Impacts of landscape and past climate on patterns of hybridization and genetic diversity in *Plestiodon tetragrammus* and *P. multivirgatus*

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Abstract

Impacts of landscape and past climate on patterns of hybridization and genetic diversity in *Plestiodon tetragrammus* and *P. multivirgatus*

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Past hybridization plays an important role in evolution of taxa. Here I test for the effects of hybridization on molecular evolution and diversity and how past climate conditions have facilitated hybridization in the four-lined skinks, *P. tetragrammus*, and the variable skink, *P. multivirgatus*. In my second chapter, I use a dataset consisting of multiple mitochondrial and nuclear genes to determine whether *P. tetragrammus* is monophyletic and suggest that past hybridization has led to capture of mitochondrial genes between *P. t. brevilineatus* and *P. multivirgatus*. In my third chapter, I show that mitochondrial genes have been introgressed between *P. multivirgatus* and *P. t. brevilineatus* with no introgression of nuclear genes. In my fourth chapter, I show that past climate conditions have led to hybridization between *P. t. brevilineatus* and *P. multivirgatus* by causing their ranges to overlap to a large degree, resulting in the patterns of genetic diversity shown in the previous sections.

Contents

I. **Introduction to hybridization and skinks**

Hybridization, which is the mating of two genetically distinct groups which leads to offspring, was not always considered an important part of evolutionary biology or speciation (Mallet, 2005). Hybridization was known to occur even by Linnaeus and Darwin (Harrison, 2012; Taylor, 1935), but it was thought to be either rare or ineffectual because hybrid offspring were less fertile. Indeed, the classic definition of a species was based on the inability of two groups to produce viable offspring-Mayr's biological species concept (Dobzhansky, 1951; Mayr, 1942). However, hybridization and gene flow between species is increasingly recognized as the rule rather than the exception in many systems (Abbott et al., 2013). The impact of this widespread hybridization on fitness can be neutral, deleterious, or positive (Abbott et al., 2013; Bock et al., 2015; Currat et al., 2008). The common genomic result of hybridization is the incorporation of genetic information from other species through backcrossing of hybrids and is termed introgression (Anderson & Hubricht, 1938). While the negative impacts of hybridization may be intuitive, the positive impact could be due to different combinations of alleles or genes which wouldn't have been possible within just one of the species (Bock et al., 2015; Rius & Darling, 2014). Beyond fitness, the evolutionary persistence of the introgressed genes in either hybridizing species can also be contingent and variable (Abbott et al., 2013; Bock et al., 2015; Good et al., 2015; Harrison & Larson, 2014). Cytoplasmic sources of DNA like chloroplasts and mitochondria are usually inherited uniparentally (usually from the mother) and thus have much smaller effective population sizes. These smaller effective population sizes lead to increased chances of fixation (Currat et al., 2008; Sloan et al., 2017; Wertheim et al., 2015).

Evidence of introgression has been found in many different species of animals, plants, even humans (Bachtrog et al., 2006; Reich et al., 2010; Toews & Brelsford, 2012). While the outcome of introgression is often clear, the conditions that lead to introgression are less well known.Potential opportunities for introgression include diverse phenomena such as range shifts or introduction of alien species (Bock et al., 2015; Good et al., 2008; Marques et al., 2017). In particular, the glacial cycling of the late Miocene/Pleistocene (2 mya to 40kya) is one of the most commonly cited causes for both bringing species together and for fostering diversity (Avise et al., 1998; Stewart & Lister, 2001). During this time-period the earth experienced periods of warming and cooling as glaciers marched and retreated multiple times. These cycles are credited with separating sympatric species and/or bringing allopatric species into sympatry allowing different lineages to diversify, as species ranges moved with changing climate (Avise et al., 1998; Hewitt, 2004). It would be useful to look at a group of organisms that have shown signs of introgression between species that live in a region with well documented prior historical climate and geographical changes in order to examine the conditions that lead to hybridization. I sought to test the role of landscape and changing climate on hybridization and introgression in a group of North American skinks.

Skinks are lizards in the order Squamata and the family Scincidae. This family of lizards comprises over 1600 species (Uetz & Hallerman, 2017), roughly a quarter of all lizard diversity, and members are found in all habitat types excluding tundra and boreal forest. Exactly how many clades or subfamilies within Scincidae is recently a hotly debated topic (Lambert et al., 2015; Linkem et al., 2016; Pyron et al., 2013), with some authors arguing for splitting of the family into smaller units (Hedges, 2014; Hedges & Conn, 2012). Skinks have been widely studies in evolutionary biology because of this diversity, but also because of several interesting

morphological characteristics such as multiple instances of limb reduction and loss (Greer, 1991), multiple reproductive strategies (oviparity, viviparity)(Blackburn, 1982; Watson et al., 2014), as well as the convergent evolution of a conspicuously colored juvenile tail for predator avoidance (Watson et al., 2012). Additional studies have also found that cryptic diversity is fairly common when studying skinks at the species level (Alfonso Silva et al., 2017; Carranza et al., 2008). Unfortunately, many groups have not been studied at the species level with current systematics tools. Almost all skinks inhabiting North America are in the genus *Plestiodon* (excepting *Scincella lateralis*), which likely crossed into North America via Beringia in the late Oligocene approximately 26-27 mya, along with many other squamate groups (Brandley et al., 2010). There are 28 currently recognized species of *Plestiodon* in North America that range from southern Ontario, Canada to southern Mexico (Brandley et al., 2012; Bryson et al., 2017). Recent studies have revealed rampant cryptic diversity and possible parallel evolution of juvenile traits (Bryson et al., 2017; Feria-Ortiz et al., 2011; Richmond, 2006). For my dissertation, I focused on a closely related group of species and subspecies of *Plestiodon*: the many-lined skink (*Plestiodon multivirgatus),* the four-lined skink (*Plestiodon tetragrammus tetragrammus*) and the short-lined skink (*Plestiodon tetragrammus brevilineatus*).

The definition and usefulness of subspecies in biology is debated (Burbrink et al., 2000; Sackett et al., 2014), and genetically distinct subspecies are often elevated to species (Burbrink, 2001; Streicher et al., 2012). The focus of my dissertation is a species complex with subspecies on either side of a potential geographic barrier (*Plestiodon tetragrammus).* This species is currently separated into two geographically disjunct subspecies, *P. t. tetragrammus* and *P. t. brevilineatus* (Lieb, 1985). The four-lined skink (*P. t. tetragrammus*) is found in Texas south of the Balcones Escarpment down the Mexican coast to northern Veracruz. The short-lined skink,

P. t. brevilineatus, is distributed north and west of the Balcones Escarpment into northern Mexico, and both western and northern Texas (Fig. 1.1). The Balcones Escarpment and Edwards Plateau are important barriers and regions of endemism in North America (Andersen & Light, 2012; Castoe et al., 2007; Chippindale et al., 2000; Edwards et al., 2004; Neiswenter & Riddle, 2010). In addition to geographic distribution, the subspecies are distinguished by external morphology (Fig. 1.2), as the length of the lateral stripes terminate at the forelimb in *P. t. brevilineatus* and extend to the hindlimb in *P. t. tetragrammus* (Lieb, 1985). The sister species of the four-lined skink is thought to be the many-lined skink, *P. multivirgatus* (Brandley et al., 2011; Richmond, 2006). One subspecies of the many-lined skink, the variable skink *P. m. epipleurotis*, is found from Arizona to western Texas. The other subspecies of the northern many-lined skink, *P. m. multivirgatus*, is found from northeastern Colorado to eastern Nebraska (Fig. 1.1). The most recent taxonomic study of *P. tetragrammus* was performed by Lieb (1985), in which he placed *P. t. brevilineatus* and *P. t. tetragrammus* as subspecies of *P. tetragrammus* instead of their own species. This was based on the lack of identifiable morphological characteristics beyond the extension of the lateral light and dark lines. No genetic data were used. The most recent taxonomic study examining relationships within *P. multivirgatus* was performed by Mecham (1957), where two varying color phases, *P. taylori* and *P. gaigei*, were synonymized into *P. multivirgatus* based on a misunderstanding in previous studies of the lightening of a dorsal line as the skinks age. The southern population was given the subspecific designation *P. m. gaigei*. This name was changed to *P. m. epipleurotis* by Axtell and Smith (2004) based on priority rules in the International Code for Zoological Nomenclature ICZN.

In the ensuing dissertation, I tested for the molecular evolution and genetic diversity consequences of introgression for both nuclear and mitochondrial genomes and how landscape

and past climate can facilitate hybridization and introgression between species. In chapter 2 (already published; Moseley et al., 2015), I use a multilocus dataset of mitochondrial DNA and nuclear DNA to test the monophyly of *P. tetragrammus* and suggest that there was past hybridization between *P. t. brevilineatus* and *P. multivirgatus*. In chapter 3, I use high-throughput genomic techniques to test whether there is evidence for hybridization in both the mitochondrial and nuclear genome. In chapter 4, I used species distribution models to test how geography and past climate have shaped the evolutionary history of hybridization and resulting patterns of genetic diversity. Finally, in chapter 5, I summarize and synthesize how my dissertation work can link landscape-level analyses to mitochondrial and nuclear genomics to understand the pattern and process of hybridization and its influence on genetic diversity and the evolution of organisms.

Chapter II. Phylogeography and lineage specific patterns of introgression in *P. tetragrammus*

This chapter has been published:

Moseley MA, Cox CL, Streicher JW, Roelke CR, Chippindale PT. 2015. Phylogeography and lineage-specific patterns of genetic diversity and molecular evolution in a group of North American skinks. *Biological Journal of the Linnaean Society* **116:** 819-833.

A. Introduction

The geographical features of a landscape can play an important role in structuring patterns of diversification in terrestrial organisms (Arbogast & Kenagy, 2001; Bermingham & Moritz, 1998; Swenson & Howard, 2005). For example, landscapes that lack significant barriers to biotic interchange can facilitate high rates of gene flow among populations, effectively limiting opportunities for diversification (Hewitt, 2001; Manel et al., 2003). In contrast, geographical features can be barriers to migration, limiting gene flow and promoting divergence between lineages (Hewitt, 2001; Manel et al., 2003). Following speciation, the landscape may further influence evolutionary trajectories by enabling or preventing interspecific gene flow between sister species (Bryson et al., 2010; Hewitt, 2001). Thus, landscapes can impact genetic diversity at multiple evolutionary tiers by generating opportunities for demographic shifts, adaptation and hybridization (Donnelly et al., 2004; Saetre et al., 2001; Toews & Brelsford, 2012).

Geography can have multiple impacts on the molecular evolution of recently diverged lineages. First, limited gene flow at the geographical range edge can lead to lower genetic diversity at the geographical range periphery (Blows & Hoffmann, 2005; Trumbo et al., 2013). This can be exacerbated during a rapid geographical range expansion, where allelic variants can increase in frequency and move with the new range front whether they are deleterious, neutral or advantageous (Excoffier et al., 2009), and can lead to the stochastic depletion of genetic variation at the geographical range front (Makowsky et al., 2009; Mattoccia et al., 2011; Streicher et al., 2012). Second, rapid adaptive evolution and selective sweeps during geographical expansion can also lead to the loss of genetic diversity (Galtier & Duret, 2007; Irwin et al., 2009). Finally, mitochondrial introgression in the geographical contact zones

between recently divergent lineages can create mitonuclear discordance and molecular evolution dynamics (Bachtrog et al., 2006; Boratyński et al., 2014; Gompert et al., 2008; Jiggins, 2003; Jones & Searle, 2015; Toews & Brelsford, 2012; Wiens et al., 2010). Introgression can also lead to the uncoupling of mtDNA and nDNA substitution rates because of divergent evolutionary history in different geographical areas and even lead to an increase in the mutation rate in the mitochondria as it adapts to the new host nuclear genome (Bryson et al., 2010; Linnen & Farrell, 2007; McGuire et al., 2007). Examining variation in molecular evolution among geographically delimited lineages can help explain how geography and evolutionary relationships can shape intraspecific patterns of genetic diversity.

We studied phylogeography and molecular evolution of taxa in south-western North America, focusing on Texas and northern Mexico. One of the most important geographical features in this landscape is the Balcones Escarpment of the Edwards Plateau, which is located in central Texas (see Fig. 1) and can act as both an important barrier to gene flow and as a foster of endemism (e.g. cotton rats: Andersen & Light, 2012; snakes: Castoe et al., 2007; salamanders: Chippindale et al., 2000; fish: Edwards et al., 2004; pocket mice: Neiswenter & Riddle, 2010; cave crickets: Weckstein et al., 2007). This geographical feature provides an excellent opportunity to characterize patterns of molecular evolution in lineages separated by a potential barrier to gene flow.

Our research focuses on the four-lined skink, *Plestiodon tetragrammus* Baird, 1859, and the many-lined skink, *Plestiodon multivirgatus* Hallowell, 1857, which are sister taxa (Brandley et al., 2012) and are distributed in south-western North America. The many-lined skink is endemic to the United States (but see unconfirmed Mexican record, Smith & Taylor, 1950) and is found in Arizona, Colorado, New Mexico, Nebraska, South Dakota, Utah, Wyoming and

Texas. A disjunct population of *P. multivirgatus* occurs in west-central Texas (Fig. 1.1). Two subspecies of the many lined skink, the northern many-lined skink (*P. m. multivirgatus*) and the variable skink (*P. m. epipleurotus*), have been named and some authors have suggested that these be recognized as separate species (Hammerson, 1999), although this is not widely accepted (Crother, 2012). The four-lined skink occurs in the United States and Mexico and is currently separated into two geographically distinctive subspecies (Lieb, 1985a). The four-lined skink, *P. t. tetragrammus*, is found in Texas south of the Balcones Escarpment down the Mexican coast to northern Veracruz. The short-lined skink, P. t. brevilineatus Cope (1880), is distributed north and west of the Balcones Escarpment of central Texas into northern Mexico, and both western and northern Texas. Beyond geographical distribution, these two subspecies can be distinguished based on external morphology (Fig. 1.2), as the lateral stripes terminate at the forelimb in *P. t. brevilineatus* and extend to the hindlimb in *P. t. tetragrammus* (Lieb, 1985).

We analysed how genetic divergence of populations across the geographical landscape impacts patterns of molecular evolution among populations of *P. multivirgatus* and *P. tetragrammus*. While the utility of subspecies in evolutionary biology is debatable (Burbrink et al., 2000; Phillimore & Owens, 2006), and genetically distinct subspecies are often elevated to species (Burbrink, 2001; Streicher et al., 2012), we treated *P. t. brevilineatus* and *P. t. tetragrammus* as potentially distinct lineages given their parapatric distributions and distinct morphologies. Using nuclear and mitochondrial loci, we reconstructed phylogenetic relationships of both subspecies of *P. tetragrammus* and southern populations of *P. multivirgatus* and analysed levels of genetic variation and substitution rates in each species. We hypothesized that geographical distribution would shape patterns of genetic diversity and molecular evolution

among lineages, and that any introgression between lineages would decouple mitochondrial and nuclear evolution among lineages.

B. Methods

Taxon and Tissue Collection

Skinks were collected by turning rocks and cover objects in appropriate habitat across their geographic range from 2008 to 2013 (Fig. 1.2). We preserved muscle, liver, or skin tissue in lysis buffer, 95% ethanol, or an RNA-preserving buffer. Specimens were deposited in the University of Texas Arlington Amphibian and Reptile Diversity Research Center. We obtained additional tissues from the Texas Museum of Natural History (TNHC collection) at the University of Texas Austin (Table S1). We used a hierarchical outgroup strategy to root our analyses, with multiple other *Plestiodon* species (*Plestiodon japonicus*, *P. septentrionalis*, *P. fasciatus*, *P. anthracinus*, *P. obsoletus*, and *P. inexpectatus*), a more distantly related skink (*Scincella lateralis*), and a lizard species from another lineage (Family Gerrhosauridae; *Gerrhosaurus major)* as the most distant outgroup.

DNA Isolation, PCR, and Sequencing

DNA was isolated using Qiagen DNeasy (Qiagen, Valencia, CA) kits following standard protocols. We amplified and sequenced three mitochondrial genes, totaling 1579 bp: a fragment of NADH dehydrogenase subunit 1 and flanking tRNAs (481 bp), 16S ribosomal RNA gene (462 bp), and 12S ribosomal RNA gene (634 bp). We amplified and sequenced four nuclear genes, totaling 1876 bp: synuclein alpha interacting protein (*SNCAIP*, 410 bp), prolactin-like

receptor (*PRLR*, 564 bp), brain derived neurotropin factor (*BDNF*, 547 bp), and oocyte maturation factor (*c-mos,* 352 bp). Primer information is given in Table S2. All mitochondrial loci were amplified with an initial 2 min denaturation cycle at 95°C followed by 35 cycles of 30 s denaturation at 95 °C, 30 s annealing at 50 °C, and a 1 min extension at 72 °C., Following these cycles, we performed a final 10 min extension at 72 °C. Nuclear loci were amplified with a touchdown procedure using an initial 2 min denaturation step at 95°C followed by 40 cycles of 30 s at 95 °C, 30s at 56–50 °C, and 1 min at 72 °C. Following these cycles, we used a final extension of 10 min at 72 °C. We used gel electrophoresis in 1% agarose to test amplification, and we cleaned PCR products for sequencing by treatment with the ExoSAP-IT kit (United States Biochemical). We used the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems Inc.) for sequencing reactions with the sequenced products precipitated using an ethanol/sodium acetate/EDTA method and rehydrated in formamide (Hi-Di). Each sample was analyzed on an ABI PRISM 3100xl Genetic Analyzer in the Genomics Core Facility at the University of Texas Arlington. We edited and assembled sequences using Sequencher (Genes Code Corp.). We obtained sequences for outgroup taxa from GenBank. Sequences were aligned using ClustalW algorithm (Larkin et al., 2007) implemented in MEGA v5.2 (Tamura et al., 2011), using default parameters, visually inspected and trimmed, then concatenated by eye. Concatenated datasets were then partitioned by gene and codon position using sequences from Brandley *et al.* (2012). Individuals that were missing complete sequence data for any genes were removed from the analysis.

Phylogenetic reconstruction

We used MEGA v5.2 (Tamura *et al.*, 2011) to perform model testing on each gene partition. The GTR+I+G model ranked in the top 3 models for each partition based on AIC and BIC scores, so we chose this model for the subsequent analyses. We used MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001) with default priors to perform Bayesian tree searches. We ran multiple tree searches with varying priors and partition schemes without major changes to topology. We ran Markov-Chain Monte Carlo searches for 10,000,000 generations, sampling every 500 generations, using 3 heated chains and 1 cold chain and checked stationarity using parameter outputs in MrBayes (Huelsenbeck & Ronquist, 2001) and the program TRACER v. 1.4 (Drummond & Rambaut, 2007). For this and all subsequent analyses we discarded 25% of the trees as burnin. For maximum parsimony (MP) analysis we used MEGA v5.2 (Tamura *et al.*, 2011) for nuclear and mitochondrial alignments, with 10,000 bootstrap replicates for nodal support. We used RAxML v7.0.3 (Stamatakis, 2006) to conduct maximum likelihood (ML) analysis using 100 ML tree searches and 10,000 bootstrap replicates on the best scoring topology to obtain nodal support values. These processes were completed separately for each concatenated mitochondrial and nuclear dataset. We then used *BEAST (Heled & Drummond, 2010) to perform coalescent species tree analysis on the combined mitochondrial and nuclear datasets using the same priors and MCMC criteria as the Bayesian tree searches. We then used DensiTree v2.1.10 (Bouckaert, 2010) to examine variation in the topologies obtained from the coalescent analysis. We used FigTree v1.3.1 (Rambaut, 2007) for tree visualization and manipulation.

Genetic diversity and testing for evidence for species-wide mitochondrial capture in sister taxa

We implemented a series of tests to compare patterns of nuclear and mitochondrial genetic variation. These tests were designed to identify mitochondrial and nuclear variation consistent with mitochondrial introgression and assume that *P. multivirgatus* and *P. tetragrammus* are sister taxa. We used the program MEGA v5.2 to generate average within group distance measures (uncorrected "p") and SYSTAT v11 (Systat Software Inc., Chicago, Illinois, USA) to conduct several non-parametric Mann-Whitney *U* tests (Mann & Whitney, 1947) of genetic diversity. We used all available sequences for each locus to calculate uncorrected "*p*" distances.

First, we tested for deviation from a well-documented pattern of molecular evolution in animals; mtDNA evolves more rapidly than nDNA (Brown, George Jr & Wilson, 1979; Eytan & Hellberg, 2010; Willett, 2012). To test this expectation, we compared mtDNA and nDNA genetic variation within *P. multivirgatus* and *P. tetragrammus*. If we failed to reject the null hypothesis of similar levels of intraspecific mtDNA and nDNA diversity we interpreted the test as evidence consistent with species-wide mtDNA introgression. Second, because sister taxa have been evolving for approximately the same amount of time, we expect that neutral (or nearly neutral) mutations will fix at similar rates. We tested this null expectation by comparing nDNA and mtDNA diversity between *P. mulitvirgatus* and *P. tetragrammus*. If this test rejected the null hypothesis of similar diversity levels between sister taxa for mtDNA but not for nDNA, we interpreted the test as consistent with species-wide mtDNA introgression. In this scenario we identified the taxon with lower levels of mtDNA diversity as the likely recipient of introgressed mtDNAs.

Testing for population size and neutral molecular evolution

Our genetic diversity comparisons of sister taxa assumed neutrally evolving loci and similar population sizes through time. To test these assumptions in *P. multivirgatus* and *P. tetragrammus* we performed codon-based selection tests (d_N /d_S) using MEGA v5.2. We examined translated amino acid sequences with both intra- and interspecific datasets (Table 1.1). Specifically, we used a Z-statistic to test the null hypothesis of strict-neutrality ($d_N = d_S$) against a model of positive selection ($d_N > d_S$). We used 1000 bootstrap pseudoreplicates to generate Z-statistics and p-values in all tests.

We used loci that displayed *dN/dS* ratios consistent with neutral or purifying selection to perform Bayesian Skyline analysis (Drummond et al., 2005) and extended Bayesian Skyline analysis (Heled & Drummond, 2008). We performed both skyline analyses using BEAST v1.7.5(Drummond et al., 2012) and restricted our analyses to nDNA loci given that, if present, species-wide mtDNA introgression may mislead our estimations of population demography. Our justification for these analyses was that, under a model of coalescence, demographic shifts should result in predictable patterns of nucleotide sequence divergence (Heled & Drummond, 2008). Based on simulations, the best strategies for estimating historical demography are achieved by sampling widely throughout the range of a species (Heller et al., 2013; Ho & Shapiro, 2011). Our sampling spanned large segments of the range of both species with samples of *P. multivirgatus* that originated from Arizona and Texas (n = 6) and samples of *P. tetragrammus* that originated from throughout Texas and Mexico (n = 16). A potential issue with our sampling was the small number of individuals of *P. multivirgatus*. We decided to estimate historical demographics from these samples because simulation studies have shown that the error rate of extended Bayesian skyline analysis is much more sensitive to the number of loci than

individuals (Heled & Drummond, 2008). Specifically, error rates from simulations run on 3–20 individuals with constant population sizes all performed with less than 20% relative error. Nonetheless, we acknowledge that demographic estimates we present for *P. multivirgatus* should be interpreted more cautiously than those we obtained for *P. tetragrammus*. In our extended Bayesian skyline analysis we focused on the population size change parameter (demographic.populationSizeChanges) to assess if variation was consistent with a constant population size through time. We ran tree searches for 10–50 million generations sampling every 1000 generations until the effective sample size of all parameters exceeded 200. Given the relatively conserved nature of nDNA sequences used in this study, we used a simple model of nucleotide evolution (HKY) and reduced the number of groups to 5 in Bayesian Skyline analyses to avoid excessive parameterization. For the remaining parameters we used default settings in all BEAST v1.7.5 runs and summarized log files in TRACER v1.4 (Rambaut & Drummond, 2007).

C. Results

Phylogenetic Relationships

Although phylogenetic trees derived from mitochondrial and nuclear loci differed, we found identical topologies and similar branch lengths with all methods of phylogenetic reconstruction within each marker type for the combined nDNA and combined mtDNA, so we present phylogenetic relationships from Bayesian analysis with nodal support (posterior probabilities) and maximum likelihood and parsimony (bootstrap proportion). We found that *P. tetragrammus* and *P. multivirgatus* form a monophyletic group that is sister to a clade of *P. anthracinus, P. fasciatus* and *P. septentrionalis,* which is broadly consistent with larger phylogenetic studies of skinks (Brandley et al., 2012).

Using phylogenetic reconstruction based on mitochondrial loci, we found that *P. t. tetragrammus* and *P. t. brevilineatus* were in separate, well-supported clades (Fig. 1.3). Moreover, there were two separate and well-supported clades within *P. t. brevilineatus* that corresponded to the eastern (northern and central Texas) and western (west Texas and the state of Coahuila) parts of their geographic range. Interestingly, *P. multivirgatus* mitochondrial haplotypes from across their geographic range (Texas-Arizona) were nested within *P. tetragrammus,* specifically *P. t. brevilineatus*.

Phylogenetic relationships based on nDNA between *P. multivirgatus, P. tetragrammus,* and the other North American were not well resolved. In contrast to the mitochondrial data, phylogenetic reconstructions using nuclear loci support a monophyletic *P. tetragrammus* (including *P. t. brevilineatus* and *P. t. tetragrammus*) while excluding *P. multivirgatus* (Fig. 1.3). Substructure within *P. tetragrammus* was neither consistent with subspecies designation nor geographically with clades that were resolved using mtDNA.

Coalescent species tree analysis with both mitochondrial and nuclear loci recovered *P. multivirgatus* and *P. tetragrammus* as monophyletic with strong statistical support (posterior prob. =1; Fig. 1.4). Additionally, this analysis recovered *P. t. brevilineatus* and *P. t. tetragrammus* as reciprocally monophyletic and sister to *P. multivirgatus*. Although the sister relationship between *P. multivirgatus* and *P. tetragrammus* received limited statistical support (posterior prob. = 0.63; Fig. 1.4), it is topologically consistent with previous studies (Brandley et al., 2012). Our findings that mitochondrial DNA support *P. multivirgatus* as nested within *P. t. brevilineatus,* and that this relationship is not supported by either phylogenetic analysis of concatenated nuclear loci or combined mitochondrial and nuclear loci in a coalescent framework

is consistent with mitochondrial introgression from *P. t. brevilineatus* into *P. multivirgatus* across its geographic range.

Mitochondrial and Nuclear Variation

We found that while *P. tetragrammus* had significantly different levels of mitochondrial and nuclear genetic variation (Mann-Whitney *U* test statistic = 9, *P* = 0.046), *P. multivirgatus* did not (*U* = 7.5, *P* = 0.184). This finding is consistent with a decoupling of mitochondrial and nuclear evolutionary rates in *P. multivirgatus* (Fig. 1.5).

Mann-Whitney U statistics indicated that levels of genetic variation of nuclear loci were not significantly different between *P. tetragrammus* and *P. multivirgatus* (*U* = 3, *P* = 0.484). This result matches our expectations for neutrally evolving sister taxa with similar population sizes. Mitochondrial levels of genetic variation, however, were significantly different (*U* = 0, *P* = 0.050), a result consistent with introgression. Although *P. t. brevilineatus* had higher levels of overall mitochondrial genetic variation than *P. multivirgatus* (Table 1.1), this difference was not statistically significant (*U* = 3, *P* = 0.184).

Evidence for neutral evolution and similar population sizes through time

All of our selection tests failed to reject the null model of neutral evolution (Table 1.2). Interestingly, the *SNCAIP* dataset of *P. multivirgatus* was the only test that received a *P*-value less than 1. In these sequences a segregating SNP revealed an amino acid difference of glutamine (polar neutral side chain) in the Texas population versus leucine (aliphatic hydrophobic side chain) in the Arizona population.

Bayesian skyline analysis revealed that collectively the four nDNA loci were more variable (inferred from the distribution of tree root heights) in *P. tetragrammus* than *P. multivirgatus* (Fig. 1.6; A). The number of population size changes inferred by our extended Bayesian skyline analysis was most frequently estimated at 0 for *P. tetragrammus* and 1 for *P. multivirgatus* (Fig. 1.6; B). However, 0 changes was the second-most frequently estimated state for *P. multivirgatus*. Thus, we cannot reject a history of constant population size for either species. A visual examination of the four nDNA alignments from *P. multivirgatus* revealed that the only polymorphisms were segregating sites between Arizona and Texas. In *PRLR*, there is a three nucleotide indel (present in the Arizona population) and *BDNF* and *SNCAIP* each feature a SNP that differentiate the populations. These differences may account for the single demographic shift suggested by our extended Bayesian skyline plot analysis (Fig. 1.6; B). Because nDNA sequences from *P. multivirgatus* featured less variation than *P. tetragrammus*, we scaled Bayesian skyline plots so that they were on the same timescale (Fig. 1.6; C). As expected given intraspecific levels of nDNA sequence variation, median population size estimates from scaled Bayesian skyline plots suggest that *P. tetragrammus* has had larger population sizes through recent time than *P. multivirgatus*. However, the 95% HPD intervals for each species feature substantial overlap which indicates that historic population sizes were likely similar. Collectively, selection tests and Bayesian skyline analyses produced evidence for (1) purifying or no selection on protein coding sequences and (2) similar population sizes through time.

D. Discussion

Geographic Patterns of Genetic Diversity

We found a well-supported phylogenetic break in the mtDNA dataset between individuals of *P. t. tetragrammus* and *P. t. brevilineatus*. This break is concordant with the Balcones Escarpment, suggesting this landscape feature may act as an obstacle to gene flow in *P. tetragrammus*. The impact of the Balcones Escarpment on lineage diversification has been found in snakes (Burbrink, 2002; Castoe et al., 2007), and mammals (Andersen & Light, 2012; Riddle, 1995), but not anurans (Lemmon et al., 2007; Mulcahy & Mendelson, 2000; Streicher et al., 2012). Beyond geographic vicariance, it is also possible that these lineages have diverged ecologically. The Balcones Escarpment divides this region into a drier, xeric habitat to the north and west (the Edwards Plateau, Trans-Pecos desert and the mixed grass prairie of the Great Plains) and a more humid, mesic habitat to the south in the Tamaulipan thornscrub and coastal habitats (Lieb, 1985b; Smith & Buechner, 1947). Additionally, any ecological divergence could have also occurred during range expansion east into both habitat types (Andersen & Light, 2012; Riddle, 1995).

We also found two well-supported clades within *P. t. brevilineatus*, one limited to the Trans-Pecos and the other to the Edwards Plateau and Great Plains ecoregions. The herpetofaunal assemblage of the Trans-Pecos is quite distinct (Ward et al., 1990), and the transition between the Trans-Pecos and the Great Plains corresponds to phylogeographic structure in some taxa (Jaeger et al., 2005; Pyron & Burbrink, 2009; Streicher et al., 2014). Both deep (between *P. t. brevilineatus* and *P. t. tetragrammus*) and shallow (between clades of *P. t. brevilineatus*) divergences among lineages follow transitions between ecological regions suggesting that ecological specialization may play an important role in driving the genetic diversity of this clade. In contrast to *P. tetragrammus*, we found low mitochondrial sequence

divergence among populations of *P. multivirgatus,* albeit with limited sampling. Although separated by hundreds of kilometers and a well described biogeographic boundary, the Cochise filter barrier (Castoe et al., 2007; Pyron & Burbrink, 2009), samples of *P. multivirgatus* from Arizona were only 0.003-0.007% divergent in mtDNA sequence from those in Texas. However, our sampling only included individuals from *P. m. multivirgatus*, and additional samples from throughout the range of *P. multivirgatus* including *P. m. epipleurotus* will be necessary to ascertain phylogenetic relationships among populations of *P. multivirgatus*.

Signatures of Introgression

Our phylogenetic trees support mitonuclear discordance, with mtDNA nesting *P. multivirgatus* within *P. t. brevilineatus*, and coalescent analysis of nDNA indicating reciprocal monophyly of both subspecies of *P. tetragrammus*, sister to a monophyletic *P. multivirgatus*. This pattern is consistent with the ancient introgression of *P. t. brevilineatus* mitochondrial DNA into *P. multivirgatus*. This interpretation suggests that introgression must have taken place after the diversification of *P. tetragrammus*, but before the two clades of *P. t. brevilineatus* diverged. Interestingly, we found *P. t. brevilineatus* mitochondrial haplotypes even in *P. multivirgatus* from Arizona, hundreds of kilometers away from the range of *P. t. brevilineatus*. While introgression has been suggested as an explanation for mitonuclear discordance in lizards multiple times (Leache & McGuire, 2006; McGuire et al., 2007; Morando et al., 2004; Ng & Glor, 2011; Wiens et al., 2010) , these studies showed introgression only in narrow hybrid zones. Based on these studies we would expect to see identical *P. t. brevilineatus* haplotypes found within *P. multivirgatus* where the two are sympatric and less frequently away from the contact

zone. In contrast, we found introgressed mitochondria throughout the range of *P. multivirgatus*, not just the individuals from the locality where they are sympatric with *P. t. brevilineatus* in Texas. There are two possible explanations for this: (1) the introgression event took place prior to a range expansion of *P. multivirgatus* into its current range or (2) a selective sweep of the introgressed mitochondria throughout the range of *P. multivirgatus*.

Our data do not currently allow us to distinguish between either of these possibilities. Our finding that all loci are under purifying selection suggests that this haplotype is being maintained as might be expected following a selective sweep. Recent research has found evidence for a rapid range expansion of rattlesnakes (genus *Crotalus*) following the last glacial maximum (Castoe et al., 2007; Schield et al., 2015), and the lack of genetic variability in our data could follow that model. Neiswenter and Riddle (2010) suggest a pattern of radiation in pocket mice (genus *Perognathus*) where multiple groups were sympatric during glacial maxima allowing for introgressive hybridization, and then followed the range expansion of arid grasslands at the conclusion of the glacial cycle. Our data are also consistent with *P. multivirgatus* following the expansion of arid grasslands after glacial maxima, while maintaining a small fraction of their range in sympatry with *P. t. brevilineatus*. The addition of more rapidly evolving nuclear markers (Toews & Brelsford, 2012) and ecological niche modeling of past distributions of *P. t. brevilineatus* and *P. multivirgatus* are needed to help identify which of these two scenarios is most likely.

Species-specific effects of introgression

We found significantly different patterns of mitochondrial diversity within *P. tetragrammus* and *P. multivirgatus*. Specifically, intraspecific mtDNA variation was much lower in *P. multivirgatus* than in *P. t. brevilineatus*, whereas intraspecific nDNA variation was similar between each species (Table 1.1, Fig. 1.5). Thus, the differences between mtDNA and nDNA variation in *P. multivirgatus* (but not *P. tetragrammus*; Fig. 1.5) run counter to expectations for most other animals studied (Brown et al., 1979). One explanation for this pattern might be that our geographic sampling for *P. multivirgatus* was not as complete as for *P. tetragrammus* (Fig. 1.2). Thus, we may have excluded a substantial proportion of extant mtDNA variation in *P. multivirgatus*. However, we note that we sampled individuals of *P. multivirgatus* from the U.S. states of Arizona, Colorado, and Texas, a geographic region that is similar in size to the entire range of *P. tetragrammus*.

We hypothesize that low mtDNA diversity in *P. multivirgatus* is related to the introgression and subsequent fixation of non-native mtDNAs, likely from an ancestral population of *P. t. brevilineatus*, based on the phylogenetic evidence (Fig. 1.3). Assuming this scenario, *P. multivirgatus* and *P. tetragrammus* have been impacted in very different ways by their historical interaction (Fig. 1.5). For example, mtDNA variation within *P. tetragrammus* has phylogeographic signal whereas mtDNA variation in *P. multivirgatus* (despite having a larger range size) does not. This potential for decoupling of mitochondrial and nuclear variation in lineages that have experienced introgression is an important reminder that the evolutionary histories of mitochondrial and nuclear genomes can be uncoupled within an entire species (as we propose here for *P. multivirgatus*). If undetected, this phenomenon can lead to incorrect inferences of divergence time and historical demography when relying on datasets consisting of mostly mtDNA loci.

Taxonomic Implications

We found that the individuals of *P. t. tetragrammus* and *P. t. brevilineatus* included in our study were reciprocally monophyletic (Fig. 1.3). However, because we did not sample individuals originating from central Texas (where these subspecies putatively overlap; Fig. 2), we refrain from suggesting taxonomic modification, in favor of testing these results using more extensive geographic and genetic sampling (Burbrink et al., 2000; Sackett et al., 2014). Regardless, our findings indicate the genetic distinctiveness of each subspecies, and further suggest complex dynamics of lineage diversification and introgression in this species complex.

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III. The genomic and geographic extent of past introgressive hybridization between *P. multivirgatus* and *P. tetragrammus*

A. Introduction

Hybridization plays an important role in the generation of diversity in animals and other organisms (Dowling & Secor, 1997). Gene pools of closely related species can act as a potential source of variation that are accessible through hybridization (Bock et al., 2015; Hedrick, 2013). As populations move into new or perturbed environments, such as through expansion, introduction, or changing climate, hybrid individuals may be more suited to the disturbed environment because of the additional variation (Abbott et al., 2013; Seehausen, 2004; Seehausen, 2013). Hybridization can also lead to portions of the genome being captured, or introgressed from a donor species to the recipient species (Mallet, 2005; Zielinski et al., 2013). The types of genes that are captured depend on several factors such as method of sex determination, the amount of selective pressure on the introgressing genes, or the ability of the species to change their ranges (Currat et al., 2008; Excoffier et al., 2009; Patten et al., 2015). Several studies in animals suggest that introgression has important effects in adaptation to new environments during range expansion (Fitzpatrick et al., 2009; Marques et al., 2017; Song et al., 2011). What is less well known is the genomic extent of introgression for many taxa.

Many cases of introgression are limited to organelles such as chloroplasts and mitochondria. There have been many proposed explanations for these trends towards biased introgression such as sex-biased dispersal (Excoffier et al., 2009). Yet most studies which evoke biased introgression of organelle DNA use only a handful of nuclear genes (Sloan et al., 2017; Toews & Brelsford, 2012). However, modern sequencing techniques are allowing for vastly increased amounts of molecular data which may reveal additional nuclear gene flow (Sloan et al., 2017). Introgressed nuclear genes could potentially be important in local adaptation (Morales et al., 2015), range expansion (Marques et al., 2017) or maintaining compatibility between nuclear

and mitochondrial genes (Bar-Yaacov et al., 2015; McKenzie et al., 2016; Sloan et al., 2017). We sought to test for introgression of nuclear and mitochondrial genes using high-throughput sequencing in our previously characterized hybridization system of *P. multivirgatus* and *P. tetragrammus*.

Our previous work (Chapter 2, Moseley et al., 2015) revealed discordance between trees derived from nuclear genes and mitochondrial genes, suggestive of mitochondrial introgression in two skink species of the central and western North America (*P. multivirgatus* and *P. tetragrammus*). Phylogenies based upon mitochondrial data suggested that *P. multivirgatus* is nested within *P. tetragrammus*, whereas analysis of nuclear DNA showed a monophyletic *P. tetragrammus* sister to *P. multivirgatus*. In order to further test for mitochondrial introgression, we compared rates of substitution in mitochondrial and nuclear genes. Genes in the mitochondria are expected to show faster rates of substitution than nuclear genes (Brown et al., 1979), yet *P. multivirgatus* had virtually the same amount of variation in both mitochondrial DNA and nuclear DNA. This potential decoupling of rates could be important in studies using mtDNA to determine demographic history or divergence timing. Interestingly, there was no signal of introgression of any of the nuclear genes in our limited dataset, yet additional nuclear data could potentially show different patterns. Importantly, our previous research used low variation protein coding genes (ranging from 0% to 0.9% within species). Thus, including more variable genetic information from the nuclear genome can reveal the robustness and extent of introgression in theses skink species.

We used high-throughput ddRADseq to generate hundreds of single nucleotide polymorphisms (SNPs) to allow us to test for genomic introgression with much higher resolution and clarify patterns of nuclear differentiation within *P. multivirgatus* and *P. tetragrammus*. We

then used these data to test the following hypotheses. First, we hypothesize that there has been past hybridization between *P. t. brevilineatus* and *P. multivirgatus* and these data allow us to characterize patterns of gene flow within and between these species. Second, we use these data to identify the regions of the genome that may have experienced introgression, in contrast to what was shown with more limited sampling. This is particularly exciting because it may reveal genes that have been important in local adaptation. Third, we hypothesize that the additional loci provided by these data allow for a better estimation of relationships within and among these species using a more robust dataset.

B. Methods

Specimens were obtained by turning cover objects in possible habitats. After removing liver or muscle tissue, specimens were then formalin fixed and entered into the Amphibian and Reptile Diversity Research Center collection at the University of Texas Arlington. Additional tissues were acquired by loans from the Texas Natural History Collection (TNHC) and from the American Museum of Natural History (AMNH). Tissue extractions were done using Qiagen DNEasy kits or through phenol-chloroform-isoamyl alchohol extraction.

We constructed double digest restriction site-associated DNA sequencing libraries for 38 samples of *P. tetragrammus* and *P. multivirgatus* along with a sample of *P. septentrionalis* and *P. obsoletus* as outgroups using the modified protocol from Peterson et al. (2012). Samples were digested using two restriction enzymes, *Sbf1* (8 bp recognition site) and *Sau3al* (4 bp recognition site), in 100 uL reactions. We then ligated indexed Illumina adapters with unique molecular identifiers (UMIs) onto the cut sites of each fragment, barcoding them for downstream sequencing. We pooled groups of samples from 8 individuals at a time, these were then cleaned using AMPure beads (Agencourt Bioscience). We targeted fragment sizes of 575-655 bp because

other studies with similar sized genomes, 2.5–2.75 gb (Olmo, 1976), have had success with this method (Schield et al., 2015) using a Blue Pippin Prep (Sage Science, Beverley, MA). We ligated primers which contained flow cell binding sequences and indices to size selected libraries allowing for multiplexing of groups and amplification via PCR with Phusion polymerase (New England Biolabs). We cleaned pooled samples using AMPure beads and verified size selection and DNA concentration on an Agilent Bioanalyzer (Agilent, Santa Clara, CA). After re-pooling samples based on molarity, they were sequenced with 100bp single-end reads on an Illumina HiSeq 2500 at the Brigham Young University Genome Core Facility.

We used the STACKS pipeline v1.37 (Catchen et al., 2013) to process raw reads. We removed PCR clones with the clone filter program in STACKS using the UMIs in our adapters, which we removed using the FASTX-Toolkit trimmer (Hannon Lab, Cold Spring Harbor Laboratory). We then demultiplexed trimmed reads into sample specific files using the radtags program in STACKS. We discarded reads that had poor quality scores or lacked barcodes or cut site sequences. We used pyRAD v.20 (Eaton, 2014) to assemble ddRADseq loci because it allows for indels when clustering sequence reads into orthologous loci. We used a clustering similarity (W_{clust}) of 88% and a minimum depth of coverage per cluster (Min_{Cov}) of 19, allowing for 50% missing data per locus. We allowed for a maximum number of four individuals with a shared heterozygous site (potential paralog)(MAX_{SH}).

To estimate a maximum likelihood phylogenetic tree, we used RAxML v8.0 (Stamatakis, 2014) employing the ASC_GTRGAMMA model of evolution, implementing the Lewis ascertainment bias correction (-asc-corr=lewis) on a concatenated dataset consisting of 12,666 unlinks SNPs. We conducted 1000 bootstrap replicates. Results were viewed using FigTree v1.4.2 (Rambaut, 2007). Population structure was estimated using the program STRUCTURE

v2.3.4 (Pritchard et al., 2000) with a dataset excluding outgroup taxa (*P. septentrionalis*, MAM055, and *P. obsoletus*, CLC298). STRUCTURE runs included MCMC chains of 125,000 (plus 25,000 burn-in) under a mixed ancestry model from K = 1-5 for dataset 1, and K = 1-4 for dataset 2. We calculated estimates of genetic differentiation between species using the diveRsity v1.9.73 (Keenan et al., 2013) package in R. Pairwise diversity measures include F_{st} (Weir & Cockerham, 1984), g_{st} (Nei & Chesser, 1983), G_{st} (Hedrick, 2005), G'_{st} (Meirmans & Hedrick, 2011), and D (Jost, 2008). Using a dataset allowing only 25% missing data per locus in the diveRsity package, we used the divBasic command, with 500 replicates, to estimate heterozygosity for *P. t. tetragrammus*, *P. t. brevilineatus*, and *P. multivirgatus*.

We performed Sanger sequencing and analysis of additional specimens of *P. multivirgatus* from Kimball County, Nebraska (Table S1) for 3 mitochondrial genes following the protocols in Moseley et al. (2015, Ch. 2). We analyzed these sequences following protocols in Moseley et al. (2015, Ch.2). Succinctly, we reconstructed phylogenies on a dataset partitioned by codon using MrBayes v3.2.6 (Huelsenbeck & Ronquist, 2001) using default parameters and the GTR + G model of evolution for 10,000,000 mcmc generations sampling every 500 generations (20,000 trees). Burnin was 25% of trees. We performed maximum likelihood reconstruction using RaxML (Stamatakis, 2006) using the GTR-CAT model of evolution, and 1,000 bootstrap replicates.

C. Results

We sequenced 13,958 loci with 45,614 SNPs (12,666 unlinked SNPs) and 1586 bp of mtDNA. We found a sister relationship between *P. septentrionalis* and *P. multivirgatus* with maximum likelihood analysis of SNPs (Fig. 2.1b) which is different from the sister relationship between *P. tetragrammus* and *P. multivirgatus* with this group sister to *P. fasciatus* and *P. septentrionalis* in

previous studies (Ch.2, Moseley et al. 2015, Brandley et al. 2012) and the analysis of mtDNA (Fig 2.2). We found that *P. tetragrammus* and *P. t. brevilineatus* were strongly supported as monophyletic using SNPs. We also found that there is half the heterozygosity in *P. t. brevilineatus* (0.06, Figure 2.3) as there was in *P. t. tetragrammus* (0.1), which is suggestive of recent range expansion. There was some additional structure within *P. t. brevilineatus* which roughly corresponds to an eastern and western clade with the exception of sample JAC26325 (Coahuila, Mexico) which is found in the primarily eastern clade. There is only marginal support for the monophyly of *P. t. tetragrammus*.

We found that *P. tetragrammus* was not monophyletic using mitochondrial DNA (Fig. 2.2) as *P. multivirgatus* is nested within *P. t. brevilineatus*. This discordance between marker types is consistent with our previous study and consistent with biased introgression of mitochondrial genes. We found that *P. t. tetragrammus* is monophyletic with strong support. We found marginal support for the sister relationship of *P. fasciatus* and *P. septentrionalis* which is consistent with other studies (Brandley et al., 2012).

We found a well-supported break between *P. tetragrammus* and *P. brevilineatus* (Fig 2.1A), using a Bayesian population clustering approach, STRUCTURE. As values for k are increased from 2-4 there are additional signatures of gene flow between *P. t. tetragrammus* and *P. t. brevilineatus* or some other non-sampled group. In only one specimen do we show signatures of gene flow or introgression between *P. t. brevilineatus* or *P. multivirgatus*, this is consistent with the mitochondrial data from the previous chapter (Moseley et al. 2015) and mitochondrial introgression. Our test of genetic differentiation (Table 2.1) consistently show a lack of admixture between *P. multivirgatus* and both *P. t. tetragrammus* and *P. t. brevilineatus*, and *P. t. tetragrammus* and *P. t. brevilineatus* are far less differentiated from one another than

they are to *P. multivirgatus*. Differentiation between *P. t. tetragrammus* and *P. t. brevilineatus* range from 0.059 (*D*) and 0.4598 (GG_{st}).

D. Discussion

We found strong evidence that past hybridization did not result in genome-wide introgression, but that introgression was limited to mitochondrial genes, despite the addition of thousands of nuclear loci. We found evidence for the introgressed *P. t. brevilineatus* mitochondrial genes by *P. multivirgatus* in individuals that are sympatric with *P. t. brevilineatus*, but also in individuals ranging from Arizona and Nebraska, albeit with limited sampling. Although high-throughput sequencing has revealed nuclear gene flow in systems previously thought to be biased introgression of mitochondrial genes (Zielinski et al., 2014; Zielinski et al., 2013), our SNP data did not reveal any gene flow between *P. t. brevilineatus* and *P. multivirgatus*. The biased introgression of mitochondrial genes is consistent with many studies which have shown mitonuclear discordance (Toews & Brelsford, 2012). This is important because many studies have relied exclusively mtDNA, and introgression could have affected those outcomes.

Our results are further evidence for the use of ddRADseq as a method for elucidation of relationships between recently diverged species (Catchen et al., 2017). The additional loci allowed for a better understanding of relationships between *P. multivirgatus* and *P. tetragrammus* and within *P. tetragrammus* (Fig. 2.1) in contrast to the nuclear data in the first chapter which was not able to elucidate relationships consistent with any geography within *P. tetragrammus*. These loci are discordant with the patterns seen in the mtDNA with respect to relationships between *P. t. brevilineatus* and *P. multivirgatus* (Fig. 2.2) consistent with previous studies (Moseley et al., 2015). This is suggestive of biased introgression of mtDNA *by P.*

multivirgatus. One specimen (CLC747) does have small amount of genomic DNA that originated from *P. tetragrammus* (Fig. 2.1), but this pattern is not seen throughout the other specimens. It does suggest the possibility of additional introgressed DNA if we had sequenced a larger portion of the genome or more individuals.

There was little structure within *P. multivirgatus* even between samples from Texas, Arizona, and Nebraska. The placement of *P. multivirgatus* as sister to *P. septentrionalis* is inconsistent with previous studies and is possibly due to the need for additional outgroup taxa or individuals in these analyses (Moseley et al. 2015, Brandley et al. 2012). The lack of structure within *P. multivirgatus*, with small sample size, is not consistent with current naming convention where the northern populations are a separate subspecies *P. m. multivirgatus*. This suggests that the northern population, if separated from the southern *P. m. epipleurotis*, has not been separated for a long period of time. This also raises the possibility that the northern group has only recently expanded into this region. This is consistent with the post glacial range expansion of grasslands (Retallack, 1997; Retallack, 2001) and many other taxa (Neiswenter & Riddle, 2010; Streicher et al., 2012).

Across thousands of nuclear loci there is general agreement in relationships that are discordant with mtDNA which is consistent with an expected pattern of mitochondrial introgression (Good et al., 2015; Sloan et al., 2017). The lack of introgression of nuclear genes in *P. multivirgatus* is interesting particularly because the introgressed mitochondrial genes are present throughout their range. It has been hypothesized that changes in the mitochondrial genome should also be followed by changes in the nuclear genome so the two can continue to work well together. It is also thought that one of the best-case scenarios in mitochondrial

introgression would be an introgression of the co-adapted nuclear markers as well (Beck et al 2015, Sloan et al 2017). However, this is not the case in the data presented here.

While our data suggest that introgression in this group has been limited to mitochondrial genes, there are many questions to address before it can be said to be adaptive in nature. There are several non-adaptive mechanisms of biased introgression which must be ruled out. Male biased dispersal is likely to lead to introgression of mitochondrial genes (Excoffier et al., 2009) yet little is known about the extent of male dispersal in skinks. However, sexual selection can lead to biased introgression of mitochondrial genes (Sloan et al., 2017). Skinks do show sexual selection as males grow large orange heads for combat (Cooper & Vitt, 1988). It is possible that this combative behavior in skinks is at least partially responsible for the introgression of mitochondrial genes we see in this system and in other skinks.

IV. Past climate conditions shape the opportunity for introgression between *P. multivirgatus* and *P. t. brevilineatus*

A. Introduction

One of the defining characteristics of a species is its geographic distribution. The distribution of a species gives insights into their ecology, biotic interactions, and evolution (Brown & Nicoletto, 1991; M. McPherson & Jetz, 2007; Smith et al., 2008). These patterns can also give insight into how the distribution of a species will react to changes in environment or climate (Brown & Heske, 1990). When species ranges change it also changes which species are interacting with one another (Smith et al., 2008). By studying theoretical shifts in geographic ranges we can better understand the consequences of these interactions.

Glacial cycling has been shown to have large impacts on the observed genetic diversity in many different taxa. Glacial cycles at the beginning of the quaternary period caused species ranges to expand, contract, and move north and south as the climate changed. This caused species or lineages that were previously allopatric to come back into contact. If the lineages had not evolved sufficient isolating mechanisms gene flow, could occur (Dowling & Secor, 1997; Schield et al., 2015). One of the possible outcomes of these interactions is interspecific hybridization and genetic introgression (Toews et al., 2014; Toews & Brelsford, 2012). However, introgression is only expected in the narrow ranges where species overlap (Currat et al., 2008; Toews & Brelsford, 2012). We need to better understand how species ranges shifted in response to glacial cycling in order to better understand how past hybridization events occurred.

Species distribution modeling allows for reconstruction of species distributions based on specimen locality and environmental characteristics (Elith et al., 2006; Elith et al., 2010). Part of the rise in use of species distribution models (SDMs) is the increased availability of climatic data and models for past time periods through sources such as WorldClim (Hijmans et al., 2005) and the digitization of museum specimen information through digital portals such as VertNet

(vertnet.org) and the Global Biodiversity Information Facility (gbif.org). There is also an increasingly popular mobile application, iNaturalist, which allows people to add species observations to these databases. Studies using methods of modelling ecological niches are common in conservation planning (Costa et al., 2010), invasive species biology (Elith et al., 2010), and evolution (Costa et al., 2010; Makowsky et al., 2010). One of the most popular uses of ecological niche modelling in evolutionary biology is looking at past distributions, particularly in the Last Glacial Maximum (LGM) approximately 22 kya (Barve et al., 2011; Waltari et al., 2007; Warren et al., 2010).

One system where SDMs could be useful is the four-lined skink, *Plestiodon tetragrammus* and the many-lined skink, *P. multivirgatus*. *Plestiodon multivirgatus* is inferred to have introgressed mitochondrial genes of *P. tetragrammus* yet their ranges only overlap in a narrow range in western Texas. Interestingly, the introgressed mitochondrial genes are found throughout the *P. multivirgatus* range. This suggests that the introgression event took place prior to a range expansion of *P. multivirgatus* into its current range, or there was a selective sweep of the introgressed mitochondria throughout the range of *P. multivirgatus*. Previous studies have also inferred phylogenetic structure consistent with the glacial cycling in this group (Lieb 1985, Moseley et al., 2015).

We hypothesize that *P. multivirgatus* and *P. tetragrammus* shared a larger portion of their ranges during the LGM and that *P. multivirgatus* has since expanded into its current range. The SDMs will allow us to determine whether glacial cycles played a factor in generating the opportunity for gene flow/introgression between *P. t. brevilineatus* and *P. multivirgatus*, by determining whether they shared glacial refugia. We hypothesized that *P. multivirgatus* had introgressed the mitochondria of *P. t. brevilineatus*; because of this we expect that *P.*

multivirgatus had a smaller range in the past and that its range overlapped much more broadly with *P. t. brevilineatus*

B. Methods

I plotted museum locality data from HerpNet (herpnet.org) and GBIF (gbif.org) and the locality information from "research grade" iNaturalist observations (iNaturalist.org), available through GBIF, for the four clades of *Plestiodon* (*P. multivirgatus, P. t. tetragrammus, P. t. brevilineatus,* and the combined *P. tetragrammus*) in DIVA-GIS. Localities were plotted and records found well outside (>200 km) known distribution were removed. The 19 biologically relevant, BIOCLIM, climate layers were downloaded from WORLDCLIM (http://worldclim.org) in the 2.5 arc-minute resolution to avoid overparameterization (Waltari et al., 2007) for the current time period as well as two models of past climate: the Community Climate Model System version 4 (CCSM, Gent et al., 2011) and the Model for Interdisciplinary Research on Climate (Hasumi & Emori, 2004). Climate data was trimmed and plotted on map. I reprojected climate layers using the SDMToolbox package (Brown & Anderson, 2014) in ArcGIS 10.5 (Environmental Systems Research Institute, Redlands, CA) into the North American Albers Equal Area Projection in order to minimize the bias associated with increasing latitude (Budic et al., 2016). I also used SDMToolbox to discard correlated variables (correlation threshold 0.8) because correlations between variables can affect how the model behaves in different time periods (Warren et al., 2014).

In order to reduce autocorrelation of climatic variables because of sampling bias, I used the R package spThin (Aiello-Lammens et al., 2015) to thin occurrence records within 10 km. To account for bias in sampling intensity, I collected occurrence data for 10 species of chiefly fossorial reptiles which are most often collected using methods which can also sample the

Plestiodon species, mostly turning cover objects. I avoided species which are commonly collected while road-cruising. These target group species (*Diadophis punctatus, P. septentrionalis, Leptotyphlops dulcis, P. obsoletus, Scincella lateralis, Tantilla gracilis, T. nigriceps,* and *Haldea striatula*) were plotted then reprojected to the equal area projection. I then performed Gaussian Kernel Density analysis using the SDMToolbox toolkit in ArcGIS 10.1, to get a layer which estimates sampling density across the landscape (Phillips et al., 2009).

I performed maximum entropy species distribution modeling in MAXENT (Phillips et al., 2006) with 5 replications using default parameters. In order to make sure that the modeling shows a reasonable estimate of the realized niche of the 3 clades of *Plestiodon* (*P. multivirgatus, P. t. tetragrammus,* and *P. t. brevilineatus*; I also plotted a combined *P. tetragrammus*), I used models of the current climate to see if the output matches the current distribution and withheld 20% of occurrence points for model testing. I then projected those estimates with all occurrences onto models of the climate variables in the LGM and the Mid Holocene. These maps were then used to determine if *P. multivirgatus* and *P. t. brevilineatus* shared a larger portion of their range in the last glacial period.

C. Results

We found that initial distribution models of *P. multivirgatus* (Fig 3.1a) fit well (average AUC 0.95) and were consistent with the current distribution. We did find some minor over-prediction to the north and in northern Mexico, where these species are not found. However, these over-predictions were consistent with some of the removed samples as well as a description of a subspecies from northern Mexico (Anderson & Wilhoft, 1959) so these over-predictions were

not removed from subsequent analysis. Mid Holocene models, both CCSM (Fig 3.1b) and MIROC (Fig 3.1c), show *P. multivirgatus* in the southern portion of their current range. Last glacial maximum models, both CCSM (Fig 3.1d) and MIROC (Fig 3.1e), showed *P. multivirgatus* in the Sierra Madre Occidental, the Mexican Plateau, and the Sonoran Desert.

Current models for *P. t. brevilineatus* (Fig 3.2a) fit well (avg. AUC 0.96) and are consistent with the current distribution, with very little over-prediction. However, there was some under-prediction in the northern extent of the range, potentially decreasing the chances for overlap with *P. t. brevilineatus*. Mid Holocene models (Figs 3.2b and 3.2c, CCSM and MIROC respectively) show *P. t. brevilineatus* in the western portion of their current range (northeastern Mexico and western Texas). Models in the LGM differed in that the CCSM model (Fig 3.2d) showed the best habitat in western Tamaulipas. The MIROC model (Fig 3.2e) shows the best habitat in much of Coahuila and western Nuevo Leon; the range projected in the MIROC model is also larger and further North than that shown in the CCSM model.

Modeling of the current distribution of *P. t. tetragrammus* was also consistent with the known distribution (avg AUC = 0.99, Fig 3.3a). Mid Holocene models with *P. t. tetragrammus* both showed the best habitat in southern Tamaulipas and Nuevo Leon, Mexico (Figs 3.3b and 3.3c, CCSM and MIROC respectively). The CCSM model (Fig 3.3d) and MIROC model (Fig 3.3e) show similar ranges with the MIROC model predicting suitable habitat slightly further north, with each predicting a range in San Luis Potosi, Tamaulipas, and Veracruz with extensions down the coast to the Yucatan.

When *P. t. tetragrammus* and *P. t. brevilineatus* were modeled together using current climate variables their range closely resembled that of expectation (Fig. 3.4a). This also shows a

lower degree of habitat suitability in the area where the ranges of each subspecies overlap in south central Texas. When modelled over time the combination of the subspecies resemble that of the individual subspecies, but vary in that some models show less area overall while others show more (Figs 3.4b-e).

To test the hypothesis of range sharing by *P. t. brevilineatus* and *P. multivirgatus* in the LGM I plotted both species together for each climate model (Figs 3.5a and 3.5b, CCSM and MIROC respectively). The two models each showed a distinct region of overlap in northern Mexico; the CCSM model (Fig 3.5a) shows this overlap smaller and slightly to the east of the range shown in the MIROC model (Fig 3.5b).

Overall the different models of past climate scenarios achieved similar results to one another, however they did have different values associated with the relative probability of occurrence. The Mid Holocene CCSM model in particular had very low values, so low that in order to get a visible plot, the maximum values had to be plotted for *P. multivirgatus* and *P. t. tetragrammus*, whereas I used the average of the 5 runs in the other plots. *Plestiodon multivirgatus* had higher relative habitat suitability than *P. tetragrammus* for all models other than the MH CCSM. For all plots I used the natural breaks option in ArcGIS 10.5 with 5 classifications.

D. Discussion

Interestingly, the model of combined distribution of *Plestiodon t. tetragrammus* and *P. t. brevilineatus* suggests a lower habitat quality in the region of overlap in central Texas. This divide in suitable habitat could be partially responsible for maintaining the distinct lineages even though they have been shown capable of interbreeding (Lieb 1985). Importantly though,

diversity within *P. tetragrammus* likely predates the crossing of the Balcones Escarpment which only occurred as recently as the Mid Holocene. Our SDMs indicate that the Balcones Escarpment is acting as a demarcation between the arid Edwards Plateau to the North and the Tamaulipan thornscrub to the south. Lieb (1985) also noted that the small hybrid zone south of the Balcones Escarpment area is an intergrade between the more riparian Tamaulipan thornscrub habitat in southern Texas preferred by *P. t. tetragrammus* and the drier habitat found in the Texas hill country of the Edwards Plateau where *P. t. brevilineatus* is found. This distinction is maintained to a small degree in the Mid Holocene and LGM if the northern cell of habitat is mostly *P. t. brevilineat*us and the southernmost is *P. t. tetragrammus* (Figs 3.4b-e). The cells of most suitable habitat are also roughly coincident with the individual runs of the subspecies though with some differences. These differences could be attributed to a larger range of features being considered suitable in the combined run. It is also possible that *P. t. brevilineatus* plays a slightly increased role in the combined analyses as it made up a larger fraction of localities, 114 localities for *P. t. brevilineatus* versus 42 for *P. t. tetragrammus*.

The post glacial northern range expansion of *Plestiodon multivirgatus* is roughly coincident with that of other arid grassland species such as rodents (Neiswenter & Riddle, 2010) and frogs (Streicher et al., 2012). As *P. multivirgatus* migrated north through the Mid Holocene it is continually associated with high elevation regions in the American southwest and is coming into the Rocky Mountains (Figs 3.1b and 3.1c). The transition from the Mid Holocene to the current distribution in Nebraska suggests this is a recent expansion into somewhat less suitable habitat (Fig. 4.1a). This recent expansion is also consistent with genetic data suggesting very little differentiation between the northern group of *P. multivirgatus* in Nebraska and those from Texas (Ch. 3). While environmental variables do model the current distribution well, additional

variables such as soil characteristics and vegetation are potentially very important for *P. multivirgatus* and other chiefly fossorial species from semi-arid grasslands.

In order for introgression to occur, the species themselves must come into contact. Mitochondrial introgression is most commonly seen in the narrow range of overlap between species. When ranges overlap over large portions of the range it becomes more common. Introgression is usually unidirectional from the species with the larger range into the species with the smaller range. However, *P. multivirgatus* seems to have introgressed the mitochondria of *P. t. brevilineatus*, even though *P. multivirgatus* has a larger range. After modeling the ranges of *P. multivirgatus* and *P. t. brevilineatus* in the LGM, this relationship doesn't change; *P. multivirgatus* still has a much larger range, but now the best habitat range of *P. t. brevilineatus* is entirely encompassed within the best range of *P. multivirgatus* regardless of climate model (Figs. 3.5a and 3.5b). This provides a good opportunity for the introgression event to have taken place, even if the gene flow went in the opposite direction than predicted (Currat et al., 2008; Toews & Brelsford, 2012).

The presence of *Plestiodon multivirgatus* in Arizona and New Mexico during the LGM is also observed in several plant species like juniper and pine that are common in the highland areas. Grasses in these areas are also thought to have been able to persist, because of their ability to persist on the warmer eastern slopes (Betancourt et al., 2001). It is possible that this strategy was employed by *P. multivirgatus* and other species.

This work suggests that species distribution modeling techniques are a useful way to use reconstructed geographic distributions and climatic condition to understand opportunities for the gene flow seen in genetic data. When combined with genetic data, this work shows that the range

of *P. t. brevilineatus* was mostly enveloped by that of *P. multivirgatus* in the LGM. The introgression of the mitochondria of *P. t. brevilineatus* by *P. multivirgatus* could have been driven by many factors. Mitochondria have been linked to thermal tolerance (Morales et al., 2015) and are subject to selective sweeps (James et al., 2016). Adaptive introgression has been suggested for some groups for these reasons and others (see Sloan et al., 2017 for review). It is also possible that this instance of introgression could have resulted from non-adaptive phenomenon such as sex biased dispersal or sexual selection (Excoffier et al., 2009). While little is known about sex biased dispersal in these skinks, they do show sexual selection in that male skinks grow large powerful jaws for combat and are very aggressive (Cooper & Vitt, 1988) and could potentially have driven the hybridization and biased introgression of mitochondrial genes.

This work could be published as a standalone study, or incorporated into the previous chapter as a larger scale work to be submitted to *Molecular Phylogenetics and Evolution*, *Molecular Ecology*, or *Evolution*, which have recently published several articles with similar designs (Schield et al., 2015; Velasco et al., 2015; Welton et al., 2013).

V. Overall conclusions and significance of research

This work highlights the importance of using an integrated approach to answer evolutionary questions. We used standard phylogenetic techniques to show that there has been past gene flow between *P. multivirgatus* and *P. t. brevilineatus* and that this gene flow can be linked to climate cycling. Our use of species distribution models was important in providing additional support for the hypothesis of recent range expansion by *P. multivirgatus* into its current range in Nebraska and South Dakota inferred from molecular work was reasonable. It also helped us determine the opportunity for introgressive hybridization to occur between *P. multivirgatus* and *P. t. brevilineatus*.

More broadly this work shows the impact of climate and geography in structuring observed genetic diversity. Past climate conditions affect the geographic distributions of species in such a way where previously allopatric taxa can come together leading to hybridization. We also show that climate cycles can bring taxa with slightly different habitat preferences across geographic regions which highlight these differences, such as the Balcones Escarpment. This work also highlights a possible interesting question in regards to biogeographical barriers, like the Balcones Escarpment. Do these barriers act as barriers in the generation of diversity, or are structures like the Balcones Escarpment simply acting as demarcation lines between habitat types? This could prove to be an interesting question in regards to several other proposed biogeographic barriers such as the Cochise filter barrier in Arizona and New Mexico.

This study also further highlights the utility of skinks as a study system in evolutionary biology. Skinks inhabit almost all terrestrial biomes and are thus useful in biology due to natural replication of patterns. Mitochondrial introgression has been found in other skink systems as well, so work on these patterns is broadly applicable throughout the system. We showed that ecology can have important evolutionary implications even when species evolve in allopatry

because species often come back into contact. Secondary contacts can be due to climate, habitat perturbations, or human introductions but genetic signatures of these interactions can follow the same patterns we've shown.

Taxonomic Implications

I refrain from making any taxonomic changes in *Plestiodon multivirgatus* because we lack extensive sampling. However future work including additional samples from the both the northern and southern populations, if it follows the patterns presented herein likely indicate that *P. m. multivirgatus* and *P. m. epipleurotis* should be synonomized.

I refrain from making any taxonomic changes on *P. tetragrammus* because we lack extensive sampling in the zone of overlap south of the Balcones Escarpment in Texas. However, our work suggests that there is a strong genetic break between the two subspecies when using extensive sampling of the nuclear genome and a large fragment of mitochondrial DNA. We also show that the subspecies have different habitat preferences which likely precludes extensive gene flow. These all suggests that elevation of the subspecies *P. t. tetragrammus* and *P. t. brevilineatus* is reasonable.

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VIII. Figures and Tables

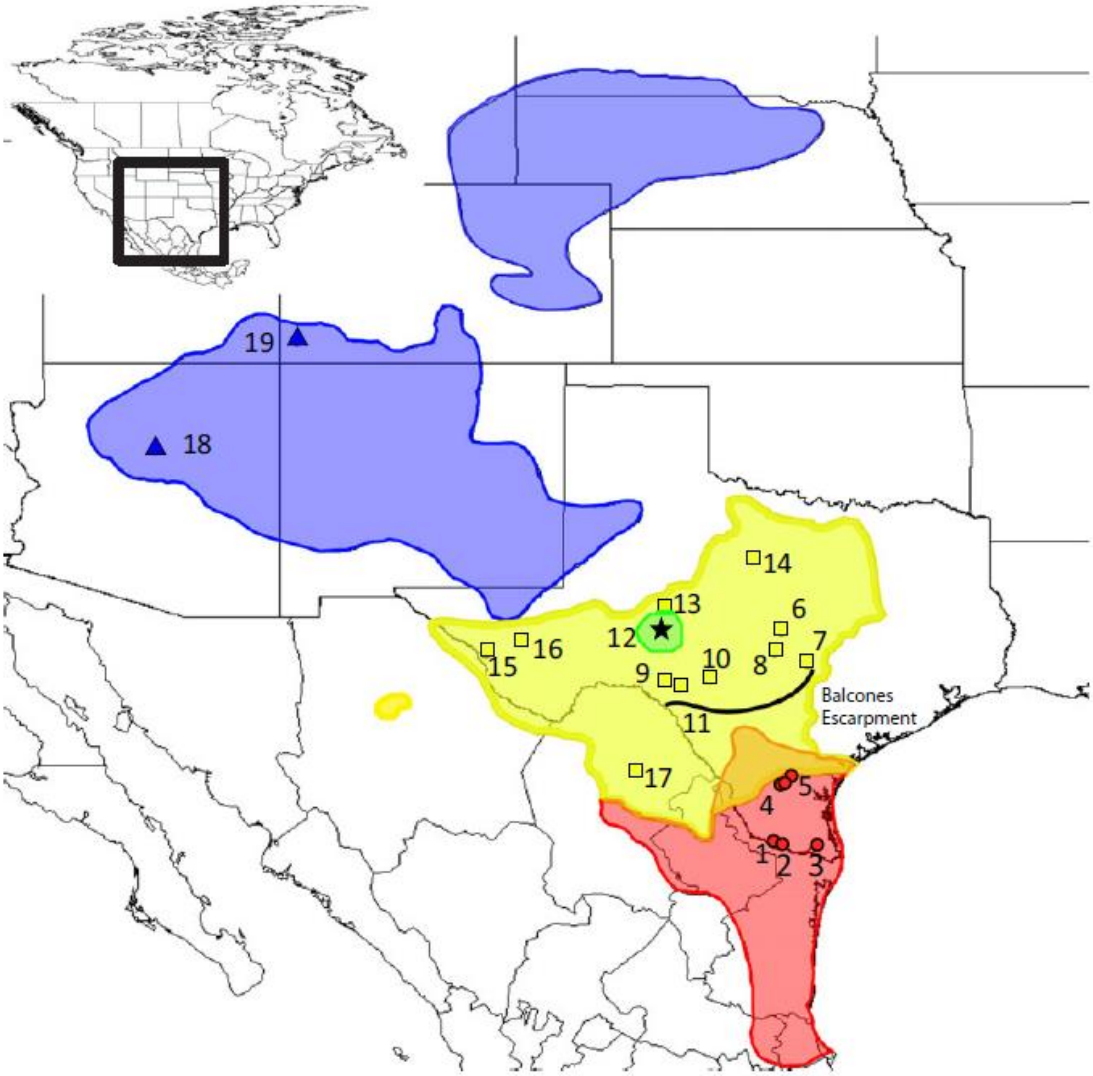


Figure 1.1. Geographical distribution of *P. t. tetragrammus* (red), *P. t. brevilineatus* (yellow) and *P. multivirgatus* (blue) based on Dixon (2013), IUCN Redlist (2007) and Lieb (1985). Green indicates range overlap between *P. t. brevilineatus* and *P. multivirgatus*. Orange indicates overlap between P. t. tetragrammus and P. t. brevilineatus. Symbols and numbers indicate collection localities used in the study. Circles: *P. t. tetragrammus*; squares: *P. t. brevilineatus*; triangles:*P. multivirgatus*. Star indicates locality where both *P. t. brevilineatus* and *P. multivirgatus* were sampled together.

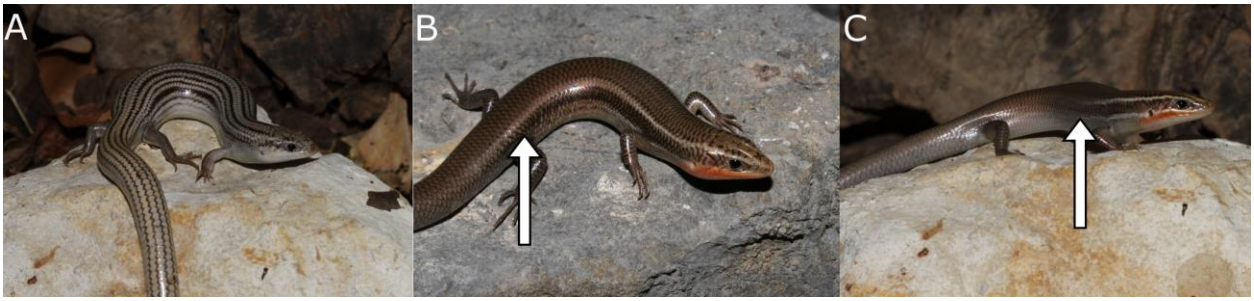


Figure 1.2. External morphology of *P. multivirgatus* (A), *P. t. tetragrammus* (B) and *P. t. brevilineatus* (C). Arrows indicate the termination of the lateral light and dark stripes in *P. t. tetragrammus* and *P. t. brevilineatus*, which is the character used to distinguish these two subspecies.

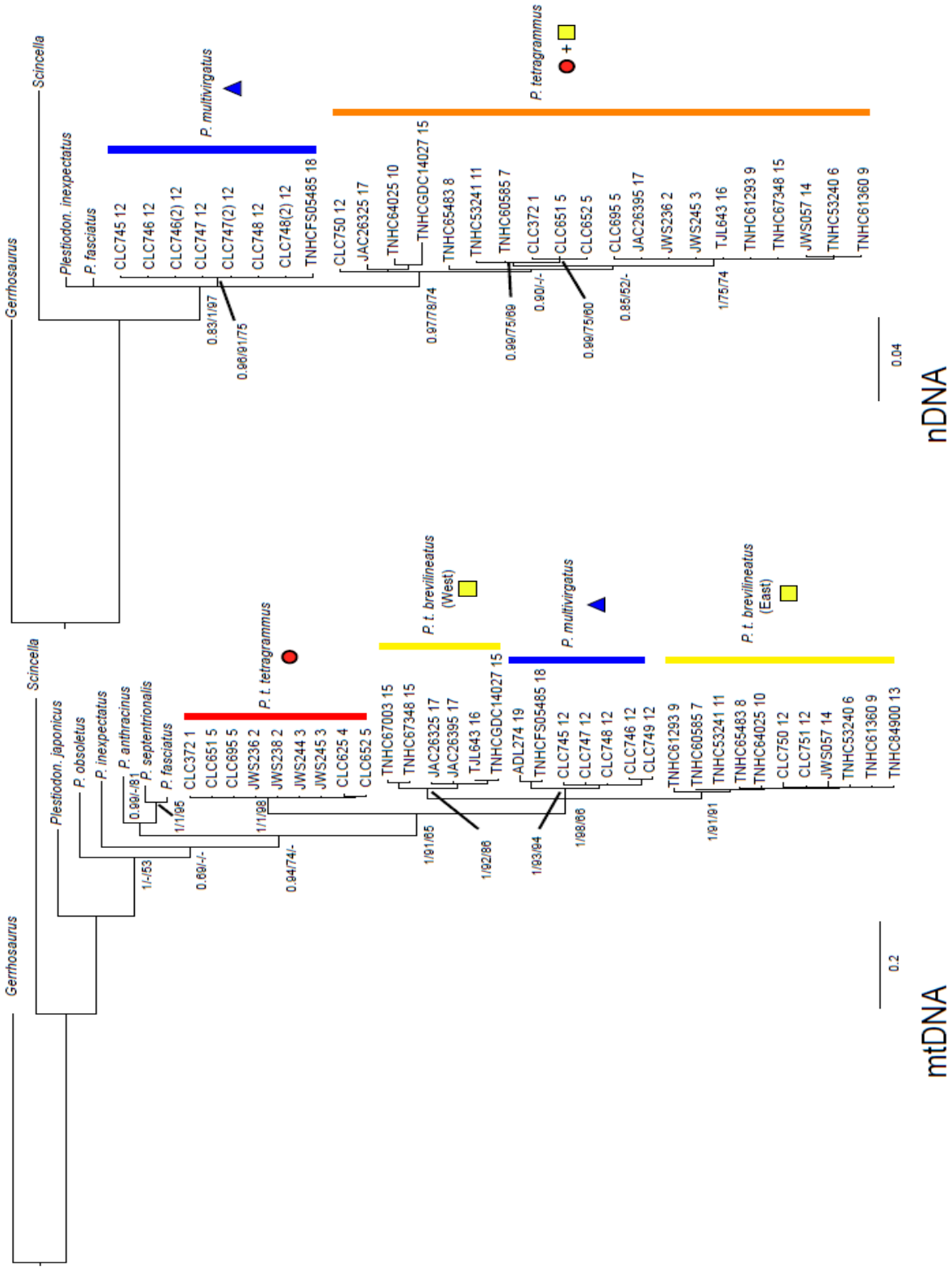


Figure 1.3. Phylogenetic reconstructions of relationships among specimens of *P. t. tetragrammus*, *P. t. brevilineatus* and *P. multivirgatus* based on mtDNA and nDNA. Trees are based on Bayesian analyses with nodal support values as Bayesian posterior probabilities, non-parametric bootstrap values using MP, and non-parametric bootstrap values using ML analysis, respectively. Symbols correspond to localities in Fig. 1.1.

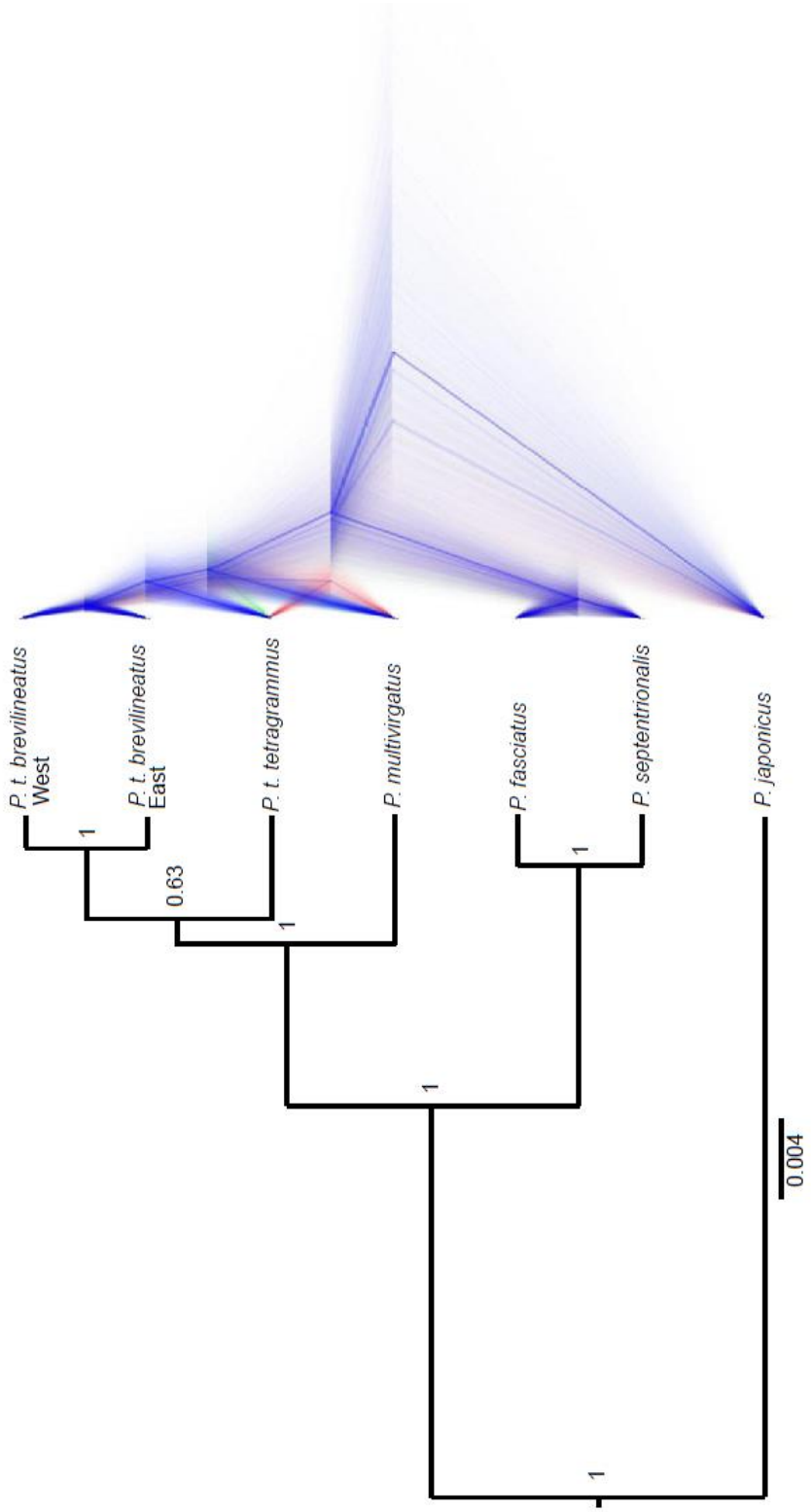


Figure 1.4. Topologies resulting from coalescent species tree analysis of combined mtDNA and nDNA data. Tree on left is a consensus topology with Bayesian posterior probabilities for nodal support. Lines on right are a DensiTree diagram of all trees recovered from coalescent analysis; the most frequently sampled topology is shown in blue with differing topologies shown in red and green revealing areas of uncertainty in the species tree topology.

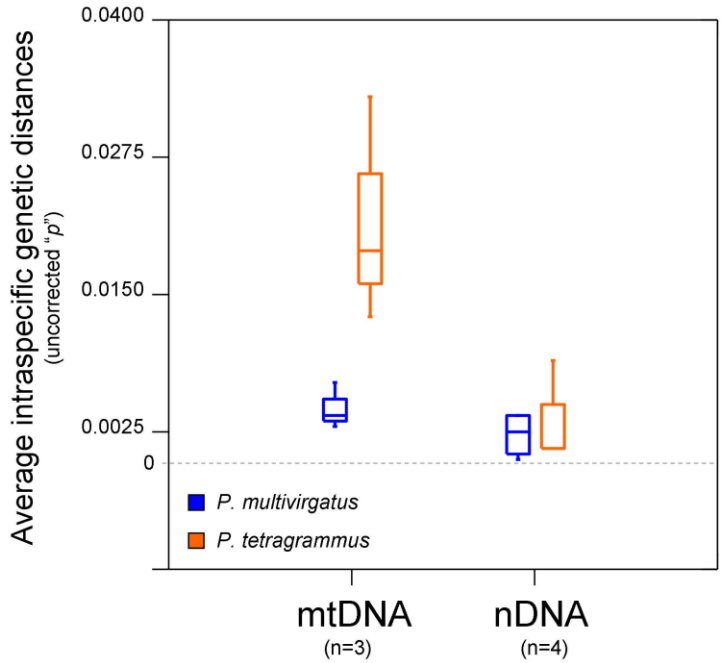


Figure 1.5. Average intraspecific genetic distances for three mitochondrial and four nuclear genes from *Plestiodon multivigatus* and *P. tetragrammus* with mtDNA on the left and nDNA on the right. Note that while *P. tetragrammus* possesses the expected pattern of mtDNA–nDNA variation for animals, *P. multivirgatus* has statistically indistinguishable levels of mtDNA--nDNA variation.

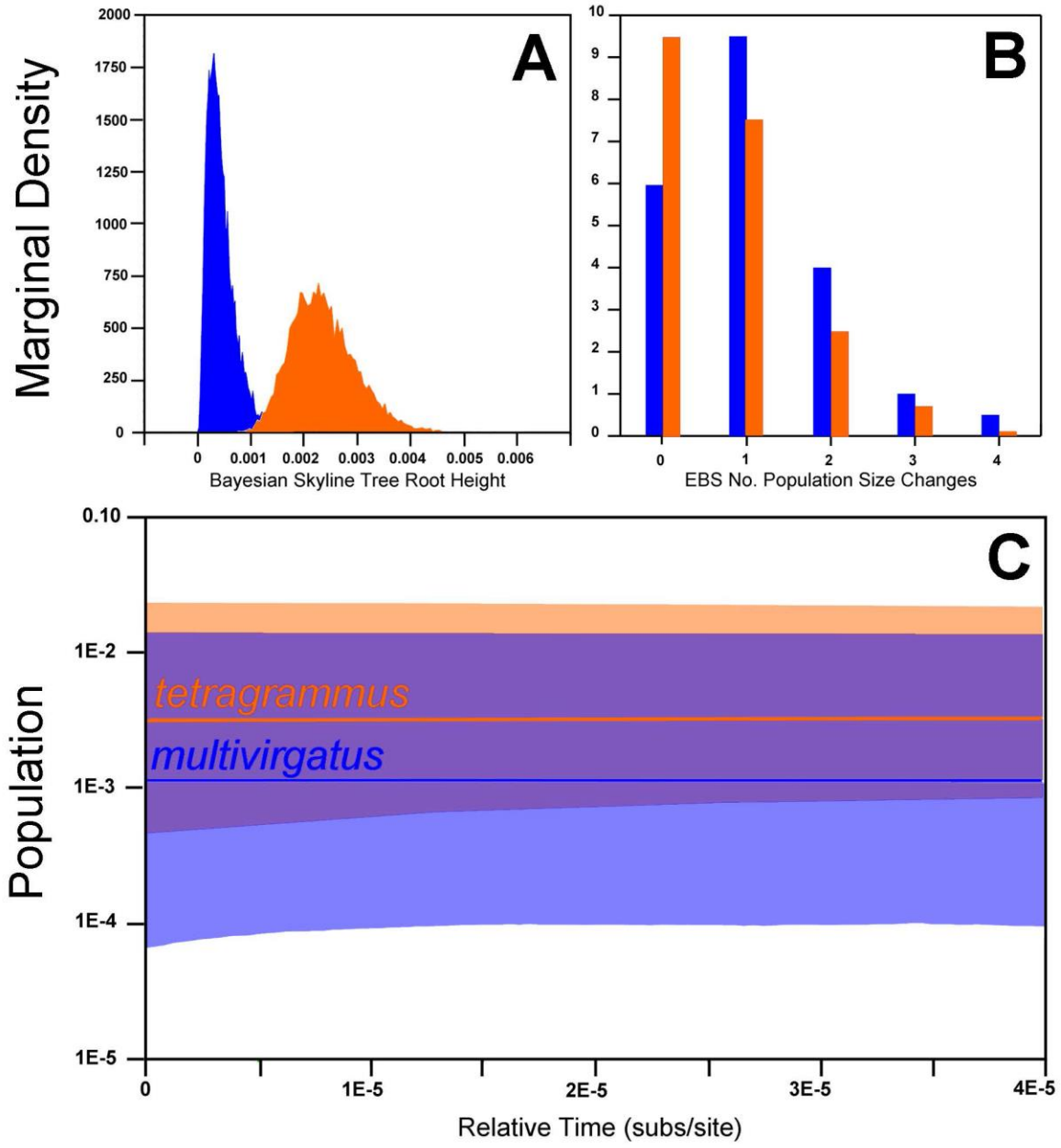


Figure 1.6. Results from extended Bayesian skyline (EBS) analyses of six *Plestiodon multivirgatus* and 16 *P. tetragrammus*: **A**, marginal densities of tree root height; **B**, marginal densities of the number of estimated population size changes; and **C**, skyline plots of population size over relative time in substitutions per site. Values for *P. multivirgatus* and *P. tetragrammus* are depicted in blue and orange, respectively and purple is where the population size values overlap.

Table 1.1 Within-species genetic distances (uncorrected "p-distances") for mitochondrial (mtDNA) and nuclear (nDNA) loci used in this study.

Locus	Genome	*tetragrammus* (both	*brevilineatus* only	*multivirgatus*
12S	mtDNA	0.019	0.012	0.003
16S	mtDNA	0.013	0.004	0.004
ND1	mtDNA	0.033	0.021	0.007
BDNF	nDNA	0.001	N/A	0.001
SNCAIP	nDNA	0.009	N/A	0.004
PRLR	nDNA	0.001	N/A	0.000
c-mos	nDNA	0.001	N/A	0.004

Table 1.2 Codon-based tests of positive selection for *Plestiodon multivirgatus* and *P. tetragrammus*. Each analysis tests the null hypothesis of strict neutrality ($d_N = d_S$) in favor of the alternative hypothesis of positive selection ($d_N > d_S$). AA = length of sequence in amino acids.

Test	locus	n	AA	Z statistic	p-value
multivirgatus	*ND1*	6	112	-2.254	1.000
multivirgatus	*BDNF*	6	182	-0.996	1.000
multivirgatus	*C-MOS*	6	117	0.000	1.000
multivirgatus	*PRLR*	6	157	-1.070	1.000
multivirgatus	*SNCAIP*	6	135	1.011	0.157
tetragrammus	*ND1*	25	112	-4.885	1.000
tetragrammus	*BDNF*	16	182	-1.005	1.000
tetragrammus	*C-MOS*	16	117	0.000	1.000
tetragrammus	*PRLR*	16	155	-1.007	1.000
tetragrammus	*SNCAIP*	16	137	-2.272	1.000
multivirgatus + *tetragrammus*	*ND1*	31	112	-5.837	1.000
multivirgatus + *tetragrammus*	*BDNF*	22	182	-1.577	1.000
multivirgatus + *tetragrammus*	*C-MOS*	22	117	-1.544	1.000
multivirgatus + *tetragrammus*	*PRLR*	22	155	-1.110	1.000
multivirgatus + *tetragrammus*	*SNCAIP*	22	137	-2.201	1.000

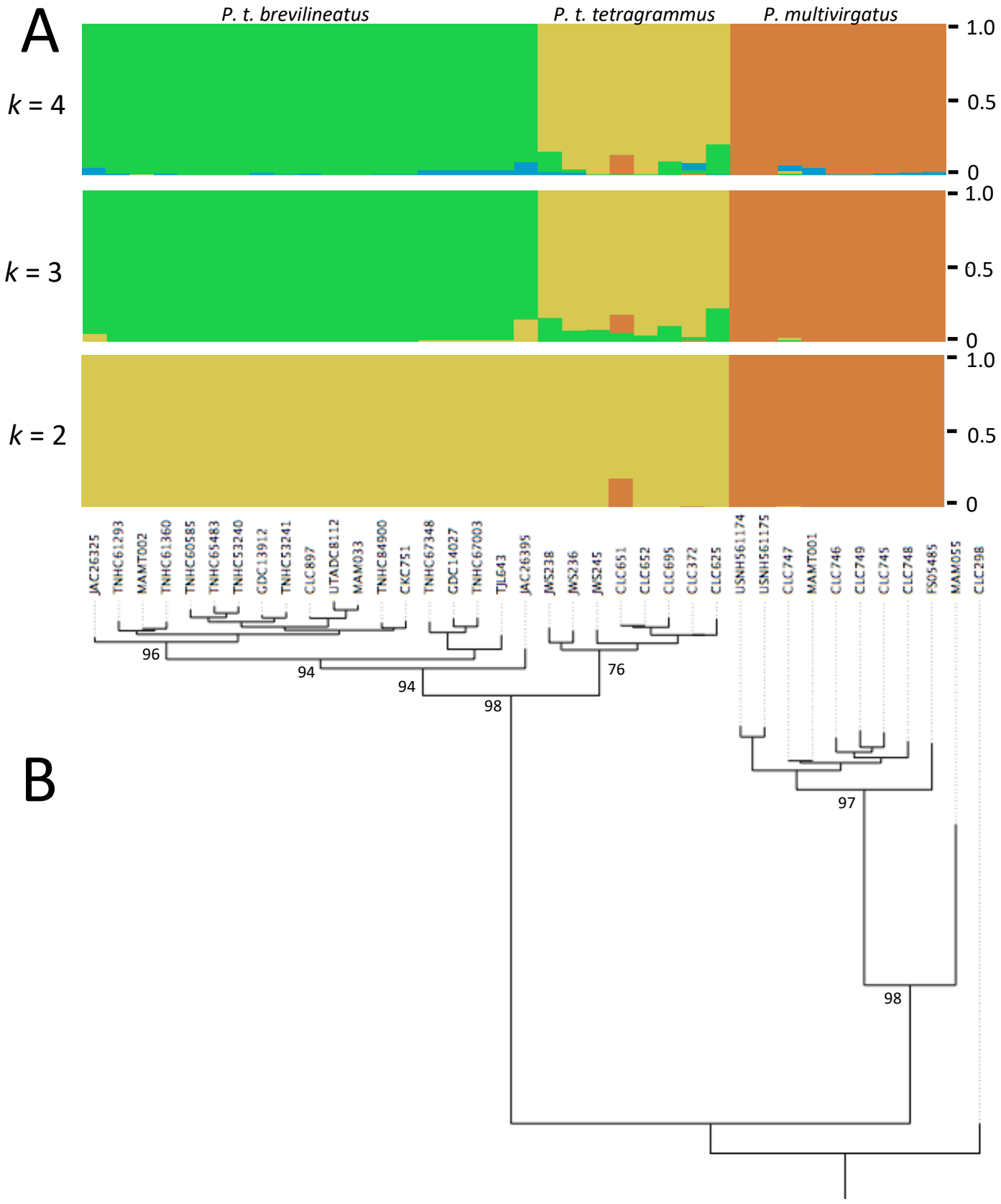

A
P. t. brevilineatus
P. t. tetragrammus
P. multivirgatus
k = 4
k = 3
k = 2
1.0
0.5
0
JAC26325
TNHC61293
MAMT002
TNHC61360
TNHC60585
TNHC65483
TNHC53240
GDC13912
TNHC53241
CLC897
UTADC8112
MAM033
TNHC84900
CKC751
TNHC67348
GDC14027
TNHC67003
TJL643
JAC26395
JWS238
JWS236
JWS245
CLC651
CLC652
CLC695
CLC372
CLC625
USNH561174
USNH561175
CLC747
MAMT001
CLC746
CLC749
CLC745
CLC748
FS05485
MAM055
CLC298
96
94
94
98
76
97
98
B

Figure 2.1 A. STRUCTURE plot of inferred origin of genome for *P. t. brevilineatus*, *P. t. tetragrammus*, and *P. multivirgatus* where k is the number of populations set prior to the analysis. All k values show a distinction between *P. tetragrammus* (yellow) and *P. multivirgatus* (brown). Analyses with k = 3 and k = 4 also show a distinction between *P. t. tetragrammus* (yellow) and *P. t. brevilineatus* (green).

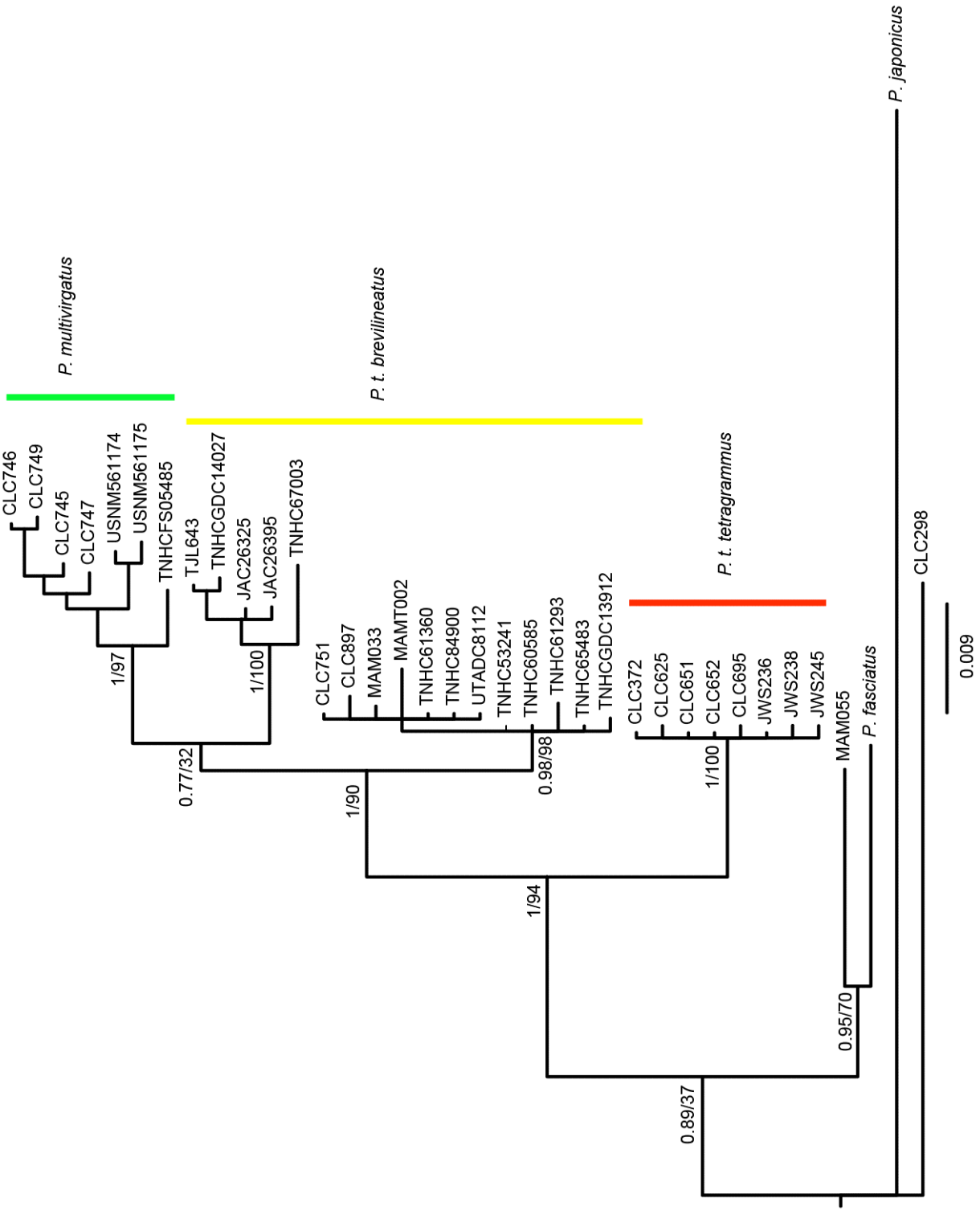


Figure 2.2 Maximum likelihood tree of mtDNA for *P. t. tetragrammus*, *P. t. brevilineatus*, and *P. multivirgatus*. Node values represent posterior probabilities from MrBayes and bootstrap support from RaxML, respectively.

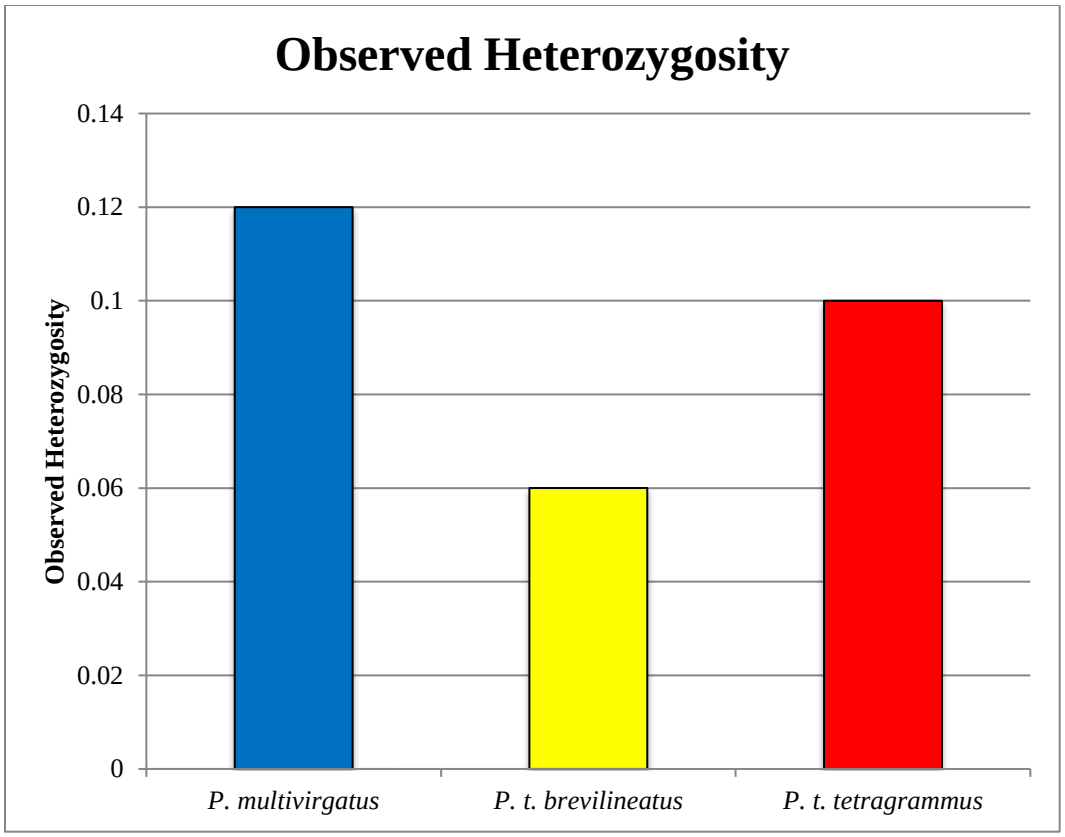


Figure 2.3 Histogram of observed heterozygosity in *P. t. tetragrammus, P. t. brevilineatus,* and *P. multivirgatus* which suggests a pattern more consistent in range expansion in *P. t. brevilineatus*.

Table 2.1 Genetic differentiation measures for comparisons between *P. t. brevilineatus*, *P. t. tetragrammus*, and *P. t. multivirgatus*. Values range from 0 for complete admixture and 1 for complete differentiation.

populations	g_{st}	G_{st}	GG_{st}	D	F_{st}
P. multivirgatus vs *P. t. brevilineatus*	0.4987	0.8641	0.9093	0.5668	0.656
P. multivirgatus vs *P. t. tetragrammus*	0.4995	0.8677	0.9117	0.5803	0.6214
P. t. brevilineatus vs *P. t. tetragrammus*	0.2094	0.3467	0.4598	0.059	0.2899

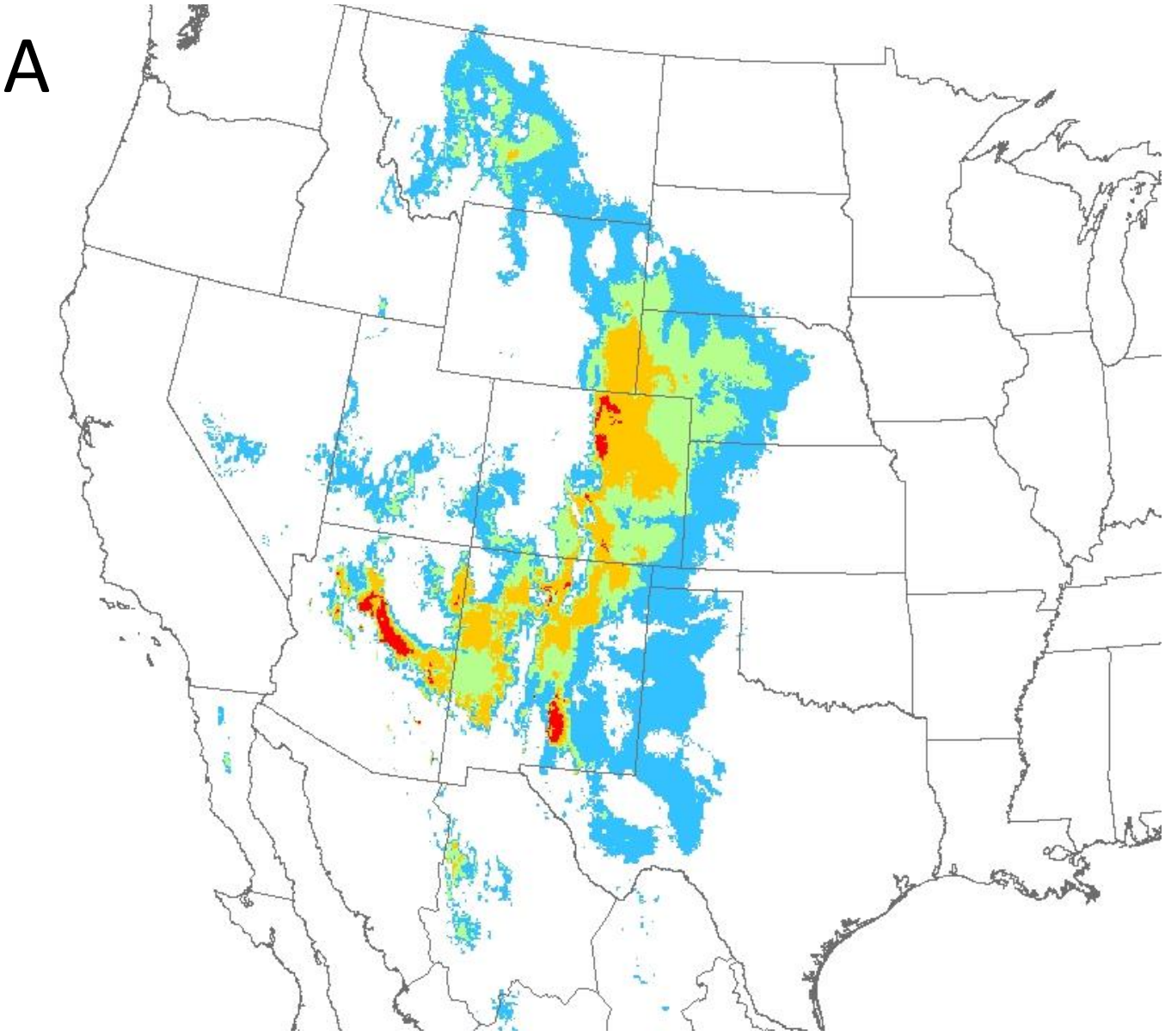
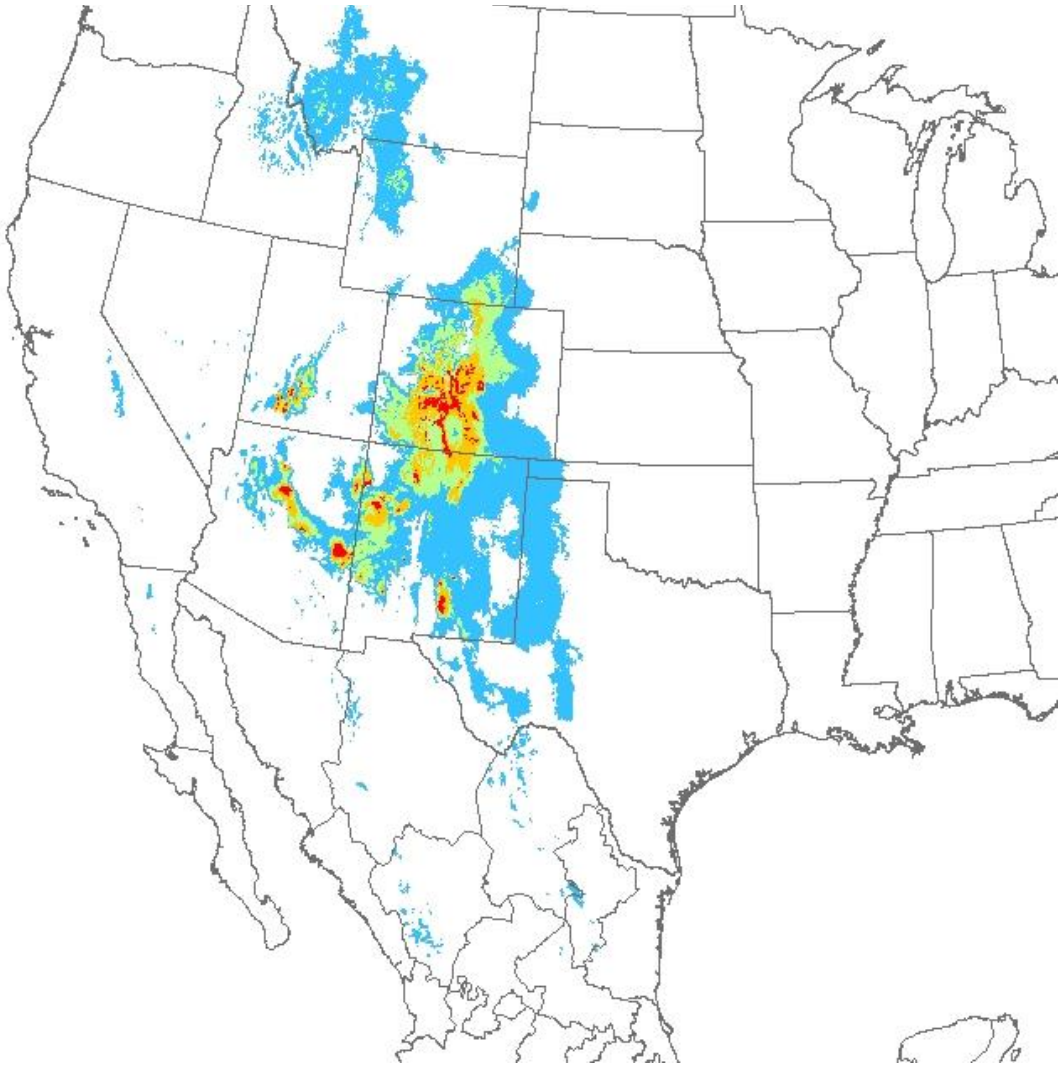


Figure 3.1 Species distribution models for *P. multivirgatus* for various timepoints. Warmer colors indicate higher relative habitat suitability. A. current time period, B. projected CCSM model of Mid Holocene ~6kya, C. projected MIROC model of Mid Holocene, D. projected CCSM model of the Last Glacial Maximum~22kya, E. projected MIROC model of the LGM.

B



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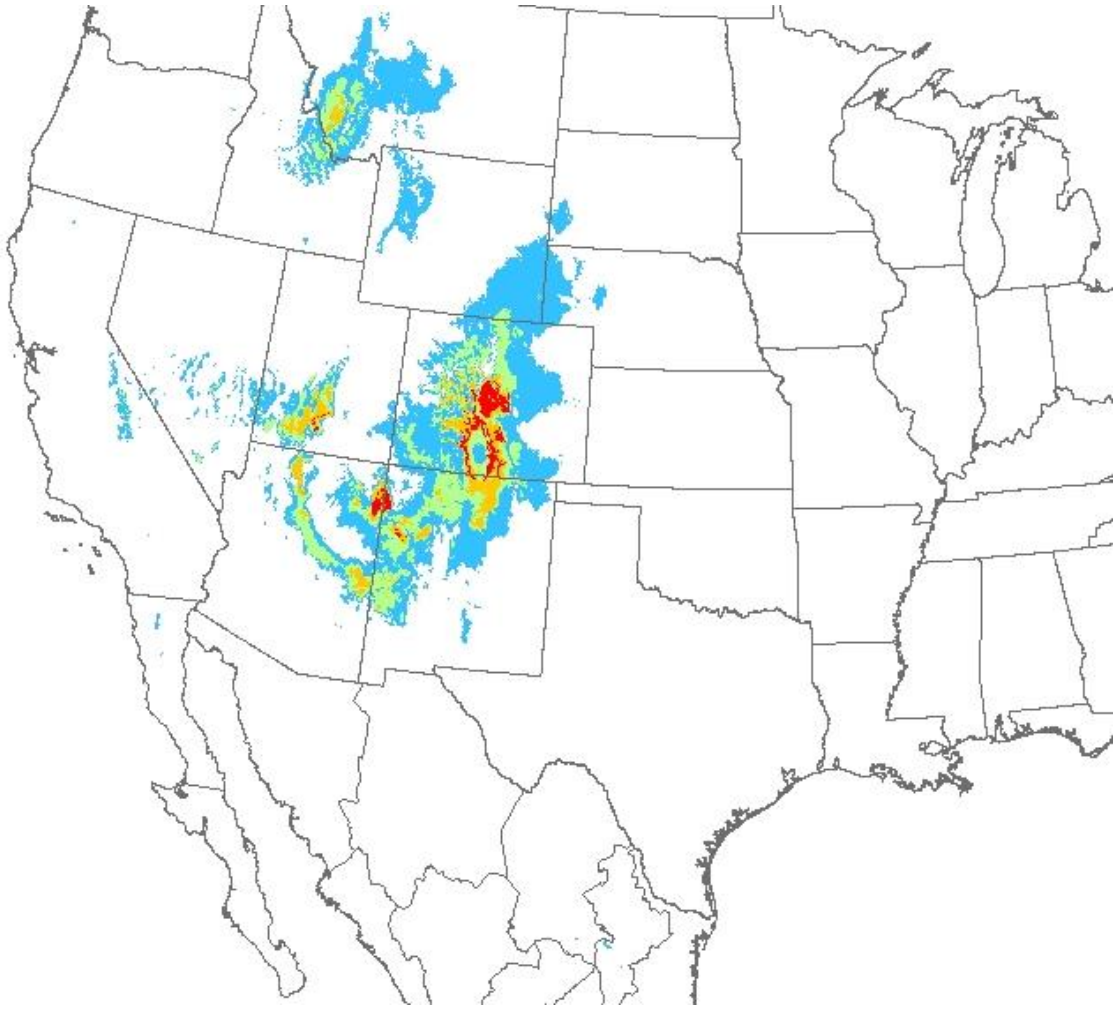

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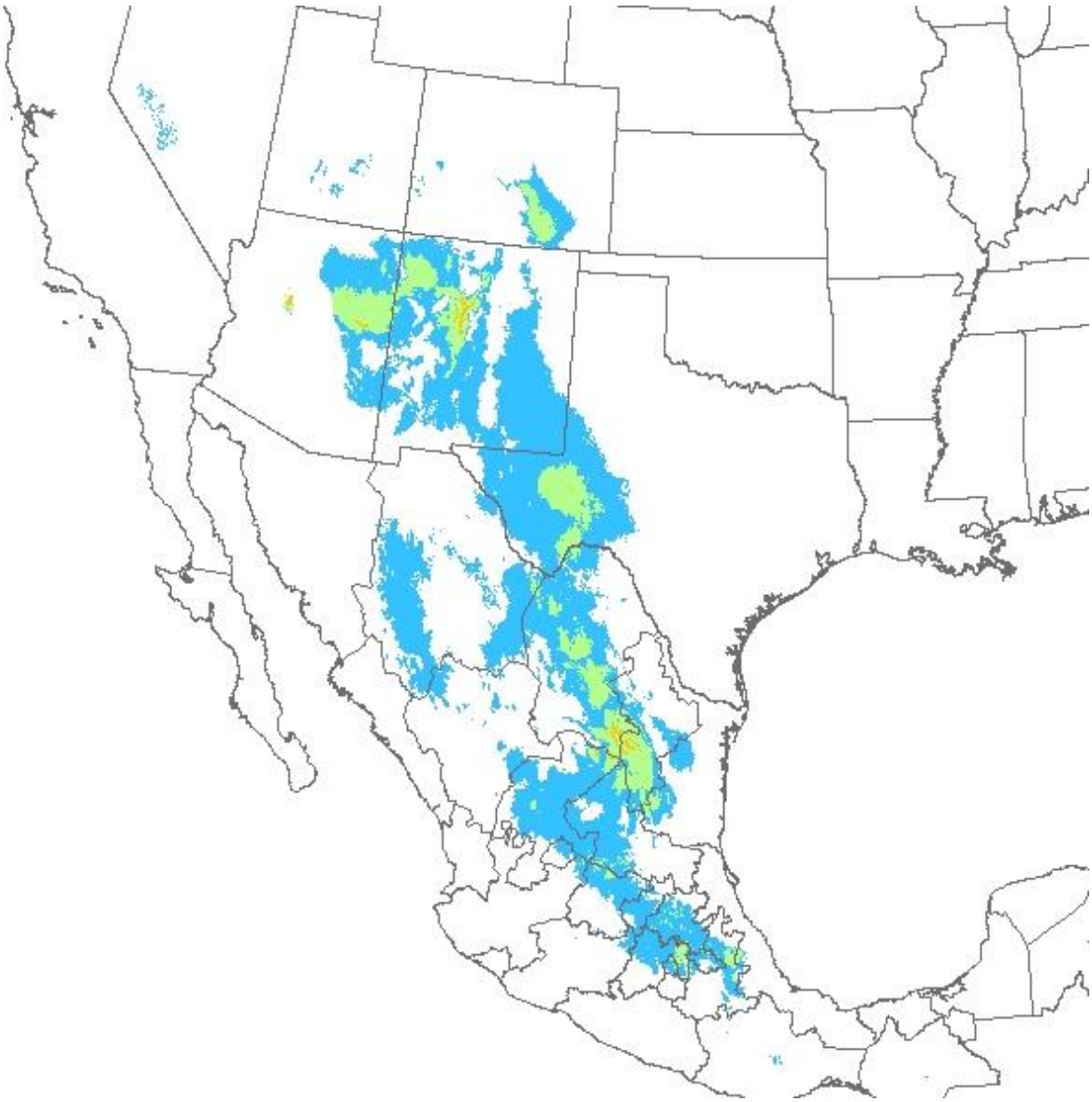

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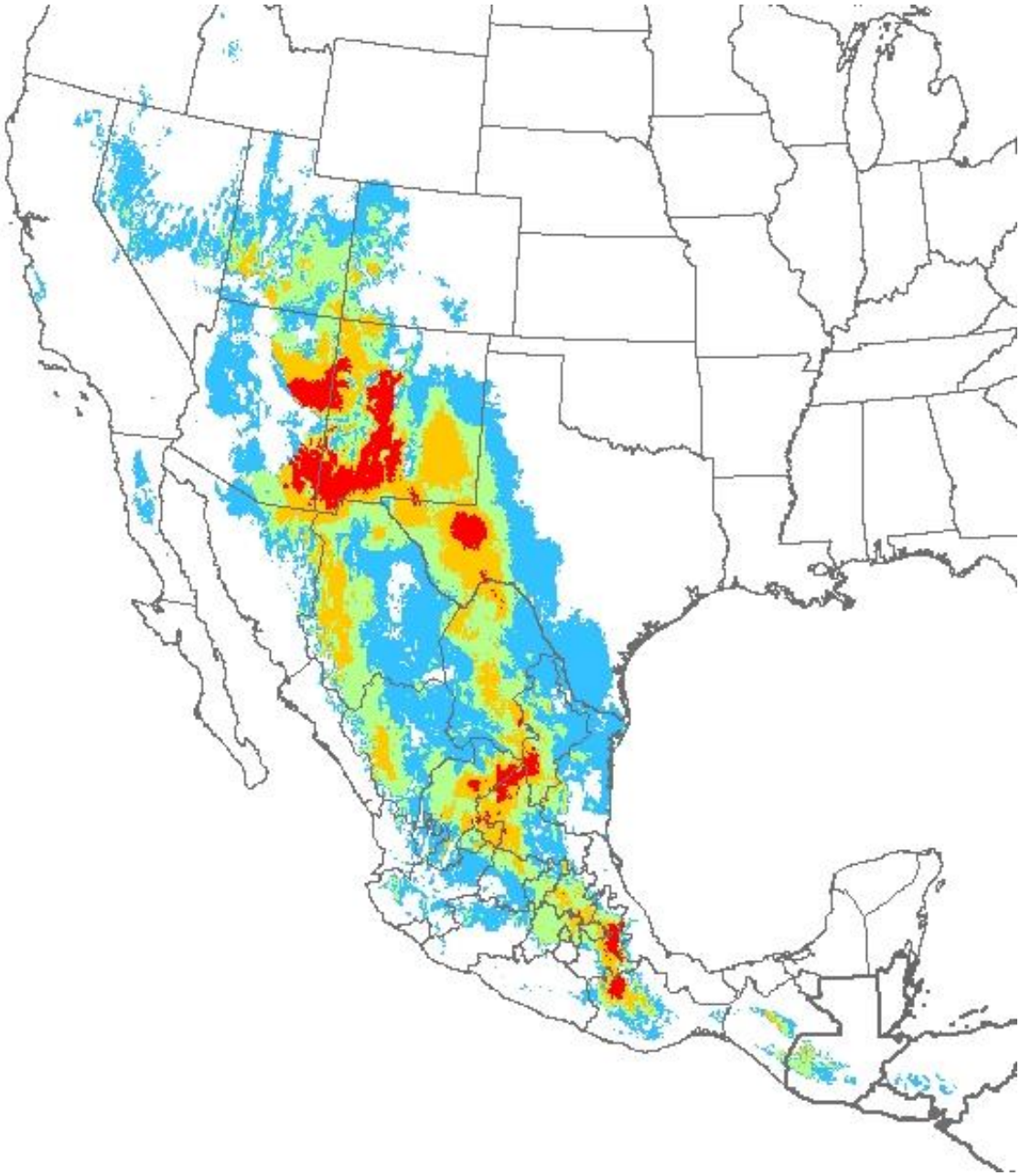


A

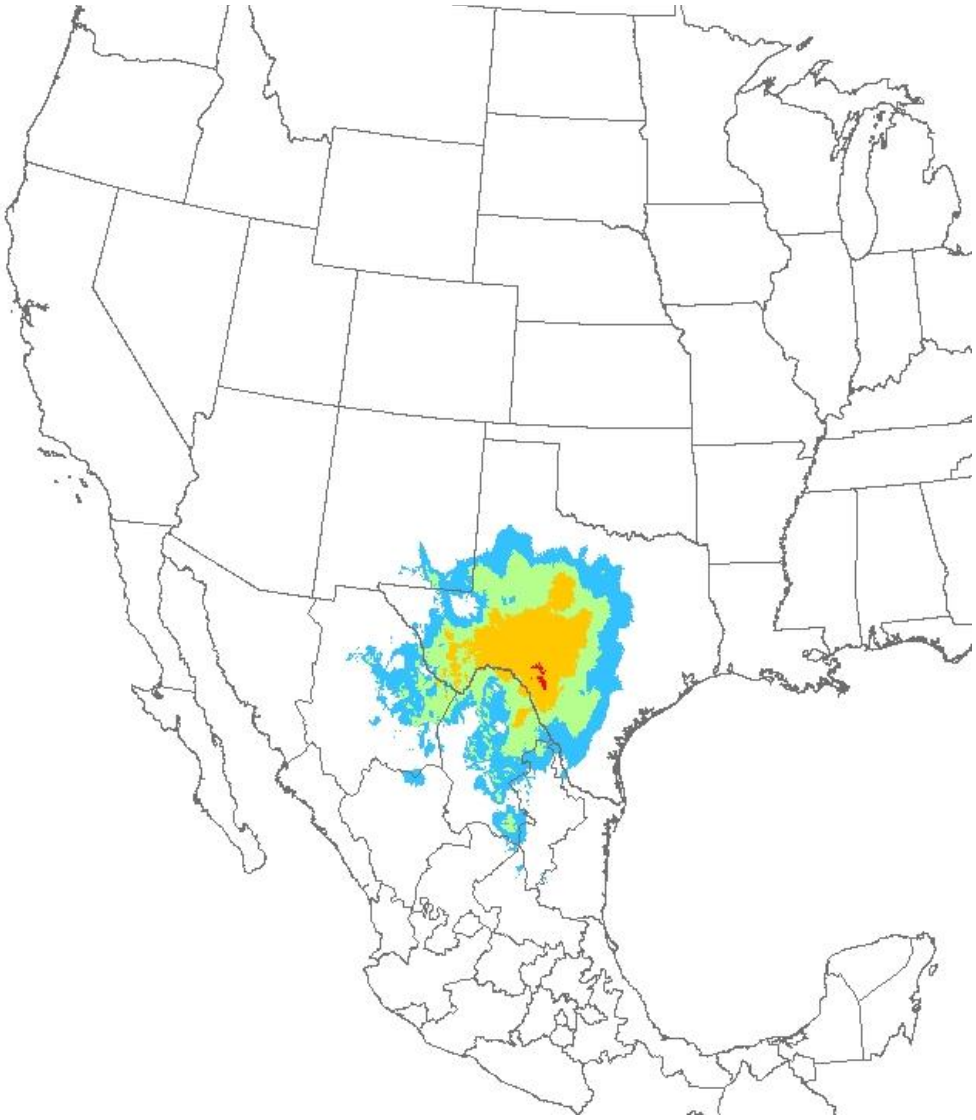
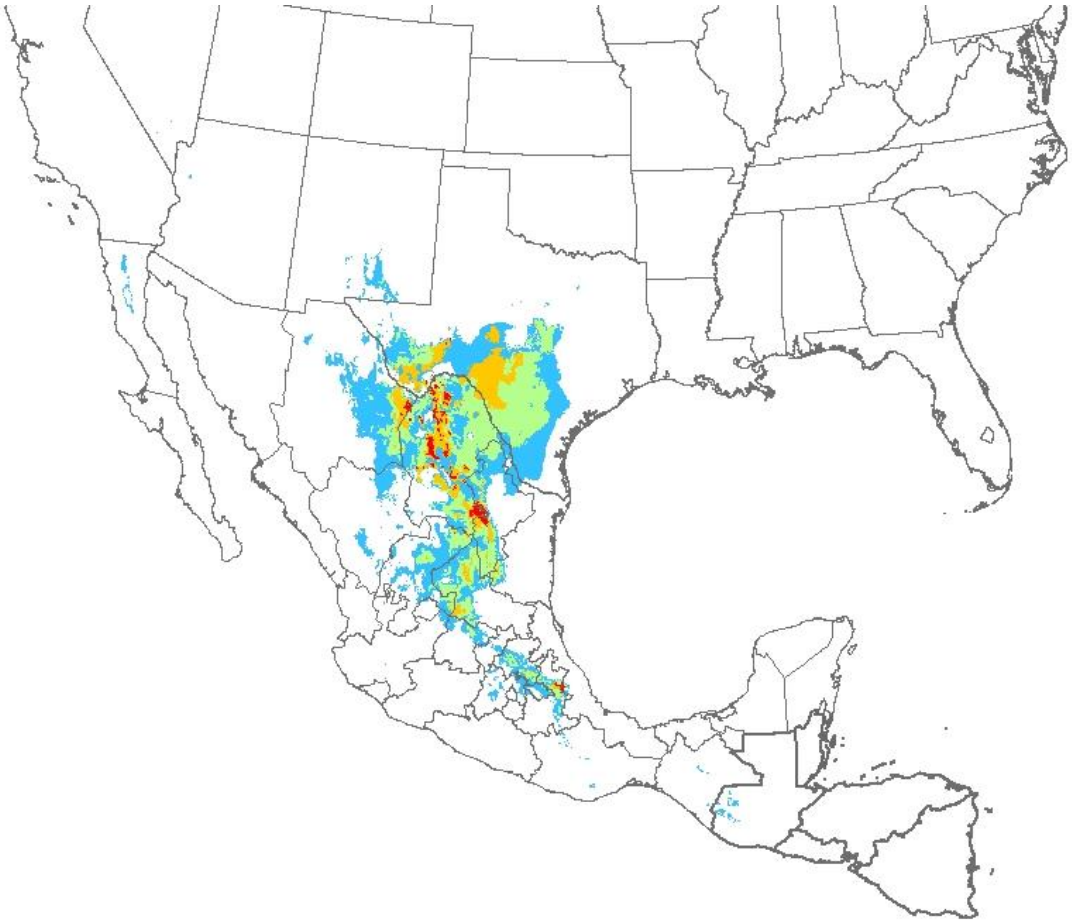
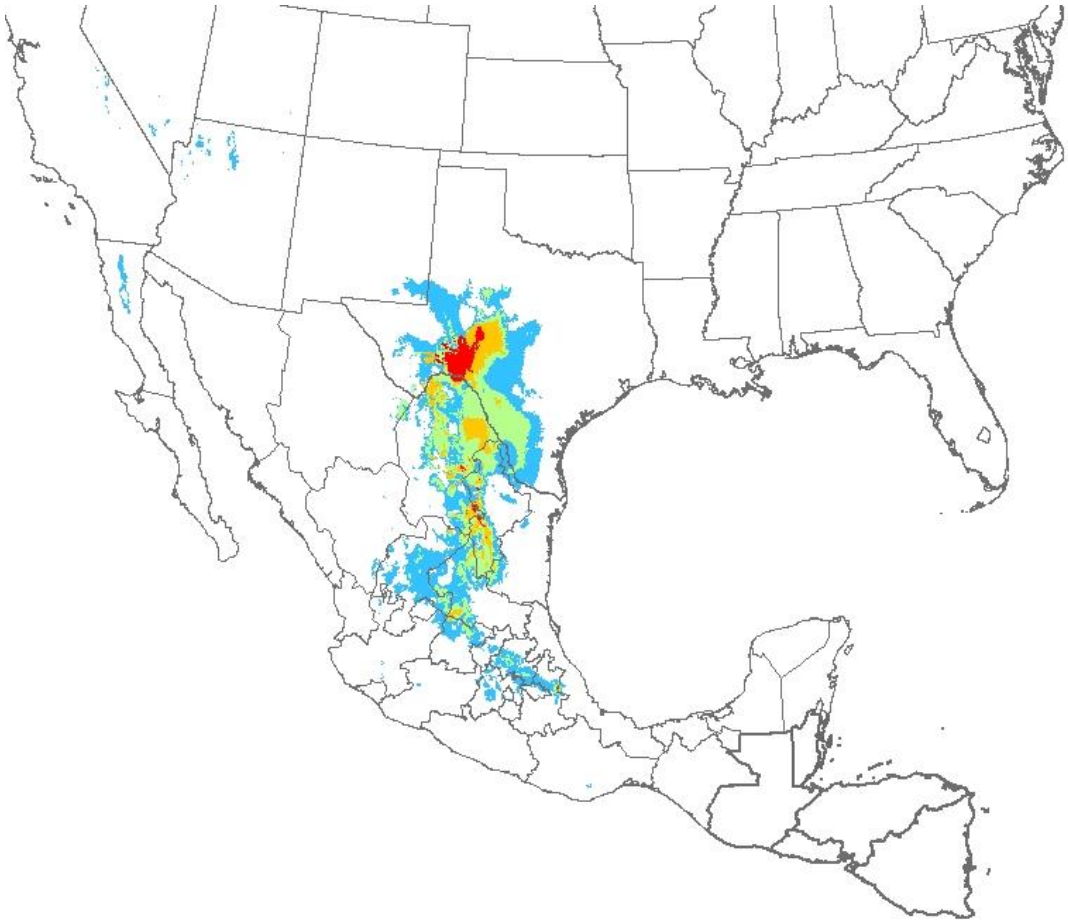


Figure 3.2 Species distribution models for *P. t. brevilineatus* for various timepoints. Warmer colors indicate higher relative habitat suitability. A. current time period, B. projected CCSM model of Mid Holocene ~6kya, C. projected MIROC model of Mid Holocene, D. projected CCSM model of the Last Glacial Maximum~22kya, E. projected MIROC model of the LGM.

B



C



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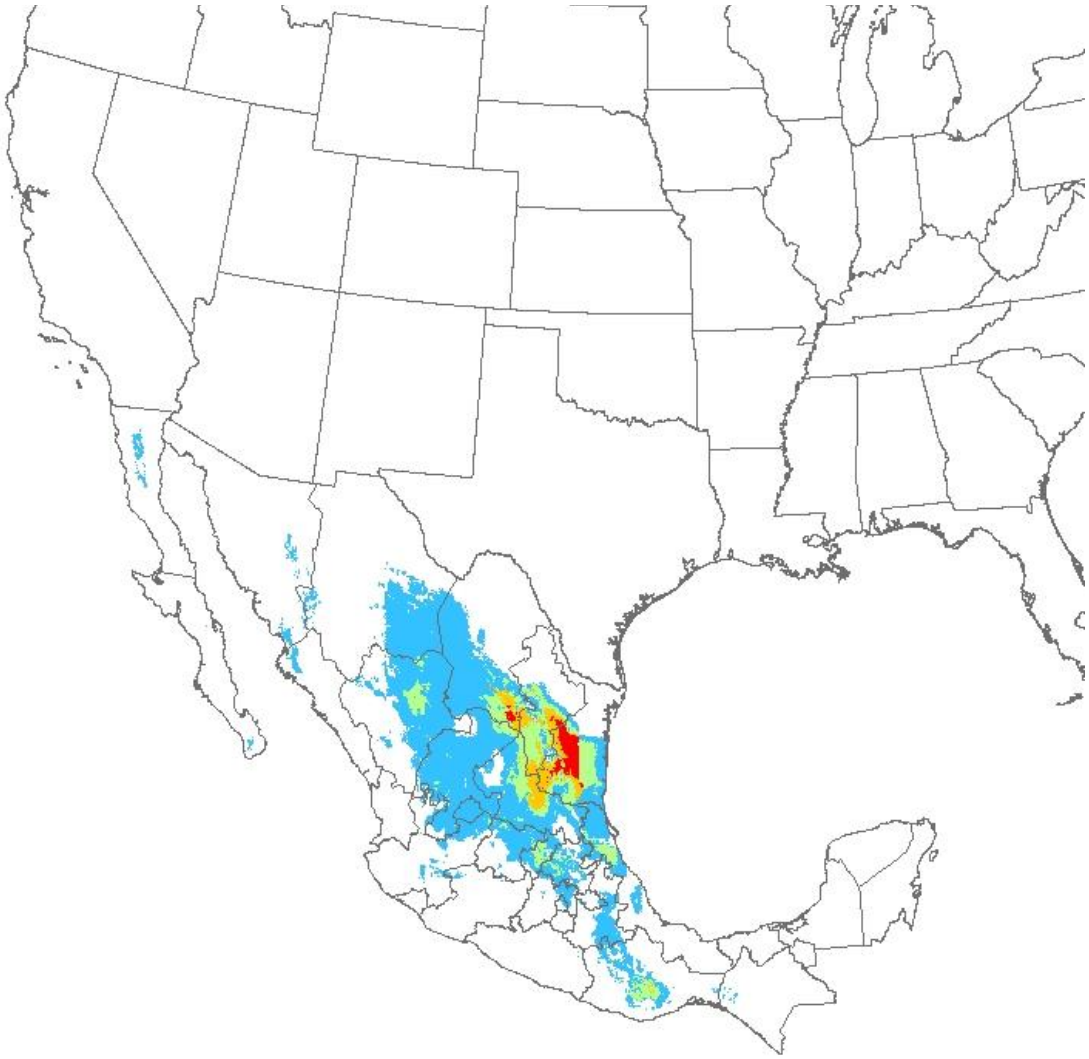

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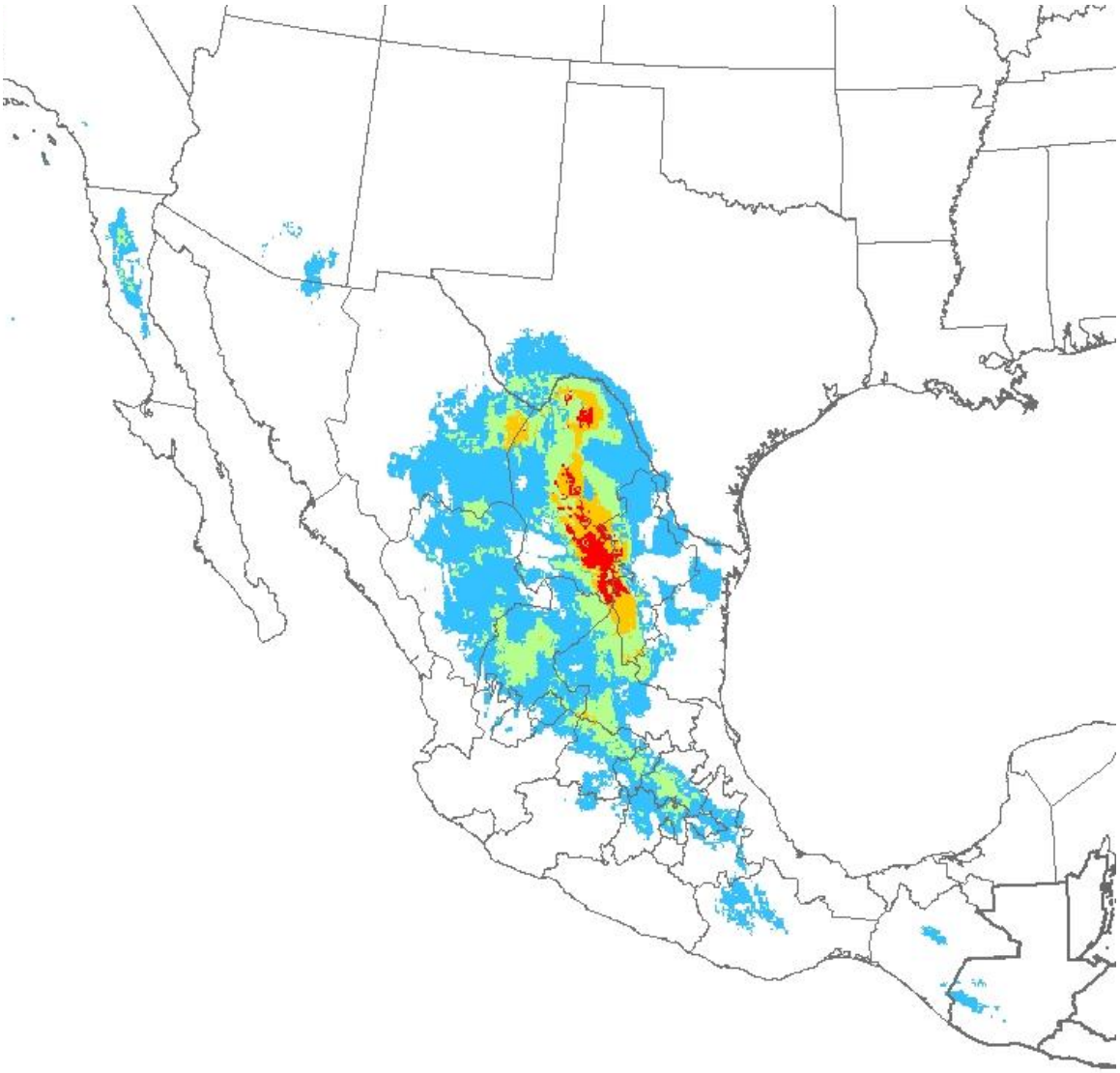


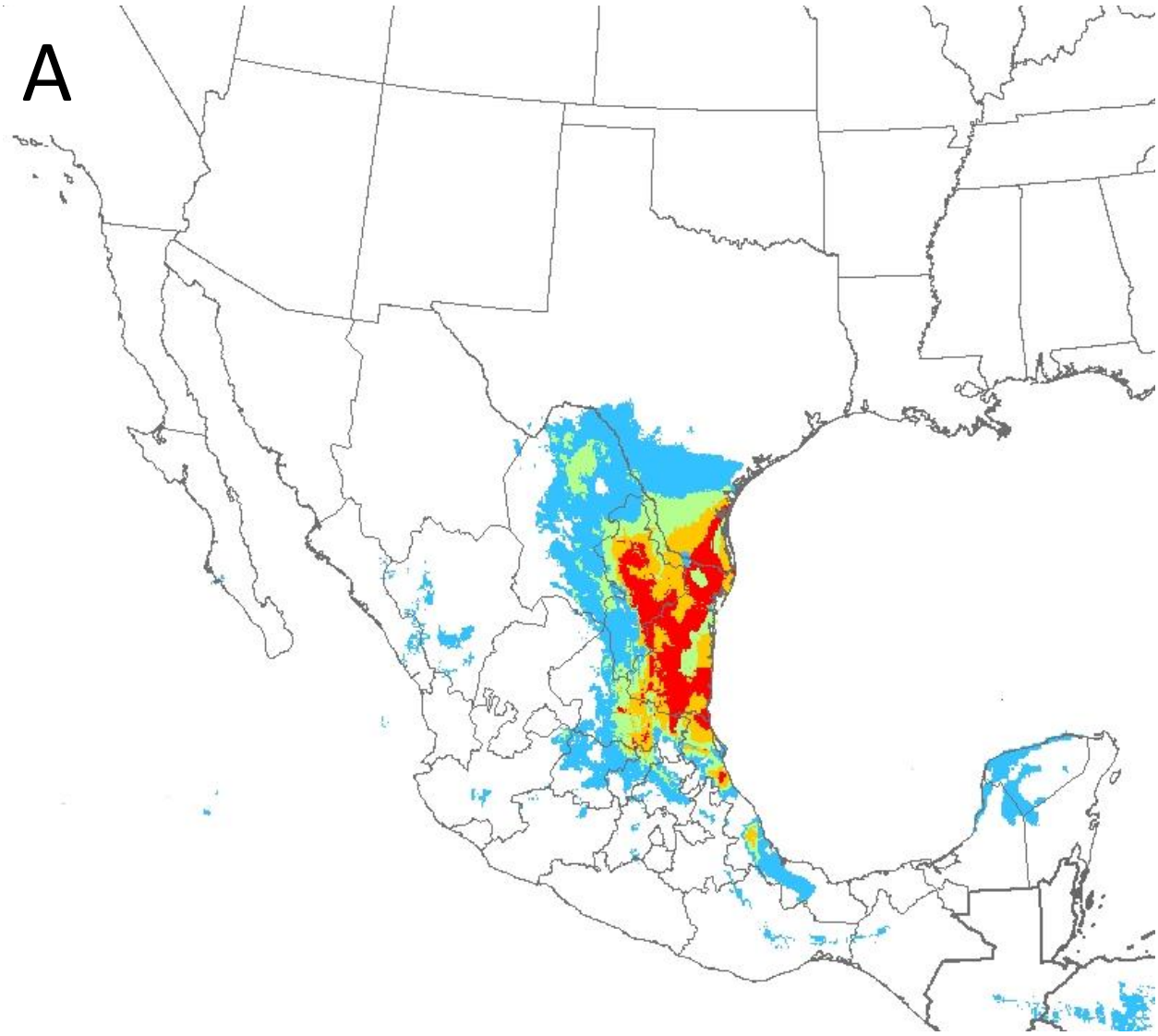
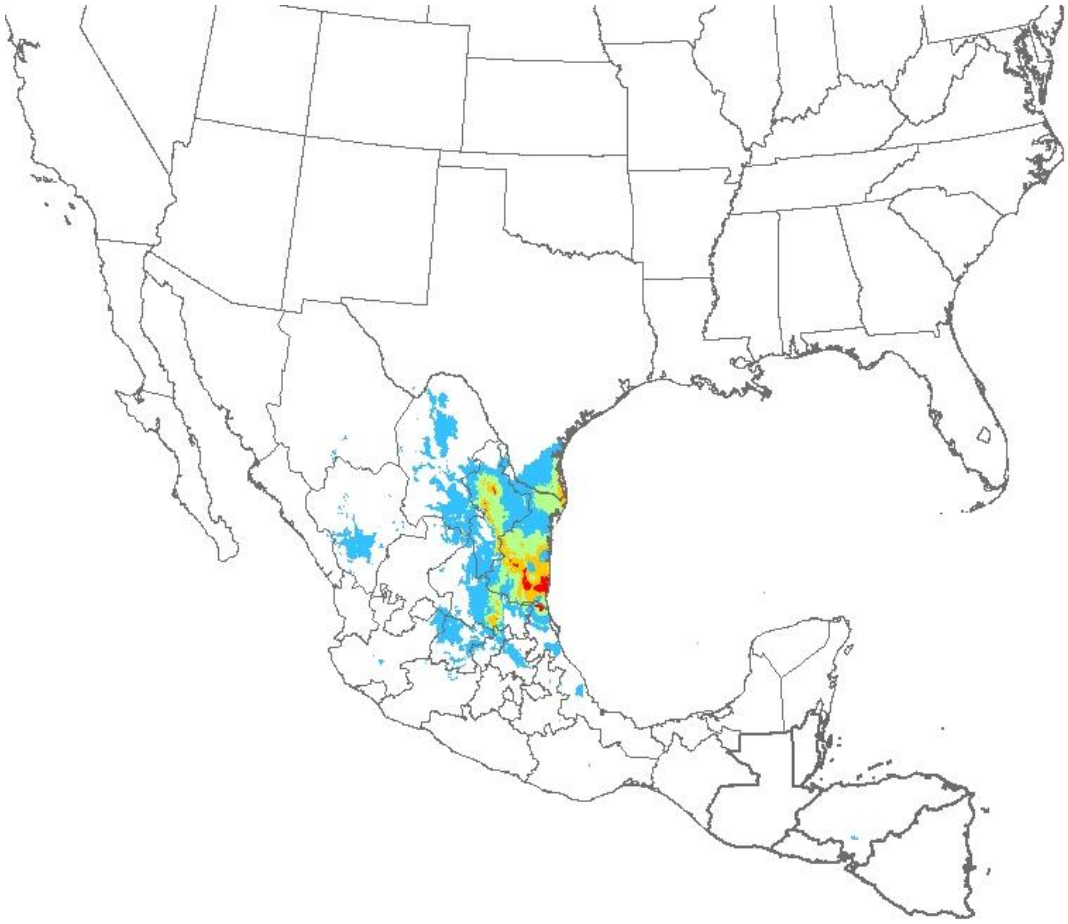
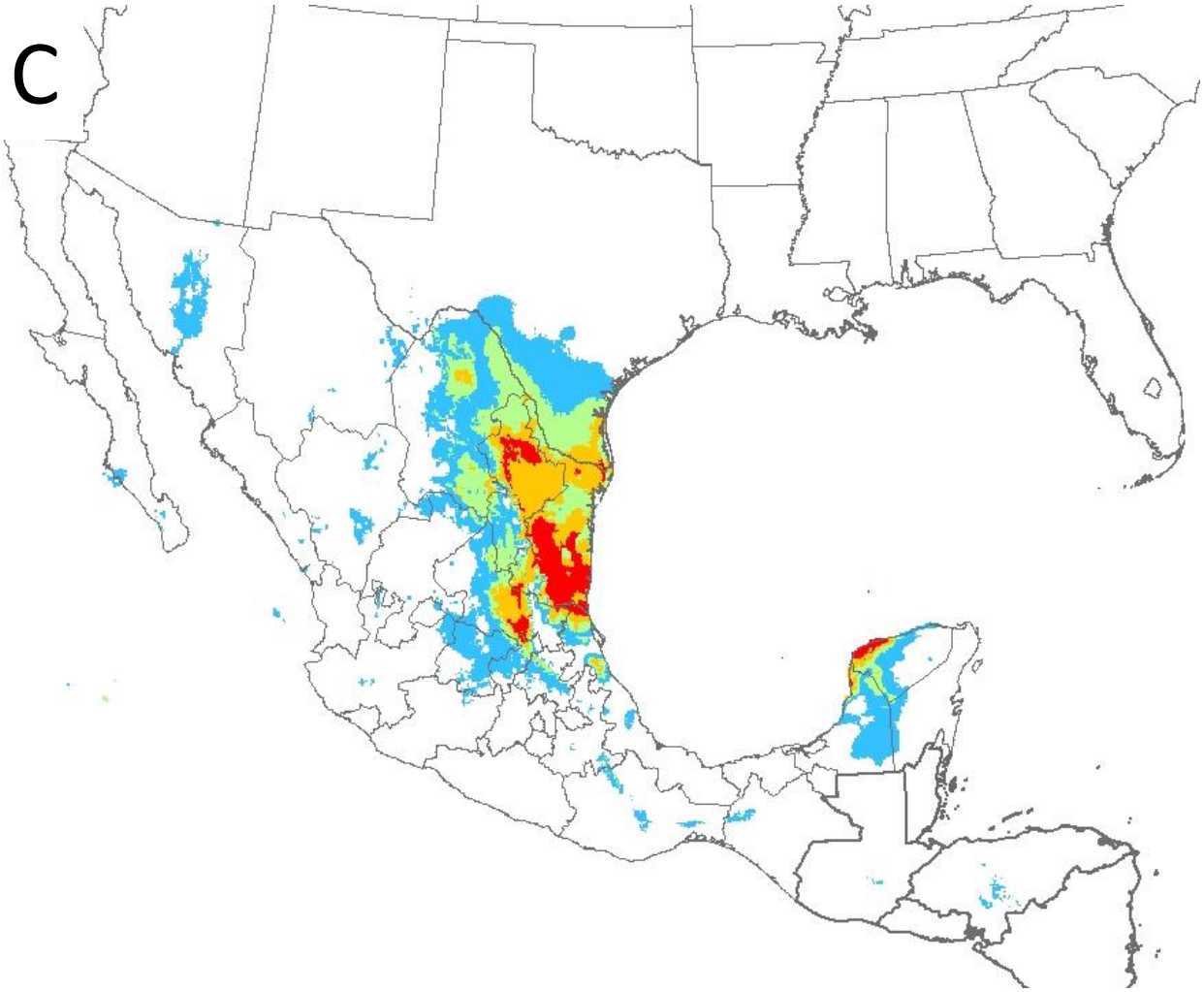


Figure 3.3 Species distribution models for *P. t. tetragrammus* for various timepoints. Warmer colors indicate higher relative habitat suitability. A. current time period, B. projected CCSM model of Mid Holocene ~6kya, C. projected MIROC model of Mid Holocene, D. projected CCSM model of the Last Glacial Maximum~22kya, E. projected MIROC model of the LGM.

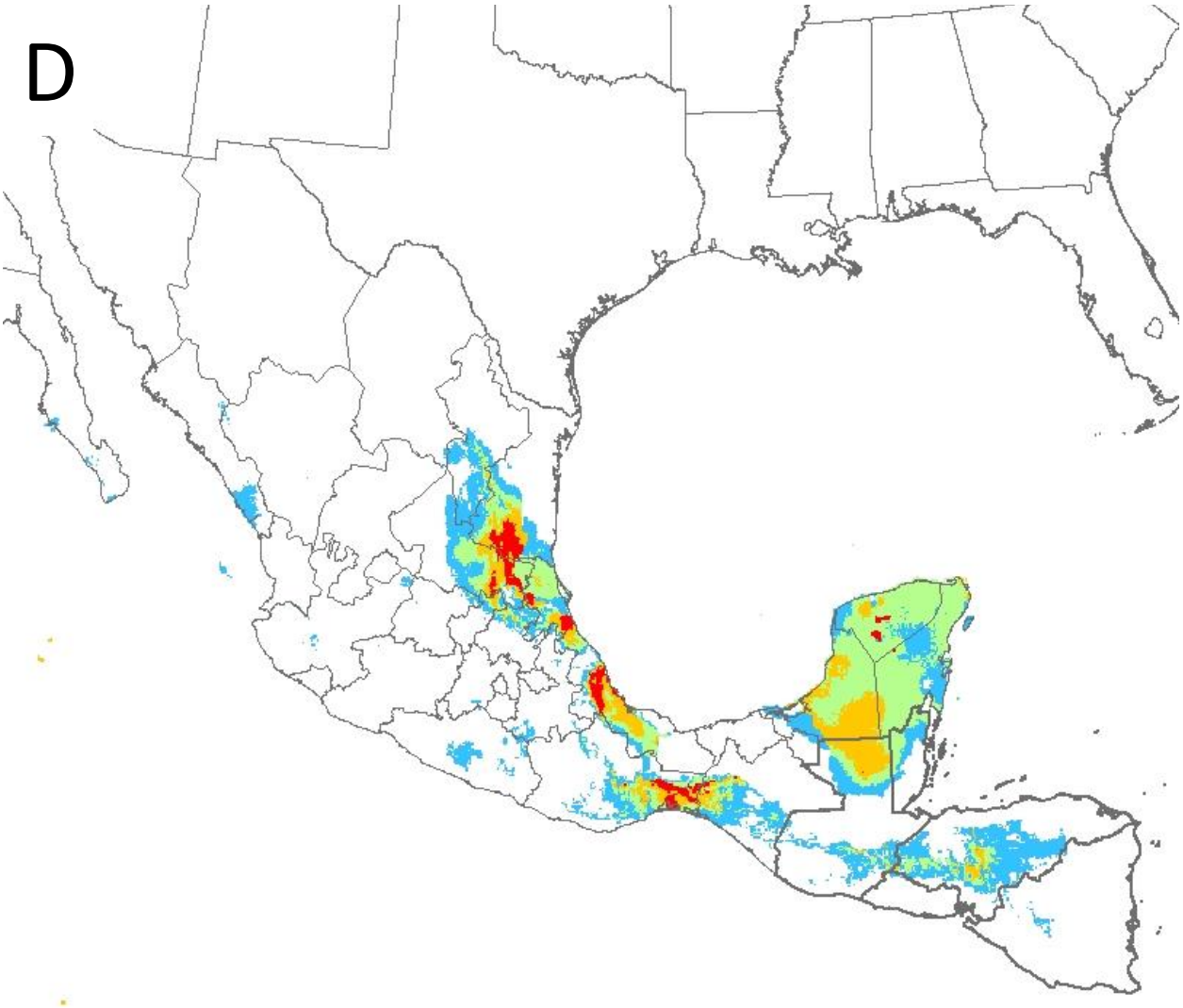
B

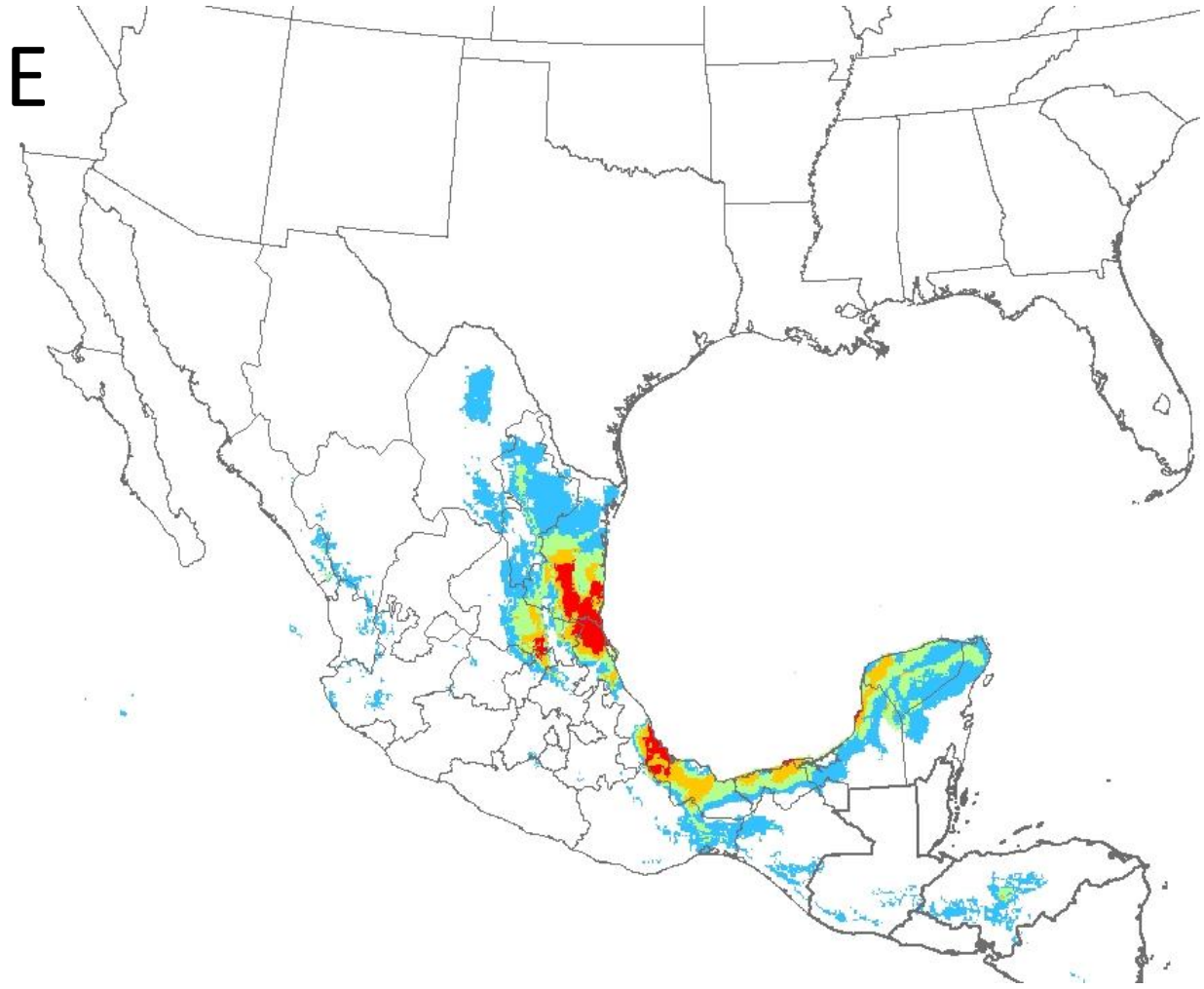


C



D




E

A

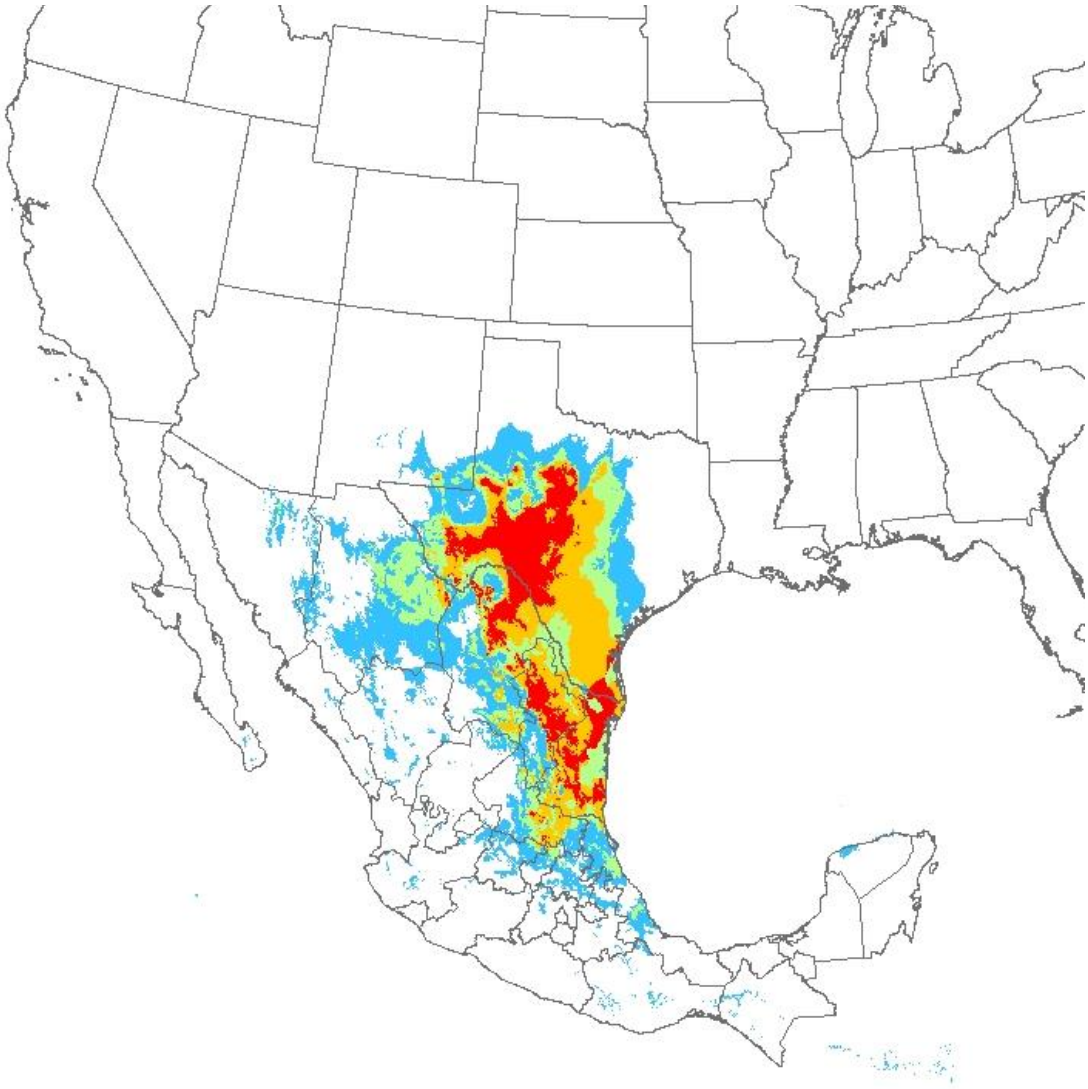
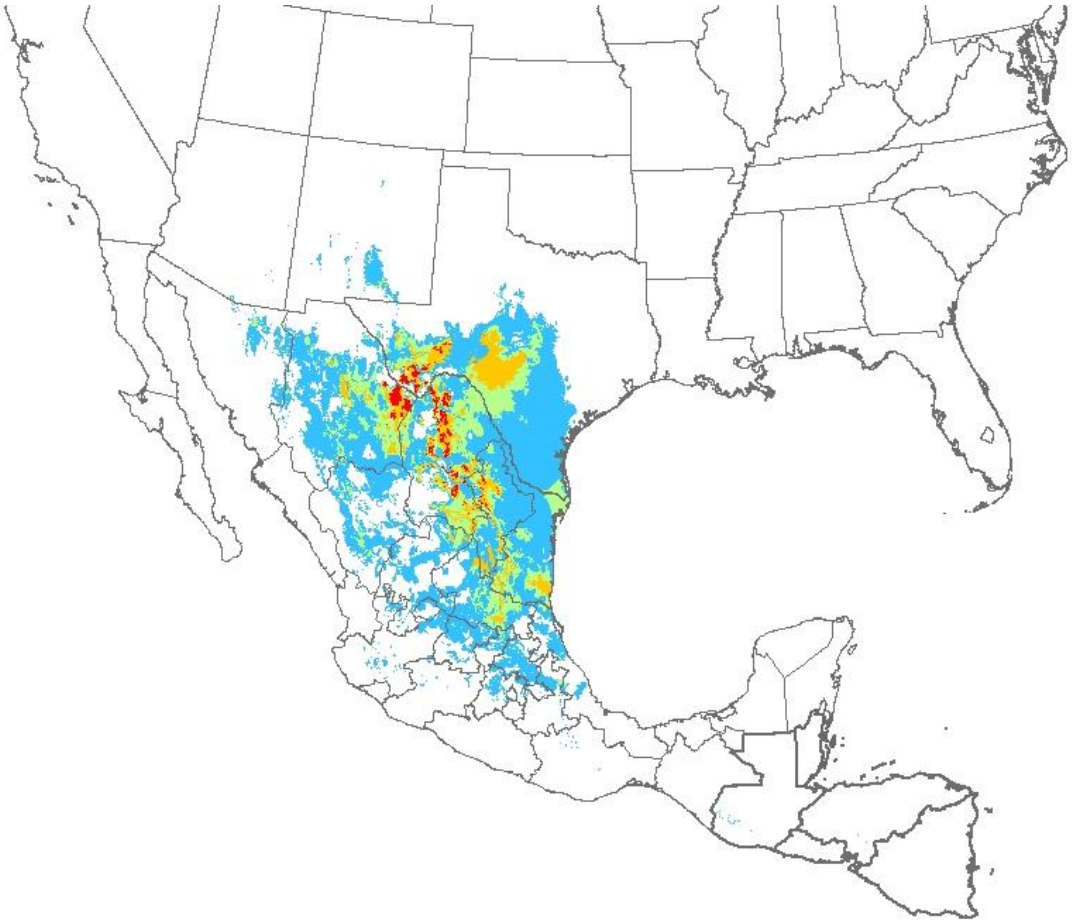
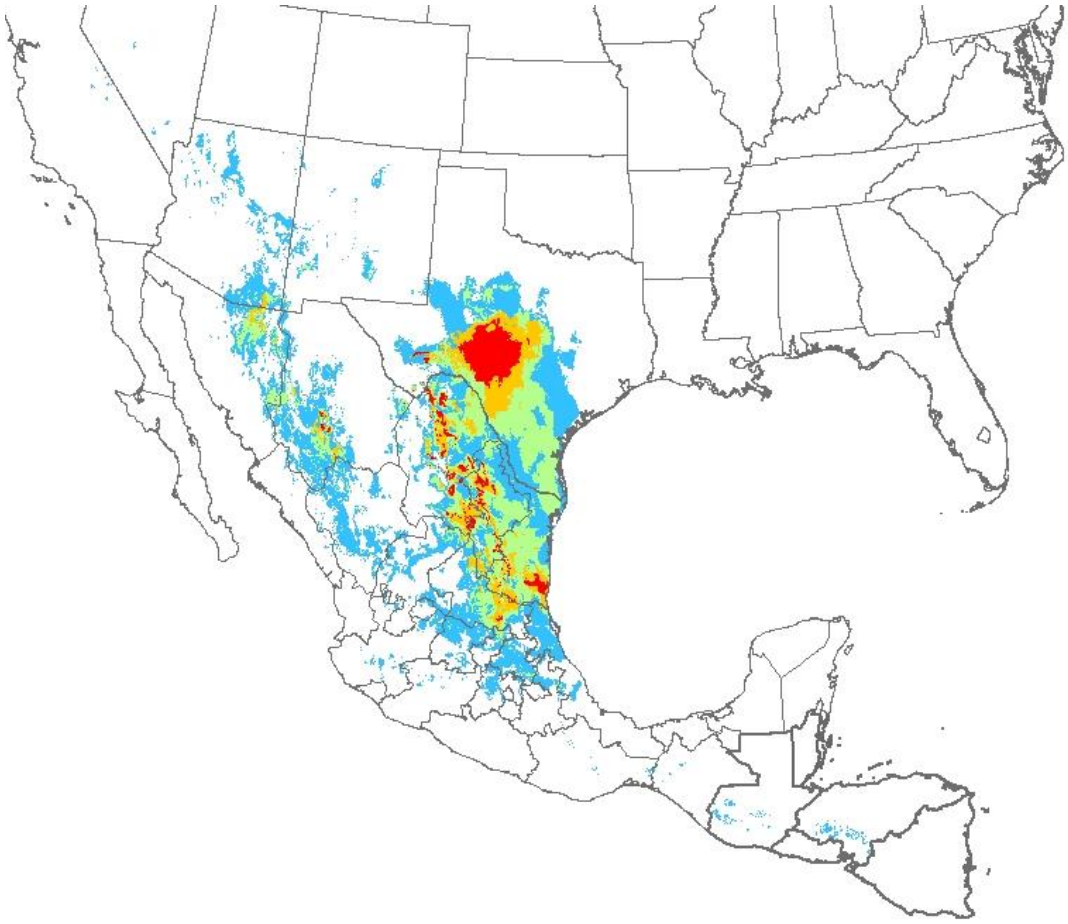


Figure 3.4 Species distribution models for combined *P. t. tetragrammus* and *P. t. brevilineatus* for various timepoints. Warmer colors indicate higher relative habitat suitability. A. current time period, B. projected CCSM model of Mid Holocene ~6kya, C. projected MIROC model of Mid Holocene, D. projected CCSM model of the Last Glacial Maximum~22kya, E. projected MIROC model of the LGM.

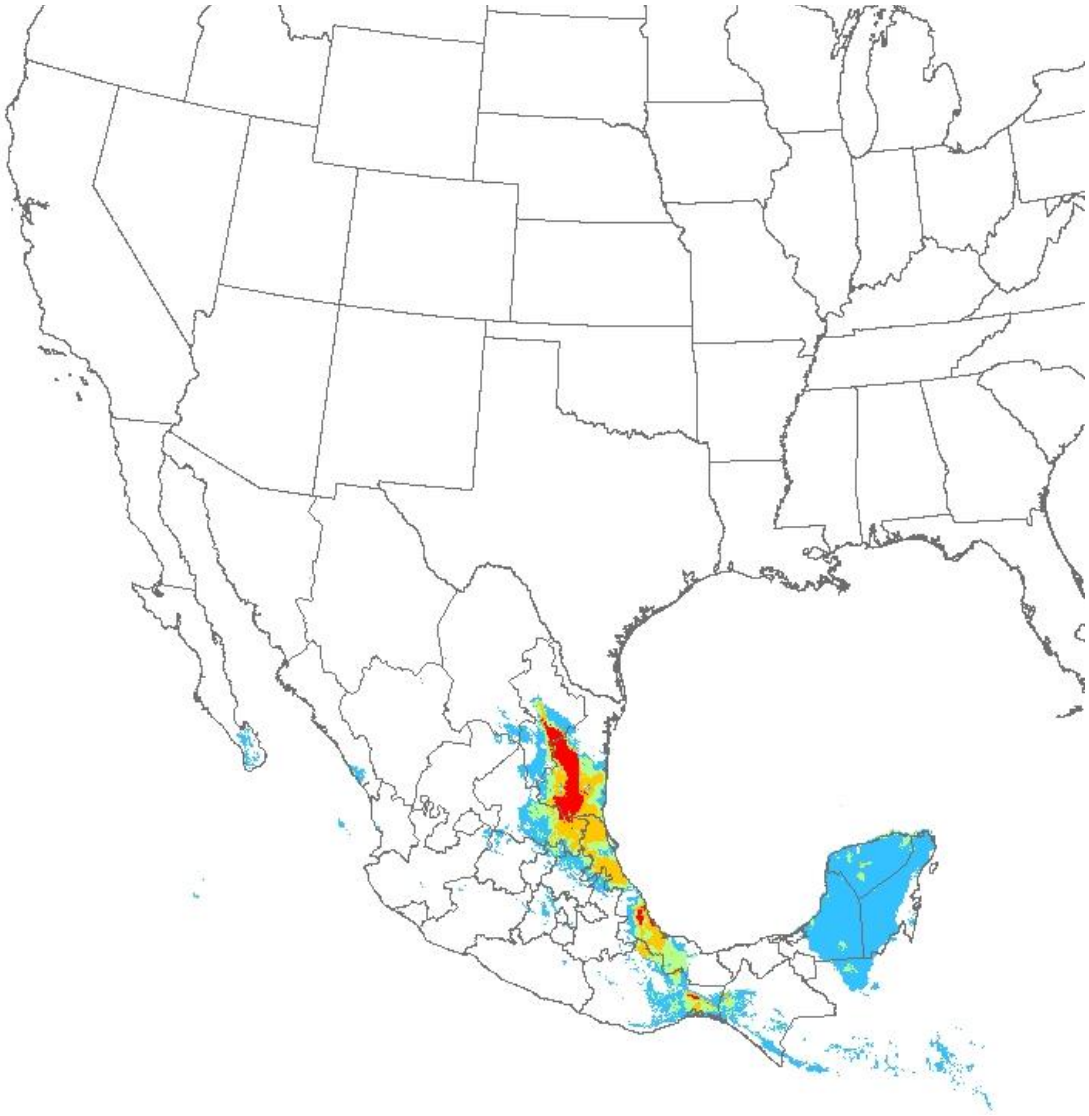
B

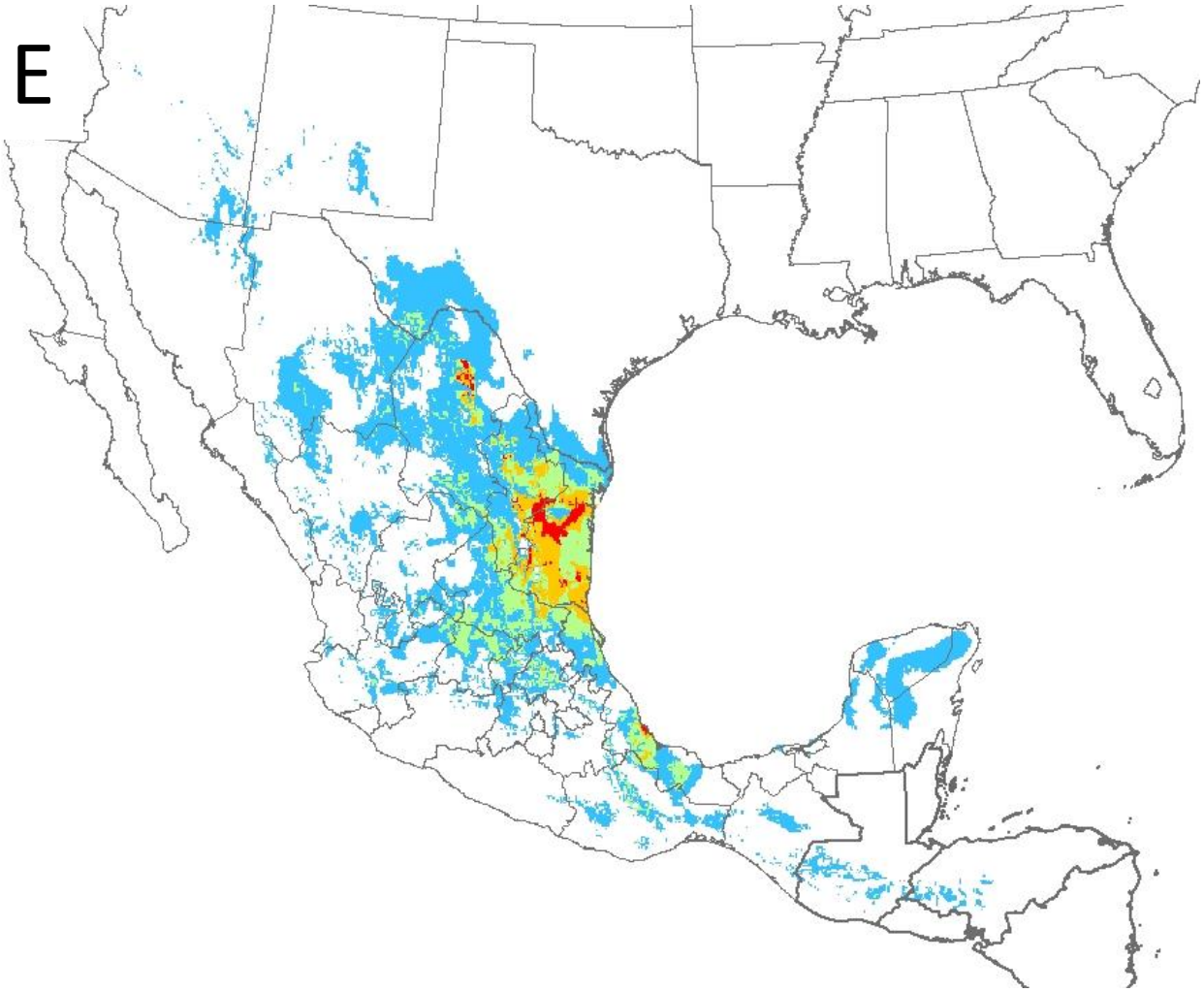


C



D




E

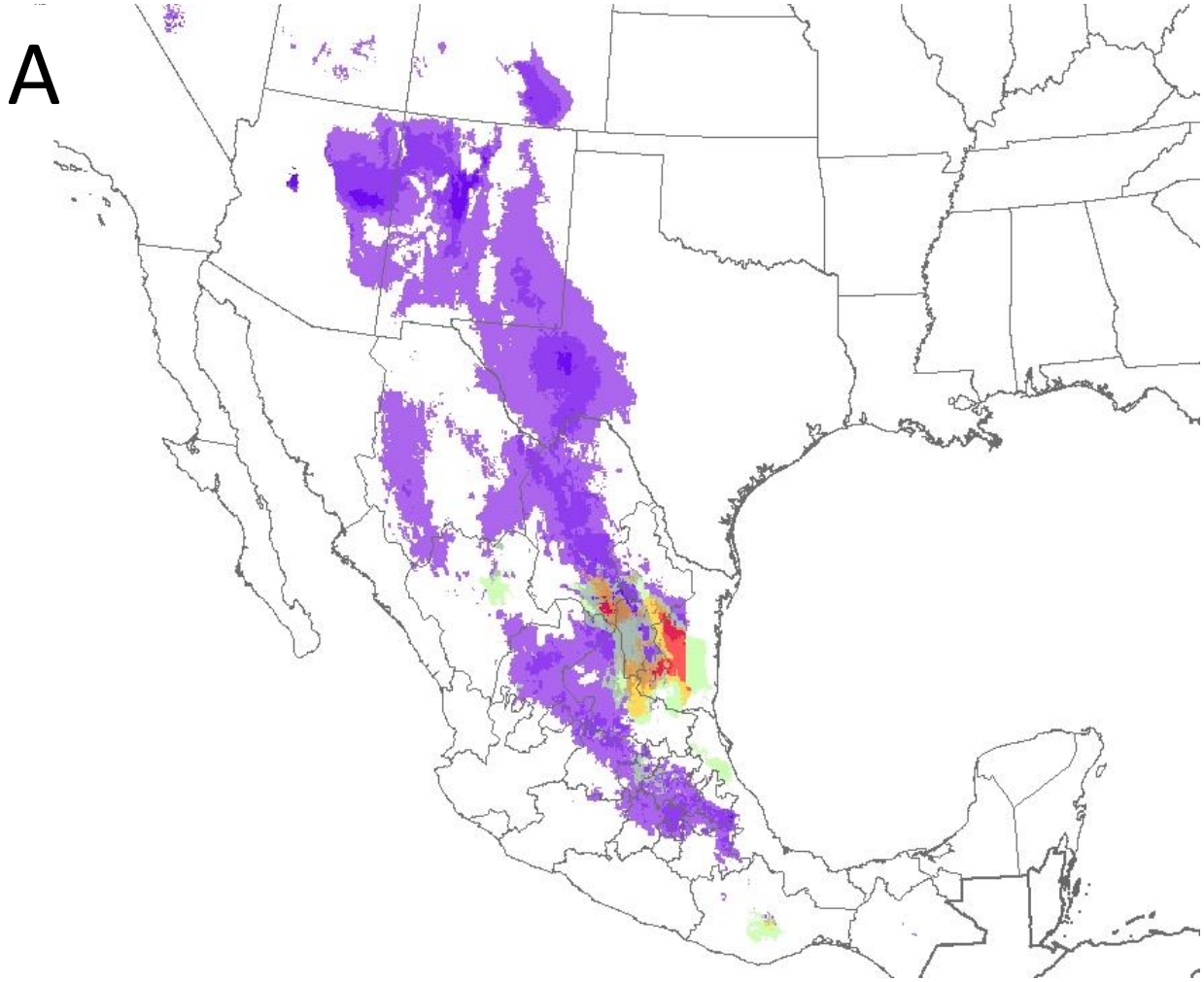


Figure 3.5 Projected species distribution models for *P. multivirgatus* (purples) and *P. t. brevilineatus* (yellow to red) in the LGM, darker colors suggest higher habitat suitability. A. CCSM model, B. MIROC model. Each show a different zone of overlap, but in each case the most suitable habitats for *P. t. brevilineatus* are almost entirely encompassed by the range of *P. multivirgatus*.

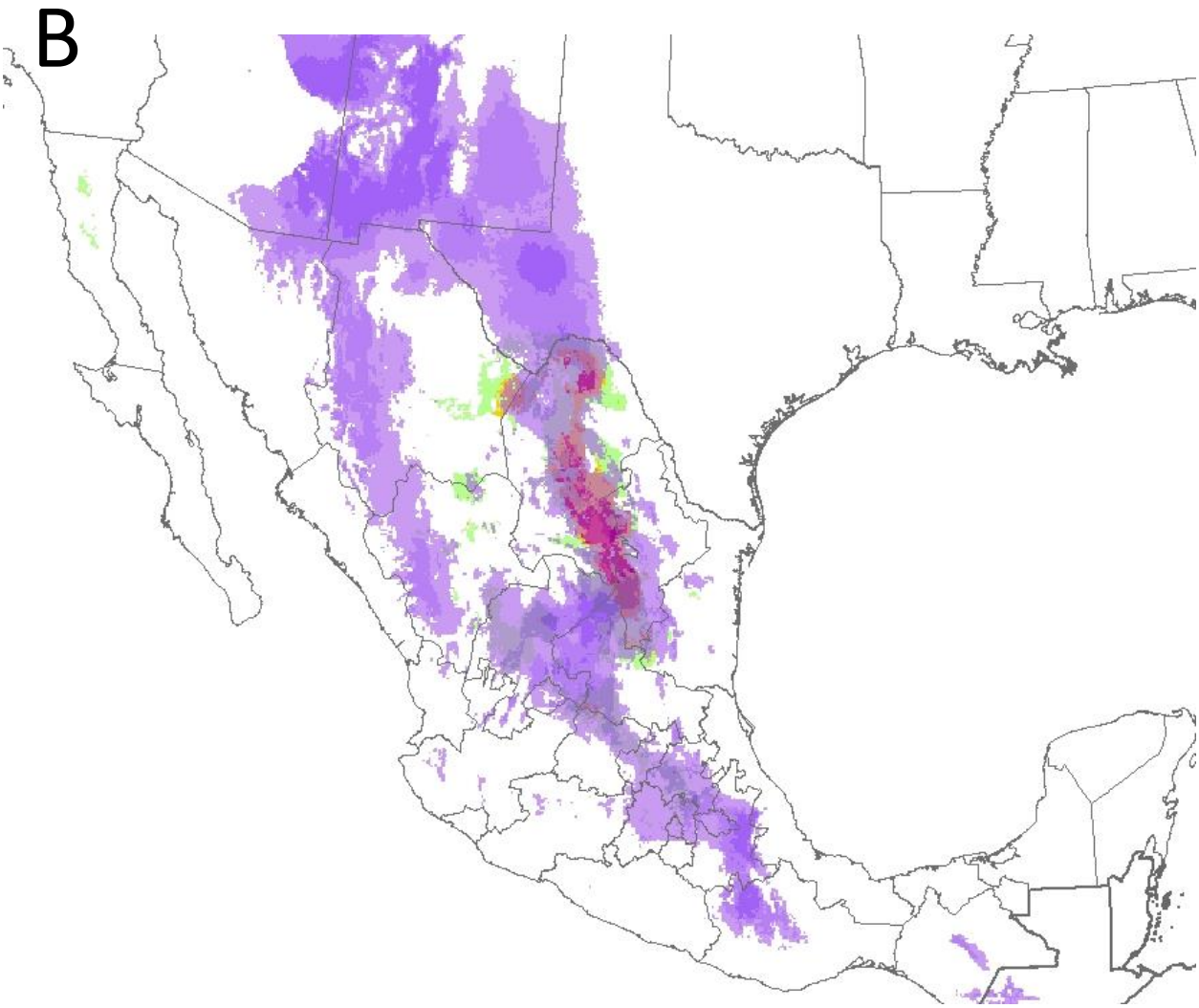

B

Table S1 Samples used in this study with collection localities corresponding to localities in Ch. 2 and Fig. 1.

Locality Number	Voucher ID	Taxon ID	Country: State	County	GPS N	GPS W
12	CLC750	*Plestiodon tetragrammus brevilineatus*	USA:TX	Crockett	31.03063	-100.99696
12	CLC751	*P. t. brevilineatus*	USA:TX	Crockett	31.03063	-100.99696
17	JAC26325	*P. t. brevilineatus*	Mexico:Coahuila		27.876098	-101.511817
17	JAC26395	*P. t. brevilineatus*	Mexico:Coahuila		27.876098	-101.511817
14	JWS 057	*P. t. brevilineatus*	USA:TX	Stephens	32.68166	-99.0514
9	TNHC61293	*P. t. brevilineatus*	USA:TX	ValVerde	29.935425	-100.943483
9	TNHC61360	*P. t. brevilineatus*	USA:TX	ValVerde	29.935425	-100.943483
11	TNHC53241	*P. t. brevilineatus*	USA:TX	Real	29.95	-99.963889
6	TNHC53240	*P. t. brevilineatus*	USA:TX	SanSaba	31.024276	-98.441927
8	TNHC65483	*P. t. brevilineatus*	USA:TX	Travis	30.29545	-97.908417
10	TNHC64025	*P. t. brevilineatus*	USA:TX	Edwards	29.79562	-100.609899
15	TNHC67003	*P. t. brevilineatus*	USA:TX	Presidio	30.530065	-104.697321
15	TNHC67348	*P. t. brevilineatus*	USA:TX	Presidio	30.549167	-104.662222
15	TNHCGDC14027	*P. t. brevilineatus*	USA:TX	Presidio	30.566416	-104.712713
7	TNHC60585	*P. t. brevilineatus*	USA:TX	Llano	30.554078	-98.603218
13	TNHC84900	*P. t. brevilineatus*	USA:TX	Sterling	31.55035	-100.9573667
16	TJL643	*P. t. brevilineatus*	USA:TX	Jeff Davis	30.811158	-103.944026
1	CLC372	*P. t. tetragrammus*	USA:TX	Hidalgo	26.23279	-98.46513
4	CLC625	*P. t. tetragrammus*	USA:TX	Duval	27.76846	-98.2472
5	CLC651	*P. t. tetragrammus*	USA:TX	Duval	27.59283	-98.46726
5	CLC652	*P. t. tetragrammus*	USA:TX	Duval	27.59283	-98.46726
5	CLC695	*P. t. tetragrammus*	USA:TX	Duval	27.60302	-98.41345
2	JWS236	*P. t. tetragrammus*	USA:TX	Starr	26.26806	-98.58724
2	JWS238	*P. t. tetragrammus*	USA:TX	Starr	26.28145	-98.62458
3	JWS244	*P. t. tetragrammus*	USA:TX	Cameron	26.21476	-97.72061
3	JWS245	*P. t. tetragrammus*	USA:TX	Cameron	26.21476	-97.72061
12	CLC744	*P. multivirgatus*	USA:TX	Crockett	31.03063	-100.99696
12	CLC745	*P. multivirgatus*	USA:TX	Crockett	31.03063	-100.99696
12	CLC746	*P. multivirgatus*	USA:TX	Crockett	31.03063	-100.99696
12	CLC747	*P. multivirgatus*	USA:TX	Crockett	31.03063	-100.99696
12	CLC748	*P. multivirgatus*	USA:TX	Crockett	31.03063	-100.99696
12	CLC749	*P. multivirgatus*	USA:TX	Crockett	31.03063	-100.99696
19	ADL274	*P. multivirgatus*	USA: CO	Montezuma	37.52	-108.70122
18	TNHCFS05485	*P. multivirgatus*	USA: AZ	Coconino	35.08886	-111.681106
	CLC897	*P. t. brevilineatus*	USA: TX	Callahan	32.14929	-99.42226
	MAM033	*P. t. brevilineatus*	USA: TX	Stephens	32.6509	-98.579072
	MAM055	*P. septentrionalis*	USA: TX	Parker	32.88902	-98.018581

MAMT001	*P. multivirgatus*	USA: TX	Crockett	31.03063	-100.99696
MAMT002	*P. t. brevilineatus*	USA:TX	Crockett	31.03063	-100.99696
USNH-561174	*P. multivirgatus*	USA: NE	Kimball	41.241111	-103.508889
USNH-561175	*P. multivirgatus*	USA: NE	Kimball	41.241111	-103.508889
UTADC8112	*P. t. brevilineatus*	USA: TX	Archer	33.431389	-98.733889

Table S2 Primer information and sources of the initial primer sequence.

Gene	Primer Name	Sequence (5'- 3')	Source
12S	tPHE	AAAGCACRGCACTGAAGATGC	Wiens and Reeder (1997)
	12e	GTRCGCTTACCMTGTTACGACT	Wiens and Reeder (1997)
16S	16aR2	CCCGMCTGTTTACCAAAAACA	Schmitz et al. (2005)
	16d	CTCCGGTCTGAACTCAGATCACGT	Reeder (1995)
ND1	INTF3	ATAATRTGRTTYATYTCNACNCTAGCAGA	Leache and Reeder (2002)
	tMET	TCGGGGTATGGGCCCRARAGCTT	Brandley et al. (2005)
c-mos	FU-F	TTTGGTTCKGTCTACAAGGCTAC	Gamble et al. (2008)
	FU-R	AGGGAACATCCAAAGTCTCCAAT	Gamble et al. (2008)
BDNF	BDNF-F	GACCATCCTTTTCCTKACTATGGTTATTTCATACTT	Brandley et al. (2011)
	BDNF-R	CTATCTTCCCCTTTTAATGGTCAGTGTACAAAC	Brandley et al. (2011)
PRLR	PRLR_f1	GACARYGARGACCAGCAACTRATGCC	Brandley et al. (2011)
	PRLR_r3	GACYTTGTGRACTTCYACRTAATCCAT	Brandley et al. (2011)
SNCAIP	SNCAIP_f10	CGCCAGYTGYTGGGRAARGAWAT	Brandley et al. (2011)
	SNCAIP_r13	GGWGAYTTGAGDGCACTCTTRGGRCT	Brandley et al. (2011)

Mitochondrial genes: 12S, 12S ribosomal RNA; 16S, 16S ribosomal RNA; ND1, NADH dehydrogenase 1. Nuclear genes: *c-mos*, oocyte maturation factor; *BDNF*, brain derived neurotropin factor; *PRLR*, prolactin-like receptor; *SNCAIP*, synuclein alpha interacting protein.