historical climate changes and associated sea level change are among the main forces driving divergence in many species. To examine how effects of climate changes may have driven patterns of intraspecific divergence, I examined Mole Skinks, *Plestiodon egregius*, a semi-fossorial lizard of conservation concern. First, I characterized *P. egregius* evolutionary history and population structure using multiple data sources: morphological characters, mitochondrial sequences (mtDNA), and genome-wide single nucleotide polymorphisms (SNPs). I determined that SNP data distinguished population structure at a finer resolution than morphology or mtDNA. From these data, I defined six conservation units within *P. egregius*, three of which are consistent with current subspecific taxonomy. Next, I used statistical phylogeography to examine how the effects of historical climate change in the southeastern United States (US) may have driven patterns of intraspecific divergence in *P. egregius*. I devised a set of alternative hypotheses regarding the historical distribution and dispersal of *P. egregius* to test using genome-wide SNP markers. I found support for a historical refugia within the southern scrub ridges in Florida followed by expansion into the Florida peninsula and mainland US. Synthesizing the results from both studies, I evaluate the current subspecific taxonomy and discuss the conservation of *P. egregius*. Overall, I conclude that *P. egregius* evolutionary history has been driven by historical sea level changes in the southeastern US, and that insular populations should be the focus of conservation efforts.

To my mom,

for always seeing the best.

ACKNOWLEDGMENTS

"If I have seen a little further, it is by standing on the shoulders of Giants," Isaac Newton. I use this quote first in the manner that Newton likely intended, none of the work presented here would have been possible without all the scientists that have come before. But, in a more personal way, the Giants I refer to are the countless people who have helped me along this journey.

First, I would like to thank my advisor and committee members. Dr. Parkinson, thank you for your mentorship and insights into the academic world. To Drs. Savage and Jenkins, for always having an open door. Also, to the Parkinson Lab family past and present for helping me grow as a scientist and as a person, notably Jason Strickland, Andrew Mason, Rhett Rautsaw, Alexa Trujillo, and Matt Lawrance. To the UCF Biology Department, especially Juana Pasco who made sure I met all requirements and deadlines.

Thank you to everyone who has given their time or energy to this work, specifically those undergraduates who helped in the lab or field: Millie Baylac, Samantha Dillon, Steffany Medina, Alex Robertson, and Chris Yanick. And to everyone who donated samples or made sampling possible, especially Paul Moler, Kevin Enge, Jonathan Mays, and Adam Emerick. Thank you to PHaSeD lab for your ideas, comments, and critiques of this research.

A HUGE thank you to BGSA and all of the friends I have made at UCF; grad school is hard and you made it easier. Thank you for being there to celebrate the ups, commiserate with the downs, or just have a beer.

Lastly, to my family, for making it possible for me to chase this dream. I couldn't have made it this far without your support: emotional, financial, or the many many times I asked you to watch Rayder. I want to thank you for always encouraging me and believing in me, not just during this chapter of my life but always.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1: INTRODUCTION

Understanding the drivers of diversity and diversification is crucial to protecting biodiversity. Historical climate change and the ramifications of those changes are one of the predominate drivers of divergence in many species (Avise, 2000; Remington, 1968). Changing climate can impact where species find suitable habitat, either directly through environmental conditions like temperature, rainfall, and humidity or through indirect effects such as sea level fluctuations, which alter the physical habitat available (Avise *et al.*, 1987). Historical changes to species dispersal and distribution will lead to patterns of diversity and divergence we can infer using morphological and molecular characters. In addition to historical effects, we have evidence that global temperature is warming at an increased rate and that warming is leading to an increase in sea level across many parts of the world (Loarie *et al.*, 2009). There is evidence that current climate change is already having an impact on species distributions (Parmesan and Gary, 2003). For all species, but especially those already recognized to be threatened or endangered, predicting the impacts of future climate change is important for conservation management. One of the primary ways that we can make better inferences about future impacts is to study what has occurred in the past.

Importantly, before we can understand how climate change or other factors have acted on any taxa, we need to have a clear characterization of the divergence and diversity in the taxon of interest. In conservation biology, characterizing divergence is often done by delineating Evolutionary Significant Units (ESUs) (Ryder, 1986; Moritz, 1994). Historically, ESUs have been defined using different data types, including morphological characters and mitochondrial sequences (mtDNA) (Waits *et al.*, 1998; Moritz, 1994). In many cases, use of these data together led to conflicting descriptions of divergence within a taxon (Rubinoff and Sperling, 2004). Recently, to combat this problem, conservation geneticists are using genome-wide single nucleotide polymorphisms (SNPs) to clarify differences between morphology and mtDNA (Peters *et al.*, 2016; Unmack *et al.*, 2017). Next-generation sequencing has allowed researchers to generate thousands of SNPs from

throughout the genome which should better reflect evolutionary history than morphology or mtDNA alone (Peterson *et al.*, 2012; Davey and Blaxter, 2010). Once we gain a clear understanding of the patterns of divergence within a taxon we are able to move on to examining what may be driving the patterns seen.

Study Species

Plestiodon egregius is a semi-fossorial lizard endemic to the southeastern United States (US) with five described subspecies (Figure 1.1) (Mount, 1965). They inhabit dry sandy substrates, including sandhill, scrub, and coastal hammock, which are rapidly disappearing in many places (Mount, 1963; Christman, 1992). Insular populations and those on the central ridge have been heavily impacted by habitat destruction (Christman, 1992). In 2016, Florida upgraded *P. e. egregius* from a Species of Special Concern to State-Threatened due to habitat fragmentation, predation from invasive species, and habitat loss from climate change associated sea level rise (Florida Fish and Wildlife Conservation Commission, 2016). At the federal level, *P. e. lividus* is listed as Threatened under the US Endangered Species Act and *P. e. insularis* is under review to determine if a petition to list is warranted (U.S. Fish and Wildlife Service, 1987, 2015). In spite of findings that *P. e. egregius* may lose up to 44% of its suitable habitat by 2060, it was recently determined not to warrant a petition to list under the US Endangered Species Act (U.S. Fish and Wildlife Service, 2017).

Plestiodon egregius taxonomic history has been described as one of vacillation and uncertainty (McConkey, 1957). The species was first described as *Plestiodon egregius* (Red-tailed Skink), from a population in Indian Key, FL (Baird, 1858). A closely related species, *P. onocrepis*, was later described based on one specimen from Brevard County, FL, stating that *P. onocrepis* was easily distinguished from *P. egregius* by the latters ornamented coloration (Cope, 1871). Cope (1875) transferred all of *Plestiodon* to the genus *Eumeces*. In 1900, *E. onocrepis* is listed in synonymy with *E. egregius* without comment (Cope). Almost four decades later, *E. e. onocrepis* was resurrected.

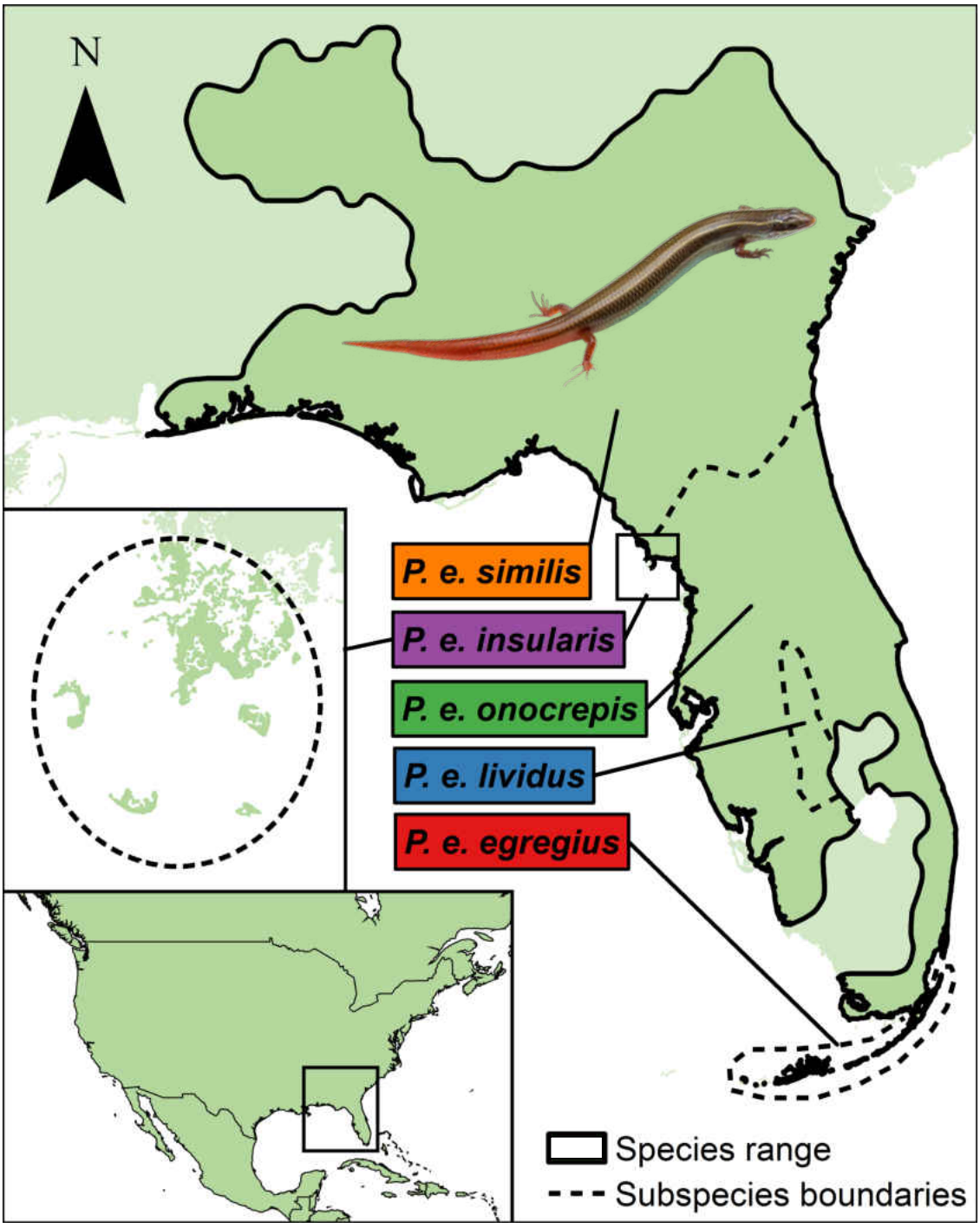

Figure 1.1: Distribution map of Mole Skinks, *Plestiodon egregius*

But, now as a subspecies of *E. egregius*, citing errors in the original description and damage to the specimen for why it was synonymized (Taylor, 1935). Carr (1940) restored *E. onocrepis* to species level, stating that no intermediaries between *E. egregius* and *E. onocrepis* could be found. In 1957, *E. onocrepis* was returned to the subspecies level, and an additional subspecies *E. e. similis* was described based on individuals from southern Georgia and northern Florida (McConkey). The five subspecies currently recognized were described in 1965. During his dissertation work, Robert Mount discovered a population of Red-tailed Skinks with blue tails, prompting him to study the variation of this species in depth (Mount, 1963) as well as change their common name from Red-tailed Skinks to Mole Skinks. Due to the patterns of variation unearthed, he described two new subspecies: *E. e. lividus*, and *E. e. insularis*. In the early 2000s, the genus *Eumeces* was split into several genera based on multiple phylogenetic studies of morphological and molecular characters (Griffith *et al.*, 2000; Schmitz *et al.*, 2004; Brandley *et al.*, 2005). The American and East Asian species were restored to the genus *Plestiodon* (Smith, 2005). For the remainder of this thesis, I will refer to Mole Skinks using their current scientific name, *Plestiodon egregius*.

Robert Mount (1965) examined 608 specimens of *P. egregius* from across their range. He examined all major external features except for appendage scalation, although only traits with geographic or sexual variation were presented. Geographic variation was found in twelve traits: tail color, body color in hatchlings, dorsolateral striping, lateral light striping, relative head to body proportions, size attained by adults, growth rate, age at which sexual maturity is attained, and number of supralabial scales, scales at midbody, midventral scales, and presacral vertebrae (Mount, 1965). Many of the patterns he found came with qualifications. For example, tail color (Figure 2) was only evaluated in individuals smaller than 45 mm. Additionally, no statistical calculations of confidence intervals or significant differences were included in his analyses. Moreover, Mount remarks in the taxonomic diagnosis that *P. e. onocrepis* appears to be an intergrade between *P. e. similis* and *P. e. lividus*. Later he states that *P. e. insularis* is practically indistinguishable from western *P. e. similis* individuals. Also interestingly, in the Reptiles & Amphibians of Alabama,

written by Mount, states there are four subspecies of *P. egregius* (Mount, 1975).

Aims

Here I used *P. egregius* as a model system to study the impact of historical climate changes on the divergence of a species. First, I characterized *P. egregius* evolutionary history and population structure with an emphasis on delimiting conservation units and evaluating the current subspecific taxonomy. I compared the results from three data types: morphological characters, mtDNA sequences, and genome-wide SNPs. Second, I used statistical phylogeography to test hypotheses regarding how historical climate changes have driven the distribution and dispersal of *P. egregius* using genome-wide SNPs. I end with taxonomic and management implications for *P. egregius*, and propose future directions of this work.

CHAPTER 2: THE ROLE OF DATA IN DELIMITING CONSERVATION UNITS: A CASE STUDY IN THE FOSSORIAL LIZARD, *Plestiodon egregius*

Abstract

Identifying and delimiting the unit-to-conserve, sometimes referred to as an Evolutionary Significant Unit (ESU), is a primary goal of conservation biology. In the history of conservation biology, different data types have been used to accomplish this task. In recent history, the data used to identify ESUs were often morphological characters and/or mitochondrial DNA sequences (mtDNA). Problematically, these two data types often led to different conclusions regarding intraspecific divergence, and therefore, different ESU's within the same taxon. An example of a taxon with conflicting signals of intraspecific divergence are Mole Skinks (*Plestiodon egregius*), which we use as a model to examine whether utilizing genome-wide single nucleotide polymorphisms (SNPs) can clarify differences in the results from the preceding two methods. We determined no substructure could be identified using morphological characters, but genetic data (mtDNA and SNPs) identified similar major phylogeographic lineages. Mitochondrial DNA, however, appeared to be biased while SNPs were able to distinguish the most fine-scale population structure. A multifaceted approach to delimit conservation units would be ideal, but based on the results of this study we recommend that genome-wide SNP data be the standard for delimiting ESUs.

Introduction

One of the fundamental tasks of conservation biology is identifying conservation units, which form the basis of planning and management. Definitions of the unit-to-conserve vary by country, legislation, and policy, but the entity for protection and curation is commonly referred to

as an evolutionary significant unit (ESU) (Ryder, 1986; Moritz, 1994). In its defining paper, an ESU is considered to be a geographic segment of a species which has one or more lines of evidence for genetic divergence (Ryder, 1986) although variations on this definition have been proposed through time (Waples, 2008). Related concepts to ESUs include: distinct population segments, independent conservation units, and management units (Waples, 2008). But, for practical purposes, most policies are developed using terminology assigned to species, subspecies, and populations (Pennock and Dimmick, 1997). Challenges arise as these taxonomic units are difficult to define. This problem is further exacerbated by historical incongruities in datatypes used to identify ESUs and each datatypes different analyses.

Morphological characters and mitochondrial DNA (mtDNA) are common datatypes used to identify ESUs in a variety of taxa and at various biological levels (Moritz, 1994; Waits *et al.*, 1998; Branch and Hokit, 2000). Morphology has historically been the most common method used to identify biodiversity and is incorporated implicitly and often explicitly in many definitions of species and subspecies (Dayrat, 2005). In contrast, the widespread use of mitochondrial DNA has been a relatively recent occurrence, and became popular due to its rapid evolution and relatively simple inheritance pattern (Brown *et al.*, 1979; Avise *et al.*, 1987). Unfortunately, these data often provide conflicting signals (Rubinoff and Sperling, 2004). An interesting example of such discordance occurs in two European newt species: *Triturus montandoni*, which is morphologically conserved, while its sister species, *T. vulgaris*, is split into seven morphologically distinct subspecies. A mitochondrial phylogeny recovered only two of the *T. vulgaris* subspecies as monophyletic and rendered *T. montandoni* polyphyletic within *T. vulgaris* (Babik *et al.*, 2005). The authors suggest that this discordance is due to both mitochondrial introgression and independent evolution of multiple traits (Babik *et al.*, 2005). As that study exemplifies, trait evolution and molecular evolution are complex processes which are not easily described. Therefore, concordance between morphology and mtDNA characterizations of divergence cannot always be assumed.

Due to the historical precedence of morphology as key diagnostic characters, many modern

classifications are rooted heavily in these data, though there are many instances where distinct morphology may arise independent of neutral genetic differentiation or be absent in the presence of genetic divergence (Barley *et al.*, 2013). In conservation, these instances frequently complicate management planning. For example, Eastern Oysters (*Crassostrea virginica*) occur in two morphologically distinct forms in Apalachicola Bay, FL, where they recently suffered a major collapse. It was unknown if these forms were genetically distinct and if they could be managed independently for recovery but differentiation was not recovered by molecular markers, and therefore individuals should be managed as one panmictic population (Lawrance *et al.*, 2017). The challenge presented in this case may be compounded in groups with small disjunct distributions, as genetic drift may act quickly to cause population structure without differential selective pressures.

One means of addressing discordant results is to harness the power of large genomic datasets. By sequencing reduced representation libraries, such as restriction-site associated DNA sequencing (RADSeq), we can characterize single nucleotide polymorphism (SNPs) from throughout the genome (Baird *et al.*, 2008; Peterson *et al.*, 2012), which presents a powerful and sensitive tool capable of detecting population-scale processes (Brumfield *et al.*, 2003; Davey and Blaxter, 2010). Moreover, SNP data are more resilient to the processes that bias interpretation of mtDNA, including incomplete lineage sorting, nuclear paralogs, and sex biased dispersal (Avise *et al.*, 1987; Zhang and Hewitt, 1996; McGuire *et al.*, 2007). Genome-wide SNPs have been used in many settings to resolve discordance among morphological and mtDNA datasets (Mims *et al.*, 2010; Brown *et al.*, 2016; Unmack *et al.*, 2017). For example, Mottled ducks (*Anas fulvigula*) are distributed in two allopatric populations, one of which is threatened by habitat loss and hybridization with *A. platyrhynchos*. These two populations lack morphological distinction and reciprocal monophyly, but their mitochondrial haplotypes cluster independently, and it was therefore unclear whether the populations represented different ESUs. SNP data indicated that greater divergence between the populations exists than would be expected from geographic distance alone, and they therefore represent two distinct ESUs (Peters *et al.*, 2016). As in this example, genome-wide SNP data may

generally provide a solution in resolving contentious patterns of intraspecific divergence.

Mole Skinks, *Plestiodon egregius* Baird (1858), are a prime example of a taxon with discordant patterns of divergence from morphology and mtDNA. These semi-fossorial lizards are endemic to the coastal plain of the southeastern United States (US) and consist of five subspecies, one of which is federally threatened, *P. e. lividus* (Figure 1.1) (Mount, 1963, 1965; U.S. Fish and Wildlife Service, 1987). This species faces several conservation challenges including habitat degradation and fragmentation; one subspecies, *P. e. egregius*, may lose up to 44% of its suitable habitat from climate change associated sea level rise by 2060 (U.S. Fish and Wildlife Service, 2017). Exacerbating this problem are inconsistencies on the evolutionary and taxonomic status of putative subspecies that have occurred as a result of different data types. Current subspecies were described based on morphological evidence including scalation, tail coloration, and dorsal stripe width (Mount, 1965) but the only molecular phylogenetic study of the species recovered none of the subspecies as monophyletic mitochondrial lineages (Branch *et al.*, 2003). Existing morphological and mtDNA evidence also show contrasting patterns of intraspecific divergence and diversity: *P. e. egregius* is morphologically similar to *P. e. similis* but most closely related to *P. e. onocrepis*, and *P. e. lividus* exhibit little morphological variation but had the highest haplotype diversity (Mount, 1965; Branch *et al.*, 2003). In recent years, *P. egregius* has become of greater interest due to two subspecies under review by USFWS to determine if a petition to list is warranted, which has highlighted the challenges of reconciling the disagreement between morphology and mtDNA (U.S. Fish and Wildlife Service, 2015). Although dispersal rates have not been directly assessed in *P. egregius*, we expect they are similar to *P. reynoldsi*, which disperse between 0.035-0.24 km and are sex-biased, with males dispersing farther than females (Penney, 2001). We expect population structuring to be high due to the low dispersal rates, therefore, SNP data should be ideal for reconciling the different signals in this system.

Despite the considerable risk *P. egregius* faces from habitat degradation, few studies examining their population structure and evolutionary history have been done, and those that

do exhibit discordant results, which complicates conservation efforts (Mount, 1965; Branch *et al.*, 2003). In order to evaluate the utility of genome-wide SNPs at reconciling the disagreement between mtDNA and morphology, we generated a morphological dataset, a mtDNA dataset, and a genome-wide SNP dataset. We asked whether the current subspecies represent distinct populations or evolutionary lineages. We placed special emphasis on evaluating the risk *P. egregius* may face due to restricted gene flow, low genetic diversity, or inbreeding. This study highlights the applicability of different data types to discern intraspecific divergence and the importance of characterizing this divergence to conservation policy.

Methods

Morphology data collection and analyses

Seven characters were measured in 116 specimens from across the range of *P. egregius* (Figure 2.1), two morphometric characters: snout-vent length and head length, and five meristic: number of midbody scales, midventral scales, middorsal scales, infralabial scales, supralabial scales. Morphometric characters were measured with digital calipers and scale counts were done by eye under a compound microscope. Characters were chosen based on previous evidence of geographic variation or their use as diagnostic characters in delimiting subspecies (Cope, 1875; McConkey, 1957; Mount, 1965). Although color was a primary character in delimiting the subspecies, it fades in ethanol preserved specimens. We were therefore not able to characterize color in these individuals. To reduce dimensionality of the morphological dataset, we performed a principal component analyses (PCA) using the five scale counts and relative head length, defined as head length divided by snout-vent length. Data were centered and scaled prior to PCA, then plotted with 95% confidence ellipses in R v3.4.2 (Wickham, 2016; R Core Team, 2017).

Genetic sample collection and DNA extraction

Tissue samples were collected from 75 individuals representing all five *P. egregius* subspecies as well as four individuals from *P. reynoldsi* to serve as an outgroup (Figure 2.2, Table S1) for use in both mtDNA and SNP data analyses. Eight tissues for *P. e. lividus* were received as loans. Skinks were captured by raking through pocket gopher mounds and by utilizing plywood cover boards or drift fences. We obtained tissue samples by pinching and lightly pulling on the distal end on the tail, causing the skink to autotomize the tip of the tail. When compared to cutting, this pinch and pull method seems to reduce trauma to the skink as evidenced by lack of bleeding. Tissues were stored at -20°C in 100% ethanol then extracted with SeraPure beads (Faircloth and Glenn, 2014).

mtDNA sequencing and analyses

Mitochondrial genes cyt-*b* (1143 bp) and NADH dehydrogenase subunit 4 (ND4) with trailing $tRNA^{His,\ Ser,\ Leu}$ (853 bp) were amplified in all samples (Arevalo *et al.*, 1994; Burbrink *et al.*, 2000). PCR reactions consisted of: 10-30 ng of template DNA, 0.6 μL of each 10 μM forward and reverse primer, 1.5 units of OneTaq DNA polymerase (New England Biosystems), 1x final concentration of OneTaq reaction buffer (New England Biosystems), and 2.4 μL of 10 dNTPs in a final volume of 30 μL. PCR conditions were as follows: an initial 30 second hold at 94°C then 35 cycles of 30 second denaturing step at 94°C, 30 second annealing step at 55°C, and a one minute extension at 68°C, all followed by a final extension at 68°C for five minutes. PCR product was cleaned using FastAP (ThermoFisher Scientific) then sequenced in both directions with amplification primers at Eurofins Genomics (Louisville, KY). Raw chromatograms were reviewed and consensus sequences determined in Geneious v10.0.3, then aligned with the Geneious alignment implementation (Kearse *et al.*, 2012).

We estimated phylogenetic relationships from the mitochondrial sequences using BEAST v2.4.7 (Bouckaert *et al.*, 2014). AICc model selection was used to determine the best partitioning

scheme and model of evolution for each partition in PartitionFinder v2.1.1; Protein coding genes were split by codon position and we assumed linked branch lengths (Guindon *et al.*, 2010; Lanfear *et al.*, 2012, 2017). This partitioning scheme was used in BEAST with a strict clock and yule tree model (Bouckaert *et al.*, 2014). In initial analyses, GTR substitution rate parameters were very low and induced long mixing times, so we altered the gamma prior such that α=2 and β=0.5. Three runs were carried out for 50 million generations sampling every 1000 generations then checked for chain stationarity and convergence in Tracer v1.6 (Rambaut *et al.*, 2013). The maximum clade credibility tree was estimated after removing the first 10% of trees as burn-in with TreeAnnotator (Bouckaert *et al.*, 2014).

Population structure was inferred in a Bayesian framework using BAPS v6.0 (Corander *et al.*, 2006, 2008; Tang *et al.*, 2009). Divergence between these clusters was assessed by calculating pairwise F_{ST} values with 1000 replicates in Arlequin v3.5.2.2 (Excoffier and Lischer, 2010). Genetic variation was estimated by calculating the number of haplotypes (h), segregating sites (S), private segregating sites (P), haplotype diversity (H_d), and nucleotide diversity (π) for each cluster in Arlequin v3.5.2.2 (Excoffier and Lischer, 2010).

SNP generation and analyses

Genomic DNA was converted into nextRAD libraries (SNPsaurus, LLC) as in Russello *et al.* (2015). Briefly, genomic DNA (40 ng) was first fragmented with the Nextera reagent (Illumina, Inc), which also ligates short adapter sequences to the ends of the fragments. Fragmented DNA was amplified for 27 cycles at 74°C, with one of the primers matching the adapter sequence and extending 10 nucleotides into the genomic DNA with selective sequence GTGTAGAGCC. Thus, only fragments starting with a sequence that could be hybridized by the selective sequence were efficiently amplified. Samples were pooled then sequenced on a HiSeq 4000 to generate 150-bp single-end reads (University of Oregon). Genotyping analysis used custom scripts (SNPsaurus, LLC) that trimmed reads using bbduk (Bushnell, 2014). Next, a *de novo* reference was created

by aligning 10 million reads, collected evenly from the samples and excluding reads with counts fewer than 7 or more then 700, to identify allelic loci and collapse allelic haplotypes to a single representative. Using this reference, all reads were mapped with an alignment identity threshold of 95% using bbmap (Bushnell, 2014). Genotype calling was done using Samtools and bcftools (Li *et al.*, 2009). The loci were then filtered to remove alleles with a population frequency of less than 3%. Loci were removed if they were heterozygous in all samples or had more than 2 alleles in a sample (suggesting collapsed paralogs).

To estimate a maximum likelihood phylogeny from the SNP data, RAxML v8.2.11 was used (Stamatakis, 2014). Indels and sites with more than 30% missing data were removed in VCFtools v0.1.14, then phased with fastPHASE v1.4 (Scheet and Stephens, 2006; Danecek *et al.*, 2011). The rapid hill-climbing mode with a GAMMA model of rate heterogeneity and ascertainment bias correction was used in RAxML. The Majority Rule Criterion was used for automatic bootstopping, up to 1,000 bootstrap replicates (Stamatakis, 2014).

The number of clusters and membership probability for each individual was estimated using Structure v2.3.4. 100,000 MCMC replicates were run after a burn-in period of 10,000 using independent allele frequencies under an admixture model. We varied the number of clusters (K) from 2 through 10 with ten replicates for each value of K (Pritchard *et al.*, 2000). The number of clusters was determined using the Evanno method in Structure Harvester (Evanno *et al.*, 2005; Earl and VonHoldt, 2012). PCA was used to examine population structure in the R package adegenet (Jombart, 2008; Wickham, 2016; R Core Team, 2017). The contribution of each allele to the PCA was visualized in a loading plot. Pairwise F_{ST} values between clusters were calculated in DnaSP v6.10.04 (Rozas *et al.*, 2017). We calculated genetic diversity metrics gene diversity (H_E), and inbreeding coefficient (F_{IS}) averaged over all loci for each population in the R package hierfstat Goudet (2005), as well as average individual heterozygosity (Danecek *et al.*, 2011).

Results

Morphology

The first two principal component axes of the PCA described over half of the variation in the morphological data, accounting for 33.2% and 24.2% of the variation, respectively (Figure 2.1). PC1 was driven by relative head length in the positive direction together with middorsal and midventral scales in the negative direction. PC2 was driven by the number of infralabial and supralabial scales, in opposing directions. There was substantial overlap of the 95% confidence ellipses for each of the five subspecies. The difference in the confidence ellipsis for *P. e. insularis*, when compared to the other subspecies is driven by a few individuals with seven infralabial scales, while most individuals have five or six. Having seven infralabial scales is not unique to this subspecies, there are individuals from *P. e. onocrepis* and *P. e. similis* that also had also seven infralabials. The 95% confidence ellipses for subspecies were all overlapping and no clustering was identified.

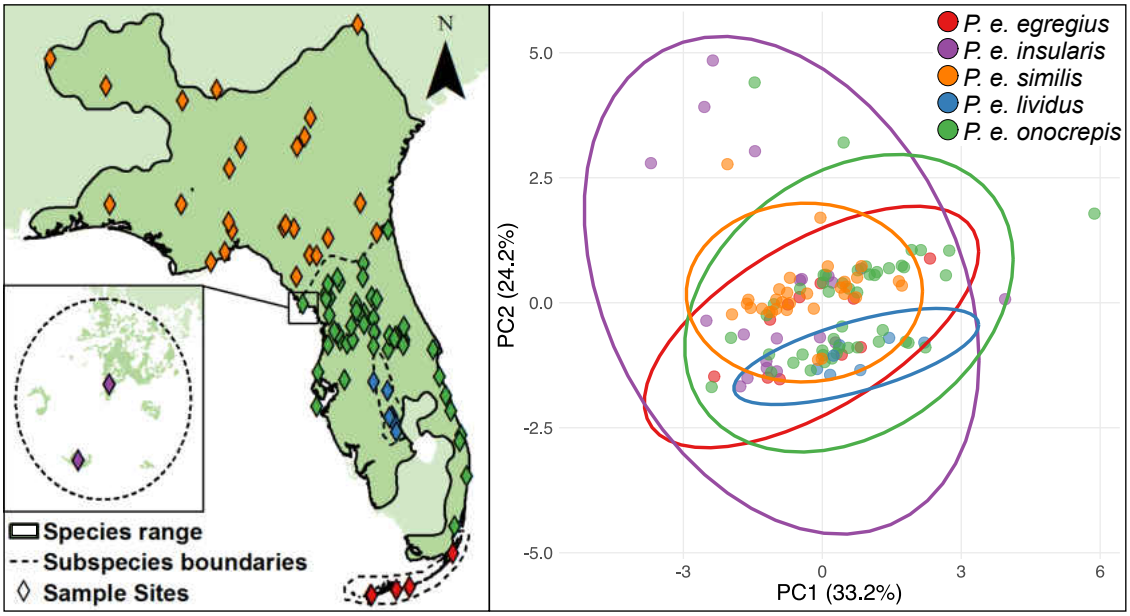

Figure 2.1: Left: Sample locations of specimens used in morphological analyses. Right: PCA of the six morphological characters, and 95% confidence intervals drawn around each subspecies.

mtDNA

We successfully amplified all 75 individuals for cyt-*b* and 73 for ND4 (Table S1). Missing data in the complete aligned sequenced matrix is less then 5%. All sequences have been deposited in GenBank (accession numbers MH259329 - MH259484).

The best partitioning scheme and model of evolution for each partition can be found in Table S2. The three independent BEAST runs converged on nearly identical estimates of the likelihood scores and had ESS values over 600 for all parameters. The maximum clade credibility tree is presented in Figure 2.2. We found strong support (>0.95 posterior probability) for *P. egregius* as monophyletic with respect to the outgroup *P. reynoldsi* and for two geographically distinct clades with sequence divergence of 8.5%. The southern clade consisted of individuals from the Lake Wales Ridge and south (*P. e. egregius*, *P. e. lividus*, Indian River and Orange County *P. e. onocrepis*). The northern clade was made up of individuals north of the Lake Wales Ridge (*P. e. similis*, *P. e. insularis*, and most of *P. e. onocrepis*). Within the two major clades, many samples from the same geographic region were non-monophyletic. Specifically, individuals from the Lake Wales Ridge (*P. e. lividus*) were polyphyletic, one lineage was most closely related to *P. e. egregius* individuals and the other lineage was most closely related to *P. e. onocrepis* individuals from Indian River and Orange County. The insular *P. e. egregius* was also polyphyletic, with a small haplotype group from Big Pine Key (BPK) sister to the rest of the southern individuals. Within the large *P. e. egregius* clade, individuals from the same key formed strongly-supported monophyletic groups. In the large northern clade, individuals from the Florida panhandle (Madison County and Liberty County) formed a strongly supported monophyletic group. The insular *P. e. insularis* was rendered paraphyletic by one *P. e. onocrepis* individual. Unlike the large *P. e. egregius* clade, *P. e. insularis* individuals sampled from the same key did not form monophyletic lineages.

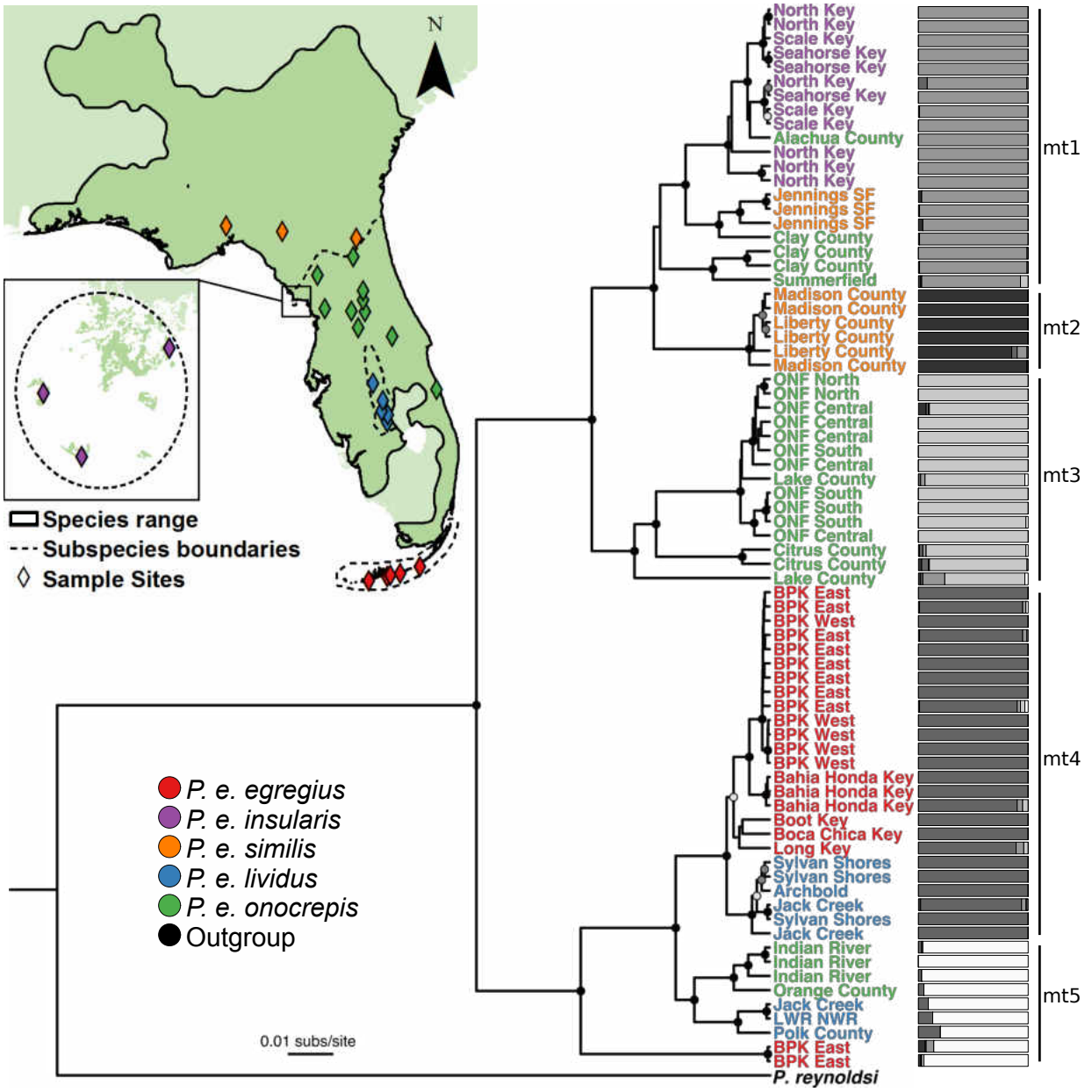

Figure 2.2: Results from mtDNA dataset. Upper left: Sample sites for genetic samples. Center: Bayesian mtDNA phylogeny run in BEAST. Posterior probability is indicated by node dot color: black is >0.98, gray is between 0.98 and 0.85, light gray is between 0.85 and 0.7, and <0.7 has no node dot. Right: Population assignment probabilities for each individual from BAPS, where each color is a different putative population. The proportion of each shade in an individuals bar represents the probability of assignment to that population.

We recovered five distinct genetic clusters, which were concordant with the phylogenetic lineages except for the small *P. e. egregius* lineage clustering with the *P. e. onocrepis* and *P. e. lividus* clade (mt5) (Figure 2.2). The remaining southern cluster consisted of the rest of *P. e. egregius* and *P. e. lividus*. Pairwise F_{ST} comparisons between the five clusters revealed moderate levels of divergence (0.061-0.182), all statistically significant at $p < 0.05$ (Table 2.1). Mitochondrial genetic diversity was high across all five clusters (Table 2.1), haplotype diversity was between 0.80 and 0.94. In the panhandle population (mt2) only about 20% of substitution sites were private compared to about half in the other four clusters. This cluster had the lowest haplotype and nucleotide diversity but also had the smallest number of individuals. The large cluster of *P. e. egregius* and *P. e. lividus* (mt4) also had low nucleotide diversity, while the smaller southern cluster including *P. e. onocrepis* (mt5) had the highest haplotype and nucleotide diversity.

Table 2.1: Pairwise F_{ST} values and genetic diversity measures for mtDNA clusters. After 1000 permutations all F_{ST} values were significant at the 0.05 level. Number of individuals (n), number of haplotypes (h), substitution sites (S), private substitution sites (P), haplotype diversity (Hd), and nucleotide diversity (π).

	mt1	mt2	mt3	mt4	n	h	S	P	Hd	π
mt1					20	9	100	47	0.83	0.016
mt2	0.182				6	4	11	2	0.80	0.004
mt3	0.119	0.123			15	11	90	47	0.93	0.025
mt4	0.166	0.178	0.118		25	9	25	13	0.84	0.007
mt5	0.118	0.122	0.061	0.116	9	7	108	59	0.94	0.038

SNPs

Sequencing of nextRAD libraries resulted in an average of 2.3 million reads per individual; after alignment and filtering, we retained 33,894 SNP loci. Outgroup individuals used in the phylogenetic analysis had considerably more missing data (65%) than *P. egregius* individuals (9%) despite starting with similar numbers of raw reads, presumably due to mutations in sites targeted by

the selective sequence. This is also consistent with other studies showing missing data to have a phylogenetic signal (Cariou *et al.*, 2013). Raw fastq files have been uploaded to NCBI Sequence Read Archive (accession number SRP145297) (Table S1).

The maximum likelihood phylogeny inferred from SNP data showed strong support (>95 bootstrap) for *P. e. lividus* as sister to the rest of *P. egregius* (Figure 2.3). Within the large clade sister to *P. e. lividus*, we recovered a similar north-south break as seen in the mtDNA tree. The southern clade was comprised of two strongly-supported lineages, one of all *P. e. egregius* individuals, and one of the Indian River *P. e. onocrepis*. As in the mitochondrial tree, *P. e. egregius* sampled from the same key formed strongly-supported monophyletic groups. The northern clade split into three lineages: one lineage of *P. e. insularis*, sister to *P. e. similis* and northern *P. e. onocrepis*, which were all together, sister to Central Florida *P. e. onocrepis*. Similar to *P. e. egregius*, *P. e. insularis* from the same key formed strongly-supported monophyletic groups.

We identified K=5 as the most likely number of clusters according to the Evanno method (Figure 2.3) (Evanno *et al.*, 2005). Individuals from *P. e. egregius* and *P. e. insularis* each formed one cluster. Individuals from north Florida (*P. e. similis* and northern *P. e. onocrepis*) formed one cluster. Central Florida individuals (*P. e. onocrepis*) formed a cluster, though a few individuals had high probabilities of assignment to the *P. e. lividus* or north Florida clusters. Individuals from Indian River clustered with *P. e. lividus* although they had some probability of assignment to the north Florida and *P. e. egregius* clusters. The first three axes of the PCA represented 32.5% of the variation in the data and showed similar clustering to the previous analysis. PC1 and PC2 clearly separated the two insular lineages, *P. e. egregius* and *P. e. insularis*, respectively (Figure 2.4). PC3 isolated *P. e. lividus* from the rest of *P. egregius*. In the PCA of *P. e. egregius*, individuals from the same key clustered together, as well as individuals from the east side and west side of Big Pine Key (Figure 2.4). Similarly, *P. e. insularis* individuals from different keys from clustered independently (Figure 2.4). The loading plot indicated that all SNPs contributed approximately equally to the variation in the data (Figure S1). F_{ST} across all populations was 0.285, and similar

between all clusters, although the two insular subspecies had the highest pairwise F_{ST} at 0.310 (Table 2.2). Insular subspecies had the lowest individual heterozygosity but high gene diversity. Insular subspecies also showed less evidence for inbreeding than the other three populations, but inbreeding coefficients were high overall (Table 2.2).

Table 2.2: Pairwise F_{ST} values and genetic diversity measures for SNP populations. Number of individuals (n), average individual heterozygosity (H_O), gene diversity (H_E), and inbreeding coefficient (F_{IS})

	pop1	pop2	pop3	pop4	n	H_O	H_E	F_{IS}
pop1					14	0.93	0.111	0.398
pop2	0.216				12	0.90	0.136	0.300
pop3	0.188	0.257			16	0.92	0.137	0.362
pop4	0.286	0.310	0.274		21	0.90	0.139	0.312
pop5	0.194	0.245	0.185	0.229	12	0.92	0.127	0.379

Discussion

Patterns of intraspecific divergence in Plestiodon egregius

This study serves as the first examination of *P. egregius* evolutionary history and population structure using multi-locus molecular data. Molecular phylogenetic analyses support a monophyletic *P. egregius* sister to *P. reynoldsi*, which conforms with previous studies at the generic level (Brandley *et al.*, 2012, 2011). Broad patterns in both molecular datasets are congruent with phylogeographic patterns of other species in the southeastern US, exhibiting a split along the Florida peninsula (Remington, 1968; Burbrink *et al.*, 2008; Ellsworth *et al.*, 1994; Strickland *et al.*, 2014). Morphological and mtDNA analyses did not recover any of the currently named subspecies, whereas SNP data support *P. e. lividus*, *P. e. egregius*, and *P. e. insularis*. The specific results from each data type differed, and each lead to different conclusions regarding the number and identity of ESUs within this species.

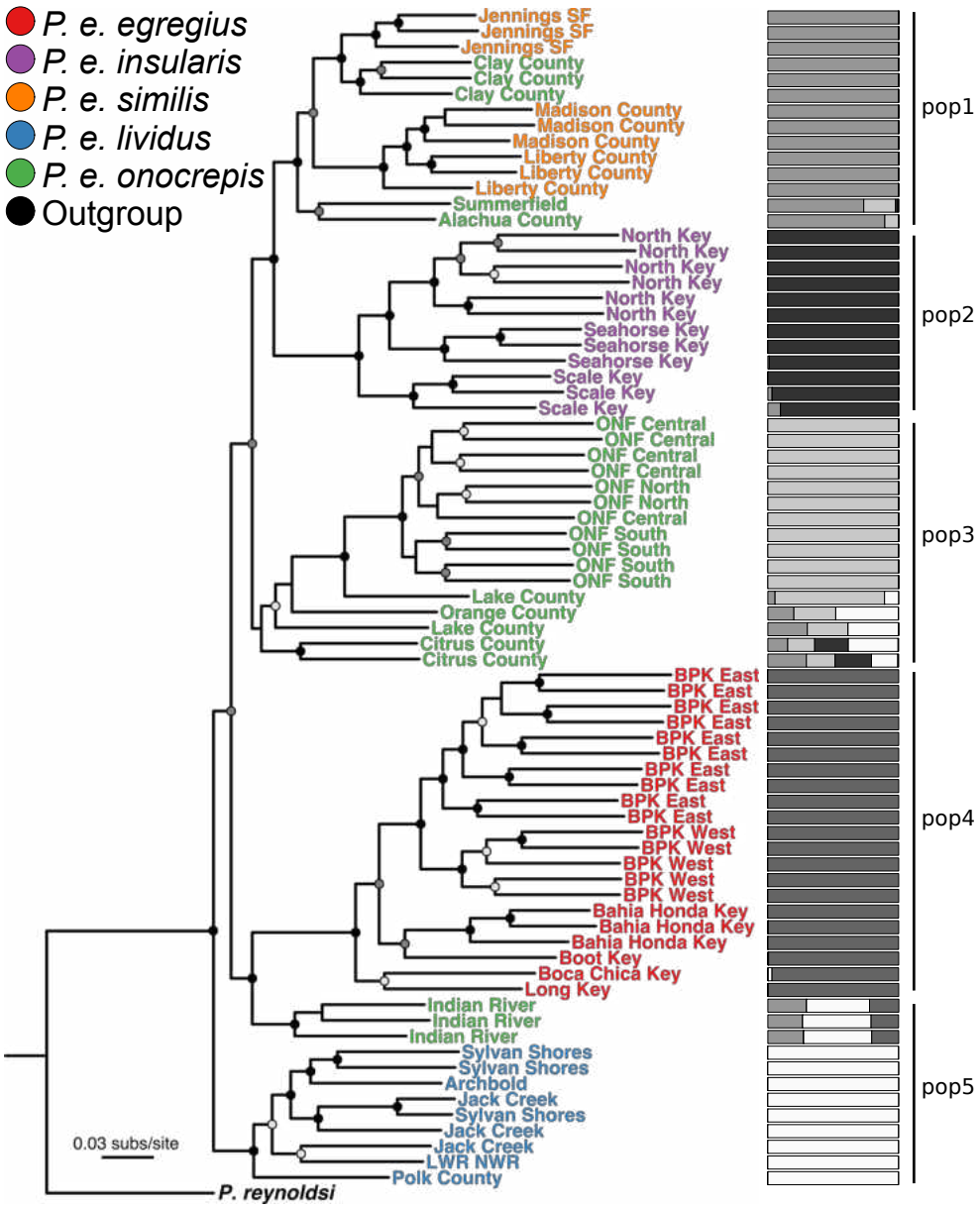

Figure 2.3: Phylogenetic and population structure results from SNP data. Left: Maximum likelihood SNP phylogeny run in RAxML. Bootstrap support is indicated by node dot color: black is >95, gray is between 95 and 80, light gray is between 80 and 60, and <60 has no node dot. Right: Population assignment probabilities for each individual inferred in structure, where each color is a different putative population. The proportion of each shade in an individuals bar represents the probability of assignment to that population.

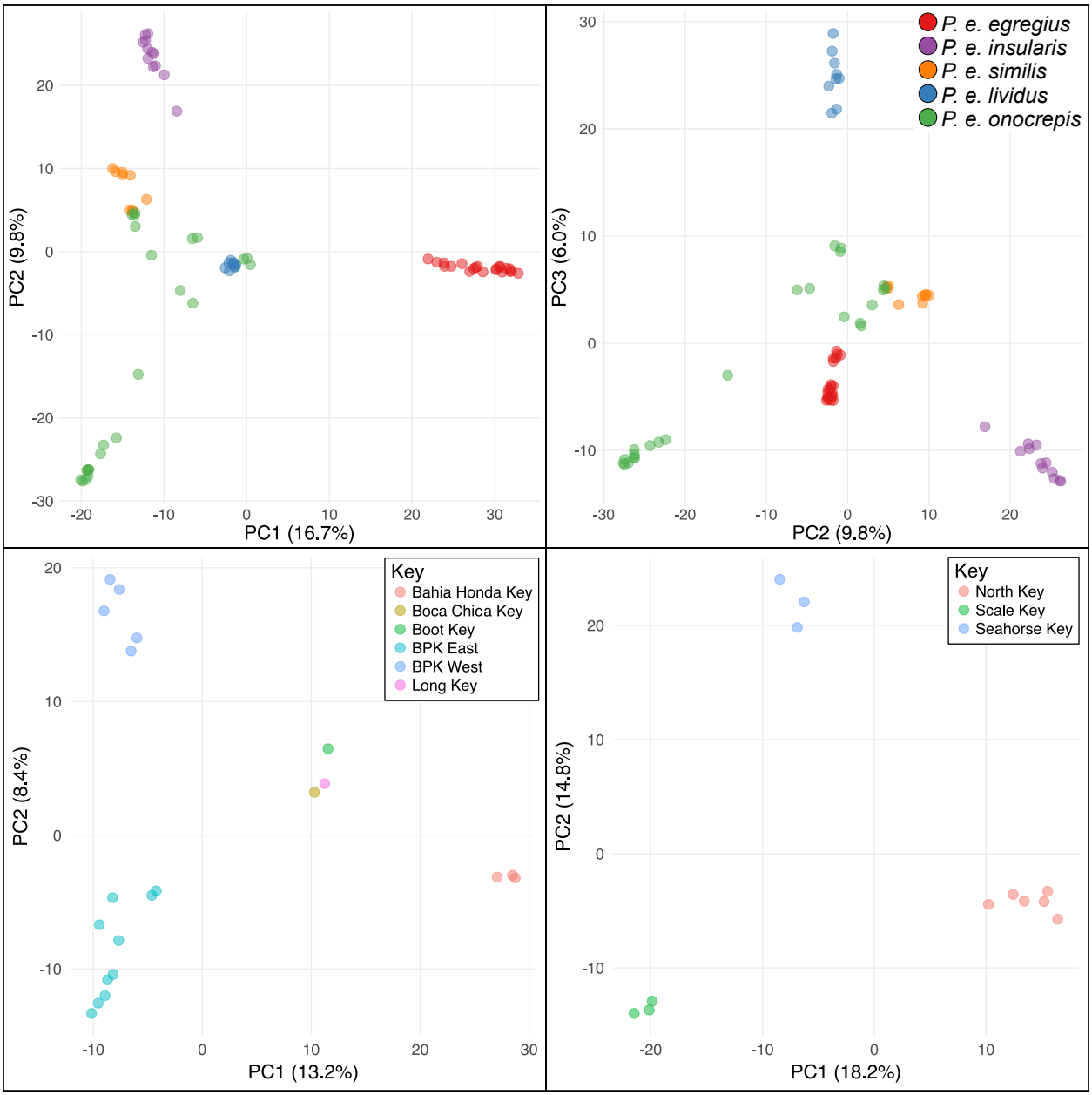

Figure 2.4: Principal component analyses using SNP data. Top left: PCs 1 and 2 of analysis including all individuals. Top right: PCs 2 and 3 of analysis including all individuals. Bottom left: PCs 1 and 2 of *P. e. egregius* individuals, explaining 21.6% of the variation in the data. Bottom right: PCs 1 and 2 of *P. e. insularis* individuals, explaining 33% of the variation in the data.

We examined seven morphological characters, including scale counts and body measurements, in *P. egregius* but were not able to identify any substructure within the species using these data. The characters used here represent only a subset of those shown to exhibit variation or those used in the subspecies descriptions. Traits we were unable to characterize in ethanol preserved specimens include tail color, hatchling color, and stripe divergence, all relying on characterizing color which fades in ethanol. These color traits may be more likely than the traits we were able to examine to be locally adapted, due to sexual selection or predation pressures. If these color traits are under selection, they are also more likely to provide signal of population structure within the species. This could explain why the previous morphological study was able to describe geographic variation using more characters.

In this study we used a total of 1,996 base pairs of mtDNA to examine *P. egregius* evolutionary history, population structure, and genetic diversity. The most basal divergence found using mtDNA, between the northern and southern clades, is similar to phylogeographic breaks in other species (Remington, 1968). In contrast, there were many geographic regions where individuals were non-monophyletic. Most apparent are the two *P. e. egregius* individuals which fall sister to the rest of the southern *P. egregius*. These individuals had sequence for both mitochondrial genes and a similar cyt-*b* haplotype was recovered from a sample in the same location in a previous study (Branch *et al.*, 2003). Sequence AF470635 is 416 bp long and only varies from the sequences in our study by 5 bp. We suspect these samples represent a low-frequency, unique mitochondrial haplotype that occurs in the Big Pine Key population and that this mitochondrial haplotype may represent an instance of incomplete lineage sorting. Additionally, individuals from the Lake Wales Ridge, Lake County, Madison County, and from the Cedar Keys were each not monophyletic. These instances are likely due, in part, to the inheritance pattern of mtDNA. We have evidence that *P. egregius* exhibits sex-biased dispersal, and since mtDNA is maternally inherited, it may not accurately represent their evolutionary history or population structure.

Lastly, we used 33,894 SNP characters to examine the intraspecific divergence and patterns

of diversity within *P. egregius*. The earliest divergence in *P. egregius* splits *P. e. lividus* from the rest of *P. egregius*. This may indicate that the Lake Wales Ridge represents the historical range of the species or that it served as a refugia during past high sea level in the Pleistocene or Pliocene. Within the large *P. egregius* clade not including *P. e. lividus*, there exists a split along the middle of the Florida peninsula with southern *P. e. onocrepis* and *P. e. egregius* forming one clade and northern *P. e. onocrepis*, *P. e. insularis*, and *P. e. similis* forming another. This break coincides with the major division in the mtDNA phylogeny, which are both similar to phylogeographic breaks in other species between the mainland US and the Florida peninsula. It is hypothesized that this phylogeographic break is due to terrestrial isolation caused by a Pliocene warm period which increased sea level (Raymo *et al.*, 2011; Dutton *et al.*, 2015). The apparent discordance in the most basal divergence between the mtDNA and SNP data may be explained by subsequent gene flow between *P. e. lividus* and southern *P. e. onocrepis* individuals.

At a smaller scale, in both the island subspecies, individuals from different islands formed unique lineages and clustered independently in the PCA. This indicates that individuals are likely not moving between islands, or migration between islands is very rare and that each island represents a separate population. Interestingly, we also found evidence for population structure within an island, in individuals sampled along a beach on Big Pine Key in the Florida Keys. This beach is approximately 2 kilometers, and skinks were collected all along the beach. At one point along the beach the coastal sandhill is interrupted by mangrove. Skinks from either side of this wetland break in the beach form monophyletic groups and clustered separately in the PCA. These individuals are separated by less than one-quarter kilometer, yet the wetlands form an apparent strong barrier to gene flow. This degree of small scale structure was not observed among population on the main land. For example, within *P. e. lividus*, individuals from the same site did not form monophyletic lineages. Samples from Jack Creek and Sylvan shores, which are separated by 10 kilometers are apparently an intermixed population. This is likely due, at least in part, to the preservation of sandhill habitat along the Lake Wales Ridge, allowing for gene flow along the ridge.

Delimiting conservation units

Disagreement between morphology and mtDNA data in delimiting intraspecific divergence is not unique to *P. egregius*. It has been seen in diverse animal taxa such as birds, fishes, mammals, amphibians, reptiles, and invertebrates (Cronin *et al.*, 1991; Fry and Zink, 1998; Babik *et al.*, 2005; Crews and Hedin, 2006; Leaché and Cole, 2007; Dibattista *et al.*, 2012). Research has suggested that these conflicts can be reconciled by using genome-wide SNP data, which should provide more detailed information and be subject to less bias than the preceding two methods. Our study shows that in *P. egregius* SNP data described more fine-scale population structure than mtDNA or morphological characters. Therefore, we described six ESUs based on the patterns of divergence seen in the SNP data. Based on Ryder (1986) ESUs are geographic units with evidence for genetic distinctiveness. In *P. egregius*, we define three ESUs which correspond to current subspecies definitions: *P. e. lividus*, *P. e. insularis*, and *P. e. egregius*. These were all monophyletic lineages and clustered independently in one of the clustering analyses. The forth ESU combines *P. e. similis* and northern *P. e. onocrepis*, while the fifth is solely central Florida *P. e. onocrepis*. These were both monophyletic and unique clusters. Lastly, we define a sixth ESU from Indian River on the Atlantic Coast of Florida. Although this is a small sample, it was a well-supported monophyletic lineage in the phylogenetic tree. Importantly, we found that the two subspecies which are already state or federally protected (*P. e. lividus* and *P. e. egregius*) are each ESUs; as is *P. e. insularis*, which is currently under review by USFWS to determine if a petition to list is warranted.

As we demonstrate here, it is important to consider what characters are being used to define ESUs. If we has used the morphological dataset to define ESUs in *P. egregius* we might infer that there exists only one ESU, which would under-represent diversity and lump potentially vulnerable populations, such as those on islands, with more stable mainland populations. Using the mtDNA results to define ESUs, we would have identified five ESUs, corresponding to populations mt1 through mt5. Some of these ESUs would lump and split geographic populations supported by the

genome-wide SNPs. For example, *P. e. lividus* would be split between two ESUs, which would complicate management given than all *P. e. lividus* occur in the same geographic region.

Conclusions

The data used in delimiting conservation units in any taxon can have a large impact on the number and identity of ESUs. It is important for conservation biologists to be aware of the advantages and disadvantages of the data types they are working with or the data types used in studies that are forming the basis of conservation decisions. We found that genome-wide SNP data was able to capture small scale population structure, in a taxon where life history traits were indicative of low dispersal. It is important also to consider the specific traits being used for each data types, for example, when using morphological characters, traits that may be under selection from local adaptation will have a stronger signal that those under less strong selection. Additionally, the signal of local adaptation may or may not accurately represent the evolutionary history and/or population structure of the taxon. We believe that given unlimited resources, it would be ideal to tackle the task of delimiting conservation units from a variety of perspectives, incorporating morphological, genetic, and ecological information of the species of interest. Given that resources are limited, we provide this case study as an example of the advantages and disadvantages of data types, and infer that, of the methods examined here, genome-wide SNP data is best suited to examine intraspecific divergence, especially in taxa where a high degree of local population structure is expected.

Acknowledgments

This work was supported by the US Fish and Wildlife Service (USFWS) Grant #24036906 and the Florida Fish and Wildlife Conservation Commission (FL FWCC) Grant #24037050. We would like to thank Aaron Schrey (Georgia Southern University) for donating tissue to this project,

the Florida Museum of Natural History Herpetology Collection at the University of Florida for loaning specimens used in morphological analyses. We would also like to thank everyone who contributed tissue samples or facilitated sample collection: Paul Moler (FL FWCC), Adam Emerick (USFWS), Kevin Enge (FL FWCC), Jonathan Mays (FL FWCC), Layne Bolen (USFWS), M. Rebecca Bolt (KSC), Matt Vance (FL FWCC), Noah Mueller (Univ of Florida), Nicole Atteberry (Zoo Miami), Jeremy Dixon (USFWS), Craig Guyer (Auburn Univ), Eric C. Soehren (AL DCNR). We would like to thank Tiffany M. Doan (New College FL) for training in scale counts. We would also like to thank the following undergraduate researchers who contributed to this project: Alex Robertson, Christopher Yanick, and Carly Grimison. Skinks were collected under the following permits: FL FWCC Scientific Collecting Permit LSSC-15-00108, Key Deer NWR SUP 2015-32, US Department of Agriculture Forest Service SUP LAK372, and University of Central Florida IACUC #15-39W.

CHAPTER 3: PHYLOGEOGRAPHY OF *Plestiodon egregius*

Abstract

Using genetic markers to identify the biogeographic factors driving divergence is a main goal of phylogeography. In statistical phylogeography, multiple *a priori* hypotheses are proposed, and then tested with statistical models to identify biotic and abiotic factors potentially driving divergence. The southeastern US has had many complex landscape changes occur due to climate and associated sea level changes. Therefore, this region has been a focus of biogeographic and phylogeographic studies. I used *Plestiodon egregius* as a model system to examine the impact of the mid-Pliocene warm period (MPWP) on terrestrial organisms in the southeastern US. I generate a genome-wide single nucleotide polymorphism data set, use these data to infer their evolutionary history, and then test four alternative hypotheses regarding the historical dispersal of *P. egregius*. The first test I used, a phylogenetic constrained topology comparison, supported isolation then expansion from the Florida scrub ridges. The second test, based on patterns of genetic diversity, were inconclusive, which is likely due to the multitude of factors that can influence genetic diversity in a species. I conclude that *P. egregius* likely found refugia along the southern scrub ridges in Florida during the MPWP.

Introduction

Phylogeography is the study of genetic lineages over space and time (Avise *et al.*, 1987). It is set apart from classical phylogenetics or population genetics by focusing on the biogeography, or the geographic distributions of species, while also serving to unite these macro and microevolutionary fields, respectively (Avise *et al.*, 1987; Avise, 2000). There are often multiple plausible explanations for observed patterns of lineage divergence because the historical events that phylogeography is concerned with cannot be observed. It is therefore useful to utilize statistical phylogeography to

test multiple alternative scenarios (Knowles, 2004; Crisp *et al.*, 2011), which also conforms with long-standing arguments for multiple working hypotheses in scientific inquiry (Chamberlin, 1890; Platt, 1964; Elliott and Brook, 2007).

The southeastern United States (US) is a fascinating region to study the phylogeography of taxa because of the changes that have occurred due to climate change and associated sea level change (Raymo *et al.*, 2011). There is evidence that the end of the Miocene (23 - 5.3 MYA) and most of the Pliocene (5.3 - 2.6 MYA) were relatively stable periods when climate was approximately 1°C warmer than present (Zachos *et al.*, 2001). However, it is postulated that the mid-Pliocene warm period (MPWP), from 3.2 - 2.8 MYA, was between 1°C and 8°C warmer than present day (Dutton *et al.*, 2015). This increased temperature drove sea level 15 - 60 m higher than current levels (Dutton *et al.*, 2015). Specifically, along the Florida peninsula sea level was estimated to be 20 - 30 m higher than present (Raymo *et al.*, 2011). During the MPWP almost all peninsular Florida was inundated, the only exception being a series of scrub ridges approximately 40 m higher than current sea level (Webb, 1990). At that time, these ridges would have been disconnected from the mainland US.

The MPWP was followed by an approximately 2°C cooling and the climate remained relatively stable until the alternating glacial events and warmer interglacial periods of the Pleistocene (Roy *et al.*, 1996). During this time, glaciers in the Northern hemisphere extended as far south as the 40th parallel and in North America, layers of permafrost extended hundreds of kilometers south (Richmond and Fullerton, 1986). These glaciers tied up huge amounts of water; global sea level fell as much as 100 m during glacial periods (Roy and Peltier, 2015). During this period, it is predicted that the Florida peninsula extended to the edge of the continental shelf. However, during interglacial periods, the estimated maximum sea level was approximately 8 m higher than today (Hearty *et al.*, 1999). The last major glacial event (LGM) occurring about 20 KYA and was followed by a gradual warming until reaching current conditions (Tushingham and Peltier, 1991).

These changes to the Florida peninsula have had impacts on the organisms residing there.

During the MPWP, terrestrial organisms inhabiting the peninsula would have experienced range retractions and isolation to the peninsular FL ridges and/or US mainland (Webb, 1990). Specifically, the two oldest ridges, the Lake Wales Ridge (LWR) and Mount Dora Ridge (MDR) (Figure 3.1) have been proposed to serve as refugia for many scrub associated species (Webb, 1990; Deyrup, 1996). During glacial periods, many organisms experienced southern range expansions and northern range constrictions (Hewitt, 2004). Because of sea level fall during the glacial periods, terrestrial organisms were able to colonize new areas which were not accessible previously, such as islands that were no longer separated by water. As sea level rose after the LGM, terrestrial organisms which had colonized these islands would now be restricted there and isolated from their mainland counterparts. Population genetic theory would predict that populations which were able to persist (on the ridges and/or mainland) acted as source populations and should have higher genetic diversity, while more ephemeral populations should be sinks and have lower genetic diversity (Avise and Hamrick, 2001).

Figure 3.1: Florida topographic map with Lake Wales Ridge in green and Mount Dora Ridge in pink. The edge of the coastal shelf is visible, in the sharp transition from light blue to dark blue.

Soltis *et al.* (2006) characterized the patterns of intraspecific divergence in the southeast US in over 140 species and identified many congruent patterns. One of the patterns identified was a break between mainland US and peninsular individuals, which is usually attributed to historical isolation due to the Suwanee Strait or other periods with high sea level (Remington, 1968). Many of the studies utilized in the meta-analysis only provided the patterns of divergence, they did not explicitly examine the timing or drivers of divergence, therefore Soltis *et al.* (2006) emphasize that although they were able to identify regions with many phylogeographic beaks, these may be pseudocongruences. The apparent concordant patterns may have different mechanisms and have occurred at different times. Consequently, it is important to examine the drivers of divergence in each species independently.

Here, I use Mole Skinks, *Plestiodon egregius*, as a model system to examine the impact of the MPWP on terrestrial organisms in the southeastern US (Figure 3.2). *Plestiodon egregius* are native to the southeastern US and are found in scrub, sandhill, and coastal hammock (Mount, 1963). Previous work based on morphological characters and mitochondrial DNA sequences hypothesized that this species originated on the LWR, but this hasn't been explicitly tested. *Plestiodon egregius* diverged from their sister species, *P. reynoldsi*, approximately 9 MYA (Brandley *et al.*, 2011) and there is evidence that this semi-fossorial skink has low dispersal rates and high local population structure (Penney, 2001). Together, this indicates that there should still be a signal of historical dispersal in their genome. I first inferred the evolutionary history of *Plestiodon egregius* and then used a statistical phylogeography framework to test four alternative hypotheses of *P. egregius* historical dispersal based on the history of the Florida peninsular and *P. egregius* natural history.

Hypotheses

H1. Snowbird hypothesis - The historical patterns of divergence within this species are based on an expansion from the mainland US after the MPWP.

H2. Southern Ridge Hypothesis - The historical patterns of divergence within this species are based

on a radiation from the southern scrub ridges (Lakes Wales Ridge, Bombing Range Ridge) after the MPWP.

H3. Northern Ridge Hypothesis - The historical patterns of divergence within this species are based on a radiation from the northern scrub ridges (Trail Ridge, Mount Dora Ridge, Orlando Ridge) after the MPWP.

H4. Multiple Refugia hypothesis - The historical patterns of divergence within this species are based on expansion from two or more isolated populations, mainland and/or ridges, after the MPWP.

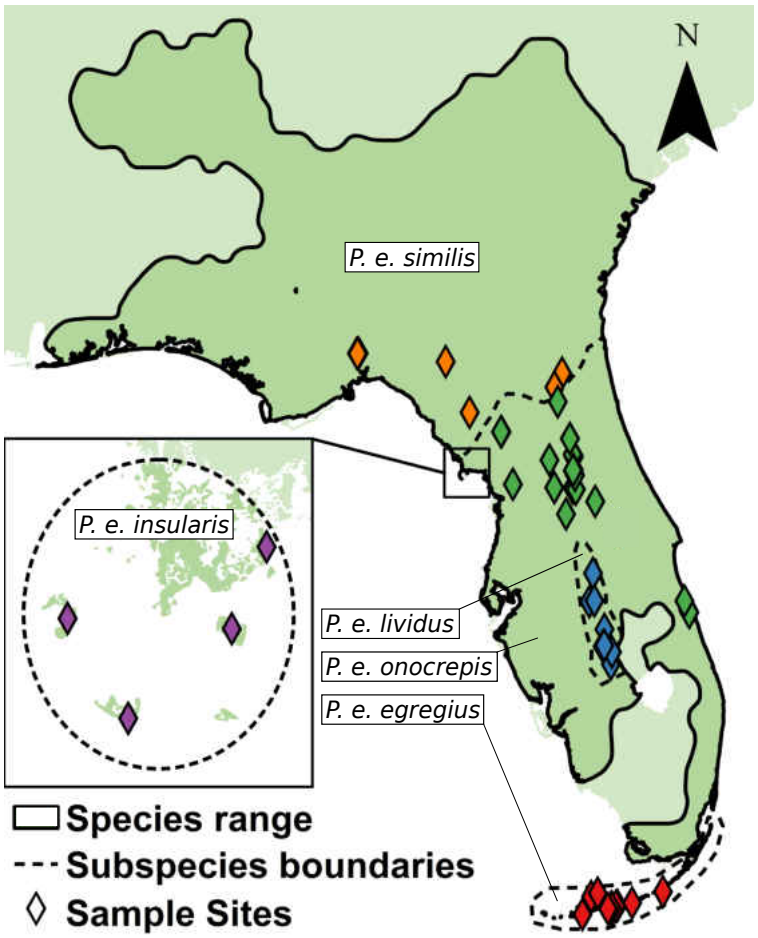

Figure 3.2: Sample locations of individuals used in this study. Distribution map of Mole Skinks, *Plestiodon egregius* based on (Mount, 1965)

I determined which of these hypotheses was most likely using two different tests, both utilizing genome-wide single nucleotide polymorphism (SNP) data. First, I made predictions of *P. egregius* evolutionary history under each hypothesis, then constrained phylogenetic trees to match those predictions and compared each tree to an unconstrained phylogenetic tree. Second, I used linear models to test the prediction that populations which had persisted longer would have higher genetic diversity. I built models for each hypothesis to test this prediction.

Methods

Tissue samples were collected from 178 individuals of *P. egregius* as well as four individuals from *P. reynoldsi* to act as an outgroup (Figure 3.2). 23 tissues for *P. e. lividus* were received as loans. Tissues were stored at -20°C in 100% ethanol then extracted with SeraPure beads following Faircloth and Glenn (2014). DNA concentration was standardized to 1ng/μL then 50ng of DNA was lyophilized and sent for library preparation.

Genomic DNA was sent to SNPsaurus, LLC for preparation as nextRAD libraries (Russello *et al.*, 2015). First, 40 ng of genomic DNA was fragmented with the Nextera reagent (Illumina, Inc), which also ligates short adapter sequences to the ends of the fragments. Fragmented DNA was amplified for 27 cycles at 74°C, with one of the primers matching the adapter sequence and extending ten nucleotides into the genomic DNA with selective sequence GTGTAGAGCC. Thus, only fragments starting with a sequence that can be hybridized by the selective sequence will be efficiently amplified. Samples were pooled then sequenced on a HiSeq 4000 to generate 150-bp single-end reads (University of Oregon). A *de novo* reference was created by aligning 10 million reads, collected evenly from the samples and excluding reads with counts fewer than seven or more then 700, to identify allelic loci and collapse allelic haplotypes to a single representative. Using this reference, all reads were mapped with an alignment identity threshold of 95% using bbmap (Bushnell, 2014). Genotype calling was done using Samtools and bcftools (Li *et al.*, 2009). The loci

were then filtered to remove alleles with a population frequency of less than 3%. Loci were removed if they were heterozygous in all samples or had more than two alleles in a sample (suggesting collapsed paralogs).

In order to test which hypotheses were supported by the phylogenetic relationships, a maximum likelihood framework was used. First, SNPs were quality filtered such that only individual sites with >5x coverage and SNPs with <30% missing data were retained (Danecek *et al.*, 2011). Next, SNPs were phased in fastPHASE v1.4 with default parameters to obtain haplotypes for each individual (Scheet and Stephens, 2006). Then, for each hypothesis I constrained the tree topology, such that it would match the expected relationships from that hypothesis (Figure 3.3) and estimated a tree in RAxML v8.2.11 with a GAMMA model of rate heterogeneity and lewis ascertainment bias correction (Stamatakis, 2014). For each hypothesis, 450 bootstrap replicates were used to derive a majority rule consensus tree. I also estimated an unconstrained tree with the same RAxML settings to act as a null hypothesis. Each constrained tree was then compared to the unconstrained tree using an unweighted and weighted Robinson-Foulds (RF) metric (Stamatakis, 2014). The RF distance can be understood as the number of clades that are unique to just one of the two trees being compared, and the weighted metric incorporates node support. Therefore, the higher the RF distances, the more unique clades are present in the two trees and the more dissimilar the two trees are. Additionally, each weighted and unweighted RF value was normalized by dividing the RF value by 2(n-3), where n is the number of taxa (Stamatakis, 2014). To visualize the differences between the unconstrained tree and best supported tree, I used the cophylo function in the R package phytools (Revell, 2012).

To further test the hypotheses, I assume that individuals closer to a historic source population will have higher genetic diversity, and that diversity should decrease moving away from the source population (Avise and Hamrick, 2001). Heterozygosity was measured in each individual by dividing the number of heterozygous loci by the total number of loci sequenced in that individual. I then used a linear model to test for a significant relationship of heterozygosity and "distance from origin" for each hypothesis. I ensured normality of the residuals using a Shapiro-Wilks test and visual

inspection of Q-Q plot in R (R Core Team, 2017). This analysis was repeated with the two island subspecies excluded.

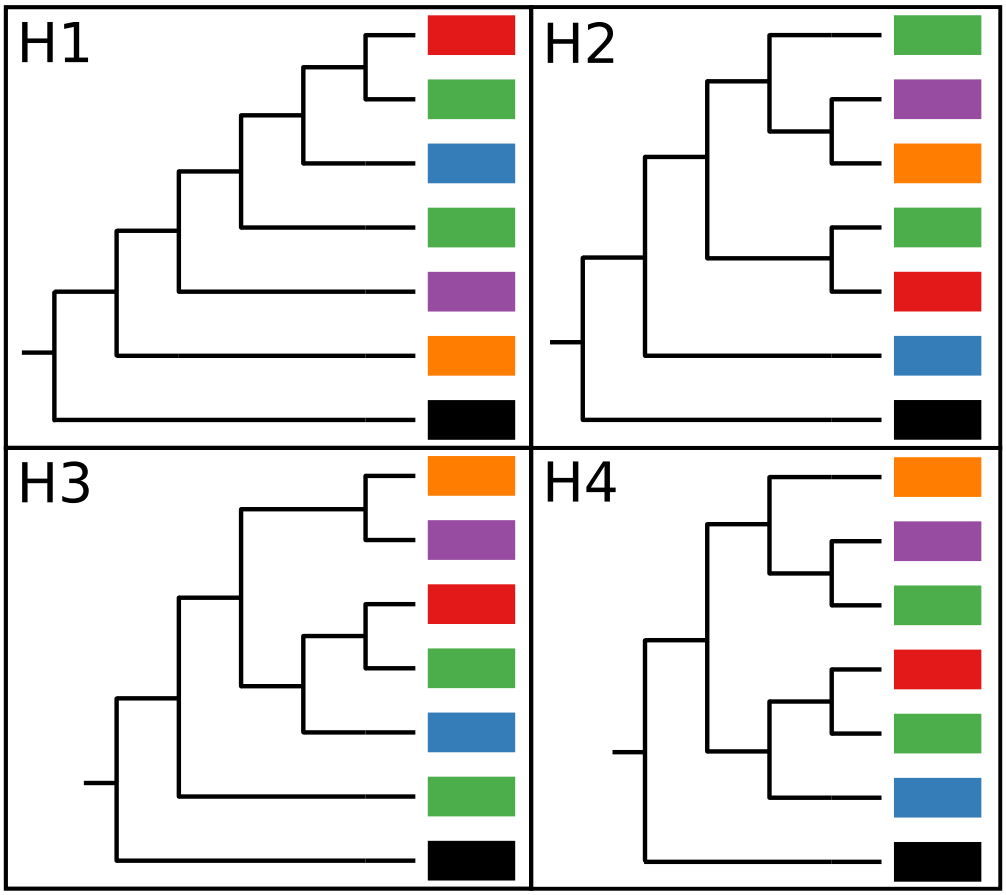

Figure 3.3: Predicted phylogenetic relationships within *P. egregius*, based on each hypothesis.

Results

After filtering of nextRAD libraries, I retained 33,898 SNPs that were used in both phylogenetic and genetic diversity analyses. The unconstrained maximum likelihood phylogeny had high support values overall, with most low supported nodes close to the tips (Figure 3.4). The most basal node within *P. egregius* split individuals from the LWR (*P. e. lividus*) and the rest of the

species. Within the large *P. egregius* clade, there was a northern and southern lineage. Within the northern clade, individuals from the Cedar Keys (*P. e. insularis*) were sister to individuals from central and north Florida (*P. e. onocrepis* and *P. e. similis*). Within the southern group individuals from the Florida Keys (*P. e. egregius*) were sister to *P. e. onocrepis* from the Indian River County on the Atlantic Coast of Florida. In both of the insular subspecies, individuals from the same key were monophyletic.

For the first test, utilizing phylogenetic analyses to test the four hypotheses, the Southern Ridge hypothesis (H2) was best supported (Table 3.1). The RF distance between the unconstrained tree and the Southern Ridge tree was 92, compared to a range of 106 to 126 for the other hypotheses. When the branch supports are taken into account the weighted RF distance is 28.2, compared to between 34.9 to 43.4 for the other three hypotheses. In both the Southern Ridge tree and the unconstrained tree, individuals from the LWR are sister to the rest of *P. egregius* (Figure 3.5) and many of the incongruities between the two trees are among shallow nodes in branches with low support values, not between major lineages.

In the second test, which compared linear models of genetic diversity and distance from origin, the results was inconclusive. I did not find support for any hypothesis (Table 3.2 & 3.3). Residuals for each model were normal when checked with the Shapiro-Wilks test and Q-Q plot. In the models representing the Snowbird hypothesis and the Southern Ridge hypothesis, distance from origin was a significant predictor of heterozygosity at the α <0.05 level. Indeed, when insular individuals are removed, all models show a significant correlation between distance and heterozygosity (Table 3.3). In both of the Southern Ridge hypothesis models (with and without insular individuals) distance and heterozygosity were negatively correlated, however the r^2 value is very low. The data points are scattered and don't fit the model well (Figure 3.6).

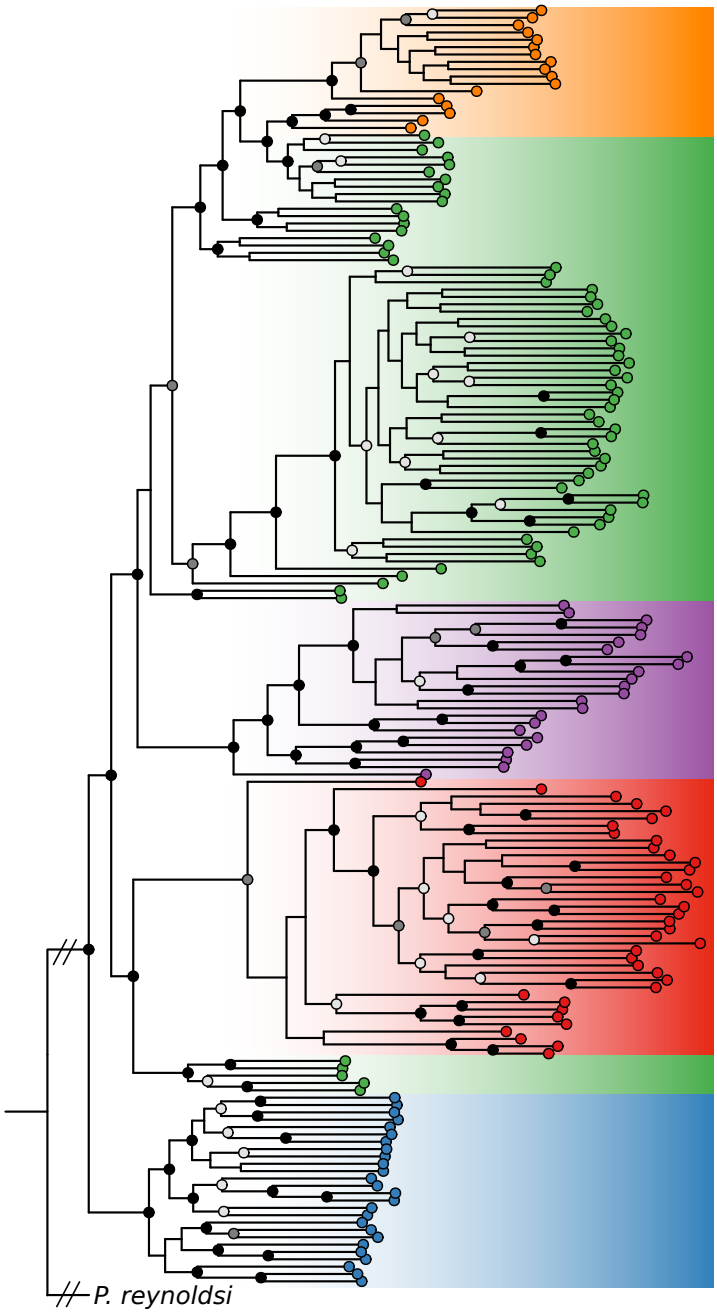

Figure 3.4: Unconstrained maximum likelihood phylogeny of *P. egregius*. Bootstrap support is indicated by node dot color: black is >95, gray is between 95 and 80, light gray is between 80 and 60, and <60 has no node dot.

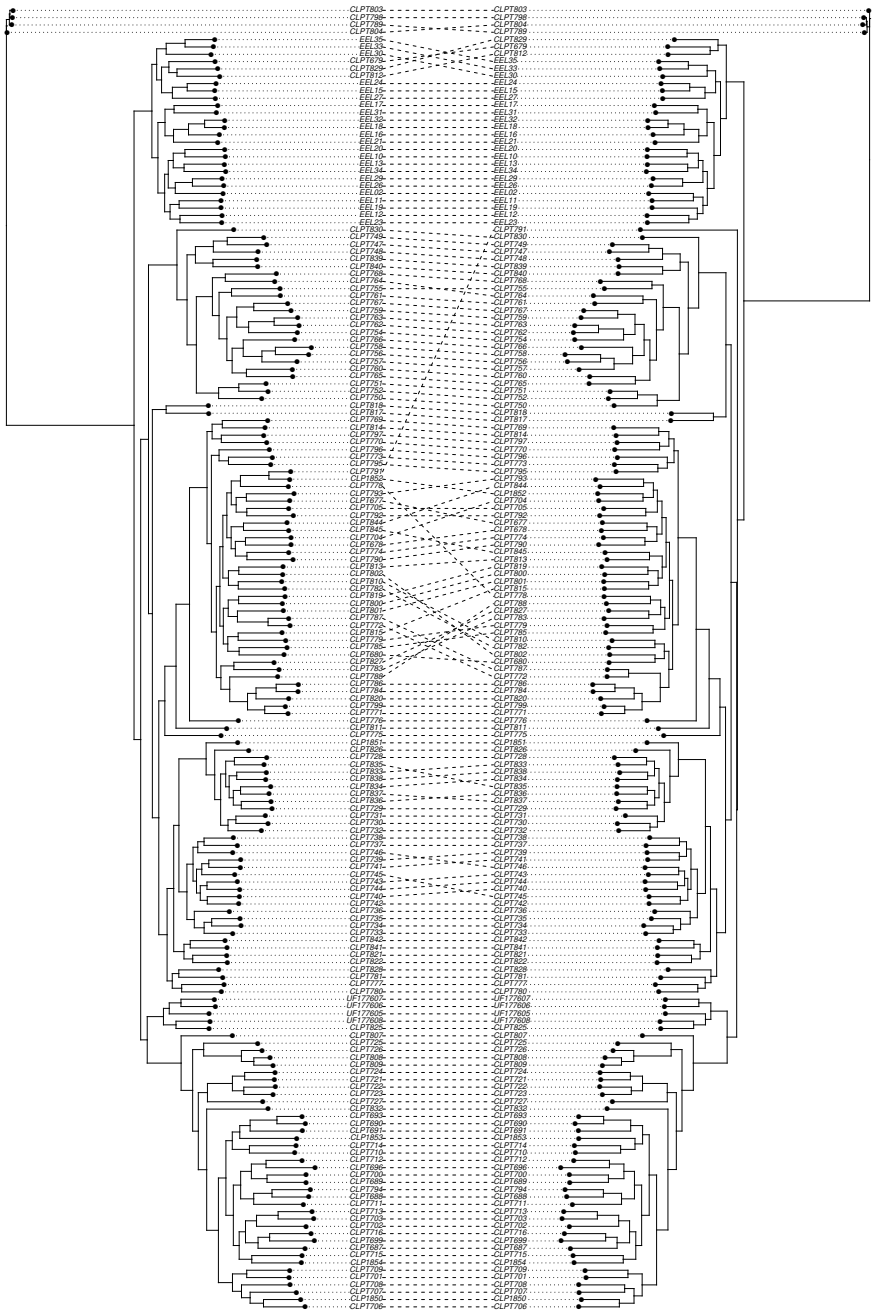

Figure 3.5: Unconstrained maximum likelihood phylogeny (left) of *P. egregius* coplot with best supported constrained tree: Southern Ridge, H2 (right).

Table 3.1: Unweighted and weighted Robinson-Foulds distances of each constrained hypothesis tree to the null, unconstrained tree.

	RF	normalized RF	WRF	normalized WRF
H1. Snowbird	126	0.36	40.9	0.12
H2. Southern Ridge	92	0.26	28.2	0.08
H3. Northern Ridge	124	0.35	43.4	0.12
H4. Multiple Refugia	106	0.30	34.9	0.10

Table 3.2: Model Comparison of genetic diversity analyses, with insular individuals included.

	intercept	slope	r^2	P value
H1. Snowbird	9.293e-01	-3.797e-05	0.03716	0.0108
H2. Southern Ridge	9.125e-01	4.775e-05	0.03965	0.0084
H3. Northern Ridge	9.139e-01	3.398e-05	0.01594	0.0969
H4. Multiple Refugia	9.171e-01	-8.561e-06	0.00064	0.7410

Table 3.3: Model Comparison of genetic diversity analyses, with insular individuals excluded.

	intercept	slope	r^2	P value
H1. Snowbird	9.465e-01	-6.957e-05	0.180	3.08e-06
H2. Southern Ridge	9.157e-01	9.195e-05	0.257	1.22e-08
H3. Northern Ridge	9.171e-01	1.019e-04	0.269	4.47e-09
H4. Multiple Refugia	9.135e-01	1.609e-04	0.190	1.55e-06

Discussion

In this study, I generated the largest genetic data set ever used to infer the evolutionary history of *P. egregius*. Previous studies examining divergence within *P. egregius* have used morphological characters, single mtDNA locus, and/or microsatellite loci (Mount, 1963, 1965; Branch *et al.*, 2003; Schrey *et al.*, 2012), but they often led to conflicting, lowly supported, results. Many of the patterns described using morphological characters in the most recent subspecies descriptions are seen here (Mount, 1965) . For example, *P. e. egregius*, *P. e. insularis*, and *P. e. lividus* are all monophyletic.

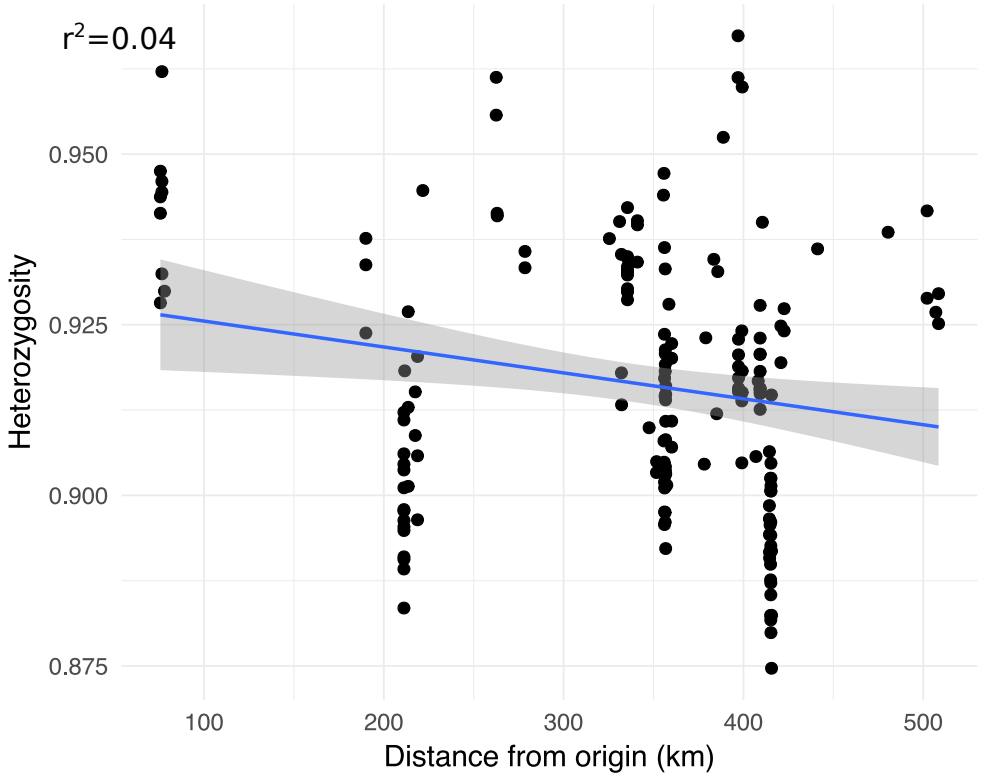

Figure 3.6: Plot of linear model corresponding the Snowbird hypothesis (H1) with insular individuals included. Best fit line in blue, with 95% confidence interval in gray.

Comparing these results to a previous mtDNA phylogeny (Branch *et al.*, 2003), we see similar branching patterns but there is overall better nodal support, and also more phylogeographic structure. Here, we utilize next-generation sequencing technology to capture variation from throughout the genome. Reduced representation sequencing methods, such as nextRAD, have shown to be more resilient to processes which can bias interpretation of mtDNA (McGuire *et al.*, 2007). Additionally, I showed in Chapter 2 that SNP data capture more fine scale structure than morphological characters or mtDNA in *P. egregius*. Therefore, I present the full unconstrained phylogenetic tree as the most up-to-date characterization of divergence within *P. egregius*.

For the phylogenetic test of the hypotheses, I found support for the Southern Ridge

Hypothesis. Although the RF metric used here is a point estimate and does not provide a confidence interval, visualizing the differences between the unconstrained and constrained trees provides a measure confidence in the metric. In the coplot of the unconstrained and best-supported constrained tree (Figure 3.5), we can see that many of the differences are between closely related individuals. This type of difference reflect nodes that are difficult to resolve, rather than differences in major lineages between the trees. These clades may lack phylogenetic signal due to sampling of closely related individuals.

In the second test of the alternative hypotheses, the genetic diversity test, results were inconclusive. This may be because of the multitude of factors that affect genetic diversity of populations. For example, habitat fragmentation may act to reduce migration and habitat loss can lead to smaller population sizes, both of which may ultimately lead to lower genetic diversity (Frankham, 1995). Much of *P. egregius* habitat, especially that on islands and the Lake Wales Ridge, is rapidly disappearing (Christman, 1992), which may have led to loss of genetic diversity in those regions. Additionally, selective pressures such as natural selection or sexual selection, may act to either increase or decrease genetic diversity (Li *et al.*, 2013). Female *P. egregius* are able to store sperm for a protracted period of time (Schaefer and Roeding, 1973), which may be a mechanism to allow females to choose which males to reproduce with. This would be sexual selection, and could act to alter genetic diversity irrespective of *P. egregius* dispersal history.

Our results support previous inferences of the LWR as the ancestral population of *P. egregius*. This pattern has been proposed based on results from mitochondrial sequence data (Branch *et al.*, 2003) as well as geographic patterns of variation in morphological characters (Mount, 1965). Here, I explicitly test this assumption and find that, based on the patterns of divergence within *P. egregius*, there is support for a historical isolation on the southern Florida ridges: the LWR and Bombing Range Ridge. Additionally, the samples of *P. e. lividus* in this study are all from the LWR, and a previous study was unable to find *P. egregius* on Bombing Range Ridge despite intensive sampling efforts (Branch and Hokit, 2000). Therefore, I may be able to narrow our conclusions to state the it

was the LWR which served as the ancestral habitat. Furthermore, a previous mtDNA study of *P. e. lividus* population structure used a nested clade analysis to show that within the LWR, the central LWR was likely the source population. Combining these inferences, it may be the central Lake Wales Ridge specifically that was the ancestral range of *P. egregius*.

More generally, this work adds to the growing body of literature of phylogeographic patterns in the southeastern US and peninsular Florida. Soltis *et al.* (2006) characterized the phylogeographic breaks of over 100 plant, animal, and fungi species in this region, and identified similar patterns in many species. One of the patterns they observed was a break between peninsular Florida and the mainland US, although they recognized that congruent patterns may be due to different processes in different species. Because the southeastern US is a large, complex landscape, and species have unique life history characters, what seem to be similar patterns may have different origins. Similarly, a recent study used hypothesis testing to examine the origin of Florida scrub species (Lamb *et al.*, 2018). They used *Arenivaga floridensis* as a model, and found evidence for a western origin in this species during the Pliocene, but add that an eastern origin is likely in other species and that the eastern vs western origin hypotheses are not mutually exclusive. Together these results emphasize the importance of species level studies to examine phylogeographic drivers, so we may more fully understand the processes and patterns of divergence in the southeastern US.

Conclusions

Overall, I find support for the Southern Ridge hypothesis. The phylogenetic analysis clearly supported the Southern Ridge hypothesis, while the genetic diversity analysis was inconclusive. There are many factors that may influence genetic diversity, the historical dispersal is not the only factor by any means. In contrast, the phylogenetic tree should be a direct result of the evolutionary history of this species, and therefore should reflect its historical dispersal. I hope this study serves as an example to consider testing *a priori* hypotheses when performing phylogeographic research.

Acknowledgments

This work was supported by the US Fish and Wildlife Service (USFWS) Grant #24036906 and the Florida Fish and Wildlife Conservation Commission (FL FWC) Grant #24037050. I would like to thank Aaron Schrey (Georgia Southern University) for donating tissue to this project. I would also like to thank everyone who contributed tissue samples or facilitated sample collection: Paul Moler (FL FWC), Adam Emerick (USFWS), Kevin Enge (FL FWC), Jonathan Mays (FL FWC), Layne Bolen (USFWS), Rebecca Bolt (KSC), Matt Vance (FL FWC), Noah Mueller (University of FL), Nicole Atteberry (Zoo Miami), Jeremy Dixon (USFWS), and Craig Guyer (Auburn University). I would also like to thank the following undergraduate researchers who contributed to this project: Millie Baylac, Samantha Dillon, Steffany Medina, Alex Robertson, and Christopher Yanick. Skinks were collected under the following permits: Florida Fish and Wildlife Conservation Commission Scientific Collecting Permit LSSC-15-00108, Georgia Department of Natural Resources Scientific Collecting Permit 29-WJH-16-244, Alabama Wildlife and Freshwater Fisheries License Scientific Permit 9157, Crocodile Lake National Wildlife Refuge SUP 41581-2016-02, Key Deer National Wildlife Refuge SUP 2015-32, Merritt Island National Wildlife Refuge SUP MI-2016-201R, US Department of Agriculture Forest Service SUP LAK372, Alabama Forever Wild Research Permit 1702, Brevard County Environmentally Endangered Lands Program Collecting Permit, Miami Dade County Natural Areas Management Permit 254, and University of Central Florida IACUC #15-39W.

CHAPTER 4: DISCUSSION

Many factors act on organisms to influence their dispersal and distribution, which therefore alters their evolutionary history and population structure. In this study I used *P. egregius* as a model to examine how the effects of historical climate change act on the evolutionary history and population structure of a species. I first characterized their evolutionary history and population structure, then examined the possible drivers of those patterns. Taking the results of these two studies together, I am able to make inferences about speciation in *P. egregius*.

Using the unified species concept (De Queiroz, 2007) I believe *P. egregius* represents one species with multiple lineages that may be in the process of diverging. Mitochondrial sequence divergence between the two major phylogeographic lineages in *P. egregius* was about 8%, which is higher than some species pairs within the genus (Kurita and Hikida, 2014; Kurita and Toda, 2017). I have presented evidence of divergence within *P. egregius* based on genetic data, but this is one line of evidence among many that can be used to support species delimitation. For example, we have no evidence that individuals from any lineage have differences in life history, ecology, or that they inhabit substantially different niches. Additionally, there is no evidence for a barrier to reproduction if individuals from these lineages were to come in contact. Here, I will define subspecies as the metapopulations within *P. egregius* having one or more lines of evidence for divergence.

I have presented evidence that two subspecies, *P. e. egregius* and *P. e. insularis*, are monophyletic and have some morphological differentiation according to previous work (Mount, 1965). We hypothesize that these subspecies were isolated as sea level rose after the last glacial maximum and have had little contact with individuals from the mainland since, although it is possible there is some gene flow due to rare rafting events. If the islands do act as barriers, then these lineages have allopatric distributions with the rest of *P. egregius*. Although these insular populations form monophyletic groups, they are not reciprocally monophyletic within *P. egregius*, and defining them as species would render *P. egregius* paraphyletic. With the available evidence,

I suggest that *P. e. egregius* and *P. e. insularis* represent subspecies of *P. egregius*. Similarly, I recommend the continued recognition of *P. e. lividus* across it's current described range. According to SNP data, *P. e. lividus* is a unique lineage and may be morphologically distinguishable according to previous work (Mount, 1965). I recommend that the range of *P. e. similis* be extended slightly south, to encompass the previously described hybrid zone between *P. e. similis* and *P. e. onocrepis* (Mount, 1975). This would result in a monophyletic *P. e. similis* that is also a distinct population. *P. e. onocrepis* as currently described is more complex. There are multiple lineages of this subspecies: individuals from central Florida being more closely related to *P. e. similis* and *P. e. insularis*, while southern individuals from Indian River along the Atlantic Coast are more closely related to *P. e. egregius*. There are individuals in this subspecies which exhibit a high degree of admixture between multiple populations, namely individuals from Indian River, Lake County, and Citrus Country. These are also sites where few individuals were sampled and apparent admixture may be a result of small sample size. Without more information I hesitate to split *P. e. onocrepis* into two subspecies, but recommend that more research be done and that those sites with small sample sizes be a focus.

Conservation

This work has significant conservation implications. Recently, *P. e. egregius* and *P. e. insularis* were both under review by USFWS to determine if a petition to list was warranted (U.S. Fish and Wildlife Service, 2015). In 2017, USFWS decided *P. e. egregius* did not warrant a petition to list, but no decision has been made regarding *P. e. insularis* (U.S. Fish and Wildlife Service, 2017). I described six ESUs within the species, closely corresponding to current subspecies descriptions, specifically *P. e. egregius* and *P. e. insularis* are each ESUs. I also found evidence that in these insular subspecies, islands are acting as barriers to gene flow and that on each island, individuals form a distinct population. Additionally, I found that individual heterozygosity was significantly lower in the island individuals than on the mainland. Maintaining genetic diversity is

key for populations to be able to sustain through changing environmental conditions (Frankham *et al.*, 2014). The insular subspecies are most likely to be impacted by future climate change and sea level rise, yet have the lowest genetic diversity.

Future directions

There are many directions this project could take into the future. As mentioned above, there is some evidence for multiple lineages within *P. egregius*, but lacking robust ecological studies there is not enough information to determine if *P. egregius* might be better characterized as multiple species. Therefore one clear line of research would be to examine the life history and ecology of the species in detail. In addition to that, another line of inquiry prompted by the taxonomic discussion would be to examine the Central Florida and Atlantic Coast populations of this species further. If more sampling could be done in these regions we may be able to gain a clearer depiction of the population structure.

Specifically, I plan to add a bootstrapping approach to the statistical phylogeography. In order to generate confidence intervals of Robinson-Foulds (RF) metric for each phylogenetic hypothesis, I will first randomly choose two individuals from each major clade in the large unconstrained tree. I will then infer new constrained and unconstrained trees using this small set of individuals and again compare each constrained tree to the unconstrained tree using the RF metric. Repeating this process with many sets of individuals will generate a set of RF metrics for each hypothesis, so that I could test for significant differences between the sets of RF metrics. Additionally, in this approach the RF metric wouldn't be inflated by closely related individuals with hard to resolve relationships. One other possible direction would be to incorporate demographic modeling into the statistical phylogeography. The relatively new method of temporally dynamic species distribution modeling (Knowles and Alvarado-Serrano, 2010; Brown and Knowles, 2012) could be used to examine how the distribution of *P. egregius* has changed since the MPWP. By coupling *P. egregius* phylogenetic

history with its past distribution changes and the specific time those changes occurred, I could more explicitly determine how geographic processes have effected *P. egregius* evolutionary history.

APPENDIX : SUPPLEMENTAL TABLES AND FIGURES

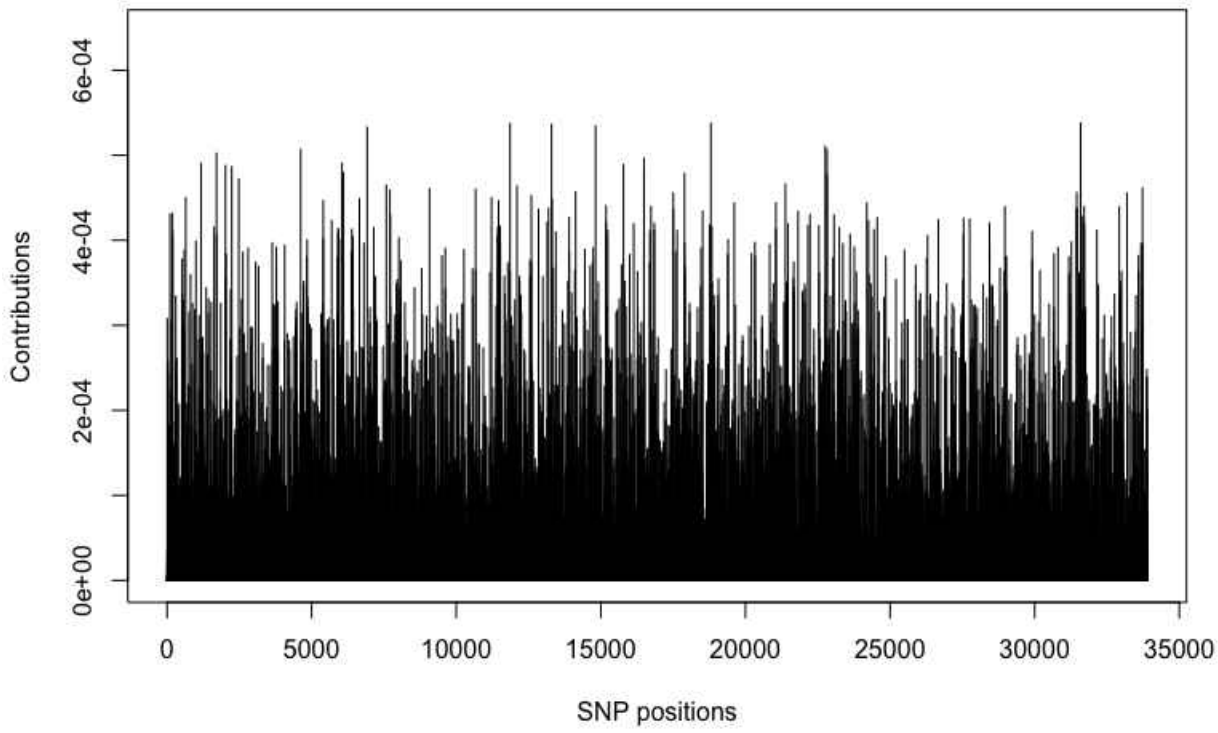

Figure S1: Contribution of each allele to the PCA of SNP loci

Table S1: Individual ID, sampling site, and accession numbers. Subspecies designation is based on sample location. Abbreviations for sample sites as follows: Ocala National Forest (ONF), Big Pine Key (BPK), State Forest (SF), Lake Wales Ridge National Wildlife Refuge (LWR NWR)

Sample ID	Organism	Site	cyt-*b* #	ND4 #	SRA accession
CLP1851	*P. e. similis*	Liberty County	MH259329	NA	SAMN09078535
CLPT619	*P. e. onocrepis*	Orange County	MH259330	MH259408	SAMN09078536
CLPT677	*P. e. onocrepis*	ONF Central	MH259331	MH259409	SAMN09078537
CLPT678	*P. e. onocrepis*	ONF North	MH259332	MH259410	SAMN09078538
CLPT679	*P. e. lividus*	Polk County	MH259333	MH259411	SAMN09078539
CLPT687	*P. e. egregius*	BPK East	MH259334	MH259412	SAMN09078540
CLPT688	*P. e. egregius*	BPK East	MH259335	MH259413	SAMN09078541
CLPT691	*P. e. egregius*	BPK East	MH259336	MH259414	SAMN09078542
CLPT696	*P. e. egregius*	BPK East	MH259337	MH259415	SAMN09078543
CLPT699	*P. e. egregius*	BPK East	MH259338	MH259416	SAMN09078544
CLPT701	*P. e. egregius*	BPK West	MH259339	MH259417	SAMN09078545
CLPT702	*P. e. egregius*	BPK East	MH259340	MH259418	SAMN09078546
CLPT703	*P. e. egregius*	BPK East	MH259341	MH259419	SAMN09078547
CLPT706	*P. e. egregius*	BPK West	MH259342	MH259420	SAMN09078548
CLPT707	*P. e. egregius*	BPK West	MH259343	MH259421	SAMN09078549
CLPT708	*P. e. egregius*	BPK West	MH259344	MH259422	SAMN09078550
CLPT709	*P. e. egregius*	BPK West	MH259345	MH259423	SAMN09078551
CLPT710	*P. e. egregius*	BPK East	MH259346	MH259424	SAMN09078552
CLPT711	*P. e. egregius*	BPK East	MH259347	MH259425	SAMN09078553
CLPT712	*P. e. egregius*	BPK East	MH259348	MH259426	SAMN09078554
CLPT721	*P. e. egregius*	Bahia Honda Key	MH259349	NA	SAMN09078555
CLPT722	*P. e. egregius*	Bahia Honda Key	MH259350	MH259427	SAMN09078556
CLPT724	*P. e. egregius*	Bahia Honda Key	MH259351	MH259428	SAMN09078557
CLPT725	*P. e. egregius*	Boca Chica Key	MH259352	MH259429	SAMN09078558
CLPT727	*P. e. egregius*	Boot Key	MH259353	MH259430	SAMN09078559
CLPT728	*P. e. similis*	Liberty County	MH259354	MH259431	SAMN09078560
CLPT729	*P. e. similis*	Liberty County	MH259355	MH259432	SAMN09078561
CLPT730	*P. e. similis*	Madison County	MH259356	MH259433	SAMN09078562
CLPT731	*P. e. similis*	Madison County	MH259357	MH259434	SAMN09078563
CLPT732	*P. e. similis*	Madison County	MH259358	MH259435	SAMN09078564
CLPT733	*P. e. similis*	Jennings SF	MH259359	MH259436	SAMN09078565
CLPT734	*P. e. similis*	Jennings SF	MH259360	MH259437	SAMN09078566
CLPT735	*P. e. similis*	Jennings SF	MH259361	MH259438	SAMN09078567
CLPT740	*P. e. onocrepis*	Clay County	MH259362	MH259439	SAMN09078568
CLPT744	*P. e. onocrepis*	Clay County	MH259363	MH259440	SAMN09078569
CLPT745	*P. e. onocrepis*	Clay County	MH259364	MH259441	SAMN09078570
CLPT747	*P. e. insularis*	Scale Key	MH259365	MH259442	SAMN09078571

CLPT748	*P. e. insularis*	Scale Key	MH259366	MH259443	SAMN09078572
CLPT749	*P. e. insularis*	Scale Key	MH259367	MH259444	SAMN09078573
CLPT750	*P. e. insularis*	Seahorse Key	MH259368	MH259445	SAMN09078574
CLPT751	*P. e. insularis*	Seahorse Key	MH259369	MH259446	SAMN09078575
CLPT752	*P. e. insularis*	Seahorse Key	MH259370	MH259447	SAMN09078576
CLPT754	*P. e. insularis*	North Key	MH259371	MH259448	SAMN09078577
CLPT755	*P. e. insularis*	North Key	MH259372	MH259449	SAMN09078578
CLPT758	*P. e. insularis*	North Key	MH259373	MH259450	SAMN09078579
CLPT759	*P. e. insularis*	North Key	MH259374	MH259451	SAMN09078580
CLPT760	*P. e. insularis*	North Key	MH259375	MH259452	SAMN09078581
CLPT761	*P. e. insularis*	North Key	MH259376	MH259453	SAMN09078582
CLPT769	*P. e. onocrepis*	ONF South	MH259377	MH259454	SAMN09078583
CLPT770	*P. e. onocrepis*	ONF South	MH259378	MH259455	SAMN09078584
CLPT773	*P. e. onocrepis*	ONF South	MH259379	MH259456	SAMN09078585
CLPT774	*P. e. onocrepis*	ONF North	MH259380	MH259457	SAMN09078586
CLPT775	*P. e. onocrepis*	Lake County	MH259381	MH259458	SAMN09078587
CLPT776	*P. e. onocrepis*	Lake County	MH259382	MH259459	SAMN09078588
CLPT777	*P. e. onocrepis*	Summerfield	MH259383	MH259460	SAMN09078589
CLPT782	*P. e. onocrepis*	ONF Central	MH259384	MH259461	SAMN09078590
CLPT783	*P. e. onocrepis*	ONF Central	MH259385	MH259462	SAMN09078591
CLPT784	*P. e. onocrepis*	ONF Central	MH259386	MH259463	SAMN09078592
CLPT785	*P. e. onocrepis*	ONF Central	MH259387	MH259464	SAMN09078593
CLPT789	*P. reynoldsi*	Outgroup	MH259388	MH259465	SAMN09078594
CLPT796	*P. e. onocrepis*	ONF South	MH259389	MH259466	SAMN09078595
CLPT798	*P. reynoldsi*	Outgroup	MH259390	MH259467	SAMN09078596
CLPT803	*P. reynoldsi*	Outgroup	MH259391	MH259468	SAMN09078597
CLPT804	*P. reynoldsi*	Outgorup	MH259392	MH259469	SAMN09078598
CLPT807	*P. e. egregius*	Long Key	MH259393	MH259470	SAMN09078599
CLPT817	*P. e. onocrepis*	Citrus County	MH259394	MH259471	SAMN09078600
CLPT818	*P. e. onocrepis*	Citrus County	MH259395	MH259472	SAMN09078601
CLPT822	*P. e. onocrepis*	Alachua County	MH259396	MH259473	SAMN09078602
EEL02	*P. e. lividus*	Archbold	MH259397	MH259474	SAMN09078603
EEL15	*P. e. lividus*	LWR NWR	MH259398	MH259475	SAMN09078604
EEL17	*P. e. lividus*	Jack Creek	MH259399	MH259476	SAMN09078605
EEL18	*P. e. lividus*	Sylvan Shores	MH259400	MH259477	SAMN09078606
EEL26	*P. e. lividus*	Sylvan Shores	MH259401	MH259478	SAMN09078607
EEL32	*P. e. lividus*	Jack Creek	MH259402	MH259479	SAMN09078608
EEL34	*P. e. lividus*	Sylvan Shores	MH259403	MH259480	SAMN09078609
EEL35	*P. e. lividus*	Jack Creek	MH259404	MH259481	SAMN09078610
UF177605	*P. e. onocrepis*	Indian River	MH259405	MH259482	SAMN09078611
UF177606	*P. e. onocrepis*	Indian River	MH259406	MH259483	SAMN09078612
UF177608	*P. e. onocrepis*	Indian River	MH259407	MH259484	SAMN09078613

Table S2: mtDNA partitions used in phylogenetic analysis and model of evolution used for each partition.

Partition	Best Model	# Sites
cyt-b_1	K80+I+G	381
$ND4_1$	HKY+I+G	227
cyt-b_2, $ND4_2$	GTR+I+G	607
cyt-b_3, $ND4_3$	GTR+G	607
$tRNA^{His}$, $tRNA^{Leu}$	HKY+I+G	101
$tRNA^{Ser}$	JC+I+G	67

REFERENCES

Arevalo, E., Davis, S.K. and Sites Jr., J.W. (1994) Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in Central Mexico. *Systematic Biology* **43**, 387–418.

Avise, J.C. (2000) *Phylogeography: The history and formation of species*. Harvard University Press, Cambridge, Massachusettes.

Avise, J.C., Arnold, J., Ball, R.M., Bermingham, E., Lamb, T., Neigel, J.E., Reeb, C.A. and Saunders, N.C. (1987) Intraspecific phylogeography: The mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics* **18**, 489–522.

Avise, J.C. and Hamrick, J.L. (Eds.) (2001) *Conservation genetics: case histories from nature*. Kluwer Academic Publishers, Norwell, MA.

Babik, W., Branicki, W., Crnobrnja-Isailović, J., Coglniceanu, D., Sas, I., Olgun, K., Poyarkov, N.A., Garcia-París, M. and Arntzen, J.W. (2005) Phylogeography of two European newt species - Discordance between mtDNA and morphology. *Molecular Ecology* **14**, 2475–2491.

Baird, N.A., Etter, P.D., Atwood, T.S., Currey, M.C., Shiver, A.L., Zachary, A.L., Selker, E.U., Cresko, W.A. and Johnson, E.A. (2008) Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PLoS ONE* **3**, 1–7.

Baird, S.F. (1858) Description of new genera and species of North American lizards in the museum of the Smithsonian Institution. *Proceedings of The Academy of Natural Sciences* , 253–256.

Barley, A.J., White, J., Diesmos, A.C. and Brown, R.M. (2013) The challenge of species delimitation at the extremes: Diversification without morphological change in philippine sun skinks. *Evolution* **67**, 3556–3572.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.H., Xie, D., Suchard, M.A., Rambaut, A. and Drummond, A.J. (2014) BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. *PLoS Computational Biology* **10**, 1–6.

Branch, L.C., Clark, A.M., Moler, P.E. and Bowen, B.W. (2003) Fragmented landscapes, habitat specificity, and conservation genetics of three lizards in Florida scrub. *Wildlife Conservation* , 199–212.

Branch, L.C. and Hokit, D.G. (2000) A comparison of scrub herpetofauna on two central florida sand ridges. *Florida Scientist* **63**, 108–117.

Brandley, M.C., Ota, H., Hikida, T., Nieto Montes De Oca, A., Fería-Ortíz, M., Guo, X. and Wang, Y. (2012) The phylogenetic systematics of blue-tailed skinks (*Plestiodon*) and the family Scincidae. *Zoological Journal of the Linnean Society* **165**, 163–189.

Brandley, M.C., Schmitz, A. and Reeder, T.W. (2005) Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of scincid lizards. *Systematic biology* **54**, 373–90.

Brandley, M.C., Wang, Y., Guo, X., Montes De Oca, A.N., Fería-Ortíz, M., Hikida, T., Ota, H., Activities, H., Brandley, M.C., Wang, Y., Guo, X., Montes De Oca, A.N., Fería-Ortíz, M., Hikida, T. and Ota, H. (2011) Accommodating heterogenous rates of evolution in molecular divergence dating methods: An example using intercontinental dispersal of *Plestiodon* (*Eumeces*) lizards. *Systematic Biology* **60**, 3–15.

Brown, J.L. and Knowles, L.L. (2012) Spatially explicit models of dynamic histories: Examination of the genetic consequences of Pleistocene glaciation and recent climate change on the American Pika. *Molecular Ecology* **21**, 3757–3775.

Brown, J.L., Weber, J.J., Alvarado-Serrano, D.F., Hickerson, M.J., Franks, S.J. and Carnaval, A.C. (2016) Predicting the genetic consequences of future climate change: The power of coupling spatial demography, the coalescent, and historical landscape changes. *American Journal of Botany* **103**, 153–163.

Brown, W.M., George, M. and Wilson, A.C. (1979) Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences of the United States of America* **76**, 1967–1971.

Brumfield, R.T., Beerli, P., Nickerson, D.A. and Edwards, S.V. (2003) The utility of single nucleotide polymorphisms in inferences of population history. *Trends in Ecology and Evolution* **18**, 249–256.

Burbrink, F.T., Fontanella, F., Alexander Pyron, R., Guiher, T.J. and Jimenez, C. (2008) Phylogeography across a continent: The evolutionary and demographic history of the North American racer (Serpentes: Colubridae: *Coluber constrictor*). *Molecular Phylogenetics and Evolution* **47**, 274–288.

Burbrink, F.T., Lawson, R. and Slowinski, J.B. (2000) Mitochondrial DNA phylogeography of the polytypic North American rat snake (*Elaphe obsoleta*): a critique of the subspecies concept. *Evolution* **54**, 2107–2118.

Bushnell, B. (2014) BBMap: a fast, accurate, splice-aware aligner. Technical report.

Cariou, M., Duret, L. and Charlat, S. (2013) Is RAD-seq suitable for phylogenetic inference? An in silico assessment and optimization. *Ecology and Evolution* **3**, 846–852.

Carr, A.F. (1940) A contribution to the herpetology of Florida. *University of Florida: Biological Science Series* **3**, 1–119.

Chamberlin, T.C. (1890) The Method of Multiple Working Hypotheses. *Science* **15**, 92–96.

Christman, S.P. (1992) Rare and Endagered Biota of Florida, volume 3. pp. 117–122.

Cope, E.D. (1871) Catalogue of Reptilia and Batrachia obtained by C. J. Maynard in Florida. *Annual Report of the Trustees of the Peabody Academy of Science* , 82–84.

Cope, E.D. (1875) Check-list of North American Batrachia and Reptilia. *Bulletin of the United States National Museum* .

Cope, E.D. (1900) *The Crocodilians, Lizards and Snakes of North America*.

Corander, J., Marttinen, P. and Mäntyniemi, S. (2006) A Bayesian method for identification of stock mixtures from molecular marker data. *Fishery Bulletin* **104**, 550–558.

Corander, J., Marttinen, P., Sirén, J. and Tang, J. (2008) Enhanced Bayesian modelling in BAPS software for learning genetic structures of populations. *BMC bioinformatics* **9**, 539.

Crews, S.C. and Hedin, M. (2006) Studies of morphological and molecular phylogenetic divergence in spiders (Araneae: *Homalonychus*) from the American southwest, including divergence along the Baja California Peninsula. *Molecular Phylogenetics and Evolution* **38**, 470–487.

Crisp, M.D., Trewick, S.A. and Cook, L.G. (2011) Hypothesis testing in biogeography. *Trends in Ecology and Evolution* **26**, 66–72.

Cronin, M.A., Amstrup, S.C., Garner, G.W. and Vyse, E.R. (1991) Interspecific and intraspecific mitochondrial DNA variation in North American bears (*Ursus*).

Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., McVean, G. and Durbin, R. (2011) The variant call format and VCFtools. *Bioinformatics* **27**, 2156–2158.

Davey, J.W. and Blaxter, M.L. (2010) RADSeq: next-generation population genetics. *Briefings in Functional Genomics* **9**, 416–423.

Dayrat, B. (2005) Towards integrative taxonomy. *Biological Journal of the Linnean Society* **85**, 407–415.

De Queiroz, K. (2007) Species concepts and species delimitation. *Systematic Biology* **56**, 879–886.

Deyrup, M. (1996) Two New Grasshoppers from Relict Uplands of Florida (Orthoptera: Acrididae). *Transactions of the American Entomological Society* **122**, 199–211.

Dibattista, J.D., Rocha, L.A., Craig, M.T., Feldheim, K.A. and Bowen, B.W. (2012) Phylogeography of two closely related indo-pacific butterflyfishes reveals divergent evolutionary histories and discordant results from mtDNA and microsatellites. *Journal of Heredity* **103**, 617–629.

Dutton, A., Carlson, A.E., Long, A.J., Milne, G.A., Clark, P.U., DeConto, R., Horton, B.P., Rahmstorf, S. and Raymo, M.E. (2015) Sea-level rise due to polar ice-sheet mass loss during past warm periods. *Science* **349**, 153–162.

Earl, D.A. and VonHoldt, B.M. (2012) STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* **4**, 359–361.

Elliott, L.P. and Brook, B.W. (2007) Revisiting Chamberlin: Multiple Working Hypotheses for the 21st Century. *BioScience* **57**, 608–614.

Ellsworth, D.L., Honeycutt, R.L., Silvy, N.J., Bickham, J.W. and Klimstra, W.D. (1994) Historical biogeography and contemporary patterns of mitochondrial DNA variation in White-Tailed Deer from the Southeastern United States. *Evolution* **48**, 122–136.

Evanno, G., Regnaut, S. and Goudet, J. (2005) Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology* **14**, 2611–2620.

Excoffier, L. and Lischer, H.E.L. (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**, 564–567.

Faircloth, B.C. and Glenn, T.C. (2014) Protocol: Preperation of an AMPure XP substitute (AKA Serapure). Technical report.

Florida Fish and Wildlife Conservation Commission (2016) Florida's Imperiled Species Management Plan: 2016-2026 .

Frankham, R. (1995) Conservation Genetics. *Annual Review of Genetics* **29**, 305–327.

Frankham, R., Bradshaw, C.J. and Brook, B.W. (2014) Genetics in conservation management: Revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. *Biological Conservation* **170**, 56–63.

Fry, A.J. and Zink, R.M. (1998) Geographic analysis of nucleotide diversity and song sparrow (Aves: Emberizidae) population history. *Molecular ecology* **7**, 1303–13.

Goudet, J.J. (2005) HIERFSTAT , a package for R to compute and test hierarchical F-statistics. *Molecular Ecology Notes* **5**, 184–186.

Griffith, H., Ngo, A. and Murphy, R.W. (2000) A cladistic evaluation of the cosmopolitan genus *Eumeces* Wiegmann (Reptilia, Squamata, Scincidae). *Russian Journal of Herpetology* **7**, 1–16.

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W. and Gascuel, O. (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Systematic Biology* **59**, 307–321.

Hearty, P.J., Kindler, P., Cheng, H. and Edwards, R.L. (1999) A +20 m middle Pleistocene sea - level highstand (Bermuda and the Bahamas) due to partial collapse of Antartic ice. *Geology* **27**, 375–378.

Hewitt, G.M. (2004) Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society B: Biological Sciences* **359**, 183–195.

Jombart, T. (2008) Adegenet: A R package for the multivariate analysis of genetic markers. *Bioinformatics* **24**, 1403–1405.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. and Drummond, A. (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**, 1647–1649.

Knowles, L.L. (2004) The burgeoning field of statistical phylogeography. *Journal of Evolutionary Biology* **17**, 1–10.

Knowles, L.L. and Alvarado-Serrano, D.F. (2010) Exploring the population genetic consequences of the colonization process with spatio-temporally explicit models: Insights from coupled ecological, demographic and genetic models in montane grasshoppers. *Molecular Ecology* **19**, 3727–3745.

Kurita, K. and Hikida, T. (2014) Divergence and Long-Distance Overseas Dispersals of Island Populations of the Ryukyu Five-Lined Skink, Plestiodon Marginatus (Scincidae: Squamata), in the Ryukyu Archipelago, Japan, as Revealed by Mitochondrial DNA Phylogeography. *Zoological Science* **31**, 187–194.

Kurita, K. and Toda, M. (2017) The role of ecological factors in determining phylogeographic and population genetic structure of two sympatric island skinks (Plestiodon kishinouyei and P. stimpsonii). *Genetica* **145**, 223–234.

Lamb, T., Justice, T.C., Brewer, M.S., Moler, P.E., Hopkins, H. and Bond, J.E. (2018) A biogeographical profile of the sand cockroach *Arenivaga floridensis* and its bearing on origin hypotheses for Florida scrub biota. *Ecology and Evolution* , 1–13.

Lanfear, R., Calcott, B., Ho, S.Y.W. and Guindon, S. (2012) PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* **29**, 1695–1701.

Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T. and Calcott, B. (2017) Partitionfinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* **34**, 772–773.

Lawrance, M.F., Mercier, K.P., Solomon, J.A., Walters, L.J. and Parkinson, C.L. (2017) Genetic diversity and population structure of oysters in Apalachicola Bay, Florida. *Florida Scientist* **80**, 145–150.

Leaché, A.D. and Cole, C.J. (2007) Hybridization between multiple fence lizard lineages in an ecotone: Locally discordant variation in mitochondrial DNA, chromosomes, and morphology. *Molecular Ecology* **16**, 1035–1054.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G. and Durbin, R. (2009) The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**, 2078–2079.

Li, M., Tian, S., Jin, L., Zhou, G., Li, Y., Zhang, Y., Wang, T., Yeung, C.K.L., Chen, L., Ma, J., Zhang, J.J., Jiang, A., Li, J., Zhou, C., Zhang, J.J., Liu, Y., Sun, X., Zhao, H., Niu, Z., Lou, P., Xian, L., Shen, X., Liu, S., Zhang, S., Zhang, M., Zhu, L., Shuai, S., Bai, L., Tang, G., Liu, H., Jiang, Y., Mai, M., Xiao, J., Wang, X., Zhou, Q., Wang, Z., Stothard, P., Xue, M., Gao, X., Luo, Z., Gu, Y., Zhu, H., Hu, X., Zhao, Y., Plastow, G.S., Wang, J., Jiang, Z., Li, K., Li, N., Li, X. and Li, R. (2013) Genomic analyses identify distinct patterns of selection in domesticated pigs and Tibetan wild boars. *Nat Genet* **45**, 1431–1438.

Loarie, S.R., Duffy, P.B., Hamilton, H., Asner, G.P., Field, C.B. and Ackerly, D.D. (2009) The velocity of climate change. *Nature* **462**, 1052–1055.

McConkey, E.H. (1957) The subspecies of *Eumeces egregius*, a lizard of the southeastern United States. Technical report.

McGuire, J.A., Linkem, C.W., Koo, M.S., Hutchison, D.W., Lappin, A.K., Orange, D.I., Lemos-Espinal, J., Riddle, B.R. and Jaeger, J.R. (2007) Mitochondrial introgression and incomplete lineage sorting through space and time: Phylogenetics of crotaphytid lizards. *Evolution* **61**, 2879–2897.

Mims, M.C., Hulsey, C.D., Fitzpatrick, B.M. and Streelman, J.T. (2010) Geography disentangles introgression from ancestral polymorphism in Lake Malawi cichlids. *Molecular Ecology* **19**, 940–951.

Moritz, C. (1994) Defining 'Evolutionarily Significant Units' for conservation. *Trends in Ecology and Evolution* **9**, 373–375.

Mount, R.H. (1963) The Natural History of the Red-Tailed Skink, *Eumeces egregius* Baird. *American Midland Naturalist* **70**, 356–385.

Mount, R.H. (1965) Variation and systematics of the scincoid lizard, Eumeces egregius (Baird). *Bulletin of the Florida State Museum* **9**, 184–212.

Mount, R.H. (1975) *Reptiles and Amphibians of Alabama*. University Alabama Press.

Parmesan, C. and Gary, Y. (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**, 37–42.

Penney, K.M. (2001) *Factors affecting translocation success and estimates of dispersal and movement patterns of the sand skink Neoceps reynoldsi on restored scrub*. Ph.D. thesis, University of South Florida.

Pennock, D.S. and Dimmick, W.W. (1997) Critique of the Evolutionarily Significant Unit as a definition for "Distinct Population Segments" under the U.S. Endangered Species Act. *Conservation Biology* **11**, 611–619.

Peters, J.L., Lavretsky, P., DaCosta, J.M., Bielefeld, R.R., Feddersen, J.C. and Sorenson, M.D. (2016) Population genomic data delineate conservation units in mottled ducks (*Anas fulvigula*). *Biological Conservation* **203**, 272–281.

Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S. and Hoekstra, H.E. (2012) Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS ONE* **7**.

Platt, J.R. (1964) Strong Inference. *Science* **146**, 347–353.

Pritchard, J.K., Stephens, M. and Donnelly, P. (2000) Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959.

R Core Team (2017) R Development Core Team. URL https://www.r-project.org/.

Rambaut, A., Drummond, A.J. and Suchard, M.A. (2013) Tracer v1.6.

Raymo, M.E., Mitrovica, J.X., O'Leary, M.J., DeConto, R.M. and Hearty, P.J. (2011) Departures from eustasy in Pliocene sea-level records. *Nature Geoscience* **4**, 328–332.

Remington, C.L. (1968) Suture-Zones of hybrid interaction between recently joined biotas. In: *Evolutionary Biology*. pp. 321–428.

Revell, L.J. (2012) phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* **3**, 217–223.

Richmond, G.M. and Fullerton, D.S. (1986) Summation of quarternary glaciations in the United States of America. *Quaternary Science Reviews* **5**, 183–196.

Roy, K., Valentine, J.W., Jablonski, D. and Kidwell, S.M. (1996) Scales of climatic variability and time averaging in Pleistocene biotas: Implications for ecology and evolution. *Trends in Ecology and Evolution* **11**, 458–463.

Roy, K. and Peltier, W.R. (2015) Glacial isostatic adjustment, relative sea level history and mantle viscosity: reconciling relative sea level model predictions for the U.S. East coast with geological constraints. *Geophysical Journal International* **201**, 1156–1181.

Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E. and Sánchez-Gracia, A. (2017) DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular Biology and Evolution* .

Rubinoff, D. and Sperling, F.A. (2004) Mitochondrial DNA sequence, morphology and ecology yield contrasting conservation implications for two threatened buckmoths (Hemileuca: Saturniidae). *Biological Conservation* **118**, 341–351.

Russello, M.A., Waterhouse, M.D., Etter, P.D. and Johnson, E.A. (2015) From promise to practice: pairing non-invasive sampling with genomics in conservation. *PeerJ* **3**, e1106.

Ryder, O.A. (1986) Species conservation and systematics: the dilemma of subspecies. *Trends in Ecology & Evolution* **1**, 9–10.

Schaefer, G.C. and Roeding, C.E. (1973) Evidence for vaginal sperm storage in the mole skink, Eumeces egregius. *Copeia* **1973**, 346–347.

Scheet, P. and Stephens, M. (2006) A fast and flexible statistical model for large-scale population genotype data: Applications to inferring missing genotypes and haplotypic phase. *The American Journal of Human Genetics* **78**, 629–644.

Schmitz, A., Mausfeld, P. and Embert, D. (2004) Molecular studies on the genus *Eumeces* Wiegmann, 1834: Phylogenetic relationships and taxonomic implications. *Hamadryad* **28**, 73–89.

Schrey, A.W., Ashton, K.G., Heath, S., Mushinsky, H.R. and McCoy, E.D. (2012) Range-wide genetic analysis of the threatened Bluetail Mole Skink identifies similar genetic structure with sympatric lizards. *Journal of Herpetology* **46**, 241–247.

Smith, H.M. (2005) *Plestiodon*: A replacement name for most members of the genus *Eumeces* in North America. *Journal of Kansas Herpetology* **14**, 15–16.

Soltis, D.E., Morris, A.B., McLachlan, J.S., Manos, P.S., Soltis, P.S., Soltis, D.E., Morris, A.B., McLachlan, J.S., Manos, P.S. and Soltis, P.S. (2006) Comparative phylogeography of unglaciated eastern North America. *Molecular Ecology* **15**, 4261–4293.

Stamatakis, A. (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**, 1312–1313.

Strickland, J.L., Parkinson, C.L., McCoy, J.K. and Ammerman, L.K. (2014) Phylogeography of *Agkistrodon piscivorus* with Emphasis on the Western Limit of Its Range. *Copeia* **2014**, 639–649.

Tang, J., Hanage, W.P., Fraser, C. and Corander, J. (2009) Identifying currents in the gene pool for bacterial populations using an integrative approach. *PLoS Computational Biology* **5**.

Taylor, E.H. (1935) Proposed Changes in the Nomenclature of the Scincoid Lizard Genus *Eumeces*. *Transactions of the Kansas Academy of Science* **38**, 345–347.

Tushingham, A.M. and Peltier, W.R. (1991) Ice-3G: a new global model of Late Pleistocene deglaciation based upon geophysical predictions of post-glacial relative sea level change. *Journal of Geophysical Research* **96**, 4497–4523.

Unmack, P.J., Sandoval-Castillo, J., Hammer, M.P., Adams, M., Raadik, T.A. and Beheregaray, L.B. (2017) Genome-wide SNPs resolve a key conflict between sequence and allozyme data to confirm another threatened candidate species of river blackfishes (Teleostei: Percichthyidae: *Gadopsis*). *Molecular Phylogenetics and Evolution* **109**, 415–420.

U.S. Fish and Wildlife Service (1987) Endangered and threatened wildlife and plants; determination of Threatened status for two Florida Lizards. *Federal Register* **52**, 42658–42662.

U.S. Fish and Wildlife Service (2015) Endangered and threatened wildlife and plant; 90-day findings on 31 petitions. *Federal Register* **80**, 37568–37579.

U.S. Fish and Wildlife Service (2017) Endangered and threatened wildlife and plants; 12-month findings on petitions to list 25 species as endangered or threatened species. *Federal Register* **82**, 46618–46645.

Waits, L.P., Talbot, S.L., Ward, R.H. and Shields, G.F. (1998) Mitochondrial DNA phylogeography of the North American Brown Bear and implications for conservation. *Conservation Biology* **12**, 408–417.

Waples, R.S. (2008) Evolutionarily Significant Units, Distinct Population Segments, and the Endangered Species Act: Reply to Pennock and Dimmick. *Conservation Biology* **12**, 718–721.

Webb, S.D. (1990) Historical Biogeography. In: *Ecosystems of Florida* (eds. R.L. Myers and J.J. Ewel). University of Central Florida Press, Orlando, pp. 70–100.

Wickham, H. (2016) *ggplot2: Elegant Graphics for Data Analysis*.

Zachos, J., Pagani, M., Sloan, L., Thomas, E. and Billups, K. (2001) Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* **292**, 686–693.

Zhang, D.X. and Hewitt, G.M. (1996) Nuclear integrations: Challenges for mitochondrial DNA markers. *Trends in Ecology & Evolution* **11**, 247–251.