Snakes on the Plain: Biogeographic Patterns in Banded Watersnakes (*Nerodia fasciata*) across the Southeastern Coastal Plain

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ACKNOWLEDGMENTS

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ABSTRACT

Barriers to dispersal often cause evolutionary divergence. In the present, identification of geographic boundaries between lineages can indicate the historic presence of biogeographic barriers. In eastern North America, these patterns often arose during periods when changes in climate accompanied the origin of topographic features that hinder gene flow through expansion of river systems, changes in sea level, and restriction of organisms to refugia. However, organisms' responses to changing landscapes is often complex. Not all taxa, even those with similar ecologies, exhibit effects on gene flow from the same changes in geographic features and climate; there is often nuance in these evolutionary shifts. In this study, I use the banded water snake, *Nerodia fasciata*, as a model to test for phylogeographic structure associated with potential biogeographic barriers. While this species is found throughout the U.S. southeastern coastal plain region, previous phylogeographic research has been limited to Florida, a deficiency I address here. I sequenced the mitochondrial gene cytochrome b (*cytb*) and the nuclear gene prolactin receptor (*PRLR*) for phylogenetic analysis across the range of *N. fasciata*. Additionally, biogeographic modeling was used to investigate the historic distributions of this group. Results indicate that this species first diversified during the late Miocene and early Pliocene and may have historically been isolated to a glacial refugium in the western part of its range and to the Florida peninsula by high sea levels. These findings are consistent with patterns observed in certain other snake species distributed throughout the American southeast. Phylogeographic breaks were not found at major river systems, while ranges of the subspecies of this group better fit the distributions of its major mitochondrial lineages. This research contributes new information on the systematics of *N. fasciata* and helps to explore how organisms respond to changing geography across continental land masses, providing a model for testing southeastern biogeography in other taxa.

INTRODUCTION

Geographic barriers are a major cause of reproductive isolation, which can lead to divergence among populations and subsequent allopatric speciation. Continental ecosystems in particular develop barriers such as mountain ranges and river systems that have a significant impact on patterns of speciation and diversification (Jensen et al., 2024; Myburgh & Daniels, 2022; Swenson & Howard, 2005). Evidence for these processes are often found in discrete geographic borders among divergent clades, also known as phylogeographic breaks (Lyman & Edwards, 2022; Soltis et al., 2006; Swenson & Howard, 2005). In eastern North America, research has documented several geographic barriers that persisted long enough to cause speciation in diverse taxa (Br&ley et al., 2010; Burbrink et al., 2000; Lemmon et al., 2007; Lyman & Edwards, 2022; Myers et al., 2020; Pyron & Burbrink, 2010; Soltis et al., 2006; Swenson & Howard, 2005). Furthermore, the life-history and ecology of organisms can have a strong influence on what features of a landscape impact phylogenetic structure (Brandley et al., 2010; Fontanella et al., 2008; Guiher & Burbrink, 2008).

Ongoing geologic and climatic processes can alter geographic barriers and their effectiveness as impediments to gene flow over extended time periods. Relatively recent examples include the Pliocene and Pleistocene epochs (approximately 5.33 million to 12,000 years ago), which were characterized by repeated cycles of glacial advance and retreat (Hobbs, 1950; Lane, 1994; Past Interglacials Working Group of PAGES, 2016). These glacial periods accompanied dips in global temperatures and sea levels falling to as much as 120 meters below present day's mark while interglacial periods saw peaks in global temperatures and rises in sea level of 25 or more meters above today (Dutton & Lambeck, 2012; Hobbs, 1950; Lane, 1994; Past Interglacials Working Group of PAGES, 2016; Rovere et al., 2015). As many as 11 of these cycles occurred in the last 800,000 years (Past Interglacials Working Group of PAGES, 2016).

Glaciation cycles often affect distribution and diversification of organisms through changing the locations of habitable climate and hence geographic barriers (Barrow et al., 2017; Lyman & Edwards, 2022; Soltis et al., 2006). This has been well-documented in North America where extreme high-sea level events of interglacial periods reduced peninsular Florida to a series of islands (Lane, 1994), isolating organisms from mainland North America and resulting in the formation of distinct Florida lineages in many organisms (Lane, 1994; Lyman & Edwards, 2022; Soltis et al., 2006). Evidence of peninsular Florida endemic linages has been found across a broad range of taxa including plants (Saeki et al., 2011), frogs (Barrow et al., 2017; Richter et al., 2014), turtles (Walker et al., 1998; Walker & Avise, 1998), insects (Marsico et al., 2015), mammals (Cullingham et al., 2008), lizards (Clark et al., 1999), and snakes (Burbrink et al., 2008; Fontanella et al., 2008; Guiher & Burbrink, 2008).

Increased global temperatures of interglacial periods had a further effect on the biogeography of the American southeast. As increasing temperatures caused a transition from glacial to interglacial periods, major river systems widened as they were inundated with newly melted glacier water (Cox & Van Arsdale, 2002; Cupples & Van Arsdale, 2014; Hobbs, 1950). At times during Pliocene and Pleistocene interglacial periods, major rivers such as the Mississippi and Apalachicola received six to eight times greater flow than today (Hobbs, 1950). Phylogeographic breaks along the Mississippi and Apalachicola Rivers are hypothesized to have originated during this time when inundated river systems served as more significant barriers to gene flow than present day (Barrow et al., 2017; Brandley et al., 2010; Burbrink et al., 2000; Martin et al., 2016). Divergence across these rivers has been documented in diverse organisms including frogs (Lemmon et al., 2007), turtles (Walker et al., 1998; Walker & Avise, 1998), insects (Hill, 2015), mammals (Cullingham et al., 2008), lizards (Leaché, 2009), and snakes (Brandley et al., 2010; Burbrink et al., 2008; 2000; Fontanella et al., 2008; Makowsky et al., 2010; Myers et al., 2020).

A further type of discontinuity that can arise due to such climate patterns are suture zones between glacial refugia (areas that maintain conditions that provide organisms with long term resistance to widespread ecological disturbance) (Barrow et al., 2017). Suture zones are areas where boundaries between divergent lineages do not result from a geographic barrier at the suture zone. In North America, and example of this has been studied in central Alabama where a suture zone likely formed during glacial periods when most latitudes became inhospitable to many taxa, especially ectotherms such as herpetofauna (Barrow et al., 2017; Newman & Rissler, 2011; Remington, 1968; Swenson & Howard, 2005). But during these periods, glacial refugia in the southern United States (specifically Florida and south Texas) maintained more moderate temperatures allowing for long term persistence of ectotherms (Barrow et al., 2017; Swenson & Howard, 2005). These allopatric refugia caused divergence in widespread species which may have retreated to these regions (Lyman & Edwards, 2022). A suture zone then formed in central Alabama during interglacial periods when divergent lineages from southwestern and southeastern refugia expanded their range until they came back into contact in central Alabama (Barrow et al., 2017; Newman & Rissler, 2011; Remington, 1968; Swenson & Howard, 2005).

While these patterns are apparent in many clades, snakes represent an intriguing model for biogeographic study as there is significant complexity in how different species and ecotypes of snake respond to biogeographic barriers. In the eastern U.S. alone, the Mississippi River has acted as a barrier to some widely distributed species (rat snakes [*Pantherophis obsoletus* complex] (Burbrink et al., 2000), corn snakes [*Pantherophis guttatus* complex] (Myers et al., 2020), North American racers [*Coluber constrictor*] (Burbrink et al., 2008), and the diamondback water snake [*Nerodia rhombifer*] (Brandley et al., 2010)), but not others (cottonmouths [*Agkistrodon piscivorous* complex] (Guiher & Burbrink, 2008), ring-necked snakes [*Diadophis punctatus*] (Fontanella et al., 2008), and the plain-bellied water snake [*N. erythrogaster*] (Makowsky et al., 2010)). Similarly, the Apalachicola River has acted as a barrier to gene flow for a subset of snake taxa (rat snakes (Burbrink et al., 2000) and ring-necked snakes (Fontanella et al., 2008)) while a lack of such evidence exists in other species (cottonmouths (Guiher & Burbrink, 2008), North American racers (Burbrink et al., 2008), corn snakes (Myers et al., 2020), and the plain-bellied water snake (Makowsky et al., 2010) suggesting a complex nature of snake dispersal in Pleistocene North America. There is a clear absence of ecological trends in predicting how different species are affected by these biogeographic barriers. For example, it is counterintuitive that the semi-aquatic diamondback water snake has been isolated by river systems while neither the semi-aquatic cottonmouth nor the small-bodied leaf litter-dwelling ring-necked snake have not undergone similar disruption from either the Apalachicola or Mississippi Rivers. In the case of peninsular Florida, cottonmouths, North American racers, and ring-necked snakes have distinct Florida clades (Burbrink et al., 2008; Fontanella et al., 2008; Guiher & Burbrink, 2008), but rat snakes present a different dynamic. The eastern lineage of this complex likely originated due to historic confinement to peninsular Florida and later spread far outside with a limit of westward dispersal at the Apalachicola River and the Appalachian Mountains further north (Burbrink et al., 2000).

Herein, I test the impacts of different phylogeographic hypotheses in the southeastern US using the banded water snake, *Nerodia fasciata*, as a study system. I investigated the effects of hypothesized biogeographic barriers on *N. fasciata*, which has a broad distribution across the southeastern United States, ranging from peninsular Florida to the Great Plains, crossing the Apalachicola and Mississippi Rivers (Fig. 1). As a semi-aquatic species, *N. fasciata* is a good model for these questions due to its wide range and because (even within semi-aquatic snakes) there is complexity in their responses to aquatic barriers. Diamondback water snakes (Brandley et al., 2010) exhibit a discontinuity across the Mississippi River whereas cottonmouths (Guiher & Burbrink, 2008) and the plain-bellied water snake (Makowsky et al., 2010) do not diverge at any of the major river systems throughout their ranges.

Furthermore, *N. fasciata* consists of three morphologically described subspecies: the broad-banded water snake (*N. f. confluens*), southern water snake (*N. f. fasciata*), and Florida banded water snake (*N. f. pictiventris*). Subspecies described morphologically without input from molecular study present preexisting hypotheses for the evolutionary history of a group that merit testing. Subspecies delineation often yields inconsistent results as molecular data can lead researchers to elevate subspecies to full species, conclude that subspecies represent groups that are monophyletic but not divergent enough to merit elevation to full species, or result from local adaptation and not evolutionary divergence (Makowsky et al., 2010; Pyron & Burbrink, 2009a, 2009b; Torstrom et al., 2014). Previous investigation of phylogeography in *N. fasciata* has found divergence between snakes in peninsular Florida and snakes in the Florida panhandle (DiMeo, 2019; Rautsaw et al., 2021; Territo, 2013). These studies were limited to Florida, whereas the goal of this study is to look at the phylogeography of this species across its range. I used sequencing and phylogenetic analysis of nuclear and mitochondrial genetic markers to test for boundaries among lineages in *N. fasciata* associated with hypothesized biogeographic barriers.

In this study, I examine the systematics and distributions of the species, including testing the monophyly of the subspecies of *N. fasciata*: the broad-banded water snake (*N. f. confluens*), southern water snake (*N. f. fasciata*), and Florida banded water snake (*N. f. pictiventris*). In doing so, I will address one additional aim of this study to test for the existence of phylogeographic breaks among the putative subspecies, including the aforementioned barriers in peninsular Florida and the Mississippi and Apalachicola Rivers.

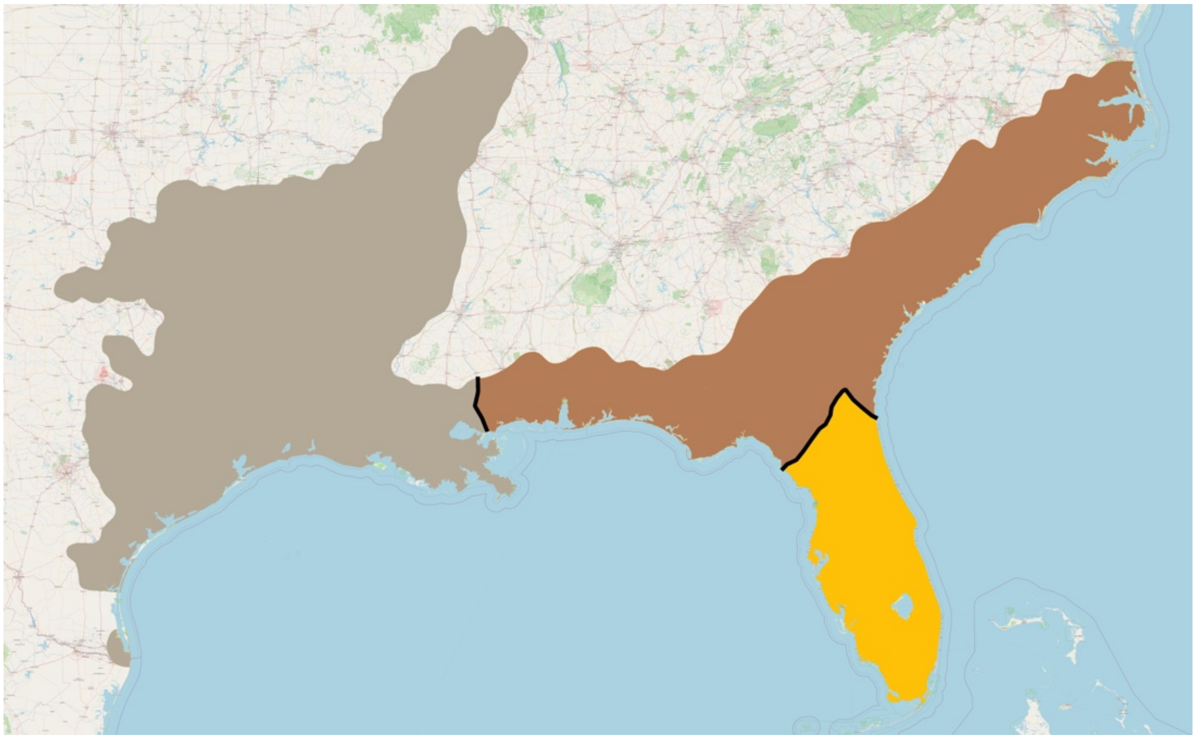

Figure 1: Range of *Nerodia fasciata*. The range of described subspecies are color coded: Grey = *N. f. confluens*, brown = *N. f. fasciata*, yellow = *N. f. pictiventris*

METHODS

Sample Collection

Tissues of *Nerodia fasciata* (*N* = 119) and outgroup taxa (*N* = 9) for this study were collected from wild snakes or by loans from natural history collections (Appendix A). Tissue samples were collected from roadkill snakes and live snakes collected by hand or with tongs before tissue samples were taken via ~ 0.5 cm tail clips under permits from the South Carolina Department of Natural Resources (permit SC-130-2024), Georgia Department of Natural Resources (permit 1001056699), Florida Fish and Wildlife Conservation Commission (permit LSSC-23-00020), and Florida Department of Environmental Protection (permit 08012335) following IACUC approved protocols (Valdosta State University 2/19/24; Appendix B; Appendix C). No live snakes were euthanized for this study.

DNA Extraction, Amplification, and Sequencing

DNA was extracted from all tissue samples using Qiagen DNeasy blood and tissue kits (Cat. No. 69506) following the manufacturer’s instructions then stored at -20° C. Analyses utilized by this study required sequencing of variable genetic markers. This study sequenced the mitochondrial gene cytochrome b (primers: *cytb*F: 5’-GACCTGTGATMTGAAAAACCAYCGTTGT-3’, *cytb*R: 5’-CTTTGGTTTACAAGAACAATGCTTTA-3’, 1080 bp amplicon) (Burbrink et al., 2000) and the nuclear prolactin receptor gene (primers: *PRLR*F: 5’-GACARYGARGACCAGCAACTRATGCC-3’, *PRLR*R: 5’ -GACYTTGTGRACTTCYACRTAATCCAT-3’, 595 bp amplicon) (Townsend et al., 2008). The thermocycler settings used for PCR were 95° C for 30s, 55° C for 45s, and 72° C for 90s. All reactions were done at 25 μL volumes containing 100 ng of DNA and confirmed by running 10μL of PCR product out on an agarose gel. PCR products were cleaned by incubation of 5 μL PCR product with 2 μL ExoSAP-IT for four minutes at 37° C then one minute at 80° C. Samples were then diluted to 10x then sequenced via Sanger Sequencing at the Biological Science Core Facility at Florida State University. Geneious Prime was used to resolve ambiguous nucleotides and align sequences in preparation for phylogenetic analysis ("Geneious | Bioinformatics Software for Sequence Data Analysis," n.d.).

Phylogenetic Analysis and Gene Network Construction

Phylogenetic analysis of sequence data was conducted in BEAST 2.6.7 using the GTR+I+Γ model with an optimized relaxed clock (Bouckaert et al., 2014). Three priors were used to date trees: a prior defining *N. fasciata* and *N. sipedon* together as a monophyletic group originating 7.52–7.27 mya, a prior defining *Nerodia* as a monophyletic group originating 16.1–15 mya, and a prior defining *Nerodia* and its sister genus *Regina*, together as monophyletic originating 15.79–15.27 mya as were found by Nuñez et al., (2023). The dataset built from sequencing tissue samples was supplemented with sequences acquired from Genbank (Appendix A). The *PRLR* sequence data obtained from Sanger sequencing was used to generate a gene network using the neighbor-net algorithm in SplitsTree 6.3.30 (Huson & Bryant, 2024).

Ancestral Area Estimation

The ancestral ranges of the different lineages of *N. fasciata* were inferred using the R package, BioGeoBEARS (Matzke, 2022, 2013). This analysis used the time-calibrated Bayesian tree generated for Fig. 2 to test the likelihood of different models of ancestral area estimation: dispersal extinction cladogenesis (DEC) (Ree & Smith, 2008), dispersal vicariance analysis (DIVALIKE) (Ronquist, 1997), and Bayesian analysis of biogeography (BAYAREALIKE)

(Landis et al., 2013). These models were tested with and without a *j* parameter allowing for the possibility of divergence resulting from founder events. The original Bayesian tree was pruned using the R package, ape (Paradis & Schliep, 2019), for the tree used for analysis leaving a single specimen from individual populations. Comparison of AIC_c values between models allowed for selection of the most likely model. The possible ancestral areas used in this analysis were chosen based on different distinct floristic regions of the Southeastern Coastal Plain with the "west of the Mississippi River" as an additional possibility (Naranjo et al., 2023; Sorrie & Weakley, 2001). For this analysis, our phylogeny was divided into three time sections: before and after the mid Pliocene warm period (3.2–3.0 mya) during which dispersal between adjacent area is allowed and the mid Pliocene warm period (3.2–3.0 mya) during which dispersal to and from the areas in peninsular Florida are coded as not possible and the Gulf Coast prairies and dunes area is excluded as a habitable location due to historically high sea levels (Fig. 5a) (Dutton et al., 2015; Dwyer & Chandler, 2008).

RESULTS

Phylogenetic analysis of *cytb* recovered three deeply divergent lineages of *Nerodia fasciata*: one lineage occurring in the eastern portion of this species' range, sister to a lineage found mostly in Florida, and a lineage found in the western portion of the range distribution as an outgroup (Figs. 2 and 3). Time calibration of *cytb* sequences gave estimates for divergence times of these lineages (Fig. 2).

Phylogenetic analysis of *PRLR* sequence data did not yield a tree with any credible posterior values so another BEAST run was attempted after increasing the number of generations to 40 million and using the TRN+I+ Γ model of evolution as previously done by Ruane et. al (2014). This did not yield a tree with any credible nodes either. The gene network generated for this data did not show any clear patterns (Fig. 4). This analysis included several outgroup species (*Nerodia clarkii*, *N. sipedon, N. erythrogaster, N. rhombifer, Thamnophis sirtalis*), none of which stood out from the *N. fasciata* sequences (Fig. 4). These findings indicate that *PRLR* alone does not provide enough variable sites to be useful for phylogenetic analysis. Biogeographic analysis in BioGeoBEARS determined that the most likely model for ancestral area estimation is DIVALIKE + J (Table 1). The base of the tree does not provide a strong inference for the geographic origin of the overall species or its western lineage but does show relatively high support for the eastern lineages of *N. fasciata* likely originating in the Apalachicola River/Florida Panhandle region (Fig. 5).

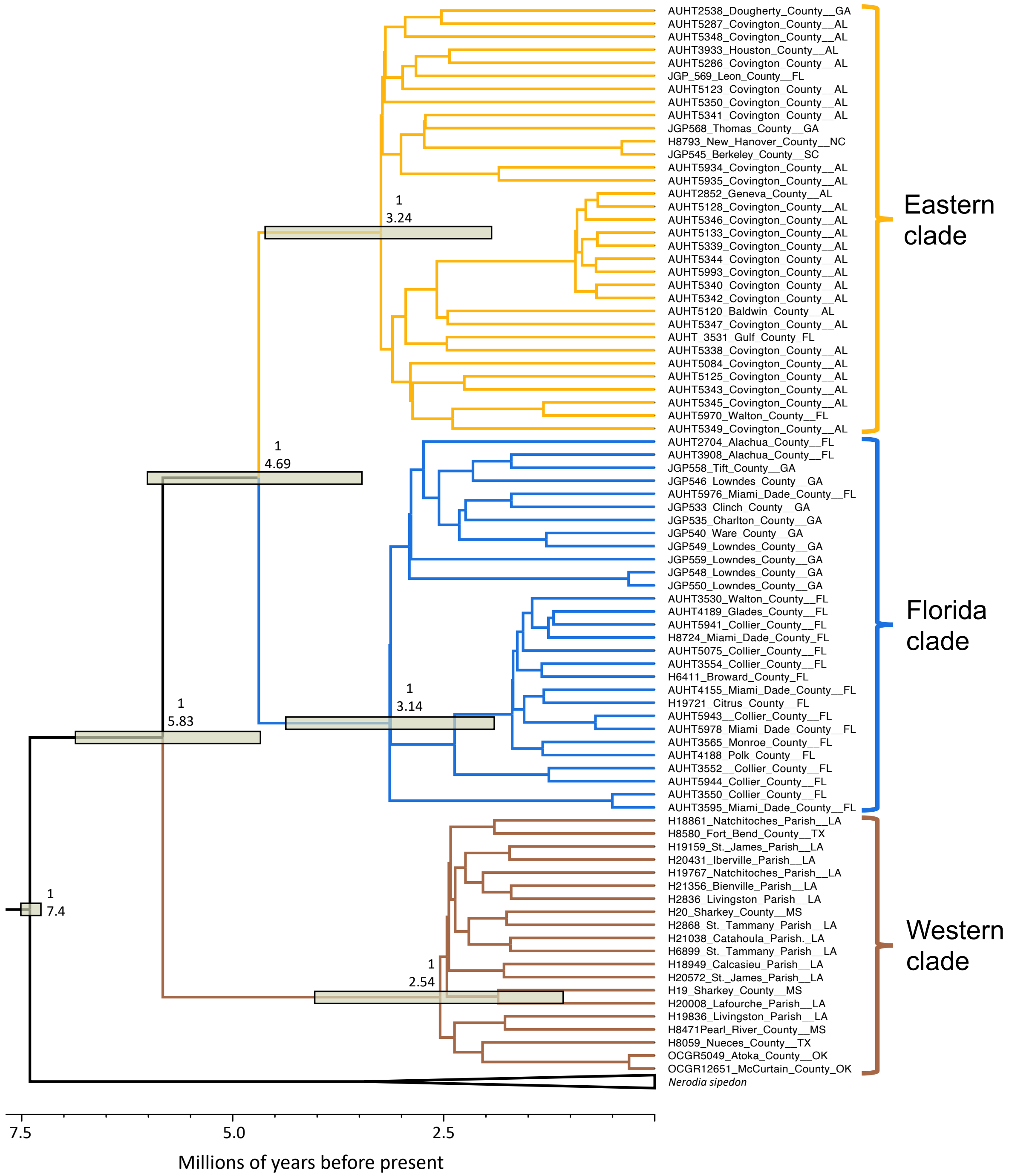

Figure 2: Cytochrome b (*cytb*) phylogeny of *Nerodia fasciata*. Bayesian reconstruction of *cytb* sequences (1098 bp) conducted in BEAST 2.6.7 with 10 million generations, 10% burn in and a GTR+I+Γ model of evolution. Major nodes (nodes with posterior probability values >0.8 that are not terminal) are labeled with posterior probability values, 95% HPD for estimated divergence times, and node bars showing 95% HPD for estimated divergence times.

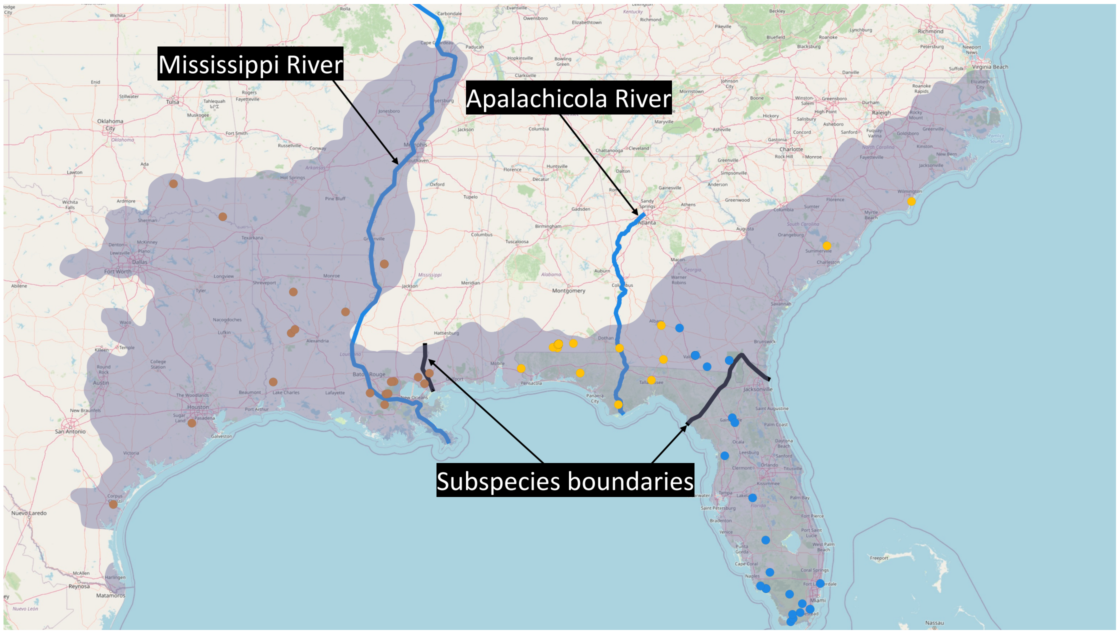

Figure 3: Map of *N. fasciata* samples used in this study. Shaded area indicates the known range of *N. fasciata*. Colors on the clades correspond to the map in Figure 1. The Mississippi and Apalachicola Rivers are labeled as well as accepted borders between the *N. fasciata* subspecies per Gibbons & Dorcas (2004)

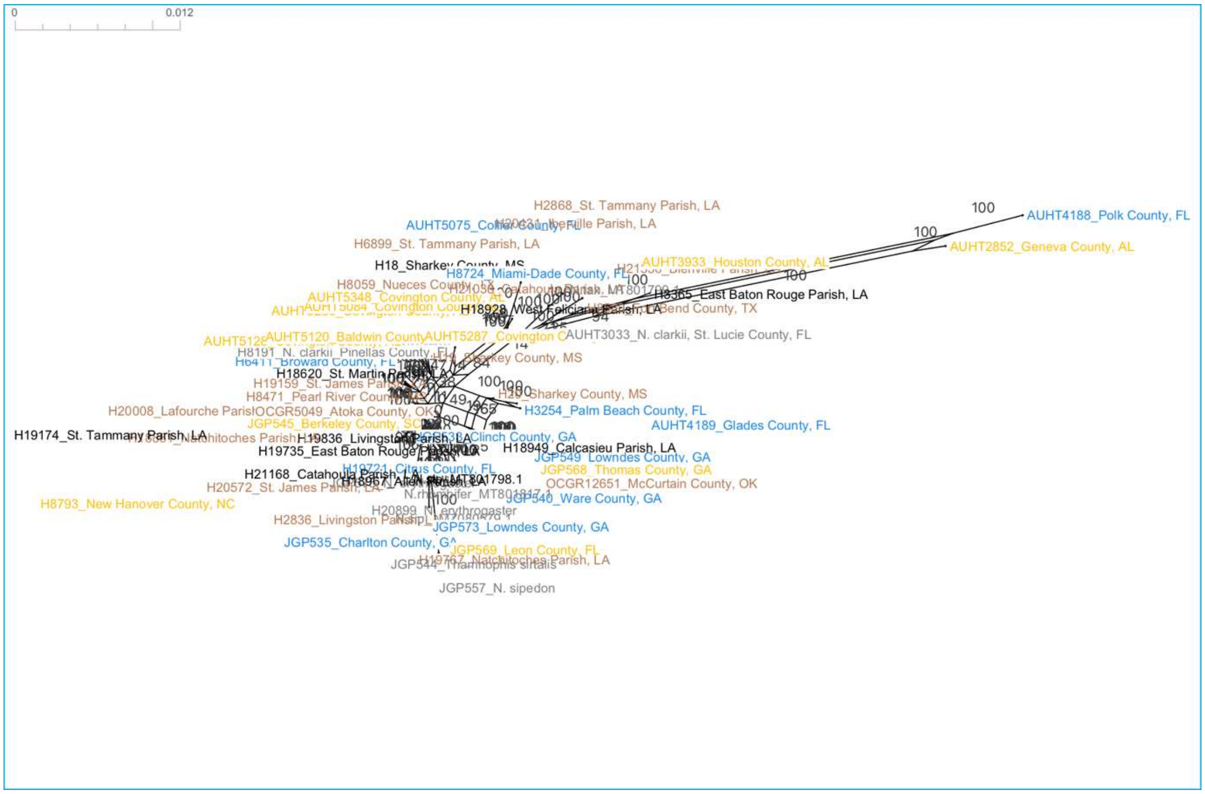

Figure 4: Gene network of prolactin receptor gene (*PRLR*) sequence data. Terminal branches and labels are colored based off cytochrome b (*cytb*) lineage (Fig. 2). Terminal branches and labels colored grey are from outgroup species and those colored black are from *N. fasciata* specimens for which *cytb* sequencing could not be done successfully.

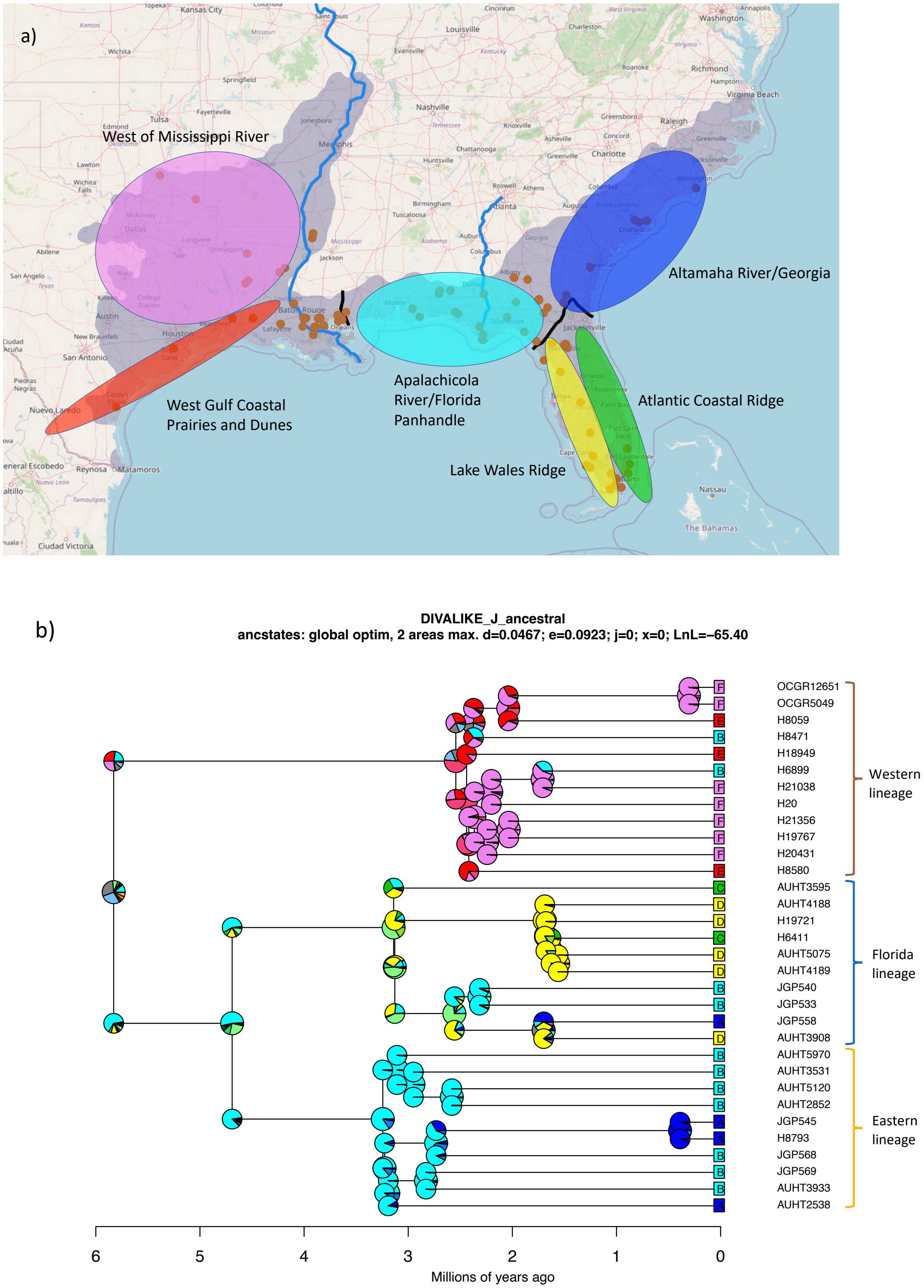

Fig. 5. a) Map of possible ancestral areas used for ancestral area estimation. b) Ancestral area estimation of most probable model (DIVALIKE + J) using time-calibrated Bayesian phylogeny of *Nerodia fasciata* exclusive cytochrome b sequence data from Fig 2. Tips used are individuals representing single populations. Node and tip colors map on to ancestral areas in a) For tip letters, A=Altamaha River/Georgia, B=Apalachicola River/Florida Panhandle, C=Atlantic Coastal Ridge, D=Lake Wales Ridge, E=Gulf Coastal Prairies and Dunes, F=West of Mississippi River.

model	AICc	AICc_wt	AICc_wt	LnL	numparams	d	e	j
BAYAREALIKE	148.2502861	4.05E-15	4.57E-16	-71.91824648	2	0.07323685	0.233793421	
BAYAREALIKE+J	83.35788838	0.499999915	0.056401258	-38.25037276	3	1.00E-07	1.00E-07	0.134820062
DEC	135.2178273	9.34E-13	3.09E-13	-65.40201711	2	0.046709453	0.092264791	
DEC+J	81.20649317	0.499999969	0.165370608	-37.17467515	3	1.00E-13	1.00E-13	0.14753683
DIVALIKE	134.6806428	7.27E-13	4.04E-13	-65.13342484	2	0.043798491	0.068798463	
DIVALIKE+J	**80.16598864**	**0.5**	**0.278228114**	**-36.65442289**	**3**	**1.00E-12**	**1.00E-12**	**0.146515528**

Table 1. Likelihood results for all models tested in BioGeoBEARS ancestral area estimation. Model with the best AIC_c value is bolded. d = dispersal, e = extinction, j = founder event divergence.

DISCUSSION

The three mitochondrial lineages of *Nerodia fasciata* roughly correlate with the subspecies hypothesis of this group's phylogeographic history. This species is made up of three morphologically described subspecies (Gibbons & Dorcas, 2004): *N. fasciata confluens* in the western portion of its range, *N. f. fasciata* in the eastern portion of its range, and *N. f. pictiventris* primarily found in Florida (Fig. 1). The distribution of the western mitochondrial lineage closely matches the distribution of *N. f. confluens* however, this analysis lacks samples from southeastern Mississippi and southwestern Alabama along the Gulf Coast so it remains unknown exactly where the barrier or contact zone between the western and eastern lineages is located (Fig. 3). This western lineage may be concordant with the previously attributed range of *N. f. confluens* but requires more extensive fine-scale sampling along the Gulf of Mexico between our western and eastern mtDNA lineages (Fig. 3).

Nerodia f. pictiventris is found primarily in Florida and its range from Gibbons & Dorcas (2004) roughly (though not exactly) matches our eastern mitochondrial lineage (Fig. 3). Our analyses found the Florida lineage extended outside the accepted range of *N. f. pictiventris* in Georgia including as far north as Tifton, Georgia (Tift County, Fig. 3). These non-Floridian mitochondrial sequences found well outside the range of *N. f. pictiventris* suggests more recent gene flow between *N. f. pictiventris* and *N. f. fasciata* outside peninsular Florida (Camper & Chick, 2010; Vogrinc et al., 2018). The exact relationship between mitochondrial lineage and subspecies would benefit from more fine-scale sampling. The main phenotypic characteristics that putatively distinguish *N. f. fasciata* and *N. f. pictiventris* are the patterning and number of the ventral scales (Gibbons & Dorcas, 2004). *N. f. fasciata* has squarish dark ventral markings and usually more than 128 ventral scales while *N. f. pictiventris* is characterized by having less than 128 ventral scales that are marked with elongated dark markings on the anterior edge (Gibbons & Dorcas, 2004). Further study could document these characteristics to determine how closely they align with the evolutionary lineages.

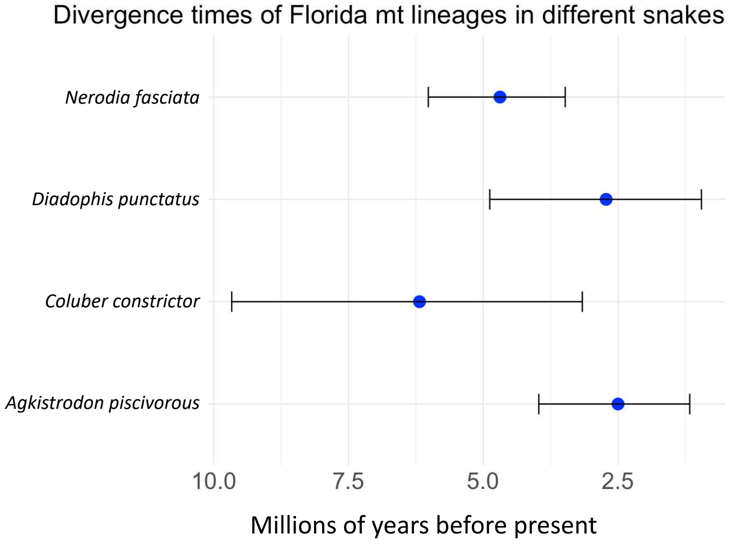

Fig. 6. Divergence times between Florida and mainland U.S. mitochondrial lineages of different snake species. Estimated divergence dates of peninsular Florida cytochrome b lineages of *Nerodia fasciata* (present study), black racer (*Coluber constrictor*, Burbrink et al., 2008), ring-necked snake (*Diadophis punctatus*, Fontanella et al., 2008), and cottonmouth (*Agkistrodon piscivorous* complex, Guiher & Burbrink 2008).

Phylogenetic analysis of *cytb* sequences found three distinct clades in *N. fasciata*: an outgroup western clade and two sister eastern and Florida clades (Fig. 2 & 3). Time calibration of the *cytb* tree finds that the mean age for the divergence of the western lineage (5.83 mya, 95% HPD: 6.87–4.69 mya) was likely in the late Miocene and not the Pliocene or Pleistocene was more common for diversification in snakes in eastern North America (Fig. 2) (Brandley et al., 2010; Burbrink et al., 2021; Guiher & Burbrink, 2008; Myers et al., 2020). Ancestral area estimation did not provide clear evidence for the geographic origin of *N. fasciata* or specifically the western lineage (Fig. 5). An early divergence date between western and eastern lineages likely does not support initial divergence being a result of confinement to glacial refugia since the late Miocene to early Pliocene is a period in Earth's marked by higher than present global temperatures cooling during the late Miocene before spiking again during the early Pliocene (Brown et al., 2022; Herbert et al., 2016; Qin et al., 2022). These warmer-than-present-day climate regimes likely would have not confined organisms to glacial refugia. An alternative explanation for this initial divergence could be embayment of the Mississippi River by high sea levels during the late Miocene/early Pliocene preventing gene flow east and west of the embayed zone (Lemmon et al., 2007). The current distribution of the western mitochondrial lineage may still have been influenced by confinement of western snakes to a glacial refugium during the glacial periods of the Pliocene and Pleistocene. Following the recession of glaciers at the end of the last glacial maximum, snakes of the western lineage may have expanded east to their current contact zone with the eastern mitochondrial lineage (Fig. 3).

Divergence between the eastern and Florida mitochondrial lineages could possibly be explained by snakes persisting in mainland North America and diverging from populations confined to Florida during periods of high sea levels (Fig. 3). Ancestral area estimation finds that these eastern snakes originated in the Apalachicola River/Florida panhandle region before the divergence of the lineage found largely in peninsular Florida (Fig. 5). Florida-specific lineages that originated presumably due to confinement to an insular Florida by historically high sea levels are found in other snakes. The time of divergence for the Florida clade of *N. fasciata* (4.69 mya, 95% HPD: 6.02–3.48) is similar to the mean divergence time of the Florida lineage (6.18

mya) of the North American racer, *Coluber constrictor* (Burbrink et al., 2008), the Florida lineage (2.50 mya) of the cottonmouth, *Agkistrodon piscivorous* complex (Guiher & Burbrink, 2008) (Fig. 6), and the Florida lineage of the ring-necked snake, *Diadophis punctatus* (Fig. 6) (Fontanella et al., 2008). The overlap between the estimated ages of these three contemporary lineages likely takes place during an early Pliocene time period of warming and high sea levels that likely isolated peninsular Florida (Fig. 6) (Dutton et al., 2015; Dwyer & Chandler, 2008; Rovere et al., 2015).

The three distinct mitochondrial lineages of *N. fasciata* do not support the hypothesis predicated on rivers serving as barriers to gene flow. The western lineage spans both sides of the Mississippi River and similarly, the eastern lineage spans both sides of the Apalachicola River (Fig. 2). Ancestral area estimation finds that it is likely that there has been gene flow without divergence across the Mississippi River in the case if *N. fasciata* (Fig. 5). This is in stark contrast to the diamondback water snake (*N. rhombifer*) which exhibits a deep mitochondrial split across the Mississippi indicating a history of limited gene flow across the river (Brandley et al., 2010). Lack of divergence correlating with major rivers is a pattern *N. fasciata* shares with the closely related plain-bellied water snake (*N. erythrogaster*) (Makowsky et al., 2010) and distantly related but similarly semi-aquatic cottonmouths (*Agkistrodon piscivorous* complex.) (Guiher & Burbrink, 2008).

It is not immediately apparent why *N. fasciata, N. erythrogaster*, and the *A. piscivorous* complex do not diverge at the Mississippi River while *N. rhombifer* does. *N. rhombifer* uses a similar range of terrestrial habitat as *N. erythrogaster* but has a significantly greater affinity for foraging in open water habitat (Laurent & Kingsbury, 2003). *N. erythrogaster* and *N. fasciata* both prefer near shore and shallow water habitats with *N. erythrogaster* having a comparatively greater usage of terrestrial habitat (Camper & Chick, 2010; Roe et al., 2004). Both of these species are able to disperse over land as evidenced by documented use of ephemeral wetlands by *N. erythrogaster* and recolonization of wetlands by *N. fasciata* where populations were extirpated by prolonged drought (Roe et al., 2004; Vogrinc et al., 2018). Stable isotope analysis shows that the diet of *N. erythrogaster* consists of a significantly greater proportion of terrestrial prey items when compared to *N. rhombifer* (Perkins et al., 2020). Evidence indicates the foraging habits of *N. fasciata* more closely resemble *N. erythrogaster* than *N. rhombifer*: eye size in *Nerodia* correlates with a preference for preying on frogs as opposed to fish and *N. fasciata* and *N. erythrogaster* are very similar in this respect with large eyes and a presumed preference for frogs (Camp et al., 2020; Perkins & Eason, 2019). It is noteworthy that gene flow seems less affected by major river systems in less aquatic taxa such as *N. fasciata* and *N. erythrogaster* (Makowsky et al., 2010) when compared to more aquatic taxa like *N. rhombifer* (Brandley et al., 2010).

Phylogenetic studies sometimes find discordance between phylogenies based on mitochondrial and nuclear data (Marshall et al., 2021; Toews & Brelsford, 2012). Such discrepancies can result from differences in dispersal behavior between sexes since mitochondrial DNA is maternally inherited while nuclear DNA is inherited from both parents (Marshall et al., 2021; Toews & Brelsford, 2012). Predicting the effects of this phenomenon is difficult in our system since few studies comparing dispersal between sexes in *Nerodia* have been conducted (Camper & Chick, 2010; Roth & Greene, 2006). Discordance between mitochondrial and nuclear data can also arise from introgression of mitochondrial genomes across nuclear lineage boundaries that are more readily maintained (Marshall et al., 2021; Toews & Brelsford, 2012). Identification of such patterns in *N. fasciata* merits further use of nuclear markers going forward. This study is limited by its use of only one mitochondrial marker. Analysis of *PRLR* sequence data did not find it to be variable enough to useful for phylogenetic analysis (Fig. 4). Further study will use RADSeq to analyze nuclear markers in these snakes to obtain enough single nucleotide polymorphisms (SNPs) to test these questions.

This study found that phylogeographic structure supports the monophyly of the morphologically described subspecies of *N. fasciata* with some evidence of range expansion of the Florida mitochondrial lineage into the range of *N. f. fasciata*. Estimation of divergence timing in this group indicates initial divergence between eastern and western lineages during the Miocene likely not because of confinement to glacial refugia as initially hypothesized. Biogeographic modeling together with this divergence date estimation finds that divergence between eastern snakes and snakes in peninsular Florida coincided with periods when high sea level during the Pliocene isolated many taxa. This phylogeographic pattern is congruent with other diverse taxa in the American southeast and evolutionary studies such as these which helps inform collective understanding of how organisms are affected by changing environments across continental land masses.

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APPENDIX A:

Tissue Samples and GenBank Sequences Used for Analysis

Sample	**Source**	**Species**	**County**
AUHT-2538	Auburn University Museum of Natural History	*N. fasciata*	Dougherty County, GA
AUHT-2662	Auburn University Museum of Natural History	*N. fasciata*	Franklin County, FL
AUHT-2704	Auburn University Museum of Natural History	*N. fasciata*	Alachua County, FL
AUHT-2852	Auburn University Museum of Natural History	*N. fasciata*	Geneva County, AL
AUHT-3502	Auburn University Museum of Natural History	*N. taxispilota*	Madison County, FL
AUHT-3521	Auburn University Museum of Natural History	*N. fasciata*	Miami-Dade County, FL
AUHT-3530	Auburn University Museum of Natural History	*N. fasciata*	Walton County, FL
AUHT-3531	Auburn University Museum of Natural History	*N. fasciata*	Gulf County, FL
AUHT-3550	Auburn University Museum of Natural History	*N. fasciata*	Collier County, FL
AUHT-3552	Auburn University Museum of Natural History	*N. fasciata*	Collier County, FL
AUHT-3554	Auburn University Museum of Natural History	*N. fasciata*	Collier County, FL
AUHT-3562	Auburn University Museum of Natural History	*N. taxispilota*	Miami-Dade County, FL
AUHT-3565	Auburn University Museum of Natural History	*N. fasciata*	Monroe County, FL
AUHT-3595	Auburn University Museum of Natural History	*N. fasciata*	Miami-Dade County, FL
AUHT-3629	Auburn University Museum of Natural History	*N. fasciata*	Walton County, FL
AUHT-3908	Auburn University Museum of Natural History	*N. fasciata*	Alachua County, FL
AUHT-3933	Auburn University Museum of Natural History	*N. fasciata*	Houston County, AL
AUHT-4138	Auburn University Museum of Natural History	*N. taxispilota*	Miami-Dade County, FL
AUHT-4155	Auburn University Museum of Natural History	*N. fasciata*	Miami-Dade County, FL
AUHT-4188	Auburn University Museum of Natural History	*N. fasciata*	Polk County, FL
AUHT-4189	Auburn University Museum of Natural History	*N. fasciata*	Glades County, FL

AUHT-5075	Auburn University Museum of Natural History	*N. fasciata*	Collier County, FL
AUHT-5084	Auburn University Museum of Natural History	*N. fasciata*	Covington County, AL
AUHT-5120	Auburn University Museum of Natural History	*N. fasciata*	Baldwin County, AL
AUHT-5123	Auburn University Museum of Natural History	*N. fasciata*	Covington County, AL
AUHT-5125	Auburn University Museum of Natural History	*N. fasciata*	Covington County, AL
AUHT-5128	Auburn University Museum of Natural History	*N. fasciata*	Covington County, AL
AUHT-5133	Auburn University Museum of Natural History	*N. fasciata*	Covington County, AL
AUHT-5177	Auburn University Museum of Natural History	*N. taxispilota*	Miami-Dade County, FL
AUHT-5286	Auburn University Museum of Natural History	*N. fasciata*	Covington County, AL
AUHT-5287	Auburn University Museum of Natural History	*N. fasciata*	Covington County, AL
AUHT-5316	Auburn University Museum of Natural History	*N. taxispilota*	Santa Rosa County, FL
AUHT-5338	Auburn University Museum of Natural History	*N. fasciata*	Covington County, AL
AUHT-5339	Auburn University Museum of Natural History	*N. fasciata*	Covington County, AL
AUHT-5340	Auburn University Museum of Natural History	*N. fasciata*	Covington County, AL
AUHT-5341	Auburn University Museum of Natural History	*N. fasciata*	Covington County, AL
AUHT-5342	Auburn University Museum of Natural History	*N. fasciata*	Covington County, AL
AUHT-5343	Auburn University Museum of Natural History	*N. fasciata*	Covington County, AL
AUHT-5344	Auburn University Museum of Natural History	*N. fasciata*	Covington County, AL
AUHT-5345	Auburn University Museum of Natural History	*N. fasciata*	Covington County, AL
AUHT-5346	Auburn University Museum of Natural History	*N. fasciata*	Covington County, AL
AUHT-5347	Auburn University Museum of Natural History	*N. fasciata*	Covington County, AL
AUHT-5348	Auburn University Museum of Natural History	*N. fasciata*	Covington County, AL
AUHT-5349	Auburn University Museum of Natural History	*N. fasciata*	Covington County, AL

AUHT-5350	Auburn University Museum of Natural History	*N. fasciata*	Covington County, AL
AUHT-5441	Auburn University Museum of Natural History	*N. taxispilota*	Miami-Dade County, FL
AUHT-5931	Auburn University Museum of Natural History	*N. fasciata*	Covington County, AL
AUHT-5932	Auburn University Museum of Natural History	*N. fasciata*	Covington County, AL
AUHT-5933	Auburn University Museum of Natural History	*N. fasciata*	Covington County, AL
AUHT-5934	Auburn University Museum of Natural History	*N. fasciata*	Covington County, AL
AUHT-5935	Auburn University Museum of Natural History	*N. fasciata*	Covington County, AL
AUHT-5941	Auburn University Museum of Natural History	*N. fasciata*	Collier County, FL
AUHT-5943	Auburn University Museum of Natural History	*N. fasciata*	Collier County, FL
AUHT-5944	Auburn University Museum of Natural History	*N. fasciata*	Collier County, FL
AUHT-5970	Auburn University Museum of Natural History	*N. fasciata*	Walton County, FL
AUHT-5976	Auburn University Museum of Natural History	*N. fasciata*	Miami-Dade County, FL
AUHT-5978	Auburn University Museum of Natural History	*N. fasciata*	Miami-Dade County, FL
AUHT-5993	Auburn University Museum of Natural History	*N. fasciata*	Covington County, AL
CAS-160716	California Academy of Sciences	*N. fasciata*	Levy County, FL
CAS-161990	California Academy of Sciences	*N. fasciata*	Santa Rosa County, FL
CAS-162017	California Academy of Sciences	*N. fasciata*	Berkeley County, SC
CAS-165426	California Academy of Sciences	*N. fasciata*	Berkeley County, SC
CAS-165789	California Academy of Sciences	*N. fasciata*	St. John the Baptist Parish, LA
CAS-214322	California Academy of Sciences	*N. fasciata*	Dixie County, FL
CAS-218697	California Academy of Sciences	*N. fasciata*	Gulf County, FL
CAS-222435	California Academy of Sciences	*N. fasciata*	East Baton Rouge Parish, LA
CAS-245625	California Academy of Sciences	*N. fasciata*	Liberty County, GA

H-18	Louisiana State University Museum of Natural Science	*N. fasciata*	Sharkey County, Mississippi
H-18620	Louisiana State University Museum of Natural Science	*N. fasciata*	St. Martin Parish, LA
H-18861	Louisiana State University Museum of Natural Science	*N. fasciata*	Natchitoches Parish, LA
H-18928	Louisiana State University Museum of Natural Science	*N. fasciata*	West Feliciana Parish, LA
H-18949	Louisiana State University Museum of Natural Science	*N. fasciata*	Calcasieu Parish, LA
H-18967	Louisiana State University Museum of Natural Science	*N. fasciata*	Allen Parish, LA
H-19	Louisiana State University Museum of Natural Science	*N. fasciata*	Sharkey County, MS
H-19159	Louisiana State University Museum of Natural Science	*N. fasciata*	St. James Parish, LA
H-19174	Louisiana State University Museum of Natural Science	*N. fasciata*	St. Tammany Parish, LA
H-19721	Louisiana State University Museum of Natural Science	*N. fasciata*	Citrus County, FL
H-19735	Louisiana State University Museum of Natural Science	*N. fasciata*	East Baton Rouge Parish, LA
H-19767	Louisiana State University Museum of Natural Science	*N. fasciata*	Natchitoches Parish, LA
H-19836	Louisiana State University Museum of Natural Science	*N. fasciata*	Livingston Parish, LA
H-20	Louisiana State University Museum of Natural Science	*N. fasciata*	Sharkey County, MS
H-20008	Louisiana State University Museum of Natural Science	*N. fasciata*	Lafourche Parish, LA
H-20431	Louisiana State University Museum of Natural Science	*N. fasciata*	Iberville Parish, LA
H-20572	Louisiana State University Museum of Natural Science	*N. fasciata*	St. James Parish, LA
H-20899	Louisiana State University Museum of Natural Science	*N. erythrogaster*	Iberville Parish, LA
H-21038	Louisiana State University Museum of Natural Science	*N. fasciata*	Catahoula Parish, LA
H-21168	Louisiana State University Museum of Natural Science	*N. fasciata*	Catahoula Parish, LA
H-21356	Louisiana State University Museum of Natural Science	*N. fasciata*	Bienville Parish, LA
H-2836	Louisiana State University Museum of Natural Science	*N. fasciata*	Livingston Parish, LA
H-2868	Louisiana State University Museum of Natural Science	*N. fasciata*	St. Tammany Parish, LA

H-3254	Louisiana State University Museum of Natural Science	*N. fasciata*	Palm Beach County, FL
H-3365	Louisiana State University Museum of Natural Science	*N. fasciata*	East Baton Rouge Parish, LA
H-6411	Louisiana State University Museum of Natural Science	*N. fasciata*	Broward County, FL
H-6899	Louisiana State University Museum of Natural Science	*N. fasciata*	St. Tammany Parish, LA
H-8059	Louisiana State University Museum of Natural Science	*N. fasciata*	Nueces County, TX
H-8471	Louisiana State University Museum of Natural Science	*N. fasciata*	Pearl River County, MS
H-8580	Louisiana State University Museum of Natural Science	*N. fasciata*	Fort Bend County, TX
H-8724	Louisiana State University Museum of Natural Science	*N. fasciata*	Miami-Dade County, FL
H-8793	Louisiana State University Museum of Natural Science	*N. fasciata*	New Hanover County, NC
JGP-533	Collected for this study	*N. fasciata*	Clinch County, GA
JGP-535	Collected for this study	*N. fasciata*	Charlton County, GA
JGP-540	Collected for this study	*N. fasciata*	Ware County, GA
JGP-545	Collected for this study	*N. fasciata*	Berkeley County, SC
JGP-546	Collected for this study	*N. fasciata*	Lowndes County, GA
JGP-548	Collected for this study	*N. fasciata*	Lowndes County, GA
JGP-549	Collected for this study	*N. fasciata*	Lowndes County, GA
JGP-550	Collected for this study	*N. fasciata*	Lowndes County, GA
JGP-556	Collected for this study	*N. erythrogaster*	Lowndes County, GA
JGP-557	Collected for this study	*N. sipedon*	Morris County, NJ
JGP-558	Collected for this study	*N. fasciata*	Tift County, GA
JGP-559	Collected for this study	*N. fasciata*	Lowndes County, GA
JGP-560	Collected for this study	*N. erythrogaster*	Lowndes County, GA
JGP-568	Collected for this study	*N. fasciata*	Thomas County, GA
JGP-569	Collected for this study	*N. fasciata*	Leon County, FL
JGP-573	Collected for this study	*N. fasciata*	Lowndes County, GA
JGP-575	Collected for this study	*N. erythrogaster*	Lowndes County, GA
JGP-584	Collected for this study	*N. fasciata*	Ware County, GA
OCGR-20971	Sam Noble Museum of Natural History	*N. erythrogaster*	Marshall County, OK
OCGR-5049	Sam Noble Museum of Natural History	*N. fasciata*	Atoka County, OK
OCRGR-12651	Sam Noble Museum of Natural History	*N. fasciata*	McCurtain County, OK

UF-105093	Florida Museum of Natural History	*N. fasciata*	Gilchrist County, FL
UF-16445	Florida Museum of Natural History	*N. fasciata*	Wakulla County, FL
UF-76288	Florida Museum of Natural History	*N. fasciata*	Hamilton County, FL
UF-92208	Florida Museum of Natural History	*N. fasciata*	Taylor County, FL
UF-93581	Florida Museum of Natural History	*N. fasciata*	Escambia County, FL
UF-93629	Florida Museum of Natural History	*N. fasciata*	Alachua County, FL
AF402918.1	GenBank	*R. grahami*	
KF258650.1	GenBank	*R. grahami*	
AF402917.1	GenBank	*R. septemvittata*	
MT80139.1	GenBank	*R. septemvittata*	
AF402914.1	GenBank	*N. taxispilota*	
MT801378.1	GenBank	*N. taxispilota*	
GQ285600.1	GenBank	*N. erythrogaster*	
GQ285601.1	GenBank	*N. erythrogaster*	
GQ285599.1	GenBank	*N. erythrogaster*	
GQ285598.1	GenBank	*N. erythrogaster*	
MZ080424.1	GenBank	*N. sipedon*	
MT801373.1	GenBank	*N. sipedon*	
MT801374.1	GenBank	*N. sipedon*	
MT801375.1	GenBank	*N. sipedon*	

APPENDIX B:

Institutional Animal Care and Use Committee (IACUC)

Institutional Animal Care and Use Committee (IACUC)
Animal Use
Protocol Modification Approval

February 19, 2024

Dr. John Phillips
Department of Biology
Valdosta State University

Dear Dr. Phillips;

Your modification request for your AUP titled, *“Population Genetics of Reptiles and Amphibians in the Southeastern United States*” (AUP-00084-2023) has been approved by the Institutional Animal Care and Use Committee (IACUC). In your annual report you will need to include the numbers used for all species. This approval is from 02.17.2024 – 03.01.2026. At that time your protocol will expire and you will need to submit a final report. You will be contacted by the Office of Sponsored Programs and Research Administration approximately one month before the final. If you would like to continue research on the project after 03.01.2026, a new Animal Use Protocol will need to be submitted to the IACUC.

Please remember that you must obtain IACUC approval before amending, or altering the scope, or procedures of the protocol. You are also required to report to attending Veterinarian, the IACUC Chair, and the IACUC Administrator any unanticipated problems with the animals that become apparent during the course, or as a result of the research, or teaching activity.

Should you have questions concerning your approved research, please contact Tina Wright, Research Compliance Specialist, at 229.253.2947, or email IACUC at iacuc@valdosta.edu.

Sincerely,

Ann
Elizabeth “Ann” Olphie
IACUC Administrator

cc: Dr. Becky da Cruz, Associate Provost for Graduate Studies and Research
Dr. Teresa Doscher, Attending Veterinarian
Dr. Robert L. Gannon, Department Head

OFFICE *of* SPONSORED PROGRAMS & RESEARCH ADMINISTRATION (OSPRA)
PHONE 229.259.5045 **FAX** 229.245.3853 **WEB** www.valdosta.edu/opsra
ADDRESS 1500 N. Patterson St. Valdosta, GA 31698 **LOCATION** Third Floor – Psychology Building
A Regional University of the University System of Georgia and an Equal Opportunity Institution

APPENDIX C:

Collection Permits

SCDNR
Scientific Collection Permit

Permit #SC-130-2024 **Date Issued: 5/16/2024**

This Permit Authorizes

Dr. John Phillips, Valdosta State University
(Permittee)

1500 N. Patterson, Valdosta, GA 31698
(Address)

Signed:

Emily Kearse, Permitting Biologist
Division of Wildlife & Freshwater Fisheries

Species: non-listed herps

Authorized to collect non-lethal genetic samples (no more than 20 per site) of non-listed herps for population genetic and systematic studies. Collected samples will be exported from South Carolina to lab at Valdosta State University. **No live animals may be exported from the state.** Specimens may be caught by hand or with minnow traps and released unharmed at the point of capture. Traps must be labeled with identifying information and checked daily. Collection gear, boots, and other equipment needs to be sterilized between sampling locales.

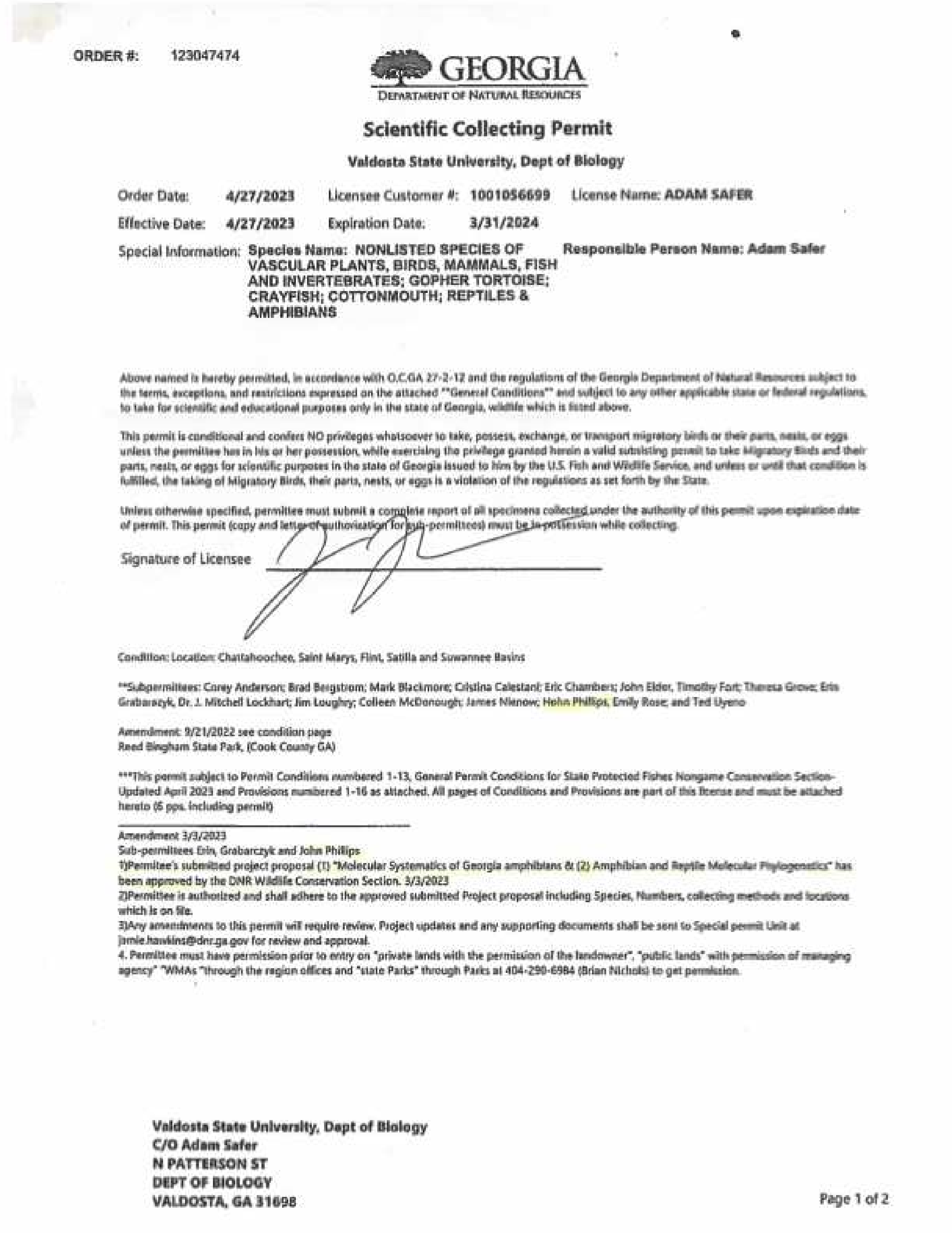
Location: Congaree Creek HP, Marsh WMA, Santee Coastal Reserve WMA, Tom Yawkey Wildlife Center, and Waccamaw River HP/WMA

Subpermittee: Ali Ashraf

Conditions: SCDNR staff must be notified by email at least 2 days prior to surveying and approve of sampling location on property. An article of international orange clothing must be worn while surveying for safety. This permit does not authorize these activities on any property, public or private, without the consent of the owner or custodian thereof. This permit, neither directly nor by implication, grants the right of trespass. No wildlife shall be collected. All collected data will be provided to SCDNR through https://scientific-collection-permits-scdnr.hub.arcgis.com/

Expiration Date: December 31, 2024

Permit Renewal Contingent on Annual Activity Summary



ORDER #: 123047474

GEORGIA
DEPARTMENT OF NATURAL RESOURCES

Scientific Collecting Permit

Valdosta State University, Dept of Biology

Order Date: **4/27/2023** Licensee Customer #: **1001056699** License Name: **ADAM SAFER**

Effective Date: **4/27/2023** Expiration Date: **3/31/2024**

Special Information: **Species Name: NONLISTED SPECIES OF VASCULAR PLANTS, BIRDS, MAMMALS, FISH AND INVERTEBRATES; GOPHER TORTOISE; CRAYFISH; COTTONMOUTH; REPTILES & AMPHIBIANS** **Responsible Person Name: Adam Safer**

Above named is hereby permitted, in accordance with O.C.GA 27-2-12 and the regulations of the Georgia Department of Natural Resources subject to the terms, exceptions, and restrictions expressed on the attached **"General Conditions"** and subject to any other applicable state or federal regulations, to take for scientific and educational purposes only in the state of Georgia, wildlife which is listed above.

This permit is conditional and confers NO privileges whatsoever to take, possess, exchange, or transport migratory birds or their parts, nests, or eggs unless the permittee has in his or her possession, while exercising the privilege granted herein a valid subsisting permit to take Migratory Birds and their parts, nests, or eggs for scientific purposes in the state of Georgia issued to him by the U.S. Fish and Wildlife Service, and unless or until that condition is fulfilled, the taking of Migratory Birds, their parts, nests, or eggs is a violation of the regulations as set forth by the State.

Unless otherwise specified, permittee must submit a complete report of all specimens collected under the authority of this permit upon expiration date of permit. This permit (copy and letter of authorization for sub-permittees) must be in possession while collecting.

Signature of Licensee

Condition: Location: Chattahoochee, Saint Marys, Flint, Satilla and Suwannee Basins

**Subpermittees: Corey Anderson; Brad Bergstrom; Mark Blackmore; Cristina Calestani; Eric Chambers; John Elder, Timothy Fort; Theresa Grove; Erin Grabarczyk, Dr. J. Mitchell Lockhart; Jim Loughry; Colleen McDonough; James Nienow; Hohn Phillips, Emily Rose; and Ted Uyeno

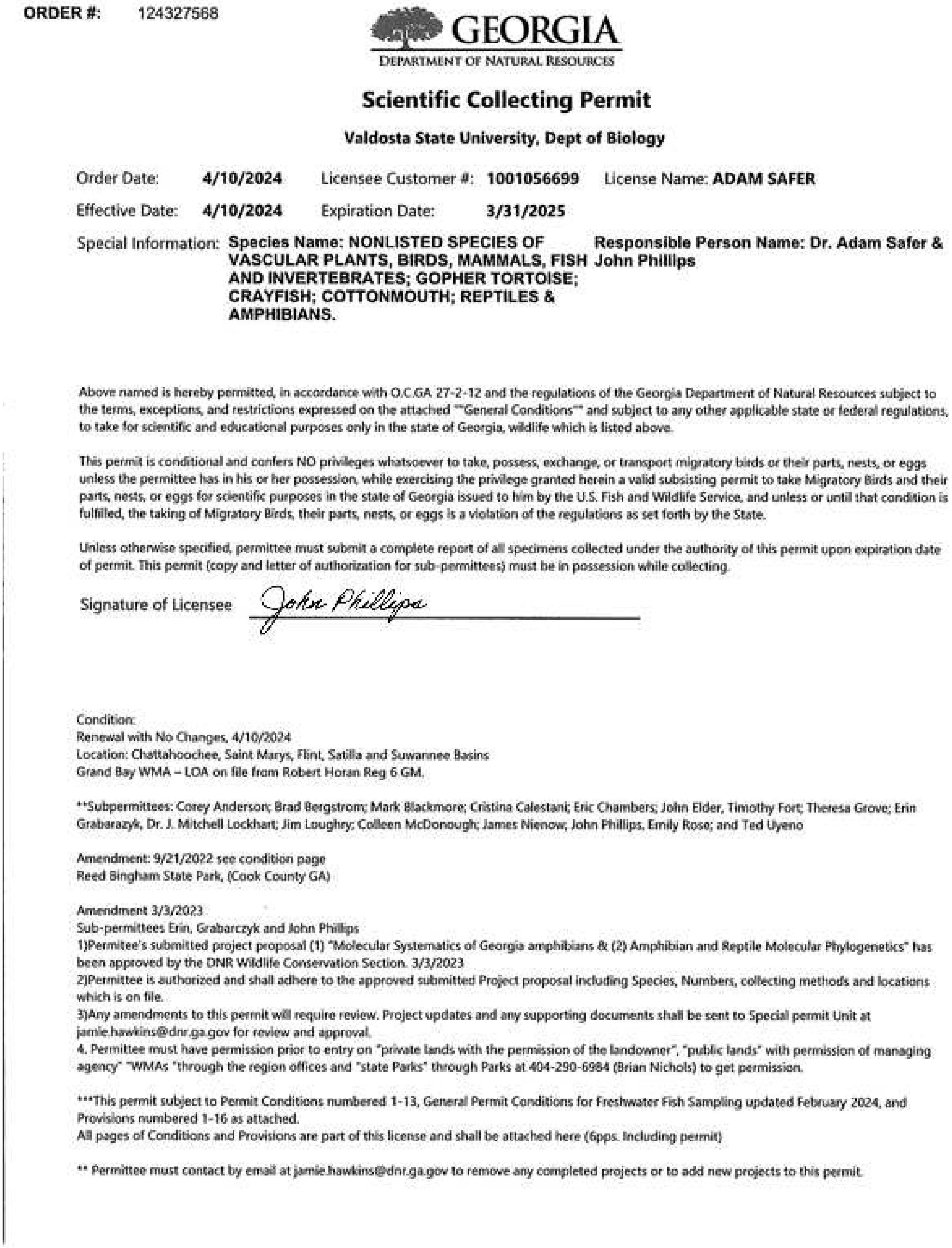
Amendment: 9/21/2022 see condition page
Reed Bingham State Park, (Cook County GA)

***This permit subject to Permit Conditions numbered 1-13, General Permit Conditions for State Protected Fishes Nongame Conservation Section-Updated April 2023 and Provisions numbered 1-16 as attached. All pages of Conditions and Provisions are part of this license and must be attached hereto (6 pps. including permit)

Amendment 3/3/2023
Sub-permittees Erin, Grabarczyk and John Phillips
1)Permitee's submitted project proposal (1) "Molecular Systematics of Georgia amphibians & (2) Amphibian and Reptile Molecular Phylogenetics" has been approved by the DNR Wildlife Conservation Section. 3/3/2023
2)Permittee is authorized and shall adhere to the approved submitted Project proposal including Species, Numbers, collecting methods and locations which is on file.
3)Any amendments to this permit will require review. Project updates and any supporting documents shall be sent to Special permit Unit at jamie.hawkins@dnr.ga.gov for review and approval.
4. Permittee must have permission prior to entry on "private lands with the permission of the landowner", "public lands" with permission of managing agency" "WMAs "through the region offices and "state Parks" through Parks at 404-290-6984 (Brian Nichols) to get permission.

Valdosta State University, Dept of Biology
C/O Adam Safer
N PATTERSON ST
DEPT OF BIOLOGY
VALDOSTA, GA 31698

Page 1 of 2



ORDER #: 124327568

GEORGIA
DEPARTMENT OF NATURAL RESOURCES

Scientific Collecting Permit

Valdosta State University, Dept of Biology

Order Date: **4/10/2024** Licensee Customer #: **1001056699** License Name: **ADAM SAFER**

Effective Date: **4/10/2024** Expiration Date: **3/31/2025**

Special Information: **Species Name: NONLISTED SPECIES OF VASCULAR PLANTS, BIRDS, MAMMALS, FISH AND INVERTEBRATES; GOPHER TORTOISE; CRAYFISH; COTTONMOUTH; REPTILES & AMPHIBIANS.**

Responsible Person Name: Dr. Adam Safer & John Phillips

Above named is hereby permitted, in accordance with O.C.GA 27-2-12 and the regulations of the Georgia Department of Natural Resources subject to the terms, exceptions, and restrictions expressed on the attached ""General Conditions"" and subject to any other applicable state or federal regulations, to take for scientific and educational purposes only in the state of Georgia, wildlife which is listed above.

This permit is conditional and confers NO privileges whatsoever to take, possess, exchange, or transport migratory birds or their parts, nests, or eggs unless the permittee has in his or her possession, while exercising the privilege granted herein a valid subsisting permit to take Migratory Birds and their parts, nests, or eggs for scientific purposes in the state of Georgia issued to him by the U.S. Fish and Wildlife Service, and unless or until that condition is fulfilled, the taking of Migratory Birds, their parts, nests, or eggs is a violation of the regulations as set forth by the State.

Unless otherwise specified, permittee must submit a complete report of all specimens collected under the authority of this permit upon expiration date of permit. This permit (copy and letter of authorization for sub-permittees) must be in possession while collecting.

Signature of Licensee John Phillips

Condition:
Renewal with No Changes, 4/10/2024
Location: Chattahoochee, Saint Marys, Flint, Satilla and Suwannee Basins
Grand Bay WMA – LOA on file from Robert Horan Reg 6 GM.

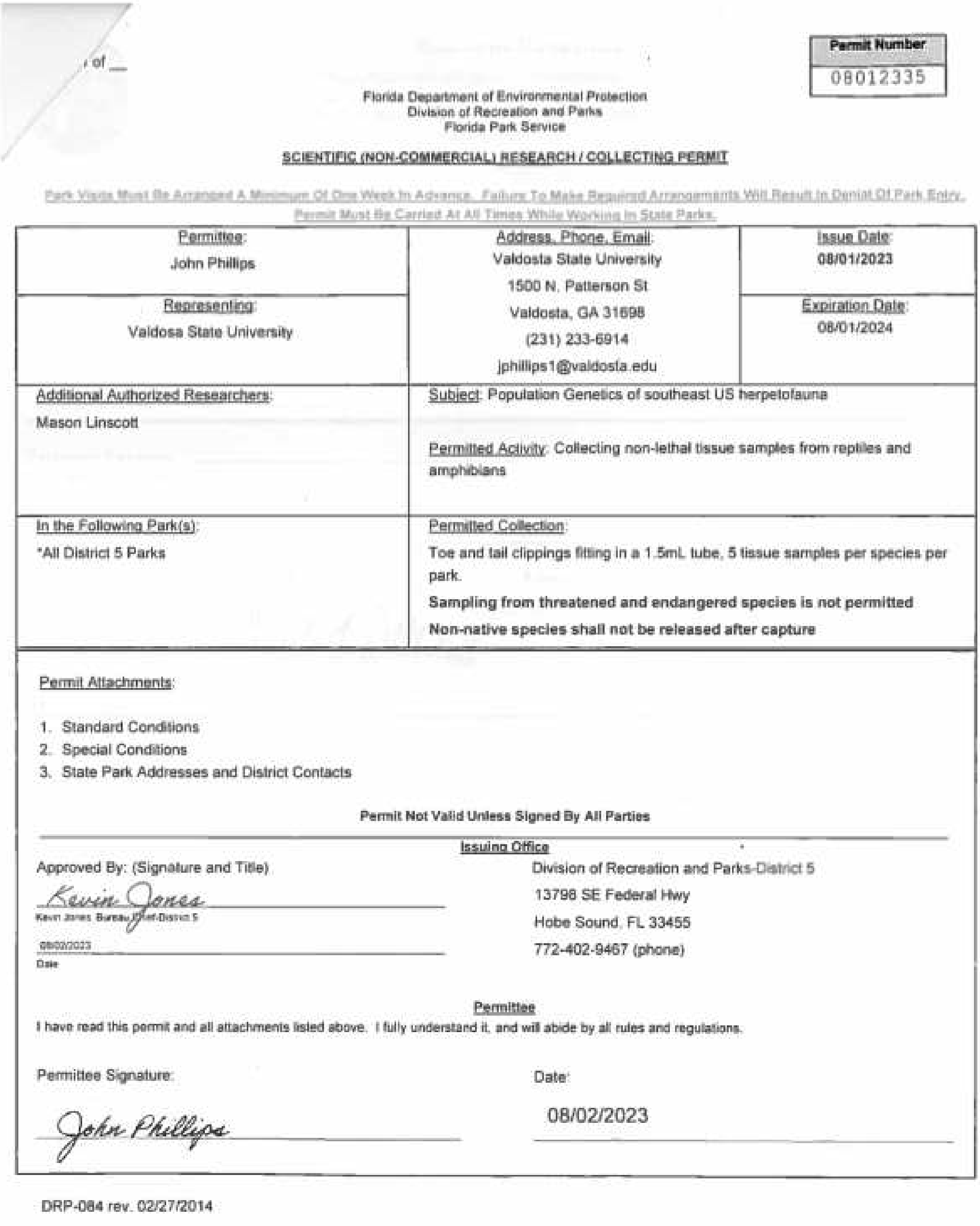
**Subpermittees: Corey Anderson; Brad Bergstrom; Mark Blackmore; Cristina Calestani; Eric Chambers; John Elder, Timothy Fort; Theresa Grove; Erin Grabarazyk, Dr. J. Mitchell Lockhart; Jim Loughry; Colleen McDonough; James Nienow; John Phillips, Emily Rose; and Ted Uyeno

Amendment: 9/21/2022 see condition page
Reed Bingham State Park, (Cook County GA)

Amendment 3/3/2023
Sub-permittees Erin, Grabarczyk and John Phillips
1)Permitee's submitted project proposal (1) "Molecular Systematics of Georgia amphibians & (2) Amphibian and Reptile Molecular Phylogenetics" has been approved by the DNR Wildlife Conservation Section. 3/3/2023
2)Permittee is authorized and shall adhere to the approved submitted Project proposal including Species, Numbers, collecting methods and locations which is on file.
3)Any amendments to this permit will require review. Project updates and any supporting documents shall be sent to Special permit Unit at jamie.hawkins@dnr.ga.gov for review and approval.
4. Permittee must have permission prior to entry on "private lands with the permission of the landowner", "public lands" with permission of managing agency" "WMAs "through the region offices and "state Parks" through Parks at 404-290-6984 (Brian Nichols) to get permission.

***This permit subject to Permit Conditions numbered 1-13, General Permit Conditions for Freshwater Fish Sampling updated February 2024, and Provisions numbered 1-16 as attached.
All pages of Conditions and Provisions are part of this license and shall be attached here (6pps. Including permit)

** Permittee must contact by email at jamie.hawkins@dnr.ga.gov to remove any completed projects or to add new projects to this permit.



of __

Permit Number
08012335

Florida Department of Environmental Protection
Division of Recreation and Parks
Florida Park Service

SCIENTIFIC (NON-COMMERCIAL) RESEARCH / COLLECTING PERMIT

Park Visits Must Be Arranged A Minimum Of One Week In Advance. Failure To Make Required Arrangements Will Result In Denial Of Park Entry.
Permit Must Be Carried At All Times While Working In State Parks.

Permittee: John Phillips	Address, Phone, Email: Valdosta State University 1500 N. Patterson St Valdosta, GA 31698 (231) 233-6914 jphillips1@valdosta.edu	Issue Date: **08/01/2023**
Representing: Valdosa State University		Expiration Date: 08/01/2024

Additional Authorized Researchers: Mason Linscott	Subject: Population Genetics of southeast US herpetofauna Permitted Activity: Collecting non-lethal tissue samples from reptiles and amphibians
In the Following Park(s): *All District 5 Parks	Permitted Collection: Toe and tail clippings fitting in a 1.5mL tube, 5 tissue samples per species per park. **Sampling from threatened and endangered species is not permitted** **Non-native species shall not be released after capture**

Permit Attachments:

1. Standard Conditions
2. Special Conditions
3. State Park Addresses and District Contacts

Permit Not Valid Unless Signed By All Parties

Issuing Office

Approved By: (Signature and Title)

Kevin Jones
Kevin Jones Bureau Chief-District 5

08/02/2023
Date

Division of Recreation and Parks-District 5
13798 SE Federal Hwy
Hobe Sound, FL 33455
772-402-9467 (phone)

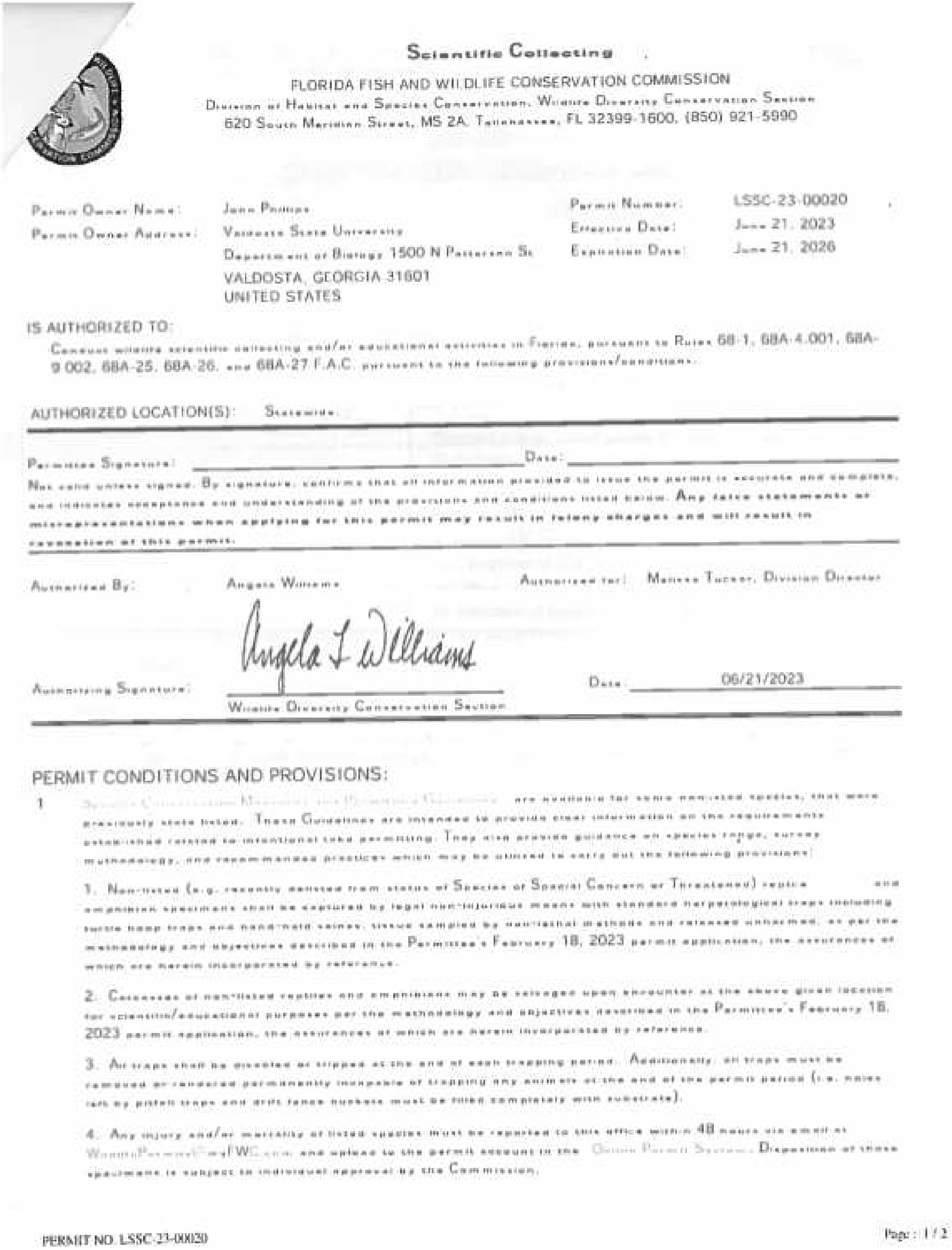
Permittee

I have read this permit and all attachments listed above. I fully understand it, and will abide by all rules and regulations.

Permittee Signature: John Phillips

Date: 08/02/2023

DRP-084 rev. 02/27/2014



Scientific Collecting

FLORIDA FISH AND WILDLIFE CONSERVATION COMMISSION
Division of Habitat and Species Conservation, Wildlife Diversity Conservation Section
620 South Meridian Street, MS 2A, Tallahassee, FL 32399-1600, (850) 921-5990

Permit Owner Name: John Phillips
Permit Owner Address: Valdosta State University
Department of Biology 1500 N Patterson St
VALDOSTA, GEORGIA 31601
UNITED STATES

Permit Number: LSSC-23-00020
Effective Date: June 21, 2023
Expiration Date: June 21, 2026

IS AUTHORIZED TO:

Conduct wildlife scientific collecting and/or educational activities in Florida, pursuant to Rules 68-1, 68A-4.001, 68A-9.002, 68A-25, 68A-26, and 68A-27 F.A.C. pursuant to the following provisions/conditions.

AUTHORIZED LOCATION(S): Statewide.

Permittee Signature: ______________________ Date: ______________________

Not valid unless signed. By signature, confirms that all information provided to issue the permit is accurate and complete, and indicates acceptance and understanding of the provisions and conditions listed below. **Any false statements or misrepresentations when applying for this permit may result in felony charges and will result in revocation of this permit.**

Authorized By: Angela Williams
Authorized for: Melissa Tucker, Division Director

Authorizing Signature: Angela L Williams
Wildlife Diversity Conservation Section
Date: 06/21/2023

PERMIT CONDITIONS AND PROVISIONS:

1 Species Conservation Measures and Permitting Guidelines are available for some non-listed species, that were previously state listed. These Guidelines are intended to provide clear information on the requirements established related to intentional take permitting. They also provide guidance on species range, survey methodology, and recommended practices which may be utilized to carry out the following provisions:

1. Non-listed (e.g. recently delisted from status of Species of Special Concern or Threatened) reptile and amphibian specimens shall be captured by legal non-injurious means with standard herpetological traps including turtle hoop traps and hand-held seines, tissue sampled by non-lethal methods and released unharmed, as per the methodology and objectives described in the Permittee's February 18, 2023 permit application, the assurances of which are herein incorporated by reference.

2. Carcasses of non-listed reptiles and amphibians may be salvaged upon encounter at the above given location for scientific/educational purposes per the methodology and objectives described in the Permittee's February 18, 2023 permit application, the assurances of which are herein incorporated by reference.

3. All traps shall be disabled or tripped at the end of each trapping period. Additionally, all traps must be removed or rendered permanently incapable of trapping any animals at the end of the permit period (i.e. holes left by pitfall traps and drift fence buckets must be filled completely with substrate).

4. Any injury and/or mortality of listed species must be reported to this office within 48 hours via email at WildlifePermits@MyFWC.com and upload to the permit account in the Online Permit System. Disposition of these specimens is subject to individual approval by the Commission.

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