Spring 2022

Florida Sand Skink and Blue-tailed Mole Skink: Expanding Geographic Coverage of Genetic Analysis for Conservation

Emma Simpson

FLORIDA SAND SKINK AND BLUE-TAILED MOLE SKINK: EXPANDING GEOGRAPHIC COVERAGE OF GENETIC ANALYSIS FOR CONSERVATION

by

EMMA SIMPSON

(Under the Direction of Aaron Schrey)

ABSTRACT

The Lake Wales Ridge is important scrub habitat that has been increasingly altered since the post-Columbian settlement in Florida. This loss of habitat has caused extreme anthropogenic fragmentation within the Lake Wales Ridge resulting in isolation among extant scrub patches. To expand the geographic scope of previous studies and answer questions concerning population connectivity, we characterized genetic diversity and differentiation using *cytochrome-b* and microsatellite genetic markers for two endemic skink species: the Florida Sand Skink (*Plestiodon reynoldsi*) and Blue-tailed Mole Skink (*Plestiodon egregius lividus*). Both species display historical isolation between central and southern Lake Wales Ridge regions with recent indication of isolation among geographically proximate sample locations. Results also indicate both focal species have low vagility based on genetic differentiation estimates. The Florida Sand Skink and Blue-tailed Mole Skink also shared similar patterns of genetic diversity within sample locations suggesting isolation by anthropogenic fragmentation is the largest threat facing both species.

INDEX WORDS: Florida Sand Skink, Blue-tailed Mole Skink, Population genetics, Florida scrub, *Cytochrome-b*, Microsatellite loci, Anthropogenic fragmentation, Lake Wales Ridge

FLORIDA SAND SKINK AND BLUE-TAILED MOLE SKINK: EXPANDING GEOGRAPHIC COVERAGE OF GENETIC ANALYSIS FOR CONSERVATION

by

EMMA SIMPSON

B.A., University of Northern Iowa, 2020

A Thesis Submitted to the Graduate Faculty of Georgia Southern University

in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

FLORIDA SAND SKINK AND BLUE-TAILED MOLE SKINK: EXPANDING GEOGRAPHIC COVERAGE OF GENETIC ANALYSIS FOR CONSERVATION

by

EMMA SIMPSON

Major Professor:	Aaron Schrey
Committee:	Michele Guidone
	Lance McBrayer

Electronic Version Approved:
May 2022

ACKNOWLEDGMENTS

I would like to thank those who helped make this study successful. First to my advisor Dr. Aaron Schrey, whom I am incredibly lucky to call my mentor. I am very appreciative of all you have taught me during my time at Georgia Southern University. I would also like to thank my committee members Dr. Michele Guidone and Dr. Lance McBrayer for your support and knowledge while I finished out my research. I am also grateful to those involved in field collections of all sample inviduals: Neil Halstead, Henry Mushinsky, Earl McCoy, Kyle Ashton, and Kurt Russell. I am also incredibly thankful to both my family and fiancé as they cheered me across this finish line.

TABLE OF CONTENTS

Page

ACKNOWLEDGMENTS.....2

LIST OF TABLES.....5

LIST OF FIGURES.....6

CHAPTER

1 INTRODUCTION……....7

Purpose of the Study…….....7

Molecular Conservation Genetics……...7

Florida Sand Skink Background…….9

Blue-tailed Mole Skink Background……..10

2 MATERIALS AND METHODS…….12

Sample Collection……..12

Cytochrome-b Methods……..12

Microsatellite Methods……...13

3 RESULTS…….....15

Florida Sand Skink *Cytochrome-b*…….15

Blue-tailed Mole Skink *Cytochrome-b*……...16

Florida Sand Skink Microsatellites…….16

Blue-tailed Mole Skink Microsatellites……..17

4 DISCUSSION……...30

Biogeography……..30

Population Structure……30

Genetic Diversity…….32

Scrub Species Comparison……..33

Conservation Implications……………………………………………………………34

REFERENCES…………………………………………………………………………….36

LIST OF TABLES

Page

Table 1: Florida Sand Skink Relative Haplotype Frequencies……18

Table 2: Focal Species Haplotype Diversity/Nucleotide Diversity by Sample Location……20

Table 3: *Cytochrome-b* Pairwise Comparisons for Florida Sand Skinks…….22

Table 4: Blue-tailed Mole Skinks Relative Haplotype Frequencies……23

Table 5: *Cytochrome-b* Pairwise Comparisons for Blue-tailed Mole Skinks……..26

Table 6: Genetic Diversity Estimates for Focal Species by Sample Location……27

Table 7: Microsatellite Pairwise Comparisons for Florida Sand Skinks…….28

Table 8: Microsatellite Pairwise Comparisons for Blue-tailed Mole Skinks……..29

LIST OF FIGURES

Page

Figure 1: Lake Wales Ridge Map with Sample Locations…….....14

Figure 2: Florida Sand Skink Phylogenetic Tree ……. ….....21

Figure 3: Blue-tailed Mole Skink Phylogenetic Tree…….….....25

CHAPTER 1

INTRODUCTION

Purpose of the Study

This study expands the geographic scope of previous work in order to test hypotheses concerning population connectivity for the Florida Sand Skink and Blue-tailed Mole Skink across the Lake Wales Ridge. Current objectives were to characterize genetic diversity and differentiation among individuals collected from previously unstudied sites at both *cytochrome-b* and microsatellite genetic markers. The current study aims to answer key questions such as: 1) are patterns of genetic diversity and differentiation similar to previous studies on these lizards? Previous studies on the Florida Sand Skink and Blue-tailed Mole Skink have found evidence for historical isolation and limited dispersal rates for both species (Branch et al. 2003, Schrey et al. 2012, Richmond et al. 2009). However, all studies have failed to detect significant genetic differentiation among sites even with patches isolated due to recent anthropogenic fragmentation. 2) What factors are more important in predicting genetic characteristics? Do sample locations continue to reflect historical connectivity? Or has isolation by anthropogenic fragmentation transformed genetic diversity and differentiation estimates? This data will increase the understanding of genetic population structure and infer relationships within these species over time and across geographic space.

Molecular Conservation Genetics

The Florida scrub is an important habitat for many endemic species of plants and animals. Characterized by patches of dry, sandy habitat, the Florida scrub consists of multiple ridge formations created by ancient shorelines (Telford 1959, Webb 1990, Branch et al. 2003). Although previous geographical studies recognized more than six ridges, recent studies show geological evidence for only five ridge systems in Florida (Hardin 2019). These ridge systems are recognized as Center Park Ridge/Atlantic Coastal Complex; Trail Ridge; Palatka Hill/Crescent City Ridge/DeLand Ridge; Mount Dora Ridge/Orlando Ridge; and Lake Wales Ridge. Evidence for a sixth, Bombing Range Ridge, is currently under consideration by the Florida Geological Survey (Hardin 2019). Lake Wales Ridge,

located in central Florida, is notable from other ridges due to age and plethora of biodiversity. This xeric upland region is approximately 186.3 km in length and includes Lake, Polk, Highlands and a small portion of Orange and Osceola counties (Weekley et al. 2008)

The Florida scrub has been increasingly altered by anthropogenic forces since the Post-Columbian settlement and more than 78% of land has been lost to the conversion of native habitat to residential, agricultural, and commercial use (Weekley et al. 2008). This has resulted in severe habitat fragmentation for several precinctive species, including the focal species of this study, the Florida Sand Skink (*Plestiodon reynoldsi)* and the Blue-tailed Mole Skink (*Plestiodon egregius lividus*). Due to the highly imperiled status of Florida scrub habitats, conservation studies on endemic species are of utmost importance.

Habitat disturbances can have profound effects on species that have restricted dispersal capabilities and require specialized habitats, which is true of both focal species. (Branch et al. 2003, Richmond et al. 2009, Schrey et al. 2012, and Tucker et al. 2014). Habitat fragmentation, especially anthropogenically-induced fragmentation, can be detrimental if populations become isolated; leading to alterations in genetic diversity (Branch et al. 2003). Small and/or isolated populations are susceptible to genetic drift and inbreeding which leads to loss of genetic diversity (Richmond et al. 2009). This loss of genetic diversity may decrease the ability of a species to adapt, thereby increasing the chance of extinction (Tucker et al. 2014, Domingues 2017). Many studies on genetic diversity of Florida scrub species provide insights on population diversity and differentiation and highlight specific risks associated with demographic change (Branch et al. 2003, Richmond et al. 2009, Schrey et al. 2011, Heath et al. 2012, Schrey et al. 2012, Tucker et al. 2014, and Schrey et al. 2015).

Although anthropogenic fragmentation is of increasing importance to species conservation, historical ecological barriers can also influence genetic diversity. The Lake Wales Ridge landscape has been historically patchy through fluctuating sea levels and natural wildfires (Webb 1990). Previous studies of this scrub region indicated significant genetic differentiation between central and southern Lake Wales Ridge groups (Branch et al. 2003, Richmond et al. 2009, Schrey et al. 2012). In all three studies, the

division between central and southern clades is due to decreased gene flow by an ecological barrier, Josephine Creek. The Josephine Creek is a remnant of ancient sea level changes and indicates that the central and southern Lake Wales Ridge were more distantly separated by water historically than what we see today (Telford 1959, Webb 1990). Fire disturbance also affects the Lake Wales Ridge. Fire can affect genetic diversity directly by causing changes in population size or indirectly by altering prey availability and habitat suitability (Ragsdale et al. 2016). This region is maintained by infrequent, high-intensity fire (scrubby flatwoods and oak-palmetto scrub: 5-20 years; rosemary scrub and sand pine scrub 15-100 years; Laessle 1958, Myers 1990, Menges 1999). Studies indicate recently burned patches have more open sand and live vegetation with increased light intensity compared to long unburned patches, which have increased ground cover, dead vegetation, and leaf litter biomass (Ashton and Knipps 2011; McCoy et al. 2010).

Florida Sand Skink

The Florida Sand Skink is precinctive to the xeric regions of central Florida. Found in 114 locations, the Florida Sand Skink has been identified as threatened by the U.S. Fish and Wildlife Service (USFWS 1999) and Florida Fish and Wildlife Conservation Commission (FFWCC 2007). The Florida Sand Skink prefers habitats of deep sand, free of excess plant roots with scattered shrubby vegetation (USFWS 1999). This allows the species to freely "swim" underneath the sand to capture prey. Habitat degradation and loss is the primary threat facing this species to date (USFWS 1999).

The Florida Sand Skink likely reproduces between late February and early May when females lay one clutch of two eggs underneath the sand (Telford 1959). These eggs will hatch between June and July and reach sexual maturity after 1-2 years (Telford, 1959, Sutton 1996). The Florida Sand Skink feeds underground, at the leaf litter surface interface, primarily on beetle larvae and termites (Sutton 1996).

Previous studies analyzed genetic differentiation of the Florida Sand Skink finding a low dispersal distance (Branch et al. 2003, Richmond et al. 2009, and Schrey et al 2012) and strong genetic differentiation between northern and southern areas of the Lake Wales Ridge due to historical separation (Branch et al. 2003 and Schrey et al. 2012). Schrey et al. (2011) also found that fire history affects genetic

characteristics. Evidence indicates that as time since fire (TSF) increases, inbreeding decreases and genetic diversity increases (Schrey et al. 2011). However, previous studies have failed to detect genetic differentiation due to anthropogenic fragmentation in the Lake Wales Ridge. Some have hypothesized this could be due to the species' long generation time and the areas' relatively recent evolutionary time frame, suggesting previous data has not yet fully revealed the negative genetic effects of fragmentation (Richmond et al. 2009, McCoy et al. 2010).

Blue-tailed Mole Skink

The Blue-tailed Mole Skink is part of a five-species complex differentiated by coloration and morphology (*Plestiodon egregius egregius, Plestiodon egregius onocrepis, Plestiodon egregius similis, Plestiodon egregius lividus and Plestiodon egregius insularis* (Branch et al. 2003). The Blue-tailed Mole Skink is also precinctive to the xeric regions of central Florida and is categorized as threatened by the U.S. Fish and Wildlife Service (USFWS 1999) and Florida Fish and Wildlife Conservation Commission (FFWCC 2007). The Blue-tailed Mole Skink is rare even in favorable conditions such as open canopies with scattered shrub vegetation and patches of loose sand (Christman 1992). Prior to this study, only 34 locations were known for the species and their distribution appears to be linked closely with surface litter, soil moisture, and prey distribution (Christman 1992). Habitat loss and fragmentation due to conversion of native habitat to residential, commercial, and agricultural use is the largest threat facing the Blue-tailed Mole Skink (USFWS 1999).

Because of the rarity of the Blue-tailed Mole Skink, little information is known about its behavior and reproductive patterns. It is assumed the Blue-tailed Mole Skink is similar to the more common Peninsular Mole Skink, *Plestiodon egregius onocrepis,* whose mating occurs during the winter months (Mount 1963). The Peninsular Mole Skink becomes reproductively active around one year of age when they will lay three to seven eggs with an incubation time of 31-51 days (Christman 1992). Typical foraging for the Blue-tailed Mole Skink is at surface level (Christman 1992).

Despite its conservation status and increasing habitat loss, only one genetic study has been conducted specifically on the Blue-tailed Mole Skink (Schrey et al. 2012). Other studies focused on the five-species

Mole Skink complex (Branch et al. 2003 and Mercier 2018) only including a small number of Blue-tailed Mole Skink samples. Branch et al. (2003), found strong population structure existed with considerable variation in Mole Skinks that supports the separation of all five sub-species. Unfortunately, Branch et al (2003) does not address population structure and genetic diversity of the Blue-tailed Mole Skink independent of the five-species complex. Schrey et al. (2012) showed distinct genetic differentiation between Blue-tailed Mole Skink populations from the northern and southern Lake Wales Ridge with some locations having significant inbreeding (Schrey et al. 2012). There was no evidence of long-distance dispersal indicating individuals do not move among remnant patches and are likely isolated by habitat fragmentation. This study also showed high genetic diversity for the Blue-tailed Mole Skink, which is notable given the isolated nature also observed. It is possible they also have a long generation time, which causes a delay in observing the negative effects of fragmentation similar to that suggested for the Florida Sand Skink (Richmond et al. 2009, McCoy et al. 2010).

CHAPTER 2

MATERIALS AND METHODS

Sample Collection

Florida Sand Skinks (N = 128) and Blue-tailed Mole Skinks (N = 53) were collected from five Florida scrub sites along the Lake Wales Ridge: Ancient Islands (AI), Collany (C), Lake Livingston (LLIV), Lake Loralin (LLOR), and Rolling Ridge (RR) (Figure 1). To date, this is the largest sample collection of Blue-tailed Mole Skinks in a single study. Individuals were captured using 18.9-L bucket traps sunk below ground level with a square cover elevated 2cm above the bucket. Tissue samples were taken from the tail of each captured individual. DNA was extracted using Qiagen DNeasy Kit (Qiagen, Valencia, California).

Cytochrome-B Methods

Cytochrome-b was used to characterize genetic diversity and genetic differentiation among sites for the Florida Sand Skink and Blue-tailed Mole Skink. Using polymerase chain reaction (PCR), a portion of the mitochondrial DNA *cytochrome-b* gene was amplified using primers Cyb-8 and Cyb-2 (Branch et al. 2003; Schrey et al. 2012). PCR was conducted following the methods outlined in Schrey et al. (2012). PCR products were sequenced on an ABI 3130XL at the Carver Biotechnology Center at the University of Illinois at Urbana-Champaign, Urbana, Illinois. DNA sequences for all individuals were aligned with MEGA4 (Tamura et al. 2007; Schrey et al. 2012). All sequences were collapsed into haplotypes, identifying relative haplotype frequencies and shared haplotypes for all sites using FABOX v. 1.6 (Villesen, 2007).

Previously collected *cytochrome-b* data for both species were incorporated into the analyses; 15 Florida Sand Skinks from Archbold Biological Station (ABS; Schrey et al 2012), eight Florida Sand Skink haplotypes (GenBank accession numbers: AF470647-AF47054; Branch et al. 2003), and 28 Blue-tailed Mole Skink haplotypes (GenBank Accession Numbers: GQ871235-GQ871262; Schrey et al. 2009).

The geographic pattern of *cytochrome-b* variation was determined by constructing a phylogenetic tree with MEGA4 using the Maximum Likelihood method and the Kimura 2-paramater model (Kimura,

1980). The Kimura 2-parameter model was used for all *cytochrome-b* analyses so that these results would be comparable to previous work (Branch et al 2003, Schrey et al 2012). Bootstraps of 1000 replicates were used, and nodes with less than 50% support were collapsed.

ARLEQUIN v 3.11 (Excoffier et al. 2005) was used to estimate haplotype diversity (*h)* and nucleotide sequence diversity (π) for all sites. ARLEQUIN was also used to characterize genetic differentiation with the Φ_{ST} estimator F_{ST} over all sites as well as pairwise between sites. Statistical significance was estimated by permutation ($\alpha = 0.05$). Sequential Bonferroni correction was used for all pairwise comparisons.

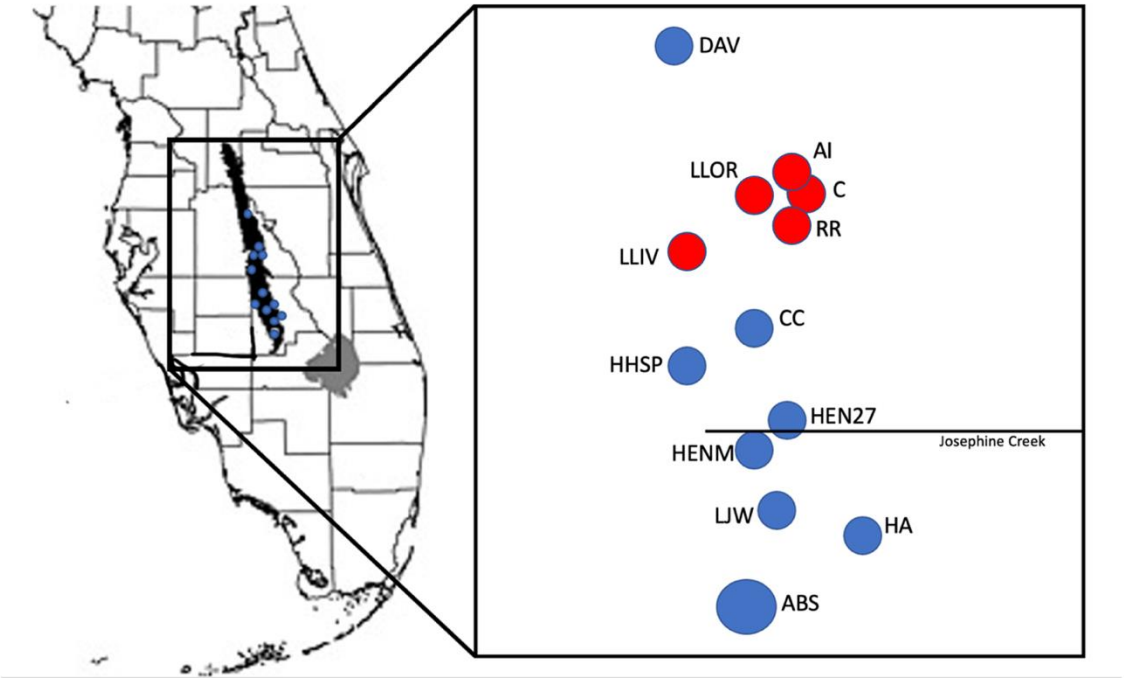
Microsatellite Methods

Seven microsatellite markers (Nr 52.2, 52.4, 52.7, 52.11, 60.5, 60.11, 60.34) developed for the Florida Sand Skink (Reid et al. 2004) were screened in the Florida Sand Skink and the Blue-tailed Mole Skink. Microsatellite loci were amplified using PCR with a final volume of 10µl following the methods outlined in Schrey et al. (2012). PCR products were then electrophoresed at the Carver Biotechnology Center at the University of Illinois at Urbana-Champaign, Urbana, Illinois. Eight previously collected Florida Sand Skinks from the Henscratch Main scrub (HENM) were incorporated into analysis (Schrey et al. 2012). Thermo-fisher's ABI online portal was used to analyze gel images and call alleles. Resultant allele size data were plotted and binned to specific allele categorized.

Genetic diversity was characterized by expected heterozygosity (H_e), observed heterozygosity (*Ho*), inbreeding coefficient (F_{IS}), and mean pairwise relatedness (MPR; Queller and Goodnight 1989) calculated at all sites with more than five individuals (FSS: AI, C, LLIV, LLOR, RR, HENM; BMS: AI, C, LLOR) using GENALEX6 (Peakall and Smouse 2012).

Genetic differentiation was estimated among sites with more than five individuals. The θ estimator of F_{ST} was calculated over sites and pairwise between sites using GENALEX6. Statistical significance was estimated by permutation ($\alpha = 0.05$). Sequential Bonferroni correction was used for all pairwise comparisons.

Figure 1: Map with locations for the Florida Sand Skink and Blue-tailed Mole Skink. Current study sites are in red; previous study sites are in blue. Site abbreviations are provided in text. Note: Location symbols are exaggerated for viewing, all study sites are extremely small and completely isolated from one another.



CHAPTER 3

RESULTS

Florida Sand Skink Cytochrome-b

The Florida Sand Skink analysis resulted in a 218-bp portion of *cytochrome-b* aligned from 55 collected individuals and 8 GenBank sequences. Thirty-three variable positions generated 22 unique haplotypes. DNA sequences for each observed haplotype will be deposited to GenBank**.** Multiple haplotypes were observed in all sites except SSr25 and SSr55 (Table 1). Only three haplotypes were shared among sites (Table 1); one between central sites AI, C, LLOR, & RR; and two between southern sites SSr1S & SSr25 and SSr1S & SSr91. Three haplotypes were also shared among GenBank population indicating all samples come from the same genetic pool with differences resulting from geographic location. Among sites, h ranged from 0.000 to 0.905 (Table 2), with the lowest h at sites SSr25 and SSr55. Among sites, π ranged from 0.000 to 0.004 (Table 2).

The Florida Sand Skink phylogenetic analysis resulted in a tree with clear partitioning of samples into two group (Figure 2). Groups are consistent with a division between central Lake Wales Ridge group (AI, C, LLOR, LLIV, and RR) and southern Lake Wales Ridge groups (SSr25, SSr55, SSr91, and SSr1S). The division between central and southern Lake Wales Ridge groups occurred between RR & SSr55 (Figure 1).

Significant genetic differentiation was detected in the Florida Sand Skink with *cytochrome-b*. Overall estimate of Φ_{ST} was 0.665 ($p < 0.001$). Pairwise Φ_{ST} ranged from 0.002 to 1.000 (Table 3). Twelve comparisons were significant. Eight comparisons were significant between new sites collected (AI & LLIV, C & LLIV, C & SSr25, LLIV & LLOR, LLIV & RR, LLIV & SSr25, LLOR & SSr25, and RR & SSr25) and four comparisons were significant between GenBank populations (AI & GB, C & GB, LLOR & GB, and RR & GB). Pairwise comparisons indicate significant differences between central and southern Lake Wales Ridge locations as well as differentiation of LLIV from other central Lake Wales Ridge sites.

Blue-Tailed Mole Skink Cytochrome-b

The Blue-tailed Mole Skink analysis resulted in a 218-bp portion of the *cytochrome-b* aligned in 51 newly sampled individuals and 28 GenBank. Thirty-nine variable positions generated 30 unique haplotypes among individuals. DNA sequences for each observed haplotype will be deposited to GenBank. Multiple haplotypes were observed in all sites except LLIV which had only two individuals (Table 4). Only three haplotypes were shared among sites (Table 4); all between centrally located sites: AI, C, & LLOR and AI, C, & LLIV. Three haplotypes were also shared among GenBank population indicated all samples come from the same genetic pool with differences resulting from geographic location. Among sites, *h* ranged from 0.000 to 0.828 (Table 2). The lowest *h* was in LLIV (0.000); all other sites ranged from 0.533 to 0.828. Among sites, π ranged from 0.000 to 0.011 (Table 2).

The phylogenetic analysis of the Bluetail Mole Skinks also resulted in a tree with obvious partitioning of samples into two groups (Figure 3). Lineages are consistent with a division between central Lake Wales Ridge group (AI, C, LLOR, LLIV, DAV, CC, HEN27, and HHSP) and southern Lake Wales Ridge group (HENM, LJW, HA, and ABS). The division between central and southern Lake Wales Ridge groups occurred near Josephine Creek, which runs immediately between HEN27 and HENM. However, one haplotype from north of Josephine Creek (HEN27) grouped in the southern cluster. The southern Lake Wales Ridge group had more strongly supported additional within-partition clustering compared to central Lake Wales Ridge group.

Significant genetic differentiation was detected in the Blue-tailed Mole Skink with *cytochrome-b*. Overall estimate of Φ_{ST} was 0.269 ($p < 0.001$). Pairwise Φ_{ST} ranged from -0.010 to 0.685 (Table 5) with the only significant comparisons occurring between AI & GenBank and C & GenBank. The small number of significant pairwise tests could be attributable to the relatively low samples sizes for all Blue-tailed Mole Skink sites.

Florida Sand Skink Microsatellites

Seven microsatellite loci were successfully amplified in the Florida Sand Skink. Genetic diversity was variable among individuals at each site (Table 6). Expected heterozygosity was slightly higher than

observed in all but one site (HENM; Table 6). The inbreeding coefficient estimates ranged from -0.121 to 0.146; with the lowest F_{is} at HENM and highest F_{is} at AI (Table 6). The observed values of F_{is} indicate that the Florida Sand Skink have a system of mating resulting in higher inbreeding than expected by chance at three centrally located sites (AI, LLIV, and LLOR; F_{is}; p = 0.001). Mean pairwise relatedness ranged from -0.002 to 0.098 (Table 6). Significantly higher relatedness than expected by chance occurred at sites LLIV, LLOR, and HENM (Table 6).

Significant genetic differentiation was detected in the Florida Sand Skink at microsatellite loci. The overall θ was 0.021 (p = 0.001). Pairwise θ estimates ranged from 0.001-0.058 (Table 7); with six significant pairwise comparisons occurring between AI & LLIV, AI & HENM, C & HENM, LLIV & LLOR, LLIV & HENM, and LLOR & HENM. Comparisons indicate strong differentiation between central and southern groups.

Blue-tailed Mole Skink Microsatellites

Amplification in the Blue-tailed Mole Skink was successful in all seven of the microsatellite loci. Genetic diversity was similar at each site (Table 6). Expected heterozygosity was slightly higher than observed in the Blue-tailed Mole Skink (Table 6). Inbreeding coefficient estimates were all positive and ranged from 0.045-0.175 (Table 6). The F_{is} estimates indicate a higher level of inbreeding than expected by chance in the Blue-tailed Mole Skink at all centrally located sites (F_{is}; p = 0.001). AI had the lowest genetic diversity and highest F_{is}. Mean pairwise relatedness ranged from -0.022 to 0.074 (Table 6). No sites showed significantly higher MPR than expected by chance.

Significant genetic differentiation was detected in the Blue-tailed Mole Skink at microsatellite loci. The overall θ was 0.021 (p = 0.007). Pairwise θ estimates ranged from 0.015-0.039 (Table 8); with two significant comparisons; C & AI and C & LLOR. This is notable as all other comparisons fail to detect significant genetic differentiation among proximate locations in central Lake Wales Ridge.

TABLE 1: Relative haplotype (Hap) frequencies within geographic locations of Florida Sand Skinks. Sites are labeled Ancient Islands (AI), Collany (C), Lake Livingston (LLIV), Lake Loralin (LLOR), Rolling Ridge (RR), Archbold Biological Sites (SSr1S, SSr25, SSr55, SSr91), and sequences obtained from GenBank (GenBank accession numbers: AF470647-AF47054; Branch et al. 2003). Haplotype diversity (*h)* for each location is provided at the bottom.

	AI	**C**	**LLIV**	**LLOR**	**RR**	**SSr1S**	**SSr25**	**SSr55**	**SSr91**	**GenBank**
Hap										
1	0.714	0.750		0.889	0.286					
2	0.143									
3	0.143									
4		0.125								
5		0.125								
6			0.778							X
7			0.111							
8			0.111							
9				0.111						
10					0.143					
11					0.143					
12					0.143					X
13					0.286					
14						0.667	1			
15						0.333			0.333	X
16								1		
17									0.667	
18										X

Table 1: Continued

19										X
20										X
21										X
22										X
h	0.524	0.464	0.417	0.222	0.905	0.667	0.000	0.000	0.667	

TABLE 2: Haplotype diversity (*h)* and nucleotide diversity (π) estimated for the Florida Sand Skink (FSS) and Blue-tailed Mole Skink (BMS) collected from Lake Wales Ridge. Location and sample size (*N*) are reported for *cytochrome-b* estimates. Current study sites: Ancient Islands (AI), Collany (C), Lake Livingston (LLIV), Lake Loralin (LLOR), Rolling Ridge (RR). Previously studied sites: Archbold Biological Station (SSr1s, SSr25, SSr55, SSr91).

Location	***N***	***h***	***π***
FSS	63		
AI	7	0.524	0.004
C	8	0.464	0.003
LLIV	9	0.417	0.015
LLOR	9	0.222	0.001
RR	7	0.905	0.001
SSr1s	3	0.667	0.003
SSr25	6	0.000	0.000
SSr55	3	0.000	0.000
SSr91	3	0.667	0.003
GenBank	8	1.000	0.039
BMS	79		
AI	29	0.828	0.011
C	14	0.648	0.006
LLIV	2	0.000	0.000
LLOR	6	0.533	0.002
GenBank	28	0.995	0.039

FIGURE 2: Phylogenetic tree depicting the relationship among *cytochrome-b* haplotypes in the Florida Sand Skink. Haplotype number is indicated on the far right. This tree was labeled by site location that possessed the specific haplotype. Numbers at the node indicate bootstrap support of >50%.

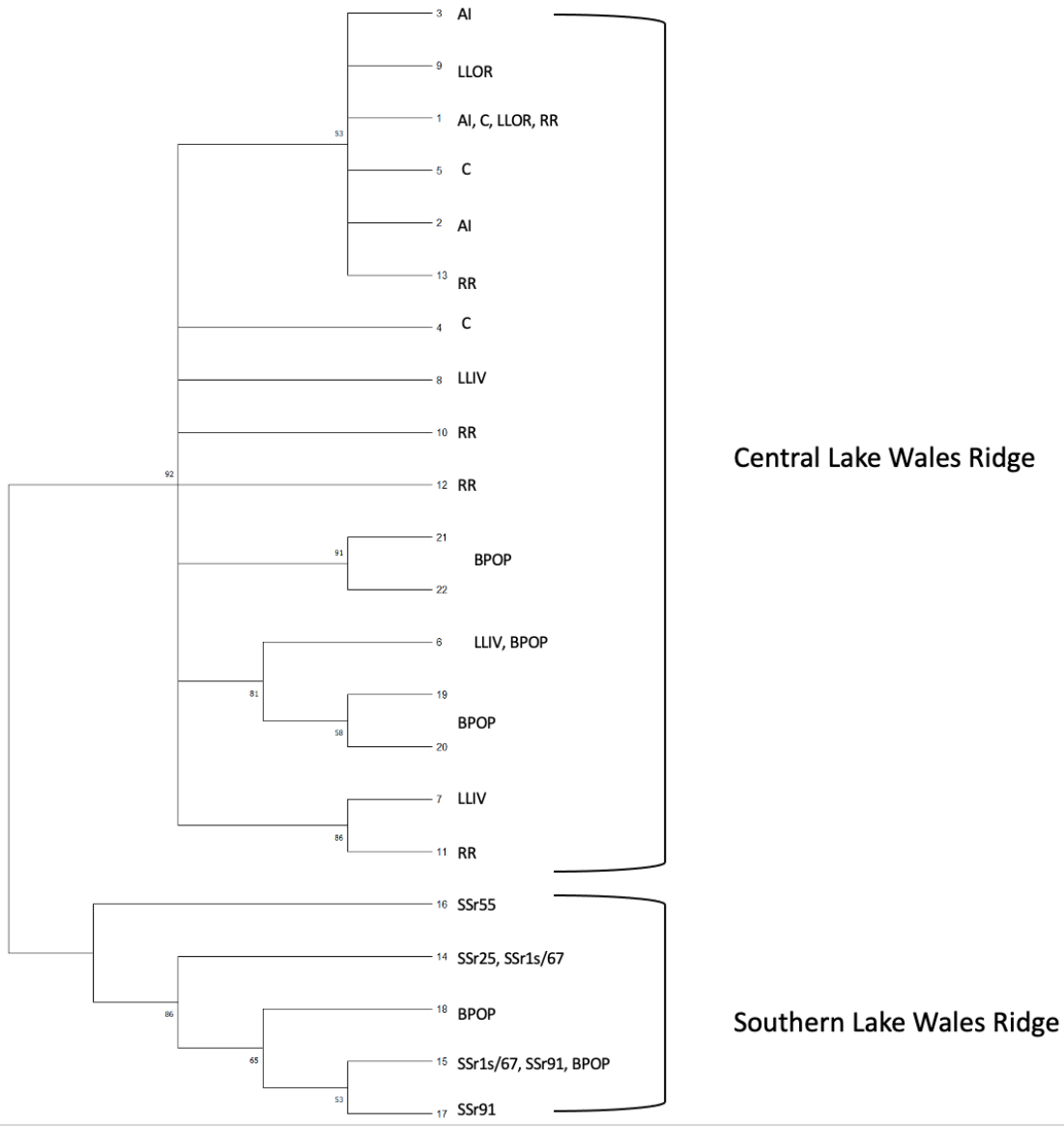


TABLE 3: Pairwise estimates of Φ_{ST} from cytochrome-b data between sites for Florida Sand Skinks (FSS). An asterisk denotes statistical significance after sequential Bonferroni correction.

	AI	**C**	**LLIV**	**LLOR**	**RR**	**SSr1s**	**SSr25**	**SSr55**	**SSr91**	**GenBank**
AI	-									
C	0.002	-								
LLIV	0.669*	0.678*	-							
LLOR	0.021	0.008	0.722*	-						
RR	0.112	0.106	0.574*	0.172	-					
SSr1s	0.911	0.919	0.719	0.964	0.797	-				
SSr25	0.946	0.950*	0.782*	0.984*	0.858*	0.250	-			
SSr55	0.927	0.934	0.767	0.979	0.810	0.924	1.000	-		
SSr91	0.923	0.930	0.755	0.969	0.825	0.502	0.880	0.942	-	
GenBank	0.343*	0.361*	0.211	0.399*	0.248	0.263	0.397	0.397	0.342	-

TABLE 4: Relative haplotype frequencies within geographic locations of Blue-tailed Skinks. Ancient Islands (AI), Collany (C), Lake Livingston (LLIV), Lake Loralin (LLOR), and sequences obtained from GenBank (GenBank Accession Numbers: GQ871235-GQ871262; Schrey et al. 2009). Haplotype diversity (*h)* for each geographic location is geographic location is provided at the bottom.

	AI	**C**	**LLIV**	**LLOR**	**DAV**	**CC**	**HA**	**ABS**	**HEN27**	**HENM**	**LJW**	**HHSP**
Hap												
1	0.241	0.214		0.667	X							
2	0.276	0.571		0.333								
3	0.034											
4	0.172	0.143	1		X							
5	0.172				X							
6	0.034											
7	0.069											
8		0.071			X							
9					X							
10					X							
11						X						
12						X						
13							X	X				
14							X					
15									X	X		
16								X				
17											X	
18												X
19												X

Table 4: Continued

20								X
21						X		X
22								X
23						X		
24							X	
25						X		
26						X		
27							X	
28							X	
29							X	
30								X
h	0.828	0.648	0.000	0.533				

FIGURE 3: Phylogenetic tree depicting the relationship among *cytochrome-b* haplotypes in the Blue-tailed Mole Skink. Haplotype number is indicated on the far right. This tree was labeled by site location that possessed the specific haplotype. Numbers at the node indicate bootstrap support of >50%.

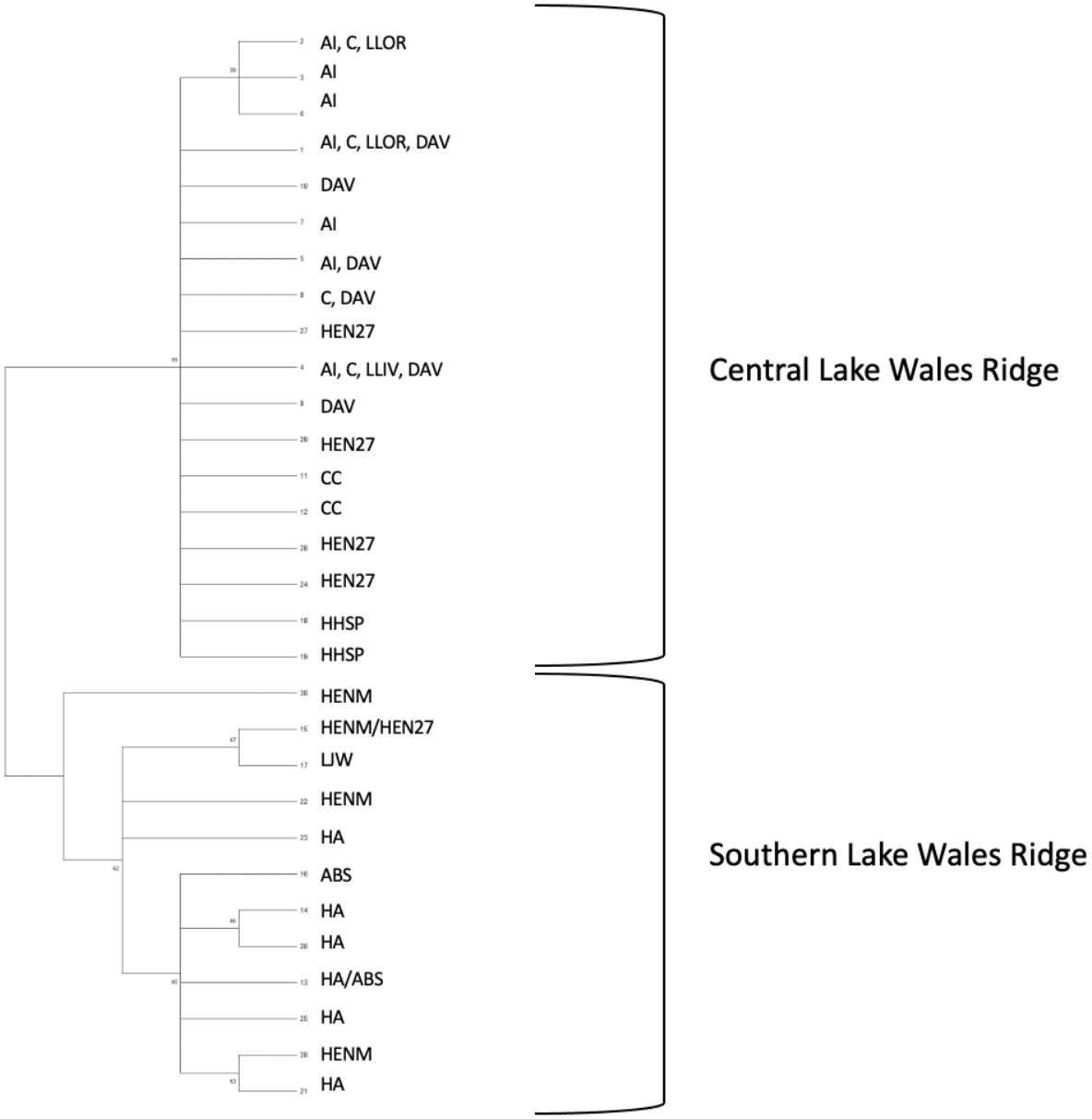


TABLE 5: Pairwise estimates of Φ_{ST} from cytochrome-b data between sites for Blue-tailed Mole Skinks. An asterisk denotes statistical significance after sequential Bonferroni correction.

	AI	**C**	**LLIV**	**LLOR**	**GenBank**
AI	-				
C	0.013	-			
LLIV	0.092	0.399	-		
LLOR	-0.010	-0.032	0.685	-	
GenBank	0.307*	0.302*	0.041	0.241	-

TABLE 6: Genetic diversity among Florida Sand Skinks (FSS) and Blue-tailed Mole Skinks (BMS) averaged across seven microsatellite loci: expected heterozygosity (H_e), observed heterozygosity (H_o), inbreeding coefficient (F_{is}), mean pairwise relatedness (MPR), number of alleles (N_a), and number of effective alleles (N_e). Location and sample size (*N)* are reported for microsatellite estimates. An asterisk denotes statistical significance after sequential Bonferroni correction.

Location	***N***	**Na**	**Ne**	**H_e**	**H_o**	**F_{is}**	**MPR**
FSS	122						
AI	41	22.714	15.714	0.942	0.794	0.146	-0.002
C	6	6.857	5.677	0.905	0.871	-0.061	0.047
LLIV	34	21.143	12.229	0.923	0.781	0.139	0.024*
LLOR	27	18.143	12.728	0.933	0.850	0.066	0.015*
RR	6	8.429	7.063	0.941	0.905	-0.061	0.011
HENM	8	7.571	6.207	0.901	0.926	-0.121	0.098*
BMS	50						
AI	31	18.429	11.234	0.894	0.734	0.175	-0.022
C	13	11.143	7.795	0.874	0.780	0.066	0.016
LLOR	6	6.571	4.949	0.838	0.762	0.045	0.074

TABLE 7: Pairwise estimates of θ_{ST} from microsatellite data between sites for Florida Sand Skinks. An asterisk denotes statistical significance after sequential Bonferroni correction.

	AI	**C**	**LLIV**	**LLOR**	**RR**	**HENM**
AI						
C	0.007					
LLIV	0.023*	0.001				
LLOR	0.006	0.011	0.020*			
RR	0.004	0.014	0.022	0.008		
HENM	0.050*	0.058*	0.051*	0.050*	0.041	

TABLE 8: Pairwise estimates of θ_{ST} from microsatellite data between sites for Blue-tailed Mole Skinks. An asterisk denotes statistical significance after sequential Bonferroni correction.

	AI	**C**	**LLOR**
AI			
C	0.020*		
LLOR	0.015	0.039*	

CHAPTER 4

DISCUSSION

Biogeography

The current data on the Florida Sand Skink and Blue-tailed Mole Skink show nearly identical biogeographic patterns. The Florida Sand Skink displayed strong partitioning into central and southern Lake Wales Ridge groups, consistent with historical isolation between the two regions. This is most likely due to ancient shore formation in Florida during the large sea level changes throughout evolutionary time (Telford 1959, Webb 1990). Stronger clustering in southern populations indicates longer-term fragmentation most likely due to smaller, more isolated patches existing historically in the southern Lake Wales Ridge. Shared haplotypes were not seen across the central-south split, further supporting historical isolation between the two regions. Among central sites, one shared haplotype occurred at four out of five locations supporting a historically large and interconnected central Lake Wales Ridge.

The Blue-tailed Mole Skink also showed strong partitioning into central and southern Lake Wales Ridge groups. This further supports the pattern of historical isolation between central and southern Lake Wales Ridge regions due to ancient shore formation. Tree topology again indicates longer-term fragmentation of southern populations with central populations displaying higher connectivity, even over relatively long distances: AI, LLOR, C, and RR and northern site: DAV. Only one haplotype was shared across the central and southern locations and occurred at the closest geographical sites to the potential dispersal barrier, Josephine Creek. This supports the historical separation of central and southern clades, with the potential for recent migration across the Josephine Creek divide. Within central Lake Wales Ridge, four haplotypes were shared among sites supporting a historical, large refugia. Current findings are congruent with previous studies on the Florida Sand Skink and Blue-tailed Mole Skink (Branch et al. 2003, Richmond et al. 2009, and Schrey et al. 2012).

Population Structure

The Florida Sand Skink and Blue-tailed Mole Skink were also similar in population structure. Notably, data for both species indicate more recent changes in central Lake Wales Ridge connectivity

most likely due to anthropogenic fragmentation of the Florida Scrub. Florida Sand Skink pairwise comparisons support historic genetic differentiation between central and southern Lake Wales Ridge. Within the southern Lake Wales Ridge, there is no genetic differentiation detected based on Φ_{ST} pairwise comparisons. This is most likely due to all the southern locations in the current study coming from Archbold Biological station. Both *cytochrome-b* and microsatellite estimates show one central site (LLIV) as genetically differentiated from other centrally located sites. Geographic distribution of sampling sites may have caused this distinction with four sites (AI, C, LLOR, and RR) clustered together and one site (LLIV) relatively further away.

Genetic differentiation in Blue-tailed Mole Skink pairwise comparisons also support a historical divide between central and southern Lake Wales Ridge. Pairwise Φ_{ST} comparisons fail to detect genetic differentiation among central Lake Wales Ridge sites. This is likely due to the geographic proximity of locations. However, pairwise θ comparisons indicate genetic differentiation between central Lake Wales Ridge (C & AI/LLOR). *Cytochrome-b* and microsatellite estimates likely differ due to microsatellite loci changing at a faster rate, and having greater statistical power in this analysis, compared to *cytochrome-b*. If so, this may indicate the more recent isolation, or genetic differentiation at a magnitude not detectable at this level of power with *cytochrome-b* for central Lake Wales Ridge sites.

The newly assayed sites had similar population structure to previous studies. Florida Sand Skink F_{st} estimates for the current study were almost identical to previous studies (Φ = 0.667, Branch et al. 2003 and θ = 0.03, Richmond et al. 2009), supporting the pattern of genetically differentiated central and southern Lake Wales Ridge. All studies have found patterns of differentiation consistent with low vagility for the Florida Sand Skink and Blue-tailed Mole Skink and current patch isolation is likely augmenting separation of individuals. Microsatellite θ estimates of F_{st} for the Blue-tailed Mole Skink were very similar to Schrey et al. 2012 (Φ = 0.689; θ = 0.029), further supporting the central-south division of Lake Wales Ridge. *Cytochrome-b* estimates were slightly lower in the current study likely due to the closer proximity of current sites compared to the geographic spread of samples in Schrey et al. (2012). Based on

all data collected, biogeography and population structure indicate similar population connectivity patterns over time and over the geographic range of the Lake Wales Ridge.

Genetic Diversity

Florida Sand Skinks had relatively low *h* for current study sites. However, due to the low sample size here, more data would be required to accurately determine *h* among these locations. Overall, *h* was slightly higher for central Lake Wales Ridge groups compared to southern groups. This supports evidence for a larger, more connected refugia in central Lake Wales Ridge. Within central Lake Wales Ridge, *h* was similar over sites with exceptions of the very high diversity seen in RR. This indicates a large population size with dispersal to/from nearby scrub patches likely in RR. This is further supported by microsatellite estimates showing a high heterozygosity estimate and mating patterns consistent with outbreeding for RR.

Microsatellite estimates displayed high genetic diversity in the Florida Sand Skink overall. Southern site, HENM showed the highest diversity with positive MPR possibly due to some samples having familial relationships. The two other sites with significant MPR estimates (LLIV and LLOR) also exhibit high F_{is} estimates, supporting patterns of inbreeding within central Lake Wales Ridge. Site AI revealed the highest F_{is} out of all collected sites, which is notable as population structure failed to detect isolation of this centrally located site. This could indicate recent isolation of proximate sample sites in central Lake Wales Ridge by habitat fragmentation.

For the Blue-tailed Mole Skink, *h* was consistently high over sampling locations, suggesting relatively large population sizes for all sites, apart from LLIV. The low *h* at LLIV is caused by the low sample size from this location ($N = 2$), resulting in a largely uninformative estimate. Microsatellite estimates also supported high diversity in the Blue-tailed Mole Skink. However, the Blue-tailed Mole Skink displayed systems of inbreeding within all three central locations. This indicates that these locations may be beginning to manifest the negative consequences of fragmentation.

In the present study, both focal species displayed high genetic diversity overall. However, Florida Sand Skinks on average had slightly higher diversity estimates than Blue-tailed Mole Skinks. This may

suggest differences between the Florida Sand Skink and Blue-tailed Mole Skink, possibly in local population size or density. Evidence of inbreeding within central Lake Wales Ridge and low dispersal rates for the species supports mating systems only including individuals in very near proximity. This has been well supported by all previous studies of these species (Branch et al. 2003, Richmond et al. 2009, Schrey et al. 2012). The current study's genetic diversity estimates highlight two ecological time frames seen in Florida Sand Skink and Blue-tailed Mole Skink. Heterozygosity estimates indicate both species had a historically large and interconnected refugia within central Lake Wales Ridge. However, inbreeding coefficients and mean pairwise relatedness indicate both species are experiencing the negative effects due to anthropogenic fragmentation. If left alone, populations will continue inbreeding within extant scrub patches and genetic diversity will decline over time for both species. This loss of diversity could potentially allow deleterious alleles to accumulate in the population and decrease the species ability to adapt to changing environments (Tucker et al. 2014, Domingues 2017).

The current study indicated some differences in genetic diversity within sites compared to previous studies. The current Florida Sand Skink data displayed slightly higher genetic diversity estimates across all sites compared to Richmond et al. 2009, indicating higher gene flow and larger population sizes for current study sites. A similar pattern of higher diversity was seen in the new Blue-tailed Mole Skink sites compared to previous studies (Schrey et al. 2012). These high diversity estimates may be due to the geographic locations of the current study sites all in the historically connected central Lake Wales Ridge compared to the geographic spread seen in Schrey et al. (2012). Haplotype diversity was very similar in the current study compared to Schrey et al. 2012. Haplotype diversity data was not sufficient to compare to previous studies by Branch et al. (2003). and Richmond et al. (2009). Overall, differences were not significant and indicate that current study sites are similar to previous sample locations across the Lake Wales Ridge.

Scrub Species Comparison

The current study focused on two threatened skink species; however, the Lake Wales Ridge is blooming with biodiversity. The current genetic data follows similar patterns from previous studies on

separate Florida scrub species. A study on the Peninsula Crowned Snake (*Tantilla relicta relicta)* showed the same pattern of biogeography to the present study with separation between central and southern Lake Wales Ridge by the area surrounding Josephine Creek (Schrey et al. 2015). This suggests most species within the Lake Wales Ridge will also show historical separation due to ancient island formation. However, evidence for migration across the Josephine Creek was also seen in the Peninsula Crowned Snake, suggesting varying effects the creek has on recent species dispersal (Schrey et al. 2015).

A study conducted on the Florida Scrub Lizard (*Sceloporus woodi)* also showed similarities to the current study (Heath et al. 2012). Because all sampling sites in the previous study were in the southern Lake Wales Ridge, no detection of a central-south division was detected in the Florida Scrub Lizard. However, evidence for genetic differentiation among southern populations of the Florida Scrub Lizard was detected. This indicates historically smaller, or more isolated populations in the southern Lake Wales Ridge similar to the Florida Sand Skink and Blue-tailed Mole Skink. Heath et al. (2012) also showed lower diversity within the Florida Scrub Lizard compared to current data on Florida Sand Skink and Blue-tailed Mole Skink. However, Florida Scrub Lizard patterns of inbreeding were very similar to the current estimates of Florida Sand Skink and Blue-tailed Mole Skink. This is consistent with a comparison of all three lizard species by Schrey et al. (2012) and again indicates the potential negative effects of recent fragmentation in the Lake Wales Ridge.

Conservation Implications

This study expanded the geographic scope of Florida Sand Skink and Blue-tailed Mole Skink population genetics over the Lake Wales Ridge. Current data on each species follow the same genetic pattern described by previous studies with one Lake Wales Ridge separated by ancient island formation into central and southern clades. However, the Florida Sand Skink and Blue-tailed Mole Skink display some characteristics of recently isolated populations, most likely due to anthropogenic fragmentation in the Florida Scrub. In order to prevent any further changes in these focal species, conservation management plans should continue to be enacted while centrally located patches show high genetic

diversity. Nevertheless, data suggests that relocation efforts could be done anywhere on the Lake Wales Ridge without serious consequence to either species' genetic structure.

Future conservation studies should expand the focus to other Florida Ridges to determine if patterns of genetic structure and connectivity seen in the Lake Wales Ridge exist within other ancient ridge formations. A nearby ridge, Mt. Dora, would make an excellent candidate to test this hypothesis. Previous studies on a similar scrub species, the Florida Scrub Lizard, have already been conducted on Mt. Dora Ridge within Ocala National Forest (Tucker et al. 2014). This study revealed similar patterns of biogeography to Lake Wales Ridge. Within this separate Florida Scrub ridge, evidence for a historical north-south split is apparent, however, no known barrier exists between the two genetically distinct regions (Tucker et al. 2014). This study also showed negative genetic effects due to anthropogenic fragmentation, however, pairwise comparisons showed no clear pattern (Tucker et al. 2014). These unanswered questions indicate the relevance for more population genetic studies on the Mt. Dora Ridge and can reveal a better understanding of historical and recent habitat fragmentation for the Florida Sand Skink and Blue-tailed Mole Skink.

REFERENCES

Ashton, K.G. and Knipps, A.C., 2011. Effects of fire history on amphibian and reptile assemblages in rosemary scrub. *Journal of Herpetology*, *45*(4), pp.497-503.

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. *Conservation Genetics*, *4*(2), pp.199-212.

Christman, S. D. 1992. Bluetail Mole Skink. In P.E. Moler (ed.), Rare and Endangered Biota of Florida, Volume III Amphibians and Reptiles, pp. 117–122. Univ. Press of Florida, Gainesville.

Domingues, R.R., Hilsdorf, A.W.S. and Gadig, O.B.F., 2018. The importance of considering genetic diversity in shark and ray conservation policies. *Conservation Genetics*, *19*(3), pp.501-525.

Excoffier, L., Laval, G. and Schneider, S., 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary bioinformatics*, *1*, p.117693430500100003.

Hardin, J.O., 2019. *A GIS-BASED SYNTHESIS OF RELICT SHORELINES IN PENINSULAR FLORIDA*.

Heath, S., Schrey, A.W., Ashton, K.G., Mushinsky, H.R. and McCoy, E.D., 2012. Contrasting genetic differentiation of a poorly dispersing lizard in connected and fragmented scrub habitats. *Journal of Herpetology*, *46*(4), pp.602-607.

Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of molecular evolution*, *16*(2), pp.111-120.

Laessle AM. 1958. The origin and successional relationship of sandhill vegetation and sand pine scrub. *Ecol Mono*. 28:361–387.

McCoy, E.D., Richmond, J.Q., Mushinsky, H.R., Britt, E.J. and Godley, J.S., 2010. Long

generation time delays the genetic response to habitat fragmentation in the threatened Florida sand skink. *Journal of Herpetology*, pp.641-644.

Menges ES. 1999. Ecology and conservation of Florida scrub. In: Anderson RC, Fralish JS, Baskin JM, editors. *Savannas, barrens, and rock outcrop plant communities of North America*. Cambridge (UK): Cambridge Uni- versity Press. p. 7–22.

Mercier, K.P., 2018. *Unearthing the Past and Present of a Semi-fossorial Lizard: Conservation Genetics, Phylogeography, and Taxonomy of Plestiodon Egregius* (Doctoral dissertation, University of Central Florida).

Mount, R. H. 1963. The natural history of the red-tailed skink, Eumeces egregius (Baird). American Midland Naturalist 70:356–385.

Myers RL. 1990. Scrub and high pine. In: Myers RL, Ewel JJ, editors. *Eco- systems of Florida*. Orlando (FL): University of Central Florida Press. p. 150–193.

Peakall R, Smouse PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28:2537-2539.

Queller, D.C. and Goodnight, K.F., 1989. Estimating relatedness using genetic markers. *Evolution*, *43*(2), pp.258-275.

Ragsdale, A.K., Frederick, B.M., Dukes, D.W., Liebl, A.L., Ashton, K.G., McCoy, E.D., Mushinsky, H.R. and Schrey, A.W., 2016. Fire increases genetic diversity of populations of six-lined racerunner. *Journal of Heredity*, *107*(7), pp.654-659.

Reid, D.T., Ashton, K.G. and Zamudio, K.R., 2004. Characterization of microsatellite markers in the threatened sand skink (Neoseps reynoldsi). *Molecular Ecology Notes*, *4*(4), pp.691-693.

Richmond, J.Q., Reid, D.T., Ashton, K.G. and Zamudio, K.R., 2009. Delayed genetic effects of habitat fragmentation on the ecologically specialized Florida sand skink (Plestiodon reynoldsi). *Conservation Genetics*, *10*(5), pp.1281-1297.

Schrey, A.W., Fox, A.M., Mushinsky, H.R. and McCOY, E.D., 2011. Fire increases variance in genetic characteristics of Florida Sand Skink (Plestiodon reynoldsi) local

populations. *Molecular Ecology*, *20*(1), pp.56-66.

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*(2), pp.241-247.

Schrey, A.W., Evans, R.K., Netherland, M., Ashton, K.G., Mushinsky, H.R. and McCoy, E.D., 2015. Phylogeography of the Peninsula Crowned Snake (Tantilla relicta relicta) on the Lake Wales Ridge in Central Florida. *Journal of Herpetology*, *49*(3), pp.415-419.

Sutton, P. E., H. R. Mushinksy, and E. D. McCoy, 1999. Comparing the use of pitfall drift fences and cover boards for sampling the threatened Sand Skink (Neoseps reynoldsi). Herpetological Review 30:149–151.

Tamura, K., Dudley, J., Nei, M. and Kumar, S., 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular biology and evolution*, *24*(8), pp.1596-1599.

Telford JR., S. R. 1959. A study of the sand skink, Neoseps reynoldsi Stejneger. Copeia 1959:110–119.

Tucker, D.B., McBrayer, L.D. and Harrison, J.S., 2014. Population structure of Florida scrub lizards (Sceloporus woodi) in an anthropogenically fragmented forest. *Herpetologica*, *70*(3), pp.266-278.

US Fish and Wildlife Service, 1999. South Florida multi-species recovery plan. U. S. Fish and Wildlife Service, Atlanta.

US Fish and Wildlife Service, 2007. 5-Year Review: Bluetail Mole Skink (Eumeces egregius lividus) and Sand Skink (Neoseps reynoldsi). *US Fish and Wildlife Service, Vero Beach, FL*.

Villesen, P., 2007. FaBox: an online toolbox for fasta sequences. *Molecular ecology notes*, *7*(6), pp.965-968.

Webb, S.D., 1990. Historical biogeography. *Ecosystems of Florida*, pp.70-100.

Weekley, C.W., Menges, E.S. and Pickert, R.L., 2008. An ecological map of Florida's Lake

Wales Ridge: a new boundary delineation and an assessment of post-Columbian habitat loss. *Florida Scientist*, pp.45-64.